

Post-doc fellowships to non-EU researchers

FINAL REPORT

Recipient: Maickel Armenteros Almanza.

Home Institute: Centro de Investigaciones Marinas, Universidad de La Habana, CUBA

Promoter: Prof. Dr. Wilfrida Decraemer

Host Unit: Section of Recent Invertebrates, Royal Belgian Institute for Natural Sciences (RBINS)

Stay duration: 11 months. Split in two periods: 2011 (February-July) and 2012 (March-July).

Introduction and research objectives

The proposed research project in 2010 was entitled “Integrative taxonomy of the family Linhomoeidae (Nematoda: Monhysterida)” and their objectives:

1. To delimit and classify the species belonging to the family Linhomoeidae.
2. To provide identification keys for the genera and species within the family.
3. To contribute to a data base of molecular identification tools for nematode taxonomy.

In agreement with my supervisor Prof. W. Decraemer, I changed the family originally proposed as focus of our research (Linhomoeidae) by the family Desmodoridae because my research in Cuba has moved to coral reefs, a typical and very diverse ecosystem distinctive of the tropical regions. The study of Desmodoridae provides us additional advantages for science and for fulfillment of the project objectives: more species can be included in the taxonomic work because the family is highly diverse (six subfamilies, 37 genera and around 243 species), individuals belonging to the family are the most abundant nematodes in coral reefs enhancing the collection, there is the intriguing feature of the ectosymbiotic relationship with bacteria (subfamily Stilbonematinae) and the phylogenetic relationships among species remain unclear. However, the integrative approach to taxonomy remains the same with the exception of the breeding experiments which are not suitable in this group because the difficulty to maintain them in culture.

Methodologies

Nematodes of the family Desmodoridae collected in a first sampling campaign (July 2010) from Cuban coral reefs were identified to species level. These nematodes were mounted in fix preparations for microscopy (glass slides) using paraffin and glycerin at Habana laboratory. Specimens were described based on morphology and measures using both interference light microscopy (LM) and scanning electron microscopy (SEM) at RBINS. For each species we took sets of photographs with LM and SEM and we draw specimens using a drawing tube coupled to the microscope. From this first group of samples no reliable molecular results could be obtained because reagents used for the preparation of the glass slides interfere with the polymerase chain reaction (PCR).

We took more samples in a second cruise (December 2011) and preserved them in DESS solution. Latter preserving solution is recommended for molecular studies. The inconvenient to work with two sets of samples is that three rare desmodorid species identified from the first sampling event could not be recorded in this second sampling (*i.e.* they could not be included in the molecular work). Conversely, other four rare species of desmodorids no previously recorded in Cuban waters occurred in the second sampling (*i.e.* they were not included in the morphological re-description). In Habana laboratory we sorted few hundreds of nematodes and keep in DESS solution. At RBINS we pick-up nematode specimens from the batch sample under the stereomicroscope. Each nematode was mounted on a temporary preparation, identified to species, photo-vouchered with a microscope-coupled camera to keep a record of the morphology and stored in the lysis buffer for DNA extraction. In the molecular laboratory each nematode was subjected to the amplification by PCR of two regions of the genome: COI (mitochondrial) and 18S (nuclear). After the PCR products were sequenced using standard procedures. This protocol was initially focused on desmodorid nematodes; other non-desmodorid species were included after we had sequenced of most of the desmodorid species typical from the studied coral reef. The inclusion of non-desmodorid species in the molecular study is because the molecular diversity of tropical marine nematodes has been poorly sampled and the obtained sequences could be valuable for phylogenetic studies at phylum-level.

Results

Morphology

We identified and described 229 nematode specimens based on the analysis of 26 morphological characters; these nematodes encompassed 15 species and three subfamilies within Desmodoridae. We selected several specimens of each desmodorid species and took

a total of ca. 270 photographs using SEM and 200 photographs using LM (Fig. 1). Fifteen plates (one per species) of drawings were made (Fig. 2). Based on this information we did the re-description of 13 already known species. Latter species were described by previous authors using only light microscopy therefore we added novel knowledge about ultrastructure and morphological variability between and within populations. We described other two new species for the science of the subfamily Stilbonematinae.

