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Morphology and Untrastructure of the Antennal Sense Organs in Tenebrionid Larvae *Tenebrio molitor* L. and *Zophobas rugipes* Kirsch (Coleoptera: Tenebrionidae)

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Abstract—The distribution, external morphology, and ultrastructure of various types of sensilla in the antennae of tenebrionid larvae *Tenebrio molitor* and *Zophobas rugipes* are studied by means of scanning and transmission electron microscopy. On the antennae of *T. molitor* there are sensilla of four basic morphological types: basiconic, styloconic, trichoid, and papillate sensilla. On the antennae of *Z. rugipes*, in addition to the aforementioned ones, there are placoid sensilla. Ultrastructure points to olfactory function of basiconic and placoid sensilla. Other sensillum types are contact chemoreceptors.

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Tenebrionids are widely represented in the fauna of many regions of the world. They are especially abundant in steppes and deserts. Some species of tenebrionids have become synathropous and occur in houses and household structures, in particular, *Tenebrio molitor* L. is a pest of food stocks. In addition, *Tenebrio molitor* L. and *Zophobas rugipes* Kirsch. are widely used objects in laboratory cultivation. At present, *Z. rugipes* seems to be a widely used insect to feed terrarium animals. A comparative investigation of sensory organs in the larvae of these two species is performed to find out to what extent synathropization is reflected in development of their sensory organs.

It is known that most sensory organs in larvae are concentrated on antennae and appendages of mouth parts—maxillary and labial palpi (Benham and Ryan, 1978; Dubinskas et al., 1997). There are data on the external structure of the antennal of the larva of *Tenebrio molitor* studied by means of light microscopy and on the structure of sensilla of some types in this species (Bloom et al., 1982a, 1982b). There are data on the electrophysiological characteristics of antennal sensilla of *T. molitor* (Albert et al., 1993; Redkozubov and Belousova, 1966). There are no data whatsoever on the morphology and ultrastructure of sensory organs of larvae of *Z. rugipes.*

MATERIAL AND METHODS

The study has been performed with reference to the antennae of larvae of the last instar of *T. molitor* and *Z. rugipes.* The larvae were taken from cultures supported at the Entomology Department. The insect cultures were kept at the optimum temperature 30 ± 1°C, relative humidity 55 ± 5%, and photoperiod 14 : 10 (light : darkness). The diet consisted of mixture of wheat bran, whole grain wheat flour, and beer yeast in a ratio of 50 : 45 : 5.

