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Ecological substrate in midwater: *Doliolula equus*, a new mesopelagic tunicate

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In the pelagic habitat, the bodies of resident animals provide much of the ecological substrate available for other organisms to attach, find shelter, and seek food. In Monterey Bay, California, the doliolid *Doliolula equus* provides substrate for multiple symbionts. These include a mutualist hydroid, commensal ciliates, and a parasitic amphipod. This new doliolid is described based on *in situ* observations from a remotely operated vehicle, and from the laboratory examinations of 43 colonies comprising hundreds of living, individual blastozooids. *Doliolula equus* differs from other members of the suborder Doliopsidina in the shape of its body, the length and configuration of its third muscle band, the size of its buccal siphon, and the position of the spiral gland. The new doliolid was found principally at depths between 300 and 400 m. This species is bioluminescent, hermaphroditic, and about one zooid in ten is peppered with orange pigment spots. A variety of other, obviously related, yet undescribed forms has been observed in the eastern Pacific.

INTRODUCTION

Increased access to mesopelagic and bathypelagic depths with manned submersibles and remotely operated vehicles has greatly expanded our understanding of the ocean's interior. One result has been a greater appreciation of this vast habitat's ecological complexity and heterogeneity (Robison, 2004). Another has been the discovery of what seems to be an inexhaustible stream of new species, as well as higher taxa. In particular, gelatinous forms, too fragile or occasionally too large to have been collected intact by nets are turning up with surprising frequency (e.g. Harbison et al., 2001; Matsumoto et al., 2003; Raskoff & Matsumoto, 2004). Likewise, the ability to observe and collect pristine specimens at depth, then bring them back to the laboratory alive and in excellent condition has enabled behavioural, physiological, and process studies that have advanced our understanding of the dynamic aspects of midwater ecology (e.g. Tamburri, et al., 2000; Raskoff, 2002; Robison, et al., 2003; Herring & Widder, 2004).

Doliolids are well known from euphotic depths where they play an important role as secondary producers, and they appear to be widespread in deep water as well (Deibel, 1998; Gibson & Paffenhöfer, 2000). They are among the gelatinous fauna whose ecology in the mesopelagic is still being resolved but it is clear that where abundant, they must be significant consumers of nanoplankton, microplankton and detritus, and substantial contributors to vertical carbon flux (Madin & Deibel, 1998). Doliolids are notorious for the complexity of their life cycle, with alternating sexual and asexual stages and a perplexing polymorphy of zooids, only some of which may be in evidence. Apparently, not all doliolid species utilize the same strategies for reproduction (Godeaux et al., 1998).

This paper describes a new doliolid and outlines its role as ecological substrate for other organisms, in a habitat where traditional benthic concepts of 'substrate' do not apply. Individually and colonially, this species is involved in at least three kinds of symbiosis. It has a mutualistic relationship with a hydroid polyp, usually attached to the doliolid's buccal opening (Raskoff & Robison, 2005). It has a commensal interaction with a population of ciliate protists, who occupy a haemocoel sinus covering its endostyle (the ciliates themselves are hosts to bacteria and flagellates). And finally, the doliolids are commonly parasitized by a hyperiid amphipod. Apart from its symbionts, the new doliolid itself is distinctly different from other described forms but is only one of a number of undescribed thaliaceans we have observed *in situ*.

Godeaux (1996) and Godeaux & Harbison (2003) proposed that the order Doliolida be divided into two suborders: the Doliolidina, which includes species with eight muscle bands; and the Doliopsidina, comprising those forms with five muscle bands. Godeaux (2003) revised the order and formalized the division into two suborders. Two families have been included within the five-banded suborder Doliopsidina, each with a single genus described to date. In the Doliopsidae the genus *Doliopsis* has a body about as high as it is long, a short third muscle band, no buccal vestibule, and an apical spiral organ (vibratile organ) behind the brain; two species are recognized. In the Paradoliopsidae the genus *Paradoliopsis* has a body longer than it is high, a long third muscle band, a buccal vestibule, and a spiral organ located to the right of and behind the brain; one species has been described (Godeaux, 2003). Within the suborder Doliopsidina, the asexual oozooid stages are unknown and only the sexual blastozooid stages (trophozooid, gonozooid, phorozooid) have been described.

MATERIALS AND METHODS

Specimens of the new doliolid collected in the type locality of Monterey Bay were acquired with 7.5 litre ‘detritus’ samplers or with variable-flow suction samplers (Youngbluth, 1984; Robison, 1993) mounted on both of MBARI’s remotely operated vehicles, ‘Ventana’ and ‘Tiburon’. In each instance, the colony was observed and recorded with a high-resolution, colour video system prior to being collected. In most cases, the specimens were returned to our laboratory ashore, where they were observed under microscopes while images of specific anatomical elements were recorded digitally and on video tape. Most of the examinations were made of specimens that were alive rather than preserved. None survived longer than four days.

From 1995 to 2004, we examined approximately 43 colonies and 320 individual zooids in the laboratory ashore. In the laboratory, specimens were maintained at *in situ* temperatures (6° to 8°C) and in the dark except when being examined. We used flourescein dye and suspensions of carmine particles to investigate water flow patterns and the actions of internal feeding structures.

