

# Giant sperm cells with accessory macrotubules in a neuropteran insect

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## Abstract

The flagellar axoneme of the atypical spermatozoa (paraspermatozoa) of *Mantispa perla* (Neuroptera, Planipennia) contains accessory microtubules or rather macrotubules that are 55 nm in diameter and that has a wall consisting of about 40 protofilaments. The sperm tail further contains two giant mitochondrial derivatives, which during spermiogenesis store an electron dense material. The mature spermatozoon has a flattened acrosome and an elliptical nucleus. These giant spermatozoa may furnish nutrients to the functional spermatozoa (euspermatozoa) when they reach the female genital tracts or/and they function in sperm competition filling the spermatheca.

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## 1. Introduction

Animals that shed their sperm in water, usually for external fertilization, have what has been called 'aquasperm' (Jamieson, 1987; Rouse and Jamieson, 1987). Typically these are small cells with a short sperm head, an even shorter mitochondrial midpiece and a long flagellum with an axoneme that in cross-sections resembles a normal  $9 + 2$  cilium. Aquasperm with these morphological characteristics are also named 'primitive spermatozoa' and believed to be plesiomorphic to other sperm categories (Franzén, 1956).

By contrast, animals that have a direct sperm transfer (with spermatophores, copulation, etc.) tend to have slender spermatozoa with an elongated midpiece and a sperm tail that is reinforced by nine so called outer dense fibers (or accessory fibers); each one of the nine microtubular doublets of the flagellum is connected to a fiber or located in correspondence to it. This kind of axoneme is designed as a  $9 + 9 + 2$  flagellum and has been described from spermatozoa of, among others, gastrotrichs (Ferraguti et al., 1995), leeches (Franzén, 1991),

fresh-water bryozoa (Franzén, 1976), gastropods (Anderson and Personne, 1969), cephalopods (Baccetti et al., 1976), lampreys (Jamieson, 1991), lizards (Furieri, 1970; Teixeira et al., 1999), snakes (Austin, 1965), passerine birds (Asa and Phillips, 1987) and mammals (Baccetti et al., 1973). These kinds of spermatozoa are called "introsperm".

Insects, velvet worms, a few polychaete worms and some priapulids provide a special case in that their sperm tails have accessory microtubules, rather than dense fibers (Dallai and Afzelius, 1999). Axonemes in the insects are also regarded as being of a  $9 + 9 + 2$  type, a designation that nonetheless hides a surprisingly large axonemal diversity, in that only a few insect orders have microtubules of the common type, thus with 13 protofilaments, whereas in other orders the cross-cut microtubules have 15, 16, 17, 18, 19 or 20 protofilaments, with 16 being the most common number found in the majority of insect orders (Dallai and Afzelius, 1990, 1994; Jamieson et al., 1999).

