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A multimodal profiling of bee foraging ecology: Contrasting palynological and melissopalynological (microscopical/optical) methods with high-throughput DNA metabarcoding

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Abstract

Honey bees (*Apis cerana indica* F.) are key pollinators in agricultural and natural ecosystems and accurate knowledge of their forage plants is essential for sustainable apiculture. However, studies on bee forage identification remain limited, many potential bee forage species remain unrecognised, restricting the identification of additional floral resources for beekeeping and potentially limiting honey production and income opportunities for beekeepers in the Coimbatore region. In addition, most of the researchers rely on conventional palynological methods that frequently identify pollen only to the genus level because of morphological similarities among closely related taxa. This study contrasted traditional palynology, melissopalynology and DNA metabarcoding to characterise the pollen composition of foraging bees and honey samples collected from six apiary sites in Coimbatore, India. Palynological analysis identified 83 plant taxa belonging to 30 families, predominantly at the genus level, whereas melissopalynological analysis of 46 honey samples identified 36 plant species from seven families. DNA metabarcoding using the rbcL, matK and trnH–psbA markers provided the highest taxonomic resolution, resolving 65 of the 83 palynologically identified genera to the species level and detecting key forage plants such as *Amaranthus spinosus* L., *Brassica juncea* (L.) Czern. and *Cucumis sativus* L.. Among the three markers, rbcL exhibited the broadest taxonomic coverage and highest amplification success, matK identified additional complementary species, whereas trnH–psbA independently resolved only two species. Overall, DNA metabarcoding outperformed conventional microscopy-based approaches in species-level pollen identification and revealed additional forage species that would otherwise remain unresolved. These findings provide a more comprehensive understanding of honey bee forage resources and offer a scientific basis for identifying additional bee forage plants to support migratory beekeeping, pollinator conservation and sustainable apiculture.

Keywords: *Apis cerana indica*; DNA metabarcoding; floral resources; Indian honey bees; melissopalynology; pollen analysis

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1. Introduction

Pollinators are crucial in the functioning of almost all terrestrial ecosystems, including those dominated by agriculture, because they are in the front line of sustainable productivity via plant reproduction (1, 2). Insects play a major role in pollination. It is estimated that in nature, 5 % of the flowers are self-pollinated and 95 % are cross-pollinated, of which 10 % depend upon wind and 85 % depend upon animal pollination (3).

The maintenance of honey bee colonies, usually in hives, by humans is known as beekeeping. Through a variety of hive products, the apiculture industry contributes significantly to job creation and rising household incomes in rural regions throughout the world. Over 250000 people in 40000 villages in India are employed part-time by beekeeping (4). Coimbatore is one of the major apiculture regions in Tamil Nadu owing to its favourable agro-climatic conditions, diverse floral resources and extensive cultivation of pollinator-dependent crops. The district supports a well-established beekeeping sector, particularly in regions such as Pollachi, Thondamuthur, Narasipuram, Pullagoundenpudhur, Vellimalaipattinam and Nallampalayam, where apiculture is widely adopted as a supplementary farm enterprise that enhances rural livelihoods through the production of honey and other hive products. In addition to its economic value, managed honey bee colonies provide essential pollination services to several agricultural and horticultural crops, particularly cucurbits, coconut, sunflower, vegetables and fruit crops, thereby improving fruit set, yield and quality. Furthermore, the close proximity of these beekeeping regions to the biodiverse foothills of the Western Ghats, together with agricultural fields, plantations, wetlands and fragmented natural habitats, creates a landscape with exceptionally high plant diversity and year-round floral resources. Despite these favourable conditions, a comprehensive understanding of the floral resources exploited by honey bees across this heterogeneous landscape remains limited. Since bees selectively forage on species that meet their nutritional and ecological needs, identifying which plants actually serve as effective bee forages becomes crucial (5). Bee forages play a crucial role in determining both the quantity and quality of honey produced. Planting the right bee-floral resources can significantly enhance honey production and, in turn, increase the income of beekeepers. By cultivating or conserving plant species that are rich in nectar and pollen and preferred by bees, hive productivity can be optimised throughout the year (6). Understanding these specific plant–bee interactions is important not only for conserving native bee diversity but also for enhancing pollination services in agriculture and maintaining overall ecosystem stability. Therefore, studying and documenting the key bee-forage plants in Coimbatore is vital for mapping interaction networks and guiding conservation and land-management practices.

Studying bee foraging requires reliable methods for identifying the floral sources of their honey as well as their pollen loads. Traditionally, this has been done through melissopalynology and palynology (7, 8). The scientific study of pollen grains and their origin, known as palynology, provides the key for comprehending the principles behind plant-pollinator interactions. For investigating the numerous aspects of pollinator behaviour, such as foraging and migration patterns, pollen is a good alternative (9). Pollen carried by bees is a powerful indicator of their foraging dynamics. By analysing the types and quantities of pollen on bee bodies, researchers can infer floral visitation patterns, resource preferences and spatial foraging ranges (10).

Melissopalynology, which involves examining pollen contained in honey and other bee products, is a valuable method for understanding where bees forage. By analysing these pollen grains, researchers can reconstruct the foraging patterns of bees and identify the specific floral sources they relied on (11). By analysing the types and proportions of pollen found in hive samples, researchers can identify the plant species visited by bees, estimate their foraging distances and track changes in pollen availability throughout the seasons (12–15). Even though these conventional palynological and melissopalynological approaches are highly time-consuming and labour-intensive, they demand substantial expertise in palynology. Interpreting results can also be challenging, as the pollen of some plant species is difficult to distinguish (16). The emergence of next-generation sequencing (NGS) technologies has expanded research possibilities by enabling detailed investigations of organism–environment interactions. As an NGS tool, DNA metabarcoding is utilised in this study to compare its outputs with those of traditional pollen analysis, helping to assess the consistency and reliability of both methods and to explain which one serves as superior in identifying bee forages.

Recently, DNA metabarcoding has emerged as an alternative approach for examining the botanical diversity present in honey and also in pollen grains collected from various sources, namely the forager's body, the corbicular load and hive-stored pollen (17, 18). By targeting specific plant DNA regions, this method enables the simultaneous sequencing and identification of numerous taxa. As a result, it serves as an efficient tool for characterizing the ecological relationships between plants and pollinators (19). Through pollen DNA barcoding, conservationists can assess a pollination network's stability and determine which species are crucial and which are most vulnerable to disturbances that could result in pollinator reductions, by researching the composition and structure of the networks (20). Although numerous studies have investigated bee foraging behaviour, floral resource utilisation and pollen identification using individual approaches such as palynology, melissopalynology, or DNA metabarcoding, these methods have largely been employed independently. In tropical Indian landscapes, where plant diversity is exceptionally high and floral resources vary across natural and agricultural ecosystems, comprehensive studies integrating conventional microscopic techniques with high-throughput molecular approaches remain scarce. Consequently, the complete spectrum of floral resources exploited by honey bees, including morphologically indistinguishable or underrepresented pollen taxa, is still inadequately understood. The lack of such multimodal investigations limits our ability to accurately characterise bee foraging ecology, assess plant–pollinator interactions and generate reliable floral resource inventories that can support sustainable apiculture, pollinator conservation and crop pollination management.

