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A new *Culeolus* species (Ascidiacea) from the NE Pacific, California

KAREN SANAMYAN[1,3], NADYA SANAMYAN[1] & LINDA KUHNZ[2]

[1]*Kamchatka Branch of Pacific Geographical Institute, Far-Eastern Branch of the Russian Academy of Sciences, Partizanskaya 6, Petropavlovsk-Kamchatsky, 683000, Russia. E-mail: ascidiacea@sanamyan.com*
[2]*Monterey Bay Aquarium Research Institute (MBARI), 7700 Sandholdt Road, Moss Landing, California 95039, USA. E-mail: lkuhnz@mbari.org*
[3]*Corresponding author. E-mail: ascidiacea@sanamyan.com*

Abstract

The paper describes *Culeolus barryi* **n. sp.** (Ascidiacea: Pyuridae), a species photographically documented in situ and collected in the northeast Pacific, off the coast of California (USA). This species of stalked tunicate inhabits deepwater (1200 m) and is characterized by numerous gonads on each side of the body. It is most similar to *C. nadejdae* from the Sea of Okhotsk and *C. sluiteri* from Aleutian Islands. A brief overview of all *Culeolus* species is provided.

Key words: Tunicata, Ascidiacea, *Culeolus*, Deep water, NE Pacific, MBARI

Introduction

Underwater remotely operated vehicles (ROVs) have advanced ecological deep sea research in recent years. High resolution photographs of deep water ascidians, whose external appearance in life was not known previously, have become available to researchers. Unfortunately only a small fraction of these ascidians are collected. Ascidians are marine invertebrates whose identification is generally not based on external features visible in photographs, so many very interesting and unusual species photographed by deep-water vehicles remain enigmatic (e.g. see Sanamyan & Hissmann, 2008, Fig. 4D). Members of the genus *Culeolus* (family Pyuridae) do have a distinctive appearance and photographed specimens are easily attributed to this genus (but not to species). All *Culeolus* spp. have ovoid sac-like bodies attached to a long stalk. The stalk is inserted at ventral side of the branchial (inhalant) aperture, which is normally wide open. There are several other genera for which some or all members resemble *Culeolus*. Members of *Fungulus* (family Molgulidae) resemble small specimens of *Culeolus*; some *Fungulus* species were initially described as *Culeolus*, (e.g. *Fungulus parvus* (Millar, 1970)). *Fungulus* is a deep-water genus comprising five species. There are no known underwater photographs a *Fungulus* species, thus its appearance in life is not known. Some stalked *Pyura* and *Boltenia* species may also resemble *Culeolus* externally (e.g. *Boltenia ovifera* (Linnaeus, 1867), see Tokioka, 1960, Pl. 25, Fig. 25), but they all have much smaller branchial and atrial apertures. Although no in situ photos are available for *B. ovifera,* it should be easily distinguishable from *Culeolus*.
The genus *Culeolus* contains 25 deep-water species which we currently treat as valid. Nearly half of them were described more than 100 years ago (Herdman, 1882, Sluiter, 1904, Ritter, 1907, Ritter, 1913, Oka, 1928), five species were added in the middle of the last century by Tokioka (1967) and Vinogradova (1962, 1970) and the remaining seven species were described by modern authors (Monniot & Monniot, 1976, 1982, 1991, 2003, Sanamyan, 1992, Sanamyan & Sanamyan, 2002). Although the original descriptions in earlier papers (especially those of Sluiter, 1904) lack significant details on the internal structures of the species (e.g. the number and the shape of gonads), many of species were subsequently recorded and redescribed. The type material of some of them was reexamined (e.g. Monniot & Monniot, 1982 reexamined some material of Herdman, 1882 and Sanamyan & Sanamyan, 2006 redescribed material of Vinogradova, 1970). Monniot & Monniot (1976) were the first authors who published a table comparing all existing descriptions of *Culeolus* species, and later Kott (2002) published a key to most (although not all) species known at that time. Kott's 2002 key, however, is mostly based on external features and would be hard to follow in practice.

