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Morphometric and mitochondrial COI-based genomic variability probation in Asian honeybees, *Apis cerana* F. (Hymenoptera: Apidae)

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Honeybees support biodiversity and agricultural productivity by pollinating native plants and important crops. The present investigation examined the morphometric and mitochondrial cytochrome oxidase subunit I (*COI*) gene-based molecular variability of *Apis cerana* F. across twenty locations in Himachal Pradesh, India, varying in agro-climatic conditions. Morphometric analysis revealed significant variations in body length (11.088–12.999 mm), head length (2.785–3.120 mm), thorax length (3.198–3.972 mm), abdomen length (4.350–6.266 mm), antennal length (4.098–4.488 mm), proboscis length (5.262–5.820 mm), forewing (8.608–8.950 mm) x (2.760–3.145 mm), hindwing (6.068–6.426 mm) x (1.665–1.795 mm), and hamuli number (17.550–19.600). Such variations may suggest the impact of environmental factors and other natural influences. The mitochondrial *COI* gene analysis revealed genetic similarity between the Himachal Pradesh population and its neighbouring Chinese *A. cerana*. Phylogenetic analysis delineated two distinct clades, reflecting population structure influenced by environmental and genetic factors. This combined approach allows for cross-species comparisons, shedding light on the evolutionary relationships between *A. cerana* and other native bees.

Keywords: *mtCOI*, Genetic variability, North Western Himalaya

Asian honey bee, *Apis cerana* F., a vital pollinator of natural and agricultural ecosystems, thrives in temperate and tropical Asia. Geographical and seasonal factors are known to significantly influence morphological and molecular alterations among regional populations[1-3]. Morphometric traits like body size, wing dimensions, and proboscis length exhibit adaptations to local environments, impacting foraging efficiency and resilience[4-9]. These morphological variations have led to the designation of multiple subspecies within *A. cerana* taxon, thereby playing a significant role in the taxonomic categorization of populations across varied geographically distinct locations[10-17].

Molecular characterization has become an extensively acknowledged approach for referencing regional diversity in a less subjective manner[18-21]. In insect molecular characterization, particularly mitochondrial genes like cytochrome oxidase I (mtCOI) are of particular interest due to maternal inheritance that provides precise insights into genetic diversity and phylogenetic relationships[22,23]. DNA extraction from various *Apis* species has been performed all over the world, such as stingless bees in Brazil[24,25]. In India, CTAB (cetyltrimethyl ammonium bromide) and, Waldschmidt method were followed during a study to check purity as well as differentiation in DNA structure taken from different body parts of *A. cerana*[26]. The study integrates morphometric and molecular approaches to investigate the population structure of *A. cerana* in Himachal Pradesh, contributing to conservation strategies and sustainable beekeeping practices.

Materials and methods

Sample collection and storage

Apis cerana worker bees foraging outside the hives were captured using sweep nets from twenty locations in Himachal Pradesh (31˚6′12′′N 77˚10′20′′E) during the summer of 2019. The selected apiaries were distinguished by their diverse altitude, latitude, and climate (Fig. 1). About 50 worker bees from 10 colonies per each location were collected and anesthetized with 5 percent formalin in a killing bottle. These bees were preserved in 70 percent

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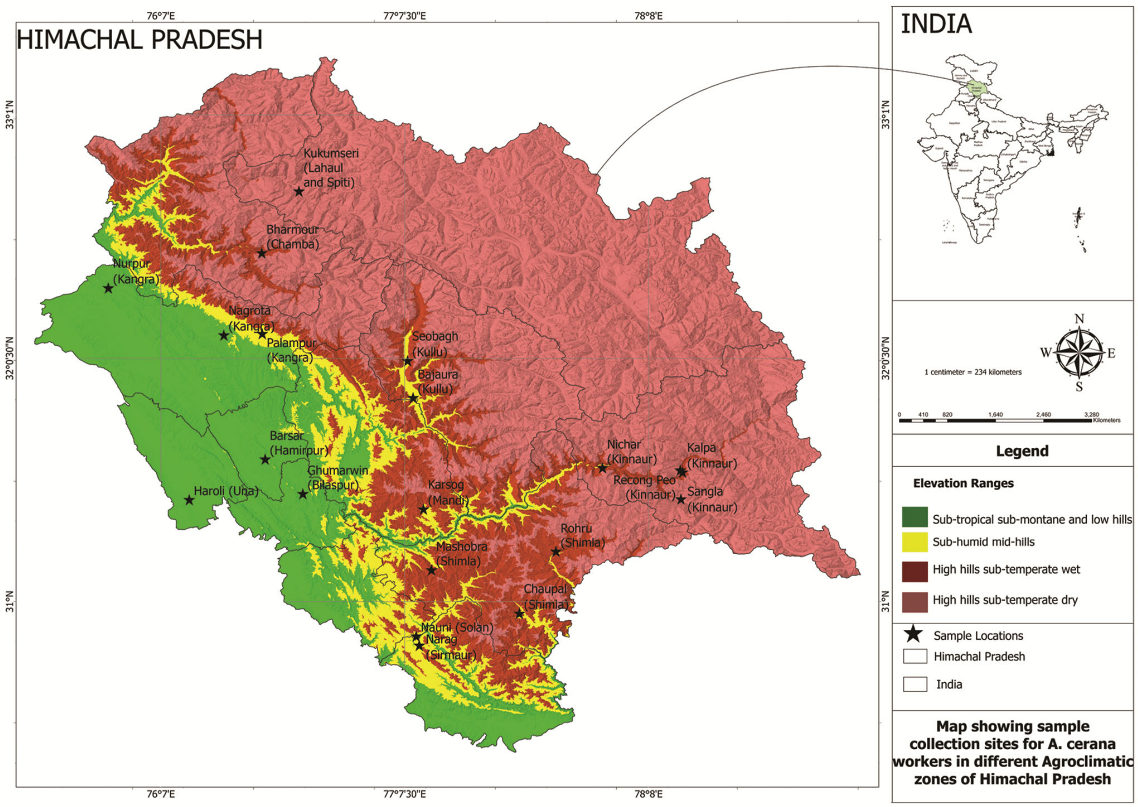

