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Light and Scanning Electron Microscopy Studies on the Parasitic Mite Affecting Honey Bee *Apis mellifera*

[1]Anurag Kumar Sonker, [2]Kamal Jaiswal, and [3]Suman Mishra*

Author's Affiliation:
[1,2,3] Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India

***Corresponding Author:**
Prof. Suman Mishra
Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India
E-mail: drsumanmishra@gmail.com

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ABSTRACT

The most significant of the social insects, honey bees *(Apis mellifera*) are the primary pollinators of domesticated plants and contribute to the stability of ecosystems. The decline in the number of bee populations due to parasitic mites has a substantial and detrimental effect on the honey bee population. Mites (Acari) have grown to be a serious global issue for beekeepers all over the world. The current study was designed to investigate the ultra-structure and morphology of the parasitic mite (*Tropilaelaps)*, an ectoparasite of the European honeybee, *Apis mellifera.* In order to conduct this study, the mites were collected from apiaries located in and around different regions of Lucknow, Uttar Pradesh. The morphological identification was done by Light Microscopy while the ultra-structural assessment was carried out with the help of a Scanning Electron Microscope (SEM). The results of this preliminary study revealed the presence of one of the main damaging genera of mites viz., *Tropilaelaps*. Light and Scanning electron microscopy revealed the dorsal shield, the ventral shields (metapodal shield, epigynal shield, and anal shield), and the genital plate on the ventral side. Peritreme, tritosternum, gnathosoma, chelicerae, and four pairs of legs were observed in detail as well as the presence of irregular setae all over the body. The study of morphological features of the *Tropilaelaps* mite will provide additional insights into the taxonomy of the parasite as well as contribute information towards further management of these parasites.

KEYWORDS: *Parasitic mite, Apis mellifera,* Acari, *Tropilaelaps, Light microscopy, Scanning Electron Microscopy.*

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INTRODUCTION

The honey bee, *Apis mellifera,* is a social insect and one of the most important pollinators of cultivated plants. It also plays a key role in ecosystem sustainability. The decline in the number of bee populations has a substantial and detrimental effect on the honey bee population. Bee populations are declining due to multiple factors, including pests, fungi, parasites,

genetically modified crops, diseases, viruses, and parasitic mites (Muli *et al*., 2014; Akimov *et al*., 2004). The parasitic mite, *Tropilaelaps*, that affects honey bees is currently one of the most destructive parasites for the honey bee colonies (Boot *et al*., 1995).

The mite of genus *Tropilaelaps* is an ectoparasite of bee brood. Morphologically, the adult *Tropilaelaps* mite is smaller (about 1 mm) in length, and light brown in color, their first pair of legs is erect in position and shows morphological similarity to the antennae of arthropods. During the development of *Tropilaelaps*, nymph stages are easily recognized by the naked eye due to their white-colored appearance. The adult female mite is mostly found inside the capped bee brood cells of worker and drone bees of infested colonies, where they easily reproduce and increase their population size (Anderson and Roberts, 2013). They appear to be moving quickly on the surface of bee broods in bee colonies where a large number of bee broods are present (Anderson and Morgan, 2007).

Morphologically adult female mites present their body shapes differently as compared to other infecting mites of the honey bee *Apis mellifera*. Additionally, there are also morphological differences between the sexes, with male mites often being smaller than females and having a sharply pointed approach to their posterior region. Female mites also have a short epigynial thoracic plate as compared to male mites. Males are considerably less prevalent as compared to females.

The parasite mite feeds on the larvae and nymphs of the honey bee resulting in deformity and death and gradually decreases the numbers of the bee population in the hives. The mature mites adhere to the adult bee and exhibit their foraging behavior, but sometimes they are not able to puncture the cuticle of an adult bee, they are unable to feed on the haemolymph of bees. Additionally, they are incapable to survive in a colony of bee brood for longer than 74 hours (Delfinado and Baker, 1961).

The mites *Tropilaelaps* take approximately one week for their life cycle and during their developmental stages, the transformation of the egg into the adult bee takes approximately 6–7 days. The mother mites and their offspring emerge from the bee brood with the help of adult bees, enter into the other bee brood cells directly and mate with the opposite sex in the bee colony. During this period, when the host queen bee does not lay the eggs, the adult *Tropilaelaps* mites enter into the phoretic stage and become attached to the bees' sclerites. After that, the queen bee survives hardly for a few days due to the inability of bee brood colonies (Koeniger and Muzaffar, 1988; Rinderer *et al*., 2001).

