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Reproductive cycle of the coral-excavating sponge *Thoosa mismalolli* (Clionaidae) from Mexican Pacific coral reefs

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Abstract. Individuals of the recently described demosponge *Thoosa mismalolli* are common on Mexican Pacific coral reefs, excavating burrows in living corals and in other calcareous substrata. To better understand the propagative abilities of this sponge, we conducted a histological study over an 18-month period (May 2007–November 2008) to identify sexual and asexual reproductive structures. Members of the species are viviparous and hermaphroditic, with various developmental stages of oocytes, spermatic cysts, and embryos co-occurring in the mesohyl for most of the year. This nearly continuous reproductive activity intensified during the warm season. Fertilization was internal, and embryos developed inside the parental sponge to produce an unciliated hoplitomella larva, characterized by a peculiar siliceous skeleton. In addition to the sexually generated larvae, adults of *T. mismalolli* formed gemmules for asexual reproduction. Gemmules occurred within the mesohyl during all months of the year, but were most abundant in the coldest months. This combination of sexual and asexual processes enables individuals of *T. mismalolli* to reproduce almost continuously. This strategy may facilitate both long-term persistence within reefs and effective dispersal between distant reefs.

Additional key words: boring sponges, reproduction, gametogenesis, larval polarity

Members of several taxonomic orders of sponges have the ability to excavate calcareous substrata. Among these bioeroding sponges, members of the family Clionaidae (order Hadromerida) are probably the most abundant, and their boring activities have serious effects on both the ecological functioning of coral reefs and the durability of reef frameworks (Goreau & Hartman 1963; Rützler & Rieger 1973; Macdonald & Perry 2003). While the ecological effects of boring sponges on corals are relatively well understood, the reproductive processes of these sponges that permit initial colonization remain poorly known.

Clionaid sponges reproduce both asexually and sexually. Asexual reproduction in these sponges may be based on both tissue fragmentation and the production of resistant structures (i.e., buds and gemmules) filled with totipotent cells (Wells et al. 1964; Rosell & Uriz 2002; Schönberg 2002). Sexual reproduction typically involves internal fertilization, after which zygotes are released into the water column where they develop into ciliated, free-swimming clavablastula larvae (Warburton 1958; Pomponi & Meritt 1990; Mariani et al. 2000, 2001; Maldonado & Riesgo 2008). However, members of two clionaid genera, *Thoosa* and *Alectona,* depart from the general reproductive pattern of the family. They also have internal fertilization, but do not discharge the zygotes for external development. Rather, they brood their embryos in the mesohyl until they are released through the aquiferous canals as unciliated, planktonic hoplomitella larvae. In addition to their lack of ciliation, hoplomitella larvae are characterized by having an unusual skeleton consisting of siliceous spicules that are not present in the adult stage (Topsent 1904; Vacelet 1999; Maldonado & Bergquist 2002). While the ciliated larvae of most clionaids (e.g., *Cliona viridis* Schmidt 1862 and *Cliona celata* Grant 1826) swim for only a few days and mostly settle near their parent sponges (Mariani et al. 2001), the hoplitomella larvae of members of the genera *Thoosa* and *Alectona* are regularly found in offshore

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plankton (Karawaiew 1896; Trégouboff 1942). To date, our understanding of the reproductive biology of members of these two ''atypical'' clionaid genera is fragmentary, with only some general histological aspects of embryo development and larval morphology having been addressed (Topsent 1904; Vacelet 1999). Adults of several species of *Thoosa* (*T. armata* Topsent 1888, *T. mollis* Volz 1939, and *T. investigatoris* Annandale 1915) are also able to produce asexual propagules, namely buds or gemmules (Annandale 1915; Volz 1939).

On the Mexican Pacific coast, members of several species of *Thoosa* are active coral excavators (Carballo et al. 2004, 2007, 2008). Individuals of one of these species, *Thoosa mismalolli* Carballo, Cruz-Barraza & Gómez 2004 (Hadromerida), occur throughout all major reef systems of the Mexican Pacific coast and are particularly common on living colonies of the corals *Pocillopora verrucosa* Ellis & Solander 1786 and *Pocillopora damicornis* Linnaeus 1758, as well as on coral rubble (Carballo et al. 2004). On living corals, the sponges appear to settle at the dead bases of branches, subsequently excavating and extending toward the tips of the coral colonies. Because of its abundance and substrate preferences, this sponge is assumed to play a crucial role in bioeroding and recycling the reef framework in Mexican Pacific reefs (Carballo et al. 2008). The aim of this research was to study the sexual and asexual reproductive cycle of *T. mismalolli* to better understand the propagative abilities of this excavating sponge. In addition, because seawater temperature has often been suggested to regulate reproductive activity in sponges (Baldaconni et al. 2007; Ettinger-Epstein et al. 2007), we examined whether the number of reproductive individuals and the production of gametes and gemmules were related to seawater temperatures.

Methods

Study site

The reproductive biology of adults of *Thoosa mismalolli* was studied in a shallow (5–9 m depth) coral community surrounding Isabel Island in Bahía Tiburon, Mexico (21°52′30″N, 105°54′54″W). Most of the sponge body occurs within a large, irregular gallery system excavated in the calcareous skeleton of its hosts, with only small pale-yellow aquiferous papillae (1.3–3.5 mm in diameter) emerging at the host surface. Adults of *T. mismalolli* are identifiable by a unique combination of spicules (amphiasters, oxyasters, spiny and smooth centrotylote oxea, and slender tylostyles; Carballo et al. 2004).

Sexual reproduction

To investigate the seasonal cycle of sexual reproduction, we collected ten branches of living corals invaded by individuals of *T. mismalolli* each month from May 2007 to November 2008. Tissue pieces were fixed in 4% formalin, decalcified (to dissolve remnants of coral skeleton) in 5% nitric acid for 2 h, rinsed in distilled water, and desilicified with 5% hydrofluoric acid for 1.5 h before dehydration. We dehydrated specimens in a graded ethanol series, cleared them in xylene, and embedded them in paraffin following standard protocols (Riesgo et al. 2007). Five-micron thick sections were produced using a Leica RM2125RT rotary microtome (Leica Microsystems, Nussloch, Germany). Sections were stained with hematoxylin–eosin–Mayer, and mounted onto glass slides. They were examined for the presence of reproductive elements (i.e., spermatic cysts, oocytes, embryos) with a Zeiss Axioplan II compound microscope (Carl Zeiss, Oberkochen, Germany) equipped with a spot-cooled color digital camera. Using the methods of Riesgo & Maldonado (2008), we took images of a total tissue area of 5.7 mm^2 per sampled individual. These digital images were used to measure sizes of spermatic cysts, oocytes and embryos over time, as well as the density of the various reproductive elements (mean number of cysts, oocytes or embryos $mm^{-2} \pm 1$ standard deviation [SD]). Oocyte diameters were measured ($n > 25$ per individual and month) only when the section intersected their nuclei. Spermatic cysts, which were nearly spherical, were measured ($n > 25$ per individual and month) across their largest diameter. To study embryonic development over time, we considered only individuals that contained embryos. In each of those individuals, 25 embryos were chosen haphazardly and classified into three developmental stages: UE (undifferentiated embryos lacking protrusion and spicules), AE (embryos showing cell differentiation and initial formation of radial protrusions that lacked spicules), and SE (embryos with longer radial protrusions and with skeletal components at their periphery). Acid-cleaned spicules of embryos were rinsed in ethanol, mounted on glass stubs, airdried, and sputter-coated with gold–palladium before scanning electron microscopy (SEM) using a JEOL JSM-5300 scanning electron microscope (Akishima, Tokyo, Japan) operating at 15.0 kV.

