Journal of Entomological Society of Iran
2008, 27(2), 1-11

Morphology and ultrastructure of chemosensory sensilla of labio-maxillary complex in the Colorado potato beetle, *Leptinotarsa decemlineata* (Col.: Chrysomelidae), larvae

H. Farazmand[1&*] and S. Yu. Chaika[2]
1. Agricultural Entomology Research Department, Iranian Research Institute of Plant Protection, P.O. Box 1454, Tehran 19395, Iran, 2. Department of Entomology, Faculty of Biology, Moscow State University, 119899 Moscow, Russia, E-mail: biochaika@mtu-net.ru.
*Corresponding author, E-mail: farazmand@ppdri.ac.ir

Abstract
The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is a destructive insect pest of potato and other solanaceous crops. The type, number and distribution of sensory receptors on the labio-maxillary complex of larvae were studied using the scanning and transmission electron microscopy. A total of 32 sensilla were identified on the maxillary and labial palps that were categorized in three basic morphological types: basiconica, trichoid, and digitiform. The maxillary palp had 16 basiconica, four trichoid and one digitiform sensilla, and the labial palp included 11 basiconica sensilla. All basiconica sensilla were on the distal apex of the palp segment. The basiconica sensilla are innervated by 2-6 neurons and function as a gustatory receptor. In addition, three trichoid sensilla were on the distal side of the second segment, one trichoid sensillum on the lateral side of the third segment and one digitiform sensillum on the lateral side of the third segment of maxillary palp. Trichoid sensilla are equipped with 4-6 receptor cells and function as chemo- and mechanoreceptors.
Key words: Colorado potato beetle, *Leptinotarsa decemlineata*, maxillary and labial palps, mechanoreceptor, chemoreceptor, gustatory receptor, sensilla, electron microscopy

چکیده

سوسک برگ‌خوار سیب‌زمینی، *Leptinotarsa decemlineata* (Say)، یکی از مهمترین آفات سیب‌زمینی و دیگر محصولات خانواده‌ی Solanaceae می‌باشد. نوع، تعداد و پراکندگی گیرنده‌های حسی روی پالپ‌های آرواره‌ای و لبی لارو سوسک برگ‌خوار سیب‌زمینی توسط میکروسکوپ الکترونی مطالعه شد. در مجموع تعداد ۳۲ گیرنده‌ی حسی شامل سه نوع بازیکونیکا (basiconica)، تریکوئید (trichoid) و دیجیتیفرم (digitiform) روی پالپ‌های آرواره‌ی پایین و لب پایین شناسایی گردید. پالپ آرواره‌ی پایین دارای ۱٦ گیرنده‌ی حسی بازیکونیکا، سه گیرنده‌ی حسی تریکوئیدی و یک گیرنده‌ی حسی دیجیتیفرم و پالپ لب پایین نیز دارای ۱۱ گیرنده‌ی حسی بازیکونیکا بود. تمام گیرنده‌های حسی بازیکونیکا روی بخش انتهایی بند آخر پالپ‌ها قرار داشتند. این گیرنده‌ها دارای ٦-۲ سلول حسی بوده و نقش گیرنده‌ی چشایی را ایفا می‌کنند. همچنین سه گیرنده‌ی حسی تریکوئیدی روی بند دوم، یک گیرنده‌ی حسی تریکوئیدی روی بند سوم و یک گیرنده‌ی حسی دیجیتیفرم نیز در بخش جانبی بند سوم پالپ آرواره‌ی پایین قرار داشت. گیرنده‌های حسی تریکوئیدی دارای ٦-٤ سلول حسی بوده و نقش گیرنده‌ی مکانیکی و شیمیایی دارند.
واژگان کلیدی: سوسک برگ‌خوار سیب‌زمینی، *Leptinotarsa decemlineata*، پالپ آرواره‌ی پایین، پالپ لب پایین، گیرنده‌ی مکانیکی، گیرنده‌ی شیمیایی، گیرنده‌ی چشایی، میکروسکوپ الکترونی

Introduction

Chemoreception is the perception of chemicals by living organisms in their external environments. Chemicals perceived are from living or nonliving sources, and include water. They are either in aqueous solution and perceived by gustatory sensilla or airborne and perceived by olfactory sensilla (Zacharuk, 1980). The chemosensory capabilities of insects are important in food selection. Phytophagous insects are known to possess receptors sensitive to various host plant chemicals, including common nutrients and secondary plant substances that may act as phagostimulants or feeding deterrents (Mitchel & Schoonhoven, 1974).