Adult bees serve as intermediate hosts, and they are responsible for the infection that occurs between healthy bees. The majority of bees are lost when they were still in the larval stage of their development, mostly drones are generally preferred, rather than worker bees (Allen and Ball, 1996; Koeniger and Muzaffar, 1988**).** The mites feed on the haemolymph of growing larvae, pupae, as well as fully grown adult bees, during their developmental phases. During times of heavy infection, the pupae of bee colonies do not mature into adult bees or may develop abnormally, resulting in shortened abdomens, undeveloped wings, deformed legs, and lower weights. A consequent result is that the lifespan of adult bees declines.

During severe infestations, the host's pupae may not mature into adult bees or they may do so in an unusual way that causes them to have shorter abdomens, malformed wings, distorted legs, and lower weights, all of which decrease their mortality (Griffiths *et al*., 1988; Dejong *et al.,* 1982**).**

Scanning electron microscopy (SEM) is generally performed to carry out the morphological identification of the mite (Bautz and Coggins, 1992), and the functional morphology of the mouthparts plays a crucial and significant role in the host-parasite relationship (Griffiths *et al*., 1988). Based on light microscopy, SEM, and transmission electron microscopy (TEM) analysis, the Russian monograph of the mite noted that the important mechanism can be revealed by morphology, systematic, and

ecology of the mite of the honeybee, *Apis mellifera* (Akimov *et al.*, 2004).

It has been determined that the mite weakens the honey bees' immune system by inhibiting the expression of genes related to immunity. As a result, it has been determined that the honey bees and their colony are seriously threatened by the mite infection. According to Akimov*et al.* (2004), the first colony losses due to mites occurred in the 1960s in China and Eastern Russia. Since then, it had been reported that the mite population had expanded quickly to all regions where *Apis mellifera* bees were present.

Keeping the importance of mites as a serious threat to the honey bee, *Apis mellifera,* this study was carried out with an aim to get detailed information regarding the morphology and microtopography of this significant ectoparasitic mite using light microscopy and SEM.

MATERIALS AND METHODS

Study area: The study was carried out in Lucknow, Uttar Pradesh, and adjacent areas where the samples were collected from the honey bee apiaries situated in Barabanki, Haidergadh, Mallihabad, Gosaiganj, and Itaunja throughout the winter and summer seasons.

Study Period: The study was carried out from April 2019 to March 2021 to collect the mite parasites from the selected apiaries. The collected mite samples were processed and stored appropriately for further detailed studies on their morphology.

Sample collection and preservation:
Infested hives were identified by inspection of a piece of white paper placed on the bottom of the hives for more than 24 hours (hrs). There was a total of 133 mites collected from the different apiaries (Barabanki, Gosaiganj, Haidergarh, Mallihabad, and Itaunja) located in different regions of Lucknow, Uttar Pradesh (Table 1). Mites were also collected from the infected broods by visual examination and brought to the Parasitology laboratory of the Department of Zoology, BBAU, Lucknow, where they were processed within 24 hrs or preserved in 70% ethanol in collecting vials and kept at -20^{0}C temperature until further analysis.

Table 1: Sample of Mite collected from apiaries located in different regions of Lucknow, Uttar Pradesh.

S.No.	Apiary visited for sample collection	Number of mites collected
1.	Barabanki	22
2.	Gosaiganj	19
3.	Haidergarh	27
4.	Malihabad	39
5.	Itaunja	26
Total		**133**

Light microscopy
The collected mites were washed properly with distilled water and were heated in 10% KOH solution at about 60° temperatures for 10-15 minutes. Mites were further washed by using distilled water and the sample was dehydrated through a series of ethanol grades (30%, 50%, 70%, 90% & 100%) for 30 minutes each. After dehydration, permanent mounting was done by using Canada balsam or DPX and observation were made under an Imaging light microscope at 10X and 40X magnification (Dietemann *et al*., 2013).

Scanning Electron Microscopy (SEM)
Mites collected were placed directly into 4% glutaraldehyde /0.01 M phosphate buffer (Millonig, 1961)at 4^{0}C temperature overnight. Mites were post-fixed by using 1% Osmium tetraoxide buffer for half hrs at room

temperature and washed properly by using phosphate buffer saline. Specimens then were dehydrated through a series of ethanol grades (30%, 50%, 70%, 90% & 100%) prepared and dried till the critical point. Specimens were mounted on the Aluminum stubs with carbon tape and then observed in the Scanning Electron Microscope available at the University Science Instrumentation Centre (USIC).

In order to identify the mite, a taxonomic identification key was consulted, which provided information regarding the genera of the mite collected (Smiley, 1991)**.** Further, molecular techniques were employed to confirm the identity of specific species of the collected mites.

