New records of ascidians from the NE Pacific: a new species of *Tridemnum*, range extension and redescription of *Aplidiopsis pannosum* (Ritter, 1899) including its larva, and several non-indigenous species

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ABSTRACT
A new species of aplousobranch ascidian, *Tridemnum alexi* n. sp., is described from the San Juan Archipelago of Washington state, USA. Colonies are smooth dark reddish brown; the largest is 8 cm in maximum width and up to 2 cm thick in some regions because of the large and complex hypozooidal cloacal canals. A thick (200-400 µm) superficial bladder cell layer is present. The spicules are 20-30 µm in diameter, irregularly stellate with short pointed rays (eight to 10 in optical equatorial plane) and scattered thinly through the tunic but absent from the bladder cell layer. Zooids have three rows of stigmata, with 12-13 stigmata per side in the first two rows in most zooids (occasionally 11 or 14) and usually one or two fewer in the third row. The tubular atrial siphon opens dorsal to the middle row of stigmata. The single testis is covered by a sperm duct with eight or nine coils. Larvae form in the basal portion of the colony, are 0.8-1.0 mm in trunk length with three adhesive papillae and usually seven pairs of lateral ampullae with curved tips in the fully formed tadpoles. A redescription of *Aplidiopsis pannosum* (Ritter, 1899) includes the morphological analysis of several larval stages. This species, though widespread in the north Pacific, was not previously known to occur south of Alaska on the west coast of North America. Range extensions are also included for several non-indigenous ascidians: *Ciona savignyi* Herdman, 1882, *Botrylloides violaceus* Oka, 1927, *Styela clava* Herdman, 1881 and *Molgula manhattensis* (De Kay, 1843).

KEY WORDS
Ascidiacea,
Aplousobranchia,
Stolidobranchia,
Phlebobranchia,
Tridemnum,
Botrylloides,
Aplidiopsis,
Ciona,
Molgula,
Styela,
NE Pacific,
non-indigenous,
new species.

RÉSUMÉ

Nouvelles mentions d'ascidies du Pacifique NE : une nouvelle espèce de Tridemnum*, extension de l'aire et redescription d'*Aplidiopsis pannosum *(Ritter, 1899) incluant sa larve, et plusieurs espèces non indigènes.*

Une nouvelle ascidie aplousobranche *Tridemnum alexi* n. sp. est décrite de l'archipel San Juan, État de Washington, USA. Les colonies sont lisses, d'un brun-rouge foncé. La largeur maximum est 8 cm et l'épaisseur 2 cm en certains endroits, due aux grands canaux cloacaux situés sous les zoides. Une épaisse couche de cellules vésiculaires (200-400 µm) est présente en surface. Les spicules de 20 à 30 µm sont irrégulièrement étoilés avec huit à 10 rayons courts et pointus en coupe optique. Ils sont dispersés dans la tunique sauf dans la couche superficielle. Les zoides ont 12 à 13 stigmates de chaque côté dans les deux premiers rangs (parfois 11 à 14) et généralement un à deux de moins dans le troisième rang. Le siphon cloacal tubulaire s'ouvre dorsalement en face du deuxième rang de stigmates. L'unique testicule est recouvert de huit à neuf tours du spermiducte. À la base des colonies les larves ont un tronc de 0,8 à 1,0 mm, trois papilles adhesives et généralement sept paires de vésicules latérales dont les extrémités sont courbées chez les têtards complètement développés. Une redescription de *Aplidiopsis pannosum* (Ritter, 1899) inclut l'analyse morphologique de plusieurs stades larvaires. Cette espèce largement répartie dans le Pacifique Nord n'était pas connue au sud de l'Alaska sur la côte ouest nord-américaine. Des répartitions géographiques sont aussi incluses pour certaines ascidies qui ne sont pas indigènes : *Ciona savignyi* Herdman, 1882, *Botrylloides violaceus* Oka, 1927, *Styela clava* Herdman, 1881 et *Molgula manhattensis* (De Kay, 1843).

MOTS CLÉS
Ascidiacea,
Aplousobranchia,
Stolidobranchia,
Phlebobranchia,
Tridemnum,
Botrylloides,
Aplidiopsis,
Ciona,
Molgula,
Styela,
océan Pacifique nord-est,
non indigène,
nouvelle espèce.

INTRODUCTION

The shallow-water ascidians of the NE Pacific around Puget Sound, the San Juan Archipelago and Vancouver Island have been studied for many years (Ritter 1900, 1913; Huntsman 1912a, b; Van Name 1945; Lambert *et al.* 1987; Lambert 1989). It is now rare to encounter a new species in this area, though a number of non-indigenous species have appeared recently (A. Cohen *et al.* 1998; Mills *et al.* 2000), probably due to anthropogenic transport *via* shipping, recreational boating and possibly also mariculture. This paper includes a description of *Tridemnum alexi* n. sp., a colonial ascidian that is most likely indigenous to the area, and a range extension and description of the larva of *Aplidiopsis pannosum* (Ritter, 1899), not previously recorded south of Alaska. Also included are new distribution records of the non-indigenous species *Ciona savignyi* Herdman, 1882, *Botrylloides violaceus* Oka, 1927, *Styela clava* Herdman, 1881 and *Molgula manhattensis* (De Kay, 1843) from artificial structures in a number of harbors; they were listed in Mills *et al.* (2000) but not by site.

MATERIAL AND METHODS

Two colonies of *Tridemnum alexi* n. sp., designated the holotype and a paratype, were collected by C. Lambert from a rope attached to a floating dock at the Friday Harbor marina, Friday Harbor, WA on 28.V.2001. The holotype was relaxed for several hours in sea water to which a few drops of menthol-saturated 95% ethanol had been added, and was then preserved in 10% seawater formalin buffered with sodium borate. The paratype was maintained alive in running sea water at the

University of Washington Friday Harbor Laboratories for several weeks before preservation. Additional paratype colonies were collected from the same rope on 24.VII.2001 and 29.VIII.2001. A colony of the same description and presumably the same species was collected two years earlier from the same docks on 5.VI.1999 but had been inadvertently discarded. A very small colony was dredged from Rock Pt off Lopez Island, San Juan Archipelago on 3.VIII.1999. The holotype and 3.VIII.1999 and 24.VII.2001 paratypes are deposited at the Muséum national d'Histoire naturelle, Paris (MNHN). The other paratypes are deposited at the California Academy of Sciences Department of Invertebrate Zoology, San Francisco (CASIZ), and at the Smithsonian Institution National Museum of Natural History, Washington DC (USNM). Small pieces of the holotype and paratypes were transferred after fixation to 95% ethanol to preserve the calcium carbonate spicules.

