APPLICATION FORM INCLUDING THE TRAINING COURSE PROPOSAL

Section 1 - Background On the Training Proposal

Project Title : Capacity building of North African countries in rapid identification of non-indigenous marine species by DNA-barcoding

Preamble:

Tunisia is a transitional zone between the two basins (the Western Mediterranean (WMED) and the central Mediterranean (CMED)), under the influence of the Sicily strait, and therefore a key area for understanding the influx of alien species in the whole Mediterranean Sea (Guidetti et al., 2010; Azzurro et al., 2014). Tunisia is under high marine bioinvasion. Actually, monitoring and control of invasive species is a national priority. Tunisia has adopted its monitoring Plan under the implementation of the Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP) of the Barcelona Convention and is starting the elaboration of a national strategy and an Action Plan to address the issue of non-indigenous species. However, taxonomists working on this topic are rare (in particular in North Africa) and identification keys are not always available. Several cases of misidentifications were reported in the literature and usually corrected with genetic analysis. DNA barcoding is considered as robust, rapid, and cost-effective approach broadly applicable for species identification. With this technology and ease of access to data via the barcode of life data System (BOLD), we can improve the research in the framework of the national and Mediterranean context in order to help North African countries fulfil their national, regional and international obligation.

Project Outline:

We plan to do an eight-day training course with 12 trainees from three countries of the southern Mediterranean (Tunisia, Algeria and Morocco) to optimize the monitoring of non-indigenous and invasive marine species at the regional level. Our choice fell on these francophone countries because the workshop will be in French. The training course will be done in 8 days with 7 working days (56 hours) of instruction and supervised work. It will operate from Monday to Sunday from 8.30 AM to 5.30 PM (GMT + 1). Ten instructors will ensure the good running of the training. The overall structure of the planned course is 30% presentations and discussions, 40% laboratory, 30% computer time. The main modules will be 1. Meeting and presentations (Day 1) where instructors will present DNA Barcoding (History of the discipline, Utility and some examples) and trainees will present briefly their expectations of the workshop, 2. Taxonomic Identification, labelling and imaging (Day 2). Several specimens of on-indigenous species will be identified and photographed. Labelling using collecting events register (date of collection, Codes (Collector, Network), GPS Coordinates, etc.) will be explained. We will work on non-indigenous and invasive marine fauna species especially fish and crustacean of the Gulf of Gabes (Southern Tunisia- area particularly affected by Bioinvasion). The Module 3 will be Molecular analysis (Days 3 and 4) namely Tissues lyses, ADN extraction and PCR in an equipped molecular laboratory. The Module 4. 'Informatics and Data Analysis' will group demonstrations and applications on several programs including Barcode of Life Data Systems, Codon Code Aligner, Basic local Alignment search tools (BLAST) and multiple sequence alignment program (Clustal W). This module will be taught in days 5 and 6 using individual computers containing the programs needed for this session. The module 5 (day7) will be Biodiversity Policy. In this module, many lectures related to International agreements such as the international

legal framework related to invasive species, Tunisian biodiversity strategy and action plan and the need for harmonization of the Tunisian legal framework with international and regional system will be explained and discussed. Explanatory courses with power point presentations, demonstrations (in the field, in the lab and in the informatics classroom), applications assisted by instructors and discussions will be dispensed at each module.

Post-Project Follow-up Activities:

* Establishment of an early warning network of non-indigenous and invasive marine species with national coordinators from the 3 countries to gain a better understanding of the spatial and temporal bioinvasion evolution in the region.

* Use citizen science and local ecological knowledge to sensitize local populations and professionals and involve them in the monitoring and control of invasive marine species (Fishing see overfishing, consumption and valorisation of the invasive marine species) by distributing brochures, multiplying the information meetings with professionals and using media and social networks.

* The use of the Barcoding DNA technique with a standard procedure from sampling to extraction and data analysis with a continuous exchange of results should become systematic as soon as possible. Thus, misidentification and confusion of non-indigenous species can be avoided in order to have a solid regional database. These data will be essential for the protection of marine and coastal resources (Aichi's 2020 targets (Aichi 9-11 and 13) for biodiversity).

* Equip national focal laboratories to facilitate analysis as soon as possible.

* Incorporate Barcoding DNA into governments strategic Plan for Biodiversity (Action: fight against invasive species).

