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Morphological and functional study of the male reproductive tract in the shrimp *pleoticus muelleri* bate (decapoda, penaeoidea)

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Summary

Anatomical relationships and the histological structure of the male reproductive tract in *Pleoticus muelleri* were described by using light microscopy. The male reproductive tract consists of paired organs: testes and vasa deferentia with the terminal ampoule, which opens at the gonopores on the coxae of the fifth pair of pereopods. Testes are located dorsally in the cephalothorax and are comprised of two lobes surrounded by laminar connective tissue. They are composed of cystic structures (with spermatogonia or primary spermatocytes) covered by connective tissue. Each vas deferens consists of four morphologically different zones: the collecting tubule, proximal, medial, and distal (terminal ampoule). The collecting tubule connects the testes with the proximal zone and is lined by a shallow epithelium. The proximal zone, lined by a columnar secreting epithelium, is internally divided into two ducts by a septum. The biggest spermatophoric duct conveys the sperm mass, and the other secretes the acellular material that supports the sperm mass in the spermatophore. In the medial zone the ducts are incompletely separated by a partial septum and the epithelial cells secrete the hyaline layer of the spermatophoric matrix. The distal zone or terminal ampoule comprises four interconnecting chambers and is externally surrounded by connective tissue and a conspicuous layer of striated muscle. The spermatophore reaches complete maturation in the terminal ampoule.

Key words: Reproduction, male, histology, Crustacea, Decapoda

Introduction

The study of reproductive biology in penaeoid shrimps has received much attention due to their commercial importance. Knowledge of the reproductive tract morphology is one of the most important aspects. Calman (1909), Heldt (1938) and King (1948) carried out the first investigations.

The male reproductive tract of decapod crustaceans comprises paired gonads, the testes, located dorsally in the cephalothorax lateral to the gut. The testes are accompanied by the vasa deferentia, which are divided into several zones: the collecting tubule, proximal, medial and distal (terminal ampoule).

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The histological structure of the male reproductive tract was described in some species of decapod crustaceans, such as *Melicertus kerathurus* (Malek and Bawab, 1974a, 1974b), *Marsupenaeus japonicus* (Bizot-Espiard, 1980), *Farfantepenaeus notialis* and *Litopenaeus schmitti* (Guitart et al., 1985) and *Macrobrachium borellii* (Verdi and Delgado, 1998). Spermatophore morphology has been studied with great interest due to its phyletic importance and also because of the development of the technology for the artificial insemination (Subramoniam, 1993).

Pleoticus muelleri is a species of opened thelycum whose distribution area in the littoral of the Atlantic Ocean extends from 20°S, Brazil, to 50°S, Argentina (Boschi, 1986). This shrimp has aroused great interest on account of its potential use in culture in temperate areas. It reaches more than 20 g weight in less than 5 months (Fenucci et al., 1990).

In this work we identify the anatomical relationships and study the histological structure of the male reproductive tract in *P. muelleri*.

Materials and Methods

Twenty adult males of *P. muelleri* from 5 g to 19 g were caught off the coast of Mar del Plata (38° 05′S 57°W, Argentina). In some individuals complete somites were fixed, and in the rest the male reproductive tract, at different stages of maturation, was dissected. The stage of maturation was determined on the basis of the presence of (1) transparent coxae (without spermatophore), (2) white coxae (with a small white or light green spermatophore), and (3) green coxae (with a completely formed olive green spermatophore). In all cases tissues were fixed in Davidson fluid (Bell and Lightner, 1988) for 24 h, dehydrated in a progressive series of ethanols and embedded in butyl-paraffin and paraffin. Sections of 5 µm were stained with hematoxylin-eosin and Mallory's triple stain.

