

Nematimermis enoplivora gen.n., sp.n. (Nematoda: Mermithoidea) from marine free- living nematodes *Enoplus* spp.

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Summary. *Nematimermis enoplivora* gen.n., sp.n. is described from the pseudocoel of marine free-living nematodes *Enoplus* spp. Infestation of the littoral and sublittoral nematodes *E. brevis* and *E. communis* respectively from Kandalaksha Bay of the White Sea by juveniles and mature females was observed during the summer seasons of 1986-1988. Four large and two smaller stichocytes were present near the oesophageal tube of parasitic juveniles. *N. enoplivora* gen. n., sp. n. grows to maturity inside the host, and almost all inner tissues are strongly degenerated in females filled with juvenile-bearing eggs. *N. enoplivora* gen. n., sp. n. is probably an aberrant marine form of the family Tetradonematidae.

Key words: mermithids, tetradonematids, *Nematimermis* gen. n., marine host.

Invasion of marine free-living nematodes *Enoplus brevis* Bastian, 1865 and *E. communis* Bastian, 1865 by juveniles and mature females of unusual parasitic nematodes was noticed during the summer seasons of 1986-1988 in the White Sea. A description of these parasitic nematodes, which belong to a new genus, and information about their ultrastructure are presented below.

MATERIALS AND METHODS

Parasitic nematodes were fixed in 4% formalin and mounted in glycerol. Specimens for scanning electron microscopy were dehydrated in a graduated ethanol and acetone series, critical point dried, coated with platinum-palladium alloy, and examined with a Hitachi S 405 A at 15 kV. Specimens for transmission electron microscopy were also dehydrated and embedded in Epon. Sectioning was done with an Ultracut ultramicrotome, using glass knives. Staining for TEM was done with uranyl acetate followed by lead citrate. Thin sections were viewed with a Jeol

JEM-100 B at 80 kV. Thick sections (2 μm) were mounted on glass slides and stained with fuchsineosin.

DESCRIPTION

Nematimermis gen. n.

Diagnosis: Tetradonematidae. Four large and two smaller stichocytes near pharyngeal tube of juveniles. Cephalic sensillae and amphids reduced. Muscle cells, hypodermis and trophosome degenerated in mature females. Vulva indistinguishable. Eggs in uteri contain stylet-bearing juveniles.

Parasites of marine free-living nematodes.

Type-species: *N. enoplivora* gen. n., sp. n.

Nematimermis enoplivora gen. n., sp. n. (Figs. 1-5)

Holotype juvenile: L= 4844 μm , D = 81 μm .

Paratype juveniles (n=7): L = 2310-4844 μm , D = 64-92 μm .

