

A brief look at the free-living Nematoda of the oxic/anoxic interface with a new genus record (*Trefusia*) for the Black Sea

by

Derya Ürkmez^{1,*}, Michael L. Brennan²,
Murat Sezgin¹, Levent Bat¹

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¹Department of Hydrobiology, Faculty of
Fisheries, Sinop University, TR57000 Sinop,
Turkey

²Graduate School of Oceanography, University of
Rhode Island, Narragansett, R1 02882, USA

* Corresponding author: deryaurkmez@gmail.com

Abstract

In order to provide the first comparative source of nematofaunal data at the oxic/anoxic interface off the Sinop Peninsula, the southern Black Sea, a survey of meiofauna and nematode fauna was conducted in August 2011 aboard the exploration vessel (E/V) Nautilus with ROV during the Black Sea Expedition NA012. Higher meiofaunal taxa and nematode composition were investigated. Free-living marine nematodes were the most abundant group at each site. A total of 84 species were found, belonging to 23 families. The suboxic zone was dominated by the nematode *Trefusia* aff. *longicauda* (42%). This is the first record of the genus *Trefusia* De Man, 1893 for the Black Sea. Although many factors are likely to influence the changes in the meiofaunal abundance and the composition of nematode assemblages, we suggest that oxygen reduction indeed affected the meiofaunal abundance and the nematode composition, however, a particular preference of several taxa for extreme conditions may be suggested.

Key words: meiofauna, Nematoda, suboxic, *Trefusia*, Turkey

Introduction

Several recent surveys conducted in the world's oceans have focused on extreme habitats, and oxygen-depleted zones are one of these areas gaining more importance due to climate changes (Levin 2002). Such unusual environments give us information on the limits of life and provide a potential for the discovery of new ecological aspects. The Black Sea is well known for its naturally occurring anoxic bottom waters and a large euxinic basin containing hydrogen sulfide and characterized by a depletion of oxygen (Luth&Luth 1998; Oğuz et al. 2001; Sergeeva&Gulin 2007; Sergeeva et al. 2013). However, a permanent suboxic zone, where O_2 and H_2S do not coexist (Murray et al. 1989; Duman et al. 2006), was also identified as a boundary between these two water layers. Similar areas in other parts of the world are referred to as oxygen minimum zones (OMZ) with their concentrations falling below 0.5 ml l^{-1} ($22 \mu\text{M}$) (Gooday et al. 2010). In these areas with low oxygen, the distribution structure of meio-, macro- and megafaunal benthic communities show large differences (Levin et al. 1991; Gooday et al. 2000; Levin et al. 2000; Neira et al. 2001) in abundance and diversity (Levin et al. 2000). Meiofauna seem to be less affected according to several studies (Gooday et al. 2000; Cook et al. 2000; Levin et al. 1991; Neira et al. 2001). Nematoda is the most abundant taxon among meiobenthic organisms and these organisms have a potential to inhabit different environments on earth (Balsamo et al. 2010; Semprucci et al. 2014). They tend to survive better under low-oxygenated, even anoxic conditions compared to other meiobenthic taxa (Levin et al. 2001; Giere 2009; Sandulli et al. 2014).

Taxonomic studies of meiobenthic organisms in the Black Sea date back to the 1880s; they were carried out on only a few groups (Vorobyeva 2003). Besides the more frequent shallow-water meiofaunal research, several studies have been published on the suboxic and anoxic meiobenthic fauna of the Black Sea. Luth and Luth (1998) examined the methane seep area southwest of the Crimean Peninsula (Ukraine) and presented the results for the meiobenthic composition of three different depths from oxic (60 m), suboxic (110 m) and anoxic (190 m) zones. Sergeeva and Gulin (2007) studied meiobenthos densities and composition

of higher taxa in an active seepage area at a depth ranging from 182 m to 252 m at the Dnieper Canyon (NW Black Sea) and stated that the abundance of meiobenthos varied between 2397 and 52 593 ind. m^{-2} with the maximum densities of nematodes and foraminifers being found at the permanent H_2S zone (220-250 m). Revkov and Sergeeva (2004) and Sergeeva et al. (2012) reported that nematodes dominated in metazoan meiobenthos at depths of 70-120 m and 70-240 m, respectively, and the next most abundant group was Harpacticoida. Luth (2004) investigated the meiofauna of a transect at a depth from 50 to 190 m in the İnebolu region where the maximum number of 10 taxa was recorded, and where nematodes were dominant at all sampling depths. According to the results of the study by Sergeeva et al. (2013), the meiobenthos occurring between 75 and 300 m is represented by 21 taxa and Nematoda was the dominant taxon. Sergeeva et al. (2013) also mentioned that the amount of meiobenthos was considerably reduced at a depth of 300 m, whereas the abundance of Nematoda showed a relative increase.

As far as we know, the previous studies from the deeper Black Sea, covering the oxic/anoxic interface, have not provided information on the nematode composition in the areas. The study of Sergeeva and Zaika (2013) considered the oxic/anoxic interface of the northwestern part of the Black Sea and reported that 69 nematode species were found at 150 m. The authors reported the presence of several genera such as *Quadricoma*, *Sabatieria*, *Theristus*, *Monhystera*, *Cobbionema*, *Linhomoeus*, *Paralinhomoeus*, *Metalinhomoeus*, *Aponema*, *Microloaimus* and *Campylaimus*.

On the other hand, studies dealing with free-living marine nematodes of the Turkish Seas are scarce and come mainly from the Black Sea coasts of Turkey. Ürkmez et al. (2011) described seasonal changes in nematode assemblages at 3 and 10 m along the Sinop coasts of the Black Sea. Ürkmez&Brennan (2013) reported a new species to science from 90 m off the Sinop coast. Çınar (2014) provided a checklist of 13 phyla from the coasts of Turkey and listed a total of 6 species of free-living marine nematodes.

This study is part of the general effort to understand the dynamics of the oxic/anoxic interface, the suboxic zone, and the internal wave action between these density boundaries (Brennan et al. 2013). This

2011 expedition included side-scan sonar mapping, ROV exploration of geological and archaeological targets as well as the bedforms from internal waves in the anoxic zone, chemical processes from sensors on the vehicle, and sediment push cores from all three zones, which were analyzed for microbiology, grain size, and geochemistry, in addition to the meiofaunal assemblages. These data compiled together will provide a greater understanding of the dynamics in this coastal zone. This preliminary study of the meiofauna from the sediment samples aims to describe and compare the nematofaunal abundance in oxic, suboxic and anoxic sediments collected from the southern Black Sea off Sinop, and to present the first findings on the community structure of free-living marine nematodes at the oxic/anoxic interface of the southern Black Sea (Sinop) sediments.

