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Ultrastructure of Sperm and Spermatogenesis of *Artioposthia* sp. (Platyhelminthes : Tricladida : Terricola)

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Abstract

This is the first reported study of the ultrastructure of sperm and spermatogenesis of a terrestrial triclad (Terricola). Processes from the cells of the testis wall envelop early germinal cells, and wall cells are ciliated in the transition zone to the sperm duct. Following meiosis of spermatocytes, spermatids develop by formation of a zone of differentiation containing peripheral microtubules, two centrioles facing in opposite directions, a prominent intercentriolar body and a semicircle of dense material around the centrioles. Centrioles become basal bodies giving rise to free flagella. Elongation of this zone, accompanied by rotation of the basal bodies around the shaft, results in flagella being carried to a distal and subterminal location, emerging on the one side of, and perpendicular to, the spermatid shaft. Nucleus and mitochondrion migrate into the shaft. *Artioposthia* is compared with other triclads and other turbellarian groups. The lack of dense bodies in sperm and of rootlets during spermiogenesis may be autapomorphies for the Tricladida. To our knowledge, the only report of splitting of flagellar tips into a number of separate threads, outside Tricladida, is from *Syndisyrinx punicea* (Rhabdocoela: Umagillidae) (Rohde and Watson 1988).

Introduction

There have been few studies of the ultrastructure of sperm and spermatogenesis of members of the Tricladida. Klima (1961) briefly examined spermatogenesis in *Dendrocoelum* (Paludicola), Franquinet and Lender (1972, 1973) made detailed studies of the process in *Polycelis nigra* and *P. tenuis* (Paludicola), while Silveira and Porter (1964) reported on the ultrastructure of the mature spermatozoa in *Dugesia tigrina* (Paludicola), *Bdelloura candida* and *B. propinqua* (Maricola). Ishida and Teshirogi (1988) and Ishida *et al.* (1991) examined mature sperm of *Bdellocephala brunnea* and *Polycelis sapporo* (Paludicola). Ishida *et al.* (1991) also examined mature sperm of *Phagocata teshirogii* (Paludicola).

Artioposthia sp. is a terrestrial triclad belonging to the suborder Terricola. Ultrastructure of spermatogenesis and sperm have not been reported for any species from this suborder. Rohde and Watson (1992) examined the ultrastructure of the protonephridia of *Artioposthia* sp., as a contribution to the phylogeny of the Platyhelminthes, and the present complementary investigation was undertaken for the same reason.

Materials and Methods

Specimens were collected from beneath rocks overlying basaltic soil in Armidale, New South Wales. The worms were cut into small pieces and fixed in a 1 : 1 mixture of 3% glutaraldehyde : 3% paraformaldehyde in 0·1 M phosphate buffer, pH 7·3 at 4°C for 2 h. After washing for 50 min in the same buffer, they were post-fixed in 1% OsO_4 in buffer, dehydrated in an alcohol series, embedded in Spurr resin and polymerised at 60°C. Sections were cut from the mid-body region on a Reichert Ultracut-E microtome, stained with uranyl acetate and lead citrate and examined under a JEOL 1200 EX electron microscope at 60 kV. Specimens have been deposited in the Queensland Museum, Brisbane, Australia.

