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Spermiogenesis in the spider *Hapalopus tripepii* (Dresco) (Araneae, Mygalomorphae): an ultrastructural analysis

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Summary

The spermiogenesis of *Hapalopus tripepii* occurs in cysts. Early spermatids are rounded cells connected by intercellular bridges. In the spherical nucleus the chromatin is comprised of thin filaments, which thicken in a subsequent stage and form a complex network. In the cytoplasm an acrosomal vesicle originates from the Golgi complex and the flagellum is precociously extruded from the spermatid. The further stages of spermiogenesis are characterised by a partial elongation of the cell. There is a slender apical part, containing a cone-shaped acrosome and an elongating nucleus, and a posterior bulbous part, which contains most of the cytoplasm. In the nucleus, chromatin filaments coalesce to form a unique block around which nucleoplasm is placed. A deep flagellar tunnel surrounds the flagellum to the centriolar zone. The spermatozoon is a small oval-shaped cell; its nucleus is a long and irregularly folded cylinder. A long perforatorium winds around the nucleus as a slender spiral, the fore part of which penetrates the subacrosomal space. The cone-shaped acrosome is the only structure which protrudes from the sperm. In the implantation fossa there are two centrioles, oriented at an angle of 45° to each other. The axoneme, which winds up into the cytoplasm for 3-4 coils, contains 9 × 2 + 3 microtubules, which is the typical pattern for the orders Araneae, Uropygi and Amblypygi.

Introduction

The sperm structure of spiders studied so far is characterised by an oval or roundish lens-like shape and by a coiled axoneme with a 9 × 2 + 3 microtubular pattern. This model is also observed in the sperm of Uropygi (Phillips, 1976) and Amblypygi (Jespersen, 1978; Tripepi & Saita, 1985). Recent ultrastructural investigations have revealed that there are some morphological differences within this basic model, especially among the three suborders (Liphistiomorphae, Mygalomorphae and Araneomorphae) of the order Araneae.

This work deals with the ultrastructure of the sperm and spermiogenesis in *Hapalopus tripepii,* a Brazilian species belonging to the suborder Mygalomorphae. This species was first described by Dresco (1984) as *Eurypelma tripepii,* but it was transferred to the genus *Hapalopus* by Raven (1985). Only one previous ultrastructural study has been reported concerning mygalomorphs (Alberti & Weinmann, 1986).

Material and methods

The testes were dissected from two male spiders, collected in the Parà region (Brazil), and fixed in 3% cold glutaraldehyde in 0.1M phosphate buffer for two hours. After several rinses in the same buffer, the specimens were post-fixed in 1% phosphate-buffered osmium tetroxide for two hours. After a phosphate rinse, there followed dehydration in increasing ethanol concentrations and pre-staining with uranyl acetate in 90% ethanol for two hours. After several rinses in propylene oxide, the testes were embedded in Epon-Araldite.

Thick sections were obtained with an Ultracut Reichert ultramicrotome and stained with toluidine blue and methylene blue-acid fuchsin. Ultrathin sections were stained with lead citrate and observed with a Hitachi HU-12A transmission electron microscope.

