Anatomy of Ascidia viridis n.sp.

by

Homer Allen Hill

Missouri State University
May 1902
Anatomy of Ascidia viridis usp.

All the observations for this description were made from specimens preserved either in formalin or picric formalin. The formalin specimens, which were left in the test, were found to be in the best condition. Those preserved in picric formalin were more or less contracted.

The material was collected by Dr. George Lyman in the harbor of Beaufort, N. C. during the summer of 1901. It was dredged up in water from three to six fathoms deep, and was found in great abundance. Frequently the individuals adhere in small clumps, but are easily separated.

In every case where examination was made the branchial sac was found to harbor a small amphipod (species undetermined).
Diagnosis of Species
Body attached, sessile
Branchial and atrial aperture
not close together; each 8 lobed
(Counter with microscope; test removed)
Test rather tough
Mantle, delicate on left side,
muscular on right side and
over siphons.
Branchial sac, not plated;
not folded on itself posteriorly.
Intestine longitudinal, bar papillae;
stipulate, straight.
Nerve ganglia and neural gland
distant from dorsal tube.
Dorsal lamina a simple
membrane, transversely ribbed.
Edge slightly crescent; it
is continued behind the
oesophageal aperture
Setae simple, numerous.
(about 40)
Alimentary Canal on left side
of branchial sac
Grows in the intestinal loops
This form in shape is not unlike that of Ascidia tenera which is given by Herdman as the shaft of a typical Ascidia. The body is globoid, flattened laterally, the posterior end is rounded, the anterior end is rather flat and slightly narrowed (Fig. 1). It is attached, often, to the inside of a fragment of Lamellibranch shell, by the posterior, left side.

The branchial aperture is circular, terminating, and median. The atrial aperture is elliptical on the dorsal border, almost half way between the anterior and posterior ends. It is directed slightly posteriorly. There are no definite lobes around either aperture, though the surface is raised somewhat, and irregular, in those regions. The entire surface is smooth but marked by irregular, and

Herdman, W. A., 1882, Report on the Tunicata collected during the Voyage of H. M. S. Challenger during the Years 1873 to 1876. Part I, Ascidiae Appendices
Irregular, pruinose and elevated.

The color is pale, whitish yellow.

The length of the body is 10.7 cm, the breadth 4.3 cm, the thickness 3.2 cm.

The test varies in thickness from 2 to 5 mm. It is a little thicker on the left side than on the right. The thickest portions are around the apertures, and the thinnest over the place of attachment to the shelf.

It is gelatinous, almost cartilaginous but translucent so that in life, the loops of the intestines may be dimly seen through the test.

Blood vessels may be clearly distinguished from the interior.

Two blood vessels from the body proper enter the test at a point just beneath the atrial siphon and quite low on the left side. From there they branch out in all directions gradually becoming smaller and terminating in coarse capillaries, each of which shows a terminal ampulla (Fig 2). The ends of the main branches thus terminated, are on the right side.
A cross section of the test shows a matrix with closely set bladder cells, and a few pigment cells (Fig. 2). Objects, such as small sand branches, annelid worms and sand particles, are found imbedded in the test. The larger portion of the surface is also covered by a film of hydroid with its roots slightly imbedded.

On splitting open the test, the body proper is easily removed. It is attached very loosely by the blood vessels, and at the siphons. The general shape is that of the outside of the test (Fig. 3). The drawing shows the siphons considerably contracted, due to the effect of the preserving fluid. The length of the body after it has been removed is 9 cm., the breadth is 2½ cm., and the thickness 1 cm. These dimensions and those given above apply to the larger specimen.

The most prominent feature of the right side is the muscular area (Fig. 3-6).
The mantle on this side and over the siphons (extending back to the intestinal loops, just the oral siphon, on left side) is composed of a network of muscles without any apparent arrangement except over the siphons where they seem to be arranged quite systematically into longitudinal and circular muscles.

