ISSN 0012-4966, Doklady Biological Sciences, 2016, Vol. 468, pp. 129–132. © Pleiades Publishing, Ltd., 2016.
Published in Russian in Doklady Akademii Nauk, 2016, Vol. 468, No. 4, pp. 470–473.

GENERAL BIOLOGY

Ultrastructural Evidence of the Excretory Function in the Asteroid Axial Organ (Asteroidea, Echinodermata)[1]

O. V. Ezhova[a, b], E. A. Egorova[a], and Corresponding Member of the RAS V. V. Malakhov[a, b]

Received January 22, 2016

Abstract—The ultrastructure of the axial organ of *Asterias amurensis* has been studied The organ is a network of canals of the axial coelom separated by haemocoelic spaces. The axial coelom is lined with two types of monociliary cells: podocytes and musculo-epithelial cells. Podocytes form numerous basal processes adjacent to the basal lamina on the coelomic side. Musculo-epithelial cells form processes running along the basal lamina. Some bundles of these processes wrapped in the basal lamina pass through haemocoelic spaces between neighboring coelomic canals. It is hypothesized that the axial organ serves for filtration of fluid from haemocoelic spaces into the axial coelom cavity, from which urine is excreted through the madreporite to the exterior.

DOI: 10.1134/S0012496616030091

The functions of the echinoderm axial organ are still discussed. It is known that this organ plays the main role in blood circulation [7, 9]. Some authors suggested that it performs the excretory function [2, 6, 10, 11]. The fine structure of axial organ has not been studied sufficiently. In the axial organ, podocytes were found, which are usually involved in the excretory function [3–5, 8, 10, 11]. We have studied the fine structure of the asteroid axial organ of *Asterias amurensis* Lutken, 1871 to understand its functions.

The material was collected in the Sea of Japan at a depth of 5–10 m (Zhirmunsky Institute of Marine Biology of the Far Eastern Branch of the Russian Academy of Sciences, Vladivostok). The axial complex of the collected animals was prepared and fixed in a 2.5% glutaraldehyde solution in 0.1 M sodium chloride buffered with 0.1 M sodium cacodylate (pH 7.35) with addition of magnesium chloride. Postfixation was performed in 1% osmium tetroxide with 0.1 M sodium chloride buffered with sodium cacodylate (pH 7.36). Decalcification was performed in 5% EDTA. Ultrathin sections were contrasted with uranyl acetate and lead citrate. The sections were examined under JEM-100S and Zeiss Libra 120 transmission electron microscopes.

In Asteroidea, the axial organ is located in the pericardial coelom and in the axial coelom. The latter is occupied by the largest part of the axial organ, the axial part [1, 2]. Anatomically, the axial organ is a network of haemocoelic spaces in the matrix between the basal laminae which separate the canals of the axial coelom.

The haemocoel of the axial organ contains flock-like material, collagen fibers, and cells (amoebocytes). Coelomic spaces look absolutely clear in sections (Fig. 1). The basal lamina is a layer of granular material about 50 nm in thickness. The lining of the axial coelom consists of two types of monociliary cells: podocytes and musculo-epithelial cells (Figs. 1a, 2).

Each podocyte bears, in the apical area, a single undulipodium surrounded by a collar of eight to ten short microvilli referred to as stereocilia (Fig. 1a). In the basal area, each podocyte forms numerous branching cytoplasmic pedicels 100–350 nm in diameter. Usually, the podocyte bodies are located far from each other (the distance between the podocyte bodies in the sections is 8–10 μm). Between the podocyte bodies, the haemocoel and the coelom are divided only by the basal lamina with the pedicels of podocytes adjacent to the basal lamina on the coelomic side (Figs. 1a, 1b, 2). The distance between adjacent pedicels is 10–15 nm. In some places, the adjacent pedicels of podocytes are connected via slit diaphragms of extracellular matrix (Fig. 1b). The

[1] The article was translated by the authors.