To prepare the calcium carbonate tunic spicules of *Trididemnum* for scanning electron microscopy (SEM), a small piece of fresh tunic was rinsed briefly in distilled water, then incinerated in a small ceramic dish over a Fisher burner for about 10 min. Five ml of bleach (5.25% sodium hypochlorite) was added to the dish for 5 min to dissolve any remaining organic matter, after which the spicules were rinsed repeatedly in 95% ethanol, then 100% ethanol. They were pipetted onto SEM stubs some of which were covered with double stick tape, and the excess ethanol allowed to evaporate. The spicules were gold sputter coated and examined at 15 kV in a JEOL 35 scanning electron microscope.

Numerous colonies of *Aplidiopsis pannosum* attached to large barnacles (*Balanus nubilus* Darwin, 1854) were obtained by dredging off Lopez I. in the San Juan Archipelago, WA, during the summers of 1999 and 2001. Representative specimens are deposited at CASIZ, USNM and MNHN. Collecting sites and dates for *Ciona savignyi*, *Botrylloides violaceus*, *Styela clava* and *Molgula manhattensis* are listed under their individual descriptions. All ascidians were preserved in 10% seawater formalin buffered with sodium borate after relaxation with menthol/ethanol in seawater.

SYSTEMATICS

Order APLOUSOBRANCHIA Lahille, 1887
Family DIDEMNIDAE Giard, 1872
Genus *Trididemnum* Della Valle, 1881

Trididemnum alexi n. sp.
(Figs 1-3)

TYPE MATERIAL. — Holotype: colony collected by C. Lambert on 28.V.2001, from a rope suspended from a floating dock at the town of Friday Harbor, WA, at an approximate depth of 2 m (MNHN A2 TRI 162). Paratype: a second colony 4 cm in greatest length collected on 28.V.2001, from the same rope, growing on a mussel *Mytilus* sp. (CASIZ 162519); companion piece from same colony in 95% ethanol (CASIZ 162520). Additional paratypes collected by C. Lambert from the same rope on 24.VII.2001, 1 colony (MNHN A2 TRI 164 24/VII/2001) and on 29.VIII.2001, 2 colonies (USNM 1006926).

OTHER MATERIAL EXAMINED. — **USA.** Friday Harbor, WA, Town dock, 5.VI.1999, 1 colony (specimen lost).
San Juan Archipelago, Rock Pt, off W side of Lopez I., dredged at 80 m, 3.VIII.1999, D. Duggins coll., 1 small colony growing around the bivalve *Mytilimeria nuttalli* Conrad, 1837 and heavily parasitized by *Botryllophyllus* sp., a red copepod with red eggs (identified by S. Ooishi); 2 nearly mature unhatched tadpole larvae found in the colony (MNHN A2 TRI 163).
Canada. British Columbia, Vancouver I., Canoe Bay Marina, 24.IX.2002, 1 colony, *c.* 15 cm depth on *Mytilus* sp. on underside of black floating boat bumper tied to a dock, no larvae, colony surface heavily infiltrated by numerous burrowing amphipods.
British Columbia, Victoria, Fisherman's Wharf floating docks, 25.IX.2002, 3 colonies, the largest 2 cm thick and 5.5 cm in greatest length, *c.* 2 m depth on sheet of white plastic tied to long rope, no larvae.

ETYMOLOGY. — It gives me great pleasure to name this species after my grandson, Alexander Ivan Coleman. I hope it will inspire him to pursue a career in biology like his parents and maternal grandparents.

DESCRIPTION

The holotype is 5 cm in greatest length, 1-2 mm thick at the edges but about 2 cm thick in the middle region. The largest paratype is 8 cm in

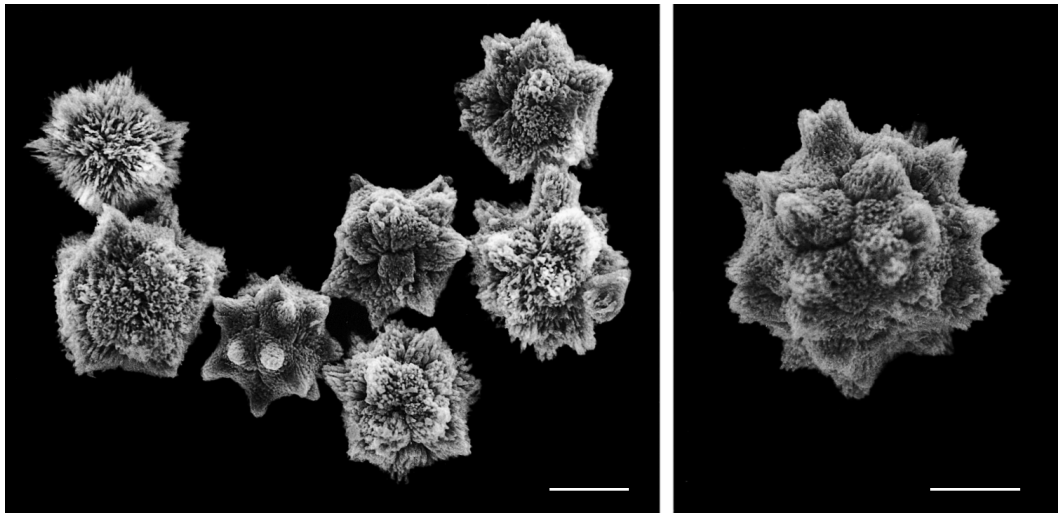

FIG. 1. — Tunic spicules of *Tridemnum alexi* n. sp. Scale bars: 10 µm.

