Heliyon 10 (2024) e33094

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Using pollen DNA metabarcoding to trace the geographical and botanical origin of honey from Karangasem, Indonesia

Saeed ullah [a], Fahrul Huyop [a,b,1,*], Roswanira Abdul Wahab [c], I Gede Arya Sujana [b], Nyoman Semadi Antara [b], Ida Bagus Wayan Gunam [b,**]

[a] Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia
[b] Bioindustry Laboratory, Department of Agro-Industrial Technology, Udayana University, Denpasar, Indonesia
[c] Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia

ARTICLE INFO

Keywords:
ITS2
eDNA
Karangasem
Pollen DNA metabarcoding
Geographical origin
Botanical origin

ABSTRACT

The unique floral fingerprint embedded within honey holds valuable clues to its geographical and botanical origin, playing a crucial role in ensuring authenticity and detecting adulteration. Honey from native *Apis cerana* and *Heterotrigona itama* bees in Karangasem, Indonesia, was examined utilizing pollen DNA metabarcoding for honey source identification. In this study, we used ITS2 amplicon sequencing to identify floral DNA in honey samples. The finding reveals distinct pollen signatures for each bee species. Results analysis showed *A. cerana* honey generated 179,267 sequence reads, assembled into Amplicon Sequence Variants (ASVs) with a total size of 485,932 bp and an average GC content of 59 %. *H. itama* honey generated 177,864 sequence reads, assembled into ASVs with a total size of 350,604 bp and an average GC content of 57 %. *A. cerana* honey exhibited a rich tapestry of pollen from eleven diverse genera, with *Schleichera* genus dominating at an impressive relative read abundance of 72.8 %. In contrast, *H. itama* honey displayed a remarkable mono-dominance of the *Syzygium* genus, accounting for a staggering 99.95 % of its pollen composition or relative read abundance, highlighting their distinct foraging preferences and floral resource utilization. Notably, all identified pollen taxa were indigenous to Karangasem, solidifying the geographical link between honey and its origin. This study demonstrates pollen DNA metabarcoding may identify honey floral sources. By using pollen profiles from different bee species and their foraging patterns, we may protect consumers against honey adulteration and promote sustainable beekeeping in Karangasem district. Future research could explore expanding the database of reference pollen sequences and investigating the influence of environmental factors on pollen composition in honey. Investigating this technology's economic and social effects on beekeepers and consumers may help promote fair trade and sustainable beekeeping worldwide.

* Corresponding author. Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia.
** Corresponding author.
E-mail addresses: usaeed@graduate.utm.my (S. ullah), fahrul@utm.my (F. Huyop), roswanira@utm.my (R.A. Wahab), igedearyasujana2@gmail.com (I.G.A. Sujana), semadi.antara@unud.ac.id (N.S. Antara), ibwgunam@unud.ac.id (I.B.W. Gunam).
[1] Current addressUNISERF Senior Research Fellow, Bioindustry Laboratory, Department of Agro-Industrial Technology, Udayana University, Denpasar, Indonesia.

https://doi.org/10.1016/j.heliyon.2024.e33094
Received 12 February 2024; Received in revised form 25 May 2024; Accepted 13 June 2024
Available online 14 June 2024

1. Introduction

The diverse flora in Bali Island plays a pivotal role in shaping the unique ecological landscape of the region, spanning coastal areas to mountainous terrain. Honey is a natural product with diverse compositions influenced by the foraging behavior of bees and the local flora. Apiculture enthusiasts and researchers have increasingly turned to molecular techniques for honey authentication, and the use of ITS2 primers for profiling plant species in honey has gained prominence [1]. This study explores the potential of using ITS2 primers to differentiate between honey produced by *Apis Cerana* and stingless bee *Heterotrigona itama* in the Karangasem region. This research focuses on utilizing Transcribed Spacer 2 (ITS2) analysis to illuminate floral origins, plant diversity, and potential applications for environmentally friendly honey production [2].

Pollen serves as a valuable tool for identifying the origins and intricate ecosystems associated with honey production in Indonesian forests [3]. Indigenous key pollinators, *A. cerana*, and *H. itama*, forage on a variety of plant and pollen species to produce honey [4]. This study aims to establish a robust model that reveals the presence and relative abundance of these plant and pollen species in the honey of *A. cerana* and *H. itama*, acting as a signature indicating the specific geographical origin of the honey. The findings will contribute to determining the geographical and botanical authenticity of honey produced by these two species in the Tenganan Dauh Tukad Manggis area in the Karangasem district.

Globally, two primary honeybee species, *A. mellifera,* and *A. cerana* honeybees, are the main contributors to honey production [5]. Notably, China, as the leading honey exporter, faces challenges related to honey fraud [6]. In China, honey from endemic honeybees *A. cerana* is often sold at a premium due to the belief that honey from rural and mountainous regions is purer and healthier. This economic discrepancy has led to fraudulent practices, with honey from the western honeybee labeled as high-end products [7]. While geographic tracing is crucial for consumers seeking local honey, traditional methods relying solely on chemistry face challenges. The composition of honey sugars naturally fluctuates, making it challenging to trace their origin accurately [8]. However, identifying pollen is a complex task. Traditional methods like Melissopalynology are time-consuming, and biochemical methods have limitations [1,2,9].

Melissopalynology - the conventional approach to identify the plant origin of honey and obtain information about its geographical origin entails morphological observation of pollen grains by optical microscope. The structure of the contained pollens is assigned to botanical taxa using a comparative approach with pollen atlas. The monofloral characteristic of nectar honey is determined by the pollen frequencies for each botanical taxon, calculated with different precautions for anemophilous, entomophilous, nectar-producing, or nectar-less plants. The classic and most widely used approach for determining the plants from which bees collect nectar will help determine the honey's regional origin using the 29 morphological characteristics of pollen present in honey (melissopalynology) [1–4, 10].

