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REPORT

Morphological organization and ultrastructural evaluation of the oocyte–sperm bundle of the Southwestern Atlantic coral *Mussismilia harttii*

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Abstract Most coral species of known reproductive traits are broadcast spawners, with about 65% being hermaphrodites that envelop their gametes together in oocyte–sperm bundles. While these bundles are crucial for the dispersion and successful cross-fertilization of gametes, research evaluating their structure in detail is rare in the literature. Here, we investigated the composition of the bundle and the arrangement of the gametes within, before and after dissociation, using histological and ultrastructural analyses. The endemic coral *Mussismilia harttii* was used, since it is one of the main reef builders in Brazil and is listed as an endangered species. Our results showed that there is no pattern to the distribution of gametes in the bundle, though there are regions of higher concentrations reaching 91 spermatozoa mm^{-2}. The dissociation of the bundle promotes important morphological changes to the oocytes, making them larger and with less dense and thicker microvilli on the surface. Spermatozoa are located within hexagonal-shaped structures, similar to the alveoli in a honeycomb. Given the morphological structural complexity observed, we believe that the release of spermatozoa occurs through the complete rupture of the walls of spermatic cysts and that such structure is a component of the spermatocytes. Once released into the gastrovascular cavity, the spermatocytes are enveloped in mucus, maintaining spermatozoa in the same organization. Our findings shed light on a poorly explored area that can assist in a better understanding of hermaphrodite broadcasting coral fertilization and provide support for future studies on reproductive biotechnologies for coral conservation.

Keywords Sexual reproduction · Coral spawning · Hermaphrodite · Gametes · Electron microscopy

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Introduction

Coral reefs are among the most biodiverse hotspots in the world and are severely threatened by anthropic activities (Hughes 2019). Understanding how coral reproduce is pivotal for better management and conservation plans. Scleractinian corals can reproduce both asexually, via fragmentation (Wallace 1985; Ayre and Resing 1986), and sexually, either via brooding their fertilized planulae internally, or broadcasting their gametes externally (Babcock et al. 1986.). About 86% of coral species with described reproductive traits are categorized as broadcast spawners (Baird et al. 2009.), a strategy that is ancestral to internal methods of fertilization (Gallego et al. 2014).

Corals can be either gonochoric, presenting colonies or polyps of a single sex (male or female), or hermaphrodites. In the latter case, a single colony presents both sexes, which can occur as hermaphrodite polyps in a whole colony or colonies with a mixture of male and female polyps (Richmond 1997; Harrison and Wallace 1990; Harrison 2011). Studies show that about 65% of known scleractinian coral species are predominantly hermaphrodites (Guest et al. 2008; Baird et al. 2009). Most hermaphrodite species envelop their gametes with a type of mucus and expel them as small floating packages (Kinzie 1996). Each bundle can contain hundreds of oocytes and billions of spermatozoa, depending on the species (Godoy et al. 2021). These oocyte–sperm bundles

have an important role in transporting gametes to the ocean surface, which minimizes sperm dilution and increases the chance of spermatozoa and oocytes from different colonies meeting. After reaching the ocean surface, the bundle dissociates apart, allowing successful cross-fertilization, and promotes genetic diversity for the coral offspring (Harrison and Wallace 1990; Richmond 1997; Baird et al. 2009; Harrison 2011).

Despite there being over 840 scleractinian corals known around the world (see Veron 2000; Madin et al. 2016; Veron et al. 2016), there are limited published studies describing their gametic morphology (Wallace 1985; Richmond 1997; Steiner 1991, 1993, 1998; Steiner and Cortés 1996; Goffredo et al. 2000; Wolstenholme 2004; Vargas-Ángel et al. 2006; Kawaroe et al. 2007; Padilla-Gamiño et al. 2011; Tsai et al. 2016; Leite et al. 2017; Lin et al. 2018). Only five of these studies present details about the oocyte–sperm bundle for ten species, with just one study (Padilla-Gamiño et al. 2011) specifically evaluated the morphology of the bundle through histological and ultrastructural analyses for *Montipora capitata*. Thus, there is a gap in the basic knowledge about the morphology and ultrastructure of the gamete bundles that drive successful fertilization in scleractinian corals.

The only biogenic reefs of the South Atlantic Ocean are found along the Brazilian coast (Castro and Zilberberg 2016). Brazilian reefs extend over more than 3,000 km (Leão et al. 2016; Pereira-Filho et al. 2019) and are characterized by low coral diversity and high endemism (Castro and Pires 2001). The main reef builders belong to the genus *Mussismilia* Ortmann 1890, which is endemic to Brazil (Leão et al. 2016) and had their reproduction features described by the pioneering studies of Pires et al. (1999). The cauliflower coral (*Mussismilia harttii*) is a simultaneous hermaphroditic species, releasing both gametes together in buoyant oocyte–sperm bundles during nights directly preceding and following the new moon from the months of September to November (Pires et al. 1999; Neves and Pires 2002; Pires et al. 2016).

With the advance of climatic alterations in the planet, bleaching and mortality events are becoming increasingly extreme, threatening the future of coral reefs (Hughes et al. 2018, 2019). *Mussismilia harttii* is already listed as an endangered species along the Brazilian coast (ICMBio 2018). Thus, understanding sexual reproduction will have a key role in the resilience of the species since the offspring of more resilient individuals will inherent genetic characteristics that can aid coral adaptation to a changing environment. Further understanding of this complex called a bundle and its possible relationships with cross-fertilization can be advantageous in conservation programs, since successful recruitment from sexual reproduction is mainly responsible for the maintenance of coral populations (Cameron and Harrison 2020).

In our study of the South Atlantic coral *M. harttii*, we evaluate the composition of oocyte–sperm bundles before and after dissociation, and the organization of the gametes within by using histological and ultrastructural analyses. The fine description of the ultrastructure of the single gametes will be presented later, in a different paper dedicated to such objective.

Material and methods

Colony sampling and legal authorizations

Forty colonies of the coral *M. harttii* were collected from around the Recife de Fora Marine Protected Area (16°24′31″S; 038°58′39″W—Bahia State, Brazil) under SISBIO license Nº 63,368–1 on fifth of September, roughly three weeks before the expected spawning period for the species (Pires et al. 1999; 2016). These colonies were taken to the Research Base of the Coral Vivo Project, where they were identified and kept in circular (1000 L) semi-closed tanks connected to the ocean.

