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Microanatomy of the cement glands and other parts of Octo
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Octolasmis lowei ~~from~~ Callinectes danae from BuanaBara Bay, Brazil
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Introduction

Since Darwin (1851) described Octolasmis lowei from the gills of crustaceans collected off the Island of Madeira, other authors made purely morphological and systematic studies. Annandale (1909) studied this genus, relating the species with the depth at which they were found. Hiro (1937) in his monograph on Cirripedia of Japan made a valuable contribution by clearly defining the differences between the species of Octolasmis. Brian and Darteville (1954) described O. lowei from Luanda and included the morphology, some systematics, and the geographic distribution. Dinamani (1963-64) extended the studies on this genus with new data on variation and orientation of O. stella in lobster gills. Newman (1961-1967) published interesting observations in comparative anatomy of O. lowei and O. cymonini. However, there is little information on microanatomy and histology of O. lowei and an opportunity presented itself to study these in numerous specimens which were constantly found in crabs, Callinectes danae from Guanabara Bay.

Approximately 90% of crabs which were collected by the ~~series~~ first author showed ~~showed~~ numerous O. lowei on various parts of the gills and on the walls of the branchial cavity (gill chamber). The study was simplified by the presence of live specimens in different phases of growth. The number of commensals per host varied and reached up to 15 specimens per host. Even this number, however, did not seem to indicate morphological damage to the crab. It was noted that the highest numbers of commensals occurred in the older crabs. A similar occurrence of higher numbers of commensals in larger (older) crabs was observed by Kujawa (1971), with up to 57 specimens of ~~with~~ O. lowei and O. geryonophila on the gills and mouthparts of Geryon quinquedens from the African coast.

O. lowei is known to occur in the gills of different species of crabs (Monod, 1956).

Materials and Methods

The crabs, Callinectes danae, were collected on the beach of Zumby, Ilha do Governador in Guanabara Bay, at a depth of 2 to 3 meters.

The cephalothorax was removed, exposing the gills and the branchial cavity filled with the commensal barnacles (Fig. 1 and 2). The latter were removed and fixed immediately in different fixatives including Bouin's modification by Dubosq-Brasil, Holms, Carnoy, Susa, calcium 10% formalin, Gendre, and Flemming fluids. Serial paraffin sections were cut at 5 and 7 microns. Staining methods employed included Delafield hematoxylin with Chromotrop 2R, Heidenhain's iron alum hematoxylin with Congo red, and nuclear fast red with naphthol green.

The specimens to be studied in whole mounts, for determining the characteristics of the mouth parts and of the calcareous plates, were stained with Congo red-orang, cleared by Spalholz method and mounted in balsam. The drawings and diagrams were prepared with a camera lucida and Orthoplan Leitz microscope. The photomicrographs were taken with Xenon illumination and automatic Leitz camera.

Acknowledgements

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Microanatomy of the Capitulum

The capitulum is the main body part of the animal (Fig. 3, CP), protected by a transparent chitinous carapace, where one observes the calcareous plates, scutum, tergum and carina (SC, TR, CA). Due to the transparent nature of the carapace one may also see the position of the prostoma (PR), of the cirri (CR), penis (PN), anus (AN), ovigerous lamellae (OL), and the mantle cavity (MT).

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Tergum

The tergum (Fig. 4) is located in the area anterior to the middle aperture of the capitulum and may vary in shape among specimens found in the same host. In general, the tergum is long and bifurcated, with one of the branches shorter and narrower than the other. The variation in the shape of the tergum may be noted in the same animal (Fig. 5): one of the branches is broad and rounded at the tip, while the opposite one is narrow and pointy.

The growth lines of the tergum are very clear and it is easy to count them under polarized light (Fig. 6 and 7). These lines are semicircular. The calcification center is very distinct. It is located in the apical region between the two branches of the tergum (Fig. 7). In general, the area from where the calcification starts is ovoid rather than round, with well defined limits and showing fine granularity of the interior.

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Dinamani (1964, 1965) relates the variable position of the tergum to the growth process of the capitulum. In histological section through the tergum in the frontal longitudinal plane (Fig. 8), the growth lines may be well identified thanks to the dense deposits of calcium crystals.

Scutum

The scutum, seen in Fig. 3 (SC), is located laterally to the aperture of the capitulum and it extends from the posterior end of the tergum to approximately the middle part of the lateral chitinous parts of the capitulum.

