Trophomera conchicola sp. n. (Nematoda: Benthimermithidae) from chemosymbiotic bivalves *Idas modiolaeformis* and *Lucinoma kazani* (Mollusca: Mytilidae and Lucinidae) in Eastern Mediterranean

Oleksandr Holovachov[1], Clara F. Rodrigues[2, 3], Magali Zbinden[2] and Sébastien Duperron[2]

[1]Department of Zoology, Swedish Museum of Natural History, Box 50007,
SE-104 05 Stockholm, Sweden; e-mail: oleksandr.holovachov@nrm.se
[2]Université Pierre et Marie Curie, UMR 7138 (UPMC CNRS IRD MNHN), Systématique, Adaptation, Evolution, 7,
quai St. Bernard, bâtiment A, 75005 Paris, France
[3]Departamento de Biologia & CESAM, Universidade de Aveiro, Campus Universitário de Santiago,
3810-193 Aveiro, Portugal

Summary. A new species of *Trophomera* Rubtzov & Platonova, 1974 is described from the chemosymbiotic bivalves *Idas modiolaeformis* and *Lucinoma kazani* collected at Amon mud volcano in the eastern part of the Central Nile Province. *Trophomera conchicola* sp. n. is particularly characterised by 23.4 mm long body; finely annulated cuticle; 13-14 µm long cephalic setae; small amphid located 54 µm from anterior end; trophosome beginning 170 µm from anterior end; straight vulva; short conoid tail 73 µm long without terminal spike. This is the first record of the genus in bivalves, in hypoxic hydrogen-sulphide rich environment, and in the Mediterranean Sea.

Key words: Amon mud volcano, deep sea, molecular phylogeny, parasitic nematodes.

The genus *Trophomera* is a member of the family Benthimermithidae and is an uncommon parasite of marine invertebrates. Only 41 nominal species of the genus *Trophomera* are described to date (Miljutin & Miljutina, 2009; Miljutin, 2011; Tchesunov & Rozenberg, 2011), most of which are known on the basis of only few, and often just a single, adult individual. These adult specimens are most commonly found free-living in sediment samples. Juveniles are rarely found, and when they are discovered by themselves, without adult individuals in the same sample, they are impossible to assign to a certain species. Due to the fact that benthimermithids parasitise as juveniles, while adults are free-living, little is known about their hosts. So far, host were established only for three species of this genus. *Trophomera australis* (Petter, 1983) Miljutin, 2006 is known to parasitise free-living nematodes of the genus *Deontosoma* (Enoplida,

Leptosomatidae) (Tchesunov, 1988), *T. bathycola* (Rubtsov, 1980) Miljutin, 2006 was described from the body cavity of the priapulid *Chaetostephanus cirratus* (Rubtsov, 1980), and *T. granovitchi* Tchesunov & Rozenberg, 2011 was found in several different species of crustaceans, i.e. in Isopoda, Amphipoda and Ostracoda (Tchesunov & Rozenberg, 2011). Other records of benthimermithids from polychaetes, sipunculids, crustaceans and echinoderms (Hope, 1977; Petter, 1980, 1983; Tchesunov, 1997) are based on unidentifiable juvenile stages only.

Several adult and juvenile specimens of the genus *Trophomera* were found inside chemosymbiotic bivalves *Idas modiolaeformis* and *Lucinoma kazani*, which were collected in 2006 during the BIONIL cruise exploring cold seep sites north of the Nile Deep-Sea Fan in the eastern Mediterranean. They are described below as a new species *Trophomera conchicola* sp. n.

MATERIALS AND METHODS

Locality and habitat. Chemosymbiotic bivalves *Idas modiolaeformis* and *Lucinoma kazani* were collected at Amon mud volcano (N 32º22, E 31º42, 1153 m depth) located in the eastern part of the Central Nile Province during the BIONIL cruise (2006; chief scientist A. Boetius). *Idas modiolaeformis* is a small mytilid found attached to several substrates (*e.g.* authigenic carbonates, tubes of the siboglinid annelid *Lamellibrachia* sp., carbonate crusts) at cold seeps in the eastern Mediterranean (Olu-Le Roy *et al.*, 2004; Lorion *et al.*, 2012). It harbours six distinct bacterial symbionts in its gills that include five Gammaproteobacteria (one methane- and two sulphur-oxidizers, a methylotroph, and a bacterium of unknown metabolism) and a representative of the Bacteroidetes (Duperron *et al.*, 2008). *Lucinoma kazani* is an infaunal clam bivalve that lives in the reduced sediment of several mud volcanoes from the Eastern Mediterranean. This bivalve associates with a sulphur-oxidizing bacterial symbiont (Salas & Woodside, 2002; Duperron *et al.*, 2007). Bivalve specimens were originally preserved in ethanol (for molecular characterisation) and subsequently fixed in 4% formaldehyde (4°C, 2-4 h) and dehydrated in an ethanol series for fluorescence in situ hybridisation (FISH). Subsequently, nematode specimens were extracted from three *Idas* and two *Lucinoma* specimens and were used for molecular studies, studies by light microscopy (LM) and scanning electron microscopy (SEM).

Light microscopy (LM). For LM, specimens were transferred to pure glycerin using Seinhorst's (1959) rapid method as modified by De Grisse (1969) and mounted on permanent slides in glycerin with paraffin wax as support for the coverslip. All measurements given in the description are in μm except for body length in mm. Abbreviations are as follows: a, body length/maximum body diameter; b, body length/pharynx length; c, body length/tail length; c', tail length/anal body diameter; V, distance from the anterior end of body to vulva/body length as %.

Semi-thin sections. One specimen was dehydrated in ethanol and propylene oxide series and then embedded in an epoxy resin (Serlabo). Semi-thin sections (600 nm) were cut using an ultramicrotome and were stained with toluidine blue for observations by LM (using an Olympus BX61 microscope).

Scanning electron microscopy (SEM). Samples for SEM were dehydrated through an ethanol series and dried in a critical-point dryer (CPD7501). They were coated with gold (scan coat six sputter coater) and observed under a SEM (Cambridge S260 at 10 kV).

