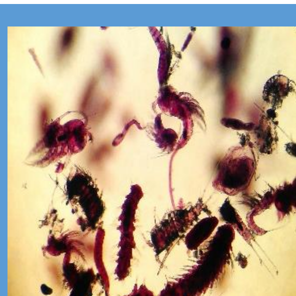
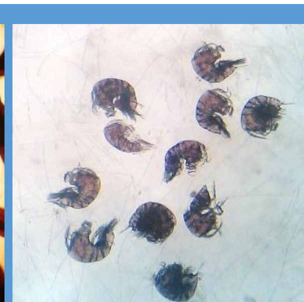
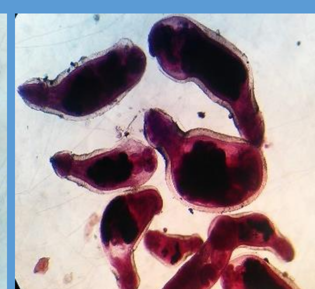
Professional Internship

First Semester, SY 2015-2016

Internship Report

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I. Internship Institute/ Company

A. Vlaams Instituut voor de Zee (VLIZ)/ Flanders Marine Institute

The Flanders Marine Institute (VLIZ) is the center for marine and coastal research in Belgium. It collaborates with different stakeholders as a partner that promotes and supports the international image of Flemish marine scientific research and international marine education. VLIZ supports thousands of marine scientists in Flanders in its capacity as a coordination and information platform by disseminating scientific information to policy makers, educators, the general public and other scientists.

As a research institute, VLIZ has five strategic objectives:

1. Promoting Flemish **marine scientific research** and related marine and coastal **education**.
2. Promoting the **international image** of Flemish marine scientific research and international marine education.
3. Acting as a **catalyst** and as a **point of contact** – also internationally – in the field of marine sciences.
4. Promoting the **visibility** of Flemish marine scientific research to the public at large (popularization and awareness raising).
5. Providing **scientific information** on the sea, the coast and tidal systems to policymakers so that it can be used for policy development and support with regard to marine affairs.

Some Core Activities of VLIZ

- Facilitating **a network of marine scientists** and other concerned parties by offering a platform (by means of the Scientific Committee), contributing to integrated projects and networks, organizing meetings, conferences and scientific awards, issuing publications, mapping relevant expertise in Flanders on a permanent basis, and promoting and presenting this expertise both in Belgium and abroad.
- Supporting marine scientific research by providing the **research vessel 'Simon Stevin'** and other research equipment and infrastructure for common use. VLIZ also contributes to the (inter)national planning, management and efficient use of marine research infrastructure.
- Managing and further developing the VLIZ data center. It collects different kinds of data, implements international standards and distributes data in Belgium and abroad. The VLIZ data center is an integral part of international networks (such as UNESCO/IOC, OBIS, European Marine Board, MarinERA, EurOcean and other European networks) and contributes to the development of international standards for managing and exchanging data and information.
- Managing the VLIZ library which is developing a collection of marine and coastal scientific literature and multimedia. The library operates both on behalf of Flemish marine scientists, universities and scientific institutions, regulatory bodies, international partners and on behalf of interested private

individuals. The VLIZ library is responsible for developing the form and contents of the Open Marine Archive of Flanders.

- By providing policy-relevant scientific information VLIZ is supporting a sustainable and scientifically founded policy for the coastal area, the marine areas and the adjacent estuaries. Within this scope, VLIZ ensures the production of the 'Compendium for Coast and Sea'.

The internship specifically took place in Marine Station Ostend (MSO). The MSO houses multifunctional Laboratories which are developed and always made available for marine scientists. One of the laboratories contains the Zooscanner for digital image analysis of plankton as part of the regular monitoring in the Belgian coast. Other relevant facts about VLIZ can be found at www.vliz.be.

B. Marine Biology Research Group – Ghent University

The Marine Biology Research Group is one of the scientific units of Ghent University. The core mission of the group is to understand the sea which includes fundamental insights in the ecology and evolution of marine ecosystems and contributes to its sustainability. The Marine Biology Research group specializes mainly in the ecology of the sea bottom, particularly bottom dwelling organisms or benthos. Studies on biodiversity, ecology, and functioning of these organisms in the ecosystems are the primary research focus of the group since 1970s. The research topics covers a wide range from the North Sea bottom and laboratory experiments to deep sea, tropical and polar environments. Currently, the wide range of topics are covered by a dynamic team of 4 professors, 8 post-docs, 20 PhD students and 9 technicians.

II. Internship Activities

The internship activities in UGhent Marine Biology for the first and second week of September 2015 include basic protocol for sample processing such as washing of fixed samples, counting and picking of meiofauna. To prepare the samples for the digital image analysis using the Zooscanner, different meiofauna such as nematodes, copepods, halacarids, tanaids, oligochaetes, polychaetes, amphipods, bivalves and others were picked and sorted into individual containers.

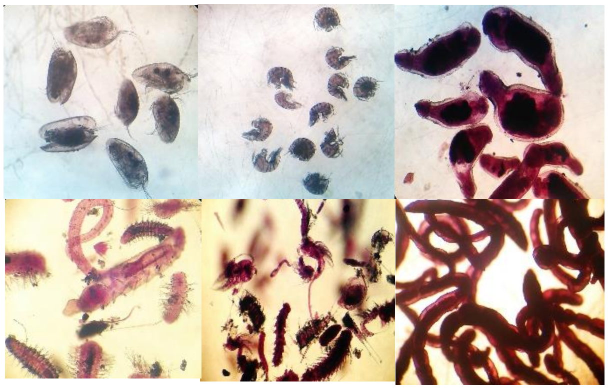

Figure 1. Different meiofauna that were individually picked in preparation for digital image analysis.

