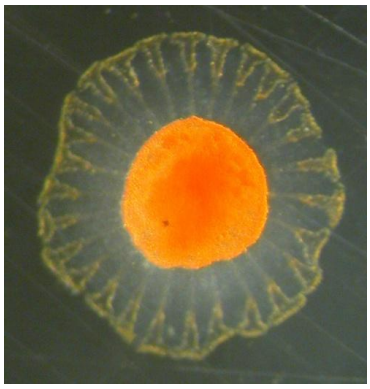
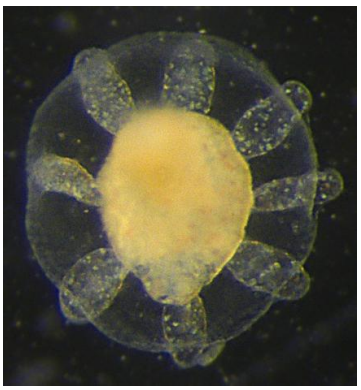
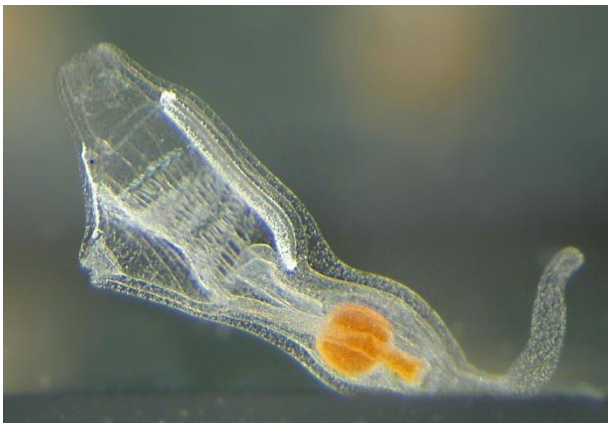
Guide to early post-settlement stages of fouling marine invertebrates in Britain







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with help from Aaron Hartnell and Christopher Dwane

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Acknowledgements

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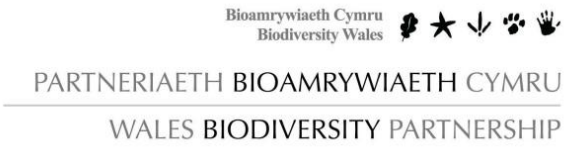

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Contents

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 hydroids and 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 generally 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. This is particularly true of 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. Many of the images used are of specimens settled onto transparent substrates (generally Petri dishes) using lighting from underneath, with either bright-field or dark-field illumination. 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. A range of online resources and booklets for the identification of the adult stage of non-native species currently fills this gap, including a guide available online at http://www.mba.ac.uk/bishop/non-native-species-guides/ 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 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: 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.

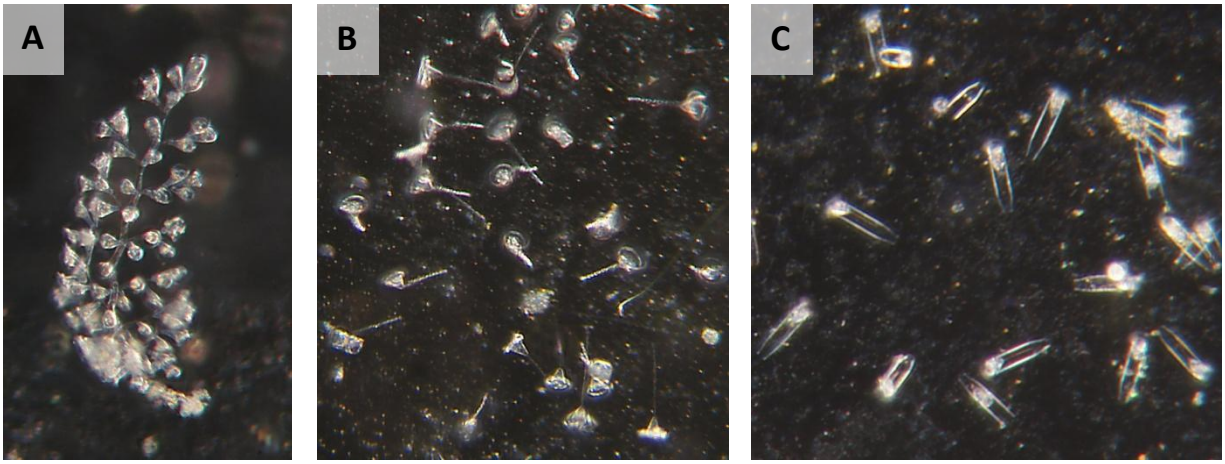
Unitary: Descriptive of species remaining as a single individual derived from the larva, not colonial (q.v.); in ascidians, also called solitary, although 'solitary' ascidians can occur in dense aggregations.

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

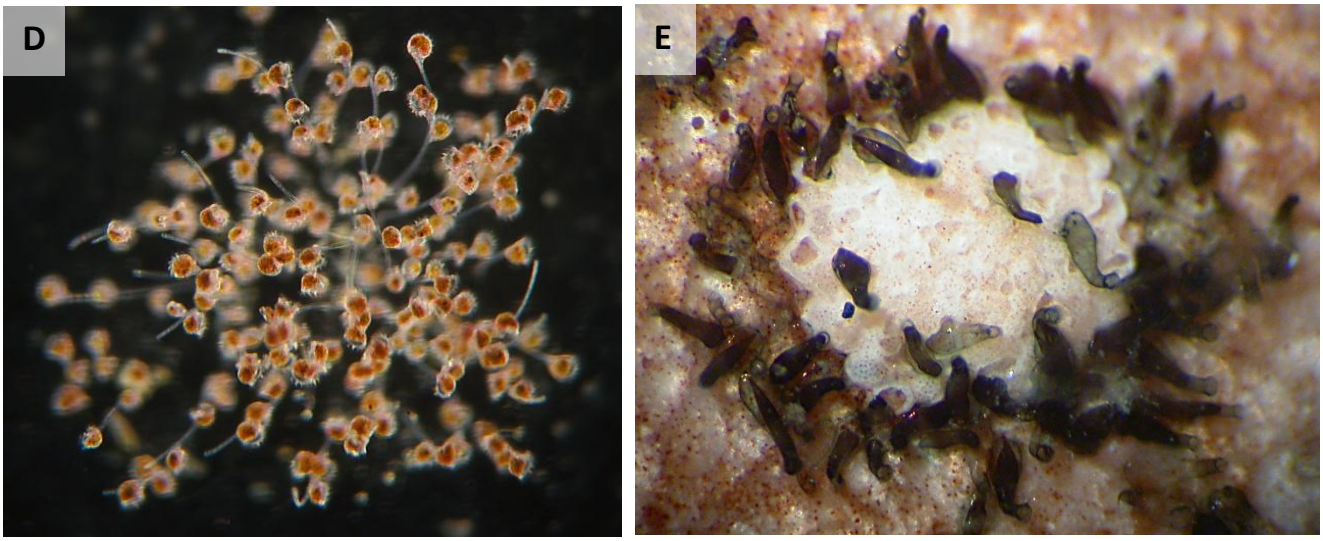
SEM = Scanning electron microscopy

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.



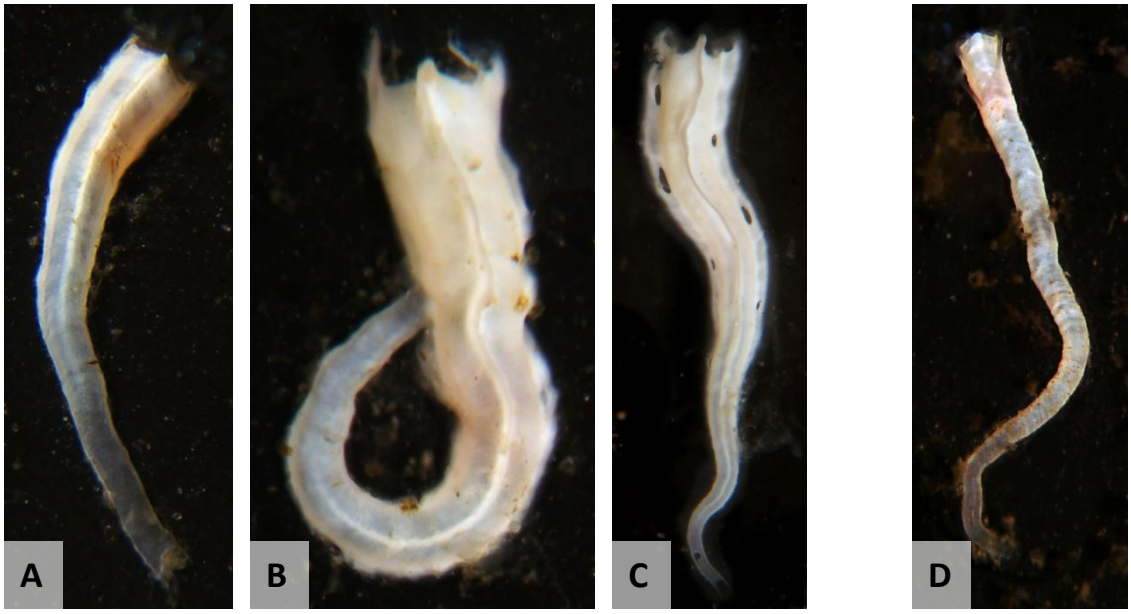
A: Colonial ciliophoran. Shown expanded; the 'stems' are strongly contractile. **B** and **C:** Unicellular ciliophorans. The tube-forming species in **C** attaches temporarily but can swim with its tube.



D: Colonial ciliophoran. **E:** Unicellular folliculinid ciliophorans growing around and on a white foraminiferan; each folliculinid possesses a bi-lobed ciliated structure extended from the flask-shaped covering (= lorica, c. 0.4 mm long) for feeding (but all are withdrawn in this photograph).

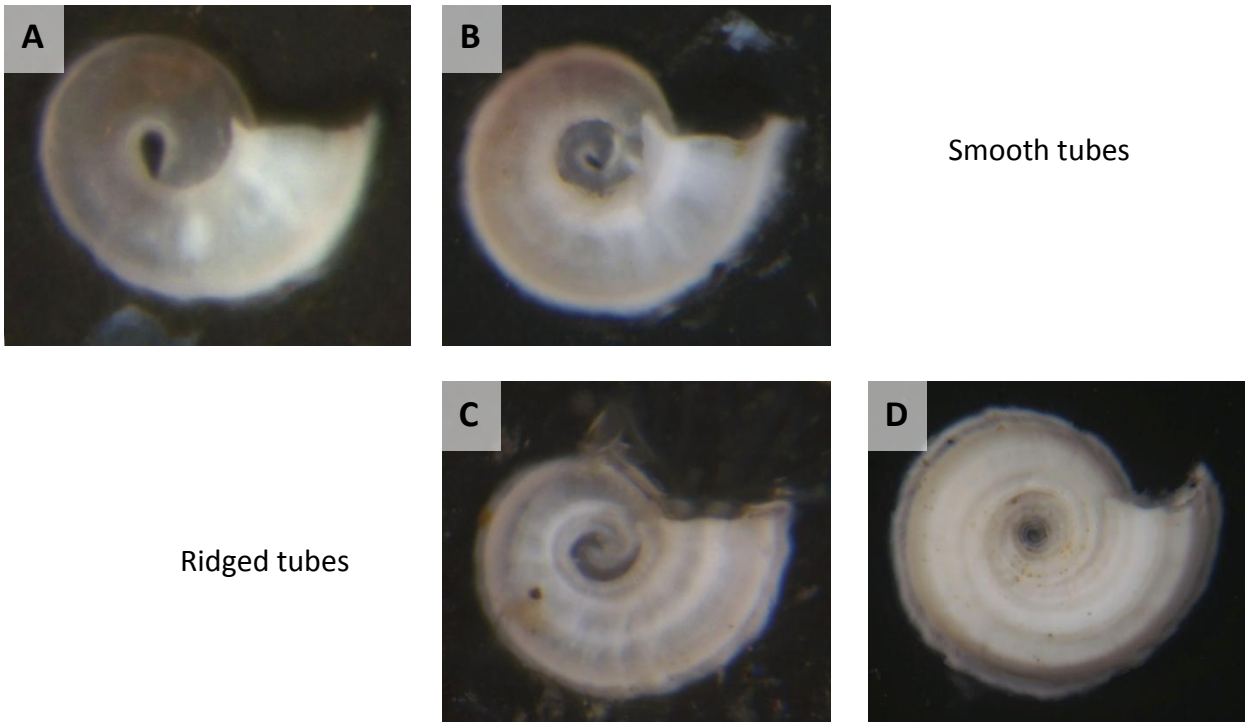
F: Foraminiferan

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

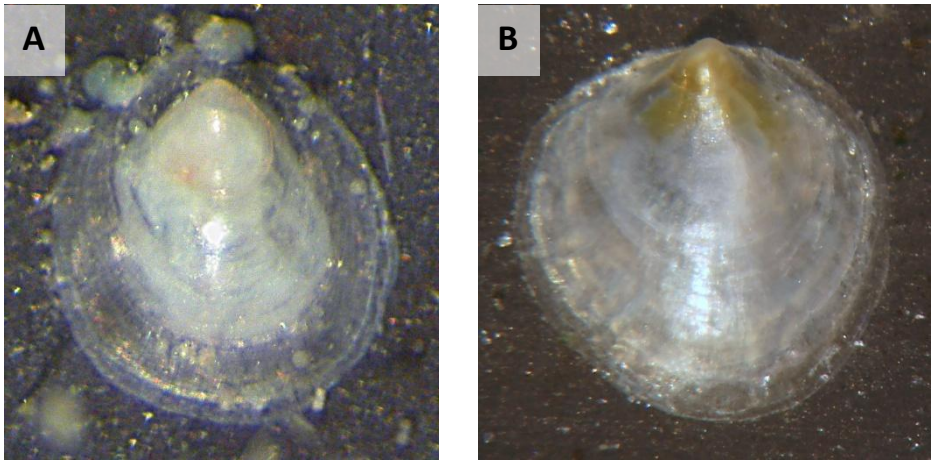


Smooth tubes

Ridged tubes

A: c. 0.3 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).

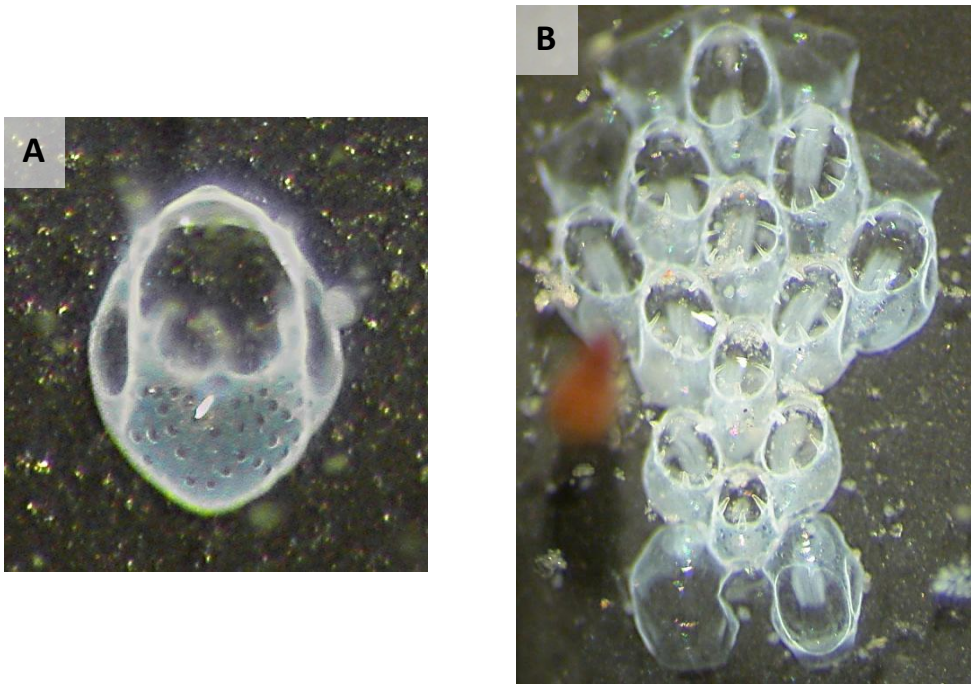
Saddle oysters (Anomiidae)



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

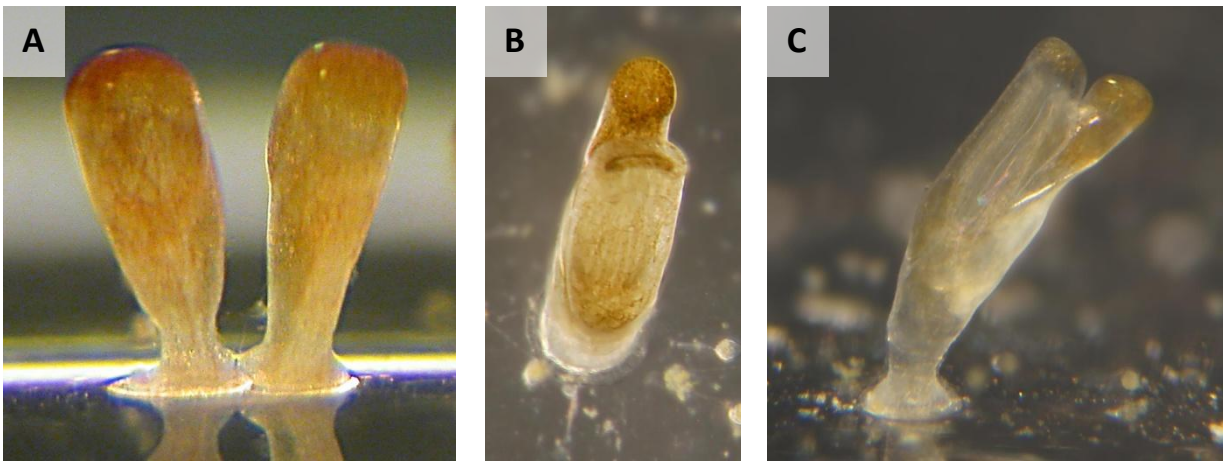
Saddle oysters are permanently attached by a calcified byssal thread passing through a deep, rounded notch in the lower valve. This means that the shell can be made to move slightly by applying gentle sideways pressure, unlike true oysters in which the lower valve is firmly cemented to the substrate.

