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Ultrastructural study of the osphradia in Polyplacophora

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ABSTRACT: The investigation of ultrastructure of osphradia in ten polyplacophoran species from suborders Acanthochitonina and Chitonina revealed a number of similar, primitive structures. In all specimens, each osphradium resembles a more or less isolated shaft or fold of specialized single-layered epithelium located in the mantle cavity immediately behind the last pair of gills. Ciliated, microvilliferous, and secretory supporting cells, as well as peripheral processes of receptor cells with several cilia forming the receptor surface of the osphradium were discovered. On the osphradial surface, there is no division into zones, which are characteristic of the osphradia in the gastropods. It is undoubtedly the organ of the chemical sense, performing the initial role of the interoreceptor and reacting to a change in the physico-chemical characteristics of the fluid in the mantle cavity (O_2, pH, osmolarity, etc.). Perhaps, osphradium acts as a distant chemoreceptor in complex behavioral acts, such as homing. However, due to the large plasticity of the molluscs, it is possible to form other sensory structures, which, especially in primitive groups (for example: Lepidopleurida), can partially perform the osphrasdial functions. If we take into account the absence of osphradia and the presence of numerous parts of sensory epithelium in the mantle cavity of the most primitive forms of chitons belonging to the order of Lepidopleurida, then probably osphradia in the more advanced chitons, the Chitonida order, as an independently developed organ, possibly not homologous to the osphradia of other molluscs (Gastropoda, Bivalvia and some Cephalopoda).
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KEY WORDS: ultrastructure, osphradium, *Acanthopleura brevispinosa, Ischnochiton hakodadensis, Tonicella marmoreal*, receptor cells.

Ультраструктурное исследование осфрадиев представителей класса Polyplacophora

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РЕЗЮМЕ: Детальное изучение осфрадиев представителей Acanthochitonina и Chitonina выявило ряд сходных, примитивных, с точки зрения сенсорной физиологии, черт

строения. У этих животных осфрадии по форме напоминают более или менее обособленный валик или складку специализированного однослойного эпителия, расположенный в мантийной полости непосредственно за последней парой жабр. Рецепторная поверхность осфрадиев образована мерцательными, микроворсинчатыми, секреторными опорными клетками, а также периферическими отростками интраэпителиальных рецепторных клеток с несколькими цилиями. На осфрадиальной поверхности не обнаружено разделения на зоны, свойственное осфрадиям Брюхоногих. Однако обнаруженный единый план строения осфрадиальных органов говорит об относительном эволюционном консерватизме этого признака, в основе которого лежат механизмы функционирования осфрадия, как сенсорного органа. Он бесспорно является органом химического чувства, выполняющим исходно роль интерорецептора и реагирующим на изменение физико-химических характеристик жидкости в мантийной полости (O_2, pH, осмолярность и т.д.). Возможно, он как дистантный хеморецептор участвует в сложных поведенческих актах, таких как хоминг. Однако, в силу большой пластичности моллюсков, возможно формирование и других сенсорных структур, которые особенно у примитивных групп (на пример: Lepidopleurida) могут частично выполнять осфрадиальные функции. Если принять во внимание полное отсутствие осфрадия и наличие многочисленных участков сенсорного эпителия в мантийной полости у наиболее примитивных форм хитонов, принадлежащих к отряду Lepidopleurida, то, вероятно, осфрадий у более продвинутых хитонов, отряда Chitonida, следует рассматривать как независимо развитый орган, возможно не гомологичный осфрадиям других моллюсков (Gastropoda, Bivalvia и некоторых Cephalopoda).

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КЛЮЧЕВЫЕ СЛОВА: ультраструктура, осфрадиум, *Acanthopleura brevispinosa, Ischnochiton hakodadensis, Tonicella marmoreal,* рецепторные клетки.

Introduction

Evolutional and systematic relations between separate groups inside of Molluscs have not been finally determined so far (Giribetetal., 2006; Salvini-Plawen, Steiner, 2014), partly due to absence of stable morphological indicators, partly due to high functional plasticity of the latter. Using TEM methods, sensory organs of some Polyplacophora were studied by G. Haszprunar (1987). Along with the canonical location of osphradia behind the last pair of gills (Fig. 1), he described other sensory structures on lateral surfaces of the mantle cavity at the base of gills and on the gills. This made some authors suppose that osphradium is not a homologous structure either within the class or within the type of Molluscs overall (Sigwart et al., 2014). Our study is devoted to morphology and ultrastructure of osphradia organs of 10 species of Polyplacophora and attempts find possible differences between them.