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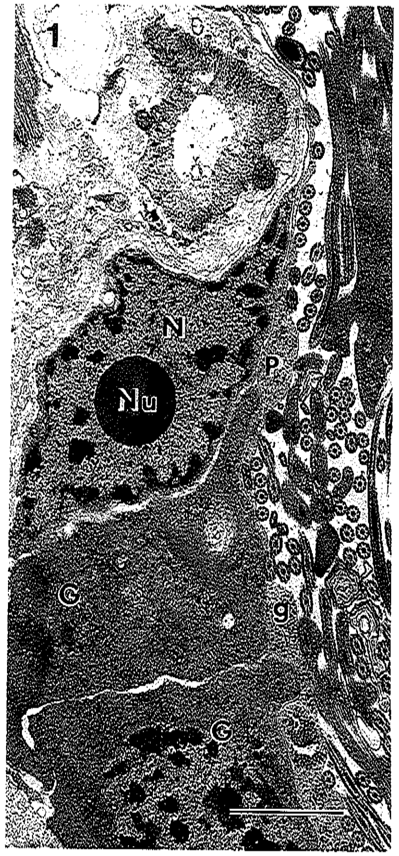
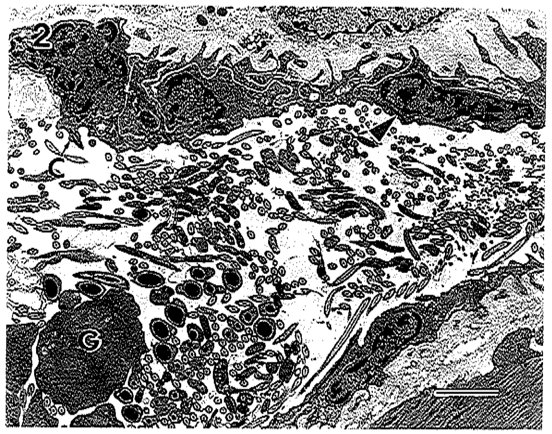
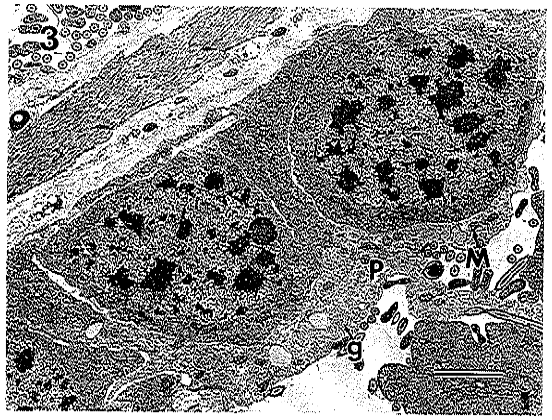






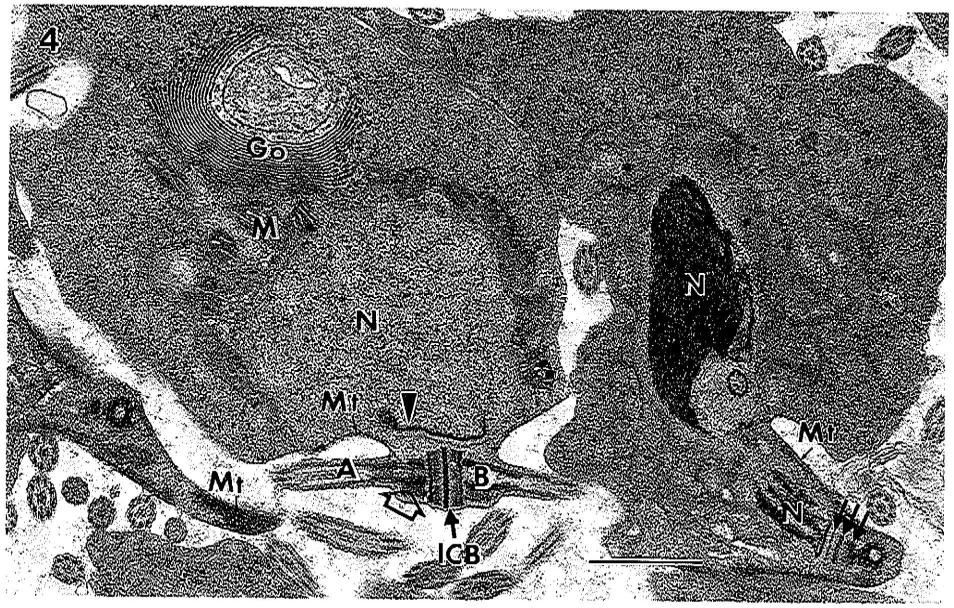
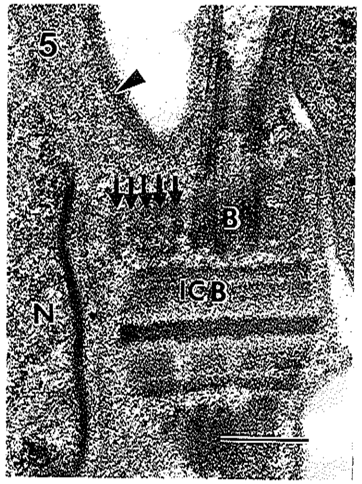


