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Diversity of plants with leaves cut by bees of the genus *Megachile* in Singapore

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Abstract. A preliminary study of leaves cut by *Megachile* leafcutter bees were documented in Singapore through photographic surveys from March to August 2014 (86 observations), pressed leaves from 2016 to 2019 (51 observations), and crowd-sourced photographs from 2017 to 2019 (24 observations). Of the 161 plant observations, 130 were identified to species (64 species), 7 to genus (five genera), and 24 undetermined. There were 67 distinct plant species or morphospecies (64 species; 3 genera— *Connarus*, *Diospyros*, and *Rosa*) from 59 genera and 21 families. The top two plant species with the highest number of observations were *Dendrolobium umbellatum* (Fabaceae; 11 observations) and *Cratoxylum cochinchinense* (Hypericaceae; 10 observations), which collectively accounted for a substantial proportion of the identified observations (15.3%; 21 out of 137). These plants are also floral associates of *Megachile* bees, thus, planting these species may augment pollinator populations. The highest proportion of plant species are from the Fabaceae (22 out of 67 species). These findings suggest that *Megachile* leafcutter bees do select for leaves of specific plants in Singapore. The majority of these plants were trees (42 out of 64 species), whereas 13 species were shrubs, and remaining nine were climbers, suggesting the bees can forage at a variety of heights. A total of 40.6% of plant species (26 out of 64 species) were exotic to Singapore, demonstrating that *Megachile* leafcutter bees utilise both native and exotic plant species. Five new bee–leaf associations at the species level were also recorded when a bee was directly observed cutting a leaf. These findings could have further implications for bioprospecting pertaining to antimicrobial properties of the leaves.

Key words. pollinators, plant–bee association, Fabaceae, bioprospecting, leaf damage, Anthophila, Megachilidae

INTRODUCTION

Bees from the family Megachilidae are important pollinators. In fact, several species are used commercially. *Megachile rotundata* is used in alfalfa pollination in the USA owing to its high pollination efficiency and ease of management (Pitt-Singer & Cane, 2011), while *Osmia cornifrons*, *Osmia lignaria*, and *Osmia cornuta* are used as pollinators for rosaceous fruit trees such as almond, apple, peach, and plum (Sedivy & Dorn, 2013). Contrary to corbiculate bees (e.g., honey bees) which store pollen in their pollen baskets (corbiculae), megachilid bees collect and store pollen on the ventral side of the abdomen (Michener, 2007). For this reason, they are efficient pollinators of plants where their scopal hairs can be in full contact with the flower's stigma (Sedivy & Dorn, 2013). In Singapore, they are floral associates of native plants such as *Cratoxylum cochinchinense*, *Dendrolobium umbellatum*, and *Memecylon* species (Ascher et al., 2016) as well as the reintroduced *Grammatophyllum speciosum* (Z. Soh et al., in preparation). Therefore, understanding the bees' natural history, such as their nesting resources, would be foundational to augment pollinator populations that are crucial to the natural ecosystem as well.

In Singapore, *Megachile* is the most speciose megachilid genus with 20 species belonging to seven subgenera (Ascher et al., 2016), as it is globally (Michener, 2007). These subgenera belong to three distinct groups: *Aethomegachile*, *Eutricharaea*, *Paracella* in group 1 (nine species), the true leafcutters; *Chelostomoda*, *Callomegachile*, and *Carinula* (placed under *Callomegachile* in Ascher et al. [2016]) in group 2 (10 species), the resin bees; and *Creightonella* in group 3 (one species), the atypical leafcutters (Michener, 2007; Praz, 2017). Here, we refer to the *Megachile* leafcutter bees as those from groups 1 and 3 (10 species). Bees from group 1 have a cutting edge on their mandibles that cuts out leaf blade pieces with clean, smooth edges. Bees from group 3 have an incomplete cutting edge on their mandibles that cuts out leaf pieces with jagged edges. The subgenus *Chelostomoda* is an exception to group 2 as it uses leaf pulp and irregularly-cut leaves to line its nest (Michener, 2007), however, they were excluded from this study as the shape of their leaf cuts are different from group 1 and 3.

By and large, female *Megachile* cut leaf blades with their mandibles to line their nests. Certain *Megachile* species also utilise petals (Zillikens & Steiner, 2004). More recently, man-made material such as plastic have also been recorded to be used (MacIvor & Moore, 2013; Allasino et al., 2019). The leafcutter bees make repeated trips to the leaf to cut and carry the leaf pieces with their mandibles (Fig. 1). *Megachile* leafcutter bees typically make their nests in the soil or rent pre-existing cavities (Michener, 2007), where their young may be vulnerable to predators or moisture that may promote microbial growth (MacIvor, 2016). In the nest, the leafcutter bee folds the cut piece of leaf blade to line its brood cell,

Fig. 1. Singaporean *Megachile* leafcutter bees of group 1 carrying the cut leaves. A, *Megachile* (*Eutricharaea*) *subrixator*; B, *M.* (*Paracella*) *tricincta*; C, *M.* (*Aethomegachile*) *conjuncta*; D, *Megachile* (*Aethomegachile*) *laticeps*. These bees are rarely seen cutting their host plant but can be photographed coming to-and-fro their nesting site. (Photographs by: Zestin W. W. Soh).