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Studies that integrate pollen-load analysis with DNA metabarcoding have become powerful tools for understanding bee foraging ecology. By analysing pollen collected directly from field-visiting bees, along with pollen stored within hives situated at specific landscape sites, researchers can accurately identify the plant species utilised by different bee communities. These approaches provide detailed insights into how bees select floral resources and how their foraging behaviour shifts in response to landscape composition, habitat fragmentation, seasonal floral availability and colony nutritional requirements.

2. Materials and Methods

Study area

The study was carried out in Coimbatore, located in the western part of Tamil Nadu, India. Coimbatore lies along the foothills of the Western Ghats and exhibits a heterogeneous landscape shaped by a mosaic of dry deciduous forests, plantations, fragmented scrub habitats, irrigated agricultural fields and semi-urban settlements. A total of six major beekeeping sites around Coimbatore district were selected for this study, viz., Pullagoundenpudhur (10.996038 ºN and 76.807039 ºE), Vellimalaipattinam (10.991618 ºN and 76.763116 ºE), Narasipuram (10.998716 ºN and 76.800541 ºE), Pollachi (10.657224 ºN and 77.010666 ºE), Thondamuthur (10.989925 ºN and 76.840881 ºE) and Nallampalayam (10.736694 ºN and 77.532269 ºE) (Fig. 1).

Pollen sampling from field bees

For collection of pollen from the field bees, floral visits by honey bees (*Apis cerana indica* F.) were recorded *in situ* through direct field observations. Surveys were conducted from 06:00 to 18:00 hr, corresponding to peak foraging hours. A plant species was considered attractive or a bee forage if more than five bees were observed visiting it within a 10 min period. Floral visitors were collected using sweep-nets and forceps (for minute insect visitors) from the experimental field. The collected floral visitors were placed in sterile water in polypropylene tubes of 1.5 mL size. Pollen and resin load on the corbiculae were examined to classify bees as pollen, nectar, or resin foragers.

Pollen sampling from hive bees

This study focused on characterising the foraging resources of the Asiatic or Indian honey bee (*A. cerana indica*). To achieve this, wooden colonies were placed within the predefined study area and two hives from each location were selected for detailed pollen analysis. Pollen grains were collected from the bee hives using pollen traps.

The study was conducted at six apiary sites in Coimbatore district, Tamil Nadu, India. Two managed colonies of *A. cerana indica* were selected at each site for pollen and honey sampling. Pollen collections were carried out three times per month over a period of six consecutive months from March–September. At each sampling occasion, pollen collected from the two colonies at each site was pooled to obtain a single representative pollen-load sample. Accordingly, a total of 108 pooled pollen-load samples (6 sites × 3 sampling occasions × 6 months) were obtained and subjected to palynological analysis. The same 108 pollen-load samples were subsequently used for DNA metabarcoding employing the *rbcL*, *matK* and *trnH-psbA* barcode markers. For each sampling interval, pollen obtained from the two managed colonies at every site was consolidated into a single representative subsample of approximately 5 g and samples were rapidly stored at –20 °C to prevent degradation prior to laboratory processing.

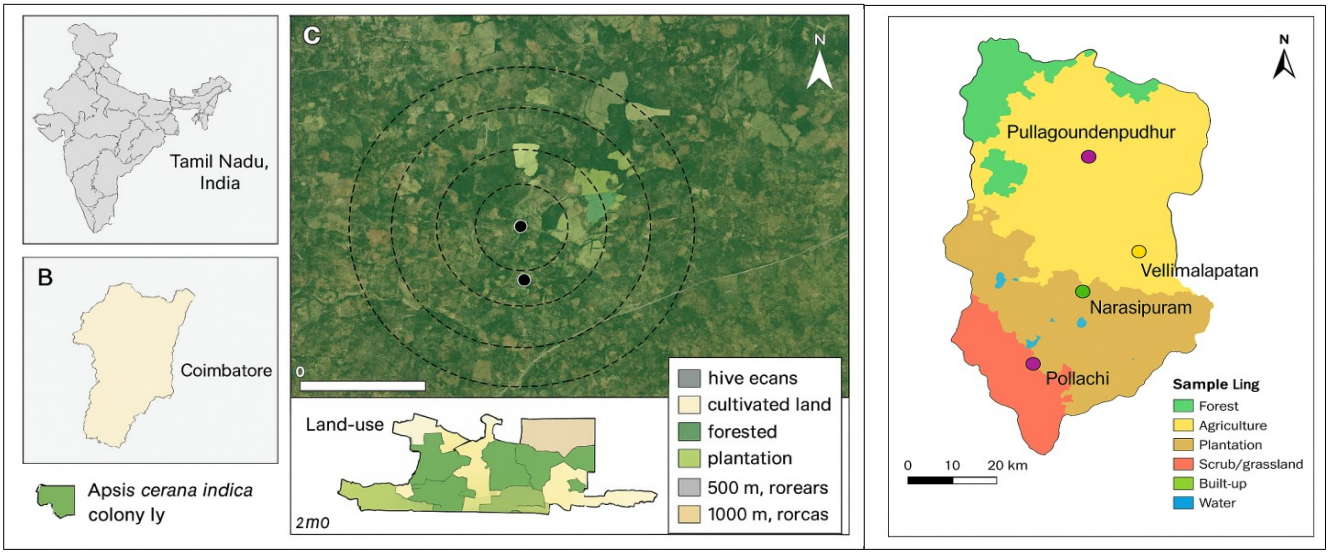


Fig. 1. Distribution map of sites where beehives for pollen collection were positioned within Coimbatore district, Tamil Nadu, India. Source: Map prepared by the authors using ArcGIS software (Esri Inc., Redlands, CA, USA). Land-use/land-cover classes are shown beneath the sampling points to illustrate the local habitat matrix.

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Honey sampling

Honey samples were collected following standardised melissopalynological guidelines to ensure representativeness and prevent contamination. Mature honey was obtained by uncapping sealed comb cells and extracting the contents using a manual or centrifugal extractor (11). From each colony, approximately 100–250 g of honey was transferred into sterile, airtight amber glass containers, using separate sterilised tools for each hive to avoid cross-contamination (21). All samples were clearly labeled with hive identification, location, date and season. Samples were subsequently stored at 4 °C and transported to the laboratory in insulated coolers to maintain physicochemical stability, in accordance with the guidelines of the International Honey Commission (22).

Palynological analysis (Acetolysis method)

The extraction was carried out following the method of Erdtman (23). Pollen grains adhering to the external surfaces of forager bees were collected by immersing the individuals in sterile water within Eppendorf tubes. The bees were vortexed for 5 min to dislodge the attached pollen grains. The suspended pollen was subsequently recovered and processed for palynological analysis to determine floral visitation and pollen diversity. Pollen collected from the pollen traps was directly subjected to pollen study.

