Contributions to Zoology, 70 (4) 229-242 (2002)
SPB Academic Publishing bv, The Hague

The colleteric glands in Sacculinidae (Crustacea, Cirripedia, Rhizocephala): an ultrastructural study of ovisac secretion

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Keywords: Cirripedia, Rhizocephala, *Sacculina carcini*, Thoracica, colleteric gland, ovisac, *Carcinus maenas*

Abstract

Ovisac secretion by the paired colleteric glands of *Sacculina carcini* and *Heterosaccus dollfusi* (Rhizocephala, Sacculinida) was documented and studied at the ultrastructural level. Preparatory to oviposition, the epithelium of each colleteric gland secretes one branched, elastic, transparent ovisac. The ovisac wall consists of a reticulated inner zone, secreted first, and a dense outer zone. After secretion, the ovisac detaches from most of the secretory epithelium but remains anchored proximally in the gland until oviposition ends. The exterior ovisac surface is predominantly smooth and impervious. Proximally, however, the surface is irregular and perforated. During oviposition the eggs enter the paired ovisacs, forcing the ovisacs through the ovipores into the maternal mantle cavity. Simultaneously the ovisac volume increases approximately 100 times. The resulting paired egg masses, branched like the ovisacs, are brooded and ventilated in the mantle cavity. Ovisacs prevent that developing embryos are lost prematurely with the ventilation current. Within two days the egg masses solidify and attach to retinacula in the mantle cavity cuticle. The ovisacs, now probably obsolete, are no longer discernible. The literature on colleteric gland morphology implies that ovisacs are secreted by all Sacculinidae and perhaps other Rhizocephala with colleteric glands. Similarities in both the secretory process and morphology suggest homology between the colleteric glands of Sacculinidae and the oviducal glands of Thoracica (Cirripedia) and between their products.

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Introduction

The Rhizocephala are aberrant cirripedians that parasitise other crustaceans, mainly Decapoda. They have lost the typical cirripedian feeding structures and instead absorb nourishment by means of a profusely branched root system, which penetrates the host's interior (Bresciani & Høeg, 2001). The part of the parasite engaged in reproduction is usually attached externally to the host abdomen. This so-called externa is of simple architecture. It includes a visceral mass containing a voluminous ovary and typically two sperm-producing receptacles. A mantle surrounds the visceral mass. Between these structures lies a mantle cavity, which functions as a brood chamber and communicates with the ambient sea by a mantle opening (Høeg & Lützen, 1985).

Most recent studies on rhizocephalan reproduction have focused on the sperm-producing tissue (reviewed by Høeg 1991; Høeg & Lützen 1995),

while the function of the female part of the reproductive organs remains insufficiently understood. In addition to the ovary, most species possess paired secretory oviducts, conventionally called colleteric glands. Gland design varies considerably and has been well-studied, as they constitute one of the few anatomical landmarks of importance for identification and separation of species. Being flat or shallow secretory epithelial pads in some groups, in other the glands are represented by simple sacs. In two families (Clistosaccidae, Sacculinidae) the glands possess richly branched secretory blind-sacs (Høeg & Lützen 1985).

Rhizocephala usually produce several broods in succession (Høeg & Lützen 1985). A study of *Sacculina carcini* Thompson, 1836 showed that the colleteric gland epithelium undergoes a secretory cycle, synchronously with the oocyte development, in preparation to oviposition (Delage 1884). Delage concluded that each colleteric gland secretes an ovisac that envelops the eggs when they pass into the mantle cavity, a conclusion that has been questioned by many authors from his own time to the present.

Comparative anatomy makes it obvious that the colleteric glands can best be compared to the oviducal glands of the non-parasitic Cirripedia. Recent ultrastructural studies have shown that the ovisacs produced by these glands are important during oviposition and fertilisation (Walley 1965; Klepal et al. 1977; Walker 1977, 1980). It was decided in this new perspective to reinvestigate the structure of the colleteric glands and their secretion. *S. carcini* was chosen in order to check Delage's results towards modern technique and because this genus represents the typical text-book Rhizocephala.

Materials and methods

S. carcini parasitising the common shore crab *Carcinus maenas* (L., 1758) were collected in August and September 1993 from eel-traps in the western part of Limfjorden, Denmark at 4 to 5 m, or as by-catch from commercial vessels fishing mussels in the same area. Additional sacculinised crabs were collected in Gullmarsfjorden, Sweden, from ell-traps in August-October 1995 and kept and observed in running sea water. Specimens of another sacculinid, *Heterosaccus dollfusi* Boschma, 1960 infesting the swimming crab *Charybdis longicollis* Leene, 1938 were collected with a beam-trawl in the Mediterranean off Palmahim, Israel, at 35 m in May 1994.

Only mature, reproducing parasites were used. Most were dissected and the colleteric glands fixed immediately after collection. Samples for TEM were fixed in modified trialdehyde (Lake 1973), postfixed in osmium tetroxide for 1 to 2 hours, embedded in Epon, ultrathin-sectioned and contrasted in uranyl acetate and lead citrate. Ultra sections were made on a LKB ultramicrotome and examined in a JEOL electron microscope (JLM-100 SX). Other glands were fixed in Bouin fixative, embedded in paraffin and sectioned for light microscopy. In order to observe the oviposition and to obtain colleteric glands during and immediately after this event a technique developed by Delage (1884) was successfully applied (Fig. 1A). Crabs hosting *S. carcini* were strapped to a coarse nylon net by 0.5 mm metal wire. The host was placed with its dorsal side against the nylon net with special care taken to immobilise chelipeds and legs to prevent the host from damaging its parasite. Finally the abdomen was strapped down exposing the parasite to *in situ* observation under dissection microscope while keeping the host and parasite in sea water. Since oviposition usually occurs 48-72 hours after nauplii-release, host animals could be strapped down well before oviposition. Ca. 48 hours after release a window (5 × 5 mm) was cut through the mantle of the parasite enabling direct observation of one of the two colleteric glands and ovipores. Hosts suffered no loss of appetite and the individual parasites survived vivisection for more than 24 hours. Egg masses were preserved at various times during and after oviposition (Figs. 1B, C, D). Empty ovisacs were examined with interference contrast optics (Nomarski).

Results

I. Anatomy of the externae of *S. carcini* and *H. dollfusi*

The bag-like externa consists of a muscular mantle surrounding a visceral mass (Fig. 1). The visceral mass contains one voluminous ovary and paired oviducts with colleteric glands situated superficially in the lateral surfaces, one on each side (orientation explained in legend to Fig. 1). Paired receptacles normally housing dwarf males and paired ovipores all open into the mantle cavity, delimited by the mantle. The mantle cavity, which may contain a pair of branched egg masses, communicates with the sea through the mantle opening.

