RAFFLES BULLETIN OF ZOOLOGY Supplement No. **31**: 250–254
Date of publication: 10 July 2015
http://zoobank.org/urn:lsid:zoobank.org:pub:0344DE27-273C-45DF-973E-7BA105968631

Spawning observed in a specimen of the shelled sacoglossan *Lobiger viridis* Pease, 1863 from Singapore (Mollusca: Gastropoda: Heterobranchia)

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Abstract. Spawning was observed in the laboratory and documented by video for a specimen of *Lobiger viridis* Pease, 1863 (Mollusca: Gastropoda: Heterobranchia: Sacoglossa) from Changi East, Singapore. The spawning animal shaped the egg mass with its mouth-area and the anterior rim of its foot. The head moved from side to side, presumably adding secretions, as the egg mass progressed. The egg mass was shaped as a more or less irregular, elongate spiral. It took approximately three hours to complete the first egg mass, and a second fertile egg mass was produced after 24 hours. The eggs were yellow when deposited but turned pale after several days as shelled veliger larvae developed. A single egg mass was estimated to contain more than 20,000 eggs. Preserved egg capsules were approximately 120 × 90 µm, and veligers had distinct statocysts but no eyes when they were ready to hatch. At this stage veliger shells had maximum diameters of about 106 µm.

Key words. Sacoglossa, *Lobiger viridis*, spawning, video documentation

INTRODUCTION

The morphology of sacoglossan egg masses has been described for many species (e.g., Baba & Hamatani, 1952; Greene, 1968; Jensen, 2001, 2003). Egg masses are somewhat flattened gelatinous ribbons, coiled in more or less regular spirals depending on the substrate. Eggs are surrounded by "albumen" (intra-capsular fluid) and wrapped individually in ovoid capsules. The string of egg capsules is sometimes coiled regularly within the egg mass, but usually egg capsules are irregularly arranged in the gelatinous coating. Egg masses of some sacoglossans, especially the superfamily Plakobranchoidea, have extra-capsular yolk (Clark et al., 1979; Boucher, 1983; Jensen, 2001). Egg size, capsule size, number of eggs per egg mass and development type, have been reviewed in a few papers (Clark & Jensen, 1981; Jensen, 2001), and poecilogony is known for a few species (Krug et al., 2007; Vendetti et al., 2012). The spawning process has been observed for a few species (Kawaguti & Yamasu, 1960, 1966; Reid, 1964; Jensen, 1986), but the present study is the first time the spawning process of a sacoglossan species has been documented by video.

Lobiger viridis Pease, 1863 is a rather large sacoglossan (preserved body length including tail 2–3 cm) with a reduced shell (length 1–1.5 cm) covering the visceral mass, a long muscular tail, and four long lobed parapodia usually held erect so they partly cover the shell. It occurs throughout the Indo-West Pacific region, but little is known about its biology and anatomy, and the synonymy with other species has been disputed (Kay, 1964; Burn, 1966; Baba, 1974; Jensen, 1985). The present study is the first record of the species from Singapore. Reproductive patterns often distinguish otherwise similar and sympatric species in the Sacoglossa, e.g., *Oxynoe antillarum* Mörch, 1863, with planktotrophic development and *O. azuropunctata* Jensen, 1980, with lecithotrophic development (Jensen, 1980), and *Alderia modesta* (Lovén, 1845), with planktotrophic development and *A. willowi* Krug, Ellingson, Burton & Valdés, 2007, with poecilogonous development (Krug et al., 2007).

MATERIAL AND METHODS

Three specimens of *Lobiger viridis* were collected at Changi East, Singapore, on 14 December 2012. One of these specimens was brought to the laboratory for photography and kept alive with some food algae, *Caulerpa* spp., in a small aquarium (12.5 × 9 cm surface area, 2.5 cm seawater depth) at ambient light and temperature conditions. Seawater was changed every other day. Photographs were taken with a Nikon D5000 digital camera equipped with an 18–200 mm lens, and video sequences of the animal during the spawning of an egg mass on 15 December 2012 were taken using a Canon Powershot G12 camera. After the first discovery of spawning in progress, videos were taken at intervals until the egg mass had been completed. A second egg mass was also photographed and subsequently preserved in formalin along with the adult specimen. Egg capsules and veliger shells of the preserved egg mass were measured by ocular

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ISSN 2345-7600 (electronic) | ISSN 0217-2445 (print)

micrometry, and the total number of eggs estimated by counting the eggs of three 1 mm sections and measuring the total length of the egg mass.