Each part of the scutum consists of two branches united at the rostral angle. The scutum is narrow and elongated. Its calcification center is located at the point of junction of the two branches, in the most flexible part of the scutum. The growth lines are not as clear as those of the tergum but nevertheless distinct (Fig.9). Variability in the scutum shape has been discussed by Barnard (1924), Newman (1960 a,b, 1967) and Dinamani (1964,1965)

Carina

The carina is an unpaired structure located at the opposite end from the tergum and scutum (Fig. 3, CA). It is long and narrow, with convex margins. At the base, next to the capitulum, the carina bifurcates at an angle which is considered characteristic for O. lowei.

The mouth parts

The mouth parts of O. lowei are relatively simple. The labrum (Fig.10) is a chitinous prominent part located dorsally in the mouth area. It is etched at the margin with small teeth (TE), short and strong, which guard the entrance to the mouth cavity.

The paired mandibular palps (PL) are permanently folded over the tip of the labrum, thus protecting this unpaired mouth part from the usual particulate impurities found in the environmental suspension. The feelers or sensory hairs (SN) of the mandibular palps are long and thin, and they select the food, filtering it out as it enters the oral cavity. In Fig. 10 one may see the mandibles (MD) with its distinctive molar teeth.

moved ^{by} ~~The labrum is composed of~~ ^{two} thick muscular sheets, which take the form of a funnel, as seen at the right angle to their axis (Fig. 11). They insert at the middle protuberance of the internal labral wall, and the attachment is found in form of a strong apodema, located on the outside of the opposite external wall. The contraction of these muscles causes the depression of the labrum during feeding. As these muscles relax, the labrum returns to its normal position.

The mandibles are located just below the labrum (Fig. 12 and 13).

Although they are small and short, they are well chitinized and possess four teeth (TE). Of the latter, 3 are heavy and the fourth is rudimentary or reduced and covered by minute spines (SP). In the lower mandibular line, close to the rudimentary tooth, one may observe long and thin sensory hairs (SN).. In the main body of the mandibles one observes numerous teeth, short and strong (STE) ~~espalhados?~~, ~~shouldered by the molar teeth.~~

Each mandible of O. lowei possesses a large mandibular palp composed of a single segment (Fig. 12 and 13). These palps remain folded over, like those mentioned in connection with the labrum. The folding is facilitated by a long notch ~~in~~ in the middle wall of the palp as seen in Fig. 13 (FD).

The external borders of the labrum penetrate through this fissure ~~per~~ permitting a better fixation of the palps in the folded position. The long and thin sensory hairs (SN) of the palps are found ~~mostly~~ at the tip, but are more abundant in the middle region. This suggests a great sensitivity of this species in the selection of food at the level of the oral cavity.

The movements ~~of~~ of the mandibular palps are accomplished by the contraction of levator muscles (LPL) which permit the palps to be raised and separated from the labrum. When the ~~mandibular~~ muscles relax, the palps return to the folded position.

The strong adductor muscles of the mandibles are actively engaged in the trituration and ingestion of the food.

The first maxillae (Fig. 14 and 15) are located ^{directly} below the mandibles. They are relatively more fragile and possess a larger number of sensory hairs (bristles) on its entire chitinized surface (fig. 14, SN). On the ~~external border of the maxilla there is one short (comprido) and some smaller spines (SP).~~ external border of the maxilla there is one short (comprido) and some smaller spines (SP).

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The musculature of this ~~part of the~~ mouth is somewhat more complex as compared with that of the mandible. The adductor muscle (fig. 14, AMX) forms a thick sheath which starts from the thickened anterior extension of the maxilla and extends up to the wall of the buccal cavity. The sheath (feixe) of the flexor muscle (FMX) is much thinner than that of the adductor, and it runs from the posterior portion of the maxilla to the wall of the mouth cavity. Next to the insertion of the flexor muscle one also finds the depressor muscles (DMX). The levator muscles (LMX) are inserted in the terminal costal zone of the maxilla, where the cuticle is particularly thick.

The first maxillae of O. lowei exhibit an interesting mechanism that maintains their correct position during movement. It involves a long apodema (AP) which maintains the correct angle during motion by passing between two cubicular structures ~~forming~~ ^{that} a bifurcation in the posterior articulating corner of the maxilla. In sections of the maxilla (Fig. 16) one observes large numbers of sensory cells ~~(hairs/structures?)~~ scattered throughout.