Material and methods

10 species of chitons were used in the work: *Ischnochiton hakodadensis* Pilsbry, 1892 (Sea of Japan, Vostok Bay), *Stenosemus albus* (Linnaeus, 1767) (Barents Sea, Dalniye Zelentsy), *Acanthopleura brevispinosa* (G.B. SowerbyII, 1840) (Madagascar), *Tonicella marmorea* (Fabricius, 1780) (Norwegian Sea, Tromsø, *T. submarmorea* (Middendorff, 1847) (Commander Islands), *Spongioradsia aleutica* (Dall, 1878)

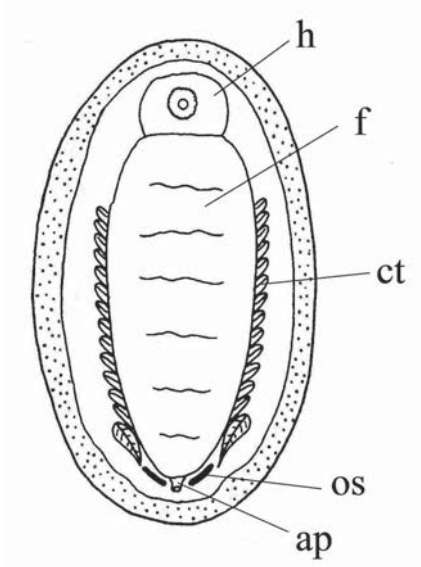

Fig. 1. Schematic structure of chiton (Chitonida); ventral view. Abbreviations: ap — anal papilla; ct — ctenidium; f — foot; os — ophradium; h — head. Scale bars 500 μm.

Рис. 1. Схема строения хитона (Chitonida) вид с брюшной стороны. Обозначения: ap — анальная папилла; ct — ктенидий; f — нога; os — осфрадий; h — голова. Масштаб 500 мкм.

(Commander Islands), *Micichiton grandispina* Sirenko, 1975 (Commander Islands), *Nanichiton deplanatus* Sirenko, 1975 (Commander Islands), *Schizoplax brandtii* (Middendorff, 1847) (Commander Islands) and *Acanthochitona rubrolineata* (Lischke, 1873) (Sea of Japan, Posyet Bay).

These specimens were studied by traditional methods of scanning (SEM) and transmission (TEM) electron microscopy. Methodological techniques, which were invented earlier for study of osphradia in Gastropoda (Kamardin, Nozdrachev, 2004), were used for identification of sensory organs and receptor cells.

Results and discussion

***Ischnochiton hakodadensis*, *Stenosemu salbu* (Chitonida – Chitonina – Ischnochitonidae)**

The order Chitonida is considered evolutionally advanced, and its representatives have progressive features of the structure of radula, egg membranes and location of gills (Sirenko, 1993). Early works of L. Plate (Plate, 1899) described pigmented combs located between the last pair of gills and the anal papilla. The paired osphradium have in the form of small shaft lying behind the last pair of gills, closer to the internal wall of the mantle fissure (Fig. 1). The osphradium surface of *Stenosemus albus* is not divided into zones. It is formed by specialized single-layered epithelium consisting of microvilli, ciliated and secretory supporting cells (Fig. 2B, C, D). Secretory cells often have a big vacuole, which is filled with homogeneous secretion and is well visible on the fissures of preparations (Fig. 2D). Receptor cells have a bipolar form with a well differentiated axon (Fig. 3A, B, D). At the same time, insufficient differentiation of the peripheral process and the intraepithelial location of receptor cells indicate the primitive structure of osphradium. Saturation with organoides, typical for peripheral processes of the gastropods, was not revealed in peripheral processes of receptor cells of ospradium of *Stenosemus albus*, which differ from supporting cells by more electron-light cytoplasm (Fig. 4A, B). There are not many small roundish mitochondria and cisterns of smooth endoplasmic reticulum and longitudinally orientated microtubules here. The apical surface is provided with microvilli of the usual length (up to 2.0 μm) and one or two cilia (Figs 2B, 4A, C). Cytoplasm of ciliated supporting cells has mitochondria, ribosomes; it is rich in glycogen granules and pigmental inclusions of up to 1.0 μm in diameter (Fig. 4B, C). One cell has up to 120 cilia on the average (Fig. 4D).

