

Morphology of the adhesive papillae of some ascidian larvae.

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Abstract : This paper presents a comparative study of the morphology of the adhesive papillae in the larvae of four ascidian species : *Clavelina lepadiformis* (Müller), *Polysyncraton lacazei* (Giard), *Diplosoma spongiforme* (Giard) and *Ecteinascidia turbinata* Herdman. The structure of the papillae varies greatly in these species, from a simple glandular epithelium to a complex structure with glandular, myoepithelial and sensory cells. The species studied cover a wide range of the variation in these organs within the group of the ascidians. Their morphology is discussed on a comparative basis, and in relation to previous studies of other species.

Résumé : Ce travail présente une étude comparative des papilles adhésives des larves de quatre espèces d'ascidies : *Clavelina lepadiformis* (Müller), *Polysyncraton lacazei* (Giard), *Diplosoma spongiforme* (Giard) et *Ecteinascidia turbinata* Herdman. La structure de ces organes est très variable selon l'espèce. La forme la plus simple est un épithélium glandulaire ; les formes plus complexes comprennent des éléments glandulaires, méiopi-théiaux et sensoriels. Ces espèces montrent la variabilité de tels organes dans le groupe des ascidies. Leur morphologie est discutée de façon comparative et en relation avec des études antérieures faites sur d'autres espèces.

INTRODUCTION

Ascidian larvae typically possess three adhesive papillae at the anterior end of the trunk. These papillae are glandular attachment organs, although they have frequently been referred to as "suckers". In some species, they form a triangular arrangement, whereas in others they are aligned along the mid-sagittal plane of the larvae. In some species, such as *Euherdmania claviformis* (Trason, 1957) and several species of the genus *Didemnum* (Lafargue, 1976), the papillary complement is reduced to two units. In addition, papillae are lacking in the Molgulidae family (Berrill, 1950) and some species of Pyuridae and Styelidae (Monniot, 1965). These are interstitial forms which are not in need of anteriorly located fixation organs ; instead, the whole larval tunic becomes sticky at the onset of metamorphosis (Grave, 1926).

The morphology of these attachment organs is not well known. A number of descriptions have been made using light microscopy, usually as short comments in research work devoted to the general larval anatomy of a single species (Grave, 1921, 1944 ; Grave & Woodbridge, 1924 ; Grave & Riley, 1935 ; Scott, 1946 ; Berrill, 1948a, 1948 b, 1949 ; Trason, 1957). Fine structure studies are scarce : they consist mainly of the works by Lane (1973), Cloney (1977), Reverberi & Arizzi (1979) and Torrence & Cloney (1983), illustrating the ultrastructure of the papillae in *Diplosoma listerianum* (Milne Edwards), *D. macdonaldi* Herdman, *Clavelina lepadiformis* (Müller) and *Distaplia occidentalis* Bancroft. Cloney (1979) studies the mechanism of papillary eversion in *Distaplia occidentalis*, adding notes on the comparative histology of certain species. Cloney (1978, 1982) and

Cloney & Torrence (1984) provide excellent reports on the events undergone by ascidian larvae at attachment and metamorphosis, and review the available information on adhesive papillae.

From the above sources, it is clear that the morphology of the papillae is very variable, although they all have glandular cells and produce a cementing substance. One possible exception is found in some Styelidae species, whose papillae have been assigned a sensory, rather than a secretory, function (Grave & Riley, 1935). Even this case can be a misinterpretation, since Cloney & Torrence (1984) found that the papillae of *Botryllus* sp. are actually sticky when probed with needles. The glandular papillae fall into two main categories (Cloney, 1978), everting and non-everting, depending on the presence or absence of the capacity for rapid eversion and exposure of the secreted adhesives. The first category includes the papillae of most of the compound species, whereas in the second category are those of solitary species and a few colonial ones. However, striking histological differences can be found within each group, and Cloney (1978) provisionally established eight groups of glandular papillae. Further work is necessary to produce a definite classification and to allow for comparisons. Moreover, data on the fine structure of these organs are desirable in order to resolve small structures of great interest (cilia, microvilli, myofibrills...).

The aim of the present work is to contribute to this issue by describing the ultrastructure of the adhesive papillae in one ascidian species with simple papillae, *Clavelina lepadiformis* (Müller, 1776), and three with complex, cup-shaped papillae with axial bodies: *Polysyncraton lacazei* (Giard, 1872), *Diplosoma spongiforme* (Giard, 1872) and *Ecteinascidia turbinata* Herdman, 1880.

MATERIAL AND METHODS

Larvae of *Clavelina lepadiformis* were obtained by dissection of the atrial cavity of mature zooids. The embryos thus released were placed in Petri dishes until some larvae broke out of their egg-membranes and began to swim actively. Mature colonies of the other three species were collected and kept in bowls of sea water where larvae were spontaneously released from the colonies. Swimming larvae were gently pipetted out and transferred to the fixative liquid.

The fixation was performed in a mixture of glutaraldehyde (2,5 %) and paraformaldehyde (2 %) in 0,1 M cacodylate buffer (pH = 7,2 ; adjusted to 960 milliosmols with sucrose) for two hours, followed by several washes in buffer and postfixation for one hour in osmium tetroxide (1 % in the same buffer). Dehydration was carried out through a graded series of acetone solutions. The specimens were then embedded in Araldite (Durcupan ACM).