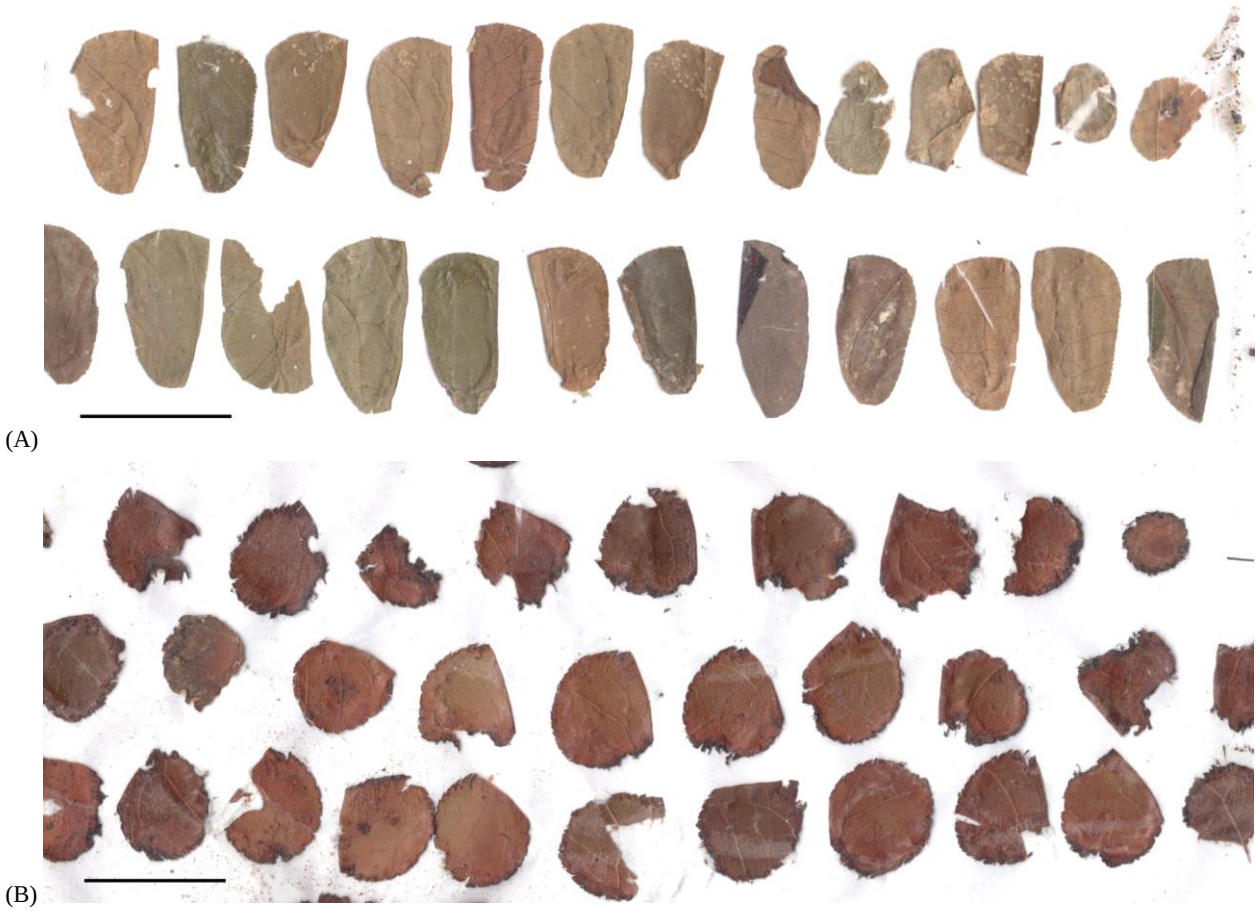
which encloses a single egg and its food provisioning (pollen and nectar; see photographs in Soh [2014]). The leaf blade pieces act as a physical barrier for the egg, larvae, or pupae, and may also offer other protective features against microbial growth.

Similar studies have documented the diversity of leaf usage by *Megachile* bees in Toronto, Canada (MacIvor, 2016); Cuba (Genero, 1996); North Malabar, Kerala, southwest India (Kambli et al., 2017); Puławy, Poland (Bilinski et al., 1980) and Arizona, USA (Sinu & Bronstein, 2018). The latest study (Sinu & Bronstein, 2018) also includes a combined analysis of the Toronto and North Malabar studies. This present study contributes to novel knowledge of *Megachile*–plant associations in the Southeast Asian tropics.

MATERIAL & METHODS

Megachile bees are not commonly observed in the act of cutting the leaves of their host plants (Soh et al., 2018), thus, cut leaves were surveyed instead. The cuts are uniquely characterised by an oblong shape (for cell lining) or a smaller, circular shape (for cell cap or nest closure) for *Megachile* group 1, and circular, jagged cuts for *Megachile* group 3 (Fig. 2). Thus, these cuts differ strikingly from other forms of leaf herbivory. There is typically more than one cut on a leaf as well, unless the leaf blade is the size of the cut. The cuts were made by leafcutter *Megachile* bees in Singapore (see Ascher et al. [2016] for a list of species).

All observations in this study were from Singapore. The first part of the study took place March–August 2014 at 13 localities by EJYS (Fig. 3, except one record of unknown locality, see Table 1). Diagnostic photographs of the plants cut by *Megachile* leafcutter bees were taken. These photographs were then identified to genus or species by HTWT. The second part of the study took place from 2016–2019, where cut leaves were collected, pressed in a book, and scanned digitally. The third part of the study comprised of crowd-sourced photographs from 2017–2019. Specimens and photographs from the second and third part were collated by ZWWS, and they were identified to species by him as well as colleagues from the National Parks Board (NParks). Each cut plant was counted only once as a proxy for a cutting event and they were considered independent observations. It is unlikely for observations to be double-counted, as each



(A)

(B)

Fig. 2. Leaves from the nest of (A) *Megachile* (*Aethomegachile*) *laticeps* from *Megachile* group 1 and (B) *M.* (*Creightonella*) *atrata* from *Megachile* group 3. Leaves used to line the side of the brood cell are all circular and jagged for *Megachile* group 3 but are not for *Megachile* group 1 which are elongate or circular with clean edges. Scale bar = 2 cm. (Images by: E. J. Y. Soh).

