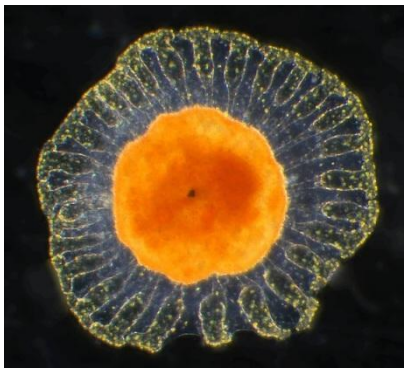
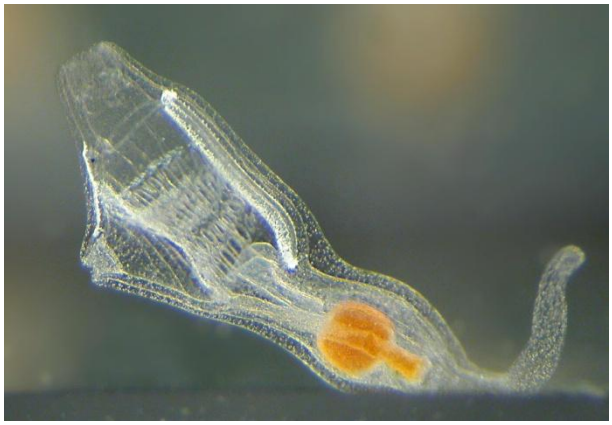
Guide to early post-settlement stages of fouling marine invertebrates in Britain







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Introduction

The early post-settlement stages of marine invertebrates often differ markedly from later stages, and may not be recognisable from conventional identification books, which typically focus on the adult stage. Yet the identification of early-stage settlers is necessary when studying recruitment onto solid structures (and its prevention) or monitoring settlement panels to detect colonization by non-native species. This guide is intended as an aid to identification of post-settlement stages of fouling species, based on those encountered in western Great Britain. It shows a bias towards ascidians and bryozoans, and to non-native species, in both respects reflecting our own interests. Some other groups are included, but others, such as sponges, are not. We hope to fill some of the gaps in later versions. We do not deal with larval (pre-settlement stages), except in some compound ascidians in which the brooded larva can be seen in colonies and helps to distinguish similar species and/or to explain the distinctive anatomy of the metamorph.

For each species, developmental stages are presented in chronological order. We have mostly illustrated living specimens. Specimens preserved in alcohol or formalin can differ considerably in appearance from live material, and it would be ideal to illustrate both conditions; some examples are shown for comparison. Differences between live and preserved specimens are particularly marked in ascidians, in which the increased opacity of preserved tissue can assist observation, while strong contraction associated with preservation can profoundly alter the shape of a specimen (see *Ciona intestinalis* figure K). Many of the images used are of specimens settled onto transparent substrates (generally Petri dishes) photographed using lighting from underneath, with either dark-field or bright-field illumination (compared in figures G and H of the bryozoan *Schizoporella japonica*). This reveals anatomical detail in partially transparent specimens, but is not a view available when scoring settlement onto opaque materials.

We deal with several species recently arrived in the NE Atlantic which may not yet be included in conventionally published identification guides, although the second edition of the Handbook of the Marine Fauna of North-West Europe (PJ Hayward & JS Ryland, Oxford UP, 2017) goes a long way to filling this gap. A range of online resources and booklets for the identification of the adult stage of non-native species is also available, including a guide that is also available as a PDF for download at http://www.mba.ac.uk/fellows/bishop-group-associate-fellow#b18 or as a waterproof booklet.

Information on early stages of NW Atlantic ascidians and bryozoans (including many species also found on the Atlantic coast of Europe) can also be found in ‘A Guide to the Larval and Juvenile Stages of Common Long Island Sound Ascidians and Bryozoans’ by Stephan Bullard and Robert Whitlatch, available at http://web2.uconn.edu/seagrant/publications/marineed/ascidian-guide.pdf .

We would be glad to receive corrections, suggestions and constructive criticism. We hope to produce further improved and expanded versions in the future.

Glossary

Ancestrula: Founding zooid of a bryozoan colony, arising from the metamorphosis of a larva.

Avicularium: Non-feeding zooid in a bryozoan colony in which the zooidal closure (operculum) is modified into a jaw-like structure.

Colonial: Descriptive of species growing from the founding individual into an array of interlinked modules sharing the same genotype (zooids or polyps; the colony's multiple 'individuals') by a vegetative budding process (cf. unitary).

Lophophore: The tentaculate feeding apparatus of a bryozoan zooid.

Metamorph: General term for the product of metamorphosis; used here for convenience where the standard terminology for the group is not applicable, e.g. the cog- or star-shaped early phase seen in the unitary ascidian *Asterocarpa humilis* and the two-zooid colony arising directly from the larva of the colonial ascidian *Diplosoma listerianum*.

Oozoid (or oozooid): Founding zooid of a tunicate (here, ascidian) colony, arising from the metamorphosis of a larva.

Operculum: The lid or closure of the zooid of a cheilostomate bryozoan, raised when the lophophore is protruded.

Unitary: Descriptive of species remaining as a single individual derived from the larva, not colonial (q.v.); in ascidians, also called solitary, although 'solitary' ascidians can occur in dense aggregations.

Zooid: module or 'individual' of a bryozoan or ascidian colony.

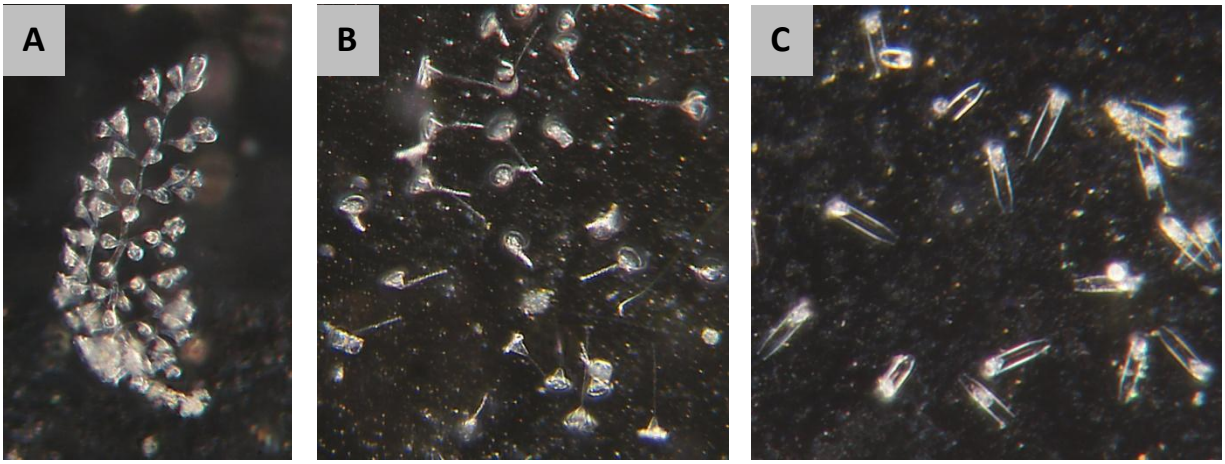
Abbreviations

LTO: Lateral-thoracic organ (producing the calcareous spicules of didemnid ascidians)

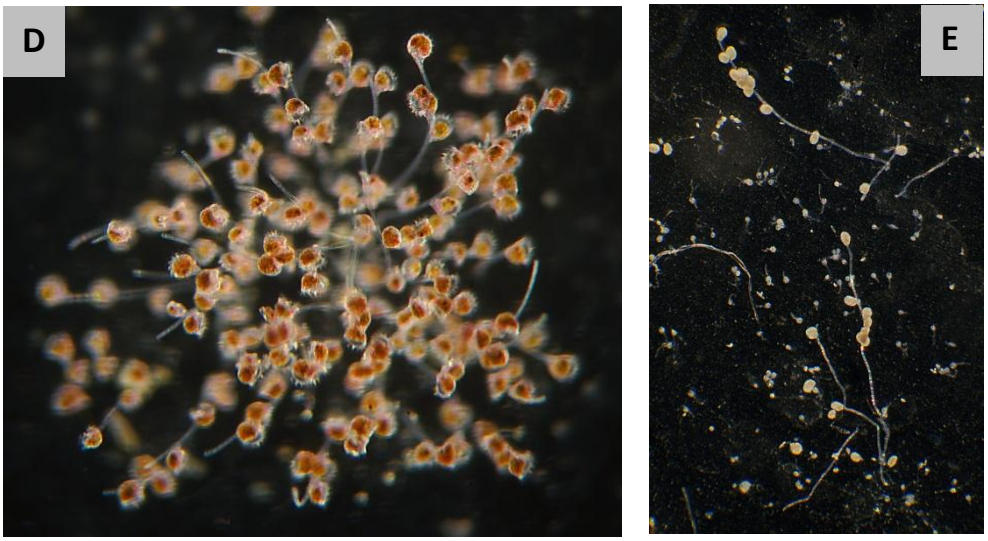
SEM: Scanning electron microscopy

Protists

Although not strictly within the remit of this guide, a small selection of fouling unicellular and colonial protists is illustrated here, because such forms can be mistaken for animal species or their propagules; for instance, the colonial form shown as **A** suggests a diminutive hydroid.

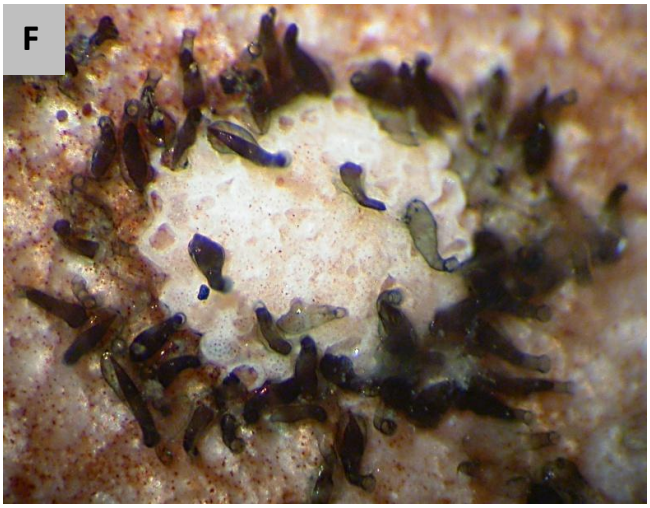


A: Colonial ciliophoran. Shown expanded; the 'stems' are strongly (and very rapidly) contractile.
B and **C:** Unicellular ciliophorans. The tube-forming species in **C** attaches temporarily but can swim with its tube.



D: Stalked colonial ciliophoran. **E:** The same species after preservation (also showing smaller, unicellular, ciliophorans); height of image represents c. 3.5mm.

Protists continued



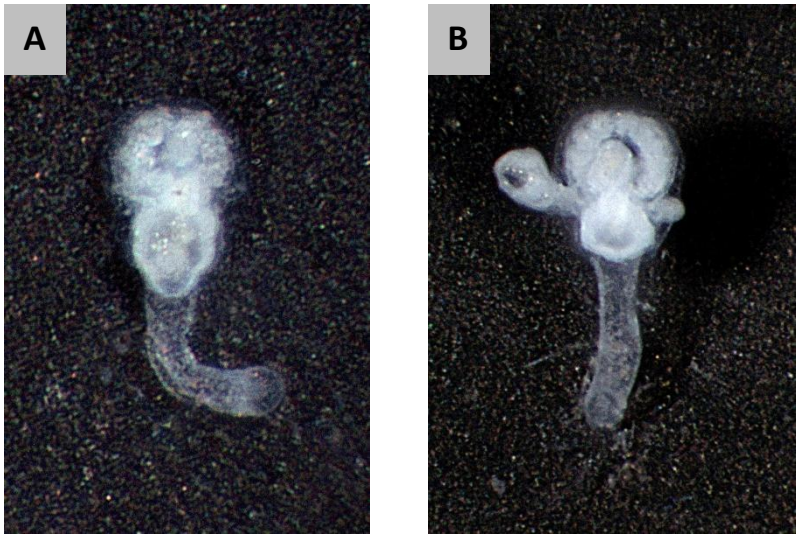
F: Unicellular folliculinid ciliophorans growing around and on a white foraminiferan; each folliculinid possesses a bi-lobed, ciliated structure that can be extended from the flask-shaped covering (= lorica) for feeding, but all are withdrawn in this photograph. **G:** Three folliculinids on a barnacle shell; these are preserved in ethanol, but may have been moribund before preservation; attached portion of lorica c. 0.35 mm.

H: Foraminiferan

Athecate hydroid

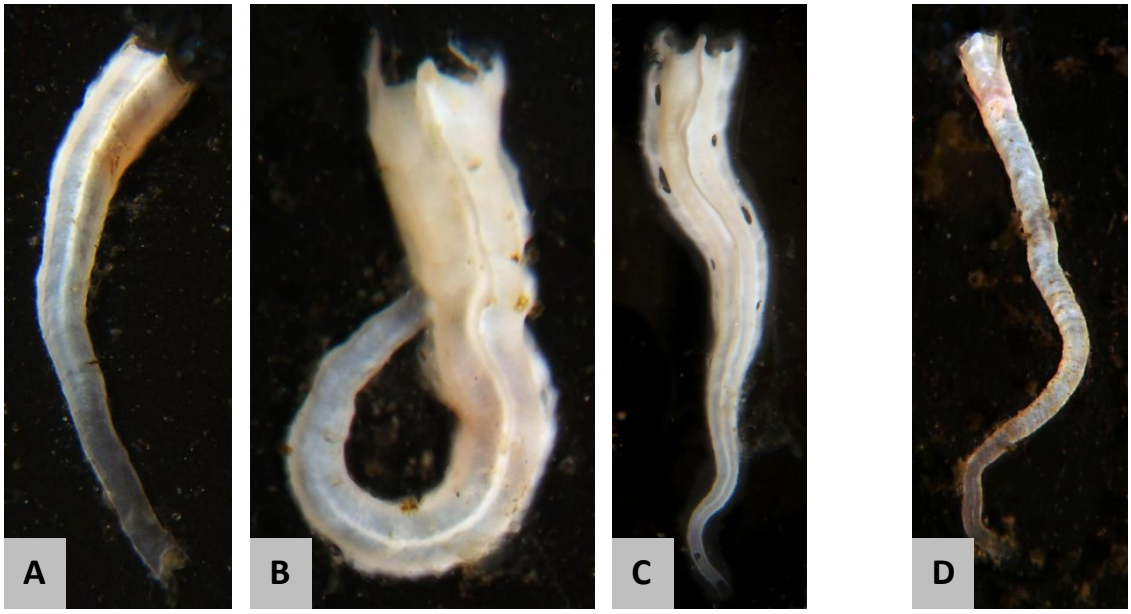
A single feeding polyp (hydranth) of an athecate hydroid, overall length c. 2.5 mm, presumably recently derived from the larva. Species unknown (specimen collected from the wild), but the capitate oral and aboral tentacles match those of the actinula larva of *Ectopleura larynx*, and annulations on the stem as seen here, occur in the grown colony of *E. larynx*.

Loxosomatid entoproct



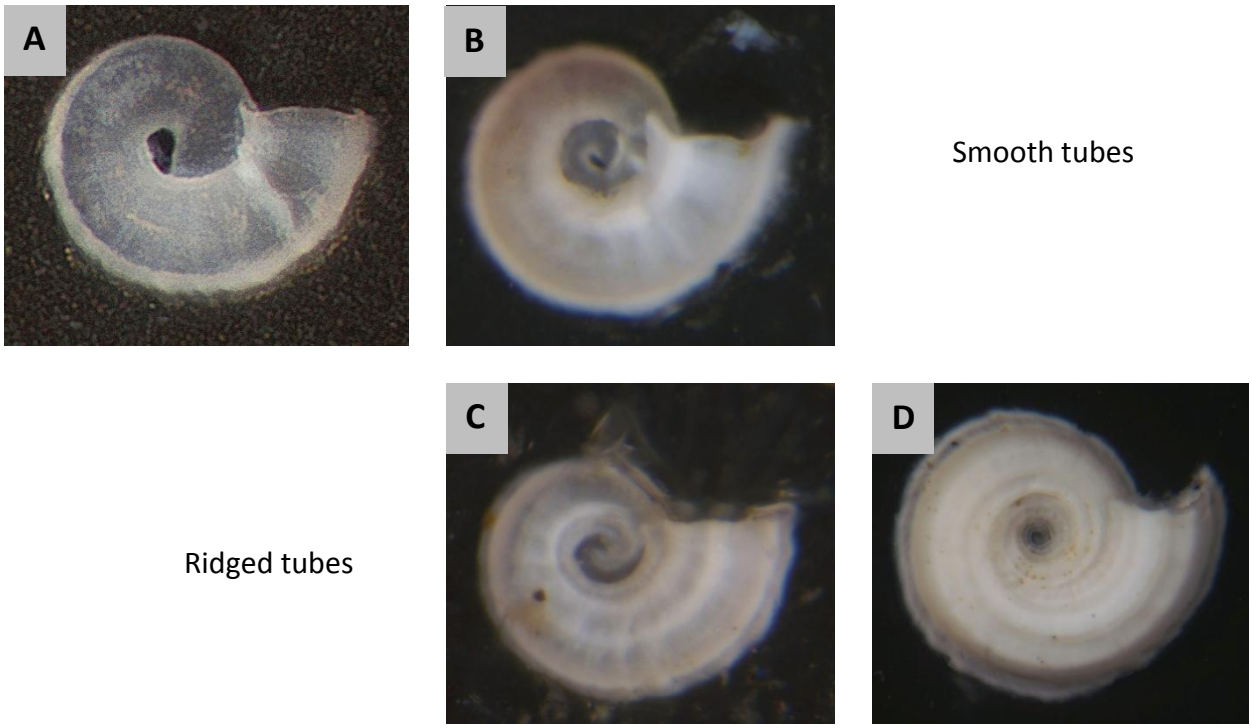
A: Presumed relatively young specimen lacking buds, length c. 0.33 mm. **B:** Presumed older specimen with two, unequal-sized, buds; length in foreshortened view c. 0.5mm. Both preserved; the tentacles in the near-circular feeding array of each specimen appear to be tightly curled up. This group typically occurs as commensals associated with other marine invertebrates, but these specimens occurred on a poorly colonized polypropylene settlement panel.

Serpulid tube worms



A: *Spirobranchus* sp., c. 1.7 mm long. **B**: *Spirobranchus* sp., c. 1.8 mm long. **C**: *Spirobranchus* sp., c. 5.2 mm long. **D:** Non-ridged tube, presumed *Hydroides* sp., c. 2.9 mm long. (Stated lengths are maximum linear span.)

Spirorbid tube worms

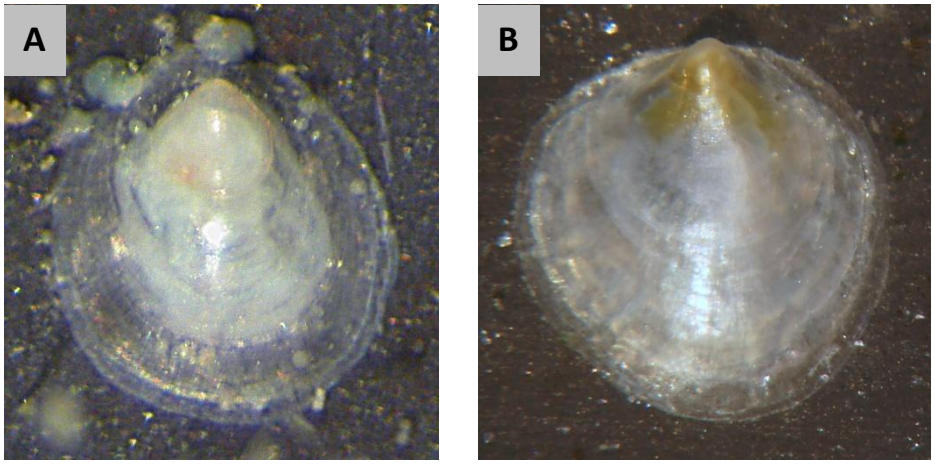


Smooth tubes

Ridged tubes

A: c. 0.26 mm wide. **B**: c. 0.6 mm wide. **C**: c. 0.5 mm wide. **D**: c. 2.0 mm wide. Provisional classification of young specimens can be made on anti-clockwise (as shown) vs. clockwise coiling; it may also be possible to classify specimens as ridged- vs. smooth-tubed at a relatively early stage (**B** cf. **C**, specimens of similar small size).

Saddle oysters (Anomiidae)



A: Early stage. **B:** Later stage, c. 1.3 mm across.

Saddle oysters are permanently attached by a calcified byssal thread passing through a deep, rounded notch in the lower valve. This means that the shell can be made to move slightly by applying gentle sideways pressure, unlike true oysters in which the lower valve is firmly cemented to the substrate.

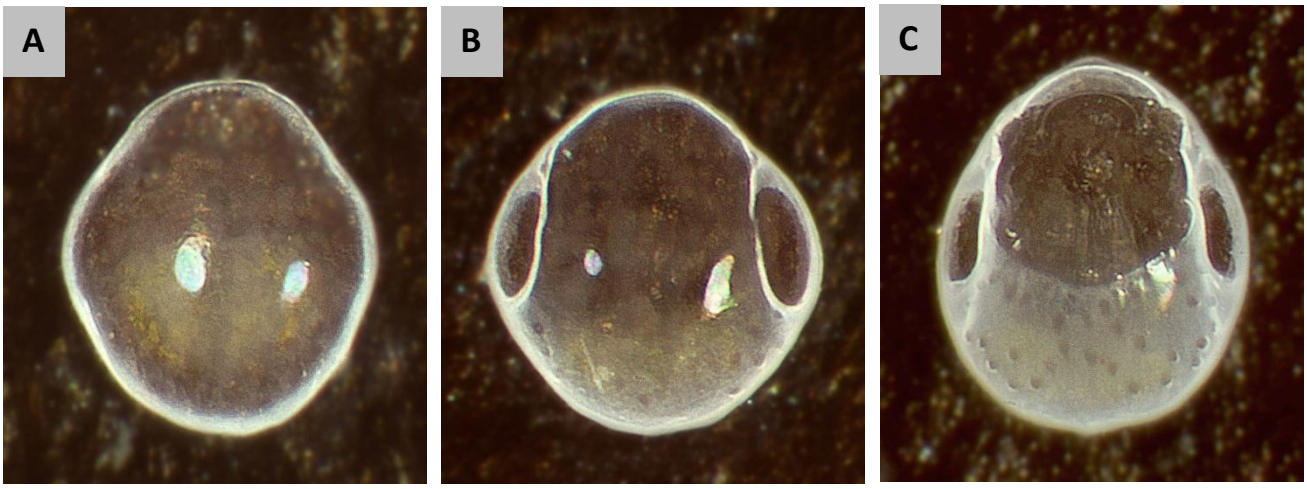
Preliminary notes on bryozoans

Bryozoa have a planktonic larval stage that eventually attaches to a substrate and undergoes metamorphosis into a small, sessile suspension-feeding animal, the ancestrula. This is the first zooid (module, or 'individual') of the bryozoan colony: after a period of feeding, the ancestrula buds one or more daughter zooids, which in turn bud, and the asexual budding process snowballs to produce a colony of many—potentially hundreds of thousands—interlinked zooids, each typically less than a millimetre long. The part of the zooid closest to the colony origin is referred to as proximal while the opposite end, closest to the extremity of the colony, is distal. The ancestrula and each of the subsequent feeding zooids (autozooids) each have a tentacular feeding organ called the lophophore, which can be extended through a distal orifice which is often delimited by a hinged operculum. In many species additional, non-feeding zooid types are specialized for reproduction, colony attachment, or defending the colony against fouling or grazing, the last being termed avicularia. Many species incubate their embryos in more or less globular structures called ovicells. Colonies often grow as a thin, typically one-zooid–thick, layer of zooids encrusting the substrate, or rise from the substrate as a branching arborescent structure attached by a narrow base. Non-calcified species are represented here by *Amathia* sp., while the remainder included are calcified to a greater or lesser degree.

