Coral Reefs

Spermatozoan ultrastructure of scleractinian corals from the eastern Pacific: Pocilloporidae and Agariciidae

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Abstract. Spermatozoa of *Pocillopora damicornis, Pocillopora elegans* (Astrocoeniina, Pocilloporidae) and *Pavona gigantea* (Fungiina, Agariciidae) from the eastern Pacific (Isla del Caño, Costa Rica) were examined using transmission electron microscopy. The hermaphroditic pocilloporids *P. damicornis* and *P. elegans* are spermio-morphologically very similar to hermaphroditic acroporids, being characterized by bullet-shaped nuclei and elongated mitochondria. Such traits have not been found in other families. Thus, the suborder Astrocoeniina, including pocilloporids and acroporids, can clearly be distinguished from other scleractinian suborders. This separation underlines the isolated position of the Astrocoeniina within the order Scleractinia following the evolutionary scheme of Wells. A conical sperm type, known from gonochoric species (sexes separate) from all families except Acroporidae and Pocilloporidae, was found in the gonochoric agariciid *Pavona gigantea*. This supports previous findings that gonochoric corals share a unique and common sperm structure regardless of which family they belong to. However, no gonochoric Astrocoeniina have ever been examined. Hence, the question whether the sperm type common to gonochorists is also represented in Astrocoeniina, which would undermine the ultrastructural distinction of Astrocoeniina and other suborders seen among hermaphrodites, as well as the systematic value of sperm structure within scleractinian corals, remains open.
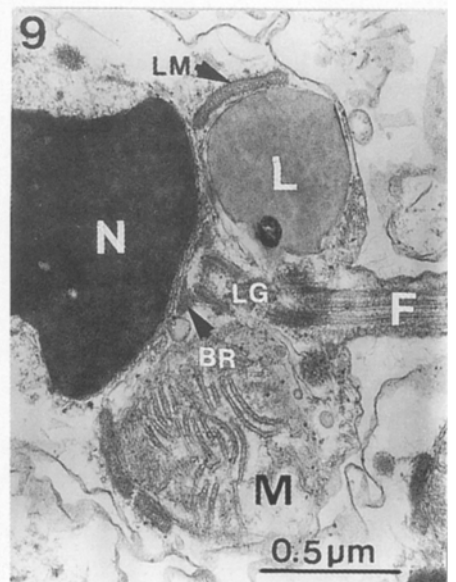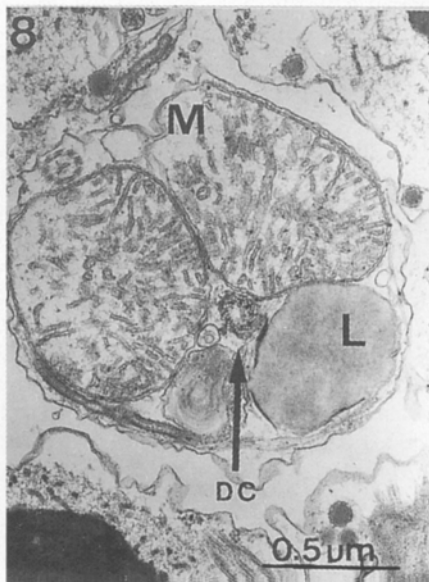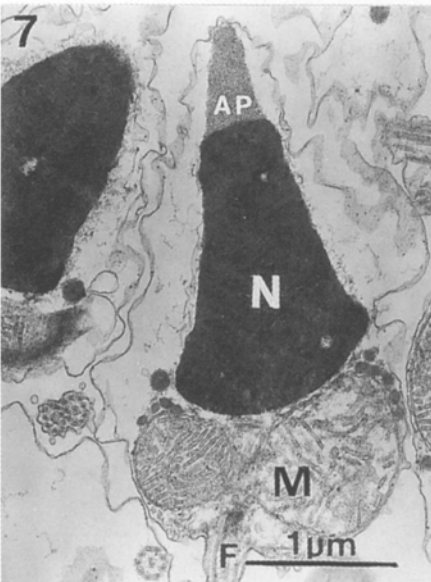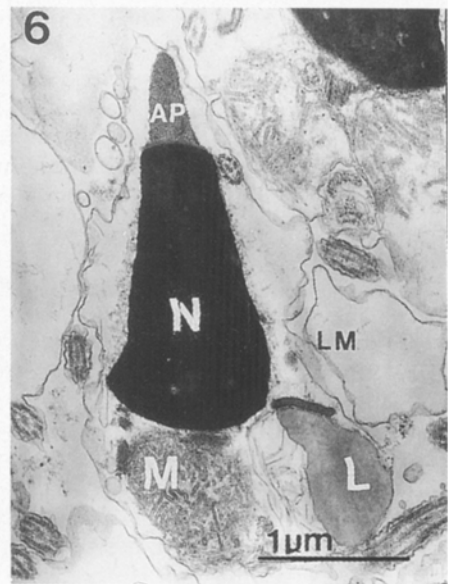
Introduction

Spermatozoa are structurally complex, morphologically species-specific and evolutionary stable cells (Franzen 1956). Based on these criteria, sperm ultrastructure has successfully been used in understanding the phylogeny of various metazoan taxa (Wirth 1984) including Anthozoa (Schmidt and Zissler 1979).

