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## Ultrastructural observations of the testicular epithelium and spermatozoa of *Pseudochordodes bedriagae* (Gordiida, Nematomorpha)

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**Abstract** Two questions are of interest concerning the male reproductive system in Gordiida: (1) is the epithelium surrounding the testis continuous or discontinuous and (2) is the type of spermatozoon as described at the transmission electron-microscopical level for the two species of *Gordius* typical for all Gordiida? An examination of the South American species *Pseudochordodes bedriagae* has allowed us to add new information to this poorly studied phylum. Testicular tubes are large, filled with spermatozoa, and surrounded by a continuous epithelium. The epithelial cells that line the posterior testes occasionally overlap, and their cytoplasm is narrow and contains dense granules, abundant endoplasmic reticulum, and vesicles. The plasma membrane possesses microvilli with many filaments. This epithelium rests on a basement membrane. The spermatozoa in *P. bedriagae* resemble the known spermatozoa of two *Gordius* species but differ in presenting a uniform halo layer of less dense chromatin that surrounds the dense chromatin in the nucleus. The finding that a similar type of spermatozoa occurs in both genera (*Pseudochordodes* and *Gordius*) makes it likely that it is present in all other Gordiida and is therefore an autapomorphy of the Gordiida.

**Keywords** Testicular epithelium · Spermatozoa · *Pseudochordodes bedriagae* · Gordiida · Nematomorpha

### Introduction

The male reproductive system of freshwater Nematomorpha (Gordiida) was originally described at the light-microscopical level (Meissner 1856; Vejdovsky 1886; Villot 1881, 1891; Montgomery 1903; Rauther 1905; Svabenik 1909; May 1919). More recently, ultrastructural studies have added new data, especially with reference to the structure of the testicular tubes and spermatozoa (Lora Lamia Donin and Cotelli 1977; Bresciani 1991; Lanzavecchia et al. 1995; Schmidt-Rhaesa 1997; Valvassori et al. 1999; Poinar 2001). Additionally, Bolek and Coggins (2002) have shown a scanning electron-microscopical image of sperm deposited on the female cuticle. These previous studies indicate the presence of internal epithelial cells lining the testes; however, no fine structural description of the testicular epithelium exists for any Nematomorpha species.

Two questions are of interest concerning the male reproductive system in Gordiida. First, is the epithelium that internally lines the testes continuous or discontinuous? This first question arises because previous studies of the testicular epithelium are contradictory (Vejdovsky 1886; Montgomery 1903; Svabenik 1909; Schmidt-Rhaesa 1997; Valvassori et al. 1999). Second, is the type of spermatozoon as described at the transmission electron-microscopical level for two *Gordius* species (Lora Lamia Donin and Cotelli 1977; Schmidt-Rhaesa 1997) typical for all Gordiida? With regards to this question, the investigation of *Pseudochordodes* is optimal because this genus and *Gordius*, from which most details are known (except for a singular image from a *Neochordodes* sperm; see Poinar 2001), represent two extremes of gordiid evolution (Bleidorn et al. 2002).

An examination of the South American species *Pseudochordodes bedriagae* has allowed us to contribute to the answers to both questions. We have also attempted to compare the spermatozoa of Gordiida with Nematoda in order to establish the differences and similarities between them.

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## Materials and methods

Seven adult male specimens of *Pseudochordodes bedriagae* were collected during the spring and summer seasons of 1998 and 1999 from the stream El Negro (38°08'00"S, 61°47'00"W) in the Sierra de la Ventana, Province of Buenos Aires, Argentina, and from an irrigation channel (40°06'00"S, 64°26'00"W) in General Conesa, Province of Rio Negro, Argentina. Adult worms collected from each location were deposited in the Natural Science Museum, La Plata, Argentina (MLP 3597, MLP 5022). They were maintained alive in non-chlorinated water, with aeration, at a temperature ranging between 16°C and 20°C.

### Transmission electron microscopy

Three specimens of *Pseudochordodes bedriagae* were anesthetized with diethyl sodium barbiturate in sterile non-chlorinated water, washed with isotonic neutral detergent, and treated with ultrasonication for 30 s. Tangential and cross sections of 0.5–1 mm in thickness were obtained from the middle and terminal end of the body of the three specimens. These pieces were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.1–7.3) at 4°C, washed (2×30 min) in 0.1 M sucrose in cacodylate-phosphate buffer, and embedded in Araldite. Ultrathin sections (70–100 nm) were stained with uranyl acetate and lead citrate and observed with a Jeol JEM 1200X II transmission electron microscope.