RESULTS

The honey bee, *Apis mellifera*, plays a crucial role in pollination and honey production across the world (Downey and Winston, 2001). A parasitic mite (*Tropilaelaps*) infestation could be recognized visually on bees or by examining bee hive debris. The above finding was based on a visual examination of adult mites exclusively taking into account the morphological features of the adult *Tropilaelaps* mite, which was smaller than the *Varroa* mite and visible to the naked eye. The literature review revealed that the *Tropilaelaps'* mite length ranged from 0.6 to 1.0 mm and that its breadth was 0.4 to 0.5 mm (Anderson and Morgan, 2007).

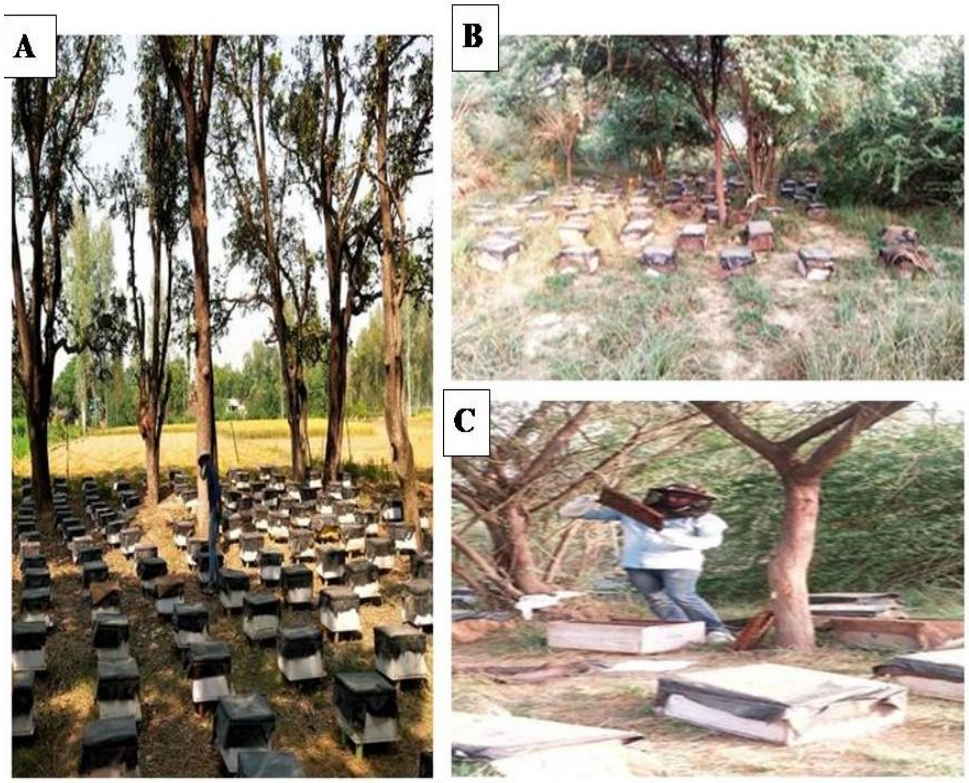

Figure 1: Different Sample collection sites **(A)** Honey bee apiary located at Barabanki (U.P.) **(B)** Bee boxes located at Gosaiganj, Lucknow (U.P.) **(C)** Sample collection from bee broods by visual examination.

Light Microscopy revealed that the adult *Tropilaelaps* mite, due to the fusion of the prosoma (equivalent to the cephalothorax) and the opisthosoma (the abdomen) into a single mass, the body of the adult mite was a light brown in color, unsegmented, and had a single visible region. This was caused by the fact that the prosoma and the opisthosoma were fused

into a single mass (Fig. 2.A & D) showing the subclass Acari. The first pair of the four pairs of legs vertically coincided, resembling the antennae of arthropods (Fig. 2.A) representing the Class Arachnida. Tritosternum and elongated peritremes were observed, suggesting the Suborder Mesostigmata (Fig. 2.A & C). The elongated epigynal plate that was posteriorly rounded or pointed in shape, and the ventrianal plate that was triangular revealed that the parasitic mite belonged to the Family Laelapidae (Fig. 2.A). It was observed that there were elongated peritremes, a tritosternum, and a reticulate sternal plate present (Fig. 2.B & C). There was a stigmata, also known as an external aperture, situated at the bottom of each peritremal tube (Fig. 2.C)**.**