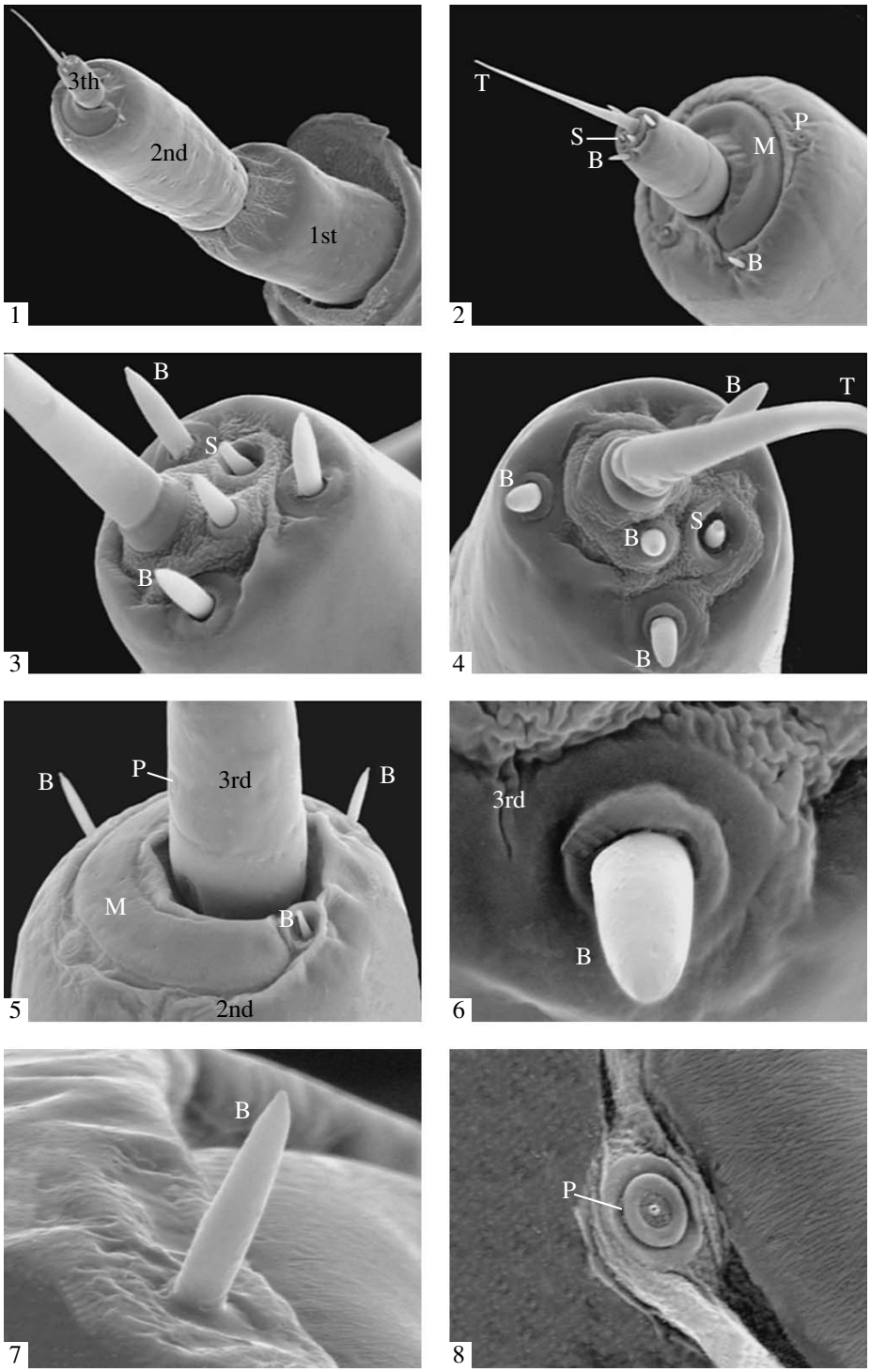
For scanning electron microscopy, the dissected head capsules of larvae were dehydrated in 75, 90, and 100% ethyl alcohol and than placed in acetone. The mounts were fixed on a stage, sputtered with a platinum–palladium mixture in approximately the 20-nm layer in vacuum. Then the preparations were examined with a Hitachi S-405A scanning electron microscope, and the image was digitally recorded.

For transmission electron microscopy, the antennae of larvae were fixed in 2.5% glutaric aldehyde in phosphate buffer (pH 7.3) for 4 h and then afterfixed in a 2% solution of osmium tetroxide for 2 h. Then the mounts were dipped in a series of alcohol with increasing concentration. For contrast, the mounts were placed in uranyl acetate. After complete dehydration the samples were embedded in a mixture of Epon resins. Ultrathin sections were stained after Reynolds and were examined with a JEM-100B transmission electron microscope at the Interdepartmental Laboratory of Electron Microscopy at the Biological Faculty, Moscow State University.

