Trimble

Fine Structure of the Sperm of the Freshwater Clam *Ligumia subrostrata* (Say, 1831)[1]

(Mollusca : Bivalvia)

BY

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(3 Plates)

INTRODUCTION

FINE STRUCTURAL STUDIES of pelecypoda sperm are sparse, and the majority of these studies have been done on sperm of the marine forms. To the authors' knowledge no reports exist on the fine structure of sperm from a freshwater bivalve. The clam, *Ligumia subrostrata* (Say, 1831), is a common inhabitant of freshwater ponds and marshes of the gulf coast area. The fine structure of the sperm of this species was studied to contribute additional information about the nature of primitive spermatozoan morphology.

MATERIALS AND METHODS

Clams used for this study were collected from a small freshwater pond in southern Louisiana. Mature sperm and sperm packets were removed from the clam by transection of the gonad. Tissue was quickly immersed in cold 3% glutaraldehyde, buffered with 0.1 M Millonig's phosphate buffer (pH 7.3), to which 1 mM $CaCl_2$ and 2% sucrose were added. After a 3 hr fix, tissue was washed overni in buffer, post fixed for 1 hr in cold 1% osmium tetrox in buffer, rinsed with distilled water, dehydrated in al hol and embedded in Spurr's epoxy. Sections were cut a Sorvall MT-2 ultramicrotome using glass knives, stai with alcoholic uranyl acetate and lead hydroxide, viewed with either an Hitachi 11A or RCA-EMU-3G e tron microscope.

Carbon replicas were prepared using glutaraldeh fixed sperm. A drop of sperm suspension was applied parlodion coated grid and a few seconds were allowed the sperm to settle out of solution onto the grid. Ex fluid was removed by blotting on filter paper. Grids w coated with about 100 A thickness of carbon and the ti digested with a solution of potassium permanganate potassium dichromate in sulfuric acid (DAWES, 19 Grids were washed in distilled water, dried and shado using a carbon-platinum pellet.

OBSERVATIONS

Sperm from *Ligumia subrostrata* measure 35 µm long possess the classical head, midpiece and tail arranger

[1] This work was supported in part from a grant from the Louisiana University Council on Research

Explanation of Figures *1* to *3*

Figure *1*: Carbon replica of mature sperm. Arrow indicates midpiece containing mitochondria × 24 000

Figure 2: Anterior tip of head of sperm showing acrosomal region (arrow) × 68 000

Figure *3*: Longitudinal section through sperm showing n (N), mitochondria (M) of midpiece, projecting into subn fossa (white arrow), ring centriole (R) and axoneme (AX) ×

