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GENERAL BIOLOGY

The Structure of Cnidosacs in Nudibranch Mollusc *Aeolidia papillosa* (Linnaeus, 1761) and Presumable Mechanism of Nematocysts Release[1]

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Abstract—The structure of cnidosacs in nudibranch mollusc *Aeolidida papillosa* (Linnaeus, 1761) before and after the discharging of kleptocnidae has been studied. In the apical zone of the cnidosac, the basal laminae of epidermis and gastrodermis are interrupted, and the muscle layers of the cnidosac and the epidermis are absent. We suggest the formation of a temporary channel during the discharging of the cnidosac. Through this channel, nematocysts move from the cnidosac to the cnidopore, which forms on the top of the ceras.

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Nudibranch molluscs (Gastropoda: Nudibranchia) lack the shell and are able to use an unusual mechanism for their defense, which is based on the sequestration of nematocysts of their prey, cnidarians. Sequestered nematocysts (or kleptocnidae) are stored in specific sacs, cnidosacs, which are placed inside the appendages (or cerata) on the dorsal side of nudibranchs. Once molluscs are threatened, kleptocnidae are extruded from the cnidosac and defend the nudibranch from enemies. This process was first discovered in the mid-19th century [1]. The structure of cnidosacs has been described in a number of publications, and it has been shown they have different morphology in different nudibranch taxa [2–7]. Nevertheless, up to date, the mechanism of the cnidosac functioning is still not entirely understood.

Aeolidia Cuvier, 1798 is a genus of large nudibranch molluscs that includes six described species according to recent data [8]. Representatives of this genus bear numerous cerata on the dorsal side with cnidosacs placed within each ceras.

The main goal of the present study is to analyze the cnidosac morphology in *Aeolidia papillosa* (Linnaeus, 1761) and to suggest a hypothesis on the mechanism of its functioning.

Representatives of *A. papillosa* were collected in Kandalaksha Bay, the White Sea, in the vicinity of the White Sea Biological Station, Moscow State University. Cerata cut off living specimens were used as the material. For the cnidosac structure study, cerata were cut off from molluscs relaxed in 7.5% magnesium chloride and then fixed. For the study of the mechanism of kleptocnidae release, the molluscs were stimulated by a contact with a needle. The cerata were cut off at the moment of their contraction and releasing of kleptocnidae. The material was preserved in 2.5% glutaraldehyde in phosphate buffer saline (PBS) for light and electron microscopic investigations. For studies using confocal laser scanning microscope, the material was fixed in 4% paraformaldehyde in PBS. The musculature was studied using the fluorescent dye Alexa Fluor® 647 Phalloidin (ThermoFisher Scientific, United States, F-actin labeling) and Propidium iodide (Sigma-Aldrich, United States, nucleus labeling). Ciliated epithelium was studied using anti-acetylated α-tubulin and anti-detyrosinated α-tubulin antibodies (Sigma-Aldrich). For amorphous chitin labeling, staining with the fluorescent dye Calcofluor White (Sigma-Aldrich) [9] was used.

Each ceras is covered with ciliated epidermis, whose cells contain numerous vacuoles with chitinous spindles (Figs. 1, 2a). At the top of cerata, the epidermis becomes wrinkled, and ciliated covering becomes sparse. The basal lamina underlying the epidermis becomes winding and thin and disappears at the top.

An endodermal channel inside the ceras forms an oblong sac in its distal part, the cnidosac (Fig. 1). Its inner lining is formed by simple endodermal epithelium (gastrodermis), which appears to be a continuation of the digestive gland epithelium. The cnidosac is surrounded by strong musculature composed of inner circular and outer longitudinal layers (Figs. 1, 2a). The