Materials and methods

Study area and sampling

The material was collected off the Sinop Peninsula during the Black Sea NA012 expedition of E/V Nautilus (operated by the Ocean Exploration Trust) in August 2011. The sampling sites are located in the southern Black Sea, at the western part of the Sinop peninsula, covering three different environments overlain by oxic, suboxic and anoxic waters (Fig. 1). Three representative locations were selected based on dissolved oxygen readings of the optode mounted on the ROV Hercules: (1) oxic zone, 69.61 μM ; (2) suboxic zone, 1.44 μM ; (3) anoxic zone, 0 μM (Table 1). Quantitative samples were obtained during three dives using plastic sediment corers (surface area of

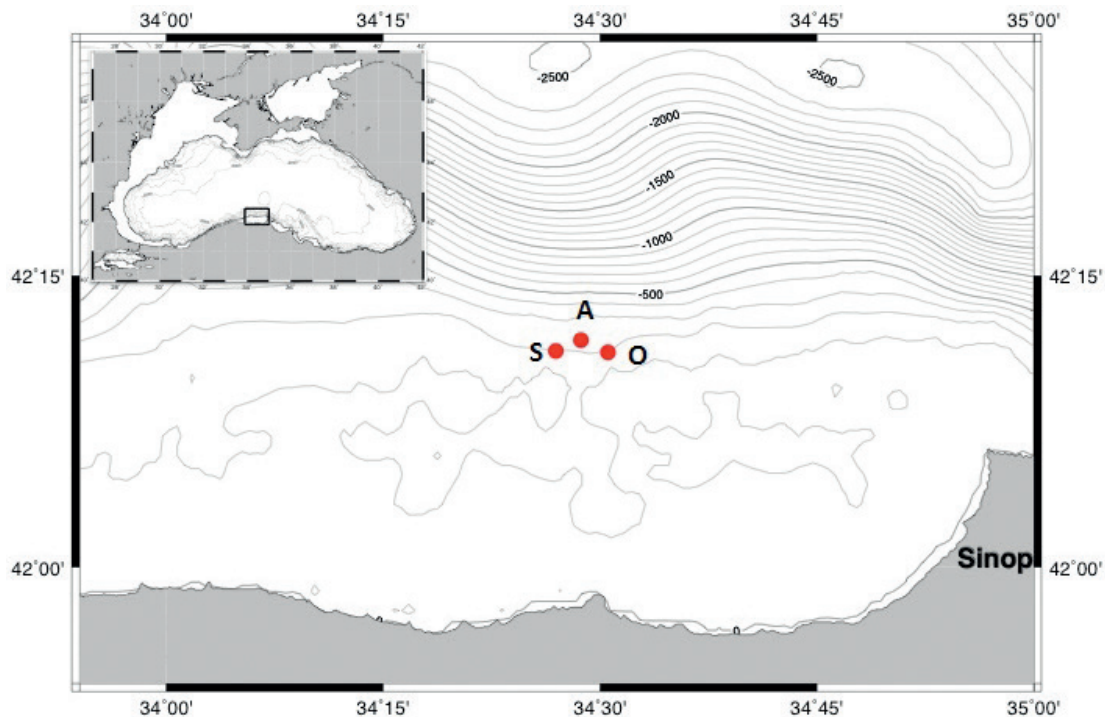


Figure 1

Study area with sampling sites located in the western part of Sinop. S: Suboxic; A: Anoxic, O: Oxic

Table 1

Sampling sites

Expedition No.	Dive No.	Date	Latitude	Longitude	Depth (m)	Site	O ₂ (μM)
NA012-003	H1152	9.8.2011	42.009.1628°N	34.030.6970°E	90	Oxic	69.61
NA012-001	H1149	5.8.2011	42.011.1197°N	34.026.9579°E	120.5	Suboxic	1.44
NA012-002	H1150	6.8.2011	42.011.6808°N	34.028.6798°E	203	Anoxic	0

32.15 cm²) pushed 10 cm into the sediment by ROV (Fig. 2). All sediment samples were fixed on board with 75% ethanol (Hulings&Gray 1971; Giere 2009; N.G. Sergeeva pers. comm.). Bottom water salinity, temperature and dissolved oxygen levels were recorded from the optode on ROV. Because of some technical problems, only three replicates were taken from the anoxic zone. Hence, the oxic zone and suboxic zones were each represented by one replicate of a sediment sample.

mesh screens (Higgins&Thiel 1988; Giere 2009) and all meiofaunal taxa retained on 63 and 500 µm meshes were examined for meiofauna. All samples were stained with Rose Bengal solution to facilitate the manual sorting of specimens from the material and sometimes to distinguish the organisms from clay or algae particles (Hulings&Gray 1971; Giere 2009). Sediments were carefully examined under a dissecting stereo microscope (Olympus SX61) using modified Bogorov counting chambers. All

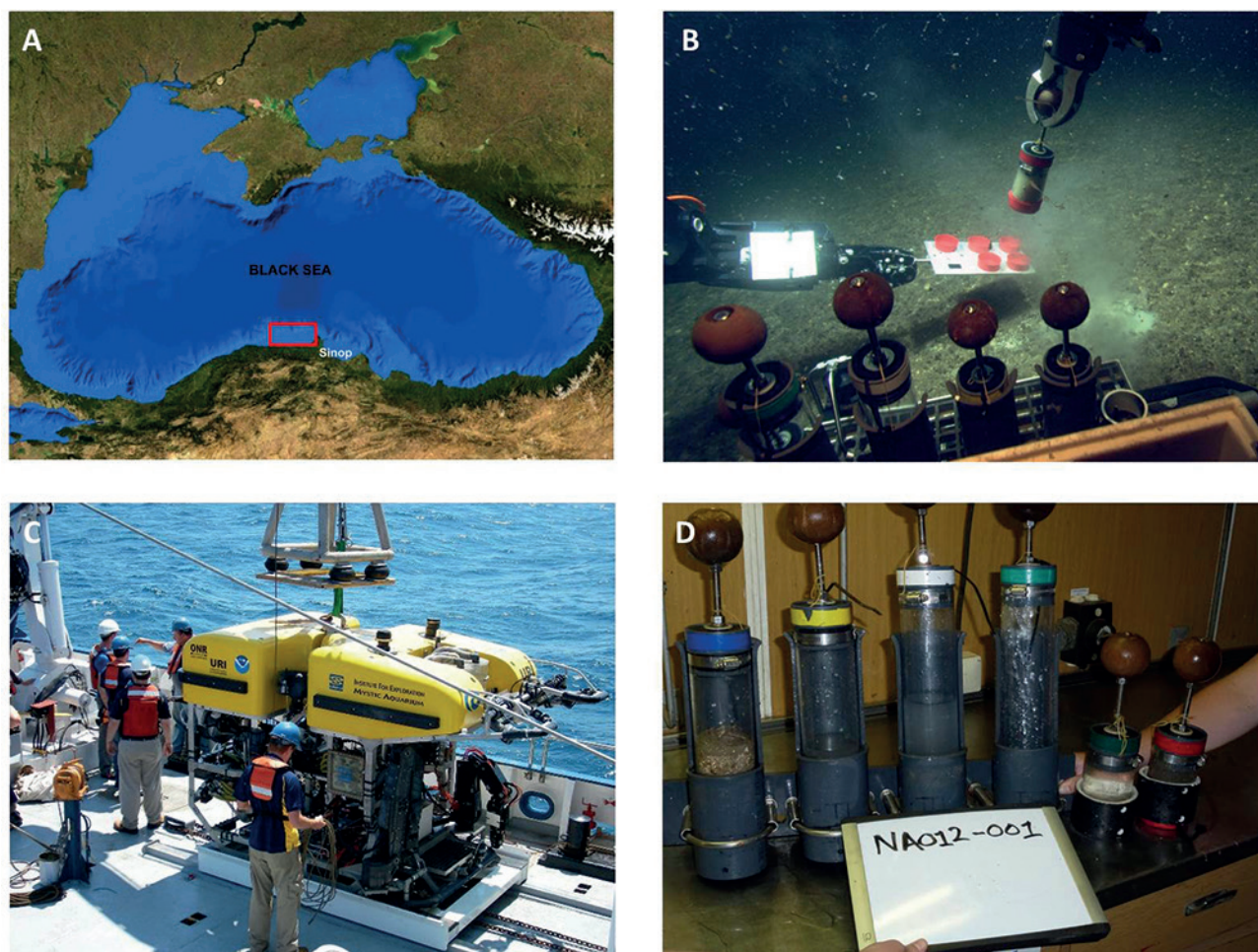


Figure 2

Sampling: A) Sampling area; B) High definition video capture of ROV during sampling; C) Remotely operated vehicle "Hercules"; D) Sediment samples retrieved onboard

