Structural specialization in the flagellum of the spermatozoon of the bloodsucking bug (Rhodnius prolixus; Hemiptera, Reduviidae)

Sônia Nair Báo
Wanderley De Souza

Abstract

Spermatozoa of the triatomideo Rhodnius prolixus possess an axoneme with a 9 + 9 + 2 microtubule pattern and two mitochondrial derivatives. Bridges occur between axoneme and mitochondrial derivatives. Two paracrystalline structures embedded in amorphous regions were observed in the mitochondrial derivative. The use of the negative staining technique shows a zig-zag profile in the mitochondrial derivatives due to infolding to the cristae, regularly spaced with approximately 50 nm. This spacing is also observed in the distribution of the strands of particles in the mitochondrial membrana as seen in freeze-fracture replicas. In the P-fracture face of the flagellar plasma membrane, a regular array of the intramembranous particles was observed. This array consists of two rows, with 12–15 particles, and occurs in the space between the mitochondrial derivatives. Thus R. prolixus spermatozoon present a membrane domain, localized in the flagellar region, and bridges between mitochondrial membrane derivatives and the plasma membrane are probably attached to the flagellar components. These membrane specializations may be related to the production of co-ordinated flagellar movement, and can contribute significantly to further phylogenetic studies.

Keywords: Flagellum; freeze-fracture; membrane domain; spermatozoon; triatomideo

Introduction

Most insects have long and filiform spermatozoa. The spermatozoon head consists of an acrosome and nucleus, and the tail, of a long axoneme flanked by two mitochondrial derivative?.. It is well-known that the typical insect spermatid mitochondria undergoes complicated changes leading to the formation in the mature spermatozoon of the mitochondrial derivatives (Pratt, 1968; 1970), which are located alongside the axoneme and which compose a larger part of the cell volume (Perotti, 1969). The presence of a prominent paracrystalline structure embedded in an amorphous matrix and precise organization of the cristae (Baccetti et al.. 1977) distinguish this organelle from typical mitochondria.

Heteropteran spermatozoa have certain characteristics in common that are not found in other insects: two or three crystalline bodies within the mitochondrial derivatives and bridges between the mitochondrial derivatives and two of the axonemal microtubules (Afzelius et al., 1976; 1985; Dallai & Afzelius, 1980; Dolder, 1988). The zig-zag profile of the mitochondrial derivatives can be related to the cristae, which are infoldings of the mitochondrial inner membrane (Rosati et al., 1976; Dolder, 1988). Using the freeze-fracture technique, a specialized membrane domain has been observed along the sperm tail. This domain appears as a zipper-line of intramembranous particles regularly arranged in rows

(Dallai and Afzelius, 1982). In the present study, the spermatozoon flagellum of Rhodnius prolixus is analyzed a combined in a correlated thinsectioning, negative staining and freeze-fracturing study, with particular emphasis on the mitochondrial derivatives structure and their interaction with the flagellar components.

Material and Methods

Adult males of the bloodsucking bug R. prolixus; Hemiptera, Reduviidae) were dissected and spermatozoa were obtained from the testes and seminal vesicles.

A drop of suspension of spermatozoa obtained from the seminal vesicle and placed in phosphate-buffered saline (PBS), pH 7.0, was smeared on a slide, coated with glass chips, and observed and photographed with a Zeiss Axiophot light microscope.

For examination of ultrathin sections, the testes and seminal vesicles were dissected and fixed in a mixture of 2.5% glutaraldehyde, 1% tannic acid and 1.8% sucrose in 0.1 M cacodylate buffer, pH 7.2, followed by block-staining in 1% uranyl acetate in distilled water (Afzelius, 1988). The tissue was dehydrated in acetone, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate.

For negative staining, spermatozoa obtained from ruptured seminal vesicles were sonicated for a few seconds with an ultrasonic apparatus and then placed on a coated grid. The preparations were negatively stained with potassium phosphotungstate, pH 6.8.