### Scanning electron microscopy

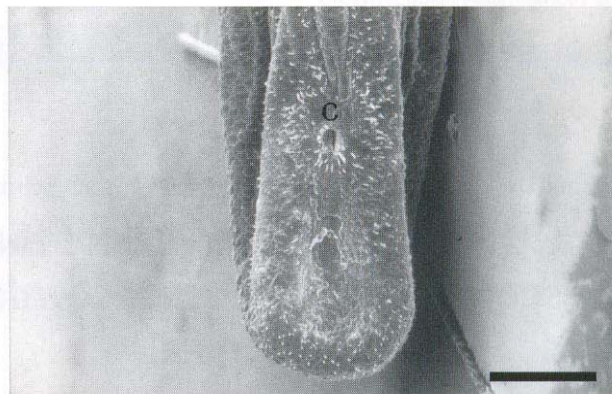
Fragments from the middle and terminal end of the body of three specimens of *Pseudochordodes bedriagae* were fixed in 95% ethanol and dehydrated in 100% ethanol, critical-point-dried, mounted on bronze blocks coated with 24-argon plasma gold, and viewed under JEOL SLM 1000 scanning electron microscope.

## Results

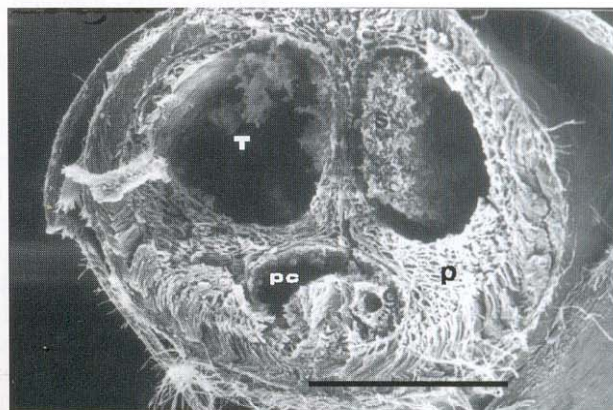
In males of *Pseudochordodes bedriagae*, two dorsolateral testicular tubes extend along the whole length of the worm, as in other species of Gordiida. At the posterior end, these testicular tubes join the intestine and lead together toward a ventral cloacal opening. The cloacal opening is externally surrounded by cuticular spiniform structures (circumcloacal spines), which are probably of importance during copulation (Fig. 1).

The testicular tubes (Fig. 2) are large and are filled with numerous spermatozoa. The parenchyma is reduced and occurs around the testicular tubes, the gut, and the ventral nerve cord.

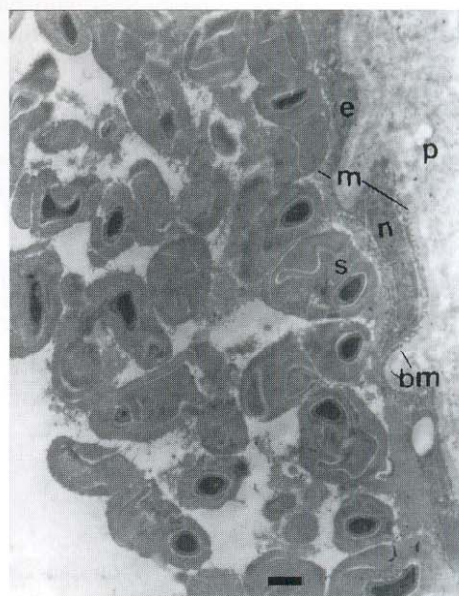
The testicular tubes (Figs. 3, 4) are surrounded by a continuous epithelium, which rests on a thick basal membrane composed of a thin dense and homogeneous layer



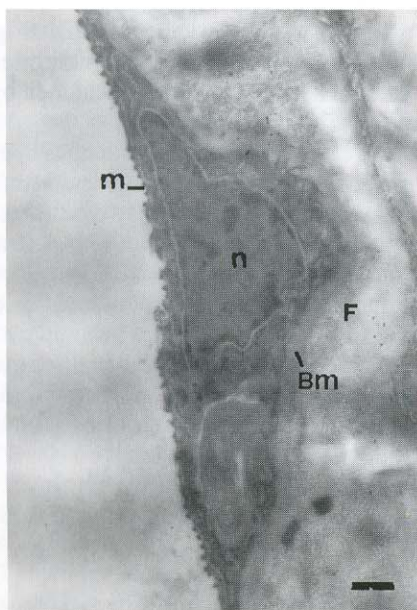
**Fig. 1** Terminal end of a male *P. bedriagae* (c cloacal opening). Scanning electron microscopy. Bar 100  $\mu$ m



