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METABARCODING IN DIET ASSESSMENT OF ADULT PARASITOID SPECIES, *Dolichogenidea metesae* (HYMENOPTERA: BRACONIDAE) TOWARDS ITS CONSERVATION AND MANAGEMENT IN THE OIL PALM PLANTATION

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ABSTRACT

The abundance of parasitoids in the oil palm plantation is greatly influenced by the host and food availability. However, studies on the main diet or food sources of the adult parasitoid wasp, Dolichogenidea metesae *of the oil palm pest,* Metisa plana *is still lacking. This study investigated the diet of adult* D. metesae *sampled from Good Agricultural Practices (GAP) and non-GAP plantations through metagenomic analysis by using a* trnL *marker. A total of 761 145 food plant* trnL *sequences were generated from 12 samples of the parasitoids collected from four plantations. No significant differences (p=0.683) were observed for diversity (Shannon-Wiener index (H') of 0.12-1.22, p=0.68) among the total 62 OTU's of the food plant species when compared between the GAP and Non-GAP plantations. Polygonaceae was the most abundant plant family (59.95%), followed by Asteraceae (10.15%) and Acanthaceae (7.51%). The* Antigonon leptopus, Paraprenanthes oligolepis, Asystasiella neesiana *and wild banana (*Musa itinerans*) were among the most dominant plant species consumed by adult* D. metesae. *We recommend that these dominant food plant species for the* D. metesae *should be planted widely in the oil palm plantations to enhance the population abundance of* D. metesae *for successful Integrated Pest Management (IPM) practices.*

Keywords: DNA, food source, genetic, natural enemy, *trnL*.

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INTRODUCTION

Dolichogenidea metesae (Hymenoptera: Braconidae) is the predominant species of parasitoids that parasitises on a gregarious pest, *Metisa plana* (Psychidae: Lepidoptera) (Halim *et al*., 2017; 2019). As a primary parasitoid of the *M. plana*, *D. metesae* seeks to infest the third to sixth instar of the host insect, and this predilection has been utilised to become one of the most effective natural or biological control methods for major pests of agricultural crops under the Integrated Pest Management (IPM) in a sustainable ecosystem (Salmah *et al*., 2006). The longevity and fecundity of the parasitoids are influenced by the availability and type of their food sources (Liu and Ueno, 2012). Thus, the biological data on the *D. metesae,* especially on its diet, food source and consumption habits in the natural ecosystem would be invaluable for a better understanding of the intricate association between the parasitoids and their hosts, the pests, pollinators and other associates in the ecosystem (Fahimee *et al*., 2021)

The destruction of ground vegetation deprives food and shelter for adult parasitoids, therefore, suitable agricultural landscape and ecological infrastructure are important for the best habitat

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management under the natural biological control system (Landis *et al*., 2000). Balmer *et al*. (2013) provided additional evidence regarding the importance of flowering weeds to the parasitoids by highlighting the effectiveness of flowering plants as intercropping plants or as companion plants that supply bountiful and nutritious resources for the natural enemies of the crop pests, including the parasitoids. Studies by Norman and Basri (2010) reported that *Asystasia gangetica*, a weed species that grew naturally in the oil palm plantations had influenced the population of the parasitoids. However, there is no evidence to prove the efficacy of all available food plants including the flowering weeds to supply their diet or to provide a host or conducive environment for the parasitoids

The implementation of Good Agriculture Practice (GAP) has had a positive impact towards environmental sustainability in Malaysia (Tan *et al*., 2009) and in neighbouring countries such as Thailand and Indonesia (Apriani *et al*., 2020; Saswattecha *et al*., 2015). The Roundtable on Sustainable Palm Oil (RSPO), a global certification standard for sustainable palm oil, requires the implementation of GAP as part of the criteria in getting the certification (Morley, 2015). GAP principles emphasize on limiting the usage of chemical inputs and minimising the detrimental environmental impacts of farming operations through the implementation IPM (Norman *et al*., 2019). As a result, IPM practices such as planting beneficial plants and weed management are used in combating the oil palm pest, *M. plana* (Wood and Norman, 2019a). Failure to follow the GAP code of practice (CoP) had resulted in a high use of chemical pesticides, which had a very significant adverse impact on weed diversity (Aini *et al*., 2007). According to Möller *et al*. (2021), the abundance of parasitoids was higher in plots with a good ground cover compared to plots treated with herbicides. Wood and Norman (2019b) and Wood *et al*. (1974) also reported that the application of chemicals had consequently disrupted the natural enemies of *M. plana* in the plantation habitats.