Thin sections were obtained with a Reichert Om U2 microtome and stained with uranyl acetate and lead citrate (Reynolds, 1963). Observations were made under a Philips EM 301 electron microscope in the University of Barcelona Microscopy Service.

RESULTS

Clavelina lepadiformis

The papillae of *Clavelina lepadiformis* are the simplest of the species studied. They are three in number, in a triangular arrangement (two dorsal and one ventral). Each papilla is formed by a thick outgrowth of the otherwise simple epithelium of the anterior part of the larva's trunk (Fig. 1). These bulbous outgrowths are about 70 μm in diameter and comprise a single layer of tall, elongated cells (Fig. 2), which rest on the basal lamina. These cells are filled with inclusions; in the lower two thirds of their length, these inclusions are large (1-4 μm) granules with a moderately electron dense and granular content. The upper third of each cell is filled with small (0,5 μm) membrane-bound secretory granules of electron dense material. The nucleus lies in a central position, and the cytoplasm of the cells, especially the lower half, is filled with cisternae of endoplasmic reticulum.

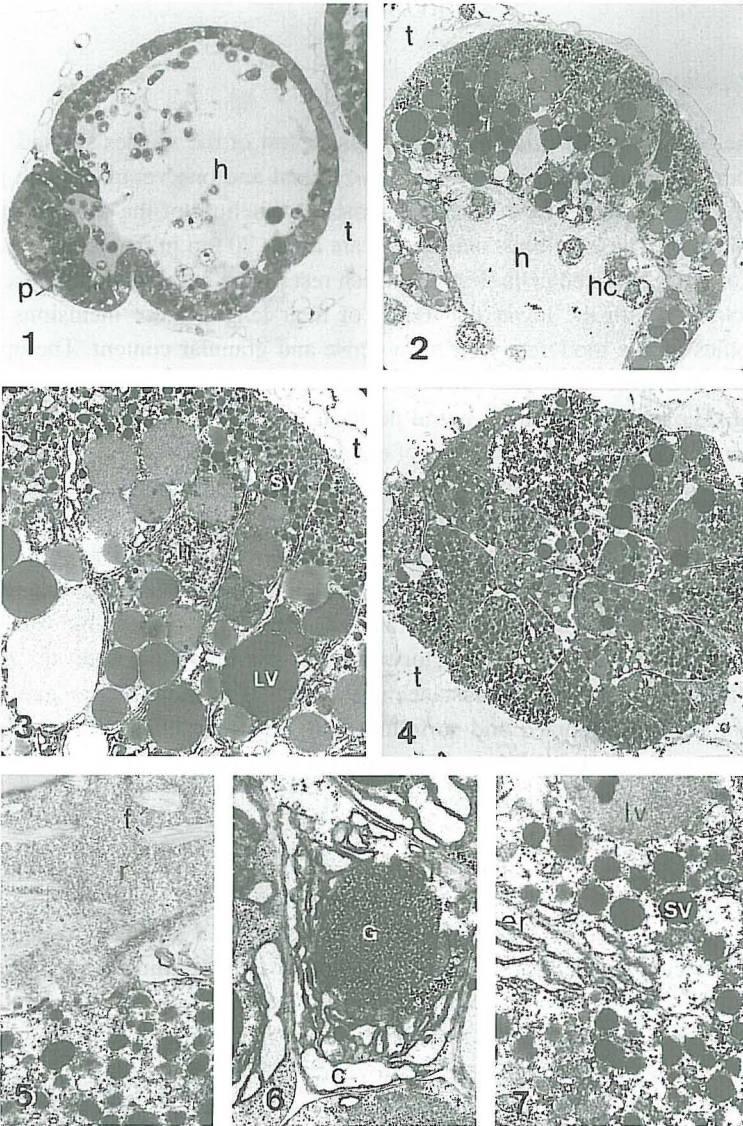
Transverse sections of the papillae (Fig. 4) reveal the somewhat polygonal profiles of 30-35 cells, which is the estimated number of cells in the papilla.

The material contained in the small, dark granules is released into the tunic (Fig. 5), where it forms a mass of reticular or fibrillar substance at the apex of each papilla, which gives the tunic a dark coloration. I assume that this material is responsible for the sticky properties of the papilla. The larger secretory granules presumably contain the precursors used in the synthesis of the cement substance in the smaller ones: the large granules often appear more or less disorganized and surrounded by cisternae in the central part of the cells, while the small, dark granules are being formed from vesicles of endoplasmic reticulum in the neighboring cytoplasm (Fig. 6, 7).

Polysyncraton lacazei

This species bears three complex papillae arranged in the larva's mid-sagittal plane (Fig. 8). They are cup-shaped, with a bulbous central body protruding from the bottom of each cup (Fig. 9). The diameters of the cup and the central body are of about 110 and 60 μm , respectively. A narrow, hollow stalk bears each papilla. The stalk is continuous with the general epidermis, and contains numerous rounded hemocoelic cells (Fig. 9).

