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STUDIES ON PARASITIC
GASTROPODS FROM ECHINODERMS

I.

ON THE STRUCTURE AND BIOLOGY OF
THE PARASITIC GASTROPOD
MEGADENUS CANTHARELLOIDES N. SP.,
WITH COMPARISONS
ON PARAMEGADENUS N. G.

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Synopsis

Two specimens, one female and one male, of a new species of Megadenus has been found in the sea cucumber *Stichopus chloronotus* from Aldabra, Indian Ocean. It is argued that the material was partially displaced when found and that the two gastropods inhabit the intestine of the host, which is pierced by the conspicuous proboscis that is further introduced into the host's body wall. The egg capsules are placed on the shell of the male and the larvae are released continuously into the host's intestine. The morphology of the larvae and the male specimen is described. A comparison with *M. holothuricola* and *M. voeltzkowi*, both parasitizing sea cucumbers, shows a close similarity, whereas it is concluded that *M. arrhynchus* Ivanov, from the sea star *Anthenoides rugolusus*, should be transferred to a new genus, *Paramegadenus* n. g.

Introduction

During phase V of the Royal Society Expedition to Aldabra, Indian Ocean, in 1968 two specimens of the parasitic gastropod *Megadenus* Rosén were taken from a sea cucumber by one of the authors (W. H.). According to Gruzov (1965) this genus occupies a central position in the phylogeny of the parasitic gastropods grouped in the family "Stiliferidae" and Ivanov (1952) has shown that *M. arrhynchus* Ivanov 1937, a parasite of sea stars, differs considerably from those species living in sea cucumbers. Owing to the sparsity of material there is little literature on the subject and save for the recent work by Ivanov (l. c.) the descriptions are relatively brief. It was therefore considered of interest to study the present material as extensively as possible, especially as the larval stages of the genus are unknown, and the biology of the parasite has been largely ignored.

The following four species have been referred to *Megadenus*:

M. holothuricola Rosén 1910, in the water lungs of *Ludwigothuria mexicana* (Ludwig) (family Holothuriidae) from Bahamas.

M. voeltzkowi Schepman & Nierstrasz 1913 in the oesophageal region of an unidentified sea cucumber (probably *Holothuria pardalis* Selenka) from Zanzibar, East Africa.

M. cysticola Koehler & Vaney 1925 living in cysts in the spines of the sea urchin *Dorocidaris tiara* Anderson (family Cidaridae) off Ceylon.

M. arrhynchus Ivanov 1937 from the dorsal surface of the sea star *Anthenoides rugulosus* Fisher (family Goniasteridae) from the Yellow Sea and, according to personal communication by David Pawson, Curator of Echinoderms, U.S. Nat. Mus., Washington, off the east coast of Mindoro in the Philippines (specimens figured by Fisher, 1919, pl. 85, fig. 4).

Furthermore, an observation by von Martens (1865) of a parasitic gastropod in the respiratory tree of an unidentified holothurian from Luzon, the Philippines, which he referred to the genus *Stilifer*, could according to his short description very well be a *Megadenus*.

During the first parts of the present work we have found it necessary to restrict any comparisons made between our material to those species living in sea cucumbers. In a final chapter conclusions are drawn as to the taxonomic and biological affinities between the holothurian-parasites and those described from other echinoderms.

The separation of the two species of *Megadenus* which have previously been described to parasitize holothurians is based on rather doubtful characters, *viz.* small and badly defined variations in shell shape and dimensions together with wide geographical separation. Although we recognize that studies on more extensive material when it becomes available may further define the division of the genus, we consider that our material represents a third species. It is distinguished from the other species parasitising holothurians by the unique and extraordinary development of a flange representing an expansion of the proboscis just distal to the pseudopallium. Owing to the likeness of this structure to a chanterelle, the new species is named *Megadenus cantharelloides* n. sp.

Material and Treatment

Two gastropods, one female and one male, were taken from a single specimen of the aspidochirote sea cucumber *Stichopus chloronotus* Brandt, collected at low water from the marine angiosperm beds adjacent to the settlement on Ile Picard, Aldabra, British Indian Ocean Territories, on October 16th 1968.

The cucumber was opened about 16 hours after collection when it had relaxed in stale water. It was cut longitudinally along the ventral surface and the intestine was found to have been autotomised at both ends and was lying free in the coelom. The significance of this treatment is discussed later.

The male was found attached by its proboscis to the interior body wall of inter-radius BC, approximately 1/7 of the distance from the anterior end. The proboscis penetrated about 3 mm into the wall but was withdrawn with only slight resistance. The shell of the male was entirely covered by a helmet of egg-mass substance, to which were attached by thin stalks the globular egg capsules. The female, with the entire proboscis detached, lay free in the coelom adjacent to the male. The animals were removed and fixed in alcoholic Bouin and consequently the calcareous material was lost. After removal of the egg capsules covering the male a perfect protoconch was found whereas this had been broken off in the female specimen.

The male was embedded in tissuemat and cut in 10 μ thick sections parallel to the columellar axis. The section series was stained in tetrachrome (Weigert's iron hematoxyline, Chlorantine fast red, orange G, and Alcian blue). A few were stained with Heidenhain's iron hematoxyline-eosin or Azan. The female was cleared in anise oil and the mantle cavity cut along its upper edge in order to disclose the organs within. A wax reconstruction based on the section series was made on a 75 \times scale to show the organization and extent of the main organ systems.

Observations were made on the empty egg capsules and those containing embryos and larvae. Full-grown larvae were studied in entire preparations cleared in anise oil; others were embedded in epon and complete 2 μ sections series prepared and stained in toluidine blue.

All material is being kept at the Institute of comparative anatomy, University of Copenhagen.

Morphology of the adult *Megadenus cantharelloides* n. sp.

External appearance. The female and male specimens are broadly similar in that they consist of a body containing several whorls and a proboscis which proximally expands into a large circular flange. Proximal to the flange is a bowl shaped fold of tissue, the pseudopallium of Rosén. Between the flange and the pseudopallium is a deep constriction and the female specimen has broken at this point. The sexes differ in the development of the pseudopallium and it is very much more extensive in the male than the female.

The shell is cyrtoconoid and globose with evenly rounded whorls that show a slight dip at the distinctly double suture lines and increase regularly in height. The shell is smooth and polished save for the presence of multitudinous fine striae, the imprint of which was reproduced on the inner surface of the egg mass helmet. The shell is delicate and transparent so that several organs, especially the dark coloured digestive glands, are clearly visible through it. The protoconch (to be fully described in a later section) forms a fragile muco of about 2 whorls succeeded by 5 adult whorls in the female and 4 in the male (including the body whorl). There is no operculum. The height and width of the shell in the female was 5.7×4.5 mm and although it was not measured in the male it was only slightly smaller. The colour of the live animal is a pale-cream except for the dark digestive glands.

The head is inconspicuous and carries two equally sized short and blunt tentacles, within the basal part of each an ocellus is located. The foot is broadly divisible into a large, fleshy foremost region of irregular shape, and a posterior semicircular fold inserted along part of the base of the visceral hump and extending to the left opening of the pallial cavity in the female (pl. I, fig. 2, pf), and even further forward in the male (text-fig. 2 B, pf). Whereas this fold is of moderate size in the female, in the male it extends upwards so as to cover the lower (left) parts of the mantle (and the shell). The anterior part of the foot is larger and more elaborate in the male than in the female; the anterior pedal gland has a slit-like opening on an extension of the anterior portion of the foot. This extension is longer in the male than in the female. On that part of the foot opposite the mantle opening, there are numerous wrinkles and deep crevices, into one of which the posterior pedal gland discharges.

In the male a comparatively short penis arises from a point to the right of the tentacles; it is entirely situated outside the pallial cavity

The pseudopallium completely surrounds the base of the proboscis as an extensive fold which is noticeably less developed in both sexes in the areas of the mantle opening. In the male the fold continues half way up the shell and in life must be able to entirely cover the shell and the attached egg capsules (text-fig. 1, ps). In the female where it is proportionally smaller it may possibly be reflected over the lower part of the shell.

The proboscis has the shape of a chanterelle owing to the modification of its proximal portion into a circular flange, the margin of which is thickened and some-

what irregular. In the male the flange envelops part of the pseudopallium. We consider the flange to be a permanent structure which has contracted only slightly during fixation. The distal end of the proboscis is deeply wrinkled and probably strongly contracted. The proboscis is darker coloured and is slightly swollen and heavily pigmented round the mouth opening. The length of the proboscis is 6 mm and the largest diameter of the flange about 7 mm.

The distal part of the proboscis is characteristically curved in relation to the flange in both specimens (text-fig. 1). At a distance of 2–3 mm from the mouth, i. e. at the point where the wrinkled foremost part of the proboscis begins to widen into the smoother surfaced flange, a distinct belt of mucous substance occurs which contains fine rust-brown granules. The belt is incomplete and more pronounced in the female. Its possible significance is discussed later.

As will be shown later, both specimens were wholly or partially displaced when found. In a following chapter we are going to demonstrate that they probably occupied a position similar to that shown in text-fig. 1: the anteriormost part of the proboscis is introduced into the host's body wall and the posterior part of that structure pierces the host's intestine in such a way that the body and pseudopallium lies within the intestine and the remaining part of the proboscis lies in the coelom.

Mantle cavity and pallial complex. The mantle cavity is fairly deep and not very spacious. The general arrangement of the organs associated with it is typical for the Mesogastropoda. Near its left entrance occurs a short osphradium, distinguishable on the sections by its tall, cylindrical and tightly packed cells containing yellowish-brown granules. Dorsal to the osphradium lies the monopectinate ctenidium which in the female carries about 45 triangular leaflets. The right half of the mantle skirt is thickened along most of its length, although separated by a longitudinal groove into two portions, on the right one of which the anus opens fairly far back. The hypobranchial gland occupies most of the thickness of the right half of the mantle roof, opening into the groove just mentioned and overlying the rectum as a single layer of mucous and mucoid cells. Posteriorly, near the floor of the mantle cavity, the gland thickens to overlie the anterior part of the kidney. Two different glandular areas may be distinguished: to the right are cells containing alcian blue-positive granules, and between them, small, distally placed supporting cells; to the left occur glandular cells, containing granules which remain unstained by the constituents of the tetrachrome.

Posteriorly the mantle cavity is much reduced in size, and protrudes back over the heart and kidney; the latter therefore opens on its floor through a simple aperture to the right of the ventricle. In the male the genital duct opens far back and continues as a ciliated gutter, stretching along the edge of the mantle skirt, protected along part of its length by a longitudinal fold.—What is probably the capsule gland extends as a thickening along the right side of the mantle cavity in the female, to open close to the mantle aperture.

