

Morphometric Characterization of *Apis mellifera* (Italian Honey Bee) Across Different agroclimatic zones of Kumaun Region of Uttarakhand

Abstract

This study investigated the morphometry of *Apis mellifera* across three diverse locations in Uttarakhand, spanning the Tarai irrigated (Pantnagar), Bhabar Irrigated (Nainital) and High hills (Almora), to explore regional adaptations and environmental influences. Significant regional differences were observed, with populations from high hills exhibiting larger dimensions, whereas populations from Tarai irrigated showed smaller size. The morphological characteristics of *Apis mellifera* worker bees were analyzed. A total of 13 morphological characters were measured using a stereo zoom microscope in combination with VUE software. The results suggest that morphometric variations can potentially distinguish intraspecific groups of honeybee colonies, identifying them as distinct ecotypes. Morphometric analysis of wings and body parts provides valuable insights into honeybee biodiversity studies. To facilitate species identification based on morphometric traits, *Apis mellifera* samples were collected. 40 worker bee samples were analyzed, and 13 morphometric characteristics were measured in the entomology laboratory at G.B. Pant University of Agriculture and Technology (GBPUA&T). The biometric data were analyzed by calculating the mean, range, standard error, and coefficient of variation.

1. INTRODUCTION

Honey bees are social insects that generate vital compounds such as honey, royal jelly, bee wax, propolis, pollen, and bee venom (Siraj et al., 2022). Beekeepers worldwide select bee traits that enhance and support production (Litvinoff et al., 2023). Honey bees are of immense economic importance and play a crucial role in the pollination of fruit crops, vegetables, and wild plants. India has a varied honey bee fauna, including significant *Apis* species such as *Apis dorsata* Fab., *A. florea* Fab., *A. cerana* Fab., and the well-known exotic *A. mellifera* L. The initial two species are still not hived, whilst the latter two species are hived, therefore used for beekeeping. Honey bee species vary in phenotypic characteristics between climates, and several ecological races are suspected. Previously, the classification of geographical races was only based on the colour and size of honey bees. Alpatov (1929) introduced biometrics to properly define honey bee races. Ruttner et al. (1978) provided a comprehensive discussion of honey bee

morphometric research. Following that, these studies were carried out all over the world on various features to account for differences between species, but precise region-specific characterizations are still required for appropriate identification and suitability. Furthermore, Kekecoglu et al., (2023) emphasized the importance of morphometric research in providing information on the structure, available races, regional and genetic variety, as well as establishing the role and importance of any organism's biometrical characteristics. . In Uttarakhand, apiculture has emerged as a profitable venture. Uttarakhand, an Indian state, is located in the northwestern Himalaya and has a diversified climate that ranges from semi-tropical in the lower hills to semi-arctic in the frigid desert parts. It is situated between Latitude 30°22'40" N to 33°12'20" N and Longitude 75° 45'55" E to 79°04'20" E, with elevations ranging from 350 to 6975 meters above sea level(masl). The current study was designed to investigate the current morphometric state of *A. mellifera*. To assess the current morphometric status of *Apis mellifera* in the Kumaun region of Uttarakhand, the present study was undertaken. To date, no study has provided a comprehensive morphometric characterization of *Apis mellifera* populations across the diverse altitudes of Kashmir. The primary objective of this research was to investigate variations in the morphological traits of *Apis mellifera* across different agroclimatic zones in the region.

2. MATERIAL AND METHODS

The morphometric studies on *A. mellifera* was carried out at IBDC Laboratory, Department of Entomology, College of Agriculture, Pantnagar, Uttarakhand in 2023. The materials used and methods employed are as follows.

2.1 Collection of *Apis mellifera* species from different altitudes

Worker bees of *A. mellifera* were gathered from natural hives and apiaries in three locations representing distinct agro-climatic zones in Uttarakhand including Tarai irrigated (Pantnagar), Bhabar Irrigated (Nainital) and High hills (Almora), (**Table 1**). At each location, 40 worker bees were collected. The captured bees were placed in a vial containing 70 per cent alcohol and preserved in deep freezer for long time storage at -20°C. This study have facilitated the study of phenotypic traits and the diversification of honey bee populations, revealing variations in body dimensions associated with latitude. Morphometry enables the discrimination of bee populations through precise numerical data obtained from measurements of

morphological traits. These measurements are used to derive colony character means, which are then subjected to statistical analyses (Irshad et al., 2022).