* Mobilize adequate financial resources for marine bioinvasion research (Detection, impact studies, fight)

Section 2 - Logic Model

Project Objectives:

1. To develop parctical skills in standard DNA Barcoding workflows ;
2. To improve regional invasive alien species early warning systems of North Africa by establishing biosurveillance checkpoints with trained field officers, a coordination ;
3. Gain the capacity to create projects of non indigenous and invasive marine species as BOLD (Barcode of Life Data System (www.barcoding life.com)) user ;
4. Use Informatic Data Analysis tools including BOLDsystems to establish phylogenetic linkages ;
5. Exchange of ideas in Invasive species detection methods and management.

Expected Project Outcomes:

1. Detection and rapid identification of non-indigenous species useful in governmental biodiversity protection strategies and essential to draft invasive alien species regulatory act;

2. Regular non-indigenous monitoring involving scientists, civil society and fishermen in the Southern Mediterranean coasts with systematic DNA Barcoding;

3. Communicate about DNA Barcoding utility in Invasive species detection and interaction with local policy makers.

4. Create a South Mediterranean database of non-indigenous and invasive marine species on BOLD with regular updates;

5. A national diagnostic laboratory based on existing laboratory facilities at National Agronomic Institute of Tunisia (to perform routine DNA Barcoding analysis by 2019).

Performance Indicators:

1. Minutes/photos from training activities, attendance lists, presentation slide decks, appreciations from trainees;

2. Create a group on facebook to discuss, meet and exchange information (new finds, interesting references ...) about the bioinvasion impacts and usefulness of DNA Barcoding. Meetings must be scheduled at least once every two months by Skype;

3. Create a regional platform with spaces for discussions, reports and publications related to the non indigenous species records and DNA Barcoding analysis. Online presence will be appreciated;

4. Creation of BOLD projects to follow the evolution of the findings and works of the whole team of trainees using CBD contacts to respective [unpublished] projects;

5. Incorporate the theme of the rapid identification by DNA Barcoding in future congresses and scientific events organized by this workshop Training Researchers (instructors and / or trainees.

Section 3 - List of Applicants and Facilitators

Lead Applicant

Name   Mouna RIFI
Address   Institut National Agronomique de Tunisie- 43, Avenue Charles Nicolle 1082 -Tunis- Mahrajène
Work phone   (+216) 71 287 110 / 71 289 431 / 71 892 785
Email   mounarifi3@gmail.com
Country   Tunisia

Co-Applicants

Name	Institution	Email
Faysal Benjeddi	Institut National Agronomique de Tunisie	benjeddi_faysal@yahoo.fr
Hechmi Missaoui	Institut National des Sciences et Technologies de la Mer	hechmi.missaoui@instm.rnrt.tn
Laassad chouba	ONG: Assosiation Tunisienne des Sciences de la Mer	lchouba@yahoo.fr
Khalil Attia	Specially Protected Areas Regional Activity center (SPA/RAC)	director@spa-rac.org

Team Members

Name	Institution	Primary Role
Mouna Rifi	Institut National Agronomique de Tunisie	Event Coordinator
Jamila Ben Souissi	Institut National Agronomique de Tunisie	Training Instructor
Françoise Denis	Faculté des Sciences et Techniques- Le Mans Université- France	Training Instructor
Monia El Bour	Institut National des Sciences et Technologies de la Mer	Training Instructor
Atef Ouerghi	Specially Protected Areas Regional Activity center (SPA/RAC)	Training Instructor
Ahmed Chouiakh	Legal Expert	Training Instructor
Mohamed Ali Ben Temessek	CBD- Focal point (Tunisia)	Training Instructor
Hazar Belli Abdelkefi	CBD- Focal point (Tunisia)	Training Instructor
Yassine Ramzi Sgaheir	Specially Protected Areas Regional Activity center (SPA/RAC)	Training Instructor
M'anouer Djemali	Institut National Agronomique de Tunisie	Training Instructor

Name		
Hanen Ben Ismail	Institut National Agronomique de Tunisie	Support staff
Fatma Ezzahra Zamouri	Institut National Agronomique de Tunisie	Support staff

