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Probable mechanoreceptor structures of osphradia in marine Caenogastropoda

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ABSTRACT. TEM and SEM electron microscopy have been used to study osphradia in 6 species of marine Caenogastropoda. The ultrastructural features of mechanoreceptor cells that perform the *Littorina* osmoreception function in osphradium organs are presented. Mechanoreception is based on a possible change in the volume of cisterns of microvilli of supporting cells, which can be transmitted by the cilia of nearby mechanoreceptor cells. These cells obviously, have mechanosensory channels on the apical surface. It has been first discovered in predatory molluscs actively searching for food, that single receptor cells with a mobile sensilla consisting of several cilium were joined together. They are located along the groove zone and follow the direction and force of the movement of water along the osphradium lamellae.

Возможные механорецепторные структуры в осфрадиях морских Caenogastropoda

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РЕЗЮМЕ. Методами ТЕМ и СЕМ электронной микроскопии исследованы осфрадии 6 видов морских Caenogastropoda. Приведены ультраструктурные особенности механорецепторных клеток, выполняющих в осфрадиальных органах литорин функцию осморецепции. Она основываются на возможном изменении объема цистерн микроворсинок опорных клеток, которые могут передаваться цилиям расположенных рядом механорецепторных клеток. Последние, очевидно, имеют на апикальной поверхности механосенсорные каналы. У хищных моллюсков, активно разыскивающих пищу впервые обнаружены одиночные рецепторные клетки с подвижной сенсиллой, состоящей из нескольких соединенных вместе цилий. Они располагающиеся вдоль зоны щели и повторяют направление и силу движения воды вдоль лепестков осфрадия.

Introduction

In the process of evolution of aquatic mollusks, a specialized sensory organ – osphradium was formed, and is most developed in Gastropoda. In the majority of members of this class, osphradium is localized in the mantle cavity in direct contact with water (Fig.1). Possessing multisensory function, osphradia of mollusks react to various external stimuli (changes in salinity, osmotic pressure, hypoxia, hypercapnia, amino acids, various odor substances) and thereby contribute to the regulation of a wide range of physiological and behavioral reactions, including respiration, nutrition, avoidance of a predator reactions, homing [Stinnakre, Tauc, 1969, Kamardin, 1988, Wedemeyer, Schild, 1995, Kamardin, 1996, Nezlin, 1997, Kamardin, Nozdrachev, 2004]. One of the necessary evidence of the receptor nature of an organ is the presence of receptor cells (Sc) and neuronal structures. The use of morphological methods, especially electron microscopy, makes it possible to investigate in detail the ultrastructure of the sensory elements of osphradia, the primary component of the chemoreceptive system in mollusks. A large number of works devoted to this exist [Benjamin, 1971, Crisp, 1973, Kamardin, Tsirulis, 1980, Kamardin, Tsirulis, 1985, Haszprunar, 1985, 1988, Kamardin, 1985, Nezlin *et al*, 1994, Kamardin, Nozdrachev, 2004]. In terrestrial pulmonary mollusks, these cells perform the function of olfaction and sense of taste [Chase, Croll, 1981, Croll, 1983, Nikitin, 2003]. For aquatic Gastropoda, osmoreception has been demonstrated many times [Stinnakre, Tauc, 1969, Jahan-Parwar, 1972, Sokolov, Kamardin, 1977, Kamardin, 1996, Kamardin *et al.*, 1999], however, the morphological type of osmoreceptors is still not identified. It is known that osmoreception can be mediated by mechanosensory cells that have mechanosensitive membrane channels studied in specialized mechanore-