The bryozoan *Electra pilosa*

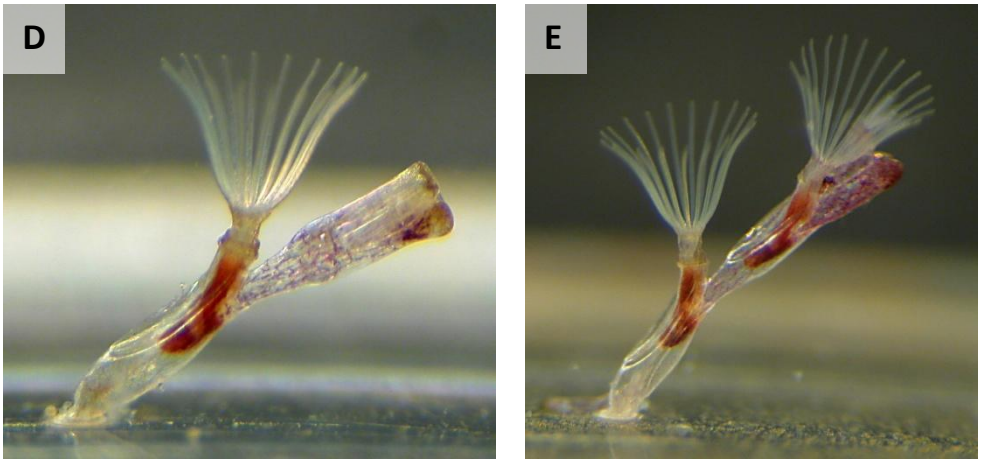


A: Ancestrula, c. 0.4 mm long. **E:** Small colony of 12 completed zooids.

The bryozoan *Bugula neritina*



A: Two developing ancestrulae. **B** and **C:** Later ancestrula with orifice, retracted lophophore and bud of daughter zooids.

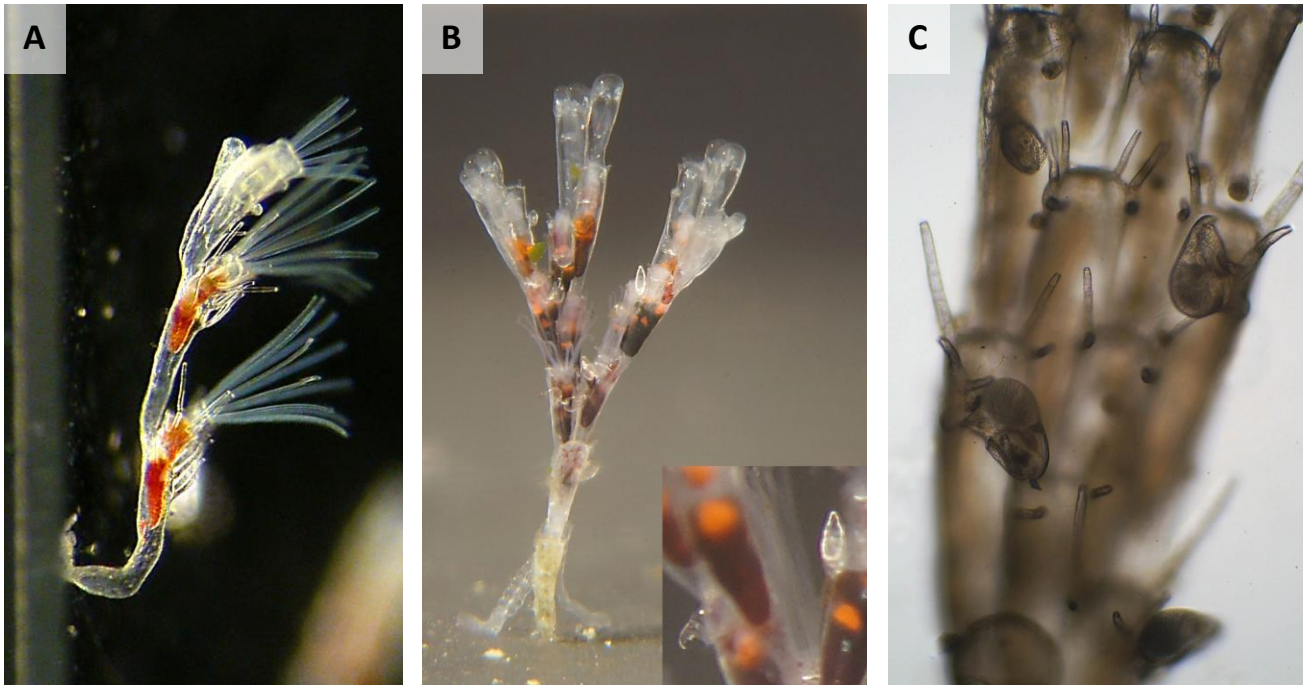


D: Ancestrula with lophophore extended and incomplete daughter zooids. **E:** Same colony 3 days later, one daughter zooid feeding.

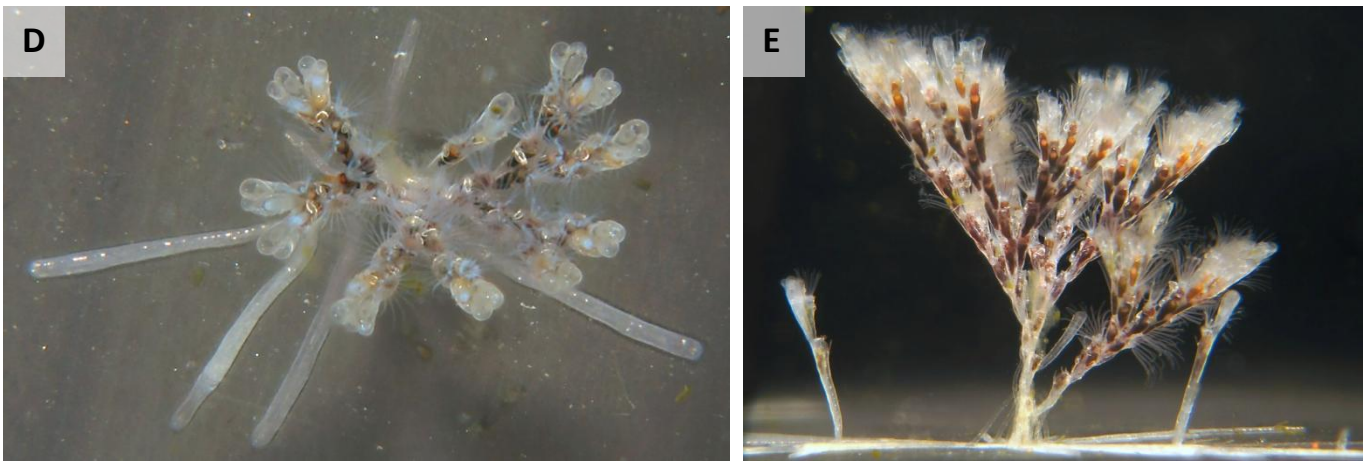
F: Cluster of young colonies resulting from co-settlement of larvae, c. 3 weeks after settlement.

Other *Bugula* species

These figures are shown as examples of a suite of *Bugula* species differing markedly from *B. 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.

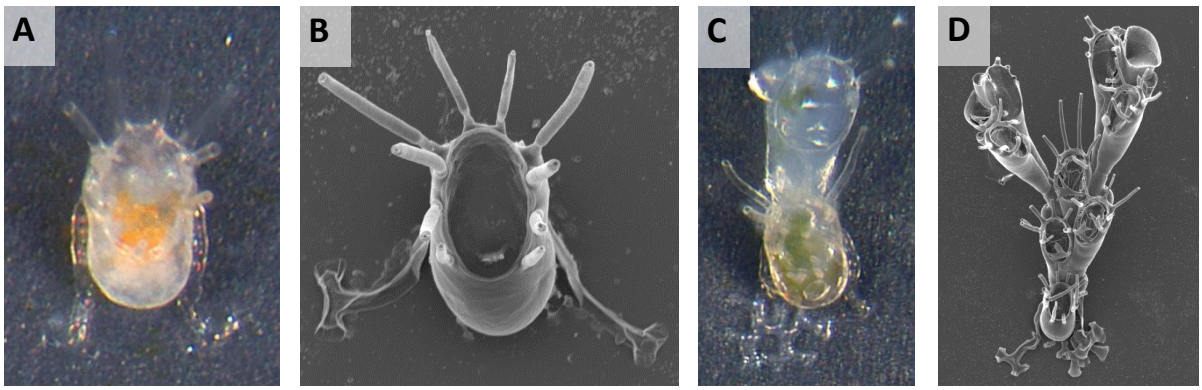


A: Ancestrula and first two generations of daughter zooids **B:** Young colony, c. 3.6 mm tall; inset, bird's-head avicularia in front and side view. **C:** Spines and bird's-head avicularia on a branch with four rows of zooids.

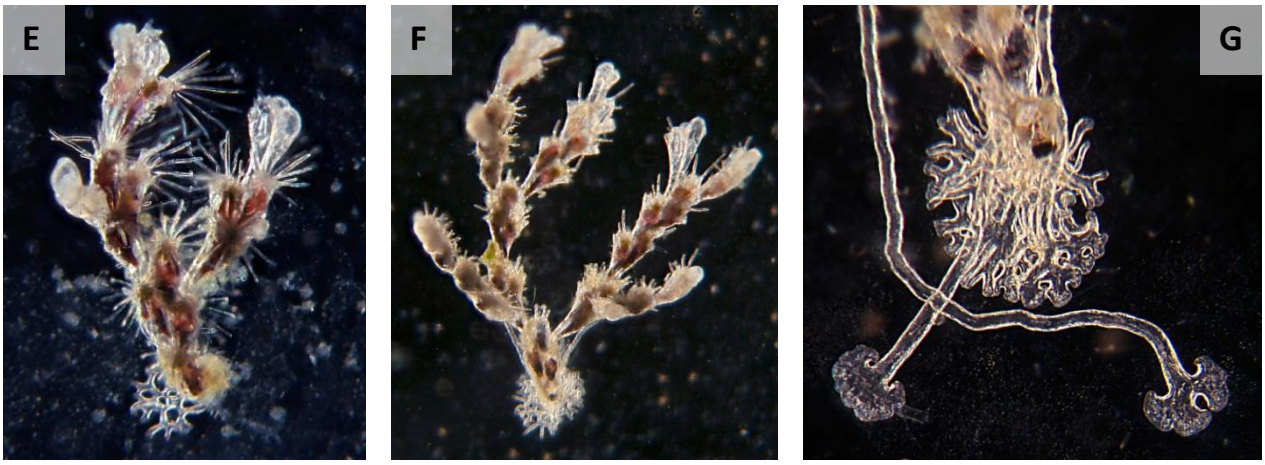


D: Small colony from above, showing attachment stolons; shining white specks are bird's-head avicularia; maximum span of stolons is c. 6.1 mm. **E:** Colony with secondary sub-colonies arising adventitiously from attachment stolons; main colony c. 5.8 mm tall.

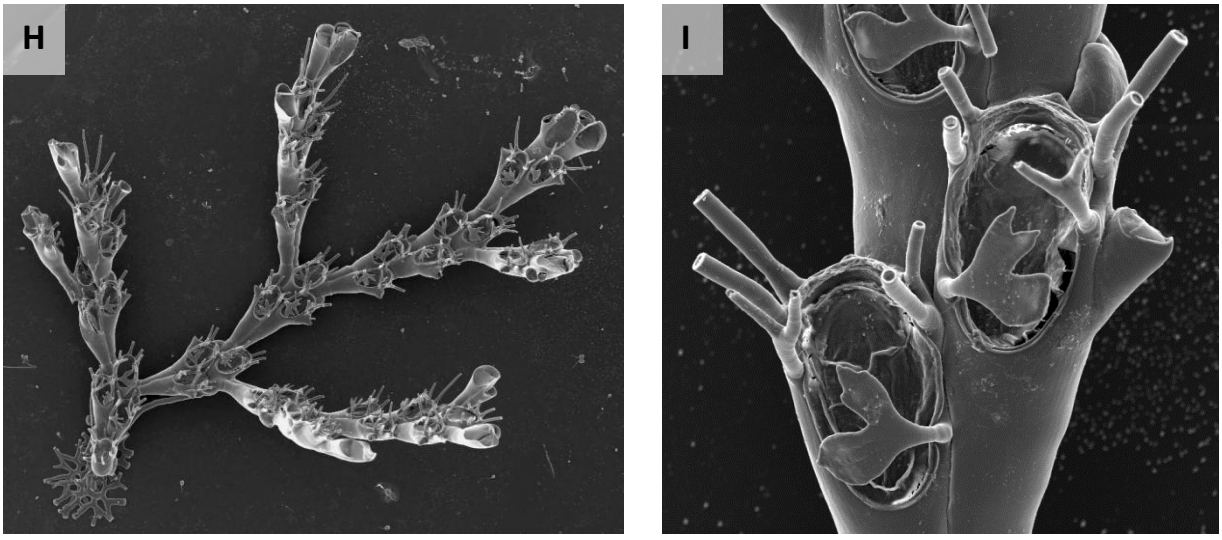
The bryozoan *Tricellaria inopinata*



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.

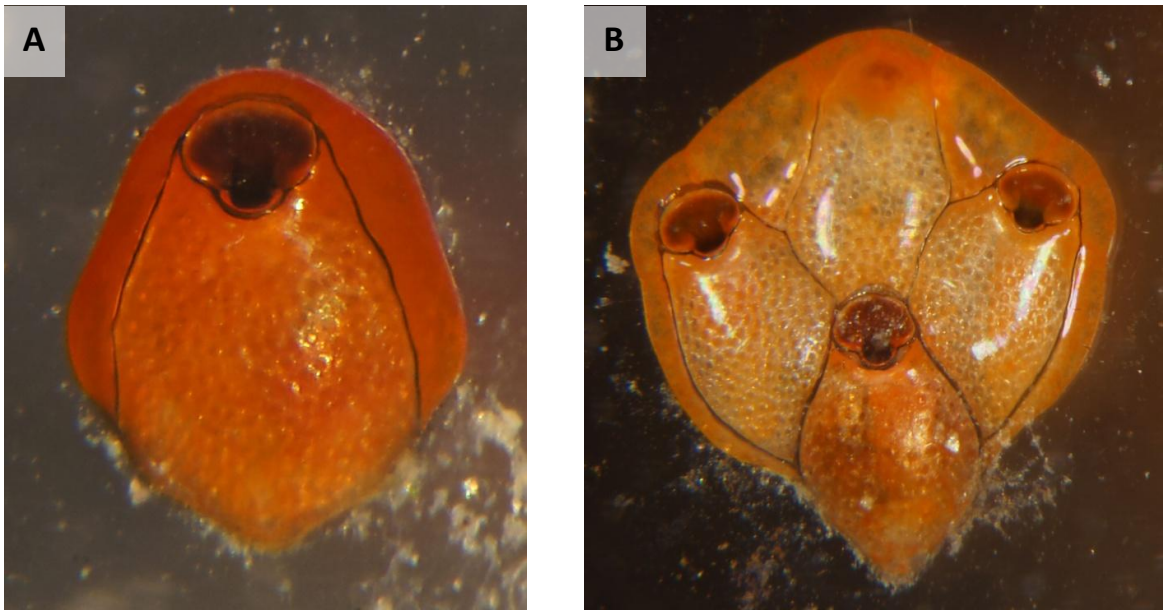


E: Young colony with first bifurcations, c. 1.2 mm across; lophophores extended. **F:** Colony with further branches, c. 2.9 mm across. **G:** attachment plaque at base of colony, with accessory attachments formed by rhizoids growing down from higher zooids; image area c. 1.4 mm across.

