ACTA ZOOLOGICA BULGARICA

Acta Zool. Bulg., 74 (4), December 2022: 505-510
Published online 28 November 2022
http://www.acta-zoologica-bulgarica.eu/2022/002633

Systematics and Phylogenetics
Research Article

First Report of *Litoditis marina* (Bastian, 1865) (Nematoda: Rhabditida) from the Hypersaline Sivash Bay, Crimea

Tatiana N. Revkova

A.O. Kovalevsky Institute of Biology of the Southern Seas, Russian Academy of Sciences, 2 Nakhimov Ave., 299011 Sevastopol; E-mail: alinka8314@gmail.com; https://orcid.org/0000−0002−3500−3776

Abstract: The marine free-living nematode *Litoditis marina* (Bastian, 1865) Sudhaus, 2011 is re-described from the hypersaline Sivash Bay with salinity 70–108‰. Males and females specimens were found in samples of bottom sediments and floating mats of the green alga *Cladophora*. This is the second finding of this species in the water body of Crimea (Azov-Black Seas basin) and the first from the hypersaline water body. The presence of a smaller number of caudal papillae on the bursa of one male was observed.

Key words: free-living nematode, taxonomy, *Litoditis*, hypersaline water body

Introduction

Hypersaline water bodies with a salinity of more than 35‰ are widespread in the world (Shadrin 2018). Despite the severity of the conditions in hypersaline lakes, there are characterised by considerable biodiversity (Anufriieva & Shadrin 2018, Shadrin et al. 2019b). Sivash Bay, also known as the Rotten Sea, is located in the western part of the Sea of Azov; is the world's largest hypersaline bay (Shadrin et al. 2018, Sergeeva et al. 2019). After the construction of the North-Crimean Canal in 1961–1971, the salinity of Sivash Bay decreased from 140‰ to 22‰ (Anufriieva & Shadrin 2020). A new increase in salinity in Sivash Bay began after closing the North Crimean Canal in 2014. During this period, changes in the zoobenthos (Shadrin et al. 2019a, 2019b) and the taxonomic structure of the nematode complex were observed (Sergeeva et al. 2019, Shadrin et al. 2019a). An important element of the Sivash ecosystem as a hypersaline water body are mats of the green filamentous alga *Cladophora*, in which the taxonomic diversity of the fauna is considerable (Shadrin et al. 2019b, Prazukin et al. 2020).

Litoditis marina is a free-living marine nematode that typically lives on dead macrophytes washed ashore or on standing macroalgae in sheltered places along coasts and estuaries (Derycke et al. 2016, Xie et al. 2020). It occurs around the world, including Arctic, Antarctic and tropical areas, having tolerance to a wide temperature range (Bastian 1865, Inglis & Coles 1961, Sudhaus 1974a, 1974c, Kito 1981, Derycke et al. 2008). It has been experimentally shown that *L. marina* can tolerate and adapt to a wide range of salinity (0–80‰), being able to reproduce in this salinity range, being the highest salinity ever tested (Tietjen et al. 1970).

For the Crimean region, the only information about *L. marina*, living on decaying algae in the Black Sea supralittoral near Sevastopol, was provided by Gagarin (2001). Our discovery of this species in hypersaline waters is the first one. This article provides the re-description of *L. marina* found

in floating mats of the green alga *Cladophora* and bottom sediments from the hypersaline Sivash Bay.

Materials and Methods

The study of the fauna of free-living nematodes of Sivash Bay was performed as part of comprehensive hydrobiological survey in different seasons (summer, autumn, winter and spring) in 2018–2019 (Shadrin et al. 2019a, 2019b). For the entire study period, *L. marina* was only detected during the winter sampling on December 19–20, 2020. Materials were collected on eight bottom stations and three stations on floating mats of filamentous green alga *Cladophora* (Fig. 1). The samples of the soft-bottom sediments were taken by a benthic tube at depths of 0.2–0.5 m. From floating mats of *Cladophora*, samples were taken from an area of 0.25 m^2.