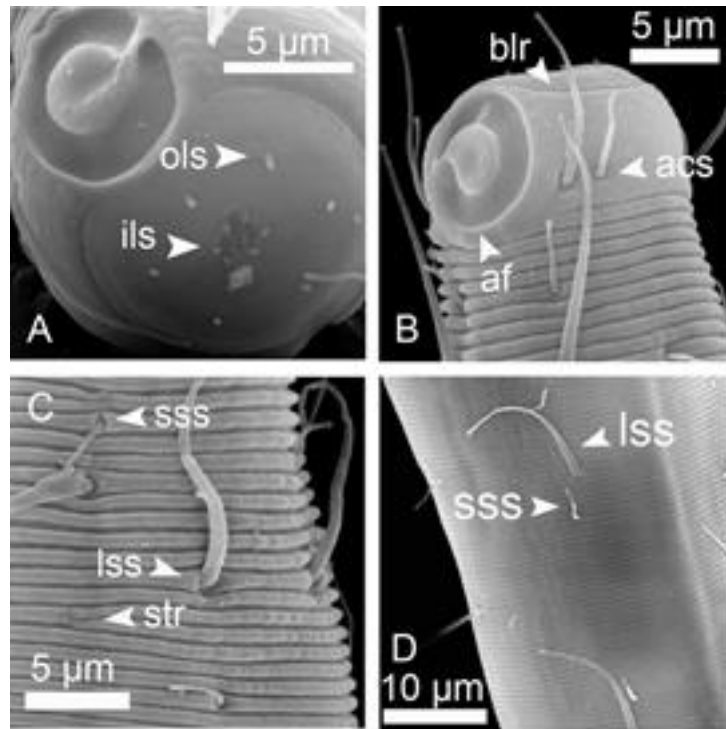


Figure 1. Example of a plate of SEM photographs of the species *Bolbonema brevicolle* with external structural features indicated.

One first article dealing with the morphological re-description of eight species of the subfamilies Desmodorinae and Spiriniinae is in preparation. A second article with the re-description of five species of the subfamily Stilbonematinae, the description of two new species for the science and a dichotomic identification key to genus is also in preparation. Both manuscripts are intended to be submitted to A1 journals and will mention the support of the BELSPO fellowship.

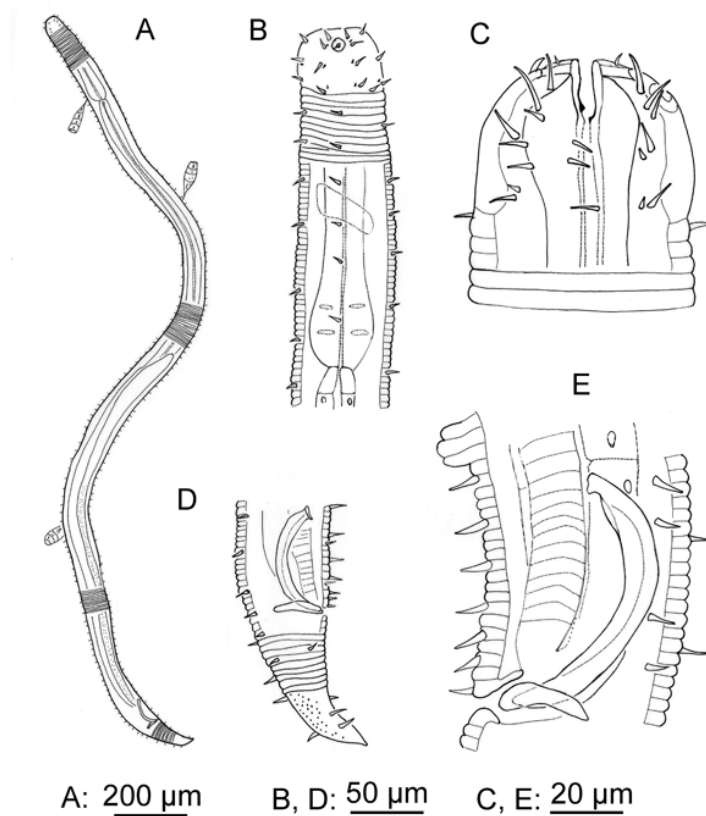


Figure 2. Example of a plate of drawings of the species *Croconema cinctum*. The habitus and most important diagnostic features are shown.