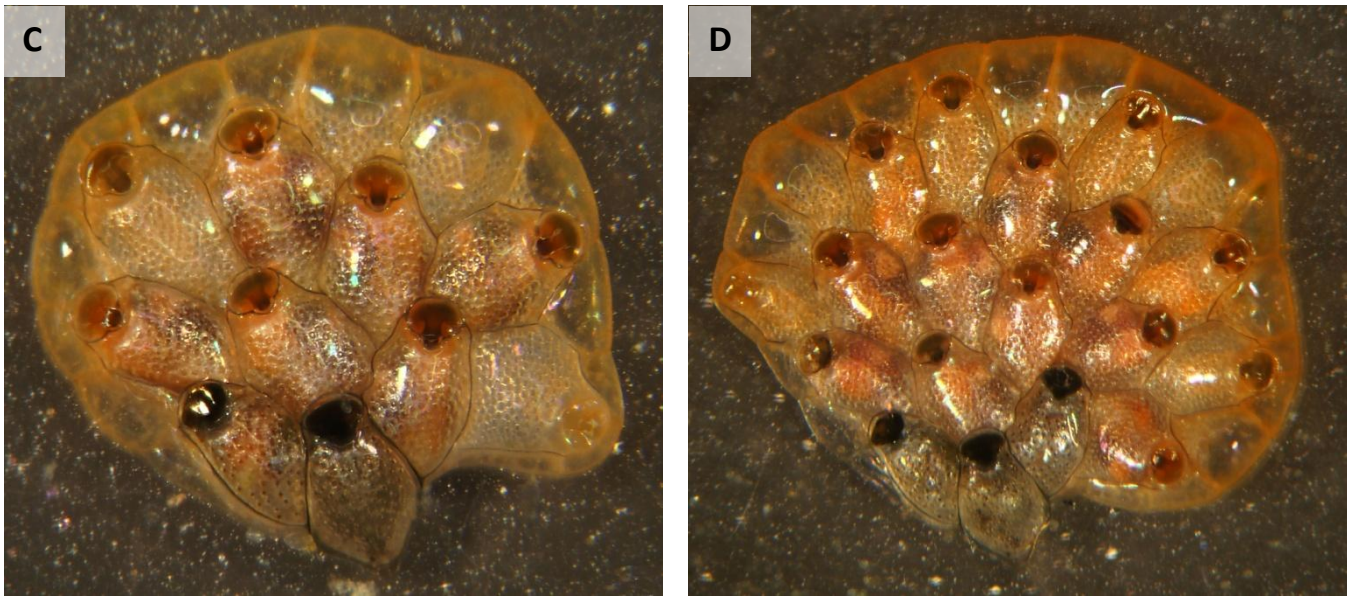


H: Colony with c. 50 zooids (SEM), maximum extent c. 3.7mm. **I:** Zooids of branch of immature colony (SEM), showing flattened spine (characteristically very variable in shape) on inner margin of zooid and bifid spine (only sometimes present) on outer margin, with triangular lateral avicularium (only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

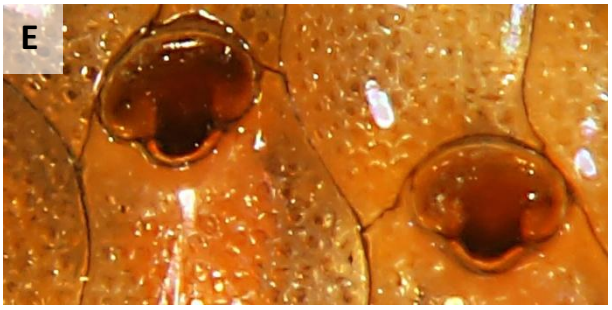
The bryozoan *Watersipora subatra*



A: Ancestrula with origins of daughter zooids, c. 0.8 mm long. **B:** Early colony, c. 1.4 mm wide.

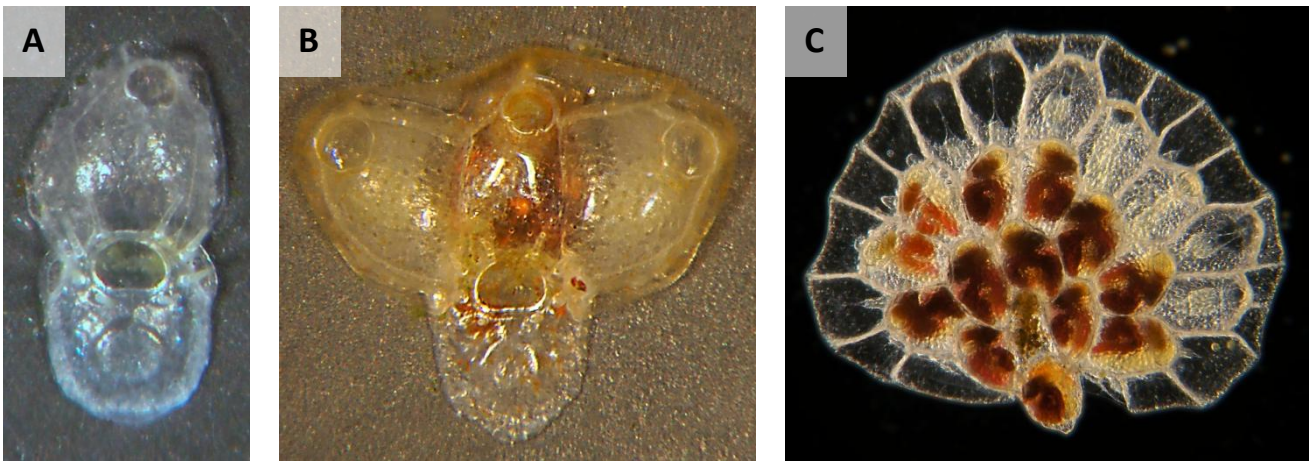


C: Colony c. 2.3 mm wide. **D:** same colony as in C but 8 days later, c. 2.9 mm wide.

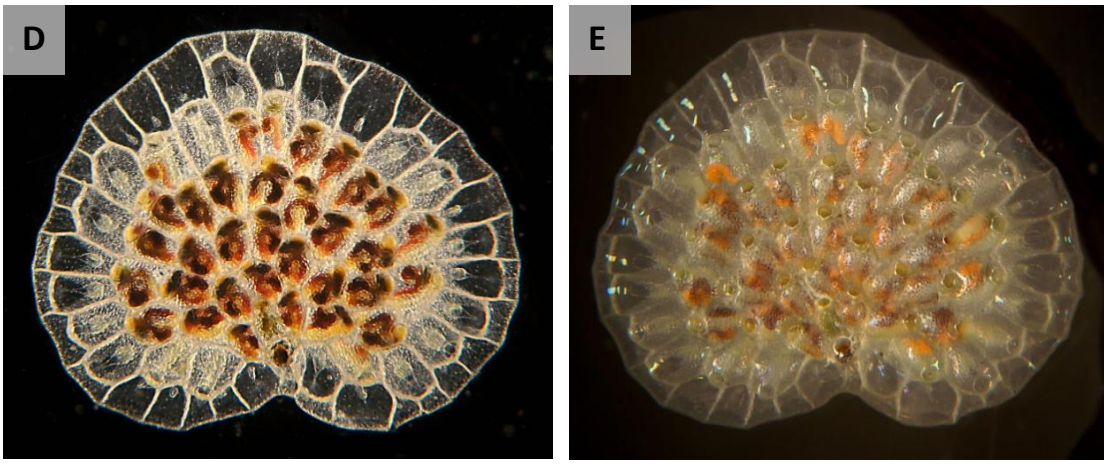


E: Detail of opercula.

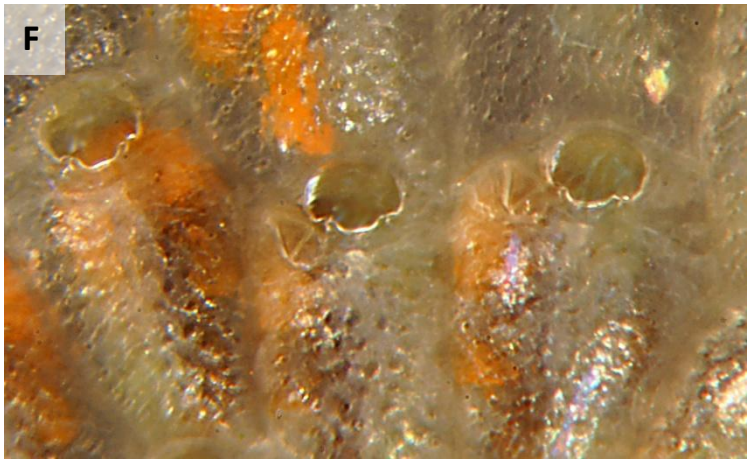
The bryozoan *Schizoporella japonica*



A: Ancestrula and first daughter zooid (dead), c. 0.8 mm long. **B:** Four-zooid colony, c. 1.0mm across. **C:** later colony, c. 2.5 mm across, showing fringe of developing zooids.



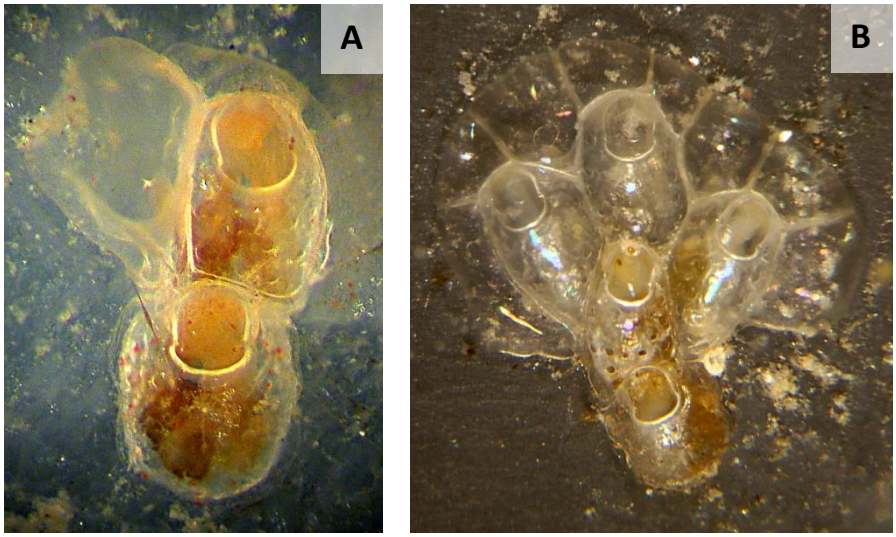
D: larger colony, c. 4.4 mm across, dark-field illumination. **E:** The same colony, incident illumination.



F: Zooids from colony shown in **D** and **E**.

Other *Schizoporella* species resemble *S. japonica*, and identification generally requires assessment of well-grown colonies for such features 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 is small colonies.

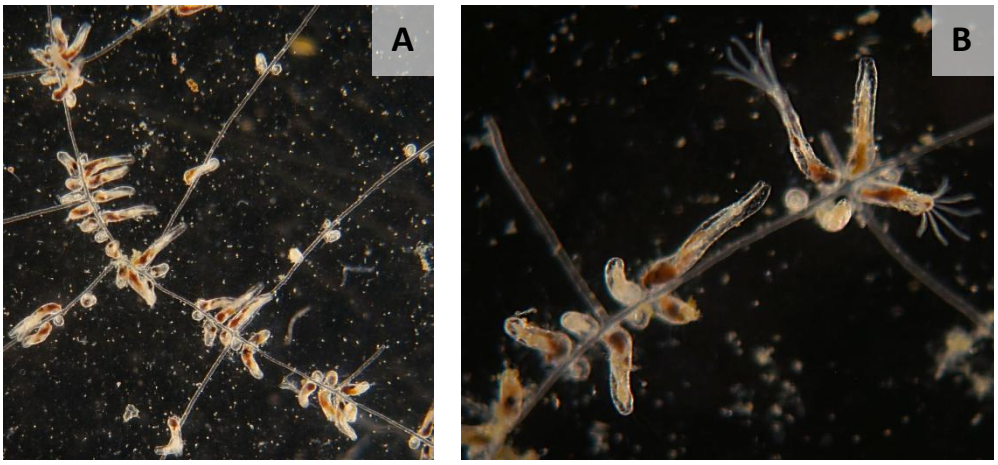
The bryozoan *Cryptosula pallasiana*



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

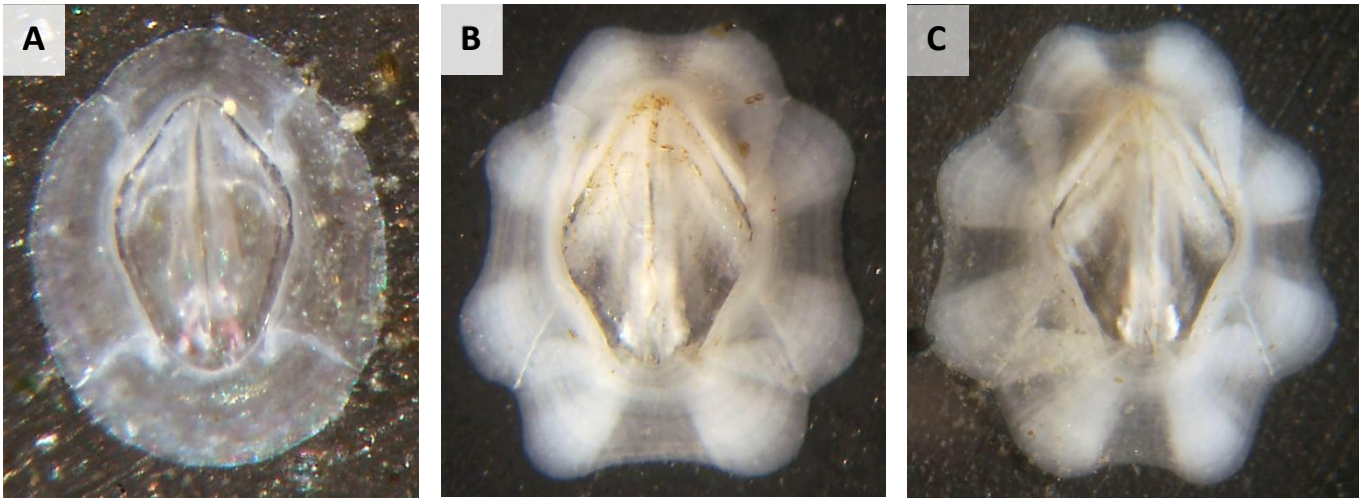
D: Colony of c. 35 zooids, c. 5.0 mm across.

The bryozoan *Bowerbankia* sp.



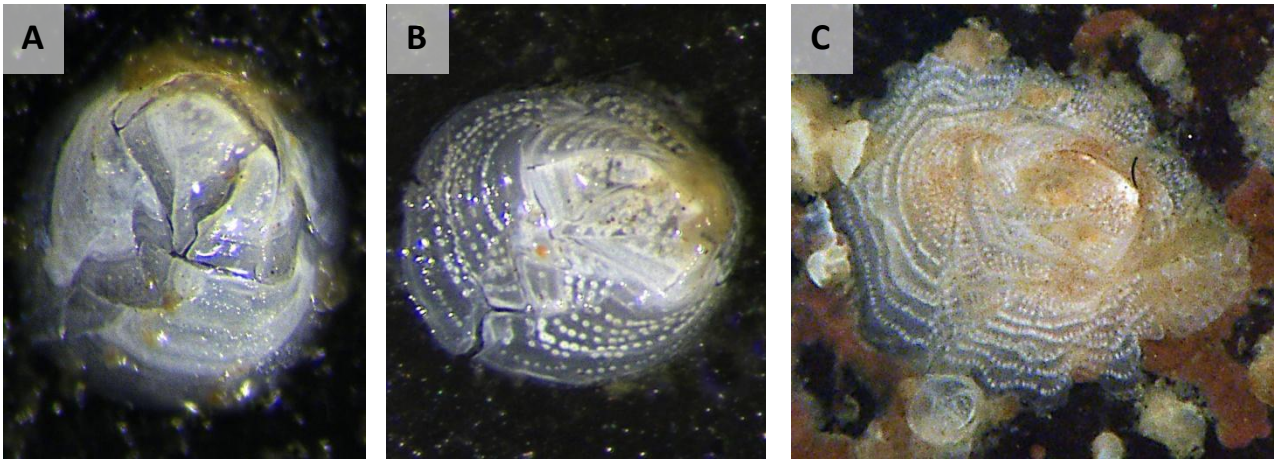
A: Section of colony. **B:** Enlargement of zooids.

The barnacle *Austrominius modestus*



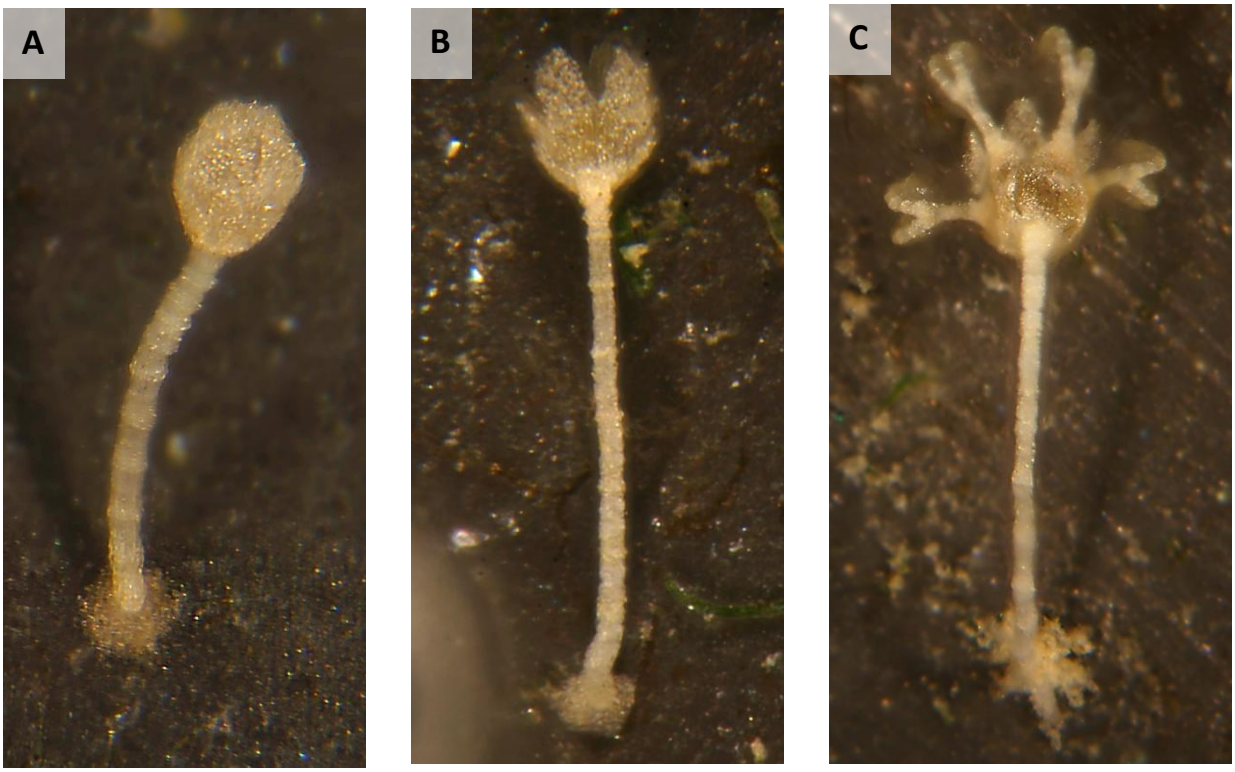
A: c. 0.8 mm long. **B:** c. 1.6 mm long. **C**: c. 2.2 mm long. Note only four (rather than the typical six) fixed plates form the outer wall, and the 'Maltese cross' outline being acquired in **C**, often seen in the absence of crowding.