In situ video recordings from MBARI’s ROVs are first annotated in real time, and then again in detail after the dive. These data are archived in a database that can be accessed for individual or cumulative information on: depth of occurrence, hydrographic conditions (e.g. O_2 level, temperature, salinity, etc.), hydrographic season, and co-occurring species. A video information management system searches for specific annotation terminology and allows the ready retrieval of video sequences for examination. These systems allowed us to re-examine our field observations, select individual video frames for counts and identification, and to search the database for depth ranges and patterns of occurrence.

RESULTS

In the field, we encountered approximately 175 colonies of an undescribed doliolid during 167 of 311 ROV dives between 1995 and 2004 in Monterey Bay. Most of these colonies were found at depths between 80 and 550 m and about half of the sightings occurred between 300 and 400 m. The number of individual zooids per colony ranged from 6 to 232, but a typical colony had 10 to 20 zooids that were mature enough to be feeding. The size of the zooids, measured from buccal siphon to atrial siphon, averaged 11.8 mm and ranged from about 3 mm to 22 mm in typical colonies. Colony length averaged about 10 cm with a range of 4 cm to 450 cm.

SYSTEMATICS

Class THALIACEA
Order DOLIOLIDA Delage & Herouard, 1898
Suborder DOLIOPSIDINA, Godeaux, 1996
Family DOLIOPSIDAE (ANCHINIDAE), Godeaux, 1996
Doliolula gen. nov.
equus sp. nov.
(Figures 1–5)

Type material

The holotype and six paratypes (all gonozooids) are deposited in the California Academy of Sciences, Department of Invertebrate Zoology & Geology collection, CASIZ nos. 168926–168930. Type locality was MBARI’s midwater time-series site at 36°42'N, 122°02'W; over the axis of the Monterey Submarine Canyon, where the water column depth is 1600 m. Capture depth for the holotype and three of the paratypes was 160 m (Ventana dive no.2557, 12 August 2004). Three paratypes were collected at 348 m (Tiburon dive no.645, 2 October 2003, at 36°20'N, 122°53'W).

Etymology

The generic name *Doliolula* was selected by the pilots of the ROV ‘Ventana’ for its euphony. The species name *equus* (Latin for horse) refers to the role of this pelagic animal as a mobile steed for its symbiotic rider, a hydrozoan polyp.

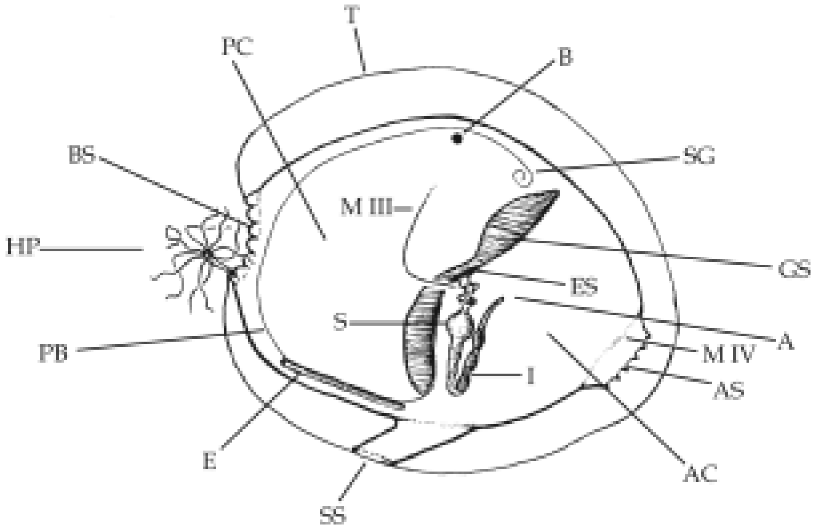

Figure 1. Line drawing of a trophozooid of *Doliolula equus* in lateral view. A, anus; AC, atrial cavity; AS, atrial siphon; B, brain; BS, buccal siphon; E, endostyle; ES, oesophagus; GS, gill slits/branchial septum; HP, hydroid polyp; I, intestine; M III, third muscle bands; M IV, fourth muscle band; PB, peripharyngeal bands; PC, pharyngeal cavity; S, stomach; SG, spiral gland; SS, stalk to stolon; T, tunic.