Over the past decade, the power of DNA barcoding has opened up new fields in taxonomic, ecological, and evolutionary research by facilitating species identification. For animals, DNA barcoding is defined as the sequencing of a standardized barcode marker (the COI

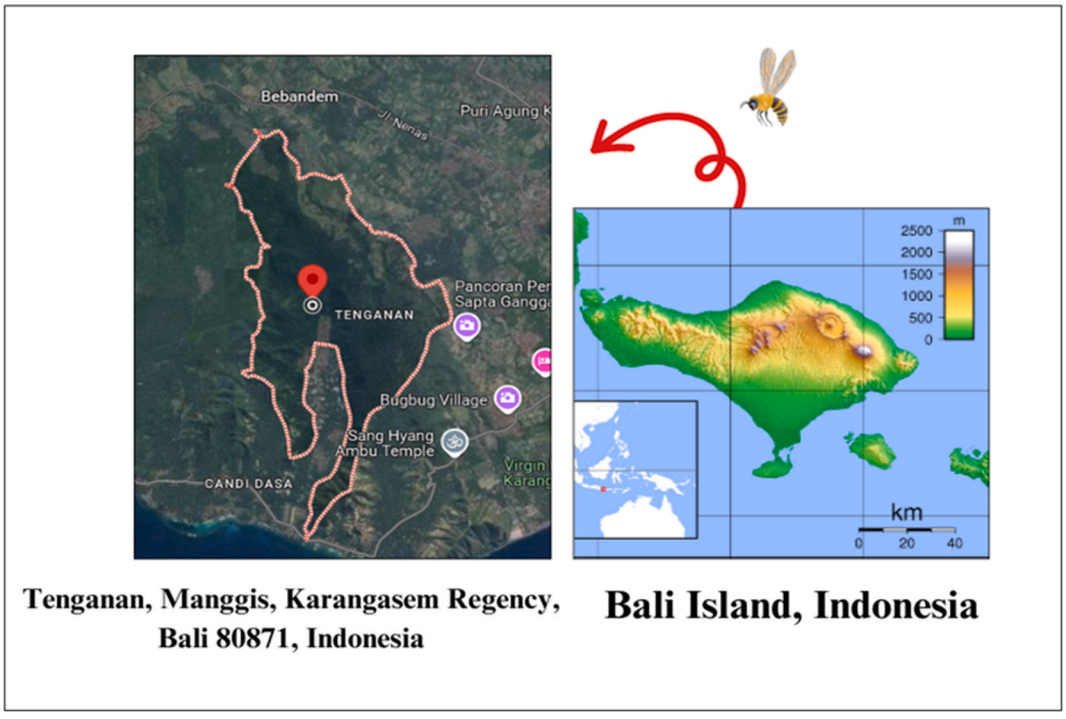

Fig. 1. Geographical location of Bali Island and sample location (Lat. 8°29′07.3″S 115°33′45.5″E).

gene region of the mitochondrial genome) that shows specificity within a species and variability between species [11]. According to diet study of honeybees that variety of pollen can have advantages, such as longer life spans and greater immune function. Depending on the resources available, the season, and the surrounding landscape structure, honeybees foraging distance, which can exceed several kilometres, may only be a few hundred metres long. Honey is a direct byproduct of honeybee foraging, making it ideal for research on the long-term nutritional requirements of bees [12].

Different plants produce nectar from different flowers, resulting in honey that ranges in texture (for instance, whether the honey stays liquid or crystallizes quickly) as well as in flavor, appearance, and scent. The health advantages of honey obtained from various flowers also vary. Due to their environmental exploration and foraging activities, honeybees are regarded as large-scale monitoring tools. Pollen and honeybee-derived products (wax, propolis, and honey) have been analyzed to detect the presence of toxins and pollutants in the environment and to pinpoint their source [8,13]. Honey has intrinsic markers that can be used to determine and track its origin at the three levels, geographical, botanical, and entomological, which in turn affects not only its economic value but also its significance in terms of sustainable production of various ecological systems and agricultural [14].

The primary objective of this research is to elucidate the botanical origins present in honey samples collected from the Bali District of Tenganan, Manggis, Karangasem Regency, in Bali, Indonesia. Specifically, is to investigate and compare the honey produced by two distinct bee species, namely *A. cerana* and *H. itama*, within this shared geographical area. The focus centers on authenticating honey through the molecular profiling of specific plant species using ITS2 primers. Through detailed analysis of pollen content in honey produced by these bee species, we aim to discern distinct plant signatures characteristic of the Karangasem region.

2. Material and methods

2.1. Sample collection

The honey samples were obtained from Tenganan, Manggis, Karangasem Regency, Bali 80871, Indonesia (Lat. 8°29′07.3″S 115°33′45.5″E) in the month of mid-December 2023 (Fig. 1). Honey samples were carefully harvested and stored at normal temperatures before processing for DNA extraction.

2.2. Sample preparation and DNA extraction

Honey is known for its high sugar content, which presents challenges in DNA extraction procedures. In this study, we adopted a modified protocol as previously described [2,15,16]. A total of 4 honey samples (two from each HB1 and SB1) were utilized, with each sample divided into four 50 mL Falcon tubes, ensuring proper representation and replication. Subsequently, 2 mL of ultrapure water was added to each tube, followed by an incubation period at 10 °C for 30 min. After centrifugation at a force of 30,000×*g* for 20 min, the liquid portion was removed, and the pellet was resuspended in a solution of 1 mL ultrapure water and 1 mL phosphate-buffered saline (PBS). This mixture was then transferred to 2 mL tubes and subjected to centrifugation at the same force for 20 min. The resulting solid residue, representing DNA material, was preserved at −20 °C until further processing.