Fig. 1 — Map showing sample collection sites for *A. cerana* workers in Himachal Pradesh, India prepared using Arc Map 10.8. [*not for legal purposes]

ethyl alcohol and stored in well-labelled jars in a laboratory refrigerator at 4ºC in containing for further analysis.

Molecular analysis

Isolation of total genomic DNA

Genomic DNA of each sampled population was extracted from internal tissues except the midgut using Qiagen DNeasy® Blood & Tissue kit (Catalogue number: 69504, a product of Germany). Electrophoresis was performed at 90 V for 1- h, and sharp bands were seen under the gel documentation system, representing the presence of good quality DNA. The Thermo Scientific NanoDrop™ 2000c spectrophotometer was used for purity and concentration evaluation of extracted DNA. The DNA samples were treated with RNase to remove the RNA contamination.

COI gene amplification

The conserved mitochondrial COI region was amplified using universal primers LCO1490 (Forward, 5′-GGTCAACAAATCATAAAGATATT GG-3′) and HCO2198 (Reverse, 5′- TAAACTTCA GGGTGACCAAAAAATCA-3′) in a thermocycler using a 25 µL reaction volume. The reaction mixture contained Ex-Taq (5 units/µL) (0.125 µL), 10× Taq polymerase buffer (2.50 µL), dNTPs mixture (2 µL), a pair of primers (1 µL each), and distilled water (17.375 µL). For the final reaction volume, 24 µL of reaction mixture and 1 µL of genomic DNA were added to sterilized PCR tubes. The PCR temperature profile consisted of an initial denaturation step of 4 min at 94 ºC followed by 35 cycles of 30 s at 94 ºC (Denaturation), 45 s at 50 ºC (Annealing), 1 min at 72 ºC (Elongation), and ending with a final extension of 72 ºC for 7 min. The separated PCR products after agarose gel electrophoresis were visualized under UV light exposure in the gel documentation system (ZENITH). The DNA fragments excised from an agarose gel were eluted and purified by using the Thermo Scientific GeneJET Gel Extraction kit.

Gene sequencing

The sequencing of the *COI* gene was performed in a 3500 Genetic Analyzer (Applied Biosystems, HITACHI). The Sanger dideoxy method was used for

