

Adaptations for living deep: a new bathypelagic doliolid, from the eastern North Pacific

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Adapting to the bathypelagic habitat imposes serious challenges for taxa that originate in shallower depths. We describe a new doliolid that has successfully made the transition into deep water. Nine specimens of *Pseudusa bostigrinus* have been found at depths between 1164 and 1890 m in three distinct regions of the eastern North Pacific. This new thaliacean has exchanged the typical doliolid body plan for one resembling a craspedote hydromedusa. This adaptation allows it to collect sinking particles by simply directing its large buccal opening upward. The development of a hydromedusa-like velum allows it to trap zooplankton prey and to propel itself with considerable force and control. While carnivory is not unknown in tunicates, this is the first report of a carnivorous doliolid. The endostyle of *P. bostigrinus* is greatly reduced, there are no ciliated bands, and there is no spiral gland; all evidence that mucus feeding filters have been abandoned by this species. Anatomy, diet, behaviour, and habitat distinguish this doliolid from all others described to date.

INTRODUCTION

Increasing access to deep pelagic habitats continues to reveal remarkable and unexpected life forms. Even in Monterey Bay, which may be the best examined pelagic region on Earth, we regularly encounter exotic higher taxa. This is particularly the case for the gelatinous fauna that for so long were routinely rendered into anatomical ambiguity by conventional sampling gear. With the advent of undersea vehicles, our appreciation of the importance and diversity of this component of the pelagic ecosystem, has grown substantially. These discoveries expand our understanding of faunal diversity, but their greatest value may be what the morphology and natural history of these animals can tell us about the ecology of the deep pelagic regions, the largest ecosystems on the planet (Robison, 2004).

Doliolids are known chiefly from euphotic depths in coastal regions. They are typically barrel-shaped suspension feeders at the second trophic level, who use ciliary action to pass water and particles into an internal mucus trap. Two mucus sheets, produced by the endostyle, intercept the particles and are wound together by the characteristic spiral gland, or volute, that channels them into the esophagus. Doliolids consume nanoplankton, microplankton and particulate

detritus, and their faecal pellets contribute significantly to vertical carbon flux out of the ocean's upper layers (Madin & Deibel, 1998; Deibel, 1998; Gibson & Paffenhöfer, 2000; Bone et al., 2003). This paper describes a remarkable new doliolid that is distinctly different from all other known doliolid forms in its anatomy, habitat, diet, and the way that it feeds.

The most recent revisions of the thaliacean order Doliolida (Godeaux, 1996; 2003; Godeaux & Harbison, 2003) divide the group into two suborders, with the principal distinctions being the number of muscle bands and the shape of the body. The suborder Doliolidina includes those species with cylindrical bodies and eight muscle bands (nine in old nurse cells). The suborder Doliopsidina encompasses globular forms with five muscle bands. Each suborder presently contains two families. In the five-banded Doliopsidina are the families Doliopsidae with two genera and three species, and the Paradoliopsidae with a single genus and one species (Godeaux, 2003; Godeaux & Harbison, 2003; Robison et al., this issue).

MATERIALS AND METHODS

Specimens of the new doliolid were collected with 7.5 litre 'detritus' samplers (Youngbluth, 1984; Robison, 1993) mounted on MBARI's deep diving,

remotely operated vehicle, Tiburon. In each instance, the individual was observed and recorded *in situ* with a high-resolution, colour digital-beta video system, prior to being collected. Captured specimens were examined, while still alive, in the laboratory aboard the R/V 'Western Flyer'. Feeding studies were conducted in a temperature-controlled, environmental chamber at sea. Digital images of specific anatomical elements were examined and recorded from both living and preserved material. We observed nine individual zooids *in situ* and collected six of them for further investigation. In the shipboard laboratory, specimens were maintained at *in situ* temperatures (3–4 °C) and in the dark, except when being examined.

Video sequences recorded at depth by MBARI's ROVs are preliminarily annotated in real time, and then again in detail ashore, after the dive. Video and environmental data are archived in a database that can be accessed for individual or cumulative information on: depth of occurrence, hydrographic conditions (e.g. temperature, O₂ level, salinity, etc.), hydrographic season, and co-occurring species. A video information management system allows searches within the database for specific annotation terminology and for the ready retrieval of video sequences for examination. These systems allowed us to re-examine our field observations, select individual video frames and series for inspection of structure and movement, and to search the database for patterns of occurrence.