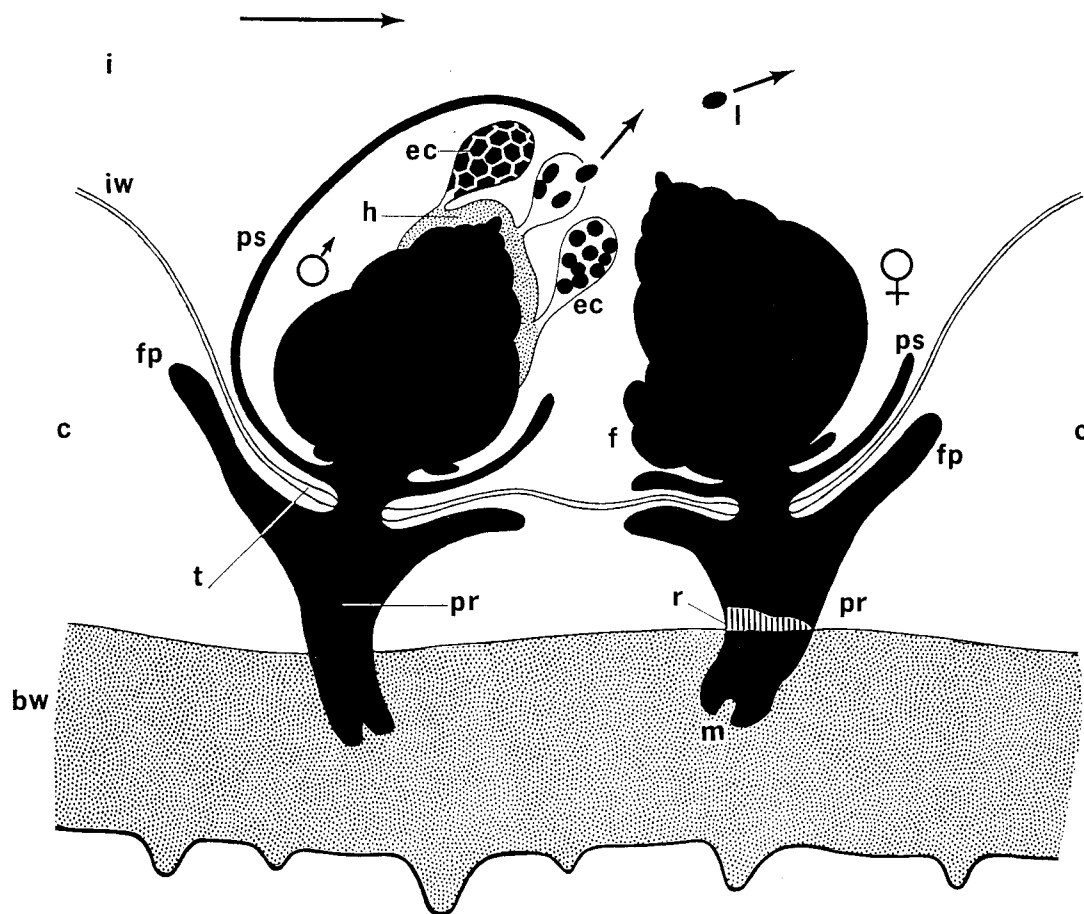


Fig. 1. Diagram showing the supposed position of female and male *Megadenus cantharelloides* n. sp. in the host. bw, body wall of host; c, coelom of host; ec, egg capsules in different stages of development; f, foot; fp, flange of proboscis; h, helmet of mucous substance covering the male shell; i, intestinal lumen of host; iw, intestinal wall of host; l, larvae released into the host intestine; pr, proboscis; ps, pseudopallium; r, ring of slime containing pigment found around the proboscis of female; t, thickening of intestinal tissue from host-parasite response. — The arrow shows the direction of movement of intestinal contents.

The skin and its glands. A ciliated columnar epithelium occurs on the margins of the deep groove marking the opening of the anterior pedal gland, and is present more diffusely on the extension of the foot onto which the gland opens. The epithelium lining all the numerous and complicated crevices of the foot, into one of which the posterior pedal glands opens, is also thickly ciliated. Both the interior and exterior aspects of the pseudopallium are ciliated. Otherwise the epidermis is mostly unciliated.

The basis of the external part of the pseudopallium forms a specialized site of mucus production. The epidermis in this region shows numerous ring-shaped folds with very tall columnar cells arising on connective tissue cores; it produces a secretion

which stains blue with alcian blue and a bright purple with Azan. The abundant supply of mucus formed here considering the morphological relations *in situ* is necessarily conveyed towards the rim of the pseudopallium. All over the pseudopallium unicellular mucous cells produce a similar secretion, which in addition to that just mentioned must aid in protecting the smooth parts from direct contact with the host's gut content (see p. 21).

The epithelium of the proboscis proper, including the flange is neither ciliate nor glandular; its tall columnar cells produce cuticular processes, which are especially long (up to one-third the length of the cells) on that part of the flange facing the pseudopallium. The proboscis, according to our interpretation, is wholly introduced into the body cavity and thus bathed in coelomic fluid, save for the tip which penetrates the body wall of the host.

The pseudopallial epithelium is also ciliated in *M. holothuricola*, and is secretory in both this species and *M. voeltzkowi*, although Rosén and Schepman & Nierstrasz were inclined to consider the inclusions found in the epithelial cells to be excretory products. A cuticular surface is present in the proximal part of the proboscis only in *M. holothuricola*, whereas Schepman & Nierstrasz picture (pl. 27, fig. 7) and describe in a most confusing way the epithelium of the distal part producing a secretion in the form of branched threads, which they claimed to attach to the host's organs, without specifying which organs.

The anterior pedal gland is too big to be accommodated wholly in the tissues of the foot and spreads into the haemocoel of the head as far as the nerve ring. Along its entire length runs a sagittal canal, crescent-shaped in transverse section. It is composed of columnar cells carrying exceptionally long cilia on the convex side, and cubical ciliated cells on the other, between which the gland cells discharge. These cells are arranged in nests within the subepithelial spaces and whereas some of them, especially those occupying a marginal and proximal location, appear to produce a mucous secretion, the staining properties of the majority suggests another production. The gland is very large and in the male occupies an estimated one-third of the portion of the foot in which it is embedded.

The posterior pedal gland is represented by an extremely narrow, unbranched and slightly coiled canal located within the substance of the foot between two major bundles of nerve fibres. Proximally it is lined with a cubical ciliated epithelium forming a non-glandular portion; distally near its opening to the exterior many, very large droplet-shaped gland cells discharge a mucous secretion into its lumen.

A conspicuous anterior pedal gland is also present in *M. holothuricola* and *voeltzkowi*, in the latter it even protrudes into the mantle cavity and is larger in the female than in the male. A posterior pedal gland is present in *M. holothuricola*, but could not be demonstrated with certainty in *M. voeltzkowi*.

Musculature and connective tissue. The columellar muscle is well-developed and most of the other muscles of the body, except those accompanying the

alimentary canal, are derivatives of it. Its fibres spread into the different part of the foot, the proboscis and the pseudopallium. In the foot and in the proboscis proper, the fibres criss-cross, whereas in the pseudopallium they establish themselves as distinct layers underlying the epithelium and radiating from its base towards the rim. The arrangement of the muscle fibres in the pseudopallium and the proboscis flange is noteworthy: In both structures a conspicuous, concentrated layer of ring muscles underlies the epithelium of the sides directed towards the body whorl, with additional ring fibres scattered in the connective tissues. Contraction of these fibres cause the pseudopallium to close around the shell; and the proboscis flange to be pressed tightly against the outer wall of the host's intestine, thus anchoring it more firmly. These fibres are antagonised in the pseudopallium by layers of radial muscles lying under the epithelium and are much more developed at the side directed away from the body; in the absence of radial muscle layers in the proboscis flange, a system of blood lacunae located there is believed to produce an antagonistic effect, possibly aided by a number of criss-crossing muscles.

It is considered significant that both radial and longitudinal muscle fibres are almost entirely absent in a ring-shaped region of the proboscis, namely at the constriction lying between the flange and the pseudopallium. The conspicuous longitudinal fibres of the columellar muscle running in an anterior direction at that point are abruptly replaced by a very dense connective tissue, except for a very few fibres lining the large blood sinus, surrounding the oesophagus. This discontinuity of the musculature starts exactly at a level where the secretory epithelium of the pseudopallium is replaced by the cuticular epithelium of the proboscis flange. The poor development of musculature here is explained by the supposition that this section is enveloped by the host's intestinal wall.

The connective tissue is vacuolated in the body whorl and vesicular blood spaces occur in the foot, especially in its hind portion. In the remainder of the foot and in the pseudopallium a dense connective tissue occurs whereas that of the proboscis is very loose, and there can be little doubt that this structure is very mobile and capable of rather sophisticated movements produced by the criss-crossing muscle fibres and shifting of the blood in its numerous sinuses. In both specimens the skin of the proboscis was much folded, indicating a state of contraction.

The alimentary canal and proboscis (text-fig. 2 A). The alimentary canal starts with what is probably a buccal cavity, a spacious and very much subdivided space, occupying the central and most of the anterior part of the proboscis. The wall of the foremost part of the buccal cavity is richly folded forming a large number of small and irregularly shaped spaces, lined with a cubical epithelium. Posteriorly the folds increase in size; the subdivisions become much larger and the cells tall and cylindrical with basal nuclei. All the cells lining the buccal cavity discharge a copious mucous secretion, which stains heavily with Alcian blue, into its lumen.

The folds of the buccal cavity are supported by connective tissue cores. Anteriorly their tissue is mainly occupied by true connective tissue, but posteriorly this is gradually replaced by muscle fibres, some of which are in continuity with the sheath of longitudinal muscles enveloping the bulbous pharynx. Two small and two large retractor muscles accompany the oesophagus and originate among these muscle fibres. A very conspicuous fold containing only a blood sinus, encircles the mid-portion of the buccal cavity.

