AN ACCOUNT OF THE STRUCTURE AND EARLY DEVELOPMENT OF A NEW SPECIES OF A NUDIBRANCHIATE GASTROPOD, EOLIDINA (EOLIDINA) MANNARENSIS*

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INTRODUCTION

A SMALL nudibranch of the family Aeolidiidae was at first obtained in the month of March 1960 from a collection of squid eggs attached to weeds in a shallow water region of the Gulf of Mannar in the vicinity of the Central Marine Fisheries Research Station at Mandapam. Subsequently the same form was found to occur in fairly large numbers creeping over the sides of some large aquaria with circulating sea water in the Research Station. The glass panes of the aquaria were kept not cleaned thereafter which promoted a vigorous growth of a few sedentary organisms like the hydroids, colonial ascidians and polychaetes amidst which the nudibranchs thrived well depositing strings of their spawn. This form has been referred to the genus *Eolidina* Quatrefages, sub-genus *Eolidina* and described as *E. (E.) mannarensis*, a species new to science. The opportunity presented by the availability of freshly laid spawn in the aquaria has enabled studying some aspects of the life-history of the species.

We have great pleasure in expressing our gratitude to Dr. S. Jones, Chief Research Officer of the Central Marine Fisheries Research Station, for his kind encouragement and valuable suggestions during the course of this study. Our thanks are due to Dr. R. Raghu Prasad for rendering translation of relevant passages of German references into English.

EXTERNAL FEATURES OF Eolidina (E.) mannarensis sp. nov.

The general appearance of the animal is acolidiform. The head is anteriorly rounded with its lateral extremities produced into a pair of long, smooth tentacles broad at the base and narrow at the distal end. A little behind the region of the tentacles is a pair of smooth rhinophores without any distinct perfoliations but presenting a slightly ringed appearance when they are contracted. Close behind the base of each rhinophore is a minute dark pigmented spot representing it. The head region in front and behind the rhinophores is narrow and is continued by an expanded dorsum, which bears on either side groups of linear cerata extending upto the region of the tail. About 6 or 7 of the anteriormost cerata on either side are grouped into a distinct cluster behind which is a little gap followed by other clusters

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not distributed in a well-defined manner. The cerata are circular in cross section ind the digestive diverticula entering into them are unbranched. The latter are in communication with tiny enidosacs which open to the exterior at the free ends of the cerata. The posterior region of the animal is produced into an elongate tapering tail. The foot is considerably broader than the dorsum. In the anterior region the former is broadly rounded and produced into a triangular flattened expansion on either side. The mouth opening is median and ventral in the head. The male genital opening in front and the female genital opening a little behind are situated beneath the level of the anteriormost group of cerata of the right side. The anal aperture is also on the right side about the level of the cerata at half the length of the animal. The general colouration is pale golden yellow speckled with orange at the tips of the tentacles and rhinophores. Faint orange colouration is present over the surface of the cerata and the dorsum on which it is more prominent in the anterior region than behind. The tips of cerata is the region of the enidosacs are slightly whitish. The digestive diverticula of the cerata seen through the transparent lining epithelium are grayish brown. The foot appears uniformly yellowish white.

A full-grown animal has a length of 20 mm. including the tentacles when fully expanded and found creeping. The tentacles and the rhinophores are 3 mm., the cerata about the same and the tail 6 mm. in length. In the region of the first group of cerata the foot has a width of 2.2 mm. and the dorsum 1.5 mm. (Text Fig. 1, a, b and c).

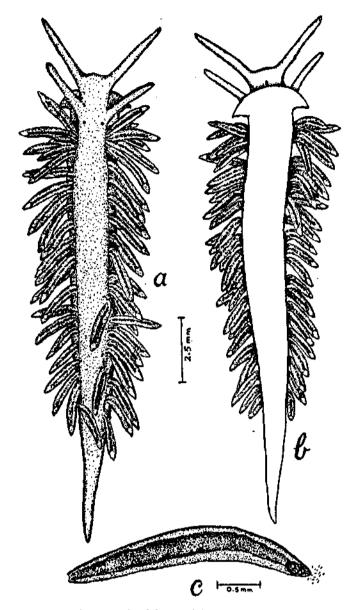
INTERNAL ORGANS OF E. (E.) mannarensis sp. nov.

The structure of the internal organs has been studied from dissections under the magnification of the binocular microscope and also from stained preparations of transverse serial sections.