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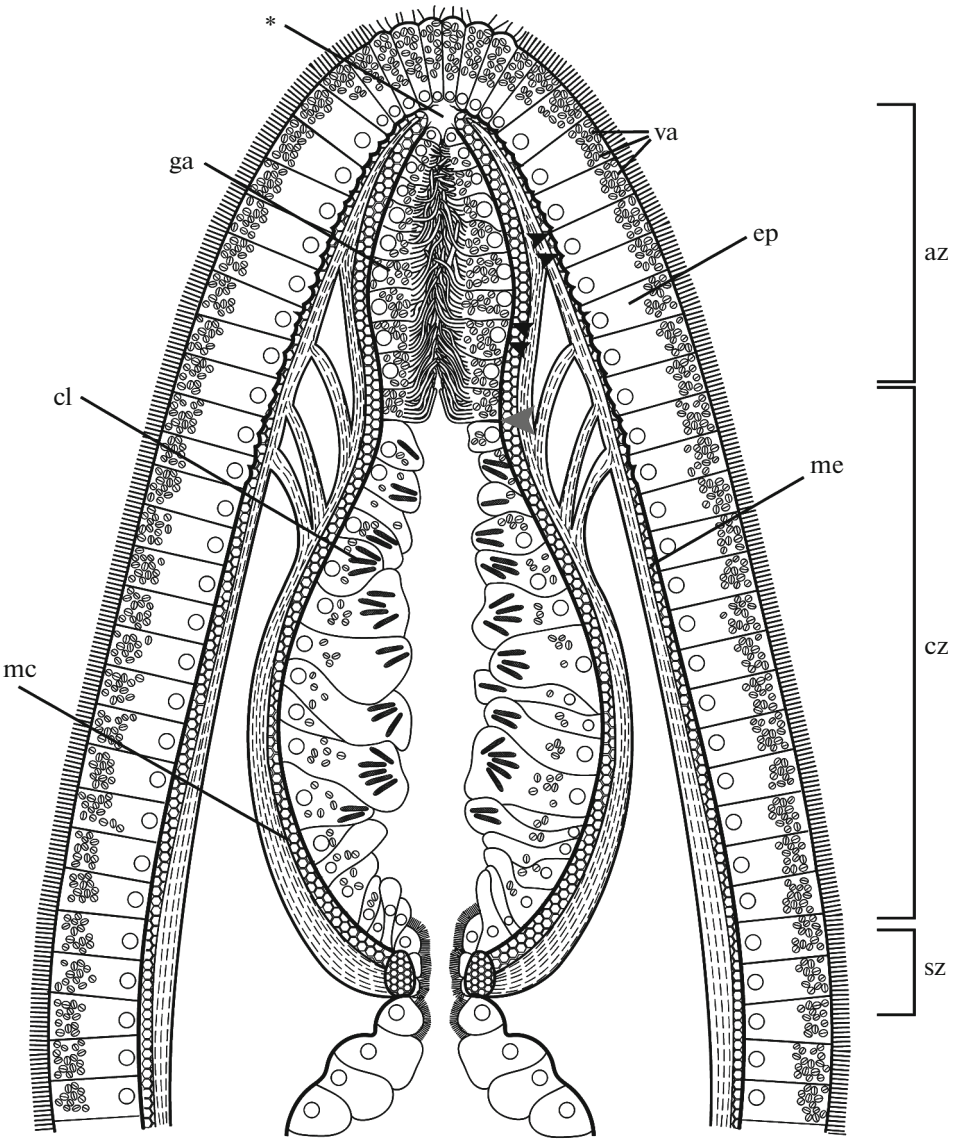

Fig. 1. Schematic overview of a longitudinal section of an *Aeolidia papillosa* ceras with an undischarged cnidosac: (az) the apical zone of the cnidosac, (cl) kleptocnidae in cnidophages, (cz) the cnidophages zone, (ep) epidermis; (ga) gastrodermis, (mc) muscular layer of cnidosac, (me) muscular layer of epidermis, (sz) the sphincter zone, (va) vacuoles with chitinous spindles. An asterisk indicates the zone of disintegrated gastrodermis at the tip of the ceras. Black arrowheads indicate the basal laminae of epidermis and gastrodermis, gray arrowheads indicate the isthmus between the apical zone of cnidosac and the cnidophages zone.

cnidosac is divided into three parts differing in morphology: the sphincter zone, the cnidophages zone, and the apical zone (Fig. 1).

The sphincter zone is characterized by a muscular ring with ciliated epithelium covering its lumen. The epithelium in the cnidophages zone is represented by large non-ciliated cells (cnidophages) containing kleptocnidae. Our electron microscopic studies have shown kleptocnidae located in vacuoles, whose membranes are adjacent closely to the kleptocnida walls. In addition, numerous vacuoles with chitinous spindles are found inside cnidophages.