Although scleractinian spermatozoa are of the primitive type (sensu Afzelius 1979), compact and lacking an acrosome (Schmidt and Zissl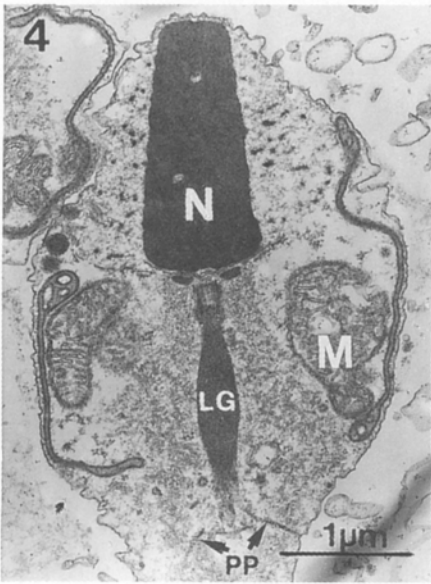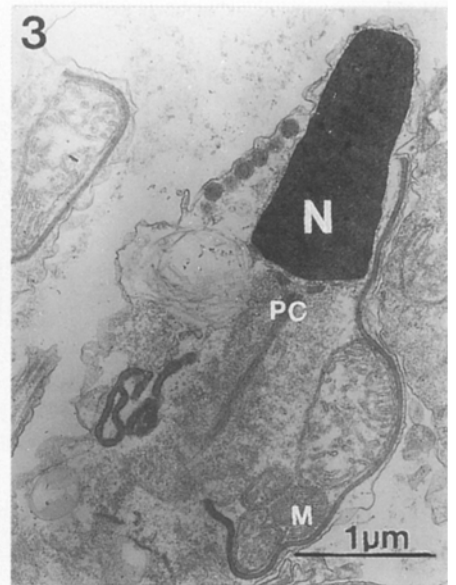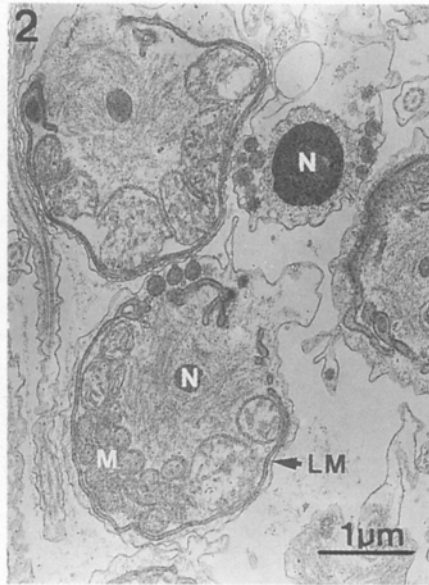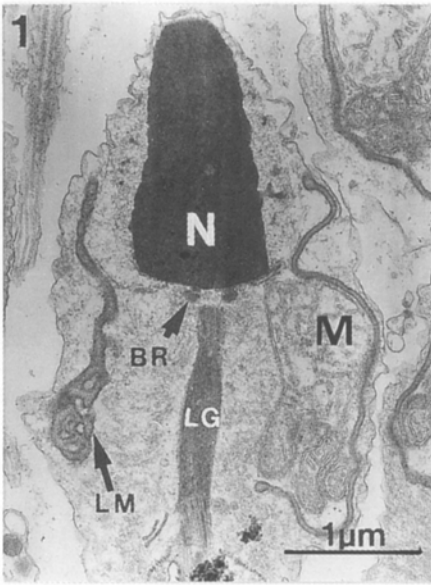er 1979), interspecific morphological variations in Australian (Harrison 1985) and Caribbean (Steiner 1991) hermatypic corals have indicated their potential importance as a soft-tissue taxonomic character. However, spermatozoan morphology is also influenced by reproductive traits (Franzen 1956), exhibited by the existence of distinctive sperm types for hermaphroditic and gonochoric corals (Harrison 1985; Steiner 1991). Thus it is essential to distinguish the phylogenetic and functional factors determining the morphology of the spermatozoon, when evaluating its use in systematics.