The barnacle *Verruca stroemia*



A: Early stage. **B, C:** Later stages . An asymmetrical barnacle with only two moveable plates.

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. **C:** Pentacrinoid, c.2.5 mm long.

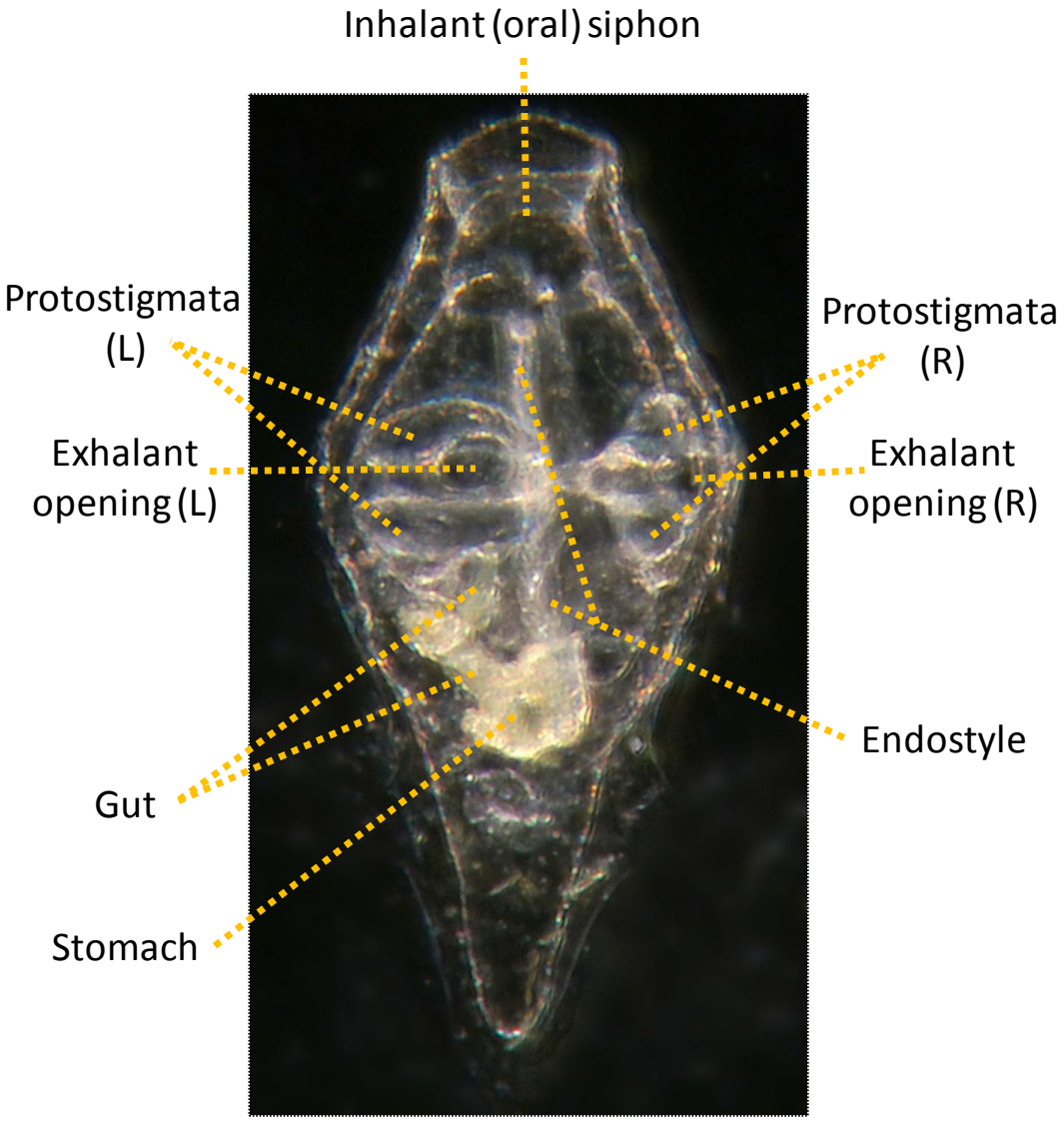
Pentacrinoid: **D**, c. 3.4mm long; **E**, c. 7.5 mm long.

(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 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 a much smaller number of openings, much larger relative to the body, with 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. The 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 protostigmata, 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 an 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*.

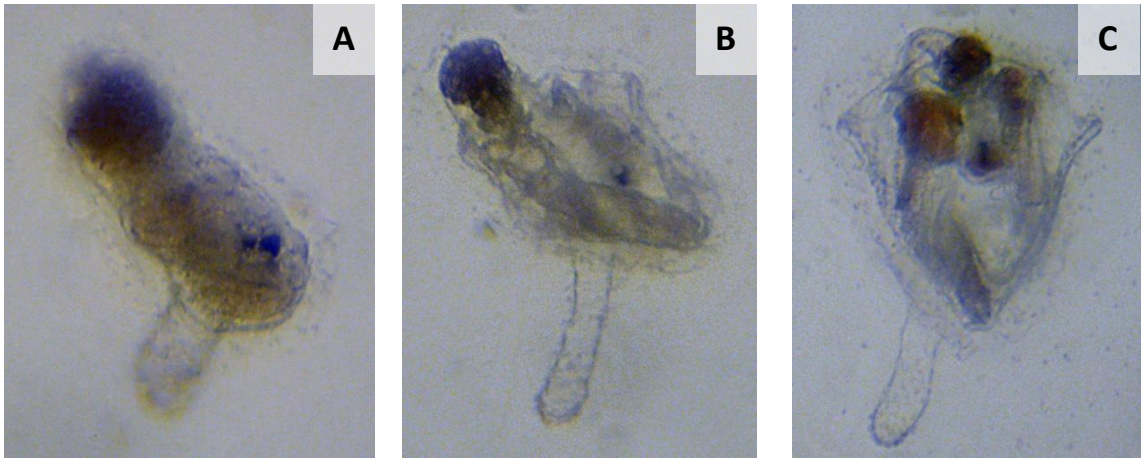
Many styelids pass through an early phase with arrays of parallel protostigmata diverging to left and right from the dorsal margin of the feeding apparatus. In combination with the underlying endostyle bisecting this array when viewed from above, a pattern reminiscent of a bird's foot is produced. This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

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.

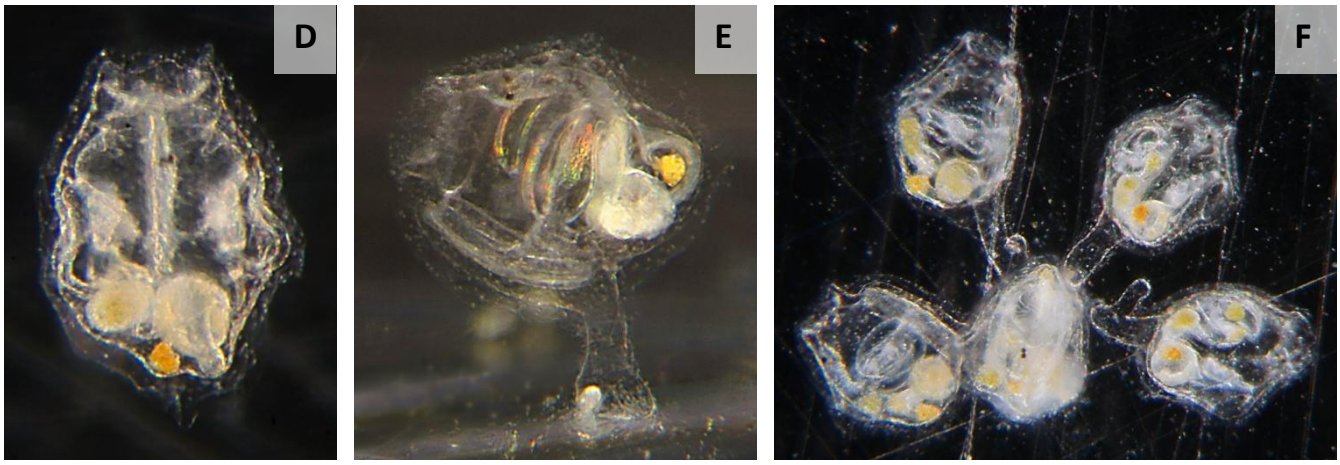
Ascidian metamorphosis involves the resorption of the larval tail into the posterior part of the trunk, creating a transitory mass of material (principally derived from the notochord) which can be seen in some of the illustrations here (e.g. *Ciona intestinalis*, dark mass in figures A-C and golden spots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

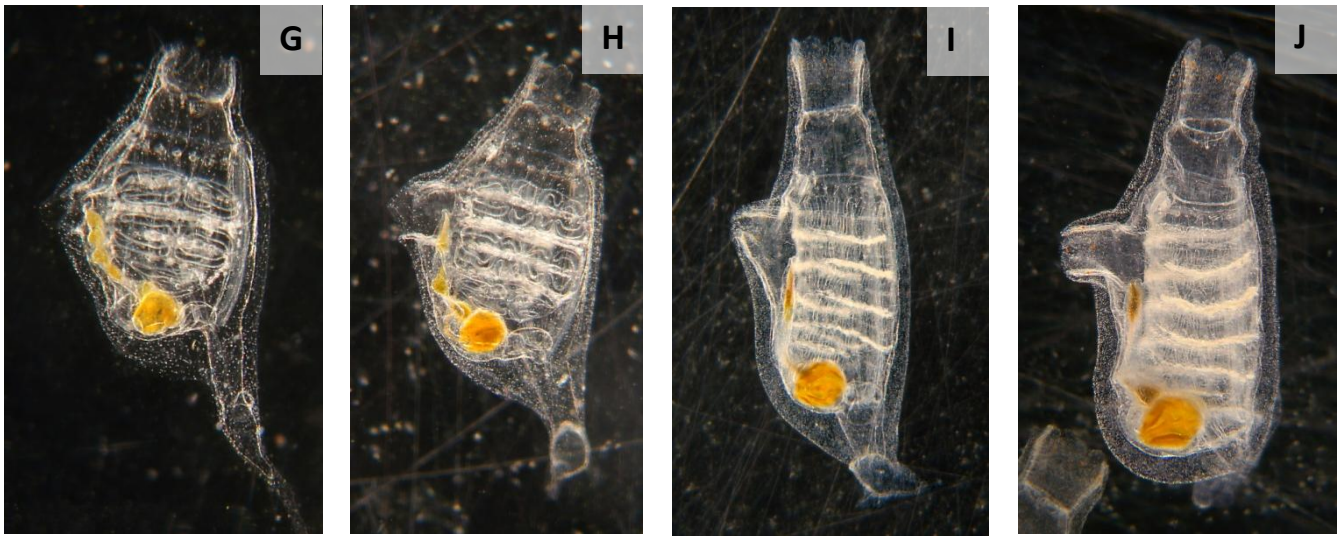
The unitary ascidian *Ciona intestinalis*



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

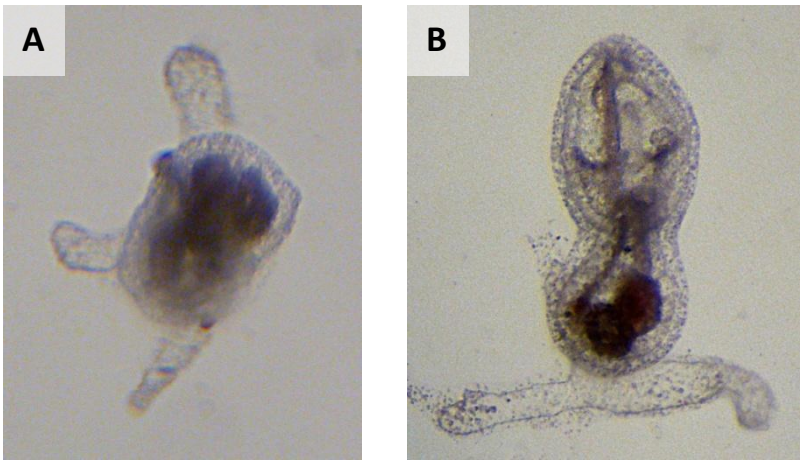


D: Stage with two pairs of protostigmata and two separate exhalant openings, c. 0.60 mm across, viewed from above with inhalant siphon at top. **E:** same stage viewed from the side, showing stalk; cilia in protostigmata iridescent, inhalant siphon to left; body c. 0.59 mm across. **F:** Same stage,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

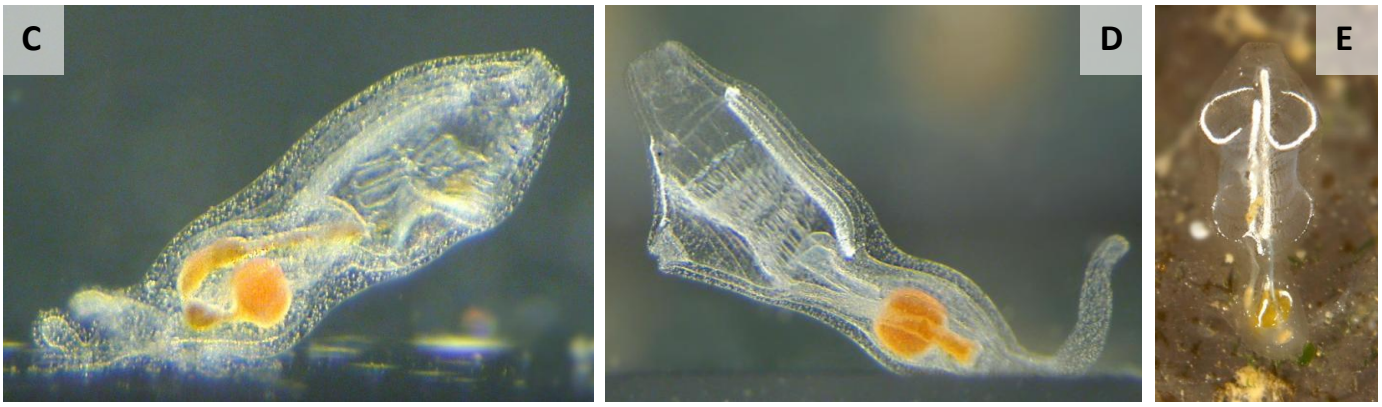


G-J: Later stages viewed from left side, showing progressive loss of stalk and elaboration of branchial basket. Heights not including stalk: **G**, c. 1.7 mm; **H**, c. 2.1 mm; **I**, c. 4.8 mm; **J**, c. 5.0 mm.

The colonial ascidian *Clavelina lepadiformis*



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

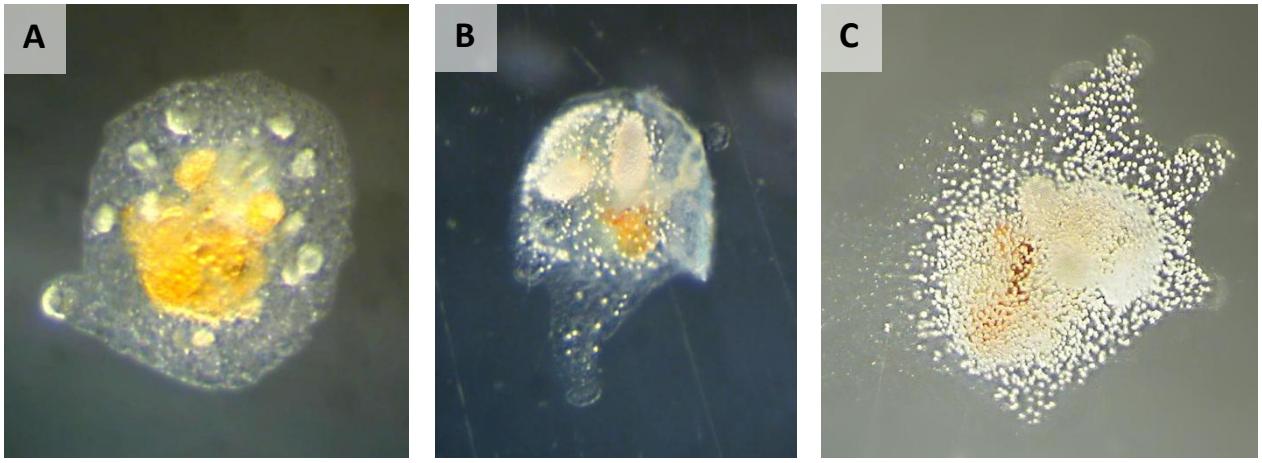


C: Oozoid c. 3 days post settlement. **D:** Oozoid c. 8 days post settlement. **E:** Single zooid on settlement panel, c. 2.1 mm wide.