Despite some previous successful studies demonstrating the relationship between flowering plants and parasitoids, e.g., *Cassia cobanenasis*, *Euphorbia heterophylla*, *Antigonon leptopus* and *Turnera subulata* (Siti Ramlah *et al*., 2005; Wood and Norman, 2019a), relatively very few studies had been undertaken to validate that these plants were consumed by the parasitoids. To our knowledge, there is no direct study investigating the various plant species foraged or consumed in the diet of adult *D. metesae*, the main parasitoid of *M. plana* in the oil palm plantation. Metabarcoding is now widely used in identifying insect diet. By using *trnL* marker, Fahimee *et al*. (2021) successfully revealed 262 plant species under 70 families foraged by stingless bee, *Heterotrigona itama*. This information benefits meliponiculture industry by recommending several food plant species to be planted such as *Momordica charantia*, *Melastoma* sp. and *Cucumis sativa*. According to Mallott *et al*. (2018), *trnL* yielded more sequences, higher resolution taxonomic identifications and identified a higher number of families in the diet of wild white-faced capuchins, *Cebus capucinus* compared to *rcbl*.

Therefore, based on the economic importance of *D. metesae* and *M. plana* in our oil palm industry (Basri *et al*., 1995; Halim *et al*., 2018), the main objective of this study was to identify the different food plant species consumed by adult *D. metesae* by conducting a metabarcoding analysis of the *D. metesae* diet using a *trnL* marker, thereby confirming the various species of plants foraged and utilised by *D. metesae* in the wild. The study also investigated how *D. metesae* diets differed between the GAP and non-GAP oil palm plantations. This information could facilitate towards a better understanding on the specific ecosystem components that could influence the introduction of *D. metesae* as an effective biological control of the main pests of oil palm as part of the IPM strategies.

MATERIALS AND METHODS

Sampling of *Dolichogenidea metesae*

Three *D. metesae* individuals (three replicates) were sampled from each plantation across Peninsular Malaysia, comprising two GAP plantations (certified with RSPO or MPOB CoGAP) and two non-GAP plantations, giving a total of 12 samples (n=12) from four sampling sites. The sampling sites were planted with specific beneficial plant species and had a history of *M. plana* infestation (*Table 1*). Samples of hymenopteran parasitoids were collected using a malaise trap and preserved in 90% ethanol for one year, from March 2020 to February 2021. All the specimens of *D. metesae* were collected and identified based on Norman *et al*. (2017) and Achterberg (1993), while only several individuals were used for the molecular analysis.

DNA Extraction

The DNA was extracted from the adults of *D. metesae* collected from four plantations with three biological replications representing each plantation. The DNA of 12 individuals of *D. metesae* were extracted from the whole body individually, using the NucleoSpin® DNA Insect protocol (Machery-Nagel, Germany) (Halim *et al*., 2018). Each sample was surface sterilised before molecular analysis was proceeded by submersing the insects in 70% ethanol and distilled water for 2-3 s for 3 times (Avanesyan, 2014; Cooper *et al.,* 2016).

TABLE 1. LIST OF *Dolichogenidea metesae* SAMPLES USED FOR METABARCODING ANALYSIS

Sample Code	Location	Grid	GAP Status	Beneficial Plant Species
Plantation A	Peninsular Malaysia. Pahang: Gambang	3.623169771292426, 103.13824123987163	GAP	*Turnera* sp. *Cassia cobanensis*
Plantation B	Peninsular Malaysia. Perak: Slim River	3.6949601778100867, 101.48563129489453	GAP	*Antigonon leptopus*
Plantation C	Peninsular Malaysia. Pahang: Kuantan	3.673757257494192, 103.14538126430287	Non-GAP	*Antigonon leptopus* *Turnera* sp.
Plantation D	Peninsular Malaysia. Johor: Segamat	2.4408761993796264, 102.6866661993966	Non-GAP	*Antigonon leptopus* *Turnera* sp.

Library Preparation

Preparation of the library was conducted by using 2-step PCR. The first PCR was performed using locus-specific sequence primers (*trnL*) with overhang adapters: g-A49425-added overhang (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GG CAATCCTGAGCCAA-3′) and h-B49466-added overhang (5′- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCATTGAGTCTCTGCACCTATC-3′). A total of 25.00 μL was prepared for PCR amplification. The mixture consisted of 5.75 μL Q5® High Fidelity DNA Polymerase (New England BioLabs Inc.), 1.25 μL of each forward and reverse primer (10 μM), 15.75 μL of water, nuclease free and 1.00 μL of DNA template. A total of 5.00 μL of DNA template was used for the sample with concentration < 2.5 ng/μL. The amplification reactions were performed under the following protocol: initial denaturation of 95°C for 3 min, followed by 30 cycles of denaturation at 98°C for 30 s, annealing at 64°C for 30 s, extension at 72°C for 30 s and a final extension at 72°C for 5 min. The second PCR was conducted to attach dual indices to the amplicon PCR by using Illumina Nextera XT Index Kit v2 following manufacturer's protocols. Even though no qPCR was conducted, however the quality of the libraries was measured using Agilent Bioanalyzer 2100 System by Agilent DNA 1000 Kit and fluorometric quantification by Helixyte Green™ Quantifying Reagent. The libraries that passed the library QC were subsequently subjected to next-generation sequencing.