***Acanthopleura brevispinosa* (Chitonida – Chitonina – Chitonidae)**

The paired osphradium of the large chiton of *Acanthopleura brevispinosa* reaching 10 cm is located near the anal papilla and has the shape of a fold or a shaft, which has reached 6–8 mm in length and up to 2 mm in diameter. Neither regular folds, nor definite zoning of location of cellular elements were revealed on the osphradium surface. Short (1.0–3.0 μm) cilia of recep-

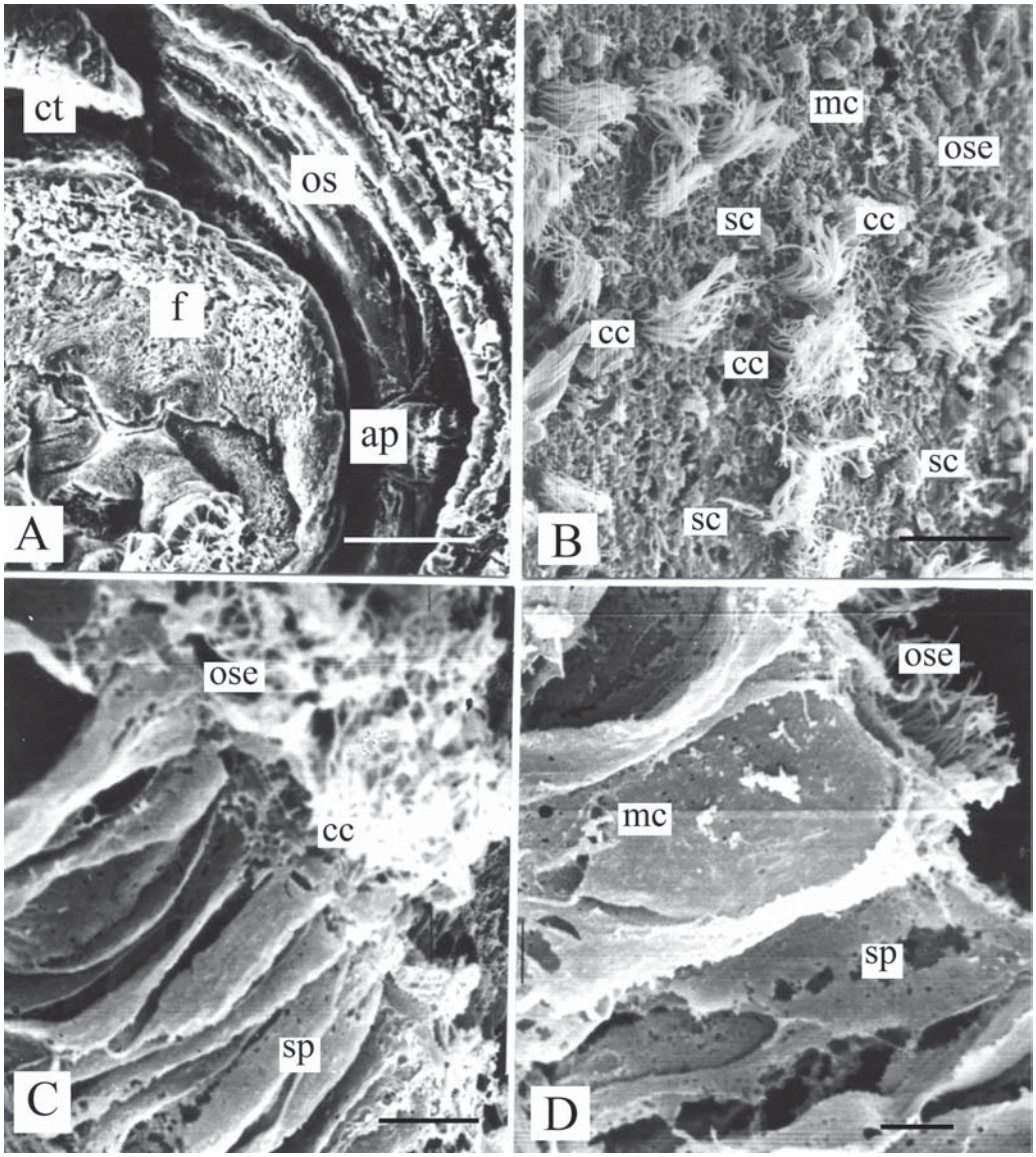

Fig. 2. General view and cellular organization of Chitonina osphradia, SEM. A — general view of the caudal part of the mantle grove of *Ischnochiton hakodadensis*. B — ciliary structures on the osphradial surface of *Stenosemus albus*. C–D — cellular structures of the osphradial epithelium *Stenosemus albus*. Clearly visible ciliary and secretory cells, which is filled with mucus.

Abbreviations: cc — ciliated cell; ct — ctenidium; mc — secretory cell; ose — apical surface of osphradial epithelium; sc — sensitive cilia; sp — supporting cell. Other abbreviations as in Fig. 1. Scale bars: A — 300 μm; B — 15 μm; C — 20 μm; D — 22 μm.

Рис. 2. Общий вид и клеточная организация осфрадиев хитонов п/отряда Chitonina, СЭМ. A — общий вид каудальной части мантийной борозды *Ischnochiton hakodadensis*. B — цилиарные структуры на осфрадиальной поверхности *Stenosemus albus*. C–D — клеточные структуры осфрадиального эпителия Stenosemus *albus*. Хорошо видны ресничные и секреторные клетки заполненные слизью.

Обозначения: mc — секреторная клетка; cc — ресничная клетка; ose — апикальная поверхность осфрадиального эпителия; sc — чувствительные реснички; sp — опорная клетка. Остальные обозначения как на рис. 1. Масштаб: A — 300 мкм; B — 15 мкм; C — 20 мкм; D — 22 мкм

