# ASSESSMENT OF FREE-LIVING MARINE NEMATODES COMMUNITY FROM THE NW ROMANIAN BLACK SEA SHELF

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**Abstract:** Diversity and distribution of abundance data for the free-living marine nematodes inhabiting the NW Black Sea shelf were assessed based on 30 samples collected from the NW Black Sea shelf in 2010. Trophic structure of nematodes populations was established in order to determine whether they form specific associations in the area influenced by the Danube input.

Key words: Black Sea, Romanian shelf, nematoda diversity, trophic structure.

## **INTRODUCTION**

With about 11,000 -12,000 species inventoried up to now and millions of individuals per square meter of sediments, free-living nematodes are amazing also for their adaptation to a variety of habitats. Recent studies show that the higher the degree of habitat microstructure, the richer the nematode community. The heterogeneous, fine sands in shallow sea bottoms with a rich food supply and an interstitial system that provides enough solute and oxygen transport may contain a huge number of species. It was noticed that, as the amount of silt and organic content increases, the occurrence of many stenotopic nematode species in the sediment is limited, and so the diversity tends to decrease. Eurytopic species show an affinity for silty or inhomogeneous sediments (Giere, 2009).

Meiofauna accounts for about 38% of the total invertebrate species identified so far in the Black Sea. The most important meiobenthic taxa are Foraminifera, Nematoda, Harpacticoida and Ostracoda. Nematodes may reach as high as 12% of total meiofauna, a total of 250 species being cited by now in the Black Sea and about 95 on the NW shelf (Vorobyova and Kulakova, 2009). Dramatic changes occurred in the NW Black Sea shelf in the late 1980s and the early '90s, caused by the eutrophication phenomena. By that time, the meiobenthic community was dominated by Foraminifera and Nematoda. Foraminifera even exceeded nematodes as abundance when the oxygenation conditions worsened. After 1995, a slight recovery of balance within meiobenthic community was recorded, which could be seen in the ratio between foraminifers and nematodes, on the one hand, and harpacticoids, on the other hand (Vorobyova and Kulakova, 2009).

Unfortunately, the references data for the nematodes community present on the Romanian shelf before the eutrophication phenomenon installation are missing. The present study represents a preliminary analysis of nematodes in a period considered relatively positive for the ecosystem recovery due to the reducing of nutrients entering the Romanian shelf through the Danube in the last 20 years. In comparison with the reference data published by Almazov (1961), phosphorus loads slightly decreased below the 1960s levels, inorganic nitrogen loads still had a higher level even if they followed a decreasing trend and silicates represented half of the reference values.

For a long time the trophic guilds (Wieser, 1953) have been used to discriminate between the functional roles of nematodes and today they still represent a valid tool used in ecological studies. The mouth conformation provides an indication about the preferences for certain food and habitats. The paper presents the trophic structure of nematodes populations in the studied area.

## MATERIAL AND METHOD

The study is based on 30 samples collected in 2010 within the Hypox Project in Portiţa and Sf. Gheorghe area situated on the NW Black Sea shelf (Fig. 1). The sampling was performed in two expeditions (14-26 May and 5-9 September) on board the R/V Mare Nigrum. The collecting instrument was Multicorrer Mark II. Regularly, 1 or 2 tubes, each having 10 cm<sup>2</sup> area were retained for nematodes collection. The first top 10 cm of sediments were washed on 90  $\mu$ m sieve and nematodes were retrieved manually from the sample. Total individuals from sample or a sub-sample were placed on slides in glycerin and species identified on microscope ZEISS "Primo Star". Total density of nematodes per sample was estimated for 1m<sup>2</sup>. For identification were used taxonomic keys from Filipjev, (1918 - 1922); Platonova, 1968.

Granulometry composition of sediments was measured in laboratory using Mastersizer granulometric laser. Statistical analysis was performed with freeware PAST version 2.15 program. There were calculated Bray-Curtis similarity (presenceabsence transformed data) between samples within the two sampling campaigns and PCA analysis for samples pattern against trophic type as variable.

The list of taxonomic composition on Sf. Gheorghe and Portița profiles is given in Appendix 1.

#### **STUDY AREA**

The Danube influence is supported by the entire NW shelf, which receives riverine inputs of inorganic nutrients and organic matter sustaining in this way high production and adding to the organic enrichment (Aubrey et al., 1996). It is extremely productive also because of the high phytoplankton standing stocks (1 to 4 mg Chl a m-3) (Barale et al., 2002), high rates of photosynthesis (20 to 350 mg C m<sup>-3</sup> d<sup>-1</sup>) (Becquevort et al., 2002) and bacterial production (15 to 60 mg C m<sup>-3</sup> d<sup>-1</sup>) (Vinogradov et al., 1998; Sorokin, 2002). The annual maximum of the discharge is usually recorded in April-May and the minimum in September-October (Cociasu et al., 2009). The study areas Portita and Sf. Gheorghe are located at the Danube mouth under its plume main influence. The samples transect ranged from shallow to deep water on Sf. Gheorghe while Portița samples were all located in shallow waters between 11 and 42 m (Fig. 1).

TOC distribution in sediments is complicated, at least in the shallow region of the continental shelf (0 – 50 -70 m), by the presence of areas with high TOC concentrations (>3% in the Danube Prodelta and the shelf area under the influence of the Danube born sediment) (Secrieru and Oaie, 2009) (Fig. 1).

Granulometry fractions measurements showed slight changes of sediments composition along the depth gradient, generally silty-clay sediments dominate. Exception was given by the PO01 station (11 m depth), with a predominant-

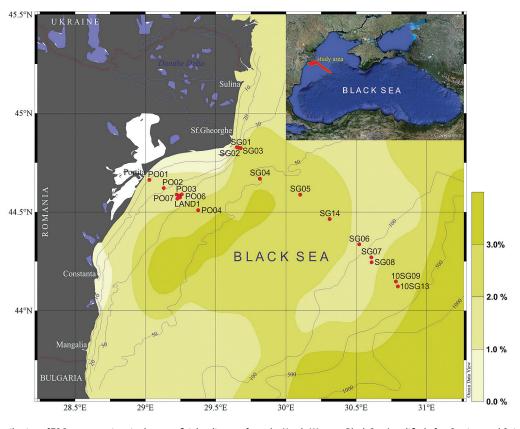


Fig. 1 Distribution of TOC concentrations in the superficial sediments from the North-Western Black Sea (modified after Secrieru and Oaie, 2009)

ly sandy substratum. In the vertical profile of sediments, the composition remained similar, the ratio between argilous and silt fraction being only slightly changed down to 11 cm depth (Secrieru, 2010). TOC contents in the finer shelf sediments (silt, argilous) may reach 2 - 3% and 3 - 5% in the abyssal ones (coccolithic ooze), with maximal values (>5% and up to 15%) in the sapropelic mud (Secrieru and Oaie, 2009), but generally both the grain size composition and the TOC concentrations of the sediments from the NW Black Sea are highly variable, influenced mainly by the "spikes" of seasonal organic input from the Danube (Fig. 1). A good correlation was recorded between the <16  $\mu$ m fraction size grain of sediments and TOC and indicates that the organic matter is associated not only to the clay size; a significant part of TOC is associated to the fine and very fine silt fractions (Secrieru and Oaie, 2009).

