



Comparative Morphometric Studies of European Honey Bee (*Apis mellifera* L.) at Different Altitudes of Kashmir Region, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2022/v12i111399

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/93225>

Original Research Article

**Received 21 August 2022
Accepted 26 October 2022
Published 02 November 2022**

ABSTRACT

European honey bee (*Apis mellifera* L.) was introduced in Kashmir during late 19th century and since, then its domestication is continued in diverse conditions of Kashmir region. It appears well adapted to unique climatic conditions of the valley. In order to analyse diversification in its characters during ecological adaptations to different altitudes and climatic conditions, morphological characterization of *Apis mellifera* was employed at different altitudes of Kashmir region. Samples of honey bees were obtained from nine locations representing three different altitudes viz., high, medium and low of Kashmir. Total of 17 characters were measured at three different altitudes. Highly significant difference ($p \leq 0.01$) in 15 characters were observed was observed among three altitudes. The three main characters viz., proboscis length, fore wing length and cubital index were compared with earlier data as reported by Ruttner (1988). The proboscis length (6.35 ± 0.06 mm) at high altitude, forewing length (9.21 ± 0.01 mm) at mid altitude, cubital index at low altitude (2.55 ± 0.08 mm) were found same or close to *A. mellifera ligustica*. However,

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other parameters did not support presence of this sub-species, therefore, it can be conclude that *A. mellifera* might have undergone degradations in its certain characters after its introduction in Kashmir region due to diverse climate conditions.

Keywords: *Diversification; proboscis; degradation; cubical index; domestication; forewing.*

1. INTRODUCTION

Honey bees are of huge economic importance, plays a vital role in pollination of fruit crops, vegetables and wild plants. They are capable of producing world's amazing sweetener, "honey" along with other important product of commercial value such as royal jelly, wax, propolis, etc. A honey bee colony comprises a queen, hundreds of drones and thousands of worker bees. The workers are always firm and perform different duties including honey, pollen collection, etc. Honeybee (*Apis mellifera* L.) is widespread in Africa, Europe and Western Asia [1] and comprises of 26 subspecies according to morphometric analyses [1,2]. The state of Jammu and Kashmir is one of the most important beekeeping areas in India with at least four agro climatic zones ranging from low altitude subtropical, intermediate, temperate and cold alpine. Kashmir lies in the Northern Hemisphere, has a moderate climate, which is largely defined by its geographic location. As a large valley with significant differences in Geo-location among various districts, the weather is often cooler in the hilly areas compared to the flat lower part. *Apis mellifera* L., was introduced in Kashmir during late 19th century and since, then its domestication is continued in diverse conditions of state. *Apis mellifera* appears well adapted to unique climatic conditions of Kashmir region. For example, foraging activities, worker and drone brood rearing, pollen storage and colony population size during the summer was significantly higher than those of other honeybee species, resulted in higher pollen and honey production. Higher pollen and honey production does not only depends upon the floral abundance of the area but also on the morphological characters of workers such as body size, proboscis length, corbicula, etc. [3]. Factors that affect worker body characteristics include pollen and nectar resources [4], content of dietary protein [5,6], geographical location [7,8,9]. Furthermore, the length of proboscis of a particular species of worker bee plays a vital role for honey production as it has positive correlation with foraging [10]. Besides these features, wing morphometry plays important role in classification of races and their size affects the flight ability of worker honey bees [11]. However,

the honey bee species in different climates may possess varied phenotypic characters and is suspected to have different ecological races. There is often a positive correlation in some characteristics, for instance, the length of the wing and altitude, the size of pollen basket and hind-leg and the size of honey. Such correlation can indicate the importance of the morphologic characteristics in the adaptation of individuals to the environment [12] which can lead to the evolution of many subspecies [13]. The study of morphometry of honey bees assumes significance for its application in species and subspecies characterization and discrimination. The honey bee races differ in their morphology, physiology and behavioural aspects in relation to their environment [14] and the use of their morphological characters is highly important in discriminating among the honey bee sub-species and in preserving the honey bee biodiversity. Worldwide, several workers [15,16,17,18] have used morphometric techniques to resolve the intraspecific taxonomy of honey bees, specifically for *A. mellifera*.

Morphometric tools along with sophisticated statistical analysis for studies of phenotypes and of the diversification of these bees made it possible to describe changes in the bee body dimensions depending on latitude [19]. Originally, discrimination of subspecies was based on descriptive methods but they proved insufficient and were replaced by morphometric methods [16]. Morphometry is therefore, a powerful tool which can discriminate populations [20,21]. This method uses numeric data resulting from exact measurements of morphological characters from which means of colony characters are obtained for statistical analyses [16].