[a] *Moscow State University, Moscow, 119992 Russia*
[b] *Far Eastern Federal University, Vladivostok, Russia*
e-mail: olga.ejova@gmail.com; olga_ejova@mail.ru

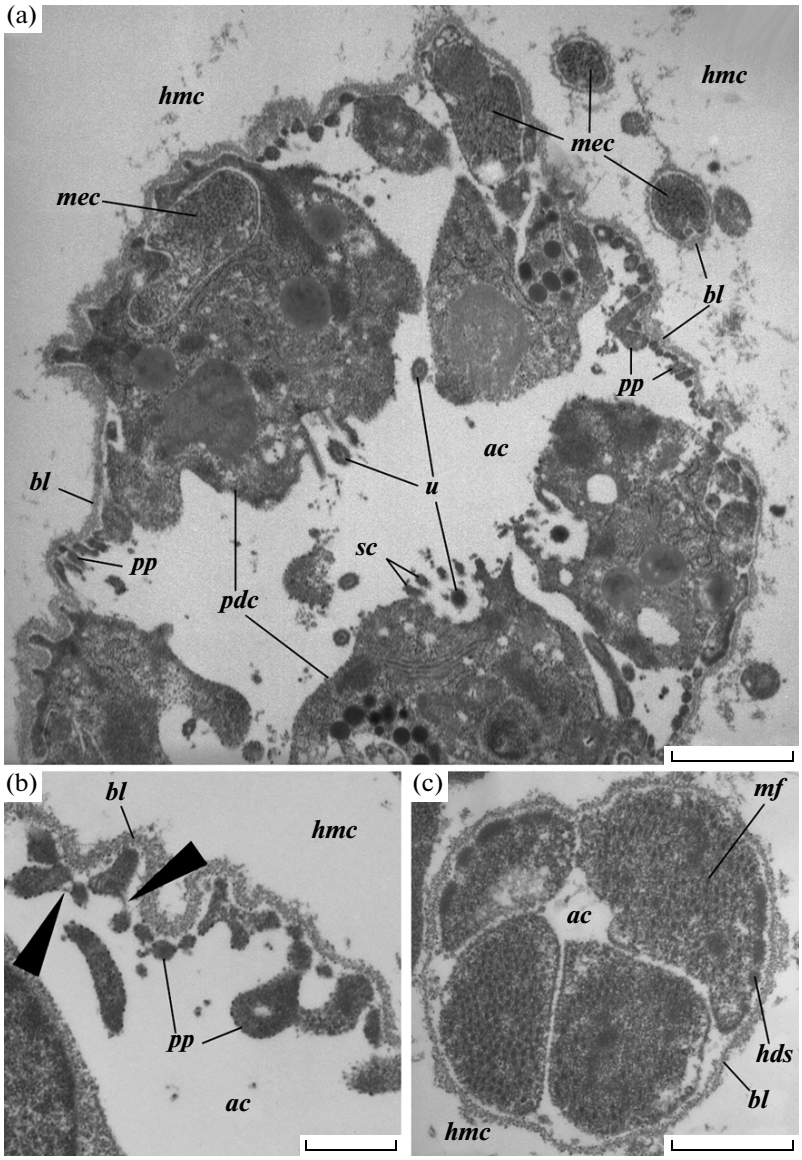

Fig. 1. A section through the axial part of the axial organ of *Asterias amurensis*. (a) Podocytes and musculo-epithelial cells in the lining of the axial coelom. (b) The area of the basal lamina covered with podocyte pedicels (the site of ultrafiltration). Arrows mark the extracellular slit diaphragms between the podocyte pedicels. (c) Sectioned musculo-epithelial cells containing myofibers. *ac*, axial coelom; *bl*, basal lamina; *hds*, hemidesmosomes; *hmc*, haemocoel; *mec*, sections of the musculo-epithelial cells; *mf*, myofilaments; *pdc*, podocytes; *pp*, podocyte pedicels; *sc*, stereocilia; *u*, undulipodium. Scale bars: (a) 1.5 μm; (b) 0.5 μm; (c) 1 μm.

podocyte pedicels are attached to the basal lamina via hemidesmosomes.