Next-Generation Sequencing

The libraries were normalised and pooled according to the protocol recommended by Illumina and then proceeded to sequencing by MiSeq platform using 150 paired-end. The next-generation sequencing was performed by Apical Scientific Sdn. Bhd. The raw data was then processed and analysed according to the bioinformatics analysis. All the read sequences were deposited in the Sequence Read Archive (SRA) of the NCBI (http://www.ncbi.nlm.nih.gov/sra) with the references number SRR20648567 (Plantation A R1), SRR20648580 (Plantation A R2), SRR20648647 (Plantation A R3), SRR20646195 (Plantation B R1), SRR20648581 (Plantation B R2), SRR20648441 (Plantation B R3), SRR20648771 (Plantation C R1), SRR20648869 (Plantation C R2), SRR20648867 (Plantation C R3), SRR20648625 (Plantation D R1), SRR20648622 (Plantation D R2) and SRR20648768 (Plantation D R3).

Bioinformatic Analysis

Sequence adaptors and low-quality reads were trimmed-off from paired-end reads using BBDuk from the BBTools package. After that, by using USEARCH v11.0.667, the forward and backward reads were merged. All sequences (sequenced on the MiSeq platform) with an average quality score of less than 150 bp or more than 600 bp were eliminated from downstream processing (Edgar, 2010). Following that, the reads were aligned with *trnL* sequences using the NCBI database. Operational taxonomic units (OTUs) were clustered with 97% similarity cut-off using UPARSE v11.0.66 (Edgar, 2013) and a single representative sequence from each OTU was picked at random. Pynast was used to construct a phylogenetic tree against NCBI database (Caporaso *et al*., 2010a) and QIIME V1.19.1 was used for taxonomic assignment of OTUs (Caporaso *et al*., 2010b). The rarefaction curve was plotted to determine the sufficiency of sequency depth by using R V3.6.2 (R Core Team, 2018). The heatmap was also created with R software to display the relative abundances of taxa (phyla) using different shades of colour, with darker colours representing greater values. Alpha diversity (Shannon-Wiener, Simpson, CHAO 1) with ANOVA showed pairwise analysis of variance between groups and its significance was annotated on the box plots with *p*-value set to 0.05. Other statistical analysis was also done in R V3.6.1. (R Core Team 2018).

RESULTS

A total of 761 145 plant *trnL* sequences were generated from 12 samples of *D. metesae*, ranging from 47 893 to 73 893 (*Table 2*). Except for the Plantation C, all samples from Plantations A, B, and D had more than 60 000 reads, with Plantation A (R3) having the most reads (73 893), while Plantation C (R1) had the lowest reads (47 893). Overall, 62 OTU's were observed, with Plantation A (R1) recording the minimum OTU's (16) while Plantation C (R1) recorded the maximum of OTU's (29). The Shannon-Wiener and Simpson index indicated a higher diversity of OTU's from Plantation D (R2) ($H' = 1.22$, $1\text{-}D = 0.68$) and the lowest from Plantation D (R3) ($H' = 0.12$, $1\text{-}D = 0.04$). Chao 1 estimator abundance showed that Plantation C (R2) has the highest value (28.75) of OTU's, while conversely, Plantation A (R2) recorded the lowest value (11) (*Table 2*).

The rarefaction curves (*Figure 1*) indicate that all the samples are almost reaching asymptote, implying that sufficient sequencing depth has been achieved. The curve rises steeply at first, and then flattens out gradually at 35 000 sequences. The *D. metesae* sample taken from Plantation C (R2) had the most diverse food plant species, since the number of different species detected was higher than in all the other samples, while the *D. metesae* sample from Plantation A (R2), had the least diverse food plant species (*Figure 1*).

Overall, Polygonaceae (59.95%) was the most common family recorded (*Table 3*), followed by Asteraceae (21.65%) and Acanthaceae (7.51%), while Arecaceae, Campanulacea, Urticaceae and Phyllanthaceae were the least recorded, accounting for less than 1.00% of the total families observed. The heatmap (*Figure 2*) showed that Polygonaceae and Asteraceae were present in all the *D. metesae* samples. High abundance of Polygonaceae was recorded from the samples collected from Plantations B, C and D, while Asteraceae was recorded in high abundance in all samples collected from Plantation A only. Meanwhile, Acanthaceae was found mostly in the samples from Plantation D, while very little of Phyllanthaceae and Malvaceae were recorded and could only be found in one *D. metesae* sample, i.e. from Plantation C (R2) (*Figure 2*).

