

## ANNEX III

### APPLICATION FORM INCLUDING THE TRAINING COURSE PROPOSAL

#### **Filling the capacity gap for application of DNA technologies in taxonomy**

##### **Section 1 - Background on the Training Proposal**

###### **Project Title**

Training for the application of DNA techniques to enable strategic implementation of the National Biodiversity Strategies and Action Plans in Bhutan.

###### **Preamble**

The training as described in this proposal will help Bhutan to start monitoring the biological quality of freshwater ecosystems and enable Bhutan to determine the presence of potential harmful alien fish. Furthermore, Bhutan is in the process of completely banning the use of pesticides and this training will help Bhutan to achieve its goal of becoming the first country in the world free of pesticides by enabling Bhutanese institutes to quickly identify (potential) agricultural pest species with special focus on grasshoppers, allowing to plan for controlling their population before they become a nuisance. The NBC has a good collection of orchids and the training will be used to make a start with barcoding Bhutanese species of orchids, a group which is used as key indicator for areas of high biodiversity value. The project is in line with the following national and international strategies:

From the National Biodiversity Strategies and Action Plan the training helps to address the National Targets listed below. Between brackets the institutes working on these subjects are listed, all of these will participate in the training.

Target 5 Mapping high biodiversity value area: the distribution of orchids is used as an indicator for areas of high biodiversity concern. DNA barcoding will facilitate correct identification even when specimens are not flowering. [NBC, RSPN]

Target 6 Baseline data development for key aquatic biodiversity: information on freshwater biodiversity in Bhutan is scarce and this lack of information is considered a key problem in the conservation of freshwater ecosystems in Bhutan. The training will make a start with a reference specimens library and a DNA library for freshwater diversity in Bhutan. The training will be used to establish a lasting cooperation between various partners in Bhutan on further completing the reference library [NBC, Uwice, CNR]

Target 7 Conservation of agriculture and forestry areas: Quick and proper identification of pest species is essential for Bhutan in the quest to become the first country free of pesticides. The reference libraries for specimens and DNA Barcodes and the related trainings will contribute to this [NBC, NPPC, Sherubtse college]

Target 8 Pollution monitoring : Bhutan aims to install a biological monitoring scheme for the quality of freshwater. The development of such a tool is hampered by the lack of proper identification tools for freshwater species. This training will make a start with reference libraries of specimens and DNA Barcodes for freshwater invertebrates and fish. [NBC, Uwice, CNR]

Target 9 Invasive species: some rivers of Bhutan are being populated by invasive alien fish. Identification of these species will be facilitated by establishing a reference collection of fishes. These vouchered specimens will provide DNA samples to develop

DNA Barcode libraries which may be used in the future for detection and monitoring of these invasive fish species with eDNA. [NBC, Uwice, CNR]

Target 12 Conservation of prioritized species: A large portion of the population of the critically endangered White Bellied Heron is restricted to Bhutan. Projects are being developed separately from the training described here. The training contributes to provide a useful method to study the diet of the endangered Heron. [NBC, RSPN]

The training furthermore contributes to Aichi Biodiversity Target 19 (knowledge, the science base and technologies relating to biodiversity are improved, widely shared and transferred, and applied) and through this to Aichi Biodiversity Targets 5, 6, 9, 11, 12 and 14.

The training increases the capacity of Bhutan regarding applied taxonomy and in line with decisions 5, 6 and 7 of the GTI Capacity Building Strategy.

One of the resolutions agreed upon during the National Water Symposium in 2017 stresses that “Research in the areas of water resources is still in its infancy in Bhutan and there is a need to strengthen research in terms of water and aquatic biodiversity.” The training provides opportunity for Bhutanese institutes to take strategic approach to undertake research that supports national biodiversity conservation.