Training instructors

Name	Area of Expertise	Instruction Topic
Mouna Rifi	Non Indigenous and Invasive Marine Species	Meeting and presentations(Day1)-Laboratory (Identification, labelling and imaging)
Jamila Ben Souissi	Non Indigenous and Invasive Marine Species	Meeting and presentations(Day1)-Laboratory (Identification, labelling and imaging)
Françoise Denis	Molecular Biology and Evolutionary Genetics of aquatic organisms	Laboratory analysis - Informatics and Data Analysis
Monia El Bour	Molecular biology of marine organisms	Laboratory analysis
Atef Ouerghi	Ecosystem Conservation	Presentation (Policy)
Ahmed Chouiakh	Non Indigenous and Invasive Marine Species	Presentations (Policy)
Mohamed Ali Ben temessek	Biodiversity - Biodiversity policy	Presentations (Policy)
Hazar Belli Abdelkefi	Bio-risk management	Presentations (Policy)
M'naouer Djemali	Evolutionary Genetics	Informatics and Data Analysis
Yassine Ramzi Sghaeir	Non Indigenous and Invasive Marine Species	Presentations

Section 4 - Background Information on Facilitators and Participants

Background of Applicants and Instructors

• The lead applicant followed the 2016 GTI training course at Guelph University in Canada and works on Invasive marine species;

• Françoise Denis has a PhD in molecular analysis; she's a Co author of 2 book chapters on the use of molecular tools for the study of biodiversity and marine eco toxicology. She had more than 20 publications in genetic diversity of marine organisms including DNA Barcoding research. She's a Co-supervisor of 9 international students PhD for molecular approaches and has more than 15 years of teaching experience in genome structure and evolution, molecular diversity, phylogeny and phylogeography for European undergraduate and postgraduate students.

• In the framework of the global warming monitoring, Jamila Ben Souissi and Mouna RIFI followed demographic and genetic structures of the gorgonians in the Marine Protected Area and conduct DNA studies on the emblematic invasive crab Portunus segnis in Tunisia.

• The lead applicant and 5 instructors are part of the steering committee of the national strategy on invasive alien species in Tunisia. Mohamed Ali Ben Temessek is an Expert in Biodiversity and Biodiversity policy.

• Lead applicant and Co-Applicants are part of several European projects related to warming effects and invasive marine species such as the Mimosa Project (Among the objectives: the genetic characterization of the corraligen) and the CoCoNet project (Invasive species tracking in marine areas protected).

Partners and Stakeholders:

*The Tunisian focal point supports this action. Moreover two members (Mohamed Ali Ben Temessek (mohamed.temessek@mineat.gov.tn) and Hazar Belli Abdelkefi (hazar.belli.abdelkefi@gmail.com) will act as instructors and will give lectures.

*The National Agronomic Institute of Tunisia, a host institute, will provide a conference room, laboratories and buses for field trips.

*The National Institute of Marine Science and Technology will provide an equipped genetic laboratory.

*The Specially Protected Areas Regional Activity Centres (RAC/SPA) will provide its expertise in international training organization and a financial support of 3500 Euros (about 4140$)

Trainees:

• Engineers and technicians in Fisheries or Marine Environment, Marine biologists, Senior technicians of specialized laboratories, Master's degree or License in Marine Biotechnology, PhD students affiliated to regulatory organizations, Post doctoral students.

• The preferred organizational affiliations: Public universities working on the theme, Research Centres and Institutions, Engineering Schools, Techno poles, Ministries (such as Tunisian Ministry of Agriculture, Fisheries and Water Resources, Ministry of Local Affairs and Environment).

Trainee Selection Criteria:

• Minimal qualification requirements for trainees will be Baccalaureat+3, BSc or MSc equivalent in biology or environmental marine science, Fisheries. A bonus will be awarded to applicants with fundamental knowledge on molecular biology and with a master's or PhD degree. Practitioners and candidates with access to laboratories equipped for genetic analysis have priority.

• Trainees should be part of organizations involved in monitoring marine species.

• Clear understanding of key biological principles (e.g. molecular mechanisms of heredity, the basics of biological systematic, taxonomy, etc.) are considered as prerequisites for this training program.

• Cover letters in addition to Curriculum Vitae will be requested from applicants to ensure the use of this approach in their work. An oral interview (via Skype) will be done for the preselected candidates.
• Candidates' files will be processed in consultation with CBD NFP Tunisia and Specially Protected Areas Regional Activity centre (SPA/RAC). The rules of illegibility will be respected.

