Spermatogenesis and spermatozoon ultrastructure in *Dugesia sicula* Lepori, 1948 (Platyhelminthes, Tricladida, Paludicola)

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ABSTRACT. We examine for the first time spermatogenesis, spermiogenesis and spermatozoon ultrastructure in *Dugesia sicula* Lepori, 1948 a sexual and diploid planarian living in Tunisian springs. This TEM-study shows that early spermatids joined by cytophores have rounded nuclei. During spermiogenesis, a row of microtubules appears in the differentiation zone beneath the plasma membrane and close to the intercentriolar body, which consists of several dense bands connected by filaments. Two free flagella (9+1 configuration) grow outside the spermatid. An apical layer of dense nucleoplasm develops and the flagellum appear, facing in opposite directions before rotating to lie parallel to each other after the intercentriolar body splits into two halves. Mitochondria are closely packed around the spermatocyte nucleus before fusing during spermiogenesis, to form a long mitochondrion, which lies parallel to the elongated nucleus along the ripe spermatozoon. The latter is thread-shaped and consists of two regions: the proximal process and a distal part. The former contains the nucleus and a part of the mitochondrion. The latter contains the rest of the mitochondrion and a tapering tail of the nucleus. Separation between these two regions is marked externally by the insertion zone of the two free flagella. The flagella extend posteriorly along the distal part of the spermatozoon. The spermatozoon nucleus consists of a lucent and a dense component coiled in a screw-like pattern around each other. The single row of peripheral microtubules consists of a maximum 40 microtubules in the middle part with an internal layer of three supplementary microtubules.

KEY WORDS: Platyhelminthes, Tricladida, Paludicola, *Dugesia sicula*, testis, ultrastructure.

INTRODUCTION

Electronic microscopy is very useful in spermatozoon and spermatogenesis studies aiming to elucidate phylogenetic relationships among Platyhelminthes (Tyler et al., 1986; Raikova, 1991; Rieger et al. 1991; Watson & Rhode, 1995, Watson, 1999, 2001). Many papers have provided knowledge already acquired on this subject with revisions on general assumptions about Platyhelminthes phylogeny (Hendelberg, 1969, 1983, 1986; Ehlers, 1985; Rohde, 1990; Watson & Rhode, 1995, Watson, 1999, 2001).

With respect to triclads, investigations have been carried out on the spermatogenesis of freshwater planarians. Klima (1961) published electronic micrographs of spermatogenesis and the spermatozoon ultrastructure in paludicolan triclads but without explaining the process of spermiogenesis. Parts of the process were studied in *Dugesia tigrina* Girard, 1850 (Silveira & Porter, 1964), *Dendrocoelum lacteum* Müller, 1774 and *Planaria torva* Müller, 1774 (Hendelberg, 1969), *Polycelis tenuis* Ijima, 1884 and *Polycelis nigra* Müller, 1774 (Franquinet & Lender, 1972, 1973), *Dugesia lugubris* Schmidt, 1861 (Farnesi et al., 1977) and other species (Ishida & Teshirogi, 1988; Ishida et al., 1991). Li et al. (1992) described for the first time the spermatozoon and the spermiogenesis ultrastructure in the terricolan triclad *Artioposthia sp.* Rhode & Watson (1995) studied the ultrastructure of sperm and spermiogenesis of the paludicolan *Romankenkius libidinosus* Sluys & Rohde, 1991 and an unidentified maricolan. The spermatogenesis of the gonochoric maricolan triclad *Sabussowia dioica* Claparède, 1863 has been studied using light and electron microscopy by Tekaya & Zghal (2001).

The Platyhelminthes spermatozoa are generally elongated and thread-like. They differ enormously from the presumed primitive and modified forms encountered in other animal groups. They lack a distinct head, an intermediate part and a tail. Flagella can be free or incorporated in the spermatozoon body, or are even lacking (as in Macrostomida and Prolecithophora). The present study reports the first ultrastructure data regarding spermatogenesis, spermiogenesis and spermatozoon structure in *Dugesia sicula* Lepori, 1948 a diploid paludicolan strain from Tunisian springs.