The macrobenthos community in the area just in front of the Danube Delta is dominated by the deposit feeders while the northern part of the shelf is characterized by low sedimentation rates being more inhabited by the macrobenthos suspension feeders' community (Wijsman *et al.*, 1999). In Sf. Gheorghe and Portiţa areas the high densities of macrobenthic fauna are given by two species of polychaeta - *Melinna palmata* and *Dipolydora quadrilobata*. Here, the organic matter accumulation in the sediments allows the massive development of the populations of polychaeta worm *Melinna palmata*. Large-sized suspension-feeders bivalves *Mya arenaria* and *Anadara inaequivalvis* reach the highest biomasses. Sampling transects cover also important biocoenosis consisting of dominant species *Mytilus* and *Modiolula*.

### **RESULTS AND DISCUSSIONS**

#### 1. DYNAMIC OF NEMATODES POPULATIONS IN PORTIȚA AREA

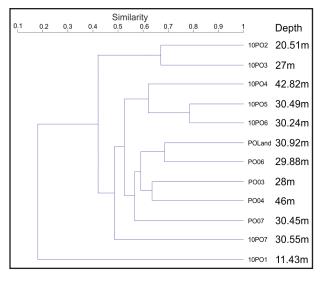
There were found 45 species in Portita area in May and September 2010. The dominant species Sabatieria pulchra, Viscosia minor, Terschellingia longicaudata and S. abyssalis formed more than 50% of the population structure (Table 1). Sabatieria pulchra dominated by far in shallow waters and reached at 11 m (St.10PO01 sample) in May up to 80% of the total population composition. Excepting this case, it did not reach densities higher than 50% of nematodes community either in May or September samples. Among the other dominant species, T. longicaudata and V. minor recorded the highest densities in May at 20 m (St.10PO02) (49,000 ind/m<sup>2</sup>, 50,500 ind/m<sup>2</sup>, respectively), representing about 30% of the nematodes community at this depth. In September, V. minor recorded the greatest densities in 28 - 30 m bathymetric interval – 36,700 ind/m<sup>2</sup> on average, 18.86 % of total, whereas T. longicaudata reached about 532 ind/m<sup>2</sup>, representing 10.78% of total population composition. Nevertheless, no significant difference was observed in the populational structure of dominant species between the two seasons, neither as distribution nor as density. It is worth mentioning the decreasing of numerical dominance of species S. pulchra in shallow waters in September, which in turn reached the highest densities in deeper water at 46 m (PO04) - 21,000

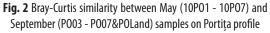
ind/m<sup>2</sup> – 52.29% of total population density. Along with them, S. *abyssalis, Eleutherolaimus longus, Halalaimus sp.* formed the core of nematode populations (Table 1).

## Table 1 Species ordination by frequency (F%) and dominance (D%) in May and September in Portiţa samples

Species	<b>F%</b>	D%
Sabatieria pulchra	100.00	40.78
Viscosia minor	91.67	14.97
Terschellingia longicaudata	83.33	10.40
Eleutherolaimus longus	75.00	1.46
S. abyssalis	75.00	9.74
Halalaimus sp.	66.67	0.63
Cobbionema acrocerca	66.67	0.56
Enoploides brevis	58.33	0.98
Paracanthonchus caecus	50.00	1.07
Axonolaimus ponticus	41.67	3.59
Mesacanthion conicum	41.67	0.66
Sphaerolaimus macrocirculus	41.67	1.18
Sphaerolaimus ostrae	33.33	1.21
Sphaerolaimus gracilis	33.33	0.68
Tripyloides marinus	33.33	0.30
Metalinhomoeus zosterae	25.00	2.58
Spirinia zosterae	25.00	1.87
Prochromadorella mediterranea	25.00	0.90
Oxystomina elongata	25.00	0.78

About overall 40% similarity between samples but 60 – 70 % similarity exists between majorities of the samples. The sample taken in May at 11.43 m is clearly different from the others due to the almost single dominance of *S. pulchra* (Fig. 2).





## 2. DYNAMICS OF NEMATODES POPULATIONS IN SF. GHEORGHE AREA

The samples on Sf. Gheorghe profile were collected at depths ranging from 16 m to 161.5 m in May, respectively from 19 to 93 m in September. A total of 78 species were found in both sampling months. Out of all samples the species S. abyssalis with only 16.67 % by dominance occupied the second place after S. pulchra, which dominated in shallow waters along with T. longicaudata (Table 2). V. minor represented in turn only 7.45% by dominance (D%) out of all samples, with the highest densities at 28 m - 32,000 ind/m<sup>2</sup>. However, a significant contribution in overall population density was at 74 m – 23.94% in May. In September, V. minor reached as high as 48.30 ind/m<sup>2</sup> at 27 m, while the greatest percentage contribution was at 64 m - 28.88%. The most common species identified in samples collected below 50 m depth were Mesacanthion conicum, Enoplus euxinus, Oncholaimus campylocercoides, Metachromadora macrouthera, Quadricoma loricata, S. abyssalis etc. Greater densities and frequencies of species Halanonchus bullatus and Eleutherolaimus longus were observed in May as compared to September, while Axonolaimus ponticus dominated in September. About 10 of the most frequent species contributed to about 50% similarity between samples (Table 2). The results of Bray-Curtis dendrogram showed the greatest similarity between samples within the same season and close bathymetric intervals. A distinct association is made by St. 10SG13 with only 7 species present (Fig. 3).