An elongate blood sinus containing the pharynx, the oesophagus and the proboscis retractors commences at the posterior margin of the buccal cavity and continues through the stalk separating the true proboscis from the pseudopallium and into the head. The pharynx is bell-shaped with a narrow, slit-like central canal and is about 700μ in diameter and 800μ in length; its anterior end projects a little into a particular, cup-shaped region of the buccal cavity. Its structure and possible function will be considered later. On entering the pharynx the epithelium lining the gut becomes very low and squamous. Otherwise its walls consist of radial muscle cells fastened to the under side of the epithelium internally and to a thin sheet of longitudinal muscle fibres and connective tissue externally. These dilator muscles are antagonised by ring muscle fibres, situated mainly on the periphery of the bulb and at regular intervals in the spaces between the radial fibres. The whole structure would form an effective pump to convey fluid from the cup-shaped part of the buccal cavity into the oesophagus.

The oesophagus originates from the inner end of the pharyngeal pump. It is a tube of uniform diameter (ab. 150μ) which coils several times on the way to the visceral hump and forms a particularly close tangle just before reaching the stomach. The constituents of the oesophageal wall are similar throughout its length. It is lined with a columnar epithelium enclosed within a sheath of longitudinal and, to the outside, of circular muscle fibres. The foremost part of the oesophagus is longitudinally folded but further back it becomes smooth-walled. The posterior part of the oesophagus contains an epithelium of cylindrical cells with very many minute granules included in their distal portions.

The small stomach, into which the oesophagus opens receives one major and one minor opening from the digestive gland, the larger draining most of that organ. The intestine arises from the extreme posterior end of the stomach. Typical stomach cells occur almost solely on the dorsal side, between the openings of the oesophagus and intestine; their structure was very badly preserved but they are distinguished

Fig. 2 A and B. Diagram showing the structure of the alimentary canal, peripheral nervous system, anterior pedal gland, and vascular system in male of *Megadenus cantharelloides* n. sp. a, anus; aa, anterior aorta; af, anterior part of foot; apg, anterior pedal gland; au, auricle; b, buccal ganglion; bc, buccal cavity; dg, digestive gland; eb, efferent branchial vessel; fp, flange of proboscis; g, gills; i, intestine; kv, accessory kidney vein; l, left tentacle; lo, left ocellus; m, mouth; mc, mantle cavity; oe, oesophagus; p, penis with open seminal groove; pa, posterior aorta; pe, pericardial cavity; pf, posterior part of foot; ph, pharynx; ps, pseudopallium; rc, right cerebral ganglion; rt, right tentacle; rv, renal vein; s, stomach; sp, supra-oesophageal ganglion; v, visceral ganglion; ve, ventricle; vv, visceral vein; y, opening of anterior pedal gland. Scale represents 2 mm.

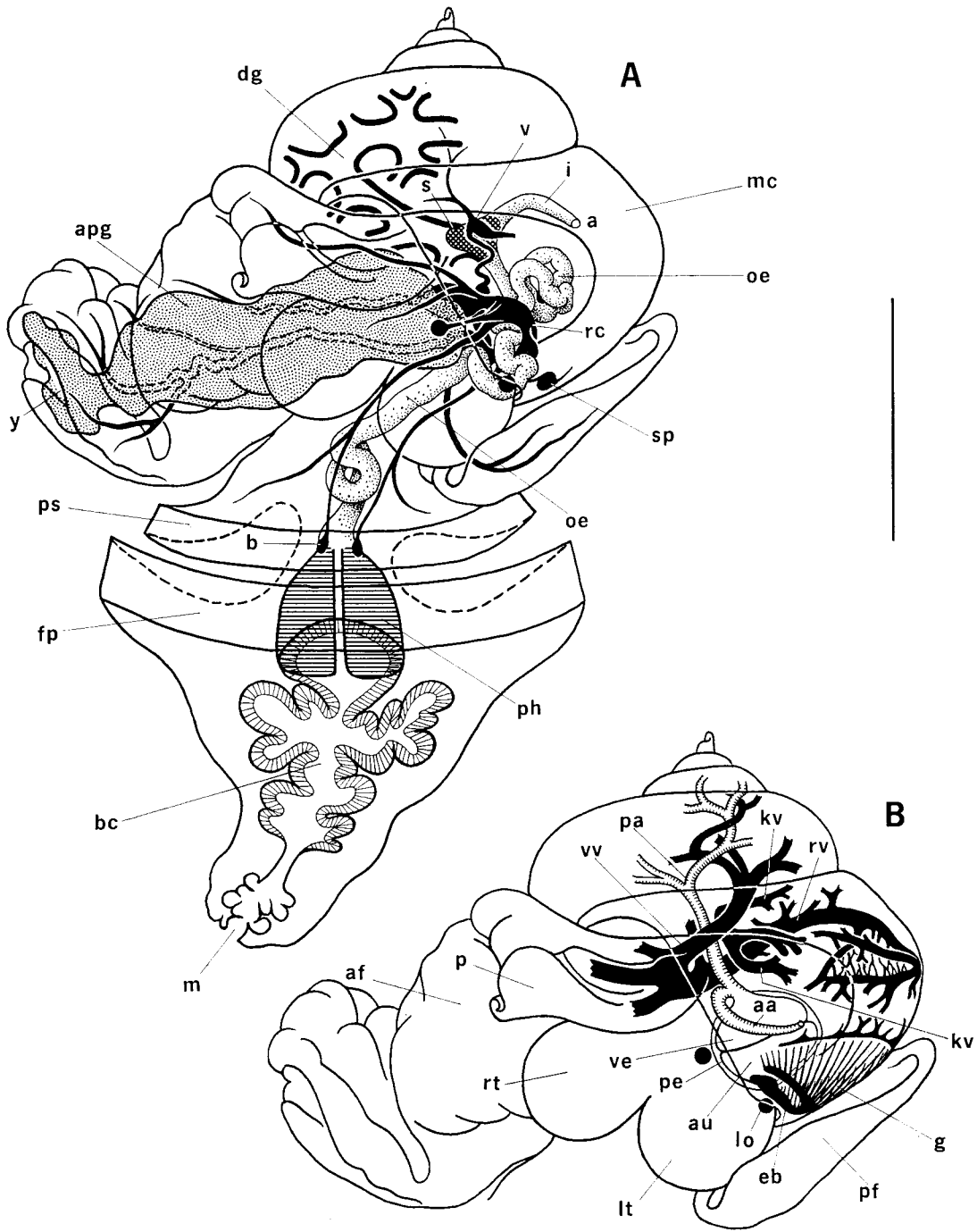


Fig. 2 A and B.

by being very tall, vacuolated and probably secretory and, in contrast to those of the oesophagus, provided with very long cilia.

The tubules of the digestive gland are aggregated in distinct groups, most of which discharge into a central tube running up the visceral hump and entering the stomach as the most prominent of the gland's two ducts. The digestive gland and the gonad constitute the major part of the visceral mass.

The posterior part of the stomach is prolonged and penetrates the kidney within which it merges with the intestine. The latter is flung into a U-bend and soon leaves the kidney to open into the anus. Its epithelium is ciliated throughout, and the walls show considerable longitudinal folding.

This description of the alimentary canal agrees well with that of *M. holothuricola* and *voeltzkowi*, except that the oesophagus in these species are not as coiled, and are accordingly shorter. Cilia were not described in the stomach cells of *M. voeltzkowi*; Rosén (1910) considers the stomach to have been completely reduced and its lumen to have been replaced by an enlargement of the combined openings of the two digestive gland ducts into the alimentary canal. Another point of difference is that four proboscis retractors exist in the present species, and only two in *M. voeltzkowi*; their presence is not mentioned in *M. holothuricola*, but only two are distinguishable in Rosén's figures (pl. 1, fig. 3; pl. 2, fig. 9).

Rosén described in a confusing manner, which was consequently misunderstood by Schepman & Nierstrasz, the structure of the cup-shaped section of the buccal cavity that is closely associated with the pharynx. Its shape in our species is shown in pl. IV, fig. 7. It is separated from the buccal cavity proper by a narrow duct, the lumen of which can be constricted and dilated respectively by the complex of muscle fibres surrounding it and those connecting its wall with the remainder of the buccal cavity. Dorsally and ventrally the cavity extends posteriorly along the sides of the muscular pharynx, which opens into its centre. Its function is connected with the pumping activity of the pharynx. When the connexion to the buccal cavity is dilated, fluid is sucked into it. With the constriction of this opening, a contraction of the radial fibres of the pharynx will dilate the pharyngeal opening and in addition probably exercise a pressure on the dorsal and ventral extensions resulting in the fluid from the antechamber being pumped through the pharynx and into the oesophagus. If the pharynx opened directly into the buccal cavity its pumping mechanism would be difficult to explain.—A similar structure is present in *M. holothuricola*, in which it seems to occupy the same position in relation to the pharynx and buccal cavity as in our species, although it is displaced ventrally. It is not reported from *M. voeltzkowi*, although an equivalent structure is said to be present in the form of a diverticulum arising from the lumen of the pharynx.

The nervous system and sense organs (text-fig. 3 A & B). The ganglia of the nervous system are mainly concentrated into a ring around the oesophagus and in which seven ganglia may be distinguished. In addition to the constituents of

this ring two buccal ganglia, a visceral ganglion and a supra-oesophageal ganglion may be observed. Both cerebral ganglia (lc and rc) are oblong bodies connected by a short commissure. From the anterior part of each cerebral ganglion arises a stout nerve which passes along the oesophagus to the two buccal ganglia (b) and which are mutually connected through a ring-shaped nerve, encircling the posteriormost part of the pharynx bulb. There are no accessory buccal ganglia. Ventro-anteriorly the cerebral ganglia connect to the larger pedal ganglia (lp and rp), which similarly so closely approximate that the commissure is hardly distinguishable. On the posterior-dorsal faces of the pedal ganglia lie the statocysts (st), containing a single calcareous statolith. Apart from some minor nerves one prominent nerve leaves the right pedal ganglion and runs along the right side of the anterior pedal gland supplying the right part of the anterior portion of the foot; from the left pedal ganglion arise three conspicuous nerves, the largest of which passes ventral to the columellar muscle to end as a thick and very much twisted bundle, ventral to the anterior pedal gland. A branch from one of the other nerves supplies the posterior folded part of the foot.

From the right cerebral ganglion arises a second connective to a small spherical (right) pleural ganglion (rpl) which connects through a prominent, curved and short nerve to a larger oval ganglion situated just beneath the osphradium, no doubt representing the supra-oesophageal ganglion (sp). No osphradial ganglion could be found.—The left pleural ganglion (lpl) is confluent with the cerebral ganglion; on its left side a stout nerve arises and runs backwards in the body; to the right it is almost confluent with the very elongate sub-oesophageal ganglion (sb); the latter connects with the single visceral ganglion (v) and is located between the stomach and the opening of the vas deferens.

