

Spermatogenesis in the archaic hydrothermal vent bivalve, *Bathypecten vulcani*, and comparison of spermatozoon ultrastructure with littoral pectinids

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Summary

Bathypecten vulcani is considered a relict species from the Paleozoic, based on shell characteristics such as the presence of calcite prisms. To date, it is the only pectinid species reported from hydrothermal ecosystems. Histological and ultrastructural studies show that spermatogenesis is identical to that of littoral pectinids. The spermatozoon has a 2.7 μm long pyriform head and a 40 μm flagellum. The four mitochondria of the mid-piece are about 1.2 μm in diameter. The nucleus contains dense chromatin fibres and possesses a wide, shallow (0.1 μm) anterior fossa and a narrow, deeper (0.2 μm) posterior nuclear fossa. Comparison of the ultrastructural characteristics of the spermatozoon of *B. vulcani* with those of littoral pectinids shows that they can be used as a diagnostic feature of this species. In particular, its acrosome characters will be a useful complement to the shell characters in the study of the phylogenetic position of this species in relation to other pectinids.

Key words: *Bathypecten vulcani*, spermatogenesis, spermatozoa, ultrastructure, phylogeny

Introduction

The super-family Pectinacea, to which belong the extant families Pectinidae, Propeamussiidae, Entoliidae and Spondylidae (Waller, 1991), has a wide geographic distribution and depth range. It contains an economically important family, the Pectinidae, from which several species are commercially fished or

extensively cultured throughout the world. As recently underscored by Healy et al. (2000), it is surprising that so little is known about the spermatozoa of such an important group.

Until recently, pectinaceans had not been reported in deep-sea reducing environments such as hydrothermal vents and subduction zones, whose bivalve

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fauna are dominated by solemyids and mytilids (Cossel and Olu, 1998). In March 1984, during the research cruise Biocyarise at the East Pacific Rise (12°48'N–103°56'W), designed 13°N, two small bivalves and shell debris collected at 2620 m depth by the submersible Cyana (Ifremer, France) were described as being a relict species of Pectinidae, *Bathypecten vulcani* (Schein-Fatton, 1985). A more detailed study of shell characters suggested the difficulty of assigning a precise taxonomic status of this organism within the Pectinacea (Schein-Fatton, 1988).

During the past 15 years, several additional specimens of *B. vulcani* were collected from the East Pacific Rise at three different sites: the Galapagos rift, 9°N (9°49'N–104°17'W) and 13°N. Preliminary anatomical studies were carried out on some of these individuals in order to better understand their nutritional strategy (Le Pennec et al., 1988). The present work focuses on spermatogenesis and sperm ultrastructure. A comparison of the spermatozoa of *B. vulcani* with those of littoral pectinid species is also made in order to gain insights into the possible relationships and taxonomy of this species as well as the Pectinidae in general.

Material and Methods

Five specimens of *B. vulcani* (Fig. 1.1) were collected from the East Pacific Rise by the research submersible "Nautile" (Ifremer, France) at 13°N (HOT 96 expedition, three individuals at 2628 m depth) and at 9°N (HOPE 99 expedition, two individuals, at 2515 and 2647 m depth). Upon arrival on board the support ship "Nadir", the specimens were field-fixed in 10% iso-osmotic formaldehyde for 24 h, and then stored in 70% ethanol.

Specimens of *Pecten maximus*, *Mimachlamys* (= *Chlamys*) *varia*, and *Hinnites pusio* (= *Chlamys distorta*) were collected in Brest harbour (Brittany, France) in October, 2000, and kept in aquaria for several hours before the gonads were dissected. Specimens of *Mizuhopecten* (= *Patinopecten*) *yessoensis* were obtained from the Ifremer hatchery in Argenton (Brittany, France). They are representatives of a laboratory-reared F3 generation, spawned from individuals obtained from the Miagy prefecture (Japan). *Aequichlamys* (= *Chlamys*) *bifrons*, *Mimachlamys* (= *Chlamys*) *asperrima* and *Pecten fumatus* were collected in the D'Entrecasteaux canal (Tasmania, Australia). Specimens of *Placopecten magellanicus* from the Bay of Fundy and *Chlamys islandica* from the St. Pierre Bank (Canada) were also

collected, as well as *Argopecten circularis* (= *Argopecten ventricosus*), from the Bay of La Paz (Baja California, Mexico).