Results

Spermiogenesis

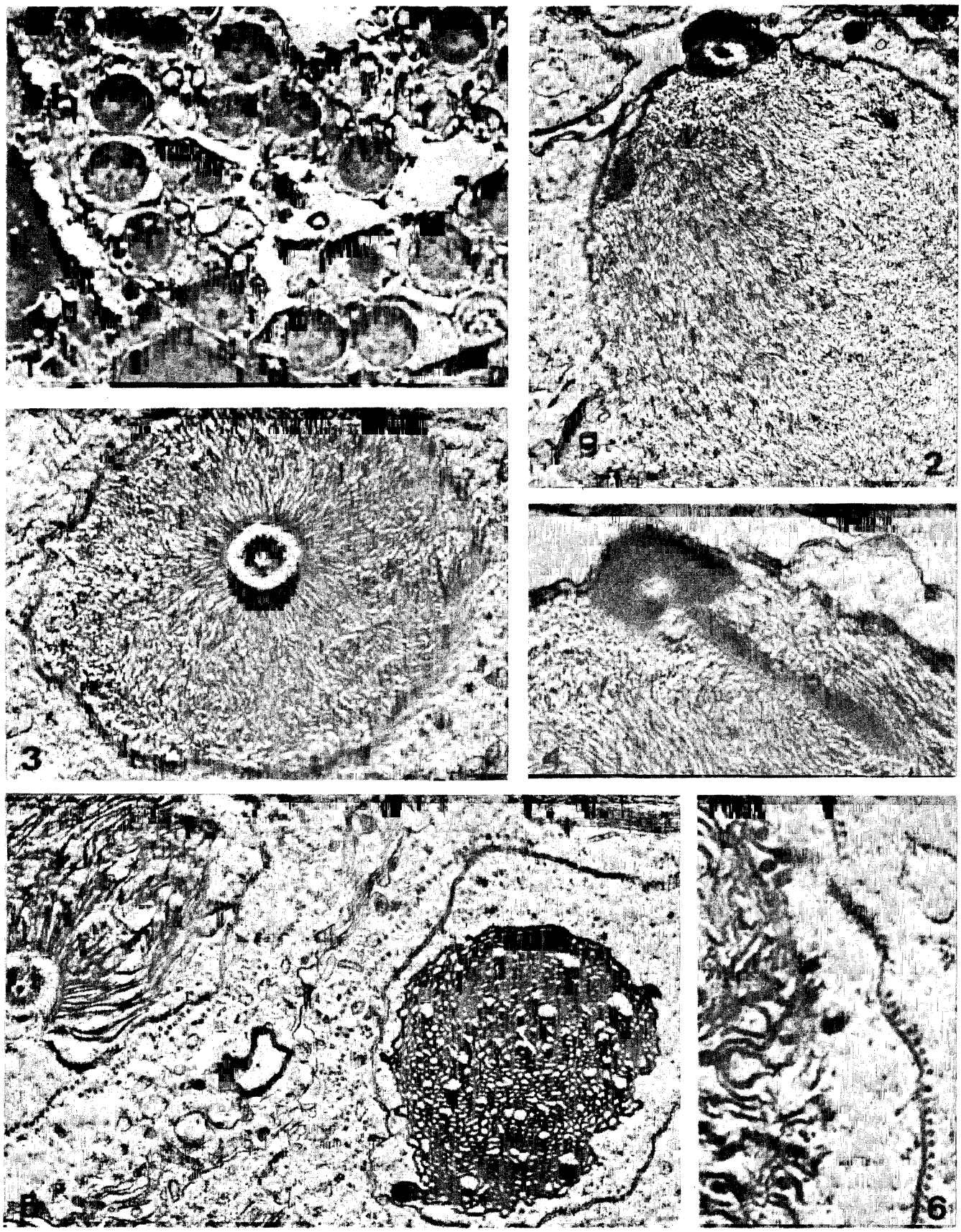
The spermiogenesis of *Hapalopus tripepii* occurs in testicular cysts, with each cyst containing germ cells at the same developmental stage.

Young spermatids (Fig. 1) are large cells with a rounded nucleus; although they begin to separate from each other, they are still connected by thin bridges.