Nowadays, there is a huge body of research on chemo-communication of insects. In this regard, the special attention is given to the study of insects sense organs located on oral appendages, particularly the ultrastructure and physiology of sensilla of cephalic appendages of beetle larvae (Ryan & Behan, 1973; Behan & Ryan, 1978; Bloom *et al.*, 1982; Albert *et al.*, 1993). Studies on labial and maxillary palps in different species of beetle larvae have revealed the presence of six different types of sensilla: basiconica, styloconica, campaniform, digitiform, ampullaceous and sensilla chaetica. Using transmission electron microscopy techniques, the role of most of these sensilla were determined; campaniform and chaetica sensilla are mechanoreceptors while basiconica sensilla are chemoreceptors; digitiform sensilla can be both mechanoreceptors and chemoreceptors (Farazmand *et al.*, 2003; Giglio *et al.*, 2003).

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is a major pest of potato in commercial products and home gardens. Larvae and adults both feed on foliage and if left untreated, complete defoliate of potato is possible (Gelman *et al.*, 2001). Studies on the mouthparts of CPB imago have shown a total of 370 sensilla on the apical tip of the maxillary palp and 130 on the labial palp. These include four morphologically different types of sensilla on the maxillary palp and three of these morphologically types on the labial palp (Sen, 1988), as well as three morphologically different types of sensilla on the galeal tip (Sen & Mitchell, 1987). Another study has revealed 13 distinct sensillar types on the antenna in three species of *Leptinotarsa* spp. (Sen & Mitchell, 2001). Furthermore, electrophysiological studies on the taste receptors of the mouthpart of CPB larva have showed at least six sensilla, chemosensory cells, on the various mouthparts of CPB larva (Mitchell & Schoonhoven, 1974).

The objective of the present work is to study the fine structure of sensory receptors on maxillary and labial palps of CPB larvae. The distribution, external morphology and ultrastructure of various types of sensilla on the labio-maxillary complex of CPB larvae are described and illustrated in detail based on scanning and transmission electron microscopy.

Materials & methods

Final instar larvae were obtained from the eggs collected from potato fields in Moscow region. The stock culture was maintained on potato fresh leaves in laboratory conditions (25 ± 1 ˚C and 70 ± 5% RH, 16: 8 L: D photoperiod).

For scanning electron microscopy (SEM), whole head capsule of larva was washed in ethanol 75%, 90%, 100% and acetone, respectively; air-dried and secured to aluminum stubs, and coated with approximately 200 Å of gold by vacuum evaporation. They were subsequently studied and photographed using a Hitachi S-405A scanning electron microscope.

For transmission electron microscopy (TEM), head capsules were prefixed in 2.5% glutaraldehyde mixed in phosphate buffer solution at a PH of 7.3 at 4˚C for 4 hr and post-fixed with 2% osmium tetroxide in phosphate buffer at room temperature for 2 hr. The tissue was dehydrated with ethanol and embedded in Epon. Serial sections with a thickness of approximately 70-80 nm were cut using an ultramicrotome with a diamond knife, mounted on Formvar coated copper girds, and stained with 2% uranyl acetate in 50% ethanol. Sections were observed using a JEM-100B transmission electron microscope.

Results and discussion

The head capsule of CPB larvae is 1.77 mm wide and includes paired antennae, mandibles, maxillae, labium and labrum (fig. 1). The observed sensillae on the labio-maxillary complex were of three basic morphological types: basiconica, trichoid, and digitiform. The basic locations of contact chemoreceptors were on the sensory disc at the tip of maxillary and labial palps. The cuticle of these discs is less sclerotized, which allows sensilla to deeply move into distal segment when the palp contacts with a substrate.

