

Limacosphaera, an Unusual Mesogastropod (Lamellariidae) Larva of the Weddell Sea (Antarctica)

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ABSTRACT

Marseniopsis conica Smith, 1915 and *M. mollis* (Smith, 1902) have planktotrophic larvae that are unique among gastropods. They cover the larval shell with a lacunous muscular mantle that can change its volume by interaction of body fluid and muscle activity. This limacosphaera larva is found in Antarctic waters and represents the most complex larval strategy within the "echinospira-group". Notes on the biology and anatomy, including histology, of this larva are presented.

Key words: Lamellariidae, *Marseniopsis*, larvae, Antarctica.

INTRODUCTION

The majority of benthic invertebrates of high-Antarctic seas brood their young or provide postspawning parental care rather than produce free-living larvae (Mileikovsky, 1971; Picken, 1980). True planktotrophic pelagic larvae are extremely rare in the 200 to 600 m deep high-Antarctic shelf areas.

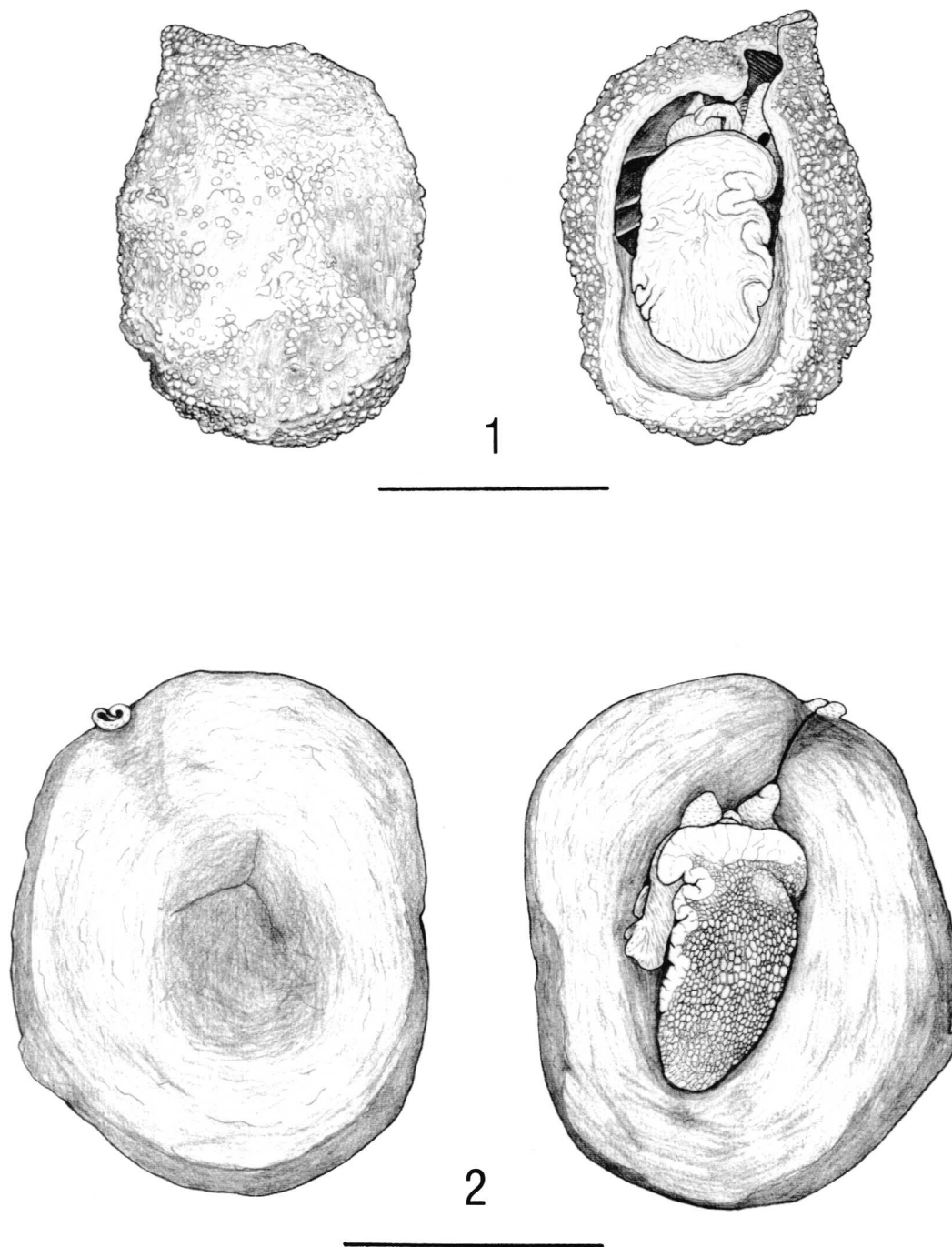
Plankton samples taken by the R/V POLARSTERN in the eastern Weddell Sea during several late winter to late summer cruises yielded only two meropelagic larvae of benthic gastropods (Piatowski, 1987; Boysen-Ennen, 1987). Both of these larval types were regularly found. One of these is *Capulus subcompressus* Pelseneer, 1903, which had first been observed by Pelseneer (1903) and is described in detail by Bandel and Hain (in preparation). The second meropelagic larvae was first observed by Simroth (1914) in material from the Davis Sea. Simroth noted anatomical similarity to a planktonic gastropod from the deep sea areas of the Indian Ocean, which