Results

The testes are paired lobe-like organs located in the cephalothorax ventrally to the heart and dorsally to the hepatopancreas and midgut (Fig. 1). A continuous layer of laminar connective tissue covers them. The histological study shows that they are composed of cystic structures (seminiferous tubules) surrounded by connective tissue and haemolymphatic sinuses. The seminiferous tubule is a solid cord of cells in early developing testes (Fig. 2). By late stage spermatogenesis, the tubule develops a lumen, filled with spermatids (Fig. 3). The germinal zone is a permanent structure with a peripheral and lateral location which contains immature germinal elements (spermatogonia). Nurse cells that nourish the spermatic cells and are involved in the formation of the seminiferous tubule lumen during the late stages of spermatogenesis cover each seminiferous tubule. The spermatic cells present a synchronous development in each cyst, with coexisting cysts being at different stages of spermatogenesis.

The vas deferens comprises four morphologically distinct zones: the collecting tubule, proximal, medial and distal (terminal ampoule). The collecting tubule connects the testis with the proximal zone of the vas deferens. This portion is narrow and lined by a shallow epithelium surrounded by a myoepithelial cells layer and connective tissue. The epithelial cells present a basal elongated nucleus that comprises nearly the entire cellular volume (Fig. 4).

The proximal zone consists of two independent lumens separated by a longitudinal septum. The larger, primary one contains the sperm mass with acellular material, and the other (secondary) contains acellular material deposited by the epithelial cells (Fig. 5). The epithelial cells in this section are active in secretion and are underlain by myoepithelial cells and loose connective tissue. The epithelium lining the primary lumen is columnar and cells possess large basophilic nuclei and a brush border. Low epithelial cells that have large basal nuclei and a brush border line the secondary lumen. A double layer of epithelial cells separated by connective tissue forms the septum.

In the medial zone of the vas deferens the lumens are partially separated; the two channels merge and allow mixing of their contents (Fig. 6). A high columnar secreting epithelium with elongated nuclei and a brush border lines this zone. A double layer of epithelial cells forms the septum. Muscle fibers and connective tissue underlie the epithelium. The mature spermatozoa (Fig. 7) are mixed with two morphologically different acellular materials, one of which stains red and the other blue when Mallory's stain is used.

The distal zone or terminal ampoule contains four interconnecting chambers (Fig. 8). This zone has an infolded glandular epithelium whose cells have elongated nuclei that occupy most of the cytoplasm and a brush border. The terminal ampoule contains a continuous layer of connective tissue and longitudinal and circular muscle fibers subjacent to the epithelium.

