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SHORT NOTES

Spermiogenesis and ultrastructure of the spermatozoon in the dioecious marine planarian *Sabussowia dioica* (Platyhelminthes, Tricladida)

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The phylogeny of the Platyhelminthes is a subject of controversy. Recently a number of attempts to reconstruct a valid phylogenetic scheme of Turbellaria-Platyhelminthes have been based on ultrastructural data of several organs (1, 2). Spermiogenesis and spermatozoon ultrastructure are often used for understanding both phylogeny and reproductive biology in the phylum (3, 4, 5). In this respect, a small number of triclads have been studied. The marine planarian *Sabussowia dioica* (Claparède, 1863) is the only known gonochoristic triclad and the rarest dioecious organism in Platyhelminthes. Males of this species transfer sperm by depositing sclerotic spermatophores onto the body wall of the female, and it is only from these capsules that sperm are discharged into the parenchyma of the partner. This sperm transfer method is unique in two respects: (a) external deposition of spermatophores is unique within the Tricladida and (b) formation of spermatophores is unique within the Maricola. Fertilization of oocytes takes place within the ovary, contrasting with the usual situation of fertilization in the proximal section of the oviductus (6). *S. dioica* also shows unusual features of the developing female germ cells and their associated cells (7).

In this study by transmission electron microscopy, we report the first data on spermiogenesis and spermatozoon ultrastructure of male specimens of *S. dioica* collected in the Gulf of Tunis. Three mitoses and the meiosis give rise to clustered structures of 32 biflagellated spermatids. Young spermatids are round shaped (Fig. 1). The spermiogenesis consists mainly of nuclear condensation and elongation (Figs 1, 2), and fusion of the numerous mitochondria of the spermatid resulting in a single giant one (Figs 4, 6).

The spermatozoon is thread-like (50µm in length). The elongated nucleus is made up of two distinct components one being filamentous, electron-dense chromatin with a helical structure (Fig. 6) and the other of uniform electron-light appearance, suggested to be residual protein (8). The nucleus and the single elongated mitochondrion are coiled around each other in a screw-like fashion. Two free flagella are coupled and lie parallel to one another (Figs 5, 7). Their axonemal microtubules are arranged in a "9+1" structure (Fig. 5). There is a single layer of peripheral microtubules running longitudinally beneath the plasma membrane (Fig. 4). By reconstruction from transverse and longitudinal sections taken through the testis and seminal vesicle, a diagrammatic drawing of the structure of the mature spermatozoon of *S. dioica* is given in Fig. 8.

This research shows that spermiogenesis and spermatozoon ultrastructure of *S. dioica* are mainly in agreement with the literature data regarding Platyhelminthes and Turbellaria Tricladida: (a) Two free flagella in the spermatids and spermatozoa are widespread in free living flatworms and should be considered the plesiomorphic structure for the Platyhelminthes (3, 4, 5). (b) Axonemal microtubules with "9+1" structure have been reported for Turbellaria and parasitic groups (4, 5, 9). (c) As in other triclads (10, 11, 12) and other Platyhelminthes (4, 5, 12), the spermatozoon of *S. dioica* lacks an acrosomal structure.

Some aspects that appear to be confined to species of Tricladida are also noted in the spermatozoon of *S. dioica*: **(a)** the occurrence of a complex nuclear structure with two distinct components coiled around each other in a screw fashion (8,13,5, the present study). **(b)** The nucleus and the single elongate mitochondrion also coil around each other (4, 5, this study). **(c)** Mature sperm lack the numerous dense bodies characteristic of many other turbellarians (4, 13, 5, this study).

Meanwhile, a particular feature of the spermatozoon of *S. dioica* is that the two free flagella are coupled and parallel to one another.