he described earlier as *Limacosphaera macdonaldi* (Simroth, 1908).

This animal is a spherical, transparent, voluminous gastropod with two openings: one to allow head and foot to come out of the shell and the other situated on the opposite side of the sphere (Simroth, 1908, 1914). Simroth called the muscular mantle covering the larval shell the deutoconcha of the limacosphaera. The posterior pore, which he (1908) called the shell tunnel (Schalengang), connects the sea water with a cavity surrounding much of the thin organic shell. Simroth (1914) also suggested the presence of glandular cells and muscle fibers in the voluminous deutoconcha that surrounds the shell. He noticed that the Antarctic forms represented larvae rather than adult gastropods as he had originally assumed when describing *Limacosphaera* as a new genus of pelagic gastropods (Pteropoda). Simroth (1914) correctly placed these larvae into the ontogeny of members of the "echinospira-group" in the genus *Marseniopsis*. The term limacosphaera was retained, now to describe a very peculiar and characteristic larva.

Simroth (1908, 1914) considered that the limacosphaera swam with the aid of their large velum. Another mode of swimming, mainly by hydrostatic effects, was considered the likely mode of propulsion in the water column by Jevdonin and Minichev (1975).

These authors observed developing spawn of *Marseniopsis conica* and found that the mantle of the embryo envelops the shell prior to hatching from an egg mass found in excavations of tunicate surfaces. Jevdonin and Minichev (1975) also noted that the mantle did not fuse completely but left an open shell pore. They seem not



Figures 1, 2. Adult animals of 1. *Marseniopsis conica* Smith, 1915, and 2. *M. mollis* (Smith, 1902). Dorsal views on left, showing mantles that are fused above the shell and cannot be retracted. Ventral views on right, show foot, parts of the head. All illustrations drawn from fixed animals. Scale bar = 5 mm for figure 1, 10 mm for figure 2.

to have been aware of the earlier descriptions of these larvae by Simroth (1908, 1914). Jevdonin and Minichev (1975) suggested that larvae with a size of about 20 mm in diameter were nearly ready to metamorphose. Their descriptions suggest that their observations had been carried out on preserved material and they most probably did not see living larvae or embryos.

We provide a new description of the histology of these

larvae integrated with observations on the living forms and their metamorphosis.

MATERIALS AND METHODS

During the cruises POLARSIRKEL 80/81, POLARSTERN ANT I, ANT III/3, ANT V/3 and ANT VII/4 larvae of the limacospaera type were caught at about 100 stations in

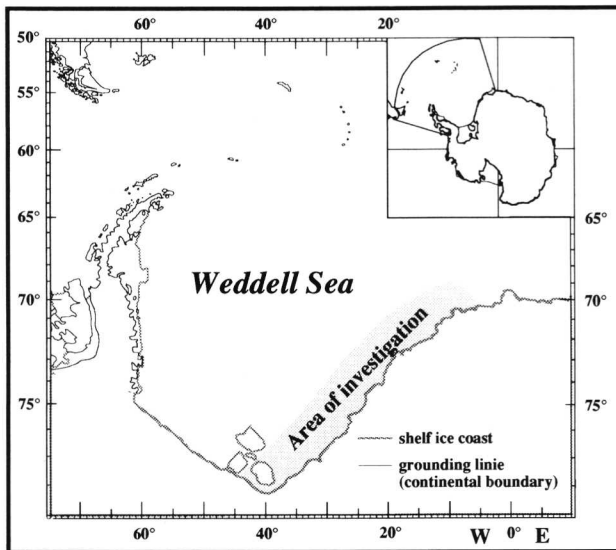


Figure 3. Map indicating location of the Weddell Sea and the area where the larvae of *Marseniopsis* were caught.

