Invertebrate Biology 127(4): 403–416.

DOI: 10.1111/j.1744-7410.2008.00137.x

# The parasitic polychaete known as *Asetocalamyzas laonicola* (Calamyzidae) is in fact the dwarf male of the spionid *Scolelepis laonicola* (comb. nov.)

Elena Vortsepneva,[1,a] Alexander Tzetlin,[1] Günter Purschke,[2] Nikolai Mugue,[3] Eva Haß-Cordes,[2] and Anna Zhadan[1]

[1] Department of Invertebrate Biology, Moscow State University, Moscow, Russia
[2] Zoologie, Fachbereich Biologie/Chemie, Universität Osnabrück, Osnabrück, Germany
[3] Koltzov Institute of Developmental Biology, Moscow, Russia

**Abstract.** The morphology of the obligately ectoparasitic polychaete *Asetocalamyzas laonicola* was studied by light and electron microscopy, and its taxonomic position was determined using molecular methods. The parasite has an extensive coelomic cavity, complete septae, and well-developed segmental nephridia, circulatory, and digestive systems. The nervous system is rudimentary and without ganglia. The parasite's anterior region penetrates the tissues of the host, and opens into the host's body cavity. The epidermal tissues of the parasite and the host are highly integrated in the area of contact, and the parasite's cuticle is continuous with that of the host. Blood vessels of the parasite and the host may interlace in the fusion zone. The dorsal side of the parasite faces the dorsal side of the host. All parasites were males, but all hosts were females. In order to elucidate the uncertain systematic position of the parasite, molecular systematic studies were conducted. Parasite and host 18S rDNA sequences were virtually identical and revealed that both belong to the spionid cluster. These sequences differed from those of *Scolelepis squamata* and *Scolelepis bonnieri* by 2.7% and 0.9%, respectively. In addition, of seven partial sequences of the mitochondrial COI gene obtained from three parasites and four hosts, six were identical, and in one host–parasite pair, COI sequences differed by one substitution. Partial ITS2 sequences from one host–parasite pair were analyzed and also found to be similar but not identical, with two indels in a 645-bp alignment. We conclude that the parasite is in fact a dwarf male of its conspecific spionid female host. Consequently, *A. laonicola* is transferred to *Scolelepis* (Spionidae), forming the new combination *Scolelepis laonicola*.

*Additional key words:* sexual dimorphism, reproductive biology, molecular systematics

*Asetocalamyzas laonicola* Tzetlin 1985 is an obligately ectoparasitic polychaete described from a single specimen found on a spionid worm host that was initially identified as *Laonice cirrata* Sars 1851. However, reinvestigation showed that host individuals belonged to a different spionid genus, *Scolelepis* (unpubl. data). Members of *A. laonicola* are known only from the subtidal zone of Kandalaksha Bay, White Sea. *Asetocalamyzas* Tzetlin 1985 was originally assigned to the family Calamyzidae Hartmann-Schröder 1971 (Tzetlin 1985). In addition to *Asetocalamyzas,* the single described species of the genus *Calamyzas* was assigned to this family—*Calamyzas amphictenicola* Arwidsson 1932, an ectoparasite inhabiting the gills of individuals of *Amphicteis gunneri* (Ampharetidae) (Arwidsson 1932). Worms of both species are small, with $\leq 20$ segments, and attach to the host by their extended anterior region. *Asetocalamyzas laonicola* was placed in Calamyzidae on the basis of similarities to *C. amphictenicola* in life style, the type of attachment to the host, the general morphology, and the small number of segments (Tzetlin 1985). However, the placement of *A. laonicola* in Calamyzidae is uncertain because it lacks chaetae and head appendages, which are important characters for generic assignment.

New collections of *A. laonicola* in the White Sea stimulated a comprehensive reinvestigation of these animals in order to clarify their systematic position, the interaction of the parasite and the host, and their

[a] Author for correspondence.
E-mail: vortcepneva@mail.ru

reproductive biology. Molecular methods seemed likely to be the most successful means of unravelling their enigmatic phylogenetic position, because the parasites have few useful morphological characters. Because 18S rDNA and mitochondrial cytochrome oxidase C subunit I (COI) sequences are frequently and successfully used molecular markers for similar questions (e.g., Martin 2001; Bleidorn et al. 2003; Jördens et al. 2004; McHugh 2005; Struck et al. 2007), they were collected here to provide evidence on the worm's systematic position.

Preliminary observations demonstrated two important facts: (1) the prevalence of the parasites, *A. laonicola,* on hosts was 100%, and (2) all hosts were females and all parasites were males. Unexpectedly, 18S rDNA and COI sequences were almost identical in individuals of both the host and the parasites. These data led us to propose the hypothesis that individuals of *A. laonicola* are the dwarf ectoparasitic males of their host spionid worms. There are no previous reports of dwarf males in the Spionidae or any closely related taxon of polychaetes. The ultrastructure of the sperm of the dwarf parasitic male was described in a separate paper by Vortsepneva et al. (2006). A detailed description of the muscle and nervous systems of this species will be given in forthcoming papers.

## Methods

### Collections

During the summer months of 1996–2005, 36 specimens of *Asetocalamyzas laonicola,* attached to their hosts, were sampled at the type locality (depths of 18–20 m near the Biological Station of the Moscow State University, Kandalaksha Bay, White Sea, Russia [WSBS; 66°34′N, 33°08′E]). Specimens were reared in an aquarium and observed alive for several days. Fixed specimens were studied using routine histology, semi-thin sectioning, and transmission and scanning electron microscopy (TEM and SEM). Observations of living worms were made with a LOMO MBS-10 dissection microscope. Photographs were obtained with an Olympus C 4000 camera.

For comparison, the holotype and two paratypes (Zoological Museum of Moscow University [ZMMU] PI818, 819, 820) from the type series of *Scolelepis matsugae* Sikorski 1994 were examined using a dissection microscope (LOMO-MBS-10). Specimens of *Scolelepis bonnieri* Mesnil 1896 were collected in eulittoral sediments of the North Sea Island of Helgoland in 2003.

### Electron microscopy

Specimens for electron microscopy were fixed in buffered 2.5% glutaraldehyde. The buffer contained 0.3–0.36 mol $L^{-1}$ sucrose and was adjusted with 0.2 mol $L^{-1}$ sodium cacodylate to pH 7.2–7.4. After rinsing in buffer, specimens were post-fixed with 1% osmium tetroxide in the same buffer, dehydrated in a graded ethanol series, and then transferred to acetone. For TEM, specimens were embedded in Epon 812 resin. Semi-thin and ultra-thin sections were made on LKB and Dupont ultramicrotomes. Semithin sections were stained with 1% toluidine blue. Ultrathin sections were stained with lead citrate (15 min) and uranyl acetate (1%, 40 min, 35°C) and examined in a Jeol JEM 100-CX transmission electron microscope.

For SEM microscopy, body fragments were dried using the critical-point method, coated with platinum–palladium, and examined with a Hitachi 400A scanning electron microscope.

### DNA extraction

Tissue samples from three pairs of parasite and host, and from one additional host worm were taken for genetic analysis. To avoid possible cross-sample contamination, the distal part of a host worm palp was cut out using a disposable razor blade, and only the most posterior parts of the body of parasite specimens were harvested to avoid possible contamination by host DNA. DNA extraction followed CTAB (Doyle & Doyle 1987; Coyer et al. 1994) or salt (Aljanabi & Martinez 1997) protocols for COI and 18S rDNA analysis (performed at the Institute of Developmental Biology, Moscow), and the protocol of Schirmacher et al. (1998) for studies of 18S DNA and the second ribosomal gene spacer (ITS2) (at Osnabrück University, Germany).

### Molecular phylogenetic analyses

APCR amplification of the COI gene was performed with the universal primers LCOI 1490 (5′-GGTCAACAAATCATAAAGATATTG G-3′) and HCOI 2198 (5′-TAAACTTCAGGGTGACCA AAAAATCA-3′) (Folmer et al. 1994). Amplification of 18S rDNA was performed using various combinations of the universal primers 18sAi (forward) (CCTGAGAAACGGCTACCACATC) and 18sBi (reverse) (GAGTCTCGTTCGTTATCGGA) (Whiting et al. 1997), and several internal primers of our own design: 18sMKI (forward) (CTTGTCTCAAAG ATTAAGCCATGC), 18sMKIIa (reverse) (CGTT GTTTTCGTCACTACCTCCCC), and 18sMKIIb

(reverse) (GATCCAAGAATTTCACCTCT). PCR was performed in 25-μL reaction volumes including 1X PCR buffer (Sileks), 2.5 mmol $L^{-1}$ $MgCl_2$, 0.8 mmol $L^{-1}$ of each dNTP, 10 μmol $L^{-1}$ of each primer, 1 μL template DNA solution, and 1.25 U of Taq-polymerase (Sileks-M, Moscow). Reactions were cycled on an MJResearch PTC200 thermocycler, starting with a 2-min denaturing step at 95°C, followed by 35 cycles of 95°C for 30 s, 45°C (COI) or 52°C (18S) for 1 min, and 72°C for 1 min 30 s. Amplifications ended with a single 72°C, 10-min elongation step. PCR products were cleaned using Wizard PCR Preps (Promega, Madison, WI, USA). Sequencing was performed from the PCR primers in both directions with the ABI PRISM® BigDye™ Terminator v. 1.1 kit on an ABI PRISM 3100 according to the manufacturer's instructions.