The digestive organs consist of a slit-like mouth leading into a small, highly muscular pharyngeal bulb, a narrow short oesophagus, a much enlarged stomach, a ramified system of digestive diverticula extending into the cerata, a short intestine and a rectum opening by the anus. The jaws are thin, chitinous, more or less triangular, hinged together in the region of the beaks and produced into cutting edges opening into the mouth. They measure about 450 μ along the long axis (Pl. I, fig. 1). The radula is short with about 11 well-developed teeth and a few others in the process of formation in the radular sac. They are broad, arched, each with an abbreviated median cusp and about 11 sharp lateral cusps mounted on an expanded wing on either side (Pl. I, fig. 2). The median cusp being small and the lateral portions very prominent, the tooth presents the appearance of two distinct halves, a feature which is very characteristic of the genus. Of the lateral cusps the first three close to the median one and the last in the series are much shorter than others. The functional teeth on the radula measure each 80 μ broad in the widest region. The general arrangement of the various organs of digestion and the disposition of the pharyngeal (ptyaline), salivary and hepatic glands are very similar to those found in most members of the family Aeolidiidae (Pl. I, figs. 3 & 4). These forms presumably feed on hydroids of which Zanclea indica occurred in fair abundance wherever they were found on the glass panes of the aquaria.

The central nervous system (Text Fig. 2 a) shows mainly a pair of cerebropheural and a pair of pedal ganglia, the former being situated close together pressing against

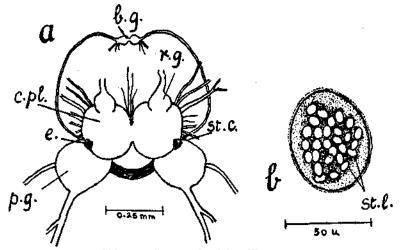
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TEXT FIG. 1. a. Dorsal aspect of *Eolidina (Eolidina) mannarensis* sp. nove; b. Ventral aspect of the animal; c. A single cerata magnified showing the discharge of the contents of the chidosac under slight pressure.

the posterior region of the pharyngeal bulb and dorsal to the oesophagus. The pedal ganglia are situated a little behind and below the cerebropleural ganglia. There are also paired rhinophoral ganglia in close association with the antero-dorsal

region of the cerebropleural ganglia, and paired buccal ganglia in the posterior region of the pharyngeal bulb ventral to the oesophagus. The commissures and connectives are similar in arrangement as in *Cuthona adyarensis* (Rao, 1952). Eyes are well developed each with a crystalline lens and a pigmented retinal cup. Paired statocysts contain several statoliths in each (Text Fig. 2 b).



TEXT FIG. 2. a. Central Nervous System of *Eolidina* (E.) mannarensis sp. nov.: b.g.—Buccal ganglion; c.pl.—Cerebro-pleural ganglion; e.—Eye; p.g.—Pedal ganglion; r.g.—Rhinophoral ganglion; st.c.—Statocyst; b. Statocyst magnified with st.l.—Statolith.

The heart, enclosed in the pericardium, has a single auricle and a single ventricle, placed dorsally behind the region of the stomach. The kidney is ventral to the pericardium and extends to some distance behind the region of the heart immediately beneath the body-wall. The anterior diverticulum of the kidney communicates with the pericardium and the rectum as revealed by the serial sections (Pl. I, fig. 5).

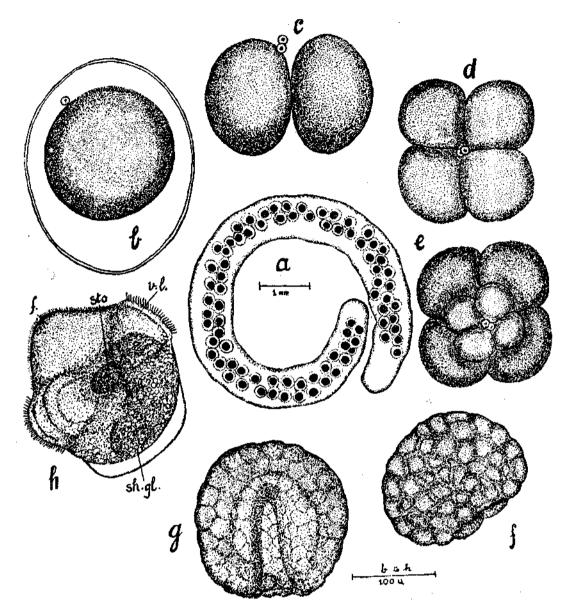
The follicles of the hermaphrodite gland are compactly packed behind the region of the heart occupying much of the body space upto the root of the tail (Pl. I, fig. 6). They have usually the male acini in the centre and the female acini at the periphery. The outgoing ducts from different follicles join into a median hermaphrodite duct which leads into an anterior genital complex. The hermaphrodite duct communicates distally with the male and female parts of the reproductive system. The former consists of a seminal ampulla, a wide coiled prostate gland, a narrow ejaculatory duct and an eversible penis in the penis sheath opening to the exterior by the male genital aperture, whereas the latter has an oviduct with a fertilisation chamber communicating with the muco-albuminous glandular passage leading out to the exterior by a short vaginal tube. The eversible penis in E. (E.) manuarensis is not provided with a stylet.

