Ruthenica, 2024, vol. 34, No. 1: 15-34.
Published online January 2, 2024.

http: ruthenica.net

Regeneration in the dorids exemplified by *Onchidoris muricata* (Gastropoda, Nudibranchia)

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ABSTRACT. Regenerative capabilities vary among different groups of invertebrates and despite being a highly abundant and diverse group of invertebrates with significant commercial and scientific value, gastropods remain relatively understudied in this respect. This work presents the first investigation of post–traumatic regeneration in the nudibranch mollusc Doridina, specifically focusing on *Onchidoris muricata*. Dorids have unique subepidermal calcite spicules that form a complex network inside the body. However, their capacity for complete or partial recovery, as well as the impact on regeneration of organs containing these spicules, has never been studied. We examined the regeneration of chemosensory organs (rhinophores) and dorsal body outgrowths (tubercle), both containing spicules and having different innervation. Our investigation explores three models of rhinophore regeneration: 1) after the removal of the apex and three lamellae of the rhinophore, 2) when the entire metameric lammelae part is removed, and 3) when the rhinophore is entirely excised. Additionally, two series of experiments were conducted to examine tubercle regeneration in the peribranchial region and in the rhinophore region. The study reveals varying regenerative abilities of these organs, likely linked to their different innervation patterns. Notably, we observed that the presence of the apex and spicules *de novo* synthesis influence the formation of the first rhinophore lamellae. The search for new patterns and mechanisms underlying the restoration of elements in the nervous system, muscular system, and solid skeleton can significantly contribute to our understanding of regenerative biology. This research expands our knowledge of nudibranch molluscs regeneration and the unique restoration of the subepidermal spicule complex. Furthermore, the regeneration of spicule-containing organs can be a model for studying the formation and structure of biomineralized structures, including their organic component.

https://doi.org/10.35885/ruthenica.2024.34(1).3

Регенерация у дорид на примере *Onchidoris muricata* (Nudibranchia, Gastropoda, Mollusca)

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РЕЗЮМЕ. Посттравматическая регенерация характерна в разной степени для всех животных. Несмотря на то, что брюхоногие моллюски представляют собой очень многочисленную и разнообразную группу беспозвоночных, имеющую значительную промысловую и научную ценность, регенеративные способности брюхоногих моллюсков изучены фрагментарно. Настоящая работа является первым исследованием посттравматической регенерации спикульных органов у голожаберных моллюсков Doridina на примере *Onchidoris muricata*. Дориды обладают уникальными субэпидермальными кальцитовыми спикулами, которые образуют сложную сеть внутри тела. Однако их способность к полному или частичному восстановлению, а также влияние на регенерацию органов, которых находятся спикулы никогда не была исследованы. Нами изучена регенерация хемосенсорных органов (ринофоров) и дорсальных выростов тела (папилл), содержащих спикулы, а также имеющих разную иннервацию. В нашей работе рассмотрены три модели регенерации ринофоров: 1) после удаления верхушки и трех складок ринофора, 2) при удалении всей метамерной складчатой части и 3) при удалении ринофора целиком. Дополнительно мы провели две серии экспериментов по изучению регенерации папилл в околожаберной области и в области ринофоров. Нами показана разная регенеративная способность этих органов, что, вероятно, связано в том числе с различной иннервацией.

Впервые показано, что наличие верхушки ринофора и синтез спикул *de novo* играют решающую роль в формировании первых складок ринофора. Поиск новых закономерностей и механизмов, лежащих в основе восстановления элементов нервной и мышечной систем, а также твердого скелета, может значительно расширить знания в области регенеративной биологии, в частности о регенерации у голожаберных моллюсков и восстановлении уникальных субэпидермальных кальцитовых спикул. Также регенерация спикульных органов может являться моделью для изучения формирования и устройства биоминерализованных структур, в том числе их органического компонента.

Introduction

Regeneration is a vital physiological process that allows for tissue renewal after various injuries, occurring in animals ranging from protostomia to humans. However, the capacity for regeneration varies across different groups [Carlson, 2007; Bely, Nyberg, 2010]. While reparative regeneration has been extensively studied in sponges [Ereskovsky *et al*., 2021], cnidarians [Bosch, 2007], flatworms, annelids and echinoderms [Egger *et al.*, 2007] among invertebrates, its understanding remains fragmentary in more complex organisms like molluscs. Molluscs, which inhabit marine, freshwater, and terrestrial environments, and is one of the most numerous and diverse groups of invertebrates [Ponder *et al.*, 2019]. These organisms hold commercial value, and many species serve as model objects for fundamental research [Mau, Jha, 2018; Chakraborty, Joy, 2020]. Despite this, the regenerative capabilities of molluscs have been relatively understudied. It is known that representatives of the Bivalvia and Gastropoda classes can regenerate both soft tissues and hard shells [Watabe, 1983; Su *et al.*, 2004]. Notably, some shell–less gastropods (Heterobranchia, Saccoglossa) demonstrate remarkable regenerative abilities, capable of regenerating an entire organism from just a single head, comparable to model organisms like planarians [Reddien, Sánchez Alvarado, 2004; Mitoh, Yusa, 2021]. The tendency to oligomerization, including the nervous system, in Heterobranchia makes these molluscs suitable for studying nervous system functionality [Carew *et al.*, 1981]. However, even for this noteworthy group, regeneration remains poorly studied.

Our research focuses specifically on the study of post–traumatic regeneration in nudibranch molluscs, which are marine shell–less representatives of gastropod subclass Heterobranchia. Nudibranchia comprises two suborders, Cladobranchia and Doridina, differing in morphology and ecology [Bouchet *et al.*, 2005; Do *et al.*, 2022]. To date, regenerative abilities have been investigated in cladobranchs [Kress, 1968; Korotkova, 1997; Maroyan, 2021], data for dorids are limited or scarce [Haefelfinger, 1961; Sekizawa *et al.*, 2018]. The study of dorid regeneration is particularly intriguing since these organisms possess spicules, distinct skeletal elements found in their body [Foale, Willan, 1987; Penney, 2008; Penney *et al.*, 2020; Nikitenko *et al*., 2021]. Calcite spicules are situated beneath the integumentary epithelium within the subepidermal space underneath the sclerocyte [Nikitenko *et al.*, 2021]. This arrangement of spicules in molluscs can only be found in heterobranch groups Acochlidiimorpha and Rhodopoidea, which are less extensively studied morphologically in comparison to dorids [Haszprunar, Künz, 1996; Neusser *et al.*, 2006; Jörger *et al.*, 2010; Neusser *et al.*, 2011]. The subepidermal localization of spicules is a unique characteristic among molluscs. The solid formations (shell of bivalves and gastropods, polyplacophores, spicules of poly- and aplacophores) are located extracellularly above the integumentary epithelium [Salvini–Plaven, 1967; Leise, 1984; Checa *et al.*, 2017]. The impact of intracellular rigid spicules on the regenerative capacity of body parts housing them, along with the ability to restore the spicule complex within organs following damage, and the potential for regeneration in cases of partial or complete spicule destruction, has not been investigated to date.

Therefore, our work aims to investigate the regenerative capability of dorid body regions containing hard subepidermal intracellular spicules using the nudibranch mollusc *Onchidoris muricata* (O.F. Müller, 1776) as model species (Fig. 1). The rhinophores and tubercles were selected as the focus of this study on regeneration. These organs selection is determined by their positioning within the mollusc body. The rhinophores on the head end of the body and the tubercle along the entire dorsal surface of the notum protrude significantly above the body surface and can be damaged, for example, during predator attacks. The external and internal morphology, functions, as well as the innervation of the rhinophores and tubercle, exhibit significant differences.

The structure of the intact rhinophore of *O. muricata* and the mechanism of its operation have been extensively studied previously [Lisova, Vortsepneva, 2022]. The rhinophores of *O. muricata* are paired ciliary chemosensory organs. The rhinophores have the ability to contract and retract through the action of the retractor muscles, which draw them into a specialized recess known as the rhinophoral pocket. The straightening of the rhinophores occurs due to hydraulic pressure generated when the lymphatic cavity within the rhinophore center is filled [Lisova, Vortsepneva, 2022]. The rhinophore is composed of two parts: a smooth base known as the rhinophore stalk, and a main section consisting of lamellae called the clavus, which end up in a rounded or conical apex (Fig. 1B, G). In adult *O. muricata*, the number of lamellae typically ranges from 7 to 10. The lamellae

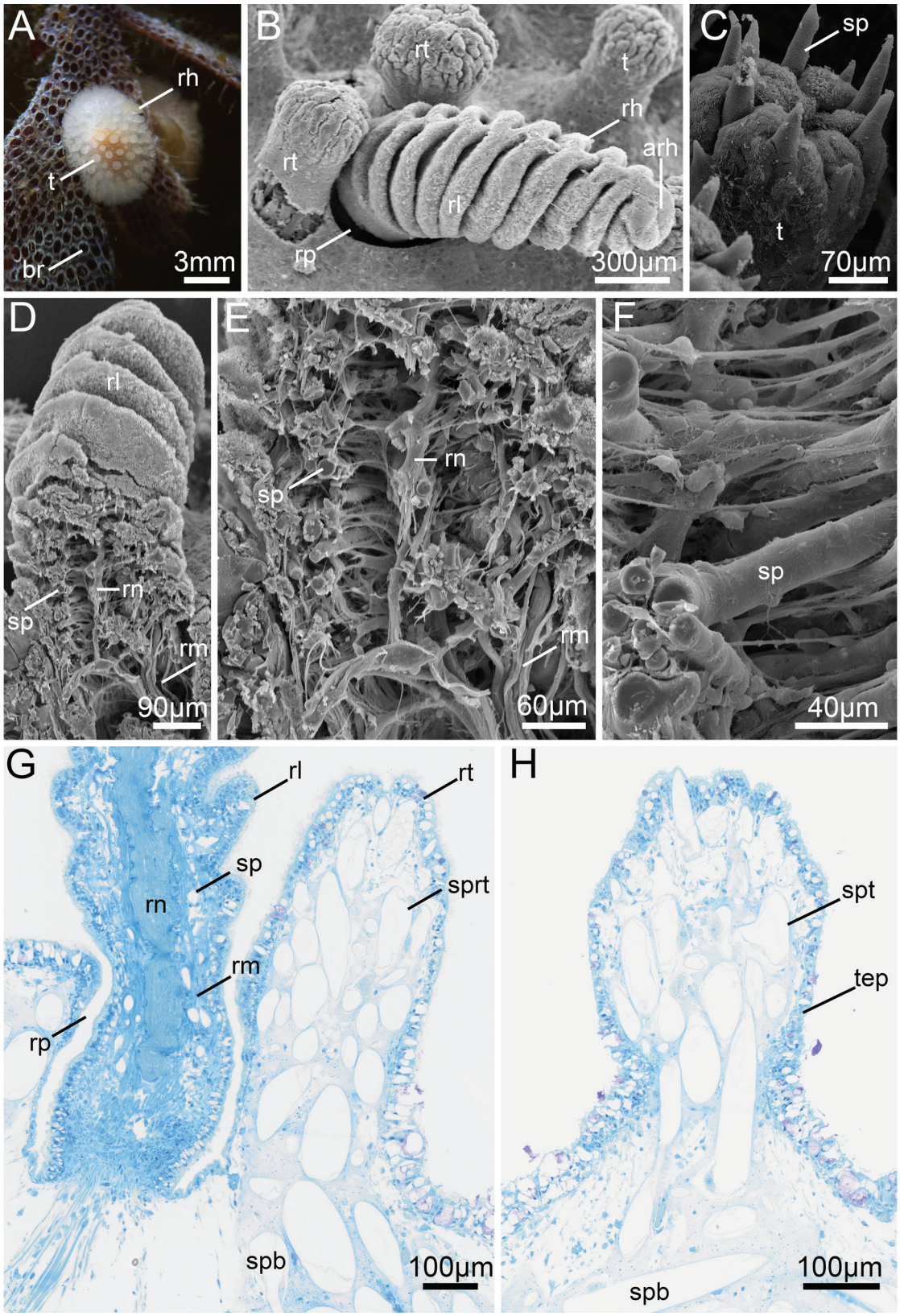

FIG. 1. General features of *Onchidoris muricata* body morphology (**A.** Living photo; **B, C, D, E, F.** SEM; **G, H.** Light microscopy). **A.** Living *O. muricata* on bryozoan. **B.** External morphology of rhinophore with rhinotubercles. **C.** External appearance of an intact tubercle. **D.** Fractured intact rhinophore. **E.** Internal structure of a fragment of an intact rhinophore upon breakage. **F.** Spicules of an intact rhinophore. **G.** Longitudinal section through a fragment of an intact rhinophore and rhinotubercle. **H.** Longitudinal section through a tubercle. Abbreviation: arh – apex of rhinophore; br – bryozoa; rh – rhinophore; rl – rhinophore lamellae; rm – retractor muscules; rn – rhinophore nerve; rp – rhinophore pocket; rt – rhinotubercle; sp – spicule; sprt – rhinotubercle spicule; spt – tubercle spicule; t – tubercle; tep – tubercle epithelium.