Amplification of the second ribosomal gene spacer (ITS2) was performed with ITS5.8S (CATCGACTT CTTGAACGC) and ITS28S (AATGCTTAAATT CAGCGGGTA) primers. PCR products were purified with QIAquick PCR Purification Kits (Qiagen 28104, Hilden, Germany). Sequencing was performed from the PCR primers using an ABI PRISM 377 (Perkin Elmer, Shelton, CT) and an ABI PRISM® BigDye™ Terminator v. 3.1 kit.

Contig assembly and alignment was performed with DNAstar software (Lasergene Inc.). All phylogenetic analyses were conducted using PAUP* version 4.0b10 (Swofford 2002). All trees were rooted using the sequence of *Nereis virens* (Phyllodocida: Nereididae). Genbank accession numbers of sequences used in the 18S rDNA analyses are listed in Table 1. For maximum likelihood (ML) analyses, a model of sequence evolution for the dataset was selected using ModelTest V 3.06 (Posada & Crandall 2001). Both hierarchical likelihood-ratio tests and the Akaike information criterion indicated that the Tamura Nei substitution model (Tamura & Nei 1993) with equal base frequencies, invariant sites, and gamma distribution (TrNef+I+C) was the optimal model. ML trees were reconstructed using tree-bisection-reconnection (TBR) branch swapping, and ten random taxon additions. The reliability of phylogenetic

**Table 1.** Terminals used in 18S rDNA analysis, including higher taxa, with GenBank accession numbers. GenBank numbers in bold indicate new sequences.

| Species | Higher taxa | | Accession # |
|---|---|---|---|
| *Nereis virens* Sars 1835 | Phyllodocida | Nereididae | Z83754 |
| *Poecilochaetus serpens* Allen 1904 | Spionida | Poecilochaetidae | AY569652 |
| *Aonides oxycephala* Sars 1872 | Spionida | Spionidae | AF448149 |
| *Boccardiella ligerica* Ferrennière 1898 | Spionida | Spionidae | AY527061 |
| *Malacoceros fuliginosus* Claparède 1863 | Spionida | Spionidae | AY525632 |
| *Polydora ciliata* Johnston 1838 | Spionida | Spionidae | U50971 |
| *Polydora giardi* Mesnil 1896 | Spionida | Spionidae | AY611455 |
| *Prionospio* sp. | Spionida | Spionidae | DQ209226 |
| *Pygospio elegans* Claparède 1863 | Spionida | Spionidae | PEU67143 |
| *Scolelepis bonnieri* Mesnil 1896 | Spionida | Spionidae | **EU084878** |
| *Scolelepis laonicola* female Tzetlin (1985) | Spionida | Spionidae | **EF569206** |
| *Scolelepis laonicola* male Tzetlin (1985) | Spionida | Spionidae | **EF569206** |
| *Scolelepis squamata* OF Müller 1789 | Spionida | Spionidae | AF448164 |
| *Magelona mirabilis* Johnston 1865 | Spionida | Magelonidae | MMU50969 |
| *Apistobranchus* sp. | Spionida | Apistobranchidae | DQ779640 |
| *Chaetopterus pugaporcinus* Osbron et al. 2007 | Spionida | Chaetopteridae | DQ209224 |
| *Chaetopterus sarsii* Sars 1851 | Spionida | Chaetopteridae | DQ209221 |
| *Chaetopterus variopedatus* Renier 1804 | Spionida | Chaetopteridae | CVU67324 |
| *Mesochaetopterus japonicus* Fujiwara 1934 | Spionida | Chaetopteridae | DQ209218 |
| *Mesochaetopterus xerecus* Petersen & Fanta 1969 | Spionida | Chaetopteridae | AJ966763 |
| *Aphelochaeta marioni* Saint-Joseph 1894 | Spionida | Cirratulidae | DQ779639 |
| *Caulleriella parva* Gillandt 1979 | Spionida | Cirratulidae | AF448151 |
| *Cirratulus cirratus* OF Müller 1776 | Spionida | Cirratulidae | DQ779645 |
| *Cirratulus* sp. | Spionida | Cirratulidae | AB106262 |
| *Cirratulus spectabilis* Kinberg 1866 | Spionida | Cirratulidae | AY708536 |
| *Cirriformia tentaculata* Montagu 1808 | Spionida | Cirratulidae | AY611456 |
| *Dodecaceria concharum* Oerstedt 1843 | Spionida | Cirratulidae | AY577891 |
| *Trochochaeta* sp. | Spionida | Trochochaetidae | DQ790097 |

nodes was estimated by 100 bootstrap replicates with one random taxon addition and TBR branch swapping.

Equal weighted parsimony analyses with a branch and bound search were also conducted for 18s DNA data set. Clade support was assessed with nonparametric bootstrapping as implemented in PAUP v4b10 (heuristic search, 1000 replicates, TBR branch swapping, and a simple addition sequence).

## Results

### Habitat and distribution

After several years of intensive sampling, *Asetocalamyzas laonicola* is still known from only one locality, a small depression a few hundred square meters in area (18–20 m in depth) on the subtidal slope in the strait between Cape Kindo and Welikiy Island, just in front of the WSBS (66°34′N, 33°08′E). Worms inhabited the sediment at this site, a nonsorted mixture of sand, clay, gravel, and stones. Individuals were never found in the upper 15 cm of the sediment, and sampling was successful only if the layer of excavated sediment was not $<25$ cm thick. The maximum density of animals observed was four per square meter. About 70 invertebrate species were found in the type habitat of *A. laonicola*. The bivalve *Modiolus modiolus* dominates (>50% of total biomass, 33–347 g $m^{-2}$); other common species include the polychaete *Nereis virens* and the echinoderms *Ophiopholis oculeata* (each ~10% of total biomass), and *Ophiura robusta* (Ophiuridae) (~5% of total biomass). The polychaetes *Amphitrite cirrata* and *Scoloplos armiger* are also common (3.5% and 3%, respectively).

Host worms inhabited flimsy mucous tubes situated ~20 cm below the surface of the bottom. The shape of the tubes is unknown. The orientation of the worms in their burrows is also unknown.

Observations made by scuba divers, photographic records of a few square meters of the bottom in the collecting site, and observations of specimens held in an aquarium showed that the hosts of *A. laonicola* never left their tubes and never extended their palps outside the openings of the tubes as many other spionid species have been observed to do.

### Morphology

**Description of free-living host.** The largest complete specimen had 75 chaetigers, and was 25 mm long and 1.8 mm wide (at the middle region of chaetiger 6, not including parapodia) when alive and relaxed.

The prostomium was rounded, and the caruncle extended posteriorly to chaetiger 2. The occipital antenna was well developed (Figs. 1A, 2A). Four eyes were present: the anterior pair round, and the second pair bean shaped. The palps had basal sheaths (Fig. 2C), and when extended posteriorly, they reached the 13th chaetiger. Ciliated bands were present along the sides of the palps (Fig. 2D). The peristomium was massive, completely separated from chaetiger 1, and had well-developed lateral wings (Fig. 1A). The horseshoe-shaped nuchal organs were located between the prostomium and the peristomium, just behind the palp bases (Figs. 1A, 2A).

The parapodia were biramous, with neuro- and notopodial postchaetal lobes (Figs. 1B–D, 2E). Chaetiger 1 bore lanceolate neuropodial and conical notopodial postchaetal lobes, and there were no notochaetae present in the first segment (Figs. 1B, 2B). Branchiae were present from the second to the 23rd–30th chaetiger; they were fused with the notopodial lobe (Figs. 1A,D, 2E,F). The edges of the branchiae were scalloped (Figs. 1D, 2B–D), and blood vessels were clearly visible in their basal parts (Fig. 2I). Branchiae on segments 23–30 were reduced in size (Figs. 1C, 2H). Ciliary lateral organs were present between noto- and neuropodia (Fig. 2F,G).