The apical zone is separated from the cnidophages zone by a visible isthmus (Fig. 1). It is lined by cuboidal endodermal epithelium. The apical surface of the cells bears numerous microvilli oriented towards the ceras tip. (Fig. 1). We have also found a large number of vacuoles with chitinous spindles in the cells of the apical zone of epithelium (Fig. 2a). Kleptocnidae are absent in these cells.

On the top of the cnidosac, the endodermal epithelium is disintegrated, the basal lamina is absent (Fig. 2a). On the way towards the apical end, the cnidosac muscles connect with the muscular layer of the ciliated epidermis of the ceras (Figs. 1, 2a). The muscular layer

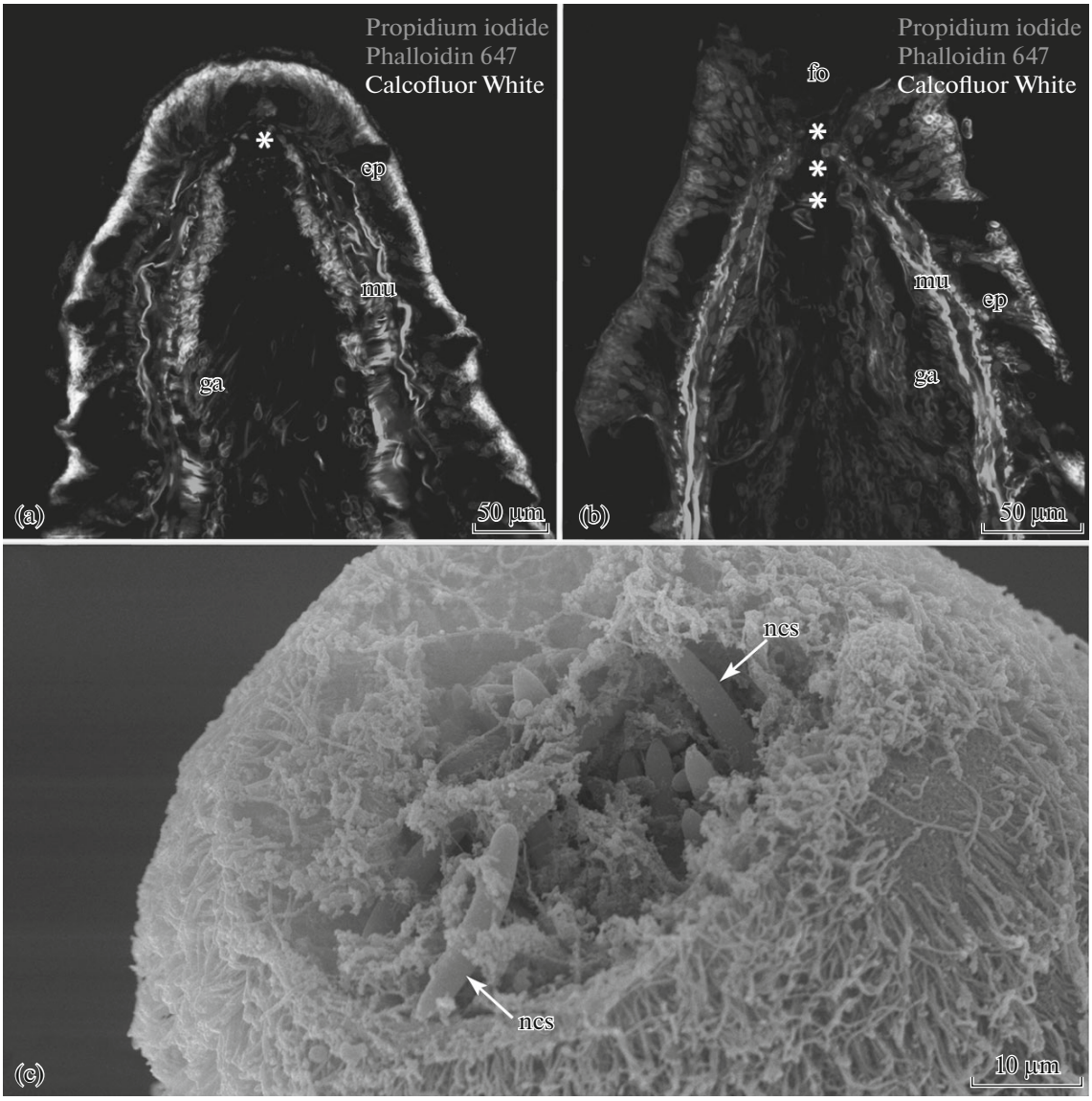

Fig. 2. The structure of ceras and cnidosac of *Aeolidia papillosa* before and after discharging. (a) A longitudinal section of the ceras with undischarged cnidosac (CLSM); (b) the same with discharged cnidosac; (c) a cnidopore at the top of cerata with extruded kleptocnidae (SEM). (fo) Funnel-shaped opening on the ceras tip, (mu) muscular layer of the apical part of the ceras, (ncs) nematocysts in the cnidopore on the ceras tip. The vertical row of three asterisks indicates a temporary channel joining funnel-shaped opening at the ceras tip and cnidosac lumen. Other designations are the same as in Fig. 1.

of the epidermis also consists of circular musculature, which is adjacent to the basal lamina of ectodermal cells, and the layer of longitudinal muscles. The musculature of ceras is significantly thinner than cnidosac muscles.

Therefore, on the top of the cnidosac, basal laminae are absent in both epidermis and gastrodermis. Since the muscular layer of the cnidosac connects with the epidermal muscular layer, muscles are also absent in this area. These features play the key role in the cnidosac functioning.

The cerata fixed during the discharging of the cnidosac have a funnel-shaped opening, which is a temporary cnidopore with kleptocnidae inside it (Fig. 2c). We also detected the connection between the cnidosac lumen and the funnel-shaped cnidopore (Fig. 2b). After the discharging, the number of vacuoles with chitinous spindles decreased significantly in both epidermis and gastrodermis of the cnidosac (Fig. 2b).

The structure of the cnidosac of *A. papillosa* studied using the light microscopy techniques has been already described [10]. In the present study, we have found some new features of cnidosac morphology in this species. The apical zone of the cnidosac is separated from the cnidophage zone by an isthmus and