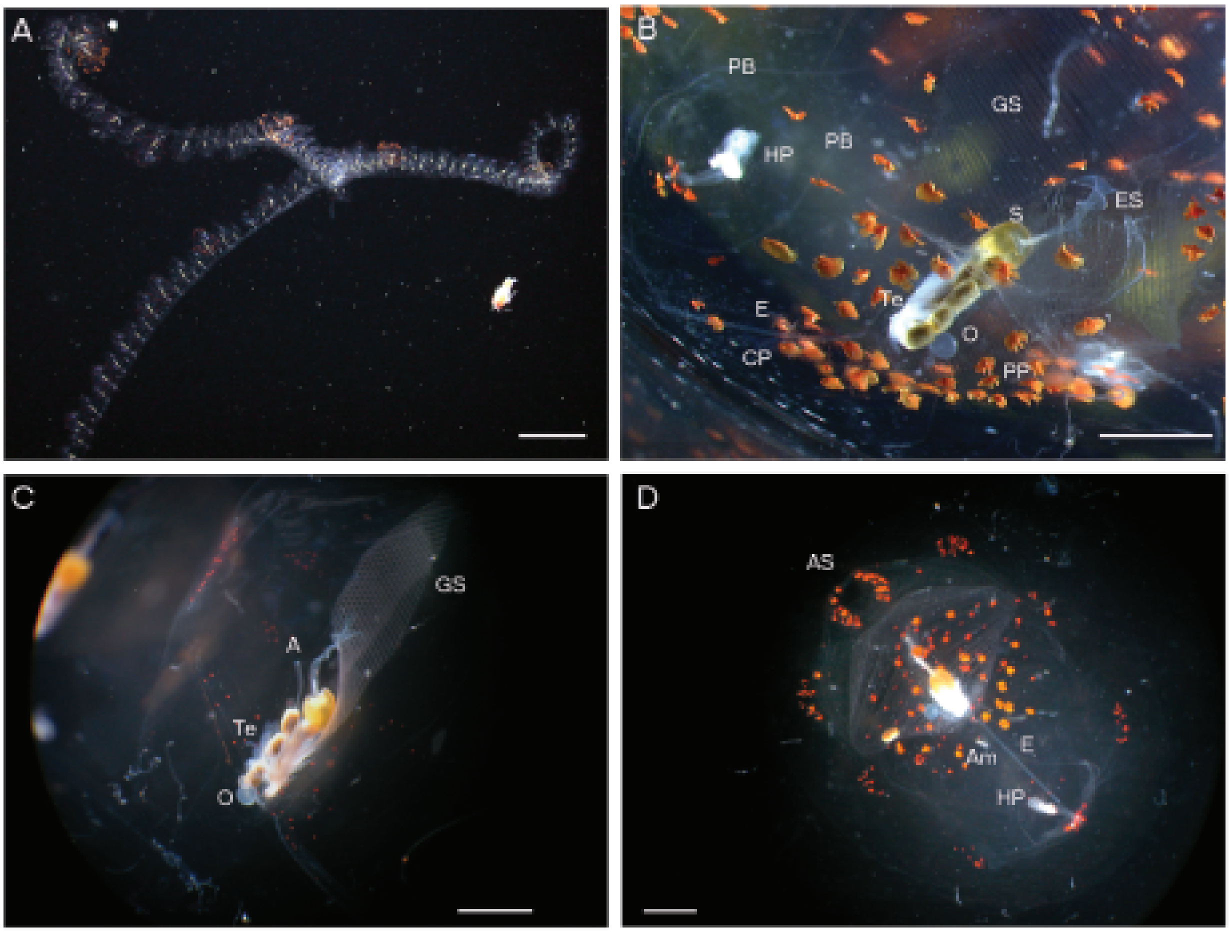

Figure 2. *Doliolula equus*. (A) A chain of zooids *in situ*, is looped on itself; (B) interior structures, viewed from below and left; (C) gut, viewed from the right side, showing well-developed oocytes and testis; (D) dorsal view showing positions of pigment patches and a hyperiid amphipod to the left of the endostyle. A, anus; Am, amphipod; AS, atrial siphon; CP, ciliate protists; E, endostyle; ES, oesophagus; GS, gill slits/branchial septum; HP, hydroid polyp; O, oocyte; PB, peripharyngeal bands; PP, pigment patches; S, stomach; Te, testis. Scale bars: A, 5 cm; B–D, 5 mm.