### **Project Outline**

The training will be used to train ten staff members of six Bhutanese institutes which are involved in relevant researches to biodiversity and agriculture. The trainees will receive training on applying DNA techniques for species identification and how to contribute to the Bhutanese barcoding library and how to employ DNA barcoding in their conservation activities. The training will include (1) fieldwork (collecting and proper labelling), (2) the proper storage of voucher specimens and maintenance and curation of collections, (3) the lab work involved in development of DNA barcodes library and uploading the information to BOLD system (including proper photographs) to share the information, globally, (4) the use of basic DNA techniques for identification and how to apply these to address research or applied questions. During the field training we aim to collect 200 samples of about 100 species of which we aim to process 150 to the stage of PCR products. The specimens will be stored in the national zoological collection of Bhutan at the NBC. This will allow the trainees to get hands on experience with the process of creating DNA barcodes. The project is part of a long-lasting cooperation between several Bhutanese institutes coordinated by the National Biodiversity Center, Bhutan and Naturalis Biodiversity Center, the Netherlands. This cooperation is aimed at increasing the capacity of Bhutan to study and safeguard its biodiversity and has a special focus on invertebrates. The workshop will help Bhutan to develop biological freshwater monitoring important for conservation of freshwater habitats and will facilitate quick identification of agricultural pest species which is important in Bhutan’s endeavour to become free of pesticides.

### **Post-Project Follow-up Activities**

In the period 2018-2021 the staff members of the six Bhutanese institutes will cooperate in applying DNA techniques for biological freshwater monitoring and for the identification of agricultural pest species. Throughout the period 2018-2021 the parties will cooperate on establishing an extensive DNA library of fishes (some of which are invasive aliens), freshwater invertebrates and agricultural pest species

because at the moment very few species of relevance to Bhutan have been barcoded and without a reference library it is difficult to implement DNA barcoding for monitoring. Therefore, two groups of species have been identified for which DNA barcoding will be of use. The funding from the CBD training call will be used to ensure that each of the participating institutes has one or two staff members able to apply DNA techniques for solving species identification problems and able to contribute to DNA barcoding libraries linked to voucher collections at NBC. For the remainder of the project we aim to secure funding from WWF Bhutan (Freshwater monitoring) and from the Bhutan Trust Fund (agricultural pest) who both have expressed their interest to support such a project. The 2018-training will be organized in such a way that at least two Bhutanese experts will have a Dutch counterpart (depending on the level of expertise, a lab technician, taxonomist or collection manager) that can provide further help. These teams will continue working on expanding the DNA library after the 2018 training is finished, aiming to have a reasonably complete DNA library for freshwater fish and invertebrates known to occur in Bhutan until 2021, so that by the end of 2021, Bhutan will be able to implement DNA barcoding for monitoring of at least freshwater biodiversity and agricultural pest species using its own DNA library. For this, a larger entomological expedition to be held in spring 2019, largely to be funded with Dutch resources, is already in preparation.

## **Section 2 - Logic Model**

### **Project Objectives**

1. Create expertise among ten teachers/researchers of NBC, Uwice, NPPC, RSPN, Sherubtse college and CNR in collecting and registering specimens from the field with a focus on priority species for conservation of aquatic biodiversity and agricultural concerns (pests) and for the purpose of training on preparation of plant specimens some samples of orchids are included;
2. Strengthen the capacity in Bhutan (primarily at NBC) to build and expand a DNA library of freshwater fauna and agricultural invertebrate pest species;
3. Increase the availability of knowledge to maintain a voucher collection in Bhutan (primarily at NBC);
4. Make a start with a DNA library for species of relevance to society (freshwater fauna, agricultural pests, orchids); and
5. Create a long-lasting exchange of expertise between the molecular labs of NBC Bhutan and Naturalis, the Netherlands for the purpose of application of DNA technologies for conservation.

### **Expected Project Outcomes**

1. Provide the GTI standardized training for 10 staff members of NBC, Uwice, NPPC, Sherubtse, RSPN and CNR in collecting, labelling, registering specimens, preserving vouchers, molecular processing (DNA extraction, amplification of barcode regions, check of amplification success), the use of the BOLD systems and some other software tools (CodonCode Aligner for sequence editing, MEGA for data analysis) The training will cover priority species of Bhutan for conservation purposes.

2. Two staff members of NBC receive additional training in the maintenance of the lab facilities and in setting up and running an efficient pipeline for DNA barcoding
3. Two staff members of NBC receive additional training regarding the maintenance of the national zoological collection at the NBC (the material will remain in Bhutan).
4. A DNA library of 100 priority species of freshwater animals, agricultural pest and orchids is available.
5. Improved access to new methodology for the molecular lab of the NBC.

