
13 ACORN-WORMS AND SEA SQUIRTS

PHYLUM CHORDATA: SUBPHYLUM UROCHORDATA (= TUNICATA)

Class Ascidiacea

The tunicate body is contained within a *test*, or *tunic*, secreted by the underlying *mantle*. This test may be thin, thick, clear, opaque, clean, or covered with sand or living organisms. Tunicates are represented on most rocky shores by the ascidians, which are sessile and either colonial or unitary. Some are indefinite in shape, and so hidden within their tunics that they superficially resemble sponges or some of the more fleshy bryozoans or cnidarians (e.g. *Alcyonidium* or *Alcyonium*). When cut open, however, they display a more complicated internal organization (Fig. 13.2), particularly a filter-feeding pharynx, or *branchial sac*, in which mucus is secreted by the ventral *endostyle*. The branchial sac contains perforations, the *stigmata*, in transverse rows. Transverse blood vessels pass between adjacent rows. In some genera the stigmata are arranged in spiral groups.

The feeding current enters the test via an *oral* (or branchial) opening or *siphon*, and passes into the pharynx through a ring of *oral tentacles*. Flow is generated by cilia, passing through the stigmata into the surrounding *atrium*. From there the water passes to the exterior via a dorsal *atrial* (excurrent) *siphon*. Positive pressure builds up inside the atrium, which becomes distended with water. On disturbance, contraction of mantle muscles forces out jets of water through the oral and atrial sphincters, hence the term 'sea squirts' popularly applied to larger ascidians. Unitary ascidians may be 1 cm long or more, and the largest British forms reach about 15 cm.

Colonial ascidians are made up of numerous zooids formed by *budding*. Each zooid is like a small unitary ascidian with few stigmata, but rarely exceeds a few millimeters in length. The colonies may be larger (sometimes as much as 10–20 cm). The zooids may arise from stolons or project from a common test, or be wholly immersed. If the zooids are embedded within the test they are generally arranged in systems; i.e. the atria do not open individually to the exterior but internally with others into a *common cloaca* (Fig. 13.3). The atrial openings may wholly lack siphons, or there may be a *languet* (tongue) projecting from its upper margin. The common cloaca communicates with the exterior through an irregular, circular, or elevated aperture.

Ascidians are hermaphroditic but the location of gonads within the body varies. In the order Enterogona (*enteron*, gut; *gone*, seed) they are essentially associated with the gut loop, though displaced into a *postabdomen* in the long, slender zooids of Polyclinidae. In the order Pleurogona (*pleura*, side) there is no abdomen and both the gut loop and gonads are attached to the mantle wall. Most unitary ascidians shed their eggs into the sea, whereas nearly all colonial species and many small unitary ones incubate their embryos. These will be found in the atria of most colonial species but in the basal test of Didemnidae. Nearly all hatch as tailed *tadpole larvae*.

Ascidians contain no skeletal parts and their identification depends upon features of their internal morphology, e.g. position of the gut and gonads, and structure of the branchial sac. Identification must therefore usually be preceded by dissection. Since contraction caused by death or fixatives distorts the body shape, narcotization is usually essential if diagnostic characters are to be observed. Dissection without fixation is usually impossible so that narcotized specimens have also to be fixed.

Collected specimens should immediately be placed in water, in plastic bags of appropriate size, to which is added a few crystals of menthol. Narcotization will usually occur within a couple of hours. Fix the specimens by adding one-tenth the seawater volume of commercial formalin (40% formaldehyde). Fixed specimens can then be stored in a less noxious preservative such as 1% propylene phenoxylol in distilled water, or transferred to seawater for immediate examination. The colour of specimens is likely to fade in preservative and should be recorded before fixing.

Note (draw if necessary) the external characteristics, particularly the arrangement of systems found in many colonial species. Unitary specimens should be dissected under water in a wax-based dish, observing through a dissecting microscope if necessary. Place the animal on its right side (i.e. its atrial siphon to your right); pin as and when necessary. Remove the test. This may be accomplished either by making a circumferential cut above the base (Fig. 13.2), so allowing the test to be turned inside out over the siphons, or by cutting with scissors in the mid-line, starting at the oral siphon, proceeding down the line of the endostyle, around the base, and up to the atrial siphon; thence back to the oral siphon. Carefully separate the left half of the test from the mantle and remove it. Cut and open the mantle by a dorsal cut from the base to the atrial siphon, carefully freeing the mantle from the branchial sac by cutting the connecting strands (Fig. 13.2).