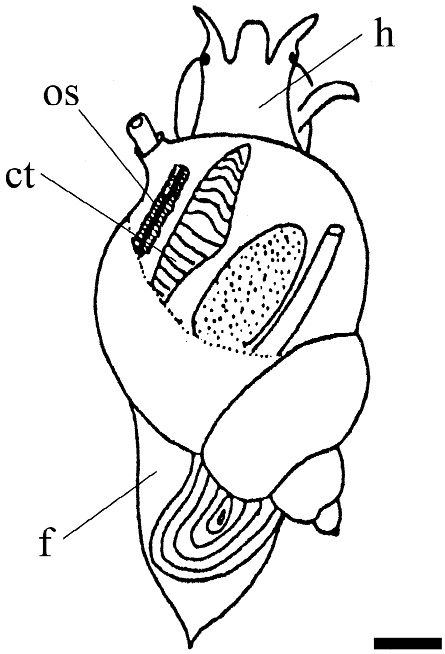

FIG. 1. The structure of Gastropoda, Caenogastropoda in a view from the dorsal side. Shell and part of the mantle removed (after Haszprunar, 1985) Abbreviations: ct – ctenidium; f – foot; h – head; os – osphradium. Scale: 500 μm.

РИС.1. Схема строения моллюска Gastropoda, Caenogastropoda вид со спинной стороны. Раковина и часть мантии удалены (из Haszprunar G. 1985). Обозначения: ct – ктенидий; f – нога; h – голова; os – осфрадий. Масштаб: 500 мкм

ceptors of gravity and hearing, as well as of volumetric detection and osmoreception [Hamill, Martinac, 2001].

Our study describes a possible osphradium osmoreceptor, which is found in almost all Caenogastropoda mollusks, and also ciliated receptor known only in predatory mollusks, which reacts to the direction of water movement along the osphradium lamellae.

Material and methods

6 species of mollusks were collected in tidal zone: *Littoraria angulifera* (Lamarck, 1822) (Guinea); *Nucella lapillus* (Linnaeus, 1758) (England); *Conus leopardus* (Röding, 1798) (Madagascar); *Nassarius globosus* (Quoy et Gaimard, 1833) (Hong Kong); *Reticunassa festiva* (Powys, 1835) (Hong Kong); *Lambis lambis* (Linnaeus, 1758) (Madagascar).

These specimens were studied by traditional methods of scanning (SEM) and transmission (TEM) electron microscopy. In some cases, using the methods of conventional transmission and scanning microscopy does not allow to get a complete picture of the three-dimensional organization of intracellular structures. The using the method of freezing-etching when the samples frozen in liquid nitrogen with subsequent etching of the cleaved surface of OsO_4 makes it possible to obtain a three-dimensional pattern of intracellular organization. Osphradium samples were fixed in a 1% solution of OsO_4 in phosphate buffer, 0.15 mol/l, pH 7.4, for 16 hours at a temperature of 40C°. After washing in the buffer, the samples of osphradium were transferred to dimethyl sulfoxide (DMSO), successively increasing its concentration from 15 to 50%. Then the samples were frozen in liquid nitrogen and splitted into small pieces with a razor blade. The thawed samples were washed in phosphate buffer and macerated in 0.1% OsO_4 in phosphate buffer, 0.15 mol/l, for 5 days at room temperature. After washing in the buffer, the samples were dehydrated in acetone and the critical point dried. The samples were coated with gold three times 30 s each with a small current value. A Jeol T-200 conventional scanning microscope and a Hitachi H800 high resolution microscope (Japan), transmission electron microscopy Hitachi H-300 were used for viewing.

Results and discussion

Littorinidae

Osphradium of *Littoraria angulifera* is a flattened ridge, which is formed by a single-layer cylindrical epithelium, having a greater thickness than the normal mantle epithelium. On the sides of the ridge there are two lateral zones formed by ciliated cells (Fig. 2A). The ciliated tufts rise above the surface by 7-10 microns. As follows from observations, the cilia of the lateral zones are in constant motion and move the fluid along the central receptor zone, while the solid carbonate chalk particles, moving along with the fluid, make spiral movements from left to right and from right to left, repeatedly crossing the receptor zone. The transport function of the ciliary zones was earlier mentioned in works performed on the Littorinidae and other gastropods [Yonge, 1947; Crisp, 1973]. A special role in the reception is attributed to a small marginal area of the osphradial surface between the ciliated and receptor zones, which often has the form of a cleft or a small groove (Fig. 2B). At the base of the groove are the modified microvilli of the supporting cells (Sp) and the peripheral processes (Pp) of the bipolar primary sensory receptor cells (Sc) in *Littoraria angulifera* (Fig. 3A,B) and *Lambis lambis* osphradia (Fig. 3D). They were previously found there in other species of molluscs [Crisp, 1973, Haszprunar, 1985, 1988].

