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Anterior muscular system of the dwarf ectoparasitic male *Scolelepis laonicola* (Tzetlin, 1985) (Polychaeta, Spionidae)

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Abstract

The muscular system of the dwarf ectoparasitic male of *Scolelepis laonicola* (Tzetlin, 1985) was reconstructed by Falloidin-TRITC technique and confocal scanning microscopy. The anterior part of the male penetrates the female. All four main longitudinal muscle strands of the male's body enter the female; they are twisted about 90°. Oblique muscle fibers form a pear-shaped structure; they are very similar to the peristomial muscles in *Prionospio cirrifera* Wirèn. The well-developed musculature of the virtually immobile parasitic male of *S. laonicola* indicates good swimming abilities of juvenile males at the presettlement stage of the free-living larva.

Key words: dwarf male, morphology, sexual dimorphism, Spionidae, Polychaeta

Introduction

Dwarf males parasitizing females of *Scolelepis laonicola* (Spionidae, Polychaeta) represent an extreme type of sexual dimorphism (Vortsepneva et al. 2008). The external morphology of the male differs considerably from that of typical spionid polychaetes. In this species, the male is an oligomerous achaetous worm attached to the dorsal side of the female; a female sometimes carries more than one male. The head region of the male is modified and lacks both palps and nuchal organs. The anterior part of the male's body penetrates the body cavity of the female. This type of interrelation between a male and female has never previously been found in the Polychaeta. Except for the nervous system, all of the male's organs (digestive tract, nephridia, body cavity, and blood vessels) are well developed. The nervous system is reduced and exhibits characteristics of both the larval and adult nervous system. Furthermore, the ventral nerve cord turns above the contact zone, which is unique for Polychaeta (Vortsepneva et al., this volume). The structure of the anterior part of the male's body inside the female's body is unclear. The intestinal lumen of the male is extremely narrow; the ciliary epithelium has numerous vacuoles and the intestinal wall contains blood vessels and myofilaments. The epidermal tissues of the male and female are highly integrated in the contact area. The contact zone contains numerous myofilaments, blood vessels, and vacuolated cells (Vortsepneva et al. 2008), but is free of the male's nerves. This previously observed peculiarity stimulated our recent studies of the muscular system of the anterior region of the male. The details of the muscular system are presented here.

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Materials and methods

Specimens of *Scolelepis laonicola* were collected from the type locality of this species at the Biological Station of the Moscow State University, Kandalaksha Bay, the White Sea, Russia (66º34' N, 33º08' E). Animals were relaxed for 20–30 min in a 7.12% $MgCl_2$ solution. After relaxation, males were separated from females so that the anterior part of the male was cut off together with a part of female's body. Subsequently, males were transferred to 4% paraformaldehyde in 0.1 M PBS (phosphate-buffered saline) (pH=7.4) for 12 hours at 4ºC. After triple rinsing in a complex solution of PBS, NaN_3 (0.05%), and Trition-X-100 (0.5 %) for 10–20 min, the males were stored in 0.1 M PBS with NaN_3 (1%) and Trition-X-100 (0.5 %) for several weeks. Before incubation in phalloidin, specimens were rinsed in PBS (0.1%) three times for 10 min each and placed in a blocking solution (PBS + Trition-X-100 [1%] + Goat Serum [5%]) for 12 hours. Specimens were incubated in phalloidin (1:500) labeled TRITC (Fluka) for an hour. Afterwards, they were rinsed in PBS and enclosed in Mowiol. A total of four males were examined with a Leica confocal laser scanning microscope. Results were processed with Leica Confocal Software (Light Version). Routine methods were used for the histology investigations (Tzetlin 1985).

Results

Position of the male in the female *Scolelepis laonicola*

The body of the smallest male was 700 µm long with seven segments. The male was attached to the dorsal side of the female. The posterior end of the male was directed toward the anterior end of the female (Fig. 1A). Another male with 11 segments and body length of 1.75 mm was also attached to the dorsal side of another female; its posterior end, however, was directed to the posterior end of the female (Fig. 1B).

