RESEARCH NOTE

Field collection of *Laevipilina hyalina* McLean, 1979 from southern California, the most accessible living monoplacophoran

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Monoplacophora remain one of the most understudied and enigmatic molluscan groups to date. Thought to exist only as a fossil group, living specimens were recovered in 1952 from abyssal Pacific waters off Costa Rica (Lemche, 1957). This sensational discovery (Yonge, 1957) energized the debate about molluscan evolution. A suite of new monoplacophoran species was subsequently described, mostly from abyssal and hadal depths worldwide (Schwabe, 2008), including the Antarctic (e.g. Schrödl, 2006; Warén & Hain, 1992), and hydrothermal vents (Warén & Bouchet, 2001), bringing the known extant diversity to 31 species (see Haszprunar, 2008). These habitats are relatively difficult to access, and provide challenges to retrieving living specimens in significant numbers and good condition. Not surprisingly, many monoplacophoran studies suffer from little and/or poorly preserved material, and a lack of knowledge about the living animals.

The shallowest known extant monoplacophoran *Laevipilina hyalina* McLean, 1979 is known from depths 174–388 m, along the Santa Rosa-Cortes Ridge in the continental borderland of southern California (McLean, 1979). It was the first monoplacophoran species to be photographed alive, and specimens were maintained in aquaria for a maximum of 25 days (Lowenstam, 1978). It is only known from a restricted range (Fig. 1), but has been collected from the type locality twice, most recently in 1977 (Lowenstam, 1978). We revisited this site to collect living specimens of *L. hyalina* for molecular studies, and to determine whether it was possible to raise larvae from collected adults maintained in aquaria.

Using the UNOLS vessel *R/V Robert Gordon Sproul*, we sampled the type locality of *L. hyalina* (32°59.0′N, 119°32.8′W) over 3 days in November 2007. We used a large Van Veen grab sampler (Kahl Scientific, volume 0.2 m^3) to collect phosphoritic nodules from depths of 367–389 m. Living monoplacophorans were found by visual examination of the nodules, with most discovered by further examination under dissecting microscopes. The prismatic nature of the shells was helpful in distinguishing the animals from the encrusting community on the nodules. We fixed animals in formalin, ethanol, paraformaldehyde, glutaraldehyde and RNA*later* (Ambion, USA), and retained some living animals in chilled water (4–8°C) to take back to Scripps Institution of Oceanography, where they were maintained on nodules in a flow-through, insulated aquarium chilled to 6°C. Vouchers were deposited in the Scripps Benthic Invertebrate Collection under SIO-BIC M11891-M11896.

Fifty-two specimens of *L. hyalina* were collected in the manner described above. Overall, deployment was efficient, with 92% of grabs successfully retrieving substrate. Most grabs retrieved phosphoritic nodules and several others contained only freshly broken-off pieces of substrate. However, even grabs that retrieved only one or two nodules often yielded *L. hyalina*. The nodules themselves were covered with a sparse invertebrate community (Fig. 2A). We found an average of 1.4 specimens of *L. hyalina* per successful grab ($n = 36$) with a maximum of nine individuals taken in a single grab (Fig. 3). The highest number of individuals collected on a single nodule was three but, when present, there was generally only one animal per nodule. Hence, most collected animals were solitary, showing no sign of congregation. However, specific habitat requirements or a patchy distribution are possible, because a small number of grabs contained many animals. Otherwise, the dominant organisms on the nodules were foraminifera and bryozoans. Some grabs included small amounts of sediment, but most contained only nodules.

Mature females of *L. hyalina* were identified by the presence of large yolky oocytes observed through the translucent foot (Fig. 2B). One female expelled an oocyte while being observed under a dissecting microscope, possibly due to thermal stress. The oocyte was initially wider at both ends and pinched narrower in middle. After a few hours it expanded to a regular ovoid shape and measured 80–120 μm. The embryo cleaved after 9 h at 12°C, but failed to continue development. This initial deformation of oocytes was previously predicted because of the narrow gonoduct and small urogenital opening (Haszprunar & Shäffer, 1997), and is confirmed by observation here.

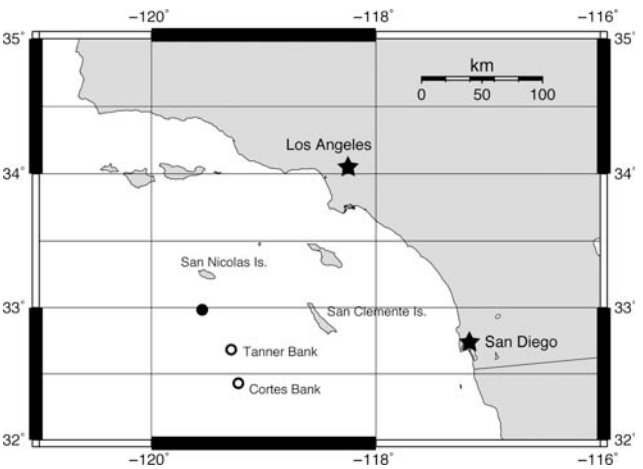

Figure 1. Distribution map of *Laevipilina hyalina*. Known samples correspond to an area bounded by San Nicolas Island, San Clemente Island and Cortes Bank. Type locality is marked by a filled circle, other sites by an open circle.