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Although the members of *Culeolus* are widely distributed in the deep waters of all oceans, the diversity of species in different regions varies significantly. In general, unlike many other deep water ascidians, they usually have rather local distribution (Kott, 2002). Most species are known from southern oceans and from the Central and North Pacific; only one species, *C. suhmi* Herdman, 1881, is thus far recorded from the North Atlantic.

Material and methods

Samples were collected using the Monterey Bay Aquarium Research Institute's (MBARI) ROV *Doc Ricketts* in December, 2017. These tunicates were present at Sur Ridge, 28 miles west of Point Sur, central California (USA) at about 1200 m water depth.

The material was preserved in 4% seawater formalin upon surfacing and then transferred to 70% EtOH for long term storage. The specimens are deposited in the Kamchatka Branch of the Pacific Geographical Institute (KBPGI).

Culeolus barryi n. sp.

(Figures 1, 2)

Material examined. Holotype. Label: MBARI D998.A6, tunicate, 19 December 2017. 36.416480°N, 122.301106°W, 1203 m, oxygen 0.583 ml/l, t=3.358°C. Registration number: KBPGI 1454/1. Paratype. Label: MBARI D998.A5, tunicate, 19 December 2017. 36.416587°N, 122.300895°W, 1207 m, oxygen 0.620 ml/l, t= 3.287°C. Registration number: KBPGI 1455/2.

Description. Dimensions of preserved specimens: Holotype: stalk 38 cm long, 4.5 mm diameter; body 10.5 cm long, about 5 cm diameter. Paratype: stalk 19 cm long, 2.5–3 mm diameter; body 6.5cm long, 3.5–4 cm wide. The test is dirty-white and opaque in preservative, internal organs (e.g. gonads) cannot be seen through it. Initially, when preserved in formalin, the test (about 3 mm thick) was rather soft and gelatinous, but after transferring it to alcohol, lost water and became somewhat more rigid and leathery. The surface of the test is free of epibionts, strongly rugose, with numerous shallow to rather deep wrinkles and folds running mainly in longitudinally. Numerous prominent wart-like papillae or tubercles with wide bases (about 5 mm) are present over the whole body and on the tunic covering upper third of the stalk. Inside each papilla there are one to several horny rods running perpendicular to the surface of the test and supporting the top of the papilla. These whitish opaque rods are clearly visible through semitransparent outer test (Fig. 1C). Mid-ventral and postero-ventral crests, which are characteristic for many *Culeolus* species, are difficult to see in photographs of live specimens, and in preserved material. On preserved specimens the mid-ventral crest is represented by an obscured line of opaque papillae forming a low longitudinal fold in the test, which is only a slightly thicker than the numerous other folds of the tunic. The postero-ventral crest is somewhat better defined, but still hard to recognize. It is composed of a row of thicker papillae and forms an open arc, encircling postero-ventral end of the body. Lateral branches of the postero-ventral crest end at lateral corners of the atrial aperture and do not extend to the dorsal side of the body. The rugosity of the test on the posterior of the body, in an area delimited by postero-ventral crest ventrally and laterally and by the ventral rim of the atrial aperture dorsally, differs somewhat from the texture on the rest of the body, being more densely papillated (Figure 1A, B).

The branchial and atrial apertures are tightly closed and hard to recognize on preserved specimens. On fully expanded specimens, as seen on underwater photographs, the branchial aperture appears to be circular, and not especially large (smaller than those seen on other *Culeolus* species in situ photographs). The atrial aperture is a wide, bilobed slit. In live specimens they are sometimes oriented so that the dorsal side is up (Figure 1A, D), and sometimes ventral side up (Figure 1B). The stalk is inserted at ventral side of the branchial siphon. It is tough, covered by tunic at its upper part (the tunic may extend one third of its length). Stalks were covered in epibionts such as hydroids and polychaete tubes. Animals are attached to hard substrate with a small lamellar disc-like structure (no root-like outgrowths are present).