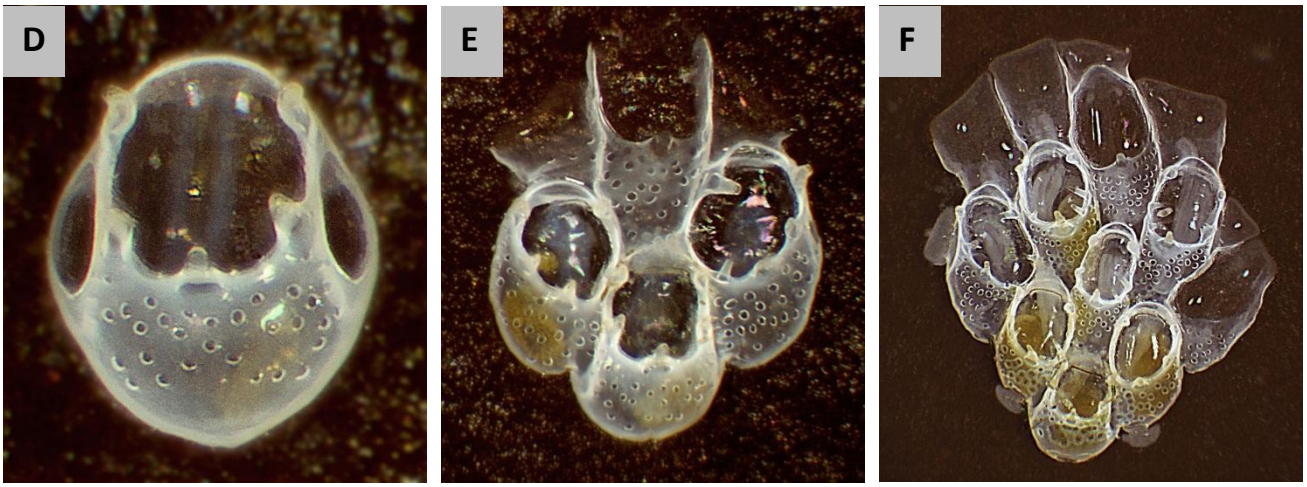
The bryozoan *Celleporella hyalina*

Small colony, c. 0.94 mm across, showing typical single-sided budding from the ancestrula and the subsequent spirally-budded pattern of autozooids in contact with the ancestrula, in which successive zooids are budded from the same side of the preceding autozooid (here, the right side).

The bryozoan *Electra pilosa*

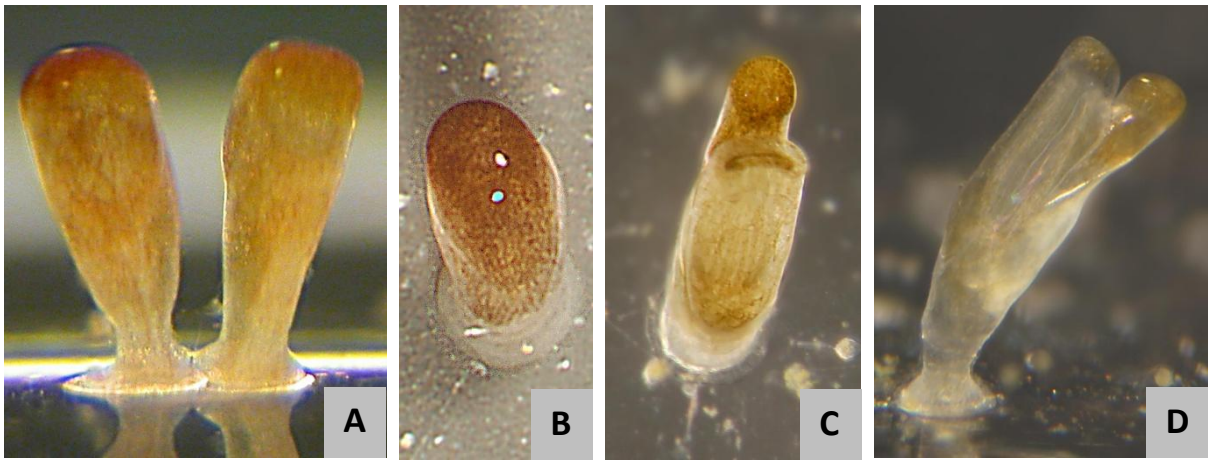


A: Early stage in development of ancestrula, c. 0.37 mm long. **B:** Later stage with paired pore chambers becoming defined, c. 0.37 mm long. **C:** Later stage with frontal calcification almost complete, c. 0.40 mm long. (The two triangular valves of the cyphonautes larva of *E. pilosa*, flattened out, may cover the early ancestrula, but are not shown here.)

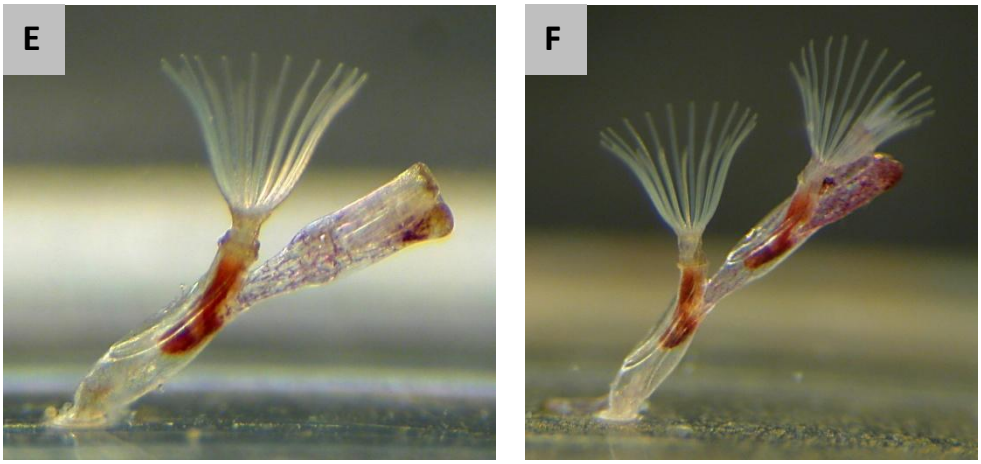


D: Completed ancestrula with lophophore retracted, c. 0.41 mm long. **E:** Ancestrula with two complete daughter autozooids budded from its lateral pore chambers, and a part-formed third autozooid; colony c. 0.69 mm across. **F:** Young colony with six completed autozooids and several more in progress; colony c. 1.3 mm across; from their proximal ends the first two daughter zooids have just initiated budding zooids which will also flank the ancestrula but will face in the opposite direction to the existing zooids, initiating a second growth axis within the colony.

The bryozoan *Bugula neritina*



A: Two developing ancestrulae, side view. **B:** Ancestrula from above; specimen c. 0.23 mm across. **C** and **D:** Later ancestrula with orifice, retracted lophophore and bud of daughter zooids.

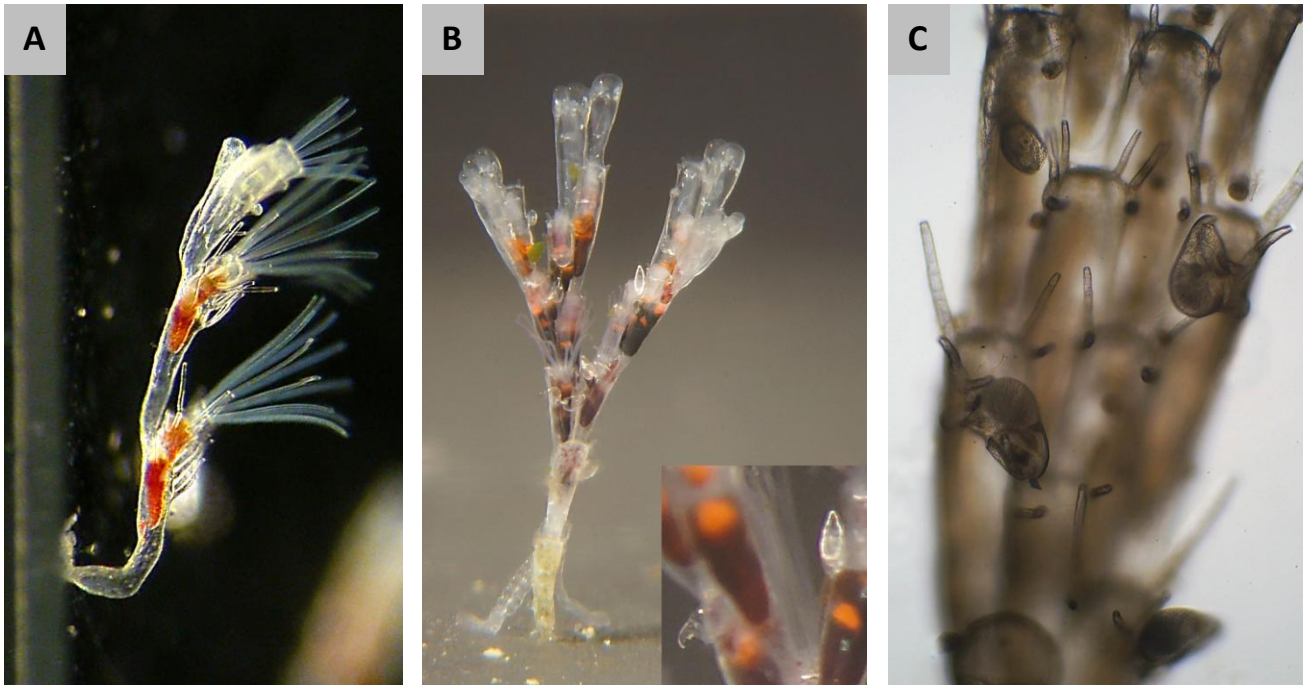


E: Ancestrula with lophophore extended, and incomplete daughter zooids. **F:** Same colony 3 days later, one daughter zooid feeding.

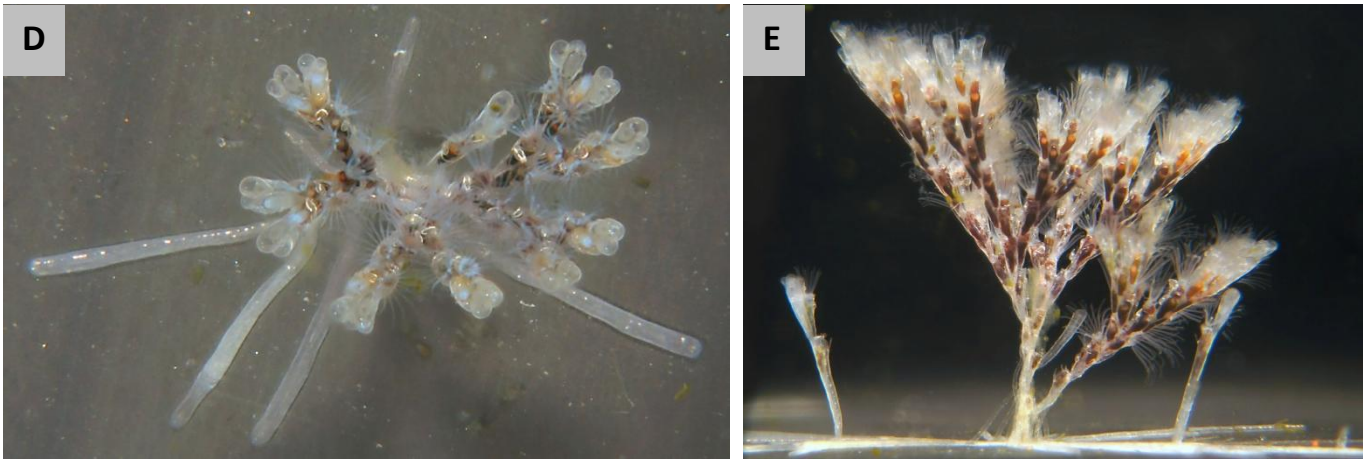
G: Cluster of young colonies resulting from co-settlement of larvae, c. 3 weeks after settlement.

Bugulina species

These figures exemplify a suite of *Bugulina* species, formerly referred to *Bugula* (along with other species now transferred to *Crisularia*), that differ from *Bugula neritina* in having: spines adjacent to the membranous area of the zooids, including the upright ancestrula; 'bird's-head' avicularia; and often, unlike *B. neritina*, colony branches with more than two rows of zooids.

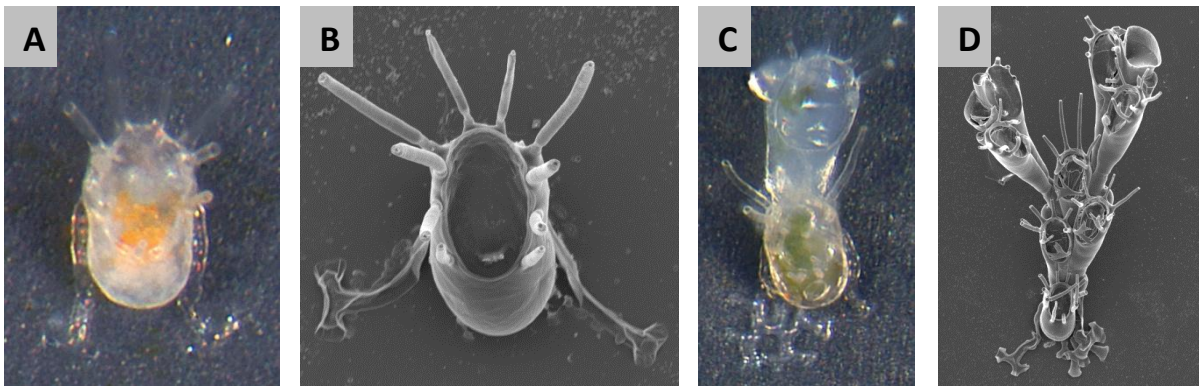


A: Ancestrula and first two generations of daughter zooids. **B:** Young colony, c. 3.6 mm tall; inset, bird's-head avicularia in front and side view. **C:** Spines and bird's-head avicularia on a branch with four rows of zooids.

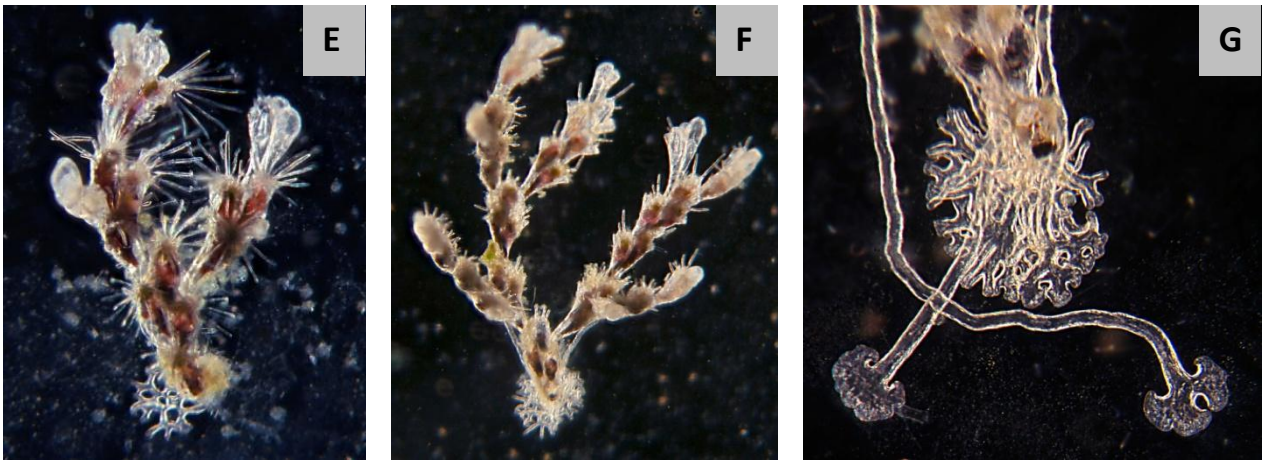


D: Small colony from above, showing attachment stolons; shining white specks are bird's-head avicularia; maximum span of stolons is c. 6.1 mm. **E:** Colony with secondary sub-colonies arising adventitiously from attachment stolons; main colony c. 5.8 mm tall.

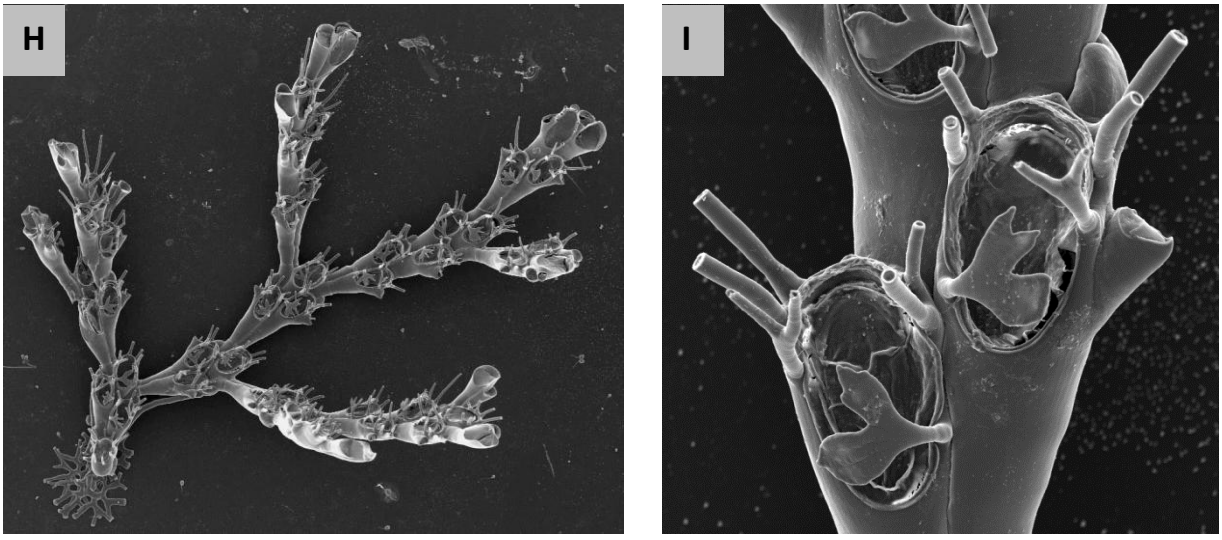
The bryozoan *Tricellaria inopinata*



A: Ancestrula, c. 0.23 mm long. **B:** Ancestrula (SEM), c. 0.23 mm long. **C:** Ancestrula and first daughter zooid, c. 0.47 mm long. **D:** Colony with first bifurcation (SEM), c. 0.72 mm across.

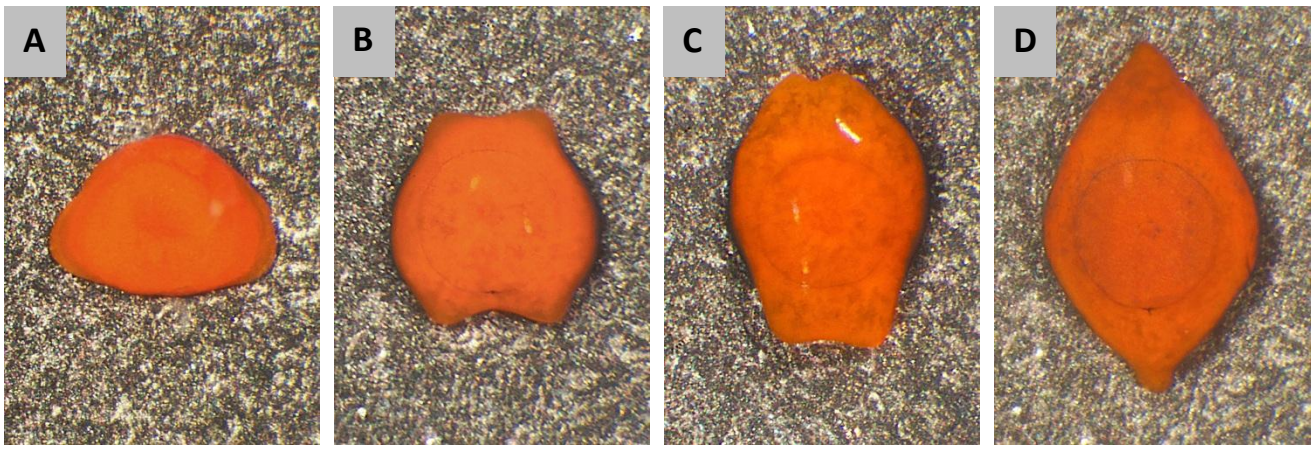


E: Young colony with first bifurcations, c. 1.2 mm across; lophophores extended. **F:** Colony with further branches, c. 2.9 mm across. **G:** Attachment plaque at base of colony, with accessory attachments formed by rhizoids growing down from higher zooids; image area c. 1.4 mm across.

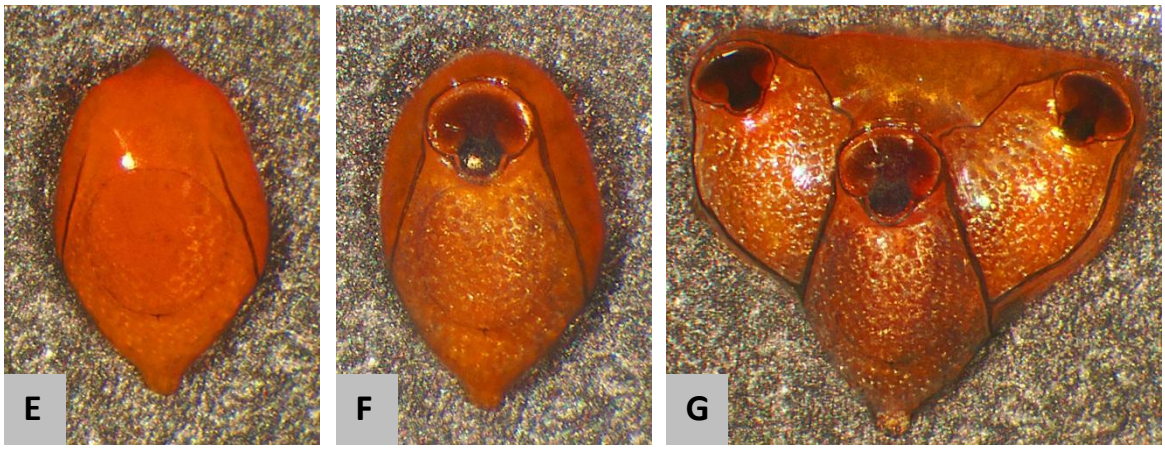


H: Colony with c. 50 zooids (SEM), maximum extent c. 3.7mm. **I:** Zooids of branch of immature colony (SEM), showing flattened spine (characteristically very variable in shape) on inner margin of zooid and bifid spine (only sometimes present) on outer margin, regular cylindrical spines, and triangular lateral avicularium (only sometimes present); branch c. 0.24 mm across.

