

Identification of Food Sources of *Apis cerana* Fabricius, 1793 Based on Pollen Analysis

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Abstract

Apis cerana, commonly known as the “Asian honey bee,” has been utilized for thousands of years across Asia, its native region, for honey production and pollination services. This species is often described as the closest “twin” of its European/African sister species, *A. mellifera*. This study aimed to identify plant species serving as pollen food sources for the honey bee *A. cerana* based on pollen morphological analysis. The research was conducted using a descriptive exploratory approach in Jononunu Village, Parigi Tengah District, Parigi Moutong Regency, Central Sulawesi, Indonesia. Pollen samples were collected from worker bees during foraging activities and subsequently analyzed through acetolysis and microscopic examination in the laboratory. Pollen identification was based on morphological characteristics, including exine ornamentation, aperture type, and pollen size. The results showed that *A. cerana* utilized pollen sources from 14 plant species belonging to 12 families, indicating a high diversity of forage resources. The identified pollen morphological characteristics comprised four types of exine ornamentation, six aperture types, and pollen sizes ranging from very small to large, with small- to medium-sized pollen being dominant. Tricolporate aperture types and psilate ornamentation were the most frequently observed characteristics. Based on plant growth form, pollen sources were predominantly derived from tree species, followed by herbs, while shrubs and bushes contributed less. These findings provide important baseline information for melissopalynological studies and may serve as a reference for the management and conservation of *A. cerana* and its forage plants.

Keywords: *Apis cerana* Fabricius 1793; acetolysis; honey bee; food sources; pollen.

INTRODUCTION

Apis cerana, commonly known as the “Asian honey bee,” has been utilized for thousands of years across Asia, its native region, for honey production and the provision of pollination services. This species is often described as the closest “twin” of its European/African sister species, *A. mellifera*. In the 1970s, *A. cerana* was deliberately introduced into Papua New Guinea, after which it continued to expand its distribution to the Solomon Islands, Vanuatu, and Australia (Rojas-Sandoval, 2022).

Indonesian populations of *A. cerana* have an average body size of approximately 2.49 ± 0.13 mm. Honey bees are capable of visiting a wide range of vegetation types, and such foraging activities have the potential to facilitate pollination, thereby increasing the likelihood of fertilization and successful generative reproduction in plants (Syayidah et al., 2024). This species collects pollen from various types of flowers, including wild plants. *A. cerana* generally prefers habitats at higher elevations with relatively low temperatures and dry

environmental conditions. Its distribution is influenced by altitude; however, in certain highland areas, *A. cerana* may be less well adapted due to limited plant diversity and temperature conditions that affect vegetation growth (Nuraini & Purwanto, 2021).

Pollen grains possess a highly stable wall layer composed of the polymer sporopollenin, which allows their morphology to remain unchanged even when carried on the bodies of honey bees. Consequently, bee forage sources can be readily observed and identified based on the morphological characteristics of the collected pollen (Kusmoro et al., 2024). Honey bees transport pollen back to the hive in the form of compact pellets, which are then stored in prepared pollen pots. Once the pots are filled, they are sealed with propolis (Masrianih et al., 2022). Pollen serves as the primary food source for honey bees, particularly as a major source of protein essential for sustaining colony life and productivity. The shape and type of pollen collected by bees vary considerably depending on their habitat. Therefore, studies measuring pollen diameter from

different plant species are necessary to identify the main pollen sources preferred by bees (Jayuli et al., 2018).

The identification of bee forage plants is conducted to determine the types of food sources utilized by honey bees. The classification of plants based on pollen characteristics represents essential data for completing the categorization of forage plants. In palynological studies, pollen grains are primarily examined based on wall characteristics. Pollen identification involves various aspects, including size, shape, and surface ornamentation, which are observed in both polar and equatorial views (Pratama et al., 2018).