The body wall is thin and transparent with well-spaced muscle bands radiating from the siphons and encircling body. There is a conspicuous concentration of circular muscles around atrial and especially around the branchial apertures (Figure 2A).

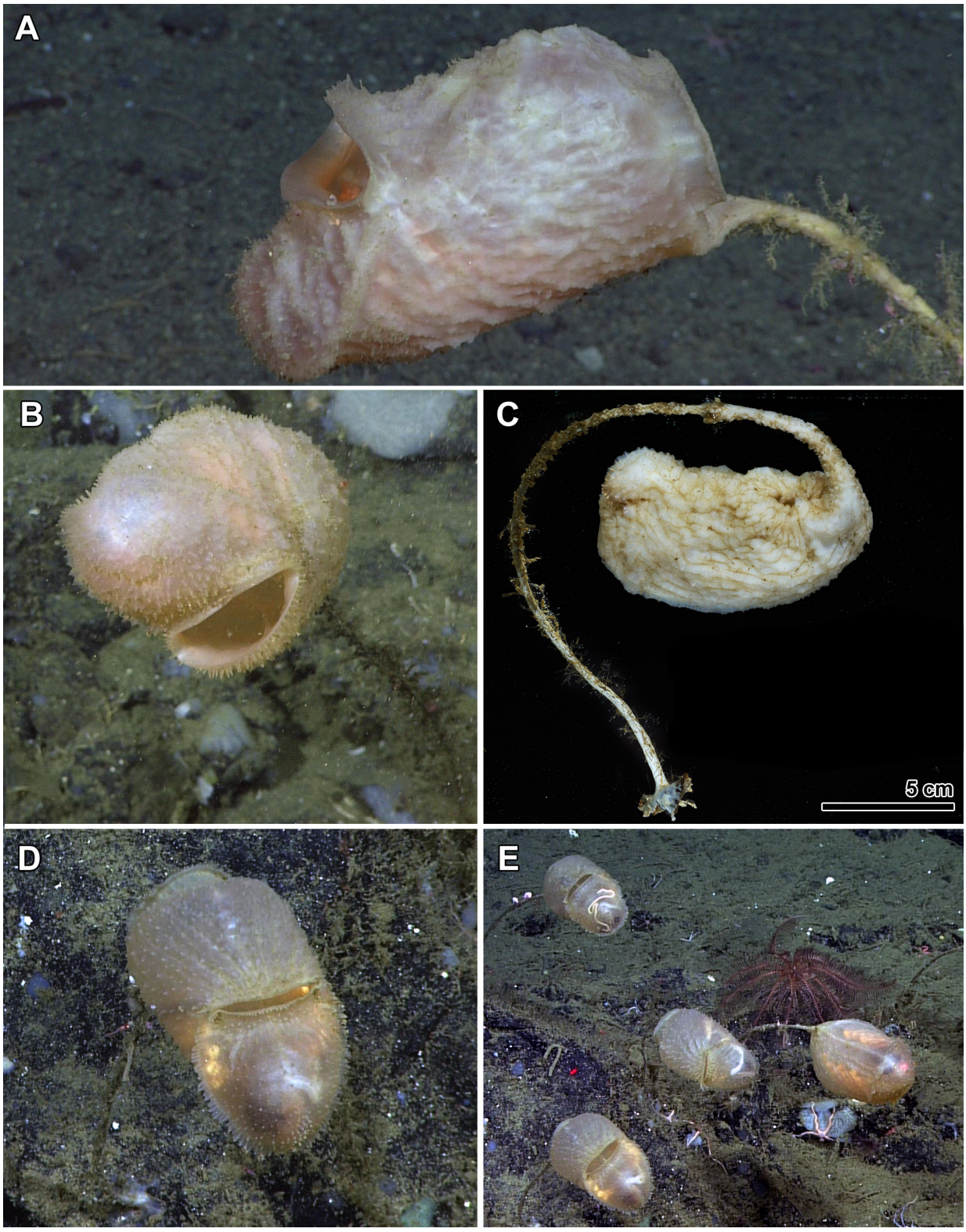

FIGURE 1. *Culeolus barryi* **n. sp.** A, paratype KBPGI 1455/2 (D998-A5), note the hydroids attached to the stalk; B, C, holotype KBPGI 1454/1 (D998-A6); D, E, not collected specimens (36.423952°N, 122.301114°W, 1375 m), note test papillae arranged in rows and commensal nemertean worm in each specimen.

