

Federal Policy Research

Final report

BOPCO-CE

MB/21/BopCo-Ce
BARCODING FACILITY FOR ORGANISMS AND TISSUES OF POLICY CONCERN – CENTRE OF
EXCELLENCE

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Published in 2024 by the Belgian Science Policy Office
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Vanderheyden A., Smitz N., Vanden Abeele S., De Meyer M., Backeljau T. **BopCo-Ce; MB/21/BopCo-Ce**. Final Report. Brussels: Belgian Science Policy Office 2024 p.25 Federal Policy Research

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ABSTRACT

Context

BopCo, initially established as the Barcoding Facility for Organisms and Tissues of Policy Concern, has transformed into a Centre of Expertise (CE) financed by BELSPO. This report presents the project activities for the period spanning from 20th of December 2021 to 15th of February 2024.

Operated jointly by the Royal Belgian Institute of Natural Sciences (RBINS) and the Royal Museum for Central Africa (RMCA), BopCo-CE functions as a centre of expertise for identifying organisms and tissues with policy implications, addressing requests from various stakeholders.

Since its foundation, BopCo-CE has experienced substantial growth in its user base, partnerships, and expertise. This growth reflects the increasing demand for precise species identification in matters of policy, upon request of various institutions and bodies such as regional and federal governmental institutions, international organizations and the private sector. In response to this demand, BopCo-CE is actively seeking to seize new opportunities and forge collaborations at national and international level.

To ensure its sustainability as a service to governmental as well as private stakeholders alike, BopCo-CE recognizes the importance of continuously enhancing its scientific expertise and expanding its range of tools.

Objectives

Aligned with the objectives of the INFRA-FED 2022 Impulse Action call (https://www.belspo.be/belspo/IMPULS/call2022_infra_en.stm), BopCo-CE aims to enhance the scope of identification services and organisms of policy concern as is required due to the expanding user demand. Therefore, BopCo-CE hopes to become a more sustainable and accessible identification facility with a wider and more diverse portfolio for all policy makers (government, private companies, general public, universities ...). Species identification requests are typically categorized into two groups: identification requests, which involve one or a few samples provided at a single instance or at regular intervals, and project requests, which involve larger batches of samples provided over a specified period – usually several years.

Conclusions

During the period covered by this report, BopCo-CE successfully finalized two projects, and two projects are ongoing. Two topics (MEMO+ and Secretariat IAS request) aimed at enhancing barcoding databases. Additionally, the team addressed 109 identification requests from 39 distinct stakeholders. During this reporting period, 13 papers were published in scientific journals and advancements were communicated at 11 (inter)national conferences (21 poster presentations and three oral presentations). BopCo-CE also informed the general public about its ongoing work and achieved milestones via blog posts on the BopCo-CE website, a video clip (Federal Minute) broadcasted on

national tv and was uploaded to YouTube, one podcast interview (Deutschlandfunk Kultur) and participation in “Open bedrijvendag” at RMCA.

Keywords

Species Identification Service, DNA barcoding, Policy concern

1. INTRODUCTION

The Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo), jointly managed by the Royal Belgian Institute of Natural Sciences (RBINS) and the Royal Museum for Central Africa (RMCA), was established in 2015 as part of the European Research Infrastructure Consortium (ERIC) “LifeWatch”, with funding provided by the Belgian Science Policy (BELSPO) under project FR/00/BO1. This initiative aimed to meet the growing demand for accurate species identifications of biological materials relevant to policymaking. Despite the recurrent societal need of BopCo's species identification services, BELSPO decided that LifeWatch, being a predominantly virtual infrastructure, was not the most suitable framework for BopCo. Therefore, BopCo was subsequently recognized as one of three priority research initiatives in support of federal governance, as outlined in contract MB/21/BopCo-CE. This contract started on 20/12/2021 and officially ended on 15/09/2023 but was extended until 15/02/2024. The present final report describes the activities of BopCo-CE (Centre of Excellence) undertaken during this contract.

The BopCo-CE team comprises eight members (employed at RBINS or RMCA), including two promoters: Dr. Marc De Meyer and Dr. Thierry Backeljau. Yet, with this report ends the promotorship of Thierry Backeljau who retired in February 2024 and who will be replaced by Dr. Frederik Hendrickx at RBINS. The RBINS team consists of Dr. Anicée Lombal (contract ended September 2023), Dr. Samuel Vanden Abeele (contract started November 2023) and Msc. Ann Vanderheyden. Additionally, two part-time collaborators, Brigitte Segers and Karin Breugelmans, are available at RBINS to support BopCo-CE in its laboratory tasks. The RMCA team includes Dr. Nathalie Smitz, Msc. Kenny Meganck (contract ended May 2023), and Msc. Fanny Kratz (contract started October 2023).

BopCo-CE specializes in identifying a wide range of biological materials, including Invasive Alien Species (IAS), CITES-listed species, agricultural pests, disease organisms and vectors, organisms within the food chain, forensic species, quarantine organisms, and species with economic and ecological impacts. Serving as a federal focal point for identifying organisms of policy concern, BopCo-CE operates as a collaborative effort between RBINS and RMCA. This strategic alliance enables BopCo-CE to access a wealth of taxonomic expertise and infrastructure, including state-of-the-art DNA laboratories, microscopy facilities, imaging equipment, libraries, and extensive natural history collections.

Species identification based on morphology and DNA-based techniques are the core expertise of BopCo-Ce team, additionally we have access to a broad network of associated taxonomic experts to aid in species identifications. DNA-based techniques allow for rapid identification procedures, particularly in cases where morphological characteristics are insufficient. DNA barcoding, a core expertise of BopCo-CE, has become an invaluable tool for implementing international legal obligations, such as CITES regulations for protected species and EU directives on invasive species and plant health. Furthermore, BopCo-CE tends to a diverse array of stakeholders, including governmental agencies, research institutions, private sector and international organizations.

In addition to its routine identification services (viz. identification requests and projects), BopCo-CE actively contributes to enhancing reference barcode libraries and DNA barcode databases for taxa of policy concern. This involves generating new DNA barcodes in accordance with international standards and maintaining reference collections following established protocols. The generated DNA sequences are made publicly available through open-access online databases, such as the Barcode of Life Database (BOLD), to facilitate broader scientific research and biodiversity conservation efforts.