neritic waters above continental shelf areas of the eastern Weddell Sea (figure 3). Samples were taken with various plankton gears (mesh sizes of 0.335–4.5 mm) in upper water layers (300–0 m) and preserved in buffered formalin.

Six larvae were kept alive for six weeks during the expedition PS ANT V/3. During this period they were fed two times with a diatom suspension. Only one of the larvae survived and metamorphosed four weeks after capture (November 1986). It was fixed in 70% ethanol.

During the expedition PS ANT VII/4 (January to March 1989) 42 living limacosphaera from RMT samples were photographed for size measurements and transferred individually to small plastic aquaria (running water system, temperature adjusted to 0 °C). The larvae were fed once a week with a suspension of Antarctic diatoms. After 8 and 13 months the photographic measurements of larvae or juveniles were repeated.

The larval shell, the thick, whitish to semitransparent tissue covering it (deutoconcha), and the juvenile shell were studied on 10 critical-point dried specimens using a SEM.

In May 1989, six living larvae were fixed for histological studies in 4% seawater-formalin or 2.5% seawater-glutaraldehyde. One limacosphaera fixed in formalin was dehydrated using isopropanol. It was then treated with benzylbenzoate, embedded in paraplast, serially sectioned (7 µm) and stained with Helioechtrubin BBL/Acidgreen 5/Acidorange 10 after Halmi (Adam & Czihak, 1964).

To detect lipids whole larvae without the deutoconcha as well as parts of the deutoconcha were treated using the oil-red-method (Romeis, 1968). These samples were embedded in glycerin and examined with a light microscope.

Another limacosphaera fixed in glutaraldehyde was osmicated in 1% osmium tetroxide, dehydrated in ace-

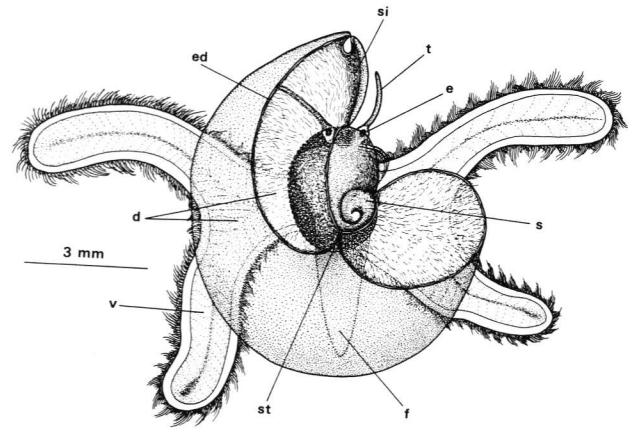


Figure 4. Larva of *Marseniopsis cf. mollis*. Schematic drawing from several photographs of living specimens. One quarter of the shell-covering tissue (deutoconcha) is removed. d, deutoconcha; e, eye; ed, epidermis; f, foot; s, shell; si, siphon; st, apical tube; t, tentacle; v, velum.

tone and embedded in Spurr's fluid. Sections were cut at ca. 80 nm to 1 µm using an ultramicrotome (Reichert). The 80 nm sections were stained with uranyl acetate and lead citrate, then examined and photographed using a TEM (Zeiss). The 1 µm sections were stained with Toluidine Blue and Pyronin and examined with a light microscope.

Voucher material is deposited at the Zoological Institute and Museum (University of Hamburg) (cat. no. M 885).