All samples were fixed with 4% formalin. The sediments and samples of mats of *Cladophora* were carefully washed on sieves with mesh sizes of 1000 µm and 63 µm. The fraction retained by the sieves was stained in rose Bengal solution before being transferred to a Bogorov chamber and sorted under a binocular microscope. For taxonomic analysis, nematodes were mounted in permanent glycerine microscope slides (Seinhorst 1959). Identification, measurements and figures were made with a Nikon Eclipse E200 microscope. Photographs were made using an Olympus BX53 microscope, GRYPHAX ARKTUR camera (JENOPTIK Optical Systems GmbH) and GRYPHAX software. Samples of this species have been deposited in the collection of A.O. Kovalevsky Institute of Biology of the Southern Seas, Russian Academy of Sciences, Sevastopol.

The following abbreviations are used: a, ratio body length/maximum body diameter; b, ratio body length/pharynx length; c, ratio body length/tail length; c', ratio tail length/anal body diameter; n, number of specimens.

Results

Taxonomy

Order Rhabditida Chitwood, 1933
Family Rhabditidae Örley, 1880
Genus *Litoditis* Sudhaus, 2011

Diagnosis (after Sudhaus 2011): Lateral field with three to seven ridges (four to eight incisures); glottis, sleeve; distinct median bulb as wide as or wider than the poorly developed terminal bulb; intestine sinched in posteriorly, forming a pre-rectum; amphidelphic; female tail conical; nine GPs, three precloacal, arrangement 1+2/3+3, GP5 and GP7 open on the dorsal surface of the velum; phasmid opening ventral at the base of GP7; gubernaculum forked; juveniles with swollen and rounded tail tip; typical for all stages disturbed is the "stay-in-place-

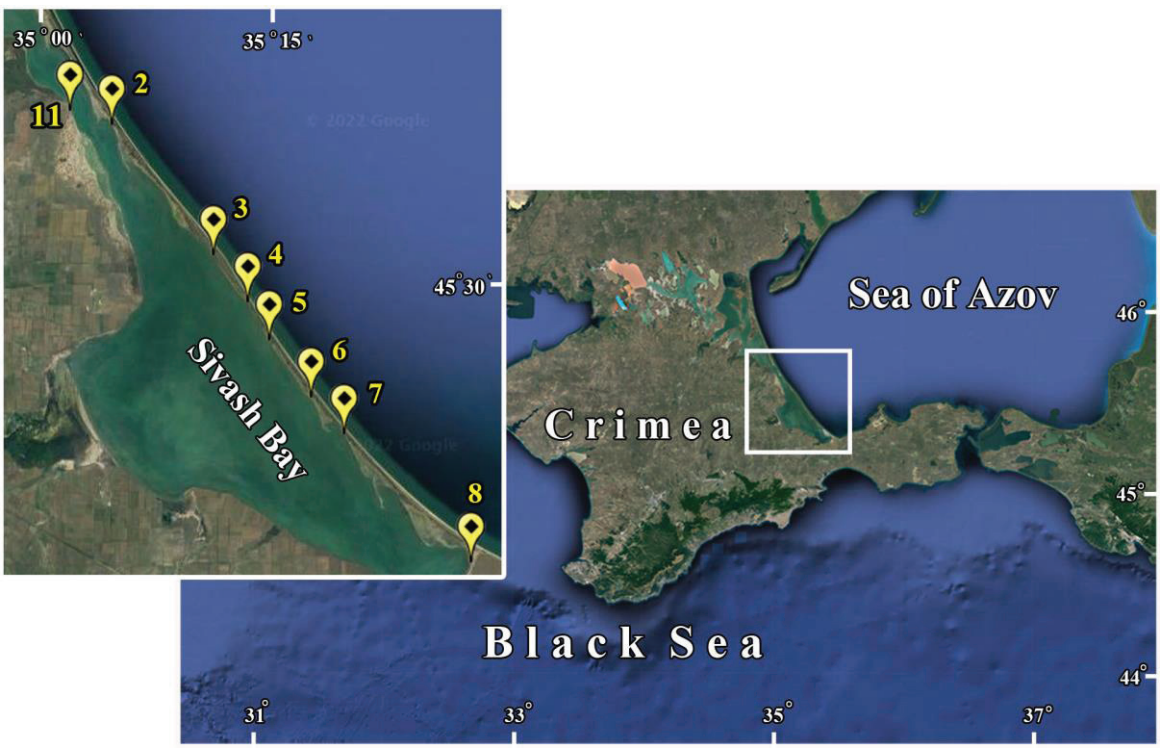

Fig. 1. Map of the Sivash Bay (Sea of Azov), with indication of sampling stations.

movement": in water, animals move both head and tail from one body side to the other, a movement which allows them to stay in place. *Habitats*: Intertidal, rotting seaweed on beaches. Type species: *Rhabditis marina* Bastian, 1865.