The pseudopallium is entirely supplied by branches of the cerebro-buccal nerves, thus proving its cephalic origin.

The observations on the nervous system of *M. holothuricola* and *voeltzkowi* are few and inadequate. They do, however, generally agree with this account of *M. cantharelloides*.

The two ocelli are embedded in the connective tissue of the tentacle basis, the least distance from the centre of the lens to the tentacle surface being ab. 100 μ . However, they are orientated so that the distance to that part of the surface from which light may reach the eye is three times this length. The eyes are oval in shape, the right being somewhat larger than the left, at least in the male, on which accurate measurements could be taken; in that sex the maximum width is 65 μ and 90 μ respectively, the length 92 μ and ab. 120 μ respectively, and the diameter of the perfectly spherical lens 42 μ and 60 μ respectively. The epithelium lining the optical vesicle forms a translucent cornea in front of the lens and a normal retina, in which occur pigmented and unpigmented cells. The retina is separated from the lens and the space is occupied by what is probably a small quantity of vitreous.

Rosén and Schepman & Nierstrasz point out that the distal part of the retinal cells are entirely pigmented in *M. holothuricola* and *voeltzkowi*, as in our species.

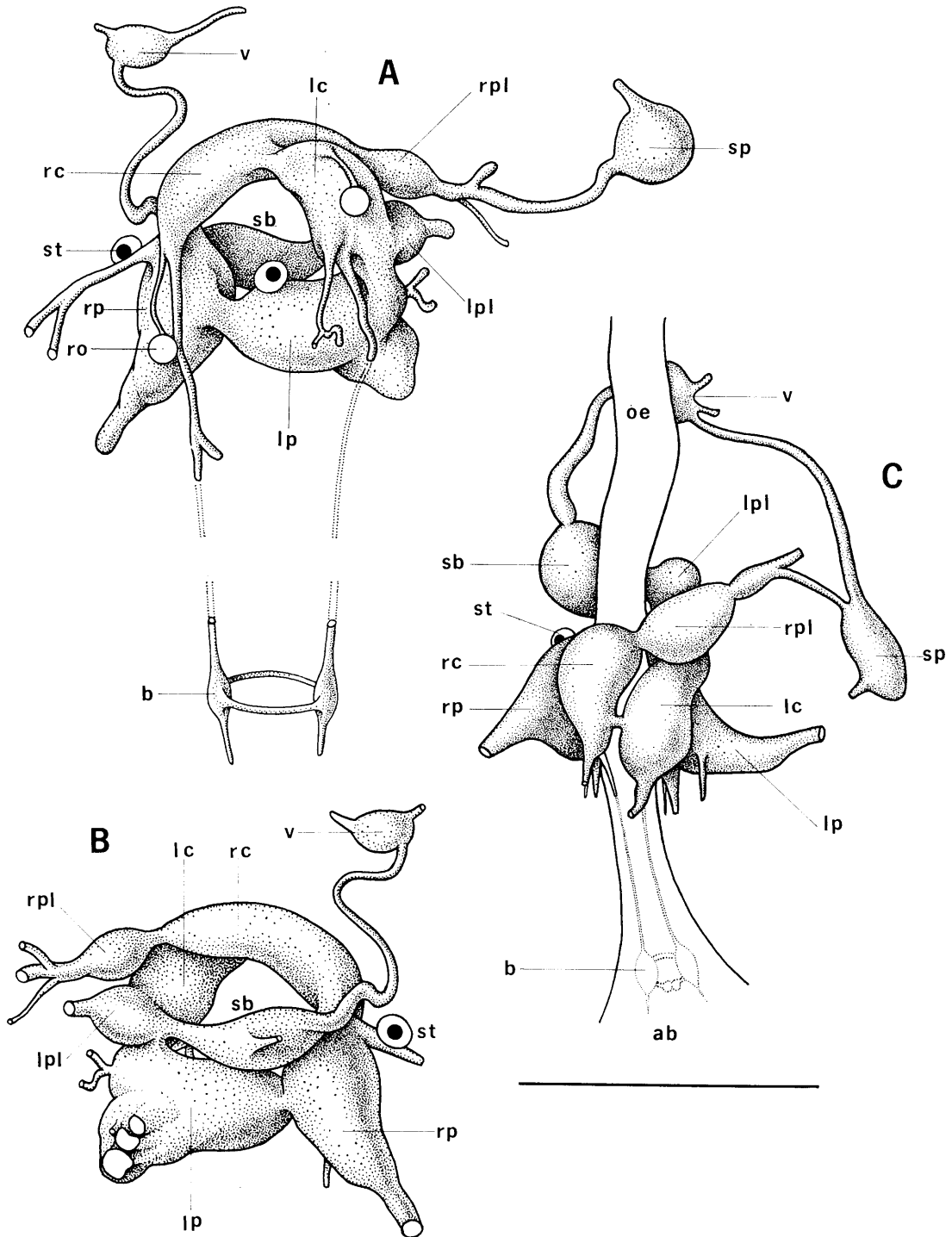


Fig. 3 A and B.

They further claim that no cornea exists, and the lens to be completely surrounded by a pigmented retina; this Rosén considers a sign of reduction and that the eyes do not function in *Megadenus*. As pl. 4, fig. 6 shows, the presence of a translucent cornea is indisputable in our species and is present in both the eyes of which the histological structure has been examined.

The arrangement of the eyes within the tentacles is similar in our form, in *M. holothuricola* and in the male of *M. voeltzkowi*. In the female of the latter species, however, only one tentacle exists, and probably the left, and the right eye has migrated deeper into the tissue than the left.

Vascular system and kidney (text-fig. 2 B). The heart lies in the pericardial cavity at the attachment of the mantle skirt on the left side, immediately proximal to the kidney. The single auricle lies to the left and slightly anterior to the ventricle, from which the anterior and posterior aorta emerge from a short common stem. The anterior aorta (pl. III, fig. 5, aa) runs forward along the left of the stomach and oesophagus and disappears, probably opening into a sinus surrounding the tangle formed by the posterior oesophagus. The posterior aorta ascends the visceral hump, on the concave side of the spiral which rests on the surface of the gonad and opens into the haemocoelic space interpenetrating the lobules of the digestive gland and gonad. This drains into an extremely large vessel running along the inner, convex side of the visceral hump. It is closely wedged between the two ducts of the digestive gland and then turns sharply to enter the kidney as the afferent renal vein; despite the latter giving off a great number of minute vessels on passage through the kidney, it emerges as a tube of almost unaltered diameter to penetrate the posterior part of the hypobranchial gland; here it diminishes in size giving rise to a series of vessels which in the roof of the mantle cavity pass to the left between the lobes of the hypobranchial gland where they ramify and anastomose among themselves. This network is visible from outside (pl. I, fig. 1, and text-fig. 2 B) to the right of the posterior ctenidial region, well in front of the kidney. From it arise the different channels, through which the blood moves to gain the ctenidial leaflets. A prominent efferent branchial vessel (pl. III, fig. 5, eb) returns the blood to the auricle.

A number of sinuses occur, some of which have been mentioned earlier. The most conspicuous of these is a cephalo-proboscideal sinus which extends from in front of the nerve ring to the buccal cavity. Posteriorly it communicates with a spacious sinus which separates the nerve ring and that part of the oesophagus lying behind it from the floor of the mantle cavity. This sinus raises the floor of the mantle cavity so that a long and broad ridge projects into the mantle cavity.

Fig. 3 A and B. Central nervous system of *Megadenus cantharelloides* n. sp. in dorsal and ventral view. C, same of *Paramegadenus arrhynchus* n. g. (from Ivanov, 1952, fig. 26 A). ab, accessory buccal ganglion; b, buccal ganglion; lc, left cerebral ganglion; lp, left pedal ganglion; lpl, left pleural ganglion; oe, oesophagus; rc, right cerebral ganglion; ro, right ocellus; rp, right pedal ganglion; rpl, right pleural ganglion; sb, sub-oesophageal ganglion; sp, supra-oesophageal ganglion; st, statolith; v, visceral ganglion. Scales represent 500 μ (A and B) and 1 mm (C).

No comparisons can be made with the vascular system in *M. holothuricola* and *voeltzkowi*, since its structure was not investigated in these species.

The kidney is a capacious sac, clearly visible from outside (pl. I, fig. 1, k). It opens into the bottom of the pallial cavity through a simple aperture in which cilia could not be detected and in which sphincters or dilators are obviously absent. The intestine forms a short loop through it. There is no nephridial gland and no renopericardial canal. The kidney is built on a conventional plan: The lumen is finely subdivided by numbers of anastomosing trabeculae, which arise exclusively from its external wall. These often widen internally to form a meshwork of irregularly shaped sinusoids. One cell type dominates, *viz.* a spherical one with a basal nucleus, a minute amount of protoplasm and a vast distal vacuole. In addition, there are scarce cup-shaped cells without vacuoles, with a distal nucleus and a stalk-like connection to the basement membrane; both types were distinguished by Rosén in *M. holothuricola*, the kidney of which is generally very similar to that of our species.

The vascularization of the kidney in *M. cantharelloides* is peculiar in that the afferent vein does not break down completely into renal capillaries, but continues through the kidney as a prominent vessel. We have been unable to detect how the blood is collected from the organ and returned to the auricle. A vein leaves either side of the superficial region of the kidney and fuse with one arriving from the intestinal region, but whether these ultimately drain into the heart, and by what course, could not be determined.