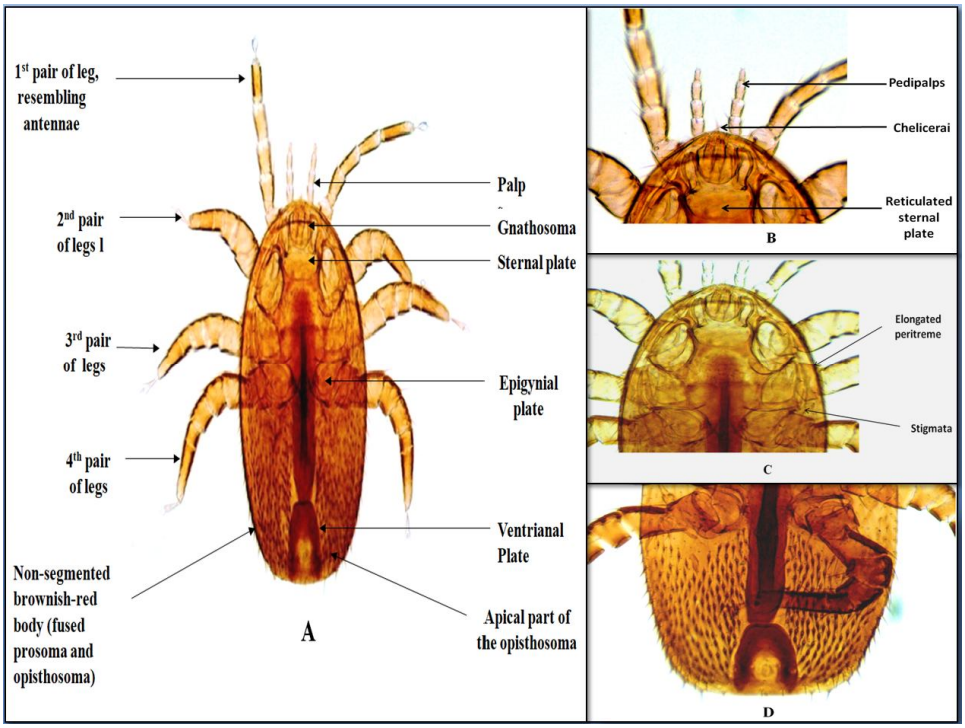

Figure 2: Showing different morphological structures**. (A)** Imaging light microscopy view (at 10X Magnification) of *Tropilaelaps* (dorsal view). **(B)** Anterior portion showing the mouth parts of *Tropilaelaps* having pair of pedipalps and chelicerae**. (C)** The anterior part of *Tropilaelaps* with an extended peritreme and stigmata (external aperture). **(D)** *Tropilaelaps* (dorsal view) have opisthosoma, coarse apical bristle thick at their base.

Scanning Electron Microscopy revealed that the body was dorsoventrally flattened, and measurements of its length and width were around 1.04 mm and 541.39 µm, respectively, revealing that it was longer than it was wider, with a dorsal and ventral shield. The dorsal shield, also known as the metapodal shield was convex in shape, giving itself a dome-shaped appearance, and it was covered with many setae of various lengths that were long, slender, and resembled fluted patterns extending along their length (Fig. 3). The Light and SEM also revealed that the flat ventral surface was made up of several plates or shields (including the epigynal shield, the anal shield, and the genital plate) present on the dorsal side (Fig. 2 & Fig. 8). The ventral structure exhibited ventral shields and had four pairs of legs. It appeared as though the mite's legs had been adapted specifically for ectoparasitism. Even though the front pair of legs (pair 1) was longer and tapered than the remaining three pairs of legs (pairs 2-4) (Fig. 2. A & Fig. 6).