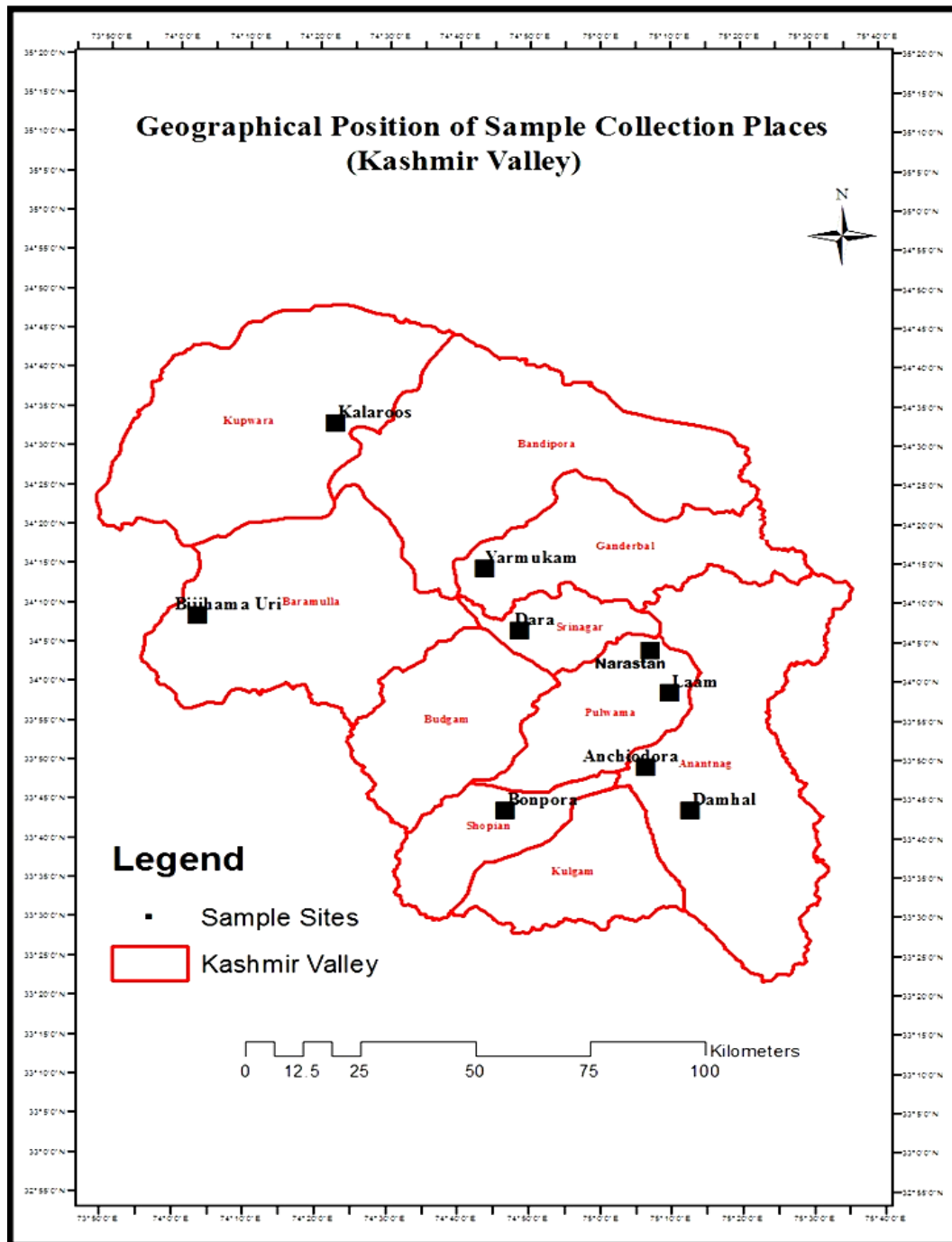
In order to analyse the present morphometric status of honey bee *Apis mellifera* in Kashmir region, the present studies were undertaken. Presently, no study has specified morphometric characterization of *Apis mellifera* found throughout Kashmir at three different altitudes [22,23]. The objective of this study was to determine difference in characters of *Apis mellifera* at different altitude under diverse climatic conditions since its introduction.

2. MATERIALS AND METHODS

2.1 Sampling of *A. mellifera* Bees from Different Altitudes

The samples of *A. mellifera* (worker bees) were obtained from nine locations viz., Narastan, Yarmukam Ganderba, Laam Tral, Damhal Anantnag, Bonpora Shopian Kalaroos kupwara, Bijihama Uri, Anchiodora Anantnag and

Dara Srinagar representing three different altitudes viz., high, medium and low of Kashmir region. Before the collection of bees, hive entrance was closed and the worker bees were handpicked gently one by one. Foraging bees were placed in killing bottles and accordingly anaesthetised with ethyl acetate. The bees were preserved in 70 per cent ethyl alcohol until morphometric analysis.



Map 1. Geographical representation of sampling sites in Kashmir region

2.2 Sample Size

Nine stationary apiaries at different locations representing three altitudes of Kashmir region were selected for sampling. Five honey bee colonies per location were taken and from each colony ten bees were collected. Total number of bees per location were fifty. One hundred fifty bees per altitude were analysed for studying different morphometric traits.

2.3 Methodology for Measurement of Different Morphological Traits of Honey Bee *Apis mellifera* L.

17 morphological parts of *A. mellifera* L. were dissected out. The dissected parts were mounted on glass slides covered with cover slips. A stereo zoom microscope (Magnus INVI) equipped with Mag-vision software calibrated with micrometre lens, was used for measurement. Some parts like head, thorax, abdomen, hind wing and forewing were measured with the help of measuring scale (mm).

2.4 Morphometric Characters of *Apis mellifera* Studied

After carefully detaching the head of *A. mellifera*, it was placed on the slide. The height was measured from the anterior most part to the origin of various appendages and the maximum head width was measured from one side of compound eye to the other. Proboscis of *A. mellifera* was detached carefully with the help of forceps, placed on slide and measured from cardo with sub-mentum up to the end of labellum. The length of flagellum and total antennal length of *A. mellifera* was measured. The sum of length of scape, pedicel and flagellum was considered as total antennal length. The thorax length was measured from cervical portion up to the first abdominal segment dorsally, whereas abdomen length were measured from anterior to posterior abdominal segment dorsally. Slides of the dissected fore wings from selected samples of bees were measured with the help of measuring scale. Length of wing was taken from the humeral angle to the apex and width was measured from coastal margin to anal angle. The cubital index of *A. mellifera* was calculated after measuring the length of two wing vein segments (A to B and B to C) of honey bees [24]. $CI = BC/AB$. BC = Distance between points B to C, AB = Distance between points A to B. The hind wing length was

measured from humeral angle to the apex while the width from costal margin to the anal angle. The number of hamuli were counted under microscope at magnification of 4x. The detached hind legs were analysed by measuring the length of femur, tibia and metatarsus. The breadth of metatarsus was also measured. The morphometric characters studied during the present investigations were based on the previous work of Ruttner et al. [21], Sakagami et al. [25] and Verma et al. [26] on adult worker bees of *Apis mellifera* L., *A. cerana cerana* F. and *Apis dorsata* F. Statistical analysis of the data was carried out by calculating colony means, standard deviation and covariance's of the morphometric characters. Further one way analysis of variance (one-way ANOVA) by SPSS software was applied for testing the significance of results. Significant results were compared on the basis of critical differences.