***Litoditis marina* (Bastian, 1865) Sudhaus, 2011**

Studied material: five males and eight females. Males mounted on slide (Meib.27.N.v. - Meib.31.N.v.1) and females (Meib.31.N.v.2 - Meib.38.N.v.) in pure glycerine.

Locality: Sivash Bay, Sea of Azov, Crimea, bottom depths 0.2–0.5 m, silt and silty-sand with fragments of mollusk shells; *Cladophora* green algal mats; water salinity 70–108 ‰. Geographical coordinates: N45°31'13.7", E35°11'12.9" (st.3, female); N45°29'04.7", E35°13'27.9" (st.4, male).

Description (Figs 2–4; Table 1): *Females*. Body cylindrical, tapering towards both extremities (Figs 2D and 3A); more tapering in posterior direction. Cuticle 2–4 μm thick, with well-defined transverse striations. Lateral field with prominent longi-

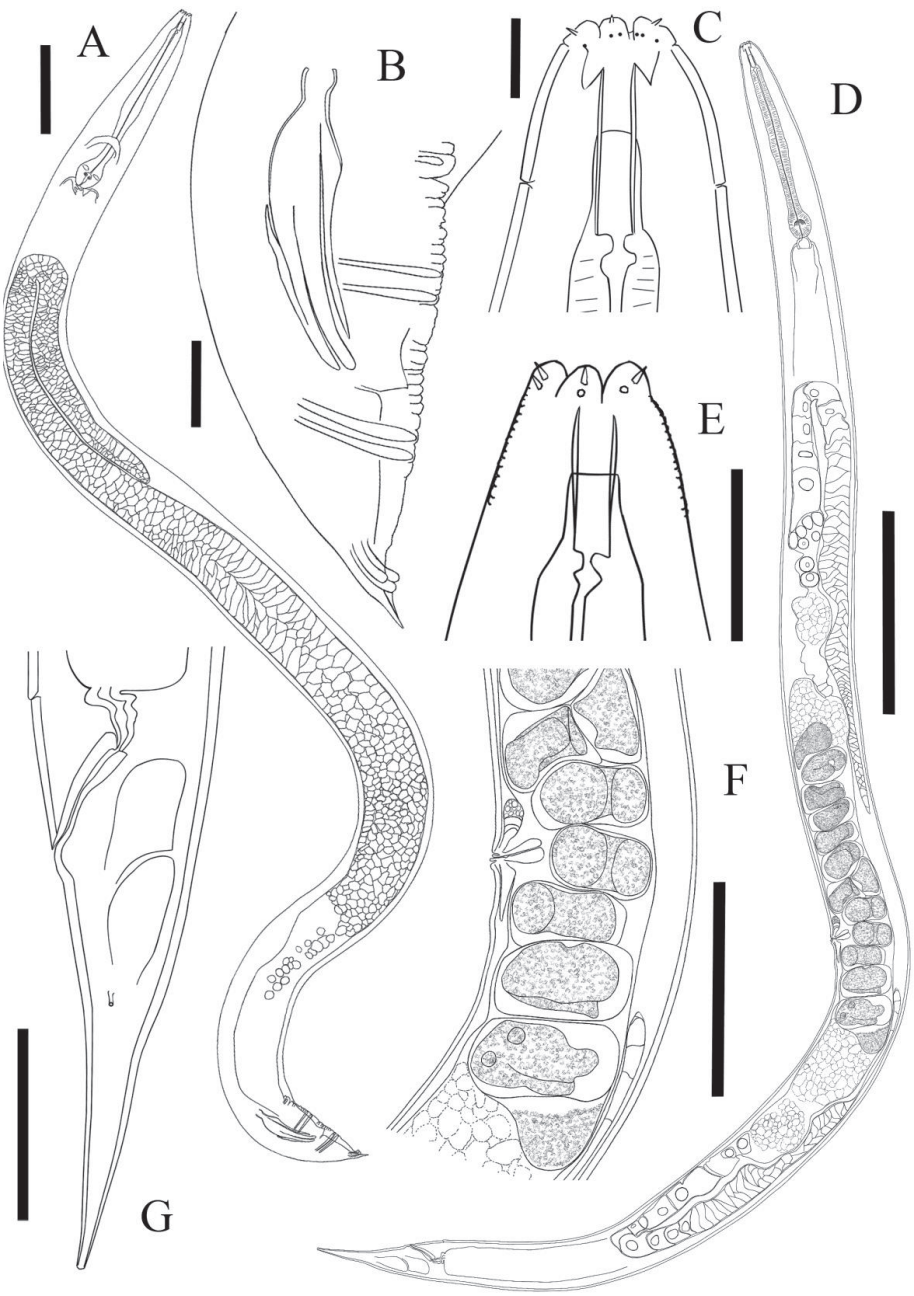

Fig. 2. *Litoditis marina* (Bastian, 1865) Sudhaus, 2011. A. Male, general view; B. Male, tail region, bursa with eight pairs of papillae; C. Female, cephalic region; D. Female, general view; E. Male, cephalic region; F. Female, vulval region; G. Female, tail region. Scale bar: 200 μm (D); 100 μm (F); 50 μm (A, G); 20 μm (B, E); 10 μm (C).

Table 1. Morphometric characteristics of *Litoditis marina* (Bastian, 1865) Sudhaus, 2011 from Sivash Bay. All measurements in μm, except for the de Man's ratios.

Characters	Males (n=5)	Females (n=8)
Body length	1273–1925	1337–2832