Each musculo-epithelial cell contains a myofiber in the basal area. The myofiber includes thick (about 25 nm in diameter) and thin (about 10 nm in diameter) filaments (Fig. 1c). The myofiber extends to form a long process running along the basal lamina. In some places, the processes pass under podocytes. The podocytes rise in the form of an arch and let the processes of musculo-epithelial cells pass (Fig. 2). The myocyte processes are connected with podocytes via desmosomes. Hemidesmosomes attach the myocyte processes to the basal lamina.

Some processes and bundles of a few myocyte processes may pierce the haemocoel and join adjacent coelomic canals. The myocyte processes are separated from haemocoel by the basal lamina (Figs. 1a, 1c).

Podocytes were found in the axial organ of the starfish *Asterias rubens* [5]. Then, the fine structure of podocytes was described for the sea urchin *Eucidaris* sp., “slit membranes” were found between the podocyte pedicels [10]. Detailed description of the

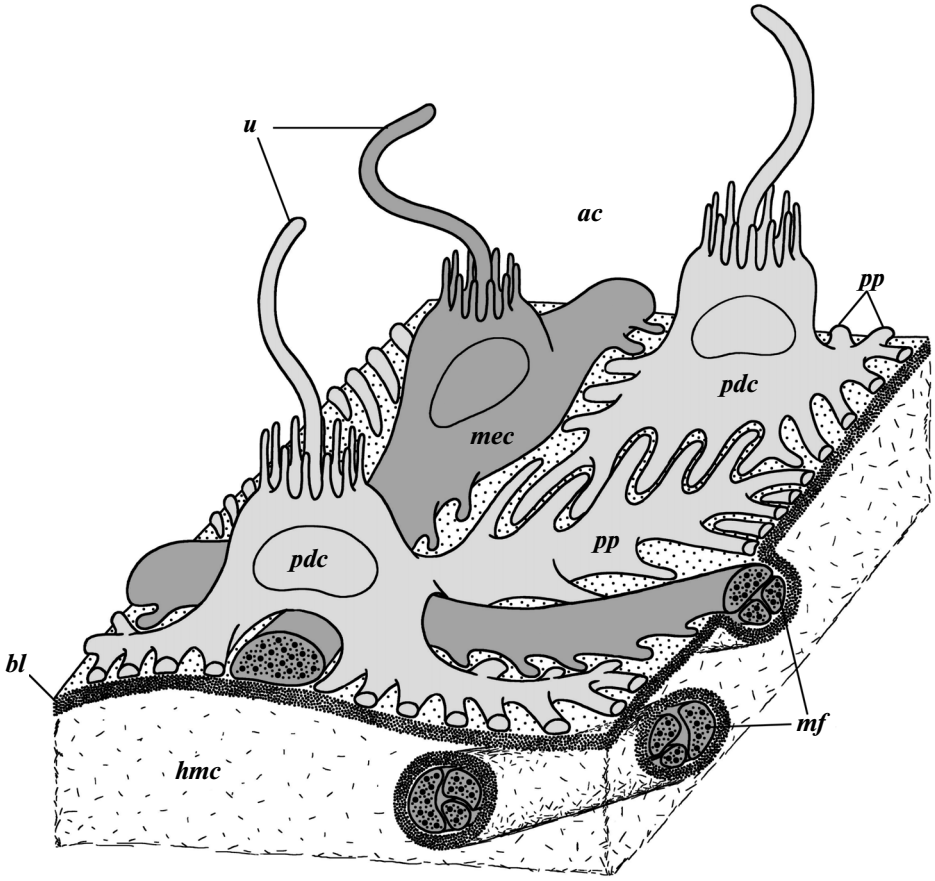

Fig. 2. Schematic diagram of the site of ultrafiltration in the asteroid axial organ. *ac*, axial coelom; *bl*, basal lamina; *hmc*, haemocoel; *mec*, musculo-epithelial cells; *mf*, myofilaments; *pdc*, podocytes; *pp*, podocyte pedicels; *u*, undulipodium.