II. Anatomy of the colleteric glands in *S. carcini*

Seen from the outside the colleteric gland occupies an oval- or heart-shaped 2 × 3 mm area distinguished from the surrounding ovary by a lighter and clearer appearance in live as well as in pre-

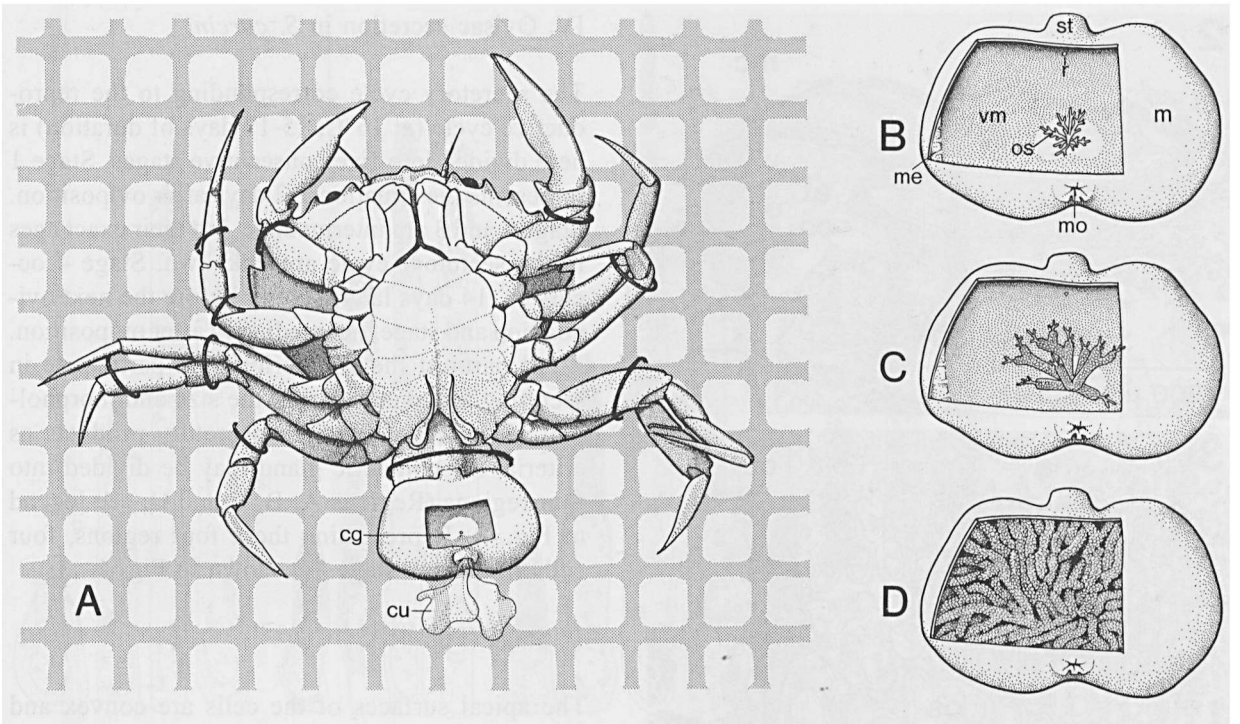


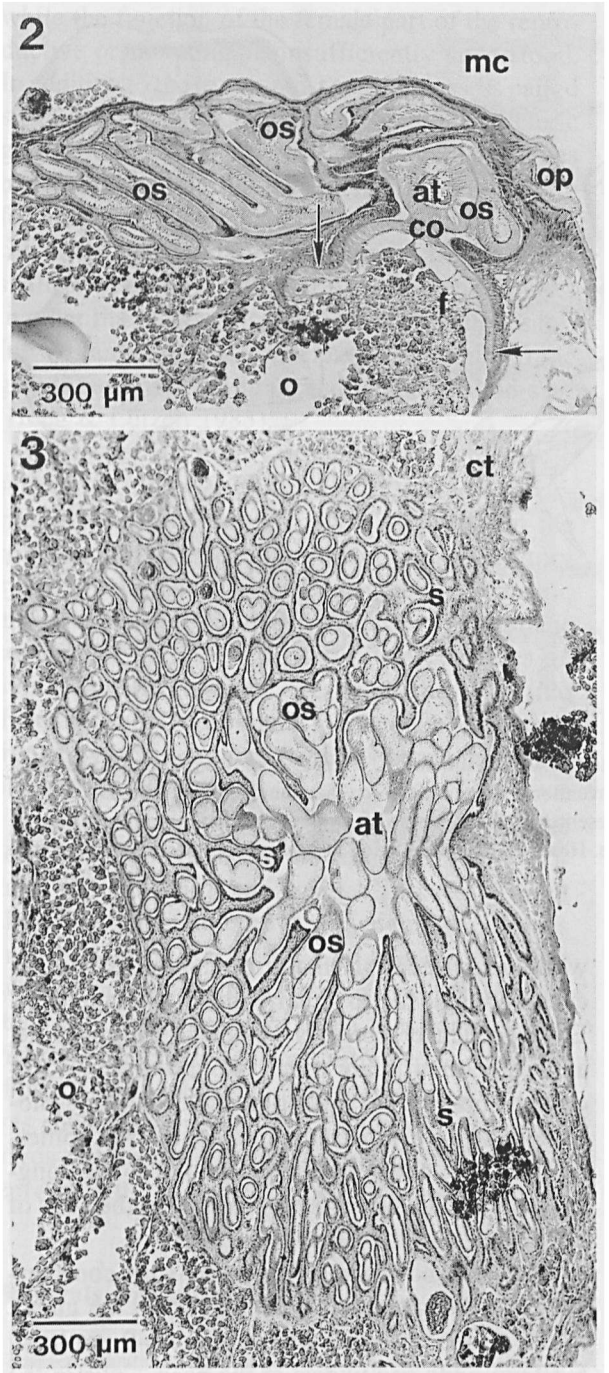
Fig. 1. Experimental set-up (modified from Delage, 1884). The immobilisation of the host, *C. maenas*, exposes the sac-like parasite, *S. carcini*. 1A-D; Steps in the oviposition (explained in text). The conventional orientation of the sacculinid externa: The stalk and the mantle opening occur posteriorly and anteriorly respectively. The mesentery connecting the visceral mass with the mantle, marks the dorsal region. Consequently, the externae are seen from the left side. However, this orientation lacks firm anatomical evidence (see Høeg & Lützen 1985). Drawn by Beth Beyerholm.

served material. The ovipore is a minute opening in the mid-anterior perimeter of the gland area. Each gland consists of three parts (Fig. 2); (i) a proximal funnel-formed part separated by a constriction from, (ii) a spherical atrium opening by way of the cup-shaped ovipore into the mantle cavity and (iii) six to eight repeatedly branching blind tubes issuing from the atrium. The branched tubes constitute the most prominent part of the colleteric gland (Fig. 3). The gland is everywhere surrounded by connective tissue. Diffuse bundles of muscles encircle the periphery of the glandular tubes laterally. Transversal muscles cross the visceral mass, but are all together absent from the glandular region.