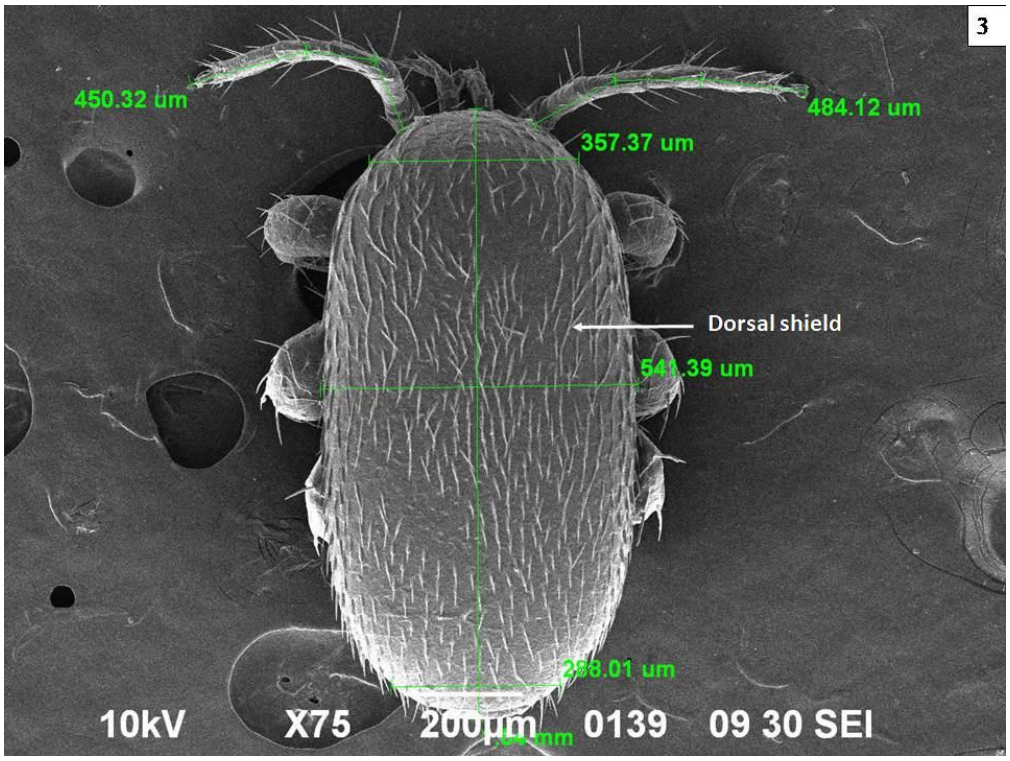

Figure 3: Scannings Electron micrographs (dorsal view) having dorsoventrally flattened dome-shaped appearance and body size of adult *Tropilaelaps*.

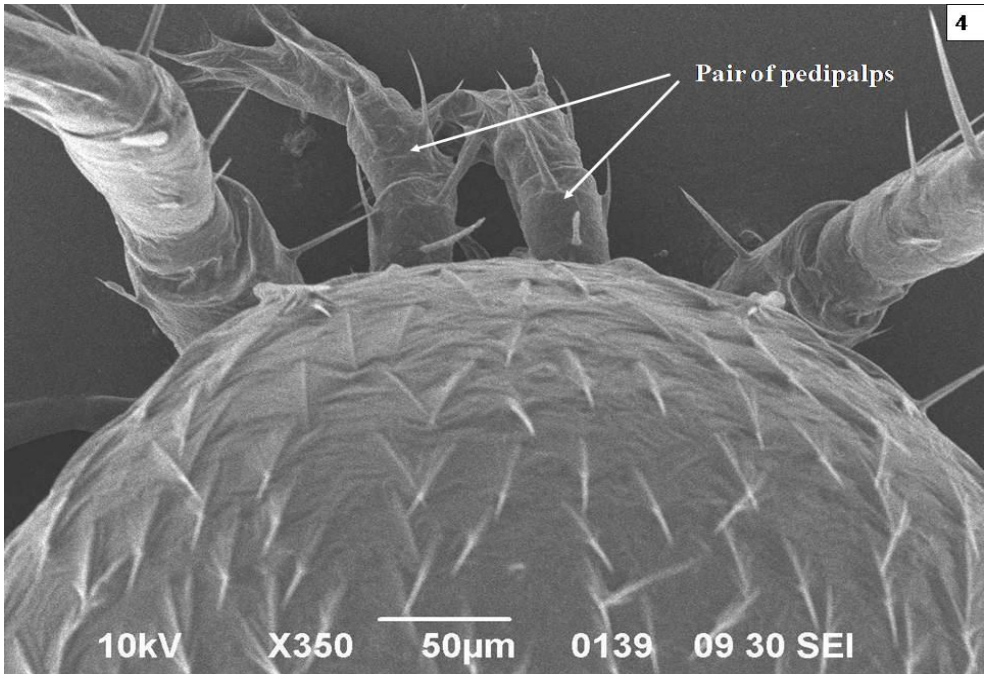

Figure 4: Anterior view of adult *Tropilaelaps* having a pair of pedipalps and a central pair of chelicerae.

