

# Data on the life cycle of parasitic benthimermithid nematodes with the description of a new species discovered in marine aquaria

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**Summary.** A species of the mostly deep-sea parasitising nematode family Benthimermithidae has been for the first time discovered alive in marine aquaria of St. Petersburg City, Russia. Parasitising juveniles, young females and a young adult male were found in small crustaceans: Ostracoda, Isopoda, Tanaidacea and Amphipoda. The nematode is described as *Trophomera granovitchi* sp. n. which is characterised by relatively large and distinctly edged amphideal fovea and is distinguished from related *T. laubieri*, *T. filiformis*, *T. petterae*, *T. pacifica*, *T. aptera* and *T. leptosoma* predominantly by the size of body and organs. The male of *T. granovitchi* sp. n., while differing from females in smaller body size, has the same type of amphideal fovea. Parasitising juveniles of *T. granovitchi* sp. n. differ from adults in having a normal pharynx with a small anterior stylet-like structure, normal midgut, tail with a vestigial terminal spinneret, and by the absence of obvious sensilla. Levels of infection of various host crustaceans by nematodes vary considerably. Adult specimens emerging from various hosts differ in body size.

**Key words:** Benthimermithidae, parasitic nematodes, taxonomy, *Trophomera*.

Parasitic benthimermithid nematodes were discovered in 1970s to the beginning of 1980s during intensive exploration of deep-sea meiofauna. The family Benthimermithidae and its type genus *Benthimermis* were established by Petter (1980); a few species were described earlier under designations “Mermithida” or “Marimermithidae” (Rubzov & Platonova, 1974; Hope, 1977; Rubzov, 1980). It was promptly ascertained (Petter, 1980, 1983A; Rubzov, 1980; Tchesunov, 1988A) that benthimermithids parasitise as juveniles in the body cavity and internal organs of a variety of deep-sea benthic invertebrates, while adults come out of the hosts into the environment where they do not feed but live at the expense of stored nutrients and reproduce. In view of such a mode of life, adult benthimermithid nematodes have no functioning mouth, their pharynx is vestigial, the midgut is transformed into a depository for nutrients being utilised during the period of adult life in the bottom sediment. At the same time, sensory organs of benthimermithids such as papillae, setae and amphids are well developed (Petter, 1981A, B, 1982A, B, 1983A, B, 1987; Tchesunov, 1988A, B).

All known 38 species and three genera of Benthimermithidae are summarised in identification keys published recently by Miljutin & Miljutina (2009). The name *Benthimermis* Petter 1980 was replaced by its senior synonym *Trophomera* Rubzov & Platonova 1974 (Miljutin, 2006). The microscopic anatomy of several species was studied in some detail on the basis of semithin and ultrathin sections (Tchesunov, 1988A, B; Miljutin & Tchesunov, 2001). It was argued on morphological grounds that benthimermithids do not relate to Mermithida and Marimermithida, two other nematode groups with similar life cycle, but constitute a separate high-ranked taxon (order) within the class Chromadorea. Benthimermithids share merely superficial similarity with species of Mermithida and Marimermithida juvenile parasites of invertebrates (Tchesunov, 1977). Benthimermithid species are recorded in all oceans; two species are known from the intertidal zone of the Kuril Islands (north-west Pacific). Other species are distributed from slopes of submerged mountains (depth 31 m) to abyssal plains (5880 m) where most of species were found within a depth range of 2000-

4000 m (Miljutin, 2004). The range of hosts includes free-living nematodes, priapulids, polychaetes, sipunculids, crustaceans (Ostracoda, Isopoda and Amphipoda), and echinoderms (Ophiuroidea, Holothuroidea) (Hope, 1977; Rubzov, 1980; Petter, 1980, 1983A; Tchesunov, 1988B, 1997; Miljutin & Miljutina, 2009).

Nevertheless, some fundamental questions still remain unresolved in this little field of nematology. Because of difficulties in obtaining rare deep-sea material, the benthimermithids are currently one of the last few high-ranked nematode taxa that does not yet have genetic data. This is an obstacle impeding determination of the position of benthimermithids in the phylogenetic tree of Nematoda. Deficiency of material means that descriptions of benthimermithid males and females are limited. Most descriptions are based either on a single specimen or several adult free-living specimens of the same sex. Both males and females are known only for two of 38 benthimermithid species, namely *Trophomera australis* (Petter 1983) and *T. petterae* (Miljutin 2004). Parasitic stages (earlier juveniles) are also poorly known; they were never found together with adults or even with late parasitic stages possessing sensilla and genital rudiments.

Therefore, our unexpected findings of a benthimermithid species in a marine aquarium at the Department of Invertebrate Zoology of St. Petersburg State University may be of great importance. The parasites were found by the second coauthor by dissecting small crustaceans. As usual, the population of marine aquaria is made up of intentionally acquired tropical fish, large spectacular invertebrates and so-called living stones, fragments of dead coral with rich epi- and infauna of minor animals. The geographical origin of these components is largely unknown; hence, the new benthimermithid species from the aquarium has no type locality. Nevertheless, a find of live benthimermithids apparently accomplishing the entire life cycle in an aquarium enables further insight into studies of diversity, biology and phylogeny of the Benthimermithidae.

## MATERIAL AND METHODS

The benthimermithid nematodes were found in two marine aquaria (35‰ salinity) in the city of St. Petersburg (Russia): 1) at the Department of Invertebrate Zoology, Faculty of Biology, St. Petersburg State University (SPbSU), and 2) Eco-Biological Center "Krestovskii Ostrov" (EBC). All specimens were collected in 2008 and 2009 from crustaceans only. Two strategies for nematode sampling were applied: 1) direct dissection of

crustaceans, and 2) raising those crustaceans, whose infection was determined beforehand during preliminary inspection under stereomicroscope (Fig. 1) in isolated microaquaria and gathering adult nematodes leaving the hosts. Artificially extracted nematodes died quickly. Specimens from infected crustacea were fixed 3-4 days after emerging from the host. A search for free-living stages in aquarial sediment and algal outwashes was unsuccessful. Nematodes were fixed either with 96° ethanol for subsequent DNA extraction and further molecular studies (Tchesunov *et al.*, 2009) or with 4% formol dissolved in sea water for morphological study using optical microscopy. Formol-fixed specimens were transferred afterwards to pure glycerol using the method of Seinhorst (1959), mounted on permanent slides and examined under the light microscope (Leica DM 5000). Type specimens are deposited in the Museum of the Helminthological collections of the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Moscow, Russia.