From all afore-mentioned species, approximately 1 mm³ gonad fragments were taken from five individuals and fixed or re-fixed in 2.5% glutaraldehyde in a sodium cacodylate buffer (pH 7.3, 1100 mosm) for 24 h at 4°C, and post-fixed in 2% osmium tetroxide in a sodium cacodylate buffer (pH 7.3, 1100 mosm) for 1 h at 4°C, prior to embedding in Epon 812 resin. Semi-thin sections (1 µm) were stained with toluidine blue, and ultra-thin sections (60 nm) were contrasted with uranyl acetate and lead citrate for observation with a JEOL 100 transmission electron microscope at 80 KV.

The gamete sizes reported are the means of five to ten examined cells.

Results

The gonad of *B. vulcani* is distinct from the visceral mass: it is a separate organ, attached to the anterior wall of the adductor muscle (Fig. 1.1). The colour of mature gonads did not permit sex identification, as they are whitish in the preserved specimens of both sexes. No simultaneous presence of male and female gametes was observed in the five individuals examined. In all specimens, the gonad acini were well developed, with all gametogenic stages, from spermatogonia to spermatozoa (Fig. 1.2–1.4) filling in so much volume that relatively little inter-acinal tissue was observed.

The spermatogonia, which chiefly adhered to the walls of the acini, are the largest cells of the germ line (Fig. 1.2). They measure up to 7×6 µm in size, and have oval-shaped, 5 µm long nuclei, with chromatin distributed in small agglomerates and fine, continuous parietal strands. Spermatocytes have nuclei measuring 4 µm in diameter; the different meiotic stages were observed (Fig. 1.3) according to the degree of chromatin condensation.

The spermatids were round, with a spherical nucleus measuring approximately 3 µm in diameter. By the end of spermatid differentiation, a loss of cytoplasm caused these cells to become pyriform (Fig. 1.3).

The spermatozoa were small cells with a headpiece (from the tip of the acrosome to the base of the nucleus) measuring 2.7±0.2 µm in length, and a maximal width of 1.6 µm (Fig. 1.4, 1.5). The nucleus was capped by a small (0.5 µm), conical acrosome consisting of two distinct structures: a horseshoe-shaped acrosomal vesicle, surrounding granular, relatively