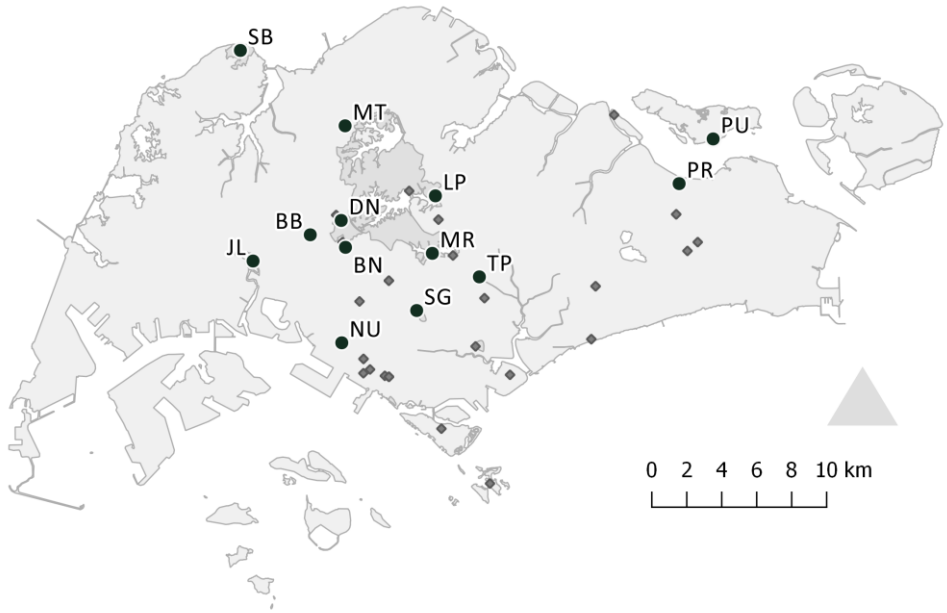


Fig. 3. Map of Singapore with 13 sampling sites from March–August 2014 (black circles), and georeferenced localities of pressed leaf specimens and crowd-sourced observations from 2016 – 2019 (grey diamonds). Legend: BB – Bukit Batok (FF), BN – Bukit Timah Nature Reserve (FR), DN – Dairy Farm Nature Reserve (FR), JL – Jurong Lake Gardens (SU), LP – Lower Peirce Reservoir Park (FR), MR – MacRitchie (FR), MT – Mandai Track 15 (FR), NU – National University of Singapore and surrounding areas (SU), PR – Pasir Ris Park (MG), PU – Pulau Ubin (IS), SG – Singapore Botanic Gardens (SU), SB – Sungei Buloh Wetland Reserve (MG), TP – Toa Payoh Town Park (SU). (Projection: SVY21; Singapore base map by: Teo Siyang).

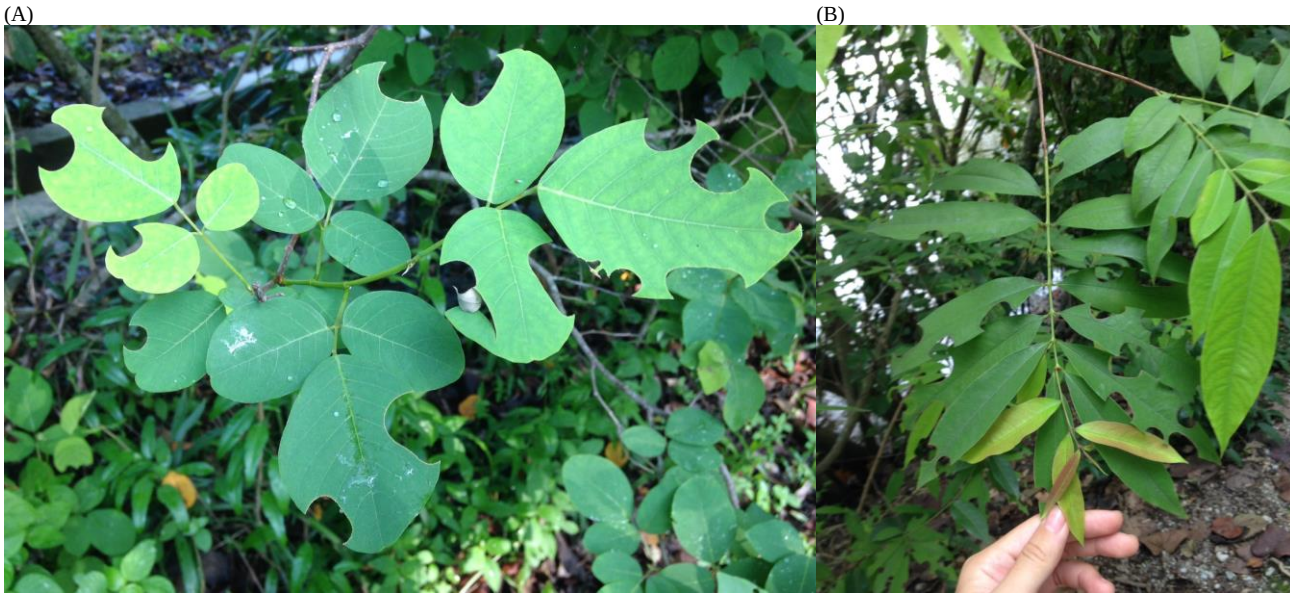


Fig. 4. Plants with the highest number of observations of cuttings. A, *Dendrolobium umbellatum* (Fabaceae); B, *Cratoxylum cochinchinense* (Hypericaceae). (Photographs by: E. J. Y. Soh).

site was only assessed for leaf damage once for each plant, or were temporally separated by more than two years. If the bee was seen cutting the leaves of the plant, the bee species was noted as well. The observations came from varied localities across Singapore, comprising urban (e.g., high-rise housing estates, community gardens), semi-urban (e.g., parks) and forest habitats as well as islands off the coast of Singapore Island (Fig. 3).

Subsequently, plant characteristics were obtained from the website, NParks Flora and Fauna Web (2019) and Chong et al. (2009). The native or exotic status of the plant species were based solely on Chong et al. (2009). Plant growth form was categorised based on its predominant growth form and consensus between NParks Flora and Fauna Web (2019) and Chong et al. (2009). No distinction was made between woody and non-woody climbers.