Phylogenetic analysis

The photo-vouchering of the desmodorid species produced 298 photographs of 54 specimens and 17 species; the photo-vouchering of the non-desmodorid species yield 243 photographs of 40 specimens and 26 species (Fig. 3). This photo-archive is an useful reference database for further taxonomic and diversity studies.

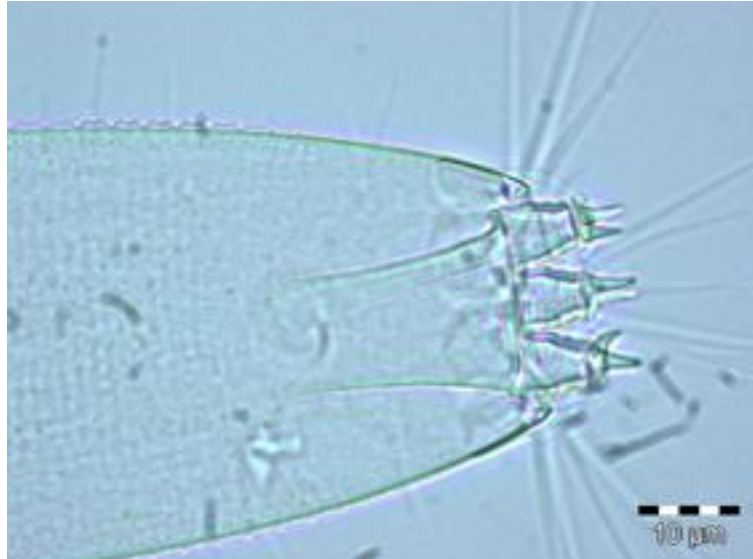


Figure 3. *Example of a photo-voucher of the buccal armature of Scaptrella sp. The photo-vouchering of diagnostic features of the species provide an useful tool for identification of further specimens and also putatively enhances the interpretation of the phylogenetic relationships based on DNA sequences of the same specimens.*

We obtained 51 DNA sequences of COI gene (31 of desmodorids and 20 of non-desmodorid species) and 40 DNA sequences of 18S gene (18 of desmodorids and 22 of non-desmodorids). Genbank has only ca. 30 species of marine nematodes with sequences of COI gene and most of the species sequenced by us are not represented in this database suggesting we will add valuable information. Currently we are editing the sequences and performing some preliminary analysis (Fig. 4). We plan to submit an article about phylogenetic relationships of marine nematodes in an A1 article

