Structure of the Adult Male Reproductive System in a Pycnogonid, *Cilunculus armatus* (Pycnogonida: Ammotheidae)

KATSUMI MIYAZAKI

Seto Marine Biological Laboratory, Kyoto University,
Shirahama, Wakayama 649-22, Japan

Abstract Structure of the male reproductive system in a pycnogonid, *Cilunculus armatus* is examined under light and scanning electron microscopes. The system consists of a U-shaped trunk testis with four pairs of diverticula (pedal testes) extending into the respective walking legs up to the second coxae and two pairs of spermiducts with respective male genital pores opening in the second coxae of the last two pairs of the legs. The testis and spermiducts are clearly distinguished for the first time in the pycnogonids based on their histological features. Male germ cells in various developmental stages, from the spermatogonia to the spermatozoa, are distributed throughout the trunk and pedal testes. Such equivalency of the testicular regions in spermatogenesis is also described for the first time in the pycnogonid testes.

Introduction

Male and female reproductive systems in some pycnogonids are known to have some unique characteristics among arthropods, such as the pedal gonads extending into the walking legs and the multiple genital pores opening on the second segments (the second coxae) of all or some pairs of the legs (King, 1973; Arnaud & Bamber, 1987). Recently, Miyazaki & Makioka (1990, 1991, 1992a, 1992b, 1993) described some histological details of the female reproductive system in some pycnogonids, and deepened the understanding of their structure-function relationships. As to the pycnogonid male reproductive system, there have been some early anatomical descriptions (Dohrn, 1881; Hoek, 1881; Helfer & Schlottke, 1935; Sanchez, 1959) and some recent ultrastructural studies on the spermatozoa (Van Deurs, 1973, 1974a, 1974b) and spermiogenesis (Van Deurs, 1974b; El-Hawawi & King, 1978, 1983; King & El-Hawawi, 1978), but no detailed histological descriptions.

In the present study, the author offers some detailed histological observations on the adult male reproductive system in a pycnogonid, *Cilunculus armatus*.

Materials and Methods

Specimens of *Cilunculus armatus* (Böhm, 1879) were collected by dredge from the sandy bottom at about 35-45 m in depth, off the coast of Shimoda, Izu Peninsula, Central Japan. After anesthetization with *l*-menthol or gradually added ethanol, adult males were fixed with the seawater-Bouin's solution (picric acid saturated in seawater: formalin: acetic acid = 25 : 5 : 1). In the fixative, walking legs of each specimen were cut off from the trunk with a razor blade. The walking legs and the trunk of each specimen were dehydrated with a graded ethanol-*n*-butanol series and embedded in paraffin. Serial sections of 5-7 μm thick were stained with Delafield's or Mayer's hematoxylin and eosin Y (H-E), or Heidenhain's azan.

For scanning electron microscopy (SEM), adult males stored in 70% ethanol were dehydrated with a graded ethanol series, transferred to isoamyl acetate, dried with a critical point drier, coated with gold or gold-palladium, and observed with scanning electron microscopes, Hitachi S-570 and JEOL JSM-T220 at 10-20 kV.

Results

Male reproductive system in *Cilunculus armatus* consists of a testis, spermiducts and male genital pores, all of which are localized in the cephalothoracic trunk and walking legs (Fig. 1).

Testis

The testis is U-shaped in the trunk region (trunk testis). Four pairs of testicular diverticula (pedal testes) extend out of the trunk testis into the respective walking legs up to the second coxae (Fig. 1). The trunk and pedal testes lie on the dorsal side of the gut, sandwiched by the dorsal and the ventral layer of the double-layered horizontal septum (Dohrn's septum) (Fig. 2). The trunk and pedal testes are tubular, communicating with each other. The testicular wall consists of a thin epithelial layer (Fig. 3).