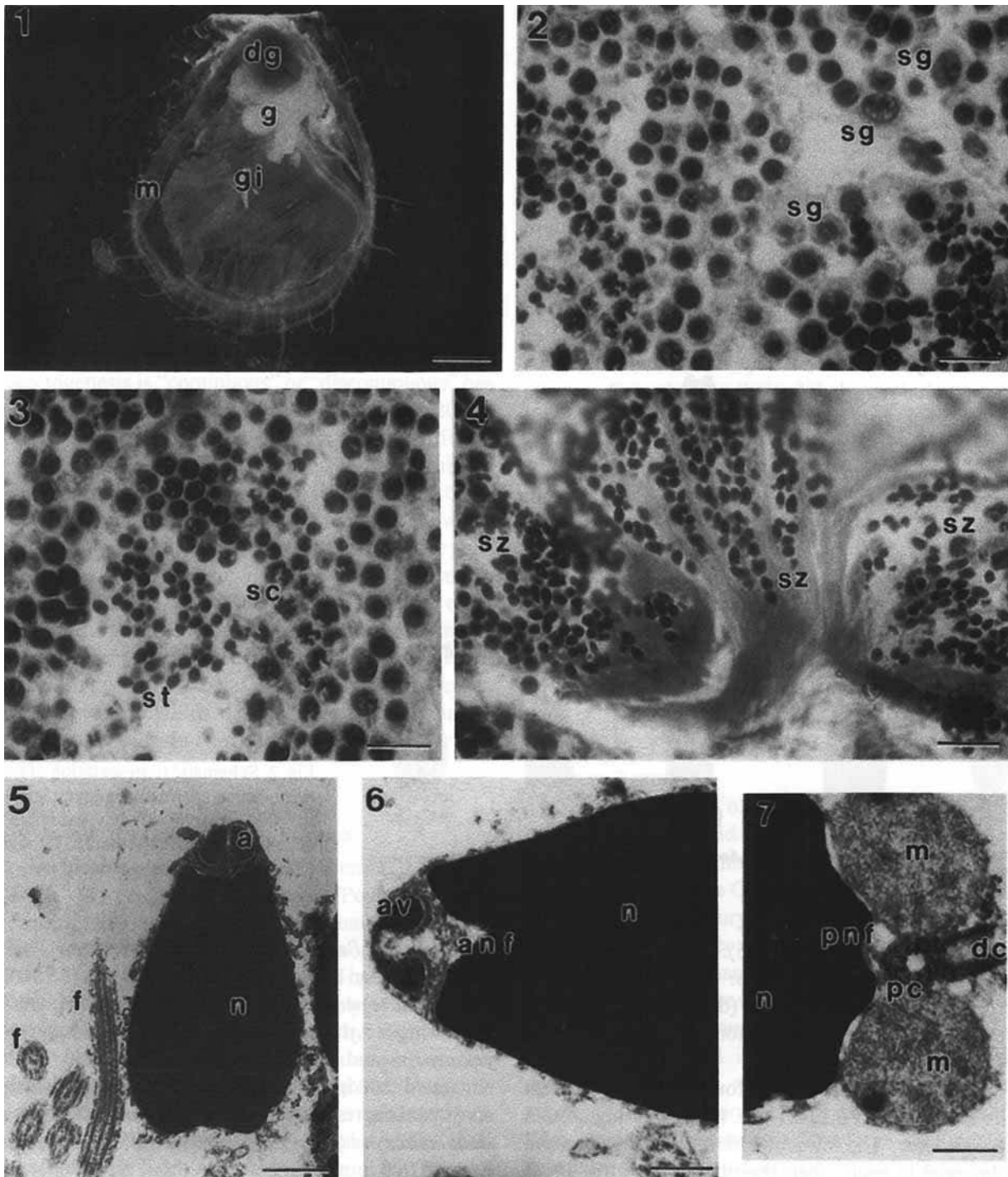


Fig. 1. Spermatogenesis in *Bathypecten vulcani*. 1: General anatomy. Dg, digestive gland; g, gonad; gi, gill; m, mantle. Scale bar: 0.30 cm. 2–4: Semi-thin sections of male gonads showing the evolution of the germ line from gonia (2) to primary spermatocytes, which go through meiosis (2, 3) and give rise to spermatids (3), which differentiate into spermatozoa (4). sg, spermatogonia; sc, spermatocytes; st, spermatids; sz, spermatozoa. Scale bar: 10 μ m. 5–7: TEM micrographs of spermatozoa. 5. Morphology of a spermatozoan head, showing the nucleus (n) and acrosome (a). Part of the flagellum is seen in transverse section and in sagittal section (f). Scale bar: 0.54 μ m. Detail of the head showing the acrosomal vesicle (av) the nucleus (n) and the anterior nuclear fossa (anf). m, mitochondria. Scale bar: 0.34 μ m. Detail of the proximal centriole (pc) and the distal centriole (dc) in orthogonal position and two mitochondria (m) of the midpiece. n, nucleus; pnf, posterior nuclear fossa. Scale bar: 0.35 μ m.