## DESCRIPTION

Order Benthimermithida Tchesunov 1995  
Family Benthimermithidae Petter 1980  
Genus *Trophomera* Rubtzov & Platonova  
1974 (= *Abos* Rutzov 1980, = *Benthimermis*  
Petter 1980)

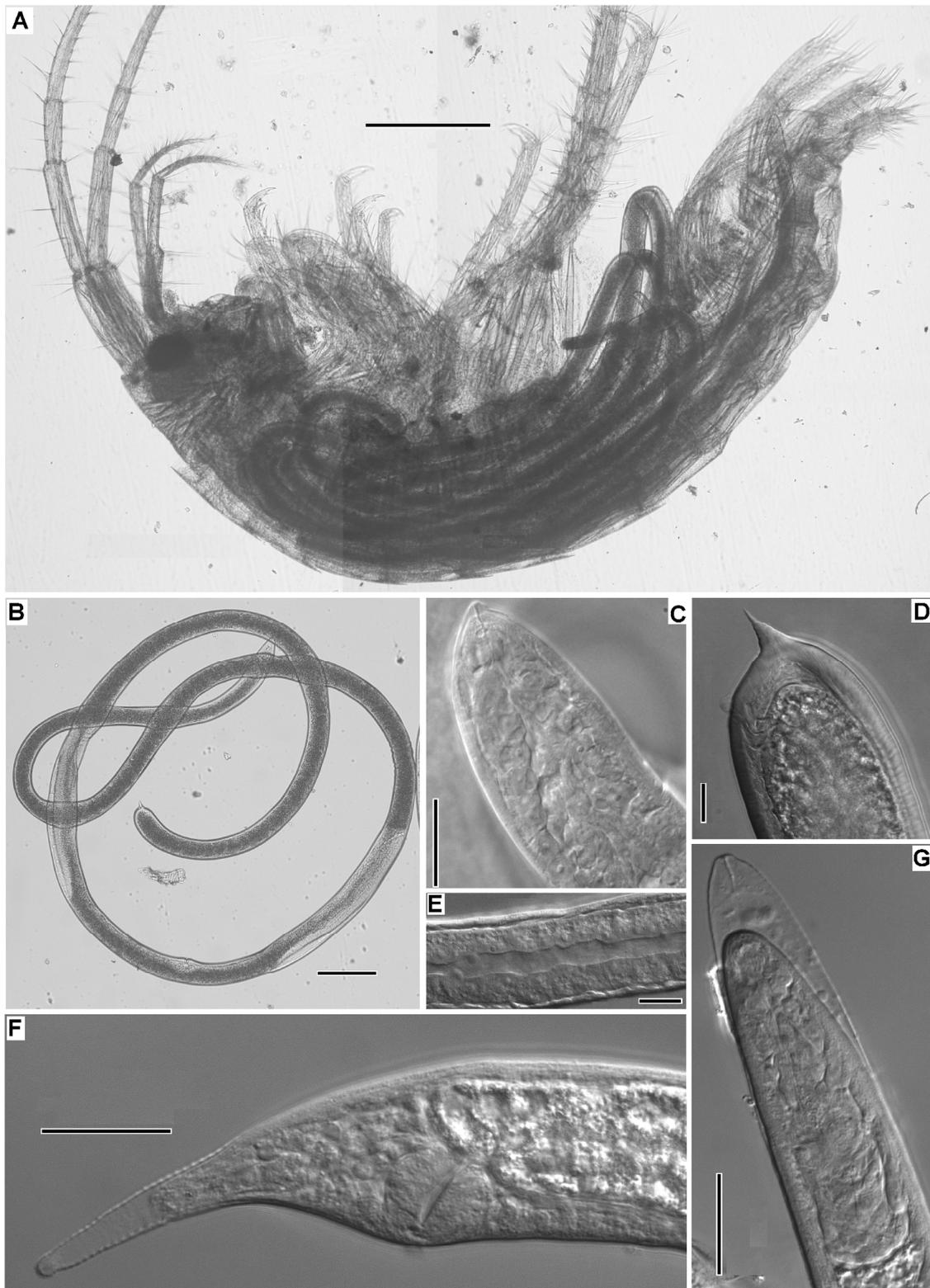
### *Trophomera granovitchi* sp. n.

**Adult females.** Figs 1 A, B, D; 2 A-C

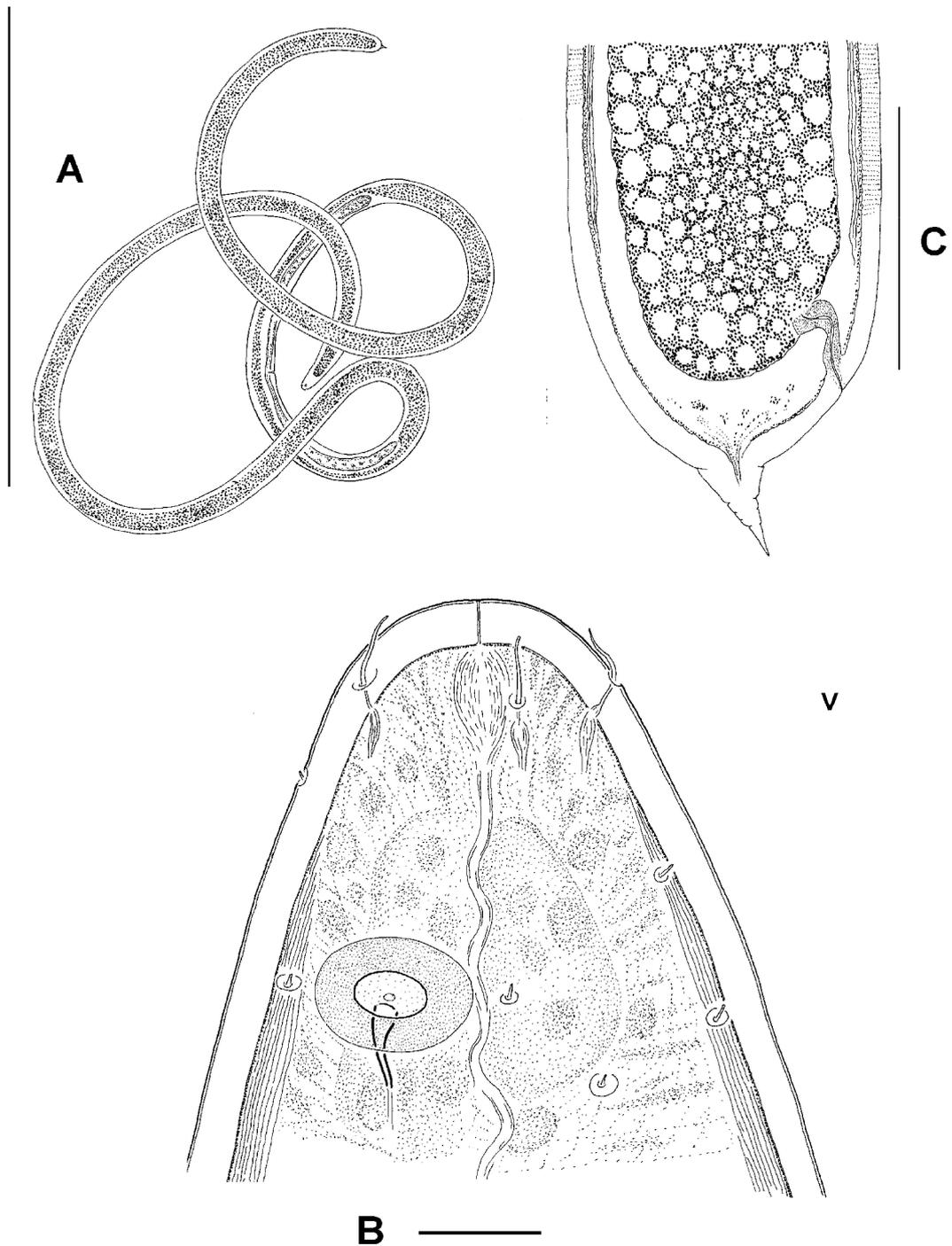
**Material:** Eight young females (one holotype and seven paratypes) with fully developed anterior sensilla and ovaries, extracted from crustacean bodies at later stage of parasitism.