Microvilli of Sp are expanded as rounded cisterns, and their membranes are tightly interconnected in the expansion region, which is clearly visible

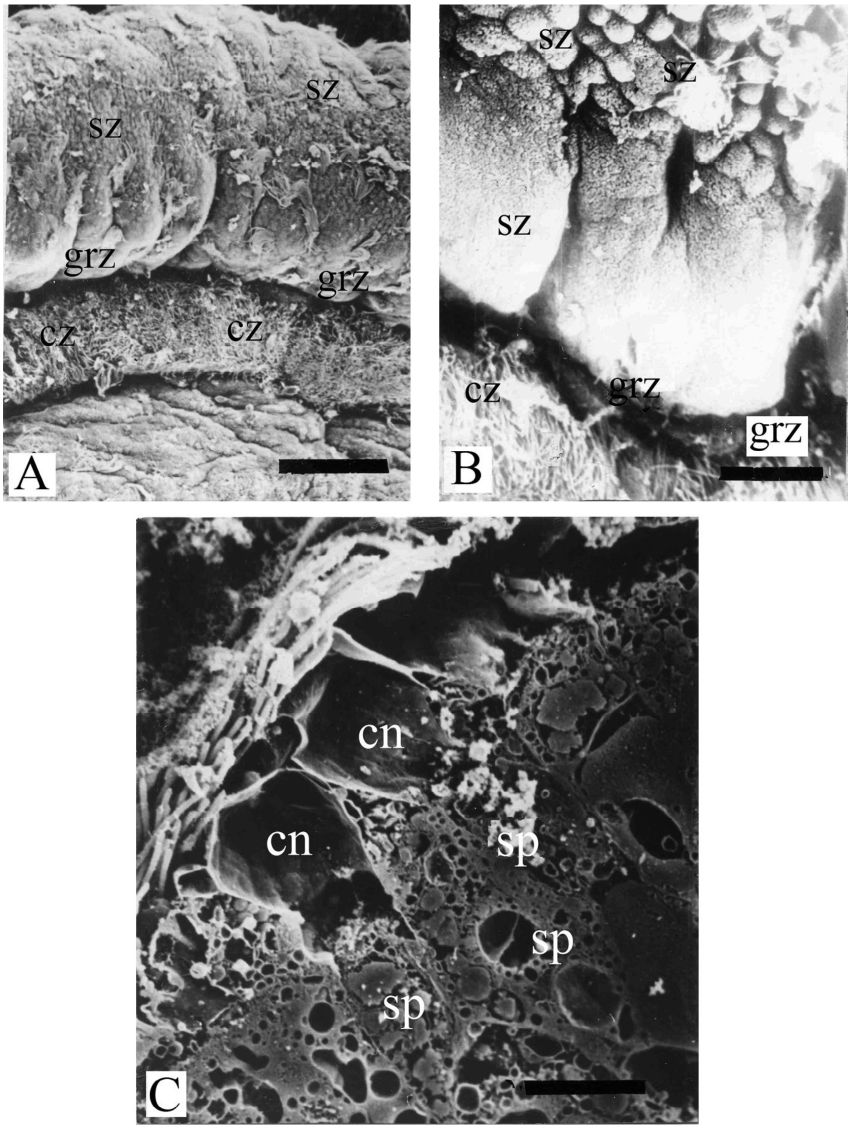

FIG. 2. The surface of *Littoraria angulifera* osphradium with indication of various zones (**A, B**), and the transverse sections of modified microvilli of supporting cells in osphradium *Nucella lapillus* by freezing-etching method (**C**). Abbreviations: cn – cistern; cz – ciliated zone; grz – groove zone; sp – supporting cell; sz – receptor zone. Scale bars: A,B – 10 µm; C – 20 µm.