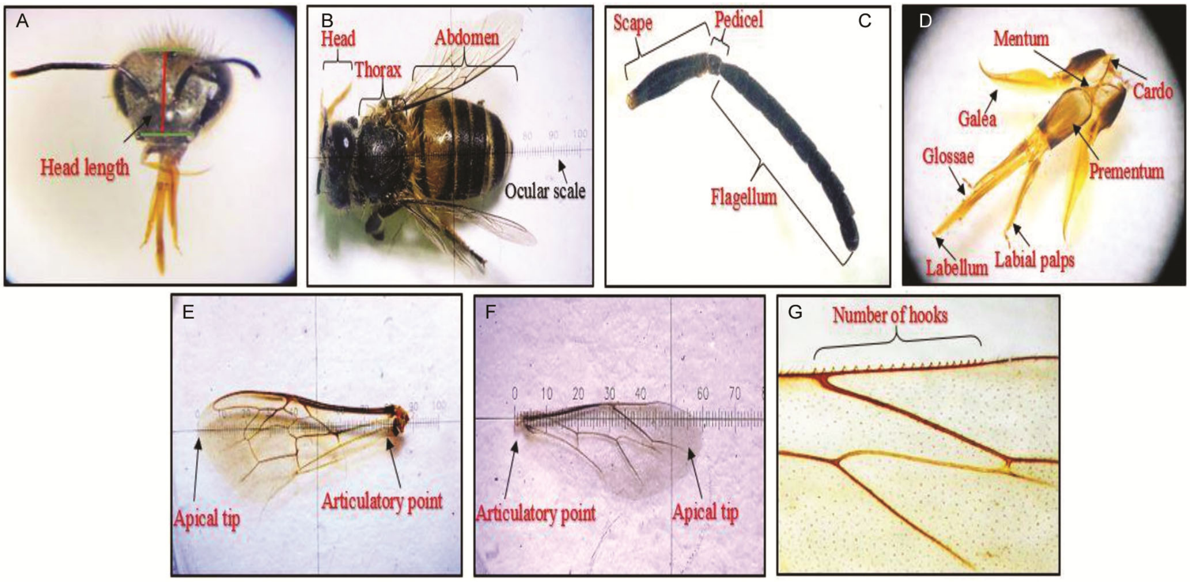

Fig. 2 — Morphological parts of *A. cerana* workers: (A) Head length, (B) Body length, (C) Antennal length, (D) Proboscis length, (E) Forewing length and width, (F) Hindwing length and width, (G) Number of hamuli.

DNA sequencing[27]. The sequencing cycle PCR reaction was performed in a thermocycler (GeneAmp PCR System 9700) using template DNA (4µL), a specific primer (forward) (4 µL), and BigDye™ Terminator v3.1 Ready Reaction Mix (6 µL) (Applied Biosystems, Lithuania). The temperature profile for sequence cycle PCR consisted of an initial denaturation step of 1 min at 96 ºC, followed by 25 cycles of 10 s at 96 ºC (Denaturation), 25 s at 52 ºC (Annealing), 4 min at 60 ºC (Elongation), and ending with a final extension at 4 ºC.

Submission of COI gene sequence to NCBI (National Centre for Biotechnology Information)

The aligned nucleotide sequences of around 600-700 bp were submitted to NCBI along with all necessary details, and accession numbers were received from NCBI for all the sequences within 2-3 days.

Phylogenetic analysis

The nucleotide sequences of *A. cerana* workers were aligned with the reference of NCBI using the BLAST tool. To trace the evolutionary patterns of sampled populations with other selected sequences at NCBI, a phylogenetic tree was constructed using the Neighbour-Joining (NJ) method in the Mega X programme[28]. The bootstrap of each branch of the phylogenetic tree was subjected to n=1000 replications. The genetic distance between the COI region of present populations was computed using the Maximum Composite Likelihood Method through the Mega X software. The Shannon Diversity Index (H′) was calculated by using DnaSP (DNA Sequence Polymorphism) software.

Morphometric analysis

The samples preserved in alcohol (i.e., twenty *A. cerana* worker bees) from each location were carefully dissected and mounted on the glass slides. Key morphological traits, including body, head, thorax, abdomen, antennae, proboscis, and wings, were measured using a stereo zoom microscope (SZ61, Olympus) with an ocular micrometer calibrated with a stage micrometer at 1× magnification (Fig. 2).

Statistical analysis

The recorded phenotypic data were analyzed statistically at 0.05 percent level of significance using Microsoft Excel and SPSS 22 statistical package (Duncan′s multiple range test).

Results

Morphometric analysis

The location-wise comparative measurements of different morphological parts viz., body length, head length, thorax length, abdomen length, antennal length, proboscis length, forewing size (length and width), hindwing size (length and width) and hamuli number of *A. cerana* are presented in Table 1.

Table 1 — Variations in different morphological characters of *Apis cerana* from different locations in Himachal Pradesh