Furthermore, BopCo-CE disseminates its findings through various channels, including scientific publications, social media, mainstream media, scientific conferences, and public events, to raise awareness and promote its services among decision-makers, the scientific community, and the general public. By fulfilling its mission to provide accurate and accessible species identifications, BopCo-CE plays a crucial role in supporting evidence-based decision-making and safeguarding biodiversity and ecosystem health.

2. MOTIVATION AND OBJECTIVES OF THE PROJECT

BopCo-CE was established with a clear motivation rooted in the pressing societal need for accurate species identification of biological materials. As such, it underpins critical aspects of biodiversity conservation, ecosystem management, public health and safety. Through our concerted efforts to enhance our identification services and engage with stakeholders, we strive to uphold our mission of advancing scientific knowledge and facilitating evidence-based decision-making in biodiversity conservation and ecosystem management. The need for species identification is an ongoing and growing imperative that is indispensable, particularly given the current decline in taxonomic expertise in Belgium/Europe. BopCo-CE plays a pivotal role as a liaison, facilitating the transfer of requests to the appropriate (often hard-to-find) specialists within the network. Therefore, the transition of BopCo-CE into a permanent species identification facility, potentially as a Center of Excellence, is paramount.

3. METHODOLOGY

Aside from the described objectives addressed in a number of Work Packages (WP) as outlined below, BopCo-CE's main function is as an identification service to all stakeholders for tissues and organisms of policy concern. Species identification assignments are requested by our stakeholders are typically categorized into two groups: identification requests, which involve one or a few samples provided at a single instance or at regular intervals, and project requests, which involve larger batches of samples provided over a specified period – usually several years.

WP1: Maintenance and update of a BopCo-CE web portal

Maintaining and updating the BopCo-CE website established at the project's inception (accessible at <https://bopco.myspecies.info/>) which serves as the primary point of contact for new stakeholders and provides comprehensive details about the services and outputs of BopCo-CE. Through standardized forms, users can submit identification requests directly on the website. Additionally, visitors can access information on the various identification methodologies employed by BopCo-CE, the request handling process, ongoing activities, and the outcomes (publications) of BopCo-CE initiatives.

WP2: Provision of reference DNA sequences of organisms of policy concern, their relatives and similar taxa

Generating reference DNA sequences with documented identity and source, utilizing voucher specimens stored in the RBINS and RMCA collections, and subsequently depositing the corresponding DNA sequences into the GenBank and BOLD DNA sequence databases. Furthermore, in conjunction with the specimen collections, retaining specific DNA extracts for potential future comparisons may be essential.

WP3: Identifying gaps in the reference databases of organisms of policy concern

This work package seeks to identify and analyse deficiencies in reference identification data for specific organism groups. It involves creating data sheets and lists for targeted organism categories of policy significance (such as invasive species on the EU list), detailing existing reference DNA barcodes, identifying taxonomic gaps in these barcodes, and providing information on the potential utility of current DNA barcodes for identification purposes.

WP4: Data management

It is imperative to protect all data produced by BopCo-CE to facilitate convenient retrieval, cross-referencing between DNA data and physical specimens, and unrestricted public access. This work package will transfer BopCo-CE data, encompassing both information directly associated with voucher specimens and/or tissues (including DNA samples) and the resultant DNA data, to the DaRWIn data management system at RBINS and RMCA (or other pertinent public repositories). Additionally, BopCo-CE will disseminate these generated DNA sequence data through the international online genetic databases (GenBank and BOLD).

WP5: Extending DNA identifications by additional expertise

Organisms and tissues of unknown identity and origin are routinely sequenced by many DNA facilities, yet the accurate interpretation of sequence data often necessitates taxonomic expertise. Hence, it is vital for BopCo-CE to serve as a central hub, where identification requests are either handled internally by BopCo-CE experts or, in cases where specific taxonomic knowledge is lacking, referred to colleagues or facilities equipped with the required expertise. BopCo-CE leverages internal taxonomic proficiency at RBINS and RMCA for tasks like morphology-based identifications and tailored client information provision. Furthermore, through Memorandums of Understanding (MoUs) and collaborative arrangements, BopCo-CE collaborates with partners such as NICC, BCCM, BGM, etc., to meet clients' diverse needs.

WP6: Development of overarching Centre of Excellence for Species Identification Services

The need for species identifications is not time limited. On the contrary, reliable species identification is a permanent and ever-expanding requirement that is essential. Hence, the evolution of BopCo-CE into a permanent species identification facility, potentially as a Center of Excellence, is paramount. WP6 endeavours to explore and actualize this notion, transforming BopCo-CE into a CE. In this framework, WPs 2, 3, and 5 will facilitate the assessment of stakeholder needs, existing tools, gaps, and available expertise within and beyond partner institutions. These evaluations will form the groundwork for the CE, mapping needs and services and fostering synergies and opportunities aligned with its objectives. Thus, the CE will progress towards a comprehensive, decentralized facility, reinforcing and expanding upon the current RBINS-RMCA axis on which BopCo-CE has previously relied.

WP7: Outreach and communication (see also WP1)

Educating prospective users (including federal/regional/local agencies and authorities, the medical component of the armed forces, customs, forensic laboratories, NGOs, environmental consultants, trade organizations, logistic companies, etc.) about the offerings of BopCo-CE is a key objective. Outreach efforts will encompass various activities such as conducting workshops and information sessions (including hands-on sessions) to familiarize users with tissue handling procedures, disseminating BopCo-CE updates through multiple channels (website, social media, printed media, radio, TV), participating in professional and scientific conferences, and contributing to scientific publications.

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

4.1. BopCo-CE - Identification Requests:

During the period 20/12/2021 – 15/02/2024, BopCo-CE has completed 109 species identification requests from 39 different applicants. These requests were handled by use of DNA-based methods, morphology-based methods (relying on our network of taxonomic experts, see below WP5), or referral to an external expert, in case no taxonomic expertise could be delivered within the BopCo-CE network.