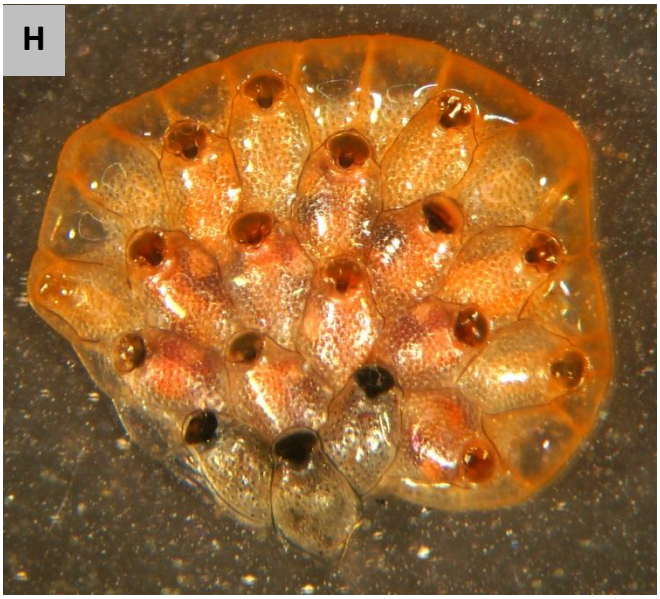
The bryozoan *Watersipora subatra*

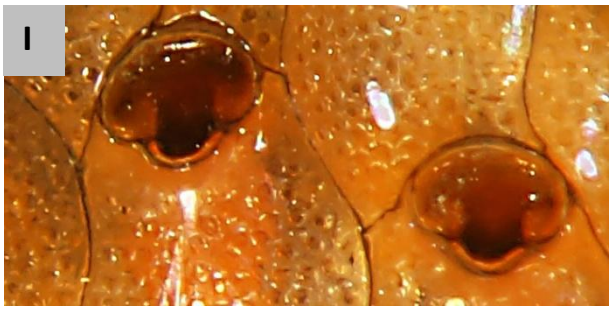


A and **B:** Initial stages completed within first 12 h following settlement. **C:** Stage often reached within 12 h of settlement and generally passed by 36 h. **D:** Stage seen 1-2 d after settlement.



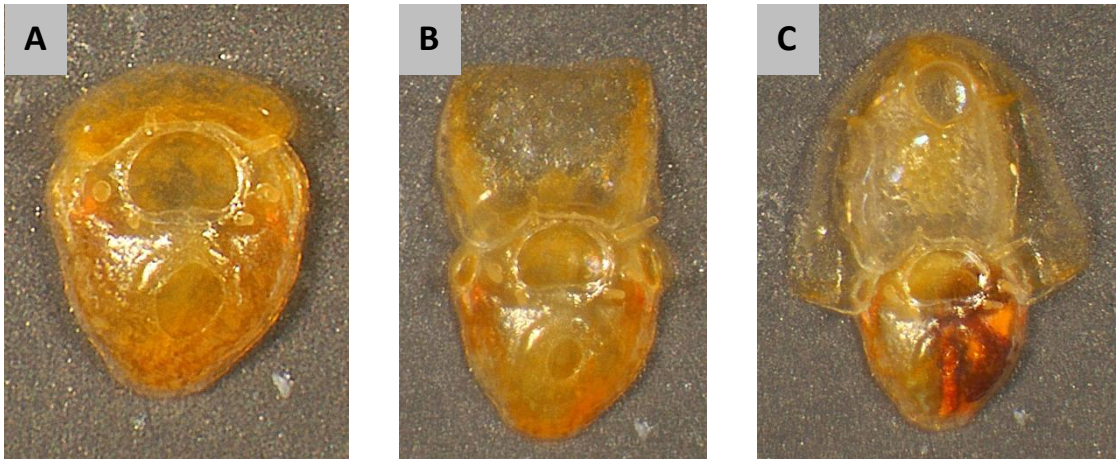
E: Stage seen 2-4 d after settlement. **F:** Completed ancestrula, reached from 3.5 d after settlement; capable of feeding; the origins of the daughter zooids are present. **G:** Young colony with first two daughter zooids completed. In **A-F**, height of image equivalent to 0.9 mm.



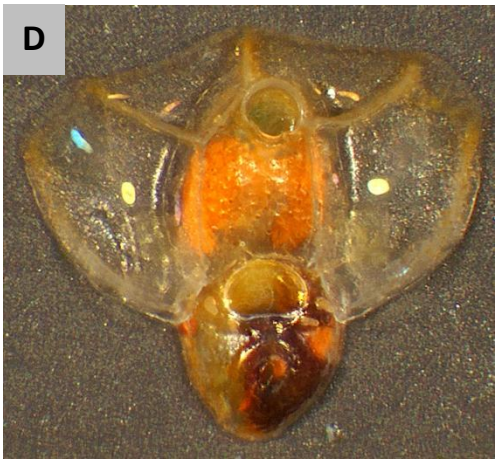


H: Colony c. 2.9 mm wide, ancestrula and first daughter zooids moribund (darkened). **I:** Detail of opercula.

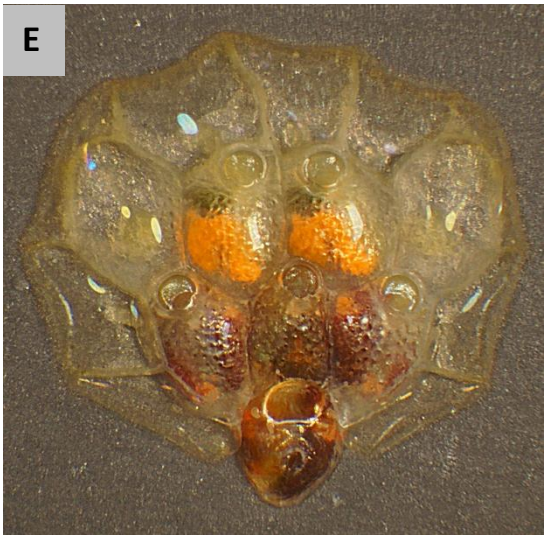
The bryozoan *Schizoporella japonica*

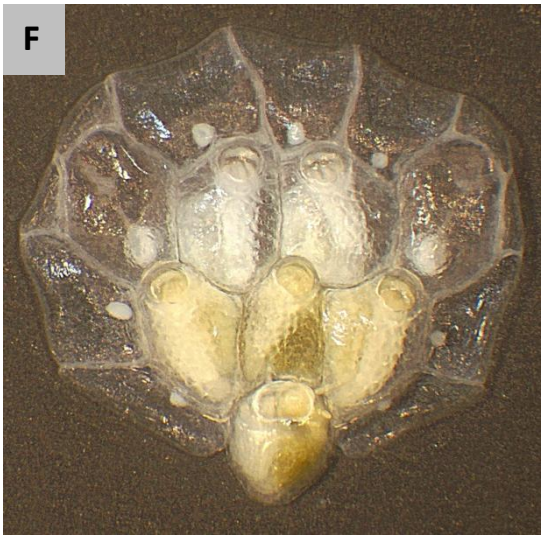


A-C: Ancestrula and developing first daughter zooid, **A** c. 0.46 mm long.



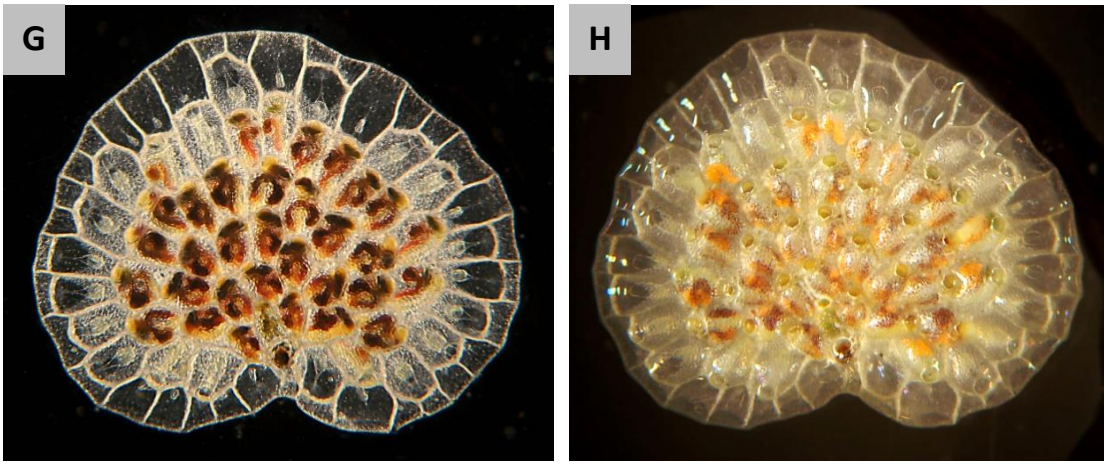
D: Later colony, with first daughter zooid complete and four further zooids initiated, c. 1.0 mm across.



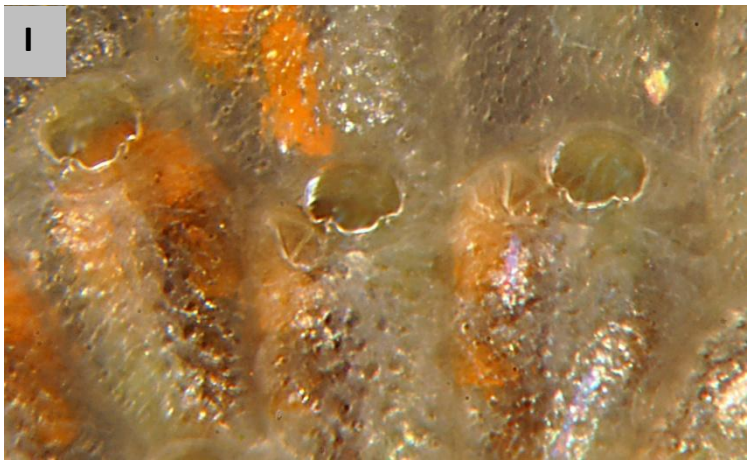


E: Later colony with five completed daughter zooids and fringe of developing zooids, live, c. 1.6 mm across. **F:** The same colony preserved in ethanol.

Schizoporella japonica continued



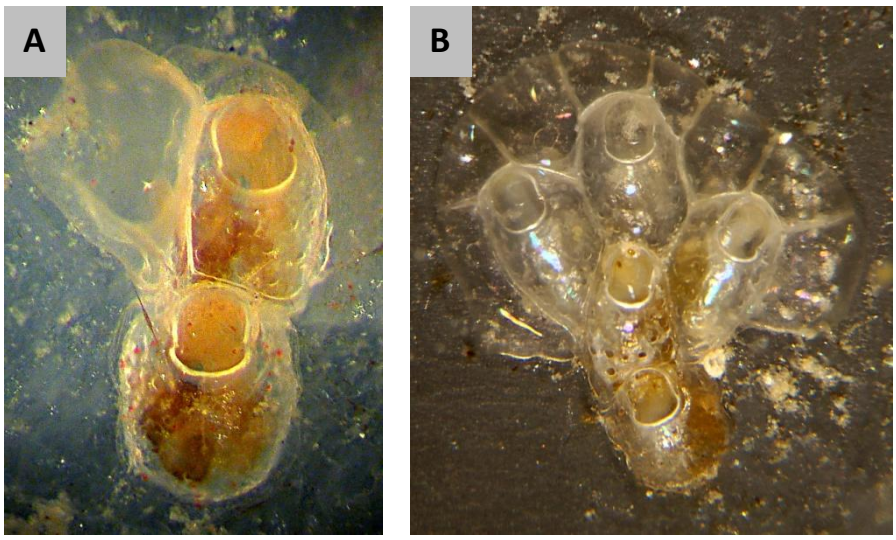
G: Larger colony on transparent substrate, c. 4.4 mm across, dark-field illumination. The ancestrula is now enclosed by developing zooids. **H:** The same colony, incident illumination.



I: Zooids from colony shown in **G** and **H**.

Other *Schizoporella* species resemble *S. japonica*, and identification generally requires assessment of well-grown colonies for details of features such as ovicells and frontal avicularia. The rather straight proximal margin of the orificial sinus often seen in *S. japonica*, and shown in **F,** is evident in small colonies.

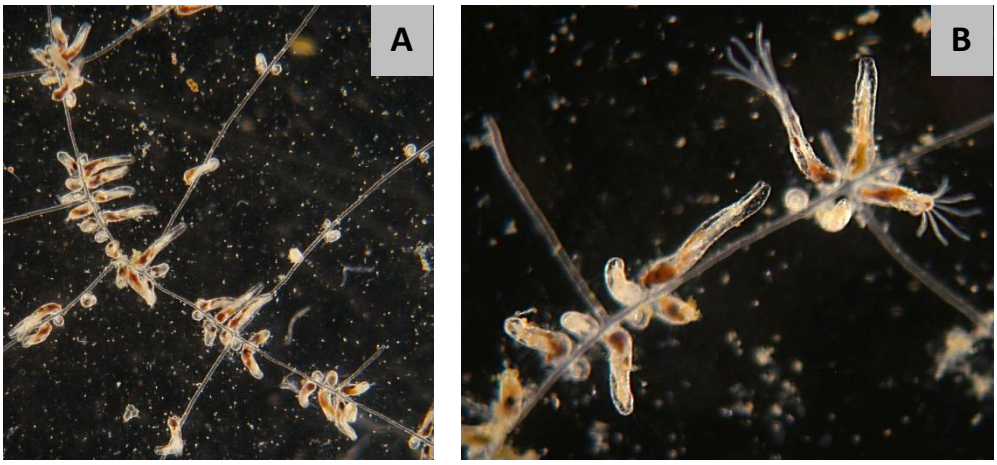
The bryozoan *Cryptosula pallasiana*



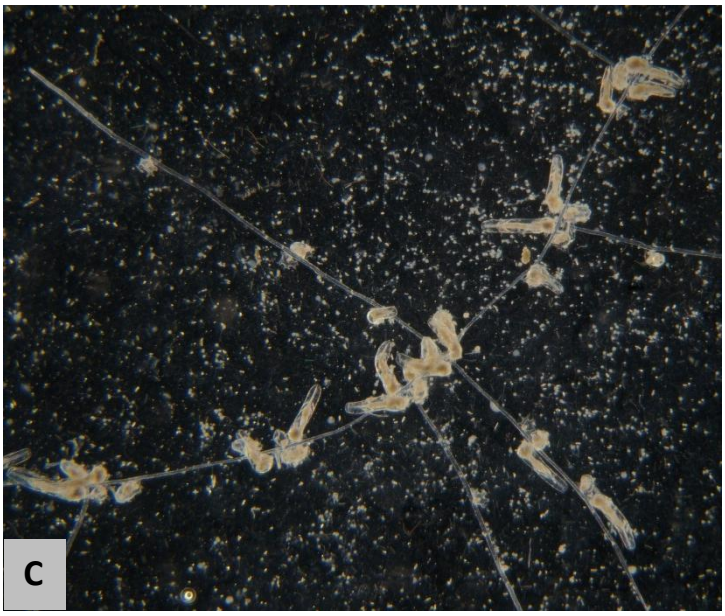
A: Ancestrula and first daughter zooid. **B:** Five-zooid colony (with additional developing zooids) c. 1.5 mm long.

C: Colony of c. 35 zooids, c. 5.0 mm across.

The bryozoan *Amathia* sp.

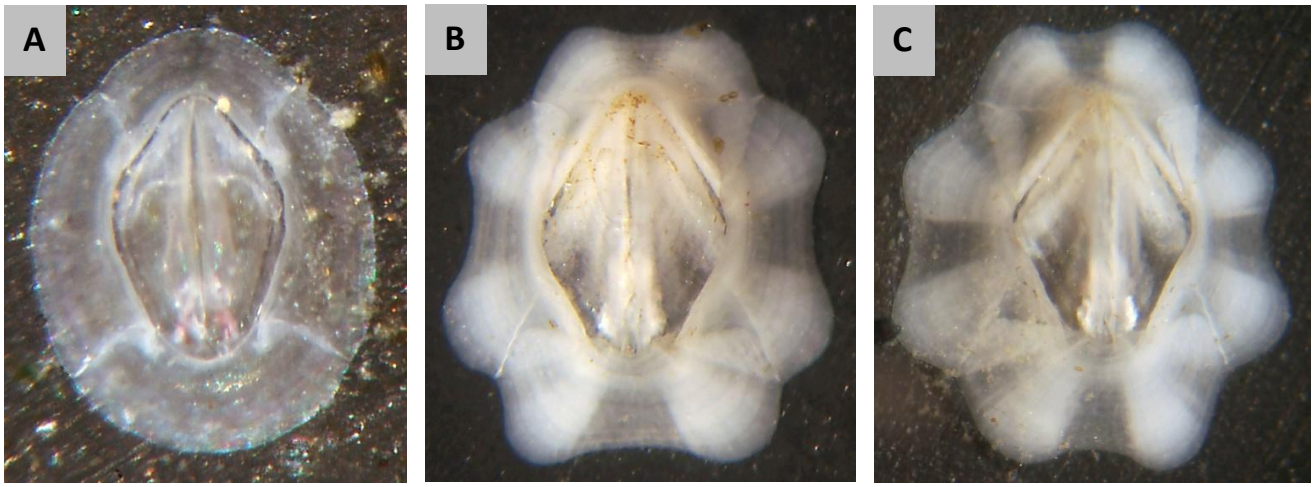


A: Part of a young colony of a creeping species of *Amathia* with the autozooids in loose groups, mostly at branches in the stolon system; photographed live. **B:** Enlargement of zooids. The presence of eight tentacles in the lophophore suggests *Amathia* cf. *gracilis*.



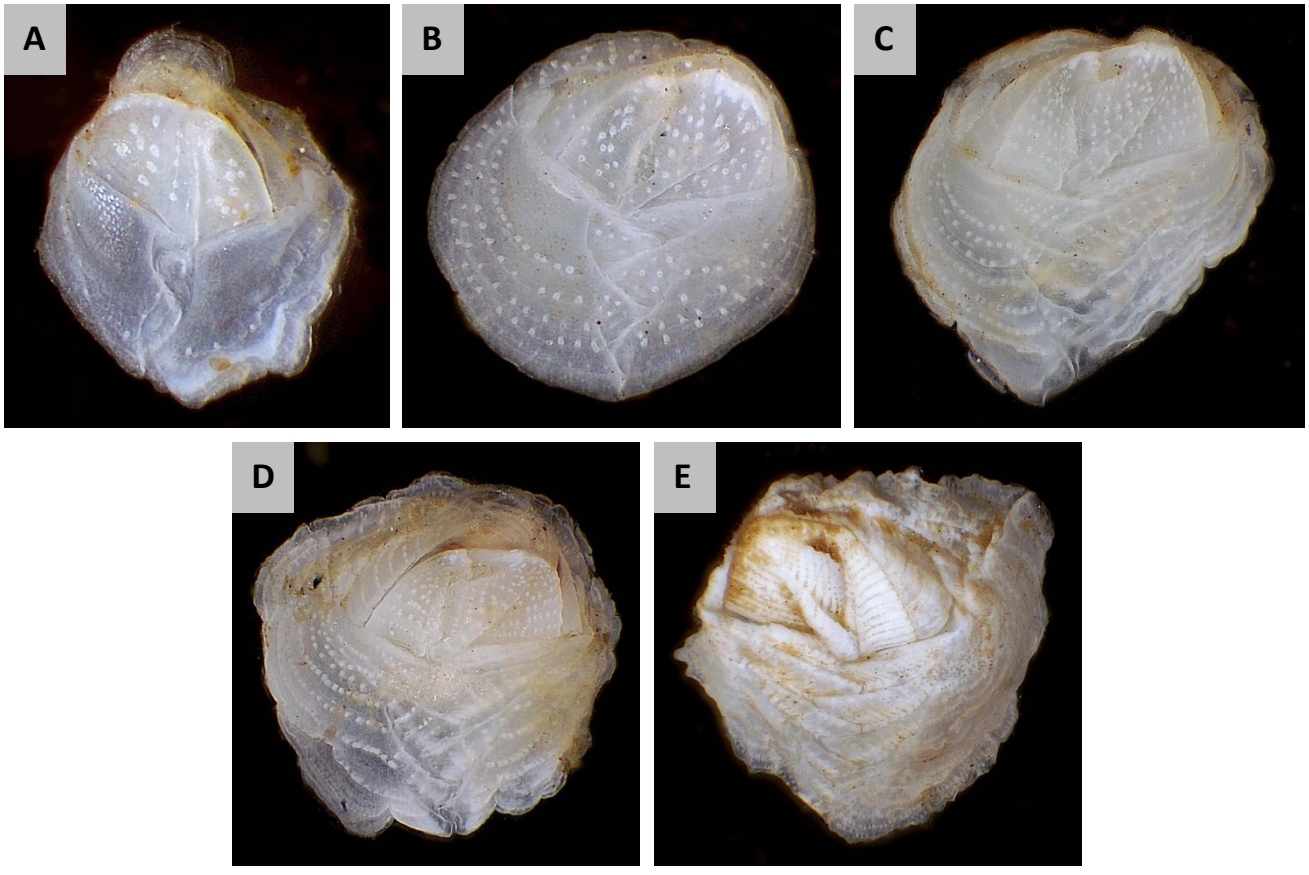
C: Part of a small colony preserved in ethanol. Numerous ciliates are also visible as pale spots.

The barnacle *Austrominius modestus*



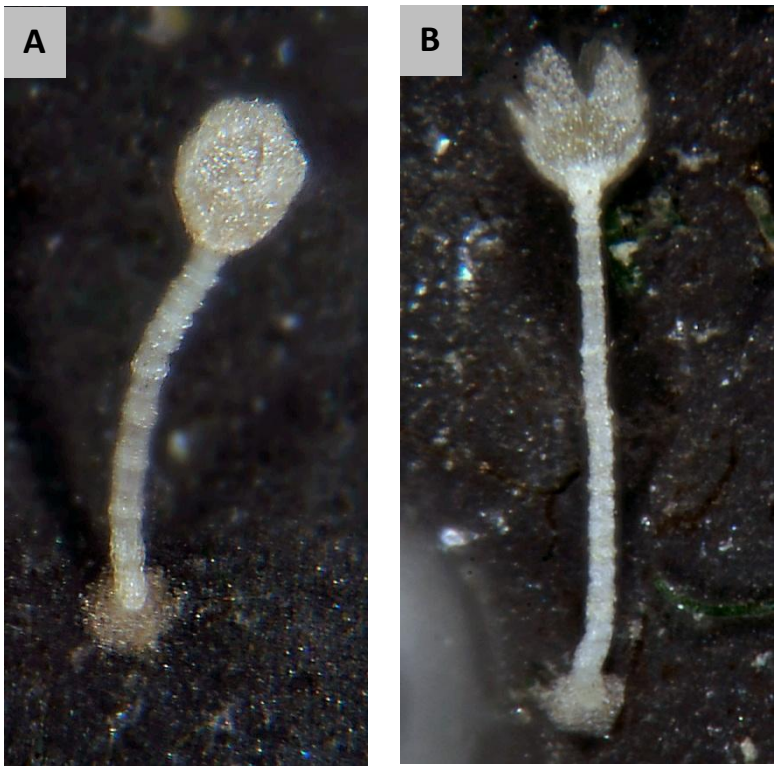
A: c. 0.8 mm long. **B:** c. 1.6 mm long. **C**: c. 2.2 mm long. Note only four plates form the outer fixed wall (rather than the six typical of other species), and the 'Maltese cross' outline being acquired in **C**, most clearly developed in the absence of crowding.