Importantly, to address technical variability and ensure robust results, DNA extraction was performed in duplicate for each honey sample. The biomass was divided into two centrifuge tubes as replicates, and DNA extraction was carried out using the standard protocols of the QIAamp PowerFecal Pro DNA Kits with minimal modification [17]. This replication strategy allows for the assessment of variability and enhances the reliability of our findings.

2.3. Library preparation & sequencing

gDNA samples were amplified with target-specific primer (ITS2-Plant). Library preparation was performed using the final PCR products. The final library was sequenced on the DNBSEQ-G400 platform to generate paired-end raw reads.

2.4. Data analysis

Adapter and PCR primer sequences from the paired-end reads were removed using Cut adapt [18]. DADA2 was used to correct sequencing errors and remove low-quality sequences and chimera errors [19]. The resulting ASVs data was used for taxonomic classification against the PLANiTS database [20]. Downstream analysis and visualizations were performed using packages in RStudio (R version 4.2.3) (https://www.R-project.org/), and Krona Tools (https://github.com/marbl/Krona).

3. Results

3.1. Data availibalty

The raw FASTA files of the honey samples from *A. cerana* and *H. itama* bees have been deposited in the National Centre for Biotechnology Information (NCBI) data repository system under project PRJNA1063524, ensuring accessibility and transparency in data sharing [Direct link: Honey Origins through eDNA profiling of the Plant Species by A. cerana and H. itama at Karangasem District, Bali Island].

3.2. Comparative analysis of ITS2 amplicon sequencing

Analysis of ITS2 amplicon sequencing results for *A. cerana* (HB1) and *H. itama* (SB1) honey samples reveals several differences. Primarily, *A. cerana* honey demonstrates a higher total base count, amounting to 485,932 base pairs (bp), in contrast to *H. itama* honey, which records 350,604 bp. Despite this divergence, both honey types yield a similar number of sequences reads, with *A. cerana* honey generating 179,267 reads and *H. itama* honey producing 177,864 reads (Table 1).

3.3. Diversity indices of pollen community

To evaluate the species diversity within each honey type, alpha diversity of pollen composition was assessed with ASVs, Shannon, Simpson and InvSimpson (Fig. 2). The ASVs described the unique taxa identified in each sample. HB1 has 131 ASVs, while SB1 has 116 ASVs. The Shannon diversity index, Simpson diversity index, and inverse Simpson diversity index are all measures of diversity that take into account both the richness (number of species) and evenness (abundance of each species) of a community. Higher values of these indices indicate greater diversity.

The Simpson diversity index revealed that the honey samples from *A. cerana* exhibited dominance and were represented by a greater variety of species compared to those from *H. itama*. Specifically, the Simpson index value was higher for *H. itama* (0.96) in contrast to *A. cerana* (0.90). Similarly, the Shannon diversity index for plant pollen was also higher for *H. itama* (3.82) compared to *A. cerana* (2.69). These findings indicate that while both honey samples contained diverse pollen, the distribution of species within the honey samples from *H. itam*a was more even compared to those from *A. cerana*.

Fig. 3 is the rarefaction curve comparing the number of species to the number of samples shows that *A. cerana* (HB1) has a higher number of detected pollen species compared to *H. itama* (SB1).

Fig. 4 is a Venn diagram a comparative analysis of honey samples from *A. cerana* (HB1) and *H. itama* (SB1) revealed a significant disparity in their associated amplicon sequence variants (ASVs). *A. cerana* (HB1) honey exhibited the highest ASV richness, harboring 126 unique ASVs, while *H. itama* (SB1) honey contained 111 ASVs. The Venn diagram depicted a minimal overlap, with only 5 ASVs shared between the two honey types. This pronounced dissimilarity, characterized by a low number of shared ASVs, suggests that the nectar sources utilized by *A. cerana* and *H. itama* honeybees are fundamentally distinct. This divergence could be attributed to various factors, including differential foraging preferences for specific plant species, each of which harbours unique pollen types that are subsequently reflected in the honey composition.

3.4. Pollen composition and taxonomy

The ITS2 marker distinguished a total of 11 at genus level in both honey samples, with 9 being identified in honey of *A. cerana* and 2 in honey of *H. itama*. This indicates the presence of unique genetic groups within the samples that were differentiated or identified using ITS2 marker (Fig. 5).

The abundance and number of genera identified varied across the two honey samples. Fig. 5 illustrates the relative abundance of identified pollen in both honey samples, along with their taxonomic breakdown at the phylum, class, family, and genus levels. As expected, *A. cerana* honey exhibited a more diverse botanical composition compared to *H. itama* honey, which was dominated by only two genera. This suggests that *A. cerana* bees foraged on a wider variety of plants/pollens as compared to *H. itama* bees.

Analysis of *A. cerana* (HB1) honey samples revealed a diverse array of pollen from nine distinct genera, all classified within the phylum Streptophyta. These genera accounted for 99.53 % of the total relative read abundance, with the remaining 0.47 % being unassigned (Fig. 5). Among these genera, *Schleichera* emerged as the pre-dominant taxon, comprising 72.8 % of the relative read abundance, followed by *Syzygium* at 26.87 %, and minor contributions from *Samanea* (0.08 %), *Dalbergia* (0.07 %), *Mangifera, Artocarpus*, and *Lepisanthes* (0.05 % each), as well as *Muntingia* (0.04 %) and *Swietenia* (0.03 %). Genera categorized as others or unassigned collectively represented of only 0.01 % of the relative read abundance. Additionally, *Lactuca* and *Planchonella* were identified as part of the *A. cerana* (HB1) honey sample's pollen composition.