The circular muscles are nearer the exterior than the longitudinal. (Figs. 485)

Each muscle of the mantle is made up of a bundle of very fine fibrillae, quite loosely enclosed in a sheath of sarcolemma. (Fig. 6)

No muscular structure has been observed. In none of the sections in which muscles were cut transversely did the fibrillae appear circular. (Figs. 18, 20, 21). A vertical section of the mantle (Figs. 20, 21) shows that the majority of the muscles are situated under the inner edge, and that the outer part is composed of connective tissue.
With blood spaces in every part and bounded by thick tissue, each aperture has eight small and unequal lobes.

The oral siphon is lined by a thin layer of test extending down to the testacular ring. The tentacles are situated at the opening into the brachial chamber at the base of the oral siphon; they are placed in one very slightly bent row around muscular ring, and extend into the cavity, but appear to be able to bend back and forth through the opening. They are simple, practically of one size, about one hundred and forty in number (Fig. 7).

The atrial siphon is slightly shorter than the oral and extends a little to the right of the median line.

In Herdman's Revised Classification of the Tunicata, the author gives a note in which he makes mention of Ascidia depressella from the Malay Archipelago, which has ten lobes on each aperture (Described by Sluiter).
At its base it is provided with quite closely arranged, strong circular, transverse muscles, the sphincter, the longitudinal muscles converge toward the orifice on the inner surface of this siphon are found scattered papillae, short, becoming longer in region of sphincter with the point directed toward the aperture (Fig 5). There are more these papillae in outer half of siphon. Many of these, as well as the tentacles in oral siphon, are filled with blood corpuscles (Fig 7) the atrial cavity is a comparatively thin, space about the large branchial sac, the branchial cavity extends throughout the entire length and breadth of the body. The branchial wall is attached on the left side by septa and blood vessels, extending across the atrial cavity to the viscera. On the right side quite strong septa connect it in the same way with the mantle.
The structure of the branched wall is complex. In the innermost plane are found the internal longitudinal bar (Fig. 8) having quite regular papillae on one side, just opposite each papilla is a short duct which connects the longitudinal bar with the transverse vessel. The transverse vessels, in a plane more external, are not of exactly equal size, but there is evidently no regularity about the arrangement of the large and small vessels. In a third and still further external plane are found some of the finer longitudinal vessels and a part of the stigmata. Pan three to five appearing in each mesh. The stigmata are not uniform either in size or shape. They are ciliated (Fig. 9) and bind with branched epithelial cells. The rest of the finer longitudinal vessels and the remainder of the stigmata are arranged in rows perpendicular to the row shown in Fig. 9. The wall is thus quite thick, and
The cut ends of all stiffening longitudinal vessels in each mesh appear in cross section, to form a quadrangle with one end open (the outer surface of itself) (Fig. 10). This section cuts across end of the transverse vessels at a very obtuse angle. At 3, it passes between the ends of the transversely arranged rows of stigmata which gives the appearance of one large vessel.

A similar section in another place would show also the external transverse vessels (Fig. 11).

A fourth plane from the inner and quit near the outer surface shows a network of blood vessels (Fig. 11) 6 and 7 in Fig. 10 and 11 are the corresponding spaces between the vessels. Between each of the external transverse vessels is a short transverse vessel connecting the two rows of stiffening vessels shown in Figs. 8, 9.
longitudinal vessels which border adjacent meshes. (Fig. 11, Fig. 10, Fig. 8) shows how the section passed through one of these short connecting vessels. There is a slight variation in the branchial wall in different regions. Sometimes three or four of these short transverse vessels occur. In every case they extend beyond the fund longitudinal vessels which the connect, somewhat resembling a capillary. One of the cut septa which connect the branchial wall with the mantle may be seen in Fig. 10 (top).

The position of the endostyle is typical. It connects alternately with the peripheral groove (Fig. 12) which extends around the oral siphon, and unites again to form the epibranchial groove in the dorsal region. It is quite a deep groove with edges free and flaring.
It extends ventrally around the posterior end of the branchial sac, then forced dorsally to the oesophageal aperture. It may be seen in the wall of the oesophagus from the exterior (test removed).