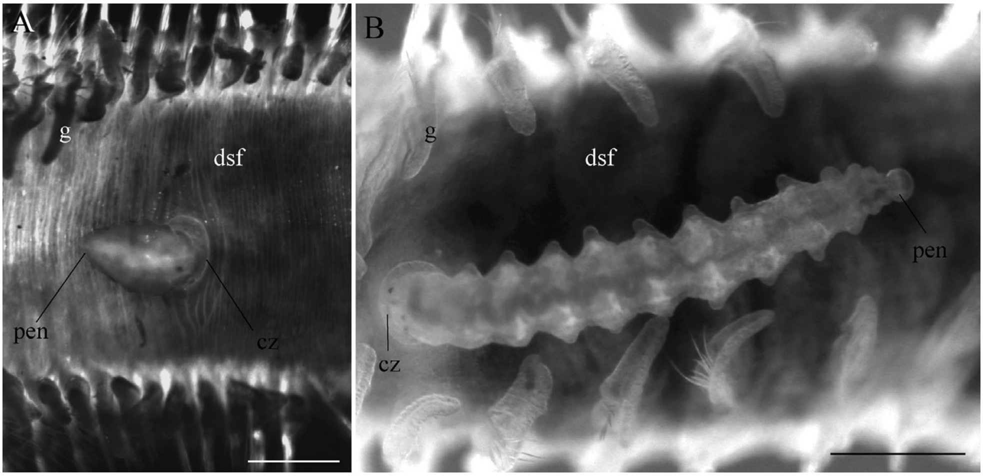

FIGURE 1. A, Smallest *S. laonicola* male with six segments attached to the dorsal side of the female. The posterior end of the male faces the anterior end of the female. Side view. Bar scale = 500 µm. B, Male with 11 segments attached to the dorsal side of the female. The posterior end of the male faces the posterior end of the female. Females' heads are directed to the left. Bar scale 500 µm. Abbreviations: cz, male-female contact zone; dsf, dorsal side of the female; g, gills of the female; pen, posterior end of the male.