In contrast, for *H. itama* (SB1) at phylum level the read were belonging to only phyla Streptophyta with relative read abundance of 50.93 % and unassigned were 49.07 %. Notably, at genus level the *Syzygium* pollen was overwhelmingly dominant in *H. itama* honey, representing almost 99.95 % of the relative read abundance, while *Schleichera* pollen made up only 0.05 % (Fig. 5).

Table 2 summarises the results of the taxonomic analysis of the ITS2 metabarcoding and reads count for the pollen identified, which showed a great variety of taxa in the honey samples. The table also provides a detailed breakdown of all detected classifications, from Phylum to Genus. Interestingly, the taxonomic makeup of the honey samples was different from one another. The honey obtained from *A. cerana* and *H. itama* in a specific region contains eDNA that encompasses a wide range of ecological diversity, as evidenced by these

Table 1
Comparison of ITS2 amplicon sequencing results for *A. cerana* (HB1) and *H. itama* (SB1) honey samples.

Attribute	*A. cerana*	*H. itama*
Total bases generated (bp)	485,932	350,604
Sequence reads	179,267	177,864
G + C content (%)	59	57

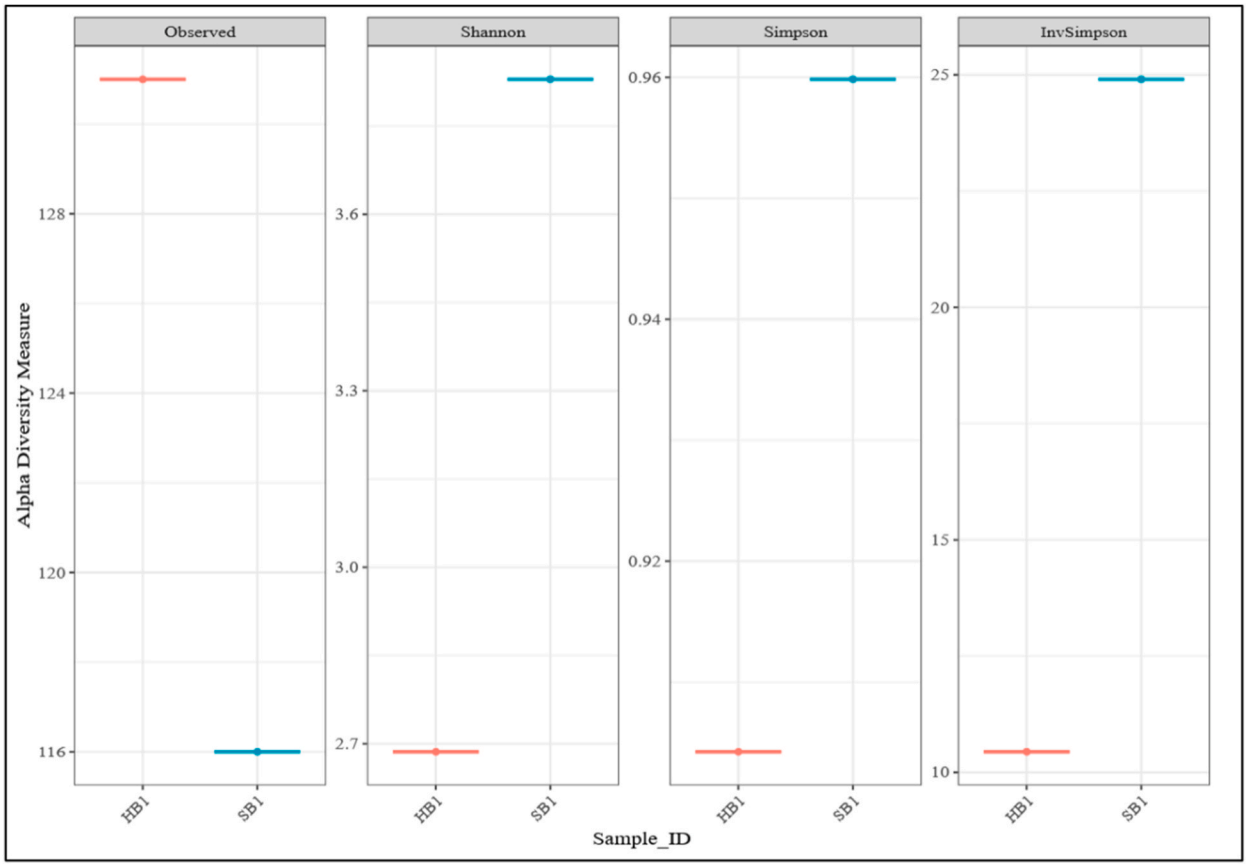

Fig. 2. Alpha diversity for both honey samples of *A. cerana* (HB1) and *H. itama* (SB1).

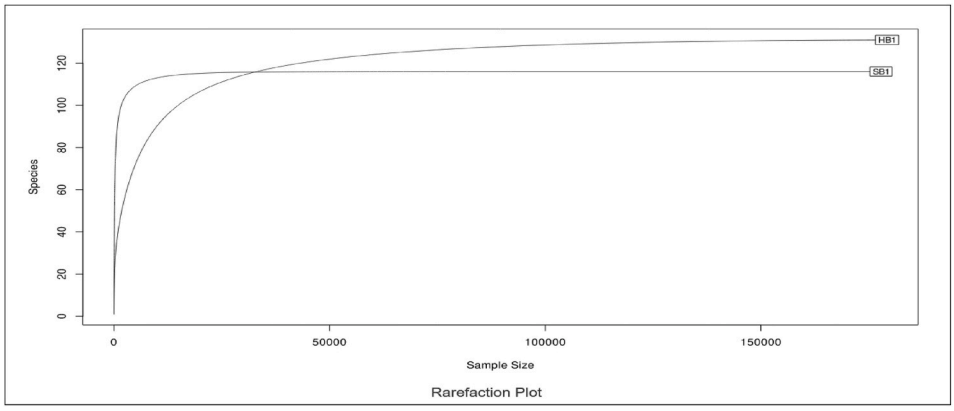

Fig. 3. Rarefaction curve of the number of annotated species for honey samples *A. cerana* (HB1) and *H. itama* (SB1).

findings.

