Fine structure of spermiogenesis in *Phalangium opilio* L. (Opiliones, Phalangiidae)

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Summary

The young spermatids of *Phalangium opilio* L. are isolated spherical cells, connected only by intercellular bridges. During spermiogenesis the nucleus becomes disc-shaped and forms at the posterior side an invagination, within which the two centrioles are included. The elimination of cytoplasm is accomplished by intercellular bridges, which convey the cytoplasm in suitable cytoplasmic drops. A flattened acrosome, surrounded by mitochondria, lies at the apex of the cell.

The mature sperm, obtained from the seminal vesicle, is an encysted cell, surrounded by a thick wall; it shows regular cristae, crossing the posterior and lateral surfaces of the sperm.

Introduction

Recent ultrastructural studies have revealed that the spermiogenesis of the Opiliones follows a common general pattern, characterised by a precise peculiarity: the formation of spermatids lacking a flagellum or axoneme, while a kinetic centre, composed of two centrioles, persists. Within this type of aflagellate spermatid there occur many different variations in the final form of the sperm and the evolution of the cellular organelles.

The following study shows that spermiogenesis in *Phalangium opilio* L. (Phalangiidae), even though it belongs to the typical pattern of the Opiliones, is characterised by some unusual features.

Material and methods

Adult male specimens of *Phalangium opilio,* collected on the mountain pass of Potame (Appennines of Calabria) during the month of September, were killed with chloroform and dissected. Small pieces of testis, vas deferens and seminal vesicle were fixed for 2 hours in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). After postfixation in 1% osmium tetroxide in the same buffer for 2 hours, the tissue was dehydrated in ethanol series, transferred to propylene oxide and embedded in epon-Araldite. Thin sections (500 Å in thickness), contrasted with uranyl acetate and lead citrate, were examined at a Hitachi HU-12 A electron microscope; semi-thin sections (1-2 μm in thickness) for light microscopy were stained with toluidine blue.

Results

The tubular testis of *Phalangium opilio,* as observed in cross-section under a light microscope (Fig. 1), is composed of several compartments delimited by flattened epithelial cells. Within each compartment all the cells are in the same developmental stage, ranging from spermatogonia to almost mature spermatozoa. In general the compartments containing the more differentiated germinal cells are located near the centre of the tubule.

The undifferentiated germinal cells (spermatogonia and spermatocytes) are large polygonal cells, adhering to one another, containing a large nucleus. Within the nucleus the chromatin is arranged in small dispersed granules that, at times, coalesce to form some masses. These cells are connected by intercellular bridges (Fig. 2); at this zone the plasma membrane is thickened. The typical organelles are present in the cytoplasm: mitochondria, ribosomes and a pair of centrioles, each formed by nine triplets of microtubules (Fig. 3). A well developed Golgi complex, composed of 5-6 flattened cisternae and several vesicles, is also present (Fig. 4).

The early spermatids are rounded isolated cells except at the level of the intercellular bridges, which represent the only point of connection (Fig. 5). In the nucleus, chromatin granules begin to migrate towards the periphery; only nucleoplasm remains at the centre of the nucleus. At high magnification the thickened cell membrane of the intercellular bridges has electron-dense material displaced in a regular pattern to form several striations, which run along the inner surface of the cell membrane (Fig. 6).

In a subsequent stage two features occur, which characterise the intermediate spermatid: the formation of the acrosome and the migration of the centrioles into the nuclear invagination. Therefore the