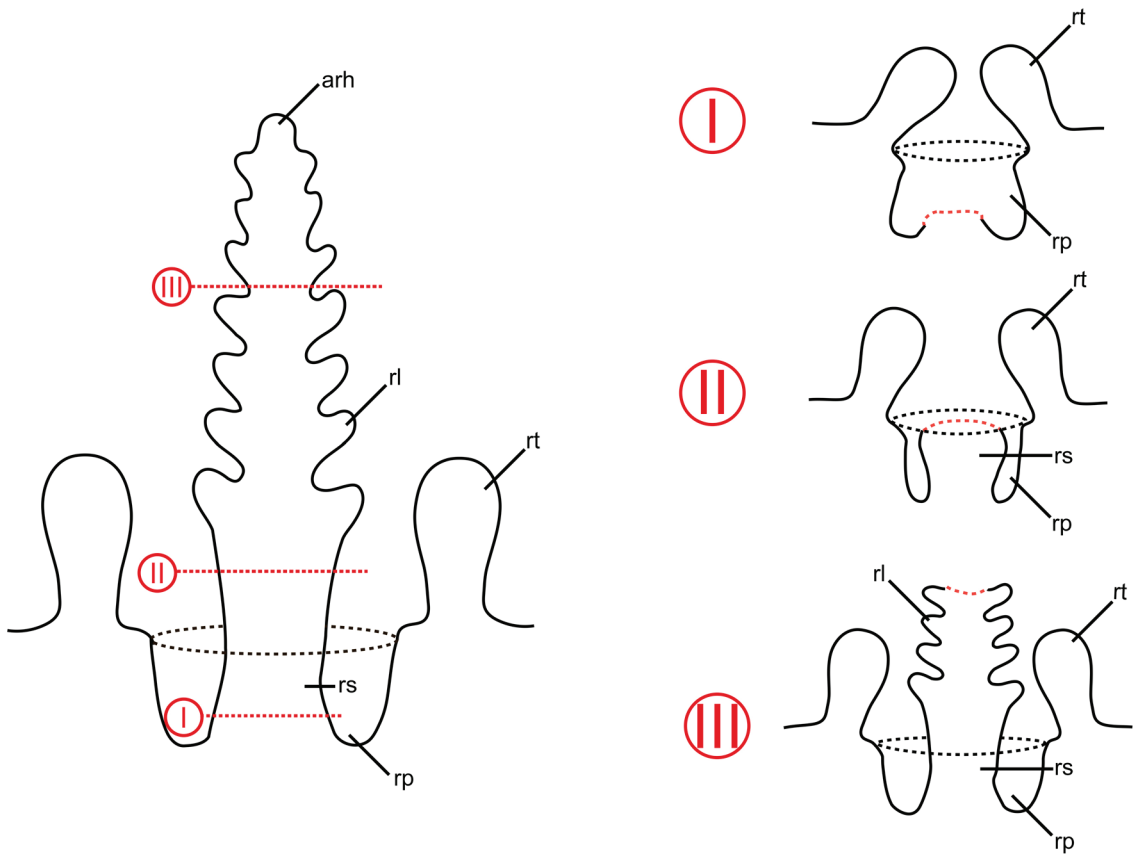

FIG. 2. Scheme of the experiment on the *Onchidoris muricata* rhinophore removal. On the left is a general view of the rhinophore. The dotted lines indicate the rhinophore cutting off sites. On the right are the experimental groups: 1) with a removed apex and three lamellae of the rhinophore, 2) a completely removed rhinophore, 3) a rhinophore with a removed lamellae part (clavus). Abbreviations: arh – apex of rhinophore; rl – rhinophore lamellae; rp – rhinophore pocket; rs – rhinophore stalk; rt – rhinotubercle.

of the rhinophore converge along its anterior side, with the right and left portions shifted relative to each other by half the length of the lamellae. They receive innervation from a large rhinophore ganglion situated in the center of the rhinophore (Fig. 1G), which extends from the cerebral ganglion. Inside the rhinophore, the nerve branches and extends into each lamellae of the rhinophore [Lisova, Vortsepneva, 2022; Nikitenko, Vortsepneva, 2023].

Tubercles are club–shaped dorsal outgrowths covering the entire notum of *O. muricata* (Fig. 1A, C, H). Tubercles, unlike the rhinophores, are immobile. In addition to body tubercles, there are specialized tubercles in *O. muricata* known as rhinotubercles, which surround the rhinophores laterally (Fig. 1B, G). The tubercle contains large subepidermal glands in the apical region and thin nerve elements that are not organized into large ganglia (Fig. 1H) [Nikitenko *et al.*, 2021; Nikitenko, Vortsepneva, 2023].

Despite the differences in the organs, both of them are reinforced with a well-organized internal spicule network. It is known that the rhinophores are strengthened by a network that resembles a log-cabin bonfire pattern [Lisova, Vortsepneva, 2022]. The morphology of the rhinophore spicules significantly differs from those found in the body. They are thinner, smaller, and exhibit lower diversity in shape. Diaxone-curved spicules dominate among the rhinophore spicules [Lisova, Vortsepneva, 2022]. The tubercle contains two rows of spicules, and spicules from the stellular tract extend into their base, connecting to provide additional support [Nikitenko, Vortsepneva, 2020; Nikitenko *et al.*, 2021; Nikitenko, Vortsepneva, 2023]. The morphology of the spicules in the tubercle is identical to the spicules found in other body parts, such as the notum and foot.

The present study aims to investigate the regeneration of organs in dorids with varying innervation and explore the potential for the restoration of the spicule complex within these organs.

Material and methods

Specimens of *Onchidoris muricata* were collected at a 12–15 m depth in the White Sea, near the Pertsov White Sea Biological Station of Moscow

State University (Kandalaksha Bay) (66°33'17"N, 33°06'02"E), by SCUBA diving. A total of 85 specimens of *O. muricata*, ranging in size from 6 to 12 mm, were studied. *O. muricata* was kept in glass cups of 500 ml in filtered sea water at a temperature of +10..+12°C. The dishes contained a substrate with prey species, such as bryozoans *Electra pilosa* (Linnaeus, 1767) on stones or red algae, along with the mollusc. The seawater was changed every two days; prey samples were changed every four days.

The study of regeneration was carried out on one rhinophore and one rhinotubercle and tubercle in the ctenidia region. The second rhinophore and other tubercles remained intact. Molluscs were relaxed in a solution of 4% $MgCl_2$ *6 H_2O 1:1 ratio with filtered seawater (FSW) for 1.5–2 hours before excising the body parts. After the operation, the molluscs were placed in normal conditions of detention in the laboratory.

Three series of experiments were carried out to study the rhinophore regenerations (Fig. 2): 1) excising the apex and three lamellae under it (in one iteration); 2) excising the whole rhinophore with its stalk (in four iteration); and 3) excising the lamellae part (the clavus) attached to the rhinophore stalk (in two iteration). Two series of experiments were conducted to study the tubercles regeneration in one iteration: 1) excising a tubercle in the ctenidia region; 2) excising one rhinotubercle surrounding the rhinophoric pocket. Each experimental group included at least 20 adult *O. muricata,* and there was also one control group with intact tubercles and rhinophores.

The condition of the individuals was monitored daily for 27 days using a Lomo MSP1, MSP2 stereomicroscopes (Lomo MA, Russia) and a Leica DM2500 microscope (Leica Biosystems Division of Leica Microsystems Inc., USA). The samples were photographed using a Leica CLS 150X, Leica M165C stereomicroscope and a Leica DM2500 microscope. Key regeneration stages were fixed every 2 days for scanning electron microscopy (SEM) studies. The fixation and subsequent treatments are described elsewhere [Nikitenko *et al.*, 2021]. The fractures of critical point-dried rhinophores and tubercles were made to study the internal morphology. The external and internal morphology of the regenerates was studied using SEM CamScan S2 (Cambridge Instrument Scientific Company, Great Britain), Hitachi S405A (Hitachi, Japan), JEOL JSM–6380L and JEOL JSM–7000 (JEOL, Japan).

To obtain semithin sections (1 μm thick) of the intact rhinophore, rhinotubercle and body tubercle, the *O. muricata* was embedded in resin using standard procedures [Nikitenko *et al.*, 2021; Nikitenko, Vortsepneva, 2023]. Sections were cut using a Leica EM UC6 ultratome (Leica Microsystems, Witzlar, Germany), stained with 1% Methylene blue (1% toluidine blue, 1% methylene blue, and 1% sodium tetraborate) for 30–60 s, then photographed using an Olympus slide scanner (Olympus Medical Systems Corp., Japan).

Results

Rhinophore regeneration

The first reaction of rhinophore excising is the compression of the edges wound and the rhinotubercles convergence (Table 1). Further recovery steps differ depending on the experimental group and will be described below.

Group 1: excising the apex of rhinophore and three lamellae under it

The rhinophore retains its mobility and can extend beyond the rhinophore pocket or be drawn into it when the rhinophore lamellae and its apex are excised (Figs 3, 5). The wound heals. The regeneration bud is formed within 24–36 hours after the operation. At this time, the rhinophore lamellae at the injury site change their position. The lamellaes approach the damaged tip of the rhinophore and cover the wound (Fig. 5B). A similar position of the distal rhinophore lamellae persists for another 2 days. Next, the apex of the rhinophore is isolated, the regeneration bud is extended, and the distal lamellae return to their normal position (Fig. 5 D–F). Subsequent recovery of lamellae and spicules does not occur within 21 days.

Group 2: excising the whole rhinophore with its stalk

There are no visible changes on the first day after excising the whole rhinophore with its stalk (Fig. 4). The wound remained closed with rhinotubercules. A transparent, convex, rounded regenerative bud 120 μm in height and 150 μm in width is formed after 24–36 hours from the excising (Fig. 6A, B; Table 1). The surface of the regeneration bud is smooth, and cilia are absent. The internal content of the bud is homogeneous. It consists of transversely oriented fibers without any inclusions. There is a small cavity in the center of the regeneration bud (Fig. 6A, B). The thickness of the muscle wall is 50 μm, the cavity diameter is 20 μm.

The rhinophore acquires an oblong–conical shape and increases in size by almost one and a half times up to 200 μm over the next 3 days (Fig. 6C, D; Table 1). Ciliary tufts are found on the surface of the rhinophore (Fig. 6B). Groups of cilia are more densely located in the middle part of the rhinophore, more rarely at the apex, and absent in the basal part.