EARLY DEVELOPMENTAL STAGES OF E. (E.) mannarensis sp. nov.

In the aquaria the copulating pair of individuals was observed to approach in opposite directions with the anterior right regions coming close together, so that the male genital aperture of one opposes the female genital aperture of the other. The duration of copulation lasts for a brief time of about a minute. Fertilization is internal. After the separation of the copulating individuals, each deposits a mucilaginous string of spawn which is either crescentic or spiral in appearance measuring about 0.8 mm. in diameter and 12 mm. in length (Text fig. 3 *a*). Large numbers of such strings of spawn were collected from the glass panes of the aquaria or from the sides of the finger-bowls in which the animals were kept under observation. Each string has about 80 to 200 eggs. When freshly deposited the egg is spherical, yellowish in colour and heavily yolk laden measuring 150 μ in diameter. It is enclosed in a spacious double-walled capsule either slightly or prominently oval, about 240 μ in the longest axis (Text fig. 3 *b*). The space between the capsule and the egg is filled with a colourless albuminous fluid. The following account is based on the development of the eggs in freshly deposited spawn kept in finger-bowls holding sea water having a varying range of salinity from $33.53^{\circ}/_{00}$ to $34.18^{\circ}/_{00}$ and a temperature from 29° C to 31° C.

Fifty minutes after the oviposition the first polar body is seen to extrude out and it is followed by the formation of the second polar body. The first cleavage which takes place 2 hours and 30 minutes after the oviposition is vertical dividing the egg into two exactly similar halves (Text fig. 3 c). The second cleavage about 4 hours after the first, is also vertical but at right angles to the first, resulting in four large cells (Text fig. 3 d). One hour and thirty minutes thereafter, the third cleavage takes place in the horizontal plane resulting in eight cells of which four are large and four others small, the megameres and micromeres respectively (Text-fig. 3 e). By about 18 hours segmentation has progressed far so as to form a blastula. The micromeres, dividing a little more rapidly than the megameres, spread over the latter (Text-fig. 3 f). Following this stage in development there is gastrulation partly by epiboly and partly by invagination (Text-fig. 3 g). At this stage the blastopore is clearly visible in the developing embryo. Later the blastopore is reduced and finally closed. A stomodaeal depression appears at the place where the blastopore has closed. It extends inwards and meets the enteric space. The antero-lateral regions of the embryo in the stomodaeal region enlarge and get demarcated into velar lobes with vibratile cilia. The region ventral to the stomodaeal depression expands into the primordium of the foot, the surface of which is covered by numerous delicate short cilia. In the aboral region the shell gland is formed secreting the larval shell. (Textfig. 3 h). At this stage which results in about 50 hours after the oviposition, the embryo begins to rotate with the help of the velar cilia.

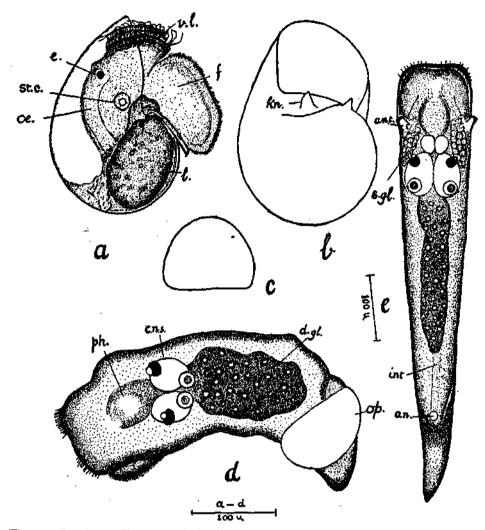
In 72 hours since the development has commenced, a fully formed veliger results (Text-figs. 4 a, b, & c). It has an ovoid shell the aperture of which is provided with a close fitting operculum. The shell structure is smooth, transparent and colourless. A very striking feature of the shell of the present form is the presence of two small peg-like projections in the region below the lower margin of the aperture. The shell measures about 230 μ in the longest axis. The soft body encased within the shell has a moderately expanded bilobed velum with powerful cilia situated dorsally and a broad median foot fringed with delicate cilia placed ventrally. Close behind the origin of the velar lobes is a pair of dark pigmented eyes and ventrally at the base of the foot are the paired statocysts. The mouth opening between the velar lobes and the foot leads into a narrow oesophagus which expands into a broad stomach at the posterior region of the shell. The intestine following the stomach runs forwards and is continued by an abbreviated rectum which opens into the mantle space. The liver mass is large, rather greenish