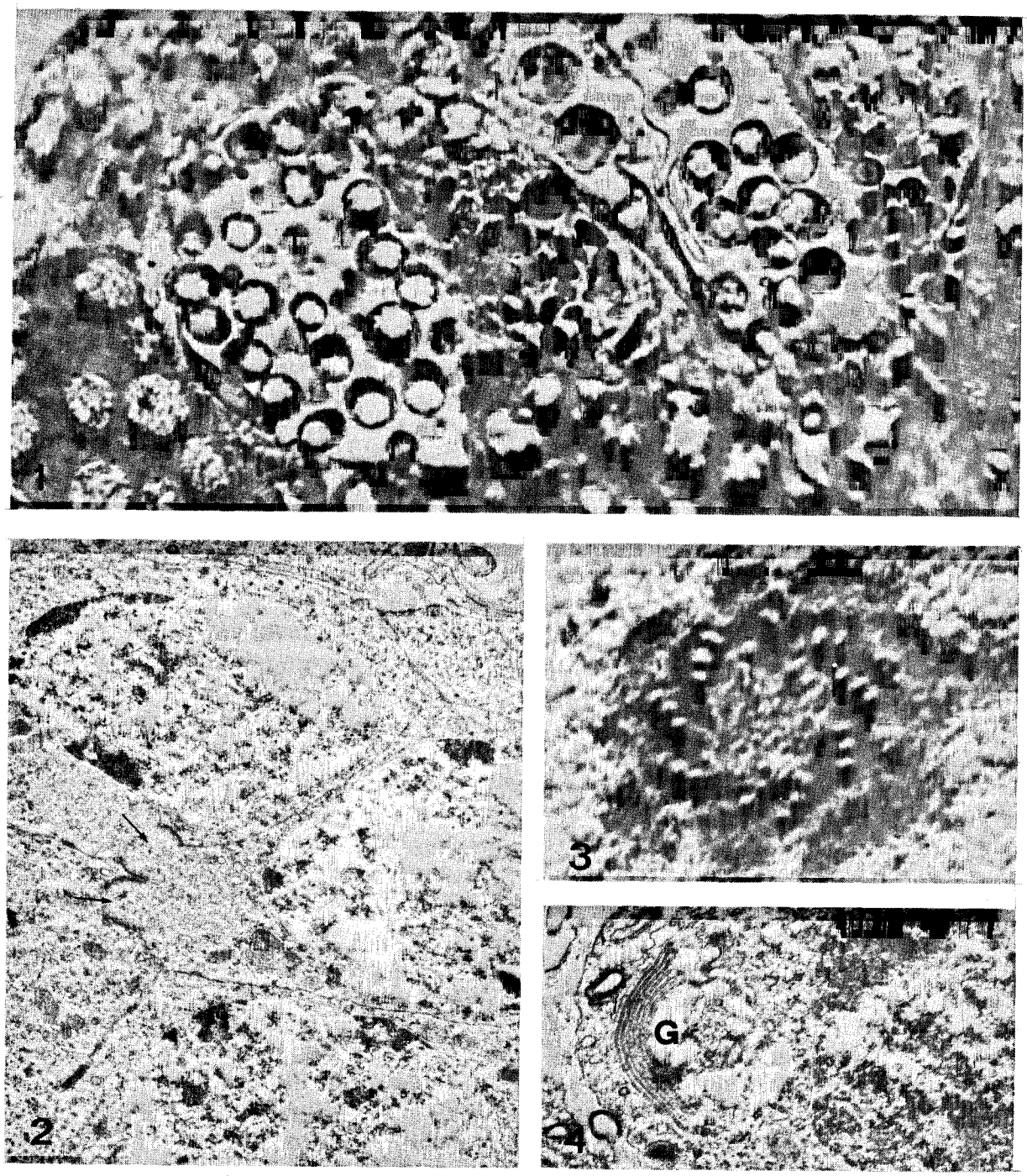
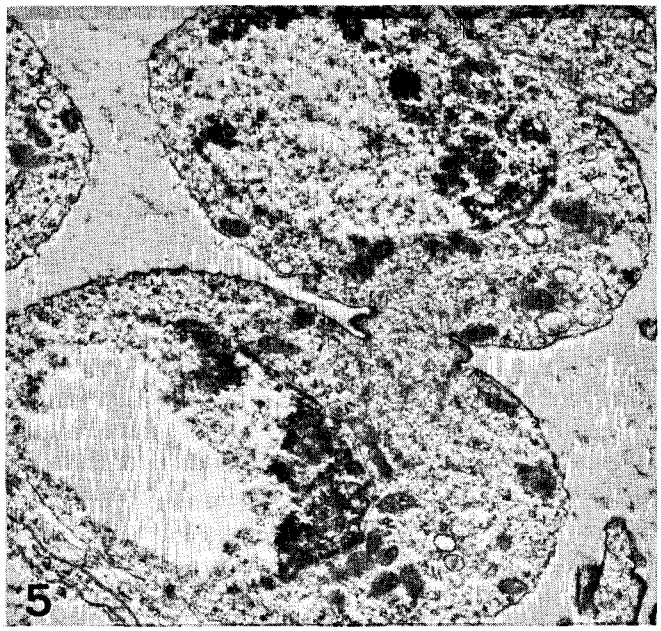