RESULTS

The spawning was first observed at approximately 1730 h on 15 December 2012. At this time the egg mass was already 2–3 cm long and had made the first turn (Fig. 1A). The animal hung upside-down with its foot-sole attached to the surface film of the aquarium by mucus continuously produced, and the egg mass was also attached to the surface film. Ten minutes later it had made a second turn. However, it looked like the animal had been disturbed, either by the photography or by underlying algal phyllodes touching the hanging parapodia, because the second length of the egg ribbon was not attached to the first one. The length of the egg ribbon before the second turn fits that required to reach the beginning of the ribbon, indicating that it would probably have formed a spiral if undisturbed. After 10 minutes more a third turn had been made. Again the animal seems to have been disturbed, changing the regular progress of the egg ribbon. Twenty minutes later a fourth turn had been made, and at this point the shape of the egg mass was quite irregular with a cross-over half way between the third and fourth turns. Two hours later, the egg mass had been completed (Fig. 1B), and the animal crawled away. Thus the entire spawning took approximately three hours. During spawning the head moved from side to side as the egg mass was shaped by the mouth area and the anterior foot margin.

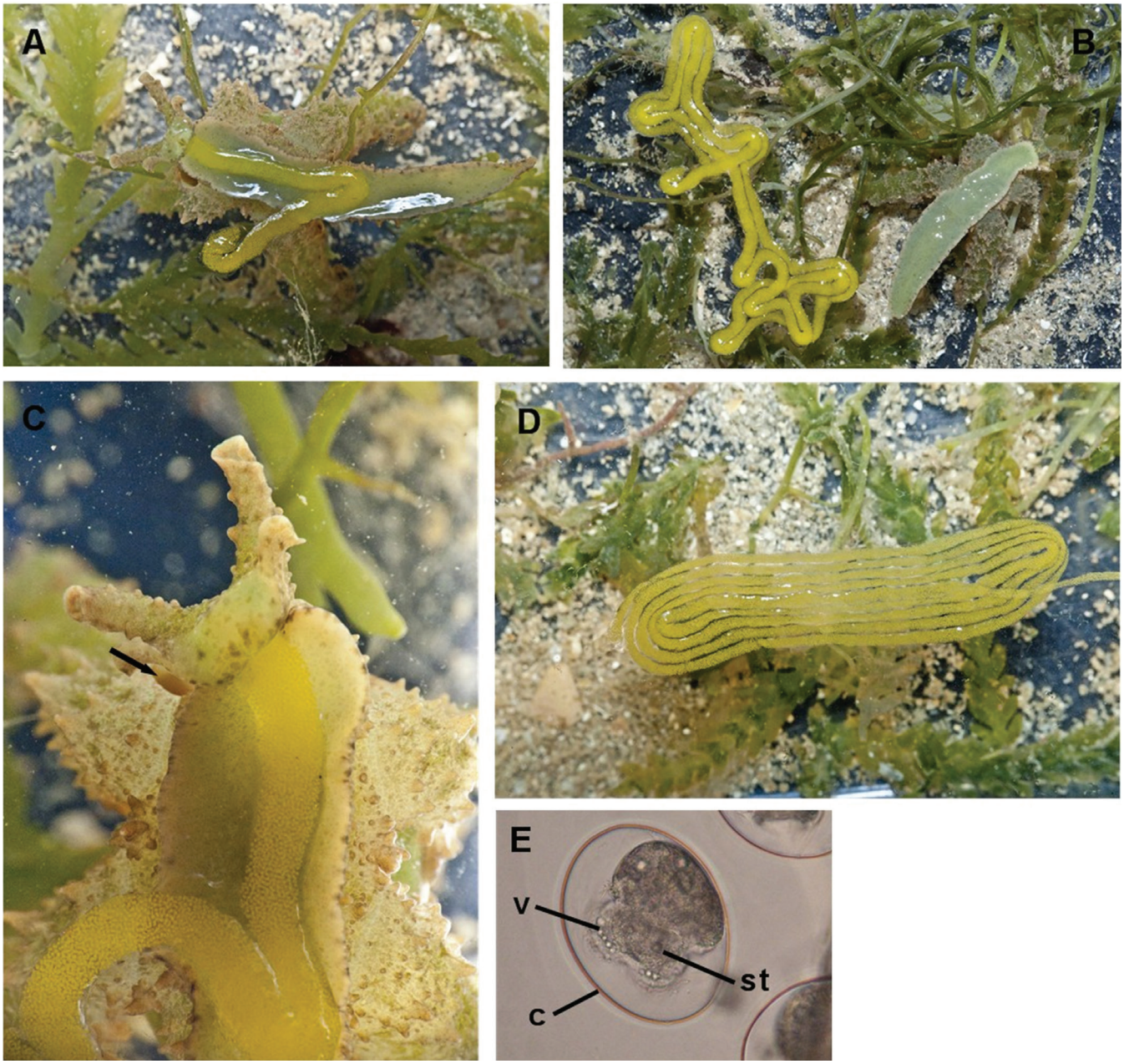

Fig. 1. Spawning *Lobiger viridis* from Singapore. A, early stage of spawning. Animal and egg mass suspended from surface film; B, after completion of egg mass animal moves away; C, close-up of spawning animal. Arrow points to cavity formed by right anterior parapodial lobe above ciliated spawn groove on right side of neck; D, Second egg mass forming a regular elongate spiral; E, Preserved veliger larva inside capsule. Legend: c = capsule wall; st = statocyst; v = velar lobe.

Presumably the eggs had been transported along the spawn groove on the right side of the neck to the groove between the foot and the mouth area. In the present animal, the anterior right parapodial lobe formed a "roof" covering the ciliated groove (Fig. 1C, arrow). The width of the egg ribbon approximately matched the width of the mouth area. As the egg mass grew it was held by the foot sole, and occasionally the edge of the foot wrapped around the edge of the egg mass (Fig. 1C). During the whole process the mouth opened and closed, and fine muscular contractions were seen as waves on the ventral surface of the head and on the foot sole. The eggs were yellow when deposited, but after a few days the embryos turned pale. No extra-capsular yolk was present, and the intra-capsular albumen appeared transparent when the eggs reached the foot-sole. After five days fully formed veligers were seen rotating inside the egg capsules.

Twenty-four hours after the first egg mass had been completed a second egg mass had been deposited. This had a more regular shape, coiled in an elongate spiral of nearly five whorls (Fig. 1D), and was also suspended from the surface film, though it was attached to the vertical aquarium wall at one end. This egg mass was preserved three days after completion. No more egg masses were produced during the five days following spawning that the specimen was kept alive, though yellow reproductive material, probably unfertilised eggs, was still visible near the female genital papilla.