The reproductive system. Since the female specimen was left unsectioned we are unable to give any details about the female genital apparatus.—The testis occupies almost all the spaces left in the visceral hump by the digestive glands and blood lacunae, and is displaced preferably to the right sides of the whorls. It consists of numerous lobes, which finally discharge into the vas deferens (text-fig. 4); the latter is fairly long and coiled into a close tangle; its walls are exceedingly flat and covered by an unciliated squamous epithelium; being narrow in its proximal section, the tube gradually widens and terminates in a much enlarged swelling. Tightly packed sperm is stored throughout the vas deferens. It opens via a narrow canal into a short cul-de sac. The cubical epithelium of this blind portion is non-glandular, but the cells of the outer part produce a mucous secretion and become cylindrical. This accessory gland, which probably represents the prostate, opens into the pallial cavity and continues as an open seminal groove (or pallial vas deferens), and still contains a few gland cells in its floor for some distance. The pallial vas deferens runs along the edge of the mantle skirt turning sharply to the left on emerging from the mantle aperture and passes, as a broad, slight depression along the right side of the head to the basis of the penis, where it again becomes grooved and heavily ciliated. It proceeds to the very tip of the penis, which is about 1.2 mm long, and 0.4 mm across, and shows a tendency to spiral. The penis contains a dense connective tissue with muscle fibres

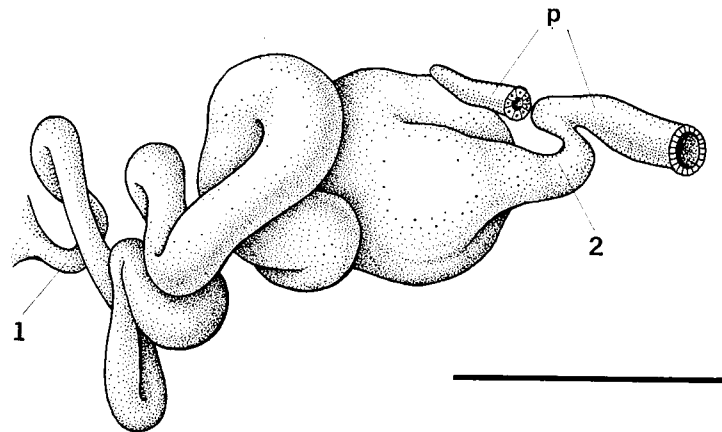


Fig. 4. Vas deferens and accessory gland in the male *Megadenus cantharelloides* n. sp. The different parts of the coils are drawn somewhat apart. 1 and 2, distal and proximal portion of vas deferens; p, prostate. Scale represents 400 μ .

and vesicular blood sinuses and is certainly capable of extension, since its surface was found to be greatly folded.

The male apparatus is unknown in *M. voeltzkowi*, except that the presence of a penis was noted. In *M. holothuricola* the structure of the vas deference was not closely described, although its walls were claimed to be ciliate, which we doubt. A prostate and a penis was also present in that species.

Morphology of the Larva

A few of the ruptured egg capsules contained full-grown larvae, which were seen to rotate. The shell of these larvae (pl. IV, fig. 9) is about 350 μ in height and 200 μ across (after fixation and included in anise oil). The apex is perfectly rounded, and there are only one and a half whorls.

The larva has a well-developed bilobed velum equipped with exceptionally long marginal cilia. The two lobes meet ventrally at the entrance to the mouth. The head carries two pointed tentacles, 35 μ long. Two equally large eyes are situated lateral to the tentacle bases. They are not, as in the adult, included within connective tissue, the unpigmented cornea being level with the skin. Almost all available space in the head is occupied by the nervous system, which comprises two fused cerebral, two pleural, two pedal, and supra- and sub-oesophageal ganglia (pl. IV, fig. 8); a small visceral ganglion lies adjacent to the stomach wall. The two statocysts are placed close together between right and left pedal ganglion. The neural mass is penetrated by the oesophagus which is ciliated throughout and connects the mouth with the stomach. The latter is spacious and almost spherical. Two-thirds of its walls is ciliated

and secretory, the remaining part secretory only. The stomach receives two outlets from the digestive gland, which is filled with yolk granules and occupies a large part of the first coil of the shell. Yolk granules pass through the two ducts to the stomach to be digested there. A ciliated intestine leads from the stomach to open far back in the mantle cavity. A small sac adjacent to the stomach lined by a squamous epithelium possibly represents the rudiment of the kidney. Neither heart nor a gonad rudiment could be demonstrated.

The foot is pointed, without a creeping sole. The anterior lobular pedal gland opens via a tubular duct on its very tip. The prominent columellar muscle spreads into the posterior part of the foot, which is provided with a shield-shaped operculum.

The mantle cavity contains a comparatively large and much differentiated hypobranchial gland. Neither an osphradium nor rudimentary gills are present.

Rosén (1910) noted that the larva of *M. holothuricola* possessed a velum, two eyes, and two statocysts. The elaboration of the velum into a powerful swimming organ and the lack of a creeping sole shows that the larvae are planktonic for some time. The apex of the adult (male) is provided with only two embryonic whorls (pl. II, fig. 4). This, in addition to the fact that the last of these suddenly increases in size indicate an early establishment in the host with its resultant ready availability of food. The larvae probably enter the host passively along with the food.

Notes on Biology

The geographical range of *Megadenus* is noted in the introduction and the present material adds a second locality to the records from the Indian Ocean. It further extends the range of holothurian hosts from the Holothuriidae to the family Stichopodidae and the parasite could perhaps profitably be searched for in all members of the Aspidochirota. Considering the relatively large size of the parasite and the few records, the infestation must be very low. The present material was found in one of 33 aspidochirotes examined of which 18 were *S. chloronotus*. No specimens were found in 19 aspidochirotes examined at Watamu Kenya. Tikasingh examined 147 individuals of *Ludwigothuria mexicana* in Curaçao and Bonaire, Netherlands Antilles, but failed to find a single *Megadenus* (personal communication). Rosén (1910) had five specimens but reported that they always occur in pairs and are exceedingly sparse. Schepman & Nierstrasz (1913) worked on a female and the proboscis of a male and said that they were of rare occurrence.

Reproduction. The female deposits her eggs in capsules which are attached by short delicate stalks to a thick mucous "helmet" almost completely covering the shell of the male (pl. II, fig. 3). A similar habit has been described in *M. holothuricola* and it probably also occurs in *M. voeltzkowi* in which species, however, the shell of the male was lost. The pseudopallial fold of the male of our species was considerably

lower on one side, and the capsules were chiefly fastened to that side (pl. II, fig. 3); this is probably the side which in life is adjacent to the female (text-fig. 1). In such a position it is possible that the anterior part of the foot of either sex may manipulate the egg capsules, and the enlarged pedal gland opening on the tip of it could perhaps at the same time produce the helmet to provide a firm attachment for the capsules. It was possible to remove the helmet as a whole together with the capsules; its inner surface showed a perfect moulding of the shell and the occurrence of fine striae perfectly comparable to those found on the fresh shell of the female. The shape of the capsules is similar to those figured by Rosén (1910, fig. I), being somewhat oviform, but their shape varies according to the stage of development of the enclosed young. Newly laid capsules are smooth contoured and the eggs tightly packed. As the embryos develop the capsules become more spacious from ab. 1.5 mm to more than 3 mm in largest diameter, and having attained the final larval stage, the individuals are free to rotate. The larvae escape through random ruptures in the wall of the capsule which then slowly breaks up leaving only the stalks in position.

Twenty-six capsules were attached to the male, of which 10 were empty, 4 were ruptured and partially empty, and 12 were entire; in addition a number of stalks were found in various stages of decay. The capsules showed all the pre-release stages in the development of the young but the contents of each capsule were in a constant stage of development. This indicates a continuous production of eggs and to substantiate this several embryos or larvae from each capsule were sectioned. Since the embryos could be arranged in a fairly continuous series according to their development as evidenced by sectioning, laying seems to be a steady process, obviously not interrupted by intervals of non-reproduction.

The contents of the whole capsules were counted, showing an average of 107 larvae or embryos with a range of 85–137.

The location of the present material in the host has been mentioned and comparing this with that of *M. holothuricola*, the only species in which the location has been described in some detail, we note that our material differs in that no direct connection with the sea existed when the animals were in their observed position. According to Rosén (1910) *M. holothuricola* occupies such a position that the body is located in the lumen of the respiratory tree and so bathed in sea water. The proboscis penetrates the wall of the tree, opens into the coelom and ingests the coelomic fluid. This location is reminiscent of that briefly described by von Martens (1865) on what we suppose is a species belonging to *Megadenus*. This apparent difference in location has resulted in the following considerations which gives a more meaningful interpretation of both our own and Rosén's observations.

Species so closely related as to be ascribed to the same genus are likely in principle to lead a similar life. Two features are of particular interest here *viz.* the release of the larvae and the nature of nutrition since our observations do not correlate on these points with those of Rosén.

In both species the female deposits her egg capsules on the shell of the male. The position of *M. holothuricola* within the respiratory tree of the host allows for the release of larvae in the exhalent water. Such a situation did not apparently occur in the male of our species, the body of which including the egg capsules were located within the coelom. It may be argued that in the absence of any coelomoducts in holothurians or other connections between the coelom and the sea the parasite relies for the release of the larvae on periodic evisceration of the host. As with many other tropical aspidochirotetes, *S. chloronotus* may eviscerate under adverse conditions as for instance in stale sea water. Whether this species eviscerates in nature is not known, but it deserves mention that in several other holothurian species belonging to the genera *Stichopus*, *Parastichopus* and *Actinopyga* a seasonal evisceration has been demonstrated (Bertolini (1932); Swan (1961); Mosher (1965), Jespersen & Lützen (1971)). We do not consider, even if seasonal evisceration does occur in *S. chloronotus* that it may explain the release of the larvae of *M. cantharelloides* since the production of the larvae has been shown to be a steady process and the opening to the sea following evisceration is of a very transitory nature. We are therefore of the opinion that the reported location of the male in *M. cantharelloides* only partially corresponds to the natural condition and we will show that the material of *M. cantharelloides* has been displaced from its original position.

An examination of the egg mass helmet revealed an entire foraminiferan and empty angiosperm mesenchymatous tissue embedded in the substance. The mucus on the distal surface of the female pseudopallium contained assorted unidentifiable debris together with a foraminiferan and a spine probably of a crustacean. No similar material was found on the proximal surface of the proboscis flange despite its close proximity to the pseudopallium. These inclusions would not be expected in the coelom of the host.

The part of the proboscis connecting the pseudopallium and the flange in *M. cantharelloides* is fairly constricted. In this region an almost complete reduction of the longitudinal musculature was noted. Accompanying this the molluscan epithelium has been sharply disrupted throughout the section series. The nature of this disruption and its restriction to this region illustrates that it is not an artefact of sectioning. It appears that something surrounding the base of the proboscis has been forceably pulled away.

The zone of disruption furthermore marks an abrupt change in the nature of the epithelium lining the proboscis. Posteriorly to the zone occurs a mucus secreting epithelium which becomes increasingly folded towards the boundary to the pseudopallial base. In the section series mucus was seen originating here and produced maximally in the region of the greatest folding. In life there would be an outward flow of mucus from this region (pl. III, fig. 5, large arrows). The proximal surface of the flange, on the contrary, is lined by a cuticularized epithelium. Such an abrupt change in the nature of these adjacent surfaces suggests that they are exposed to different environmental conditions in life.

Throughout the section series an unbroken ring of tissue was found to surround the zone of epithelial disruption (pl. III, fig. 5, iw). Its structure is strongly reminiscent of the connective tissue found in the intestine of a sea cucumber, and a careful examination revealed that it was not derived from the gastropod.