For freeze-fracture, glutaraldehyde fixed spermatozoa were washed in 0.1 M cacodylate buffer, pH 7.2, and gradually impregnated for 30min with glycerol in cacodylate buffer up to a concentration of 30%, in which they were left for periods varying from 3 to 12 hr. Specimens were mounted on Balzer's support disks and rapidly frozen in the liquid phase of partially solidified Freon 22 cooled by liquid-nitrogen. Freeze-fracture was carried out at -115°C in Balzer's apparatus. After fracturing, the specimens were platinum-shadowed and carbon-coated. Replicas were cleaned with disodium hypochloride, washed with several changes of distilled water and mounted on 300 mesh grids.

All preparations were examined in a Jeol 100 C transmission electron microscope.

Results

The spermatozoon of the bloodsucking bug (R. prolixus) showed a threadlike appearance, approximately 350 pm long, only 30 pm of which belong to the head (Fig. 1).

The sperm tail of the R. prolixus was formed by an axoneme with 9(outer singlets) + 9(intermediate doublets) + 2(central singlets) pattern of microtubules flanked by two mitochondrial derivatives. The two traits that are believed to be characteristic for the heteropteran spermatozoa could be distinguished: bridges that connect the mitochondrial derivatives to axonemal microtubules 1 and 5, and two separate paracrystalline structures in each mitochondrial derivative (Fig. 2). These paracrystalline structures differ in size and in the regular spatial distribution of their subunits. The bridges between axonemal microtubules and mitochondrial derivatives occur in the region of the small paracrystalline structure (Figs 2, 8). The paracrystalline structure is embedded in an amorphous material and at the periphery the mitochondrial inner membrane presents a series of deep regular invaginations (Figs 3, 4).

After negative staining, the mitochondrial derivatives presented a zig-zag profile (Figs 5, 6). This profile could be related to the cristae. which are infoldings of the inner membrane of the mitochondrial derivative (Fig. 5). The spacing observed between cristae was regular, approximately 50nm. When surrounded by the mitochondrial outer membrane, the expansions of the derivative may be observed to correspond to the infoldings of the cristae (Fig. 6).

In longitudinal sections and in the negative staining preparations, striations exhibiting a periodicity and an undulated disposition were observed in the paracrystalline structure (Figs 3-6).

In cross and oblique sections of the sperm tail that small bridges occurring between membrane outer derivatives and plasma membrane (Figs 7-9) were observed. The distance between bridges was perfectly regular and apparently corresponds to spacing observed between cristae in longitudinal sections and in the negative staining.

In freeze-fracture preparations, the zig-zag arrangement of the cristae was also evident (Figs 10-12). Where the fracture plane had passed between the mitochondrial cristae and plasma membrane, along the mitochondrial outer membrane, regularly spaced particles. with a periodicity of about 50nm, could be seen. These particles, with a size of about 9nm, were aligned in parallel in the region corresponding to the space between the infoldings of the cristae (Fig. 11). When the fracture plane entered deeper into the mito chondrial cristae, transverse indentations with about 25 nm wide, which alternated with projecting bands of approximately 50 nm, were observed (Figs 12 and 13). In this region of the fracture, particles associated with indented and projecting bands were not observed.

