



Morphometrics of *Apis mellifera* after Five Decades of its Introduction in North-Western Himalayan Region of India

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ABSTRACT

A total of 17 characters were taken for morphometric studies on *Apis mellifera* L. and samples were collected from four locations representing two different agro-climatic zones of Himachal Pradesh during 2012. The findings revealed that the significant differences were observed between the populations for all characters except abdomen length, fore wing width and length of trochanter and tibia. Overall, the mean of populations for head height $\times$ width, proboscis length, thorax length, abdomen length, fore wing length $\times$ width, cubital index, hind wing length $\times$ width, number of hamuli, coxa length, trochanter length, femur length, tibia length and metatarsus length $\times$ width were found to be $3.19 \pm 0.10 \times 3.68 \pm 0.09$ mm, 6.29 ± 0.06 mm, 4.26 ± 0.20 mm, 5.91 ± 0.93 mm, $9.13 \pm 0.18 \times 3.00 \pm 0.08$ mm, 2.20 ± 0.36 , $6.38 \pm 0.14 \times 1.80 \pm 0.05$ mm, 20.88 ± 1.32 , 1.10 ± 0.09 mm, 0.79 ± 0.10 mm, 2.45 ± 0.10 mm, 2.91 ± 0.13 mm and $1.92 \pm 0.07 \times 1.08 \pm 0.07$ mm. The mean values of important characters like, proboscis length, fore wing length and cubital index were compared with available data of *A. mellifera*. The mean proboscis length and fore wing length were found to be near to *A. mellifera ligustica* and *A. mellifera meda* whereas mean cubital index was near to *A. mellifera caucasica* and *A. mellifera anatoliaca*.

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Authors' Contributions

MMI performed the experiment, YSC guided to conduct the experiment. Anil conducted the analysis and prepared the manuscript.

Key words

Honey bee, Proboscis length, Fore-wing length, Cubital index.

INTRODUCTION

Honey bees are social insects which produce important substances like honey, royal jelly, bee wax, propolis, pollen and bee venom besides playing a major role in the field of agriculture by pollination (Alburaki and Alburaki, 2008). India has diverse honey bee fauna with major *Apis* species like, *Apis dorsata* Fab., *A. florea* Fab., *A. cerana* Fab. and well established exotic *A. mellifera* L. The former two species are still not hived while later two species are hived, thus suitable for beekeeping.

The honey bee species in different climates possess varied phenotypic characters and it is suspected to have different ecological races. Earlier, the description of geographical races was exclusively based on colour and size of honey bees. The biometrics was introduced by Alpatov (1929) and Goetze (1940) to describe accurately the races of honey bees. The morphometrical studies of honey bees were comprehensively discussed by Ruttner *et al.* (1978). Thereafter, these studies were continued throughout the world on different characters for the variations among the species but the detailed region wise characterizations are

still awaited for their proper identification and suitability (Singh *et al.*, 1990). Further, Kekecoglu *et al.* (2007) also emphasized that the morphometric studies are the important tools to provide information on the structure, available races or geographical and genetic variability and defining the role and importance of the biometrical characters of any organism.

It is the possibility that *A. mellifera* might have undergone certain morphological changes or degeneration in its characters during ecological adaptation to different climatic conditions after its introduction in India. Himachal Pradesh, a northern Indian state, is situated in the lap of north-western Himalaya and blessed with diverse climate that varies from semi tropical in lower hills to semi arctic in the cold deserts areas. It is located between Latitude $30^{\circ}22'40''$ N to $33^{\circ}12'20''$ N and Longitude $75^{\circ}45'55''$ E to $79^{\circ}04'20''$ E with altitude ranges from 350 to 6975 m above sea level. In Himachal Pradesh, *A. mellifera* was established during 1964-65 and since then, its domestication is continued in diverse conditions of the state. Therefore, the present study was devised to analyse the present morphometric status of *A. mellifera* being domesticated in the state.

MATERIAL AND METHODS

The investigations on morphometrics of *A. mellifera*

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were carried out at Post Graduate Laboratory, Department of Entomology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur during the year 2012. The details of materials used and methods employed are as under.

Collection of bees

Worker bees of *A. mellifera* were collected from apiaries at four locations representing different agro-climatic zones of Himachal Pradesh (Table I). At each location, a total of 50 worker bees were collected from three random colonies of the apiary. For the collection of bees, hive entrance was closed and the worker bees were handpicked gently one by one. The collected bees were killed in boiling water and further preserved in 75 per cent alcohol.