Sampling and separation of gametes

During spawning monitoring (from 27 to 29 of September), the water flow of the tank was closed, and then, the bundles containing oocytes and spermatozoa were collected from the water surface of the tanks. A total of 16 colonies spawned over the three nights. Three bundles were sampled from each colony ($n = 6$) and immediately transferred to 10 mL tubes containing fixing solutions ($n = 18$). Another 15 bundles were collected from the same six colonies (total = 90) and placed in 50 mL tubes (three bundles per tube) containing seawater, where they remained still until their complete dissociation with the separation of oocytes and spermatozoa. The oocytes floated and occupied the surface of the tube, while the dense mucus encasing the sperm sank. The oocytes were then removed from the tube using a Pasteur pipette, rinsed in filtered seawater to remove any trace of sperm and mucus, and transferred to a fixing solution. Aliquots (± 1 mL) containing spermatozoa were collected from the bottom of the tube and transferred to the fixing solution.

Scanning electron microscopy (SEM)

For the scanning electron microscopy, 12 bundles were fixed in 3% glutaraldehyde with a 0.1 M phosphate buffer solution, following the methodology described by Graham and Orenstein, (2007). The fixed materials were rinsed three times (30 min each) in the same buffer. Dehydration was conducted through a series of increasing concentrations of acetone (30%–100%) for 10 min each, while desiccation of

materials occurred using a critical point device (Critical Point Dryer, Leica EM CPD030, Germany). For metallization, the material received conduction layers of gold and platinum using a Super Cool Sputter Coater (Leica EM SCD050, Germany) and was then observed in a scanning electron microscope (Jeol JSM 6060, USA and Zeiss Evo 50, Germany).

Transmission electron microscopy (TEM)

For the transmission electron microscopy analysis, samples (6 bundles) were fixed in a solution containing 2.5% glutaraldehyde, 2% paraformaldehyde and 0.1 M phosphate buffer (Karnovsky 1965). The pre-fixed material was rinsed three times (30 min each) in the same buffer. Post-fixing was carried out in 2% OsO_4 with a 0.2 M phosphate buffer (45 min), followed by three soaks in the same buffer (15 min each). Dehydration was conducted through a series of increasing concentrations of acetone (30%–100%) for 10 min, and pre-embedding was carried out in soaks, mixing the dehydrant with resin (Epoxy Embedding Kit) in gradual and increasing proportions, with a minimum time of 2 h in each soak. Embedding was conducted with a resin soak (100%) for 24 h, and inclusions were in silicon molds with pure resin in an incubator (60 °C) for 72 h. Ultrathin Sections (80 nm) were collected from the ultramicrotome (Leica, EM UC6, Germany) and deposited on grids and contrasted with an aqueous solution uranyl acetate and lead citrate (2% each), observed in a transmission electron microscope (FEI COMPANY, Tecnai G^2 20 S-TWIN, USA).

Light microscopy (LM)

For histological analyses, samples were fixed and dehydrated following the same procedure as described for samples analyzed through TEM. After dehydration, samples were included in glycol methacrylate (Historesin, Leica, Germany) and cut in sections measuring 3 µm in thickness using a rotary microtome (Leica, RM2255, Germany) with glass blades. The cuts were adhered to glass slides and stained with toluidine blue and hematoxylin–eosin (HE). Samples were mounted with Entellan (Merck, Frankfurt, Germany) and photodocumented with an Olympus BX53 photomicroscope (Tokyo, Japan) using a coupled Olympus DP73 camera.

Image analysis

The obtained images were digitized using Adobe Photoshop CS3 v10.0, where focus, contrast, brightness and gray gradations were adjusted. Qualitative and morphometric studies were performed on these images, collecting information on the various components of the bundles and gametes. For the morphometric analyses, the ImageJ digital analysis program (Microsoft Java 1.1.4.) was used, with quantitative data presented as mean ± standard deviation. The following equation was used to determine oocyte and microvilli diameter (D):

$$D = \frac{larger\ diameter + smaller\ diameter}{2}$$

Results

Spawning and dissociation of the oocyte–sperm bundle

Spawning of *M. harttii* occurred between 17:30 h and 19:30 h over the course of three days (27–29 September 2019) during the new moon. Approximately 5 min before the spawning happened, it was possible to observe an increase in the size of the oral disk and shortening of the tentacles of spawning polyps (Fig. 1A). About 30 s before spawning,

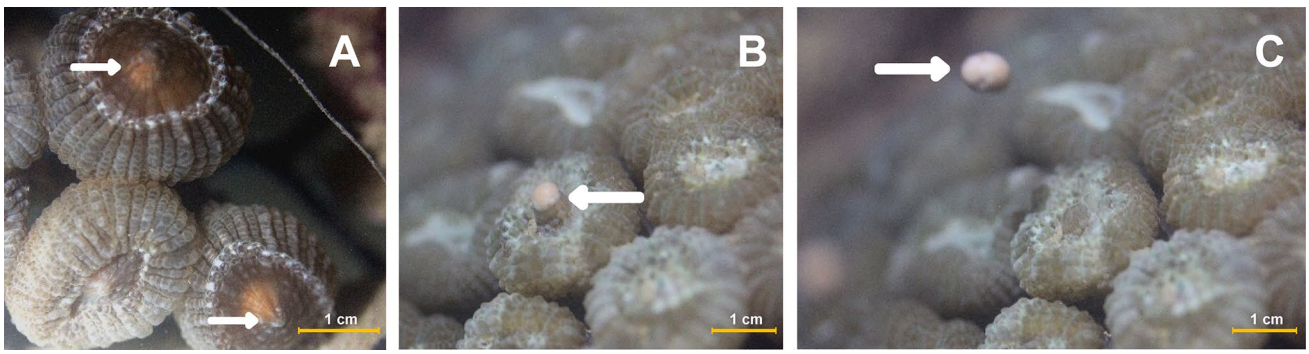

Fig. 1 Sequence of oocyte–sperm bundle release during spawning of *Mussismilia harttii*. **A** spawning polyps with shortened tentacles and increased oral disk. **B** spawning polyps with the gamete bundle visible in the oral disk. **C** bundle released into the water. Photographs by Leandro F. A. Santos/Coral Vivo Project

the oocyte–sperm bundle became visible under the oral disk (Fig. 1B) and was then released into the water (Fig. 1C).