A	17.5–22.7	14.9–19.9
B	5.8–8.1	5.9–10.1
C	28.1–33.8	12.4–20.5
c'	1.2–1.7	2.3–3.2
Pharynx length	201–238	205–285
Stoma length	19–23	19–30
Nerve ring from anterior end	151–170	140–210
Tail length	44–57	108–140
Anal body diameter	32–48	38–61
Maximum body diameter	67–95	86–168
Spicule length	60–71	
Gubernaculum length	37–45	
Distance from anterior end to vulva		702–1570
Vulval body diam.		86–156

tudinal lines evenly spaced from each other: about 14 in mid-body and 4 in posterior body region. Head with six lips, separate, continuous with body contour; with minute inner labial papillae (Figs 2C, 3C) and two circles of short cephalic setae. Amphidial apertures oval, small, open at level of outer cephalic setae (Fig. 3B). Stoma of rhabditoid type, 3–5 μm wide. Cheilostoma not cuticularised, gymnostom culticularised, stegostom (pharyngeal collar) conspicuous, surrounding about 50 % of stoma length. Oesophagus with a distinct middle bulb and a posterior basal bulb. Nerve ring located at 71.3–75.1 % of pharyngeal length. Secretory-excretory pore not observed.

Reproductive system amphidelphic. Ovaries paired, reflexed; anterior branch on right and posterior on left side of intestine with former usually smaller than latter. Vulva located slightly posterior to mid-body, at 49.9–57.7 %. Six to twelve intrauterine eggs, 55–61 × 26–40 μm. Small gelati-

