

Notes on *Apis dorsata* Food Source Using Metabarcoding Techniques in Cameron Highland

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Abstract— *Apis dorsata* or lebah tualang has been recognized as a pollinator agent in Malaysia. This species travels from northern to southern part of Malaysia yearly and pollinating the trees along their migrating way. Study of *A. dorsata* has been less concern due to several limitations such as their nesting habitat. Thus, this research objective was to assess their food source using metabarcoding technique to understand their foraging behavior. Three individual has been sampled in Cameron Highland and extracted to assess plant DNA inside their body. Total of 56901 plants DNA from 18 family and 32 species has been identified during this project. Fabaceae has been identified the most abundance species taken by *Apis dorsata* followed by Zingerberaceae, Poaceae, Myrtaceae and Rubiaceae and *Acacia koa* was the most plant DNA taken by *A. dorsata*. Thus, this result can be a baseline data for *A. dorsata* research in Malaysia. In addition this data also can used to regulate the *A. dorsata* management plan from decline.

Keywords— *Apis dorsata*, food source, metabarcoding

I. INTRODUCTION

There are three species of wild honey bees in Malaysia namely *Apis dorsata*, *Apis florea* and *Apis cerana*. Among these three, *Apis dorsata* was the largest species and habitually nesting in *Koompassia excelsa* or Tualang tree. Each colony of *Apis dorsata* consists of ± 35000 workers with one queen (Dyer & Seeley, 1994) and construct massive single comb. They also can migrate more than 1000 km since their range of habitat from India to Southeast Asia (Robinson, 2012). However, in one movement of searching of new nesting site, they will flight within 200km from the previous nest and nesting in the new place for 3 to 4 months (Kahono et al., 1999). In addition, their movement was relying on flowering seasons in Malaysia (Sahebzadeh et al., 2012), which starts from the north to the south. However, some of the colony has been eliminated when the *A. dorsata* nested in the residential areas. For example there is a case of *A. dorsata* has been eliminated in KLIA 2 when nested in the walkaway bridge (Khan et al., 2021).

However, their behavior of migrating normally will be same from the past decade and nesting site are reoccupied again and again. Interestingly, they will find the same place and took the same route. Based on workers life span was less than 2 months (Michener, 2007) and some researcher suggested that the queen played a major role in the migrating process. Besides that, the migrating has been identified was followed by the climate pattern and flowering seasons in Malaysia

due to the existence of food. *Apis dorsata* has been identified to collect plant with dominating sugar compared to others bees (Abrol, 2016) such as Citrus, Bottlebrush Lemon (Neupane et al., 2012) *Coryandra viscosa*, and *Syzygium cumini* (Huda et al., 2023).

Identification of plant taken by *A. dorsata* was challenging due their nesting habitat and hard to sample the individual with pollen on the nest. However, the development of science and technology has facilitated to access the *A. dorsata* food source using Metabarcoding techniques. This method was using the individual of bees without pollen on their hind legs and extracted to get the plant DNA inside their body. There are several research has been conducted to identify the bees food source using metabarcoding such as *Heterotrigona itama* and the result was more comprehensive if compared with other methodology. Thus, this research objective was to identify the *A. dorsata* food source using metabarcoding technique.

II. MATERIALS AND METHODS

A total of three *Apis dorsata* were sampled and collected from Cameron Highland. The sampled were dissected and the DNA was extracted from the whole body using NucleoSpin® DNA Insect protocols (Macherey-Nagel, Germany). Methodology by Fahimee et al. 2022 has been used in this protocol from DNA sample QC until data analysis. The first PCR was carried out with trnL primers PCR forward primers forward primer (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACA

G) and PCR reverse primers (5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AG) then the second PCR is using Illumina sequencing adapter with normal protocols.

