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**STYLOTHERISTUS PARAMUTILUS SP. NOV. (NEMATODA: XYALIDAE),
A NEW NEMATODE SPECIES FROM THE BLACK SEA**

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Stylotheristus paramutilus sp. nov. from bottom sediments sampled in shallow-water and deep-sea habitats in the Black Sea is described and illustrated. The new species is characterized by well-developed lip region; 12 setiform cephalic sensilla in female and 16 in male; cervical setae present; spicules 0.6–0.9 anal body diameters long and expanded proximally; gubernaculum plate-like slightly curved; conico-cylindrical tail of 4.5–5.8 anal body diameters (except for one male with it equal to 12.9 anal body diameters); and 3 terminal setae. The present study provides the first *Stylotheristus* species record in the Black Sea. *S. paramutilus* sp. nov. is characterized by a wide spatial and bathymetrical (2–250-m depths) distribution in the Crimea region and the Istanbul Strait's (Bosphorus) outlet area of the Black Sea. However, in future, molecular analysis is required to confirm the identity of these specimens from different Black Sea habitats.

Keywords: Monhysterida, free-living marine nematodes, taxonomy, distribution, deep-sea, shallow-water

Free-living nematodes are among the most numerous and widespread multicellular organisms in the World Ocean. The study of meiobenthos in various areas of the Black Sea provided extensive data on the taxonomic diversity of free-living nematodes. In total, the species richness of the nematode fauna of the entire Black Sea is about 350 species and morphotypes identified only down to a genus or family level. In the region of Turkey alone, the nematode fauna includes 255 species [personal communication of PhD Derya Ürkmez]; for the Crimea region, about 230 species of nematodes are known [Sergeeva, 2003; Revkova, unpublished data].

The family Xyalidae includes 50 genera [Nemys, 2023; Venekey et al., 2014]. Out of them, 7 genera are registered in the Black Sea: *Valvaelaimus* Lorenzen, 1977, *Theristus* Bastian, 1865, *Daptonema* Cobb, 1920, *Steineria* Micoletzky, 1922, *Paramonohystera* Steiner, 1916, *Cobbia* de Man, 1907, and *Amphimonhystera* Allgén, 1929 [Mureşan, 2012, 2014; Revkova, 2015; Sergeeva, 2003; Sergeeva et al., 2021; Shnyukov, Yanko-Hombach, 2020; Vorobyova, Kulakova, 2009; Yanko et al., 2017].

Representatives of the genus *Stylotheristus* (the family Xyalidae) are widely distributed in the World Ocean: in the North and Mediterranean seas, in the Pacific, Atlantic, and Indian oceans [Nemys, 2023; OBIS, 2023], and in the Sea of Japan [our unpublished data]. Most of them were identified only down to a genus level. According to Nemys database [2023], two valid *Stylotheristus* species are known: *S. mutilus* described from the depth of 27–28 m in the North Sea and *S. multipapillatus* described from the depth of 5 m on the coast of Portugal.

In the present study, we provide a taxonomic description, illustrations, and data on distribution of a new *Stylotheristus* species for the Black Sea.

MATERIAL AND METHODS

The material was sampled in different years in the coastal and deep-sea areas of the Black Sea (Fig. 1, Table 1).