Zones	Locations	BL (mm)	HL (mm)	ThL (mm)	AbL (mm)	AL (mm)	PL (mm)	FWL (mm)	FWW (mm)	HWL (mm)	HWW (mm)	HN (No.)
Sub-Tropical Sub Mountain and Low Hills	Haroli (Una)	11.314^{f} ± 0.067	2.838^{f} ± 0.002	3.246^{l} ± 0.008	5.230^{h} ± 0.018	4.230^{fg} ± 0.016	5.420^{ef} ± 0.029	8.786^{def} ± 0.045	2.840^{e} ± 0.007	6.178^{ef} ± 0.014	1.690^{i} ± 0.009	18.356^{e} ± 0.092
	Ghumarwin (Bilaspur)	11.272^{f} ± 0.039	2.785^{f} ± 0.013	3.310^{k} ± 0.029	5.177^{hi} ± 0.015	4.285^{e} ± 0.023	5.382^{f} ± 0.037	8.744^{efg} ± 0.066	2.854^{e} ± 0.017	6.204^{e} ± 0.023	1.708^{h} ± 0.003	18.460^{e} ± 0.125
	Nurpur (Kangra)	11.306^{f} ± 0.032	2.835^{f} ± 0.005	3.242^{l} ± 0.006	5.223^{h} ± 0.008	4.265^{ef} ± 0.010	5.425^{ef} ± 0.011	8.830^{bcde} ± 0.014	2.905^{cd} ± 0.015	6.126^{fg} ± 0.005	1.758^{de} ± 0.010	18.550^{de} ± 0.150
	Nagrota (Kangra)	11.360^{f} ± 0.032	2.802^{f} ± 0.008	3.198^{l} ± 0.010	5.360^{g} ± 0.023	4.306^{de} ± 0.020	5.458^{e} ± 0.015	8.608^{h} ± 0.018	2.868^{de} ± 0.007	6.048^{h} ± 0.012	1.692^{i} ± 0.004	18.654^{cde} ± 0.096
	Barsar (Hamirpur)	11.327^{f} ± 0.077	2.826^{f} ± 0.016	3.402^{i} ± 0.005	5.228^{h} ± 0.013	4.266^{ef} ± 0.014	5.402^{ef} ± 0.006	8.728^{fg} ± 0.033	2.886^{cde} ± 0.006	6.206^{e} ± 0.013	1.728^{g} ± 0.008	18.506^{de} ± 0.037
Sub-Humid Mid Hills	Bajaura (Kullu)	12.360^{c} ± 0.006	2.908^{e} ± 0.002	3.972^{a} ± 0.002	5.483^{f} ± 0.002	4.345^{cd} ± 0.006	5.386^{f} ± 0.017	8.765^{efg} ± 0.013	2.925^{c} ± 0.013	6.426^{a} ± 0.003	1.783^{abc} ± 0.010	19.250^{ab} ± 0.189
	Nauni (Solan)	11.088^{g} ± 0.007	2.815^{f} ± 0.003	3.928^{ab} ± 0.002	4.350^{j} ± 0.004	4.168^{h} ± 0.003	5.262^{g} ± 0.003	8.625^{h} ± 0.005	2.760^{f} ± 0.006	6.354^{bcd} ± 0.004	1.665^{j} ± 0.009	18.350^{e} ± 0.171
	Narag (Sirmaur)	11.393^{f} ± 0.010	2.803^{f} ± 0.002	3.458^{h} ± 0.005	5.133^{i} ± 0.005	4.183^{gh} ± 0.004	5.626^{d} ± 0.009	8.680^{gh} ± 0.006	2.840^{e} ± 0.013	6.068^{gh} ± 0.015	1.745^{ef} ± 0.014	18.900^{bcd} ± 0.238
	Seobagh (Kullu)	12.974^{a} ± 0.022	3.060^{bc} ± 0.008	3.950^{a} ± 0.010	5.960^{de} ± 0.017	4.388^{bc} ± 0.000	5.710^{bc} ± 0.010	8.815^{cdef} ± 0.013	2.970^{b} ± 0.014	6.412^{ab} ± 0.007	1.730^{fg} ± 0.017	19.600^{a} ± 0.000
	Palampur (Kangra)	11.576^{e} ± 0.007	2.835^{f} ± 0.003	3.356^{j} ± 0.000	5.383^{g} ± 0.008	4.270^{ef} ± 0.002	5.623^{d} ± 0.007	8.865^{abcd} ± 0.005	2.985^{b} ± 0.021	6.132^{fg} ± 0.000	1.730^{fg} ± 0.015	17.550^{f} ± 0.250
High Hill Temperate Wet Zone	Karsog (Mandi)	12.909^{ab} ± 0.017	2.996^{d} ± 0.010	3.942^{a} ± 0.008	5.971^{de} ± 0.017	4.488^{a} ± 0.026	5.602^{d} ± 0.018	8.938^{a} ± 0.031	3.138^{a} ± 0.011	6.328^{cd} ± 0.019	1.768^{cd} ± 0.005	18.680^{cde} ± 0.074
	Rohru (Shimla)	12.892^{ab} ± 0.120	3.010^{d} ± 0.008	3.948^{a} ± 0.006	5.934^{e} ± 0.040	4.398^{b} ± 0.002	5.710^{bc} ± 0.035	8.942^{a} ± 0.027	2.998^{b} ± 0.009	6.346^{bcd} ± 0.022	1.782^{abc} ± 0.011	18.902^{bcd} ± 0.109
	Mashobra (Shimla)	12.999^{a} ± 0.006	3.003^{d} ± 0.005	3.943^{a} ± 0.002	6.058^{c} ± 0.011	4.187^{gh} ± 0.005	5.727^{bc} ± 0.003	8.950^{a} ± 0.008	3.145^{a} ± 0.013	6.358^{abcd} ± 0.007	1.795^{a} ± 0.005	19.150^{b} ± 0.050
	Bharmour (Chamba)	12.104^{d} ± 0.084	3.074^{ab} ± 0.013	3.832^{de} ± 0.014	5.390^{g} ± 0.021	4.286^{e} ± 0.015	5.606^{d} ± 0.029	8.898^{abc} ± 0.021	3.012^{b} ± 0.013	6.288^{d} ± 0.020	1.726^{g} ± 0.008	19.110^{b} ± 0.042
	Chaupal (Shimla)	12.096^{d} ± 0.063	2.988^{d} ± 0.012	3.892^{bc} ± 0.014	6.016^{cd} ± 0.044	4.298^{de} ± 0.020	5.702^{c} ± 0.039	8.910^{abc} ± 0.050	3.126^{a} ± 0.015	6.336^{cd} ± 0.036	1.774^{bc} ± 0.005	18.998^{bc} ± 0.056
Dry Temperate High Hills	Nichar (Kinnaur)	12.902^{ab} ± 0.046	3.102^{ab} ± 0.006	3.868^{cd} ± 0.009	5.932^{e} ± 0.036	4.192^{gh} ± 0.022	5.802^{a} ± 0.036	8.916^{abc} ± 0.041	3.134^{a} ± 0.017	6.304^{cd} ± 0.053	1.708^{h} ± 0.005	19.010^{bc} ± 0.115
	Kalpa (Kinnaur)	12.846^{ab} ± 0.060	3.018^{cd} ± 0.017	3.662^{f} ± 0.015	6.266^{a} ± 0.043	4.108^{i} ± 0.011	5.798^{a} ± 0.021	8.950^{a} ± 0.023	3.142^{a} ± 0.011	6.310^{cd} ± 0.036	1.788^{ab} ± 0.008	19.002^{bc} ± 0.111
	Recong Peo (Kinnaur)	12.766^{b} ± 0.052	3.002^{d} ± 0.008	3.620^{fg} ± 0.010	6.144^{b} ± 0.026	4.204^{gh} ± 0.024	5.776^{ab} ± 0.005	8.868^{abcd} ± 0.015	2.988^{b} ± 0.012	5.996^{h} ± 0.006	1.708^{h} ± 0.009	18.908^{bcd} ± 0.051
	Sangla (Kinnaur)	12.098^{d} ± 0.066	3.024^{cd} ± 0.009	3.590^{g} ± 0.021	5.484^{f} ± 0.027	4.110^{i} ± 0.018	5.820^{a} ± 0.035	8.932^{ab} ± 0.013	3.002^{b} ± 0.014	6.372^{abc} ± 0.015	1.734^{fg} ± 0.012	19.068^{bc} ± 0.120
	Kukumseri (Lahaul and Spiti)	12.912^{ab} ± 0.046	3.120^{a} ± 0.006	3.802^{e} ± 0.035	5.990^{de} ± 0.018	4.098^{i} ± 0.024	5.698^{c} ± 0.016	8.902^{abc} ± 0.074	3.014^{b} ± 0.017	6.008^{h} ± 0.021	1.781^{abc} ± 0.009	19.152^{b} ± 0.085
C.D.		0.148	0.026	0.039	0.067	0.045	0.064	0.092	0.037	0.059	0.027	0.356