First, the pollen was fixed in acetone and stored in small vials for a minimum of 12 hr before further processing. The acetone-preserved specimens were then transferred to polythene centrifuge tubes and gently crushed with a needle. The resulting dispersed pollen material was carefully collected for subsequent analysis. The dispersed material was collected and concentrated by centrifugation and the alcohol was decanted. The residue was washed with glacial acetic acid and subsequently treated with freshly prepared acetolysis mixture, consisting of nine parts acetic anhydride and one part concentrated sulfuric acid. The tube containing the mixture was placed in a water bath and heated for 3–5 min until the acetolysis mixture turned medium brown. After centrifugation, the mixture was decanted, leaving a brownish residue containing pollen and other organic debris. The residue was washed repeatedly with distilled water through centrifugation to obtain clear, neutral preparations. The prepared samples were examined using both phase-contrast microscopy and scanning electron microscopy (SEM) to assess pollen morphology and to identify pollen in detail. To document the SEM image, the dusted and vortexed specimens were sputter-coated with gold-palladium and images were acquired in environmental scanning electron microscopy (ESEM) mode (low vacuum) with an electron beam spot size of 3.0 under different magnifications from 1000×–4000× at the Department of Nanotechnology, Tamil Nadu Agricultural University, Coimbatore. Morphometrics of pollen samples were analysed using FEI Quanta 250 (Netherlands) software.

Melissopalynological analysis

Approximately 2 g of honey was mixed with 40 mL of 0.5 % sulfuric acid solution and incubated in a water bath at 80 °C for 5 min. The samples were then filtered through a 5 µm filter, washed with 8 mL of glacial acetic acid for dehydration and centrifuged at 800 g for 2 min. The resulting pellet was resuspended in 1 mL of acetolysis mixture and heated in a water bath at 80 °C for 10 min. After a second centrifugation step, the pellet was again resuspended in 1 mL of acetolysis mixture (24–26). A few drops of the final suspension were mounted on microscope slides and examined under a light microscope (Magnus, India) at 40× magnification. For each sample, an average of 300 pollen grains were counted and identified to the lowest possible taxonomic level.

DNA extraction, amplification and sequencing of barcoding regions

Genomic DNA was extracted from pollen using a modified cetyltrimethylammonium bromide (CTAB) protocol. Pollen was suspended in 500 µL CTAB buffer, homogenised and incubated at 65 °C for 1–2 hr, followed by cooling and centrifugation (12000 rpm, 10 min, 4 °C). The supernatant was extracted with chloroform alcohol (24 : 1) and the aqueous phase was precipitated with ice-cold isopropanol at –20 °C overnight. DNA pellets were recovered by centrifugation, washed with 70 % ethanol, air-dried and resuspended in 20–40 µL Tris–EDTA (TE) buffer. DNA integrity was evaluated on 1 % agarose gels prepared in 1× TAE buffer and visualised under UV illumination, while concentration and purity were assessed using a NanoDrop spectrophotometer and standardised to 50 ng/µL for downstream analyses.

DNA amplification was carried out using multiple plant barcode loci to ensure broad taxonomic resolution. The chloroplast *rbcL* region was amplified using the primers *rbcLa*-F and *rbcLa*-R, while the *matK* locus was targeted with the universal plant primers 3F_KIM (F) and 1R_KIM (R). In addition, the highly variable *trnH–psbA* intergenic spacer was amplified using the primer pair *trnH-psbA*-F and *trnH-psbA*-R. Each reaction was prepared in a 25 µL volume containing 50 ng of genomic DNA, 1× polymerase chain reaction (PCR) buffer, magnesium chloride ($MgCl_2$), deoxynucleoside triphosphates (dNTPs), primers and Thermus aquaticus DNA polymerase (Taq polymerase). The primer combination used for PCR amplification and sequencing of *rbcL* was *rbcLa*-F: ATGTCACCACAAACAGAGACTAAAGC and *rbcLa*-R: GTAAAATCAAGTCCACCRCG and the thermocycling began with an initial denaturation at 94 °C for 5 min, followed by 36 cycles of denaturation at 94 °C for 40 s, annealing at 57 °C for 45 sec and extension at 72 °C for 2 min. A final extension was performed at 72 °C for 10 min, after which reactions were held at 4 °C.

The primer combination used for PCR amplification and sequencing of *matK* was 3F_KIM (F):

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CGTACAGTACTTTTGTGTTTACGAG, 1R_KIM (R): ACCCAGTCCATCTGGAAC TTGGTC and here for *matK*, the PCR amplification was conducted with an initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 sec, annealing at 53 °C for 40 sec and extension at 72 °C for 1 min. A final extension step was performed at 72 °C for 10 min, after which reactions were held at 4 °C. The primer combination used for PCR amplification and sequencing of the *trnH–psbA* intergenic spacer was *trnH-psbA*-F: CGAAATCGGTAGACGCTACG, *trnH-psbA*-R: GGGGATAGAGGGACTTGAAC. The PCR reactions were initiated with a primary denaturation step at 94 °C for 5 min, followed by 36 amplification cycles consisting of denaturation at 94 °C for 20 sec, primer annealing at 59 °C for 1 min and strand extension at 72 °C for 1.45 min. The cycling programme concluded with a terminal extension at 72 °C for 10 min, after which the products were maintained at 4 °C until further processing.

Amplified PCR products of *rbcL*, *matK* and *trnH–psbA* regions were subjected to gel electrophoresis. Unpurified PCR products (35 µL), together with their forward and reverse primers (10 µL each), were sent for Sanger sequencing at Eurofins Genomics India Pvt. Ltd. (Bangalore). The obtained sequences were reverse-complemented when required and aligned to generate consensus reads. These sequences were compared with those in the National Center for Biotechnology Information (NCBI) database using basic local alignment search tool (BLAST) and pollen taxa were identified based on the highest similarity and query coverage.

3. Results and Discussion

The palynological and melissopalynological analyses together provide complementary perspectives on the floral resources utilised by honey bees within the study landscape. Palynological examination revealed a broad floral spectrum consisting of 83 plant taxa across 30 families, most of which were identifiable only up to the genus level, with herbs forming the dominant life-form category, followed by shrubs, trees and climbers. Palynological analysis identified pollen belonging to 23 plant families. Among these, Cucurbitaceae constituted the highest proportion (11 %), followed by Asteraceae (9 %), Fabaceae (7 %) and Acanthaceae, Brassicaceae and Caesalpiniaceae (6 % each). Euphorbiaceae, Malvaceae, Amaranthaceae, Polygonaceae, Rosaceae, Solanaceae and Verbenaceae were represented at moderate frequencies (4–5 %), whereas the remaining families each contributed 1–2 % of the total taxa. Fig. 2 and Table 1 contributing an intermediate proportion of taxa. This indicates that the surrounding vegetation contains highly diverse polleniferous plants. While many present diagnostic traits, fine-scale morphology enables species-level identification for only a specific subset. In contrast, melissopalynological analysis of 46 honey samples demonstrated a more selective foraging pattern, with honey bees predominantly utilising 36 plant species from only seven families, most of which were identifiable at the species level due to their characteristic pollen signatures. Although reduced in taxonomic breadth, the honey-based dataset strongly mirrored the dominance of Asteraceae, Cucurbitaceae and Brassicaceae, reaffirming their role as core foraging families.