Overview identification requests (20/12/2021 – 15/02/2024) N= 109

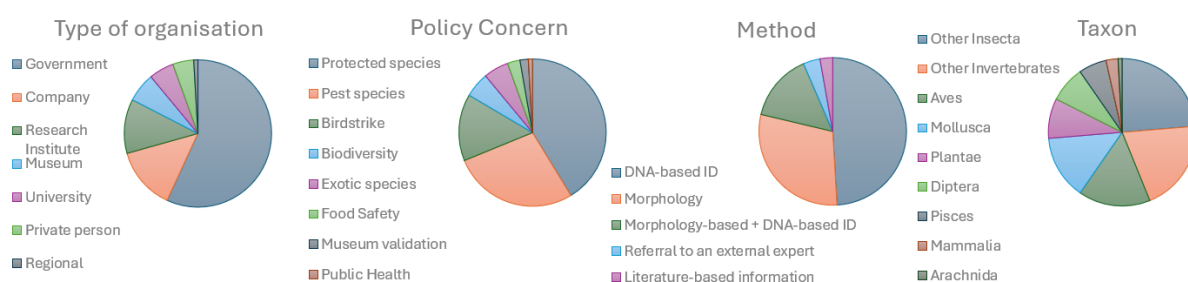


FIGURE 1: Overview of the numbers of species identification requests (N = 109) performed during the period 20/12/2021 – 15/02/2024. Data from projects are not included.

BopCo-CE addressed identification requests from 39 different stakeholders. This included six requests from four different companies, encompassing the identification of pest species and validation of products intended for the food chain. Additionally, 14 requests were received from 11 research institutions, spanning policy concerns related to pest species and biodiversity. Six universities submitted requests primarily focused on pest species identification, while eight requests for pest species identification came from within RBINS and RMCA. Moreover, BopCo-CE processed three requests from private individuals seeking identifications of pest species. However, the majority of identification requests originated from governmental agencies, including the Atomic Energy Commission (Bangladesh), FAVV & FPS HFE (Belgium), IAS Secretariat (Belgium), Plant Protection Agency (Lybia), Lybian Ministry of Agriculture (Lybia), NATO, and the Non-native Species Inspectorate - Department for Environment - Food and Rural Affairs (Defra) (United Kingdom). Among these, recurrent requests were predominantly from FPS HFE (n = 45).

In accordance with FAVV & FPS HFE, BopCo-CE assisted in identifying intercepted organisms suspected to be Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)-listed under. According to the current framework agreement (renewed in November 2023 for 10000 euro) BopCo-CE acts as an identification service for all suspected CITES species intercepted by FPS HFE. These identifications were based on morphology and/or DNA methods. As such, 29 requests involved the morphological identification of corals and shells, while 16 requests involved a DNA-based identification of eels, plants, birds and wild meat. Within the reporting period seven identification

requests were performed for FPS HFE ($N_{\text{samples}} = 17$), wherein suspicious plant samples were confiscated of which 10 samples were verified as American ginseng (*Panax quinquefolius*) which is a CITES-listed species and therefore a protected species. Six eel identification requests were performed for FPS HFE ($N_{\text{samples}} = 91$), targeting the illegal trade of the CITES listed European eels (*Anguilla anguilla*). Intercepted juvenile eels (glass eels) are typically transported alive in large quantities and need to be processed very quickly. The INBO holds these shipments until BopCo-CE confirms their identification as *A. anguilla*, after which they are eventually released into the wild. This measure has been the interest in at least four news articles:

- De Standaard (https://www.standaard.be/cnt/dmf20230302_98057263)
- Knack (<https://www.knack.be/nieuws/zes-arrestaties-voor-handel-in-glasaaltjes-het-ivoor-van-de-zee/>),
- Het Nieuwsblad (https://www.nieuwsblad.be/cnt/dmf20230227_97349536)
- VRT News (<https://www.vrt.be/vrtnws/nl/2023/02/25/zes-mensen-gearresteerd-op-brussels-airport-voor-smokkel-van-gla/>)

Finally, 16 bird strike identification requests came from the Aviation Safety Directorate (ASD), the Belgian Air Force, and Brussels Airport Company (BAC). The 14 different bird species involved were the red kite (*Milvus milvus*), common pigeon (*Columba livia*), stock pigeon (*Columba oenas*), song thrush (*Turdus philomelos*), European robin (*Erithacus rubecula*), goldcrest (*Regulus regulus*), common gull (*Larus canus*), barn swallow (*Hirundo rustica*), Mediterranean gull (*Ichtyaetus melanocephalus*), common swift (*Apus apus*), Eurasian skylark (*Alauda arvensis*), wild duck (*Anas platyrhynchos*), meadow pipit (*Anthus pratensis*), and black-headed gull (*Chroicocephalus ridibundus*).

BopCo-CE requests are related to WP2 and 5 as further highlighted below under the respective WPs.

4.2. BopCo-CE - Projects:

BopCo-CE projects differ from standard identification requests in that they typically involve a substantial number of samples to be processed within a predefined timeframe and are usually guided by specific questions that go beyond the basic species identification service offered for standard identification requests. As such, BopCo-CE has contributed to four projects, of which two were finalized (Wildmeat WWF and eDNA Crayfish) and two are still ongoing (MEMO+ and INTERCEPT).

In projects, BopCo-CE usually acts as subcontractor (e.g. MEMO and MEMO+, Monitoring of Exotic Mosquitos in Belgium - <https://www.itg.be/en/research/projects/monitoring-of-exotic-mosquitos-in-belgium-memo>) or participates as promotor or collaborator (e.g. INTERCEPT). In the MEMO+ project BopCo-CE collaborates with ITM and Sciensano. It provided DNA-based species identifications of 211 mosquito samples (mainly eggs), involving 159 samples of the invasive exotic Tiger mosquito (*Aedes albopictus*), seven of the invasive exotic Asian Bush mosquito (*Aedes japonicus*) and 35 of the native tree hole mosquito (*Aedes geniculatus*), for 10 samples no species identification could be provided.