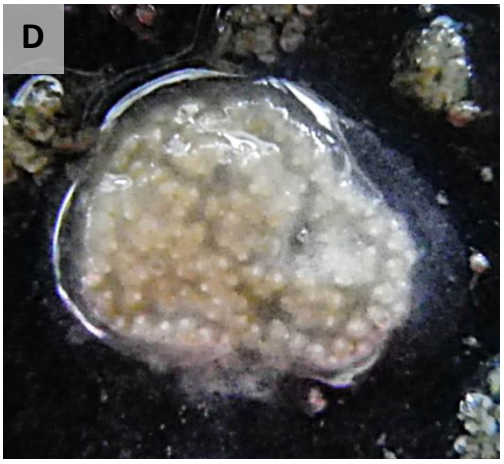
Presumed polyclinid ascidian

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*

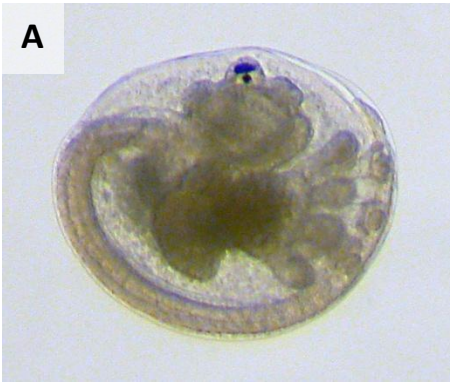


A: Very early post-settlement stage; two orange, oval lateral thoracic organs (LTOs) flank the developing branchial basket of the oozoid. **B:** Early oozoid, the two LTOs are white and producing calcareous spicules that are spreading into the tunic. **C:** Later oozoid, spicules numerous. It is very doubtful whether *D. vexillum* can be distinguished from other didemnid species at this stage (see next page).



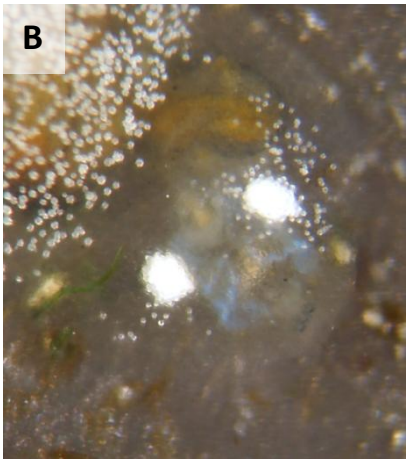
D: Small didemnid colony on settlement panel at site known to host *D. vexillum*. The dark (relatively spicule-free) water channels between zooids and the separation of zooids into small clumps bordered with white dots (dense spicules where the zooidal orifices are tightly closed in this specimen out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

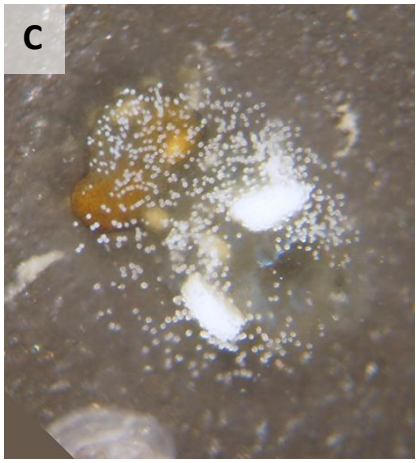
Spicule-producing didemnids other than *D. vexillum*

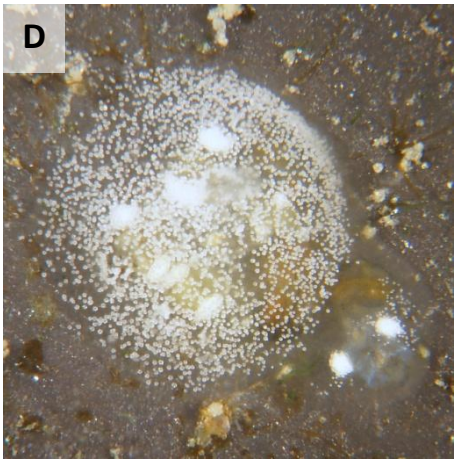


A: Unhatched larva clearly not belonging to *Didemnum vexillum*. This larva, with the tail still wrapped aroung the body, has three adhesive papillae (by which the larva will initially attach), seen here on the extreme right, and four pairs of darker, rounded vascular ampullae just behind them. *D. vexillum* 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



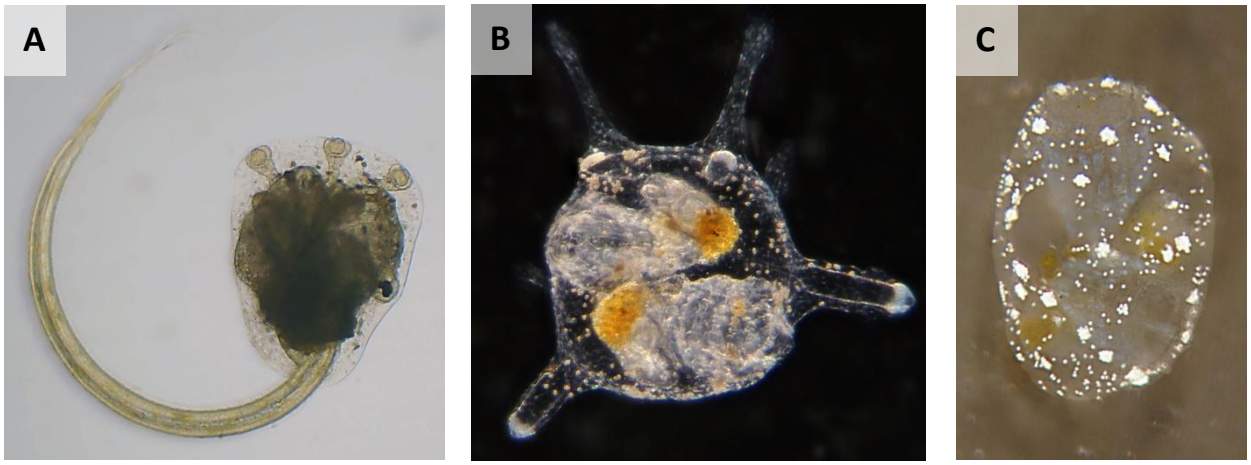




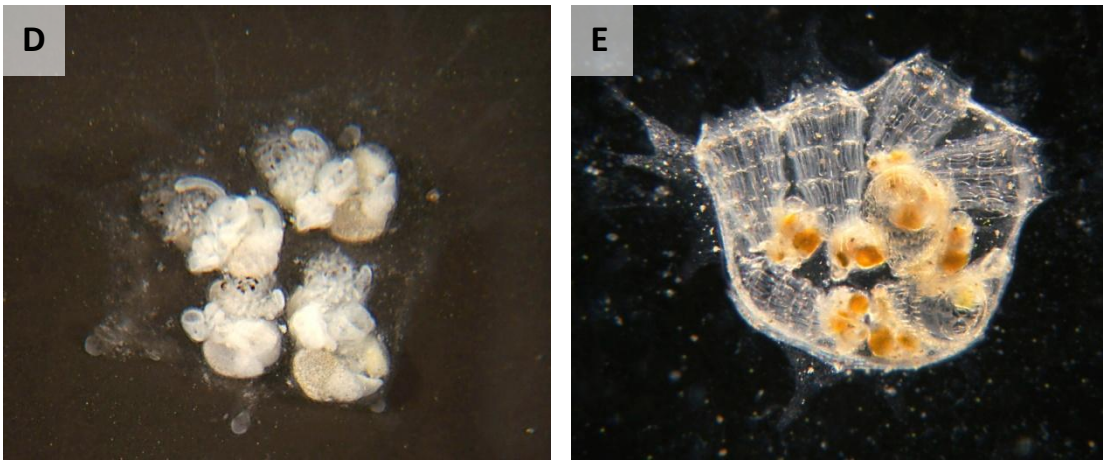
B: Oozooid (adjacent to larger colony, as shown in **D**), c. 0.9 mm across, a few spicules entering tunic from white LTOs; branchial basket bluish. **C:** slightly later stage with spicules spread further, c. 1.2 mm across. **D:** Later three- or four-zooid stage, c. 2.0 mm across.

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

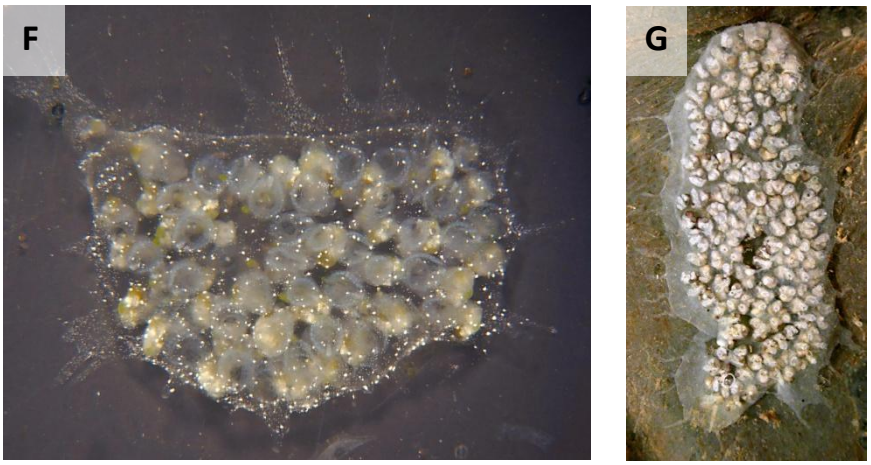
The colonial ascidian *Diplosoma listerianum*



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.

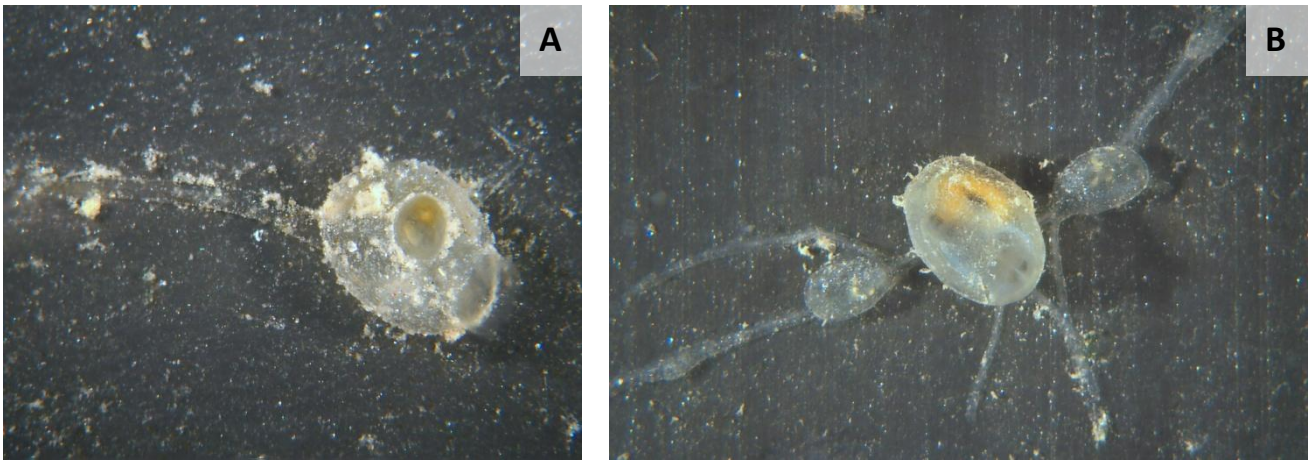


D: Alcohol-preserved four-zooid colony, c. 1.7 mm across zooids, incident illumination. **E:** Live seven-zooid colony, dark-field illumination.

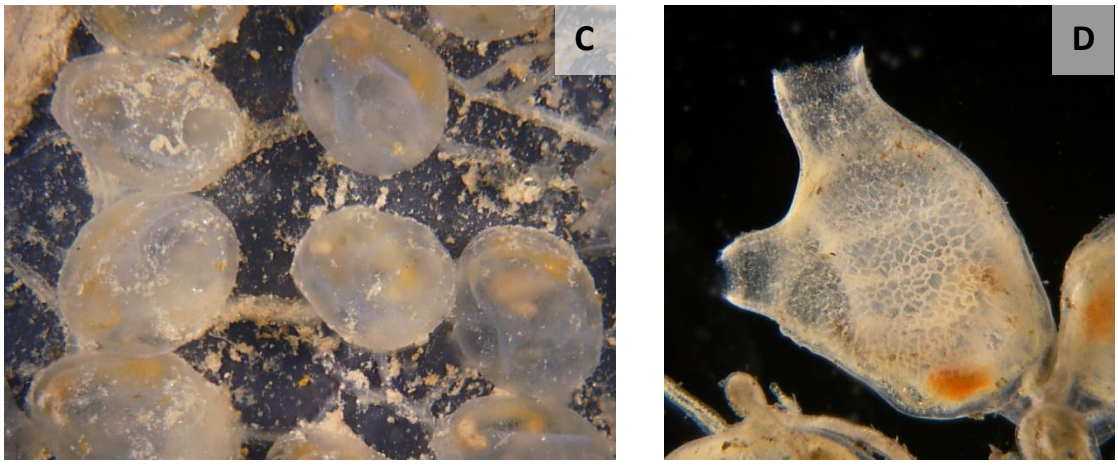


F: Live colony of c. 40 zooids, c. 9.5 mm across; incident illumination. **G**: Alcohol-preserved colony of *Diplosoma* sp., c. 130 zooids, c. 14.5 mm across; central oval hole in tunic is exhalant opening.

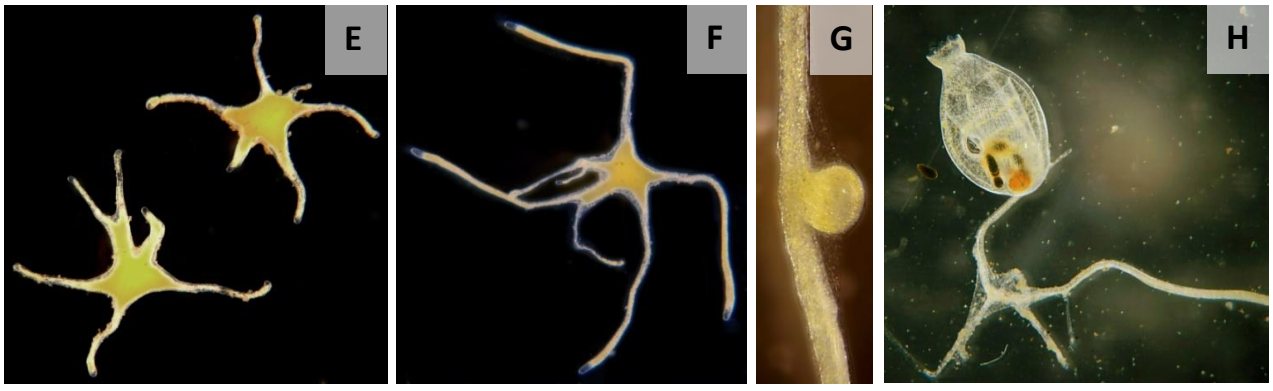
The colonial ascidian *Perophora japonica*



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.

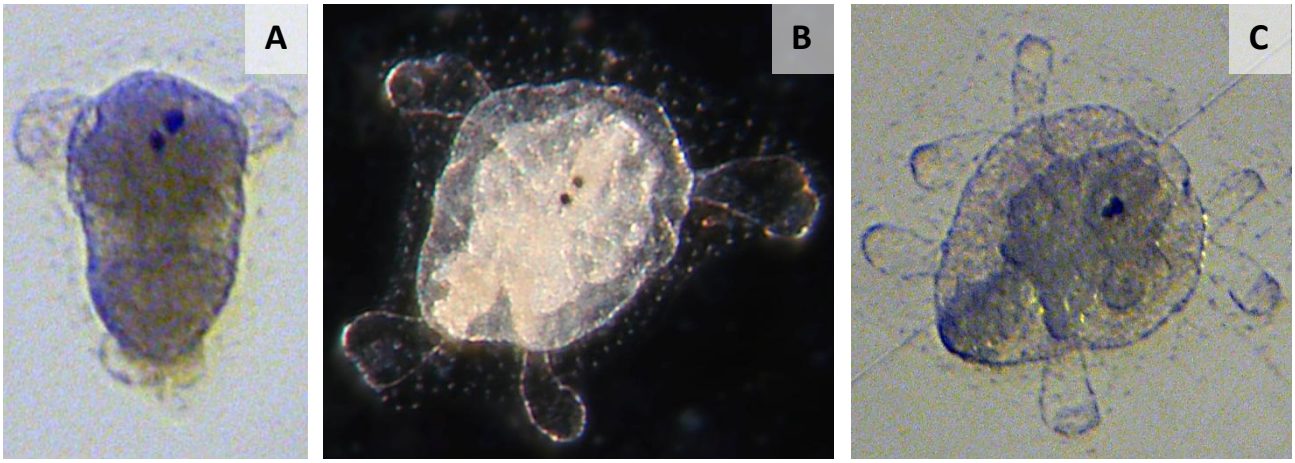


C: Portion of a young colony on a settlement panel, siphons closed; region shown c. 8.8 mm from top to bottom. **D:** Single zooid of colony, c. 5.0 mm tall.