Asexual reproduction

Histological sections were used to assess the production of internal asexual propagules (i.e., gem-

mules). We measured the density of gemmules in sponge tissue (mean number $mm^{-2} \pm 1$ SD) as well as gemmule size (maximum diameter, $n > 25$ per individual and month). Gemmule structure was described using a combination of light microscopy and SEM. For light microscopy, the material was processed as described above. For SEM, gemmules dissected from sponge tissue were fixed in 5% glutaraldehyde, rinsed in ethanol, mounted on glass stubs, airdried, and finally sputter-coated with gold–palladium before SEM.

Environmental control of reproduction

To examine the potential relationships between temperature and (1) the timing of gamete production, (2) the rate of embryonic development, and (3) the timing of production of asexual gemmules, we measured seawater temperature every 6 h for the entire study period using a HOBO data logger (Onset Computer Corporation, Bourne, MA, USA) placed at 6 m depth. Monthly averaged temperature and variables quantifying reproductive development were plotted against time of year. Spearman's rank correlations were used to assess relationships between the numbers of individuals with reproductive elements, numbers and sizes of reproductive elements, and water temperature.

Results

Adults of *Thoosa mismalolli* were hermaphroditic and viviparous, with oocytes and spermatic cysts at various stages of development co-occurring in the mesohyl. Gametes and embryos were never found clumped in particular areas of the mesohyl. Rather, we found that reproductive elements were relatively erratically distributed through the mesohyl.

Water temperature varied seasonally, with the highest temperatures found during the summer (30.7°C in August) and the lowest during the winter (21.2°C in February). The number of individuals with reproductive elements increased significantly with seasonal increases in water temperature ($r = 0.7$, $p < 0.01$), peaking in August 2007 and May 2008 (Fig. 1). Gametogenesis (both spermatogenesis and oogenesis) and embryogenesis occurred simultaneously in the mesohyl of the sponges during most of the year, except for January and February, when no sponges with spermatic cysts or embryos were found (Figs. 1, 2).

Oogenesis

The production of oocytes was detected during every month of the study (Fig. 2), with embryos and spermatic cysts co-occurring in the mesohyl (Fig. 3A). The lowest average density of oocytes (1 oocyte mm^{-2}) was observed in January and February, coincident with winter minimums in seawater temperature (21.2°C) (Fig. 2A). The highest oocyte densities (18 and 13 oocytes mm^{-2}) occurred in spring each year, 1 month before the peak of spermatic cyst density (Fig. 2A). The smallest oocytes that we were able to identify by light microscopy measured ~16 μm in diameter and were slightly ovate and amoeboid, with a cytoplasm lacking obvious yolk bodies (Fig. 3B). When oocytes reached 30–40 μm in diameter, they were surrounded by nurse cells, and the production of yolk was initiated (Fig. 3C). The largest detected oocytes were also

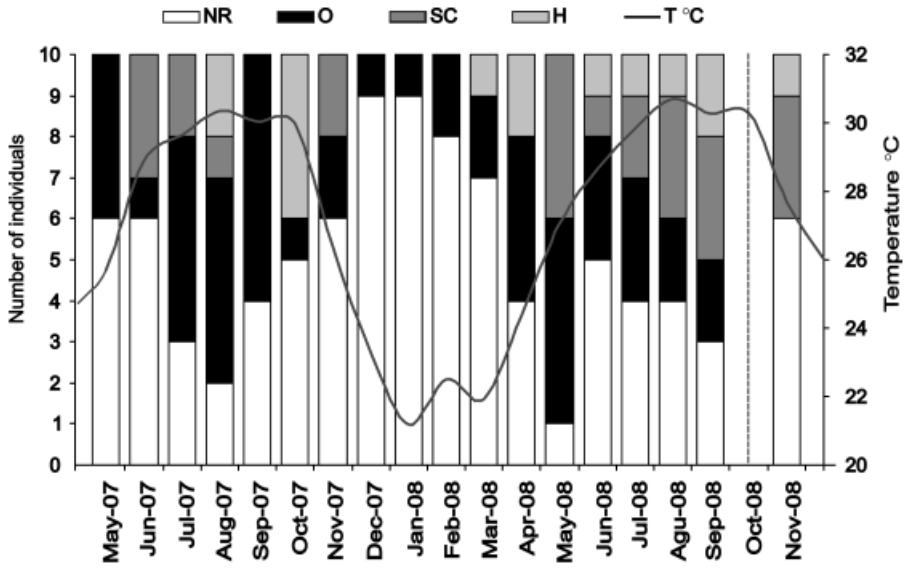

Fig. 1. Sexual reproduction in the population of *Thoosa mismalolli* from May 2007 to November 2008 ($n = 10$ samples per month). Reproductive activity was assessed on the basis of the number of individuals bearing oocytes (O), spermatic cysts (SC), both (H), or no reproductive elements (NR). No data were available for October 2008 (dashed line). Sea surface temperature is shown as the curve.