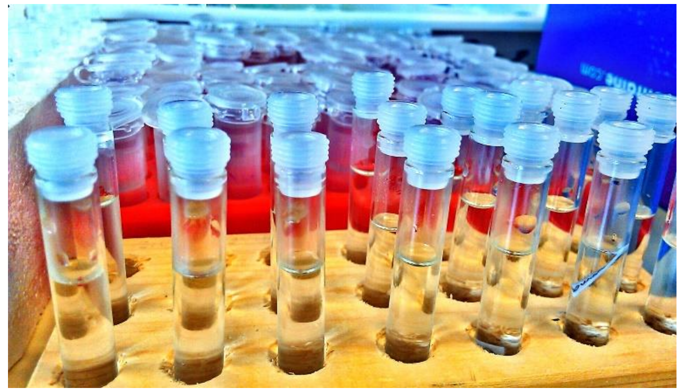

Figure 2. Sorted meiofauna in individual containers.

In VLIZ, the digital image analysis using the Hydroptic Zooscan system. The technical specification of the machine is given below. Aside from this, three computer software were used, Vuescan to operate the scanner, ImageJ as the main user interface, and PlanktonIdentifier for identification and quantification of the different taxa.

Dimensions (LxWxH): 60 x 54 x 36 cm (Cover closed)
Weight: 25 Kg
Input voltage : 110 to 230 VAC, 50 to 60 Hz
Interface: USB 2.0
Resistant to salt water, formaldehyde and ethanol.
Specifications: Samples
ZooSCAN is designed to handle and digitize liquid samples
Sample volume: 0.2litre to 1 liter
Non-destructive with safe sample recovery
Specifications: Images
High resolution, optimized for objects above 200µ equivalent spherical diameter
Image resolution: up to 4800 dpi (dots per inch)
Each image is 14,150 x 22,640 pixels and may contains hundreds, even thousands, of individual animals.
Each image is processed as a single frame of 24.5cm x 15.8cm
Bottom illumination to allow sample dispersion
Optimized lighting system to enhance image quality and contrast
Supplied with transparent frames for good quality images at borders
Computer Support
ZooSCAN is supported by a number of open-source computer programmes.

Figure 3. .Hydroptic Zooscanner

For the digital image analysis, the following activities were performed:

1. **Sample Preparation for Scanning**
   The sorted meiofauna were rinsed with tap water in a 32µm sieve to remove formalin.
   The rinsed samples were placed in a beaker. As the samples were clean, samples were no longer split. Splitting of samples may be necessary if in case of numerous debris or particles.

2. **Scanning**
   A background scan, consisting only of clean water was made as a control. After scanning following the protocol given, individual sorted meiofauna, one of each group was poured into the scanner. The samples were carefully checked and if bubbles appeared, these were removed using plastic needle. It should be made sure that the meiofauna sink at the bottom of the scanning platform to ensure clarity of the images after scanning. Important information was recorded in the ImageJ.

After scanning that would take about 10-15 minutes, the samples were retrieved and fixed with 4% formalin. Using the same software, the ImageJ converted and processed the scanned images. The process took around 30-60 minutes or more.

3. **Data Processing**

After scanning all the different meiofauna taxa, using the PlanktonIdentifier, a learning file was created. Five basic steps were done using the software, Learning, Evaluation, Prediction, Validation and Compilation. It is important to give logical names to the folders and files created as to avoid confusion. A learning set was created for each of the individual meiofauna taxon. The accuracy and precision of the learning sets were evaluated when mixed meiofauna samples were scanned. Prediction allows the automatic recognition of the samples to the different identification groups created. When validated files were created, the folders were individually checked to determine correctness of the prediction. If images were misplaced in a different identification folder, images were cut and transferred to the correct folder.

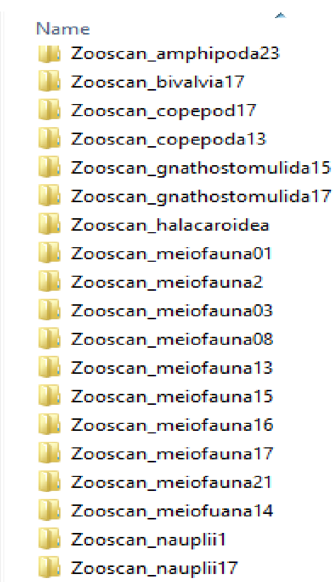

Figure 4. Folders containing scanned and processed images.

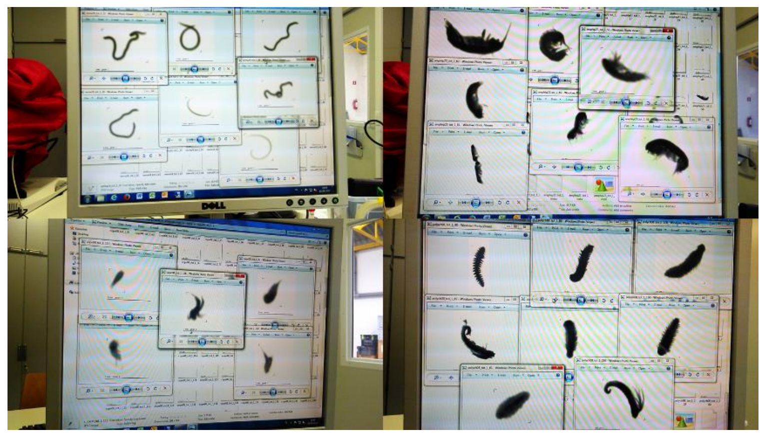

Figure 5. Samples of processed images generated by the ImageJ.