**Fig. 2** Cross section from the middle region of *P. bedriagae* (p parenchyma, pc peri-intestinal cavity, T testis, s spermatozoa). Scanning electron microscopy. Bar 100  $\mu$ m



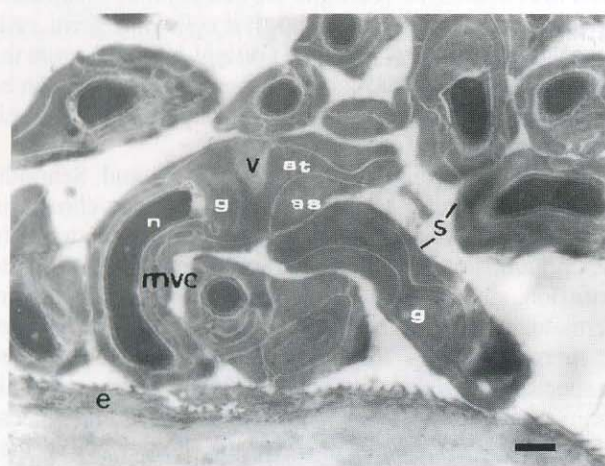
**Fig. 3** Testis from the middle region of the body of *P. bedriagae* (e epithelial cell, n nucleus, m microvilli, p parenchyma, s spermatozoa). The continuous testicular epithelium rests on a thick basal lamina (bm). Transmission electron microscopy. Bar 1  $\mu$ m



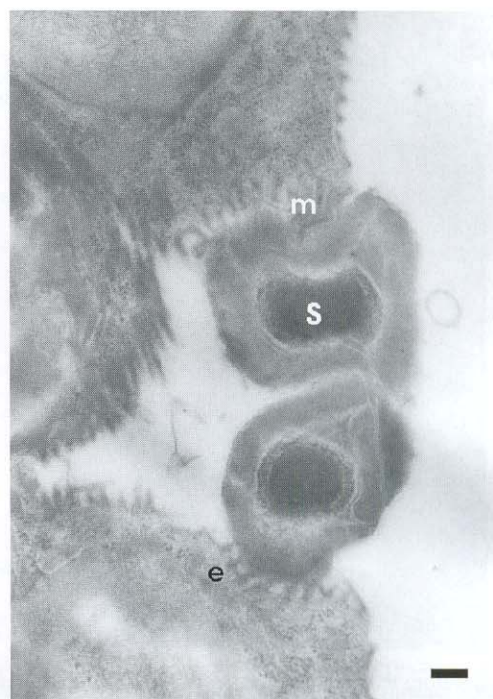
**Fig. 4** Testicular epithelium cell from the middle region of the body of *P. bedriagae* (*Bm* basal lamina, *F* fibrillar matrix, *n* nucleus, *m* microvilli). Transmission electron microscopy. Bar 500 nm

and a fibrillar layer (Fig. 4). Beneath this basal membrane there are the parenchyma cells, each surrounded by a thick layer of fibrillar extracellular matrix.

The epithelium that lines the testicular cavity internally is formed by a layer of flat irregular cells that occasionally overlap (Figs. 4, 5), especially in the posterior part of the body. Cellular junctions have not been observed. The cells possess an irregular nucleus with abundant heterochromatin surrounded by a nuclear membrane with nuclear pores and a perinuclear cisterna. The nuclear zone protrudes toward the parenchymal extracellular matrix, which is separated from the basal lamina (Fig. 4). The cytoplasm is narrow and contains dense granules (probably glycogen), abundant cisternae of the endoplasmic reticulum, and nu-