3. RESULTS

3.1 Head Height and Head Width

The data on head height and head width of *Apis mellifera* at different altitudes of Kashmir region is presented in Table 1. The perusal of data on head height revealed highest mean (3.27 ± 0.01 mm) at Narastan of high altitude and lowest mean (3.14 ± 0.07 mm) was recorded at Achidora at low altitude. Highest mean head width (3.71 ± 0.08 mm) was recorded at Damhal at mid altitude and lowest mean (3.61 ± 0.01 mm) was at Achidora at low altitudes. At three altitudes the head height and head width ranged from 3.10-3.15 mm and 3.28-4.03 mm respectively. The data revealed highly significant difference in head height (CD=0.0101, $p \leq 0.01$) and significant difference in head width (CD=0.0101, $p \leq 0.05$) among three altitude. Non-significant variation in head height and head width was observed within the locations at each altitude.

3.2 Proboscis Length, Flagellum Length and Antennal Length

Same trend was followed by proboscis length, flagellum length and antennal length of *Apis mellifera* where highest proboscis length (6.37 ± 0.02 mm), flagelleum length (3.81 ± 0.01 mm), antennal length (6.11 ± 0.01 mm) was recorded at Narastan at high altitude, respectively (Table 2). Lowest mean proboscis length (6.22 ± 0.07 mm), flagellum length (5.63 ± 0.03 mm) antennal length (3.31 ± 0.03 mm)

was measured at Uri Bijiham, Dara and Anchidora at low altitude, respectively. At three altitudes, the proboscis length ranged from 6.20-6.76 mm, flagellum length from 3.22-3.89 mm and antennal length ranged from 5.44-6.12 mm. The perusal of data revealed highly significant difference in proboscis length (CD=0.0101, $p \leq 0.01$), flagellum length (CD=0.0305, $p \leq 0.01$) and antennal length (CD=0.0143, $p \leq 0.01$) among three altitudes.

3.3 Thorax and Abdomen

The data on thorax length and abdomen length of *Apis mellifera* is presented in Table 3. Highest thorax length (4.30±0.01 mm) was measured at Narastan at high altitude. The lowest mean (4.15±0.07 mm) was recorded at Anchidora. Highest abdomen length (6.08±0.04 mm) was measured at yarmukaam at high altitude and lowest mean (5.55±0.02 mm) was recorded at Anchidora. At three altitudes, thorax length and abdomen length ranged from 3.89-4.56 mm and 5.26-6.89 mm, respectively. Highly significant difference in thorax length (CD=0.0143, $p \leq 0.01$) and abdomen length (CD=0.0305, $p \leq 0.01$) among three altitude was observed.

3.4 Fore Wing Length, Fore Wing Width, Cubital Index, Hind Wing Length, Hind Wing Width, Number of Hamuli

The data on forewing length, fore wing width, cubital index, hind wing length, hind wing width, number of hamuli of *Apis mellifera* at different altitudes in Kashmir region is presented in Table 4. The data revealed highest average forewing length (9.32±0.02 mm) of *A. mellifera* at Laam followed by Yarmuqam and Narastan (9.29±0.07 and 9.23±0.07 mm) at high altitude. The lowest mean (9.17±0.06 mm) was recorded at Dara at low altitude. At three altitudes, the forewing length ranged from 8.24-9.67.15 mm. Forewing width was recorded highest (3.06±0.02 mm) at Laam at high altitude and lowest mean (2.96±0.28 mm) was recorded at Bonpora at mid altitude. The forewing width ranged from 2.09-3.31 mm. Cubital index, highest mean (2.55±0.08 mm) was recorded at Uri bijiham at low altitude and lowest mean (2.19±0.02 mm) at Bonpora at mid altitude. At three altitudes, the cubital index ranged from 2.05-2.55 mm. Highest hind wing length (6.59±0.03 mm) and hind wing width (1.82±0.08 mm) was recorded at Narastan at high altitude and lowest mean (6.19±0.06 mm) and (1.71±0.02 mm) was recorded at Uri

bijiham and Anchidora, respectively. At three altitudes, the hind wing length ranged from 6.01-6.99 mm and hind wing width ranged from 1.34-1.98 mm. Number of hamuli of *Apis mellifera* at different altitudes of Kashmir region revealed highest mean (23.88±0.06) at Dara at low altitude and lowest mean (18.64±0.28) was recorded at Damhal. At three altitudes, the number of hamuli ranged from 15-23. The perusal of data revealed highly significant difference in fore wing length (CD=0.0203, $p \leq 0.01$), significant difference in fore wing width (CD=0.0305, $p \leq 0.05$), highly significant difference in cubital index (CD=0.0380, $p \leq 0.01$), hind wing length (CD=0.0635, $p \leq 0.01$), hind wing width (CD=0.0176, $p \leq 0.01$) and number of hamuli (CD=0.04016, $p \leq 0.01$) among three altitude.

