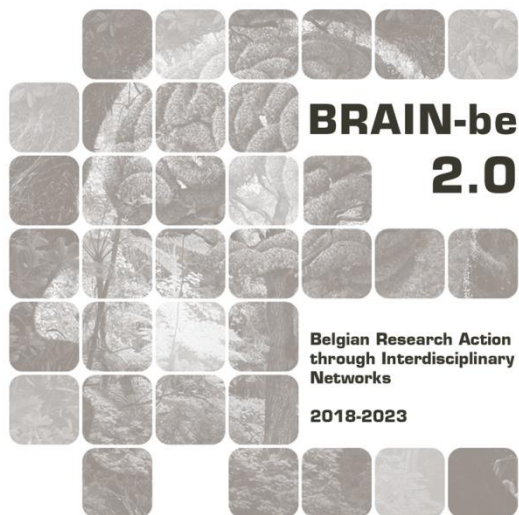


## **BIODIV-AFREID-Top-up**

### **Biodiversity changes in African forests and Emerging Infectious Diseases: should we worry? Top-up**

ERIK VERHEYEN (Institute of Natural Sciences) – SOPHIE GRYSSELS (Institute of Natural Sciences)

Pillar 1: Challenges and knowledge of the living and non-living world



NETWORK PROJECT

## **BIODIV-AFREID-Top-up**

**Biodiversity changes in African forests and Emerging Infectious Diseases: should we worry? Top-up**

**Contract - B2/191/P1/BIODIV-AFREID-Top-up**

## **FINAL REPORT**

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## **1. INTRODUCTION**

This project extension was funded to extend the original project application to study the possible emergence of human coronaviruses (CoV). The original BIODIV-AFREID project proposed to investigate how biodiversity conditions (dis)promote the transmission of infectious agents from small mammals to human populations in African forests (in DR Congo and Côte d'Ivoire). The project originally focused on Ebola and Monkeypox viruses, but exploratory studies on other virus groups were also already part of the original project: arenaviruses, coronaviruses and hantaviruses. With the provided additional support, BIODIV-AFREID top-up was enabled to expand its efforts to target coronaviruses in its screening.

## **2. STATE OF THE ART AND OBJECTIVES**

The objective of this project was to expand the zoonotic virus surveillance effort of the original project to include a comprehensive testing of newly collected samples (from the fieldwork incorporated of the original project) for the presence of coronaviruses, as well as of an extensive collection of suitable archived samples collected during the past decades via previous projects from a larger geographic range within Africa. In this context it was the RBINS' role to assist the BIODIV-AFREID project partners by making available a larger number of samples from the existing tissue collections from a wider geographic area than foreseen in the initial project application. These samples were to be provided as subsamples to the other members of the BIODIV-AFREID team after consultation and in mutual agreement with all consortium partners, and in function of their respective expertise.

### 3. METHODOLOGY

The contribution for partner RBINS in this BioDiv-AFREID Top-up project consisted of appointing personnel for 7 months to conduct the following tasks:

1. Selecting and sorting of existing tissue collections according to criteria to be agreed upon when the project starts (such as the preservation methods, the targeted taxa and their geographical origin).
2. Taking subsamples of the selected samples in separately labelled vials.
3. Distribution of the vials among the partners responsible for their analyses.

A new staff member was hired to carry out these tasks. The vacancy notice (NL/FR/EN) was published online on 29 November 2021 on the RBINS website. The closing date for sending in applications was 11 December 2021 (midnight). The following competences were requested in the vacancy:

- Master's degree in sciences, biology, genomics, biotechnology
- Experience maximum 3 years after obtaining your university degree
- Experience working in a molecular genetics laboratory
- Experience processing and reporting DNA data in GenBank

We received 12 applications, 2 of which were inadmissible on the basis of either excessive seniority (>3 years elapsed after obtaining the MSc degree) or were overqualified (because they held a PhD). We screened the remaining candidates based on their CVs to draw up a shortlist who would be invited for an interview. The judges were Erik Verheyen (RBINS; coordinator of the Biodiv-Afreid project for RBINS), Nicolas Laurent (RBINS scientist recruited on the Biodiv-Afreid project), Sophie Gryseels (half-time staff Fed-tWIN scientist at KBIN and UAntwerpen), and Léa Joffrin (postdoctoral researcher on the Biodiv-Afreid project at UAntwerpen). After compiling the individual evaluations of the judges, a shortlist of the five candidates who were invited for an interview was drawn up: Cooreman Tine, Van Craenenbroeck Lore, Snoeks Jante and Chkiri Ismaël.

The online interviews were organised on 15 December 2021 and lasted about 20 minutes per candidate. They were interviewed by Erik Verheyen, Léa Joffrin, Nicolas Laurent and Sophie Gryseels. Based on the analysis of the CVs and interviews, the jury concludes that Tine Cooreman is narrowly the most suitable candidate for this vacancy. She obtained her MSc degree from the University of Antwerp in 2021. Based on her knowledge of lab techniques related to DNA/RNA extraction, she was ranked almost ex-aequo with the others. But since, in the context of the short period that her recruitment concerns, and the fact that laboratory work is difficult to start in times of covid, she was chosen because she has already worked in the sister laboratory (UAntwerpen). As a result, we judge, we can save a lot of time with the activities that need to be learnt/performed in a short period of time, because she is already familiar with many of these procedures. The three other candidates have equivalent CVs, but for the sake of efficiency in these difficult times (as argued above) they seem just a little less suitable to recruit them for this project.