RESULTS

We encountered individuals of this new doliolid on nine occasions between 2001 and 2004. All were observed singly, and within the depth range of 1164 to 1890 metres. Four specimens were seen at our deep, midwater survey site off Monterey Bay, California; one was found in the waters of the Pescadero Basin in the Gulf of California, Mexico; and four occurred west of the California Current, on a transit between Monterey Bay and Hawai'i. The size range of the specimens was between 5.1 and 6.6 cm in greatest dimension, which for this species is height (equivalent to the maximum diameter of the buccal opening). Six of the nine individuals observed had attached buds. All of the specimens appear to be phorozoids.

SYSTEMATICS

Class THALIACEA

Order DOLIOLIDA Delage & Herouard, 1898

Suborder DOLIOPSISIDINA, Godeaux, 1996

Family DOLIOLUNIDAE fam. nov.

Pseudusa gen. nov.

bostigrinus sp. nov.

(Figures 1–3)

Type material

The holotype and two paratypes are deposited in the California Academy of Sciences, Department of Invertebrate Zoology & Geology collection, CASIZ

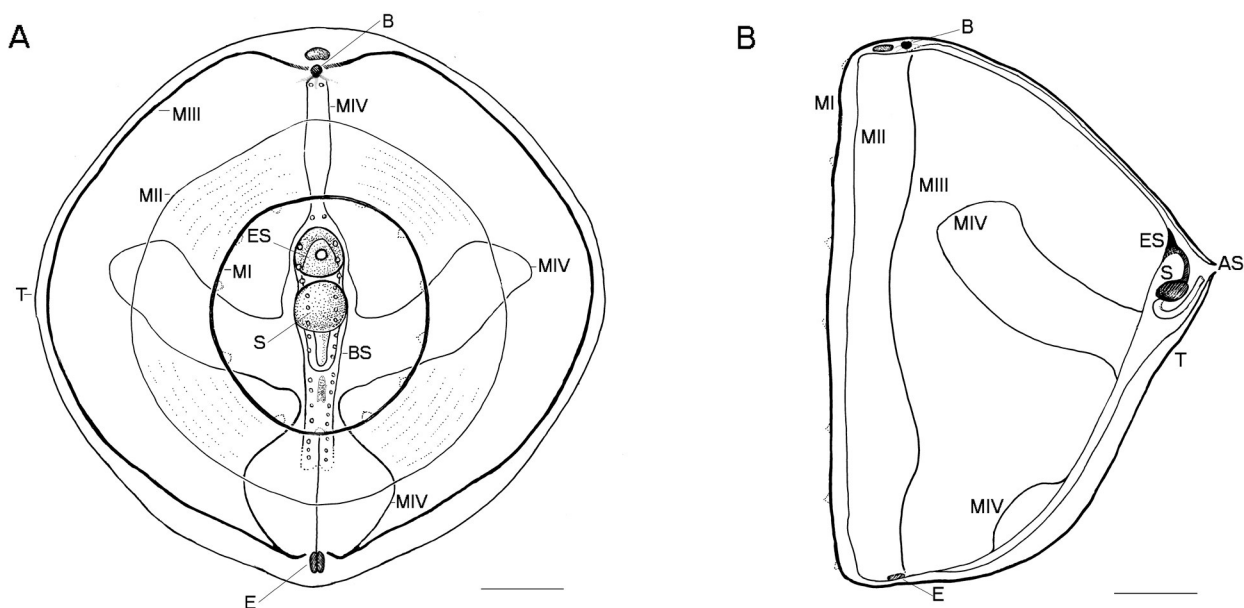


Figure 1. Line drawing of *Pseudusa bostigrinus*. (1A) frontal view with the buccal aperture half open; (1B) lateral view with the buccal aperture wide open. AS, atrial siphon; B, brain; BS, branchial septum; E, endostyle; ES, esophagus; I, intestine; M I–IV, first through fourth muscle bands; S, stomach; T, tunic. Scale bars: 1 cm.

numbers 170948-170950. Type locality was MBARI's deep (3500 m) midwater site at 36° 18'N 122° 54'W; off Monterey Bay, at a depth of 1583 m (Tiburon dive #687, 6/8/04). One paratype was collected at 21° 26'N 158° 24'W, at 1408 m, near Hawai'i (Tiburon dive #271, 3/27/01), and the second was collected at 24° 18' N, 109° 12'W in the Gulf of California, at 1514 m (Tiburon dive #541, 3/24/03). Both of the paratypes have well-developed buds.

