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THESIS RESUME

TAXONOMIC STRUCTURE AND ECOLOGY OF FREE LIVING MARINE NEMATODES LIBERE FROM THE ROMANIAN BLACK SEA SHELF

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SUMMARY

The thesis comprises 284 pages and it is structured according to criteria enforce in two parts: the documental part of 53 pages (5 tables and 18 figures) and the experimental part of 154 pages (10 tables and 114 figures). Bibliography comprises 502 titles.

INTRODUCTION

Keywords: Black Sea**,** free-living nematodes, taxonomy, quantitative structure

I proposed myself to study a part of unseen world, overlooked by great explorers, which nevertheless still occasions glory moments to humanity, and revelations about our body. The researchers S. Brenner, H. R. Horvitz and J.E. Sulston found into nematode *Caenorhabditis elegans* genes' the "life and death secret…" as the poet would say, discovery for which they were awarded with the Nobel Prize in 2002.

A question comes out: is there any creature without its mystery or meaning in the Universe? We should not to ignore either the question or the nematodes. They are omnipresent in our life and are the key of essential ecosystemic functions within the environment. The fossils are little known, but some still remained from Cambrian (in geological formations named chondrites). The nematodes' reputation is that they are able to survive more than any other metazoans to harsh conditions from the oceanic abyssal plain, desert, Antarctic ice or toxic sulphurous environments. The Movile Cave special habitats are already famous for the chemosynthesis specialized organisms thriving there, among which nematodes (Muschiol, 2009).

In the light of early studies of free-living nematodes at the Romanian littoral started by Groza - Rojancovski and Paladian in 50's - 60's, Onciu in 70's and 2000's, I tried to carry on their studies, having the chance to extend my researchers up to 200 m depth, in the periazoic zone. The latter has been very little studied, only one bibliographic reference of the Romanian researchers exists related to the nematodes living there (Băcescu et al., 1971). This privilege was offered to me by Prof. Univ. Dr. M.-T. Gomoiu, Member of the Romanian Academy, to whom I am very grateful. I am indebt to my colleagues Dr. Tatiana Begun and Dr. Adrian Teacă, enthusiastic benthic researchers, an example of friendship and competence.

Nematodes represent a phylum that is posing a lot of questions, daring hypothesis and remarkable discoveries regarding their taxonomic and genetic diversity, physiological and habitat adaptations, the origin and phylogeny, functional role in the ecosystem. The Black sea, due to so many particularities of abiotic environment: reduce salinity (brackish basin), freshwater and nutrients periodically input, high productivity comparing with other seas, strong stratification and the existence of anoxic habitats is itself a laboratory, and unique environment for nematodes. About 25 % (Kulakova, 2002) are pontic endemic species. This is capital information which reclaims the continuation of taxonomic studies and mapping of their genome, as a main objective in the near future. Recently, Ürkmez and Brennan, (2012) have announced the discovery of new species, and even more of new genus in the prebosforic area, at 200 m depth, in anoxic sediments.

Nematodes are at the base of trophic chain in sedimentary substrata, being part of microbial loop and a dominant unit of meiobenthos, as well a trophic component of macrobenthos and fishes. They are selective or unselective primary consumers of vegetal detritus and organic matter as well as predators upon bacterial community, other nematodes or micro and meiobenthic organisms.

As researcher within the benthic studies team at GeoEcoMar, the topic of the thesis comes to complete the integrated studies of ecology, sedimentology and geochemistry realized by the institute. A part of the studies of meiobenthic communities was performed within the project FP 7 HYPOX that was carried out between 2009 and 2012, coordinator from GeoEcoMar's side being Prof. Univ. M.-T. Gomoiu. The subject of the project, of high scientific interest, dealt with seasonal and permanent hypoxia in the Black Sea, the causes of its occurrence and the benthic indicators. As key organisms of meiobenthos, permanently found in sediments, the nematodes are proper testing organisms as indicators for early detecting of ecosystem disturbances.

Having in view the above, I consider the study of nematodes at the Black Sea a scientific milestone. Even that the technology has made a great leap from microscopic identification, difficult and time consuming, to genomic mapping, the eye of taxonomist will always be the one who will evenly weigh, analyze and decide whether the taxonomic diagnosis is right. Therefore, the identification effort realized in this paper, using traditional methods, based on analysis of morphological characters is, undoubtedly, essential for extending our researches beyond.

CHAPTER 1. HISTORICAL REFERENCES AND CRITICAL ANALYSIS OF KNOWLEDGE OF FREE-LIVING NEMATODES

In the first subchapter it is presented the evolution of nematology at international level, highlighting the milestones and scientific literature in the field. In the second subchapter, there are reviewed the contributions of scientists from the Black Sea and especially of Romanian ones at development of diversity and ecological studies of free-living nematodes.

CHAPTER 2. GENERAL MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF NEMATODES

Taking into account that many anatomical elements of nematodes are considered in taxonomic diagnosis, in this chapter is made a general presentation of their external and internal morphology, as well as of the most important physiological functions (locomotion, feeding, and reproduction).

CHAPTER 3. TAXONOMIC DIVERSITY

Taxonomic diversity of nematode group still constitutes a subject of debate, impelling the studies focused on identification and discovery of new species.

This chapter comprise an analysis of evolution of systematic classifications, proposed by different authors (Filipjev, (1918/1921), Cobb, (1919), De Coninck and Schuurmans Stekhoven, (1933), Maggenti, (1963,

1970), Chitwood and Chitwood (1950), De Coninck (1965), Gadea, (1972, 1973), Andrassy, (1976), Lorenzen, (1981), De Ley and Blaxter, (2002), a short characterization of subclasses (Enoplia şi Chromadoria) of Nematoda phyllum, and of morphological characters used in taxonomic diagnosis.