Tine Cooreman started the position 1/02/2022 and continued until 31/08/2022.

In agreement with the partners that task for the RBINS was redefined as follows:

- 1) To sort out, inventorize and aliquot all the following earmarked samples of the existing collections at RBINS and UAntwerpen:
  - a) For DNA/RNA extraction and PCR-based virus screening at UAntwerp (coordinator BioDiv-AFREID):
    - i) All small mammal tissue samples from Yangambi, DR. Congo (2012-2015): 1042 samples;
    - ii) Frozen tissue samples from various locations in Rwanda (1984): 34 samples
    - iii) Frozen tissue samples from Kikwit, DRC (1995): 224 samples
- 2) For serological screening at IRD, Montpellier (partner BioDiv-AFREID):
  - a) All dried blood samples collected during the first field campaign of the Biodiv-AFREID project in Inkanamongo, DRC (2021): 1355 samples;

- 3) To carry out the initial laboratory work for the 1042 samples from Yangambi, DRC (collection 2012-2015):
  - a) RNA extractions (N=516) - two individuals were pooled by genus (and if possible by locality)
  - b) Reverse transcription (cDNA)
  - c) Filovirus PCR
- 4) Travel to Institut National de Recherche Biomédical, Kinshasa, DRC, for two months to coordinate the processing of small mammal specimens collected in Likati, DR. Congo (2017) and stored at Institut National Recherche Biomedical (INRB), Kinshasa, DR Congo: 230 rectal swabs; 1200 organ samples. During two months, Tine helped sorting out the samples, trained INRB researchers and participated in the laboratory work (RNA and DNA extraction, PCRs to detect coronaviruses, paramyxoviruses, filoviruses, hepaciviruses, mpox). The lab work is currently further ongoing at INRB with support from BioDiv-AFREID partner IRD (Martine Peeters).
- 5) Making of a digital inventory all dried blood samples kept as frozen filter paper from mammals from different African countries in the collections of RBINS and UAntwerp: 304 envelopes of dried blood samples containing samples of >5000 dried specimens.
- 6) Writing a publication of coronavirus surveillance of zoo animals. This work was part of the BIODIV-AFREID original and Top-up project of coordinator H. Leirs (UAntwerp). As RBINS staff T. Cooreman conducted much of the laboratory work for this study during her Master's thesis, she also worked on finalizing the manuscript.



#### **4. SCIENTIFIC RESULTS AND RECOMMENDATIONS**

The nature of the work at RBINS for this top-up was to make mammal samples available for further research by the other partners of the BioDiv-Afreid project and by INRB at Kinshasa, DRC. About 2500 samples were curated and aliquoted and shared with partners, and for ~1500 samples RNA was already extracted. The laboratory work by the other partners is still ongoing. It has a.o. led to the detection of several paramyxoviruses and a novel lineage of shrew coronaviruses (likely representing a new genus). The analyses and publication of the generated results are (according to their areas of expertise) underway under the lead of the UAntwerpen (genetic virus detection) en IRD Montpellier (indirect serological virus detection).

The surveillance study of mammals in the Antwerp and Plankendael zoo was published in *Veterinary Sciences*.

## 5. DISSEMINATION AND VALORISATION

A total of 4075 archival tissue samples, originally collected for different purposes throughout the past decades and since stored at RBINS and UAntwerp, were re-valorised to be used for zoonotic pathogen screening.

We disseminated BIODIV-AFREID project findings via:

- regular posts on twitter: <https://twitter.com/BiodivAfreid>
- making and participation of two outreach videos available on youtube:
  - o **“Expedition Inkanamongo - In search of the source of zoonoses in DR Congo”**  
[https://youtu.be/A1\\_jWaa89S0?si=ivMMmubkADTE1cL4](https://youtu.be/A1_jWaa89S0?si=ivMMmubkADTE1cL4)
  - o **“Coronaviruses in the Wild – a One Health perspective”**  
<https://youtu.be/2Qk3RBpVdoM?si=zrYekalWC6nqPL2>
- Opinion piece about the emergence and global spread of Mpox virus in newspaper De Standaard **“Van het woud naar de stad naar de rest van de wereld”**.  
[https://www.standaard.be/cnt/dmf20220523\\_97625654](https://www.standaard.be/cnt/dmf20220523_97625654)

## **6. PUBLICATIONS**

Joffrin L, Cooreman T, Verheyen E, Vercammen F, Mariën J, Leirs H, Gryseels S. (2023) SARS-CoV-2 Surveillance between 2020 and 2021 of All Mammalian Species in Two Flemish Zoos (Antwerp Zoo and Planckendael Zoo). *Veterinary Sciences*.10(6):382.

Several other publications in the pipeline.