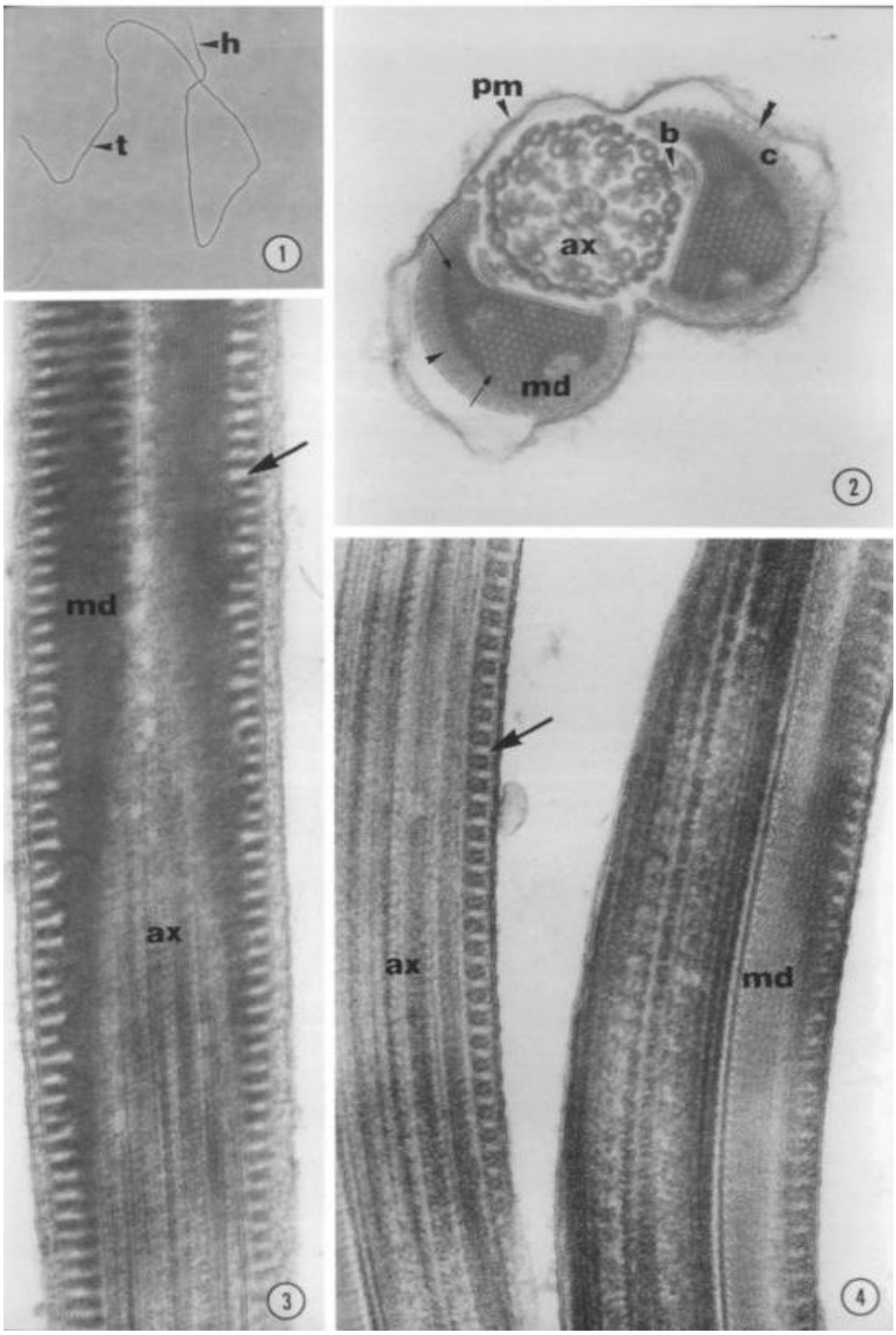