3.5 Femur Length, Tibia Length, Metatarsus Length, Metatarsus Breadth

The data on Femur length, tibia length, metatarsus length, metatarsus breadth of *Apis mellifera* at different altitudes in Kashmir region is presented in Table 5. Femur length of *Apis mellifera* at different altitudes of Kashmir region revealed highest mean (2.55±0.09 mm) at Narastan. The lowest mean (2.33±0.08 mm) was recorded at Uri bijiham at low altitude. At three altitudes, the femur length ranged from (2.21-3.35 mm). Tibia length was measured highest (2.94±0.01 mm) at Laam at high altitude. The lowest mean (2.80±0.08 mm) was recorded at Bonpora. At three altitudes, the tibia length ranged from (2.70-3.00 mm) Metatarsus length of *Apis mellifera* was recorded highest (1.96±0.02 mm) at Narastan. The lowest mean (1.78±0.08 mm) was recorded at Anchidora at low altitude. At three altitudes, the Metatarsus length ranged from (1.34-1.98 mm). Metatarsus breadth of *Apis mellifera* was also found highest mean (1.13±0.07 mm) at Narastan. The lowest mean (1.03±0.02 mm) was recorded at Dara at low altitude. Damhal, Bonpora and kalaroos of mid altitude recorded (1.07±0.04, 1.07±0.03, and 1.08±0.03 mm), respectively. At three altitudes, the metatarsus breadth ranged from (1.02-1.23 mm). The perusal of data revealed highly significant difference in femur length (CD=0.0203, $p \leq 0.01$), tibia length (CD=0.0143, $p \leq 0.01$), metatarsus length (CD=0.0176, $P \leq 0.01$) and metatarsus breadth (CD=0.0101, $p \leq 0.01$) among three altitude. Non-significant variation in femur length, tibia length, metatarsus length and breadth was observed within the locations at each altitude.

Table 1. Variability in dimensions of head of *Apis mellifera* L. at different altitudes in Kashmir region

| Parameters | Altitudes | Locations | Mean±S.E (mm) | Range | Over all mean | C.V | C.D (P=0.05) | P value |
|-------------|-----------|--------------|------------------|-----------|------------------|------|-----------------|------------------|
| Head height | High | Narastan | 3.27±0.01 | 3.21-3.35 | 3.23±0.01 | 1.70 | 0.0101 | 0.005** <0.01 |
| | | Laaam | 3.21±0.04 | 3.21-3.34 | | 6.81 | | |
| | | Yarmukaaam | 3.22±0.09 | 3.13-3.34 | | 1.48 | | |
| | Mid | Damhal | 3.18±0.05 | 3.12-3.23 | 3.18±0.04 | 0.82 | | |
| | | Bonpora | 3.18±0.04 | 3.12-3.23 | | 0.70 | | |
| | | Kalaroos | 3.18±0.04 | 3.12-3.23 | | 0.72 | | |
| | Low | Uri Bijihama | 3.15±0.07 | 3.10-3.23 | 3.14±0.03 | 1.15 | | |
| | | Anchidora | 3.14±0.07 | 3.10-3.23 | | 1.26 | | |
| | | Dara | 3.15±0.01 | 3.10-3.31 | | 1.66 | | |
| Head width | High | Narastan | 3.64±0.02 | 3.28-3.81 | 3.67±0.20 | 3.33 | 0.0101 | 0.023* <0.05 |
| | | Laaam | 3.71±0.02 | 3.50-4.03 | | 3.05 | | |
| | | Yarmukaaam | 3.68±0.01 | 3.50-4.00 | | 2.39 | | |
| | Mid | Damhal | 3.71±0.08 | 3.60-3.76 | 3.70±0.03 | 1.19 | | |
| | | Bonpora | 3.70±0.08 | 3.60-3.76 | | 1.10 | | |
| | | Kalaroos | 3.70±0.09 | 3.60-3.76 | | 1.25 | | |
| | Low | Uri Bijihama | 3.62±0.01 | 3.45-3.78 | 3.62±0.08 | 2.68 | | |
| | | Anchidora | 3.61±0.01 | 3.45-3.76 | | 2.58 | | |
| | | Dara | 3.64±0.02 | 3.45-3.91 | | 3.46 | | |

Table 2. Variability in dimensions of proboscis, flagellum and antenna of *Apis mellifera* L. at different altitudes in Kashmir region