As seen in the bar chart (*Figure 3*), *A. leptopus* (Polygonaceae) was the most dominant species of plant food recorded from Plantations B, C and D, accounting for more than half of the total plant species consumed by *D. metesae*. In contrast, *P. oligolepis* was the most dominant plant food recorded in the *D. metesae* samples collected from Plantation A, although it also occurred in all the other 12 samples. Likewise, Plantations A and D seemed to record a higher number of plant species (seven) compared to Plantations B and C, which recorded only four species among the dominant plant foods of *D. metesae*.

According to the Venn diagram (*Figure 4*), both GAP and non-GAP plantations shared 34 OTU's out of 62 OTU's. A Venn diagram is an illustration of the relationships between and among groups of samples with their own unique OTUs and those that share OTUs in common. Our study shows that alpha diversity metrics as given in the box plot (*Figure 5*) reveals no significant difference (Chao 1, $p = 0.23$; Simpson, $p = 0.45$; Shannon, $p = 0.68$) between the GAP and non-GAP plantations.

DISCUSSION

The metabarcoding analysis has been conducted to obtain the information on the diet of adult *D. metesae.* The results obtained from *D. metesae* samples collected from the GAP and non-GAP plantations are very significant and comparatively valuable in determining the diversity and abundance of *D. metesae* in oil palm plantations adopting different agricultural practices (Saswattecha *et al*., 2015).

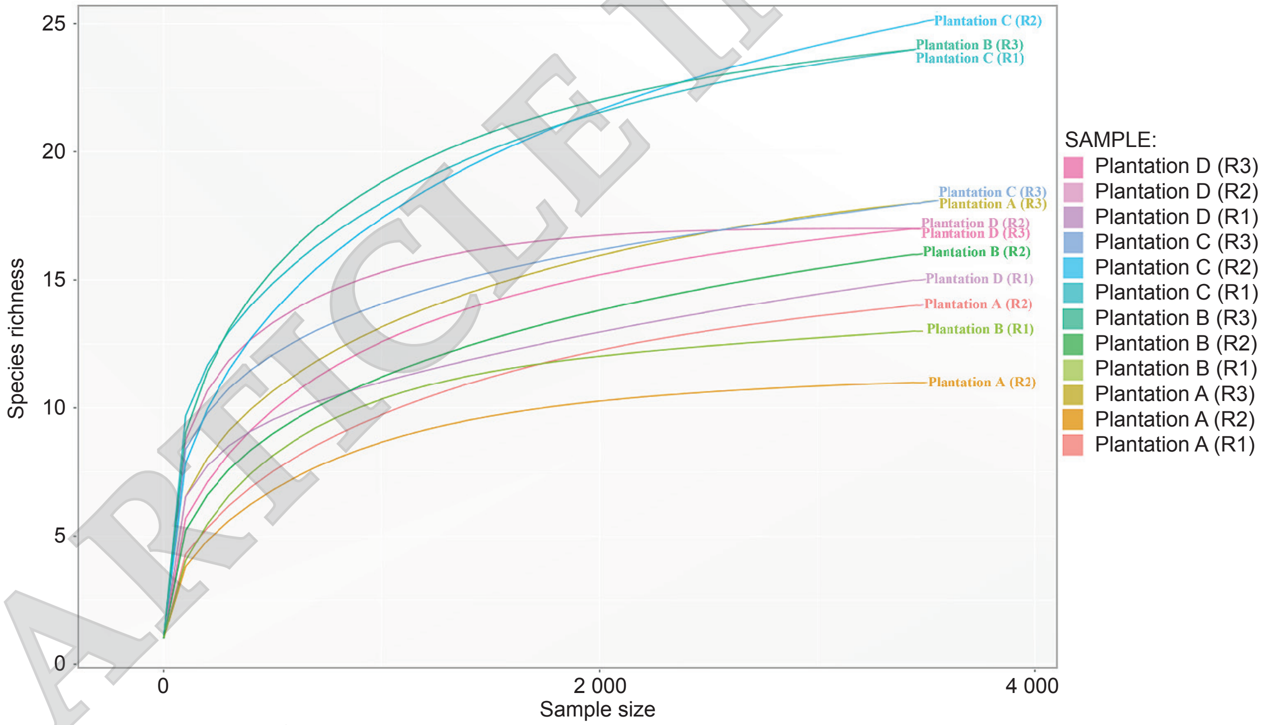
The rarefaction curve *(Figure 1)* indicates that the sequencing depth has almost reached asymptote, implying that the maximum number of OTU's have been collected in deriving at the conclusion in this study (Cayuela *et al*., 2015). The family Polygonaceae, belonging to the flowering plant group of angiosperms (Vandebroek *et al*., 2018), was recorded as the most abundant family (59.95%) found in the *D. metesae* diet, whereas several other families such as Asteraceae (21.65%), Acanthaceae (7.51%), Musaceae (6.29%) and Poaceae (2.47%) constitute the top five families consumed by the *D. metesae*. Both families, *i.e.*, Asteraceae and Acanthaceae are classified as weeds (Ali *et al*., 2021) and are abundantly found in the diet of *D. metesae*. Notably, the families Musaceae and Arecaceae (fruit plant species) also occurred in the *D. metesae* diet.

