Testicular morphology and histochemical of *Pseudonannolene tocaiensis*

Fontanetti, 1996 (Diplopoda, Pseudonannolenidae).

[1]Vanessa Cabreira de Freitas, [1]Carmem Silvia Fontanetti

[1]Department of Biology, Institute of Biociences, Paulista State University (UNESP),

Rio Claro, SP, Brazil.

ABSTRACT

The testicle of *Pseudonannolene tocaiensis* Fontanetti, 1996, consists of a large deferent duct composed by a simple secretory epithelium, to which numerous testicular vesicles are attached by a short pedicle. The testicular vesicles are formed by an epithelial wall; the cells of this wall give rise to the gametocytes that gradually fill the vesicles' lumen. There is a synchronism in the development of the cells inside each testicular vesicle, that is, only one developmental stage (either spermatozoa, spermatids, spermatocytes, or spermatogonies) is found occupying a single vesicle. The mature testicular vesicles, those that carry mature spermatozoa, present a very peculiar constitution: a) a peripheric portion, b) a central portion with typical secretory characteristics, and c) between these two portions, there is an intermediary portion where the spermatozoa can be found. Histochemical data are the first time reported in the testicles of the group. The results suggest that the testicular vesicles and the deferent duct are responsible for the production of the spermatic liquid in species of *Pseudonannolene*.

Key Words: Diplopoda, histochemistry, morphology, testicular vesicle.

Correspondence to: Dr. Carmem S. Fontanetti
Departamento de Biologia, Instituto de Biociências,
Universidade Estadual Paulista (UNESP)
Av. 24A, n°1515, CP 199, CEP 13506-900,
Rio Claro, SP, Brasil.
Tel: (55) 193526-4139, Fax: (55) 193534-0009
e-mail: fontanet@rc.unesp.br

INTRODUCTION

Studies concerning the reproductive system of millipedes are scarce and most of the existing ones date of old. The first studies on this subject date from the 19th century (Treviranus, 1817 *apud* [2, 12]) and present superficial results regarding the histology and cytology of the reproductive system.

A few other studies regarding the male reproductive system of polydesmoid millipedes were published by Miley [10, 11], Seifert [15], and Warren [16]. While studying five species of diplopods, Warren [16] described three types of male reproductive system: the reticular, the scalariform, and the arthrospheroid. The reticular type presents the deferent ducts in the form of a reticule. The arthrospheroid type presents only a few transversal connections at the anterior portion, thus causing the two ducts to be parallel and independent at the region were the testicular vesicles attach. The scalariform type presents connections between the ducts throughout the testis.

Supported by the observations made by Warren [16], Kanaka & Chowdaiah [7] suggested that during the course of evolution of the reproductive system of diplopods, the most primitive and complex is the reticular type, from which derived the scalariform, and from this derived the simplest and most advanced type, the arthrospheroid. A fourth morphological type was observed by Fontanetti [3, 5] in species of *Pseudonannolene* and *Rhinocricus*, in which there is a single deferent duct and, therefore, is considered the most derived type of male reproductive system.

West [17] studied the internal anatomy of the reproductive apparatus of *Scytonotus virginicus* (Polydesmidae) and observed a synchronism in the cellular development inside a single testicular vesicle, meaning that only one developmental stage (either spermatozoa, spermatids, spermatocytes, or spermatogonies) is found within any single vesicle. Cecchi & Chelazzi [1], studying *Epibolus pulchripes bravensis*, and Fontanetti [3], in a study of three Brazilian species, agreed with the results proposed by West in relation to the testicular cycle.

Fontanetti [4] observed a very peculiar morphology in the mature testicular vesicles of *Pseudonannolene tricolor* (Spirotreptida), in which a drastic morphological change occurs in order to accommodate the spermatozoa.

The present work presents the morphology, histology, and histochemistry of the testicular vesicle of adult males of *Pseudonannolene tocaiensis* Fontanetti, 1996.

MATERIAL AND METHODS

Adult males (n= 10) of *Pseudonannolene tocaiensis* Fontanetti, 1996, were collected in the cave Caverna da Toca, Itirapina, São Paulo State, Brazil, during the months of February and August of 2003 by Freitas *et col*.