MATERIALS AND METHODS

Specimens of *D. sicula* were collected from a spring located in the Serj Mountain northwest of Tunisia. There is a large sexual population of sexually mature, young and newly hatching individuals raised from cocoons deposited

under stones. For transmission electron microscopy (TEM), parts of specimens were fixed in 3% glutaraldehyde in 0.2 M sodium-cacodylate buffer (pH 7.4) for about 4h at 4°C, washed in buffer for 30 min at 4 °C, post fixed in 4% OsO_4 in cacodylate buffer for 1h at room temperature. After dehydration through graded ethanol and propylene oxide, pieces were embedded in Epox. Ultrathin sections through testes, sperm ducts and seminal vesicle were stained with uranyl acetate and lead citrate and examined under a JEOL GEM-1010 at 80 kV.

RESULTS

Spermatogenesis

The testicular follicles of *D. sicula* are situated dorsally throughout the body. They are rounded or oval shaped and can reach 45µm diameter. At least one layer of parietal cells, as they have been called by Hendelberg (1983), delimits the testis follicles sharply from the surrounding parenchyma. These parietal cells display lobed nuclei, prominent nucleoli, numerous mitochondria, well-developed endoplasmic reticulum (ER), lipid droplets and electron-dense granules. All stages of germ cells from spermatogonia to spermatozoa are present at the same time. They extend from the peripheral side to the lumen. Clusters of spermatogonia and spermatocytes are close to the parietal cells, whereas groups of spermatids and spermatozoa are free in the gonad lumen.

Spermatogonia have almost the same shape as neoblasts: little cytoplasm and large nuclei containing granular and fibrillar chromatin (Fig. 2). Spermatocytes are connected by cytoplasmic bridges. Within these germ cells, mitochondria are rich in cristae and increase in number (Fig. 3). Annulate lamellae of various sizes appear and prominent Golgi complexes become very close to the nuclei (Fig. 3). Spermatids are maintained together by cytoplasmic bridges consequent upon incomplete cell division during spermatogenesis. Anucleate pieces of residual cytoplasm following spermatid detachment are also present in the lumen (Fig. 1).

Spermiogenesis

Within early spermatids, the rounded nucleus occupies the distal end of the cell and becomes gradually condensed. Small dense granules appear inside the nucleus in close contact with chromatin (Figs 4, 5). Nuclear pores are prominent in the region closest to the differentiation zone (Fig. 5). The latter is a small cytoplasmic protrusion that develops distal to the nucleus and where an intercentriolar body (ICB) is built to support the two flagella growing out in opposite directions (Figs 6, 8). Spermatids change their spherical shape to become pear-shaped and the nuclei become increasingly elongated and filiform. During the first spermiogenesis stages, mitochondria encircle the nucleus, a row of microtubules appears under the plasma membrane in the differentiation zone, and a dense layer of nucleoplasm develops in the apical region of the nucleus (Figs 6-8). The ICB appears initially with an irregular outline; it contains dense granules and some translucent regions (Fig. 6). The final ICB consists of five bands connected by fine filaments; one dense and thick central band, two intermediate interrupted bands on both sides of it and finally and more externally two thin and continuous ones (Figs 7, 8). It appears that both flagella, lengthening in opposite directions outside the spermatid, are not directly fixed to the ICB. On the contrary, their basal bodies are separated by a small space from the external bands of it. However, these basal bodies are attached with the help of small dense plates to the plasma membrane, and with the help of rootlets to the nearest nuclear membrane (Fig. 8). During advanced stages of spermiogenesis, spermatids start to elongate and their shafts grow in the distal direction. The nucleus elongates too and cytoplasm containing mitochondria and other inclusions migrates alongside the nucleus. Thus, the cell attains gradually the filiform shape of the ripe spermatozoon. The ICB splits at its central band allowing both flagella to rotate in order to approach and to lie parallel to each other (Fig. 9). Each flagellum remains attached with its basal body to one of the basal parts of the intercentriolar body.