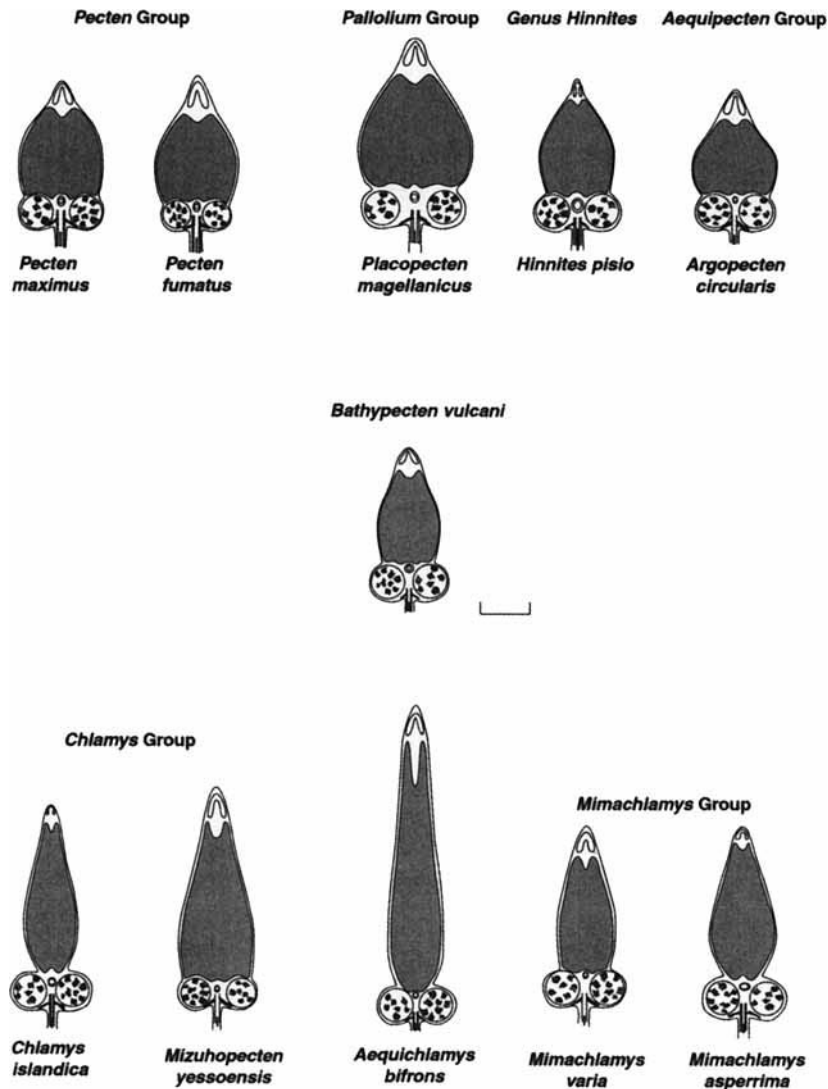


Fig. 2. Schematic representation of the pectinacean spermatozoa investigated in the present study, assembled according to Categories and to Groups (Waller, 1991). Scale bar: 1.10 μm .

homogenous matter (Fig. 1.6). The dense nucleus presented two invaginations: the anterior, sub-acrosomal nuclear depression (depth of 0.2 μm) and the posterior nuclear depression (depth of 0.1 μm) (Fig. 1.5–1.7).

The midpiece consisted of four mitochondria, each with a diameter of $0.8 \pm 0.1 \mu\text{m}$, forming a ring around the centrioles. The mitochondria and centrioles were orthogonal to each other; the proximal centriole was found under the posterior nuclear fossa, while the distal centriole, which gave rise to the flagellum, extended dense projections to the cell membrane (Fig. 1.7). The flagellum, measuring approximately 40 μm in length, had a classical 9+2 microtubule arrangement.

Based on transmission electron micrographs of the sperm headpieces of several pectinacean species, sketches were made to allow morphological and anatomical comparisons (Fig. 2). *B. vulcani*, *Pecten*

maximus and *Pecten fumatus* had spermatozoa with small, globular nuclei, measuring between 2.7 and 3 μm . *Placopecten magellanicus* had a headpiece measuring 3.5 μm . *Chlamys islandica*, *Mizuhopecten yessoensis* and *Aequichlamys bifrons* had more elongated headpieces, and more pronounced sub-acrosomal depressions than the three previous species. *Aequichlamys bifrons* had the narrowest (1.3 μm) and longest (6.8 μm) headpiece of the 11 pectinaceans studied. *Hinnites pusio* had small spermatozoa, similar to those of the *Pecten* species. *Mimachlamys varia* and *Mimachlamys asperrima* had elongated spermatozoa, measuring about 3.2 μm in length for a maximal nuclear diameter of 1.5 μm . *Argopecten circularis* had the stoutest spermatozoa (2.5 μm max. width).

At the species level, spermatozoa showed distinct differences in the shape of the acrosome and the shape and depth of the nuclear depressions.

Discussion

Spermatogenesis

The presence of exclusively male or exclusively female gametes in the gonad acini indicates that this species is either gonochoristic or a successive hermaphrodite. Many more specimens will need to be collected at different times of the year and at different sites in order to confirm this observation and to distinguish between the two possibilities.

Spermatogenesis in *B. vulcani* resembles that previously described in the Pectinidae (Masson, 1958; Lucas, 1965; Naidu, 1970; Dorange and Le Pennec, 1989). The difficulty of determining whether gametogenesis is “continuous” or “discontinuous” has been underscored by Beninger and Le Pennec (2000). The simultaneous presence of germ cells of different developmental stages, as evidenced by the acini and the thin inter-acinal connective tissue, suggests an active, continuous reproduction, as has been shown to occur in other bivalves from the East Pacific Rise such as *Bathymodiolus thermophilus* (Herry and Le Pennec, 1987). Additional collections of *B. vulcani* from other times of the year are necessary in order to confirm this interpretation; several recent studies have documented the existence of a sexual resting period in certain hydrothermal mytilids (Le Pennec and Beninger, 1997), and notably in *Bathymodiolus azoricus* (Comtet et al., 1999).