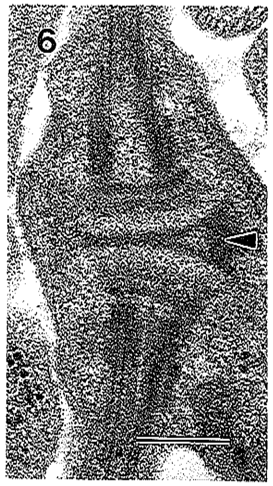
Fig. 1. Wall of the testis. Note nucleus (N) of wall cell with prominent nucleolus (Nu), fine cell processes (P) containing granules (g) around testis and around germinal cells (G). **Fig. 2.** Transition region between testis (on the left) and sperm duct. Note cilium of wall cell (C), junction (arrowhead), germinal cell probably spermatid (G). **Fig. 3.** Two spermatogonia adjacent to testis wall and surrounded by processes (P) of wall cell. Note mitochondria (M) and granules (g) abundant in cell processes. **Fig. 4.** Early spermatids. In central spermatid (earliest) note pale nucleus (N) surrounded by mitochondria (M), prominent Golgi apparatus (Go), dense layer in nucleus (arrowhead), basal body (B) of axoneme (A), surrounded by a semicircle of dense material (open arrowhead), intercentriolar body (ICB), microtubules (Mt) along apical cell membrane. In spermatid on the right note partially condensed chromatin of nucleus, elongation of zone of differentiation, dense bars (arrows) between the tip of the nucleus and the centriole, microtubules beneath the cell membrane. In the spermatid on the left, note extension of spermatid distal to the centriole, also lined with microtubules. Scale lines: *1–3*, 2 μm; *4*, 1 μm.