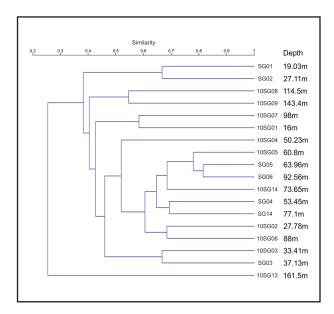


Fig. 3 Bray-Curtis similarity between May (10SG01 - 10SG14) and September (SG01 - SG14) samples on Sf. Gheorghe profile **Table 2** First ranked species ordination by frequency (F%) and dominance (D%) in May and September in Sf. Gheorghe samples

Species	F%	D%
Sabatieria abyssalis	88.89	16.67
S. pulchra	77.78	28.38
Viscosia minor	77.78	7.45
Halalaimus sp.	66.67	0.99
Terschellingia longicaudata	61.11	14.50
Odontophora angustilaimus	55.56	1.73
Mesacanthion conicum	50.00	0.67
Quadricoma loricata	50.00	0.27
Enoplus euxinus	50.00	2.04
Axonolaimus ponticus	44.44	4.27
Metachromadora macroutera	44.44	0.96
Eleutherolaimus longus	44.44	0.27
Sphaerolaimus macrocirculus	38.89	2.61
Metalinhomoeus zosterae	38.89	2.57
Enoploides brevis	38.89	0.74
Paralinhomoeus ostrearum	38.89	0.28
Paracanthoncus caecus	33.33	0.91
Oxystomina elongata	33.33	0.41
Oncholaimus campylocercoides	27.78	1.56
Cobbionema acrocerca	27.78	0.37
Terschellingia pontica	27.78	0.20
Halanonchus bullatus	22.22	0.78
Spirinia zosterae	22.22	0.73
Prochromadorella mediterranea	22.22	0.35
Sphaerolaimus gracilis	22.22	0.58
Daptonema sp.	22.22	0.47

# Trophic structure of nematodes populations in Portița area

In May, the contribution of non-selective deposit feeders (1B) in Portița areas covered more than 50 - 60% of the population structure due mainly to *S. pulchra*, excepting the stations at 30 m depth, where prevailed the omnivores/predators (St.10PO06 – 56.58% of density and St.10PO07 – 71.22% of density) (Fig.4). Their greatest contribution as number of species, representing about 60%, was recorded at 27 m (St. 10PO03) (Fig. 5). A negative relation between the abundance of deposit feeders and predators could be observed (Fig. 4). The latter were mainly represented by *Viscosia minor, Sphaerolaimus macrocirculus* and *Enoploides brevis*.

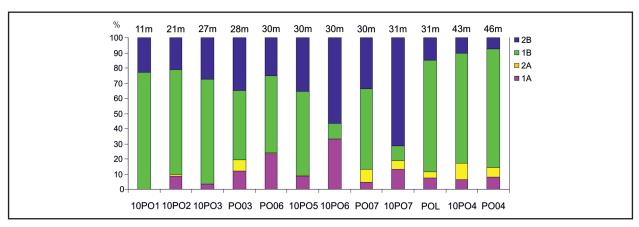


Fig. 4 Percentage representation of species abundance according to trophic groups within samples in Portița area in May and September (1A- selective deposit feeder; 2A – epigrowth feeders; 1B – non-selective deposit feeder; 2B – omnivore/predator) (POL = POLand)

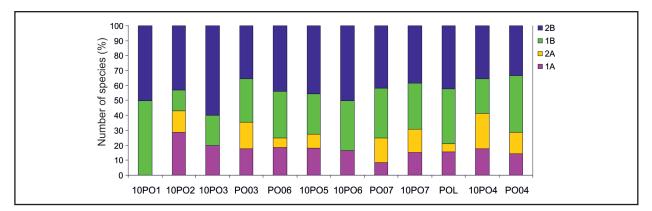
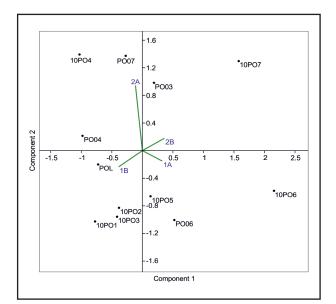


Fig. 5 Percentage representation of species number according to trophic groups within samples in Portiţa area in May and September (1A- selective deposit feeder; 2A – epigrowth feeders; 1B – non-selective deposit feeder; 2B – omnivore/predator)



**Fig. 6** PCA applied on May and September samples from Portița area (variables: 1A- selective deposit feeder; 2A – epigrowth feeders; 1B – non-selective deposit feeder; 2B – omnivore/predator)

In September, the greatest percentage of non-selective deposit feeders was found at 46 m - 78.11%, respectively at about 31 m – 73.71%. In fact, non-selective deposit feeders prevailed, followed by omnivores/predators, represented mainly by *C. acrocerca, Paracanthonchus caecus, Mesacanthion conicum, Sphaerolaimus macrocirculus, Viscosia minor.* Apparently, the trophic offer is limited for the epigrowth feeders, which were found in low abundances and number of species (Fig. 5).

Trophic type was used in PCA analysis to discriminate between samples association (Fig. 6). No significant relation was detected between omnivores/predators (2B) and selective deposit feeders (1A), although both groups were important as density in samples from 30m (St.10PO06, St.PO06 and St.10PO05). This could suggest a stronger influence of predators on non-selective deposit feeders also present in the samples. Long, slender, fast-moving selective deposit feeders (*T. longicaudata, Halalaimus sp., Oxystomina sp.*) might not be an attractive food for the predators from the energetic point of view. Mihaela Mureşan – Assessment of free-living marine nematodes community from the NW Romanian Black Sea shelf

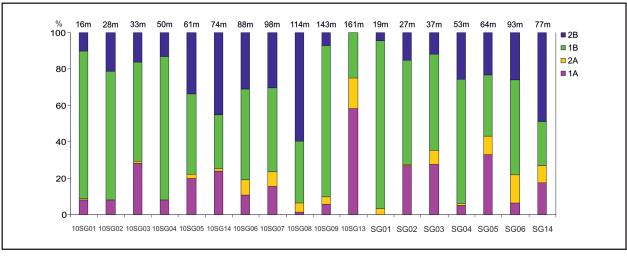


Fig. 7 Percentage representation of species abundance according to trophic groups within samples in Sf. Gheorghe area in May and September; 1A – selective deposit feeder; 2A – epigrowth feeders; 1B – non-selective deposit feeder; 2B – omnivore/predator)

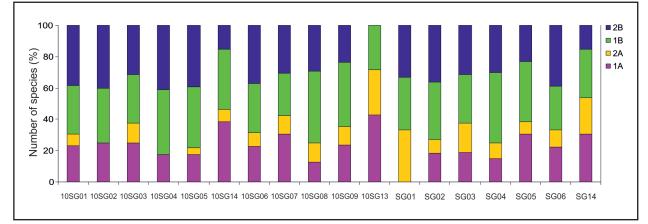
The samples St.10PO07 and St. PO03 were different due to the great proportion of predators (71.22%, respectively 35.59%). The latter is the opposite of sample from May (St.10 PO03 – 27 m) in which the non-selective deposit feeders predominated as density. Nevertheless, the number of predator species exceeded the number of deposit feeders.

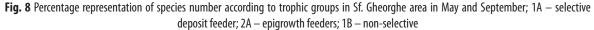
The samples situated between 11 and 30 m depth were all dominated by non-selective deposit feeders. A similar functional structure of populations was identified at 42 m in *Mytilus* biocoenosis (St.10 PO04 - in May and St.PO04 – in September), mentioning that the percent of epigrowth feeders slightly increased. The explanation could reside in the substrate provided by the mussels' shells.