CHAPTER 4. DRIVING ECOLOGICAL FACTORS AND ROLE OF FREE LIVING NEMATODES

This chapter summarizes the findings of international ecological studies conducted in different brackish or marine environments regarding the role and influence of environmental factors on distributional aspects related to diversity, abundance and behaviour of nematodes populations. The ecological factors analyzed were: salinity, substrate, temperature, oxygen concentration in sediments and vertical distribution of nematodes, and pollution. Also, there are presented few considerations regarding the importance of nematodes in marine ecosystems.

CHAPTER 5. THE SCOPE AND THE OBIECTIVES OF THE THESIS

The paper has as main scope the knowledge of taxonomic diversity, populational structure, distribution and role of free-living nemtodes on the Romanian Black Sea shelf.

The objectives:

1. Taxonomic inventory of nematodes collected within the research area.

1.1. *Taxonomic identification up to* species level or as *much as possible hierarchically down,*

1.2. *Taxonomic description and critical analysis of systematic position of specimens identified on the Romanian shelf.*

2. Identification of nematodes populations' distribution and associations.

2.1. *Qualitative and quantitative assessment of nematodes populations,*

2.2. *Determination of populational distribution after bathymetric gradient and benthic floors, transects, oxic gradient and morpho-physiologic features*.

CHAPTER 6. MATERIAL AND METHODS OF WORK

6.1. Planning and the calendar of activities

Sampling of meiobenthos was carried out during 4 oceanographic expeditions, which took place between 14 - 26 May, 5 - 10 September 2010, 1 - 10 April 2011, and in March 2012, respectively. The stations were set along 4 perpendicularly transects on shore oriented V - E (Sf. Gheorghe, Portița, Constanța, and Mangalia) from10 to 200 m depth. For nematodes analysis purpose, there were selected 69 quantitative samples.

6.2. Selection of study areas

The samples collected covered the main habitats of the Romanian shelf (Fig. 1).

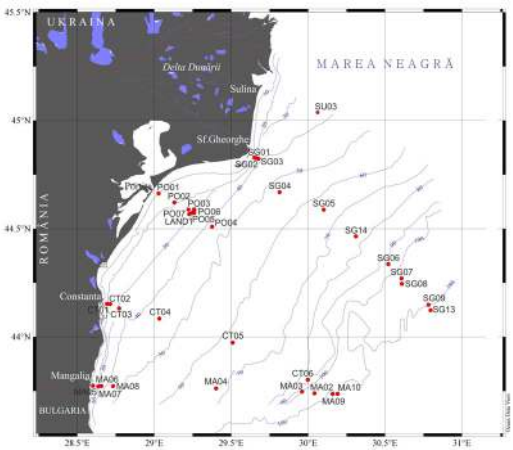

Fig. 1. The stations map of meiobenthos samples from the Romanian continental shelf, collected between 2010 - 2012.

6.3. Collecting and analysis of samples

The samples were collected with Multicorrer „Mark II", equipped with 4 collected tubes, displayed in rosette, deployed from R/V Mare Nigrum board (Fig. 2).

Fig. 2. Field sampling of meiobenthos - general aspects.

From collecting tubes (from 1 or 2 regularly), there were cut up 5 or 10 cm from the top of the sedimentary column. The tube surface was of 0.0075 m^2. The washing of collected material was done by subsequent sieving through 3 mesh sizes (500 μm, 125 μm, 90 μm). After preliminary washing, the samples were preserved with sea water buffered formaldehyde 4 - 5 %.

6.4. Preparation and analysis of samples in laboratory

The nematodes were manually sorted and retrieved from entire or partially sample under stereomicroscope. Then, the individuals were countered and the density per square meter was estimated by extrapolation (number of individuals in the sample multiplied by a factor). For identification, the nematodes were placed between slide and lamella in anhydrous glycerine and then examined under microscope model ZEISS PrimoStar, with magnifying power of 10X100.

The pictures were taken at microscope model ZEISS AxioLab.A1, using a digital photocamera Canon *PowerShot A640,* with resolution of 10 Mpx, attached on the trinocular head of the microscope. It was also used a dedicated camera model ZEISS AxioCam ERc 5s with resolution of 5 Mpx (Fig. 3).

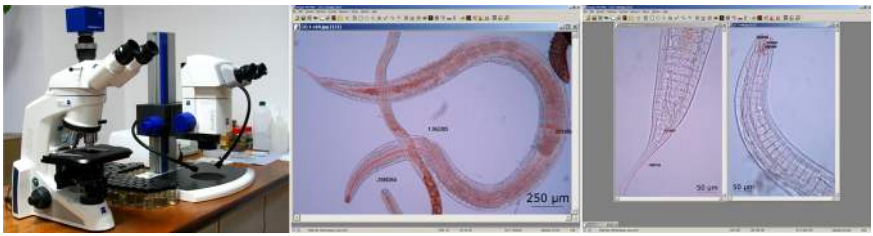

Fig. 3. Identification and biometric analysis of nematodes.

The relevant details for taxonomic identification were completed by specific measurements, according to methodology in use. These were realized by helping of micrometric scale (10 divisions = 1 mm) of microscope ocular, intercalibrated with the measurement software (Image - ProPlus v. 4.5).

6.5. Statistical analysis of data

The quantitative data (abundances) were analysed with statistical programmes PRIMER v.5.0. and PAST v.2.15. There were calculated several univariate indices such as: abundance (A), average density (D_{avg}), frequency (F %), dominance (DD %), ecological significance indices (WD) (Gomoiu and Skolka, 2001), as well as diversity indices (Shannon-Wiener index: H` (Log base = e), Margalef, Simpson (1-lambda) and equitability (Pielou's - J`). The Ocean DataView programme was used for graphical representation of distributional maps of nematodes populations according to oxygen gradient.