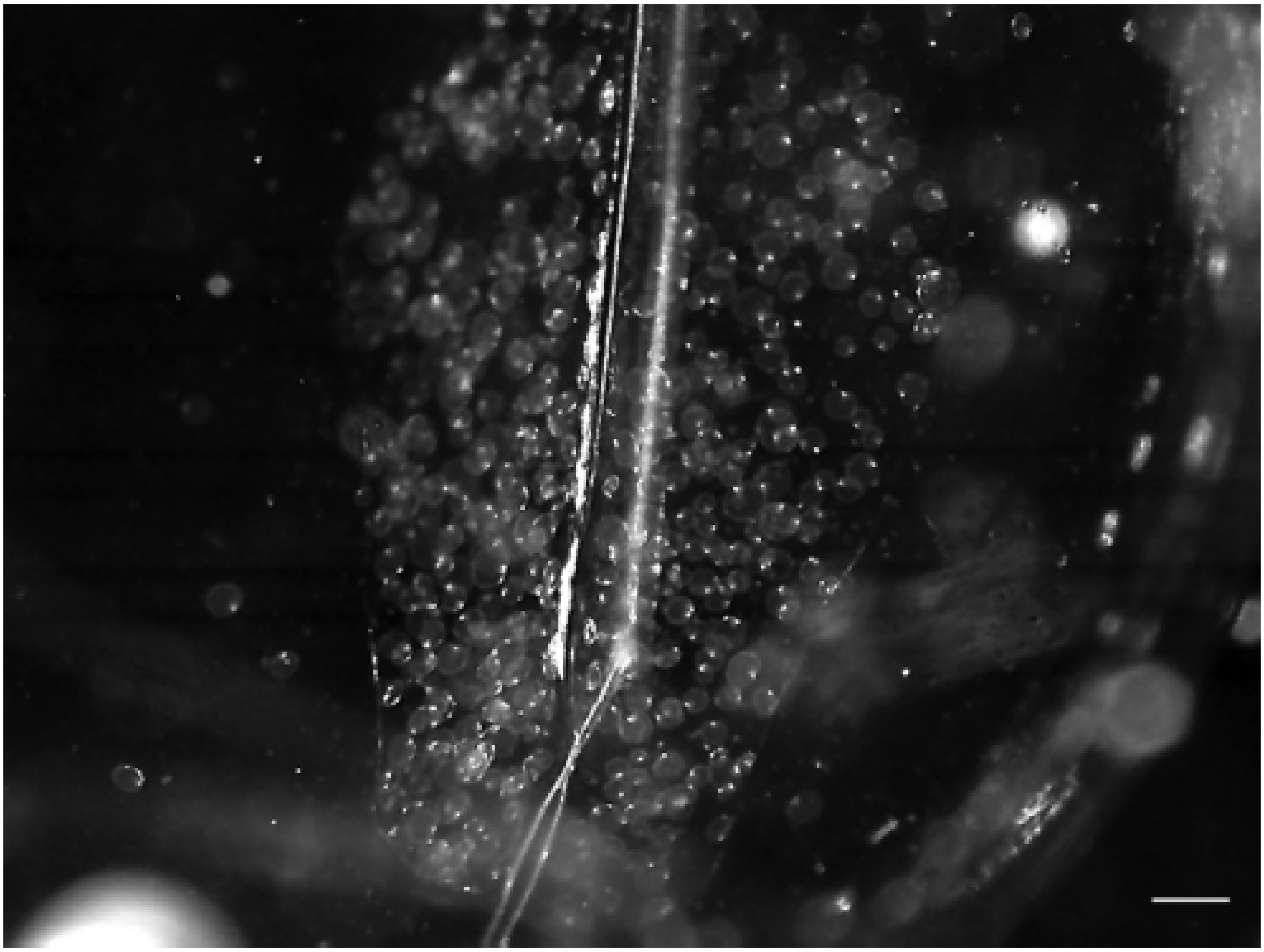

Figure 3. *Doliolula equus*; anterior portion of the endostyle, showing the peripharyngeal bands, and the overlying pouch filled with ciliate protists. Scale bar: 0.5 mm.

Diagnosis

Doliolula equus is an ovate form of doliolid, with five girdling muscle bands. The third band consists of two, L-shaped segments that are not connected to each other dorsally or ventrally. All other muscle bands are complete rings. The spiral organ is posterior to the neural ganglion (brain), the endostyle is long, and the gut is U-shaped (Figure 1). As is the case with other species in the suborder Doliopsidina (Godeaux, 2003), only blastozooids are known. Individual zooids occur along the stolon (lace) without apparent pattern to their size or type. Gonozooids are hermaphroditic.

Description of blastozooids

Trophozooids

All specimens occurred initially as chain-like colonies of zooids (Figure 2A), each attached to a common stolon by a stalk protruding from the ventral portion of the body, beneath the gut. Living specimens have a nearly spherical shape, slightly longer than high, with the keel-like endostyle shaping the anterior ventral surface beneath the pharyngeal chamber. The tunic is thick, except in the area of the buccal siphon (branchial aperture).

Upper and lower portions of the buccal siphon are separated by an enlarged lobe (flap) on each side. Along the upper arch there are seven adjacent lobes, while the lobes of the lower arch usually number five. The medio-dorsal and medio-ventral lobes are largest. The lower arch typically bears a symbiotic, hydroid polyp. The upper half of the buccal opening commonly protrudes forward, beyond the lower. The atrial siphon (aperture) is smaller than the buccal siphon and is undivided. Several (14–16) elongate lobes ring the atrial opening. These lobes are much smaller than those of the buccal siphon and are separated from each other by gaps.