# TROPHIC STRUCTURE OF NEMATODES ON SF. GHEORGHE PROFILE

The trophic structure was dominated by far by the nonselective deposit feeders as abundance, while their influence decreased in deeper stations (St.10SG14 -73.65 m; St.10SG13 – 161.5 m; St.10SG07 – 98 m) (Fig.7). Excepting the proportion of species within the epigrowth feeders group, the other groups were relatively equitably represented in all samples (Fig 8).

The omnivores/predators were missing at 161.5 m, the selective deposit feeders on bacterial mats (Halalaimus sp., Quadricoma sp. and Tricoma platycephala) and the non selective deposit feeders (S. abyssalis, Linhomoeus sp.) came on the first places. Remarkably different from Portița profile samples is the increase of proportion of selective deposit feeders (1A) in all samples, excepting the sample St. SG01 from 19.03 m, where the non-selective deposit feeders clearly dominated. T. longicaudata, Oxystomina clavicaudata and O. elongata constituted the main core of population of selective deposit feeders at 33 m in May. As well, T. longicaudata dominated along with Halalaimus sp. and Quadricoma media at 37 m in September. At 114.5 m the omnivores/predators proportion (60%) overwhelmed the other trophic groups. The Modiolula biocoenosis must have contributed to the richness of the predators, of which





Oncholaimus campylocercoides and Halanonchus bullatus reached the greatest densities (34.50 ind/m<sup>2</sup>, 16.23 ind/m<sup>2</sup> respectively). Among non-selective deposit feeders at this depth were linhomoneids species, Odontophora angustilaimus, S. abyssalis, S. pulchra etc., attracted here by the rich organic sediments. It is not excluded to be a source of food for the predators also, although not the only ones. The bacterial communities are likely to play a role and also the necrophagous activity of some species (e.g. O. campylocercoides). An almost equitable structure between selective deposit feeders, non selective deposit feeders and omnivores/predators was noticed in St.SG05 (63.96 m) and 10SG05 (60.8 m depth) pointing out to the dynamical equilibrium of the functional structure of nematodes populations in mussels biocoenosis.

The samples pattern association against each trophic type (variables) analyzed with PCA (Fig. 9) showed a positive correlation between selective deposit feeders (1A) and epigrowth feeders (2A). This relation was best expressed within samples St. SG03 (37 m), St.10SG03 (33 m), St.SG05 (64 m). The sample St.10SG13 (161 m) was different due to high proportion of 1A and 2A. Samples St.10SG05 (61 m), St. 10SG06 (88 m), St.10SG07 (98m), SG06 (93 m) were associated with omnivore/predators (2B). St.10SG14 (74 m), St. SG14 (77 m) and St. 10SG08 (114 m) were distinct due to the highest omnivores/ predators abundance. The shallowest samples (St. SG01 - 19 m and St.10SG01 - 16 m) and the sample St.10SG09 situated at about 143 m depth were all grouped around non-selective deposit feeders (1B). Generally, a negative correlation exists between the abundance of non-selective deposit feeders, on the one hand, and selective feeders and epigrowth feeders, on the other hand.

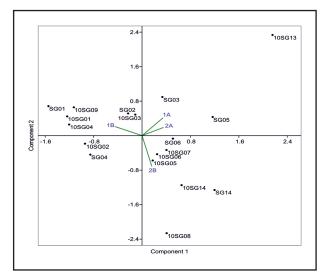


Fig. 9 PCA applied on May and September samples from Sf. Gheorghe area (variables: 1A- selective deposit feeder; 2A – epigrowth feeders; 1B – non-selective deposit feeder; 2B – omnivore/predator)

#### DISCUSSIONS

The non-selective deposit feeders typically found on muddy substrate in shallow waters and also in deeper waters constitute the greatest part of the trophic assemblages on the Romanian shelf. Their importance as dominance decreases on heterogeneous substrata provided by Mytilus and Modiolula biocoenosis, where the diversity of nematodes community and especially the complexity of trophic functional groups increase. Keeping in mind also the particularly limited distribution of metazoan life in the Black Sea up to 170 - 180 m depth because of the high hydrogen sulfide concentration, our results are in accordance with the main literature findings regarding the distribution and tolerance of the dominant species (S. pulchra, S. abyssalis, T. longicaudata) in organic enriched sediments, and also related to the tolerance to hypoxic conditions. Thus, species specific responses of nematode assemblages to organic enrichment, documented by Schratzberger and Warwick (1998a) and Schratzberger and Warwick (1998b), Armenteros et al., (2010) reported S. pulchra, S. parasitifera, Terschellingia.communis, T. gourbaultae and T. longicaudata being discriminant species between different levels of organic enrichment. Sabatieria is typically present in enriched muddy sediments all over the world, and shows generally low abundances in sand (Warwick, 1984, Warwick and Gee, 1984; Jensen, 1987; Ott et al., 1991; Soetaert et al., 2002). Indeed, in Constanța and Mangalia area (out of the main influence of the Danube) with higher fraction of coarser grains, Sabatieria pulchra was less abundant and frequent, prevailing instead S. abyssalis. This could be also related to low salinity tolerance of S. pulchra. We presume that the stout body conformation of *S. pulchra* could be a specialization for muddy sediments; a greater quantity of organic matter could be ingested in the voluminous intestine and nutritionally processed. An increased resistance against compact silty sediments could also be supposed; nevertheless, rapid mobility could be the reason for its strategy life developing. Sabatieria sp. is often the only remaining species in the most stressed situations, such as under high pollution pressure, or towards the centre of shallow, cold seeps (Jensen, 1986; Thiermann et al., 1994).

The genera *Terschellingia* is among the dominant taxa in urbanized areas that are characterized by organic enrichment (Moreno *et al.*, 2008, 2009, 2011) and it is also very resistant and spread in muddy sediments rich in hydrogen sulphide, where it is known as a representative of the "thiobios" (Warwick and Gee, 1984; De Leonardis *et al.*, 2008). It is a typical inhabitant of the deeper sediment horizons (Steyaert *et al.*, 2007) in the shallow water sediments. This points out to a preference for suboxic or anoxic environments, where a substantial fraction of the organic matter becomes incorporated below the oxic zone of the sediment (Soertat and Heip, 1995). The slender body and the long tail like a whip help the species to have an increased mobility in compact sediments. A review of literature (Armenteros *et al.*, 2010) recording the response of nematodes species to different levels of organic loading in experimental studies, emphasized the resistance over long periods of *T. longicaudata* to high levels of organic input originating from different sources. For example, Schratzberger and Warwick (1998a) found that phyto-detritus added in portions of 100, 200 and 400 g C m<sup>-2</sup> several times, did not produce much effect on this species even after 62 days. Armenteros *et al.*, (2010) using 25 and 50 mg C g<sup>-1</sup> DW (eq. to 400 and 800 g C m<sup>-2</sup>) of phyto-detritus added one time in experimental field induced depletion and changes in the general composition of nematodes compared to the control site, which led to the increasing of dominance of species: *T. longicaudata*, and *Spirinia parasitifera*.