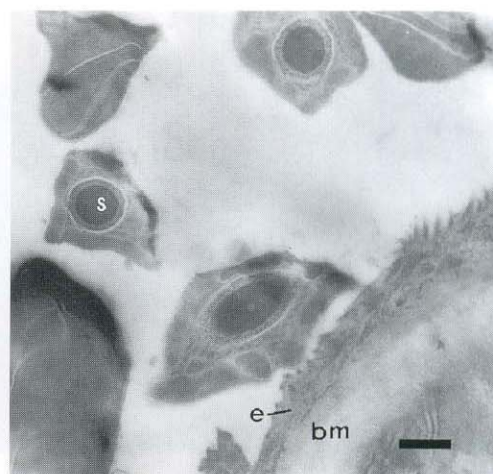


**Fig. 5** Testis from the posterior region of the body of *P. bedriagae* (*as* acrosomal sheath, *at* acrosomal tube, *v* acrosomal vesicle, *e* epithelium with some overlapping of epithelial cells, *n* nucleus, *g* Golgi-like complex, *mvc* multivesicular complex, *s* spermatozoa). Transmission electron microscopy. Bar 500 nm

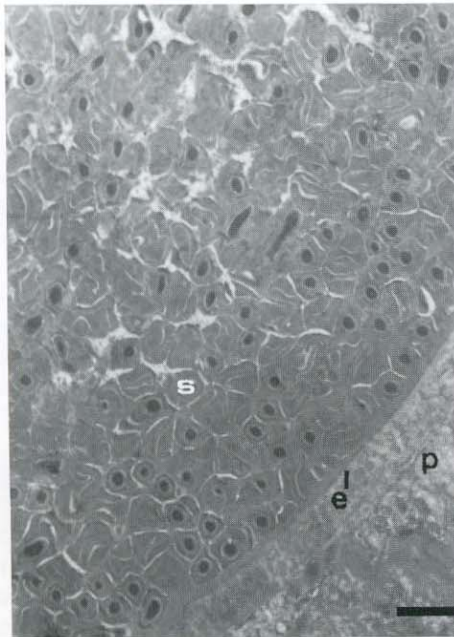


**Fig. 6** Testis from the middle region of the body of *P. bedriagae*. Association of epithelial cells and spermatozoa (*e* epithelium, *m* microvilli, *s* spermatozoa). Transmission electron microscopy. Bar 200 nm

merous vesicles. Mitochondria have not been observed. The plasma membrane possesses short microvilli over its whole surface, the microvilli containing abundant filaments that are more highly developed toward the lumen of the tube (Figs. 3, 4, 6). Close contacts between the microvilli of the epithelial cells and the spermatozoa (Fig. 6) can be observed in the anterior and medial regions of the testis, but intercellular junctions have not been seen. In the posterior region of the animal, the epithelium is reduced, being represented by a thin layer of cytoplasm and of basal membrane (Figs. 7, 8).

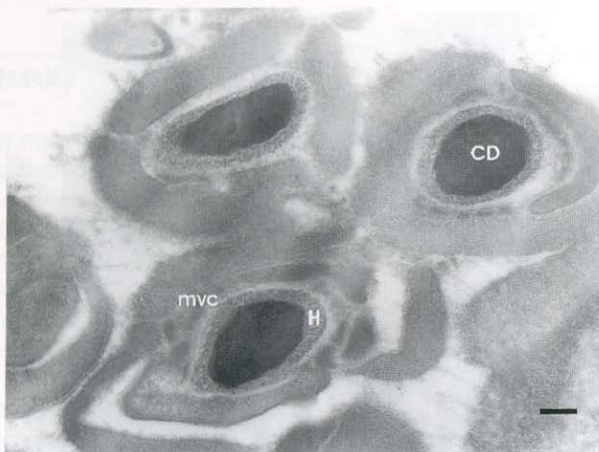


**Fig. 7** Testicular epithelium from the posterior region of the body (*e* epithelium, *bm* basal lamina, *s* spermatozoa). Transmission electron microscopy. Bar 500 nm



**Fig. 8** Testis from the posterior region of the body of *P. bedriagae*. Flat continuous testicular epithelium and sperm packed more densely in the peripheral zone (*e* epithelium, *p* parenchyma, *s* spermatozoa). Transmission electron microscopy. Bar 2  $\mu$ m

The mature spermatozoa in the lumen of the testicular tubes resemble those described earlier for the two *Gordius* species, *G. panighettensis* (Lora Lamia Donin and Cotelli 1977) and *G. aquaticus* (Schmidt-Rhaesa 1997). The spermatozoa consist of a broader anterior part and a slender posterior region (Fig. 5). The anterior part contains the acrosomal complex composed of an acrosomal tube surrounded by an acrosomal sheath. The acrosomal tube is dilated at its tip, thereby forming the acrosomal vesicle, which shows greater density at its apex. This structure appears to be a bundle of filaments or fibrils probably representing a perforatorium. A Golgi-complex-like space occupied by membranes and vesicles can be observed be-