Etymology

The familial name, Doliolunidae, reflects the moon-like shape of this new form. The generic name *Pseudusa* stems from the false impression this animal gives, from both its behaviour and configuration, that it is a medusa. The specific name refers to the unusual, carnivorous nature of this doliolid, a cow that became a tiger.

Diagnosis

Pseudusa bostigrinus (Figure 1) is a bell-shaped (medusoid) doliolid with four muscle bands. The buccal opening is very large and the atrial opening is quite small. The pharyngeal chamber comprises almost the entire interior of the animal. The atrial chamber is a small capsule containing the gut, with narrow dorsal and ventral extensions running forward, to the mid-body. The endostyle is very small and the spiral organ, typical of all other known doliolids, is absent. The branchial septum and gill slits are greatly reduced and lie in a narrow strip along the centreline of the pharyngeal chamber. Ciliated peripharyngeal bands are absent. Overall, this animal is highly modified from the typical doliolid form.

Description

All known specimens have occurred as solitary individuals, the majority with buds. Living specimens have a medusoid shape yet are bilaterally symmetrical. The buccal opening is very large and highly elastic, capable of expanding to the full height (diameter) of the animal, and of contracting to less than 5% of height. The margin of the opening has 12–13 small, widely spaced, triangular lobes. The pharyngeal chamber is also very large, equal to about 90% of the interior volume. The atrial chamber is correspondingly small, just large enough to surround the gut. The atrial opening is very small and without apparent lobes, although the upper and lower margins have a V-shaped notch. The tunic is thin, particularly near the buccal and atrial openings.

Three of the four muscle bands are annular and encircle the anterior portion of the body (Figure 2A). Muscle I is thick and serves as a sphincter for the buc-

cal opening. Muscle II is slender and located just posterior to muscle I when the body is relaxed. The tissue between muscles I and II is highly elastic and functions like the velum of hydromedusae. When contracted, it folds into annular pleats; when extended, it contorts longitudinally. Muscle III is a broad, ribbon-like, circular band that is discontinuous both dorsally and ventrally.

Muscle IV runs a convoluted course along the ventral, lateral, and dorsal walls of the pharyngeal chamber (Figures 1A & 2B). It begins as a slender branch of muscle III, on either side of the ventral gap. From each side, it runs laterally beside muscle III for a short distance then sweeps outward in an arc that brings it back toward the ventral midline. The band continues posteriorly to the rear of the chamber, then curves up and outward again to form a large loop along the lateral wall. The loop returns toward the center of the chamber then turns again and runs anteriorly along its dorsal arc toward the buccal opening, and ends just before reaching the dorsal gap in muscle III.

The endostyle is very small and lies just posterior to the ventral gap in muscle III. The branchial septum common to other doliolids is reduced to a narrow strip along the midline of the pharyngeal chamber (Figure 2C). It runs from behind the endostyle to the rear of the chamber and upward in front of the gut, where it bears the opening to the esophagus. Then it continues up along the dorsal arc of the chamber, ending just behind the brain. The ciliated gill slits usually found on the septum are limited to two series of up to 26 circular holes in the ventral and central portions of the strip. Beneath the ventral portion of the strip is a shallow pouch that runs posteriorly and connects to the atrial chamber, just below the gut. A tubular structure, which may be the heart, occupies the posterior region of the pouch.

The two sections of muscle IV that run anteriorly from the gut and along the dorsal arc toward the brain, border the dorsal portion of the vestigial branchial septum. The narrow septum encloses a soft tube that connects with the atrial cavity just above the gut. At the anterior end are two circular holes, widely separated yet clearly part of the series of perforations in the central and ventral parts of the septum.

The gut is located behind the narrow, perforated branchial septum, near the posterior apex of the bell (Figures 1B & 2C,D). The gut has a sigmoid shape. A broad collar, without a spiral whorl, surrounds the opening of the esophagus, which faces anteriorly. There is a long neck that travels posteriorly and then down into the elongate stomach. The intestine curves downward and then upward, ending in the anus, which empties posteriorly out into the atrial chamber, next to the small atrial siphon.