Characters studied

The sampled bees were brought to the laboratory

and dissected for studying different appendages under Stereozoom microscope. Various characters namely, head height (HH), head width (HW), proboscis length (PL), thorax length (TL), abdomen length (AL), fore wing length (FWL), fore wing width (FWW), cubital index (CI), hind wing length (HWL) and width (HWW), number of hamuli (NH) on hind wing, length of coxa (CL), trochanter (TrL), femur (FL), tibia (TiL), and metatarsus length and width on hind leg (ML and MW) were studied (Fig. 1). In addition, cubital index was also calculated as per the following formula by Goetze (1930).

$$\text{Cubital Index} = \text{BC}/\text{AB}$$

Where,

BC is distance between points B to C and AB is distance between points A to B.

Table I.- Details of apiaries used for sampling in Himachal Pradesh, India.

Location	District	Agro-climatic zone	Latitude (°N)	Longitude (°E)	Altitude (m) ASL
Dhaulakuan	Sirmour	Sub-Montane and Low Hills Sub-Tropical	30° 36' 09.85"	77° 11' 14.65"	644
Nagrota Bagwan	Kangra	-do-	32° 06' 22.35"	76° 22' 46.72"	861
Bajaura	Kullu	Mid Hills Sub-Humid Zone	31° 50' 54.25"	77° 09' 51.60"	1087
Palampur	Kangra	-do-	32° 06' 39.10"	76° 32' 10.51"	1268

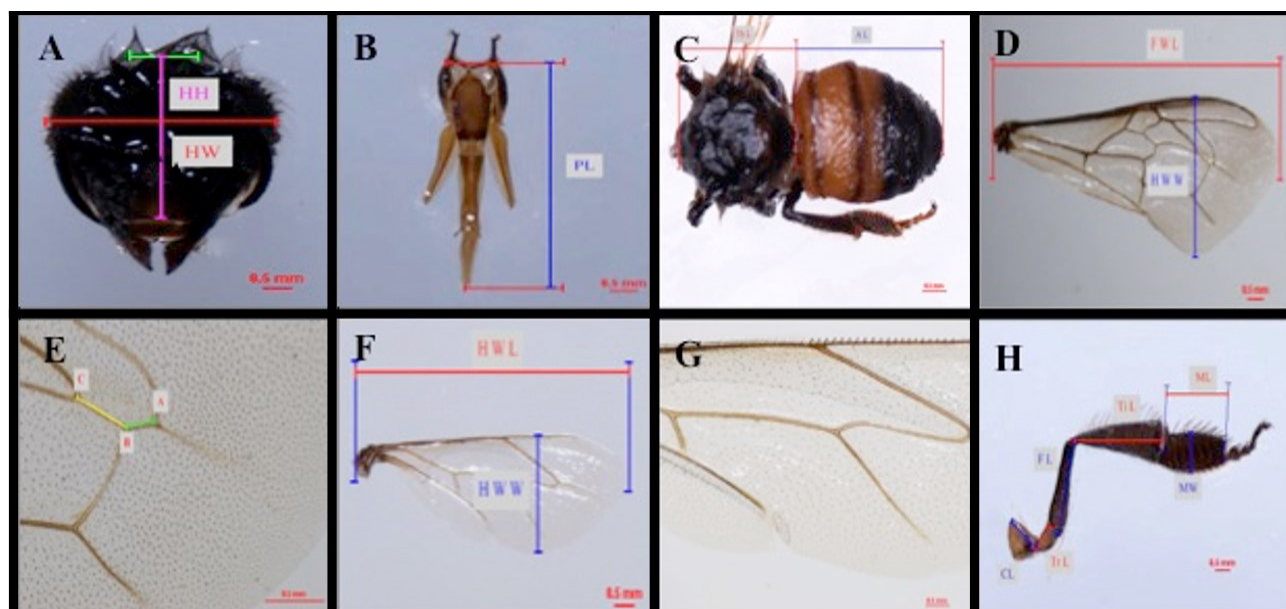


Fig. 1. Measurements of different characters (A) head, (B) proboscis, (C) thorax and abdomen, (D) fore wing, (E) cubital index, (F) hind wing, (G) hamuli, (H) hind leg. HH, head height; HW, head width; PL, proboscis length; TL, thorax length; AL, abdomen length; FWL, fore wing length; FWW, fore wing width; HWL, hind wing length; HWW, hind wing width; CL, coxa length; TrL, trochanter length; FL, femur length; TiL, tibia length; ML, metatarsus length; MW, metatarsus width.