III. Self-evaluation of the Internship.

The following are relevant evaluations for the internship:

1. The use of digital image analysis is a promising tool for ecological research especially in meiobenthology particularly in nematology. The tool can offer a faster and reliable alternative to conventional methods such as manual counting and measurements. The internship is a good start to test the feasibility of this technique as it has never been used in nematology.
2. Internship in general is a good practice to expose the students to different techniques done in different laboratories or institutions. In my case, the skills learned can be used for my thesis. Internship will also allow the students to understand the goals and mission of a particular institute most especially its research direction and focus.
3. The internship also creates a venue for new collaborations and linkages. These collaborations can be of great help for future research endeavors. My supervisors were very helpful during the whole duration of the activity.

4. It can help develop critical thinking and develop new ideas on possible research topics in the future.
5. If budget warrants, the expenses during the internship can be subsidized. This can be done maybe if the internship is done under a funded research project.
6. In my own opinion, the internship is quite short. There can be lots of things to be explored and learned. Part of the internship also overlaps with the classes.

IV. Case Study

Based on the result of the internship, it is important to do a study on meiofauna digital image analysis: an automated identification using the ZooScan integrated system or other digital imaging devices. Ecological research on meiofauna such as biodiversity, impact assessment and ecosystem functioning have been investigated extensively in the past years. To study them, population counts can be made and individual measurements can be obtained for example to obtain biomass. These methods have been proven effective but could entail work and time constraints. Different methods have been proposed to separate organisms from other components of the benthic samples. However, sorting these organisms can still impose time-related issues. Therefore, there is a need to develop techniques that can possibly reduce processing time and could allow an easier way to store and retrieve basic information which can be provided by image analysis. With image analysis, the semi-automated or automated technique for identification can increase spatial and temporal resolution and would in turn result in processing more samples with less time. This method could transform alpha taxonomy into a much more accessible, testable and verifiable science. The digitalization will also allow the storage and retrieval of valuable information preventing loss of information (Bachiller and Fernandes, 2011).

To do this, it is important to determine the kind of digital system to be used. One of the most popular integrated system is the ZooScan. It has been used numerous zooplankton studies (Gorsky et al. 2009) and even in identification of fish eggs (Lelievre et al. 2012). However, during the course of internship, a problem in the contrast and resolution of the images were encountered especially the organisms with smaller sizes. The Zooscanner was able to not able to generate a clear image of organisms with size of less than 200µm even at the highest scanning resolution of 4800 dots per inch (dpi). To address this issue, we can use a digital camera in addition to the scanner. This would increase the resolving power of up to 8500 dpi as mentioned in Bachiller and Fernandes (2011). With good images, we can create a more meaningful learning data sets that can be used to assign specific taxon into their own "identification folder". As meiofauna is very diverse, with high morphological diversity even within the same group like the nematodes, it is necessary to create a huge learning sets containing diverse forms. Aside from taxonomic identification of meiofauna, the image analysis will also be used to perform morphometric measurements and biomass. The Zooscan analysis provide sensitive measures of body size which can be converted into size spectra.

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Pictorial Guide to Scanned Meiofauna

1. Amphipoda

Taxonomic Classification

Kingdom Animalia

Phylum Arthropoda

Subphylum Crustacea

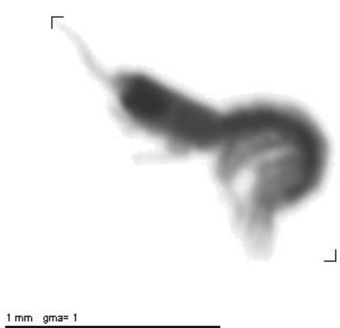
Class Malacostraca

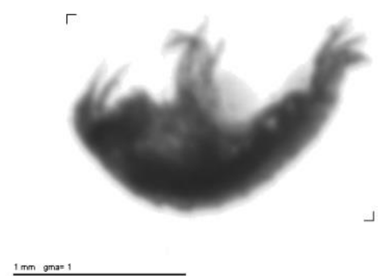
Superorder Peracarida

Order Amphipoda

Taxon Description

Amphipods belong to a diverse group of crustaceans under Class Malacostraca including common groups such as crabs, lobsters and shrimps. The order is part of the superorder Peracarida which unites a diverse groups of shrimp-like taxa that brood their young in a pouch, with no independent larval dispersal stage. Amphipods are characterized by having three pairs of pleopods and three pairs of uropods. This taxon range from a millimeter in length to 340 mm (in some species like *Alicella gigantea*). They can be found in all marine habitats and are also known to colonize freshwater and terrestrial habitats. Amphipods are important herbivores, detritivores, micropredators and scavengers in aquatic ecosystems (Horton et al. 2015)





