AN INVESTIGATION OF *PYURA STOLONIFERA* (TUNICATA) FROM THE CAPE PENINSULA

R. W. DAY

Zoology Department, University of Cape Town *

ABSTRACT

The paper presents a detailed anatomical description of the ascidian *Pyura stolonifera*, a dominant zone form on South African coasts which is ideal for student dissections. Notes on the ecology of the animal and new observations on the function of the branchial folds in feeding are included, as these lend significance to the structural specialisations of the animal and the pattern of evolution within the tunicates.

INTRODUCTION

Pyura stolonifera (Heller 1878) is a large simple ascidian which is distributed over the whole South African coastline and is a dominant zoning form at the sublittoral fringe on the rocky shores of the south and east coasts. Its large size and abundance have led to its use as bait by fishermen, and also render it an ideal animal for student dissection and study, yet no detailed account of its anatomy has been available.

The species was first described from 'The Cape of Good Hope' (the Cape Peninsula) by Heller in 1878, as *Cynthia stolonifera*. His description deals largely with the external appearance, and is apparently based on a single rather juvenile specimen, 30 mm in diameter, with club-shaped extensions of the test called tubercles. The tubercles, and other features described by Heller, are seldom found in the much larger adults. The anatomical changes which occur during growth have led to the description of a number of synonyms, recognised as such by Michaelsen (1923).

The Australian 'cunjevoi', described by Heller (1878) as *Cynthia praeputialis* may or may not be the same species as the South African *P. stolonifera*. Michaelsen (1923, 1927, 1928) considered them to be conspecific, whereas Millar (1962, 1963) suggests that they are distinct species. Millar points out that the horseshoe-shape of the neural canal opening has its blind side towards the branchial siphon in *P. stolonifera* from South Africa (Figure 2), whereas the ends of the horseshoe face the siphon in Australian specimens. Most Australian specimens also have very small siphons, even when extended, so that the dorsal area looks flattened or sunken, but this is a variable character. They are obviously very closely related, and work on the Australian animal has been referred to here, using the name *Pyura praeputialis* for clarity. A comparison of the Australian and South African animals is planned.

For this study, fresh specimens ranging in size from 25 to 110 mm maximum diameter were collected from intertidal and sublittoral rocks in False Bay, Table Bay, and the west coast of the Cape Peninsula; and fixed in 5% formalin before dissection. In addition preserved material

* Present address: School of Biological Sciences, University of Sydney, Australia.

from the collections of the University of Cape Town, including specimens identified by Michael-
sen, were examined. Two specimens were dissected alive in seawater to trace mucus movements
in the pharynx.

<div align="center">THE TEST AND BODY WALL</div>

External Appearance

Large specimens growing tightly packed together tend to be pear-shaped with a cylindrical base
widening out around the internal body. Solitary specimens from below tide marks are usually
hemisperical (Figure 1) but littoral specimens are more stunted and more variable. Specimens
have been recorded from South African estuaries which have rootlike extensions of the test to
anchor them in the sand (MacNae & Kalk 1958), but their identification as *P. stolonifera* needs
confirmation (J. H. Day, pers. comm.). Such forms are well documented (Kott 1952) for *P.
praeputialis* in Australia.

The only juveniles seen by the author were found fixed to the sides of adults in colonies,
and growing in the sand-filled cracks between the larger individuals. These juveniles are tuber-
culate, typically with a ventral root extending down between their large neighbours. On the dorsal
surface, and especially on the siphons, the tubercules are prominent and effectively camouflage
the siphons.

In adults the tubercles are usually absent, and the siphons are prominent and long when the
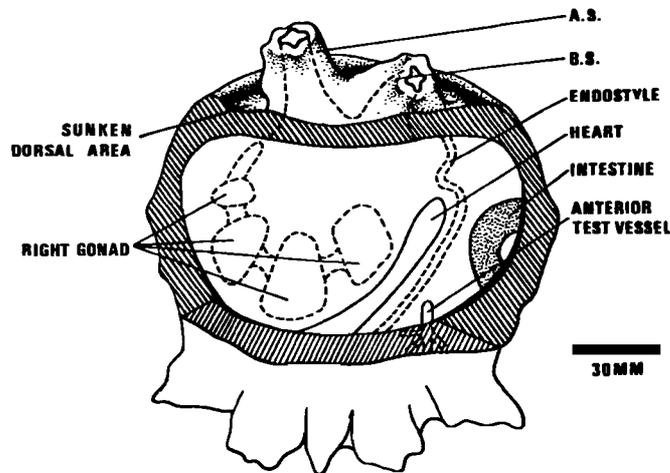animal is feeding. However when the siphons retract (as they do during fixation) the dorsal area

FIGURE 1

WHOLE ANIMAL

The right side and anterior part of the test have been cut away to show the position of the interior organs through
the body wall.

B.S.: Branchial siphon (anterior).

A.S.: Atrial siphon (posterior).

becomes sunken, and the siphons are then often camouflaged by epiphitic algae. A very constant feature is that the atrial siphon projects directly upwards, whereas the branchial siphon is always bent so as to open anteriorly.

The Structure of the Test

The test of *P. stolonifera* has an outer surface or cuticle (terminology of Saint-Hilaire 1931) which is packed with sand grains and bits of shell, especially in juveniles. It appears to protect the animal from predation by fish, and also crabs (Endean 1955b). The cuticle is coloured blue-black or brown; and in littoral specimens the dorsal area is darker. The interior of the test is translucent and gelatinous with a network of fibres following the surface contours. An opaque layer of densely packed fibres surrounds the cavity of the test. In *P. praeputialis* the test has the same structure, and the fibres are apparently all aggregates of very thin polysaccharide fibres, which are formed from globules in the iron-rich blood cells called ferrocytes (Endean 1955b). The formation of the test during regeneration as described by Endean (1955c) involves a migration of ferrocytes from the blood-vessels through the test towards the injury. Sea-water causes lysis of the globules and the fibres thus formed enmesh sand particles and detritus.

The interior of the test is laced by an extensive network of blood-vessels branching from the anterior and posterior test vessels, which each have major branches to all sections of the test. Endean (1955b) feels that these two vessels form independant blood supplies to the test of *Pyura praeputialis* and that there is no connection between them. He describes the smaller vessels as branching so extensively, and having such small interior diameters that he doubts whether a significant interchange of blood between the two systems of major vessels would occur. This seems to be a questionable argument. A large number of small vessels can support as high a flow as a few large ones, and a linking of the two systems via large vessels would in fact, be surprising.

Vascular ampullae were not found in the test although Berrill (1950) describes them in other pyurids. In some species the ampullae are concentrated in the tubercles (Ritter 1909), and the siphonal areas and ampullae are rich in receptor cells and nerve cells in many pyurids (Das 1936). In *Pyura stolonifera* the tubercles of juveniles and the few adults which possess them occur mainly on the siphons, which are sensitive to stimulation. Round cellular structures were found near the surface of the tubercles, but these do not appear to be nervous tissue, and there were no ampullae.

The Mantle or Body Wall

The mantle, which lies inside a strong membrane lining the cavity in the test, is formed between the outer epidermis and the lining of the peribranchial cavities. It contains three muscle layers embedded in an orange-red mantle tissue. The outermost muscle layer is a series of horizontal encircling fibres which are very thin and do not extend very far ventrally. They are most strongly developed at the base of the siphons, but do not extend onto the siphons themselves (Figure 3A).

The strongly developed radial, or longitudinal muscle fibres are grouped into bands spanning the thickness of the mantle. They run down from the tips of the siphons to radiate over the dorsal siphonal area, where they are thickest, and then pass down the sides of the body becoming thinner ventrally (Figure 3A). The innermost muscles are the circular muscles in the siphons

(Figure 3B). There is a fairly distinct sphincter at the base of each siphon, and near the tips of the siphons are four longitudinal muscles, which close the four siphon lobes (Figure 1).

The orange-red mantle tissue consists of small lobules of very small round cells with a certain amount of interstitial tissue between the lobules. This tissue extends between the radial muscle bands and covers them internally. It is absent in very young individuals. It appears first around the heart and gut, and in large specimens it becomes very extensive particularly in the ventral body wall, and partly covers the gonads, heart, and gut.