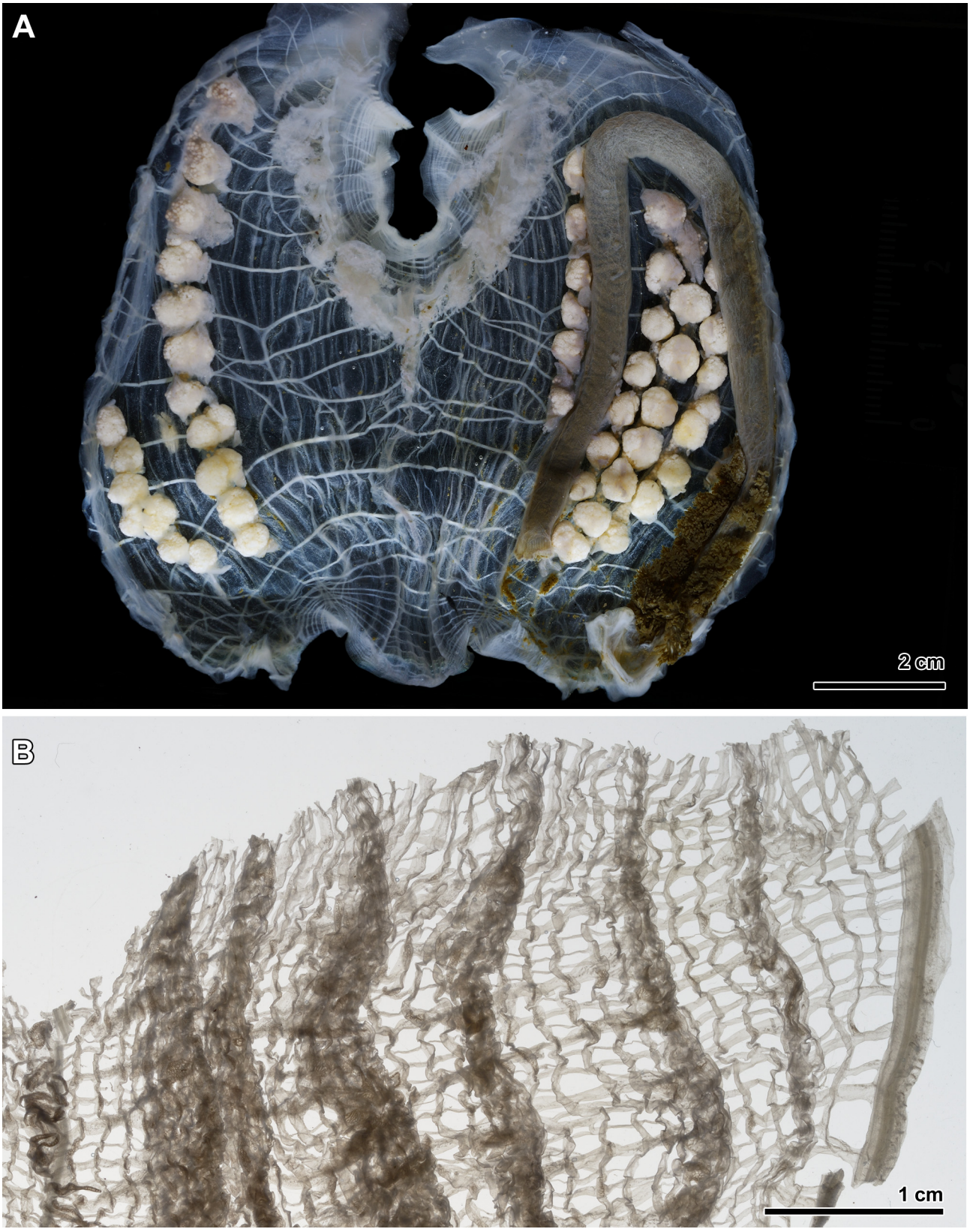

FIGURE 2. *Culeolus barryi* **n. sp.**, holotype. A, opened ventrally; B, left side of the branchial sac.