Previous studies have demonstrated that pollen availability is a critical factor for honey bee survival, particularly in urban forest conservation areas in Bandung Regency, West Java. Pollen is the main protein source for honey bees and plays a crucial role in the development of hypopharyngeal glands, royal jelly production, and larval growth; thus, both pollen quality and quantity strongly determine colony health (Liang et al., 2025). Earlier research identified 27 plant species belonging to 20 families, with *Ageratum conyzoides* as the dominant pollen source (44.28%), while *Ceiba pentandra* and *Pinus merkusii* showed the lowest percentages (0.12%) (Kusmoro et al., 2024). This study aims to identify plant species that serve as pollen food

sources for the honey bee *Apis cerana*, particularly in Central Sulawesi. The results of pollen morphological analysis are expected to contribute to scientific knowledge, especially in the field of melissopalynology (the study of pollen in bees), by providing references on forage plants of *A. cerana*. Furthermore, this study is expected to enhance understanding of the relationship between pollen morphological characteristics and forage origin determination, thereby serving as a reference for future studies.

MATERIALS AND METHODS

Study area

This study was conducted in November 2025 in Jononunu Village, Central Parigi District, Parigi Moutong Regency, Central Sulawesi, Indonesia (Figure 1). The research site was located in a managed *Apis cerana* honey bee farming area surrounded by various vegetation types, including cultivated plants and wild flora that potentially serve as forage resources. The environmental conditions of the study area support honey bee foraging activities, making this location representative for investigating the diversity of pollen food sources utilized.

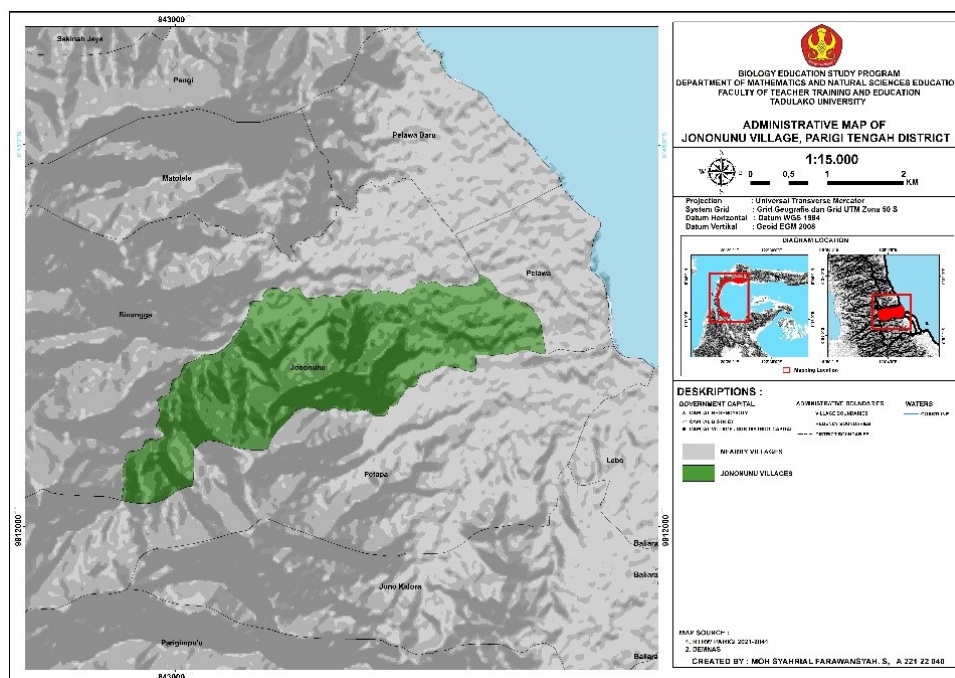


Figure 1. Map of the research location in Jononunu Village, Central Parigi District, Parigi Moutong Regency, Central Sulawesi, Indonesia.

Procedures

Sample Collection

Sampling of *Apis cerana* worker bees was carried out at 06:00 WITA, when bees began their foraging activities. Collected bees were placed into clean, closed plastic containers to allow the pollen attached to their bodies to

fall off. The pollen accumulated in the containers was then transferred into sterile 1.5 mL microtubes. Each sample tube was properly labeled with information on the sampling time and location to facilitate subsequent identification and analysis.