Five days after the operation, the height of the rhinophore slightly increased to 225 μm (Table 1). There are also groups of cilia on the surface of the rhinophore. The rhinophore apex is conical (Fig. 6E). The first spicule is revealed, located at the top

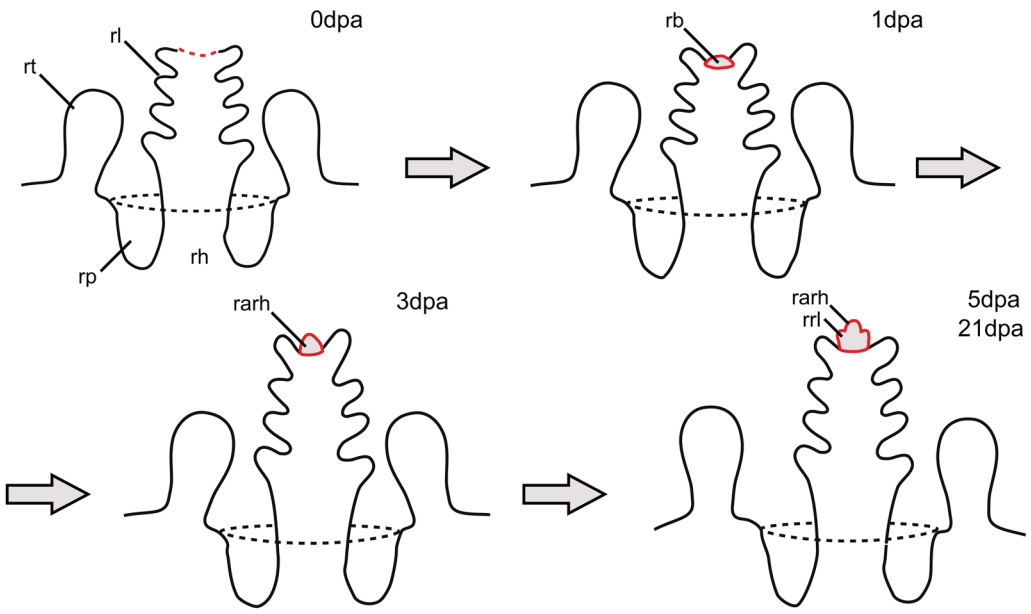

FIG. 3. Scheme of the regeneration of *Onchidoris muricata* rhinophore after cutting off the apex and three lamellae. Abbreviations: dpa – day post amputation; rarh – regenerating rhinophore apex; rb – regenerating bulb; rh – rhinophore stalk; rl – rhinophore lamellae; rp – rhinophore pocket; rrl – regenerating rhinophore lamellae; rt – rhinotubercle.

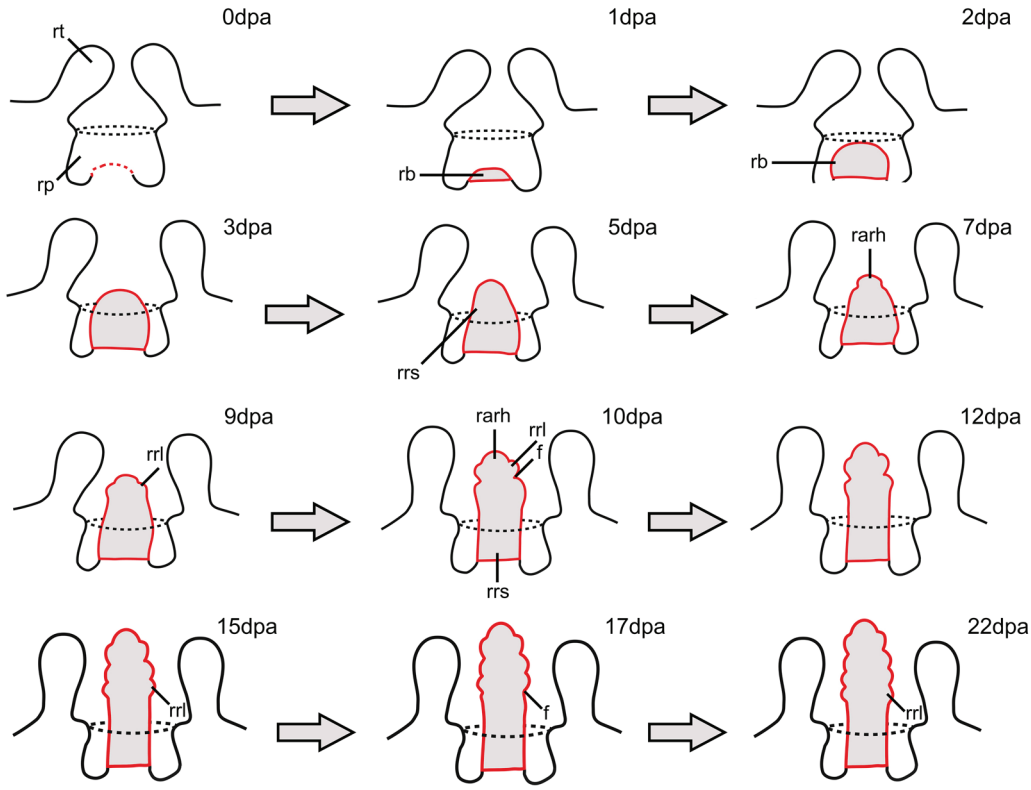

FIG. 4. Scheme of the regeneration of *Onchidoris muricata* rhinophore after cutting off the entire rhinophore. Abbreviations: f – furrow; rarh – regenerating rhinophore apex; rb – regeneration bulb; rh – rhinophore stalk; rl – rhinophore lamellae; rp – rhinophore pocket; rrl – regenerating rhinophore lamellae; rt – rhinotubercle.

Table 1. Transformation of the regenerating rhinophore of *Onchidoris muricata* after complete removal.

Таблица 1. Преобразование регенерирующего ринофора *Onchidoris muricata* после его полного удаления.

Time post amputation	Change in the external structure of the regenerate (shape, size)	Changes in the cillia cover	Change in the internal structure	Spicule detection
Immediately post amputation (0 hours post amputation (hpa))	Mechanical compression of the wound edges	No	No	No
24–36 hpa	Formation of a regenerative spherical bud	The regenerate surface is smooth, the cilia are absent	Regenerate with powerful muscular walls. There is a central cavity inside the regenerative bud	No
36–64 hpa	The regenerating bud oblong conical shape. The bud increases in size	Bunches of cilia are densely located in the middle part of the rhinophore, less often at the apex and absent in the basal part	No	No
5 day post amputation (dpa)	The bud increases in size slightly	Bunches of cilia are located over the entire surface of the rhinophore	No	The first spicule was detected at the top of the regenerate. The spicule is inclined in the transverse plane of the rhinophore
7 dpa	Elongated regenerate, rounded apex is wider than base of rhinophore	The surface of the rhinophore apex is abundantly covered with cilia	The base of the rhinophore is composed of powerful longitudinal muscle bundles; the rhinophore cavity is absent	Spicules have a monolithic or mixed internal structure, 10–15 μm in diameter. Spicules are located one above the other
8–10 dpa	regenerating lamellae are separated. A pronounced apex is formed	The apex of the rhinophore is abundantly covered with cilia. Islands of cilia are located along the length of the rhinophore	No data	Spicules are located on the sides of the regenerate wall
12 dpa	Cutting furrows appear under the formed lamellae. The regenerate slightly increases in size.	Similarly 8–10 dpa	No data	No data
15 dpa	Additional lamellae of the regenerating rhinophore are formed	Similarly 8–10 dpa	Two bundles of longitudinal muscles are well developed. The central cavity is poorly expressed	The number of spicules is increases. The spicules are located closer to each other in the basal part of the regenerating rhinophore than in the apical part
18 dpa	The shape of the rhinophore is elongated. The lamellae are symmetrically. The isolated apex is not expressed	Cilia form a uniform cover over the entire rhinophore	Two bundles of longitudinal muscles are well developed. The central cavity is formed. It is free of muscles, spicules and soft tissues	The spicules are also arranged one below the other, but more ordered, 10–15 μm in diameter.
22 dpa	regenerating rhinophore have four lamellae	Cilia form a uniform cover over the entire rhinophore		The number of spicules increase. It is difficult to determine the exact amount

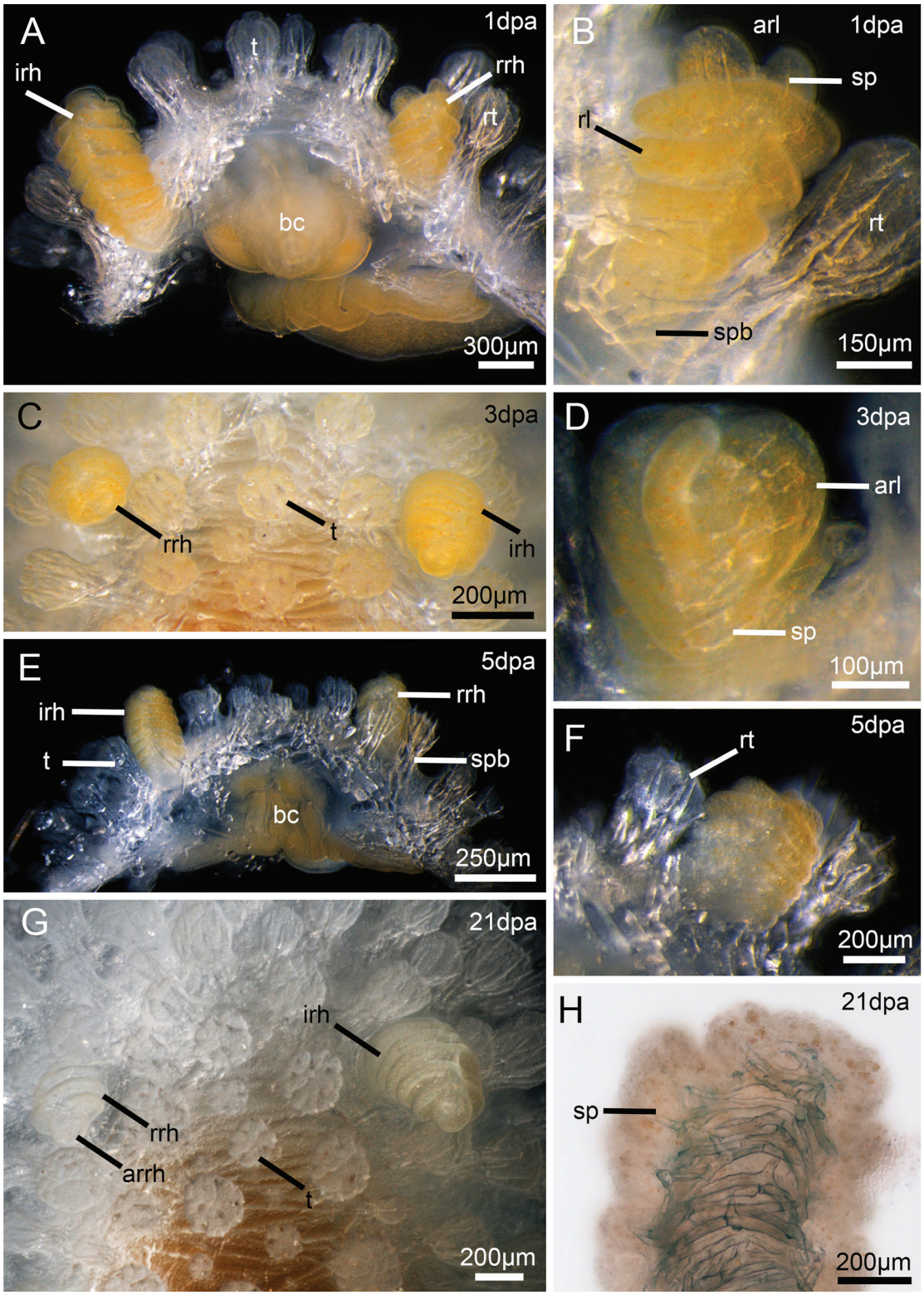

FIG. 5. Rhinophore regeneration after removal of the apex and three rhinophore lamellae (light microscopy). **A.** Frontal section through the front of the body. Left – intact rhinophore, right – rhinophore 1 day after amputation of the apex and two rhinophore lamellae (1dpa). **B.** Regenerating rhinophore (1 dpa). The apical lamellae are everted. **C.** Top view of the rhinophores at 3 dpa. On the left – regenerating rhinophore, on the right – intact rhinophore. **D.** External morphology of the regenerating rhinophore at 3 dpa. **E.** Frontal section through the front of the body. Left – intact rhinophore, right – rhinophore 1 day after amputation of the apex and two rhinophore lamellae (5 dpa). **F.** Regenerating rhinophore (5 dpa). Apical lamellae return to normal position. **G.** Top view of the rhinophores 21 days after amputation. On the left – regenerating rhinophore, on the right – intact rhinophore. The formed tip of the rhinophore is visible. **H.** Regenerating rhinophore with internal spicules (21 dpa). Abbreviation: arl – apical rhinophore lamellae; arrh – apex of regenerating rhinophore; bc – buccal complex; irh – intact rhinophore; rl – rhinophore lamellae; rrh – regenerating rhinophore; rt – rhinotubercles; sp – spicule; spb – spicule of body; t – tubercle.