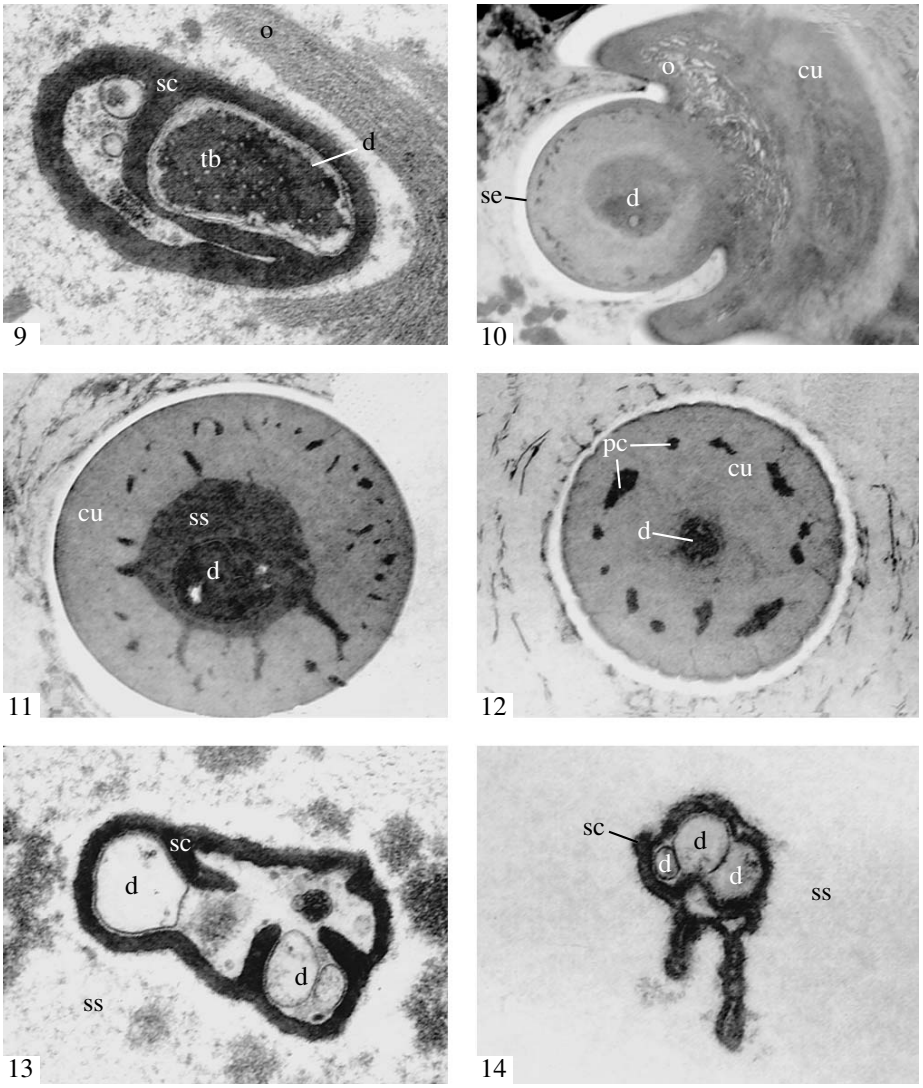
RESULTS AND DISCUSSION

(a) Tenebrio molitor

The antennae of larvae of the last instar consist of three segments: a strong chitinized basal segment, a longer pedicel, and a large flagellum or third segment beginning from the dorsolateral side of the antennal pedicle. The size of antennal segments is (length × diameter at the base): the first segment 190 × 150 μm,



Figs. 1–8. Chemoreceptor organs on antennae of *T. molitor*. (1) General view of the antenna (250×); (2) apex of antenna (600×); (3, 4) sensilla of the third segment (3000×); (5) sensilla of the second segment (1500×); (6) basiconic sensillum of the third segment (10000×); (7) basiconic sensillum of the second segment (3800×); (8) sensory papilla of the second segment (5000×). Abbreviations in figures: 1st—the first segment of the antenna; 2nd—the second segment of the antenna; 3rd—the second segment of the antenna; a—axons; B—Basiconic sensillum; bd—branches of dendrites; h—hemocel; cu—cuticular region; d—dendrites; M—multiporous plate; n—nucleus; o—accessory cells; P—sensory papilla; pc—pore canals; PL—placoid sensillum; po—pore; S—styloconic sensillum; sc—scolopoid envelope; se—sensillum; ss—sensillar cavity; T—trichoid sensillum; tb—tubular body.



Figs. 9–14. Ultrastructure of chemoreceptor sensilla on the antennae of larvae of *T. molitor.* (9) Section through dendrites of receptor cells of trichoid sensillum (40000×); (10) cuticular region of a basiconic sensillum (1500×); (11) section through dendrites ofstyloconc sensillum (25000×); (12, 13) sections through dendrites of a basiconic sensillum (40000×); (14) section through dendrites of a basiconic sensillum (50000×).