greatest length and also up to 2 cm thick. The structure is complex because the colony has low rounded thickened lobes, is somewhat folded and contains many large ramifying hypozooidal cloacal canals that meander through the tunic. The reddish-brown tunic is smooth, soft and easily torn, with a thick (200-400 µm) superficial bladder cell layer containing pigment granules but lacking spicules. Different colonies vary somewhat in the intensity of coloration, depending on the concentration of pigment. Under the superficial layer the tunic contains small irregularly stellate spicules (Fig. 1), 20-30 µm in diameter, with eight to 10 short rays in the equatorial plane. Though the spicules are numerous, they are not densely packed but scattered thinly throughout all layers of the tunic except the bladder cell layer. The pigment and spicules render the tunic opaque so that only the oral openings of the zooids can be seen in an undissected colony. The zooids are located in the upper stratum (above the cloacal canals) and the larvae in the basal zooid-free stratum (below the cloacal canals), with thick columns of tunic here and there connecting the two layers. The zooids do not appear to be in definite systems. Each oral siphon opens separately on the tunic surface. The spicules in the oral lobes are denser in three of the tunic lobes than in the other three. The infrequent common cloacal openings are smooth-edged, large, round and far apart, bordered by thin spicule-free tunic and very little pigment.

The zooids are about 1.8-2.2 mm long in the preserved state, and heavily pigmented the same reddish brown hue as the tunic. The oral siphon has six short pointed lobes. It was not possible to count the oral tentacles in most zooids, but in one well relaxed individual there were 16 oral tentacles of two sizes, with four longer ones evenly spaced around the base of the oral siphon separating four groups of three shorter tentacles. The three rows of stigmata are elongate, with a large unperforated area just posterior to the peribranchial region (Fig. 2A, B). There are usually 12-13 stigmata per side in row 1, 12 in row 2, and 10-12 in row 3. Two backward-curved dorsal languets are present. The unlobed atrial siphon, large and tubular or slightly flaring, is directed dorsally or posteriorly, and is located at the level of the middle row of stigmata. It opens into the large hypozooidal common cloacal cavity. The spicule-forming lateral organs of the thorax (Kniprath & Lafargue 1980) are large, densely filled with very small spicules, and located between the second and third rows of stigmata or over the third row (Fig. 2B, dashed circle).

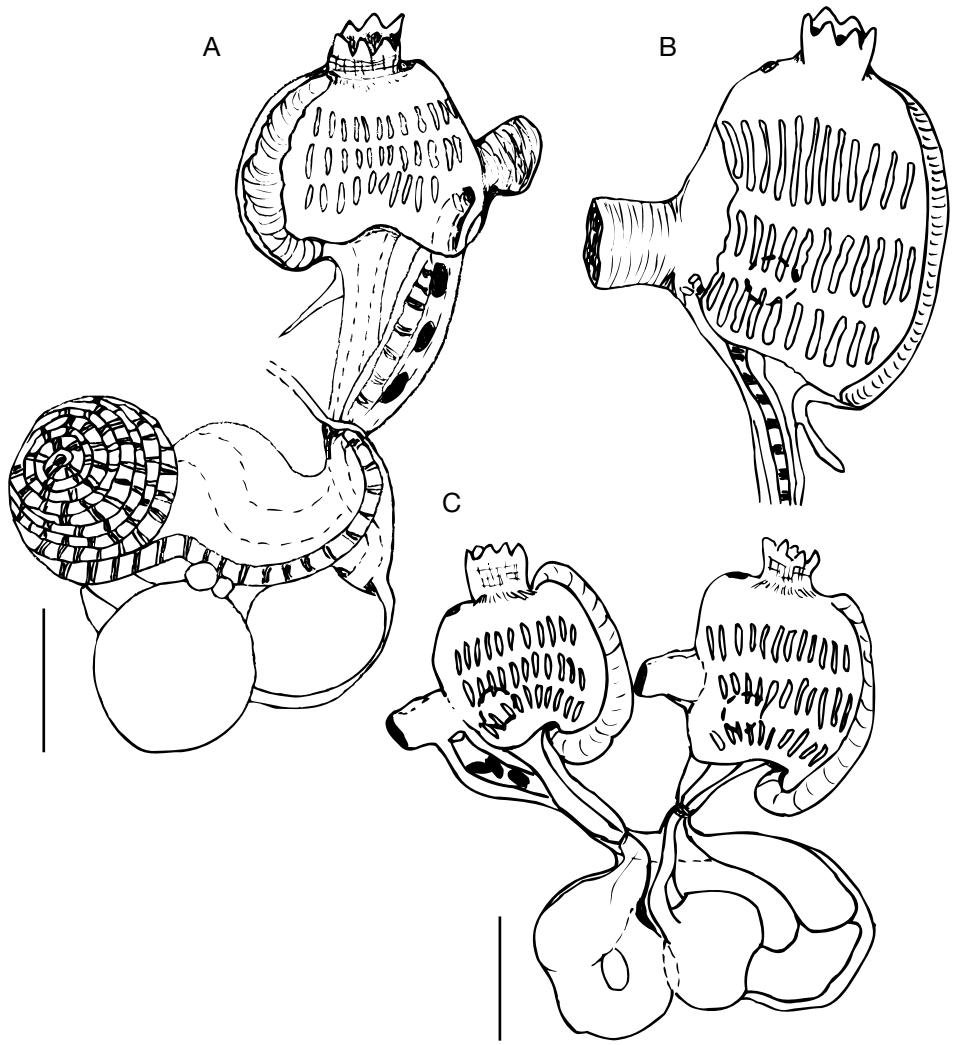

FIG. 2. — *Tridemnum alexi* n. sp., zooids; **A**, left side of whole zooid; **B**, right side of thorax, dotted circle indicates position of right spicule-forming structure; **C**, zooid at late stage of budding. Scale bars: A, 0.4 mm; B, C, 0.5 mm.