The wall of each cup is made up of an infolding of the epithelial layer forming the stalk of the papilla. Thus, the wall is a two-layered formation, with a narrow hemocoelic space separating the two layers (Figs. 10, 11). The epidermal cells forming the marginal layer of the cup are elongated, with a nucleus in the upper part. Occasionally, cilia can be seen arising from some cells in the proximal half of the marginal layer. The inner layer (parietal layer) of the wall is formed by typical secretory cells (Fig. 9). Their cytoplasm is dark, filled with rough endoplasmic reticulum, and large vesicles containing inclusions of granular material are produced and released into the tunic (Figs. 11, 12). All the cells rest on a basal lamina. Occasionally, free blood cells are found in the hemocoelic space between the layers of the cup (Fig. 10), which is broader at the basal part, narrowing and finally collaps-



Figs. 1-7 : *Clavelina lepadiformis* (Müller).

Fig. 1 : Frontal section through the anterior ampulla of the larva. h: hemocoel ; p: papilla ; t: tunic. x 350. Fig. 2 : Longitudinal section through one papilla. h: hemocoel ; hc: hemocoelic cells ; t: tunic. x 1200. Fig. 3 : Papillar cells showing the different types of secretory granules. lv: large vesicles ; n: nucleus ; sv: small vesicles ; t: tunic. x 3900. Fig. 4 : Transverse section of an adhesive papilla. t: tunic. x 2400. Fig. 5 : An image of the material in the small vesicles being released into the tunic, forming zones of reticular (r) and fibrillar (f) appearance. x 11900. Fig. 6 : Central region of one papillar cell, showing a large inclusion of granular content (g), surrounded by cysternae (c) of endoplasmic reticulum. x 11900. Fig. 7 : Central region of another papillar cell ; large (lv) and small (sv) secretory vesicles, together with rough endoplasmic reticulum (er) are visible. Note the different stages of formation of the small vesicles. x 11900.

sing towards the apex of the wall. Papillary nerves can be seen extending from the stalk and entering the wall between the cellular layers (Fig. 9).

The central body is formed by a group of some forty axial cells and a parietal layer of about thirty secretory cells, much like those in the papillary wall (Fig. 13, 14). The granules secreted are released into the tunic, where they become disorganized into fibres. The granules coming either from the wall or the central body appear to "feed" a continuous layer of fibrillar material (Fig. 12) extending along the sulcus between the wall and the central body and over the latter (Fig. 9), just below a fenestration in the cuticle of the tunic (Fig. 8).

The core of the central mass is occupied by slender axial cells. Its cytoplasm is filled with rough endoplasmic reticulum and small vesicles. At the anterior end of the cells some short, broad processes can be seen entering the tunic (Fig. 13).

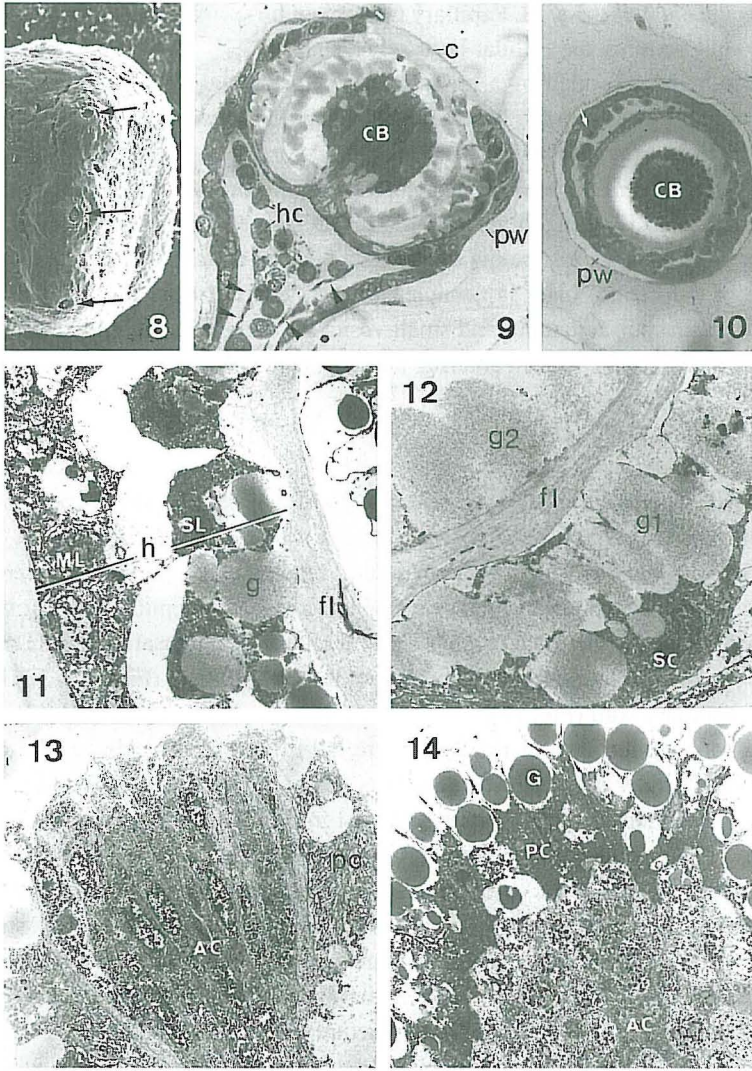
Diplosoma spongiforme

The three papillae are aligned in the sagittal plane, and their general appearance is similar to that of the previous species : cup-shaped organs (80 μm in diameter) each with a bulbous central body (Fig. 15). However, several differences can be readily observed. To begin with, the wall of the cup is not formed by a true infolding of the epithelium, but by a ring of elongated overlapping cells, each originating at the underlying basal lamina. The secretory cells in this species are found at the periphery of the central body (Figs. 15, 16) but are not found in the papillary wall (Fig. 17).