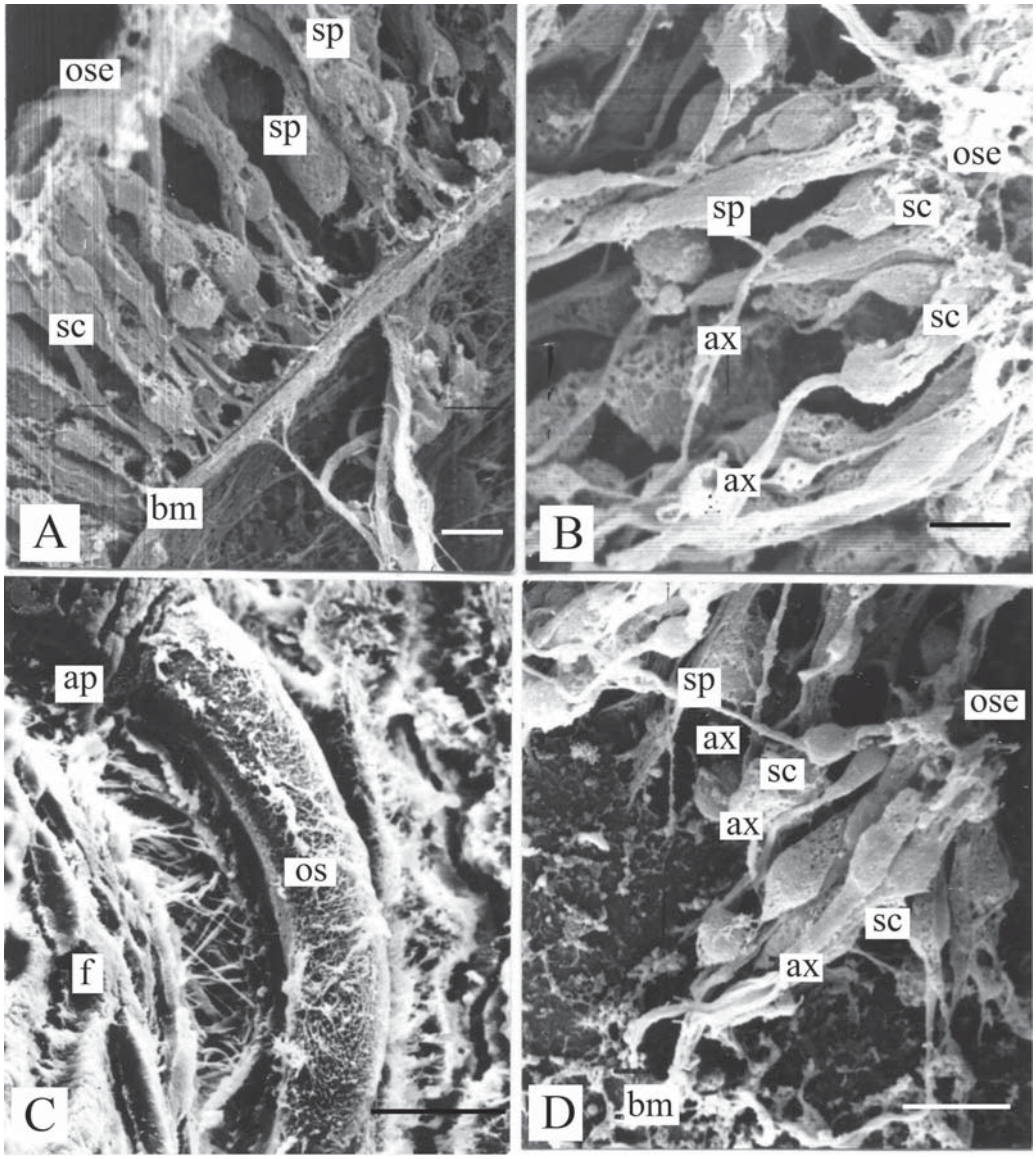

Fig. 3. General view and cellular organization of chiton osphradia, SEM. A — fine structure of the osphradial epithelium *Ischnochiton hakodadensis*, support and receptor cells are visible. B — small bipolar receptor cells with well differentiated axons. C — general view of the osphradia of *Acanthochitona rubrolineata*. D — group of sensory cells, the axons of which are directed to the basal membrane.

Abbreviations: ax — axon; ap — anal papilla; bm — basal membrane. Scale bars: A — 10 μm; B — 12.5 μm; C — 80 μm; D — 15 μm.

Рис. 3. Общий вид и клеточная организация осфрадиев хитонов, СЭМ. А — тонкое строение осфрадиального эпителия *Ischnochiton hakodadensis.* Видны опорные и рецепторные клетки. В — видны мелкие биполярные рецепторные клетки с хорошо дифференцированным аксоном. С — общий вид осфрадия *Acanthochitona rubrolineata*. D —хорошо заметны объединенные в группы чувствительные клетки, аксоны которых направляются к базальной мембране.

Обозначения: ax — аксон; ap — анальная папилла; bm — базальная мембрана. Остальные обозначения как на рис. 1. Масштаб: А — 10 мкм; В — 12,5 мкм; С — 80 мкм; D — 15 мкм