Expected Number of Trainees

The number of candidates will be limited to 12. And, as it will be a regional workshop with 3 countries (4 Tunisians, 4 Algerians and 4 Moroccans).

Section 5 - Training Venue and Logistics

Hosting Institution:

•National Agronomic Institute of Tunisia (INAT, web site: www.inat.tn) is a governmental high education and research institution, created since 1898. It is the dean of engineering university in Africa. The total staff of the institute comprises 147 professors and teachers-researchers, 31 technicians and 160 administrative employees. The laboratories are well equipped for environmental studies and field experiments;

• The workshop staff will consist of 12 directly and 6 (administrative agents, drivers) indirectly members;

•An officer of national and international projects will be responsible for project administration and financial management;

• The annual operating budget of the Institute in 2017 was 370000 $, externally-funded projects are about 800000 $. The National Institute of Agronomy in Tunisia, an ethatic Institute, is a tax-exempt public institution;

• Specially Protected Areas Regional Activity center (SPA/RAC) will provide a financial support of 3500 Euros.

Training Venue:

• The trainees will be hosted at the National Agronomic Institute of Tunisia (INAT) for presentations in a conference room, for taxonomic identification and imaging in an equipped laboratory and for bioinformatics analysis in a computer room. Molecular analyses will be done at the National Institute of Marine Sciences and Technologies in an equipped laboratory.

•Analyses and treatments will be done on samples stored in 95 ° Ethanol. For photos, there are: a camera (high resolution) and a binocular loupe connected to the computer with the Leica Application Suite;

•Molecular analysis will be processed in laboratory with the necessary equipment (incubator, centrifuge, thermocyclers, electrophoresis set-up, vortex mixer) and some reagents will be purchased for this purpose;

• Bioinformatics analysis will be done with existing samples data. The trainees will practice on BOLD systems, Codon Code Aligner and other softwares (BLAST, ARLEQUIN, MEGA, ClustalW) under the guidance of 2 instructors. Most of the software used are downloadable for free.

• The sequences of the samples processed in the training will be transmitted to the students by e-mail. Trainees should treat these sequences with distance instructor assistance. This exercise will be considered as a proficiency test.

Training Activities

Day 1. Presentations and lectures (DNA Barcoding especially a brief History; Invasive Marine species in Tunisia, Specific identification: the level of molecular variability of the good markers : Jamila Ben Souissi, Mouna Rifi, Françoise Denis, Yassine Ramzi Sghaeir); Day 2. Taxonomic identification, labelling and imaging of non indigenous specimens (Jamila Ben Souissi -Mouna RIFI); Day 3. Laboratory treatments: Tissue subsampling, Tissue Lyses, DNA Extraction (Monia El Bour - Françoise Denis); Day 4. PCR amplification, E-Gel running (Monia El Bour - Françoise Denis) ; Day5. Informatics and Data Analysis: Introduction to BOLD and Codon code (Françoise Denis), Day6: Data analysis using some softwares (BLAST, ARLEQUIN, MEGA and Clustal W) (Françoise Denis) and Day 7: Policy with Lectures about National biodiversity strategy and action plan, The status of the national regulatory framework related to IAS, The international legal framework related to IAS (Mohamed Ali Ben Temessek, Hazar Belli Abdelkefi, Ahmed Chouiakh, Atef Ouerghi). Use of the DNA Barcoding will be discussed with the Policy makers. Certificates will be distributed in the evening.

Project Logistics

• Communication –by e-mail and Skype: with event participants prior to and post-workshop, including invitation and application procedures for trainees;

• Transportation for facilitators, instructors and participants (local and international travels – flights will be reserved and paid by the staff support; Hotel will be reserved and paid by the staff support; For the trip Airport / Hotel, INAT car will be used; The hotel is close to the host institute (walk: 10 minutes); For long distances (to the Institut des Sciences et Technologies de la Mer), a bus will be available to trainees);

• Airfare and Hotel of Ms. Francoise Denis (a French instructor) will be reserved and paid,

• Meals and catering arrangements will be per-diem allowance;

• A financial report will be drawn up at the end of the workshop;

• Presentations (PowerPoint presentations) and lecture notes (laboratory techniques, software usage guide, etc.) will be distributed to trainees on USB sticks;

• Relevant publications, including Web presence.