III. Ultrastructure of the gland cells in *S. carcini*

A secretory epithelium forms the inner surface of the gland. The secretory cells have ellipsoid nuclei normally situated near the basal membranes while rough endoplasmatic reticulum (RER) occurs more apically together with Golgi complexes. Electron dense secretory vesicles, 0.2 µm wide, and mitochondria are usually found near the apical membrane. Cell junctions include belt desmosomes, septate junctions and hemidesmosomes. Microtubuli, chiefly running parallel to the long-axis of the cells, are most prominent in the cells of the atrium and the funnel.

The size and shape of the cells vary considerably according to their position within the gland. In the peripheral tubes the cells are 6-9 µm high, 5-10 µm wide and widest at the base. Cells in the wider part of the tubes and the atrium are columnar, 4-5 µm wide and reach a height of 30 µm. A maximum height of 50-60 µm is reached near the constriction and followed by a gradual decrease in the funnel to 15 µm near the ovary. The cell height tends to be lower at the end of each secretory cycle.



Figs. 2 & 3. S. carcini. Anatomy of the colleteric gland.
2. Section of the colleteric gland along the long-axis of the externa show ovipore, atrium, funnel formed part of oviduct and branching gland tubes. The gland contains one completed ovisac only attached in the funnel (arrows). *3.* Section parallel to the mantle cavity cuticle. The gland tubes constitute most of the colleteric gland.

IV. Ovisac secretion in *S. carcini*

The secretory cycle corresponding to the reproductive cycle (at 16°C, 15-17 days of duration) is here divided into five consecutive stages. Stage 1 represents the situation 1-2 days after oviposition. Stage 2 and 3 are intermediate, but their exact ages in the secretory cycle are unknown. Stage 4 occurs ca. 14 days later, shortly before the next oviposition and stage 5 immediately after oviposition.

All parts of the colleteric gland participate in secreting the ovisac. Using the size and morphology of the cells and their secretory products as criteria, the colleteric gland may be divided into four regions (Regions A, B, C and D; see legend to Fig. 4). Representing these four regions, four cells from each stage are shown in Fig. 4.

1st Stage (Fig. 4)

The apical surfaces of the cells are convex and equipped with microvilli. The microvilli are 2-3 µm long in region A and reach 6 µm in regions B, C and D. Mitochondria and RER are both relatively sparse and the latter is found throughout the cells. There is usually one Golgi complex per cell. Every cell contains secondary lysosomes and few secretory vesicles. Discharged contents of the vesicles refound as electron dense 0.1-0.3 µm granules among the microvilli (Fig. 5), mark the initiation of ovisac secretion.

2nd Stage (Fig. 4)

In all regions all cells have numerous microvilli, and the cells in regions C and D each have a 7.5 µm long apical extension. Compared to stage 1, the RER is now organised in relatively tight assemblies. Also more mitochondria and Golgi complexes (up to 4 per cell) are found (Fig. 8), and the number of secretory vesicles has increased to 5-15 per section in regions A and D, and 15-30 in region B and C. An electron dense unit has been secreted on the apex of each gland cell. All units are of a reticulated structure with hollow bases, which accommodate the convex apices of the cells. The principal sites of secretion seem to be the apical extensions and the main apical cell surfaces, but

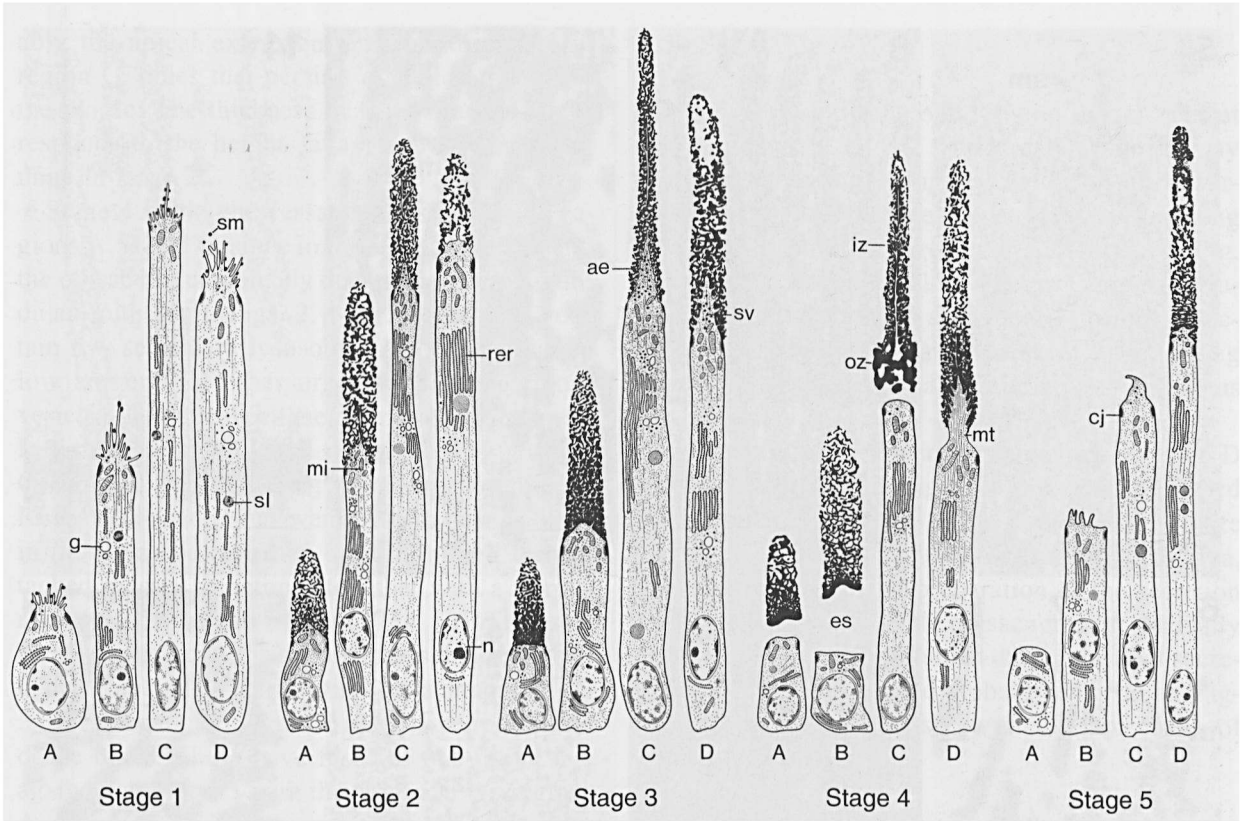


Fig. 4. S. carcini. Schematic representation of cells from five stages in the ovisac secretion (Stage 1-5). Each stage is illustrated by four cells respectively from the: A) ultimate glandular tubes; B) broadest glandular tubes; C) constriction between atrium and funnel and D) attachment area in the funnel. Drawn by Beth Beyerholm.