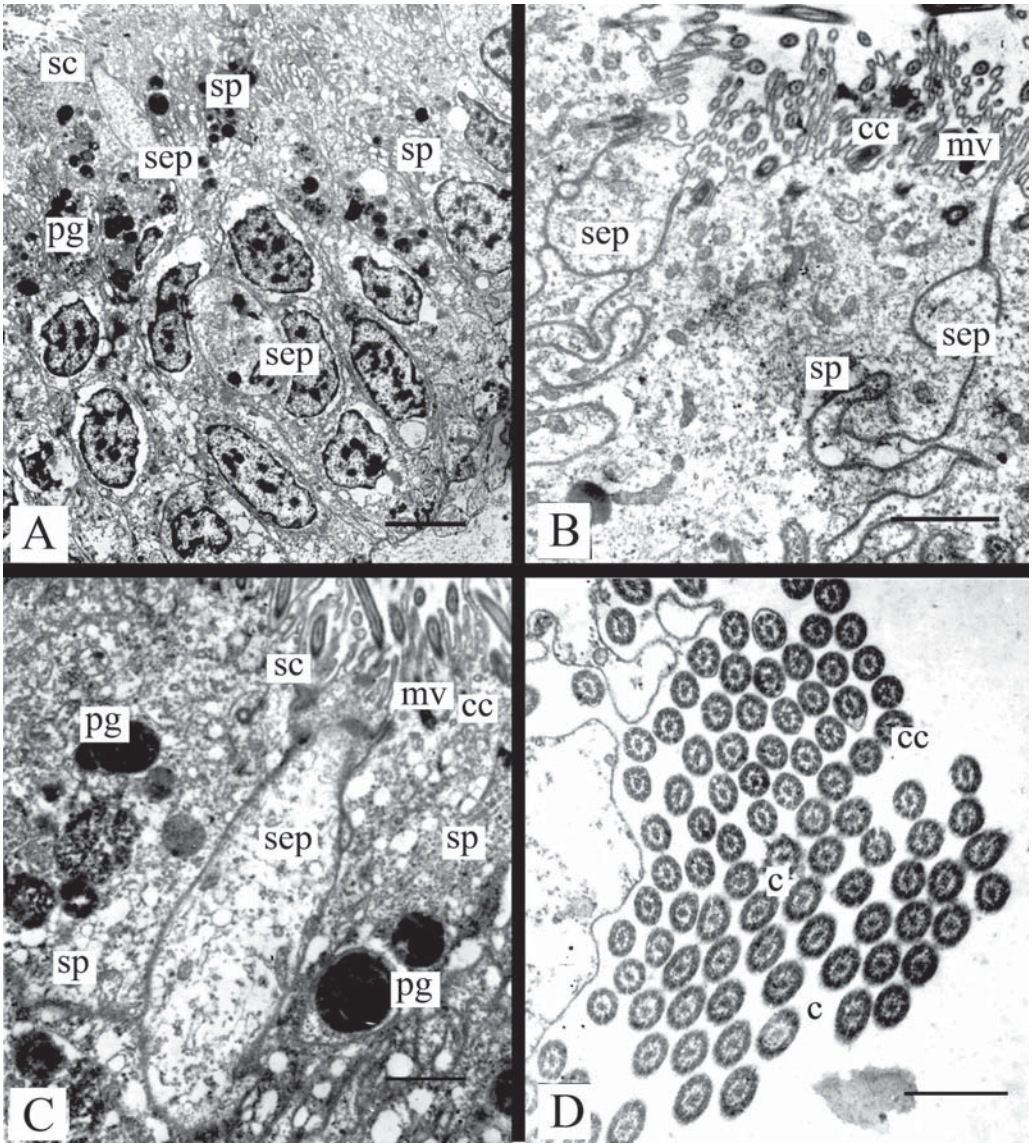

Fig.4. Details of the fine structure of *Stenosemus albus,* TEM. A — transverse section of osphradium with supporting cells and peripheral processes of receptor cells. B — apical region of ciliary supporting cell. C — peripheral processes of the receptor cell isolated by supporting cell with pigment granules. D — transverse section of cilia belonging to one supporting ciliary cell.

Abbreviations: c — cilium; mv — microvilli; pg — pigment granules; sep — peripheral process of the receptor cell. Scale bars: A— 10 μm; B — 2.5 μm; C — 1.2 μm; D — 1.0 μm.

Рис.4. Детали тонкого строения осфрадиев *Stenosemus albus* по данным ТЕМ. А — поперечный срез осфрадия с опорными клетками и периферическими отростками рецепторных клеток. В — апикальная область ресничной опорной клетки. С — хорошо виден периферический отросток рецепторной клетки изолированный опорными с пигментными гранулами. D — поперечный срез ресничек принадлежащих одной опорной ресничной клетке.

Обозначения: с — ресничка; mv — микроворсинки; pg — пигментные гранулы; sep — периферический отросток рецепторной клетки. Масштаб: А — 10 мкм; В — 2,5 мкм; С — 1,2 мкм; D — 1,0 мкм.

tor cells and tufts of longer (up to 10.0 μm) cilia belonging to supporting cells rise above the organ formed by microvilli of supporting cells.

***Tonicella marmorea, Tonicella submarmorea* (Chitonida – Acanthochitonina – Tonicellidae)**

Representatives of the suborder of Acanthochitonina are united by the abanal location of gills and large and rare appendages of secondary egg membrane. These structures have more significance in taxonomy than the previously utilized signs using shell plates (Sirenko, 1993). The paired osphradium of *Tonicella marmorea* and *T. submarmorea* are located near the anal papilla and have the shape of small shaft with the length of 1.2 mm and the diameter of about 100 μm. The osphradium shaft surface is organized in the same way as the osphradium surface of *Acanthopleura*. No zoning of location of cellular elements was detected on it. Receptor cells differ from supporting ones by more electron-light cytoplasm and a small nucleus of a roundish shape. The distal part of the peripheral process is marked by the characteristic mace-like dilation with microvilli and one to three cilia.