The five most abundant plant food species found in the *D. metesae* samples were *A. leptopus*, *P. oligolepis*, *A. neesiana*, *M. itinerans*, and *Cocos nucifera (Figure 3).* The *A. leptopus* was introduced into the oil palm plantations as a beneficial plant (Hariyadi and Syahlan, 2021) however, *P. oligolepis* and *A. neesiana* existed naturally as weeds in Malaysia (Shi *et al*., 2011). Both species *i.e., M. itinerans* and *C. nucifera* were naturally pollinated by animals such as the Long-tongued Fruit Bat (*Macroglossus sobrinus*), the nectar-feeding bird, Little Spiderhunter (*Arachnothera longirostris*) and the Indian Palm Squirrel, *Funambulus palmarum* (Chakravarthy and Thyagaraj, 2012; Liu *et al*., 2002). However, our own observations indicated that both species *i.e., M. itinerans* and *C. nucifera* were very sparse in all the four plantations. Additionally, the *C. nucifera* that belongs to the Palmae was not suggested to be planted in the plantation due to its potential to attract pest, Red Palm Weevil (RPW), *Rhyncophorus ferrugenius* (Aman-Zuki *et al*., 2021). According to Mathews *et al*. (2007) from a total of 333 plant species

TABLE 2. NUMBER OF OBSERVED OTU'S, ALPHA DIVERSITY INDICES FOR THE PLANT DNA FROM 12 SAMPLES OF *Dolichogenidea metesae*

Localities	Sample	Sequences		OTU's	Chao-1	Shannon-Wiener	Simpson	Evenness
		Before	After			(H')	(1-D)	(E^HS)
Plantation A	R1	458 2582	66 511	14	15.5	0.72	0.43	15.55
	R2	496 5272	66 931	11	11.0	0.70	0.49	11.29
	R3	593 4902	73 983	18	18.6	0.68	0.35	20.37
		Mean ± SD		14.33 ± 3.51a	15.03 ± 3.82b	0.70 ± 0.02a	0.42 ± 0.07 a	15.74 ± 4.55 a
Plantation B	R1	4 394 041	62 425	13	14.00	0.51	0.32	13.79
	R2	4 649 663	62 582	16	18.00	0.67	0.38	19.95
	R3	5 192 020	68 353	24	24.75	0.98	0.56	25.63
		Mean ± SD		17.67 ± 5.69a	18.92 ± 5.43a	0.72 ± 0.24a	0.42 ± 0.123 a	19.79 ± 5.93 a
Plantation C	R1	3 685 638	47 893	24	25.50	0.49	0.22	26.68
	R2	4 592 931	57 067	25	28.75	0.41	0.20	30.76
	R3	4 153 120	56 846	18	24.00	0.68	0.32	22.08
		Mean ± SD		22.33 ± 3.79a	26.08 ± 2.43a	0.52 ± 0.14a	0.24 ± 0.07a	26.51 ± 4.34 a
Plantation D	R1	5 411 881	72 422	15	17.00	0.81	0.47	22.45
	R2	4 778 117	63 958	17	17.00	1.22	0.68	17.00
	R3	4 691 056	62 174	17	18.00	0.12	0.04	18.87
		Mean ± SD		16.33 ± 1.16a	17.33 ± 0.58a	0.72 ± 0.56a	0.40 ± 0.33 a	19.44 ± 2.77 a

Note: Mean ± SD value with a different letter indicates a significant difference (ANOVA, $p < 0.05$).



Note: R1-R3 were replicates.

Figure 1. The rarefaction curve of trnL *gene sequence for 12 samples of* Dolichogenidea metesae.

in 83 families collected in an oil palm plantation in Malaysia, 279 of them were angiosperms. The abundant flowers of the angiosperms would attract beneficial insects by providing nectar and sugar as a source of food and energy (Heimpel and Jervis, 2005; Tompkins *et al*., 2010). Our results have also proven that the angiosperm species serve as the main food source for *D. metesae* (Patt *et al*., 1997) providing for adult longevity and fecundity of the parasitoids (Olson *et al*., 2005).