RESULTS & DISCUSSION

Diversity of plant species. A total of 161 plant observations were made, comprising 130 identified to species (64 species), 7 to genus (five genera), and 24 undetermined (Table 1). These were based on photographic surveys for the period of March–August 2014 (86 observations), pressed leaves from 2016 to 2019 (51 observations) and crowd-sourced photographs from 2017 to 2019 (24 observations) across Singapore (Fig. 3). The plant species with the largest number of observations included *Dendrolobium umbellatum* (Fabaceae) and *Cratoxylum cochinchinense* (Hypericaceae), with 11 and 10 observations respectively (Fig. 4, Table 1) and accounted for 15.3% (21 out of 137) of the identified observations in this study. Since *Megachile* are flower visitors of *Cratoxylum cochinchinense* and *Dendrolobium umbellatum* in tropical Asia (Kato et al., 2008; Soh, 2014), it is possible that they also use the same plant to cut its leaves.

Of these observations, there were 67 distinct species or morphospecies in 59 genera and 21 families (Fig. 5, Table 2). All species were angiosperms except a single gymnosperm (*Gnetum gnemon*). The highest proportion of species (22 out of 67) were from the family Fabaceae (Table 2) from four subfamilies (Caesalpinioideae, Detarioideae, Papilionoideae, and Cercidoideae; LPWG [2017]). *Megachile* leafcutter bees are known to cut the leaves of the Fabaceae (Bilinski et al., 1980; MacIvor, 2016; Sinu & Bronstein, 2018), Fabales (Genaro, 1996), or Rosaceae (Chang et al., 1989; MacIvor, 2016). The family Rosaceae is not speciose in Singapore (~8 species) though ornamental *Rosa* species were observed to be cut in two instances. Conversely, there are more species of the Fabaceae in Singapore (~258 plant species; Chong et al. [2009]). *Megachile* species are known floral associates of the Fabaceae in tropical Asia (Momose et al., 1998; Kato et al., 2008) and these floral associates would possibly also be their leaf associates if leaves are favourable for cutting. However, certain Fabaceae species with cut leaves (e.g., *Senna alata*, *Phanera kockiana*, and *Phanera sembifida*) are not floral associates of Singapore *Megachile* species as their flowers are non-papilionaceous (Z. W. W. Soh, pers. obs.), suggesting that Fabaceae leaves are selected for their leaves owing to the plants' leaf traits as well. There were no cut leaves of the Asteraceae in this study, which is similar to findings in Sinu & Bronstein (2018) even though the bees are floral associates of some species in Singapore (e.g., *Bidens pilosa*, *Pluchea indica*, *Tridax procumbens*, *Sphagneticola trilobata* [Insect Diversity Laboratory, unpublished data]), though MacIvor (2016) found three Asteraceae species in his study. Only a subset of plants at a site were observed to have their leaves cut (E. J. Y. Soh & Z. W. W. Soh, pers. obs.). These suggest that these bees do select for the leaves of particular plant species. The species list in this study is also likely

Table 1. Number of observations for each plant species that was cut by *Megachile* leafcutter bees from first, second and third part of the study. The first study is further broken down into sites with habitat types in brackets. For the sites, the following abbreviations apply: BB – Bukit Batok (FF), BN – Bukit Timah Nature Reserve (FR), DN – Dairy Farm Nature Reserve (FR), JL – Jurong Lake Gardens (SU), LP – Lower Peirce Reservoir Park (FR), MR – MacRitchie (FR), MT – Mandai Track 15 (FR), NU – National University of Singapore and surrounding areas (SU), PR – Pasir Ris Park (MG), PU – Pulau Ubin (IS), SG – Singapore Botanic Gardens (SU), SB – Sungei Buloh Wetland Reserve (MG), TP – Toa Payoh Town Park (SU), U – unknown locality. For habitat types, the following abbreviations apply: FR – Forest reserve, FF – Forest fragment, MG – Mangrove, CS – Coastal scrub, IS – Island, SU – Semi-urban, UR – Urban.

		Part 1															
Species	**N records**	**BN (FR)**	**DN (FR)**	**LP (FR)**	**MR (FR)**	**MT (FR)**	**BB (FF)**	**PR (MG)**	**SB (MG)**	**PU (IS)**	**JL (SU)**	**NU (SU)**	**SG (SU)**	**TP (SU)**	**U**	**Part 2**	**Part 3**
Undetermined	24	10			3	3	1		1							6	
Identified to genus	7	2				1										2	2
1. *Syzygium* (Myrtaceae)	2	1				1											
2. *Rosa* (Rosaceae)	2																2
3. *Connarus* (Connaraceae)	1	1															
4. *Diospyros* (Ebenaceae)	1															1	
5. *Dalbergia* (Fabaceae)	1															1	
Identified to species	130	7	5	1	4	1	1	3	14	2	2	18	5	1	1	43	22
1. *Dendrolobium umbellatum* (Fabaceae)	11								1	1		2	1			4	2
2. *Cratoxylum cochinchinense* (Hypericaceae)	10	1				1			3							5	
3. *Mallotus paniculatus* (Euphorbiaceae)	6				1				1			3					1
4. *Phanera kockiana* (Fabaceae)	6						1					3				1	1
5. *Archidendron clypearia* (Fabaceae)	5	2		1												1	1
6. *Caesalpinia crista* (Fabaceae)	5								4							1	
7. *Phanera semibifida* (Fabaceae)	5	1	1										1			1	1
8. *Senna alata* (Fabaceae)	5							1			1					2	1
9. *Macaranga heynei* (Euphorbiaceae)	3	1			1												1
10. *Clitoria ternatea* (Fabaceae)	3											1				1	1
11. *Vitex pinnata* (Lamiaceae)	3								1			1				1	
12. *Neolitsea cassia* (Lauraceae)	3															3	
13. *Syzygium polyanthum* (Myrtaceae)	3											1				2	
14. *Maranthes corymbosa* (Chrysobalanaceae)	2															1	1
15. *Terminalia catappa* (Combretaceae)	2								1							1	
16. *Albizia saman* (Fabaceae)	2											1					1
17. *Archidendron jiringa* (Fabaceae)	2															1	1
18. *Bauhinia galpinii* (Fabaceae)	2		2														
19. *Pterocarpus indicus* (Fabaceae)	2								1			1					
20. *Cratoxylum formosum* (Hypericaceae)	2											1				1	
21. *Cinnamomum iners* (Lauraceae)	2									1						1	
22. *Litsea umbellata* (Lauraceae)	2															2	
23. *Pternandra echinata* (Melastomataceae)	2	1			1												
24. *Breynia disticha* (Phyllanthaceae)	2															1	1
25. *Quisqualis indica* (Combretaceae)	1											1					
26. *Shorea curtisii* (Dipterocarpaceae)	1															1	
27. *Shorea foxworthyi* (Dipterocarpaceae)	1																1
28. *Erythroxylum cuneatum* (Erythroxylaceae)	1							1									