Figure 1

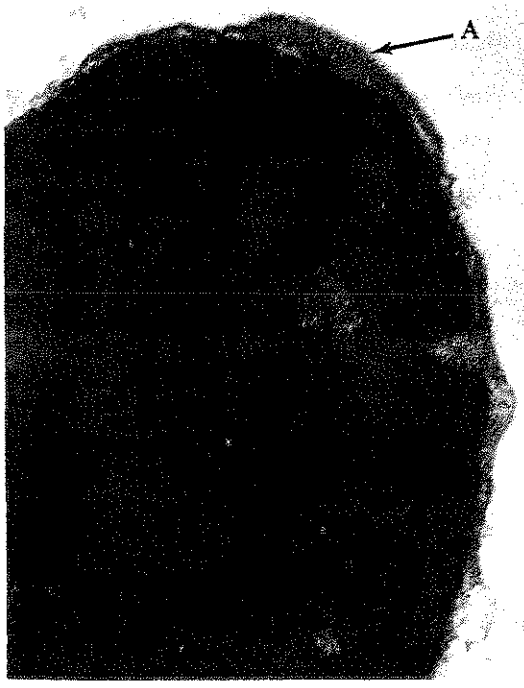

Figure 2

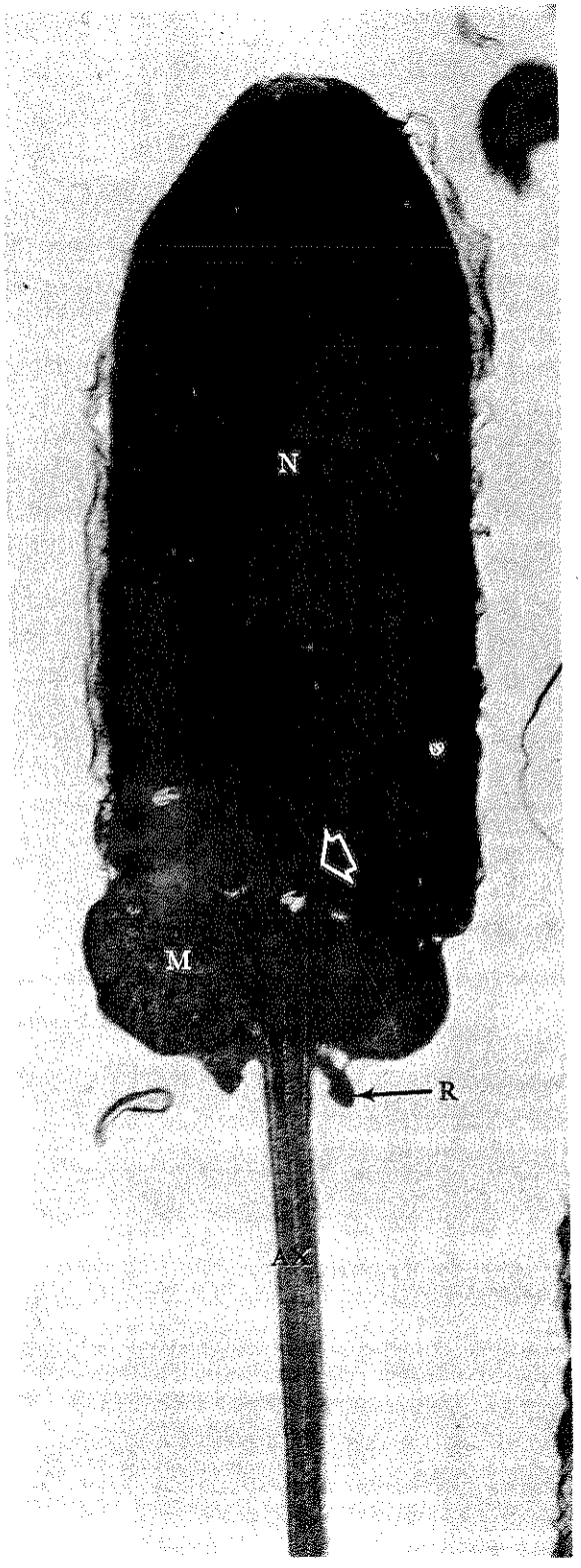

Figure 3

Figures *1* and *2*). The head region contains the conical nucleus which averages 2.5 µm long by 1 µm wide. Associated with the anterior region of the nucleus is a vesicle believed to be the acrosome (Figure *2*).

A short midpiece measuring 0.5 µm long by 1 µm wide consists of five mitochondria arranged symmetrically around the centriole and axonemal complex (Figure *6*). The midpiece fits into a depression in the base of the nucleus, the subnuclear fossa (Figure *3*). This pattern of the midpiece is best illustrated by following the sequential appearance of each of these structures as seen in transverse section proceeding from the nucleus posteriorly through the sperm to the tail. The dense nucleus is surrounded by a loose fitting nuclear envelope (Figure *4*). Five symmetrically arranged mitochondria appear at the level of the proximal centriole with blocks of nuclear material interspersed (Figure *5*). At a level just posterior to the distal centriole (Figure *6*), five mitochondria of about equal size are arranged around the tubules of the anterior portion of the axonemal complex. At this level, no central pair of tubules within the axoneme is present. Posterior to this level (Figure 7), the mitochondria lose their association with each other and the axonemal complex now exhibits a classical 9 + 2 tubular arrangement. The posterior portion of the midpiece consists of a cytoplasmic collar or ring centriole surrounding the axoneme (Figure *8*). The axoneme changes shape posterior to this region becoming oblong in appearance. The matrix of the axoneme is electron dense and contains no other structure besides the axonemal tubules (Figure *10*). The core of each tubule is hollow and completely void of any accessory material. The 9 pairs of peripheral tubules and pair of central tubules are equal in diameter. Near the end of the sperm tail, the central pair of tubules is the first to disappear followed by the peripheral tubules (Figure *10*).

DISCUSSION

The primitive sperm of *Ligumia subrostrata* exhibits the typical morphology of a broadcast fertilizer. In general appearance, it has the same basic architectural format as sperm from unrelated animals such as the Cnidaria (Hinsch & Clark, 1973).