Pollen Acetolysis

Pollen samples stored in 1.5 mL microtubes were processed using the acetolysis method. Approximately 1 mL of acetolysis solution, consisting of acetic anhydride (C₄H₆O₃) and sulfuric acid (H₂SO₄) in a 9:1 ratio, was added to each sample. The mixture was allowed to stand for approximately 5 minutes to ensure adequate reaction. The samples were then heated in a water bath at 80°C for 5 minutes and centrifuged at 3,500 rpm for 10 minutes. After centrifugation, the supernatant was discarded, and the pollen residue was washed by adding 1 mL of distilled water, followed by centrifugation under the same conditions. This washing step was repeated two to three times until the solution became clear. The sedimented pollen was then dried in an oven at 60°C overnight.

Pollen Preparation

Pollen samples stored in 1.5 mL microtubes were processed using the acetolysis method. Approximately 1 mL of acetolysis solution, consisting of acetic anhydride (C₄H₆O₃) and sulfuric acid (H₂SO₄) in a 9:1 ratio, was added to each sample. The mixture was allowed to stand for approximately 5 minutes to ensure adequate reaction. The samples were then heated in a water bath at 80°C for 5 minutes and centrifuged at 3,500 rpm for 10 minutes. After centrifugation, the supernatant was discarded, and the pollen residue was washed by adding 1 mL of distilled water followed by centrifugation under the same conditions. This washing step was repeated two to three times until the solution became clear. The sedimented pollen was then dried in an oven at 60°C overnight.

Pollen Type Identification

Prepared pollen slides were examined under a light microscope (Olympus CX31LEDRFS1) equipped with an Indomicro camera connected to a computer. Images were captured for documentation and identification purposes. Pollen identification was based on morphological characteristics, including (1) exine ornamentation, (2) aperture type, and (3) pollen size, following Priambudi et al. (2021) and supported by reference data from the Australian Pollen and Spore Atlas (APSA).

Data analysis

Pollen identification was conducted using two approaches: comparison with reference pollen collected from plants surrounding the bee hives and comparison

with pollen databases from the Australian Pollen and Spore Atlas (<https://a.psa.anu.edu.au/>) and Pollen Flora of Taiwan. According to Pollen Flora of Taiwan, pollen grains were classified into five size categories: very small (<10 µm), small (10–25 µm), medium (25–50 µm), large (50–100 µm), and very large (100–200 µm). Exine ornamentation was categorized into echinate, scabrate, psilate, and reticulate types, while aperture types were classified as tricolporate, monosulcate, syncolpate, tricolpate, triporate, and tetracolpate. Quantitative pollen analysis was performed to determine dominant, secondary, important minor, and minor pollen types. A total of 200–300 pollen grains were counted per sample following the methods of Louveaux et al. (1987) and Louveaux (1990). Pollen grains were counted individually using a hand counter to avoid duplicate counts. Finally, the identified forage plants were grouped based on growth form into four categories: trees, herbs, shrubs, and bushes.

RESULTS AND DISCUSSION

Pollen Morphology

The results of pollen morphological identification collected by the honey bee *Apis cerana* revealed a variety of plant species serving as forage sources. Based on the analysis, a total of 14 plant species belonging to 12 families were identified (Figure 2). These species included *Ageratum conyzoides*, *Cocos nucifera*, *Elaeis guineensis*, *Mimosa pudica*, *Calophyllum inophyllum*, *Cratogeomys formosum*, *Elaeocarpus ganitrus*, *Pternandra coerulea*, *Symplocos adenophylla*, *Impatiens balsamina*, *Myrica javanica*, *Sphagneticola trilobata*, *Lantana camara*, and *Mischocarpus sondaicus*. The presence of diverse plant species indicates that *A. cerana* exploits a wide range of pollen sources to fulfill its nutritional requirements. During foraging activities, *A. cerana* tended to collect small-sized pollen grains. This preference suggests that *A. cerana* favors pollen types that allow the bees to carry a larger number of pollen grains per foraging trip. Such a strategy is likely associated with foraging efficiency and colony nutritional demands. This finding is consistent with the study by Kusmoro et al. (2024), which reported that among various plant species identified as honey bee forage, small- to medium-sized pollen grains were the most dominant types observed.