| Parameters | Altitudes | Locations | Mean±S.E (mm) | Range | Overall mean | C.V | C.D (P=0.05) | P value |
|------------------|---------------|--------------|---------------|-----------|------------------|------|--------------|------------------|
| Proboscis length | High altitude | Narastan | 6.37±0.01 | 6.23-6.67 | 6.35±0.06 | 1.46 | 0.0101 | 0.001** <0.01 |
| | | Laaam | 6.34±0.02 | 6.21-6.74 | | 2.12 | | |
| | | Yarmukaaam | 6.35±0.02 | 6.23-6.76 | | 1.93 | | |
| | Mid altitude | Damhal | 6.27±0.08 | 6.24-6.45 | 6.26±0.03 | 0.71 | | |
| | | Bonpora | 6.26±0.06 | 6.21-6.34 | | 0.52 | | |
| | | Kalaroos | 6.26±0.09 | 6.23-6.43 | | 0.74 | | |
| | Low altitude | Uri Bijihama | 6.22±0.07 | 6.20-6.34 | 6.22±0.03 | 0.56 | | |
| | | Anchidora | 6.23±0.07 | 6.21-6.34 | | 0.57 | | |
| | | Dara | 6.23±0.07 | 6.32-6.43 | | 0.76 | | |
| Flagellum length | High altitude | Narastan | 3.81±0.02 | 3.67-3.87 | 3.79±0.08 | 1.66 | 0.0394 | 0.001** <0.01 |
| | | Laaam | 3.78±0.01 | 3.71-3.89 | | 1.55 | | |
| | | Yarmukaaam | 3.79±0.01 | 3.71-3.87 | | 1.44 | | |
| | Mid altitude | Damhal | 3.60±0.02 | 3.56-3.76 | 3.60±0.01 | 1.83 | | |
| | | Bonpora | 3.58±0.01 | 3.54-3.66 | | 0.94 | | |
| | | Kalaroos | 3.64±0.03 | 3.55-3.87 | | 2.71 | | |
| | Low altitude | Uri Bijihama | 3.64±0.03 | 3.22-3.45 | 3.42±0.01 | 3.62 | | |
| | | Anchidora | 3.33±0.02 | 3.22-3.45 | | 3.20 | | |
| | | Dara | 3.31±0.02 | 3.45-3.91 | | 2.61 | | |
| Antennal length | High altitude | Narastan | 6.11±0.01 | 6.01-6.12 | 6.09±0.03 | 0.52 | 0.0305 | 0.001** <0.01 |
| | | Laaam | 6.09±0.01 | 6.01-6.12 | | 0.52 | | |
| | | Yarmukaaam | 6.09±0.01 | 6.01-6.23 | | 0.90 | | |
| | Mid altitude | Damhal | 5.95±0.01 | 5.87-5.98 | 5.94±0.05 | 0.78 | | |
| | | Bonpora | 5.93±0.01 | 5.87-5.98 | | 0.80 | | |
| | | Kalaroos | 5.94±0.01 | 5.87-5.99 | | 0.80 | | |
| | Low altitude | Uri Bijihama | 5.67±0.03 | 5.45-5.87 | 6.09±0.03 | 1.95 | | |
| | | Anchidora | 5.63±0.03 | 5.44-5.78 | | 1.97 | | |
| | | Dara | 5.64±0.02 | 5.45-5.68 | | 1.27 | | |

Table 3. Variability in dimensions of thorax and abdomen of *Apis mellifera* L. at different altitudes in Kashmir region

| Parameters | Altitudes | Locations | Mean±S.E (mm) | Range | Over all mean | C.V | C.D P=0.05 | P value |
|----------------|-----------|--------------|------------------|-----------|------------------|------|---------------|------------------|
| Thorax length | High | Narastan | 4.30±0.01 | 4.2-4.42 | 4.28±0.08 | 1.51 | 0.0143 | 0.000** <0.01 |
| | | Laaam | 4.27±0.01 | 4.2-4.44 | | 1.48 | | |
| | | Yarmukaaam | 4.28±0.01 | 4.2-4.56 | | 1.80 | | |
| | Mid | Damhal | 4.24±0.02 | 3.89-4.44 | 4.23±0.03 | 2.54 | | |
| | | Bonpora | 4.24±0.09 | 4.12-4.33 | | 1.15 | | |
| | | Kalaroos | 4.23±0.02 | 3.98-4.44 | | 2.47 | | |
| | Low | Uri Bijihama | 4.16±0.05 | 4.14-4.23 | 4.15±0.05 | 0.69 | | |
| | | Anchidora | 4.15±0.04 | 4.14-4.23 | | 0.57 | | |
| | | Dara | 4.16±0.06 | 4.12-4.3 | | 0.80 | | |
| Abdomen length | High | Narastan | 6.03±0.03 | 5.89-6.92 | 6.02±0.03 | 3.09 | 0.0431 | 0.001** <0.01 |
| | | Laaam | 5.96±0.05 | 5.26-6.92 | | 4.79 | | |
| | | Yarmukaaam | 6.08±0.04 | 5.89-6.8 | | 3.75 | | |
| | Mid | Damhal | 5.81±0.04 | 5.45-6.04 | 5.88±0.04 | 3.44 | | |
| | | Bonpora | 5.90±0.05 | 5.45-6.78 | | 4.74 | | |
| | | Kalaroos | 5.95±0.04 | 5.67-6.98 | | 3.86 | | |
| | Low | Uri Bijihama | 5.58±0.02 | 5.45-5.98 | 5.56±0.08 | 2.53 | | |
| | | Anchidora | 5.55±0.02 | 5.45-5.98 | | 2.25 | | |
| | | Dara | 5.57±0.02 | 5.45-5.98 | | 2.29 | | |

Table 4. Variability in dimensions of fore wing and hind wing of *Apis mellifera* L. at different altitudes in Kashmir region