The maxillary palps of CPB larvae consist of three segments (fig. 2). Twenty one sensilla were observed on each maxillary palp, including 16 basiconica, four trichoid and one digitiform (figs 3-5).

All basiconica sensilla were on the distal apex of third segment (figs 4-6). These sensilla were on average of 3.5 μm length with a basal diameter of 3 μm and a tip diameter of 1.1 μm. Their cuticular membrane was smooth and thick with a pore at the distal section. The unusual structure of basiconica sensilla is characterized by a thin cuticular cylinder that surrounded their bases. These sensilla have apical finger-like cuticular projections. The number of these finger-like filaments, which surrounded the terminal pore, reaches to 16-17. These finger-like filaments were 1 μm length with a basal diameter of 0.28 μm and a tip diameter of 0.15 μm (figs 7 and 19). Basiconica sensilla as contact chemoreceptors are characterized by innervations to several receptor cells (in the present study by 2-6 receptor cells), lack of dendrites branching in a cuticular section lumen, presence of a well-developed scolopidial sheath surrounding dendrites (figs 13-18). Thus, for one basiconica sensillum, a single scolopidial sheath encloses all sensillum dendrites (d = 0.06 μm), but other sensilla between dendrites have shell invaginations. The thickness of cuticular layer is 0.3 μm. However the presence of different number of receptor cells can be representative of differences in the function of these outwardly similar sensilla. All basiconica sensilla function as gustatory receptors.

In addition to basiconica sensilla, there were four trichoid sensilla on each maxillary palp, of which three sensilla (20 μm length) were on the lateral side of the second segment and one sensillum (8 μm length) on the ventral side of the third segment (figs 3 and 5). The examination of cross-sections through dendrites of these sensilla showed that at least one sensillum acted as a mechanoreceptor, as it was innervated by a single receptor cell, and the dendrite of mechanoreceptor cell contained a tubular body and terminates at the hair base. Other trichoid sensilla were innervated by 5 receptor cells, of which one dendrite contained a tubular body. The absence of a pore along the hair shaft indicates a possible gustatory role. These sensilla have a structure of chemo- and contact mechanoreceptors (fig. 20) (Ivanov, 2000).

There was also one digitiform sensillum (15.2 μm length) on the lateral side of each maxillary palp. This sensillum appears externally as elongate finger-like pegs, which lies in individual longitudinal grooves in the lateral wall of the distal segment of maxillary palp (figs 8 and 21). Digitiform sensillum is innervated by a single bipolar neuron, as it has previously been documented (Zacharuk *et al.*, 1977). The distal process or dendrite consists of a distal microtubular and a proximal expanded cytoplasm. The distal portion branches at the base of the peg and the dendritic branches extend to the tip of the lateral chamber. This is the characteristics of mechanoreceptors. The digitiform sensillum responds only to mechanostimulation including contact and vibratory stimuli (Zacharuk *et al.*, 1977).