РИС. 2. Поверхности осфрадия *Littoraria angulifera* с указанием различных зон (**A, B**), и вид видоизмененных микроворсинок опорных клеток в зоне щели осфрадия *Nucella lapillus* в методике freezing-etching (**C**). Обозначения: cn – цистерна; cz – ресничная зона; grz – зона щели; sp – опорная клетка; sz – рецепторная зона. Масштаб: A, B – 10 мкм; C – 20 мкм.

on freezing - etching preparations (Fig. 2C) and in the transverse section of the groove zone (Fig. 3B, C). Thus, they isolate a small space above the apical surface of the cells from the environment (Fig. 3C). In this space there are short microvilli and modified cilia of numerous peripheral processes of Sc in *Littoraria angulifera* (Fig. 3A,B) and *Lambis lambis* osphradia (Fig. 3D). The ultrastructure of the apical region of Pp corresponds to that known for peripheral processes of Sc. It differs from Sp electron-transparent cytoplasm and has longitudinally oriented microtubules, elongated mitochondria which are connected with desmosomes (Fig. 3A, D). The basal bodies of cilium lack of roots (Fig. 3B) and have a modified set of peripheral tubular fibrils (8x2+2) (Fig. 3A, D). The length of the cilium is only 1.5-2.0 microns (Fig. 3D). It is assumed that the Pp of Sc located next to the cisterns can, in a complex, perform the function of osmoreception. Large microvilli cistern of Sp obviously play the role of osmotic sensors (the simplest one consists of a semi-permeable membrane filled with a solution, in our case the cytoplasm), which vary in volume when osmotic pressure of the medium is changed. These changes can be perceived by Sc (Fig. 3A, D), usually, have on the apical surface