### **Performance Indicators**

1. During the workshop at least 200 samples are collected, labelled, correctly preserved and registered by the trainees.
2. A lab training protocol is written in accordance with the GTI standardised DNA Barcoding training procedures. It will describe the procedures to be followed when material arrives from the field at the lab and all steps required to obtain DNA barcodes
3. A manual on establishing and maintaining specimen collections is written by the collection staff on how fieldworkers should label and preserve the material submitted to the voucher collection
4. About 150 samples (about 100 species freshwater fauna, agricultural pest, orchids) are processed to the stage of PCR product ready for sequencing.
5. An agreement on further cooperation of the molecular labs of NBC and Naturalis becomes part of an updated MoU between NBC and Naturalis in order to sustain the outcomes of the training for long-term collaboration in application of the DNA technologies for conservation in Bhutan.

### **Section 3 - List of Applicants and Facilitators**

#### **Lead Applicant**

<b>Name</b>	Kencho Dorji
<b>Institution</b>	National biodiversity Center, Ministry of Agriculture and Forests
<b>Address</b>	Serbithang, Thimphu, PO Box # 875
<b>Work phone</b>	+97517634846
<b>Email</b>	kencho.185@gmail.com
<b>Country</b>	Bhutan

### Co-Applicants

Name	Institution	Email
Vincent Kalkman	Naturalis Biodiversity Center	vincent.kalkman@naturalis.nl
Tashi Yangzome Dorji	National Biodiversity Center, Ministry of Agriculture and Forests	tyangzome@moaf.gov.bt
Sangay Dema	National Biodiversity Center, Ministry of Agriculture and Forests	sangaydema@moaf.gov.bt
Dhan Gurung	College of Natural Resources	<a href="mailto:chokig@gmail.com">chokig@gmail.com</a>
Norbu Wangdi	Department of Forests and Park Services of Bhutan (UWICE)	nwangdi@uwice.gov.bt

### Team Members

Name	Institution	Primary Role (Select from Drop down)
Choki Gyeltshen	National biodiversity Center, Ministry of Agriculture and Forests	Support staff

### Training Instructors

Name	Are of Expertise	Instruction topic
Dr. Vincent Kalkman (Naturalis)	Molecular techniques and conservation/DNA barcoding Odonata	Outline of a monitoring scheme of biological water quality based on molecular techniques
Dr. Oscar Vorst (Naturalis)	Data management	Correct procedures for registration of voucher and DNA material; Lab training: DNA extraction and PCR amplification
Berry van Hoorn (Naturalis)	Freshwater biodiversity	Application of DNA techniques for conservation planning
Dr. Barbara Gravendeel (Naturalis)	Genomics & museomics	Applied research using DNA techniques
Dr. Luc Willemse (Naturalis)	Head Entomological collection Naturalis; specialist pest Orthoptera of the Indo-Malayan region	Collection maintenance and registration
Dhan Gurung (CNR)	DNA barcoding fishes	Building a DNA library of fishes
Kencho Dorji (NBC)	DNA barcoding	Lab training: DNA extraction and PCR amplification
Tshewang (NBC)	Lab Technician	Lab equipment operation and reagent preparation

## **Section 4 - Background Information on Facilitators and Participants**

### **Background of Applicants and Instructors**

The lead applicant Mr. Kencho Dorji has acquired a month-long DNA barcoding training from the Biodiversity Institute of Ontario, Canada in 2015 and he is the trained trainer of DNA barcoding techniques in Bhutan. In the next few years he will coordinate the work on DNA barcoding in Bhutan. Two of the team members of Naturalis have extensive experience with DNA lab-work and will assist him in the lab-training (Vorst, Gravendeel). The other instructors from Naturalis have extensive experience with collection management (Willemse), the application of molecular techniques for freshwater monitoring (Dijkstra, Kalkman) or the application of DNA-techniques for applied research (Gravendeel). All instructors from Naturalis have extensive experience with the proper labelling of material (vouchers and DNA), with registration of collected material and with the production of proper photographs. In the past few years Naturalis contributed over 50,000 sequences to BOLD. The institute has extensive experience with barcoding of a wide range of taxa and is one of the leading institutes regarding the application of eDNA. Dhan Gurung is a fish taxonomist and Dean of the Science Department of the CNR, as such he has a good overview of the needs and possibilities of applying DNA techniques in Bhutan.