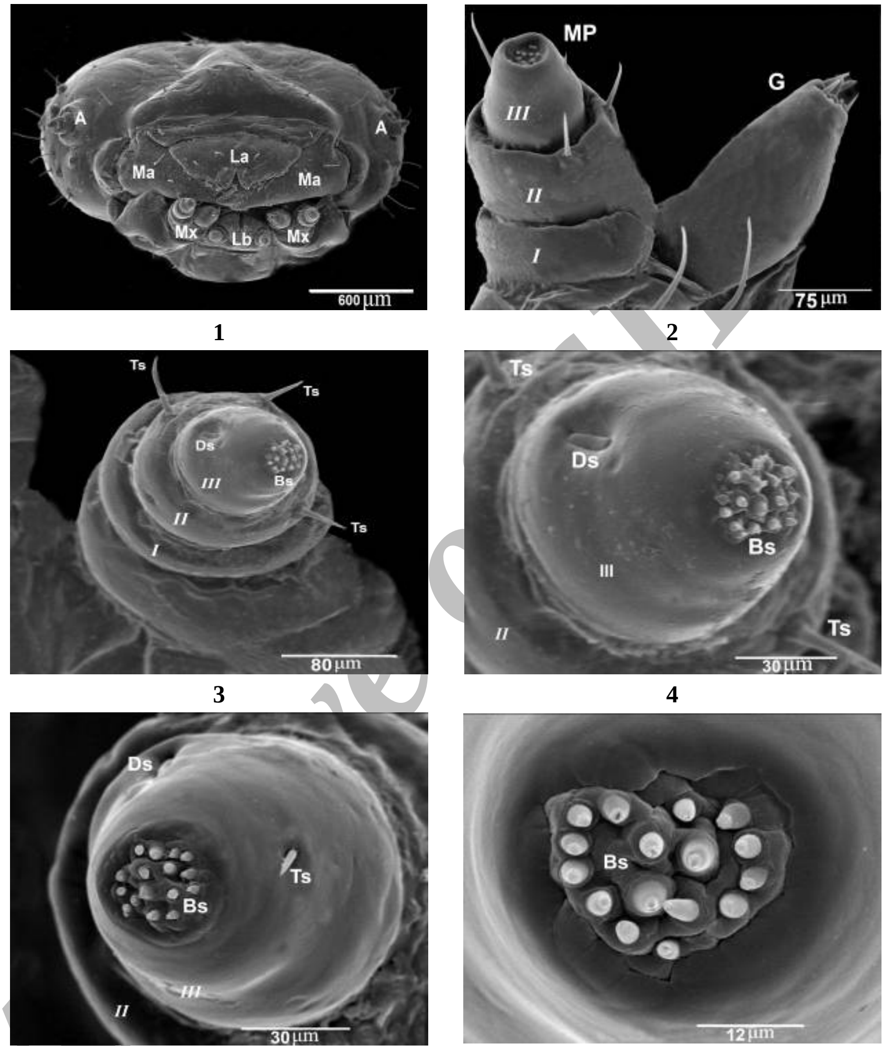
Labial palp of CPB larvae consists of two segments (fig. 9). Eleven basiconica sensilla were observed on each labial palp (figs 10-11). All the sensilla were on the distal apex of the second segment. Basiconica sensilla were 4.5 μm in length and 3 μm in diameter at the base. The structure of these sensilla is similar to that of basiconica sensilla located on the maxillary palp. There were also cuticular outgrowths at the distal apex of basiconica sensilla, however, outgrowths were smaller in sensilla located on maxillary palps. Their cuticular membrane was thick and smooth with a pore at the distal section. Similar to basiconica sensilla on maxillary palps, basiconica sensilla on labial palps had apical finger-like cuticular projections and were innervated by 2-6 receptor cells (figs 12 and 22-24).

The present study showed that chemoreceptors on mouthpart appendages of CPB larvae are mainly located on the terminal disc of the distal segment of maxillary and labial palps. Such a sensillum arrangement is consistent to the localization of chemoreceptors at coleopteran larvae. As it was observed in the experiments, the number of sensilla per larva is likely to be constant during larval development.