4. Discussion

Alongside conventional melissopalynology, DNA analysis of honey samples is gaining importance for determining the origin and microorganisms or potentially allergenic species. The primers ITS2 successfully amplified the extracted DNA and allowed analysis of the botanical composition of all the honey samples by *A. cerana* and *H. itama*. Many studies showing the plant material can be used to authenticate honey origin and differentiate between honey samples [2,3].

Our results show that pollen eDNA amplicon sequencing can produce detailed information regarding the floral sources of Bali,

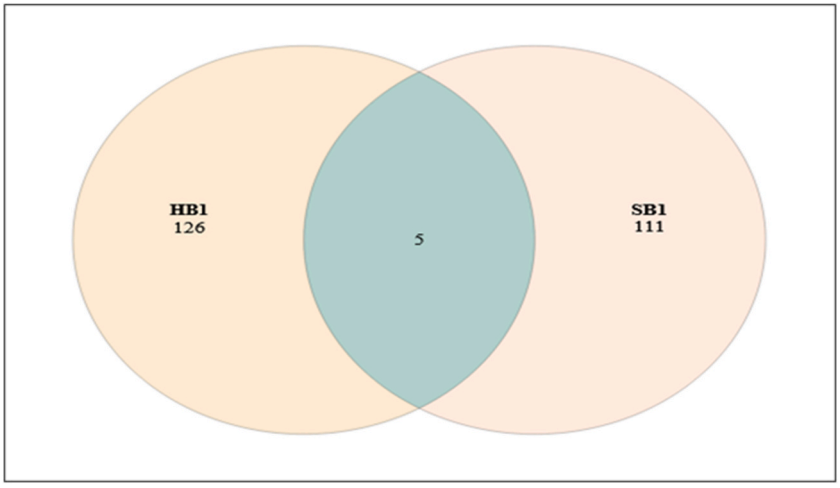

Fig. 4. Venn diagram showing the total number of ASVs identified in both honey samples and the number of ASVs shared between honey samples.

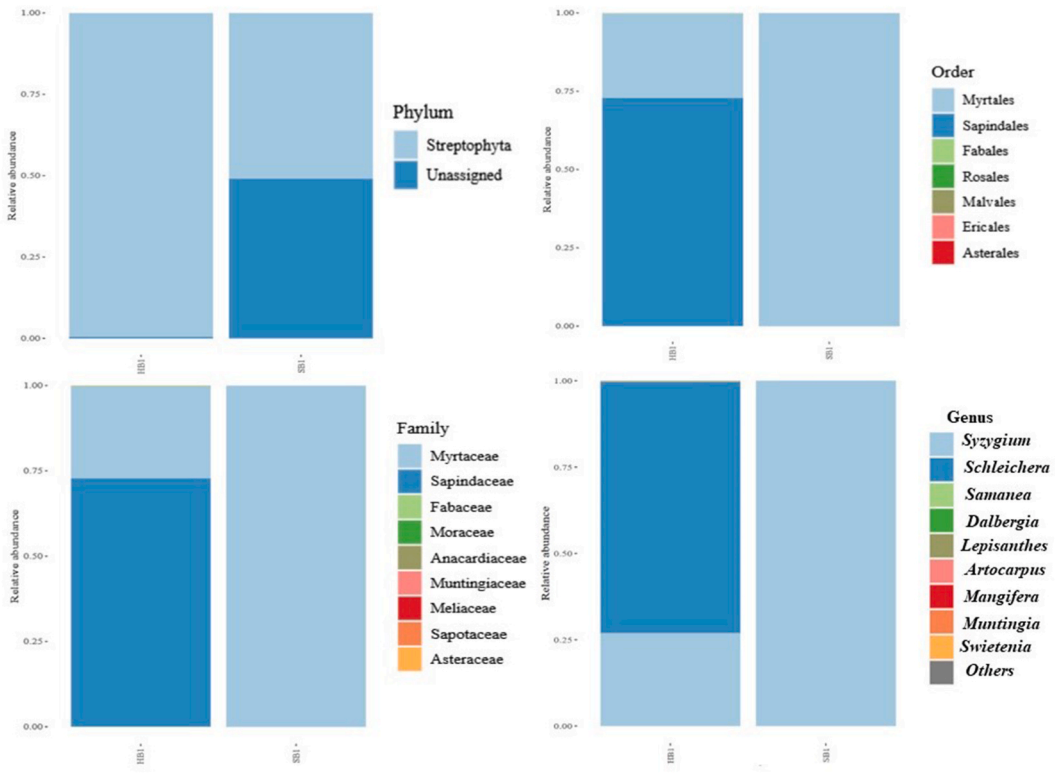

Fig. 5. Relative abundance (%) of pollen identified in both honey samples, obtained from *A. cerana* (HB1) and *H. itama* (SB1) showing different levels of taxonomy from Phyla to Genus.