The barnacle *Verruca stroemia*

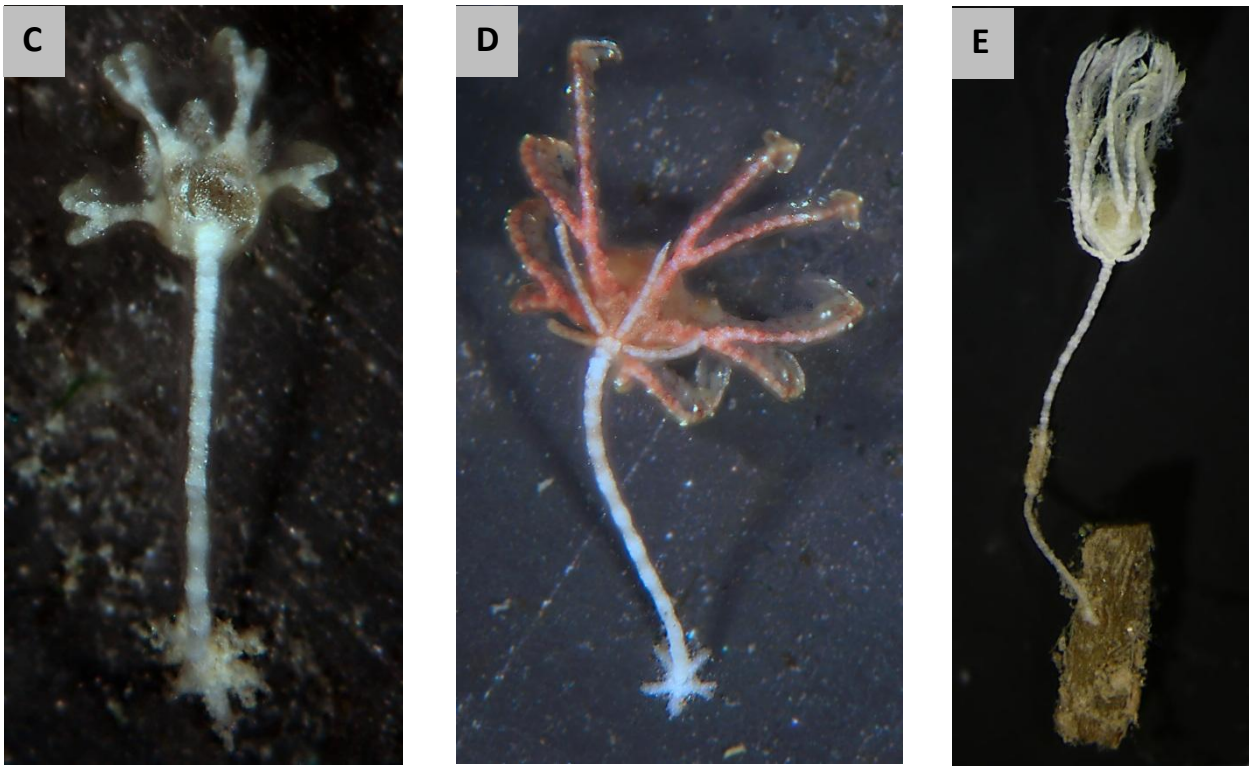


A: c. 0.75 mm across. **B:** c. 1.1 mm across. **C:** c. 1.7 mm across. **D:** c. 2.1 mm across **E:** c. 3.6 mm across. An asymmetrical barnacle (with only two moveable plates) which occurs in two states of 'handedness'; specimens **A** and **E** are opposite in symmetry to **B**, **C** and **D**.

Sessile stages of a crinoid, presumed to be *Antedon bifida*



Cystidean or early pentacrinoid. **A**: c. 1.0 mm long. **B**: c. 1.5 mm long.



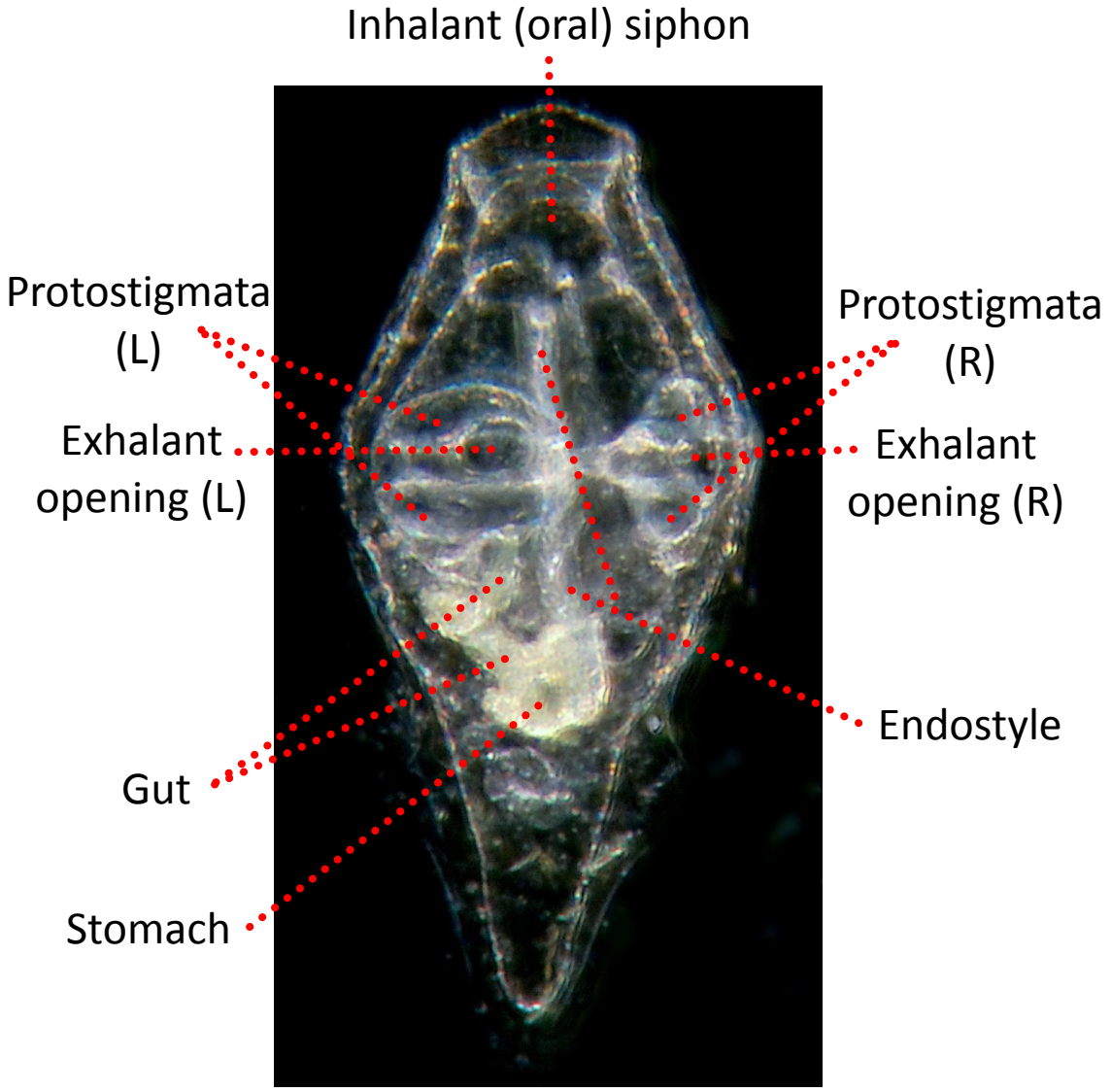
Pentacrinoid. **C:** c.2.5 mm long. **D**: c. 3.4mm long. **E:** c. 7.5 mm long.

(**A-D** living, **E** preserved.)

Preliminary notes on ascidians

The exhalant (or atrial) siphon of ascidians is anatomically dorsal and the inhalant (or oral) siphon is anterior. The endostyle, which produces a mucous feeding net, marks the mid-ventral line. In early growth the endostyle is very substantial, forming a straight or slightly curved rod-like shape running most of the length of the body.

The branchial basket (feeding apparatus) of adult suspension-feeding ascidians is penetrated by numerous minute openings, the stigmata, through which the feeding current passes; there is extensive variation and elaboration, but the usual orientation of the stigmata is with the long axis longitudinal (anterior-posterior). In early post-settlement growth, water passes through many fewer openings, which are much larger relative to the body and have a transverse (dorsal-ventral) orientation; these are referred to as protostigmata, and ultimately proliferate and divide into the stigmata.



First feeding stage of a phlebobranch ascidian, with two pairs of protostigmata, each pair served by a separate exhalant opening. This animal is attached ventrally, with the endostyle parallel to the substrate and more-or-less central viewed from above. The gut leads into the atrial chamber above the left-hand protostigma, while in this view the stomach overlays or is slightly left of the endostyle.

In the unitary phlebobranch species included here (*Ascidiella aspersa*, *Phallusia mammillata* and *Corella eumyota*), the first feeding stage possesses a pair of protostigmata on each side of the body, each pair overlain by a separate atrial (exhalant) chamber with its own exhalant opening. The gut vents via the left-hand atrial chamber (see figure on previous page). This early stage lays flat in all three species and considerable similarity exists between the three versions. However, *C. eumyota* initially does not lay squarely on its ventral surface but leans slightly towards the right side, so that from above the endostyle is slightly to the left (moving further left as development proceeds). This means that in the initial, four-protostigmata, stage of *C. eumyota* the gut crosses the endostyle when viewed from above (*C. eumyota* figures D and E). *A. aspersa* lays squarely on its ventral surface or leans towards the left side, and *P. mammillata* is mainly attached on its left. Thus in *A. aspersa* the stomach may overlie the endostyle but the gut (and often the stomach) is to the left of it (*A. aspersa* figures D and E), while in *P. mammillata* the entire gut and stomach are generally left of the endostyle (*P. mammillata* figures C and D). In development of all three species following the four-protostigmata stage, the left and right openings move together and fuse to leave a single atrial space and exhalant opening (the exhalant or atrial siphon). Because of their respective postures, the siphon is on the left side of the body as seen from above in *A. aspersa* and *P. mammillata*, and slightly to the right in *C. eumyota*.

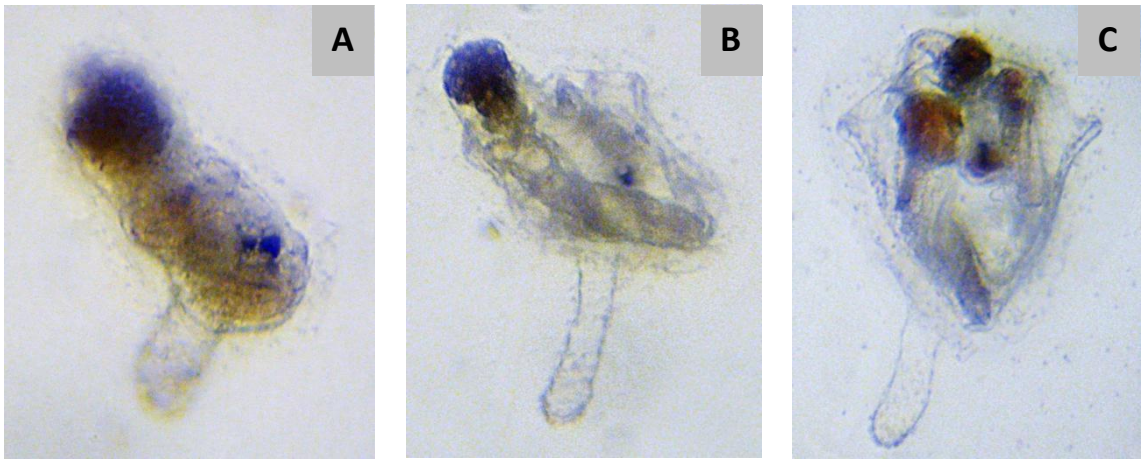
Many styelids pass through an early phase with arrays of parallel protostigmata diverging to left and right from the dorsal margin of the feeding apparatus. In combination with the underlying endostyle bisecting this array when viewed from above, a pattern reminiscent of a bird's foot is produced. This is particularly noticeable in the oozoid of *Botryllus schlosseri* (figures A-D), but is also seen, for example, in *Botrylloides diegensis* (figure E) and *Asterocarpa humilis* (figure H).

The process of division of the protostigmata can include phases when the perforations of the branchial basket are C-shaped or otherwise curved even in species that ultimately have longitudinally-arranged stigmata (e.g. *Ciona intestinalis*, figure H). Of those included here, members of the genera *Corella* and *Molgula* retain curved stigmata into adulthood, arranged into spirals which, in *Molgula*, occupy conical indentations (the infundibula) of the branchial basket.

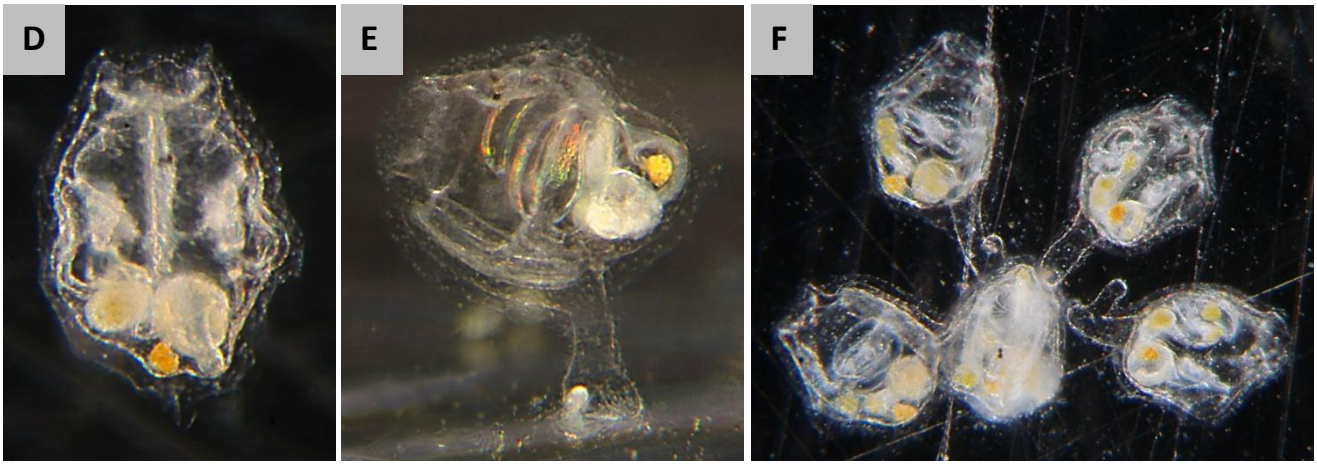
Ascidian metamorphosis involves the resorption of the larval tail into the posterior part of the trunk, creating a transitory mass of material (principally derived from the notochord) which can be seen in some of the illustrations here (e.g. *Ciona intestinalis*, dark mass in figures A-C and golden spots in figures D-F; *Corella eumyota* figures B-D).

Informative line drawings of early post-settlement stages of several ascidian species are included in N.J. Berrill's Ray Society volume (No. 133) 'The Tunicata, with an Account of the British Species' (1950, 354 pp.).

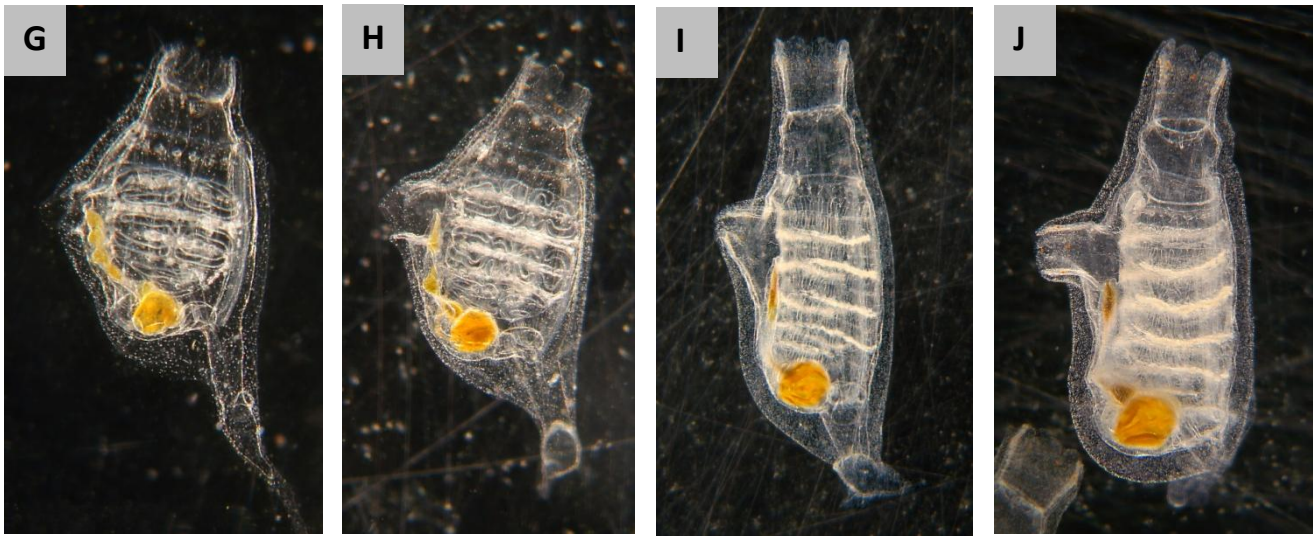
The unitary ascidian *Ciona intestinalis*



A: Metamorph c. 1 day after settlement. **B:** Metamorph c. 2 days after settlement. **C:** Metamorph c. 3 days after settlement.



D: Stage with two pairs of protostigmata and two separate exhalant openings, c. 0.60 mm across, viewed from above with inhalant siphon at top. **E:** same stage viewed from the side, showing stalk; cilia in protostigmata iridescent, inhalant siphon to left; body c. 0.59 mm across. **F:** Same stage, cluster of five individuals after co-settlement of larvae, image c. 1.7 mm across.

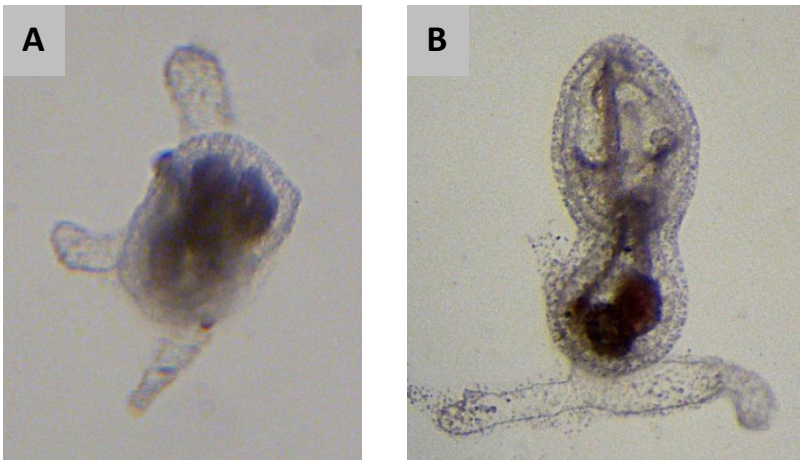


Later stages viewed from left side, showing progressive loss of stalk and elaboration of branchial basket. Heights not including stalk, **G**: c. 1.7 mm; **H**: c. 2.1 mm; **I**: c. 4.8 mm; **J**: c. 5.0 mm.

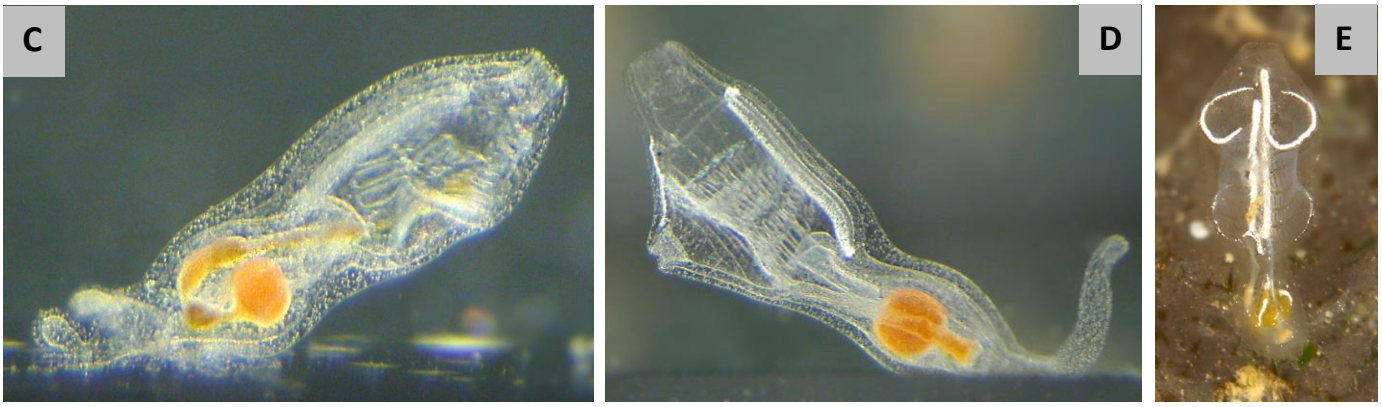
Ciona intestinalis continued

K: Preserved specimen showing the intense contraction by this species unless narcotized before preservation; the clear outer material is the tunic. This individual is at a development stage similar to the live individual in figure **I**.

The colonial ascidian *Clavelina lepadiformis*



A: Very early post-settlement stage. **B:** Oozoid c. 2 days post-settlement.

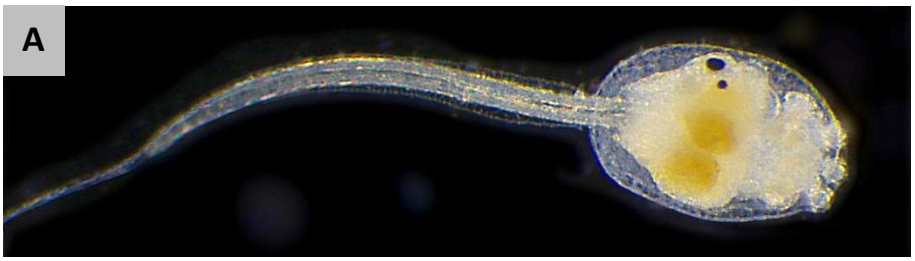


C: Oozoid c. 3 days post settlement. **D:** Oozoid c. 8 days post settlement. **E:** Single zooid on settlement panel, c. 2.1 mm wide.