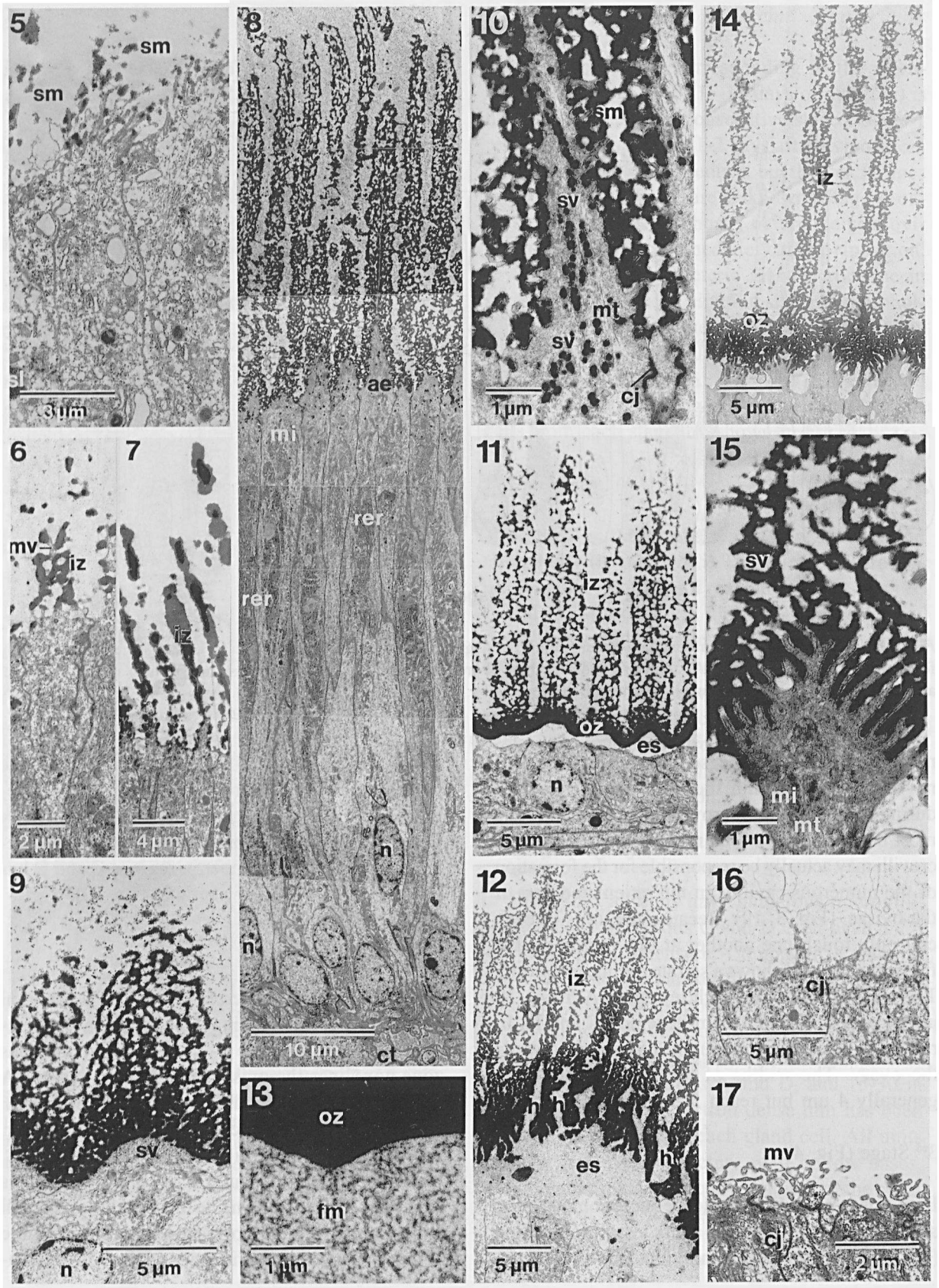
not the microvilli since secretory vesicles were never observed within the microvilli. However, during secretion the microvilli always occupy the most basal cavities of the reticulation and the microvilli may actually be responsible for the formation of the numerous cavities in the reticulated part of the ovisac. (Fig. 8, 19). Because the units of each cell only touch one another at the very base, the future ovisac wall shows a serrate outline in section. The units are 5-10 μm high in region A, reach 20 μm in region B, and 30 μm in region C and D. In region D the height gradually decreases towards the ovary. The widths of the secreted units are generally 4 μm but reach 5-8 μm in region A.

3rd Stage (Fig. 4)

In regions A and B more dense secretion has been added basally, resulting in units less porous bases (Fig. 9). Consequently, individual units increasingly merge into one continuous structure, the future ovisac. The porosity of the secretion regions C and D is unchanged. The number of secretory vesicles in regions A and B has decreased noticeably, whereas their number in regions C and D has increased to 30-50 per section (Fig. 10).

4rth Stage (Fig. 4)

The condensation of secretory material in the basal part of the ovisac has increased further. As a result, an electron dense 1-2 μm thick non-porous zone now lines the ovisac externally in regions A and B (Fig. 11). Now also dense, the outer zone in region C reaches a thickness of 5 μm. However, this zone is not impervious as in regions A and B, but penetrated by irregular 0.5-1.5 μm wide holes (Fig. 12). There appears to be one hole per cell and of a distribution corresponding to that of the apical extensions seen in stages 2 and 3. Presum-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ably the apical extensions imprint the holes in region C, holes that persists after detachment of the ovisac. The thickness of the ovisac wall corresponds to the heights attained by the secreted units in stage 2.

Stage 4 marks the cessation of secretion in regions A, B and C, since in these parts of the gland the ovisac has completely detached from the glandular epithelium (Figs. 2, 11, 12). The cells contain few secondary lysosomes and comparatively low amounts of other organelles and secretory vesicles. The apical cell membranes have flattened, lost their microvilli and occasionally ruptured. Cellular debris including membrane fragments, RER, mitochondria and even nuclei frequently occur in the space between the epithelium and the detached ovisac, sometimes together with a fibrous material of unknown function (Fig. 13). The presence of cellular debris demonstrates that some cell damage accompany the ovisac detachment.