Out of 53 scleractinian species from the Indo-Pacific (Harrison 1988) and the Caribbean (Steiner 1993), the majority of hermaphrodites have pear-shaped and ovoid spermatozoa. With a single exception gonochorists have conical spermatozoa. A spherical sperm type was found in three Caribbean species, one hermaphroditic brooder, one hermaphroditic broadcaster and one gonochoric brooder (Steiner and Klepal 1991). *Galaxea fascicularis* (Oculinidea), whose breeding population is composed of female colonies and hermaphrodites, exhibits an "intermediate" sperm type, characterized by a short ovoid head (Harrison 1985).

The decisive influence of sexual character on sperm morphology is so strong that species of the same sex character from different families are often more alike than members of the same family but of different sexual character (Steiner 1993). Hence, even though each family is predominantly hermaphroditic or gonochoric (Harrison 1985), it is difficult to separate families on a spermio-morphological basis. This illustrates the inadequacy of spermatozoa used as a taxonomic criterion for families; this problem also prevails in lower taxonomic categories. For example, the hermaphroditic sibling species *Montastraea annularis* and *Montastraea faveolata* cannot be distinguished spermio-morphologically, but differ strongly from their gonochoric congener *Montastraea cavernosa* (Steiner in preparation). Clarification of the systematic value of scleractinian spermatozoa will require new ultrastructural examination of other families and groups with multiple reproductive patterns.

1 — N, BR, LG, LM, M; 1μm

2 — N, M, LM; 1μm

3 — N, PC, M; 1μm

4 — N, LG, M, PP; 1μm

5 — N, BR, M; 0.5 μm

6 — AP, N, LM, M, L; 1μm

7 — AP, N, M, F; 1μm

8 — M, L, DC; 0.5 μm

9 — LM, N, L, LG, F, BR, M; 0.5 μm
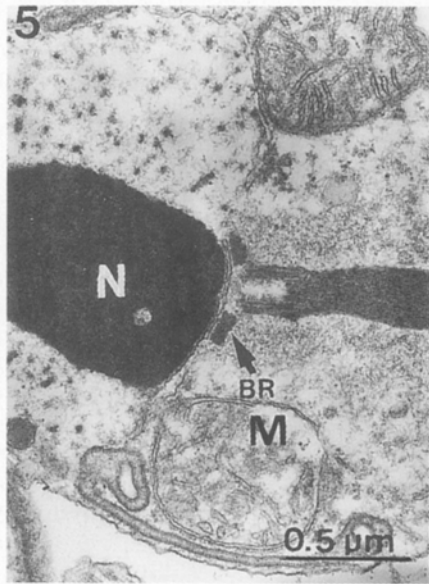
The present study offers the first ultrastructural information on the spermatozoa of the Pocilloporidae *Pocillopora damicornis* and *Pocillopora elegans* and the Agriciidae *Pavona gigantea* from the eastern Pacific. *Pocillopora damicornis* is hermaphroditic and broods planulae over most of its Indo-Pacific range, but the eastern Pacific populations of *Pocillopora damicornis* and *Pocillopora elegans* are probably broadcast spawners (Glynn et al. 1991). *Pavona gigantea* has a mixed sexuality with high percentages of hermaphroditic and gonochoric broadcast spawners (Glynn et al. in preparation, 1995).

Materials and methods

Samples of *Pocillopora damicornis*, *Pocillopora elegans* and *Pavona gigantea* were collected on June 23, 1994 (full moon) at Isla del Caño, Costa Rica. This date was selected following previous studies on the reproductive cycles of pocilloporids by Glynn et al. (1991) and *Pavona gigantea* (Glynn et al. in preparation, 1995) of the eastern Pacific. Samples were pre-fixed in 3% glutaraldehyde for 2 h and then stored in phosphate buffer for 4 days. While gonad-containing mesenteries of *P. damicornis* and *P. elegans* were extracted after decalcifying the coral skeleton in EDTA (60 h), mesenteries of *Pavona gigantea* were dissected directly from the sample. Once extracted, all mesenteries were rinsed in phosphate buffer three times (15 min), post-fixed in 2% osmium tetroxide (30 min), rinsed again three times (15 min) in phosphate buffer (15 min), dehydrated in a (30%-50%-70%-80%-90%-100%-100%) ethanol series, and a 3:1, 1:1 and 1:3 ethanol:propylene oxide mixture and finally in propylene oxide. For the infiltration of samples we used mixtures of resin (Spurr) and propylene oxide with the ratios 1:3, 2:2, 3:1, as well as pure resin (2X) in which mesenteries were kept 15 min, 2 h, 2 h and 2 h (2X) respectively. Embedded samples were polymerized for 48 h at 90 °C. 70–65 nm thick sections were made on a Leica Ultracut Ultra-microtome, stained with uranyl acetate and lead citrate, and viewed on a Hitachi H-7100 transmission electron microscope at the Unidad de Microscopia Electronica, Universidad de Costa Rica.