Measurement and statistical analysis

Stereo-microscope (Nikon SMZ 745T) equipped with Image Analysis System (NIS elements D 3.1) was used for the measurement studies of the characters. Data thus obtained were analyzed following standard statistical procedures suggested by Gomez and Gomez (1984).

RESULTS*Head*

The head height in worker bees of *A. mellifera* varied from 3.14 ± 0.05 to 3.22 ± 0.03 mm between different locations of Himachal Pradesh where minimum and maximum values corresponded to Nagrota Bagwan and Palampur, respectively and differed significantly (Table II). However, head height of other locations was at par. The head width varied from 3.64 ± 0.04 – 3.72 ± 0.04 mm among the different populations.

The head width of *A. mellifera* collected from Bajaura and Nagrota Bagwan (both being at par) was significantly different from the populations of Palampur and Dhaulakuan (both being at par). The mean head height and width of *A. mellifera* was found to be 3.19 ± 0.10 and 3.68 ± 0.09 mm, respectively.

Proboscis length

Proboscis length is of extreme importance in honey production. Bees with a long proboscis such as Carniolan, Caucasian and Italian can work on red clover, while race with short proboscis cannot. In the present studies, the proboscis length of *A. mellifera* collected from different locations showed significant differences between the populations of Dhaulakuan (6.24 ± 0.01 mm) (at par with Nagrota Bagwan) and Bajaura (6.32 ± 0.03 mm, at par with Palampur) (Table II). The minimum proboscis length of *A. mellifera*, was recorded in the population of Dhaulakuan (6.24 ± 0.01 mm) whereas, it was maximum in Palampur (6.34 ± 0.03 mm).

The mean of proboscis length in different populations of *A. mellifera* was calculated to be 6.29 ± 0.06 mm.

Thorax and abdomen length

Among different populations of *A. mellifera*, the thorax length was minimum (4.14 ± 0.09 mm) in Dhaulakuan (being at par with Palampur) which differed significantly from Nagrota Bagwan (4.30 ± 0.07 mm) and Bajaura (4.38 ± 0.07 mm) (Table II). The mean thorax length of different locations of *A. mellifera* was 4.26 ± 0.20 mm. The abdomen length in *A. mellifera* varied from 5.54 ± 0.36 to 6.08 ± 0.36 mm, but without any significant differences between the populations of different locations. The mean abdomen length of *A. mellifera* of different locations was found to be 5.91 ± 0.93 mm.

Fore wings

The minimum fore wing length (8.88 ± 0.08 mm) in *A. mellifera* was observed for Dhaulakuan, whereas it was recorded to be 9.18 ± 0.05 , 9.19 ± 0.08 and 9.25 ± 0.08 mm in the populations of Bajaura, Nagrota Bagwan and Palampur (all being statistically at par), respectively (Table III). The mean wing length of populations from different locations was recorded to be 9.13 ± 0.18 mm. However, no significant differences were found in fore wing width among different populations of *A. mellifera* which ranged from 2.97 ± 0.05 to 3.02 ± 0.03 mm. The mean of fore wing width was calculated as 3.00 ± 0.08 mm.

The minimum cubital index (2.02 ± 0.13) was calculated in the population of Bajaura, which was significantly different from the populations of Dhaulakuan (2.25 ± 0.17), Nagrota Bagwan (2.25 ± 0.13) and Palampur (2.28 ± 0.14). The mean cubital index was 2.20 ± 0.36 .

Hind wings

The data on the length and width of hind wing and number of hamuli were taken in account (Table III).

Table II.- Morphometrics of head, proboscis, thorax and abdomen of *A. mellifera*.

