Morphometrics of *Apis mellifera* in North-Western Himalayan region of Himachal Pradesh, India

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**Abstract**

Morphological traits of the queen, workers, and drones of *Apis mellifera* were analyzed from district Kangra, Himachal Pradesh (India). A total of 10 characters of queen, worker, and drones of *Apis mellifera* were measured. There were some significant differences observed between the populations of all characters. The head, proboscis, thorax, abdomen, fore wing, cubital index, hind wing, coxa, trochanter, femur, tibia, meta tarsus were found to be 2.45±0.10× 3.62±0.09mm, 4.51±0.06mm, 3.72±0.20mm, 6.63±0.93mm, 9.27±0.18× 2.98±0.08mm, 2.28±0.36mm, 6.20±0.14mm× 1.82±0.08mm, 1.10±1.32mm, 1.43±0.99mm, 2.96±0.10mm, 3.14±0.10mm, 1.79±0.07mm× 1.22±0.07mm which shows a significant difference (p<0.05) between the morphological traits of the queen, worker and drones. The observation revealed the relationship between the morphological traits of queen, worker and drones of *Apis mellifera*.

**Keywords:** *Apis mellifera*, morphology, morphometrics, insects, morphological traits, queen bee, worker bee

**Introduction**

Honey bees are social insects. They produce various substances like honey, propolis, royal jelly, beeswax, and bee venom besides playing a major role in the field of agriculture by pollination [1]. Four species of honey bee are *Apis cerana indica*, *Apis mellifera*, *Apis florea* and *Apis dorsata*. Two commonly domesticated species of honey bee are *Apis cerana indica* and *Apis mellifera*. *Apis mellifera* is available in every continent of the world except Antarctica. In Himachal Pradesh *Apis mellifera* was established during 1964-1965 and since then, its domestication is continued in diverse conditions of the state. The honey bee species have varied phenotypic characters in different climatic conditions. Biometrics was introduced by Alpatov and George (1940) [2] to describe accurately races of honey bees. The morphometrical study of honey bees was discussed by Ruttner et al. (1978) [3]. Further Kekecoglu, (2010) [4] described morphometric studies are important tools to provide information on structure, geographical and genetic variability. *Apis mellifera* might have undergone various morphological changes in its characters due to different climate conditions after its introduction in India. Himachal Pradesh is a North-Indian state which is situated in the North-western Himalayas. It is located between Latitude 30°22’40” N to 33°12’20” N and Longitude 75°45’55” E to 79°04’20” E with altitude ranges from 350 to 6975m above sea level. In this study, we aimed to determine the present morphometric status of *Apis mellifera* being domesticated in Himachal Pradesh.

**Materials and Methods:** The investigation on morphometrics of *Apis mellifera* were carried out at Post Graduate Laboratory, Department of Entomology, YSPUHF Neri, Hamirpur and Department of Zoology, Sri Sai University Palampur during the year 2021. The details of materials used and methods employed are as follow.

Bees of *Apis mellifera* (queen, worker, drone), beekeeping jars(100ml), Ethanol, Dibutyl phthalate Polystyrene Xylene (DPX), Glass slides, Coverslips, Samples of honey, Digital Vernier caliper, Compound microscope, Stereo-zoom microscope, Dissecting box, pH meter& electrical conductivity meter, blotting paper.

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Methods
1. Collection of bees: Morphologically, molecularly identified cultured bees of *Apis mellifera* were collected from Bee Research Station Nagrota Bagwan District Kangra of Himachal Pradesh. Total 45 workers, 30 drones, 10 queens were collected.
2. Preservation: Bees were preserved in 70% ethanol in beekingup jars. Then the bees were kept in the jars for about 24 hours.
3. Characters studied: The collected bees were dissected for studying various characters with the help of a compound microscope (40X), Stereo-zoom microscope (10x-20x) and Digital Vernier caliper. Various characters of bees were studied Head height, head width, proboscis length, thorax length, abdomen length, fore-wing length, fore-wing width, cubital index, hind-wing length, hind-wing width, coxa length, trochanter length, femur length, tibia length, metatarsus length, metatarsus width.
4. Measurements: Apparatus used: Digital Vernier Caliper, Compound microscope, Stereo-zoom microscope. The data obtained were analyzed following standard statistical procedure [5].

