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# INTEGRATING MORPHOMETRIC AND GENETIC ANALYSES OF POPULATION DIVERGENCE IN APIS CERANA INDICA (INDIAN BEE) FROM PAPANASAM TALUK, TAMIL NADU, INDIA

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**ABSTRACT:** Taxonomic resolution within the genus Apis is often complicated by morphological variability and phenotypic plasticity, with obscure species and subspecies boundaries. This study aimed to establish clear taxonomic evidence for cavity-nesting Apis cerana indica from Papanasam Taluk, Tamil Nadu, by integrating classical and geometric morphometrics with mitochondrial DNA barcoding. Forty-five worker bees from three colonies were analysed for 34 morphometric traits along with forewing shape using 19 landmarks with multivariate ordinations. Molecular identification was performed through COI gene sequencing and phylogenetic analysis. Morphometric data revealed strong inter-colony similarity with conserved wing shape, while COI sequences showed high conservation (0.94) and tight clustering within A. cerana indica (0.00–0.17), with clear divergence from A. dorsata, A. florea, and A. mellifera (10-13%). Regional populations from Tamil Nadu, Orissa, and Calicut were homogeneous, whereas Thrissur, Bangalore, and Shillong exhibited moderate divergence (4.7-5.5%). Concordant morphometric and molecular evidence confirms the regional identity of A. cerana indica, providing a robust baseline for breed purity, genetic conservation, and future ecological and applied research in tropical agroecosystems.

**KEYWORDS:** Apis cerana indica; Morphometrics; COI barcoding; Taxonomy; Genetic diversity; Tropical agroecosystem.

## INTRODUCTION

Honeybees are globally acknowledged for their cardinal role in providing nourishments through their by-products, rendering pollination services and for maintaining a stable ecosystem. This petal courtiers are employed in various disciplines of research such as Robotics and Computer vision (Rizzi et al., 1998b), study of astronomical orientation and time sense (Renner, 1960), Electric ecology (Clarke et al., 2017), Environmental health and epidemiological research (Mair et al., 2023), Forensic research (Morice et al., 2020), Nanotechnology and Bioengineering (Khalifa et al., 2024), Space Science (Poskevich et al., 1989), Microbiome research (Wang et al., 2018) and in Military operations (Bhumika & Singh, 2011). Based on morphological features, Maa (1953) divided honeybees into 3 genera Micrapis, Megapis and Apis. The genus Apis comprises over a thousand species with noticeable differences in their morphology within a single group due to which they pose a risk of exacerbation for the taxonomists, hampering the identification of a particular specimen whether it belongs to one species or another (Gupta, 2014). Ruttner (1988) defined Apis cerana as a single species based on his investigations on their morphometric characteristics. He further divided them into four subspecies A. cerana cerana, A. cerana japonica, A. cerana himalaya and A. cerana indica. Despite being considered as a single species this Indian cavity nesting bees (A. cerana) inhabits various regions exhibiting variations in their phenotypes and behavioral patterns (Zhang et al., 2025). The changes in climatic types, food sources, altitude and latitude greatly account for the variations in population size, nutritional status, morphological characters , reproductive potential and genetic status in A. cerana (Sousa et al., 2016; Ji et al., 2023).The ability of an individual to change their behavior , morphology or physiology when exposed to changing environmental cues is called phenotypic plasticity (Duncan et al., 2022).Bees display remarkable instances of phenotypic plasticity (Duncan et al., 2020) making it difficult for the taxonomists to identify and classify them. Accurate identification of the bee species is crucial for maintaining the breed purity which helps in preserving their genetic diversity. It also serves as a critical component in discrimination, conservation, and preservation of different species, subspecies or races for future research (Syromyatnikov et al., 2018). Morphometrics in bee research uses numerical data obtained from measuring physical/ morphological characters to study species differentiation, subspecies classification and geographical variations. It also helps to identify intraspecific variations in bees. The database established by Ruttner (1988) with 38 standard morphometric characteristics serves as an international blueprint for the characterization and classification of bees. Though morphometrics aids in the identification of bees it has some limitations such as lack of experts in resolving cryptic species, sexual dimorphism and variations due to

phenotypic plasticity. Molecular identification is more sensitive and accurate to identify species by matching unknown genes with already identified ones (S et al., 2024). Cytochrome c oxidase I (COI) gene of mitochondrial DNA and many other molecular markers have been used to detect and differentiate various species of Coleoptera, Diptera Ephemeroptera, Hemiptera, Hymenoptera, and Lepidoptera as it provides high resolution to identify cryptic species (Karthika et al.,2016). Although few works were reported on morphometrics of A. cerana in Papanasam Taluk of Thanjavur District in Tamil Nadu it lacked proper molecular proves and no new work on it were carried out in last decade. Integration of both morphometric and molecular approaches provides a comprehensive understanding of phenotypic traits and genetic diversity in Apis cerana cerana populations (Dong et al., 2026). The present study focused on the morphometrics and molecular identification of the bees in Papanasam taluk to provide clear data on the taxonomical identification of bees present in this region for subjecting them to further research.

## MATERIALS AND METHODS

### Description of the Study Area

Papanasam taluk is one of the eight taluks of Thanjavur district in Tamilnadu. It is circumscribed on the Northeast by Kumbakonam taluk, East and Southeast by Valangaiman taluk, South by Orathanadu taluk Southwest by Thanjavur taluk and West by Thiruvaiyaru taluk and the Northern part by Ariyalur taluk. The area is bound between 10.9233ºN and 79.2864ºE with elevation of about 39 m above sea level. It occupies an area of about 129.82 sqkms (Fig.1). The land use types present in this taluk are farm land (mixed plantations), fallow land, scrub land, barren land, water bodies and settlements. Nestled in the Cauvery delta region the taluk's physical and climatic conditions are favorable for cultivation, with paddy being the dominant crop. Other crops grown include sugarcane, oilseeds, pulses, and cotton. It has three cropping seasons: Samba (single crop), Kuruvai (double crop) and Thaladi (major paddy cropping). This region has a tropical wet and dry climate. It experiences hot, dry summers and receives rainfall primarily during the monsoon season, especially from the southwest monsoon. The area also experiences a northeast monsoon season, which contributes significantly to rainfall.

