



Nervous system of the dwarf ectoparasitic male of *Scolelepis laonicola* (Polychaeta, Spionidae)

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Abstract

The nervous system of the ectoparasitic male of *Scolelepis laonicola* (Tzetlin, 1985), which attaches to the dorsal side of the female, was investigated by immunohistochemical methods in combination with confocal laser scanning microscopy. The male's nervous system is reduced; no ganglia are found in the central nervous system. The circumesophageal connective is split into dorsal and ventral roots. Two median and one paramedial nerve run along the midventral axis of the male. The peripheral nervous system is well developed. Five commissures arise from each main cord per segment. There are two dorsolateral and two ventrolateral longitudinal nerves. The biggest segmental transverse nerve runs to the parapodia and diverges into a few fine nerves at the top of the parapodia. The nerve cord turns 90° in the male-female contact zone. Well-developed peripheral nerves, the presence of lateral nerves, and the absence of differentiated ganglia indicate the progenetic origin of the *S. laonicola* male. Well-developed parapodial nerves in immobilized mature males suggest an important role of the parapodia prior to settlement and possible presence of chaetae in the larva.

Key words: dwarf male, nervous system, sexual dimorphism, Polychaeta, Spionidae

Introduction

Scolelepis laonicola (Tzetlin, 1985) is a spionid polychaete with vividly pronounced sexual dimorphism (Vortsepneva et al. 2008). During the summer months of 1996–2005, 28 females and 36 males attached to females were collected at the type locality in the White Sea (depth 18–20 m). Female *S. laonicola* are large spionid polychaetes about 2 cm long; they inhabit flimsy mucous tubes found about 20 cm below the surface of the bottom. In all investigated females, oocytes were accumulated in the parapodial cavities of the middle region of the body (starting at segments 22–34 and ending at segments 35–55). The males are oligomerous polychaetes (up to 14 segments) lacking chaetae in the parapodia and appendages in the head region. The males are oriented along the longitudinal axis of the female (Fig. 1A) (Vortsepneva et al. 2008). The anterior part of the male's body penetrates into the female's dorsal tissues and into its body cavity. The epidermal tissues of the male and the female are highly integrated in the area of contact. It was difficult to define whether epidermal cells and vessels belonged to the male or the female. Septae of the female form a chamber around the anterior region of the male (Fig. 1B) Vortsepneva et al. (2008). The coelomic cavity of the male's body is filled with spermatids and mature spermatozoa (Vortsepneva et al. 2006).

Among Spionidae, sexual dimorphism is found in *Pygospio elegans* (Schlötzer-Schrehardt 1987, 1991; Hartmann-Schröder 1996), Autolytinae (Syllidae), *Paralvinella* (Alvinellidae), certain Polynoidae, and *Ophryotrocha* (Dorvilleidae) (Åkesson 1975; Zal 1994; Jolliver et al. 2000; Zhadan et al. 2000; Nygren 2003; Tzetlin & Purschke 2005). In a number of protandrous hermaphrodites, e.g., *Myzostoma glabrum* and *M. alatum* (Myzostomida), males are noticeably smaller than females (Graff 1877; Eeckhaut & Jangoux 1991; Grygier 2000).

Dwarf males also occur in several taxa of Annelida. They are known for *Osedax* (Siboglinidae) (Rouse et al. 2004), Dinophilidae (Westheide 1990), and Bonellidae (Echiura) (Zenkevitch 1966; Schuchert & Rieger 1990; Rouse & Pleijel 2006). Male *Dinophilus gyrociliatus* are trochophore-like spherical animals having a prototroch, paired protonephridia, testes, and a penis (Windoffer & Westheide 1988). However, sexual dimorphism with a dwarf ectoparasitic male has never previously been reported for Spionidae nor for any other polychaete. In this paper we describe the nervous system of male *S. laonicola*.