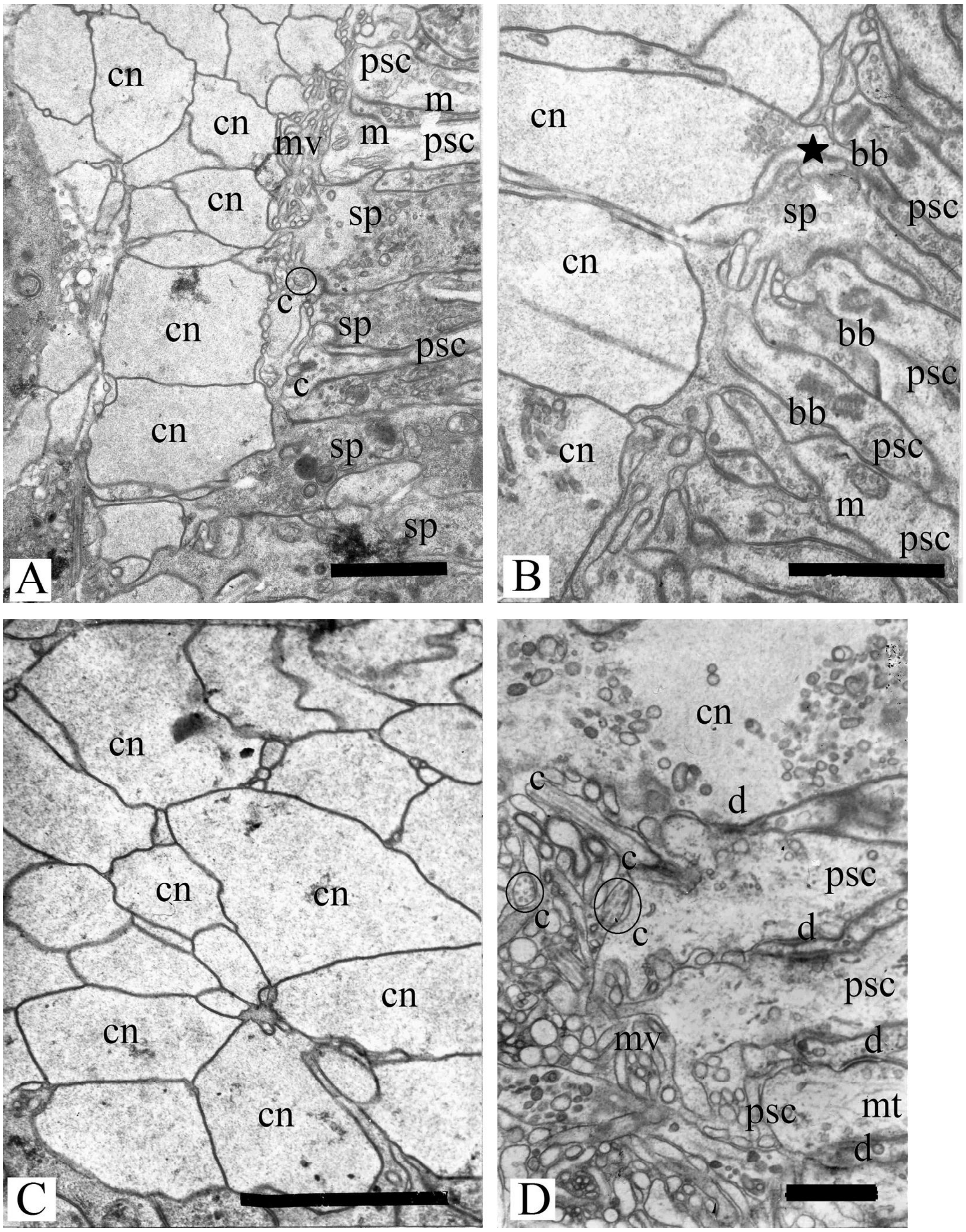

FIG. 3. Transverse sections of the groove zone of osphradia of *Littoraria angulifera* (**A; B; C**) and *Lambis lambis* (**D**). Cisterns (modified microvilli of supporting cells) and numerous peripheral processes of receptor cells with 1–2 cilia are visible. Abbreviations: bb – basal body of cilia; c – cilium; cn – cistern; d – desmosomes; m – mitochondria; mv – microvilli; psc – peripheral process of the receptor cell; sp – supporting cell. ★– microvilli expansion space. Scale bars: A, C – 5 μm ; B – 3 μm; D –1 μm.

РИС. 3. Поперечные срезы зоны щели осфрадия *Littorina angulifera* (A;B;C) и *Lambis lambis* (D). Видны цистерны (видоизмененные микроворсинки опорных клеток) и многочисленные периферические отростки рецепторных клеток с 1–2 цилиями. Обозначения: bb – базальное тельце реснички; c – цилия; cn – цистерна; d – десмосомы; m – митохондрии; mv – микроворсинки; psc – периферический отросток рецепторной клетки; sp – опорная клетка. ★ – место расширения микроворсинки. Масштаб: A, C – 5 мкм; B – 3 мкм; D – 1 мкм.

mechanoreceptor channels [Hamill, Martinac, 2001]. Excitation is transmitted to the central nervous system, where one or other reflex arcs close. Under the conditions of free behavior and non-invasive registration of cardioactivity, we were able to demonstrate a significant decrease in heart rate in *Littoraria littorea* when injecting 1 ml of hypotonic seawater (50% sea water) into the mantle cavity [Kamardin *et al.*, 2012]. Reactions to inulin, sucrose, and NaCl were recorded electro-physiologically from