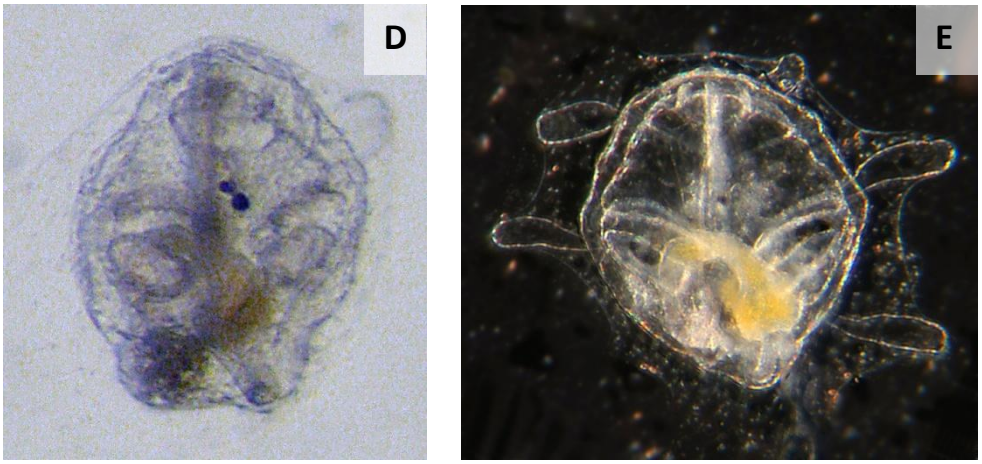


Additional to sexually produced larvae, *P. japonica* also has asexual propagules. Bright yellow 'terminal buds', typically produced at the edge of the colony, detach and are dispersed by water movements. If a bud eventually sticks to a surface the 'arms' elongate as stolons which bud the zooids of a 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.

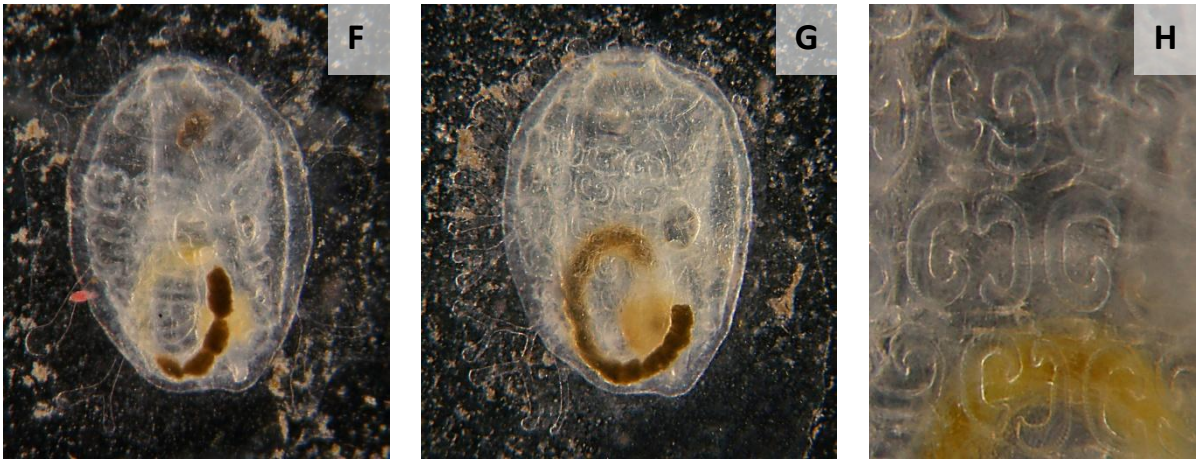
The unitary ascidian *Corella eumyota*



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.

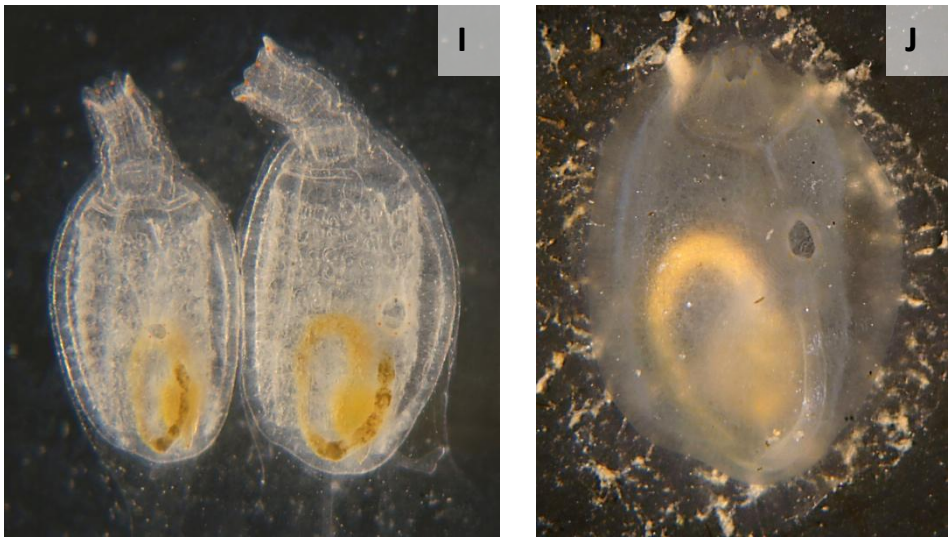


D: 2 days post-settlement. **E:** 3 days post settlement, c. 0.55 mm long. **D** and **E** both possess 2 pairs of protostigmata with separate left and right exhalant openings.



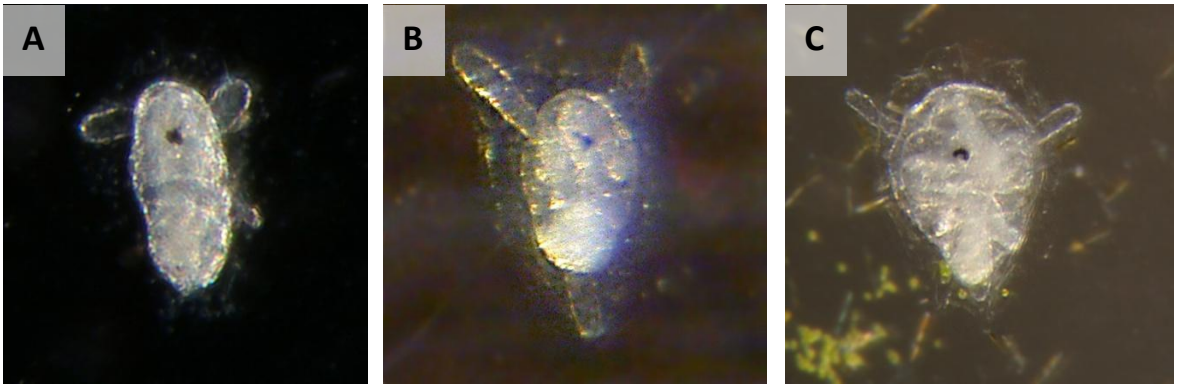
F: Later stage, c. 1.5 mm long, still with transverse protostigmata; the two exhalant openings are approaching the dorsal midline and the left-hand opening is larger than the right. **G:** Later stage, c. 2.0 mm long, with a single exhalant opening and longitudinally aligned C-shaped stigmata; the animal 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

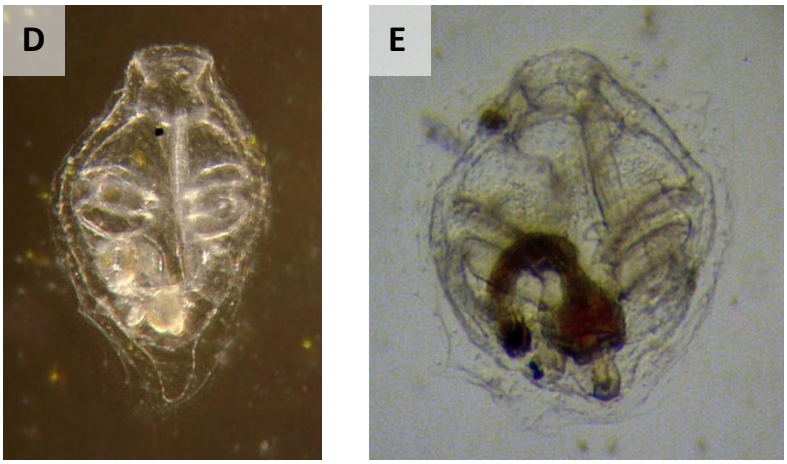


I: Two specimens grown in culture c. 4.1 and 4.7 mm long. **J:** Specimen 6.3 mm long on a settlement panel; the animal is surrounded by a fringe of tunic (incorporating sediment). Note the spiral gut leading from the stomach to the anus just inside the exhalant opening. The long inhalant siphons and 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.

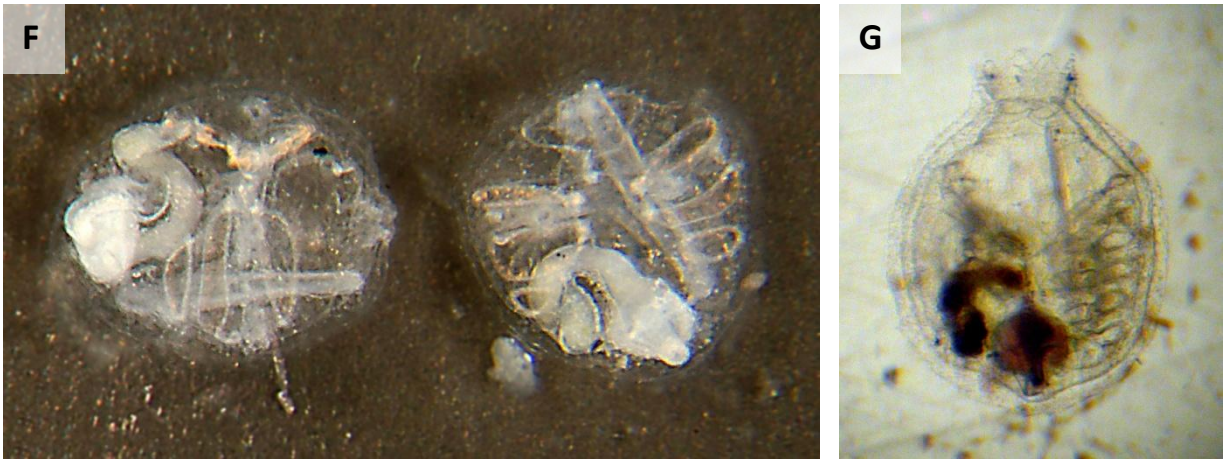
The unitary ascidian *Ascidiella aspersa*



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.

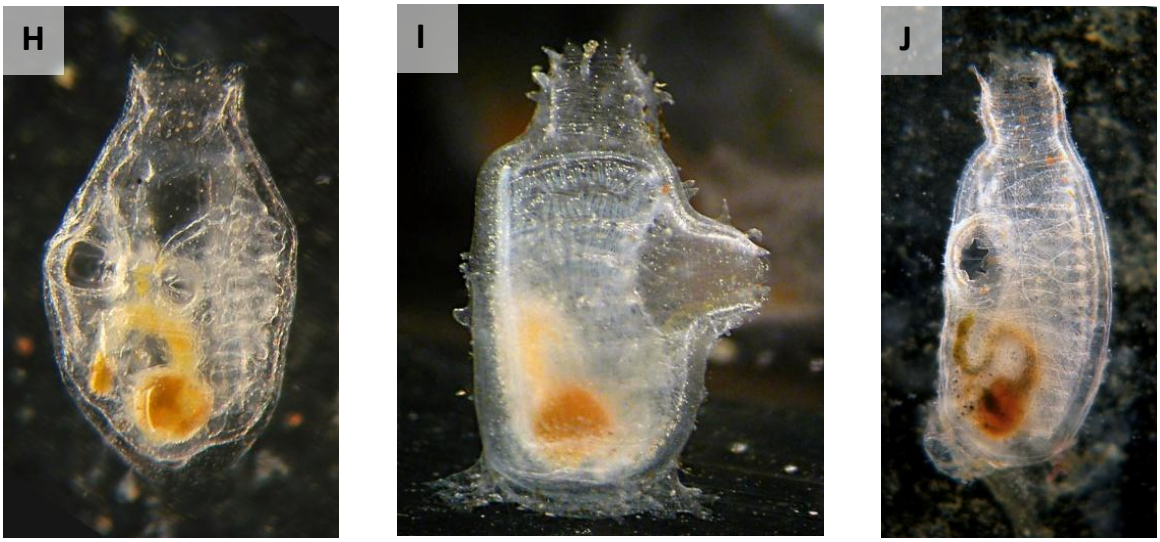


D: First feeding stage, with two pairs of protostigmata and two atrial openings, c. 0.65 mm across, dark-field illumination. **E:** Slightly later stage; bright-field illumination, gut appears dark.



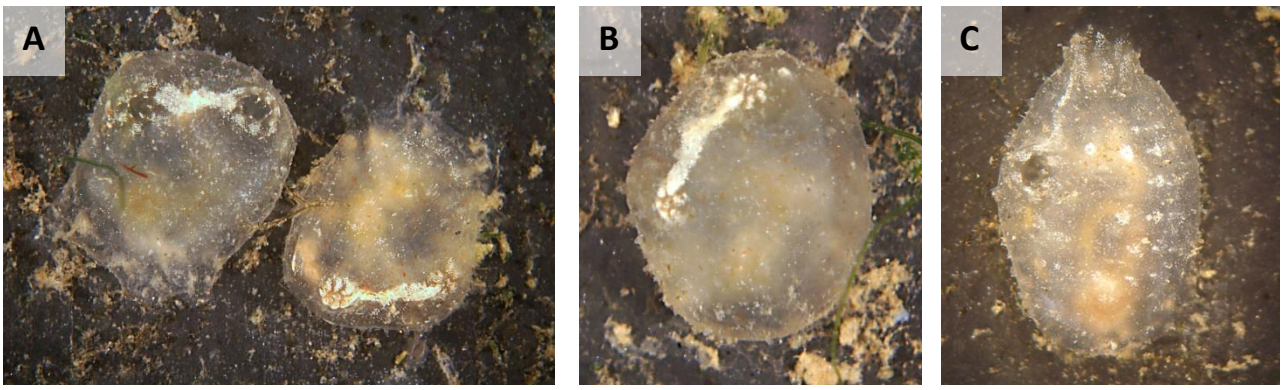
F: Preserved specimens on a settlement panel, similar stage to **E**, c. 0.6 mm long; third pair of protostigmata forming (protostigmata show as pinkish, guts pale, endostyle rod-like). **G:** later stage with protostigmata dividing.

Ascidiella aspersa continued



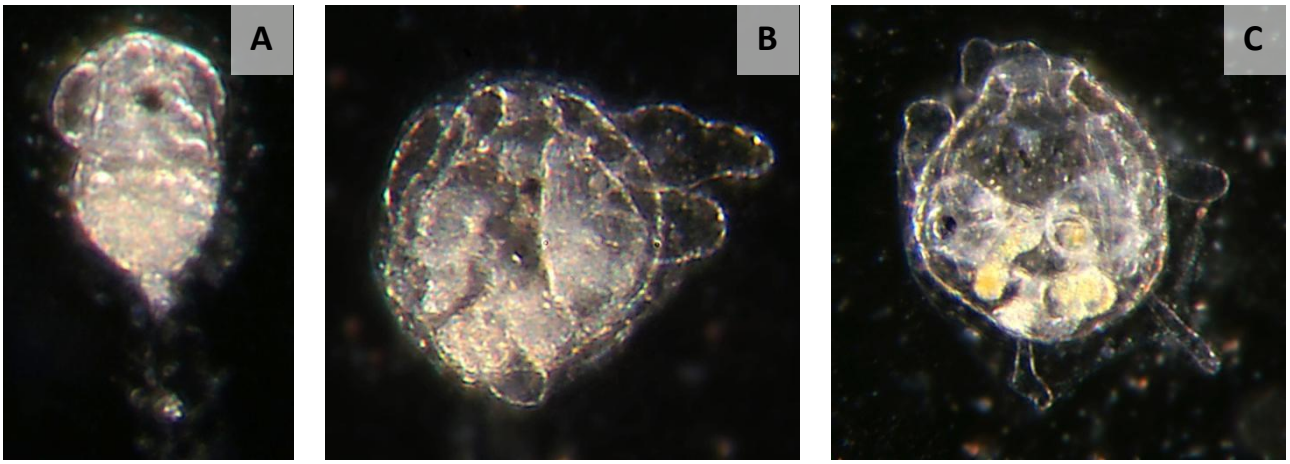
H: Specimen showing atrial openings approaching each other prior to fusing, c. 1.5 mm long. **I:** Stage with well-developed exhalant siphon and several rows of numerous longitudinally-oriented stigmata, here growing almost upright, view of left side; lab-reared specimen. **J:** Stage with c. 15 rows of stigmata; lab-reared specimen, c. 7.4 mm long.

Ascidiella spp.