| Parameters | Altitudes | Locations | Mean±S.E (mm) | Range | over all mean | C.V | C.D (P=0.05) | P value |
|------------------|-----------|--------------|---------------|-----------|------------------|------|--------------|------------------|
| Fore wing length | High | Narastan | 9.23±0.07 | 8.24-9.83 | 9.28±0.02 | 4.29 | 0.0203 | 0.003** <0.01 |
| | | Laaam | 9.32±0.02 | 9.01-9.67 | | 1.44 | | |
| | | Yarmukaaam | 9.29±0.07 | 8.24-9.91 | | 4.27 | | |
| | Mid | Damhal | 9.20±0.02 | 9.12-9.19 | 9.21±0.01 | 0.15 | | |
| | | Bonpora | 9.21±0.03 | 8.24-9.19 | | 2.02 | | |
| | | Kalaroos | 9.21±0.01 | 8.78-9.34 | | 0.98 | | |
| | Low | Uri Bijihama | 9.18±0.06 | 9.12-9.23 | 9.17±0.05 | 0.36 | | |
| | | Anchidora | 9.17±0.08 | 9.10-9.25 | | 0.44 | | |
| | | Dara | 9.17±0.06 | 9.23-9.21 | | 0.35 | | |
| Forewing width | High | Narastan | 3.03±0.02 | 2.90-3.50 | 3.04±0.08 | 3.36 | 0.0305 | 0.047* <0.05 |
| | | Laaam | 3.06±0.02 | 2.99-3.50 | | 4.36 | | |
| | | Yarmukaaam | 3.04±0.01 | 2.99-3.50 | | 3.27 | | |
| | Mid | Damhal | 2.97±0.05 | 2.55-3.09 | 2.97±0.01 | 2.75 | | |
| | | Bonpora | 2.96±0.28 | 2.34-3.09 | | 4.74 | | |
| | | Kalaroos | 3.00±0.07 | 2.94-3.09 | | 1.22 | | |
| | Low | Uri Bijihama | 3.04±0.04 | 2.90-3.89 | 3.00±0.02 | 7.20 | | |
| | | Anchidora | 3.00±0.04 | 2.09-3.31 | | 7.01 | | |
| | | Dara | 2.97±0.01 | 2.67-3.10 | | 2.41 | | |
| Cubital index | High | Narastan | 2.30±0.01 | 2.20-2.55 | 2.29±0.03 | 3.58 | 0.0380 | 0.001** <0.01 |
| | | Laaam | 2.29±0.01 | 2.16-2.55 | | 4.17 | | |
| | | Yarmukaaam | 2.29±0.01 | 2.20-2.55 | | 3.52 | | |
| | Mid | Damhal | 2.19±0.08 | 2.05-2.23 | 2.19±0.03 | 2.01 | | |
| | | Bonpora | 2.19±0.02 | 2.08-2.34 | | 2.33 | | |
| | | Kalaroos | 2.20±0.06 | 2.21-2.25 | | 1.50 | | |
| | Low | Uri Bijihama | 2.55±0.08 | 2.42-2.55 | 2.55±0.08 | 1.71 | | |
| | | Anchidora | 2.55±0.01 | 2.08-2.55 | | 3.95 | | |
| | | Dara | 2.55±0.02 | 2.21-2.55 | | 4.70 | | |

| Parameters | Altitudes | Locations | Mean±S.E (mm) | Range | over all mean | C.V | C.D (P=0.05) | P value |
|------------------|-----------|--------------|---------------|-----------|-------------------|------|--------------|------------------|
| Hind wing length | High | Narastan | 6.59±0.03 | 6.11-6.99 | 6.55±0.01 | 3.00 | 0.0635 | 0.001** <0.01 |
| | | Laaam | 6.55±0.03 | 6.20-6.98 | | 3.00 | | |
| | | Yarmukaaam | 6.53±0.01 | 6.33-6.78 | | 1.95 | | |
| | Mid | Damhal | 6.35±0.02 | 6.06-6.55 | 6.35±0.05 | 1.81 | | |
| | | Bonpora | 6.36±0.02 | 6.06-6.56 | | 1.66 | | |
| | | Kalaroos | 6.35±0.02 | 6.06-6.55 | | 1.88 | | |
| | Low | Uri Bijihama | 6.19±0.06 | 6.01-6.23 | 6.20±0.06 | 0.52 | | |
| | | Anchidora | 6.21±0.09 | 6.01-6.24 | | 0.75 | | |
| | | Dara | 6.21±0.09 | 6.01-6.24 | | 0.78 | | |
| Hind wing width | High | Narastan | 1.82±0.08 | 1.72-1.92 | 1.81±0.06 | 2.40 | 0.0176 | 0.001** <0.01 |
| | | Laaam | 1.80±0.04 | 1.67-1.88 | | 2.89 | | |
| | | Yarmukaaam | 1.82±0.01 | 1.56-1.98 | | 4.80 | | |
| | Mid | Damhal | 1.75±0.08 | 1.67-1.87 | 1.75±0.03 | 2.34 | | |
| | | Bonpora | 1.76±0.07 | 1.68-1.87 | | 2.08 | | |
| | | Kalaroos | 1.75±0.01 | 1.56-1.98 | | 4.03 | | |
| | Low | Uri Bijihama | 1.73±0.01 | 1.56-1.89 | 1.72±0.05 | 3.52 | | |
| | | Anchidora | 1.71±0.02 | 1.34-1.87 | | 7.79 | | |
| | | Dara | 1.72±0.01 | 1.56-1.73 | | 3.03 | | |
| No. of Hamuli | High | Narastan | 20.64±0.18 | 20-23 | 20.66±0.02 | 4.39 | 0.4016 | 0.001** <0.01 |
| | | Laaam | 20.64±0.18 | 20-23 | | 4.39 | | |
| | | Yarmukaaam | 20.72±0.18 | 20-23 | | 4.51 | | |
| | Mid | Damhal | 18.64±0.28 | 15-21 | 18.86±0.2 | 7.72 | | |
| | | Bonpora | 18.68±0.24 | 15-21 | | 6.68 | | |
| | | Kalaroos | 19.28±0.23 | 15-21 | | 6.08 | | |
| | Low | Uri Bijihama | 23.44±0.19 | 21-24 | 23.58±0.14 | 7.72 | | |
| | | Anchidora | 23.44±0.15 | 22-24 | | 3.27 | | |
| | | Dara | 23.88±0.06 | 23-24 | | 1.38 | | |