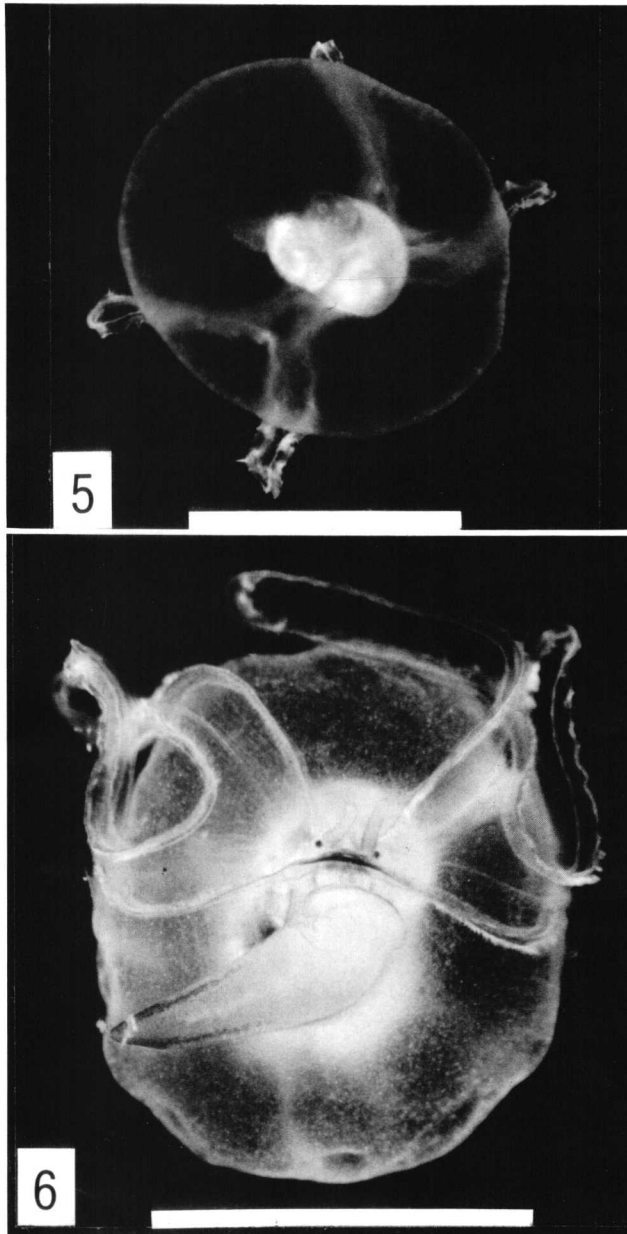
RESULTS

THE LIVING LARVA

Description: The shape of the limacosphaera (figures 4–6) is globular with an overall diameter ranging from 1.8 to 20 mm. The actual larval shell is covered by the deutoconcha. Some of the larvae have granular deposits in the outer layer of the deutoconcha. On the ventral side of the larva (figure 6) there is a slit-like opening for the foot and the four large velar lobes. This opening is extended anteriorly by a siphon-like, semicircular groove. A second, small, tunnel-like excavation extends from the apical region of the shell to the surface of the deutoconcha. Observations on living larvae sorted from plankton samples show that both openings can be closed and that the deutoconcha produces much hyaline mucus.

During metamorphosis the shell is still barely calcified (figure 7), becoming solidly calcified right after metamorphosis (figure 8). The larval shell reaches 2.3 whorls with fine spiral threads on the first 1.5 whorls. Earliest growth lines are visible after 0.8 whorls (figure 9 arrow). In well fed larvae, the greenish visceral mass can be seen through the shell and the deutoconcha.

All studied larvae had a well developed foot. The eyes are at the base of the tentacles. In contrast to Simroth's original description of *Limacosphaera macdonaldi* from