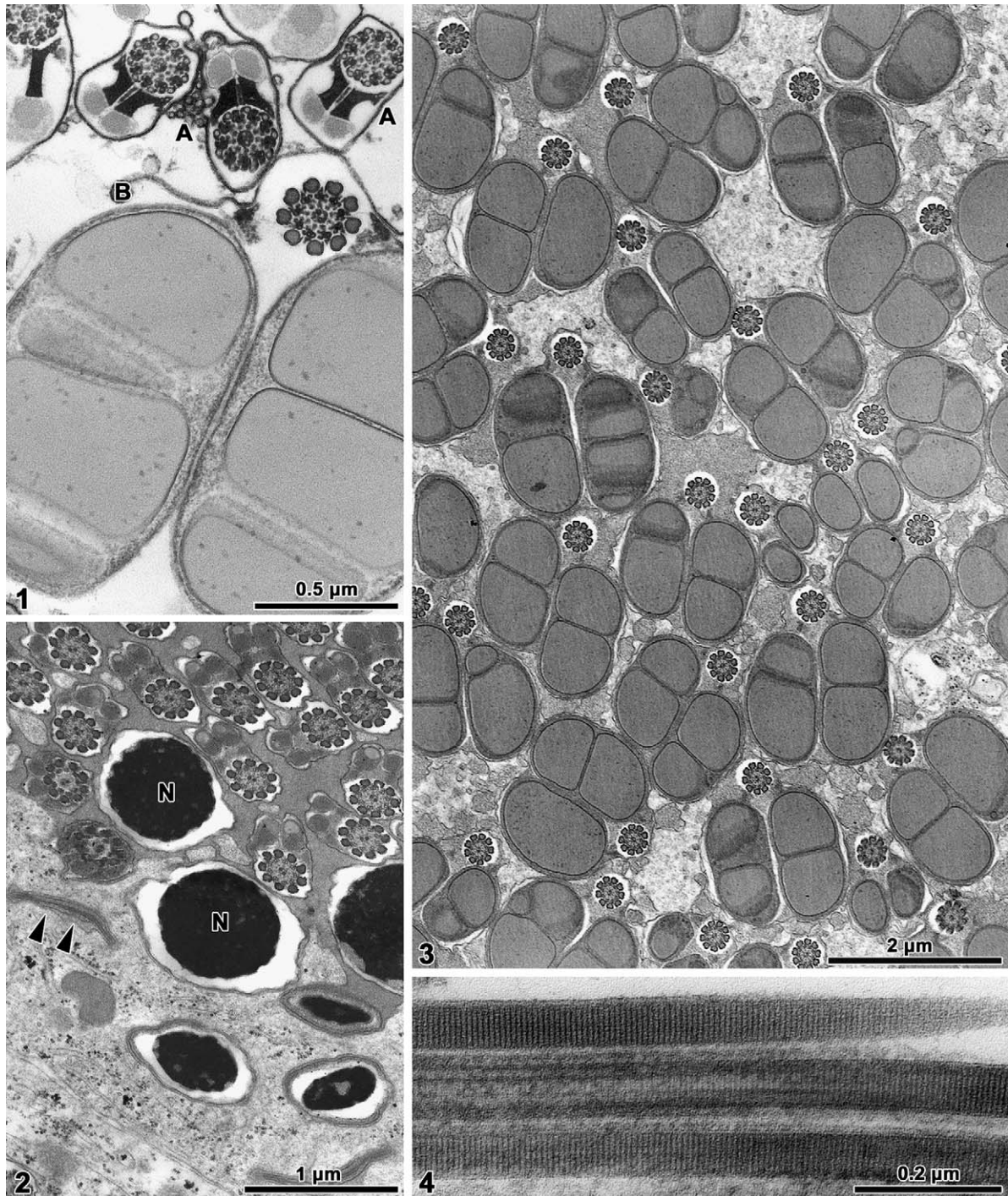
In this paper, we show that the axoneme of the atypical spermatozoa (paraspermatozoa) of the neuropteran *Mantispa perla* is of  $9 + 9 + 2$  type, as is common in insects, although the accessory units are neither regular fibers, nor normal microtubules, but are of a kind that is termed

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macrotubules (cf. Unger et al., 1990). The purpose of this communication was to describe this unique feature, which, at first glance, recalls the structure of accessory fibers of other organisms.