2.2 Methodology for Measuring Morphological Traits of *Apis mellifera* L

Forager bees were captured using a net during the daytime in locations where colonies were challenging to locate. Bees were directly collected from colonies in the evening at locations where hives were easily accessible. Bees were stored in 70% ethanol. Thirteen morphological parts of *Apis mellifera* L. were dissected and mounted on glass slides with cover slips for analysis. A stereo zoom microscope equipped with VUE software, calibrated using a micrometer lens, was employed for precise measurements. Key structures, including the head, thorax, abdomen, hindwings, forewings, forelegs, midlegs, and hindlegs, were measured using a measuring scale (mm).

Table 1: Collection of *Apis mellifera* from different altitudes of Uttarakhand

Districts	Agro climatic zones	latitude	longitude	Altitude	Date of collection	Species collected
Pantnagar	Tarai irrigated	29.0222°N	79.4908° E	244 masl	8 August 2023	<i>Apis mellifera</i>
Nainital	Bhabar Irrigated	29.3924° N	79.4534° E	2,084 masl	21 April 2024	<i>Apis mellifera</i>
Almora	High hills	29.5892° N	79.6467° E	2116masl	16 July 2024	<i>Apis mellifera</i>

2.3 Statistical Analysis

The morphometric variation of *Apis mellifera* populations across Uttarakhand was analyzed using Principal Component Analysis (fig. 1). These statistical methods were employed to identify patterns and groupings within the dataset, elucidating variations and similarities among populations from diverse geographic and ecological regions. PCA was utilized to reduce the dimensionality of the dataset while retaining the most significant traits contributing to variability.

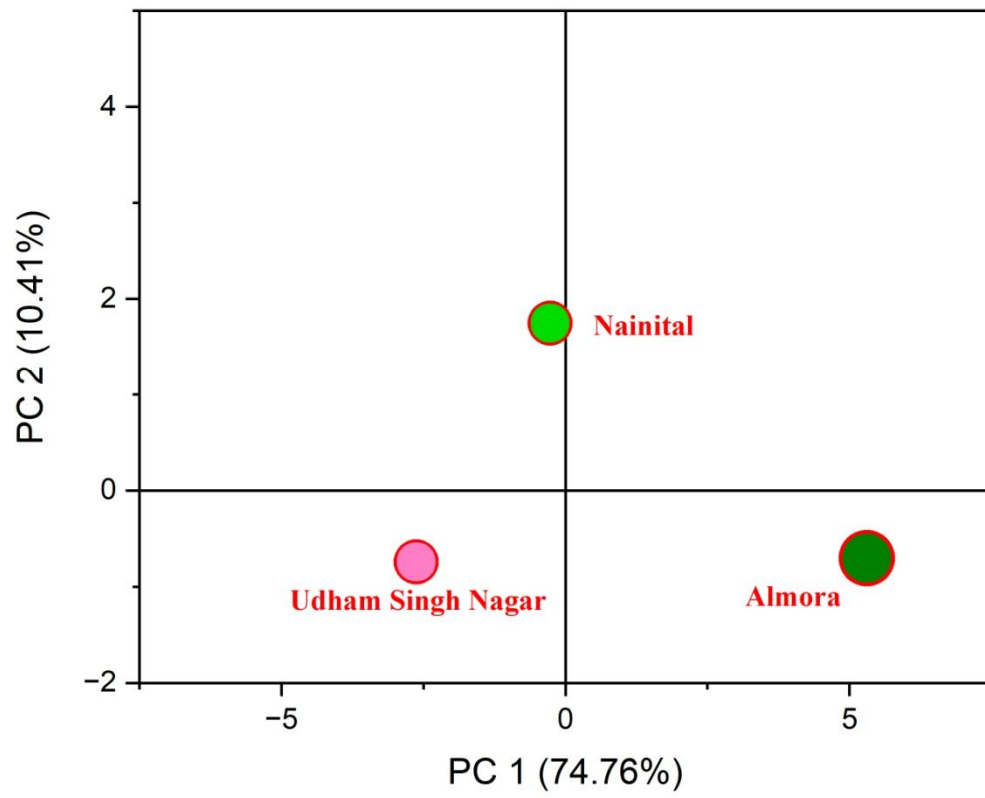


Fig 1. Component loading of morphometric variables

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3. RESULTS

3.1 Head length, Thorax length ,Abdomen length

The data on Head length, Thorax length ,Abdomenlengthof*Apis mellifera* at different altitudes of kumaun region is presented in Table 2.The maximum head length (3.280 ± 0.012 mm) was observed in the population from Almora, followed by Nainital (3.228 ± 0.012 mm) and Udham Singh Nagar (3.220 ± 0.013 mm). The overall mean head length was 3.270 ± 0.012 mm.The highest thorax length (4.454 ± 0.015 mm) was observed in honey bee samples collected from Almora, which was followed closely by samples from Nainital (4.423 ± 0.023 mm) and Udham Singh Nagar (4.404 ± 0.023 mm). The maximum abdomen length (6.197 ± 0.022 mm) was recorded at Almora, followed by Nainital (6.169 ± 0.028 mm) and Udham Singh Nagar (6.119 ± 0.032 mm), with an overall mean of 6.174 ± 0.027 mm and a coefficient of variance 1.35 per cent.