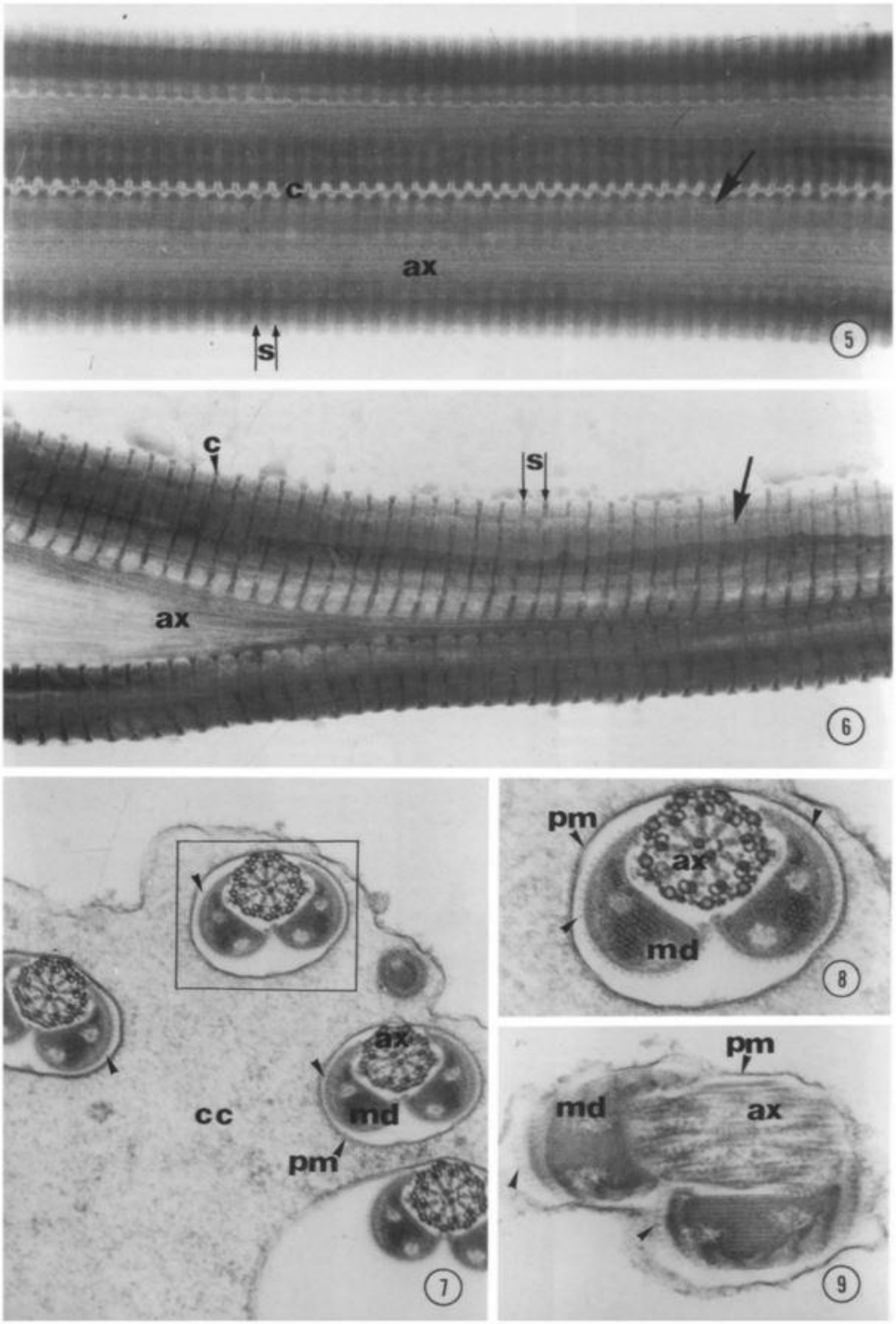