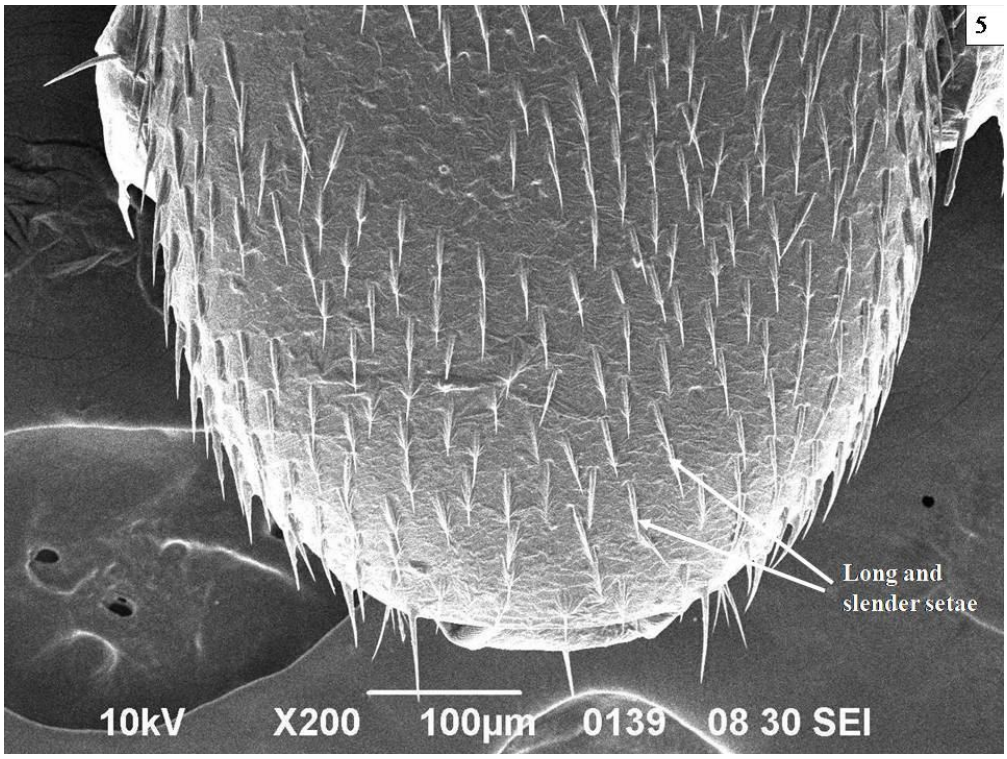

Figure 5: Posterior view of adult *Tropilaelaps* exhibits a dorsal shield and the setae, present are long and slender and have a fluted pattern.

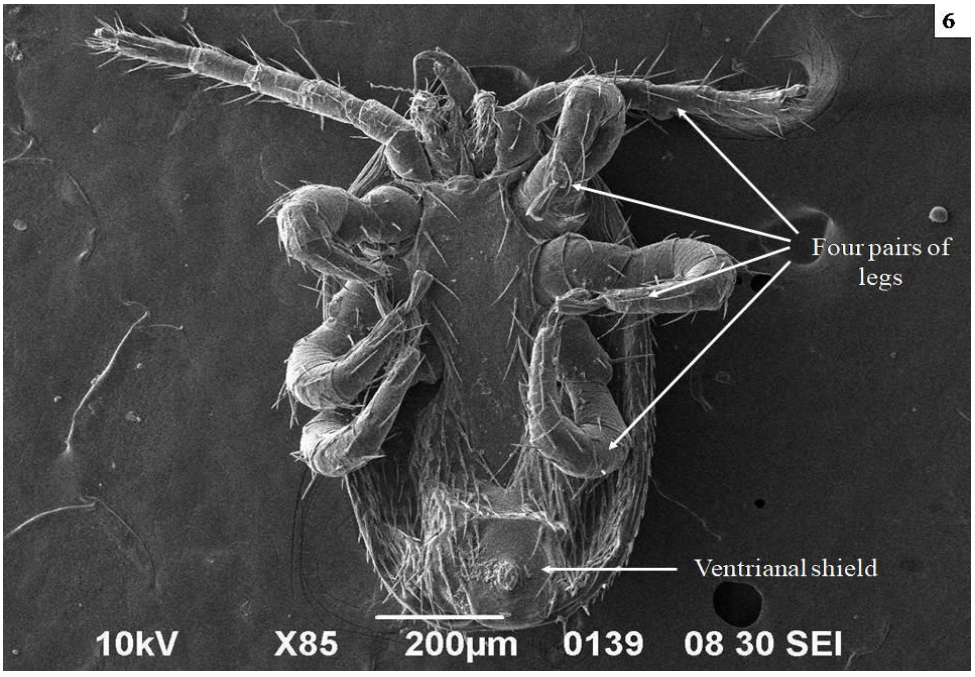

Figure 6: Ventral view of adult *Tropilaelaps* revealed the ventral shield, series of sclerotized plates, and four pairs of legs