Molecular analysis. The DNEASY kit (Qiagen, Valencia, CA) was used to extract total DNA from three nematode specimens. About 900 bp of 18S were amplified using the primer combination MN18F and Nem_18S_R (Bhadury *et al.*, 2006). PCR mixtures contained (total 20 μl, molecular-grade water) 0.4 pmol μl^{-1} primers, 1 μl of DNA template, 1 × reaction buffer (Bioline), 1.5 mM MgCl$_2$, 1.5 U BioTaq DNA polymerase (Bioline) and 0.25 mM each dNTP. Reactions were held at 95°C for 5 min, followed by 37 cycles of 95°C for 1 min, 54°C for 1 min and 72°C for 2 min, and finally one cycle of 2 min at 55°C with a final extension of 8 min at 72°C. PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN, Valencia CA) and sequenced biodirectionally at GATC Biotech (Germany). Only 802 and 831 bases of the 5' end of the 18S rDNA gene were sequenced successfully.

Phylogenetic analysis. The newly determined SSU rDNA sequences of *T. conchicola* n. sp. (GenBank accession numbers HE820056 and HE820057) were analysed against a background of selected sequence data available in public databases for the phylum Nematoda (Table 1). The secondary structure alignment from Holovachov *et al.* (2009) was modified by adding sequences from the public databases and newly generated ones and edited by eye with the JAVA-based secondary structure alignment editor 4SALE (Seibel *et al.*, 2006) to maximise apparent homology and minimise gaps. The resulting alignment was analysed with Bayesian phylogenetic inference using the *mcmcphase* program of the PHASE package (Version 2.0; Gowri-Shankar & Jow, 2006). The alignment was partitioned using nucleotide substitution model REV (Tavare, 1986) for non-paired sites, and RNA7A (Higgs, 2000) for paired sites. For Bayesian inference, chains were allowed to burn in for 1 million generations, followed by 10 million generations during which tree topologies, branch length and model parameters were sampled every 200th generation. Two datasets were analysed, one dataset was 880 bases long with sequences trimmed to the size of *T. conchicola* sp. n. sequences, and another dataset was 1784 bases long (nearly complete 18S rDNA). Placement of *Trophomera* was identical in both analyses; however, the general support of the tree topology was lower for the trimmed dataset. Therefore, the discussion in this paper is based on the phylogenetic analysis of the nearly complete 18S rDNA dataset.

Table 1. List of nematode SSU ribosomal RNA gene sequences obtained from GenBank.

Order	Species name	Accession №	Habitat
Araeolaimida	*Axonolaimus* sp.	EF591331	marine, brackish
Araeolaimida	*Cylindrolaimus communis*	AY593939	terrestrial
Araeolaimida	*Cylindrolaimus* sp.	FJ969121	terrestrial
Araeolaimida	*Diplopeltula* sp.	EF591329	marine
Araeolaimida	*Odontophora* sp.	FJ040459	marine
Araeolaimida	*Sabatieria pulchra*	EF591335	marine
Araeolaimida	*Sabatieria pulchra*	FJ040466	marine
Chromadorida	*Achromadora ruricola*	AY593941	freshwater
Chromadorida	*Choanolaimus psammophilus*	FJ040467	terrestrial
Chromadorida	*Chromadorita leuckarti*	FJ969119	freshwater
Chromadorida	*Ethmolaimus pratensis*	AY593942	freshwater
Chromadorida	*Ethmolaimus pratensis*	FJ040475	freshwater
Chromadorida	*Halichoanolaimus* sp.	EF591338	marine
Chromadorida	*Paracanthonchus caecus*	AF047888	marine
Chromadorida	*Paracyatholaimus intermedius*	FJ969133	freshwater
Chromadorida	*Ptycholaimellus* sp.	FJ040472	marine
Chromadorida	*Punctodora ratzeburgensis*	FJ969138	freshwater
Chromadorida	*Synonchiella* sp.	FJ040468	marine
Desmodorida	*Desmodora ovigera*	Y16913	marine
Desmodorida	*Draconema japonicum*	FJ182217	marine
Desmodorida	*Epsilonema* sp.	FJ182218	marine
Desmodorida	*Eubostrichus topiarius*	Y16917	marine
Desmodorida	*Laxus oneistus*	Y16919	marine
Desmodorida	*Metachromadora* sp.	EF591339	marine
Desmodorida	*Paradraconema jejuense*	FJ182220	marine
Desmodorida	*Stilbonema majum*	Y16922	marine
Desmoscolecida (?)	*Cyartonema elegans*	AY854203	marine
Desmoscolecida	*Paratricoma* sp.	FJ460255	marine
Desmoscolecida	*Tricoma* sp. 1	FJ460256	marine
Desmoscolecida	*Tricoma* sp. 2	FJ460257	marine
Dorylaimida	*Cryptonchus* sp.	FJ040479	freshwater
Dorylaimida	*Cryptonchus tristis*	EF207244	freshwater
Monhysterida	*Astomonema* sp.	DQ408759	marine
Monhysterida	*Daptonema procerum*	AF047889	marine
Monhysterida	*Desmolaimus* sp.	EF591333	marine
Monhysterida	*Diplolaimella dievengatensis*	AJ966482	marine
Monhysterida	*Diplolaimelloides meyli*	AF036611	marine
Monhysterida	*Eumonhystera filiformis*	AY593937	freshwater
Monhysterida	*Geomonhystera villosa*	EF591334	terrestrial, freshwater
Monhysterida	*Halomonhystera disjuncta*	AJ966485	marine
Monhysterida	*Monhystera riemanni*	AY593938	freshwater
Monhysterida	*Theristus acer*	AJ966505	marine

3

Table 1. List of nematode SSU ribosomal RNA gene sequences obtained from GenBank (**continued**).