Body long, cylindrical. Body length varies greatly from 2590 to 7005  $\mu\text{m}$ ;  $a=46.3-79.1$ ;  $V=50.6-62.5\%$ . Body diameter at the level of: cephalic setae - 14-20  $\mu\text{m}$ , amphideal fovea - 28-47  $\mu\text{m}$ , anterior end of the trophosome - 41-66  $\mu\text{m}$ , midbody - 53-100  $\mu\text{m}$ , rectum - 42-70  $\mu\text{m}$ . Cuticle thick and seemingly smooth under optical microscope. Thickness of the cuticle apically 5  $\mu\text{m}$ , at the anterior end of the trophosome 4  $\mu\text{m}$ , at the midbody 4-5  $\mu\text{m}$ , at the posterior end of the trophosome 5-6  $\mu\text{m}$ , at the base of terminal spike 7-8  $\mu\text{m}$ .

There is a very thin axial canal instead of mouth opening. Neither inner nor outer labial sensilla visible; four thin cephalic setae 4.5-5.5  $\mu\text{m}$  long inserted in small pits. There are a few minute papillae or very short setae distributed irregularly anterior to the trophosome.



**Fig. 1.** *Trophomera granovitchi* sp. n., details: A: – a gammarid amphipode specimen heavily infected by nematodes; B: – adult female (paratype) extracted from the host; C: – anterior body of a parasitic juvenile; D: – tail of a paratype adult female; E: – midgut of a parasitic juvenile; F: – tail of a parasitic juvenile; G: – anterior body of a moulting parasitic juvenile. Scale bars: A = 500  $\mu\text{m}$ ; B = 200  $\mu\text{m}$ ; C = 20  $\mu\text{m}$ ; D = 15  $\mu\text{m}$ ; E = 20  $\mu\text{m}$ ; G = 20  $\mu\text{m}$ .



**Fig. 2.** *Trophomera granovitchi* sp.n., holotype female: A: – entire; B: – anterior end; C: – tail. Scale bars: A = 1 mm; B = 10 μm; C = 50 μm.

Amphideal fovea transversally oval, 6-8  $\mu\text{m}$  wide, encircled with a cuticular bolster or halo with an indistinct outer contour. Distance from the cephalic apex to the fovea is 17-30  $\mu\text{m}$ . Amphideal nerve comes out from the center of fovea as a funnel-shaped structure and extends posteriad and deep into the body.

Pharynx short and vestigial, appearing as a fuzzy-shaped string. There is a slightly coiled internal lumen in the anterior part of the string; the lumen disappears posteriorly. The pharyngeal string widens posteriorly into conical mass, which appears to be glandular. The string is entirely devoid of muscular striation. There is a very fine transverse fibrous structure at the posterior end of the pharyngeal string, a possible nerve ring. Length of the pharynx (distance from the cephalic apex to the trophosome) is 67-79  $\mu\text{m}$ .

Trophosome with distinct cell borders; the cells filled densely with spherical inclusions. No internal lumen was observed within the trophosome. The trophosome squeezed into a thin string at the level of gonads. There is a distinct cuticular rectal tube at the posterior end of the trophosome.

Ovaries are paired and reflected. Vulva is encircled with a distinct sphincter.

Posterior end is rounded and possess a conical acute spike 10-23  $\mu\text{m}$  long which in some specimens seems somewhat two-jointed.

**Male.** Fig. 3 A, B

**Material:** Single specimen with missing posterior body part, with remains of exuviae of the previous stage over the cuticle. Posterior body has been lost during dissection of the ostracode host.

Body length (without posterior part) is 1214  $\mu\text{m}$ . Body diameter at the level of: cephalic setae - 9.5  $\mu\text{m}$ , amphideal fovea - 15.5  $\mu\text{m}$ , anterior and of the trophosome - 22.5  $\mu\text{m}$ , midbody - 23  $\mu\text{m}$ . Body cylindrical, slender. Cuticle thin, with fine transversal striation without a lateral differentiation.

Cephalic end rounded, with an obscure apical pore instead of the mouth. Inner and outer labial sensilla not visible. Four cephalic setae 2.5  $\mu\text{m}$  long. There is a tiny papilla visible on the left body side at half distance from the cephalic apex to the amphideal fovea. No other sensilla anterior or posterior to the amphideal fovea. No lateral cuticular ridge in the anterior region.

Amphideal fovea small, round, with distinct cuticular rim, 4  $\mu\text{m}$  wide, distance from the cephalic apex to the fovea is 18  $\mu\text{m}$ . Amphideal nerve extends out deep and posterior from the fovea.

Distance from the cephalic apex to the trophosome is 76  $\mu\text{m}$ . Rather narrow and slightly curved pharyngeal string with narrow internal lumen

extends from the apical pore posteriad. The string gets lost at distance from the trophosome. Coarse granularity within the string core might correspond to the dorsal pharyngeal gland. The string widens and loses distinct delineation to the posterior region; inner lumen disappears in the posterior third of pharynx. There is a possible nerve ring at two thirds distance from the cephalic apex to the trophosome.

Trophosome filled with coarse granules and large vacuoles with light transparent content; neither internal lumen nor cell borders were observed in the trophosome.

