



EUROPEAN UNION OF AQUARIUM CURATORS

REPORTING FORM

FOR *IN SITU* CONSERVATION PROJECTS FUNDED

2021

1 TITLE OF PROJECT

Fish Net Madagascar- Using environmental DNA metabarcoding to assess presence/absence and the distribution of critically endangered fish species

2 NAME OF APPLICANT

Charles Fusari¹/ Brian Zimmerman²/Quentin Mauvisseau³/Tsilavina Ravelomanana⁴

INSTITUTION

¹Aquarium tropical du palais de la Porte Dorée

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³Oslo Natural History Museum

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DATE OF REPORT:

01/12/2022

PLEASE SEND YOUR REPORT TO ISABEL KOCH, SECRETARY-GENERAL OF EUAC

(ISABEL.KOCH@WILHELMA.DE)

AND COPY TO

João Falcato : jfalcato@oceanario.pt; Brian Zimmerman: bzimmerman@bzsociety.org.uk

3 LOCATION OF PROJECT (REGION & COUNTRY)

Madagascar, Marotandrano, Mandritsara district

4 PROJECT START AND END DATES:

Project started in September 2021

5 PROJECT CO-ORDINATOR, ADDRESS AND INSTITUTIONAL AFFILIATION

(IF DIFFERENT FROM APPLICANT)

Charles Fusari charles.fusari@palais-portedoree.fr ; Aquarium tropical du Palais de la porte Dorée

6 PROJECT TYPE

(TICK ANY COMPONENTS THAT APPLY)

BIOLOGICAL/ECOLOGICAL RESEARCH

VETERINARY/CONSERVATION MEDICINE

ANIMAL WELFARE

CAPTIVE BREEDING

RE-INTRODUCTION/RE-STOCKING/TRANSLOCATION

HUMAN-WILDLIFE CONFLICT

EDUCATION/PUBLIC AWARENESS

TRAINING/WORKSHOPS

COMMUNITY-BASED/SOCIAL POLICY

ECOTOURISM/SUSTAINABLE DEVELOPMENT

SUSTAINABLE USE

WARDENING/LAW ENFORCEMENT

PROTECTED AREAS MANAGEMENT

OTHER: _____

7 FOCAL SPECIES (COMMON AND SCIENTIFIC NAME)

- *Rheocles derhami*
- Mangarahara Cichlid (Joba mena) - *Ptychochromis insolitus*
- Lamena – *Paretroplus nourissati*
- Melemsiska – *Paretroplus gymnopreopercularis*
- Killifish - *Pachypanchax sp. Sofia*
- Vily – *Sauvagella robusta*

8 IUCN RED LIST STATUS (OR OTHER THREAT LISTING) OF FOCAL SPECIES

- *Rheocles derhami* **Critically endangered**
- *Ptychochromis insolitus* **Critically endangered**
- *Paretroplus nourissati* **Endangered**
- *Paretroplus gymnopreopercularis* **Critically endangered**
- *Pachypanchax sp. Sofia* **not evaluated**
- *Sauvagella robusta* **Endangered**

CITES YES **NO**

APPENDIX

9 PROJECT BACKGROUND

This project is part of the Fish Net Madagascar programme which focuses on the Amboaboia River basin, an important Key Biodiversity Area and the last known watershed for *Ptychochromis insolitus*, *Paretroplus gymnopreopercularis* and *Rheocles derhami*, making the catchment a critical habitat and priority for conservation efforts. Since 2013, only one *Rheocles derhami* specimen has been observed (caught) despite the subsequent expeditions in 2014, 2016, 2017 and 2018 which represent a total of 32 sampling sessions along the river. This Critically Endangered species endemic to the Amboaboia River has no *ex-situ* population established within the trade industry or zoological institutions which makes it at high risk of extinction. The traditional sampling methods used until now were unsuccessful to establish an estimation of its area of occupancy and validate its presence in the river. This current project aims to investigate the use of molecular-based detection as a reliable monitoring tool. By detecting the DNA traces left by organisms in the system, this non-invasive method will allow an efficient assessment of the fish species present in low abundance. The efficiency of this monitoring method in a similar case was further demonstrated by a recent study conducted in Greece in collaboration with ZSL, HCRM and the University of Derby.

