DEFENSIVE MECHANISMS IN SOME NUDIBRANCHS

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Abstract of thesis on "Defensive mechanisms in some nudibranchs"

In a typical mollusc, the shell is the most important defensive mechanism. But the shell does not protect the mollusc from all predators, and it is for this reason that secondary defensive mechanisms have evolved in many species. In some cases, these secondary mechanisms become more important than the shell as a means of protection from predators, and where this occurs selection may favour the reduction and even the total loss of the shell. The shell has been lost independently in each of the nine orders of the Opisthobranchia except the Thecosomata, and in the Sacoglossa and the Nudibranchia it may have been lost two or three times. In all of these cases where the shell-less or nudibranch condition has evolved, the animal must be adequately protected by defensive mechanisms other than the shell. This thesis studies the defensive mechanisms of a number of nudibranch molluscs.

In the Sacoglossa there is a series of forms from the primitive Arthessa, with a well-developed defensive shell, through Oxynoë and Lobiger, with reduced shells, to the nudibranch Stiliger on the one hand and to the bivalved Berthelinia on the other. The defensive mechanisms of Berthelinia and Stiliger are compared. It is shown that even in Berthelinia, which is protected by a tightly closing bivalved shell, there is a secondary defensive mechanism in the form of a secretion from the hypobranchial gland. In Stiliger, the defensive mechanisms are located in the cerata.

Three types of gland are present of which at least one, and probably all, are defensive. In addition the cerata can be autotomised and regenerated, and this may also be of defensive importance. It is also shown that the ceratal glands of Stiliger and the hypobranchial gland of Berthelinia are muscle-operated. It is concluded that in both Stiliger and Berthelinia the defensive system involves two or three defensive mechanisms; and there is evidence that these mechanisms act in series just as do the defensive mechanisms of many tropical insects.

The defensive mechanisms of the Doridacea are discussed. It is concluded that camouflage is of widespread occurrence, but that there is as yet no evidence for the occurrence of warning coloration. Many bright colours are deflection marks, but the importance of other bright colours is still not known. Dorsal papillae with a probable defensive function are present in some species of the Doridacea, and they are usually supplied with glands. The function of the caryphyllidia of certain species is not known. Autotomy of papillae has not been described, but autotomy of the mantle edge occurs in the Discodoridinae. Defensive glands are of widespread occurrence in the Doridacea. One type of defensive gland that has not been described before in dorids is the acid gland of Discodoris pusae and Anisodoris stellifera. Acid secretion is well known from certain other opisthobranch and prosobranch molluscs, and the discovery of its occurrence in dorids means that it has evolved independently at least four times in the Mollusca. In D.pusae and

A.stellifera, the acid glands are large subepidermal pits with a mucous plug and a muscular sphincter. The acid is inorganic and contains sulphate ions. It may register pH 1 or 2 close to the skin of the mollusc. Another species of Discodoris was found to secrete acid of a similar pH. In this species acid-secretion is from the ordinary epidermal cells, and subepidermal glands are totally absent. In the light of these results, the evolution of acid-secretion is discussed.

In all four suborders of the Nudibranchia and in the Sacoglossa, species with dorsal papillae or cerata have evolved. In all cases where these have been studied, they have been found to be of defensive importance. Cerata usually contain glands, are mobile, can be autotomised without harm to the mollusc, and may have yet other defensive mechanisms. The Eolidacea are the best known of these groups with defensive dorsal papillae. In the eolids, some species are camouflaged, but none has been shown to possess warning coloration. Cerata may be brightly coloured to direct attacks away from the head, and the behaviour of the mollusc supports this view. Cerata can be autotomised and regenerated, but the ease with which autotomy occurs varies between different species. Cerata are especially sensitive at their tips, and it is shown that this is because there is a high concentration of neurosensory cilia in this region. Stimulation, by touch, of these cilia can cause ejection of nematocysts or of glandular secretions as a defensive response.

Ceratal glands are described for a number of species of eolid. The role of the cellules spéciales is discussed, and it is concluded that these are not of defensive importance. Their content of RNA and of protein suggests that they synthesize protein, and their high glycogen content suggests that they are storage cells. They may have a duct in some species, and could thus be excretory.

Simple, unicellular mucous glands occur in probably all eolids, but they may be epidermal or subepidermal in different species. They probably protect the animal from abrasion. They may also be responsible for the mucin cuticle, or for the presence of a mucous film just outside the cuticle which is continuously being carried off the tips of the cerata by ciliary action. Mucous glands are the only glands present in some species, for example Eolis M and Tergipes despectus. In other acleioproct eolids there are glands which have a defensive function: the species of Catriona studied have two types of defensive gland, whilst the eubranchids studied have three and possibly five types of defensive gland. These defensive glands are usually concentrated at the tips of the cerata and are exuded when the animal is violently disturbed. Some of them are muscle-operated and are proteinaceous, others contain mucopolysaccharides or mucoproteins. In the smaller species of both Catriona and Eubranchus, the surface area of ceratal epithelium is limited, and the defensive glands are packed so as to utilize all available space. The method of packing the glands is different in the two genera,

suggesting that it is a case of parallel evolution. It is further suggested that the defensive glands of Calma, Catriona and the Eubranchidae are convergent. In the cleioprocts studied, no such concentration of glands at the ceras tip was found, but all species possess two or more types of ceratal gland. There is evidence that some of these may be defensive in function.

Nematocysts are used in defence by many eolids, but for them to be effective, they must be ejected from the cnidosac and an appreciable percentage of them must explode. It is shown that whilst nematocysts are frequently ejected and exploded as a defensive reaction, there is considerable variation between different species. In Calma, glands are of considerable defensive value, but nematocysts are totally absent; whilst in Tergipes despectus, defensive glands are absent and nematocysts are important in defence. Other species utilize both defensive mechanisms, but to varying extents. In the Cleioprocta generally, and particularly in the Aeolidiidae, glands are not of great importance in defence whilst nematocysts are; but in the Eubranchidae and in Catriona glands are well-developed and of considerable defensive importance. There is even variation within one genus. Thus Catriona tina rarely uses glands but frequently uses nematocysts in defence, whilst C.perca rarely uses nematocysts but frequently uses glands. This interspecific variation in behaviour is probably related to the different predators which different species of eolid are likely to encounter.

The predators of nudibranchs in the sea are not well known,

but laboratory experiments have demonstrated that many nudibranchs are relatively unpalatable to many species of fish. However, some species of fish are not deterred from eating eolids by nematocysts, and it is likely that it is with relation to these predators that glandular defensive mechanisms have evolved. Thus nematocysts may be protective against one set of predators whilst glands are protective against another set of predators.

Nematocysts may be damaging not only to potential predators of eolids, but also to the eolid itself when it crawls over coelenterates. The mechanism by which eolids escape damage from nematocysts is not known, but there is evidence that the vesicular structure of the epidermis is involved.

It is concluded that in many nudibranchs the defensive system involves several distinct mechanisms which come into action in series. There is some evidence that certain mechanisms are adapted to specific predators. The eolidiform condition is a particularly efficient defensive adaptation since it concentrates several mechanisms into that part of the mollusc which is expendible, and which is the first to be encountered by a potential predator.

Three papers are included in the appendix. The first describes the occurrence of Polycera elegans (Bergh) in Britain, and discusses its taxonomy. The second gives notice of a new species of bivalved gastropod from Jamaica, and the third is a description of this animal.

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INTRODUCTION

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INTRODUCTION

The typical mollusc is more or less completely covered by a thick calcareous shell which presents a number of problems not found in shell-less animals. The most important of these problems are :

1) Mechanical. The shell (in gastropods) is asymmetrical and hence lopsided. It is also heavy, even under water; and thus it impedes movement and renders a rapid escape from predators an impossibility.

2) Sanitary. Since the molluscan shell covers most of the body, the parts from which the anal and renal ducts can discharge are limited. It is not uncommon for the reproductive ducts to open in close proximity to the anal and renal ducts (in Calliostoma, for example), and clearly this could be detrimental if the eggs were to be harmed by faecal and renal material. Eales (1950) discusses how many molluscs have overcome these sanitary problems.

3) Respiratory. Since the shell covers most of the body surface, gaseous exchange can only occur in limited parts. These parts are the gills, and it is important that there should be a regular flow of clean water over their lamellae.

The shell has only one advantage to outweigh these important disadvantages: it is protective. Very few molluscs escape

by movement, but they can retract into their shells and remain out of reach of most predators until danger has passed.

The molluscan shell, although it is hard and impenetrable, does not protect the animal from all predators. The barrier of the shell is penetrated in at least four different ways, for instance:

1) Some animals, such as flatfish, swallow molluscs whole, and dissolve the shell in their acid stomach.

2) The skates and rays have powerful batteries of teeth and they crush molluscs before swallowing them.

3) Starfish evert their stomachs into the shells of molluscs, digest and dissolve the soft parts, and then suck up the resultant fluid.

4) Shell borers drill holes through the shell and then suck up the soft parts inside. This group includes the gastropods Nucella, where boring is entirely mechanical, and Natica, in which boring is facilitated by the secretion of acid (Graham, 1952).

It is probably because the shell has proved ineffective as a barrier to so many predators that secondary defensive adaptations have evolved in many molluscs. The most widespread of these is secretion of slime by simple mucous glands (Graham, 1957). Defensive protein glands may also be present on the sides of the foot, at the mantle edge, or in the hypobranchial gland. Muscle-operated glands have been found in Acmaea, Calyptraea, and Crepidula (Graham, 1954;

Fretter and Graham, 1962) Thompson (1960,a;1961) describes defensive secretion of inorganic acids in Lamellaria, Velutina and Trivia. Another type of defensive adaptation is camouflage, but a shell is exceedingly difficult to camouflage since it normally has a sharp outline. This outline is broken up by ridges and spines in Murex, and by a permanent covering of the mantle in Lamellaria which resembles in colour and texture the sponges and tunicates on which it is found. Secondary defensive mechanisms may be directed towards one specific predator, particularly the starfish. For example, Nassa responds to a starfish by a series of violent jerks caused by sudden contractions of the foot. Physa responds similarly to the fresh-water leech Glossiphonia. In Natica, the foot covers the shell making it impossible for a starfish to grip it; and the lamellibranch Pecten swims by clapping its valves when touched by a starfish. Russell (1934) discusses these examples and gives references.

Thus the molluscan shell does not protect the animal from all predators, and even shelled molluscs often possess accessory defensive mechanisms. In the opisthobranchs, the shell is reduced or absent, and is rarely of defensive value. Bulla and Haminoea have glands similar to those of the prosobranchs Acmaea and Calyptraea (Fretter and Graham, 1962), but the shell is still of protective value. Aplysia secretes a purple fluid when it is disturbed; Pleurobranchus, Berthella and Philine all secrete inorganic acids (Thompson, 1960,a); other species, such as Scaphander, possess glands which are probably of

defensive value (Thompson, 1960,b). In Onchidella the shell is completely lost, and protection is presumably entirely by means of glandular secretions. In those molluscs which are protected by glandular secretions, the shell may become a positive disadvantage, since it may no longer be of defensive value, and it gives rise to mechanical, sanitary and respiratory problems. In such animals, selection will favour reduction of the shell, and in some cases it may be lost altogether. The shell has been completely lost in this way at least seven times in the Opisthobranchia (eight if we include the Onchidiidae, as is recommended by Fretter, 1943). Table 1 illustrates that loss of the shell has occurred in every group of the Opisthobranchia except the Thecosomata; it has probably occurred several times independently in both the Sacoglossa and the Nudibranchia.

Table 1. Opisthobranch groups in which the shell has been lost. The classification is that of Horton (1958), but with the addition of the Onchidiacea (Fretter, 1943; Marcus, 1961).

Order	Families with shell-less genera
Cephalaspidea	Runcinidae
Anaspidea	Aplysiidae
Thecosomata	- none -
Gymnosomata	- all -
Sacoglossa	Hermaeidae, Elysiidae, Limapontiidae
Acochlidiacea	- all -
Onchidiacea	- all -
Notaspidea	Pleurobranchidae
Nudibranchia	- all -

Two possibilities are open to the shell-less or nudibranch mollusc which are closed to shelled molluscs.

1) The nudibranch mollusc can occupy habitats such as crevices which are too narrow to take a shell, or exposed places where a shelled mollusc would be very conspicuous. Shelled molluscs can of course live in exposed places, but they cannot easily be camouflaged, and are therefore an easy prey for molluscan predators.

2) The nudibranch mollusc can develop appendages on the dorsal surface in the position formerly occupied by the shell. These may perform several functions: they may be respiratory, they may contain branches of the liver for digestion and assimilation, or they may be of defensive importance.

Both of these possibilities can only be utilised if the mollusc is adequately protected from predators. The defensive systems of nudibranch molluscs may involve protective coloration, defensive glands, and the presence of expendable papillae which can be regenerated. This thesis investigates some of the defensive mechanisms which are used by shell-less molluscs, and attempts to show how in some species the complete defensive system is composed of several distinct mechanisms.

In Chapter 2, the Sacoglossa are examined, and the defensive mechanisms of Stiliger are described. The Sacoglossa contains many shell-less or nudibranch molluscs, but these are quite distinct from the order Nudibranchia, and the shell-less condition is convergent. Pelseneer (1906) classified the elysioid sacoglossans in his suborder

Nudibranchia, but this convergence is now recognised, and the classification followed in this thesis is based on that of Odhner (1939). Morton (1958) compares the two systems of classification. The Sacoglossa are a uniform group as regards feeding methods, but are exceedingly diverse in external morphology. The defensive mechanisms of both shelled and shell-less genera of this order are compared, and it can be seen that Stiliger and Berthelinia represent two extreme types; the first with no shell and well developed ceratal defensive mechanisms, and the second with a pair of bivalved shells completely enclosing the body, and no cerata.

Chapter 3 is an introductory section to the Nudibranchia. It discusses the radiation into a number of distinct types that has occurred in this order, particularly the evolution along several independent lines of the eolidiform condition. The defensive mechanisms of the Dendronotacea and the Arminacea are summarised, but our knowledge of these groups is very incomplete.

Chapter 4 describes the defensive mechanisms of the Doridacea, and includes work on acid secretion in Anisodoris and Discodoris. There is, however, little direct evidence of the defensive nature of most of the mechanisms discussed in this chapter.

Chapter 5 discusses the defensive mechanisms of the Eolidacea, and describes how the different mechanisms are utilised to a varying extent in different species. In some eolids nematocysts are important in defence, in others glands are, and in yet others both nematocysts and glands are equally important. In the Eubranchidae and the

Cuthonidae the defensive glands are well developed and some of them are muscle-operated.

Chapter 6 summarises our knowledge of predation in nudibranchs. Very little is known, but it is likely that most nudibranchs are unpalatable to most species of fish.

General conclusions are presented in Chapter 7. It is shown that many nudibranchs have several lines of defence, and that some defensive mechanisms are probably directed towards specific predators. Attention is drawn to the fact that parallel evolution has occurred in the naked opisthobranchs with respect to several characters, one of which is the eolidiform condition. It is concluded that the eolidiform condition is one which concentrates the defensive mechanisms of the mollusc into the cerata, and that the possession of cerata has been responsible at least in part for the success of these animals.

Chapter 2

DEFENSIVE ADAPTATIONS OF THE SACOGLOSSA

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Introduction to the Sacoglossa

The most important taxonomic character shared by the different genera of the Sacoglossa is the presence of a force-pump mechanism for piercing and sucking algae. This mechanism includes a muscular, suctorial pharynx, and dagger-shaped radular teeth. The shape of the pharynx and its mode of action has probably evolved in each species with respect to one or a few species of alga. Gascoigne (1956) shows how the pharyngeal morphology in the genera Limapontia, Acteonia and Alderia varies with the type of alga preferred by each particular species, and Edmunds (1963, in the appendix to this thesis) gives evidence that the same applies to the genus Berthelinia.

Defensive mechanisms of the Sacoglossa

Since there is this close relationship between the sacoglossan and its food plant, we might expect the defensive system of the mollusc to become adapted to the alga, particularly as regards colour. Thus whilst most sacoglossans are green, and hence inconspicuous on the green algae on which they feed, for example Elysia viridis (Montagu) (Garstang, 1889; Hecht, 1896; Tchang Si, 1931), Hermaea dendritica (Alder and Hancock) (Garstang, 1889; Evans and Evans, 1917, Lobiger serradifalci (Calcara) (Tchang Si,

1930) and Berthelinia caribbea Edmunds (personal observation), other species which feed on non-green algae correspond to their food in colour. For example, the red Hermaea bifida (Montagu) has been recorded from four species of red alga (Griffithsia by Alder and Hancock, 1845-1855; Renouf, 1935; and Miller, 1961; Delesseria by Garstang, 1889; Bornetta by Cornet and Marche Marchard, 1951; and Heterosiphonia by Plymouth Marine Fauna, 1957), and one or two unidentified species of brown Stiliger have been found on a brownish alga (personal observation). The crypsis is brought about by the colour of the food plant persisting in the liver of the mollusc in the cases of Elysia and the Hermaeidae, but the green is found elsewhere in the body of Berthelinia. Most green sacoglossans would be cryptic on any green alga, so there is no specific resemblance to a particular organism as occurs in some of the dorid nudibranchs. These are all examples of a general resemblance in colour. Thus it would appear that colour is of some importance in the defensive systems of the Sacoglossa, but since the resemblance is not specific, it is unlikely to be the only defensive mechanism present.

The defensive mechanisms of the Sacoglossa have not received the attention that has been paid to the eolid or the dorid nudibranchs. They can be listed under six headings:

1) Camouflage. This is widespread in the group, but is probably not universal. Examples have already been given.

2) The Shell. Arthessa has a bulloid shell into which it can retract completely. Lobiger and Oxynoë possess small shells which

may also be of some protective value. The bivalved gastropods, Berthelinia and Julia, possess two shells into which the animal can retract when it is molested. There can be little doubt that the bivalved shell is of defensive importance in B.caribbea since the animal is very sensitive to tactile stimuli, and responds immediately by withdrawing between its valves. After a few seconds, the valves open slightly, and the animal starts to emerge (Edmunds, 1963; and unpublished observations). Whilst retracted, the mollusc retains a hold on its alga by means of a fine byssus-like thread.

3) Papillae. Species of the Hermaeidae have well developed mobile dorsal papillae or cerata which can be autotomised. It is possible that a predator that attacked a Stiliger would obtain a meal of cerata whilst the mollusc crawled away unharmed. The missing cerata can probably be regenerated. Sacoglossan cerata are thus important in defence, but they may also have a digestive function in the Hermaeidae, whilst in Alderia they are respiratory (Evans, 1953). Lobiger has four tentacle-like appendages which are also of defensive importance although they do not contain branches of the liver and are hence not similar to the cerata of the Hermaeidae. Tchang Si (1930; 1931) describes how they are moved when the animal is irritated, and how they may be autotomised.

4) Glands. Subepidermal glands which are probably defensive have been described from Elysia and Hermaea by Hecht (1896). Hecht

believes that H.bifida has a protective smell of hydrogen sulphide, but this has not been confirmed. In H.dendritica, there are apparently two types of subepidermal gland, one of which is blackened with osmium fixatives. Defensive glands are concentrated in the cerata where these are present (for example, in Hermaea). In Berthelinia, the hypobranchial gland is of defensive importance. When the animal is violently irritated, it exudes a viscous white fluid from the hypobranchial gland (Burn, 1960; Edmunds, 1963). Some of the gland cells contain mucus, but others are responsible for the white threads which disperse gradually in the water. Muscles run between the gland cells in B.caribbea, and presumably these are responsible for exudation (Fig.1).

5) Leathery skin. Evans (1953) describes how the skin of Alderia modesta (Lovén) is tough, and bound to the underlying viscera like the skin of a leech. This is probably an adaptation to prevent desiccation, but it may protect the animal from some predators as well.

6) Unpalatability. Thompson (1960,b) found that Elysia viridis is unpalatable to some species of fish. Inedibility may thus be an important defensive adaptation.

Defensive systems of shelled and shell-less species

There is a series of forms in the Sacoglossa from the bulloid Arthessa through Oxynoë and Lobiger with reduced shells, to the

nudibranch species on the one hand, and to the bivalved gastropods on the other. The defensive systems of sacoglossans are not well known. In this section I shall attempt to describe and compare the defensive systems of three species of sacoglossan. All are found on the alga Caulerpa.

Lobiger serradifalci (Calcara).

In Lobiger, the shell only protects the mantle region of the body, but the mollusc is also protected by its colour and by the possession of mobile and detachable appendages (Tchang Si, 1930; 1931). It is not known if glandular secretions are important in defence. Colour change according to background has been reported from Lobiger viridis (Hornell, 1922) but has not been recorded in L.serradifalci.

Berthelinia caribbea Edmunds.

Berthelinia caribbea is difficult to detect on Caulerpa because it is camouflaged and because it often rests deep amongst the branches of the alga. The bivalved shell can completely enclose the animal except for a small opening at the posterior end - the anal siphon (Edmunds, 1963, appendix to this thesis). One might expect that with a bivalved shell, other defensive mechanisms would be unnecessary, and would be poorly developed or absent. But this is evidently not the case in Berthelinia since violent disturbance, such as when some object is inserted between the valves preventing

their closure, elicits the response of a copious secretion of a viscous white fluid from the hypobranchial gland. The fact that the gland cells have muscle fibrils round them for rapid and efficient exudation suggests that glandular secretion is important in defence in Berthelinia, but there is no direct evidence that this in any way deters potential predators.

Stiliger vanellus Marcus

A single specimen of this species was found with Berthelinia caribbea on the green alga Caulerpa verticillata Agardh growing in the mangrove swamps at Port Royal, Jamaica. Although the body of the living animal is silvery-grey with cream, black, greenish brown and crimson markings on it, the animal is inconspicuous to the human eye because of its small size. The living animal was only 3.5 mm. long, with the rhinophores and the largest cerata reaching 1 mm. On Caulerpa, however, S.vanellus is certainly not camouflaged. Violent disturbance results in autotomy of cerata, as in other species of Stiliger (personal observation of two undescribed species). On two occasions when the animal was prodded with forceps, large, white, subepidermal glands in the cerata were seen to eject their contents as a cloud of discrete droplets which disperse in the water after a few minutes. Each gland exuded a single large white droplet. Similar white glands can be seen in the living animal on the rhinophores, the foot, and the back. Since they are exuded when the animal is violently disturbed, it is reasonable to suppose that they are defensive in

function. The white glands can be seen most easily at the tips of the cerata where there is no black pigment. They may also occur all over the cerata, but the epidermal black pigment obscures them.

The animal was fixed in Heidenhain's "Susa", and sections in paraffin were cut at 6μ. The cerata have an epidermis about 3μ in thickness, with a cuticle of about 0.5μ. In young cerata the epidermal nuclei are almost spherical; but in larger cerata, where the epidermis is apparently somewhat stretched, the nuclei are oblong and 4 - 5μ long. The epidermis is packed with pigment granules of about 0.5μ in diameter, and over much of the ceratal surface the nuclei are completely obscured by them (Figs. 6 and 7). The ceras tip has many fewer pigment granules, and since the tip is the only part that is not blackish-green in life, these granules would appear to be responsible for the blackish-green colouration. Scattered cells in the epidermis are ciliated, and these have few or no pigment granules. In the fixed animal, the black colour is preserved; but in vivo there are many minute whitish or transparent specks in the black of the cerata. These are probably the ciliated cells which lack pigment. No epidermal glands were detected.

The bulk of the ceras (Figs. 2 and 3) is filled up with a loose connective tissue in which are three types of gland. In the centre of the ceras is a diverticulum of the liver which is somewhat lobed, but does not reach to the tip of the ceras as it does

in the eolid nudibranchs. The cerata are mobile, and their shape varies in the living animal. The muscles that bring about contraction of the cerata are large longitudinal ones passing from halfway up the ceras down into the body of the animal. They are 5μ in diameter, and the nuclei, 10 - 12μ long, lie inside the contractile cylinder. The only other muscles detected in the cerata with Masson's trichrome are small fibrils in the region of 0.5μ diameter which appear to be associated with large mucous glands (Figs. 5 and 7). These glands are unicellular, with an elongated nucleus up to 10μ long compressed at the side of the cell. The secretion, which completely fills the rest of the cell, is strongly positive to PAS and weakly positive to dialysed iron, so is probably an acid mucopolysaccharide. The size of the gland varies with the size of the ceras, but a large ceras (0.5 mm. long in fixed sections) has mucous glands near its tip which may reach 65μ in length. When one of these glands is cut obliquely through its margin, the fine muscle fibrils mentioned above can be seen. They seem to be arranged irregularly round the mucous glands, but usually closely invest them in a fine sheath.

The other two types of gland are the granular and the non-granular glands. The granular glands (Figs. 4, 5 and 7) are up to 34μ in length, with a small basal nucleus of about 6μ. There is a duct through the epidermis for discharge. With Masson's trichrome, the contents of these glands may be lightly stained with xylidène

red and completely filling the cell, or deeply staining and slightly pulled away from the wall of the cell as a large globule. They are negative to the P.A.S. test and to Hale's dialysed iron, and so do not contain mucopolysaccharides. With Sakaguchi's test for arginine, they are strongly positive, and the chief constituent of them thus appears to be protein.

The non-granular glands, when mature, are up to 33μ long, with a slightly oblong basal nucleus of about 9μ in length. Residual cytoplasm is present round the nucleus and extending up towards the orifice of the gland as a thin layer close to the wall (Figs. 4, 6, and 7). The rest of the gland appears empty in "Susa" preparations apart from a residue of material which is weakly basophilic - it takes up green with Masson's trichrome. This residue is negative to dialysed iron, and only weakly positive to P.A.S., so if mucopolysaccharides are present, they must be neutral and not acidic (Pearse, 1960; Casselman, 1959). Proteins appear to be absent from the residue (Sakaguchi's test), but are present in the cytoplasm. Young non-granular glands lie deeper (Fig.7) and have as yet no duct to the epidermis. The granular cytoplasm completely fills the cell, and the 9μ diameter nucleus is spherical. There is no central cavity, and no basophilic residue. A 4μ nucleolus is present in young and mature glands which is positive to Sakaguchi's test for arginine. Intermediate stages develop a small cavity and residue, and the nucleus gradually becomes more elongate. Young glands are easiest

to find in young cerata, and are absent in large cerata, indicating that in the oldest cerata they have all become mature.

Both granular and non-granular glands also occur on the foot of Stiliger, and are more or less as abundant as the white glands seen in the living animal. Mucous glands are much more abundant on the foot than are the white glands, and in any case mucous glands are not usually white in colour. Thus it is not known whether the white defensive glands of S.vanellus are the granular or the non-granular glands of fixed preparations. White glands are often osmiophil, but lack of material made it impossible to check this. Bürgin-Wyss (1961) reports red, yellow, green and blue glands from S.costai, but such brightly coloured glands were not observed in S.vanellus.

The granular and the non-granular glands do not appear to be muscle-operated when examined with an oil immersion lens and ordinary illumination. But with polarised light, the walls of both these and the mucous glands can be seen to contain birefringent fibrils. The mucous gland muscle-fibrils seen with ordinary illumination are similarly birefringent when viewed under polarised light, so it is likely that all these types of gland are muscle-operated. The mucous secretion is probably important in defence as well as the white fluid, since mucous glands are well developed in the cerata and are muscle-operated. Thus two and probably all three of the ceratal glands of Stiliger vanellus are involved in the defensive system of the mollusc.

Discussion and conclusions

Thus *Lobiger serradifalci*, *Berthelinia caribbea* and *Stiliger vanellus* each possess two or more defensive mechanisms. No single mechanism is capable of protecting the mollusc from all of its predators, not even when a second shell is present. As in the tropical insects studied by Kettlewell (1959), these molluscs have several lines of defence. In *Lobiger*, the first line is camouflage, but if the animal is discovered, the appendages are waved and may be autotomised when grabbed by the predator. In *Berthelinia*, the first line of defence is again camouflage, but should the mollusc be detected, it is still protected by its shell. If the shells are forced open, there is yet a third line of defence in the form of the secretion of fluid from the hypobranchial gland. Finally, in *Stiliger vanellus*, should the fluid from the ceratal glands not deter a predator, the cerata can be autotomised and left with the predator whilst the mollusc crawls away. Further investigation will probably show that other sacoglossans have a similar range of defensive mechanisms.

These results also give an indication of how the nudibranch condition could have evolved. If the shell of a shelled sacoglossan failed to give sufficient protection, selection would favour the enlargement of other defensive mechanisms which are more effective. These mechanisms might be papillae or glands, but they would already have been present in the shelled form. As they enlarged, so selection would favour the reduction and loss of the shell since then the glands or

papillae could occupy the whole of the dorsal surface of the animal. This has evidently occurred in the Sacoglossa at least once, and presumably analogous changes have taken place in the Nudibranchia and in the other shell-less opisthobranchs.

Figure 1. Section of hypobranchial gland of Berthelinia caribbea stained with Masson's trichrome. e., epidermis; g., unicellular gland; m., muscle fibril.

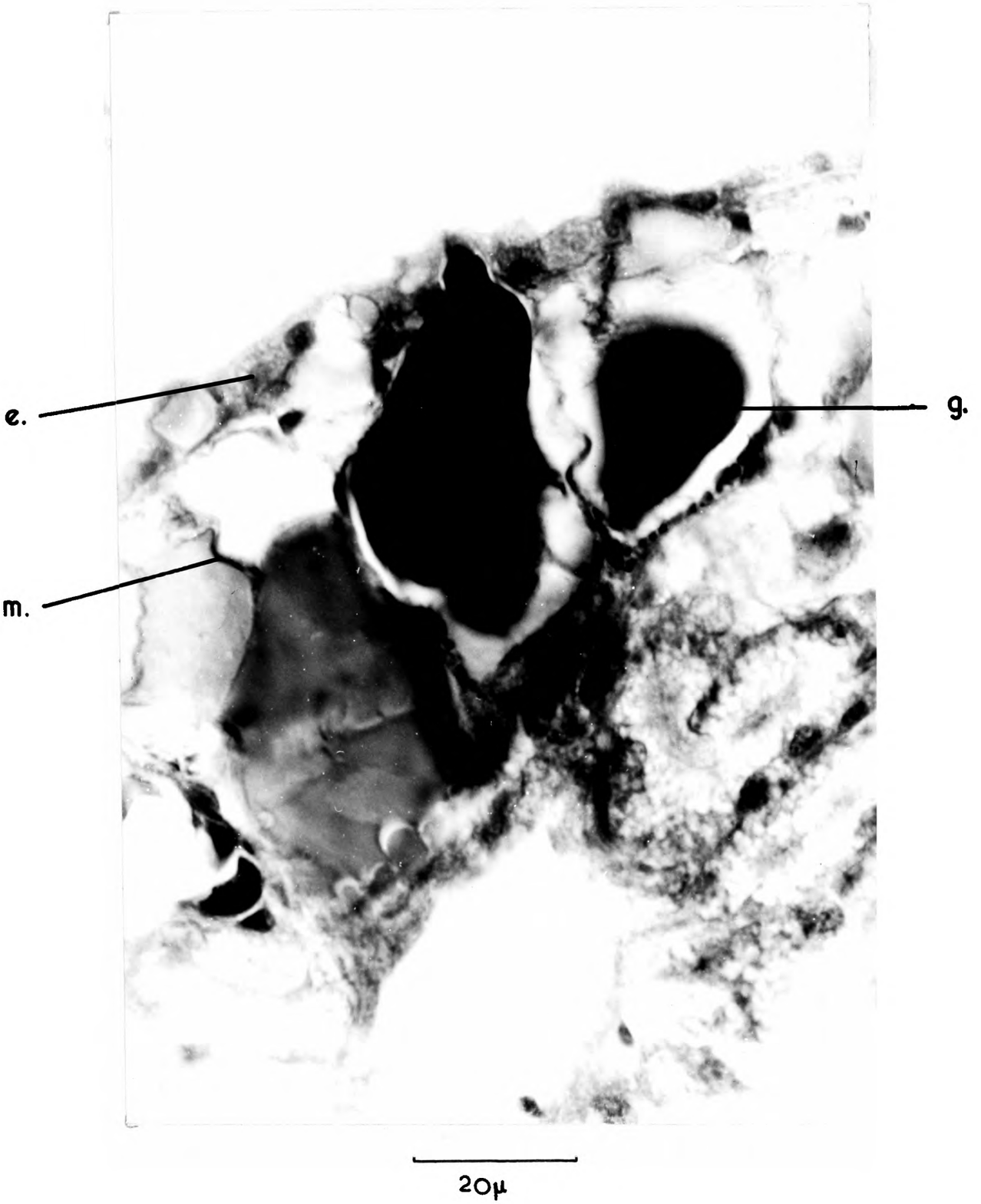
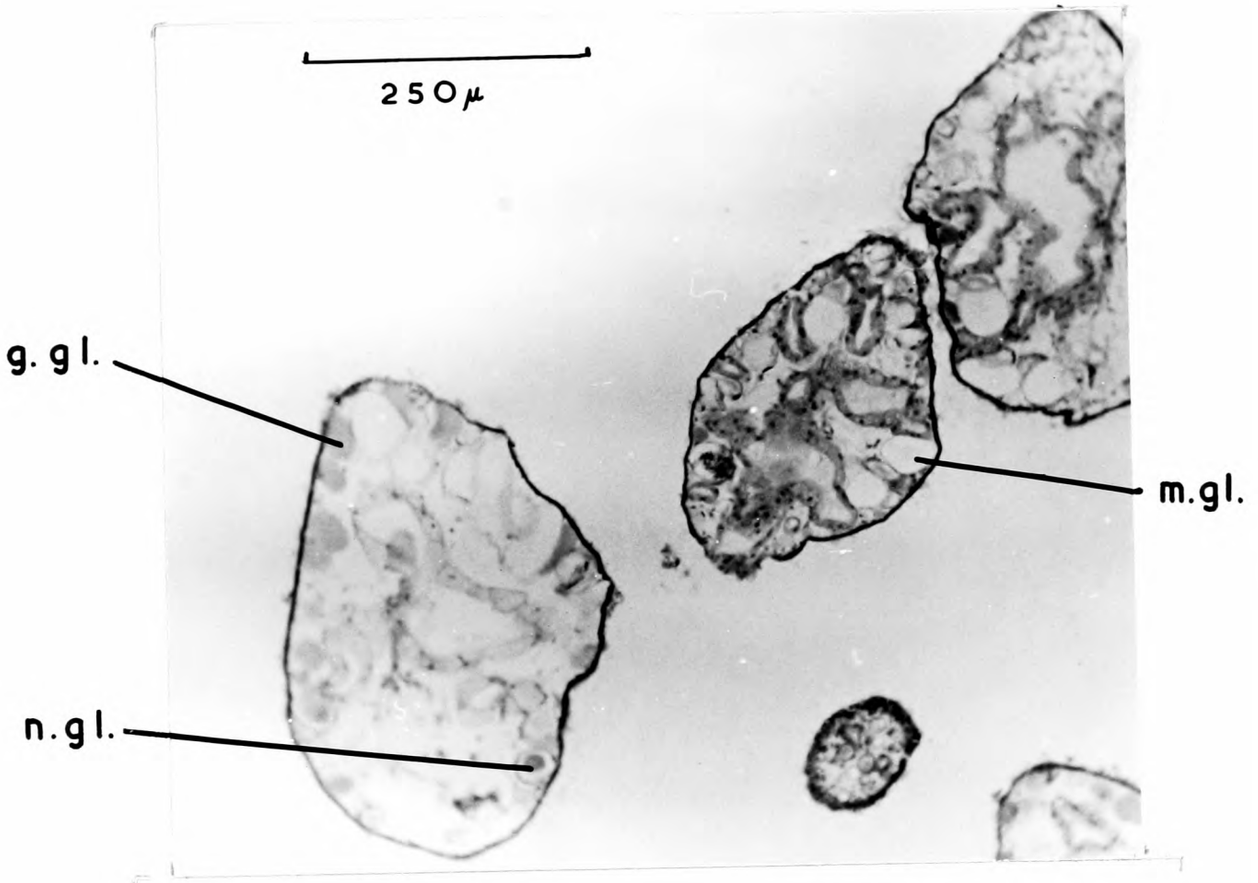
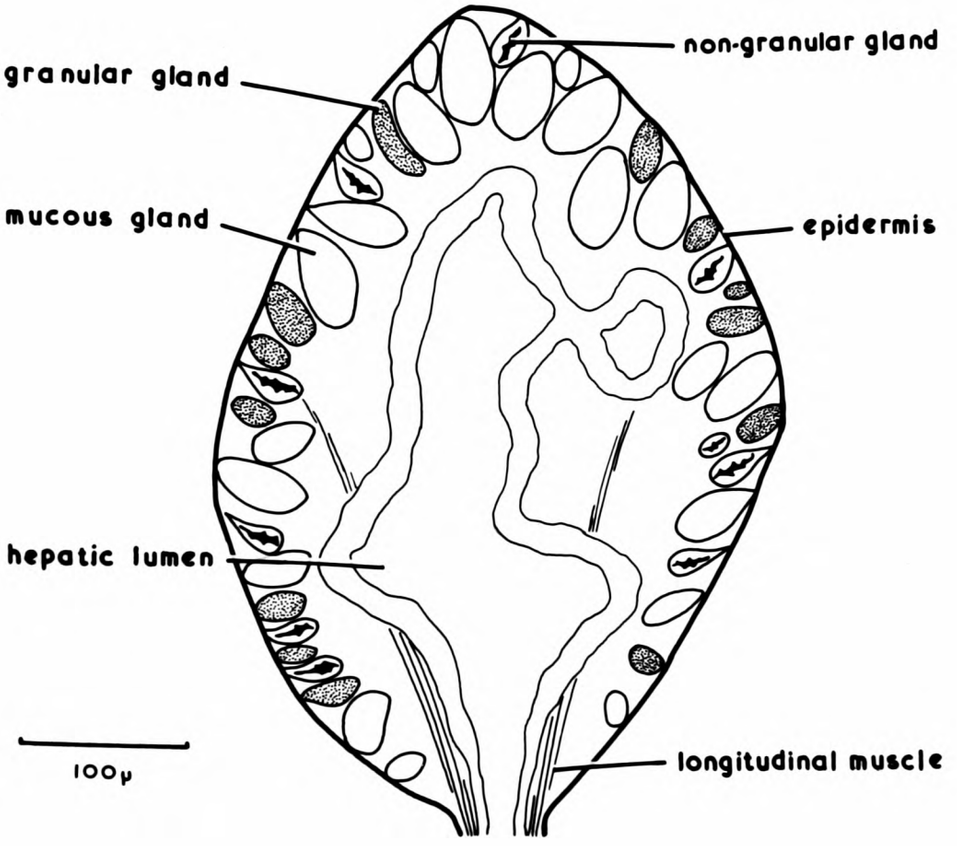

e.
g.
m.
20μ

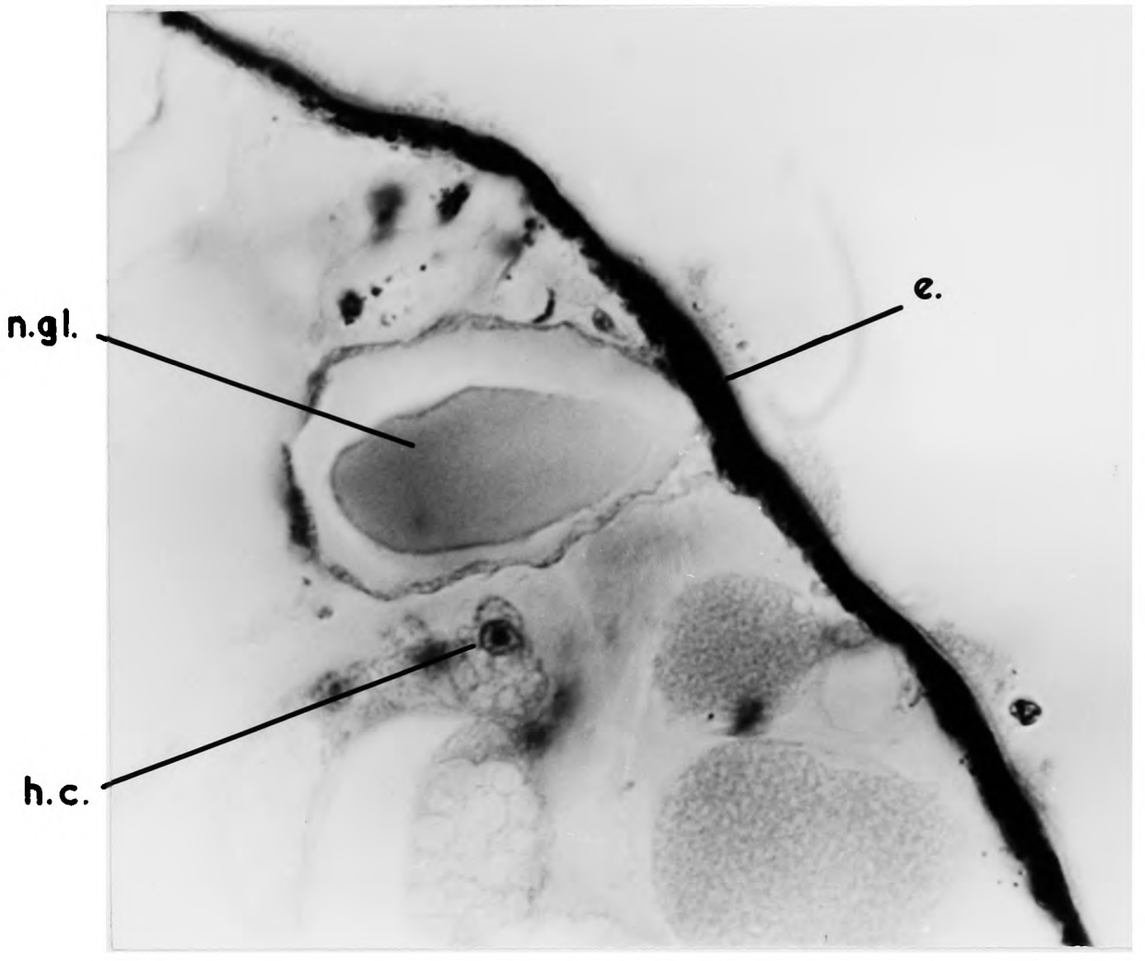
Figure 2. Cerata of Stiliger vanellus in longitudinal section, stained with Masson's trichrome. g.gl., granular gland; m.gl., mucous gland; n.gl., non-granular gland.

Figure 3. Diagram of ceras of Stiliger vanellus in longitudinal section.


250μ
g. gl.
m.gl.
n.gl.


non-granular gland
granular gland
mucous gland
epidermis
hepatic lumen
100μ
longitudinal muscle

Figures 4 and 5. Sections of cerata of Stiliger vanellus stained with Masson's trichrome. e., epidermis; g.gl., granular gland; h.c., hepatic cell; m.gl., mucous gland; n.gl., non-granular gland.


n.gl.
e.
h.c.