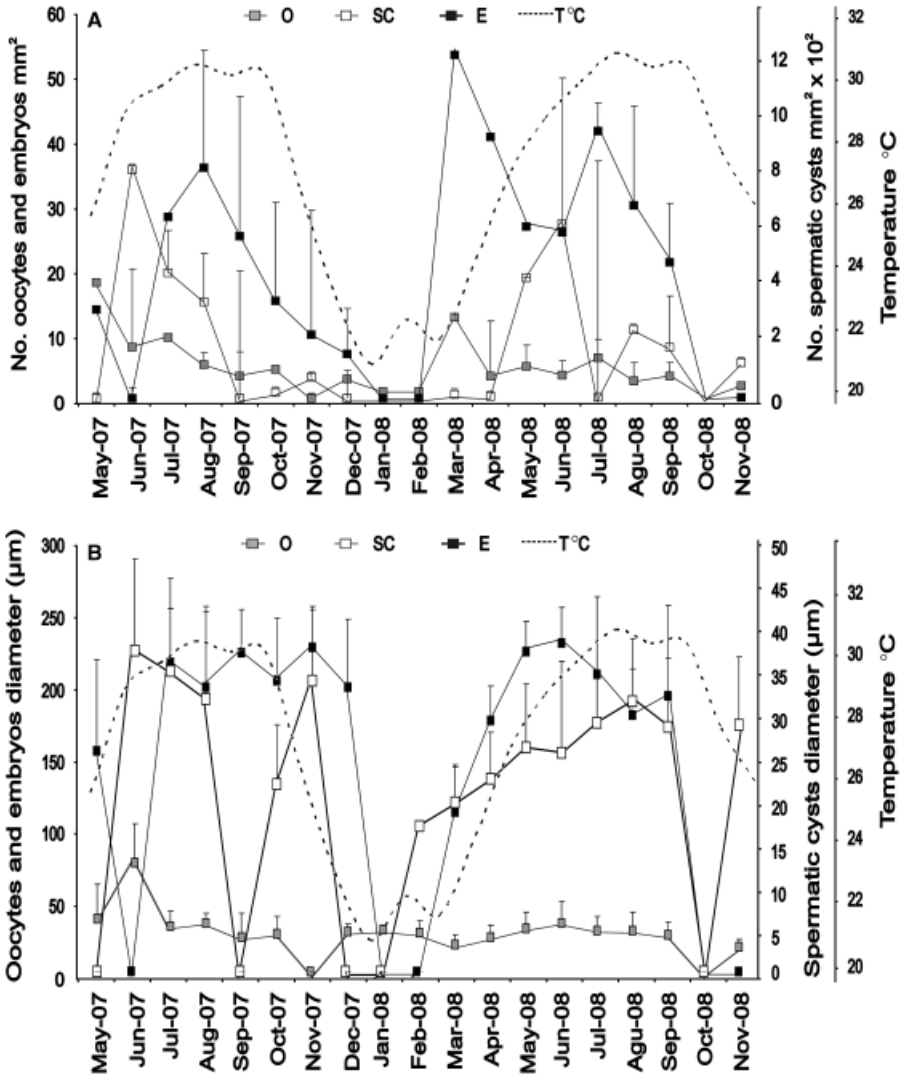

Fig. 2. A. Mean density (+1 standard deviation [SD]) of reproductive elements in tissue of *Thoosa mismalolli* ($n = 10$ samples per month). **B.** Mean diameter (+1 SD) of reproductive elements in tissue of *T. mismalolli* ($n = 10$ samples per month). Note that for some data points, error bars are smaller than the size of the symbols. Sea surface temperature is shown as the dashed curve. E, embryos; O, oocytes; SC, spermatic cysts.

somewhat amoeboid, 70–120 µm in diameter, with a large nucleus (16–23 µm) and abundant yolk bodies (Fig. 3D).

Spermatogenesis

Low to moderate densities of spermatic cysts occurred during most months, with the exceptions of December 2007 and January 2008, when the water temperature was at or near annual minimums (23.2°C and 21.1°C, respectively). In 2007, spermatogenesis was first observed in June and proceeded for 4 months; this was a period of high seawater temperatures (28–30°C). In 2008, spermatic density increased from ~13 spermatic cysts mm^{-2} in March–April to 640 spermatic cysts mm^{-2} in June, and then decreased progressively to 131 spermatic cysts mm^{-2} (Fig. 2A). The average diameter of spermatic cysts ranged from 36.8 ± 10.5 µm in June to 21.5 ± 6.8 µm in October (Fig. 2B). In 2008, spermatic cyst size increased from 16.7 ± 1.2 µm in February to 30.0 ± 7.2 µm in August, concurrent with the seasonal increase in water temperature (Fig. 2B). These changes were also significantly correlated with water temperature, though the association between the variables was weak (Fig. 2B; $r = 0.54$, $p < 0.05$).

Cysts were rounded or oval in section and were haphazardly distributed in the mesohyl (Fig. 3E). Spermatic cysts in different stages of spermatogenesis often co-occurred in close proximity to each other (Fig. 3F). Our observations by light microscopy suggest that the maturation of spermatozoa within a cyst was synchronous. Initially, the spermatic cysts contained large cells (spermatocytes) measuring ~2.5–3 µm in diameter, which became more numerous and smaller in size during spermatogenesis (Fig. 3F). Late-stage cysts showed numerous and slightly elongated spermatids or spermatozoa (1.4–1.7 µm head

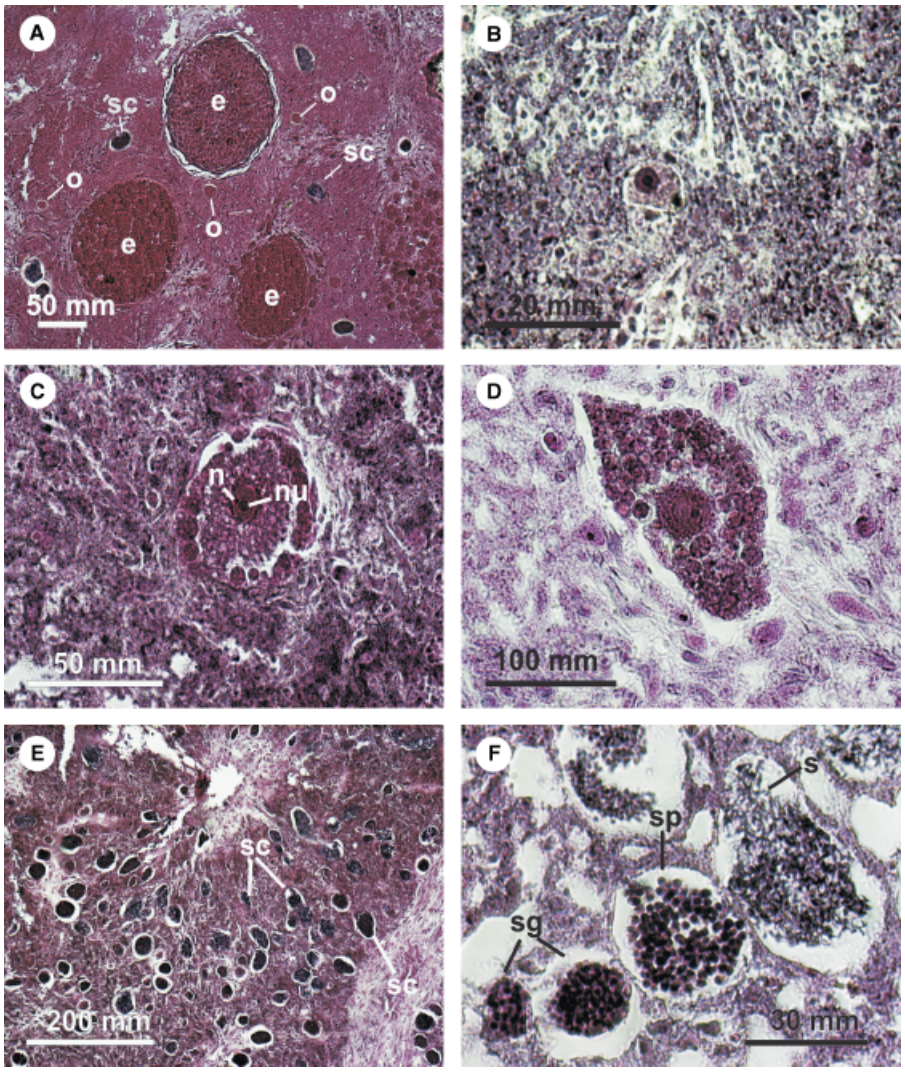

Fig. 3. Gametogenesis in specimens of *Thoosa mismalolli*. **A.** Co-occurrence of early-stage oocytes, embryos, and spermatic cysts in the mesohyl. **B.** Early-stage, previtellogenic oocyte. **C.** Mature oocyte in early vitellogenesis, and a nucleus with a nucleolus. **D.** Late-stage oocyte in late vitellogenesis. **E.** Occurrence of spermatic cysts in the mesohyl. **F.** Asynchronous spermatic cysts showing a maturation gradient, consisting of spermatogonia, spermatocytes, and nearly mature spermatozoa. e, embryos; n, nucleus; nu, nucleolus; o, oocytes; s, spermatozoa; sc, spermatic cysts; sg, spermatogonia; sp, spermatocytes.

diameter) with a visible and large flagellum. We could not resolve whether spermatocytes and spermatozoa had a particular orientation within the cysts.