In 2022, BopCo-CE was solicited by the BRAIN pillar 3 INTERCEPT project, run in cooperation of by RMCA, RBINS (<https://www.naturalsciences.be/en/science/research/biodiversity-in-a-changing-world/projects/intercept>) UAntwerp, Belgian Biodiversity Platform and Sciensano (<https://www.sciensano.be/en/projects/monitoring-import-exotic-animals-wild-meat-and-pathogens-they-carry>). This project focuses on monitoring the trade of exotic animals and the pathogens they may harbour. The aim of the project is to develop a fully operational and robust data collection workflow for the long-term monitoring of the import of exotic animals and wild meat into Belgium and evaluate the associated health risks. As promotor in the project, BopCo-CE will 1) optimise field protocols to safely take samples suited for molecular analyses, 2) train officers in the field to perform the sampling and collect the required metadata, 3) optimise a laboratory protocol for the species-level identification of the traded animals and meat samples, and 4) co-organize workshops to transfer the knowledge. The final aim is to establish an efficient and durable work programme for monitoring the import of legal and illegal exotic animals and animal products. In this context, more than 300 vertebrate meat samples were collected during 12 Baggage Control actions at Brussels International airport and identified using DNA-based techniques.

In 2021 BopCo-CO was asked to identify 112 tissue samples of imported wild meat for WWF-France. To this end, BopCo-CE generated sequences for four mitochondrial DNA markers (12S, 16S, COI, and Cytb) using MinION technology. In this way all samples were reliably identified (89 species were CITES-listed species) and the 315 generated DNA barcodes were deposited in a specific database. The project aimed to address the increasing wild meat consumption and trade in Europe, which poses significant threats to both hunted species conservation and public health. The final report for this project was completed in June 2022 (https://assets.website-files.com/61e6902f6bfd78f1ce2c2d2f/63cea704f4a5c3991bca2036_Wild%20meat_EN_HD_Page.pdf).

During 2023, BopCo-CE was hired to investigate the presence of four invasive crayfish species (*Procambarus clarkii*, *Procambarus virginalis*, *Faxonius limosus* and *Pontastacus leptodactylus*) in 50 ponds in Brussels. These surveys were performed upon the request of Brussels Environment (<https://environment.brussels/>) as part of the LIFE RIPARIAS project (<https://www.riparias.be/nl/>) (LIFE19 NAT/BE/000953). In this project 50 ponds in Brussels were investigated for the presence of four invasive crayfish species (*Faxonius limosus*, *Pontastacus leptodactylus*, *Procambarus clarkii* and *Procambarus virginalis*) using for the first time “environmental DNA” (eDNA) methodology via qPCR. *Faxonius limosus* was detected in four different ponds and *P. leptodactylus* was detected in one pond in Brussels, a result that is in line with the live capture data of Brussels Environment. This project was completed in December 2023. The report was finalized in February 2024 and will be published soon on the RIPARIAS website (<https://www.riparias.be/nl/>) (see further below WP5)

BopCo-CE projects are related to WP2, 3 and 5 as further highlighted below under the respective WPs.

4.3. Work packages:

WP 1: Maintenance and update of a BopCo-CE website

The BopCo-CE website (<https://bopco.myspecies.info/>) is regularly updated including the addition of new team members, links to published papers (13 published papers within report time frame, see reference list *Section 6. Publications*), news items, conference attendances (11 conferences attended within report time frame, see list below WP7) and links to videos and other scientific outreach projects BopCo was involved in (see below WP7). In preparation for the discontinuation of Scratchpad (current website platform), BopCo-CE has established a taskforce to build a new website using WordPress.

WP 2: Provision of reference DNA sequences of organisms of policy concern, their relatives and similar taxa

To enhance the identification capacity for birds involved in strikes with aircrafts, BopCo-CE, in collaboration with RMCA ornithologist Alain Reygel, sampled and barcoded various bird species, including species belonging to the following genera: *Falco*, *Larus*, and *Ichthyaetus*. These 40 barcodes, along with voucher information, were archived on BOLD.

In response to the publication of our invasive alien species (IAS) Factsheets (see below WP3) on the BopCo-CE website (<https://bopco.myspecies.info/content/invasive-alien-species-ias-factsheets>), the national Secretariat of IAS (Secretariat IAS) requested the sequencing of collected tissues from four invasive crayfish species (*Faxonius virilis*, *Procambarus clarkii*, *Pontastacus leptodactylus*, and *Procambarus acutus*) and one invasive plant species (*Salvinia modesta*). The factsheets illuminate the gaps in online DNA reference databases (BOLD and GenBank) and the associated problems regarding species identification. In total, 13 barcodes were generated for the five respective species, which will facilitate their identification in the future.

During the MEMO and MEMO+ projects (see *Section 4.2*), barcodes from native and invasive mosquito species were generated, in close collaboration with ITM and Sciensano. Tissue material and dried DNA from these specimens were added to the institutional collections (RBINS: IG32776; RBINS: IG34179) for long-term preservation, with the goal of establishing a reference collection of mosquito species occurring in Belgium. The generated DNA barcodes (n = 3350 (MEMO); n = 201 (MEMO+), representing 33 taxa) were -or are in the process of being, deposited in GenBank and linked to the NCBI BioProject ID PRJNA837425. The BioProject (i.e. collection of data generated under MEMO and MEMO+) is progressively populated with new barcodes, along with new scientific peer-reviewed publications.

WP 3: Identifying gaps in the reference databases of organisms of policy concern

To illuminate the problems concerning incomplete online reference databases, genetic markers and misidentified sequences BopCo-CE generated factsheets covering the invasive plant and animal species listed in the original EU Regulation (2016/1141) and its first update (2017/1263)

(<https://bopco.myspecies.info/content/invasive-alien-species-ias-factsheets>). Additionally, BopCo-CE developed 15 new factsheets upon request of the Secretariat of IAS (<https://www.iasregulation.be/nl>). These 15 factsheets include species that were recently added to the list of IAS of EU concern, including *Faxonius rusticus*, *Lampropeltis getula*, *Solenopsis invicta*, *S. geminata*, *S. richteri*, *Wasmannia auropunctata*, *Koenigia polystachya*, *Fundulus heteroclitus*, *Gambusia holbrooki*, *G. affinis*, *Amerius melas*, *Morone americana*, *Channa argus*, *Limnoperna fortunei*, and *Callosciurus finlaysonii*. The factsheets were finalized and sent to Secretariat of IAS as well as displayed on the BopCo-CE website along with the existing 53 IAS factsheets already published on the website (<https://bopco.myspecies.info/content/invasive-alien-species-ias-factsheets>).