The body was divided into two regions due to the structure of the muscles and intestine. The anterior region was characterized by well-developed body-wall muscles, and the intestine in these chaetigers was cream-colored (in living specimens), with a covering of muscles. This part of the intestine never contained any food. In the posterior region of the body (24th–30th chaetiger), the intestine was black with a thin wall. In this region, the body wall was thin as well (Fig. 3A). Worms often broke at the border of these two regions. The pygidium was disk-shaped without any appendages, and the anus terminal (Fig. 1E).

In all the host specimens investigated, oocytes accumulated in the parapodial cavities of segments between chaetigers 22–34 (anteriorly) and 35–55 (posteriorly). The diameter of oocytes was ~120–130 μm (Fig. 3D).

**Description of parasites.** Parasites had nine to 14 segments, and were 0.17–0.5 mm in width (first segment, without parapodia) and 0.75–2.55 mm in length. Their bodies were dorsoventrally flattened (Figs. 3A–C, 4A). On average, the length of parasites was 1/10th that of their hosts. There were no appendages in the head region (Fig. 4A). Several specimens had five eyes—red or black pigmented spots along the perimeter of the head. We observed no nuchal organs in parasites. Their parapodia were

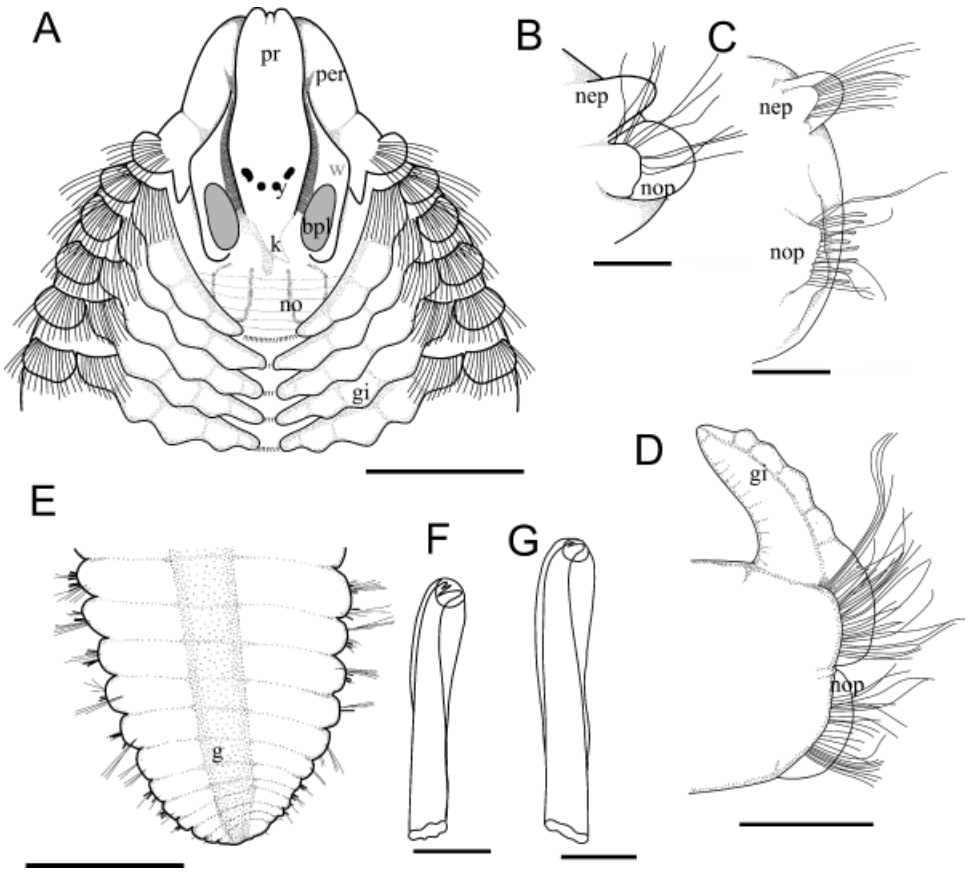


**Fig. 1.** Female of *Scolelepis laonicola*. **A.** Anterior end, dorsal view, palps removed. Scale bar, 1 mm. **B.** First parapodium, anterior view. Scale bar, 500 µm. **C.** Chaetiger 48, left parapodium, lateral view. Scale bar, 500 µm. **D.** Chaetiger 19, left parapodium, lateral view. Scale bar, 1 mm. **E.** Pygidium and some chaetigers, anterior view. Scale bar, 1 mm. **F.** Hooded hook from chaetiger 22, lateral view. Scale bar, 10 µm. **G.** Hooded hook from chaetiger 22, lateral view. Scale bar, 10 µm. Abbreviations: bpl, base of palps; g, gut; gi, gills; k, caruncle; nep, neuropodium; no, nuchal organ; nop, notopodium; per, peristomium; pr, prostomium; w, wings of peristomium; y, eyespots.

conical and uniramous, without chaetae and aciculae (Figs. 3A, 4B). The pygidium lacked anal cirri or papillae, and the anus was terminal (Fig. 4B,C). Parasites had an extensive coelomic cavity, well-developed septae, and segmental nephridia. The ventral nerve cord consisted of two separated nerves without ganglia; cerebral ganglia were not developed. Perikaria were randomly distributed along the nerves (unpubl. data). The circulatory and digestive systems of the parasite were well developed (Fig. 5); some intestinal content can be seen in TEM photos (Fig. 6A–C). The coelomic cavity of the trunk segments was full of spermatids and mature spermatozoa (Figs. 3E, 4D,E).

**Distribution of parasites.** Hosts sometimes carried more than one parasite; a total of 28 hosts carrying 36 parasites were examined (Figs. 3A–C, 4A). Up to four specimens could be found on one host (Fig. 3A–C). The parasites were oriented parallel to the longitudinal axis of the host (Fig. 3A), with their ventral sides (recognized by the ventral nerve cord) facing upwards. The dorsal side of the parasite was adjacent to the dorsal side of the host (Fig. 3E). The parasites were frequently located in the middle part of the host (Fig. 7A).

**Contact zone.** The anterior region of the parasite penetrated host tissues and opened into its body cavity (Fig. 3E,F). The parasite's intestinal lumen in this region was extremely narrow (Figs. 5, 6F,G). The intestinal epithelium was ciliated, and the cells contained numerous vacuoles; the intestinal wall contained blood vessels and myofilaments (Fig. 6A,F,G). The epidermal tissues of the parasite and the host were highly integrated in the area of contact. The contact zone contained numerous myofilaments, blood vessels, and vacuolated cells. It was difficult to define whether these cells and vessels belonged to the host or the parasite (Fig. 6E,H,I). The cuticle of the parasite was continuous with the cuticle of the host (Fig. 4F). Septae of the host formed a chamber around the anterior region of the parasite (Figs. 3F, 5).

## Molecular data

We obtained partial sequences of the 18S rDNA gene from three hosts and one parasite, and one 18S rDNA sequence of *Scolelepis bonnieri*. All four sequences of hosts and parasite were identical in an alignment of 1807 bp (GenBank #569206). On the