The extent and the development of the intestine and the digestive gland in *M. cantharelloides* does not suggest an animal living on the very dilute simple organic foods available in the coelomic cavity, such as sugars and aminoacids, as suggested by Rosén (1910) for *M. holothuricola*. Gastropod parasites feeding on dilute media and simple organic compounds as e.g. nutrients from the coelomic fluid tend to show considerable modification to the digestive system; the endoparasitic family Entoconchidae provides a classical example of this (Ivanov, 1945).

The evidence presented leads us to the conclusion that the field observations on *M. cantharelloides* were an artefact of the autotomy of the intestine in the host prior to it being examined. We believe, that the parasite inhabits the intestine of the host, probably, according to the arrangement of the gut in *S. chloronotus*, in the oesophageal region, although the anterior ventral loop of the large intestine is also adjacent to the area where the male was attached. The entire proboscis lies in the coelom so that the wall of the intestine lies between the pseudopallium and the flange of the proboscis. It is probably partially introduced in the body wall of the host (text-fig. 1) as was actually demonstrated by the male specimen. In this situation the parasite can maintain its position in the alimentary canal without the expenditure of much energy, for the flange of the proboscis will prevent the displacement of the animal. The proboscis is now free to attach in different adjacent regions of the body wall without the parasite being displaced. The discontinuity in the musculature referred to above indicates that the proboscis is not functioning as an attachment organ, rather as a food gatherer. The copious mucus production will provide a radial flow of mucus which will clear the area of debris from the host gut content and will provide a degree of protection from digestive fluids of the host in a region where it is particularly vulnerable.

The egg mass on the male must now lie in an exposed position within the lumen of the alimentary canal of the host. The extreme development of the pseudopallium in the male (pl. II, fig. 3) and its position even in the preserved material suggest that in life it covers the egg mass and protects the cocoons from damage. In this position the larvae are able to pass through the gut with the food, or while it is relatively empty in the early morning (Yamanouchi, 1956). That larvae of parasitic gastropods are able to pass the intestine without being digested is well known in members of the family Entoconchidae (Schwanwitsch, 1917).

It follows that although much of the evidence is derived from the male specimen, the female must inhabit the same position as the male so that the egg capsules can be laid on the latter. To support this there is the evidence of the tissue debris restricted to the external side of the pseudopallium and possibly comparable to the ring of tissue around the proboscis of the male. More significant, perhaps, is the earlier men-

tioned presence of a brownish belt of a mucous substance surrounding the proboscis probably indicating the level at which this structure is introduced in the host's body wall (text-fig. 1, r). Observations on live specimens of *S. chloronotus* show that the interior body wall is lined by a delicate mucus which is slightly pigmented; it appears dark when concentrated as for example by scraping an instrument across the surface of the coelomic wall.

It is considered that Rosén's observations were correct save that the proboscis of his specimens of *M. holothuricola* had become detached from the body wall. In the absence of any consideration of the morphology of the alimentary tract he considered that the parasite fed on coelomic fluid.

The observations on the present material in the field are artefacts of the autotomy of the intestine and its subsequent contraction. In this situation with the parasites attached to the body wall a strain would be exerted in a direction opposite to that occurring naturally. The wall of the intestine disrupted leaving the male parasite attached to the body wall and at the same time dislodging the female and breaking her at the base of the proboscis, the weakest point. The subsequent contractions of the intestine expelling the body of the female into the coelom where it was found.

Conclusions

Comparisons between the structure of *M. cantharelloides* and the two other species known to parasitize sea cucumbers have been given earlier in this account. It is distinguished from both of them by the peculiar flange-like portion of the proboscis, the presence of functional eyes and four, rather than two retractor muscles. It furthermore differs from *M. holothuricola* in possessing a small, but typical stomach, and from *M. voeltzkowi* by having two tentacles in the female instead of one. We consider that these differences are sufficient for establishing a new species. On the other hand, it should be emphasized that the three species mentioned are extremely similar in so many fundamental respects that a very close kinship can not be doubted.

It would therefore be of interest to compare the structure of this group of three species with the remaining two species referred to that genus.

Megadenus cysticola Koehler & Vaney, a parasite provoking cysts in the spines of the cidaroid *Dorocidaris tiara*, was unfortunately described rather briefly and it remains therefore difficult to compare this species with those living in sea cucumbers. It is apparently similar to these in several respects, but the lapidary description does not allow one to decide whether these similarities are real or not. *M. cysticola* (figured in Grassé, 1968, p. 216) has a mucronate shell, which is whitish, polished, and brittle. Sexual dimorphism is well-expressed, the male being much smaller than the female, both of which live together in the cysts. The egg capsules are deposited within the cysts but it is not known whether they are attached to the male's shell or to the interior of the cyst. There is a well-developed proboscis, from the base of which arises a so-called pseudopallium, which shows greater development in the male than

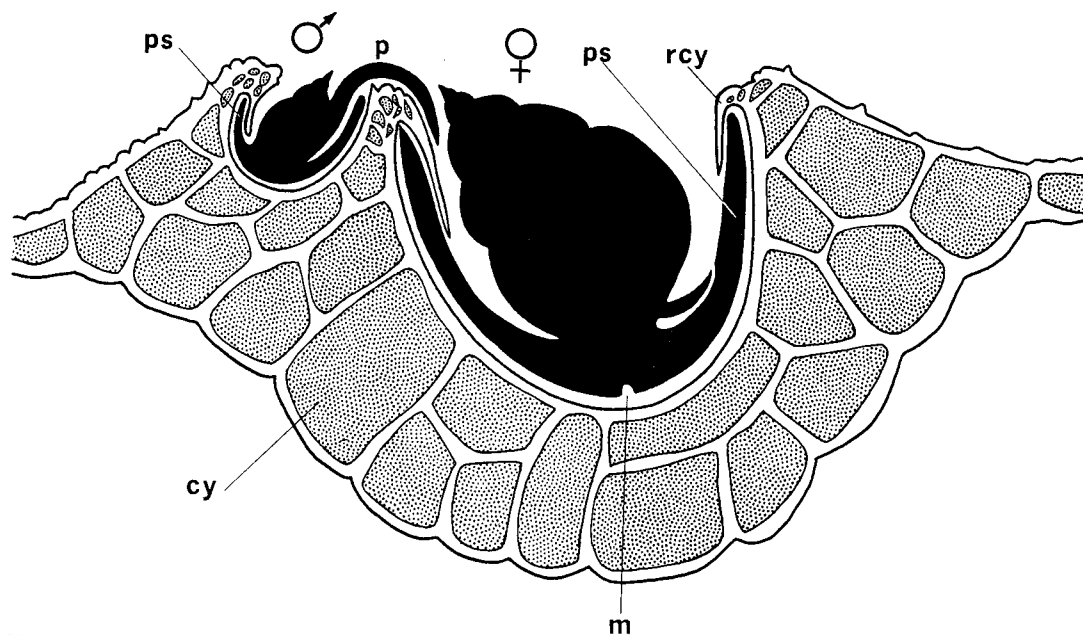


Fig. 5. Diagram showing the position of *Paramegadenus arrhynchus* (Ivanov) in the skin of *Anthenoides rugulosus*. cy, cyst of host's skin plates; m, mouth opening; p, penis; ps, pseudopallium; rcy, rim of cyst enveloping margin of pseudopallium. (From Ivanov, 1952, fig. 4).

in the female. The cephalic region and the foot are said to be reduced, and there is no operculum. Eyes and statocysts are present, and the anterior pedal gland is very prominent.

The most profoundly examined species referred to *Megadenus* is *M. arrhynchus* Ivanov, on the structure of which an extensive report has been given (Ivanov, 1952). Ivanov's material consists of two sea stars of *Anthenoides rugulosus*¹ from the Yellow Sea, each of which were parasitized by three snails and preserved in formalin. The parasites were placed in fairly deep depressions, or cysts, in the skin of the aboral side of the sea star's interradial plates, the plates of which thereby had become considerably thickened and more or less irregularly deformed (text-fig. 5). The gastropod body

¹ As pointed out by Ivanov, the species was first discovered and pictured by Fisher (1919, pl. 35, fig. 4), although not mentioned in his text. On request, Dr. David Pawson (U.S. Nat. Mus. Wash.) has kindly told us that these gastropods were on the holotype of *A. rugulosus* (USNM catalogue no. 30561), from 9 miles southeast of Malabrigo Light, east coast of Mindoro, Philippines, 108 faths., mud. Actually the specimen had four gastropods on it, the two shown in Fisher's plate, and two more (also close together) lying near the margin of an arm about two-thirds of the arm length away from the center of the disc. — The Zoological Museum in Copenhagen has one alcohol-fixed specimen of *Anthenoides granulatus* parasitized by a male and a female of *M. arrhynchus*, taken on the Th. Mortensen Expedition to Java and S. Africa (Java Sea, 7°35' S, 114°42' E, 200 m), and 3 dried specimens of *Anthenoides* sp. (according to Dr. F. J. Madsen, Zoological Museum, Copenhagen, these differ from *A. rugulosus*) with one female, one female, and two pairs respectively, taken by the Th. Mortensen Pacific Expedition (W. of Kyushu, Japan, 32°17' N, 128°11' E, 208 m). Except for one of the pairs, which was placed in the middle of a radius, the others were located interradially and very close to the center of the disc.

is sunk obliquely into the host's skin so that 3 or 4 body whorls may be seen from the outside. In the complete absence of a proboscis, the contact with the host is entirely accomplished by a formation, which Ivanov calls a pseudopallium; all around its margins fit closely into a circular groove formed from the rim of the cyst.

The species is relatively little modified by its parasitic way of life. Besides the presence of a pseudopallium and the absence of a proboscis and an operculum, it is distinguished mostly by specialization of the foot and accompanying structures. The head is small and poorly defined. There are two very large and stout tentacles. An ocellus is included in the base of each. The mouth is a short slit placed eccentrically in the pseudopallium.

The pseudopallium is formed from the front part of the head and has the shape of a large fold of skin reflected to form a cavity which is widely open aborally. Owing to the oblique position of the body, it is largest to the right, whereas its lesser development to the left leaves the whorls uncovered and visible at that side.