Spermatozoon structure

The ripe spermatozoon examined in the testis lumen and vasa deferentia is thread-like and shows two parts: a proximal main body containing the nucleus and the mitochondrion, and a distal process containing mainly the mitochondrion. The elongated nucleoplasm consists of two components; one dense and filamentous (chromatin) and another more lucent (probably protein) coiled around each other in a screw-like pattern (Fig. 10). Mitochondria fuse end-to-end to form a single elongated mitochondrion lying alongside the nucleus of the mature sperm. In some sections, the mitochondrion and the nucleus appear coiled around each other in a screw-like fashion (Fig. 10). Both free flagella are subterminal and emerge together from one side of the spermatozoon between the proximal and the distal parts (Figs 11, 12). We distinguished fusion between flagella only for a short distance just after the insertion zone (Fig. 12). The axoneme pattern is of 9+1; many dense granules (probably glycogen) are present between the nine external microtubule doublets and the central complex (Fig. 13). Flagellar tips split into long microvilli containing few microtubules (Fig. 13).

A single row of peripheral longitudinal microtubules with a maximum number of 40 surrounds the nucleus and the mitochondrion along the entire sperm shaft (Figs 14-17). A short row of three inner microtubules extends beside the mitochondrion and the nucleus along the sperm shaft before disappearing toward both ends (Figs 14, 15). Within vasa deferentia, cross sections through ripe spermatozoa permitted distinction between proximal and distal parts. The nucleus tapers in sections through the distal part where we can see mainly the mitochondrion surrounded by peripheral microtubules (Fig. 16). Very close to the proximal end, the mitochondrion becomes very small in cross sections, and we can see only the nucleus surrounded by peripheral microtubules decreasing in number (Fig. 17).

