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Spermatozoan ultrastructure in the trigonioid bivalve *Neotrigonia margaritacea* Lamarck (Mollusca): comparison with other bivalves, especially Trigonioida and Unionoida

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ABSTRACT: Spermatozoa of the trigonioid bivalve *Neotrigonia margaritacea* (Lamarck) (Trigoniidae, Trigonioida) are examined ultrastructurally. A cluster of discoidal, proacrosomal vesicles (between 9 to 15 in number) constitutes the acrosomal complex at the nuclear apex. The nucleus is short (2.4–2.6 µm long, maximum diameter 2.2 µm), blunt-conical in shape, and exhibits irregular lacunae within its contents. Five or sometimes four round mitochondria are impressed into shallow depressions in the base of the nucleus as is a discrete centriolar fossa. The mitochondria surround two orthogonally arranged centrioles to form, collectively, the midpiece region. The distal centriole, anchored by nine satellite fibres to the plasma membrane, acts as a basal body to the sperm flagellum. The presence of numerous proacrosomal vesicles instead of a single, conical acrosomal vesicle sets *Neotrigonia* (and the Trigonioida) apart from other bivalves, with the exception of the Unionoida which are also known to exhibit this multivesicular condition. Spermatozoa of *N. margaritacea* are very similar to those of the related species *Neotrigonia bednalli* (Verco) with the exception that the proacrosomal vesicles of *N. margaritacea* are noticeably larger than those of *N. bednalli*.

INTRODUCTION

The Trigonioida constitute an important and ancient order of marine bivalves which are perhaps best known from the numerous species and genera occurring in Jurassic and Cretaceous horizons (Cox, 1952; Fleming, 1964; Newell & Boyd, 1975; Stanley, 1977, 1984). Today, only a few species survive, all belonging to the genus *Neotrigonia* (family Trigoniidae) and confined to the Australian continental shelf (Fleming, 1964; Tevesz, 1975; Habe & Nomoto, 1976; Darragh, 1986). The origins of the Trigonioida and their relationship to other bivalve orders, especially the Unionoida, have been extensively debated in the literature (see Neumayr, 1889; Douville, 1912; Cox, 1952; Fleming, 1964; Newell, 1969; Gould & Jones, 1974; Newell & Boyd, 1975; Morris, 1978; Stanley, 1978, 1984; Smith, 1983; Morton, 1987; Purchon, 1987). In a previous account, the author demonstrated that the long-held concept of a close relationship between the Trigonioida and Unionoida is supported by the occurrence of a highly unusual arrangement of multiple acrosomal vesicles in the spermatozoa of both of these groups. During the course of a survey of bivalve spermatozoa, the author had the opportunity to examine sperm ultrastructure in a further member of the Trigonioida, *Neotrigonia margaritacea*

(Lamarck, 1804), notable for being the first discovered living trigonioid. This species is also of some taxonomic interest because the designated type species of *Neotrigonia*, *Trigonia pectinata* Lamarck, 1819, is in fact an objective synonym of *N. margaritacea* (see Darragh, 1986) . In this paper, the spermatozoon of this important species are described and compared with results published on other species of *Neotrigonia* (*N. bednalli* [Verco], *N. gemma* Iredale) (Healy, 1989) and the spermatozoically similar Unionoida (Higashi, 1964; Trimble & Gaudin, 1975; Healy, 1989, 1995; Peredo et al., 1990; Rocha & Azevedo, 1990; Lynn, 1994).