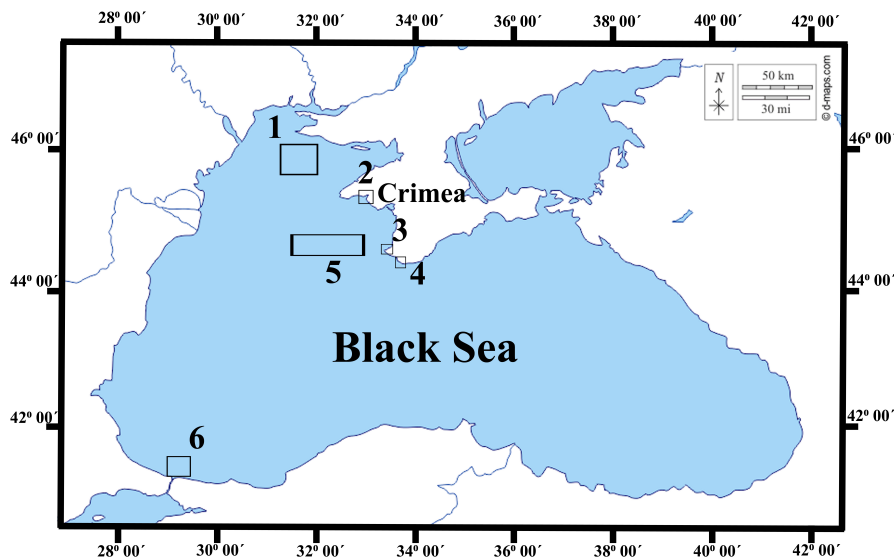


Fig. 1. The study areas where *Stylotheristus paramutilus* sp. nov. nematodes were found: 1, the Northwestern Crimea, the Zernov's *Phyllophora* Field (2010); 2, the Donuzlav Bay (2019); 3, the Kruglaya (Omega) Bay (2010); 4, the Laspi Bay (2017); 5, the Southwestern Crimea (2010); 6, the Istanbul Strait's (Bosphorus) outlet area (the Black Sea) (2009 and 2010)

To study the meiobenthos, in the coastal areas of the Crimea, the Kruglaya (Omega), Donuzlav, and Laspi bays, material was sampled at various depths using push cores (sample area of 18.1 cm²; height of 5 cm) at each station by a scuba diver. In the Kruglaya Bay, material was sampled at 1 station in different seasons in 2009–2010 [Zaika et al., 2011]; in the Donuzlav Bay, at 10 stations in its southern area in 2019 [Revkov et al., 2021]; and in the Laspi Bay, at 19 benthic stations in 2017 [Sergeeva et al., 2023]. To analyze meiofauna on the Zernov's *Phyllophora* Field, 18 sediment cores were taken by subcoreing the sediment sampled with an Okean-25 bottom grab during the cruise No. 68 of the RV "Professor Vodnyanitsky" in 2010. A cylindrical corer with inner diameter of 4.8 cm was used [Sergeeva et al., 2013].

In the deep-sea areas, material was sampled at the oxic/anoxic interface (82–363-m depth) in the northern Black Sea off the Crimean Peninsula during the research cruise No. 15/1 of the RV "Maria S. Merian" (Germany) (April–May 2010). In the Istanbul Strait's (Bosphorus) outlet area of the Black Sea (93–300-m depth), sampling was carried out during two research cruises: the cruise of the RV "Arar" of the Istanbul Technical University (November 2009) and the cruise No. 15/1 of the RV "Maria S. Merian" (April 2010). In the deep-sea areas, bottom sediments were sampled with a multiple corer (diameter of 9.6 cm), push corer or geological corer (diameter of 7.3 cm), and devices that provide obtaining virtually undisturbed samples. The sediment cores were sliced into 1-cm-thick layers down to a depth of 5–10 cm in order to study the vertical distribution of the fauna [Sergeeva et al., 2017, 2021].

Table 1. Station coordinates and sampling depth and time (the Black Sea)