Two male gonads, both directed anteriorly; the testes are connected by slender vas deferens. There is a long row of 18 midventral supplementary organs; the anteriormost organ is situated at the level of the anterior testis. The supplements are shaped as minute papillae or inconspicuous conical projections with apical pores; secretory drops appear from some supplements.

**Parasitic juveniles.** Fig. 1 C, E-G; 4 A-D

**Material:** Ten specimens extracted from isopods *Carpias* sp. and ostracods. Most specimens are presented by fragments.

Body cylindrical. Body length 1305-1331  $\mu\text{m}$ , a = 32.9-46.8, b = 18-19, c = 20.6-22.5. Body diameter at the level of: nerve ring - 37-38  $\mu\text{m}$ , posterior end of the pharynx - 40-45  $\mu\text{m}$ , midbody - 42-48  $\mu\text{m}$ , rectum - 42-53  $\mu\text{m}$ . Cuticle very thin, with nearly indiscernible striation except for posterior narrowed portion of the tail, which has clear and rather broad annulation. Thick epidermis is present beneath the cuticle. No muscular longitudinal striation discernible. Anterior end conical. No sensilla detected on the head and body. There is a small "stylet", a 4-7  $\mu\text{m}$  long refractive stick-like structure located apically within the cephalic end. The structure may actually be a narrowed vestigial stoma or sclerotised terminal duct of a pharyngeal gland. Vestigial pharynx gland-like enlarged anteriorly just posterior to the stick-like structure, then continues as a slightly curved string narrowed in the middle and widens again in the posterior third. Nerve ring situated in the middle of the pharynx. Midgut with distinct cell borders and internal lumen; midgut cells are filled with numerous large vacuoles with light transparent content and smaller refractive inclusions. Vestigial rectum as a bulb-like structure with a thin cuticular canal within. Primordia of the anterior and posterior gonads are situated at opposite lateral sides of the midgut. Tail conical, its terminus attenuated into a hyaline mucro. The posteriormost portion of the tail is an empty cuticular sheath. Caudal glands not

developed as such, but there is a very thin terminal duct, probably a rudiment of a spinneret.

**Diagnosis.** Female body length 2590-7005  $\mu\text{m}$ ,  $a = 46-79$ . Cuticle smooth. Distance from the cephalic apex to the amphideal fovea is 17-30  $\mu\text{m}$ , distance from the cephalic apex to the trophosome is 67-79  $\mu\text{m}$ . Cephalic setae thin, 4.5-5.5  $\mu\text{m}$  long. Sparse minute pre- and postamphideal papilla present. Amphideal fovea transversally oval, 6-8  $\mu\text{m}$  wide. Ovaries reflexed. Tail very short, rounded and with an acute terminal spike 10-23  $\mu\text{m}$  long.

Male body length >1214  $\mu\text{m}$ ,  $a > 53$ . Cuticle with very fine cross striation. Distance from the cephalic apex to the amphideal fovea is 18  $\mu\text{m}$ , distance from the cephalic apex to the trophosome is 76  $\mu\text{m}$ . Cephalic setae thin, 2.5  $\mu\text{m}$  long. A few pre- and postamphideal papillae present. Amphideal fovea round, with distinct cuticular rim, 4  $\mu\text{m}$ . No lateral ridge along the anterior body end. Two successive testes directed anteriorly. Series of tiny midventral preanal supplementary papillae  $\geq 18$  in number.

**Differential diagnosis.** *Trophomera granovitchi* sp. n. is close to *T. laubieri* (Petter, 1987), *T. filiformis* (Petter, 1987), *T. petterae* (Miljutin, 2004) and *T. pacifica* Miljutin & Miljutina, 2009 in the tail shape of females. However, *T. granovitchi* differs from all these species by the shape of transversally oval amphideal fovea with a halo, and also from each individual species in the following characteristics: from *T. laubieri* by distance from the apex to the amphideal fovea 17-30  $\mu\text{m}$  vs 48-60  $\mu\text{m}$ , midbody diameter 68-100 vs 120-200  $\mu\text{m}$ , length of the caudal spike 10-23 vs 70-100  $\mu\text{m}$ ; from *T. filiformis* by distance from the apex to the amphideal fovea 17-30  $\mu\text{m}$  vs 100  $\mu\text{m}$ , length of the caudal spike 10-23 vs 38-54  $\mu\text{m}$ ; from *T. petterae* by length of the caudal spike 10-23 vs 70-100  $\mu\text{m}$ ; from *T. pacifica* by distance from the apex to the amphideal fovea 17-30 vs 57  $\mu\text{m}$ , midbody diameter 68-100 vs 115  $\mu\text{m}$ , length of the caudal spike 10-23 vs 81  $\mu\text{m}$ .

The new species males resemble *T. aptera* (Petter, 1982) and *T. leptosoma* (Petter, 1981) among the 16 species with males described. *T. granovitchi* is more similar to *T. aptera* due to absence of lateral cuticular alae or ridges on the anterior body end, the same construction of gonads and similar number of preanal supplementary organs, but differs in having shorter cephalic setae (2.5  $\mu\text{m}$  vs 3.5  $\mu\text{m}$ ), well-defined circular amphideal fovea and possibly shorter body length (>1214 vs 4200-6600  $\mu\text{m}$ ). *Trophomera granovitchi* differs from *T. leptosoma* in lacking lateral cuticular preamphideal ridge (vs present).

**Molecular data.** The nucleotide sequence of the 18S rRNA gene from females of *T. granovitchi* has been deposited in the GenBank under accession number JN625216. A separate paper discussing the position of *T. granovitchi* in the nematode molecular phylogenetic tree is in preparation (V.V. Aleshin and coworkers).

**Etymology.** The species is named in honor of Prof. Dr Andrei I. Granovitch, director of the Department of Invertebrate Zoology of St. Petersburg State University, who maintains the aquarium in his office where the benthimermithids were discovered.

## DISCUSSION

For the first time, three known stages or semaphoronts, namely postparasitic adult females, adult males and parasitising juveniles, surely belonging to the same species, have been found in one place simultaneously. Until now, no concurrent finds of conspecific parasitising juveniles and free-living adults have been recorded, and 36 of 38 discovered benthimermithid species were described either from males (14 species) or females (22 species). Both males and females are described only for two species, *T. australis* (Petter, 1983) and *T. petterae* (Miljutin, 2004). Unification of males and females under the same specific name was based on morphological similarity when both sexes were found in the same sample of sediment (Petter, 1983a; Miljutin, 2004). In our case, the male differs significantly from females in size of the body and organs, so much that they perhaps would not be unified in the same species even if found in the same sample from the sea. However, both females and male share a common structural character, a relatively large amphideal fovea that is evidence of their conspecificity. Probably, the smaller size of the male *T. granovitchi* is because it was extracted from the host prematurely; if it not been extracted it would have continued feeding and growing for a normal period of time within the host after its last moult. The other reason for the smaller size of the male may be that the nematodes living in such small-bodied hosts (ostracods) are not able to grow as big as those living in large-bodied hosts (isopods and amphipods).