The cells forming the wall of the papilla are tall myoepithelial cells, each reaching the basal lamina by slender processes. The nucleus lies on the enlarged upper part of the cells and arrays of myofilaments are visible, scattered in the cell body, but better organized in parallel lines in the tapered basal processes (Fig. 17).

Near the apex of the wall, there is a pair of cells in most sections hereafter called "anchor cells" (following the name given by Cloney, 1978, to very similar structures in *Distaplia occidentalis*). They are darker than the surrounding epithelial cells ; they are pear-shaped (Figs. 18, 19), with a process at the anterior end that protrudes into the tunic, bearing a simple cilium (of 9 + 2 axonematic structure) and microvilli that penetrate the tunic (note that the cuticle of the tunic is very close to this part of the wall). Each anchor cell possesses a tapered process or axon (see discussion) at the basal end. Axons from different anchor cells have been traced to the base of the papillary wall, forming a bundle that intermingles with the processes of the myoepithelial cells and enters the hemocoelic space of the stalk, forming the papillary nerve. The cytoplasm of each anchor cell is filled with small vesicles, mitochondria, glycogen granules, smooth reticulum and microtubules (longitudinally arranged in the axonal processes) (Figs. 18, 19).

The solid central body (50 μm in diameter) of each papilla is constituted by two cellular types : the axial and the secretory cells (Figs. 15, 16). Transverse sections of papillae consistently show 9-11 and 18-20 cells of each category, respectively. The large secretory cells are located at the periphery of the central body ; their cytoplasm is dark, with plenty of



Figs. 8-14 : *Polysyncraton lacazei* (Giard).

Fig. 8 : Scanning electron microscope image of the anterior end of a larva. The three papillae are visible, each with one cuticular fenestration (arrows). x 85. Fig. 9 : Longitudinal section of a papilla. Arrowheads point to the papillar nerves. c : cuticle ; cb : central body ; hc : hemocoelic cells ; pw : papillar wall. x 480. Fig. 10 : Transverse section of a papilla. Arrow marks free hemocoelic cells between the two layers of the papillar wall. cb : central body ; pw : papillar wall. x 300. Fig. 11 : Papillar wall, transverse section. The marginal (outer) (ml) and secretory (inner) (sl) layers are separated by hemocoelic space (h). fl : fibrillar layer of the tunic ; g : granule of secretion. x 3270. Fig. 12 : Papillar wall, longitudinal section. The granules (gl) formed by the secretory cells of the wall (sc) and those (g2) secreted by the central body converge to form the fibrillar layer (fl) of the tunic inside the papillar cup. x 3000. Fig. 13 : Longitudinal section of the central body, illustrating the axial cells (ac) and the peripheral, secretory cells (pc). x 1530. Fig. 14 : Central body, transverse section. ac : axial cells ; g : granule of secretion ; pc : peripheral cells. x 3100.

rough endoplasmic reticulum, cisternae with granular material and large vesicles (up to 4-5 μm in diameter) with a dark inclusion surrounded by granular material (when they are fully developed) (Fig. 16). The contents of these vesicles are released into the tunic, where the electron dense inclusions retain their individuality and move upwards forming a dome over the central body and under the cuticle of the tunic (Fig. 15), which appears broken in this zone, forming a hole or cuticular fenestration.

The core of the central body is occupied by axial columnar cells. Their nuclei are located in the upper third of the cells; below the nuclei, the cytoplasm is filled with bundles of microfilaments (Fig. 20), whereas the cytoplasm above the nuclei lacks these filaments and features developed Golgi complexes, rough endoplasmic reticulum and small vesicles (Fig. 21). The anterior end of these cells form apical processes that are much longer and narrower than in the previous species. They protrude into the tunic layer below the dome of granules (Fig. 15).