Materials and methods

Specimens of male *Scoelepis laonicola* (Tzetlin, 1985) were collected at the Biological Station of the Moscow State University, Kandalaksha Bay, the White Sea, Russia (66°34'N, 33°08'E). The nervous system of four males was investigated by routine histology (Tzetlin 1985) and by immunohistochemical methods in combination with confocal laser scanning microscopy (Voronezhskaya et al. 2002).

Live animals were relaxed for 20–30 min in a 7.12 % $MgCl_2$ solution. Subsequently, males were transferred to 4% paraformaldehyde in 0.1 M PBS (phosphate-buffered saline) (pH=7.4) for 12 hours at 4°C. After triple rinsing in a complex solution of PBS, NaN_3 (0.05%) and Triton-X-100 (0.5 %) for 10–20 min, the males were stored in 0.1 M PBS with NaN_3 (1%) and Triton-X-100 (0.5 %) for several weeks. Specimens were rinsed in PBS (0.1%) three times for 10 min each and placed in a blocking solution (PBS + Triton-X-100 [1%] + Goat Serum [5%]) for 12 hours.

Specimens were placed in the primary antibodies (anti-FMRF-amide (Diasorin) 1:1800, anti-5HT (Diasorin) 1:1800, and anti-acetyl-tubulin (Sigma) 1:1000) for 24 hours at 4°C. After several rinses (three times during 10 min in 0.1 % PBS, pH 7.4), males were incubated with secondary antibodies (Goat Anti-Rabbit (Alexa 488) 1:750, Goat Anti-Mouse (Alexa 633) 1:700) for 24 hours at 4°C. Finally, the specimens were washed in PBS and mounted between two cover slides in Mowiol. Preparations were examined with a Leica confocal laser scanning microscope. Results were analyzed with Leica Confocal Software (Light Version).

Results

General morphology of the nervous system

The nervous system of male has a typical polychaete appearance, although it is poorly developed. It retains an intraepithelial position. The ventral nerve cord (VNC) runs along the upper side of the male attached to the female (Fig. 1C). This position of the VNC indicates that the dorsal side of adult male faces the dorsal side of female. A circumesophageal nerve connective is positioned above the male-female contact zone (Fig. 1D).