III. DATA ANALYSIS

Paired-end reads were first removed of sequence adaptors and low-quality reads using BBDuk of the BBTools package. After this, the forward and reverse reads were merged using USEARCH v11.0.667 (<http://www.drive5.com/usearch>). All sequences that were shorter than 150 bp or longer than 600 bp were removed from the downstream processing. Reads were then aligned with 16S rRNA (SILVA Release 132) or UNITE ITS database. After these quality assessment steps, reads were clustered de novo into Operational Taxonomic Units (OTUs) at 97% similarity using UPARSE v11.0.667; rare OTUs with less than 2 reads (doubleton) which were often spurious, were deleted from down-stream processing. A single representative sequence from each OTU was randomly cho-sen, and Pynast was used to align and construct a phylogenetic tree against the SILVA 132 16S rRNA database. Taxonomic assignment of OTU was achieved using QIIME V1.9.1 (<https://qiime.org>) against the Silva database 16S rRNA database (release 132). All statistics analysis was done in R package V3.6.1

IV. RESULTS AND DISCUSSION

Total of 56901 plants DNA from 18 family and 32 species has been identified during this project. Fabaceae has been identified the most abundance species taken by *Apis dorsata* followed by Zingerberacea, Poaceae, Myrtaceae and Rubiaceae (Table 1). Fabaceae was a family has been taken by bees as a pollen source during larvae stages ([Haider et al., 2014](#)). As a legume trees with more than 19 500 species around the world ([Raj et al., 2022](#)), this family of tree provide a unlimited pollen to the bees. In addition, legume family can be easily found cascading in the Malaysia landscape and indicated that this family resilience in any weather condition. Furthermore, high nitrogen compound such as alkaloids and amines also make this family as a food source by bees ([Wink, 2013](#)).

Meanwhile, family of Zingerberaceae also has been identified as a *A. dorsata* in this research. Solitary bees

species such as halictid and blue-banded bees has been recognized as a Zingerberacea pollinator ([Appalasamy et al., 2023](#)). Based on this family flowering behavior, they will start anthesis at 1000H and fully bloomed on 1100H until 2000H ([Arumugam et al., 2021](#)). Thus, this period has been proven that the active foraging by the *A. dorsata* and can be concluded that *A. dorsata* food range was diverse compared to other honey bees ([Raffiudin et al., 2024](#)). Poaceae family also has been found in this research as *A. dorsata* food source. Poaceae is a grasses family and easily found in Malaysia landscape. Some of this species such as *Gigantochloa scortechinii* has a mutual associated with ants ([Kaufmann et al., 2001](#)). This family produce great number of pollen with ranged 14 500 to 22 000 000 pollen grains per inflorescence ([Prieto-Baena et al., 2003](#)). With this great number of pollen and unlimited make this family becomes a bees food source.