**Fig. 9** Cross section of the posterior region of spermatozoa of *P. bedriagae* (*CD* dense chromatin, *H* halo of low-density chromatin, *mvc* multivesicular complex). Transmission electron microscopy. Bar 200 nm

tween the acrosomal tube and the nucleus (Fig. 5). The posterior end of the sperm consists of an elongated nucleus, with densely condensed chromatin, surrounded by a halo of less dense chromatin and separated from the cytoplasm by a zone of lower electron density. No nuclear membrane is present (Figs. 3, 5, 6, 9). The nucleus is surrounded by a multivesicular complex, formed by two or three compartments of granules or vesicles of various sizes that surround the nucleus in an asymmetric fashion. In transverse sections, these granules or vesicles are rounded or elongated, whereas in sagittal section, they always appear elongated (Figs. 5, 9). The components of the multivesicular complex are separated from the rest of the cytoplasm by a condensation of the granular material of which they are composed.

## Discussion

Different points of view exist regarding whether the testes of adult forms of the various Gordiida species are internally bounded by discontinuous or continuous epithelium. Valvassori et al. (1999) consider that the testicular epithelium in *Gordius villoti* is continuous in the initial stages of spermatogenesis, and that, when mature gametes fill the testicular cavity, the epithelium becomes discontinuous. Schmidt-Rhaesa (1997) maintains that this epithelium in *Gordius aquaticus* is discontinuous and that the cells rarely contact each other, being completely absent in some individuals. On the other hand, Vejdovsky (1886) in *Gordius preslii*, Montgomery (1903) in *Paragordius varius* and Svabenik (1909) in *Gordius montenegrinus* have reported that the testes are limited by an epithelium along their whole length. Our observations allow us to confirm that, at least in *P. bedriagae*, the testicular epithelium is constituted by a continuous layer of flat cells that interdigitate and that remain present in the zone near the cloacal opening.

Contacts between epithelial cell microvilli and spermatozoa in *P. bedriagae* resemble the associations established between nongerminal or supportive cells and germ cells observed in *Capillaria hepatica* (Wright 1991). Despite the absence of intercellular junctions, these contact sites can be considered sites of exchange between the testicular epithelium and spermatozoa.

Lora Lamia Donin and Cotelli (1977) and Schmidt-Rhaesa (1997) refer to the halo of less dense chromatin surrounding the dense chromatin as perinuclear cisterna. In our opinion, this term is inappropriate and might lead to confusion, since no nuclear membrane is present in mature spermatozoa. Even though many of the characteristics of the spermatozoa in *P. bedriagae* resemble those described for species of *Gordius*, viz., *G. panighettensis* (Lora Lamia Donin and Cotelli 1977), *G. aquaticus* (Schmidt-Rhaesa 1997) and *Neochordodes occidentalis* (Poinar 2001), they differ in presenting a uniform halo of less dense chromatin surrounding the dense nuclear chromatin.

*P. bedriagae* and the three *Gordius* species represent two extremes of gordiid evolution. According to a phylogenetic hypothesis based on 18S rRNA and morphological data

(Bleidorn et al. 2002), the genus *Gordius* represents the basalmost taxon in Gordiida, whereas *Pseudochordodes* belongs to the derived Chordodinae. The finding that a similar type of spermatozoa occurs in both genera makes it likely that this type is present in all other Gordiida and is therefore an autapomorphy of Gordiida.

The spermatozoa of Gordiida show differences and similarities with those of Nematoda. Nematode spermatozoa lack an acrosome, which is present in nematomorphs, but they share the presence of aflagellate spermatozoa and membranous vesicles (Baccetti et al. 1983) and also share the absence of a nuclear envelope, except in Enoplea (Nematoda; Justine and Jamieson 1999). Even though the spermatozoa of *Gastromermis* sp. (Nematoda: Mermithidae) exhibit some resemblance to those of Gordiida (Poinar and Hess-Poinar 1993), they differ in that the nucleus of *Gastromermis* sp. is surrounded by membranous organelles, whereas in Gordiida, it is surrounded by a multivesicular complex. In the Nematoda species *Caenorhabditis elegans* (Wolf et al. 1978) and *Heterakis gallinarum* (Lee 1971), the nucleus presents, as in *P. bedriagae*, a halo of less dense chromatin around the dense nuclear chromatin.

Gordiida spermatozoa thus have an unique combination of features that appears to have no equivalent in the animal kingdom.

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