Order	Species name	Accession №	Habitat
Monhysterida	*Theristus agilis*	AY284695	marine
Plectida	*Anaplectus grandepapillatus*	AY284697	terrestrial, freshwater
Plectida	*Camacolaimus* sp.	EF591325	marine
Plectida (?)	*Ceramonema altogolfi*	JN815320	marine
Plectida (?)	*Ceramonema inguinispina*	JN815319	marine
Plectida (?)	*Ceramonema reticulatum*	FJ474095	marine
Plectida	*Chronogaster boettgeri*	AY593931	freshwater
Plectida	*Chronogaster typica*	FJ040456	freshwater
Plectida (?)	*Creagrocercus drawidae*	HM064453	parasite of terrestrial Oligochaeta
Plectida	*Domorganus macronephriticus*	FJ040454	symbiont of terrestrial Oligochaeta
Plectida	*Leptolaimus* sp.	EF591323	marine
Plectida	*Paraplectonema pedunculatum*	EF591320	freshwater
Plectida	*Plectus acuminatus*	AF037628	terrestrial
Plectida	*Plectus aquatilis*	AF036602	terrestrial, freshwater
Plectida	*Procamacolaimus* sp.	EF591326	marine
Plectida	*Setostephanolaimus spartinae*	EF591321	marine
Rhabditida	*Acrobeloides* sp.	AF034391	terrestrial
Rhabditida	*Anguillicola crassus*	DQ118535	parasite of aquatic Vertebrata
Rhabditida	*Ascaris lumbricoides*	U94366	parasite of terrestrial Vertebrata
Rhabditida	*Brumptaemilius justini*	AF036589	parasite of terrestrial Diplopoda
Rhabditida	*Gnathostoma turgidum*	Z96948	parasite of terrestrial Vertebrata
Rhabditida	*Leidynema portentosae*	EF180073	parasite of terrestrial Arthropoda
Rhabditida	*Oxyuris equi*	EF180062	parasite of terrestrial Vertebrata
Rhabditida	*Rhigonema thysanophora*	EF180067	parasite of terrestrial Diplopoda
Rhabditida	*Teratocephalus lirellus*	AF036607	terrestrial
Rhabditida	*Truttaedacnitis truttae*	EF180063	parasite of freshwater Vertebrata
Triplonchida	*Tobrilus gracilis*	AJ966506	freshwater

DESCRIPTION

Trophomera conchicola sp. n.
(Figs 1-3)

Female from *Idas* (Figs 1A, D). Body very long (L = 23.4 mm) and thin (a = 113), cylindrical over most of its length, tapering anteriorly in pharyngeal region and posteriorly on tail. Body 208 µm wide at vulva. Cuticle thick, 7 µm thick at midbody, with fine transverse striation most distinctly visible along the anterior end (Fig. 2B). External lateral alae (lateral field) absent. Somatic sensilla small papilliform, most prominent along the pharyngeal body region; arranged in four longitudinal rows, one dorsal, one ventral and one lateral on each body side (Figs 2B-C). Somatic sensilla are connected to large epidermal glands. Lip region rounded, continuous with body contour, lips absent/undeveloped. Inner labial sensilla indistinct. Outer labial sensilla small, arranger in a circle close to the oral opening, their nerve endings are visible penetrating the head cuticle on the sides of the cheilostom. Cephalic sensilla setiform, 13-14 µm long, arranged in a circle 14 µm from anterior end (Fig. 2F). Subcephalic and cervical sensilla and ocelli absent. Amphidial fovea small transversely oval slit, 2 µm wide, situated 54 µm from anterior end. Nerve ring surrounding pharynx at about its posterior third. Secretory-excretory system not seen. Buccal cavity small cylindrical, 9 µm long. Cheilostom undifferentiated, clearly demarcated by the cuticle. Pharyngostom undifferentiated and very short. Pharynx short (b = 138) and vestigial, 170 µm long, subcylindrical anteriorly, gradually widening posteriorly; devoid of musculature; with weakly developed lumen visible only in its anterior part.
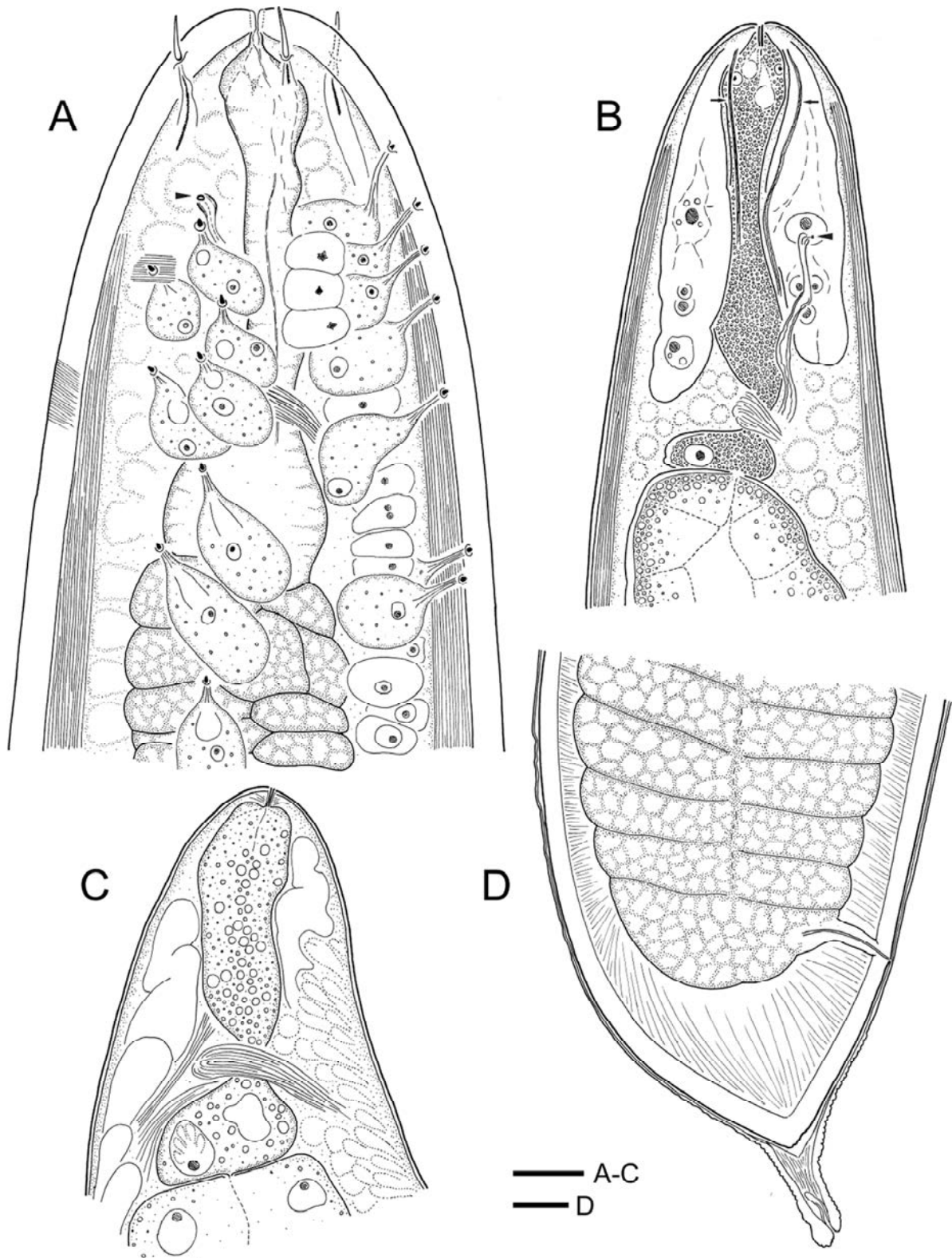
4