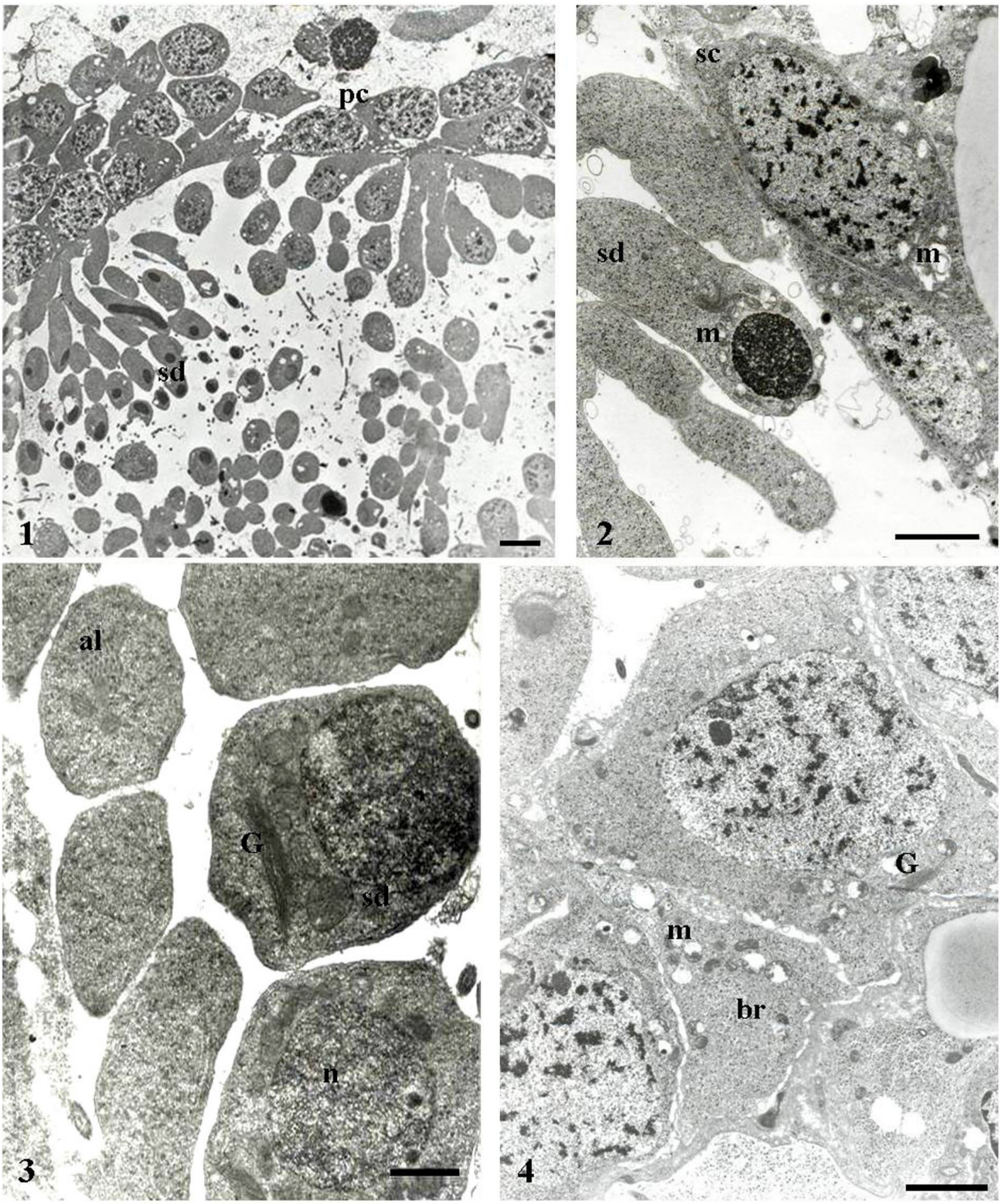

Fig. 1-4. – Spermatogenesis of Dugesia sicula. 1. - Electron micrograph of a section through a testis follicle showing parietal cells (pc) and different stages of male germ cells in the testis lumen. Scale bar = 5µm. 2. - Spermatocytes (sc) are connected to each other by cell processes and their cytoplasm is rich in mitochondria (m). Within young spermatids (sd), mitochondria are rich in cristae and increase in number. Scale bar = 2 µm. 3. - Annulate lamellae (al) of various sizes appear and prominent Golgi complexes (G) become very close to the nuclei (n). Scale bar = 1 µm. 4. - Spermatocytes are connected to each other by cytoplasmic bridges (br). Scale bar = 2 µm.