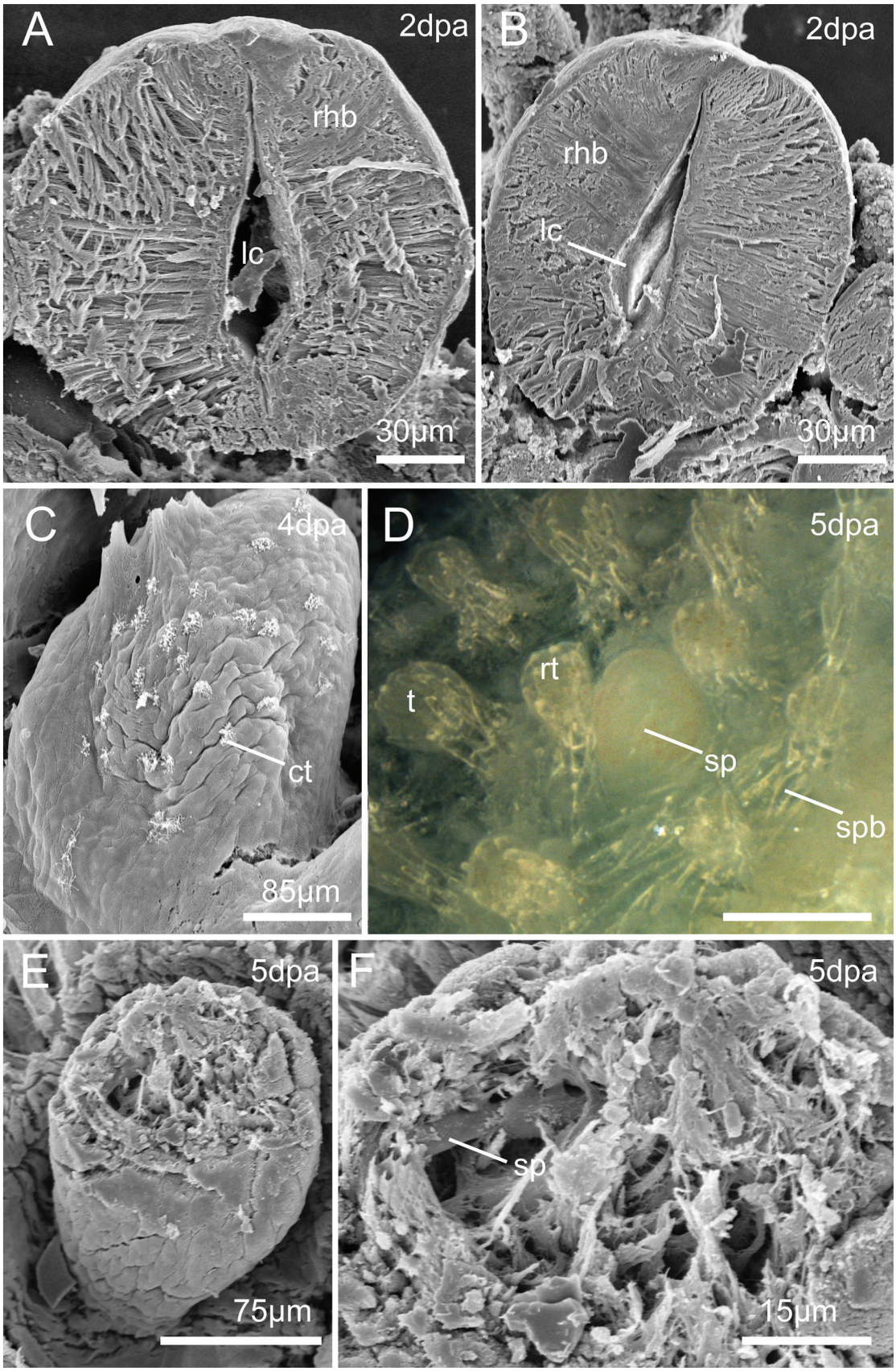

FIG. 6. Regenerating rhinophore morphology (SEM, light microscopy). **A–B.** Longitudinal section trough the regenerating rhinophore, one day post amputation (dpa). **C.** External morphology of the regenerating rhinophore with cilia tufts (4 dpa). **D.** Spicule are into the regeneration rhinphore (5 dpa). **E.** Scrapped of the apical part of regenerating rhinophore (5 dpa). **F.** The spicule in the apical parts of the regenerating rhinophore (5 dpa). Abbreviation: ct – cilia tuft; lc – lymphatic cavity; rhb – rhinophore regenerating bud; rt – rhinotubercle; sp – spicule; spb – spicule of body.

at an angle in the transverse plane of the rhinophore. (Fig. 6F).

The regenerating rhinophore has an elongated shape after 7 days post–removal. The apex is rounded, and its width exceeds the rhinophore base (Fig. 7 A–E; Table 1). The surface of the rhinophore apex is abundantly covered with cilia. The granules of a yellowish pigment are visible in the light. The base of the rhinophore is composed of powerful longitudinal muscle bundles (Fig. 7D). The cavity of the rhinophore is absent. The four fractures of spicules 10–15 μm in diameter are revealed on a longitudinal section through the central part of the rhinophore (Fig. 7 D–E). The spicules are located one above the other (Fig. 7E). The internal structure is monolithic or mixed (with a very loose center and a harder periphery). Isolation of spicules at this stage is not possible. The standard method of isolation with bleach spicules along with soft tissues; mechanical extraction also fails due to the loose structure.

Regeneration of the rhinophore lamellae is observed 8–10 days after excising (Figs 7 F–H, 8; Table 1). The lamellae recovery occurs both symmetrically on both sides of the rhinophore (Fig. 8A, B) and asymmetrically, with one of the lamellae being larger than the other (Fig. 7G, H). The formation of a pronounced apex of the rhinophore also occurs approximately at the stage of lamellae separation. Symmetrical lamellae with a rounded apex (Fig. 7F), secondary lamellae with a non–delimited apex (Fig. 8), as well as lamellae with an asymmetric non–delimited apex, are formed (Fig. 9). At this stage, the regenerating rhinophore becomes covered with a continuous layer of cilia. The top of the rhinophore is abundantly covered with them. There are also bunches of cilia along the length of the rhinophore, as in the previous stages (except for the basal part) (Fig. 8). The thickness of the lamellae ranges from 20 μm to 30 μm. The spicules located on the rhinophore sides are detected inside the rhinophore (Fig. 7H).

The well–defined notches and grooves appear under the newly formed rhinophoral lamellae on the 12th day after the operation (Fig. 8C, D; Table 1). Previously formed lamellae increase in size to almost 40–50 μm.

Over the next three days, 15 days after injury (Table 1), additional rhinophore lamellae are formed (Fig. 9). Lamellae can be formed asymmetrically. The first lamellae are formed on one of the lateral sides and then on the other. By day 15, a total of three rhinophore lamellae are formed, 40–50 μm thick. The distribution of the ciliary cover remains unchanged. A longitudinal section through the lateral side of the rhinophore shows that the number of spicules in the rhinophore also increases (Fig. 9B). The diameter of the spicules is 15 μm. The internal structure of the spicules is mixed: in the center there is a cavity filled with monolithic content, then radial layers follow, and the periphery of the spicules is monolithic. The spicules are located one above the other and closer to each other in the basal part than in the apical part.

The regenerating rhinophore acquires a symmetrical shape on the 17th day after the experiment start (Fig. 9C, D; Table 1). The lamellae number reaches three on each side. Cilia evenly cover the entire rhinophore (Fig. 9C). Two well–developed bundles of longitudinal muscles are found in the basal part of the rhinophore (Fig. 9D). The sides of the rhinophore have spicules 10–15 μm in diameter (Fig. 9E). The internal structure of the spicules is mixed: in the center of the spicule there is a cavity filled with a homogeneous “loose” content, and the peripheral part of the spicules is monolithic (Fig. 9E). The spicules are also located one above the other, but in a more orderly manner.

Further changes in the regenerate are associated with an increase in the size of the rhinophore lamellae and spicules in them. So, by day 22, the rhinophore has four lamellae; by day 25, it is even more extended (Fig. 10).

Group 3: excising the lamellae part (the clavus)

Rhinophore stalks devoid of rhinophore lamellae contain up to 3 spicules (Fig. 11A). The site of excising is tightened, and a regeneration bud is formed in the first 24–36 hours, similarly to other groups (Fig. 11B, C). In contrast to group 1, the mobility of the rhinophore at this time is reduced; as a rule, it is drawn into the pocket of the rhinophore and remains covered by rhinotubercles. The bud expands (Fig. 11D, E) and stretches (Fig. 11F) after regeneration. By day 10 post–amputation, the regenerating rhinophore already has detached lamellae (Fig. 11G, H) containing spicules (Fig. 11H, I). Further, there is an increase in the number of lamellae of the rhinophore and its size, similar to regenerating when it is completely removed (Fig. 12).

Tubercles regeneration

A wound completely filled with spicules is formed when a tubercle is removed in the ctenidia region (Fig. 13A, C). Wound epithelialization occurs within 24 hours of injury (Fig. 13B, D). Further, no visible changes after tightening the wound occur. Thus, the regenerative ability of the tubercle of the dorsal part of the body is not expressed.

The rhinotubercle elongates within 27 days in contrast to the tubercle in the ctenidia region (Fig. 13E, F). The regenerating tubercle exhibits a cone-shaped protrusion, which is covered with cilia on the surface.