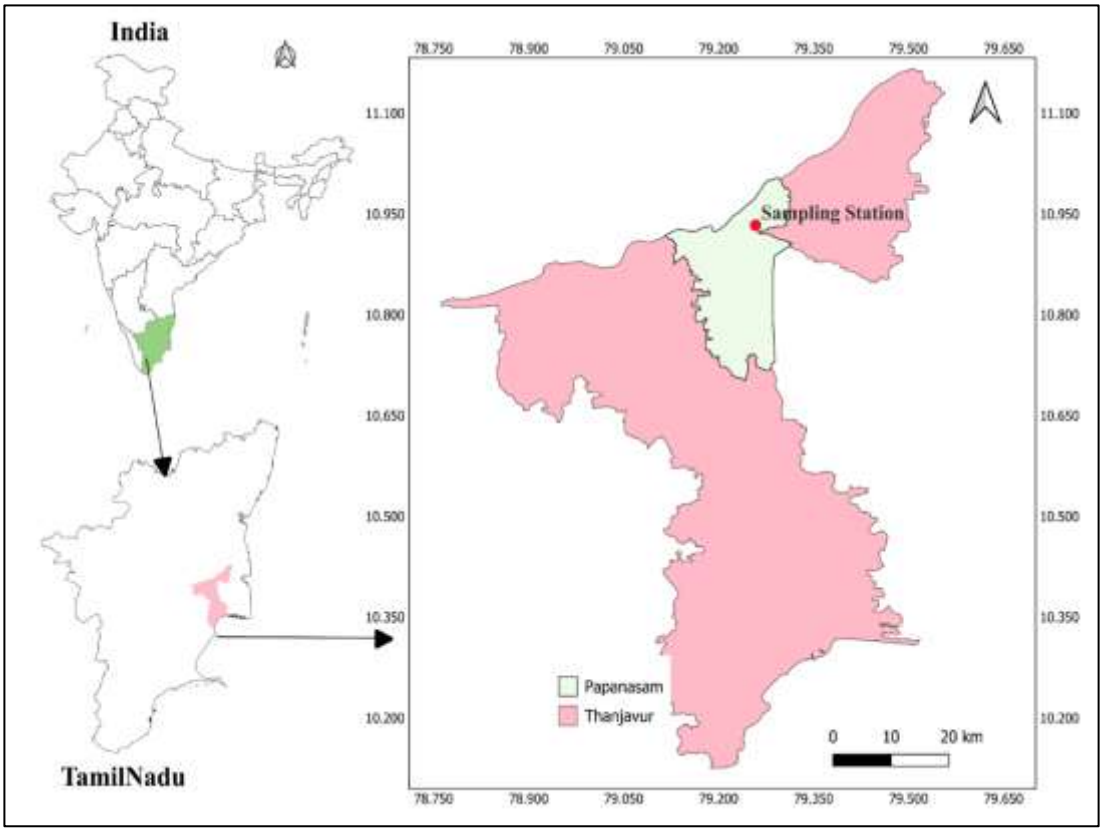


**Fig. 1 Study area map**

### Sample Collection

To minimize ecological disruption and avoid unnecessary depletion of local bee populations, a minimal-impact sampling approach was adopted. A total of 45 worker bees were collected during peak foraging hours from three randomly selected healthy colonies (15 bees per colony) maintained in Newton's hives at a local bee farm. Bees were carefully handled using soft-tipped forceps to prevent damage to delicate body parts, immediately transferred into 15 mL centrifuge tubes containing 90 % ethanol, and stored at -20 °C until analysis.

### Classical Morphometrics

Specimens were dissected and measurements recorded following the protocols of Ruttner et al. (1978). Larger anatomical structures were measured manually using a digital Vernier caliper, while smaller structures such as wing angles and

indices were photographed with an Adcom AD 12x/24x Macro Mobile Camera Lens and analysed digitally using ImageJ software following Dadgostar et al. (2020).
A total of 34 morphometric characters were assessed, including 20 size parameters (Fig.2), 11 wing angles A4, B4, D7, E9, G18, J10, J16, K19, L13, N23, O26 as documented by Kitnya et al. (2022) (Fig. 3), 2 wing indices and the number of hamuli on hindwings. All size parameters were expressed in millimetres (mm).

**Fig.2 Morphometric measurements of Apis cerana indica worker bee body parts**

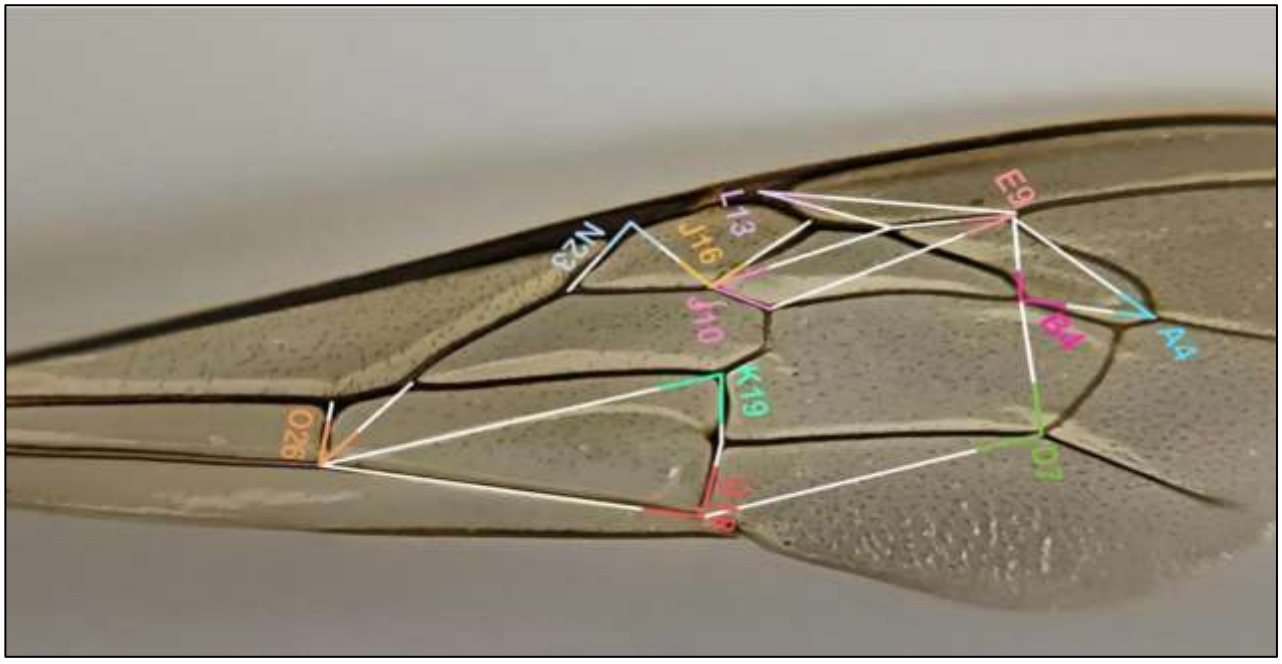


**Fig.3 Wing venation angle analysis of the forewing in Apis cerana indica**

**Geometric Morphometrics of Wings**
The right forewings of 15 bees from each colony (total 45) were dissected, mounted on plain white sheets, and photographed using an Adcom AD 12x/24x Macro Mobile Camera Lens. Images were converted into TPS files using tpsUtil32 v1.83 and digitized in tpsDig232 v2.31 by marking 19 landmark coordinates. Landmark data were aligned and averaged within colonies using Generalized Procrustes Analysis (GPA) in Morpho J v1.08. After scaling, translation, and rotation against the consensus configuration, wing shape variation was visualized. Wing size was quantified using log-transformed centroid size, calculated as the square root of the sum of squared distances between the centroid and each of the 19 landmarks (Aglagane et al., 2022).

**Statistical Analysis**

Descriptive statistics (mean, standard deviation, standard error, minimum, maximum, range, and correlation) for classical morphometrics were calculated using MS Excel. Geometric morphometric data were analysed using GPA, Principal Components Analysis (PCA), and Canonical Variate Analysis (CVA) in MorphoJ v1.08. These analyses enabled evaluation of morphometric variation in terms of size and shape, population differentiation, and evolutionary interpretation.

**Molecular Examination**

**DNA Isolation, PCR Amplification, and Gel Electrophoresis**

Genomic DNA was extracted from bee tissue using the DNeasy Blood and Tissue Kit (Qiagen, Cat. No. 69504) following the manufacturer's protocol. Samples were rinsed with PBS, air-dried, homogenized, and lysed with Buffer ATL and Proteinase K at 56 °C for 3 h. After adding Buffer AL and ethanol, lysates were loaded onto spin columns, washed with Buffers AW1 and AW2, and eluted with Buffer AE. DNA quality was checked by agarose gel electrophoresis. The mitochondrial COI gene was amplified using primers M13F-LCO1490 and M13R-HCO2198 (Folmer et al., 1994) in a 20 µl PCR reaction containing Emerald Amp GT Master Mix. Cycling conditions were: 95 °C for 2 min; 30 cycles of 95 °C for 40 s, 52 °C for 40 s, 72 °C for 45 s; and a final extension at 72 °C for 7 min. PCR products (5 µl) were resolved on 1% agarose gel with ethidium bromide in 1× TAE buffer at 120 V for 45 min. A 100 bp DNA ladder was used as marker, and gels were visualized under a UV documentation system (Enfys Lifesciences, Cochin, Kerala).