### **Partners and Stakeholders**

The organising partners are: National Biodiversity Center (NBC), Ugyen Wangchuk Institute of Conservation and Environmental Research (UWICE), College of Natural Resources (CNR), Naturalis. NBC: see information under hosting institution. UWICER is a Government based research and training institute. It strives to foster better stewardship of the natural heritage – land, water, air and species therein – through rigorous science-based research and transfer of cutting-edge science results to field practitioners, environmental leaders and policy makers. The CNR is a college under the Royal University of Bhutan that serves as a centre that generates knowledge through research in agriculture, natural resources management and rural development. Naturalis is among the largest natural history museums in the world with a staff of over 120 scientists and housing over 30 million specimens. The institute has a large DNA lab and is actively involved in applied research in the field of biological monitoring. In the past few years Naturalis contributed over 50,000 sequences to BOLD. The institute has extensive experience with barcoding life from the fieldwork to the actual upload of sequences to BOLD. In addition, Naturalis has been involved in the training of Bhutanese counterparts for the past few years and is dedicated to support DNA barcoding work in Bhutan in the coming years. Other stakeholders are the Royal Society for Protection of Nature (RSPN), Sherubtse college, WWF Bhutan, National Environment Commission Secretariat (NECS) and the National Plant Protection Center (NPPC).

### **Trainees**

All trainees are from Bhutan and represent six institutes which are housed in different parts of Bhutan. The two persons attending from NBC are responsible for the DNA lab and the voucher collection. The eight persons of UWICE, CNR, NECS, Sherubtse, and the RSPN are field workers and/or taxonomical experts. The six institutes are currently cooperating on a larger programme focussed on establishing a biological water quality monitoring scheme for Bhutan and will remain in contact after the training (they keep in contact by using e-mail and skype). In addition, they are likely to participate in

entomological fieldwork scheduled for spring 2019 where they will continue their work on DNA-barcoding.

### **Trainee Selection Criteria**

The trainees are selected based on their current work and their involvement in biodiversity conservation and NBSAPs implementation in Bhutan. They include teachers/researchers from the two main colleges of Bhutan (CNR, Sherubtse), science officers from RSPN, NECS and from the NPPC, fieldworkers from UWICER and staff from the NBC. Most of the selected trainees will be involved in a large project between NBC Bhutan and Naturalis focussed on monitoring and conservation of freshwater biodiversity and invertebrates of agricultural relevance. Gender balance in participation will be considered, as appropriate.

### **Expected Number of Trainees**

We will provide the GTI standardized DNA Barcoding training for 10 staff members of NBC, NPPC, CNR, UWICER, RSPN and Sherubtse College, NECS (including one lab technician, one collection manager and eight researchers of the different institutes).

## **Section 5 - Training Venue and Logistics**

### **Hosting Institution**

The National Biodiversity Center (NBC) under the Ministry of Agriculture and Forests is mandated to implement the National Biodiversity Strategies and Action plan of Bhutan, 2014 to achieve relevant targets by 2020. The Center has a molecular lab with basic facilities for performing DNA extraction and PCR amplification that would accommodate a maximum of six trainees. The equipment in the lab are: one Gradient Thermal Cycler PCR, a gel analyser, two centrifuges, a biosafety cabinet, two PAGE electrophoresis units and one submarine electrophoresis unit and several deep freezers for storing tissue and DNA samples. There are two technicians working full time with the molecular analysis of domestic animal germplasm of Bhutan using non-CTAB extraction of DNA, and one of them will be available as an instructor and facilitator during the training period. The Center also has a lecture room for taking any other classes related to the training. During the training period these facilities and resources will be made available to the trainees. NBC is the only institution in the country with basic molecular laboratory facility and it provides molecular related services to all the partner institutions within the country. Further, it has storage facility for both voucher specimens and DNA samples for future use with provision for further expansion. It collaborates with several academic and research institutions in the country as well as few international institutions in conducting molecular studies of Bhutanese biodiversity. It aims to build DNA barcode reference library for some of the selected biodiversity groups with support from the partner institutions for implementing DNA barcoding as a monitoring tool for conservation and sustainable food and agriculture.