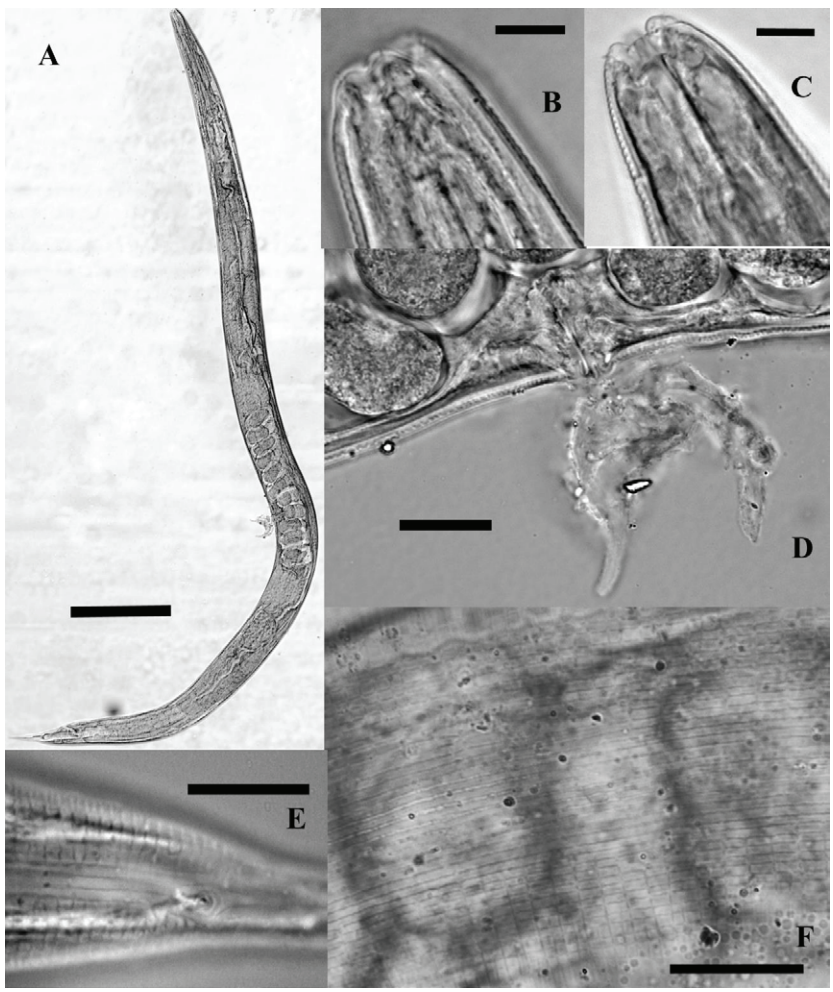

Fig. 3. *Litoditis marina* (Bastian, 1865) Sudhaus, 2011. A. Female, general view; B. Female, anterior end; C. Female, stoma; D. Female, vulva and copulatory plug; E. Female, phasmid on the tail; F. Female, cuticular lateral ridges in the vulval region. Scale bar: 200 μm (A); 20 μm (D, E, F); 10 μm (B, C).

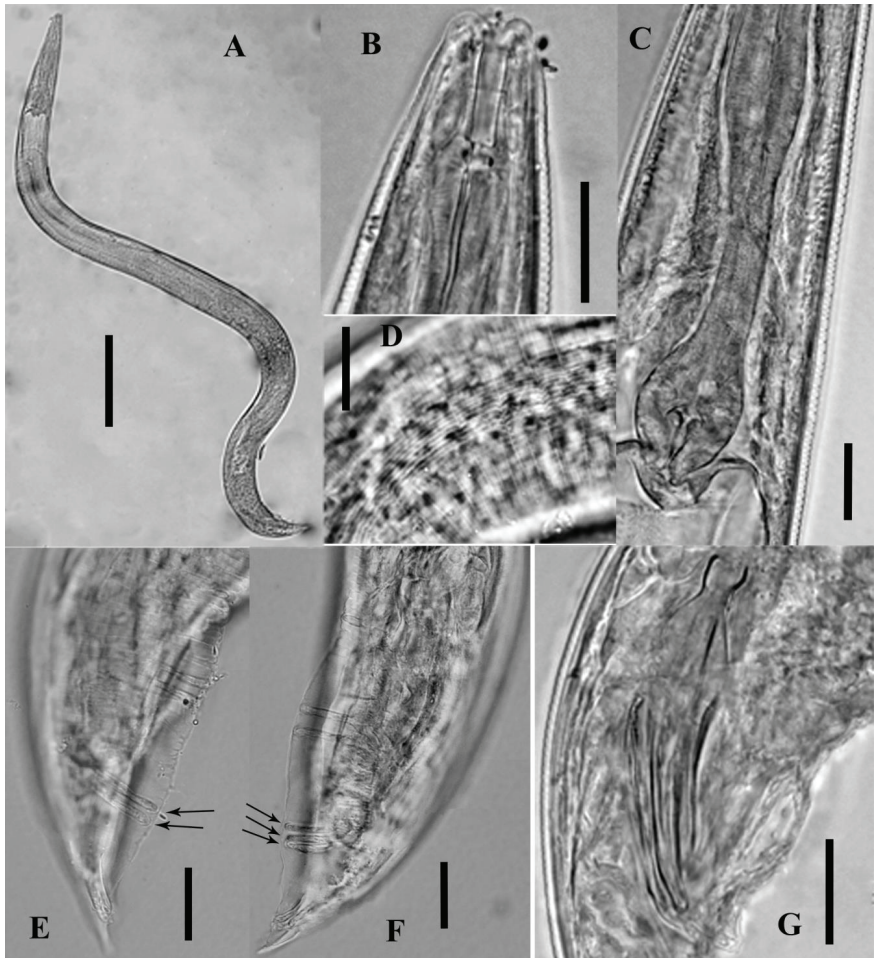

Fig. 4. *Litoditis marina* (Bastian, 1865) Sudhaus, 2011. A. Male, general view; B. Male, stoma; C. Male, bulbus; D. Male, cuticular lateral ridges in front of anus; E. Male, bursa with eight pairs of papillae; F. Male, bursa with nine pairs papillae; G. Male, spicule. Scale bar: 200 μm (A); 20 μm (B-G).