THE ALIMENTARY CANAL

Siphons

Both siphons are lined by inversions of the epidermis, and the atrial siphon has a thin inner lining of black, or sometimes red, tunicin with a frilled edge. This lining almost reaches the anus
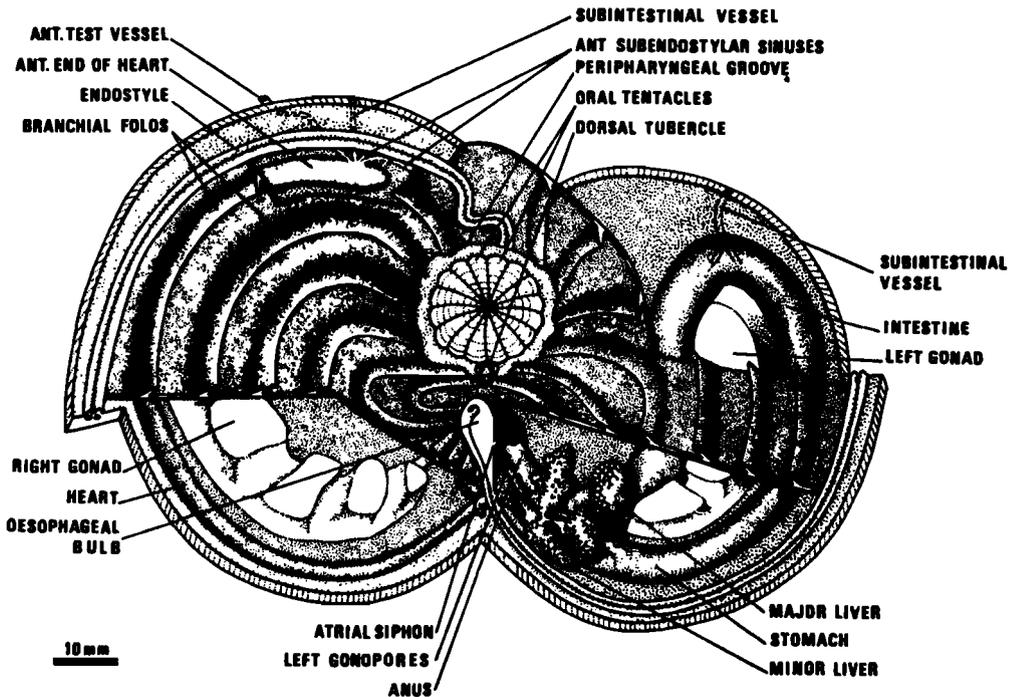

FIGURE 2

GENERAL DISSECTION

The mantle and pharynx have been cut open ventrally along the middle line, with the anterior end uppermost. The interior of the pharynx is shown, and portions of the pharyngeal walls have been removed to show the organs in the peribranchial cavities. The oral tentacles are schematic to clarify the figure.
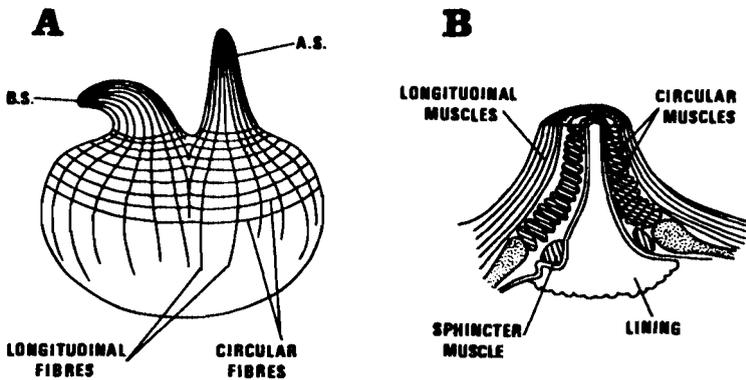
Ant.: Anterior.

FIGURE 3

MUSCULATURE

A. Animal with the test removed, showing the orientation of the muscle bands. Anterior to the left.

B. A longitudinal section through the atrial siphon to show the arrangement of the muscles.

B.S.: Branchial siphon (anterior).

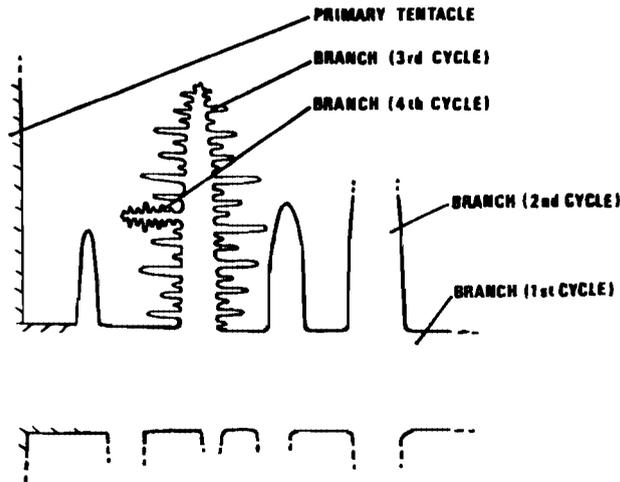*A.S.*: Atrial siphon (posterior).

FIGURE 4

THE PATTERN OF THE ORAL TENTACLE BRANCHES

Four cycles of branching are diagrammatically illustrated. The sub-branches of only one branch in each cycle have been shown.

and gonopores and partly covers the oesophagus where it runs across the base of the atrial siphon. The branchial siphon lining may also be red, but is usually grey on the inside and silver on the hidden side next to the muscles. It is folded into a series of teeth in a single or double ring near the base.
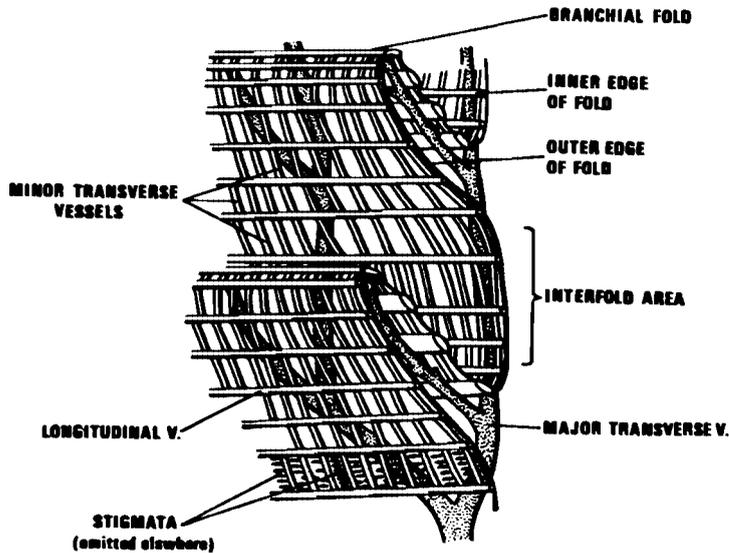

FIGURE 5

BRANCHIAL WALL

A plan of the branchial wall of a juvenile is shown. In the adult the longitudinal vessels are closer together especially on the folds, and the stigmata are longer and more tightly packed.

V.: Vessel.

The Pharynx

The pharynx is enclosed by the branchial walls and contains the ventral endostyle, the dorsal lamina, the dorsal tubercle, the oral tentacles, and the oesophageal bulb (Figure 2). It is curved into a U-shape which narrows progressively during growth. Thus the endostyle is very long and the dorsal lamina very short, and the oesophageal opening is dorsal and immediately below the atrial siphon. In the oesophageal bulb surrounding the oesophagus, there is a spiral 'infundibulum' formed by the posterior end of the endostyle as it runs into the oesophagus. At its anterior end the endostyle has a sharp S bend before dividing to form the peripharyngeal grooves, which run around the oral tentacles and dorsal tubercle. These grooves join at the beginning of the dorsal lamina area (Figure 7 A and B), which is attached to the body wall. The posterior end of the dorsal lamina area is divided from the oesophageal bulb by a deep crevice (Figure 7 A and B).

The oral tentacles encircle the base of the oral siphon and form a closed tentacular cage

(Figure 2). Although the tentacles in front of the dorsal tubercle are reduced there is no gap at this point. The tentacles each have branches and sub-branches arising from a main axis, and up to five cycles of such sub-branching can be found. Each set of branches consists of branches of different sizes arranged regularly, larger alternating with smaller ones, and still smaller branches growing between these. The tentacles thus conform to the pattern of growth described so well by Ritter (1909) in *Halocynthia johnsoni*. In *P. stolonifera* as in *H. johnsoni* the largest secondary tentacles are in the middle of the primary tentacles and the same is true for further cycles (Figure 4).