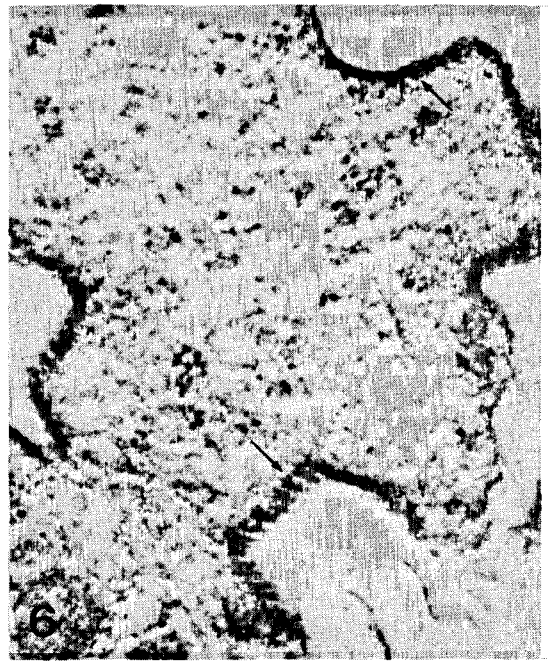
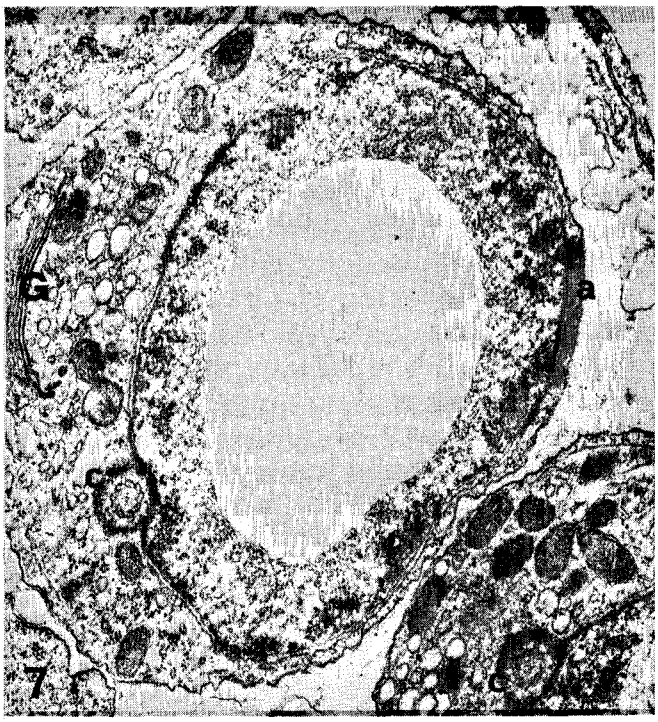