Fig. 1. Mature spermatozoon of Rhodniur prolixus seen with the phase contrast microscope. h, head; t, tail. X250. Fig. 2. Cross section of the spermatozoon tail. Bridges (b) may be seen extending between the axoneme (ax) and the mitochondrial derivatives (md), which are formed by paracrystalline structures (arrow) embedded in amorphous material (arrowhead). Cristae (c) are observed in the cortical region. Small bridges (arrowhead double) appear distributed regularly binding the mitochondrial derivatives at plasma membrane (pm). X 165,000. Figs 3, 4. Longitudinal sections of the spermatozoa flagellum showing the mitochondrial derivatives (md) with the typical wave pattern of the paracrystalline structure. The mitochondrial inner membrane exhibit invaginations (arrows) regularly spaced. ax, axoneme. Fig. 3, X 70,000; Fig. 4, x100,000.



Figs 5, 6. Mitochondrial derivatives after negative staining. Infolding of the cristae (c) apparently produce a zig-zag profile of the mitochondrial derivatives. The spacing (s) of 50 nm between cristae is evident. The typical wave pattern of the paracrystalline structure is clearly visible (arrows). ax. axoneme. Fig. 5, x56,GOO; Fig. 6, x75.000. Fig. 7. Cross section of the spermatozoa tail. Small bridges (arrowheads) are observed between the mitochondrial derivatives (md) and the plasma membrane (pm). ax. axoneme: cc. cystic cell. X50.000. Fig. 8.