10 WAS THE OVERALL PROJECT PURPOSE FULFILLED?

Yes, A population of *Rheocles derhami* has found in the Amboaboia River with more than 200 individuals sampled in 2 different locations

11 WHAT OBJECTIVES WERE MET?

All *Rheocles derhami* sampled in the Amboaboa river were caught using traditional sampling method (mosquito net) by locals.

Another population morphologically identical to *R. derhami* but genetically different has been discovered in a nearby river.

Another new species of *Rheocles* has been discovered in a river flowing east from the Marotandrano reserve.

WHAT OBJECTIVES WERE NOT MET?

none

12 WHAT PROJECT ACTIVITIES WERE UNDERTAKEN?

In total, 14 sampling stations were visited. They concern four rivers, three of which are tributaries of the Sofia, namely: the Amboaboa River, the Anjombony River, the Manambendrana River and the Manampaneva River, which are both tributaries of the Anjombony River. Finally, the fourth river is called Vakoany, which is a tributary of the Simianona River to the east of the Marotandrano Reserve.

At each location, a representative water sample was collected and then filtered using an eDNA sampling kit. Traditional monitoring of the fish population using various local fishing techniques occurred after eDNA sampling to avoid any cross contamination between sites from the fishing gears. Fin-clips from each different fish species collected along the sampled location were collected to build a genetic reference database allowing the identification of fish species detected in the metabarcoding analysis. Reference database building was performed at the Natural History Museum in Oslo following a dedicated metabarcoding analysis pipeline. eDNA collected at each location will be sequenced and these sequences will be compared to the reference database built at the early stage in this project. This will allow identification of the full fish community present at each location and compare the efficiency between both eDNA and traditional monitoring.

13 WHAT OUTCOMES WERE ACHIEVED DURING THE COURSE OF THE PROJECT?

Discovery of healthy populations of *Rheocles derhami* in the Amboaboa River.

Discovery of a 2 potential new species of *Rheocles*

ARE ANY ONGOING?

No

DID ANY EXPECTED OUTCOMES FAIL?

No

14 DID LOCAL PEOPLE/COMMUNITIES PARTICIPATE IN THE PROJECT? IF SO, HOW MANY AND WILL CONTINUED CONTACT BE MADE?

2 local park rangers from Madagascar National Parks took part in the field work by doing water quality analysis. Local Fishermen/women also participated in the sampling sessions. Contact is maintained with them for future field work

15 DID THE GOVERNMENT OF THE HOST COUNTRY RECEIVE INFORMATION ON THE PROJECT'S RESULTS?

Not yet

16 HOW DID THE RELATIONSHIP WITH OTHER NGOS WORK? WERE THERE ANY ISSUES?

The relation were good with all NGOs and stakeholders. No issues

17 TOTAL PROJECT BUDGET AND EXPENDITURE (IN EUROS)

18 AMOUNT OF MATCHING FUNDS

SPENT:

- Project manager time - 3,500€
- eDNA researcher time – 2,700€
- Lab renting (bench fees) – 800 €

19 AMOUNT SPENT FROM EUAC FUNDS:

8368€ (everything)

20 EXPENDITURE BREAKDOWN (IN EUROS)

Field work expenses	Fieldwork &travel	Per diem	4857
	admin	admin and data analysis before and after field work	
	Travel	Gasoil vehicle hire	
	Travel	Taxi fees in Tana	
Lab work	Equipment	Kit control negative (+ analyse)	30€
	Lab work	eDNA extraction	360€
	Lab work	PCRs and sequencing prep for all eDNA samples	500€
	Lab work	Sequencing MiSeq (Metabarcoding analysis)	2500€
sp ID confirmation	Lab work	Tissues DNA extraction	432€
	Lab work	PCR	216€
	Lab work	Barcoding sequencing	600€
	Shipping sampling cost		325
Total			9820

21 PUBLICATIONS PRODUCED AS A RESULT OF THE PROJECT

2 publications should be produced in 2023.