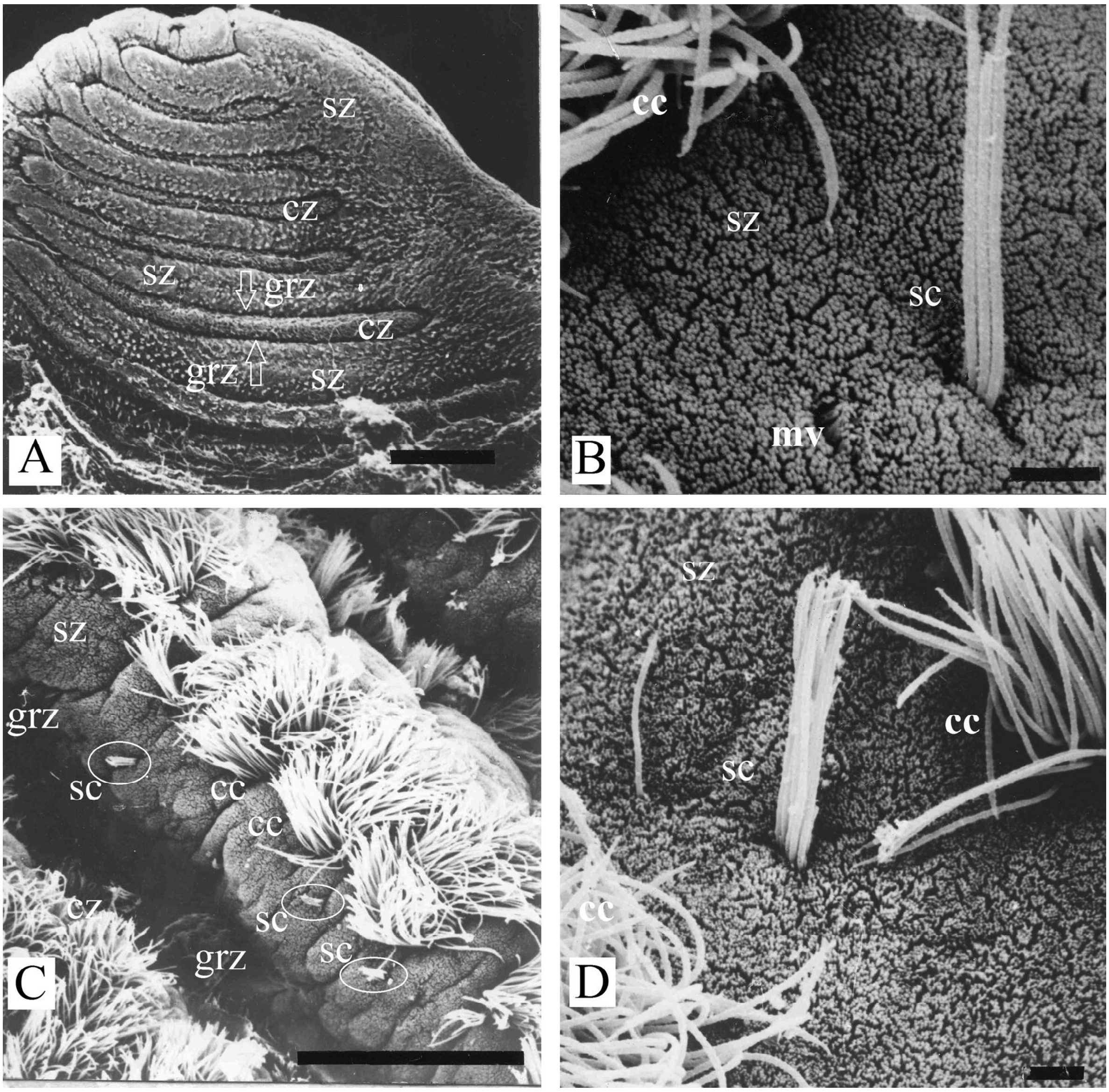

FIG. 4. The surface of osphradium of *Conus leopardus* with different zones (A,D), separate mechanoreceptor sensilla (D) are visible. The finger-shaped protrusion of the ciliary zone of osphradia of *Nassarius globosus* (B,C). The same orientation of the neighboring sensory sensillae is clearly distinguishable (C). Abbreviations: cc – ciliated cell; sc – mechanoreceptor sensilla; arrows indicate areas of the groove in the finger-shaped protrusion of the ciliated zone. Other abbreviations as in Fig. 2. Scale: A, C – 100 μm; B, D –1 μm.