| Station No. | Latitude, N | Longitude, E | Depth, m | Date |
|---|-------------|--------------|----------|------------|
| <i>Zernov's Phyllophora</i> Field, the cruise No. 68 of the RV "Professor Vodyanitsky" | | | | |
| 21 | 45°45'24" | 31°21'28" | 41 | 13.11.2010 |
| 25 | 46°4'3" | 31°35'5" | 20 | 13.11.2010 |
| The Donuzlav Bay | | | | |
| 1 | 45°18'59" | 33°1'11" | 2 | 11.07.2019 |
| 3 | 45°19'13" | 33°0'50" | 2 | 11.07.2019 |
| 4 | 45°19'27" | 33°0'60" | 2 | 11.07.2019 |
| The Kruglaya (Omega) Bay | | | | |
| 5 | 44°36'11.0" | 33°26'34.3" | 8.8 | 28.01.2010 |
| The Laspi Bay | | | | |
| 1 | 44°25'05" | 33°41'43" | 14.5 | 15.09.2017 |
| 15 | 44°25'10" | 33°42'13" | 9 | 16.09.2017 |
| 17 | 44°25'04" | 33°42'22" | 10 | 16.09.2017 |
| 18 | 44°25'04" | 33°42'28" | 9 | 16.09.2017 |
| The Southwestern Crimea and the Bosphorus Strait outlet area, the cruise No. 15/1 of the RV "Maria S. Merian" | | | | |
| 235 | 41°29'37" | 29°15'12" | 159 | 15.04.2010 |
| 372 | 44°37'14" | 32°53'49" | 163 | 25.04.2010 |
| 405 | 44°37'21" | 32°54'9" | 155.5 | 28.04.2010 |
| 425 | 44°47'09" | 31°58'05" | 163.2 | 30.04.2010 |
| The Bosphorus Strait outlet area, the cruise of the RV "Arar" (Istanbul Technical University) | | | | |
| 3 | 41°24'01.2" | 29°03'12.6" | 82 | 12.11.2009 |
| 4 | 41°24'01.2" | 29°03'12.6" | 88 | 15.11.2009 |
| 5 | 41°23'17.4" | 29°12'14.4" | 103 | 15.11.2009 |
| 7 | 41°26'51.6" | 29°12'57" | 160 | 15.11.2009 |
| 9 | 41°28'59.4" | 29°15'08.4" | 250 | 15.11.2009 |

All sediment sections were fixed with 75% alcohol to preserve morphological structures without distortion. In a laboratory, all sampled sediments were washed through two stacked sieves with mesh size of 1 mm and 63 µm in series and stained with rose bengal for at least 24 h.

The stained samples were placed in a Bogorov chamber; meiofaunal organisms were identified to major taxa and counted out under a binocular microscope. Nematode specimens were transferred to pure glycerin and mounted on wax–paraffin ring permanent slides [Ryss, 2002]. All measurements, photographs, and drawings were taken under Carl Zeiss Axiostar Plus and Olympus BX53 light microscopes. Holotype and paratypes were deposited in IBSS collection (Sevastopol).

Abbreviations are as follows: a, ratio of body length / maximum body diameter; b, ratio of body length / pharynx length; c, ratio of body length / tail length; c', ratio of tail length / anal body diameter; cbd, corresponding body diameter; and abd, anal body diameter.

RESULTS

Taxonomy. Order Monhysterida Filipjev, 1929. Family Xyalidae Chitwood, 1951. Genus *Stylotheristus* Lorenzen, 1977.

Diagnosis (emended from [Fonseca, Bezerra, 2014]). Cuticle transversely striated. Somatic setae usually present. Anterior sensilla arranged in two crowns with the number of setae in the second crown depending on the sex and life stage: (6 + 4) setae in juveniles; (6 + 4) or (6 + 6) in females; and (6 + 10) in males. Amphidial fovea circular or oval. Buccal cavity conical. Pharyngeal muscles

well-developed around the buccal cavity. Females with one outstretched ovary, located to the left side of intestine. Males with single anterior outstretched testis to the right or left of intestine. Spermatheca can be present. Spicules short (< 1 abd). Gubernaculum narrow, without apophysis. Precloacal supplements present or absent. Three caudal glands opening through separate pores. Tail conico-cylindrical, with three terminal setae.

Type species. *Stylotheristus mutilus* (Lorenzen, 1973) Lorenzen, 1977.

List of valid *Stylotheristus* species:

- *Stylotheristus mutilus* (Lorenzen, 1973) Lorenzen, 1977;
- *Stylotheristus multipapillatus* Pinto & Neres, 2020;
- *Stylotheristus paramutilus* sp. nov. (Figs 2, 3, 4, 5, Tables 2, 3).

Table 2. Measurements of *Stylotheristus paramutilus* sp. nov. from different areas of the Black Sea. All values are in µm unless otherwise stated, except for the ratios a, b, c, and c'. All curved structures were measured along the arc