Discussion

For the first time, this work demonstrates the ability to regenerate body parts with spicules through

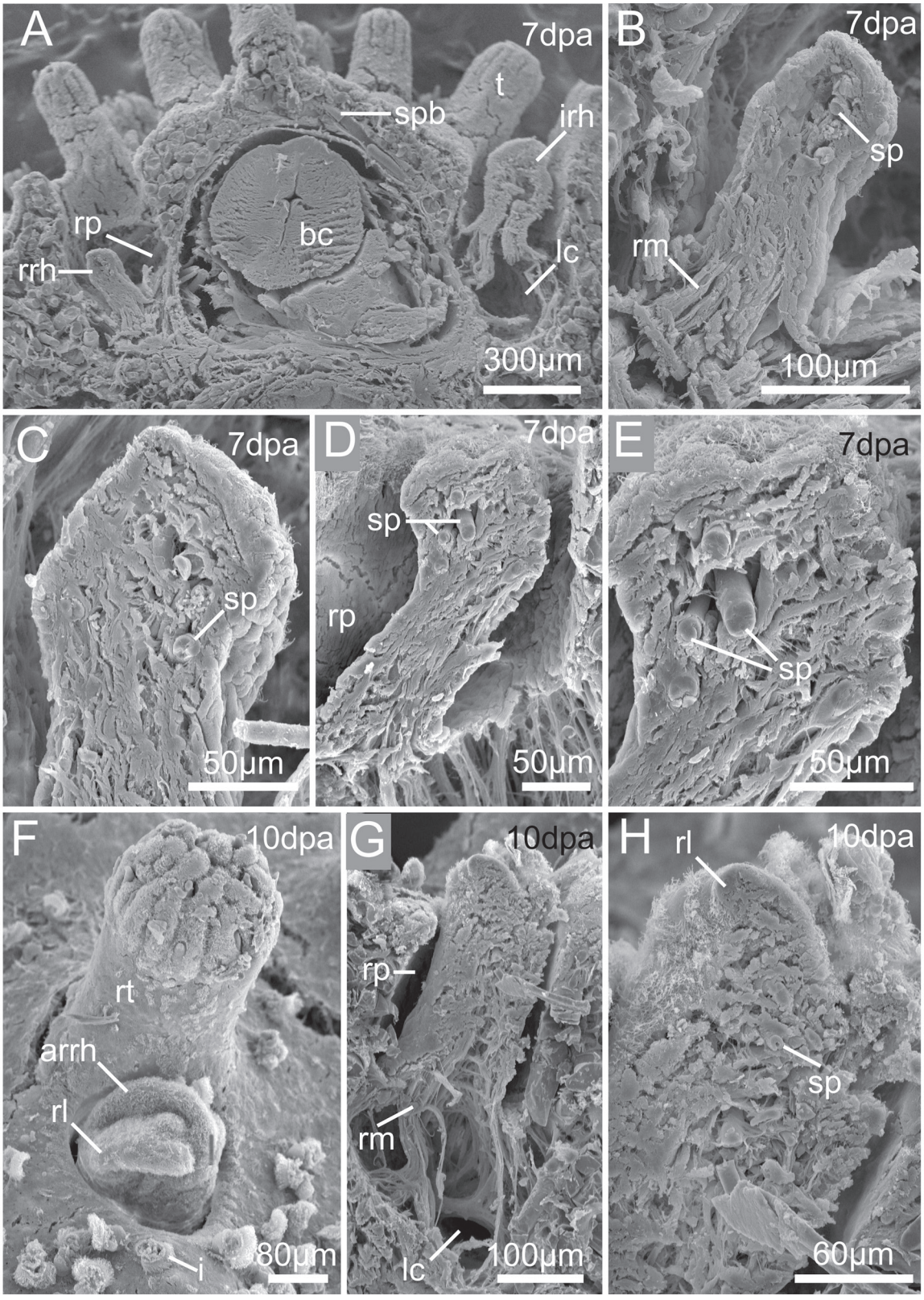

FIG. 7. Regenerating rhinophore morphology (SEM). **A–E.** 7 days after amputation (dpa). **F–H.** 10 days after amputation. **A.** Longitudinal section through the regeneration and intact rhinophores. **B–E**. Longitudinal section through the regenerating rhinophore with a dense muscular layer and spicules in the apical part. **F.** External morphology through the regenerating rhinophore with first lamellae and a well–shaped apex. **G.** Longitudinal section through the regenerating rhinophore with a lymphatic cavity at its base. **H.** Longitudinal section through the apical part of the rhinophore with spicules. Abbreviation: arrh – apex of regenerating rhinophore; bc – buccal complex; i – infusoria; irh – intact rhinophore; lc – lymphatic cavity; rl – rhinophore lamellae; rm – retractor muscles; rp – rhinophore pocket; rrh – regenerating rhinophore; rt – rhinotubercle; sp – spicule; spb – spicule of body.

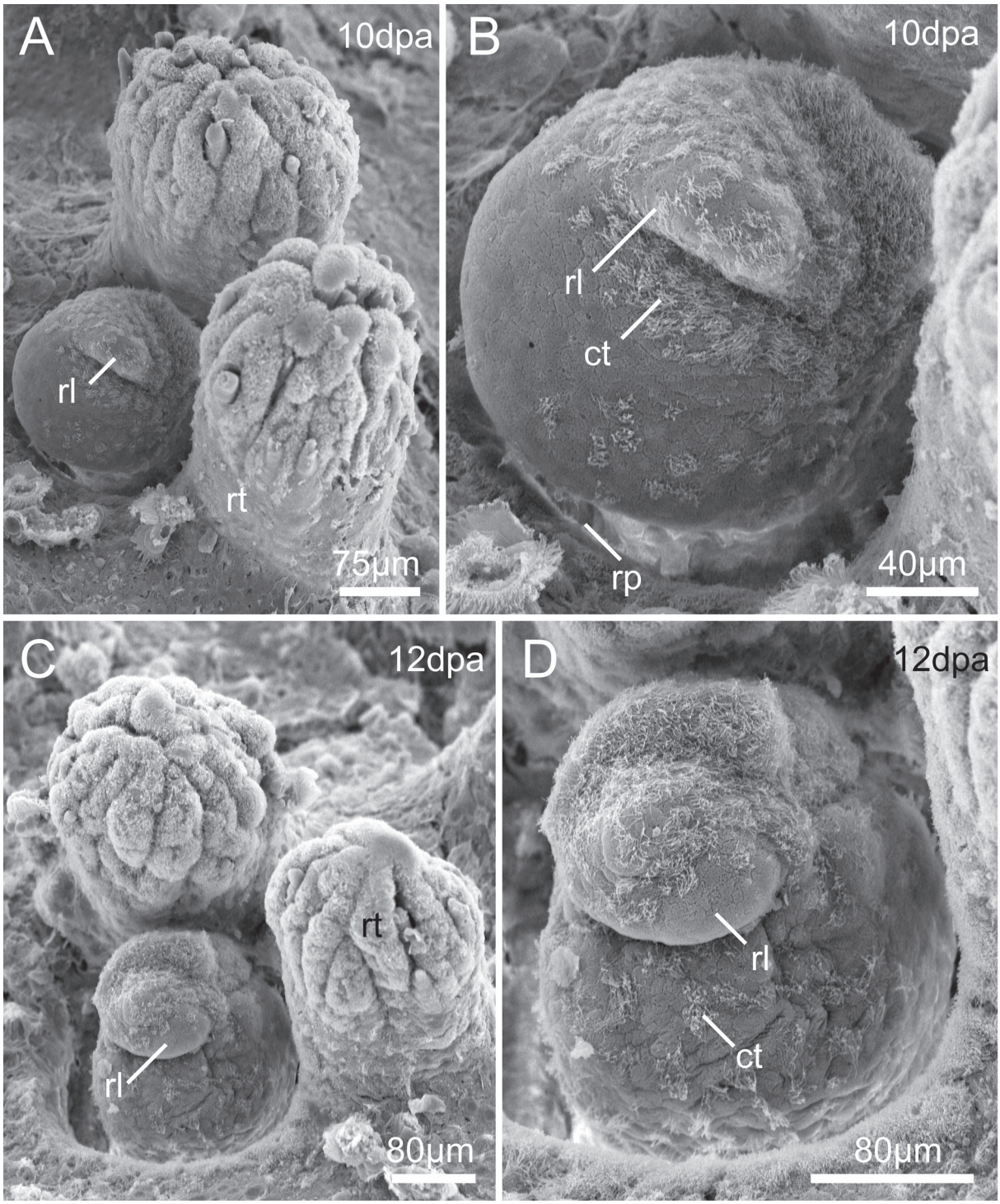

FIG. 8. Regenerating rhinophore morphology (SEM). **A–B.** 10 days after amputation. **C–D.** 12 days after amputation. The apical part of the regenerating rhinophore with lamellae is abundantly covered with cilia. Abbreviation: ct – cilia tuft; rl – rhinophore lamellae; rp – rhinophore pocket; rt – rhinotubercle.

the study of *Onchidoris muricata*. The regenerative capacity varies across different body parts and is influenced by factors such as location, function, and the extent of injury. Notably, the experiment inflicted damage to the rhinophore and tubercle, but this did not affect the vital activities of *O. muricata*.

The response to excision of the rhinophore, rhinotubercles, and tubercles differed. When the rhinophore or its parts were removed, there was mechanical constriction observed at the wound edges. In the case of complete rhinophore removal, the rhinophore pocket constricted in order to prevent the loss of hemolymph, a phenomenon also observed in other molluscs [Korotkova, 1997]. This constriction

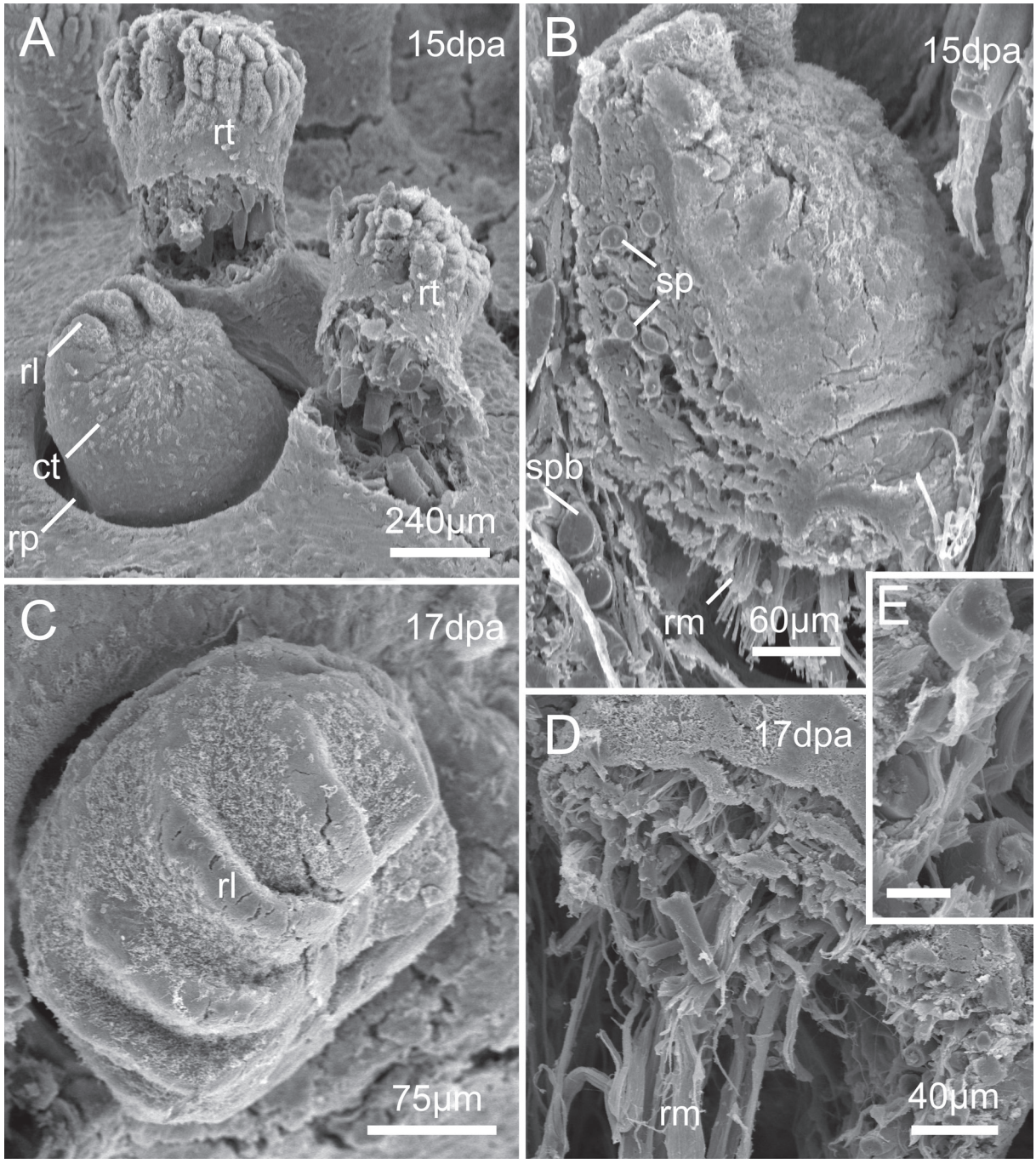

FIG. 9. Regenerating rhinophore morphology (SEM). **A–B.** 15 days after amputation. **C–E**. 17 days after amputation. **A.** External view of a rhinophore with three lamellae. **B.** Scrapped rhinophore with spicules. **C.** External view of the rhinophore with 5 symmetrical lamellae along the anterior side. The ciliary cover is uniform over the entire surface of the rhinophore. **D.** Breakage of the basal part of the regenerating rhinophore with retractor muscles and spicules. **E.** Spicules with monolithic and inhomogeneous internal structure. Abbreviation: ct – cilia tuft; rl – rhinophore lamellae; rm – retractor muscles; rt – rhinotubercle; sp – spicule; spb – spicule of body.

was achieved through the action of annular muscles in the rhinophore lamellae and the powerful annular muscles surrounding the opening of the rhinophore pocket.