Parasitic juveniles differ considerably from adults, first, by having a pharynx that is not reduced as much (or less vestigial) with a solid stick-like anterior structure, normal midgut, tail with a vestigial spinneret, and, second, by total absence of visible sensilla. The first set of features is inherited from any ancestral free-living nematodes which belong to Chromadorea according to molecular data

(Tchesunov *et al.*, 2009) and even to Camacolaimidae (Plectida) on morphological grounds (Tchesunov, 1997). Lack of sensilla is related to the endoparasitic mode of life. Species identification of parasitic juveniles is impeded because of absence of necessary features, *i.e.*, sensilla.

## BIOLOGY

### Hosts of the aquarial benthimermithids.

1) Isopod crustaceans *Carprias* cf. *algicola* (Miller, 1941) (Crustacea, Peracarida, Isopoda, Asellota, "Janiridae"). Body length of adults is 2-4.5 mm. All the infected specimens were pubescent. Infected isopod specimens contribute *ca* 5% of the populations as observed *via* mass analysis of live crustaceans (310 individuals in total). The real number may be a little greater as small juvenile nematodes are difficult to observe without dissection. Crustaceans of this species complex are distributed in the tropical zones worldwide – Red Sea, India, Madagascar, Hawaii, Caribbean Sea and Gulf of Mexico, from the tidal zone to about 20 m deep, on algae and coral debris (Pires, 1982; Kensley & Schotte, 2002). In aquaria, these crustaceans are rather abundant (in the absence of fish) and live on walls, stones and *Caulerpa* algae.

2) Isopod crustaceans of the family Stenetriidae (Crustacea, Peracarida, Isopoda, Asellota), possibly a new genus and species. The life style seems to be strongly epiphytal. Infected specimens were not found (12 individuals analysed).

3) Tanaid crustaceans (Crustacea, Peracarida, Tanaidacea, Apseudomorpha). Body length 1.5-7.5 mm. Five out of seven infected specimens were less than 4 mm long and apparently juveniles (18 specimens analysed). The species lives in soft sediment in contrast to epifaunal *Carprias*.

4) Amphipod crustaceans (Crustacea, Peracarida, Amphipoda, Gammaridae). Only a single infected specimen was found, but that one was heavily infected (Fig. 1A).

5) Ostracod crustaceans (Crustacea, Ostracoda). Two unidentified species. Many infected specimens.

### Some notes on locality, movement and coming out.

Nematodes lie in the body cavity of the hosts, coiled in a few turns, usually ventrally from the intestine. The nematodes can stretch out themselves at half body length and coil again. They also can move within the thoracic body tagma and even get into the abdominal region. Usually there is a single

parasite per host, but cases of multiple infection by up to four juveniles of different sizes were observed.

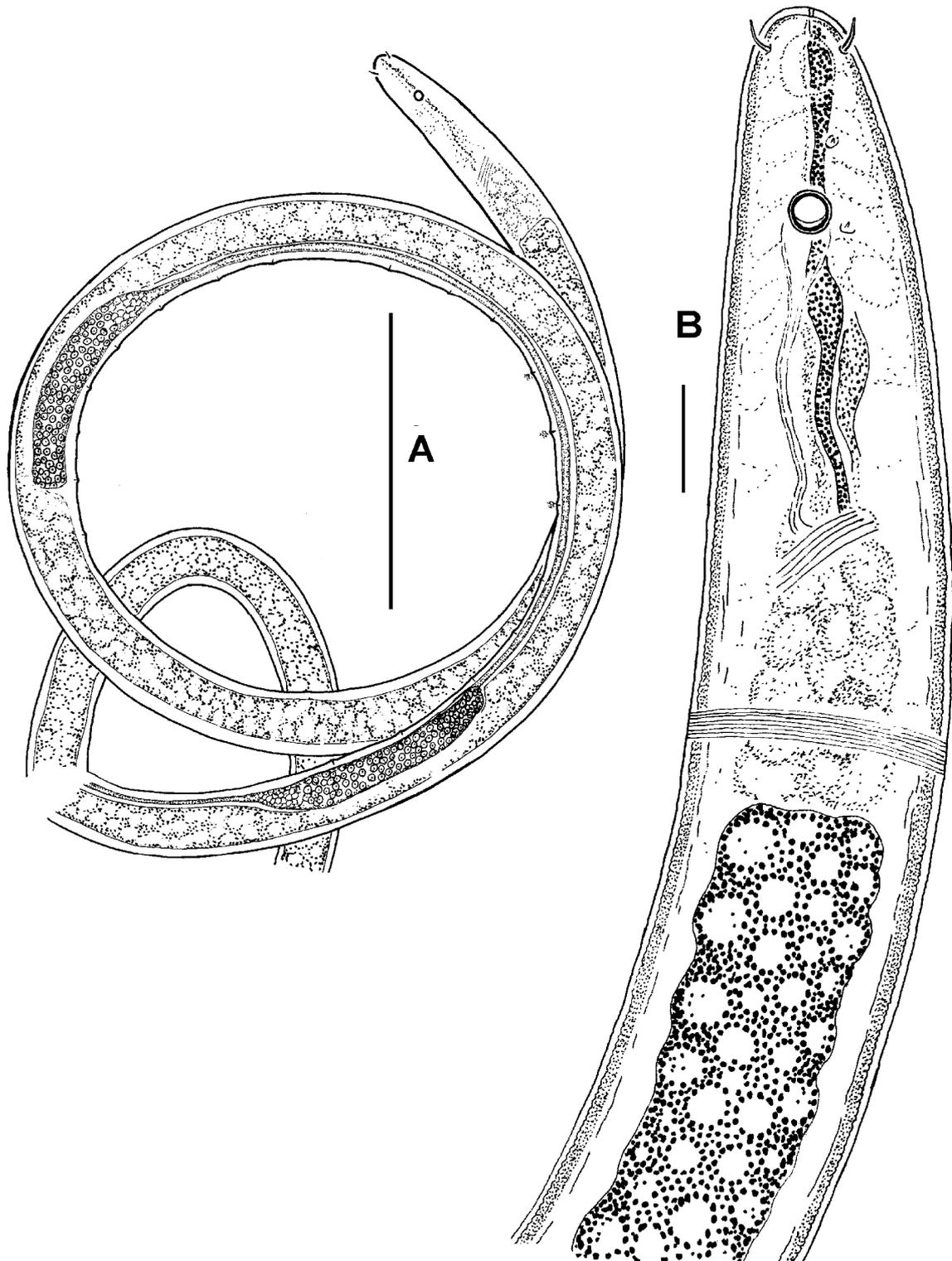
In *Carprias* cf. *algicola*, adults of both sexes can be present. In the tanaids, infection seems to be predominantly juveniles. Crustacean specimens containing nematodes show no indication of parasitic castration. Infected *Carprias* specimens show normal oo- and spermatogenesis, and they can copulate; infected females often have eggs in marsupium. Nevertheless, infected peracarids weaken and cease moving several hours before the parasite emerges. That event invariably leads to the host's death after a short time.