2. Bivalvia

Taxonomic Classification

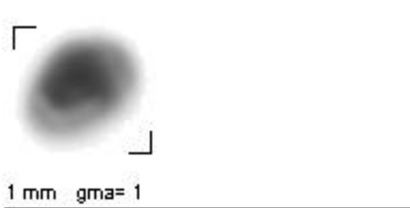
Kingdom Animalia

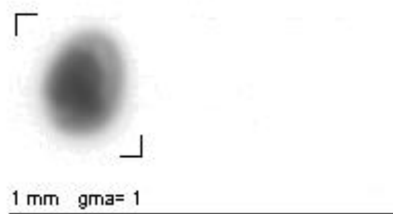
Phylum Mollusca

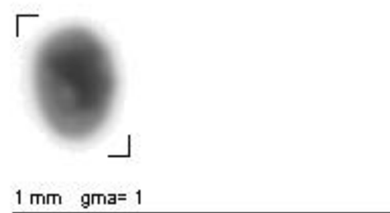
Class Bivalvia

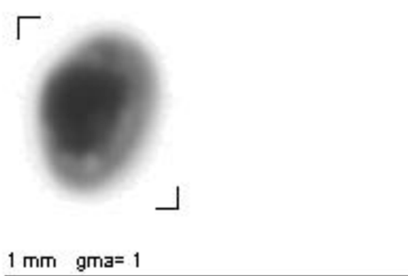
Taxon Description

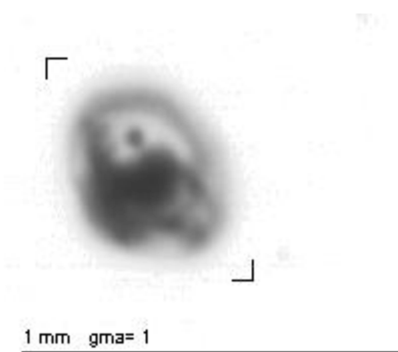
The bivalves include clams, scallops, mussels and oysters. The Class Bivalvia is one of the largest groups of invertebrates. The bivalve body plan is quite simple. It has lost a defined head end with pharyngeal glands and radula. Tactile, chemosensory, and visual receptors where present, have migrated to the mantle at the open edges of the shell. In preserved samples, bivalve larvae are often shriveled while the shell remains intact (Shanks, 2001).