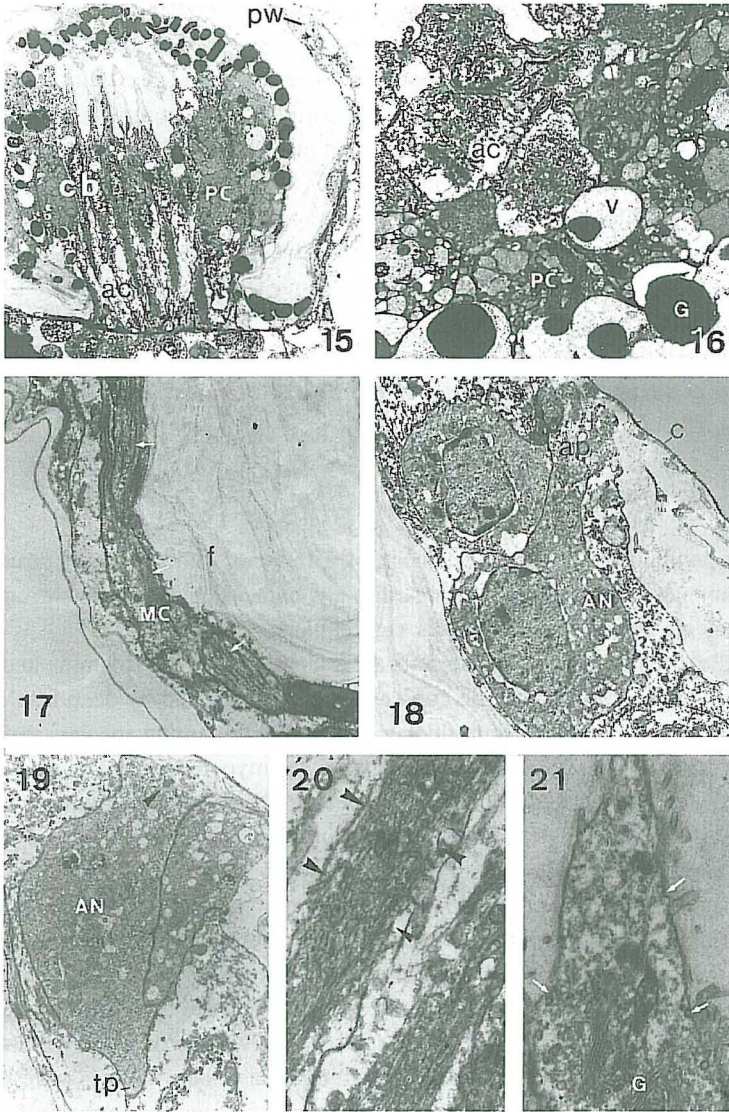
Ecteinascidia turbinata

The three papillae of this species consist of a flattened saucer-shaped structure (about 120 μm in diameter) with a prominent central body, higher than the cup's surrounding walls (Fig. 22). They are arranged in the larva's sagittal plane.

As in all the species studied, all the cells are attached to the basal lamina and the epithelium remains simple throughout. The cup's walls are not formed by deep infoldings of the epithelium. However, these walls are thicker than in the remaining species, and are formed by two types of cells (Fig. 23): the external ones are myoepithelial, and the internal ones are secretory. The outer surfaces of the cells contacting the tunic have numerous microvilli, and sometimes cilia (of 9 + 0 axonemal structure) are seen arising from deep invaginations of the plasmalemma of some of the outer layer's cells (Fig. 24). The myoepithelial cells are tall and columnar, forming three-four overlapping layers in the external part of the wall. Their nuclei are located in the lower half of the cells, and a tapering process from each cell reaches the basal lamina near the central body. Myofilaments are scarce and loosely arranged, except in the slender basal processes, where they are organized in parallel arrays (Fig. 25). The cells in the rim of the cup send extensive microvillous formations to the tunic, which reach the external cuticle. Although no cilia or basal bodies have been seen in these cells, they are similar in position and perhaps also in function to the anchor cells described above.

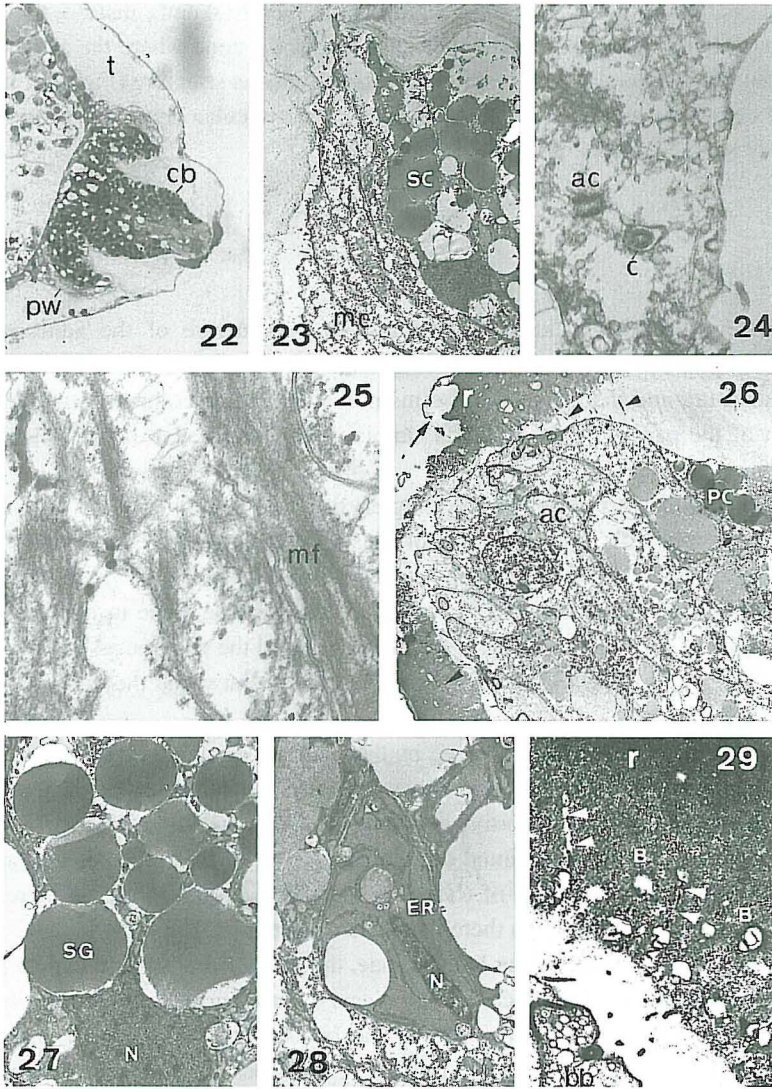
The inner side of the wall, the bottom of the papillae and the periphery of the central body are occupied by large secretory cells (Figs. 22, 23, 26). They are electron dense, with the cytoplasm filled with rough endoplasmic reticulum and secretory granules of varying size (Figs. 27, 28). The mature granules contain inclusions of electron-dense material which are released into the tunic, where they form a reticular mass of dark appearance in front of the central body (Figs. 26, 29).

