



**MOLECULAR ANALYSIS**  
**For**  
**Protecting and increasing the value of**  
**Mediterranean fisheries resources**

**FINAL REPORT PROJECT**

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## Indice

### Sommario

<b>1. Abstract</b> .....	<b>3</b>
<b>2. Objectives</b> .....	<b>4</b>
<b>3. Work planning</b> .....	<b>5</b>
3.1 FIRST year: .....	5
3.2 SECOND year: .....	5
<b>4. MEETING REPORT</b> .....	<b>6</b>
4.1 1° Meeting Report: 16th to 19th October, 2018 – LEBANON.....	6
4.2 2° Meeting Report: 26th to 30th of May, 2019 – ITALY .....	7
<b>4. Conclusion</b> .....	<b>8</b>

## **1. Abstract**

This project proposal is focused on marine sciences and brings in some process and product-related scientific innovations that can be relevant for the Lebanese and the Italian fisheries sectors. In particular, knowledge sharing and expertises on molecular analyses tools applied to marine resources and seafood authentication represents the objective of this project proposal.

Specific tools will be developed to improve traceability of Mediterranean resources. Among molecular analysis, DNA authentication technologies will be applied to sea-food certification and marine resources management.

## 2. Objectives

According to the CNR/CNRS-L agreement, a research project entitled: “DNA IDENTIFICATION AND AUTHENTICATION OF MEDITERRANEAN FISHERIES RESOURCES (DIAMETER)” is being implemented between scientists in the National Centre for Marine Sciences – CNRS (NCMS/CNRS-L) in Lebanon and the Institute for the Study of Anthropogenic impacts and sustainability in marine environment (IAS-CNR) in Italy.

Participants:

CNR IAS – SS DI CAPO GRANITOLA CAMPOBELLO DI MAZARA (TP)

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Antonino Adamo - Project management and international cooperation

Aldo Nicosia - Researcher

Marcello Tagliavia - Researcher

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NCMS – CNRS-L – LEBANON

Sharif Jemaa – Project coordinator

Myriam Lteif – Researcher

Gaby Khalaf – Senior Consultant

Milad Fakhry – Director of the National Centre for Marine Sciences – CNRS-L

### 3. Work planning

The work took two years.

#### 3.1 FIRST year:

Target species will be selected for food certification on the basis of their price and market desirability. They will include groupers, red mullets, anchovy, sardines and two species of cartilaginous fishes. A pilot action to increase traceability of selected species from chilled markets and supermarkets, both sliced or filleted, will be carried out. Identification of molecular analyses tools will be also achieved.

- State-of-the-art analysis.
- Context analysis: selecting species and identifying key issues.
- Identification of molecular methods and develop new protocols: a suite of molecular markers, including CO I, CytB and D-Loop. These studies will highlight different populations inhabiting the Mediterranean basin. Common analysis will be developed through shared protocols.
- Search of planning tools for identifying interventions and further funding.
- 1<sup>st</sup> workshop in Lebanon: visits to fish markets, preliminary report on basic needs will be issued.

#### 3.2 SECOND year:

- Protocol exchange and mutual validation.
- Visit for training on the identified methods in the IAMC CNR Laboratory. Exchange of samples and validation of results.
- A comparison on data achieved by both Institutions will be carried out with the aim to verify the opportunity of setting resources management plans and to promote the selected species.
- Search of planning tools for identifying possible interventions and further funding.
- Second workshop to be held in Lebanon gathering both Italian and Lebanese scientific experts. Methods and protocols standardization will be launched. The opportunities of scientific submissions to selected ISI indexed journals will be investigated.
- A final report on the results of the cooperation will be issued: further opportunities of scientific cooperation will be highlighted.

## 4. MEETING REPORT

### 4.1 1° Meeting Report: 16th to 19th October, 2018 – LEBANON

According to the CNR/CNRS-L agreement, a research project entitled: “DNA IDENTIFICATION AND AUTHENTICATION OF MEDITERRANEAN FISHERIES RESOURCES (DIAMETER)” is being implemented between scientists in the National Centre for Marine Sciences – CNRS (NCMS/CNRS-L) in Lebanon and the Institute for the Study of Anthropogenic impacts and sustainability in marine environment (IAS-CNR) in Italy. In the framework of this project, visits involving both parties are previewed to be executed. The first workshop/meeting took place in Lebanon from the 16th to the 19th of October

#### Results

During the visit of the Italian party, several meetings took place at the National Center for Marine Sciences in Jounieh and Batroun, where the Italian team was introduced to the centres and staff. Then, the Italian scientists were allowed to visit several fish markets in Beirut and Tripoli, where they had the chance to create a full image of the local commercial fish in Lebanese fish markets.

The overall meeting plan focused on: i) ecology and fisheries of Lebanon species; ii) issues of food safety and mislabelling; iii) DNA based species identification; iv) shared protocol for genetic analyses; v) population ecology of small pelagic fish.