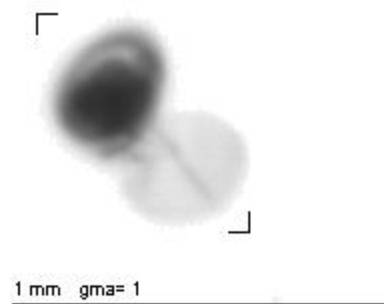


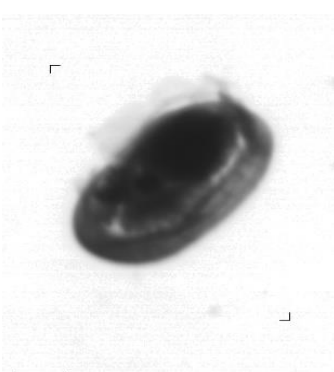


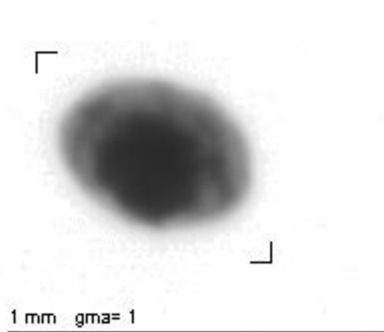












3. Copepoda

Taxonomic Classification

Kingdom Animalia

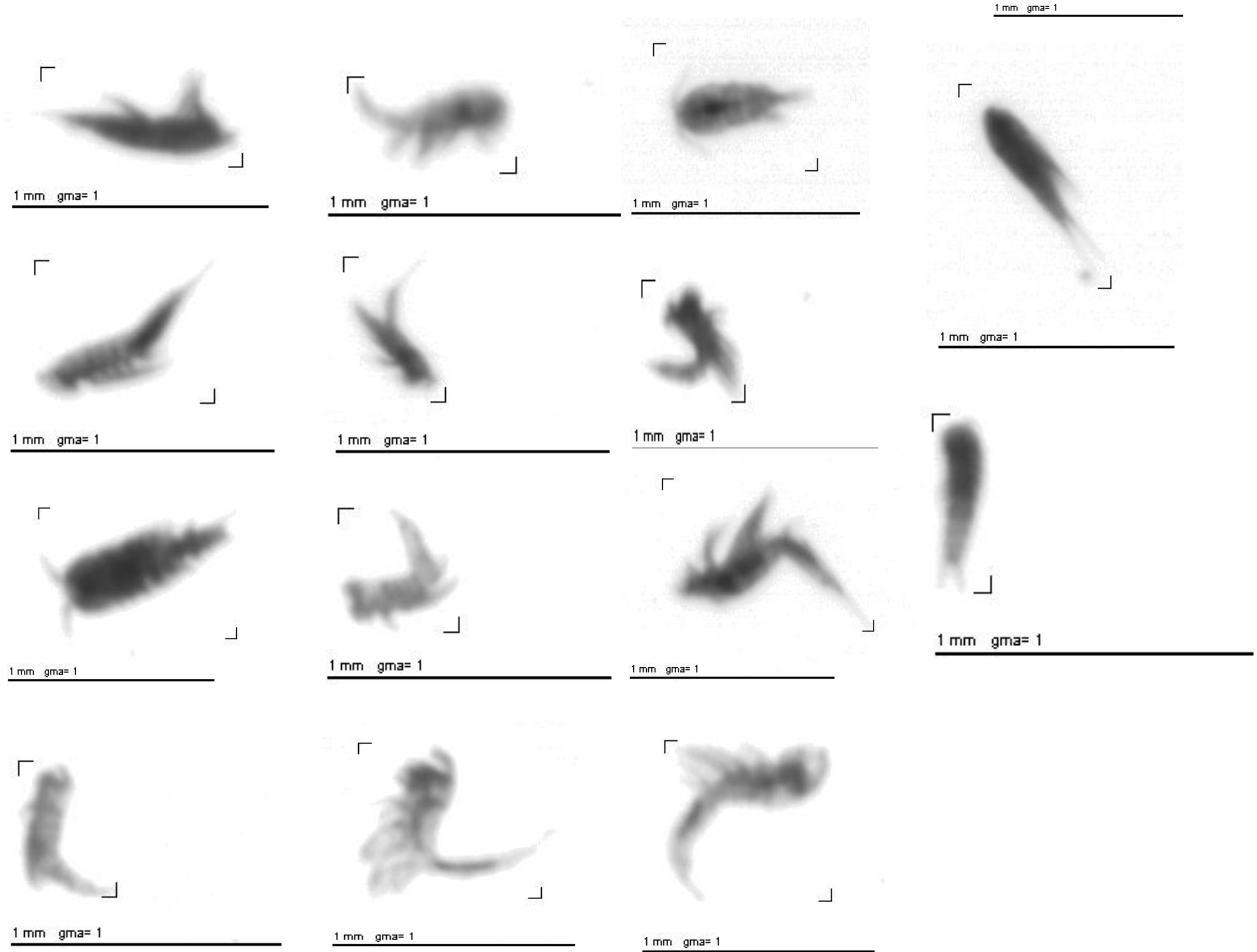
Phylum Arthropoda

Subphylum Crustacea

Class Copepoda

Taxon Description

Copepods are small, diverse aquatic crustaceans and are the most numerous metazoans in the water community. Copepods are found in a range of habitats, from freshwater to hypersaline conditions and in sediment layers in open ocean. The usual adult copepod size range is 1-2mm but some species may be as short as 0.2mm while others can be as long as 10mm. Ecologically the planktonic copepods are important links in the aquatic food chain (Walter and Boxshall, 2015).



4. Gnathostomulida

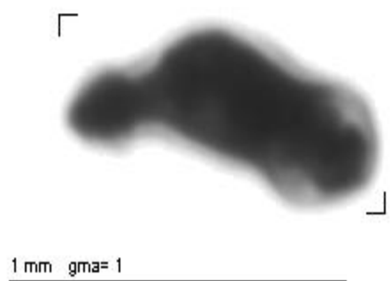
Taxonomic Classification

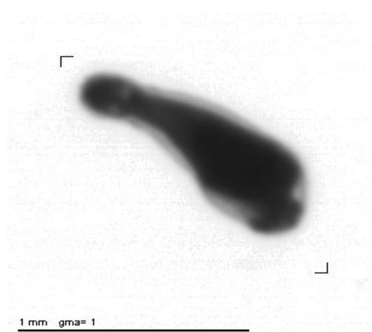
Kingdom Animalia

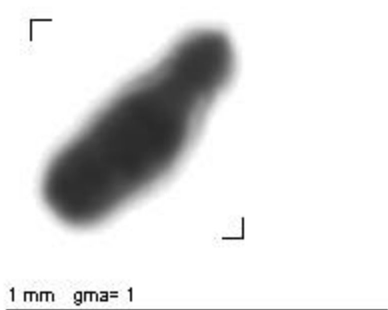
Phylum Gnathostumulida

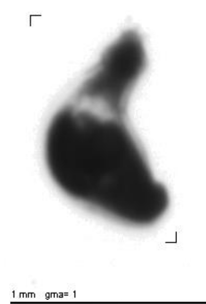
Taxon Description

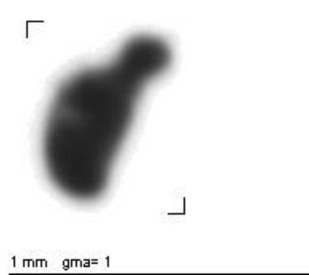
Gnathostomulids are translucent marine worms with muscular pharynx armed with forceps-like jaws in nearly all species. The average body length is 2 mm but size ranges from 0.3 to 3.5 mm. The outer epithelial cells of this organism each have single cilia for movement in the sand. Gnathostomulids are hermaphroditic with only a single ovary.

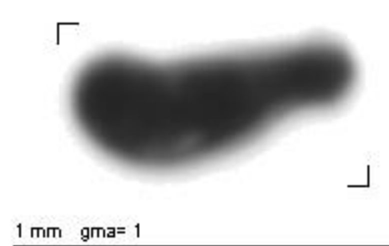












5. Halacaroidea

Taxonomic Classification

Kingdom Animalia

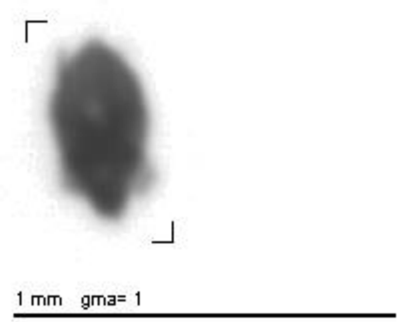
Phylum Arthropoda

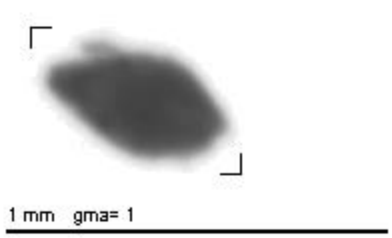
Class Arachnida

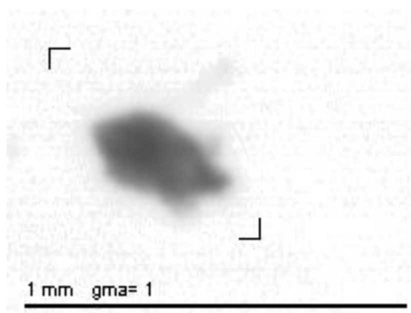
Superfamily Halacaroidea

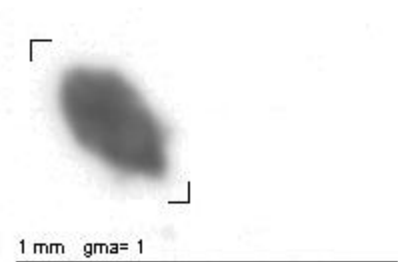
Taxon Description

Halacarid mites are meiobenthic organisms which are mostly marine and only a few are restricted to freshwater habitats. These organisms are often present from tidal areas to the deep sea and are completely adapted to life in the sea. Most species are less than 1 mm in length. Halacarid fecundity is considered low with rarely more than 20 eggs per female (Barstch, 1989).