Results
- **Head**: The head height of *Apis mellifera* in worker bees was 2.45±0.10 mm, in drones 2.88±0.10mm and queen 3.92±0.10mm. The head width of the worker bee was 3.62±0.09mm, in drones 4.37±0.09mm and the queen was 3.69±0.09mm.
- **Proboscis**: In present studies, the proboscis’s length of *Apis mellifera* in worker bees was 4.51±0.06mm, in drones 2.91±0.06mm and the queen was 2.92±0.06mm. Proboscis’s length is of extreme importance in honey production.
- **Thorax and abdomen length**: The mean thorax and abdomen length of *Apis mellifera* in worker bees were found to be 3.72±0.20mm, 6.63±0.93mm; in drones, 4.81±0.20mm, and 7.01±0.93mm and in queen was 4.83±0.20mm, 8.08±0.93mm.
- **Fore-wings**: The minimum fore-wing length in *Apis mellifera* was observed in worker bees which were 9.27±0.18mm, where it was recorded in drones to be 10.01±0.18mm and in queen, it was recorded as 10.2±0.18mm. However, no significant differences were observed in fore-wing width among all the traits of *Apis mellifera* which ranged from 2.98±0.08mm to 3.44±0.08mm. The minimum cubital index (2.28±0.36mm) was observed in worker bees than in queen (2.98±0.36mm) and then in drones (3.2±0.36mm).
- **Hind-wings**: The worker bees of *Apis mellifera* showed minimum hind-wing length as 6.20±0.14mm and hind-wing width 1.82±0.05mm. The hind-wing length and width in drone bees were 6.48±0.14mm and 1.99±0.05mm. In queen bees, it had been observed as 7.50±0.14mm and 2.20±0.05mm.
- **Hind-legs**: The various parts of the hind legs of *Apis mellifera* were summarized in table3. The various parts of the hind legs were coxa, trochanter, femur, tibia, and metatarsus. The mean length of coxa in worker, drone, and the queen was observed as 1.1±0.09, 1.18±0.09, 1.13±0.09mm. The mean length of trochanter in worker, drone, and the queen was observed as 1.42±0.10, 1.89±0.10, 2.34±0.10mm. The mean length of the femur in the worker, drone, and the queen was observed as 2.96±0.10, 3.04±0.10, 3.42±0.10mm. Similarly, the mean length of the tibia in the worker, drone, and the queen was observed as 3.14±0.13, 2.89±0.13, 2.45±0.13mm. The mean length of metatarsus in the worker, drone, and the queen was observed as 1.79±0.07, 1.97±0.07, 2.02±0.07mm.

Table 1: Showing morphometric values of head, proboscis, thorax and abdomen. ±Standard Error (n=10).

| Bees         | Head (in mm) | Proboscis | Thorax | Abdomen |
|--------------|--------------|-----------|--------|---------|
|              | Length       | Width     | Length  | Width   | Length  | Width  |
| Worker (mean)| 2.45±0.10    | 3.62±0.09 | 4.51±0.06 | 3.72±0.20 | 6.63±0.93 |
| Drone (mean) | 2.88±0.10    | 4.37±0.09 | 2.91±0.06 | 4.81±0.20 | 7.01±0.93 |
| Queen (mean) | 3.92±0.10    | 3.69±0.09 | 2.92±0.06 | 4.83±0.20 | 8.08±0.93 |

Table 2: Showing morphometric values of fore-wing, hind-wing, cubital index. ±Standard Error (n=10).

| Bees (Mean) | Fore-wing(mm) | Hind-wing(mm) | Cubital(index(mm) |
|-------------|--------------|---------------|------------------|
|             | Length       | Width         | Length           | Width   | Length  |
| Worker      | 9.27±0.18    | 2.98±0.08     | 6.20±0.14        | 1.82±0.05 | 2.28±0.36 |
| Drone       | 10.01±0.18   | 3.08±0.08     | 6.48±0.14        | 1.99±0.05 | 3.2±0.36 |
| Queen       | 10.26±0.18   | 3.44±0.08     | 7.50±0.14        | 2.20±0.05 | 2.98±0.36 |

Table 3: Showing morphometric values of hind legs of the queen bee, worker bee and drone.