		Part 1															
Species	**N records**	**BN (FR)**	**DN (FR)**	**LP (FR)**	**MR (FR)**	**MT (FR)**	**BB (FF)**	**PR (MG)**	**SB (MG)**	**PU (IS)**	**JL (SU)**	**NU (SU)**	**SG (SU)**	**TP (SU)**	**U**	**Part 2**	**Part 3**
29. *Macaranga conifera* (Euphorbiaceae)	1	1															
30. *Adenanthera pavonina* (Fabaceae)	1																1
31. *Amherstia nobilis* (Fabaceae)	1															1	
32. *Baphia nitida* (Fabaceae)	1												1				
33. *Bauhinia tomentosa* (Fabaceae)	1															1	
34. *Crotalaria pallida* (Fabaceae)	1																1
35. *Cynometra malaccensis* (Fabaceae)	1															1	
36. *Dalbergia latifolia* (Fabaceae)	1													1			
37. *Dalbergia rostrata* (Fabaceae)	1															1	
38. *Derris trifoliata* (Fabaceae)	1								1								
39. *Dialium platysepalum* (Fabaceae)	1																1
40. *Lonchocarpus cyanescens* (Fabaceae)	1															1	
41. *Phyllodium longipes* (Fabaceae)	1															1	
42. *Sindora wallichii* (Fabaceae)	1															1	
43. *Gnetum gnemon* (Gnetaceae)	1														1		
44. *Ceiba pentandra* (Malvaceae)	1												1				
45. *Sterculia oblongata* (Malvaceae)	1																1
46. *Taliparitiliaceum* (Malvaceae)	1								1								
47. *Memecylon edule* (Melastomataceae)	1											1					
48. *Plinia cauliflora* (Myrtaceae)	1															1	
49. *Psidium guajava* (Myrtaceae)	1							1									
50. *Rhodamnia cinerea* (Myrtaceae)	1																1
51. *Rhodomyrtus tomentosa* (Myrtaceae)	1											1					
52. *Tristaniopsis whiteana* (Myrtaceae)	1															1	
53. *Cleistanthus malaccensis* (Phyllanthaceae)	1																1
54. *Cleistanthus polyphyllus* (Phyllanthaceae)	1																1
55. *Antigonon leptopus* (Polygonaceae)	1															1	
56. *Mussaenda glabra* (Rubiaceae)	1										1						
57. *Pertusadina eurhyncha* (Rubiaceae)	1				1												
58. *Unicaria gambir* (Rubiaceae)	1																1
59. *Clausena excavata* (Rutaceae)	1											1					
60. *Allophylus cobbe* (Sapindaceae)	1															1	
61. *Guioa pleuropteris* (Sapindaceae)	1															1	
62. *Lepisanthes alata* (Sapindaceae)	1												1				
63. *Nephelium lappaceum* (Sapindaceae)	1		1														
64. *Pometia pinnata* (Sapindaceae)	1		1														
Total	161																