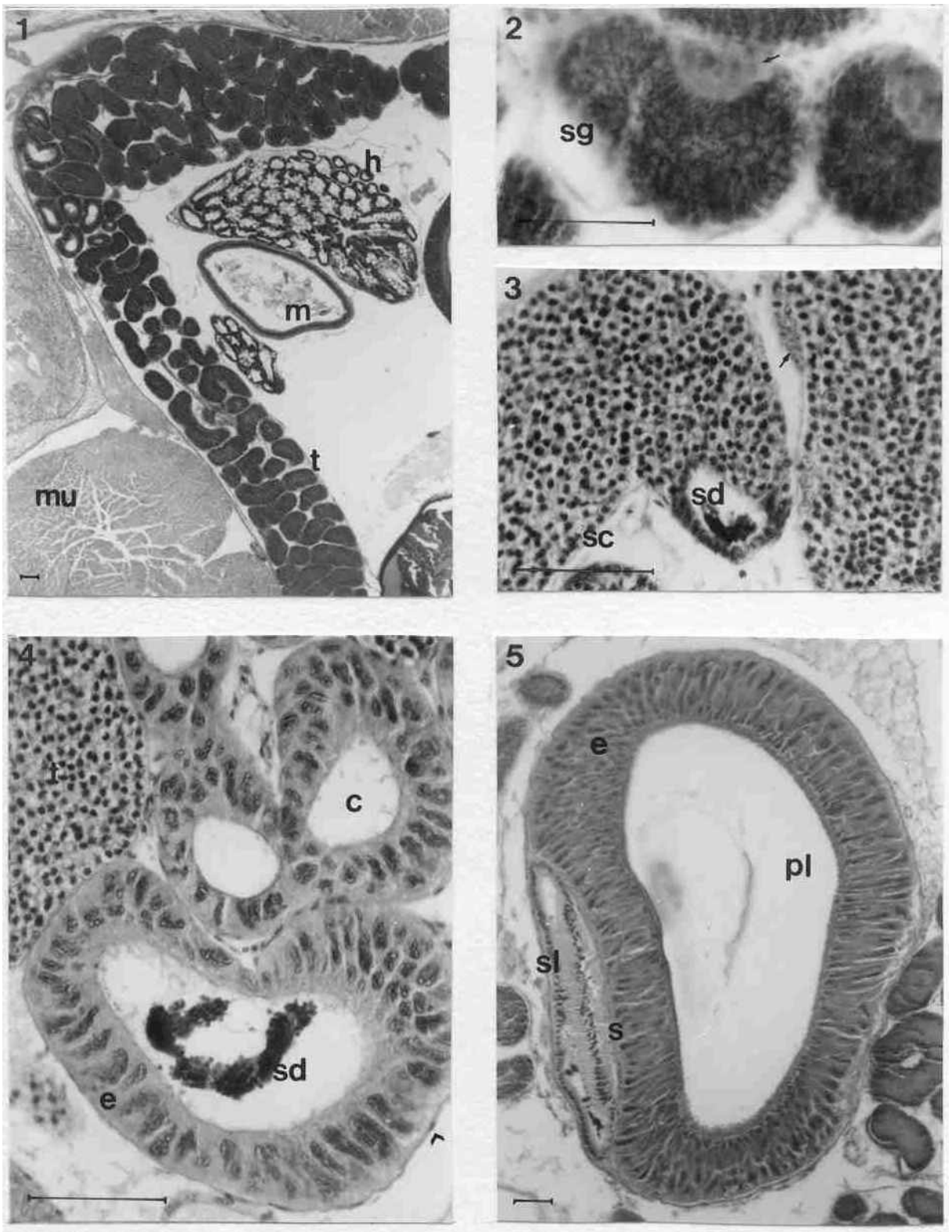

Fig. 1. Transversal section of thoracic somite. h: hepatopancreas; m: midgut; mu: muscle; t: testis. Fig. 2. The testis showing spermatogonia (sg) and germinal zone (arrow). Fig. 3. The seminiferous tubule contains spermatocytes (sc) and in the lumen spermatids (sd) are present. Arrow: germinal zone. Fig. 4. Transversal section showing testis (t) and collecting tubule (c) filled with spermatids (sd) and surrounding by myoepithelial cells (arrowhead). e: elongated epithelial cells. Fig. 5. Vas deferens. Proximal zone. e, epithelial cells; pl, primary lumen; s, septum; sl, secondary lumen.