Table 5. Variability in dimensions of hind leg (femur, tibia, metatarsus length and breadth) of *Apis mellifera* L. at different altitudes in Kashmir region

| Parameters | Altitudes | Locations | Mean±S.E (mm) | Range | Over all mean | C.V | C.D (P=0.05) | P value |
|-------------------|-----------|--------------|---------------|-----------|------------------|------|--------------|-----------------|
| Femur length | High | Narastan | 2.55±0.09 | 2.50-2.67 | 2.53±0.01 | 1.92 | 0.0203 | .001** <0.01 |
| | | Laaam | 2.53±0.01 | 2.34-2.66 | | 2.46 | | |
| | | Yarmukaaam | 2.51±0.01 | 2.34-2.66 | | 2.76 | | |
| | Mid | Damhal | 2.38±0.01 | 2.22-2.45 | 2.41±0.05 | 2.81 | | |
| | | Bonpora | 2.42±0.01 | 2.22-2.52 | | 3.01 | | |
| | | Kalaroos | 2.43±0.01 | 2.22-2.56 | | 3.10 | | |
| | Low | Uri Bijihama | 2.33±0.08 | 2.21-2.45 | 2.35±0.05 | 1.91 | | |
| | | Anchidora | 2.38±0.04 | 2.21-3.35 | | 8.7 | | |
| | | Dara | 2.34±0.01 | 2.21-2.45 | | 2.16 | | |
| Tibia length | High | Narastan | 2.93±0.06 | 2.87-2.98 | 2.93±0.03 | 1.03 | 0.0143 | .001** <0.01 |
| | | Laaam | 2.94±0.01 | 2.78-3.00 | | 1.70 | | |
| | | Yarmukaaam | 2.93±0.07 | 2.87-2.99 | | 1.26 | | |
| | Mid | Damhal | 2.82±0.01 | 2.75-2.98 | 2.81±0.05 | 1.83 | | |
| | | Bonpora | 2.80±0.01 | 2.71-2.94 | | 1.93 | | |
| | | Kalaroos | 2.81±0.08 | 2.75-2.89 | | 1.47 | | |
| | Low | Uri Bijihama | 2.86±0.01 | 2.76-2.94 | 2.87±0.03 | 2.15 | | |
| | | Anchidora | 2.87±0.01 | 2.70-2.99 | | 2.80 | | |
| | | Dara | 2.88±0.01 | 2.76-2.98 | | 1.74 | | |
| Metatarsus length | High | Narastan | 1.96±0.02 | 1.95-1.98 | 1.95±0.05 | 0.52 | 0.0176 | .001** <0.01 |
| | | Laaam | 1.95±0.05 | 1.87-1.98 | | 1.34 | | |
| | | Yarmukaaam | 1.94±0.05 | 1.86-1.98 | | 1.51 | | |
| | Mid | Damhal | 1.83±0.08 | 1.67-1.89 | 1.83±0.01 | 2.31 | | |
| | | Bonpora | 1.83±0.01 | 1.67-1.98 | | 3.77 | | |
| | | Kalaroos | 1.82±0.08 | 1.67-1.88 | | 2.37 | | |
| | Low | Uri Bijihama | 1.78±0.09 | 1.67-1.89 | 1.78±0.03 | 2.52 | | |
| | | Anchidora | 1.78±0.08 | 1.74-1.98 | | 2.23 | | |
| | | Dara | 1.79±0.08 | 1.74-1.88 | | 2.30 | | |

| Parameters | Altitudes | Locations | Mean±S.E (mm) | Range | Over all mean | C.V | C.D (P=0.05) | P value |
|---------------------------|-----------|--------------|---------------|-----------|------------------|------|--------------|---------|
| Metatarsus breadth | High | Narastan | 1.13±0.07 | 1.1-1.23 | 1.12±0.05 | 3.20 | 0.0101 | 0.001** |
| | | Laaam | 1.12±0.08 | 1.05-1.23 | | 3.64 | | |
| | | Yarmukaaam | 1.11±0.04 | 1.08-1.23 | | 2.38 | | |
| | Mid | Damhal | 1.07±0.04 | 1.2-1.09 | 1.07±0.03 | 2.01 | | |
| | | Bonpora | 1.07±0.03 | 1.04-1.10 | | 1.41 | | |
| | | Kalaroos | 1.08±0.03 | 1.04-1.12 | | 1.51 | | |
| | Low | Uri Bijihama | 1.04±0.03 | 1.02-1.09 | 1.04±0.00 | 1.66 | | |
| | | Anchidora | 1.04±0.03 | 1.02-1.09 | | 1.70 | | |
| | | Dara | 1.03±0.02 | 1.02-1.08 | | 1.29 | | |

S.E = Standard error, C.V= Coefficient of variation, C.D = Critical Difference, P=threshold of Significance, P≤0.01(**) difference highly significant, P≤ 0.05(*) difference significant, P≥0.05 ns (Non-Significant)