After being released, the bundles floated to the surface. Total dissociation of bundles happened within 10 min, marked initially by the presence of visibly isolated and positively buoyant oocytes. When inside the bundles the oocytes were compressed, showing irregular shape and measured 90.5 ± 0.02 µm in diameter ($n = 76$ measured; Fig. 2A, B). After dissociation of the bundle, 78% of the oocytes became rounded and measured 155.8 ± 0.02 µm in diameter (Fig. 2C, D). However, even after the dissociation of the bundle, it was possible to observe that some oocytes (22%) presented an ovoid morphology, measuring 260 ± 1.21 µm in diameter ($n = 209$ measured; Fig. 2E, F).

Morphology and ultrastructure of oocyte–sperm bundles

The gamete bundles released by *M. harttii* measured 2.95 ± 1.45 mm and held 296 ± 26 oocytes. Immotile spermatozoa and oocytes were randomly distributed in the bundles (Fig. 3A, B). The light microscopy observations indicate that the peripheral regions of the bundle as well as the spaces between oocytes were enveloped in mucus (Fig. 4A–C). This mucus was amorphous and did not show a structural composition. It seems that the mucus was extracted from the samples during the preparation for electron microscopy analyses, and therefore, we could observe empty spaces that used to be occupied by mucus (Fig. 4).

The size of interaction area among spermatozoa and oocytes in the bundle was variable. Regions where spermatozoa and oocytes were in close proximity without any delimitating feature were visible (Fig. 5A), as well as empty regions that used to have large amount of mucus isolating the gametes (Fig. 5B).

We observed morphological differences in the surface microvilli of oocytes before and after their dissociation from the bundle (Fig. 6). While oocytes were associated with the bundle, microvilli were near one another and thinner (0.61 ± 0.08 µm; Fig. 6A). Once dissociated from the bundle, oocytes presented less dense and thicker microvilli measuring 1.08 ± 0.26 µm in diameter (Fig. 6B). The TEM observations revealed cortical vesicles in the oocytes (Fig. 4C), which seem to release granules from the cytoplasm to the extracellular region, since it was possible to see vesicles fused either to the plasma membrane of the oocytes or near the membrane.

Spermatozoa were distributed across the bundle but not in a uniform manner. Some regions had higher concentrations of spermatozoa organized in lines, in which it was possible to count up to 91 spermatozoa mm^{-2} (Fig. 7 A, B), and while in regions of lower concentration (38 cells mm^{-2}), they were dispersed and disorganized (Fig. 7C, D). The analysis of ultrastructural aspects showed, in spermatozoa located in the most peripheral areas, the presence of more than two mitochondria, possibly indicating cell immaturity. In turn, spermatozoa from more central regions presented two separated mitochondria, which could indicate maturity (Fig. 7D–red triangle detail). Spermatozoa are found together and covered by a mucus similar to that coating the entire bundle (Fig. 8), sometimes distributed near the oocytes (Fig. 8A) and other times isolated (Fig. 8B). The spermatozoa are located within hexagonal-shaped structures, similar to the alveoli of a honeycomb (Fig. 8C, D). These structures seem to be made by mucus, were randomly found in the bundle and were in contact with the oocytes. The spermatozoa of *M. harttii* measured 6.25 ± 1.34 µm in total length, with an oval head (0.40 ± 0.11 µm) and a flagellum (5.53 ± 1.25 µm) that represents 88.38% of its total length (Fig. 9A, B).

Discussion

The present study brings new information about the morphology of oocyte–sperm bundles of the coral *M. harttii*. We evaluated the composition of these bundles, the layout and organization of gametes within, before and after their dissociation, using histological and ultrastructural analyses.

The pre-dissociation time of the oocyte–sperm bundle was short in *M. harttii*, with dissociation occurring within 10 min after bundles were released in the water. According to Wolstenholme (2004), for the corals *Acropora samoensis* and *A. digitifera,* the maximum dissociation time of bundles was higher (30 min) after contact with seawater, and while in *Diploria labyrinthiformis,* it exceeded 30 min (Grosso-Becerra et al. 2021). This is an important characteristic from a practical point of view, since the rapid dissociation of the bundles would not allow, for example, in situ collections.

We observed irregular oocytes in smaller amounts, from different colonies, after the dissociation of bundles. This observation can be related to the packaging process and also associated with environmental stressors of human origin, such as sea temperature rise. In fact, in the first semester of 2019, approximately 80% of the colonies of *M. harttii* suffered intense bleaching in the Marine Park of Recife de Fora, which lasted six months (Godoy et al. 2021). When studying the potential effects of bleaching in the reproduction of the corals *Fungia sutaria* and *Montipora capitata*, Hagedorn et al. (2016) observed that 5.2% of oocytes of *F. sutaria* presented irregular shape, reaching up to 50% in some situations. Studies about the effect of mass bleaching in the Australian Great Barrier Reef in 1998 showed that colonies of the species *Acropora aspera*, *A. palifera*, *A. pulchra* and *Montipora digitata* that suffered bleaching had a significant decrease in their number of oocytes (Ward et al. 2002). These were smaller when compared to oocytes