## 2. Materials and methods

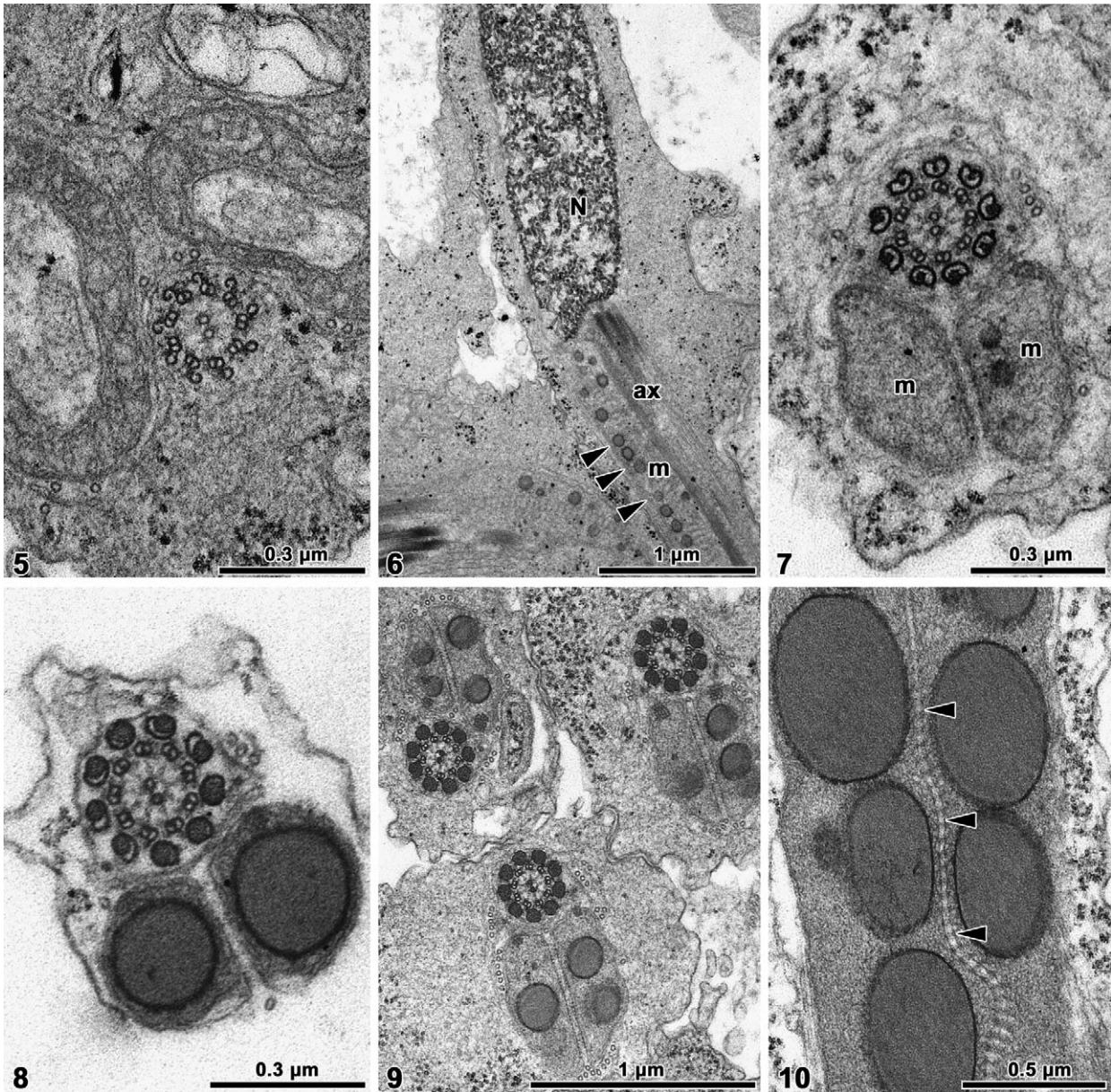
A male of the rare neuropteran *M. perla* (Poll.) (Planipennia) was caught in the neighbourhood of L'Aquila (Central