3.2 Total body length

The data on Total body lengthof*Apis mellifera* at different altitudes of kumaun region is presented in Table 2. The maximum total body length (13.931 ± 0.006 mm) was recorded at Almora, followed by Nainital (13.821 ± 0.033 mm) and Udham Singh Nagar (13.743 ± 0.038 mm). The overall mean total body length across all locations was 13.891 ± 0.020 mm.

3.3 Total antennal length

The data on Total antennal length of *Apis mellifera*at different altitudes of kumaun region is presented in Table 2The maximum total antennal length (5.133 ± 0.030 mm) was recorded at Almora, followed by Nainital (5.031 ± 0.018 mm) and Udham Singh Nagar (4.997 ± 0.029 mm).

3.4 Proboscis length

The data on Proboscis lengthof*Apis mellifera* at different altitudes of kumaun region is presented in Table 2. The maximum proboscis length (6.483 ± 0.023 mm) was observed at Hawalbagh, followed by Jyolikot (6.441 ± 0.079 mm) and Pantnagar (6.419 ± 0.024 mm).

3.5 Fore wing length and Fore wing width

The data on Fore wing length and Fore wing width of *Apis mellifera* at different altitudes of kumaun region is presented in Table 2. The maximum fore wing width (3.430 ± 0.013 mm) was found at Nainital, followed by Almora (3.413 ± 0.024 mm) and Udham Singh Nagar (3.407 ± 0.012 mm). The maximum fore wing length (9.312 ± 0.047 mm) was recorded at Almora, followed by Nainital (9.289 ± 0.032 mm) and Udham Singh Nagar (9.279 ± 0.031 mm).

3.6 Hind wing length and hind wing width

The data on Hind wing length and hind wing width of *Apis mellifera* at different altitudes of kumaun region is presented in Table 2. The maximum hind wing length (6.655 ± 0.028 mm) was recorded at Almora, followed by Nainital (6.648 ± 0.028 mm) and Udham Singh Nagar (6.633 ± 0.022 mm). The maximum hind wing width (1.805 ± 0.012 mm) was observed at Almora, followed by Nainital (1.801 ± 0.011 mm) and Udham Singh Nagar (1.796 ± 0.011 mm).

3.7 Cubital index

The data on Cubital index of *Apis mellifera* at different altitudes of kumaun region is presented in Table 2. The maximum cubital index (2.120 ± 0.020) was found at Udham Singh Nagar, followed by Nainital (2.090 ± 0.010) and Almora (2.055 ± 0.007), with an overall mean of 2.109 ± 0.013 and a coefficient of variance of 1.93 per cent.

3.8 Total fore leg length

The data on Total fore leg length of *Apis mellifera* at different altitudes of kumaun region is presented in Table 2. The maximum total fore leg length (5.748 ± 0.007 mm) was observed at Almora followed by Nainital (5.687 ± 0.028 mm) and Udham Singh Nagar (5.650 ± 0.050 mm). The overall mean total fore leg length was 5.726 mm, with a coefficient of variance 1.48 per cent.

3.9 Total mid leg length

The data on Total mid leg length of *Apis mellifera* at different altitudes of kumaun region is presented in Table 2. The maximum total mid leg length (6.083 ± 0.020 mm) was recorded at Almora, followed by Nainital (6.024 ± 0.047 mm) and Udham Singh Nagar (5.991 ± 0.043 mm). The overall mean total mid leg length across all locations was 6.063 ± 0.020 mm with a coefficient of variance 1.79 per cent.

3.10 Total hind leg length

The data on Total hind leg length of *Apis mellifera* at different altitudes of kumaun region is presented in Table 2. The maximum total hind leg length (8.859 ± 0.049 mm) was recorded at Almora, followed by Nainital (8.759 ± 0.027 mm) and Udham Singh Nagar (8.217 ± 0.050 mm). The overall mean total hind leg length across all locations was 8.690 ± 0.020 mm with a coefficient of variance 1.30 per cent.