A higher magnification of the flagellum detached in the Figure 7. x 100,000. Fig. 9. Oblique section of the spermatozoon flagellum showing the small bridges (arrowheads) between the mitochondrial derivative (md) and the plasma membrane (pm). ax. axonome. x 100,000

In the tail region of the spermatozoon, intramembranous particles (IMPS) were observed throughout the plasma membrane (Figs lS15). A regular array of the IMPS was observed in a few regions of the P-fracture face. This array consisted of short double rows, with 12-15 particles. The particles had a diameter of about 15 nm (Figs 13, 14). Our observations suggest that the particle array observed in the tail portion of the spermatozoon was located in the space between the mitochondrial derivatives. associated with the P-fracture face of the plasma membrane (Fig. 15). In the E-fracture face the lining of the tail portion of the sperm IMPS were randomly distributed throughout the plasma membrane.

Discussion

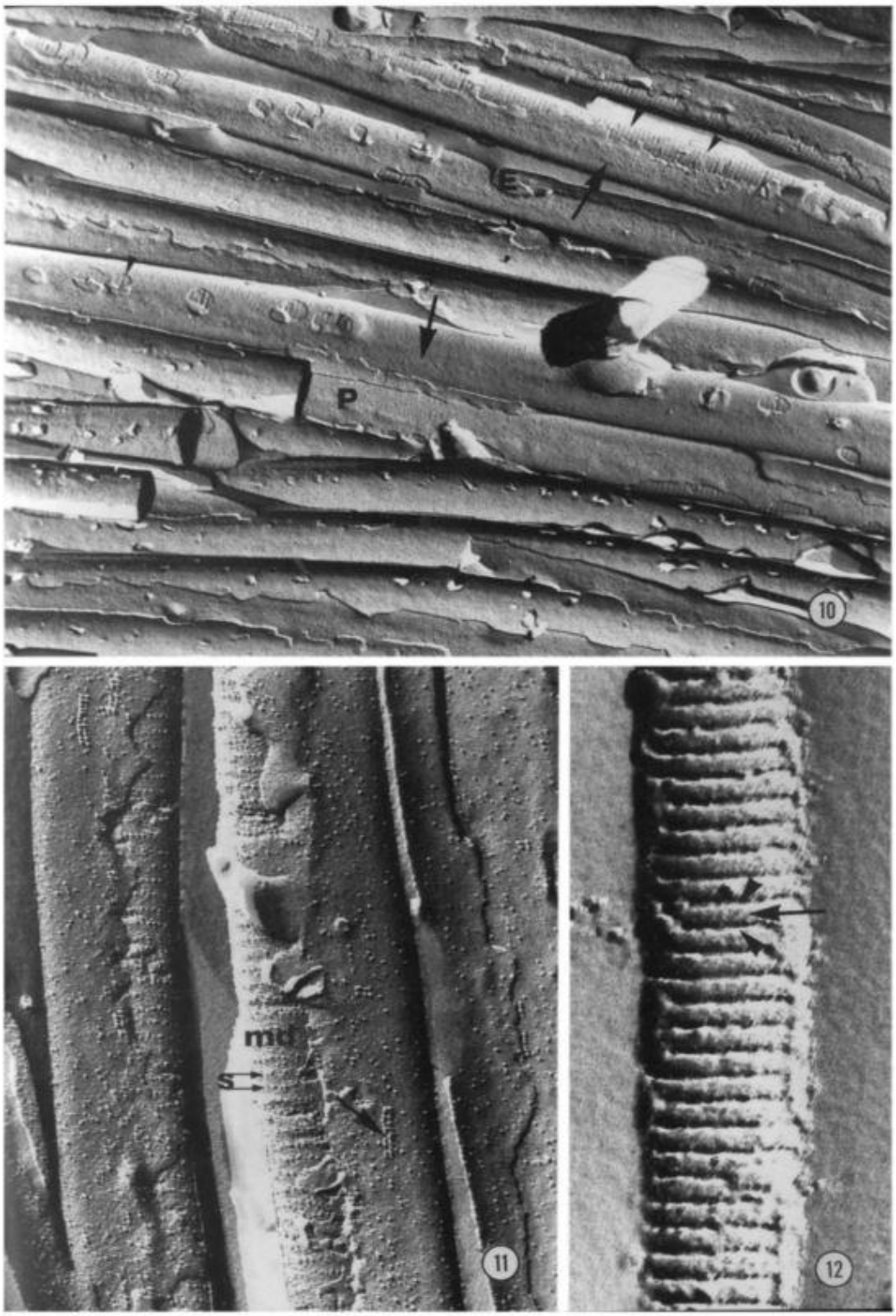
Two features are described here as characteristic of the spermatozoon of the bloodsucking bug (R. yrolixus): (1) bridges between the axoneme and mitochondrial derivatives, and (2) two paracrystalline structures in the mitochondrial derivatives. The same features have been described in spermatozoa of other heteropteran species (Phillips, 1970; Afzelius et al., 1976; 1985; Dallai and Afzelius, 1980; Dolder, 1988). These characters are synapomorphic and hence useful in phylogenetic studies.