The endostyle in cross-section shows a thick lining of epithelial pad which reaches nearly to the free edge of the sides (Fig. 13). This epithelial pad, on each side of the groove, is thrown into short transverse folds (Fig. 12, 13). Long flagella arise from the bottom of the groove. Short cilia are found on the sides for a short distance, about half way down and over the free edges. Branchial epithelium begins where the epithelial pad leaves off and continues over the outside to the point of union.

This condition is possibly artificial, as was observed, however, in both the formalin and picro-solutions material.
With the branchial wall and then continues ventrally around the endostyle (Fig. 13).

The ventral edge of the endostyle forms the upper wall of the atrial cavity in this region.

From the extreme posterior, median point to the oesophagus the endostyle is considerably modified; the epithelial pad is wanting; there are no long flagellated. The sides are mucosal, short, without cilia, while the very bottom is lined with short cilia (Fig. 14).

The dorsal lamina is a narrow membrane, extending from the posterior end of the oesophageal groove to the posterior end of the branchial sac. It becomes narrowest back of the oesophageal aperture. The lamina and endostyle meet at this point where the endostyle becomes modified. The left edge of the endostyle continues forward as the lamina; the right edge
Continues forward as the right and longer side of the modified endostyle, the other (left) side of the modified endostyle arises between these two at the point of divergence. (Posterior median foramen branchiodes)

Near the base of the dorsal lamina is a short fold which, with the lamina on one side, forms a more or less irregular groove, a canal. These two grooves continue forward to the oesophagus, parallel to each other, each one forming the boundary of a large dorsal groove (Fig. 14). This dorsal groove is lined with a slightly modified branchial epithelium. Short cilia are found on some side of the fold near base of lamina, and extending out from this a short distance (Fig. 11).

Continua to the oesophagus, affecting the lamina is broader, but the dorsal groove is not so wide and it is not bounded on either side by a smaller groove.
(Fig. 1) A few short cilia are found near base of laminae and also on one side of a small fold at the digital edge from laminae. The dorsal lamina is not a perfectly straight membrane but is curved to the right. The transverse cords are split left or convex side. The central portion of the rib does not extend quite half way to the free edge but a much lamellated fold or rib does continue (Fig. 13, 16). The free edge is not plain but slightly crenate.

The heart is similar to the heart of other aspidia. It is a long circular tube, extending on the ventral edge, from a point just below the end of the anterior loop of the intestine, back around the posterior end of the body to a position even with the dorsal end of the stomach.

Most of the heart is visible only on the left side, but a small portion may be seen only on the right side (Fig. 3, 8, 8a).
The wall presents an irregular, spiral or folded appearance. Although it is not the same, yet it is similar in appearance to the heart of Polyophaga antennata described by William Prittie. Outside of the heart is a light colored spherical mass (sometimes two) which moves freely from one end to the other. Apparently forced along by the pulsations. The nature of this body was not determined.

In general the blood vessels are quite well defined tubes. Numerous of these vessels are cut across in removing the branches from the vessels on the left side. Two of the largest vessels in the body lead from a point near the anterior end of the heart out into thetest (Fig. 3, a). A central vessel is

2 Glimpse of the Pacific Coast of North America. Periplan apparatus 27 (Page 37) (Issued Sept. 28, 1893)
situated beneath the endostyle (Fig. 13) and a partially defined dorsal vessel above the lining and dorsal groove (Fig. 14, 15). There are, however, blood spaces in every part of the body where the blood flows freely without being confined to definite channels. Such blood spaces are in the mantle (Figs. 18, 20, 21); in the wall of the stomach and intestines (Figs. 26 & 27); throughout the gonads, stomach, intestines, and among the glands of the reproductive organs. Throughout all these spaces and in the course of all the blood vessels appear innumerable blood corpuscles. These corpuscles are light green and, in life, in account of their great numbers, they give the body a decided green color. It is possible that they are not blood corpuscles at all, but a host of green algae, living symbiotically...
in the blood. They appear singly and collected in groups of six or more (Figs. 14, 15, 26 & 37). Many of them show nuclei. In others no nucleus can be made out. They are round but not all of equal size. Under the high power of immersion each one appears to be a colony. Probably they represent a form of protozoa.