Fig. 1. *Trophomera conchicola* sp. n.: A: holotype female, anterior end (arrowhead points to amphid); B: pre-adult juvenile, anterior end (arrows point to nerve endings of cephalic sensillae, arrowhead points to amphid); C: early stage juvenile, anterior end, D: holotype female, posterior end. Scale bars: A-C = 20 μm; D = 25 μm.
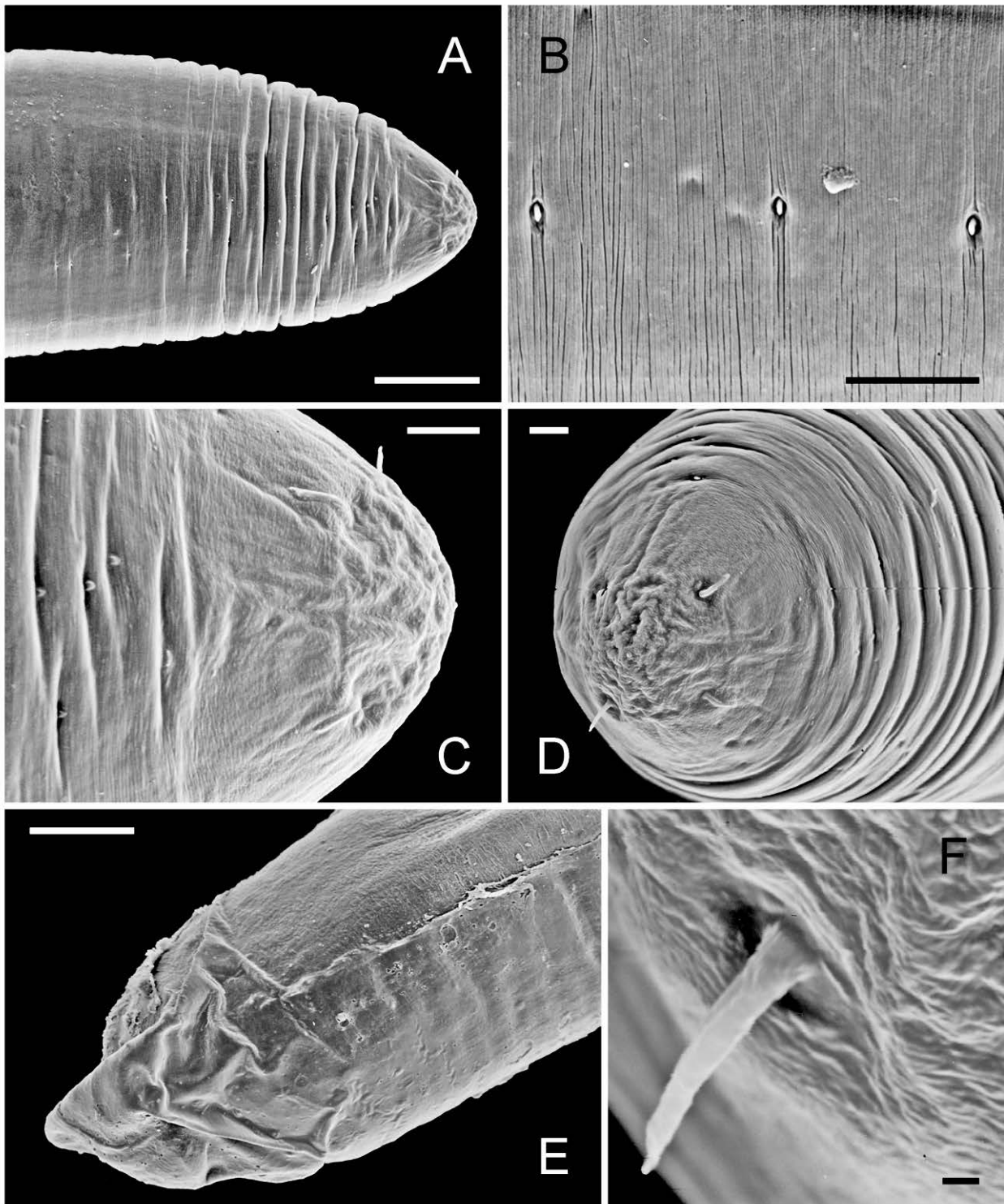
Fig. 2. *Trophomera conchicola* sp. n. (SEM): A: anterior end; B: somatic sensilla; C-D: labial region; E: posterior end; F: cephalic setae. Scale bars: A, E = 50 µm; B-D = 10 µm; E = 1 µm.

Pharyngeal glands indistinct. Trophosome with distinct cell borders but with rudimentary internal lumen (Figs 3A and 3C). There is a distinct cuticular rectal tube 43 μm long at the posterior end of the trophosome. Reproductive system didelphic, amphidelphic; not fully developed. Vulva located at about the mid body (V = 52.3 %). Vagina straight, equal to 1/4 of the vulval body diameter. Intrauterine eggs not seen. Tail 73 μm long, conoid, very short (c = 321, c' = 0.56), still enveloped in the cuticle from the previous developmental stage. Caudal glands and spinneret absent.

Pre-adult parasitic juveniles from *Idas* (Fig. 1B). Body very long and thin (cannot be measured), cylindrical over most of its length, tapering anteriorly in pharyngeal region and posteriorly on tail. Body 205 μm wide. Cuticle thinner than in adults, appears smooth under the light microscope. External lateral alae (lateral field) absent. Somatic sensilla and epidermal glands absent. Lip region rounded, continuous with body contour, lips absent/undeveloped. Inner and outer labial sensilla indistinct. Cephalic sensilla indistinct but their nerve endings are very distinct, extending along the sides of the pharynx towards the nerve ring. Subcephalic and cervical sensilla and ocelli absent. Amphidial fovea small porelike, situated 59-61 μm from anterior end; amphidial nerves also very distinct, extending towards the nerve ring. Nerve ring surrounding pharynx at about its posterior fifth. Secretory-excretory system not seen. There are four sets of large glandular cells located just under cuticle of pharyngeal region: one ventral one dorsal and two (left and right) lateral. Their function is unclear but they appear to be connected to the pharyngeal region (stoma). Buccal cavity narrow, tubular, spear-like. Pharynx short and vestigial, 130-155 μm long, fusiform anteriorly, narrowing at the level of nerve ring and pyriform posteriorly; devoid of musculature; pharyngeal lumen indistinct. Dorsal pharyngeal gland strongly developed. Intestine with distinct internal lumen. Rectum 22 μm long. Primordia of the anterior and posterior genital branches are developed, one of the juveniles with developing vagina. Tail 135 μm long, conoid, with subcylindrical terminal part (c' = 1.3). Caudal glands poorly developed, spinneret present.