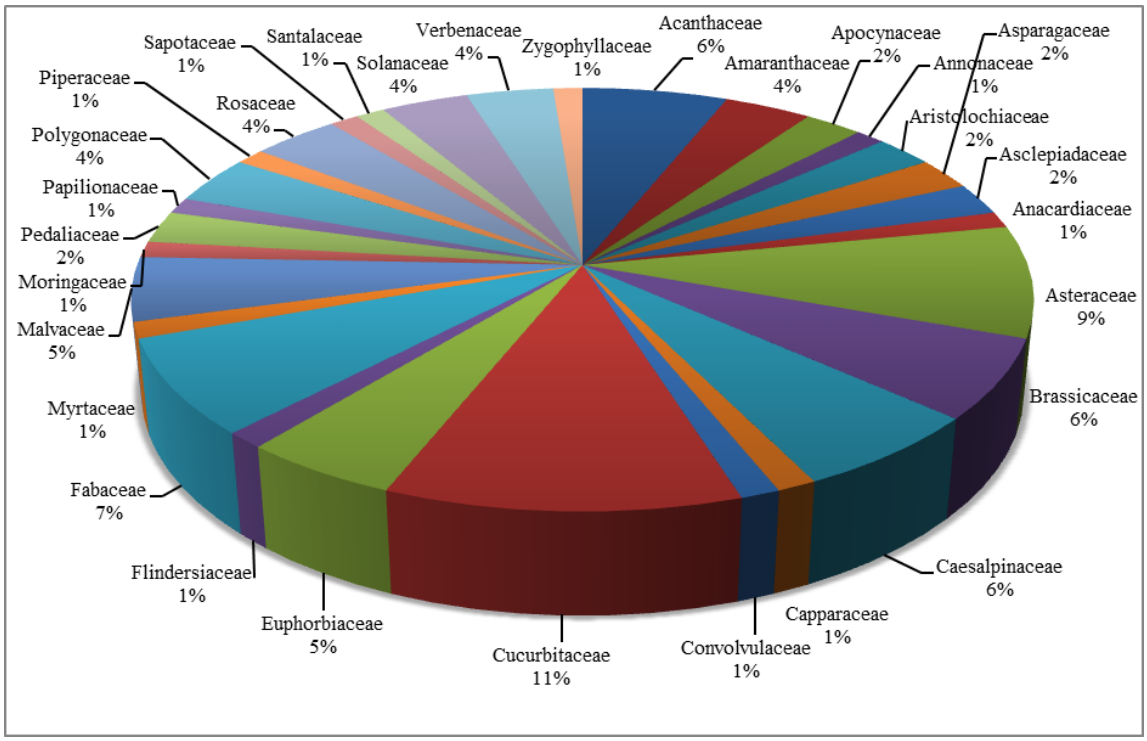


Fig. 2. Family-wise distribution of bee forage plants identified through palynological analysis.

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Table 1. Comparative identification of plant taxa using palynology, melissopalynology and DNA metabarcoding

Sl. No	Family	Plant taxon	Palynology	Melissopalynology	Metabarcoding		
					rbcL	*matK*	*trnH–psbA*
1.	Acanthaceae	*Andrographis* sp.	✓	-	-	-	-
2.		*Adhatoda vasica* Nees	✓	-	✓	-	-
3.		*Asystasia* sp.	✓	-	-	-	-
4.		*Hygrophila* sp.	✓	-	-	-	-
5.		*Strobilanthes* sp.	✓	-	-	-	-
6.	Amaranthaceae	*Amaranthus spinosus* L.	✓	✓	✓	✓	-
7.		*Amaranthus blitum* L.	✓	-	✓	✓	-
8.		*Amaranthus viridis* L.	✓	✓	✓	✓	-
9.	Apocynaceae	*Alstonia scholaris* (L.) R.Br.	✓	-	-	✓	✓
10.		*Carissa* sp.	✓	-	-	-	-
11.	Annonaceae	*Annona squamosal* L.	✓	-	✓	✓	✓
12.	Aristolochiaceae	*Aristolochia* sp.	✓	-	-	-	-
13.		*Aristolochia indica* L.	✓	-	✓	-	-
14.	Asparagaceae	*Asparagus* sp.	✓	-	-	-	-
15.		*Asparagus racemosus* Willd.	✓	-	✓	-	-
16.	Asclepiadaceae	*Calotropis gigantean* (L.) W.T. Aiton	✓	-	-	✓	-
17.		*Gymnema* sp.	✓	-	-	-	-
18.	Anacardiaceae	*Mangifera indica* L.	✓	-	✓	✓	-
19.	Asteraceae	*Guizotia abyssinica* (L.f.) Cass	✓	✓	✓	✓	-
20.		*Vernonia* sp.	✓	-	-	-	-
21.		*Parthenium hysterophorus* L.	✓	✓	✓	-	-
22.		*Ocimum sanctum* L.	✓	✓	✓	✓	✓
23.		*Chrysanthemum sp.*	✓	✓	✓	-	-
24.		*Helianthus annuus* L.	✓	✓	✓	✓	-
25.		*Tridax procumbens* L.	✓	✓	✓	-	-
26.	Brassicaceae	*Brassica alba* (L.) Rabenh.	✓	-	✓	✓	✓
27.		*Brassica campestris* L.	✓	-	✓	✓	✓
28.		*Brassica juncea* (L.) Czern	✓	✓	✓	✓	✓
29.		*Brassica nigra* Koach.	✓	✓	✓	✓	✓
30.	Brassicaceae	*Raphanus sativus* L.	✓	✓	✓	✓	✓

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31.		*Bauhinia* sp.	✓	-	-	-	-
32.		*Saraca* sp.	✓	-	-	-	-
33.	Caesalpinaceae	*Cassia fistula* L.	✓	-	✓	-	-
34.		*Cassia* sp.	✓	-	-	-	-
35.		*Tamarindus* sp.	✓	-	-	-	-
36.	Capparaceae	*Capparis* sp.	✓	-	-	-	-
37.	Convolvulaceae	*Ipomoea pulchella* Roth.	✓	-	-	-	-
38.	Cucurbitaceae	*Cucumis sativus* L.	✓	✓	✓	✓	✓
39.		*Cucumis melo* L.	✓	✓	✓	✓	✓
40.		*Cucurbita maxima* Duchesne	✓	✓	✓	✓	✓
41.		*Cucurbita moschata* Poit.	✓	✓	✓	✓	✓
42.		*Cucurbita pepo* L.	✓	✓	✓	✓	✓
43.		*Lagenaria vulgaris* Ser.	✓	✓	✓	✓	✓
44.	Cucurbitaceae	*Luffa acutangula* L.	✓	✓	✓	✓	✓
45.		*Momordica charantia* Descourt.	✓	✓	✓	✓	✓
46.		*Trichosanthes cucumerina* L.	✓	✓	✓		
47.		*Bridelia* sp.	✓	-	-	-	-
48.	Euphorbiaceae	*Mallotus philippensis* (Lam.) Müll.Arg.	✓	-		✓	-
49.		*Phyllanthus emblica* L.	✓	✓	✓	✓	-
50.		*Ricinus communis* L.	✓	-	-	✓	✓
51.	Flindersiaceae	*Chloroxylon* sp.	✓	-	-	-	-
52.		*Butea monosperma* (Lam.) Taub.	✓	-	-	-	✓
53.	Fabaceae	*Mimosa pudica* L.	✓	✓	✓		✓
54.		*Cajanus cajan* L.	✓	✓	✓	✓	✓
55.		*Cicer arietinum* L.	✓	✓	✓	✓	✓
56.		*Pisum sativum* L.	✓	-	-	✓	✓
57.		*Phaseolus mungo* L.	✓	✓	-	✓	✓
58.		*Phaseolus vulgaris* L.	✓	✓	-	✓	✓
59.	Myrtaceae	*Eucalyptus* sp	✓	-	-	-	-
60.	Malvaceae	*Abelmoschus esculentus* (L.) Moench	✓	✓	✓	✓	✓
61.		*Gossypium hirsutum* L.	✓	✓	✓	✓	✓
62.	Malvaceae	*Hibiscus cannabinus* L.	✓	✓	✓	✓	✓
63.		*Abutilon hirtum* (Lam.) Sweet	✓	✓	✓	✓	✓