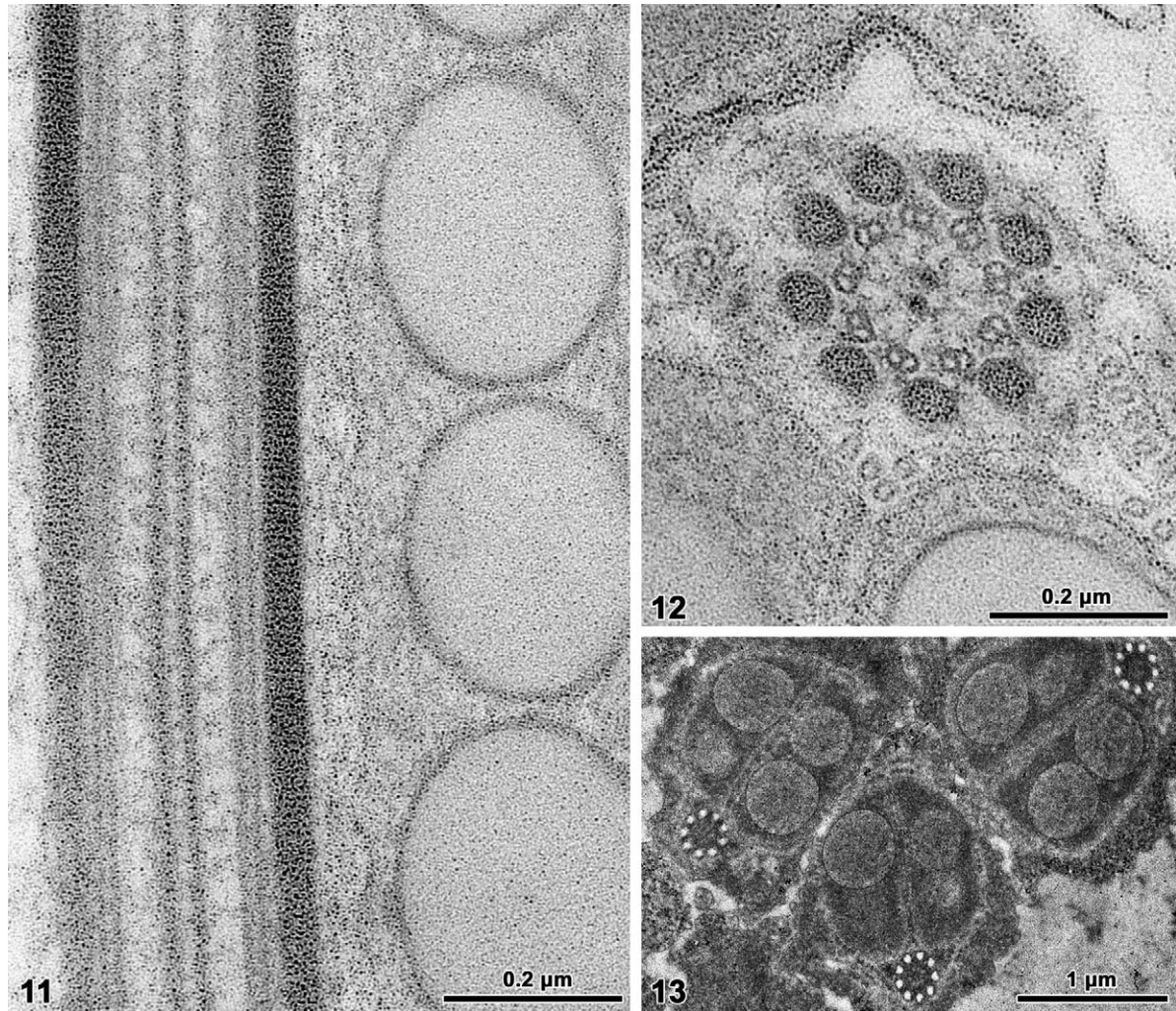
Figs. 1–4. (1) Cross-section through a bundle of *Mantispa perla* spermatozoa showing the euspermatozoa (A) and the paraspermatozoa (B), the latter with both large accessory tubules and mitochondrial derivatives. (2) Cross-section through a cyst of paraspermatozoa to show their acrosome (arrowheads) and nucleus (N). (3) Cross-section through a cyst of paraspermatozoa showing the large mitochondrial derivatives. (4) Longitudinal section through the accessory macrotubules to show the periodicity of the material filling the tubules.

Italy), was dissected in phosphate buffer (PB) to isolate testes and deferent ducts. Part of the material was fixed for 2 h at 4 °C in 2.5% glutaraldehyde in 0.1 M PB pH 7.2, to which 1.8% sucrose was added; after rinsing with PB, the material was post-fixed for 1 h in 1% osmium tetroxide, then rinsed in PB, dehydrated in ethanol and embedded in Epon-Araldite. Polysaccharides were detected according to [Thiéry's method \(1967\)](#); for the visualization of proteins enzymatic digestions

by 0.5% pepsin in 0.1N HCl at 37 °C were performed for 5–15 min ([Behnke and Forer, 1967](#)). Part of the material was fixed according to [Dallai and Afzelius \(1990\)](#), thus in a fixative containing 1% tannic acid (but omitting the osmium tetroxide step), then followed by en bloc staining in 1% uranyl acetate and rinse in PB. Ultrathin sections, obtained with a Reichert Ultracut II E ultramicrotome, were routinely stained and observed with a Philips CM 10 electron microscope oper-