Compound ascidians should be handled in a Petri dish or watch-glass on the stage of a stereomicroscope. If there are separate zooids, open them with fine scissors, cutting down from the oral siphon. In others (e.g. many polyclinids) zooids are embedded in, but easily dissected out of, the common test. Handling small zooids requires the use of fine needles, needle knives, or sharp, small-bladed scalpels, and watch-maker's forceps; spring-bow scissors are also a help. The minute, close-packed zooids of didemnids are best recovered from a few vertical slices rather than by individual dissection. Didemnid colonies may contain densely aggregated granules: some should be saved for high-power microscopy but the colony may be cleared without the generation of gas bubbles using EDTA (ethylene diamine tetra acetic acid). This is prepared as a solution of 250 g in 1750 ml distilled water buffered to pH 7 by addition of sodium hydroxide pellets.

There are two orders of Ascidiacea: Enterogona and Pleurogona. The former has two suborders.

Order 1 ENTEROGONA

The larva has left and right atrial openings which meet and fuse medially during metamorphosis to form the atrial siphon. Gonads are associated with the intestinal loop, or are postabdominal.

Suborder Aplousobranchia

Branchial sac without internal longitudinal vessels (*haploos*, simple), except in families Cionidae and Diazonidae. Body divided into thorax, abdomen, and, sometimes, postabdomen. Gut abdominal; gonads abdominal or postabdominal. Colonial except Cionidae.

Suborder Phlebobranchia

Branchial sac with internal longitudinal vessels (*phleps*, vein). Body not divided into thorax and abdomen. Gut loop and gonads beside the branchial sac, left or right. Unitary except for Perophoridae. *Perophora* has stolonate colonies and rather small zooids; rows of papillae project from the transverse vessels into the lumen of the pharynx. In other genera the papillae bifurcate and fuse with their neighbours fore and aft, to form the inner longitudinal vessels. These vessels are evenly distributed around the walls of the pharynx, which are not longitudinally folded.

Order 2 PLEUROGONA

Suborder Stolidobranchia

The atrial opening forms as a single, median invagination. Gut loop alongside the pharynx; gonads also lateral, developing in the mantle wall, usually on both sides. Branchial sac (except botryllines) with longitudinal folds (*stolidotos*, hanging in folds) and with inner longitudinal vessels usually distributed unevenly, mostly along the lateral infoldings. Unitary or colonial.

KEY TO SPECIES (Corrected August 2008)

1. Unitary ascidians occurring singly or in dense aggregations 2
 - Colonial ascidians without systems (i.e. each zooid having two externally opening apertures); zooids joined at the base by test or stolons 21
 - Colonial ascidians with systems (i.e. oral apertures opening to the surface but atria leading to an internal cavity opening by one or more common cloacal apertures); zooids completely embedded in common test 25
2. Ascidian attached to a firm substratum (e.g. rock, seaweed) 3

Ascidian in or on sand or other soft bottom **18**

3. Ascidian with soft, translucent test; loop of gut below the long branchial sac

Ciona intestinalis (p. 697)

Test firm, commonly rather opaque; loop of gut alongside the branchial sac

4

4. Ascidian somewhat rectangular in outline, laterally flattened; test transparent; gut loop to the right of the branchial sac

Corella parallelogramma (p. 705)

Shape and test not as above; gut loop to the left of the branchial sac

5

5. Ascidian elongate, tapering below to a long, slender attachment stalk; test leathery; southern Britain

Styela clava (p. 707)

Ascidian not as above

6

6. Oral siphon with 6–8 lobes; atrial siphon six-lobed; test often thick, cartilaginous; branchial sac without folds; gonads in the gut loop; oviparous (Phlebobranchia) **7**

Oral siphon with 4–6 lobes; atrial siphon four-lobed; test leathery or thin; branchial sac generally with folds; gonads in the body wall; oviparous or larviparous (Stolidobranchia) **12**