Fig. 1: Cross section of testis observed under light microscope. x 1,200.
Fig. 2: Undifferentiated germinal cells, connected by intercellular bridges (arrows). x 12,000.
Fig. 3: Centriole. x 150,000.
Fig. 4: Golgi complex (G). x 25,000.







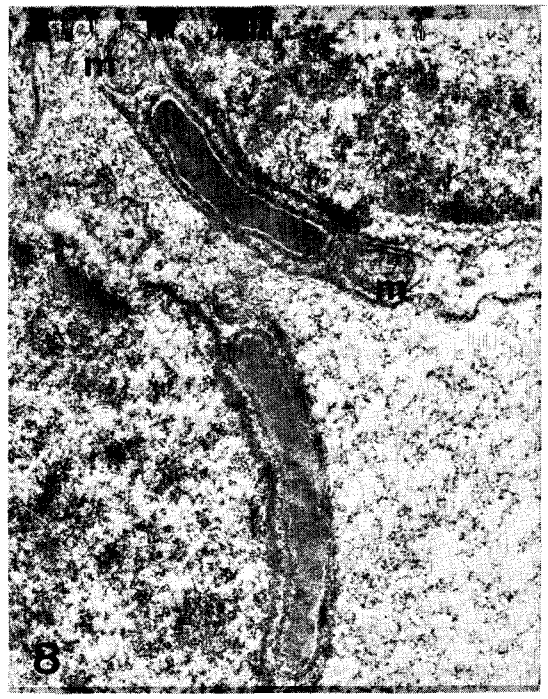


Fig. 5: Two early spermatids connected by an intercellular bridge. x 10,000.
Fig. 6: Intercellular bridge, showing electron-dense striations (arrow). x 55,000.
Fig. 7: Intermediate spermatid; a = acrosome, c = centriole, G = Golgi complex. x 21,000.
Fig. 8: Two acrosomes, surrounded by mitochondria (m). x 45,000.

intermediate spermatid is a cell which displays a polarity: the apical part is composed of a flattened acrosome, located between the nuclear and cell membranes, while the posterior part is represented by the centriolar zone (Fig. 7).

A bilayered membrane surrounds the acrosomal dense material; some mitochondria are located around the acrosome (Fig. 8). Behind the acrosome the nucleus still maintains its rounded conformation; chromatin granules continue to coalesce, forming flattened masses, which become localised on the inner surface of the nuclear envelope (Fig. 9). The centrioles are located behind the nucleus and are surrounded by a manchette of dense fine granular material (Figs. 7, 9).

During the last stages of spermiogenesis most of the cytoplasm is eliminated by the intercellular bridges, which convey the cytoplasm and its organelles, e.g. most of the mitochondria, vesicles, etc., towards suitable cytoplasmic droplets (Fig. 11). The spermatids become disc-shaped cells; in the nucleus the chromatin is fully condensed in peripheral blocks. The nuclear centre is for the most part occupied by nucleoplasm but it may contain several chromatin filaments, arranged parallel to one another, forming a paracrystalline-like structure (Fig. 10). The centrioles are invaginated by the nucleus (Fig. 11).

The observation of mature sperms, obtained from the seminal vesicles, has presented some difficulties: a general increase of opacity in the sperm and the difficulty of fixatives in penetrating two barriers, represented by the epithelium of the seminal vesicle and by the wall of the encysted sperm. In spite of this we have obtained a fair micrograph, in which the disc-shaped sperm appears to be surrounded by the thick wall of the cyst. Between the sperm and the wall a clear space is interposed. The acrosome maintains its flattened shape, but it becomes electron transparent. In the nucleus densely packed chromatin alternates with zones of clear nucleoplasm. The lateral and posterior surfaces of the sperm are crossed by regular cristae, which are clearly evident on the posterior side of the sperm (Fig. 12).

Discussion

An extensive analysis of spermiogenesis in Opiliones has been made by Juberthie & Manier (1978); we are only adding some new ultrastructural elements that have emerged from the present study, along with some general considerations.

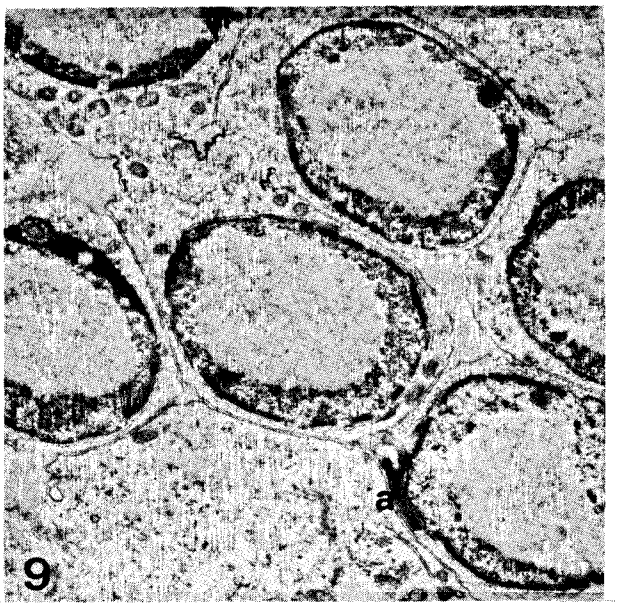
The spermiogenesis of *Phalangium opilio* shows some features that have been observed in other Opiliones; the process of sperm shaping and the acrosome conformation are very similar to those reported in *Odiellus gallicus* (Roewer), another member of the family Phalangiidae, but they differ from those observed in *Leiobunum* sp. (Reger, 1969), another species belonging to the same family (Juberthie & Manier, 1978).