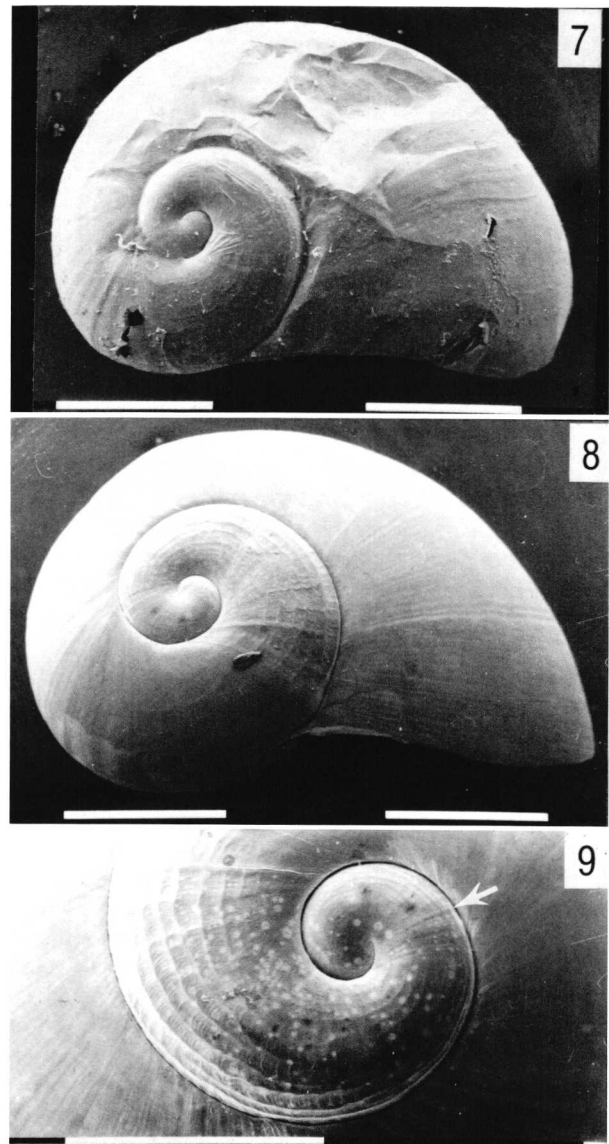


Figures 5, 6. Living larvae of *Marseniopsis cf. mollis*. **5.** Dorsal view. **6.** Ventral view, with foot, velum, tentacles and eyes visible. Scale bars = 5 mm for both photographs.

the tropics, the operculum is absent in the Antarctic specimens.

BIOLOGY

Deposition of egg capsules in the tests of compound ascidians is known for temperate lamellarian species (Fretter & Graham, 1962). Antarctic species have the same spawning habit (Jevdonin & Minichev, 1975; P. Dayton, personal communication). One compound ascidian with approximately 20 egg capsules was obtained in February 1989. Although maintained in an aquarium, the eggs failed to develop.



Figures 7–9. Shell of *Marseniopsis cf. mollis*. **7.** During metamorphosis, the shell is barely calcified and therefore, flexible. **8.** After metamorphosis, shell solidly calcified and has reached 2.4 whorls. **9.** Same specimen as in fig. 8. First growth lines (arrow) are visible at 0.7 whorls of the shell. The spiral threads end at 1.4 whorls. Scale bars = 1 mm in all photographs.

Larval size, as well as the time and location of sampling, indicates that hatching of larvae occurs from late winter to austral summer in the shelf areas of the eastern Weddell Sea.

The smallest larvae found have a diameter of 1.8 mm. We suggest that this size is reached shortly after the larvae have hatched and risen from the bottom. Aquarium observations indicate that the larvae rise by buoyancy. The large velar lobes are not used or have only secondary function. The buoyancy control mechanism is unknown.

In the northern shelf areas of the Weddell Sea the amount of food ($0.07 \mu\text{g}$ chlorophyll-*a*/liter) is more

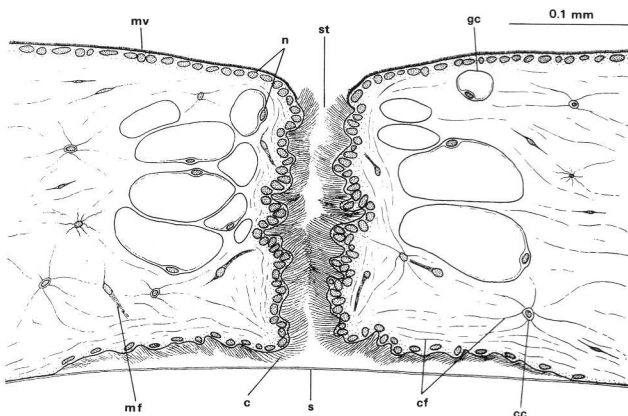


Figure 10. Schematic drawing of the apical tube (shell tunnel) of *Marseniopsis cf. mollis*. c, cilia; cc, collagenous cell; cf, collagen fibers; gc, gland cell; mf, muscular fibers; mv, microvilli; n, nuclei; s, shell; st, apical tube.

limited even during phytoplankton blooms. In open waters or polynyas (ice free areas), the phytoplankton concentration barely reaches $1.5 \mu\text{g chl}\cdot\text{a/liter}$. It is suspected that growth of larvae in these areas takes a much longer time.