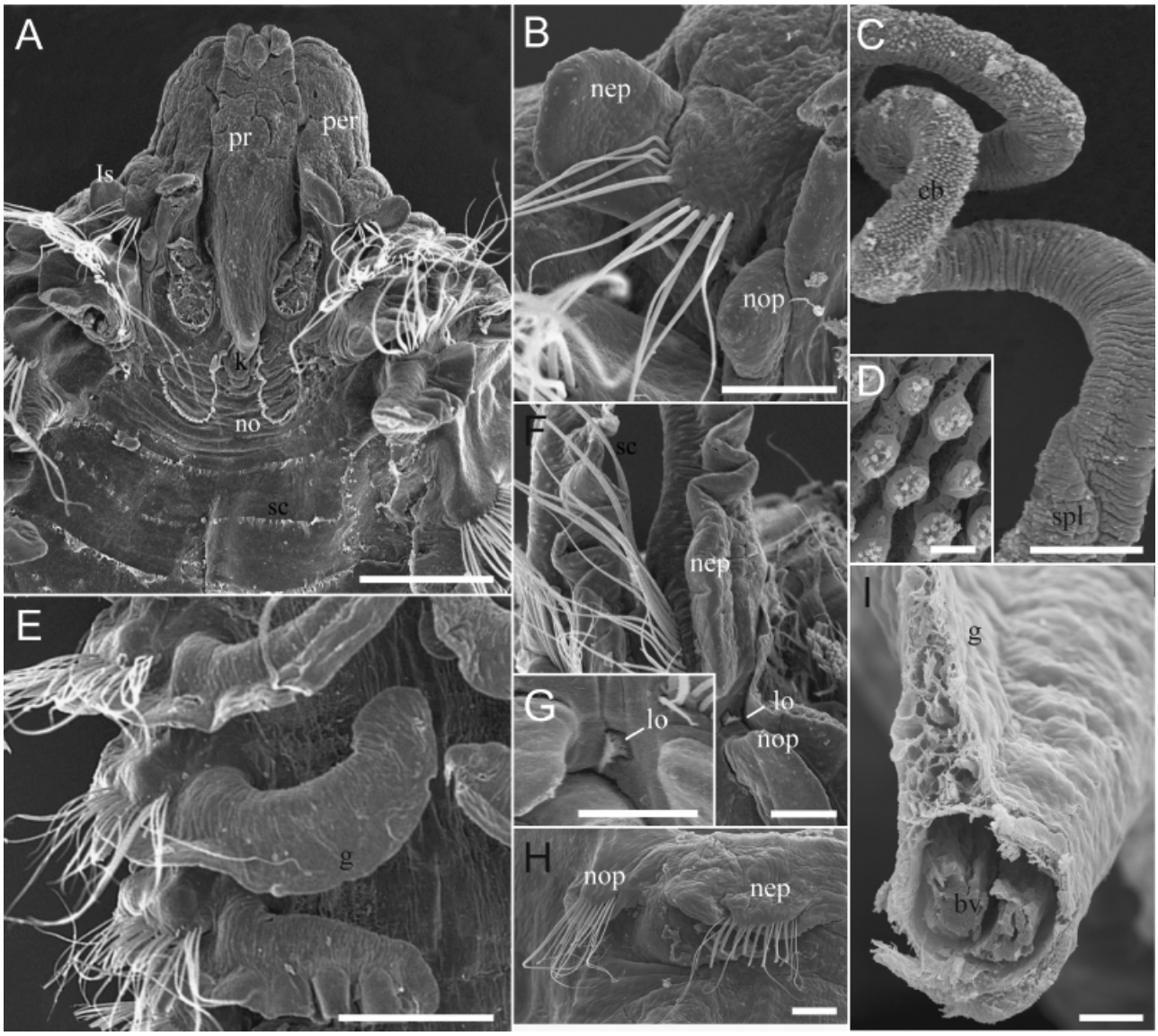


**Fig. 2.** Female of *Scolelepis laonicola*. **A.** Anterior end, dorsal view, palps removed. Scale bar, 300 µm. **B.** First parapodium, anterior view. Scale bar, 60 µm. **C.** Palp, anterior view. Scale bar, 300 µm. **D.** Ciliated band of palps. Scale bar, 12 µm. **E.** Middle region of the body, dorsal view. Scale bar, 400 µm. **F.** Chaetigers 22–23, left parapodium, lateral view. Scale bar, 300 µm. **G**. Lateral organ, chaetigers 22–23, lateral view. Scale bar 150 µm. **H.** Chaetiger 48, right parapodium, lateral view. Scale bar, 1 mm. **I.** Transverse section across the posterior region of the gill. Scale bar, 300 µm. Abbreviations: Is, first chaetiger; bv, blood vessel; cb, ciliated band; g, gill; k, caruncle; lo, lateral organ; nep, neuropodium; no, nuchal organ; nop, notopodium; per, peristomium; pr, prostomium; sc, sensory cilia; spl, sheath of palp.

other hand, sequences of both the hosts and the parasite had clear differences with that of *S. bonnieri* (GenBank # EU084878, 14 substitutions and one indel [0.9%] of 1711 aligned sites), as did the hosts and parasite with the previously published 18sRNA sequence of *Scolelepis squamata* OF Müller 1789 (GenBank #AF448164, 18 substitutions and three indels of 1807 aligned sites). Phylogenetic analysis of the above sequences as well as others from GenBank (Table 1) revealed that both the parasite and the host belonged to the spionid cluster and, of the taxa included in the analysis, were most closely related to *S. squamata* and *S. bonnieri*. The positions of both the parasites and the hosts in the spionid clade, and particularly within the genus *Scolelepis,* have 100% bootstrap support (Fig. 7B).

Of seven COI sequences (three from parasites and four from hosts), six were identical (GenBank #EF569202), but the sequence from one specimen (parasite 2, GenBank #EF569203) differed from the others by a single substitution out of 647 bp; the

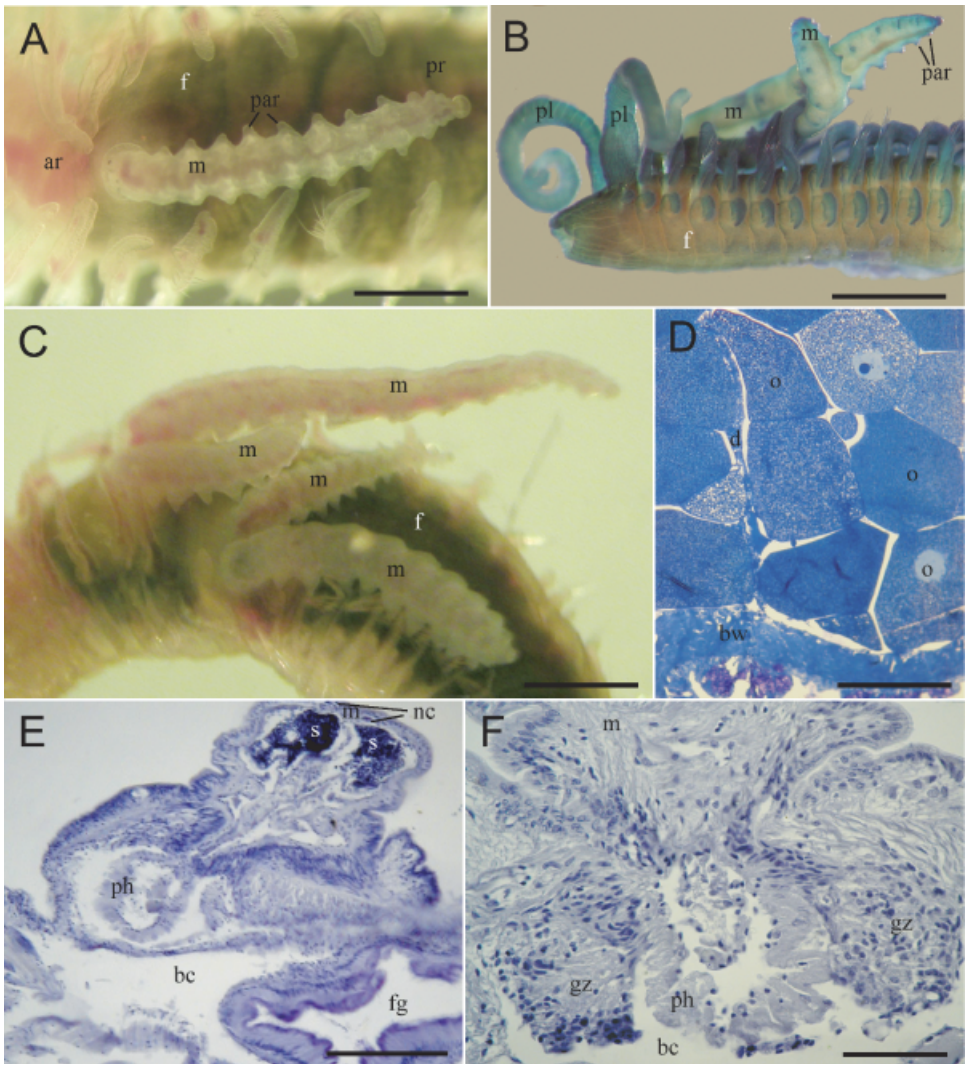


**Fig. 3.** *Scolelepis laonicola*. **A.** One male attached at the border between two body regions of female, view from above. Scale bar, 500 µm. **B.** Two males attached to chaetigers I and II of female, lateral view. Scale bar, 1 mm. **C.** Four males on adjoining segments of female, lateral view. Middle region of female. Scale bar, 500 µm. **D.** Transverse semi-thin section of female in the middle region of the body. Scale bar, 150 µm. **E.** Longitudinal thick section of male attached to female. Scale bar, 1 mm. **F.** Transverse thick section of male's pharynx in the coelomic cavity of female. Scale bar, 1 mm. Abbreviations: ar, anterior region; bc, female coelomic cavity; d, female septae; f, female; fg, gut of female; gz, fusion zone tissue; m, male; o, oocytes; par, parapodia; ph, pharynx of male; pl, palps; pr, posterior region; s, male gametes; vnc, ventral nerve cord.

difference was at a first position site and resulted in an amino acid change from valine to isoleucine. To make sure that this substitution did not represent a PCR or sequencing error, DNA was extracted again from parasite 2. The sequence obtained from this second DNA extract confirmed that parasite 2 and host 2 had different mitochondrial haplotypes.