Trunk testis

The testicular lumen is filled with male germ cells in various developmental stages from the spermatogonia to the spermatozoa (Fig. 3). The male germ cells in more advanced developmental stage are present more dorsally in the testicular lumen, and the spermatozoa are always in the most dorsal region (Fig. 3). Some mitotic figures are seen among the spermatogonia (Fig. 3). The mitotic figures are irregularly distributed throughout the testis.

Pedal testes

The pedal testes in the first and the second pair of the walking legs are blind, but those in the third and the fourth pairs of the walking legs are connected with the respective spermiducts at their distal ends (Fig. 1). The testicular lumens of the pedal testes are narrower than that of the trunk testis (Figs. 2, 4). The pedal testes are always closely

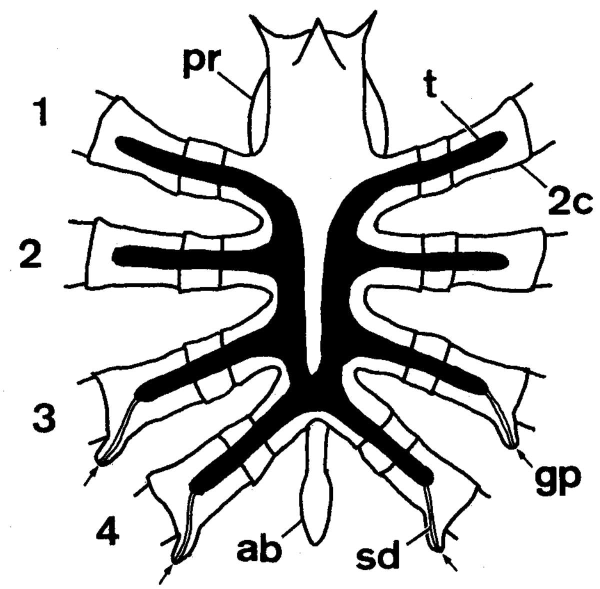
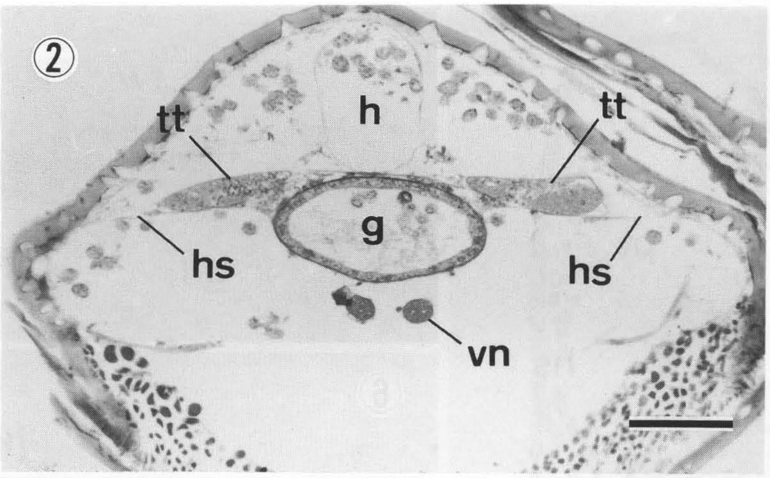


Fig. 1. Diagrammatic representation of adult male reproductive system in *Cilunculus armatus* (dorsal view). ab, abdomen; gp (arrows), genital pore; pr, proboscis; sd, spermiduct; t, testis; 1–4, first–fourth walking legs; 2c, second coxa.