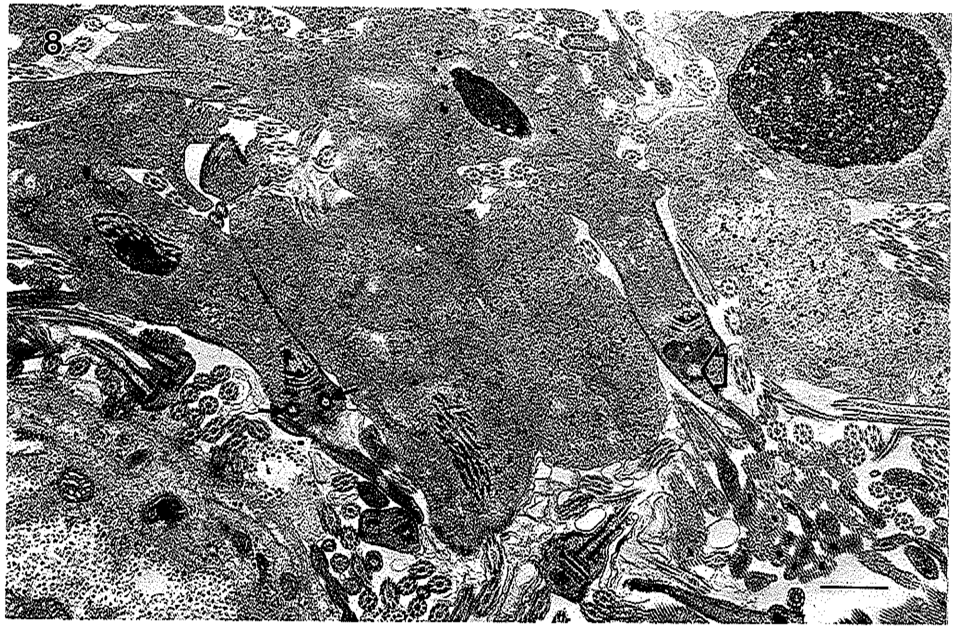
Results

The wall of the testis of *Artioposthia* sp. is formed by cells with a large nucleus containing a prominent nucleolus and dark aggregations of chromatin (Fig. 1). Mitochondria, polyribosomes and clusters of granules presumed to be glycogen are abundant in the cytoplasm. Processes (sometimes extremely thin) from these parietal cells extend around the testis, forming its wall, and into it, surrounding groups of developing germ cells, including spermatogonia (Figs 1, 3) and spermatocytes. Developing spermatids are not enveloped in this manner. In the transition area between testis and sperm duct, epithelial cells bear numerous cilia and there are septate junctions between adjacent cells (Fig. 2).

Spermatogonia lie adjacent to the testis wall and have a large, oval nucleus with prominent nucleolus and chromatin aggregations, and a high nucleus:cytoplasm ratio (Fig. 3). A few mitochondria and polyribosomes were also observed. Primary spermatocytes, characterised by synaptonemal complexes, are also in close proximity to the testis wall. Their cytoplasm contains a number of mitochondria, a large Golgi complex, endoplasmic reticulum and numerous polyribosomes. Following meiosis, spermatids remain connected by cytoplasmic bridges. The nucleus of the spermatid moves to one side of the cell and mitochondria become aggregated around it except where it is closest to the plasma membrane (Fig. 4). A prominent, distinctive Golgi apparatus also lies adjacent to the nucleus (Fig. 4), and a dense layer forms in the nucleus beneath the nuclear membrane nearest to the plasma membrane (Figs 4, 5). In a protrusion adjacent to this region, two centrioles appear which become basal bodies and give rise to two free flagella growing in opposite directions to each other (Figs 4, 5). Axonemes are of the 9+'1' arrangement and have the complex central element characteristic of Trepaxonemata (Figs 1, 2, 4, 10, 15). A prominent intercentriolar body consisting of at least 5 bands is present between the bases of the two centrioles and an incomplete cylinder of dense material partially encircles the centrioles (Figs 4, 5). Between the centrioles and the nucleus this material appears as several separate plates (Fig. 5). Microtubules line the plasma membrane in this zone of differentiation (Figs 4, 5). The next stage of spermiogenesis involves condensation of nuclear chromatin and elongation of the zone of differentiation. A short region of spermatid shaft extends distal to the flagella and is lined with microtubules (Figs 4, 7–9). This results in the eventual subterminal insertion location of flagella in the mature spermatozoa. Basal bodies rotate around the extending shaft (Fig. 7), accompanied by an apparent splitting of the intercentriolar body (Fig. 6), to eventually lie adjacent and parallel to each other (Figs 8, 9). This results in flagella emerging together, on one side of, and perpendicular to, the main shaft (Fig. 8). The nucleus follows close to the centrioles along the growing shaft, appearing to be anchored to them by bands of dense material (Figs 4, 7, 8). A row of microtubules encircles the shaft just beneath the plasma membrane (Figs 4, 7–9). A single mitochondrion is seen in cross sections of spermatids and sperm (Figs 10, 11, 15); thus it is presumed that fusion results in one or several longitudinally arranged mitochondria (Fig. 14) that extend(s) into the shaft alongside the nucleus, for much of its length.