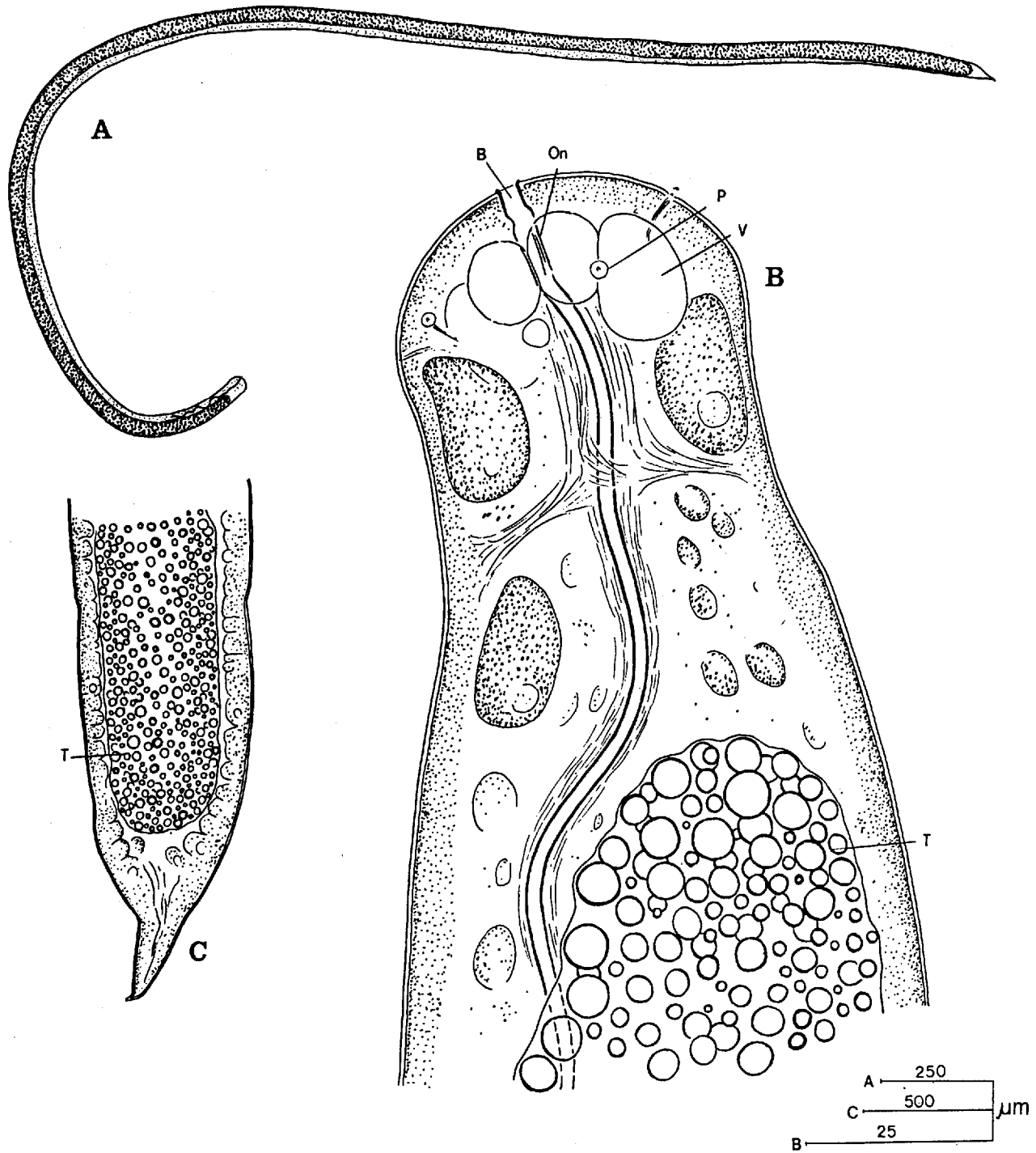


Fig.1. *Nematimermis enoplivora* gen.n., sp.n. Holotype. A: Total view; B: Head end; C: Caudal end. B - buccal cavity, On - onchiostyle, P - cephalic papilla (presumably), T - trophosome, V - vacuoles inside head.