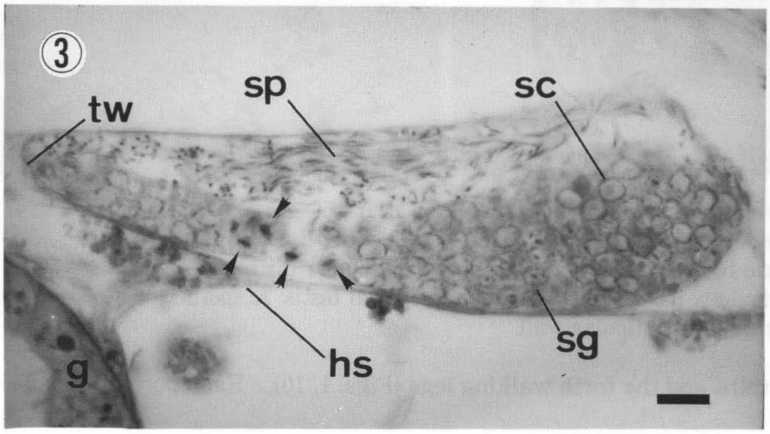


Fig. 2. Cross section of cephalothoracic trunk. H-E. Bar=100 μm.
Fig. 3. Cross section of trunk testis. Arrowheads showing mitotic figures. H-E. Bar=10 μm. g, gut; h, heart; hs, horizontal septum; sc, spermatocyte; sg, spermatogonium; sp, spermatozoon; tt, trunk testis; tw, testicular wall; vn, ventral nerve cord.

contacted with the pedal gut-diverticula (Fig. 4). The male germ cells of various developmental stages are present in the same manner as those in the trunk testis. Spermatozoa (Fig. 5) and spermatogonial mitotic figures (Fig. 6) are found even near the distal ends of pedal testes.

Spermiduct

In the second coxae of the third and the fourth walking legs, the pedal testes are connected with respective spermiducts at their distal ends (Fig. 1). The structure of the wall of the duct is similar to that of the testis, but the lumen is much narrower and includes no developing germ cells (Figs. 7-9). The spermiduct leaves the pedal testis (Fig. 7), passes downward across the pedal gut (Fig. 8), and reaches the male genital pore (Fig. 9). The inner surface of the spermiduct is cuticulated near the genital pore (Fig. 9).

Male genital pore

The male genital pores are situated at the tip of the spurs in the ventral side of the second