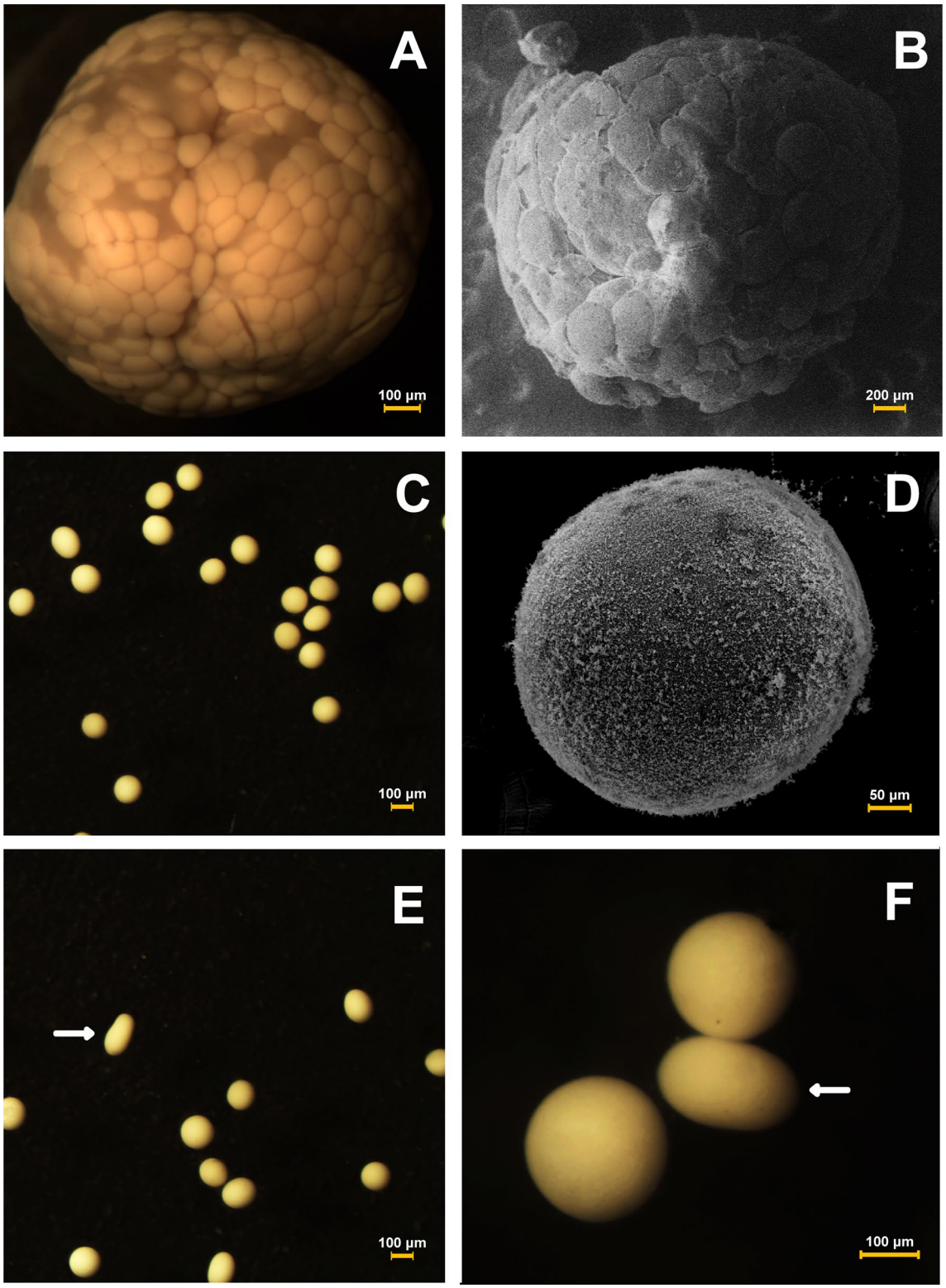

Fig. 2 Organization and morphology of the oocytes of *Mussismilia harttii*. **A** stereomicroscopy (StM) of the cluster of oocytes with irregular morphology when contained within the bundle. **B** scanning electron microscopy (SEM) showing the arrangement of oocytes and their irregular shape within the bundle. **C** StM showing the rounded morphology of the oocytes after dissociation of the bundle. **D** SEM showing the rounded shape of the oocyte after dissociation of the bundle. **E** StM highlighting the presence of oocytes of ovoid morphology even after dissociation of the bundle (white arrow). **F** StM enlarged to highlight the presence of an ovoid oocyte after dissociation of the bundle (white arrow)

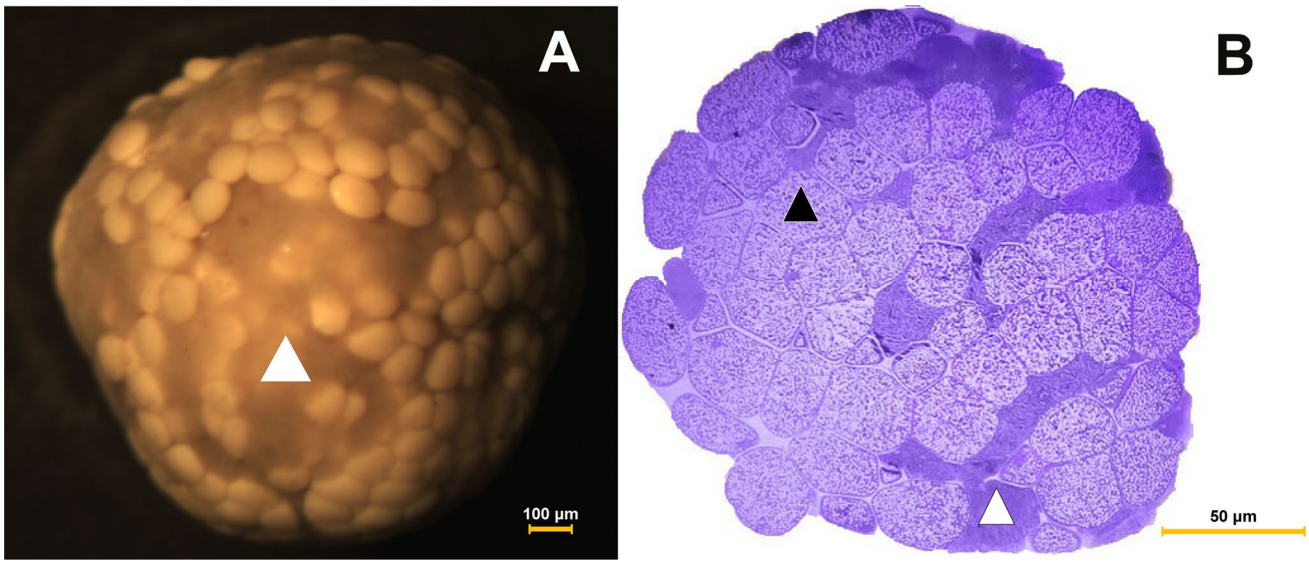

Fig. 3 Arrangement of the gametes in the bundles of *Mussismilia harttii*. **A** Stereomicroscopy highlighting the areas where the spermatozoa can be found (white triangle). **B** Light microscopy section of the bundle showing the arrangement of the gametes. The spermatozoa (white triangle) are located in the most densely stained areas. The least stained and rounded elements are the oocytes (black triangle). Sample was stained with toluidine blue in 2% borax