Embryogenesis

Embryos occurred during most months of the study, but peaked in abundance during the warmer period of each year (Fig. 2A). Density of embryos was not significantly correlated with temperature. In 2007, the average density increased from 14 ± 2 embryos mm^{-2} in May to 36 ± 22 embryos mm^{-2} in late August 2007. During 2008, the highest embryo density (54 embryos mm^{-2}) was recorded in March, decreasing gradually in subsequent months (Fig. 2A). This pattern suggests that new embryos were produced almost continuously over the year, except for a 2-month break during the coldest period (January–February; Fig. 2A).

Embryo development was asynchronous both within and between sponge individuals (Fig. 4). Often, small round embryos (80–150 μm) consisting of undifferentiated blastomeres (UE) co-occurred in the sponge mesohyl with mid-size embryos (160–230 μm) showing cell differentiation and initial formation of radial protrusions that lacked spicules (AE), and late-stage embryos (240–289 μm) with longer radial protrusions and with skeletal components at the periphery of the embryonic body and within the protrusions (SE) (Fig. 5). In 2007, the average diameter of embryos (all developmental stages pooled together) increased from May (150.8 ± 60.2 μm) to a maximum mean size in November (222.7 ± 25.7 μm; Fig. 2B). In 2008, size increased from March (109.5 ± 31.5 μm) to a maximum mean size in June (226.1 ± 24.3 μm). When developmental stages were considered separately, it was clear that the percentage of late embryos (SE) progressively increased from early

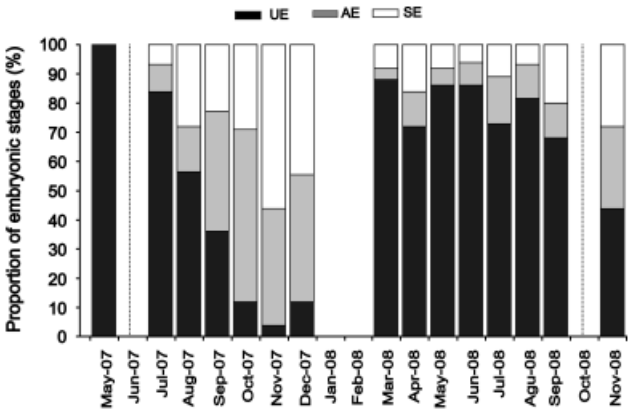

Fig. 4. Average monthly proportion of embryonic stages observed during the study. Each month, 25 embryos from each of ten individuals were categorized as undifferentiated embryos (UE), aspiculate embryos bearing small radial protrusions (AE), or spiculate embryos (SE). Missing data are represented by dashed vertical lines.

spring to autumn, with a corresponding parallel decrease in early-stage embryos (UE, AE) (Fig. 4).

During development, a marked change in the organization and architecture of embryos was noticed (Fig. 5). The first cleavage of a zygote heavily charged with large yolk bodies (Fig. 5A) produced two elongated blastomeres that became separated by a thick band of collagen fibrils (Fig. 5B). In the subsequent cleavages, these between-blastomere collagen bands thinned, and yolk bodies (6–13 μm diameter) progressively decreased in size (2.8–5 μm diameter) and density (Fig. 5C). During these early cleavage stages, all blastomeres were of similar sizes; differentiation into macromeres and micromeres was not seen. Early embryonic stages still lacking cellular differentiation (i.e., UE and AE stages) ranged from 80 to 230 μm in diameter, suggesting a 23-fold increase in volume from the UE to the AE stage.

After the AE stage, some level of cellular differentiation was detected (Fig. 5D). Embryonic cells became smaller, were separated by larger intercellular spaces, and started reorganizing their relative position, some differentiating distinctive cytoplasmic features. Around the embryo, which at this stage was composed exclusively of pseudo-spherical yolk-filled blastomeres, a follicle-like envelope of flattened cells appeared (Fig. 5E). The cytoplasm of some blastomeres (mostly those in the periphery) stained more intensely than others, suggesting that cell differentiation had begun at this stage. Intercellular collagen bands were no longer obvious (Fig. 5F).

In a later stage, groups of small, elongated cells appeared at several equidistant locations around the periphery of the embryo (Fig. 5F,G). Each group was formed of cells (possibly sclerocytes) that radiated from a central point (Fig. 5H). Subsequently, these elongated cells proliferated in number and migrated outwards to produce small, radial protuberances at points equidistant from the embryo's surface (Fig. 5H,I). At the surface between the protuberances, the outermost cells flattened to form an epithelium-like structure. The subepithelial cells also became slightly flattened and oriented parallel to the epithelium, whereas most of the internal cells became slightly elongated and radially oriented (Fig. 5J). In the innermost region of the embryos, cell density was very low, with many intercellular spaces, reminiscent of a central cavity. At this stage, embryos averaged 226.1 ± 24.3 μm in total diameter (Fig. 5K). After the radial protuberances were formed, production of siliceous spicules began (Fig. 5L). A monolayer cover of flat, oval, disk-like spicules (i.e., discotriaenes; 65×30 μm on average) was secreted from the periphery of the embryos (Fig. 5L,M). Spicules were not obvious in the histological micrographs because they had been dissolved in HF before tissue sectioning and the space they occupied was vacant (Fig. 5L); however, the SEM analysis confirmed the presence of discotriaenes (Fig. 5L,M). Tiny amphiasters (20.3 ± 3 μm length) also occurred in the developing embryos. These microscleres were occasionally located among the discotriaenes that reinforced the peripheral skeletal layer. Each radial protrusion contained one (occasionally two) needle-like long spicules, characterized by rounded ends (i.e., strongyles). These spicules constituted the internal skeletal axis of the protrusions, and were oriented radially (Fig. 5N). A thin cell layer covered these long strongyles and formed small accumulations of organic tissue at the outer spicule end (Fig. 5O,P). The spicules protruded 250–270 μm beyond the embryo surface, similar to the skeletal components of radiolarians. Examination of embryos dissected intact from the mesohyl indicated that the number of protuberances was ≥ 10 per embryo. At this stage of development, the diameter of the embryonic body (minus the spicules) averaged 290 ± 40 μm. To migrate from the mesohyl to the aquiferous canals before release, these "radial" embryos (Fig. 5N) underwent a remarkable reshaping, involving reorienting the radial spicules toward a putative "posterior" embryonic pole (Fig. 5O) and suggesting the existence of an anterior–posterior axis that would otherwise have been unnoticed.