[*The superscript letters are used to indicate that means for the same characteristics followed by different letters among locations are significantly different (P<0.05) according to one-way analysis followed by Duncan′s multiple range tests, where BL: Body length; HL: Head length; ThL: Thorax length; AbL: Abdomen length; AL: Antennal length; PL: Proboscis length; FWL: Forewing length; FWW: Forewing width; HWL: Hindwing length; HWW: Hindwing width; HN: Hamuli numbers]

Molecular analysis

Phylogenetic analysis

Phylogenetic trees of *A. cerana* from twenty different sites revealed two major clades (Fig. 3):

Clade I: Populations from Recong Peo (OR123215), Nichar (OR123214), Kalpa (OR123213), Sangla (OR123212), Kukumseri (OR122464), Narag (MT027921), Mashobra (MT027916), Rohru (OR123209), Karsog (OR123208), Nauni (MT027919), Haroli (OR123204), Barsar (OR123206), Nurpur (MT027905), Nagrota (OR123205), Ghumarwin (OR123207), Chaupal (OR123210), and Bharmour (OR123211) were clubbed in clade I.

Clade II: Populations from Seobagh (MT027917), Palampur (MT027920), and Bajaura (MT027915) were clustered together in clade II due to their proximity. The pairwise genetic differentiation (F_{ST}) between the twenty tested populations of *A. cerana* varied from 0.000 to 0.009. The Shannon Diversity Index (H′ ≈ 1.95) reflects considerable haplotype diversity, highlighting a robust level of genetic variability among the sampled *A. cerana* populations.

Cluster analysis with reference sequences retrieved from NCBI

The *mtCOI* gene-based cluster analysis revealed five different clades (Fig. 4). The twenty identified