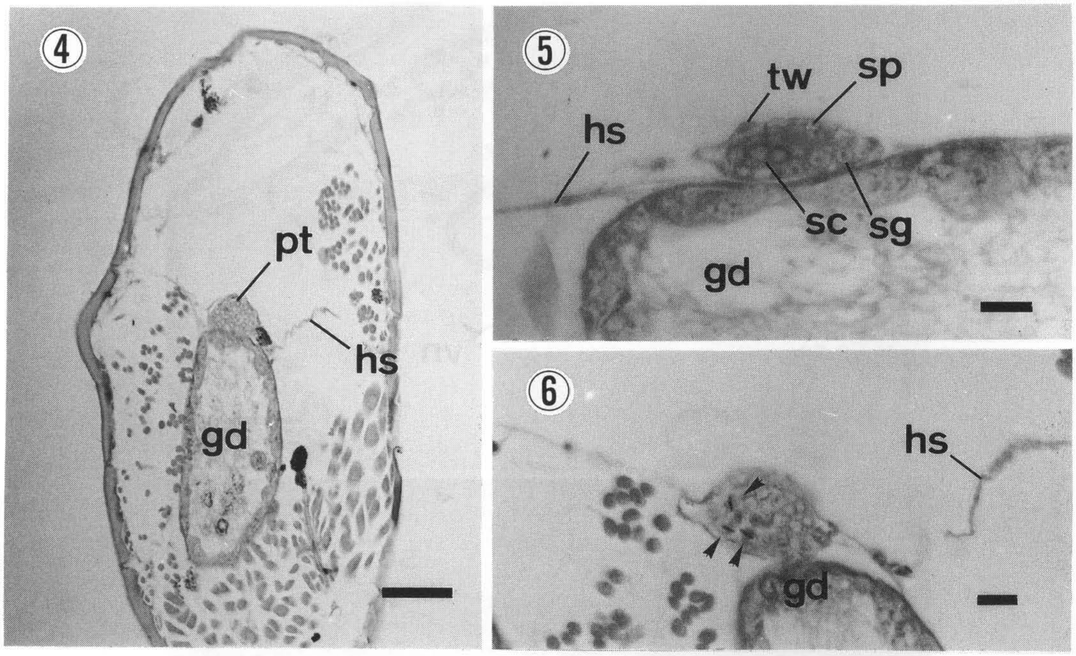


Fig. 4. Cross section of first walking leg. H-E. Bar=50 μm.
Fig. 5. Cross section of pedal testis near distal end. H-E. Bar=10 μm.
Fig. 6. Mitotic figures (arrowheads) in pedal testis near distal end. H-E. Bar=10 μm.
gd, gut-diverticulum; hs, horizontal septum; pt, pedal testis; sc,spermatocyte; sg, spermatogonium; sp, spermatozoon; tw, testicular wall.

coxae of the third and the forth walking legs (Figs. 1, 10). Each pore opens as a crescent slit (Fig. 11).

Discussion

In most of the pycnogonids so far studied, the trunk testes are U-shaped (Helfer & Schlottke, 1935; King, 1973; Arnaud & Bamber, 1987) just like that in *Cilunculus armatus*. Only exception has been reported in *Pycnogonum litorale*, whose trunk testis is a continuous sheet (King & El-Hawawi, 1978).

Some variations have been reported in the position and the length of pedal testes (Helfer & Schlottke, 1935): Some species have the pedal testes in all the walking legs like *Cilunculus armatus*, while the others have them only in the legs bearing genital pores. Most species have the pedal testes extending up to the second pedal segments as in *C. armatus*, but some have longer ones up to the third, fourth, or even to the sixth segments. These variations seem to have no phylogenetic significance, as they show inter- and intrafamily irregularities.

On the connection between the testis and the male genital pore, there has been some confusion among the previous studies: Hoek (1881) described a duct connecting the pedal testis with the male genital pore in *Colossendeis proboscidea*, but Sanchez (1959) denied the existence of such a duct. Other authors did not mention such a duct, as if they considered that the testes were directly connected with the genital pores. In the present study, the author has found spermiducts connecting the pedal testes with the male genital pores. The spermiduct is a passage of the spermatozoa and does not produce male germ cells in its lumen. The cuticular lining of the distal part of the spermiduct indicates that the part

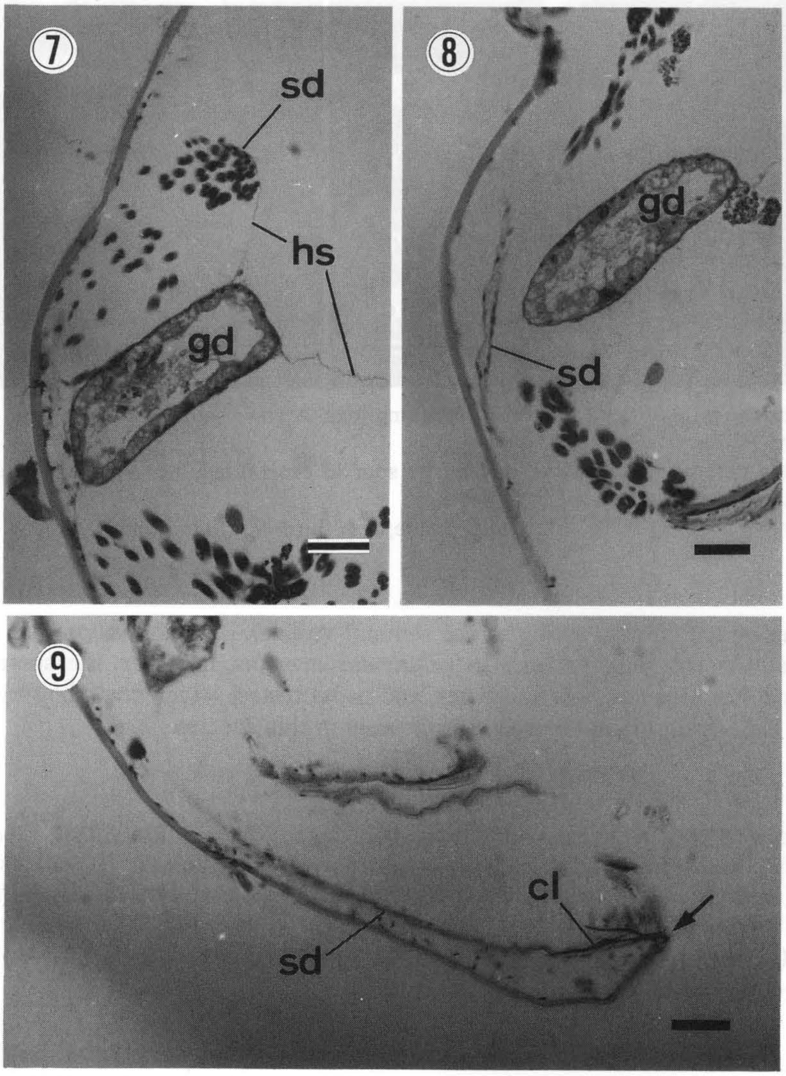
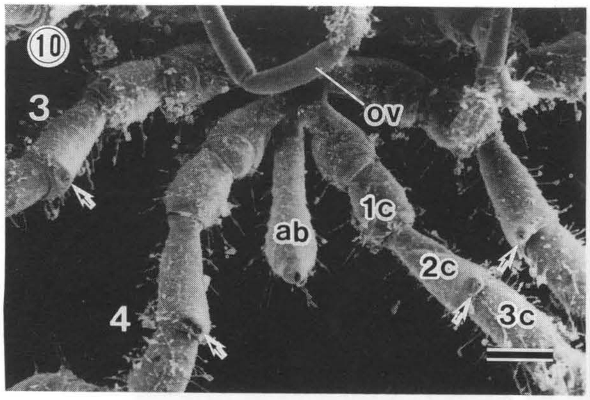