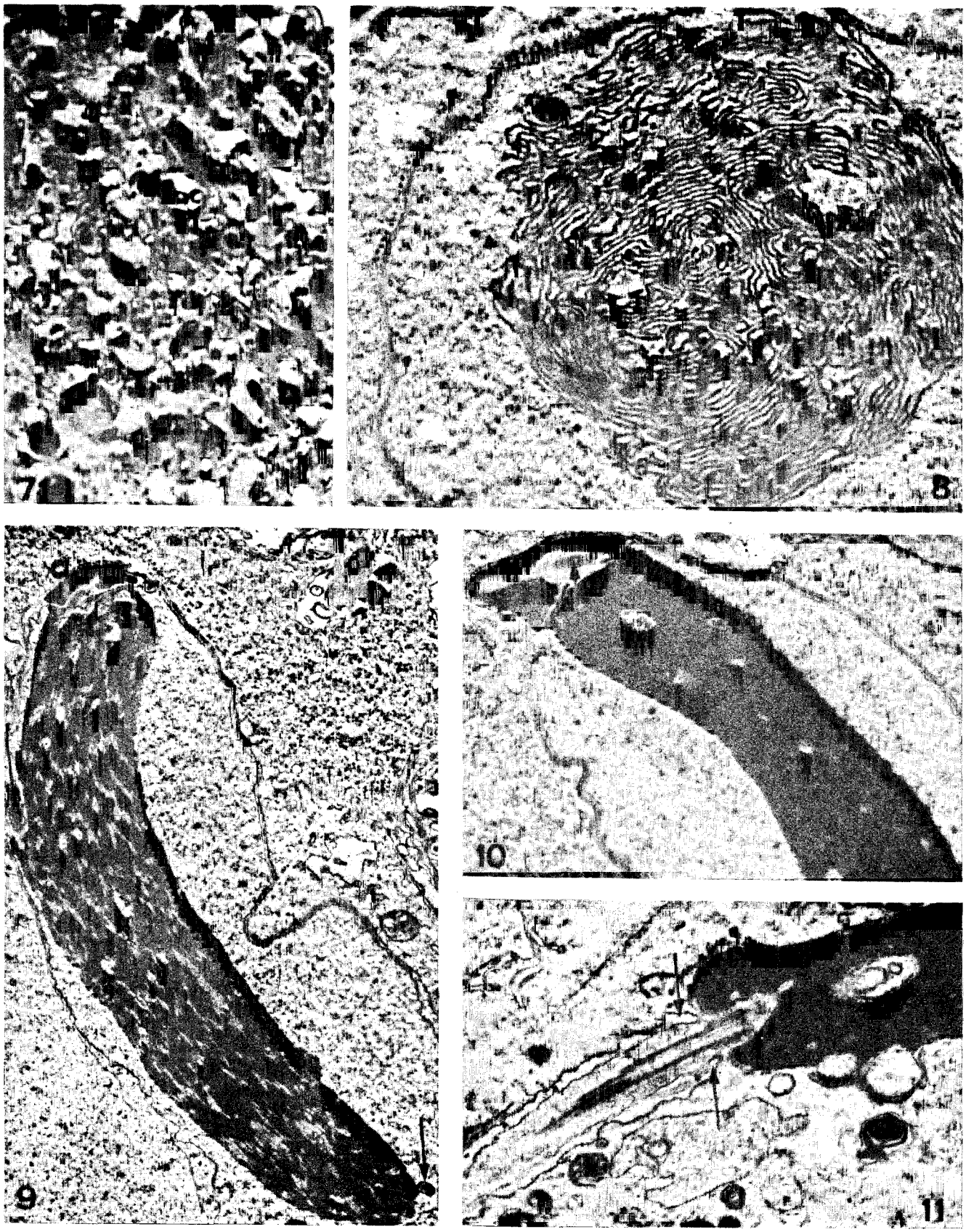
During the elongating stage of spermatid formation an acrosomal vesicle develops early on the nuclear tip and protrudes from the cell body (Fig. 2). This acrosomal formation shows a conspicuous indentation which contains a long rod-like perforatorium penetrating the apical part of the nucleus (Fig. 4); the perforatorium contained within the nucleus is surrounded by a nuclear envelope forming a nuclear canal around it. The chromatin material is arranged in thin filaments, disposed longitudinally to the long axis of the nucleus. These filaments can be seen arising from the nuclear envelope area, which delimits the implantation fossa (Fig. 3) and is where the centrioles are located. The microtubules originate in a central cytoplasmic area, almost equally disposed between the nuclear envelope and plasmalemma; the microtubules are arranged in several groups of 10-20 aligned units (Fig. 5). Later in spermiogenesis they migrate towards the nuclear envelope to form a monolayered manchette surrounding the nucleus (Figs. 6, 8).

The chromatin filaments of the next spermiogenetic stage become thicker and interconnected, so as to form a complex network (Fig. 5). Later, dense chromatin granules appear among the thickened filaments (Figs. 6, 8) and thus a granular structure characterises the last stages of chromatin condensation.