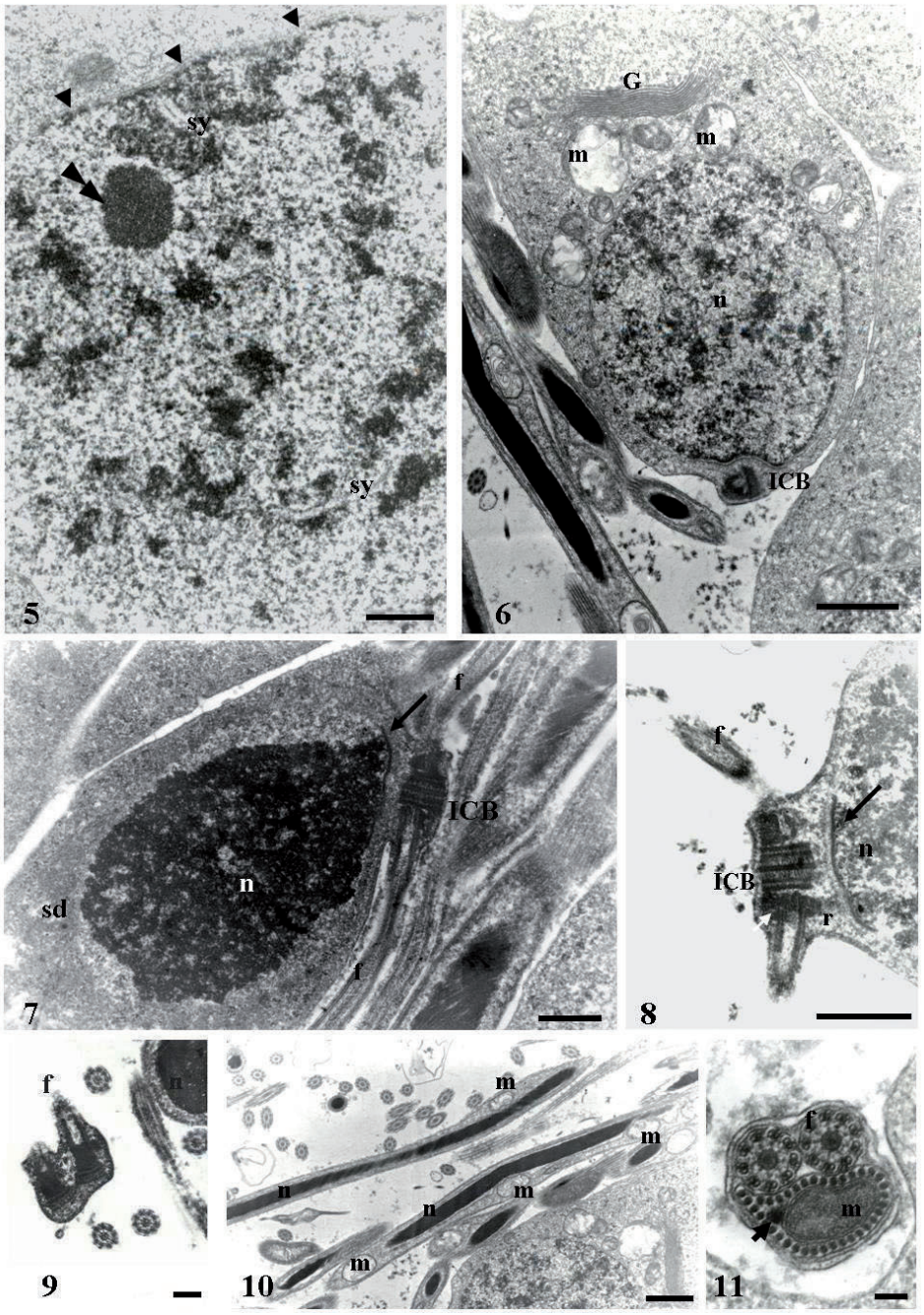

Fig. 5-9. – Spermiogenesis of *Dugesia sicula.* **5. -** Early spermatid, Small dense granules are in close contact with chromatin inside the nucleus (double arrow head), synaptonemal complexes (sy) appear and nuclear pores are prominent (arrow heads). Scale bar = 1 µm. **6. -** Early spermatid, the rounded nucleus occupies the distal end of the cell and becomes gradually condensed, a differentiation zone appears distal to the nucleus as a small protrusion of cytoplasm where the intercentriolar body (ICB) develops to support the two flagella. G; Golgi complexes, m: mitochondrion. Scale bar = 1 µm. **7-8. -** An apical layer of dense nucleoplasm develops (black arrows). The final ICB consists of five bands connected by fine filaments; one thick central and dense band, two intermediate interrupted bands on both sides of it and finally and more externally two thin and continuous ones. Both flagella (f) grow in opposite directions outside the spermatid (sd). Their basal bodies are attached with the help of small dense plates to the plasma membrane (white arrow), and with the help of rootlets (r) to the nearest nuclear membrane. n; nucleus. Scale bars = 500 nm. **9. -** The ICB splits at its central band allowing both flagella to rotate and to lie parallel to each other. Scale bar = 200 nm.

Fig. 10-11. – Spermatozoa of *Dugesia sicula* examined in the testes lumen. **10. -** The elongated nucleoplasm consists of one dense filamentous component and another more lucent coiled around each other in a screw-like pattern. The single elongated mitochondrion (m) lies alongside the nucleus; they appear coiled around each other in a screw-like fashion. n; nucleus. Scale bar = 1 µm**. 11. -** Transverse section in mature spermatozoa showing two free flagella (f), mitochondrion (m), a part of the nucleus (arrow) and microtubules. Scale bar = 100 nm.