Five annular muscle bands gird the body. Muscle I is at the branchial opening. Muscle II is just behind it and is complete, although it is sometimes difficult to see the thin, dorsal portion of this closed ring. Muscles III are a symmetrical pair, on the left and right sides of the body. They are L-shaped and short, running forward from behind the branchial septum at mid-

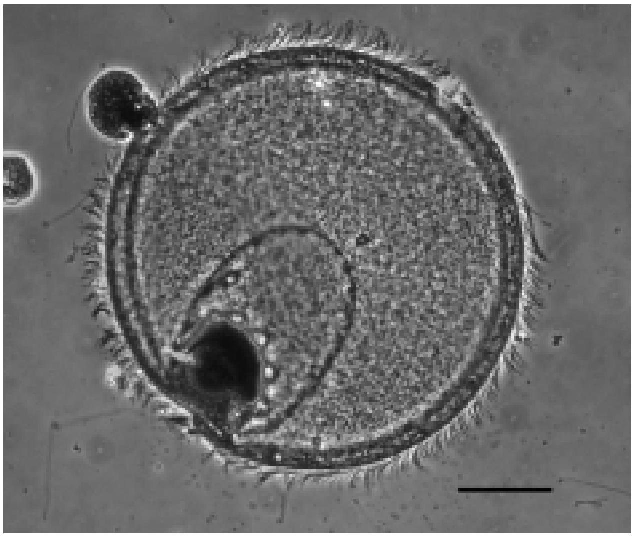

Figure 4. Disc-shaped ciliated protist from *Doliolula equus*. The cell margin nearest the nucleus is characteristically flattened, and with larger specimens this trait is more pronounced. Scale bar: 10 µm.

body level, then turning sharply upward, terminating well below the dorsal apex, and leaving a large dorsal gap. Muscle IV is complete and terminates the atrial cavity, bordering a short vestibule. Muscle V is the sphincter of the atrial siphon. The distance between muscles IV and V is much greater than that between muscles I and II.

The pharyngeal cavity (chamber) is about twice the volume of the atrial (cloacal) cavity. They are separated by a branchial septum that is perforated by two continuous tiers of parallel, transverse, ciliated gill slits (stigma), which increase in number with the size of the individual, but are always at least 20 and can be more than 60. The two sets of gill slits are separated by a narrow, vertical band of epithelium. When viewed from above, the branchial septum, bearing the gill slits, has the shape of a shallow V.

The gut is U-shaped, and begins between the two wings of the branchial septum at about mid-body level (Figure 2B). The outer surface of the oesophageal tube bears a pronounced spiral whorl. There is a short neck, then a bulbous stomach, and then the intestine descends to the floor of the atrial cavity before ascending back to the level of the atrial aperture, albeit below the level of the oesophagus.

The endostyle is long and runs along the midline of the lower part of the pharyngeal cavity, beginning just forward of the branchial septum. Above and draped over the endostyle is a flat, shallow chamber, rounded at the front, tapered and subdivided at the rear, that is probably a haemocoel sinus. Frequently this chamber contains a population of ciliate protists. The heart (cardiopericardium) is a tubular structure lying longitudinally, to the right rear of the endostyle, beneath and anterior to the gut and branchial septum. As with other thaliaceans, the flow is bi-directional, with the peristaltic motion frequently but irregularly reversing direction.

Ciliated peripharyngeal bands extend forward from the endostyle, they diverge to encircle the buccal opening while crossing the plane of muscle II, then sweep upward and back, converging to pass to the right of the brain, and forming the spiral gland between muscle III and the branchial septum. A mucus cord extends from the ciliated funnel beneath the spiral gland, to the oesophagus. Short retropharyngeal bands project posteriorly from the endostyle, pass upward in front of the gut, along the inner margins of the two tiers of gill slits in the branchial septum, and ultimately to the oesophagus.

The brain (neural ganglion) is quite small (about 200 µm). It sits near the apex of the dorsal arc of the body, just to the left of and above the paired, ciliated bands and forward of the spiral organ that they ultimately form. Slender nerve fibers radiate out from the brain to the muscle bands. Two pairs of latero-anterior nerves are prominent between muscles II and III.

Gonozooids

Except for the presence of gonads, gonozooids are indistinguishable from trophozooids. Gonozooids are hermaphroditic. The oocytes appear to form first, however protogyny is unlikely because sexually mature zooids possess both viable sperm and mature oocytes simultaneously. Both ovary and testis (testicular ceca) originate near the curve of the U-shaped gut; testis on the left, ovary on the right (Figure 2C). The translucent ovary grows in a semi-spiral with the smallest oocytes located proximally, increasing in size distally. Oocytes up to 1150 µm have been measured and larger ones have been observed. The white testis is connected by a thin filament to the ovary, and stretches like blunt fingers around the gut, reaching up toward the stomach. The testis branches and may have ten or more projections that eventually envelop the gut. The sperm of *D. equus* possess a defined, elongate acrosome, tapered at each end and slightly flared in the middle. In several of the colonies we examined, all of the individuals were gonozooids of similar size, and all had mature gonads of both types.