The brain is relatively large (about 430 μm). It sits in the dorsal gap of muscle III with multiple nerve fibres radiating outward (Figure 2E). Anterior to the brain is a rounded translucent patch consisting of a single layer of granular, cell-like tissue. Anterior to the patch is a small trumpet-shaped structure, connected to the patch and brain by neurons. In small buds there is a pronounced anterior process of the brain, which flares out at its terminus and then pinches off as the bud grows larger. The patch appears between the trumpet-shaped structure and the brain, and occurs only in free-swimming adults. The function of these structures is unknown.

Buds

Gonads were not apparent in any of the adult specimens we examined. However, six of the nine individuals had buds growing from a ventro-caudal peduncle, just below the gut (Figure 2F). The largest of these buds pulsed occasionally, independent of the parent, while all of the smaller buds were immobile. The buccal openings of the smaller specimens were clearly plugged with transparent tissue. Three of the adult specimens had two buds present, one large and one small.

In the buds, a thin strip of tissue runs anteriorly from the gut to the endostyle (Figure 2B). The strip divides and flanks the endostyle then each branch curves up one side of the pharyngeal chamber and they meet again near the brain. Unlike the muscle bands, which are clearly within the tissue of the body wall, the strip appears on the inner surface of the pharyngeal chamber. We assume that these strips are vestiges of the ciliated, peripharyngeal bands found in all other doliolids, and while faint traces of them may be seen in some adults, they do not appear to be functional.

Laboratory observations

One living specimen, examined immediately after capture, was observed to have a crustacean in its gut,

located just beyond the stomach. Over a period of several hours this prey item moved slowly through the intestine and eventually disappeared. We later presented this same doliolid with a copepod, placed into the pharyngeal chamber with forceps. The copepod adhered to the area around the opening of the esophagus, probably due to mucus, and was ingested. During the next few hours the copepod passed slowly through the stomach and intestine in a manner similar to the naturally-ingested prey. A second doliolid, presented with a euphausiid, trapped it with mucus and moved it to the opening of the esophagus, but did not ingest the prey before the experiment had to be terminated.