The dorsal tubercle, the epibranchial groove, the nerv. ganglion, and the intestinal organ are all present (Fig. 7). The distance from the posterior end of the epibranchial groove to the anterior end of the ganglion is not quite equal to the distance from the same point to the dorsal tubercle. Which latter distance is the length of

One objection to considering them protozoa is the fact that after the material has been inserted, sectioned, and mounted, with staining and dehydrating process, the green color persists.
The epibranchial groove.

The dorsal tubule is situated near the tentacular ring and at the place where the pedunculated groove enters the epibranchial. It is shifted a little to the right of a line drawn longitudinally through this groove (Fig. 13). The linings of the tubules had an epithelial lining and are ciliated (Fig. 18). The character of the epithelium and the length of the cilia vary in different regions. The duct of the neural gland leads directly into the tubule dorsally. It is imbedded in the mantle near the lower surface below the anterior nerves (Fig. 13). It has an epithelial lining throughout its entire length.

The ganglion is situated dorsally about one third of the distance between the siphons. The relative position of the ganglion and neural gland is somewhat
similar to that in Ascidamentula.

The gland lies beneath the ganglia and projects beyond it at each end and on each side. (Fig. 9)

But it is asymmetrically situated: the ganglia does not lie over the median line of the gland. At the anterior end the gland projects on the left side, and not on the right, while at the posterior end the condition is just reversed: the larger mass of the gland is on the right side.

A transverse section (Fig. 10) through the anterior end of ganglia shows the gland medially on the left side, extending only to the median longitudinal line.


A similar section through the posterior end would show the same relation, but reversed position.

Blood sinuses completely surround the gland and ganglia. They are also enclosed by the atrial epithelium which lines the mouth (Fig. 20). The large duct of the neural gland is situated close to the ventral side of the ganglia; part of it lying beneath the anterior duct and also extending back beyond the posterior end (Fig. 21). The figure does not show a median section. Blood vessels of the branchial wall appear beneath and at the side of the gland (Fig. 20). The duct of the neural gland is continuous with this lumen extending directly beneath the base of the nerve ganglia. I have not been able to demonstrate a nephridial duct posteriorly, either by longitudinal or transverse sections. The nerve ganglia is elongated.
With its long axis in the long axis of the body (Fig. 19), the oesophagus is quite freely attached to the peritoneum by connective tissue. Four nerves are given off anteriorly and four posteriorly, these do not originate in the same transverse plane at either end. The ganglia extend further anteriorly on the left, and posteriorly on the right side (Fig. 17). The same general description is true for the retractor gland with regard to its shape. Fig. 22 shows the relative position of the anterior nerves, duct and gland. The different size of the nerves in this section clearly is understood by an inspection of Fig. 19.

The epibranchial groove is calcified (Fig. 18). Its inner surface of the groove is not regular but is thrown into longitudinal folds, which are apparently continuations of the perichondral bands.
The digestive tract is visible only on the left side. The intestine is large and makes a double loop which occupies all of the space dorsally and nearly all of the space antero-posteriorly, having the shape of an large S turned backward.

The oesophagus and anus are placed dorsally. The anus opens into the atrial cavity near base of the atrial siphon.

The oesophagus is situated back of the atrial siphon, a little to the left of the median line, about 2/3 of the distance from the anterior to the posterior end of the body. The oesophageal aperture opens out of the branchial cavity and becomes much smaller just outside of the branchial wall.