6. Isopoda

Taxonomic Classification

Kingdom Animalia

Phylum Arthropoda

Subphylum Crustacea

Class Malacostraca

Order Isopoda

Taxon Description

Isopods are the most diverse in form and the most species-rich crustaceans of the superorder Peracarida. Isopods are common inhabitants of nearly all environments. Isopods are characterized by having an abdomen primitively consisting of 5 segments which are commonly used for swimming and respiration. These organisms have compound eyes, two pairs of antennae and four sets of jaws (Brusca, 1997)

7. Nematoda

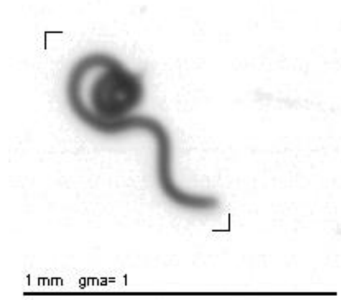
Taxonomic Classification

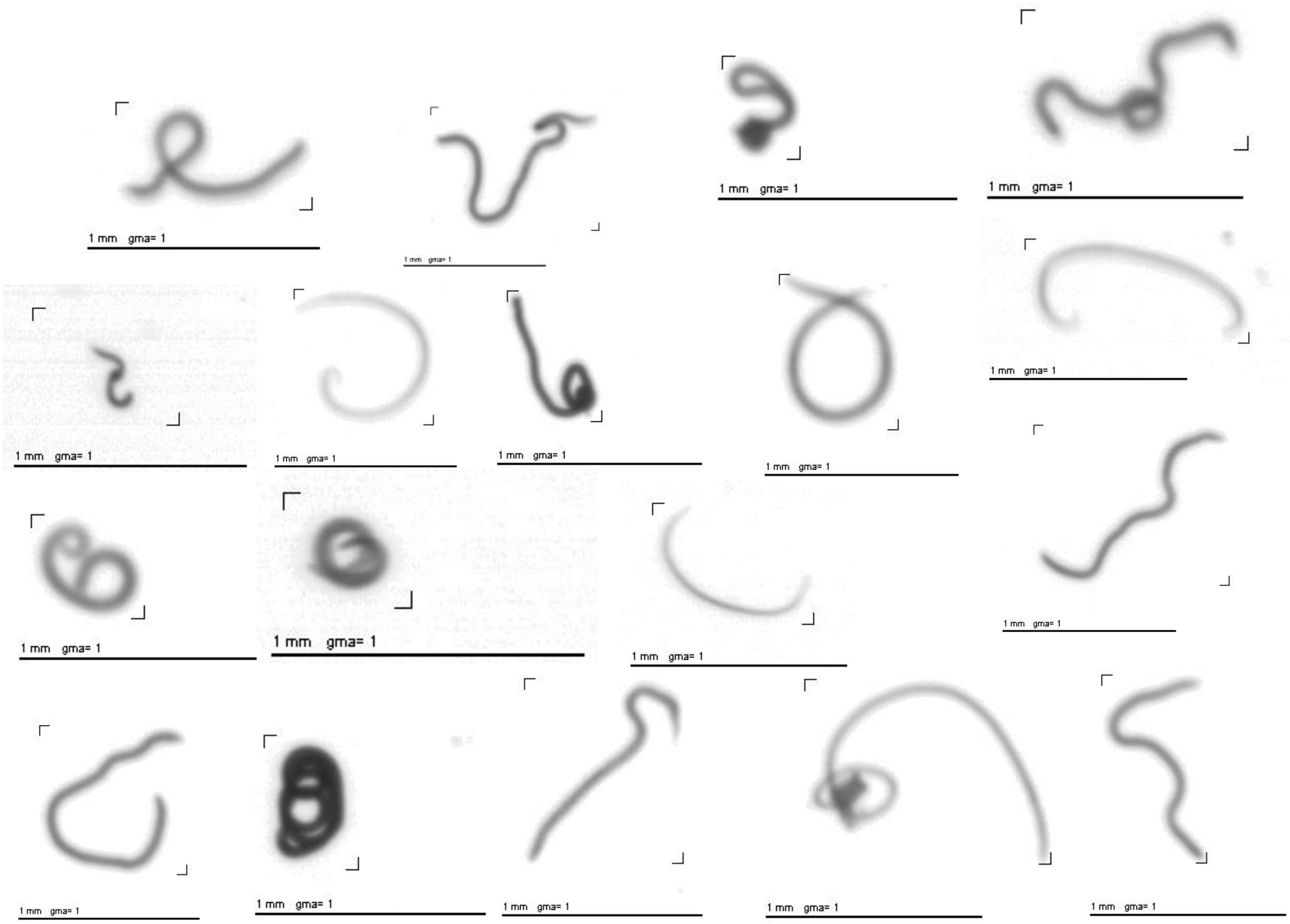
Kingdom Animalia

Phylum Nematoda

Taxon Description

Nematodes are one of the most abundant metazoans found in all substrata and sediment types. The group contains a fairly large number of species with ecological importance. Marine nematodes vary greatly in morphology with no single species can be considered a true representative. Most nematodes have elongated cylindrical body of about 1 to several millimeters long. Some nematodes can be slender with spindle-shaped bodies (Tahseen, 2012).