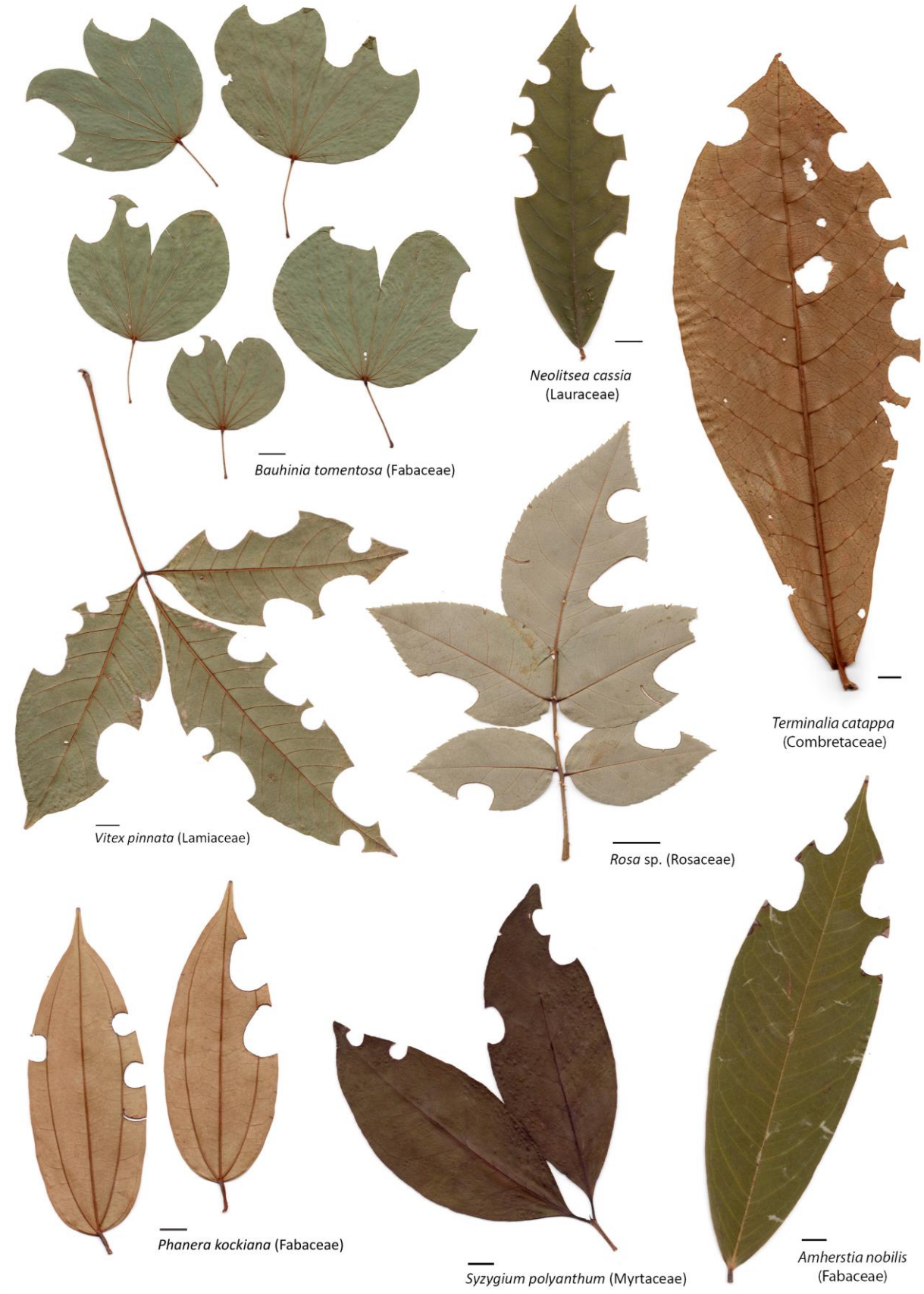


Fig. 5. A selection of pressed leaves cut by *Megachile* leafcutter bees in Singapore. Plant species come from a variety of leaf shape and sizes. All the leaves comprise of cuts of *Megachile* group 1 except for the *Terminalia catappa* leaf that has cuts from *Megachile* group 3. *Megachile* group 1 cuts are characterised by circular and elongate cuts as opposed to group 3 which have jagged edges and are only circular. Scale bar = 1 cm. (Images by: E. J. Y. Soh).

Table 2. Number of plant species with leaves cut by *Megachile* leafcutter bees in 21 families.

Family	No. of species
Fabaceae	24
Myrtaceae	6
Sapindaceae	5
Euphorbiaceae	3
Lauraceae	3
Malvaceae	3
Phyllanthaceae	3
Rubiaceae	3
Combretaceae	2
Dipterocarpaceae	2
Hypericaceae	2
Melastomataceae	2
Chrysobalanaceae	1
Connaraceae[+]	1
Ebenaceae[+]	1
Erythroxylaceae	1
Gnetaceae	1
Lamiaceae	1
Polygonaceae	1
Rosaceae[+]	1
Rutaceae	1
Total	**67**

[+]Plant family with only one genus and one morphospecies

to be preliminary and incomplete, as there is a great diversity of plants (3,941 Angiosperm and 40 Gymnosperm species) found in Singapore (Chong et al., 2009).

The large majority (42 out of 64 species) of the plants were trees, whereas the rest were shrubs (13 species) or climbers (nine species). *Megachile conjuncta* and *Megachile* (*Eutricharaea*) sp. 1 were observed cutting leaves of *Clitoria ternatea* and *Crotalaria pallida*, respectively on the 7th and 9th storeys of high-rise buildings. These observations suggest that *Megachile* species can forage for leaves at different heights though previously shown not to prefer great heights, i.e., more than 6 storeys of a building (MacIvor, 2015). Overall, 40.6% of (26 out of 64) plant species were exotic to Singapore, indicating that *Megachile* species can utilise both native and exotic species, similar to findings of Sinu & Bronstein (2018) where 32–45% of plant species whose leaves were cut by *Megachile* were exotic.

Leaf traits. Existing studies have suggested *Megachile* species avoid leaves that are high in saponines (Morato & Martins, 2006) but preferred glabrous leaf blades, leaves with a lower water content (as a proxy for leaf toughness) and no leaf latex (Sinu & Bronstein, 2018). The two plants with the highest number of observations in this study, *Cratoxylum cochinchinense* and *Dendrolobium umbellatum*, have glabrous leaf blades. The leaves or leaflets were also of varying toughness, from soft (*Dendrolobium umbellatum*) to relatively tough (young leaves of *Terminalia catappa*). We did not make observations for leaf sap in this study. Sinu & Bronstein (2018) also noted that leaf or leaflet shape and size were not important traits while in this study, varying leaf shapes were observed (Fig. 5). Small leaflets of *Clitoria ternatea* were cut, suggesting that leaf or leaflet size is not crucial unless it is too small (e.g., *Mimosa pudica*).

Future studies. To summarise, crucial leaf traits likely include the presence of chemicals used in plant defence (e.g., saponines or tannins; see Sinu & Bronstein [2018] for further discussion), glabrous nature of the leaf blade, leaf blade toughness and presence of leaf sap. Leaf size, leaf blade shape, plant growth form (e.g., tree, shrub, climber), height of plant and woody nature of plant are not likely to be important factors (MacIvor, 2016; Sinu & Bronstein, 2018). Softer leaf blades may be preferred but bees may be able to use tougher ones as well. Future work comparing traits of plant species with leafcutter bee cut leaves and without cuts could be done to test these hypotheses. In addition, incorporating phylogeny of the diverse Fabaceae (LPWG, 2017) could shed light on particular clades and their shared foliar and floral traits that the *Megachile* prefer.

Testing for antimicrobial properties (i.e., antifungal and antibacterial properties) of species used by *Megachile* may also result in the discovery of novel antimicrobial compounds that may be useful in medicine. Messer (1985) showed the resinous nest lining of *Megachile pluto* had antifungal properties while MacIvor (2016) showed that all, except 6 out of 48 plant species utilised by *Megachile* species, have at least one antimicrobial compound.

Fig. 6. Bracts (modified leaves) of *Bougainvilllea* species cut by Megachile leafcutter bee, observed by Jacqueline Chua at a rooftop community garden, at Jurong East St 32 on 2 May 2019. (Photograph by: Jacqueline Chua).