The total lipid content of larvae caught in February (end of austral summer) is low (3.5% dry weight) in comparison to other planktonic organisms. Seventy percent of these lipids are triacylglycerols (Hagen, 1988).

Although fed only twice, one limacosphaera caught in October 1986 metamorphosed four weeks later. During PS ANT VII/4 small larvae with a diameter of 3.9 mm began to metamorphose in the aquaria after 10 to 24 hours, while specimens of 10 mm diameter remained in the larval stage for up to 8 weeks. One specimen caught in the end of February 1989 was still in the larval stage when it died in August 1990.

Predatory pressure on the larvae seems to be very low. The shell-covering tissue of juveniles and adults is known to produce acidic secretions.

HISTOLOGY

OBSERVATIONS WITH LIGHT MICROSCOPE

The deutoconcha of the limacosphaera consists of tissue that is composed of an outer epidermis, a central, cavernous connective tissue, and an inner epidermis covering the shell.

The outer epidermis is composed of a single layer of cells with flattened nuclei and immersed, large, light, glandular cells with a single nucleus. Below it is a voluminous, spacious connective tissue, consisting of collagenous and muscular fibers with few cells suspended in it. The inner epidermis is a single layer of cells supported by connective tissue and muscle fibers. These cells are stretched in length, and their nuclei are even more flattened than those of the outer epidermis.

The deutoconcha of the limacosphaera is connected

to the inner mantle of the apertural region of the shell by an especially strong muscular bridge of tissue. The outer mantle rests on the shell near the aperture and forms the connection to the inner mantle covering the visceral mass and the pallial cavity. Outer and inner mantle are "divided" from each other by the periostracal gland zone characterized by the presence of many nuclei.

On the apical side of the deutoconcha the external mantle is pierced by a tube-like canal (figure 10, st) connecting sea water and shell surface. This canal is lined with a rugged, ciliated epithelium (figure 10, c) that continues along the inner mantle for about 0.4 mm, to form a small cavity between shell and mantle (figure 10). The tissue below the ciliated epithelium of the canal contains an agglomeration of large light epithelial glandular cells.

The inner side of the deutoconcha connects to the organic shell that is composed of a double-layered periostracum. This shell in its apical portion is filled with the visceral mass containing the large digestive gland. Its diverticula consist of large endodermal cells with basal nuclei and small cavities. The apical portion of the digestive gland is glandular and contains three different types of stored substances. The most basal portion contains proteins, somewhat above lie a large number of spherical bodies. Scale-like storage material that is weakly acidophilous is suspended between these.

The spherical bodies ($6\text{--}19 \mu\text{m}$ in diameter) are lipids (analysed by oil-red method). The spheres of lipids were arranged like strings of pearls of different length throughout the entire digestive gland of a larva caught in February 1989 during the phytoplankton bloom.

The outer surface of the deutoconcha was also tested for the presence of lipids, but was found to lack them. This test was repeated on another larva that had been caught prior to the beginning of the phytoplankton bloom (mid-November 1986). It lacked lipids in either the mantle or the digestive gland.

Of the other organs of the larva, the nerve ring proved to be quite large. The eyes were well-developed, with lens-like light collectors. The foot is intensely ciliated, almost to the same degree as the large velum. The radula is well developed and functional.

OBSERVATIONS WITH ELECTRON MICROSCOPE

The epidermis of the deutoconcha (figure 11) is covered by a $2 \mu\text{m}$ high rim of microvilli. These microvilli are differentiated into a narrow basal portion with many filaments and a light, partly bubble-like expanded upper portion.

These "bubbles" are secretory vesicles, usually linearly arranged and attaining 2–4 times the width of the microvilli. They are produced by glandular cells at the base of the rim of microvilli and are secreted at the outside of the rim. "Bubbles" open up at the outside of the rim as could be seen in some sections.

The surface of the rim of microvilli is covered with fine fibrillar material containing small dark granules.

Between microvilli, larger vesicles with a small internal membrane are often present. Some larger vesicles are as high as the microvilli and are connected to the glandular vesicles of the epidermis.

The epithelium of the deutoconcha consists of a single layer of flattened cells resting on a distinct basal membrane. The cells are up to 10 μm in size, and are connected to each other by apical contact zones. Cell boundaries are strongly folded and intercalated.