Direct evidence for DOM uptake from sediments among nematode species was early provided by Chia and Warwick (1969), Tietjen and Lee (1975) and Lopez et al., (1979). Riemann and Helmke (2002), after reviewing a wide literature source and based on their studies over years concerning the topic of symbitiotic relationships between bacteria, agglutinating nematodes and detritical material from sediments, concluded that the "perpetual accretion of detritical organic particles to sediment agglutinations by nematodes is an adaptation for operating an enzymatic reactor for the production of dissolved nutrients". A β-glucosidase enzyme sharing was evidenced as involved in the cellulase system. Proteolithic enzymatic system of microbial communities associated was found to provide the nematodes with aminoacids, which, otherwise, would not be accessible. Montagna (1984) experimentally showed DOM uptake by a meiofauna community in which nematodes formed a major component. Although his study did not distinguish the types of nematodes utilizing DOM, the results proved direct meiofaunal uptake without bacterial involvement. Jensen (1986) suggested a trans-epidermal uptake among thiobiotic nematodes (mainly deposit feeders) dwelling in the microenvironmental gradient between oxic and anoxic processes and where microbial activity is high. The significantly higher body surface to body volume ratio in thiobiotic nematodes was viewed as an adaptation for DOM uptake from the environment and for respiration. From a view of resource partitioning, Jensen (1986) found that it is very unlikely that a thiobiotic nematode dwelling in this potential source of food would not utilize it in the same way as is known from other marine benthic soft-bodied animals, mainly polychaetes (Jorgensen and Kristensen, 1980 a, b). Organisms suggested, being solely dependent on DOM such as the gutless pogonophores (Southward, 1982) and possibly also gutless oligochaetes (Giere et al., 1982) are known to utilize  $CO_2$  as a carbon source with the fixation of  $CO_2$  by the Calvin cycle intermediated through internal symbiotic chemoautotrophic bacteria (Southward et al., 1981; Felbeck et al., 1981). That could be the case of large community of species having long, slender bodies, found in deeper parts of the sea, and also in the turbulent area influenced by the high input of organic matter; these species are representatives of the Families Linhomoeidae (Linhomoeus sp, Terschellingia longicaudata, Eleutherolaimus longus, Sphaerocephalum sp.

etc.), Oxystominidae (Halalaimus sp., Oxystomina elongata), Trefusiidae (Halanonchus bullatus).

However, the content of organic matter in sediments could partially explain the spatial patterns of distribution of free-living nematodes in some habitats (Ólafsson and Elmgren, 1997; Schratzberger *et al.*, 2006).

Nematode communities are looked upon also as grazers of microalgae and bacteria (Montagna, 1995). Microphytobenthos carbon is routed to nematodes via other pathways than mere grazing, in accordance with results from the enrichment experiments. Experimental studies demonstrated by using isotopic labeling of algal detritus (13C-labeled) added in sediment microcosms the nematodes' ability to rapidly utilize settling organic matter. Incorporation of carbon from phytodetritus by subsurface nematodes in enrichment studies was higher compared to carbon utilization by surface individuals, and showed time-lags largely consistent with sediment mixing rates. A high feeding selectivity has been suggested for estuarine nematodes grazing on bacteria and microphytobenthos (Moens et al., 1999, 2002). Studies performed on meiobenthic representatives from Baltic Sea using radiolabeled Skeletonema costatum (Olafsson et al., 1999) revealed an unexpected result; the highest uptake of labeled carbon-specific activity among nematodes was recorded for Axonolaimus spinosus, used to be considered a non-selective deposit feeders, because it has not any teeth in the buccal cavity and obviously is not adapted to piercing or cracking diatoms (Olafsson et al., 1999). On the Romanian shelf, Axonolaimus ponticus was ubique in Portița and Sf. Gheorghe area between 27 - 77 m depth in September, whereas its distribution was reduced in May to only St. 10SG03 sample (33 m) -51,700 ind/m<sup>2</sup> and 10SG09 (143 m) - 1,816 ind/m<sup>2</sup>. Frequent associations formed with non-selective deposit feeders like linhomonaeids, omnivores/predators and selective deposit feeders. This may suggest the abilities of colonizing all places that might provide a source of food even if this would mean a changing in the food regime. Chromadorids considered in fact the real consumers of microphytobenthos were very rare and in low densities on both transects and seasons. However, in September, on both profiles their density and diversity were higher not only in shallow waters but also in deeper waters (80 - 115 m).

A combination of observed natural isotope signatures and experimental results suggests that tidal flat nematodes preferentially utilize labile organic carbon derived from microphytobenthos or settling phytoplankton while the organic matter from terrestrial or riverine origin does not significantly contribute to the diet of nematodes.

In an experimental work, nematode assemblages from naturally enriched sediments suffered deleterious changes when they were exposed to different loads of phyto-detritus in microcosms, possibly due to the accumulation of byproducts of bacterial metabolism. Dominant nematode species showed a different degree of sensitivity to reduced conditions, with *S. parasitifera* as the least sensitive species, followed by *T. longicaudata, Metalinhomoeus filiformis,* and *S. pulchra*. Instead, the reaction of the deep-sea metazoan nematodes is relatively slow (Ruhl *et al.*, 2008) and uptake of <sup>13</sup>C-labelled phytodetritus was always limited (Moodley *et al.*, 2002; Ingels *et al.*, 2010) or absent (Ingels *et al.*, 2010; Nomaki *et al.*, 2005). This implies that nematodes that dominate abundance, biomass and probably local species richness amongst the metazoan meiofauna (Heip *et al.*, 1985, Lambshead *et al.*, 1983) display a trophic specialization and/or slow biological processes that avoid competitive displacement (Giere, 2009).

Direct utilization of microphytobenthic exopolymer secretions is another possible food-web link. In microcosmos experiments, nematodes diet behavior showed a preferential uptake of food. The studied species firstly consumed microphytobenthos and its extracellular secretions via direct grazing and via predation on herbivores, while later on recycled carbon may have become a prominent source for the nematode community. Montagna (1995) suggested that nematode grazing on microphytobenthos may have a significant impact on primary production. Some authors have even found grazing to (temporarily) exceed primary productivity (Blanchard, 1991, Montagna and Yoon, 1991).