Indonesian honey, including inferences about its regional provenance. It can also be used to gain insights into the floral resources available to honeybees in the surrounding area. However, due to the lack of complete plant reference databases and poor taxonomic resolution of some taxa, the interpretation of metabarcoding results requires careful validation [12]. Notably, all identified pollen taxa were native to Karangasem, solidifying the geographical link between honey and its origin.

4.1. Identification of pollen revolved honey origin

ITS2 results reveal the honey sample's botanical makeup. The molecular technique identifies honey-producing plant species by

Table 2
Plant pollen classifications (Phylum, Class, Order, Family, and Genus) and reads identified in honey produced by *A. cerana* and *H. itama.*

No.	Honey from:	Phylum	Order	Family	Pollen/plant Genus	Habitat	References
01	*H. itama*	Streptophyta	Myrateles	Myrtaceae	*Syzygium* sp.	Secondary rainforest	[21]
02		Streptophyta	Sapindales	Sapindaceae	*Schleichera* sp.	Dry deciduous	[22]
01	*A. cerana*	Streptophyta	Sapindales	Sapindaceae	*Schleichera* sp.	Dry deciduous	[22]
02		Streptophyta	Rosales	Moraceae	*Artocarpus* sp.	Terrestrial	[23]
03		Streptophyta	Fabales	Fabaceae	*Dalbergia* sp.	Terrestrial	[24]
04		Streptophyta	Sapindales	Sapindaceae	*Lepisanthes* sp.	Secondary rainforest	[25]
05		Streptophyta	Sapindales	Anacardiaceae	*Mangifera* sp.	Terrestrial	[23,26]
06		Streptophyta	Malvales	Muntingiaceae	*Muntingia* sp.	Tropical lowland areas	[27]
07		Streptophyta	Fabales	Fabaceae	*Samanea* sp.	coastal bushland	[28]
08		Streptophyta	Fabales	Fabaceae	*Swietenia* sp.	coastal bushland	[29]
09		Streptophyta	Myrateles	Myrtaceae	*Syzygium* sp.	Secondary Rainforest	[21]

focusing on ITS2 variability. This information enhances honey authentication by verifying botanical or geographical provenance. The accurate ITS2 analytical data improves the honey supply chain's transparency and reliability, assuring consumers obtain products that match their claims and boosting honey market trust and authenticity. Pollen/plant species identified in *H. itama* honey samples belonging to local flora origin of Karangasem district of Bali Island. The dominant species *Syzygium samarangense* [21], known locally as Jambu klampok in Indonesia and Jambu Mawar in Malaysia. Another species *Schleichera oleosa* locally known as Kesambi wood in Indonesia, both the pollen/plant is overlap in both honey samples [22].

The majority of pollen/plant species identified in the honey of *A. cerana* and *H. itama* hail from the indigenous flora of Tenganan, Manggis, within the Karangasem Regency of Bali, as extensively documented by Wijana et al., [23]. These plants, known locally as *Syzygium* (Jambu), *Schleichera* (Kusum), *Artocarpus* (Marang), *Dalbergia* (Sonokeling), *Lepisanthes* (Mertajam), *Mangifera* (Pohon Kweni mango), *Muntingia calabura* (Cherry), *Samanea* (Ki Hujan or Pokok Hujan), and *Swietenia* (Pohon Mahogany), are deeply rooted in the region's ecosystem (Table 2). By comparing our pollen identification results with previously published work, we have substantiated the geographical origin of the honey and affirmed its authenticity as originating within the Karangasem Regency of Bali. This meticulous analysis not only highlights the indigenous botanical diversity of the surveyed area but also establishes a reliable methodology for confirming the geographical origin of honey procured from this specific locale.

4.2. Comparative analysis of Malaysian-Indonesian honey pollen

This comparative investigation serves as an exemplar for discerning between honey samples originating from two distinct geographical regions, produced by two prevalent local honeybee species. The pollen taxa identified within each honey sample serve as distinctive markers for their respective origins, facilitating sample differentiation. In Fig. 6, we present the comparison of pollen/plant genera identified via the ITS2 gene marker in honey samples sourced from Malaysia and Indonesia. The honey produced by *A. cerana* and *H. itama* in the present study was juxtaposed with that produced by *A. dorsata* and *H. itama* [30]. This comparison conclusively revealed that all plant species contributing to the honey were indigenous to Peninsular Malaysia, with a particular emphasis on the East Coast region of Terengganu, Malaysia.

At level phyla the honey samples from Indonesian honey were thoroughly represented by phyla Streptophyta while Malaysian honey was represented by two different phyla namely Magnoliophyta and Spermatophyta suggesting its localities. At the genus level, the pollen composition of honey samples exhibited distinct variations from one another. Only *Syzygium* appears in as overlapping floral source of honeybee, highlighting a common nectar source despite regional differences Indonesia and Malaysia. Floral source of Malaysian honey for Giant honeybee (*A. dorsata)* represented by *Corynandra viscosa*, *Syzygium cumini*, *Scaevola taccada*, *Syzygium claviflorum* and *Mallotus paniculatus,* while stingless be (*H. itama*) floral source represented by *Mallotus paniculatus*, *Cleome rutidosperma, Acacia mangium, Mimosa pudica, Eleucine indica, Richardia brasiliensis,* and *Ludwigia hyssopifolia* [30].