The testes were dissected in physiological solution, fixed in 4% paraformaldehyde, and processed according to routine techniques for inclusion in historesin. The material was sectioned in a Sorvall JB-4 BIO-RAD microtome. The sections were hydrated, arranged on

glass slides, and stained with hematoxiline and eosin following routine histological techniques.

Histochemical tests for the detection of proteins (Bromophenol Blue [13] and Xylidine Ponceau [9]), lipids (Nile Blue [8] and Sudan Black [6]), polysaccharides (PAS [6]), and calcium (Von Kossa Method [6]) were applied.

RESULTS

Morphology

The testis of *P. tocaiensis* (Figure 1) consists of a large deferent duct to which numerous testicular vesicles are attached by a short pedicle. The deferent duct is constituted by a columnar simple epithelium (Figure 2, 4). The nucleus positioned at the basal region of the cell. Various secretory vesicles of the apocrine type were observed being liberated into the lumen (arrows in Figure 4). The pedicle presents numerous cells undergoing division (Figure 3).

The testicular vesicles are formed by an epithelial wall, enveloped by an evident peritoneal sheath. Apparently, the gametocytes originate from the cells of the epithelial wall and they gradually fill the lumen of the testicular vesicles (Figure 5). The gametocytes appear much larger than the epithelial cells that originate them (Figure 6).

A synchronism in the development of the cells inside a single testicular vesicle was observed, in other words, there was only a single cellular type of the germ line within any given vesicle; the testicular vesicles present only spermatozoa, spermatids, or spermatocytes (Figures 6 and 7).

The mature testicular vesicles that possess mature spermatozoa, or completely differentiated spermatozoa, present a very peculiar constitution. These vesicles were composed by: a) a peripheric portion; b) a central portion with typical secretory characteristics; and c) an intermediary portion between the peripheric and the central portions filled with spermatozoa. This intermediary portion presents the characteristics of a lumen in which the spermatozoa appear completely differentiated (Figure 7).

Histochemistry

The tests for the detection of proteins (Bromophenol Blue and Xylidine Ponceau) evidenced a strong reaction in the epithelium of the deferent duct (Figure 8) and in the mature testicular vesicles (Figure 9). The secretion liberated through the epithelial cells of the duct reacted strongly positive to the tests (arrows in Figure 8 and detail).

Part of the secretion at the central portion of the testicular vesicles that contain spermatozoa was consisted of proteins (Figure 9).

Neutral polysaccharides were observed in small amounts at the apical region of the epithelial cells of the deferent duct (Figure 10). In the mature testicular vesicles we observed that the central portion presents a considerable amount of neutral polysaccharides (Figure 11).

The peritoneal sheath that envelops the duct and testicular vesicles reacted strongly positive to the tests for proteins and polysaccharides (Figures 8, 9, and 10).

A low amount of lipids was detected in the testicular vesicles and in the deferent duct. A few droplets of this element were observed in the peritoneal membrane of both structures.

The total absence of calcium and acid polysaccharides was evidenced in the testicular vesicles and in the deferent duct of *P. tocaiensis*.