Figs. 5–10. (5) Cross-section through an early spermatid of paraspermatozoa showing the formation of the accessory microtubules from outgrowths of microtubular doublets. (6) Longitudinal section through the aged spermatid of paraspermatozoa to show the condensing-nuclear material (N), the axoneme (ax) and the mitochondrial derivatives (m) that start to be filled with droplets of dense material (arrowheads). (7) Cross-section through an aged spermatid of paraspermatozoa with kidney-shaped accessory tubules and a beginning of dense material in their lumen. m, mitochondrial derivatives. (8) Cross-section through the posterior region of an old spermatid of paraspermatozoa with accessory macrotubules almost filled with dense material and a diameter greater than usual. The mitochondrial derivatives at this level show a large globular inclusion. (9) Cross-section through old spermatids of paraspermatozoa with the accessory macrotubules filled with dense material. (10) Longitudinal section through the mitochondrial derivatives filled with large dense globular units. Note the ladder-like junction between the two membrane surfaces (arrowheads).



Figs. 11–13. (11) Longitudinal section through the paraspermatozoa axoneme after Thiéry method for polysaccharides. Note the positive reaction of the material in the lumen of the accessory macrotubule, which shows a longitudinal repeat; and in that of the two central tubules. (12) Cross-section through the paraspermatozoa axoneme after Thiéry method for polysaccharides. The positive reaction is localized on accessory macrotubules and on the two central tubules. (13) Cross-sections through paraspermatozoa after pepsin digestion. The accessory macrotubules are removed by the enzymatic digestion.

ated at 80 kV. *Eledone cirrhosa* Lamarck (cephalopod) and human spermatozoa fixed according to Dallai and Afzelius (1990) were used for comparison.