| Character | The Donuzlav Bay | | | The Bosphorus Strait area | | The Laspi Bay |
|------------------------------|------------------|----------------------|------------------------|---------------------------|-----------------|---------------|
| | Male holotype | Male paratype, n = 5 | Female paratype, n = 4 | Male paratype, n = 2 | Female paratype | Male paratype |
| Body length | 1,767 | 1,545–1,752 | 1,613–1,799 | 1,854–1,875 | 1,820 | 1,565 |
| a | 49.1 | 40.6–48.7 | 38.2–46.1 | 51.5–52.1 | 45.5 | 36.4 |
| b | 9.7 | 8.2–9.8 | 8.7–9.8 | 10.4–11.2 | 10.5 | 8.5 |
| c | 5.1 | 4.6–5.5 (12.9*) | 4.8–5.7 | 4.5 | 4.5 | 5.8 |
| c' | 12.6 | 12.2–13.3 (3.3*) | 12.2–14.3 | 12.9 | 16.2 | 9 |
| V (%) | | | 51.9–56.6 | | 49.1 | |
| Vulval body diameter | | | 32–43 | | 35 | |
| Maximum body diameter | 36 | 36–40 | 35–47 | 36 | 40 | 43 |
| Pharynx length | 181 | 165–188 | 172–196 | 165–180 | 173 | 184 |
| Buccal cavity length | 20 | 18–21 | 20–24 | 20–21 | 17 | 20 |
| Buccal cavity diameter | 15 | 15–20 | 12–14 | 17–20 | 12 | 19 |
| Amphid width / cbd (%) | 32 | 29.6–36 | 31.1–33.3 | 33.3–34.8 | 38.1 | 26.7 |
| Amphid from anterior end | 17 | 17–20 | 20–21 | 12–15 | 11 | 18 |
| Nerve ring from anterior end | 86 | 81–98 | 81–105 | 82–95 | – | – |
| Nerve ring cbd | 35 | 32–35 | 31–38 | 30–32 | – | – |
| Tail length | 353 | 317–345 (120*) | 316–345 | 412 | 405 | 270 |
| abd | 28 | 26–31 | 22–27 | 29–30 | 25 | 30 |
| Spicule length | 22 | 17–22 (27×) | | 22 | | 21 |
| Gubernaculum length | 14 | 10–12 | | 12 | | 9 |

Note: * denotes a male (Meib.44. N.p.) with a very short tail; × denoted a male (Meib.43. N.p.) with longer spicules.

The latter species was previously recorded in the Bosphorus Strait outlet area (the Black Sea) as *Daptonema* sp. [Sergeeva et al., 2021].

Type material. Nine males and six females. Male holotype mounted on slide Meib.39. N.h. Male paratypes mounted on slides: Meib.40. N.p. – Meib.45. N.p., Meib.50. N.p. Female paratypes in pure glycerin: Meib.41. N.p., Meib.46. N.p. – Meib.49. N.p.

Type locality. The Black Sea, the Donuzlav Bay, 45°19'13"N, 33°0'50"E, sta. 3, silt with the smell of hydrogen sulfide, sediment depth of 2 m.

Etymology. The species name means “close to *mutilus*,” “similar to *mutilus*.”

Description. Male. Body cylindrical and gradually tapering towards posterior end. Cuticle striated. Somatic setae scattered along the body, 4–11 µm long. Cervical setae thin, 9–14 µm long. Lips well developed, high. Six short inner labial conical papillae (3 µm long) and a circle with 16 cephalic setae: 12 long setae (11–18 µm long) and 4 short setae (6–9 µm long). Stoma funnel-shaped. Cheilostoma with thin, smooth walls.

Pharyngostoma funnel, with weakly cuticularized walls. Pharynx muscular, almost cylindrical. Cardia 15–21 µm long, surrounded by intestine. Amphids circular, 8–9 µm in diameter. Pharynx cylindrical, about 8.9–12.2% of total body length. Nerve ring situated near middle of pharynx (45–52.8%). Secretory-excretory pore not observed. Reproductive system monorchic. Testis outstretched, situated to the right of intestine.

Spicules short (0.6–0.9 abd), slightly curved and expanded proximally. In proximally part of spicules, visible ejacular canal. Gubernaculum plate-like, slightly curved, about 42.9–63.6% of spicule length. Sperm cells globular, 11–15 µm wide. Tail conico-cylindrical, with elongated filiform portion; three terminal setae, 8–11 µm long, on the tail tip.