Our observations show the presence in the mature spermatozoon of two mitochondrial derivatives of similar size and shape, flanking the axoneme along its length. Two distinct regions may be seen in the mitochondrial derivatives. The major portion is occupied by two paracrystalline structures which is embedded in a region containing amorphous material. It is interesting to note that paracrystalline structures of different sizes were observed. The bridges that occur between the axoneme and mitochondrial derivatives are always located in the region of the small paracrystalline structures and microtubules doublets 1 and 5. Hence, these bridges established a structural and functional relation between the flagellar components. The presence of bridges which strongly bind mitochondrial derivatives to the axoneme is essential in flagellar motion, to produce coordinated movement. Bridges with regular distribution between the flagellar organelles has been shown in spermatozoa Triatoma infestam (Dolder, 1988). Previous studies suggest that the mitochondrial derivatives and their association with the other flagellar components play some role in the control of the movement of the axoneme (Tokuyasu, 1975), and regulation of the wavelength (Phillips, 1974).

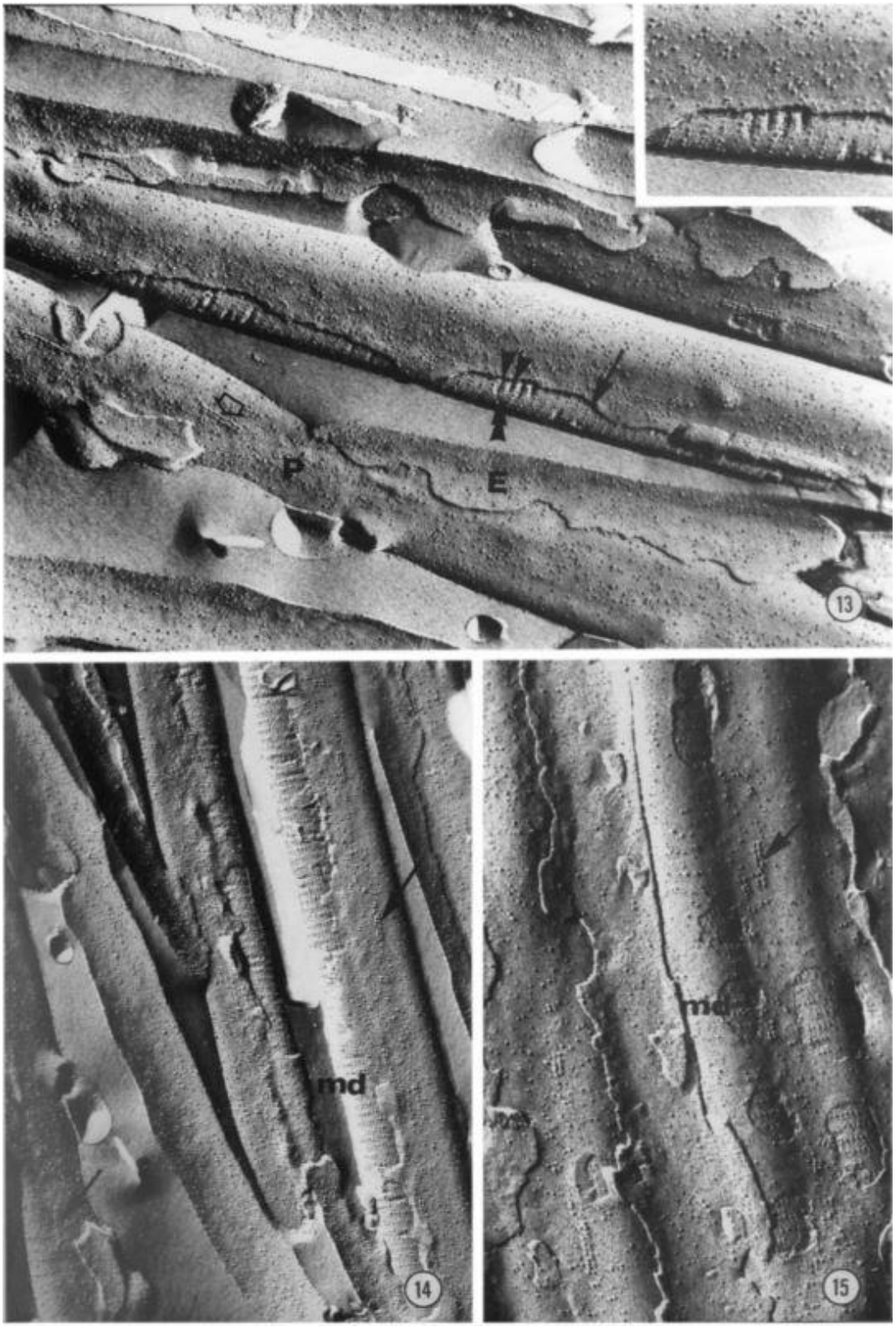
The reorganization of the membranes in the peripheral region of the mitochondrial derivatives establishes a zig-zag profile, due to infoldings to the cristae which clearly correspond to expansions of the mitochondrial derivative, while the membrane extending between the infolding. This pattern was better visualized after negative staining. Studies carried out on insect spermatozoa have shown that the reorganization of the mitochondrial inner membrane leads to an helicoidal arrangement of this structure (Rosati et al., 1976).

Small bridges are regularly distributed between the mitochondrial outer membrane and the plasma membrane, possibly establishing a strong attachment of the mitochondrial derivatives to the plasma membrane. They were analyzed in more detail in cross sections of spermatozoa fixed in a glutaraldehyde solution containing tannit acid. Tannic acid acts as a mordant and binds to the protein molecules of the bridges, giving contrast to them. This pattern of contrast observed in the small bridges is similar to that reported in microtubules (Afzelius et al., 1991) and in the paracrystalline structure of mitochondrial derivatives (Baa et al., 1992).