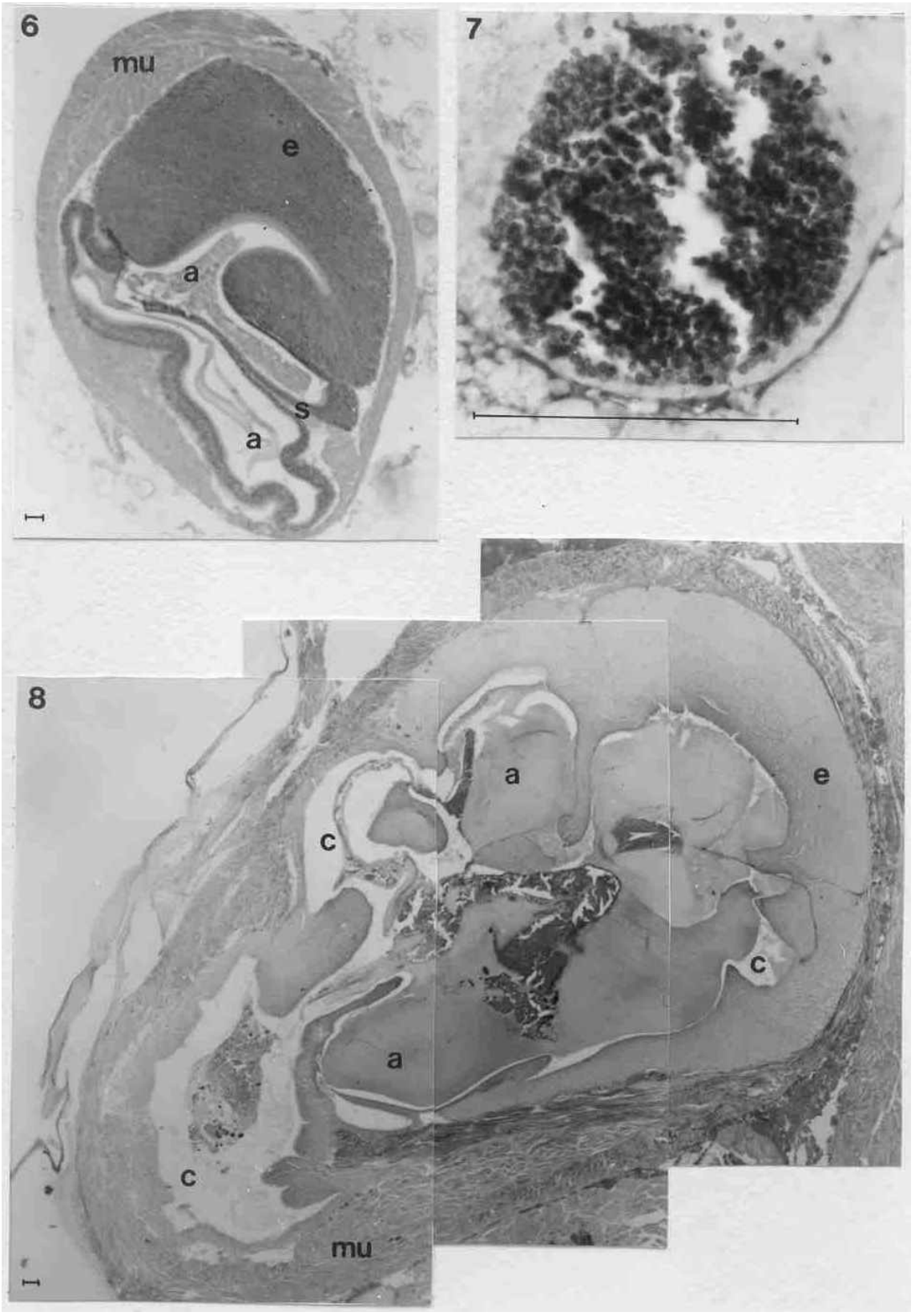

Fig. 6. Vas deferens. Medial zone. a, acellular materials; e, epithelial cells; mu, muscle; s, septum. Fig. 7. Mature sperm from the terminal ampoule. Fig. 8. Terminal ampoule with spermatophore in early developmental stage; Mallory's stain. a, acellular materials; c, chambers; e, epithelial cells; mu, muscle.

Discussion

The male reproductive organs of decapod crustaceans are located in the cephalothorax. The testes in *P. muelleri* are situated dorsal to the hepatopancreas and midgut and ventrally to the heart. This location is similar to that described for other decapod species (Guitart et al., 1985; Verdi and Delgado, 1998).

The external morphology of the testes varies among the different groups of decapods. In penaeoids, testes are comprised of several lobes each independent of the other which are connected to the vas deferens through a short and narrow connective tubule (Ro et al., 1990). In *F. notialis* there are seven pairs of lobes; in *L. schmitti* eight pairs (Guitart et al., 1985). In contrast to other species, in the shrimp *P. muelleri* the testes are formed by seminiferous tubules surrounded by a continuous layer of connective tissue. The testes do not posses a muscle layer in this species; this was also reported for other species of decapods (King, 1948; Bell and Lightner, 1988; Verdi and Delgado, 1998).