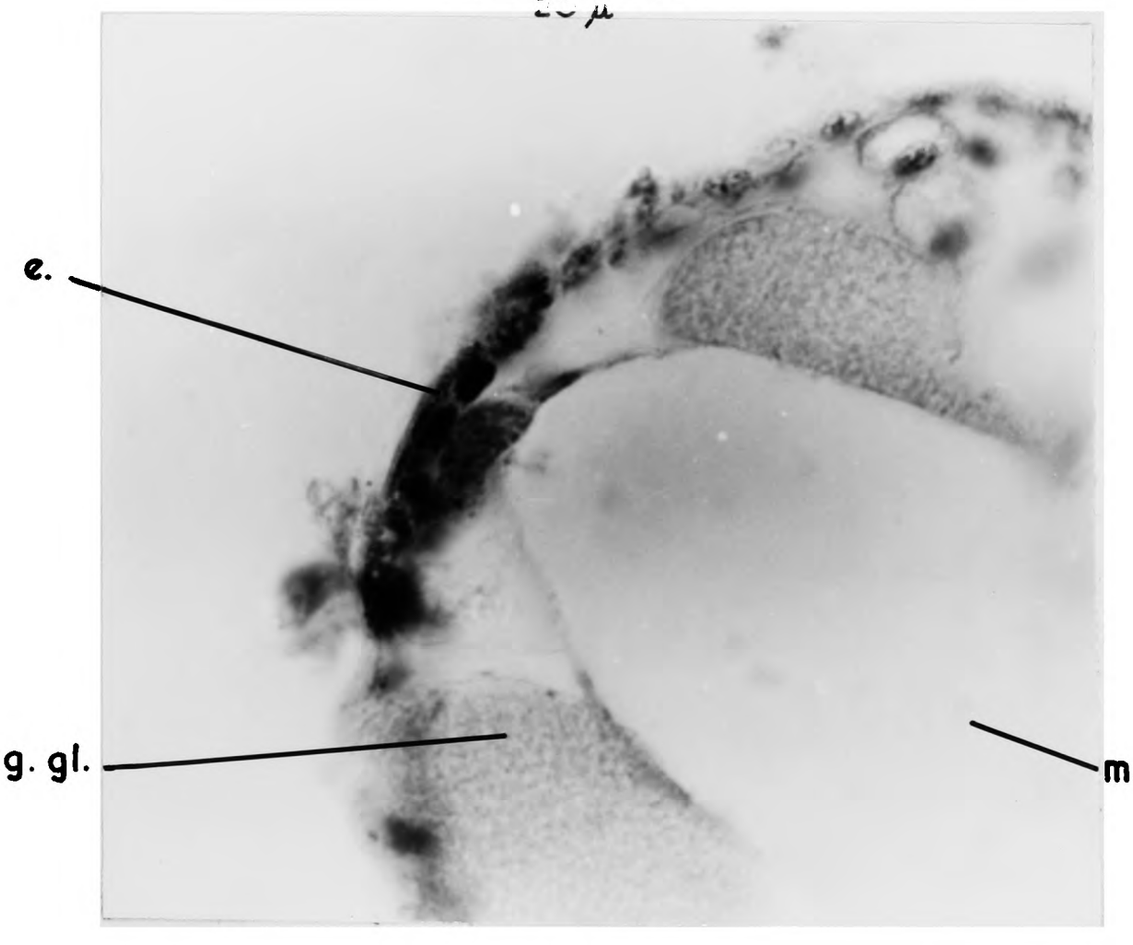
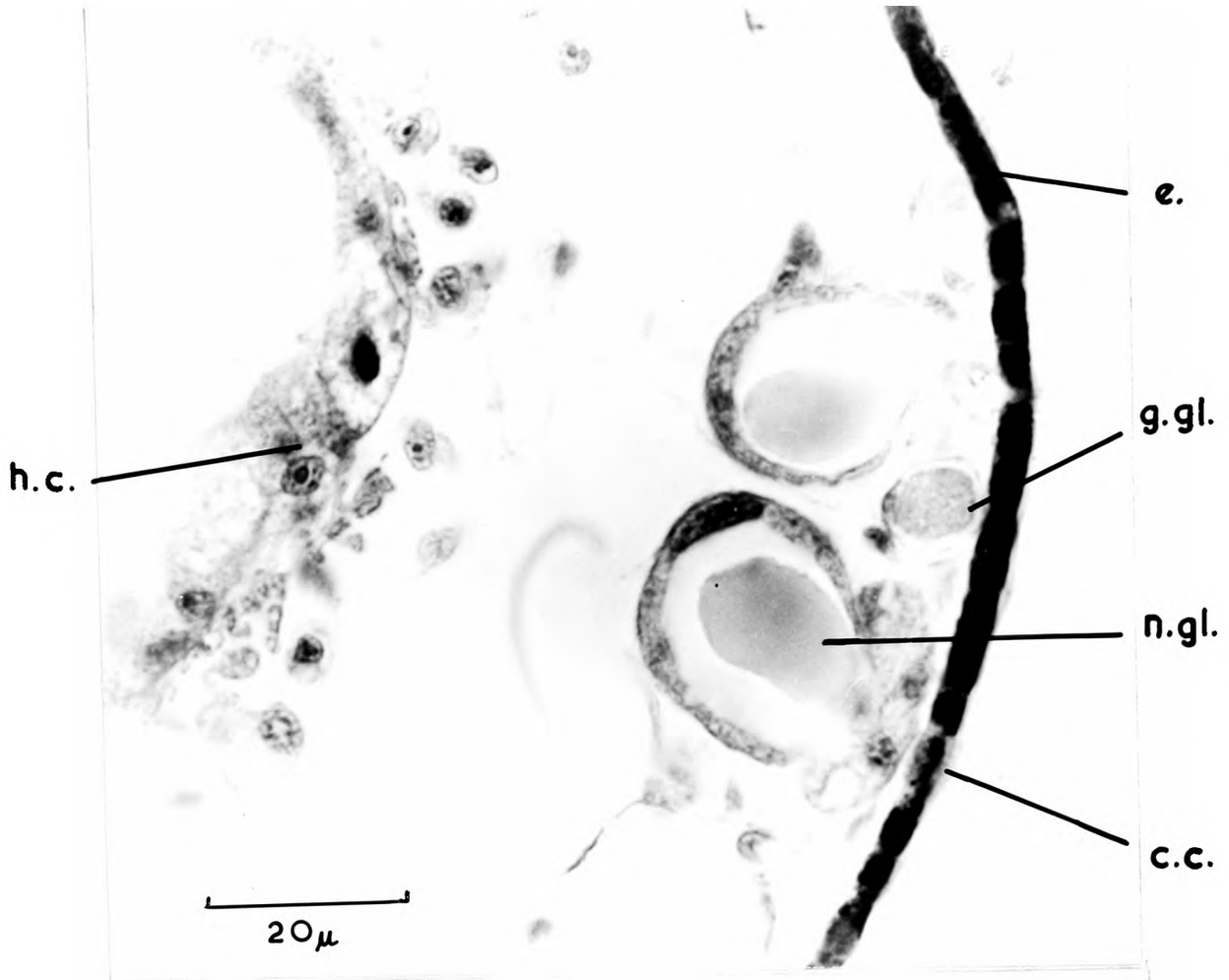
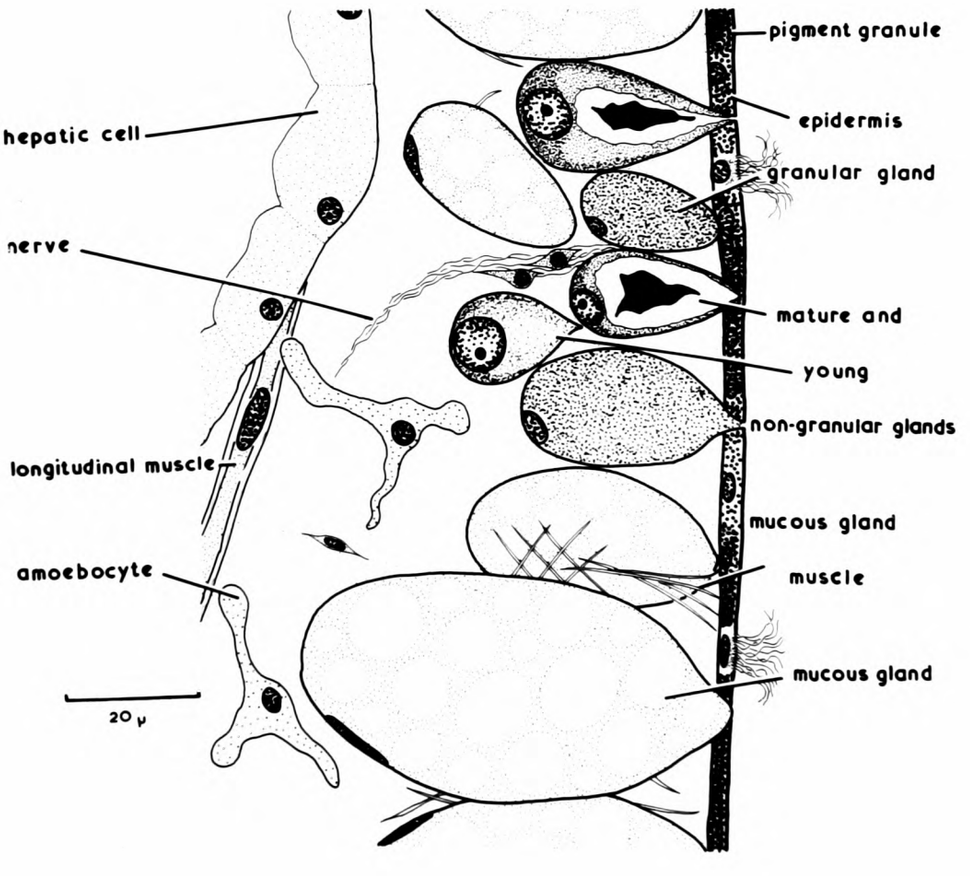

e.
g. gl.
m.gl.

Figure 6. Section of ceras of Stiliger vanellus stained with Masson's trichrome. c.c., ciliated cell; e., epidermis; g.gl., granular gland; h.c., hepatic cell; n.gl., non-granular gland.

Figure 7. Diagrammatic section of ceras of Stiliger vanellus.


e.
g.gl.
h.c.
n.gl.
c.c.
20μ


pigment granule
epidermis
hepatic cell
granular gland
nerve
mature and
young
non-granular glands
longitudinal muscle
mucous gland
amoebocyte
muscle
mucous gland
20 μ

Chapter 3

DEFENSIVE ADAPTATIONS IN THE ORDER NUDIBRANCHIA : INTRODUCTION

Chapter 3

DEFENSIVE ADAPTATIONS IN THE ORDER NUDIBRANCHIA : INTRODUCTION

The order Nudibranchia contains molluscs of several very different types. Odhner (1939) classifies them into four suborders: the Eolidacea, Arminacea, Dendronotacea and Doridacea. This is a considerable advance towards a phylogenetic classification compared to the system of Pelseneer (1906), discussed by Morton (1958), but Thompson (1962) believes that some of Odhner's suborders may themselves be polyphyletic. The presence of jaws in the Gnathodoridacea suggest that the Doridacea are derived from early tritoniid Dendronotacea, but an alternative view is that they evolved from the Notaspidea. These hypotheses are discussed by Morton (1958), Marcus and Marcus (1962), and Thompson (1962). The affinities of the Arminacea and the Eolidacea remain obscure - they could be related to the Dendronotacea, or they could equally well be independent derivations from shelled opisthobranchs.

Parallel evolution seems to have occurred in each of the four suborders of the Nudibranchia (Morton, 1963). The parallel that I wish to stress here is the eolidiform condition, in which the dorsal surface of the mollusc is covered with papillae or cerata. I hope to show that these papillae are of defensive importance and that they are a highly efficient defensive system. In the Eolidacea,

each ceras contains a branch of the liver and this opens at the tip into a sac, the cnidosac. In Chapter 5 I shall describe the ways in which different species of eolid use the cnidosac and the nematocysts inside it as a means of defence; here it suffices merely to mention that the eolid ceratal defensive system may involve nematocysts, glands, autotomy, colour and behaviour.

Among the Arminacea, both Dirona and Janolus are eolidiform. The cerata contain branches of the liver, but there is no cnidosac and no nematocysts. Glands which are probably of defensive importance occur at the tips of the cerata in Proctonotus mucronifera (Hecht, 1896) and Janolus comis (Marcus, 1958). In both of these species, special neurosensory cells are associated with the ceras tip. These presumably have a similar function to the neurosensory cells described in Chapter 5 for certain eolids, but they have a very different structure. P.mucronifera and J.comis readily autotomise their cerata when they are irritated. I have studied J.cristatus at Plymouth and can confirm that in this species too, cerata are autotomised as a result of prodding the animal with forceps. As in the eolids, when J.cristatus is prodded, the cerata are waved violently to and fro, and it is possible that glandular exudation occurs, but this was not observed.

Some further genera of the Arminacea are doridiform, but I can find no trace in the literature of their defensive adaptations.

The Dendronotacea are characterised by possessing rhinophores

which are retractile into turret-like sheaths. Appendages with the appearance of gills are often present, for example in Tritonia, Dendronotus and Melibe, but there is no evidence that they are actually respiratory. Probably they facilitate gaseous exchange because they give an increased surface area, but are not primarily respiratory as are the gills of dorids. Defensive glands are present in these appendages (Agersborg, 1921; Thompson, 1960,b); and in the pelagic Melibe and Tethys, these glands are believed to emit a protective smell (Crossland, 1911; Agersborg, 1921; Tchang Si, 1931). Crypsis in pelagic Dendronotacea involves transparency (Agersborg, 1921). Other genera of this group possess appendages which closely resemble eolid cerata. For example, in Doto and Hancockia, the cerata contain hepatic branches, and Hecht (1896) describes epidermal and subepidermal glands in Doto whose function is probably defensive. I have found that prodding several species of Jamaican Doto results in exudation of white material from epidermal glands of the cerata, and this again suggests a defensive function. Autotomy of papillae occurs readily in Doto, Hancockia and Tethys (Hecht, 1896; Tchang Si, 1931), but regeneration is only described for Doto. Hancockia even has cnidosacs, but how these function is not known (Marcus, 1957).

The Doridacea have a pair of rhinophores which are usually retractile into sockets, a median anus, and a circlet of gills which are usually retractile into a branchial cavity. Typically, the dorsal

surface is smooth, or spicular, and without papillae. Defensive adaptations of these dorids are discussed in Chapter 4. However, in the nonsuctorian Polycera and in the goniodorids Ancula and Okenia, eolidiform dorsal papillae are present. I have found several individuals of Okenia impexa in which some of the papillae were missing or broken off, so in some cases they may be detachable, and hence of use in defence. Defensive glands are concentrated in the papillae of Ancula and Polycera (Herdman and Clubb, 1890; Thompson , 1960,b).

Thus all four suborders of the Nudibranchia have evolved species possessing dorsal papillae or cerata. Usually these papillae contain branches of the liver, and in all cases which have been examined carefully, glands or nematocysts are present whose function is probably defensive. Usually, also, the papillae can be autotomised and regenerated. In Chapter 2 similar papillae were described for the sacoglossan Stiliger. Thus eolidiform papillae have probably evolved separately at least five times in shell-less molluscs - once in the Sacoglossa, and at least once in each of the four suborders of the Nudibranchia. The original function of these papillae may have been defensive, respiratory or to increase the absorptive area of the liver; but clearly the most important function now is that of defence. It would seem that the eolidiform condition is one in which the defensive system of the mollusc is concentrated in the cerata. Since a ceratal defensive system is present in so many unrelated nudibranch molluscs, it would seem likely that it gives very efficient protection from potential predators.

Chapter 4

DEFENSIVE ADAPTATIONS OF THE NUDIBRANCHIA: DORIDACEA

Chapter 4

DEFENSIVE ADAPTATIONS OF THE EUBRANCHIA: DORIDACEA

Introduction

Early work on the defensive adaptations of the Doridacea is described by Garstang (1889) and Herdman 1890). They find that there are two categories of dorids: forms with smooth or spicular dorsal surfaces, which are cryptic; and forms with dorsal papillae tipped with glands, which are not usually camouflaged. Other defensive mechanisms are now known, and these can be listed under six headings: camouflage, warning coloration, unpalatability, dorsal papillae, autotomy and glands. In the first section of this Chapter the evidence for the defensive importance of each of these six mechanisms is discussed in turn, but it should be emphasized that any one species of dorid may possess two or three of these mechanisms. For example, the defensive systems of most of the eight species of the Doridiдae and the Onchidoridiдae studied by Thompson (1960,b) include three mechanisms - crypsis, unpalatability, and defensive glands. The only other comprehensive study of the defensive system of a dorid nudibranch is that of Crozier (1916; 1917) for Chromodoris zebra. The second section of this Chapter describes work on one of these defensive mechanisms, acid secretion, and suggests how it may have evolved in dorids.

Section 1: Defensive mechanisms in the Doridacea

1) Camouflage

It is probably true that most of the Dorididae and the Onchidoridídae are cryptic in colour and texture, but the best known examples belong to the genera Archidoris and Rostanga. References to crypsis in other genera can be found in the papers of Garstang (1889), Herdman (1890), and Thompson (1960,b). Many of these dorids are normally found associated with the sponges on which they feed, but their resemblance to a sponge is so close that even when they are crawling slowly on a bare rock they may derive some protection. In some cases the resemblance may be of this general type, for example, in the Onchidoridídae which feed principally on Polyzoa (Miller, 1961); but other dorids have a specific resemblance to one particular type of sponge. In such a case the sockets for the gills and the rhinophores may resemble the oscula of the sponge, and the spicular texture of the dorid may be almost identical with that of the sponge. The behaviour of dorids is of the type one would expect to find in cryptic animals (Thompson, 1960,b). They are slow-moving, and when disturbed the rhinophores and gills are retracted whilst the animal remains motionless exposing the spicular and glandular notum.

Rostanga pulchra MacFarland is usually found on the sponge Oplitaspongia pennata Lambe which it resembles in colour and texture (Cook, 1962). Cook shows that R.pulchra finds O.pennata by chemotaxis in the laboratory, and prefers this species of sponge to four

other sponges tested. Cook reports it, in nature, from four other species of red sponge, but there is no evidence that it was feeding on them, and in any case these may have represented second choices in the absence of O.pennata. Similarly, R.rufescens Iredale and O'Donoghue is usually found on the sponge Microciona atrasanguinea Bowerbank to which it bears a close resemblance (Garstang, 1889; Hecht, 1896; Plymouth Marine Fauna, 1957; personal observation), but it is occasionally found on another red sponge Politasponcia seriata (Grant) (Bolgan, 1911; Yonge, 1949). Archidoris tuberculata Cuvier is often found on its principal food Halichondria panicea (Pallas) (Giard, 1888; Garstang, 1889; Herdman, 1890; Plymouth Marine Fauna, 1957), but it is also found away from this sponge. The red form of A.tuberculata (var.flammea Alder and Hancock) is usually found on red sponges, but this, too, is not invariable (Fisher, 1934). Cook (1962) shows that the closely related form A.montereyensis (Cooper) feeds on H.panicea in the laboratory when given a choice of five species of sponge, but that it does not find H.panicea by chemotaxis. In my experience, A.tuberculata is much more frequently found away from its preferred food than is R.rufescens, and it is likely that this is because it must spend much more time searching for its food than R.rufescens since it lacks a chemotactic food sense.

We can conclude that species of the Dorididae and the Onchidoridiidae are often cryptic to the human eye in their natural surroundings, especially when on their preferred food. They are probably

also cryptic to predators, but there is no evidence for this. In the case of the sponge-feeding Doridiidae, they may be guided to their preferred food by chemotaxis.

2). Warning Coloration

Although most dorids are dully coloured and may be cryptic, genera from four families have species which are often brilliantly coloured. It has been suggested that these are cases of warning coloration, and I shall therefore consider them in some detail. Hecht (1896) supposes that Triopa clavigera (Müller) and Polycera quadrilineata (Müller) possess warning coloration, and Crossland (1911) believes that species of Chromodoris are aposematically coloured. Thompson (1960,b) has recently studied the brightly coloured P.quadrilineata. Both this species and Ancula cristata (Alder) studied by Herdman and Clubb (1890) are conspicuous in their natural surroundings, and their behaviour when disturbed is to retract the rhinophores and gills, but to erect the dorsal papillae. These papillae contain glands (Herdman and Clubb, 1890) that are probably of defensive importance. However, Thompson stresses that there are insufficient grounds for regarding this as warning coloration. In cases of true warning coloration, the predator must learn to associate inedibility with colour. There is no evidence that any predator of these animals is capable of this type of behaviour, and without it, selection would clearly not favour the evolution of conspicuous recognition marks. Another possible function of these

bright colours is discussed by Garstang (1889), with reference to eolid colours. He suggests that bright colours on the papillae may direct attack towards the expendible processes and divert it away from the vulnerable body. This may be the case in Polycera quadrilineata, Ancula cristata, and Triopa clavigera, but it cannot be so for Polycera elegans (Bergh) and for the chromodorids because they lack dorsal papillae. P.elegans can be exceedingly conspicuous (Edmunds, 1961 - see appendix) but may not be so in its normal habitat. It is unknown whether or not it has any defensive glands.

The chromodorids are well known for their brilliant colours, and Crossland (1911) provides some evidence for warning coloration in C.reticulata Pease. Crossland fed various species of fish on the viscera of other fish and on the mollusc Margaritifera which had been preserved in formalin. These were readily eaten, but when specimens of C.reticulata and C.diardii were offered, they were refused, and were rarely even tasted. Crossland could find no repugnant smell, and concludes that this is a case of warning coloration. However, although Crossland describes C.reticulata as being conspicuous, Eliot (1911) finds it to be inconspicuous under normal circumstances since it prefers to live under stones. Crozier (1916) doubts whether warning coloration does in fact occur in chromodorids. He finds that C.zebra is conspicuous, but not normally eaten by fish. But he is also able to show that it is not colour that causes rejection, since red stained specimens are avoided just as are the normal blue ones. Instead, he believes that rejection is due to an unpleasant smell.

In Crozier's view, the bright colours of Chromodoris are immunity colours - colours that have arisen by chance as a result of selection acting on internal characters. My own experience is that the chromodorids Cadlina sp. and Glossodoris neona Marcus from Jamaica are conspicuous, but lack of an abundant supply of material rendered a thorough investigation impossible.

There is, therefore, insufficient evidence to conclude that warning coloration occurs in the Doridacea. The coloured papillae of Polycera and Ancula may be deflection marks, but the significance of the bright colours of the chromodorids is still not known. There is some evidence that it is aposematic, but there is also the suggestion of Crozier that it is of no selective importance at all.

3) Unpalatability

The work of Crossland (1911), Crozier (1916) and Thompson (1960,a and b) has already been referred to. They have shown that many dorids are not eaten by several species of fish, and may therefore be described as inedible. Inedibility is a relative term except in the case of powerful poisons, and Thompson found that damaged specimens are sometimes eaten. Further examples of this are given in Chapter 6.

4) Papillae

Mobile dorsal papillae occur in several genera of dorids, for example Polycera, Triopa, Ancula and Okenia. These papillae usually contain glands whose function is probably defensive. They have been

discussed in Chapter 3 and also in section 2 of this Chapter, so here I shall merely summarize these conclusions. The behaviour of dorids with papillae when they are disturbed is to erect the papillae. This probably directs attacks away from the head and body and towards the glandular papillae. The papillae are probably the most repellent part of the mollusc since they contain a high concentration of supposedly defensive glands, but this has not been thoroughly investigated.

Many other dorids possess a second type of appendage on the dorsal surface. Labbé (1929; 1933) describes these papillae which he calls caryophyllidia. They occur in Rostanga, Acanthodoris and Jorunna. Each caryophyllidium consists of a ring of long spicules standing almost vertically up from the notum in the centre of which is a depression containing a button. The button contains gland cells and sense cells, and a large nerve passes from the button down the stem of the caryophyllidium. Labbé regards these papillae as being sensory in function. He suggests that they may detect light or heat, but offers no evidence for these ideas. I have examined a specimen of Rostanga rufescens and can confirm Labbé's description of the caryophyllidia of this species which form a close pelt over the dorsal surface, almost completely obscuring the notum itself. I have not been able to study the living animal, but it would seem likely that caryophyllidia are of defensive importance. The central nerve could have a motor as well as, or instead of, a

sensory function, and the glands could be of use in defence, Labbé regards the tubercles and papillae of other dorids as being of a different nature to caryophyllidia, but Marcus (1958) shows that the small non-spicular pads of epithelial cells of Cadlina are probably of the same nature as the papillae of Rostanga. Thus it is possible that the caryophyllidia of certain dorids are of defensive importance, but there is as yet no convincing evidence of this.

5) Autotomy

Autotomy of papillae when they are grabbed by a predator or on severe disturbance is well known for eolid and certain other nudibranchs (see Chapter 3). It has not to my knowledge been recorded for the papillae of any dorid nudibranch, but it may occur. Autotomy of the mantle edge is, however, well known for a number of species, particularly amongst the Discodoridinae. Marcus (1955) describes it for Discodoris evelinae Marcus, and gives references to its occurrence in other species of Discodoris. I have observed it myself in an unidentified species of Discodoris in Jamaica. Autotomy of the mantle edge is probably of defensive value since it may mean that a potential predator is satisfied with a small portion of the mollusc whilst the otherwise intact animal crawls away.

6) Glands

Glandular secretions are probably important in the defensive systems of most species of dorid. Mucous glands occur scattered

over the dorsal surface of many species, and these may play some part in defence as well as performing a cleansing and lubricating function. Non-mucous glands which are probably defensive are described by Thompson (1960,b). In Acanthodoris pilosa (Müller), these glands are epidermal, but in Onchidoris pusilla (Alder and Hancock) they are subepidermal. Baba (1937) describes glands from Okadaia elegans Baba which appear to exude a granular fluid, and these could be defensive. Crozier (1917) describes the defensive glands of Chromodoris zebra. These occur on the ventral surface of the mantle and are subepidermal. He describes a pore and a sphincter to each gland, but these are not figured. Crozier finds that if C.zebra is disturbed, it erects the mantle edge and secretes an oily white fluid from these glands. This fluid can be secreted from most of the mantle surface, but is especially concentrated in these glands. He also finds that whilst the internal parts of the animal are palatable to fish, the glandular mantle edge is not palatable (Crozier, 1916). This is fairly convincing evidence of the defensive value of the glands. However, I have failed to elicit exudation from similar glands in two Jamaican chromodorids, Cadlina sp. and Glossodoris neona, so it may be that a very violent stimulus is necessary before exudation results.

I have found two other types of glandular secretion which may be of defensive importance. The first is based on a single observation, and may have no defensive function at all. One individual of a species of Dendrodoris exuded a cloud of granular white

material from the branchial glands when it was prodded with forceps. But this was not repeatable either on the same individual or on another one, so requires confirmation before we can record it as definitely of defensive importance. The second glandular secretion is an acid of a low pH, and since this has not been recorded before from a dorid nudibranch, it is described in greater detail in section 2 of this chapter.

Section 2 : Acid secretion in dorid nudibranchs

Occurrence of acid secretion in the Mollusca

Defensive acid secretion is known to occur in a variety of opisthobranch and prosobranch molluscs. It is first described by Garstang (1890) for the opisthobranch Pleurobranchus membranaceus (Montagu). Thompson and Slinn (1959) confirm this and find that the acid is of pH 1. It is apparently secreted from the cells of the mantle, as well as from a specific acid-gland. Thompson (1960,a) describes defensive acid secretion from four other species of mollusc; Berthella plumula (Montagu), Philine quadripartita Ascanius, Lamellaria perspicua (L.), and Velutina velutina (Müller). In all cases the acidity, measured on the surface of the animal, is pH 1 or lower. Trivia arctica (Montagu) and T.monacha (da Costa) secrete a fluid of stronger acidity than pH 2 (Thompson, 1961), and I have myself detected secretion of a similar fluid from the Caribbean cowrie Cypraea spurca acicularis Gmelin. Thus acid secretion is known from the Pleurobranchidae and the Philinidae amongst the opisthobranchs, and from the Lamellariidae and the Cypraeidae amongst the

prosobranchs. In this section I shall describe acid secretion from yet another family, the nudibranchiate Doridiidae.

Material and Methods

The material used in this investigation was collected at Port Royal, Jamaica, between October 1961 and March 1962. Tests for acid secretion were performed using BDH indicator paper applied to the surface of the animal immediately after removal from water. Sulphate was tested for using barium chloride with the animal placed in an isotonic saline solution. Parts of the mantle of the dorids were fixed in Heidenhain's "Susa", and in Lewitsky-saline. Staining techniques used include those of Masson and Mallory; also safranin and light green, and dialysed iron. Some histological detail was lost, however, due to carbon dioxide liberated from the spicules by the fixative.

Identification of Material

During the course of this investigation four individual dorids which secreted acid were found, three at Port Royal, and one at Plymouth. Unfortunately I have only been able to identify the British Anisodoris stellifera and one of the Jamaican dorids with certainty; this latter is Discodoris pusae Marcus. Since the identity of the other animals is in doubt, I shall describe my specimen of D.pusae, and compare it with the original description of Marcus (1955).

Marcus's description of D.pusae is based on an examination

of 12 individuals from Brazil. The size, in life, is up to 35 x 20 mm. with the foot projecting from the notum when crawling. The colour is light orange with irregular brown blotches on the tuberculate notum and superficial light granules giving the animal a whitish hue. The underside is paler, and is without spots. Cutaneous glands on the notum have spicules concentrated round their openings, and in some specimens the openings are marked with dark pigment. The border of the branchial cavity is smooth; that of the rhinophorial grooves is lobulate. The rhinophores have a long shaft and 15 to 20 slanting perfoliations. The oral tentacles are not grooved, and the bilabiate foot is notched in all but one specimen. The cuticle of the lips bears 20 - 50µ high rodlets which arise from basal pits. The radular formula is 21-25 x 8-12. 27. 0 . 27 . 8-12.

The specimen which I have identified as D.pusae was found on 22nd of February 1962, on a buoy at Port Royal. In life it is 35 x 17 mm. in size, with the tail projecting behind the notum by about 5 mm. The notum is yellow, but this is largely obscured by brown, especially on the low tubercles. There is blackish brown on the tail and on the ventral surface of the mantle. The notum is covered with a fine meshwork of white lines. The foot is orange yellow, without brown. Cutaneous glands are marked by brown rings. The rhinophores have 20 blackish brown lamellae, with white blotches at the tip. The 8 tripinnate gills are also blackish brown, with white on the anterior ones. The oral tentacles are not grooved,

and the bilabiate foot is notched. The labial cuticle and the radular teeth are identical with the figures of Marcus, and the radular formula is 23 x 11-13 . 27 . 0 . 27 . 11-13. The oldest row has only 14 instead of 27 lateral teeth. Some of the first lateral teeth have a small projection as described by Marcus, but rather less well developed.

It can be seen that my specimen agrees in almost every particular with the description of the type material of Discodoris pusae, and there can be no doubt as to the certainty of the identification.

The second acid-secreting dorid was found under a stone in shallow water at Port Royal on 28th January, 1962. In life it is 12 x 5 mm, with the tail just projecting when crawling. The notum is basically grey, but this is largely obscured by brick-red pigment except at the mantle edge and the rhinophore bases. The notal tubercles are sometimes tipped with white, and there are fine white lines all over the notum as in the typical D. pusae. There are 12 lamellae to the rhinophores, which are white with red-brown dots. There are 6 bi- or occasionally tri-pinnate gills, and these too are white with red-brown dots. The rhinophorial pit is tuberculate, but the branchial pit is not, just as in D. pusae. The anal papilla has a white ring round it. The labial cuticle and the radular teeth resemble those of D. pusae, but the formula is only 13 x 10 . 11 . 0 . 11 . 10 . This low count does not necessarily

imply that this animal is distinct from D.[illegible], since the oldest teeth of the Jamaican D.pusae described above have only 14 laterals. It is not improbable that a 12 mm. slug with 11 lateral teeth might grow to a 35 mm. slug with 27 lateral teeth. It is clear from this description that this 12 mm. slug could also be Discodoris pusae; it differs from typical pusae in colour, which varies anyway in most dorids, and in the structure of the gills. However, this animal readily sheds the mantle edge when disturbed, as does D. evelinae Marcus (Marcus, 1955). Marcus does not describe this for D.pusae, nor did I observe it with the D.pusae just described. Certainly if this is not D.pusae, it is a very closely related species.

The third acid-secreting dorid from Port Royal was found 70' down on the coral reef by Dr I.Goodbody on 8th October, 1961. In life it is 8 mm. long, and the colour is brick-red as in the second dorid described above. There are scattered white dots, and three pairs of tubercles on the back are prominent with white tips and bases and brown in between. The white tipped brown rhinophores have 12 lamellae, and the 8 gills are uni- or occasionally bi-pinnate. The front two are brown with white tips, the others are rufous. The radular formula is 17 x 10-13 . 9 . 0 . 9 . 10-13. The lateral teeth are finely serrate, unlike those of D.pusae. The labial cuticle is similar to that in D.pusae. This animal thus has a different type of radular tooth from D.pusae. This is not in itself sufficient grounds for supposing it to be a different species, but,

as I shall describe later, it also lacks the large cutaneous glands of D.pusae. This animal must therefore remain unidentified, but it certainly belongs to the Discodoridinae.

The fourth acid secreting dorid is Anisodoris stellifera (Vayssière), of which a single specimen was found at Plymouth on 20th April, 1961. In life it is 51 mm. long, and in both colour and size it agrees with the description given by Eliot (1910), of A.stellifera, and differs from that of Geitodoris planata (Alder and Hancock). The radula, too, is that of stellifera, and differs from Geitodoris in lacking marginal teeth. The formula is 24 x 38 . 0 . 38, but there may be a few more lateral teeth per row in the youngest row. Pruvot-Fol (1954) gives a formula of 30 x 45 . 0 . 45, and Eliot gives this as a maximum. There can thus be little doubt that this animal is Anisodoris stellifera.

Acid secretion of living specimens

All four animals were tested for acid secretion by removing them from water and applying BDH indicator paper to the mantle. All four showed an immediate reaction of pH 2. They may have been even more acid, but the paper used does not register below pH 2. In A.stellifera, I also used another indicator paper which registers to pH 1 (checked by comparison with 10% HCl), and in this species at least, the acid is of pH 1. In all animals, acid secretion occurs from the dorsal and ventral surfaces of the mantle, but does not occur from the foot. The large D.pusae even registered an acidity of pH 2

under water, when roughly stimulated, so either the acid is of a much lower pH, or it is not rapidly dispersed and diluted by sea water.

After D.pusae was blotted dry and placed in isotonic saline solution, barium chloride ejected from a pipette close to the animal did not give a precipitate; but when the animal was prodded, an immediate precipitate formed. This strongly suggests that sulphate ions are released by the animal. No other inorganic ions would give a precipitate under these conditions, and few organic acids would give a pH of 1 or 2. Fontaine (1961) found acid mucopolysaccharides of pH 1 in Ophiocomina nigra Abildgaard, so the possibility of this being an acid mucin cannot be eliminated. An attempt was made to fix the acid in situ by narcotising this animal in $MgCl_2$ and $BaCl_2$, but there is no trace in such material, fixed with either "Susa" or Lewitsky-saline, of crystals of barium sulphate. It is possible that the sphincter muscles described below would prevent entry of the barium ions, and so a precipitate only forms when acid is released into the water.

Histology of the acid glands

Thompson and Slinn (1959) and Thompson (1960,a) describe the characteristic empty appearance of the acid secreting tissues of the gastropods they studied. The acid sulphated mucopolysaccharide described by Fontains (1961) stains with the usual mucin stains. In both D.pusae and A.stellifera, the histology of the mantle edge is

identical. In the epidermis there is a single type of mucous gland (Fig.12) which resembles the mucous glands of other nudibranch molluscs, and presumably has a lubricative function. The non-mucous epidermal cells are not particularly empty in sections, but are vacuolar. It is possible that they secrete acid, but I have no evidence for this. In the subepidermal parts of the mantle, however, there are numerous large glands of an empty appearance. These open on the dorsal surface of the animal by small pores (see Figs. 8, 9). Each gland is up to 1 mm. in length in fixed preparations, and is surrounded by a layer of non-spicular connective tissue. This probably acts as a compensatory device according to the state of secretion of the large glands. If they are full of liquid, there is little of this loose connective tissue; but if they are empty, then intercellular fluid probably flows in and fills up this area of loose connective tissue. The wall of these glands is composed of a layer of squamous epithelium (see Fig.12), and this is invested with a thin muscular sheet. The muscle fibrils run in many directions round the glands, and presumably their contraction expels liquid from the orifice which is less than 50μ in diameter. At the orifice of the gland there is a prominent plug of mucous gland cells Figs. 10, 11, 12, 13) similar in staining reactions to the mucous glands of the epidermis. The duct which leads from this plug to the surface of the body has a second concentration of glands along its length; but between the two, the epidermis is very

low, and is constricted by 10 or 15 circular muscle fibrils which act as a sphincter. Presumably, in life, the sphincter prevents leakage of fluid from the gland except under certain conditions when it can be violently expelled by contraction of the muscles in the gland wall.

In two cases in D.pusae I have found small glands of exactly the same structure as the large ones just described, which open into the ducts of these large glands (see Fig.12). They are only 30 to 50 μ in length, but have muscle fibrils and a small sphincter just as do the large glands. It is thus possible that these glands are formed by invagination of the epidermis, and that new ones may regularly bud off from mature ones.

It is difficult to get direct evidence as to the function of these subepidermal glands. Their highly developed musculature and the concentration of mucous glands at their orifices suggests that they have some important secretory function. But the curious nature of the epithelium is unlike that of most glands. They could be for storage of some secretion produced by the mucous glands of the plug, but if this were so, one would expect to find the glands to be full of mucus in a fixed preparation, and this is not the case. The only reasonable alternative is that they are acid glands, and that the squamous epithelium secretes acid. This hypothesis would account for the musculature and the sphincter to regulate the flow of acid, and for the mucous glands which no doubt give the acid a viscous

medium so that it is not rapidly dispersed and diluted in the sea. In A.stellifera I treated an excised piece of mantle with liquid BDH wide-range indicator. Almost immediately, the inner surface of the mantle registered pH 2 in scattered blobs in the dermis. These blobs are clearly the glands just described.

There can thus be little doubt that the large, subepidermal glands of Discodoris pusae and of Anisodoris stellifera are responsible for the production of acid, and that one of the constituents of this acid is sulphuric acid.

The picture with respect to the other dorids is less clear. The 12 mm. slug which sheds its mantle shirt in defence was not sectioned, but it certainly belongs to the genus Discodoris. The 8 mm. slug with the finely serrate lateral teeth is closely related to Discodoris, but may belong to another genus. Nevertheless, we should expect to find in it the same histological features that are present in the two acid secreting species already described. The animal is fixed in "Susa", and although the fine detail of the epidermis is lost due to the bubbling of carbon dioxide, there can be no doubt that large subepidermal glands are totally absent. The acid must therefore be secreted from the epidermal cells.

Discussion

The occurrence of acid secretion from the epidermis of one species of dorid suggests that this may be true also for D. pusae and A.stellifera. But in these species, the chief source of acid

is undoubtedly the subepidermal glands, as the experiment with BDH indicator on A.stellifera proves. These facts do, however, suggest how such complex glands may have evolved :

1. Originally, acid secretion was probably confined to certain epidermal cells. It may have been in the form of some acid mucopolysaccharide, but later the secretory product was modified to simple inorganic acids. Mucopolysaccharides are often of defensive value in nudibranchs, and an acid component would no doubt make the secretion more repellent to fish. Bateson (1890) shows that pure acid is distasteful to fish, so each change in this sequence would be advantageous.

2. Special acid-secreting areas developed, perhaps in small depressions of the skin, and thus the acid produced was localised and hence concentrated. Rapid dispersal would be prevented by the mucus from other glands.

3. The acid pits deepened into large subepidermal glands. Acid secretion may persist in the ordinary epidermal cells (as is possible in D.pusae and A.stellifera), but this is trivial compared to the acid produced by the large glands. Muscles for regulating the flow of acid developed, and mucous glands concentrated near the openings of the glands. Each stage in this sequence has some advantage over the preceding one such that a slug of stage 3 can secrete a considerable quantity of concentrated acid that will not readily disperse; but secretion is strictly regulated, and it will not occur at all unless the animal is violently stimulated.

Taxonomic considerations

Pruvot-Fol (1954) places Anisodoris Bergh in the Dorididae together with Doris L.and Archidoris Bergh; whilst the Discodorididae form a completely separate family. Odhner (1939), followed by Marcus (1961), have separate subfamilies of the Dorididae for Archidoris and Discodoris (the Archidoridinae and the Discodoridinae), and Anisodoris is placed in the Discodoridinae. Since D.pusae and A. stellifera both have identical acid glands which are absent from Archidoris tuberculata (Thompson,1960,b; and personal observation), this can be used as additional evidence for the close relationship of Discodoris and Anisodoris. Marcus (1955) likens these glands to those of Geitodoris patagonica Odhner, so it is possible that this species too secretes acid.

Conclusions

Defensive acid secretion has thus evolved at least four times independently in the Mollusca: in the Lamellariidae and the Cypræidae, in the Philinidae, in the Pleurobranchidae, and in the Dorididae. In the Dorididae, however, it is not widespread, and occurs in only one subfamily. Table 2 lists the dorids that have been tested for acid secretion, and illustrates this point.

All of the dorids studied by Thompson in Table 2, and also Ancula cristata (Herdman and Clubb, 1890), possess non-mucous defensive glands, but neither of the two acid-secreting dorids possesses such glands. It is clear that in the Doridacea, glands

play an important part in defence; but whilst some species have evolved acid glands, others have evolved defensive glands of a different nature.

Table 2. Distribution of acid secretion in the Doridacea.

Underlined species secrete acid
Others do not. References are T = Thompson, 1960, b; E - this paper

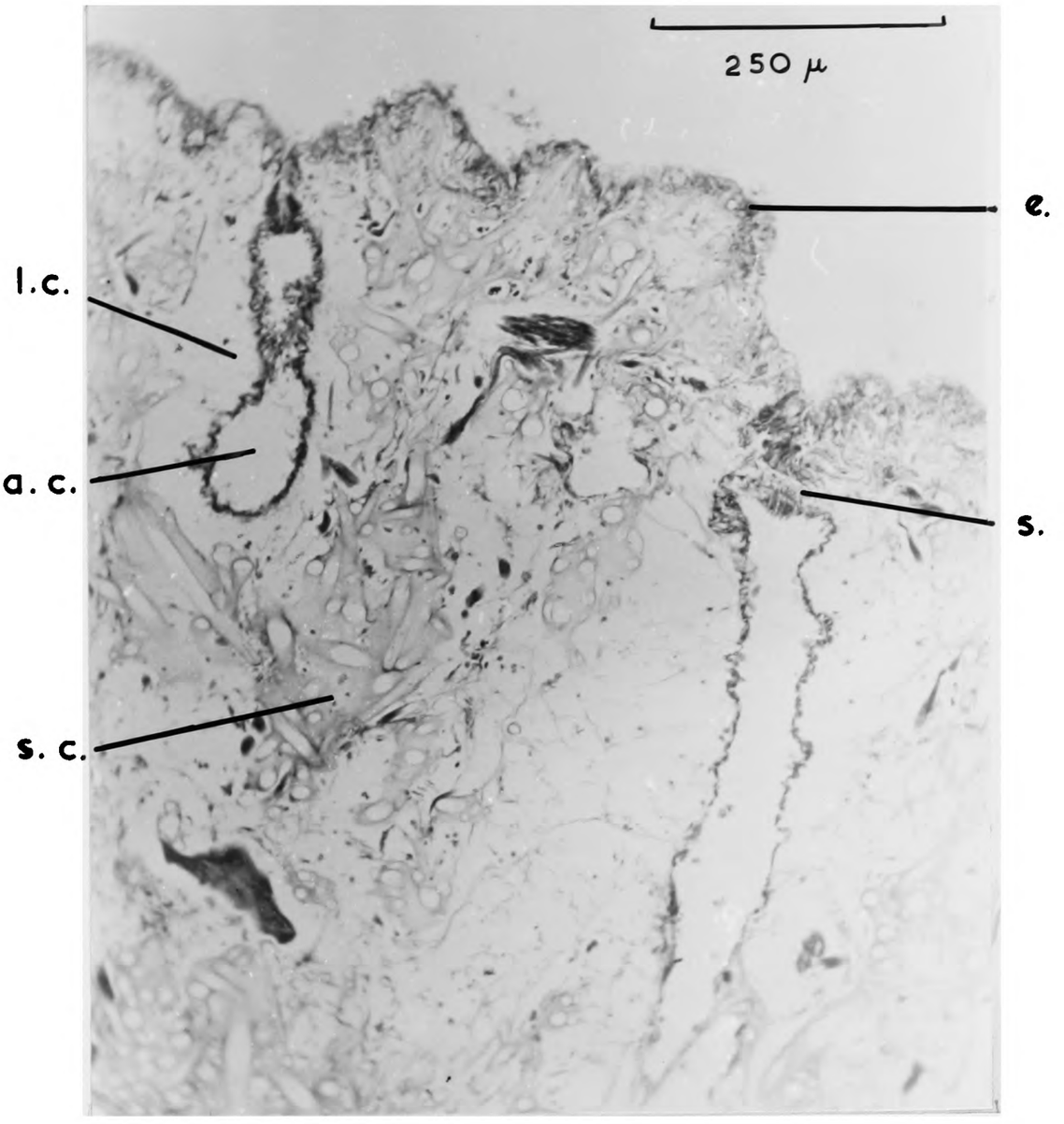
Endoridacea	Cryptobranchia		Doridídae	
			Glossodoridinae	
			Cadlina sp.	E
			Glossodoris neona Marcus	E
			Thorunninae	
			Rostanga rufescens Iredale & O'Donoghue	E
			Jorunna tomentosa (Cuvier)	T
			Archidoridinae	
			Archidoris tuberculata Cuvier	T
			Discodoridinae	
			Anisodoris stellifera (Vayssiere)	E
			Discodoris pusae Marcus	E
	Phanerobranchia	Non-suctoria	Notodorididae	
			Aegires punctilucens (d'Orbigny)	E
			Polyceridae	
			Polycerella conyna Marcus	E
			Polycera quadrilineata (O.F.Müller)	T
			Polycera elegans (Bergh)	E
		Suctoria	Onchidorididae	
			Acanthodoris pilosa (O.F.Müller)	T
			Onchidoris pusilla (Alder & Hancock)	T
			Onchidoris fusca (O.F.Müller)	T
			Onchidoris muricata (O.F.Müller)	T
			Adalaria proxima (Alder & Hancock)	T
			Goniodoridae	
			Goniodoris nodosa (Montagu)	T
			Goniodoris castanea Alder & Hancock	E
			Okenia aspersa (Alder & Hancock)	E
			Okenia impexa Marcus	E
Porostomata			Dendrodorididae	
			Dendrodoris sp.	E

Summary

The Doridacea have at least six types of defensive mechanisms, but any one species has usually two, three or four in its defensive system. Camouflage is of widespread occurrence, and involves colour, texture and behaviour. Warning coloration has not been established, but some brightly coloured papillae are probably deflection markings. Many species are relatively inedible to fish. Papillae with defensive value are present in some species, but the function of caryophyllidia is unknown. Autotomy of the mantle edge and acid secretion occur in some of the Discodoridinae, and non-mucous defensive glands are of widespread occurrence. However, the evidence for the defensive value of some of these mechanisms is unconvincing; and much further work is necessary before we have a comprehensive picture of the defensive mechanisms of the Doridacea.

Figure 8. Section of notum of Discodoris pusae at right angles to the surface, stained with Masson's trichrome. a.c., acid gland; e., epidermis; l.c., loose connective tissue; s., sphincter; s.c., spicular connective tissue.

Figure 9. Diagrammatic section of the glands at the edge of the notum of Discodoris pusae.


250 μ
e.
l.c.
a. c.
s.
s. c.

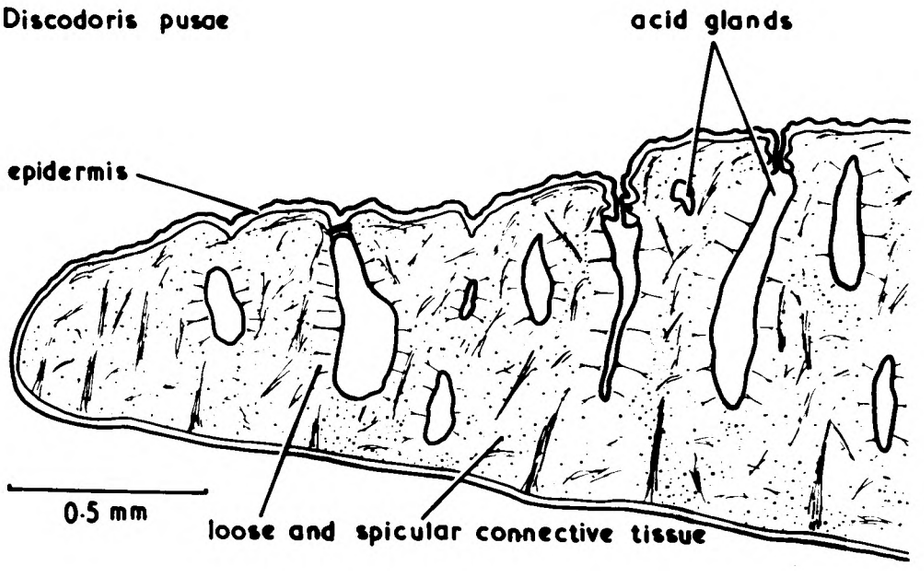

Discodoris pusae
acid glands
epidermis
0·5 mm
loose and spicular connective tissue

Figure 10. Section of mouth of acid gland of Discodoris pusae passing through the discharge canal. e., epidermis; l., lumen of acid gland; m.gl., mucous glands; s., sphincter muscle.