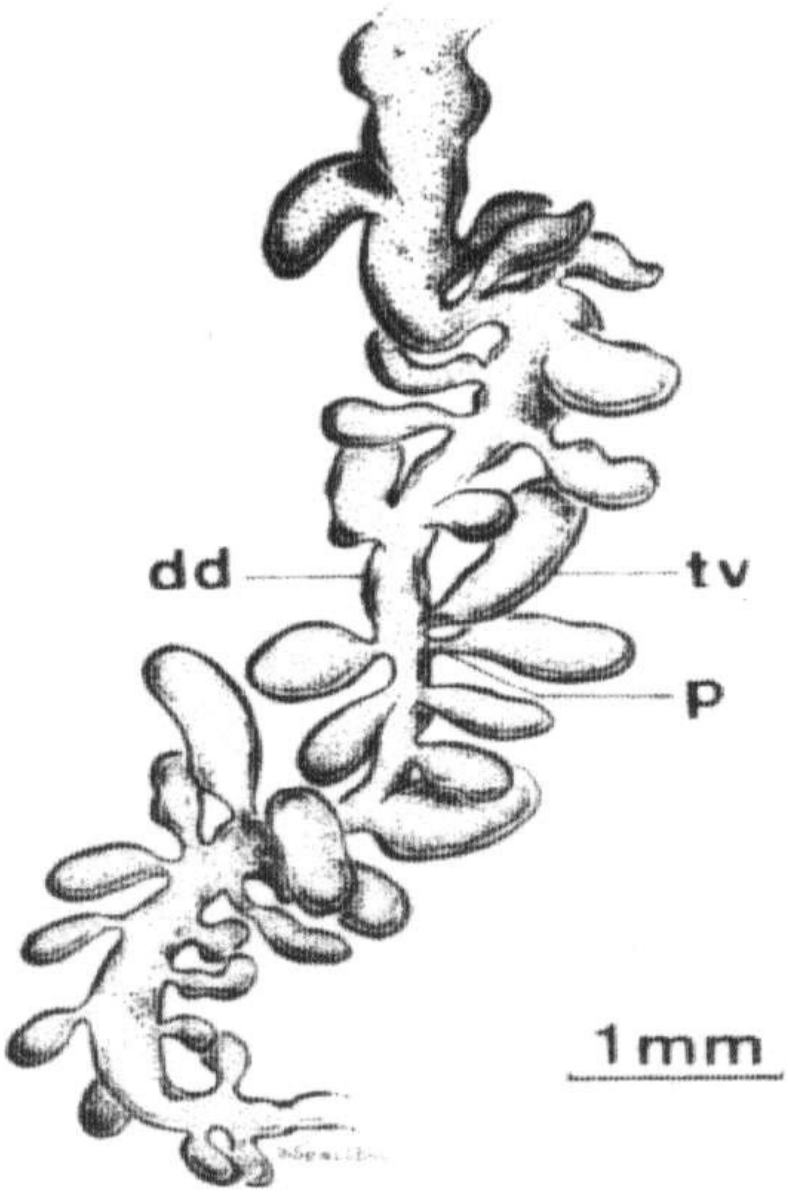

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Figure 1: Diagram of the testicle of *P. tocaiensis.*
dd = deferent duct; p = pedicle; tv =testicular vesicle.