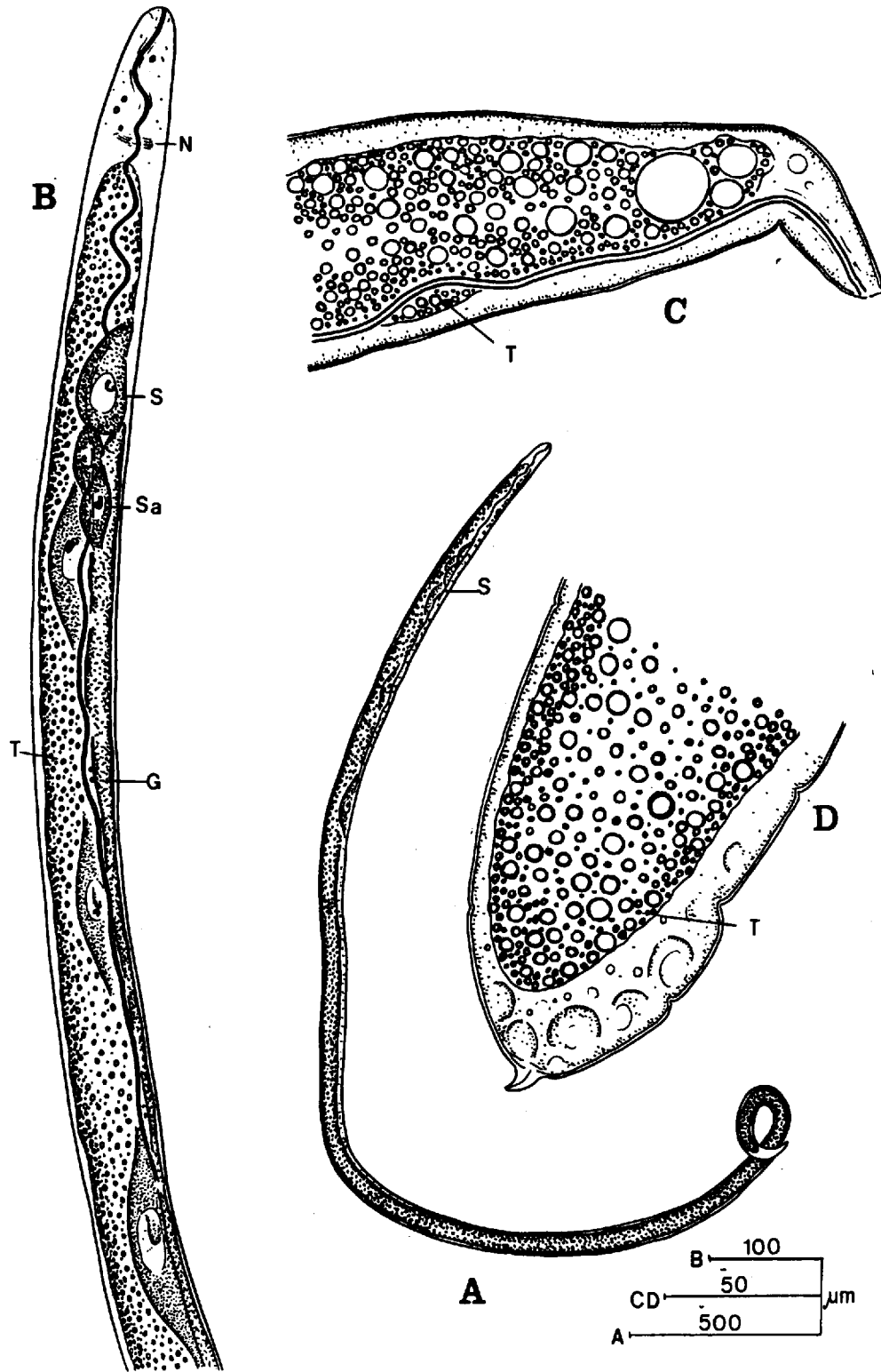


Fig. 2. *Nematimermis enoplivora* gen.n., sp.n. juvenile morphology. A: Total view of juvenile with prominent stichocytes; B: Anterior end of the same juvenile; C: Head end of smaller juvenile; D: Caudal end of smaller juvenile. G - genital primordium, N - nerve ring, S - stichocyte, Sa - small stichocyte.

Paratype females (n=3): L = 3470-5020 μm , D = 140-160 μm .

Juveniles. Body short, cylindrical, with anterior end slightly swollen, narrowing in the neck region. Some specimens with head distinctly deformed due to compression inside host. Tail shape conical to rounded always with pointed terminus. Larval cuticle with slight annulations and microscopic appendages on the surface - microvilli length 0.9-1.5 μm , diameter 0.13-0.2 μm . Cuticle consists of two layers: epicuticle of three sublayers (two dark osmiophilic divided by electron-bright one) and homogenous inner layer with granular, sometimes striated structure. Cuticular microvilli on the surface are simple protuberances of both main layers - granular core coated with epicuticle. Total thickness of cuticle 0.17-0.21 μm , for epicuticle solely - 0.029-0.032 μm . Cephalic papillae and amphids not found, sometimes separate papilla-like structures can be observed in subcuticular position. Oral opening slightly displaced ventrally; and in some specimens encircled by groove. Narrow buccal cavity curved in the middle, divided into two parts: anterior with thickening in the walls near the oral opening; posterior cylindrical with rudiment of onchiostyle merging with dorsal lining of lumen. Buccal cavity without any clear dividing border (length about 10-12 μm , diameter 1-2 μm) turns into pharyngeal tube of 130-210 μm length. Large cells with granular cytoplasm situated around anterior part of pharyngeal tube, along with vacuoles and indistinct nerve ring. Row of 4 large and 2 small cells (stichocytes) with granular cytoplasm and nucleus with distinct nucleolus can be observed near posterior part of pharyngeal tube at level of trophosome. Hypodermis 2-3 times thicker than cuticle, with 6 ridges: two broad lateral ones; ventral and dorsal ridges of medium size; narrowest are the lateroventral. Myocytes are divided by hypodermal ridges in 6 fields and separated from cuticle by voluminous subcuticle with scattered myofilaments in bundles without order. Pseudocoel filled by coarse electron-dense material. Trophosome with entirely syncytial cylindrical body without lumen or cell

boundaries and filled with spherical homogenous globules which occupy the main space of this body. Germinal primordium between trophosome and body wall.

Females. Body thicker than in juveniles, covered by adult cuticle and loose sac of juvenile cuticle. Both of two main layers: epicuticle and homogenous inner layer. Adult cuticle 0.15-0.17 μm thick and juvenile one of 0.03 μm on average. Space between them filled by flakes of electron-dense material. Sublayers of juvenile epicuticle less distinct than adult or juvenile cuticles. Concentric striations visible in some sections of inner layer of juvenile cuticle. Head conical, rounded without visible sensillae. Stomatal cavity and pharyngeal tube 2 μm in diameter. Trophosome completely depleted in adulthood, hypodermis and musculature also on the edge of degeneration. Two parallel uteri with eggs containing embryos in later stages of development. Some cell structure (nuclei, cytoplasm) can be observed in the walls of uterus. Juveniles within eggs with stylet. Egg shell 0.15 μm thick with tripartite uterine layer and broad vitelline one. The middle electron-dense sublayer surrounded by two bright ones in uterine layer. Vitelline layer with osmiophilic granules in the middle. Spherical egg shell about 30-32 μm .