The foot has obviously lost any locomotory power. Its foremost margin is strongly dislocated and lies adjacent to the right antenna. The foot is subdivided into three parts, the two first of which are shovel-shaped, whereas the third, and posterior one, is a muscular, ciliated fold, that surrounds the base of the visceral mass almost reaching the entrance to the pallial cavity. The two pedal glands open onto the first and second parts respectively. The function of these parts remains somewhat obscure, whereas it is supposed that the third part serves to lead sea water into the mantle cavity, thus forming a specialized siphon of pedal origin.

The shape of the shell appears from Ivanov's fig. 5. It has a well expressed larval shell and 3.5–4 adult whorls. The larval shell is transparent and 400 μ high, whereas the adult whorls are shining, smooth, rather thick and opaque, with almost invisible striae. The sutures are very distinct and whitish. Height and width of shell: 6.4–6.5 and 4.9 mm (females); 1.5 and 1.5 mm (males).

The interrelations between *M. arrhynchus* and the three other species of that genus (ignoring the poorly described *M. cysticola*) deserve the closest attention. Gruzov (1965) has provided very convincing evidence that the ancestors of *Asterophila japonica* Randall & Heath, an internal gastropod parasite of various sea stars were structurally reminiscent of *Megadenus*. He has proposed an evolutionary trend, which starts with *Megadenus* (i. e. the species parasitizing sea cucumbers) and ending with *Asterophila* and in which *M. arrhynchus* occupies an intermediate position (Gruzov, 1965, fig. 49). We feel that the relations between the forms in question are better expressed when *M. arrhynchus* is given a generic status of its own.

The general structure of *M. arrhynchus* is very similar to that of the species of *Megadenus* living in holothurians. Reliable indications of this relationship are seen in those specializations common to all such as a pseudopallium of purely cephalic origin, the structure and location of the enormously enlarged anterior pedal gland, the reduced size of the posterior pedal gland, and the elaboration of the foot, particularly its posterior, folded portion. To these one may possibly add the similar plan

of the nervous system and reproductive apparatus and the identical histological structure of the eyes. This combination of characters is no doubt expressive of a true affinity. *M. arrhynchus*, on the other hand, is characterized by a number of peculiarities, which separate it sharply from the other species of that genus to an extent which justifies the raising of a new genus. This we propose to name *Paramegadenus* n.g., accomodating only one species, *P. arrhynchus* (Ivanov). The name *Megadenus* should be restricted to the three species which live in sea cucumbers, *viz.* *holothuricola*, *voeltzkowi*, and *cantharelloides* n. sp.

Among the most distinctive characteristics of *Paramegadenus* is the complete absence of a proboscis, a feature accompanied by simplification of the buccal cavity, which is lined with a simple, cubical epithelium. Another important distinguishing character is that the males are considerably smaller than the females, although not simple pygmies. Differences also exist in the larval shell which in *Paramegadenus* has 3 whorls, and, as we shall see, probably in the mode of reproduction. Peculiarities of minor importance are that a posterior pedal gland is found only in the female, and the hind part of the foot is muscular and ciliated.

The position of *P. arrhynchus* in the phylogenetic scheme was considered by Gruzov (1965) to be one leading from *Megadenus* towards *Asterophila*. It might very well be too early to say whether *Paramegadenus* occupies a more advanced stage than *Megadenus*. Some of the peculiarities separating it from the latter are obviously directly related to differences in the mode of feeding; thus, the lack of a protruding proboscis and the rudimentary state of the buccal cavity in *P. arrhynchus* might well be considered original features, and the elaboration of these parts in *Megadenus* as later aquisitions. The presence of two proboscis retractors in *Paramegadenus* and *Megadenus* (four in *M. cantharelloides*) and the reduced condition of the stomach in both genera do not lend support to either theory.

Perhaps the most conspicuous differences separating *Paramegadenus* from *Megadenus* are that the former exhibits a pronounced male-dwarfism. That the number of ctenidial leaflets is smaller in the male than in the female, that no posterior pedal gland exists in the male, and the apparent large size of the penis in *Paramegadenus* is explained by the great reduction in size of the male. Male dwarfism is often regarded as an indication of an advanced position in an evolutionary series. Among parasites, however, such tendencies usually occur the more intimate the parasite-host relationships become. Since *P. arrhynchus* depends upon its host no more than does *Megadenus*, one may just as well turn the problem the other way round and ask why the latter has not aquired this characteristic also. This question may probably, we believe, be related to differences in the mode of reproduction. The fact that there is no information on *Paramegadenus*' reproduction other than Ivanov's observation of an egg capsule in the capsule gland of a female, may itself offer the most simple explanation. A total of 18 specimens of *P. arrhynchus* (males and females together) have been observed, compared with half that number, also of both sexes, in the three species of *Megadenus*. Nevertheless, egg capsules have been commonly observed

attached to the male shell in the latter. It is probable that in *Paramegadenus* the egg capsules are immediately liberated into the sea. Perhaps, supporting this, is the condition prevailing in *Asterophila*, where the single egg capsule rests unattached in the female pseudopallial cavity (Gruzov, 1965). That the species of *Megadenus* living in the host's intestine retain their capsules is understandable. If liberated they have to pass the entire alimentary tract of the host unprotected from the digestive juices and attrition. The operculate larvae, on the other hand, able as they are to withdraw into their shell, are excellently equipped for the process of escape. To retain the capsules, however, demands a firm substrate on which to glue them, and in the particular environment which these parasites inhabit, this is provided only by the shells of the adults. For some reason unknown, the female is unable to attach them to its own shell and for that purpose depends upon the male's. The lack of sexual dimorphism in these species could therefore be accounted for by the male requiring a large size in order to anchor and protect the egg capsules. If this is correct then the lack of dwarf males in *Megadenus* becomes a dubious evolutionary indicator.

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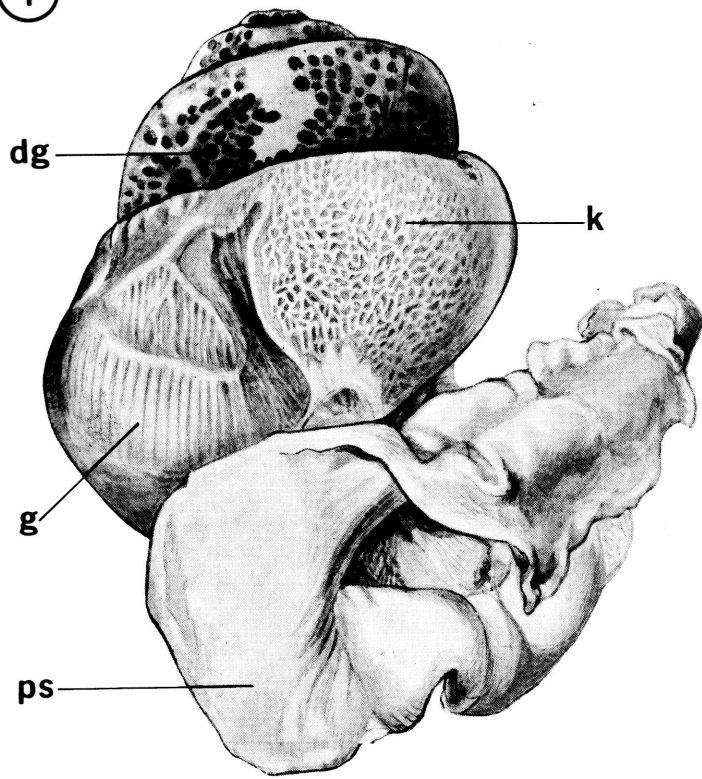
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PLATES

PLATE I

Fig. 1 and 2. Female *Megadenus cantharelloides* n. sp. af, anterior part of foot with opening of anterior pedal gland; cg, capsule gland; dg, digestive gland; g, gills; k, kidney; pf, posterior part of foot; ps, pseudopallium; rt, right tentacle. Scale represents 2 mm.

