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Ultra structural comparison of tongue in *Apis cerana* (Hymenoptera: Apidae): Plains and hill populations

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Abstract
Tongue of honey bees is modified for collecting nectar. Regarding their nutritional requirements, taste perception plays vital role in the life of honey bees. Honey bee biodiversity with respect to sensory structures on tongue can be analyzed by Scanning electron microscopy. This technique offers an advanced diagnostic tool to study honey bee biogeography and to determine adaptive variations to native flora. Mouth parts have been considered as an important morphological character for beneficial exploitation under the electron microscope. This aspect has, however, not been sufficiently exploited. In the present investigation, scanning electron microscopic studies on tongue of *Apis cerana* plains and hill populations have been performed and significant differences in the arrangement of spines in the middle region of tongue have been observed. It is therefore imperative that before intraspecific hybridization further complicates the scenario, modern taxonomic tests which can supplement the already existing information on honey bee biosystematics are exploited and the taxa placed on firm footing.

Keywords: *Apis cerana*, plains, hills, tongue, scanning electron microscopy

1. Introduction
The mouth parts of honey bees are of chewing and lapping type. They can act upon solid material as well as lap up liquids. The mandibles are of chewing type. Mandibles, also used in moulding of wax are attached on the sides of the head and the proboscis or tongue is made up of the maxillae and the labium. The maxillae and labium are developed into a series of flattened elongate structures to form a proboscis [1]. The glossa of labium is greatly elongated, covered with hair and ends in a small rounded lobe, the flabellum forming a flexible tongue [2]. The glossa, which is a muscular tube on coming in contact with the nectar at the bottom of a corolla tube by capillary action draws nectar up to its base [3]. The glossa is also important for pollen collection [4]. Liquids are absorbed by the flabellum which is present at the tip, into the mouth by a narrow channel [2]. The present study determined differences in the tongue of native species *viz.*, *A. cerana*- plains and hill populations of honey bees at electron microscopic level.

2. Materials and Methods
Study area and sample collection
Cavity-dwelling species *A. cerana* F. was taken for the present study. The study material was collected during the spring season. *A.cerana* (plains type) was collected from apiaries in Chandigarh and that of hilly region was taken from the high-hills of Distt. Kinnaur in Himachal Pradesh.

Scanning electron Microscopy
Preservation
The collected material of *A.cerana* was preserved in 5% glutaraldehyde and the protocol given by [5] was followed for electron microscopy.

Preparation of material for scanning electron microscopy
The tongue was carefully excised from the freshly collected worker bees of *A.cerana*. These were then washed with phosphate buffer 2 to 3 times and then dehydrated through graded series of acetone and dried in a critical point drier. Dehydrated samples were mounted on slides in the desired orientation with the help of double side adhesive tape under binocular

microscope. The samples were attached in such a way that they became visible from all sides. The stubs were placed inside the sputter for gold coating to overcome the problem of "charging" and "beam damage". The sputtered specimens were examined in Jeol JS-6100 scanning electron microscope operated at an acceleration voltage of 10KV at Regional sophisticated instrumentation centre, Panjab University, Chandigarh. The results of scanning were preserved as photographs used in this presentation.

3. Results

Apis cerana (plains population) - In the basal region, the proximal part of tongue of *A.cerana* showed an arrangement of short, spinous structures on the ridges in an irregular fashion (Fig 1).

Fig 1: SEM of tongue of *A.cerana* (plains population) (Bar= 100 µm).

Middle region formed sucking apparatus by creating suction with the help of unevenly arranged hair. The hair were long and covered about half the length of the tongue (Fig 2).

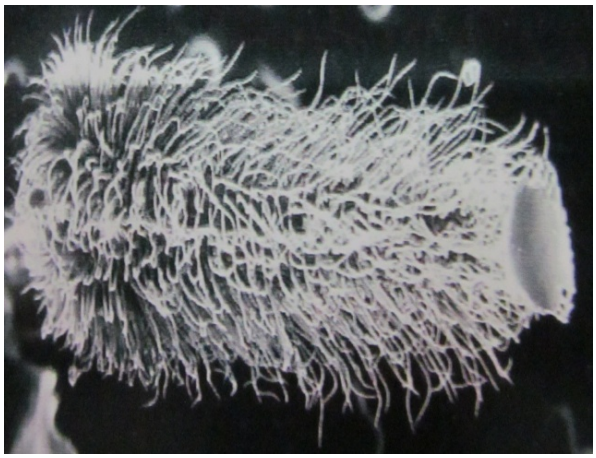

Fig 2: SEM of middle part of tongue of *A.cerana* (plains population) (Bar= 100 µm).

The distal portion, the flabellum *i.e.* the fluid absorbing organ was rhomboidal in shape. The distal margin of it was fringed with row of distinct processes and tips of these were further divided in a characteristic pattern showing several branches (Fig. 3).

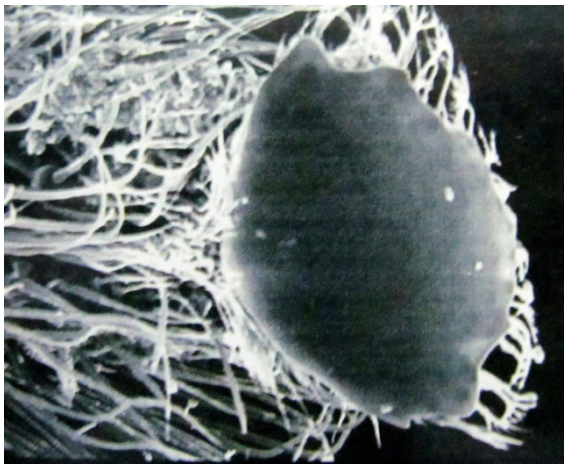

Fig 3: SEM of flabellum of *A.cerana* (plains population) (Bar=10 µm).