The branchial walls stretch between the dorsal lamina area, the peripharyngeal grooves, and the semicircular endostyle lying embedded in the mantle. They are thrown into six concentric semicircular folds on each side, centred on the dorsal lamina area. In living material the folds stand out but they collapse during fixing. They begin anteriorly at the peripharyngeal grooves and end very close together around the oesophageal bulb (Figure 2). A set of interior longitudinal blood vessels run parallel to the branchial folds (Figure 5). They project into the pharynx, and have thickened bands on their inner edges, which are presumably the ciliary bars described by Berrill (1950) for other ascidians. The small straight stigmata run parallel to the longitudinal vessels and are arranged in irregular transverse rows (Figure 5). They increase in size during growth.

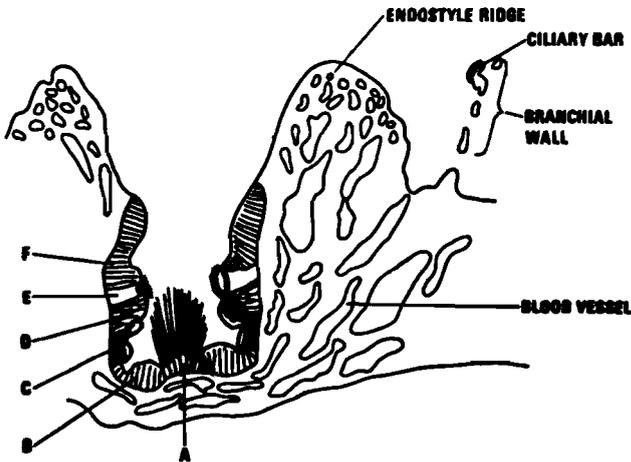The endostyle (Figure 6) has eleven bands of cells, arranged in pairs around a central band of

FIGURE 6

TRANSVERSE SECTION OF THE ENDOSTYLE

Cell bands: A. Median basal flagellate cells.
B. Basal glandular cells.
C. First ciliated epithelium band (here thickened, perhaps glandular).
D. Second glandular band.
E. Second ciliated epithelium band.
F. Third (outer) glandular band.

flagellate epithelial cells. It differs slightly, however, from the general ascidian pattern described by Berrill (1950), as the second pair of bands from the centre is thickened and probably glandular, rather than being a band of simple ciliated epithelial cells.   There are thus four pairs of glandular bands and one pair of ciliated bands, not three and two pairs, respectively. Possibly this is related to the method of feeding, described below.

The sides of the endostylar walls are extremely vascular (Figure 6), possibly because a good blood supply is necessary to support the high metabolic activity of the endostyle, or more likely as M. Webb suggests (personal communication), it is to maintain a turgid condition of the endostylar walls, thus keeping the groove open.

The dorsal lamina is reduced in the adult and the tentacle-like lappets are partly or completely lost, as noted by both Hartmeyer (1911) and Michaelsen (1928). This is a result of the progressive closing of the U-shape of the pharynx during growth. For example juveniles of 20 mm and 25 mm maximum diameter had 14 and 9 lappets respectively, whereas lappets were absent in almost all the large individuals examined. Two examples are shown in Figure 7. In the juveniles the lappets begin alongside a tongue-shaped groove formed from the united peripharyngeal grooves and end at the oesophageal bulb. In progressively larger specimens the tongue-like groove is extended and widened while the lappets are reduced in number, so that in large specimens only a short bare area remains between the groove and the oesophageal bulb (Figure 7B).

Feeding

The feeding process in *P. stolonifera* differs from that outlined by Berrill (1950) for *Ciona* and other ascidians, and demonstrates the rôle of the pharyngeal folds in feeding.

The mucus produced by the endostyle moves rapidly out over the folds in continuous sheets. Perhaps this movement is caused by a transverse waving of the longitudinal vessels as described in *Ascidiella* by Berrill (1950). At the edge of each fold part of the mucus sheet is drawn posteriorly as a string along the edge of the fold, while part continues up the pharynx as a sheet. There is thus a string of mucus moving along the thickened edge of each fold. At the posterior end of the folds the mucus strings arrive at the oesophageal bulb. They are then drawn slowly down the edge of the folds into a groove, in which the mucus moves to the front of the oesophageal bulb. The mucus accumulates in front of the bulb, and eventually becomes attached to the infundibulum or conical opening of the oesophagus. It is then drawn very rapidly into the oesophagus. The rapid movement pulls the slow-moving mucus out of the groove around the oesophageal bulb and gradually strips the strings off the edges of the folds.

In this way the mucus moving along the edge of each fold becomes detached at some point along the fold and forms a string which stretches directly into the opening of the oesophagus. Indeed there are typically several strings from each fold. Since mucus moves posteriorly along the edge of each fold, perhaps mucus is supplied to the anterior end of each fold from the peripharyngeal grooves. This could not be confirmed, but the peripharyngeal grooves are continuous with the anterior end of the endostyle so that mucus might be passed into them.

The Digestive Tract

The enormous pharyngeal sac is followed by a narrow oesophagus leading into the stomach.

A

ENDOSTYLAR GROOVE

OESOPHAGEAL BULB

FOLD

CREVICE

LAPPET

LONGITUDINAL VESSEL

FOLD

SPIRAL INFUNDIBULUM

MINOR TRANSVERSE VESSEL

PERIPHARYNGEAL GROOVE

B

OESOPHAGUS

CREVICE

LAPPET

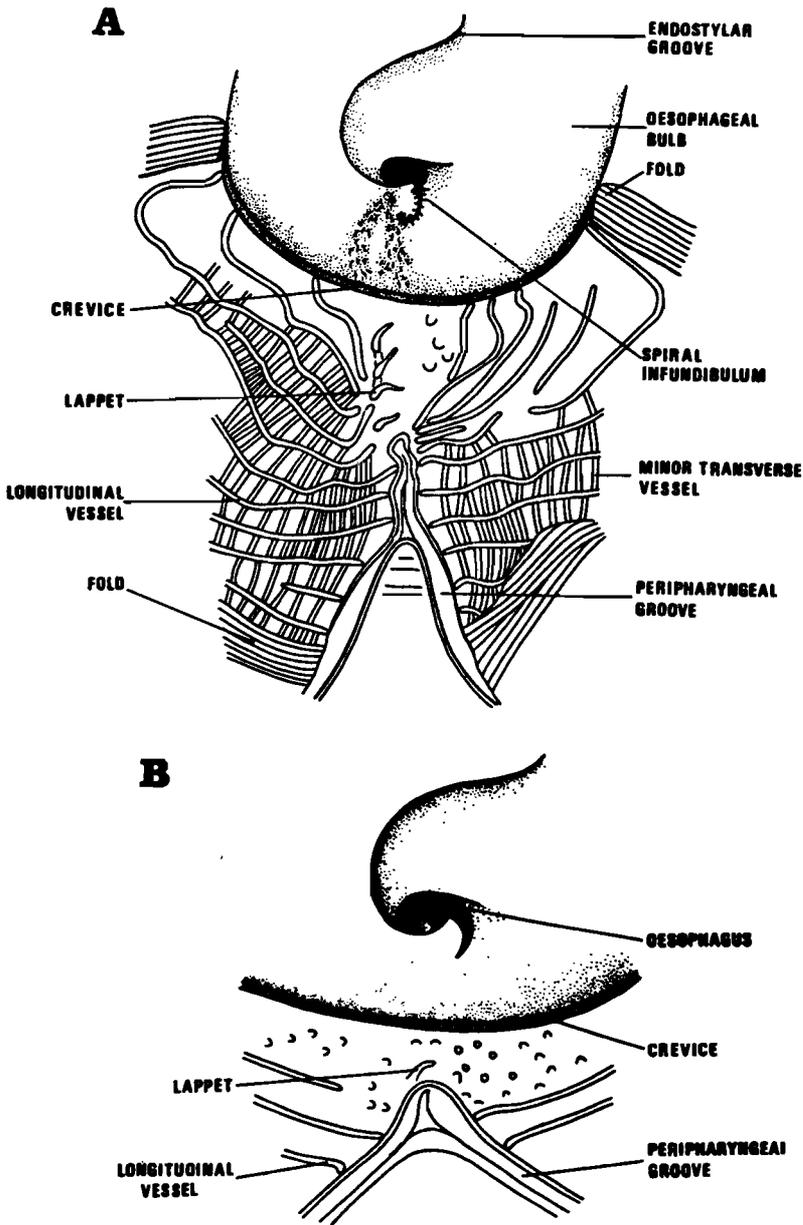LONGITUDINAL VESSEL

PERIPHARYNGEAL GROOVE

FIGURE 7

DORSAL LAMINA AREA

A view of the interior of the pharynx between the peripharyngeal grooves and the oesophagus.