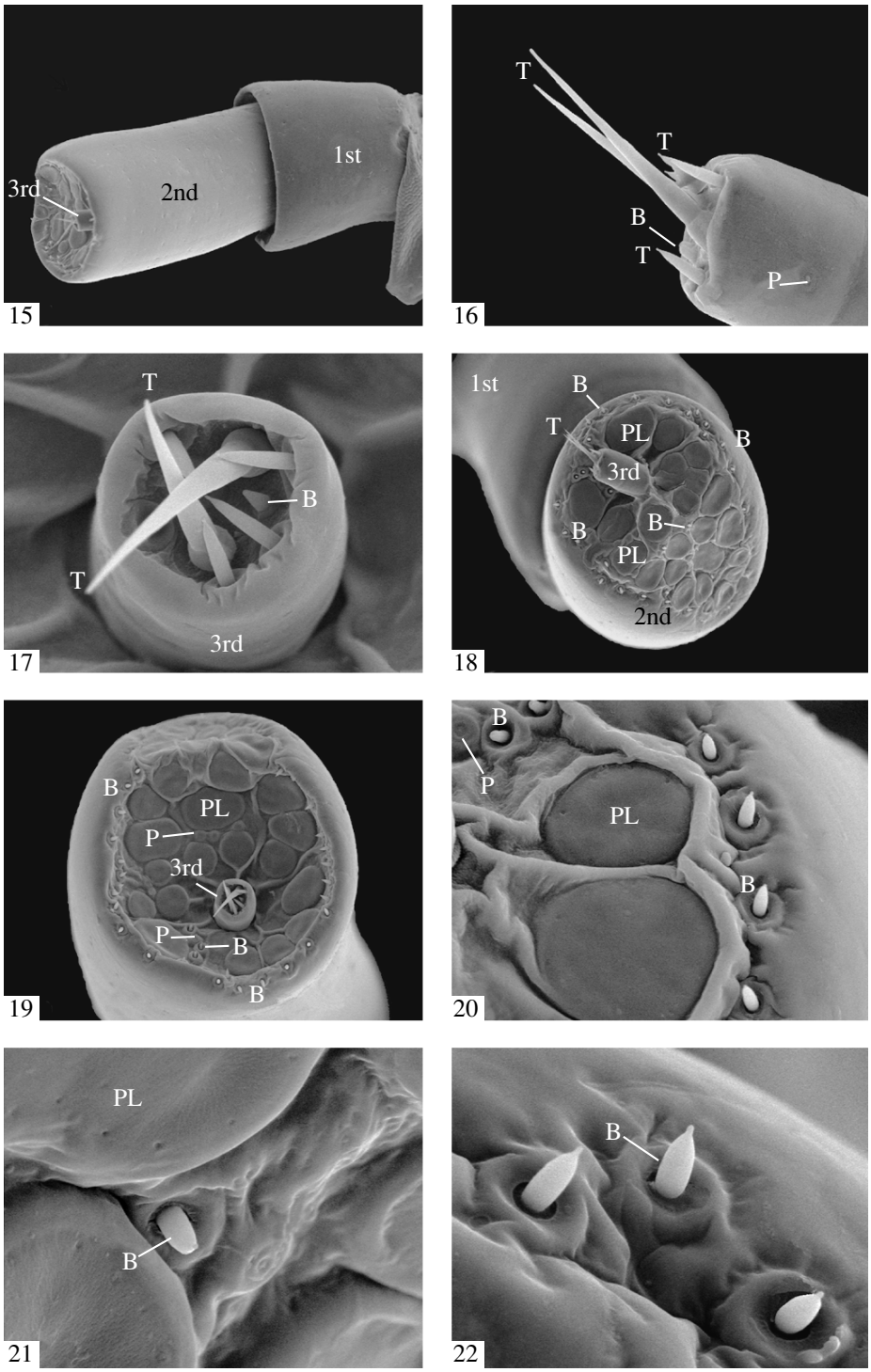
the second segment 270 × 100 μm, and the third segment 80 × 40 μm (Figs. 1, 2).

On each antenna there are only 12 chemoreceptor sensilla, represented by 7 basiconic, 2 styloconic, 1 trichoid sensilla, and 2 papillae. These sensilla are placed only on the apex of the second segment and on the third segment of the antennae. In addition, on all three segments, mechanoreceptor campaniform sensilla of various size are found, and on the third segment, a mechanoreceptor scolopoform sensillum.

A long (96 μm) and slightly bent trichoid sensillum begins from the apex of the third segment. The cuticle of wall of this hair is 0.7 μm thick. The cuticle is perforated with numerous pores (Fig. 3). The trichoid sensillum is innervated with two neurons. These neurons are represented by typical bipolar receptor cells. The distal regions of peripheral outgrowths are situated in the cavity of the cuticular region and do not branch (Fig. 9).

Basiconic sensilla are situated on the second and third segment of the antennae. On the second segment there are three basiconic sensilla 14 μm in height and 2.5 μm at the base. On the third segment there are four basiconic sensilla of somewhat smaller size—10.5 μm in height and 2.5 μm in diameter (Figs. 3–7). They have smooth cuticular walls. There is a pore on the apex of the hair. These sensilla rae innervated with two to six receptor cells (Figs. 10, 12–14).