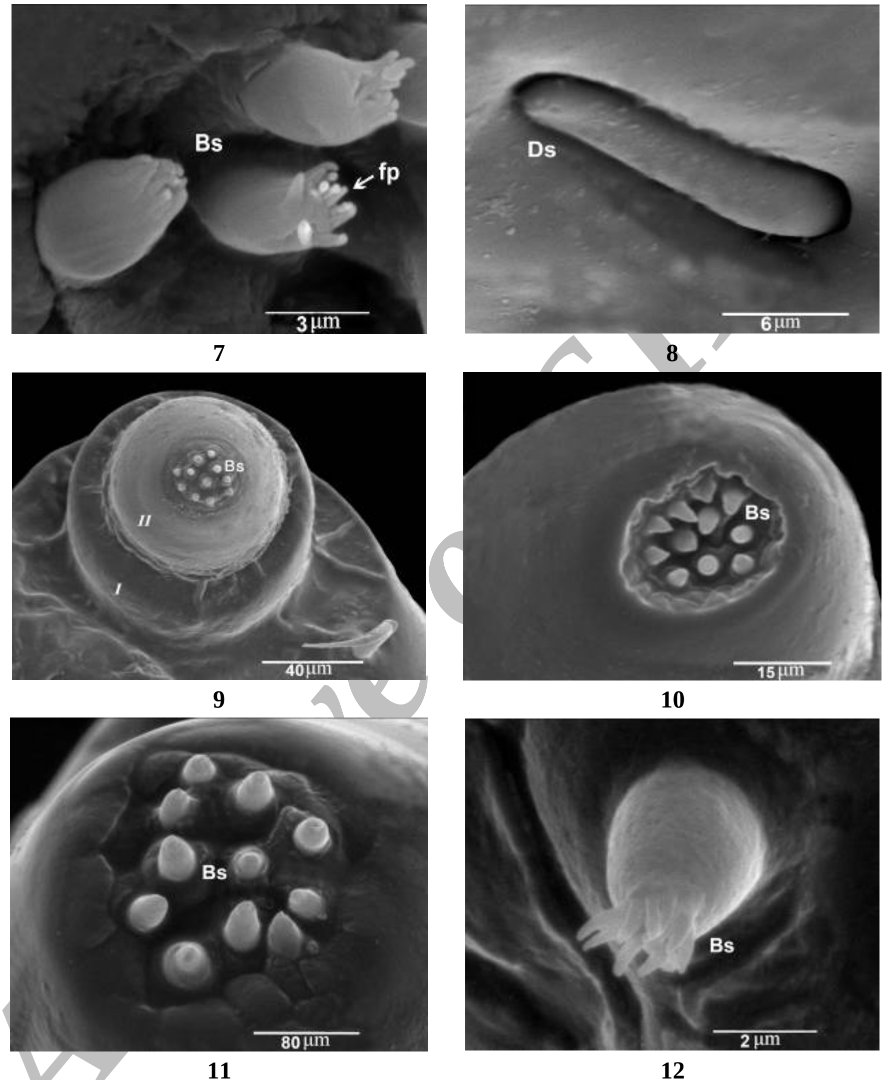
The results of the ultrastructural study showed that basiconica sensilla were the basic chemoreceptor organs on maxillary and labial palps of larvae. Basiconica sensilla were innervated into several receptor cells that likely to be due to their specialization to different food stimuli. These sensilla function as a gustatory receptor. The presence of these sensilla in chemoreceptive cells, which are sensitive to various phagostimulants, suggests that these sensilla are important in the larval feeding habits.

Trichoid sensilla are equipped with 1-5 receptor cells and function as chemo- and mechanoreceptors. The dendrite of mechanoreceptor cell contains a tubular body and terminates at the hair base. Digitiform sensillum, located on the third segment of maxillary palp, probably function as mechanoreceptor.