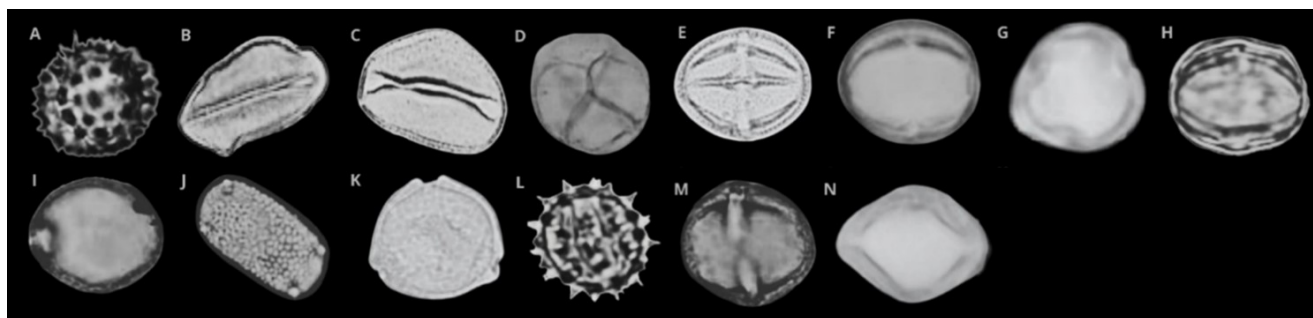


Figure 2. Pollen morphology. A. *Ageratum conyzoides*, B. *Cocos nucifera*, C. *Elaeis guineensis*, D. *Mimosa pudica*, E. *Calophyllum inophyllum*, F. *Cratoxylum formosum*, G. *Elaeocarpus ganitrus*, H. *Pternandra coerulescens*, I. *Symplocos adenophylla*, J. *Impatiens balsamina*, K. *Myrica javanica*, L. *Sphagneticola trilobata*, M. *Lantana camara*, and N. *Mischocarpus sundaicus*.

Exine Ornamentation, Aperture Type, and Pollen Size

The identification in this study focused on morphological characteristics of pollen, including exine ornamentation, aperture type, and pollen size (Table 1). The results revealed considerable variation in pollen morphological characteristics among the 14 plant species identified as forage sources for the honey bee *A. cerana*. This diversity indicates that *A. cerana* does not rely on a single flowering plant species but instead utilizes a wide range of plant species as primary pollen sources to fulfill its nutritional requirements. Based on the identification of the 14 plant species, four categories of exine ornamentation were recorded, namely echinate, scabrate,

psilate, and reticulate. Exine ornamentation represents structural features of the outermost pollen wall and exhibits species-specific characteristics, making it an important diagnostic trait for pollen identification. Moreover, variation in exine ornamentation may reflect different adaptive strategies related to pollination mechanisms and interactions with pollinators. Six aperture types were identified among pollen sources utilized by *A. cerana*, including tricolporate, monosulcate, syncolpate, tricolpate, triporate, and tetracolporate. The presence of diverse aperture types further indicates a high taxonomic diversity of forage plants exploited by *A. cerana* at the study site.