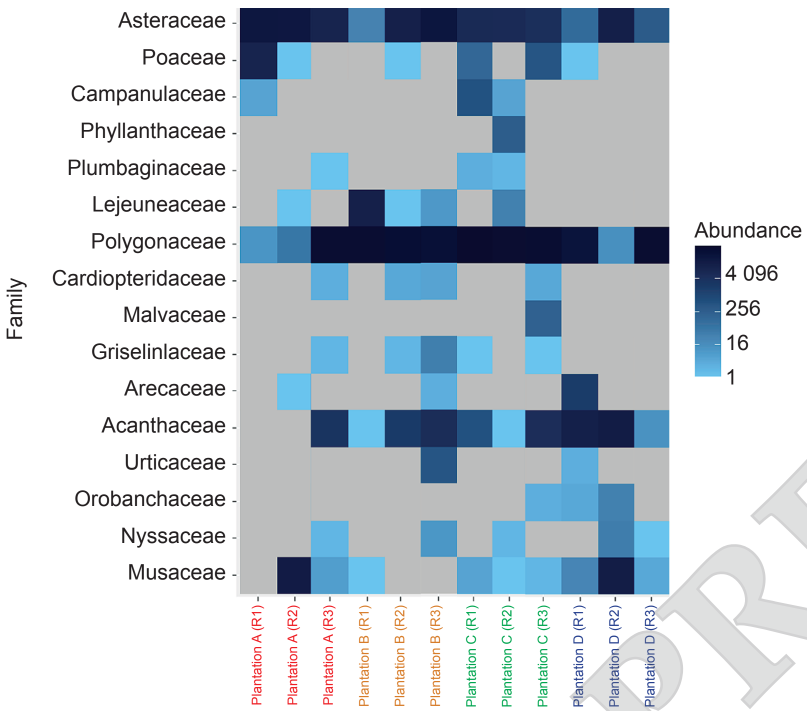


Figure 2. Heat analysis at the family level. The darker colour represents a correspondingly greater value of abundance.

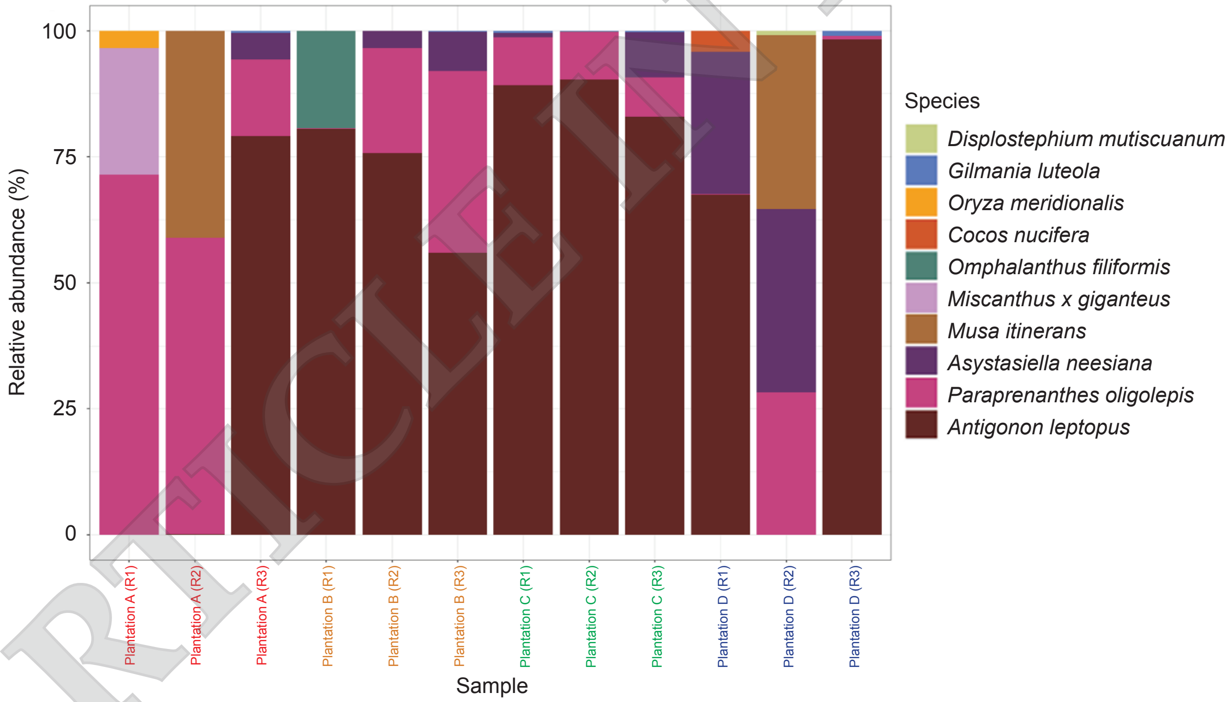


Figure 3. Relative abundancy bar chart at the species level. The ten most abundant food plant species recorded in all the 12 samples of Dolichogenidea metesae.