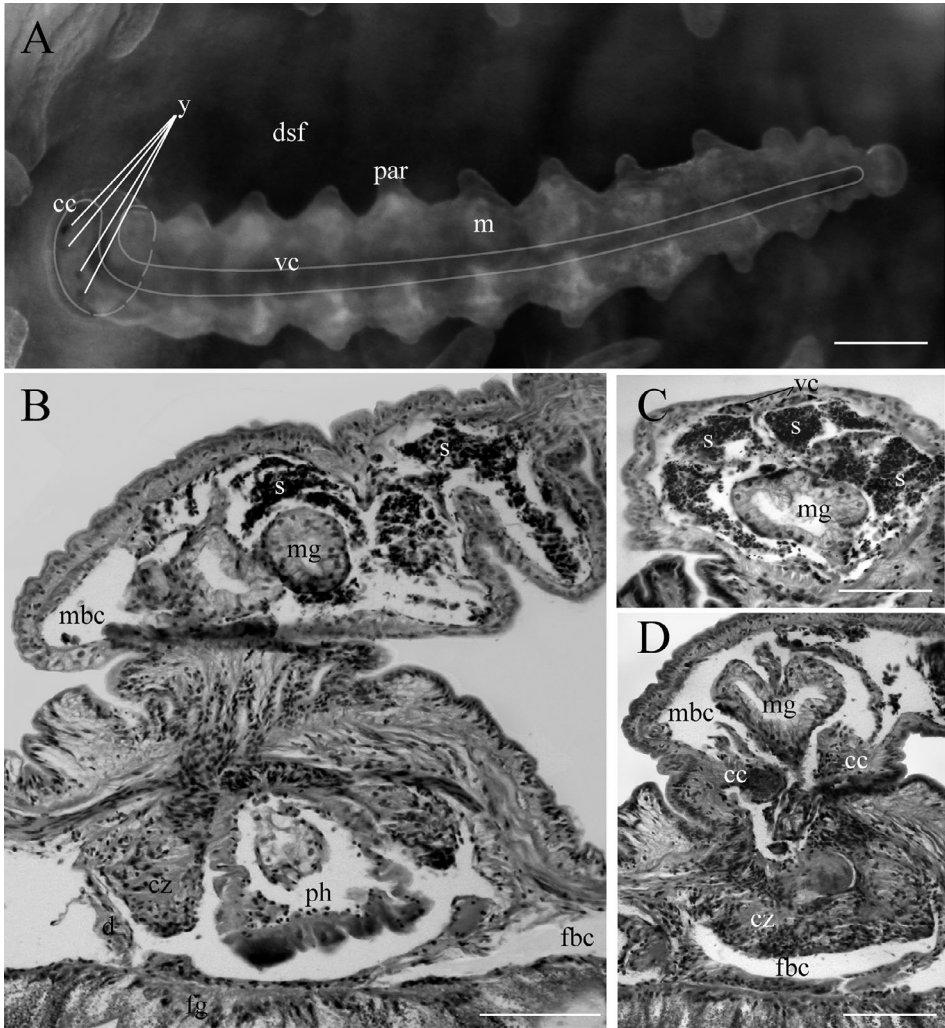


FIGURE 1. A, Male *Scolelepis laonicola*, photo made through a binocular microscope. The grey line is the reconstruction of the nerve cord. Bar scale = 300 μm . B, Male *S. laonicola*. Sagittal section through the anterior part. Bar scale = 100 μm . C, Male *S. laonicola*. Transverse histological section through the body. Bar scale = 100 μm . D, Male *S. laonicola*. Transversal histology section through the anterior part. Bar scale = 100 μm . Abbreviations: cc – circumesophageal connective, cz – contact zone, d – septa, dsf – dorsal side of female, fbc – body cavity of the female, fg – gut of the female, m – male, mbc – body cavity of the male, mg – gut of the male, par – parapodia, ph – anterior part of male inside the female body cavity, s – body cavity of male with spermatozoa, vc – ventral nerve cord, y – eyespot.

Immunocytochemistry

Incubation with acetylated- α -tubulin antibody resulted in extensive staining of part of the VNC,

peripheral nervous system, and cilia bundles. 5-HT and FMRF-amid staining patterns were also applicable to defining the position of the central nervous system in males.

5-HT and FMRF-amid IR. These fibers are visible in the circumesophageal connectives, in the VNC, and in the parapodia (Fig. 2B–D, F).