РИС. 4. Поверхности осфрадия *Conus leopardus* с различными зонами (A,D), видны отдельные механорецепторные сенсиллы (B; C; D). Пальцевидное выпячивание ресничной зоны осфрадия *Nassarius globosus* (B,C). Хорошо различима одинаковая ориентация соседних сенсорных сенсилл (C). Обозначения: cc – ресничная клетка; sc – механорецепторные сенсиллы; стрелки указывают на зоны щели в пальцевидном выпячивании ресничной зоны. Остальные обозначения как на Рис. 2. Масштаб: A, C – 100 мкм; B, D – 1 мкм.

the osphradial nerve of the pond snail [Sokolov, Kamardin, 1977], and a small input current response to NaCl was detected in single osphradium neurons by patch-clamp method [Kamardin *et al.*, 1999à, Kamardin, Nozdrachev, 2004].

A fundamental similarity is found between the structure of osmoreceptors in kidney or liver parenchyma and osphradium osmoreceptors. Both receptors represent a volume-modifying structure: cistern-like microvilli of supporting cells interacting with receptor cells, or capillaries in the parenchyma of the kidneys and liver, whose walls contain immunocytochemically identified nerve endings of the peripheral nervous system [Lechner *et al.*, 2010]. The discovered ultrastructure of the groove zone of *Littoraria angulifera* is also found in marine mollusks belonging to families Littorinidae, Naticidae, Muricidae, Buccinidae, etc.