TEXT FIG. 3. a. Freshly laid spawn of *Eolidina* (E.) mannarensis; b. Egg with the polar body just extruded; c. Two-cell stage; d. Four-cell stage; e. Eight-cell stage; f. Blastula; g. Gastrula; h. Early veliger: f.—Foot; sh.gl.—Shell gland; sto.—Stomodaeum; v.l.—Velar lobe,

and bilobed in appearance. At the posterior region of the shell are visible the retractor muscles of the velum. The fully formed veligers have been observed to move vigorously within the egg capsules for a considerably long time before they are

liberated at the end of the eighth day. The larva has only a short duration of pelagic life lasting about 24 hours after which it has been observed to settle down at the bottom and creep with the help of the fully expanded foot. The velar lobes undergo



TEXT FIG. 4. a. Freshly hatched veliger of *Eolidina* (E.) mannarensis; e.-Eye; f.-Foot; l.-Liver; oe.-Oesophagus; st.c.-Statocyst; v.l.-Velar lobes. b. Empty shell; kn.-knob-like projection; c. Operculum; d. Early stage short creeping form with operculum still attached; c.n.s.-Central nervous system; d.gl. -Digestive gland; op.-Operculum; ph.-Pharynx; e. Late stage of creeping form with elongate body: an.-Anal aperture; an.t.-Anterior tentacle; int.-Intestine; s.gl.-Salivary gland.

a gradual diminution in size. The internal organs are squeezed out as it were from the shell and the operculum is dropped. Often the creeping form devoid of shell may still be seen dragging the operculum attached to the posterior region (Text-fig.

4 d). The earliest creeping stage about 350 μ long is rather clumsy-looking, short and a bit stumpy bearing traces of the velar cilia in front, a distinct mouth opening leading into an expanded pharyngeal bulb, paired ganglionic swellings behind the latter, dark pigmented eyes on either side and a pair of very clear transparent statocysts each containing a single statolith. Behind the ganglionic swellings is the greenish mass of the liver. It is devoid of pigmentation which is characteristic of *Embletonia pallida* (Rasmussen 1944). Within a day after this stage the young one has become distinctly long about 680 μ , and assumed more or less a flattened appearance resembling a planarian. By now the rudiments of the oral tentacles have been formed, the rhinophoral ganglia in the central nervous system have been demarcated, the mass of liver has very much elongated and an intestine makes its appearance with an anal opening situated posteriorly. No further changes were, however, observed even though they survived for about a week in the glass bowls in which they were reared. In spite of frequent changes of water in the bowls ciliates in large numbers appeared attaching themselves on the young ones and smothering them. Before any further development could take place all the forms gradually perished. Under artificial conditions the non-availability of the specific items of food of which so little is known is one of the reasons for the failure of the young ones to complete their metamorphosis.

SYSTEMATIC POSITION OF E. (E.) mannarensis sp. nov.

History of the genus Eolidina :--Alder and Hancock (1845-1855) in their Monograph on the British Nudibranchiate Mollusca included under the genus Aeolis a number of very dissimilar forms which were subsequently ascribed to different genera by themselves in their synopsis to the species in the appendix to their publication and also by other workers. Thus the forms originally described as *Aeolis angulata, Ae. alderi,* and *Ae. glauca* were ascribed to the genus *Aeolidiella* Bergh with the following characteristics as given by Eliot (1910 a): 'Body somewhat elongate and depressed. Cerata somewhat flattened. Corners of the foot produced into short processes. Rhinophores not perfoliate but sometimes wrinkled or furrowed. Jaws not denticulate. Teeth pectiniform, but divided into two halves with more or less developed median denticle. Ptyaline glands present.' Under the family Aeolidiidae and sub-family Eolidinae the generic name *Eolidina* Quatrefages 1843 has undoubted priority over *Aeolidiella* Bergh 1867, both being identical (Thiele 1931). The closely allied genera, viz., *Spurilla* Bergh 1864 and *Berghia* Trinchese 1877 are now being considered as subgenera under *Eolidina*. In all, the genus has three sub-genera, *Eolidina s.s.* in which the rhinophores are without perfoliations *E. (Spurilla*) in which the rhinophores are perfoliated and *E. (Berghia*) in which the rhinophores are perfoliated and also bear small knobs.

