Botrylloides violaceus

A colonial ascidian, or tunicate

Phylum: Chordata, Tunicata

Class: Ascidiacea

Order: Stolidobranchia

Family: Styelidae

Description

Size: Colonies range in size from several to 50 cm across (Abbott and Newberry 1980; Saito et al. 1981; Carver et al. 2006); individual zooids are 1-2 mm long (as seen from above) and about half as wide as they are long (Fig. 1). Zooids occur in ladder-like rows or "systems" and dozens to hundreds of individual zooids make up a flat, encrusting colony (Carver et al. 2006). Each zooid has an independent siphon opening into a common cloacal cavity between rows (Fig. 1) (Carver et al. 2006).

Color: Can be purple, pink, yellow, or orange (Epelbaum et al. 2009a) and color may possibly be light dependent (Berrill 1947). Current specimen (Coos Bay) light yellow-orange to red. "Test" (see below) clear.

Zooids: Oblong, more or less free (Lambert 2003) and each with a raised oral aperture. Cloaca shared by zooids across row. With one large ovary on each side of body: genus *Botrylloides* (Van Name 1945). Asexual buds develop on zooid walls or from ampullae (vascular buds) at colony edges (Figs. 1a, 3). Incubating pouches develop from ovaries: genus *Botrylloides* (Abbott and Newberry 1980).

Tunic or Test: An external connective tissue, transparent in these specimens.

Mantle: The true body wall: a thin, sac-like membrane inside test, containing muscle and blood vessels, and enclosing the internal parts (Van Name 1945) (Fig. 3a).

Ampullae: Enlarged, finger-shaped, blind blood reservoirs around edges of tunic. Can give rise to new zooids by vascular budding. Ampullae also have a respiratory function.

Oral Aperture: Round, raised and smooth-edged on anterior surface with small simple tentacles and four-lobed siphon inside (Fig. 1).

Tentacles: Simple; four large and several small (these specimens) (Fig. 1).

Cloaca: Common, between rows of zooids (Figs. 1a, 2a). Atrial apertures of zooids are below surface of colony (Fig. 3a).

Pharynx: Branchial sac or pharyngeal basket, which posteriorly leads to esophagus, stomach, and intestine. This structure contains stigmata (stilts) and cilia for filtering food. It has 3 inner long vessels or bars, but no longitudinal folds separating stigmata (Berrill 1947) (Fig. 3a). Species lacks an abdomen and body not divided as in some elongate solitary ascidians (Fig. 3).

Endostyle: A deep groove on ventral side of pharynx (side opposite atrial siphon), containing long glandular bands which produce mucus used for feeding (Berrill 1947) (Fig. 3a).

Dorsal Lamina: A membranous ridge, projecting inward from the dorsal midline of the pharynx (atrial siphon side) (Fig. 3a). Dorsal lamina rolls mucus sheets into a cord, after receiving them from endostyle across sac walls (Goodbody 1974).

Stigmata: Groups of slits in pharynx walls between longitudinal vessels (Figs. 1, 3, 3a).

Atrium: Cavity surrounding pharynx. Water enters atrium via stigmata, and exits by atrial siphon (Fig. 3a).

Gonads: One ovary on each side of zooid (Fig. 3a) (Saito et al. 1981). Egg fertilized and embryo develops in brood pouch in ovum (Mukai et al. 1987). Brood pouch eventually detaches from atrial epithelium and is taken

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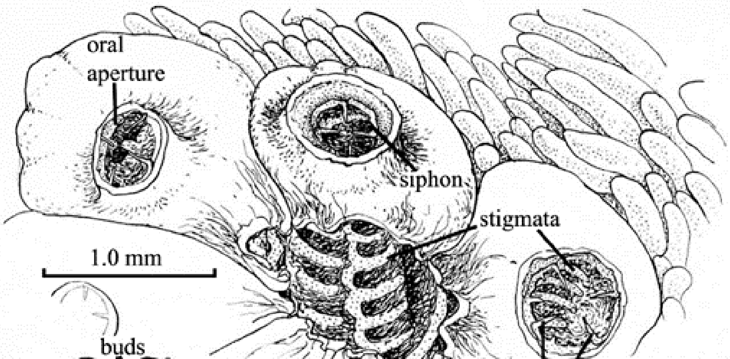
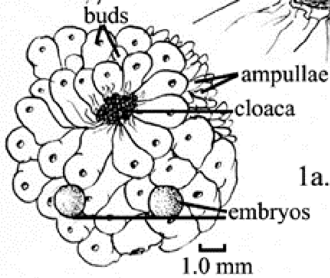
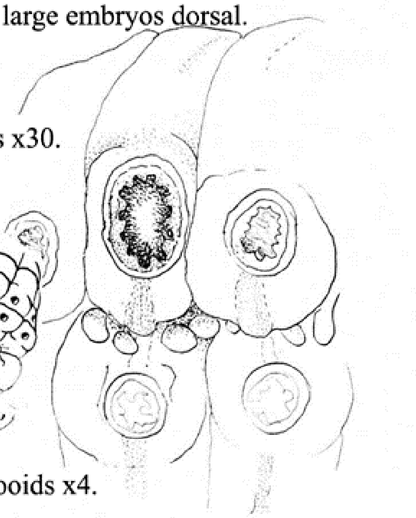