The core of the central body is again occupied by axial columnar cells (Fig. 26). Their nuclei are located in the distal third of the cells, and the cytoplasm is rich in reticulum and



Figs. 15-21 : *Diplosoma spongiforme* (Giard).

Fig. 15 : Longitudinal section through one papilla. ac : axial cells ; cb : central body ; pc : peripheral cells ; pw : papillar wall. x 1320. Fig. 16 : Transverse section through the central body of the papilla. ac : axial cells ; g : granule of secretion ; pc : peripheral cells ; v : vesicle. x 6000. Fig. 17 : Longitudinal section of the papillar wall, showing elongated myoepithelial cells (mc) with arrays of myofilaments (arrows). f : fibrillar contents of the tunic inside the papilla. x 6260. Fig. 18 : Paired anchor cells (an) near the rim of the papillar wall. In one of them is visible an anterior process (ap) penetrating the tunic near the cuticular layer (c). x 6520. Fig. 19 : Another pair of anchor cells (an), showing the beginning of the posterior, tapered process (tp). Arrowhead points to the basal body of a cilium. x 9000. Fig. 20 : Longitudinal section of the lower region of the axial cells, with bundles of microfilaments (arrowheads). x 11900. Fig. 21 : Upper end of the axial cells. The developed Golgi bodies (g) produce small secretory granules that contact the plasmalemma, releasing their contents to the tunic (arrows). x 14680.



Figs. 22-29 : *Ecteinascidia turbinata* Herdman.

Fig. 22 : Longitudinal section of a papilla. cb : central body ; pw : papillar wall ; t : tunic. x 300. Fig. 23 : Papillar wall, longitudinal section showing its two components : myoepithelial (mc) and secretory (sc) cells. x 2000. Fig. 24 : Enlarged view of a cell in the outer layer of the wall with a cilium (c) arising from a invagination of the plasmalemma. ac : accessory centriole. x 1650. Fig. 25 : Basal processes of the myoepithelial cells, showing arrays of myofilaments (mf). x 20000. Fig. 26 : Transverse section of the distal part of the central body. Axial (ac) and peripheral cells (pc) are visible, as well as the cuticular fenestration (arrow) and the reticular zone of the tunic (r). Arrowheads point to microvilli emerging from the axial cells and entering the reticular zone. x 2500. Fig. 27 : Secretory cell of the central body. n : nucleus ; sg : secretion granule. x 6000. Fig. 28 : Secretory cell of the papillar wall. er : endoplasmic reticulum ; n : nucleus. x 6000. Fig. 29 : Reticular zone of the tunic (r) in front of the central body. Arrowheads mark microvilli coming from the axial cells and ending by a bulbous structure (b). bb : basal body in an axial cell. x 11660.

granular material. They do not produce digitations at the distal end ; instead, the apical surfaces produce long microvilli which enter the tunic in the zone where the accumulation of reticular material occurs. Each microvillus ends in a bulbous structure (Fig. 29). In addition, each axial cell bears a basal body near its apex. The cuticular layer of the tunic over the central body is fenestrated (Fig. 26).

DISCUSSION

The above descriptions illustrate the variation in structure of the adhesive papillae among three aplousobranch and one phlebobranch species. Fig. 30 schematically summarizes the structure and disposition of the main elements. One common feature is that the epithelium of the papillae is in all cases simple, never stratified. All the cells rest on the basal lamina.

Clavelina lepadiformis possesses the simplest type : a thickening of the epidermal tissue formed by secretory cells. No contractile or sensory elements have been found. Nor have I observed the banded bodies pointed to by Reverberi & Arizzi (1979). In that work, the authors failed to appreciate the exact structure of the papillae : due to observations made only on transverse sections at several levels, they reported the papillae as being a multilayered organ. The longitudinal sections studied here clearly show that they consist of a single layer of columnar cells.

The other three types are characterized by their complex structure, which involves a surrounding wall and a solid central body. However, there is much variation with regard to the disposition of secretory and supportive elements, as well as in the structure of the cup wall and the axial cells. It must be pointed out that the larvae of these species are larger (0,8 - 1 mm of trunk length) than those of *Clavelina lepadiformis* (0,3 mm). An increased complexity of the attachment organs is therefore to be expected.

Even among a single family, the Didemnidae, the wall of the papillae can be formed by overlapping elongated epidermal cells (*Diplosoma spongiforme*) or by a deep infolding of the epithelium forming an inner (secretory) and outer layers separated by an hemocoelic space (as in *Polysyncraton lacazei*). The first type agrees with previous descriptions of *Diplosoma macdonaldi* (Torrence & Cloney, 1983) ; the second type closely resembles the structure of the papillae of one Polycitorid species, *Distaplia occidentalis*, as reported by Cloney (1977).

