Short Term Scientific Mission (STSM)

Scientific Report

Bridging Lab Science with Policy: Molecular taxonomy and evolutionary relationships of deep-sea octocorals of the NE Atlantic Ocean

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Host:	Sérgio Stefanni
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Purpose of the STSM

The main purpose of this STSM was to start the 3rd year of my PhD project by learning molecular techniques applied to the discrimination of octocoral species in the deep-sea of the Atlantic Ocean. My four years PhD project combines three approaches: 1) morphology, 2) genetics and 3) zoogeography, with the aim of identifying species and coral gardens, communities at high risk of anthropogenic impact. Consequently, these communities are defined as priority in conservation and management of deep-sea seabed resources.

Horizon 2020 ATLAS is the project housing my research. In ATLAS, partners from both sides of the Atlantic Ocean are gathering efforts in the development of knowledge about deep-sea ecosystems in order to advise a sustainable exploitation of Atlantic marine biological resources. My PhD contributes to ATLAS by identifying octocorals species (including new records and new species) from coral gardens considered Vulnerable Marine Ecosystems (VMEs) and Ecologically or Biologically Significant Marine Areas (EBSAs) in both national and international waters (Areas Beyond National Jurisdiction, ABNJ).

The opportunity offered by the STSM grant was to have access to the molecular laboratory of the Department of Biology and Evolution of Marine Organisms (BEOM) at the Stazione Zoologica Anton Dohrn (SZN) in Napoli, and learn suitable techniques essential for the development of my work in molecular taxonomy applied to octocorals. This work was done with the support of Dr. Sérgio Stefanni who has the expertise of integrating molecular approaches with classical morphological taxonomy. As a result, I have applied DNA Barcoding tools in support to the morphological identification and description of cold-water octocorals of the Northeast Atlantic Ocean sampled in three biodiversity reference collections. Furthermore, phylogenetic analyses will give insights on their evolutionary history. Also, biodiversity patterns on octocoralian fauna may be revealed by mapping their distribution in space and depth. Finally, species in higher risk of anthropogenic impacts will be signalized in the NE Atlantic Ocean.

Description of the work carried out during the STSM

From the 20th of January to the 11th of February I visited the SZN to develop the goals proposed on the STSM 38491. I have learned and have gained confidence in all the laboratory methods needed for the barcoding approach to be used in octocorals: extraction of DNA, measurement of quantity of DNA, PCR, gel electrophoresis and sequencing.

Mitochondrial Cytochrome Oxidade subunit I (COI), an octocoral-specific mitochondrial proteincoding gene (msh1) and the adjacent intergenic region (igr1), are genes commonly screened for octocoral barcoding projects. Other sequences of interest to discriminate among some octocorals are the ITS2 and ND2 and specific primers for octocoral species have been selected after a throughout research of the specific scientific literature applied to the families of octocorals Plexauridae and Coralliidae.

Total genomic DNA was extracted from selected specimens. Samples recently collected and stored in ethanol versus samples from museums possibly stored in formaldehyde were tested for DNA extraction. The DNA extracted were observed on agarose gel electrophoreses runs and the

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amount/quality measured with a Nanodrop Spectrophotometer ND1000. It was decided to clean the genomic DNA with RNAase to reduce the interference of RNA during the amplification of DNA in PCRs' reactions. As a result, some of the samples revealed a clear band of DNA and were used as positive control in future tests. Second extraction of genomic DNA and RNAse treatment was applied to 20 recently collected samples. Finally, the E.Z.N.A.® Mollusc DNA Kit (OMEGA bio-tek) was used on the extraction of DNA of 6 samples of octocorals recently collected and of 6 samples of octocorals from museums, following manufacturer instructions.