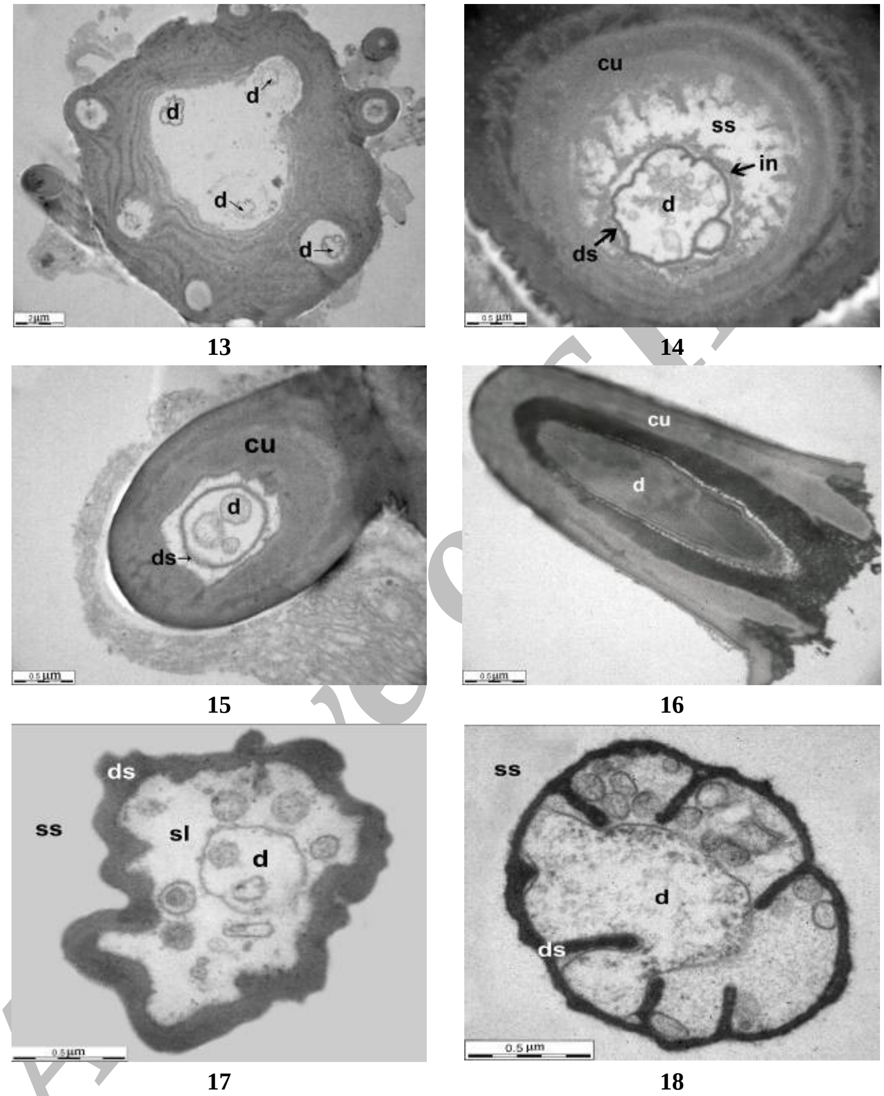
In a morphological study on taste receptors of CPB larvae using scanning electron microscope, Mitchell & Schoonhoven (1974) found 16 and 11 uniporous peg sensilla on the tip of each maxillary and labial palp, respectively. Here, in detailed morphological and ultrastructural examinations using both the scanning and transmission electron microscopy, we identified 16 basiconica sensilla (functioning as a gustatory receptor), three trichoid sensilla (functioning as a chemo-mechanoreceptor) and one digitiform sensillum (functioning as a mechanoreceptor) on the each maxillary palp and 11 basiconica sensilla (functioning as a gustatory receptor) on the each labial palp of larvae.



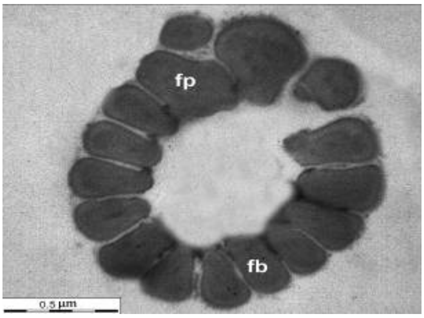
Figures 1-6. 1. SEM micrograph of the head capsule of CPB larvae; 2-6, SEM micrographs of the maxillary palp of CPB larvae: 2. maxillary palp, 3. distribution of sensilla on the maxillary palp, 4 & 5. sensilla on the 2nd and 3rd segments, respectively, 6. sensilla on the distal apex of the third segment. *I* = 1st segment, *II* = 2nd segment, *III* = 3rd segment, A = antenna, Bs = basiconica sensilla, Ds = digitiform sensillum, G = galea, La = labrum, Lb = labium, Ma = mandibule, MP = maxillary palp, Mx = maxilla, Ts = trichoid sensilla.