Nematodes leave a host through the sites of thin cuticle where extremities join. The nematodes leave hosts that had been isolated in separate vials four-five days after detection. The emerging nematodes are very active; they wriggle, swiftly coiling and uncoiling.

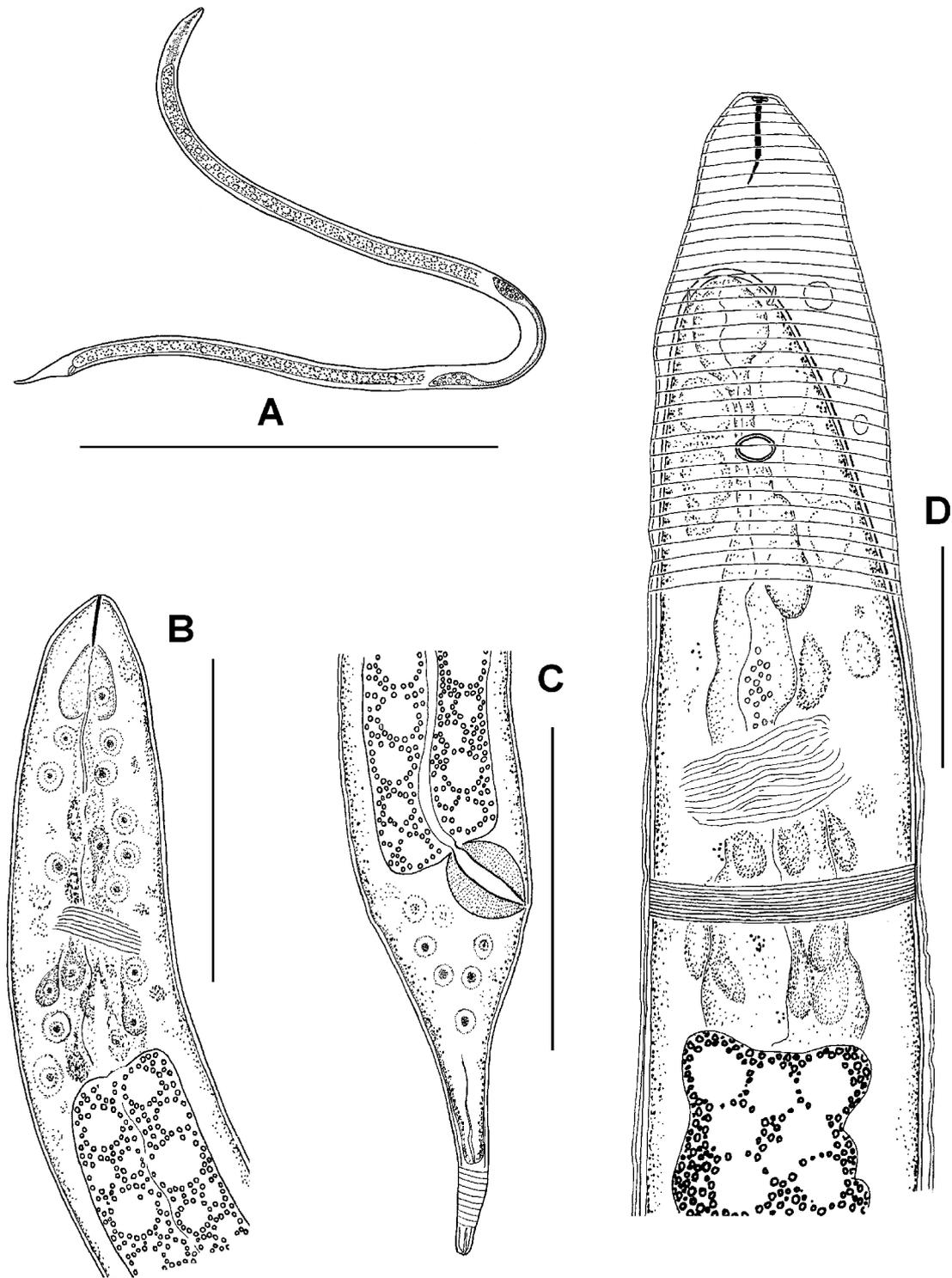
In the population of *Carprias* cf. *algicola* (the most intensively inspected host species) several specimens harbouring another parasite with a similar life cycle were found. That is apparently a fecampiid plathelminth (the group is already known to infect isopods: Christensen, 1981; Blair & Williams, 1987), probably of a new species, showing localisation similar to that of benthimermithids. At least one case of co-occurrence of specimens of both parasites inside one host individual was observed.

**Fragmented and wounded nematodes.** There are a few specimens in our collection which have evident injuries of the body. Thus, one female is missing a considerable part of the posterior body just behind the vulva. This female possesses only an anterior functioning gonad, thus becoming prodelphic. The wound is healed and the newly formed cuticle covers the remaining stump (Fig. 5 C-E). Even more striking specimens were found in one *Carprias* individual (Fig. 5 A-B). These are short pieces of the nematode body with healed wounds on both ends.