A. Juvenile with four lappets.

B. Adult with one remaining lappet.

The intestine forms the rest of the descending limb as well as the entire ascending limb of the gut, and terminates at the anus at the base of the atrial siphon (Figure 2). From individuals killed after being fed carmine particles it seems that the mucus strings, which are not broken during digestion, reach the stomach within half an hour of the food entering the siphons but they are still present in the rectum one or two days later.

The oesophagus leads from the oesophageal bulb across the base of the atrial siphon to the posterior body wall, and widens sharply into the stomach. A common duct enters the first part of the stomach from the three lobes of the 'minor liver' which is the first of two digestive glands. The second digestive gland or 'major liver' is larger, and has two lobes. Its duct opens into the last part of the stomach, about midway down the descending limb of the gut. Both openings are in a groove or gutter which runs the length of the stomach on the right or pharyngeal side (Figure 8).
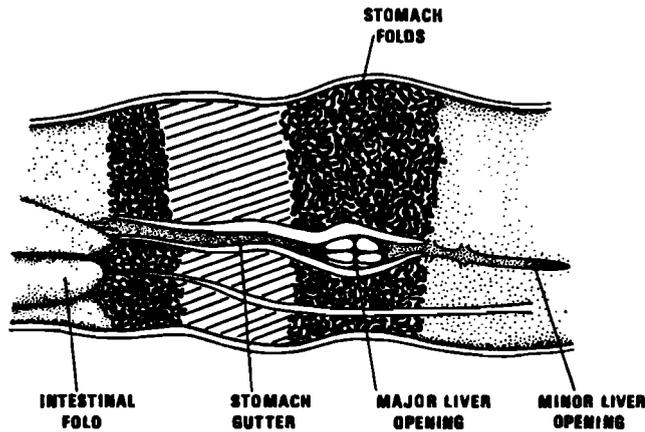

FIGURE 8

INTERIOR OF THE STOMACH

The stomach and the beginning of the intestine have been slit open laterally, and are shown with the oesophageal end to the right. The stomach folds are incompletely illustrated.

The two livers are both closely applied to the branchial wall, being connected to it by blood vessels. The number of lobes very occasionally varies from three and two for the minor and major livers respectively. One of the minor liver lobes lies to the right of the pharynx and its duct passes beneath the endostyle to join the stomach. The livers differ in colour and shape. The major liver lobules are round and grape-like, and are orange-red in juveniles and dark-brown in old specimens. The minor liver lobules, by contrast, are flattened and pointed, rather like arrowheads, and are bright-orange in young specimens and yellow in large individuals. Berrill (1950) describes two liver masses for *Pyura microcosmus* but does not mention any difference between them.

Beyond the opening of the major liver duct the interior lining of the stomach is thrown into

small irregular folds (Figure 8). The end of these folds marks the beginning of the intestine, which is almost as wide as the stomach. It has a single large fold projecting into the lumen on the left, or body wall side (Figure 8), which runs the full length of the intestine and contains a large blood vessel.

The pyloric gland which is present in all other ascidians, but is of uncertain function (Berrill 1950), could not be found in *P. stolonifera*. The gland consists of ampullae distributed over the intestine, each linked to a system of canals. In most ascidians these join together and cross a strong ligament to the pyloric region of the stomach, where the canal opens into the stomach lumen. In the Pyuridae the pyloric gland has no ligament and extends over the whole gut with ampullae on the stomach and liver as well as on the intestine (de Lacaze-Duthiers & Delage 1889). The canals are associated with blood vessels and the gland opens into the gut lumen about halfway down the descending limb. Both the ampullae and the canals are extremely small (15–20μ and 1–2μ respectively in one species described by de Lacaze-Duthiers & Delage 1889).

THE REPRODUCTIVE SYSTEM AND LARVA

There is one pair of hermaphrodite gonads. These lie in the lateral mantle walls and project into the peribranchial cavities, and each consists of a single series of lobes or polycarps through the middle of which run the gonoducts (Figure 9 A and C). The left gonad usually has four polycarps and lies in the loop of the gut, except for the most posterior polycarp which is usually on top of the intestine (Figure 9C). Almost all of the right body wall is covered by the right gonad, which has between five and seven polycarps. Each polycarp contains both ovary and testes arranged radially. The polycarps and the gonoducts linking them are clearly visible through the body wall in juveniles, but in adults they become progressively obscured by the red mantle tissue and parietal vesicles which grow over the gonads (Figure 9 A and C).

The gonoduct may simply be a common duct partitioned by folds into sperm and ova channels, but there are separate male and female gonopores at the base of the atrial siphon (Figure 9 B and D). These may be arranged side by side (Figure 9 A and D) or one behind the other (Figure 9B). The left gonopores may be either medial (Figure 9C) or lateral (Figure 9D) to the anus. When they are lateral to the anus the left gonoduct runs between the stomach and intestine and then bends to pass beneath the anus in the body wall.

J. H. Day and C. R. Richards (unpublished) have cultured the tadpole larvae. They found some reproductive activity in June, but more marked activity in December, when the round eggs were 0,55 mm to 0,7 mm in diameter and surrounded by a clear chorion, well-separated from the cytoplasm. The tadpole larvae develop within 24 hours and possess both an ocellus and an otolith. The head is 0,75 mm long and 0,5 mm wide and the tail is 0,17 mm wide tapering to a point over a length of 2,46 mm. There are three slightly tapered adhesive papillae on the head, with cup-shaped ends.
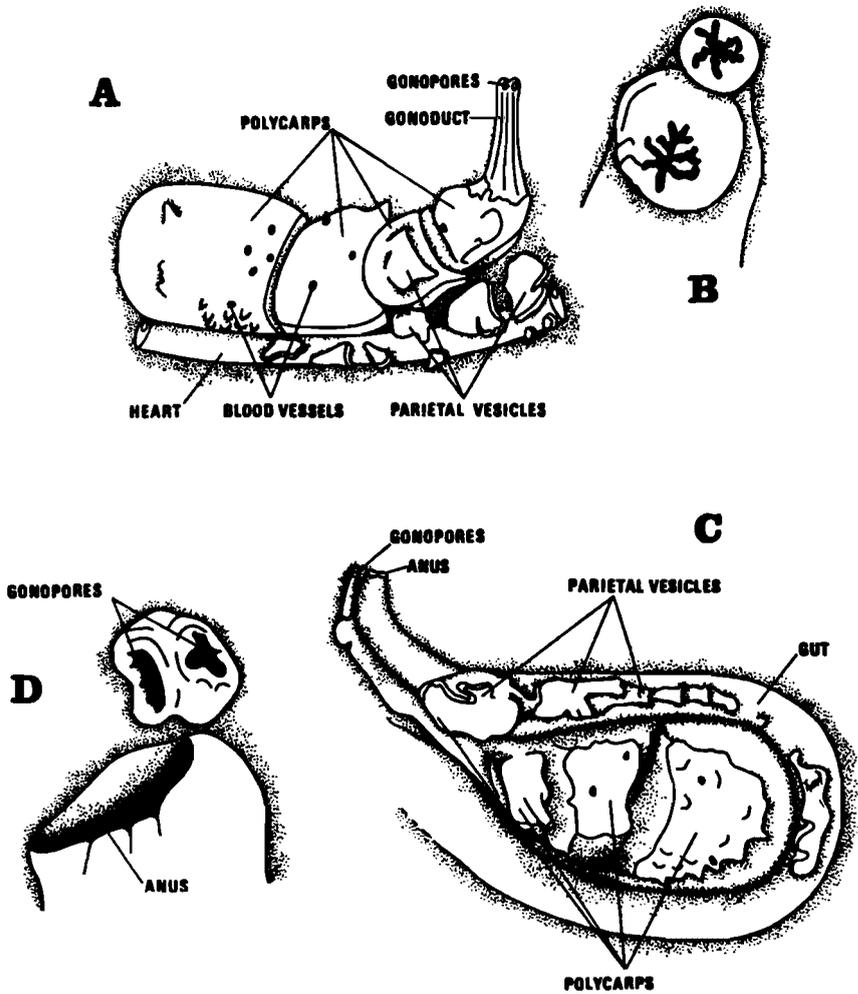
FIGURE 9

GONAD AND PARIETAL VESICLES

A. The right gonad and a section of the heart both covered by parietal vesicles.
B. Right gonopores, shown lying one behind the other.
C. The left gonad in the loop of the gut, both covered by parietal vesicles.
D. Left gonopores, shown lying lateral to the anus.