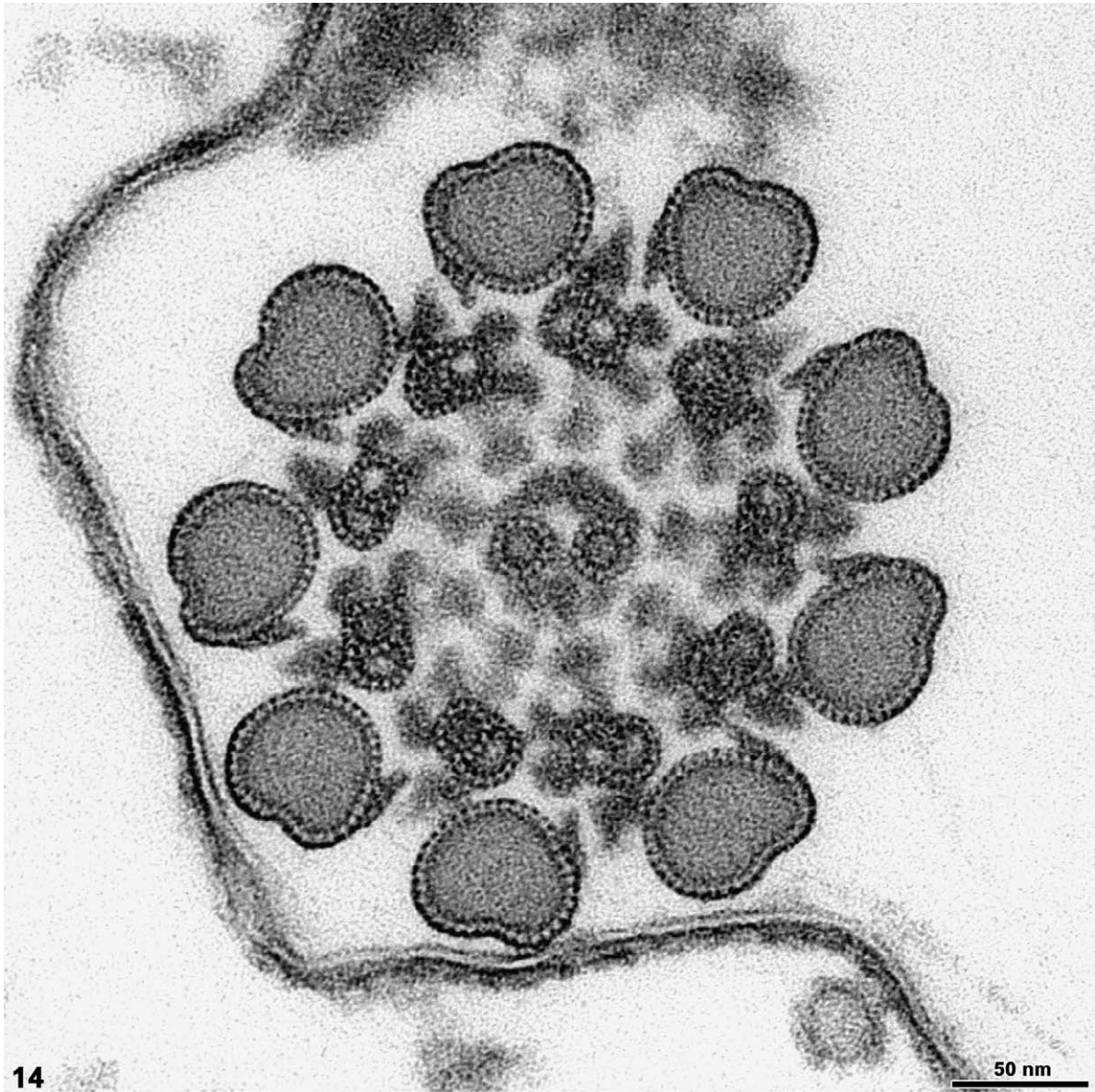
### 3. Results

*M. perla* spermatozoa are of two kinds: euspermatozoa (typical) and paraspermatozoa (atypical), as occurs also in a few other insect orders (Jamieson et al., 1999) (Fig. 1). The structure of paraspermatozoa, is here described, with emphasis on the flagellar axoneme.

The paraspermatozoon in cross-section is 2.5–3 times wider than euspermatozoon. It consists of a flat acrosome, a nucleus with elliptic cross-section, 4–5 times wider than its equivalent in normal spermatozoon and a relatively long flagellum (Fig. 2). Paraspermatozoa are formed in gametic cysts, the number of which is lower than those containing the euspermatozoa: in a cross-section through the testis, a few cysts only of paraspermatozoa are visible among several of eu spermatozoa.

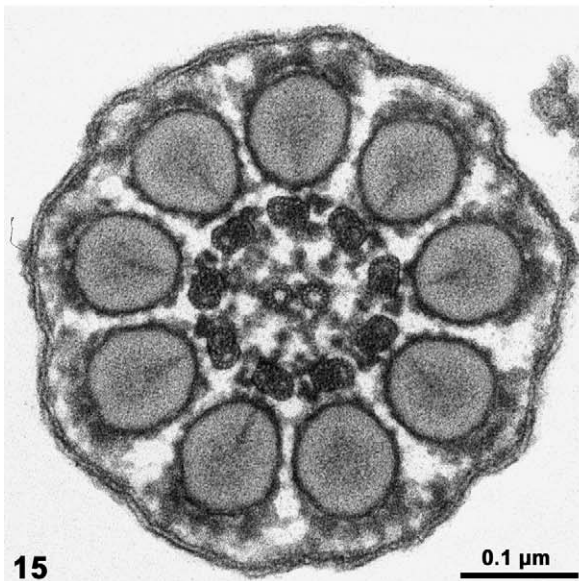
The flagellar axoneme of paraspermatozoa consists of an axoneme and two giant mitochondrial derivatives (Figs. 3 and 11). The axoneme exhibits a 9+9+2 pattern,

Figs. 14–16. (14) Cross-section through the flagellar axoneme of *Mantispa perla* paraspermatozoa. Note the large size of the accessory macrotubules which have 40 protofilaments in their tubular wall. The inner 9+2 unit is of the well-known type, thus with the nine microtubular doublets carrying both outer and inner dynein arms and the spokes (or radial links) extending from the doublets to the central microtubules. (15) Cross-section through the *Eledone cirrhosa* sperm flagellum. The 9+2 unit is surrounded by nine similar accessory fibers. (16) Cross-section through a man sperm flagellum. The 9+2 unit is surrounded by nine accessory fibers of different size.