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64.	Moringaceae	*Moringa oleifera* Lam.	✓	✓	-	✓	-	
65.	Pedaliaceae	*Sesamum indicum* L.	✓	-	-	✓	✓	
66.		*Pedalium* sp.	✓	-	-	-	-	
67.	Papilionaceae	*Desmodium* sp.	✓	-	-	-	-	
68.	Polygonaceae	*Antigonon leptopus* Hook. & Arn.	✓	✓	✓	-	-	
69.		*Fagopyrum* sp.	✓	-	-	-	-	
70.		*Polygonum glabrum* Willd.	✓	✓	✓		✓	
71.	Piperaceae	*Piper betle* L.	✓	-	-	-	-	
72.	Rutaceae	*Citrus* sp.	✓	-	-	-	-	
73.	Rosaceae	*Rubus elipticus* Sm.	✓	-	-	✓	✓	
74.	Solanaceae	*Glycosmis* sp.	✓	-	-	-	-	
75.		*Mimusops* sp.	✓	-	-	-	-	
76.	Santalaceae	*Santalum album* L.	✓	-	-	-	-	
77.		*Solanum torvum* Sw.	✓	-	✓	✓	✓	
78.		*Solanum trilobatum* L.	✓	-	✓	✓	✓	
79.		*Solanum melongena* L.	✓	✓	✓	✓	✓	
80.		*Gmelina asiatica* L.	✓	-	✓	✓	✓	
81.	Verbenaceae	*Lantana camara* L.	✓	-	-	-	-	
82.		*Vitex* sp.	✓	-	-	-	-	
83.	Zygophyllaceae	*Tribulus terrestris* L.	✓	-	✓	-	-	

✓ indicates detection of the taxon by the respective method; – indicates non-detection.

Palynology and melissopalynology are morphology-based and may not reliably resolve closely related taxa to species level. DNA metabarcoding resolution depends on barcode marker amplification and reference database availability.

Additional contributions from Fabaceae, Malvaceae, Lamiaceae and Solanaceae suggest that nectar availability, pollen release efficiency and floral accessibility substantially shape actual bee foraging decisions. The dominance of nectariferous and pollen-rich crops and ruderal species (e.g., *Guizotia abyssinica* (L.f.) Cass., *Cucumis* spp., *Brassica* spp., *Phaseolus* spp.) indicates that bees preferentially exploit species that combine high pollen output with consistent nectar secretion (Table 1). The predominance of these plant families also reflects the nutritional requirements and foraging behaviour of *A. cerana indica*. Honey bees preferentially exploit plant species that provide abundant, easily accessible nectar together with protein-rich pollen, thereby maximising energetic efficiency during foraging. Large floral displays, prolonged flowering periods, high flower density and mass flowering events characteristic of families such as Asteraceae, Brassicaceae and Cucurbitaceae further increase their attractiveness to foraging bees (10). The repeated detection of these families across palynological, melissopalynological and DNA metabarcoding analyses therefore indicates that they constitute the principal floral resources (4, 7). Species-level identification is essential for accurately characterising bee forage resources, as it enables precise determination of the plant species visited by bees. However, in melissopalynology, species-level identification is often limited because many closely related plant species produce morphologically similar pollen grains, restricting reliable identification to the genus or family level.

The Asteraceae, Cucurbitaceae, Brassicaceae, Fabaceae and Malvaceae reflects the combined influence of seasonal flowering phenology, landscape heterogeneity, agricultural practices and the foraging

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preferences of *A. cerana indica*. The study area comprises a mosaic of cultivated fields, vegetable farms, plantations, wastelands and semi-natural vegetation, providing a continuous succession of flowering resources throughout the sampling period. Cultivated crops such as cucurbits, brassicas, pulses, cotton and okra contribute abundant nectar and pollen during their flowering seasons, while wild herbs and weeds belonging to Asteraceae and Amaranthaceae maintain floral continuity when cultivated crops are not in bloom. This complementary distribution of cultivated and spontaneous vegetation ensures a relatively stable supply of floral resources for honey bees across seasons.

Pollen mixtures obtained from beehives at the study localities over sampling periods generated DNA sequences exhibiting minimal nucleotide divergence. These sequences were rigorously compared against curated reference databases to infer the taxonomic composition of plant sources represented in each pollen mixture. The resulting plant identifications and their relative occurrence across samples are summarised in Table 1. DNA metabarcoding of honey samples and pollen collected from pollen traps using the *rbcL*, *matK* and *trnH–psbA* chloroplast markers revealed a diverse spectrum of plant taxa foraged by honey bees, providing higher taxonomic resolution than traditional palynology. Palynological analysis initially identified 83 plant genera across all samples. However, this approach is constrained by its reliance on pollen morphology, which often limits identification to the genus level. Closely related taxa frequently exhibit highly similar pollen features, particularly spine morphology, making species-level discrimination difficult under phase-contrast light microscopy (Fig. 3). Even with higher-resolution SEM, species-level identification remained largely inconclusive due to overlapping morphological characteristics (Fig. 4). While DNA metabarcoding resolved many of these genera to the species level, detecting key taxa such as *Amaranthus spinosus* L., *Amaranthus viridis* L., *Brassica juncea* (L.) Czern., *Brassica nigra* (L.) K.Koch, *Raphanus sativus* L., *Cucumis sativus* L., *Cucurbita maxima* Duchesne, *Ocimum sanctum L.* and *Solanum melongena L.*. Overall, 65 of the 83 palynologically identified genera were resolved to species by DNA metabarcoding, highlighting its high taxonomic precision (Table 1).