fine structure of the axial organ was given for the feather star *Comactinia meridionalis* [4]. Those authors described a layer of podocyte pedicels on the surface of the basal lamina that divides the haemocoel and the coelom. In some places, the podocyte pedicels are bridged laterally by a thin layer of extracellular matrix ("slit diaphragms") as described above for *A. amurensis.*

Our data and observations of the cited authors allow us to suppose that the wide areas between the podocyte bodies are sites of ultrafiltration. Myocyte basal processes in the wall of coelomic canals and muscle bundles passing through haemocoel contract and produce the pressure for fluid ultrafiltration from haemocoelic spaces of the axial organ into the axial coelom. The fluid is driven from the haemocoel through the basal lamina, and, as a result, primary urine is formed. The podocyte pedicels bridged by slit diaphragms of the extracellular matrix may serve as an additional filter. Primary urine passes through this filter and becomes definitive urine.

It is well known that the axial coelom communicates with the exterior via the madreporite [6, 7, 9]. Cuénot [6] introduced vital stain into the asteroid axial coelom and observed excretion of the stain through the madreporite. Presumably, definitive urine is in the same manner excreted from the axial coelom through the madreporite to the exterior. All this confirms the hypothesis that the echinoderm axial organ fulfills the excretory function and the madreporite is the excretory pore.

ACKNOWLEDGMENTS

Collection and fixation of the material were supported by the Russian Foundation for Basic Research (grant no. 14-04-00366-a). The fine structure was studied in the Far East Center of Electron Microscopy, Institute of Marine Biology, Far Eastern Branch, Russian Academy of Sciences; this study was supported by the Russian Science Foundation (grant no. 14-50-00034).

REFERENCES

1. Ezhova, O.V., Lavrova, E.A., and Malakhov, V.V., *Biology Bulletin*, 2013, vol. 40, no 8, pp. 643–653.
2. Ezhova, O.V., Lavrova, E.A., and Malakhov, V.V., *Russian Journal of Marine Biology,* 2014, vol. 40, no. 3, pp. 153–164.

3. Bachmann, S. and Goldschmid, A., *Cell Tiss. Res.*, 1978, no. 193, pp. 107–123.

4. Balser, E.J. and Ruppert, E.E., *Acta Zool.* (Stockholm), 1993, vol. 74, no. 2, pp. 87–101.

5. Bargmann, W. and von Hehn, G., *Z. Zellforsch. Mikrosk. Anat. Histochem.*, 1968, vol. 88, pp. 262–277.

6. Cuénot, L., in *Anatomie, éthologie et systématique des Échinodermes, Traité de zoologie*, Paris: Masson et Cie, 1948, vol. 11, pp. 3–272.

7. Goldschmid, A., in *Spezielle Zoologie*, vol. 1: *Einzeller und Wirbellose Tiere*, Stuttgart: Gustav Fischer, 1996, pp. 778–834.

8. Holland, N.D., *Tissue Cell*, 1970, vol. 2, no. 4, pp. 625–636.

9. Ruppert, E.E., Fox, R.S., and Barnes, R.D., *Invertebrate Zool.*, 2004, vol. 28, pp. 872–929.

10. Welsch, U. and Rehkamper, G., *J. Zool.* (London), 1987, vol. 213, pp. 45–50.

11. Ziegler, A., Faber, C., and Bartolomaeus, T., *Front. Zool.*, 2009, vol. 6, no. 10, pp. 1–31.