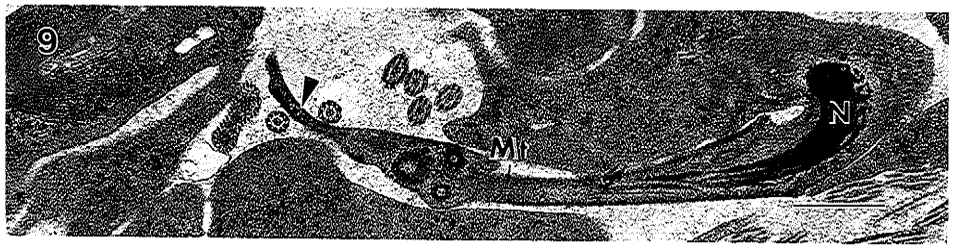


Fig. 5. Early zone of differentiation of spermatid. Note microtubules (arrowhead), intercentriolar body (ICB) and dense bars (arrows) between basal body (B) and nucleus (N). **Fig. 6.** Intercentriolar body appears to be splitting (arrowhead) as flagella rotate around the spermatid shaft. **Fig. 7.** Rotation of flagella around the shaft. Note dense bars (arrows) between nucleus (N) and basal bodies, microtubules (Mt) distal and proximal to the basal body region. **Fig. 8.** Developing spermatids. Upper right, note early stage of nuclear condensation. In later spermatids note basal bodies now almost parallel with each other, dense bars between nucleus and basal bodies (arrows), dense circular area (open arrowhead) adjacent to basal bodies (may be remnant of intercentriolar body). **Fig. 9.** Later spermatid with basal bodies parallel with each other. Note nucleus (N), microtubules (Mt) and distal process (arrowhead). Scale lines: *5–7*, 200 nm; *8*, *9*, 1 μm.

Mature sperm in the testis and adjacent sperm duct have the following distinctive features. A short distal process, surrounded by microtubules, is distal to the area of attachment of flagella. Flagella emerge together, perpendicular to the main shaft. The tips of flagella lose the central element (Fig. 10), taper to nine single microtubules, and then split into thin processes containing one or more microtubules (Fig. 15). The main body, also surrounded by longitudinal microtubules, contains a nucleus only in one region (Figs 10, 13), and both nucleus and mitochondrion in the remainder (Figs 10, 11, 15); it was not determined which represented the most proximal region. Where only the nucleus is present, it occupies most of the cross-sectional area and usually shows two distinctive components—an electron-dense lamellar component and a more homogeneous, more electron-lucent component (Figs 12, 13). The two elements are arranged in a regular, spiralling formation. Where both nucleus and mitochondrion are present, the nucleus exhibits mainly the lamellar component. In no region were any electron-dense bodies, characteristic of many other turbellarian spermatozoa, seen.

Discussion

The parietal cells forming the wall of the testis of *Artioposthia* extend processes around groups of developing germ cells, in particular spermatogonia and, less consistently, spermatocytes. They contain conspicuous clusters of granules which are presumed to be glycogen and thus could perform a nutritive function for the differentiating cells. Franquinet and Lender (1973) observed a similar enveloping arrangement in two species of *Polycelis* (Paludicola) and noted that their cytoplasmic contents suggested both a supportive and secretive role. Farnesi *et al.* (1977) also commented on processes of the testis wall entering between germinal cells in *Dugesia lugubris* (Paludicola). They also observed that cells in the transition zone between testis and sperm duct bear numerous cilia, as we have noted in *Artioposthia*. There are few other observations in the literature concerning ultrastructure of the cells forming the wall of the testis in Turbellaria (see Hendelberg 1983; Williams 1984; Noury-Sraïri *et al.* 1989).