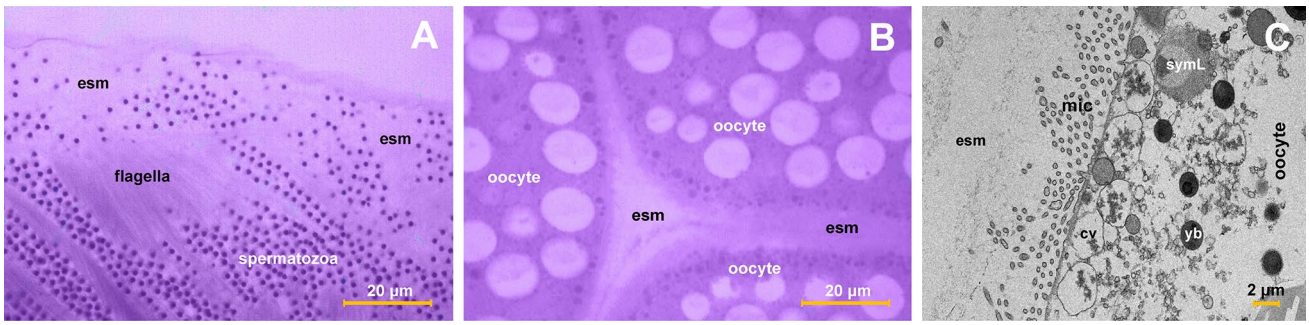

Fig. 4 Morphological analysis of gamete bundles of *Mussismilia harttii*. **A** Light microscopy (LM) showing the presence of empty spaces that were occupied by mucus (esm) in the peripheral area of the bundle and near spermatozoa. **B** LM highlighting the empty space previously filled by mucus around the oocytes in the bundle. **C** Transmission electron microscopy (TEM) showing the empty space that was occupied by mucus. It is possible to observe microvilli (mic) in dots shape due to the angle of the histological cut. Within the oocyte, we found the presence of *Symbiodinium*-like cells (symL), cortical vesicle (cv) and yolk bodies (yb)

of healthy colonies. Moreover, a decrease in the number of male mesenteries was also observed for these species.

Histological analyses of the mucus showed that it carries out an important structural role in the maintenance of the bundle, protecting gametes until they are released into the water. The mucus completely involves the gametes, maintaining a disorganized pattern in the bundles, with oocytes interlayered with spermatozoa clusters. A characteristic reinforcing that the cortical vesicles in the oocytes seem to release granules from the cytoplasm to the extracellular region is associated with the similarity of electron density in the granules present in the cytoplasm and in the inner region of the cortical vesicles. Thus, we believe that bundle formation may consist in part by the release of granules from the oocytes through the cortical vesicles. The release of cortical vesicles has been observed in oocytes of marine crustaceans. For the shrimps *Penaeus monodon* (Pongtippatee-Taweepreda et al. 2004) and *Penaeus aztecus* (Clark et al. 1980), cortical secretions produced within the oocytes are released to the extracellular region once the oocyte comes in touch with seawater.

Histological and histochemical studies seeking to identify mucus-secreting cells in corals have shown the presence of a single type of cell able to produce and secrete mucus, the mucocyte (Goldberg 2002). These cells are found in abundance in both ectoderm and gastrodermis

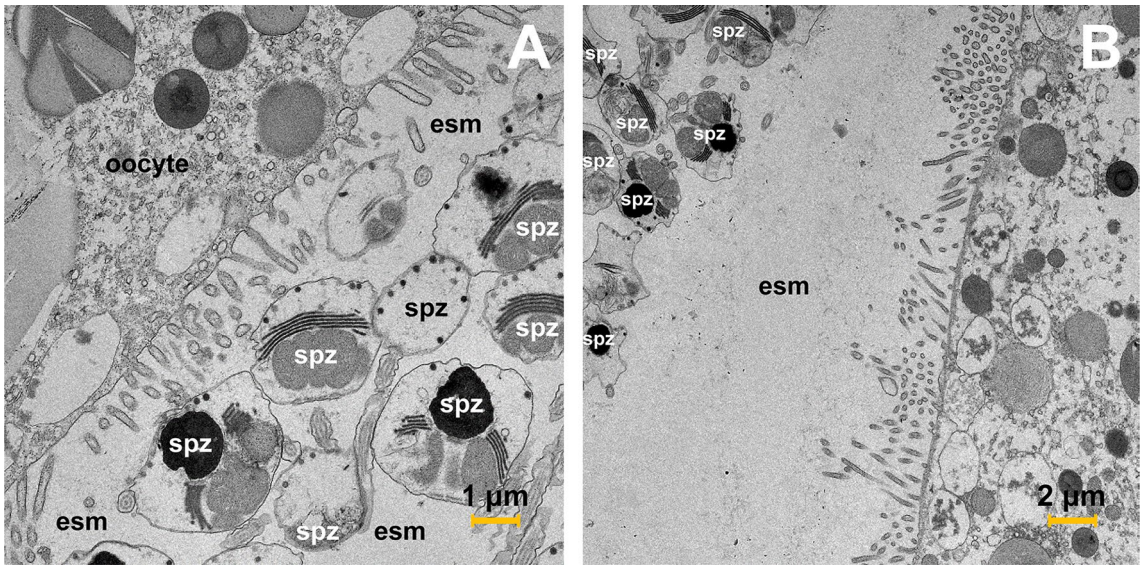

Fig. 5 Transmission electron microscopy images showing the different arrangements of spermatozoa and oocytes in the bundle. Spermatozoa (spz) could be in close contact with the oocytes **A** or with some distance (**B**), separated by an empty space (esm) that used to be a mucous layer among the gametes