However, wound constriction did not occur when rhinotubercles and body tubercles were excised (Fig. 13). The base of these tubercles is fortified with spicules, which prevent mechanical compression of the wound. Additionally, a thick layer of spicules was observed to prevent hemolymph loss in these cases (Fig. 13C).

An interesting observation was made when the apex and three upper lamellae of the rhinophore were excised. In response, the intact lamellae moved closer to each other, effectively covering the wound. This similar reaction was also observed in the rhinotu-

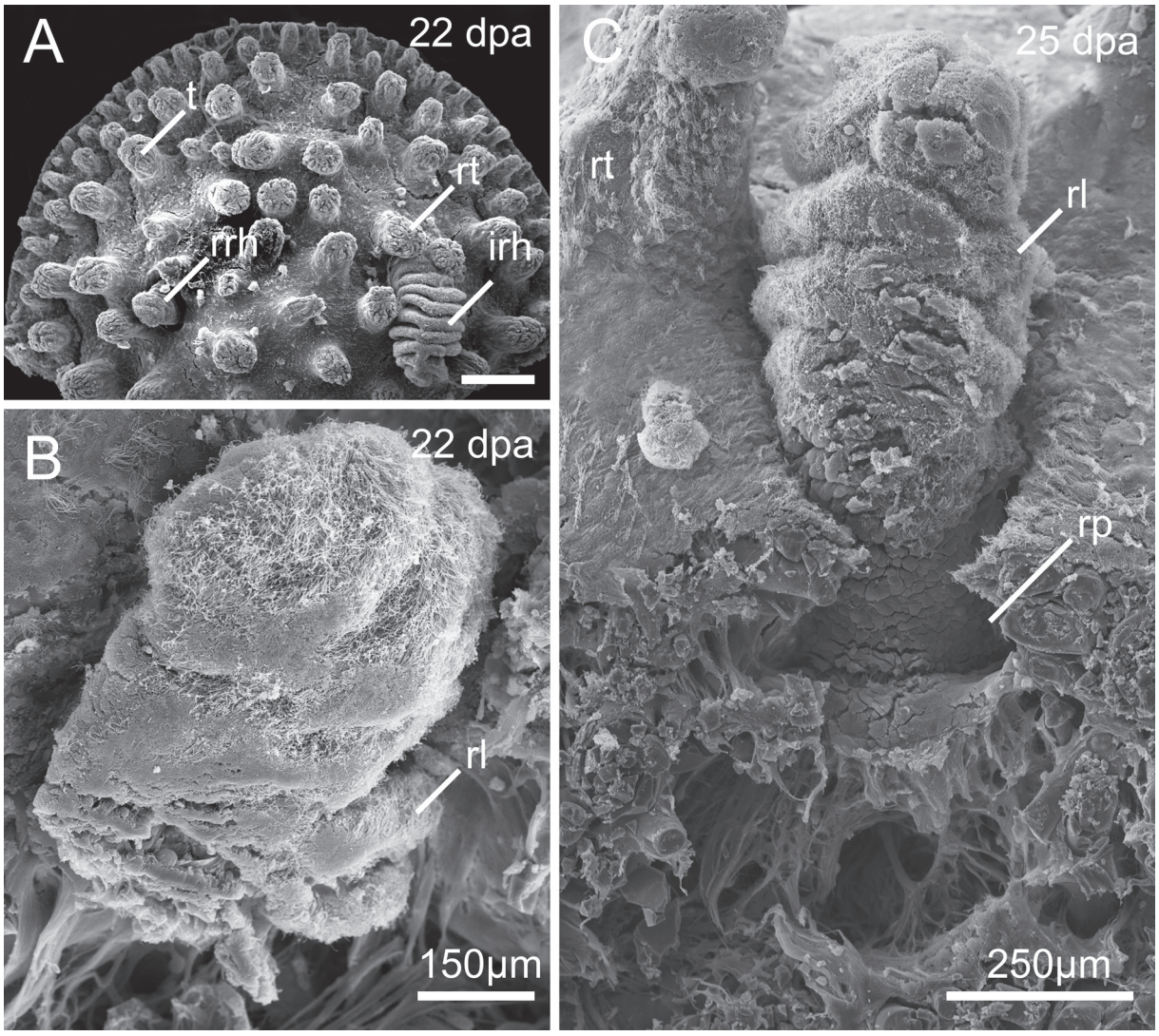

FIG. 10. Regenerating rhinophore external morphology (SEM). **A–B.** 22 days post amputation. **C.** 25 days post amputation. **A.** Dorsal view of the anterior part of the notum with rhinophores. **B.** Side view of a regenerating rhinophore. **C.** Rear view. Abbreviation: irh – intact rhinophore; rl – rhinophore lamellae; rrh – regenerating rhinophore; rp – rhinophore pocket; rt – rhinotubercle; t – tubercle. Scalebar: A – 400µm.

bercles when the entire rhinophore was removed. It is suggested that such a reaction of the lamellae and rhinotubercles acts as a barrier, protecting the site of damage from physical impacts.

Rhinophore and their spicules regeneration

Rhinophores are tentacles found on the head of nudibranchs, which are homologous to eye tentacles in Caenogastropoda, "lower" Heterobranchia, and Panpulmonata [Staubach, Klussmann–Kolb, 2007; Brenzinger *et al*., 2021]. The regeneration process of the smooth part of the rhinophore in dorid *O. muricata* is similar to that of the Cladobranchia rhinophore (*Doto* Oken, 1815) [Kress, 1968]. It is also similar to the regeneration of the smooth eye stem of *Achatina fulica* (Bowdich, 1822), *Helix aspersa* O.F. Müller, 1774, marine Caenogastropoda representatives [Gibson, 1984; Sidelnikov, 1991; Gorbushin *et al.*, 2001].

In the regeneration process, there is an initial bulge at the site of damage, followed by the formation of undifferentiated tentacles. The ability of *O. muricata* to regenerate its rhinophore depends on the degree of injury. While the three lamellae and the removed apex do not regenerate, this is primarily due to the minimal damage caused and the ongoing functionality of the rhinophore. There is no fundamental difference between removing the entire rhinophore or just the rhinophore lamellae, leaving the stalk intact (Figs 6–12). In the first five days after complete rhinophore excision, the regeneration process focuses on rebuilding a smooth rhinophore stalk (Fig. 6). The formation of lamellae occurs similarly in both cases.

The restoration of rhinophore motility occurs after the formation of the lymphatic cavity, muscles,

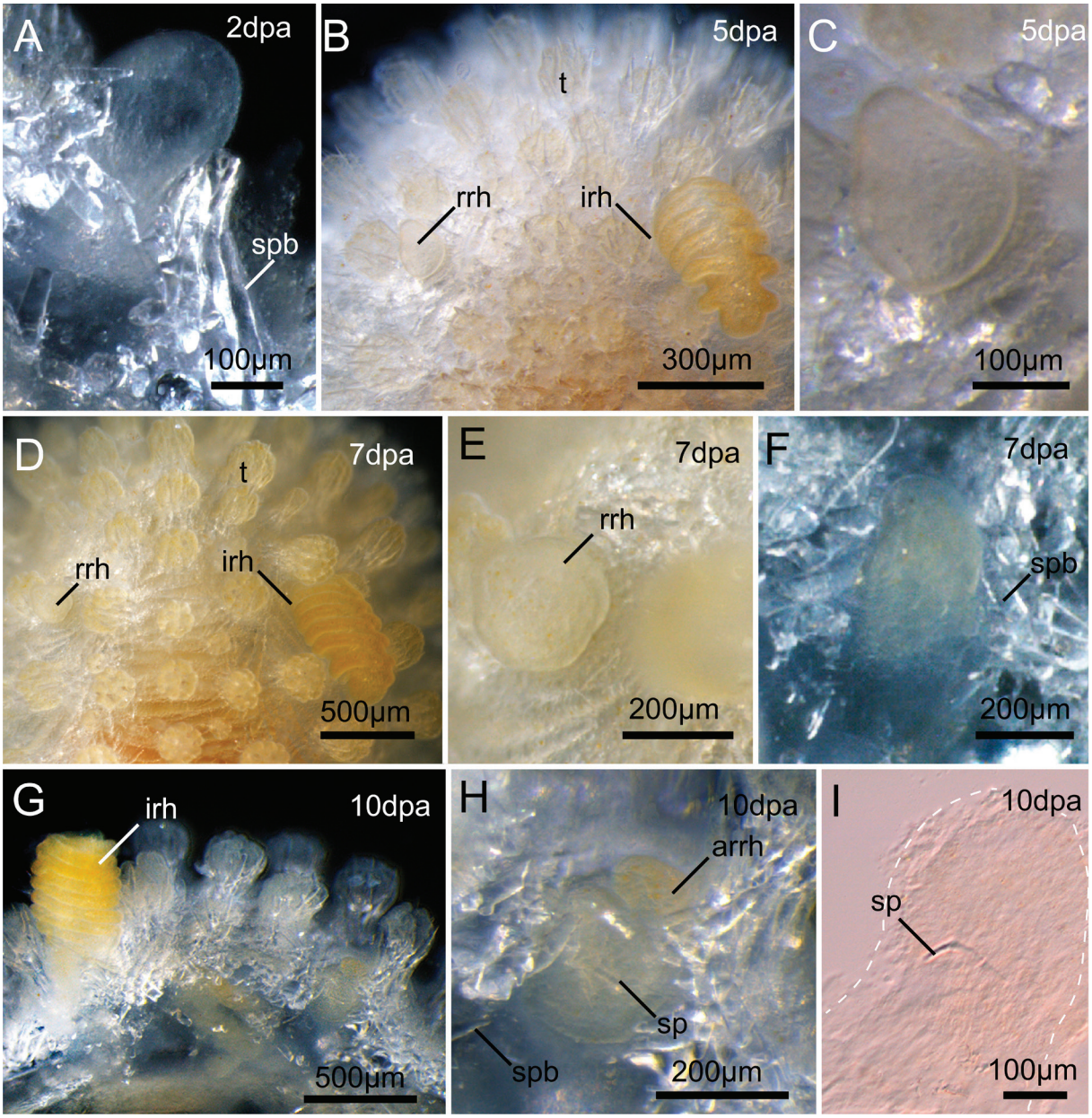

FIG. 11. Rhinophore recovery after removal of the clavus with lamellae (light microscopy). **A.** Appearance of the regenerate on the 2nd day post amputation, front view. **B, C**. Regeneration bud on the surface of the regenerate and intact rhinophore, dorsal view. **D–F.** The appearance of the rhinophore on the 7th day after injury from the dorsal side (D, E) and from the front (F). **G–H.** Appearance of the regenerate with the first lamellae, isolated apex and spicules inside. **I.** Spicule of the regenerate on the 10th day post amputation. Abbreviation: arrh – apex of regenerating rhinophore; irh – intact rhinophore; rrh – regenerating rhinophore; sp – spicule; spb – spicule of body; t – tubercle.

and cilia on the surface. The cilia play a sensory role in the rhinophore's functioning. Upon irritation, the rhinophore walls constrict, leading to the outflow of lymphatic fluid and rhinophore contraction [Lisova, Vortsepneva, 2022]. Once the cavity relaxes, fluid is pumped back in, indicating the restoration of nervous system elements and rhinophore mobility.