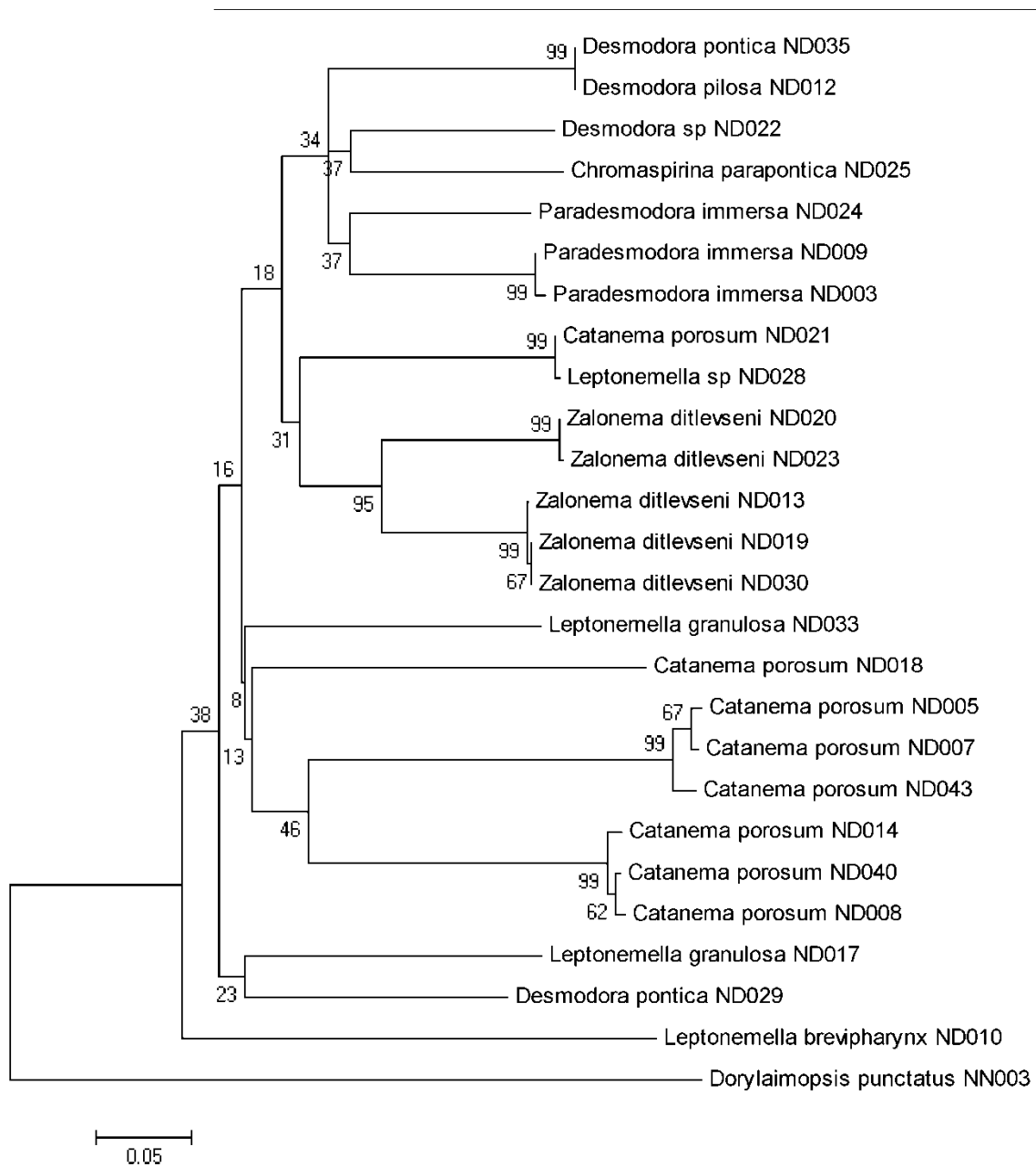


Figure 4. Maximum likelihood phylogenetic tree of desmodorid nematode species based on DNA sequences of partial COI locus. Numbers on the branches indicate Bootstrap support value (1000 permutations). Low support values of deeper relationships suggest COI has a limited value to resolve the systematic of Desmodoridae.

Collaboration and networking

The results of this investigation strongly strength the bilateral collaboration in the field of Nematology between the Belgian partners represented by Prof. W. Decraemer and the Cuban team. Further steps in the functioning of the research network are ongoing (e.g. VLIR

project of South Collaboration) and this postdoctoral stay has been a key aspect for that. We address the collaborative links we have made or strength, during this stay, with the Molecular Unit at RBINS and with both the Faculty of Science and the Marine Biology Section at Ghent University.

The personal training I have received in molecular techniques is a very important step in my scientific career and now I can also spread my know how to other nematologists and students in my university.

List of publications, missions and scientific meetings

- Presentation in Nematology Seminar Day PINC & EUMAINE at Ghent University with the talk entitled “Belgian – South collaboration in Nematology: A case study about free-living nematodes in Cuba”.
- Presentation of a poster in the Second International Symposium of Nematodes as Bioindicators held in Ghent (5-6 July, 2012).
- One article entitled “Depth-related diversity patterns of benthic assemblages (macroalgae, megazoobenthos and free-living nematodes) in tropical rocky shores” has been re-submitted to the journal *Marine Ecology*.
- Two manuscripts are in preparation:
 - 1) The family Desmodoridae (Filipjev, 1922) (Nematoda: Desmodorida). I: re-description of eight known species of Desmodorinae and Spiriniinae from Cuban coral reefs. *To be submitted to Zootaxa*.
 - 2) The family Desmodoridae (Filipjev, 1922) (Nematoda: Desmodorida). II: description of five known and two new species for science of Stilbonematinae from Cuban coral reefs. *To be submitted to the Zoological Journal of the Linnean Society*.
- Molecular data are being processed to write a third manuscript about phylogenetic relationships of free-living marine nematodes.