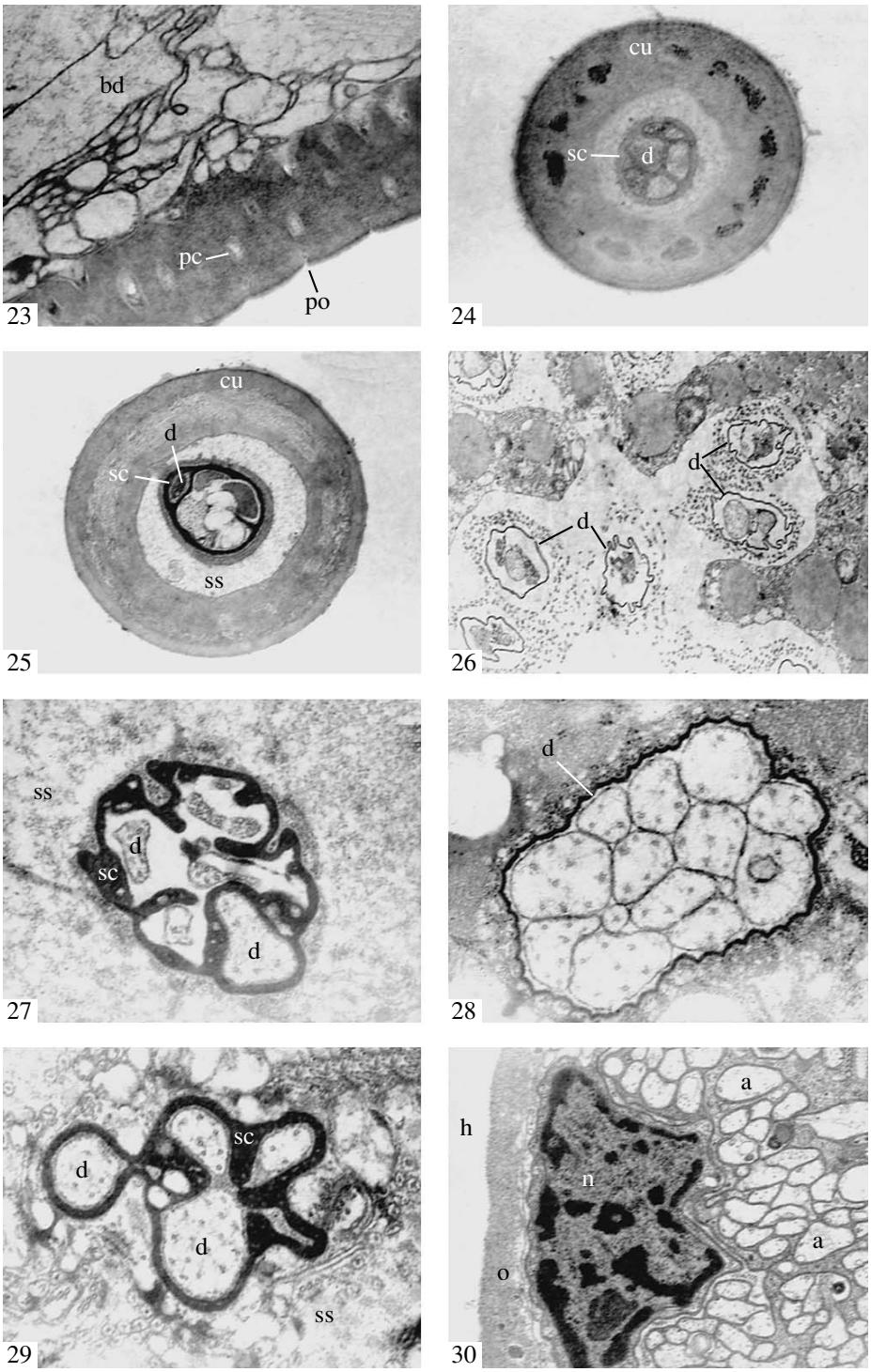
On each antenna there are two styloconic sensilla, one 4.3 μm in height on the apex of the third segment and the other, on the ventral side of the second segment, 7.14 μm in height. There is a rather large pore on the apex of the sensillum. The surface of the cuticle is



Figs. 15–22. Chemoreceptor organs on the antennae of larvae of *Z. rugipes.*
(15) General view of the antenna (200×); (16) Apex of the third segment of the antenna (1500×); (17) Sensilla of the third segment (2000×); (18, 19) Apex of the second and third segments of the antenna (350×); (20) Sensilla of the second segment (1500×); (21, 22) Parts of the sensory field of the second segment (3000×).

finely corrugated and bears furrows. The transverse section through the cuticle of the sensilla shows longitudinal canals. From these canals numerous thin pore canals begin. Styloconic sensilla are innervated by three or four neurons. In all investigated sensilla, two neurons ascend to the apex of the hair without branching (Fig. 11).