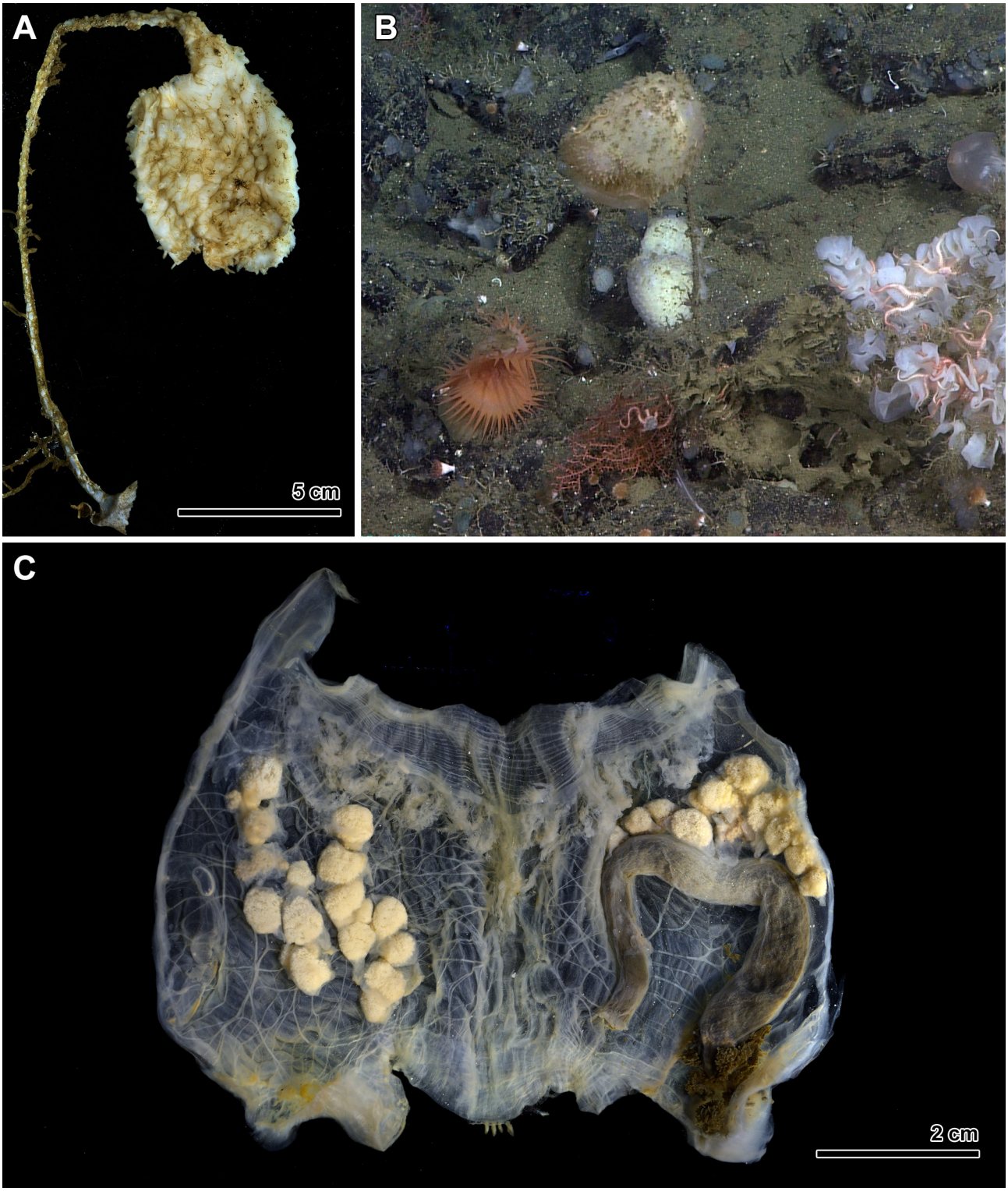

FIGURE 3. *Culeolus* sp., D998-A8, this may be an aberrant specimen of *Culeolus barryi* **n. sp.** A, preserved; B, in situ; C, opened ventrally