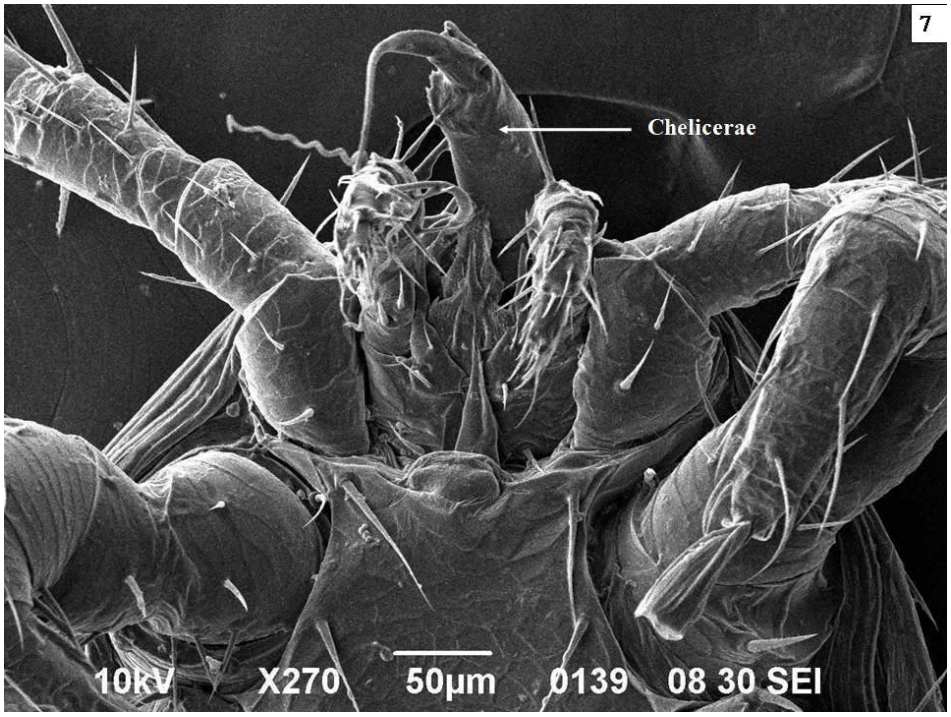

Figure 7: Anterior portion represents the Gnathosoma, mouthparts (pedipalp, chelicerae, and hypostome) through which the animal feed.

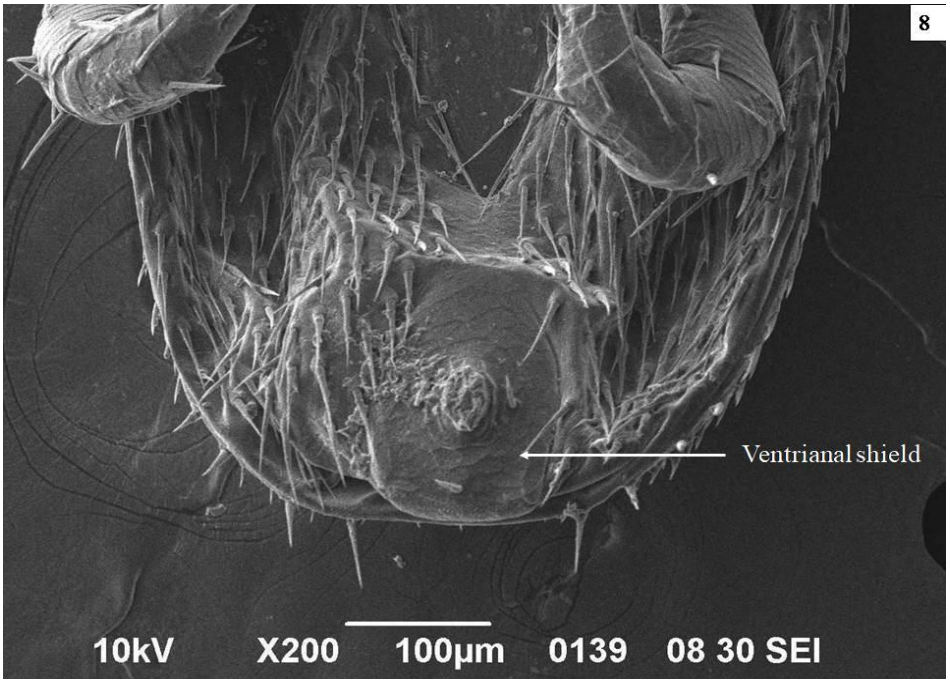

Figure 8: Posterior view having the opisthosoma with coarse bristles and thick at the base of the ventrianal shield.