The extraction of gametes by decalcification is not dependent on spawning, but it should be noted that this is a compromise and that processing spawned gametes is less tissue damaging and results in a better quality of samples. Logistics and limitations in field time forced us to use this technique.

Results

Pocillopora damicornis (Pocilloporidae)

Ultrastructurally the spermatozoon of *P. damicornis* is approximately 4.5 μm long and 2.5 μm wide, excluding the flagellum (Figs. 1, 3, 10). The nucleus is the only organelle of the sperm head and is bullet-shaped with an indentation or fossa at the center of its base. This basal fossa is surrounded by a ring shaped structure originating from the proximal centriole. It links the nucleus with the centriolar complex consisting of the proximal and distal cen-

triole (Fig. 1, 10). The centrioles are positioned in a tandem position along the central longitudinal axis and are connected by a fibrillar ligament (1, 5 μm) attached at their facing ends (Figs. 1, 3, 10). The distal centriole lies at the base of the midpiece and forms the basal body of the flagellum. It consists of nine microtubule triplets embedded in a dense matrix and is the source of nine pericentriolar processes radiating from the matrix between the triplets. Each primary process forks into secondary processes extending to the outer plasma membrane and thus delineates the collar from the cytoplasm of the midpiece. Up to four elongated mitochondria and a varying number of smaller spherical mitochondria are arranged around the centriolar complex. While the elongated mitochondria lie parallel to the central axis of the spermatozoon, the smaller spherical ones tend to lie at the base of the midpiece (Figs. 1, 10). A single electron-dense and sheet-like lamella encompasses the organelles of the midpiece (Figs. 1, 2, 10). Some portions are structured in a net-like manner, others pinch off towards the lateral base of the nucleus, forming small vesicles (Figs. 1, 10).

Pocillopora elegans (Pocilloporidae)

Although the spermatozoa from our samples of *P. elegans* are not fully mature, showing uncondensed chromatin, the arrangement of organelles and cell structures does not seem to differ from those in *P. damicornis* (Figs. 4, 5). Mature samples may reveal slight variations in size and shape of cell components, but we assume that the general sperm type is the same in both *P. damicornis* and *P. elegans*.

Pavona gigantea (Agariciidae)

With lengths of 2.5 μm for the sperm head and 1.5 μm for the midpiece, the proportions of *P. gigantea* differ considerably from *Pocillopora spp*. The conical nucleus with a central indentation consists of a truncated cone with condensed chromatin and the anterior process or Spitzenkoerper of electron-translucent chromatin (Figs. 6, 7, 10). The arrangement of the centriolar complex and its link to the cell wall is the same as in *P. damicornis*. However, the fibrillar ligament is much shorter because the midpiece itself is only one third of the total length of head and midpiece combined (Fig. 9). The terminal collar around the attachment of the flagellum is 1 μm deep. No more than 3 mitochondria with approximate diameters of 1 μm were observed. A lipid body in the same plane as the mitochondria is a constant constituent of these sperm cells (Figs. 6, 8, 9, 10). An electron-dense lamella without any intra-lamellar spacings fills the gap between the cell wall and the bulk of organelles.