**Data Analysis**

Sequences obtained were trimmed and manually edited using BioEdit software. Edited sequences were compared with reference sequences available in the NCBI database. Nucleotide composition, substitution patterns, genetic distances, and phylogenetic relationships were analysed using MEGA v11 software.

**RESULTS**

**Classical Morphometrics**

The morphometric analysis of worker bees from three colonies revealed a high degree of similarity across all measured characters (Table 1). The mean body length was 10.19 ± 0.087 mm, with values ranging from 10.11 to 10.28 mm. Head length and width recorded mean values of 3.00 ± 0.013 mm and 3.13 ± 0.040 mm, respectively, while antenna length showed minimal variation (3.01 ± 0.015 mm). Abdomen length and width averaged 4.50 ± 0.134 mm and 3.18 ± 0.109 mm, respectively. The Proboscis length exhibited comparatively higher variability (3.46 ± 0.369 mm; range = 0.70 mm), whereas proboscis width remained constant at 0.20 mm across colonies. Thoracic measurements indicated a mean thorax length of 3.35 ± 0.140 mm and thorax width of 3.09 ± 0.025 mm. Forewing length averaged 7.52 ± 0.011 mm, while forewing width measured 2.51 ± 0.020 mm. Hindwing length and width recorded mean values of 5.09 ± 0.112 mm and 1.56 ± 0.052 mm, respectively. The mean number of hamuli was 18.64 ± 0.367. Leg morphometry showed limited variation, with femur length averaging 2.52 ± 0.034 mm and tibia length 2.50 ± 0.007 mm. Femur width, tibia width, and metatarsus width remained nearly constant across colonies. The cubital index showed a mean value of 2.68 ± 0.182, while the Hantel index averaged 1.14 ± 0.027.

Wing venation angles demonstrated moderate variation among colonies. Angle A4 had a mean of 29.38° ± 0.920, B4 measured 107.95° ± 1.662, and D7 averaged 95.47° ± 0.532. Other angles recorded were: E9 (20.31°), G18 (88.67°), I10 (42.76°), I16 (96.33°), K19 (72.61°), L13 (12.43°), N23 (80.90°), and O26 (33.87°), all showing relatively low standard deviations (Table 1).

**Table 1 Descriptive statistical measures of different morphometric characters of Apis cerana indica from three colonies of the same geographical location (n=45).**

| Morphological Characters | Mean | S. D | S. E | Min | Max | Range |
|---|---|---|---|---|---|---|
| Body length | 10.19 | 0.08 | 0.05 | 10.11 | 10.28 | 0.17 |
| Head length | 3.00 | 0.01 | 0.01 | 2.98 | 3.01 | 0.03 |
| Head width | 3.13 | 0.04 | 0.02 | 3.10 | 3.17 | 0.07 |
| Antenna length | 3.01 | 0.01 | 0.01 | 3.00 | 3.03 | 0.03 |
| Abdomen length | 4.50 | 0.13 | 0.08 | 4.41 | 4.65 | 0.24 |
| Abdomen width | 3.18 | 0.10 | 0.06 | 3.11 | 3.31 | 0.19 |
| Proboscis length | 3.46 | 0.36 | 0.21 | 3.17 | 3.87 | 0.70 |
| Proboscis width | 0.20 | 0.00 | 0.00 | 0.20 | 0.20 | 0.00 |
| Thorax length | 3.35 | 0.14 | 0.08 | 3.19 | 3.45 | 0.26 |
| Thorax width | 3.09 | 0.03 | 0.01 | 3.06 | 3.11 | 0.05 |
| Forewing length | 7.52 | 0.01 | 0.01 | 7.51 | 7.53 | 0.02 |
| Forewing width | 2.51 | 0.02 | 0.01 | 2.49 | 2.53 | 0.04 |
| Hindwing length | 5.09 | 0.11 | 0.06 | 5.00 | 5.21 | 0.21 |
| Hindwing width | 1.56 | 0.05 | 0.03 | 1.50 | 1.60 | 0.10 |
| No. of Hamuli | 18.64 | 0.37 | 0.21 | 18.40 | 19.07 | 0.67 |
| Femur length | 2.52 | 0.03 | 0.02 | 2.50 | 2.56 | 0.06 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Femur width | 0.50 | 0.01 | 0.00 | 0.49 | 0.50 | 0.01 |
| Tibia length | 2.50 | 0.01 | 0.00 | 2.49 | 2.50 | 0.01 |
| Tibia width | 0.80 | 0.00 | 0.00 | 0.80 | 0.80 | 0.00 |
| Metatarsus length | 1.53 | 0.06 | 0.03 | 1.50 | 1.60 | 0.10 |
| Metatarsus width | 0.80 | 0.00 | 0.00 | 0.80 | 0.80 | 0.00 |
| cubital index | 2.68 | 0.18 | 0.11 | 2.52 | 2.88 | 0.36 |
| Hantel index | 1.14 | 0.03 | 0.02 | 1.12 | 1.17 | 0.05 |
| Angle A4 | 29.39 | 0.92 | 0.65 | 28.12 | 30.26 | 2.14 |
| Angle B4 | 107.95 | 1.66 | 1.18 | 106.47 | 110.27 | 3.81 |
| Angle D7 | 95.48 | 0.53 | 0.38 | 94.80 | 96.10 | 1.30 |
| Angle E9 | 20.31 | 0.18 | 0.13 | 20.06 | 20.49 | 0.42 |
| Angle G18 | 88.68 | 0.25 | 0.18 | 88.46 | 89.03 | 0.57 |
| Angle I10 | 42.77 | 1.55 | 1.09 | 40.62 | 44.21 | 3.59 |
| Angle I16 | 96.34 | 0.39 | 0.28 | 95.79 | 96.63 | 0.84 |
| Angle K19 | 72.62 | 1.44 | 1.02 | 71.59 | 74.65 | 3.06 |
| Angle L13 | 12.43 | 0.33 | 0.23 | 12.03 | 12.84 | 0.80 |
| Angle N23 | 80.91 | 0.88 | 0.62 | 79.67 | 81.61 | 1.94 |
| Angle O26 | 33.87 | 0.81 | 0.57 | 32.97 | 34.93 | 1.96 |

**Geometrical Morphometrics**

A total of 19 landmarks were used to represent key venation junctions. Visual comparison of the superimposed configurations indicated a high degree of similarity in overall wing shape among colonies. The general venation framework remained conserved, with only minor positional deviations observed at specific landmarks. Slight displacement was noticeable in the distal landmarks, particularly around the apical and radial vein regions near the wing tip. Minor variation was also observed in the medial region of the wing; however, the basal landmarks remained largely stable across colonies. The overlapping configurations demonstrated that inter-colony variation in wing shape was minimal, with no pronounced structural deformation or major shift in venation pattern. Overall, geometric morphometric analysis confirmed strong shape conservation among the studied colonies (Fig. 4). Principal Component Analysis (PCA) was performed to evaluate patterns of morphometric variation among the three colonies. The distribution of individuals along the first two principal components (PC1 and PC2) revealed partial clustering with noticeable overlap among colonies. Colony-wise grouping showed that individuals largely occupied overlapping regions within the morphospace, indicating limited inter-colony differentiation. Although slight dispersion was observed along PC1 for one colony and along PC2 for another, the clusters were not distinctly separated. The convex hull boundaries further demonstrated substantial overlap among the three colonies. The absence of clear segregation along the principal component axes suggested that the measured morphometric characters did not support strong structural divergence among colonies. Instead, the PCA indicated overall morphological similarity, with only minor variation distributed continuously rather than forming discrete groups (Fig. 5).