On each antenna there are two sensory papillae: one on the third segment (3.6 μm in diameter), another on



Figs. 23–30. Ultrastructure of chemoreceptor sensilla on the antennae of larvae of *Z. rugipes*. (23) Section through a sensory papilla (25000×); (24, 25) Transverse section through the cuticular region of a basiconic sensillum (30000× and 15000×); (26) Section through dendrites (15000×); (27, 29) Section through dendrites of a basiconic sensillum (40000×); (30) Section through antennal nerve (15000×).

the apex of the second segment (4.2 μm in diameter) (Figs. 5, 8). Each of the investigated papillae are innervated by five bipolar sensory neurons. Dendrites from three or four of them are situated within the cavity of the hair.

(2) Zophobas rugipes

The antennae of larvae of the last instar also consist of three segments, cylindrical in form. The basal segment is weakly sclerotized (230 μm long and 217 μm

in diameter).The second segment (360 μm long and 200 μm in diameter) and the third segment (62 μm long and 35 μm in diameter) are considerably sclerotized. The third segment is attached to the lateral edge of the apical surface of the second segment (Figs. 15, 18, 19).

On the apex of the third segment of the antennae there are six trichoid and two basiconic sensilla, and one sensillum in a more proximal position. Analysis of transverse sections through cuticular regions of trichoid and basiconic sensilla shows that they belong to contact chemoreceptors (Fig. 16). These sensilla belong to the category of thick-walled ones. The diameter of wall in the base of hairs reaches 1.5 μm, and more distally, 0.5–0.9 μm. In the cuticle of sensillae there are no lateral pores characteristic of olfactory receptors. Dendrites of receptor cells extend to the cavity of cuticular regions of these sensilla without ramification and are surrounded by the cuticular scolopoid envelope.

On the apical end of the third segment there are two long thrichoid sensilla 51 μm in height and 6.2 μm in diameter at the base and four small trichoid sensilla 12.4 μm in height and 2.4 μm in diameter. The cuticular region of these sensilla contains pores (Fig. 17).

The trichoid sensilla are innervated by five receptor cells. One is a mechanoreceptor cell, other four, chemoreceptor cells. Usually, the dendrites of chemoreceptor cells along their way to the terminal pore are surrounded by the general scolopoid envelope. However, in this species the dendrite of one of the receptor cells is separated from the other dendrites. This dendrite may belong to the mechanoreceptor cell, usually ending at the base of the hair.

Basiconic sensilla are present on the second and third segments. On the second segment there are 32 sensilla 20 μm long and 7.2 μm n diameter at the base. On the third segment there are only two basiconic sensilla 4.8 μm in height and 2.1 μm in diameter (Figs. 20–22).

The basiconic sensilla are innervated with two-six receptor cells. In contrast to trichoid sensilla, in the cuticle of basiconic sensilla there are about 12 longitudinal canals, not connected with the external surface of sensilla. All dendrites in the cuticular region are surrounded by the general scolopoid envelope (Figs. 24–29).

In the proximal region, before they enter the cavities of hairs, the dendrites are also surrounded by scolopoid envelopes. Some dendrites are completely separated from each other, others just partly.

On each antenna there are 13 papillae, one on the middle of the third segment, and others, on its apex.

The apex of the second of antennae is transformed into a sensitive platform with rather closely situated 24 placoid sensilla. On the outside, each sensilla is surrounded by a cuticular ridge. The cuticle in this part of the segment is less sclerotized and is capable of submersion inside the segment. This may protect the sensilla from mechanical damage, both the sensilla of this segment and those of the distal segment of the antennae (Fig. 18–20).