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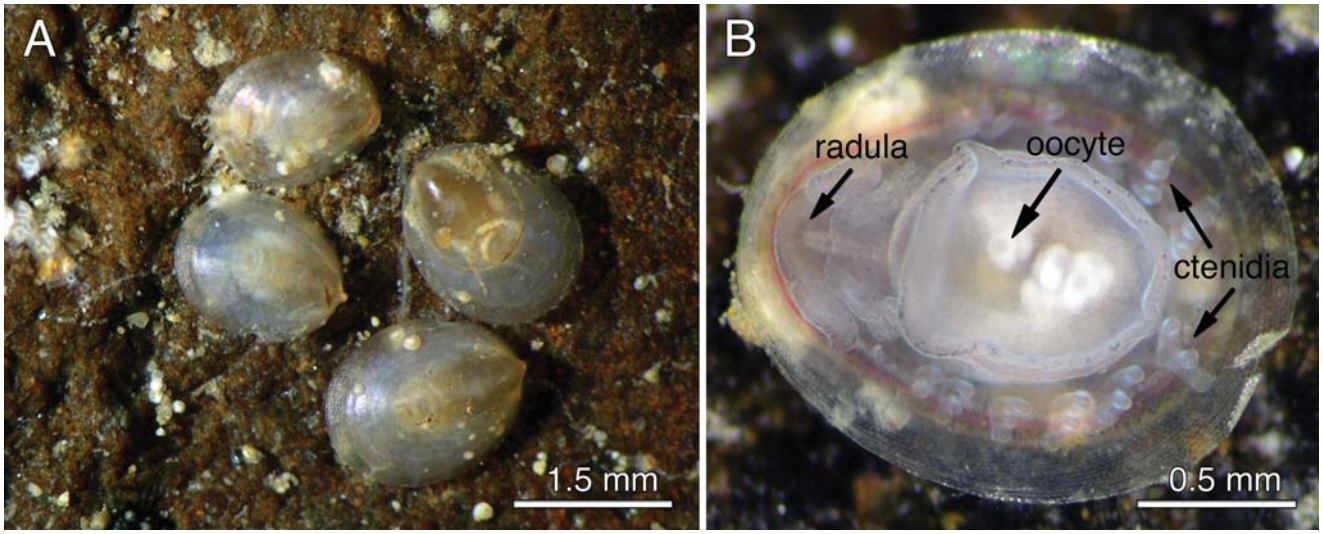

Figure 2. Living specimens of *Laevipilina hyalina*. **A.** Artificially arranged group of four individuals on nodule surface. **B.** Ventral side of female; arrows indicate radula, oocytes and ctenidia.

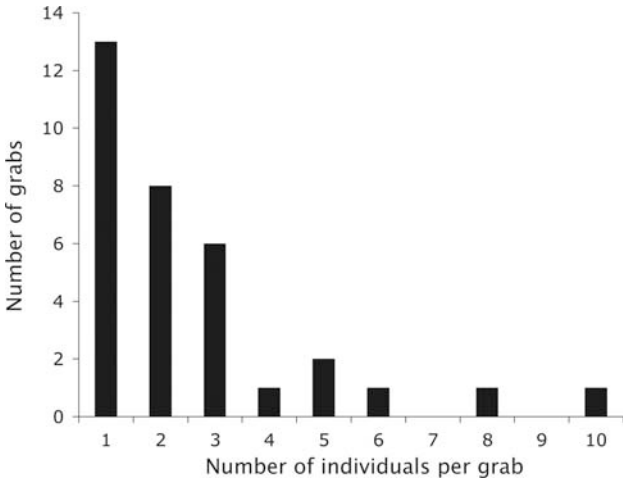

Figure 3. Histogram of Van Veen grab success at the type locality of *Laevipilina hyalina*.

Ten animals were kept alive and transferred to the laboratory (mixed adult and juveniles) with the nodules on which they were collected. The coiled gut was easily observed through the transparent shell, and we observed all animals to have empty guts after approximately 24 h following collection. Although suggested to be either generalists that feed on detritus (Warén & Hain, 1992), including foraminiferans and radiolarians (Lemche & Wingstrand, 1959), or specialists on xenophyophore protozoans (Tendal, 1985), no feeding was observed in the laboratory, and their digestive system remained empty. The two smallest individuals died after nine days. With the remaining specimens showing no indication of feeding, we decided to fix them for other studies.

The accessible population of *L. hyalina* occurs off southern California, and has been sampled three times over a 40-year period from a small area surrounding the type locality. A note added in proof by McLean (1979) indicates that attempts to resample at Cortes Bank and Tanner Bank were unsuccessful, although further details are not given. The type locality of the species, south of San Nicolas Island, seems to host relatively high densities of specimens.

Mature female specimens of *L. hyalina* contained six or more oocytes at a similar stage of development and a number of others at an earlier vitellogenic stage (Fig. 2B). This number may be comparable to that in its congener *L. antarctica*, but is more than that in *L. cachuchensis* (Urgorri, García-Álvarez & Luque, 2005) and the brooding *Micropilina arntzi* (Haszprunar & Shäffer, 1997). Haszprunar & Shäffer (1997) posited lecithotrophic development for all neopilinids, and possible external fertilization based on sperm structure (Healy *et al*., 1996), and we found nothing to contradict these inferences. Major gaps exist in knowledge of the biology of Monoplacophora, and the important question of their larval development has far reaching implications for molluscan evolution. Future studies on these enigmatic molluscs will no doubt benefit by having such a relatively accessible population available. We hope that this report motivates other researchers to address these, and other, outstanding questions in monoplacophoran biology.

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