The second maxillae (fig. 17 and 18) are smaller, more fragile and morphologically different from the first pair. They consist of two flat parts, not very chitinized ~~and~~ and lacking teeth, but entirely covered by long sensory bristles (fig. 17, SN). In the middle portion there is an unpaired structure, fine and long, corresponding to buccal cone (CB), the margin of which is serrated and covered with minute spines (SP). This part probably serves to triturate the food. The musculature of this mouth part is simple, with adductor muscles (ADM) inserting in the internal wall of the second maxilla and attaching to the strong reinforcement in the middle wall of the same. The abductor muscles (ABM) insert in the lateral basal cuticle of the second maxilla and attach directly on top of the insertion of the adductor muscle. The unpaired part between the two maxillary parts, the buccal cone (CB), has

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has a long muscle which is involved in the movement during mouth part action (MUC). The dilated (wider) part of the second maxilla ~~shows~~ reveals many sensory structures in histological sections.

~~The first~~ There are six pair of cirri, as in ~~other~~ crustaceans. The first pair of cirri (Fig. 20 and 21) is much smaller than the others and shows some striking differences in the microscopic structure. The coxopodite (fig. 20 CX) is elongated. It is followed by a narrow basopodite (BS) which supports the branched ~~exo-~~ and endopodite. The first pair of cirri act as accessory mouth parts. Both of their branches are profusely covered with sensory structures over their entire surface of the six segments.

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The flexor muscles of the first pair of cirri are arranged differently than those of the other cirri. For each segment of the ~~branch~~ branch, the flexor muscles (fig.20 FED and FEX) send out muscle fibers, and these insert at the end of each segment. Only in the segment before last the flexor muscles appear simple, and the last segment is devoid entirely of musculature.

In the basopodite (BS) we find the basal flexor muscle of the exo-and endopodite. In the coxopodite (fig.20 CX) we find the flexor muscles of the basopodite (FBS) in addition to the Extensor intermedium muscle (EKT), which extend from the basopodite to the lateral wall of the coxopodite. In sections at the level of the mouthparts and of the first pair of cirri (Fig.22) one sees the structure of the first and second maxillae, as well as of the first pair of cirri.

The remaining pairs of cirri (fig. 22) consist of 9 or 10 segments. They are ~~much~~ longer and thinner than the first pair. The distribution of the sensitive hairs is completely different than in the first pair of cirri. The long sensory bristles are found on the inner part of the branch (fig. 24, SN), five on each segment. On the opposite side there are tufts of bristles at the basal margin of each segment (TF). Only the last segment is covered

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entirely at the tip by long sensory structures. According to Pilsbry (1911) these are the so-called ctenopod cirri.

The flexor muscles (fig.24, MFL) of the branches of the exo- and endopodites are simple, without ramifications and extend up to the segments before last. In the basopodite one sees a flexor basal muscle of the cirrus (FBC) which is relatively small, and in addition the part of the extensor intermedius (EXT). The latter inserts in the prolonged apodema (AP) of the basal margin of the basopodite, on the inside of the coxopodite. In the coxopodite there are flexors of the basopodite (FBS). Parts of these muscles may be seen in the photograph (Fig.22) taken with polarized light.

The fifth pair of cirri has a ~~xxxxxx~~ basopodite well convex on the inner surface of the cirrus. The sixth pair of the cirri (fig.25, BS), besides this form of basopodite also has a pair of caudal appendices in the coxopodite (CX). These two long filaments (fig.25, APC). with reduced articulation, ~~xxxx~~ are covered at the ends by sensory hairs oriented in one direction. ~~xxxxxx~~ The structure of the tips suggests that these filaments are highly sensory in function, and that this is probably related to the function of the copulatory organ during the reproductive cycle of the animal.

Penis

The ~~form~~^{shape} of the penis in the genus Octolasmis was described by various authors as a diagnostic structure. Newman (1962) cites some differences between the penis structure in O. californiana and O. lowei, and the similarities between the first and O. ayonamini geryonophila. The shape of the penis, the distribution of the sensory structures and the presence or absence of the "laquet" ? (Lq) at the tip, represent the characteristics ~~of~~ described in papers of systematic nature.