Type host and locality. *Enoplus brevis* collected in Kandalaksha Bay of the White Sea, 13 August 1987.

Type specimens. Holotype juvenile (slide Jc 362) deposited in the Collection of Moscow State University Zoological Museum; paratype females with eggs (slide Jc 363) at the same collection.

Type locality and habitat. *N. enoplivora* was found at several sites of Kandalaksha Bay. The highest level of infection was observed in *Enoplus communis* collected from silty sublittoral sand at 4 m depth in Velikaja Salma Straight (3 infected from 100 examined hosts in August 1986 and 6 infected hosts from 50 examined in August 1988). Infestation of *Enoplus brevis* collected from littoral sand was only

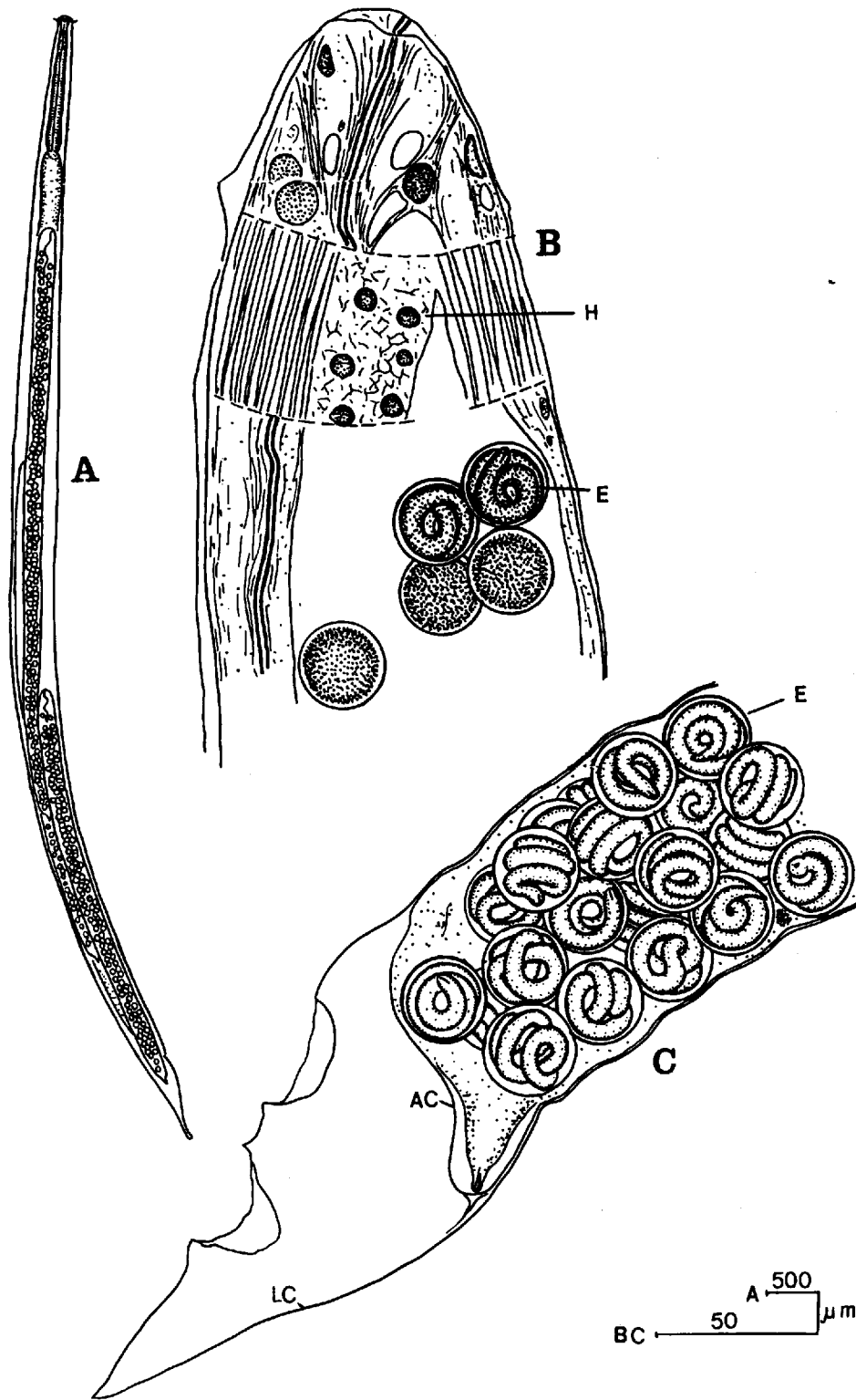


Fig. 3. *Nematimermis enoplivora* gen.n., sp.n. A: Three parasites inside *Enoplus communis*; B: Head end of the mature female; C: Caudal end of the same female with remnants of juvenile cuticle. AC - adult cuticle, H - hypodermal cord with nuclei, E - eggs with juveniles, LC - juvenile cuticle.

occasional: one juvenile was found during dissection of hundreds of *E. brevis* in Chupa fjord and two during a similar survey in Velikaja Salma Straight.