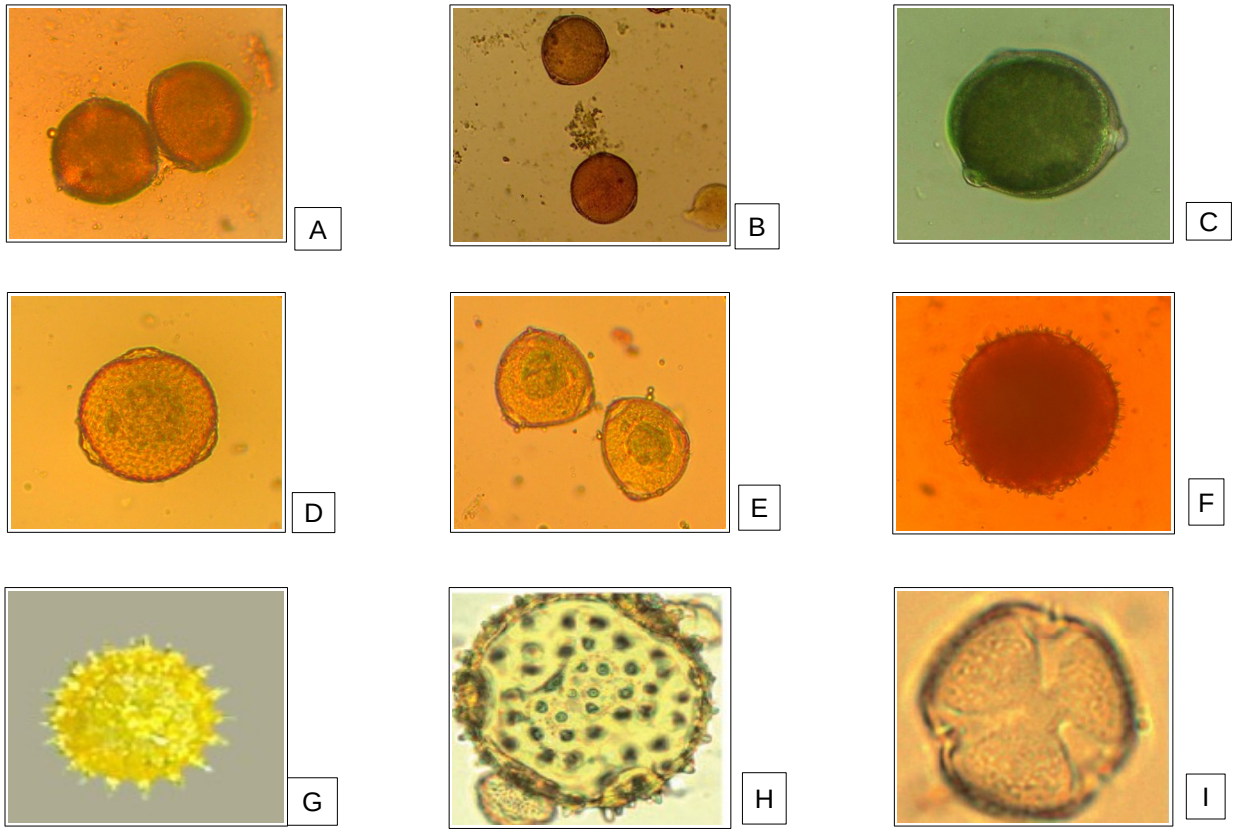


Fig. 3. Representative palynological images of pollen taxa from bee-foraged plants under light microscopy. (A) *Momordica charantia*; (B) *Luffa acutangula*; (C) *Lageneria siceraria*; (D) *Trichosanthes cucumerina*; (E) *Cucumis Sativus*; (F) *Cucurbita pepo*; (G) *Abelmoschus esculentus*; (H) *Gossypium hirsutum*; (J) *Brassica nigra.*

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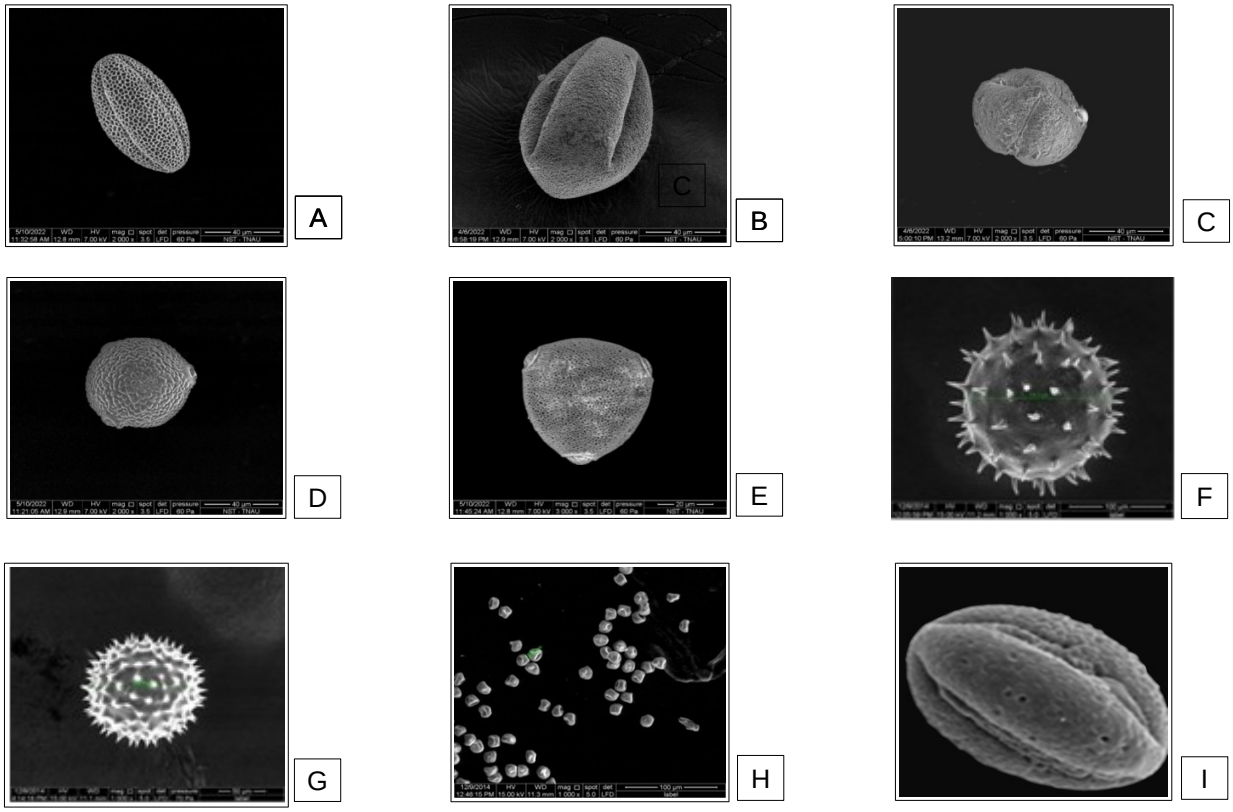


Fig. 4. Representative scanning electron micrographs of palynological features of pollen taxa from bee-foraged plants. (A) *Momordica charantia*; (B) *Luffa acutangula*; (C) *Lageneria siceraria*; (D) *Trichosanthes cucumerina*; (E) *Cucumis Sativus*; (F) *Cucurbita pepo*; (G) *Abelmoschus esculentus*; (H) *Gossypium hirsutum*; (J) *Brassica nigra*.

A comparative analysis of the three approaches revealed both shared and method-specific detection of floral taxa (Fig. 5). Overall, 86 plant taxa were identified across all methods. Among these, 31 taxa were commonly detected by palynology, melissopalynology and DNA metabarcoding, indicating strong agreement in identifying the major floral resources utilized by *A. cerana indica*. Palynology uniquely identified 30 taxa, while DNA metabarcoding uniquely detected seven taxa. Melissopalynology identified only one unique taxon. Pairwise comparisons showed that 14 taxa were shared exclusively between palynology and DNA metabarcoding, two taxa were shared between melissopalynology and DNA metabarcoding and one taxon was shared exclusively between palynology and melissopalynology.

The heatmap also illustrates the distribution of plant taxa detected by palynology, melissopalynology and DNA metabarcoding (Fig. 6). Several taxa, including *O. sanctum*, *B. juncea*, *R. sativus*, *C. sativus*, *C. maxima*, *Luffa acutangular* (L.) Roxb., *Momordica charantia* L., *Cajanus cajan* (L.) Huth, *Cicer arietinum* L., *Abelmoschus esculentus* (L.) Moench, *Gossypium hirsutum* L. and *S. melongena*, were consistently detected by all three methods, indicating their importance as major floral resources for *A. cerana indica*. Palynology detected the widest range of taxa, while DNA metabarcoding provided additional species-level identifications that were not recovered through microscopy-based methods. In contrast, melissopalynology identified comparatively fewer taxa. Overall, the heatmap demonstrates both the overlap and method-specific detection patterns, highlighting the complementary nature of conventional and molecular approaches for comprehensive bee forage characterisation.