4. DISCUSSION

Earlier too, morphometric studies were carried out by different Indian workers. Kshirsagar [27] on *Apis cerana* (Pune), Ananda [28] on *A. cerana* (Bangalore), Mujumdar and Kshirsagar [29] on *A. dorsata* (Pune), Makhmoo [30] on four honey bee species (Jammu), Ibrahim et al. [31] on *Apis mellifera* (Himachal Pradesh) and Makkar et al. [32] on *Apis mellifera jemenitica* (Yemen). Our findings are in conformity with all these above mentioned studies. Sharma [33], measured head height of *A. mellifera* as 3.19 mm which is near to average head height at mid altitude in Kashmir region as per the present findings. The proboscis length of various geographical races of honey bees has shown considerable variations. Ruttner [16] worked on proboscis length of different races of *A. mellifera* viz., Australia (6.40 mm), Hungary (6.43 mm), Romania (6.39 mm) and Greece (6.57 mm) which substantiate the present findings on proboscis length wherein slight variations might be due to different geographical races. On subspecies level, the proboscis length was reported to be 6.07±1.3, 6.41±0.7, 6.35±0.79, 7.06±1.5, 6.64±0.70, 6.28±0.89, 6.43±1.7 and 6.41±1.80 mm in *A. mellifera mellifera*, *A. mellifera carnica*, *A. mellifera ligustica*, *A. mellifera caucasica*, *A. mellifera armeniaca*, *A. mellifera meda*, *A. mellifera anatoliaca* and *A. mellifera pomonella*, respectively [34]. The proboscis length observed at High altitude as 6.35±0.06 in the present investigations is in close proximity of *A. mellifera ligustica*. In India, different workers have reported the proboscis length of *A. mellifera* as 6.37±0.05 [33] and 7.00 mm [35]. Ajao et al. [36] reported the average antenna length of *A. mellifera* as 5.48±0.015 which is near to average antenna length at mid altitude in Kashmir region. Dyer and Seeley [37] reported the fore wing length in *A. mellifera* of different attitudes between 7.64 to 9.70 mm. Jevtic et al. [38] reported fore wing length ranged from 7.81 to 10.37 mm and width from 2.12 to 3.75 mm from different parts of Serbia. Alattal et al. [39] reported the length of forewing of 3 clusters of local Saudi honey bees as 8.79 ± 0.22, 8.31 ± 0.19 and 8.04 ± 0.12 and width of fore wing as 3.05 ± 0.22, 2.86 ± 0.06 and 2.76 ± 0.05 mm. Our findings are in congruence with the report of Bouzeraa et al. [40] who measured cubital index of *Apis mellifera* at three different sites of Algeria as 2.08±0.20, 2.50±0.34 and 2.53±0.33. The mean cubital index 2.55±0.08 mm of low altitude in Kashmir region was found same as *A. mellifera ligustica*. AL-Kahtani and Taha [41]

reported that with the exception of the number of hamuli, worker Yemeni bee body size and morphometric parameters related to the colony productivity were smaller than Carniolan bees under environmental conditions of the study region. Earlier, Sharma [33] reported the femur length in *A. mellifera* as 2.41±0.05 mm which is same to the average femur length at mid altitude of the present findings (2.41±0.05 mm). Bouzeraa et al. [40] measured femur length at three different sites of Algeria as 3.87±0.16, 3.71±0.09 and 3.86±0.12 mm which is higher than present findings. According to their results, values obtained are closer to giant bee (*Apis dorsata*). However, El-Aw et al. [42] found smaller length (2.22 mm) of femur in Egypt in same bee species. The higher length of femur ranging from 2.60– 2.73 mm has also been reported for different races of *A. mellifera* [43,44]. The tibial length in *A. mellifera* has been reported by different workers as 3.01 ± 0.10 for Indian population [33], 2.83±0.08 mm for Egyptian population and 3.03- 3.22 mm for Iranian population [44] and 3.16 to 3.18 mm for bees population in Turkey [43]. In other studies the dimension of metatarsus were also observed to be (length 2.00 to 2.44 mm, width 1.08 to 1.19 mm) for the same bee species [45,44,42]. Intraspecific variation in body size may reflect quality of the environment Banaszak et al. [46] and worker body weight is altered by the availability of nectar and pollen resources [4,47] and season [48]. Ahmad and Bilal [49] who found positive correlation of bee size with increase in altitude. Narayanan et al. [50] also observed a definite trend of variation in *A. cerana* from plains to hills. These observations lend support to present investigation that altitude has impact. Fernando [51] observed tongue length of bees significantly longer in mountainous districts than lowlands. Present findings also draw the support from Kshirsagar [52] who reported the bigger size of antenna at higher elevations. This is because of the fact that bees found at the higher elevations require longer antenna in order to have better sense perception, so as to cope with the larger environmental disturbances at these altitudes [53]. Mattu and Verma [54,55] reported significant and positive correlation of length of postmentum with altitude. Narayanan et al. [56,57] reported smaller number of hooks in bees at higher altitudes. Hepburn et al. [58] also found smaller number of hooks from Northeast Himalayan region. Size of hind leg especially metatarsus affect the pollen carrying capacity of honey bee [16]. In

Kashmir region, significant differences were observed only in length of femur and breadth of metatarsus of *Apis cerana*. Bees of mountainous zone also showed significantly higher values for these characters as compared to those of sub mountainous zone of Kashmir region [49]. Bouzeraa et al. [40] reported highly significant effect concerning the length of femur, tibia, metatarsus ($p \leq 0.001$) and significant effect concerning to the width of metatarsus ($p \leq 0.05$) on bees coming from three different sites of Algeria. Verma et al. [26] also found bigger size of hind leg at higher elevations as compared to the plain area.

5. CONCLUSION

The morphometric variations found in *Apis mellifera* L. in Kashmir with respect to altitude particularly, body size, tongue length, length of antenna, number of hamuli, length of femur could have a significant impact in future bee breeding programmes. The mean proboscis length (6.35 ± 0.06 mm) of high altitude, mean forewing length (9.21 ± 0.01 mm) of mid altitude, mean cubital index of low altitude (2.55 ± 0.08 mm) were found same to *Apis mellifera ligustica* when compared with data as reported by Ruttner [16]. The other characters, however, were different in measurement which imply presence of subspecies other than *A. mellifera ligustica* at different altitudes. Hence, *Apis mellifera* L. differing in their morphology in relation to their environment and the use of their morphological characters is highly important in discriminating among the sub-species and in preserving the honey bee biodiversity.

ACKNOWLEDGEMENTS

The authors wish to acknowledge to the Head, Department of Entomology, Sher-e-Kashmir University of Agriculture Science and Technology, Wadura, Sopore, Jammu and Kashmir, for providing necessary facilities to carry out the research. Also we wish to acknowledge the bee keepers of Valley for allowing us to collect bees from their bee hives.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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