In order to discriminate between nematodes populations distribution according to different variables, I used SIMPER and MDS analysis, included in the PAST programme (Hammer et al., 2001).

CHAPTER 7. TAXONOMICAL STRUCTURE OF NEMATODES FROM THE STUDY AREA

This chapter approaches in a critical manner the systematic and taxonomy of nematodes species identified on the Black Sea Romanian shelf. The presented aspects evince the evolution of researches concerning the methodologies and theoretical and practical concepts that influenced the changes occurred at systematic level.

The identification of species of nematodes from the Black Sea presents a series of difficulties because of scarce bibliography and reduced number of studies. The attempt of actualization of biodiversity list of free living nematodes should be done by looking first of all at the original descriptions and eventually the holotypes and more of that a considerable effort must be dedicated to comparing with the synonymies and identification of changes of their systematic. This is necessary in order to complete and integrate the biodiversity list of nematodes from the Black Sea into the World Register of Marine Species (WoRMS) or in specialized databases on nematodes (e.g., NeMys (www.nemys.ugent.be)), facilitating the communication with other specialists.

In this chapter are described 53 species and in most of the cases the orders and families they belong to. I used for documentation about 70 bibliographic sources and the existing biodiversity databases.

CLASS ADENOPHOREA

SUBCLASS CHROMADORIA

1. ORD. ARAEOLAIMIDA

a. **Commesomatidae** Filipjev, 1818

Genus *Sabatieria* De Rouville, 1903 (= Parasabatieria De Man, 1907)

Representative species for the Romanian Black Sea Shelf:

- *Sabatieria pulchra* (Schneider, 1906)
- *S. abyssalis* (Filipjev, 1918)

- *S. longicaudata* Filipjev, 1922

b. **Axonolaimidae** Filipjev, 1918

Genus *Axonolaimus*

Representative species for the Black Sea NW Shelf:

- *Axonolaimus ponticus* Filipjev, 1918
- *A. setosus* Filipjev, 1918
- *Odontophora angustilaimus* (Filipjev, 1918)

c. **Tripyloididae** Filipjev, 1928

Representative species for the Black Sea NW Shelf:

- *Bathylaimus australis* Cobb, 1894
- *B. cobbi* Filipjev, 1922
- *Tripyloides. marinus* (Bütschli, 1874)

2. ORD. MONHYSTERIDA

a. **Fam. Linhomoeidae** Filipjev, 1929

Representative species for the Black Sea NW Shelf:

- *Linhomoeus filiformis* Filipjev, 1918

Genus Anticyclus: a new genus at the Black sea?

The species presented bellow has morphological features of Anticyclus genus from Linhomoeidae, but the difficulty of identification of some characters at the exemplars had, make us to insist for a better description of it, for species identification.

Anticyclus sp. (Fig. 4) - undistinguished stoma with denticles inside, much reduced vestibule, formed from invagination of anterior region, the labial papillae could not be observed, setae disposed in the labial region and on entire body almost, round amphida (13 μm) with central pore, representing 36 % of head diameter, fine annulated cuticle, thick oesophageal ring situated in the anterior third of oesophagus, upper than the ventral gland position, oesophagus is widening posterior, without forming a bulb, cardia short, roundly - elongated, flanked by two oesophageal glands, spicula arched, long (145 μm),

without gubernaculum, the anterior part pointed, short apophysa (16 μm). The seventeen equidistant supplementary organs are rounded, low, conoid papillae separated by distances about equal to their own diameters, and occupy a distance from two to three times as great as the corresponding body-diameter. The tail is filiform, its length is 11.64 times the diameter in the anal region.

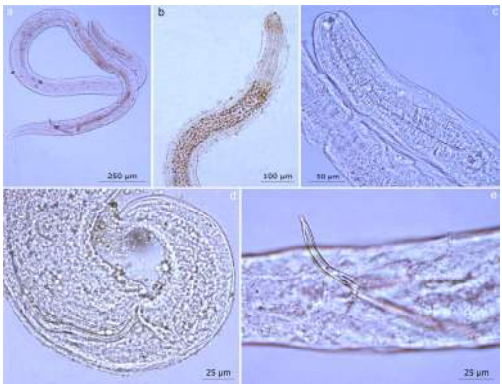

Fig. 4. *Anticyclus sp.*- male. a - general aspect, b - anterior region, c - detail oesophageal region, d, e - detail spicula and preanal supliments.

Genus *Paralinhomoeus*

Representative species for the Black Sea NW Shelf:

- *Paralinhomoeus (Linhomoeus) ostrearum*

Note: the species belonging to genus *Paralinhomoeus* from this study could not be identified at species level, their description is based on author's measurements after available exemplars.

Genus *Terschellingia* de Man, 1888

Representative species for the Black Sea NW Shelf:

- *Terschellingia pontica* Filipjev, 1918
- *T. longicaudata* de Man, 1907

Genus *Sphaerocephalum* Filipjev, 1918

Genus *Prosphaerolaimus* Filipjev, 1918

Representative species for the Black Sea NW Shelf:

- *Prosphaerolaimus eurypharynx* Filipjev, 1918

Genus *Eleutherolaimus* Filipjev, 1922

- *Eleutherolaimus longus* Filipjev, 1922

b. **Fam. Xyalidae** Chitwood, 1951

Genus *Daptonema* Cobb, 1920

Genus *Theristus* Bastian, 1865

Genus *Paramonhystera* Steiner, 1916

Representative species for the Black Sea NW Shelf:

- *Paramonhystera elliptica* Filipjev, 1918
- *P. setosa* (Filipjev, 1918)

c. **Fam. Sphaerolaimidae**

Representative species for the Black Sea NW Shelf:

- *Sphaerolaimus gracilis* de Man, 1876
- *S. dispar* Filipjev, 1918
- *S. ostreae* Filipjev, 1918
- *S. macrocirculus* Filipjev, 1918

d. **Monhysteridae** De Man, 1876

3. ORD. CHROMADORIDA

a. **Fam. Chromadoridae** Filipjev, 1917

Representative species for the Black Sea NW Shelf:

Genus *Dichromadora* Kreis, 1929

- *D. gracilis* (Kreis, 1929)
- *D. cephalata* (Steiner, 1916)

Genus *Chromadora* Bastian, 1865

- *C. nudicapitata* (Bastian, 1865)

Genus *Neochromadora* Micoletzky, 1924

- *N. poecilosomoides* Filipjev, 1918

Genus *Prochromadorella* Micoletzky, 1924

- *P. mediterranea* **(**Micoletzky, 1922)

Genus *Chromadorina* Filipjev, 1918

- *C. obtusa* Filipjev, 1918

Genus *Chromadorita* Filipjev, 1922

b. **Fam. Cyatholaimidae** Filipjev, 1918

4. ORD. DESMODORIDA

a. **Fam. Desmodoridae** Filipjev, 1922

Genus *Desmodora* de Man, 1889

Genus *Spirinia* Gerlach, 1963

Representative species for the Black Sea NW Shelf:

- *Spirinia sabulicola* Filipjev, 1918
- *S. zosterae*

Genus *Chromaspirina* Filipjev, 1918

The species found by us is probably a new species on the Romanian shelf, but is still need more observations and detailed measurements for establishment of taxonomic identity. The main features that differentiate our species of *C. pontica* Filipjev, 1918, but resemble the species *C. gerlachi* Blome, 1982, are: the cephalized head and lenght of spicula (39 μm) and lenght of oesophagus (197 μm).

Genus *Metachromadora* Filipjev, 1918

Representative species for the Black Sea NW Shelf:

- *M. macroutera* Filipjev, 1918

b. **Fam. Microlaimidae** Micoletzky, 1922

Genus *Microlaimus* de Man, 1880

Representative species for the Black Sea NW Shelf:

- *M. kaurii* Wieser, 1954

Fam. Selachinematidae Cobb, 1915

Genus *Cobionema* Filipjev, 1923

Representative species for the Black Sea NW Shelf:

- *Cobionema acrocerca* Filipjev, 1922

Genus *Halichoanolaimus* de Mann, 1886

Representative species for the Black Sea NW Shelf:

- *Halichoanolaimus robustus* (Bastian, 1865) syn. *H. clavicauda* Filipjev, 1918

5. ORD. PLECTIDA

a. **Fam. Camacolaimidae** Micoletzky, 1924

Genul *Camacolaimus* de Mann, 1889

Representative species for the Black Sea NW Shelf:

- *Camacolaimus bathycola* Filipjev, 1922
- *C. dolichocercus* Filipjev, 1922

b. **Fam. Leptolaimidae** Örley, 1880

Genul *Halaphanolaimus* Southern, 1914

Representative species for the Black Sea NW Shelf:

The species identified by us, unlike *H. pellucidus* and *H. sergeeva* sp. nov, newly described (Ürkmez and Brennan, 2012) presents 9 preanal hooks and only one in the oesophageal region (unlike 2 - 3 oesophageal and 6 - 7 preanals - *H. pellucidus*, or 12 - *H. sergeevae*), spicula greater than those of *H. pellucidus* (74 - 75 μm instead of 47 - 52 μm, but comparable: 68 - 70 μm - with those of *H. sergeevae*). The lenght of the body is close to that of *H. pellucidus* (1.6 - 1.7 mm vs. 1.5 mm). Ratio Lenght spicula/Lenght tail smaller than that of *H. pellucidus* (1.52 – 1.61 vs. 2.7), but comparable with that of *H. sergeevae* sp. nov. (1.6).

SUBCLASS ENOPLIA

1. ORD. ENOPLIDA Filipjev, 1929

a. **Fam. Oncholaimidae** Filipjev, 1916

Genus *Oncholaimus* Dujardin, 1845

Representative species for the Black Sea NW Shelf:

- *Oncholaimus brevicaudatus* Filipjev, 1918
- *O. dujardinii* de Man, 1876

- *O. campylocercoides* De Coninck & Schuurmans Stekhoven, 1933

Genus *Metoncholaimus* Filipjev, 1918

Representative species for the Black Sea NW Shelf:

- *Metoncholaimus demani* (Zur Strassen, 1894)
- *M eberthi*

Genus *Prooncholaimus* Micoletzky, 1924

Representative species for the Black Sea NW Shelf:

- *Prooncholaimus mediterraneus* (Fig. 5)

The representatives of this genus have never been reported on the Romanian shelf. In the present study, 2 females were found. The taxonomic features are as follow: the oral cavity well developed (Length = 33 μm; Width = 14 μm), with 3 strong teeth, the subventral one being the tallest, the other two subdorsal almost of equal size; the labial papillae distinct; subcephalic setae (3.2 μm); oesophagus length: 355 μm; germinal zone large, anterior ovary more developed; large cells, trabecular in the pseudocoelomic cavity, giving the annulated epidermis aspect; the tail tapers in the anterior third (tail length = 117 μm); 3 caudal glands, terminated with spinneret.

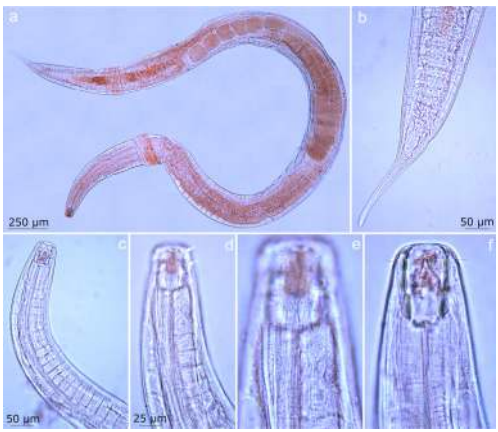

Fig. 5. *Prooncholaimus mediterraneus* - female. a - general aspect, b - caudal region with epidermic trabecules and caudal glands, c - anterior region, d - cephalic region with labial papile and amphid, e, f - detail cephalic capsula, cephalic seta and tooth.