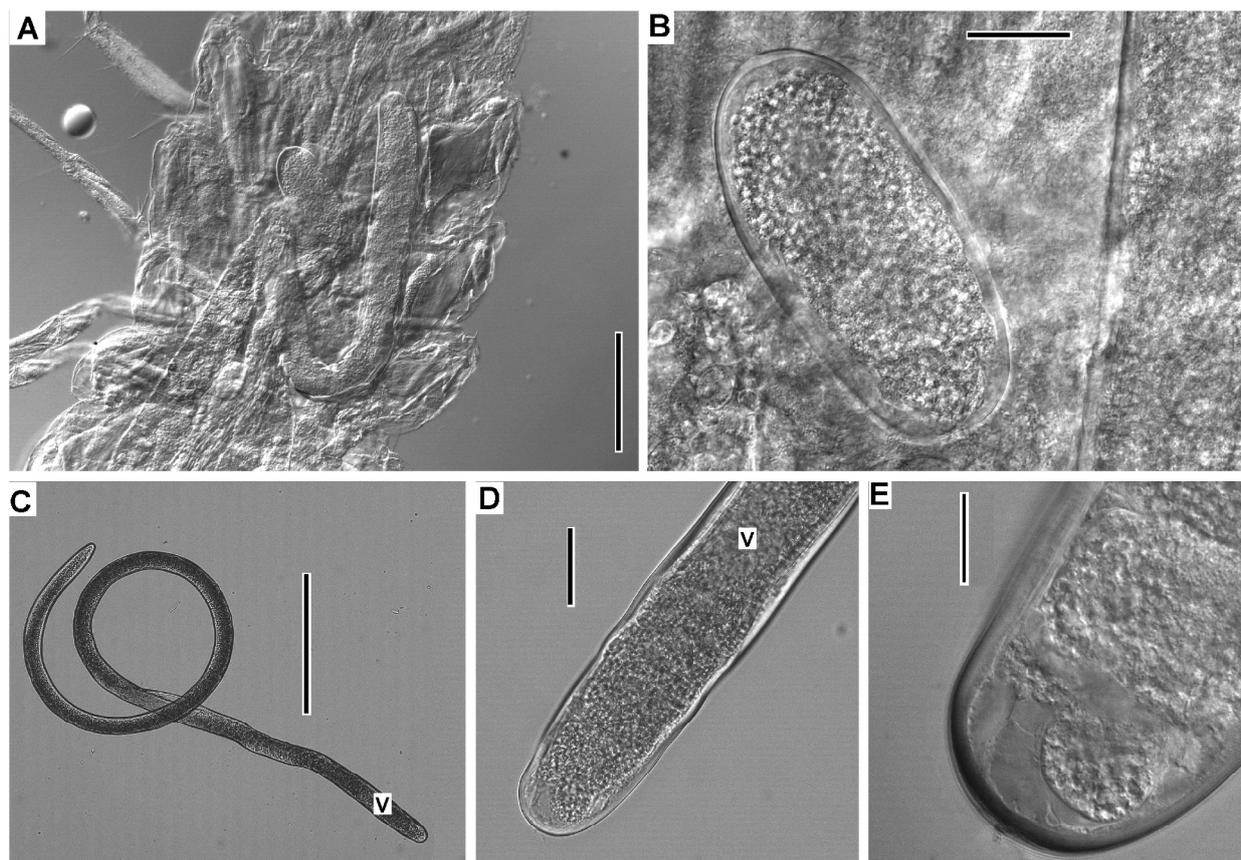
Perhaps the nematodes could be injured while emerging from the host or being damaged inside the live agile crustacean. We did not inspect the fragments closely; however, we suppose the short body pieces can possibly survive for some time within the host absorbing dissolved nutrients through the cuticle and body wall as normally do intact benthimermithid nematodes with vestigial alimentary tracts.



**Fig. 3.** *Trophomera granovitchi* sp. n., paratype male. A: – entire (posterior body lost); B: – anterior end. Scale bars: A = 100  $\mu$ m; B = 10  $\mu$ m.



**Fig. 4.** *Trophomera granovitchi* sp. n., parasitic juveniles. A: – entire; B: – anterior body; C: – tail; D: – juveniles moulting to the adult stage, anterior body. Scale bars: A = 500  $\mu\text{m}$ ; B = 50  $\mu\text{m}$ ; C = 50  $\mu\text{m}$ ; D = 20  $\mu\text{m}$ .



**Fig. 5.** *Trophomera granovitchi* sp. n., fragmented specimens. A: – an isopod crustacean *Carpias* cf. *algicola* with nematode fragments in the body cavity; B: – a small fragment of the nematode body with healed wounds on both body ends; C: – adult female with missing posterior body (“v” indicates the position of vulva); D: – postvulvar stump (“v” indicates the position of vulva); E: – healed wound with restored cuticle. Scale bars: A = 300  $\mu\text{m}$ ; B = 50  $\mu\text{m}$ ; C = 500  $\mu\text{m}$ ; D = 50  $\mu\text{m}$ ; E = 20  $\mu\text{m}$ .

## DISCUSSION

All but two species of Benthimermithidae known up to now live in the deep sea or at least in cold waters. Only *T. litoralis* and *T. iturupiensis* were found in the intertidal zone of the Kuril Islands, north-east Pacific Ocean. Since all initial components of the St. Petersburg aquaria, *i.e.*, living stones with their crustacean infauna, come from warm-water regions, *T. granovitchi* may be considered as the first shallow-water tropical or subtropical benthimermithid species, despite its exact living area being unknown. The known ecological range of the Benthimermithidae is thus broadened.

The new find of *Trophomera* in the marine aquaria fits the presumed scheme of the benthimermithid life cycle suggested earlier based on incomplete finds of individuals in samples of

deep-sea sediments. The assortment of hosts of *T. granovitchi* in aquaria is rather interesting; parasites infest four peracarid crustacean species, isopods, tanaids and amphipods, as well as taxonomically and dimensionally different ostracods. The life cycle of *T. granovitchi* could not be studied completely. No free-living adult specimens were found in the sediment or on algae of either aquaria. Meanwhile, the persistence of infection in host populations for several years strongly indicates that the parasite is able to perform the whole cycle in artificial conditions. Nematodes leave their host at the very beginning of the adult stage and seem to breed amphimictically as specimens of both sexes were found. There is a sharp sex-ratio in favour of females, a phenomenon already known for another species caught in several samples, *T. megala*, for which no males were found yet (Miljutin & Tchesunov, 2001). The route of infection and the infectious stage are unknown, but such a great

variety in host habits seems to indicate that the infection occurs passively *via* the digestive tract.

Healing of wounds is a very rare and poorly documented phenomenon in nematodes. Loss of the hind body with subsequent healing occurs in some marine free-living species of the family Linhomoeidae (Hendelberg, 1977), but as far as we know, phases of healing and restoration of cuticle and tissue has not yet been studied thoroughly.

