

ANNEX III

APPLICATION FORM INCLUDING THE TRAINING COURSE PROPOSAL

Section 1: Background on the Training Proposal

Project Title

Hands-on training on DNA barcoding for threatened species and regulation of illegal wildlife trade addressed to governing agencies and academics

Preamble

Colombia is known for its remarkable high biodiversity even though different drivers underlie its decline. In 2017 the Ministry of Environment (ME) released an updated list of 1300 species (plants and animals) with a category of Vulnerable, Endangered or Critically Endangered. This list orientates efforts to generate insightful information to conserve these species. Two of the main drivers of species vulnerability are illegal wildlife trade and invasive species. Illegal wildlife trade is a practice still largely undocumented in the country as a consequence of limit capacity of environmental authorities to rapidly and accurately identify confiscated species. Besides, accurate and rapid identification of invasive species is a major need to prevent biodiversity loss and impact in agriculture productivity. This project seeks to implement DNA barcoding to assist public servants and researchers in identifying taxa. We will focus on species listed as threatened by ME and in the CITES Appendices, which are currently preserved in a tissue biorepository hold by the Humboldt Institute. This project fits under the umbrella of the AICHI targets goal C “to improve the status of biodiversity by safeguarding ecosystems, species and genetic diversity” and two of the Colombia’s National Biodiversity Strategies and Action Plans: “(i) conservation and care of nature, iv) management of knowledge, technology and information”.

Project Outline

The training course will run for two weeks, Monday to Friday from 8AM-6PM. It will include 10 days of instruction, supervised work and discussion. There will be a one-day symposium event before the course (The Fifth National Symposium on DNA Barcoding) which will be focused on the power of DNA barcoding, accomplishments and challenges, CITES and IUCN categories and normativity. This symposium will be an attractive venue to bring together a large public of students, researchers and other citizens, interested in the initiative and whom may not be able to be part of the hands-on training. The program will then proceed into the first day of the course, a maximum of 13 students will be selected, they are expected to come from institutions that address issues related to illegal trade or conservation management, in the case they are associated to an academic institution their study subject must be related to threatened species. First week (20% presentations, 10% Field and Collection data managing, 40% Molecular Lab, 30% Discussions) will be dedicated to students orientation, introduction of participants (interest, expectations and challenges faced in their institutions), field sampling and collection data managing of specimens and tissues. Hands-on molecular training (tissue lysis, DNA extraction, polymerase chain reaction (PCR), PCR quality scoring using gel-electrophoresis, lab health and safety, introduction to DNA barcode protocols and laboratory processes. If feasible, training course participants are encouraged to bring their own specimens for processing during the training. The second week of training (20% presentations, 50% Bioinformatics and computer time, 30% Discussions) will involve exploration using different bioinformatic tools (e.g. MEGA), BOLD platform, bioinformatic analyses (e.g. BINS, NJ trees, genetic distances), properties for uploading and downloading data in BOLD, searching, filtering and aggregating BOLD data. This week will also include analyses on platforms different from BOLD and

discussion on next steps to reach the goal of characterizing genetic diversity for threatened species and implementation of DNA routines from environmental authorities to fight illegal trade.

Note: For extraction and PCR every participant will have the possibility to work on 6 samples (that they bring or provided by us). For the bioinformatic module we will provide sequences that has been previously generated.

Post-Project Follow up Activities

- Consolidation of the national network iBOLColombia (ibolcolombia.org.co) by integrating new members (see "Trainees" description in section 4) that will continue to enrich the DNA barcode library of Colombia biodiversity.
- Communication will be maintained with trainees to orientate any proposal that might emerge after the training and for which they require orientation.
- A virtual meeting after 6 months and then after 18 months will be held to discuss complete design for specimen processing pipelines and evaluate projects progress from trainees or other members of the institutions that they represent.
- Checklist of endangered species of Colombia generated in BOLD to see the continuous progress in the generation of DNA barcodes for these target species
- Proposal on barcoding the 1300 species considered as threatened written up to 8 months after the training for seeking further funding. We plan to present this proposal to the Environmental Police Authorities, Ministry of Environment and Colciencias (National Agency for Science and Technology)
- Establishment of a pilot project for environmental agencies to implement barcoding in confiscated specimens with preliminary results two years after the training