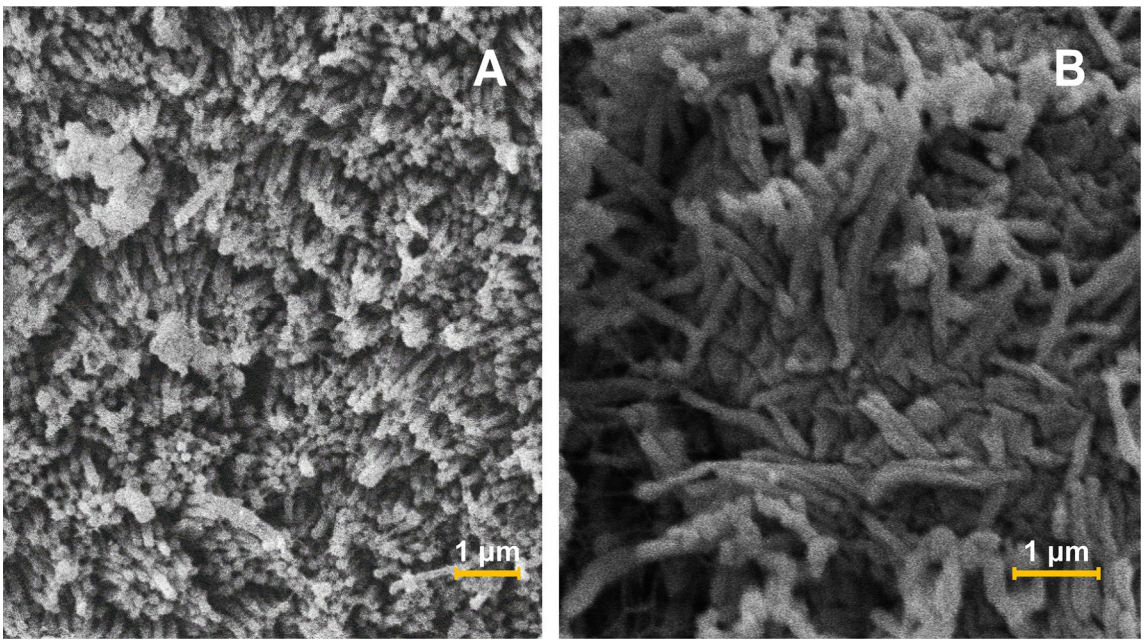

Fig. 6 Scanning electron microscopy (SEM) showing the morphology of surface microvilli in the oocytes of *Mussismilia harttii* before **A** and after **B** dissociation of the gamete bundle

(Marshall and Wright 1993). The mucus produced by these cells is basically composed of carbohydrates, proteins and small amounts of lipids (Coffroth 1990; Wild et al. 2004, 2005; Brown and Bythell 2005). More than half the macronutrients that compose this mucus is known to dissolve in a short period of time after contact with seawater (Wild et al. 2004). This information reinforces the hypothesis that the mucus produced by mucocytes can aid in the formation of oocyte–sperm bundles, which are easily dissociated when in contact with the water. Therefore, we can assume that the mucus produced by mucocytes can be released within the gastrovascular cavities, assisting in the final construction of the oocyte–sperm bundles, where gametes are enveloped.

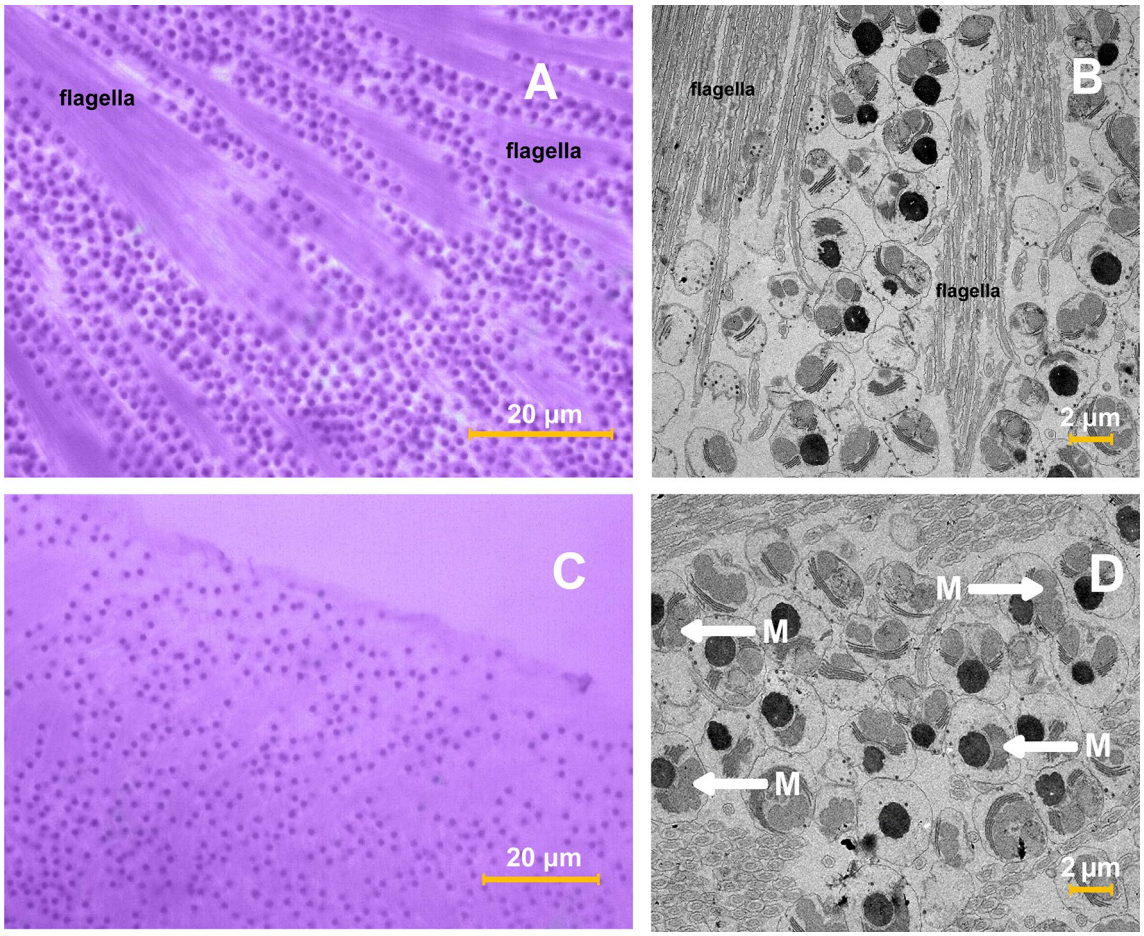

Fig. 7 Distribution and organization of *Mussismilia harttii* spermatozoa in the bundle. **A, B** Light microscopy (LM) and transmission electron microscopy (TEM) showing spermatozoa organized in lines within the bundles. **C, D** LM and TEM showing spermatozoa disorganized in the bundle. White arrows indicate mitochondria in the head of spermatozoon