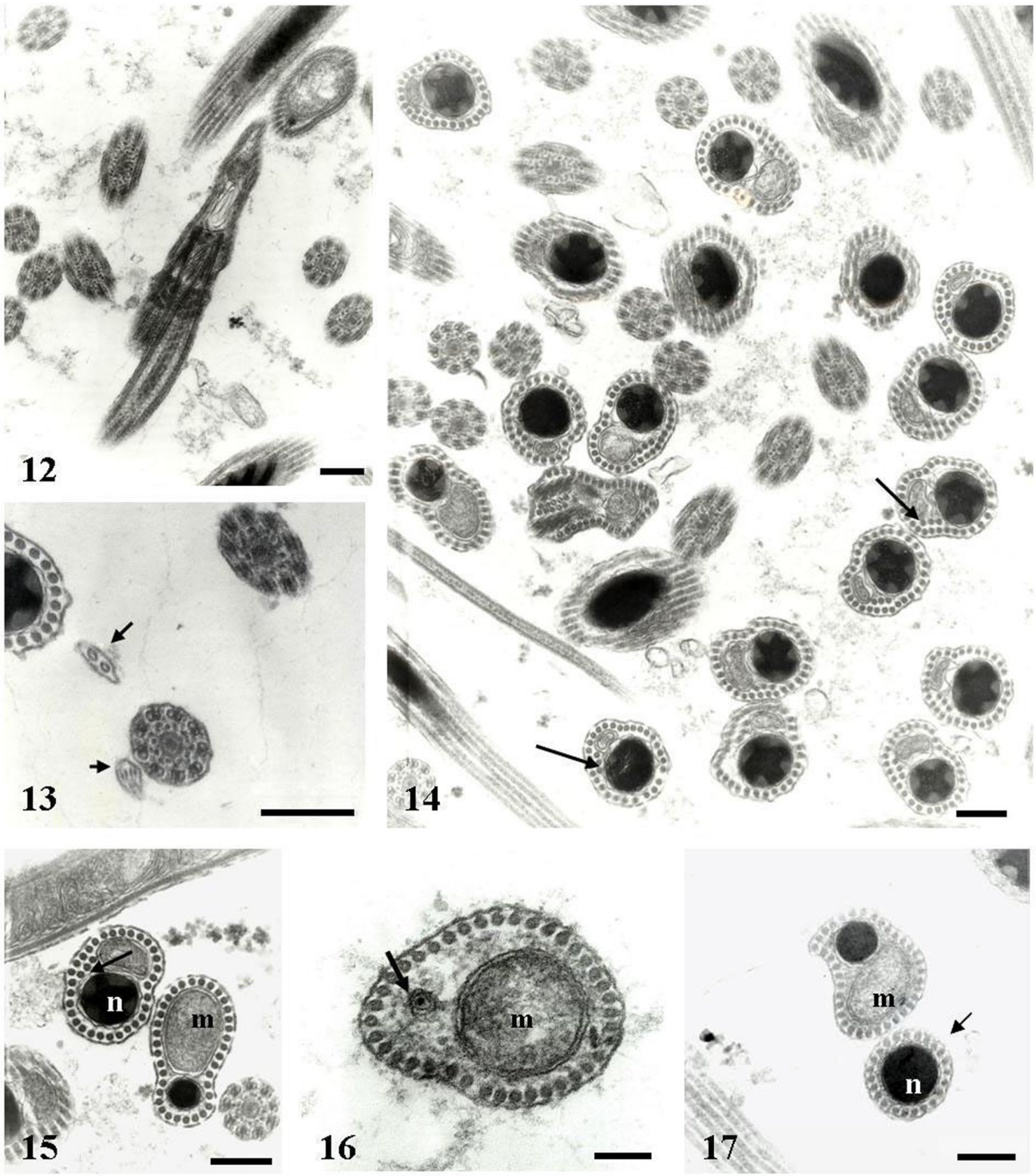

Fig. 12-17. – Spermatozoa of *Dugesia sicula.* Mature spermatozoa in the seminal vesicle. **12. -** Both flagella are free, subterminal and emerge together from one side of the spermatozoon; they fuse for a short distance just after the insertion zone before lying parallel to one another. Scale bar = 200 nm. **13. -** The axonemal pattern is of 9+1, many dense granules are present between the nine external microtubule doublets and the central complex. Tips of flagella split into long microvilli showing few microtubules in transverse sections (arrows). Scale bar = 100 nm. **14-15. -** Portion of the seminal vesicle showing different sections at different levels of spermatozoa. Flagella are free and we note the occurrence of three inner microtubules (arrows) close to the nucleus (n) and the mitochondrion (m). Scale bars = 200 nm. **16. -** Section through the distal part of the spermatozoon showing the mitochondrion (m) surrounded by peripheral microtubules and a tip of the elongated nucleus (arrow). Scale bar = 100 nm. **17. -** Cross section of the proximal end of the spermatozoon (arrow) showing only the nucleus (n) surrounded by peripheral microtubules. Scale bar = 100 nm.

DISCUSSION

In comparison with previous studies carried out on triclads, we enumerate the characteristics of spermatogenesis and spermiogenesis in the Tunisian sexual strain of *D. sicula*:

- Spermatids present an apical layer of dense nucleoplasm opposite to the differentiation zone. It has been interpreted as a basal plate by Ishida et al. (1991) and it has been found in paludicolan, maricolan and terricolan triclads (Franquinet & Lender, 1972; Ishida et al., 1991; Li et al., 1992; Rohde & Watson, 1995).
- Apart from in triclads, the presence of the rootlets has been mentioned for some Proseriates (Sopott-Ehlers, 1986, 1989, 1993) and many other taxa belonging to Trepaxonemata (see Watson & Rohde, 1995).
- During spermiogenesis, dense plates are associated with the ICB at the opposite side of the rootlets and around the basal bodies. These structures were observed in other triclads (Franquinet & Lender, 1972; Li et al., 1992; Rohde & Watson, 1995).