The dorsal groove, described above, is in direct communication with the funnel shaped oesophageal opening. After this constriction, the
Oesophagus gradually widen again as it enters the stomach (Fig. 3. a)

The stomach is an elongated circular sac, situated in the posterior part on the left side of the body, only the heart being more posterior (Fig. 3. a) The long axis is placed dorso-ventrally with either end constricted, dorsoventrally into the oesophagus, ventrally into the intestine. The posterior loop of the intestine lies over the anterior edge of the stomach. Thus giving the stomach from the exterior a crescent shaped appearance.

The inner surface of the stomach is thrown into longitudinal folds. These folds are large on the right side of the folds just described converge into the end of the pylorus. The cardiac arises at the beginning of the intestine and continues throughout its entire length. It is placed ventrally except in the loops
of the intestine, where it is situated slightly up and closer to the wall on the inside of the loop. It stops just back of the anal opening. No specialized epithelium of the stomach or intestine can be demonstrated, the anal opening is plain and not surrounded by any mucous (Fig. 2.)

In all of the observed specimens the stomach and intestine were filled with foetal matter.

Reproductive Organs

The reproductive organs are easily distinguished in the loops of the intestine (Fig. 3.) The larger space is in the anterior loop. By sectioning and examining with the microscope the anterior loop is found to contain ovaries, the posterior loop being filled with testes. Also, the gonads are imbedded in the wall of the intestine, which is made up of rather coarse, fibrovascular & connective tissue (Fig. 2.) Male gonads are also found in the wall of the
Stomach (Fig. 26)
In the wall of the intestine between the loops, both male and female gonads are found together. (Fig. 24)
The ova appear in all stages a layer of follicle cells surrounds each ovum which clearly shows the nucleus and nucleolus. (Fig. 25).
The male lobules are filled with spermatozoa. (Figs. 24, 25, 26)
The spermatozoa, seen in a cross section, of the testicles are very small, elongated and provided with a minute short flagella.

The ovary arises from a mass of small tubes which ramify among the ovaries, and follows the posterior loop of the intestine around to the anus. (Fig. 39)
The ovary differs from males in the right wall of the intestine at about the point where the ovary first becomes visible. The ova descend then
follows the course of the
oviduct, out a little below
and slightly in front of it. This
may be seen after careful
inspection.

These terminate at the
same place near the end
of nerves and have fluted
pipes (Fig. 24). In several individuals
observed the oviduct was filled with spermatozoids
and the oviduct filled with eggs. These partly
both moving at the same time.

The wall of the stomach is filled with
spherical bodies, its seminal vesicle
containing concretions. These are more
abundant in the right wall and over
the end of the typhlosole. In this region
named, after the stomach has been
stained in borax Carmin, these vesicles
are so numerous and so closely set
that they render the wall quite opaque.

Dr. Jeffrey demonstrated in the
that self-fertilization is possible
in this animal, and embryos thus
produced were raised into larvae.

The proportion of eggs fertilized in this
way is not as great, however, as in cross-
fertilization, and more abnormalities occur.
In the left wall of the stomach they appear in great numbers, but are more scattered (Fig. 27). They are also throughout the entire length of the intestine even among the glands wherever these occur (Fig. 28).

I have made only a cursory examination of the sections. Evidently the treatment given the sections containing them was not adapted to the bringing out of the details of the gland structure.

Their general appearance is that of large round vesicles with a darker staminal body enclosed. Frequently more than one capsule may be seen within a single vesicle. The surface of each vesicle shows polygonal cells of pavement epithelium (Fig. 29). Fig. 26 shows a characteristic appearance of the neural connective tissue in section.
the general structure is that of concentric rings, which, in some cases, lie so close together as to leave no space between them as seen in the figure. They do not all show a double center.

Class - Tunicata
Order - Ascidiaea
Suborder - Ascidian Simple
Family - Ascididae
Subfamily - Ascididae
Genus - Ascidia
Species - A. vindis

Methods, For total preparations, formalin was stained in boro carmin. The sections were stained with hemalun.

For Fig 19, a slight differentiation of nerve and muscle was afforded by staining with boro carmin with through washings, and afterwards staining with 10% aqueous safranin and washing out in water.
Explanations of Plates

All of the figures excepting 1, 3, 8, 9, 10 were outlined with the Camera lucida.