Gemmulation

Although production of gemmules in *T. mismalolli* was a continuous process, intensity increased from June to November 2007, when a decrease in water

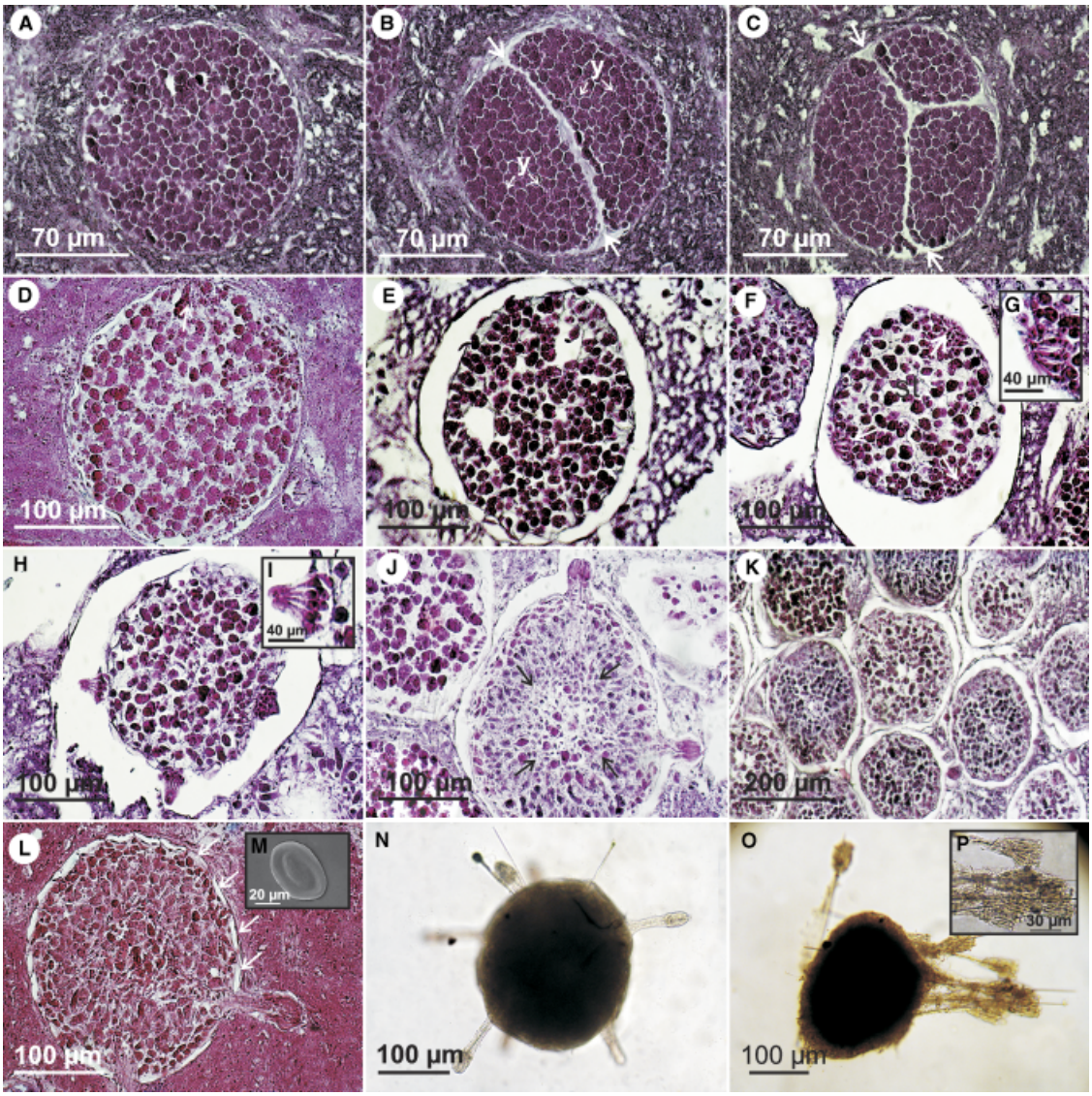

Fig. 5. Early embryonic development. **A.** Early embryo (UE). **B.** First division of a zygote heavily charged with large yolk bodies (y). Note the thick band of collagen fibrils that separate the two blastomeres (arrows). **C.** A later stage embryo. Division furrows (arrows) are filled with collagen fibrils. **D.** Embryo stage (AE) in which the internal cell arrangement becomes less dense and some cell differentiation starts in the peripheral blastomeres. **E.** Embryo at an early stage of cell differentiation. **F.** Embryo that has developed elongated cells, probably sclerocytes (st), at several equidistant regions of the outer epithelium. **G.** Details of likely sclerocytes. **H.** Embryo showing elongated cells that are migrating toward the outer epithelium to initiate formation of the radial protrusions. **I.** Details of radial protrusions. **J.** Embryo with initial protrusions. Note that the most internal cells became slightly elongated and radially arranged (arrows). **K.** High abundance of AE embryos in the sponge mesohyl. **L.** Late-stage embryo (SE) showing the peripheral skeleton (arrows) of discotriaenes and development of radiating protrusions. **M.** Scanning electron micrograph of discotriatene spicule from periphery of embryo. **N.** Hoplitomella larva dissected from an aquiferous canal, showing the complete spicule skeleton. **O.** Migrating hoplitomella larva dissected from the mesohyl, showing the orientation of the formerly radial spicules toward a putative "posterior" embryonic pole. **P.** Detail of accumulation of organic tissue at the outer ends of the larva's spicules. St, sclerocytes; y, yolk bodies.

temperature occurred (Fig. 6). Highest gemmule density (8 ± 4 gemmules mm^{-2}) occurred in November. In 2008, density was comparatively low (2 ± 1 gemmules mm^{-2}) during most of the year except for January and February (3 and 5 gemmules mm^{-2}, respectively). However, no significant correlation was found between gemmule production and seawater temperature ($r = 0.006$, $p > 0.74$). The average diam-