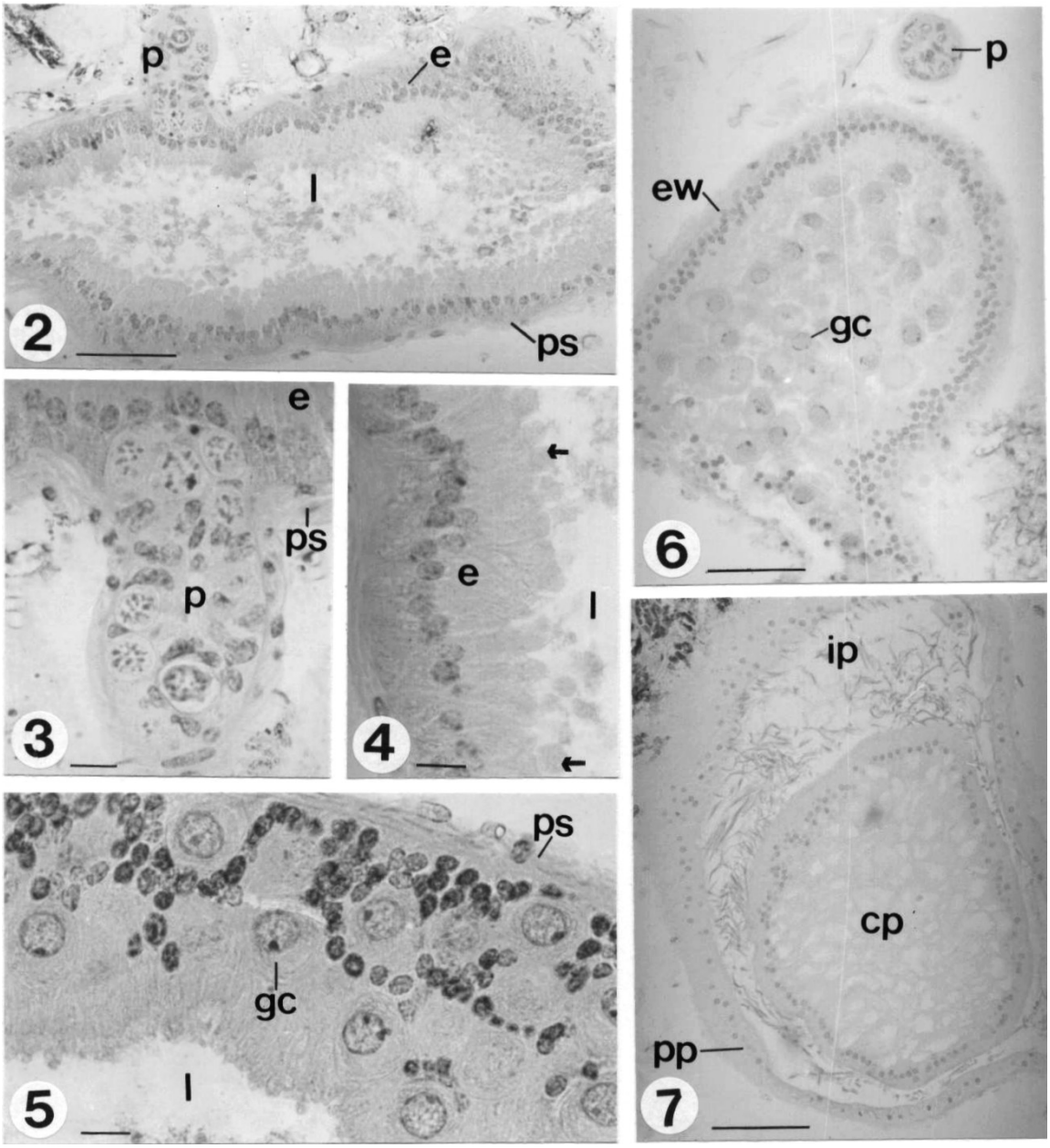

Figure 2-7: Histological sections of the testicle of *P. tocaiensis* Hematoxylin-Eosin staining – deferent duct (figure 2), pedicle (figure 3), epithelium of deferent duct (figure 4), epithelial wall of testicular vesicle (figure 5) and testicular vesicles (figures 6 and 7).
cp = central portion; e =epithelium; ew = epithelial wall; gc = germinative cells; ip = intermediary portion; l = lumen; p = pedicle; pp = peripherical portion; ps= peritoneal shealth; arrows = secretory vesicles.