We also anecdotally noted that *Megachile* groups 1 and 3 have differing plant preferences (Fig. 2). Circular cuts with jagged edges, indicative of *Megachile* group 3, had been observed for *Psidium guajava* (Myrtaceae), *Terminalia catappa* (Combretaceae), and *Archidendron jiringa* (Fabaceae). Individuals of *Megachile* (*Creightonella*) *atrata*, the only Singapore species in group 3, were also observed nesting in mud lobster mounds of the landward side of mangrove forest sites at Pasir Ris Park in 2014 (J. S. Ascher & E. J. Y. Soh, pers. obs.). Thus, it is likely that their nesting requirements would differ from the cavity renters of group 1, and could be hypothesised that they would likely prefer leaves that are more hydrophobic (e.g., with waxy surfaces). Future studies could include elucidating the nesting substrate of each *Megachile* species as it is currently not known for all species. For example, an individual of *Megachile* (*Eutricharaea*) species (group 1) was only recently ascertained to nest in soil (Ang, 2019). After nesting substrates are better documented, comparing the leaf traits of cavity-renters from group 1 and group 3 *Megachile* ground-nesters would be possible.

Lastly, we studied the bee–plant interactions at the genus level of the bee. To better understand the requirements and preferences of nest lining resources for the *Megachile* leafcutter bees, species-level interactions are required as current observations may be driven by commonly-occurring *Megachile* species (see Table 3, no. of records). Firstly, this has particularly important implications for forest-associated species which may exhibit a greater specialisation on particular plant species. Secondly, the converse is so for urban-adapted species whose nest linings may extent to atypical resources as well. For example, towards the end of the study, cut bracts (modified leaves) of *Bougainvilllea* species were observed by Jacqueline Chua at a rooftop community garden, at Jurong East St 32 on 2 May 2019 (Fig. 6). Thirdly, we observed

Table 3. List of *Megachile* leafcutter bees and their associated plant species whose leaves they cut where known. Further information on *Megachile* leafcutter species such as intertegular distance (ITD), site and habitat information (records from 1979 to 2018) are provided as ancillary information from Ascher et al. (in preparation, forthcoming paper on checklist and conservation assessment of bees in Singapore). All data presented are based on specimens or observations from Singapore. For habitat type, the following abbreviations apply: FR – Forest reserve, FF – Forest fragment, MG – Mangrove, CS – Coastal scrub, IS – Island, SU – Semi-urban, UR – Urban.

				Habitat type							
Species	**ITD (mm)**	**No. of records**	**No. of sites**	**FR**	**FF**	**MG**	**CS**	**IS**	**SU**	**UR**	**Plant species**
Group 1											
Megachile (*Aethomegachile*) *borneana*[32]	2.550	5	3	2	1						Unknown
Megachile (*Aethomegachile*) *conjuncta*	3.643	25	17	1	2	3		1	5	5	*Clitoria terneata*
Megachile (*Aethomegachile*) *laticeps*	3.641	196	34	7	4	2	1	2	10	8	*Dendrolobium umbellatum* (Soh et al., 2018), *Neolitsea cassia*, *Rosa* sp.
Megachile (*Aethomegachile*) *ramera*[18]	4.160	5	2	2							Unknown
Megachile (*Aethomegachile*) nr. *borneana*	2.880	5	3	2	1						*Uncaria* sp. (Ascher et al., 2016)
Megachile (*Aethomegachile*) sp. [*fusciventris* group]	3.760	19	4	3	1						Unknown
Megachile (*Paracella*) *tricincta*	2.724	47	7	2				2	2	1	Unknown
Megachile (*Eutricharaea*) *subrixator*	2.260	155	30	4	4	2		4	10	6	*Antigonon leptopus*
Megachile (*Eutricharaea*) sp. 1[+]	2.325	65	16			1		2	8	5	*Crotalaria pallida*
Group 3											
Megachile (*Creightonella*) *atrata*	4.680	61	14	1	1	1		4	4	3	Unknown

* Indicated as 1854 to 1856 in records

[+]See Ascher et al. (2016) for information on species

a high number of cuts for two plant species, suggesting a graded preference exhibited at a species level. It has been shown that *Megachile rotundata* exhibits extreme preference for two of 11 plant species it cuts (Horne, 1995). Practically, it may be more feasible to conduct molecular barcoding of the leaf pieces as was done by MacIvor (2016) than to observe leaf-cutting in action. However, a DNA barcode library of plants in Singapore is also necessary to do so. Further, nesting sites in the wild can be challenging to locate as they are highly camouflaged and difficult to find in tropical Singapore though using trap-nests may potentially overcome this issue. Despite these challenges, we can make educated guesses of the bee species based on the size of the leaf cuts that correspond to the size of the bee and habitat as some *Megachile* leafcutter bees show high fidelity to specific habitats (Table 3).

Implications for management. In light of bee conservation and management, planting the floral-associates of the bees (i.e., plants whose flowers these bees visit) is frequently recommended for enhancement of pollinator habitats (Keith, 2014; Million Pollinator Garden Challenge, 2016; Mizejewski, 2017). However, some emphasis should also be placed on nesting sites (e.g., cavities or suitable ground) and nesting materials such as leaves (as in this study). A high proportion of leaf cutting observations in this study were of the native plants *Dendrolobium umbellatum* and *Cratoxylum cochinchinense* (Fig. 3). Planting such species may further augment populations of these specialist pollinators.

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LITERATURE CITED

Allasino ML, Marrero HJ, Dorado J & Torretta JP (2019) Scientific Note: First Global Report of a Bee Nest Built Only with Plastic. Apidologie. https://doi.org/10.1007/s13592-019-00635-6. (Accessed 13 April 2019).

Ang J (2019) Hymenopterist Forum. A Female Leafcutter, *Megachile* (*Eutricharaea*) sp. https://www.facebook.com/groups/HymenopteristsForum88/1542672442533277/. (Accessed 11 April 2019).