    (A and C represent a large individual with well-developed parietal vesicles.)

ACCRETORY SYSTEM

The parietal vesicles of the Pyuridae are thought to be organs of accretion, as they contain large numbers of nephrocytes, in which solid xanthine and urate are formed (Berrill 1950). The vesicles are usually not evident in juvenile *P. stolonifera*, and develop during growth, appearing first on the polycarps of the gonads. In large individuals they become numerous and prominent and occur on various parts of the gut, pericardium and body wall (Figure 9 A and C). They project into the atrial cavity against the walls of the pharynx and the gaps between them maintain flow-channels in the atrial cavity. In young specimens this function is served by the polycarps of the gonads. Possibly the gonads of many of the Pleurogona are polycarp for this reason.

The nephrocytes in the parietal vesicles are believed to be homologous with the renal sac of the Molgulidae (Berrill 1950). The nephrocytes in *P. praeputialis* are about 20µ in diameter (Endean 1955a).

CIRCULATION

The detailed features of the circulation, especially in the branchial sac are here tentatively described, as the vessels are delicate and diffuse, and the necessary breaking of the trabecular vessels across the peribranchial cavities during dissection renders an injection technique very difficult. There are no capillaries, and the blood sinuses in the viscera may fade into a mass of spongy tissue.

The heart consists of a tubular invagination along the long crescent-shaped pericardium embedded in the right body wall. The muscular structure and contraction of the heart in *Pyura praeputialis* has been described by Goddard (1972). The blood vessels in ascidians cannot be termed arteries and veins as the direction of the heart-beat reverses periodically. For clarity, however, the circulatory system is described below as if blood always flowed out of the anterior end of the heart. The major vessels from the anterior end of the heart (Figure 2) are the anterior test vessel, the anterior subendostylar sinuses, the double posterior subendostylar sinus and the subintestinal vessel. At its posterior end the heart crosses the endostyle in the region of the oesophagus and gives rise to a hepatic vessel, a gastric vessel and an oesophageal vessel (Figure 10B).

From the anterior end of the heart then, blood flows into the subendostylar sinuses, and then through the minor transverse vessels at the edges of the endostyle to the branchial walls. The left anterior subendostylar sinus leads into the tentacular vessel (Figure 10C) in the siphonal area. Branches lead to the branchial and atrial siphons, the body wall and the oral tentacles (Figure 10C). A large branch extends up the centre of each tentacle with sub-branches to each of the secondary and subsidiary tentacles.

The complementary blood-vessels run down the outside edges of the tentacles to join the dorsal vessel (Figure 10C). Blood from the branchial siphon and oesophageal bulb also flows into the dorsal vessel, which passes close to the cerebral ganglion and leads to the dorsal sinus of the pharynx. Blood from the atrial siphon returns directly to the posterior end of the heart (Figure 10B).

Blood from the anterior end of the heart also flows into the anterior test vessel, and thus to the test. The theory that the anterior and posterior test vessels are separate systems (Endean
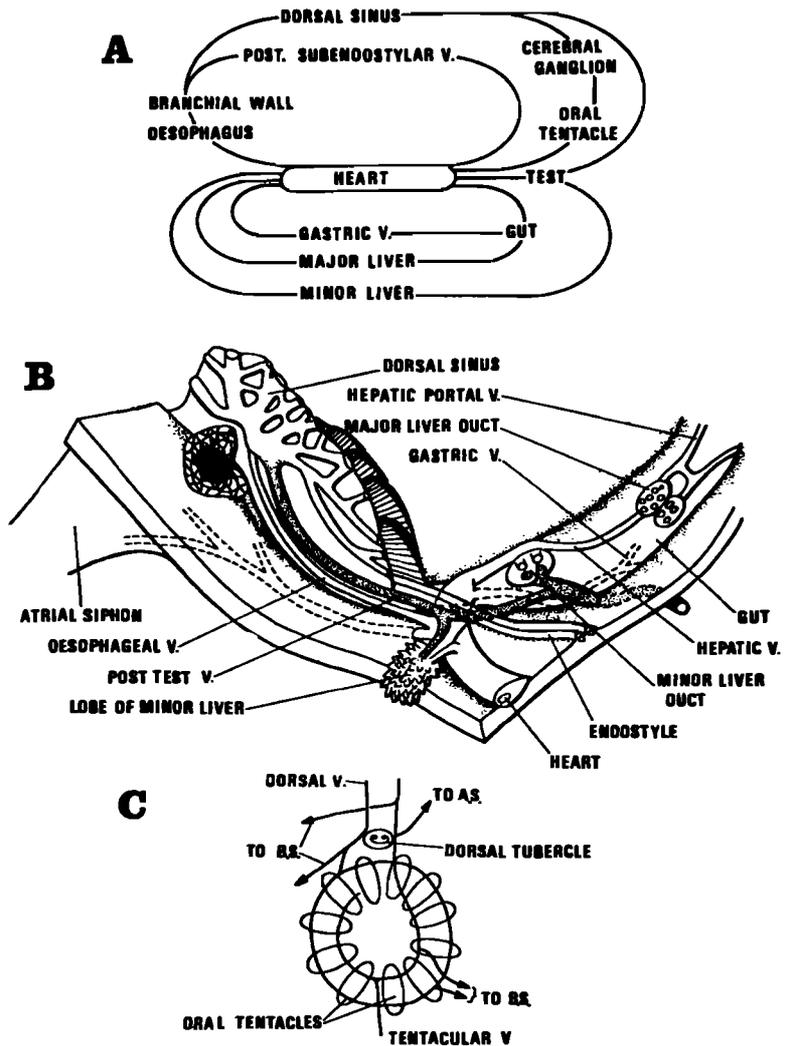
A

DORSAL SINUS

POST. SUBENDOSTYLAR V.

CEREBRAL GANGLION

BRANCHIAL WALL

OESOPHAGUS

ORAL TENTACLE

HEART

TEST

GASTRIC V.

GUT

MAJOR LIVER

MINOR LIVER

B

DORSAL SINUS

HEPATIC PORTAL V.

MAJOR LIVER DUCT

GASTRIC V.

ATRIAL SIPHON

OESOPHAGEAL V.

POST TEST V.

LOBE OF MINOR LIVER

GUT

HEPATIC V.

MINOR LIVER DUCT

ENDOSTYLE

HEART

C

DORSAL V.

TO A.S.

TO B.S.

DORSAL TUBERCLE

TO B.S.

ORAL TENTACLES

TENTACULAR V

FIGURE 10

CIRCULATORY SYSTEM

A. Schematic diagram of the circulation.

B. Vessels around the posterior end of the heart.

C. Diagram of the vessels supplying the oral tentacles.

Post.: posterior, *V.*: vessel, *B.S.*: branchial siphon, *A.S.*: atrial siphon.

1955b) has been discussed earlier. It is here assumed that blood from the anterior test vessel flows out of the test through the posterior test vessel during this phase of blood-flow. The posterior test vessel leads to the dorsal sinus which thus receives blood from the siphonal area and test.

There is another distinct sinus around the oesophageal bulb, linked to the posterior end of the heart via the oesophageal vessel. The two sinuses appear to supply distinct sets of major transverse vessels (Figure 10B and Figure 5), which radiate over the outside of the pharynx. The major vessels supply minor transverse vessels between the rows of stigmata (Figure 5). On the inner side of the pharyngeal walls the minor transverse vessels connect to the longitudinal vessels running parallel to the branchial folds.

The longitudinal vessels on the dorsal or inner side of each fold are larger than the remainder (Figure 5). All longitudinal vessels are linked anteriorly to very small vessels running along the peripharyngeal grooves, but posteriorly their connections become lost in the spongy mass of the oesophageal bulb.