Figs. 1–9. TEM micrographs: sections through spermatozoa;
Figs. 1–3. *Pocillopora elegans*
Figs. 4–5. *Pocillopora damicornis*
Figs. 6–9. *Pavona gigantea*
Abbreviations for all figures: *AP* anterior process, *BR* basal ring, *DC* distal centriole, *F* flagellum, *L* lipid vesicle, *LG* ligament, *LM* lamellae, *M* mitochondria, *N* nucleus, *PC* proximal centriole, *PP* pericentriolar processes
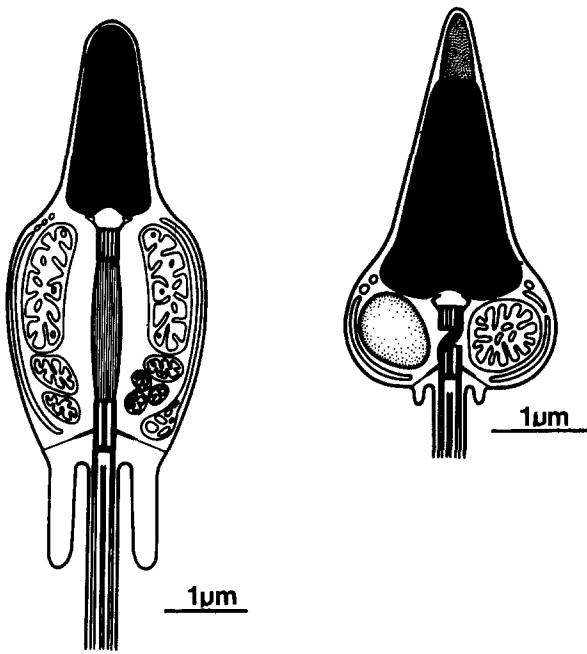
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Fig. 10. Schematic drawings of sagittal cross sections of spermatozoa, *Pocillopora elegans* and *P. damicornis* (left) *Pavona gigantea* (right)

Discussion

Pocilloporidae

Spermatozoa of pocilloporids share many structural characteristics with acroporids examined by Harrison (1988) and Steiner (1991). There is a similar size proportion of head versus midpiece, a bullet-shaped nucleus, elongated mitochondria and a deep terminal collar. The only distinctive difference is in the visicular lamellae, which occur in several layers in Acroporidae and in a single layer in Pocilloporidae. This morphological proximity can be regarded as an indication of their common roots in the sub-order of Astrocoeniina following Wells (1956). Sperms of hermaphroditic congeners *Pocillopora damicornis* and *P. elegans* do not differ in shape and arrangement of cell structures. Similar observations were made for hermaphroditic congeners of *Acropora* (Harrison 1988; Steiner 1993).

All species studied in the families Pocilloporidae and Acroporidae of sub-order Astrocoeniina are characterized by hermaphroditism and a distinctive spermatozoon with bullet-shaped nucleus as described above and named type C2 in an earlier study of *Acropora* (Steiner 1991). This clearly separates them ultrastructurally from hermaphrodites of other sub-orders like Fungiina, Faviina, Caryophylliida and Dendrophylliida, which have spermatozoa with a pear-shaped and ovoid nucleus. The question of whether this separation between sub-orders can also be detected among gonochorists remains open, because ultrastructural information on gonochoric Astrocoeniina is lacking.

Comparing the distinct sperm morphology of Astrocoeniina hermaphrodites to all other scleractinian sub-

orders, one could speculate that gonochoric representatives of Astrocoeniina also follow this trend and make the independent position of Astrocoeniina within the evolutionary scheme of Wells (1956) even stronger. If the opposite were found, the superiority of "sexual character" in determining the morphology of spermatozoa that persists in all scleractinian suborders could be inferred.

Comparison of the brooding *P. damicornis* from Indo-Pacific (Stoddart and Black 1985; Richmond and Hunter 1990) and the presumably broadcasting (Richmond 1985, 1987; Glynn et al. 1991) *P. damicornis* examined in this study will show whether such variations in the reproductive mode are also reflected in sperm morphology. This also applies to the comparison of *P. elegans* from the eastern Pacific, which is a broadcast spawner (Glynn et al. 1991), and *P. elegans* from Enewetak where it is reported to brood.

Agariciidae

The conical sperm type of *Pavona gigantea* also occurs in Rhizangiidae (Szmant-Froelich et al. 1980), Fungiidae, Poritidae, Faviidae, Caryophylliidae and Dendrophylliidae (Harrison 1985) and Faviidae, Meandrinidae and Caryophylliidae (Steiner 1993, unpublished data). The occurrence of this C3 sperm type (Steiner 1991) coincides with gonochorism in all scleractinian suborders except possibly the Astrocoeniina in which gonochorists have not been reported.

Within the suborder Fungiina, *Pavona gigantea* (Agariciidae) resembles *Goniopora lobata* (Poritidae) and *Fungia scabra* (Fungiidae). With the existence of spherical nuclei in sperm of hermaphroditic poritid *Porites astreoides* and the gonochoric Siderastreidae *Siderastrea sidera* (Steiner 1991), the suborder Fungiina is morphologically less uniform than Astrocoeniina. *P. gigantea* has a mixed sexuality (Glynn et al. in preparation 1995), but does not have an intermediate sperm type like *Galaxea fascicularis* (Oculinidae) which can be a female or hermaphroditic type (Harrison 1988). In purely spermio-morphological terms, the gonochoric character of *Pavona gigantea* from Isla del Caño (Costa Rica) is thus more dominant than the hermaphroditic one.

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