All previously investigated spermatozoa of species closely related to *Ligumia* differ by possessing a more highly developed acrosome. The oyster, *Crassostrea virginica* (Gmelin, 1791), possesses a prominent highly oxmiophilic acrosome which caps a bilobed nucleus (Galtsoff & Philpott, 1960: 244; figure 3). An acrosomal rod and inner acrosomal membrane are present and the midpiece consists of four mitochondria. In the related mussel, *Mytilus edulis* Linnaeus, 1758, a complex spear shaped acrosome is present with an axial rod running from the base of the acrosome to the midpiece dividing the nucleus into two equal lobes (Longo & Dornfeld, 1967: 477; figure 25). Five mitochondria are present in the midpiece.

Spisula solidissima (Dillwyn, 1817), the surf clam, has a barrel shaped nucleus capped by an elaborate acrosomal complex containing a prominent rod (Longo & Anderson, 1969: 441; figure 7). The midpiece of the mature sperm is composed of four ellipsoid mitochondria resulting from the fusion of the numerous smaller ones during differentiation of the sperm. This pattern of development is characteristic of most primitive sperm (Potswald, 1966; Fawcett, 1970). The acrosome is far less complex than seen in other bivalves, and it contains no Golgi-proacrosomal granules as reported for certain primitive hydrozoan sperm (Hinsch & Clark, 1973). *Ligumia* is similar in possessing five mitochondria arranged circumferentially around the centriole complex.

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Explanation of Figures *4* to *7*

Figure *4*: Transection through nucleus of sperm × 46 000
Figure *5*: Transection of sperm at level of the proximal centriole (arrow). Mitochondria (M) are arranged symmetrically with blocks of nuclear material (N) interspersed × 40 200
Figure *6*: Transection of sperm at level below distal centriole. At this level the central pair of tubules (arrow) is not yet present × 42 000
Figure *7*: Transection of sperm at level where the axoneme shows a 9+2 tubular pattern (arrow) × 48 000

Explanation of Figures *8* to *10*

Figure *8*: Transection of sperm at level of ring centriole (R) × 84 500
Figure *9*: Longitudinal section of axoneme, showing the parallel tubule pattern (arrow) × 65 000
Figure *10*: Transection of axoneme illustrating the tubular pattern at various levels × 60 000