The number of eggs in the second egg mass was estimated to be more than 20,000 (total length of egg ribbon approximately 200 mm and density approximately 110 eggs per mm). The egg capsules measured 118.2 ± 4.2 µm by 93.4 ± 3.0 µm (N = 15). When the second egg mass was discovered the eggs had already begun dividing, and hence we could not measure uncleaved eggs. However, the large number of eggs and the rather small egg capsules indicate planktotrophic development. The preserved encapsulated veliger larvae had well-developed velar lobes with long cilia and refractive bodies at the margins. The statocysts were distinct, but other organs could not be identified (Fig. 1E). Veliger shells were of the typical shape for sacoglossans, sinistrally coiled with slightly over one whorl, and with a distinct operculum. Maximum diameter of the veliger shell was 106 ± 3.3 µm (N = 20).

We have selected a few video sequences from various stages of spawning of the first egg mass to be included as supplementary material. Three digital video files are available for viewing at http://lkcnhm/rbz/Lobiger01.wmv; http://lkcnhm/rbz/Lobiger02.wmv; and http://lkcnhm/rbz/Lobiger03.wmv

DISCUSSION

Spawning has been observed in a few shelled sacoglossans, e.g., *Berthelinia* (*Tamanovalva*) *limax* (Kawaguti & Baba, 1959) and *Julia japonica* Kuroda & Habe, 1951 (see Kawaguti & Yamasu, 1960, 1966). These two species are small and produce relatively small egg masses. The present observations of spawning in *Lobiger viridis* are the first in this genus, and also this is the first time the process has been documented by video for any sacoglossan. In all of the above species the egg string passes from the female genital opening along a ciliated groove on the right side of the neck to the groove between the anterior foot margin and the mouth area. Although we cannot document that secretions from the mouth area or foot margin are added to the egg mass, the texture of the egg mass changes from a single file string in the ciliated groove to a coiling ribbon with the egg capsules irregularly arranged inside, and the movements of the mouth area during the spawning indicates that secretions are added at this point. *Lobiger viridis*, like other sacoglossans, has conspicuous glands surrounding the mouth opening (oral glands) as well as lining the anterior foot margin (pedal glands). The shape of egg masses deposited in the laboratory may not be the same as in the field. Both egg masses of the present study were suspended from the surface film, something often observed in laboratory spawning (e.g., Jensen, 1986). However, most sacoglossans produce a more or less regular spiral egg mass attached to the substrate, often the food algae. Photos of egg masses of *L. viridis* from other localities also show that it forms an irregular spiral, e.g., on the Sea Slug Forum (http://www.seaslugforum.net/find9589).