nous copulatory plug is deposited to seal the vulva (Fig. 3D). Up to 30 embryos within uteri in female (Meib.35N.v.). Tail conical, gradually tapering to a fine point. Phasmids pore-like, located behind anus at 38.5 % to 51.9 % of tail length.

Males. General morphology similar to that of females. Testis single, anterior and reflexed ventrally. Spicules paired (1.5–2.1 anal body diameter), identical and strongly cuticularised. Gubernaculum parallel to spicules, about 61.4–65.6 % of spicule length. Tail short, sharply tapering off to a point. Bursa peloderan, anteriorly open. Genital papillae nine pairs, three pre-cloacal and six post-cloacal, arranged in groups of 1+2/3+3 (Fig. 4F). One male (Figs 2B and 4E) with eight papillae, arranged in 1+2/2+3. First (GP1) short; second and third (GP2, GP3) pairs narrow, spaced, precloacal; six post-cloacal papillae (GP4, GP5 and GP6) grouped very closely together; and final group (GP7, GP8 and GP9) of three shorter pairs just anterior to the posterior tip of the tail.

Remarks. Specimens from the Sivash Bay agree well with the original description by Bastian (1865) and the redescription by Inglis & Coles (1961) in general measurements. However, one male from Sivash had fewer caudal papillae arranged in 1+2/2+3. Japanese specimens (Kito 1981) also have different numbers of caudal papillae between the right (1+2/2+3) and left (1+2/3+3) caudal alae.

Habitat and distribution. In the Sivash Bay, the bottom sediments and floating mats of *Cladophora* are two distinct biotopes for the development of meiobenthic organisms, in particular for nematodes. Bottom sediments are represented by silt and sandy silt with shell hash. *Cladophora* was found in Sivash Bay before but now it forms extensive floating mats along the coast. *Cladophora* mats attract the bulk of the fauna and now are a key element in the Sivash ecosystem (Shadrin et al. 2018, 2019a, 2019b, Prazukin et al. 2020).

Female specimens of *L. marina* were only found at three (st. 3, 4, 11) of the eight bottom sta-

tions; one specimen per station. Male and female specimens of *L. marina* were found in all three samples of floating mats of the alga *Cladophora* – st.2 (2 ind./10g; one male only), st.3 (14 ind./10g; 2 males, 8 females, 1 juv. male and 2 juv. females), st.4 (23 ind./10g; 2 males, 5 females).

Acknowledgments: This study was supported by the Russian Science Foundation (grant 18–16–00001). The author thanks Mr. A.A. Latushkin and Dr. E.V. Anufriieva, who performed sampling, to Dr. N.V. Shadrin for helpful advice, and to Mrs. L.F. Lukyanova for her help with processing bottom sediment samples.

References

Anufriieva E. V. & Shadrin N. V. 2018. Diversity of fauna in Crimean hypersaline water bodies. Journal of Siberian Federal University, Biology 11 (4): 294–305. DOI: 10.17516/1997–1389–0073.

Anufriieva E. & Shadrin N. 2020. The long-term changes in plankton composition: Is Bay Sivash transforming back into one of the world's largest habitats of *Artemia* sp. (Crustacea, Anostraca)? Aquaculture Research 51 (1): 341–350.

Bastian H. C. 1865. Monograph of the Anguillulidae or free nematodes, marine, land and fresh-water, with descriptions of 100 new species. Transactions of the Linnean Society of London 25: 73–184: 9–13.

Derycke S., Remerie T., Backeljau T., Vierstraete A., Vanfleteren J., Vincx M. & Moens T. 2008. Phylogeography of the *Rhabditis (Pellioditis) marina* species complex: evidence for long–distance dispersal, and for range expansions and restricted gene flow in the northeast Atlantic. Molecular Ecology 17: 3306–3322.