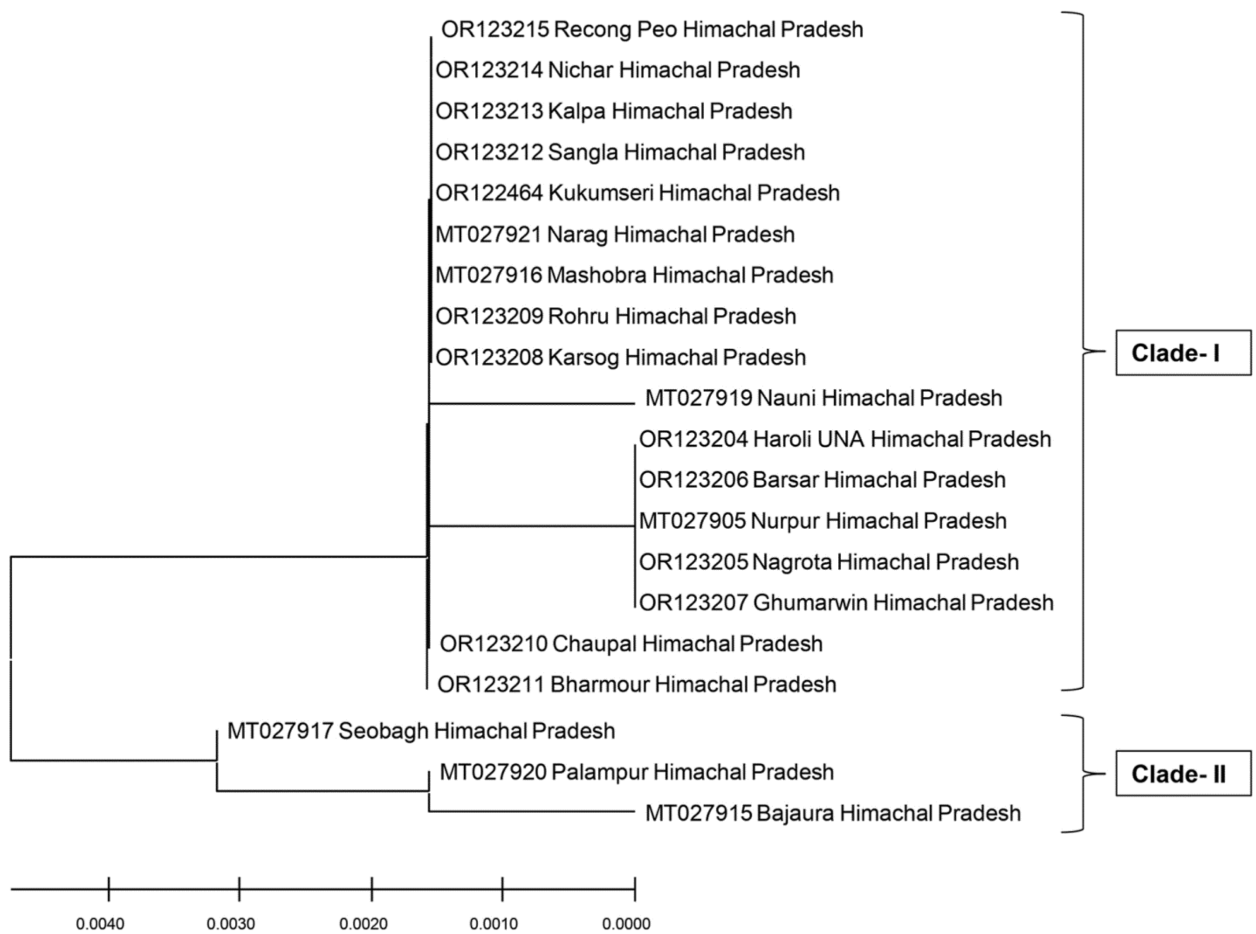

Fig. 3 — Phylogenetic analysis of *COI* gene sequences of *A. cerana* collected from different sites in Himachal Pradesh.

samples of *A. cerana* and the other 23 closely related sequences based on percent NCBI identification were constructed into a phylogenetic tree. Two groups, *viz.,* East-Asian isolates from China and North Indian clade representing populations of the present study, are monophyletic groups sharing common ancestry. Analysis implied that *A. cerana* populations from Himachal Pradesh (North Indian Clade) were genetically closest to the Chinese *A. cerana* population, suggesting shared ancestry.

Discussion

In terms of the biological differences that lead to intraspecific variation, many taxonomic studies have demonstrated the importance of strong isolation mechanisms, based on selection or genetic drift. The species exhibits a significant increase in the length of the body, head, thorax, and abdomen size as the altitude of the region rises in comparison to low and mid hills. This suggests that the species has successfully adapted to the challenging conditions of high-altitude regions over several years, making it native to and thriving in such environments. The length of the proboscis in these extreme areas highlights the species' ability to utilize the limited flora found in cold regions. Larger body size and wing dimensions at higher altitudes enhance flight efficiency and foraging capacity, leading to their compulsion to forage over larger areas in search of suitable habitats and food sources, particularly while migrating for resources during harsh winters. This extensive travel necessitates the development of stronger veins and larger wing muscles. Smaller dimensions observed in subtropical regions are consistent with adaptations to relatively stable and resource-rich environments.