Table 2: Morphological characters of *Apis mellifera* collected from different agroclimatic zones of Uttarakhand

Table 3. Eigenvalues of the correlation matrix

Districts	Almora	Nainital	Udham Singh Nagar	CV%
Characters				
Head length	3.280 ± 0.012	3.228 ± 0.012	3.220 ± 0.013	1.19
Thorax length	4.454 ± 0.015	4.423 ± 0.023	4.404 ± 0.023	2.04
Abdomen length	6.197 ± 0.022	6.169 ± 0.028	6.119 ± 0.032	1.35
Total body length	13.931 ± 0.006	13.821 ± 0.033	13.743 ± 0.038	1.65
Proboscis length	6.483 ± 0.023	6.441 ± 0.079	6.419 ± 0.024	2.20
Total antennal length	5.133 ± 0.030	5.031 ± 0.018	4.997 ± 0.029	1.65
Fore wing length	9.312 ± 0.047	9.289 ± 0.032	9.279 ± 0.031	1.22
Fore wing width	3.413 ± 0.024	3.430 ± 0.013	3.407 ± 0.012	1.55
Hind wing length	6.655 ± 0.028	6.648 ± 0.028	6.633 ± 0.022	1.29
Hind wing width	1.805 ± 0.012	1.801 ± 0.011	1.796 ± 0.011	2.25
Cubital index	2.055 ± 0.007	2.090 ± 0.010	2.120 ± 0.020	1.93
Total fore leg length	5.748 ± 0.007	5.687 ± 0.028	5.650 ± 0.050	1.48
Total mid leg length	6.083 ± 0.020	6.024 ± 0.047	5.991 ± 0.043	1.79
Total hind leg length	8.859 ± 0.049	8.759 ± 0.027	8.217 ± 0.050	1.30

Table 2. Eigenvalue and correlation matrix

Eigenvalue	Percentage of Variance	Cumulative
10.46696	74.76%	74.76%
1.45805	10.41%	85.18%
0.78796	5.63%	90.81%
0.5488	3.92%	94.73%
0.28831	2.06%	96.79%
0.1669	1.19%	97.98%
0.11995	0.86%	98.84%
0.06735	0.48%	99.32%
0.03425	0.24%	99.56%
0.02346	0.17%	99.73%
0.01849	0.13%	99.86%
0.01355	0.10%	99.96%

4. DISCUSSION

4.1 Comparison with previous studies

Earlier, morphometric studies were conducted by various Indian researchers. Frunze et al. (2022) reported a total body length of *Apis mellifera* as approximately 13.90 mm, proboscis length was 6.20 mm, the forewing was 9.24 mm, and the width of the forewing was 3.09 mm, which was closer to the value obtained in our study. The proboscis length of various geographical races of honey bees has shown considerable variation. Ruttner (1988) reported the proboscis length of different *A. mellifera* races, including 6.40 mm in Australia. In the present investigation, the proboscis length observed at high altitudes was 6.483 ± 0.023 mm, closely matching that of *A. mellifera ligustica*.

Ibrahim et al., (2017) studied *Apis mellifera* populations in Himachal Pradesh, while Sharma (1990) measured the head length of *A. mellifera* as 3.19 mm, which closely aligns with the average head height observed at Kumaun region of Uttarakhand.

Siraj et al. (2022) found that the antennal length of *Apis mellifera* from mid-altitude was 5.94 mm.

Ajao et al., (2014) reported an average antenna length of *A. mellifera* as 5.48 ± 0.015 mm, which was closer to the average antenna length observed at high altitudes (5.133 ± 0.030) mm in the Kumaun region. Jevtic et al. (2007) recorded forewing lengths ranging from 7.81 to 10.37 mm and widths from 2.12 to 3.75 mm across different parts of Serbia.

Additionally, our findings align with those of Bouzeraa et al., (2016), who measured the cubital index of *A. mellifera* at three sites in Algeria as 2.08 ± 0.20 , 2.50 ± 0.34 , and 2.53 ± 0.33 mm.

Abou-shaara et al., (2012) reported an average hind wing length and hind wing width of *Apis mellifera* as 6.13 ± 0.05 and 1.82 ± 0.08 mm which was closer to the average Hind wing length (6.655 ± 0.028) and hind wing width (1.805 ± 0.012) observed at high altitude in the Kumaun region. Sawadogo et al. (2022) found that the cubital index of *Apis mellifera* was 2.02.

5. CONCLUSION

Bergmann's rule states that geographic races tend to have larger body sizes in the north or cooler climates compared to those in the south or warmer climates. In our results, we observed differences in the body size of honeybees. *Apis mellifera* was collected from different agroclimatic zones of Uttarakhand, and it was found that the individuals of the *Apis mellifera* species belonging to higher altitudes were larger in size compared to those from other regions that means environmental conditions also have an effect on the size and characteristics of the *Apis mellifera* species.

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1.

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