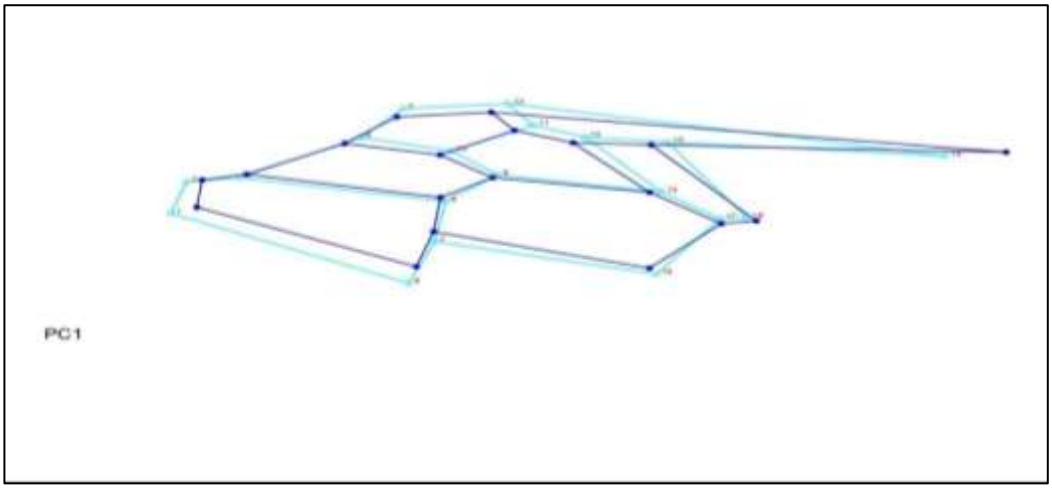


**Fig. 4 Wireframe representation of superimposed wing landmark configurations showing shape variation among three colonies.**

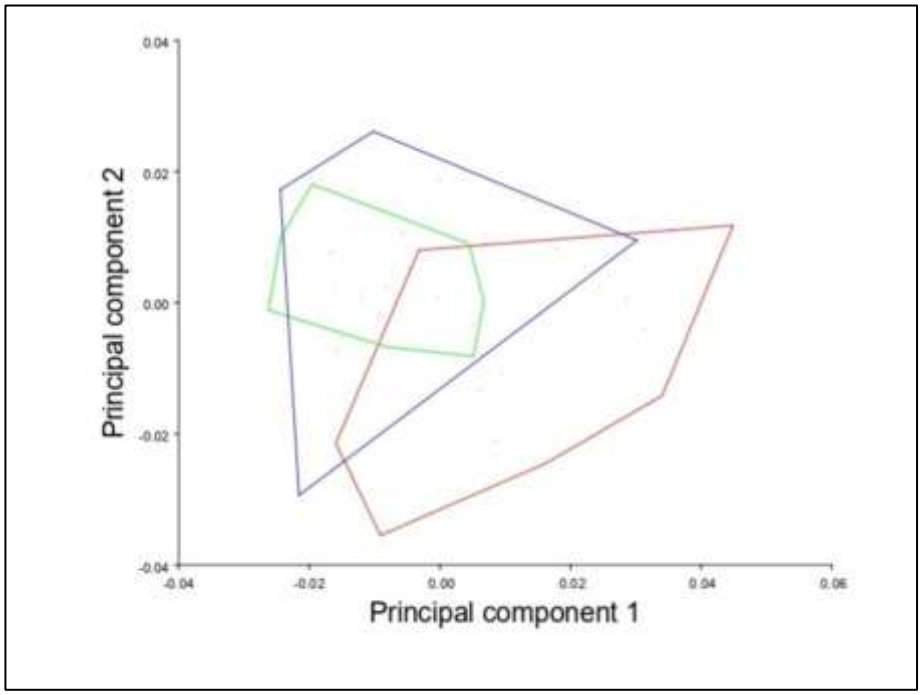


**Fig. 5 Principal Component Analysis (PCA) plot showing the distribution of individuals from three colonies based on morphometric characters.**

**Molecular Analysis**

The sequences were submitted in the NCBI (GenBank), the accession numbers obtained are PQ333121.1 and PQ620158.1

**Nucleotide Composition and Maximum Likelihood Estimate of Transition/Transversion Bias**

Nucleotide composition T (U) 43.5, C 13.8, A 33.0, G 9.7. The estimated Transition/Transversion bias (R) is 1.37. Substitution pattern and rates were estimated under the Kimura (1980) 2-parameter model (+G+I) [1]. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories, [+G], parameter = 0.0500). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 29 % sites). The nucleotide frequencies were A = 25.00%, T/U = 25.00%, C = 25.00%, and G = 25.00%. For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was -712.136. This analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There was a total of 421 positions in the final dataset.

**Genetic Conservation and Haplotype Diversity**

The analysis of the conserved DNA regions in Apis cerana indica revealed a high level of sequence conservation across the 421 sites examined. Out of these, 22 sites were variable, resulting in an overall conservation value of 0.94, which indicates strong genetic stability within the selected region. Two highly conserved regions were identified as region 1 (positions 175 - 229) and region 2 (positions 309 - 367). Both regions showed complete conservation (1.00), full homozygosity (1.00) and statistically significant p-values (0.04 and 0.03 respectively). The conserved motifs within these regions TATTATTATTAAGAAATTTATTTTATCCAAGACCAGGAACAGGATGAACAGTA and TCATCAATTA TAGGATCATTAAATTTAATAGTTACAATTATAATAATAAA AAATTTTTC represent highly stable genetic sequences that may play important roles in maintaining functional integrity across populations. These findings highlight that despite the presence of polymorphic sites, Apis cerana indica retains strongly conserved DNA stretches, which could be critical for species-specific adaptation and evolutionary stability.

**Estimation of inter specific sequence divergence**

The genetic distance analysis among Apis cerana indica and other honeybee species reveals clear intra-species clustering and strong inter-species divergence. Within Apis cerana indica, sequences PQ269815.1, PQ269790.1 and PV362628.1 show very low divergence (0.00 - 0.17), indicating close genetic similarity and forming a conserved cluster. PV358402.1 (Apis cerana) is slightly more divergent (0.17- 0.35), but still within the same species-level grouping. In contrast, Apis dorsata (MZ420495.1, MZ420494.1) shows ~10.14 -10.34 % divergence from Apis cerana indica, confirming species-level separation. Apis florea (KU752356.1, MN163113.1, KU666428.1) were even more distinct, with divergence values ranging from ~11.16 - 13.67 %, highlighting its evolutionary distance. Apis mellifera (MN563104.1, MN563102.1) also shows substantial divergence (~10.38 - 12.64 %) consistent with its classification as a separate species. Overall, the results demonstrate that Apis cerana indica populations are highly conserved with minimal intra-species variation, while Apis dorsata, Apis florea and Apis mellifera are clearly distinct, showing 10 - 13 % divergence. This confirms strong genetic clustering within Apis cerana indica and clear species boundaries among the different honeybee taxa analyzed.