Some nuclei are strongly heterochromatic, others are large, less densely packed and of irregular shape. All cells hold large, light vesicles often containing membranous extensions. These vesicles form a loosely connected system that is in contact with the vesicles extruded into the rim of microvilli. Newly extruded vesicles hold dense granules.

A voluminous connective tissue is present below the epidermis and fills the space between inner and outer layer of the deutoconcha. Within a homogeneous basal substance, individual, transversally striped fibers of collagen connect the basal membrane of cells within the tissue to the epidermis.

Large cavities lie within the connective tissue near the epidermis. These cavities are lined with a thin, conspicuously foamy cellular layer resting on a basal membrane. The nuclei are flattened and elongated.

Nerves, consisting of several axones within a glial cell, and smooth muscular fibers with connections to the collagen fibers are distributed within the connective tissue.

The intermediate layer between outer and inner epithelia (gelatinous layer, Simroth, 1908) is shown to contain a network of collagenous fibers connected to muscle and epithelial cells. This layer functions together with interconnected blood lacunae as a hydroskeleton that can change the outline, shape and width of the limacosphaera.

The apical tube (figures 4, 10, st) of the deutoconcha is ciliated, in contrast to the remaining surface of the mantle. This ciliation continues into the small cavity at the proximal end of tube above the shell. This cavity is less extensive than assumed by Simroth (1908) and does not surround the entire shell surface.

Simroth (1908) noted a folded surface of the shell, which is an artifact of preservation.

A rim of microvilli on a unilayered epithelium surrounding a structured connective tissue represents a large surface for resorption as well as for secretion by many glandular cells that come together in canals opening into pores. The type of secretion produced by these glands is unknown. The secretion expelled between the microvilli probably represent neutral mucus material.

DISCUSSION

The larvae metamorphosed into juveniles that had either a smooth surface or a tuberculated body surface. Both juvenile forms were analysed by gel-electrophoresis (1D-DISK-SDS-PAGE) at the Alfred-Wegener-Institut (Dr. T. Stadler). The results were compared to gel-electro-

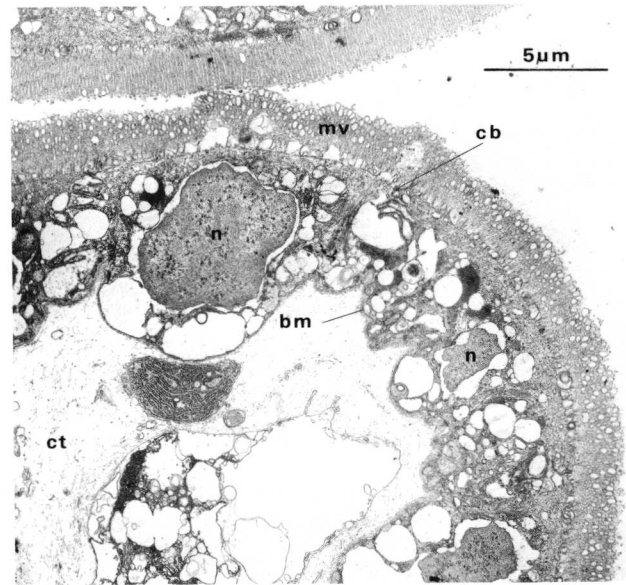


Figure 11. Transmission Electron Micrograph of the deutoconcha epidermis of *Marseniopsis cf. mollis*. bm, basal membrane; cb, cell boundary; ct, connective tissue; mv, microvilli; n, nucleus.

phoresis (same method) analyses of adult *Marseniopsis conica* and *Marseniopsis mollis*. Differences in the data indicate that the predominantly smooth juveniles represent *M. mollis* and the tuberculated ones *M. conica*. Thus it can be concluded that both species have very similar larvae. It was not possible to section and electrophoretically analyse the same individual.

The course of a limacosphaera's development depends on a variety of factors, including currents in the upper water layers, melting of sea ice, and patchiness of phytoplankton production. Larval survival in captivity for 1.5 years indicates that a limacosphaera could remain in the plankton for over a year if conditions were not favorable for metamorphosis.