Differentiation of spermatids in *Artioposthia* follows a similar course to that documented in the brief descriptions of Klima (1961), Silveira and Porter (1964), Farnesi *et al.* (1977), and the more detailed studies of Franquinet and Lender (1972, 1973) for other triclad species. The following stages of development occur in common with other turbellarian groups that have long, thin mature sperm with two free flagella. The nucleus first moves to one side of the cell and a zone of differentiation appears; this contains two centrioles facing in opposite directions, with an intercentriolar body between them. Microtubules line the cell membrane in this protruding region. The centrioles become basal bodies for two free flagella that grow directly out from the protrusion. This subsequently elongates, carrying the flagella a considerable distance from the main spermatid mass. Elongation of the distal region beyond the flagella insertion point results in their subsequent subterminal location. Condensation of nuclear chromatin, and nuclear and mitochondrial migration into the shaft, eventually result in the formation of a long, thin mature spermatozoon, with two free flagella. Longitudinal microtubules beneath the cell membrane extend throughout the sperm body. Subterminal location of flagella has been observed in many other turbellarian species by light (and fewer by electron) microscopy (see reviews by Henley 1974; Hendelberg 1983).

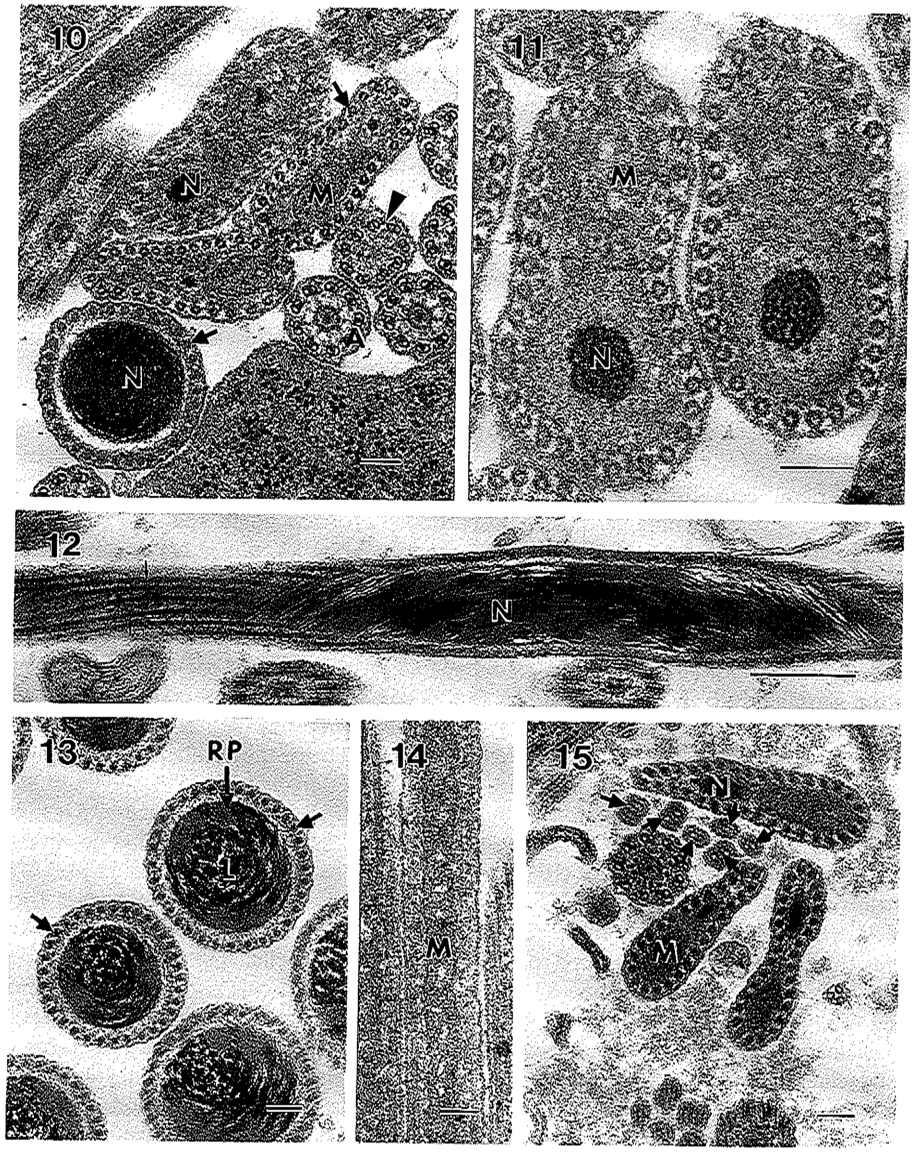


Fig. 10. Cross sections of sperm in the testis. Note peripheral microtubules (arrows), nucleus (N), mitochondrion (M), axoneme (A). Note also central element missing from one axoneme (arrowhead). **Fig. 11.** Cross sections of sperm in region with nucleus (N) and mitochondrion (M). **Fig. 12.** Longitudinal section of sperm showing parallel longitudinal microtubules (arrows) and spiralling nuclear components (N). **Fig. 13.** Cross sections of sperm in the region of two nuclear components. Note lamellar chromatin (L) and 'residual protein' (RP), microtubules (arrows). **Fig. 14.** Longitudinal section of a sperm showing single mitochondrion (M). **Fig. 15.** Cross sections of sperm in the region of nucleus (N) and mitochondrion (M). Note tip of one flagellum split into separate strands containing one or two microtubules each (arrows). Scale lines: *10*, *11*, *13–15*, 100 nm; *12*, 500 nm.