Specific characters of E. (E.) mannarensis sp. nov:—Body is aeolidiform; head bears a pair of tentacles and a pair of rhinophores of which the latter is slightly ringed when contracted; dorsum is bare along the middle but with linear cerata in loosely arranged clusters on either side; foot in front is broadly rounded with triangular lateral expansions and is drawn behind into a tapering tail; anus is dorsolateral on the right side about the middle of the length of the body; cutting edges of the jaws are without any trace of denticulation; radula is uniseriate with about 11 to 14 teeth, each with a short median cusp and two lateral expansions bearing on either side about 12 acute cusps of which the inner ones adjoining the median one and the last are smaller than the rest; statocysts are with several statoliths in each; penial armature is absent and general colouration is golden yellow, speckled with light orange on the dorsum, tentacles, rhinophores and cerata.

Type locality: Indian Ocean, Gulf of Mannar near Mandapam.

E. (E.) mannarensis and other species under the genus :--E. (E.) mannarensis is distinguishable from the members of the sub-genera, Spurilla and Berghia in the nature of the rhinophores as described earlier. Of the Atlantic and Mediterranean nudibranchs under the genus, E. (E.) glauca (A. & H.) from Torbay is characterised by the body being pale red and the branchiae stout, depressed, vermicular, tapering towards the apex, sage-green, speckled with brown and white, frequently with reddish tinge near the apices and their rows extending forwards round the base of the rhinophores. E. (E.) alderi (Cocks) from Falmouth has a body with opaque white spots and subconical branchiae, arranged in 12 to 14 rows extending round the head in front, the first two rows of them being opaque white and the rest gray marbled with purplish brown. In E. (E.) angulata (A. & H.) from Cullercoats the body is depressed terminating abruptly behind and the branchiae are in about 10 to 12 close set rows on either side, cylindrical in shape, obtusely pointed, orange red in colour with apices and surface having white blotches ; the foot is produced in front into angles at the sides and tapers suddenly behind into a fine point. E. (E.) sanguinea (Norman) has an attenuated body and numerous transverse rows of branchiae having blood red colouration (Alder & Hancock 1845-1855 and Eliot 1910 a). In E. (E.) occidentalis (Bergh) the general colouration is grayish brown with whitish lines running almost parallel to one another, transversely on the head and the back but vertically on the neck and the sides (Engel 1925). The radular teeth in E. (E.) glauca and E. (E.) alderi with numerous cusps in the lateral halves as given by Alder and Hancock (1855, Pt. 7, Pl. 47 Sup., figs. 5 & 6) are different from those met with in E. (E.) mannarensis which has a restricted number of cusps on their lateral expansions.

Of the species from the Pacific and the Indian Oceans, E. (E.) orientalis (Bergh) has a general reddish colouration of the body with elongate whitish speckles on the posterior half of the dorsum, the foot in front is rounded with a characteristic marginal furrow and the teeth resemble those of E. (E.) glauca. In E. (E.) drusilla (Bergh) the papiliary rows are free only in the anterior quarter of the back but converge behind running close to each other for the rest of the length of the dorsum In E. (E.) indica (Bergh) the papillary rows converge on the posterior half of the back, cerata have alternating light and dark longitudinal stripes and the radula resembles that of E. (E.) glauca. In E. (E.) faustina (Bergh) the cerata are arranged in front on a horse-shoe shaped ridge and in oblique rows behind; the basal region of the tooth is much smaller than in other species. In E. (E.) japonica (Eliot), the papillary rows are simple; the lateral halves of the teeth are unusually broad and undulating; the middle denticle is large and the lateral denticles about 65 on each side (Engel 1925). It may thus be seen that, the present form differs from all the above species in general colouration of the body and from some of them in the arrangement of the cerata and structure of radular teeth.