MATERIALS AND METHODS

Specimens of *Neotrigonia margaritacea* (Lamarck, 1804) were dredged at a depth of 10–15 m in Port Philip Bay, Victoria, Australia. Testis pieces were excised and fixed immediately in ice-cold 3 % glutaraldehyde for 2 h (fixative prepared in 0.1M phosphate buffer containing w/v 5 % sucrose). Following glutaraldehyde fixation, tissues were rinsed thoroughly in phosphate buffer (sucrose-adjusted) then further fixed in a 1 % osmium tetroxide solution (prepared in sucrose-adjusted phosphate buffer) for 80 min. After another rinse in buffer, the tissues were gradually dehydrated using an ascending series of ethanols (beginning at 20 %), then infiltrated and embedded in Spurr's epoxy resin. All stages of processing, up to and including 70 % ethanol, were conducted at 0 °–4 °C, and thereafter at room temperature. Tissue blocks were sectioned using an LKB 2128 IV Ultrotome, collected on uncoated 200 µm mesh copper grids and stained using Daddow's (1986) contrast-enhancing technique. Specimen grids were stained for 30 min in 10 % uranyl acetate and for 10 min in Reynold's lead citrate. All sections were examined using a Hitachi A 300 transmission electron microscope operated at 75 kV. Light microscopic observations were carried out using a Wild M20 microscope adjusted for phase-contrast viewing. Voucher specimens of *N. margaritacea* have been deposited with the Queensland Museum, Brisbane, under the registered number QMMO 55783.

RESULTS

Mature spermatozoa of *Neotrigonia margaritacea* consist of a thin acrosomal complex (featuring multiple proacrosomal vesicles), a short nucleus, a midpiece composed of five (sometimes four) mitochondria and two centrioles positioned at the base of the nucleus, and a single flagellum (Fig. 1A).

The acrosomal complex is composed of between 9 to 15 disc-shaped, proacrosomal vesicles, which collectively form a thin cap over the nuclear apex (Fig. 1A–E). Each vesicle is membrane bound and has a diameter of 0.25–0.3 µm and thickness of approximately 0.08 µm (Fig. 1C, D). Vesicle contents are moderately electron-dense with the exception of a denser, plate-like core zone. An electron-lucent peripheral zone separates the dense contents from the vesicle membrane (Fig. 1C, D). The proacrosomal vesicles are not contained by a common membrane nor do they appear to be associated with any obvious subacrosomal deposit. Longitudinal sections through the acrosomal complex show between three and five vesicles – the larger number perhaps being typical of sections close to sagittal. The entire complex has a maximum diameter of about 0.8 µm. The nucleus is blunt-conical, 2.4–2.6 µm long and with a maximum diameter of approxi-