- The spermatozoon of *D. sicula* consists of two main parts: the proximal part, which contains the nucleus and the mitochondrion, and the distal process, which contains mainly the mitochondrion. Two free flagella emerge from the same side between the distal process and the proximal part. This spermatozoon form is known in several paludicolan and maricolan triclads (Silveira & Porter, 1964; Franquinet & Lender, 1972; Ehlers, 1985; Ishida & Teshirogi, 1988; Ishida et al., 1991; Rohde & Watson, 1995; Tekaya & Zghal, 2001).
- The organisation of the dense and lucent components of the nucleoplasm in *D. sicula* sperm was observed in other triclads too (Silveira & Porter, 1964; Ishida & Teshirogi, 1988; Ishida et al., 1991; Li et al., 1992; Rohde & Watson, 1995; Tekaya & Zghal, 2001).
- During spermiogenesis mitochondria fuse to form a single long mitochondrion, which lies parallel to the spermatozoon nucleus in a screw-like fashion in the proximal part before extending into in the distal part. Such organisation characterizes many triclads (Franquinet & Lender, 1972; Ishida et al., 1991; Rohde & Watson, 1995; Tekaya & Zghal, 2001).
- The internal layer of three microtubules present in *D. sicula* has been described in some other paludicolan and maricolan triclads (Farnesi et al., 1977; Ishida et al., 1991; Rohde & Watson, 1995).
- Flagella of *D. sicula* contain dense granules as in other turbellarians (Silveira & Porter, 1964; Li et al., 1992; Rohde & Watson, 1995).
- In mature sperm, tips of the flagella split into microvilli containing few microtubules as described in other triclads (Franquinet & Lender, 1972; Farnesi et al., 1977; Ishida & Teshirogi, 1988; Ishida et al., 1991; Li et al., 1992; Rohde & Watson, 1995).
- We notice that *D. sicula* sperm, along with some other turbellarian species and all Neodermata, lack the dense bodies characterizing several turbellarians (Ehlers, 1985).

In conclusion, this ultrastructural study carried out for the first time on the spermatogenesis and spermiogenesis of *D. sicula* enriches the knowledge in this field and shows that they are in conformity with previous studies on Platyhelminthes and especially in triclads.

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