BopCo-CE is hired as subcontractor on the INTERCEPT project (see *Section 4.2*). The project aims to counter the illegal import of wild meat into Belgium. To increase the species identification accuracy of wild meat samples, which usually lack morphological characteristics, BopCo-CE has started generating DNA reference barcodes from voucher material stored at the museums and Belgian universities, focusing first on the heavily hunted *Thryonomys*, *Philantomba* and *Cephalophus* genera. For species from these genera, DNA reference sequences were often not available in public databases. So far 36 sequences from voucher specimens (representing seven species) were generated.

WP 4: Data management

All DNA sequence data generated by BopCo-CE are stored in the public reference databases GenBank and BOLD, whenever approved by the client. This way the sequence data are reliably archived for the future and permanently freely available for any potential (public) user. Other documents generated by BopCo-CE (reports, raw DNA sequence files, images, etc.) are stored on a shared Dropbox account, which is backed-up monthly on two physical hard drives (one stored at RBINS, one at RMCA).

BopCo-CE aims to ensure efficient coordination and standardized project management procedures among team members. This involves maintaining shared documents and lab books, conducting regular meetings for evaluation and problem-solving, and adhering to established workflows for reporting and communication.

WP 5: Extending DNA identifications by additional expertise

BopCo-CE is a species identification facility that provides identification services utilizing both DNA-based techniques and morphology-based methods. During the reporting period, 33 requests were successfully fulfilled using species identification methods based solely on morphology (see Figure 1). Among these, 13 identification requests were completed using only images provided by clients. Additionally, 16 requests were completed through the combined utilization of DNA and morphology-based identification methods. Finally, four requests were passed on to other experts at our hosting institutes, leading to successful species identification.

As reported in the last paragraph of 4.2, BopCo-CE applied in 2023 for the first time eDNA methodology to detect four invasive crayfish species in Brussels. In this way, BopCo-CE has extended

its portfolio of identification techniques with eDNA methodology, an important approach for which there is an increasing demand by many stakeholders.

In 2021 BopCo-CE contributed to a project investigating the trade of wild meat in Europe, led by WWF France. Herein, BopCo-CE identified 112 wild meat specimens collected by the University of Antwerp and RBINS. For each specimen, four genes were amplified in multiplex and subsequently sequenced on an Oxford Nanopore Technologies MinION Sequencer. This sequencing method allowed us to efficiently generate a large amount of data. The DNA sequence processing pipeline is currently being optimized, which will allow us to process these kinds of large datasets more time and cost efficiently. The final report for this project, as well as the contributions from BopCo-CE (Dr. Sophie Gombeer, previous BopCo member), were published in January 2023 (https://assets-global.website-files.com/61e6902f6bfd78f1ce2c2d2f/63cea704f4a5c3991bca2036_Wild%20meat_EN_HD_Page.pdf)

WP 6: Development of overarching Centre of Excellence for Species Identification Services

BopCo-CE aims to broaden its user base and foster collaborative relationships with other research infrastructures, with the goal of establishing a comprehensive framework for species identification. This entails mapping existing expertise and infrastructure, developing joint work plans, conducting regular evaluations, and providing annual reports. A preliminary meeting was held on 17/11/2023 between BopCo-CE and ENFORCE (a new Belgian center of expertise for forensic wood research at RMCA, <https://enforce.africamuseum.be/>) to discuss the platform for highlighting this overarching structure, the unified identification services, the centre's name, and potential collaborations. It was decided that BopCo-CE will take the lead in creating a website serving as a primary contact point. This platform will offer an overview of various federal species identification services available in Belgium and direct users to the appropriate facility based on their specific needs.

WP 7: Outreach and communication

The outcomes of BopCo-CE activities were displayed at 11 international conferences. During these conferences, BopCo-CE team members and/or partners delivered a total of three oral presentations and presented 20 posters (please see the publication list in section 6 for more details).

- 22nd International Conference on Aquatic Invasive Species. 18-22 April 2022, Ostend, Belgium.
- International Conference on DNA Barcoding and Biodiversity. 25-27 May 2022, Sofia, Bulgaria.
- 5th International Workshop on *Aedes albopictus*. 11-13 May 2022, Montpellier, France.
- The Danish Society of Parasitology Symposium. 21-26 August 2022, Denmark, Copenhagen.
- 7th European *Dirofilaria* and *Angiostrongylus* Days. 23-24 September 2022, Madrid, Spain.
- 22nd European ESOVE conference. 11-14 October 2022. Sofia, Bulgaria.
- 15th International Congress on the Zoogeography and Ecology of Greece and Adjacent Regions (ICZEGAR). 12-15 October 2022, Mytilini, Lesvos.

- Xth EMCA Workshop. 28-29 November 2022, Mendrisio, Switzerland.
- World Biodiversity Forum. 26 June – 1 July 2022, Davos Congress Centre, Switzerland.
- Benelux Congress of Zoology 2022 - Diversity of Model Organisms and Model Organisms from Diversity. 22-23 September 2022, Leuven, Belgium.
- Benelux Congress of Zoology. 30-31 May 2023, Leiden, the Netherlands.

The BopCo-CE team meets every other week to coordinate and cooperate requests and projects. In addition, two annual meetings (21/06/2022 and 05/12/2023) were held with all BopCo-CE promoters and the BopCo-CE team to discuss and evaluate all aspects of the functioning of BopCo-CE.

In order to keep in contact with our stakeholders BopCo-CE attended over twenty meetings with Dissco Flanders, BioScan, INBO, ENFORCE team, INTERCEPT steering committee and Brussels Environment to make sure that our services are adjusted and finetuned in function of our stakeholders' requirements.

While BopCo-CE has put in effort to communicate the scientific contributions to the scientific public, we also aim to inform the general public about our services and projects. BopCo-CE contributed to the "Open Company Day" held at RMCA in honour of 125 years anniversary of the institute (October 1st, 2023). Where a small tour of the molecular lab was held, and a demonstration was given on DNA-extraction (Dipteran pollinators and fruit fly pests).