The major transverse vessels, taken together, are larger, and supply a larger area of the pharynx than the subendostylar sinuses. It is therefore suggested that the blood flow does not follow the typical pattern for ascidians described by Berrill (1950) from the subendostylar sinus to the dorsal sinus through the pharynx. Perhaps the blood flows from the dorsal sinus to the oesophageal vessel via the pharyngeal vessels described above.

Blood from the dorsal sinus may also flow into the trabecular vessels crossing the peri-branchial cavities to the livers, the gonads, and the intestine. A subintestinal vessel (Figure 2) from the anterior end of the heart enters the intestine at the bottom of the intestinal loop. It divides to run in both limbs of the gut in the intestinal fold, with branches to the left gonad and body wall.

Blood from the intestine and left gonad is collected by a 'hepatic portal vessel' which lies on the medial side of the intestine (Figure 10B). Branches of this vessel lead to the stomach and the lobes of the major liver. The gastric and hepatic vessels (Figure 10B) drain the stomach and liver and join to the posterior end of the heart. Branches of the hepatic vessel run from the minor liver, which is supplied with blood from the posterior test vessel (Figure 10B).

The pattern of blood flow, which is described above as if it were unidirectional, is shown diagramatically in Figure 10A.

THE NERVOUS SYSTEM

The elongate cerebral ganglion lies just above (dorsal to) the dorsal tubercle and has four main nerve trunks (Figure 11). One of these leads to the base of the oral siphon and another to the trunk and tip of the oral siphon. The atrial siphon is similarly innervated by the other two nerves. Histological preparations would no doubt reveal further nerves but are beyond the scope of the present work.

The ascidian heart has a separate nervous supply (Berrill 1950) with a ganglion at each end controlling the heart-beat and its reversal, but these are usually only histologically demonstrable. Goddard (1972) discusses the initiation of the heart-beat in *Pyura praeputialis*.

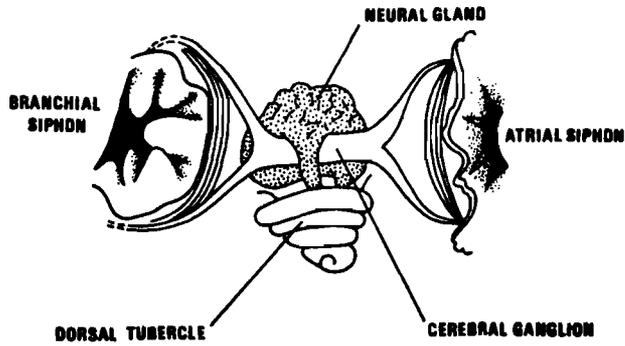The neural gland, which lies between the cerebral ganglion and the pharynx in *Ciona* and

FIGURE 11

NERVOUS SYSTEM

The cerebral ganglion and neural gland of a juvenile are shown, viewed from the inside of the animal, after removal of the pharynx.
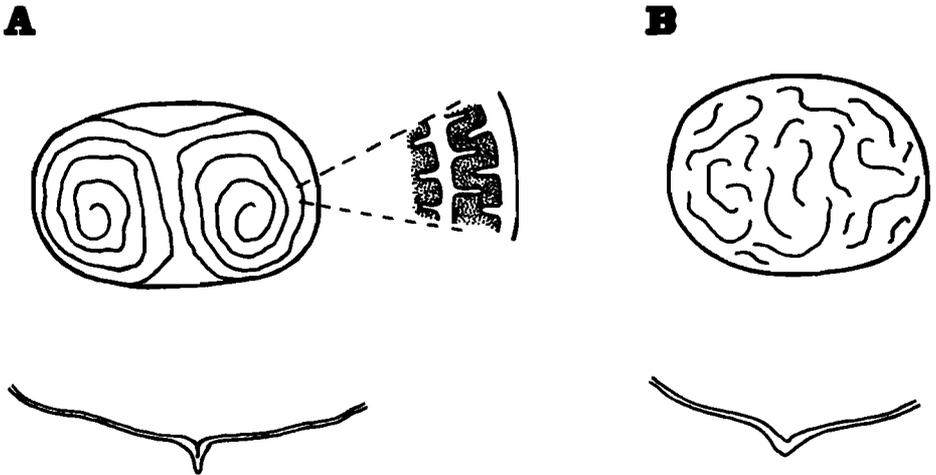
FIGURE 12

DORSAL TUBERCLE

The dorsal tubercle is shown together with part of the perioesophageal grooves, as viewed from the pharynx. Anterior is uppermost.

A. Normal horshoe spiral pattern, with part enlarged to show the detailed shape of the opening.

B. Broken pattern found in some large adults.

other ascidians, is dorsal to the ganglion in *P. stolonifera*. The dark gland is two or three times as large as the ganglion. It has two horns, which pass down on either side of the ganglion and join between the dorsal blood vessel and a branch of the tentacular vessel leading to the oesophageal bulb. The junction marks the beginning of the neural canal which opens into the pharynx on the dorsal tubercle. Various shapes of the canal opening are described in the taxonomic literature; this is because the shape changes as the animal grows and also varies from specimen to specimen.

The initial shape of the opening is a horseshoe with spirally inrolled ends. The inrolling of the ends of the horseshoe is progressive during growth, so that in larger individuals the rolled ends form two conical spires with many turns (Figure 12A). Occasionally three spires are found, and often the pattern is very irregular and may break down to become complex and disjointed in large individuals (Figure 12B).

In the South African *P. stolonifera* the opening of the horseshoe, when this is distinct, always faces away from the branchial siphon (Figure 2), as noted by Millar (1962, 1963).

ECOLOGY

Distribution (see Gazetteer on p. 56)
In southern Africa *P. stolonifera* reaches as far as Lüderitzbucht in South West Africa, but is absent from Rocky Point (Penrith & Kensley 1970a, b). It stretches along the entire South African coast (unpublished records of the University of Cape Town), and in Moçambique it is recorded as far as Inhaca Island, where it is described as abundant (Macnae & Kalk 1958), but the author found it to be rare if present at Jangamo Reef.

The species has also been recorded from Dakar (Monniot 1965), Rabat in Morocco (Sluiter 1927), and Australia (for references see Kott 1952). It would not be surprising if there were three species involved here.

Habitat
In the western Cape colonies of *P. stolonifera* form dense sheets on rocks from about mean low water springs to about four metres below. Morgans (1959) records scattered clumps and single individuals from mean low water neaps to about twelve metres depth. This zone is much the same as that of the kelp *Ecklonia maxima*, but in any particular area only one of these species is abundant. *Pyura stolonifera* is dominant in warmer waters such as those of the south and east coasts of South Africa and *Ecklonia* is dominant on the cold west coast (see Stephenson 1944). The change in dominance occurs near Cape Agulhas (Eyre 1939). In False Bay *Pyura* is dominant on the Cape Peninsula from Oatlands Point northwards (Morgans 1959) presumably because the shallow northern parts of the bay have greater temperature extremes.

In areas where both species are common they presumably compete for attachment sites. In such places *Ecklonia* appears to dominate where there is more shelter and well-aerated water (Morgans 1959), whereas *Pyura* appears to be especially prevalent where prolonged and severe shear forces are encountered from the waves. This may explain why Morgans (1959) found that *Ecklonia* was dominant at mean low water springs in an area of maximal exposure. The site Morgans examined faced the swell and was not subjected to the side on shearing action of the waves. The author has examined a similar site at Castle Rocks, False Bay.

Perhaps strong currents assist *P. stolonifera* in feeding. The extended atrial siphon may act as a suction chimney by an application of Bernoulli's principle, thus drawing water into the short anteriorly facing branchial siphon. Kott (1969) points out that many ascidians have the branchial siphon directed down towards the sediment to catch stirred-up detritus, but this would not be applicable to the extensive banks of *Pyura* covering sublittoral rocks. Specimens on the shore at low tide always have closed siphons and they extend them only after some minutes of immersion.

Pyura stolonifera has been found in various places in False Bay in loose rounded masses which roll around on the sandy bottom. These are known as 'sand bait'. They have presumably been torn loose from nearby rocks, but continue to grow. Another form of the species is found on the intertidal sandbanks of some estuaries (Macnae & Kalk 1958) and has a weaker test, with root-like extensions which anchor it in the mud. The author has not examined these forms, but the Australian *P. praeputialis* has a marked estuarine form, described by Kott (1952).