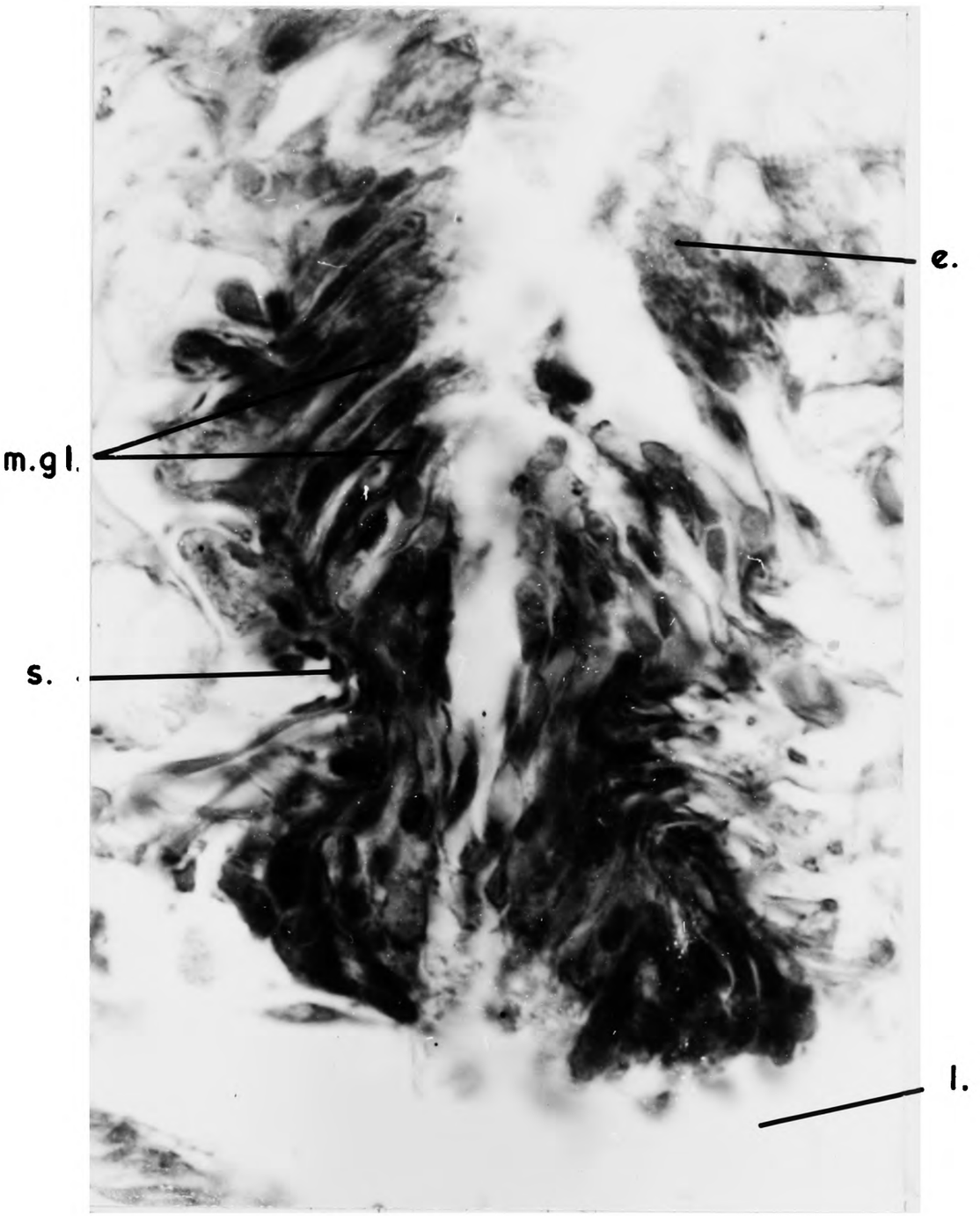

e.
m.gl.
s.
l.
20μ

Figure 11. Section of mouth of acid gland of Discodoris pusae to show the sphincter muscle. This is the same gland as that in Fig. 10, but is the neighbouring section. e., epidermis; l., lumen of acid gland; m.gl., mucous glands; s., sphincter muscles; w., wall of acid gland.

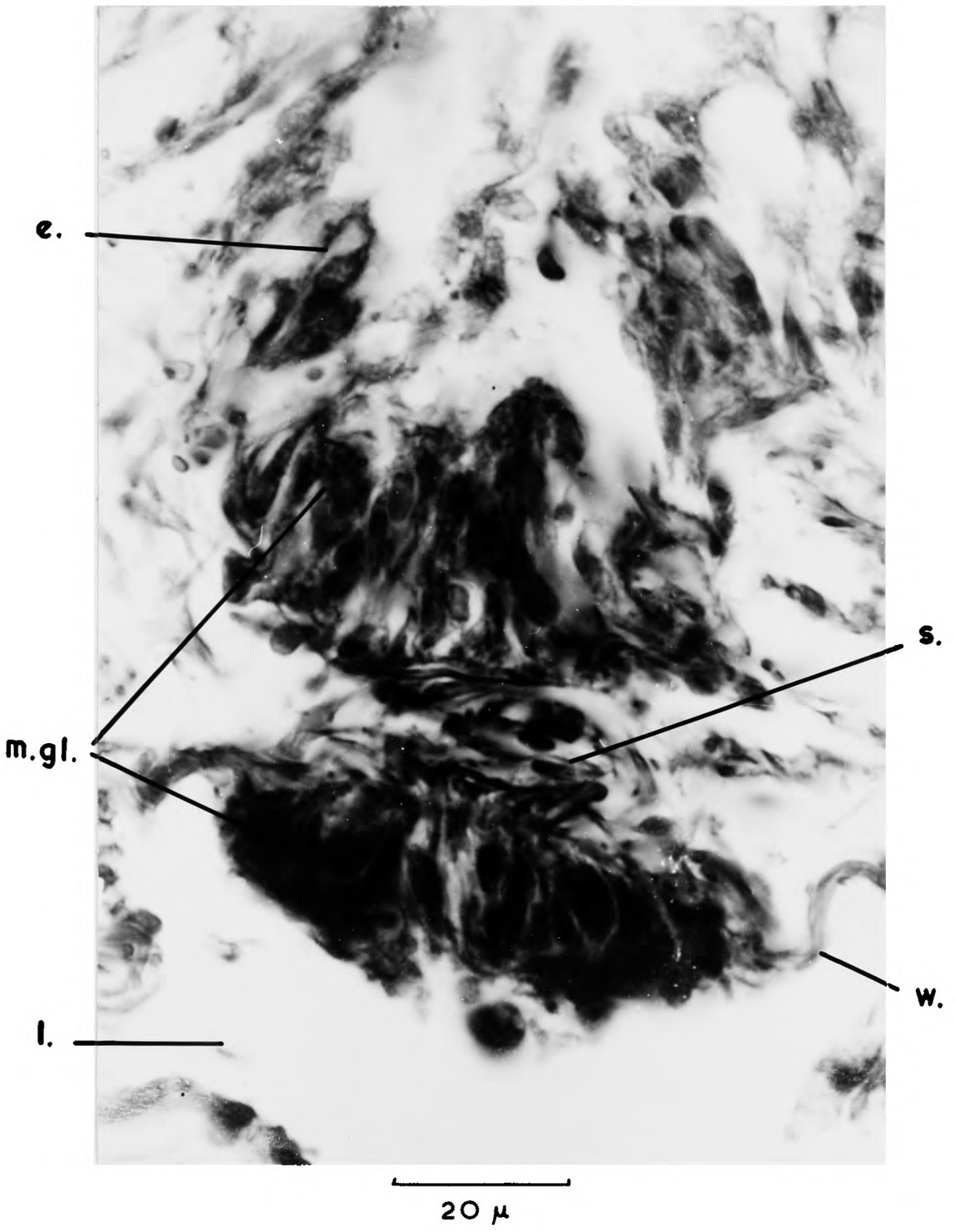

e.
s.
m.gl.
l.
w.
20 μ

Figure 12. Diagram to show the structure of the acid gland of *Discodoris pusae*.

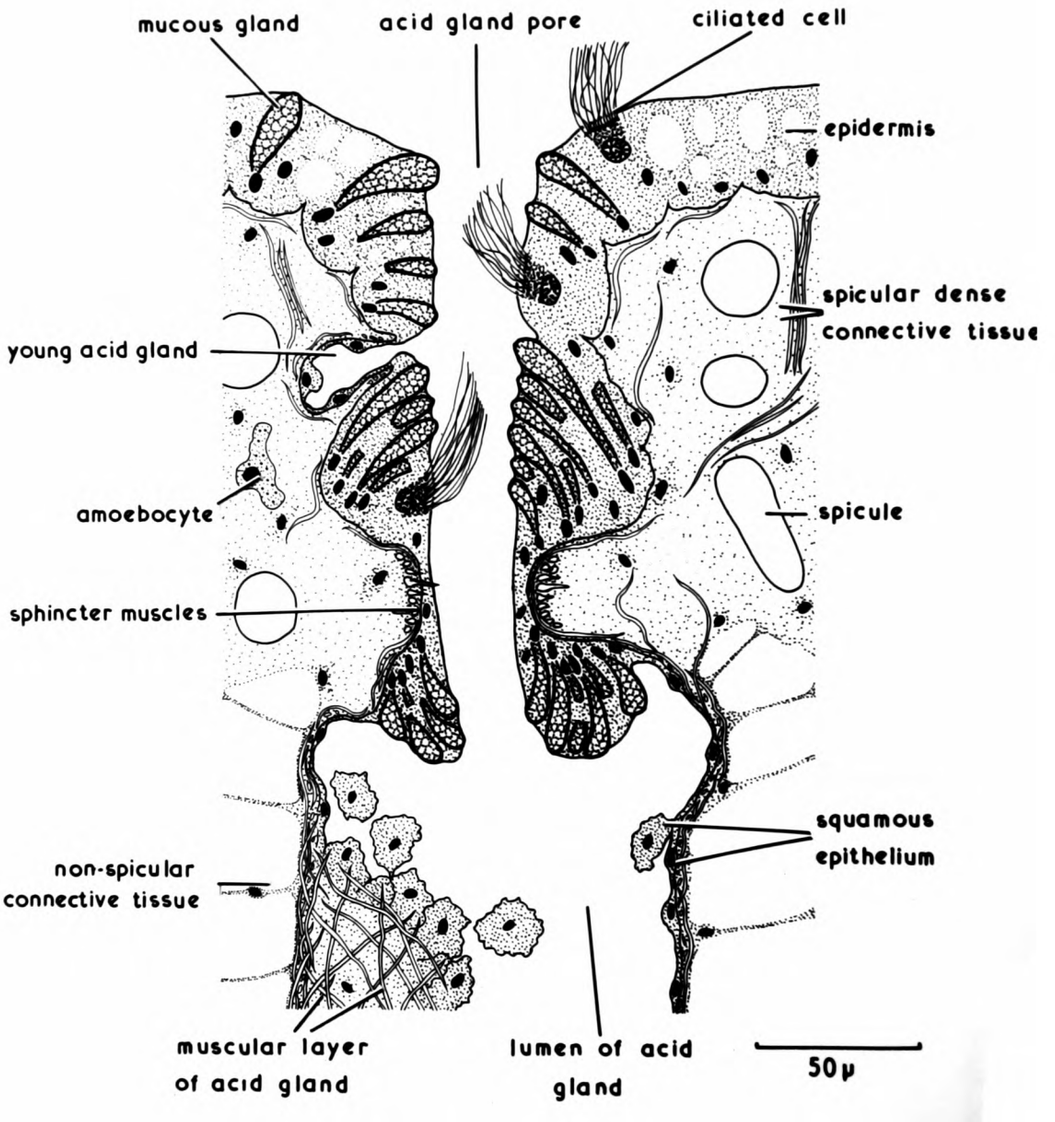
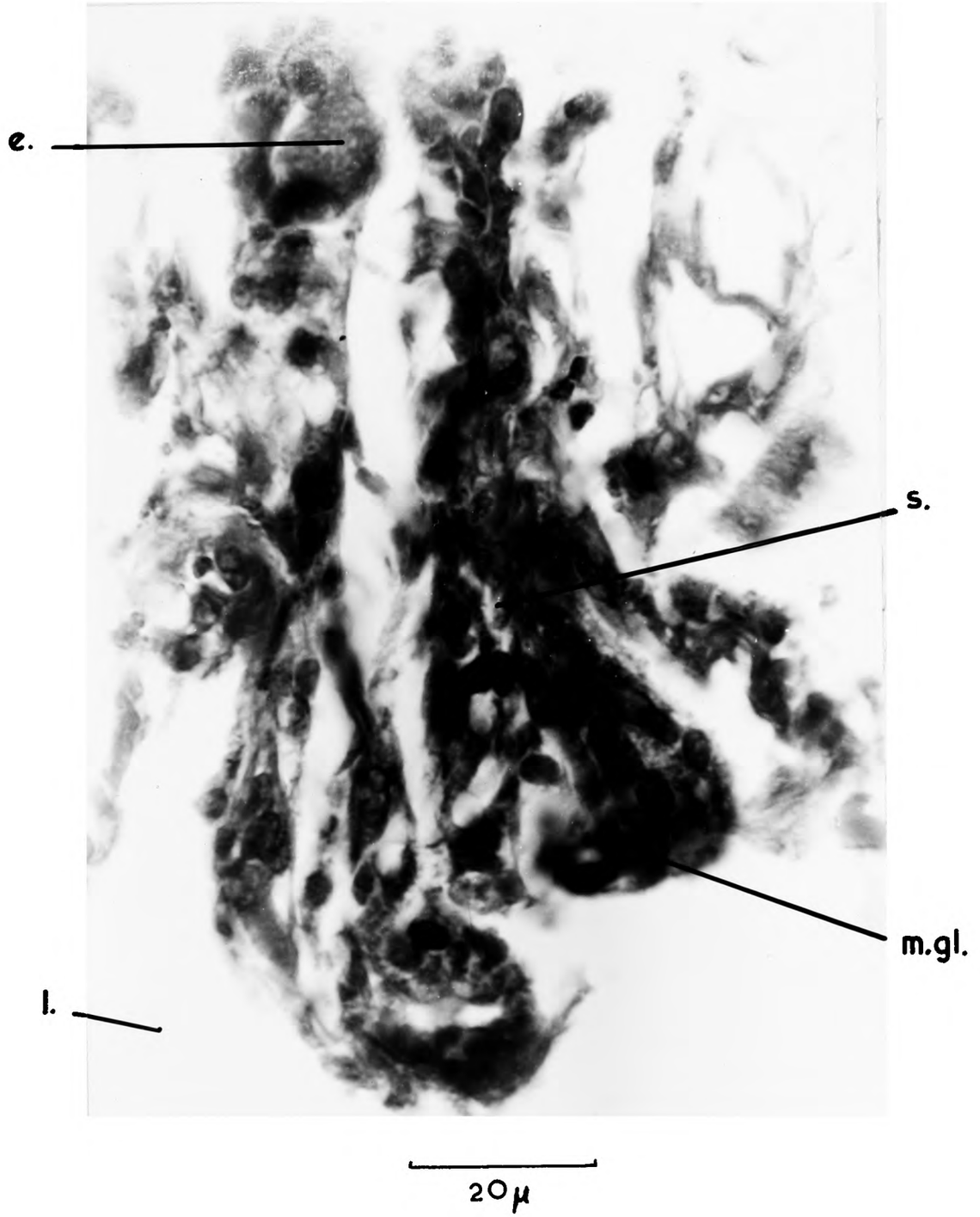

mucous gland
acid gland pore
ciliated cell
epidermis
spicular dense
connective tissue
young acid gland
amoebocyte
spicule
sphincter muscles
squamous
epithelium
non-spicular
connective tissue
muscular layer
of acid gland
lumen of acid
gland
50μ

Figure 13. Section of mouth of acid gland of Anisodoris stellifera passing through the discharge canal. e., epidermis; l., lumen of acid gland; m.gl., mucous glands; s., position of sphincter muscle.


e.
s.
m.gl.
l.
20μ

Chapter 5

DEFENSIVE MECHANISMS IN THE EOLIDACEA

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DEFENSIVE MECHANISMS IN THE EOLIDACEA

INTRODUCTION

More is probably known of the defensive mechanisms of the eolids than of any of the other three groups of the Nudibranchia. Despite this, the complete story is not yet known, and results in the past have often been conflicting. There are two statements that are often made without sufficient evidence, and these I wish to discuss in some detail. The first is that eolid nudibranchs have warning coloration; and the second is that they are protected by means of nematocysts derived from their prey. I hope to show that there is no evidence for the first of these statements, and that the second is true - with certain reservations.

The defensive systems of eolid nudibranchs comprise up to six mechanisms; colour and behaviour, autotomy, inedibility, epidermal structure, glands, and nematocysts. Each mechanism will be discussed in turn, and then the interaction of some of these mechanisms in the overall defensive systems of different species will be discussed.

1. COLOUR AND BEHAVIOUR

Garstang (1889) and Herdman and Clubb (1890) find that eolids are not eaten by a variety of species of fish, and suggest that the brilliant colours of many eolids are warning colours. They assume that the nematocysts in the cerata are responsible for the unpalatability

of the molluscs, and that the brightly coloured cerata divert attacks away from the palatable body and towards the unpalatable cerata. The whole behaviour of the animal is adapted to directing attacks towards the cerata. When the animal is disturbed, it contracts the body and rhinophores whilst the cerata are erected and waved about. Should a potential predator attempt to eat such an eolid, it will probably get a cluster of the easily detached cerata whilst the body of the animal remains unharmed. However, there is no evidence at all that any fish can learn to associate colour with inedibility, and if this does not occur it is impossible for warning coloration to evolve.

Although Herdman (1890) believes that all eolids possess warning coloration, Garstang and later workers modified his views. Garstang (1890) finds that Eubranchus exiguus is cryptic, and Hecht (1896) lists E.exiguus, E.cingulatus, Tergipes despectus and Catriona foliata as being cryptic on hydroids. I can confirm that all of these species are often inconspicuous to the human eye on hydroids, particularly T.despectus in which the alternating single cerata closely resemble the hydranths of Laomedea and Obelia on which it feeds. No other eolid has alternating single cerata, so this is almost certainly a case of a specific resemblance of an eolid to its food. Crypsis on land implies resemblance to a plant or to some inanimate object, but in these examples, it is a resemblance of one animal to another animal. Such a resemblance is normally classified as mimicry, but since the model is motionless and resembles a terrestrial plant in habit, it can equally be called crypsis. Hence

the distinction of Garstang (1890) between the mimicry of Aeolidiella glauca to the anemone Sagartia and the crypsis of E.exiguus to the hydroid Halecium is not clear. Presumably the first implies that Aeolidiella resembles a complete anemone whilst E.exiguus resembles only a part of Halecium. Other examples of mimicry or crypsis are Calma glaucoides to fish eggs (Hecht, 1896), Aeolidia papillosa to Sagartia troglodytes (Giard, 1888), Spurilla neapolitana to Anemonia sulcata (Tchang Si, 1931), and Precuthona peachii to Hydractinia echinata (Beaumont, 1900). Thus there is evidence that many of the commoner European eolids are inconspicuous and cryptic when in their normal habitat. Warning coloration is supposed to occur in Eubranchus tricolor and Facelina coronata (Hecht, 1896) which are conspicuously coloured. My observations on these species are too few to be dogmatic, but F.coronata at Plymouth frequently feeds on Tubularia amd Coryne, and on both of these it is difficult to detect. Catriona aurantia is another bright red species, very conspicuous on most backgrounds, but exceedingly well camouflaged on Tubularia on which it is almost invariably found in my experience.

Colour and behaviour are therefore important in the defensive systems of many eolids which are cryptic, but there is no evidence for the occurrence of warning coloration. Even brightly coloured species are often cryptic on their preferred food. However, it is probable that a number of species remain which are often very conspicuous: Facelina coronata when not on Tubularia, for example;

or Phidiana lynceus, which I found to be very common in Jamaica and very easy to see. The importance of colour in these species is not known - it could be warning, or it could be for some other purpose, but much more evidence is required before we can be certain.

2) AUTOTOMY

As already mentioned, the cerata of eolids are capable of being autotomised and regenerated. Hecht (1896) found that in some species, such as Eubranchus exiguus, Catriona foliata and Tergipes despectus, cerata are very readily autotomised; whilst in others, such as Aeolidia papillosa and Facelina coronata, it occurs only rarely. My observations are similar, and I have found autotomy to occur very readily on rough handling, or on pinching (but not pulling) a ceras, in Catriona aurantia, maua and perca, and in Eubranchus pallidus and exiguus. But it occurs rarely with rough handling in the Facelinidae, the Favorinidae and the Aeolidiidae. In these groups autotomy on pinching a ceras usually occurs readily, but pulling is required in Dondice occidentalis, and in Aeolidia papillosa breaking often occurs before autotomy. Cerata are thus readily shed in the acleioproct eolids, but less readily in the Cleioprocta. The significance of these differences to the animals in nature is not known, but it may be related to attacks from different types of predator. Thus for a fish, which takes food into its mouth, tastes it, then rejects it if it is inedible, cerata which are not readily autotomised could be an advantage since they protect the soft parts

dorsally. On the other hand, a crab tends to grab its food with a chela and tear small pieces off it. In this case cerata which are readily shed could be an advantage since the initial grab might then merely take off the cerata and leave the body of the eolid untouched. As can be seen from Chapter 6, our knowledge of the predators of eolids is practically nil, so this is pure speculation; but certainly there must be some reason for the differences between eolids with respect to autotomy.

3. UNPALATABILITY

Eolids are not eaten by a variety of species of fish and invertebrates. This is clearly of defensive importance. It is more fully discussed in Chapter 6.

4. STRUCTURE OF THE EPIDERMIS

General

The epidermal histology of eolid nudibranchs is described in detail by Henneguy (1925). He describes the peculiar vacuolar cytoplasm, and mentions that several types of mucous gland may be present in each species. The glands are dealt with in section 5 of this Chapter; this section describes the histology of the non-glandular epidermal cells, and how these may be of defensive importance. To avoid repetition, the paragraph on material and methods applies to both section 4 and section 5.

Material and Methods

The material used in this investigation was collected at Plymouth, England, and at Port Royal, Jamaica. Specimens for sectioning were narcotised in 1:1 7½% $MgCl_2$: sea-water, the exact time depending on the size of the animal. Heidenhain's "Susa" and Lewitsky-saline proved the best fixatives for most species, but where material was available, Bouin, Zenker, formol-saline and formol-calcium were also used. Sections were cut at 6µ in 52° or 60°C paraffin or in polyester wax. Gelatine embedding was used for the lipid tests. Masson's trichrome technique proved to be the most useful histological stain, although Lewitsky-saline fixed material had first to be decoloured by 10 minutes in acidified 0.05% $KMnO_4$ followed by 5 minutes in 2% oxalic acid and thorough washing in tap water. The usual counterstains for some of the histochemical tests could not be used since they also stained the glands under investigation - in particular carmalum and most haematoxylin preparations stain some forms of mucin. A new counterstain was devised for use with alcian blue and dialysed iron preparations which only affects the colour of a very few types of mucin. After staining, the slides are dipped for one minute in 1% basic fuchsine and then direct to alcoholic picric acid for about 10 seconds (either of the two used in Metzner's method proved suitable). It is necessary to pass very rapidly through two changes of absolute alcohol if the stains are not to be washed out, and then through xylene to a synthetic resin.

The structure of the non-glandular epidermal cells

Henneguy (1925) describes how the cells of the gut and of the epidermis of eolid nudibranchs are packed with empty-looking vesicles 4 - 10μ long, and ellipsoidal in shape. Each vesicle has a line across its longest diameter which is sometimes stained with basic dyes - green with Masson's trichrome and blue with Mallory's triple stain. According to Henneguy, the cytoplasm in life is filled with vacuoles in each of which floats a balloon-like vesicle. After fixation, the vesicle collapses and forms the basophilic line across the vacuole. Henneguy supposes that the function of this peculiar cytoplasm is to cushion the body against contact, and hence presumably damage, from hard bodies. Graham (1938) shows that the structure of the vesicles is the same in living material as in fixed preparations, and that the line across the ellipsoidal vesicle is in fact a complete partition. The junction between the partition and the walls of the vesicle can occasionally be made out. Bürgin-Wyss (1961) finds that in Facelina species it is possible to see the vesicles in living material, but in Catriona coerulea the appearance of fixed and living material is quite different. My own observations support Graham's conclusions. In living cerata of Catriona aurantia stained with neutral red or with toluidine blue (or thionine), the epidermal cells are packed with deeply staining crescents, horseshoes, rings and drumsticks (Fig.14c). This is very similar to the appearance of "Susa" fixed material stained with dialysed iron (Fig. 14a). The deeply staining part is probably the central partition at

its junction with the wall of the vesicle, hence the rings represent the vesicle in side view and the drumsticks represent vesicles end-on. Why the edges of the partition should stain more readily than the central part is not clear. The appearance with Mallory staining is quite different (Fig.14,b), but apart from the staining, Figures 14a and b have undergone identical treatment. Figure 14d is of vesicles from Lewitsky-saline fixed Eubranchus pallidus stained with dialysed iron. In this case rings, drumsticks and fine partitions are all present. These rings and drumsticks are not so easy to demonstrate vitally in other species, but I have seen them in Coryphella lineata, Facelina coronata and Eubranchus exiguus. In some species, the vital dye is weakly taken up by the vesicles in the form of evenly staining circles and plates, for example, in E.exiguus, E.pallidus, Tergipes despectus, Aeolidia papillosa and C.lineata. These evenly staining circles and ellipses are of the same size as the fixed vesicles, and are clearly the same structures. Thus the vesicles are present in living as well as in fixed material, but they may react to stains in a different way, and hence appear to be of a different structure.

The chemical nature of the vesicles is not known.. Colouring with vital dyes in this case is probably due to an acid-base reaction since the vesicles take up basic dyes when fixed and are therefore themselves acidic. They also stain with alcian blue and with dialysed iron. This is further evidence for the acidic nature of the vesicles since these dyes usually stain only acid structures (Casselman, 1959).

The vesicles are negative, or only as positive as cytoplasm from other cells, to the periodic acid-Schiff reaction for carbohydrates, Best's test for glycogen, and the Sakaguchi test for arginine (Casselman, 1959). They are negative to Sudan black for lipid. Thus they consist of an acidic compound, possibly a mucoprotein.

The function of the epidermal vesicles

Graham (1938) found an interesting correlation between the occurrence of epidermal vacuoles in both gut and ectoderm and of a coelenterate diet. All those species of nudibranchs which feed on coelenterates, such as Tritonia, Dendronotus, Doto and the eolids, possess these vacuoles; but species which do not feed on coelenterates such as the Sacoglossa, the dorids and the aberrant eolid Calma glaucoides, do not possess these vacuoles. It seems reasonable to suppose that this vacuolar structure in some way protects the eolid (or other nudibranch) from damage resulting from discharge of nematocysts. The fact that many nematocysts pass through the stomach and are ingested by cells in the cnidosac and the digestive gland without being exploded does not necessarily mean that no nematocysts at all are exploded in the gut. It seems likely that the vacuolar cytoplasm of the alimentary canal in some way protects the animal from damage due to nematocysts which may explode in the gut. Similarly, it seems likely that the ectodermal vacuoles protect the eolid from explosions of nematocysts triggered off by the animal crawling over hydroids. Certainly as eolids move over hydroids they frequently brush against the tentacles of hydranths. This

often results in discharge of nematocysts by the hydroid, although this is only easily seen in species with large nematocysts such as Tubularia with F.coronata or the anemone Anemonia sulcata, with A.papillosa. Whilst it is probably true that the eolid is immune to the poisonous and penetrative powers of these nematocysts, this does not mean that it is entirely unaffected by it. In fact, in many species of eolid, contact of the oral tentacles with hydranth tentacles results in immediate retraction of the oral tentacles and even of a momentary withdrawal of the whole animal. The eolid may then crawl on to the hydroid and even eat it, but clearly it has been affected by the nematocysts even if no actual injury has been sustained. This is particularly clear with Aeolidia papillosa feeding on Anemonia sulcata. On one occasion I watched the eolid rasping at a tentacle of this anemone in its mouth whilst the anemone bent other tentacles towards the eolid and discharged a number of nematocysts on contact with the cerata or the oral tentacles. The eolid retracted the oral tentacles and the rhinophores so that they were out of reach of the anemone but was otherwise apparently unaffected. Table 3 summarises those species of eolid which I have seen to retract as if stung from certain species of coelenterate. It is possible that such retraction only occurs with relation to species of coelenterate which do not represent the preferred diet, but I have insufficient evidence to be conclusive on this point. It is true that each species of eolid will feed on a variety of different

hydroids as Miller (1961) found; but some species at least have

Table 3

Species of eolid	Found eating	Withdrew from
Eubranchus pallidus	Campanularia	Campanularia
E.cingulatus	Plumularia	Tubularia
E.exiguus	Coryne	Coryne Plumularia Bougainvillea Tubularia
Coryphella pedata		Bougainvillea
Catriona foliata	Tubularia	Coryne Tubularia
Facelina coronata	Tubularia Coryne	Tubularia
Aeolidia papillosa	Anemonia sulcata Actinia equina	Anemonia sulcata

Species of coelenterate from which various eolids withdrew. The second column indicates species of coelenterate which particular eolids were actually observed to be feeding on: it is not intended to suggest that these are the only or even the preferred foods.

very pronounced food preferences. Thus Catriona aurantia has a preference for Tubularia indivisa L. (Braams and Geelen, 1953). I have never found this eolid on any other hydroid unless Tubularia was nearby, and if left overnight with a variety of hydroids it always chooses to rest on Tubularia. I have not seen C. aurantia retract from Tubularia. Facelina coronata feeds at Plymouth on Tubularia and Coryne, but I have only once seen it retract from Tubularia in many observations. We can conclude, then, that nematocysts are sometimes discharged by coelenterates when eolids crawl over them. Although we have no direct evidence, it seems likely that the cytoplasmic vacuoles are in some way associated with the immunity of eolids to nematocysts; but we cannot satisfactorily explain why eolids may be unaffected by some hydroids whilst showing signs of being mildly stung by others.

Ciliated cells

Cytoplasmic vacuoles are present in all non-glandular epithelial cells of the ceratal ectoderm. Scattered cells, often about one in every four, are ciliated over the entire free border. Henneguy (1925) states that there are two cilia for each basal granule, but in none of the species studied here can one be so dogmatic because even with an oil-immersion objective it is not possible to resolve every basal granule and cilium and to correlate the two. In each ciliated cell there is a meshwork of fibrils running from the basal granules to the nucleus. These fibrils run

between the vacuoles, and never pass through them. In some species, such as Catriona aurantia, there is a network of fibrils throughout the cell at the junction of the vacuolar cytoplasm with the 1μ thick clear layer at the outer border of the cell. This is at the same level as the basal granules. Whether this system is part of a co-ordinating system is not known; but it could ensure that the cilia from different cells beat in the same metachronal phase.

Some species of eolid have, in addition to these ciliated cells, special neurosensory ciliated cells. These are particularly clear in Catriona species, and the following description is based on C.aurantia. Similar structures occur in other genera, and these are listed in Table 4. They may occur in all species of eolid, but are often small and difficult to find. On the surface of a ceras in addition to the rapidly beating cilia, there are a number of what appear in life to be bristles. These may be more or less motionless, or they may vibrate due to the beating of the cilia. They have a broad base in life, and narrow to a fine point. They occur scattered over the ceras, but are more abundant towards the tip (Fig.15). Around the orifice of the cnidosac they are particularly abundant. In fixed preparations, these bristles can be resolved into tight bunches of cilia or flagella projecting about 10μ from the epidermis. Just below the border of the cell, where one might expect to find basal granules, there is a stainable layer, but I cannot be certain that this does in fact represent basal granules. The fibrils from the cilia then pass down through the epidermal cell, narrowing in a cone from about 4μ at the border to a

fine thread of rather less than 1μ in diameter. This thread passes through the basement membrane into the connective tissue and becomes part of a neurone Figs. 16, 17). These neurosensory cells are particularly abundant at the tip of the ceras, and several large nerves run from them down to the central nervous system in the body of the animal.

These structures have been described only once before, by Bürgin-Wyss (1961). However, it is necessary to describe them again

Table 4. List of species of eolid which possess neuro sensory cilia at the tips of the cerata.

ACLEIOPROCTA	Eubranchidae	Eubranchus exiguus
		E.pallidus
		E.tricolor
		Capellinia conicla
	Cuthonidae	Catriona aurantia
		C.foliata
		C.maua
		C.perca
		C.tina
		Eolis M
CLEIOPROCTA	Facelinidae	Learchis poica
		Phidiana lynceus
	Favorinidae	Favorinus auritulus
		F.branchialis
		Dondice occidentalis
		Eolis K
	Aeolidiidae	Spurilla neapolitana

since she is of the opinion that the cilia are connected to the longitudinal muscle layer just below the epidermis, and not to the nerves. What the function of this curious system might be she does not suggest. It is true that the neurones are very close to the muscles, but in some preparations of C.aurantia it is clear with an oil immersion objective that they are not actually connected. These muscles are strongly birefringent, so it is possible to confirm this using a polarising microscope. The cilia themselves are not usually birefringent, nor is the process leading down through the epidermis to the neurone, but the cone of fibrils from the cilia is strongly birefringent in some species. With this technique I have found that in at least some of the species that possess neurosensory ciliated cells, there is definitely no connection between the cilia and the muscles.

This system of ciliated nerve cells does appear to have an important function related to the defensive system of the eolid. The tips of the cerata are particularly sensitive in eolids. Thus if a ceras is lightly touched near its base, or half-way up, there will often be no response. But if it is touched at or near the tip, the whole ceras is usually waved about, and nematocysts or glandular secretions, or both, may be extruded. It is likely that it is stimulation of these neurosensory bristles that causes this response. It may be a reflex response not involving the central nervous system at all since, in C.aurantia, prodding the tip of

an autotomised ceras often results in extrusion of nematocysts. Normally, stimulation of one ceras (by pinching it, for example) results in extrusion by a large number of adjacent cerata which bend towards the forceps. Clearly this response does involve the central nervous system; so nematocyst extrusion can be caused either by a simple reflex arc involving the neurosensory bristles, or by nerve pathways not involving an external stimulation of the particular ceras responding.

5. CERATAL GLANDS

Introduction

Defensive glands have not been described from the Eolidacea except from the aberrant Calma glaucoides. Herdman (1890) gives a drawing of the ceras of Eubranchus pallidus, but the only glands he shows are a few mucous glands. Hecht (1896) and Henneguy (1925) state that mucous glands occur scattered over the ceratal epithelium of all the eolid species they studied, but they find a concentration of glands at the ceras tip only in Calma glaucoides. Both of these authors studied species of Eubranchus and Catriona, but did not find any concentration of glands at the ceras tip. This section describes for the first time concentrations of defensive glands at the ceras tip in species of Eubranchus and Catriona. Glands which may have a defensive function are also described from several species of cleioproct eolids. Most of these glands are epidermal and are either simple mucous glands or are

(in all probability) derived from simple mucous glands. There is, however, another type of gland of a different nature. This is the "cellule speciale" of Hecht (1896), which can be translated as peculiar cell, or enigmatical cell. These are probably not of defensive importance, but since in some cases they have a duct through the epidermis, and hence may secrete some fluid, they are discussed here. For convenience, these enigmatical cells are discussed first, then the other ceratal glands are described for each family in turn.

Cellules speciales

Hecht (1896) describes the cellules speciales, or enigmatical cells, from several species of eolid and from the dendronotacean Doto. They lie in the connective tissue of the cerata and are characterized by the darkly-staining, granular cytoplasm, and the large nucleus. Hecht finds no excretory canal and concludes that they are neither secretory nor nervous in function. Evans (1922) studied the cellules speciales of Calma glaucoides. He regards them as modified connective tissue cells which undergo a secretory cycle correlated with the feeding activities of the animal. This species feeds on fish eggs when these are available, and fasts for long periods when they are absent. When the animal has recently fed, the cellules speciales swell up, their contents stain deeply and a clear sphere forms in the nucleolus. As the animal fasts, so the reserves in these cells are used up, and they diminish in size. Evans regards the cellules speciales as being protein storage cells for use during periods of fasting and to provide material at

this time for the gonads. Henneguy (1925) also describes cellules spéciales, but he fails to find the clear sphere in the nucleolus described by Evans.

In this work, the cellules spéciales have been studied in order to determine whether they may be of defensive importance. Table 5 shows that they occur in all of the acleioprocts studied, and also in a few cleioprocts. Most cleioprocts, however, do not possess cellules speciales. Bürgin-Wyss (1961) finds no cellules spéciales in the body of the eolid, but I have found this is not always the case. Favorinus auritulus, for example, has all of its cellules spéciales in the body, and none in the cerata.

I have found that there is evidence for a secretory or a storage cycle; sometimes the cellules spéciales are large with well-developed nucleolar vesicles, whilst in other individuals of the same species they are small and apparently without vesicles in the nucleolus.

In Calma, there is a regular alternation between feeding and fasting which correlates with this cycle of the cellules spéciales (Evans, 1922), but no such alternation occurs in most other eolids. Consequently it is impossible to correlate these changes in the cellules spéciales with the condition of the animal without a more detailed investigation - this does not come within the scope of the present thesis. However, there is direct evidence for the theory that the cellules speciales are storage cells. The darkly staining

Table 5. To show the occurrence of cellules spéciales in the Eolidacea.

E - present investigation; 1922 - Evans, 1922; 1896 - Hecht, 1896; 1925 - Henneguy, 1925.

Family	Cellules spéciales present	Cellules spéciales absent
Eubranchidae	Eubranchus cingulatus 1896 E.exiguus 1896, E E.pallidus E E.tricolor 1896, E Capellinia conicla E	
Cuthonidae	Catriona aurantia E C.maua E C.perca E C.tina E Eolis M. E Tergipes despectus 1896, 1925, E	
Facelinidae		Facelina coronata E Learchis poica E Phidiana lynceus E Eolis D. E
Favorinidae	Favorinus auritulus E (in body only) Favorinus branchialis E Dondice occidentalis E (very few, small)	
Aeolidiidae	Berghia coerulescens E	Eolis K. E Aeolidia papillosa E Aeolidiella glauca E Spurilla neapolitana E
Calmidae	Calma glaucoides 1896, 1925, 1925	
Fionidae	Fiona pinnata 1925	
Dotoidae	Doto coronata 1896, 1925, E Doto cinerea 1925	

cytoplasm contains glycogen and proteins (strongly positive to Best's carmine and to the Sakaguchi test). It is also sometimes positive, though not always strongly, to the periodic acid and Schiff's test for carbohydrates. These results are listed in Table 6. Whilst most of these conclusions are based on experiments on Catriona aurantia and Eubranchus exiguus, it can be seen that the cellules spéciales of other species have similar reactions. The pyronine and methyl green test for RNA was performed on C.aurantia (Jordan and Baker, 1955). The cytoplasm and the nucleolus stain red, suggesting the presence of RNA, whilst the nucleus stains green, suggesting DNA, and the vesicle in the nucleolus remains colourless. A section treated with 1% ribonuclease is identical except that the cytoplasm and the nucleolus are colourless, thus confirming the presence here of RNA.

These results strongly suggest that the cellules spéciales may have a storage function, as Evans (1922) finds to be the case in Calma. However, in some individuals there appears to be a duct from the cellule spéciale through the epidermis. This duct is present in probably every cell of Eubranchus tricolor, and shows clearly in Figs. 18 and 19. In Catriona aurantia it is present only in some of the cellules spéciales, particularly those in the body of the animal at the base of the cerata. In E.exiguus a careful search through sections of four animals revealed only a very small number of ducts, and none at all could be found in Tergipes despectus or Favorinus branchialis. It is probable that this duct is also cyclic

in its occurrence since it is absent in some individuals of C.aurantia but present in others. This would account for the fact that previous workers failed to find a duct. The presence of this duct strongly suggests that the cellules speciales, at some stage of their cycle, secrete or excrete material from the body. They do not contain mucus (negative to alcian blue, dialysed iron, and toluidine blue), and there is no evidence that the glycogen and protein that they contain is passed through this duct. The cytoplasmic RNA suggests that protein synthesis occurs in these cells, but this is unlikely to be of use in defence since the ducts are often absent. Thus the contents of the cellules spéciales strongly suggest that their chief function is that of storage, but the occasional presence of a duct indicates that they may be excretory as well. They are almost certainly not of defensive importance.

Table 6. Histochemical reactions of Cellules spéciales

Species	Cytoplasm					Nucleus		Nucleolus	
	Mucin tests:- Alcian blue Dialysed Iron Thionine	Carbohydrates PAS	Glycogen Best's Carmine	Arginine Sakaguchi	RNA:- Pyronine Methyl-Green	Sakaguchi	Pyronine Methyl-Green	Sakaguchi	Pyronine Methyl-Green
C.aurantia	0	++	+++	++	+++	+++	0	++	+++
C.perca	0	++	+++	+++					
C.maua	0	0	+++	+++					
C.tina	0		+++						
T.despectus	0	+	+++						
E.pallidus	0	+	+	+		+++		++	
E.tricolor	0	0	+						
F.exiguus	0	++	+++	++		+		++	
F.branchialis	0	+	+++	+					

Key: +++ strongly positive

++ positive

+ weakly positive

0 negative

Figure 14. Epidermal vesicles from eolid nudibranchs. a. - Catriona aurantia stained with dialysed iron, fixed in "Susa"; b. - C. aurantia, Mallory's triple stain, fixed in "Susa"; c. - C. aurantia stained vitally with thionine; d. - Eubranchus pallidus stained with dialysed iron, fixed in Lewitsky-saline.

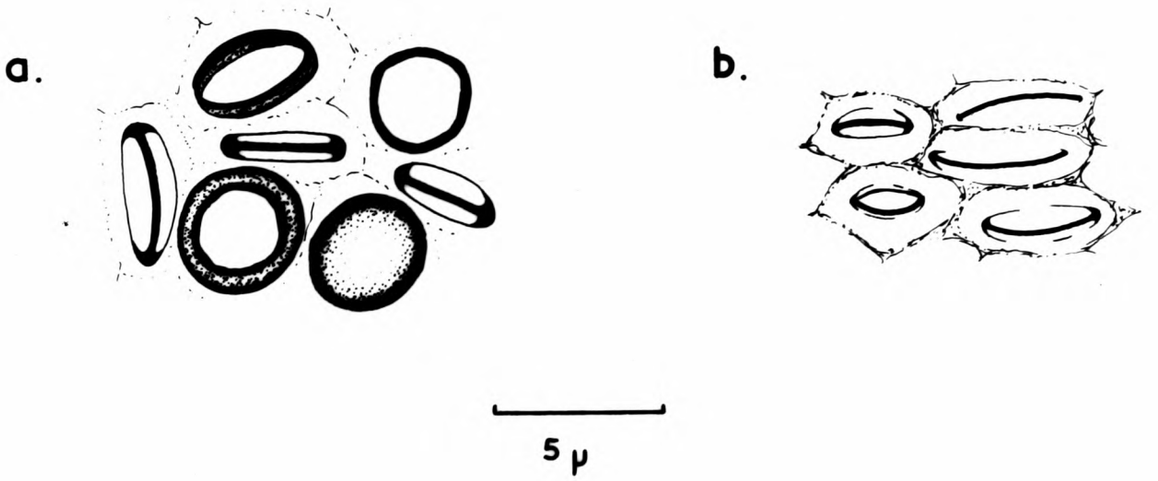

a.
b.
5 μ
c.
d.

Figure 15. Diagram of cerci tip of Catriona aurantia to show distribution of sensory bristles.