Fig. 7. Spermiduct near connecting point with pedal testis of fourth walking leg. H-E. Bar=25 μm.
Fig. 8. Spermiduct at more distal position than that in Fig. 7. H-E. Bar=25 μm.
Fig. 9. Spermiduct at connecting with male genital pore (arrow). H-E. Bar=25 μm.
cl, cuticular lining; gd, gut-diverticulum; hs, horizontal septum; sd, spermiduct.

originates from the ectodermal invagination.

El-Hawawi & King (1978, 1983) and King & El-Hawawi (1978) described that the male germ cells in *Acelia echinata*, *Nymphon gracile*, and *Pycnogonum litorale* developed only in the trunk testis in the region of the first trunk segment (the region corresponding to the germarium), the premature and mature spermatozoa were found in the region of the second and third segments, and only mature spermatozoa were found in the region of the fourth



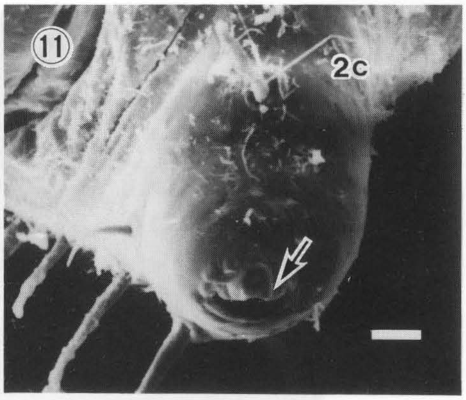


Fig. 10. Ventral view of third and fourth walking legs. Arrows showing male genital pores. SEM. Bar=500 μm.
Fig. 11. Male genital pore (arrow) opening on spur in ventral side of second coxa of right third walking leg. SEM. Bar= 25 μm.
ab, abdomen; ov, oviger; 1c-3c, first-third coxae; 3-4, third-fourth walking legs.

segment (the region corresponding to the seminal vesicle). These authors did not describe the germ cells in the pedal testes. In *Cilunculus armatus*, however, the spermatozoa are produced and stored throughout the trunk and pedal testes. Thus the whole regions of the testis plays the same role in the spermatogenesis in this species.

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