Processing

Samples (sediment column with a height of 10 cm) taken to the benthic ecology laboratory at Sinop University were wet sieved through 500 and 63 µm

meiofaunal taxa were sorted into higher taxa and counted for quantitative analysis. Nematodes were put into glycerol via the slow evaporation method (Seinhorst 1959) and mounted on glass slides, which were then identified to the lowest possible taxonomic

rank with Nomarski's Differential Interference Contrast (DIC) system attached to a Nikon Eclipse Ni-U research microscope. All measurements are expressed in micrometers and curves are measured along the arc. The specimens were measured and photographed using Nikon Imaging Software (NIS) Elements. Identifications were based on generic pictorial keys of Warwick et al. (1998), Platt and Warwick (1983, 1988), and the NeMys data base (Vanaverbeke et al. 2015). The material is deposited in the museum of Sinop University, Faculty of Fisheries, Sinop, TURKEY(SNUFF/NEM).

Data analyses were performed with the software package PRIMER 5. In the present study, nematode species richness, diversity and evenness were measured using Margalef's (d), Shannon's (H'), Pielou's (J') indices.

Results

Bottom water properties of the sampled sites

Salinity and temperature measurements were collected with a CTD mounted on Hercules and dissolved O_2 was measured with an optode also on the vehicle. Plots of the ROV's descent through the water column above the locations of the suboxic and anoxic sediment core samples sites are shown in Figures 3 and 4. At both sites, temperature and salinity reach minimums between a depth of 50 and 60 m depth, while oxygen becomes depleted between 100 and 120 m. The suboxic core location was selected based on the O_2 sensor level $<5 \mu\text{M}$ and was therefore taken at 120.5 m, with the estimate that this depth would be above the onset of hydrogen sulfide. The anoxic core was collected at 203 m, well into the anoxic zone with a complete lack of oxygen. The differences between these environments were also evident in the sediment types comprising the seabed at each location. The suboxic core was taken in an area of soft sediment with shells and evidence of other bioturbation at the surface. The anoxic core was taken from a sediment surface composed of fine mud with no visible biology, and also in an area of bedforms originating from the activity of internal waves across the chemocline (for additional information about the sampling site please see Brennan et al. 2013 where part of this study was published).

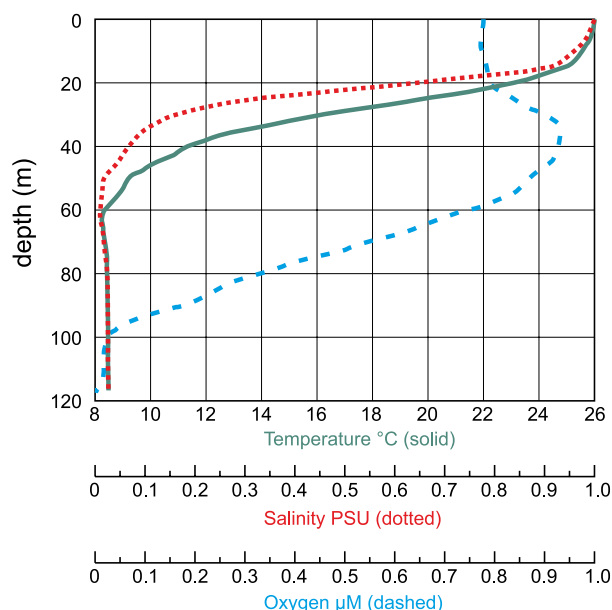


Figure 3

CTD graph of the suboxic site showing salinity, temperature and dissolved oxygen versus depth through the water column

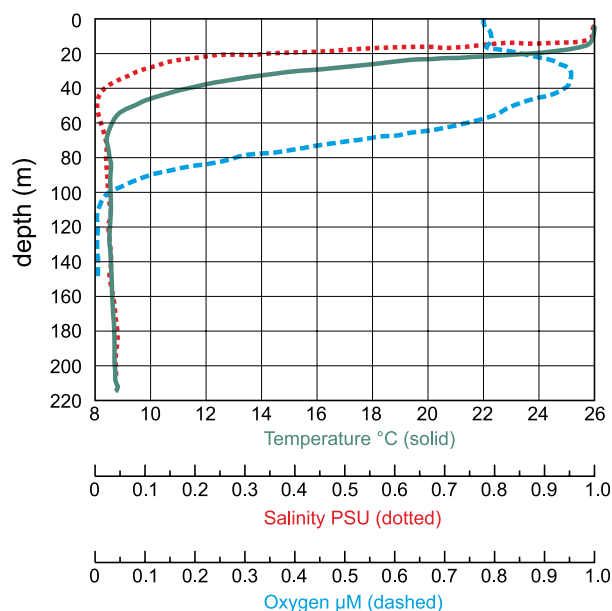


Figure 4

CTD graph of the anoxic site showing salinity, temperature and dissolved oxygen versus depth through the water column

Composition of Meiofauna

Meiobenthos was represented by a total of 13 higher taxa and the total abundance ranged from 1141 ind. m⁻² to 217 078 ind. m⁻² with the highest abundance recorded in the oxic sample (Brennan et al. 2013). Nematoda was quantitatively the most abundant meiofaunal taxon at three sites. The abundance of higher meiofaunal taxa excluding nematodes in the area are shown in Figure 5. Unknown organisms were assigned to the group “others”. At the oxic site, Harpacticoida was the second most abundant group with 24 880 ind. m⁻². Polychaeta ranked third in terms of abundance and occurred with a density of 7153 ind. m⁻², and was followed by juvenile Bivalvia (4976 ind. m⁻²). A rare taxon, Kinorhyncha, was also present in the sample.

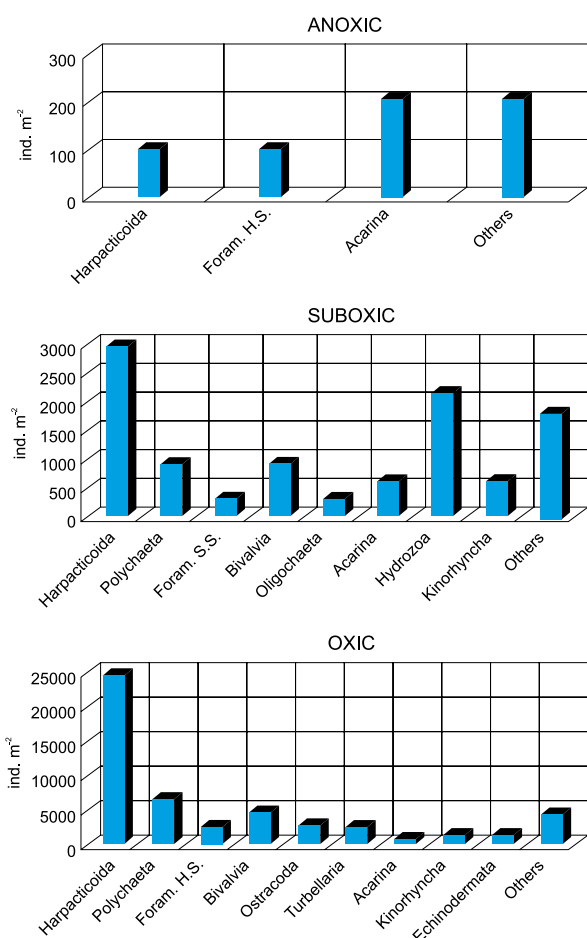


Figure 5

Abundance of higher meiofaunal taxa excluding Nematoda at three sites (Foram.: Foraminifera, H.S.: hard-shelled, S.S.: soft-shelled)

Turbellaria, Ostracoda and Echinodermata were only recorded at the oxic site.