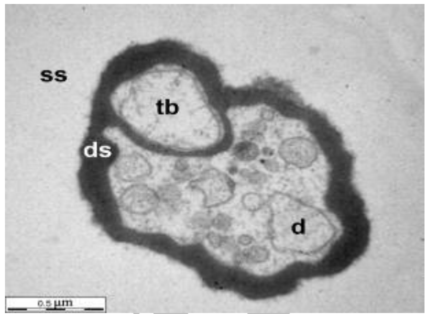
Figures 7-12. 7-8, SEM micrographs of the maxillary palp of CPB larvae: 7. basiconica sensilla with apical finger-like projections, 8. digitiform sensillum on the third segment; 9-12, SEM micrographs of the labial palp of CPB larvae: 9. labial palp, 10 & 11. sensilla on the distal apex of second segment, 12. basiconica sensilla on the tip of palp with apical finger-like projections. *I* = 1st segment, *II* = 2nd segment, Bs = basiconica sensilla, Ds = digitiform sensillum, fp = finger-like projections on basiconica sensilla.



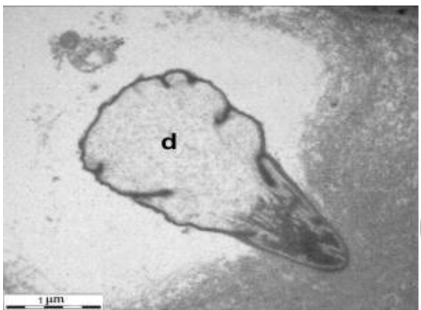
Figures 13-18. TEM micrographs of the maxillary palp of CPB larvae: 13. cross-section through the distal region of the 3rd segment, 14. cross-section of basiconica sensilla along the hair shaft, 15 & 16. longitudinal sections of basiconica sensilla at the socket region, 17 & 18. cross-sections of the distal dendritic process of basiconica sensilla. cu = cuticle, d = dendrite, ds = dendritic sheath, in = inner sheath cell, sl = sensillar lumen, ss = sensillar sinus.



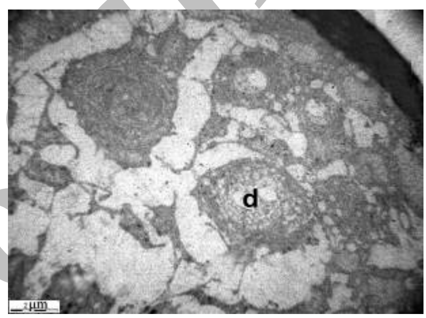
19



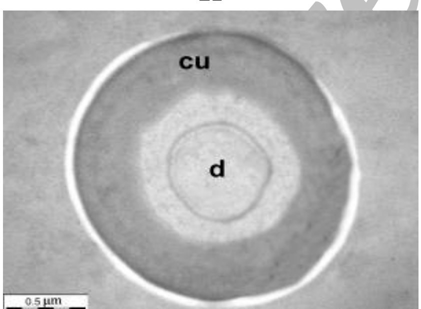
20



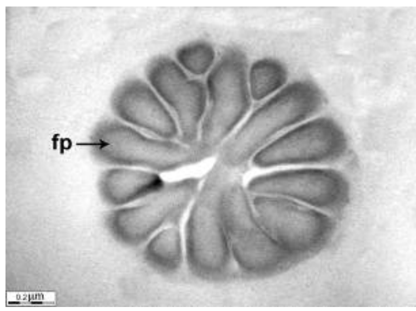
21



22



23



24

Figures 19-24. 19-21, TEM micrographs of the maxillary palp of CPB larvae: 19. cross-section of the tip of basiconica sensilla, 20. cross-section of the distal dendritic process of trichoid sensilla, 21. cross-section of a digitiform sensillum; 22-24, TEM micrographs of the labial palp of CPB larvae: 22. cross-section through the base of basiconica sensilla at the ciliary region, 23. cross-section of a basiconica sensilla with a thick wall on the distal end of palp, 24. cross-section of the tip of basiconica sensilla. cu = cuticle, d = dendrite, ds = dendritic sheath, ss = sensillar sinus, fp = finger-like projections, tb = tubular body.