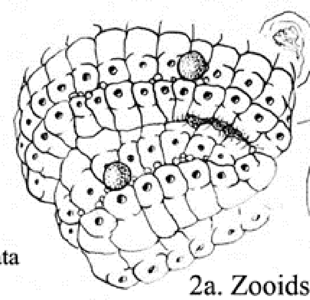
1. *Botrylloides violaceus* (L: 1.7mm, W: 1.0mm) x30: zooids; round oral aperture with four-lobed siphon, simple tentacles; common cloacal cavity; zooid systems in rows or crowded; many dozen zooids possible; ampullae at margins or between rows; buds, large embryos dorsal.



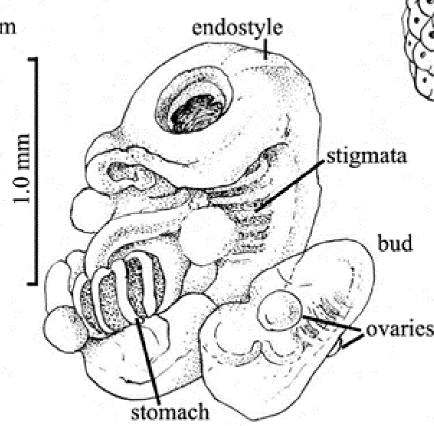
1a. A crowded colony x4.



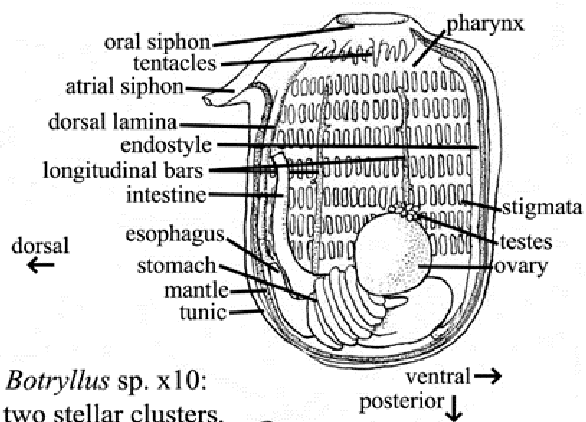
2. Zooids in rows x30.



2a. Zooids x4.

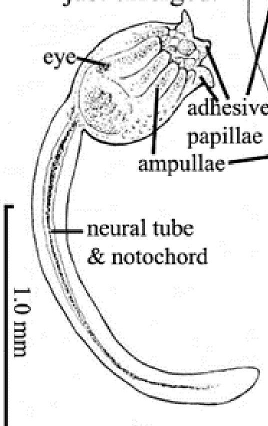


3. Zooid and bud lateral view x40.

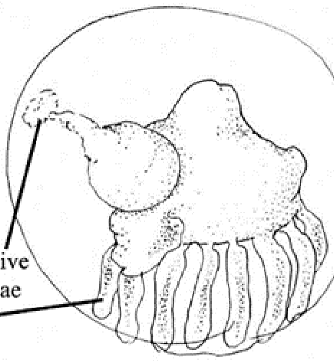


3a. Zooid, schematic,

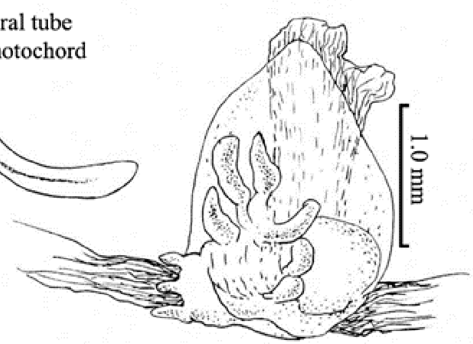
4. Larvae.



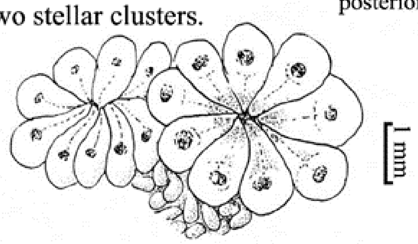
4a. Tadpole x40: just emerged.