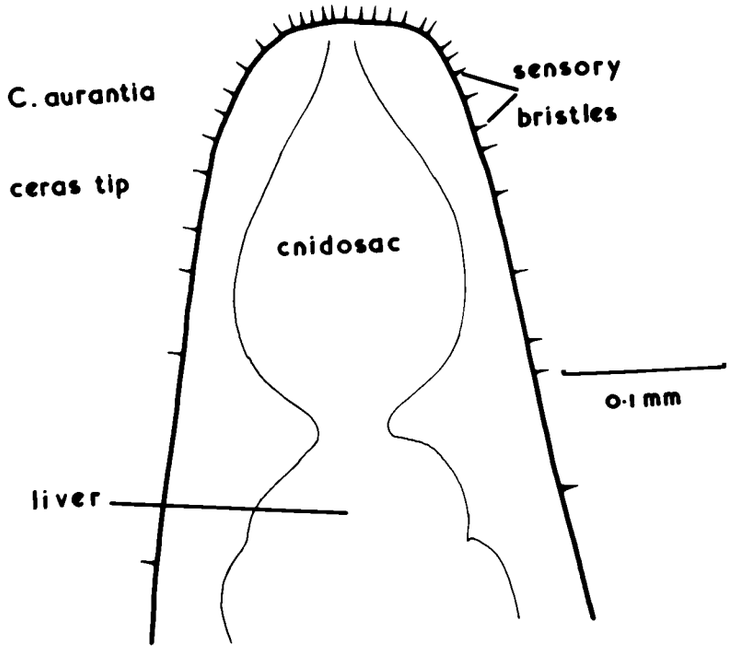
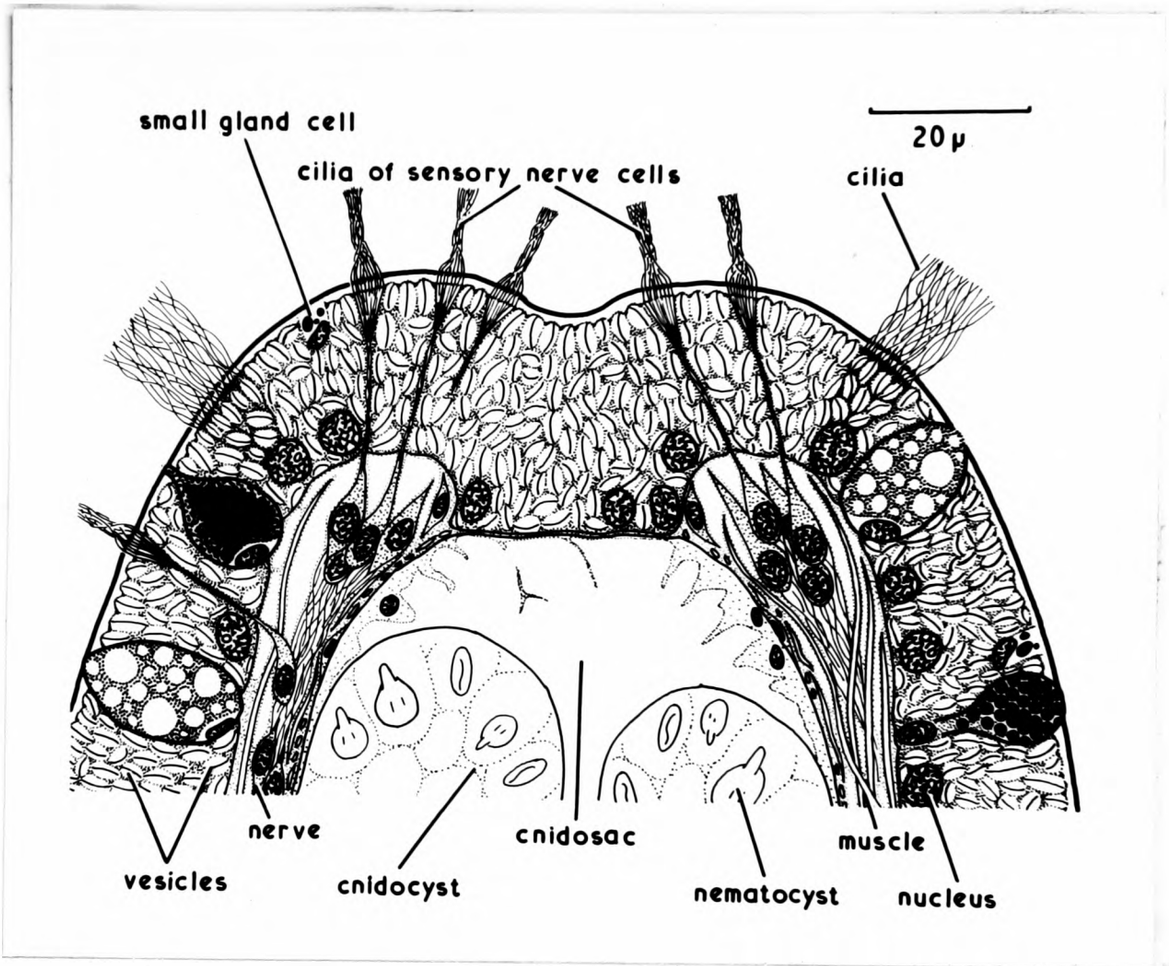
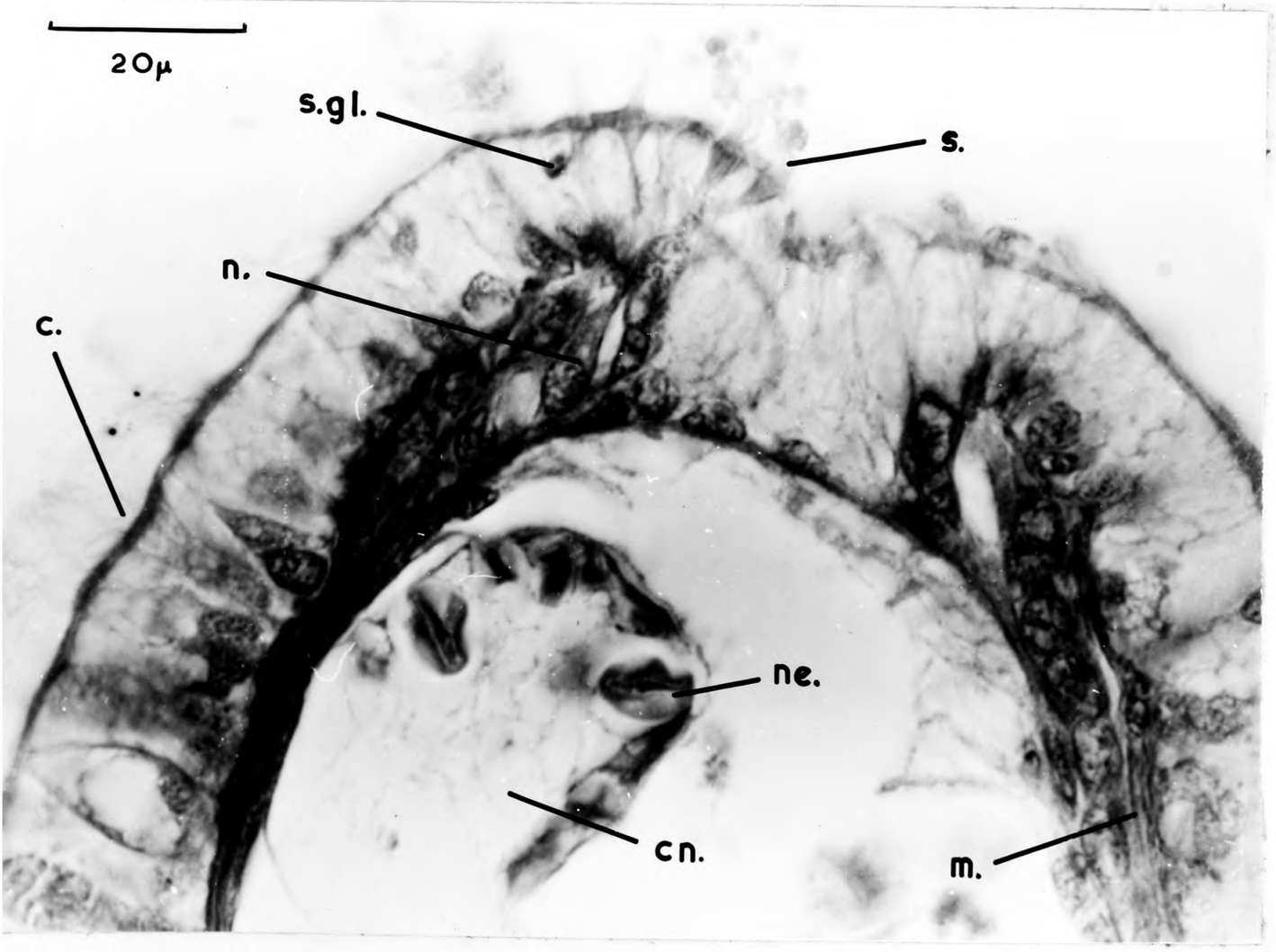

C. aurantia
ceras tip
sensory
bristles
cnidosac
0·1 mm
liver

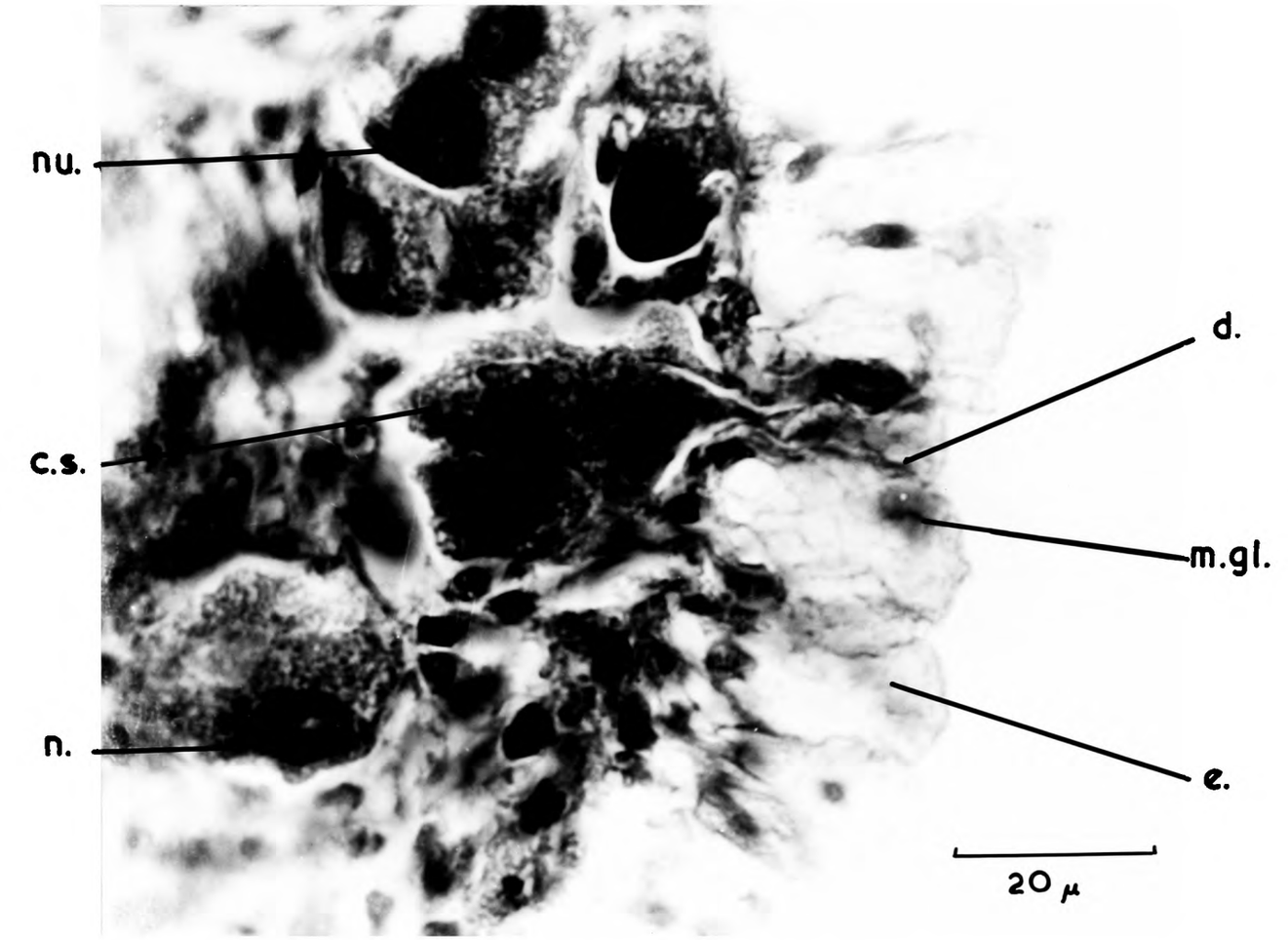
Figure 16. Semi-diagrammatic section of ceras tip of Catriona aurantia to show the relation between sensory cilia and nerve fibres.

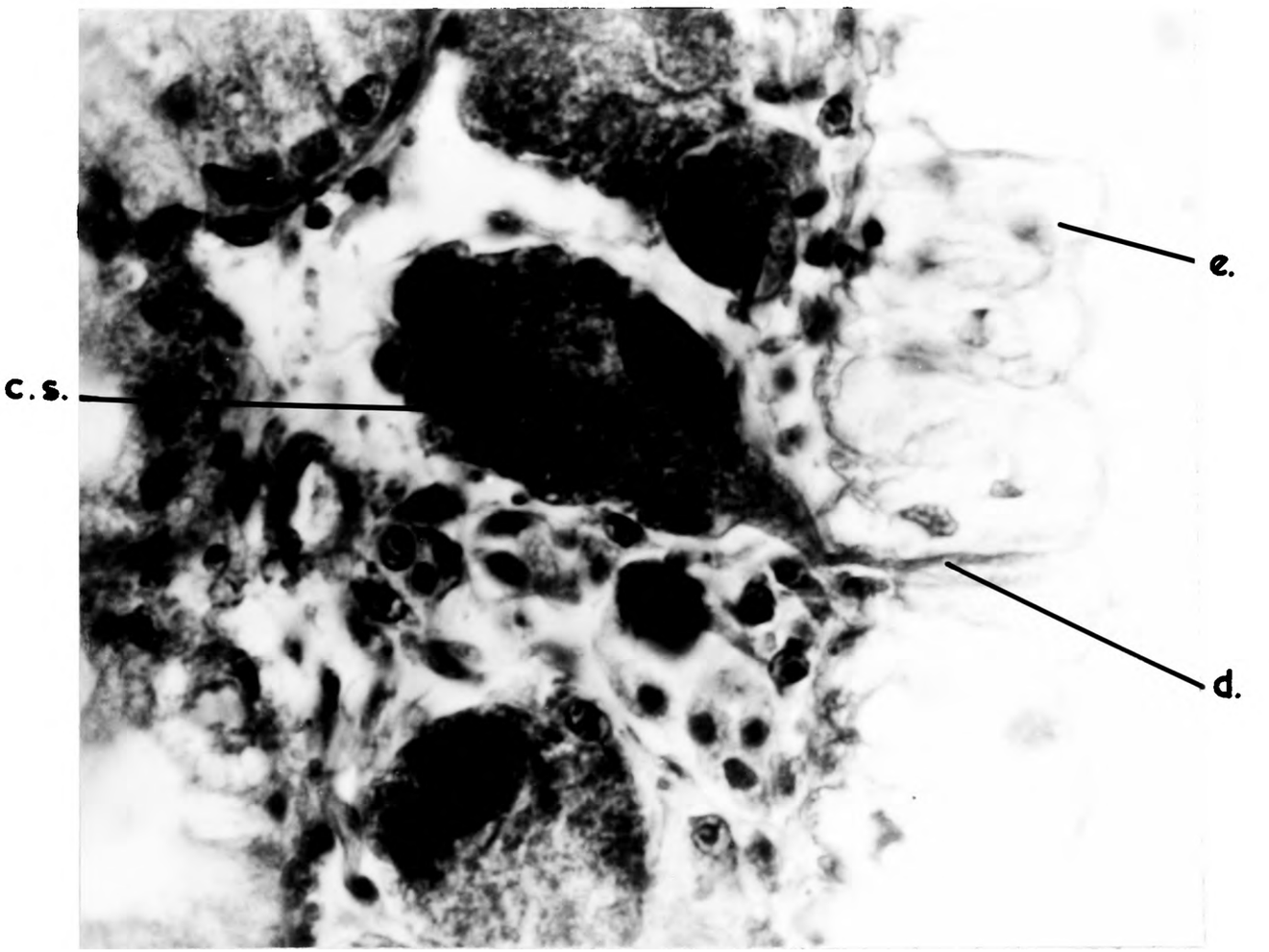
Figure 17. Longitudinal section of ceras tip of Catriona aurantia fixed in "Susa" and stained with Masson's trichrome. c., cilia; cn., cnidocyst; m., muscle; ne., nematocyst; s.gl., small gland cell of unknown function; s., cilia., and n., cell body of sensory nerve cells.


small gland cell
cilia of sensory nerve cells
cilia
20μ
nerve
cnidosac
muscle
vesicles
cnidocyst
nematocyst
nucleus


20μ
s.gl.
s.
n.
c.
ne.
cn.
m.

Figures 18 and 19. Sections of cerata of Eubranchus tricolor fixed in "Susa" and stained in Masson's trichrome. c.s., cellule spéciale; d., duct of cellule spéciale; e., epidermis; m.gl., small mucous gland; n., nucleus, and nu., nucleolus of cellules spéciales.


nu.
d.
c.s.
m.gl.
n.
e.
20 μ


e.
c.s.
d.

Ceratal glands of the Eubranchidae

Introduction to the Eubranchidae

The Eubranchidae can be defined as acleioproct eolids in which the radular formula is 1.1.1. Of the four species studied, Eubranchus pallidus (Alder and Hancock), E.tricolor Forbes, and E.exiguus (Alder and Hancock) are all British, and Capellinia conicla Marcus is Jamaican. None of these species was found in large numbers, so the behavioural conclusions are necessarily somewhat tentative.

The genus Capellinia is very close to Eubranchus, but differs in the possession of an armed penis (Marcus, 1958). The two genera are considered together here because the ceratal glands are very similar. Solely on the basis of ceratal glands, E.exiguus is more similar to C.conicla than it is to either of the other two species of Eubranchus.

Ceratal glands of Eubranchus pallidus

In Eubranchus pallidus, glands are present all over the surface of the cerata but are most abundant in the region of the cnidosac (Figs. 20 and 21). Six types of gland can be detected, three being confined to the tips of the cerata, and three occurring all over the dorsal surface of the animal but usually absent from the tips of the cerata. Four of these glands consist mainly of mucin, the other two are negative to tests for mucopolysaccharides. These glands, for convenience, will be designated with the letters A to F.

A. White osmiophil glands. These are abundant in the cnidosac region, but the epithelium bordering the cnidopore is free of glands (Fig.21). Each gland is unicellular and flask-shaped, between 20 and 32μ long and about 15μ broad, with a basal, ellipsoidal nucleus about 3 to 4μ in length. The base of the cell does not project noticeably below the level of the bases of the neighbouring epidermal cells. In life, the cells contain an opaque white substance which is blackened by osmium fixatives and then appears to be in the form of a loose coil (Figs. 22 and 24). The black substance probably consists of large numbers of minute granules, less than 0.4μ in diameter, which can occasionally be seen. After decolouring with permanganate, the osmium is bleached out and the secretion takes up basic dyes, green with Masson's trichrome and blue with Mallory's triple stain. It is negative to alcian blue, and only as positive to dialysed iron as is the cytoplasm of most non-mucoid cells. When fixed in "Susa", these glands have a characteristic empty appearance with only a very small residue at the bottom (Fig.23). Lack of material made it impossible to try other fixatives and hence to perform certain other histochemical tests. The osmium presumably attaches to unsaturated bonds in the secretion, but this secretion is not a simple lipid in the similar osmiophil glands of Catriona. Table 7 shows that the secretion is negative to tests for carbohydrates generally (PAS) and in particular to glycogen and mucin; but it is strongly positive to the Sakaguchi test for arginine and hence contains protein.

Observations of living cerata confirm that these white glands do in fact exude their contents, either as globular blobs, or as threads up to 100μ long by about 3μ in diameter. Occasionally a gland is fixed in a partially exuded state, as shown in Fig.24. It is not known whether each gland exudes all of its secretion at one time and then is incapable of further exudation until more of the osmiophil substance has been formed, or if only a part of the secretion is lost at any one time. The latter seems the more likely as no completely empty glands were found in the Lewitsky-saline fixed animal, and no possible stages in a secretory cycle were found.

B. Granular mucous glands. Like the white osmiophil glands, the granular mucous glands occur only in the region of the cnidosac. They are less numerous than the white glands and are not easy to detect in Masson preparations. They are elongate, vase shaped, and only 8 or 9μ broad with a basal nucleus about 2.5μ diameter. The cell is closely packed with spherules 0.4 - 0.5μ diameter (Figs. 23 and 24) which give the characteristic reactions of acid-mucopolysaccharides, blue with alcian blue and dialysed iron, and metachromatic with toluidine blue or thionine (see Table 7). The spherules are weakly positive for protein, so mucoproteins may be present. Since the granular mucous glands occur in exactly the same region of the ceras as the white osmiophil glands, it is pertinent to consider if they might not possibly be the same gland in different stages of its secretory cycle. The evidence for their being distinct is:-

1. No intermediates can be found between the two types although with histological, as opposed to histochemical, stains they may have similar staining reactions.

2. They respond differently to histochemical tests, and because of (1), they cannot therefore be stages in the secretory cycle of the same type of gland.

3. The related Eubranchus exiguus and Capellinia conicla have similar glands with similar staining reactions (see later). In these species also there are no distinct intermediates between the two glandular types. In addition, in these species, each type 'A' gland is closely associated with a type 'B' gland such that the 'B' gland is invariably towards the base of the ceras with respect to its corresponding 'A' gland (see Fig.31). If these were merely the same type of gland functioning alternately, one would expect to find some cerata or some individuals showing the reverse condition, with the 'A' gland below the 'B' gland. However, in four specimens of E. exiguus and two of C.conicla, the 'B' gland is always below the 'A' gland in its position on the ceras; so we can conclude that the two glands are distinct.

C. Large mucous glands. These are unicellular, epidermal glands occurring just below the cnidosac region of the ceras, and just below the glands of types 'A' and 'B' (Fig.21). They are ovoid in shape, 25 to 32μ long by about 13 to 18μ broad, with a compressed, elongate nucleus 4 to 5μ long at the base of the cell (Figs. 22, 23, 24). As in the white glands, there is very little cytoplasm, the bulk of the

gland being taken up with the secretion. The appearance of the secretory product depends on the fixative employed and probably also on the time for which it was fixed, as Fontaine (1962) found for ophiuroids. With "Susa", the contents form a reticulum which takes up basic dyes and is positive to all the mucin tests employed (Table 7). With Lewitsky-saline, the contents are more evenly dispersed, and react similarly to histochemical tests and to dyes except that they do not stain at all readily with Masson's trichrome. In the "Susa" preparations, the contents of the cell are often partially exuded. It is not clear whether this is due to swelling with one particular fixative, or whether it merely indicates that the animal concerned was less effectively narcotised than the animal fixed in Lewitsky-saline. The latter is the more likely because the homologous glands of the other species to be discussed were never found partially exuded in fixed preparations.

D. Small mucous glands. These glands are scattered throughout the dorsal and lateral surfaces of the body except in the region of glands of types 'A', 'B', and 'C' (Fig.21). They consist of a spherical mass of mucus about 10μ in diameter, not reaching the base of the cell (Figs. 25 and 26). Cell boundaries are difficult to detect, so it is not easy to be certain which nuclei belong to these gland cells. However, the nuclei at the base of these mucous secretions are usually in the region of 2.5 to 3.5μ diameter, and slightly ellipsoidal. The non-glandular part of the cytoplasm is vacuolated

just as it is in the neighbouring epidermal cells. The secretion stains evenly and has similar staining reactions to the mucin of the type 'C' glands, except that there is some protein present in these glands (Table 7).

E. Subepidermal mucous glands. These are far less abundant than any of the other types of gland, but they may occur both in the cnidosac region and in the rest of the dorsal and lateral surfaces of the body (Fig.21). They are difficult to detect in Masson preparations as the epidermal part resembles a type 'D' mucous gland, and the subepidermal part is only weakly stained by light green. They stain with basic fuchsin and dialysed iron, and are metachromatic with toluidine blue. The oval nuclei are 4 to 5μ long, and are surrounded by an area of cytoplasm. The rest of the cell is packed with mucin granules 0.5 to 1.0μ in diameter (Fig.26). A duct leads from the subepidermal part of the cell through the epidermis to the free border, and there is usually a small reservoir in this region. As with glands of types 'B', 'C' and 'D', the staining reactions indicate that the mucin is some form of acid-mucopolysaccharide.

F. Granular non-mucous gland. This type of gland is abundant in the epidermis of the dorsal and lateral surfaces of the body except in the cnidosac region (Fig.21). Each gland is unicellular and flask-shaped, with the base bulging into the subepidermal layer. A typical gland is 15μ long by 8μ broad, with a spherical or oblong nucleus of 3 to 4μ towards the base of the cell (Figs. 25 and 26).

Table 7. Histochemical reactions of the ceratal glands of the Eubranchidae.
0 - negative, + - weakly positive, ++ - positive, +++ - strongly positive, ? - indicates that no glands could be found, but if they had been strongly coloured, they would have been detected. The 'A' and 'F' glands are weakly positive to dialysed iron in E.pallidus, but so under the same conditions is the cytoplasm of most non-mucous cells. Some species are generally responsive to certain reagents whilst others are not; for example E.tricolor to mucicarmine, or E.exiguus to PAS.

Test \ GLAND	A			B			C			D			E		F		
	pallidus	tricolor	exiguus	pallidus	tricolor	exiguus	pallidus	tricolor	exiguus	pallidus	tricolor	exiguus	pallidus	tricolor	pallidus	tricolor	exiguus
Carbohydrates: PAS	0		0	0		+	0		+	+		+++	?		0		
Glycogen: Best's carmine	0	0	0	0	0	0	0	0	0	0	0	0	?	?	0	0	
Mucins: Alcian blue	0	0	0	++		++	++	++	++	+++	+++	+	++		0	0	0
Dialysed iron	+	0	0	+++	+++	++	+++	+++	+++	+++	+++	++	+++	+++	+	0	0
Mucicarmine	0	0		+	+		++	+++	+	++	+++		+++	+++	0	0	
Safranin (metachromatically)	0		0	+++			+++			+++			+++		0		
Thionin (metachromatically?	0	0	0	++	++	++	+	+	+	+	+	+	+++	+++	0	0	
Proteins: Sakaguchi	+++			+			0		0	+					++		

In fixed preparations the cell appears empty apart from a cluster of granules 0.5μ in diameter which take up basic fuchsin, xylidine red (Masson), and orange G (Mallory). They are not coloured by mucin stains, but do contain protein.

Ceratal glands of Eubranchus tricolor

Only one individual of this species was found, and it was fixed in "Susa". The general histology of the epidermis is very much as it is in E.pallidus, but cell boundaries are easier to detect. The distribution of glands is similar to that found in E.pallidus (Figs. 21 and 27). The osmiophilic type 'A' glands are exceedingly difficult to detect as they have very little residual secretion not dissolved by the fixative. The granular glands of type 'F' are also less conspicuous, and have smaller granules, rather less than 0.5μ diameter. The reactions of these glands to the histochemical reagents employed closely resemble those of E.pallidus (Table 7).

Ceratal glands of Eubranchus exiguus

This species is much smaller than either of the preceding species, and shows some interesting differences in its histology. The cytoplasmic vesicles are smaller, in the region of 2μ instead of 3 to 4μ, and they have therefore been omitted from Figs. 31 and 32 for clarity. Small mucous glands, of type 'D', are far less abundant than they are in the other two species of Eubranchus, and the subepidermal mucous glands, type 'E', are totally absent (see Figs. 21 and 28). The granular type 'F' glands are less frequent, and are of a different shape. They are considerably elongated, with

a basal nucleus, and the granules or droplets are concentrated near the outer surface of the cell (Fig.32). The most interesting differences, however, are in the cnidosac region. The large mucous glands (type 'C') are similar to those of E.pallidus, but the osmiophil type 'A' glands are much swollen and project down into the subepidermal connective tissue. The residue in "Susa" fixed animals is quite considerable, and in Lewitsky-saline material it is not strongly osmiophil. Each gland has at its base one, or possibly more, muscle cells whose 10 to 20 fibrils, each less than 1μ in diameter, insert at half to three-quarters of the way up the gland (Fig.31). These are presumably involved in the ejection of the secretion from the cell. The granular mucous glands of type 'B' occur alternately with the osmiophil glands such that each type 'A' gland is nearer to the tip of the ceras than is the corresponding 'B' gland (Figs. 29 and 30). The pores of each pair of glands are adjacent, and it seems reasonable to suppose that they are both secreted at the same time. Some of the muscle fibrils appear to insert on the wall of the type 'B' glands, although it is difficult to be certain of this. Certainly when these muscle fibrils contract it is likely that secretion is forced out from both types of gland. The chemical reactions of these glands are summarised in Table 7, and are similar to those of E.pallidus.

Ceratal glands of Capellinia conicla

The appearance of Capellinia conicla is very similar to that

of E.exiguus. Both have small, frequently knobbed cerata, with small cnidosacs rarely more than 100μ in length. The ceratal glands of C.conicla are almost identical with those of E.exiguus in both type and location (Fig.21), although as the cerata are slightly smaller, there are relatively fewer glands. The osmiophil glands show practically no shrinkage with "Susa" fixation, unlike those in E.pallidus and tricolor; and the largest of them do not project far below the level of the basement membrane of the neighbouring cells, unlike in E.exiguus (Fig.33). A further similarity to E.pallidus is that there are no conspicuous muscle fibrils forming a basket round the glands, although birefringent fibrils can be seen in all species with polarised light. As in E.exiguus, the type 'A' and the type 'B' glands occur in pairs, such that the 'A' gland of each pair is towards the tip of the ceras. Lack of material prevented many histochemical tests from being performed, but the type 'B' glands contain granules of mucin (alcian blue and dialysed iron) as in the species of Eubranchus.

Mode of secretion of the ceratal glands of the Eubranchidae

Secretion of a gland may be brought about in either of two distinct ways: it may secrete continuously, in which case no particular mechanism is necessary to cause exudation; or it may secrete a large quantity of substance at one particular time when stimulated, in which case some contractile mechanism is necessary to ensure that exudation occurs at the appropriate time. Since the osmiophil type 'A'

glands and the granular type 'B' mucous glands of E.exiguus are surrounded by a basket-work of muscle fibrils, it would appear likely that they are of the second type. Observation of the living animal confirms this with respect to the white osmiophil glands which are only exuded when the animal is violently disturbed. Similarly, the white glands, and either the B or the C type mucous glands, of E.pallidus are also only exuded when the animal is disturbed. In this species, no basket-work of muscle fibrils can be seen with an oil immersion objective. However, in E.exiguus, these muscle fibrils are strongly birefringent in polarised light, and by this means it is possible to detect the presence of fine fibrils round the osmiophil glands of E.pallidus, E.tricolor and C.conicla. They are few in number, and of very small diameter. Because they are in the same position as the much larger fibrils of E.exiguus which clearly stain as do muscle fibrils, and because they are birefringent, it is probable that they too are muscle fibrils. Thus in all of the Eubranchidae studied, the white glands are muscle-operated. The granular mucous glands may also be muscle-operated, but this is not certain. The remaining glands are probably of the first type which ooze continuously, although it is possible that for some of them contraction of the muscles in the dermal layer causes exudation.

Functions of the ceratal glands of the Eubranchidae

Observations were made on living animals of Eubranchus

pallidus and E.exiguus. In E.exiguus, if the animal is violently disturbed, the cerata are waved about, and if the tips touch a solid object (e.g. forceps or the sides of a glass dish) a copious white secretion often emerges from near the tips of the cerata. When a ceras is pinched, it is usually autotomised, and if the tip has been kept clear of all objects that might adhere to the white substance and hence remove it, it is often found that the large white glands near the tip of the ceras have been exuded as white globules. The thread-like nature of this secretion is easily demonstrated if a coverslip is used to compress the ceras on a slide. The white material then oozes out in the form of a thread. On the intact animal, the white blobs adhere to objects and are pulled out into long, sticky threads. These white thread-like glands are the osmiophil type 'A' glands of fixed material, since both occur in the same position on the ceras, and both are of the same size. Because they are only exuded when the animal is prodded in a manner simulating attack by a predator, it is reasonable to conclude that these glands are of defensive value. No comparable ejection of the secretions of the 'B' and 'C' mucous glands was observed, but this may have been masked by the white secretion, and if the mucus is easily dispersed in water it could have been overlooked. Since the granular type 'B' glands occur in close association with the white glands, they are almost certainly exuded at the same time, and it may be that the combined secretion from

these two glands is more repellent than either fluid alone. On this hypothesis, the function of the large type 'C' mucous glands would be to provide a medium in which the defensive fluids are effective but not too rapidly dispersed and hence diluted. Hecht (1896) believes that the ceratal mucous glands are defensive, and he also noticed that the ceratal cilia of eolids create a continuous current from the base to the tip of the ceras. I have seen this current, in E.exiguus, carrying the glandular secretions away from the tip of each ceras towards the forceps, and presumably, in nature, towards the potential predator. On eleven occasions, a ceras of E.exiguus was pinched with a pair of forceps and autotomised. At least seven times autotomy was accompanied by exudation of white substance either from the ceras being pinched or from neighbouring ones which bent towards the forceps. This is further discussed on page 120 and the results are listed in Table 9.

E.pallidus also secretes white threads from the osmiophil glands when it is prodded with forceps, and in addition it often secretes colourless globules from either the 'B' or the 'C' mucous glands. In ten cases of pinching and causing autotomy of a ceras, either white, or mucous, or both types of gland were exuded. As with E.exiguus, the fact that these glands are situated near the tips of the cerata and are exuded only on prodding the animal, indicates that they are defensive in function. It is still not certain, however, exactly what part is played by the 'B' and 'C' mucous glands. They may be repellent in themselves or they may

provide a viscous medium for the white glands.

The functions of the remaining types of gland are even less clear. The small type 'D' mucous glands probably secrete fluid continuously. Henneguy (1925) describes a thin cuticle covering the ceratal epidermis of eolids except for the ciliated cells. This thin layer is positive to tests for mucins (alcian blue, dialysed iron, mucicarmine, PAS) and stains similarly to the small mucous glands. It seems likely that this mucous film is secreted by these small mucous glands, and so is of a different nature from the cuticle of many other invertebrates where protein or chitin is present. As Hecht (1896) points out, this film of mucus probably protects the animal from abrasion. However, E.exiguus and C.conicla have many fewer type 'C' glands in relation to the surface area of the cerata than either E.pallidus or E.tricolor, yet if this were the only function of these glands, one would have expected them to have approximately the same number. Desiccation is unlikely to be a serious problem, since these animals live just below the lowest tide levels. Mucus apparently facilitates gaseous exchange since most gilled animals have the gills covered with mucus; but this is unlikely to be important in such small and slowly-moving animals as these with such a large surface area. A clue to their function is that in a living ceras, a strong ciliary current passes from the base of the ceras towards the tip, as was first described by Hecht. It seems likely that the mucous cuticle performs a cleansing function, and since it is continuously being

removed at the ceras tip any obnoxious chemicals will tend to be wafted away from the animal. As we have seen already, this current probably aids in defence in relation to the glands at the tip of the ceras, and it may be that the secretion from the small mucous glands forms a suitable medium for the repellent secretions of the glands at the tip.

The subepidermal 'E' type mucous glands are not abundant and clearly cannot be responsible for the mucous cuticle since they are absent from E.exiguus and C.conicla. They probably produce some potent secretion, possibly defensive in function, which is distributed and diluted by the stream from the small mucous glands. Indirect evidence for this is that they are absent from the two species in which the tip defensive glands are mosthighly developed (E.exiguus and C.conicla), and it therefore seems likely that they are of defensive importance in the two species which possess them.

The non-mucous, granular, type 'F' glands could be straightforward defensive glands with a proteinaceous secretion, but they do not always appear to have a permanent pore which one would expect if they are defensive. A second possibility is that they are excretory. They may be coelomocytes, but they do not resemble the coelomocytes of other invertebrates, nor have I found them in the blood spaces. They have not the appearance of amoeboid cells, and therefore are unlikely to be cells which travel round the body before settling down in the epidermis. They could perhaps be

secretory rather than excretory, but in Berghia, discussed on page 104 similar cells do appear to be coelomocytes.

A further possibility should be mentioned, although there is no evidence either for or against it. One or several of these glands may perform some function related to nematocyst explosion. On this hypothesis, they would produce some secretion that facilitates the explosion of nematocysts extruded from the cnidosac. Glands with the converse action might be supposed to occur in the mouth region or in the salivary glands to prevent the explosion of nematocysts whilst the animal is feeding. If such chemicals do occur, they are probably protein derivatives rather than mucopolysaccharides since enzymes and some hormones are proteins, and so the glands that could be involved are the granular type 'F' glands and the osmiophil 'A' glands. However, this is pure speculation, and I must stress again that there is no evidence for it.

Ceratal glands of the Cuthonidae

Introduction to the Cuthonidae

The Cuthonidae is a large family of acleioproct eolids with a single tooth in each radular row. Four species of Catriona are included in the present work, C.aurantia (Alder and Hancock) from Plymouth, and C.maua Marcus, C.perca Marcus, and C.tina Marcus from Jamaica. At least a dozen individuals of each species were found, and the results of histochemical tests performed on their glands are summarised in Table 8. Marcus (1958) lists over 50 species

of the genus Catriona, the most important taxonomic features being the radula and the hepatic diverticula. C.aurantia and C.maua are evidently closely related (Marcus and Marcus, 1960). Both have a long radular ribbon with the central cusp much receded, and the reddish cerata are arranged in much the same way. Both have stenotele nematocysts in the cnidosac indicating that tubularian hydroids are important in their diet, but whereas C.aurantia feeds almost entirely on Tubularia indivisa (see pages 50 and 59), C.maua has several other types of nematocyst in its cerata some of which do not occur in Tubularia, and is probably less restricted in its diet, at least in Jamaica. C.perca has very different radular teeth from C.aurantia and C.maua, indicating a different type of food, and it is clearly not closely related to them. C.tina is a very distinct species showing some affinities to the British C.foliata (Forbes and Goodsir) (Marcus and Marcus, 1960).

Two other species of Cuthonidae were also studied, but since their glands are quite different from those of the four species of Catriona, they will be described separately. The first is Tergipes despectus (Johnston) which at times is common at Plymouth. Tergipes differs from Catriona in that the cerata are arranged alternately on the back and there is only one ceras per row. The second animal is a new species from Jamaica which is referred to as Dolis M. It is probably a new genus, but is close to Catriona. It differs from Catriona in that the radular cusp projects, and from Cuthona in the armed penis. Only three specimens were found, and of these two

were sectioned, one fixed in "Susa", the other in Bouin's fluid.

Ceratal glands of Catriona aurantia

Specimens of C.aurantia were fixed in the fluids of Bouin, Zenker and Altmann; in Heidenhain's "Susa", Lewitsky-saline and formol-calcium; and in two methanol-acetic mixtures of the composition: Absolute methanol - acetic acid - formalin - 8 : 1 : 1 ; and 70% methanol - acetic acid - 19 : 1. Three types of gland were found, two confined to the cnidosac region and containing mainly proteinaceous material, and the third present all over the dorsal and lateral surfaces of the body and containing acidic mucopolysaccharides. These glands will not be given reference letters as this might be thought to imply homology or distinctiveness from the glands of Eubranchus, but it is worth noting that there are similarities between the first two types described here and types 'A' and 'B' of the Eubranchidae. In addition, there is a fourth possible gland in Catriona species, particularly clear in C.aurantia, but not definitely seen in C.tina. These cells resemble the special epidermal cells that Henneguy (1925) describes for Facelina. They are oval, with a basal nucleus, and the base of the cell does not reach the basement membrane of neighbouring cells. The clear cytoplasm contains one or two droplets which take up basic dyes and are hence acidic. They are negative to all the histochemical tests performed on the other glands. Henneguy suggests they may have a sensory function, but finds no nervous connexion to them. It is more likely that they are glandular since there is a globular

secretion, but nothing is known of their possible function, and they will not therefore be considered further. They are illustrated in Fig.16 as small gland cells.

White osmiophil glands. These occur only in the cnidosac region (Figs. 34 and 35), and are absent from the immediate vicinity of the cnidopore (Fig.16). Each gland is unicellular and flask-shaped, between 20 and 40μ long depending on the thickness of the epidermis which varies between individuals and between different parts of the same ceras. The basal nucleus is from 4 to 7μ long, and the base of the cell does not project noticeably below the level of the base of the epidermis (Figs. 36, 37 and 38). With Heidenhain's "Susa", Zenker's and Bouin's fixatives, the contents of the cell are contracted to a small residue which colours only faintly with dialysed iron and with the periodic acid and Schiff reaction (PAS). The methanol-acetic fixatives give a variety of appearances, but usually there is a granular material in the glands. The two osmium fixatives colour the secretion black, and this deposit of osmium is located in minute granules, mostly 0.5μ diameter, but occasionally up to 1μ with heavy staining. Unlike in the osmiophil glands of E.pallidus, there is never a coiled appearance to the secretion, although when material is squeezed out of a living gland by a coverslip, it is frequently in the form of a white thread. When Lewitsky-saline material is decoloured with permanganate, the granules are positive to dialysed iron, though not strongly so, and only very weakly

positive to PAS. The secretion is positive to Sakaguchi's test for arginine, so it appears to consist either of a mucoprotein, or of a protein together with some form of mucopolysaccharide to give it a fluid but viscous consistency.

Granular glands. These occur only in the cnidosac region of the cerata (Fig.35), and are packed with granules about 1μ in diameter which stain strongly with the xylidine red of Masson's trichrome. The nucleus is usually 4 to 5μ long, as in most non-glandular cells of this species (Fig.38). The histochemical reactions of these granules or globules are shown in Table 8. As with the osmiophil glands, they appear to contain carbohydrate and protein, either separately or in some form of mucoprotein. Since these two glands occur in the same region of the ceras and have similar histochemical reactions, I give here the evidence by which I conclude that they are distinct and are not merely stages in the secretory cycle of the same gland:

1) With most of the fixatives employed there is no trace of any cell that could be interpreted as being in any way an intermediate between the two types. It is difficult to distinguish them with the methanol-acetic fixatives, and I am unable to explain this; but if any of the cells here are intermediates one would expect to see them also in tissues fixed with more usual fixatives.

2) Cells that could be young stages of the osmiophil glands can be found (Fig.38), and they are simply small editions of the mature gland. They in no way resemble the granular glands.

Test	Osmiophil glands				mucous glands				granular glands				C.perca glands
	a	m	p	t	a	m	p	t	a	m	p	t	p
Carbohydrates PAS	+	0	0		+++	++	+		+++	+	+++		0
Best's for glycogen	0	0	0	0	++	+	+	+	0	0	0	0	0
Alcian blue for mucins	0	0	0	+	+++	+++	+++	+++	0	0	0	+	0
Dialysed iron for mucins	+		+	+	+++	+++	+++	+++	+	++	+	++	0
Mucicarmine for mucins	0	0	0	0	0	+	+++	++	0	0	0	0	0
Safranin - metachromatically for mucins	0	0	0	0	+++	+++	+++	++	0	0	0	+	+
Thionine for mucins	0	0	0	0	+++	+++	+++	+++	0	0	0	0	0
Proteins Sakaguchi for arginine	+	0	+		0	0	0		++	+	+		0
Lipids Sudan black	0	0	0		0	0	0		0	0	0		0

Table 8 to show the histochemistry of the ceratal glands of Catriona species. C.aurantia - a; C.maua - m; C.perca - p; C.tina - t.
0 - negative, +++ - strongly positive, ++ - positive, + - weakly positive

3) Different individuals of C.aurantia have different proportions of the two types of gland. If one were merely a stage in the secretory cycle of the other , one would expect the relative proportions of the two to be constant.

4) Two types of gland can be seen in the cnidosac region of a living ceras: transparent glands which exude on disturbing the animal, and white glands which can be squeezed out under a coverslip, but which exude naturally in C.maua and C.perca. Both glands are thus capable of exudation, and neither is likely to be immature.

5) In C.maua, perca and tina there are no intermediates between these same two types of gland. In C.tina, most of the ceras tip glands occur in pairs such that the granular gland is towards the base of the ceras in each pair. If they represent stages in the secretory cycle of the same gland, one would expect about half of the pairs to be in the reversed position.

The only important evidence against this is the appearance of the glands with methanol-acetic fixatives already referred to. Possible intermediate stages can be found, but none is conclusive, and in view of the overwhelming evidence presented, I can only conclude that these two types of gland are in fact distinct and are not stages in the secretory cycle of the same gland.

Mucous glands. These occur all over the dorsal and lateral body surfaces, but are not so abundant in the cnidosac region of the cerata (Fig.35). They are from 10 to nearly 40μ in length depending on the thickness of the epidermis, and the nuclei are elongated and

up to 8μ in length (Fig.38). The secretion (Table 8) consists of acid mucopolysaccharides, and is positive to Best's carmine for glycogen.

Ceratal glands of Catriona maua

The distribution of ceratal glands in C.maua is very similar to that in C.aurantia (Fig.35); the chief differences are that there are fewer mucous glands, and the cnidosac glands are restricted to a much smaller area towards the tip of the ceras. The cytological structure and the histochemical reactions of the glands are closely similar in the two species (Fig.39 and Table 8). The cnidosac is only about half the length in C.maua that it is in C.aurantia. This is not simply due to the fact that C.aurantia is the larger animal; cnidosac size is not correlated with ceras size, and a young ceras has a cnidosac of approximately the same size as an older, and much larger ceras. Below the small cnidosac (which is usually 120 to 165μ in length in fixed animals), C.maua has an accessory sac in which nematocysts are taken up and stored. This is really just a modified part of the hepatic epithelium in which there are no glandular cells at all, and it is sharply demarcated from the typical liver. The length of this accessory sac is variable even within one individual in different cerata, but it often equals the cnidosac in length. There is no muscular or other constriction separating the accesory sac from the glandular part of the liver.

Ceratal glands of Catriona perca

The distribution of ceratal glands in C.perca is very similar to that in C.maua; the cnidosac is of approximately the same size, but there is no accessory sac. The absolute size of the ceras indicated in Fig.35 is not a valid difference since the four animals that were sectioned and from which this was drawn were not the largest available. C.perca is of approximately the same size as C.maua, and has cerata of much the same size. C.perca differs from the other species of Catriona studied in that it has a fourth type of gland (Fig.35) scattered all over the cerata, but not as abundant as the mucous glands. The secretion is often fixed so that part is exuded as a globule. It does not react to any of the stains and histochemical reagents employed. It has the appearance of one or two large, transparent but slightly yellowish globules, that fill the entire thickness of the epidermis (8 to 30μ). It seems unlikely that these can represent the final stage in the development of the mucous glands, since no intermediates occur, and they are absent from the other species of Catriona which have identical mucous glands.

Ceratal glands of Catriona tina

Catriona tina is a very small species with much smaller cerata than in the three preceding species. The cnidosac is small compared to in the other species, but large in relation to the size of the ceras. The glands are distributed as are those of C.maua,

except that, as already mentioned, at the ceras tip they occur in pairs such that the granular gland of each pair is towards the tip and the white gland is towards the base of the ceras (Figs. 40 and 41). This arrangement is not invariable: one can occasionally find isolated glands of one type or the other, but it is usually the case. Strangely enough, two of the four individuals sectioned did not possess any granular glands at all. White glands were also apparently absent in fixed material of these two animals, but these are easily overlooked in "Susa" or Bouin fixed material when the neighbouring cells compress the walls of the gland which has practically no residue. The reactions of the glands are similar to those in the other species (Table 8), but the granular glands are more strongly positive to acid mucopolysaccharides, and the white glands also are weakly positive to alcian blue. The fact that the mucous glands are positive to mucicarmine here and in C.perca but are only weakly so in the other two species may have little importance. Mucicarmine appears to be unreliable on its own as a guide to the mucoid nature of a secretion both in Catriona and in the Eubranchidae.

Mode of secretion of the ceratal glands of Catriona species

Muscle fibrils, such as occur in the glands of Eubranchus exiguus, could not be seen in any of the species of Catriona with routine Masson preparations and an oil immersion objective. With a polarising microscope, however, fine birefringent fibrils can be seen round the white glands, and these are particularly clear

in C.aurantia. They are not present round either the granular or the mucous glands in any of the four species studied. Since the white glands are often exuded on stimulation in C.maua and perca, it is probable that these birefringent fibrils are muscle fibrils and are responsible for exudation. The mucous glands probably ooze continuously and hence require no muscle fibrils, but the mode of secretion of the granular glands is not known.

Functions of the ceratal glands of species of Catriona.