7. Surface of test with rounded swellings; southern coasts only

Phallusia mammillata (p. 707)

Surface of test smooth, rough, with irregular swellings, or with attached particles

8

8. Siphons close together; ascidian upright **9**

Siphons well separated, the atrial siphon at least one-third way down body; ascidian often recumbent **10**

9. Test cartilaginous, opaque, white to pink; branchial test vessels papillate at intersections *Ascidia virginea* (p. 705)

Test semi-transparent, often showing red pigmentation; branchial sac vessels without papillae at intersections

Ascidiella scabra (p. 705)

10. Test translucent, greyish, rough (finely papillate); branchial sac vessels without papillae at intersections
Ascidella aspersa (p. 705)
- Test not as above; branchial sac papillate at intersections **11**
11. Test thick, smooth surfaced, often pink
Ascidia mentula (p. 705)
- Test thin, greenish; smooth, rough, or covered with particles
Ascidia conchilega (p. 705)
12. Both siphons four-lobed; oral tentacles simple; folds in branchial sac four or fewer each side; stigmata straight **13**
- Oral siphon six-lobed; oral tentacles branched; 5–7 folds each side of branchial sac; stigmata in spirals **16**
13. Ascidian flat (depressed), its test tessellate (with mosaic-like markings)
Pyura tessellata (p. 709)
- Ascidian flask-shaped or, if flat, its test not tessellate **14**
14. Ascidian flat or flask-shaped; siphons usually disposed more or less symmetrically; gonad extensive, on the right side only, with large (almost 0.5 mm) red eggs
Dendrodoa grossularia (p. 707)
- Ascidian flask-shaped; siphons usually disposed asymmetrically; gonads subdivided into ‘tear-drops’, in both walls; eggs small **15**
15. Surface of test tough, leathery, wrinkled; not predominantly red
Polycarpa pomaria (p. 707)
- Test smooth, reddish; southern and western coasts only
Polycarpa rustica (p. 707)
16. Lobes of siphons fringed; ovaries (on each body wall) with short oviduct directed anteriorly (toward the six-lobed oral siphon)
Molgula complanata (p. 709)
- Lobes of siphons entire; oviduct directed posteriorly (toward the four-lobed atrial siphon) **17**
17. Branchial sac with six folds each side; oviparous
Molgula tubifera (p. 711)
- Branchial sac with seven folds each side; larviparous
Molgula citrina (p. 709)

18. Ascidian elongate, club-shaped *Pelonaia corrugata* (p. 707)
 Ascidian rounded or flask-shaped **19**
19. Body up to 80 mm; siphons well separated; test covered with particles except between the siphons *Molgula oculata* (p. 711)
 Body not exceeding 30 mm; siphons rather close **20**
20. Oviduct (on the body wall) directed posteriorly (toward the four-lobed atrial siphon); test fibrils with attached particles; may occur in aggregations *Molgula tubifera* (p. 711)
 Oviduct directed anteriorly (toward the six-lobed oral siphon); test completely covered with particles *Molgula occulta* (p. 710)
21. Zooids minute (<5 mm high), lentil-shaped, shortly stalked, well separated, linked by stolons; test translucent; gut loop beside the branchial sac *Perophora listeri* (p. 705)
 Zooids taller than wide, joined basally, translucent; gut below the branchial sac **22**
 Zooids upright or flattened, joined by stolons or lateral expansions of test; test opaque, yellow to reddish **24**
22. Zooids up to 20 mm high, united only at the very base by short stolons *Clavelina lepadiformis* (p. 697)
 Zooids arising from, and partially immersed in, an encrusting base; free portion <20 mm **23**
23. Basal mass thick, colonies often >10 cm across; oviparous *Diazona violacea* (p. 697)
 Basal incrustation up to about 5 mm thick, colonies small; larviparous *Pycnoclavella aurilucens* (p. 697)
24. Colony encrusting; zooids closely packed, usually depressed *Distomus variolosus* (p. 709)
 Colony composed of more or less separated, upright zooids budded from stolons *Stolonica socialis* (p. 709)