Discussion. *N. enoplivora* was found in a marine host but has the characteristic appearance of the Mermithidae - the soil and fresh water group of parasitic nematodes of invertebrates. *Benthimermis australis* was described from the marine free living nematode *Deontostoma* sp. (Tchesunov, 1988), but this nematode like all other members of the families Benthimermithidae Petter, 1981 and Marimermithidae Rubtsov et Platonova, 1974 has a distinctive morphology. Both families have no phyletic links with Mermithoidea (Petter, 1983; Tchesunov, 1988). The resemblance of *N. enoplivora* gen. n., sp. n. to soil and freshwater members of the parasitic Enoplia (superfamily Mermithoidea) is supported by its general morphology, the structure of head and tail ends, presence of syncytial trophosome without lumen and very typical stichosome, pharyngeal tube and onchiostyle.

Data on Mermithoidea infesting other nematodes are scarce, and refer to infestations of mononchids and dorylaimids. These reports are usually incomplete because they deal only with the juvenile stages of the parasite (Tjepkema, 1969; Andrassy, 1973, 1985). The single description of mature mermithids from a nematode host is that of *Nanomermis nemicola* (Cobb, 1925). *Nanomermis* is considered to be a genus of uncertain systematic position between mermithids (Rubtsov, 1978). Due to the short description of these small nematodes (length of gravid female only 140 μ m) they may be included in Mermithoidea, but their morphology strongly differs from those in our material.

The above mentioned mermithid features of *Nematimermis enoplivora* gen. n., sp. n. put them into the Mermithoidea, which is divided into the families Mermithidae and Tetradonematidae. The latter unites the Mermithoidea which mature inside the host, although recently such forms were also found in Mermithidae - *Heleidomermis* (Rubtsov, 1978). The family Tetradonematidae is considered sometimes as

an artificial grouping of mermithids evolved independently to neoteny (Kaiser, 1983). In the present contribution we use existing nomenclature and diagnoses. As adult *Nematimermis enoplivora* gen. n., sp. n. can be found inside the host, it is included in the Tetradonematidae. This is supported by the nematode having four large stichocytes and two smaller additional stichocytes. The presence of four large stichocytes (tetrad) is reported for several genera in this family: *Tetradonema* Cobb, 1920, *Trichonema* Rubtsov, 1978, *Crassinema* Rubtsov, 1978 and *Heterogonema* Waerebeke & Remillet, 1973. The absence of a vulva in first generation females was observed in *Heterogonema ovomasculis* (Waerebeke Van & Remillet, 1973), which resembles the absence of a prominent vulva in *Nematimermis*. In the general shape of the body *N. enoplivora* gen. n., sp. n. differs from the thick *Heterogonema ovomasculis* and is closer to the thread-like *Tetradonema*, *Trichonema* and *Crassinema*. Other thread-like tetradonematids (*Aproctonema* Keilin, 1917; *Mermithonema* Goodey, 1941; *Corethrellonema* Nickle, 1969) have differently structured stichosome which excludes them from comparison with *Nematimermis* (Nickle, 1969; Poinar, 1977; Rubtsov, 1978). Other genera reported as members of Tetradonematidae (*Bissonema* Rubtsov, 1978; *Brevinema* Rubtsov, 1978 and *Paraproctonema* Rubtsov, 1978) were described based on larval stages only. The descriptions of these nematodes lack the main feature of all tetradonematids - the presence of the adult stage inside the host. Therefore they can not be considered as valid representatives of this family.