Section 2: Logic Model

Project Objectives

1. To train public servants and researchers that can apply DNA barcoding to identify specimens confiscated from illegal wildlife trade and invasive species.
2. To improve the identification of threatened species (plants, insects and vertebrates), listed in the last official checklist from the Ministry of Environment (2017).
3. To improve the identification of agricultural-related specimens (e.g. plants, seeds, adult and juvenile insects) in order to detect the presence of invasive species.
4. To consolidate the national network of institutions and local researchers interested in the development of targeted reference barcode libraries to inventory Colombian biodiversity.
5. Design a strategy with the academic sector and environmental authorities with the ultimate goal of establishing diagnostic laboratories for DNA-based species detection in Colombia.

Expected Project Outcomes

1. Thirteen trainees in capacity to implement DNA barcoding to improve the conservation of endangered species and the early detection of invasive species, including regional environmental authorities.
2. Checklist of endangered species in Colombia created in BOLD with the continuous progress of the genetic information that will be generated.
3. Checklist of species categorized as potentially invasive in Colombia created in BOLD with the continuous progress of the genetic information that will be generated.
4. New members in the national network of iBOLColombia and outreach in the media (e.g. newsletters).
5. A document with the strategy will be generated with the trainees in the following months after the course and advances will be reported by the end of 2018.

Performance Indicators

1. Attendance lists, presentation slides and testimonials from trainees. This information will be gathered upon course completion.
2. Generated barcodes for 95 samples from threatened and invasive species.
3. Trainees and their institutions will be part of the national network iBOLColombia (ibolcolombia.org.co).
4. The event and the results of the project will be communicated to the scientific community through different channels such as the iBOLColombia webpage, Facebook and Twitter accounts of the Humboldt Institute.
5. Meetings organized with partners and potential funding agencies where the strategy will be socialized

Section 3: List of Applicants and Facilitators

Lead Applicant:

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Co-Applicants

Name	Institution	Email
Paola Pulido-Santacruz	Instituto Alexander von Humboldt	ppulido@humboldt.org.co
Gerardo Gallego	International Centre for Tropical Agriculture (CIAT)	g.gallego@cgiar.org

Team members

Name	Institution	Primary role
Mailyn Gonzalez	Alexander von Humboldt Research Institute	Event coordinator
Paola Pulido	Alexander von Humboldt Research Institute	Event coordinator
Eduardo Tovar	Alexander von Humboldt Research Institute	Support staff
Felipe Villegas	Alexander von Humboldt Research Institute	Training Instructor
Maria Piedad Baptiste	Alexander von Humboldt Research Institute	Training Instructor
Rodrigo Moreno	Alexander von Humboldt Research Institute	Training Instructor
Gerardo Gallego	International Centre for Tropical Agriculture (CIAT)	Stake holder/Sponsor
Jairo Rodriguez	International Centre for Tropical Agriculture (CIAT)	Training Instructor

Patricia Zapata	International Centre for Tropical Agriculture (CIAT)	Support staff
Juan Fernando Diaz	EAFIT University	Training Instructor
Sandra Uribe	Universidad Nacional de Colombia-Medellín	Training Instructor

Training Instructors

Name	Area of Expertise	Instruction Topic
Mailyn Gonzalez	All steps for implementing DNA barcoding initiatives. Background in plants, insects and vertebrates	Field sampling, Molecular laboratory (Extraction, PCR and Sequencing), BOLD processing data
Paola Pulido	Molecular biology. Background in vertebrates	Sampling, Molecular laboratory (Extraction, PCR and Sequencing), BOLD and data analyses
Eduardo Tovar	Molecular biology. Background in plants, insects and vertebrates	Molecular laboratory (Extraction, PCR and Sequencing)
Felipe Villegas	Photography and communication	Specimen Imaging and workshop communication outreach
Maria Piedad Baptiste	CITES and IUCN policy	CITES and IUCN policy. Illegal wildlife trade in Colombia
Rodrigo Moreno	Biodiversity Policy	Nagoya protocol and national normativity for collecting, exporting and bioprospecting biological resources
Jairo Rodriguez	Background in insects-Agriculture pests	Field sampling and specimen processing
Patricia Zapata	Molecular biology. Background in plants.	Molecular laboratory (Extraction, PCR and Sequencing), DNA barcoding applications
Juan Fernando Diaz	Taxonomy and molecular biology of mammals. Use of MINION technologies	Use of DNA on identifying confiscated mammals species
Sandra Uribe	Systematic biology. Background in insects.	DNA barcoding applications