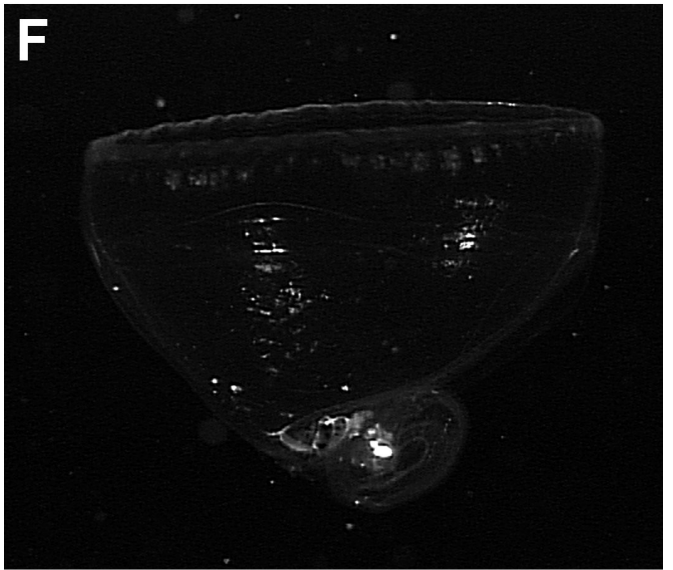
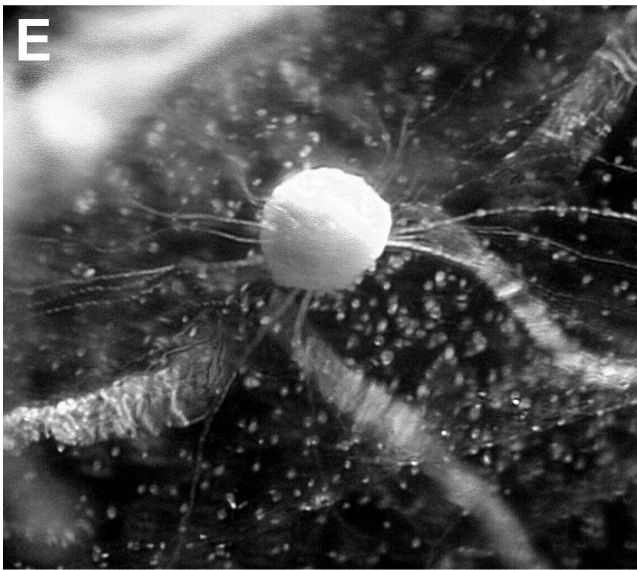
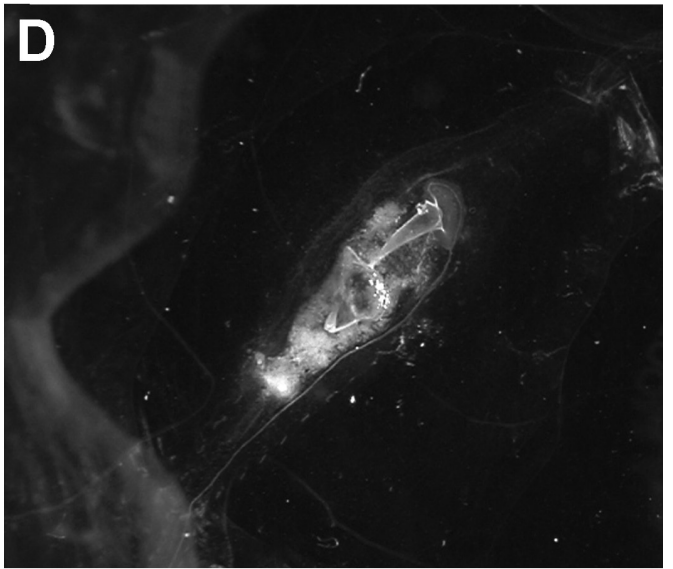
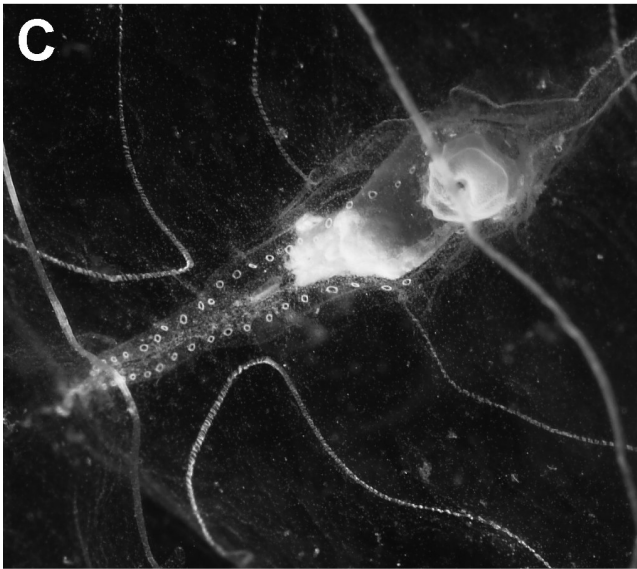
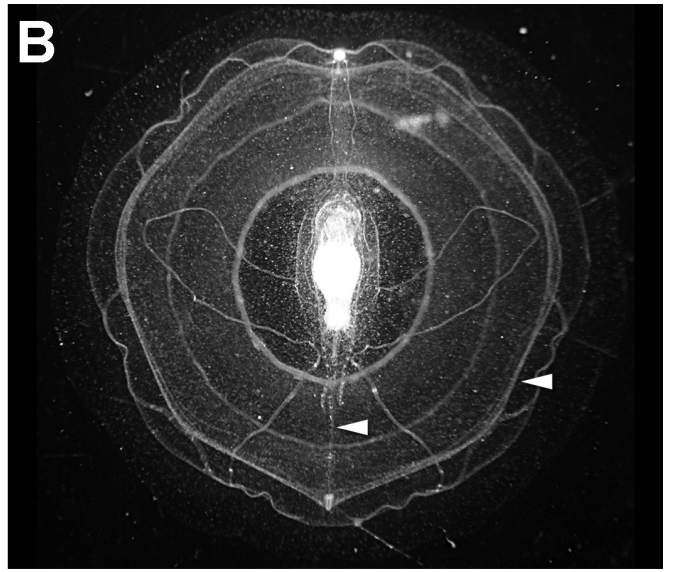
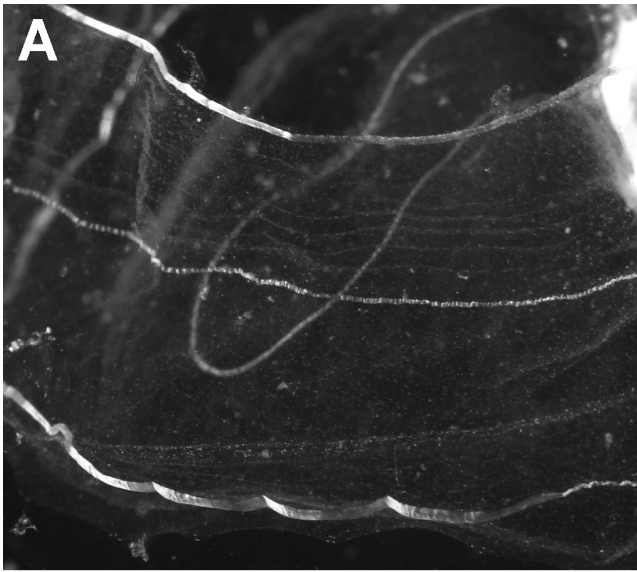
We examined the stomach contents of a third specimen and found a variety of crustacean remains including eyes, legs, antennae, and pieces of carapace. In addition, the stomach contained several eggs, faecal pellets, protists, and a volume of flocculent material that may be aggregated marine snow. Other than the brain, the stomachs of *Pseudusa* are its only pigmented structures. Colours ranged from pale yellow to orange and dark red. Atypical of most doliolids, faecal pellets have not been observed in the intestines of any specimens of *Pseudusa*.