The fully-developed elongated spermatids (Fig. 7) show a nucleus and an acrosome protruding from a posterior bulbous part. In the nucleus an evident separation can be observed between the condensing chromatin and the nucleoplasm, which is pushed towards the posterior nuclear area. The microtubular manchette is still present (Fig. 9) and a long spiral perforatorium, the fore part of which is located in the



Figs. 1-6: **1** Testicular cyst, containing young spermatids, observed with light microscope, × 1,500; **2** Young spermatid, observed with transmission electron microscope; at this stage chromatin condensation occurs as thin filaments; a = acrosome, g = Golgi apparatus, × 14,000; **3** Transverse section, showing the nuclear base of a young spermatid; note the sunbeam-like arrangement of chromatin filaments, disposed around the implantation fossa, × 24,000; **4** Acrosomal vesicle observed in longitudinal section; p = perforatorium, × 25,000; **5** Groups of aligned microtubules (left) migrate towards the nuclear envelope to surround the nucleus; note the network arrangement of chromatin filaments at a later stage (right), × 27,000; **6** Monolayered manchette of microtubules surrounding the nucleus, × 38,000.

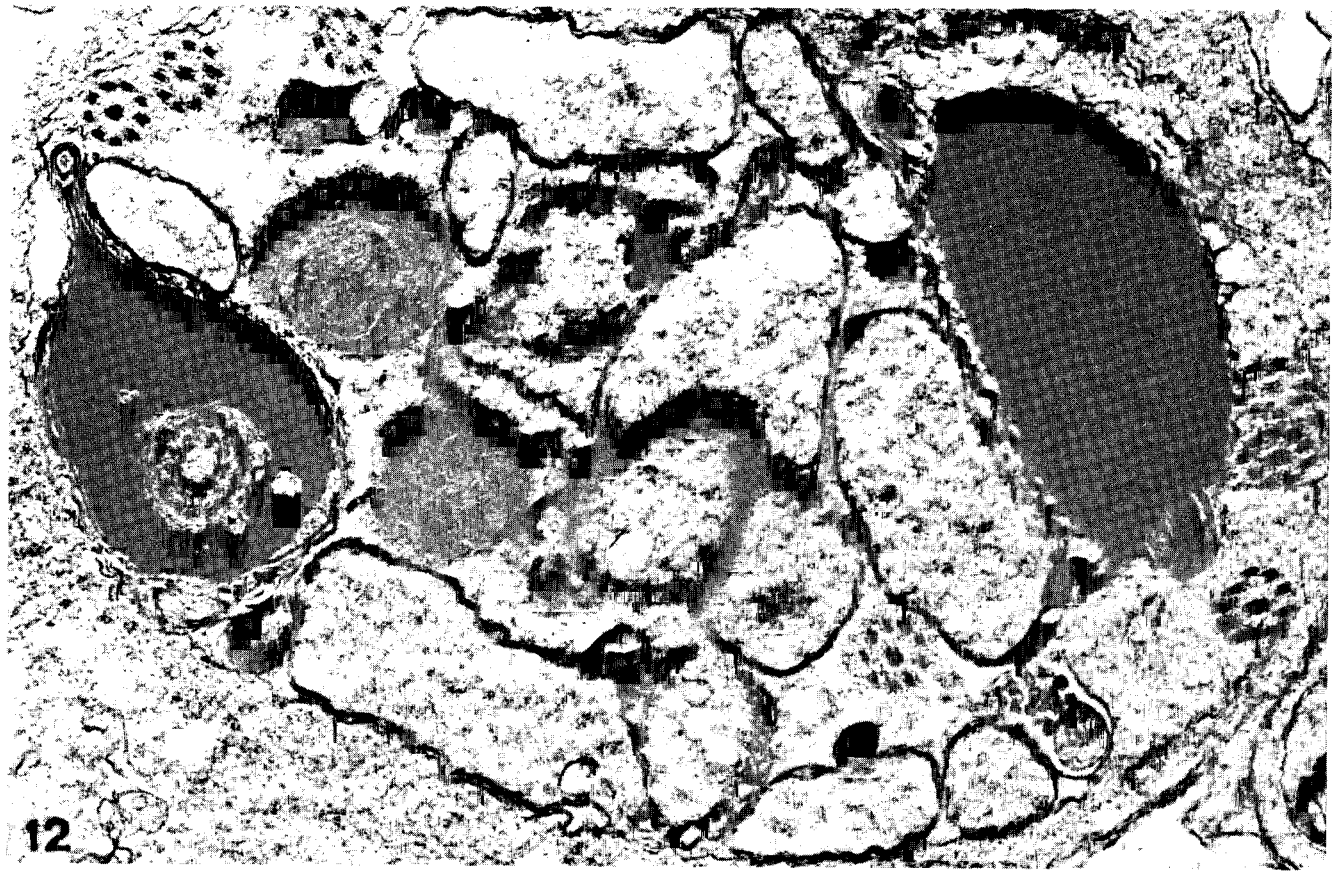


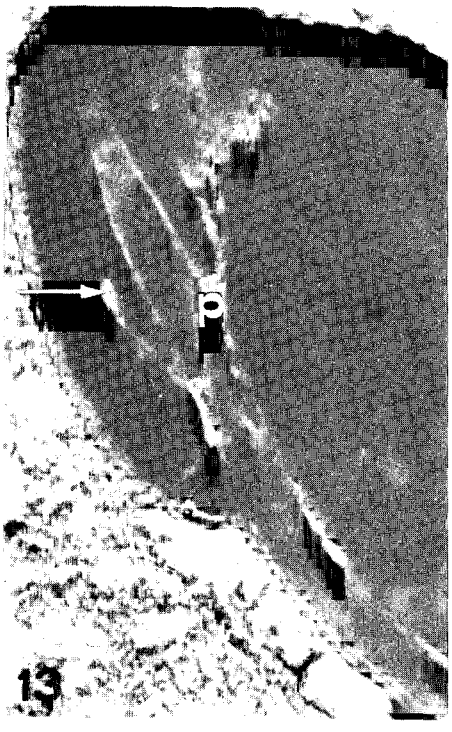