Partial ITS2 sequences from a single parasite and its host were analyzed. These sequences were not identical, with two indels and one substitution in the 645 bp alignment. Sequences are deposited in GenBank (EF569207, EF569208).

## Taxonomic account

*Scolelepis laonicola* Tzetlin 1985 comb.n.

**Material examined.** Twenty-eight female specimens and 36 male specimens, White Sea, Kandalaksha Bay, 66°34′N, 33°08′E, 20 m; holotype (male) is a series of sections deposited in the Zoological Museum of Moscow University (No PL 307), voucher No Pl-976 (female with two males) (96% alcohol) in the Zoological Museum (Moscow, Russia).

**Female.** Paratype with 70 chaetigers. Total 21 mm in length and 3.02 mm in width, without parapodia, in sixth chaetiger.

Chaetiger 1 with capillary chaetae present only in neuropodia 6–11 (Figs. 1B, 2B). Chaetiger 2 with 10–16 capillary chaetae in neuropodia and 12–14 capillary chaetae in notopodia. In the following segments, the number of chaetae increases and hooded hooks appear starting with segment 27–40 in notopodia and with 23–30 in neuropodia. There are four to nine hooded hooks in the neuropodia and two to seven in notopodia (in segments 30–45). Hooded hooks with three teeth: two apical and one main large tooth. In frontal view, hooks to two teeth

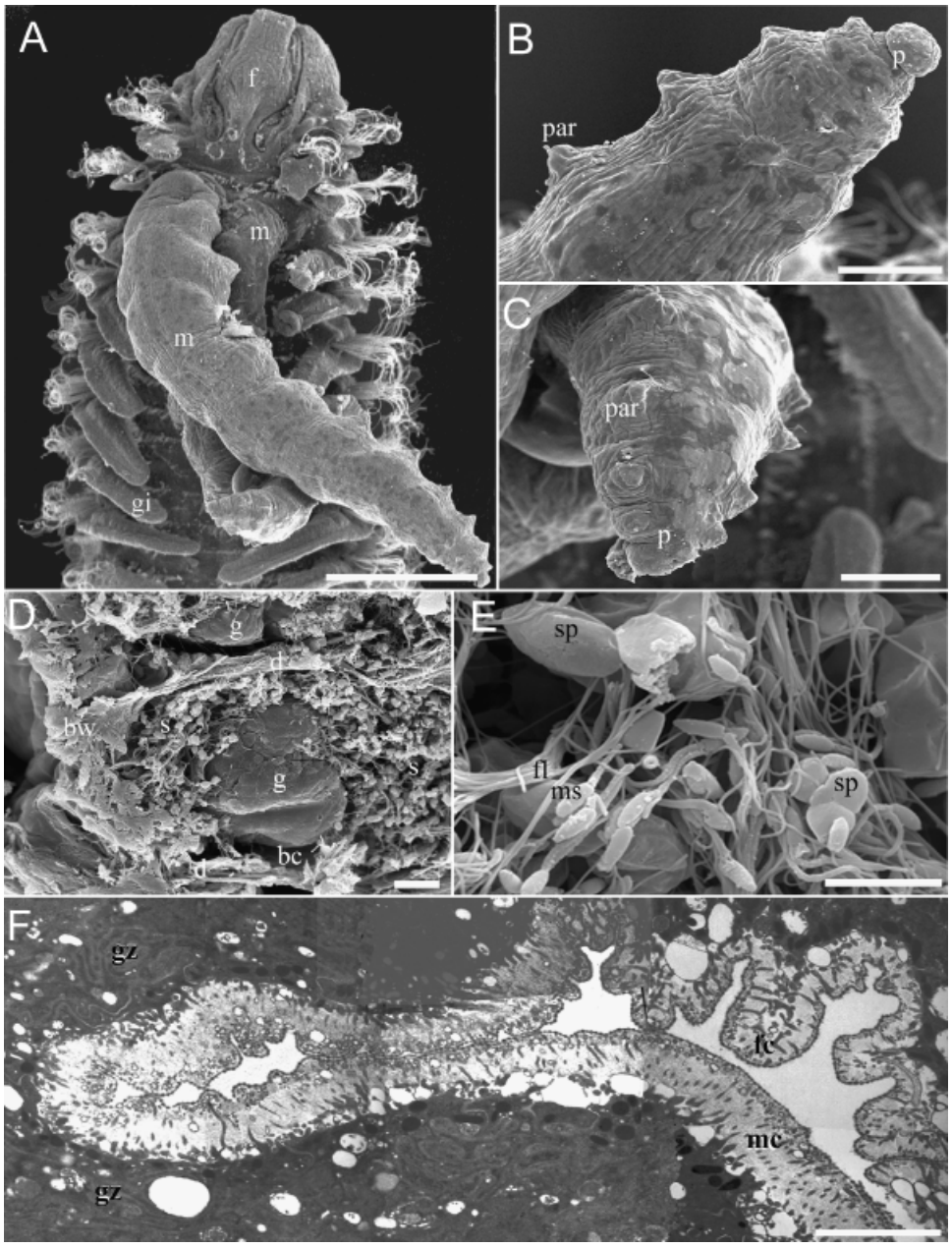


**Fig. 4.** *Scolelepis laonicola*. **A.** Anterior end of female with two males; palps of female removed. Scale bar, 800 μm. **B.** Posterior end of male, lateral view. Scale bar, 100 μm. **C.** Pygidium of male. Scale bar, 100 μm. **D.** Parasagittal section of the middle region of male showing septae and spermatids. Scale bar, 30 μm. **E.** Different stages of spermatogenesis. Scale bar, 600 μm. **F.** Transverse section through the attachment zone of male and female. Cuticles of both specimens fused. Scale bar, 30 μm. Abbreviations: bc, coelomic cavity; bw, body wall; d, septae; f, female; fc, female cuticle; fl, flagellum of spermatozoon; g, gut; gi, gills; gz, fusion zone tissue; m, male; mc, male cuticle; ms, mature spermatozoon; p, pygidium; par, parapodia; sp, spermatids.

(Fig. 1F,G). Several posterior segments closely related with a few hooded hooks and capillary chaetae. For further details, see the above results under ''Morphology.''

**Male.** Two male paratypes attached to female paratype. One male attached to segment 22 of female comprising 14 segments, 0.37 mm in width (first segment without parapodia) and 2 mm in length. Second male attached to female in segment 60, with 12 segments 0.33 mm in width and 1.6 mm in length. Male dorso-ventrally flattened. For further details, see the above results under ''Morphology.''

## Discussion

Our initial assumption was that the parasite and host worms belonged to different species and even to different families (Tzetlin 1985). To unravel the systematic position of these animals, the 18S rDNA gene and the COI region from one host–parasite pair were initially sequenced. Host and parasite DNA were identical at each of the two loci, leading us to conclude that host and parasite worms are conspecific.

Two hypotheses were developed to explain these findings: (1) the parasite is a dwarf male, attached

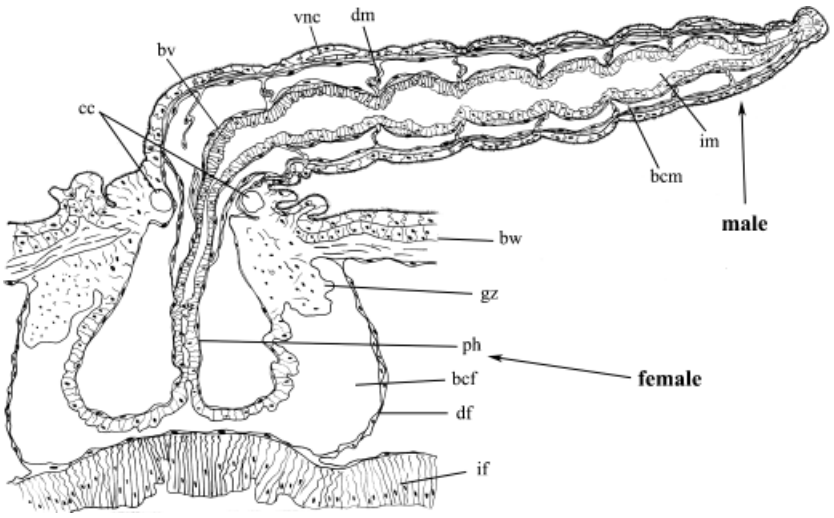


**Fig. 5.** *Scolelepis laonicola*. Reconstruction of the internal morphology of male and fusion zone tissue. Sagittal section. Scale bar, 500 μm. Abbreviations: bcf, coelomic cavity of female; bcm, coelomic cavity of male; bv, blood vessel; bw, body wall; cc, circum oesophageal connective; df, female septae; dm, male septae; gz, fusion zone tissue; if, female intestine; im, male intestine; ph, anterior region of intestine; vnc, ventral nerve cord.

to the host (female) during an early stage of the life cycle, and (2) the parasite is a ''generative bud'' carrying male gonads and originated as a vegetative protrusion of the female.