The holotype has 27 large branchial tentacles comprising approximately three different sizes ranges. They are inserted at a rim of strongly muscular velum. The tentacles of dirrefent size orders are distributed without apparent order; most ventral tentacle on the left side of the body and one dorsal tentacle appear to be larger than the rest. The tentacles are flattened and bear numerous primary, secondary and tertiary branches. The prepharyngeal band consists of two thick lamella and runs just along the branchial velum. It makes an obvious, but not deep V around the dorsal tubercle. A round dorsal tubercle has a C-shaped slit-like opening with both ends turned outward and an

open interval facing to the right side of the body. The neural ganglion is long, and situated close (just posterior) to the dorsal tubercle at one forth distance between the branchial and the atrial apertures.

The dorsal lamina is composed of about 40 languets. The branchial sac has a common *Culeolus* structure, with the inner longitudinal and the outer transverse vessels forming rectangular meshes and having no ciliated stigmata (Figure 2B). It has six folds on each side, all are well developed. The branchial formula for the holotype is: E4(5)3(8)5(9)7(9)4(6)4(9)4DL3(7)4(7)5(11)5(7)5(7)3(4)4E.

The isodiametric gut forms a long and almost straight U-shaped loop with the ascending limb running along the whole left side of mid-ventral line. The stomach is of the same diameter as the intestine. It occupies one third of the ascending limb of the gut loop. Hepatic diverticula are in a form of dense bushes of branched cylindrical or slightly flattened lobes sitting in two series along the stomach. Anal margin has many small lobes.

Gonads are distributed as follows:

The holotype has two gonads on the right and four on the left. The right gonads are almost parallel to the endostyle, the anterior one has 11 follicles and is significantly longer than posterior, which has seven follicles. On the left, three gonads occupy nearly the whole space within the gut loop and the most anterior gonad is outside of the gut loop, running along external side of the intestine (Figure 2A). Four left gonads are composed of 6, 7, 7 and 6 follicles respectively.

The paratype has four gonads on the right (10, 8, 7 and 8 follicles) and seven gonads on the left, some of which are short (2, 1, 8, 2, 3, 1, 5 follicles). One gonad on the left lying outside of the gut loop (as in holotype) and the rest occupy the whole space in the gut loop.

We did not detect spicules in any tissues. Each specimen had one large commensal nemertean worm and several parasitic copepods in the peribranchial cavity.

The material collected by MBARI included a third *Culeolus* specimen (label: MBARI D998.A8, tunicate, 1206 m, 19 December 2017. 36.416506°N, 122.301045°W, 1203 m, 0.580 ml/l, t=3.371°C) (Fig. 3). It was collected with the two specimens described above, and in most features is identical with them, being only a little smaller (stalk 15 cm long, 2–3 mm diameter; body 8 cm long, 3–3.5 cm diameter) (Fig. 3A, B). However, the position of its gonads is different: it has two gonads located in the middle of the right side of the body (not along the endostyle as in above described material) and three gonads on the left, all of which are outside of the gut loop (Fig. 3C). Such position of the left gonads, behind the pole of the gut loop, is very unusual and not known in other specimens belonging to *Culeolus*. We believe that this is an aberrant specimen of *Culeolus barryi* **n. sp.** rather than a distinct taxon. However, for safety we prefer to label it as *Culeolus* sp., rather than *Culeolus barryi* **n. sp.**

Etymology. The species name is in honor of James P. Barry for his many contributions to deep-sea ecology.

