# **RESEARCH NOTE**

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The first ITS2 sequence data set of eDNA

(Apis dorsata) and stingless bees

from honey of Malaysian giant honeybees

(Heterotrigona itama) reveals plant species

## Abstract

diversity

**Objectives** Pollen is a useful tool for identifying the provenance and complex ecosystems surrounding honey production in Malaysian forests. As native key pollinators in Malaysia, Apis dorsata and Heterotrigona itama forage on various plant/pollen species to collect honey. This study aims to generate a dataset that uncovers the presence of these plant/pollen species and their relative abundance in the honey of A. dorsata and H. itama. The information gathered from this study can be used to determine the geographical and botanical origin and authenticity of the honey produced by these two species.

**Results** Sequence data were obtained for both A. dorsata and H. itama. The raw sequence data for A. dorsata was 5 Mb, which was assembled into 5 contigs with a size of 6,098,728 bp, an N50 of 15,534, and a GC average of 57.42. Similarly, the raw sequence data for *H. itama* was 6.3 Mb, which was assembled into 11 contigs with a size of 7,642,048 bp, an N50 of 17,180, and a GC average of 55.38. In the honey sample of A. dorsata, we identified five different plant/pollen species, with only one of the five species exhibiting a relative abundance of less than 1%. For H. itama, we identified seven different plant/pollen species, with only three of the species exhibiting a relative abundance of less than 1%. All of the identified plant species were native to Peninsular Malaysia, especially the East Coast area of Terengganu.

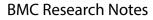
Data description Our data offers valuable insights into honey's geographical and botanical origin and authenticity. Metagenomic studies could help identify the plant species that honeybees forage and provide preliminary data for researchers studying the biological development of A. dorsata and H. itama. The identification of various flowers from

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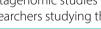
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the eDNA of honey that are known for their medicinal properties could aid in regional honey with accurate product origin labeling, which is crucial for guaranteeing product authenticity to consumers.

Keywords Honey, ITS2, Metabarcoding, Sequencing, OTU, NCBI

## Objective

Pollen is a useful tool for identifying the provenance and complex ecosystems surrounding honey production in Malaysian forests. As native key pollinators in Malaysia, *A. dorsata* and *H. itama* forage on various plant/pollen species to collect honey. This study aims to generate a dataset that uncovers the presence of these plant/pollen species and their relative abundance in the honey of *A. dorsata* and *H. itama*. The information gathered from this study can be used to determine the geographical and botanical origin and authenticity of the honey produced by these two species.

## **Data description**

This dataset contains eDNA sequence information from honey samples of *A. dorsata* and *H. itama*, collected from the East Coast area of Terengganu, Malaysia in June and July 2022. The samples were located at 4° 57' 6.48" N and 103° 20' 25.44" E. Individual DNA sequencing and FASTQ files for both samples are available through the National Centre for Biotechnology Information (NCBI) data repository system. The ITS2 nuclear gene region was amplified using previously described primers [1]. The filtered reads were clustered based on k-mer frequency profile using NanoCLUST [2], followed by consensus generation and error correction with Racon and Medaka v.1.4.1 [3].

For *A. dorsata* honey eDNA, a total output of 5 Mb was generated, which assembled into 5 OTUs. For *H. itama* honey eDNA, we obtained 5 contigs with a size of 6,098,728 bp, an  $N_{50}$  of 15,534, and a GC content of 57.42. The operational taxonomic unit (OTU) and FASTA file for this sample are accessible via NCBI (https://dataview.ncbi.nlm.nih.gov/object/SRR21831607) (Table 1). For *H. itama*, the raw sequence data shows a total size of 6.3 Mb, assembled into 11 contigs with a size of 7,642,028 bp, an N50 of 17,180, and a GC content of 55.38, based on the NCBI genome annotation pipeline.

**Table 1** General features of *A.dorsata* and *H. itama* predicted by

 NCBI genome annotation pipeline

<b>A. dorsata</b> 6,098,728	H. itama
6 098 728	7 4 40 000
0,000,720	7,642,028
05	11
15,534	17,180
57.42	55.38
SRR21831607	SRR21831606
SAMN31155927	SAMN31155926
PRJNA887189	PRJNA887189
	15,534 57.42 SRR21831607 SAMN31155927

The operational taxonomic unit (OTU) and FASTA file for this sample are accessible via NCBI (https://dataview. ncbi.nlm.nih.gov/object/SRR21831606).

The relative abundance (Ra) of the identified plant and pollen species, along with their taxonomical classification levels (Phylum, Class, Order, Family, Genus, and Species), are presented in Table 2. Each plant species' individual sequences underwent MEGABLAST analysis to identify highly similar sequences with nearly 100% identity. The complete sequences of selected species were downloaded in FASTA format for subsequent analysis.

The eDNA sequence analysis of honey from A. dorsata revealed frequent identification of plant species such as Corynandra viscosa (42.02%) and Syzygium cumini (40.11%). C. viscosa, locally known as Maman pasir, is an erect herb that can reach a height of 1.2 m. It features attractive yellow-colored flowers with a petiole length of 4.5 cm [4]. On the other hand, the genus Syzygium comprises over 1,200 species of trees or shrubs with sessile flowers ranging from 7 to 12 cm in height [5]. Every pollen species detected in the honey sample belonged to flowering plants, except for Mallotus paniculatus (known locally as Balik Angin), which accounted for less than 1% compared to other flowering plants/pollen species. Additional identified species included Scaevola taccada (10.17%), known locally as Merambong, and Syzygium claviflorum (7.66%), known locally as Bangkoh. It is worth noting that the identified pollen species in the eDNA sequence are native flowering plants found in the Peninsular Malaysia region where the sample was collected. These species have been previously reported in various studies, such as C. viscosa [6], S. cumini [7], and S. taccada [8].