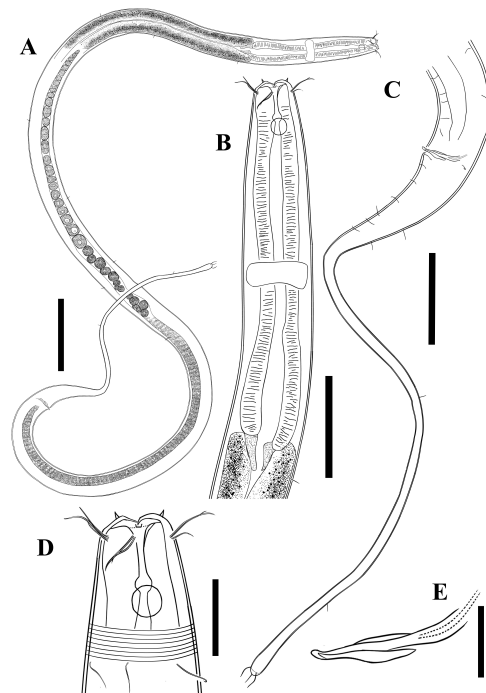


Fig. 2. *Stylotheristus paramutilus* sp. nov. Male holotype. A, general view; B, pharyngeal region; C, tail region; D, head; E, spicule. Scale bar: A, 100 µm; B, C, 50 µm; D, 20 µm; E, 10 µm

Female. Similar to male in general morphology. Cephalic sensilla arranged in a circle with 12 cephalic setae (10–18 μm long). Nerve ring at 50–57.4% of pharynx length from anterior. Reproductive system monodelphic. Ovary outstretched and on the left side of intestine. Vulva directed anteriorly, situated slightly posterior to mid-body (861–1,015 μm). Small copulatory plug visible to seal the vulva. Vagina long, with muscular walls. Spermatheca absent. Mature egg (127 \times 43 μm) and spermatozoa present in uterus. Caudal glands not visible. Tail long, with three terminal setae (8–9 μm long).

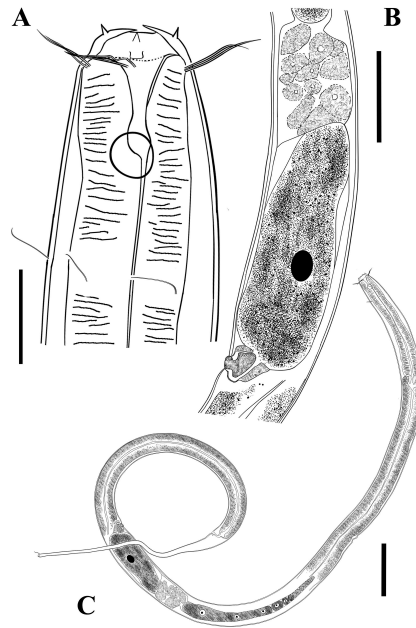


Fig. 3. *Stylotheristus paramutilus* sp. nov. Female paratype. A, head; B, vulval region; C, general view. Scale bar: A, 20 μm ; B, 50 μm ; C, 100 μm