Additionally, in April 2023 BopCo-CE was selected to promote the identification services of the federal government as part of a series of videos as a message to the general public (Federal Minute) in collaboration with LDV production (La Une, RTL TVI, Eén and VTM). Source: https://www.belgium.be/nl/nieuws/2023/de_federale_minuut_het_koninklijk_belgisch_instituut_voor_natuurwetenschappen_bestrijdt. Also, BopCo-CE was interviewed by German journalists (Deutschlandfunk Kultur) in the context of the INTERCEPT project in order to contribute to a podcast documentary about wild meat consumption in Africa and by the African diaspora in Europe (<https://www.deutschlandfunkkultur.de/affenfleisch-im-koffer-illegaler-buschfleisch-handel-und-seine-folgen-dlf-kultur-5f7d7266-100.html>).

Finally, Between October and December 2023, BopCo-CE participated in a training course, "Video Pitching", at the Science Communication Academy (<https://www.scicomm-academy.eu/>). Through interactive workshops participants improved their skills to convey complex ideas to broader audiences. At the end of this training, each participant produced a 3-minute video. These videos will be made available on the Sci Comm website (<https://www.scicomm-academy.eu/pages/showcase>) upon their release in 2024.

5. DISSEMINATION AND VALORISATION

I. Vision and Mission of BopCo-CE

BopCo-CE represents the practical application of taxonomic expertise and service capabilities from RBINS and RMCA, offering species identification services crucial for societal needs, including policy support.

II. Unique Value Proposition of BopCo-CE

Having access to two well-equipped DNA laboratories at RBINS and RMCA, two extensive libraries, rich zoological reference collections, and high-resolution imaging infrastructures (e.g. SEM, μ CT-scanning, stacking photography), BopCo-CE delivers highly accurate species identifications, supported by solid classical taxonomic expertise.

III. Pricing Policy

BopCo-CE operates as a project-based, non-profit public service funded by BELSPO, with unrestricted access to RBINS and RMCA facilities. It provides services to diverse stakeholders, including governmental bodies. While BopCo-CE is a non-profit service, its services are not free of charge.

Price per Unit

Ad hoc requests, which typically include a few specimens (1-50) and standard DNA markers, are priced at € 50.00/sample for government entities and BopCo-CE partners and € 70.00/sample for companies. Yet, pricing depends on operational conditions, requested deliverables, and required lab consumables.

Market Comparison

Biome-id (Germany): € 30.00/sample (minimum 48 samples)

e-biom (Belgium): € 78.60/sample (single direction), €99.00/sample (both directions)

1st Base (Singapore): € 55.00/sample

Genelethu (RSA) Animals: € 20.40/sample

CCBD (Canada): € 15.50/sample (minimum 96 samples)

Allgenetics (Spain): € 70.70/sample

Sales Strategy

Tailored pricing for non-standard requests, recurring services, large sample volumes, subcontracts, or project participations. So far, there has been no decision on an established pricing for NGS or eDNA techniques. Free advice provided when requests fall outside BopCo-CE's scope.

IV. Weakness

Operating as a project rather than a permanent, structurally financed, service poses challenges, including the lack of long-term funding perspectives and non-flexible accounting rules due to the implementation of budget neutrality.

V. Long-term Vision

Although BopCo-CE cannot operate in a self-sustainable way, it does explore revenue-generating opportunities, focusing on anchoring services within Belgian federal and regional public sectors. The overarching goal is to establish BopCo-CE as part of a "Centre of Excellence for Fundamental and Applied Taxonomy," supported by BELSPO funding.

6. PUBLICATIONS

Scientific peer-reviewed publications:

D'hondt, B., Meganck, K., Van Acker, K. and De Beer, D. 2024. Genetic confirmation for the presence of the rare moss *Atrichum angustatum* (Brid.) Bruch & Schimp. in Belgium. *Dumortiera* 123: 5-8. [Link](#)

Pauwels, S.G.O., Brecko, J., Baeghe, D., Venderickx, J., Vanderheyden, A. and Backeljau, T. 2023. Morphological, acoustic and genetic identification of a reproducing population of the invasive African clawed frog *Xenopus laevis* (Anura, Pipidae) recently discovered in Belgium. *ZooKeys* 1184: 41-64. <https://doi.org/10.3897/zookeys.1184.103702>

Dekoninck, W., Smits, N., Vanderheyden, A., Grootaert, P. and Backeljau, T. 2023 DNA-based species identification of mosquitoes collected with Malaise Traps in the Botanical Garden Jardin Massart (Diptera: Culicidae). *Belgian Journal of Entomology* 134: 247-255.

Edmunds, S.C., Fouque, F., Copas, K.A., Hirsch, T., Schigel, D., Shimabukuro, P.H.F., Andrade-filho, J.D., Marceló, C., Morales, C.A., Lesmes, M.C., Fuya, P., Méndez, S., Cadena, H., Ávila-Díaz, A., Santamaría, E., Južnič-Zonta, Z., Eritja, R., Palmer, J.R.B., Bartumeus, F., dos Santos-Conceição, M., Chahad-Ehlers, S., Silva-Inácio, C.L., Lozovei, A.L., de Andrade, A.J., Paull, S., Miranda, M.A., Barceló, C., Schaffner, F., Della-Torre, A., Brosens, D., Dekoninck, W., Hendrickx, G., Van Bortel, W., Deblauwe, I., Smits, N., Brilhante, A.F., Ceccarelli, S., Balsalobre, A., Vicente, M.E., Curtis-Robles, R., Hamer, S.A., Landa, J.M.A., Rabinovich, J.E. and Marti, G.A. 2022. Publishing data to support the fight against human vector-borne diseases. *GigaScience* 11: giac114. doi: 10.1093/gigascience/giac114

Chomchoei, N., Backeljau, T., Segers, B., Wongsawad, C., Butboonchoo, P. and Nantararat, N. 2022. Morphological and molecular characterization of larval trematodes infecting the assassin snail genus *Anentome* in Thailand. *Journal of Helminthology* 96, e52: 1-11. <https://doi.org/10.1017/S0022149X22000463>

Ibáñez-Justicia, A., Smits, N., Blom, R., Vanderheyden, A., Jacobs, F., Meganck, K., Gombeer, S., Backeljau, T., Koenraadt, C.J.M., Griep, J.S., De Meyer, M. and Stroo, A. 2022. *Anopheles maculipennis* complex in the Netherlands: First record of *Anopheles daciae* (Diptera: Culicidae). *Diversity* 14(8):636. <https://doi.org/10.3390/d14080636>