Laboratory observations

Hydroid polyps

Mature (feeding) zooids in virtually all of the *D. equus* colonies we collected, had single, hydroid polyps attached to their buccal siphons (Figures 1 & 2B). Twice, we observed zooids with two polyps on the buccal siphon, and on one occasion we found a polyp that was

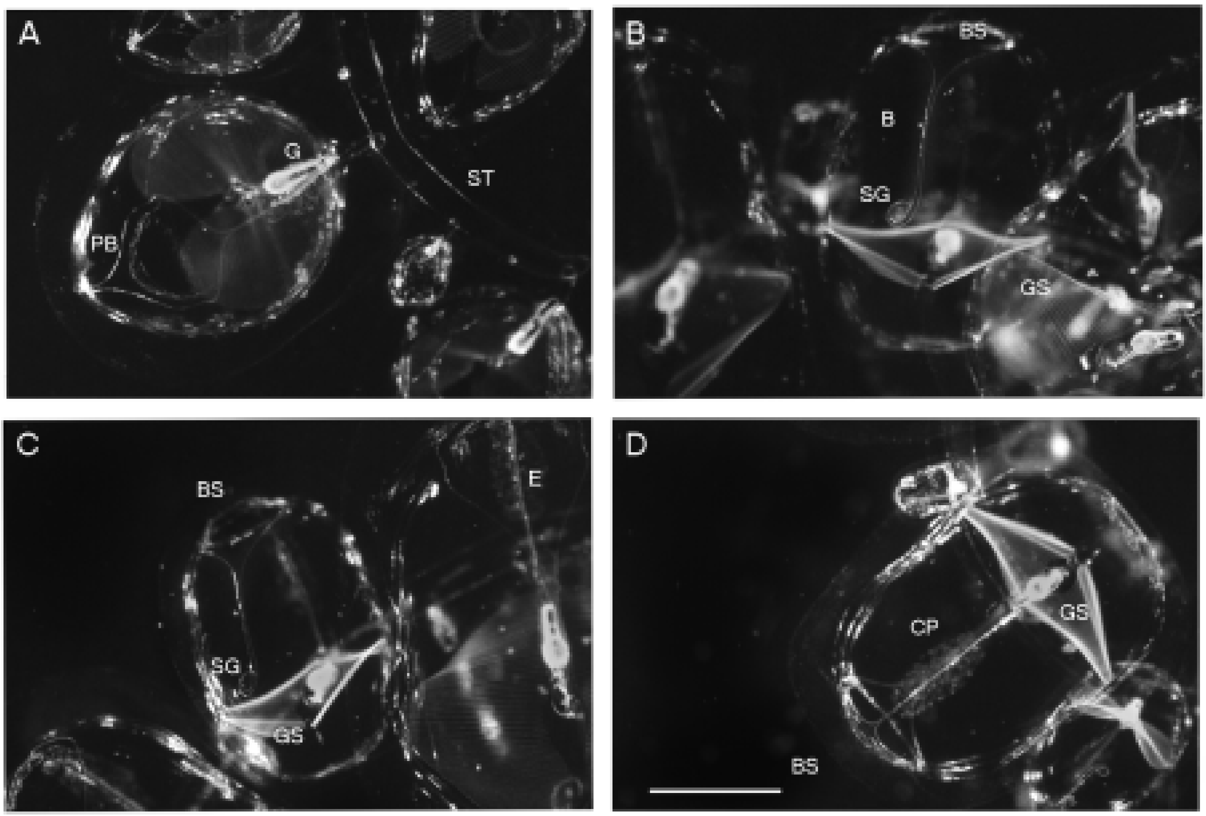

Figure 5. Shots of colonial chain segments, showing the differences in size among zooids. (A) Stolon segment with adjacent large and small zooids, and an incipient zooid just above the largest one; (B) the brain can be seen to the left of the peripharyngeal bands of the central zooid; (C) the position of the gut, just behind the gill slits can be seen in the central zooid; (D) ventral view of the detached larger zooid, dorsal view of the smaller one, each with a ciliate-filled pouch above the endostyle. B, brain; BS, buccal siphon; CP, ciliate protists; E, endostyle; G, gut; GS, gill slits/branchial septum; SG, spiral gland; ST, stolon; PB, peripharyngeal bands. Scale bar: 5 mm.

attached at the atrial siphon, but such anomalies were rare. In addition, autonomous tentacles released by the hydroids, were abundant on the tunics of all zooids in most colonies. The polyps are a new species, *Bythotiara dolioeques*, described by Raskoff & Robison (2005). The doliolid and the hydroid co-exist in a mutualistic symbiosis. In this relationship, the doliolid gains some measure of protection from predators by the presence of the nematocyst-laden tentacles scattered on its surface, and from the cluster of hydroid tentacles in its buccal region. Also, food and faecal boluses, egested by the polyp, are quickly trapped by the doliolid's feeding web and ingested. Finally, the polyp acts as a preliminary filter, preventing large food items from damaging the delicate mucus feeding web of the doliolid. Advantages for the hydroid are: access to a favourable substrate for settlement, access to feeding currents generated by the doliolid, the opportunity to exploit pelagic food sources unavailable to benthic forms, and a reduction of exposure to predators adapted to feed on benthic polyps. This novel relationship and the implications of asexual reproduction by the autonomous tentacles are further described by Raskoff & Robison (2005).