Nematimermis enoplivora gen. n., sp. n. resemblance to several tetradonematids is based on individual features: general shape of body and presence of stichocytes. All other morphological peculiarities of *N. enoplivora* gen. n., sp. n. are affected by the degeneration of the adult female non-reproductive organs. Other tetradonematids have full adult morphology, which is difficult to compare with the sac like females of our species. Also, all tetradonematids were described from insect hosts

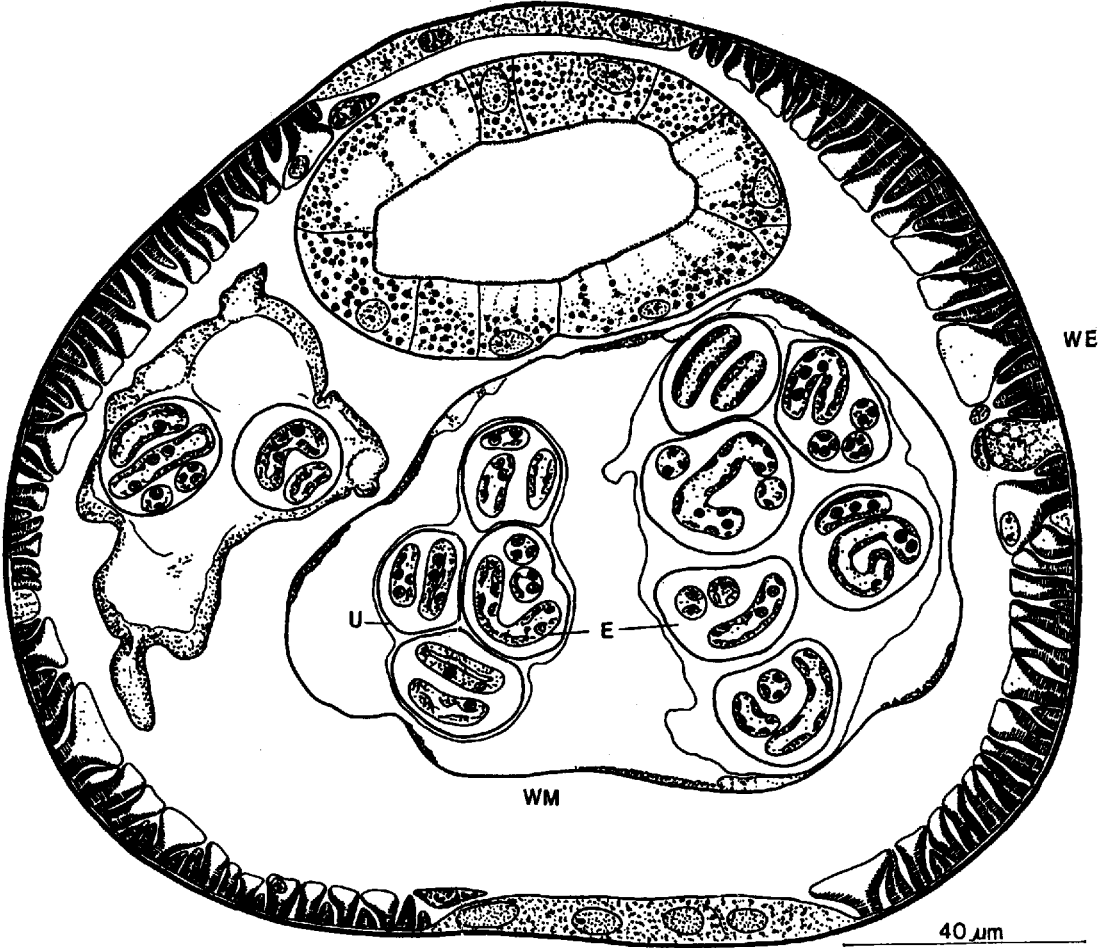


Fig.4. *Nematimermis enoplivora* gen.n., sp.n. Cross section through parasitized host. U - uterus walls, WE - body wall of host, WM - body wall of mermithid.