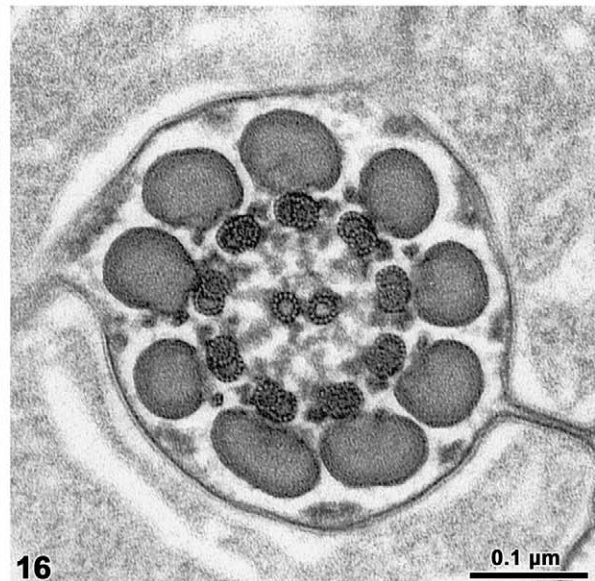
14

50 nm



15

0.1 μm



16

0.1 μm

in which the central 9 + 2 units have normal structure, in that the microtubular doublets are provided with both dynein arms and radial links. The nine accessory units have a larger size compared with the accessory microtubules in other insect orders. In cross-section, they are kidney-shaped and have a diameter of about 55 nm, a tubular wall consisting of about 40 protofilaments, which are retained for the entire tubular length, and a dense material in their lumen (Figs. 4 and 11). A scarce intertubular material is present and it is organized in two small densities: one adheres to the tubular wall of the accessory tubules and the second one to the B-subfiber of each doublet (Fig. 11). The accessory tubules are formed as they usually do in insects: during spermiogenesis, a projection emerges from the B-tubule of each microtubular doublet (Fig. 5), it extends and bends inward to close into a tubular structure which has a lobate rather than a circular cross-section (Figs. 7–9). Thereafter, the lumen begins to store a dense material, which progressively fills the tubule (Figs. 7–11). This material, in longitudinal section, shows a periodicity of about 6 nm (Fig. 4); polysaccharides and proteins were detected at the level of the material present in the accessory macrotubules (Figs. 11–13). Lateral to the axoneme, near the plasma membrane two small densities are evident (Fig. 1). Early spermatids of paraspermatozoa do not show any trace of accessory bodies, which are instead evident in euspermatozoa.

The flagellum also contains two mitochondrial derivatives, which in early spermatids of paraspermatozoa are similar to those in the typical spermatids. During spermiogenesis, however, a small area of crystallization becomes evident in the mitochondrial matrix (Figs. 2 and 9) and small droplets of dense material are stored here (Fig. 6). These droplets fuse to form large inclusions, which in cross-sections appear in two overlapping layers (Figs. 1 and 3). As a consequence, hereof, the mitochondrial derivatives develop into two giant structures. In longitudinal sections, the spheroidal inclusions are seen as a series of several units (Fig. 10). The two membrane surfaces of the mitochondrial derivatives join by a ladder-like junctions reminiscent of a septate junction, with cross-bridges that have a periodicity of about 34 nm.

#### 4. Discussion

The paraspermatozoa of *Mantispa perla* are giant sperm cells, due to the great dimensions of both their axoneme and mitochondrial derivatives. Giant spermatozoa have been described in other insect orders, but the combination of the two enlarged sperm characters—mitochondrial derivatives and accessory microtubules is a novel feature. Giant spermatozoa with giant axonemes made of a huge number of microtubular doublets are present in several gall-midge species Cecidomyiidae and Sciaridae (Phillips, 1966; Baccetti and Dallai, 1976; Dallai, 1988; Dallai et al., 1996a,b); analogously, giant spermatozoa provided with unusually large mitochondria have been described in some bugs (Afzelius et

al., 1976; Jamieson et al., 1999) and beetles (Mazzini, 1976). The close association of the two facing outer mitochondrial membranes gives rise to a ladder-like junction reminiscent of that described in a chilopodan spermatozoon (Reger et al., 1980).