Taxonomic and phylogenetic insights

In bivalves, descriptions of spermatozoa have helped to elucidate phylogeny (Popham, 1979; Franzén, 1983) and taxonomic designation of species (Hodgson and Bernard, 1986; Hodgson et al., 1987, 1990; Healy, 1989; Reunov and Hodgson, 1994; Beninger and Le Pennec, 1997; Healy et al., 2000). However, as recently underscored by Healy et al. (2000), despite the diversity, economic importance, and wide geographic distribution of the Pectinacea, the cytology of their spermatozoa has received little attention; the ultrastructural description of the male gametes of *P. maximus* (Dorange and Le Pennec, 1989) is the only complete description available for any species.

The most species-distinct structural elements of spermatozoa are the acrosome and the nucleus (Popham, 1979). The spermatozoa of *B. vulcani* are similar to the primitive type common in bivalves (Franzén, 1955). This type is characterized by a conical acrosomal vesicle, a small nucleus, a midpiece with a single ring of mitochondria and two centrioles with

triplet structures, and a simple flagellum (9+2 axoneme pattern), shorter than 50 μm . Spermatozoa of the primitive or ect-aquasperm type (Rouse and Jamieson, 1987) are broadcast, and fertilization is external.

From the morphological comparison of the sperm head pieces of 10 pectinid species, it seems that we can distinguish two broad categories (Fig. 2): those spermatozoa with small, globular heads and those with more elongated heads. In the first group *Pecten* spp., *Placopecten*, *Hinnites*, and *Argopecten* could be placed. The round shape of these spermatozoa is considered as a more primitive condition than that found in the second group, comprising the *Chlamys*, *Mimachlamys*, *Mizuhopecten*, and *Aequichlamys* species. According to their global morphology, each category can be further subdivided into groups corresponding to those of Waller (1991): the *Pecten*, *Aequipecten*, and *Palliolum* groups in Category 1, and the *Chlamys* and *Mimachlamys* groups in Category 2.

In addition to headpiece morphology, a clear ultrastructural discriminant may be found in the anterior nuclear depression. In the species of Category 1, this depression is wide and shallow (usually less than 0.2 μm deep), whereas in the species of Category 2, this structure is quite deep (up to 1 μm in *A. bifrons*).

Taken together, the spermatozoa data of the present study thus could confirm the more primitive status of the Category 1 groups and the more recent status of the Category 2 groups.

The spermatozoa of *B. vulcani* are distinct from both of these pectinid groups. The anterior nuclear depression is approximately 0.2 μm deep, placing it closer to the primitive Category 1 pectinids than to the more recent Category 2 species. However, the headpiece is slimmer than that of the Category 1 species, but stouter than that of the Category 2 species. This dissonance echoes the problem of placement of the genus *Bathypecten* within the Pectinacea. The genus is represented in the Recent by two known species: *B. vulcani*, which has only been found surrounding hydrothermal vents in the East Pacific Rise, and *B. eucymatus*, found on sedimentary substrates at bathyal and abyssal depths in the Eastern and Western Atlantic (Schein-Fatton, 1988). According to the latter author, the valves of *B. vulcani* show archaic characters, including prismatic calcite on both valves, evoking certain fossils from the Paleozoic. Moreover, the shell characters appear to be intermediate to those of the families Pectinidae, considered more evolved, and the Propeamussiidae, considered more primitive; *B. vulcani* could be closely related to the common ancestor of these two families

(Schein-Fatton, 1988). Although *B. vulcani* has been considered to be a Pectinidae (Schein-Fatton, 1985; Le Pennec et al., 1988), there are indications that it does not belong to this family, notably (1) the absence of a ctenolium in *B. vulcani*, universally present in the Pectinidae; and (2) the presence of homorhabdic gills in *B. vulcani*, typical of the Propeamussidae but unreported in the Pectinidae, which have heterorhabdic gills (Waller, 1978; Beninger et al., 1988). The morphological difference between the spermatozoa of *B. vulcani* and the 10 pectinid species reported in the present work also argue for a non-pectinid designation. Further studies of the anatomical and genetic characteristics of *B. vulcani*, in comparison to those of the Pectinidae, and especially the Propeamussiidae, would greatly improve our understanding of the taxonomy and phylogeny not only of *B. vulcani*, but also of the Pectinacea as a whole.

The results obtained should encouraged further work on other deep-sea pectinaceans such as those discovered in the Bay of Biscay during the Ecofer cruise (1989, Ifremer). A comparison of the biology of these deep-sea pectinaceans would allow a better placement in the phylogenetic trees suggested in the literature, and a better understanding of the strategies evolved by those bivalves to remain in these reducing or non-reducing environments.

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