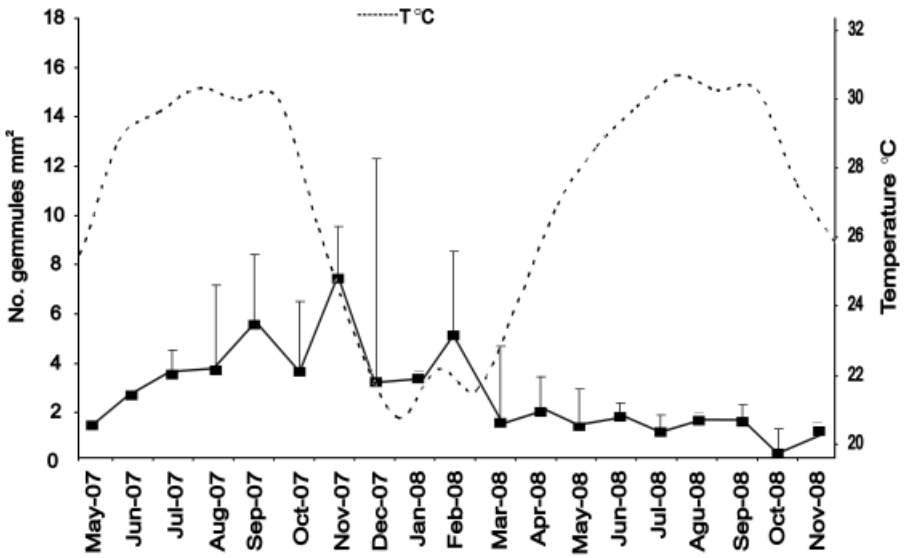

Fig. 6. Mean density (+1 SD) of asexual reproductive elements in tissue of *Thoosa mismalolli* ($n = 10$ samples per month). Note that for some data points, error bars are smaller than the size of the symbols. Sea surface temperature is shown as the dashed curve.

eter of gemmules increased from April 2007 ($204.8 \pm 22\,\mu m$) to a maximum mean size ($249.7 \pm 120.9\,\mu m$) in May 2007 and from February 2008 ($168.6 \pm 95.3\,\mu m$) to a maximum mean size ($269.4 \pm 146.1\,\mu m$) in May 2008.

Numerous internal gemmules (three to eight per coral erosion chamber) co-occurred with gametes and embryos during the annual cycle (Fig. 6). They were either spread throughout the sponge mesohyl, or attached to the ectosome that lined the erosion chambers (Fig. 7A,B). The gemmules were bright yellow, subspherical to lenticular, with a 3–8.5-μm-thick coat when viewed with light microscopy (Fig. 7C,D). Both light microscopy (Fig. 7A,B) and SEM (Fig. 7E) observations suggested that this dense, protective coat consisted of a collagenous matrix that contained multiple layers of small, interlocking amphiasters (Fig. 7E,F). In the innermost region, the gemmules contained a small group of cells. The largest region of the gemmule, from the central cellular zone to the external coat, was acellular and cavernous. It consisted of homogenous material (possibly collagenous matrix) with irregular spaces and was without spicules (Fig. 7D).

Discussion

Sexual reproduction

The current study demonstrates that individuals of *Thoosa mismalolli* are hermaphroditic and viviparous, with the processes of gametogenesis and embryogenesis occurring simultaneously during most months of the year. In contrast, other clionaid sponges, such as members of *Cliona* and *Pione,* are characterized by short periods of gametogenesis and a single, brief event of gamete release, typically restricted to one or two days a year (Pomponi & Meritt 1990; Mariani et al. 2000, 2001; Fromont et al. 2005). We found oocyte production in our population of *T. mismalolli* during most parts of the year, in a pattern similar to that reported for populations of the Mediterranean sponge *Corticium candelabrum* Schmidt 1862 (Riesgo et al. 2007; Riesgo & Maldonado 2008), and without a post-reproductive recovery period as reported for other demosponges (Turón et al. 1999). Although the period of spermatogenesis was shorter than that of oogenesis, it was relatively long when compared with other clionaids, where it usually lasts one to a few weeks (e.g., Mariani et al. 2000, 2001). Riesgo et al. (2007) suggested that prolonged spermatogenesis at the population level may increase the chances of fertilization when mature oocytes are available over several months. Prolonged periods of either spawning or larval release may decrease the risk of larval mortality by spreading the risk of all larvae encountering the same unfavorable conditions (e.g., Ilan & Loya 1990; Ettinger-Epstein et al. 2007; Riesgo et al. 2007).

The timing and duration of reproduction may be determined by a combination of internal physiological processes and environmental factors such as water temperature (or one of its possible correlates, e.g., food availability). For many sponges, reproductive period has been associated with increasing (e.g., Usher et al. 2004; Fromont et al. 2005; Badalcconi et al. 2007; Riesgo & Maldonado 2008) or decreasing seawater temperatures (e.g., Riesgo & Maldonado 2008; Maldonado & Riesgo 2009). In our study, only the number of individuals with reproductive elements

and spermatic cyst size were positively (but weakly) correlated with water temperature. The lack of a significant correlation between water temperature and gametogenic activity seems to be evident because both spermatogenesis and oogenesis occurred simultaneously in the mesohyl of the sponges during most of the year. However, despite this lack of correlation, it appears that temperature is important to the reproductive cycle of this species, because the highest production of oocytes and spermatic cysts seems to be coupled with a seasonal rise in water temperature during late spring and summer (Fig. 2), a pattern previously reported in other viviparous sponges (e.g., Baldaconni et al. 2007; Whalan et al. 2007). Data from additional reproductive seasons will be needed to gain a complete understanding of the effect of temperature on the reproduction of *T. mismalolli* and to formally test this hypothesis.

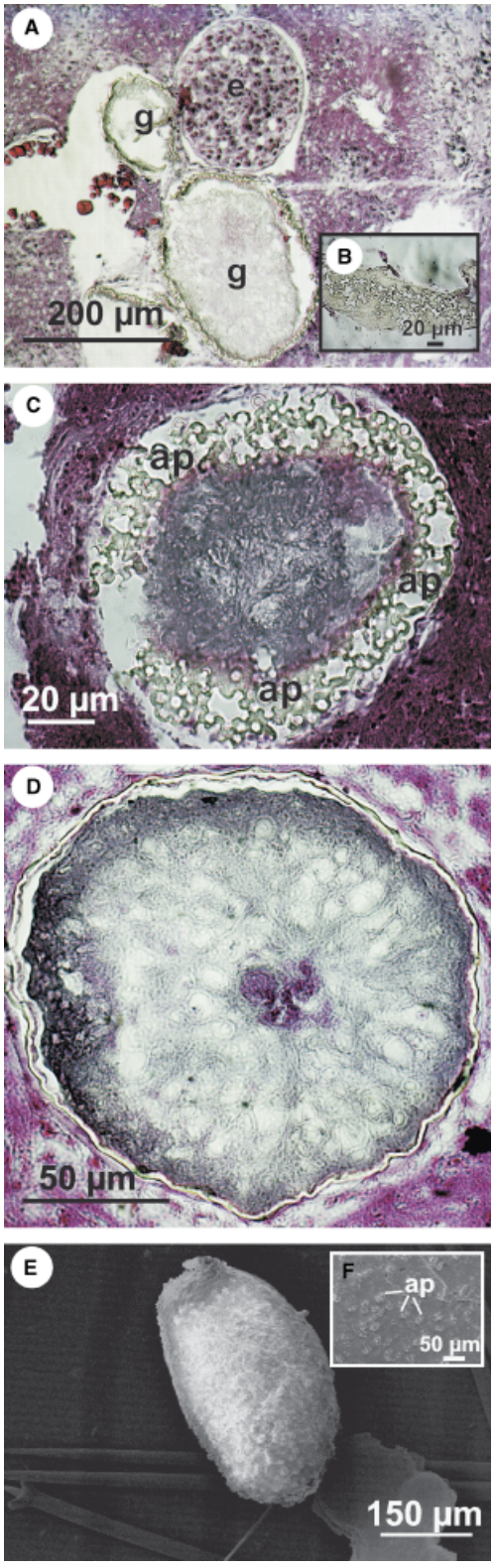

Fig. 7. Gemmules of *Thoosa mismalolli*. **A.** Co-occurrence of embryo and gemmules in the mesohyl. **B.** Detail of gemmule coat, which includes numerous amphiasters (ap). **C.** Gemmule with a dense coat of amphiasters. **D.** Internal details of gemmule. **E.** Scanning electron micrograph of a gemmule. **F.** Detail of (E), showing the gemmule coat with numerous amphiasters in an irregular arrangement. ap, amphiasters; e, embryo; g, gemmule.