Table 1. Pollen exine ornamentation, aperture type, and pollen size

No	Species	Size (μm)	Exine Ornamentation	Aperture
1.	<i>Ageratum conyzoides</i>	15-20 μm	Echinate	Tricolporate
2.	<i>Cocos nucifera</i>	20-55 μm	Scabrate	Monosulcate
3.	<i>Elaeis guineensis</i>	30-40 μm	Psilate	Syncolpate
4.	<i>Mimosa pudica</i>	<10 μm	Psilate	Tricolpate
5.	<i>Calophyllum inophyllum</i>	35-55 μm	Reticulate	Tricolporate
6.	<i>Cratoxylum formosum</i>	10-20 μm	Psilate	Triporate
7.	<i>Elaeocarpus ganitrus</i>	10-20 μm	Psilate	Tricolporate
8.	<i>Pternandra coerulescens</i>	10-20 μm	Psilate	Triporate
9.	<i>Symplocos adenophylla</i>	15-20 μm	Psilate	Triporate
10.	<i>Impatiens balsamina</i>	20-30 μm	Reticulate	Tetracolporate
11.	<i>Myrica javanica</i>	20-30 μm	Psilate	Tricolporate
12.	<i>Sphagneticola trilobata</i>	15-20 μm	Echinate	Tricolporate
13.	<i>Lantana camara</i>	20-30 μm	Reticulate	Tricolporate
14.	<i>Mischocarpus sundaicus</i>	15-20 μm	Psilate	Tricolporate

Pollen Providing Plants

Based on the results of this study, a total of 12 plant families were identified as pollen forage sources for the *A. cerana*. These families include Asteraceae, Arecaceae, Fabaceae, Calophyllaceae, Hypericaceae, Elaeocarpaceae, Melastomataceae, Symplocaceae, Balsaminaceae, Myricaceae, Verbenaceae, and Sapindaceae (Table 2). The diversity of plant families indicates that *A. cerana* exploits pollen resources from a wide range of taxonomic groups, encompassing both wild and cultivated plants available in the surrounding environment of the study area. Several of these plant

families were represented by more than one species. For example, the family Asteraceae was represented by two species, *Ageratum conyzoides* and *Sphagneticola trilobata*, which are flowering plants characterized by relatively long flowering periods and abundant pollen production. Similarly, the family Arecaceae was represented by *Cocos nucifera* and *Elaeis guineensis*, woody palm species known for their substantial pollen output and importance as forage resources for honey bees in tropical regions. The presence of multiple species within the same family highlights the significant contribution of these plant families to pollen availability.

Table 2. Pollen providing plants

No	Species	Family	Order	Local Name
1.	<i>Ageratum conyzoides</i>	Asteraceae	Asterales	Babadotan
2.	<i>Cocos nucifera</i>	Arecaceae	Arecales	Kelapa
3.	<i>Elaeis guineensis</i>	Arecaceae	Arecales	Kelapa Sawit
4.	<i>Mimosa pudica</i>	Fabaceae	Fabales	Putri Malu
5.	<i>Calophyllum inophyllum</i>	Calophyllaceae	Malpighiales	Nyamplung
6.	<i>Cratoxylum formosum</i>	Hypericaceae	Malpighiales	Kemutun
7.	<i>Elaeocarpus ganitrus</i>	Elaeocarpaceae	Oxalidales	Ganitri
8.	<i>Pternandra coerulescens</i>	Melastomataceae	Myrtales	Memeteng
9.	<i>Symplocos adenophylla</i>	Symplocaceae	Ericales	Kambos
10.	<i>Impatiens balsamina</i>	Balsaminaceae	Ericales	Pacar Air
11.	<i>Myrica javanica</i>	Myricaceae	Fagales	Picisan
12.	<i>Sphagneticola trilobata</i>	Asteraceae	Asterales	Wedelia
13.	<i>Lantana camara</i>	Verbenaceae	Lamiales	Tahi Ayam
14.	<i>Mischocarpus sundaicus</i>	Sapindaceae	Sapindales	Bangkongan

Growth Forms of Pollen Providing Plants

Based on the results of this study, the growth forms of pollen-providing plants utilized by the honey bee *Apis cerana* exhibited a relatively high level of diversity. Analysis of plant growth forms revealed that tree species constituted the highest proportion of pollen sources, accounting for 50%, followed by herbaceous plants at 35%. In contrast, bushes and shrubs contributed smaller and relatively equal proportions, each accounting for 7.5%. The collected data indicate that tree species provide the greatest contribution as pollen sources for *A. cerana*, with a total of seven species identified. Herbaceous plants were represented by five species, whereas bushes and shrubs were each represented by only one species (Table 3).