Early stage parasitic juvenile from *Lucinoma* (Fig. 1C). Body very long (L = 10 mm) and thin (a = 77), cylindrical over most of its length, tapering anteriorly in pharyngeal region and posteriorly on tail. Body 130 μm wide. Cuticle thinner than in adults, 1.5 μm thick at midbody, appears smooth under the light microscope. External lateral alae (lateral field) absent. Somatic sensilla and epidermal

glands absent. Lip region rounded, continuous with body contour, lips absent/undeveloped. Inner and outer labial sensilla indistinct. Cephalic sensilla and amphids indistinct. Subcephalic and cervical sensilla and ocelli absent. Nerve ring surrounding pharynx at about its posterior fifth. Secretory-excretory system not seen. Buccal cavity narrow, tubular, spear-like. Pharynx short (b = 94) and vestigial, 106 μm long, fusiform anteriorly, narrowing at the level of nerve ring and pyriform posteriorly; devoid of musculature; pharyngeal lumen indistinct. Dorsal pharyngeal gland strongly developed. Intestine with distinct internal lumen. Rectum indistinct. Primordia of the anterior and posterior genital branches are obscure. Tail 136 μm long, conoid, with subcylindrical terminal part (c = 74, c' = 1.5). Caudal glands poorly developed, spinneret present.

Diagnosis. *Trophomera conchicola* sp. n. is particularly characterised by 23.4 mm long body; finely annulated cuticle; 13-14 μm long cephalic setae; small amphid located 54 μm from anterior end; trophosome beginning 170 μm from anterior end; straight vulva; short conoid tail 73 μm long without terminal spike.

Relationships. *Trophomera conchicola* sp. n. is most similar to *T. iturupiensis* (Rubtzov & Platonova, 1974) Miljutin, 2006 in large body size (23.4 mm in *T. conchicola* vs 26-32 mm in *T. iturupiensis*); however, the new species differs from *T. iturupiensis* in having shorter cephalic setiform sensilla (13-14 μm long *vs* 30-32 μm in *T. iturupiensis*), narrower amphid (2 μm wide *vs* 5 μm wide in *T. iturupiensis*) and somatic sensilla arranged in four rows only (*vs* eight rows in *T. iturupiensis*). The new species should also be compared with other large *Trophomera* species with short conoid or hemispherical tail, such as *T. granovitchi* Tchesunov & Rozenberg, 2011 and *T. longiovaris* Miljutin, 2011. From *T. granovitchi* the new species differs in having somewhat longer body (23.4 mm *vs* 2.6-7.0 mm in *T. granovitchi*), longer cephalic setae (13-14 μm long *vs* 4.5-5.5 μm long in *T. granovitchi*), narrower amphid (2 μm wide *vs* 6-8 μm wide in *T. granovitchi*) and tail without acute spike (*vs* with spike in *T. granovitchi*). From *T. longiovaris* the new species differs in having somewhat longer body (23.4 mm *vs* 7.9-15.4 mm in *T. longiovaris*), longer cephalic setae (13-14 μm long *vs* 7 μm long in *T. longiovaris*) and shorter tail.

Molecular phylogenetic analysis. The first phylogenetic analyses of Benthimermithidae were based on the nearly complete 18S rDNA sequence of *T. granovitchi* (Tchesunov *et al.*, 2009) and partial 28S rDNA sequence of *Trophomera* sp. (Mardashova *et al.*, 2011) and support a close relationship with
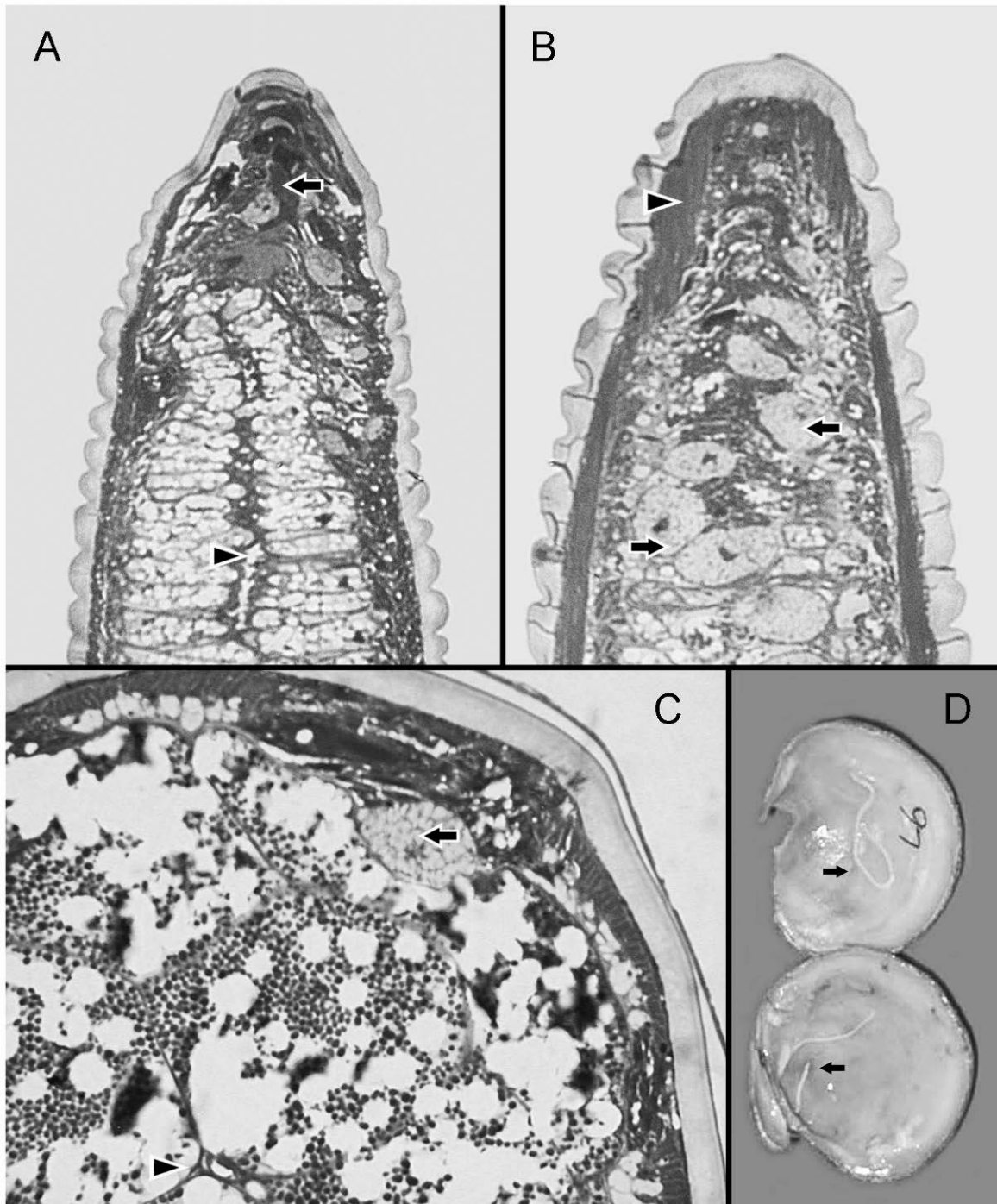
Fig. 3. Semi-thin sections of *Trophomera conchicola* sp. n. (A-B) and dissected *Lucinoma kazani* showing cavities (arrows) from which nematodes were extracted (D): A: Mid-sagittal section showing vestigial pharynx (arrow) and rudimentary lumen of the trophosome (arrowhead); B: Para-sagittal plane showing longitudinal muscules (arrowhead) and epidermal glands (arrows); C: Transverse section at the level of trophosome showing epidermal gland (arrow) and rudimentary lumen of the trophosome (arrowhead). Not to scale.