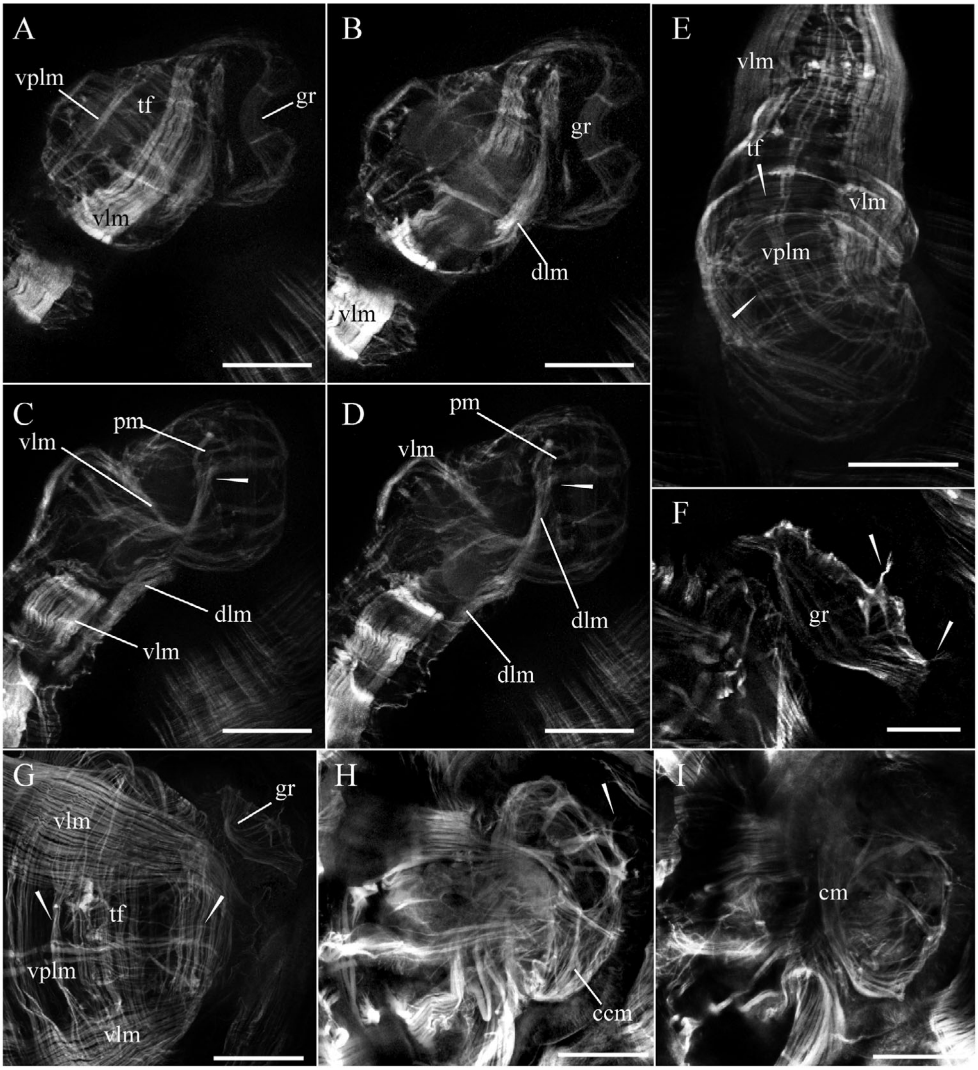

FIGURE 2. Male *S. laonicola*: F-actin muscle of anterior region. A–D. First examined male specimen. Side view. Bar scale = 400 µm. A, Two longitudinal fibers (one ventral and one dorsal) turn and run into the female's body; part of anterior region penetrates the female; B, Two fibers of the first parapodia lie under the body wall; C, Upper layer, anterior part of male is pear-shaped, paired thin fibers connect the dorsal longitudinal strands; D, Lower layer, ventral longitudinal fiber turns 90°; E, Second examined male, male turns 90° above the contact zone; transversal thin fibers covering two ventral longitudinal fibers and a paramedial fiber. Bar scale = 300 µm. F–I. Third examined male, photo of the lower layers. F, View from above the wavy crest and fiber tentacles of the anterior part of male growing into female's body. Bar scale 300 µm. G–I. The fiber crest and circumesophageal fiber complex are seen. Bar scale = 300 µm. Abbreviations: ccm, circumesophageal fiber complex; cm, circum fibers; dlm, dorsal longitudinal fibers; gr, wave crest; pm, fibers connecting with the fibers of body wall; vlm, ventral longitudinal fiber; vplm, ventral longitudinal paramedial fiber.

Muscular system of the anterior end of male *S. laonicola*

The muscles of the male's anterior end penetrate the body cavity of the female (Figs. 2A– D, 3A). Inside the female's body cavity, the following groups of muscle fibers can be distinguished: ventral and dorsal longitudinal strands of the body wall and transversal and longitudinal fibers of the anterior end (Fig. 3A–B).