Epibionts

The test becomes covered by a great variety of epibionts, and in colonies of *Pyura* there is a diverse community associated with the ascidians. Eyre (1939) gives a short list of the common species, most of which are crevice dwellers, or merely occupy the same zone. However two species warrant particular mention.

The bivalve *Lanistina cuneata*, reaching a length of about 10 mm, was found on about 20 per cent of the animals examined, always embedded in the test of the siphons. It has also been recorded in association with *Ascidia sydneyensis*, another large tunicate in the same region (Day 1969). Substrate-attached bivalves such as mytilids are found partly or completely over-grown by the test, but *Lanistina* is never completely enclosed in the test.

Barnard (1964) records the species as occurring free, usually under stones, or in the crevices between *Pyura*, or embedded in the test.

Koch (1949) described a dwarf form of the limpet *Patella barbara* associated with *Pyura*. Branch (1971) concludes that the ascidian occurs in the middle of the settling zone of the limpet, which is usually associated with *Lithothamnion*, an alga which is often found on *Pyura* tests. The limpet develops a curved shell margin to fit the test and is apparently then restricted to it and the small food supply on the test.

Parasites and Commensals

Barnard (1955) lists the parasitic copepods *Doropygus pulex* and *Gunenotophorus globularis* var *giganteus* as infesting *Pyura stolonifera*. The first is a cosmopolitan species and is found in other ascidians. The second species is European, but all the South African specimens known are of the variety *giganteus* and have been found in *Pyura* from Lüderitzbucht to Plettenberg Bay. The author has found both species in the folds of the pharynx on the mucus sheets. In addition amphipods of the genus *Podocerus* were found occupying a gap in the cage formed by the oral tentacles. They may use the ascidian only as a sheltered crevice, but possibly they ingest particles retained by the oral tentacles. Members of the genus are usually free-living.

DISCUSSION

Tunicate evolution and the position of Pyura

A discussion of this subject seems relevant because the Pyuridae together with the Molgulidae represent the peak of one line of tunicate evolution, and because many of the features of *Pyura stolonifera* assume significance when viewed in the light of the evolutionary trends in the group. Evolution within the tunicates has been considered by Berrill (1950), Millar (1966) and Kott (1969). Monniot (1965) discusses evolutionary lines within the Pyuridae.

Two evolutionary trends have occurred in the tunicates. The first is towards colony forma-tion, and is best developed in the aplousobranchiate families, but is also seen in the Perophoridae and some Styelidae. The individual becomes smaller, vegetative reproduction is enhanced and there is a trend to oviparity, with the peribranchial cavities used as a brood pouch (Berrill 1950, Kott 1969).

Kott (1969) has pointed out that this is an adaptation to turbulent shallow water and inter-tidal zones, where the gametes and larvae would be quickly dispersed. The colony ensures sperm will be available to fertilize the eggs produced. The larvae of these animals have become speci-alized as agents of short range dispersal, with the disappearance of sensory structures (ocellus and otolith).

The second evolutionary tendency is towards a larger body size, and the peak of this line is seen in the Pyuridae and Molgulidae. In large ascidians the gonads are larger, and thus more gametes can be produced at once. In the Pleurogona, in fact, there is a pair of gonads lying in the mantle walls rather than one gonad in the gut loop, and the gonads may even be replicated on each side. Because each individual can produce a large number of gametes there is an increased chance that cross fertilization will occur even when there is a low density of individuals or very extreme water movement.

Pyura stolonifera has only two gonads, each with a single row of polycarps, but the dense concentrations in which the species lives would alleviate the problem of gamete dispersal. The retention of the sensory structures, the ocellus and otolith, in the larva of *P. stolonifera* and of the other pyurids investigated (Berrill 1950) may enable the larvae to select a restricted range of site-types, thus maintaining a high density of sessile adults while retaining a high dispersal potential.

The test of *P. stolonifera* represents a very advanced protective device against predation and wave action. Monniot (1965) notes that the protective spines, tubercles and plates of many pyurids are replaced in the most advanced members of the genus *Pyura* by the cuticle of embed-ded foreign particles described earlier. Possibly the tubercles are retained in young *P. stolonifera* as protective structures while the test is thin.

After the pyurids, the Molgulidae show the most elaborate test characters. The ability of *P. stolonifera* to form rootlike projections of the test for anchorage on mud exactly parallels the characteristic adaptation of the Molgulidae to this habitat.

Evolution in the solitary ascidians is most clearly reflected in the evolution of the feeding organ, the pharynx. Yet nothing has been previously recorded of the feeding mechanism of the largest forms such as *Styela*, *Molgula*, and *Pyura*, where the pharynx is most complex.

Berrill (1950) points out that the longitudinal vessels, the first step in pharyngeal elaboration,

have ciliary bars on their edges which aid in moving the mucous sheets up the pharyngeal walls. The pharyngeal folds in the Pleurogona obviously increase the surface area of the pharynx. In *Pyura stolonifera* the folds are very prominent, and provide sites at their edges for the formation of a number of mucous strings which pass into the oesophagus concurrently, whereas in other ascidians such as *Ciona* (Berrill 1950) there is only a single string formed at the dorsal lamina.

The retention of the primitive condition of the dorsal lamina as a series of lappets (Berrill 1950) and the shortening and eventual disappearance during growth of the lamina in *P. stolonifera* falls in line with the reduced use made of the dorsal lamina to carry the mucous strings to the oesophagus.

Pyura stolonifera has a wide digestive tract and an indistinct stomach, in common with other large solitary ascidians. The stomach wall in *P. stolonifera*, however, is thrown into numerous small folds which may increase its efficiency. The digestive gland or liver, which appears only in the Pyuridae and Molgulidae, is derived from the simple diverticulum of the Styelidae (Berrill 1950). Clearly the two well-differentiated digestive glands of *P. stolonifera* represent an advanced stage of this line of development.

The absence of the pyloric ligament in *P. stolonifera*, as in all the Pyuridae, is probably associated with the spread of the ampullae over the stomach, but may also be due in part to the assimilation of its functions by the subintestinal vessel described earlier. The pyloric gland canals are associated with the blood sinuses (De Lacaze-Duthiers & Delage 1889).

Comments on the Circulatory System

The structure of the oral tentacles, and their situation suggests that considerable respiratory exchange may occur in the tentacles as well as in the branchial wall, since the vessels running into the dorsal vessel from the tentacles are very thin-walled, and in this way the dorsal vessel could carry oxygenated blood during both cycles of blood movement. This may be significant as the dorsal vessel supplies the cerebral ganglion and subneural gland.

The present study reveals some other aspects of the circulation pertinent to the reversal of blood flow. It is noteworthy that in ascidians the branchial circuit does not follow any pattern of counter current flow so that there is no advantage to be gained from an unidirectional blood flow. Due to the structure of the body and the flow of water in the branchial and peribranchial cavities it is possible that respiration may not be the most important function of the blood flow, except in the test, and its main function may be the distribution of food substances from the gut. The schematic diagram of the vascular system (Figure 10A) shows two main circuits, a branchial circuit and a nutritional circuit. The heart appears to act as a mixing chamber for these circuits, and the connection from the branchial wall to the gut via the trabecular vessels, which is not shown, would further improve this mixing. Furthermore if blood flow was in one direction only, each of the organs would have to be 'in parallel' whereas Figure 10A shows, for instance, the gut and the major liver in series, so that one would receive blood depleted in oxygen or food if the flow was unidirectional. It would seem that a more rigidly ordered system would be required for blood flow in one direction only. The argument, however, does not necessarily apply beyond this species, and certainly does not seem to be applicable to the blood flow of *Ciona* as described by Millar (1953).

APPENDIX: PRACTICAL CLASS USAGE

For a practical class in comparative anatomy *Ciona intestinalis* demonstrates the primitive features of the tunicates (Millar 1953) and is most easily related to the chordate body form. *Pyura stolonifera* is a rather generalised member of the very advanced Pyuridae and if compared with *Ciona* demonstrates the evolutionary specialization of the group and particularly the differences between the Enterogona and the Pleurogona.
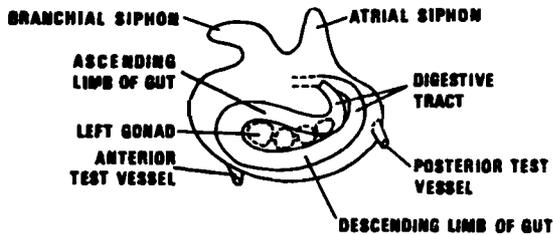

FIGURE 13

DISSECTION GUIDE

The animal is shown from the left, with the test removed, to illustrate the position of the gut, the left gonad, and the anterior and posterior test vessels. Anterior to the left.