8. Oligochaeta

Taxonomic Classification

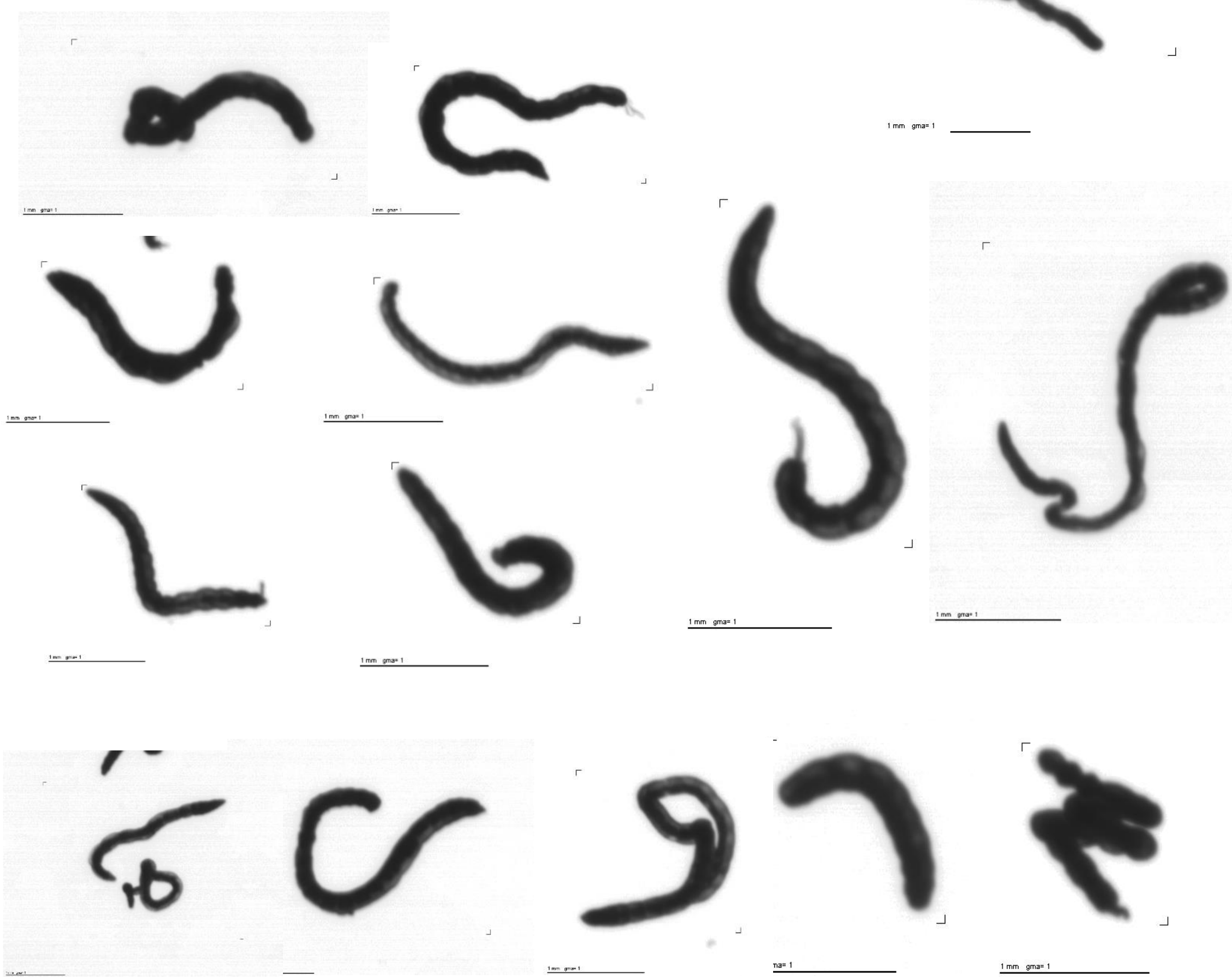
Kingdom Animalia

Phylum Annelida

Class Oligochaeta

Taxon Description

Marine oligochaetes are characterized by having no appendages and segmented body which is usually shorter than 10 cm with tiny hairs on every segment which are used both as basic touch sensors and anchors in the sediment to facilitate movement. Oligochaetes have both female and male reproductive parts, and most species rely on copulation with another individual for reproduction



9. Ostracoda

Taxonomic Classification

Kingdom Animalia

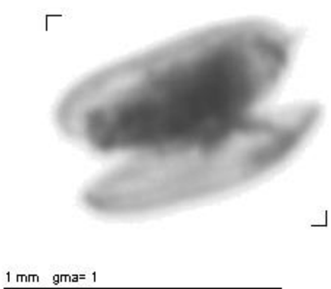
Phylum Arthropoda

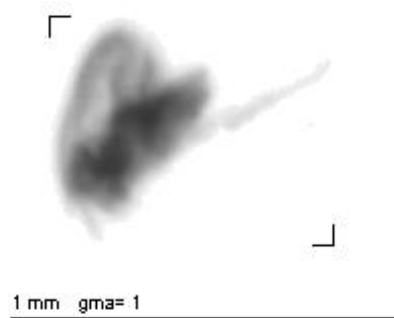
Subphylum Crustacea

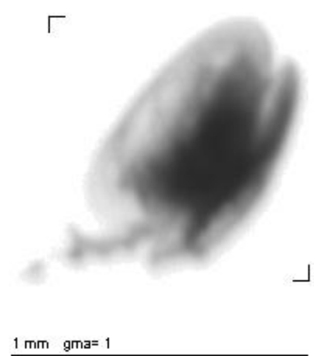
Class Ostracoda

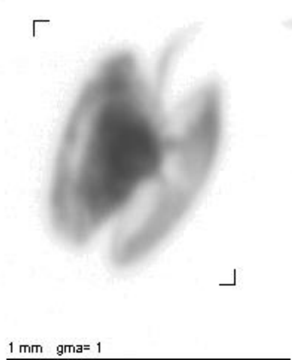
Taxon Description

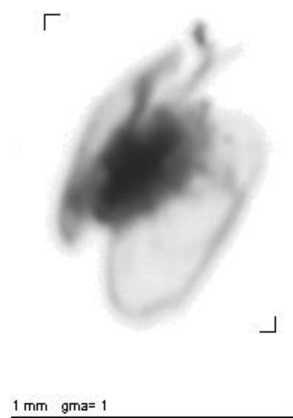
The Ostracoda are a Subclass of small crustaceans of about 0.3-30 mm long which occur in particularly every aquatic environment. They can be found in different habitats like estuaries, lagoons, freshwater lakes, ponds, streams and oceans. These organisms are free-swimming or commonly can be benthic living among aquatic plants. The most distinctive feature of the ostracods is the calcareous bivalve carapace which can totally envelop the body and limbs, but from which various appendages are protruded for locomotion, feeding and reproduction (de Kluijver and Ingalsuo, 2015).