Findings from previous researchers[9,12,29-32] on morphological data of *A. cerana* coincided with the variations recorded in different samples of our study. Mitochondrial DNA studies offer insights into genetic variation, whereas morphometric methods have been

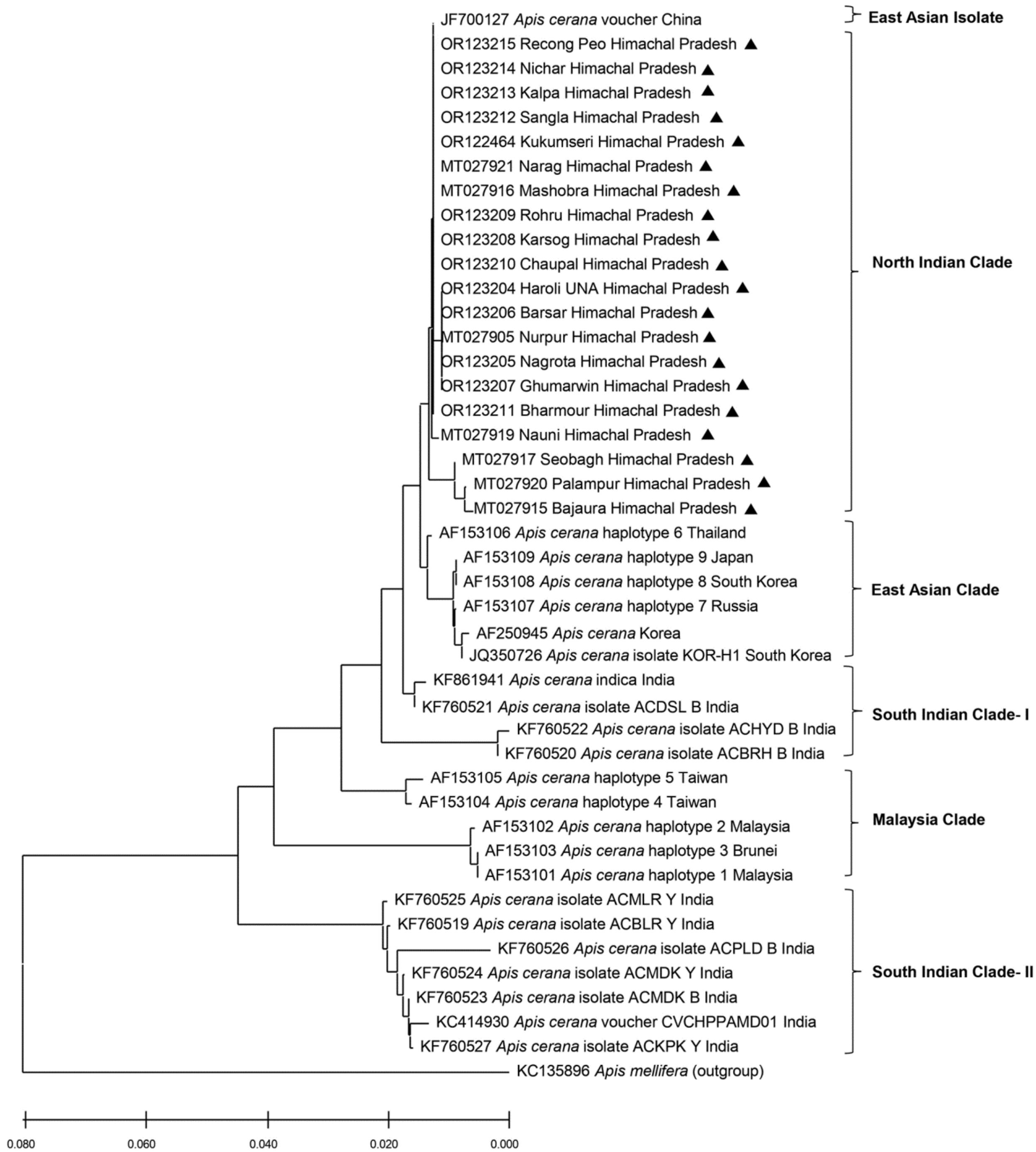

Fig. 4 — Clustering analysis of *A. cerana* based on *mtCOI* gene using Mega X programme. (where *A. mellifera* has been used as an outgroup)

applied to better understand morphological differences among *A. cerana* populations. The diversity of this species is influenced by geographical and climatic conditions, underscoring the need for regional conservation measures to sustain the genetic resources of this important pollinator. It has been observed that several factors other than altitudes, such as subspecies, climate, collection season, bee age, rainfall, and temperature, contribute to morphometric variations within the *A. cerana* population. Radloff *et al.*[33]