Genus *Viscosia* De Man, 1890

Representative species for the Black Sea NW Shelf:

- *Viscosia minor* Filipjev, 1918
- *V. cobbi* Filipjev, 1918
- *V. elongata* Filipjev, 1922
- *V. glabra* (Bastian, 1865) de Man, 1890

b. **Fam. Enchelidiidae** Filipjev, 1918

Genus *Eurystomina* Filipjev, 1921

Representative species for the Black Sea NW Shelf:

- *Eurystoma assimilis* (de Man, 1876)

c. **Fam. Enoplidae** Dujardin, 1845

Genus *Enoplus*

Representative species for the Black Sea NW Shelf:

- *Enoplus euxinus* Filipjev, 1918
- *E. maeoticus* Filipjev, 1916
- *E. littoralis* Filipjev, 1918

d. **Fam. Thoracostomopsidae** Filipjev, 1927

Genus *Enoploides* Saveljev, 1912

Representative species for the Black Sea NW Shelf:

- *Enoploides brevis*
- *E. cirrhatus* Filipjev, 1918
- *E. amphioxi* Filipjev, 1918

Genus *Mesacanthion* Filipjev, 1927

Representative species for the Black Sea NW Shelf:

- *Mesacanthion conicum* (Filipjev, 1918)

e. **Fam. Anticomidae** Filipjev, 1918

Genus *Anticoma*

Representative species for the Black Sea NW Shelf:

- *Anticoma acuminata* (Eberth, 1863)

f. **Fam. Leptosomatidae** Filipjev, 1916

Genus *Leptosomatum* Bastian, 1865

Representative species for the Black Sea NW Shelf:

- *Leptosomatum sabangense* Steiner, 1915

g. **Fam. Rhabdodemaniidae** Filipjev, 1934

Genus *Rhabdodemania* Baylis şi Daubney, 1926

Representative species for the Black Sea NW Shelf:

- *Rhabdodemania pontica* Platonova, 1965

h. **Fam. Pandolaimidae**

Genus *Pandolaimus* Allgén, 1929

Representative species for the Black Sea NW Shelf:

- *Pandolaimus ponticus*

i. **Fam. Oxystominidae** Chitwood, 1935

Genus *Halalaimus* de Man, 1888

Representative species for the Black Sea NW Shelf:

- *Halalaimus ponticus* Filipjev, 1922

Genus *Oxystomina* Filipjev, 1918

Representative species for the Black Sea NW Shelf:

- *Oxystomina elongata* Bütschli, 1874
- *O. clavicauda* (Filipjev, 1918)

Genus *Thalassoalaimus* de Man, 1893

Body length ranges from 0.7 to 1 mm. The buccal cavity reduced, 12 cephalic setae displayed in two circles and 4 cervical setae longer than the previous. Oesophagus presents a reduced bulb. The amphidial aperture - a slit like shape, large fovea - pocket like. The ovaries reflex, with a small prevulvar sack. The males usually present precloacal papilliform supplements. The caudal tip has thick margin, like a caudal capsule (Fig. 6).

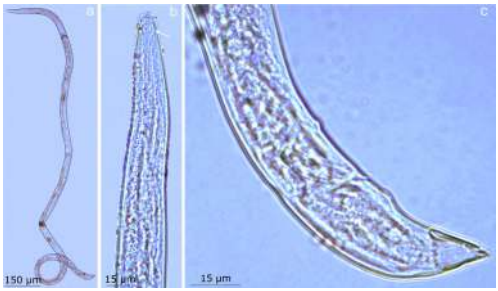

Fig. 6. *Thalassoalaimus* sp. - male. a - general aspect, b - anterior region and amphidial detail, c - spicula detail and terminal capsule.

j. **Fam. Trefusiidae** Gerlach, 1966

Genus *Halanonchus* Cobb, 1920

Representative species for the Black Sea NW Shelf:

- *Halanonchus bullatus* Gerlach, 1964

2. ORD. DESMOSCOLECIDA

a. **Fam. Desmoscolecidae** Shipley, 1896

Genus *Quadricoma* Filipjev, 1922

Representative species for the Black Sea NW Shelf:

- *Quadricoma loricata* Filipjev, 1922
- *Q. media* (Reinhard, 1881)
- *Q. nematodoides*
- *Q.pontica* Filipjev, 1922

Genus *Tricoma* Cobb, 1894

Representative species for the Black Sea NW Shelf:

- *Tricoma platycephala* Filipjev, 1922

CHAPTER 8. STRUCTURE AND ABUNDANCE OF FREE-LIVING NEMATODES ON BATHIMETRIC INTERVALS AND BENTHIC LEVELS FROM THE ROMANIAN SHELF OF THE BLACK SEA

The assessment of structure of nematodes populations was based on 69 samples collected along 4 transects (Sf.Gheorghe (SG), Portița (PO), Constanța (CT) and Mangalia (MA), between bathimetric interval 11 - 200 m.

The total number of taxa identified were 93, belonging to 28 families and 7 orders, the most representatives after number of species being: Enoplida - 30.11 %, Monhysterida - 21.51 % and Chromadorida - 18.28 % (Fig. 7).