Remarks. All *Culeolus* spp. can be divided into following groups:

1. Seven species having entire (not lobed) gonads, or gonads composed of a maximum of two lobes: *C. recumbens* Herdman, 1881, *C. quadrula* Sluiter, 1904, *C. easteri* Tokioka, 1967, *C. longipedunculatus* Vinogradova, 1970, *C. robustus* Vinogradova, 1970, *C. pinguis* Monniot C. & Monniot F., 1982 and *C. likae* Sanamyan & Sanamyan, 2002. Only C. *recumbens* has been recorded several times; it is a distinctive species with a test covered by sand that cannot be confused with other *Culeolus* species (Monniot & Monniot, 1982, 1991, 2003, Sanamyan & Sanamyan, 1999, Monniot & Lopez-Legentil, 2017). All other species in this group have only been recorded once and are known from the original descriptions only. The paratypes of *C. robustus* and *C. longipedunculatus* were redescribed by Sanamyan & Sanamyan (2006). These species can all be easily distinguished from *C. barryi* **n. sp.** by the shape of the gonads.

2. Six species which usually have one gonad divided into several lobes on each side: *C. moseleyi* Herdman, 1881, *C. gigas* Sluiter, 1904, *C. thysanotus* Sluiter, 1904, *C. pyramidalis* Ritter, 1907, *C. antarcticus* Vinogradova, 1962 and *C. caudatus* Monniot C. & Monniot F., 1991. Among them *C. moseleyi*, *C. thysanotus* and *C. caudatus* are based on original descriptions only. The type specimen of *Culeolus moseleyi* was reexamined by Monniot & Monniot (1982, p.117) who said that "most likely it will never be possible to identify the type with specimens in good condition", so it should be probably considered as *incertae sedis*. *Culeolus thysanotus* has a distinctive exterior (covered by hair-like outgrows of the tunic) and for new records it would be easy to identify. *Culeolus gigas* and *C. pyramidalis* were redescribed by Monniot (1998) based on newly recorded specimens from the east and NE Pacific. *Culeolus antarcticus* is well documented and often recorded from the Antarctic (Vinogradova, 1962, Monniot & Monniot, 1982, 1985, Sanamyan & Sanamyan, 2002, Monniot *et al.*, 2011).

3. Six species usually having two gonads on each side, each divided into several lobes: *C. suhmi* Herdman,

1881, *C. wyvillethomsoni* Herdman, 1881, *C. herdmani* Sluiter, 1904, *C. inversus* Oka, 1928, *C. anonymus* Monniot F. & Monniot C., 1976 and *C. hospitalis* Monniot F. & Monniot C. 2003. In this group *C. inversus* is based on only one specimen which had a strange position of the atrial opening (on the ventral side of the body). It is possible that its structure was incorrectly interpreted by its author (Oka, 1928), and if so it would not be possible to identify any new material as this species in the future. *Culeolus anonymus* and *C. wyvillethomsoni* are probably conspecific: according to Monniot & Monniot (1982) the type specimen of the latter may be an abnormal specimen of *C. anonymus.* This species, and also *C. herdmani* and, especially, *C. suhmi* were often recorded and are well documented (e.g. Monniot & Monniot, 1976, 1982, 1985, 1991, 2003, Sanamyan & Sanamyan, 2002, 2005, 2006).

4. Species which may have more than two gonads on either or both sides, each divided into several lobes: *C. murrayi* Herdman, 1881, *C. sluiteri* Ritter, 1913, *C. tenuis* Vinogradova, 1970, *C. elegans* Monniot C. & Monniot F., 1991, and *C. nadejdae* Sanamyan, 1992. All these species were described from the Pacific region (including Sea of Okhotsk). With this new description, *C. barryi* n.sp. belongs to this group which we further describe in detail.

Culeolus elegans is known from the original description based on five specimens from New Caledonia, in 1530–1480 m water depth (Monniot & Monniot, 1991). It usually has four, or, in one specimen, three gonads on the right and two or one gonad on the left. All left gonads are inside the gut loop. Externally this species differs from *C. barryi* n.sp., in that the peduncle is very thin (0.5 mm) and the test is thin and transparent with all internal organs clearly visible through it in preserved specimen (Monniot & Monniot, 1991a, Fig. 31B). It is interesting that Monniot & Monniot (1991a) mentioned in their discussion photographs of a *Culeolus* species with three gonads from Californian coasts which at that time were never collected.