Table 3. Growth forms of pollen providing plants.

No	Species	Habitus
1.	<i>Ageratum conyzoides</i>	Herbs
2.	<i>Cocos nucifera</i>	Tree
3.	<i>Elaeis guineensis</i>	Tree
4.	<i>Mimosa pudica</i>	Herbs
5.	<i>Calophyllum inophyllum</i>	Tree
6.	<i>Cratoxylum formosum</i>	Tree
7.	<i>Elaeocarpus ganitrus</i>	Tree
8.	<i>Pternandra coerulescens</i>	Herbs
9.	<i>Symplocos adenophylla</i>	Shrubs
10.	<i>Impatiens balsamina</i>	Herbs
11.	<i>Myrica javanica</i>	Tree
12.	<i>Sphagneticola trilobata</i>	Herbs
13.	<i>Lantana camara</i>	Bushes
14.	<i>Mischocarpus sundaicus</i>	Tree

Discussion

Based on the identification results, a total of 14 plant species belonging to 12 families were recorded. The high diversity of plant species indicates that the range of primary forage preferences of the honey bee *Apis cerana* is remarkably broad. This diversity is closely related to environmental conditions that support species adaptation and reproductive success. In this context, pollen morphological characteristics such as size, exine

ornamentation, and aperture type play a crucial role in shaping plant–pollinator interactions (Sukapti & Dewi, 2023). The foraging range of *Apis cerana* typically extends from 200 to 500 m from the nest, with visual detection capabilities estimated to exceed 500 m. Therefore, the distance between pollen-producing plants and bee colonies strongly influences forage selection by honey bees (Koetz, 2013).

In natural environments, honey bees collect nectar from surrounding flowers; however, pollen grains are simultaneously gathered and stored in the pollen baskets on their hind legs. In addition, some pollen grains adhere to the fine hairs covering the bee's body (Priambudi et al., 2021). One of the key factors influencing pollen attractiveness to bees is pollen size. Larger pollen grains tend to be more difficult for individual bees to transport, whereas smaller pollen grains are easier to collect and carry in greater quantities. Nevertheless, pollen selection by bees is not solely determined by size but is also influenced by floral scent, flower color, and the distance between the pollen source and the nest. Honey bees are sensitive to ultraviolet, yellow, and blue wavelengths, making them more attracted to brightly colored flowers. Moreover, bees tend to prefer flowers with open pollen aperture structures, which facilitate pollen release and collection (Yanto et al., 2016).

Exine ornamentation represents the outermost layer of the pollen wall and is characterized by species-specific sculpturing patterns (Purba et al., 2024; Erdtman, 1986). Pollen ornamentation types are classified based on observations of the outer exine surface (Erdtman, 1952). In general, bees tend to prefer pollen with rough or spiny ornamentation, such as reticulate and echinate types, because these surfaces adhere more easily to the bee's body. However, the present study revealed that psilate ornamentation was the most dominant type. This dominance is likely influenced by factors such as plant availability, spatial distribution, and the predominance of tree species producing psilate pollen in the study area (Konzmann et al., 2019). The dominance of psilate exine ornamentation in pollen collected by *Apis cerana*

suggests that smooth pollen surfaces may be ecologically advantageous for transport and collection by bees. Psilate pollen grains are generally smoother and lighter, allowing them to detach easily from anthers and be efficiently dispersed through mechanical contact and the movement of generalist pollinators such as honey bees. Several palynological studies have reported that smooth ornamentation is often associated with plants exhibiting less specialized pollination strategies, enabling interactions with a wide range of pollinators and increasing pollen transfer efficiency (Sannier et al., 2009). This finding supports the observation that *A. cerana*, a polylectic species, frequently collects psilate pollen due to its abundance and ease of transport during foraging activities in heterogeneous vegetation landscapes.