S*pongioradsia aleutica*, *Micichiton grandispina*, *Nanichiton deplanatus*, *Schizoplax brandtii* (Chitonida – Acanthochitonina – Tonicellidae)

A small strip of specialized epithelium with the length of up to 200 μm and the width of 60 μm, beginning near the base of the last pair of gills was detected for these species. According to SEM data, the surface of this structure is formed by microvilli supporting cells and peripheral appendices of receptor cells carrying one to two cilia with the length of up to 10 μm.

Thus, three representatives (S*pongioradsia aleutica*, *Micichiton grandispina*, *Schizoplax brandtii)* show low degree of differentiation of osphradia (absence of isolated, developed osphradium shaft), but the general plan remains (a section of specialized epithelium with cells having one to two cilia).

***Acanthochitona rubrolineata* (Chitonida – Acanthochitonina – Acanthochitonidae)**

Osphradium of *Acanthochitona* in the shape of a shaft with the length of about 300 μm and the diameter of 70 μm (Fig. 3C) is located immediately behind the last pair of gills. Its surface is smooth, and we did not manage to detect any signs of zoning. However, a secretory zone and a sensory fissure of *Acanthochiton communis* are known (Haszprunar, 1987). The osphradium is formed by microvilli of supporting cells and a small number of receptor cells, long (up to 6 μm) cilia of which are located parallel to the surface. One cilium or more seldom two cilia happen to be on the apical surface of the receptor cell.

We did not manage to reveal essential differences in the ultrastructure of osphradia of Acanthochitonina and Chitonina, and the existing likeness in the structure and location of osphradium organs of chitons can be interpreted in different ways (Table 1). At the same time, numerous additional, possibly "sensory" formations, on lateral surfaces of the mantle cavity, in the base of the head lobes and on the tops of gills of representatives of Lepidopleurida (Sigwart et al., 2014) can also partially perform the osphradium function — analysis of chemical environmental in the mantle cavity, as it was stated earlier (Plate, 1901). If we take into account complete absence of osphradium of most primitive forms of chitons belonging to the order of Lepidopleurida (Sigwart et al., 2014), then probably osphraduim in more advanced chitons of the order of Chitonida should be considered as an independently developed organ, possibly not homologous to the osphradia of other molluscs (Gastropoda, Bivalvia and some Cephalopoda).

In this respect we agree with the conclusion of Lindberg and Sigwart (Lindberg, Sigwart, 2015), that it is difficult (due to absence of certain data, especially for representatives of Polyplacophora) to assert absence or presence of homology among various structures described as "osphradium". However, similar plan of osphradia discovered by us for Acanthochitonina

and Chitonida and detected earlier for Bivalvia of primitive Gastropoda entitles us at least to say about similarity of these structures that is connected with performance of the same chemosensory function.

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Table 1. Location, size, shape and cellular structure of Chitonida osphradia.
Таблица 1. Положение, размер, форма и клеточное строение осфрадиев Chitodina.

Species	Location	Size (mm)	Form	Supporting cells		Receptor cells	
				ciliary	microvilli	position	presence of cilia
Chitonina: *Ischnochiton hakodadensis*, *Stenosemus albus*	in mantle groove, behind last pair of ctenidium	0.1 × 0.6	shaft without zones	120, cilia, 15–20 μm	microvilli length 1 μm	intra-epithelial	1–3, typical structure, up to 5 μm
Chitonina: *Acanthopleura brevispinosa*	in mantle groove, behind last pair of ctenidium	2 × 8	fold without zones	100, cilia, 15–20 μm	microvilli length 1 μm	intra-epithelial	1–5, modified, up to 5 μm,
Acanthochitonina: *Tonicella marmorea*, *Tonicella submarmorea*	in mantle groove, behind last pair of ctenidium	0.1 × 1–2	shaft without zones	no	microvilli length 1–3 μm	intra-epithelial	1–2, modified, up to 6 μm
Acanthochitonina: *Spongioradsia aleutica*, *Micichiton grandispina*, *Namichiton deplanatus*, *Schizoplax brandtii*	in mantle groove, behind last pair of ctenidium	missing or up to 0.01	unformed strip	no	microvilli length 3 μm	intra-epithelial	1–2, long up to 10 μm