Location	Head height (mm)	Head width (mm)	Proboscis length (mm)	Throax length (mm)	Abdomen length (mm)
Dhaulakuan	3.20 ± 0.03	3.72 ± 0.03	6.24 ± 0.01	4.14 ± 0.09	5.96 ± 0.30
Nagrota Bagwan	3.14 ± 0.05	3.65 ± 0.04	6.25 ± 0.04	4.30 ± 0.07	6.07 ± 0.51
Bajaura	3.19 ± 0.04	3.64 ± 0.04	6.32 ± 0.03	4.38 ± 0.07	5.54 ± 0.36
Palampur	3.22 ± 0.03	3.71 ± 0.04	6.34 ± 0.03	4.23 ± 0.09	6.08 ± 0.36
Mean	3.19 ± 0.10	3.68 ± 0.09	6.29 ± 0.06	4.26 ± 0.20	5.91 ± 0.93
LSD (P = 0.005)	0.06	0.05	0.04	0.11	NS

Average of 25 bees followed by Standard Error; NS, Non-significant.

Table III.- Morphometrics (fore and hind wing), cubital index and number of hamuli of *A. mellifera*.

Location	Fore wing length (mm)	Fore wing width (mm)	Cubital index	Hind wing length (mm)	Hind wing width (mm)	No. of hamuli
Dhaulakuan	8.88±0.08	3.02±0.02	2.25±0.17	6.20±0.06	1.82±0.02	22.00±0.47
Nagrota Bagwan	9.19±0.08	3.00±0.03	2.25±0.13	6.41±0.06	1.79±0.02	20.48±0.54
Bajaura	9.18±0.05	2.97±0.05	2.02±0.13	6.41±0.05	1.78±0.02	20.52±0.56
Palampur	9.25±0.08	3.02±0.03	2.28±0.14	6.48±0.07	1.82±0.02	20.52±0.59
Mean	9.13±0.18	3.00±0.08	2.20±0.36	6.38±0.14	1.80±0.05	20.88±1.32
LSD (P = 0.005)	0.11	NS	0.20	0.08	0.03	0.75

Average of 25 bees followed by Standard Error; NS, Non-significant.

Among the populations of *A. mellifera*, Dhaulakuan showed the minimum wing length (6.20±0.06 mm) which was significantly different from the populations of all other locations *i.e.* Bajaura (6.41±0.05 mm), Nagrota Bagwan (6.41±0.06 mm) and Palampur (6.48±0.07 mm), all being statistically at par. The hind wing width varied from 1.78±0.02 to 1.82±0.02 mm among different populations of *A. mellifera*. The population from Bajaura showed minimum hind wing width (1.78±0.02 mm) which was significantly different from Palampur and Dhaulakuan (1.82±0.02 mm each). The wing width of bees (1.79±0.02 mm) from Nagrota Bagwan and Dhaulakuan was significantly at par. The mean wing length and width of different location was found to be 6.38±0.14 and 1.80±0.05 mm, respectively.

The population of *A. mellifera* collected from Dhaulakuan was found to have significantly higher number of hamuli (22.00±0.47) as compared to Nagrota Bagwan, Palampur and Bajaura (all being statistically at par). The mean number hamuli in *A. mellifera* populations were counted to be 20.88±1.32.

Hind legs

The various parts of hind legs of *A. mellifera* worker bees were summarized in Table IV and discussed as under:

Coxa

The coxa length in *A. mellifera* varied from 1.06±0.04 to 1.18±0.04 mm in different locations of Himachal Pradesh. The maximum value (1.18±0.04 mm) was observed in population of Dhaulakuan which was significant higher than other samples while, the minimum value was recorded in Bajaura population, being statistically at par with populations of Nagrota Bagwan and Palampur. The mean coxa length in the populations of *A. mellifera* was found to be 1.10±0.09 mm.

Trochanter

No significant variations in the length of trochanter could be found in the bee collected from different locations of Himachal Pradesh and it ranged from 0.76±0.04 to 0.81±0.04 mm.

Femur

The maximum femur length (2.55±0.04 mm) was recorded from Palampur population which was significantly different from other populations, whereas the minimum value (2.41±0.04 mm) was recorded from Dhaulakuan population. Femur length of the bee from Dhaulakuan, Bajaura and Nagrota Bagwan were at par. The mean of femur length in different populations was found to be 2.45±0.10 mm.

Tibia

No significant differences were observed in tibia length of different populations in *A. mellifera*. It was found to be ranging from 2.87±0.08 to 2.96±0.04 mm. The mean of tibial length was found to be 2.91±0.13 mm.