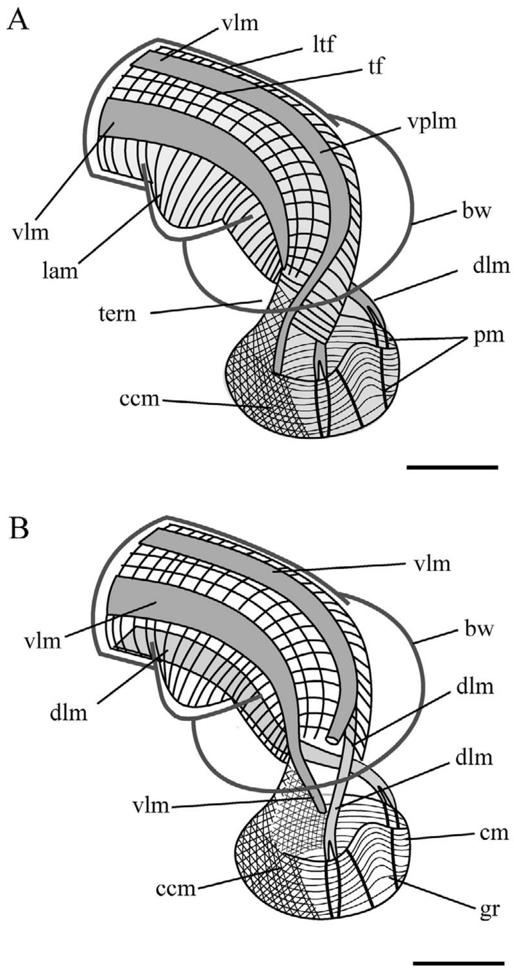

FIGURE 3. Reconstruction of the muscular system in the anterior part of the male's body. Grey lines indicate body wall. Bar scale = 300 µm. A, Two ventral longitudinal and ventral longitudinal paramedial fibers run to the female's body and turn 90° inside it; a circumesophageal complex develops between ventral longitudinal strands in the anterior part; wavy crest develops between dorsal longitudinal strands. B, Reconstruction of the muscles without upper layer. Abbreviations: bw, body wall; ccm, circumesophageal fiber complex; cm, circum fibers; dlm, dorsal longitudinal fibers; gr, fiber crest; ltf, lateral transversal fibers; pm, fibers connecting with the fibers of body wall; tf, transversal fibers; vlm, ventral longitudinal fiber; vplm, ventral longitudinal paramedial fiber.

Two thick ventrolateral and ventral paramedian longitudinal muscles extend to the anterior end (Figs. 2A–B, 3A). Transverse thin fibers run from one ventrolateral longitudinal muscle strand to another (Fig. 2A, E, G). The ventral muscles turn twice before they enter the male-female contact zone. At first, the ventral muscles turn horizontally 90° left (Figs. 2E, 3A–B), then they make a vertical turn down to about 45° (Figs. 2D, 3B). Ventrolateral longitudinal strands of the male's body wall extend into the female's body. Ventral paramedian longitudinal and transverse fibers disappear before they reach male-female contact zone (Figs. 2E, G; 3A).

Two dorsolateral longitudinal muscles extend to the anterior end. These fibers turn twice before they enter the contact zone in the same manner as the ventrolateral strands and extend into the female's body (Figs. 2D–E; 3A–B). The dorsolateral longitudinal muscles fork on the ends (Fig. 2C, D).

The male's muscles form a pear-shaped structure inside the female's body (Figs. 2D, H; 3A). The following fibers can be distinguished: circumbuccal muscle complex, transverse muscles, longitudinal muscles, and fibers protruding into the contact zone. The circumbuccal muscle complex is located between ventrolateral strands and consists of transverse and oblique fibers (Fig. 2H). Transverse muscles extend from one ventrolateral strand to another across dorsolateral strands (Fig. 2I). Transverse fibers form a wave-shaped crest between dorsolateral strands (Figs. 2A–B, F; 3B). Six longitudinal fibers comprise the pear-shaped structure (Figs. 2C–D, 3B). Two pairs of fibers extend into the dorsolateral longitudinal muscles and two fibers are located on the sides of the wave crest. Thin filaments run from the transverse muscles and protrude into the contact zone (Fig. 2F, H).

Discussion

The anterior part of male *Scolelepis laonicola* is modified. In the original description (Tzetlin 1985), the part of the male's body located inside the female's body cavity was termed "pharynx" by analogy with the pharynx of *Calamyzas amphictenicola* Arwidsson, 1932. *C. amphictenicola* is an ectoparasitic polychaete with a suctorial pharynx that penetrates into the host's body (Arwidsson 1932). Spionids usually have dorsolateral folds and, sometimes, a ventral pharyngeal organ in the pharynx (Purschke & Tzetlin 1995). The anterior region of male *S. laonicola* lacks such structures; the muscles located there look like those in the prostomium of *Prionospio cirrifera*. The prostomium of *P. cirrifera* contains the following muscles: ventral and dorsal longitudinal muscles of the trunk and circumbuccal muscles (Filippova et al. 2005). Similar structures can be distinguished in the anterior part of male *Scolelepis laonicola* (Fig. 3A–B). Thus we supposed that the anterior part of the male's body could be a prostomium rather than a pharynx. The ventral and dorsal strands of trunk muscles extend into the female body and turn there. Such a type of male-female relationship has not been previously reported for polychaetes.