### **Training Venue**

Lectures on DNA barcoding workflow will be given at the Center's lecture room using PowerPoint presentations and training manuals. The trainees will be taken in the field where there are agriculture fields and water bodies for collection of specimens. They will be provided with specimen collection kit, data recording forms and GPS. The data on the collected specimens will be aggregated in a BOLD compliant format. Imaging of specimens will be done in the field using DSLR camera. Hands-on tissue sampling of

the specimens collected from the field will be done in the lab. The trainees will be trained in preparation of reagents for molecular processing of plants and animals (buffers for DNA extraction, primer dilution, PCR mixes, agarose gels, ). DNA extraction of only a part (150 specimens) of the entire collection (200 specimens??) resulted from fieldwork during training will be done at the NBC for the training purpose and tissue samples of the unprocessed specimens will be sent to Naturalis for extraction and sequencing as an in-kind support from the Naturalis to NBC. Voucher specimens of both animals and plants will be stored in Bhutan (NBC). The trainees will have to prepare agarose gel for electrophoresis and will determine the presence of PCR product. They will be given hands-on operation of all the available equipment in the lab. The bioinformatics training for the trainees will be given using DNA sequences of biological material previously collected in Bhutan. The trainees will also be introduced to BOLD systems and some other relevant software tools (CodonCode Aligner and MEGA) used in barcoding. The extracted DNA samples will be divided into two sets and one set will be taken to the Naturalis Biodiversity Center for sequencing and the other set of DNA extract will be stored at the NBC. One of the DNA barcoding experts from the Naturalis will be available during the lab work in addition to the GTI trained trainer and one of the lab technicians of the Center.

### **Training Activities**

**Saturday 20 October:** arrival, arranging visa, meeting with NBC staff

**Sunday 21 October:** preparation of workshop, discuss the procedures used and agree on the format used for the registration of vouchers and DNA-samples and fine tune the manual for fieldworkers.

**Monday 22 October:** Lectures on DNA barcoding, DNA-techniques, biological water monitoring, preservation of collection material and registration. Lectures presented by Kencho Dorji (present & future molecular work in Bhutan), Oscar Vorst (molecular work flow from field to upload in BOLD), Berry van Hoorn (overview of possible application of DNA-barcoding), Luc Willemse (collection management and DNA-barcoding). In addition, the CBD National Focal Point and the WWF project manager on freshwater will be invited to present their views on how DNA-barcoding should contribute to the implementation of the NBSAP in Bhutan. Participants will be asked to give a presentation on how they will use DNA-barcoding in their work in 2018-2020. Discussions on the Nagoya protocol and its implications for DNA barcoding will be included.

**Tuesday 23 – Wednesday 24 October:** Fieldwork (collecting and labeling of material and when possible taking photographs)

**Thursday 25 October – Saturday 27 October:** Split into two groups. Group 1 continues fieldwork. Group 2 starts with lab work.

**Sunday 28 October – Tuesday 30 October:** Group 2 resumes fieldwork. Group 1 starts with lab work.

**Wednesday 31 October – Friday 2 November:** Training in CodonCode Aligner, MEGA software and BOLD system. The Friday will be used for a wrap-up session during which the training is reviewed and to develop a plan for future cooperation among Bhutanese institutes trained and Naturalis, regarding application of DNA technologies, specimen management and contribution of training outcomes to conservation of



nature in Bhutan The outcome of the wrap-up session will be incorporated in the report of the training in details.

### **Project Logistics**

Bhutan is a small country with limited resources. For this reason, we do not aim at training a large number of persons but instead focus on a tailor-made training for a small number of persons of which we know that they will remain active in the field of biodiversity and conservation. The total group will consist of ten persons from the NBC, Uwice, NPPC, RSPN, Sherubtse and CNR and five persons from Naturalis. The training will take place at the NBC in Thimphu where we can use the lecture room and the DNA-lab. The DNA-lab is small for which reason the practical part of the lab-work will be split into two sessions of three days. NBC will arrange food and lodging (in Thimphu). The fieldwork will take place in the vicinity of Thimphu and Paro. For the transport from the city to the research station and for the field work we will need to hire cars. Thimphu is easily accessible from the nearby airport of Paro (Naturalis will cover the international flights). The four organizing institutes are already closely cooperating and communication will be straightforward.