Observations were made on eight or more individuals of each species of Catriona, but even with these numbers it is difficult to draw many definite conclusions. The behaviour of an animal depends on its age and health, on how long it has been in captivity, and on other unknown factors. Nevertheless, we can draw some conclusions as to the functions of the various types of gland even if the differences between the species are not clear. In C.maua and C.perca, if the animal is prodded violently with forceps, the secretion from the white glands is sometimes exuded in the form of a white cloud which slowly diffuses away. Exudation was not observed on pinching and autotomising a ceras. These results are listed in Table 9. Similar behaviour on prodding the animal may have been overlooked in C.aurantia, and in C.tina no exudation was found, although sought. Exudation of glandular material does, however, occur regularly in C.aurantia on pinching and autotomising a ceras. This material is apparently colourless, although in view of subsequent work on C.maua and perca where only the white glands were seen to exude,

this requires confirmation. Pressure of a coverslip on a ceras did on one occasion cause exudation of a white secretion. Thus in C.aurantia, it is possible that the granular glands are more important in defence than the white glands. The granular glands of the other species are probably also important in defence, although they may be more readily miscible with water than they are in C.aurantia, and hence less easy to detect.

The mucous glands of Catriona probably have the same functions as the small mucous glands of the Eubranchidae. They may be responsible for secreting the cuticle, but it is not clear whether the current caused by cilia is a movement of this mucin cuticle itself or of a film of mucus outside this layer. The mucus probably protects the animal from damage due to chemicals, abrasion, and particles settling on the animal. The current also tends to carry the secretions of the defensive granular and white glands towards any potential predator. The function of the globular glands of C.perca is not known.

Ceratal glands of Eolis M and Tergipes despectus

Eolis M and Tergipes despectus differ from the other acleioprocts in possessing only one type of ceratal gland. There are no glands confined to the cnidosac region (Figs. 42 and 43). In the eubranchids and catrionids, the glandular epidermis of the cnidosac region is much thicker than the epidermis over the rest of the ceras, but in these two species it is, if anything, slightly thinner over

the cnidosac. The shape of the ceras tip, too, is different; in T.despectus and Eolis M it is rounded over the bulging cnidosac, but in typical acleiprocts it is contracted just below the tip.

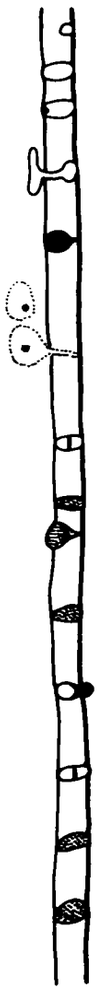
The mucous glands of Eolis M are often 10μ in diameter and reach the level of the base of the neighbouring cells,as in Catriona species. The secretion is positive to tests for acid mucopolysaccharide - dialysed iron, toluidine blue and safranin - and also takes green with Masson's trichrome. About half of the glands appear to be empty. This may be an artifact, or it may mean that they have exuded all of their secretion.

The glands of T.despectus all appear to have lost their contents before fixation or during subsequent treatment. It is just possible that the secretion is present, but nothing stains with Masson's trichrome, Mallory's triple stain, dialysed iron, alcian blue, PAS, and Best's carmine. Such a secretion could be a neutral mucopolysaccharide, but it is more likely that the secretion has been lost, so its reactions are unknown.

These glands of T.despectus and Eolis M probably have the same functions as the mucous glands of other eolids. They may secrete the mucin cuticle, and in addition, or instead of this, they may secrete mucus with a general protective function. They are unlikely to be of direct defensive importance, but they may carry nematocysts from the cnidosac towards a potential predator in the ciliary current.

Figure 20. Key to eolid gland diagrams. This applies to Figures 21, 35, 43, 44, 45, 46, and 47. For further explanation, see text.

Key to eolid gland diagrams



Generai

epidermal mucous glands

subepidermal mucous gland

osmiophil gland

'cellules spéciales'

Eubranchus

granular mucous gland

granular non-mucous glands

Catriona

granular gland

Catriona perca gland

Favorinus

'empty' gland

Eolis D

granular gland

Berghia

granular gland (coelomocyte?)

Figure 21. Diagrams to show the distribution of cecetal glands in the Eubranchidae. Each diagram represents a 6μ thick longitudinal section. For a key to the glands, see Fig. 20.

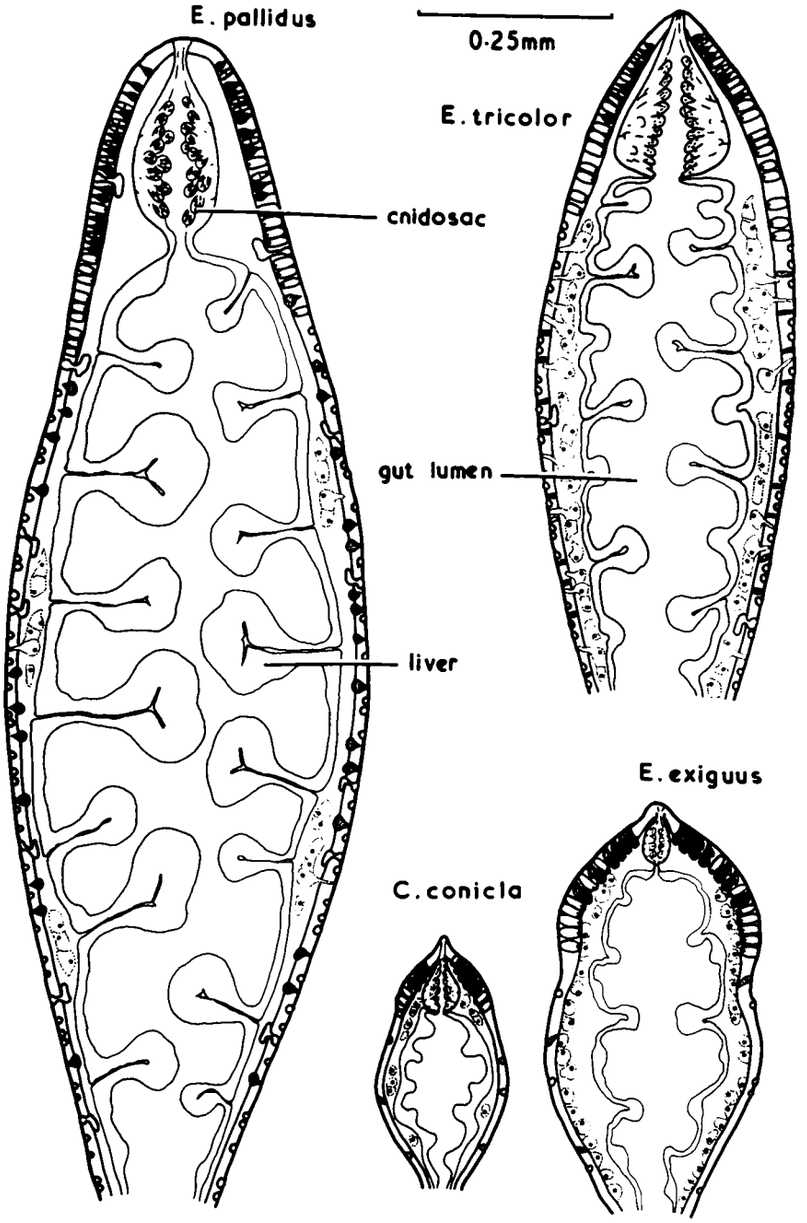
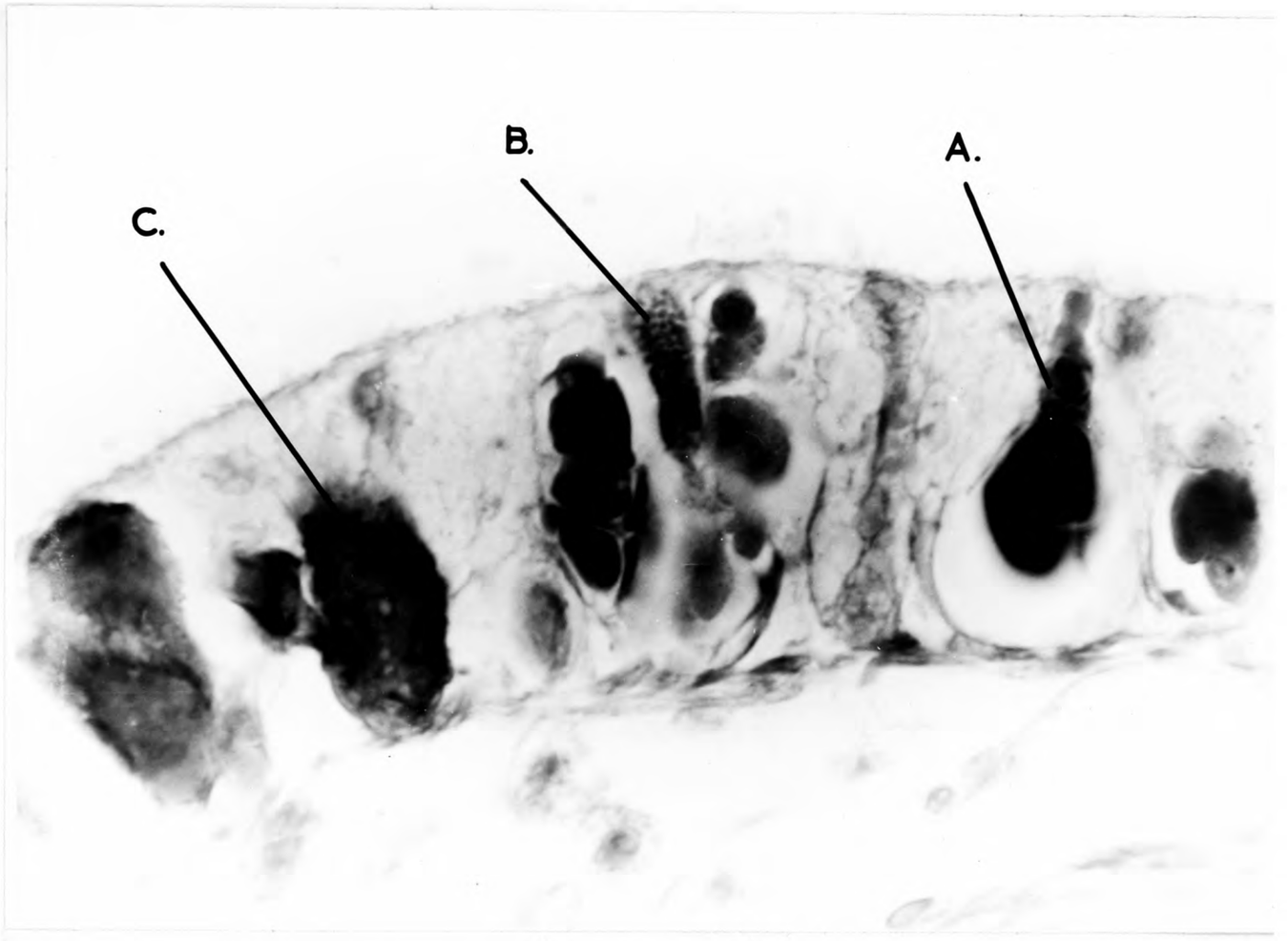

E. pallidus
0·25mm
E. tricolor
cnidosac
gut lumen
liver
E. exiguus
C. conicla

Figure 22. Section of cerata1 epidermis of Eubranchus pallidus fixed in Lewitsky-saline, decoloured, and stained with Safranin and Light Green. A., osmiophil gland; B., granular mucous gland; C., large mucous gland.

Figure 23. Section of cerata1 epidermis of Eubranchus pallidus fixed in "Susa" and stained with dialysed iron, basic fuchsin and picric acid. Lettering as for Fig. 22. The epidermal vesicles, v., are particularly clear.


B.
A.
C.
20 μ

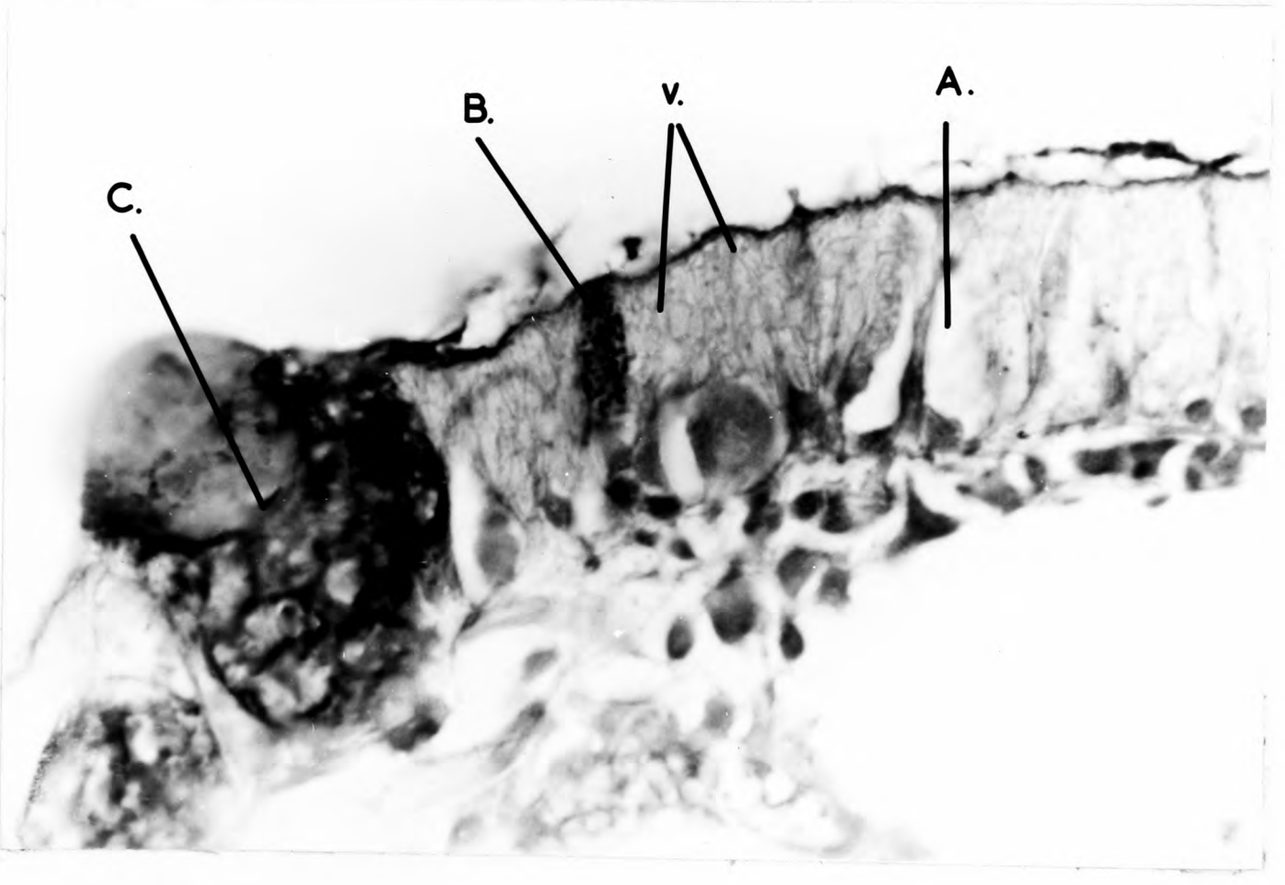

B.
V.
A.
C.

Figure 24. Diagram to show the glands of the ceras tip region of Eubranchus pallidus.

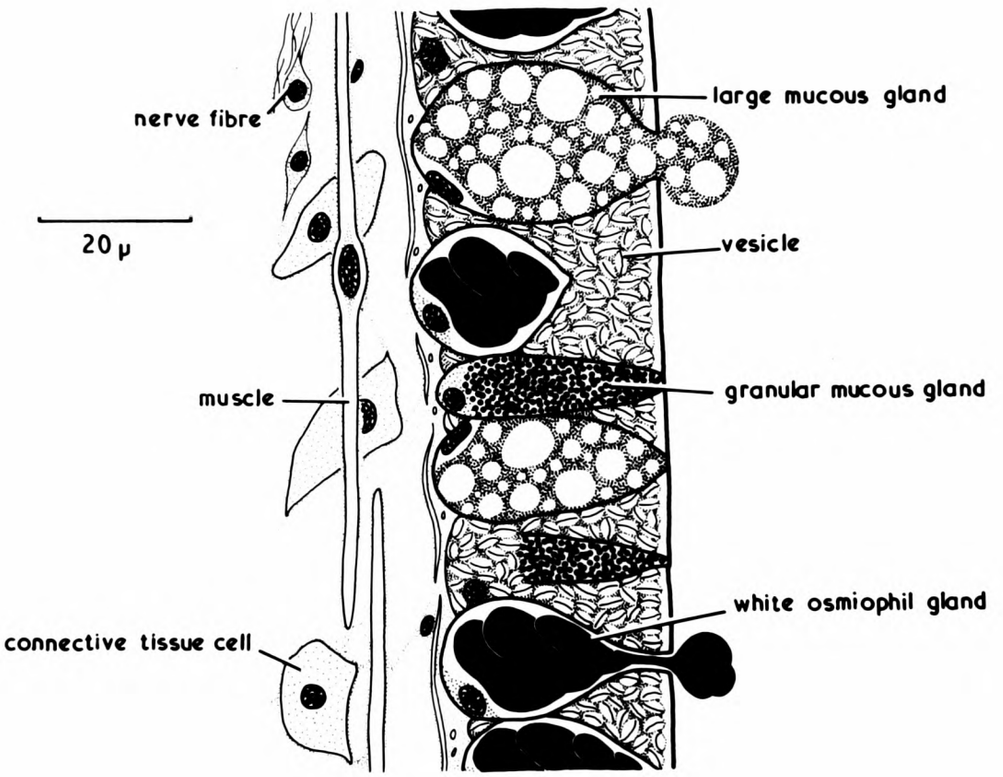
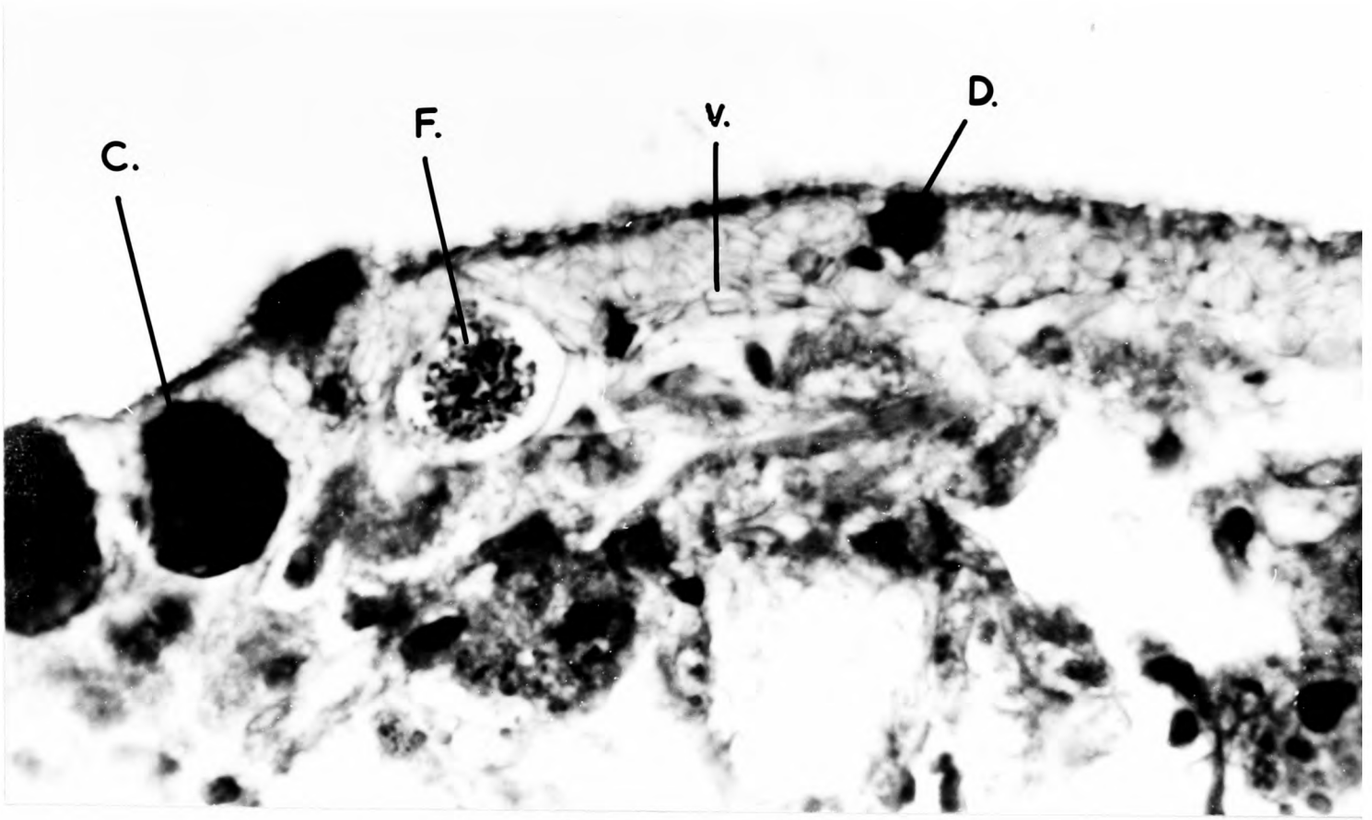

nerve fibre
20 μ
muscle
connective tissue cell
large mucous gland
vesicle
granular mucous gland
white osmiophil gland

Figure 25. Section of cerus of Eubranchus pallidus fixed in Lewitsky-saline and stained with dialysed iron, basic fuchsin and picric acid. C., large mucous gland; D., small mucous gland; F., granular non-mucous gland; v., vesicles.

Figure 26. Diagrammatic section of cerutal epidermis of Eubranchus pallidus showing glands present.


C.
F.
V.
D.
20μ

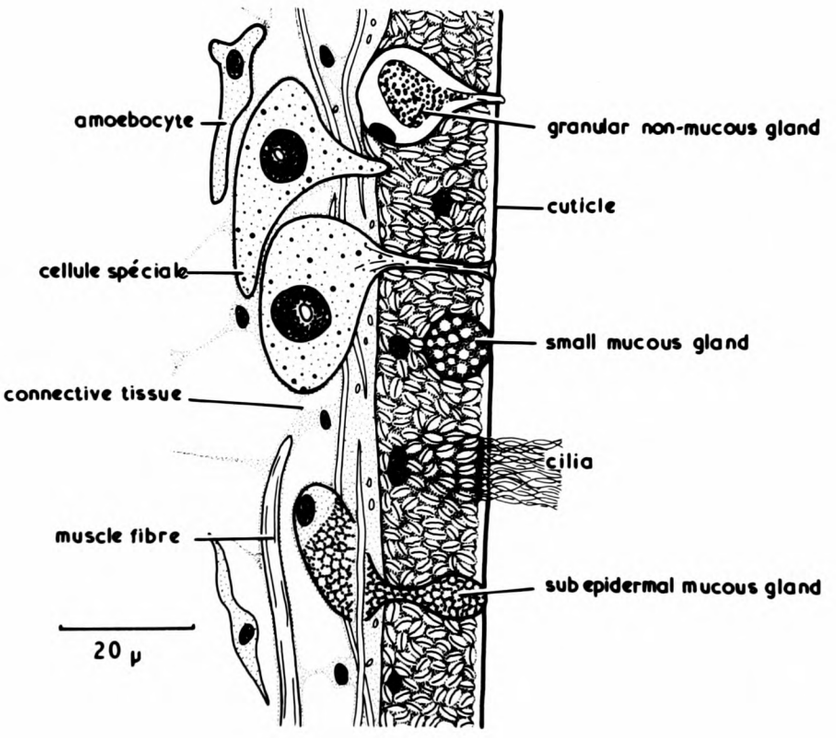
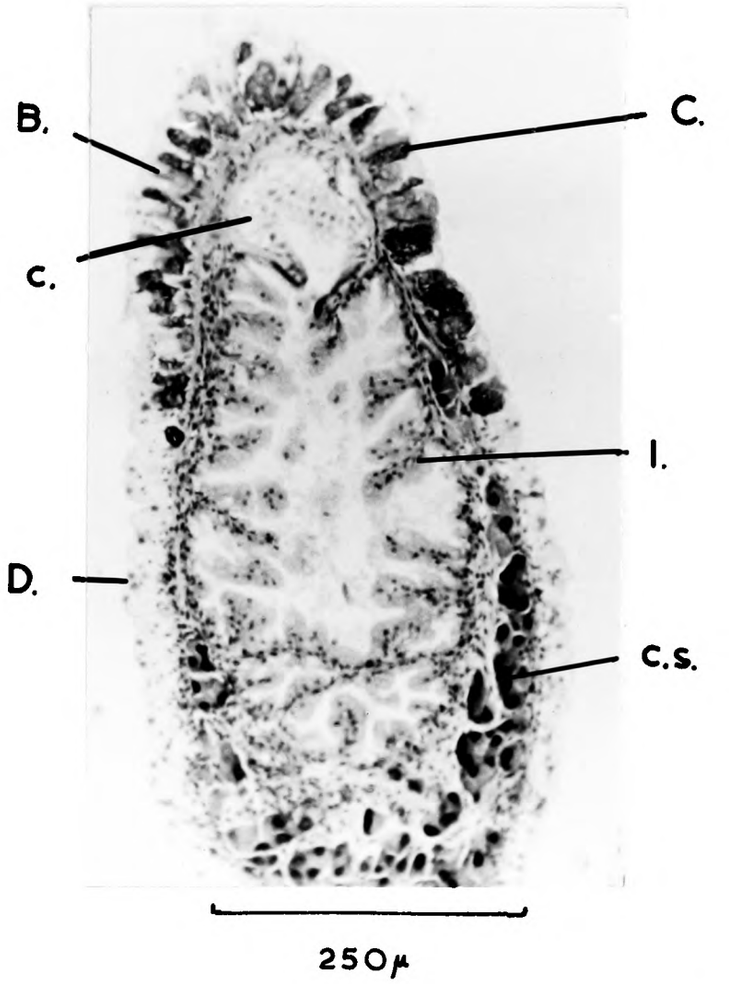
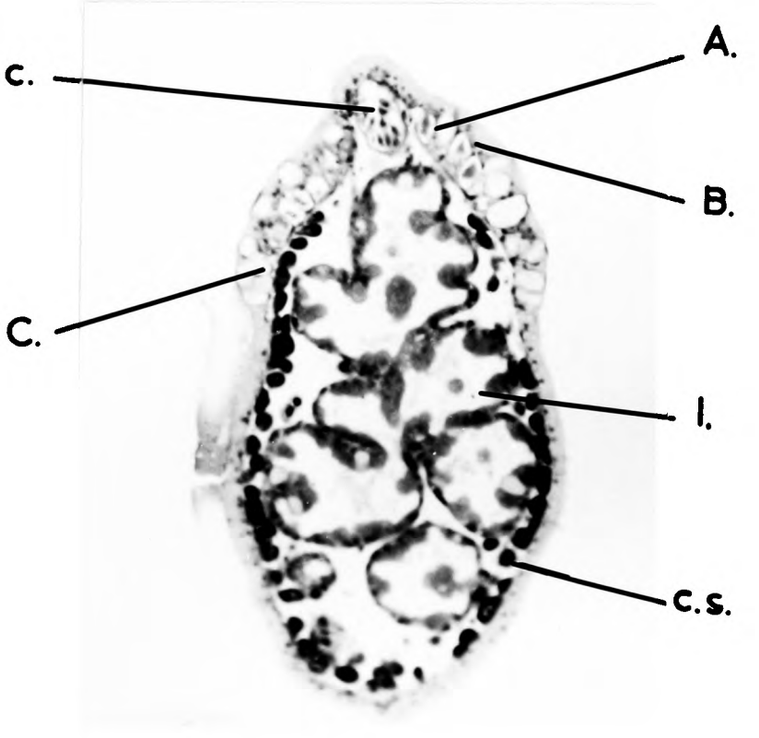

amoebocyte
granular non-mucous gland
cuticle
cellule spéciale
small mucous gland
connective tissue
cilia
muscle fibre
sub epidermal mucous gland
20 μ

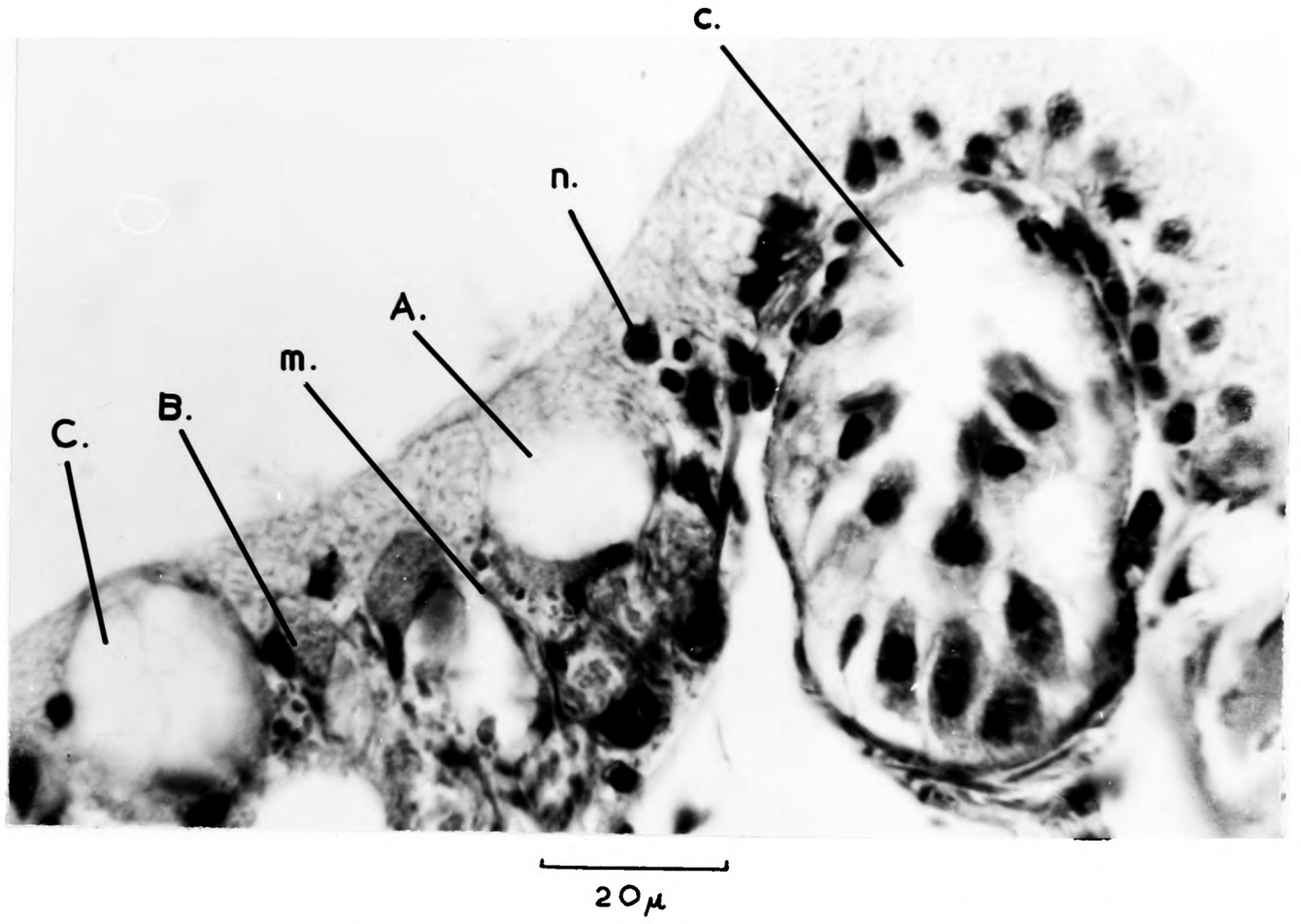
Figure 27. Longitudinal section of a ceras of *Eubranchus tricolor* fixed in "Susa", stained with Ehrlich's haematoxylin and Southgate's mucicarmine. *B.*, granular mucous gland; *C.*, large mucous gland; *D.*, small mucous gland; *c.*, cnidosac; *c.s.*, *cellule spéciale*; *l.*, liver.

Figure 28. Longitudinal section of ceras of *Eubranchus exiguus* fixed in Zenker's fluid, stained with Masson's trichrome. Lettering as for Figure 27. *A.*, white osmiophil gland.


B.
C.
c.
I.
D.
c.s.
250μ


A.
c.
B.
C.
I.
c.s.

Figures 29 and 30. Ceras tip glands of Eubranchus exiguus, treated as was the ceras in Fig. 28. A., osmiophil gland; B., granular mucous gland; C., large mucous gland; c., cnidosac; h.c., hepatic cell; m., muscle fibril; n., nucleus; p., pigment granule; v., vesicles.


c.
n.
A.
m.
B.
C.
20μ

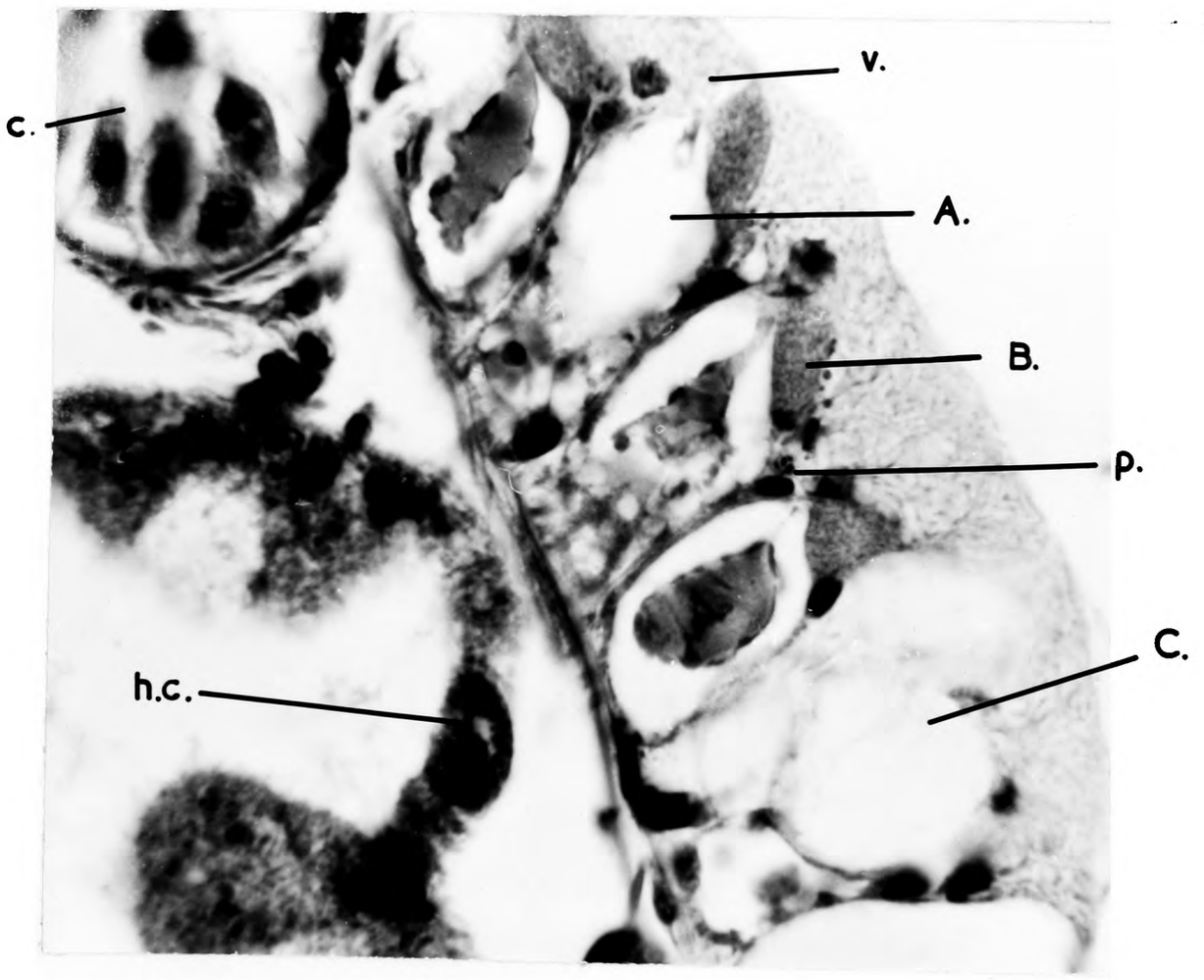

v.
c.
A.
B.
p.
C.
h.c.

Figure 31. Diagrammatic section of cerus tip cerotal glands of Eubrachchus exiguus. Vesicles have been omitted for clarity.

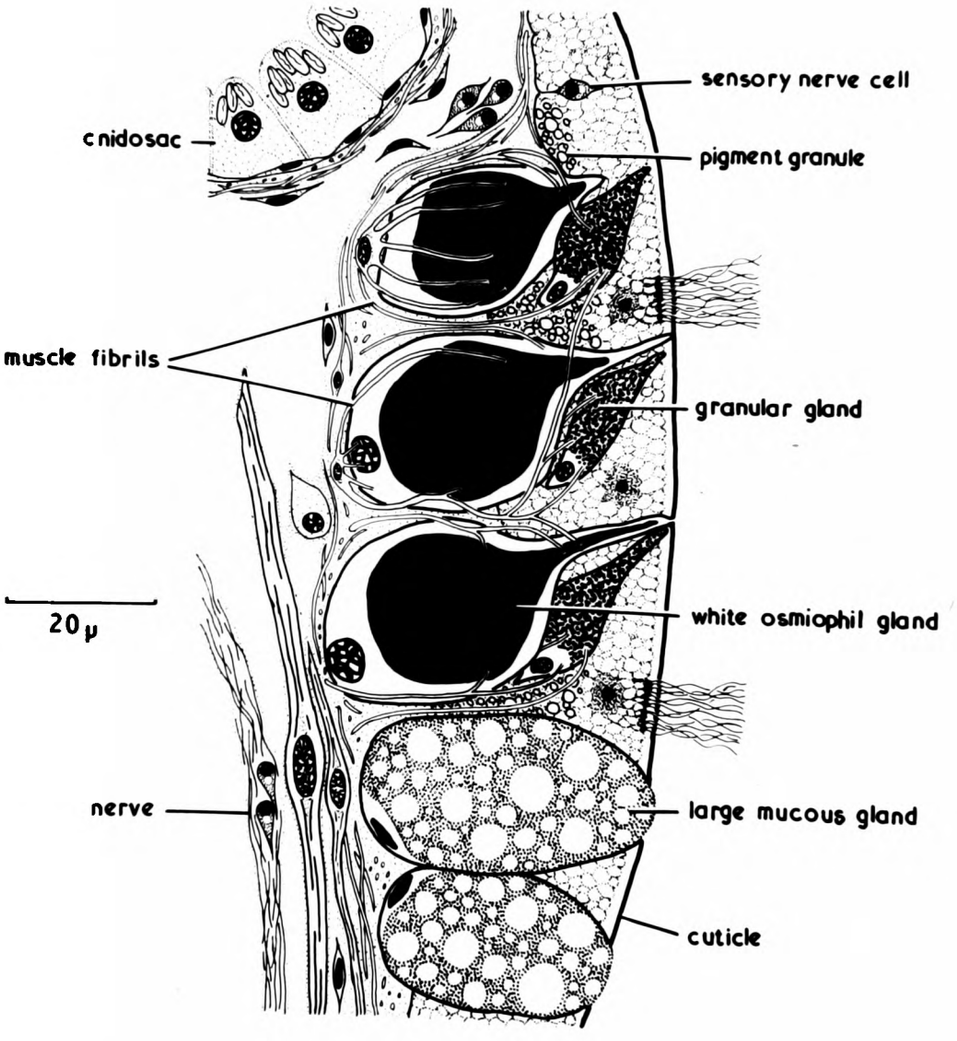
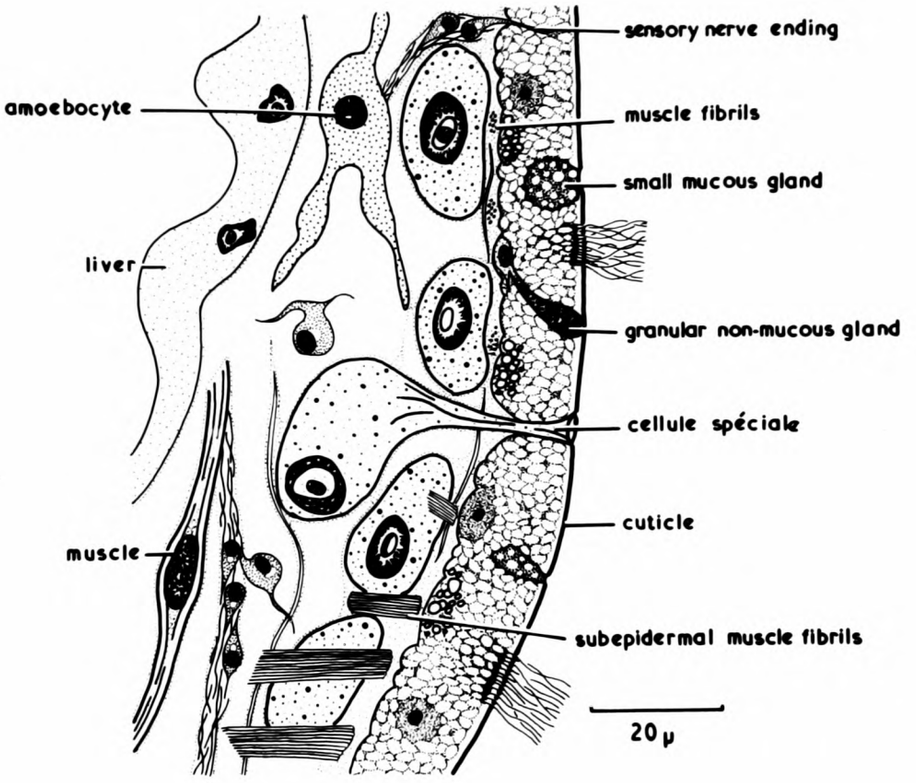

sensory nerve cell
cnidosac
pigment granule
muscle fibrils
granular gland
20 μ
white osmiophil gland
nerve
large mucous gland
cuticle

Figure 32. Semi-diagrammatic section of ceratal epithelium of Eubranchus exiguus. The upper part of the diagram is at right angles to the surface of the ceras, and the muscles are cut transversely; the lower part is more oblique, and the muscles are cut in longitudinal section.

Figure 33. Section of ceras of Capellinia conicla fixed in "Susa" and stained with Masson's trichrome. A., white osmiophil gland; B., granular mucous gland; C., large mucous gland; c., cnidosac; c.s., cellule speciale; l., liver.


sensory nerve ending
amoebocyte
muscle fibrils
small mucous gland
liver
granular non-mucous gland
cellule spéciale
cuticle
muscle
subepidermal muscle fibrils
20 µ

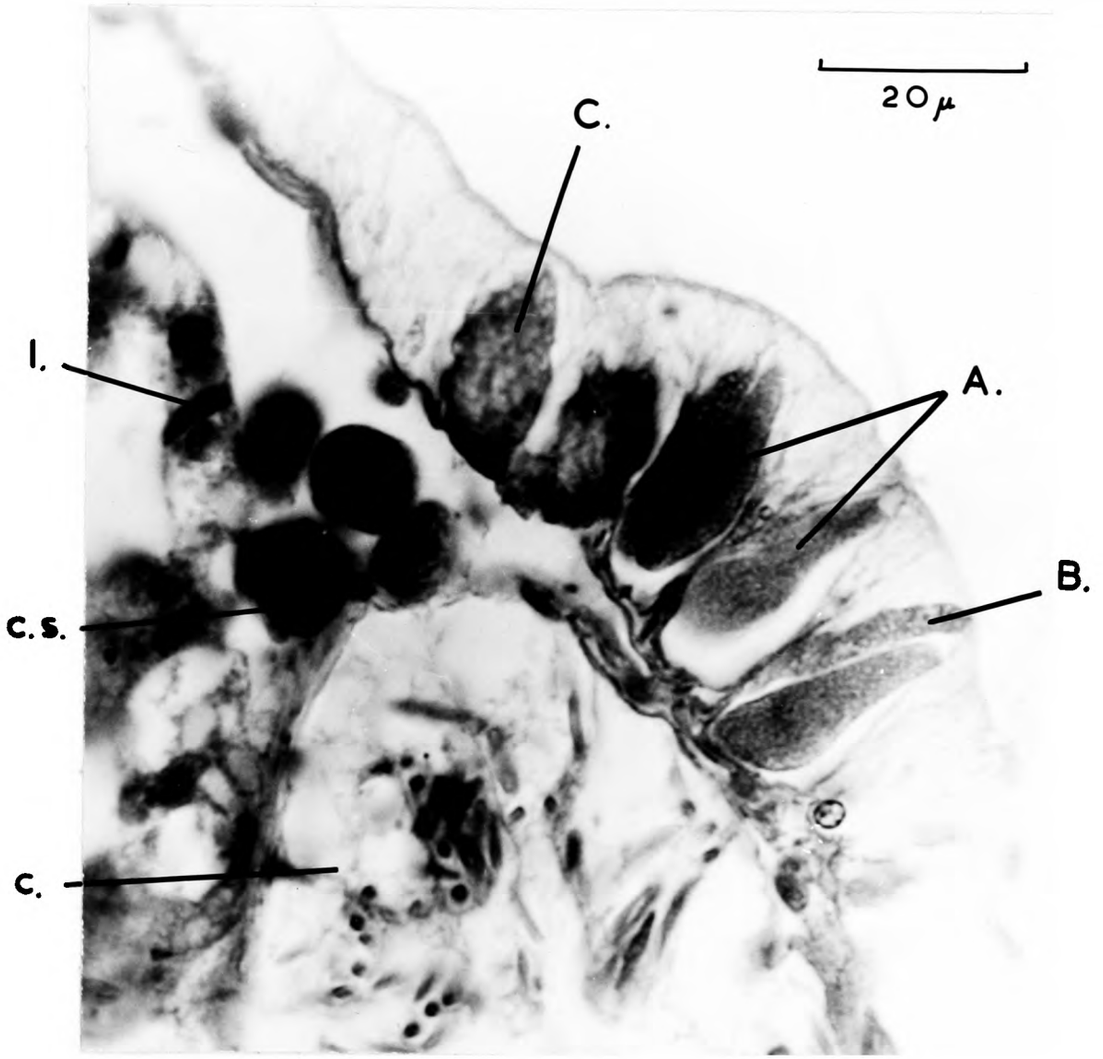

20 µ
C.
I.
A.
B.
c.s.
c.