In region D the secreted units have reticulated dense bases. These have fused to one lining that again is continuous with the ovisac of the regions A, B and C. The lining of region D remains in close contact with the secretory cells (Fig. 14) and now serves as the only area of attachment for the entire ovisac (Fig. 2). In region D, the cells are rich in mitochondria and especially microtubuli and their surfaces form fern or spruce-like structures (Fig. 15) that project into the secreted lining.

5th Stage (Fig. 4)

After oviposition the ovisac is no longer present in region A, B and C. The glandular lumen may appear collapsed having cells with flat apical surfaces (Fig. 16) or appear open with 2-4 μm long microvilli emerging from the cell surfaces (Fig. 17). The cells contain low amounts of most cellular elements including secondary lysosomes. Secondary lysosomes bear witness of an on-going intracellular digestion and reparation in stage 5 as well as in stage 1 and 4.

Part of the ovisac remains attached in region D after the ovipostion (Fig. 18). This remnant is shed before secretion starts anew liberating the entire epithelial surface, including the attachment area, to form microvilli in preparation of the secretion of a new ovisac. The old ovisac remnant gradually decays in the oviduct funnel during the next secretory cycle (Fig. 30) and probably ends up as fragments in the new ovisac with the next batch of eggs.

V. Ovisacs and oviposition in *S. carcini*

The transparent and colourless empty ovisac forms a ca. 2 mm wide branched bush (Fig. 22). The external surface of the empty ovisac is decorated with 4-8 μm wide polygons (Figs. 23, 24). Each polygon obviously corresponds to one secreted unit produced by one cell within the colleteric gland (Fig. 19).

←

Figs. 5-17. Ovisac secretion, TEM. Figs. 5 & 8-17; *S. carcini*. Figs. 6 & 7; *H. dollfusi*.

Fig. 5. Secreted material deposited between the microvilli (stage 1).

Fig. 6. Secreted material initially forms units on the central part of the apical cell surfaces (Stage intermediary of stages 1 and 2 from *S. carcini*).

Fig. 7. Same intermediate stage as in Fig. 6. Cells in the atrium secrete slender units.

Fig. 8. Near the constriction, the columnar cells have slender apical extensions (stage 2).

Fig. 9. The secreted units have acquired dense bases (stage 3).

Fig. 10. Numerous secretory vesicles within the apical part of cells in the atrium (stage 3).

Fig. 11. Gland tube with complete ovisac. The ovisac wall consists of an inner zone of reticulated units and an impervious outer zone. Apical membranes are flat (stage 4).

Fig. 12. At the constriction the detached ovisac has holes that enter and sometimes traverse the outer zone. Apical cell membranes are flat (stage 4). Note that the shape and size of the holes are similar to the apical extensions in Figs. 8 and 10.

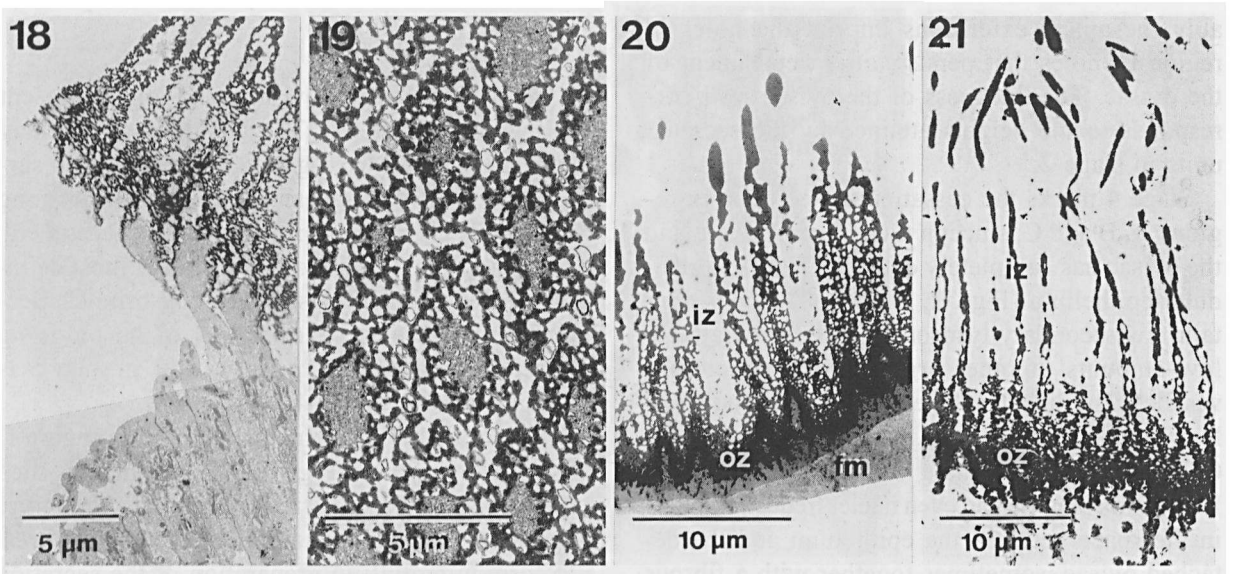
Fig. 13. Fibrous material between ovisac and epithelium (stage 4).

Fig. 14. Ovisac attached to epithelium in the funnel (stage 4).

Fig. 15. Apically fern shaped cells from the attachment area (stage 4).

Fig. 16. After oviposition some cells have flat apical membranes (stage 5).

Fig. 17. Same gland as Fig 16; Some cells have reformed microvilli (stage 5).