The suboxic site showed much lesser abundance of Harpacticoida with 3110 ind. m⁻². It was followed by Hydrozoa (single polyps) with 2177 ind. m⁻² and representatives of this taxon were only available at the suboxic site. Polychaeta and juvenile Bivalvia occurred with similar densities (933 ind. m⁻²). Kinorhyncha was still present at this site despite of a lesser abundance (622 ind. m⁻²) compared to the oxic site. Specimens of soft shelled Foraminifera were only found at the suboxic site. Higher taxa recorded at the anoxic site, following Nematoda, were Acarina, Harpacticoida and hard shelled Foraminifera with reduced densities.

Composition of Nematode fauna

In total, 775 individuals, belonging to 23 families were recorded from the two sites (Table 2). The nematode individuals from the anoxic sample were juveniles, in poor condition, and therefore could not be identified to a lower taxonomic level. These individuals were excluded from this discussion on the nematofauna at the sites. Nematodes were dominant at three sites and made its highest contribution (90%) to the sample associated with the suboxic zone. The highest nematode density (175 093 ind. m⁻²) was recorded at the oxic site (Fig. 6).

At the oxic site, nematode diversity was found to be higher with 53 species. They belong to 22 families, of which Comesomatidae (22.91%), Linhomoeidae (15.28%), Desmodoridae (14.39%) and Enoplidae (11.90%) were dominant (Table 2). *Sabatieria* was the most abundant genus (21.1%) followed by *Terschellingia* (12.1%), *Desmodora* (11.9%) and *Enoplus* (12.1%) (Fig. 7, Table 3). Representatives of some other families were also found in notable amounts. A new species, *Halaphanolaimus sergeevae*, was previously described from the specimens collected at this oxic site (Ürkmez&Brennan 2013). A total of 21 families and 31 species were recorded at the suboxic site with a distinctively high dominance of Trefusiidae (49.5%) represented mostly by *Trefusia* aff. *longicauda* (42.5%) compared to its extremely lower contribution (1%) at the oxic site. Other most abundant families were Rhabdodemanidae (8.49%), Comesomatidae (7.08%), Chromadoridae (6.60%) and Desmodoridae (6.13%) (Table 2). The dominant

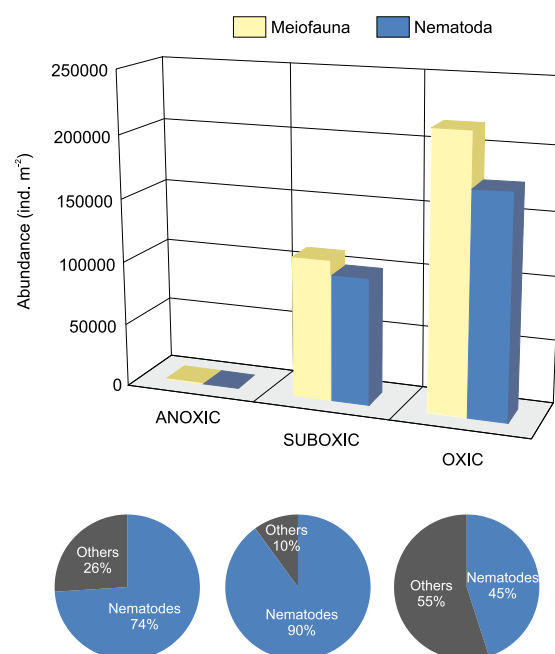
Table 2

Taxonomic composition of free-living nematodes with the number of species and relative abundance (%) of the families found at the oxic and suboxic sites

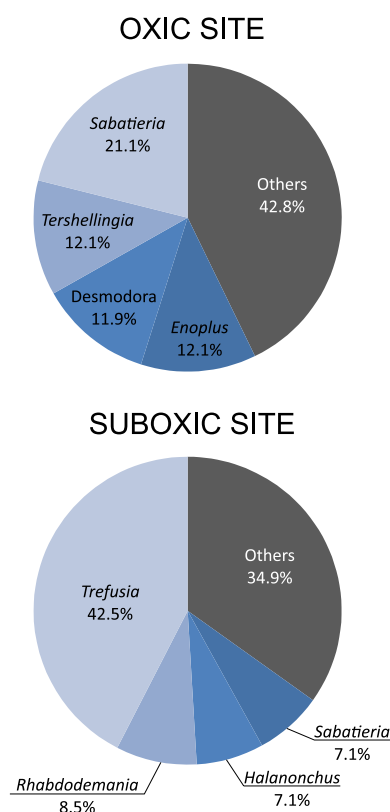
		OXIC		SUBOXIC	
		%	Species	%	Species
ENOPLA	Enoplidae	11.90	1	0.47	1
	Thoracostomopsidae	0.53	2	0.00	0
	Leptosomatidae	0.36	2	0.47	1
	Oxystominidae	5.15	3	4.25	2
	Oncholaimidae	0.36	1	0.94	1
	Tripyloididae	0.00	0	0.47	1
	Rhabdodemaniidae	0.18	1	8.49	1
	Pandolaimidae	3.73	1	1.42	1
	Trefusiidae	3.37	2	49.53	2
CHROMADORIA	Chromadoridae	3.37	1	6.60	2
	Cyatholaimidae	0.18	1	0.94	1
	Selachinematidae	0.36	1	2.36	3
	Comesomatidae	22.91	3	7.08	2
	Desmodoridae	14.39	6	6.13	2
	Microlaimidae	1.24	2	0.94	1
	Leptolaimidae	1.42	2	0.94	2
	Desmoscolecidae	1.95	2	2.36	1
	Monhysteridae	0.18	1	0.47	1
	Xyalidae	2.84	6	0.47	1
	Sphaerolaimidae	2.31	1	0.00	0
	Linhomoeidae	15.28	10	0.94	2
	Axonolaimidae	7.28	3	3.30	1
	Diplopeltidae	0.71	1	1.42	2
	TOTAL	100.00	53	100.00	31

genera were *Trefusia* (42.5%), *Rhabdodemania* (8.5%), *Halanonchus* (7.1%) and *Sabatieria* (7.1%) (Fig. 7, Table 3).

The number of species within particular families varied from 1 to 10 at the two sites. The maximum number of species was distributed within the family Linhomoeidae at the oxic site, where in general a higher nematode species diversity was recorded (Table 3). Families found at the suboxic site showed the lowest number of species (generally 1 or 2 species). Margalef's species richness had a higher value of 8.2 at the oxic site. Shannon Wiener's diversity (H') was lower at the suboxic site (3.3) compared to the oxic site (4.4).