Plectida, but the complete data were not yet published. Our phylogenetic analysis supports the idea of close relationships between Plectida and *Trophomera*; however, the length of the *Trophomera* sequences available to us is not sufficient (Fig. 4). It is not impossible that the use of full-length 18S rRNA sequence of *Trophomera* in future phylogenetic analysis will alter the current topology of the tree. Therefore, we consider our results preliminary and recommend that they are treated with caution.

Type material. Holotype female (slide № 8411) deposited in the invertebrate type collection of the Department of Zoology, Swedish Museum of Natural History, Stockholm, Sweden. Holotype female was sectioned.

Additional material. Four parasitic juveniles deposited in the general invertebrate collection of the Department of Zoology, Swedish Museum of Natural History, Stockholm, Sweden. Three of the juveniles were collected in *Idas*. They are not complete: two individuals have their posterior ends missing, and one female is without the anterior end. A single complete juvenile specimen was found in *Lucinoma*.

Type locality and habitat. *Idas modiolaeformis* and *Lucinoma kazani*, Amon mud volcano (N 32º22', E 31º42'), eastern Mediterranean Sea. Specimens that were extracted during the cruise occurred in the pallial cavity of *Idas* and were alive and active after the dissection. The specimen from *Lucinoma* was located between the mantle tissue and the shell, and was partly trapped into a cavity inside the shell (Fig. 3D).

Etymology. Species name is derived from a combination of Latin words "concha" (mollusc) and "colo" (inhabit) and means "one who lives in the mollusc".

DISCUSSION

Types of association between marine nematodes and marine molluscs are insufficiently studied. The majority of known cases include the symbiotic or parasitic relationship between nematodes and marine bivalves, with only one case of commensalism between nematodes and deep-sea gastropod (Holovachov *et al.*, 2011). Moreover, most of the nematode species found in association with molluscs belong to the taxa with mostly free-living representatives, who either seek shelter in the host shell or feed on its dead tissues, and the effects of nematodes on their host remain unstudied. This is the first report of a species of the highly specialised parasitic genus *Trophomera* associated with bivalve

molluscs. Other members of this group are known to infest marine nematodes, priapulids, polychaetes, sipunculids, crustaceans and echinoderms (Hope, 1977; Petter, 1980, 1983; Rubtsov, 1980; Tchesunov, 1988, 1997; Tchesunov & Rozenberg, 2011). Further investigation might thus extend the range of metazoan groups parasitised by *Trophomera* species.

All *T. conchicola* specimens were collected from a single site, the Amon mud volcano, during a single cruise in 2006. Other cruises exploring this and similar sites in the eastern Mediterranean took place in 2003, 2007 and 2009, and no additional specimens of *Trophomera* were ever collected. This suggests that the nematodes are rare, and possibly that localised outbreaks of infestation can occur during certain periods. Whether infestation is detrimental to the hosts is not clear, as no tissue lesion was visible. However, given the small size of *Idas modiolaeformis* (around 1 cm shell length, infested or not), and the rather large size of *Trophomera*, one can expect that *T. conchicola* extracts a significant fraction of resources from the hosts, yet it remains to be investigated how the host is affected (for example, growth, health, reproduction etc.).

The mussel *I. modiolaeformis* lives attached to carbonate crusts at cold seeps in the eastern Mediterranean while *L. kazani* lives buried in the sediment (Duperron *et al.*, 2007, 2008). In both cases, the environment is hypoxic, and reduced compounds such as methane and hydrogen sulphide are more enriched compared to normal seawater. This indicates that *T. conchicola* can withstand relatively low levels of oxygen, and high levels of the toxic compound hydrogen sulphide, similarly to for example the polychaete *Branchipolynoe seepensis*, which lives inside the pallial cavity of mussels at many seeps and vents, and probably experiences similar conditions. It is not clear whether the relationship between polychaete and mussels is commensal or parasitic (Jollivet *et al.*, 1998). As far as it is known, this is the first record of *Trophomera* from this type of habitat.