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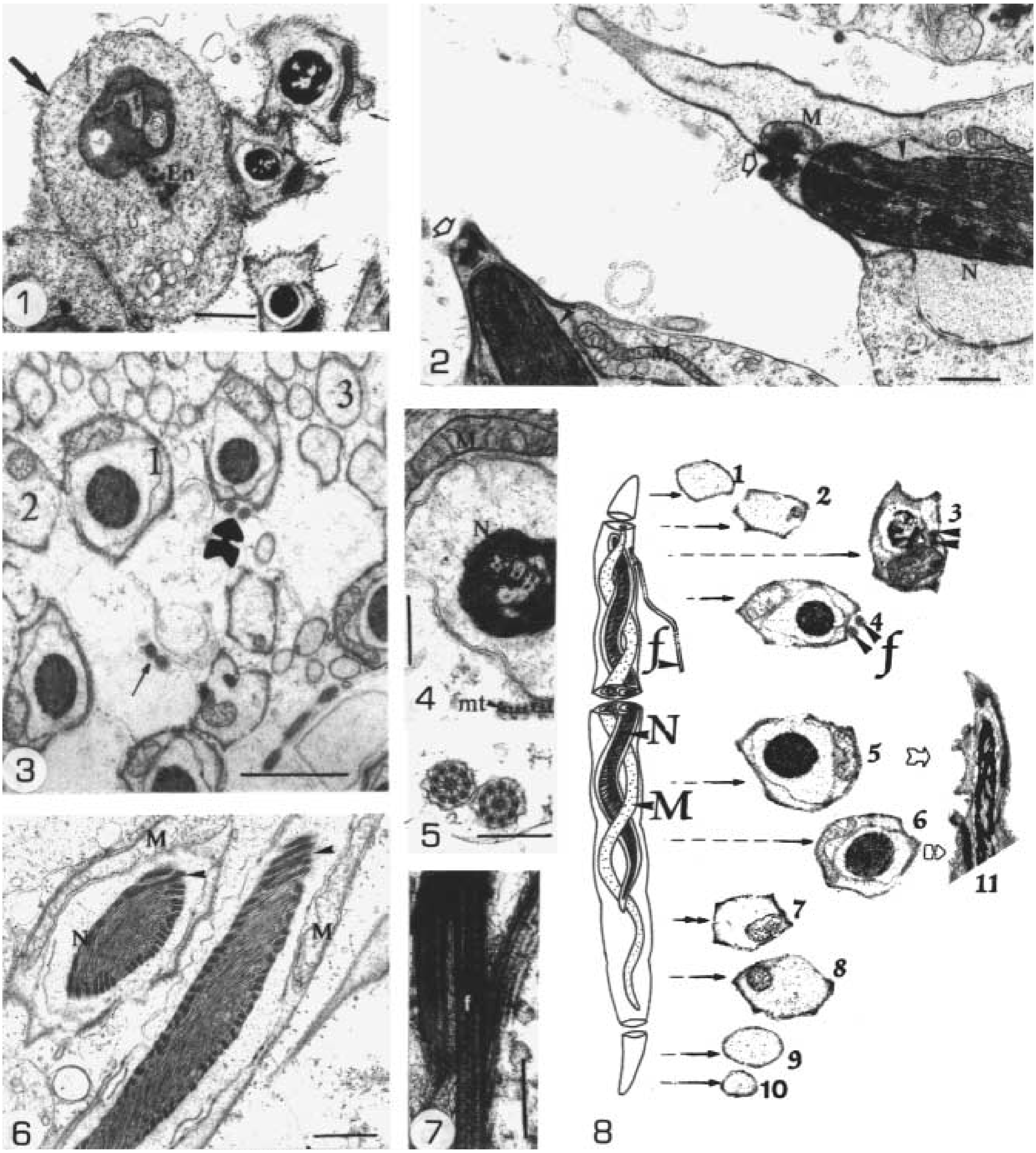

Fig. 1. – A young spermatid (strong arrow) showing nuclear emissions (En) and three sections of spermatozoa (small arrows) in the testis; scale bar = 1 µm.

Fig. 2. – Spermiogenesis of two spermatids showing elongation of the condensed nuclear material (heads of arrows) and departure of the two flagella (light arrows); M: mitochondria; N: nucleus; scale bar = 1 µm.

Fig. 3. – Transverse sections of spermatozoa in the seminal vesicle; three section levels (1: nucleus & mitochondrion; 2: mitochondrion; 3: 0); arrows: two flagella; scale bar = 2 µm.

Fig. 4. – Transverse sections of a spermatozoon showing the complex nuclear structure (N) and peripheral microtubules (mt); scale bar = 0.5 µm.

Fig. 5. – Transverse section of the two coupled flagella with “9+1” structure; scale bar = see 0,5 µm.

Fig. 6. – A transverse (on left) and a longitudinal (on right) section of spermatozoa showing the nucleus (N) with spiral-shaped condensed chromatin (heads of arrows) and the giant mitochondrion (M); scale bar = 1 µm.

Fig. 7. – Longitudinal section of the two coupled and parallel flagella (f); scale bar = 0.5 µm.

Fig. 8. – Reconstruction of *S. dioica* spermatozoon structure (on left) showing the nucleus (N), the mitochondrion (M) and the two free flagella (f); transverse (1-10) and longitudinal sections (11). Heads of arrows at profile 3 indicate two dense structures where the flagellar axonemes originate in the shaft of the spermatozoon.

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