**Estimation of intra specific sequence divergence**

The genetic distance matrix among Apis cerana indica populations shows clear clustering with some regional differentiation (Table 2). Most Tamil Nadu samples (PQ333121.1, PQ620158.1, MW093739.1, PQ810048.1) were identical, with a distance of 0.00, indicating strong genetic homogeneity. The Coimbatore sample (PQ269812.1) shows slight divergence (0.24) from other Tamil Nadu and Orissa populations, suggesting minor regional variation. Similarly, the Orissa sample (PQ809994.1) clusters closely with Tamil Nadu and Calicut Kerala (KM230116.1), showing distances of 0.00 - 0.24, reinforcing their genetic similarity. In contrast, the Thrissur Kerala sample (OP050121.1) and Bangalore, Karnataka sample (PQ197639.1) exhibit higher divergence (4.68 - 4.93) from Tamil Nadu, Orissa and Andhra Pradesh populations, indicating distinct haplotype grouping. The Shillong, Meghalaya sample (OR608006.1) was the most divergent, with genetic distances of ~ 5.19 - 5.46 compared to other populations, highlighting its unique genetic identity. Overall, the results suggest that Apis cerana indica populations from Tamil Nadu, Orissa and Calicut Kerala form a highly conserved genetic cluster, while Thrissur Kerala, Bangalore in Karnataka and Shillong in Meghalaya represent more differentiated lineages. This pattern reflects both strong conservation within core populations and localized genetic structuring in certain regions, particularly Kerala and Meghalaya which may be shaped by ecological or geographical isolation.

**Neighbor-Joining (NJ) tree by Maximum likelihood Method**

The Neighbor-Joining (NJ) tree constructed from the genetic distance data provides a clear visualization of the evolutionary relationships among Apis species (Fig.6). Within Apis cerana indica, sequences PQ269815.1, PQ269790.1 and PV362628.1 cluster tightly together, marked with red dots, reflecting very low divergence (0.00 - 0.17) and indicating strong genetic conservation. Apis cerana (PV358402.1) forms a nearby branch, showing slight divergence (0.17- 0.35) but still closely related to A. cerana indica. In contrast, Apis dorsata (MZ420495.1, MZ420494.1), Apis florea (MN163113.1, KU752356.1, KU666428.1) and Apis mellifera (MN563104.1, MN563102.1) form distinct, well-separated clades with longer branch lengths, reflecting higher genetic distances (0.04 - 0.06). The scale bar (0.01) emphasizes the magnitude of divergence, highlighting that while A. cerana indica populations are highly conserved, other Apis species show clear evolutionary separation. Overall, the NJ tree underscores both intra-species stability within A. cerana indica and inter-species divergence across the genus Apis, supporting species-level differentiation and localized genetic structuring.

**Neighbor-Joining Phylogenetic Tree of Apis cerana indica Populations Across India**

The Neighbor-Joining tree illustrates the genetic relationships among Apis cerana indica populations collected from diverse regions of India (Fig. 7). Two sequences from the present study (PQ269815.1 and PQ269790.1) are marked with red dots, clustering closely with samples from Tamil Nadu (Madurai, Chidambaram, Coimbatore), Orissa and Calicut Kerala, reflecting strong genetic conservation and minimal divergence. In contrast, populations from Thrissur Kerala and Bangalore Karnataka form distinct branches, showing moderate divergence (~4.68 - 4.93), while the Shillong Meghalaya population is the most divergent (~5.19 - 5.46), forming a separate lineage. The scale bar (0.01) indicates genetic distance, with branch lengths highlighting evolutionary divergence among populations. Overall, the NJ tree demonstrates that A. cerana indica populations from Tamil Nadu, Orissa, and Calicut Kerala were genetically homogeneous, whereas Kerala, Karnataka, and Meghalaya populations exhibit localized differentiation, underscoring both conservation and regional structuring within the species.

**Table 2 Estimation of Intraspecific sequence variation**

| **Species** | | **Accession Number** | **State** | **1** | **2** | **3** | **4** | **5** | **6** | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Apis cerana indica | 1 | PQ269815.1 | Sample | | | | | | | | | | | | |
| | 2 | PQ269790.1 | Sample | 0.00 | | | | | | | | | | | |
| | 3 | MW093739.1 | Madurai, Tamil Nadu | 0.00 | 0.00 | | | | | | | | | | |
| | 4 | PQ810048.1 | Chidambaram, Tamil Nadu | 0.00 | 0.00 | 0.00 | | | | | | | | | |
| | 5 | PQ269812.1 | TNAUCoimbatore, Tamil Nadu | 0.24 | 0.24 | 0.24 | 0.24 | | | | | | | | |
| | 6 | MW093739.1_ | Tamil Nadu | 0.00 | 0.00 | 0.00 | 0.00 | 0.24 | | | | | | | |
| | 7 | PQ809994.1 | Orissa | 0.00 | 0.00 | 0.00 | 0.00 | 0.24 | 0.00 | | | | | | |
| Apis cerana | 8 | OP050121.1 | Kerala | 4.93 | 4.93 | 4.93 | 4.93 | 4.68 | 4.93 | 4.93 | | | | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 9 | KM230116.1 | Kerala | 0.00 | 0.00 | 0.00 | 0.00 | 0.24 | 0.00 | 0.00 | 4.93 | | | | |
| | 10 | PQ835611.1 | Andhra Pradesh | 0.24 | 0.24 | 0.24 | 0.24 | 0.48 | 0.24 | 0.24 | 4.68 | 0.24 | | | |
| | 11 | PQ197652.1 | Karnataka | 0.24 | 0.24 | 0.24 | 0.24 | 0.48 | 0.24 | 0.24 | 4.68 | 0.24 | 0.00 | | |
| | 12 | PQ197639.1 | Karnataka | 4.93 | 4.93 | 4.93 | 4.93 | 4.68 | 4.93 | 4.93 | 0.00 | 4.93 | 4.68 | 4.68 | |
| Apis cerana indica | 13 | OR608006.1 | Meghalaya | 5.46 | 5.46 | 5.46 | 5.46 | 5.19 | 5.46 | 5.46 | 0.48 | 5.46 | 5.19 | 5.19 | 0.48 |