Apis cerana (hills population) – The tongue of *A.cerana* of hills population showed the same three principal divisions and their ultra-structural details are presented below- The basal region possessed ridges and spines were present on these ridges which gave it a rough surface (Fig.4).

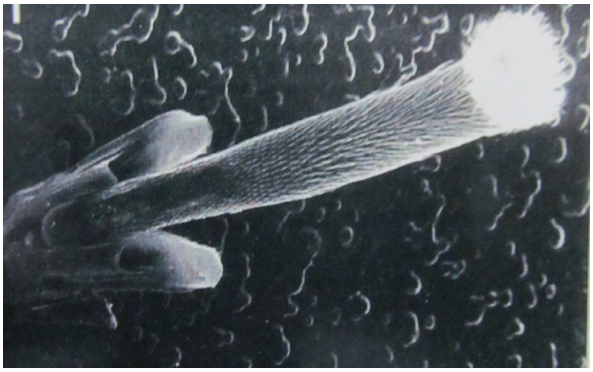

Fig 4: SEM of tongue of *A.cerana* (hill population) (Bar= 100 µm).

The middle grooved region formed sucking portion along with evenly arranged hair. The hair were observed to be long, stiff and unbranched structures (Fig 5).

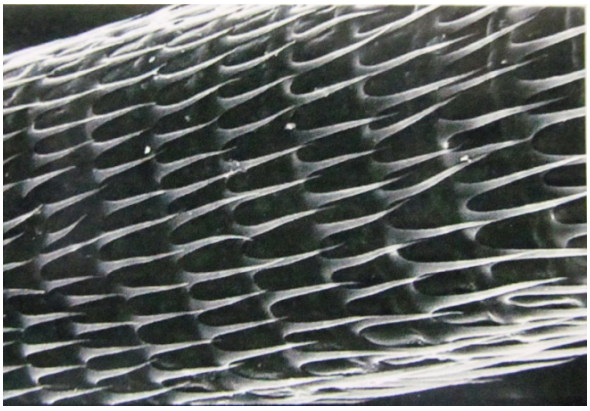

Fig 5: Higher magnification of middle part showing arrangement of hair (Bar= 100 µm).

The distal region forming the flabellum which worked for the absorption of liquid fluid was rhomboidal in shape. The distal margins showed distinct processes, branched at the tips (Fig 6).

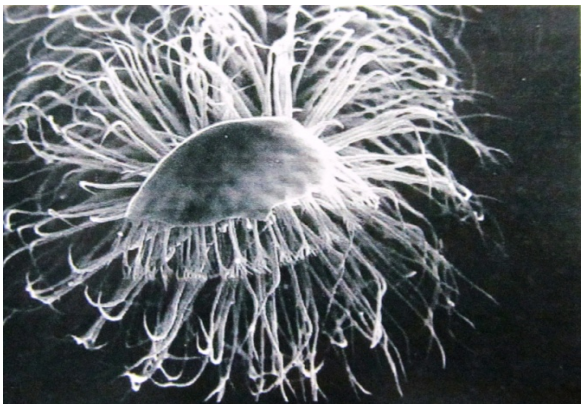

Fig 6: SEM of flabellum showing branched processes (Bar= 100 µm).

4. Discussion

[6] Performed SEM studies on *A.mellifera* L. and observed gustatory sensilla in the form of hair (chaetic sensilla) or pegs (basiconic sensilla). [7] While working on the mouth parts of worker *A.mellifera* reported the sensilla and their distribution on the labrum, mandibles and maxillae. Differences in the shape of the sensory structures of tongue of *A.dorsata* F. from Nurpur and Jaipur have been reported by [8]. They reported different types of sensilla found on prementum, labial palps, galeae and glossa and attributed these to different environmental conditions.

Several variants of the Asian honey bee *A.cerana* have been reported from time to time. [9, 10, 11, 12] have been able to distinguish 2 ecotypes in sub-species of *A.c.cerana*. On the basis of computer-based biometric data [13, 14, 15] has distinguished 4 races/sub-species of *A.cerana viz., A.c.cerana, A.c. himalaya, A.c. indica* and *A.c. japonica.* [16] Collected together much scattered information and reported the presence of 4 distinct sub-species of *A.cerana.* [17, 18, 19] identified *A.c. cerana* ecotypes: high-hill, mid-hill and foot-hill on the basis of biological and behavioral parameters. Even each geographic race has been shown to have locally adapted populations called ecotypes which differ from each other in several biological and economic traits [20, 21, 22, 23, 11, 10, 24, 13, 19]. [25] Identified characteristic ultra-structural variations in different parts of tongue of two cavity-nesting species- *A.cerana* and *A.mellifera*. Scanning electron microscopic studies on tongue of open-nesting honey bee's *A. dorsata* F. and *A. florea* F. have been performed by [26]. They revealed differences in proximal region, middle region and flabellum of the tongue in these two species accounting for the variation.

5. Conclusion

The findings during the present investigation are interesting and helped to identify characteristic ultra-structural variations in different parts of the tongue of the plains and hill populations of *A.cerana*. The arrangement of spines on the sucking plate differed in the two populations reflecting variation under the influence of native flora. The present findings are illuminatory and emphasize advantage of applying and incorporating SEM data for separating the two populations of *A.cerana*.

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