In gastropods, the sensory function and subsequent differentiation into chemosensory rhinophores or ophthalmic stalks are only possible if the ganglion remains intact [Sidelnikov, 1991]. Regenerates have sensory cilia bundles that are located in patches (Fig. 6C, E). The same arrangement of sensory cilia is observed in the rhinophores of veligers of *Rostanga pulchra* MacFarland, 1905 [Chia, Koss, 1982]. Therefore, we suggest that the formation of sensory areas corresponds to that of the veliger.

The metamerism of the rhinophore, manifested in the form of lamellae, does not appear immediately during regeneration. Initially, there is a separation of the apex and the formation of regenerates spicules, which then determine the site of cutting furrow formation and the subsequent lamellae's location. Each subsequent lamella is associated with rhinophore

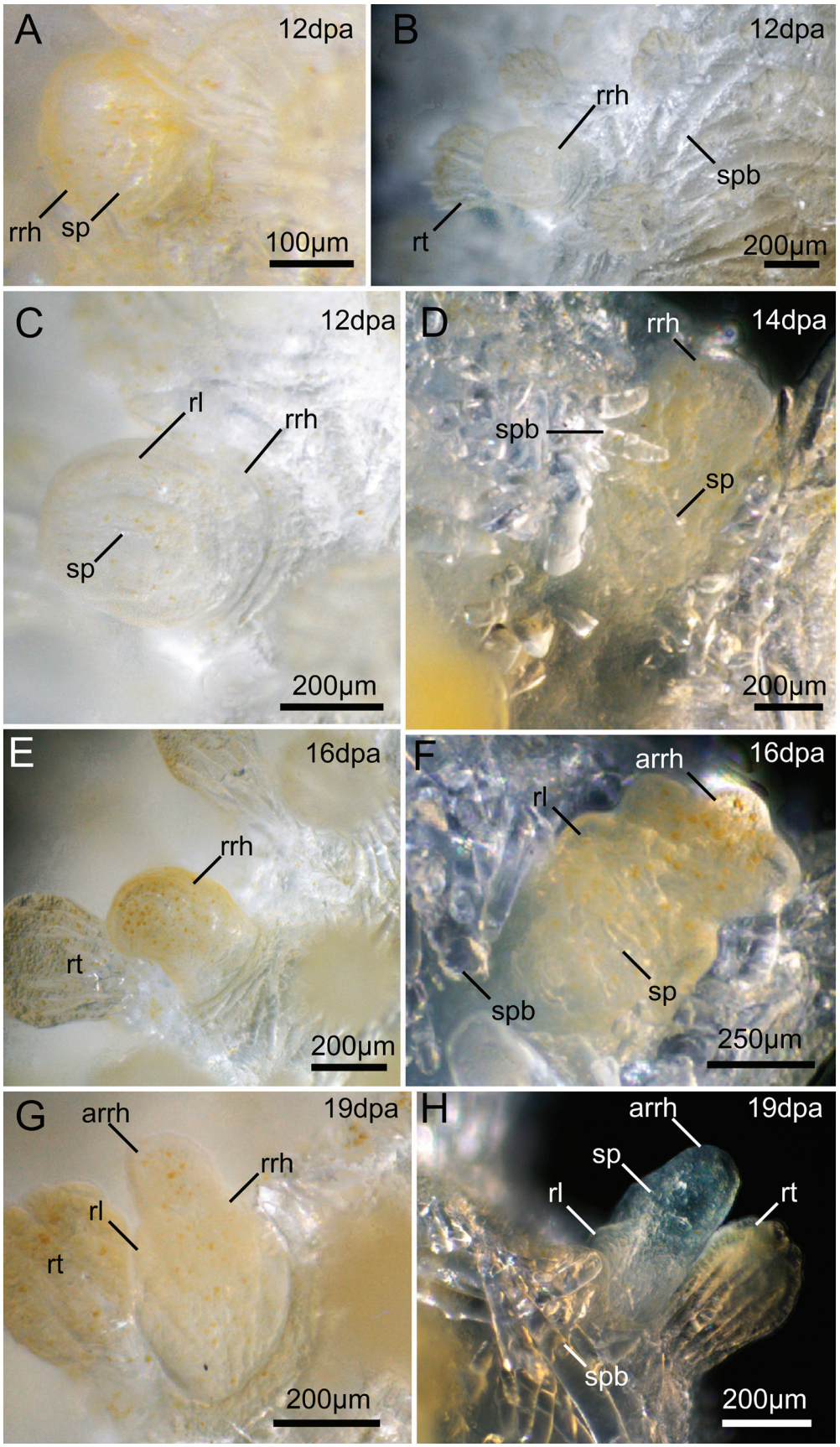

FIG. 12. Regenerating rhinophore external morphology after removal of the clavus with lamellae (Light microscopy). An increase in the number of rhinophore lamellae and spicules inside it. **A, B, C, E, G.** Dorsal view. **D, F, H.** Front view. Abbreviation: arrh – apex of regenerating rhinophore; rl – rhinophore lamellae; rrh – regenerating rhinophore; rt – rhinotubercle; sp – spicule; spb – spicule of body.

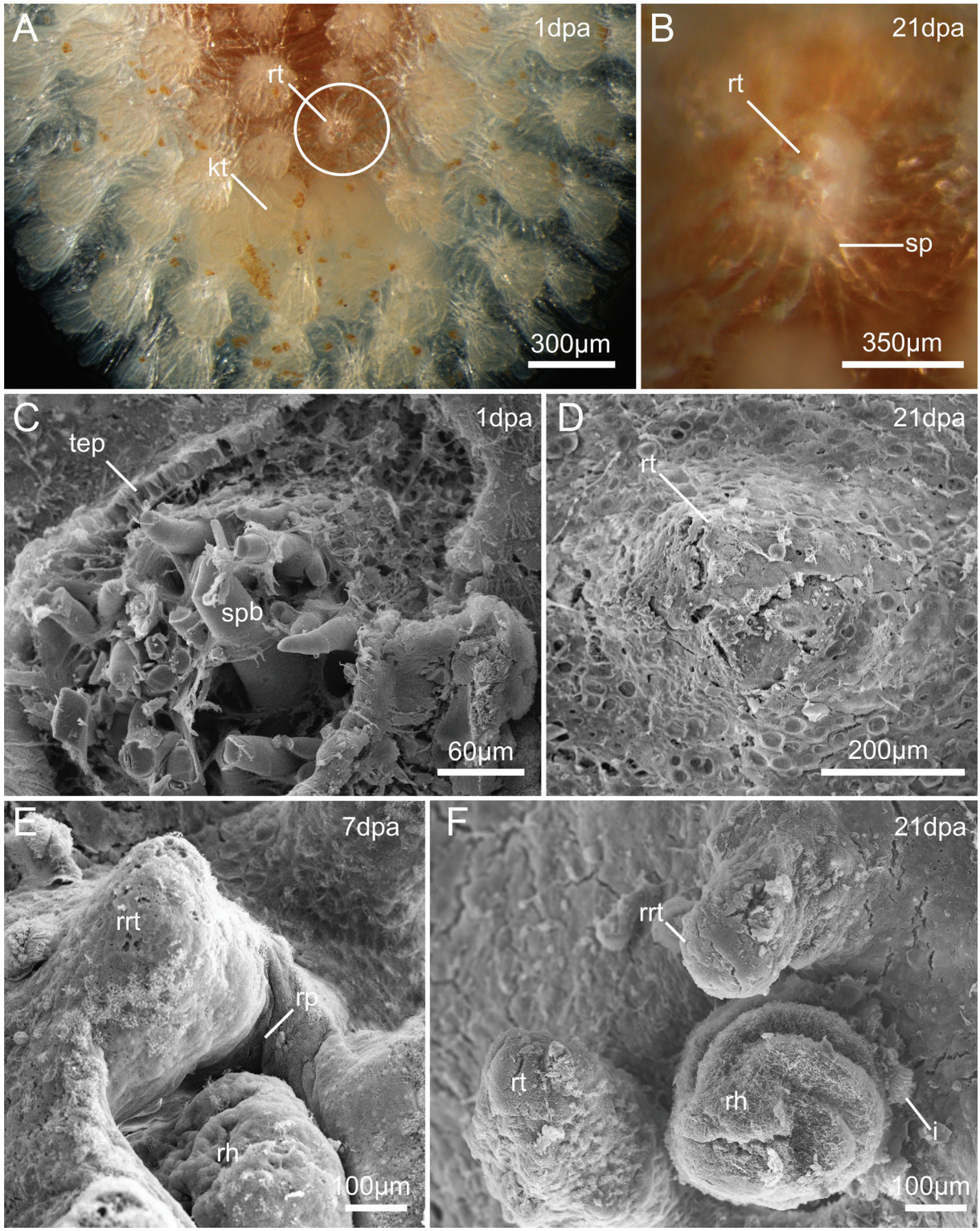

FIG. 13. Regeneration of tubercles after removal. **A, B.** Light microscopy. **C–F.** SEM. **A, C.** Damaged tubercle in the ctenidia region. **B, D.** Epithelialization of the wound, tubercle recovery does not occur. **E.** Rhinotubercle on the 7th day post amputation. **F.** Rhinotubercle at the rhinophore 21 days post amputation. Abbreviation: i – infusoria; kt – ctenidia; rh – rhinophore; rp – rhinophore pocket; rt – regenerating tubercle; rrt – regenerating rhinotubercle; t – tubercle; tep – tubercle epithelium.

spicules (Fig. 7 C–E) and forms intercalary at the border between the smooth rhinophore stalk and the clavus. Although only four rhinophore lamellae were observed in our study, this could be due to the experiment's short duration. However, the external morphology and metamerism of the regenerate were comparable to those of intact rhinophores.

Using *O. muricata* as an example, we have demonstrated the *de novo* formation of spicules in adult dorids for the first time. The morphology of these new

regenerates spicules differs significantly from those of intact rhinophores [Lisova, Vortsepneva, 2022]. Regenerate's spicules synthesized *de novo* are short and have blunt ends (Figs 11H; 12), while intact spicules are sharp and curved [Lisova, Vortsepneva, 2022]. The regenerate spicules dissolve very quickly in bleach when trying to isolate them from the regenerate. This turned out to be completely impossible. Intact spicules stand out and remain undamaged under the same exposure to bleach. This suggests a higher content of organic matter in the regenerate spicules compared to the intact ones. The diameter and size of spicules in regenerates are smaller than those in intact rhinophores. According to previous data [Lisova, Vortsepneva, 2022], the diameter of the spicules of an intact rhinophore is from 15 to 20 μm, while the spicules of a regenerate are from 8 to 14 μm. The internal structure of spicules in regenerates appears looser (Fig. 9E). Which also may indicate a higher content of organic contents. The data obtained support the hypothesis about the formation of spicules *de novo*. However, further studies are required to fully understand the details of spicule formation.

Deviations

During our observations, we noted both symmetrical (Figs 8; 9C, D; 11; 12) and asymmetric (Fig. 9A, B) recoveries. While asymmetric wrinkle repair may not be typical, repeated experiments are necessary to clarify this aspect.

Tubercle regeneration

In this study, we have demonstrated that the regenerative capacities differ within the dorid body itself. Our findings reveal that *O. muricata* rhinophore regeneration was more successful compared to tubercle regeneration (Figs 5–12; 13), indicating variations in the ability to restore tubercles in different body parts (Fig. 13). Specifically, rhinotubercles were able to recover (Fig. 13E, F), whereas tubercles in the ctenidia region could not (Fig. 13 A–D), despite the assumption that both locations serve an important protective function. In instances where rhinophores and rhinotubercle were completely or partially removed, the cerebral ganglion remained intact, and only a partial removal of the rhinophoral nerve, stemming from it, occurred. Notably, the tubercle in the ctenidia region lack substantial nerve elements. Hence, the potential for successful regeneration appears to be linked to the innervation of these organs. This corroborates previous research, indicating that the nervous system plays a crucial role in influencing the success of regeneration. Impaired innervation is recognized to potentially impede the recovery process or even result in its failure [Chase, Kamil, 1982; Gorbushin *et al.*, 2001; Maroyan, 2021].