Based on Light and Scanning Electron microscopy it was observed that the gnathosoma is present at the anterior side of the body which helps to feed on the haemolymph of the honeybee. A pair of modified chelicerae was present ventral to the pedipalps. A preoral trough was formed by the inner surface of the chelicerae and the hypostome, which is used by the animal for feeding (Fig. 2 & Fig. 7).

Thus, based on the observations obtained by Light and SEMicroscopy, consulting the available literature and referencing in light of the available standard taxonomic keys it was concluded that the mite collected during this study from the different apiaries of the honeybee (*Apis mellifera)* belonged to the genus *Tropiolaelaps,* which is an ectoparasite. It was easily observed in the hives of the honey bee as it is visible to the naked eye. The present study concludes that the mite *Tropilaelaps* belongs to the Phylum Arthropoda, Class Arachnida, Subclass Acari, Order Parasitiformes, Suborder Mesostigmata, and Family Laelapidae, which is in consonance with reports of other workers (Smiley, 1991).

DISCUSSION

In this present brief study of mite *Tropiolaelaps* using Light microscopy and Scanning Electron Microscopy (SEM), it was observed that the dorsal shield is convex in shape and is covered by numerous setae. Light Microscopy revealed the external morphology of the mite. The Scanning Electron Microscopy study revealed morphological details of the parasitic mite, *Tropiolaelaps* and revealed their dorsal shield and ventral shields that showed a series of sclerotized plates, and four pairs of legs, as well as some of their external morphological characteristics. The adult mite's body was dorsoventrally flattened, measuring roughly 1.04 mm in length and 541.39 um in width. The dorsal shield setae were fluted along their length and are long and slender.

The dorsoventrally flattened shape of the adult mite provides for the mite to attach themselves in between the sclerites of bees and help in feeding. This shape also provides and helps to create wind resistance when the bee is in flight. In general, the shape of *Tropilaelaps* was similar to general mite morphology, although its body is more flattened.

Scanning electron microscopy also revealed that elongated peritremes, tritosternum, and gnathostoma are present. The gnathosoma consisted of modified pair of chelicerae and a hypostome was present on the anterior side of the body through which the animal feeds. The ventral structures consisted of four pairs of legs and the first pair of legs was vertically aligned, bearing visual similarity to the antennae of arthropods.

The epigynial plate was elongated and posteriorly rounded or pointed; the ventrianal plate was triangular and the reticulated sternal plate was also seen. The flat ventral surface was composed of a series of sclerotized plates. The legs of this mite appeared to be modified for ectoparasitism. Thus, the overall morphology revealed in this study of *Tropilaelaps* resembles the general mite morphology, although its body was dorsoventrally flattened.

The structure of the legs appears to be another modification to a parasitic way of life. The four pairs of legs of adult mite were concealed by the dorsal shield. The posterior three pairs of legs are modified for walking, but the anterior pair acts as antennae. The leg's empodium is structurally unusual and likely designed for grasping as reported by Liu(1982).

The gnathosome appears similar to that of other parasitic mites. The functional morphology and specialization of these mouthparts were studied by Griffiths *et al.* (1988) who reported this type of functional mouthpart pattern in other specialized arthropod blood feeders. The penetration of the intersegmental membrane by the chelicerae, allows the mite to insert its hypostome to feed on haemolymph.

Apis mellifera, the European honey bee, has developed behavioral defenses against the mite and to remove and eliminate the mites, the *Apis mellifera* performs self-grooming, grooming dances, and collective cleaning activities (Peng *et*

al., 1987), however, these studies were beyond the scope of the present work.

During the collection of samples for this study, it was observed by the random survey that the mite, *Tropilaelaps* is considered one of the most damaging pests of honey bee colonies by the farmers. During their developmental stages, it was seen that the mites feed on haemolymph of their larval and pupal stages also, as well as on the mature adult bees. Although the *Tropilaelaps* evolve to have multiple morphological modifications that show its parasitic lifestyle, it is not as well adapted to European honey as to the Asian honey bee.

Thus, the studies made in the current investigation of the ectoparasitic mite *Tropilaelaps* of honey bee *Apis mellifera* showed their morphological characteristics as aligned with major studies reported. The results may reveal additional insights into the taxonomy of the parasite as well as provide for future parasite management technologies.

CONCLUSION

This study provides morphological structures of the mite *Tropilaelaps* revealed by a Light and Scanning Electron microscope. These results will help to provide insights into the taxonomy of the parasite as well as information for the further management of these parasites, which have a significant role in improving the bee population and role in maintaining biodiversity. The study revealed the morphological attributes of mites as well as their ultrastructural identification that concerned well with the available literature. The results of the current observation have implications for future research on epidemiology, parasite infection diagnostics, and honey bee control strategies.

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