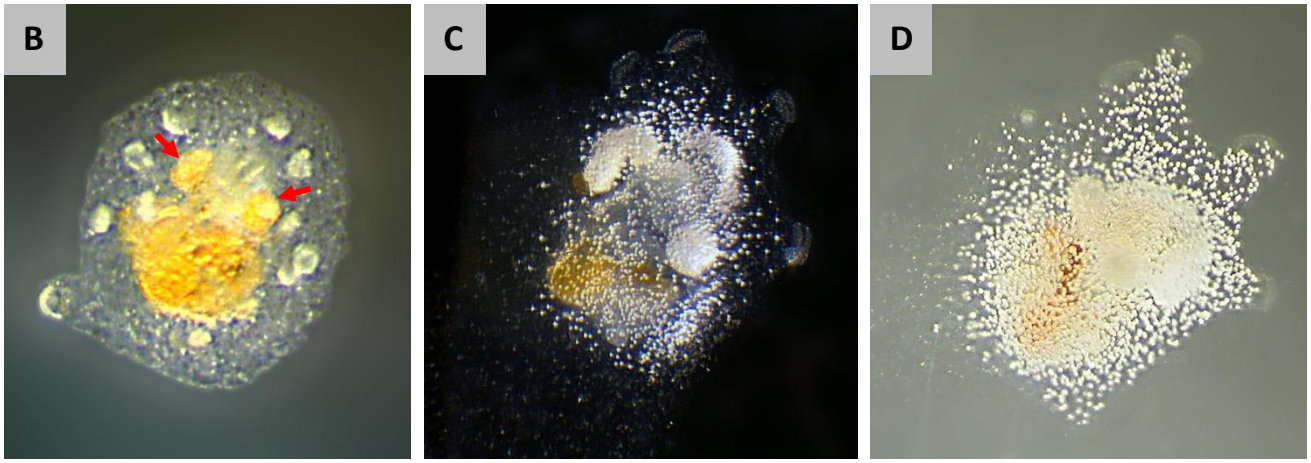
Presumed polyclinid ascidian

The long post-abdomens of the zooids in this small colony on a settlement panel suggest it is very probably a polyclinid; these zooids are lying flat, but the long zooids would be upright in the grown colony. The ridged stomach just visible in the zooid second from the right indicates an *Aplidium* species.

The colonial ascidian *Didemnum vexillum*



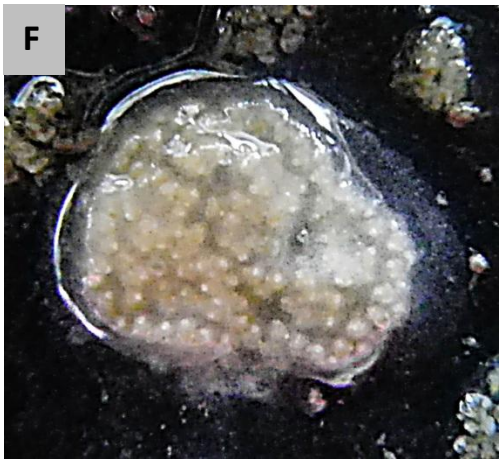
A: Hatched larva, length c. 1.6 mm, with three adhesive papillae (by which the larva will initially attach) seen here on the extreme right, with a set of vascular ampullae just behind them. In *D. vexillum*, the larva has six pairs of vascular ampullae, which are rather crowded and not individually discernible in this image. The remaining, more posterior, organs of the larval trunk form a round-cornered triangle which seems rather characteristic of *D. vexillum*, with the rudiments of the oozoid poorly differentiated. Compare with the unhatched larva of a different spicule-producing didemnid shown on page 31.



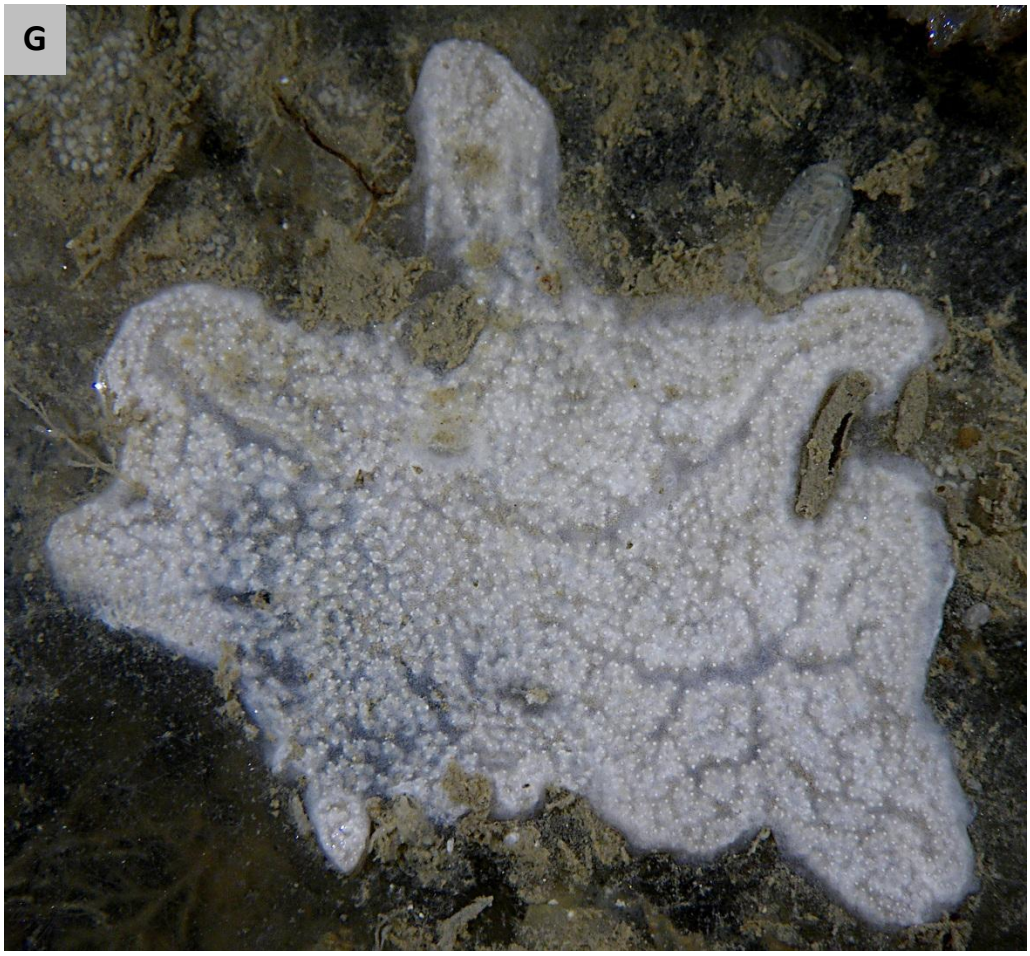
B: Very early post-settlement stage surrounded by clear tunic; two small, orange, oval lateral-thoracic organs (LTOs) (red arrows) flank the developing branchial basket of the oozoid. **C:** Early oozoid, c. 0.8 mm across, the two LTOs are now white and producing calcareous spicules that are spreading into the tunic. **D:** Later oozoid, spicules numerous. In **B-D** the gut and stomach appear orange. It is very doubtful whether *D. vexillum* can be distinguished from other spicule-producing didemnid species at the oozoid stage (see page 31).

E: Young colony of c. 10 zooids; contracted, with only c. 5 zooids having their inhalant orifices partially open (showing a triradiate pattern).

Didemnum vexillum continued

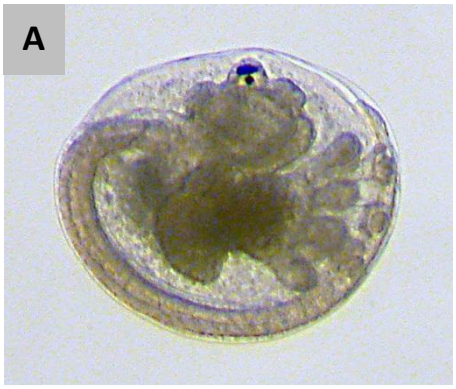


F: Small didemnid colony of c. 100 zooids on settlement panel at site known to host *D. vexillum*. The dark (relatively spicule-free) water channels between zooids and the separation of zooids into small clumps incorporating white dots (dense spicules where the zooidal orifices are tightly closed in this specimen out of water) suggest it is *D. vexillum*.



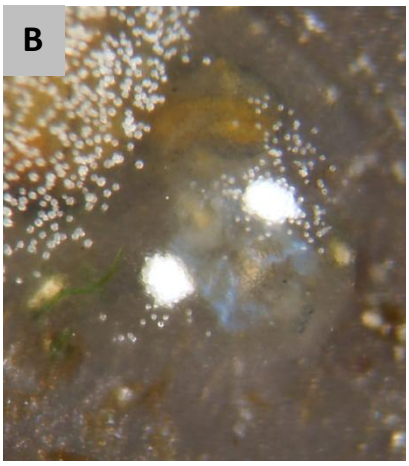
G: A 40 mm-wide preserved young colony of *D. vexillum*, identifiable from the water channels separating clusters of white-dotted zooids, as noted for the much smaller specimen in **F**.

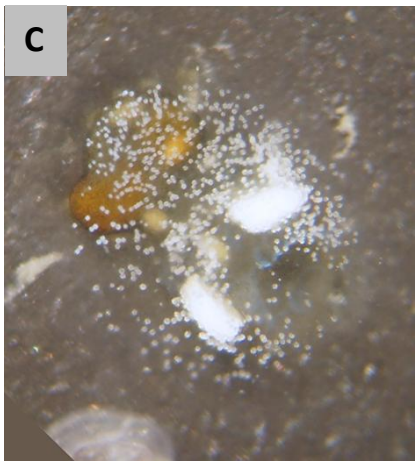
Spicule-producing didemnids other than *D. vexillum*

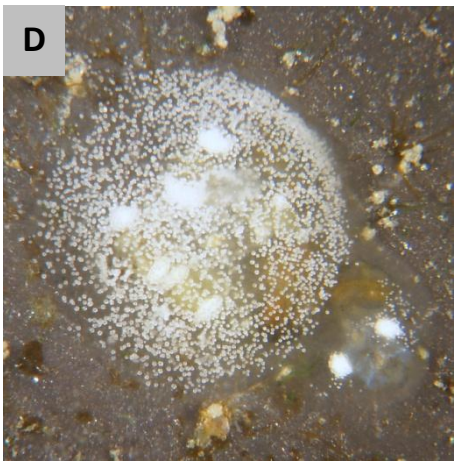


A: Unhatched larva clearly not belonging to *Didemnum vexillum*. This larva, with the tail still wrapped around the body, has three adhesive papillae, seen here on the extreme right, and four pairs of darker, rounded vascular ampullae just behind them. *D. vexillum* (page 29, figure **A**) also has three adhesive papillae but six pairs of vascular ampullae (as do various other didemnid species). The rudiments of the oozoid are also much clearer in this species than in the larva of *D. vexillum*, with the branchial basket, siphon and endostyle clearly discernible just below the black sense organs.

B-E below are from sites where *D. vexillum* is not recorded



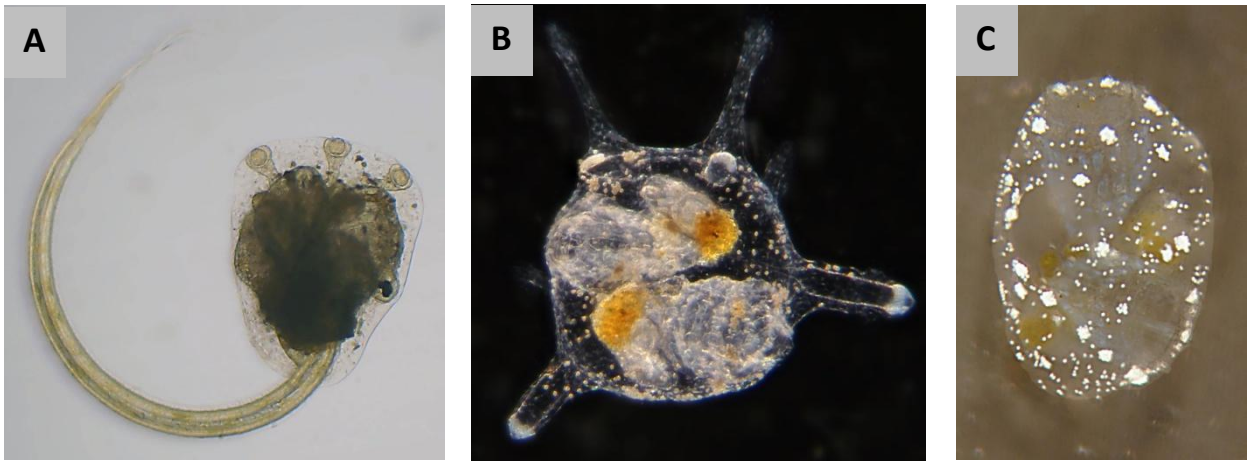




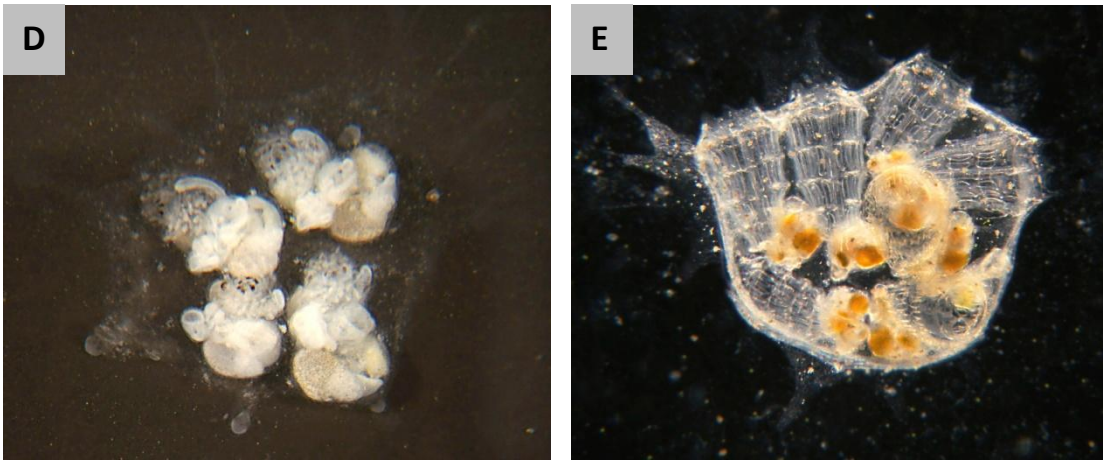
B: Oozoid (adjacent to larger colony, as shown in **D**), c. 0.9 mm across, a few spicules entering tunic from white LTOs; branchial basket bluish. **C:** slightly later stage with spicules spread further, c. 1.2 mm across. **D:** Later three- or four-zooid stage, c. 2.0 mm across.

E: Colony of c. 20 zooids, image c. 7.5 mm across, faint circles mark closed zooidal orifices.

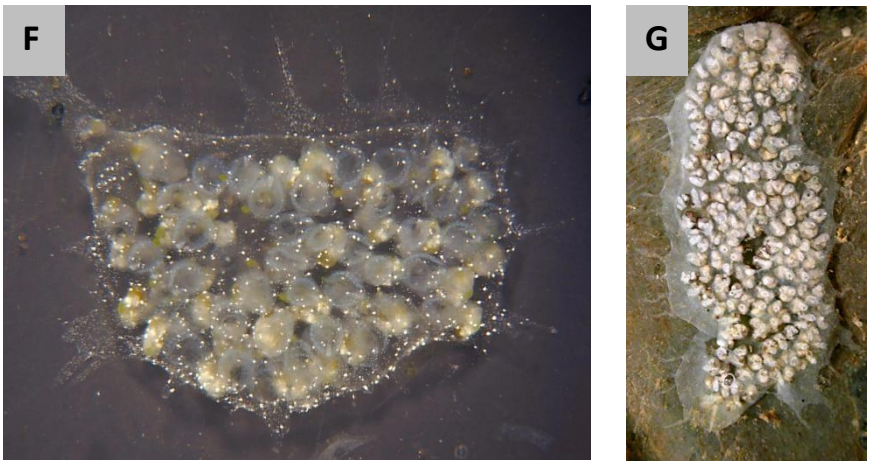
The colonial ascidian *Diplosoma listerianum*



A: Larva; the trunk contains two developing zooids. **B:** Metamorph, c. 0.8 mm across, with the two zooids present in the larva; tunic transparent. **C:** Metamorph, c. 0.9 mm across; bright white spots are pigment granules in the tunic (calcareous spicules are absent); branchial baskets bluish; clear oval exhalant opening mid-left.

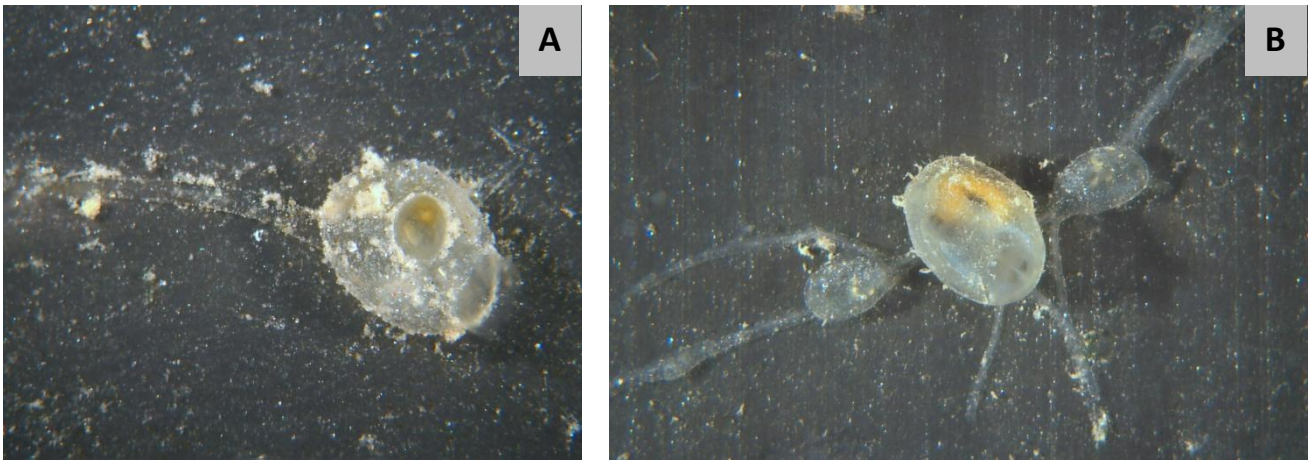


D: Alcohol-preserved four-zooid colony, c. 1.7 mm across the zooids; incident illumination. **E:** Live seven-zooid colony, dark-field illumination.

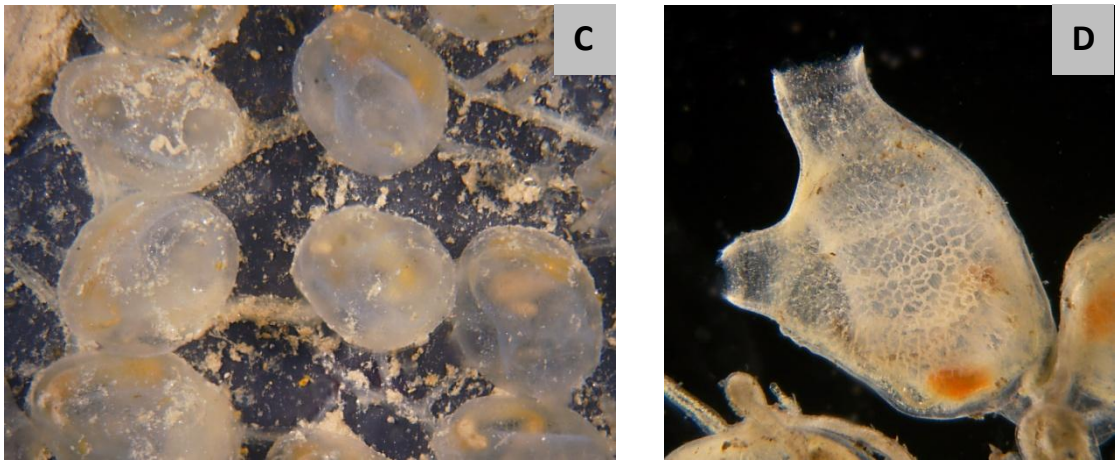


F: Live colony of c. 40 zooids, c. 9.5 mm across; incident illumination. **G**: Alcohol-preserved colony of *Diplosoma* sp., c. 130 zooids, c. 14.5 mm across; central oval hole in tunic is exhalant opening.

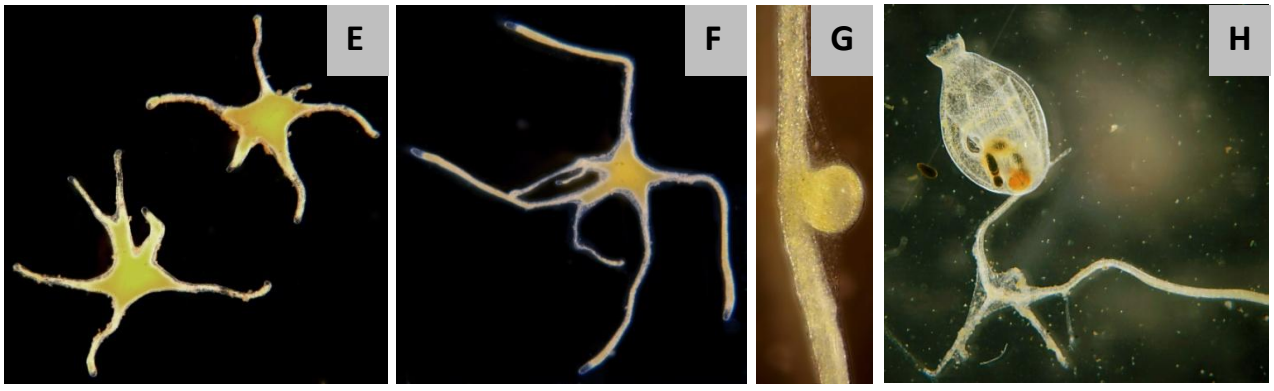
The colonial ascidian *Perophora japonica*



A: Oozoid, c. 1.4 mm across, with first stolon (to left) and beginning of second. **B:** Later oozoid, c. 2.7 mm across, with more extensive stolons and first two daughter zooids.

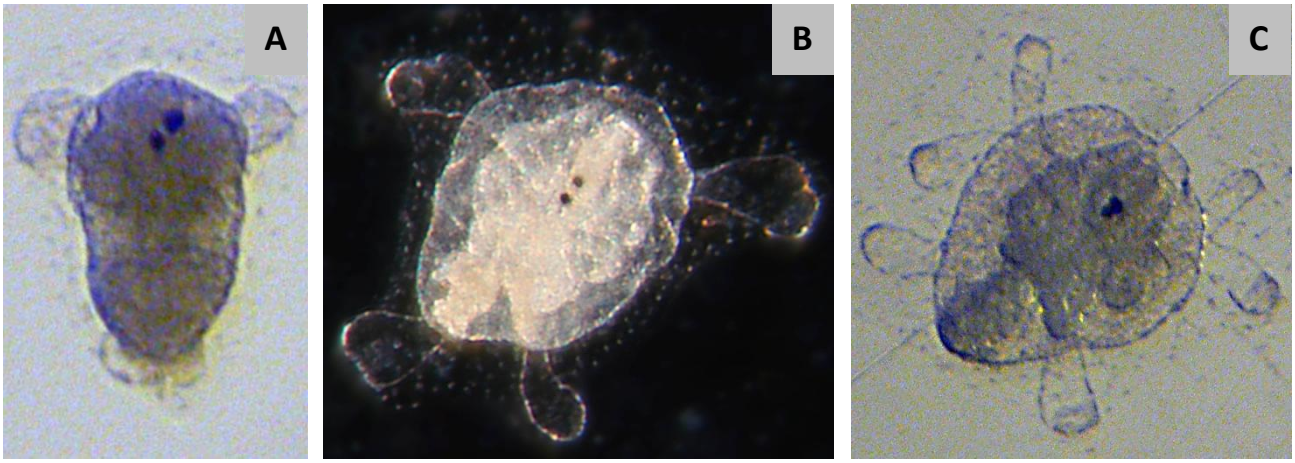


C: Portion of a young colony on a settlement panel, siphons closed; region shown c. 8.8 mm from top to bottom. **D:** Single zooid of colony, c. 5.0 mm tall.