This study provides the first evidence that *A. leptopus* (Polygonaceae) is highly consumed by *D. metesae* as a source of diet (*Figure 3*). Yusdayati *et al*. (2014) reported that parasitoid wasps *Brachymeria carinata* and *D. metesae* could be found in higher abundance on the plant foods *C. cobanensis* and *Turnera* sp. compared to *A. leptopus*. However, our findings revealed that *D. metesae* consumed more *A. leptopus*, while there was no record of *C. cobanensis* and *Turnera* sp. in the diets despite their presence in the plantation. The use of metabarcoding here is novel since the protocol has not been used previously to confirm the diet of parasitoids. This information is important since nectar, pollen, and water are all necessary for the adult parasitoids to survive and flourish (Damien *et al*., 2020).

Although previous research works had shown that the common weed locally known as Chinese violet (*A. gangetica*: Achantaceae) could increase parasitoid diversity in the oil palm plantations

TABLE 3. RELATIVE ABUNDANCE OF THE TOP 10 FAMILIES OF PLANTS RECORDED IN THE 12 SAMPLES OF *Dolichogenidea metesae*

Family	Relative abundance (%)
Polygonaceae	59.95
Asteraceae	21.65
Acanthaceae	7.51
Musaceae	6.29
Poaceae	2.47
Lejeuneaceae	1.62
Arecaceae	0.35
Campanulaceae	0.07
Urticaceae	0.05
Phyllanthaceae	0.04

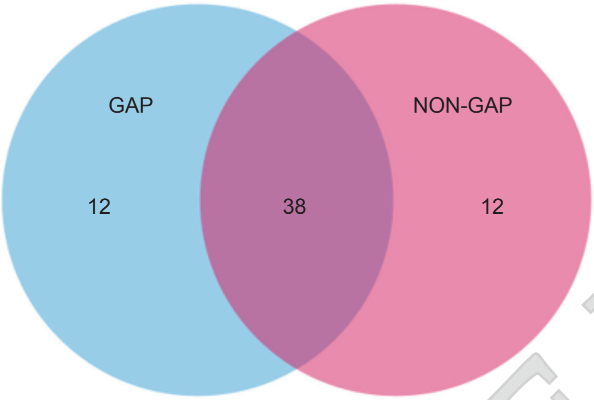


Figure 4. Comparison of the Venn diagrams between the GAP and non-GAP plantations.

(Norman and Basri, 2010; Tiong, 1982) our study data had shown that this weed species was not consumed or foraged by *D. metesae* despite its abundant presence in the sampling sites. In contrast, the weedy herbs *P. oligolepis* (Asteraceae) and *A. neesiana* (Acanthaceae) were detected in almost all *D. metesae* samples in this study *(Figure 3)*. Based on literature information, the herbaceous species *P. oligolepis*, which is a synonym to *Melanoseris oligolepis* (Shi *et al*., 2011) and *A. neesiana*, which is a synonym to *Mackaya neesiana* (Wall.) (POWO, 2021), can both be found in Malaysia, although these two weedy species are not yet recorded so far in our oil palm plantations.

The differences in *D. metesae* diet shown in *Figure 2* could be due to food availability in the respective areas (Folgarait and Gilbert, 1999; Jervis *et al*., 1992;). In an agroecosystem, two important resources (i.e., host and food) for the parasitoids are rarely located within the same site, hence, the adult parasitoids must be able to fly regularly between the host site and the plant food site in order to reproduce successfully (Lewis *et al*., 1998). Furthermore, a study by Coudrain *et al*. (2014) found that the parasitoid species richness was higher in areas surrounding the forests than in isolated sites due to higher plant variability (richness) in the former. The importance of plant species such as *M. acuminata* and *C. nucifera*, in which the DNA of both have been detected as plant foods of *D. metesae* in this study cannot be overlooked, since both species can be planted as side crops or ornamentals in the oil palm plantations (Mathews *et al*., 2007). The intercropping strategy has been applied in many