**Figure 6**

Nematoda/meiofauna abundance and percentage of nematodes at three sites

**Figure 7**

Percentage of the most abundant genera at oxic site and suboxic site

Table 3

Univariate indices for nematofauna assemblages for the two sampling sites: the number of species (S), Margalef's species richness (d), Pielou's evenness (J'), Shannon Wiener's diversity (H')

Sampling Site	Diversity indices				Dominant genera (%)
	S	d	J'	H' (log ₂)	
Suboxic	31	5.6	0.68	3.38	<i>Trefusia</i> (42.5) <i>Rhabdodemania</i> (8.5) <i>Halanonchus</i> (7.1) <i>Sabatieria</i> (7.1)
Oxic	53	8.21	0.76	4.4	<i>Sabatieria</i> (21.1) <i>Terschellingia</i> (12.1) <i>Desmodora</i> (11.9) <i>Enoplus</i> (12.1)

Systematics

Family TREFUSIIDAE Lorenzen, 1981

Description (from Leduc, 2013)

Cuticle smooth or with faint striations. Metanemes absent. Amphid either spiral or non-spiral. Outer labial sensillae and cephalic setae usually in two separate circles (except in *Trefusialaimus*); jointed cephalic setae. Buccal cavity funnel- or barrel-shaped, teeth absent. Males usually with two testes (except *Trefusialaimus*) and females usually with two ovaries.

Genus *Trefusia* De Man, 1893

Type species *Trefusia longicauda* De Man, 1893

Trefusia aff. *longicauda* De Man, 1893 (Fig. 8 and 9, Table 4)

Description: Body long and slender, tail filiform. Cuticle smooth. Amphideal fovea pocket shaped 25-30% of the corresponding body diameter in width, located at a distance more than the height of the amphid from the anterior end. Buccal cavity conical and small. Cephalic setae jointed and approximately 0.7 head diameters long. Four subcephalic setae at level of amphid, more than a half length of the cephalic setae. Pharynx slender and cylindrical. Nerve ring and ventral gland not seen. The reproductive system is monorchic, with one anterior testis. Spicules 1-1.2 anal diameters long, paired, proximally expanded. Central lines near proximal ends. Gubernaculum present. Preloacal

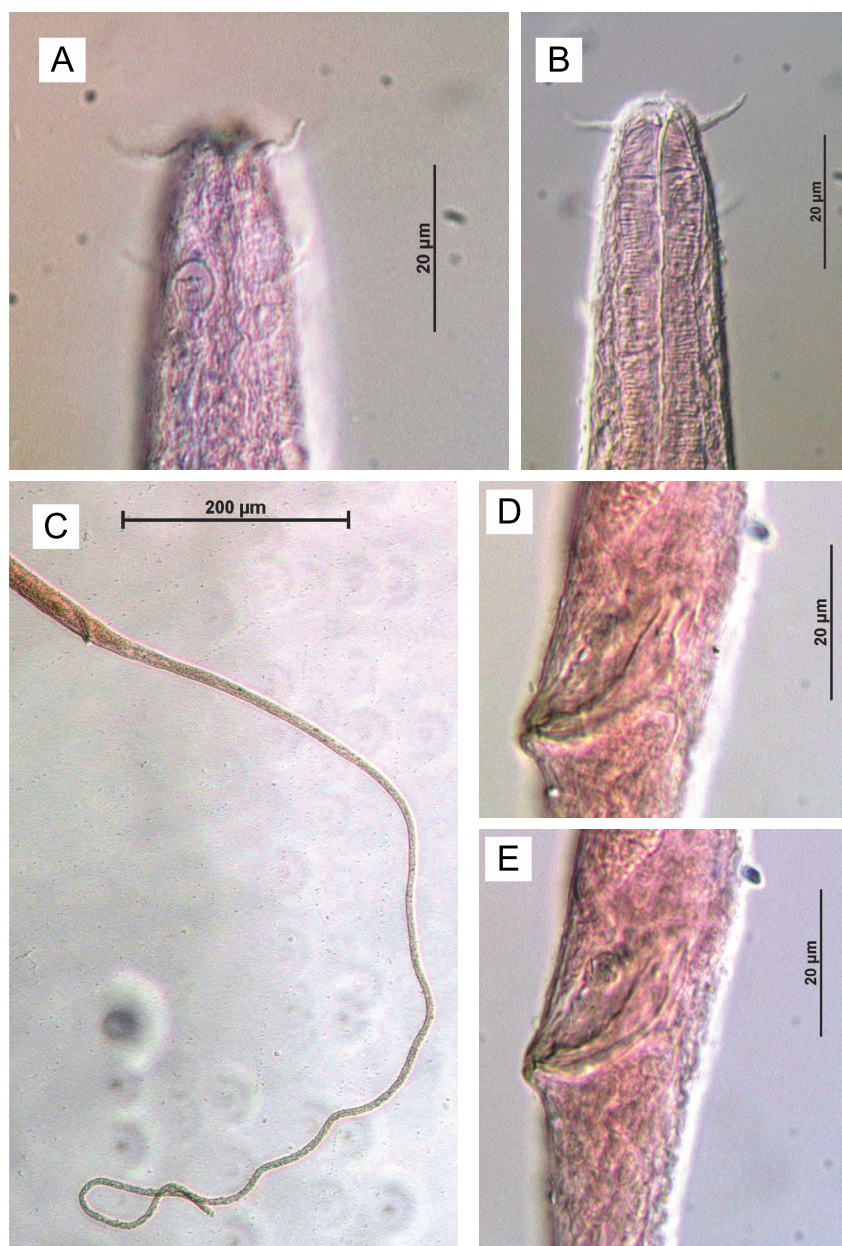
supplements and setae not observed. Most of the tail is filiform and it is not a reliable feature as it may be broken in many specimens. Females with two ovaries.

Material examined: A total of 96 individuals with 33 males, 43 females, and 20 juveniles were counted. Morphometric measurements of 5 males and 2 females were provided. Specimens were deposited in the museum of Sinop University, Faculty of Fisheries (SNUFF/NEM), Turkey.

Remarks: Genus *Trefusia* was recently reviewed by Leduc (2013) who provided an updated key. This new record for the Black Sea has a close resemblance to *Trefusia longicauda* with most of its characteristics, including its general appearance and spicules with a distinct median rib, however, setiform postamphidial papillae were not observed. The studied specimens were determined as *T. aff. longicauda* due to the lack of cervical papillae at the amphid level.

There are 20 valid species of the genus *Trefusia* in the world's oceans and the last updated key was provided by Leduc (2013). *Trefusia longicauda* de Man, 1893 was recorded from the North Atlantic, the North Sea and from France (Vincx 2015). Our specimens are closely related to the original description of *Trefusia longicauda* de Man, 1893, however, no cervical papillae were observed at the amphid level. The studied specimens were determined as *T. aff. longicauda* due to the lack of well-preserved material.