Affinities :—In possessing a slender delicate build of the body, paired smooth rhinophores and tentacles, cerata being arranged in clusters on either side of the dorsum and the dorso-lateral disposition of the anus, members of the genus *Eolidina* have a superficial resemblance to those of *Cuthona* A. & H. In both the radula is

uniseriate. Yet, species of *Eolidina* are distinguishable from those of *Cuthona* upon close examination of certain external and internal characters. In the former the antero-lateral regions of the foot are expanded into triangular lobes, the cutting edges of the jaws are without denticulations and the teeth of the radula are pectinate, each with a short median cusp and broad lateral expansions on either side. In the latter the foot is rounded in front, jaws are with well-marked denticulations on the cutting edges and the tooth with a prominent median cusp and a small number of lateral cusps on either side. In the structure of the radular teeth Eliot (1910 b) finds in *Cuthona annandalei* from the Chilka Lake a certain amount of resemblance to that of *Aeolidiella* (Syn. *Eolidina*). He, therefore, suggests the probability of the dentition in *Eolidina* having been derived from that of *Cuthona*.

SUMMARY

1. A small acolidiform molluse from the Gulf of Mannar in the vicinity of Mandapam has been referred to the genus *Eolidina*, sub-genus *Eolidina* and described as a new species, *E. (E.) mannarensis*.

2. A narrow dorsum, a tapering tail, a broad foot with prominent anterolateral expansions, paired tentacles and rhinophores, the latter without distinct perfoliations, linear cerata arranged in lateral rows but not grouped into distinct clusters, jaws without denticles, pectinate radular teeth, a dorso-lateral anus, statocysts with several statoliths in each, a penis without a stylet and a general golden yellow colouration of the body speckled with orange are the salient features of the species.

3. Spawning behaviour and the early developmental stages have been observed. The first cleavage of the egg results in two cells of equal size. The succeeding cleavages follow the same pattern as in other nudibranchs. The larval shell is characterised by the presence of two peg-like projections below the lower edge of the aperture. The duration of the pelagic life of the larva is very short. The creeping form, after discarding the shell, has been described. Further stages in metamorphosis could not be followed as the young under observations perished in about a week due to lack of specific food materials and infestation by ciliates.

4. The distinctive features of the genus *Eolidina* and of its sub-genera are dealt with. A comparative account of the specific characters of the present form with those of the hitherto known species under the genus has been given. The superficial resemblances of the form with certain members under the genus *Cuthona* have been pointed out.

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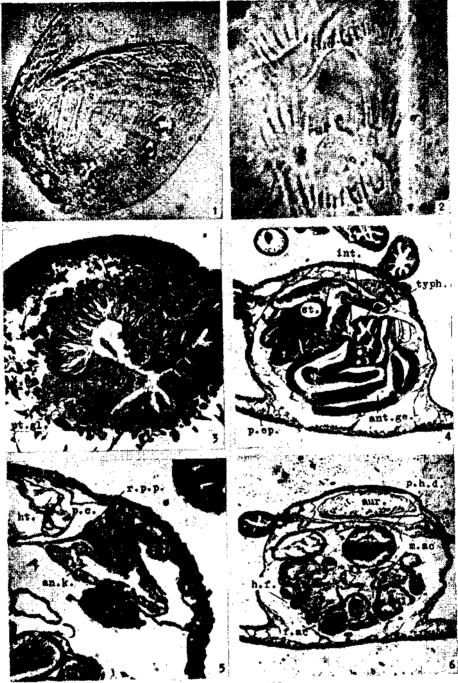
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Fto. 1. Jaw of Eolidina (E.) manuarcasis sp. nov. x 135.
Fto. 2. Radular teeth. x 550.
Fto. 3. T.S. through pharyngeal passage : pt. gl.—Ptyaline glands. x 140.
Fto. 4. T.S. through the region of the stomach : ant. ge.—Anterior genitalia : int. -Intestine ;
p.e.p.—Pedal epithelium ; st.—Stomach ; typh.—Typhlosole. x 50.
Fto. 5. T.S. through the region of the pericardium and renal organs : an.k.—Anterior diverticulum of kidney ; ht.—Heart ; p.c.—Pericardium ; r.p.p.—Reno-pericardial opening, x 150.
Fto. 6. T.S. through the region of the hermaphrodite follicles : aur.—Auricle ; t.ac.—Female acini ; h.f.—Hermaphrodite follicles ; m.ac.—Male acini ; p.h.d.—Posterior hepatic diverticulum, x 50.