Dissection Guide

The atrial siphon opens dorsally, whereas the branchial siphon faces anteriorly, parallel to the flattish dorsal area around the siphons. This facilitates orientation. The test is removed by shaving one of the sides away close to the dorsal 'siphonal area' down to the mantle wall. The body core is then extracted by cutting around the test from this point, severing the anterior and posterior test vessels which pass from the ventral surface of the mantle into the test, and removing the bottom half of the test. As the inner lining of the siphons is continuous with the test, the test must be cut away at the tips of the siphons.

The vessels in the test may be followed by pumping air into them. They are then readily visible in thick sections of the test.

For rapid identification of an animal the mantle and pharynx are cut open together along the line of the heart (Figure 1) to expose the dorsal tubercle and other pharyngeal characters. To avoid destroying the heart for anatomical study the digestive tract is first dissected out by piercing the left mantle wall anteriorly and just below the dorsal area (Figure 13), cutting the mantle wall around the line of the gut, and severing the trabecular vessels joining the hepatic tubules to the branchial wall. The endostyle with its subendostylar vessels, and the posterior test vessel are very close to the descending limb of the gut.

The right gonad can be dissected out similarly, leaving the heart and the endostyle lying together, and the pharynx is opened by cutting either branchial wall close to and parallel to the

endostyle. Note that figure 4 does not demonstrate this method. A sagittal section is necessary to reveal the cerebral ganglion and the associated neural gland in large specimens, although they are visible from the pharynx in juveniles. Endean (1955b) describes for *P. praeputialis* a useful technique to reveal the blood vessels by keeping the animals alive in sea-water to which $1:10^4$ parts of neutral red are added, for 48 hours. The blood vessels are stained red.

GAZETTEER

Cape Agulhas	35°S	20°E
Cape Peninsula	34°S	18°E
Dakar	15°N	17°W
False Bay	34°S	18°E
Inhaca Island	26°S	33°E
Jangamo	24°S	36°E
Lüderitzbucht	27°S	15°E
Plettenberg Bay	34°S	23°E
Rocky Point	19°S	12°E
Rabat, Morocco	34°N	07°W
Table Bay	34°S	18°E

REFERENCES

BARNARD, K. H. 1955. South African parasitic Copepoda. *Ann. S. Afr. Mus. 41*: 223–312.
BARNARD, K. H. 1964. Contributions to the knowledge of South African Mollusca. Part 5, Lamellibranchiata. *Ann. S. Afr. Mus. 47*: 401–402.

BERRILL, N. J. 1950 *The Tunicata*. Ray Soc. London.

BRANCH, G. M. 1971. The ecology of *Patella* Linnaeus from the Cape Peninsula, South Africa. 1. Zonation, movements and feeding. *Zool. afr. 6*: 1–38.

DAS, S. M. 1936. On the structure and function of the ascidian test. *J. Morph. 59*: 539–601.

DAY, J. H. 1969. *A guide to marine life on South African shores*. A. A. Balkema, Cape Town.

ENDEAN, R. 1955a. Studies of the blood and tests of some Australian ascidians. 1. The blood of *Pyura stolonifera* (Heller). *Aust. J. mar. Freshwat. Res. 6*: 35–59.

ENDEAN, R. 1955b. Studies of the blood and tests of some Australian ascidians. 2. The test of *Pyura stolonifera* (Heller). *Aust. J. mar. Freshwat. Res. 6*: 139–156.

ENDEAN, R. 1955c. Studies of the blood and tests of some Australian ascidians. 3. The formation of the test in *Pyura stolonifera*. *Aust. J. mar. Freshwat. Res. 6*: 157–164.

EYRE, J. 1939. The South African intertidal zone and its relation to ocean currents: Part 7. An area in False Bay. *Ann. Natal Mus. 9*: 283–306.

GODDARD, C. K. 1972. Structure of the heart of the ascidian *Pyura praeputialis* (Heller). *Aust. J. biol. Sci. 25*: 645–647.

HARTMEYER, R. 1911. Die Ascidien der deutschen Südpolar-Expedition 1901–1903. *Dt. Südpol-Exped. 12* (Zool. 4): 554–560.

HELLER, C. 1878. Beiträge zur nähern Kenntnis der Tunikaten. *Sber. K. Akad. Wiss. Wien Math-natur. 77*: 83–110.

KOCH, H. J. 1949. A review of the South African representatives of the genus *Patella* Linnaeus. *Ann. Natal Mus. 11*: 487–517.

KOTT, P. 1952. The ascidians of Australia. 1. Stolidobranchiata, Lahille and Phlebobranchiata, Lahille. *Aust. J. mar. Freshwat. Res. 3*: 205–332.

KOTT, P. 1969. *Antarctic ascidacea*. (Antarctic research reports, 13) Amer. Geophys. Union. Washington D.C.

LACAZE-DUTHIERS, F. J. H. DE & DELAGE, Y. 1889. Études anatomiques et zoologiques sur les Cynthiadées. *Archs Zool. exp. gén.* (2), *7*: 519–534.

MACNAE, W. & KALK, M. 1958. *A natural history of Inhaca Island, Mocambique*. Witwatersrand Univ. Press, Johannesburg.

MICHAELSEN, W. 1923. Neue und altbekannte Ascidien aus dem Reichmuseum zu Stockholm. *Mitt. zool. StInst. Hamb. 40*: 50–52.

MICHAELSEN, W. 1927. Einige neue westaustralische Ptychobranchiate Ascidien. *Zool. Anz. 71*: 193–203.

MICHAELSEN, W. 1928. *in:* HARTMEYER, R. & MICHAELSEN, w., Ascidiae Diktyobranchiae und Ptychobranchiae. *Fauna Südwest-Aust. 5*: 433–435.

MILLAR, R. H. 1953. *Ciona*. (L.M.B.C. Memoir 35). Liverpool Univ. Press.

MILLAR, R. H. 1962. Further descriptions of South African ascidians. *Ann. S. Afr. Mus. 46*: 113–221.

MILLAR, R. H. 1963. Australian ascidians in the British Museum (Natural History). *Proc. zool. Soc. Lond. 141*: 689–746.

MILLAR, R. H. 1966: Evolution in Ascidians. *in:* Barnes, H., ed. *Some contemporary studies in marine science*. Allen and Unwin, London.

MOLINA, J. 1782. *Saggio sulla storia naturale del Chili: 4. Animali del Chili*. Bologna.

MONNIOT, C. 1965. Étude systématique et évolutive de la famille des Pyuridae (Ascidiacea). *Mém. Mus. natn. Hist. nat., Paris (A)*, *36*: 1–203.

MORGANS, J. F. C. 1959. The benthic ecology of False Bay, Part 1. *Trans. R. Soc. S. Afr. 35*: 387–442.

PENRITH, M.-L. & KENSLEY, B. F., 1970a. The constitution of the intertidal fauna of rocky shores of South West Africa. Part 1. Lüderitzbucht. *Cimbebasia, 1*: 191–239.

PENRITH, M.-L. & KENSLEY, B. F., 1970b. The constitution of the fauna of rocky intertidal shores of South West Africa. Part 2. Rocky Point. *Cimbebasia, 1*: 243–268.

RITTER, W. E. 1909. *Halocynthia johnsoni* n.sp. A comprehensive inquiry as to the extent of law and order that prevails in a single animal species. *Univ. Calif. Publs Zool. 6*: 65–114.

SAINT-HILAIRE, K. 1931. Morphogenetische Untersuchungen des Ascidienmantels. *Zool. Jb.* Jena (Anat.), *54*: 435–608.

SLUITER, C. P. 1927. Les ascidies de la côte atlantique du Maroc. *Bull. Soc. Sci. nat. phys. Maroc, 7*: 50–99.

STEPHENSON, T. A. 1944. The constitution of the intertidal fauna and flora of South Africa, part 2. *Ann. Natal Mus. 10*: 261–358.