The esophagus is very long and may be constricted by a blood vessel or ectodermal appendage that wraps around the zooid at this point and extends out into the tunic (Fig. 2A). The retractor process is present in only some of the zooids and may be very short or almost as long as the esophagus; it originates from the base of the thorax alongside the top ventral side of the esophagus (Fig. 2B). The stomach is large, globular, and smooth. A single large egg is present in most zooids. The sperm duct is a flattened cap over the single testis, with eight or nine coils in most zooids; a few have seven and two were observed to have 10. Many of the zooids are forming a bud: the adult thorax forms a new abdomen, while the adult abdomen forms a new thorax (Fig. 2C).

In the basal layer of tunic are the developing embryos (Fig. 3A, B), some in an advanced stage apparently ready to be released. The embryos do not appear to be connected to any zooids. The fully developed unhatched tadpoles are 0.8-1.0 mm in

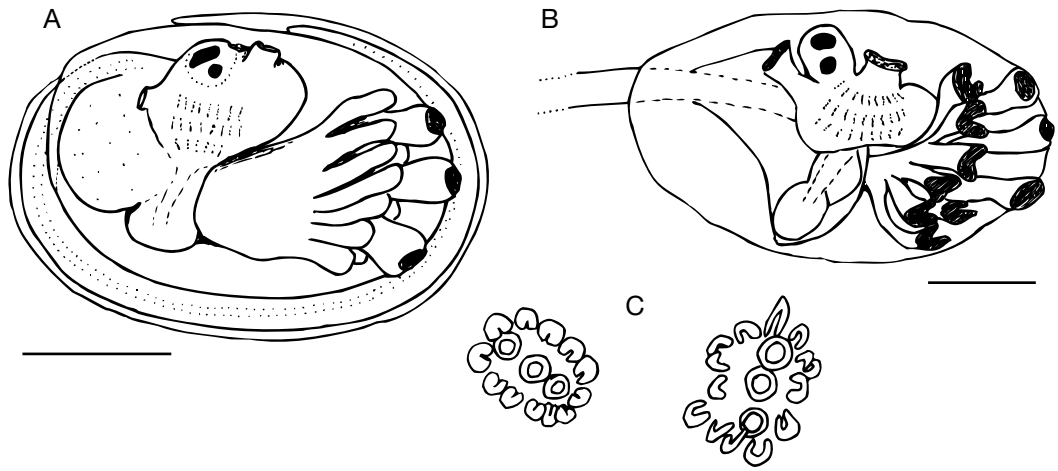

FIG. 3. — *Tridemnum alexi* n. sp., larvae; **A**, middle stage of development; **B**, mature hatched larva; **C**, view of the ends of the three median adhesive papillae and the lateral ampullae corresponding to the developmental stages in A and B respectively. Scale bars: A, B, 250 µm.

trunk length, with three adhesive papillae and in most cases 14 long stout ampullae, seven on each side. Rarely there is a 15th median ampulla anterior to the papillae. Both ocellus and otolith are present, and three faint rows of stigmata can be seen in the developing branchial sac. A hatched tadpole that was still in the cloacal canal and a fully mature unhatched tadpole had a trunk length of 1 mm. In these two largest tadpoles the lateral ampullae are not circular and cylindrical at their tips but are curved (Fig. 3B, C), similar to those described and illustrated by Sanamyan (1999) for *T. tenerum* (Verrill, 1871).

REMARKS

The only other species of *Tridemnum* known from the San Juan Archipelago is *T. opacum* (Ritter, 1907), a common species that is more thinly encrusting and grey or a pale flesh color when alive. It has much denser stellate spicules throughout the tunic with longer points than in the new species, the atrial siphon is posterior to the third row of stigmata, and there are only about six coils of the sperm duct (Table 1). It is possible that *T. alexi* n. sp. is the same species as Huntsman's (1912a) *Tridemnum* sp. A from the east coast of Vancouver Island in the Departure Bay area; he wrote that "a very dark *Tridemnum* with few or occasionally no (?) [his question mark] spicules was taken several times in the dredge". This specimen has apparently been lost, as persistent searching of several museums has yielded no trace of his specimen.

The new species resembles *Tridemnum strangulatum* (Ritter, 1901) from Alaska in its overall shape and the long constricted esophagus, a character present in most *Tridemnum* species and thus considered by Van Name (1945) of no taxonomic importance. However, Ritter described *T. strangulatum* as translucent and "ashen" and with only four coils of the sperm duct. He did not measure the spicules, stating only that they were "short and blunt". Van Name (1945) identified as *T. strangulatum* a colony from Chignik Bay, Alaska, with 20 µm spicules but did not include other morphological details; he did not examine Ritter's specimens. Van Name indicated that his specimen is in the USNM, but it could not be located to examine for this study, nor could Ritter's type specimen. Sanamyan (1999) tentatively identified as *T. strangulatum* two colonies from Sanak Island, Gulf of Alaska; however in his specimens the spicules are large (up to 50 µm) with "prominent conical rays" and twice as many coils of the sperm duct as in Ritter's specimens. *Tridemnum tenerum* (Verrill, 1871) is considered a circumbo-

TABLE 1. — Morphological features of NE Pacific *Tridemnum* spp. References: [1]Ritter 1901; [2]Ritter 1907; [3]Van Name 1945; [4]Sanamyan 1999.

	T. alexi n. sp.	*T. opacum* (Ritter, 1907)	*T. strangulatum* (Ritter, 1901)	*T. tenerum* (Verrill, 1871)
Color of living colony	reddish brown	dull brown with traces of green[2]; grey or flesh colored[3]	ashen, translucent[1]	translucent, grey[3, 4] or yellowish[4]
Colony thickness	up to 2 cm	10-15 mm[3]	2-3 mm[4]	2.0-2.5 mm[4]
Spicule morphology	20-30 µm; short rounded rays	30-35 µm; long conical points, sometimes truncated[2, 3]	20-30 µm; short rounded rays[3]	? not known for type[3]; up to 65 µm[4]
Branchial tentacles	16	16[3]	?[1, 3]	8 or 16[3]
Stigmata/half row	12-13 (see text)	11-12[2, 3]	?[1, 3]; (figure suggests about 12[1])	10-12[3]; 10[4]
Atrial siphon position	2nd stigmatal row	posterior to 3rd stigmatal row[2, 3]	2nd stigmatal row in contracted specimen[1]	3rd stigmatal row[3, 4]
Retractor process	short, usually present	short, usually present[3]	absent? (not in figure)[1]	short, usually present[3, 4]
Sperm duct spirals	usually 8-9	6[3]	4?[1]	8-10[3]; 6-7[4]
Total larval ampullae	13-15 (usually 14)	?	?	?[3]; 8-9[4]