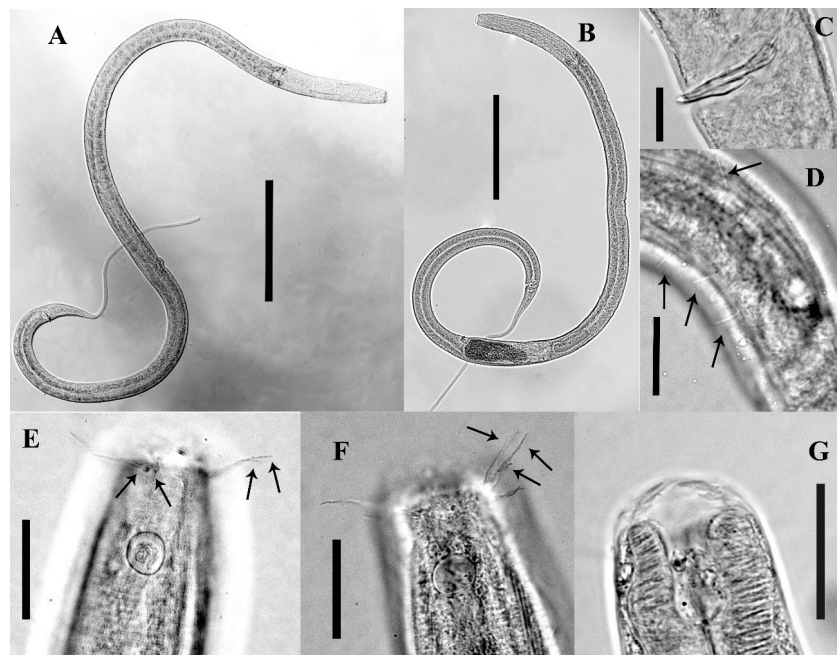


Fig. 4. *Stylotheristus paramutilus* sp. nov. A, male holotype, general view; B, female paratype, general view; C, male holotype, spicule; D, male paratype, preanal somatic setae; E, female paratype, head; F, male paratype, head; G, female paratype, buccal cavity. Scale bar: A, B, 200 μm ; C, 10 μm ; D–G, 20 μm

Diagnosis. *S. paramutilus* sp. nov. is characterized by body length of 1,545–1,875 μm ; 12 setiform cephalic sensilla in female; cervical setae present; spicules short and widening proximally; gubernaculum plate-like slightly curved; and tail 270–412 μm long (except for one male paratype, 120 μm long).

Differential diagnosis. *S. paramutilus* sp. nov. differs from all valid species (see Table 3) by number of cephalic setae in female (12 vs. 10); relatively shorter body in males (1,545–1,875 μm vs. 1,830–2,330 μm in *S. mutilus* and 1,968–2,052 μm in *S. multipapillatus*) and in females (1,657–1,820 μm vs. 1,970 μm in *S. mutilus* and 2,100–2,240 μm in *S. multipapillatus*); structure of the spicular apparatus (expand proximally vs. thin all over in *S. multipapillatus* and *S. mutilus*); and longer tail (c value of 4.5–5.8 [except for one male with 12.9] vs. 5.8–6.6 in *S. mutilus* and 6.8–8.6 in *S. multipapillatus*). *S. paramutilus* sp. nov. is similar in the body structure to *S. mutilus*, but differs from it by wider body in males (a value of 36.4–52.1 vs. 55–61). The new species differs from *S. multipapillatus* by precloacal supplements (absent vs. present).

Variability of body size and copulatory organs. Specimens from different areas of the Black Sea have significant variability in body and tail lengths; there are also slight differences in the shape and length of spicules (see Fig. 5, Table 2). Specimens from the Bosphorus Strait area are much longer and slightly slenderer than those from the Donuzlav and Laspi bays. One male (Meib.42. N.p.), from the Bosphorus Strait area, has straight spicules, and another male (Meib.41. N.p.) has proximally curved spicules (Fig. 5D, E). The third male paratype (Meib.43. N.p.), from the Donuzlav Bay, has longer spicules [27 μm (0.9 abd) vs. 17–22 μm (0.6–0.8 abd)] and ratio of spicule length to gubernaculum length (2.25 vs. 1.6–1.9) compared to other specimens (Fig. 5B). The fourth male paratype (Meib.44. N.p.), from the Donuzlav Bay, has shortened tail (3.3 abd vs. 9–13.3 abd) (Fig. 5A).