The penis in O. lowei (fig.26) shows tuft-like arrangements of long ~~xx~~ thin bristles at the terminal part of the copulatory organ (SN). In this region there is also a deep fissure (notch), at the end of which one finds the characteristic "laquet" (Lq). The spines are short and thick (SP), and are arranged around

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the entire terminal part of the organ. Other sensory structures, longer and thin, are found on the entire surface of the penis (SN²).

The longitudinal musculature of the penis (MUL) extends to the third part of the organ, however the circular musculature (MUC) continues to the end of this structure except at the tip, where the region is entirely covered by sensory structures and spines.

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Microanatomy of the peduncle and of the cement glands

The peduncle of O. lowei (Fig.27) is cylindrical and transparent. In polarized light one may observe a dense muscular network and the ovaries, which are reddish when mature.

The ovarian follicles, containing oocytes in different stages of development, occupy almost one-third of the peduncle. Connective tissue fills the interfollicular spaces up to the longitudinal and circular muscles. In Fig. 27 some developing embryos may be seen within the ovigerous ~~sac~~ ^{sac}.

According to Dinamani (1964) and Arudpragasam (1967) the distribution of Octolasmis on the gills of crustaceans is determined by the adequate space and favorable water current. In Callinectes danae we were able to study the attachment of the peduncle of O. lowei in the gills and the cement glands of the species, not described to date by other authors.

The cement glands in O. lowei are located in the anterior part of the peduncle (Fig.27) and are especially numerous at the pedunculo-capitular zone. The glands form two lateral groups between the ovarian follicles and the muscular wall of the peduncle (Fig.28). The number of the glands in each group varies, but they are relatively small.

The cytological structure of these cement glands is simple as compared with the cement glands in Lepas anatifera (Lacombe and Liguori, 1969), but quite distinct from the cement glands in balanid barnacles (Lacombe, 1966, 1967 a, b, and 1970). In some cases, the chain of the cement glands reaches the wall of the ovarian follicle, however, even then the principal canal of the gland remains separate from the follicle, supported only by the connective tissue.

The cytoplasm of the cement gland cells is dense and homogeneous in the area where the secretion is formed (Fig.30). In the region which corresponds to the pole at which cement secretion accumulates, the secretion appears in the form of small vacuoles, oriented towards the principal canals.

Due to the orientation of the glandular chain, the principal canal is a simple long tube (Fig. 31), which extends down to the basal disc of the peduncle and carries the secretion which serves for the attachment of the animal to the gills of the host.

Further cytological details of these glands are shown in figs. 32 and 33. In mature cells the cytoplasm is granular, but not very dense and reveals minute vacuoles. The nucleus is ^opoor in chromatin, with most of the chromatin at the periphery close to the nuclear membrane. The nucleoli vary in number from 1 to 4, but are relatively large, well defined and dense as compared to the caryolymph. ^{nucleolar} Some/vacuoles show densely packed granules; some of these granules appear double (fig. 33) suggesting a stage anticipating nucleolar division.

The principal canals, through which the cement flows from the secretory cells, open to the outside close to the point of attachment of the cypris larva. In transverse sections (fig. 34) a dense cement layer may be seen surrounding this area.

The secretion spread through the entire base of the peduncle. In polarized light one sees clearly the interphase between the cement and the basal peduncular chitin in the zone of attachment. The cement infiltrates the fills of the decapod and provides firm attachment for O. lowei. At this point one observed some changes in the gills, which apparently affects to some extent the respiratory gas exchange. Should the high incidence of O. lowei cause the involvement of the major part of the gill surface, the crab would probably dies from suffocation. However, since the peduncular attachment are is actually minute and secretes a small amount of cement, Octolasmis are benign commensals of Callinectes and other crustaceans, without causing serious damage to the host. ^A Annandale (1906) and Broch (1924) suggested that Octolasmis living in the gills of crustaceans are symbionts, and that through the action of their cirri they eliminate the foreign organisms that penetrate into the gill chamber of the crustacean. (end p. 15 of manuscript)