real species occurring in both the north Atlantic and north Pacific. Van Name (1945) reexamined Verrill's specimens and gives a detailed description. Verrill described living colonies as translucent and did not mention any color. The zooids apparently resemble *T. opacum* and *T. alexi* n. sp. morphologically but no accurate description of the spicules is available. Van Name (1945) described the holotype as having needle-like radiating crystals; this is surely a sign of the original spicules having dissolved in the preservative and then the calcium carbonate recrystallized in a needle-like pattern. This is not an unusual phenomenon in didemnids if the preservative is not properly buffered, and happened to some of my own samples occasionally in years past. I have never seen this type of spicule in fresh living colonies, only in fixed material. Sanamyan (1999) tentatively identified a colony from Kamchatka as *T.* aff. *tenerum*, with large (50+ µm) spicules with rounded rays and also spicules with needle-like rays; the latter may have formed by dissolution and recrystallization within the spicular envelopes. The larvae are much larger than *T. alexi* larvae and have a total of only eight or nine lateral ampullae. Thus *Tridemnum alexi* n. sp. is distinguished from all known NE Pacific *Tridemnum* species by the following constellation of characters, as listed in Table 1: smooth tunic and zooids dark reddish brown with abundant pigment granules; colony up to 2 cm thick with large ramifying hypozooidal canals and basal portion containing the larvae; irregularly stellate spicules 20-30 µm in diameter with short pointed rays (eight to 10 in the equatorial plane) thinly scattered through the tunic but absent from the 200-400 µm thick superficial bladder cell layer; thorax with 12-13 stigmata per half row in the first two rows on average and one or two fewer in the third row; atrial siphon at the level of the second row of stigmata; single testis covered by the sperm duct with eight or nine coils; larvae 0.8-1.0 mm in trunk length with 13-15 (usually 14) lateral ampullae with curved tips when fully mature.

Genus *Aplidiopsis* Lahille, 1890

Aplidiopsis pannosum (Ritter, 1899) (Figs 4; 5)

Polyclinum pannosum Ritter, 1899: 519, figs 17, 18 (type locality: Alaska).

FIG. 4. — *Aplidiopsis pannosum* (Ritter, 1899), zooid 14 mm in length with incubated larvae in the atrial chamber.

Polyclinum globosum Ritter, 1899: 518, figs 14-16.

Aplidiopsis pannosum – Hartmeyer 1924: 187. — Van Name 1945: 66-68, fig. 27. — Tokioka 1960: 194, 195. — Nishikawa 1990: 80, 81. — Sanamyan 1998: 107.

Aplidiopsis helenae Redikorzev, 1927: 382.

MATERIAL EXAMINED. — **USA.** Alaska, dredged off Point Barrow, 42 m, 9.IX.1948, G. E. MacGinitie coll., ident. D. P. Abbott, 2 small colonies (USNM 10893); 43m, 15.IX.1948, G. E. MacGinitie coll., ident. D. P. Abbott, 4 small colonies (USNM 10964); 37 m, 16.IX.1948, G. E. MacGinitie coll., ident. D. P. Abbott, 2 small colonies (USNM 10894).
San Juan Archipelago, Rock Pt, off W side of Lopez I., dredged at 74 m, 9.VI.1999, C. Lambert & D. Duggins coll., 3 colonies, larvae present (USNM 1006924); same data, 1.VII.1999, D. Duggins coll., 8 colonies, the largest 4.6 cm in diameter with 2 cormidia, attached to a sabellarid tube, larvae present (MNHN A1 APL A 21); same data, 3.VIII.1999, D. Duggins coll., 11 colonies, the largest 2 cm in diameter, no larvae (CASIZ 162517) together with numerous large colonies of *Distaplia occidentalis* full of mature larvae (CASIZ 162516, 3 colonies); 80 m, 29.VI.2001, D. Duggins coll., 11 colonies, the largest 16 mm in diameter, resting stage, no larvae (CASIZ 162518).
Pt Caution, San Juan Channel, dredged at 96-114 m, mud, sand, shell bottom, 8.VIII.2001, D. Duggins coll., 1 small colony 16 mm in diameter, 20 mm tall, no larvae (USNM 1006925).

DISTRIBUTION. — Japan Sea (Nishikawa 1990); Kurile I. (Sanamyan 1998); Bering Sea (Ritter 1899; Sanamyan 1998); Point Barrow (USNM specimens); Montague I., Gulf of Alaska (Van Name 1945); Sea of Okhotsk (Redikorzev 1927; Sanamyan 1998); Kamchatka (Redikorzev 1927; Tokioka 1960; Sanamyan 1998); off Lopez I., San Juan Archipelago, WA (present study). Depth: intertidal to 535 m; usually less than 100 m.

DESCRIPTION

Colonies are globular or irregularly capitate, attached basally by a very short stalk or peduncle that is not always apparent. The tunic is smooth, clean, translucent and without any embedded sand. Colonies may be whitish or pale yellow, mostly due to the color (or lack of color) of the zooids; the tunic may contain a few scattered reddish brown pigment granules. The largest colony collected is 4 cm in diameter across the upper surface and 3.4 cm in height including a stalk length of 1.4 cm.
The zooids are arranged in approximately circular systems around the common cloacal openings; the largest colonies contain many systems. Zooids are 19-20 mm in length if not contracted, though many are only 10-12 mm in preserved colonies. The oral siphon is short with six lobes and bears numerous fine circular muscles. The non-lobed atrial opening is surmounted by a wide, cowl-like broad languet with many circular muscles and with three small teeth at its tip (see Ritter 1899: fig. 16). There are about 16-18 irregular longitudinal muscles on each side of the thorax. The majority of mature zooids (Fig. 4) have 12 rows of stigmata, with up to about 28 stigmata/half row in the middle rows of the largest zooids; those at the ends of the rows are very small. The number of stigmata/half row is