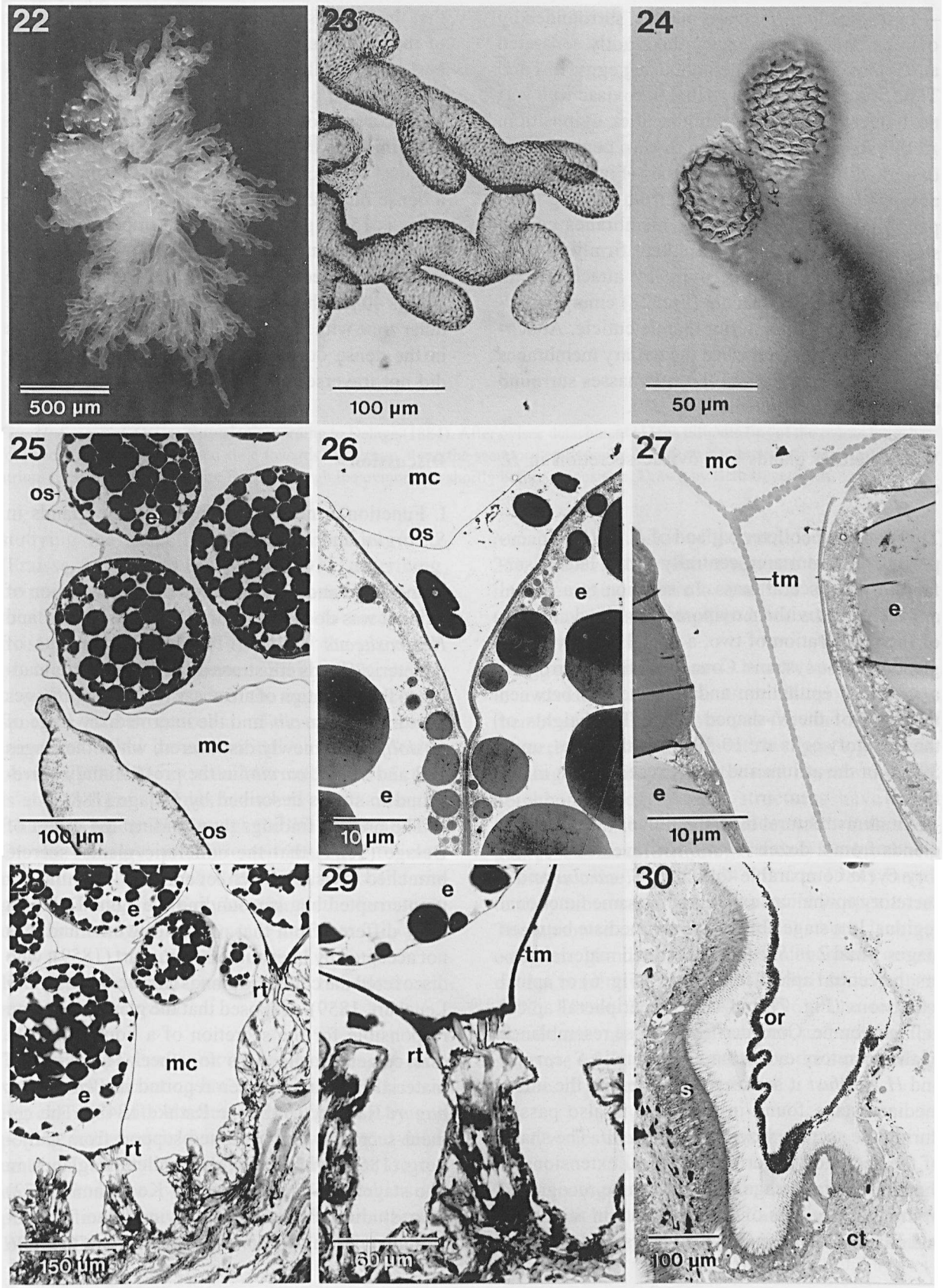
Figs. 18-21. TEM.
Fig. 18. S. carcini. Ovisac remnant in attachment area after oviposition (stage 5).
Fig. 19. S. carcini. Section parallel to the glandular surface; secreted units with polygonal outlining.
Fig. 20. H. dollfusi. Ovisac wall secreted in the gland tubes.
Fig. 21. H. dollfusi. Ovisac wall secreted near the constriction.

The mantle cavity always molts (indicated in Fig. 1A) one or two days before oviposition. Vigorous repeated contractions in the visceral mass precede and probably cause the extrusion of the branches of the ovisacs through the corresponding ovipores (Fig. 1B). Minutes later eggs pour into the basal part of each ovisac (Figs. 1C, 22); later as more eggs arrive, the eggs are forced into the ultimate branches (Fig. 1D). Hereby the ovisac is strongly distended yet it maintains the branched structure. As a result each egg mass surrounded by an ovisac, forms a branched bush, originating from the ovipore. Occasionally one ovisac is filled before eggs start entering the second. Oviposition lasts ca. 5 minutes.

After oviposition the egg mass occupies a volume of approximately 10 × 20 × 2 mm within the mantle cavity and the diameter of each branch has increased up to ten times. This increase in volume by ca. 2 orders of magnitude clearly demonstrates the elastic properties of the ovisac. During this stretching of the ovisac the reticulated units on the interior wall disappear (Fig. 25) and the thickness of the ovisac wall is reduced to 0.15 µm (Fig. 26).

→

Figs. 22-30. S. carcini. The ovisac.
Fig. 22. One branching ovisac. The basal part (seen to the left) distended by eggs.
Fig. 23. Tips of ovisac branches.
Fig. 24. Polygonal ovisac surface. Nomarski technique.
Fig. 25. Section of ovisac during oviposition. In the empty part of the ovisac, reticulated units of the inner zone are clearly seen. Another part of the ovisac, distended by eggs, has lost such structures.
Fig. 26. Section of egg mass, immediately after oviposition shows two adjacent eggs in the undivided compartment of the ovisac, TEM.
Fig. 27. Section of egg mass 48 hours after oviposition. Adjacent eggs appear separated; each egg clearly enclosed in a compartment formed by its own tertiary membrane. The ovisac is no longer discernible. TEM.
Fig. 28. Egg masses attached to retinacula in the mantle cavity cuticle.
Fig. 29. Detail from Fig. 28. Retinaculum partly surrounded by tertiary membrane of attached egg.
Fig. 30. The ovisac remnant from the attachment area lies detached in the gland lumina and a new secretory cycle has been entered. The apically pointed shape of the cells in the epithelium is typical of actively secreting cells.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In the newly formed egg masses, surrounded by ovisacs, individual eggs can be readily separated and removed from the neighbouring eggs. LM and TEM observations showed that the ovisac wall was no longer visible ca. 48 hours after oviposition. At this stage each egg lies in its own compartment delimited by tertiary membranes (Fig. 27). Eggs are no longer easily removed from the egg mass perhaps because the tertiary membranes cohere strongly. The egg masses are kept firmly in position within the mantle cavity by attachment to numerous spiny retinacula (Fig. 28) emerging everywhere from the interior mantle cuticle. Attachment is accomplished when the tertiary membranes in the peripheral parts of the egg masses surround the tips of the retinacula (Fig. 29).

VI. Colleteric glands and ovisac secretion in *H. dollfusi*

The V-shaped colleteric gland of *H. dollfusi* occupies a 3 × 4 mm area centrally in the lateral surface of the visceral mass. In addition to a funnel and an atrium with an ovipore each gland consists of the ramification of two, a dorsal and a ventral, glandular tube systems. Connective tissue surrounds a secretory epithelium and fills the gap between the arms of the V-shaped gland. The heights of the secretory cells are 10-30 µm in the tubes, up to 30 µm in the atrium and may exceed 50 µm in the funnel.