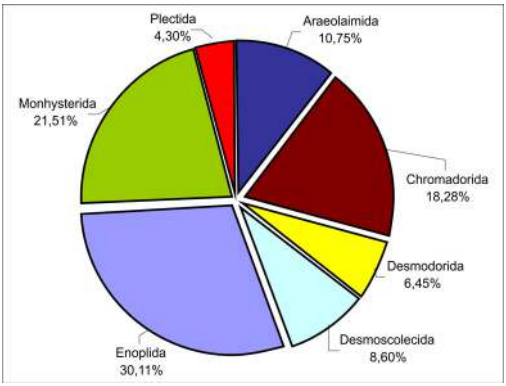

Fig. 7. Taxonomic structure of nematodes populations according to % species within each order from the Romanian shelf of the Black Sea.

8.1. Structure and abundance of nematodes populations according to benthic levels

The benthic levels were considered according to Peres and Picard (1964) methodology and adapted by Băcescu et al., (1971), for the Black Sea.

8.1.1. Infralittoral - upper circalittoral

The greatest specific diversity (Margalef) of all 9 stations was recorded at 17 m, on the Mangalia profile, and at 20 m, on the Portiţa profile, respectively. The abundances of nematode populations ranged between 7,500 and 323,000, being 10 times greater in the northern sector than in the south (Mangalia and Constanța).

8.1.2. Upper circalittoral (26 - 50 m)

The number of species within 25 samples collected varried between 4 and 31. The average density was 200,000 ind.m^{-2}.

8.1.3. Lower circalittoral (56 - 100 m)

The average number of species was 18, and an average density of 36,374 ind.m^{-2}.

8.1.4. Periazoic level (101 - 150 m)

An average of 17 species was found between 100 - 115 m comparatively with 9 species found deeper than 140 m. Although most of the samples were collected from Sf.Gheorghe transect, the number of species is comparable with that from Mangalia profile. The densities decreased on both profiles deeper than 140 m.

8.2. Nematodes contribution to formation of specific associations

The structure of nematodes populations in terms of density distribution on bathymetric intervals was compared using the multi dimensional analysis. The results showed an evident resemblance between the structure of populations found within 26 - 55 m and 56 - 100 m, bathymetric intervals corresponding to location of *Mytilus* and *Modiolula* communities. Bellow 150 m, the resemblance between populations is not so evident, the nematodes having a more uneven distribution of populations density (Fig. 8).

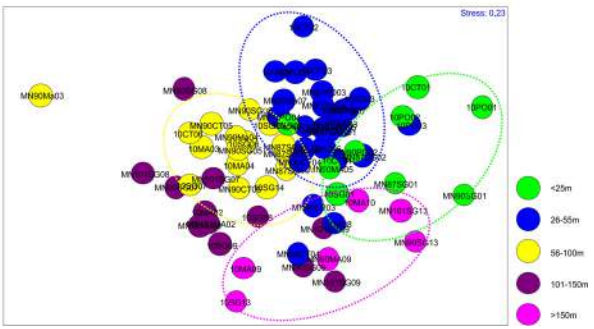

Fig. 8. The MDS Diagram (Multidimensional Scalling) based on densities (logarithmic data) of nematodes populations on bathymetric intervals.

8.3. Analysis of nematodes distribution on Sf. Gheorghe, Portița, Constanţa and Mangalia transects

There were no major differences between transects regarding the distribution of number of species of nematodes from each order. From densities point of view, it is observed the dominance of species from Araeolaimida and Monhysterida, while the Enoplida and Monhysterida are well represented as number of species. It is observed the small number of Chromadorida on Constanța and Mangalia transects on one hand, and the greater number of Desmodorida, in south, on the other hand. Similarly, the densities and number of species from Plectida were greater in south (Fig. 9).

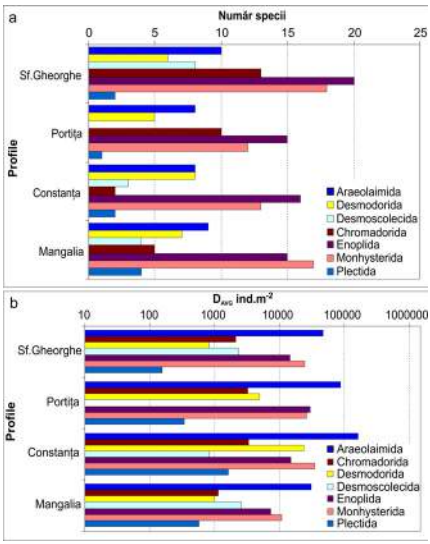

Fig. 9. a. - The structure of nematodes communities after number of species within each order, on each transects, b. - The structure of nematodes communities after density, on each transect from the Romanian shelf of the Black Sea.

8.4. Nematodes in relation with abiotic factors (oxygen concentration)

The diversity and densities recorded by populations of nematodes species on the Romanian shelf do not show major differences, neither in oxygen normal conditions nor of deficit. Anyway, a selection is seen regarding

the distribution of some species (opportunistic species), which were found in great densities even in oxic deficit conditions unlike those which usually inhabit the poor oxygenated habitats (periazoic zone), but have reduced populations and different adaptation strategies.

8.5. Structure and distribution of nematodes populations according to the substrate

Analyzing the distribution of species on different types of substrate, it was noticed that 62 % of them formed associations on phaseolinus substrate (or phaseolinic mud), 70 % on cocolithic one, 24 % on mud, 64 % on coarse sediments, 42 % on sandy mud, while 59 % of them on muddy sand.

The ANOVA test showed (after that normality test was applied to density values distribution) significant differences ($p < 0.005$) between populations inhabiting muddy sand and phaseolinic mud, on one hand, and muddy sand and cocolithic substrate, on the other hand. Also, significant differences ($p < 0.005$) in populations' distribution were noticed in case of both sandy mud and mud substrates comparing with muddy sand.