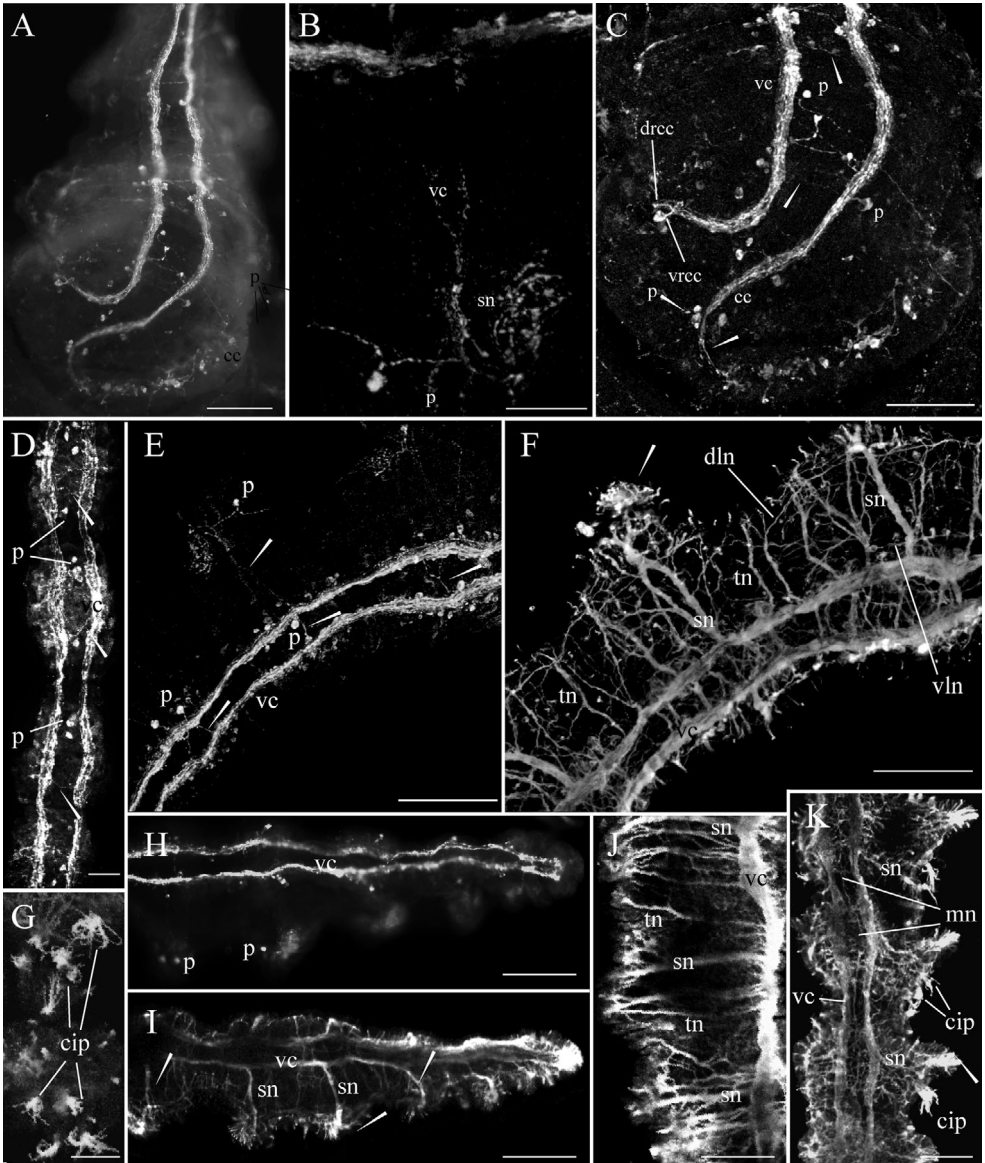


FIGURE 2. A–E, H, serotonin IR and FRFM-amid IR; F, G, I–K, acetylated- α -tubulin. A, Top view. Anterior region of male, circumesophageal connective divided into dorsal and ventral roots. Bar scale = 150 μ m. B, Top view. Nervous system turned 90°. Perikarya located along the ventral nerve cord. Bar scale = 300 μ m. C, Innervations of parapodia. Bar scale = 200 μ m. D, Middle segments of the body, side view. Perikarya located between the trunks of ventral nervous cord. Bar scale = 200 μ m. E, Middle segments of the body, side view. Perikarya located along the ventral nervous cord. Bar scale = 100 μ m. F, Middle segments of the body, side view. Two longitudinal nerves run along the lateral side. Bar scale = 100 μ m. G, Middle segments of the body, side view. Cilium bunches located in the lateral side. Bar scale = 100 μ m. H, The last six segments. Bar scale = 200 μ m. I, The last six segments. One thick nerve runs to the parapodium per segment. Bar scale = 200 μ m. J, Three body segments, side view. Perikarya located along the ventral nervous cord. Bar scale = 200 μ m. K, Four body segments, top view. Bar scale = 200 μ m. Abbreviations: cc – circumesophageal connective, cip – cilia bunch, dln – dorsolateral longitudinal nerve, drcc – dorsal root of circumesophageal connective, mn – median nerve, p – perikarya, sn – segmental nerve, tn – transversal nerve, vc – ventral nerve cord, vln – ventrolateral longitudinal nerve, vrcc – ventral root of circumesophageal connective, y – eyespot.