4b. Oozoid x50: about to settle.



4c. Oozoid, settling x25.



5. *Botryllus* sp. x10: two stellar clusters.

Dorning, S.L. 2017. *Botrylloides violaceus. In:* Oregon Estuarine Invertebrates: Rudys' Illustrated Guide to Common Species, 3rd ed. T.C. Hiebert, B.A. Butler and A.L. Shanks (eds.). University of Oregon Libraries and Oregon Institute of Marine Biology, Charleston, OR.

up by colonial tunic (Zaniolo et al. 1998). Testes mulberry-like, anterior to ovum (Fig. 3a). (Not visible in zooid in Fig. 3.)
Digestive Tract: To left of branchial sac, with narrow loop at base (Berrill 1947) (Figs. 3, 3a.)
Larva: Large, up to 3mm in length, "tadpole" type, with long posterior tail containing notochord and slender neural tube. Body contains photolith, a balance and light organ near eye, and 24-34 ampullae (Fig. 4a) (Saito et al. 1981, Lambert 2003).

Possible Misidentifications

The family Styelidae contains both solitary and compound forms. Family characteristics include square or four-lobed apertures, simplfiliform tentacles, a continuous dorsal lamina (Fig. 3a), and straight longitudinal stigmata. Some genera have four curved longitudinal folds in the pharynx, but *Botrylloides* and the closely related *Botryllus* and *Metandrocarpa* do not (Van Name 1945). Four other genera also lack these longitudinal folds, but do not occur in our area: *Symplegma, Kukenthalia, Polyzoa* and *Alloeocarpa* (Van Name 1945).

Of the three local encrusting colonial Styelidae, *Metandrocarpa* (dura) is usually reddish, with large zooids (5-6 mm). It is not arranged in systems: each zooid has a separate atrial siphon. Zooids are more separate and distinct, being embedded in the tunic. Zooids can seem to be in rows and laterally fused, but are only connected basally.

Botryllus spp., a cosmopolitan genus, is often found with *Botrylloides* on floats, and the two can be difficult to distinguish. *Botryllus* always forms circular or star-shaped clusters or systems and never has more than 20 zooids in a system. *Botrylloides* forms systems composed of long double rows or clumps of zooids, and often has several dozen zooids in a system. Because of the shape of the colony, *Botryllus* zooids tend to be tear-shaped, with a "languet" or tongue-shaped atrial end and a narrow, pointed end directed inward (Van Name 1945) (Fig. 5). *Botrylloides* zooids are usually oval-shaped, with the narrow end pointing outward (Carver et al. 2006). *Botryllus* individuals lack the brood pouch of *Botrylloides* and their young develop in the atrium before being extruded. A further difference between the two species is that *Botryllus* has the ovaries anterior to the testes (the reverse of *Botrylloides*), and can have one or several ovaries; *Botrylloides* has one large ovary on each side.

Botrylloides violaceus has been confused with the European *B. leachi*, *B. aureum,* as well as *B. diegensis*, a southern California form, with brown and purple zooids and test vessels. This has made the invasion of this species difficult to track. Van Name (1945) discusses *Botrylloides magnum* from Alaska, but it is very large, and poorly described. Cohen and Carlton (1995) documented *B. violaceus* in the San Francisco Bay as early as 1973. *Botrylloides violaceus* has been established in Coos Bay since the at least the late 1980s, when it was documented in a comprehensive study of macro-fouling fauna in the bay (Hewitt 1993).

Ecological Information

Range: Genus worldwide
Local Distribution: Coos Bay: Charleston Inner and Outer Boat Basins, South Slough, Coos Bay city docks. Population introduced to Isthmus Slough via transplanted dock in 1990, no longer present (Hewitt 1993).
Habitat: On floating docks (Coos Bay); in bays and harbors (Abbott and Newberry 1980). Ascidians represent a significant percentage of the fouling organism community (Miller 1971, Simkanin et al. 2012).
Salinity: Tolerance range 20-32, optimal growth at 32 (Epelbaum et al. 2009b). Collect-

ed at 15.6-37.8 (Coos Bay, Dorning pers. obs.).

Temperature: Global temperature range of 0.6-29.3°C (Zerebecki and Sorte 2011). 10-18 °C (Coos Bay, Dorning pers. obs.). Geographical variation in maximum temperature tolerance (West coast: 25°C) (Sorte et al. 2011).

Tidal Level: Low intertidal and shallow subtidal (Abbott and Newberry 1980). Restricted to depths less than 50m (Carver et al. 2006).