Metatarsus

The metatarsus length varied from 1.86±0.03 to 1.98±0.03 mm. Samples from Bajaura showed minimum metatarsus length (statistically at par with Nagrota Bagwan) which was found significantly different from Dhaulakuan samples (being at par with Palampur). Similarly, the maximum metatarsus width (1.12±0.04 mm) was recorded from the population of Palampur and it was statistically at par with populations of Dhaulakuan and Nagrota Bagwan but differed significantly from the samples of Bajaura. The populations of Bajaura and Nagrota Bagwan were statistically at par in their metatarsus width. The mean values of length and width of metatarsus of *A. mellifera* were observed to be 1.92±0.07 and 1.08±0.07 mm, respectively.

Table IV.- Morphometrics of different parts of hind leg of *A. mellifera*.

Location	Coxa length (mm)	Trochanter length (mm)	Femur length (mm)	Tibia length (mm)	Metatarsus length (mm)	Metatarsus width (mm)
Dhaulakuan	1.18±0.04	0.76±0.04	2.41±0.04	2.90±0.06	1.96±0.03	1.09±0.02
Nagrota Bagwan	1.06±0.04	0.81±0.04	2.43±0.04	2.87±0.08	1.89±0.02	1.08±0.02
Bajaura	1.06±0.03	0.80±0.04	2.42±0.04	2.89±0.04	1.86±0.03	1.04±0.02
Palampur	1.11±0.04	0.79±0.05	2.55±0.04	2.96±0.04	1.98±0.03	1.12±0.04
Mean	1.10±0.09	0.79±0.10	2.45±0.10	2.91±0.13	1.92±0.07	1.08±0.07
LSD (P = 0.005)	0.05	NS	0.06	NS	0.04	0.04

Average of 25 bees followed by Standard Error; NS, Non-significant.

DISCUSSION

A honey bee colony comprises of a queen, hundreds of drones and thousands of worker bees. The workers are the dedicated individuals and perform different duties including honey, pollen collection, etc. The collection of pollen and production of honey not only depend upon the floral abundance of the area but also on the morphological characters of workers such as body size, proboscis length, corbícula, etc. (Alburaki and Alburaki, 2008). 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 (Mostajeran et al., 2006). Body size and hind leg length affect pollen carrying capacity of honey bees (Morimoto, 1968). Corbícula size, fore and hind wing areas have the direct correlation with the production potential of the honey (Szabo, 1990). Wing morphometry is important for classification of races and their size affects the flight ability of worker honey bees (Horowitz, 1983).

In India, *A. mellifera* was firstly introduced at Bee Research Station, Nagrota Bagwan (Himachal Pradesh). For the purpose, *A. mellifera* and its hybrid were obtained from USA and Italy between the years 1962 and 1964. Presently, the populations available in India have its genetic pool from the blood of various races and the race of the bee species is considered as *A. mellifera ligustica* (Mishra, 1995). After five decades of its introduction and establishment in north-western Himalayan region of India, the morphometric studies on worker bees were conducted and compared with available literature in India and the World.

In present investigation, the mean head height and width was found to be 3.19±0.10 and 3.68±0.09 mm, respectively. These parameters have not been much studied earlier for *A. mellifera*, except the report of Sharma (1990) who observed the mean head height (3.19 mm) and width (3.78 mm) of *A. mellifera* in India which is near to the present findings. The proboscis length of various geographical races of honey bees has

shown considerable variations. Ruttner (1988) 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 (6.29±0.06 mm) wherein slight variations might be due to different geographical races. Further, Atwal and Sharma (1968) also concluded that *A. mellifera* in India is a Californian strain. 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 armenica*, *A. mellifera meda*, *A. mellifera anatoliaca* and *A. mellifera pomonella*, respectively (Sheppard and Meixner, 2003). The proboscis length observed in the present investigations is in close proximity of *A. mellifera meda* and *A. mellifera ligustica*. In India, different workers have reported the proboscis length of *A. mellifera* as 6.37±0.05 (Sharma, 1990) and 7.00 mm (Jagannadham and Goyal, 1983). Information on variability in thorax and abdomen length of *A. mellifera* are not available in the literature. Present findings add information on mean thorax length and abdomen length in *A. mellifera*. The mean fore wing length and width was recorded to be 9.13±0.18 and 3.00±0.08 mm in present investigation. Earlier, the fore wing length and width have been reported to be variable (Ruttner, 1988; Jevtic et al., 2007; Adl et al., 2007) which can be attributed to altitude or geographical races. Dyer and Seeley (1987) reported the fore wing length in *A. mellifera* of different attitudes between 7.64 to 9.70 mm. Jevtic et al. (2007) 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. In India, Sharma (1990) examined the morphometrics of the hind wings of *A. mellifera* and observed the length and width as 6.436±0.084 and 1.925±0.060 mm, respectively which is slightly higher compared to the present results (6.38±0.14 and 1.80±0.05 mm, respectively). Elsewhere in the world, variable morphometrics of hind wings has been

Table V.- Morphometrics (proboscis and fore wings), cubital index (fore wing) of *A. mellifera* races (Ruttner, 1988).