If the first hypothesis is true, one would expect sequence differences between a parasite and its host, at least within some host–parasite pairs. If the second hypothesis is true, a parasite and its host should be genetically identical at any locus sequenced.

Additional analyses revealed one host–parasite pair (number 2) with COI sequences that differed by one substitution. The differences observed were obtained in repeated analyses. However, differences were only found in one individual pair, whereas all others were identical. To confirm these findings, ITS2 was sequenced in one additional host–parasite pair, and two substitutions were found. Although the ITS region is present in multiple copies in a given organism, and any sequence differences could be attributed to intra-organismic polymorphism, we have never obtained more than one sequence from a given individual (e.g., Westheide & Hass-Cordes 2001).

These findings provided evidence that the parasite is not a ''generative bud'' produced asexually, but rather an independent individual that attached to the host at some point in its life cycle. Thus, the data are consistent with hypothesis 1 (''dwarf male''), while hypothesis 2 (''generative bud'') must be rejected. We thus argue that the parasites and hosts are males and females of the same species.

## Systematic position of *Scolelepis laonicola*

Because the dwarf males have a simplified morphology, lacking diagnostic features for placement into a given family taxon (e.g., chaetae and aciculae in the parapodia, and appendages in the head region), the systematic position of the species can only be determined by the morphology of the female or by molecular data. However, from the morphological characters alone there are no doubts that the female belongs to Spionidae. It possesses all characters typical of the family: an elongated prostomium is situated on the dorsal surface of the peristomium, an occipital papilla on the caruncle, one pair of long palps situated dorsally on the peristomium close to the prostomium, and well-developed biramous parapodia with parapodial lobes, branchia, capillary chaetae, and hooded hooks. This placement is confirmed by our 18S rDNA data.

Both external morphology and molecular data show that the free-living females belong to the genus *Scolelepis*. Members of *Scolelepis* possess hooded hooks in noto- and neuropodia, and branchiae fused with the postchaetal lobe. Furthermore, in members of the genus, there is a correlation between the sizes of branchiae and the notopodial postchaetal lobe (Imajima 1992; Hartman-Schröder 1996). Most species of *Scolelepis* have a pointed prostomium (Hartman-Schröder 1996). The only species described from the Kara and the Barents Sea, *Scolelepis matsugae,* has a rounded prostomium like the free-living females in our material (Sikorski 1994). Both species have tridentate-hooded hooks. Furthermore, the structure of the first parapodia (without notochaetae and branchiae on the 1st chaetiger of female), the shape of the branchiae and the postchaetal noto- and neuropodial lobes, and the number of the chaetigers on which hooded hooks appear would allow combining these two species. However, individuals of

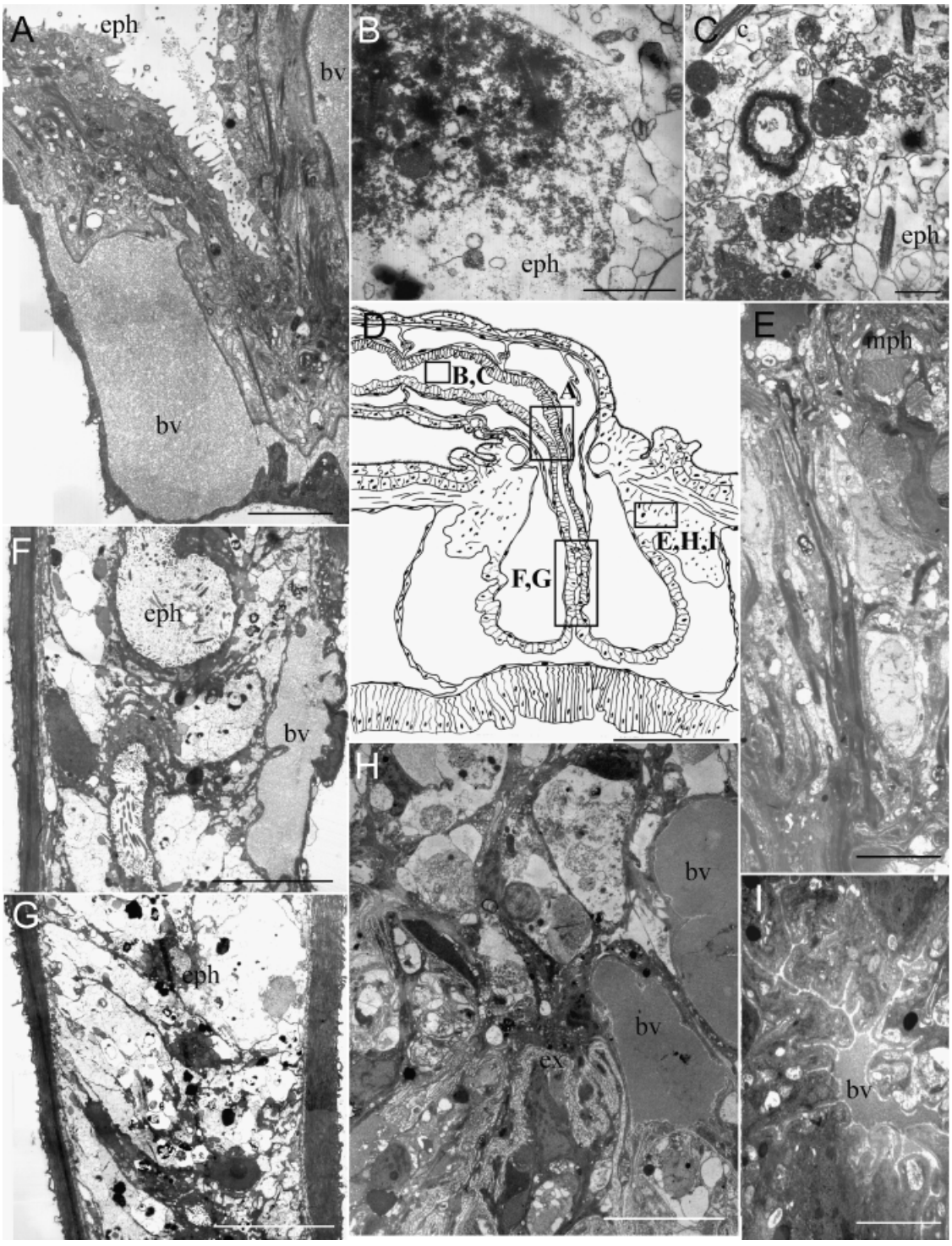


**Fig. 6.** *Scolelepis laonicola*. **A.** Region of male's intestine near the place of attachment male to female. There are two large blood vessels and unclear contents of intestine. Scale bar, 3 μm. **B, C.** Contents of male intestine. Scale bar, 2 μm. **D.** Reconstruction of the attachment zone of male. Frames and letters correspond to transmission electron micrographs. **E, H, I.** Tissues of fusion zone with blood vessels. Scale bar, 500 μm. **F, G.** Intestine of male inside the female's coelomic cavity. Scale bar 500 μm. Abbreviations: bv, blood vessel; c, cilia of intestine; eph, pharynx; ex, extracellular matrix.