Figure 34. Longitudinal section of ceras of Catriona aurantia fixed in Lewitsky-saline and stained with safranin and light green. c., cnidosac; c.s., cellule spéciale; g.gl., granular gland; l., liver; m.gl., mucous gland; o.gl., osmiophil gland.

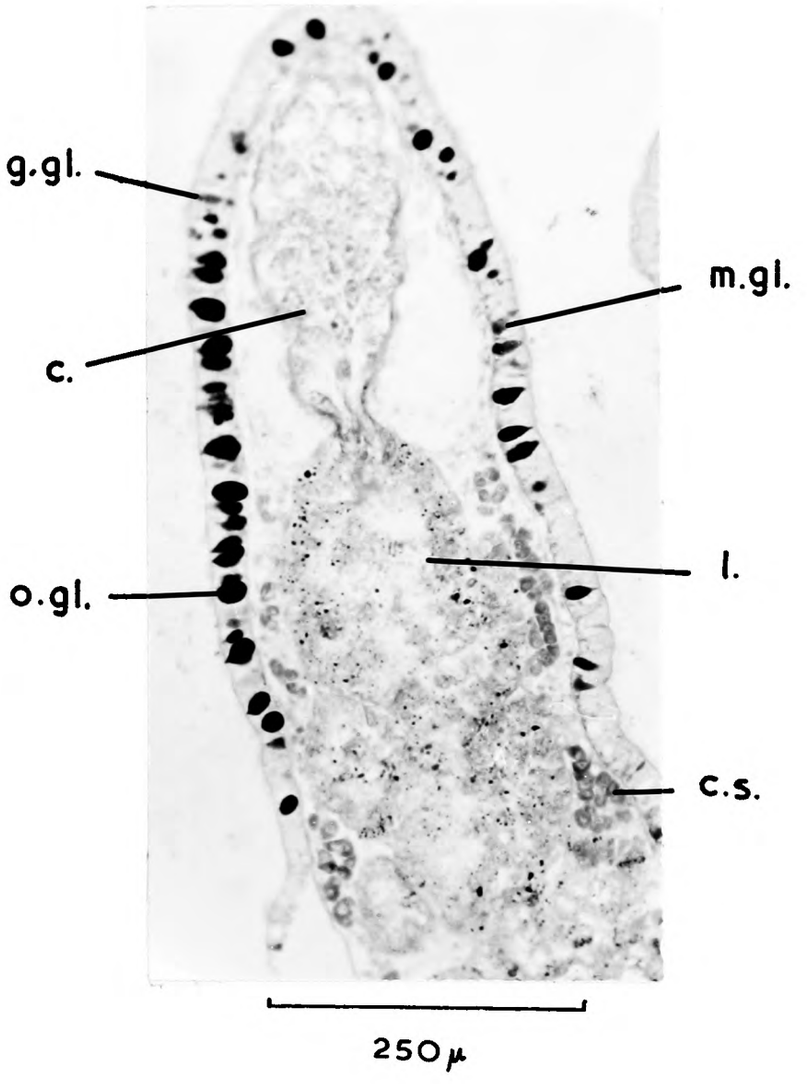

g.gl.
m.gl.
c.
o.gl.
l.
c.s.
250 μ

Figure 35. Diagrams to show the distribution of ceratal glands in species of Cetriona. Each diagram represents a 6μ thick longitudinal section. For a key to the glands, see Fig. 20.

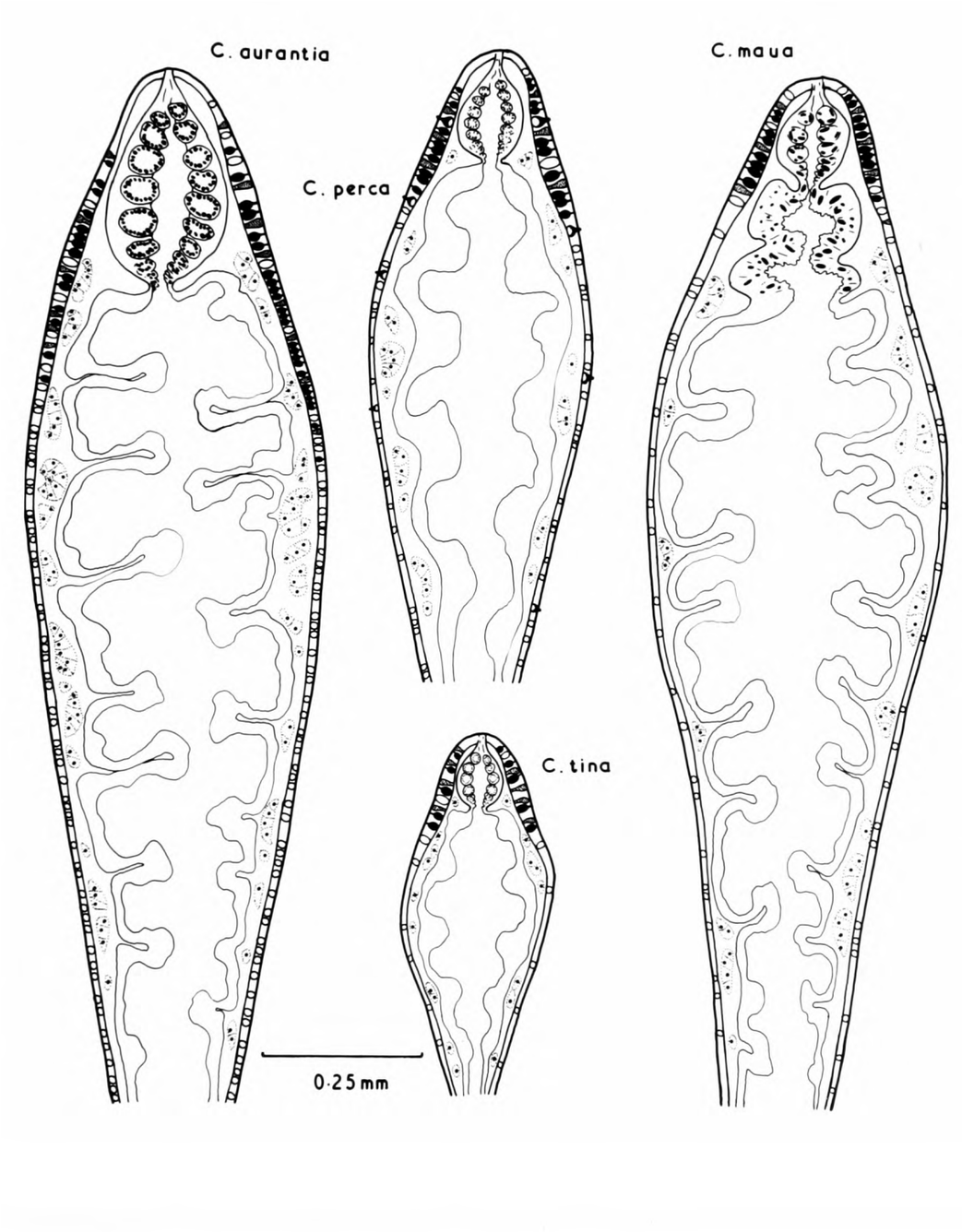
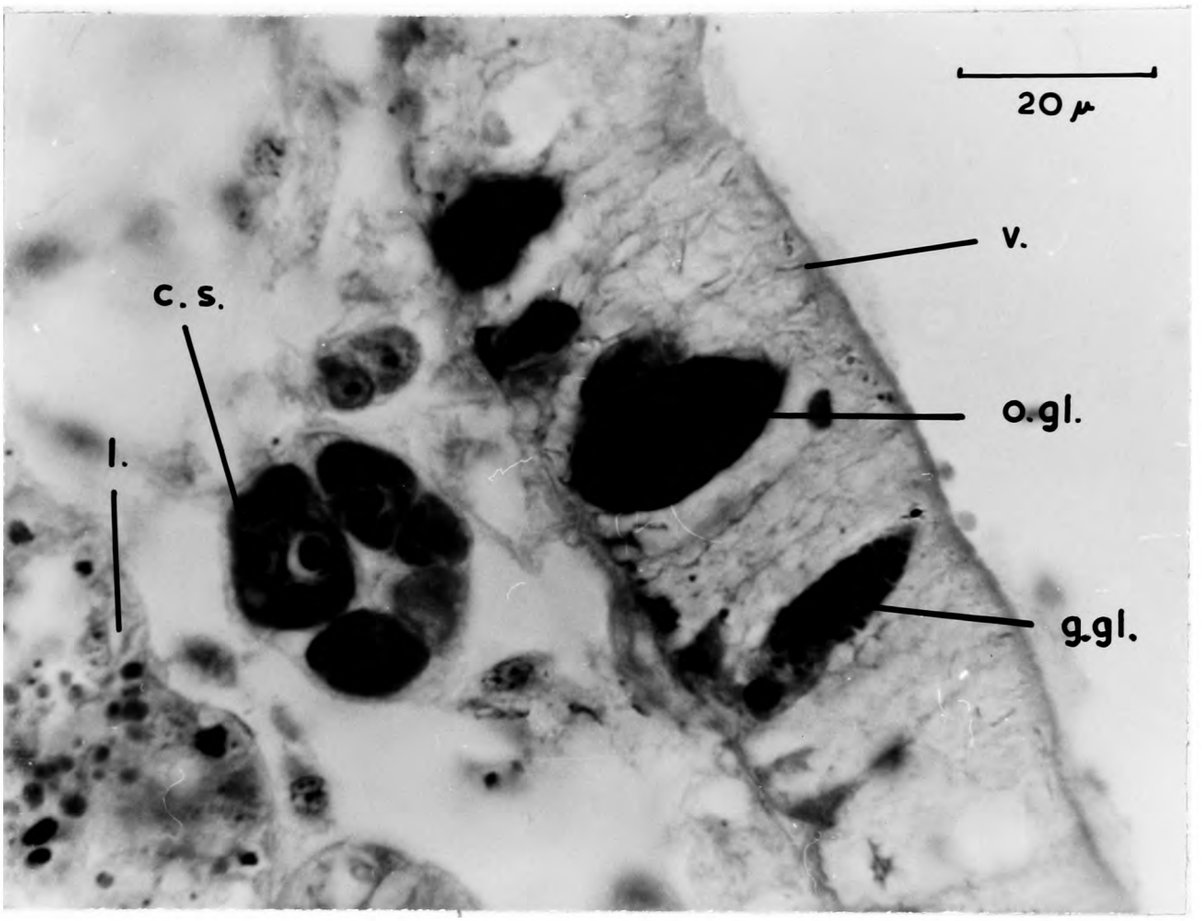

C. aurantia
C. perca
C. maua
C. tina
0·25 mm

Figure 36. Section of ceras of Catriona aurantia fixed in Lewitsky-saline, stained with safranin and light green. c.s., cellule spéciale; g.gl., granular gland; l., liver; o.gl., osmiophil gland; v., vesicles.

Figure 37. Section of ceras of Catriona aurantia fixed in "Susa", stained with Masson's trichrome. Lettering as for Fig. 36; also c., cnidosac.


20 μ
v.
c. s.
o.gl.
l.
g.gl.

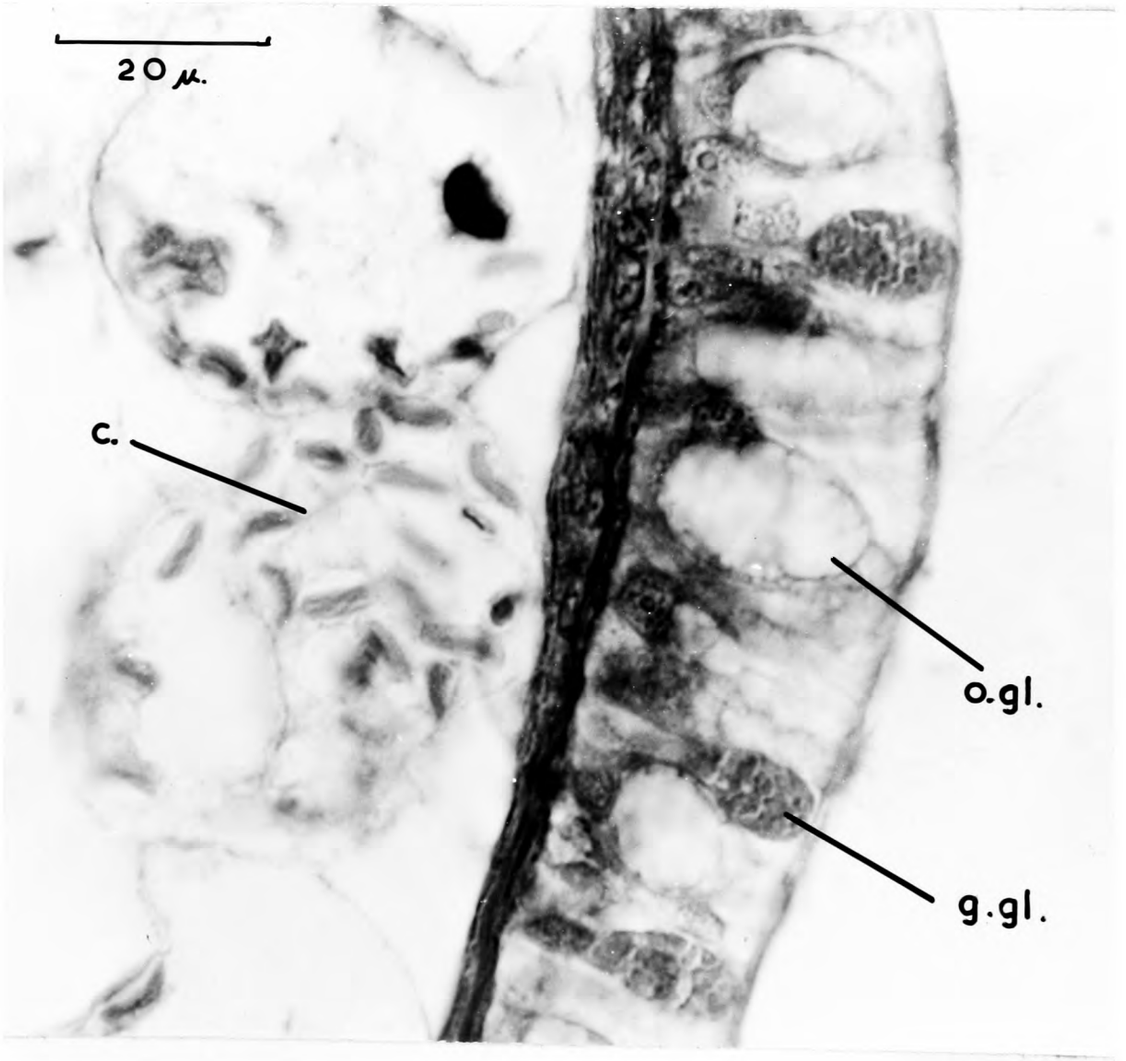
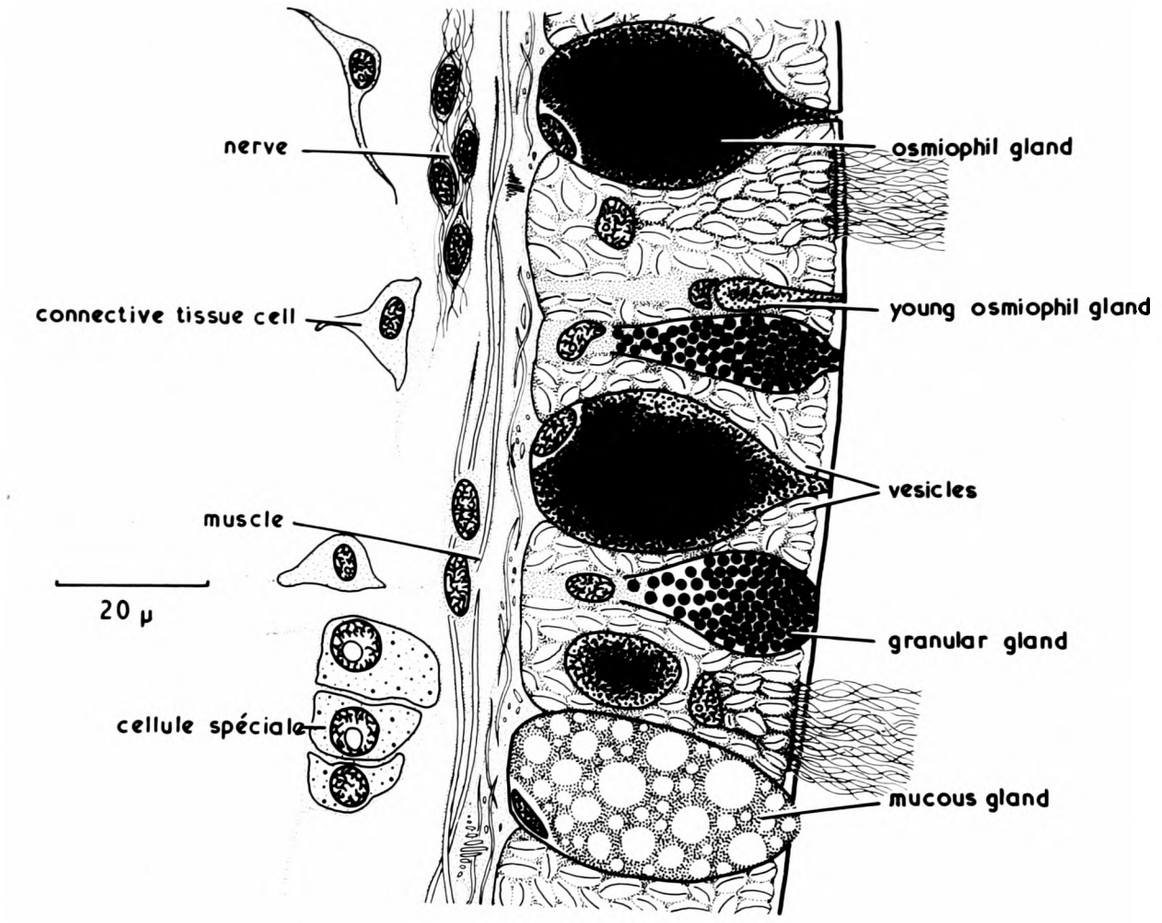

20 μ.
c.
o.gl.
g.gl.

Figure 38. Diagrammatic section of ceras of Catriona aurantia .

Figure 39. Section of ceras of Catriona maua, fixed in "Susa", stained with Masson's trichrome. For key to lettering, see Fig. 36.


nerve
connective tissue cell
muscle
20 µ
cellule spéciale
osmiophil gland
young osmiophil gland
vesicles
granular gland
mucous gland

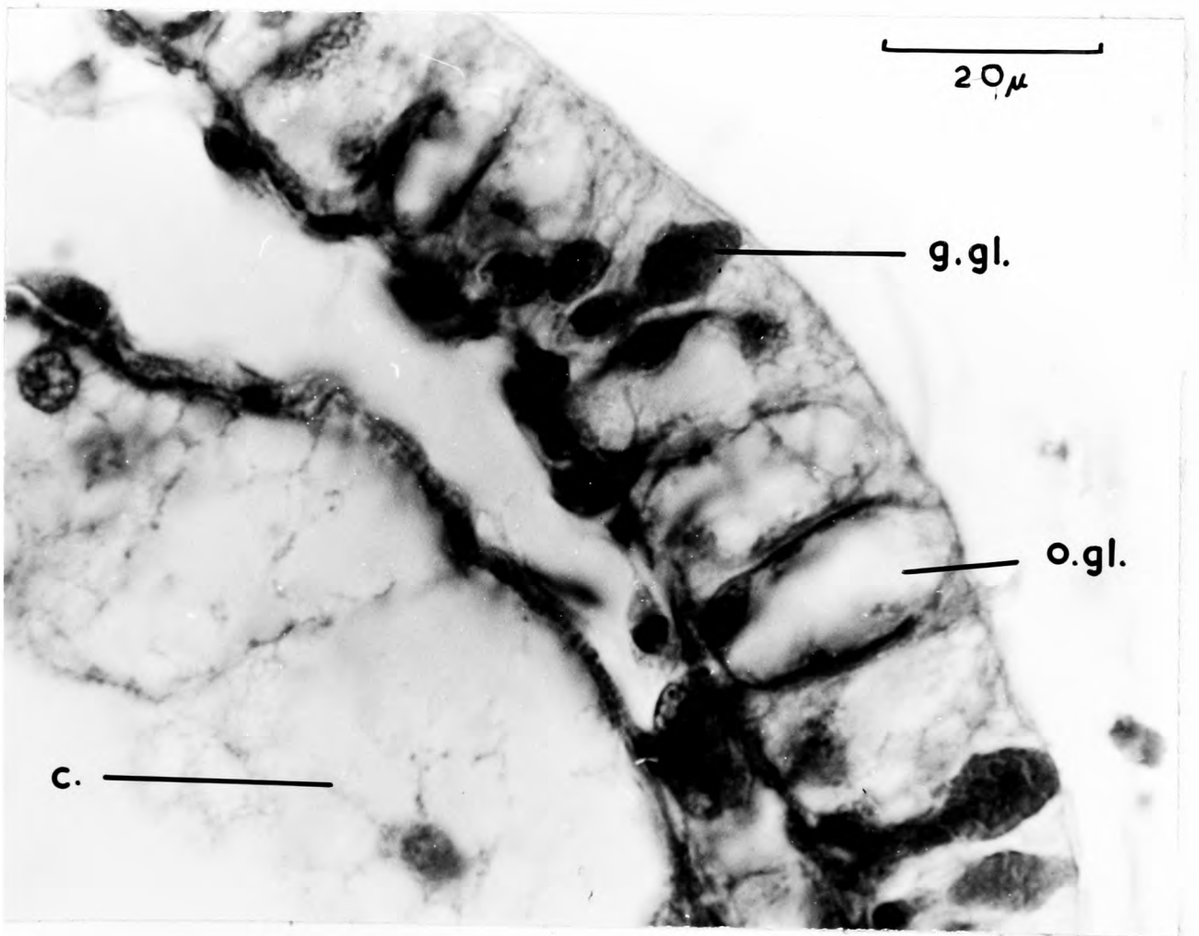
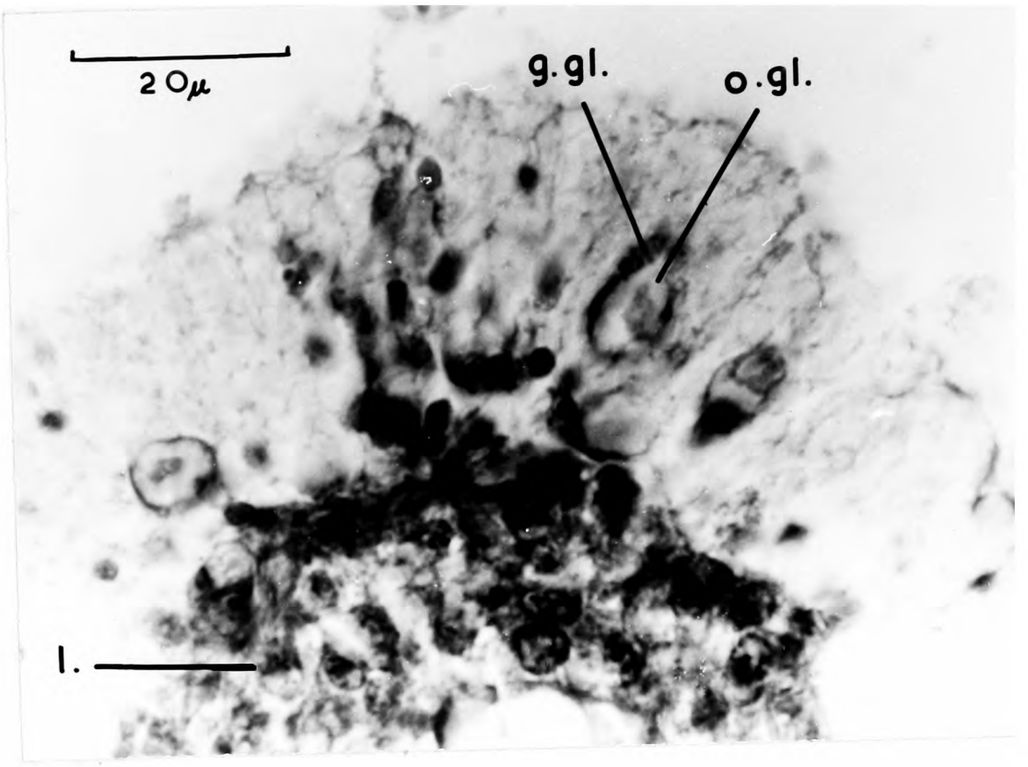

20µ
g.gl.
o.gl.
c.

Figure 40. Section of ceras of Catriona tina fixed in "Susa", stained with Masson's trichrome. Lettering as for Fig. 36.

Figure 41. Semi-diagrammatic section of ceras of Catriona tina.


20μ
g.gl.
o.gl.
l.

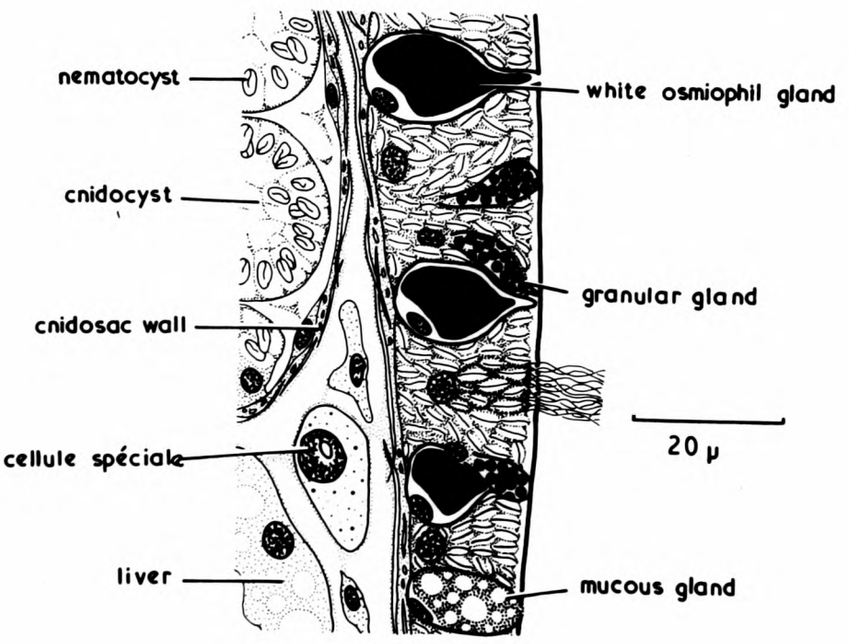
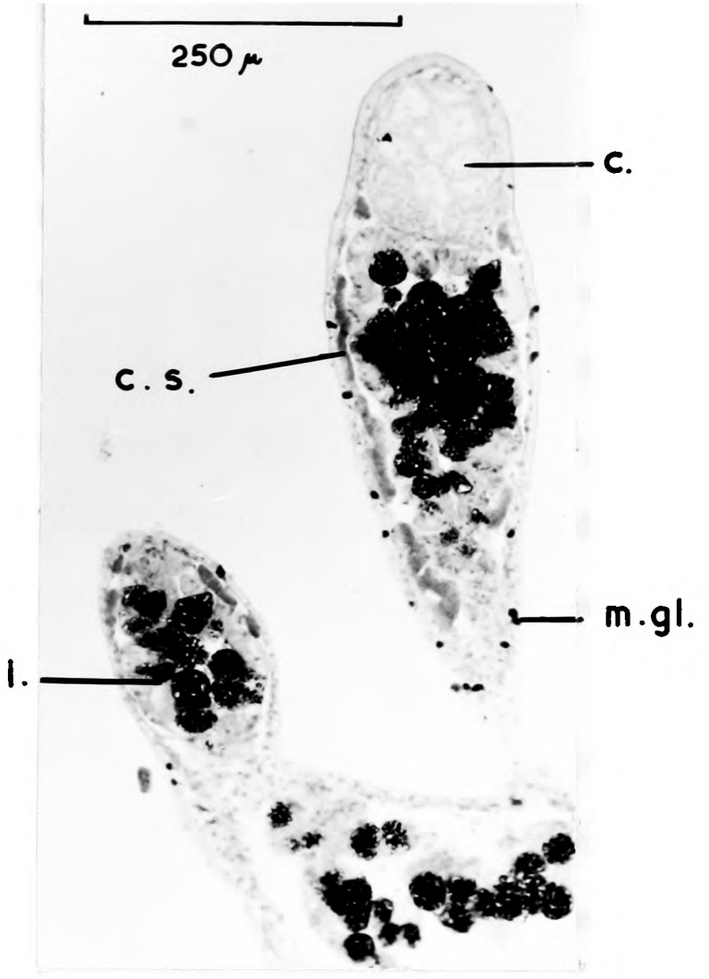
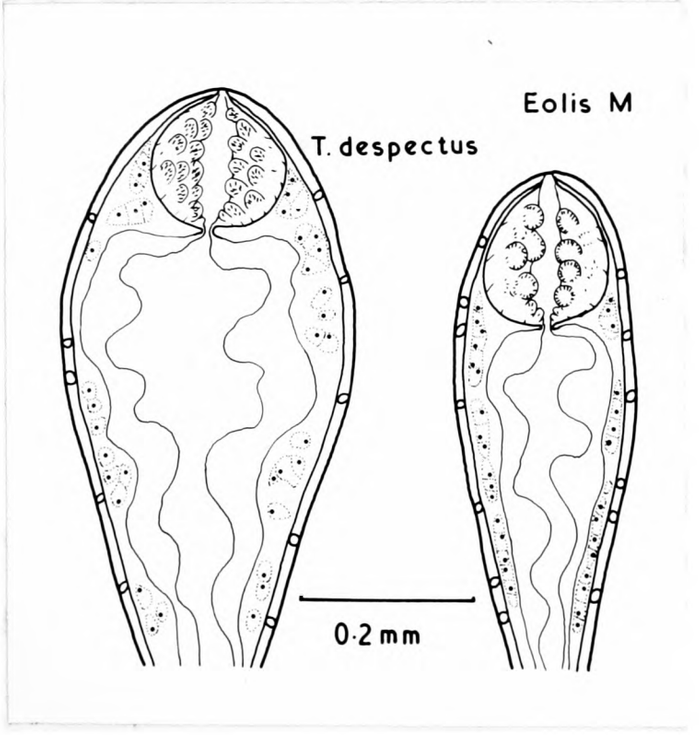

nematocyst
cnidocyst
cnidosac wall
cellule spéciale
liver
white osmiophil gland
granular gland
20 μ
mucous gland

Figure 42. Longitudinal section of ceras of Eolis M fixed in "Susa", stained with safranin and light green. c., cnidosac; c.s., cellule speciale; l., liver; m.gl., mucous gland.

Figure 43. Diagram of ceratal glands of Eolis M and Tergipes despectus as seen in a 6μ thick longitudinal section.


250 μ
c.
c. s.
m.gl.
l.


Eolis M
T. despectus
0·2 mm

Ceratal glands of the Cleioprocta

Introduction

Of the three main families of the Cleioprocta, the Aeolidiidae are anemone feeders with pectinate radular teeth, and the Facelinidae and the Favorinidae are hydroid feeders with cuspidate radular teeth. The Facelinidae and the Favorinidae are distinguished by the mode of liver branching (Odhner, 1939; Macnae, 1954), but they may not be phylogenetic units. The Aeolidiidae are probably a natural group, and this family is reviewed by Marcus (1961).

The histology of the ceratal epidermis of the cleioproct eolids is basically similar to that of the acleioprocts. All of the species examined have at least two types of ceratal gland, osmiophil non-mucous, and acidic mucopolysaccharide. In the osmiophil glands, the secretion usually has a coiled appearance, just as in Eubranchus pallidus. With non-osmium fixatives, these glands may have a small residue in the middle of an otherwise empty cavity, or they may retain a coiled appearance to the secretion. There is no direct evidence that these glands are white in life, but in view of the similar features to the osmiophil glands of Catriona and of Eubranchus, this seems probable. White markings are usually present on cerata of most eolids, and some of these could be due to the osmiophil glands.

The mucous glands of cleioprocts may be epidermal or subepidermal. The subepidermal ceratal glands resemble those of

E.pallidus, but there is usually even less cytoplasm than in that species - the mucin occupies practically the entire cell apart from the nucleus. The epidermal mucous glands may or may not reach the level of the basement membrane of neighbouring cells, according to the species; but otherwise they are uniform throughout the species studied, and resemble the mucous glands of Eubranchus and of Catriona. Unless otherwise stated, all mucous glands are metachromatic to toluidine blue, and positive to alcian blue and to dialysed iron. They are usually, but not always, positive to the PAS test for carbohydrates. Thus in all of the mucous glands the contents consist of some form of acid mucopolysaccharide.

The distribution of glands on the ceratal surface is shown by means of diagrams. Each diagram represents a median longitudinal section of a ceras of a particular species. The number of glands shown is approximately the number seen in a 6μ thick section. No account is taken of the fact that one surface of a ceras may be rich in glands whilst another is poor; these diagrams represent approximate averages. All cerata are drawn to the same scale but with a maximum length of 1.5 mm. In Phidiana and Berghia, cerata may grow to a length of several millimetres. Since the cindosac is of much the same size in large and in much smaller cerata, in the case of these species, small cerata have been drawn.

Ceratal glands of the Favorinidae

A single individual of Favorinus branchialis (Müller) was found at Plymouth and fixed in "Susa". Glands of similar appearance

to the osmiophil glands of F.auritulus are present, but without Lewitsky-saline material it is impossible to be certain that these are in fact osmiophil. Both these apparent osmiophil glands, and epidermal mucous glands are distributed all over the ceratal surface (Fig.44). Some of the mucous glands reach the level of the basement membrane of neighbouring cells, but others do not. All intermediates occur, and clearly they are all of the same type. At the tip of the ceras, in the cnidosac region, is a third type of gland. Each gland is oval, 20μ long, and reaches the inner border of the epidermis. The contents of these glands are either not fixed or have been lost in the subsequent treatment of the specimen, so the nature of the secretion is unknown. These "empty" glands are, however, quite distinct from the similarly shaped mucous glands in which the secretion is almost always preserved.

Favorinus auritulus Marcus is a small species from Jamaica with similar ceratal glands to F.branchialis (Fig.44). Typical osmiophil glands are present scattered over the ceratal surface, and the glands at the tip have no stainable contents with either "Susa" or Lewitsky-saline, just as in F.branchialis. The mucous glands are of two distinct types,unlike in F.branchialis, and no intermediates occur. The large epidermal mucous glands are up to 20μ long, and resemble the large mucous glands of Eubranchus; whilst the small mucous glands are only 4 to 7μ in diameter, and resemble the small mucous glands of Eubranchus.

Eolis K is an undescribed species from the Caribbean. One specimen was found at Jamaica, and seven more were found on a pier at Miami, Florida, in April 1962. The arrangement of the first group of cerata in a double arch clearly demonstrates that K is a favorinid of the subfamily Facalininae (Marcus, 1958). Because of the value of the animals as type material, little was done in the way of experimentation on the living animal. One animal was fixed in Heidenhain's "Susa" and sectioned, the others were fixed in Bouin's fluid, one being sectioned. Two types of ceratal gland are present (Fig.45). In the cnidosac region, but absent elsewhere, are a few glands with a coiled appearance in Bouin's fluid, and these are probably osmiophil. Mucous glands are present all over the ceratal surface, but unlike in Favorinus, they are all subepidermal.

Eleven specimens of Dondice occidentalis (Engel) were found at Jamaica in 1961-1962. Three types of ceratal gland are present. Coiled, osmiophil glands are scattered over the surface of the ceras; and epidermal mucous glands with the mucin reaching the base of the cell occur all over the cerata including the cnidosac region. Subepidermal mucous glands are present in the cnidosac region only (Fig.45), and they have identical staining reactions to the epidermal glands.

Ceratal glands of the Facelinidae

Eolis D is an undescribed facelinid from Jamaica which is

one of the commonest species present at Port Royal. The cerata are narrow and pointed, unlike in most other species of eolid. There are three types of ceratal gland (Fig.44). Osmiophil glands are distributed all over the ceratal surface, but they are only weakly osmiophil, and the contents are not distinctly coiled. Epidermal mucous glands up to 12μ in diameter are present over the entire ceratal surface. They all appear to reach the level of the inner border of the epidermis, and can probably all be assigned to a single type although there is considerable variation in the shape and staining reactions of the contents. In the cnidosac region only there is a third type of gland, the granular gland, which appears as a number of minute droplets less than 0.5μ in diameter. It does not appear to contain mucin (negative to alcian blue, dialysed iron and toluidine blue), carbohydrates (PAS), or protein (Sakaguchi's test).

Learchis poica Marcus was found in small numbers in Jamaica. Two types of gland are present (Fig.46), both being distributed more or less evenly over the ceratal surface. The osmiophil glands are only weakly osmiophil, but clearly have a coiled secretion. The mucous glands are subepidermal, but it is just possible that some of them are epidermal. All have identical histochemical and staining reactions, and there is clearly only one type of gland even if sometimes there is no subepidermal part.

Phidiana lynceus Bergh is the commonest eolid in the mangroves at Port Royal, Jamaica. It is a very large species and reaches 60 mm.

in life. Three types of ceratal gland are present (Fig.46); and, in addition, scattered over the ceras especially in the distal half, are cells similar to the special epithelial cells described by Henneguy (1925) from Facelina, but also described from Catriona aurantia on page 87. Since the function of these cells is unknown - they may be sensory or they may be glandular - they have been omitted from the diagram. Two types of gland are found over the whole of the ceratal surface: coiled, weakly osmiophil glands, and epidermal mucous glands. In the cnidosac region is a third type, the subepidermal mucous gland. These are absent from the rest of the ceratal surface, except that some of the lateral cerata have them over their entire surface. Both types of mucous gland stain for acid mucopolysaccharides, but they stain differently with histological stains. Thus the subepidermal glands stain green with Masson's trichrome and blue with Mallory's triple stain, but do not take up basic fuchsine readily; whilst the epidermal glands are a very pale green with Masson's and a pale blue with Mallory's stain, but do take up basic fuchsine. The subepidermal glands contain minute granules which take red colours with both Masson's and Mallory's stains. These granules are absent from the epidermal mucous glands.

Facelina coronata (Forbes) from Plymouth is another species with osmiophil and epidermal mucous glands scattered all over the ceratal surface, but the material was inadequate to be certain whether there is a second type of mucous gland at the tips of the cerata.

Ceratal glands in the Aeolidiidae

Spurilla neapolitana (Delle Chiaje) is the commonest anemone-feeding eolid at Port Royal. Two types of ceratal gland are present, both being distributed over the entire ceratal surface (Fig.47). Osmiophil glands with coiled contents are more abundant in Spurilla than in most other species in this study, and the more numerous mucous glands are all subepidermal.

Berghia coerulescens (Laurillard) was found on two occasions at Port Royal. The ceratal glands are quite different from those of S.neapolitana (Fig.47). The mucous glands over most of the ceratal surface, but absent from the cnidosac region, are epidermal and not subepidermal. But in the cnidosac region and extending to almost half the length of the ceras, are subepidermal mucous glands. Both types of gland stain in a similar way with the reagents used, and it is likely that one type is derived from the other. The epidermal glands sometimes, but not always, reach the level of the basement membrane of neighbouring cells. Coiled osmiophil glands are present scattered over the ceratal surface. There are also some cells in the epidermis which take up the xylidine red of Masson's trichrome and the orange G of Mallory's triple stain. Similar cells are present in the subepidermal tissues, and it is likely that these are excretory coelomocytes. They resemble the type 'F' glands of Eubranchus species.

Observations on sections of Aeolidia papillosa (L) and Aeolidiella glauca (Alder and Hancock) var. angulata (Alder and Hancock) show that in these two species the common mucous glands

are all epidermal. It is therefore possible that the nature of the mucous glands, whether they are epidermal or subepidermal, may be of importance in the taxonomy of the Aeolidiidae.

Functions of the ceratal glands of the Cleioprocta

In none of the cleioprocts studied is there as great a concentration of glands in the cnidosac region as is commonly found in the acleioprocts. Consequently it is difficult to observe glandular secretion because the quantity of fluid exuded, if any, is very small. In addition, the cerata are frequently long and mobile, and their violent waving means it is often impossible to see if exudation has or has not occurred. Thus glandular secretion is not recorded for many species of cleioprocts. On one occasion, when a ceras of F.branchialis was pinched and autotomised, small transparent globules were seen to be exuded from the empty glands at the ceras tip. These glands may therefore have a defensive function. Dondice occidentalis was observed on three occasions to exude clear globules from glands in the cnidosac region when cerata were autotomised. These globules, 11 to 17μ in diameter, take up vital dyes just as do the mucous glands over the rest of the ceratal surface in this species. They are red with neutral red, pink with janus green, and turquoise with toluidine blue (non-exuded mucous glands did not take up toluidine blue). I was unable to distinguish epidermal from subepidermal mucous glands by means of vital dyes, but this may have been because the

dyes did not penetrate below the epidermis. It is not clear from which of the two types of mucous gland the exuded globules come, but there is indirect evidence that it is from the subepidermal glands. The cerata of Dondice are very slimy, much more so than are those of Catriona or Eubranchus, and an autotomised ceras tends to stick to the forceps. When stained vitally with neutral red or toluidine blue, a thin mucous sheath can be seen completely enveloping the ceras, and almost doubling its apparent diameter. This sheath has evidently been secreted by the epidermal mucous glands, and it may in life be removed continuously from the ceras tip by ciliary action. Since the epidermal mucous glands have this function, it would seem likely that the subepidermal ones are responsible for the secretion of the globules. Both glands are probably of defensive importance.

In Eolis D, on three occasions, prodding of an animal elicited the secretion of many minute white droplets from all over the cerebral surface. This white cloud rapidly disperses, but it almost certainly comes from the osmiophil glands. The granular glands in the cnidosac region could also be defensive, but exudation of them was not observed. A similar opaque cloud was once observed coming from a ceras of Learchis poica after prodding, but it is not known if it came from osmiophil or from mucous glands.

In all of these cases, glandular exudation occurs when the animal is violently disturbed, and it is therefore probable that the glands involved are of defensive importance. It is likely

that the ceras tip glands of other species, for example Phidiana, are of defensive importance, but their violent waving renders it almost impossible to check this. Not all species, however, have defensive ceratal glands. Eolis K possesses just one type of mucous gland with, presumably, the usual cleansing functions, and a few osmiophil glands, of possible defensive function; and in the acleioprocts Eolis M and Tergipes despectus, osmiophil glands are absent.

In the Aeolidiidae, defensive glandular secretion was only seen once, in Spurilla neapolitana. In this case it was clear globules from mucous glands. The mucous glands of other species may also be important in defence because of the copious quantity of mucus produced.

None of the cleioprocts examined has any trace of muscle fibrils round its glands, either with oil immersion or polarised light, so presumably glandular secretion is by means of contraction of subepidermal muscles, or is a continuous exudation.

Thus although the evidence is not as conclusive as is the case with the acleioprocts, there is evidence that some of the glands of many cleioprocts are of defensive importance.

Discussion

Defensive glands at the ceras tip have been described only once, by Hecht (1896) for Calma glaucoides. According to Hecht, the glands in this species are large cells, up to 55μ in length -

considerably larger than in any of the species described here which reach only 40μ in C.aurantia and E.exiguus. Hecht describes three appearances to these cells with osmium fixation: a black, opaque mass; an empty appearance; and staining as do mucous glands with carmalum. The empty glands no doubt are merely cases where the secretion has been lost by exudation or during fixation and sectioning, but it seems much more likely that the other two appearances are in fact two different types of gland, and not, as Hecht supposes, merely stages in the secretory cycle of the same gland. The black appearance with osmium suggests a gland of a similar nature to the white, osmiophil glands of Eubranchus and Catriona. The mucous glands could be similar to the granular mucous glands or the large mucous glands of Eubranchus. Calma is a cleioproct eolid, and is almost certainly not closely related to the acleioprocts described in this Chapter. Yet the similarities between the glands of Calma and of these species is very striking. It is almost certainly the result of parallel evolution since similar batteries of ceratal glands are absent from cleioprocts generally. It is also probable that the ceratal glands of Catriona and Eubranchus are the result of convergence. The osmiophil glands of the two genera are very similar, but the other ceras tip glands are quite different. Also the cuthonids T.despectus and Eolis M, which are closely related to Catriona, lack ceratal glands. This is either a secondary loss, or else the glands of Eubranchus and Catriona are definitely the result of parallel evolution.

Another parallel can be drawn between the Eubranchidae and the species of Catriona. In the larger species of both genera, there is no clear arrangement of the osmiophil and the granular glands - they appear to be spaced at random with relation to each other. But in the smaller species of both genera they are arranged in a very definite way in pairs. Here, however, the parallel ends, because in Eubranchus exiguus and Capellinia conicla, the osmiophil gland of each pair is towards the ceras tip, whilst in Catriona tina it is towards the base of the ceras. The significance of this arrangement is twofold: it enables each pair of glands to function as a single unit, which may be important if the combined fluid is more effective a deterrent than either fluid alone; and it results in an economy of space such that more glands can be packed into a given area than if the arrangement were less regular. In E.exiguus and C.conicla, the osmiophil gland of each pair bulges below the granular mucous gland. In C.tina the osmiophil gland has a broad base and a narrow neck, whilst the granular gland has a narrow base and a broad neck. In all three species, each pair occupies only as much surface area of ceratal epithelium as would one gland in the same position. In small species it is evidently of great importance to make efficient use of all available space.

We can conclude that there is very strong evidence that many of the ceratal glands of the Acleioprocta are primarily

defensive in function. This is also true for Calma glaucoides. The evidence for the defensive function of ceratal glands in the Cleioprocts is less conclusive, but even here, in some species, certain types of gland are concentrated at the tips of the cerata, and in some cases there is evidence that some glands are exuded as a defensive reaction. Hecht (1896) regards all ceratal glands as of defensive value, but we can now go further and consider the mucous glands of all eolids to perform cleansing and mechanical protective functions whilst some of the other glands perform a protective function directed towards certain predators.