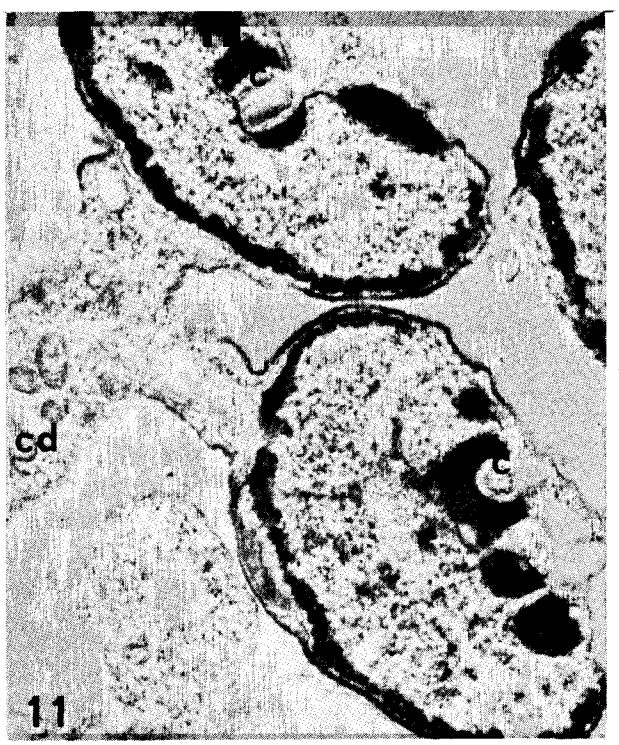
The nuclear shaping occurs in the absence of a microtubular apparatus, as observed in the other Opiliones Palpatores; on the contrary a microtubular plate, located in a posterior part of the sperm, is present during the spermiogenesis of all Opiliones Laniatores so far investigated (Juberthie & Manier, 1977a). The gradual thickening of the nuclear envelope, well observed in other Opiliones, is less evident here.

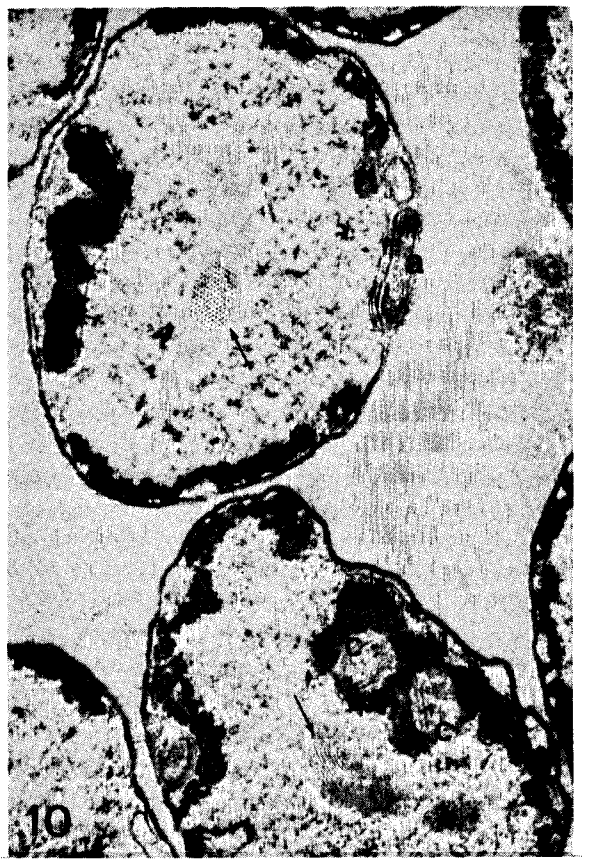
The remarkable characteristics in the spermiogenesis of *Phalangium opilio,* when compared with other species studied, are the elimination of cytoplasm and the modification of the mature sperm.