The present study revealed intramembranous particles regularly arranged in rows when the fracture plane occurs presumably along the mitochondrial outer membrane. These rows are regularly spaced, with a periodicity of 50nm, which corresponds to the space between the infoldings of the cristae. Our suggestion is that this specialized domain observed in the mitochondrial membrane, probably participates in the attachment of the mitochondrial derivatives to the plasma membrane. Previous studies have revealed examples of special arrays of membrane particles in sites involving association of cytoplasmic components with the plasma membrane of spermatozoon, as is the case of the helical arrangement of the mitochondrial cristae of insect (Afzelius et al., 1976; Dallai and Afzelius, 1985); the longitudinal particle lines along the free portion of vertebrate sperm tail (Olson et al., 1977; Suzuki and Nagano, 1980); and the helically wound particle arrays overlying the midpiece mitochondria in mammalian spermatozoa (Friend and Fawcett, 1974; Friend and Heuser, 1981; Cieciura et al., 1986).



Figs 10-12. Freeze-fractured Rhodnius prolixus spermatozoa. Fig. 10. The replica shows the P and E-fracture faces of the plasma membrane of several spermatozoa. Along the P-face of the plasma membrane short double rows of intramembranous particles (arrows) are visible. Particles are aligned in rows (arrowheads) in the mitochondrial outer membrane. x 18,000. Fig. 11. The regularly arranged particles of mitochondrial derivatives (md) is visible, which display a spacing (s) of 50 nm between the rows. Short double rows of particles (arrow) may be seen in the P-face of the plasma membrane. X 52,000. Fig. 12. The fracture in the plane of the mitochondrial cristae show transverse indentations (arrowheads) alternating with projecting bands (arrow). X 92,000.



Figs 13-15. Freeze-fractures Rhodnius prolixus spermatozoa Fig. 13. The replica shows intramembranous particles randomly distributed throughout the E-face of the plasma membrane, while in the P-face, short double rows of particles are observed (arrow. open). Rows of particles (arrow) along the mitochondrial outer membrane, and indentations (arrowheads) alternating with projecting bands (arrowhead, double) of the mitochondrial cristae may be seen. Note that the particle rows are localized in the region corresponding to projectin bands (inset). ~40,000; Inset, x64,GQO. Fig. 14. The short double rows of

intramembranous particles (arrows) associated with the Pface of the plasma membrane are distributed along the flagellum. There is an apparent lack of regularity in the spacing between these. md, mitochondrial derivative. x 33,000. Fig. 15. The short double rows of intramembranous particles (arrow) is associated with the P-face of the plasma membrane in the space between the mitochondrial derivatives (md). x 46.000.

Our present observations show that the spermatozoon of R. prolixus does not present membrane specializations similar to those found in other insect spermatozoa (Baccetti er al., 1971; Afzelius et al., 1976; Dallai and Afzelius, 1982; 1985; Báo and de Souza, 1992). However, a regular arrangement of two rows, with about 12-15 particles, was seen on the P-face of the sperm plasma membrane lining the tail. These membrane domains occur in the plasma membrane region between the two mitochondrial derivatives. These double rows of intramembranous particles are probably elements that relate to stability of the flagellum.

Specialized domains have been found in the plasma membrane of some insects. A longitudinal row of membrane particles was previously observed in the plasma membrane of a fruit-fly sperm (Baccetti et al., 1971). On specialized domains characterized by different patterns of intramembranous particles is displayed in paired spermatozoa of the dytiscid beetles (Dallai and Afzelius, 1985). The zipper-line was located in the tail region in hemipteran spermatozoon (Dallai and Afzelius, 1982); and in the post-acrosomal region in Culex quinquefasciutus spermatozoon (Baa and de Souza, 1992).

Therefore, the R. prolixus spermatozoon displays a membrane domain, the double row of 12-15 particles, localized between mitochondrial derivatives in the flagellar region, and regularly spaced rows of particles in the mitochondrial membrane. These structures in conjunction with the bridges between axoneme and mitochondrial derivatives may be related to the production of coordinated flagellar movement. These membrane specializations can contribute significantly to further phylogenetic studies.

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