A new record of the genus *Trefusia* in the Black Sea, exclusively dominant at the suboxic site, is a significant result of this study. This finding is in agreement with the suggestion that an important feature of nematode populations is that many species inhabit a single habitat (Nicholas&Hodda 1999). Although some other factors may affect the distribution of deep sea nematodes, we know that permanently low oxygen levels may lead to organic matter accumulation and this organic matter is of better quality due to a less advanced degradation process inhibited by the burial factor (Middelburg&Levin 2009), which in turn may support large populations of specific taxa. Previous records of *Trefusia* from extreme habitats, such as manganese deposits (Miljutin et al. 2010) and sea mounds (Van Gaever et al. 2004), are also available in literature. In suboxic sediments, nematodes with a longer body can overcome oxygen deficiency more

**Figure 8**

Trefusia aff. *longicauda* male: A) Anterior of male with amphid and cervical setae; B) Stoma and cephalic setae of male; C) Posterior of male with a filiform tail; D) Spicule of male showing the central line on the proximal end; E) Spicule of male showing the gubernaculum

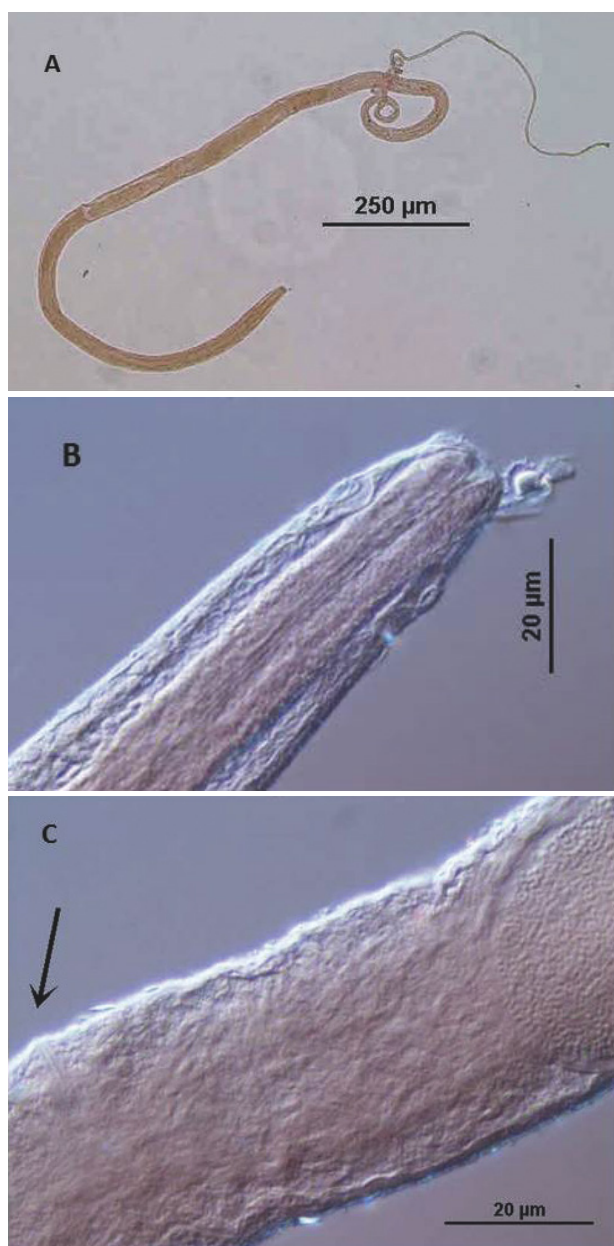
easily, they have a slow respiration rate and hence these conditions are in favor of slender nematodes (Braeckman et al. 2013), as in the case of *Trefusia* aff. *longicauda*. Our results reveal that *T. aff. longicauda* is dominant in nematode assemblages inhabiting suboxic sediments off Sinop.

Discussion

The results of this preliminary study of meiobenthos in three different zones off Sinop shores are in accordance with the results of studies previously conducted at corresponding depth ranges in the Black Sea by several authors. According to Sergeeva (2003), a benthic fauna that adapted to limited oxygen concentrations occurs at depths of 130-150 m. Soft-shelled foraminifers, large numbers of nematodes, polychaetes and hydrozoans were the main groups of this community, in parallel to the results of our study. Additionally, Revkov and Sergeeva (2004) reported that Hydrozoa was the subdominant group in terms of biomass at a depth of 142 m. They also recorded only four groups, namely Nematoda, Turbellaria, Ostracoda and Acarina from a depth of 230-235 m. Similar to our results, they stated that Harpacticoida was the second subdominant group between 70 and 120 m (Sergeeva et al. 2012).

The number of main meiofaunal taxa decreased at the anoxic site. The absence of many groups at this site suggests that oxygen deficiency may have an influence on the meiofauna composition at higher taxonomic levels, as in oxygen minimum zones (OMZ) worldwide (Neira et al. 2001). However, the sites were not represented well due to the limited sampling. Harpacticoids

are known to have a low tolerance to decreased oxygen levels and reported to occur in the upper oxygenated bottom layer (Wells 1988, Wetzel et al. 2011, Frontalini et al. 2011, Semprucci et al. 2013). Supporting this suggestion, harpacticoids in this study occurred with the highest abundance at the oxic site. However, we also found harpacticoids at the

**Figure 9**

Trefusia aff. *longicauda* female: A) Total body of female showing the filiform tail; B) Head of female showing the mouth, cephalic setae and amphideal fovea; C) Midbody of female with the arrow showing the vulva

anoxic site and, consistent with our study, differences were observed in the tolerance of Harpacticoida to the lack of oxygen in other parts of the Black Sea (Sergeeva&Zaika 2013).

The present study has provided us with the first insights into the nematode assemblage of the oxic/anoxic interface in the Southern Black Sea (Sinop)

Table 4

Measurements of *Trefusia* aff. *longicauda* in µm. (M = male;

F = Female; a = body length/maximum body diameter; abd = anal body diameter; b = body length/pharynx length; c = body length/tail length; cbd = corresponding body diameter; %V = (Vulva distance from the anterior end of the body × 100) / the total body length)

Morphometrics	M1	M2	M3	M4	M5	F1	F2
L	3092	3449	3352	3167	2580	3206	3555
a	118.9	114.6	111.7	106.6	80	61.1	93.5
b	*	10.1	11.3	13.4	10.7	11.6	12.3
c	1.9	2.4	2.3	2.1	2.25	2.3	2.3
Head diameter	12	12.5	14.6	12.7	12.7	14.5	12
Length of cephalic setae	*	8.6	9.1	8	8.9	*	8
Length of subcephalic setae	*	5.4	*	*	4.8	*	6.6
Amphid height	9	9.5	*	*	*	12.6	*
Amphid width	5.2	5	*	5	5.1	*	5
Amphid width/cbd%	30	25	*	29.5	25.2	*	26
Amphid from anterior end	16.4	15.8	*	16.7	15.9	16.5	14
Pharynx length	*	340	296	236	241	275	287
Max body diameter	26	30	32	29.7	32	52.4	38
Spicule length	26.7	27.5	27	25.2	27	–	–
Gubernaculum length	16	19	19	17.6	17.7	–	–
Anal body diameter	22.2	25.2	27	21.7	25	27	*
Tail length	1555	1432	1418	1504	1142	1370	1542
Tail length/abd	70	56.8	52.5	69.3	45.7	50.7	–
Vulva from anterior	–	–	–	–	–	1034	1067
%V	–	–	–	–	–	32	30
Vulval body diameter	–	–	–	–	–	47.5	38.7

*Not observed or not calculated due to a missing parameter.

and showed the dominance of particular families (Comesomatidae, Linhomoeidae and Desmodoridae at the oxic site; Trefusiidae at the suboxic site) and species (*Sabatieria* spp. and *Terschellingia* spp. at the oxic site; *Trefusia* aff. *longicauda* at the suboxic site) at each site. Non-selective feeding *Sabatieria* and selective feeding *Terschellingia* are generally found at habitats with organic material (Bouwman et al. 1984). The oxic and suboxic sites differed in terms of dominant species. Sergeeva and Gulin (2007) mentioned that the hypoxic zone is characterized by particularly adapted benthic groups. Sergeeva and Zaika (2013) reported that 38 species and 6 genera were confined to areas of hypoxia in the

northwestern part of the Black Sea.