Our data on dorid regeneration indicate that the restoration of damaged body parts varies among representatives of the two groups in the order. Cladobranchia, known for their ability to regenerate lost body parts like papillae [Kress, 1968], exhibit a higher regenerative capacity. The papillae of Cladobranchia perform a very important function – they carry the ducts of the digestive gland [Kress, 1968, 1981]. It is known that they are able to regenerate after damage [Korotkova, 1997]. The tubercle of dorids perform only a structural and protective function, carrying only a group of spicules inside [Penney, 2008; Penney *et al.*, 2020]. We propose that the disparity in tubercle restoration between the Doridina and Cladobranchia groups is also linked to the functional demands placed on the organ.

Conclusion

This study provides the first description of the regenerative capacity of dorids, specifically *Onchidoris muricata*, following disruption of the integrity of rhinophores and tubercles that contain hard spicules. Notably, this is the first observation of the potential restoration of rhinophores and rhinotubercules in dorid nudibranchs. Moreover, we have obtained unique data on the *de novo* recovery of calcite subepidermal spicules. Our findings significantly contribute to the understanding of post–traumatic regenerative processes in molluscs and invertebrates in general, particularly regarding the maintenance of innervation in the regenerated structures through the rhinophoral and cerebral ganglia.

The rhinophores of *O. muricata* serve as valuable model objects for further in–depth investigations into regeneration and post–traumatic spiculogenesis at the histological, cellular, and molecular levels.

Acknowledgements

We are thankful to our colleagues of Pertsov White Sea Biological Station and Zoology department of Lomonosov Moscow State University: A.A. Semenov, D.A. Ozerov, A.L. Mikhlina, G.D. Kolbasova, V.L. Semin, A.I. Chava, G.D. Zakharov for the helping with the sampling. We would like to express our gratitude to V.V. Kozin for discussing the regeneration process. We are thankful to I.A. Kosevich, E.N. Temereva, B.V. Osadchenko, A.I. Lavrov, F.V. Bolshakov and M.A. Petrova for helping with microscopy methods. We also would like to thank G. Davidovich and A. Bogdanov and the Electron Microscopy Laboratory of the Shared Facilities Center of Lomonosov Moscow State University sponsored by the RF Ministry of Education and Science and Research. The authors would also like to thank S. Mitelev and G. Bykov (I.D. Papanin Institute for the biology of inland waters Russian Academy of Sciences) for helping us with the electron microscopy. We also express our gratitude to the reviewers and editor for enhancing our work.

Funding

Support for research within the Interdisciplinary Scientific and Educational Schools of Moscow University No. 23-ШI04-29.

References

Bely A. E., Nyberg K. G. 2010. Evolution of animal regeneration: re–emergence of a field. *Trends in Ecology and Evolution*, 25(3): 161–170. DOI:10.1016/j.tree.2009.08.005

Bosch T.C.G. 2007. Why polyps regenerate and we don't: towards a cellular and molecular framework for *Hydra* regeneration. *Developmental Biology,* 303: 421–433.

Bouchet P., Frýda J., Hausdorf B., Ponder W.F., Valdés Á., Warén A., Rocroi J.P. 2005. Working classification of the Gastropoda. *Malacologia*, 47: 239–283.

Brenzinger B., Schrödl M., Kano Y. 2021. Origin and significance of two pairs of head tentacles in the radiation of euthyneuran sea slugs and land snails. *Scientific Reports*, 11(1): 21016.

Carew T.J., Walters E.T., Kandel E.R. 1981. Classical conditioning in a simple withdrawal reflex in *Aplysia californica. Journal of Neuroscience,* 12: 1426–1437.

Carlson B.M. 2007. *Principles of regenerative biology.* Elsevier, Amsterdam, The Netherlands. 379 p.

Chakraborty K., Joy M. 2020. High-value compounds from the molluscs of marine and estuarine ecosystems as prospective functional food ingredients: An overview. *Food Research International*, 137: 109637.

Chase R., Kamil R.A. 1983. Morphology and odor sensitivity of regenerated snail tentacles. *Journal of Neurobiology*, 14(1): 43–50.

Checa A.G., Vendrasco M.J., Salas C. 2017. Cuticle of Polyplacophora: structure, secretion, and homology with the periostracum of conchiferans. *Marine Biology*, 164: 1–17.

Chia F.S., Koss R. 1982. Fine structure of the larval rhinophores of the nudibranch, *Rostanga pulchra*, with emphasis on the sensory receptor cells. *Cell and Tissue Research*, 225*:* 235–248.

Do T.D., Jung D.W., Kim C.B. 2022. Molecular phylogeny of selected dorid nudibranchs based on complete mitochondrial genome. *Scientific Reports*, 12: 18797.

Egger B., Gschwentner R., Rieger R. 2007. Free–living flatworms under the knife: past and present. *Development genes and evolution*, 217: 89–104.

Ereskovsky A., Borisenko I.E., Bolshakov F.V., Lavrov A.I. 2021. Whole–body regeneration in sponges: diversity, fine mechanisms, and future prospects. *Genes*, 12(4): 506.

Foale S.J., Willan R.C. 1987. Scanning and transmission electron microscope study of specialized mantle structures in dorid nudibranchs (Gastropoda: Opisthobranchia: Anthobranchia). *Marine Biology*, 95: 547–557.

Gibson B.L. 1984. Cellular and ultrastructural features of the regenerating adult eye in the marine gastropod *llyanassa obsoleta*. *Journal of Morphology*, 180(2): 145–157.

Gorbushin A.M., Levakin I.A., Panchina N.A., Panchin Y.V. 2001. *Hydrobia ulvae* (Gastropoda: Prosobranchia): a new model for regeneration studies. *Journal of Experimental Biology*, 204(2): 283–289.

Haefelfinger H.R. 1961. Beiträge zur kenntnis von *Peltodoris atromaculata* Bergh 1880 (Mollusca, Opisthobranchiata). *Revue suisse de zoologie,* 68: 331–343.

Haszprunar G., Künz E. 1996. Ultrastructure and systematic significance of the epidermis and haemocoel of *Rhodope* (Gastropoda, Nudibranchia). *Journal of submicroscopic cytology and pathology*, 28: 485–497.

Jörger K.M., Stöger I., Kano Y., Fukuda H., Knebelsberger T., Schrödl M. 2010. On the origin of Acochlidia and other enigmatic euthyneuran gastropods, with implications for the systematics of Heterobranchia. *BMC evolutionary biology*, 10: 1–20.

Korotkova G.P. 1997. *Regeneration in animals.* Saint–Petersburg University Press, Saint–Petersburg. 297 p. [In Russian].

Kress A. 1968. Untersuchungen zur Histologie, Autotomieund Regeneration dreier Doto–Arten *Doto coronata*, *D. pinnatifida*, *D. fragilis* (Gastropoda, Opisthobranchiata). *Revue suisse de Zoologie,* 75: 235–303.

Kress A. 1981. A scanning electron microscope study of notum structures in some dorid nudibranchs (Gastropoda: Opisthobranchia). *Journal of the Marine Biological Association of the United Kingdom*, 61(1): 177–191.

Leise E.M. 1984. Chiton integument: metamorphic changes in *Mopalia muscosa* (Mollusca, Polyplacophora). *Zoomorphology*, 104(6): 337–343.

Lisova E.D., Vortsepneva E.V. 2022. New data on nudibranchs rhinophore morphology and their spicule complex in *Onchidoris muricata* (Doridina, Gastropoda). *Zoologischer Anzeiger*, 296: 58–70.

Maroyan A. 2021. The time course and neuroanatomy of rhinophore regeneration in the nudibranch *Berghia stephanieae*. *Masters Theses.* University of Massachusetts Amherst, 1119: 37 p. https://doi.org/10.7275/24368366.0

Mau A., Jha R. 2018. Aquaculture of two commercially important molluscs (abalone and limpet): existing knowledge and future prospects. *Reviews in Aquaculture*, 10(3): 611–625.

Mitoh S., Yusa Y. 2021. Extreme autotomy and whole–body regeneration in photosynthetic sea slugs. *Current Biology*, 31(5): R233–R234.

Neusser T.P., Heβ M., Haszprunar G., Schrödl M. 2006. Computer-based three-dimensional reconstruction of the anatomy of *Microhedyle remanei* (Marcus, 1953), an interstitial acochlidian gastropod from Bermuda. *Journal of Morphology*, 267(2): 231–247.

Neusser T.P., Fukuda H., Jörger K.M., Kano Y., Schrödl M. 2011. Sacoglossa or Acochlidia? 3D reconstruction, molecular phylogeny and evolution of Aitengidae (Gastropoda: Heterobranchia). *Journal of Molluscan Studies*, 77(4): 332–350.

Nikitenko E.D., Vortsepneva E.V. 2020. Spicule complex of three Onchidorididae species (Gastropoda: Doridina) from the White Sea. *Invertebrate Zoology,* 17(1): 44–58. [In Russian].

Nikitenko E., Ereskovsky A., Vortsepneva E. 2021. Ontogenetic dynamics of the subepidermal spicule complex in Nudibranchia (Gastropoda): the case of *Onchidoris muricata. Zoology,* 144: 125886.

Nikitenko E.D., Vortsepneva E.V. 2023. Ontogenetic dynamics of the nudibranch epithelium in *Onchidoris muricata* (O.F. Müller, 1776). *Zoology*, 161: 126129.

Penney B.K. 2008. Phylogenetic comparison of spicule networks in cryptobranchiate dorid nudibranchs (Gastropoda, Euthyneura, Nudibranchia, Doridina). *Acta Zoologica*, 89(4): 311–329.

Penney B.K., Ehresmann K.R., Jordan K.J., Rufo G. 2020. Micro-computed tomography of spicule networks in three genera of dorid sea-slugs (Gastropoda: Nudipleura: Doridina) shows patterns of phylogenetic significance. *Acta Zoologica*, 101(1): 5–23.

Ponder W.F., Lindberg D.R., Ponder J.M. 2019. *Biology and Evolution of the Mollusca.* Vol. 1. CRC Press. 899 p.

Reddien P.W., Sánchez Alvarado, A. 2004. Fundamentals of planarian regeneration. *Annual Review of Cell and Developmental Biology*, 20: 725–757.

Salvini–Plawen von L. 1967. Neue scandinavische Aplacophora (Mollusca, Aculifera). *Sarsia*, 27(1): 1–63.

Sekizawa A., Yama R., Nakashima Y. 2018. Biological differences between *Chromodoris reticulata* and *Chromodoris tinctoria* (Opisthobranchia: Nudibranchia). *Venus (Journal of the Malacological Society of Japan)*, 76(1-4): 45–52.

Sidelnikov A.P. 1991. Regeneration of the eye tentacle in the giant African snail *Achatina fulica*. *Izvestiya Academii Nauk SSSR*. *Seria biologia*, 5: 716–725 [In Russian].

Staubach S., Klussmann–Kolb A. 2007. The cephalic sensory organs of *Acteon tornatilis* (Linnaeus, 1758) (Gastropoda Opisthobranchia) – cellular innervation patterns as a tool for homologisation. *Bonner Zoologische Beitrage*, 55(3/4): 311.

Su X.W., Zhang D.M., Heuer A.H. 2004. Tissue regeneration in the shell of the giant queen conch, *Strombus gigas*. *Chemistry of Materials*, 16(4): 581–593.

Watabe N. 1983. Shell repair. In: Saleuddin A.S.M., Wilbur K.M. (Eds.) *The Mollusca, Vol. 4: Physiology,* 289–316.