Apertures are thinner regions of the pollen wall that serve as germination sites for pollen tubes and facilitate fertilization by enabling rapid pollen tube emergence on the stigma surface (Purba et al., 2024). In this study, the tricolporate aperture type was the most dominant, occurring in seven plant species. Tricolporate pollen possesses three apertures, which confer advantages in terms of fertilization speed and efficiency (Hesse et al., 2009). This result is consistent with findings by Triyadi et al. (2023), who also reported tricolporate pollen as the most frequently encountered aperture type. The dominance of tricolporate pollen is likely associated with the high availability of flowering plant species in the surrounding environment, which provides abundant nectar and pollen resources for honey bees (Wappler et al., 2015). Morphologically, tricolporate pollen grains are generally small to medium in size and possess exine structures that facilitate adhesion to the bee's body and efficient storage in pollen baskets. Furthermore, tricolporate apertures provide a larger germination surface and more efficient water imbibition, increasing the likelihood that pollen grains detach from anthers and adhere to pollinators during floral contact. Studies on pollen morphology and insect-pollen interactions indicate that aperture types with physiologically responsive germination features are more frequently represented in pollen loads carried by actively foraging honey bees (Romaschenko et al., 2004).

Beyond serving as a primary food source for *A. cerana*, pollen plays a critical ecological role within ecosystems. Honey bees contribute substantially to pollination processes, enhancing plant reproductive success and productivity, thereby establishing mutualistic relationships between plants and pollinators. Consequently, understanding pollen sources is essential as a foundational reference for the management, cultivation, and conservation of both honey bees and pollen-producing plants within their ecosystems (Sarah et al., 2017). The growth forms of plants utilized as pollen sources by *A. cerana* exhibited high diversity, comprising seven tree species, five herbaceous species,

one subshrub species, and one shrub species. Tree species provide particular advantages by offering relatively stable and consistent pollen supplies for honey bees (Indriyani et al., 2025). The dominance of tree-derived pollen indicates that honey bees preferentially exploit woody vegetation, which generally produces large quantities of pollen within the bees' foraging range. Meanwhile, the substantial contribution of herbaceous plants reflects the importance of understory vegetation as accessible supplementary pollen sources.

The low proportion of pollen derived from shrubs and other growth forms is likely related to limited flower availability or lower visitation rates by bees. The predominance of woody plants as pollen sources has significant implications for honey bee foraging behavior and colony sustainability. Landscapes with high vegetation diversity, particularly those containing abundant woody plants, have been shown to enhance pollen availability over time, positively influencing honey bee nutritional health and population stability. Flowering trees provide more consistent pollen resources across seasons compared to herbaceous plants, which are often seasonal, thereby ensuring a reliable protein supply essential for larval development and overall colony maintenance (Parreño et al., 2024). Similar patterns have been observed in tropical landscapes, where woody plants contribute disproportionately to bee bread composition despite being less abundant than herbaceous vegetation, indicating that honey bees preferentially exploit tree-based pollen resources when available in agricultural and fragmented forest landscapes (Cannizzaro et al., 2022).

CONCLUSIONS

This study identified 14 plant species belonging to 12 families as pollen forage sources for *Apis cerana*. The identified pollen morphological characteristics comprised four types of exine ornamentation, six aperture types, and pollen sizes ranging from very small to large, with small-to medium-sized pollen being dominant. Tricolporate aperture types and psilate ornamentation were the most frequently observed characteristics. Based on plant growth form, pollen sources were predominantly derived from tree species, followed by herbs, while shrubs and bushes contributed less.

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writing original draft preparation, Moh Syahrial Farawansyah S, Lestari M.P Alibasyah, and Manap Trianto; writing review and editing, All authors.

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