Figs. 7-11: **7** Elongating spermatids, observed with light microscope, × 2,100; **8** Dense granules are formed among the thick filaments of condensing chromatin, × 24,000; **9** Elongating spermatid; a = acrosome, arrows (left and bottom right) indicate perforatorium, × 15,000; **10** Apical portion of an elongate spermatid; chromatin is almost fully condensed; a = acrosome, arrow indicates perforatorium, × 14,000; **11** Implantation fossa of an elongate spermatid; a flagellar tunnel (arrowed) surrounding flagellum, deeply indents the cytoplasm, almost reaching the nuclear base, × 18,000.

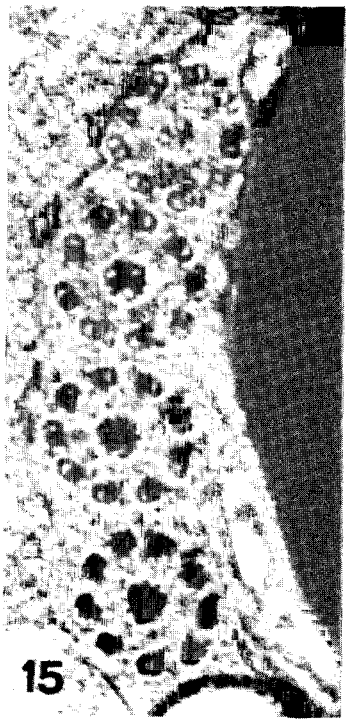
subacrosomal space, coils peripherally around the nucleus (Figs. 9, 10). At this stage the flagellum is still displaced from the cell body, and cytoplasm surrounds its anterior part to form a flagellar tunnel (Fig. 11).

Spermatozoon

The spermatozoon is a small oval-shaped cell with the major axis 5-6μ long, into which the nucleus and flagellum are reabsorbed. The nucleus is a long







Figs. 12-15: **12** Spermatozoon, × 36,000; **13** Acrosomal region of the sperm; note perforatorium (p) and subacrosomal diverticula (arrowed), × 45,000; **14** Implantation fossa of the sperm; note the centrioles arranged at an angle of 45°, × 48,000; **15** Coiled axoneme; the amount of dense material, seen within the central triplet and within the A tubules, decreases from the first coil (bottom) to the last (*top*), × 60,000.