An ultrastructural investigation of the colleteric glands from a dozen *H. dollfusi* revealed a secretory cycle comparable to that of *S. carcini* and a secretory epithelium exhibiting the same functional regions. In a stage obviously intermediate between stages 1 and 2 in *S. carcini*, secreted material covers the central apical cell surface (Fig. 6) or apical extensions (Fig. 7), but not the peripheral apical cell membrane. Considering the close resemblance of the secretory events and products in *S. carcini* and *H. dollfusi* it seems reasonable that the intermediary stage found in the latter is also passed during the secretory cycle in *S. carcini*. The shape of the secretion covering the apical extensions in the intermediary stage (Fig. 7), can be recognised in the ultimate tops of secreted units in regions C and D in *H. dollfusi* (Fig. 21). This demonstrates that the initial secretion eventually forms the tops of the mature units, and, thus, that the innermost part of the wall is secreted first.

At the end of a secretory cycle each colleteric gland contains one ovisac attached to an area within the funnel. As in *S. carcini* the ovisac of *H. dollfusi* consists of reticulated secreted units, connected in a dense outer zone. The ovisac wall in the gland tubes is 15-30 µm thick with an impervious 2 µm thick dense outer zone (Fig. 20). Near the constriction between the atrium and the funnel, the wall is 40 µm thick including a 5-7 µm thick dense outer zone with an irregular surface (Fig. 21). Holes in the dense outer zone were observed, but they did not traverse the ovisac wall.

Discussion

I. Functional morphology of colleteric glands in *S. carcini* and *H. dollfusi*

Using TEM and light microscopy, the secretion of ovisacs was documented in *Sacculina carcini* and *Heterosaccus dollfusi*. The obtained stages of colleteric glands illustrate a secretory cycle leading to the formation of an ovisac (Figs. 5-18). Stages 2 and 5 of *S. carcini* and the intermediary stage of *H. dollfusi* are newly discovered, while the stages 1, 3 and 4 of *S. carcini* in the present study correspond to stages described by Delage (1884).

The present findings thus confirm the report of Delage (1884) that the colleteric glands secrete branched ovisacs, each eventually forming an uninterrupted bag surrounding the eggs. Delage's view differed from that of earlier works and was not accepted by later authors. Leuckart (1859), who discovered the colleteric glands (in *Sacculina inflata* Leuckart, 1859), proposed that the gland cells were responsible for the secretion of a liquid material that cemented the eggs together. This kind of material had already been reported in *Peltogaster paguri* Rathke, 1842 (see Rathke 1842). The cement-secretion theory gained support from Lilljeborg (1861) who discovered colleteric glands in two stages in *S. carcini* and by Kossmann (1872) who studied the anatomy of indo-pacific Sacculinidae. It was further advocated by Smith (1906

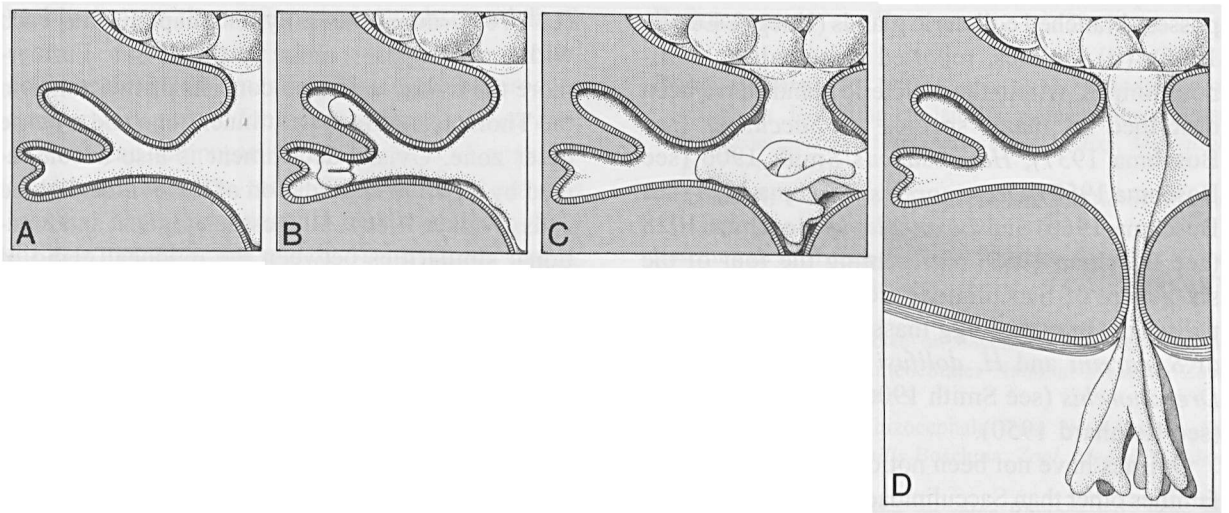

Fig. 31. S. carcini. Ovisac extrusion according to Delage (1884). After ovisac detachment (1) the ultimate tips of the ovisac branches fold back upon themselves and slide towards the atrium along the secretory epithelium (2). The ovisac branches concentrate in the atrium (3). Ovisac branches are forced through the ovipore (4) shortly before oviposition. Drawn by Beth Beyerholm.

studying the sacculinid *Drepanorchis neglecta* (Fraisse, 1877)) and by Ramult (1935, describing oviposition in *S. carcini*). Both favoured the cement-secretion theory and refused to accept the ovisac theory of Delage. Believing the glandular product to be a liquid cement Smith (1906, p. 26) claimed that each branch of the egg masses corresponded "to a diverticulum of the ovary", a statement not based on anatomical evidence. Smith obviously failed to explain how each batch of eggs is able to form complex branched egg masses, as it happens repeatedly through the life of sacculinids. On the contrary, prefabricated branched ovisacs molded on the branched ducts of the colleteric glands offer a simple explanation to the formation of branched egg masses.

II. Oviposition and formation of branched egg masses in *S. carcini*

The pre-ovipository molt of the mantle cavity provides the forthcoming batch of eggs with a brooding chamber with a reduced infection risk by expelling fouled cuticle, unfertilised eggs and dead embryos. Simultaneously, cuticular plugs are removed from the ovipores by the molt and it supplies the mantle cavity with new retinacula (Smith 1906). The reticulated inner part of the ovisac remains on the inside of the extruded ovisac (Fig. 25). The ovisac branches seem to be transferred into the atrium before oviposition. Fig. 31 illustrates how this is accomplished, according to Delage (1884).

Egg masses older than 48 hours consists of eggs in individual compartments produced by tertiary membranes. It seems obvious that the substance formerly believed to cement the eggs together is nothing but the fused tertiary membranes of neighbouring eggs. Such structures have been observed in many other Rhizocephala whether the glands are simple (Peltogastridae) or branched (Clistosaccidae) (Rathke 1842; Lützen 1981). Fused tertiary membranes are also present in species of Thompsoniidae and Polysaccidae, both lacking colleteric glands, proving the independent origin of the cementing structure (Lützen & Jespersen 1992; Lützen & Takahashi 1996). The tertiary membranes mechanically support and sustain the structure of the egg masses once the surrounding ovisac, if present, has vanished.