Embryogenesis and larva formation

To date, the development of hoplitomella larva was known only from observations on members of the genus *Alectona* (Vacelet 1999). Our study provides the first developmental data for the genus *Thoosa*. Embryogenesis and the morphology of larvae of *T. mismalolli* showed some similarities to those of *Alectona wallichii* Carter 1874 and *Alectona mesatlantica* Vacelet 1999 (Vacelet 1999), and some special characteristics were also detected. Cleavage was total and equal in all species, producing a stereoblastula characterized by blastomeres filled with large yolk bodies and abundant intercellular collagen. During early cleavage stages the blastomeres were separated from each other by a thick collagen layer that thinned progressively in subsequent cleavage steps. Abundant secretion of collagen by early blastomeres appears to be characteristic for embryos of *Thoosa* and *Alectona,* and highly unusual in the embryos of other sponges.

The most peculiar traits of the hoplitomella larvae were the absence of cilia and the secretion of a unique larval skeleton. Larval skeletogenesis began after some blastomeres differentiated into sclerocytes, and involved remarkable changes in the internal and external embryonic architecture. Although the secretion of siliceous spicules by sponge larvae has been described in many sponges, the larvae of *Alec-*

tona and *Thoosa* are unusual because the larval spicules are completely different from those found in the adult stage. In larvae of *A. wallichii* and *A. mesatlantica,* three spicule types occur simultaneously (thin styles, amphiasters, and discotriaene-like plates; Vacelet 1999). Three spicule types were also reported from the larvae of *Thoosa armata* by Topsent (1904), and three spicule types also occurred in larvae of *T. mismalolli:* long, thin strongyles protruding radially from the larval body, rare tiny amphiasters, and numerous plate-like structures, which are interpreted as discotriaenes with missing rhabdomes. These spicules do not occur in the adult stage. Maldonado (2004) suggested that the occurrence of discotriaenes in the larvae of *Thoosa* and *Alectona* indicated that these genera do not belong to the family Clionaidae (order Hadromerida) and suggested transferring them to the order Astrophorida. The striking differences in embryonic development and larval architecture between members of *Thoosa* and *Alectona* and the remaining clionaids also support such a reallocation. Further, a genetic study based on 28S rDNA sequences by Borchiellini et al. (2004) corroborated the suggestion that *Alectona millari* (Carter, 1879) was more closely related to members of the order Astrophorida than to members of the Hadromerida.

Another peculiar trait in the development of embryos of *T. mismalolli* was the reorientation of the radial spicules toward a putative ''posterior'' embryonic pole when migrating from the mesohyl to the excurrent canals before larval release. We suggest that such a skeletal rearrangement may facilitate the movement of the embryo through the mesohyl to reach the canals, and also prevent canal clogging in the case of many larvae being released simultaneously. A similar reorientation of the radial spicules was described in embryos of *T. armata* dissected from a brooding sponge by Topsent (1904). In contrast, the planktonic larvae of *T. mismalolli* collected from the study location (unpubl. data) had a radial arrangement of spicules. A radial arrangement has also been reported from hoplitomella larvae drifting in the plankton (e.g., Karawaiew 1896; Trégouboff 1942). In radiolarians, similar radially protruding skeletal pieces are postulated to serve as flotation devices (e.g., Afanasieva 2007). By slightly modifying the particular spatial arrangement of the protruding spicules and the extent of protrusion the surface area, the strength of the viscous forces of seawater acting on the larval body vary, allowing the larva to have a rough control of its vertical position in the water column (Trégouboff 1942; Vacelet 1999; Maldonado & Bergquist 2002; Maldonado 2006). Our observations also revealed a thin cell layer covering the protruding strongyles, as well as small accumulations of cells at the outer spicule end (Fig. 5O,P). This cellular cover does not appear to allow for movement of these spicules, which might potentially act as paddles. However, we have no evidence that these larvae are able to perform any rapid movement of their radial protrusions. Instead, we suggest that this cell layer is involved in the process of secreting and dissolving silica to slowly modify the length and orientation of the spicules during larval life, particularly during the skeletal resorption that occurs at the end of larval life.

Asexual reproduction

Gemmules are propagules for asexual reproduction known from many freshwater and some marine demosponges. Within the family Clionaidae gemmules have been reported in members of the genera *Pione, Cliona,* and *Thoosa* (e.g., Volz 1939; Wells et al. 1964; Rützler 1974; Rosell & Uriz 2002; Schönberg 2002). Continuous gemmule production similar to that found in adults of *T. mismalolli* is known in the Mediterranean sponge *Pione vastifica* Hancock 1849 (Topsent 1900). In other members of the genus *Thoosa,* such as *T. mollis* from the Adriatic Sea, gemmules were found only during mid-July and late October (Volz 1939). Recent work by Schönberg (2002) suggests that the gemmules of *Pione lampa* De Laubenfels 1950 provide a crucial adaptation to persist in sabellariid worm reefs in Florida, where the sand-grain framework built by the worms can easily be disintegrated by frequent storms and hurricanes. Some coral communities from the Eastern Pacific region where populations of *T. mismalolli* are found are also exposed to nearly continuous disturbance due to the concurrence of a variety of physical perturbations though the year (i.e., severe storms, hurricanes, droughts, etc.). Such disruptive processes commonly involve coral fragmentation, disintegration of the reef framework and exposure to extreme environmental conditions, with drastic shifts in salinity and temperature, high sedimentation rates, and intense siltation (Rogers 1990; Cortés 1997). The continuous risky conditions experienced by populations of *T. mismalolli* may have selected for production of gemmules over the entire year, as a mechanism to compensate for the loss of adults and facilitate recruitment. It is noteworthy that the highest production of gemmules occurred during the coldest months of the year, a period during which the production of sexual larvae was at a minimum. Therefore, it appears that populations of *T. mismalolli* combine the mechanisms of sexual and asexual reproduction to

function as continuous sources of propagules. Such a reproductive strategy ensures population persistence in an ever-changing environment and facilitates effective between-reef dispersal.

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References

Afanasieva MS 2007. Radiolarian skeleton: morphology of spines, internal framework, and primary sphere. Paleontol. J. 41: 1–14.