Culeolus murrayi was originally described from the NW Pacific, west of Japan, 35°41'N, 157°42'E, 4186 m. Subsequently Kott (1969) identifies several *Culeolus* species from the Antarctic as *C. murrayi* and Vinogradova (1970) described many specimens from Kuril-Kamchatka Trench under this name. Both of these records are based on incorrect identifications: Monniot & Monniot (1982) examined the holotype of this species and showed that it has no relation to any Antarctic species, and Sanamyan & Sanamyan (2006) showed that the specimens identified by Vinogradova (1970) would be more accurately assigned to *C. suhmi*, which is very similar to *C. murrayi,* except that it usually has two gonads on each side; the holotype of *C. murrayi* has three. Externally *C. murrayi* is very different from *C. barryi* **n. sp.**, as it has a smooth leathery test with a complete ring of papillae encircling the posterior end of the body. According to Monniot & Monniot (1982, p. 117) the posterior crest "is not made of a single row of papillae, but a ring which constitutes a kind of beard". This is clearly shown on the original figure of Herdman (1882, Pl. 8, Fig. 1).

Culeolus sluiteri was described from the Aleutian Islands, at 515 m depth. The original figure of the preserved specimen (Ritter, 1913, Pl. 34, Fig. 14) resembles preserved specimens of *C. barryi* **n. sp.** externally: the test is opaque, greatly folded and wrinkled, with numerous irregular papillae. As in *C. barryi* **n. sp.** the mid-dorsal crest is not discernible and postero-ventral crest is poorly defined (but may be recognized). Ritter's (1913) description of the gonads is puzzling; he misinterpreted a commensal nemertean worm as an ovary, but it appears that the species has one gonad on the right and three on the left, and all left gonads are within the gut loop. The main difference separating it from *C. barryi* **n. sp.** is the number of branchial folds: Ritter (1913) reported only five folds in his specimen. We do not think this is an incorrect observation as Ritter (1913) was aware that most (although not all) *Culeolus* species have six branchial folds and used this feature to separate his species. Further, the numbers of longitudinal vessels on the branchial folds reported by Ritter (1913) are rather high (higher than in *C. barryi* **n. sp.**) and this excludes the possibility that the branchial sac was imperfectly developed.

Culeolus tenuis was originally described from Kuril-Kamchatka Trench at 5035–6282 m, where it is common (Vinogradova, 1970). Sanamyan & Sanamyan (1998) recorded it in the Aleutian trench. Material of Vinogradova (1970) was reexamined and redescribed by Sanamyan & Sanamyan (2006). The external appearance of this species differs significantly from *C. barryi* **n. sp.** It has smooth leathery test without any traces of papillae, but with a well-defined lamellar postero-dorsal crest forming an open arc (see Sanamyan & Sanamyan, 2006, Fig. 12). This species has three gonads on each side. Two of the left gonads are outside the gut loop and one is inside.

Culeolus nadejdae was described from the Sea of Okhotsk, at 1050–1040 m. Internal features, especially the number and position of the gonads are very similar to those of *C. barryi* **n. sp.** There are minor differences, for which the significance is hard to establish having limited specimens of both species: the number of the branchial tentacles is 27 in *C. barryi* **n. sp.** while only 14 were reported in *C. nadejdae*, the horns of dorsal tubercle are turned inside in C. *nadejdae*, but outside in *C. barryi* n.sp. The test surface of *C. nadejdae* was described as

"smooth, without folds or wrinkles" (Sanamyan, 1992). In general, the texture of the test surface in *Culeolus* (unlike many other solitary ascidians) does appear to be a rather stable species-specific feature. Having a limited number of specimens collected in geographically distant locations (off California and in the Sea of Okhotsk), we prefer not to lump them together and thus describe the Californian specimens as a new species.

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