①



②

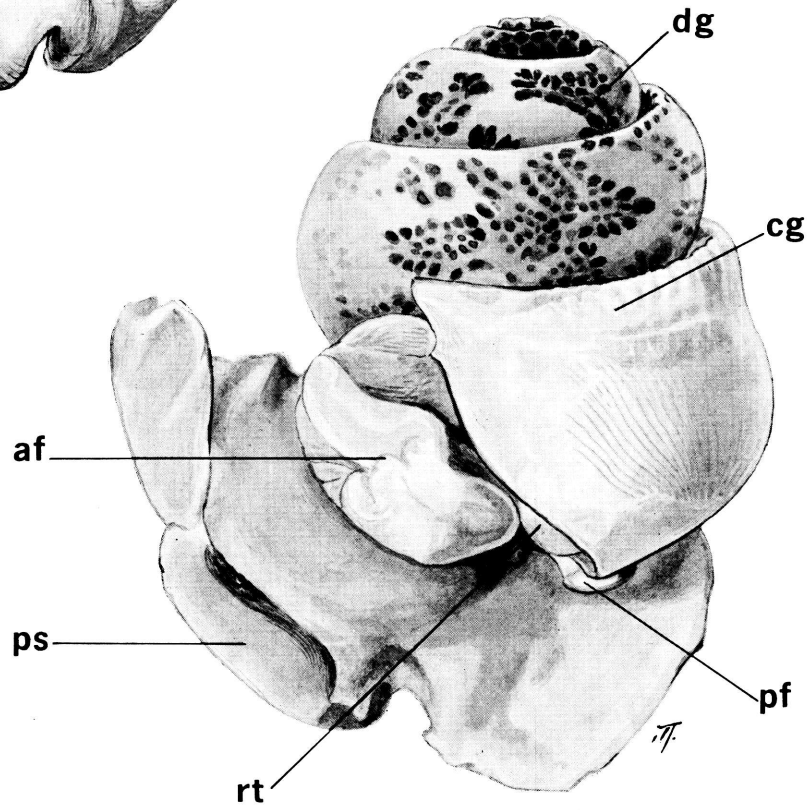
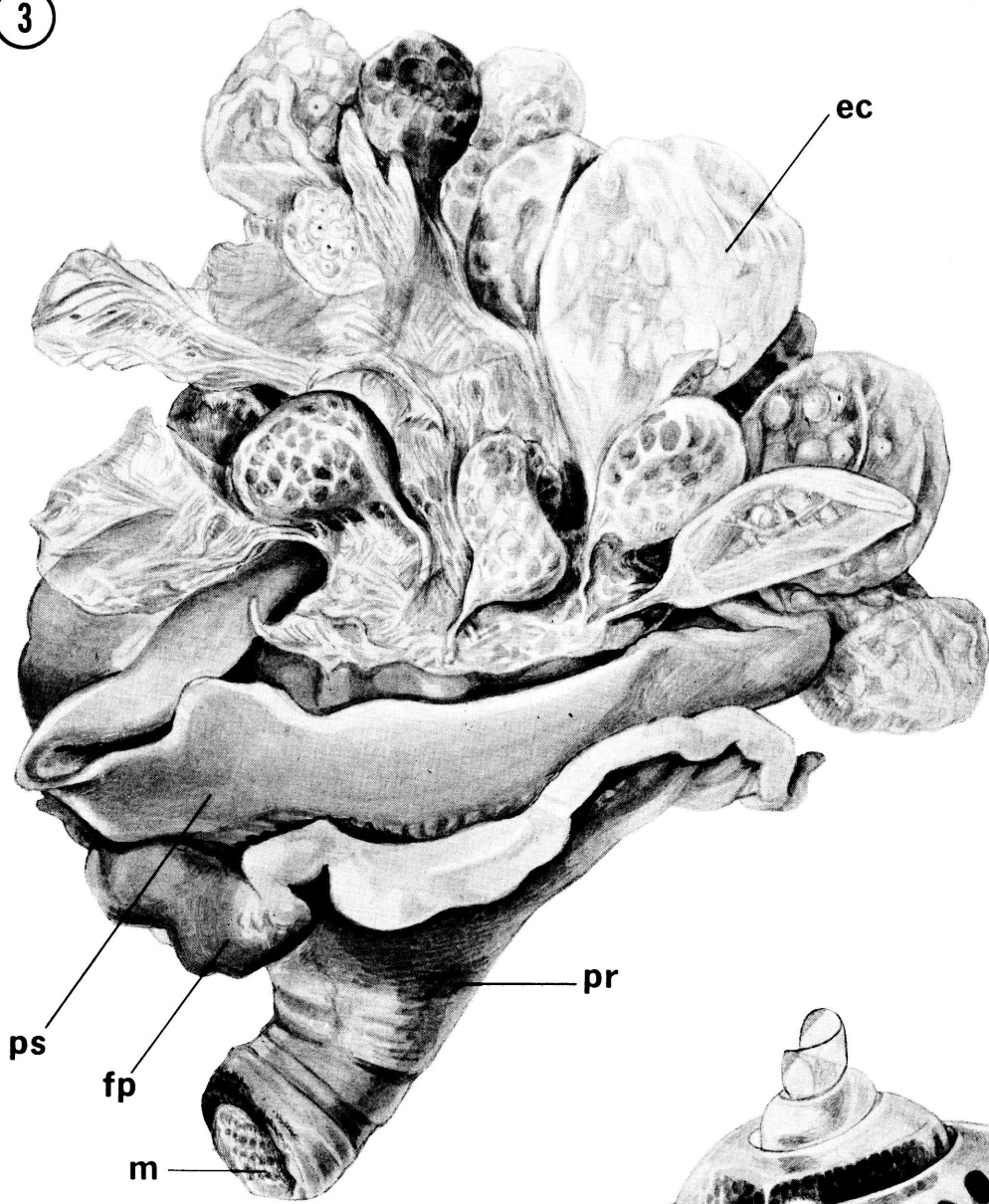


PLATE II

Fig. 3 and 4. Male *Megadenus cantharelloides* n. sp. The mucous helmet and egg capsules have been removed in fig. 4. ec, egg capsules; m, mouth opening; fp, flange of proboscis; pr, proboscis; ps, pseudopallium. Scale represents 2 mm in fig. 3 and 500 μ in fig. 4.

3



4

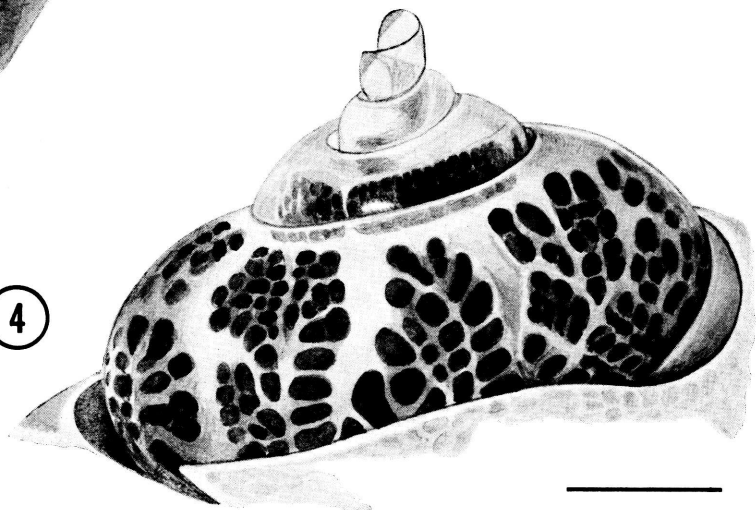


PLATE III

Fig. 5. Section parallel to columellar axis through male *Megadenus cantharelloides* n. sp. aa, anterior aorta; af, anterior part of foot; bc, buccal cavity; dg, digestive gland; eb, efferent branchial vessel; fp, flange of proboscis; hg, hypobranchial gland (posteriormost part); i, intestine; iw, remains of intestinal wall of host; k, kidney; m, mouth; mc, mantle cavity; oe, oesophagus; pf, posterior part of foot; ph, pharynx (cut tangentially); ps, pseudopallium; r, retractor muscle; t, testis; v, visceral ganglion; vd, vas deferens; x, opening of prostate into mantle cavity; y and z, openings of anterior and posterior pedal glands. -- Large arrows illustrate a radial flow of mucus produced by the glandular epithelial folds. The small arrows indicate the discontinuity of the muscle layer and the transition between epithelia of glandular and cuticular nature.

Scale represents 500 μ . Tetrachrome, 10 μ .

5

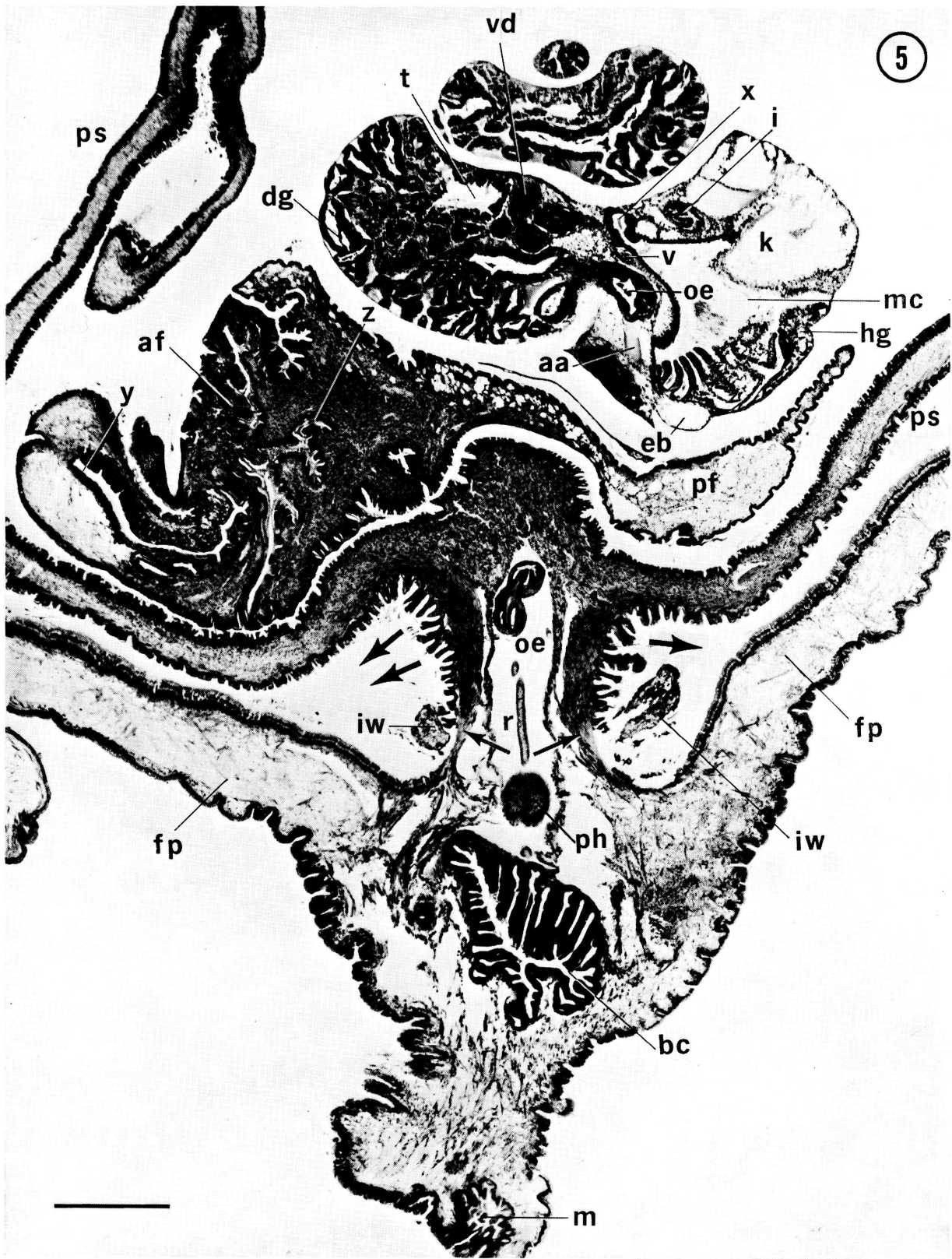


PLATE IV

Megadenus cantharelloides n. sp.

Fig. 6. Left ocellus in longitudinal section. c, cornea; l, lens; on, optic nerve; rc, retina; the arrows mark the boundary between the pigmented cup and the cornea. Scale represents 50 μ . Tetrachrome, 10 μ .

Fig. 7. Slightly oblique longitudinal section through pharynx (ph) and buccal cavity (bc). b, buccal ganglion; iw, remains of intestinal walls of host; oe, oesophagus; r, retractor muscle; the arrows indicate a disruption of the epithelium. Scale represents 500 μ . Iron hematoxyline-eosin, 10 μ .

Fig. 8. Longitudinal section through larva. cm, columellar muscle; dg, digestive gland; f, muscular portion of foot; lc, left cerebral ganglion; lp, left pedal ganglion; mc, mantle cavity; oe, oesophagus; op, operculum; rp, right pedal ganglion; s, stomach; st, statocyst; x, duct of digestive gland into stomach. Scale represents 100 μ . Toluidine blue, 2 μ .

Fig. 9. Larval shell. Scale represents 100 μ .