TEM observations of the interaction areas between gametes showed regions of proximity and regions of distance between spermatozoa and oocytes, which were separated by an empty space that used to be occupied by mucus. Preliminary tests carried out by our research group have so far shown that the spermatozoa contained in the bundle remain inactive (no flagellar movement), suggesting that biochemical blockages may be involved and maintained by the microenvironment created within the bundle. According to Oliver and Babcock (1992), the spermatozoa of the hermaphrodite corals *Montipora digitata, Favites pentagona* and *Platygyra sinensis* remain inactive when they are still concentrated within the bundles, unable to carry out any movement during the initial stages after bundle dissociation. Investigations on the synchronous spawning of 13 species of stony corals conducted by Babcock et al. (1986) showed that no sign of fertilization was observed before the dissociation of oocyte–sperm bundles. The first signs of fertilization were only observed 2.5 h after total dissociation. This information reinforces our previous finding of gamete inactivity when contained within the bundle.

Observations through SEM on the surface of oocytes (either free or contained within the bundles) revealed morphological differences in the microvilli. These differences may be associated with changes in the oocyte's cytoplasmic pH and, consequently, with the stimuli that act on the formation and elongation of microvilli (see Spiegel and Spiegel 1977; Begg et al. 1982). Microvilli extension in sea urchin eggs was controlled in two ways: first by increasing the cytoplasmic pH of the oocyte due to interaction with seawater and second after contact of the oocyte with spermatozoa during fertilization (Begg et al. 1982). Studies have also shown that microvilli are involved in the starfish fertilization process (Matsunaga et al. 2002). In the present study, the oocyte microvilli before bundle dissociation were close to each other and thin. It is likely that the low cytoplasmic pH may cause the oocytes not to emit intracellular stimuli to generate changes in the conformation of the microvilli. On the other hand, the oocytes that were free in the

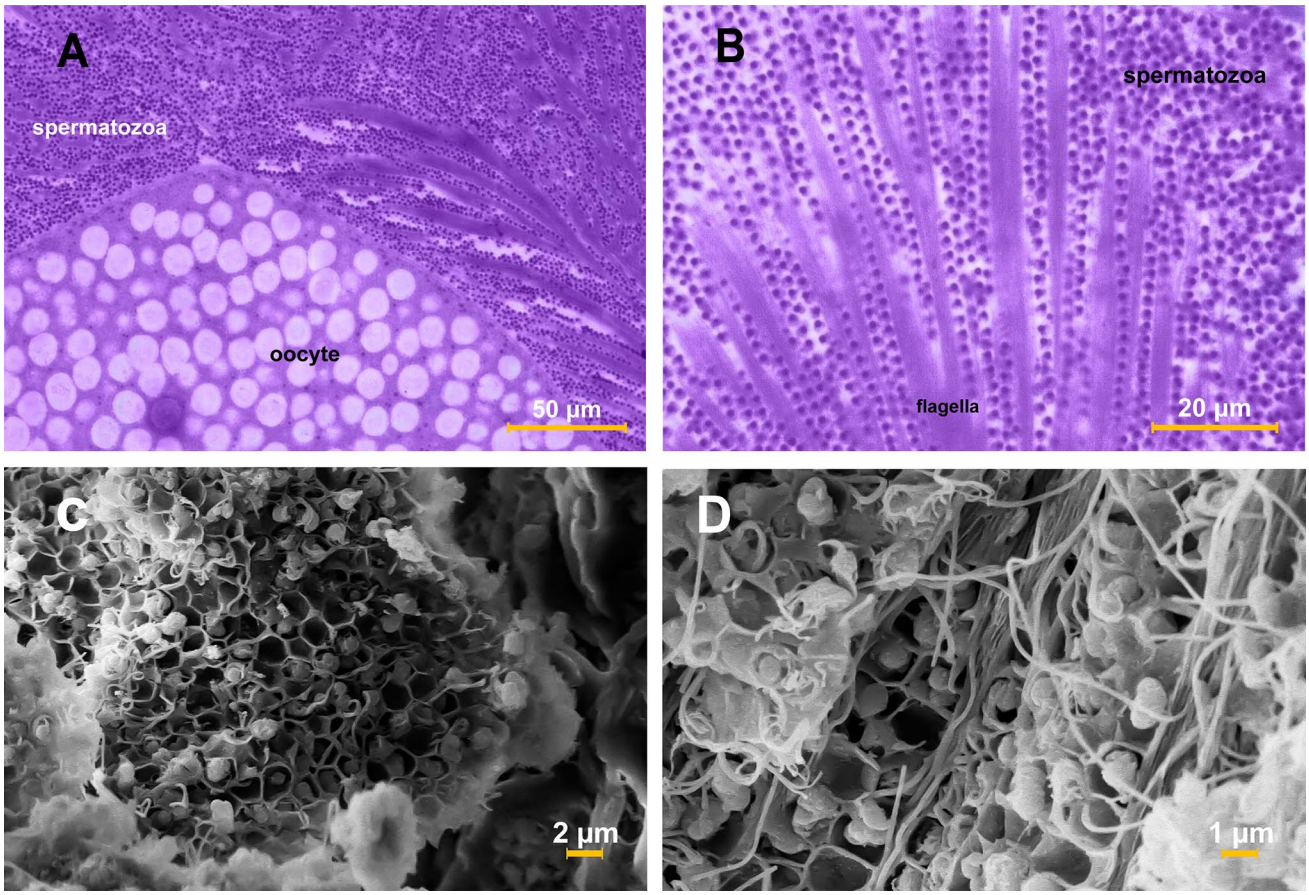

Fig. 8 Location and orientation of spermatozoa in the bundle of *Mussismilia harttii*. **A** light microscopy (LM) of the bundle showing spermatozoa disorganized in the mucus. **B** detail (LM) of spermatozoa with their flagella extended laterally, promoting organization in lines. **C, D** spermatozoa located within hexagonal-shaped structures, similar to the alveoli in a honeycomb