Derycke S., De Meester N., Rigaux A., Creer S., Bik H., Thomas W. K. & Moens T. 2016. Coexisting cryptic species of the *Litoditis marina* complex (Nematoda) show differential resource use and have distinct microbiomes with high intraspecific variability. Molecular Ecology 25: 2093–2110. doi: 10.1111/mec.13597

Gagarin V. G. 2001. A new species of nematodes found in rotting seaweed on the shore of the Black Sea. *Russian Journal of Marine Biology* 27 (4): 259–261.

Inglis W. G. & Coles J. W. 1961. The species of *Rhabditis* (Nematoda) found in rotting seaweed on British beaches. Bulletin of the British Museum (Natural History) Zoology 7: 320–333.

Kito K. 1981. Studies on the free–living marine nematodes from Hokkaido, IV. Journal of the Faculty of Science, Hokkaido University, Series 6 – Zoology 22(3): 250–278.

Prazukin A., Shadrin N., Balycheva D., Firsov Y., Lee R. & Anufriieva E. 2020. *Cladophora* spp. (Chlorophyta) modulate environment and create a habitat for microalgae in hypersaline waters. European Journal of Phycology 56 (3): 231–243.

Seinhorst J. W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. Nematologica 4: 67–69. http://dx.doi.org/10.1163/187529259X00381

Sergeeva N. G., Shadrin N. V. & Anufriieva E. V. 2019. Long–term changes (1979–2015) in the nematode fauna in Sivash Bay (Sea of Azov), Russia, worldwide the largest hypersaline lagoon, during salinity transformations. Nematology 21(4): 337–347.

Shadrin N. V. 2018. Hypersaline lakes as the polyextreme habitats for life. In: Zheng M., Deng T. & Oren A. (Eds.). Introduction to salt lake sciences. Beijing: Science Press, pp.180–187.

Shadrin N. V., Anufriieva E. V., Kipriyanova L. M., Kolesnikova E. A., Latushkin A. A., Romanov R. E. & Sergeeva N. G. 2018. The political decision caused the drastic ecosystem shift of the Sivash Bay (the Sea of Azov). Quaternary International 475: 4–10.

Shadrin N., Kolesnikova E., Revkova T., Latushkin A., Chepyzhenko A., Drapun I., Dyakov N. & Anufriieva E. 2019a. Do separated taxa react differently to a long–term salinity increase? The meiobenthos changes in Bay Sivash, largest hypersaline lagoon worldwide. Knowledge and Management of Aquatic Ecosystems 420: 36. DOI: 10.1051/kmae/2019028

Shadrin N. V., Kolesnikova E. A., Revkova T. N., Latushkin A. A., Chepyzhenko A. A., Dyakov N. N. & Anufriieva E. V. 2019b. Macrostructure of benthos along a salinity gradient: The case of Sivash Bay (the Sea of Azov), the largest hypersaline lagoon worldwide. Journal of Sea Research 154: 101811.

Sudhaus W. 1974a. Nematoden (insbesondere Rhabditiden) des Strandanwurfs und ihre Beziehungen zu Krebsen. Faunistisch–ökologische Mitteilungen 4: 365–400.

Sudhaus W. 1974c. Zur Systematik, Verbreitung, Okologie und Biologie neuer und wenig bekannter Rhabditiden (Nematoda) 2. Teil. Zoologische Jahrbücher. Abteilung für Systematik, Ökologie und Geographie der Tiere 101: 417–465.

Sudhaus W. 2011. Phylogenetic systematisation and catalogue of paraphyletic "Rhabditidae" (Secernentea, Nematoda). Journal of Nematode Morphology and Systematics 14 (2): 113–178.

Tietjen J. H., Lee J. J., Rullman J., Greengart A. & Trompeter J. 1970. Gnotobiotic culture and physiological ecology of the marine nematode *Rhabditis marina* Bastian. Limnology and Oceanography 15: 535–543.

Xie Y., Zhang P., Xue B., Cao X., Ren X., Wang L., Sun Y., Yang H. & Zhang L. 2020. Establishment of a marine nematode model for animal functional genomics, environmental adaptation and developmental evolution. BiorXiv. DOI: 10.1101/2020. 03.06.980219.

Received: 21.03.2022
Accepted: 02.11.2022