6. NEMATOCYSTS

Historical

When Garstang (1889) and Herdman (1890) first suggested that some eolids have warning coloration, they believed that the repellent properties of these animals causing them to be rejected by potential predators are located in the cerata. They both showed that eolids are rejected as food by several species of fish (Poulton, 1890; Herdman and Clubb, 1890), and the behaviour of the eolid when disturbed suggested to both that the cerata might be particularly repellent. Since nematocysts occur in the cerata, it seemed reasonable to suppose that these are responsible, at least in part, for the distastefulness of the eolid. The behaviour of a fish when rejecting an eolid after having taken it into the mouth, is to spit it out again almost immediately. Clearly the cause of this

rapid rejection is something located on the surface of the eolid. But the nematocysts are located inside the ceras in a sac; so if they are important in defence, then either the cerata must be squeezed or broken, or the nematocysts must be ejected from the cnidopore. Since a healthy eolid is apparently unharmed after passage through the mouths of several individual fishes, it is clear that even when it is tasted by a fish, cerata are not broken or destroyed, although they may be autotomised. To be effective, then, nematocysts must be ejected from the cnidopore. This does in fact occur, as Hecht (1896) found. He describes the ejection of white blobs from the tips of cerata of Aeolidia papillosa. These blobs soon disperse in water, but he was able to show that they consist of a large number of nematocysts.

The theory of nematocyst function in coelenterates depends on the principle that the nematocyst is harmless when it is in the unexploded condition, and only becomes harmful to other organisms the moment it explodes. It would seem likely, then, that in eolids also it is the explosion of the nematocyst that causes rejection by other animals. Abric (1904) concludes that nematocysts are not important in the defence of eolids because they do not readily explode, and because they are not normally exploded during ejection. This is contradicted, however, by the observations of Grosvenor (1903) and of myself (see pages 115 and 116), and may have been based on observation of an aberrant individual or an aberrant species.

Grosvenor states that nematocysts usually explode immediately following ejection, and he regards this as the experience of most workers.

Grosvenor (1903) and Cuénot (1907) find that nematocysts are rarely ejected when the eolid is prodded with a glass rod, except in one individual of Spurilla neapolitana, but that a violent stimulation such as plucking off a ceras results in a massive ejection. Such a stimulation might occur in the mouth of a fish. Grosvenor points out that one should not expect nematocysts to be very readily ejected, since if they were, then ejection and explosion would occur before the predator was sufficiently close to the eolid for the nematocysts to be at all effective. On the other hand, if ejection only occurs after violent stimulation, such as being taken into the mouth by a fish, then the explosions of the nematocysts would occur in close contact with the delicate lining of the mouth. This would cause the maximum degree of irritation, and would probably result in complete rejection by the fish. Cuénot, however, concludes that nematocysts are of little defensive value. He believes that mucous secretions and the taste of the eolid as a whole are the chief causes of their unpalatability. Glaser (1910) confirms this when he finds that eolids with no cerata, and hence no nematocysts, are rejected by fish just as are intact eolids. However, even if the whole of the eolid is unpalatable, this does not necessarily imply that the nematocysts are of no defensive value.

Methods and Results

In order to decide between the opposing theories of Grosvenor and of Cuénot, it seemed important first of all to collect more data on the conditions under which different species of eolid will eject nematocysts, and on whether or not these nematocysts are exploded. The results obtained depend on the species used, and there is also a certain amount of individual variation. Animals which have been kept for several days in aquaria tend to be less reactive than fresh ones. Because of this variability in behaviour, where observations have been based on only one or two individuals, the results should be viewed with caution, for example with Berghia coerulescens and Favorinus branchialis. Table 9 summarises the results obtained from Plymouth and from Jamaica for seventeen species of eolid. The first six columns are concerned with nematocysts. Column 2 gives the number of times in which ejection of nematocysts occurred when the animal was prodded on the back with a pair of fine forceps. Column 3 gives the number of times in which ejection occurred when a ceras was pinched with forceps and plucked off. The number of individuals used for obtaining the figures in columns 2, 3, and 7 to 10 is given in column 1. The behaviour of an eolid when a ceras is pinched, and whether autotomy occurs, has been discussed on page 51.

Column 5 gives the number of nematocysts which are exploded out of the total number ejected, and column 6 gives this as a percentage. The number of animals used to obtain these figures is given in column 4.

Species	Nematocysts						Glands			
	No. of animals	Ejection on prodding animal—	Ejection on pinching ceras	No. of animals	Number of nematocysts exploded	% exploded	Exudation on prodding animal		Exudation on pinching ceras	
							white	clear	white	clear
	1	2	3	4	5	6	7	8	9	10
Eubranchus pallidus	4	✓	3/14	1	6/20		✓		3/6	8/9
E. exiguus	10	✓	3/5				✓		7/11	
Catriona aurantia	9	11/13	28/28	7	106/430	25%				8/10
C. maua	19	5/17	4/15	3	63/335	19%	5/33		0/15	
C. perca	8	0/18	0/4	2	13/101	13%	5/22		0/4	
C. tina	15	9/21	2/5	2	195/275	71%	0/17			
Tergipes despectus	12	11/22	9/17	6	178/255	70%				
Eolis M	2	2/7								
Favorinus branchialis	1		2/12	1	20/30					✓
Dondice occidentalis	8	6/22	1/13	2	139/178	78%				✓
Eolis D	12	17/29	6/10	7	463/517	89%	✓			
Learchis poica	7	2/13	1/12	4	321/1572	20%		✓		
Phidiana lynceus	15	1/25	0/12							
Berghia coerulescens	2	3/4	1/2	1	145/300	48%				
Spurilla neapolitana	12	12/35	7/11	5	553/782	70%				✓
Aeolidia papillosa	5	9/12	8/22	5	343/753	45%				
Aeolidiella glauca	2	3/8	2/3	1	64/88					

Table 9. Behavioural reactions of eolids. ✓ - a positive reaction. For further explanation, see text.

From columns 2 and 3, we can conclude that nematocysts are usually ejected (over 50%) on appropriate stimulation in Catriona aurantia, Tergipes despectus, Eolis D, Spurilla neapolitana and Aeolidia papillosa. They may also be regularly ejected in Eubranchus exiguus, Catriona tina, Eolis M, Aeolidiella glauca and Berghia coerulescens, but there is insufficient data to be certain of this. Nematocyst ejection also occurs occasionally in all of the other species studied, except Catriona perca and Phidiana lynceus inwhich it is rare or non-existent. It is probable that nematocyst ejection is more frequent when the stimulus is more violent, but with violent prodding, the water is disturbed and the cerata are waved vigorously about so that any ejection is obscured. Phidiana is a particularly difficult species in this respect. Although it is one of the largest eolids, and is very abundant in Jamaica, the cerata are moved rapidly to and fro at the slightest provocation, and it is very difficult to be certain whether or not ejection has occurred. All doubtful records have been omitted from Table 9.

From column 6, we can conclude that of the ejected nematocysts, a high percentage are exploded in Tergipes despectus, Eolis D, Spurilla neapolitana and Aeolidia papillosa, and probably also in Catriona tina, Dondice occidentalis and Berghia coerulescens where only one or two individuals were examined. Comparatively few are exploded in the other species of Catriona and in Learchis poica. Where less than 100 nematocysts were counted from only one or two cerata, the figures have

been ignored as they may have been due to chance variation. Hancock and Embleton (1845) find that if a ceras is compressed slowly many of the nematocysts explode. If the water in which nematocysts have been ejected is agitated, more explosions occur. Therefore it is important to standardise conditions for these results to have any relevance. In every case, a ceras was pinched and autotomised, and then placed on a slide in a drop of water. The counts were made after a coverslip had been lowered, except in the case of T.despectus. In the case of Dondice occidentalis, of 74 nematocysts ejected naturally, 68 were exploded; whilst of 75 squeezed out by means of pressure exerted on the coverslip, 68 were exploded. So in this species at least, extra pressure does not appear to affect the number of nematocysts exploded. There is a certain amount of individual variation in the behaviour of eolid nematocysts, but in the case of Spurilla neapolitana, the results are often repeatable. From one animal, five cerata were removed, and of 100 nematocysts examined from each ceras, 61, 64, 69, 80 and 80 were exploded. Cerata from two other individuals gave counts of 61 and 82. Thus individual variation does occur, but if several hundred nematocysts from a number of individuals are examined, the results should be comparable for different species. Another point is that in the figures given, no account has been taken of the different types of nematocyst present. Kepner (1943) describes the nematocysts of Catriona pilata (Gould). This species feeds on Pennaria tiarella which has five types of nematocyst, large, medium and small stenoteles (or penetrants), 33,

15 and 7.5μ long), desmonemes (or volvents, 3μ long), and microbasic mastigophores (15μ long). The large stenoteles and the mastigophores are rare, the others are common. In C.pilata, Kepner describes how all nematocysts are destroyed by the eolid in the stomach and the cnidosac, except the mastigophores. These are stored in high concentration in the cnidosac, and are used in defence. Whilst Cuénot (1907) and Glaser (1910) believe that the defensive value of nematocysts is slight, Kepner holds that their defensive value is great, and that this selection of nematocyst types is such that the types stored in the cnidosac are those that are most repellent to potential predators. It is certainly true that selection of nematocyst types does occur in many species, but it is not easy to show that the types stored are the most repellent to predators. Bedot (1896) finds that spirocysts do not occur in eolids, and in the specimens of Berghia, Spurilla, Aeolidia and Aeolidiella that I have examined, I have seen only one spirocyst in a specimen of Spurilla. Since each cnidosac may contain 10,000 nematocysts in A.papillosa (personal estimation), and perhaps even more in Berghia, it is clear that mastigophores have been selectively taken up, and that spirocysts have been eliminated. In Eolis D, the only type of nematocyst present is a form of stenotele with an elongate capsule 25 to 30μ long. Since few hydroids have only one type of nematocyst (Hyman, 1940), it is clear that here too selection of nematocyst types has occurred. In Catriona tina, Tergipes despectus, Eolis M, Eolis K, and probably Eubranchus exiguus and Catriona perca as well, the nematocysts are almost all of the same

type, usually 3 to 5μ long isorhizas. The hydroids on which these species feed are not well known, so it is not possible to correlate this data with the nematocysts present in the food. On the other hand, other species have several types of nematocyst, for example Dondice occidentalis, Learchis poica, Facelina coronata and Catriona aurantia. Different types of nematocyst tend to explode under different conditions. Thus with L.poica, there are the same five types of nematocyst as are initially taken up by C.pilata. All are present in the cnidosac, and all are functional. 5μ long volvents make up 1/4 to 1/3 of the nematocysts, and of 218 examined, 148 (68%) were exploded. Microbasic mastigophores are uncommon, making up only 4 or 5% of nematocysts present, and only 5 out of 51 (10%) were exploded. Stenoteles make up the rest of the nematocysts, most being small or medium in size (6 to 15μ long) with a few large ones (up to 40μ). But of 1303 stenoteles, only 168 (13%) were exploded, and this includes one ceras from which 87 out of 93 stenoteles were exploded. This suggests that either nematocyst selection has not occurred in L.poica, or that it has produced a very different result from that found in other species of eolid. It is difficult to see how volvents could be effective deterrents, yet in L.poica it is volvents that are the most likely to explode.

Conclusions

These results demonstrate that different species of eolid utilize their nematocysts in different ways. One cannot generalize and say that nematocysts are or are not ejected or exploded in eolids -

the result obtained depends on the species studied. This does not give any direct evidence on whether or not nematocysts are of use in defence, but it strongly suggests that they are. It is difficult otherwise to explain why some species selectively take up certain types of nematocyst but reject others, or why nematocysts should be ejected at all as a result of stimulation by a vigorous prod.

INTERACTION OF NEMATOCYST AND GLANDULAR DEFENSIVE MECHANISMS

The previous two sections have established that glandular defensive mechanisms exist in many acleioproct eolids, that glands which may be defensive exist in cleioprocts, and that nematocysts are ejected as a defensive reaction in most species of eolid. In this section I shall attempt to show how nematocysts and glands are used together or separately to a varying extent in different species. Columns 7 to 10 of Table 9 relate to the behaviour of white and clear (transparent) glands when the animal is treated as for columns 2 and 3. In the Eubranchidae and the genus Catriona, where defensive glands are concentrated in the cnidosac region, glandular extrusion is often visible with a low power dissecting microscope (or even with the naked eye in E.exiguus). But in the cleioprocts, and in the case of clear glands in the acleioprocts, exudation is often only seen when a ceras has been mounted under a microscope with a 16 mm. lens. Since it is probable that some of these secretions disperse readily, the fact that there are many blanks in the table does not necessarily indicate that glandular

exudation is non-existent; it merely means that it was not observed.

Eubranchidae

In Eubranchus pallidus, nematocysts, white thread-like glands, and colourless glands are all regularly ejected when the animal is molested, but from the frequency of their exudation, it would seem that glandular secretions are perhaps more important than nematocysts in the defensive system of this species. In both E.pallidus and E.exiguus, there are at least three types of defensive gland (pages 70 to 85). In E.exiguus, nematocysts and white thread-like glands are both regularly ejected, and it is probable that both are important in the defensive system of this species also. In E.exiguus the glands are much larger and better developed than they are in E. pallidus, and the muscles of the muscle-operated glands are clearly visible with an ordinary light microscope, but there is no evidence as to whether or not the glands are a more efficient deterrent because of this. More observations are wanted, too, on the ejection and explosion of nematocysts in these species.

Cuthonidae

The four species of Catriona studied all have at least two types of defensive gland (pages 85 to 96). The white glands are muscle-operated and are often exuded on stimulation, but the secretion of the granular clear glands may be too readily dispersed in water to be easily detected. The absence in C.aurantia of observations on secretion of white glands may not be significant - such

secretion may have been overlooked. All four species probably utilise both nematocysts and glands in defence, despite the absence of nematocyst ejection in C.perca and of glandular exudation in C. tina from Table 9. The nematocysts of C.perca examined for column 4 were extruded when an autotomised ceras was prodded, so it is likely that nematocysts are extruded under certain circumstances in this species. For C.tina, since it possesses glands similar to the defensive glands of other Catriona species, it is likely that they are sometimes used in defence here as well. Thus the four species of Catriona all have similar glands, and all use both glands and nematocysts in defence. But whereas in C.perca it is glands that seem to be the more important of the two mechanisms, in C.tina it is nematocysts. The difference between $^{0}/_{18}$ for the nematocysts of C.perca and $^{9}/_{21}$ for C.tina is significant at the 1% level (using the 2 X 2 contingency table of Armsen, 1955), and that between $^{5}/_{22}$ and $^{0}/_{17}$ for glands is significant at the 5% level. C.maua and C. aurantia are intermediate and utilize both nematocysts and glands, but whilst in C.maua prodding does not often elicit a response, in C.aurantia it almost invariably does. Another point is that in C. tina alone amongst these species are there an appreciable percentage of nematocysts exploded. These conclusions are somewhat tentative and more observations are necessary to be certain, but it does seem that the defensive systems of these four species are quite different even though they all belong to the same genus.

Tergipes despectus and Eolis M both rely entirely on nematocysts

for defence. Defensive cerebral glands are completely absent (pages 96 and 97), but the cnidosacs are much larger than they are in, for example, Catriona tina, which is of about the same size. Only a small number of observations were made on Eolis M, but for T.despectus, nematocysts are frequently ejected on prodding, and are almost certainly of defensive importance.

Favorinidae

In the cleioprocts, there is no great concentration of glands at the cerata tip (except in Calma), and in none of the species studied here are any of the glands muscle-operated. In the Favorinidae, it is probable that both nematocysts and glands are utilized in defence, but there is adequate evidence for this hypothesis in only one species, Dondice occidentalis. The poor development of glands in Eolis K suggests that here they are not important, but direct observations on this species are lacking.

Facelinidae

In the Facelinidae, too, both nematocysts and glands are probably used in defence. Learchis poica is a good example, although in this species few of the nematocysts are exploded. In Phidiana lynceus the absence of observations may be due to the mobile cerata masking nematocyst or glandular ejection. Eolis D uses both glands and nematocysts in defence, but is unique in two respects: each cnidocyst of this species contains only a single nematocyst, and there is a long narrow duct leading from the cnidosac to the

cnidopore of exactly the same diameter as are the nematocysts. The importance of this arrangement is not known, but it is probable that the cnidosac is adapted to storing and ejecting one particular type of nematocyst which explodes readily (89%) and is a particularly effective deterrent to predators.

Aeolidiidae

In the Aeolidiidae, glandular secretions may be of some defensive importance, but the most important defensive mechanism is undoubtedly the nematocysts. In all species studied there is evidence that nematocysts are frequently ejected as a defensive reaction and that many of these are exploded. A single ceras of A. papillosa frequently contains 400 cnidocysts, and each cnidocyst may have 25 or more nematocysts. Spurilla may have 300 cnidocysts in one cnidosac, and each cnidosac has about 30 nematocysts. Berghia has an even larger cnidosac. Thus the supply of nematocysts is colossal, and even if only 50% explode, the deterrent effect of them is probably very great.

Calmidae

Calma glaucoides is an example of an eolid in which nematocysts are of no defensive importance, but glands are developed to a high degree. Nematocysts and cnidosac are completely absent in this species (Evans, 1922), but the glands closely resemble those described for Eubranchus and Catriona, and presumably function in a similar way.

CONCLUSIONS

In the overall defensive system of eolids, each particular mechanism comes into action in series:

1. Crypsis. For those eolids that are cryptic, this is obviously the first mechanism to prevent predation.

2. Behaviour. Should an eolid be discovered by a potential predator, its immediate reaction is to erect and wave the cerata. This diverts a possible attack towards the expendible cerata. At this stage, should an attack occur, any or all of three mechanisms may occur:

3a. Cerata may be autotomised.

3b. Glandular secretions may be exuded.

3c. Nematocysts may be ejected.

4. Unpalatability. The ultimate protective mechanism should all of these other mechanisms fail is that the eolid is comparatively unpalatable.

Although this is the general pattern of the defensive system of eolids, there are many variations. Some species are not cryptic, for example, and the ease with which different species autotomise their cerata varies. Finally there is variation in the importance in different species of nematocysts and glands: in some the nematocysts are important whilst defensive glands are absent; in others glands are important whilst nematocysts are absent; and in yet others both glands and nematocysts are utilised. The relative importance of these two mechanisms may even vary within one genus.

Figure 44. Diagrammatic longitudinal sections of cerata of Favorinus auritulus, F. branchialis and Eolis D. Each of these diagrams, and of Figs. 45, 46, and 47, represents the glands seen in a 6μ thick median longitudinal section of a ceras of up to 1·5mm. overall length. For explanation of glands, see Fig. 20.

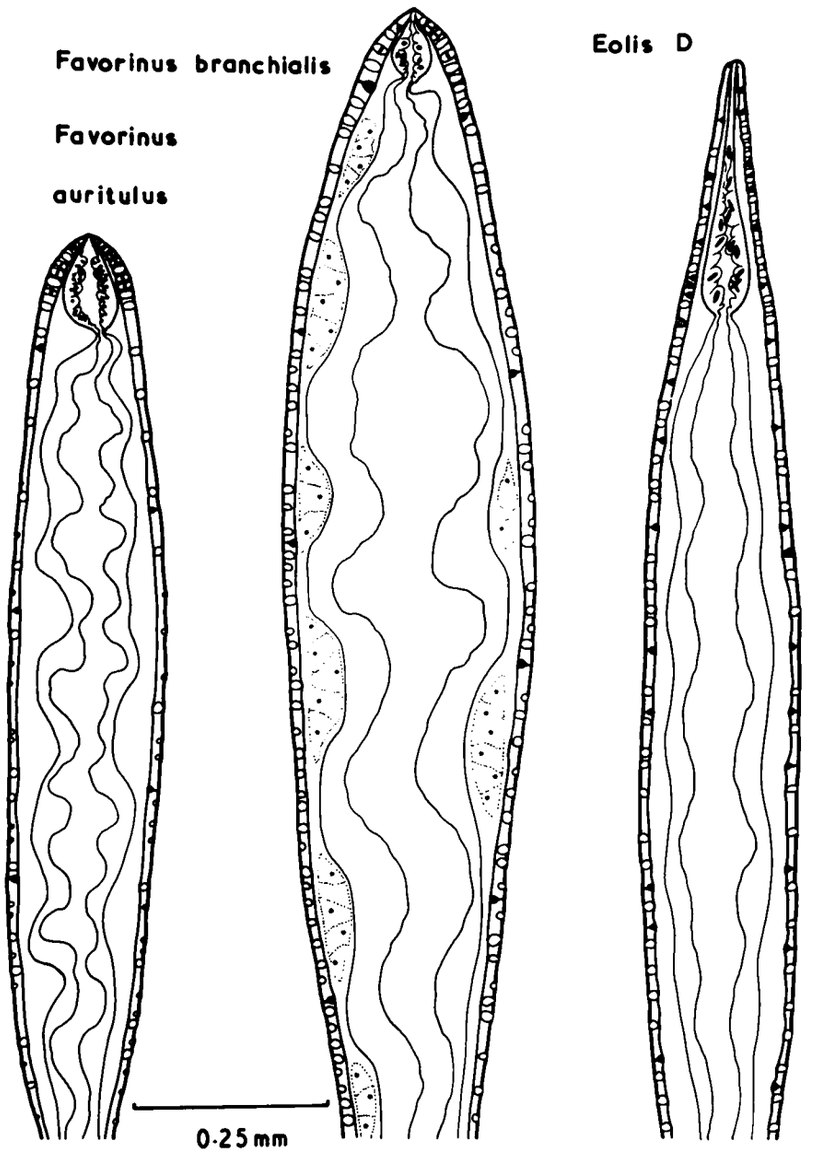

Favorinus branchialis
Favorinus auritulus
Eolis D
0·25 mm

Figure 45. Diagrammatic longitudinal sections of cerata of Tolis K and Dondice occidentalis.

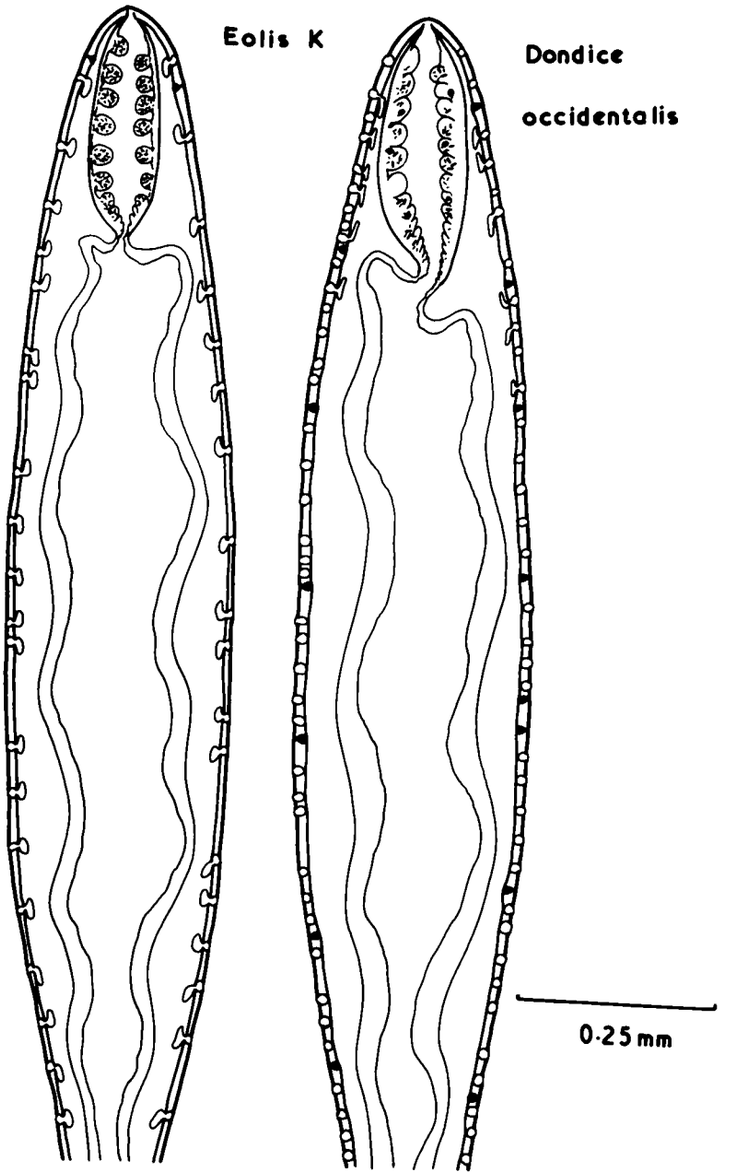

Eolis K
Dondice
occidentalis
0·25 mm

Figure 46. Diagrammatic longitudinal sections of cerata of Learchis poica and Phidiana lynceus.

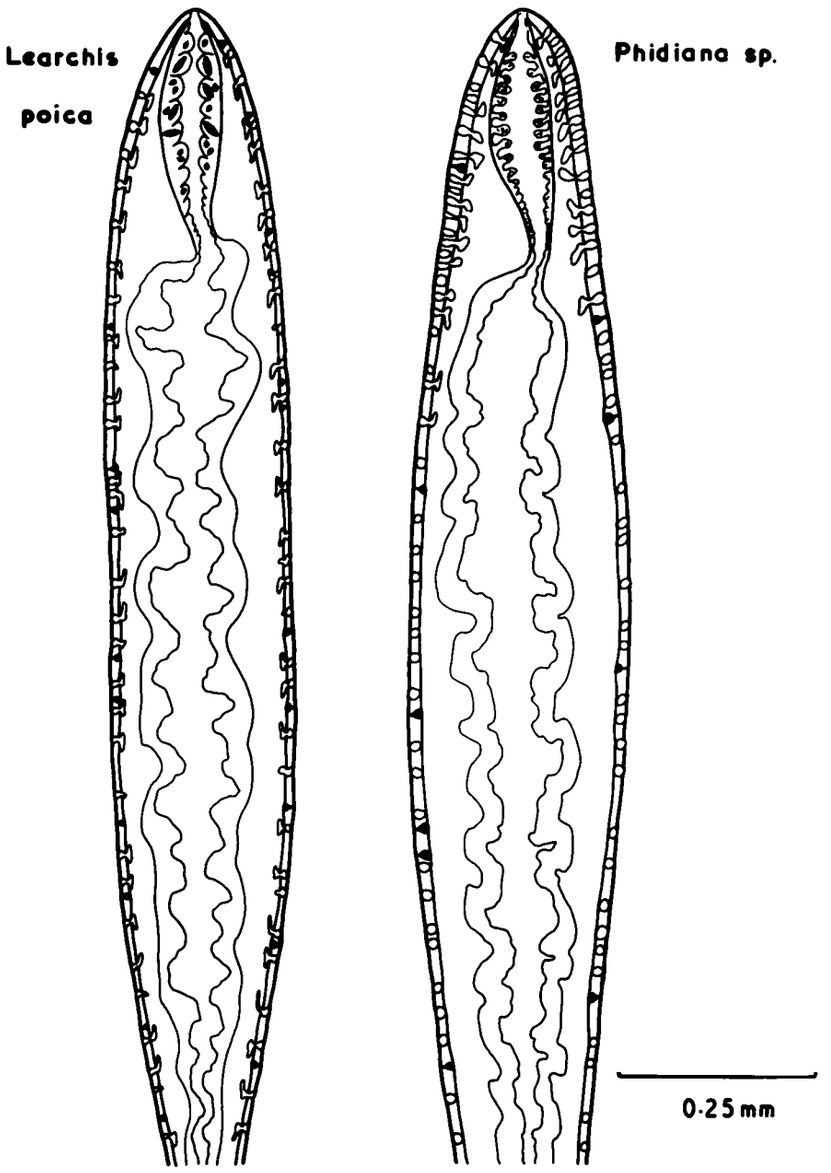
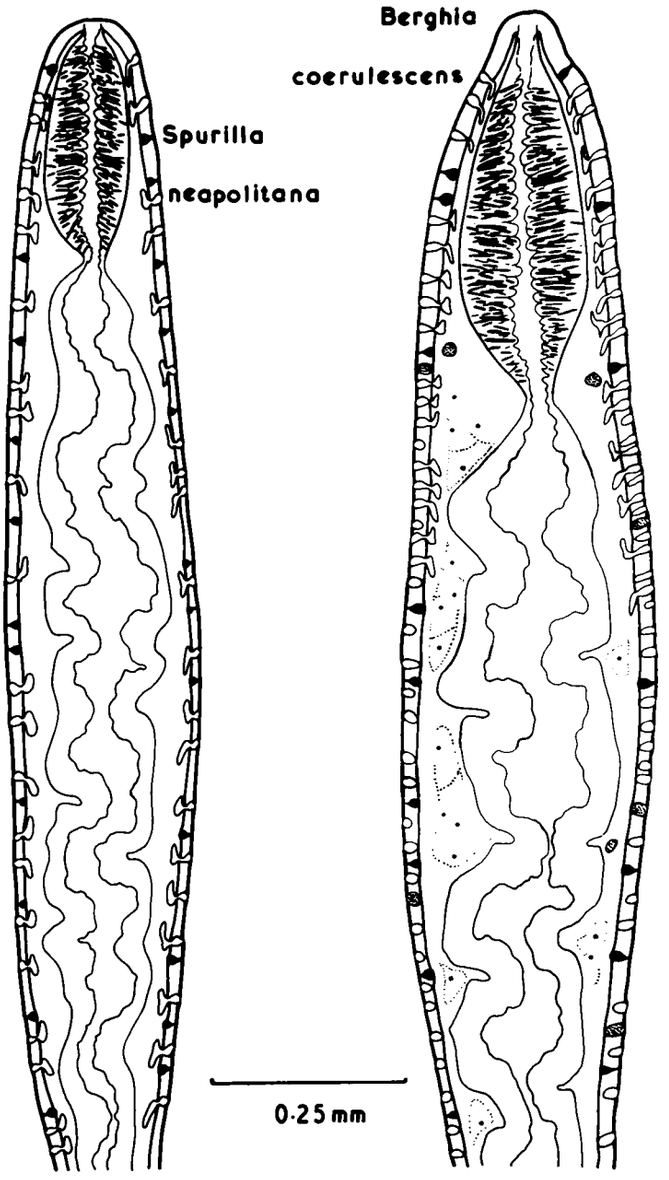

Learchis
poica
Phidiana sp.
0·25 mm

Figure 47. Diagrammatic longitudinal sections of cerata of *Spurilla neapolitana* and *Berghia coerulescens*.


Berghia
coerulescens
Spurilla
neapolitana
0·25 mm

Chapter 6

PREDATION OF NUDIBRANCHS

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Historical

Throughout the previous four chapters defensive adaptations have been described from many nudibranchs, but nothing has been said about the predators to which these adaptations are directed. Our knowledge of the predators of nudibranchs in the sea is very small, but several workers have tried to feed nudibranchs to various species of fish and invertebrates in aquaria. The results with invertebrates are inconclusive. Crabs are the most suitable of the invertebrates to experiment with, but even with these Thompson (1960,b) finds that they invariably ignore the test nudibranchs. Experiments with fish are easier to perform since if the test nudibranch is thrown into the water, aquarium fish usually inspect and snap at it - they are usually fed by food thrown into the water, and therefore behave as if the nudibranch too were food. Bateson (1890) describes how most species of fish taste food by taking it into the mouth, and if it is unpalatable they spit it out. Experiments of this type can be expected to demonstrate whether a particular species of nudibranch is palatable or not to a particular species of fish. They do not give any information on whether or not fish eat nudibranchs in the sea. However, if these experiments show that nudibranchs are unpalatable, then they are probably unpalatable also in the sea; but if they

show that they are palatable, then it must also be shown that they are eaten in nature before we can regard fish as being important predators.

Herdman and Clubb (1890) experimented with over a dozen species of fish. They find that the dorids Lamellidoris fusca and Ancula cristata are rejected, and are therefore unpalatable to most species of fish. Similarly, the eolids Coryphella rufibranchialis, Eubranchus pallidus and Facelina coronata are rejected by Blennius pholis L. and by other species of fish. Dendronotus frondosus, however, is eaten by blennies (B.pholis), and may be palatable to other species also. Garstang (in Poulton, 1890) finds that Eubranchus tricolor is unpalatable to pollack (Gadus pollachius L.). McIntosh, however, (in Grosvenor, 1903) finds that Aeolidia papillosa is eaten by cod (Gadus callarias L.). Crossland (1911) and Crozier (1916) find that the chromodorids Chromodoris reticulata, diardii and zebra are unpalatable to several species of fish. Thompson (1960, a and b) has recently reinvestigated the problem. He used eight species of fish, and his results show conclusively that Elysia viridis, Tritonia hombergi, Dendronotus frondosus, Eubranchus tricolor, Facelina auriculata and seven species of dorid are all unpalatable. Hero formosa is also usually rejected, but one was eaten. Thompson concludes that whilst dead or damaged opisthobranchs may be eaten by fish, healthy ones are always unpalatable. The only evidence that nudibranchs are eaten by fish in the sea is that jaws of Tritonia

hombergi have been found in the stomach of dogfish (Scyliorhinus canicula L.) (Thompson, 1960,b; and D.Nichols, personal communication), and Aeolidia papillosa has been reported from the stomachs of young haddock (Gadus aeglefinus L.) (Homans and Needler, 1944). In this case A.papillosa was evidently exceptionally abundant, and was eaten by a number of individual fish. It is possible that the fish concerned were exceptionally hungry, and that they were therefore prepared to eat any food, however unpalatable it might be. The results of Cuénot (1907) and Glaser (1910) support this view. Cuénot finds that three species of fish will taste Spurilla neapolitana several times and reject it before finally swallowing it. Glaser describes similar results.

Discussion

The evidence from laboratory experiments thus conclusively suggests that most species of nudibranch are unpalatable to fish. However, nudibranchs are occasionally eaten by fish in nature, and it is probable that in these cases the fish were so hungry that they were not deterred by the unpleasant properties of the mollusc. We should expect there to be two selection pressures acting on the system, one tending to favour those nudibranchs which are most unpalatable or best protected in other ways, the other favouring those fish that have some immunity to the repellent properties of the nudibranchs. A balance would result such that nudibranchs would be rejected by most fish but taken by a few starved fish. Selection pressure would be incapable of pushing

the system further than this point. One would expect that different species of nudibranch and different species of fish would find this balance at different points. Thus Cuénot finds that whilst three species of fish behave as described already (eventually eating S.neapolitana), a fourth species, Gobius niger, always rejects Spurilla neapolitana. Without a very large supply of one or two species of eolid and extensive work with fishes, it is impossible to draw further conclusions, and these suggestions should be regarded as tentative.

The source of this unpalatability of eolids was thought by Garstang (1889) and Herdman (1890) to be the nematocysts. Nematocysts are almost certainly responsible for the apparent inedibility of coelenterates which are not normally eaten by fish in aquaria or in the sea. Since many eolids regularly eject nematocysts as a defensive reaction (Chapter 5), it is reasonable to conclude that nematocysts are of defensive importance here as well. There are, however, a few species of fish which do eat coelenterates (Cuénot, 1907), and obviously these species would not be deterred from eating eolids by nematocysts. It is probably with relation to these species of fish that defensive glandular secretions have evolved in eolids. It is likely that nematocysts are protective against one set of predators whilst glands are protective with relation to another set of predators. Invertebrate predators are probably even more variable in their behaviour towards eolids than are fish, and it is likely that some eolid defensive mechanisms are

specifically adapted towards certain invertebrate predators. Until more is known of the predators of eolids and of other nudibranchs it is impossible to draw further conclusions, but there is sufficient evidence to conclude that predation of nudibranchs does exist, and that many nudibranchs have evolved a series of defensive mechanisms directed towards some of these predators.

Chapter 7

DISCUSSION AND CONCLUSIONS

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Chapters 2, 3, 4 and 5 of this thesis have described the defensive mechanisms of nudibranchiate molluscs, and how the overall defensive system of any one species of nudibranch may comprise several different mechanisms. Some of these mechanisms have not been described before and have therefore been discussed in considerable detail. These include the hypobranchial gland of Berthelinia caribbea, the ceratal glands of Stiliger vanellus, acid secretion in dorids, and ceratal glands of several species of eolid. Other defensive mechanisms are also discussed, and the evidence supporting the hypothesis for the defensive value of some of these mechanisms is reviewed. Two important conclusions can be drawn from this work. First, in the Sacoglossa and the Eolidacea, and perhaps in other groups as well, several lines of defence occur in any one species, and these may operate in series. The situation is similar to that described in Brasilian moths by Kettlewell (1959), but the adaptations are not so complex. The second conclusion is more tentative since so little is known of the predators of nudibranch molluscs. It is that some of the different mechanisms present in some species of eolid may be directed towards specific predators. There is evidence that different species of fish vary in their readiness to eat eolids, and this is due in some cases to an ability to tolerate nematocysts. The final stage in this argument is that the defensive glands are adapted

to repelling those predators which are not repelled by nematocysts. This is speculation, nevertheless it does go some way towards explaining why some eolids use glands alone, some use nematocysts alone, and some use both methods of defence.

This work has also shown that some of the defensive mechanisms have evolved several times independently in different groups of the naked opisthobranchs. It is important to recognise such parallel evolution if we are to obtain a true picture of the phylogeny of the group. In the case of epidermal vesicles, which have probably evolved independently in the Eolidacea, Dendronotacea and Arminacea (Graham, 1938), this is a mechanism directed towards protecting the animal from its prey rather than from a potential predator. The other cases of convergence are all directed towards protection from predators. One case that has not been mentioned before is the neurosensory cilia of eolids which I have shown to be involved in the mechanism of glandular secretion and nematocyst ejection. The nerves and sense cells of Janolus probably have a similar function, and it is possible that the same also applies to the nervous structures in the caryophyllidiae of dorids.

Defensive glands have certainly evolved many times in the opisthobranchs, but it is not easy to be certain which examples are in fact parallels. In some cases, however, we can be certain. Thus the ceratal glands of Stiliger and those of the eolids are clearly convergent, and it is interesting that in Stiliger they are all subepidermal whilst in the eolids they are nearly all epidermal.

Within the Eolidacea, the defensive glands of the Cuthonidae and the Eubranchidae are almost certainly convergent, and the glands of the cleioprocts may be epidermal or subepidermal irrespective of affinity. Our knowledge of the chemical composition of the secretions of some of these glands is limited at present, but osmiophil glands occur in many eolids and in some sacoglossans. Acid glands have evolved independently many times in the Mollusca, including at least once in the Doridacea. Muscle-operated glands occur in the Sacoglossa, the acid secreting dorids, and in the eolid genera Catriona and Eubranchus.

The nematocyst storing habit has also evolved at least twice, as it occurs in the Eolidacea and in the Dendronotacean Hancockia.

Finally, and perhaps most important of all, the eolidiform condition has evolved several times in the opisthobranchs. It has probably evolved independently in the Sacoglossa, the Polyceridae, the Triophidae, the Goniodoridae, the Dendronotacea (perhaps twice), the Arminacea and the Eolidacea. The eolidiform condition has several important advantages over forms without papillae. Papillae or cerata serve to deflect attacks away from the vital regions of the body and to direct them towards the papillae themselves which are usually expendable and can be rapidly regenerated. They also enable the defensive mechanisms of the animal to be concentrated in the most prominent and least vital region of the body, such that any repellent substances will

be taken by the potential predator at the earliest opportunity, and before the body of the animal has been harmed. The cerata of nudibranchs represent a highly successful defensive device, and they are without doubt responsible to a considerable degree for the success of the group.

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POLYCERA ELEGANS BERGH : A NEW SPECIES TO BRITAIN AND DISCUSSION OF ITS TAXONOMY.

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(*Read* 10 *March* 1961)

Bergh (1894) describes a new genus and species of nudibranch from Rovigno in the Adriatic under the name of *Greilada elegans* Bergh. Odhner (1941) describes several individuals of a species found on *Zostera* at Messina (in the Mediterranean) in 1888. He concludes that the genus *Greilada* should be abandoned and regarded as a section of the genus *Polycera,* and these specimens he names *P. messinensis* Odhner. A single individual of *G. elegans* is described from Banyuls (in the Mediterranean) by Pruvot-Fol (1951). On the evidence of more material from Arcachon, Biscay, Pruvot-Fol (1955) tentatively concludes that the genus *Greilada* should be abandoned. She also concludes, provisionally, that the Arcachon material be classified as a separate, new species : *P. atlantica* Pruvot-Fol. The purpose of the present note is to record *P. elegans* from Plymouth, and to discuss the taxonomy of the group.

Polycera elegans at Plymouth.

On the 27th June 1960, Mr. G. R. Forster found an orange nudibranch about 26 metres down at Stoke Point rocks (Hilsea Pit), Plymouth, whilst aqua-lung diving. Dr. D. P. Wilson identified the animal as *G. atlantica,* and he has kindly lent me the specimen for examination of the radula and jaws. This individual was 4.25 cm. long when extended, of a brilliant orange colour with several ultramarine blue spots on the head, back and sides. The spots, in life, had a darker rim and several minute carmine spots in the blue. The narrow border, separating the back from the flanks, was covered with minute white spots, interspersed with fewer blue spots. There were slight thickenings in the border and at times slightly raised papillae were visible. There are 12 complete rings to the rhinophores, 9 orange digitations to the oral veil, and 5 gills. Whilst in captivity the animal spawned. The coil was of an orange colour and about 3 mm. wide.

On the 19th October 1960 I found two more individuals of *P. elegans* at Plymouth. The smaller one, 1.2 cm. long, was found on a rope about five metres below low water on the breakwater. A number of ascidians including *Ciona* were growing on the rope. The larger specimen, 2.6 cm. long, was caught in a handnet on a colony of *Ciona intestinalis* (L.) growing on a pontoon in Millbay docks. This individual (Fig. 1) is very similar to that found by Mr. Forster, but differs in a number of features. There are 7 digitations to the oral veil, 7 tripinnate gills, and 15 complete rings to the rhinophores. The border carries white, blue, and black flecks, and there are irregular thickenings on it, including a pair of short papillae behind the gills. There is no trace of carmine either on the gills or in the spots.

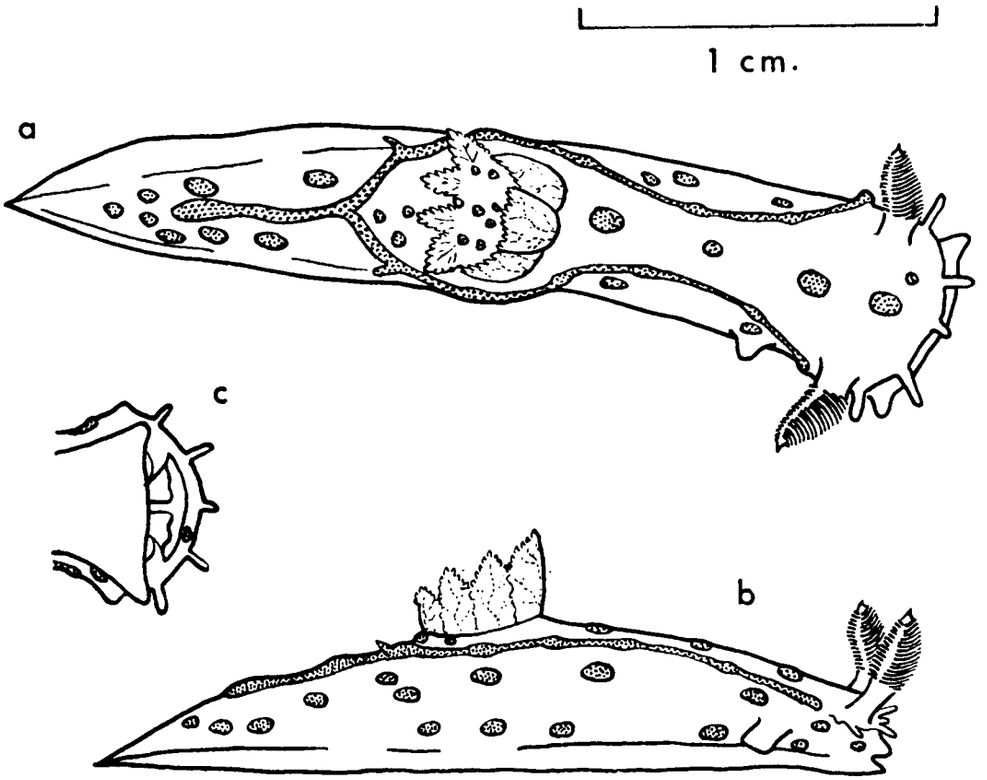

FIGURE 1.—*Polycera elegans* from Millbay docks, Plymouth, drawn from life
Stippled areas appear ultramarine blue in life.
(a) from above.
(b) from right side.
(c) head from below.

The taxonomy of *P. elegans*.

These two individuals, though not identical, are very similar to those described by Bergh (1894). His were 0.9 cm. long, and orange with blue spots. They had 6 simple pinnate gills, 15 to 20 rings to the rhinophores, and 4 digitations to the oral veil. There were no dorsal papillae or thickenings to the border, but there was a rose tinge to the rhinophores and the gills.

Odhner's *P. messinensis* had 7 tripinnate gills, but was otherwise identical in externals with Bergh's *P. elegans*. The Banyuls specimen carried pronounced dorsal papillae, and the blue border extended in front of the rhinophores (Pruvot-Fol, 1951). The Arcachon specimens had each spot surrounded by a carmine line, tripinnate gills, definite dorsal papillae, and 6 to 8 digitations to the oral veil (Pruvot-Fol 1955).