Acknowledgments

We thank G. N. Davidovich (the SEM & TEM units of Biology Faculty, Moscow State University) for technical assistance and use of the facilities, and J. Karimzadeh for comments on the draft manuscript.

References

Albert, P. J., Zacharuk, R. Y., Weaver, D. K. & Mcfarlane, J. E. (1993) Electrophysiological and behavioural responses to lactic acid stimuli in larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and permeability of antennal sensilla. *Physiological Entomology* 18, 329-335.

Behan, M. & Ryan, M. F. (1978) Ultrastructure of antennal sensory receptors of *Tribolium* larvae (Coleoptera: Tenebrionidae). *International Journal of Insect Morphology and Embryology* 7(3), 221-236.

Bloom, J. W., Zacharuk, R. Y. & Holodniuk, A. E. (1982) Ultrastructure of the larval antenna of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae): structure of the trichoid and uniporous peg sensilla. *Canadian Journal of Zoology* 60(7), 1528-1544.

Farazmand, H., Chaika, S. Yu. & Sinitsina, E. E. (2003) Sense organs of the labio-maxillar complex of larvae in tenebrionid beetles: *Tenebrio molitor* and *Zophobas rugipes* (Coleoptera, Tenebrionidae). *Proceedings of the II International Conference of Zoocenos Role in Natural Ecosystems, Denepropetrovsk, Ukrine*, 182-183.

Gelman, D. B., Bell, R. A., Liska, L. J & Hu, J. S. (2001) Artificial diets for rearing the Colorado potato beetle, *Leptinotarsa decemlineata*. *Journal of Insect Science* 1(7), 1-11.

Giglio, A., Ferrero, E. A., Perrotta, E., Tripepi, S. & Brandmayr, T. Z. (2003) Ultrastructure and comparative morphology of moith-oart sensilla in ground beetle larvae (Insecta, Coloeoptera, Carabidae). *Zoologischer Anzeiger* 242(3), 277-292.

Ivanov, V. P. (2000) *The sensory organs of insects and other arthropods*. 1st ed. 280 pp. Nauka, Moscow. [In Russian].

Mitchell, B. K. & Schoonhoven, L. M. (1974) Taste receptors in Colorado beetle larvae. *Journal of Insect Physiology* 20(9), 1787-1789.

Ryan, M. F. & Behan, M. (1973) The sensory receptors of *Tribolium* larvae. *Physiological Zoology* 46(3), 238-244.

Sen, A. (1988) Ultrastrucure of the sensory complex on the maxillary and labial palpi of the Colorado potato beetle, *Leptinotarsa decemlineata*. *Journal of Morphology* 195(2), 159-175.

Sen, A. & Mitchell, B. K. (1987) Ultrastructure of the galeal sensory complex in adults of the Colorado potato beetle, *Leptinotarsa decemlineata*. *Physiological Entomology* 12, 81-90.

Sen, A. & Mitchell, B. K. (2001) Olfaction in the Colorado potato beetle, *Leptinotarsa decemlineata*: ultrastructure of antennal sensilla in *Leptinotarsa* sp. *Journal of Biosciences* 26(2), 233-246.

Zacharuk, R. Y. (1980) Ultrastructure and function of insect chemosensilla. *Annul Review of Entomology* 25, 27-47.

Zacharuk, R. Y., Albert, P. J. & Bellamy, F. W. (1977) Ultrastructure and function of digitiform sensilla on the labial palp of a larval elaterid (Coleoptera). *Canadian Journal of Zoology* 55, 569-578.

Received: 2 July 2006
Accepted: 26 February 2007