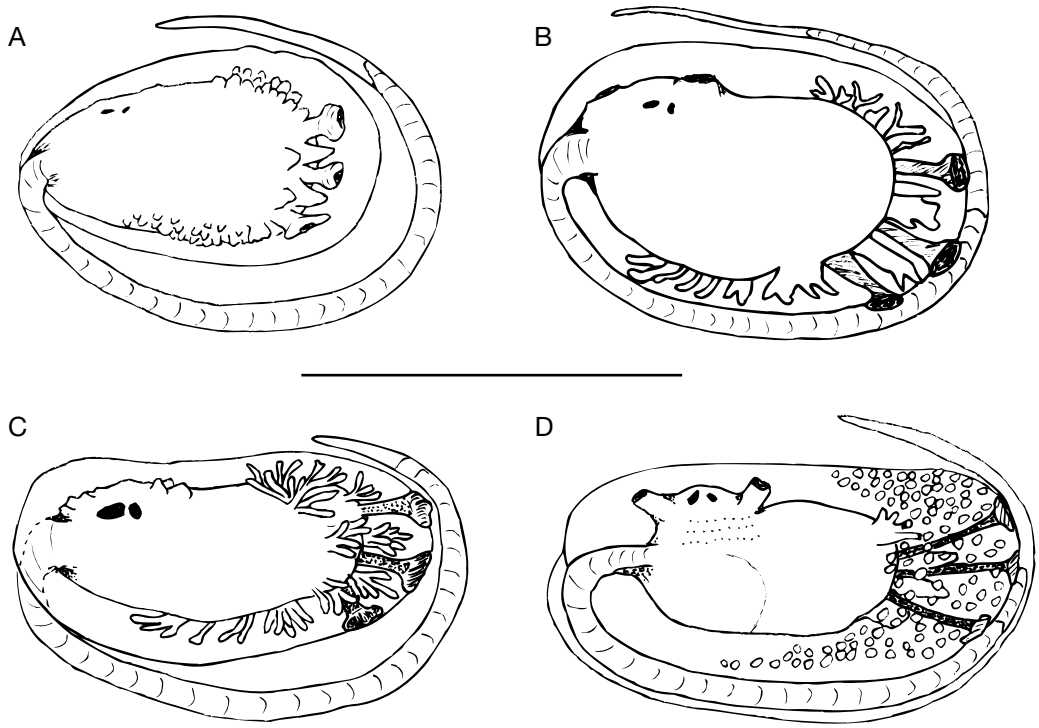

FIG. 5. — *Aplidiopsis pannosum* (Ritter, 1899), unhatched larvae from early to hatching stage of development. Scale bar: 0.5 mm.

quite variable. Papillae are absent from the transverse vessels. The dorsal languets are long, broad and triangular and do not extend as far across the branchial sac as described by Ritter (1899) except in contracted zooids; they originate to the left of mid-dorsal. The esophagus is short, funnel-shaped, and twisted to enter the smooth globular stomach on its dorsal side (Fig. 4) (Ritter 1899: fig. 18). A prominent typhlosole extends from the entrance of the esophagus to the posterior end of the stomach. An abruptly narrowed post-stomach about as long as the stomach itself ends equally abruptly in the widened intestine that forms the posterior loop and then extends anteriorly into the atrium to the level of the sixth row of stigmata. The end of the rectum is flared out and bilobed (see Ritter 1899: fig. 18). In living zooids the stomach and proximal region of the intestine are a dark yellowish orange, but the post-stomach is colorless. The postabdominal ovary and testis are not developed in any of these specimens. The heart is at the posterior end of the postabdomen and has two beating chambers in living zooids.

In those colonies with larvae, eight to 10 could be found in the atrial chamber of a single zooid (Fig. 4), the most mature being closest to the atrial opening. Rather than exhibiting a continuous series of developmental stages, there are groups of two or three at approximately the same stage of development: the three closest to the atrial opening are mature or nearly so, three at mid-atrial chamber are at early tailbud with small otolith and ocellus beginning to form, and three at the base of the chamber are at pre-tailbud stage. This may indicate that on average three eggs or embryos are released into the atrial chamber each day or at a single spawning. Some mature swimming larvae were released in the laboratory in July 1999. They are large, with an elongate trunk up to 1.3 mm long (Fig. 5) and three large adhesive organs each on a long stalk.

There are large lateral and ventral ampullae with multiple fingerlike branches. In addition, numerous tiny ectodermal vesicles are scattered over most of the lateral, anterior and ventral areas in a pattern corresponding to the "multiple arc of vesicles" in some *Aplidium* species (Kott 1992: 508). The tadpoles have a wide tail fin. The siphons appear to be open in the most advanced larvae even before hatching. A large ocellus and otolith are present.

Matching Ritter's (1899) description of his specimens, the post-abdomens are filled with opaque mesenchymatous cells and the gonads have apparently regressed as the larvae matured. Parasitic copepods were present in several of the colonies from Rock Pt, and one in the stomach of a zooid from the Pt Caution colony.

REMARKS

Although this is the first record of this species in the NE Pacific south of Alaska, the colonies are easily mistaken for pale-colored *Distaplia occidentalis* Bancroft, 1899 until examined under a microscope. The collection of 3.VIII.1999 contains numerous *D. occidentalis* and only a few *A. pannosum*. The region of collection has been a popular dredge site for the Friday Harbor Laboratories for many years for *Balanus nubilis*, so it is likely that at least a few colonies of *A. pannosum* were collected over the years along with *D. occidentalis*. Huntsman (1912a: 115) wrote that "Dall has remarked that the fauna of the inner channels of the British Columbian archipelago is of a distinctly more northern character than that of the open coast. This is well shown in the ascidians. The list from Departure Bay includes arctic forms that are not represented at Ucluelet and among the Ucluelet species are a number of southern forms that do not occur at Departure Bay".