In situ observations

In six of the seven instances when we came upon undisturbed individuals, they were motionless, stationary, and oriented with the buccal siphon wide-open and directed upward (Figure 2F). In this position *Pseudusa* is able to intercept sinking particles without the typical doliolid energy expenditure of ciliary action to create water flow through the body and mucus filter. When live prey is encountered the buccal opening can be snapped shut very quickly (<650 msec) to enclose active prey and prevent escape.

Pseudusa propels itself through medusoid pulsations of its bell-shaped body and by constriction of the buccal aperture (Figure 3). In a fully relaxed state, the pharyngeal chamber is expanded, with the buccal

Figure 2. (*Opposite*) *Pseudusa bostigrinus*. (A) frontal view of muscles I, II, and III respectively, arcing from the top to the bottom of the frame. Concentric pleats are visible in the velum-like tissue between I & II. A portion of muscle IV appears as a loop in the background. (B) frontal view of a separated bud, showing all three annular muscle bands and the full configuration of muscle IV. The brain is at the top, the gut in the centre, and the endostyle at the bottom. The arrows point to the thin strip of tissue that may be a vestige of the peripharyngeal bands found in other doliolids. In buds, muscle III is typically convoluted, as it is here. (C) frontal view of the branchial septum, its perforations, and the esophageal opening to the gut. (D) frontal view of the gut showing the atrial capsule, the esophageal collar and neck, the stomach, and the intestine. (E) dorso-posterior view of the brain and its radiating nerve fibers. Running diagonally upward, right to left, are the parallel, dorsal bands of muscle IV, near their anterior terminus. Arcing downward from right to left is muscle III, showing its dorsal gap at the brain. (F) *in situ* photograph of an adult phorozooid with a well-developed bud. This individual is in the typical orientation, with the buccal aperture open wide and directed upward.