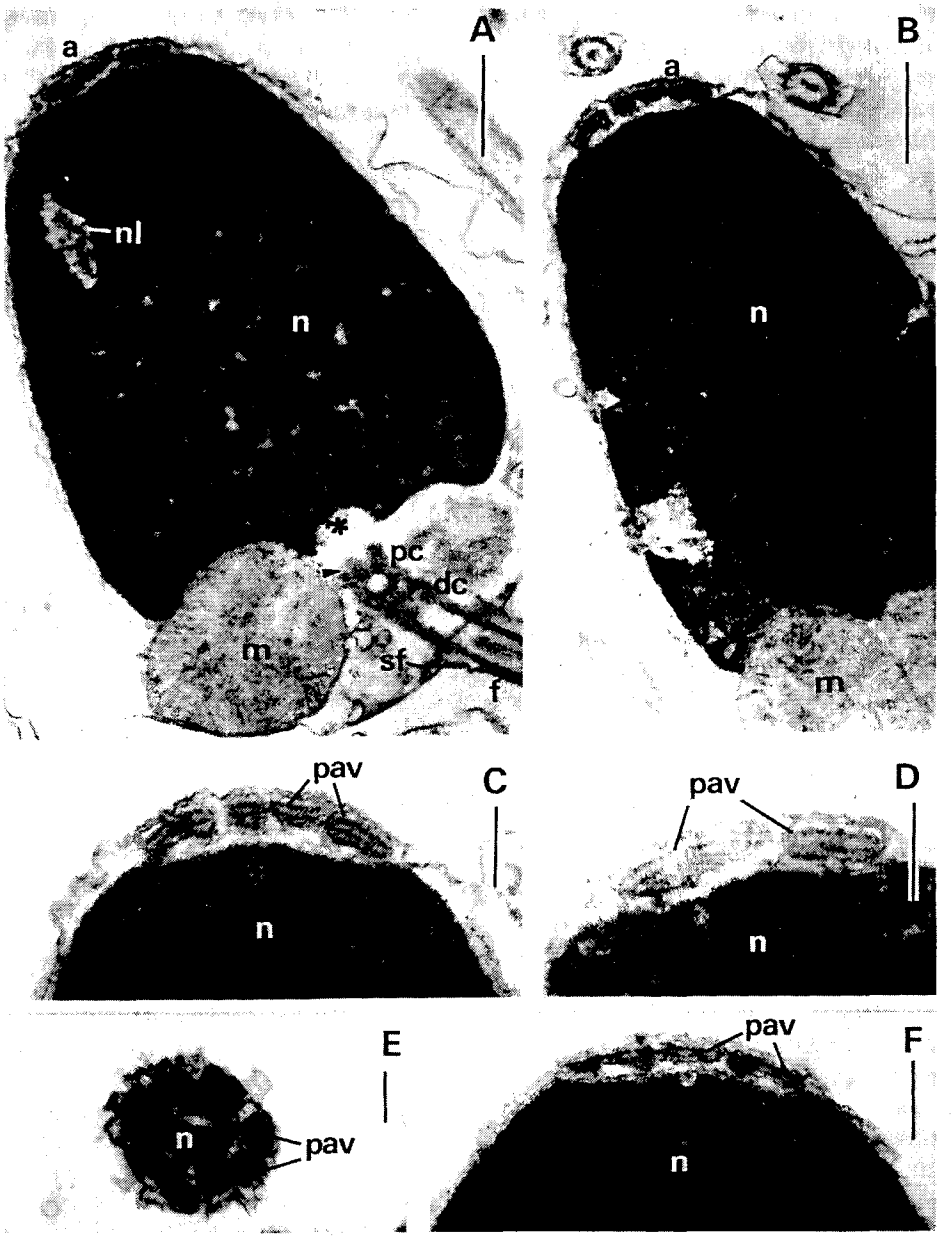

Fig. 1. A–E: *Neotrigonia margaritacea* (Lamarck). F: *N. bednalli* (Verco). A, B: Longitudinal sections of sperm acrosomal complex, nucleus, midpiece and proximal portion of flagellum. Note also centriolar fossa (*) and dense material (arrowhead) associated with proximal centriole. C, D: Longitudinal sections of acrosomal complex and nuclear apex. E: Transverse section through acrosomal complex, glancing nuclear apex. F: Longitudinal section of acrosomal complex and nuclear apex of *N. bednalli*. Abbreviations: a, acrosomal complex; dc, distal centriole (basal body); f, flagellum; m, mitochondrion; n, nucleus; nl, nuclear lacuna; pav, proacrosomal vesicles of acrosomal complex; pc, proximal centriole; sf, satellite fibres. Scale bar: A, B = 0.5 μm; C–F = 0.25 μm

mately 2.2 μm (near the base of the nucleus). Prominent nuclear lacunae are usually visible within the otherwise highly electron-dense, granular contents (Fig. 1 A, B). Five or more rarely four, dish-shaped depressions in the base of the nucleus cup the anterior portion of the midpiece mitochondria (Fig. 1A, B). A small, curved centriolar fossa occurs at the centre of the ring of nuclear depressions (See * in Fig. 1A). The midpiece consists of a pair of orthogonally arranged centrioles surrounded by five (sometimes only four) round mitochondria (Fig. 1A). Each mitochondrion exhibits radiating cristae. A loose deposit of dense material observed attached to the proximal centriole (see Fig. 1A arrowhead) possibly serves some purpose in anchoring the centriolar complex to the small fossa at the base of the nucleus. Nine radially arranged satellite fibres connect the distal centriole to the plasma membrane (Fig. 1A). The distal centriole acts as a basal body to the single 9+2 microtubular pattern flagellum. As Figure 1A indicates, the central microtubules of the flagellar axoneme commence posterior to the distal centriole. Light microscopic observations give a length of 48–55 μm for the flagellum.