highly differs from the latter in its morphology: the cells of the apical zone lack kleptocnidae and bear long microvilli oriented towards the ceras tip. There is no such differentiation of the apical zone found in cnidosacs of other Aeolididae and representatives of the families Flabellinidae, Fionidae, and Facelinidae, and the cnidophages zone commonly occupies all the cnidosac lumen [3–6]. We have discovered vacuoles with chitinous spindles in the endodermal epithelium of the cnidosac, in the cells of both the cnidofages and the apical zone. Vacuoles with chitinous spindles were previously described in the stomach cells and also in the epidermis of cerata and rhinophores of nudibranch molluscs [11].

According to current data, we can suggest the mechanism of the functioning of *A. papillosa* cnidosac. Kleptocnidae are extruded from cnidophages' cytoplasm and move by the contraction of cnidosac muscles to its apical zone. Microvilli in the apical zone, oriented towards the ceras tip, act as a valve that allows only unidirectional movement of kleptocnidae. The epidermis of the ceras is disrupted and forms a funnel-shaped opening due to the contraction of the ceras musculature (Figs. 2b, 2c). Kleptocnidae move into this opening, passing through the temporary channel that connects the opening with the cnidosac lumen (Fig. 2b). In this area, the endodermal epithelium is disintegrated and the muscular layer is absent; therefore, there are no barriers for the movement of kleptocnidae. The contraction of muscular layers of both cnidosac and the ceras plays the key role in kleptocnidae releasing. This contraction is most likely to be controlled by the nervous system of the nudibranch.

It was suggested that kleptocnidae discharge when contact the sea water [3, 5, 6, 10]. Recently it has been shown that Aeolidiida ceratal epidermis contains numerous vacuoles with chitinous spindles that protect the epidermis from the damage of discharged kleptocnidae [11]. We have found a large number of these vacuoles not only in the epidermal cells, but in the gastrodermis of the cnidophage zone and the apical zone. After the cnidosac discharging, the number of vacuoles decreases significantly in both epidermis and gastrodermis. It is most likely that the chitin provides the protection of both epithelial layers, being extruded at the same time as kleptocnidae.

It is still unclear how nematocysts appear in the cnidosac lumen, because they are located in the cnidophages' cytoplasm in an undischarged cnidosac. In a discharged cnidosac, there is no evidence of disruption of the cnidophage layer. In addition, in the cnidopore we did not find any cell material, which would have been found together with nematocysts if cnidophages were damaged. These unresolved questions open a prospect for further research.

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