III. Ovisacs in other Rhizocephala

The existing data on sacculinid morphology suggests that branched ovisacs are produced in all Sacculinidae: (i) All known species of Sacculinidae

possess branched colleteric glands (Høeg & Lützen 1996). (ii) Ovisacs, reported as so-called 'chitinous linings' within the colleteric glands have been described in many species of *Sacculina* (see Boschma 1937), *Heterosaccus* Smith 1906 (see Boschma 1950), *Drepanorchis* Boschma 1927 (see Boschma 1960) and *Loxothylacus* Boschma 1928 (see Boschma 1955) representing the four of the six genera of Sacculinidae richest in species. Finally, (iii) branched egg masses resembling those of *S. carcini* and *H. dollfusi* are also formed in *Drepanorchis* (see Smith 1906) and *Loxothylacus* (see Reinhard 1950).

Ovisacs have not been noticed in rhizocephalan families other than Sacculinidae. However, the short duration (apparently less than 48 hours) of the ovisac combined with their extreme delicacy in *S. carcini*, could explain the failure to discover them in other families. A secretion in many respects similar to the ovisac in the colleteric gland of Sacculinidae is produced in the glands of *Peltogaster paguri* (Peltogasteridae) (unpublished). Secretion also occurs in the branched colleteric glands of *Clistosaccus paguri* Lilljeborg 1860 and *Sylon hippolytes* M. Sars 1870 (both Clistosaccidae) (see Boschma 1928). Interestingly, Clistosaccidae egg masses are branched, indicating the existence of a mechanism responsible for shaping the egg masses. It is therefore possible that ovisacs are produced in most, if not all, rhizocephalans with colleteric glands.

IV. Morphological comparison between Sacculinidae and Thoracica

The non-parasitic Thoracica is considered the sistergroup of Rhizocephala (Jensen et al. 1994; Spears et al. 1994). The thoracican oviducts are paired and include distal oviducal glands (Walker 1992).

1. Oviducal gland morphology

The oviducal glands in Thoracica secrete ovisacs in many ways similar to that of *S. carcini* and *H. dollfusi* although much simpler in shape. The thoracican ovisacs are also colourless, transparent and elastic (Nussbaum 1890; Walley 1965; Walker 1980). In the thoracican *Semibalanus balanoides* (L. 1767), numerous polygon-shaped units each with a central pore, make up the ovisac. Furthermore the ovisac wall also consists of two zones in the Thoracica; a flocculent inner zone and a dense outer zone. Ovisac detachment is also accompanied by a partial degradation of the oviducal gland cells (Walker 1980). All these anatomical and functional similarities between the oviducal gland of the Thoracica and the colleteric gland of the Sacculinidae strongly suggest that these glands are homologous.

2. Oviposition and fertilisation

After detachment, the ovisac of *S. balanoides* remains anchored to a limited proximal part of the oviduct until the oviposition comes to a hold. Arriving oocytes distend each ovisac through the ovipores into the mantle cavity, greatly reducing the ovisac wall thickness of the resulting egg mass (Walley 1965). In *S. carcini*, the ovisac must also stay attached during oviposition to allow the funnelling of eggs into the elastic ovisac.

In Thoracica, spermatozoans are deposited in the mantle cavity and subsequently pass through the ovisac wall by way of the pores and fertilise the oocytes inside the ovisac (Walker 1977; Klepal et al. 1977). Fertilisation has not been observed in Sacculinidae. The irregular holes in the ovisac of *S. carcini*, produced in a manner similar to the pores in the thoracican ovisac, may fulfill the same role. Since the spermatozoans of *S. carcini* are ca. 0.5 µm in diameter (Pochon-Masson 1971), the holes are sufficiently wide to allow passage.

3. Function of the ovisacs

The ovisacs of *S. balanoides* provide the initial control over the newly laid eggs allowing the eggs to make contact and cohere (Walley 1965) and form solidified egg masses (Walley et al. 1971). At this stage the solid egg masses in Thoracica are either attached to special structures, so-called ovigerous frena, or simply to large to be lost through the mantle opening (Walker 1992). The now unneeded ovisacs deteriorate. The estimated duration of the ovisac in a range of Thoracica is at least 10 days (Walley 1965; Barnes & Barnes 1977) and thereby consid-

erably longer than the two days observed in *S. carcini*. A premature loss of eggs in *S. carcini* could be inflicted by mantle contractions that produce a respiratory current for the same eggs. In *S. carcini*, ovisacs can temporarily prevent loss of eggs, allowing the eggs to touch, cohere and form egg masses. Then the retinacula take over by locking the egg masses to the cuticle of the mantle cavity. The short duration of the ovisacs in *S. carcini* may allow the developing brood to respire more efficiently.

Acknowledgements

I gratefully thank Dr. Jørgen Lützen for patient support and supervision. Thanks also to Beth Beyerholm for graphic work; to Else Schiøtt Hansen, Tina Olesen and Morten Schiøtt for technical assistance and to Dr. Henrik Glenner and Dr. Jens Høeg for stimulating discussions (all above are colleagues from the Department of Zoomorphology, Zoological Institute, University of Copenhagen, Denmark). Further thanks are due to Dr. Ib Svane (Kristineberg Marine Research Station, Sweden) for supervision, to Dr. Bella Galil (Israel Oceanographic & Limnological Research Ltd.) for help and hospitality during collection in the Mediterranean and to Mr. Frits Lange, skipper of 'Annuai', for participation in collection in Limfjorden, Denmark. This study was supported by the Nordic Academy for advanced study (NorFa grant No. 96.30.039-O).

Abbreviations

ae apical extension – at atrium – cg colleteric gland – cj cell junction – co constriction between atrium and funnel of oviduct – ct connective tissue – cu molted cuticle of the mantle cavity – e egg – es extracellular space between epithelium and ovisac – f funnel formed part of oviduct – fm fibrous material – g Golgi complex – h hole in ovisac wall – iz inner zone of ovisac wall – m mantle – mc mantle cavity – me mesentery – mi mitochondrium – mo mantle opening – mt microtubuli – mv microvilli – n nucleus – o ovary – op ovipore – or ovisac remnant – os ovisac – oz outer zone of ovisac wall – r opening of receptacle – rer rough endoplasmatic reticulum – rt retinaculum – s secretory epithelium – sl secondary lysosome – sm released secretory material – st stalk – sv secretory vesicle – tm tertiary membrane – vm visceral mass

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Received: 5 November 2001