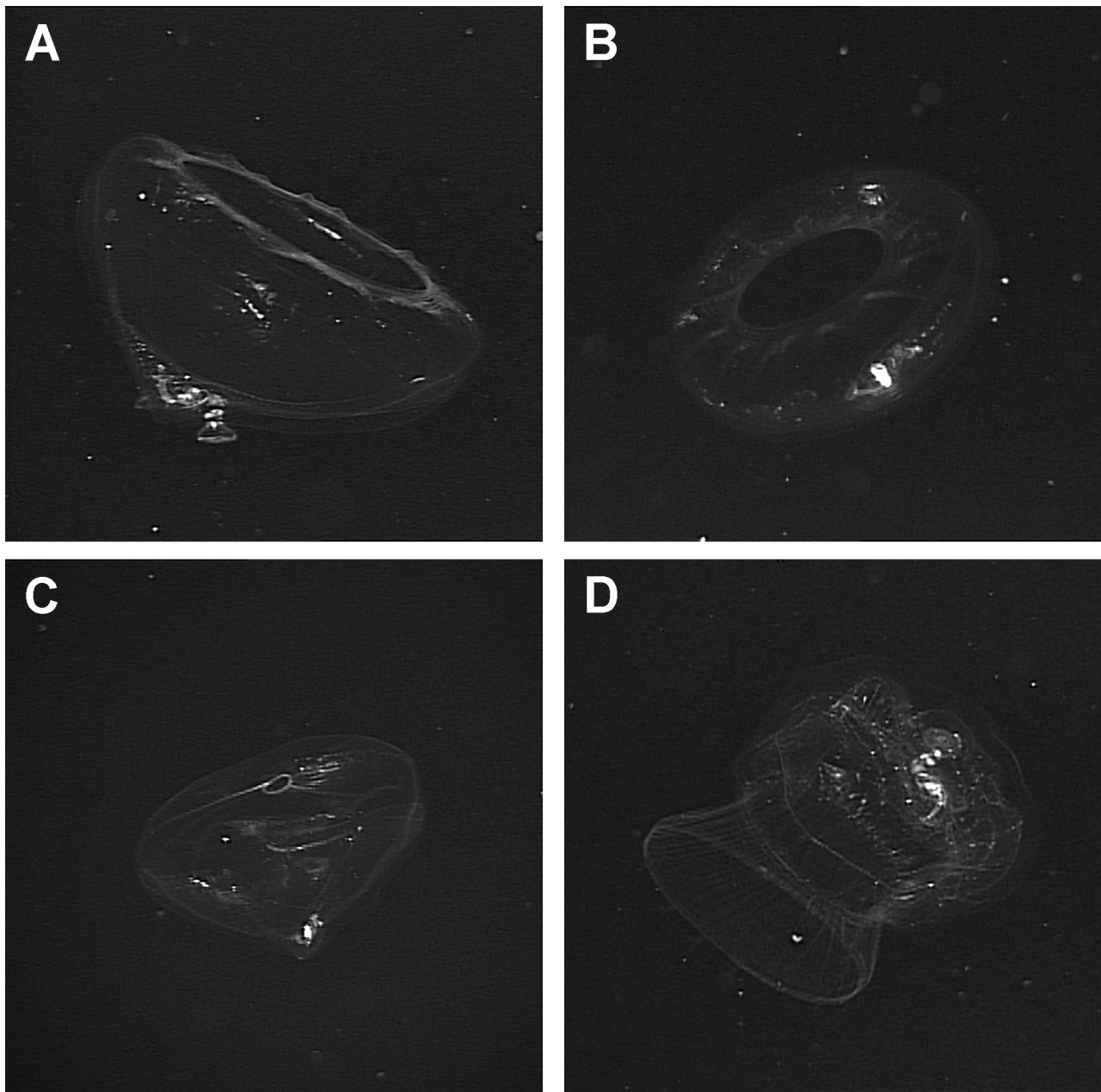


Figure 3. *Pseudusa bostigrinus*. (A) specimen with the buccal aperture beginning to close. A small bud is located ventral to the atrial chamber and gut. (B) a second specimen, with the buccal aperture about half-closed. (C) specimen with the buccal aperture fully contracted. Even when muscle I is fully cinched, there is always a small opening. (D) specimen swimming actively, with the body contorted by the contracted muscle IV, and the elastic tissue between muscles I and II forming the velum-like structure in which longitudinal pleats are evident.

aperture open wide. The unique shape of muscle IV provides both longitudinal (radial) and circular (circumferential) contraction of the pharyngeal chamber, forcing water out of the concavity. The annular muscles (I–III) assist with the expulsion and increase its velocity by reducing the size of the buccal aperture. This creates a propulsive jet that pushes the animal backward. During contractions the body wall contorts between the lateral extensions of muscle IV, and after

contraction the original shape is restored. During strong pulsations, the velum-like tissue between muscles I and II forms an elongate, narrow nozzle that trails behind the animal (Figure 3D).