identified a morphocluster I named "Northern *cerana*", which spans from northern Afghanistan and Pakistan to northwest India, southern Tibet, northern Myanmar, China, and north easterly into Korea, far eastern Russia, and Japan[33]. Within this morphocluster, six subclusters or populations can be discerned morphometrically (a) an "Indus" group in Afganistan, Pakistan and Kashmir; (b) a "Himachali" group in Himachal Pradesh, India; (c) an "Aba" group in southern Ganshu and central and northern China Sichuan provinces in China, northern China and Russia (larger bees); (d) a subcluster in central and eastern China; (e) a "southern" *cerana* subcluster in southern Yunnan, Guangdong, Guangxi and Hainan in China; and (f) a "japonica" group in Japan and Korea.Morphocluster I bees have been previously named as follows: *A. skorikovo, A. c. abansis, A. c. abanensis, A. c. bijjieca, A. c. cathayca, A. c. cerana, A. c. fantsum, A. c. hainana, A. c. hainanensis, A. c. heimifeng, A c. indica, A. c. japonica, A. c. javana, A. c. kweiyanga, A. c. maerkang, A. c. pekinga, A. c. peroni, A. c. skorikovi, A. c. shankianga and A. c. twolareca.* Ji *et al* collected 140 *A. cerana* colonies from 9 places on the Qinghai-Tibet Plateau, 3 on islands, and 5 on plains, and utilized conventional and geometric morphometrics to estimate diversity. Their findings demonstrated greater variation in Qinghai-Tibet Plateau and island populations than in reference populations from plain regions. Morphometric features (hair length, wing size, colour) correlated with altitude, latitude, and longitude, mostly associated with foraging adaptations, cold tolerance, and survival in a variety of regions. Traits likely relate to adaptations for foraging, cold tolerance, and survival in diverse climates[34]. Zhang *et al.*[35] examined 40 morphometric parameters on China's Qinghai-Tibet Plateau. Bees with longer hind legs and a broader abdomen were demonstrated to capture pollen and produce honey more efficiently. Distant populations collected together, most likely due to physical barriers (mountains, rivers) that influenced gene flow more than distance[35].

The genetic distance across the studied populations revealed a significant amount of genetic diversity across all the samples examined. In a previous study by Chalapathy *et al.*, the genetic distance between different sequences of mitochondrial *COI* genes was explored, leading to the discovery of notable genetic heterogeneity among Indian honey bee groups[36]. The findings suggest that the observed genetic variation could potentially stem from divergence in the genes responsible for the adaptation to various factors like climate, genetic recombination, geographical isolation, mutation, or human-aided migratory beekeeping[37,38]. Another research effort analyzed the complete mitochondrial genome of *A. cerana* from Borneo. The findings indicated higher genetic diversity in Bornean populations compared to mainland Asian populations, emphasizing the region's role as a genetic reservoir for the species[39]. Shinmura *et al.*[40] determined the complete mitochondrial genome of the Taiwanese honeybee, providing insights into its genetic distinction. The mitochondrial genome's structure and organization were found to be consistent with other *Apis* species, but specific genetic markers highlighted its uniqueness[40]. *A. cerana* populations from far East, South Korea and Vietnam analyzed using seven microsatellite loci and mitochondrial *tRNAleu-COII* sequences identified 14 haplotypes, with "Japan1" being the predominant haplotype. Microsatellite data revealed two distinct clusters: one comprising samples from Vietnam (tropical climate) and another combining samples from South Korea and the Russian Far East (temperate climate)[41]. In six Sumatran provinces, an investigation was conducted at various altitudes utilizing mitochondrial genes (*Cox1* and *Cox2*) and intergenic spacers. It was discovered that specific haplotypes were more common in the lowlands, whereas intra-colony variation was greater in the highlands[42].

The current findings imply that *A. cerana* populations found in diverse agro-climatic loci of Himachal Pradesh in Northern Indian clade are genetically closer to the Chinese *A. cerana* population (JF700127, obtained from NCBI). These findings align with previous studies indicating gene flow between geographically proximate populations due to historical connectivity and ecological similarities. Befitting the present study, Yu *et al.,* suggested that the obstruction of gene flow among different populations of the valley might be a consequence of selection and genetic drift caused by long-term population isolation[43]. These genetic differences might be responsible for variations in traits such as behaviour, reproduction, and resistance to diseases. The integration of morphological and molecular data offers valuable insights into population structure and evolutionary history, supporting conservation and management of this crucial pollinator species. Therefore, it is crucial to gain a comprehensive understanding of the genetic factors behind these variations in order to develop and implement effective conservation and management strategies for Indian honey bee populations.

Conclusion

This investigation marks the initial endeavour in Himachal Pradesh to elucidate the phylogenetic tree of *A. cerana* based on morphometric analysis and examination of the conserved COI mitochondrial region. Traits like proboscis length and wing size directly influence foraging strategies, while genetic variations provide the raw material for adaptation to changing environments. This dual approach highlights the evolutionary significance of *A. cerana* in maintaining ecological balance and supporting agricultural productivity in diverse habitats. The development of two discrete clades within the twenty test populations as a result of observed variations in genetic structures could be attributed to the resilience and environmental adaptability of *A. cerana*. Understanding genetic diversity and phenotypic adaptations can guide strategies to protect and manage *A. cerana* and other indigenous bees, ensuring the preservation of their ecological roles. This could be achieved by the involvement of government bodies in formulating and enforcing strict laws to protect the habitat from harmful agricultural practices, regulating pesticide use, promoting organic and sustainable farming, funding innovative research, and engaging the community in pollinator conservation programs.

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Conflict of interest

The authors declare that they have no known competing financial interests.

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