Kennedy (1994) found that the nematodes community in muddy sediments can take up to 7.04g C y<sup>-1</sup>, while the nonselective deposit feeders consume about 55% of all. Comparing with the muddy biotopes, non-selective deposit feeders from sand contributed only 16.1% to the carbon uptake, while the omnivores/predators had about 57.4%.

### CONCLUSIONS

The great bulk of non-selective deposit feeders (mostly from Commesomatidae and Linhomoeidae) in shallow waters highly influenced by the Danube and also in deeper waters where an autochthonous sulphourous bacterial production is added up, pleads for the existence of a specialized community of nematodes in organic enriched sediments. Vorobyova and Kulakova (2009) found on the NW shelf 50 species of nematodes on the muddy substrate, their densities and biomasses increasing with the silty (muddy) fraction. In summer, for example, it reached 377, 429  $\pm$  83.69 ind/m<sup>2</sup> average, comparing with densities on coarse sediments: 650  $\pm$  110 ind/m<sup>2</sup>.

Due to the high and variable sources of organic and inorganic input on the NW Black Sea shelf, nematodes community structure represents a good indicator of sediments state. It was demonstrated by many studies that their feeding habits play one of the most important role in organic carbon conversion helping the mineralizing processes and bacterial production in sediments. Even at small scales, their bio-irrigation activity at the interface of oxic/anoxic layer enhances the sediments oxygenation, influencing organic matter recycling.

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### REFERENCES

- ALMAZOV, N.M., (1961). Stok rasverennykh solej i biogennykh veshchestv kotorye vynoseatsya rekami SSSR v Chernom More. Naukovi Zapiski Odess. Biol St., 3: 99-107
- ARMENTEROS, M, PÉREZ-GARCÍA, J. A., RUIZ-ABIERNO, A., DÍAZ-ASENCIO, L., HEL-GUERA, YUSMILA, VINCX, MAGDA, DECRAEMER, WILFRIDA, (2010). Effects of organic enrichment on nematode assemblages in a microcosm experiment. Marine Environmental Research xxx 1-9; doi:10.1016/j.marenvres.2010.08.001
- AUBREY, D., MONCHEVA, S., DEMIROV, E., DIACONU, V., DIMITROV, A., (1996). Environmental changes in the western Black Sea related to anthropogenic and natural conditions. Journal of Marine Systems 7, 411–425.
- BARALE, V., CIPOLLINI, P., DAVIDOV, A., MELLIN, F., (2002). Water constituents in the north–western Black Sea from optical remote sensing and in situ data. Estuarine, Coastal and Shelf Science 54, 309–320.
- BECQUEVORT, S., BOUVIER, T., LANCELOT, C., CAUWET, G., DELIAT, G., EGOROV, V.N., POPOVICHEV, V.N., (2002). The seasonal modulation of organic matter utilization by bacteria in the Danube–Black Sea mixing zone. Estuarine, Coastal and Shelf Science 54, 337–354.

- BLANCHARD, G.F., (1991). Measurement of meiofauna grazing rates on microphytobenthos—is primary production a limiting factor? J Exp Mar Biol Ecol 147: 37–46
- CHIA, F.S., WARWICK, R.M., (1969). Assimilation of labelled glucose from seawater by marine nematodes. Nature 224, 720 – 721; doi:10.1038/224720a0
- COCIASU, A., VARGA, L., LAZAR, L., VASILIU, D., (2009). Recent data concerning evolution of the eutrophication level indicators in Romanian seawater. Journal of Environmental Protection and Ecology 10, No 3: 701–731.
- DE LEONARDIS, C., SANDULLI, R., VANAVERBEKE, J., VINCX, MAGDA, DE ZIO, S., (2008). Meiofauna and nematode diversity in some Mediterranean subtidal areas of the Adriatic and Ionian Sea. Sci Mar 72(1): 5–13.
- FELBECK, H., CHILDRESS, J. J., SOMERO, G. N., (1981). Calvin-Benson cycle and sulphide oxidation enzymes in animals from sulphide-rich habitats. *Nature* 293: 291–293.

- FILIPJEV, I. N., (1918-1922). Free-living Nematodes of the Sevastopol area // Trudy osob. zool. lab. Sevastop. biol. St. Vol. (2) 4: 1-614 (in Russian).
- GIERE, O., (2009). Meiobenthology: the microscopic motile fauna of aquatic sediments. 527 p.
- GIERE, O, LIEBEZEIT, G, DAWSON, R, (1982). Habitat conditions and distribution pattern of the gutless oligochaete Phallodrilus leukodermatus. Mar Ecol Prog Ser 8: 291–299
- HEIP, C.H.R., VINCX, MAGDA, VRANKEN, G., (1985). The ecology of marine nematodes. Oceanogr Mar Biol Annu Rev 23: 399–489.
- INGELS, J., BILLETT, D. S. M., VAN GAEVER S., VANREUSEL, A., (2010). An insight into the feeding ecology of deep-sea canyon nematodes - Results from field observations and the first *in-situ*<sup>13</sup>C feeding experiment in the Nazaré Canyon. J Exp Mar Biol Ecol 396: 185–193.
- JENSEN, P., (1986). Nematode fauna in the sulphide-rich brine seep and adjacent bottoms of the East Flower Garden, NW Gulf of Mexico. Mar Biol 92: 489–503
- JENSEN, P., (1987). Differences in microhabitat, abundance biomass and body size between oxybiotic and thiobiotic free-living marine nematodes. Oecologia 71: 564–567.
- JORGENSEN, N.O.G., KRISTENSEN, E., (1980a). Uptake of amino acids by three species of Nereis (Annelida: Polychaeta). I. Transport kinetics and net uptake from natural concentrations. Mar. Ecol. Prog. Ser.3: 329-340.
- JORGENSEN, N.O.G., KRISTENSEN, E., (1980b). Uptake of amino acids by three species of Nereis (Annelida: Polychaeta). 11. Effects of anaerobiosis . Mar. Ecol. Prog. Ser.3: 341-346.
- KENNEDY, A.D., (1994). Carbon partitioning within meiobenthic nematode communities in the Exe Estuary, UK. Mar Ecol Prog Ser 105: 71–78
- LAMBSHEAD, P.J.D., PLATT, H.M, SHAW, K.M., (1983). The detection of differences among assemblages of marine benthic species based on an assessment of dominance and diversity. J Nat Hist 17: 859–874
- LOPEZ, G., RIEMANN, F, SCHRAGE, M., (1979). Feeding biology of the brackish-water Oncholaimid nematod *Adoncholaimus thalassophygas*. Mar Biol 54: 311-318
- MOENS, T., GANSBEKE, D.V, VINCX, MAGDA, (1999). Linking estuarine nematodes to their suspected food. A case study from the Westerschelde estuary (south-west Netherlands). J Mar Biol Ass UK 79: 1017–1027
- MOENS, T., VERBEECK, L., MAEYER, A.D, SWINGS, J., VINCX, MAGDA, (1999). Selective attraction of marine bacterivorous nematodes to their bacterial food. Mar Ecol Prog Ser 176: 165–178
- MOENS, T., LUYTEN, C., MIDDELBURG, J.J., HERMAN, P. M. J., VINCX, MAGDA, (2002). Tracing organic matter sources of estuarine tidal flat nematodes with stable carbon isotopes; Mar Ecol Prog Ser, Vol. 234: 127–137
- Montagna, P.A., (1984). Competition for dissolved glucose between meiobenthos and sediment microbes. J Exp Mar Biol Ecol 76: 177–190
- MONTAGNA, P.A., (1995). Rates of metazoan meiofaunal microbivory: A review. Vie Milieu 45: 1–9