| Bees (mean) | Coxa (mm) | Trochanter (mm) | Femur (mm) | Tibia (mm) | Metatarsus (mm) |
|-------------|-----------|-----------------|------------|------------|-----------------|
| Worker      | 1.1±0.09  | 1.42±0.10       | 2.96±0.10  | 3.14±0.13  | 1.79±0.07       |
| Drone       | 1.18±0.09 | 1.89±0.10       | 3.04±0.10  | 2.89±0.13  | 1.97±0.07       |
| Queen       | 1.13±0.09 | 2.34±0.10       | 3.42±0.10  | 2.45±0.13  | 2.02±0.07       |
Discussion
Honey bee colonies consist of three caste’s queen, workers and drones. All of them perform certain functions. The workers are dedicated individuals and perform different duties like pollen collection, honey etc. Body size and hind leg length affect the pollen carrying capacity of honey bees discussed by Morimoto, (1968) [6]. Wing morphometry is important for the classification of races and their size affects the flight ability of the honey bees. The production of honey and collection of pollen not only depend upon the floral abundance of that area but also on the morphological characters of the worker bees such as proboscis length, body size. In India, Apis mellifera was firstly introduced at Bee Research Station, Nagrota Bagwan (Himachal Pradesh). For this purpose, Apis mellifera and its hybrids were obtained from the USA and Italy between the years 1962 and 1964. After five decades of its introduction and establishment in the north-western Himalayan region of India, the morphometric studies on worker bees were conducted by Ibrahim et al. (2017) [7]. Ibrahim & Y.S. Chandel (2019) [8]. The present investigation on the morphometrical study was conducted and compared with available literature in India and the world. In the present investigation, the mean head height and width was found to be 2.45±0.10mm and 3.62±0.09mm. These parameters are studied earlier of Apis mellifera in the report of Sharma (1990) [9] who observed head height (3.19mm) and width (3.78mm) of Apis mellifera in India which is agree with our study. Ruttner, (1978) [13] worked on proboscis length of Apis mellifera. 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±1.80mm in Apis mellifera mellifera, A. mellifera carnica, A. mellifera ligustica, A. mellifera caucasia, A. mellifera armeniaca, A. mellifera mela, A. mellifera anatolica and A. mellifera pomonella, respectively Ruttner, (1978) agreed with our study. The morphometric value of proboscis length is 4.52±0.06mm is an agreement with the study described in the morphometric study done by Kauhausen-Keller et al. 1997 [10]; De Souza et al. 2015 [11]; Meixner et al. 2007 [12]; Ibrahim et al. 2017 [7]. Present findings add information on thorax length and abdomen length. The fore-wing length and width had been recorded as 9.27±0.18mm and 2.98±0.08mm, agreed with measurements discussed by Ruttner, (2013) [13] that is 9.33mm which is almost similar. Earlier the fore-wing length and width have been reported to be variable [14, 15, 16, 17, 18 & 19] which can be attributed to geographical races. Dyer & Seeley (1987) [20] reported the fore-wing length in A. mellifera of different altitudes between 7.64 to 9.70mm. In India, Sharma, (1990) [21] observed the morphometrics of hind-wing length as
6.43±0.084 and width as 1.925±0.060mm respectively agreed with the present findings 6.20±0.14mm and 1.82±0.05mm. Sharma, (1990) [21] measured the coxal length as 1.09±0.088mm which was similar to the present findings which were 1.10±0.09mm. Earlier Sharma also reported femur length, tibia length and metatarsus length as 2.41±0.05mm, 3.01±0.10mm, 2.00 to 2.44 mm which were similar to present findings which were 2.96±0.10mm, 3.14±0.13mm and 1.79±0.07mm. In the present investigation, the cubital index was found to be 2.28±0.36mm. All these differences might be due to varying geographical conditions and different races. We analyzed differences in morphometric parameters at P<0.05 using one-way analysis of variance (ANOVA), which indicated a significant difference between the traits.

Conclusion
This study showed that the population of Apis mellifera domesticated in Himachal Pradesh shows variation in morphometrical characters within queen bee, worker bee and drone. The above study shows a significant variation in the population of the queen bee, worker bee and drone. According to morphometrics the height of the head in queen bee is larger than drone and worker bee. Similarly, the height of the thorax and abdomen is more in the case of queen bees compared to drone and worker bees. But in the case of proboscis, the worker bee consists of large proboscis than the queen and drone this may be due to the worker bee perform the function of nectar collection. The fore-wing and hind-wing are also long in queen bees than drones and worker bees, the queen has to take strong nuptial flight so their hind and fore wings are stronger. There is a very slight difference in the hind legs of queens, drones, workers. But in case of tibia length worker bees has long tibia than drone and queen, due to the presence of pollen baskets in the tibia segment of metathoracic legs of worker bees they have long tibia than other castes. This study can infer that the population of A. mellifera (queen bee, worker bee and drone) of district Kangra, Himachal Pradesh showed a variation in morphometrics characters.

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