Kurucz, K., Zeghib, S., Arnoldi, D., Marini, G., Manica, M., Michelutti, A., Montarsi, F., Deblauwe, I., Van Bortel, W., Smits, N., Pfitzner, W.P., Czajka, C., Jost, A., Kalan, K., Susnjar, J., Ivović, V., Kuczmog, A., Lanszki, Z., Toth, G.E., Somogyi, B.A., Herczeg, R., Urban, P., Bueno-Mari, R., Soltesz, Z. and Kemenesi, G. 2022. *Aedes koreicus*, a vector on the rise: Pan-European genetic patterns, mitochondrial and draft genome sequencing. *PlosONE* 17(8): e0269880. <https://doi.org/10.1371/journal.pone.0269880>

Vanderheyden, A., Smits, N., De Wolf, K., Deblauwe, I., Dekoninck, W., Meganck, K., Gombeer, S., Vanslebrouck, A., De Witte, J., Schneider, A., Verlé, I., De Meyer, M., Backeljau, T., Müller, R. and Van Bortel, W. 2022. DNA identification and diversity of the vector mosquitoes *Culex pipiens* s.s. and *Culex torrentium* in Belgium (Diptera: Culicidae). *Diversity* 14(6): 486. <https://doi.org/10.3390/d14060486>

Deblauwe, I., Brosens, D., De Wolf, K., Smits, N., Vanslebrouck, A., Schneider, A., De Witte, J., Verlé, I., Dekoninck, W., De Meyer, M., Backeljau, T., Gombeer, S., Meganck, K., Vanderheyden, A., Müller, R. and Van Bortel, W. 2022. MEMO: Monitoring of exotic mosquitoes in Belgium. GIGabyte: 1-9. <https://doi.org/10.46471/gigabyte.59>

Harbi, A., Abbes, K., Elimem, M., Lazheri, H., Meganck, K. and Chermiti, B. 2022. The seedcorn maggot *Delia platura* (Diptera: Anthomyiidae): An emerging pest of garlic crops in Tunisia. EPPO Bulletin 52: 149– 153. DOI:10.1111/epp.12841.

Deblauwe, I., De Wolf, K., De Witte, J., Schneider, A., Verlé, I., Vanslebrouck, A., Smits, N., Demeulemeester, J., Van Loo, T., Dekoninck, W., Krit, M., Madder, M., Müller, R. and Van Bortel, W. 2022. From a long-distance threat to the invasion front: invasive *Aedes* mosquito species in Belgium between 2007 and 2020. Parasites & Vectors 15:206. 10.1186/s13071-022-05303-w

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Mariën, J., Laurent, N., Smits, N. and Gombeer, S. 2022. First observation of *Aedes albopictus* in the Tshuapa province (Boende) of the Democratic Republic of the Congo. African Entomology 30: e11932. <https://doi.org/10.17159/2254-8854/2022/a11932>

Poster presentations at (inter)national conferences:

De Meyer, M., Meganck, K., Dermauw, W., Lombal, A. & Witters, J. 2023. Development of surveillance program and identification tools for non-European fruit flies (Diptera: Tephritidae) in Belgium. Benelux Congress of Zoology 2023, Leiden, the Netherlands.

Lombal, A., Dekoninck, W., Smits, N., Vanderheyden, A., Grootaert, P. & Backeljau, T. 2023. DNA-based species identification of mosquitoes collected with Malaise traps in the botanic garden Jean Massart (Diptera: Culicidae). Benelux Congress of Zoology 2023, Leiden, the Netherlands.

Schols, R., Smits, N., Vanderheyden, A. & Huyse, T. 2023. Swimmer's itch in Belgium: A first record of *Trichobilharzia regenti*. The Danish Society of Parasitology Symposium, Denmark, Copenhagen.

Dimzas, D., Di Cesare, A., Morelli, S., Iorio, R., Backeljau, T., Vanderheyden, A., Lombal, A., De Meyer, M., Meganck, K., Smits, N., Kassari, N., Traversa, D. & Diakou, A. 2022. Metastrongyloid parasites of felines in naturally infected gastropods in Greece. 15th International congress on the Zoogeography and ecology of Greece and adjacent regions. ICZEGAR, Mytilini, Lesvos.

Deblauwe, I., De Wolf, K., Smits, N., Vanslebrouck, A., Schneider, A., Dekoninck, W., De Meyer, M., Backeljau, T., Müller, R. & Van Bortel, W. 2022. Finding the balance between efficiency and budget: preventive invasive mosquito species (IMS) surveillance. 22nd European ESOVE conference. Sofia, Bulgaria.

Smits, N., Lombal, A., De Wolf, K., Deblauwe, I., Kampen, H., Schaffner, F., De Witte, J., Schneider, A., Verlé, I., Vanslebrouck, A., Dekoninck, W., Meganck, K., Gombeer, S., Vanderheyden, A., De Meyer,

M., Backeljau, T., Werner, D., Müller, R. & Van Bortel, W. 2022. Population genetic structure of the exotic Asian bush mosquito, *Aedes japonicus*, in Belgium suggests multiple introduction pathways. 22nd European ESOVE conference. Sofia, Bulgaria.

Chomchoei, N., Backeljau, T., Segers, B., Lombal, A., Vanderheyden, A., Wongsawad, C., Butboonchoo, P. & Nantararat, N. 2022. Survey of Parasitic Larval Trematodes in the Assassin Snails *Anentome helena* and *A. wykoffi* from Thailand. 22nd European ESOVE conference. Sofia, Bulgaria.

Vanderheyden, A., Lombal, A., Smitz, N., Meganck, K., Kassari, N., Dimzas, D., Diakou, A., Di Cesare, A., Traversa, D., De Meyer, M. & Backeljau, T. 2022. DNA barcoding and identification of intermediate terrestrial host gastropods of metastrongyloid cat parasites in Greece. 22nd European ESOVE conference. Sofia, Bulgaria.