Ascher JS, Risch S, Soh ZWW, Lee JXQ & Soh EJY (2016) *Megachile* leaf-cutter and resin bees of Singapore (Hymenoptera: Apoidea: Megachilidae). Raffles Bulletin of Zoology, Supplement 32: 33–55.

Bilinski M, Gosek J, Kuna K, Kaczmarska K & Jablonski B (1980) Rosliny wykorzystywane przez miesiarki (*Megachile* Latr.). Pszczelnicze Zeszyty Naukowe, 24: 97–114.

Chang Y, Chen H & Qi R (1989) Ornamental pest—studies on leafcutting bees *Megachile subtranquilla* Yasumatsu. Acta Agriculturae Universitatis Pekinensis, 15: 208–213.

Chong KY, Tan HTW & Corlett RT (2009) A Checklist of the Total Vascular Plant Flora of Singapore: Native, Naturalised and Cultivated Species. Raffles Museum of Biodiversity Research, National University of Singapore, Singapore, 273 pp. Uploaded 12 November 2009. https://lkcnhm.nus.edu.sg/app/uploads/2017/04/flora_of_singapore_tc.pdf. (Accessed 29 March 2019).

Genaro J (1996) Plantas usadas por abejas del genero *Megachile* para construir las celdillas de sus nidos (Hymenoptera: Megachilidae). Carribean Journal of Sciences, 32: 365–368.

Horne M (1995) Leaf area and toughness: Effects on nesting material preferences of *Megachile rotundata* (Hymenoptera: Megachilidae). Annals of the Entomological Society of America, 88: 868–875.

Kambli SS, Manoj A, Varma S, Asha G, Sinu TP, Allesh R & Sinu PA (2017) Leaf foraging sources of leafcutter bees in a tropical environment: implications for conservation. Apidologie, 48: 473–482.

Kato M, Kosaka Y, Kawakita A, Okuyama Y, Kobayashi C, Phimminith T & Thongphan D (2008) Plant-pollinator interactions in tropical monsoon forests in Southeast Asia. American Journal of Botany, 95: 1375–1394.

Keith J (2014) Gardening for Native Bees. The American Gardener. https://www.ahsgardening.org/uploads/pdfs/Native_Bees_TAG_ND14.pdf. (Accessed 8 April 2019).

LPWG (2017) A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. Taxon, 66: 44–77.

MacIvor J & Moore A (2013) Bees collect polyurethane and polyethylene plastics as novel nest materials. Ecosphere, 4(12): 1–6.

MacIvor J (2015) Building height matters: nesting activity of bees and wasps on vegetated roofs. Israel Journal of Ecology & Evolution, 62. https://doi.org/10.1080/15659801.2015.1052635 (Accessed 11 April 2019).

MacIvor J (2016) DNA barcoding to identify leaf preference of leafcutting bees. Royal Society Open Science, 3. https://doi.org/10.1080/15659801.2015.1052635 (Accessed 11 April 2019).

Messer A (1985) Fresh dipterocarp resins gathered by megachild bees inhibit growth of pollen-associated fungi. Biotropica, 17: 175–176.

Michener C (2007) The Bees of the World. 2nd Edition. The Johns Hopkins University Press, Baltimore, Maryland, 953 pp.

Million Pollinator Garden Challenge (2016) Million Pollinator Garden Challenge: Three Simple Steps. http://millionpollinatorgardens.org/wp-content/uploads/2016/06/MPGN-Customizable-OneSheet.pdf. (Accessed 8 April 2019).

Mizejewski D (2017) Six Tips for Pollinator Gardens in Small Spaces. National Wildlife Federation Blog. https://blog.nwf.org/2017/06/six-tips-for-pollinator-gardens-in-small-spaces/. (Accessed 8 April 2019).

Momose K, Yumoto T, Nagamitsu T, Kato M, Nagamasu H, Sakai S, Harrison RD, Itioka T, Hamid AA & Inoue T (1998) Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. Characteristics of the plant-pollinator community in a lowland dipterocarp forest. American Journal of Botany, 85: 1477–1501.

Morato EF & Martins RP (2006) An overview of proximate factors affecting the nesting behavior of solitary wasps and bees (Hymenoptera: Aculeata) in preexisting cavities in wood. Neotropical Entomology, 35: 285–298.

National Parks Board (2019) Flora and Fauna Web. https://florafaunaweb.nparks.gov.sg/. (Accessed 11 April 2019).

Pitt-Singer TL & Cane JH (2011) The alfalfa leafcutting bee, *Megachile rotundata*: the world's most intensively managed solitary bee. Annual Review of Entomology, 56: 221–237.

Praz CJ (2017) Subgeneric classification and biology of the leafcutter and dauber bees (genus *Megachile* Latreille) of the western Palearctic (Hymenoptera, Apoidea, Megachilidae). Journal of Hymenoptera Research, 55: 1–54.

Sedivy C & Dorn S (2013) Towards a sustainable management of bees of the subgenus *Osmia* (Megachilidae; *Osmia*) as fruit tree pollinators. Apidologie, 45: 88–105.

Sinu PA & Bronstein JL (2018) Foraging preferences of leafcutter bees in three contrasting geographical zones. Biodiversity Research, 24: 621–628.

Soh EJY (2014) Diversity and Trap-nesting Studies of Singaporean *Megachile* Bees to Inform Monitoring and Management of Tropical Pollinators. Honours Year Thesis, Department of Biological Sciences, National University of Singapore, Singapore, 110 pp.

Soh EJY, Ng DJJ, Ascher JS, Soh ZWW, Yap KKL & Chua JLE (2018) Leafcutter bee, *Megachile laticeps*, cutting leaf of *Dendrolobium umbellatum*. Singapore Biodiversity Records, 2018: 23–24.

Zillikens A & Steiner J (2004) Nest architecture, life cycle and cleptoparasite of the Neotropical leaf-cutting bee *Megachile* (*Chrysosarus*) *pseudanthidioides* Moure (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society, 77: 193–202.