Associates: *Obelia*, caprellid amphipods, *Corophium* amphipods, nereid polychaetes, spirorbid polychaetes, *Eudistylia*, *Botryllus schlosseri.* Found overgrowing *Schizoporella unicornis*, *Watersipora subtorquata, Balanus* spp. and *Mytilus* spp (Hewitt 1993; Dorning pers. obs.). Can overgrow and be overgrown by *Halichondria bowerbanki.* Ascidians are commensal hosts to notodelphid copepods, amphipods, and host to some specific parasitic copepods (Miller 1971), but resistant to epibiotic larval recruitment (Hewitt 1993).

Abundance: Locally common on floating docks, especially in summer. Peak settlement abundance observed in April (Point Adams Jetty and North Jetty) (Hewitt 1993).

Life-History Information

Reproduction: Hermaphroditic and ovoviviparous. Asexual budding also occurs. Sexual fertilization internal, embryos develop in ovary (one to a zooid, 1-1.5mm diameter), and emerge as tadpoles after a one-month gestational period (Takeuchi 1980; Saito et al. 1981, Zaniolo et al. 1998). Parent zooids disintegrate 5 days after ovulation and only brood pouches containing larvae remain (Mukai et al. 1987). Larvae develop quickly, settle soon (within minutes to hours) and metamorphose to form functional oozooids, from which blastozooids develop asexually (Fig. 4) (Takeuchi 1980; Saito et al. 1981). Sexual reproduction occurs year-round, with highest recruitment in late spring and early summer. Prolonged periods of warmer temperatures extends reproductive period by initiating earlier onset of recruitment (Powell 1970; Ross and McCain 1976; Stachowicz et al. 2002; Epelbaum et al. 2009a; Dijkstra et al. 2011). Larval settlement in Coos Bay peaks between July and September (Hewitt 1993) during periods of low recruitment by native species (Stachowicz and Byrnes 2006). Larvae release determined by light and released between 8am-12pm in Charleston harbor (Marshall et al. 2006).

Asexual reproduction can happen in two ways. Buds can develop from parent zooids via palleal/propagative budding (Fig. 3), which occurs continuously to replace transient zooid structures and to grow laterally. In the absence of adult zooids, and in adverse environmental conditions, growth can occur at the bases of vascular ampullae (Nakauchi 1982; Carver et al. 2006; Kurn et al. 2011). Numerous buds of all types abort during development, and one colony may be comprised of adult zooids, primary palleal buds, and secondary buds connected to primary buds (Carver et al. 2006). Fragmentation of colonies results in reattachment to substrate and subsequent asexual budding as multiple colonies (Agius 2007). Contact between two adjacent colonies can result in fusion to form a single chimera (Cima et al. 2004).

Larva:

Juvenile:

Longevity: An ascidian colony may live more than three years and an individual zooid for five to seven days before undergoing apoptosis and being replaced by new asexual buds (Berrill 1947; Brown et al. 2009; Kurn et al. 2011).

Growth Rate: First generation of blastozooids develop to form a functional colony after 7-10 days. Asexual growth rate highly variable (Epelbaum et al. 2009a); oozooids observed to grow 1.9 mm within a week before producing buds asexually (Yamaguchi 1975). Growth rate increases at higher temperatures (19.1-23.3°C) (Stachowicz et al. 2002); coolest peak growth at 14°C (Lord and Whitlatch 2015).

Food: Ciliary mucus feeders, filtering plankton through the tentacles.

Predators: No natural predators observed, possibly due to chemical unpalatability. Only vulnerable to potential predation a short period after settlement (days to a week), (Pisut and Pawlik 2002; Tarjuelo et al. 2002). Experimental exclusion of potential chiton, gastropod and flatworm predators does not affect *B. violaceus* recruitment or abundance (Grey 2010). Potential sea star, crab, nudibranch, and urchin predators prefer natural prey to *B. violaceus* (Epelbaum et al. 2009b). Ascidian predators include fish, crab, polychaetes, sea stars; especially prosobranch molluscs, opisthobranchs, nudibranchs, turbellarian flatworms and the grey seal (Scotland). Used by man for food (Japan, Mediterranean, Chile) and bait (Australia, South Africa. Destroyed as a pest in oyster beds and commercial fishing grounds (Miller 1971).

Behavior: Zooids are sessile. Tadpole larvae can swim, but tend to settle near parents, attaching to substrate with adhesive papillae, perhaps due to chemical induction of settlement by adult colonies (Railkin 2004). Not particularly competitive in native habitat (Japan) but competitively dominant in Coos Bay, overgrowing most native species (Hewitt 1993).

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