Vanderheyden, A., Smitz, N., De Wolf, K., Deblauwe, I., Dekoninck, W., Meganck, K., Lombal, A., Vanslebrouck, A., De Witte, J., Schneider, A., Verlé, I., De Meyer, M., Backeljau, T., Müller, R. & Van Bortel, W. 2022. DNA identification and diversity of the vector mosquitoes *Culex pipiens* s.s. and *Culex torrentium* in Belgium. 22nd European ESOVE conference. Sofia, Bulgaria.

Lombal, A., Meganck, K., Vanderheyden, A., Smitz, N., Backeljau, T. & De Meyer, M. 2022. BopCo, a barcoding facility for organisms and tissues of policy concern, and its role in the identification of vector species. 22nd European ESOVE conference. Sofia, Bulgaria.

Dimzas, D., Di Cesare, A., Morelli, S., Iorio, R., Backeljau, T., Vanderheyden, A., Lombal, A., De Meyer, M., Meganck, K., Smitz, N., Kassari, N., Traversa, D. & Diakou, A. 2022. Natural intermediate hosts of *Angiostrongylus chabaudi* in Greece. 7th European *Dirofilaria* and *Angiostrongylus* Days. Madrid, Spain.

Dimzas, D., Di Cesare, A., Morelli, S., Iorio, R., Backeljau, T., Vanderheyden, A., Lombal, A., De Meyer, M., Meganck, K., Smitz, N., Kassari, N., Traversa, D. & Diakou, A. 2022. Metastrongyloid parasites of felines in naturally infected gastropods in Greece. 15th ICZEGAR. Mytilini, Greece.

Vanderheyden, A., Lombal, A., Meganck, K., Smitz, N., Backeljau, T. & De Meyer, M. 2022. A Barcoding Facility for Organisms and Tissues of Policy Concern. Benelux Congress of Zoology 2022 - Diversity of model organisms and model organisms from diversity. Leuven, Belgium.

Deblauwe, I., Hermy, M.R.G., Schneider, A., Vanslebrouck, A., Geebelen, L., Smitz, N., De Meyer, M., Vanderheyden, A., Backeljau, T., Müller, R., Lernout, T., Rebolledo, J. & Van Bortel, W. 2022. Multiple detections of *Aedes albopictus* in Belgium through Citizen Science. Xth EMCA workshop. Mendrisio, Switzerland.

Vanderheyden, A., Smitz, N., Deblauwe, I., De Wolf, K., Gombeer, S., Meganck, K., Lombal, A., Schneider, A., De Witte, J., Dekoninck, W., Backeljau, T., De Meyer, M., Müller, R., Rebolledo Gonzalez, J. & Van Bortel, W. 2022. MEMO and MEMO+: Identification of introduction pathways and surveillance of exotic *Aedes* mosquito species in Belgium. International Conference on DNA Barcoding and Biodiversity. Sofia, Bulgaria.

Meganck, K., Gombeer, S., Vanderheyden, A., Smitz, N., Lombal, A., Backeljau, T. & De Meyer, M. 2022. The Belgian Barcoding facility for organisms and tissues of policy concern. International Conference on DNA Barcoding and Biodiversity. Sofia, Bulgaria.

Deblauwe, I., De Wolf, K., Schneider, A., De Witte, J., Vanslebrouck, A., Dekoninck, W., Smitz, N., De Meyer, M., Backeljau, T., Müller, R. & Van Bortel, W. 2022. From a long-distance threat to the invasion front: a review of the invasive *Aedes* mosquito species in Belgium between 2007 and 2020. 5th international workshop on *Aedes albopictus*. Montpellier, France.

Gombeer, S., Meganck, K., Vanderheyden, A., Smitz, N., Pauwels, O., Brecko, J., De Meyer, M. & Backeljau, T. 2022. Detecting *Xenopus laevis* in Belgium using eDNA and qPCR. 22nd International Conference on Aquatic Invasive Species. Ostend, Belgium.

Aydin, M., Biltekin, D., Breugelmans, K., Vanderheyden, A., Gombeer, S., Meganck, K., Smitz, N. & Backeljau, T. 2022. First record and DNA identification of the Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), in the southern Black Sea. 22nd International Conference on Aquatic Invasive Species. Ostend, Belgium.

Oral presentations at (inter)national conferences:

Smitz, N., Meganck, K., Lombal, A., Vanderheyden, A., De Meyer, M. & Backeljau, T. 2022. DNA-barcoding, a tool to identify invasive alien species: strengths and limits of the technique. World Biodiversity Forum 2022, Davos Congress Centre, Switzerland.

Vanderheyden, A., Gombeer, S., Gryseels, S., Chaber, A., Meganck, K., Smitz, N., Nebesse, C., Musaba, P., Ngoye, S., Backeljau, T., De Meyer, M., & Verheyen, E. 2022. Using MinION Nanopore sequencing to simultaneously identify multiple, illegally imported wild meat samples in Brussels, Belgium. International Conference on DNA Barcoding and Biodiversity. 25-27 May 2022, Sofia, Bulgaria.

Gombeer, S., Meganck, K., Vanderheyden, A., Smitz, N., De Meyer, M. & Backeljau, T. 2022. Identifying EU-listed Aquatic Invasive Species by DNA-barcoding using currently available sequence data. 22nd International Conference on Aquatic Invasive Species. 19-22 April 2022. Ostend, Belgium.

7. ACKNOWLEDGEMENTS

We acknowledge the input of all RBINS and RMCA staff who have contributed or assisted to any identification requests by providing their expertise or advice. We extend our sincerest gratitude to our partners whose contributions and/or collaboration have been instrumental in the finalization of projects; JEMU, ITM, Sciensano, Brussels Environment as part of the LIFE RIPARIAS project (LIFE19 NAT/BE/000953), INTERCEPT, WWF France. We would like to express our appreciation to BAC, ASD, Belgian Defense, INBO, FPS HFE, FAVV for the opportunity to collaborate on recurring requests such as identification of bird strikes and the identification of protected species. Also, we are grateful to National Scientific Secretariat of IAS, ENFORCE, INBO, all companies, universities and private people who have requested identification requests from BopCo-CE. This research would not have been possible without the funding of BELSPO, collective efforts and commitment of all our partners, and we look forward to continued collaboration in the future.