Supplemental figures and video images are posted at: <http://www.mbari.org/midwater/pseudusa>

DISCUSSION

Contrary to all other known pelagic tunicates (Madin & Deibel, 1998), *Pseudusa bostigrinus* has abandoned filter feeding. Its apparent feeding mode is to direct its broad buccal opening upward to intercept sinking particles and to use its elastic buccal opening to entrap living zooplankton that enter the bell. In this respect they resemble some benthic tunicates, ascidians like *Megalodicopia hians* Oka, which have eschewed suspension feeding, ceded their mucus filter nets, and become macrophagous carnivores (Monniot & Monniot, 1978; Bone et al., 2003). *Pseudusa's* vestigial endostyle, branchial septum, and gill slits are clear evidence that it has relinquished the typical doliolid feeding mechanism. Likewise, the undeveloped peripharyngeal bands seen in the buds and the trumpet-shaped structure, which may be an uncompleted spiral gland, reflect its evolutionary past.

The most striking adaptation by *Pseudusa* to its bathypelagic lifestyle is the re-shaping of its body to that of a craspedote hydromedusa and the consequent change in its mode of propulsion. Medusae are archetypal gelatinous predators and thus it is not too surprising that the medusoid form was achieved, particularly when we note that an inverted, bell-shaped body also serves well for collecting settling particles. In medusae, the velum shapes the subumbrellar aperture, thus controlling the water jet produced by bell contractions during propulsion (Colin & Costello, 2002). In *Pseudusa*, the muscle bands and tissue at the buccal opening form an analogous structure that clearly enhances the doliolid's swimming abilities. In addition, the utility of this structure to quickly seal off the pharyngeal chamber and trap prey, goes beyond the capabilities of any medusa's velum.

In general, bathypelagic animals have reduced metabolic rates when compared to their shallow-living counterparts. This trend has been attributed to decreases in temperature and food, and to reduced pressure from visually-cued predators (Childress, 1995). *Pseudusa* also shows a reduced level of activity when compared with its shallower relatives. Doliolids that live higher in the water column continually pass water through the body and produce mucus filters to trap particles. The constant ciliary activity necessary to sustain this action must be considerably more costly in energetic terms than waiting quietly for food to drop in. In addition to its anatomical adaptations to depth, *Pseudusa* has adopted an energy conserving, sit-and-wait feeding strategy, like anglerfish, lobate ctenophores, and many other bathypelagic predators.

Additional steps in *Pseudusa's* life cycle are still unknown. Because they are budding and no gonads

are present, we assume that we have encountered phorozoids. The absence of incipient gonads in even the largest buds suggests that they may also be phorozoids. Whether or not this is a continuing cycle remains to be seen. The shallow water doliolid *Doliolum nationalis* Borgert exhibits a 'short cycle' reproductive adaptation in which phorozoids bud new phorozoids in response to favourable conditions (Godeaux et al., 1998). At bathypelagic depths where meals may be few and far between, this reproductive strategy may also be advantageous.

Pseudusa is apparently widespread, it occurs in all three regions of the eastern North Pacific where we have deployed the ROV 'Tiburon' for midwater work at depths below 1000 m. While conditions near the surface may vary considerably, at bathypelagic depths there is relative uniformity (Angel, 2003; Robison, 2004). Temperatures at the capture depths were all within a narrow range between 2.2° and 3.4°C, and in each case the animals were well below the core of the regional oxygen minimum, at concentrations above 0.5 ml/L.

While we are convinced that *Pseudusa bostigrinus* is indeed a doliolid, it is so different from all others that we must recognize the possibility that it might be something else. Carnivorous, benthic tunicates like *Situla* and *Megalodicopia*, and the deep-living Sorberaceans share a number of similar modifications with *Pseudusa* (Monniot & Monniot, 1978). Given the evolutionary plasticity of these animals, we might just be dealing with an ascidian that has slipped its leash. We have placed *Pseudusa* in the *Doliopsidina* because of its reduced number of muscle bands, and its greater difference from the barrel-shaped forms than from the globular species. The puzzle of its origins, and the question of whether the new form deserves subordinal status, should yield to a molecular approach.

We thank Kim Reisenbichler, Steve Haddock and Karen Osborn for their assistance at sea and ashore. The skilled pilots of the ROV 'Tiburon' not only collected these tiny animals while flying through 3-dimensional space at the end of a 2 kilometer string, they also gave us the time we needed for undisturbed observations *in situ*. We also thank the captain and crew of the R/V 'Western Flyer' for our lab and home base at sea. Kirsten Carlson created the line drawings of *Pseudusa*. Supported by the David and Lucile Packard Foundation.

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