Even the substrate is relatively homogenous, the enclaves which are forming under the influence of different factors, such as: the quantity of terrigenous material carried from the land, the sediments transporting correlated with the bottom currents force, the surface occupied with biota, oxygen saturation, depth, could differently influence the distribution of nematodes populations, sometimes "hiding" their interdependency relation of substrate.

8.6. Relation between the trophic type, substrate and morphological parameters of nematodes species from the Romanian Black Sea shelf

The trophic types 2A (epidetritivores) (27.31 %) and 2B (omnivore-predator) (33 %) have dominated as number of species the qualitative composition of nematodes populations from the study area, whereas the types 1A (detritivor selective) with 18 %, and 1B type (detritivore non-selective) with 22 %, balanced the functional structure of nematodes populations.

However, the great abundances of detritivore species contribute most at the characterization of trophic profile of communities (e.g., only species from *Sabatieria* genus constitute more than 50 % from the quantitative structure of nematodes populations). It is probably due to dominance of silty fraction in all types of sediments and to capacity of detritivores (*Sabatieria sp*., Monhysteridae, Chromadoridae, Linhomoeidae) to ingest more efficiently the dissolved organic matter.

8.7. Analysis of nematodes morphological parameters related to their distribution in different habitats

In this subchapter it was analyzed the distribution of percentage and cumulative frequencies of nematodes species on bathymetric intervals, according to morphological type of body and tail by helping of histograms.

It is evinced the dominance (over 60 % cumulative frequency) of medium size species (about 50 μm) and length of tail exceeding 150 μm, between 25 - 55 m, comparative with the sizes of nematodes found deeper, when it increases the frequency of efilate species (> 100 μm) and tail size species > 250 μm.

CONCLUSIONS AND GENERAL RECOMMENDATIONS

The study is based on 69 quantitative samples, collected on the Romanian shelf, along 4 transects (Sf. Gheorghe, Portiţa, Constanţa, Mangalia), in the period 2010 - 2012.

Main results obtained according to objectives:

Obiectiv I. Inventory of taxonomic diversity of free-living nematodes within the study area.

1.1. Taxonomic identification at species level or as lowest hierarchical level as possible

- There were 93 nematodes taxa identified on the north-western shelf, in majority at species level. They are belonging to 1 class, 2 subclasses, 7

orders, and 28 families. Out of all, 28 species are included in Enoplia Subclass, whereas 65 species in the Chromadoria Subclass;

- In majority, the species (12) belong to Fam. Chromadoridae, followed by Fam. Desmoscolecidae and Oncholaimidae (each with 8 species). In turn, the species with greater frequency and abundance pertain to Fam. Comesomatidae, Oncholaimidae, Linhomoeidae.

1.2. Taxonomic description and critical analysis of systematic hierarchy of species identified from the Romanian shelf of the Black Sea

Many taxonomical changes that occurred in nematodes systematic due to knowledge evolution, introduction of molecular techniques and performing equipments used led to its revision both to superior and specific hierarchical level.

Synthetically presented, these changes highlighted by our study, are:

- Shift of Families Comesomatidae and Axonolaimidae from Chromadorida Order, and Monysterida, respectively to Ord. Araeolaimida, based on the evidences provided by molecular sequential analyses and revision of role of some morphological characters used in taxonomical identification;
- As a result of differentiation between Monhysterida and Chromadorida Orders based on reconsideration of genital organs' taxonomical importance, the Linhomoeidae Family is contained now into Order Monhysterida. However, there are still a lot of uncertainties with the taxonomical position of the Family's members;
- The most recent molecular discoveries put back the Tripyloidae Family into the Order Enoplida, unlike the Order Araeolaimida, according to WoRMS database (WoRMS, (2013) Tripyloides.);
- The systematic classification of Trefusiidae Family within Enoplia is quite unclear in the present, being in accordance with Lorenzen, 1981 classified in Order Trefusiida or according to other classifications (WoRMS (2013). Trefusiidae Gerlach, 1966), in the Order Enoplida;

- The Xyalidae's taxonomy remains unsolved in great part at both specific and generic level. The Daptonema and Theristus genus are hyper diverse (many of species being now in process of revision), while the others are not enough researched and known. Long time the Daptonema genus has been considered either synonym of Theristus or subgenus of it (Wieser, 1956), only recently their differentiaton being done;
- The ambiguity or difficulty of distinguishing the taxonomic characters of some species belonging to genus from the Family Cyatholaimidae have deepen the crisis of establishing the group's systematic;
- The Family Camacolaimidae Micoletzky, 1924 needs further molecular researchers in order to be clarified its position either in the Order Plectida or Araeolaimida, or as subfamily within the family Leptolaimidae (Lorenzen, 1994) or as Family within Superfamily Camacolaimoidea, respectively;
- According to Lorenzen (1994), the desmocolecids used to be grouped within the Superfamily Desmoscolecoidea from Order Chromadorida, suborder Desmoscolecina, based on type of straight ovaries. De Ley and Blaxter (2002), based on molecular analysis, rise the Suborder Desmoscolecina at rank of Order Desmoscolecida;
- The genus Quadricoma needs to be revised because it is not well distinguished of genus Tricoma.

From genus and species level point of view, the main results of this study are:

- Identification of **three genus cited for the first time** on the Romanian shelf:
  - ***Genus Anticyclus*** (Fam. Linhomoeidae) is very little known in the present, no more than 8 - 10 species being described at international level. In this study, in spite of small number of individuals found, I was able to identify the morphological characters of the genus Anticyclus from Linhomoeidae, but the difficulty of describing some features in case of individuals had, impose the continuation of their study;

- ***Genus Thalassoalaimus*** (Fam. Oxystominidae) was found in two stations, on Sf. Gheorghe profile, at 110 m, and 177 m depth respectively. We suppose that the species belonging to the genus is *Th. mediterraneus*, a representative of enoplids (Ord. Enoplida), being characterized by typical amphid of pocket shape, presence of weak cephalic capsule, small and round buccal cavity, lamellar with velum spicules. It presents two preanal protuberances with setae. The tail has characteristic terminal capsule;
- ***Genus Prooncholaimus*** (Fam. Oncholaimidae) - two female exemplars found on Mangalia profile, deeper than 100 m depth. After morphologic characteristics analysis, we consider that the species is *P. mediterraneus,* but for the identification certainty, we need to examine also the male individuals' characteristics, especially the spicules, which are very important for species diagnosis;

- Presentation of taxonomic keys and photos for most of the described species.