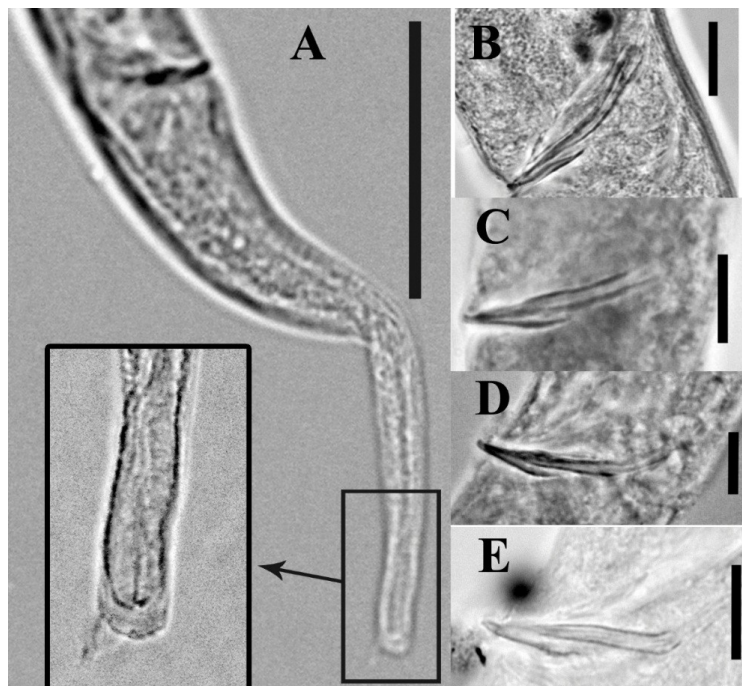


Fig. 5. *Stylotheristus paramutilus* sp. nov. A, male paratype, tail (the Donuzlav Bay); B–E, variation of shape of spicules in male paratypes: B, the Donuzlav Bay; C, the Laspi Bay; D–E, the Bosphorus Strait area. Scale bar: A, 50 μm ; B–E, 10 μm

Table 3. Morphological characters of *Stylotheristus* species. All values are in μm unless otherwise stated, except for the ratios a, b, c, and c'

| Character | <i>Stylotheristus paramutilus</i> sp. nov. | | <i>Stylotheristus mutilus</i> | | <i>Stylotheristus multipapillatus</i> | |
|--------------------------|---|-------------|-------------------------------|--------|---------------------------------------|-------------|
| | males | females | males | female | males | females |
| Body length | 1,545–1,875 | 1,613–1,820 | 1,830–2,330 | 1,970 | 1,920–2,052 | 2,100–2,240 |
| a | 36.4–52.1 | 38.2–46.1 | 55–61 | 45 | 56.9–68.9 | 43.7–56.45 |
| b | 8.2–11.2 | 8.7–10.5 | 9.2–9.6 | 8.7 | 8–9 | 8.2–8.9 |
| c | 4.5–5.8 | 4.5–5.7 | 5.9–6.6 | 5.8 | 7.5–8.6 | 6.8–7.6 |
| c' | 9–13.3 | 12.2–16.2 | 11.2 | 14.3 | 7.4–9.8 | 9.7–11 |
| Number of cephalic setae | 16 | 12 | 16 | 10 | 16 | 10 |
| V (%) | | 49.1–56.6 | | 55 | | 57–63 |
| Spicule length | 17–22 | | 18–20 | | 15.5–25 | |
| Number of supplements | absent | | absent | | 11–15 | |

It can be assumed that such morphological variability is determined by the adaptation of the species to different conditions of the waterbody. On the other hand, it is possible that in future, genetic methods applied to study individuals from various habitats will show the existence of different species of *Stylotheristus* genus in the Black Sea.

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**STYLOTHERISTUS PARAMUTILUS SP. NOV. (NEMATODA: XYALIDAE),
НОВЫЙ ВИД НЕМАТОД ИЗ ЧЁРНОГО МОРЯ**

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Приведены иллюстрации и описание *Stylotheristus paramutilus* sp. nov. из сборов донных осадков мелководных и глубоководных зон Чёрного моря. Новый вид характеризуется хорошо развитой губной областью, 12 щетинковидными головными сенсиллами у самки и 16 у самца; наличием шейных щетинок; спикулами (0,6–0,9 анального диаметра), расширяющимися проксимально; пластинчатым рульком, слегка изогнутым; конико-цилиндрическим хвостом, равным 4,5–5,8 анального диаметра (кроме одного самца, значение у которого составило 12,9 анального диаметра) и 3 щетинками на кончике хвоста. В настоящем исследовании описана первая находка рода *Stylotheristus* в Чёрном море. *S. paramutilus* sp. nov. характеризуется широким пространственным и батиметрическим (глубины от 2 до 250 м) распространением в Чёрном море — как в различных районах Крыма, так и на выходе из пролива Босфор. Сделано заключение о необходимости проведения в будущем молекулярного анализа для подтверждения принадлежности черноморских представителей из разных местообитаний к одному виду.

Ключевые слова: Monhysterida, свободноживущие морские нематоды, систематика, распределение, глубоководный, мелководный