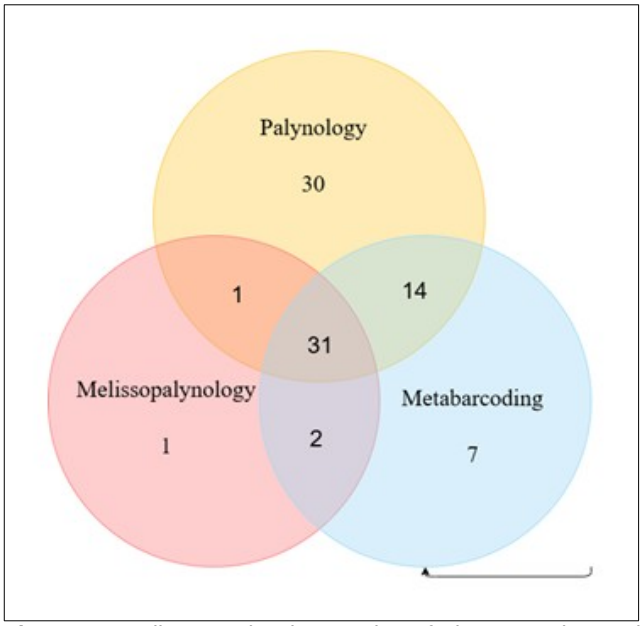


Fig. 5. Venn diagram showing overlap of plant taxa detected using three chloroplast barcode markers (*rbcL, matK, trnH–psbA*) in DNA metabarcoding analysis.

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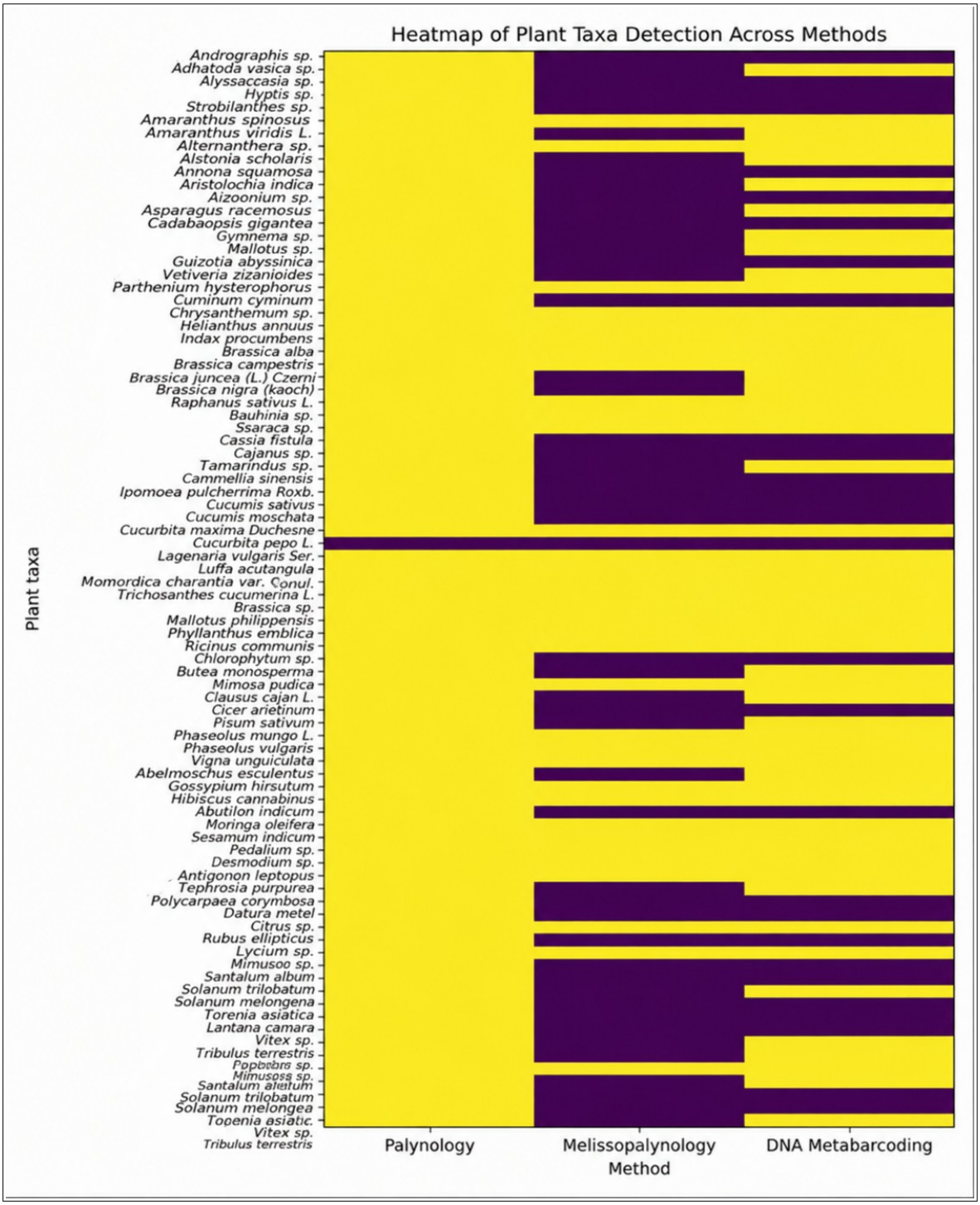


Fig. 6. Heatmap illustrating plant taxa detected in bee foraging samples using palynological,melissopalynological and DNA metabarcoding approach.

The rbcL marker captured the broadest taxonomic range across families such as Brassicaceae, Cucurbitaceae, Fabaceae, Malvaceae and Solanaceae due to its universality and high amplification efficiency. The *matK* marker provided complementary resolution, detecting additional species not recovered by *rbcL*, including *Adhatoda vasica* Nees, *Amaranthus blitum* L. and *Alstonia scholaris* (L.) R.Br.. The *trnH–psbA* marker further enhanced species-level identification, amplifying taxa such as *Brassica alba* (L.) Rabenh. and *Brassica campestris* L., despite lower amplification efficiency. The partial overlap among markers underscores the value of a multi-locus metabarcoding approach for maximising taxonomic coverage. Several taxa identified by palynology, including *Strobilanthes kunthiana* (Nees) T.Anderson ex Benth., *Capparis zeylanica* L. and *Tribulus terrestris* L., were not detected by any DNA marker, likely due to low pollen abundance or primer limitations, highlighting the complementary nature of morphological and molecular methods. Overall, DNA metabarcoding provided high-resolution species-level identification, with Cucurbitaceae, Brassicaceae,

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Asteraceae, Fabaceae, Malvaceae and Solanaceae emerging as the most frequently represented families, reflecting honey bee foraging preferences and the floral composition of the surrounding landscape.

The results from the conventional palynological analysis in the present study predominantly enabled identification at the genus level, with species-level resolution achieved only for a limited number of taxa. This limitation is well recognised and arises from the conservative nature of pollen morphology, where closely related species often produce morphologically indistinguishable pollen grains, coupled with pollen damage, deformation and observer-dependent interpretation (24, 27). As a result, routine palynology generally provides reliable information on overall floral diversity but remains constrained in resolving fine-scale species composition.