The circumesophageal connective is located above the male-female contact zone (Figs. 2A, C; 3A). Male nerve fibers are not found inside the contact zone nor in the male tissues penetrating the female's body. The circumesophageal connective is divided into dorsal and ventral roots. Four perikarya are found near the place where the circumesophageal connective splits into two roots. Ten perikarya are located frontally along the circumesophageal connective (Fig. 2C).

The male nervous system turns 90° immediately above the male-female contact zone (Figs. 1A; 2A, C).

Two strands of the ventral cord are widely spaced (up to 10 μ m between strands) (Fig. 2D). One to three thin transverse fibers per segment connect the strands of the ventral cord (Fig. 2 C–E). Ten to 14 perikarya are found lateral to the nerve strands in every segment, and about 2–4 perikarya are located inside the strands. These perikarya do not form ganglia and are spread along the length of the segment (Fig. 2 D–E).

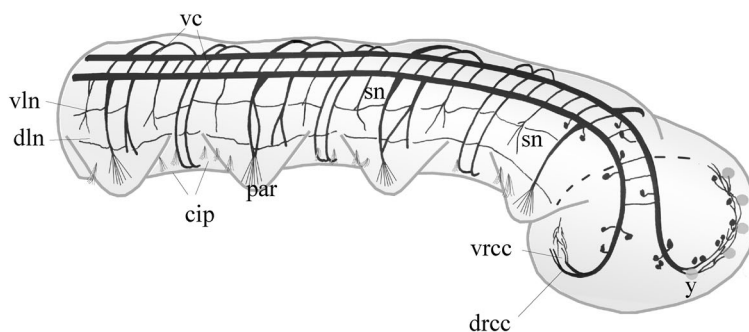
A pair of segmental transverse nerves runs from the ventral cord to the top of parapodia (Fig. 2B, E). There are one or two perikarya in the distal part of parapodia. Thin nerve fibers form the nervous plexus in the parapodia (Fig. 2 B, E).

Acetylated- α -tubulin. *Central nervous system.* The ventral cord of male *S. laonicola* consists of two main nerve strands and a thin median unpaired nerve (Fig. 2F, I, K). The nerve strands are connected to each other by 5–6 thick commissures per segment and numerous thin transverse nerves (Fig. 2F).

Peripheral nervous system. The main nerve strands give rise to 4–6 transverse segmental nerves, on each side of the ventral nerve cord. One pair of these nerves, the parapodial ones, is much better developed than the others. Segmental nerves branch and form a plexus on the lateral sides (Fig. 2F, G). The parapodial nerve branches at its distal end at the top of the parapodium (Fig. 2F, I, K). In addition to the ventral nerve cord, two pairs of longitudinal nerves (dorsolateral and ventrolateral) run along the body. Numerous thin nerves form a plexus on the ventrolateral side of the body (Fig. 2F, K).

Non-neuronal structures. One to three bunches of cilia per segment are seen in the parapodial zone and the same number are located between the parapodia of each segment (Fig. 2G, K)