The size of the egg capsules of *L. viridis* is slightly smaller than measured for *L. sagamiensis* Baba, 1952 from Hong Kong (142 × 108 µm), but the number of eggs per egg mass (6000 for *L. sagamiensis*) is higher (Jensen, 1985; present study). The spawning animal in this study was larger (shell length 9 mm) than the specimens studied in Hong Kong (shell length 7 mm). Egg masses of the Mediterranean *Lobiger serradifalci* (Calcara, 1840) contained between approximately 12,000 and 48,500 eggs (Thibaut & Meinesz, 2000). The estimated number of eggs in one of the present egg masses of 20,000+ eggs falls within that range. In the Caribbean *L. souverbii* Fischer, 1856, the number of eggs per egg mass is much lower (1630) and also the egg capsules are smaller (99 × 88 µm) (DeFreese & Clark, 1983). With the few egg masses of each species studied, it is not possible to determine whether these differences are actually species-specific characters or whether they might be intraspecific variation. *Lobiger sagamiensis* has often been considered a junior synonym of *L. viridis* and some, e.g., Kay (1964), even consider both junior synonyms of the Caribbean *L. souverbii*. This was accepted by Baba (1974), but later disputed by Jensen (1985). However, taxonomic problems of the genus are beyond the scope of the present paper. We simply point out that there are differences in number of eggs per egg mass as well as capsule size. In the Mediterranean *Oxynoe olivacea* Rafinesque, 1819, intraspecific variation in egg capsule size is related to size of the spawning animals (Gianguzza et al., 2005), whereas a larger specimen of *L. viridis* produced smaller egg capsules than the smaller *L. sagamiensis*.

The veliger larvae of *Lobiger viridis* are similar to those of other sacoglossans (see e.g., Rasmussen, 1951; Hamatani, 1960; Jensen, 1997), and agree with type 1 larval shells

of the classification by Thompson (1961). The size of the veliger shell is consistent with that of other sacoglossans with planktotrophic development (Hamatani, 1960; Greene, 1968).

Life history parameters are important for dispersal, distribution, evolution and speciation in the Sacoglossa (Clark & Jensen, 1981; Jensen, 2001; Gianguzza et al., 2005; Krug et al., 2007). In the present study we have described the spawning process, size and shape of egg masses, number of eggs and egg capsule measurements for just one specimen of *Lobiger viridis* kept under laboratory conditions for several days after collection. Although laboratory conditions may have affected spawning behaviour, our observations agree with egg masses observed in the field and with published observations on spawning in other species of sacoglossans. The fact that only two egg masses were produced the first two days after collection and none during the following 5 days indicates that stored allosperm had been depleted. Previous studies have shown that egg capsule size is an important indicator of development type in sacoglossans (Clark & Jensen, 1981; Jensen, 2001). This in combination with the large number of eggs per egg mass, the small size of the veliger larvae, lack of eyes and propodium at hatching indicates that *L. viridis* has planktotrophic development. Future studies should focus on fertility, capacity to store allosperm, embryonic development, duration of planktonic stage, and observations on copulatory behaviour.

ACKNOWLEDGEMENTS

The Johor Straits marine biodiversity workshop on Pulau Ubin, Singapore was organised by the National Parks Board and National University of Singapore and held from 15 October to 2 November 2012 at Outward Bound School. The workshop, as part of the Comprehensive Marine Biodiversity Survey (CMBS) was supported by generous contributions from Asia Pacific Breweries Singapore, Care-for-Nature Trust Fund, Shell Companies in Singapore and The Air Liquide Group. We also wish to thank the management and staff of the Outward Bound School for kindly accommodating our special needs for a successful workshop. We are grateful to the National Biodiversity Centre of the National Parks Board of Singapore and to Toh Chay Hoon of the Lee Kong Chian Natural History Museum, National University of Singapore for assistance in the field and to Ong Joo Yong and Lee Yen-ling from the Tropical Marine Science Institute, National University of Singapore for help with specimen preservation. We would also like to thank Ria Tan for organizing the field trip to Changi East.

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