On the other hand, melissopalynological analysis yielded comparatively improved species-level identification. This enhanced resolution can be attributed to the selective foraging behaviour of honey bees and the compositional nature of honey, which predominantly incorporates pollen from highly polleniferous and nectariferous plant species that are intensively visited during nectar collection. Such dominant forage plants typically produce abundant pollen with distinctive morphological features, facilitating more confident species-level identification under light microscopy (11, 28, 29).

Moreover, pollen from low-abundance, wind-dispersed or morphologically ambiguous taxa is often underrepresented or absent in honey, further biasing melissopalynological assemblages toward easily identifiable species. This methodological contrast explains the relatively higher species-level resolution observed in honey samples and underscores the complementary but unequal taxonomic resolving power of the two microscopy-based approaches. In contrast, DNA metabarcoding substantially overcame these limitations by enabling consistent species-level identification across complex pollen mixtures, independent of pollen morphology or abundance. The molecular approach resolved most palynologically identified genera to the species level, providing higher taxonomic precision and reducing observer bias (30–32). Overall, the DNA barcoding approach enabled reliable species-level taxonomic assignment for nearly all pollen mixtures obtained from the selected locations in Coimbatore. Nevertheless, the choice of barcode marker remains critical for ensuring methodological standardisation and compatibility with international genetic reference repositories, in accordance with the guidelines established by the Consortium for the Barcode of Life (CBOL).

Among the three chloroplast barcode markers evaluated (*rbcL*, *matK* and *trnH–psbA*), *rbcL* emerged as the most effective and reliable marker for characterising the plant species composition of pollen mixtures collected by honey bees. The superior performance of *rbcL* is primarily attributable to its high universality, robust amplification success and extensive representation in global reference databases. In the present study, *rbcL* consistently amplified across a wide range of taxa and enabled accurate species-level identification for the majority of pollen samples, confirming its suitability as a core barcode marker for complex mixed-pollen analyses. These findings are in strong agreement with Consortium for the Barcode of Life (CBOL) recommendations, which recognise *rbcL* as a standard plant barcode due to its high amplification efficiency and cross-taxon comparability (33).

The *matK* marker provided complementary resolution by identifying additional taxa not detected by *rbcL*, reflecting its higher substitution rate and improved discriminatory power among closely related species. However, its relatively lower amplification success, particularly in degraded or low-template DNA typical of environmental pollen samples, limited its standalone effectiveness. Similar constraints on *matK* performance have been reported in previous metabarcoding and barcoding studies, especially when applied to mixed or trace DNA samples (30, 34).

In contrast, *trnH–psbA* exhibited the lowest overall performance, independently identifying only two taxa when used alone. Although this intergenic spacer is known for high interspecific variability and can provide fine-scale resolution in specific plant groups, its utility is often constrained by inconsistent amplification, length polymorphisms and alignment difficulties (35, 36). In mixed-pollen contexts, these limitations substantially reduce its effectiveness as a primary barcode marker. Consequently, *trnH–psbA* functioned mainly as a supplementary marker in this study, contributing marginally to species discrimination but offering limited standalone diagnostic value.

Crucially, the species-level foraging preferences revealed through DNA metabarcoding offer actionable insights for apicultural management. An important ecological insight emerging from the present study is the detection of pollen from several underreported floral resources, particularly members of the family Acanthaceae, including *Andrographis* sp., *A. vasica* and *Asystasia* sp. Although these species are not commonly recognised as major bee forage plants in tropical agroecosystems, their consistent detection indicates that they contribute to sustaining honey bee populations. Likewise, the occurrence of pollen from weeds such as *A. spinosus* and *C. gynandra* highlights the ecological significance of non-crop vegetation in supporting pollinator foraging. These findings broaden the current understanding of bee forage diversity in southern India and emphasise the value of conserving spontaneous vegetation surrounding agricultural landscapes. By identifying key nectar- and pollen-producing plant species preferentially exploited by honey bees, these findings provide a scientific framework for optimising floral resource management in and around apiaries. Targeted cultivation and conservation of bee-preferred plant species have the potential to enhance forage continuity, improve colony health and ultimately increase honey yield.

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A limitation of the present study is that sampling was restricted to six apiary sites over a six-month period within the Coimbatore region. Consequently, the observed floral resource composition and bee foraging patterns may not fully represent other geographical regions or annual climatic variations. Furthermore, DNA metabarcoding was based on chloroplast markers (*rbcL*, *matK* and *trnH–psbA*), which may have limited discriminatory power for certain closely related plant taxa. In addition, sequencing read abundance was used only for taxonomic identification and should not be interpreted as a direct measure of pollen abundance because PCR amplification efficiency, primer bias and differences in chloroplast copy number can influence read counts. Future studies should incorporate broader spatial coverage, multi-seasonal and multi-year sampling, additional molecular markers with higher taxonomic resolution and quantitative approaches to further validate and extend the applicability of the present findings.

Although DNA metabarcoding enhanced species-level identification and detected taxa that were not recovered by microscopy, its results should be interpreted with caution. The accuracy of metabarcoding may be influenced by primer bias, PCR amplification bias, incomplete or unevenly represented reference sequence databases and variation in chloroplast copy number among plant species, all of which can affect taxon detection and relative representation. Similarly, microscopy-based methods may be constrained by morphological similarity among pollen grains and the requirement for taxonomic expertise. Therefore, rather than considering one method superior, the present findings demonstrate that palynology, melissopalynology and DNA metabarcoding provide complementary information. Integrating these approaches enables a more comprehensive and reliable characterisation of honey bee floral resources than any single method alone.

4. Conclusion

In conclusion, the present study demonstrated that both traditional palynological and melissopalynological (microscopical/optical) methods and high-throughput DNA metabarcoding are valuable tools for understanding bee foraging ecology. Microscopic techniques provide direct observation of pollen morphology and relative abundance, whereas DNA metabarcoding offers greater sensitivity and accurate identification of a wider range of plant taxa, including morphologically similar or rare species. The combined use of these approaches provides a more comprehensive and reliable assessment of bee–plant interactions, floral diversity and foraging preferences. Such knowledge can help beekeepers identify major nectar and pollen sources, improve colony management, enhance honey quality and authenticity and ultimately increase honey production and economic profit. Therefore, integrating conventional and molecular approaches can significantly support pollinator research, biodiversity conservation and sustainable apiculture practices. Future research should evaluate seasonal and landscape-level variation in bee forage resources across diverse agroecosystems, establish comprehensive regional DNA barcode reference libraries to improve species-level identification and investigate the nutritional and ecological significance of the previously underrecognised forage plants detected in this study. Integrating quantitative DNA metabarcoding with long-term ecological monitoring will further enhance our understanding of plant–pollinator interactions and support evidence-based conservation and sustainable apiculture.

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Authors' contributions

MR conducted bee foraging studies, pollen analysis and manuscript preparation. AP and GD contributed to ecological and environmental interpretation of the study area. TS, VAA and MK contributed to the interpretation of plant health and disease-related aspects associated with floral resources. KTM and AJ assisted in the analysis of economic significance and beekeeping profitability. DVP and MS contributed to the study by reviewing the manuscript, providing critical intellectual inputs and revising the article for important intellectual content. All authors have read and approved the final version.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

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