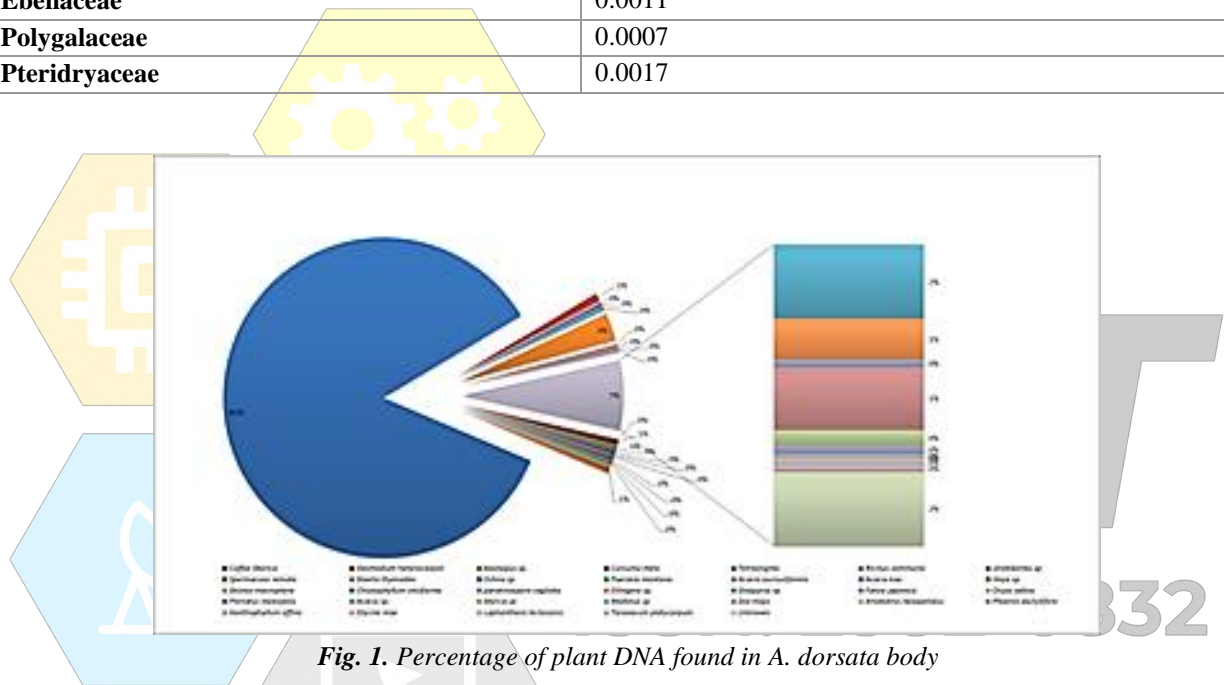
The higher plants DNA were comes from *Acacia koa* (85%) followed by *Etlingera sp.* (2.8%), *Acacia sp.* (1.7%) and *Zea mays* (1.4%). Meanwhile, there was 1.69% unidentified plant DNA has been found (Figure 1). *Acacia* species produce unlimited honey from the young bud leaves and taken by the insect such as ant and bees as a food ([Adgaba et al., 2016](#)). In Malaysia, *Acacia* plantation has been started from 1932 for forest rehabilitation process ([Arifin et al., 2012](#)). In addition, some of *Acacia* plantation has been integrated with honey bees (*Apis mellifera*) for honey production ([Warui et al., 2018](#)). Thus, the unlimited nectar and pollen from *Acacia* species also attract *A. dorsata* and will influence the number of *A. dorsata* colony with more than 21% ([Pribadi, 2020](#)).

This data constructed the *A. dorsata* food source without collected the pollen from their pollen basket. The DNA of plant inside their body can be said that is the plant they consumed and crucial for them to sustain their life. In some cases, we did not know that the pollen carrying back to the colony has been used as a food source or others function. Thus, with this data, the plant can be identified as a crucial food source for the *A. dorsata* in conserved them from decline. Therefore, metabarcoding technique has been confirmed to assess the *A. dorsata* food source and easily to use and saves a time.

Table 1. Relative abundance of plant DNA family from Apis dorsata.

Family	Relative Abundance
Acanthaceae	0.0017
Annonaceae	0.0034

Araliaceae	0.0035
Dipterocarpaceae	0.00062
Malpighiaceae	0.0013
Myrtaceae	0.0092
Poaceae	0.0195
Sapotaceae	0.0034
Asteraceae	0.00169
Zingiberaceae	0.0309
Euphorbiaceae	0.0026
Rubiaceae	0.004
Menispermaceae	0.0033
Apocynaceae	0.0082
Fabaceae	0.882
Ebenaceae	0.0011
Polygalaceae	0.0007
Pteridryaceae	0.0017



V. CONCLUSION

This study has been conducted in Cameron Highland and show that species has been taken by *A. dorsata* as a food source. Family of Fabaceae show most diverse compared to other family. However, this result so far away to conclude that this species and family as a major food source of *A.dorsata* since the sampling size was only in Cameron Highland. Further assessment must be done from other sites such as in northern region and southern region of Malaysia to complete this issue. This data also indicate *A. dorsata* flight range will start from the north and stop by Cameron Highland before move to southern Malaysia.

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