For *H. itama* the eDNA sequence of honey analysis revealed a significant presence of various plant species. The most abundant species were M. paniculatus (Balik angin) (42%) and Cleome rudisperma (41%), locally called Maman ungu. M. paniculatus is a medicinal plant native to the East Coast of Malaysia [9]. C. rudisperma, on the other hand, is a flowering plant reported to be native to Malaysia [10]. Additional plant species identified in the eDNA analysis included *Richardia brasiliensis* (0.53%) [11], Ludwigia hyssopifolia (0.42%) (known locally as Lakum air), Eleucine indica (0.56%) (known locally as Rumput sambau) [12], Mimosa pudica (2.46%) (known locally as Semalu) [13], and Acacia mangium (14.49%) (known locally as Manga hutan) [14] (Table 2). Apart from our findings, another study reported a higher abundance of pollen from the phylum Spermatophyta [15].

Table 2 Numbers of plant/pollen species identified from honey samples A. dorsata and H. itama

Honey sample	Phylum	Order	Family	Pollen/plant species	Ra (%)	Habitat	References
A. dorsata	Magnoliophyta	Brassicales	Cleomaceae	Corynandra viscosa	42.02	deciduous forests	[4]
	Magnoliophyta	Myrateles	Myrtaceae	Syzygium cumini	40.11	secondary rainforest,	[16]
	Spermatophyta	Asterales	Goodeniaceae	Scaevola taccada	10.17	coastal forest	[17]
	Magnoliophyta	Myrateles	Myrtaceae	Syzygium claviflorum	7.66	terrestrial	[18]
	Magnoliophyta	Malpighiales	Euphorbiaceae	Mallotus paniculatus	0.04	secondary rainforest	[19]
Spe	Magnoliophyta	Malpighiales	Euphorbiaceae	Mallotus paniculatus	42	Secondary Rainforest	[19] [9]
	Spermatophyta	Brassicales	Cleomaceae	Cleome rutidosperma	41	ruderal habitat	[20]
	Spermatophyta	Fabales	Fabaceae	Acacia mangium	14.49	coastal tropical lowlands	[21] [22]
	Spermatophyta	Fabales	Fabaceae	Mimosa pudica	2.46	terrestrial	[13]
I	Spermatophyta	Poales	Poeceae	Eleucine indica	0.56	riverside, beaches	[23]
	Magnoliophyta	Myrtales	Rubiaceae	Richardia brasiliensis	0.53	any open places	[24]
							[25]
							[26]
	Spermatophyta	Myrtales	Onagraceae	Ludwigia hyssopifolia	0.42	wetlands	[27]

Note: Ra: Relative abundance or percentage of pollen based on plant species foraged by Apis dorsata and Heterotrigona itama

Specifically, four species, namely *Garcinia oblongifolia*, *Muntingia calabura*, *Mallotus pellatus*, *and Pinus squamata*, were found to occur abundantly and were consumed by *H. itama* in all populations.

### Limitations

Sample size: A small sample size may not be representative of the larger population and may limit the generalizability of the findings.

Regional specificity: The study focuses on honey samples from the Peninsular Malaysia region, which may limit the generalizability of the findings to other regions or countries.

Identification methods: The study uses eDNA sequencing and pollen analysis to identify plant species in the honey samples.

Honey production: honey was collected from multiple hives in one area. This could affect the diversity and abundance of plant species present in the honey samples.

Honey age: The age of honey can affect the diversity and abundance of plant species present in the sample.

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#### Authors' contributions

Nurul Huda: Resources, Writing – review & editing, Funding acquisition; Saeed Ullah: Data curation, Formal analysis, Investigation, Software, Writing – original draft; Roswanira Abdul Wahab: Supervision, Validation, Resources, Writing – review & editing; Mohd Nizam Lani: Data curation, Supply raw materials; Nur Hardy Abu Daud: Conceptualization, Software, Writing – review & editing; Amir Husni Mohd Shariff: Data curation, Formal analysis, Investigation, Methodology; Norjihada Izzah Ismail: Data curation, Formal analysis, Investigation, Methodology; Azzmer Azzar Abdul Hamid: Supervision, Conceptualization, Software, Writing; Mohd Azrul Naim Mohamad: Resources, Writing – review & editing; Fahrul Huyop: Supervision, Funding acquisition, Project administration, Validation, Writing – review & editing.

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#### Data Availability

https://www.ncbi.nlm.nih.gov/sra/SRX17820767. https://www.ncbi.nlm.nih.gov/sra/SRX17820766. https://www.ncbi.nlm.nih.gov/sra/SRX17820765.

#### Declarations

**Ethics approval and consent to participate** Not applicable.

#### **Consent for publication**

Not applicable.

#### **Competing interests**

The author declared that they have no known competing financial interest that could have appeared to influence the work presented in this paper.

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