Plate I

Fig. 1. Whole specimen, A. cinctivincis (photograph).

Fig. 2. Cross section of test. X 52.5.

Fig. 3. Body removed from test; natural size. a, left side; b, right side.

Fig. 4. Portion of wall of oral siphon seen from inside, with the test lining removed. X 50.

Figs 5–6. Portion of atrial siphon, seen from the interior, X 8.5.

Fig. 6. Broken end of muscle from mantle, on right side. X 8.5.

Fig. 7. Pharyngeal opening and inter-biphalal organs; the oral siphon, above the tentacle, has been removed. The tentacle was then cut and spread out, with the tentacles directed outward. X 10 (seen from the interior).
Fig. 8. Portion of branchial wall seen from interior x 85
Fig. 9. Small portion of branchial wall (tangential section) x 85
Fig. 10. Transverse section branchial wall x 70
Fig. 11. Tangential section of same seen outer wall x 85

Plate II

Fig. 12. Union of endostyle and peripharyngeal groove x 70
Fig. 13. Cross section endostyle x 85
Fig. 14. Cross section, dorsal origin of branchial sac, back of esophagopharyngeal aperture x 85
Fig. 15. Cross section of same, anterior to esophagopharyngeal aperture x 85

Fig. 16. Dorsal lamina x 70
Fig. 17. Dorsal tubercle, inversion x 70

Fig. 18. Longitudinal vertical section of dorsal tubercle, showing cut side of epibranchial groove with folds (continuing peripharyngeal bands) x 70
Fig. 19. Nerve ganglion and seaweed gland, section from entering branchial wall (part of the muscles removed) X 50

Plate III

Fig. 20. Transverse section of ganglion and gland, showing also thickness of the muscle X 70

Fig. 21. Longitudinal, vertical section of ganglion and gland. (The section is not a median section) X 70

Fig. 22. Section same as Fig. 20, only more anterior. (Details not filled in) X 70

Fig. 23. Cross section of wall of stomach, showing folds on right side (of) inner surface X 70

Fig. 24. Cereus, also the end of oviclict & vas deferens X 50

Fig. 25. Portion of cross section wall of intestines, showing male & female glands in trabecula of connective tissue X 84
Reference Letters

a. = ammus
a.ep. = atrial epithelium
a.v. = ampullated blood vessel in test
a.s. = atrial siphon
b.c. = blood corpuscle
b.ep. = branchial epithelium
b.s. = blood sinus
b.v. = blood vessel
b.d. = connecting duct; internal longitudinal
b.m. = circular muscles of siphon
d. = duct of mesal gland
d.b.s. = dorsal blood sinus
d.l. = dorsal lining
d.t. = dorsal tubercle
eot. = ectoderm
e.g. = epibranchial groove
e.n. = endostyle
e.p. = epithelial pad
e.t.v. = external transverse vessel branchial
t. = fibrolle of muscle bundle
f.l.v. = first longitudinal vessel branchial
f.s. = flagellar
f.w. = nerve ganglion
f.g. = neural gland
f.h. = heart
i.l. = internal longitudinal bar of branchial wall
Reference Letters (Continued)

int. = intestine
l.m. = longitudinal muscle
de. = dorsal muscle gland
vit. = ventral muscle
n. = nerve
o. = ovary
or. = oesophagus
o.s. = oral siphon
ouv. = oviduct
p. = papilla
p.e. = pigment cell
p.p.g. = periphero-gland groove
r.c. = renal ciliation
s. = stigma of branch of wall
sel. = selengia
st. = stomach
t. = testis or testes
tcm. = transverse muscle
tn. = tentacle
t. r. = transverse vein, dorsal line
r. v. = transverse vessel of branchial wall
v.d. = vas deferens
v.s. = vessel of neural connective
v.u. = ventral vessel (blood)
This thesis is never to leave this room. Neither is it to be checked out overnight.