A



B

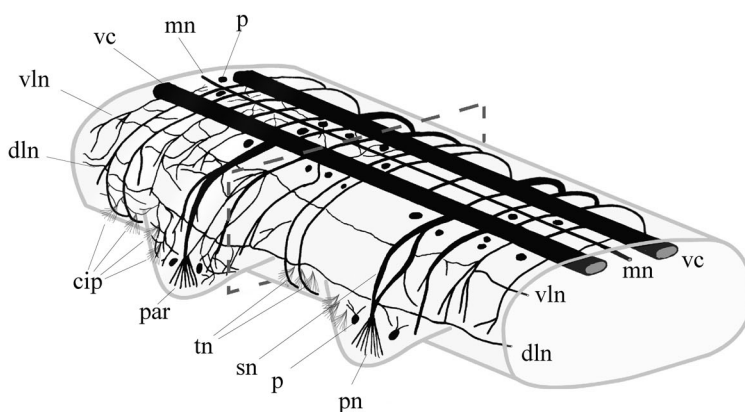


FIGURE 3. A, Reconstruction of the male nervous system (anterior region). Grey lines indicate the border of the male body. Two trunks of ventral nervous cord make a 90° turn in front of the place where the anterior part of male body protrudes into the female body. Bar scale = 300 μm. B, Reconstruction of the nervous system in two body segments. Grey line indicates the border of the body, dotted line separates more detailed layout. Bar scale= 300 μm. Abbreviations: cip – cilia bunch, dln – dorsolateral longitudinal nerve, drcc – dorsal root of circumesophageal connective, mn – median nerve, p – perikarya, par – parapodium, pn – parapodial nerve, sn – segmental nerve, tn – transversal nerve, vc – ventral nerve cord, vln – ventrolateral longitudinal nerve, vrcc – ventral root of circumesophageal connective, y – eyespot.

Discussion

The nervous system of the male lacks either cerebral ganglia or a brain and is generally reduced as a consequence of its parasitic life and attachment to female.

The ventral nerve cord runs along the upper side of the male and turns 90° immediately above the male-female contact zone. The eyespots take up a lateral position. Consequently, the dorsal side of the male faces the dorsal side of the female and the male ventral side (recognized by the ventral nerve cord) is turned upwards (Fig. 1A). The circumesophageal connectives of the male are located above the male-female contact zone. At the same time, the male's nerves are not found in the contact zone nor in the male tissues inside the female's body. This twist of the nervous system has never before been found in annelids and is presumably a consequence of the very specific sedentary lifestyle.

The central nervous system of the male is reduced. Two groups of perikarya (four in each) are situated in the places where circumesophageal connectives split into dorsal and ventral roots. With respect to their localization, these groups are very similar to the commissural ganglia found by Orrhage (1964) in another *Scolecopsis* species and other Spionidae. Only these two groups of nerve cells could be considered as ganglia. About ten perikarya found in the frontal part of the circumesophageal loop are spread far apart. Segmental ganglia are not found in the VNC; solitary perikarya are located along the ventral nervous cord. The nervous system of adult representatives of several Spionidae species was studied in detail by Orrhage (1964, 1974) and Orrhage & Müller (2005). Meanwhile, little is known about the nervous system in Spionidae larvae and juveniles (Blake & Arnofsky 1999). Almost no data are available for direct comparison between the nervous systems of spionid larvae and dwarf parasitic males of *Scolecopsis laonicolae*.

The nervous system of polychaete larvae was traditionally described as a centralized nervous system with a cerebral ganglion (Hay-Schmidt 1995; Lacalli 1984; Eeckhaut et al. 2003), e.g., in the larvae of *Polygordius lacteus* (Hay-Schmidt 1995), metatrochophore of *Myzostoma cirriferum* (Grygier 2000), trochophore of *Lopadorhynchus* (Åkesson 1967), trochophore of *Spirobranchus* (Lacalli 1984), and *Ophryotrocha dimorphica* (Zavarzina & Tzetlin 1991). At the same time, the absence of ganglia in the VNC, perikarya spread along the VNC, widely spaced-apart strands, a well-developed nerve plexus, and intraepithelial position of the entire nervous system are evidence of progenetic conditions (Westheide 1990).

The typical peripheral nervous system of polychaetes is well developed and consists of four lateral longitudinal nerves, 3–5 segmental transverse nerves per segment, one thick parapodial nerve, and numerous thin oblique nerves constituting a nerve plexus. The number of segmental nerves in polychaetes ranges from none (*Trilobordius hermaphroditus*) to numerous (*Protodrilus* sp.). The peripheral nervous system of male *S. laonicolae* is similar to that of *Protodrilus* sp. (Orrhage & Müller, 2005). Moreover, the parapodial nerve of male *S. laonicolae* is thick and splits at the end as is typical for the polychaetes with normal parapodia (Orrhage & Müller 2005). Mature males lack chaetae and acicula (Vortsepneva et al. 2006). We suggest that well-developed innervations of parapodia with the absence of locomotory functions in mature males indicate that the juveniles have chaetae in the parapodia and actively move before settlement.

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