10.Polychaeta

Taxonomic Classification

Kingdom Animalia

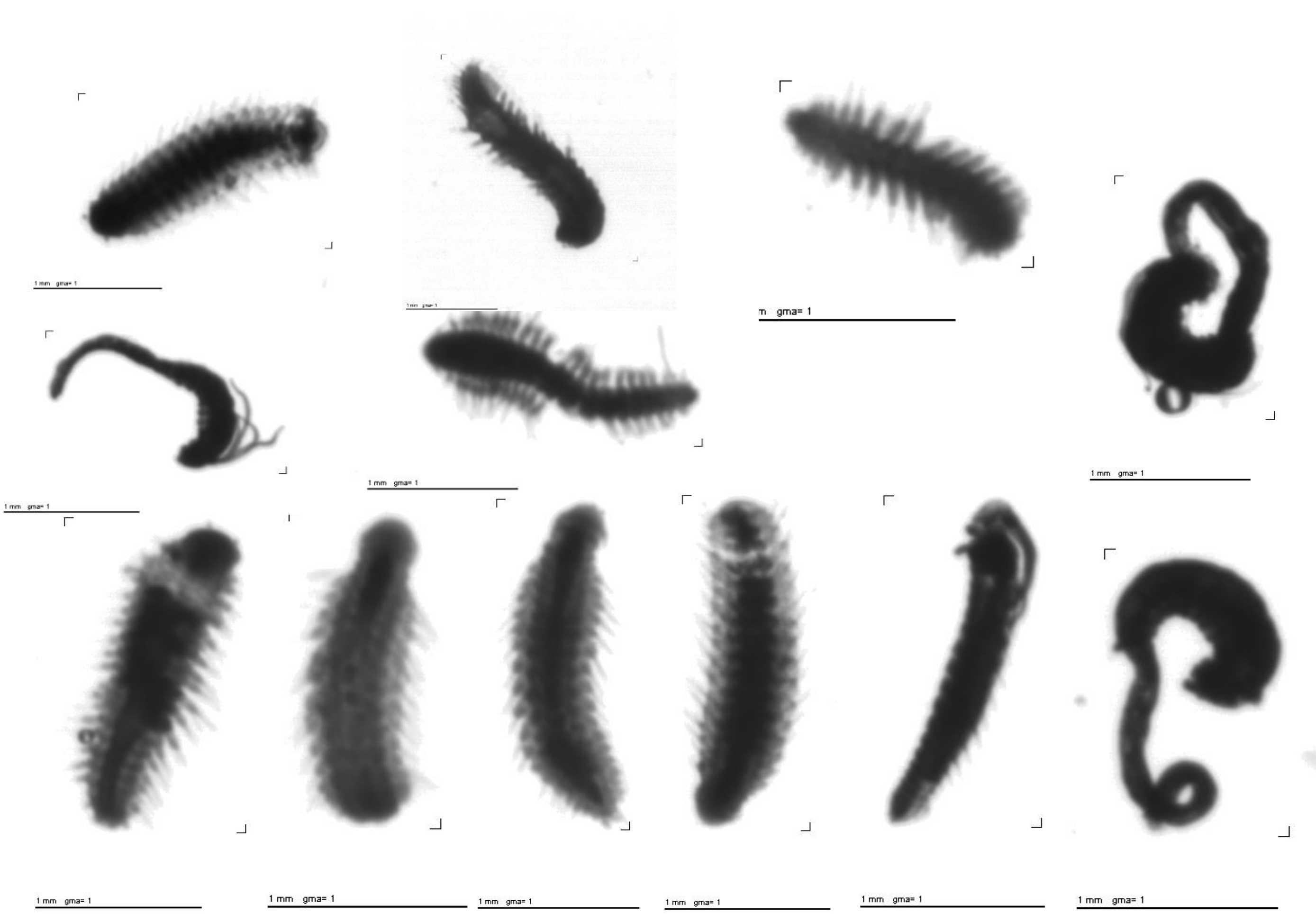
Phylum Arthropoda

Subphylum Crustacea

Class Copepoda

Taxon Description

The polychaetes or bristle worms are considered the largest group of aquatic annelids which are almost entirely marine. Polychaetes can be free-living, commensals and very few are parasitic. Species show a variety of morphological characteristics. Sedentary polychaetes like those living in tubes, the body is often divided into two or more regions and the prostomial sense organs are reduced. In many errant species, the segments making up the trunk are similar and the head is bearing relatively few short appendages. Polychaetes in general have well-developed parapodia and many chaetae.



11.Tanaidacea

Taxonomic Classification

Kingdom Animalia

Phylum Arthropoda

Subphylum Crustacea

Class Malacostraca

Order Tanaidacea

Taxon Description

Tanaids are small, shrimplike crustaceans measuring 1 to 20 mm. Both pairs of antennae in tanaids are branched. The compound eyes are located at the tip of stalks. The cephalothorax which is covered by a shield-like carapace, joins the head and the first two segments of the body.