Analyses of lipid content suggest that larvae either use most food-energy for growth or they store energy in form of other biochemical metabolites (e.g., proteins). The vertical distribution and the stomach contents of larvae caught during mid-October at 72°S indicate that they feed under the sea ice where the phytoplankton concentration (0.07 $\mu\text{g chl}\cdot\text{a/liter}$) was seven times larger than in deeper water (Scharek, personal communication). Due to the southwesterly water currents near the surface, the majority of larvae will be transported to southern parts of the study area, where very high phytoplankton concentrations (50–150 $\mu\text{g chl}\cdot\text{a/liter}$) were measured directly under the sea ice in October 1989. By utilization of this enormous food resource, larvae living in that area could grow very rapidly. Due to the fact that the macrozoobenthos community of the southern Weddell Sea is totally different from that of the eastern Weddell Sea (Voss, 1988) and therefore not suitable for survival of benthic stages of *Marseniopsis*, there must be a way of

returning pelagic stages (metamorphosing larvae, juveniles or adults) back to the hatching grounds. This may be accomplished by counter-currents running near the bottom.

Piatkowski (1987) and Boysen-Ennen (1987) reported the absence of larvae in the oceanic domain between the tip of the Antarctic peninsula and the north-eastern part of the Weddell Sea. *Marseniopsis* populations of the peninsula are likely zoogeographically separated from populations in the eastern Weddell Sea. Nevertheless some of the larvae could be transported by eastward wind drift of surface water layers near the ice edge from eastern-Antarctica to the Weddell Sea. Between February and March 1983, the mean abundance of larvae in the eastern Weddell Sea was 24 specimens per 1,000 m³ (Boysen-Ennen, 1987), with maximum densities of 65 larvae per 1,000 m³ in the southern part of the study area (Piatkowski, 1987). Most larvae were found in water layers of 200 to 50 m (63%) and 50 to 0 m (35%), only 2% were reported from 300 to 200 m depth (Boysen-Ennen, 1987). The major function of the deutoconcha is that of a buoyancy organ. Altering the volume of the outer mantle may vary the speed of sinking of the limacosphaera. According to Stoke's formula (Tiemann & Betz, 1979) this speed depends on the specific weight, which again is a function of the diameter of the limacosphaera. Histological analyses of the limacosphaera showed that the deutoconcha contains very little organic material and is thus very close to the density of sea water. The deutoconcha can enlarge the diameter and thus the volume of the larva without decreasing its weight in the water. A general calculation indicated that the voluminous outer mantle of the limacosphaera decreases the speed of its sinking to about one half of what it would be without such a cover around the shell.

The ability to float was observed in aquaria for all developmental stages. Even large adult specimens could float near the bottom after disturbance (diving observations at the Antarctic peninsula, W. Wägele, personal communication).

The function of the deutoconcha's apical tube and cavity is still unknown. It is evident that water can be pumped into the cavity and expelled out by ciliary currents as well as by muscular movements of the deutoconcha. It is also evident that glands can secrete substances into the lumen of tube and cavity.

Histological examination of the limacosphaera for the most part confirms the observations of Simroth (1908, 1914). His assumption that muscular cells are present within the deutoconcha is substantiated.

The prolific mucus secretion of the entire deutoconcha, as well as its large size may serve as defensive mechanisms against carnivorous planktonic groups like copepods or krill.

The shell size of hatching larvae can only be interpreted from the embryonic and the larval shell (figure 8). There are two possibilities. The normal case would be that the embryo hatches shortly after the beginning of growth lines (the mantle becomes free from the shell)

(figure 9, arrow). This happens after 0.8 whorls and a diameter of the shell of 0.65 mm.

The second possibility is that the embryo does not hatch before reaching 1.4 whorls (with spiral threads). The shell then has a diameter of 1.5 mm. To reach this size the embryo would have to feed on extra yolk.

It is proposed that the embryo hatches when the shell has reached 1.4 whorls. At this time the spiral threads disappear. This is probably the consequence of the mantle fusing above the shell. There is no difference in sculpture between the subsequent part of the larval shell and the teleoconch of the investigated species of *Marseniopsis*.

ACKNOWLEDGEMENTS

This study was carried out with financial support of the Deutsche Forschungsgemeinschaft (DFG) in the frame of the Antarctic-research grant and science grant Ba 675/6-1. We have been greatly aided by our colleagues of the Alfred-Wegener-Institut (Bremerhaven), Zoological and Geological-Paleontological departments of the University of Hamburg. We are grateful to Dr. T. Stadler who carried out the gel-electrophoresis analysis. Dr. M. G. Harasewych greatly improved style and grammar of the manuscript.

To all persons and institutions involved, we express our sincere thanks.

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