The position of the male on the female was unclear before its muscular system was studied. The dorsal side of the male faces the dorsal side of female and the male's ventral side (recognized by the ventral nerve cord) is turned upwards (Fig. 4). The turns of the muscular and nervous systems mark peculiar turns of the male's body (Fig. 4). We can only speculate upon the prerequisites of the male's position relative to the female body. Both larval development and settlement of *S. laonicola* have never been observed. All known females have males attached to them, with the posterior ends of the males directed toward the posterior ends of the females (Vortsepneva et al. 2008*)* (Fig. 5C–D). However, the position of the smallest male with six segments was the opposite: the posterior end of its body faced the female's head (Figs. 1A, 5A–B). Therefore, we can propose the following scenario for the process of the male's attachment to female, resulting in the described positions of the male's muscle and nervous systems. A larval male attaches to the dorsal side of female in such a way that its posterior end faces the anterior end of the female's body (Fig. 5A–B). The larva's head end then turns horizontally 90° and, subsequently, its body makes a vertical turn to 180°, so that the posterior end of the male turns toward the posterior end of the female. Fusion of male and female tissues must occur over the entire period of settling and attachment (Fig. 5 C–D). The reasons for such a complicated twist of the male relative to the female are still vague and require additional investigation.

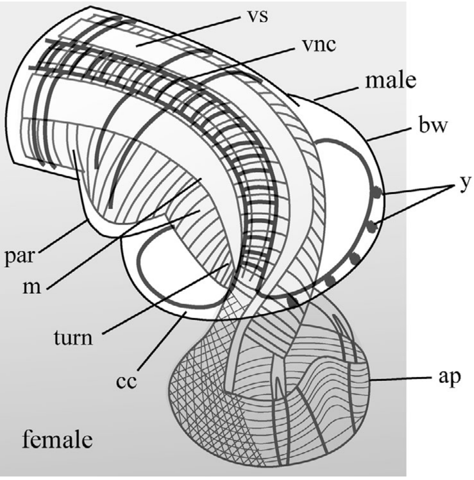

FIGURE 4. Layout of the twists of the male's muscular and nervous systems. The structure of the nervous system is modified from Vortsepneva et al. (2008). The anterior end of male is inside the female's body. Abbreviations: ap, anterior end of the male. bw, body wall; cc, circumesophageal connective; m, muscular system of the male; par, parapodia; vnc, ventral nervous cord of the male; vs, ventral side of the male body; y, eyespots of the male.

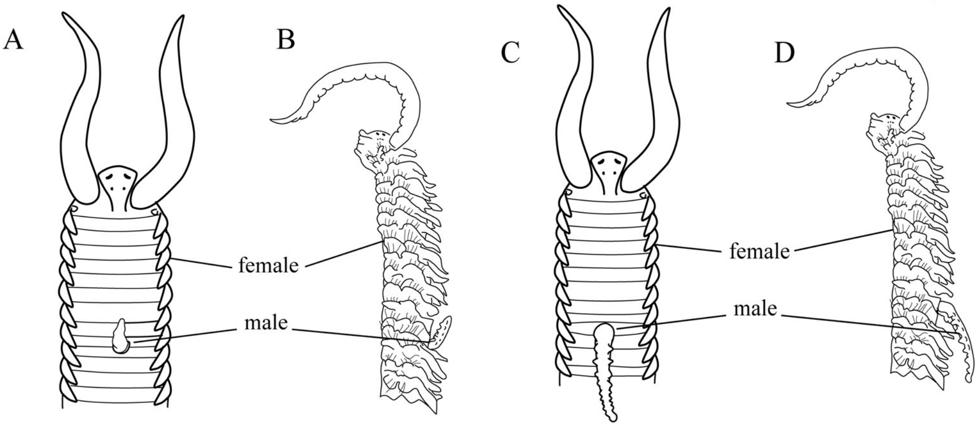

FIGURE 5. Phases of the male's attachment to the dorsal side of the female. A, C, top view; B, D, side view. A–B, First stage of attachment; posterior end of the male faces the head end of female. C–D, Last stage of attachment; posterior end of male faces the posterior end of female.

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