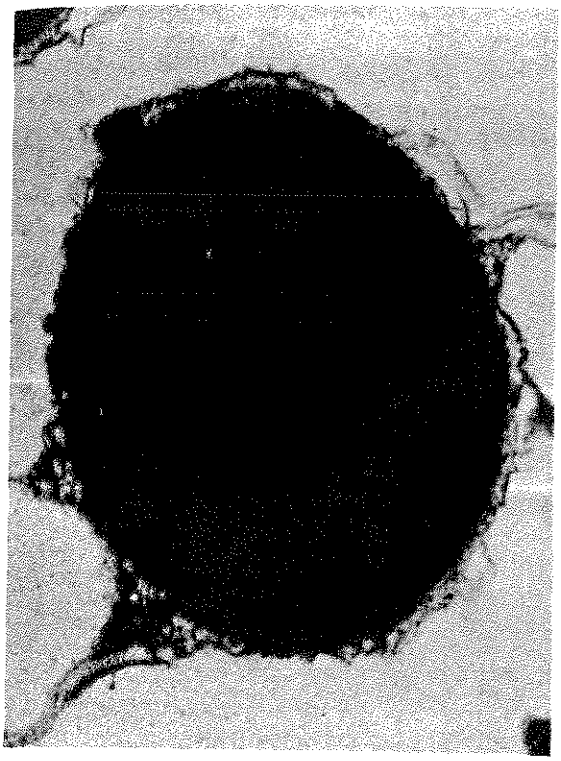

Figure 4

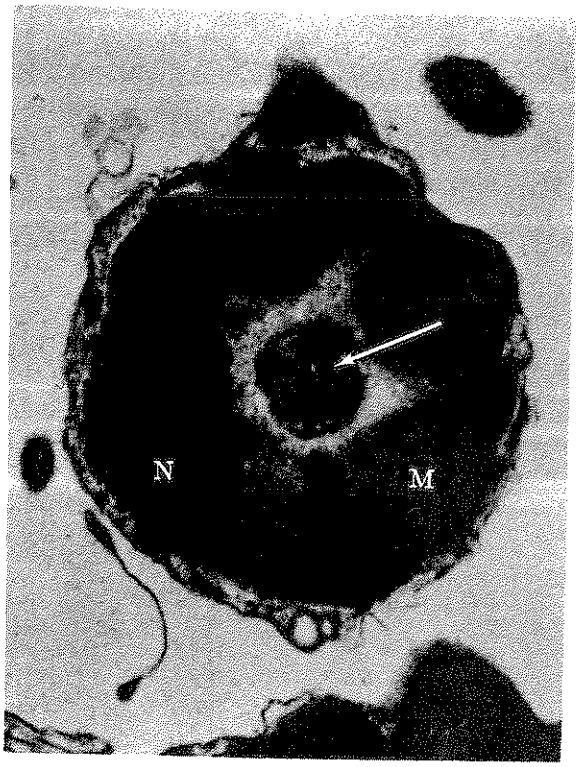

Figure 5

Figure 6

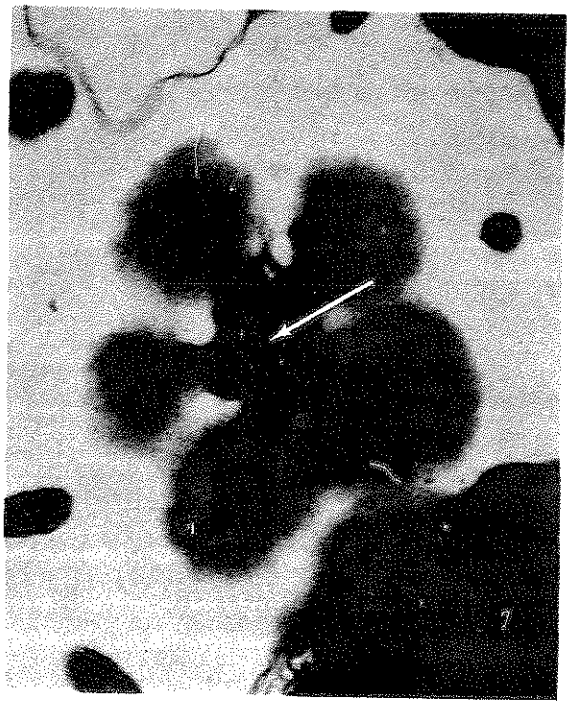

Figure 7

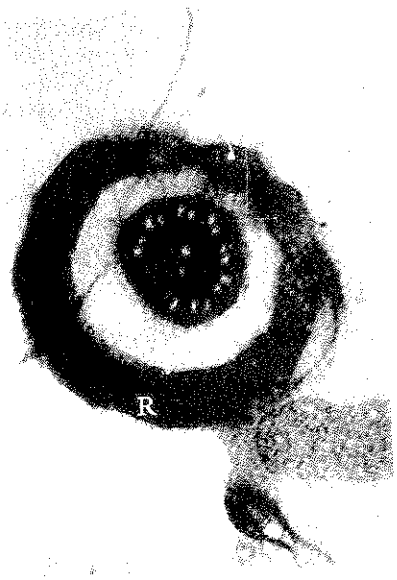

Figure 8

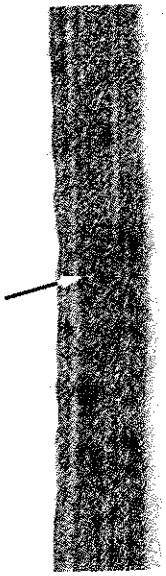

Figure 9

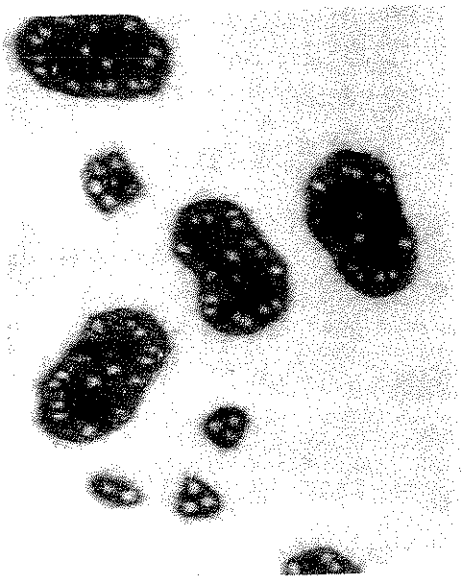

Figure 10

Figure 8: Transection of sperm at level of ring centriole (R) × 84 500
Figure 9: Longitudinal section of axoneme, showing the parallel tubule pattern (arrow) × 65 000
Figure 10: Transection of axoneme illustrating the tubular pattern at various levels × 60 000