The secretory cells in each of the three species with complex papillae are much alike, although the disposition of the secretory elements is variable (Fig. 30). We have not found, as did Cloney (1977) for *Distaplia occidentalis*, more than one type of secretory cell in each species (except for a possible secretory role of the axial cells of *D. spongiforme*, see below). It is assumed that the secretions of these cells form the adhesive substance that is exposed directly through the cuticular fenestration to the substratum at the onset of the attachment processes.

- SECRETORY ZONES
- ▨ CONTRACTILE ZONES
- NERVOUS AND SENSORY? ELEMENTS

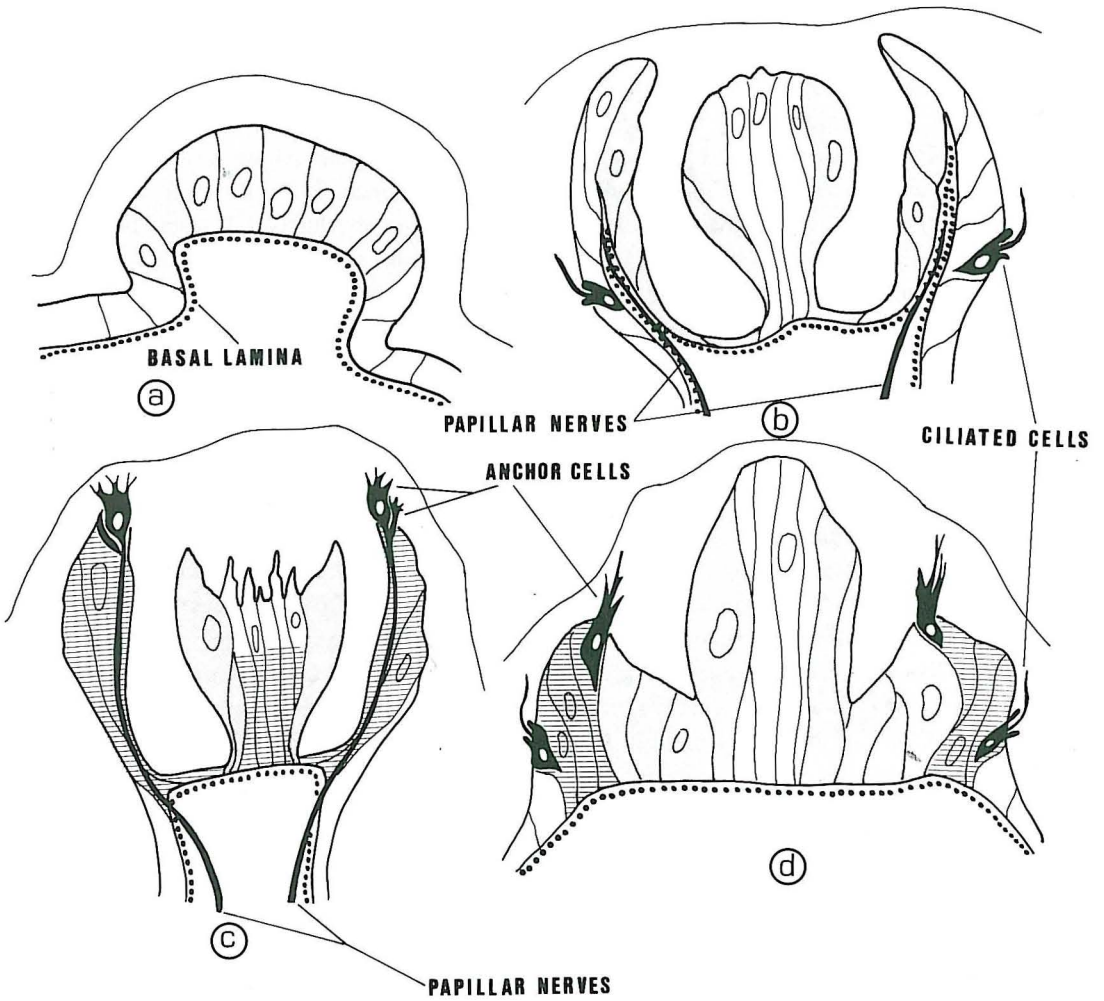


Fig. 30 : Schematic sagittal sections of the adhesive papillae of the species studied (approximately at the same scale) ; a : *Clavelina lepadiformis*, b : *Polysyncraton lacazei*, c : *Diplosoma spongiforme* d : *Ecteinascidia turbinata*.

Although no studies or observations on papillary eversion have been made in this work, it is assumed that papillae with a complex structure, such as those of *Polysyncraton lacazei*, *Diplosoma spongiforme* and *Ecteinascidia turbinata* are capable of eversion in order to rapidly expose the adhesive substance to the substratum (Cloney, 1978, 1982). Contraction of myoepithelial cells and/or hydrostatic pressure transmitted through the stalk have been claimed as the possible driving forces provoking papillary eversion. We have found evidence for organized cytoplasmic filaments in the cells of the papillae of *Diplosoma spongiforme* and *Ecteinascidia turbinata* and we have thus given these cells the name of myoepithelial, assuming that they have contractile properties, a fact demonstrated in other species, like *Distaplia occidentalis* (Cloney, 1979). However, myofilaments have not been clearly demonstrated in *Polysyncraton lacazei*; perhaps they are scarce and disorganized and only appear visibly at the time of the contraction (as found, for instance, in the epidermis of the tail of some ascidian species, Cloney, 1966).