- MONTAGNA, P.A., YOON, W.B., (1991). The effect of freshwater inflow on meiofaunal consumption of sediment bacteria and microphytobenthos in San Antonio Bay, Texas USA. Estuarine and Coastal Shelf Science 33: 529-547.
- MOODLEY, L., MIDDELBURG, J.J, BOSCHKER, H.T.S., DUINEVELD, G. C. A., PEL, R, *ET AL.*, (2002). Bacteria and Foraminifera: key players in a short-term deep-sea benthic response to phytodetritus. Mar Ecol Prog Ser 236: 23–29.
- MORENO, M., FERRERO, T.J., GALLIZIA, I., VEZZULLI, L., ALBERTELLI, G., FABIANO, M., (2008). An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbour through the analysis of meiofauna and nematode assemblages. Estuarine, Coastal and Shelf Science 77, 565e576.
- MORENO, M., ALBERTELLI, G., & FABIANO, M., (2009). Nematode response to metal, PAHs and organic enrichment in tourist marinas of the mediterranean sea. *Marine Pollution Bulletin*, Vol. 58, pp. 1192-1201.
- MORENO, M., SEMPRUCCI, F., VEZZULLI, L., BALSAMO, M., FABIANO, M., ALBERTELLI, G. (2011). The use of nematodes in assessing ecological quality status in the Mediterranean coastal ecosystems. Ecological Indicators, Vol. 11, pp. 328-336.
- Nomaki, H., Heinz, T., Nakatsuka, T., Shimanaga, M., Kitazato H., (2005). Species-specific ingestion of organic carbon by deep-sea benthic foraminifera and meiobenthos: In situ tracer experiments. Limnol Oceanogr 50: 134–146.
- ÓLAFSSON, E., ELMGREN, R., (1997). Seasonal dynamics of sublittoral meiobenthos in relation to phytoplankton sedimentation in the Baltic Sea. Estuar Coast Shelf Sci 45: 149–164
- OLAFSSON, E., MODIG, HELENE, VAN DE BUND, W. J., (1999). Species specific uptake of radio-labelled phytodetritus by benthic meiofauna from the Baltic Sea. Marine Ecology Progress Series Mar Ecol Prog Ser, Vol. 177: 63-72
- OTT, J.A, NOVAK, R., SCHIEMER, F., HENTSCHEL, U., NEBELSICK, M., ET AL., (1991). Tackling the sulfide gradient: a novel strategy involving marine nematodes and chemoautotrophic ectosymbionts. PSZN I: Mar Ecol 12: 261–279.
- PLATONOVA, T.A., (1968). Round worms class Nematoda/Key for Black and Azov Sea fauna (in russian). Nauk Dumka: 111 – 183
- RIEMANN, F., HELMKE, ELISABETH, (2002). Symbiotic relations of sediment agglutinating nematodes and bacteria in detrital habitats: the enzyme sharing concept. Marine Ecology 23(2): 93-113
- RUHL, H.A., ELLENA, J.A, SMITH K.L. JR., (2008). Connections between climate, food limitation, and carbon cycling in abyssal sediment communities. Proc Nat Acad Sci USA 105: 17006–17011.
- SCHRATZBERGER, M., WARWICK, R.M., (1998a). Effects of physical disturbance on nematode communities in sand and mud: a microcosm experiment. Mar Biol 130: 643–650
- SCHRATZBERGER, M., WARWICK, R.M., (1998b). Effects of the intensity and frequency of organic enrichment on two estuarine nematode communities. Mar Ecol Prog Ser 164: 83–94
- SCHRATZBERGER M., BOLAM, S., WHOMERSLEY, P., WARR, K., (2006). Differential response of nematode colonist communities to the intertidal placements of dredged material. J Exp Mar Biol Ecol 334: 244–255

- SECRIERU, D., (2010). Monitoringul geoecologic al platoului continental românesc, Contract nr.: PN 09 41 01, faza nr. 6: Finalizarea analizelor de laborator. Prelucrarea şi analiza datelor. Elaborarea documentațiilor de specialitate, 1-72.
- SECRIERU, D., OAIE, G., (2009). The Relation between the Grain Size Composition of the Sediments from the NW Black Sea and their Total Organic Carbon (TOC) Content. Geo-eco-marina 15/2009: 5-11
- SOETAERT K, MUTHUMBI A, HEIP C., (2002). Size and shape of ocean margin nematodes: morphological diversity and depth-related patterns. Mar Ecol Prog Ser 242: 179 – 193
- SOROKIN, Y.I., 2002. The Black Sea: Ecology and Oceanography. Biology of Inland Waters. Backhuys Publishers, Leiden. 875 pp.
- SOUTHWARD, A. J., E. C. SOUTHWARD, P. R. DANDO, G. H. RAU, H. FELBECK, AND H. FLUGEL., (1981). Bacterial symbionts and low 13C/12C ratios in tissues of *Pogonophora* indicate unusual nutrition and metabolism. *Nature* 293: 616–620.
- SOUTHWARD, E. C. (1982). Bacterial symbionts in Pogonophora. J. mar, biol. Ass. U.K. 62: 889-906
- STEYAERT M, MOODLEY L, NADONG T, MOENS T, SOETAERT K, ET AL., (2007). Responses of intertidal nematodes to short-term anoxic events. J Exp Mar Biol Ecol 345: 175–184.
- THIERMANN F, WINDOFFER R, GIERE O., (1994). Selected meiofauna around shallow water hydrothermal vents off Milos (Greece): ecological and ultrastructural aspects. Vie Milieu 44(3/4): 215–226.