Meiobenthic organisms in the Black Sea are of particular importance due to their ability to tolerate or exclusively live under extreme conditions. Our results demonstrate that severe oxygen depletion affects the meiobenthos and nematode abundance; moreover, such conditions may actually support the abundance of several taxa. This may be attributed to a probable cause of their tolerance to suboxic conditions, along with the reduction of predation at these O₂ depleted levels. A considerable portion of the anoxic fauna in the Black Sea could not be identified even to the phylum level (Sergeeva 2003). Further sampling with more replicates including one for granulometric analysis, particularly from this special ecosystem is required for more detailed taxonomical and ecological analysis of meiofaunal and nematofaunal composition, and for a better understanding of special communities under these permanent conditions of oxygen deficiency and hydrogen sulfide availability.

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References

- Balsamo M., Albertelli G., Ceccherelli V.U., Coccioni R., Colangelo M.A., Curini-Galletti M., Danovaro R., D'Addabbo R., Leonardis C., Fabiano M., Frontalini F., Gallo M., Gambi C., Guidi L., Moreno M., Pusceddu A., Sandulli R., Semprucci F., Todaro M.A., Tongiorgi P. (2010). Meiofauna of the Adriatic Sea: current state of knowledge and future perspectives. *Chemistry and Ecology*, 26: 1, 45-63.
- Bouwman, L.A., Romeyn K., Kremer D.R. & Es F.B. (1984). Occurrence and feeding biology of some nematode species in aufwuchs communities. *Cahiers de Biologie Marine* 25: 287-303.
- Braeckman, U., Vanaverbeke J., Vincx M., van Oevelen D. & Soetaert K. (2013). Meiofauna metabolism in suboxic sediments: currently overestimated. *PLOS One* 8(3): e59289. DOI: 10.1371/journal.pone.0059289.
- Brennan, M.L., Davis D., Roman C., Buynevich I., Catsambis A. et al. (2013). Ocean dynamics and anthropogenic impacts along the southern Black Sea shelf examined through the preservation of pre-modern shipwrecks. *Continental Shelf Research* 53: 89-101. DOI: 10.1016/j.csr.2012.12.010.
- Cook, A.A., Lambshead P.J., Hawkins L.E., Mitchell N., Levin L.A. (2000). Nematode abundance at the Oxygen Minimum Zone in the Arabian Sea. *Deep-Sea Research II* 47:75-85. DOI: 10.1016/S0967-0645(99)00097-1.
- Çınar, M.E. (2014). Checklist of the phyla Platyhelminthes, Xenacoelomorpha, Nematoda, Acanthocephala, Myxozoa, Tardigrada, Cephalorhyncha, Nemertea, Echiura, Brachiopoda, Phoronida, Chaetognatha, and Chordata (Tunicata, Cephalochordata, and Hemichordata) from the coasts of Turkey. *Turkish Journal of Zoology* 38: 698-722. DOI: 10.3906/zoo-1405-70.
- Duman, M., Duman S., Lyons T.W., Avci M., Izdar E., Demirkurt E. (2006). Geochemistry and sedimentology of shelf and upper slope sediments of the south-central Black Sea. *Marine Geology* 227:51-65. DOI: 10.1016/j.margeo.2005.11.009.
- Frontalini F., Semprucci F., Coccioni R., Balsamo M., Bittoni P., Covazzi-Harriague A. (2011). On the quantitative distribution and community structure of the meio and macrofaunal communities in the coastal area of the Central Adriatic Sea (Italy). *Environmental Monitoring and Assessment*, 180:325-344. DOI: 10.1007/s10661-010-1791-y.
- Gooday, A.J., Bernhard J.M., Levin L.A. & Suhr S. (2000). Foraminifera in the Arabian Sea oxygen minimum zone and other oxygen deficient settings: taxonomic composition, diversity and relation to metazoan faunas. *Deep-Sea Research II* 47:25-54. DOI: 10.1016/S0967-0645(99)00099-5.
- Gooday, A.J., Bett B.J., Ecobar E., Ingole B. & Levin L.A. (2010). Habitat heterogeneity and its influence on benthic biodiversity in oxygen minimum zones. *Marine Ecology An Evolutionary Perspective*, Blackwell Verlag GmbH, 31:125-147. DOI: 10.1111/j.1439-0485.2009.00348.x.

- Giere, O. (2009). *Meiobenthology. The Microscopic Motile Fauna of Aquatic Sediments*. 2nd ed. Berlin, Heidelberg, Springer-Verlag. DOI: 10.1007/b106489.
- Hulings, N. & Gray J.A. (1971). *Manual for the study of meiofauna*. Smithsonian Contributions to Zoology.
- Leduc, D. (2013). Two new free-living nematode species (Trefusiina: Trefusiidae) from the Chatham Rise crest, Southwest Pacific Ocean. *European Journal of Taxonomy* 55:1-13. DOI: 10.5852/ejt.2013.55.
- Levin, L.A., Huggett C.L. & Wishner K.F. (1991). Control of deep-sea benthic community structure by oxygen and organic-matter gradients in the eastern Pacific Ocean. *Journal of Marine Research* 49:763-800. DOI: 10.1357/002224091784995756.
- Levin, L.A., Gage J.D., Martin C. & Lamont P.A. (2000). Macrobenthic community structure within and beneath the oxygen minimum zone, NW Arabian Sea. *Deep-Sea Research II* 47: 189-226. DOI: 10.1016/S0967-0645(99)00103-4.
- Levin, L.A., James D.W., Martin C.M., Rathburn A., Harris L., Michener R. (2001). Do methane seeps support distinct infaunal assemblages? Observations on community structure and nutrition from the northern California slope and shelf. *Marine Ecology Progress Series*. 208: 21-39.
- Levin, L.A. (2003). Oxygen minimum zone benthos: adaptation and community response to hypoxia. *Oceanography and Marine Biology* 41:1-45.
- Kiseleva, M.I. (1963). On the fauna of Polychaeta in the Black Sea. *Trudy Sevastopolskoy Biologicheskoy Stanstsi* 15:75-89 (In Russian).
- Luth, U. & Luth C. (1998). Benthic meiofauna and macrofauna of a methane seep area South-west of the Crimean Peninsula, Black Sea. In Luth U., Luth C. & Thiel H. (Eds.), *MEGA-SEEPS- Methane Gas Seeps Exploration in the Black Sea* (pp. 59-77). Berichte aus dem Zentrum fuer Meeres und Klimaforsch, Hamburg.
- Mare, M.F. (1942). A study of a marine benthic community with special reference to the microorganisms. *Journal of Marine Biology Association of the United Kingdom* 25:517-554.
- Middleburg, J.J. & Levin L.A. (2009). Coastal hypoxia and sediment biogeochemistry. *Biogeosciences* 6: 1273-1293.
- Miljutin, D.M., Gad G., Miljutina M.M., Mokievsky V.O., Fonseca-Genevois V. et al. (2010). The state of knowledge on deep-sea nematode taxonomy: how many valid species are known down there? *Marine Biodiversity* 40:143-159. DOI: 10.1007/s12526-010-0041-4.
- Murray, J.W., Jannasch H.W., Honjo S., Anderson R.F., Reeburgh W.S. et al. (1989). Unexpected changes in the oxic/anoxic interface in the Black Sea. *Nature* 338: 411-413. DOI:10.1038/338411a0.
- Neira, C., Sellanes J., Levin L.A. & Arntz W.E. (2001). Meiofaunal distributions on the Peru margin: relationship to oxygen and organic matter availability. *Deep Sea Research I* 48:2453-2472. DOI: 10.1016/S0967-0637(01)00018-8.
- Neira, C., King I., Mendoza G., Sellanes J., De Ley P. et al. (2013). Nematode Community Structure along a central Chile margin transect influenced by the oxygen minimum zone. *Deep Sea Research I* 78:1-15. DOI:10.1016/j.dsr.2013.04.002.
- Nicholas, W.L. & Hodda M. (1999). The free-living nematodes of a temperate, high energy, sandy beach: faunal composition and variation over space and time. *Hydrobiologia* 394:113-127. DOI: 10.1023/A:1003544115600.
- Oguz, T., Murray J.W. & Callahan A.E. (2001). Modeling redox cycling across the suboxic-anoxic interface zone in the Black Sea. *Deep Sea Research I* 48:761-787.
- Platt, H.M. & Warwick R.M. (1983). *Freeliving marine nematodes. Pt.1. British Enoplids. Pictorial key to world genera and notes for the identification of British species*. Cambridge: Cambridge University Press.
- Platt, H.M. & Warwick, R.M. (1988). *Freeliving marine nematodes. Pt.2. British Chromadorids. Pictorial key to world genera and notes for the identification of British species*. Leiden: Brill/Backhuys.
- Revkov, N.K. & Sergeeva, N.G. (2004). Current state of zoobenthos at the Crimean shores of the Black Sea. In: Öztürk B., Mokievsky V.O., Topaloğlu B. (Eds) *International Workshop of the Black Sea Benthos*, 18-23 April, İstanbul, Turkey: 189-217.
- Sandulli R., Semprucci F., Balsamo M. (2014). Trends of taxonomic and functional biodiversity of free-living nematodes from a Blue Hole (Maldives, Indian Ocean). *Italian Journal of Zoology*, 81: 508-516. DOI: 10.1080/11250003.2014.952356.
- Semprucci F., Frontalini F., Covazzi-Harriague A., Coccioni R., Balsamo M. (2013). Meio- and Macrofauna in the marine area of the Monte St. Bartolo Natural Park (Central Adriatic Sea, Italy). *Scientia Marina*, 77(1): 189-199.
- Semprucci F., Balsamo M., Frontalini F. (2014). The nematode assemblage of a coastal lagoon (Lake Varano, Southern Italy): ecology and biodiversity patterns. *Scientia Marina*, 78: 579-588. DOI: 10.1080/02757541003705492.
- Sergeeva, N.G. (2003). Meiobenthos in the region with the methane gas seeps. In: Eremeev V.N., Gaevskaya A.V. (Eds.), *Present Time Conditions of Biological Diversity in the Nearshore Zone of Crimea Peninsula (the Black Sea sector)*. *Ecosi-Gidrophizika*, Sevastopol: 258-267 (In Russian).
- Sergeeva, N.G. & Gulina, M.B. (2007). Meiobenthos from an