Bars in 2, 6 and 7 = 50μm

Bars in 3-5 = 10μm

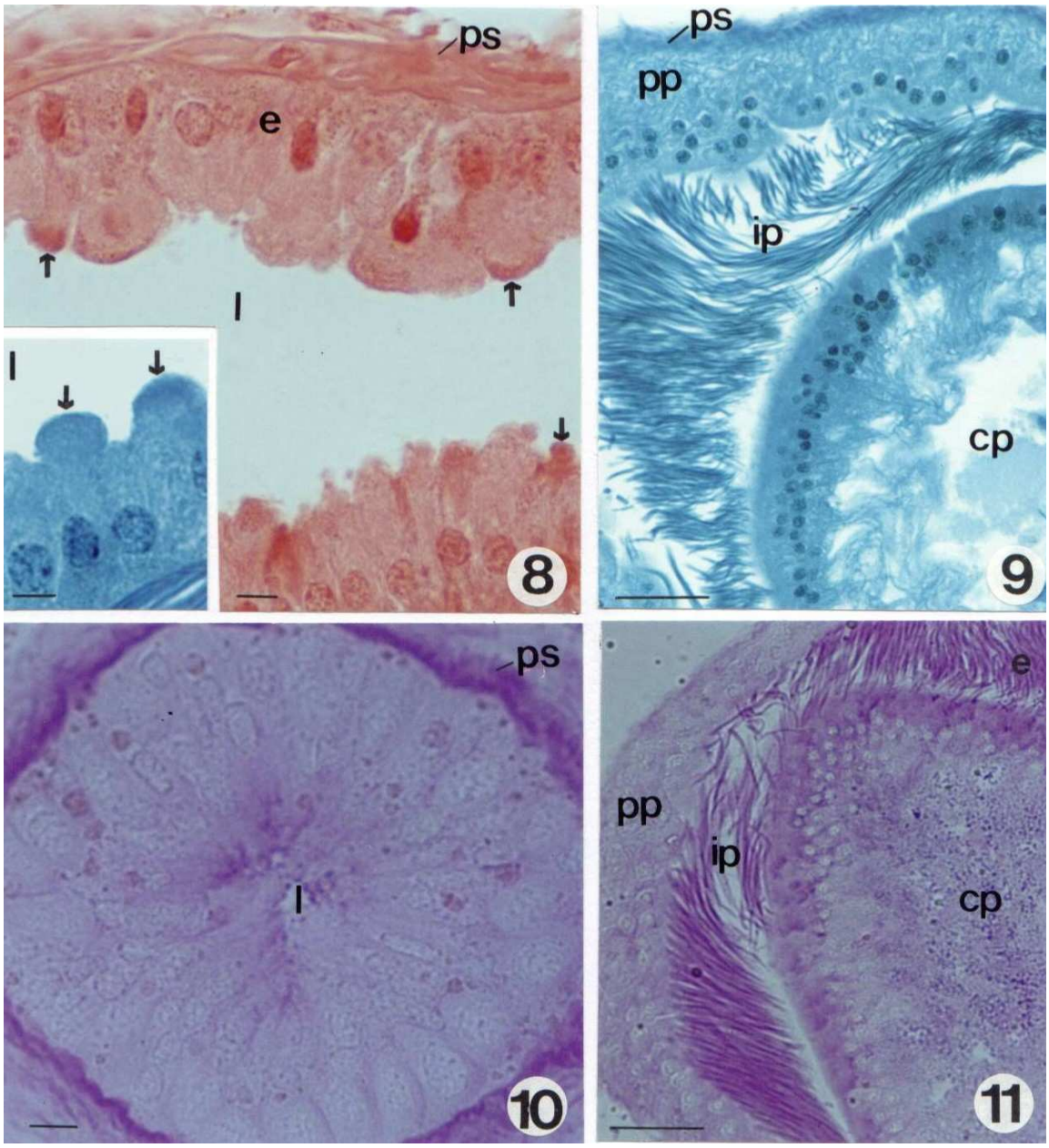

Figure 8-11: Histological sections of the testis of *P. tocaiensis* subjected to the tests of Xylidine Ponceau (8), Bromophenol Blue (9 and detail in 8), and PAS (10 and 11) – deferent duct (Figures 8 and 10) and mature testicular vesicles (Figures 9 and 11).
cp = central portion; e =epithelium; ip = intermediary portion; l = lumen; pp = peripherical portion; ps = peritoneal sheath; arrows = secretory vesicles.
Bars in 8 and 10 = 50µm
Bars in 9 and 11 = 10µm

DISCUSSION

The testicular morphology observed in Brazilian species of the genus *Pseudonannolene*, including the species studied in the present work, is characterized by the elimination of the connection tubules among the deferent ducts and by the complete union of the ducts, which were originally paired, into a single deferent duct. This morphological type has been considered the most simplified system and, therefore, the most derived among millipedes. This morphological type is also observed in the genus *Rhinocricus* [5].

The mature testicular vesicles, those that possess completely differentiated spermatozoa, present the same morphology observed by Fontanetti (1998) in *Pseudonannolene tricolor*. The process of the testicular vesicles maturation is the same too.

Other authors have also observed the synchronism in the development of the germ cells inside the testicular vesicles in different species [1, 3, 14, 17]. The origin of the germinative cells in the group is obscure; our data demonstrated one origin from cells of the epithelial wall but the exactly determination these cells is still obscure.

The difference in the degree of cell maturity observed among the testicular vesicles is related to the fact of the cells starting the meiotic cycle separately; most of the posterior vesicles are formed sometime after the meiotic activity is initiated at the anterior region [17]. In *Polydesmus angustus* (Polydesmida), the gametes are subjected to a maturation gradient that proceeds in an anteroposterior direction [14].

Histochemical data of the testes of millipedes have not been published previously.

The testicular vesicles and the deferent duct must supply the lack of accessory glands for the production of the sperm liquid in the species of *Pseudonannolene*; the histochemical observations evidenced a glycoproteic constitution of the compounds liberated by these structures.

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