cylinder, about 1.5μ thick, irregularly folded and containing a unique condensed block of chromatin material, with the cytoplasm fully eliminated (Fig. 12). The short and cone-shaped acrosome is the only structure which protrudes from the sperm body. The subacrosomal space comprises a central canal, in which lies the fore part of the perforatorium, plus some narrow lateral diverticula (Fig. 13). The perforatorium extends towards the nuclear base, lying in the nuclear canal just under the nuclear envelope. Its three-dimensional pattern seems to have a spiral but irregular arrangement. In the posterior part of the nucleus the perforatorium disappears and only the nuclear canal is present. A well-developed implantation fossa is evident containing the proximal centriole which is arranged obliquely to the distal one at an angle of 45° (Fig. 14). The fossa is surrounded by dense material and the microtubules contained within the proximal centriole are arranged in doublets and not in the typical triplet formation. The distal centriole is crossed by the central microtubules of the axoneme. The axoneme coils for 4 turns in the cytoplasm and presents a 9 × 2 + 3 microtubular pattern. The A tubules are filled with dense material in the first two coils, but the amount of dense

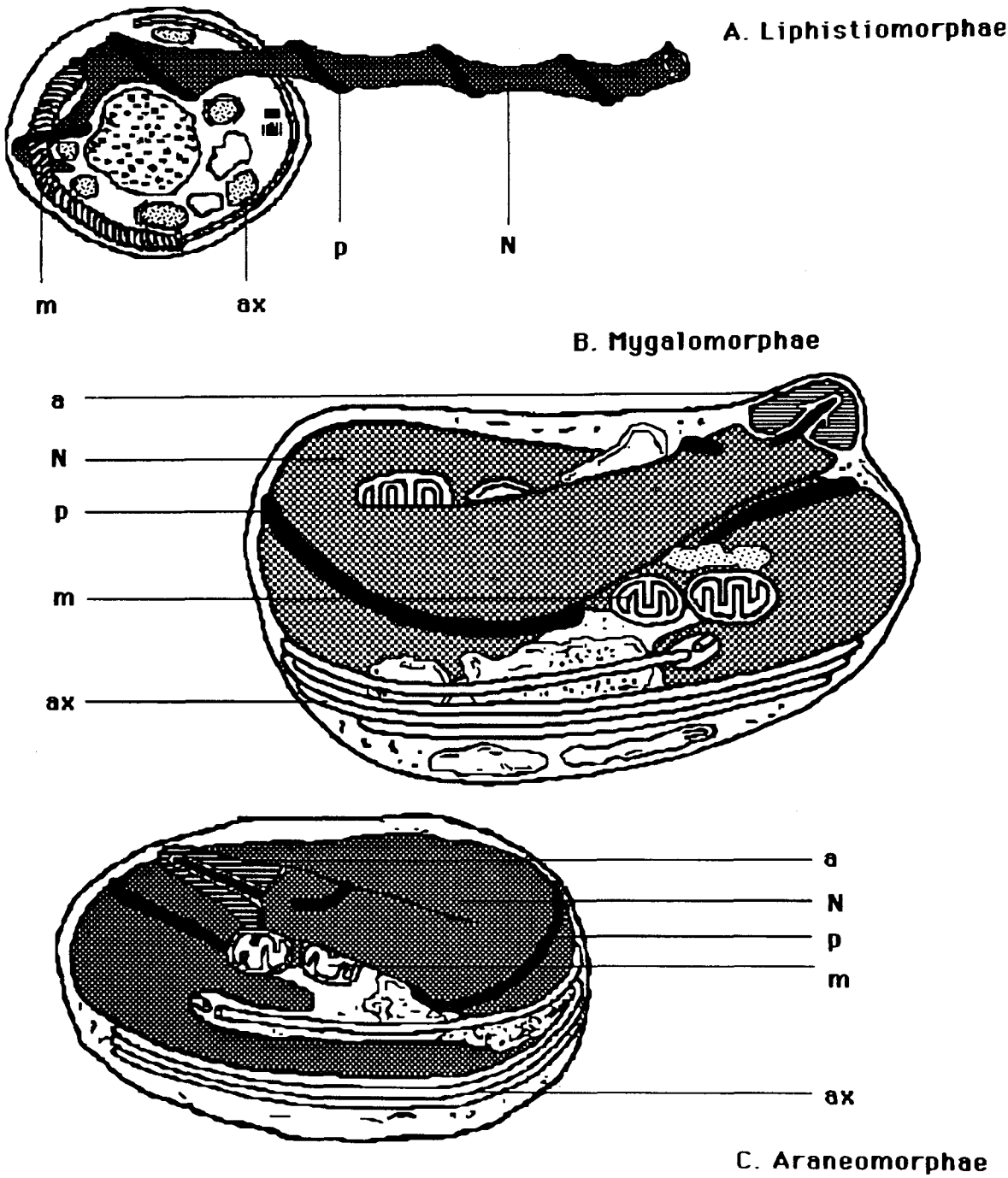


Fig. 16: Evolution of spermatozoa in spiders. **A** Liphistiomorphae. The nucleus and the acrosomal region (not represented) protrude out of the cell body. The nucleus is spiral-shaped and the mitochondria form a helical sheath surrounding the axoneme (modified from Osaki, 1969). **B** Mygalomorphae. Only the acrosome protrudes out of the cell body. A few large mitochondria are located in the cytoplasm. **C** Araneomorphae. All cellular organules are located in the cell body of the sperm. a = acrosome, ax = axoneme, m = mitochondria, N = nucleus, p = perforatorium.

material within the central triplets decreases from the first coil to the third, finally disappearing in the last coil (Fig. 15). There are also regularly-developed dynein spokes protruding from the A tubule.

The cytoplasm is filled with many vesicles of various size and form, and the few large mitochondria are round with cristae not easily distinguishable.

Discussion

Spermiogenesis