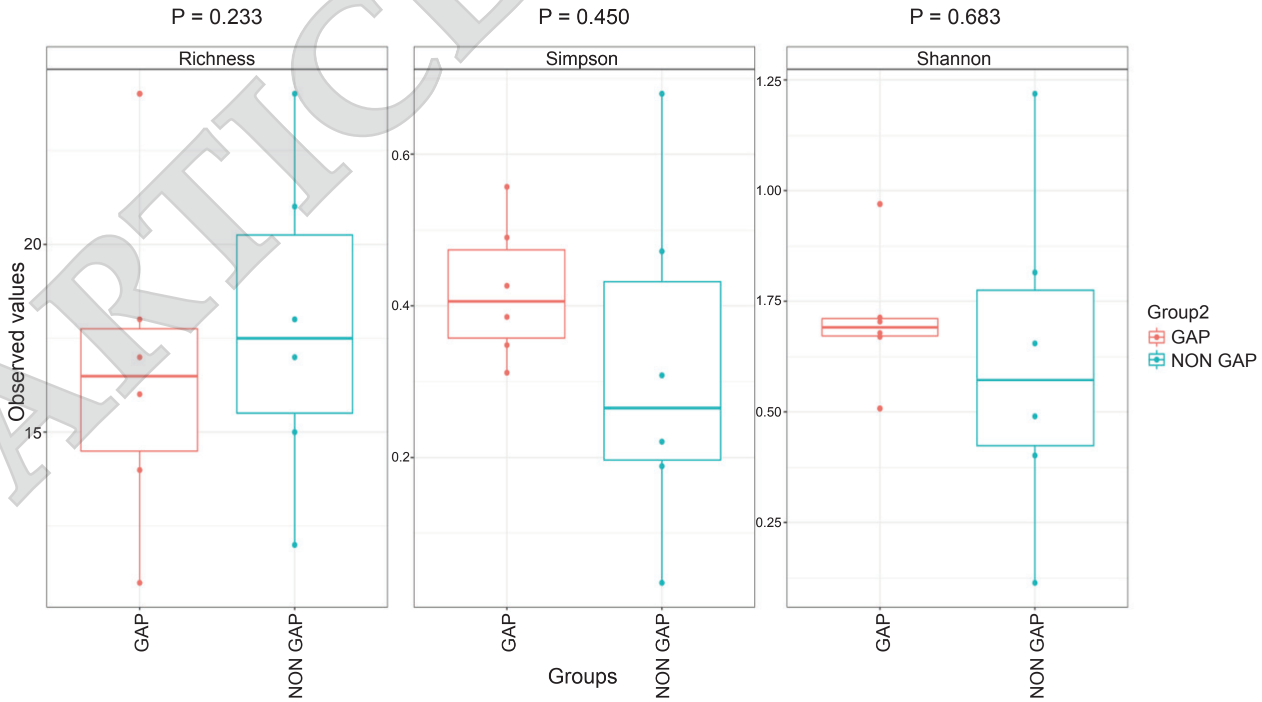


Figure 5. Comparison of the alpha diversity box plots between the GAP (Red) and non-GAP (Blue) plantations. The p-value threshold for significance is set to 0.05.

plantations for many purposes. One of it is to reduce or control the infestation of crop pests such as okra and legumes (Singh *et al*., 2017), pickerelweed and rice (Xiang *et al*., 2021) by increasing the population abundance and diversity of parasitoids (Amala and Shivalingaswamy, 2018; Devi *et al*., 2020), and the second objective is to provide two-in-one crop production (Nchanji *et al*., 2016; Rocha *et al*., 2020).

In this study, the molecular data and the mathematical analysis showed that there was no significant difference in plant diversity in the *D. metesae* diet between the GAP and non-GAP plantations *(Figure 5)*, even though the agricultural practices differed significantly between the two in terms of chemical application (Saswattecha *et al*., 2015), which would have had very significant impacts on the diversity of weeds and the associated biota (Darras *et al*., 2019). This might be because in our study, the weed species commonly found in the oil palm plantations such as *A. gangetica*, *Clidemia hirta* and *Drymaria cordata* (Ali *et al*., 2021; Satriawan and Fuady, 2019) could not be traced or detected in the diet of *D. metesae (Figure 2)*. However, *A. gangetica* had been documented in other studies as having an important role in the abundance of parasitoids other than *D. metesae* (Wood and Norman, 2019a). Although this study showed that there was no significant different in terms of plant species consumed by *D. metesae* in GAP and non-GAP plantations, Fuat *et al.* (2022) found that the parasitoid abundance was significantly higher in GAP plantations compared to non-GAP plantations.

CONCLUSION

This is the first study to use metabarcoding analysis in determining the plant communities consumed by the *D. metesae* as their food sources in the wild. Overall, the natural diet of *D. metesae* was dominated by the Coral vine or Bride's Tears (*A. leptopus*), a common creeper with abundant pink flowers and rich in nectar. Therefore, it is highly recommended that this beneficial plant should be planted in the oil palm plantations to attract more *D. metesae*, the dominant parasitoid species of *M. plana* as a control measure for bagworm infestation.

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