Both host bivalve species harbour chemosynthetic bacteria in their gill tissue, which ensure a significant part of their nutrition (Duperron *et al.*, 2007, 2008). They benefit from bacterial primary production, which favour higher growth rates and biomasses than expected for other organisms at similar depths. Because of this, they appear as excellent niches for parasites. The fact that the *Trophomera* specimen found in *L. kazani* was partly trapped into a cavity in the thickness of the shell is intriguing (Fig. 3D), since other species of this
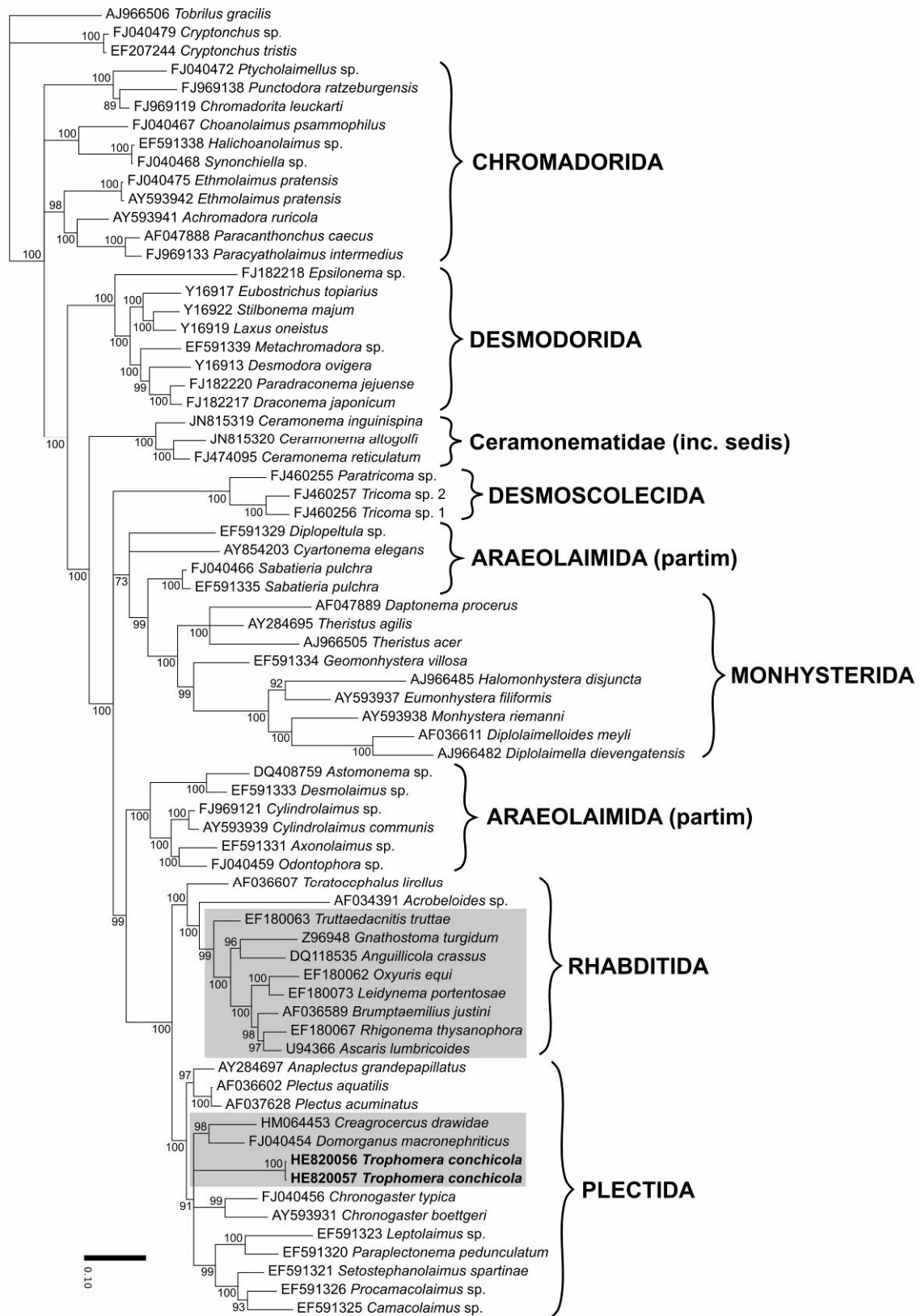
Fig. 4. Majority-rule consensus tree of the Bayesian phylogenetic analysis of Chromadoria including *Trophomera conchicola* sp. n. rooted using *Tobrilus gracilis*. Branch length represent the mean posterior estimates of the expected number of substitutions per site. Animal parasitic taxa are shaded.

genus were found inside the body cavity of their hosts. It could result from the specimen digging inside the shell, which would require that specific substances (such as acids) are produced, but most probably it is a consequence of the shell carbonate precipitating around the worm as a possible defence response of the host.

Like certain free-living gutless marine nematodes of the genus *Astomonema* (Ott *et al.*, 1982), *Trophomera* does not have a well developed pharynx for most of its life. However, unlike free-living *Astomonema* that thrives with the help of bacterial symbionts (Giere *et al.*, 1995; Tchesunov *et al.*, 2012), it is generally assumed that parasitic stages of *Trophomera* feed by absorbing nutrients through cuticle (osmotrophy), while free-living adults do not feed at all and rely on the reserves accumulated in the trophosome (Tchesunov, 1997). This model is further supported by the fact that we failed to amplify any bacterial marker genes from the tissues of the nematode (16S rRNA) despite several attempts, and we did not observe bacterial cells in the nematode sections (Fig. 3C).

ACKNOWLEDGEMENT

REFERENCES

BHADURY, P., AUSTEN, M.C., BILTON, D.T., LAMBSHEAD, J.D., ROGERS, A.D. & SMERDON, G.R. 2006. Development and evolution of a DNA-barcoding approach for the rapid identification of nematodes. *Marine Ecology Progress Series* 320: 1-9.

DE GRISSE, A.T. 1969. Redescription ou modifications de quelques techniques utilisées dans l'etude des nematodes phytoparasitaires. *Mededelingen Rijksfakulteit Landbouwwetenschappen Gent* 34: 351-369.

DUPERRON, S., FIALA-MEDION, I.A., CAPRAIS, J.C., OLU, K. & SIBUET, M. 2007. Evidence for chemoautotrophic symbiosis in a Mediterranean cold seep clam (Bivalvia: Lucinidae): comparative sequence analysis of bacterial 16S rRNA, APS reductase and RubisCO genes. *FEMS Microbiology Ecology* 59: 64-70.

DUPERRON, S., HALARY, S., LORION, J., SIBUET, M. & GAILL, F. 2008. Unexpected co-occurrence of six bacterial symbionts in the gills of the cold seep mussel *Idas* sp. (Bivalvia: Mytilidae). *Environmental Microbiology* 10: 433-445.

GIERE, O., WINDOFFER, R. & SOUTHWARD, E.C. 1995. The bacterial endosymbiosis of the gutless nematode, *Astomonema southwardorum*: ultrastructural aspects. *Journal of the Marine Biological Association of the UK* 75: 153-164.