Another interesting point is the relationship between the papillae and the nervous system. The anterior location of the papillae seems the appropriate place for sensory elements; besides, eversion is known (Cloney, 1982; Cloney & Torrence, 1984) to occur simultaneously, which indicates the presence of some synchronization mechanism. It would be reasonable to expect to find in the papillae the sensory elements capable of recognition of the stimuli (mechanical, chemical) from the substratum which trigger the attachment reactions.

Papillary nerves do exist in *Diplosoma spongiforme* and *Polysyncraton lacazei* (in *Ecteinascidia turbinata* they have not been found, perhaps due to unfavourable sectioning), and some of the structures described may be sensory. In particular, the anchor cells found here in *Diplosoma spongiforme* are primary sensory neurons (Torrence & Cloney, 1983). Their basal processes are actually axons that join together to form the papillary nerve. We have retained the original name given by Cloney (1977) to these cells with regard to another possible role: the mechanical anchoring of the papillar wall to the tunic (due to their microvilli complement reaching the cuticle of the tunic, these cells are able to remain in place during the eversion). In the similarly located cells in *Ecteinascidia turbinata*, no basal processes or cilia have been encountered; their role may be strictly supportive.

The cells in the core of the axial body, especially in the case of *Ecteinascidia turbinata* (see below) may also have a sensitive function. Occasionally, some cilia have been seen in the outer surface of the papillar wall in *Polysyncraton lacazei* and *Ecteinascidia turbinata* (Fig. 24). These cilia arise from deep invaginations of the plasmalemma and resemble the basal cells described by Torrence & Cloney (1983) as primary sensory neurons. However, the connections between these cells and the papillar nerves have not been observed in any of the sections examined. Specific studies must be devoted to ascertaining the sensory capabilities of the adhesive papillae. It is noteworthy that in some genera (*Botryllus*, *Dendrodoa*, *Symplegma*), these organs are ganglionated and in some cases, as in *Botryllus*, they have been claimed to be sensory, rather than glandular, organs (Grave & Riley, 1935; Berrill, 1949).

The interpretation of the axial cells is problematic (Cloney, 1977). They differ in structure in the species studied here. Their role may be strictly supportive, as proposed by

Torrence & Cloney (1983) who reported similar cells in *Distaplia occidentalis*. However, the abundance of microfilaments regularly arranged in these cells in *Diplosoma spongiforme* (Fig. 20) strongly suggests a contractile function in this species. Bundles of microtubules have been reported in the axial cells of *Distaplia occidentalis* (Cloney, 1977). They may function in the papillary retraction observed after papillary eversion in ascidian settlement. This retraction is inhibited by cytochalasin B (Cloney, 1979). A sensory function for the axial cells is also possible (Cloney, 1977) : in all the species studied these cells bear microvilli or digitiform processes which extend towards the apical zone of the papillae ; in *Ecteinascidia turbinata*, together with long microvilli with bulbous terminations (Fig. 29), these cells also bear a single basal body, although cilia have not been observed in the sections studied. However, the lack of evidence of innervation of these cells prevents us from assigning them definite sensory capacities. In addition, a secretory function cannot be ruled

TABLE 1

Characteristics of the papillae in the species studied and in others from the literature. Question marks indicate that there is a reasonable doubt concerning these points due to the incompleteness of the evidence available.

	Type (*)	Cup wall double-layered	Myoepithelial cells in cup wall	Microfilaments in axial cells	Innervation	Anchor cells	Ciliated cells in cup wall
Fam. Polycitoridae							
<i>Clavelina lepadiformis</i>	s	-	-	-	-	-	-
<i>Distaplia occidentalis</i> (1)	c	+	+	+	+	+	-
Fam. Didemnidae							
<i>Polysyncraton lacazei</i>	c	+	-	-	+	+	+
<i>Diplosoma spongiforme</i>	c	-	+	+	+	+	-
<i>Diplosoma macdonaldi</i> (2)	c	-	+	+	+	+	+
<i>Diplosoma listerianum</i> (3)	c	-	?	?	?	?	?
Fam. Perophoridae							
<i>Ecteinascidia turbinata</i>	c	-	+	-	?	+	+

(1) from Cloney (1978)

(2) from Torrence & Cloney (1983)

(3) from Lane (1973)

(*) s : simple coniform papilla ; c : cup-shaped papilla with axial body.

out, since small vesicles appear in *Diplosoma spongiforme* in contact with the apical surfaces in some cases (Fig. 21), and release some type of hyaline material into the tunic.

As seen above, many questions arise from a morphological study of the adhesive papillae of the ascidians, even from a limited sample. Table I summarizes some of the features observed in the species studied, together with data from the other three species of ascidians whose ultrastructure is well documented in the literature. Notable differences are evident at the family level, and many points remain unclear. Further comparative studies are necessary to uncover the variability in structure and function of these organs, thus enabling us to make a complete classification of the papillae of this group.

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