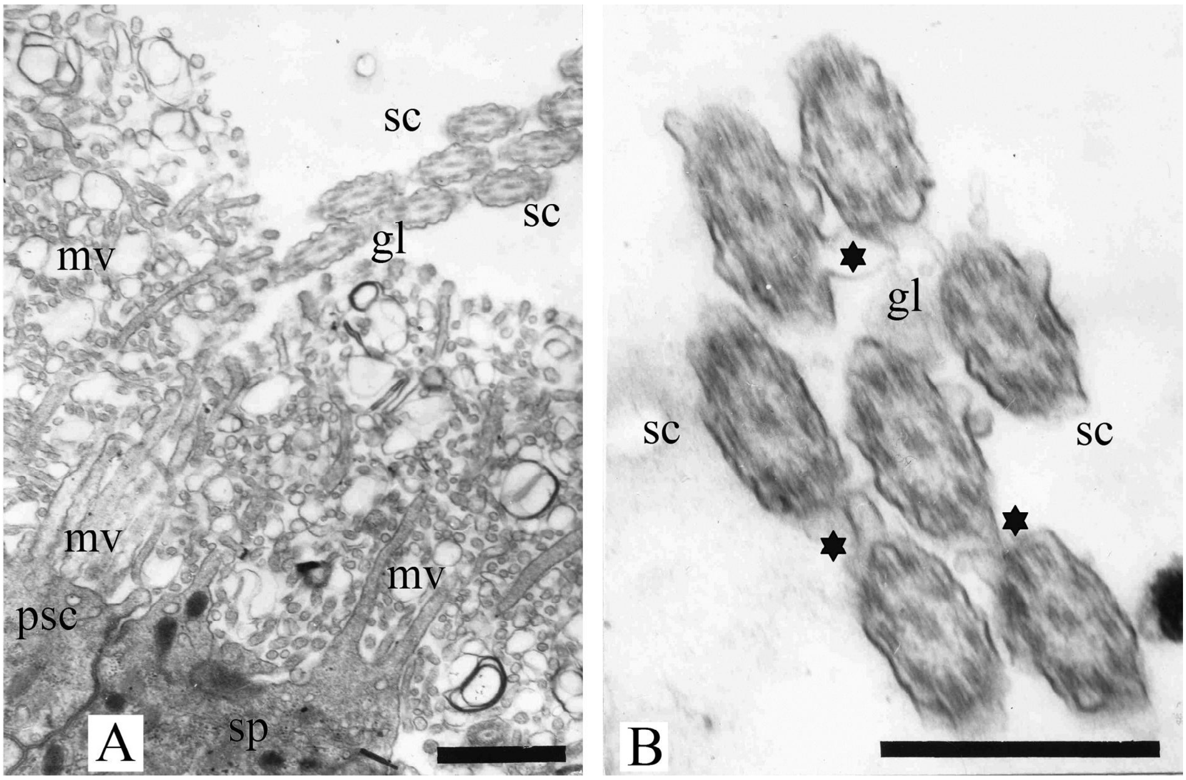

FIG. 5. Transverse section of the receptor zone of *Nassarius festivus* with the apex of the peripheral process (A) of the receptor cell with cilia combined into a mechanoreceptor sensilla (B). Abbreviations: gl – glycacalix; ✶ – outgrowths of the outer membrane of cilia. Other abbreviations as in Fig. 3. Scale: A – 1.0 µm; B – 0.5 µm

РИС. 5. Поперечный срез рецепторной зоны осфрадия *Nassarius festivus* с вершиной периферического отростка (А) рецепторной клетки с цилиями, объединенными в механорецепторную сенсиллу (В). Обозначения: gl – гликакалис; ✶ – выросты наружной мембраны цилий. Остальные обозначения как на Рис. 3. Масштаб: А – 1 мкм; В – 0,5 мкм.

Conidae

Predatory marine mollusks of the families Conidae, Nassariidae (*Nassarius globosus* and *Conus leopardus*, etc.) actively search for their prey by smell. They have the most sophisticated osphradium of the ctenidial type. Numerous (up to 13) finger-shaped invaginations of the ciliated zone and a groove zone in the receptor zone are formed on the side surfaces of the lamellae of osphradium. Along the lamellae on the lateral surfaces and on the ventral edge of the lamellae, in the receptor zone, individual, equally oriented ciliated structures were found, consisting of 3-10 cilia, joined together with a normal set of tubular elements (Fig. 4B, C, D; Fig. 5B). For the first time, such receptors were found in 1992 [Taylor, Kamardin, 1992]. The group of united cilia belongs to single receptor cell. The apical part of this cell has a more electron-transparent cytoplasm, as compared to supporting cells (Fig. 5A). Cilia do not have roots, and are interconnected by the membrane processes and are additionally glued with a glycacalyx-like substance (Fig. 5B). The orientation of sensilla follows the direction of fluid moved by beating of the cilia of the ciliated zone along the groove zone on the lateral surfaces of the osphradium lamellae (Fig. 4C). Comparing the ultrastructural organization of the basal part of the sensilla of these receptors and the receptor cells of the statocysts of mollusks, we can conclude that the cilia found by us can perform a mechanoreceptor function [Budelmann, 1976]. Wind-oriented sensilla are known for insects [Tishchenko, 1977]. By analogy with insects, it can be assumed that the ciliated Sc found in osphradium is sensing the direction and force of water movement along the lamellae of the osphradial organ of predatory mollusks. Otherwise, the ultrastructure of Sp and Sc osphradium of predatory mollusks is similar to that of *Littoraria*, *Natica* and *Murex*.

Thus, primary sensing, intraepithelial receptor cells of osphradia of molluscs, along with chemosensory function, can perform the function of osmoreceptors and mechanoreceptors of the direction of fluid movement along osphradium. Both cells and other cells have mechanosensory membrane channels on the apical surface of the Sc, which, when deformed, change the permeability of the membrane, leading to depolarization [Hamill, Martinac, 2001]. The apical surface of mechanoreceptors of water movement have 3-10 cilia united in a single sensilla, which increases their surface. They resemble trichocid sensilla of insects oriented in the wind and reacting with excitation to the force and direction of the movement of water.

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