DISCUSSION

As observed in *Neotrigonia bednalli* (see Fig. 1F), spermatozoa of *Neotrigonia margaritacea* exhibit multiple, flattened proacrosomal vesicles at the nuclear apex. Although other species of the genus remain to be studied, the close conchological similarities between these species (Darragh, 1986) suggest that they too will also show this unusual style of acrosomal complex (acrosomal region not preserved in available material of *N. gemma*). Nevertheless it was observed that the proacrosomal vesicles of *N. margaritacea* are larger than those of *N. bednalli* (compare Fig. 1C [*N. margaritacea*] with Fig. 1F [*N. bednalli*]: same magnification). Other features of the spermatozoa of *N. margaritacea*, such as the morphology of the nucleus and midpiece, and flagellar length, are essentially as observed in *N. bednalli* (and *N. gemma* from examination of museum material). With few exceptions the midpiece of other bivalves is similar to that occurring in *Neotrigonia* spp. (see Gharagozlou-Van Ginneken & Pochon-Masson, 1971; Popham, 1979; Franzén, 1983; Healy, 1989, 1995; Eckelbarger et al., 1990; Hodgson et al., 1990), that is, composed of round mitochondria surrounding a pair of orthogonally arranged centrioles (an arrangement characteristic of aquasperm in general; cf. Jamieson, 1987).

Within the Mollusca, the presence of multiple, unfused proacrosomal vesicles in mature spermatozoa is observed only in the Trigonioida and the Unionoida, thus providing what seems to be a valuable synapomorphy for the subclass Paleoheterodonta (for a detailed discussion of this aspect see Healy, 1989). All other bivalves possess a single, conical acrosomal vesicle, although during spermatogenesis in most of these species, multiple proacrosomal vesicles (which fuse to form the definitive acrosomal vesicle) are produced by the Golgi complex (see Longo & Dornfeld, 1967; Longo & Anderson,1969; Popham, 1979; Franzén, 1983; Healy, 1989, 1995; Hodgson & Bernard, 1986; Eckelbarger et al., 1990; Hodgson et al., 1990). The morphology of the mature acrosome of unionoids has been studied by various workers but with differing interpretations. Trimble & Gaudin (1975) reported a small acrosome in *Ligumia rostrata* (Say): their Figure 3 showing what appear to be two small vesicles embedded in a finely granular material. Healy (1989) demonstrated unequivocally that multiple vesicles were consistently present in spermatozoa of *Velesunio ambiguus* Iredale and drew attention to the close morphological

similarity of these vesicles to those of *Neotrigonia*. An acrosome is said to be absent in the unionoid species *Hyriopsis schlegelii* (by Higashi, 1964) or *Diplodon chilensis chilensis* (by Peredo et al., 1990), but this is understandable given the small size and seemingly fragile nature of unionoid proacrosomal vesicles (see Healy, 1989). Rocha & Azevedo (1990) have claimed that the proacrosomal vesicles of the unionoid *Anodonta cygnea* fuse to form a single acrosomal vesicle at a very late spermatid stage. Unfortunately, the actual fusion event was not demonstrated by Rocha & Azevedo, and only a single micrograph showing what appears to be only a single acrosomal vesicle in the mature sperm of *A. cygnea* was presented (two proacrosomal vesicles are shown in a micrograph of a spermatid). The presence of multiple proacrosomal vesicles in the mature sperm of unionoids and trigonioids could either be (1) a primitive state (e.g. as in sperm of some Cnidaria – see Baccetti & Afzelius, 1976) or (2) more likely be the result of a failure or extreme delay of these vesicles to fuse into a single acrosomal vesicle. While the author has never observed fusion of the proacrosomal vesicles in his own studies of unionoid or trigonioid spermatids, Rocha & Azevedo's findings for *Anodonta cygnea*, if verifiable by further study, would support option (2). However, the most recent study of mature sperm ultrastructure in unionids (Lynn, 1994) clearly demonstrates, in longitudinal and transverse sections (using transmission electron microscopy), that multiple acrosomal vesicles are present in the mature sperm of *Anodonta grandis*. Certainly the topic of acrosomal development in unionoids is worthy of further examination, ideally using a variety of species and genera. At present, however, the author believes that there is strong evidence in favour of multiple proacrosomal vesicles being present in mature unionoid spermatozoa, as is clearly the case in the Trigonioida.

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