Ciliate protists

Disc-shaped ciliate protists were frequently found within the zooids of *D. equus*. They were occasionally observed spinning or tumbling slowly in the atrial cavity of recently captured specimens, but were most numerous within the flat, pouch-like chamber located above the endostyle (Figure 3). In some specimens, the protists were concentrated at either end of the pouch in what appears, because of the clustered protists, to be a pigmented area. The pouch itself lies directly above and laterally to either side of the endostyle, with paired lobes at the posterior. The ciliates, which are most likely in the class Phyllopharyngea (D.W. Coats, personal communication), spread out to fill the entire cavity as they grow in both number and size. Larger ciliates range from about 150 μm to 225 μm in diameter. At their external margin, closest to the nucleus, the ciliates are flattened (Figure 4). DAPI-stained individuals show a prominent nucleus and micronuclei. Bacteria (both rod and coccus), that coat the outside of the protists are prominent and flagellates are also present externally.

The appearance and location of the protists in *D. equus* correspond very closely with the 'cloud of scattered white pigment dots' in the descriptions of *Doliopsis bahamensis* and *Paradoliopsis harbisoni* (Godeaux, 2003; Godeaux & Harbison, 2003). In our material the protists turned opaque and white over time. If these are the same, then the pigment cloud is probably not a useful diagnostic character for these latter species.

Parasites

Single, hyperiid amphipods were often found within large individual zooids in colonies of *D. equus* (Figure 2D). When the affected zooid was in good condition, the amphipod was typically located low in the branchial cavity, apparently feeding on the mucus strand and its trapped food particles. When the zooid was in poor condition (not feeding), the amphipod was invariably located on the branchial septum, feeding on the stigma.

Coloration

The tunic and much of the body wall were transparent. In about half of the larger, living zooids we observed, the gill slits appeared red. Those of the remaining large individuals and in most of the smaller zooids were colourless. Likewise, some specimens had red-coloured peripharyngeal and retropharyngeal ciliated bands. The oesophagus was always translucent while the stomach was most often yellow-orange and the intestine was yellow-brown. Gonads were translucent and without colour. Patches of bright orange pigment occurred on the body and around the buccal and atrial regions of some individuals, both trophozooids and gonozooids (Figure 2B&D). Individual patches were small, irregularly shaped and flat. Overall, only about one zooid in ten was substantially pigmented. In the smaller zooids, when pigment was present, it was usually limited to spots on the the buccal and atrial lobes. In larger specimens the patches also occurred within the body wall, particularly in places where it bulged into the tunic. Shrinkage or expansion of individual patches was never observed directly, however, the size of patches did change over time.

Bioluminescence

Viewed in complete darkness under a microscope, with a low-light video camera, or with the naked eye, *D. equus* produced a diffuse, blue glow upon mechanical stimulation. The light outlined the body and appeared to originate in the tunic rather than the interior of the body. In most cases the light pulses were of short duration, lasting only a few seconds after the stimulus. However, with continuous stimulation the glow persisted and the intensity of the output appeared to be related to the level of the stimulus. Rarely, a sustained glow from a single individual in a colony lasted for more than 30 seconds after stimulation. No light was seen to emanate from the stolon, nor from the symbiotic hydroid polyps or the ciliates. Light production was apparently not coordinated between the individuals of a colony, in that not all of the members luminesced every time there was stimulation.

In situ *observations*

All of our observations of *Doliolula equus* in its natural habitat have been of colonial chains. Individual zooids have not been seen, although the size of mature zooids is well within the resolution capabilities of our cameras, and their shape is distinctive and familiar. Colonies showed considerable variability in length and also in the size ranges of the individual zooids within a chain (Figure 5). In some cases, all of the individuals were about the same size. In others, there were orders of magnitude difference in zooid size, even between adjacent zooids. In general, only a few of the individuals in a colony showed conspicuous pigmentation and these were invariably the larger zooids. With no nurse to propel them and their axial thrust not in-line, chains of *D. equus* appear to be passive drifters. The stolon is elastic and quite flexible.

Within its depth range in Monterey Bay, *D. equus* occupies a temperature envelope of about 5° to 10°C, oxygen concentrations from 0.5 to 2.5 ml l^{-1}, and salinities from 33.5 to 34.0. The daytime light regime it inhabits is dim and monochromatic. At these depths transparency and visual trickery are commonly employed as anti-predation tactics (Robison, 1999; Johnsen, 2001). A number of known and likely predators of *D. equus* are abundant at mesopelagic depths in Monterey Bay. These include tentaculate predators such as the narcomedusae *Solmissus marshalli* Agassiz & Mayer and *S. incisa* Fewkes (Raskoff, 2002) and the giant siphonophores *Praya* spp. and *Apolemia* spp. Several visually-cued midwater fish consume pelagic tunicates in their diets (e.g. *Leuroglossus stilbius* Gilbert, *Bathylagus pacificus* Gilbert, *Stenobrachius leucopsarus* Eigenmann & Eigenmann), as do the ctenophores *Beroe cucumis* Fabricius and *B. abyssicola* Mortensen (Harbison, 1998). There was no strong seasonal pattern in the abundance of *D. equus* between 1995 and 2004, although occurrence during the period from February to June was quite variable. Peak abundance was in March, the month that is usually associated with the onset of local upwelling.