- TIETJEN J., H, LEE J., J., (1975). Axenic cultures and uptake of dissolved organic substances by the marine nematode *Rhabditis marina* Bastian. Cah Biol Mar 16: 685–693
- VINOGRADOV, M.E., SHUSHKINA, E.A., LEBEDEVA, L.P., NEZLIN, N.P., (1998). Structural and functional analyses of planktonic communities of the Black Sea. In: Ivanov, L.I., Oguz, T. (Eds.), Ecosystem Modeling as a Management Tool for the Black Sea. NATO ASI Series. Kluwer Academic Publishers, Amsterdam, pp. 237–255
- VOROBYOVA, L., V., KULAKOVA, I., I., (2009). Contemporary state of the meiobenthos in the western Black Sea, pp. 126
- WARWICK, RM., (1984). Species size distribution in marine benthic communities. Oecologia (Berl) 61: 32–41
- WARWICK RM, GEE JM (1984). Community structure of estuarine meiobenthos. Mar Ecol Prog Ser 18: 97–111.
- WIESER, W., (1953). Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nernatoden. Ark. Zool. 4: 439-484
- WUSMAN, J.W.M., HERMAN, P.M. J., GOMOIU, M.-T., (1999). Spatial distribution in sediment characteristics and benthic activity on the northwestern Black Sea shelf. Mar Ecol Prog Ser 181: 25-39

# ANNEX 1

Taxonomic composition of nematods populations on profiles Sf.Gheorghe and Portița in 2010

No. crt.	Taxonomic composition on profiles Sf.Gheorghe and Portița	Profi	Profiles	
		Sf. Gheorghe	Portița	
1	Anoplostoma viviparum (Bastian. 1865)		Х	
2	Araeolaimus sp.	Х	Х	
3	Axonolaimus ponticus Filipjev. 1918	Х	Х	
4	Bathylaimus australis Cobb. 1894	Х		
5	Bathylaimus cobbi Filipjev. 1922	Х	Х	
6	Camacolaimus de Man. 1889	Х		
7	Chromadora cricophana	Х	Х	
8	Chromadora nudicapitata (Bastian. 1865)		Х	
9	Chromadorita gracilis (Filipjev. 1922)	Х		
10	Chromadora Bastian. 1865	Х		
11	Chromadorina obtusa Filipjev. 1918	Х	Х	
12	Chromadorita Filipjev. 1922	Х		
13	Chromaspirina pontica Filipjev. 1918	Х	Х	
14	Cobbionema acrocerca Filipjev. 1922	Х	Х	
15	Daptonema Cobb. 1920	Х		
16	Daptonema elegans (Kreis. 1929)	Х	Х	
17	Daptonema oxycerca (de Man. 1888)	Х		
18	Dichromadora gracilis (Kreis. 1929)	Х		
19	Desmodora de Man. 1889	Х		
20	Desmolaimus bulbulus Lorenzen. 1969	Х		
21	Desmoscolex minutus Claparède. 1863	Х		
22	Eleutherolaimus longus Filipjev. 1922	Х	Х	
23	Enoploides amphioxi Filipjev. 1918	Х	Х	
24	Enoploides cirrhatus Filipjev. 1918	Х		
25	Enoploides brevis	Х	Х	
26	Mesacanthion conicum (Filipjev. 1918)	Х	Х	
27	Enoplus euxinus	Х	Х	
28	Enoplus maeoticus Filipjev. 1916		Х	
29	Halalaimus de Man. 1888	Х	Х	
30	Halaphanolaimus pellucidus Southern. 1914	Х	Х	
31	Halanonchus bullatus	Х		
32	Halichoanolaimus clavicauda	Х	Х	
33	Halichoanolaimus filicauda (Filipjev. 1918)	Х		
34	Linhomoeus filiformis	Х	Х	
35	Linhomoeus hirsutus Bastian. 1865	Х		
36	Linhomoeus Bastian. 1865	Х		
37	Metalinhomoeus de Man. 1907	Х		
38	Metalinhomoeus zosterae Filipjev. 1918	Х	Х	
39	Metachromadora macroutera Filipjev. 1918	Х	Х	
40	Metoncholaimus demani (Zur Strassen. 1894)	Х		
41	Microlaimus de Man. 1880		Х	
42	Monhystera Bastian. 1865	Х		

	Taxonomic composition on profiles	Profiles	
No. crt.	Sf.Gheorghe and Portița	Sf. Gheorghe	Portița
43	Nemanema filiforme (Filipjev. 1918)	Х	
44	Neochromadora Micoletzky. 1924		Х
45	Odontophora angustilaimus (Filipjev. 1918)	Х	
46	Oncholaimus brevicaudatus Filipjev. 1918		Х
47	Oncholaimus campylocercoides De Coninck & Schuurmans Stekhoven. 1933	Х	
48	Oncholaimus dujardinii (De Man. 1878)	Х	Х
49	Oxystomina clavicauda (Filipjev. 1918)	Х	Х
50	Oxystomina elongata Bütschli. 1874	Х	Х
51	Quadricoma loricata Filipjev. 1922	Х	
52	Quadricoma media (Reinhard. 1881)	Х	
53	Quadricoma pontica Filipjev. 1922	Х	
54	Quadricoma Filipjev. 1922	Х	
55	Quadricoma nematodoides	Х	
56	Paralinhomoeus ostrearum	Х	
57	Paracanthonchus caecus (Bastian. 1865)	Х	Х
58	Ptycholaimellus ponticus (Filipjev. 1922)	Х	
59	Paramonhystera elliptica	Х	Х
60	Paramonhystera setosa	Х	Х
61	Paroncholaimus zernovi	Х	
62	Prochromadorella mediterranea (Micoletzky. 1922)	Х	Х
63	Prosphaerolaimus eurypharynx Filipjev. 1918	Х	
64	Rhabdodemania pontica Platonova. 1965	Х	
65	Sabatieria abyssalis (Filipjev. 1918)	Х	Х
66	Sabatieria longicaudata Filipjev. 1922	Х	Х
67	Sabatieria pulchra (Schneider. 1906)	Х	Х
68	Sphaerolaimus gracilis de Man. 1876	Х	Х
69	Sphaerolaimus macrocirculus Filipjev. 1918	Х	Х
70	Sphaerolaimus ostreae Filipjev. 1918	Х	Х
71	Sphaerolaimus dispar Filipjev. 1918	Х	
72	Spirinia sabulicola Filipjev. 1918	Х	Х
73	Spirinia zosterae	Х	Х
74	<i>Terschellingia sp.</i> de Man. 1888	Х	
75	Terschellingia communis de Man. 1888	Х	
76	Terschellingia pontica Filipjev. 1918	Х	Х
77	Terschellingia longicaudata de Man. 1907	Х	Х
78	Theristus moeoticus	Х	
79	Theristus longicaudatus Filipjev	Х	Х
80	Theristus latissimus Filipjev. 1922	Х	
81	Tricoma platycephala Filipjev. 1922	Х	
82	Tripyloides marinus (Bütschli. 1874)	Х	Х
83	Viscosia elongata Filipjev. 1922	Х	Х
84	Viscosia minor Filipjev. 1918	Х	Х
Total no.of	taxa	78	45