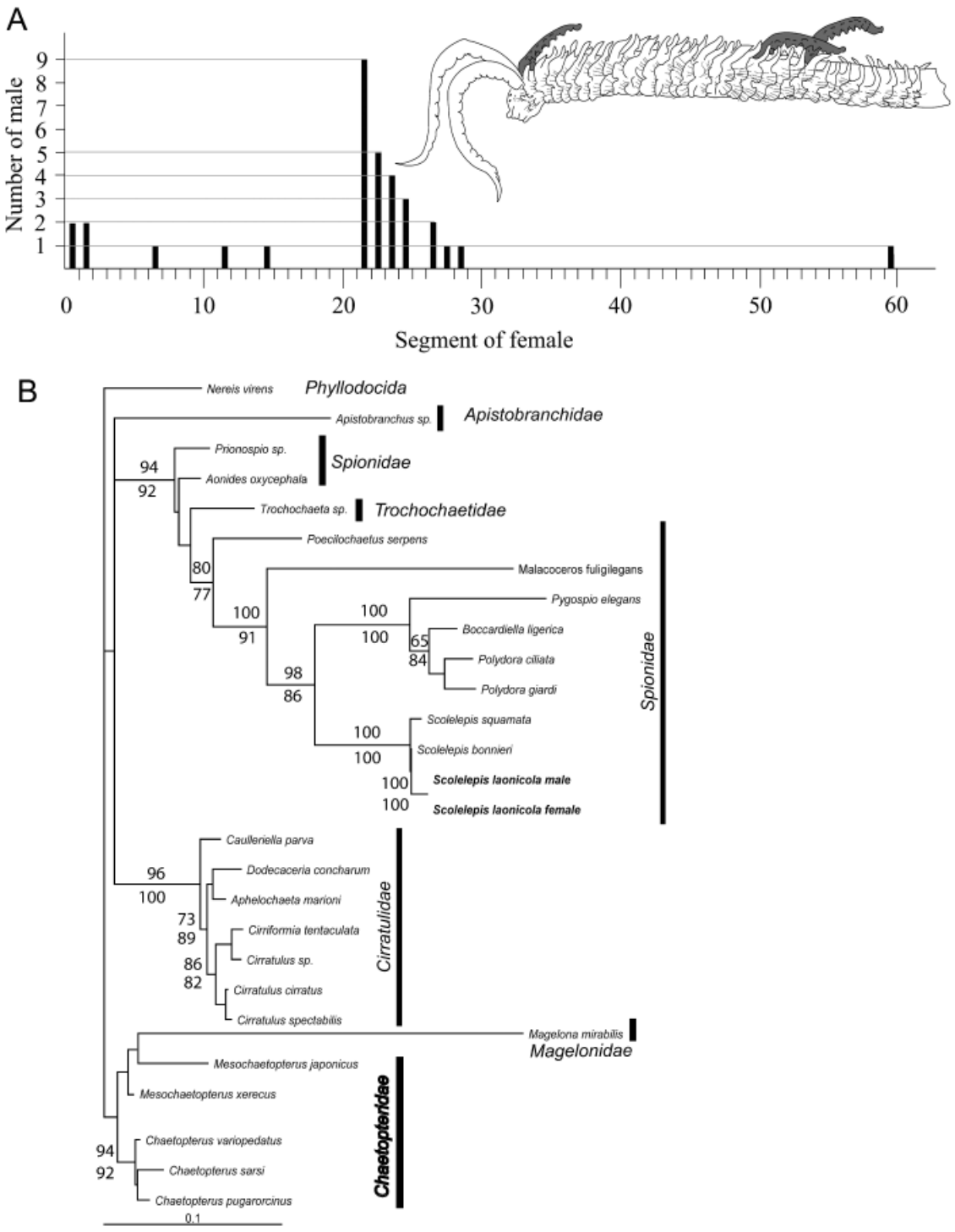


**Fig. 7.** *Scolelepis laonicola.* **A.** Distribution of males along the females' body. **B.** Strict consensus of three most parsimonious trees. Bootstrap frequencies under ML and MP criteria are given above and below the branches. Abbreviations: f, female; m, male.

*S. matsugae* are smaller than our worm. The females in our material have a well-developed pair of nuchal organs and an occipital antenna, which we did not observe in the holotype of *S. matsugae*. Furthermore, the biology of our worms differs from that of *S. matsugae,* which inhabits another type of sediment

and is not sexually dimorphic. This allows us to consider the material of the present study as a separate species of *Scolelepis,* closely related to *S. matsugae*. As a result, we place the male worm (*Asetocalamyzas laonicola*) and its female in *Scolelepis laonicola* (comb. n.). Thus, *Asetocalamyzas* is a junior synonym of *Scolelepis*. Because taxon sampling in Spionidae and probably related groups is still rather poor, no further conclusions regarding the monophyly and relationships of these taxa can be drawn from the current analysis (see also Rousset et al. 2007).

## Biology

The dwarf males parasitic on the females of *S. laonicola* represent a very extreme type of sexual dimorphism. The males are firmly attached to the females and their tissues are completely fused in the zone of contact. Because the males have no mouth opening to the exterior, they must feed at the expense of the females. Two modes of feeding are suggested for the male individuals. They can consume nutrition directly from the blood of the female through the contact zone. Although blood vessels are present in this zone, it is not clear whether the blood vessels of the male and female interlace and form a structure resembling a placenta. Such a type of male–female relationship is only known for oceanic angler-fishes (Teleostei, Ceratioidea) (Munk 2000), and has never been reported previously in polychaetes. The other possibility is that the parasite swallows the female's coelomic liquid. The well-developed intestine of the males, with some contents, and the mouth opening situated inside the body cavity of the female are consistent with this second hypothesis. It is possible that males combine both modes of feeding.

*Scolelepis laonicola* represents an extreme case of sexual dimorphism in the polychaetous Annelida. Less extreme sexual dimorphism, with representatives of the different sexes only differing in shape, size, and arrangement of the outer appendages, is quite common among polychaetes. For instance, among Spionidae, sexual dimorphism is found in *Pygospio elegans* (Schlötzer-Schrehardt 1987, 1991; Hartman-Schröder 1996); in Syllidae, among the Autolytinae; in the Alvinellidae, in *Paralvinella;* in certain Polynoidae; and in the dorvilleid *Ophryotrocha* (Åkesson 1975; Zal et al. 1994; Jollivet et al. 2000; Zhadan et al. 2000; Nygren 2003; Tzetlin & Purschke 2005). In a number of protandrous hermaphrodites, males are smaller than females, for example in *Myzostoma glabrum* and *Myzostoma alatum* (Myzostomida) (Graff 1877; Eeckhaut & Jangoux 1991; Grygier 2000).

Dwarf males also occur in several other taxa of Annelida, including the Dinophilidae, the Bonellidae (Echiura), and the Siboglindae (Zenkevitch 1966; Schuchert & Rieger 1990; Westheide 1990; Rouse et al. 2004; Blake 2006). Males of *Dinophilus gyrociliatus* are trochophore-like spherical animals, having a prototroch, paired protonephridia, testes, and penis (Windoffer & Westheide 1988). They release sperm into the tissues of the larger female juveniles inside the egg cocoons (Westheide 1990). Males of Bonellidae are small turbellarian-like worms covered by cilia. They also show an acoelomate trochophore-like level of organization. These dwarf males of different bonnelid species inhabit the nephridia of the females and can be found in a special area between the two nephridiopores or the surface of the basal part of the introvert. This type of sexual dimorphism is known for all species of Bonellidae (Stephen & Edmonds 1972). Recently described dwarf males of *Osedax rubiplumus* and *Osedax frankpressi* (Siboglinidae) appear to be very similar to the trochophores of Siboglinidae (Rouse et al. 2004). In this case the dwarf males inhabit the tubes of the females. Very likely, all these dwarf males are of neotenic or progenetic origin. Their cilia are used for locomotion; they are unsegmented and possess an acoelomate body cavity and protonephridia. Some of them (Dinophilidae) are lecithotrophic; some are parasites or symbionts of their large females (Bonellidae and *Osedax*). None of them is able to feed on its own.

In contrast to the dwarf males of the different annelids mentioned above, the parasitic males of *S. laonicola* show a typical polychaete-like organization. Their body comprises up to nine to 14 segments with parapodia, a well-developed digestive tract, a circulatory system, segmental metanephridia, well-developed septae, etc. Besides the number of segments they differ from typical spionid polychaetes only in the absence of chaetae and palps, and their reduced nervous system and sense organs.

Both larval development and settlement in *S. laonicola* are still unknown. The species has a modified thread-like spermatozoon, the nucleus of which comprises two depressions, a long midpiece, and a long flagellum (Vortsepneva et al. 2006). On the basis of these characteristics, the spermatozoon of *S. laonicola* may be referred to as introsperm (Jamieson & Rouse 1989). It allows us to assume either pseudocopulation or internal fertilization for *S. laonicola*. Because no copulatory organs were found in *S. laonicola,* they most probably transfer sperm by pseudocopulation that occurs inside the female's tube.

A few cases of impregnation of females with sperm of the introsperm type are known in Annelida

(Myzostomida [Grygier 2000]). Given that no penile structures were found in the dwarf males, production of spermatophores appears to be the most probable mode, and transfer by pseudocopulation is likely, as it is the type of fertilization present in most other spionids (Blake & Arnofsky 1999). Most probably, the male and the female spawn synchronously.

**Acknowledgments.** We thank Dr. T. Struck for discussions and suggestions. We express our gratitude to: P. Rybnikov (†), I. Kosevich, G. Kolbasov, K. Soloviev, I. Kronberg, O. Savinkin, Ò. Britaev. A. Dmitriev, N. Budaeva, S. Pyataeva, E. Sineva, V. Marinocheva, T. Kuzmina, V. Britaeva, and M. Pluscheva for help in SCUBA diving, sampling, and processing of the numerous samples. Furthermore, we thank A. Volkov for help with molecular studies, and G. Davidivich, N. Agalakova, A. Bogdanov (Laboratory of electron microscopy of Moscow State University), and D. Gulyaev and E. Citrin (Institute of Developmental Research, Russian Academy of Science) for help with electron microscopy. We are very grateful to V. Mokiewsky for help in analyzing the bottom communities. This contribution was supported in part by the Russian Foundation for Basic Researches, Grant N-04-04-48785.