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### REFERENCES

- BLAIR, D. & WILLIAMS, J.B. 1987. A new fecampioid of the genus *Kronborgia* (Platyhelminthes: Turbellaria: Neorhabdocoela) parasitic in the intertidal isopod *Exosphaeroma obtusum* (Dana) from New Zealand. *Journal of Natural History* 21: 1155-1172.
- CHRISTENSEN, A.M. 1981. The geographical and bathymetrical distribution of the Fecampiidae (Turbellaria, Rhabdocoela). *Hydrobiologia* 84: 13-16.
- HENDELBERG, M. 1977. *Paralinhomoeus gerlachi* (Linhomoeidae), a new marine nematode from Bermuda. *Zoon* 5: 79-86.
- HOPE, W.D. 1977. Gutless nematodes of the deep sea. *Mikrofauna des Meeresbodens* 62: 307-308.
- KENSLEY, B. & SCHOTTE, M. 2002. New species and records of Asellota from the Indian Ocean (Crustacea: Peracarida: Isopoda). *Journal of Natural History* 36(12): 1421-1461.
- MILJUTIN, D.M. 2004. New findings of deep-sea nematodes of genus *Benthimermis* Petter, 1980 (Nematoda, Benthimermithidae) with description of seven new species. *Zoosystema* 26: 21-48.
- MILJUTIN, D.M. 2006. The genus *Trophomera* Rubtsov & Platonova, 1974 with description of *T. litoralis* sp. n. (Nematoda: Benthimermithidae) from the tidal zone of the Kuril Archipelago and proposal of *Benthimermis* Petter, 1980 as a junior synonym. *Nematology* 8: 411-423.
- MILJUTIN, D.M. & MILJUTINA, M.A. 2009. Description of *Bathynema nodinauti* gen. n., sp. n. and four new *Trophomera* species (Nematoda: Benthimermithidae) from Clarion-Clipperton Fracture Zone (Eastern Tropic Pacific), supplemented with the keys to genera and species. *Zootaxa* 2096: 173-196.
- MILJUTIN, D.M. & TCHESUNOV, A.V. 2001. On the histological anatomy of *Benthimermis megala* Petter, 1987, a giant nematode from Norwegian deep-sea (Nematoda: Benthimermithidae) *Nematology* 3: 491-502.
- PETTER, A.-J. 1980. Une nouvelle famille de Nématodes parasites d'Invertébrés marins, les Benthimermithidae *Annales de Parasitologie Humaine et Comparée* 55: 209-224.
- PETTER, A.-J. 1981A. A Description des mâles d'une nouvelles espèce de nematode marin de la famille des Benthimermithidae. *Annales de Parasitologie Humaine et Comparée* 56: 285-295.
- PETTER, A.-J. 1981B. Description des mâles de trois nouvelles espèces de Nématodes de la famille des Benthimermithidae. *Bulletin du Muséum National d'Histoire Naturelle, Paris, Sér.4* 3(A)2: 455-465.
- PETTER, A.-J. 1982A. *Benthimermis gracilis* n. sp., nouveau mâle de la famille des Benthimermithidae (Nematoda). *Bulletin du Muséum National d'Histoire Naturelle, Paris, Sér.4* 4(A)1-2: 71-74.
- PETTER, A.-J. 1982B. Description de deux nouveaux mâles de la famille des Benthimermithidae (Nematoda). *Bulletin du Muséum National d'Histoire Naturelle, Paris, Sér.4* 4(A)3-4: 397-403.
- PETTER, A.-J. 1983A. Description d'un nouveau genre de Benthimermithidae (Nematoda) présentant des utérus munis de glandes annexes. *Annales de Parasitologie Humaine et Comparée* 58: 177-184.
- PETTER, A.-J. 1983B. Quelques nouvelles espèces du genre *Benthimermis* Petter, 1980 (Benthimermithidae: Nematoda) du Sud de l'Océan Indien. *Systematic Parasitology* 5: 1-15.
- PETTER, A.-J. 1987. Quelques nouvelles espèces de femelles du genre *Benthimermis* Petter, 1980 (Benthimermithidae, Nematoda) des grands fonds de la mer de Norvège. *Bulletin du Muséum National d'Histoire Naturelle, Paris, Sér.4* 9(A)3: 565-578.
- PIRES, A.M.S. 1982. Taxonomic revision of *Bagatus* (Isopoda, Asellota) with a discussion of ontogenetic polymorphism in males. *Journal of Natural History* 16: 227-229.
- RUBZOV, I.A. 1980. The new marine parasitic nematode, *Abos bathycola*, from priapulids and a taxonomic position of the family Marimermithidae in the class Nematoda. *Parazitologiya* 14: 177-181 [in Russian, English summary].
- RUBZOV, I.A. & PLATONOVA, T.A. 1974. A new family of marine parasitic nematodes. *Zoologicheskii Zhurnal* 53: 1445-1458 [in Russian, English summary].
- SEINHORST, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4: 67-69.
- TCHESUNOV (LAPS. CHESUNOV), A.V. 1988A. A case of nematode parasitism in nematodes. A new find and redescription of a rare species *Benthimermis australis* Petter, 1983 (Nematoda: Marimermithida:

- Benthimermithidae) in South Atlantic. *Helminthologia* 25: 115-128.
- TCHESUNOV, A.V. 1988b. [New finds of deep-sea nematodes of the family Benthimermithidae in South Atlantic with description of a new species.] *Vestnik zoologii* 6: 12-22 (in Russian).
- TCHESUNOV, A.V. 1997. [Marimermithid nematodes: anatomy, position in the nematode system, phylogeny.] *Zoologicheskii Zhurnal* 76(11): 1283-1300 (in Russian). English translation in: *Russian Journal of Zoology* 1: 440-455 (Tchesunov A.V. /laps. Chesunov/, "Marimermithids (Nematoda): Anatomy, Taxonomy, and Phylogeny").
- TCHESUNOV, A.V., KUZMIN, A.A. & ALESHIN, V.V. 2009. Position of Benthimermithidae (parasites of marine invertebrates) as derived from morphological and molecular data. *Russian Journal of Nematology* 17: 162.
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**А.В. Чесунов, А.А. Розенберг.** Данные по жизненному циклу и описание нового вида паразитических бентимермитид, обнаруженных в морских аквариумах.

**Резюме.** Новый вид обычно глубоководных паразитических нематод сем. Benthimermithidae впервые обнаружен в морских аквариумах в Санкт-Петербурге. Паразитические личинки, молодые самки и самцы были найдены в мелких ракообразных: Ostracoda, Isopoda, Tanaidacea и Amphipoda. Эти нематоды описаны как *Trophomera granovitchi* sp. n. и характеризуются относительно крупными размерами, четко очерченным карманом амфидов, и отличаются от близких *T. laubieri*, *T. filiformis*, *T. petterae*, *T. pacifica*, *T. aptera* и *T. leptosoma* главным образом по размерам тела и отдельных органов. Самцы *T. granovitchi* sp. n., отличаясь от самок меньшими размерами, имеют амфиды того же строения. Паразитические личинки *T. granovitchi* sp. n. отличаются от взрослых наличием нормально развитого фаринкса с небольшой стилетовидной структурой в передней части, нормально развитой средней кишкой, хвостовым концом с зачаточной терминальной спиннеретой и отсутствием различных сенсилл. Зараженность существенно варьирует у различных видов хозяев-ракообразных. Взрослые нематоды, выходящие из разных хозяев, разнятся также и по размерам тела.

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