Section 4: Background Information on Facilitators and Participants

Background of Applicants and Instructors

The Humboldt Institute is part of the National Environmental System in Colombia and as such has experience working with international cooperation. It is currently working in projects with PNUD and BID funds. It has major experience organizing and conducting workshops. Mailyn Gonzalez (PhD) leads the Conservation Genetics Laboratory at Humboldt and coordinates the national network iBOLColombia. She is currently involved in DNA barcode projects for plants, insects and vertebrates and has taken the 2015-2016 GTI training course at Guelph University. Paola Pulido (PhD) is currently working on all operations linked to DNA barcode analysis at

Humboldt. Gerardo Gallego (PhD) researcher at CIAT works on biotechnology and plant genetics applied in sustainable agriculture. He has extensive experience coordinating inter-institutional events. Sandra Uribe (PhD) senior researcher from the National University has been leading the invertebrate DNA barcoding agenda in Colombia. Maria P. Baptiste (MSc) leads the group on assessing species risk of extinction at Humboldt and is part of the national group of experts for CITES and IUCN. Felipe Villegas (MSc) is a researcher at Humboldt in charge of photography and communication outreach. Jairo Rodriguez (MSc) is an entomologist with expertise in field sampling and use of DNA for species identification. Juan Fernando Diaz (PhD) is a researcher at EAFIT University and is leading projects to identify confiscated species of mammals.

Partners and Stakeholders

The national CBD focal point (Maria Claudia Velez Crismatt from Ministry of Foreign Affairs maria.velez@cancilleria.gov.co and Jessika Carvajal from Ministry of Environment jecarvajal@minambiente.gov.co) has endorsed this proposal by presenting a letter of support attached to this document. The General Director of Alexander von Humboldt Biological Resources Research Institute has provided a letter of endorsement in support for this proposal for the implementation of DNA barcoding agenda in biodiversity conservation. The International Centre for Tropical Agriculture (CIAT) is an external sponsor that will provide matching funds -in-kind and is also presenting a letter of support. Finally, the invited instructors from national universities are part of the iBOLColombia network and with their expertise in DNA barcoding projects they will enrich the theoretical and practical component of the training.

Trainees

Target trainees will belong to: (1) Regional environmental authorities (CARs), (2) Environmental police (3) Ministry of Environment: the department of genetic resources is in charge of leading CITES policy (4) Institutions of the Natural Environmental Systems (SINCHI, IAP, INVEMAR)-they focus on Amazonia, Chocó and marine diversity respectively and lead conservation agenda in these particular areas. (5) National Parks- they represent more than 10% of the country and have the potential to become leaders in sampling threatened species and used DNA barcoding biodiversity management plans (6) Colciencias (Colombian Science Foundation)- being the principal funding agency for research in the country their involvement in training will be suitable to seek further funded initiatives. Humboldt has direct communication with all the mentioned partners above. We will prioritize their participation. If remaining places are available, we would train researchers from academy working on threatened species. Direct invitations will be sent to the six mentioned Institutions and remaining places will be published in the Humboldt and iBOLColombia websites. We expect that all the trainees will become part of the iBOLColombia network guaranteeing future communication.

Trainee Selection Criteria

Trainees will be in leading positions in their institutions with the possibility to propose and establish pilot projects using DNA barcoding to enrich the DNA inventory of threatened species, regulate illegal trade or to identify potential invasive species. Trainees will fill a form with questions on their experience, interest and expected outcomes. Participants from different institutions and geographic area of influence will be prioritize.