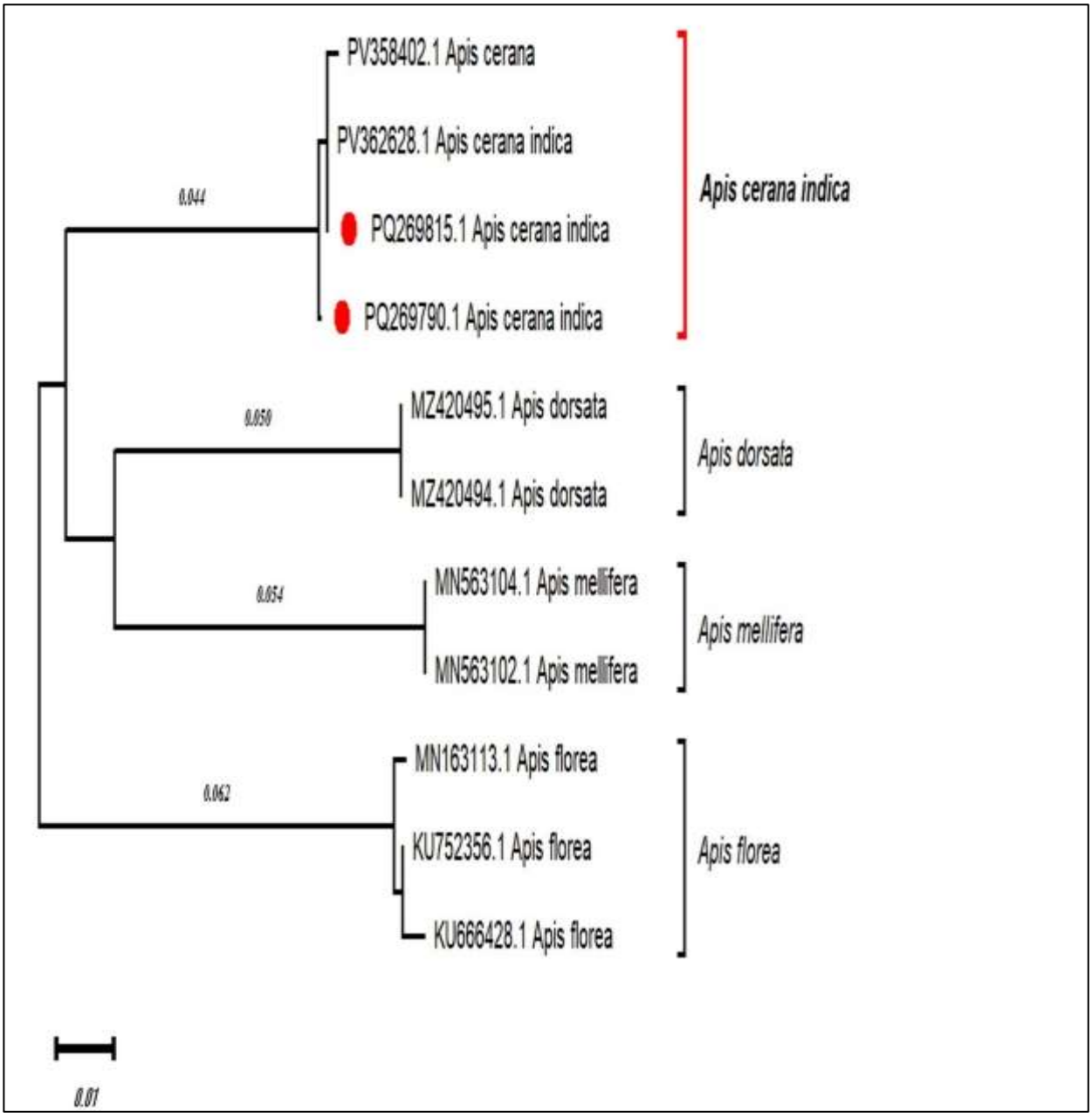


**Fig. 6 Neighbor-Joining (NJ) tree by Maximum likelihood Method**

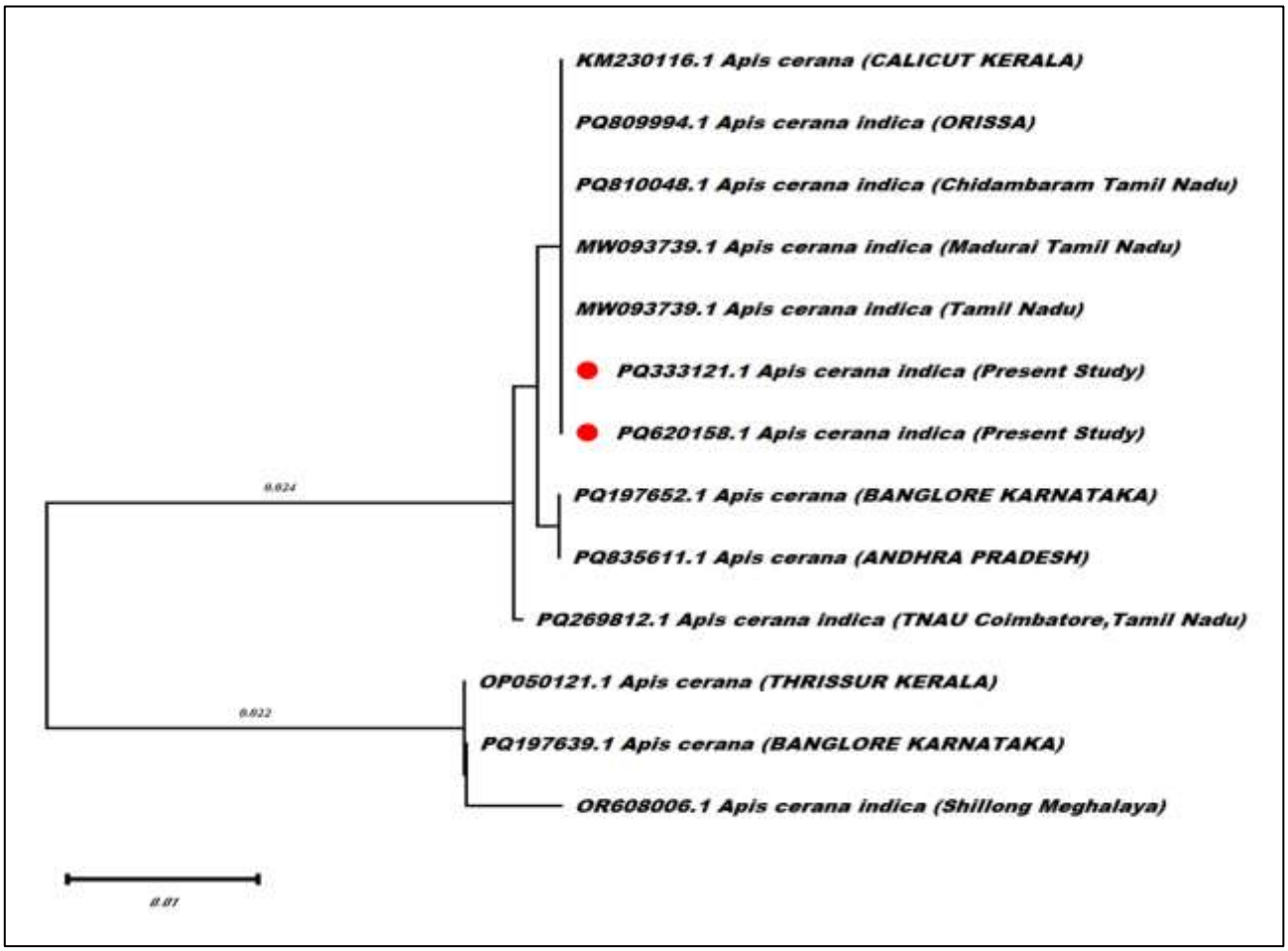


**Fig. 7 Neighbor-Joining Phylogenetic Tree of Apis cerana indica Populations Across India**

## DISCUSSION

Morphometric variation in the Asian honey bee, Apis cerana indica, has been widely documented across ecological gradients, seasons, and geographic regions in India, with its distribution spanning low-elevation plains to high mountain areas, tropical to temperate climates, and humid coastal to arid or semi-arid environments (Ji et al., 2023). Several bee taxonomists have conducted diversity analyses on this species across diverse regions based on morphological variations and prevailing environments (Radloff et al., 2010), yet the present study reveals relative uniformity in morphometric characters among colonies from Papanasam Taluk, Tamil Nadu, where morphometrics aids taxonomic classification, species description, and analysis of regional differences within populations (Hepburn et al., 2001; Shaibi et al., 2009; Rattanawannee et al., 2010). Studies comparing hill and plain populations show clear ecological influences, as Lalremliana et al. (2024) reported significant differences in Tamil Nadu, with hill bees exhibiting larger tongue length, antenna length, forewing length and width, hindleg dimensions, and cubital index adaptations likely enhancing flight efficiency and nectar extraction in high-altitude conditions with extended distances and diverse flora. Colour-based morphs also differ, with Chalapathy et al. (2024) finding black (hill-associated) morphs having longer proboscises for deep-corolla flowers and yellow (plain) morphs showing more hamuli for flight mechanics, though most traits remain similar, while broader analyses across 29 parameters reveal greater differentiation with expanded datasets (Lalremliana et al., 2024). Habitat influences morphology, with urban bees displaying larger bodies, abdomens, and forewings due to better nutrition (Bhatta & Kumar, 2022; Baltz et al., 2025), wild bees longer hindwings, and rural bees broader tibiae (Rowe et al., 2021), and uniform conditions in this study minimized such divergence; seasonal effects further contribute, as summer/autumn bees have longer forewings, radial cells, femora, and tibiae from peak nutrition (Mattu and Verma, 1984; Nicholls et al., 2021 and Shawer et al., 2021). Key adaptive traits include longer tongues and hindlegs in hills (Fernando, 1979; Kapil, 1956; Lalremliana et al., 2024), larger forewings in hills/urban areas (Hepburn et al., 2001; Bhatta & Kumar, 2022), and more hamuli in plains (Chalapathy et al., 2024). Overall, these findings support that while A. cerana indica shows considerable plasticity across gradients, lowland populations under similar conditions exhibit stable morphology.