The new finding deals, however, with the presence of accessory microtubules of an unusually large diameter. Insects usually show accessory tubules with 16 protofilaments and a diameter of about 26–28 nm (Jamieson et al., 1999). Accessory tubules with a greater number of protofilaments have been observed in spermatozoa of a few caddisflies, their tubules consist of 19–20 protofilaments (Dallai and Afzelius, 1994, 1999) and have a diameter of about 34 nm. Their protofilaments were skewed rather than straight (Lanzavecchia et al., 1994) as predicted by Langford (1980) and Wade and Chrétien (1993). The skewness of protofilaments permits the accommodation of extra tubulin molecules arranged in each turn (Wade et al., 1990). If the macrotubules with 40 protofilaments in their tubular wall have a similar skewness is matter of future studies.

The 9 + 9 + 2 axoneme of the *Mantispa* is quite similar to the sperm axonemes of the cephalopods or mammals (compare Figs. 14–16). Its central portion – the 9 + 2 unit – is identical in these three animal species or nearly so – any minor differences that may be found, such as the pattern exhibited by the electron dense matter inside the lumen of the A-subtubule of the doublets, might be caused by minor differences in the specimen preparation technique.

Since the accessory fibers in the three phyla are obviously independent inventions, the outer dense fibers of cephalopods, mammals, or of *Mantispa* are to be regarded as analogous structures; the three types of sperm axonemes represent good examples of what is known as ‘convergent characters’: similar features present in phylogenetically unrelated organisms.

The accessory macrotubules of *M. perla* are true insect accessory microtubules, as they are formed as outgrowths from the B-subtubules, in conformity with the accessory microtubules in other insects (Dallai and Afzelius, 1993). Nevertheless, they are unusual in that:

- (a) They are about 55 nm rather than 25–34 nm in diameter, as are microtubules in general; for this reason, we call them ‘macrotubules’ (Unger et al., 1990).
- (b) Their cross-sectional shape is kidney-shaped. In this respect, they differ from their equivalents in other insects. They also differ from the fibers in cephalopods and mammals, whilst they resemble the outer dense fibers in the leech, except that the concave side is facing away from the doublets, rather than facing towards it as in the leech (Franzén, 1991).
- (c) They contain a dense material, consisting of proteins and polysaccharides, which in longitudinal sections show a repeat of 6 nm. Accessory tubules in other insects are filled by material containing polysaccharides and proteins (Baccetti et al., 1970) but a substructure of this

material is not evident (Dallai and Afzelius, 1999). Outer dense fibers of cephalopods and mammals show a weak repeat of about 20–25 nm (Baccetti et al., 1973, 1976). A longitudinal periodicity has also been observed in the outer dense fibers of the pulmonate snail (Anderson and Personne, 1969), and in gastrotrichs (Ferraguti et al., 1995).

The function of the giant sperm cells of *M. perla* is a matter of speculation; we can only suggest that they aid the functional spermatozoa in sperm transfer to the female genital tract, believed to occur in Lepidoptera (Friedländer and Gitay, 1972), or/and furnish nutrients when they reach the female spermatheca (Fain-Maurel, 1966). The presence of polysaccharides and proteins in the material filling the lumen of macrotubules fits well with this hypothesis. Alternatively they could be involved in the sperm competition acting by filling the spermatheca, thus preventing in the case of multiple matings, female remating (Parker, 1970; Eberhard, 1996). They could also act as a copulatory plug, as it occurs for the giant spermatozoa of pentatomids (Ladle and Foster, 1992), the extraordinary long spermatozoa of some *Drosophila* species (Pitnick and Markow, 1994), and the polyploid spermatozoa of some beetles (Bouix, 1963).

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