Supplemental figures and video images are posted at: http//:www.mbari.org/midwater/doliolula.htm

Key for the genera of Doliopsidina

1. (a) Body substantially longer than it is high, long third muscle band, buccal vestibule present, spiral gland to the right of the dorsal midline, muscle IV open ventrally………*Paradoliopsis* Godeaux (1996)
   (b) Body higher than long or spherical to slightly elongate, short third muscle band, no buccal vestibule, spiral gland on or to the left of the dorsal midline, muscle IV is a complete ring....……………………………………..……2
2. (a) Body higher than long, third muscle band sigmoid, no atrial vestibule, spiral gland on dorsal midline, buccal siphon small (<15% of height), ...……….……*Doliopsis* Vogt (1854)
   (b) Body rounded and slightly elongate, third muscle band L-shaped, no endodermal fold associated with muscle III, a small atrial vestibule, spiral gland slightly to the left of dorsal midline, buccal siphon moderate (>25% of height............... ....*Doliolula* gen. nov.

DISCUSSION

Clearly there is much yet to be learned about the taxonomy and relationships of the deep-water doliolids. Our material, while abundant, does not fit readily into either of the genera or families described by Godeaux (2003) and Godeaux & Harbison (2003). Given the complexity of doliolid life cycles and the dearth of mesopelagic specimens from other areas, we choose not to erect a new family to include this unique form from Monterey Bay. Rather we will tentatively assign it to the Doliopsidae, which it resembles more than the Paradoliopsidae, and we will wait until a clearer picture of the higher taxa emerges.

Oozooids have not been reported for any member of the doliolid suborder Doliopsidina (Godeaux & Harbison, 2003; Godeaux, 2003). This situation is particularly puzzling given the large number of colonies we have observed since 1995. Just once, we collected what we initially believed was a colony of *Doliolula equus*, with an oozooid nurse. When we examined the specimens, we were not convinced that the blastozooids were *D. equus* because of their shorter endostyles, thicker tunics, and different pigmentation. The 'nurse', however, was quite remarkable and may shed some oblique light on the puzzle. At the terminus of the colony was an individual fully twice as large as any other doliopsid we have seen. It had a radially symmetrical pattern of orange pigment spreading outward from the posterior region of its ventral surface. This individual had a substantial chain of zooids attached, yet it still contained a functional endostyle, ciliated bands, branchial stigma, and gut. Most unusual was what appeared to be a sturdy, orthogonal branch of the endostyle, which arched up the left side of the branchial cavity to about the position of the spiral gland, where the colonial stolon was attached, dorsally! A continuous thread of tissue ran along the branch, into, and through the length of the stolon. We are unaware of any such configuration ever described for a doliolid.

We have occasionally encountered a number of specimens of other, unusual doliolids, similar to *D. equus*, but different enough in proportion or structure or pigmentation to indicate that they represent undescribed species within the suborder Doliopsidina. Among these is a form without symbiotic polyps or protists but with a pronounced, triangular, postero-dorsal projection. This feature, along with the lobes around the buccal siphon, were heavily pigmented a bright orange. Unlike *D. equus*, where only some of the zooids in a colony are pigmented, in this one, all individuals in a colony were brightly coloured in this fashion. At a depth of 2384 m in Monterey Bay we found a large, rounded, individual zooid that had a symbiotic polyp at its buccal siphon, but it also had both buccal and atrial vestibules. Another variant was collected, in chains of zooids, during dives by the Johnson-Sea-Link submersible around the Galapagos Islands. This form, like *D. equus*, also carried a symbiotic hydroid polyp attached to the ventral lip of its buccal siphon. However, it differs from *D. equus* in that its body was much taller than long, it had a proportionally longer endostyle, and its tunic was much thicker.

The principal characters that distinguish the two suborders of the Doliolida are body shape, the number of muscle bands, and the relative position of the brain. To these traits we may someday add, mutability of the life cycle. The suborder Doliolidina shows relatively little variability in life cycle stages. The principal exception is *Doliolum nationalis* Borgert, which shifts to a cycle of phorozooids budding new phorozooids under certain environmental conditions (Godeaux et al., 1998). In contrast, every described species in the suborder Doliopsidina shows significant variance, if only in the apparent absence of oozooids. Unlike most shallow-living doliolids, the trophozooids and gonozooids of *D. equus* occurred together, attached to a single stolon. No phorozooids were apparent. Godeaux & Harbison (2003) have shown that *Paradoliopsis harbisoni*, another deep-living doliopsidine with no known nurse, produces gonophorozooids. It may be that *D. equus* has similarly revised the doliolid life cycle.

A common misconception about the mesopelagic habitat has been that there is no counterpart to the solid ecological substrate found at the sea-floor. In fact, direct observations from undersea vehicles have shown that the bodies of many mesopelagic animals provide significant substrate; used by other species in a variety of ways to settle, find shelter, and seek food (e.g. Robison, 1983; Larson et al., 1988; Harbison, 1998; Drazen & Robison, 2004).

To this growing list we can add the multiple habitation of *Doliolula equus* by organisms from three different phyla. Thus the physical structure of this habitat is determined at one level by patterns of hydrographic parameters, and at another level by the animals who live there.

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