GOWRI-SHANKAR, V. & JOW, H. 2006. *PHASE: a software package for Phylogenetics and Sequence Evolution.* University of Manchester, Manchester (UK), 60 pp.

HIGGS, P.G. 2000. RNA secondary structure: physical and computational aspects. *Quarterly Revue of Biophysics* 33: 199-253.

HOPE, W.D. 1977. Gutless nematodes of the deep sea. *Mikrofauna des Meeresbodens* 62: 307-308.

HOLOVACHOV, O., BOSTRÖM, S., MUNDO-OCAMPO, M., TANDINGAN DE LEY, I., YODER, M., BURR, A.H.J. & DE LEY, P. 2009. Morphology, molecular characterisation and systematic position of *Hemiplectus muscorum* Zell, 1991 (Nematoda: Plectida). *Nematology* 11: 719-737.

HOLOVACHOV, O., BOSTRÖM, S., REID, N., WARÉN, A. & SCHANDER, C. 2011. Description of *Endeolophos scaneae* sp.n. (Chromadoridae) – a free-living nematode epibiotically associated with deep-sea gastropods *Skenea profunda* (Skeneidae). *Journal of the Marine Biological Association of the UK* 91: 387-394.

JOLLIVET, D., COMTET, T., CHEVALDONNÉ, P., HOURDEZ, S., DESBRUYÈRES, D. & DIXON, D. 1998. Unexpected relationship between dispersal strategies and speciation within the association *Bathymodiolus* (Bivalvia) *Branchipolynoe* (Polychaeta) inferred from the rDNA neutral ITS2 marker. *Cahiers de Biologie Marine* 39: 359-362.

LORION, J., HALARY, S., NASCIMENTO, JD., SAMADI, S., COULOUX, A. & DUPERRON, S. 2012. Evolutionary history of *Idas* sp. Med (Bivalvia: Mytilidae), a cold seep mussel bearing multiple symbionts. *Cahiers Biologie Marine* 53: 77-87.

MARDASHOVA, M.V., ALEOSHIN, V.V. & NIKITIN, M.A. 2011. The origin of marine parasitic nematodes. *Russian Journal of Nematology* 19: 196.

MILJUTIN, D.M. 2006. The genus *Trophomera* Rubtzov & 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. 2011. Deep/sea parasitic nematodes of the genus *Trophometra* Rubtzov et Platonova, 1974 (Benthimermithidae) from the Equatorial Atlantic, with the description of two new species. *Helgoland Marine Research* 65: 245-256.

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.

OLU-LE ROY, K., SIBUET, M., FIALA-MÉDIONI, A., GOFAS, S., SALAS, C., MARIOTTI, A., FOUCHER, J.P. & WOODSIDE, J. 2004. Cold seep communities in the deep eastern Mediterranean Sea: composition, symbiosis and spatial distribution on mud volcanoes. *Deep-Sea Research I* 51: 1915-1936.

OTT, J., RIEGER, G., RIEGER, R. & ENDERES, F. 1982. New mouthless interstitial worms from the sulphide system: symbiosis with procaryotes. *Marine Ecology* 3: 313-333.

PETTER, A.-J. 1980. Une nouvelle famille Nématodes parasites d'Invertébrés marins, les Benthimermithidae. *Annales de Parasitologie Humaine et Comparée* 55: 209-224.

PETTER, A.-J. 1983. Description d'un nouveau genre de Benthimermithidae (Nematoda) présentant des utérus munis de glandes annexes. *Annales de Parasitologie humaine et Comparée* 58: 177-184.

SALAS, C. & WOODSIDE J. 2002. *Lucinoma kazani* n. sp. (Mollusca: Bivalvia): evidence of a living benthic community associated with a cold seep in the Eastern Mediterranean Sea. *Deep-Sea Research I* 49: 991-1005.

SEIBEL, P.N., MÜLLER, T., DANDEKAR, T., SCHULTZ, J. & WOLF, M. 2006. 4SALE – A tool for synchronous RNA sequence and secondary structure alignment and editing. *BMC Bioinformatics* 7: 498.

SEINHORST, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4: 67-69.

RUBTZOV, 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* 145: 177-181 (in Russian, English summary).

RUBTZOV, I.A. & PLATONOVA, T.A. 1974. [A new family of marine parasitic nematodes]. *Zoologicheskii Zhurnal* 53: 1445-1458 (in Russian, English summary).

TAVARE, S. 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 17: 262-272.

TCHESUNOV (LAPS. CHESUNOV), A.V. 1988. 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. 1997. Marimermithid nematodes: anatomy, position in the nematode system, phylogeny. *Zoologicheskii Zhurnal* 76: 1283-1300 [in Russian, English summary].

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.

TCHESUNOV, A.V. & ROZENBERG, A.A. 2011. Data on the life cycle of parasitic benthimermithid nematodes with the description of a new species discovered in marine aquaria. *Russian Journal of Nematology* 19: 139-150.

TCHESUNOV, A.V., INGELS, J. & POPOVA, E.V. 2012. Marine free-living nematodes associated with symbiotic bacteria in deep-sea canyons of north-east Atlantic Ocean. *Journal of the Marine Biological Association of the UK*, doi: 10.1017/ S0025315411002116.

O. Holovachov, C.F. Rodrigues, M. Zbinden, S. Duperron. *Trophomera conchicola* sp. n. (Nematoda: Benthimermithidae) из хемосимбиотических двустворчатых моллюсков *Idas modiolaeformis* и *Lucinoma kazani* (Mollusca: Mytilidae и Lucinidae) из восточной части Средиземного моря.

Резюме. Новый вид рода *Trophomera* Rubtzov & Platonova, 1974 описан из хемосимбиотических двустворчатых моллюсков *Idas modiolaeformis* и *Lucinoma kazani,* собранных на грязевом вулкане Амон в восточной части Центральной Нильской провинции Средиземного моря. *Trophomera conchicola* sp. n. характеризуется длиной тела, равной 23.4 мм, тонкой кольцевой исчерченностью кутикулы, головными щетинками длиной 13-14 мкм, небольшими амфидами, расположенными в 54 мкм сзади от головного конца, трофосомой, начинающейся в 170 мкм от переднего конца, прямой вульвой и коротким коническим хвостом длиной 73 мкм без терминального шипика. Это первое сообщение о паразитировании нематод этого рода в двустворчатых моллюсках, в среде с недостатком кислорода и избытком H_2S, и из Средиземного моря.