Expected Number of Trainees

13 (there is not a quota for the number of trainees per institution but if feasible we would like to have 2 participants per institution). We would expect to guarantee the participation of 7 women and 6 men.

Section 5: Training Venue and Logistics

Hosting Institution

The Humboldt Institute mission is to promote, coordinate and carry out research that contributes to the conservation and sustainable use of the biological diversity in Colombia. The Humboldt will be the lead institution administering the funds. The Humboldt Institute works in collaboration with the International Centre for Tropical Agriculture (CIAT) that is part of the Consultative Group on International Agricultural Research (CGIAR) and focuses its research on global food security and agro-diversity systems resilience. Laboratory space, classrooms and computer laboratories will be provided by CIAT and Humboldt. Three staff members from Humboldt will be directly involved in the event organization and other four staff members, from CIAT and Humboldt, will be involved as staff support. Both Humboldt and CIAT had previous experience organizing trainings in molecular biology for up to 20 students.

Training Venue

All activities will be held at the Centre for Tropical Agriculture (CIAT) in Palmira, Valle del Cauca (30min away from Cali city). The molecular biology laboratory of the Humboldt Institute is based in CIAT since 1998. CIAT will provide participants with exceptional facilities; from spacious auditoriums, exhibition space and catering facilities, to state-of-the-art meeting rooms, computer facilities and on-site laboratories for practical activities. Molecular genetics laboratories are fully equipped with thermocyclers, pipettes, gel electrophoresis chambers, UV room, incubators, autoclave, reagents, etc. Specimen processing laboratories are very well equipped with microscopes, insect boxes, tissue arrays, etc; as well as software for image processing. All training room facilities come equipped with LCD full screen projectors, plasma flat screens, video conferencing tools, high-speed internet service and 30 desktop computers. For bioinformatic analyses the following software packages will be installed: Geneious, MEGA, RStudio, BEAST, SpeciesIdentifier (Taxon DNA), among others.

Training Activities

One-day symposium event focused on talks related to DNA barcoding power, accomplishment and challenges and also on CITES and IUCN categories and normativity.

Day 1: Morning: introduction of participants (their interest, expectations and challenges faced in their institutions). Afternoon: field sampling, labelling and collection data managing of specimens and tissues.

Day 2: Imaging, introduction to laboratory health and safety, introduction to DNA barcode protocols and laboratory processes

Day 3: DNA extraction

Day 4: DNA amplification (PCRs) and PCR quality scoring using gel-electrophoresis

Day 5: PCR products will be cleaned up and sent for sequencing using an external service provider. For extraction and PCR every participant will have the possibility to work on 6 samples (that they bring or provided by us)

Weekend

Day 6: Talks on different sequencing technologies. Sequence editing exercises and presentation of specimen's data spreadsheets. We will provide sequences that have been previously generated.

Day 7: Introduction to BOLD platform, properties for uploading and downloading data, searching, filtering and aggregating BOLD data

Day 8: Analyses on BOLD platform: BINS, NJ trees, genetic distances, etc.

Day 9: Analyses external to BOLD

Day 10: Discussion on next steps to reach the goal of characterizing genetic diversity for threatened species and implementation of DNA routines to fight illegal trade and identification of invasive species. Workshop feedback.

Project Logistics

- Communication – course will be advertised on the Humboldt Institute and iBOLColombia website, on Twitter and Facebook. Event coordinator will contact participants by email prior to the course and during the course. Application for each participant will be done on Google Forms. Language will be Spanish. We will have a staff in charge of photography's on the event and generation of contents and at the end of the workshops audio-visual support will be published in social networks of Humboldt and CIAT.
- Invited speakers and trainers from Humboldt will travel from Bogotá to Cali or Medellin to Cali.
- Ground transportation from main city (Cali) to CIAT will be included for facilitators, instructors and participants.
- Hotel accommodation for instructors and trainees will be included in Cali, Colombia.
- Meals arrangements will be made at CIAT for instructors and trainees to receive lunch and coffee breaks.
- Support materials with protocols, bagging and certificates will be provided to assistants.
- Laboratory materials for practical hands-on will be provided to trainees including laboratory coats.