The nucleotide composition analysis of the COI gene region in Apis cerana indica revealed an AT-biased genome (A+T = 76.5%), typical of insect mitochondrial DNA, with a transition/transversion bias (R = 1.37) under the Kimura 2-parameter model (+G+I), reflecting standard evolutionary dynamics in hymenopteran mtDNA (Kaskinova et al., 2022). According to the 10X rule, the percentage of nucleotide divergence between intraspecies should be less than 3%, while interspecies divergence should exceed 3 % (S et al., 2024). The sequence analyzed in this study showed significant inter-species variability based on nucleotide sequences and the intraspecific divergence was high enough to distinguish between individuals. The mitochondrial DNA variations provide the phylogenetic relationship of various insect groups at

a generic level. The high sequence conservation motifs underscore genetic stability within A. cerana indica, likely preserving critical functional elements for species-specific adaptation, while the 22 variable sites enable detection of subtle population-level polymorphisms (Chauhan et al., 2014; Ji et al., 2023). Interspecific divergence analyses confirm clear species boundaries, with A. cerana indica clustering tightly against A. dorsata, A. florea and A. mellifera aligning with established mtDNA thresholds for Apis taxonomy and validating COI as a reliable barcode (Hepburn et al., 2001). Intraspecific patterns reveal a highly conserved southern Indian cluster contrasting with divergent northern or eastern lineages suggesting historical gene flow in lowland plains disrupted by ecological barriers like the Western Ghats or Himalayan foothills, consistent with prior morphometric stability in uniform lowlands and the "Indian Plains cerana" morpho cluster (Hepburn et al., 2001).

## CONCLUSION

In conclusion, Apis cerana indica shows clear morphometric plasticity shaped by altitude, habitat type, and seasonal conditions, with traits such as tongue length, wing dimensions, hindleg size, and hamuli number serving as reliable indicators of ecological adaptation. Colonies from Papanasam Taluk exhibited relative uniformity, reflecting stable lowland conditions, while comparative studies highlight divergence in hill, urban, and seasonal populations. Molecular analysis further confirmed strong genetic conservation with localized haplotype differentiation, underscoring the combined importance of morphometric and molecular approaches in understanding population diversity and ecological adaptation in this pollinator species.

## DECLARATIONS

**Conflicts of Interest:** The authors declare that there are no conflicts of interest regarding the publication of this paper.

**Ethical Approval:** Ethical approval was not required for this study because it involved invertebrates (bees) and did not include human participants or vertebrate animals, in accordance with institutional and international guidelines.

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## REFERENCES

1. Aglagane, A., Tofilski, A., Er-Rguibi, O., Laghzaoui, E., Kimdil, L., Mouden, E. H. E., Fuchs, S., Oleksa, A., Aamiri, A. and Aourir, M. (2022) Geographical Variation of Honey Bee (Apis mellifera L. 1758) Populations in South-Eastern Morocco: A Geometric Morphometric Analysis. Insects, 13(3), 288. https://doi.org/10.3390/insects13030288.
2. Baltz, L. M., De Vastey, J., Gardein, H., Klaus, F., Greil, H., Paxton, R. J. and Theodorou, P. (2025) Local floral resources and edge density within the urban ecosystem promote larger and less variable body size in the great banded furrow bee, Halictus scabiosae. BMC Ecology and Evolution, 25(1), 75. https://doi.org/10.1186/s12862-025-02416-5.
3. Bhatta, V. R. and A, N. K. (2022b). Morphometric Characters of Apis cerana indica Worker Bees under Urban, Rural and Wild Habitats. Applied Ecology and Environmental Sciences/Applied Ecology and Environmental Science, 10(10), 614–621. https://doi.org/10.12691/aees-10-10-3
4. Bhumika, N. and Singh, B. Z. (2011) Six-legged soldiers: using insects as weapons of warfare. Medical Journal Armed Forces India, 67(4), 325. https://doi.org/10.1016/s0377-1237(16)30016-8.
5. Chalapathy, C. V., Sivaram, V., Seetharam, D. and Patil, S. J. (2024) Comparative morphometric studies between black and yellow strains of Indian honeybee - Apis cerana. ENTOMON, 49(4), 559–564. https://doi.org/10.33307/entomon.v49i4.1348.
6. Chauhan, R., Singh, D. and Chauhan, S. (2014). A pilot study on genetic diversity in Indian honeybees-Apis cerana. Journal of Entomology and Zoology Studies, 2(3), 196–200.
7. Clarke, D., Morley, E. and Robert, D. (2017) The bee, the flower, and the electric field: electric ecology and aerial electroreception. Journal of Comparative Physiology A, 203(9), 737–748. https://doi.org/10.1007/s00359-017-1176-6.
8. Dadgostar, S., Nozari, J. and Tahmasbi GH. (2020) Wing characters for morphological study on the honey bee (Apis mellifera L.) populations among six provinces of Iran. Arthropods, 9:129–138.
9. Dong, T., Lv, Q., Wu, L., Yang, L., Liang, Z., Miao, C., Zhang, Y., Wang, K., Niu, Q., Ji, T., & Lin, Z. (2026). Morphological and Genetic Variation of the Chinese Honey Bee (Apis cerana cerana Fabricius, 1793) in Wanyuan, Southwest China. Insects, 17(2), 189. https://doi.org/10.3390/insects17020189.
10. Duncan, E. J., Cunningham, C. B. and Dearden, P. K. (2022) Phenotypic Plasticity: What Has DNA Methylation Got to Do with It? Insects, 13(2), 110. https://doi.org/10.3390/insects13020110.
11. Duncan, E. J., Leask, M. P. and Dearden, P. K. (2020) Genome Architecture Facilitates Phenotypic Plasticity in the Honeybee (Apis mellifera). Molecular Biology and Evolution, 37(7), 1964–1978. https://doi.org/10.1093/molbev/msaa057.
12. Fernando, E.F.W. (1979). Some biometrical features of Apis cerana F. from Sri Lanka. Indian Bee Journal, 41: 5-8.
13. Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology, 3(5), 294–299.