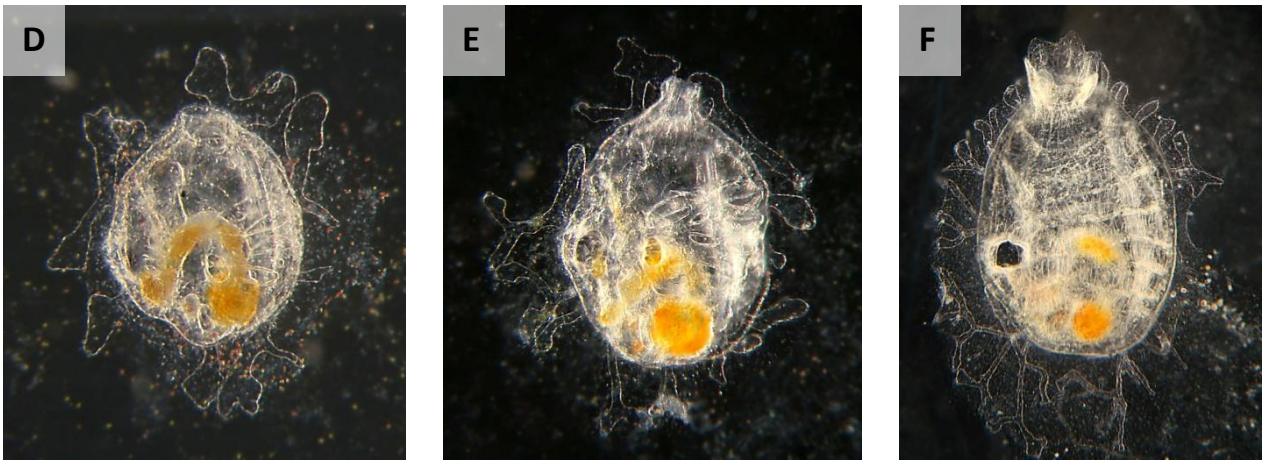


Very small *Ascidiella* on settlement panels. The extensive pigment between the siphons in the three smallest specimens seen in **A** and **B**, and their rounded shape, suggest that these are *A. scabra*. The slightly larger specimen seen in **C** is more questionable: the pigment between the siphons 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.

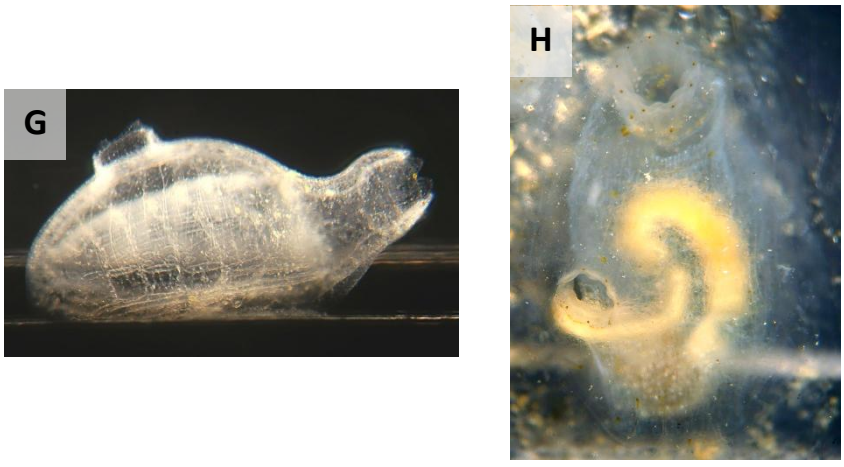
The unitary ascidian *Phallusia mammillata*



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.

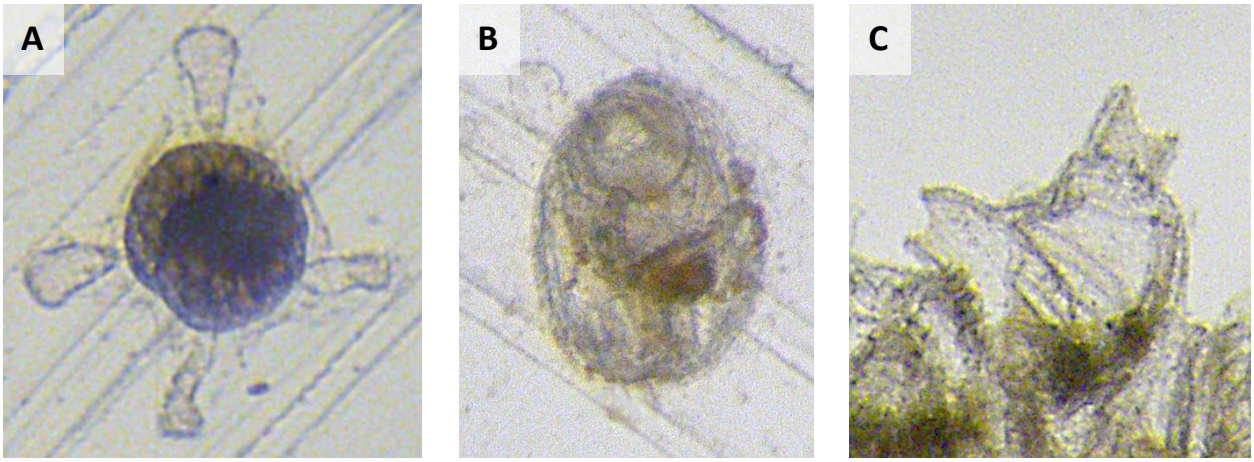


D: Stage with several protostigmata, right-hand exhalant opening displaced to the left; c. 0.77mm long. **E:** Later stage with more protostigmata, right-hand exhalant opening further left. **F:** Stage with single exhalant opening on extreme left of upper surface, numerous stigmata; c. 3.0 mm long.

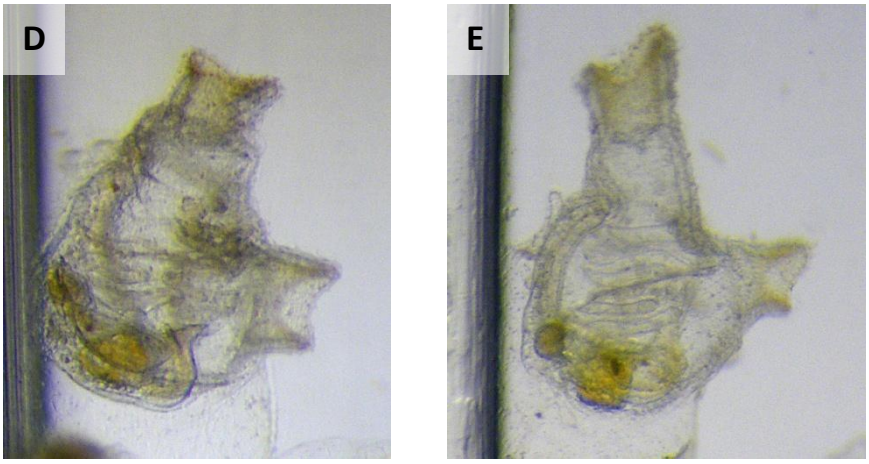


G: Stage similar to **F**, viewed from side. c. 2.9 mm long; prostrate early posture contrasts with upright stance of adult *P. mammillata*. **H:** Juvenile on settlement panel, c. 4.6 mm long, showing thick tunic, strongly meandering gut and large distance between siphons.

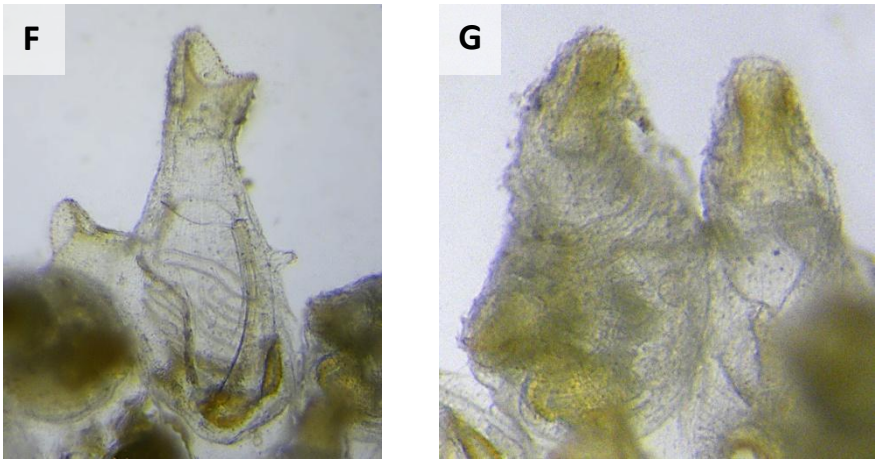
The unitary ascidian *Styela clava*



A: Very early post-settlement stage. **B:** Later stage. **C:** Stage with two pairs of protostigmata.



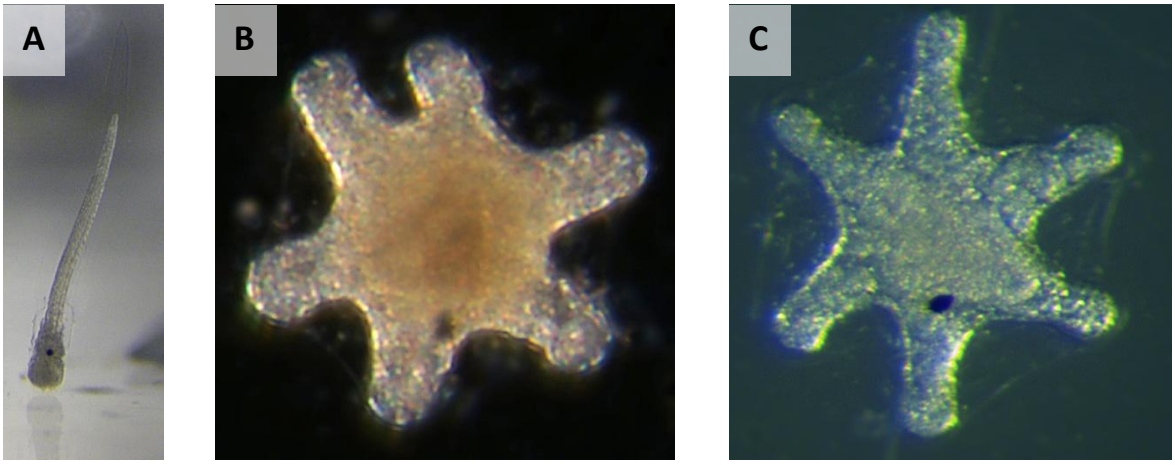
D: Later stage. **E:** Stage with at least three pairs of protostigmata.



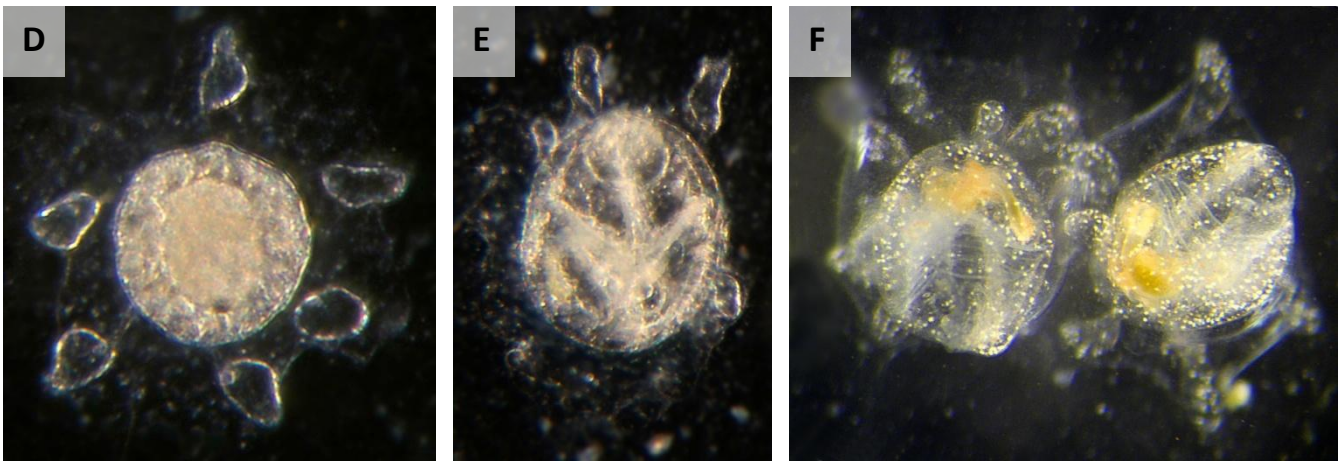
F: Stage with four protostigmata on the left side at least. **G:** Same stage as **F** but contracted; the two 'lips' of the siphons close together as if hinged where they join, and the body becomes wrinkled.

There seems very little to link these juvenile forms with adult *Styela clava*. Dense settlement was obtained, but the young did not subsequently thrive in laboratory culture: these photographs were obtained over almost a month and growth beyond the last stage shown here was not obtained.

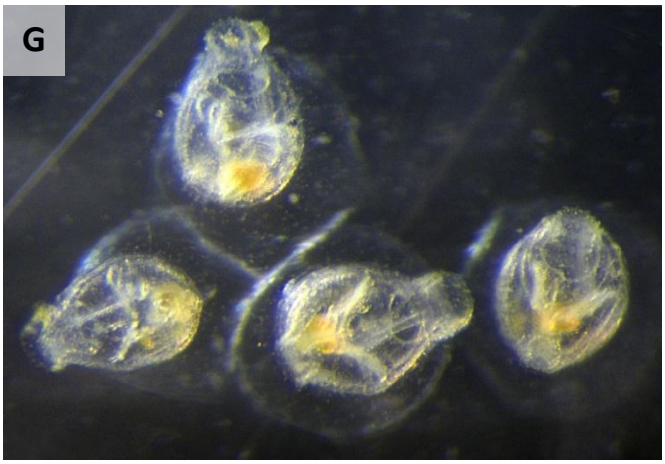
The unitary ascidian *Asterocarpa humilis*



A: attached larva. **B** and **C:** early cog- or star-shaped metamorph, c. 0.3mm across.

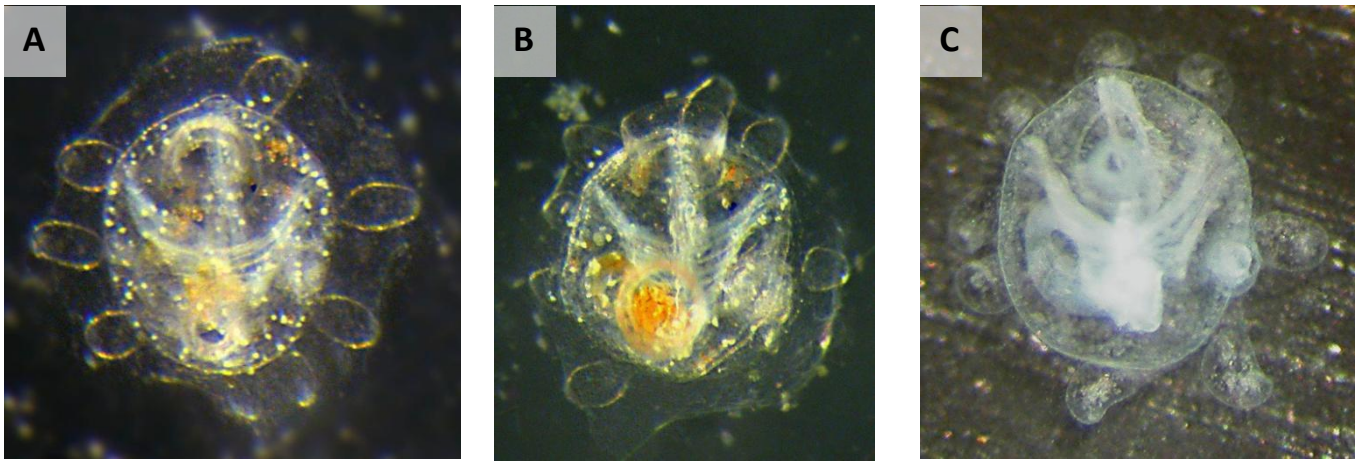


D: later rounded-off metamorph, c. 0.3 mm across. **E:** early feeding stage, c. 0.4 mm long.
F: Two specimens at later stage.

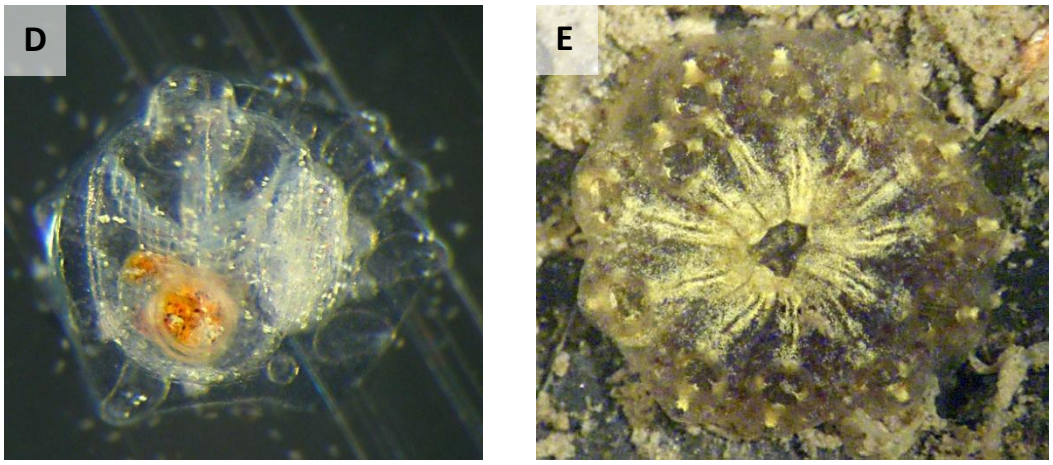


G: cluster of 4 juveniles, with tunics touching.