Huntsman did not collect any *Aplidiopsis pannosum* from the Departure Bay area but it is apparently abundant and widespread in the North Pacific (see Distribution). The northernmost record is the samples from Point Barrow collected in 1948. These differ from the present samples in having sand grains embedded on and in the tunic, though Ritter (1899) states that there may or may not be sand present. This is a variable character in the NE Pacific species *Aplidium californicum* (Ritter & Forsyth, 1917) and could also be variable in *Aplidiopsis pannosum*.

The larvae described by Sanamyan (1998) are somewhat different from those in the present collections and are probably immature, based on the appearance of his groups of epidermal vesicles and lateral ampullae, as well as the lack of siphons in his figure 1C and the very small siphons in his figure 1D. In the 9.VI.1999 colonies of the present study the siphons are very prominent in the most mature larvae. In an examination of various larval stages I found that the ampullar blocks of the immature larva subdivide at their tips during maturation, forming fingerlike projections that remain joined at the base (Fig. 5B, C). There are two discrete clumps of vesicles in immature larvae similar to those illustrated by Sanamyan (1998); these spread out anteriorly and laterally as the larva matures (Fig. 5B, C). Epidermal vesicles form at the ampullar tips and are pinched off late in larval development, breaking the connection between vesicle and ampulla and leaving the vesicles free in the tunic (R. Cloney pers. comm.) (Fig. 5D). Larval morphology, now a crucial part of species identifications, was not considered important by the early taxonomists and thus there are very rarely any comments about the larvae other than possibly their presence or absence. The above observations on morphological changes that occur as the larvae mature show the necessity of having fully mature larvae available, a requirement that is unfortunately often lacking.

Japan Sea specimens that have been assigned to this species have a higher number of stigmatal rows and lower number of longitudinal muscles than the present specimens (Nishikawa 1990). In the present collections, some small immature colonies have zooids with fewer than 12 rows of stigmata but none have more than 12 rows. Curiously, the largest colony from 1.VII.1999, with the largest cormidium 4 cm in diameter, contains zooids that appear to be immature or may have regressed. Gonads are lacking, the post-abdomen is not well developed, and some zooids

have an empty gut and intestine indicating that they were not feeding.

Order PHLEBOBRANCHIA Lahille, 1887
Family CIONIDAE Lahille, 1887
Genus *Ciona* Fleming, 1822

Ciona savignyi Herdman, 1882

Ciona savignyi Herdman, 1882: 236, 237 (type locality: Japan). — Hoshino & Nishikawa 1985: 69-71, figs 1D-G, 3. — Nishikawa 1991: 33. — Cohen *et al.* 1998: 31, 33. — Lambert & Lambert 1998: 675-688. — Mills *et al.* 2000: 135.

MATERIAL EXAMINED. — **USA.** S of Seattle, WA, Des Moines Marina, on ropes on covered floating docks, 1 m, C. Lambert coll., 1-8.IX.1998, 3 specimens (CASIZ 162514), 2 specimens (USNM 1006927), 3 specimens (MNHN P1 CIO 77).
Puget Sound, WA, Brownsville Yacht Club float, 10.IX.1998, 3 large specimens in sabellid/mussel clumps.
N of Seattle, Edmonds Marina, on covered floating docks, IX.1999, numerous individuals of all sizes.
Tacoma, WA, Yacht Club adjacent to Pt Defiance ferry dock, only on covered floats, 23.IX.2001, many.

DISTRIBUTION. — *Ciona savignyi* is apparently native to Japan (Nishikawa 1991). It was first recorded from the Pacific coast of North America (as *C. intestinalis* (Linnaeus, 1767)) by Ritter (1913) from a collection made in 1903 in Loring, Alaska (USNM 5633). A second specimen (American Museum of Natural History 1427) was collected from British Columbia in 1937 (also identified as *C. intestinalis*). Hoshino & Nishikawa (1985) reexamined these two museum specimens and determined that they were actually *C. savignyi*. Lambert & Lambert (1998) first noted *C. savignyi* on the US west coast in 1985 in Long Beach Harbor, CA. Their first Pacific NW sightings were at Des Moines Marina south of Seattle in 1998, where the species was very abundant (A. Cohen *et al.* 1998; Mills *et al.* 2000). Though absent in 1998 at Edmonds Marina north of Seattle, it appeared there in large numbers in 1999. It remains abundant at these two marinas and now extends south to Tacoma (unpublished observations).

DESCRIPTION
See Hoshino & Nishikawa (1985) for a detailed comparison of *C. savignyi* with *C. intestinalis*. Individuals are long and slender (up to 15 cm), with usually five (but may be four to six) strong longitudinal muscle bands on each side showing through the translucent yellowish/green tunic. White pigment flecks are scattered in the body wall. As indicated by Hoshino & Nishikawa (1985) for this species, there is no endostylar appendage at the base of the endostyle and no red pigment spot at the end of the sperm duct, though both are present in US west coast *Ciona intestinalis*. Also agreeing with the description by Hoshino & Nishikawa (1985), there is a pair of large pharyngeo-epicardiac openings very close to the opening of the esophagus, one on each side of the mid-ventral line. The siphons, both anterior, are long and divergent. The oocyte follicle cells of *C. savignyi* contain multiple small refringent droplets as compared with the single droplet per follicle cell in *C. intestinalis* (Byrd & Lambert 2000).

REMARKS
This species favors shaded locations such as covered floats. Even if it is abundant on these surfaces it is nearly always absent from adjacent uncovered floats.

Order STOLIDOBRANCHIA Lahille, 1887
Family STYELIDAE Sluiter, 1895
Genus *Botrylloides* Milne-Edwards, 1841

Botrylloides violaceus Oka, 1927

Botrylloides violaceus Oka, 1927: 608, 609 (type locality: Japan). — Saito *et al.* 1981: 360-364, figs 3, 4, 5b, redescription.

MATERIAL EXAMINED. — **North America.** Many marinas in Puget Sound, the San Juan Archipelago and southern Vancouver Island (specific sites available from the author), VIII.1998-IX.2001, numerous samples.
USA, 16 km N of Seattle, WA, Edmonds Marina, 0.5-2.0 m, 21.VIII.1998, several colonies with larvae on floats and ropes (CASIZ 162515, 3 colonies) (MNHN S1 BOT A 32, 3 colonies).
Willapa Bay, WA (A. Cohen *et al.* 2001), on oysters and marina floats, 22.V.2000, many.