14. Gupta, R. K. (2014) Taxonomy and Distribution of Different Honeybee Species. In: Beekeeping for Poverty Alleviation and Livelihood Security (Ed. R. K. Gupta, et al), Springer Science+Business Media, Dordrecht pp. 63–103). https://doi.org/10.1007/978-94-017-9199-1_2.
15. Hepburn, H. R., Smith, D. R., Radloff, S. E., & Otis, G. W. (2001). Infraspecific categories of Apis cerana: morphometric, allozymal and mtDNA diversity. Apidologie, 32(1), 3–23. https://doi.org/10.1051/apido:2001108.
16. Ji, C., Shi, W., Tang, J., Ji, T., Gao, J., Liu, F., Shan, J., Chen, X. and Chen, C. (2023) Morphometrical analyses revealed high diversity of the eastern honey bee (Apis cerana) in mountains and islands in China. Journal of Apicultural Research, 62(4), 647–655. https://doi.org/10.1080/00218839.2023.2205670.
17. Karthika, P., Krishna Eni, N., Vadivalagan, C., Murugan, K., Nicoletti, M. and Benelli, G. (2016) DNA barcoding and evolutionary lineage of 15 insect pests of horticultural crops in South India. Karbala International Journal of Modern Science, 2(3), 156–168. https://doi.org/10.1016/j.kijoms.2016.03.006.
18. Khalifa, S. a. M., Shetaia, A. A., Eid, N., El-Wahed, A. a. A., Abolibda, T. Z., Omri, A. E., Yu, Q., Shenashen, M. A., Hussain, H., Salem, M. F., Guo, Z., Alanazi, A. M. and El-Seedi, H. R. (2024) Green Innovation and Synthesis of Honeybee Products-Mediated Nanoparticles: Potential Approaches and wide Applications. Bioengineering, 11(8), 829. https://doi.org/10.3390/bioengineering11080829.
19. Kitnya, N., Otis, G. W., Chakravorty, J., Smith, D. R. and Brockmann, A. (2022) Apis laboriosa confirmed by morphometric and genetic analyses of giant honey bees (Hymenoptera, Apidae) from sites of sympatry in Arunachal Pradesh, North East India. Apidologie, 53(4). https://doi.org/10.1007/s13592-022-00956-z.
20. Lalremliana, J., Srinivasan, M. R., Saminathan, V. R., Chitra, N., Mohankumar, S., Sabatina, P., Suroshe, S. S. and Kumaranag, K. M. (2024). Variation in the Morphological Characters of the Hill and Plain Populations of Indian Honey Bee, Apis cerana indica (Fab.) in Tamil Nadu, India. Sociobiology, 71(4), e11298. https://doi.org/10.13102/sociobiology.v71i4.11298.
21. Maa T.C. (1953) An inquiry into the systematics of the tribus Apidini or honeybees (Hym.). Treubia 21: 525–640.
22. Mair, K. S., Irrgeher, J. and Haluza, D. (2023) Elucidating the role of honey bees as biomonitors in environmental health research. Insects, 14(11), 874. https://doi.org/10.3390/insects14110874.
23. Mattu, V. K. and Verma, L. R. (1984) Morphometric Studies on The Indian Honeybee, Apis cerana indica F. Effect of Seasonal Variations. Apidologie, 15(1), 63–74. https://doi.org/10.1051/apido:19840106.
24. Morice, B. D., Lord, W. D., Barthell, J. F., Jourdan, T. H. and Morris, T. L. (2020) Necrophagy in Honey Bees (Apis mellifera L.); A Forensic Application of Scent Foraging Behavior. Journal of the Kansas Entomological Society, 92(2), 423. https://doi.org/10.2317/0022-8567-92.2.423.
25. Nicholls, E., Rossi, M. and Niven, J. E. (2021) Larval nutrition impacts survival to adulthood, body size and the allometric scaling of metabolic rate in adult honeybees. Journal of Experimental Biology, 224(14). https://doi.org/10.1242/jeb.242393.
26. Poskevich D. (1989) A comparison of honeycomb structures built by Apis mellifera (SE82_17). In: Jackson JT, Christie NW, comps. Shuttle Student Involvement Program (SSIP) Final Reports of Experiments Flown. Houston TX: National Aeronautics and Space Administration, Lyndon B. Johnson Space Center; JSC 24005. Available from https://beeculture.com/the-mind-of-the-honey-bee/ (Accessed on 17 March,2026).
27. Radloff, S. E., Hepburn, C., Hepburn, H. R., Fuchs, S., Hadisoesilo, S., Tan, K., Engel, M. S. and Kuznetsov, V. (2010) Population structure and classification of Apis cerana. Apidologie, 41(6), 589–601. https://doi.org/10.1051/apido/2010008.
28. Rattanawannee, A., Chanchao, C. and Wongsiri, S. (2010) Gender and species identification of four native honey bees (Apidae:Apis) in Thailand based on wing morphometic analysis. Annals of the Entomological Society of America, 103(6), 965–970. https://doi.org/10.1603/an10070.
29. Renner, M. (1960) The contribution of the honey bee to the study of Time-Sense and Astronomical Orientation. Cold Spring Harbor Symposia on Quantitative Biology, 25(0), 361–367. https://doi.org/10.1101/sqb.1960.025.01.037.
30. Rizzi, A., Bianco, G. and Cassinis, R. (1998b) A bee-inspired visual homing using color images. Robotics and Autonomous Systems, 25(3–4), 159–164. https://doi.org/10.1016/s0921-8890(98)00045-1.
31. Rowe, L., Gibson, D., Landis, D. A. and Isaacs, R. (2021) Wild bees and natural enemies prefer similar flower species and respond to similar plant traits. Basic and Applied Ecology, 56, 259–269. https://doi.org/10.1016/j.baae.2021.08.009.
32. Ruttner, F. (1988) Biogeography and taxonomy of honeybees. Springer Berlin, Heidelberg,284pp. https://doi.org/10.1007/978-3-642-72649-1.
33. Ruttner, F., Tassencourt, L. and Louveaux, J. (1978) Biometrical-Statistical Analysis of The Geographic Variability of Apis Mellifera L. I. Material and Methods. Apidologie, 9(4), 363–381. https://doi.org/10.1051/apido:19780408.
34. S, Y., P, G., R, V. and R, V. (2024) Studies on Molecular Taxonomy and Morphological Characteristics of Haematobia irritans exigua (de Meijere, 1903); A New Range Extension in the Bargur Hills of Erode District in Tamil Nadu, India. Uttar Pradesh Journal of Zoology, 45(15), 394–405. https://doi.org/10.56557/upjoz/2024/v45i154256.
35. Shaibi, T., Fuchs, S. and Moritz, R. F. (2009) Morphological study of Honeybees (Apis mellifera) from Libya. Apidologie, 40(2), 97–105. https://doi.org/10.1051/apido/2008068.
36. Shawer, M. B., Taha, E. A., Mousa, K. M., Khan, K. A., Ibrahim, S., Hassan, S. and Elnabawy, E. M. (2021) Seasonal variations of colony activities linked to morphometric and glands characterizations of hybrid Carniolan honey bee (Apis mellifera carnica Pollmann) workers. Journal of King Saud University - Science, 33(6), 101543. https://doi.org/10.1016/j.jksus.2021.101543.

37. Sousa, A., Araújo, E., Gramacho, K. and Nunes, L. (2016) Bee's morphometrics and behavior in response to seasonal effects from ecoregions. Genetics and Molecular Research, 15(2). https://doi.org/10.4238/gmr.15027597.
38. Syromyatnikov, M. Y., Borodachev, A. V., Kokina, A. V. and Popov, V. N. (2018) A Molecular Method for the Identification of Honey Bee Subspecies Used by Beekeepers in Russia. Insects, 9(1), 10. https://doi.org/10.3390/insects9010010.
39. Wang, X., Zhang, X., Zhang, Z., Lang, H. and Zheng, H. (2018) Honey bee as a model organism to study gut microbiota and diseases. Drug Discovery Today Disease Models, 28, 35–42. https://doi.org/10.1016/j.ddmod.2019.08.010.
40. Zhang, X., Lu, J., Qu, X. and Chen, X. (2025) An Evaluation of Morphometric Characteristics of Honey Bee (Apis cerana) Populations in the Qinghai–Tibet Plateau in China. Life, 15(2), 255. https://doi.org/10.3390/life15020255.