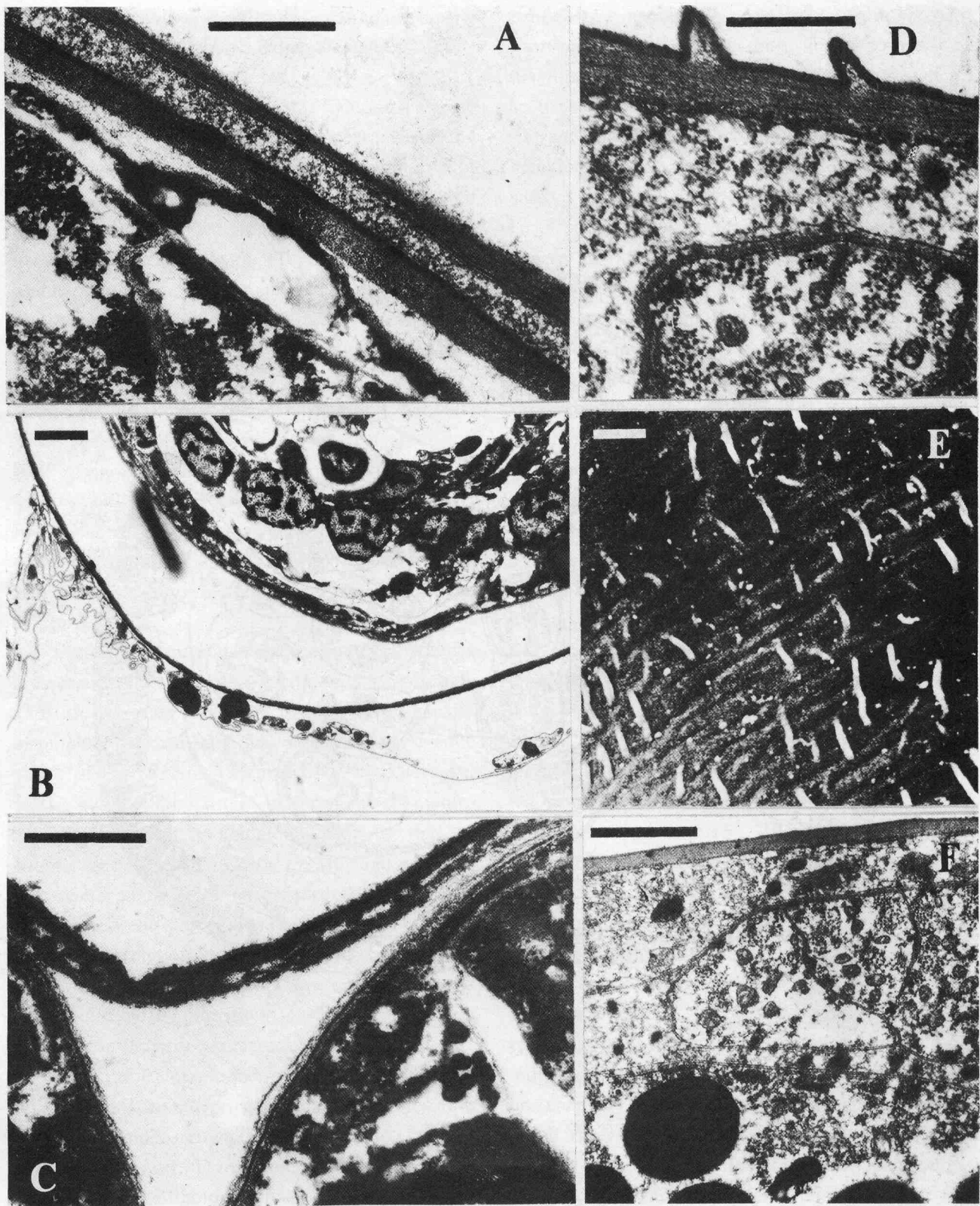


Fig. 5. *Nematimermis enoplivora* gen.n., sp.n. A: TEM, cuticle of mature female covered by juvenile cuticle. Electron dense flackes between the cuticles; B: TEM, uterus wall and egg containing juvenile; C: TEM, tripartite egg costella. D: TEM, juvenile cuticle surface with microvilli; E: SEM, juvenile cuticle surface and microvilli; F: TEM, parasitic juvenile body section. (Bars : A,D,C - 0,5 μ m; B,E,F - 1 μ m.)

(Diptera and Coleoptera) and both males and females were discovered in the host. We propose to include *N. enoplivora* gen. n., sp. n. in Tetradonematidae as an isolated aberrant form.

The juvenile cuticle of *Nematimermis enoplivora* gen. n., sp. n. is comparatively simple in structure. With its voluminous hypodermis and slightly differentiated muscle cells it resembles the morphology of parasitic larva of *Gastromermis boophthorae* (Batson, 1979). The thin cuticle and the thick, organellae-rich hypodermis were considered in the latter species as a pathway for transcuticular osmotic transport of nutrient organic compounds from the host. Cytoplasmic digitations entering the cuticle basal layer were reported for parasitic stages of *Gastromermis boophthorae* and *Romanomermis culicivorax* (Poinar & Hess, 1977). Channels running from the surface to the cuticle basal layer were also observed in the latter species and considered as the pathways of the same mode of nutrition. No such structures were observed in *N. enoplivora* gen. n., sp. n., however hypodermal digitations and cuticular channels also were absent in some true Mermithoidea (Webster & Gordon, 1974). On the contrary the cuticular microvilli observed in *N. enoplivora* gen. n., sp. n. seem to be unique for nematodes of invertebrates. Superficial protuberances were observed in *Bradynema* (Riding, 1970) but because of the lack of a cuticle in *Bradynema* females they are considered to be part of the hypodermis.