Spermatogenesis is the development of germinal cells from spermatogonias to spermatozoa. This process can be synchronous or not so in decapods, depending on the species. *Procambarus clarkii*, *Geryon maritae*, *Callinectes sapidus*, *Panulirus argus* and most other penaeid shrimps show synchronous development, which is evidenced by the presence of one or two stages of development in a segment of the seminiferous tubule (Lu et al., 1973; Johnson, 1980; Melville-Smith, 1987). This pattern concurs with that observed in *P. muelleri* where there are cysts with primary spermatocytes and others with secondary spermatocytes.

In penaeoid decapods the vas deferens is a tubular duct, sinuous in some segments, which conveys the spermatozoa from the testes to the gonopore. The wall of this duct is formed by a glandular epithelium surrounded by connective tissue; in some species there is a muscle layer between the epithelium and the connective tissue (Adiyodi and Anilkumar, 1988). The vas deferens comprises from five to ten distinct zones depending on the species (McLaughlin, 1983). King (1948) distinguished four zones in *Litopenaeus setiferus*, which were named segments I, II, III and IV; the last segment was also called the terminal ampoule. Malek and Bawab (1974a) divided the vas deferens of *M. kerathurus* into three functionally different zones: the medial zone (ascending and descending), the distal zone and the terminal ampoule. In *Aristaeomorpha foliacea* and *Aristeus foliacea*, the vas deferens is differentiated into three zones: an initial, a medial and a distal (ampoule) (Tunesi, 1987; Orsi Relini and Tunesi, 1987).

The vas deferens epithelium has a variety of functions such as gamete transport, maintenance of an appropriate environment and the production of the protecting coat of the spermatophore. Ro et al. (1990) reported that epithelial cells in *L. setiferus* have numerous mitochondria and the organelles associated with protein synthesis and secretion of the material that surrounds the sperm. The contraction of the muscle layer contributes to sperm transport through the tract.

In the proximal vas deferens the epithelium secretes the matrix responsible for packing and lining up the sperm. Besides the secretory function, this segment stores the developing spermatophore up to the moment at which it is conducted to the terminal ampoule to complete maturation. In *P. muelleri*, as in other penaeoid shrimps (Malek and Bawab, 1974a; Guitart et al., 1985; Ro et al., 1990; Krol et al., 1992), this segment consists in a dorsal primary lumen and a ventral secondary one separated by a longitudinal septum. Large cells with big nuclei and fibrous structures in the cytoplasm compose the epithelium and secrete the material forming the primary spermatophore layer.

The medial zone of the vas deferens conveys the partially formed spermatophore to the terminal ampoule where its formation is completed. Spermatophore structure in decapods is highly variable, and it is not surprising to find great variability in the anatomy of the medial zone where acellular components of the spermatophore are deposited. In *P. muelleri* this section is formed by two partially separated compartments in which the sperm mass mixes with the acellular secretions, and the glandular epithelium has elongated nuclei and a brush border in the apical zone. These characteristics correspond to those reported for other species such as *L. setiferus* (Ro et al., 1990) and *Litopenaeus stylirostris* (Krol et al., 1992). However, in *L. stylirostris* there is a longitudinal fold in the secondary lumen that increases the area.

In penaeid shrimps, the distal zone or terminal ampoule is the site for the complete maturation of the spermatophore, and deposition and organisation of the spermatophore components also occur here. Its structure in *P. muelleri* is similar to that described for other penaeoid species (Guitart et al., 1985; Krol et al., 1992; Subramoniam, 1993), and comprises four interconnecting chambers lined by an infolded glandular epithelium. The epithelial cells have a brush border and secrete mucus and adhesive substances that facilitate spermatophore insertion into the female.

There is a general agreement that the reproductive quality of males plays an important role in the productivity of broodstock of captive penaeoids. The present study provides the basis at the anatomical and light microscope levels of the normal structure of the male reproductive tract.

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