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Taxonomic study of *Apis cerana* (Fabricius, 1793), collected from *Prunus persica* (L.) Stokes flowers from different areas of Himachal Pradesh

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Abstract
Apis cerana is an important insect pollinator of *Prunus persica* plant and samples were collected from eleven localities of Himachal Pradesh i.e. Mashobra (2146 m), Summer Hill (2100 m), Naldera (1887 m), Jatoli (1464 m), Kandaghat (1425 m), Jaladi (508 m), Hamirpur (786 m), Gheori (786 m), Bathra (503 m), Bilaspur (673 m) and Rajgarh (1555 m). During present investigation, the samples of *Apis cerana* was identified on the basis of morphological features, wing venation and external genitalia. Study of morphological variations, wing venation and genitalia of insects help in identify the relationship between morphology, and ecology and also provide the information about their evolution. The taxonomic and morphological features of insects helpful in their classification, proper identification and conservation of many endemic insect species.

Keywords: *Apis cerana*, *Prunus persica*, wing venation, genitalia

Introduction
Bees, given their major role as pollinators, are important both economically and ecologically. Nearly three-fourths of all the cultivated crop species depend on insects including bees for pollination. Geographical and ecological variations are the key factors in the evolution of bees (Ruttner, 1978) [8]. Study of morphological variations of animals help in identify the relationship between morphology, and ecology and also provide the information about their evolution (Losos, 1990; Ricklefs and Miles, 1994) [5, 7]. But, many closely related species shows similar morphological character and are difficult to identify at subspecies level, such species differentiated by studying their wing venation and genitalia (Dias *et al*., 2010) [3]. In insects wings vary in size, shape, markings, and venation pattern, and these characteristics can be used to identify species up to the subspecies level. The pattern of venation in insects also provides information about their phylogenetic and developmental process. In the morphological and taxonomic perspective, genitalia study has gained significant interest during the past few years. Zander (1900, 1901, 1903) [13-15] suggested a common genitalia structural plan for various insects.

Materials and Methods
The samples of *Apis cerana* were collected from different areas of Himachal Pradesh i.e. Mashobra (Shimla), Summer Hill (Shimla), Naldera (Shimla), Jatoli (Solan), Kandaghat (Solan), Jaladi (Hamirpur), Hamirpur, Gheori (Kangra), Bathra (Kangra), Bilaspur and Rajgarh (Sirmaur) (Table 1). The collected specimens were killed with the help of benzene vapors and then pinned by entomological pins. For the examination of genitalia, the abdomen of *Apis cerana* was detached and then put into 10% KOH solution. The material was then washed with distilled water and was dissected in 10% alcohol. Genitalia were removed carefully with the help of fine needles and analyzed under NIS elements imaging software with the help of a digital camera (Nikon SMZ1270). For wing venation, wings were detached from the thorax of the insect specimens and transferred to the higher grades (80% and 90%) of ethyl alcohol for 5 to 10 minutes, followed by staining in alcohol eosin. After proper dehydration in alcohol, the wings were mounted on the glass slide in Canada balsam. The wing veins were named according to Comstock and Needham scheme (Comstock and Needham, 1899; Wootton, 1979) [2].

Table 1: Physiographic details of different sites selected for the collection of *Apis cerana*

S. No.	Locality	District	Altitude (Meter)	Latitude (North)	Longitude (East)
1.	Mashobra	Shimla	2146 m	31°-12′96	77°-22′83
2.	Summer Hill	Shimla	2100 m	31°-11′46	77°-13′99
3.	Naldera	Shimla	1887 m	31°-18′39	77°-18′69
4.	Jatoli	Solan	1464 m	30°-87′96	77°-12′66
5.	Kandaghat	Solan	1425 m	30°-97′02	77°-10′54
6.	Jaladi	Hamirpur	508 m	31°-77′85	76°-34′44
7.	Hamirpur	Hamirpur	786 m	31°-68′62	76°-52′13
8.	Gheori	Kangra	559 m	31°-85′02	76°-18′90
9.	Bathra	Kangra	503 m	31°-88′18	76°-21′46
10.	Bilaspur	Bilaspur	673 m	31°-34′07	76°-68′75
11.	Rajgarh	Sirmaur	1555 m	30°-85′00	77°-29′94

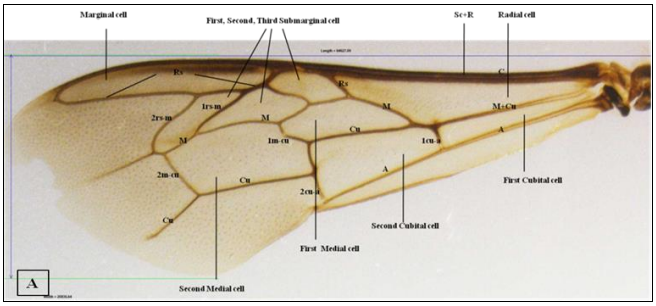
Results and Discussion

Material Examined: 2ex., 22.ii.19**,** Gheori; 4ex., 25.ii.19, Bathra; 2ex., 27.ii.19, Jaladi; 3ex., 1.iii.19, Hamirpur; 3 ex., 4.iii.19, Bilaspur; 2ex., 7.iii.19, Rajgarh; 4ex., 9.iii.19, Jatoli; 2ex., 13.iii.19, Kandaghat; 5ex., 16.iii.19, Mashobra; 3ex., 20.iii.19, Summer Hill; 4ex., 24.iii.19, Naldera; 4ex., 20.ii.21**,** Gheori; 5ex., 23.ii.21, Bathra; 3ex., 25.ii.21, Jaladi; 2ex., 3.iii.21, Hamirpur; 2 ex., 6.iii.21, Bilaspur; 3ex., 9.iii.21, Rajgarh; 3ex., 12.iii.21., Jatoli; 3ex., 15.iii.21, Kandaghat; 3ex., 19.iii.21, Mashobra; 3ex., 21.iii.21, Summer Hill; 2ex., 26.iii.21, Naldera (Collector: Poonam Dhiman).