5. Conclusion

The ITS2 analysis conducted in this study has successfully elucidated the floral source diversity inherent in honey produced by *A. cerana* and *H. itama*. Our findings established the distinctiveness of honey types originating from these two bee species, as evidenced by their disparate pollen sources obtained during foraging activities, thereby facilitating the discrimination of honey samples. Furthermore, all identified floral sources in our investigation are native to Karangasem Regency, Bali, thus enhancing the authenticity and provenance determination of the honey samples. Moreover, the comparative analysis of pollen compositions in Malaysian and Indonesian honey samples highlights the botanical diversity and foraging behavior variations among honeybee populations in different geographical regions. This highlights the importance of taking into account the regional floral diversity in both honey production and authentication efforts. For future research, efforts should focus on refining DNA-based techniques and expanding reference databases to enhance our ability to accurately analyze honey composition and origin. By incorporating multiple DNA barcodes and expanding reference databases, researchers can improve the accuracy and reliability of floral source identification in honey samples. Such advancements will not only bolster consumer confidence in local honey products but also contribute to global

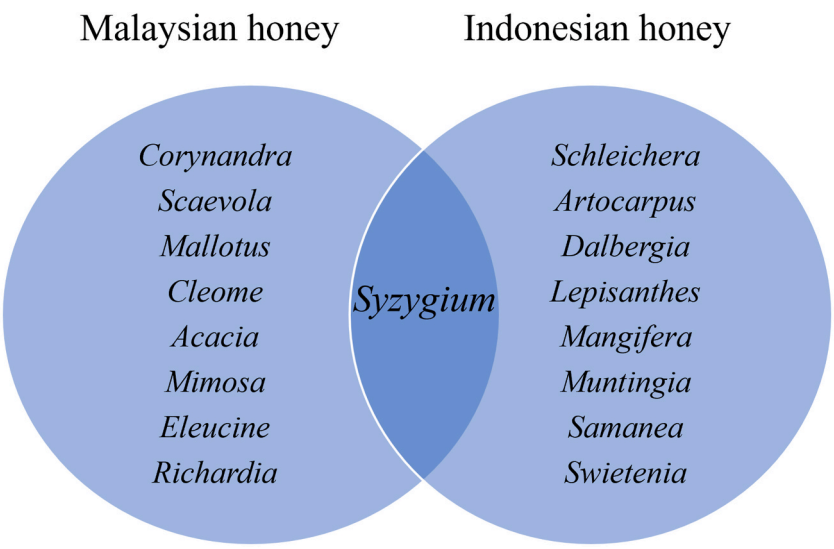

Fig. 6. Comparison of pollen composition at the genus level between honey samples from Malaysia [30] and Karangasem, Bali, Indonesia (present study). Each represents particular pollen genus in the honey samples. The distinct pollen composition highlights the differences in floral sources utilized by bees in these two geographical locations.

efforts aimed at ensuring the integrity and authenticity of honey sources.

Ethics statement

The current research work does not involve studies with animals and humans.

Data availability

The data supporting the findings of this study are available in the SRA (NCBI) repository under accession number PRJNA1063524. They can be accessed directly via the following link: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1063524. Additionally, Supplementary 1 contains data for Taxonomy Classification, and Supplementary 2 contains data for Relative Abundance at the Genus Level.

CRediT authorship contribution statement

Saeed ullah: Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Fahrul Huyop:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Roswanira Abdul Wahab:** Writing – review & editing, Supervision, Methodology, Conceptualization. **I Gede Arya Sujana:** Writing – review & editing, Data curation, Conceptualization. **Nyoman Semadi Antara:** Methodology, Data curation, Conceptualization. **Ida Bagus Wayan Gunam:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors sincerely thank the private suppliers, Honeybee Farm & Luwak Coffee Agrotourism, Tenganan, Manggis, Karangasem Regency, Bali 80871, Indonesia. We also extend our gratitude to Udayana University for their support through the UNISERF Program, providing research funding (Reference number: B/775-4/UN14.4A/PT.01.03/2023). The research permit was awarded by BRIN under Letter of Research Permit: 620/SIP/IV/FR/10/2023. The invaluable assistance and support significantly contributed to the successful completion of this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33094.

References

[1] S. Soares, J.S. Amaral, M.B.P. Oliveira, I. Mafra, Improving DNA isolation from honey for the botanical origin identification, Food Control 48 (2015) 130–136, https://doi.org/10.1016/j.foodcont.2014.02.035.

[2] H. Wirta, et al., DNA traces the origin of honey by identifying plants, bacteria and fungi, Sci. Rep. 11 (2021) 1–14, https://doi.org/10.1038/s41598-021-84174-0.

[3] B. Chiara, et al., Exploring the botanical composition of polyfloral and monofloral honeys through DNA metabarcoding, Food Control 128 (2021) 108175, https://doi.org/10.1016/j.foodcont.2021.108175.

[4] D. Pathiraja, J. Cho, J. Kim, I.-G. Choi, Metabarcoding of eDNA for tracking the floral and geographical origins of bee honey, Food Res. Int. 164 (2023) 112413, https://doi.org/10.1016/j.foodres.2022.112413.

[5] Y.-Z. Zhang, et al., Authentication of *Apis cerana* honey and *Apis mellifera* honey based on major royal jelly protein 2 gene, Molecules 24 (2019) 289, https://doi.org/10.3390/molecules24020289.

[6] P.M. Ferrier, Detecting origin fraud with trade data: the case of US honey imports, Aust. J. Agric. Resour. Econ. 65 (2021) 222–245, https://doi.org/10.1111/1467-8489.12412.

[7] S. Liu, et al., Tracing the origin of honey products based on metagenomics and machine learning, Food Chem. 371 (2022), https://doi.org/10.1016/j.foodchem.2021.131066.

[8] C. Santos-Buelga, A.M. Gonzalez-Paramas, Chemical composition of honey, in: J.M. Alvarez-Suarez (Ed.), Bee Products-Chemical and Biological Properties, Springer International Publishing, Cham, 2017, pp. 43–82. ISBN : 978-3-319-59688-4.