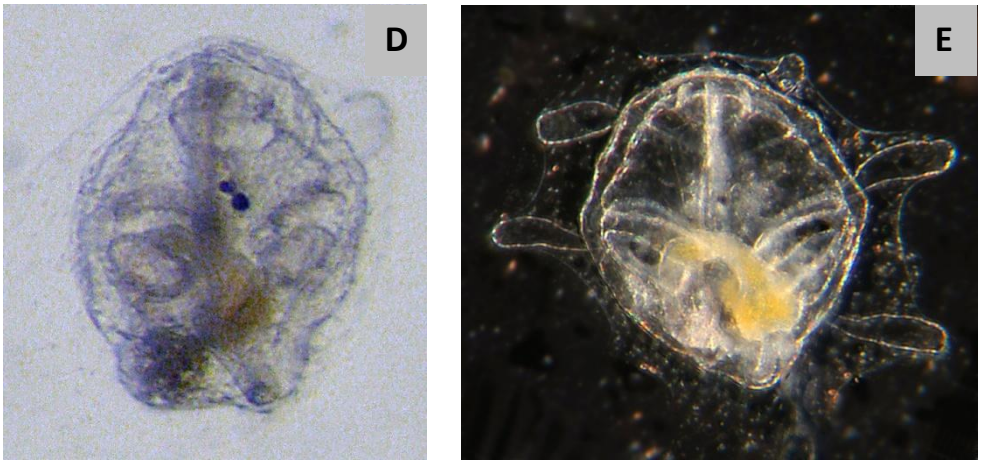


Additional to sexually produced larvae, *P. japonica* also has asexual propagules. Bright yellow 'terminal buds', typically produced at the edge of the colony, detach and are dispersed by water movements. If a bud eventually sticks to a surface the 'arms' elongate as stolons which bud the zooids of a new colony. **E:** Recently released terminal buds; the lower bud is c. 5.0 mm across its greatest span. **F:** Stolons extending from a reattached terminal bud, c. 7.5 mm across greatest span. **G:** Bud of zooid developing on stolon. **H:** Zooid, c. 3.6 mm tall, arising from stolon of terminal bud.

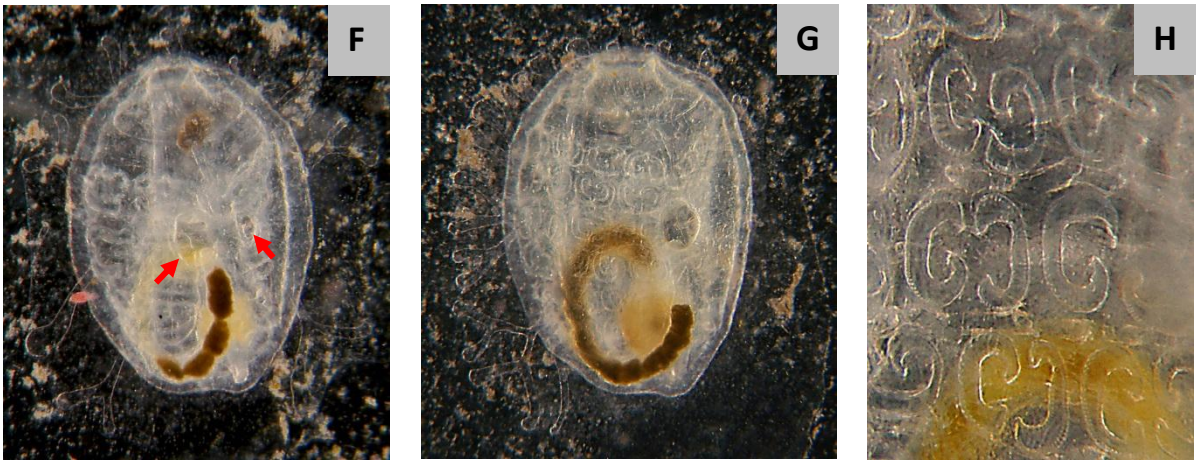
The unitary ascidian *Corella eumyota*



A: Very early post-settlement stage. **B:** c. 1 day post settlement, c. 0.33 mm long (not including the vascular ampullae). **C:** Same stage as B, note bifurcations of the vascular ampullae.

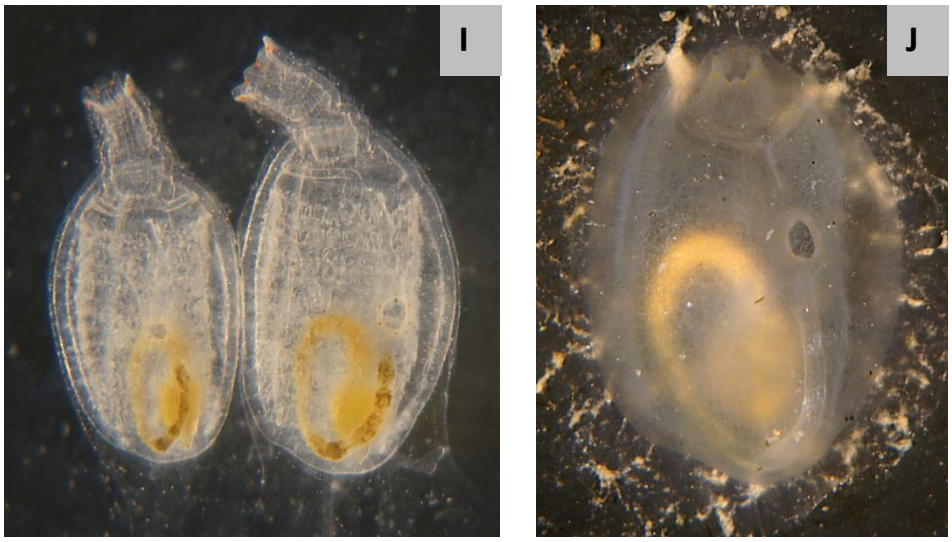


D: 2 days post-settlement. **E:** 3 days post settlement, c. 0.55 mm long. **D** and **E** both possess 2 pairs of protostigmata with separate left and right exhalant openings.



F: Later stage, c. 1.5 mm long, still with transverse protostigmata; the two exhalant openings (red arrows) are approaching the dorsal midline and the left-hand opening is larger than the right. **G:** Later stage, c. 2.0 mm long, with a single exhalant opening and longitudinally aligned C-shaped stigmata; the animal is attached leaning onto its right side, so the exhalant opening is seen slightly to the right and the endostyle, seen through the body, is towards the left. **H:** Close-up of C-shaped stigmata; image shows area c. 0.95 mm from top to bottom.

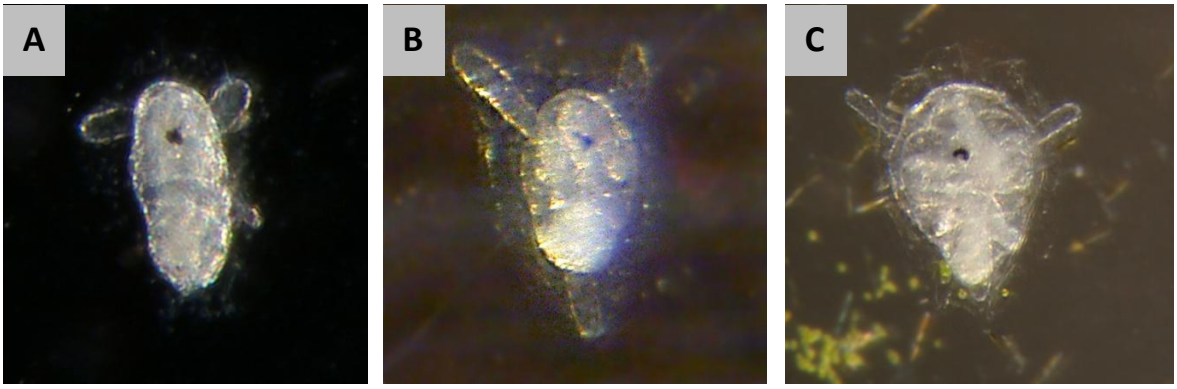
Corella eumyota continued



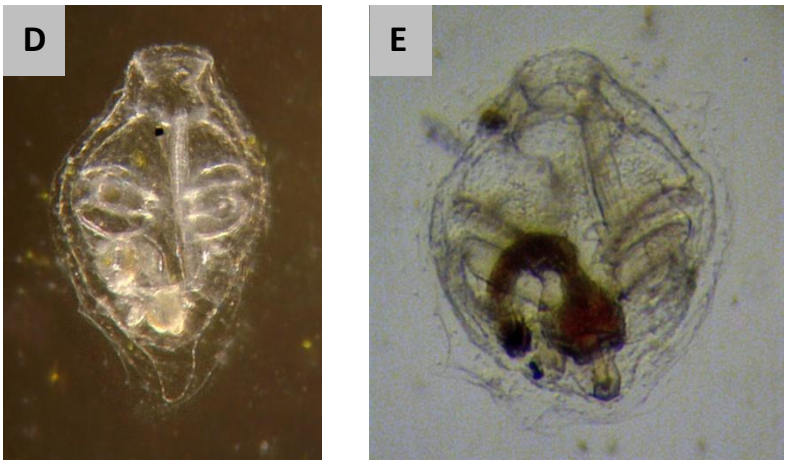
I: Two specimens grown in culture c. 4.1 and 4.7 mm long. **J:** Specimen 6.3 mm long on a settlement panel; the animal is surrounded by a fringe of tunic (incorporating sediment). Note the spiral gut leading from the stomach to the anus just inside the exhalant opening. The long inhalant siphons and thinner tunic of the specimens in **I** and shorter siphon and much thicker tunic in **J** at least in part reflect the different levels of water movement in their respective growth environments.

K: Specimen grown in culture and preserved at a similar stage to those shown live in figure **I**, c. 3.4 mm long overall; the siphons are contracted; the spiral gut leading to the exhalant opening, which lies slightly to the right, help to identify the animal; the curved stigmata may also be visible without dissection if the tunic is as clear as in this specimen. Although they were lying flat, the peculiarly square-ended tunic was frequent in this batch of specimens growing on a smooth substratum.

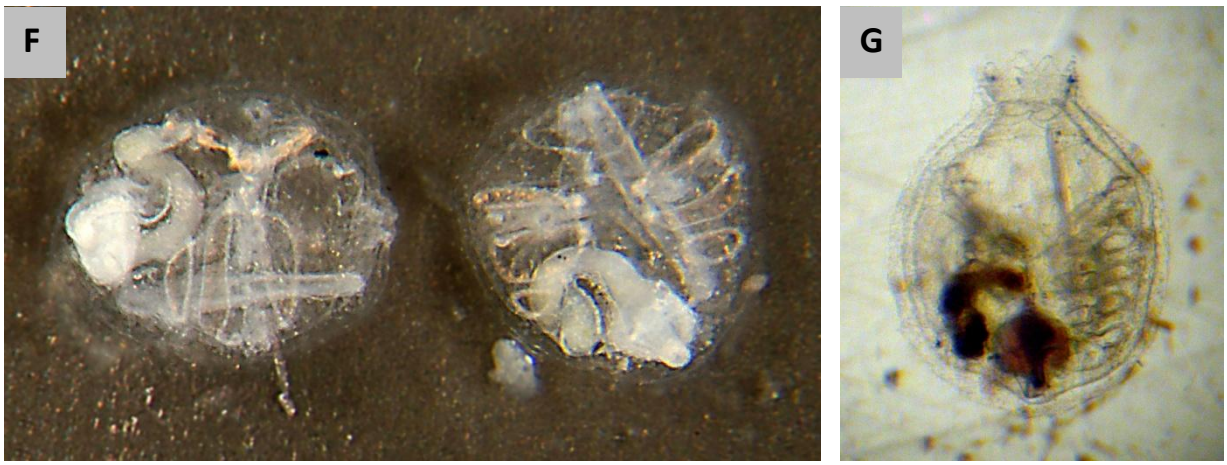
The unitary ascidian *Ascidiella aspersa*



A: Early post-settlement stage, c. 0.26 mm long. **B:** Similar stage. **C:** Slightly later stage with organ systems differentiating, c. 0.31 mm long.

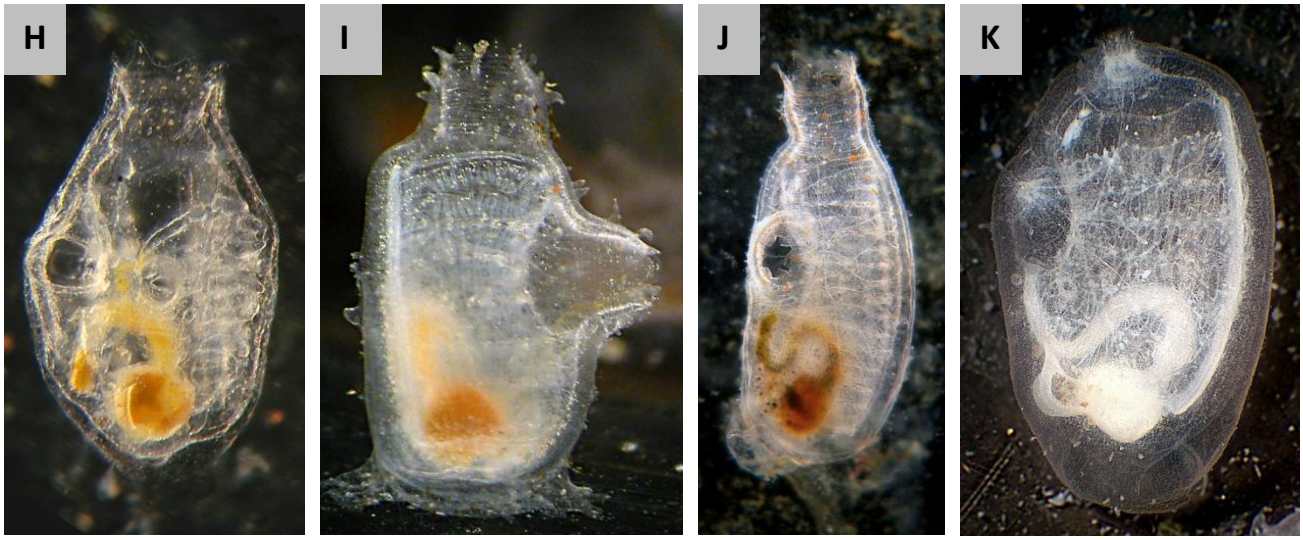


D: First feeding stage, with two pairs of protostigmata and two atrial openings, c. 0.65 mm across, dark-field illumination. **E:** Slightly later stage; bright-field illumination, gut appears dark.



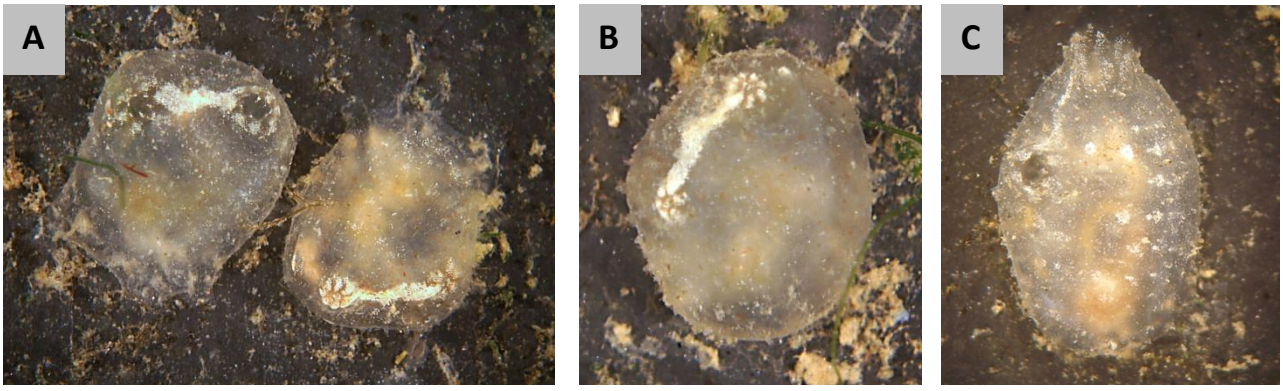
F: Preserved specimens on a settlement panel, similar stage to **E**, c. 0.6 mm long; third pair of protostigmata forming (protostigmata show as pinkish, guts pale, endostyle rod-like). **G:** Later stage with protostigmata dividing.

Ascidiella aspersa continued



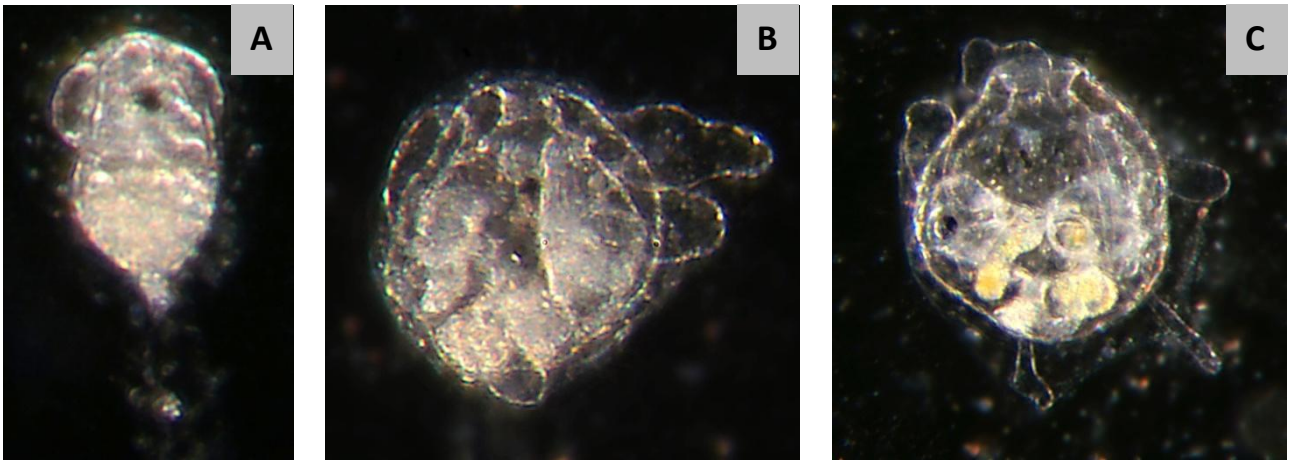
H: Specimen showing atrial openings approaching each other prior to fusing, c. 1.5 mm long. **I:** Stage with well-developed exhalant siphon and several rows of numerous longitudinally-oriented stigmata, here growing almost upright, view of left side; lab-reared specimen. **J:** Stage with c. 15 rows of stigmata; lab-reared specimen, c. 7.4 mm long. **H-J** photographed alive. **K:** Preserved specimen, siphons tightly closed, at stage similar to **I**, viewed from right side.

Ascidiella sp(p).

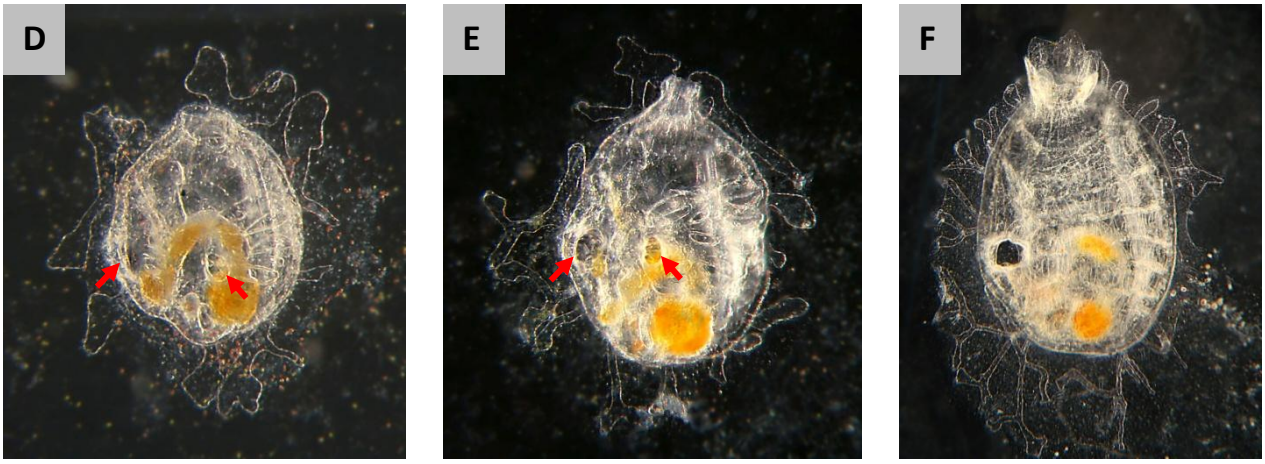


Very small *Ascidiella* on settlement panels. The extensive pigment between the siphons in the three smallest specimens seen in **A** and **B**, and their rounded shape, suggest that these are *A.* cf. *scabra*. The slightly larger specimen seen in **C** is more questionable: the pigment between the siphons is much fainter and the more elongate shape of the body and general appearance suggests *A. aspersa*. Specimens in **A** 3.4 and 3.1 mm across, **B** 3.0 mm across, **C** 4.5 mm across.

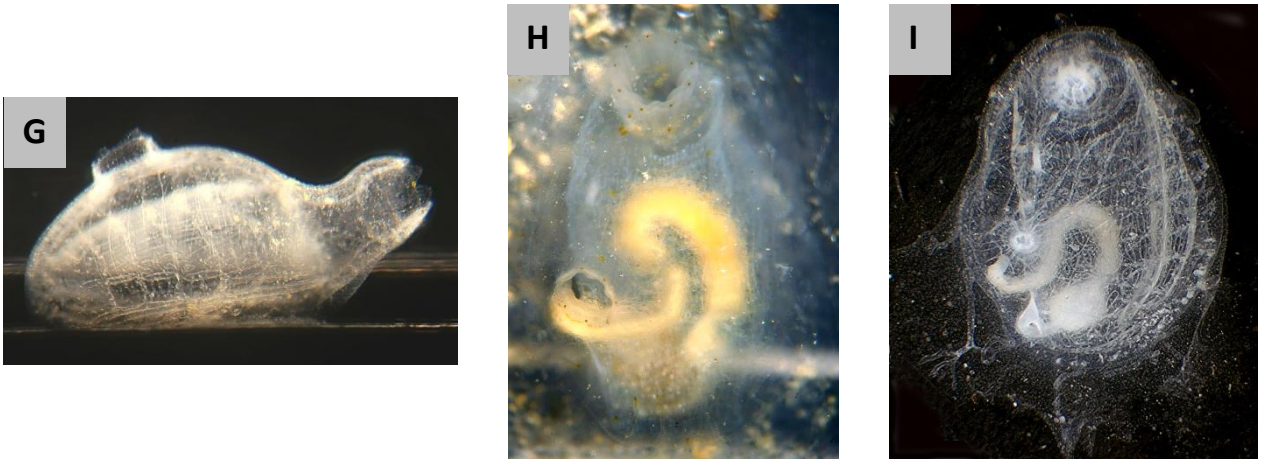
The unitary ascidian *Phallusia mammillata*



A: Very early post-settlement stage, c. 0.21 mm long; remains of larval tail visible. **B:** Later stage undergoing organ development, c. 0.28 mm long. **C:** Stage with two pairs of protostigmata and two separate exhalant openings, c. 0.36 mm across; the endostyle is already well to the right, indicating that the animal is attached by its left side.