Subspecies	Distribution	Proboscis length	Cubital index	Fore wing length
<i>mellifera</i>	West Mediterranean and North Europe	6.05	1.84	9.33
<i>carnica</i>	Central Mediterranean and South-east Europe	6.40	2.59	9.40
<i>ligustica</i>	Central Mediterranean and South-east Europe	6.36	2.55	9.21
<i>caucasica</i>	Western and South-western part of the Caucasus are available from Abkhazia and Circassia and Near East	7.05	2.16	9.51
<i>meda</i>	Near East	6.33	2.56	9.07
<i>anatoliaca</i>	Near East	6.46	2.24	9.27
<i>pomonella</i>	Central Asia	6.41	2.24	-

reported in *A. mellifera* (Rahimi and Asadi, 2009; El-Aw et al., 2012). Work on the hamuli count in *A. mellifera* is not available under Indian conditions thus present findings add information on number of hamuli in *A. mellifera*. Sharma (1990) measured the coxal length as 1.09 ± 0.088 mm in *A. mellifera* which substantiate the present findings (1.10 ± 0.09 mm). The information on variability of trochanter length is not available in the literature. Earlier, Sharma (1990) reported the femur length in *A. mellifera* as 2.41 ± 0.05 mm which is in close proximity of the present findings (2.45 ± 0.10 mm). However, El-Aw et al. (2012) 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* (Gencer et al., 2004; Adl et al., 2007). The mean of tibial length was found to be 2.91 ± 0.13 mm in present investigation. The tibial length in *A. mellifera* has been reported by different workers as 3.01 ± 0.10 for Indian population (Sharma, 1990), 2.83 ± 0.08 mm for Egyptian population and 3.03– 3.22 mm for Iranian population (Adl et al., 2007) and 3.16 to 3.18 mm for bees population in Turkey (Gencer et al., 2004). In present investigation, the mean length and width of metatarsus were observed to be 1.92 ± 0.07 and 1.08 ± 0.07 mm, respectively. Earlier, Sharma (1990) found higher dimensions of metatarsus in *A. mellifera* from Himachal Pradesh (India). In other studies the dimension of metatarsus were also observed to be higher (length 2.00 to 2.44 mm, width 1.08 to 1.19 mm) for the same bee species (Alqarni et al., 2011; Adl et al., 2007; El-Aw et al., 2012).

Further, the morphometrics of three characters namely, proboscis length, fore wing length and cubital index of *A. mellifera* were compared with the earlier reports of Ruttner (1988) presented in Table V. In the present investigations, proboscis length, cubital index and fore wing length of *A. mellifera* were found to be ranging from 6.24 ± 0.01 to 6.34 ± 0.03 , 2.02 ± 0.13 to 2.28 ± 0.14 and 8.88 ± 0.08 to 9.25 ± 0.08 mm, respectively. The mean proboscis length (6.29 ± 0.06 mm) and fore wing length

(9.13 ± 0.18 mm) were near to *A. mellifera meda* and *A. mellifera ligustica*. However, the mean cubital index (2.20 ± 0.36 mm) was found near to *A. mellifera caucasica* and *A. mellifera anatoliaca*. These differences might be due to many factors such as varying geographical conditions and presence of different races. Besides, bee samples collected during different seasons even at the same place also have remarkable effect on the biometry of honey bees (Mattu and Verma, 1984).

CONCLUSION

It can be inferred that the populations of *A. mellifera* at different locations of Himachal Pradesh (India) showed variations in morphometrics characters. Overall, *A. mellifera* populations in Himachal Pradesh showed reduced proboscis length, fore wing length and cubital index when compared with earlier report on *A. mellifera ligustica*.

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Conflict of interest statement

We declare that we have no conflict of interest.

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