[9] S. Mohamadzade Namin, M.-J. Kim, M. Son, C. Jung, Honey DNA metabarcoding revealed foraging resource partitioning between Korean native and introduced honey bees (Hymenoptera: *Apidae*), Sci. Rep. 12 (2022) 14394, https://doi.org/10.1038/s41598-022-18465-5.

[10] I. Bruni, et al., A DNA barcoding approach to identify plant species in multiflower honey, Food Chem. 170 (2015) 308–315, https://doi.org/10.1016/j.foodchem.2014.08.060.

[11] K.L. Bell, et al., Pollen DNA barcoding: current applications and future prospects, Genome 59 (2016) 629–640, https://doi.org/10.1139/gen-2015-0200.

[12] L. Milla, et al., Pollen DNA metabarcoding identifies regional provenance and high plant diversity in Australian honey, Ecol. Evol. 11 (2021) 8683–8698, https://doi.org/10.1002/ece3.7679.

[13] S. Bovo, et al., Shotgun sequencing of honey DNA can describe honey bee derived environmental signatures and the honey bee hologenome complexity, Sci. Rep. 10 (2020) 1–17, https://doi.org/10.1038/s41598-020-66127-1.

[14] V.J. Utzeri, A. Ribani, L. Fontanesi, Authentication of honey based on a DNA method to differentiate *Apis mellifera* subspecies: application to Sicilian honey bee (*A. m. siciliana*) and Iberian honey bee (*A. m. iberiensis*) honeys, Food Control 91 (2018) 294–301, https://doi.org/10.1016/j.foodcont.2018.04.010.

[15] S. Bovo, et al., Shotgun metagenomics of honey DNA: evaluation of a methodological approach to describe a multi-kingdom honey bee derived environmental DNA signature, PLoS One 13 (2018) e0205575, https://doi.org/10.1371/journal.pone.0205575.

[16] P. Vit, et al., A novel integrative methodology for research on pot-honey variations during post-harvest, Sociobiology 69 (2022), https://doi.org/10.13102/sociobiology.v69i4.8251 e8251-e8251.

[17] S.S. Wei, et al., Gut microbiome and metabolome of sea cucumber (*Stichopus ocellatus*) as putative markers for monitoring the marine sediment pollution in Pahang, Malaysia, Mar. Pollut. Bull. 182 (2022) 114022, https://doi.org/10.1016/j.marpolbul.2022.114022.

[18] M. Martin, Cutadapt removes adapter sequences from high-throughput sequencing reads, EMBnet. journal. 17 (2011) 10–12, https://doi.org/10.14806/ej.17.1.200.

[19] B.J. Callahan, et al., DADA2: high-resolution sample inference from Illumina amplicon data, Nat. Methods 13 (2016) 581–583, https://doi.org/10.1038/nmeth.3869.

[20] E. Banchi, et al., PLANiTS: a curated sequence reference dataset for plant ITS DNA metabarcoding, Database 2020 (2020), https://doi.org/10.1093/database/baz155.

[21] S. Sutomo, I.D.P. Darma, A. Priyadi, R. Iryadi, Trees species diversity and indicator species in Bedugul forest ecosystem, Bali, Indonesia, Biodiversitas Journal of Biological Diversity 19 (2018) 2213–2218, https://doi.org/10.13057/biodiv/d190629.

[22] W. Sujarwo, I.B.K. Arinasa, Diversity of plants naturally grow in tropical dry forest (T-df) of Bukit Jimbaran Bali, J. Text. Inst. 36 (2013) 87–96.

[23] N. Wijana, I.G.A.N. Setiawan, Mapping and distribution of useful plant species in bukit kangin forest, pegringsingan village, Karangasem, Bali, in: 3rd International Conference on Innovative Research across Disciplines (ICIRAD 2019), Atlantis Press, 2020, https://doi.org/10.2991/assehr.k.200115.015.

[24] K. Yulita, et al., Population genetic of the Indonesian rosewood (*Dalbergia latifolia*) from Java and West Nusa Tenggara revealed using sequence-related amplified polymorphism, For. Sci. Technol. 18 (2022) 172–181, https://doi.org/10.1080/21580103.2022.2123051.

[25] L. Knorr, H. Phatak, H. Keifer, Web-spinning eriophyid mites, J. Wash. Acad. Sci. (1976) 228–234.

[26] P. Suwardike, I. Rail, R. Dwiyani, E. Kriswiyanti, DNA polymorphism and genetic diversity of mango (Mangifera sp.) Germplasm in tropical islands, Int J Biosci Biotechnol 7 (2019) 45, https://doi.org/10.24843/IJBB.2019.v07.i01.p05.

[27] F.X. Sudaryanto, et al., Daily activity, diet and habitat of Bali myna (*Leucopsar rothschildi*) in nusa Penida, Bali, Indonesia, Biodiversitas Journal of Biological Diversity 21 (2020), https://doi.org/10.13057/biodiv/d211002.

[28] R.J. Hesturini, Antihyperlipidemic activity of trembesi leaf extract (*Samanea saman*) against total and LDL cholesterol levels in hypercholesterolemic mice, Jurnal EduHealth 14 (2023) 1270–1275.

[29] M. Ervina, The recent use of *Swietenia mahagoni* (L.) Jacq. as antidiabetes type 2 phytomedicine: a systematic review, Heliyon 6 (2020) e03536, https://doi.org/10.1016/j.heliyon.2020.e03536.

[30] N. Huda, et al., The first ITS2 sequence data set of eDNA from honey of Malaysian giant honeybees (*Apis dorsata*) and stingless bees (*Heterotrigona itama*) reveals plant species diversity, BMC Res. Notes 16 (2023) 211, https://doi.org/10.1186/s13104-023-06495-9.