On the basis of biological matrices, selected procedures for DNA purification and genetic characterization were planned accordingly. The expertise in genetic analyses was summarized by the Italian researchers, and applied at samples typology. Similarly, recommendations for sampling (geographically referred in absence of cross- contamination) were given by the Lebanese researchers noting their higher experience in Lebanese fish markets. Five groups of commercially exploited fish (that are either thought to be misidentified and mislabeled) will be included in the sampling procedure (sharks, rays, *Epinephelus* spp., *Seriola dumerili*, and *Tunnidae*). On the basis of procedures developed by Italian researchers a specific protocol for PCR based fish identification, the first step of sample lysis and rapid DNA recovery will be performed in NCMS laboratory; while molecular analyses will be carry out at IAS-CNR laboratory. In addition, regarding the species identification phase, three species of small pelagic fishes (*Sardinella aurita*, *Engraulis encrasicolus* and *Engraulis albidus*) will be considered for genetic analyses.

Finally, the main goal of the DIAMETER project were defined also in the frame of internationally relevant projects to be developed. Taking into account such analyses, a plan set was defined including activities to be performed and the second meeting to be held in Italy (scheduled in Spring 2019).

## 4.2 2° Meeting Report: 26th to 30th of May, 2019 – ITALY

In the framework of the Agreement for Scientific Cooperation between the Italian CNR and the Lebanese CNRS-L, a research project entitled: “DNA IDENTIFICATION AND AUTHENTICATION OF MEDITERRANEAN FISHERIES RESOURCES (DIAMETER)” is being implemented between scientists in the National Centre for Marine Sciences – CNRS (NCMS/CNRS-L) in Lebanon and the Institute for the Study of Anthropogenic impacts and sustainability in marine environment - (IAS-CNR) in Italy. In the framework of this project, visits involving both parties are expected to be carried out.

### Results

During the visit of the Lebanese party, several meetings took place at the IAS-CNR (Capo Granitola and Palermo), where the CNRS-L team was introduced to the centres and staff. Moreover, the Lebanese scientists had the possibility of taking advantage of the EuroMarine Working Group on “Enhancement of Human resources: a focus on fisheries, aquaculture, and seafood processing” (EHUSEA), being held at IAS-CNR in Capo Granitola, 27-29 May 2019, and attended some sections of this Working Group.

The overall activity plan focused on: i) introduction to laboratory activities; ii) sharing protocols for DNA analyses; iii) planning future activities; iv) planning a new proposal for the CNR/CNRS-L 2020-2021 joint call.

During the visit to the laboratories (both at CNR and University of Palermo, where the latter shares some instruments and facilities with CNR researchers), the Lebanese scientists were introduced to basic molecular procedures and equipment, useful for the project purposes.

Lebanese researchers, as planned since the first meeting held in Lebanon, provided more than 100 fish samples belonging to the previously selected groups, mainly recovered in Lebanese markets and fisheries. Samples had been prepared and stored according to protocols previously shared by the Italian party with Lebanese colleagues.

Selected samples were further processed, according to the aforementioned protocols, so as to test their suitability for genetic analyses.

In particular, samples underwent PCR amplification using universal primers suitable for species identification by DNA sequencing. After the reaction, amplified DNA was analysed by agarose gel electrophoresis and visualized under UV light.

All samples showed the expected PCR product, many of them yielded excellent amplicons (specific and abundant), whereas some showed to require some reaction optimization.

Results were shared with Lebanese colleague, who were introduced to their interpretation, and discussed.

Next operative steps have been discussed, where PCR amplification of most Lebanese samples will be carried out by the Italian party and sequenced (in service) by the Lebanese one.

DNA sequencing results will be read and elaborated by CNR researchers, then shared with CNRS-L. Moreover, Italian researchers will evaluate the possibility of developing a molecular test for the direct, DNA sequencing independent identification/discrimination of some selected species.

## 4. Conclusion

Next operative steps have been discussed, where PCR amplification of most Lebanese samples will be carried out by the Italian party and sequenced (in service) by the Lebanese one.

DNA sequencing results will be read and elaborated by CNR researchers, then shared with CNRS-L. Moreover, Italian researchers will evaluate the possibility of developing a molecular test for the direct, DNA sequencing independent identification/discrimination of some selected species.

The third meeting has been planned to be held in Lebanon (scheduled in November 2019).

Very unfortunately we were unable to hold the meeting in person due to serious events.

First of all the pandemic caused by Covid, unfortunately still in place, and also the very serious accident in Lebanon, in Beirut, where a factory exploded with very serious environmental consequences and great risk for the population.

All this did not allow us to meet personally, however we kept in touch via the media to try to find new opportunities for our project.

In this period all the samples (100) brought by the Lebanese colleagues were extracted and the amplicon 3 '5' and rev created for the sequencing of the species.

The species to be analyzed were: *Engraulis encrasicolus*, *Sardinella aurita*, *Sardina pilchardus*, *Raya raya*.

We are very determined to finish and deepen what we had proposed and we agree to find economic resources to finish the analyzes that would allow us to publish the results obtained so far.

We continue with the scouting of project opportunities and we hope in new calls for international collaboration, also because the project has created a truly cohesive team.