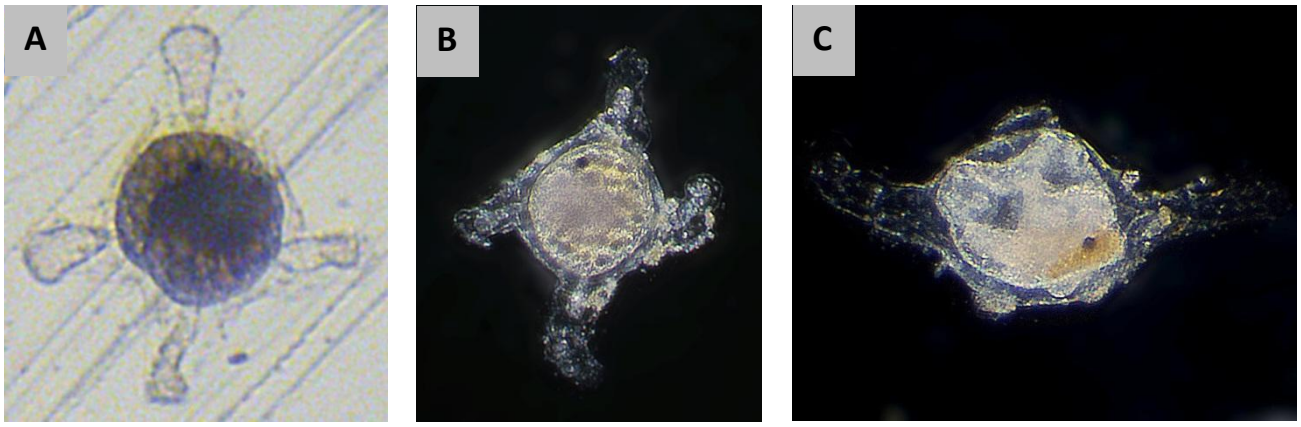


D: Stage with several protostigmata, c. 0.77mm long; right-hand exhalant opening displaced to the left. Openings indicated by red arrows. **E:** Later stage with more protostigmata, right-hand exhalant opening further left. **F:** Stage with single exhalant opening on extreme left of upper surface, numerous stigmata; c. 3.0 mm long.

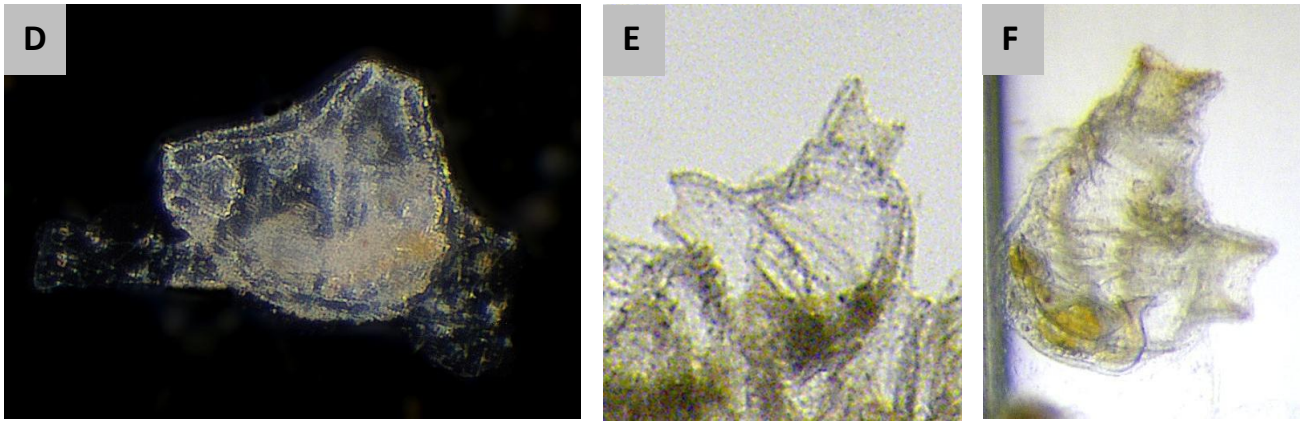


G: Stage similar to **F**, viewed from side. c. 2.9 mm long; prostrate early posture contrasts with upright stance of adult *P. mammillata*. **H:** Juvenile on settlement panel, c. 4.6 mm long, showing thick tunic, strongly meandering gut and large distance between siphons. **I:** Cultured specimen preserved at stage similar to **F** and **G**, c. 2.9 mm long.

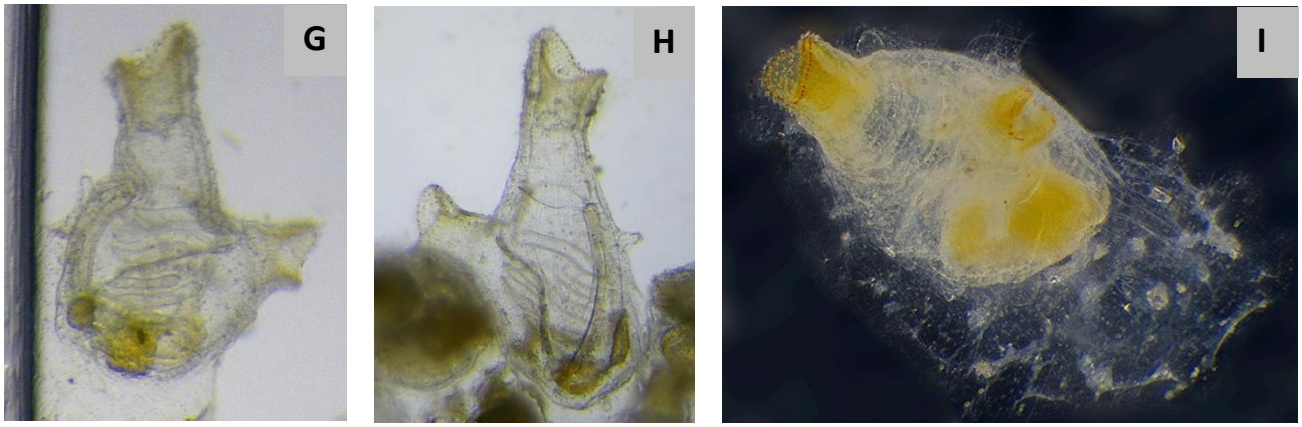
The unitary ascidian *Styela clava*



A and **B:** Very early post-settlement stage; **B** c. 0.2 mm across (not counting tunic extensions). **C:** c. 2 d after settlement, c. 0.3 mm long (excluding tunic extensions); siphons and other organs forming.

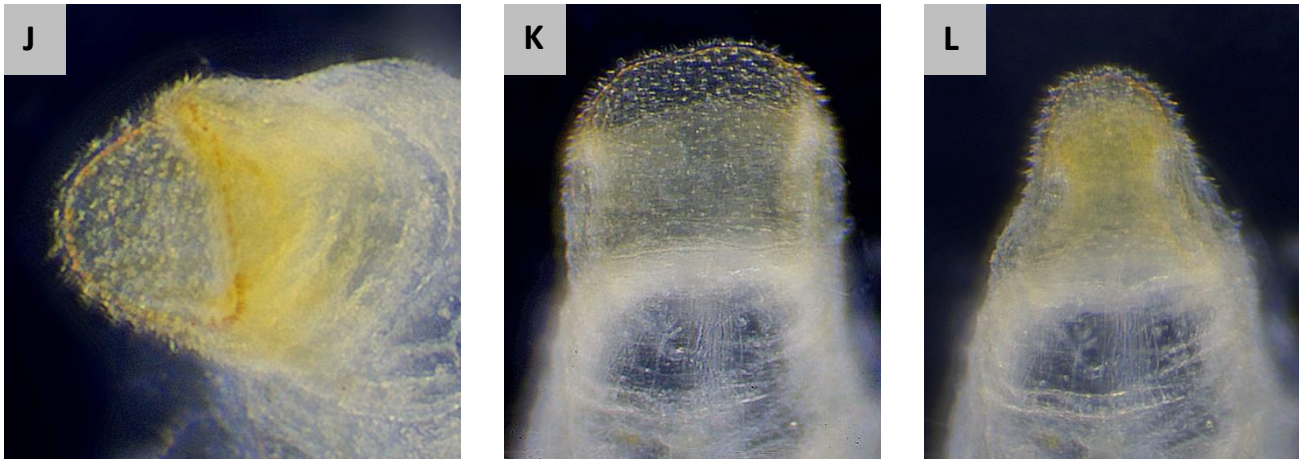


D: c. 4 d after settlement, c. 0.4 mm long (excluding tunic extensions); siphons open. **E:** Stage with two pairs of protostigmata discernible and siphons distinctly 'two-lipped'. **F:** Later stage.

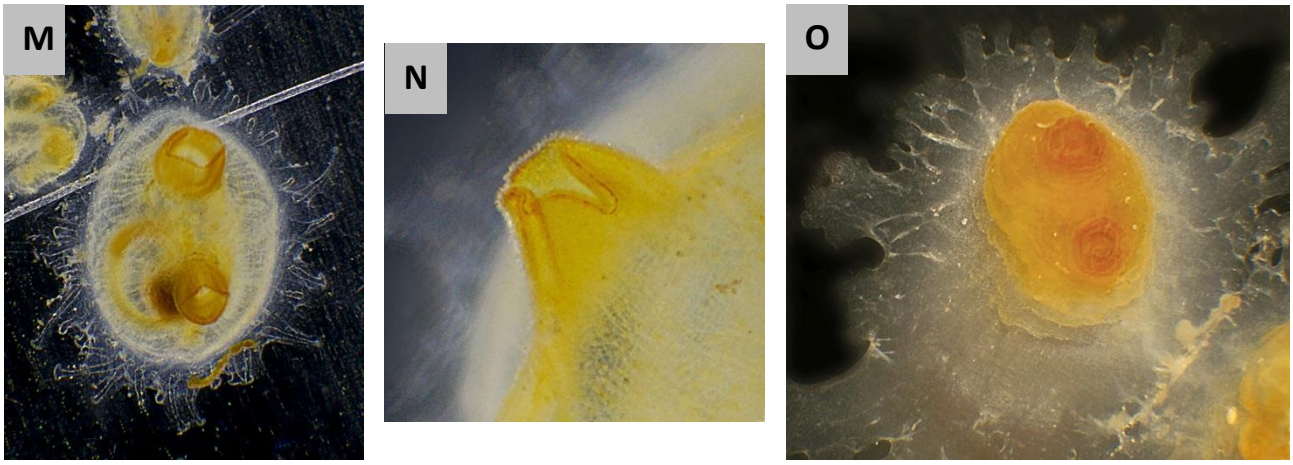


G: Stage with at least three pairs of protostigmata. **H:** Stage with four protostigmata on the left side at least. **I:** Later stage, body c. 0.9 mm long (including the inhalant siphon but not the tunic), with extensive area of tunic forming attachment; the siphons are still 'two-lipped'.

Styela clava continued



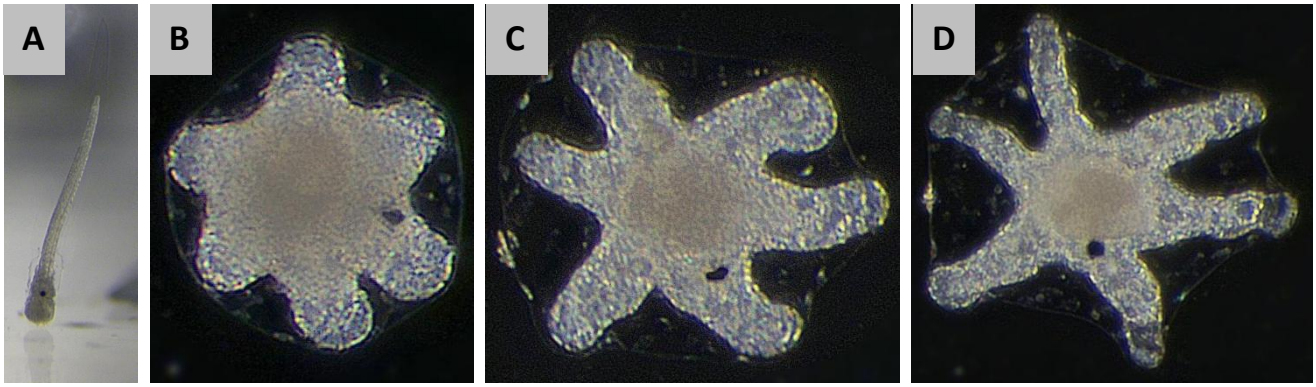
J: 'Two-lipped' siphon (here the atrial siphon) of the same stage as shown in **I**; the two 'lips' of the siphons close together as if hinged where they join. **K** (open) and **L** (closed) atrial siphon, viewed from above, showing the narrowing of the lips into a conical shape that accompanies closure; specimen at a similar stage to that shown in **I**. The siphons are spinous and have a very narrow terminal red band which sometimes appears double.



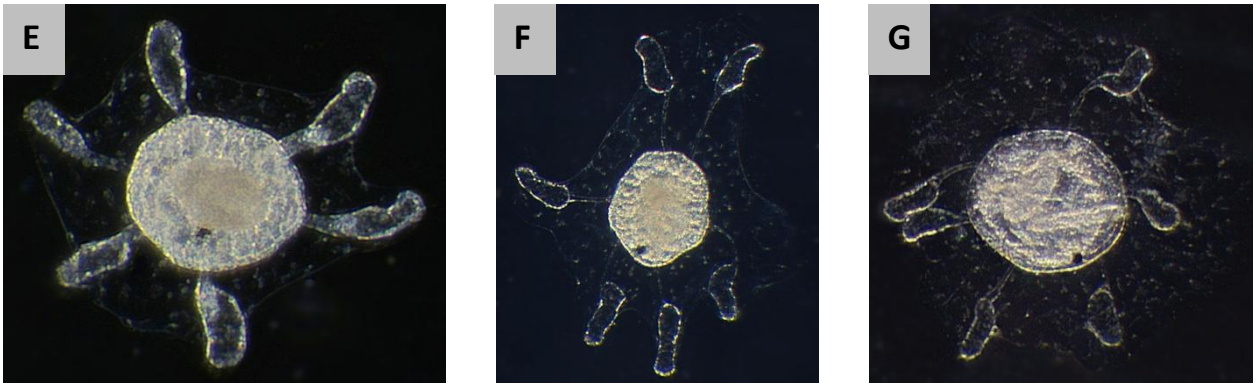
M: Specimen 2.1 mm across (with two adjacent smaller specimens); the siphons are now approximately quadrangular at their ends (with two additional inflections, on the midline), but still fold closed between their lateral angles (figure **N**, partly closed siphon), much as in the earlier 'two-lipped' form. **O:** Later stage, c. 1.9 mm across the body, not counting the extensive tunic attachment, a precursor of the holdfast of the stalked adult form; the specimen is live but contracted and the siphons are tightly closed; the tunic abuts that of another individual in the bottom right of the image.

A later juvenile stage, c. 7mm long but still lacking a stalk, is shown on page 42 in a comparison with *Asterocarpa humilis*.

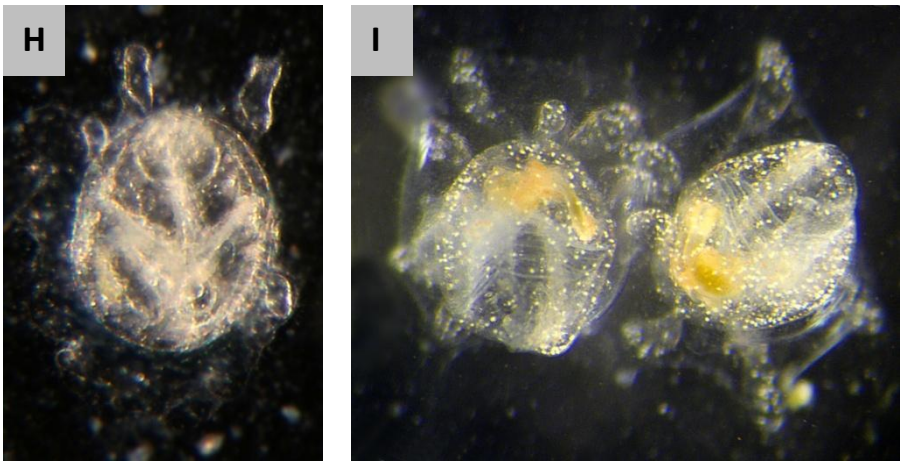
The unitary ascidian *Asterocarpa humilis*



A: Attached larva. **B, C** and **D:** Early cog- or star-shaped metamorph, c. 0.25 to 0.45mm across, within transparent tunic; **B** was a larva eight hours before the photo was taken.

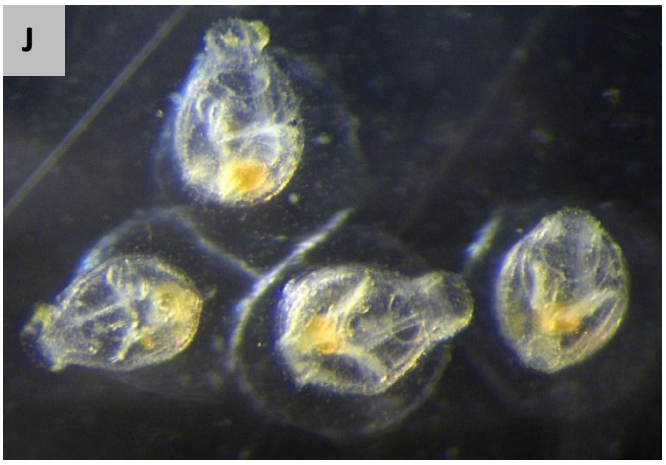


E, F and **G:** Later, rounded-off metamorphs, 'body' c. 0.25 to 0.35 mm across; the six earlier protuberances have resolved into vascular ampullae with narrow stems; in **G**, organs are becoming discernible, including a rod-like endostyle .



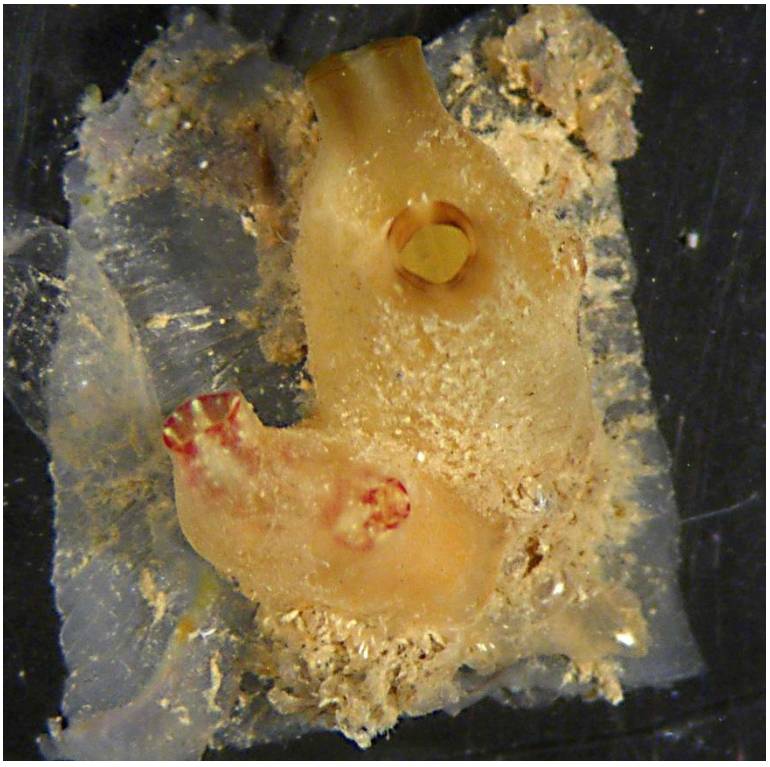
H: Early feeding stage, c. 0.4 mm long. **I:** Two specimens at later stage.

Asterocarpa humilis continued



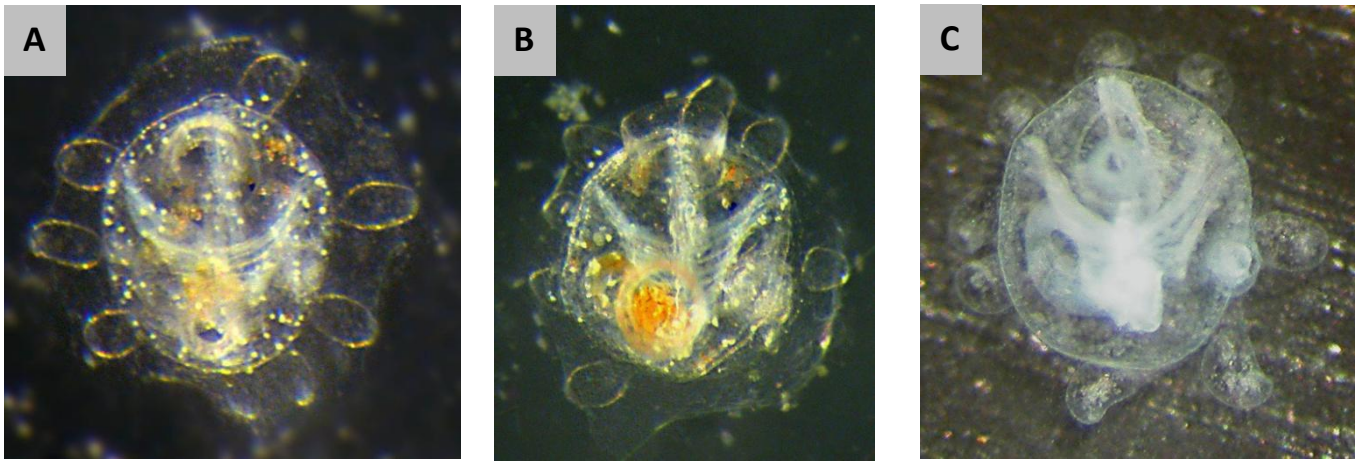
J: Cluster of 4 juveniles, with tunics touching.

Styela clava and *Asterocarpa humilis*

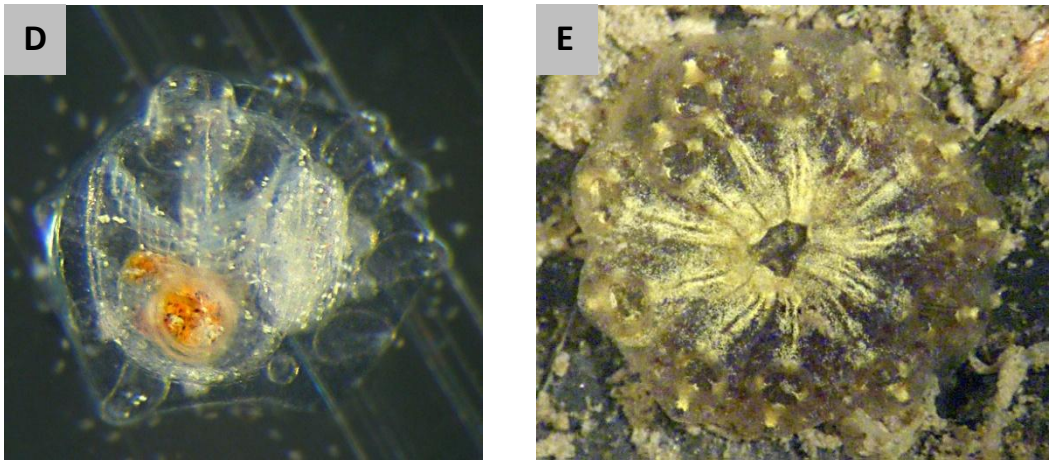


Young specimens of *Styela clava* and *Asterocarpa humilis* growing together on a trimmed substrate about 1cm long. They are already separable on the basis of the distinctive colouration of the siphons: brown stripes (*S. clava*) vs. reddish with internal cream/white 'compass' marks (*A. humilis*), as in the respective adults. At this size, *S. clava* lacks a stalk.