## References

Åkesson B 1975. Reproduction in the genus *Ophryotrocha* (Polychaeta, Dorvilleidae). Pubb. Staz. Zool. Napoli 39: 377–398.

Aljanabi SM & Martinez I 1997. Universal and rapid salt-extraction of high quality gnomic DNA for PCR-based techniques. Nucl. Acids Res. 25: 4692–4693.

Arwidsson I 1932. *Calamyzas amphictenicola,* ein ektoparasitischer Verwandter der Sylliden. Zool. Bidr. Upps. 14: 153–218.

Blake JA 2006. Spionida. In: Reproductive Biology & Phylogeny of Annelida. Rouse GW & Pleijel F, eds., pp. 565–638. Science Publishers, Enfield, NH.

Blake JA & Arnofsky PL 1999. Reproduction and larval development of the spioniform Polychaeta with application to systematics and phylogeny. Hydrobiologia 402: 57–106.

Bleidorn C, Vogt L, & Bartolomaeus T 2003. A contribution to sedentary polychaete phylogeny using 18S rRNA sequence data. J. Zool. Syst. Evol. Res. 41: 186–195.

Coyer JA, Robertson DL, & Alberte RS 1994. Genetic variability within a population and between diploid/haploid tissue of *Macrocystis pyrifera* (Phaeophyceae). J. Phycol. 30: 545–542.

Doyle JJ & Doyle JL 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Eeckhaut I & Jangoux M 1991. Development and behavior of *Myzostoma alatum* and *Pulvinomyzostomum pulvinar,* two myzostomid symbiotes of the comatulid *Leptometra phalangium* (Echinodermata). In: Echinoderm Research. Scalera-Liaci L & Canicatti Ñ, eds., pp. 229–236. A.A. Balkema, Rotterdam, the Netherlands.

Folmer O, Black M, Hoeh W, Lutz R, & Vrijenhoek R 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3: 294–299.

Graff L v 1877. Das Genus *Myzostoma* (F. S. Leuckart). Wilhelm Engelmann, Leipzig, Austria 82 pp.

Grygier M 2000. Class Myzostomida. In: Polychaetes and Allies: The Southern Synthesis. Fauna of Australia. Vol. 4A Polychaeta, Myzostomida, Pogonophora, Echiura, Sipuncula. Beesly PL, Ross GJB, & Glasby CJ, eds., pp. 297–329. CISRO Publishing, Melbourne, Australia.

Hartman-Schröder G 1996. Annelida, Borstenwürmer, Polychaeta. Tierwelt Deutschlands 58: 1–648.

Imajima M 1992. Spionidae (Annelida, Polychaeta) from Japan. VIII. The genus *Scolelepis*. Bull. Nat. Sci. Mus. 18: 1–34.

Jamieson BGM & Rouse GW 1989. The spermatozoa of the Polychaeta (Annelida): an ultrastructural review. Biol. Rev. 64: 93–157.

Jördens J, Struck T, & Purschke G 2004. Phylogenetic interference regarding Parergodrilidae and *Hrabeiella periglandulata* (''Polychaeta'', Annelida) based on 18S rDNA, 28S rDNA and COI sequences. J. Zool. Syst. Evol. Res. 42: 270–280.

Jollivet D, Empis A, Bake MC, Hourdez S, Comtet T, Jouin-Toulmond C, Desbruyeres D, & Tyler PA 2000. Reproductive biology, sexual dimorphism, and population structure of the deep sea hydrothermal vent scaleworm, *Branchipolynoe seepensis* (Polychaeta: Polynoidae). J. Mar. Biol. Assoc. UK 80: 55–68.

Martin P 2001. On the origin of the Hirudinea and the demise of the Oligochaeta. Proc. R. Soc. London B 268: 1089–1098.

McHugh D 2005. Molecular systematics of polychaetes. Hydrobiologia 535/536: 309–318.

Munk O 2000. Histology of the fusion zone between the parasitic male and the female in the deep-sea anglerfish *Neoceratias spinifer* Pappenheim, 1914 (Teleostei, Ceratioidei). Acta Zool. 81: 315–324.

Nygren A 2003. Autolytinae: molecules, morphology, and reproduction. PhD thesis, Department of Marine Ecology, University of Gothenburg, Gothenburg. 250 pp.

Posada D & Crandall KA 2001. Selecting the best-fit model of nucleotide substitution. Syst. Biol. 50: 580–601.

Rouse GW, Goffredi SK, & Vrijenhoek RC 2004. *Osedax*: bone-eating marine worms with dwarf males. Science 305: 668–671.

Rousset V, Pleijel F, Rouse GW, Erséus C, & Siddall ME 2007. A molecular phylogeny of annelids. Cladistics 23: 41–63.

Schirmacher A, Schmidt H, & Westheide W 1998. RAPD-PCR investigations on sibling species of terrestrial

*Enchytraeus* (Annelida: Oligochaeta). Biochem. Syst. Ecol. 26: 35–44.

Schlötzer-Schrehardt U 1987. Ultrastructural investigation of the nuchal organs of *Pygospio elegans* (Polychaeta) II. Adult nuchal and dorsal organs. Zoomorphology 107: 169–179.

——— 1991. Ultrastructural differentiation of nuchal and dorsal organs during postembryonic and sexual development of *Pygospio elegans* Claparède (Polychaeta: Spionidae). Ophelia 5 (Suppl.): 633–640.

Schuchert P & Rieger RM 1990. Ultrastructural observations on the dwarf male of *Bonellia viridis* (Echiura). Acta Zool. 71: 5–16.

Sikorski AV 1994. New arctic species of *Scolelepis* (Polychaeta, Spionidae). In: Actes de la 4éme Conférence internationale des Polychètes. Dauvin JC, Laubier L, & Reish DJ, eds., 279–289. Mem. Mus. Natn. Hist. Nat. 162.

Stephen AC & Edmonds SJ 1972. The Phyla Sipunculida and Echiurida. Br. Mus. (National History), London, UK. 102 pp.

Struck TH, Schult N, Kusen T, Hickman E, Bleidorn C, McHugh D, & Halanych K 2007. Annelid phylogeny and the status of Sipuncula and Echiura. BMC Evol. Biol. 7: 57.

Swofford DL 2002. PAUP—Phylogenetic Analysis Using Parsimony, Version 4.0. Sinauer Associates, Sunderland, MA.

Tamura K & Nei M 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10: 512–526.

Tzetlin AB 1985. *Asetocalamyzas laonicola* gen. et sp. n., a new ectoparasitic polychaeta from the White Sea. Zool. Zh. 64: 296–298 [in Russian].

Tzetlin AB & Purschke G 2005. Pharynx and intestine. Hydrobiologia 535/536: 199–225.

Vortsepneva E, Zhadan A, & Tzetlin A 2006. Spermatogenesis and sperm ultrastructure of the aberrant polychaete *Asetocalamyzas laonicola,* an ectoparasite of *Scolelepis* sp. (Polychaeta, Spionidae). Sci. Mar. 70 (Suppl. 3): 343–350.

Westheide W 1990. Polychaeta: interstitial families. Synopsis Br. Fauna, New Series 44: 1–152.

Westheide W & Hass-Cordes E 2001. Molecular taxonomy: description of a cryptic *Petita* species (Polychaeta: Syllidae) from the island of Mahé (Seychelles, Indian Ocean) using RAPD markers and ITS2 sequences. J. Zool. Syst. Evol. Res. 39: 103–111.

Whiting MF, Carpenter JC, Wheeler QD, & Wheeler WC 1997. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst. Biol. 46: 1–68.

Windoffer R & Westheide W 1988. The nervous system of the male *Dinophilus gyrociliatus* (Annelida: Polychaeta). I. Number, types and distribution pattern of sensory cells. Acta Zool. (Stockholm) 69: 55–64.

Zal F, Desbruyeres D, & Jouin-Toulmond C 1994. Sexual dimorphism of *Paralvinella grasslei,* a polychaete annelid from deep-sea hydrothermal vents. C. R. Acad. Sci. Ser. 3317: 42–48.

Zenkevitch LA 1966. The systematics and distribution of abyssal and hadal (ultraabyssal) Echiuroidea. Galathea Rep. 8: 175–184 [in Russian].

Zhadan AE, Tsetlin AB, & Safronova MA 2000. Anatomy of some representatives from the family Alvinellidae (Polychatea, Terebellida) from the Pacific hydrothermal habitats. Zool. Zh. 79: 141–160 [in Russian].