The process of cytoplasm elimination could be compared to that observed in the Opiliones Palpatores Ischyropsalidae (Juberthie & Manier, 1976) and Nemastomatidae (Juberthie & Manier, 1977b), in which the cytoplasm is pushed towards the posterior part of the cell. At the end of this process the cytoplasm is connected to the cell body by means of a cytoplasmic bridge, which progressively narrows to cause the separation of the cell body from the cytoplasm. In *Phalangium opilio,* however, the elimination of cytoplasm is obtained by the intercellular bridges, which connect one or more spermatids to a cytoplasmic drop to which the cytoplasm of each spermatid gradually migrates. The intercellular bridges, observed during the spermiogenesis of other Opiliones, especially *Leiobunum* sp. and *Trogulus nepaeformis* (Scop.) (Juberthie & Manier, 1977c), are not differentiated like those observed in *Phalangium.* They do not persist until the last stage of spermiogenesis and do not seem to play a role in the elimination of cytoplasm.

The formation of encysted sperm has already been







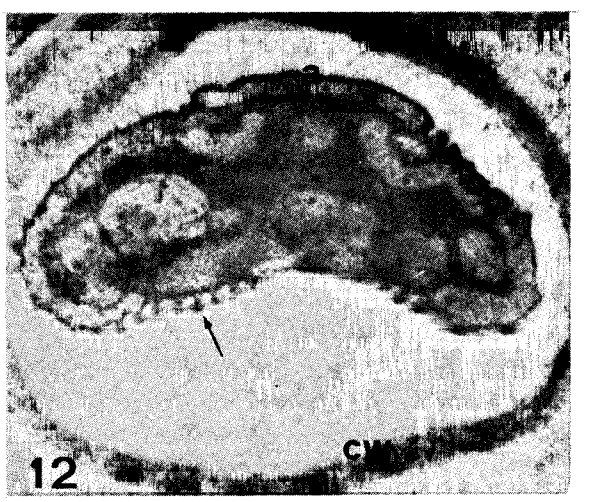


Fig. 9: Intermediate spermatids, connected by intercellular bridges; a = acrosome. x 12,000.
Fig. 10: Advanced spermatids, showing regularly arranged chromatin filaments (arrows); a = acrosome, c = centrioles invaginated in the nucleus. x 20,000.
Fig. 11: Cytoplasm of advanced spermatids is conveyed by intercellular bridges to a cytoplasmic droplet (cd); c = centrioles. x 20,000.
Fig. 12: Encysted sperm, showing regular cristae (arrow); a = acrosome, cw = cyst wall. x 30,000.

reported by Legg (1973) in Pseudoscorpiones; but, while in this case the cyst envelope adheres to the cell membrane, in the sperm of *Phalangium opilio* there is an electron-transparent space between sperm and cyst wall.

The regular cristae of the encysted sperm are a peculiarity of *Phalangium* and were not observed in other species of Opiliones. The microvilli of the sperm in Cyphophthalmes (Juberthie, Manier & Boissin, 1976) and the protuberances of the sperm in Trogulidae are too different in size and in structure to be compared with these cristae.

Thus the spermiogenesis of Opiliones Laniatores and Palpatores differs from that reported in Cyphophthalmes, since the latter includes a fully flagellate stage. This fact lends some support to the recent views of a few authors, who consider the Cyphophthalmes as a distinct order (Savory, 1977). Within the family Phalangiidae, some resemblances exist between the spermiogenesis of *Phalangium opilio* and that of *Odiellus gallicus,* especially if we consider the general structure of the advanced spermatid and the disposition of mitochondria around the acrosome. The striking differences which exist between the sperm structure of *Phalangium* and that of *Leiobunum* could indicate that it would be right to carry out the proposed subdivision of the family Phalangiidae (Savory, 1977).

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