The colonial ascidian *Botryllus schlosseri*



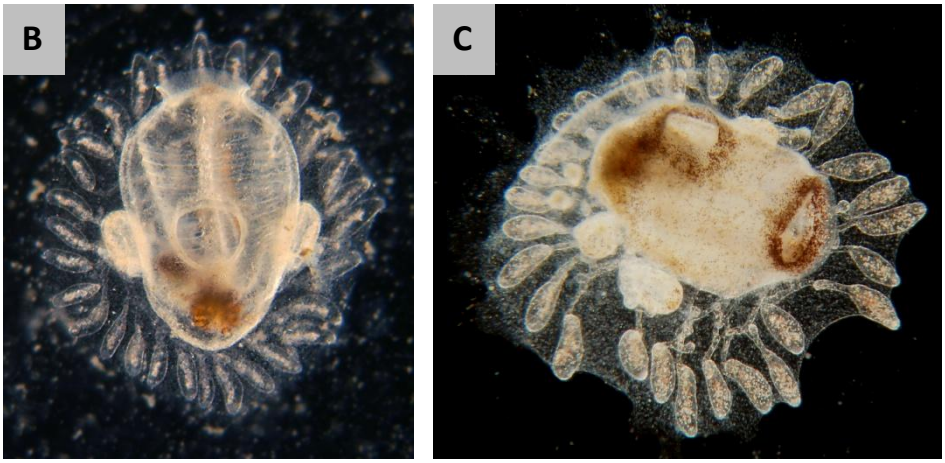
A: Early oozoid with very small bud on right and eight vascular ampullae. **B:** Slightly later oozoid with larger bud on right; note the five or more pairs of parallel protostigmata in V-configuration with endostyle passing centrally below them, giving impression of bird's foot. **C:** Similar stage, specimen on settlement panel, preserved in ethanol.



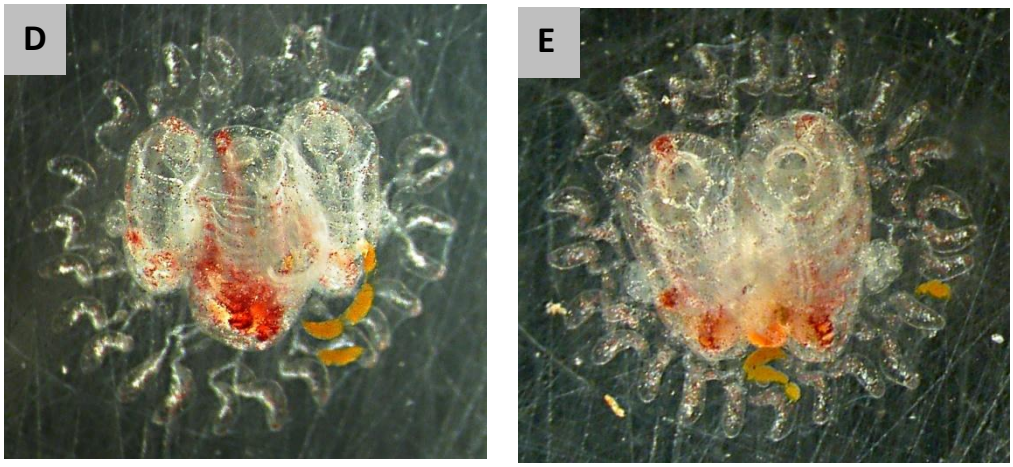
D: Zooid with bud on right side. **E:** Much later growth, forming 'system' of zooids, inhalant openings on the periphery and shared central exhalant opening in the centre.

The colonial ascidian *Botrylloides violaceus*

A: Larva, very large (trunk c. 1.3 mm long, plus tail) with complete ring of 25-35 forward-facing vascular ampullae.

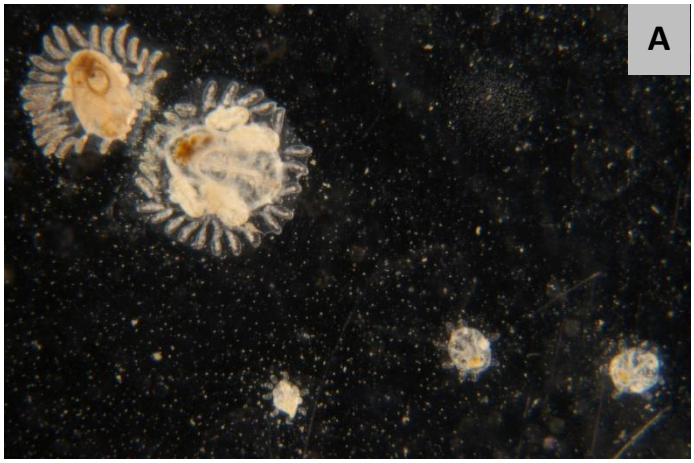


B: Oozoid, c. 3.9 mm across including vascular ampullae, with two small buds. The numerous vascular ampullae of the larva have radiated out and surround the oozoid. **C:** Oblique view of larger oozoid, with three buds.

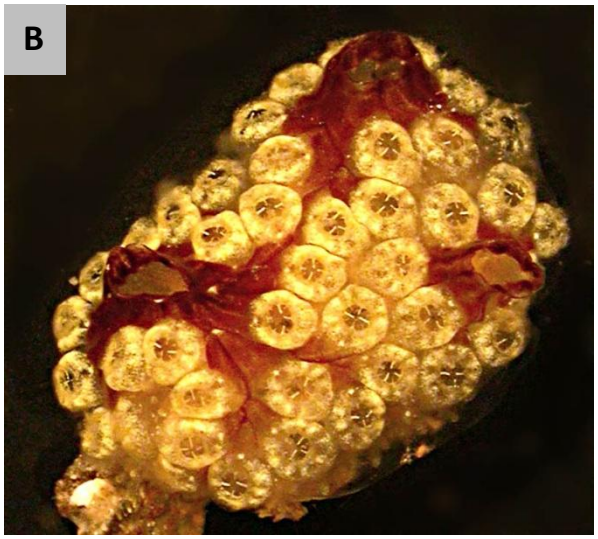


D: Oozoid with two daughter zooids, 7 days after settlement, cultured in the laboratory. **E:** The same colony 1 day later; the oozoid (centre) is being resorbed.

Comparison of *Botrylloides* and *Botryllus*

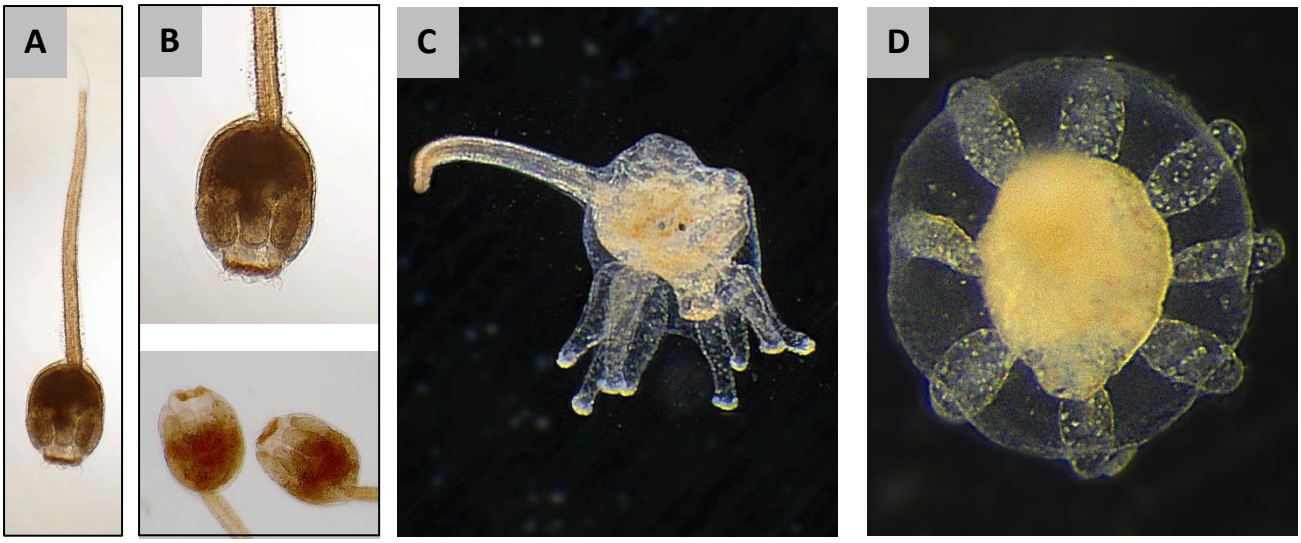


A: Comparison of size of oozoids of *Botrylloides violaceus* (two individuals top-left) and *Botryllus schlosseri* (two individuals bottom-right). The smaller fifth individual appears to be *B. schlosseri* in the process of metamorphosis, showing remnants of the larval tail. The large size of the larva and oozoid is a distinctive feature of *B. violaceus*; in *Botrylloides diegensis* and *B. leachii* (see below), the larva and early oozoid are much smaller and possess only eight vascular ampullae, as in *B. schlosseri*.



B: Small colony of *Botrylloides* sp. with three dark exhalant openings. Note that some zooids discharge into an enclosed water channel leading to an exhalant opening rather than reaching the edge of the opening and discharging directly into it. **C:** Eight-zooid 'system' of *Botryllus schlosseri* colony for comparison; all zooids reach the exhalant opening and contribute to its edge (red-rimmed in this colony) and thereby discharge directly into the opening. (See also figure **E** on *Botryllus schlosseri* page.) This difference from *Botryllus schlosseri* is generic rather than relating specifically to *Botrylloides violaceus*. In very small colonies of *Botrylloides,* the zooids can sometimes all be arranged around a central exhalant opening, much as in *B. schlosseri*, but this arrangement does not persist as the number of zooids increases, some zooids losing direct contact with the opening within elongated 'systems' incorporating water channels. In both genera, an extensive network of blood vessels links the zooids and forms a fringe of vascular ampullae around the colony.

The colonial ascidian *Botrylloides diegensis*

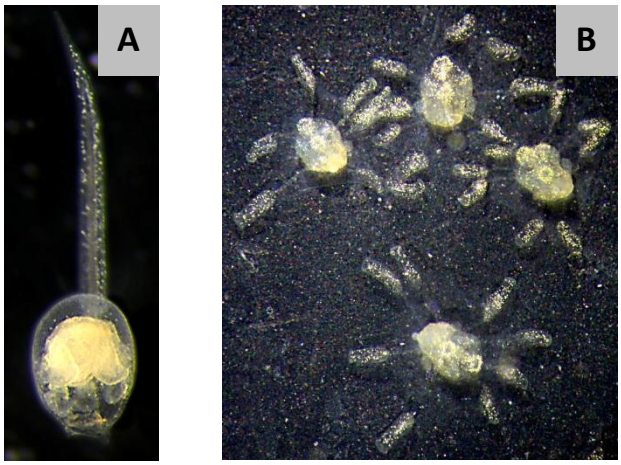


A: Larva. **B:** Larval trunks, showing central adhesive organ and outer ring of eight vascular ampullae. **C:** Larva with vascular ampullae expanded and separated, possibly the result of delayed metamorphosis. **D:** Initial attached phase.

E: Feeding oozoid c. 2 days after settlement, c. 1.1 mm long excluding vascular ampullae. **F:** Later oozoid with developing bud on right side. **G:** Side-view (left side) of stage similar to that shown In **F**, inhalant siphon to left of image.

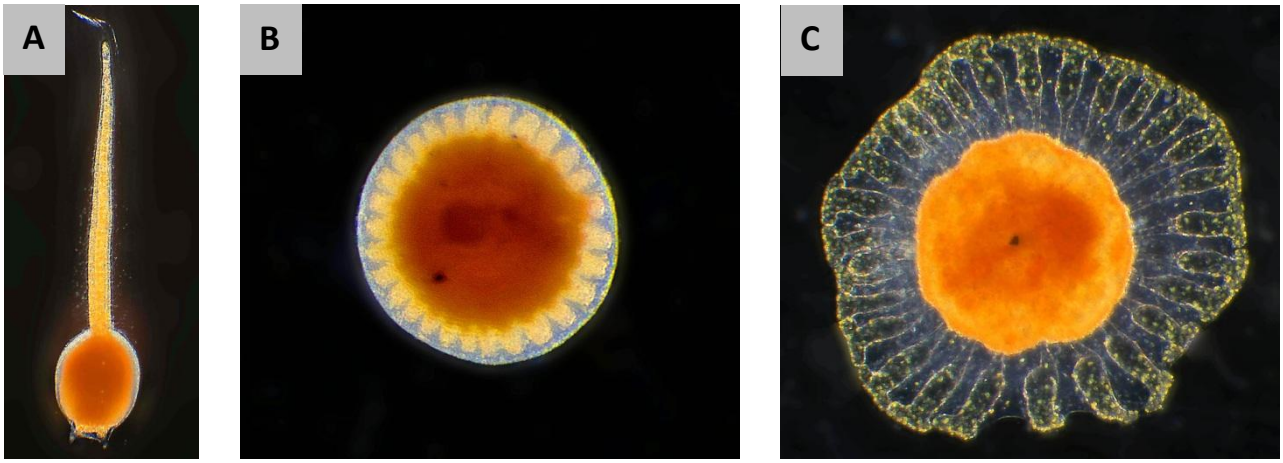
H: Daughter zooid (the first blastozooid of the incipient colony) budded by the oozoid, which was then resorbed; the daughter zooid, c. 2.7 mm long excluding vascular ampullae, now has a bud itself.

The colonial ascidian *Botrylloides leachii*

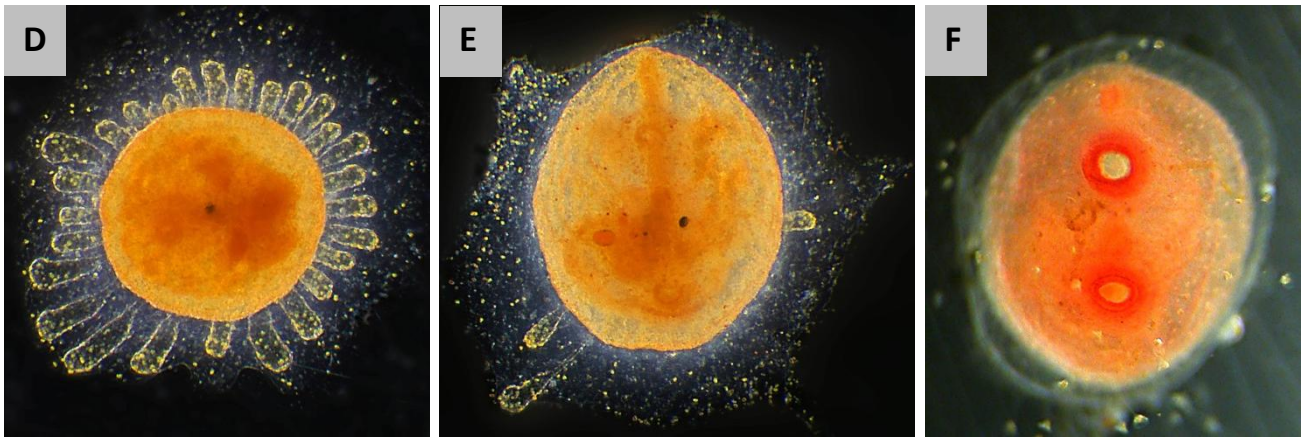


A: Larva. **B:** Four oozoids showing the eight large vascular ampullae.

The unitary ascidian *Dendrodoa grossularia*

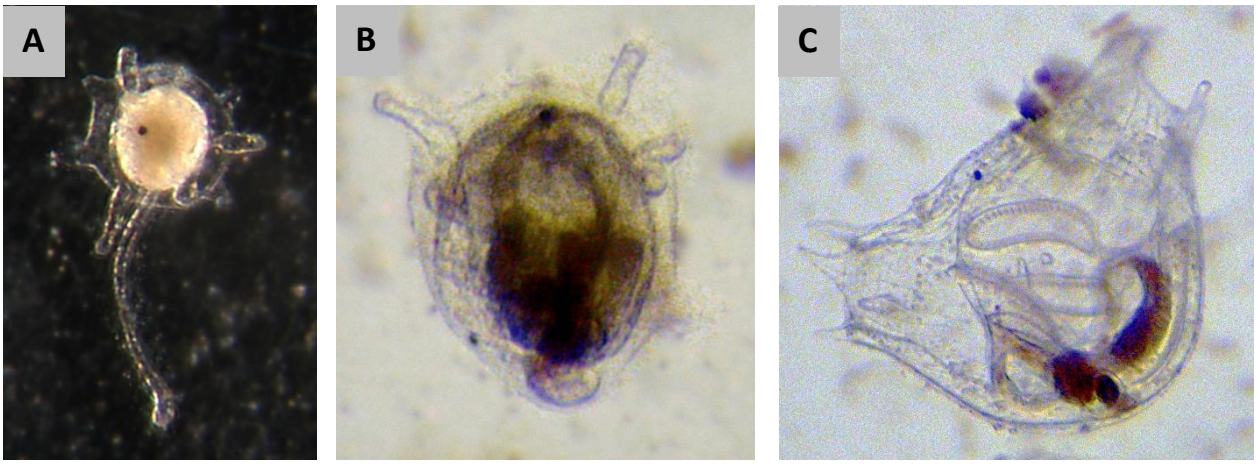


A: Larva. **B:** Very early post-settlement stage c. 0.57 mm across; almost 30 vascular ampullae are arrayed around the periphery. **C:** The numerous vascular ampullae, expanded at the tips, have formed an almost continuous border; central part (excluding ampullae) c. 0.60 mm.

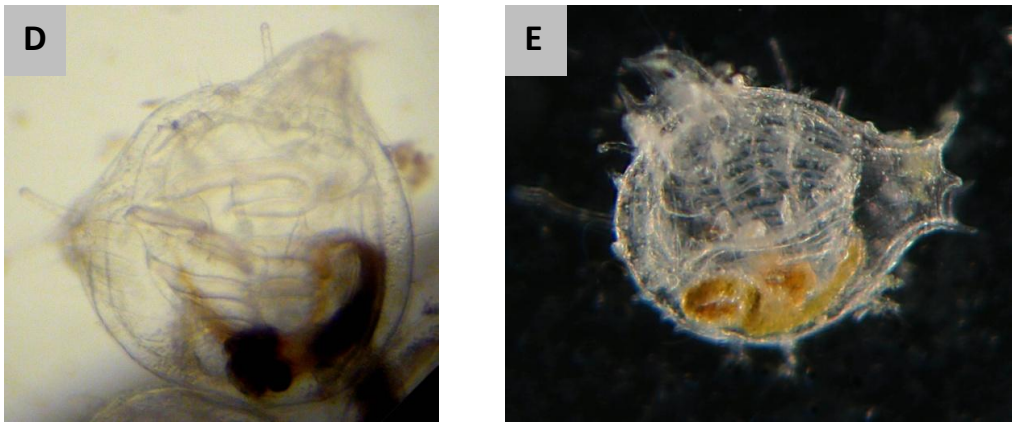


D: Vascular ampullae being withdrawn, leaving tunic behind; central part c. 0.62 mm across. **E:** Later stage, attached predominantly by tunic, c. 0.83 mm long, small siphons and endostyle discernible. **F:** Juvenile ascidian; the red colouration of the tissues persists throughout life. **C, D** and **E** depict the same specimen.

The unitary ascidian *Molgula* sp.



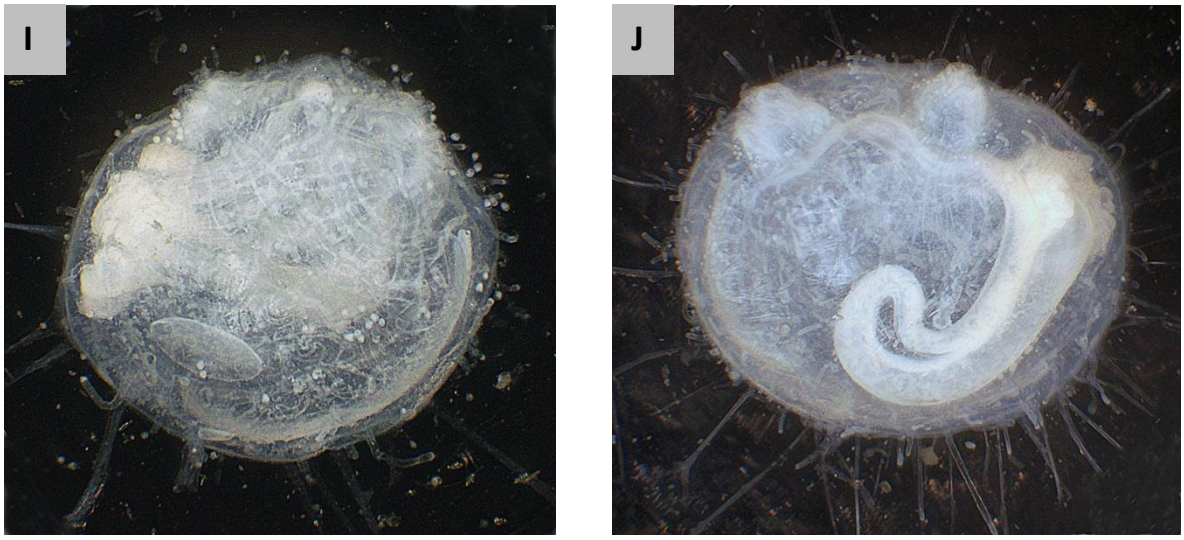
A: Very early post-settlement stage, c. 0.17 mm across excluding vascular extensions; the irregular nature of the extensions, often including at least one very long example, appears typical. **B:** 3 days after settlement, organs developing. **C:** Stage with two pairs of protostigmata; atrial siphon well developed (single opening, contrasting with phlebobranch species); dark oval renal vesicle adjacent to gut.



D: Later stage with original four protostigmata curling back dorsally at ventral (endostyle) end, creating hook shape, prior to division. **E:** Stage with at least five pairs of parallel protostigmata; c. 1.1 mm across including atrial siphon.

F: C-shaped stigmata during elaboration of branchial basket. **G:** Left side of specimen with infundibula forming in branchial basket; note hairpin gut-loop. **H**: Right side of similar specimen showing liver (below exhalant siphon) and renal vesicle (bottom centre; characteristic of molgulids).

Molgula sp. continued



I: Preserved specimen, c. 1.9 mm across, viewed from right side, showing oval renal vesicle (lower-left) and curved endostyle (lower right). **J:** Preserved later specimen, c. 4.0 mm across, viewed from left side, showing prominent hairpin gut-loop ending adjacent to the contracted exhalant siphon. In both images, the prominent, lobed liver is seen just below the exhalant siphon, and attachment fibrils radiate from the tunic.

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