Description of Figures

- Fig. 1-2 Octolasmis lowei on the gill filaments of Callinectes danae
- Fig. 3 Schematic drawing of O. lowei
 AN - anus, CA - carina, CP - capitulum, CR - cirrus, MT - mantle cavity, OL - ovigerous lamella, PD - peduncle, PN - penis, PR - prossoma, SC - scutum, TR - tergum
- Fig. 4-8 Tergum : Fig. 4 general view in polarized light
 Fig. 5 shape
 Fig. 6 growth lines
 Fig. 7 calcification center
 Fig. 8 longitudinal section (frontal) showing growth lines
- Fig. 9 Scutum, in polarized light
- Fig. 10 Labrum, schematic drawing
 FD - notch, ND - mandible, PL - mandibular palp, SN - sensory hair, TE - teeth
- Fig. 11 Section through a region including the labrum and the mandibular palps. Iron alum hematoxylin and Chromotrop 2R
- Fig. 12 Mandible, with teeth and mandibular palp
- Fig. 13 Mandible, schematic drawing of parts
 Ap - apodema, AMD - mandibular adductor muscle, FD - notch of the mandibular palp, LPL - adductor muscle of the mandibular palp, PL - mandibular palp, SN - sensory hair, SP - spines, STE - short teeth, TE - molar tooth
- Fig. 14 First maxilla, schematic drawing of parts
 AMX - adductor muscle, AP - apodema, DMX - depressor muscle, FMX - abductor muscle, FR - furca, LMX - levator muscle, SN - sensory hair, SP - spine

- Fig. 15 First maxilla, showing part of the musculature in polarized light
- Fig. 16 Longitudinal section at the level of the mandible and of the first maxilla. Ehrlich hematoxylin with Congo red and Orange G.
- Fig. 17 Second maxillae, schematic drawing of parts
 ABM - abductor muscle, ADM - adductor muscle, CB - buccal cone,
 SN - sensory hair, SP - spines
- Fig. 18 Second maxilla, general view
- Fig. 19. Section through the second maxilla. Delafield hematoxylin with Congo red.
- Fig. 20 First cirrus, schematic drawing including the musculature
 BS - basopodite, CX - coxopodite, EN - endopodite, EX - exopodite,
 EXT - extensor intermedius muscle, FBC - basal flexor muscle of the cirrus, FBS - basal flexor muscle of the basopodite, FED - flexor of the endopodite, FEX - flexor of the exopodite
- Fig. 21 First cirrus, general view showing the coxopodite and the basopodite
- Fig. 22 Longitudinal section through the first cirrus, with parts of the first and second maxillae. Nuclear fast red with naphthol green
- Fig. 23 Cirri, longitudinal section. Heidenhein iron hematoxylin with Congo red.
- Fig. 24 Second cirrus, schematic drawing of parts
 AP - apodema, BS - basopodite, CX - coxopodite, END - endopodite,
 EXO - exopodite, EXT - extensor intermedius muscle, FBC - basal flexor muscle of the cirrus, FBS - flexor muscle of the basopodite,
 MFL - flexor muscle of the endo- and exopodite, SN - sensory hair
- Fig. 25 Sixth cirrus, schematic drawing of parts
 APC - caudal appendix, BS - basopodite, CX - coxopodite,
 END - endopodite, EXO - exopodite, SN - sensory hair, TF - tuft
- Fig. 26 Penis parts
 FD - notch, LQ - "linquet" ?, MUC - circular muscle, MUL - longitudinal muscle, SN₁-long sensory hair, SN₂-short sensory hair, SP - spine

Fig. 27 Peduncle and a small portion of the ~~oviferous~~ **oviferous sac, section.**

Nuclear fast red and naphthol green

EM - embryos, GL - cement gland, MUC - circular muscles,

MUL - longitudinal muscles, OV - ovary, TC - connective tissue

Fig. 28 Cement glands, detail of fig. 27

GL - cement gland, MUL - longitudinal muscles, OV - ovary

Fig. 29 Cement gland in relation to the ovarian follicle, detail of fig. 27

Fig. 31 Cement glands and the principal canal (arrow). Section, Nuclear fast red and naphthol green.

Fig. 30 Cement glands, general view, detail of fig. 27

Fig. 32 Cement gland, higher magnification than in fig. 31. Note the nucleoli. Iron hematoxylin and Chromotrop 2R

Fig. 33 same as in Fig. 32

Fig. 34 Cement of O. lowei infiltrating the branchial lamellae (gill filaments) of Callinectes danae. Delafield hematoxylin and Congo red.

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