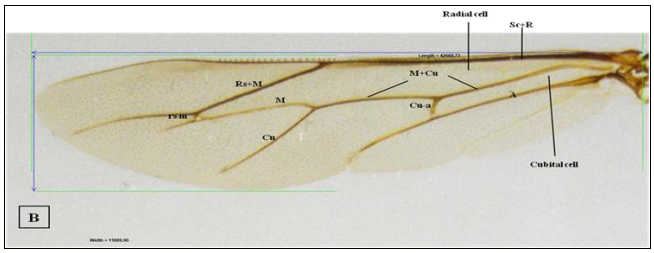
Diagnostic Features: Worker: Head and thorax black, and of same size; ovate eyes; elongated clypeus. Thorax with fore and hindwings; forewing longer and narrow; body covered with plumose branched hair. Abdomen with four abdominal strips, truncate and convex at base; stoulish legs with pollen basket; spine present at anterior and intermediate tibiae; posterior tibiae without spines and shiny; curved sting.

Apis cerana Fabricius

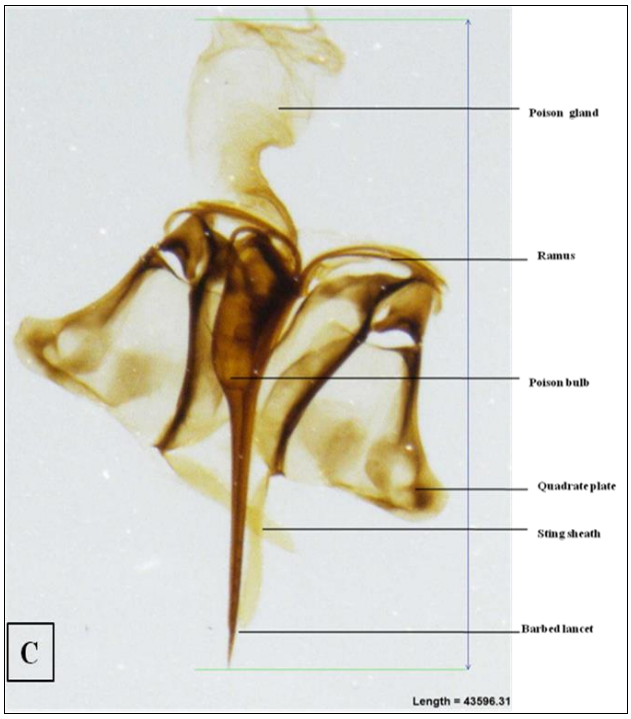
Forewing: Larger and narrow than hindwings with dark brown veins. Costa (C), first longitudinal vein; dark brownish; arise from the axillary area and running upto apical margin. Below costa subcosta (Sc) fused with radius (Sc +R), subcosta (Sc) running upto the middle of wings. Radius (R) vein moved forward upto the apical margin and bifurcated, formed a rounded marginal cell and three submarginal cells. Radius vein (R) connected to media vein (M) by two cross veins i.e. 1rs-m and 2rs-m. A thick, dark vein M+Cu emerges from middle of axillary area, moved upto the middle of wings and bifurcated into two individual veins i.e. Media (M) and cubitus (Cu). Two cross veins i.e. 1m-cu and 2m-cu connected the media (M) with the cubitus (Cu) and formed two medial cell. Anal vein arises from anal margin of the wing, connected to cubitus by 1cu-a and 2cu-a (Fig. A).



Forewing: *Apis cerana*



Hindwing: *Apis cerana*



Genitalia: *Apis cerana* Worker Bee

Hindwing: Subcosta (Sc) fused with radius vein (Sc+R), Sc ends upto the middle of wing, radius vein running forward upto apical margine. A cross vein rs-m connected the radius (R)with media (M) and formed a irregular redial cell. Media (M) and cubitus (Cu) fused (M+Cu), moved forward upto the middle and bifurcated into individual veins. A dark brown, convex anal vein (A) emerges from the anal margin and connected to cubitus by single cross vein i.e. cu-a (Fig. B).

Genitalia: Workers ovary reduced and ovipositor modified into sting apparatus which consists of poison gland, poison bulb, quadrate plates, sting sheath and barbed lancet. A large and oval shaped poison gland attached at the apex of sting apparatus. Barbed lancet arises from middle of sting, quadrate plates large and present at both the sides (Fig. C).

These results support the previous findings of Sakagami *et al*. (1980) [9], who reviewed morphological traits that varied between *Apis laboriosa* and *Apis dorsata*. Mendes *et al*. (2007) [6] and Francoy *et al*. (2008) [4], who suggested study of wing venation as an important tool for identification of various honeybees species and subspecies. Similarly, Aytekin *et al*. (2007) [1] studied the wing morphology and venation pattern in the wings of *Apis cerana*, *Apis mellifera* and in bumble bee species. Tan *et al*. (2008) [11] examined the wing venation pattern in *Apis cerana* and observed adventitious distal abscissa in the forewing arising from posterior edge. These results are similar to the findings of Surendra *et al*. (2012) who studied the morphology of sting apparatus of four species of *Apis* i.e. *Apis dorsata*, *Apis cerana*, *Apis florea* and *Apis mellifera* and observed significant variation in the length and width of sting apparatus between different *Apis* species.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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