25. Colony cushion-, mound-, or club-shaped; zooids long and slender (the key now uses characters of zooids, which should be dissected out with cuts through the test parallel to them) **26**
- Colony flat, encrusting (rarely a lobe); zooids short **35**
26. Colony a flat cushion or mound, *not* pear-shaped with a narrow base or a distinct stem **27**
- Colony pear- or club-shaped, narrowed at the base or with a distinct stem; or of lobes that are taller than wide **32**
27. Zooids comprising thorax and abdomen; embryos brooded in an embryo sac (diverticulum of the atrium); oral siphon with six shallow lobes
- Distaplia rosea* (p. 698)
- Zooids comprising thorax, abdomen, and postabdomen, with gonads in the postabdomen; embryos brooded in the atrium; oral siphon with six or eight triangular lobes **28**
28. Oral siphon with eight lobes *Sidnyum elegans* (p. 699)
- Oral siphon with six lobes **29**
29. Stomach surface smooth *Polyclinum aurantium* (p. 699)
- Stomach surface tuberculate *Synoicum pulmonaria* (p. 702)
- Stomach with linear folds **30**
30. Colonies to 60 mm diameter, with three or four well-defined systems of zooids, each surrounding a common cloacal aperture *Aplidium nordmanni* (p. 699)
- Colonies not >40 (usually not >20) mm across, each cushion comprising a single system of zooids **31**
31. Colony heavily encrusted with sand, occurring on rock faces; atrial opening of zooid overhung by a triangular flap (languet) *Aplidium densum* (p. 698)
- Colonies mostly free of sand, generally on algae; atrial opening without languet *Aplidium pallidum* (p. 699)
32. Oral siphon with six lobes **33**
- Oral siphon with eight lobes **34**

33. Stomach surface smooth *Polyclinum aurantium* (p. 699)
- Stomach with six linear folds *Aplidium punctum* (p. 699)
- Stomach with about 30 vertical columns of tubercles *Aplidium proliferum* (p. 699)
34. Colony of reddish capitate lobes up to at least 40 mm in length; stomach with prominent tubercles *Morchellium argus* (p. 699)
- Colony a cluster of short lobes, each not >20 mm when intertidal; stomach with linear folds, these continuous or broken into short lengths *Sidnyum turbinatum* (p. 699)
35. Colony soft or gelatinous, not containing small, spherical, calcareous granules; the zooids often visible internally or of contrasting colour to the test (beware of some forms of *Trididemnum tenerum* which are mauve-purple and almost devoid of granules) **36**
- Colony containing small, spherical, calcareous granules, often so densely packed that the colony is uniformly or streakily white **39**
36. Colony greyish, translucent, often soft **37**
- Colony firm, the zooids often vividly contrasting in colour to the test **38**
37. Colony thin (<2 mm), transparent; intertidal and just below, often on algae *Diplosoma listerianum* (p. 702)
- Colony thicker (4 mm), opaque; subtidal, especially on rock surfaces *Diplosoma spongiforme* (p. 702)
38. Zooids arranged in small, star-shaped clusters *Botryllus schlosseri* (p. 709)
- Zooids arranged in sinuous parallel lines *Botrylloides leachii* (p. 709)
39. Colonies white to violet, densely packed with granules; oral openings of zooids not very apparent; zooids with four rows of stigmata; larvae with two fixatory papillae (the indistinguishable *D. coriaceum* has larvae with three fixatory papillae) *Didemnum maculosum* (p. 702)
- Colonies white, densely packed with granules; oral openings of zooids conspicuous at the surface; zooids with four rows of stigmata; larvae with three fixatory papillae *Lissoclinum perforatum* (p. 703)

Colonies variable in colour (depending on the abundance of granules), whitish through mauve to black, with granules generally few (when abundant the colony resembles *Didemnum maculosum*); oral openings of zooids not very apparent; zooids with three rows of stigmata; larvae with three fixatory papillae

Trididemnum cereum (p. 704)

(The species included in this triplet can be difficult to separate.)

This is the corrected key from Chapter 13 of Hayward & Ryland (1995) *Handbook of the Marine Fauna of North-West Europe*. OUP, Oxford.