- active methane seepage area in the NW Black Sea. *Marine Ecology Progress Series*, 28:152-159. DOI: 10.1111/j.1439-0485.2006.00143.x.
- Sergeeva, N.G., Gooday, A.J. & Mazlumyan, S.A. (2012). Meiobenthos of oxic/anoxic interface in the south-western region of the Black Sea: abundance and taxonomic composition-In: A.V. Valtenbach et al. (Eds.): *Anoxia: Evidence for eukaryote survival and paleontological strategies, cellular origin, life in extreme habitats and astrobiology*, 21:369-402. DOI: 10.1007/978-94-007-1896-8_20.
- Sergeeva, N.G., Mazlumyan, S.A., Çağatay, N. & Lichtschlag, A. (2013). Hypoxic Meiobenthic Communities of the Istanbul Straits's (Bosporus) Outlet Area of the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences* 13:33-41. doi:10.4194/1303-2712-v13_1_05.
- Sergeeva, N.G. & Zaika, V. (2013). The Black Sea Meiobenthos in Permanently Hypoxic Habitat. *Acta Zoologica Bulgarica* 65 (2):139-150.
- Somerfield, P.J., Warwick, R.M. & Moens, T. (2005). Meiofauna techniques. In: Eleftheriou A., McIntyre A. (Eds). *Methods for the study of marine benthos*. Oxford: Blackwell.
- Ürkmez, D., Sergeeva N.G., Sezgin M. (2011). Seasonal changes of nematodes from Sinop coasts of the Black Sea. *Sixth International Conference "Environmental Micropaleontology, Microbiology and Meiobenthology", EMMM-2011*, 279-282.
- Ürkmez, D. & Brennan M.L. (2013). A new species of *Halaphanolaimus* (Nematoda: Leptolaimidae) from the southern Black Sea (Turkey) with a modified key for species identification. *Zootaxa* 3691 (2): 220-228. <http://dx.doi.org/10.11646/zootaxa.3691.2.2.0>.
- Vanaverbeke, J., Bezerra T.N., Braeckman U., De Groote A., De Meester N. et al. (2015). NeMys: World Database of Free-Living Marine Nematodes. Accessed at <http://nemys.ugent.be> on 2015-02-01.
- Van Gaever, S., Vanreusel A., Hughes J.A., Bett B. & Kirikoulis K. (2004). The macro- and micro-scale patchiness of meiobenthos associated with the Darwin Mounds (north-east Atlantic). *Journal of the Marine Biological Association of the United Kingdom* 84:547-556. <http://dx.doi.org/10.1017/S0025315404009555h>
- Vincx, M. (2015, March). *Trefusia longicauda* de Man, 1893. In: Vanaverbeke J, Bezerra TN, Braeckman U, De Groote A, De Meester N, Deprez T, Derycke S, Gilarte P, Guilini K, Hauquier F, Lins L, Maria T, Moens T, Pape E, Smol N, Taheri M, Van Campenhout J, Vanreusel A, Wu X, Vincx M (2015). NeMys: World Database of Free-Living Marine Nematodes. Accessed through: Vanaverbeke J, Bezerra TN, Braeckman U, De Groote A, De Meester N, Deprez T, Derycke S, Gilarte P, Guilini K, Hauquier F, Lins L, Maria T, Moens T, Pape E, Smol N, Taheri M, Van Campenhout J, Vanreusel A, Wu X, Vincx M (2015). NeMys: World Database of Free-Living Marine Nematodes. Retrieved 2015-03-31, from <http://nemys.ugent.be/aphia.php?p=taxdetails&id=122268>.
- Vorobyeva, L.V. (2003). Meiobenthos of the Black Sea (Essay of the History of Its Study). *Hydrobiological Journal*. Vol. 39, No. 2. DOI: 10.1615/HydrobJ.v39.i2.120.
- Wells, J.B.J. (1988). Copepoda. In: Higgins, R. P. and H. Thiel. (Eds.). *Introduction to the Study of Meiofauna*. Washington, D.C.: Smithsonian Institution Press.
- Wetzel, M.A., Fleeger J.W. & Powers S.P. (2001). Effects of hypoxia and anoxia on meiofauna: A review with new data from the Gulf of New Mexico, Coastal and Estuarine Studies, *Coastal Hypoxia: Consequences for living resources and ecosystems*, edited by: Turner, N.N.R.A.R.E., AGU, Washington, DC.: American Geophysical Union. DOI: 10.1029/CE058p0165.