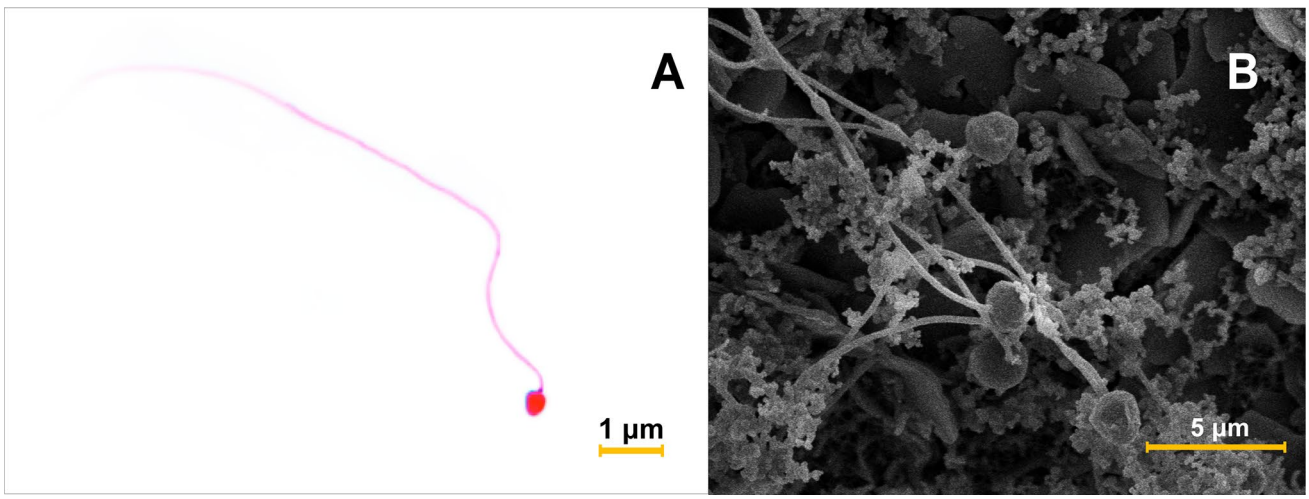

Fig. 9 Spermatozoa of *Mussismilia harttii* observed through **A** light microscopy and through **B** scanning electron microscopy

water presented long and thick microvilli, suggesting that the microvilli underwent alterations in their conformation probably due to the increase in the cytoplasmic pH after the interaction with sea water, apart from the proximity of the oocytes with the free spermatozoa from other bundles and colonies. Investigating the role of microvilli can provide important information to understand the dynamics of coral fertilization.

In a study about the reproductive patterns of three hermaphrodite species of the genus *Mussismilia*, Pires et al. (1999) showed that immature spermatozoa were concentrated in the peripheral area of the spermatic cysts, while mature spermatozoa were located in central regions, forming lines and with their flagella extended toward the narrow extremity of the spermatic cysts, forming bouquet-shaped arrangements. Similar data were presented in studies considering the black corals *Cupressopathes pumila* and *Antipathella subpinnata*, in which mature spermatozoa were found in the central regions of spermatoceles, organized in lines, while the immature spermatozoa were in the peripheral areas in a disorderly distribution (Gaino and Scoccia 2009; 2010). In addition to the position of spermatozoa in the spermatic cysts, the identification of their degree of maturity was described in relation to organelle organization. Immature spermatozoa presented free axonemes, their cytoplasm was rich in small mitochondria, and the nucleus showed decondensed chromatin (Gaino et al. 2008; Gaino and Scoccia 2009). The histological and ultrastructural analyses of the interior of the gamete bundles of *M. harttii* allowed the identification of regions with high concentration of spermatozoa organized in lines and regions with dispersed and disorganized spermatozoa, demonstrating similar patterns as previously described. Based on the organization of organelles and the position of cells similar to what had been previously found for the spermatocysts of the genus *Mussismilia* and in black corals, we believe that this distribution of spermatozoa in the bundles is related to cell maturity. However, future histological and ultrastructural studies of the male mesentery of the species can bring information about the organization and maturity of spermatozoa in the spermatic cysts.

The evaluation of spawning patterns for the black coral *Cirrhipathes* sp. and *Cupressopathes pumila* demonstrated that spermatic cysts were released to the gastrodermis cavity through the rupture of mesentery walls, with mesenteric cells identified in the spermatic cysts present in the gastrodermis (Gaino et al. 2008; Gaino and Scoccia 2009). This information can justify the observations we made using SEM in the *M. harttii* bundles. Spermatozoa were identified in the present study within the hexagonal structures, which are similar to honeycomb alveoli. Given the structural complexity observed, we believe that the release of spermatozoa in *M. harttii* occurs through the complete rupture of the walls of spermatic cysts and that the hexagonal-shaped structure is a component of the spermatic cysts. When released to the gastrovascular cavity, the spermatic cysts are enveloped in mucus, maintaining spermatozoa in this organization. According to Wallace (1985), the formation of oocyte–sperm bundles occurs a few hours before the spawning period. Based on this information, we believe that the release of spermatic cysts can be an adaptive strategy that allows reducing the amount of time spent forming the oocyte–sperm bundles in the gastrovascular cavities.

Here, we bring new and crucial knowledge about the morphology and ultrastructure of the gamete bundles that drive successful fertilization in scleractinian corals. Considering the severity of the global climate crisis and current pressures on coral reef survival, a deeper understanding of sexual reproduction will have a key role in species resilience since the offspring of more resilient individuals will inherent genetic characteristics that can aid coral adaptation to such a changing environment. This pioneer study sheds light on a poorly explored area and can provide support for future studies on reproductive biotechnologies for coral conservation such as assisted evolution, in vitro fertilization and biobanking.

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Author contributions WV, JASZ and LG designed the study; WV, AGG and LG collected the biological material; WV performed both electron and light microscopies and morphometric analysis; WV and LG performed graphic illustration; WV, AGG and LG drafted the manuscript; all authors contributed to the manuscript revisions.

Data availability Data are available upon request.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval The authors declare that all applicable international, national and/or institutional guidelines for sampling, care and experimental use of animals for the study have been followed, all and necessary approvals by the Chico Mendes Institute for Biodiversity Conservation–ICMBio (SISBIO N° 63368-1) and by the Environmental Office of the municipality of Porto Seguro (Authorization No. 01/2019) have been obtained.

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