However, some aspects of spermiogenesis and mature sperm appear to be confined to species of Tricladida. Thus the nucleus of mature sperm has two distinct components, one being filamentous, electron-dense chromatin and the other of uniform, less electron-dense appearance, suggested by Silveira and Porter (1964) to be residual protein. The two components spiral around each other along the longitudinal axis of the sperm. In all triclads studied by electron microscopy both flagella emerge together on one side of the sperm body, whereas in most other turbellarians free flagella emerge on opposite sides. Dense bodies typical of most other free-living Turbellaria (see Hendelberg 1986) have not been observed in the triclads studied by electron microscopy (Silveira and Porter 1964; Franquinet and Lender 1972, 1973; Farnesi *et al.* 1977; Ishida and Teshirogi 1988; Ishida *et al.* 1991). The tips of flagella in *Artioposthia* and in several species of freshwater triclads (Ishida and Teshirogi 1988; Ishida *et al.* 1991) split into a number of separate threads, eventually containing a single microtubule in each; this was also observed in *Syndisyrinx punicea* by Rohde and Watson (1988). During spermiogenesis, a well-developed intercentriolar body is present in all triclads studied but typical rootlets have not been observed. Instead, a series of dense plates is present between each centriole and the nucleus, and an additional semicircle of dense material partially surrounds each centriole in the early stages before elongation of the zone of differentiation. Distinctive splitting of the intercentriolar body, as occurs in *Artioposthia*, has also been described in freshwater planarians (Franquinet and Lender 1972), accompanying the rotation of basal bodies around the spermatid shaft. A similar splitting was also described during spermiogenesis of *Temnocephala novaezealandiae* (Williams 1984). Thus, although in general the events of spermiogenesis resemble those seen in many other turbellarian groups, there are some features distinctive to triclads.

Proseriata and Tricladida are united in the taxon Seriata (see Sopott-Ehlers 1985; Sluys 1989). Spermiogenesis has been studied in several species of proseriates (see Ehlers 1985; Sopott-Ehlers 1989, 1990) but there do not appear to be any characteristics of spermiogenesis or mature sperm that are synapomorphic for triclads and proseriates. Proseriates studied have well-defined intercentriolar bodies and distinct rootlets during spermiogenesis and conspicuous dense bodies in the mature sperm in common with many other free-living turbellarian groups. Thus it is suggested that the lack of rootlets and dense bodies in triclads is due to secondary loss, and these features would therefore represent autapomorphies for the Tricladida. It is claimed (Sopott-Ehlers 1985) that Bothrioplanida are more closely related to the Tricladida than to the other members of the Proseriata. However, neither spermatogenesis nor sperm have been studied in this group.

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