The DNA was then amplified by standard PCR reactions. The first PCR was performed using 6 samples for all the 6 pairs of primers selected according to PCR conditions reported in literature. Another PCR with different settings for annealing (lower temperature and longer time) was done for 5 pairs of primers that did not work previously. Then, a gradient PCR was performed for all the molecular markers with a positive control, a sample cleaned with RNAse. This PCR had the goal of understanding what were the best PCR conditions for each pair of primers not amplifying in accordance with the conditions described in the literature. All the following PCRs were performed for the 4 selected markers using the best performing annealing temperature. Another set of PCRs tested the other two molecular markers in those samples that did not amplify. After extracting DNA with the E.Z.N.A. kit, PCRs were repeated for the 4 selected molecular markers. Furthermore, for the samples that did not produced any amplification under the conditions described above, new PCRs were performed using four different Taq polymerases. All products derived from successful amplifications were purified and sequenced.

Description of the main results obtained

During three weeks I was able to improve my laboratory skills and learn the basics of the molecular techniques that are essential to the development of an integrative approach on the study of cold-water octocorals of the Atlantic Ocean.

Extraction of DNA of octocorals

For a better performance on the digestion of the samples by the proteinase K, the samples of octocorals should be sectioned into smaller pieces. RNAse treatment is needed to clean the DNA extraction product from RNA of octocoral samples. As expected, recently collected samples have more quantity of DNA than museum samples stored for decades and putatively fixed with formaldehyde. However, we were able to amplify DNA from some museum samples. A comparison between a traditional DNA extraction method versus the E.Z.N.A. kit to extract genomic DNA revealed to be more efficient. The kit is easy to use, includes an RNAse step to clean RNA and, despite resulting in less amounts of genomic DNA, the DNA has higher quality.

PCR in octocoral samples

PCR amplifications performed better using a low amount of template as most probably the PCR inhibitors carried along with the extraction had a minor effect on the amplification of DNA of octocorals. Among 4 Taq polymerase options, the Phusion® High-Fidelity DNA Polymerase (New England BioLabs) performed more efficiently for the first set of primer pair. The best PCR settings considering annealing temperature and time was achieved for 6 pairs of primers. The 4 selected pair of primers were able to amplify DNA of octocoral samples but not consistently. Amount of DNA and

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size of target fragment must be limiting a consistent result. Recently collected samples DNA extracted with the kit worked in most of the 4 pairs of primers. Nonetheless, the 5th pair of primers is amplifying easily most of the samples. ITS2 is the best performing molecular marker for DNA of octocorals, even with small amounts of template. The reason may be due to the universality of the primers and the short length of the fragment compared to the others. It can work as a link between museum samples with recently sampled specimens when the genetic octocoral database is completed and, if the inter-specific variability is clear among species or coupled to morphology. The first sequences of ITS2 were tested for quality by comparison with the sequences deposited in GenBank using Blast. They proved to be within the family Plexauridae by being similar to Caribbean plexaurid octocorals in which this gene was previously sequenced (Sánchez et al., 2003: Sánchez et al., 2007).

Future Collaborations

By working at the SZN, the collaboration with Dr. Sérgio Stefanni on the molecular taxonomy of octocorals has started. At the same time, on the laboratory of BEOM all the team working with molecular evolution of marine organisms including Dr. Elio Biffali, Dr. Pasquale De Luca, Raimondo Pannone and Elvira Mauriello were essential for the development of this STSM project and are available for the continuation of this work. Dr. David Stankovic from the National Institute of Biology of Slovenia was and will continue to be in straight contact with us for the development of bioinformatic analyses when the sequences of octocorals are obtained.

The SZN museum harbors an old well-preserved collection of cold-water octocorals from the Gulf of Naples. Also the library has historical literature about these animals, like the book where the species caught during Challenger expedition where Darwin traveled all over the world, are described (Wright & Studer, 1889). Despite my lack of time to visit the museum as initially planned, due to laboratory work, I met Dr. Andrea Travaglini which is responsible for the museum. A new collection from the same location is planned for the near future and a collaboration on the identification of octocoral species and sharing of knowledge and samples may occur.

Dr. Marcello Calisti from University of Pisa was visiting the SZN while I was there during the STSM. He is developing a new robot PoseiDRONE to sample the Ocean with less disturbance of the seafloor. As I have expertise gained by participation in deep-sea cruises, I shared my opinion in terms of application, limitations and benefits of ROVs on the exploration of the deep-sea. A future collaboration is forthcoming.

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