The pattern of spermiogenesis in *Hapalopus tripepii* is similar to that reported for other Mygalomorphae (Alberti & Weinmann, 1986) and in particular to that of *Eurypelma californicum* Ausserer. The size and shape of the various spermatid stages, presence of intercellular bridges, nuclear elongation pattern, acrosome and perforatorium formation obtained for the *Eurypelma californicum* spermiogenesis were also encountered in *Hapalopus tripepii.* Furthermore, in this study a regular arrangement of chromatin filaments around the implantation fossa, and the formation of microtubules were observed. Groups of 10-30 aligned microtubules are formed in the middle of the cytoplasm and then migrate to surround the nucleus, forming a monolayered manchette, suggesting that the microtubules may play an important role in the nuclear shaping.

Spermatozoon

The coiled oval spermatozoon of *Hapalopus tripepii* is similar to that observed in most spiders, and although a great structural similarity exists among the sperms of these species, some differences can be noted. Compared with the other mygalomorphs studied, the nucleus of the mature sperm in *Hapalopus tripepii* contains less nucleoplasm. The double layer of electron-lucid filaments, which is present in the periphery of the nucleus of the sperm of other Mygalomorphae, was not observed in this study. *Hapalopus tripepii,* furthermore, possesses a more complex subacrosomal space and a different orientation of the two centrioles at an angle of 45°, compared with almost 90° observed in the other Mygalomorphae.

Evolution of sperm in spiders

The evolution of sperm in all spiders (from Liphistiomorphae to Araneomorphae) is characterised by a progressive reabsorption of the nucleus into the cell body. This tendency, illustrated in Fig. 16, can be summarised as follows:

(1) A primitive model, represented by the sperm of Liphistiomorphae (Mesothelae), in which most of the elongated corkscrew-shaped nucleus protrudes from the cell body and a conspicuous middle-piece is present (Osaki, 1969). A corkscrew-shaped nucleus and a well-developed middle-piece are also present in the sperm of Amblypygi (Jespersen, 1978; Tripepi & Saita, 1985).

(2) Mygalomorphae present a transition model, in which the middle-piece is lacking, the mitochondria are dispersed into the cytoplasm and the nucleus is irregularly coiled into the cell. Only the acrosome protrudes from the sperm profile.

(3) A highly evolved model is represented by the sperm of all Araneomorphae species studied so far (Alberti & Weinmann, 1985; Juberthie *et al.,* 1981; Lopez & Boisson, 1976; Lopez *et al.,* 1983; Osaki, 1972; Reger, 1970). All of these spermatozoa are oval-shaped cells with the middle-piece lacking and with the acrosome incorporated within the cell body.

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