DISTRIBUTION. — NE Pacific: Alaska to Baja California (Lambert & Sanamyan 2001); NW Pacific: Vladivostok (Sanamyan 2000); NW and NE Atlantic, Mediterranean (Zaniolo *et al.* 1993), Japan (Nishikawa 1991).

DESCRIPTION

All specimens agree in every respect with the detailed description given by Saito *et al.* (1981) for the Japanese colonies. The zooids are aligned vertically in the tunic, in round, oval or elongated systems or sometimes crowded together so that the systems are obscured. The colonies may be purple, light lavender, any shade of yellow or orange, brown, or even nearly colorless, but in all cases the entire colony is all of one color. The tunic is soft, easily torn, and the zooids are then easily freed from the tunic. There are 10-11 rows of stigmata; the second row of stigmata is incomplete on both sides. The most distinguishing character is the large larva (about 1 mm trunk length) with 24-32 lateral ampullae. The larvae are brooded in a separate brood sac that is nourished by the vascular system while the parent zooid dies.

REMARKS

Botrylloides violaceus was described from Japan by Oka in 1927 and redescribed by Saito *et al.* (1981). Tokioka (1953) characterized it as "the commonest botryllid in Japanese waters". It appeared on both the US Atlantic and Pacific coasts at least 30 years ago. The earliest confirmed description of it was by G. Freeman (cited by Berrill 1975) who observed the unusual larva in colonies collected in San Diego but misidentified it as *Botryllus schlosseri* (Pallas, 1766). Fay & Vallee (1979) also observed it in southern California but misidentified it as *Botrylloides diegensis* Ritter & Forsyth, 1917. *B. violaceus* has a very long breeding season and is a precocious species that begins to produce larvae when the colony is still quite young and small. Following the classification of Saito *et al.* (2001), the genus *Botrylloides* is retained here for those botryllids in which the testes are located anterior to the ovaries. An additional non-indigenous species, *Botryllus schlosseri*, is also common in many areas of Washington state from Olympia to the Canadian border at Blaine (Mills *et al.* 2000). Locations can be found in C. S. Cohen *et al.* (1998) and the unpublished reports by A. Cohen *et al.* (1998, 2001).

Genus *Styela* Fleming, 1822

Styela clava Herdman, 1881

Styela clava Herdman, 1881: 70 (type locality: Japan). — Abbott & Johnson 1972: 95-105. — Kott 1985: 115. — Lambert & Lambert 1998: 675-688.

Styela barnharti Ritter & Forsyth, 1917: 452. — Van Name 1945: 309.

MATERIAL EXAMINED. — **Canada.** Vancouver I., French Creek Marina, 1993-2002, numerous individuals. — Nanoose Bay, on floating dock, 4.IX.1998, 1 small individual. — Nanaimo, Brechin Pt, boat launch floats, 24.IX.1998, 2 individuals. — Maple Bay, 9.XII.2000, a small population was observed by Bill Austin near Duncan at Birds Eye Cove.
USA. Blaine, WA, Drayton Harbor, floating docks, 3.IX.1998, 11.IX.1998, 7.IX.2001, many.
Olympic Peninsula, WA, Neah Bay, Neah Bay Marina, 18.VIII.2001, many.

DISTRIBUTION. — Described from dredged specimens collected near Kobe, Japan, it now occurs worldwide in temperate waters, including Europe, the UK, Australia, Asia, and the east and west coasts of North America. It is considered to have been introduced to these regions *via* hull fouling or other anthropogenic transport (see Abbott & Johnson 1972; Lambert & Lambert 1998).

DESCRIPTION

See Van Name (1945, under *S. barnharti* Ritter & Forsyth, 1917) and Abbott & Johnson (1972) for detailed descriptions with photos and illustrations. This large (up to 8-9 cm or more in length) cylindrical stolidobranch has a tough but thin brownish tunic and short broadly tapering posterior stalk. Both siphons are short and close together at the anterior end. The tunic is tubercular anteriorly around the siphon bases, while posteriorly it is folded into longitudinal ridges and grooves. There are two to five slender long gonads on the left and four to nine on the right, with numerous small testes attached along the length of the sinuous ovaries.

Family MOLGULIDAE Lacaze-Duthiers, 1877

Genus *Molgula* Forbes & Hanley, 1848

Molgula manhattensis (De Kay, 1843)

Ascidea manhattensis De Kay, 1843: 259 (type locality: NE United States).

Molgula manhattensis – Verrill 1871: 54. — Van Name 1945: 385-389, figs 271-273. — Monniot 1969: 191-196, figs 7-9 (for a detailed description and extensive synonymy). — Kott 1985: 379, 380.

MATERIAL EXAMINED. — **Canada.** Vancouver I., French Creek Marina, B.C., 4.IX.1998, 4 small specimens on tire.
USA. Puget Sound, Shelton Yacht Club floats, 9.IX.1998, many, all sizes.
Willapa Bay, WA (see A. Cohen *et al.* 2001), 22.V.2000, several from 3 sites (not abundant).

DISTRIBUTION. — Worldwide now in temperate waters: Japan, Australia, US east and west coasts, Europe.

DESCRIPTION

Globular, usually 4 cm or less in diameter, with a soft, thin, translucent, colorless tunic through which some of the internal organs can occasionally be seen. Usually, however, the tunic is covered by greyish fine sediment trapped in the numerous small tunic hairs. The divergent siphons are fairly close together at the anterior end; as in other molgulids the oral siphon has six lobes, the atrial four. There are six branchial folds per side. The spiral stigmata are usually quite broken up and irregular. The intestine makes a closed deep loop, somewhat U-shaped but more pronounced; "the whole loop is bent in a curve of about three-fourths to four-fifths of a circle" (Van Name 1945). This species is a free spawner; the embryos form swimming larvae.

REMARKS

See Van Name (1945), Monniot (1969) and Kott (1985) for a detailed description of the morphology of this species and its probable routes of anthropogenic transport. Individuals often occur by the millions in dense clumps on artificial structures in harbors, especially where the salinity is 27-30 parts per thousand.

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