Annandale N 1915. Indian boring sponges of the family Clionidae. Rec. Indian Mus. 11: 1–24.

Baldaconni R, Nonnis-Marzano C, Giano E, & Corriero G 2007. Sexual reproduction, larval development and release in *Spongia officinalis* L. (Porifera, Demospongiae) from the Apulian coast. Mar. Biol. 152: 969–979.

Borchiellini C, Alivon E, & Vacelet J 2004. The systematic position of *Alectona* (Porifera, Demospongiae): a tetractinellid sponge. Boll. Mus. Istit. Biol. dell'Univ. Genova 68: 209–217.

Carballo JL, Cruz-Barraza JA, & Gómez P 2004. Taxonomy and description of clionaid sponges (Hadromerida, Clionaidae) from the Pacific Ocean of Mexico. Zool. J. Linn. Soc. 141: 353–387.

Carballo JL, Hepburn L, Nava HH, Cruz-Barraza JA, & Bautista-Guerrero E 2007. Coral boring *Aka*-species (Porifera: Phloeodictyidae) from Mexico with description of *Aka cryptica* sp. nov. J. Mar. Biol. Assoc. UK 87: 1477–1484.

Carballo JL, Bautista-Guerrero E, & Leyte-Morales GE 2008. Boring sponges and the modeling of coral reefs in the east Pacific Ocean. Mar. Ecol. Prog. Ser. 356: 113–122.

Cortés J 1997. Biology and ecology of eastern Pacific coral reefs. Coral Reefs 16: S39–S46.

Ettinger-Epstein P, Whalan WS, Battershill ChN, & Nys R 2007. Temperature cues gametogenesis and larval release in a tropical sponge. Mar. Biol. 153: 171–178.

Fromont J, Craig R, Rawlinson L, & Alder J 2005. Excavating sponges that are destructive to farmed pearl oysters in Western and Northern Australia. Aquac. Res. 36: 150–162.

Goreau TF & Hartman WD 1963. Boring sponges as controlling factors in the formation and maintenance of coral reefs. In: Mechanisms of Hard Tissue Destruction, Vol. 75. Soggnaes RF ed., pp. 25–54. American Association for the Advancement of Science Publications, Washington, DC, USA.

Ilan M & Loya Y 1990. Sexual reproduction and settlement of the coral reef sponge *Chalinula* sp. from the Red Sea. Mar. Biol. 105: 25–31.

Karawaiew W 1896. Über ein neues Radiolar aus Villafranca. Zool. Anz. 19: 501.

Macdonald IA & Perry CT 2003. Biological degradation of coral framework in a turbid lagoon environment, Discovery Bay, north Jamaica. Coral Reefs 22: 523–535.

Maldonado M 2004. Choanoflagellates, choanocytes, and animal multicellularity. Invertebr. Biol. 123: 231–242.

——— 2006. The ecology of the sponge larva. Can. J. Zool. 84: 175–194.

Maldonado M & Bergquist PR 2002. Phylum Porifera. In: Atlas of Marine Invertebrate Larvae. Young CM, Sewell MA, & Rice ME, eds., pp. 21–50. Academic Press, San Diego, CA, USA.

Maldonado M & Riesgo A 2008. Reproduction in the phylum Porifera: a synoptic overview. In: Biologia de la reproducció. Durfort M & Vidal F, eds., pp. 29–49. Societat Catalana de Biologia, Barcelona, Spain.

——— 2009. Gametogenesis, embryogenesis, and larval features of the oviparous sponge *Petrosia ficiformis* (Haplosclerida, Demospongiae). Mar. Biol. 156: 2181–2197.

Mariani S, Uriz MJ, & Turon X 2000. Larval bloom of the oviparous sponge *Cliona viridis*: coupling of larval abundance and adult distribution. Mar. Biol. 137: 783–790.

Mariani S, Piscitelli MP, & Uriz MJ 2001. Temporal and spatial co-occurrence in spawning and larval release of *Cliona viridis* (Porifera: Hadromerida). J. Mar. Biol. Assoc. UK 81: 565–567.

Pomponi SA & Meritt DW 1990. Distribution and life history of the boring sponge *Cliona truitti* in the Upper Chesapeake Bay. In: New Perspectives in Sponge Biology. Rützler K, ed., pp. 384–390. Smithsonian Institution Press, Washington, DC, USA.

Riesgo A & Maldonado M 2008. Differences in reproductive timing among sponges sharing habitat and thermal regimen. Invertebr. Biol. 127: 357–367.

Riesgo A, Maldonado M, & Durfort M 2007. Dynamics of gametogenesis, embryogenesis, and larval release in a Mediterranean homosclerophorid demosponge. Mar. Freshw. Res. 58: 398–417.

Rogers CS 1990. Responses of coral reefs and reef organisms to sedimentation. Mar. Ecol. Prog. Ser. 62: 185–202.

Rosell D & Uriz MJ 2002. Excavating and endolithic sponge species (Porifera) from the Mediterranean: species descriptions and identification key. Org. Divers. Evol. 2: 55–86.

Rützler K 1974. The burrowing sponges of Bermuda. Smithson. Contrib. Zool. 165: 1–32.

Rützler K & Rieger G 1973. Sponge burrowing: fine structure of *Cliona lampa* penetrating calcareous substrata. Mar. Biol. 21: 144–162.

Schönberg CHL 2002. *Pione lampa,* a bioeroding sponge in a worm reef. Hydrobiologia 482: 49–68.

Topsent E 1900. Étude monographique des spongiaires de France 3. Monaxonida (Hadromerina). Arch. Zool. Exp. Gén. 8: 1–331.

Topsent T 1904. Spongiaires des Açores. Rés. Camp. Sci. Monaco 25: 109–111.

Trégouboff G 1942. Contribution à la connaissance des larves planctoniques d'éponges. Arch. Zool. Exp. Gén. 82: 357–399.

Turón X, Uriz MJ, & Willenz P 1999. Cuticular linings and remodelisation processes in *Crambe crambe* (Dempsogongiae: Poecilosclerida). Mem. Qld. Mus. 44: 617–625.

Usher KM, Sutton DC, Toze S, Kuo J, & Fromont J 2004. Sexual reproduction in *Chondilla australiensis* (Porifera: Demospongiae). Mar. Freshw.. Res. 55: 123–124.

Vacelet J 1999. Planktonic aromoured propagules of the excavating sponge *Alectona* (Porifera: Demospongiae) are larvae: evidence from *Alectona wallichii* and *A. mesatlantica* sp. nov. Mem. Qld. Mus. 44: 627–642.

Volz P 1939. Die Bohrschwämme (Clioniden) der Adria. Thalassia 3: 1–64.

Warburton FE 1958. Reproduction of fused larvae in the boring sponge, *Cliona celata* Grant. Nature 181: 493–494.

Wells HW, Wells MJ, & Gray IE 1964. Ecology of sponges in Hatteras Harbor, North Carolina. Ecology 45: 752–767.

Whalan S, Battershill C, & de Nys R 2007. Sexual reproduction of the brooding sponge *Rhopaloeides odorabile*. Coral Reefs 26: 655–663.12