The cuticle of placoid sensilla is 2.2 μm thick and in perforated with numerous pore canals whose density is 8–10 canals/μm^2. The diameter of pore canals decreases distally. On the inner side of the cuticle of placoid sensilla it reaches 0.2 μm, and on the outer side, only 0.04 μm. The structure of the cuticular region of placoid sensilla and its relationship with dendrites of receptor cells is rather unusual. The mounts clearly show the places where dendrite membranes (or their thinnest branches) enter the pore canals. This not observed in the olfactory sensillae of other insects. Usually, the pores are connected to dendrite membranes by pore tubes, which are absent in the investigated placoid sensilla. In the cavity of pore canals, the membranes from a sheathlike structure.

Under the cuticular region of placoid sensilla there is a rather large cavity separated by cuticular outgrowths of the antenna wall into two interconnected chambers. Both chambers comprise thin branches of dendrites of receptor cells innervating these sensory organs.

Placoid sensilla are innervated by a large group (up to 12) of receptor cells. The dendrites of receptor cells of each sensillum are surrounded by a scolopoid envelope 0.03 μm thick.

Taking into consideration the traits of inner organization of the pore system it may be assumed that these sensory organs perform the olfactory and probably other functions: hygro- and/or thermoreceptory.

Over the periphery of the sensory platform of the second segment, 32 small basiconic sensilla are distributed. On the lateral side there are 12 papillae. Most of these sensory organs are innervated by two receptor cells.

The axons of receptor cells of all sensilla are combined into a rather large nerve. On the outside, the nerve is surrounded by a homogeneous envelope of connective tissue, 0.5 μm in diameter. Within the nerve, the diameter of axons is 0.2–0.6 μm. In axons there are microtubules, and in larger axons—mitochondria too. The nerve comprises also Schwann cells, occupying a marginal position.

Comparison of sensory organs of the antennae of the two investigated species revealed numerous sensilla on the antennae of *Zophobas* larvae: the total number of sensilla on the antenna of *Z. rugipes* is 77, while in *T. molitor* there are only 12. The types of sensilla in these two species are also different. On the antenna of *T. molitor*, styloconic sensilla are present, but they are absent in *Z. rugipes*. The latter possesses placoid sensilla.

The more developed sensory apparatus of the antennae in *Z. rugipes* than in *T. molitor* is related to differences in ecology of these two species. The beetles *T. molitor* are synanthropous insects living directly in various food substrata. The initial search fir a food substratum is made by females which lay eggs into the substrate. The beetles *Z. rugipes* live in free nature, thus for

the search of food substrata the larvae have to possesses sufficiently well developed sensory organs, including chemoreceptors.

The difference in the assortment of functional types of chemoreceptory sensilla should be also noted. In larvae of *T. molitor*, contact chemoreceptory sensilla prevail, while in *Z. rugipes*, olfactory sensilla are also widely represented along with contact chemoreceptors.

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REFERENCES

Albert, P.J., Zacharuk, R.Y., Weaver, D.K., and McFarlane, J.E., Electrophysiological and Behavioural Responses to Lactic Acid Stimuli in Larvae of *Tenebrio molitor* L. (Coleoptera, Tenebrionidae) and Permeability of Antennal Sensilla, *Physiol. Entomol.*, 1993, vol. 18, pp. 329–335.

Bloom, J.W., Zacharuk, R.Y., and Holodniuk, A.E., Ultrastructure of the Larval Antenna of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae): Structure of the Trichoid and Uniporous Peg Sensilla, *Can. J. Zool.*, 1982a, vol. 60, pp. 1528–1544.

Bloom, J.W., Zacharuk, R.Y., and Holodniuk, A.E., Ultrastructure of the Larval Antenna of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae): Structure of the Blunt-Tipped Peg and Papillate Sensilla, *Can. J. Zool.*, 1982b, vol. 60, pp. 1545–1556.

Benham, M. and Ryan, M.F., Ultrastrucutte of Antennal Sensory Receptors of *Tribolium* Larvae (Coleoptera: Tenebrionidae), *Int. J. Insect Morphol. Embryol.,* 1978, vol. 7, pp. 221–236.

Dubinskas, V.K., Sinitsina, E.E., and Chaika, S.Yu., Sensory Organs of Mouth Parts of larvae of *Dermestes frischi* Kul. and *Tenebrio molitor* L., *Vest. Mosk. Univ., Ser. Biol.*, 1997, no. 1, pp. 41–45.

Redkozubov, A.E. and Belousova, T.A., Mechanoactivated Receptor Currents in Chetoid Sensilla of the Antennae of *Tenbrio molitor*, *Sensornye systemy*, 1996, vol. 10, no. 1, pp. 77–79.