Objectiv II. Identification of nematodes distribution pattern and of specific associations.

In the chapters dedicated to ecological aspects there were approached the qualitative and quantitative structure of populations and the ecological drivers influencing it.

2.1. Qualitative and quantitative estimation of populational structure

- There were identified 93 taxa, belonging to 28 families and 7 orders, of which the most representatives were: Enoplida - 30,11 %, Monhysterida - 21,51 % and Chromadorida - 18,28 %;
- As abundance the dominant were the species belonging to: Ord. Araeolaimida, Monhysterida and Enoplida. As the depth increases (45 - 50 m) the proportion of species from Ord. Desmoscolecida and Desmodorida while the populations of Plectida and Chromadorida orders are relatively reduced as abundance;

- The species from Linhomoeidae such as *Terschellingia longicaudata* and the species from *Linhomoeus* genus could be considered common for the Romanian shelf, having more than 50 % frequency and numerical dominance. The species of Families Desmoscolecidae and Xyalidae are not numerous, being more important in the deep habitats, between 90 and 130 m. The species which form the nucleus of the nematodes communities belong to the Families Comesomatidae, Oncholaimidae, Oxystominidae and Enoplidae.

2.2. Estimation of nematodes distribution:

a) after bathimetric interval and benthic floor

- Between 10 and 25 m depth (the infralittoral and superior limit of circalittoral) the density of nematde populations varied between 7,500 and 323,000 indv.m^{-2}, almost ten times greater in the north than in the Mangalia and Constanta stations. The most numerous species were: *Sabatieria pulchra* (53.108,8 ind.m^{-2}), *S. abyssalis* (23.433,3 ind./m^2), *Viscosia minor* (10.417,2 ind.m^{-2}), *Terschellingia longicaudata* (8.026,4 ind.m^{-2});
- Between 26 - 50 m (superior circalittoral), the number of species varied between 4 and 31. The average density was 200,000 indv.m^{-2}.
- Between 56 - 100 m (inferior circalittoral), the nematode populations were well representative as diversity and abundance comparative with the macrobenthic communities. The greatest density was recorded on the Sf .Gheorghe and Mangalia profiles, at 90 m depth. An average of 18 species and 36,374 indv.m^{-2} were recorded here;
- Between 101 - 150 m (inferior circalittoral - periazoic), an average of 17 species were found between 100 - 115 m, 9 species at 140 m, respectively.

b) after transects location, the following characteristic distribution was evinced:

- Among all species, *S. abyssalis* and *S. pulchra* have distinguished with greater densities in the northern sector (Portiţa and Sf. Gheorghe transects) than in the southern one (Mangalia and Constanţa transects);

- The contribution to the disimilarity between north and south sectors of *V. minor* and *T. longicaudata* was smaller than 20 %, reason for why these species cannot be considered illustrative for discrimination of their preferences for one of the two sectors.

c) after oxygen saturation in the superficial water layer above the sediments in the analysed periods (May and September 2010, April 2011 and March 2012)

- In **May 2010,** the oxygen concentration varied between 6.47 and 8.92 mg/L, within the depth interval 14.5 - 33 m. An average of 8.37 mg/L O_2 was recorded within the deep mussel community (42 - 63 m), while the average concentration was of 4.88 mg/L within the phaseolinic mud substrate (76 and 98 m). Bellow 100 m, the hypoxia ($[O_2] < 2$ mg/L) was recorded. The number of nematodes species was 10 in average within the littoral and offshore zones, while within the mussels and *Modiolula* community there were 15 species in average. The greatest number (25 species) of species was recorded in the northern sectors bellow 70 m depth. As the depth increased the densities of nematodes decreased of about ten times;
- In **September 2010,** a dramatic change of oxic regime occurred throughout of shelf, disregarding the "normal hypoxic" values bellow 80 m. the number of nematodes species was 16 in average in the south sector and 12 in the northern sector respectively. The maximum number recorded was of 18 within 40 - 70 m interval depth and only 14 in the periazoic;
- In **April 2011** and **March 2012,** the oxygen concentration was high even at 60 -70 m depth. At 107 m, in spite of very poor oxygen level within the superficial water column, there were found 17 species of nematodes and more of that four of these species (*S. pulchra, Linhomoeus filiformis, T. longicaudata* şi *Axonolaimus ponticus*) were still identified at 171 m depth, in a medium completely anoxic and with traces of H_2S.

d) after substrate type

- About 62 % of species formed associations on the phaseolinic subsrate, 70 % on the cocolithic one, 24 % on the muddy substrate, 64 % on the coarse substrate, 42 % on the sandy mud sediments, and 59 % on the muddy sand sediments. The most abundant popuations were encountered on the muddy sand (62 %) and coarse sediments (24.7 %), respectively.

e) after morpho-physiologic particularities of nematodes

o *after trophic type*

- The epidetritivores (27.31 %) and the predators (33 %) were the dominant trophic categories according to the number of species;

o *after the morphological type*

- The dominant morphological types after frequency belonged to small (> 30 - 50 μm) and big species size (over 70 μm) and tail lenght of 150 - 250 μm and > 300 μm, respectively.

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