The colonial ascidian *Botryllus schlosseri*



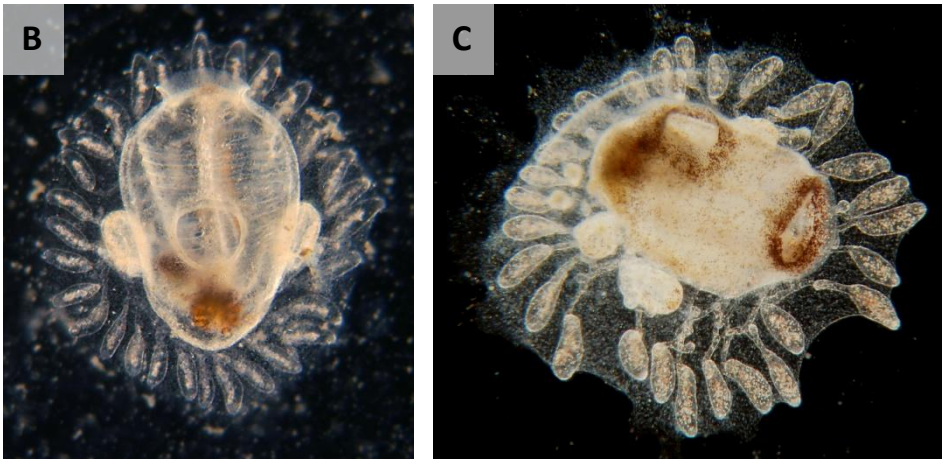
A: Early oozoid with very small bud on right and eight vascular ampullae. **B:** Slightly later oozoid with larger bud on right; note the five or more pairs of parallel protostigmata in V-configuration with endostyle passing centrally below them, giving impression of bird's foot. **C:** Similar stage,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



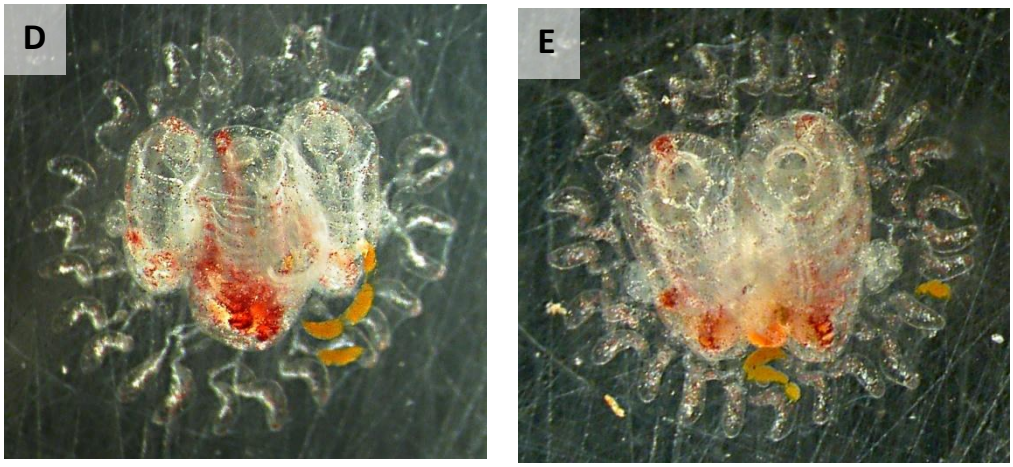
D: Oozoid with larger bud on right side. **E:** Much later growth, forming 'system' of zooids, inhalant openings on the periphery and shared central exhalant opening in the centre.

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.

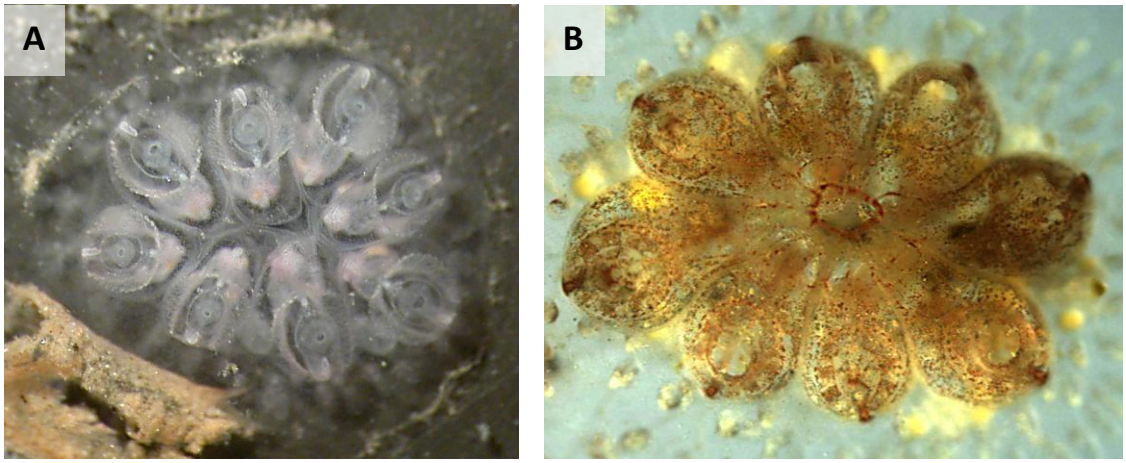


B: Oozoid, c. 3.9 mm across including vascular ampullae, with two small buds. The numerous vascular ampullae of the larva have radiated out and surround the oozoid. **C:** Oblique view of larger oozoid, with three buds.

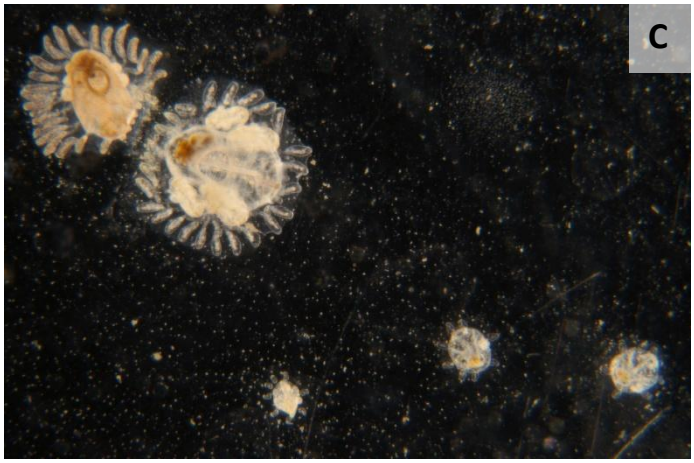


D: Oozoid with two daughter zooids, 7 days after settlement, cultured in the laboratory. **E:** The same colony 1 day later; the oozoid (centre) is being resorbed.

Comparison of *Botrylloides* and *Botryllus*

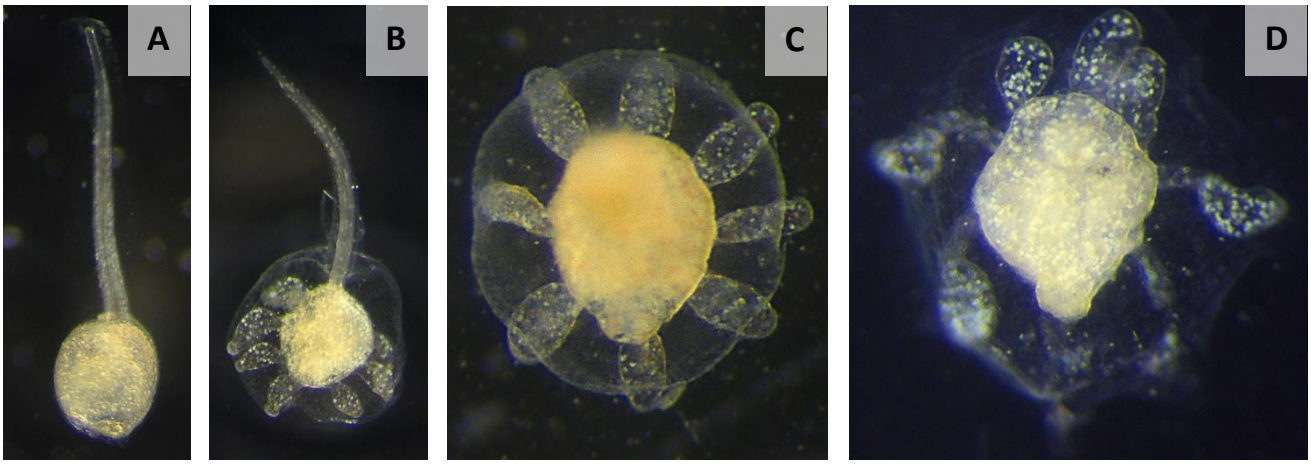


A: Eight-zooid colony of *Botrylloides* sp. on settlement panel. Note the relatively erect posture of the zooids compared to earlier stages, and the fact that not all zooids reach the margin of the common exhalant opening. **B:** Eight-zooid 'system' of *Botryllus schlosseri* colony for comparison; all zooids reach the exhalant opening and contribute to its edge (red-rimmed in this colony). (See also figure **E** on *Botryllus schlosseri* page.) This difference from *Botryllus schlosseri* is generic rather than relating specifically to *Botrylloides violaceus*. In 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'. In both genera, an extensive vascular network links the zooids and forms a fringe of ampullae around the colony.

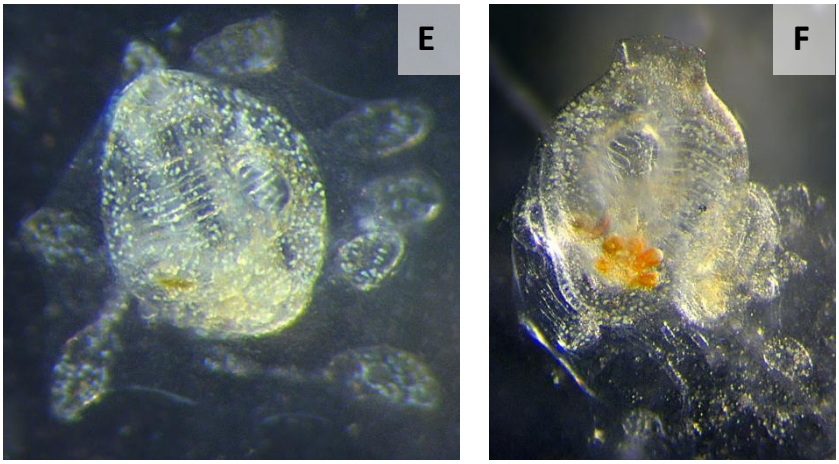


C: Comparison of size of oozoids of *Botrylloides violaceus* (two individuals top-left) and *Botryllus schlosseri* (two individuals bottom-right). The smaller fifth individual appears to be *B. schlosseri* in the process of metamorphosis, showing remants of the larval tail. The large size of the larva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

The colonial ascidian *Botrylloides diegensis*

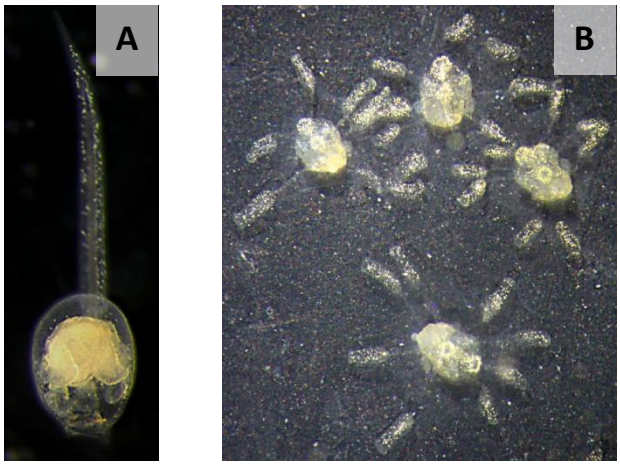


A: Larva. **B:** Early metamorphosis, the eight vascular ampullae expanded. **C:** Initial attached phase. **D:** Later pre-feeding stage.



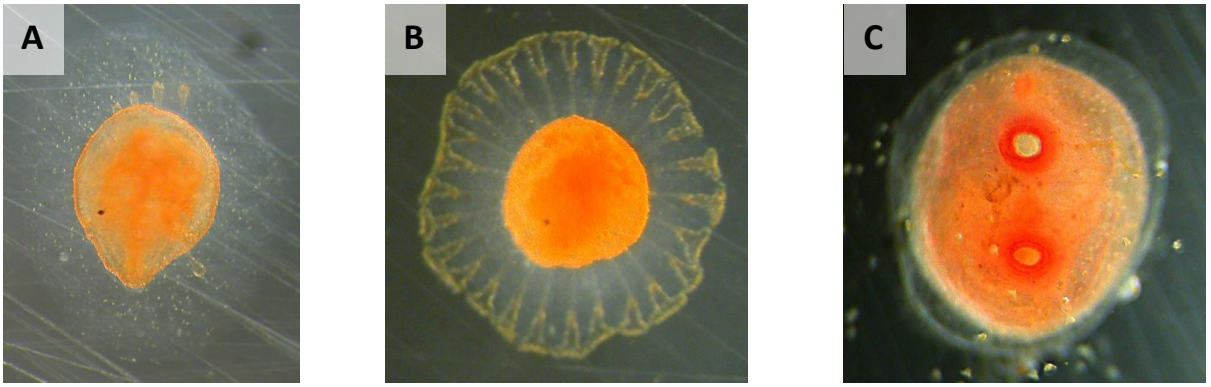
E: Feeding oozoid c. 2 days after settlement. **F:** Later oozoid with developing bud.

The colonial ascidian *Botrylloides leachii*



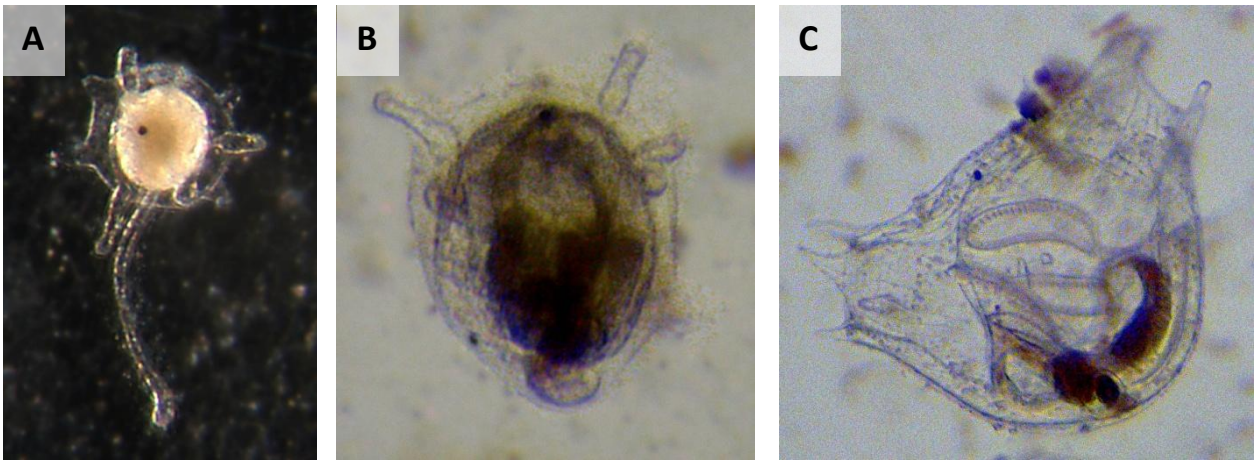
A: Larva. **B:** Four oozoids showing the eight large vascular ampullae.

The unitary ascidian *Dendrodoa grossularia*

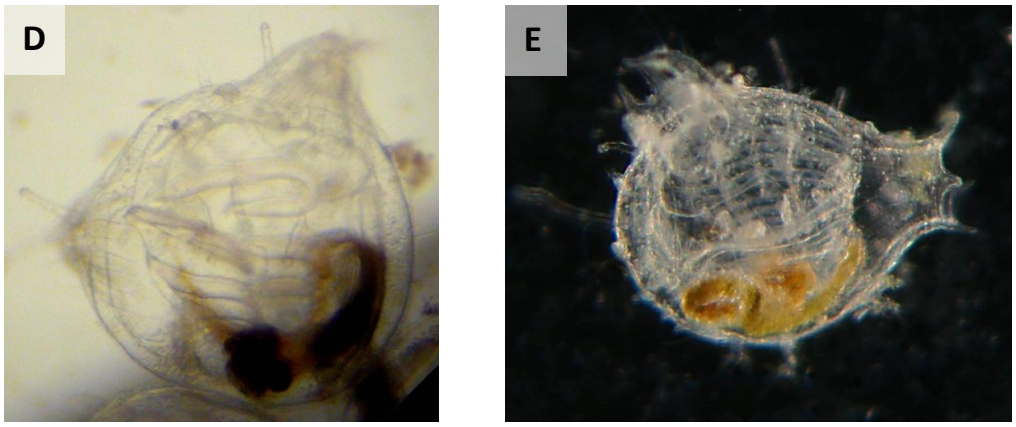


A: Very early post-settlement stage; the vascular ampullae are starting to spread. **B:** The numerous vascular ampullae have formed a complete border. **C:** Juvenile ascidian with inhalant and exhalant siphon.

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).

Notes

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