We can conclude from the external features that there appears to be continuous variation within one species rather than a dichotomy into two distinct species (see table 1). Thus there may be 5, 6 or 7 simple or tripinnate gills, and there may be 4 to 9 digitations to the oral veil. There may or may not be dorsal papillae. The spot distribution, and the amount of rose or carmine and of blue is variable. A comparable

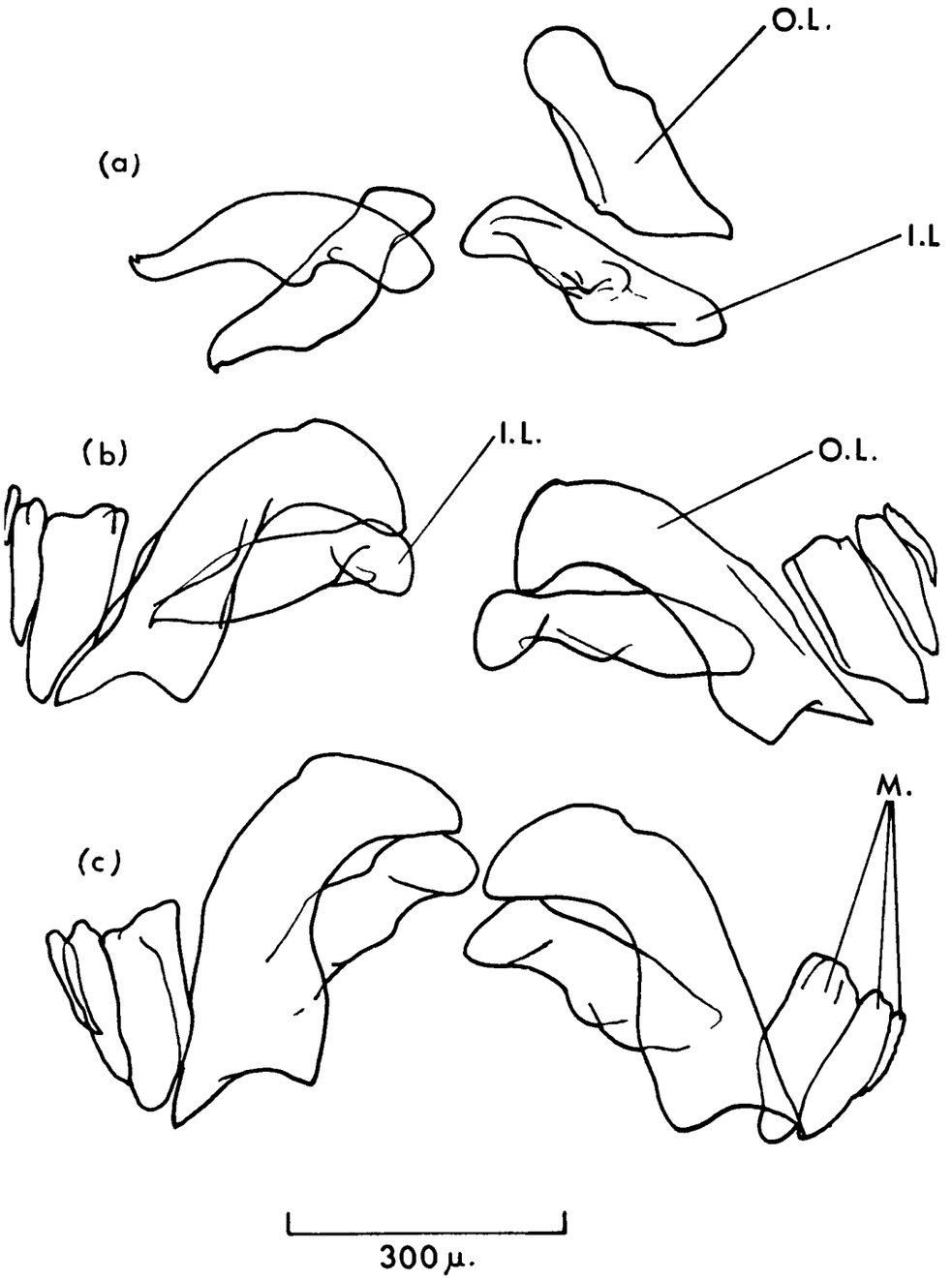

FIGURE 2.—*P. elegans* (Stoke Point, Plymouth): radula, transverse rows of teeth.

(a) 1st row, much worn.
(b) 3rd row.
(c) 7th row.

M = marginal teeth; IL = inner lateral tooth; OL = outer lateral tooth.

degree of variation is known from the closely related species *P. quadrilineata* Müller which may have 5 to 9 simple, bi- or tripinnate gills, 1 to 13 oral digitations, and simple or branched dorsal papillae (Alder and Hancock, 1845-55, plate 22; Eliot, 1910, page 3; Elmhirst, 1908).

The radula of *P. elegans* has 15 rows of teeth according to Bergh (1894), whilst that of *P. messinensis* has only 10 rows (Odhner, 1941), and that of *P. atlantica* has only 6 to 8 rows of teeth (Pruvot-Fol, 1955). Pruvot-Fol does not attach much importance to these differences, and with this I am in complete agreement. The radula from the first Plymouth specimen (Fig. 2) has 11 rows of teeth, and the formula: 3—1.1—0—1.1—3. Anteriorly the marginal teeth may be reduced to 2 or 0. The shape of the lateral teeth is similar to, but not identical with, those depicted by Bergh, Odhner, and Pruvot-Fol. Such differences as do exist are due in part to the angle of viewing which it is difficult to standardise and impossible to alter in permanent preparations.

The genus *Greilada,* according to Bergh, differs from *Polycera* in that it has 6 gills which are simple pinnate; there are no papillae behind the gills; and the jaws are joined above and below by intermediary pieces. Pruvot-Fol (1955) has pointed out that *P. faeroensis* also has intermediary pieces to the jaws, and all the other features of *Greilada* have been shown to be very variable. Thus the generic name *Greilada* should be abandoned (Odhner 1941, Pruvot-Fol 1955). In view of the differences between two individuals at Plymouth, there does not seem to me to be sufficient justification for denoting each group of specimens from a different locality by means of a different name—either as separate species or as varieties. The species first described by Bergh is then *P. elegans* Bergh, of which *P. messinensis* Odhner and *P. atlantica* Pruvot-Fol are synonyms.

Ecology of *P. elegans.*

As to the ecology of *P. elegans,* very little is known. Two individuals were found on or near *Ciona,* so they may have been feeding on tunicates. Dorids usually appear to be cryptically coloured (Garstang, 1890), and *P. dubia* Sars is usually well camouflaged (personal observation). But *P. quadrilineata* is stated by Thompson (1960) to be "never inconspicuous." The (presumably) defensive glands of *P. quadrilineata* are concentrated in the dorsal papillae, which are held erect when the animal is disturbed. *P. elegans* also appears to be conspicuous but may lack these papillae, and some other source must be sought for its defensive mechanism. It does not secrete acid (personal observation) as does *Pleurobranchus* (Thompson and Slinn, 1959), and merely contracts when disturbed. *P. elegans* has been found on only five or six occasions in over 70 years, yet it is a conspicuous animal, and several individuals have usually been found on each occasion. It appears to occur not only in the Mediterranean

and in Biscay, but also in the English Channel. Three explanations, not necessarily mutually exclusive, are plausible :

(a) that *P. elegans* lives in inaccessible places. *Okenia elegans** Leuckart has been found embedded in the tunic of *Styela* (Alder and Hancock, 1845-55, plate 27), and I would suggest that *P. elegans* may have a similar habit with respect to *Ciona* and other ascidians. The viscera of *Ciona* and *Styela* are often identical in hue with the orange of *Polycera,* so the colour of this nudibranch may be fortuitous, due to its food, and of no allaesthetic significance since it is not normally exposed to the eyes of would-be predators. But although two individuals at Plymouth were found on or near tunicates, the Messina specimens were found on *Zostera,* and *P. elegans* is much more active than one would expect a commensal to be.

(b) that the individuals found of *P. elegans* represent spasmodic irruptions of an otherwise extremely rare species.

(c) that *P. elegans* is a local species that is spreading its range northwards.

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I am grateful to the Director and staff of the Marine Biological Association's laboratory at Plymouth and to Prof. Sir Alister Hardy, F.R.S. for the use of laboratory facilities. In particular, my thanks are due to Dr. D. P. Wilson for his advice, and for permission to publish some of his material; to Mme. A. Pruvot-Fol for her advice; and to Dr. D. Nichols for his invaluable help in the preparation of the manuscript. The work was carried out whilst holding a research studentship of the Department of Scientific and Industrial Research, to whom due thanks are given.

Summary

(1) The occurrence of *Polycera elegans* at Plymouth is described.

(2) The Plymouth individuals of *P. elegans* are compared with Mediterranean forms, and with *P. messinensis* and *P. atlantica.* It is concluded that these should all belong to the single species *Polycera elegans* Bergh.

(3) The ecology of *P. elegans* is discussed, and explanations are suggested to account for the rare occurrence of the species.

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* The *Okenia elegans* of this paper is described by Alder & Hancock as *Idalia elegans* Leuckart. It is referred to as *Okenia elegans* Leuckart in Winckworth's 1932 The British Marine Mollusca (*J. Conchol.,* **19**, 211-52). It is included in the 1957 Plymouth Marine Fauna under *O. quadricornis* Montagu, and in Pruvot-Fol's Mollusques Opisthobranches (1954, Faune de France **58**, 460 pp., Paris) under *O. leachii* Alder & Hancock.

Species	Authority	Locality	No. of rings to the rhinophore	No. of digitations to the oral veil	No. of gills	Gills—no. of times pinnate	No. of dorsal papillae	No. of rows to the radula
P. elegans	Bergh 1894	Rovigno	15-20	4	6	1	0	15
P. messinensis	Odhner 1941	Messina	12-15	4	7	3	0	10
P. elegans	Pruvot-Fol 1951	Banyuls		4			2	
P. atlantica	Pruvot-Fol 1955	Arcachon		6-8	7	3	2	6-8
P. elegans	This paper	Plymouth (Stoke Point)	12	9	5	3	2	11
P. elegans	This paper	Plymouth (Millbay docks)	15	7	7	3	2	

Table 1 : to show variation in the *Polycera elegans* group.

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Bivalved Gastropod from Jamaica

THE bivalved gastropod *Berthelinia* Crosse (= *Tamanovalva* Kawaguti and Baba) has been reported from Japan[1], Australia[2], and California[3]. A second genus, *Midorigai* Burn, has been described from Australia. The purpose of this communication is to record *Berthelinia* from Jamaica. The first specimen was found on *Caulerpa verticillata* Agardh growing on rocks in an open part of a mangrove channel near Port Royal on January 8, 1962. Small quantities of algæ collected on January 11 and 16 yielded fifty more specimens, so in this small area *Berthelinia* can be described as common. To my knowledge, this is the first time that living bivalved gastropods have been taken from the Atlantic.

The shell is almost identical with that of *B. typica* Gatliff and Gabriel[4]. The present species certainly belongs to this genus, although further work is necessary to decide whether or not it is distinct from those species already described.

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Appendix iii.

BERTHELINIA CARIBBEA N. SP., A BIVALVED GASTROPOD FROM THE WEST ATLANTIC

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(Accepted for publication September 1962, in J.Linn.Soc.)

INTRODUCTION

Since the discovery by Kawaguti and Baba (1959) of living bivalved gastropods in Japan, intensive search has resulted in several species being found in Australia (Burn, 1960a, b, c), in Baja California (Keen, 1960), and in Hawaii (Kay, 1962). A preserved specimen of Julia Gould from the Mariana Islands has been described by Morrison (1961), and it now seems likely that related species may be found throughout the world, at least in warm waters. The present paper describes Berthelinia caribbea n.sp. from Jamaica. This is believed to be the first record of a living bivalved gastropod from the Atlantic (Edmunds, 1962), although related fossil forms are known from the area (Gardner, 1926), and Julia gardnerae Woodring actually lived in Jamaica during the Miocene (Woodring, 1925).

CLASSIFICATION

The classification used is that of Keen and Smith (1961). As more is discovered about bivalved gastropods, this will no doubt have to be revised; in particular, it may be necessary to raise the

Berthelliniinae to the rank of family, and the reinstate Anomalomya Edenttellina, Ludovidia, Midorigai and Tamanovalva as full genera.

Subclass OPISTHOBRANCHIA Milne-Edwards, 1848

Order SACOGLOSSA Von Ihering, 1876

Suborder TAMANOVALVIDA Kawaguti and Baba, 1959

Family JULIIDAE Dall, 1898

Subfamily BERTHELINIINAE Beets, 1949

Shells lenticular in shape, ovate to quadrate, with a weak hinge; spiral protoconch on one valve retained in the adult; adductor muscle scar central, undivided, circular.

The subfamily Juliinae differs principally in having a thicker, cordate shell, a heavy hinge, a prominent tooth-like ridge in one valve, and a centrally constricted adductor muscle scar.

Genus BERTHELINIA Crosse, 1875

Left valve with spiral protoconch; right valve slightly smaller, without spiral apex; outline of shell somewhat quadrate. Colour of shell greenish to yellowish.

Berthelinia caribbea n.sp.

Diagnosis. Shell ovate-trigonal, sharply narrowed but rounded posteriorly; protoconch large, of 1¼ whorls, inclined at an angle to the horizontal, and situated at the latter one-third of the shell length. Umbos not prominent. Shell green or grey-green, often tinged brownish, with more or less distinct yellow rays. Hinge with well developed posterior teeth. Ligament white. Mantle with irregular horizontal bands of dark reddish-brown or yellowish-brown showing clearly through the transparent shell in life, and persisting

for at least several months in alcohol. A mid-dorsal brown line is present on the neck which bifurcates at the eye turret, one branch running to the inner side of each rhinophore. Oral tentacles reduced to two slightly raised ridges. Pharynx heart-shaped, with a dorsal pouch and two large crops. Radula with 5-6 teeth in the ascending, and 19-26 in the descending series, not counting the pre-radular tooth. Larger teeth with 60-70 fine denticulations on each side, entire at the tip.

Locality. Over 100 individuals were collected between 6 January and 13 March 1962 from the alga Caulerpa verticillata J.Agardh growing on stones in the lighter and more open parts of the mangrove beds near Port Royal, Jamaica.

Types. The holotype, No.1962261 W, and paratypes Nos.1962262-3 W, are in the British Museum (Natural History); paratypes are in collections at the University Museum, Oxford; Academy of Natural Sciences, Philadelphia; Smithsonian Institution, Washington; Marine Laboratory, University of Miami; University of the West Indies; Institute of Jamaica; University of Okayama; and the National Museum of Victoria, Australia.

FURTHER DESCRIPTION AND COMPARISON WITH RELATED SPECIES

Shell. The largest shell is 3.73 mm. long. The height varies from 60 to 73 per cent of the length, with a mean at 65.6 per cent. The protoconch lies at a slight angle to the horizontal, and is situated at two-thirds of the shell length. The distance from the anterior edge of the shell to the inner whorl of the protoconch (Fig.1a)

varies from 63 to 77 per cent of the shell length, with an error in measurement of up to 4 per cent. The mean of 14 young shells (1.36-2.0 mm. shell length) is 67.9 per cent. and of 16 older shells (3.0-3.5 mm.) is 70.6 per cent. Shells of intermediate size have means of 68-69 per cent. 67.9 differs significantly from 70.6 ($P < 0.01$ for 28 degrees of freedom), but not from the means of shells of intermediate size. Thus although the protoconch of B.caribbea is usually situated at approximately two-thirds of the shell length, in older specimens it is closer to three-quarters. Now B.limax, chloris, australis and babai (this is the Edenttellina typica Gatliff and Gabriel of Burn, 1960b, redescribed as Tamanovalva babai by Burn, in press) have the protoconch at the latter one-third of the shell length, whilst B.typica Gatliff and Gabriel has it at the latter one-fourth (Baba, 1961,a). I have been able to measure four shells of B.limax Kawaguti and Baba which give figures of 66, 66, 71 and 76; three shells of B.australis Burn which give figures of 69, 70 and 73; and two shells of B.typica Gatliff and Gabriel which give figures of 81 and 81. Clearly B.caribbea is in the limax-australis group, and distinct from typica; but these results also suggest that the position of the protoconch may not be a good taxonomic character unless a number of individuals of varying ages is available for study.

Considerable variation was noted in shell shape, but no convenient method could be devised to quantify it. In particular, the posterior end sometimes tapers considerably as in B.chloris Dall (Keen and Smith, 1961 Figs. 24, 32), whilst in other shells of the

same size it may be more evenly rounded. The colour of the ligament and the colour of the rays in the shell may prove to be good taxonomic characters. The ligament is white in B.caribbea, dark brown in typica and in babai, ashy-yellow in limax, ashy-white in chloris, and ashy-brown in australis (Baba, 1961,a). Yellow shell rays occur in caribbea (Fig.2,a) and sometimes in limax (Baba, 1961,b) and typica (personal observation); green rays are present in australis and chloris (Burn, 1960,b; Keen and Smith, 1961; Baba, 1961,a). The yellow rays in caribbea lie immediately above dark brown blotches at the edge of the mantle. The hinge teeth are similar to those of B.typica, and are better developed than in limax and chloris. In particular, the posterior cardinal of the right valve is large, and fits into a socket between two teeth of the left valve (Fig.2,c), only one of which is described for limax (Baba, 1961,b). The anterior cardinal of the right valve is very small and inconspicuous. The hinge itself is long and straight, and is marked by the white ligament scar. The periostracum is transparent brownish, and the protoconch either creamy-white or brownish.

External features of soft parts. The colour of the living animal is green or yellowish-green. White dots are scattered over the head and neck, and are particularly abundant on the dorsal margin of the foot, on the rhinophores, and on either side of the mid-dorsal brown stripe. On the mantle there are scattered white dots with clusters at the edge alternating with the radial blackish-brown

blotches. Some of this white persists for a time in alcohol or formalin, and this is indicated in Fig.1a and b. The sides of the neck are suffused with yellow-brown, and the anterior margin of the foot may be tinged with blue. Brown pigment is also scattered over the dorsal and lateral parts of the body, but not in sharply marked areas as it is on the mantle or the dorsal stripe (Plate 1). The longitudinally grooved foot is rounded anteriorly, and occasionally shows a slight notch. The rhinophores are deeply grooved on the outer surface (Fig.1c); oral tentacles are absent, but two curved ridges can be regarded as these organs. In life, the mantle projects slightly from the edges of the valves except posteriorly where there is a small anal siphon. At this point the valves never close completely. Water is carried in by a ciliary current dorsally at the front and passes out of the anal siphon (Fig.1a). The gill hangs from the right mantle, and in one animal which I dissected out has 29 parallel lamellae.

Alimentary canal. The alimentary canal resembles that of B.limax (Baba, 1961,b), but there are important differences in the pharyngeal region (Fig.3). The pharynx is heart shaped with the mouth receded between the two anterior lobes (w). There is a ventral lobe containing the old radular teeth, and a large thick-walled dorsal pouch (x). A pair of voluminous pharyngeal crops (y) open into the pharynx, one on either side, at the base of the dorsal pouch (x); and the long, coiled salivary glands open immediately dorsal to the crops. Radulae from five individuals were examined. Animals

of shell lengths 2.8 and 3.3 mm. both have 25 teeth, and one of 3.5 mm. has 31 teeth. The teeth (Fig.4) are very similar to those described for B.limax, chloris and australis.

Nervous system. The nervous system is similar to that found in B.limax (Baba, 1961,b), but it is more concentrated anteriorly (Fig.3). There is more fusion to the cerebro-pleural-pedal ring, and the supra-and infra-intestinal connectives are relatively shorter than in limax.

Reproductive system and development. The reproductive system, studied by means of serial sections, is similar to that of B.limax (Baba, 1961,b; Kawaguti and Yamasu, 1961), but there are several differences. The hermaphrodite duct (Fig.5,H) from the ovotestis (O) leads into the ampulla (A) which is divided into two parts, both apparently functioning as seminal vesicles. The first part is a tube with lateral pouches, some of which are filled with spermatozoa, whilst those nearer to the ovotestis may remain empty. The second part is a swollen tube. The spermoviduct (S.o) from the ampulla runs across to the right side of the animal before dividing into the vas deferens (V.d) and the small oviduct (O.s). There is no seminal pouch at the base of the vas deferens as occurs in B. limax. The vas deferens passes through the prostate (Pr) before running forward to the penis (P). The small oviduct receives a duct from the albumen gland (A.gl) before it passes into the mucous gland (M). A pouch at the junction of the albumen duct with the small oviduct as occurs in B.limax was not found. The albumen gland

is much larger compared to the mucous gland in B.caribbea than it is in B.limax. The large oviduct (L.o) emerges from the mucous gland and opens into the mantle cavity. Two ducts enter the large oviduct; one connects with the small oviduct and is probably the fertilization canal (F), the other is the vagina (V) which opens into the large spermatheca (S.t) and the somewhat smaller spermatocyst (S.c). Spermatozoa probably travel from the spermatocyst or the spermatheca via the fertilization canal to reach the unfertilized eggs.

The penis of an animal of 2.3 mm. shell length is 1 mm. long, and an individual of only 1.75 mm. was seen to copulate. Yellowish egg masses were frequently laid, usually with 40 to 80 eggs in each mass. The veliger resembles that of B.limax at the time of hatching (Kawaguti, 1959; Kawaguti and Yamasu, 1960,b), but metamorphosis was not observed. The spiral shell is of Thompson's type 1 as in other Sacoglossa (Thompson, 1961).

Ecology. B.caribbea was invariably found on Caulerpa verticillata, usually amongst the bases of the stipes. C.sertularioides (Gmelin) Howe was also present, but no bivalved gastropods were found on it. When feeding, a branch of the delicate alga is sucked into the pharynx and pierced by the radula. The juice from a small area is drained out, and the branch is released. Pale green patches on the alga indicate areas which have been sucked out by these animals. The only other species apparently feeding on C.verticillata was the sacoglossan Stiliger vanellus Marcus. Also of common occurrence on

Caulerpa were the holothurian Synaptula hydriformis Lesueur, the platyctenid ctenophore Vallicula multiformis Rankin, and various species of caprellids. The predators of Berthelinia are not known, but it is likely that the bivalved shell is of defensive importance. It is interesting to note that in addition to possessing a bivalved shell, Berthelinia has a second defensive mechanism in the form of a secretion from the well-developed hypobranchial gland. When disturbed, the animal retracts between its valves, but remains attached to Caulerpa by a transparent byssus-like thread. Violent disturbance, such as when a needle is inserted between the valves, results in extrusion of a viscous white fluid from the hypobranchial gland near the anal siphon, and also occasionally all round the mantle edge.

B.caribbea was found in waters from 3-4 inches to 3-4 feet in depth. Two places in which it occurs in the Kingston area of Jamaica are subject to periods of reduced salinity following heavy tropical rains. Under these conditions, there is often a mass mortality of the invertebrate fauna as described by Goodbody (1961). Once the salinity has returned to normal, recolonization takes only a few weeks. B.caribbea was first found on 8 January 1962, at Goodbody's station A (Goodbody, 1961). In late October and early November 1961 there was a mass mortality in this area following heavy tropical rains. Unfortunately it is not known whether B. caribbea can tolerate such a reduction in salinity, but this is possible as it may have been present but overlooked before 8 January. Alternatively, recolonization could have occurred from veligers of

open water colonies not decimated by the fresh water - B.limax may take as little as 7½ weeks to reach maturity from hatching (Kawaguti and Yamasu, 1960,a).

DISCUSSION

Berthelinia caribbea differs from B.limax in possessing pronounced hinge teeth, a white ligament, brown markings on the head and mantle, a heart-shaped pharyngeal bulb with a dorsal pouch, a more concentrated nervous system, a bipartite ampulla, and no pouch to the vas deferens. B.chloris and babai can be separated because of their less pronounced hinge teeth and lack of brown stripes. B.typica has the protoconch at three-quarters of the shell length instead of at two-thirds, and notched radular teeth without denticulations. B.australis, with the protoconch lying below the level of the umbos, is clearly distinct. Each species is probably associated with one or possibly two species of the alga Caulerpa: thus B.limax lives on C.okamurai (Kawaguti and Baba, 1959), B.chloris lives on C.sertularioides and C.racemosa (Forskall) var. turbinata (Agardh) Eubank, and B.caribbea has only been found so far on C.verticillata. It seems likely that the pharyngeal sucking mechanism has evolved in each species of Berthelinia with respect to one particular species of Caulerpa. Hence we might expect the pharynx to give good taxonomic characters at the specific level.

Mr R.Burn (personal communication) has pointed out to me the resemblance of B.caribbea to Anomalomya corrugata Cossmann and to the Edenttellina typica of Hedley (1920) and Verco (1916), which

differ from typica Gatliff and Gabriel. The chief points of similarity are the straight hinge, the well developed hinge teeth, and the corrugate appearance. However, the shell is not actually corrugate in B.carribea, the corrugate appearance being due to pronounced growth lines, and the hinge teeth closely resemble those of B.typica Gatliff and Gabriel. In any case the differences from limax as regards shell characters are small, not sufficient at the present time, in my opinion, to warrant generic separation.

B.caribbea also has a number of distinctive soft-part characters, for example the coloration, the concentrated nervous system, and the pharyngeal morphology. These may indicate that caribbea deserves generic separation, but they cannot be used as evidence in favour of its being an Anomalomya since this genus is based on a fossil form whose soft parts are unknown. When more species of the family are described, it may indeed be necessary to split the genus Berthelinia, but until then I prefer to regard these characters as of only specific rank, and to follow Keen and Smith who include Anomalomya in the genus Berthelinia.

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SUMMARY

Berthelinia caribbea n.sp. is described from the alga Caulerpa verticillata growing in the mangrove swamps near Port Royal, Jamaica. The most important characters which distinguish it from related species are the position of the protoconch at two-thirds of the shell length; the straight hinge with well developed hinge teeth; the white ligament; yellow shell rays; brown stripes on the head and mantle; the heart-shaped pharyngeal bulb with a dorsal pouch; entire, denticulate radular teeth; the concentrated nervous system; and the ampulla divided into two parts. The resemblance to Anomalomya corrugata Cossmann is discussed, but it is concluded that, for the present, caribbea should remain in the genus Berthelinia. It appears to feed entirely on C.verticillata to which it remains attached by a byssus-like thread even when the valves are closed. When irritated, it secretes a white fluid from the hypobranchial gland, but the deterrent value of this to potential predators is not known. B.caribbea may be able to survive in brackish water, although the evidence for this is inconclusive.

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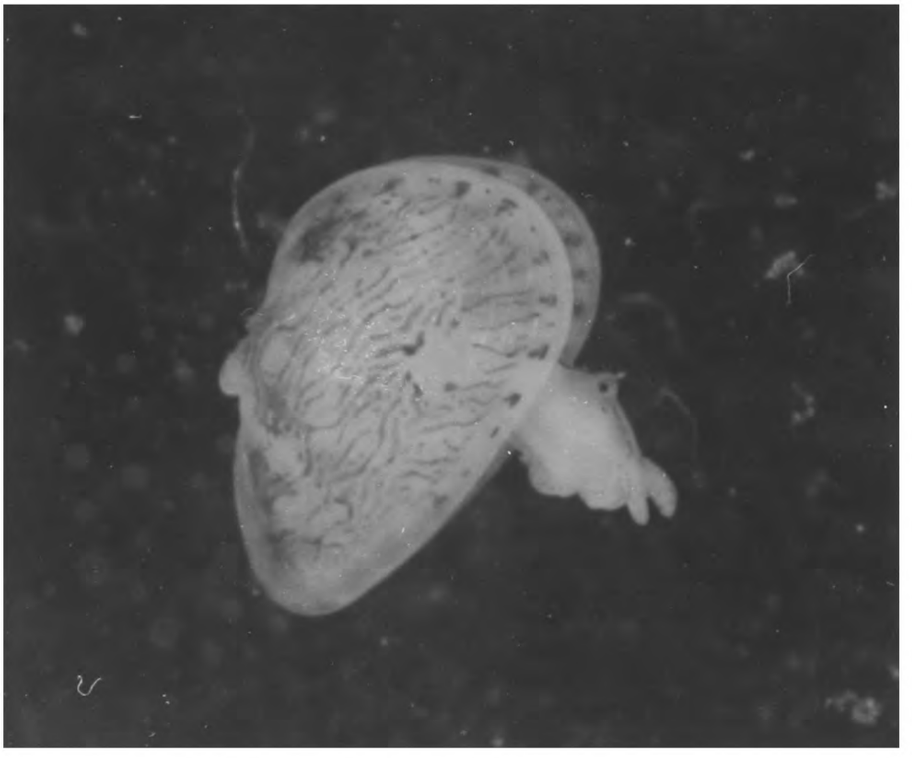
Plate 1. Right (upper) and left side views of two individuals of Berthelinia caribbea, narcotized in Magnesium chloride and alcohol (added drop by drop to sea water), fixed for two hours in neutral formalin, and preserved in 70% alcohol.



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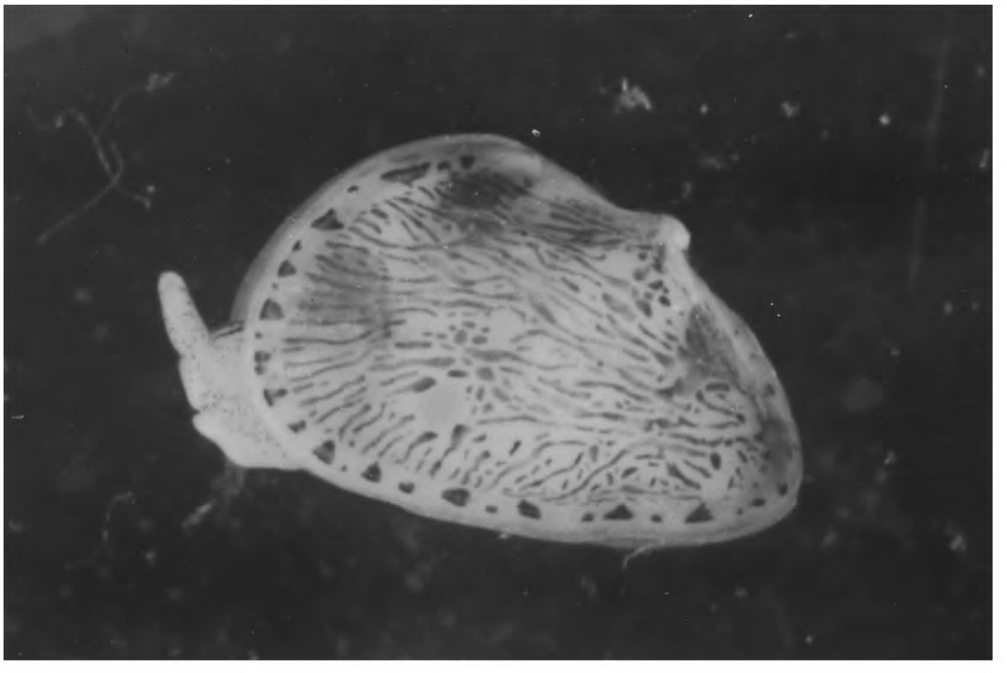


Fig. 1. Berthelinia caribbea n. sp.

a) Dorsal view of living animal. Arrows indicate water currents. A-B is the measurement used from the anterior margin of the shell to the inner whorl of the protoconch.

b) Preserved animal from right side. Mantle edge shown retracted from the margin of the shell. Position of adductor muscle is indicated by a circle.

c) Head of living animal from right side to show grooved rhinophore, and oral ridges (O).

Solid black and stippling indicate areas of brown pigment. Patches in (a) and (b) surrounded by dotted lines represent white areas which persist for a time in neutral formalin or alcohol. The upper 1mm. scale refers to (a) and (b); the lower 0·5mm. scale refers to (c).

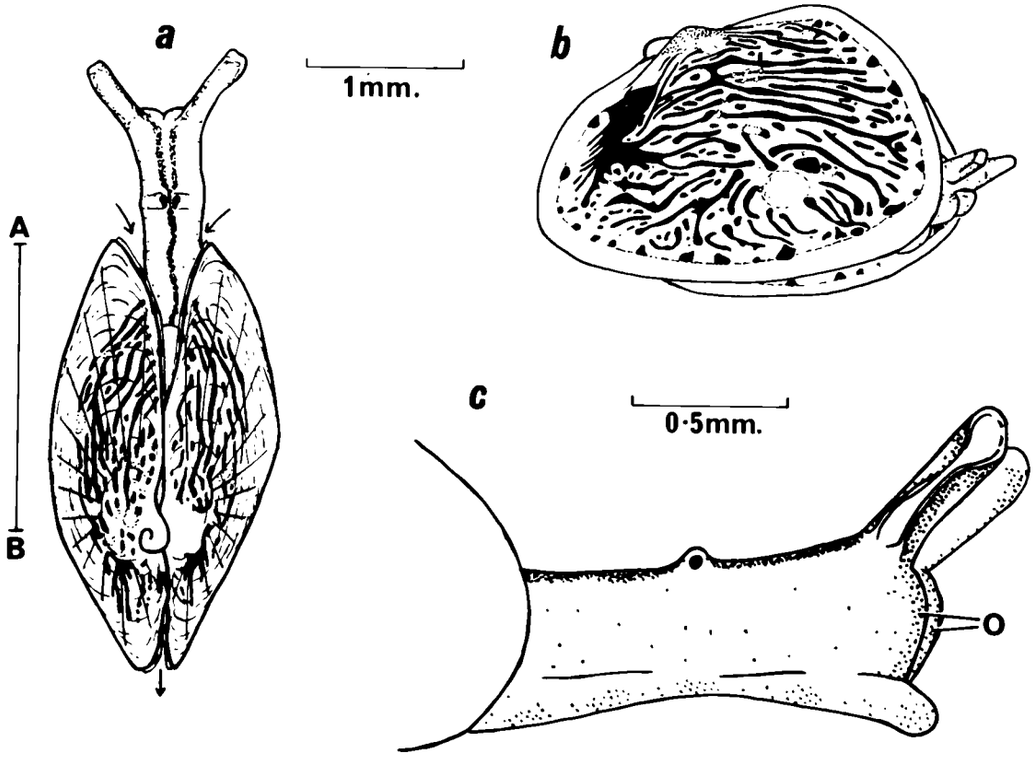

a
1mm.
A
B
b
c
0·5mm.
O

Fig 2. Shells of B. caribbea.

a) Right valve from outside. Yellow rays are dotted.

b) Protoconch of left valve from left side.

c) Details of hinge structure of right (upper) and left valves. A - posterior cardinal of right valve; B - small cardinal of left valve; C - large cardinal of left valve; D - anterior cardinal of right valve. The ligament is dotted.

The 1mm. scale to the left refers to (a); the 0·25 scale to the right refers to (b) and (c).

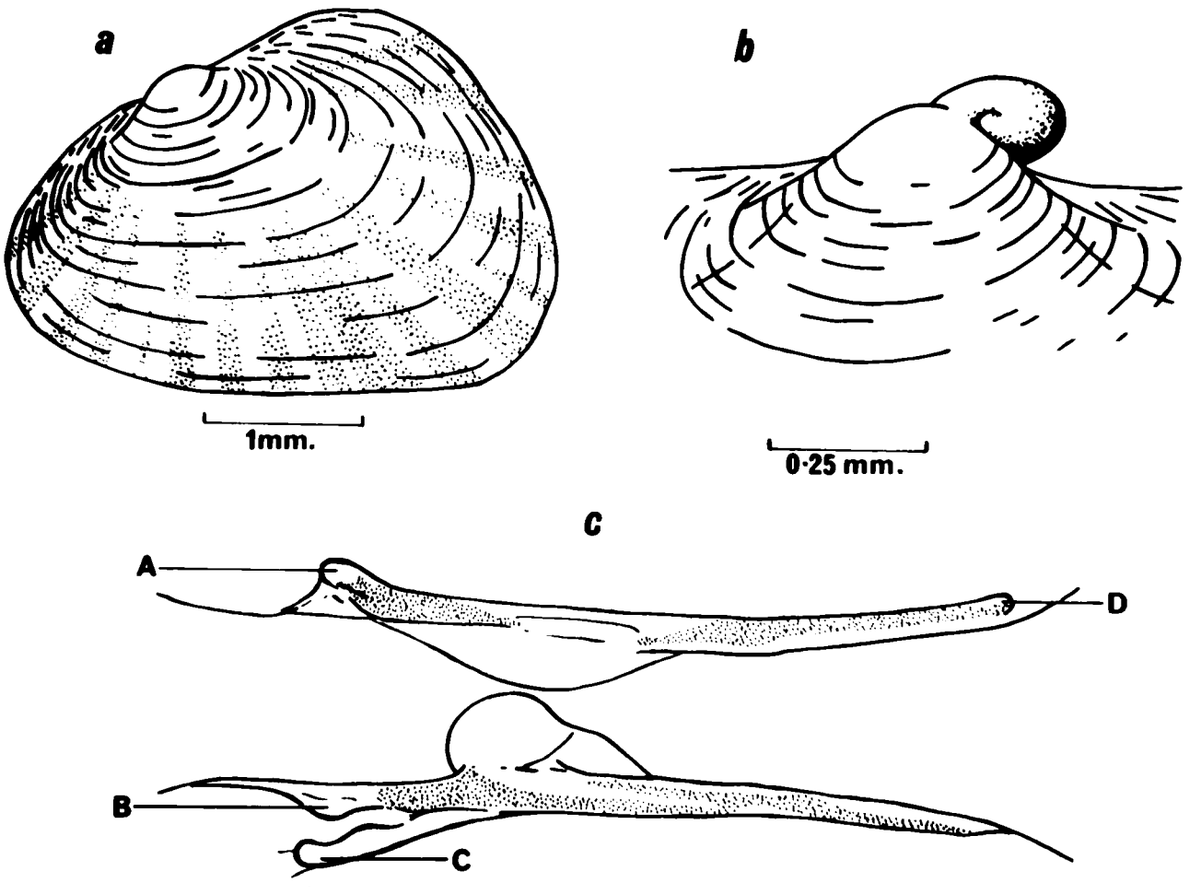

a
1mm.
b
0·25 mm.
c
A
B
C
D

Fig. 3. Pharyngeal bulb and nervous system of *B. caribbea* dissected out. Details of the pedal nerves and of the visceral, osphradial and genital ganglia added from serial sections. The salivary glands have been omitted for clarity.
a) Dorsal view. b) Lateral view. c) Ventral view of nerve ring. b - buccal ganglion; c - cerebro-pleural ganglion; d - oesophageal diverticulum; g - genital ganglion; i - infra-intestinal ganglion; m - mouth; o - osphradial ganglion; p - pedal ganglion; r - radula; s supra-intestinal ganglion; w - lateral lobe of pharynx; x - dorsal pharyngeal pouch; y - pharyngeal crop; z - oesophegus.

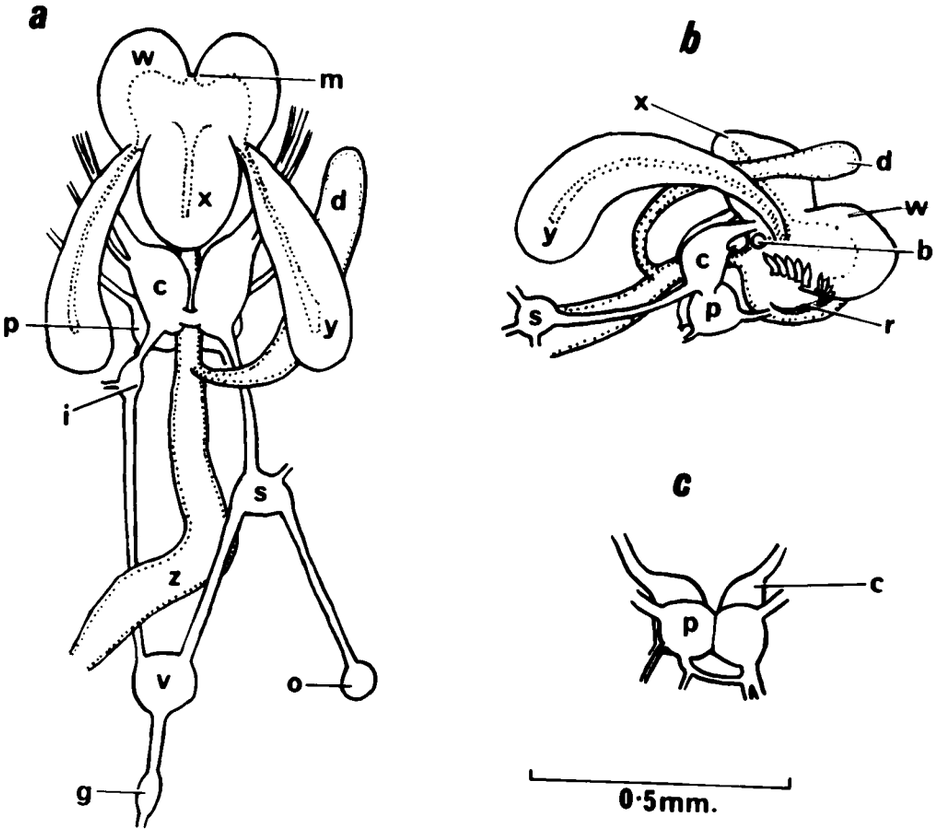

a
w
m
x
d
c
p
y
i
s
z
v
o
g
b
x
d
w
y
b
c
s
p
r
c
c
p
0·5mm.

Fig. 4. Radular teeth of B. caribbea.

a) Pre-radular tooth (P) and first two teeth of a 3·5mm. animal.

b) 31st tooth of the same animal.

c) 22nd tooth of another animal.

The upper 25μ scale refers to (a); the lower 50μ scale refers to (b) and (c).

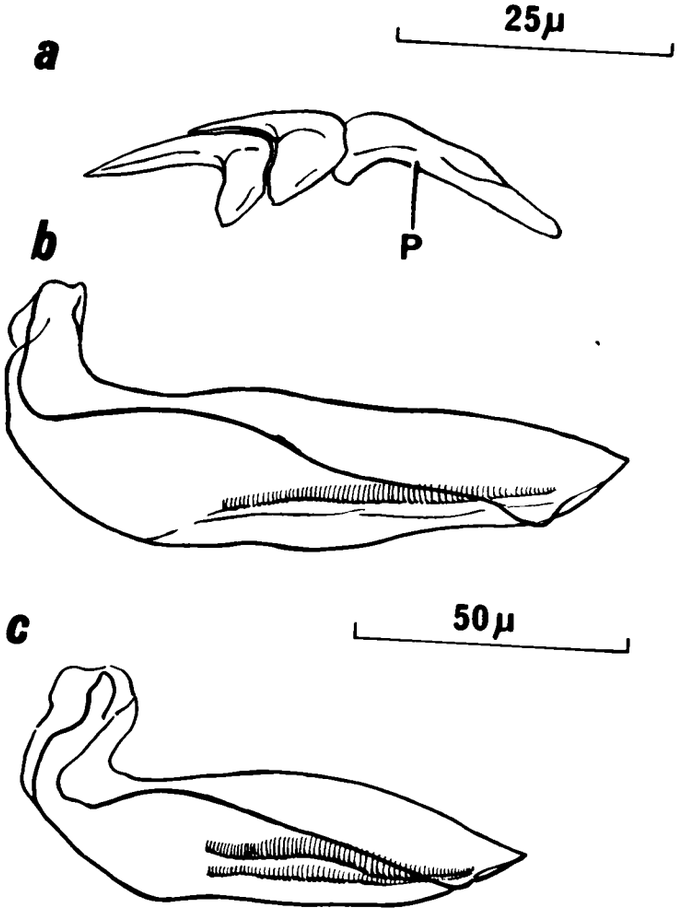
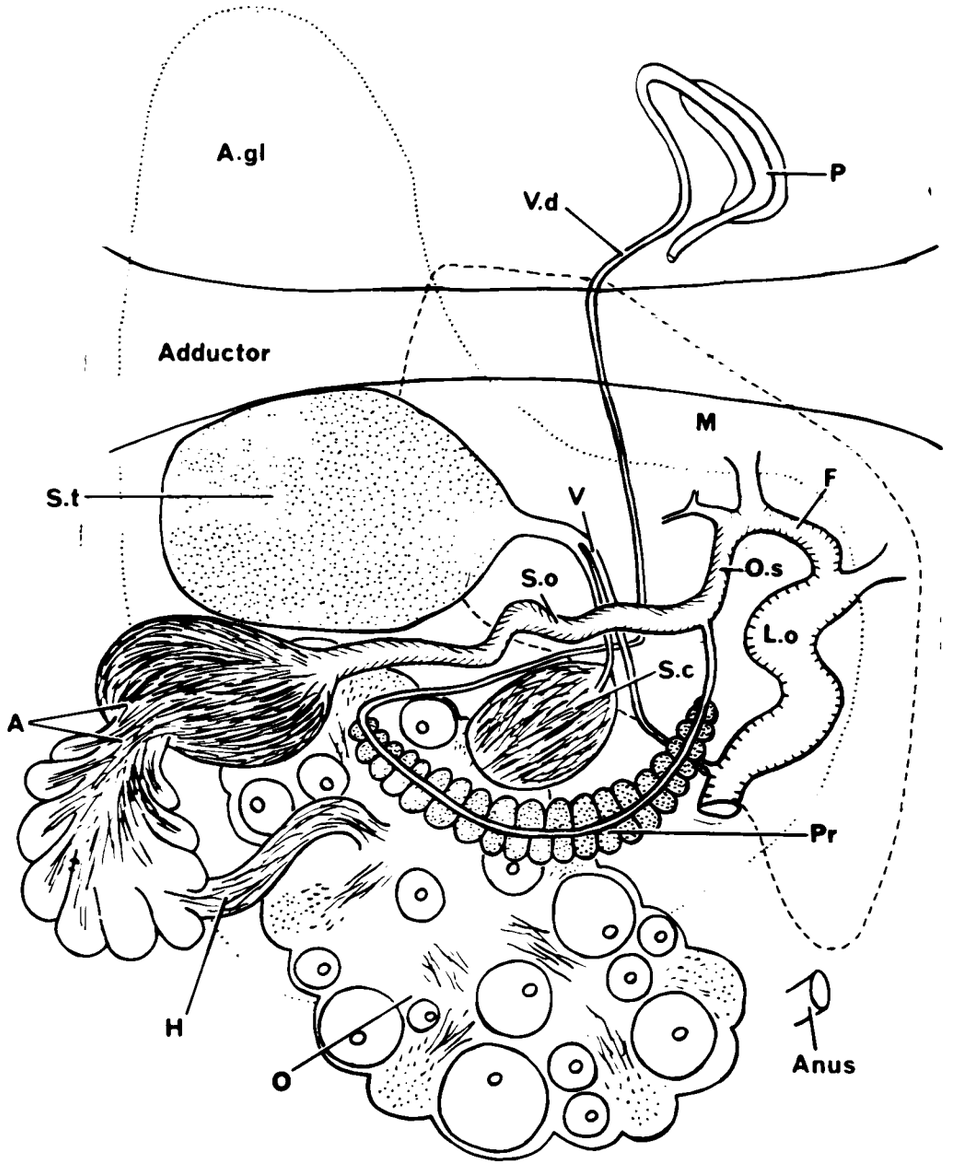

25μ
a
P
b
50μ
c

Fig. 5. Reproductive system of B. caribbea as reconstructed from serial sections and viewed from the dorsal aspect. The ampulla and the large oviduct have been pulled out laterally for clarity, and the adductor muscle and the anus have been drawn in to give an indication of the position of the various organs in the body. A - ampulla; A. gl - albumen gland; F - fertilisation canal; H - hermaphrodite duct; L.o - large oviduct; M - mucous gland; O - ovotestis; O.s - small oviduct; P - penis; Pr - prostate; S.c - spermatocyst; S.o - spermoviduct; S.t - spermatheca; V - vagina; V.d - vas deferens.


A.gl
P
V.d
Adductor
M
S.t
V
F
O.s
S.o
L.o
S.c
A
Pr
H
O
Anus