The life cycle of *Nematimermis enoplivora* gen. n., sp. n. is not known. The presence of stylet-bearing juveniles inside eggs in the uteri is common with *Mermis* sp. Infestation of new hosts occurs when *Mermis* eggs are swallowed. A similar method of infestation is likely to occur with *N. enoplivora*, because a direct penetration of *Enoplus* cuticle appears difficult. General degeneration of the body wall in adult females of *N. enoplivora* indicates that they do not leave the host, therefore the mode of egg distribution into the environment is unclear. Little is known about the reproduction of *N. enoplivora*. Males were not found but they may exist, e.g. dwarf males in *Tetradonema*. However the absence of any discern-

ible vulva suggests self-fertilization or parthenogenetic development. Protherandric hermaphroditism was reported for *Gastromermis bretschkoi* (Kaiser, 1986) and parthenogenesis is common (Rubtsov, 1978) in soil mermithids.

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REFERENCES

- Andrassy, I. 1973. In Nematoden parasitierende Nematoden. *Helminthologia* 14: 451-457.
- Andrassy, I. 1985. Nematological notices, 2.1. Once again: nematodes parasitic in nematodes. *Opuscula Zoologica, Budapest* 19-20: 109.
- Batson, B. S. 1979. Ultrastructure of the trophosome, a foodstorage in *Gastromermis boophthorae* (Nematoda: Mermithidae). *International Journal of Parasitology* 9: 505-514.
- Cobb, N. A. 1925. A new mermithid infesting another nema. *Journal of Parasitology* 11: 217-218.
- Kaiser, H. 1983. Phylogenetic relationships in the Mermithidae (Nematoda) based on traditional and physiological evidence. In: *Concepts in Nematode Systematics*. (A. R. Stone, H. M. Platt & L. F. Khalil. Eds.) pp.249-259. London, Academic Press.
- Kaiser, H. 1986. Die Mermithiden-Fauna von Hochgebirgseen (Vorderer Finstertaler See, 2237 m über N. N., Hinterer Finstertaler See, 2256 m über N. N., Tirol, Austria). *Archiv für Hydrobiologie* 74: 61-96.
- Nickle, W. R. 1969. *Corethrellonema grandispiculosum* n. gen., n. sp. and *Aproctonema chapmani* n. sp. (Nematoda: Tetradonematidae), parasites of the dipterous insect genera *Corethrella* and *Culicoides* in Louisiana. *Journal of Nematology* 1: 49-54.
- Petter, A. J. 1983. Quelques nouvelles especes du genre *Benthimermis* Petter, 1980 (Benthimermithidae: Nematoda) du Sud de l'Océan Indien. *Systematic Parasitology* 5: 1-15.
- Poinar, G. O. Jr. 1977. CIH key to the groups and genera of nematode parasites of invertebrates. *Commonwealth Agricultural Bureau Bulletin* 4: 1-43.
- Poinar, G. O. Jr. & Hess, R. 1977. *Romanomermis culicivorax*: morphological evidence of transcuticular uptake. *Experimental Parasitology* 42: 27-33.
- Rubtsov, I. A. 1978. [Mermithids. Classification, Importance,

- Application*]. Nauka, Leningrad, 207 pp.
- Riding, J. L. 1970.** Microvilli on the outside of a nematode. *Nature* 226: 179-180.
- Tchesunov, 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.
- Tjepkema, J. P. 1969.** Parasitism of aporcelaimid nematodes by mermithid nematodes. *Journal of Nematology* 1: 29.
- Wacrebeke Van D. & Remillet M. 1973.** Morphologie et biologie de *Heterogonema ovomaculis* n. g., n. sp. (Nematoda: Tetradonematidae) parasite de Nitidulidae (Coleoptera). *Nematologica* 19: 80-92.
- Webster, J. M. & Gordon, R. 1974.** The cuticle structure of larval *Mermis nigrescens* and its possible function. *Journal of Nematology* 6: 154.
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Чесунов А. И., Спиридонов С. Э. *Nematimermis enoplivora* gen. n., sp. n. (Nematoda: Mermithoidea) от морских свободноживущих нематод рода *Enoplus*.

Резюме. *Nematimermis enoplivora* gen. n., sp. n. описан по паразитическим личинкам и зрелым самкам, обнаруженным в полости тела морских свободноживущих нематод *Enoplus* spp. Заражение литторального *Enoplus brevis* и сублитторального *E. communis* в Кандалакшском заливе Белого моря было отмечено в летние сезоны 1986-1988. Рядом с пищеводной трубкой паразитической личинки располагаются четыре крупных и два мелких стихоцита. *Nematimermis enoplivora* gen. n., sp. n. развиваются внутри тела хозяина до половозрелого состояния. Внутренние органы половозрелых самок подвергаются значительной дегенерации за счет заполнения их множеством содержащих личинок яиц. Предполагается, что *Nematimermis enoplivora* gen. n., sp. n. представляет собой aberrантную форму тетрадонематид.
