

A BARCODING FACILITY FOR ORGANISMS & TISSUES OF POLICY CONCERN

NATHALIE SMITZ, KENNY MEGANCK, ANN VANDERHEYDEN, ANICEE LOMBAL,
THIERRY BACKELJAU & MARC DE MEYER



EMPOWERING BIODIVERSITY CONFERENCE

24-25 MAY 2022

bopco@naturalsciences.be

<http://bopco.myspecies.info/>



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BOPCO JOINTLY RUN
BY RBINS AND RMCA

FEDERAL CONTRIBUTION
TO THE ERIC LIFEWATCH



A BARCODING FACILITY FOR ORGANISMS & TISSUES OF POLICY CONCERN



- Aim: Supplying identifications of policy concern organisms and derived products

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- Objective: Provide access to knowledge (taxonomic expertise) and infrastructure (laboratory) necessary to identify biological samples of policy concern

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- For whom: All stakeholders who deal with biological materials of policy concern and who need an accurate identification
- Conditions: Compliance with BopCo scope & Costs may be charged

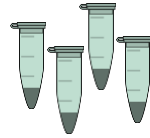
- Policy concern organisms including endangered species, invasive alien species, human and veterinary disease organisms or their vectors, species of forensic relevance, agricultural pest species, organisms of the human food chain, etc.



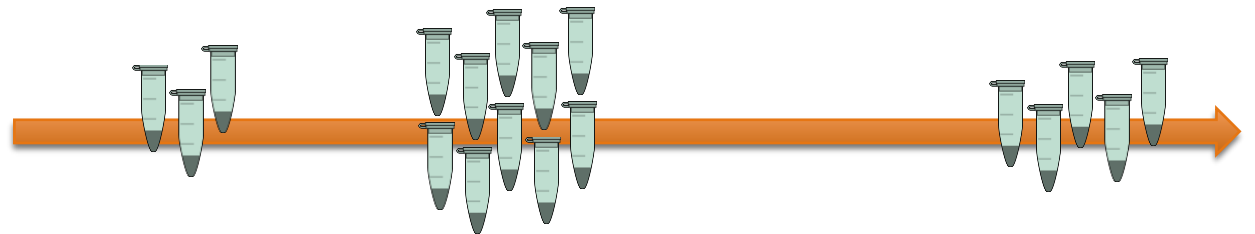
IDENTIFICATION REQUESTS AND PROJECTS



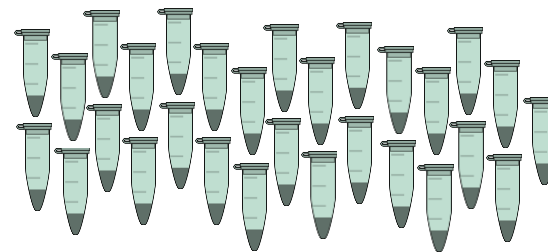
- Punctual identification requests



- Recurrent identification requests



- Identification projects & filling the gaps



Morphological characteristics

Monographs, identification keys, scientific periodic



Microscopy

Network of in-house and external taxonomic experts



Specimen collections

Morphological characteristics

Monographs, identification keys, scientific periodic



Microscopy

Network of in-house and external taxonomic experts



Specimen collections

Meretrix meretrix



Excluding CITES-listed species by partial ID



Hippopus hippopus



All species of the genus *Tridacnidae* (giant clams) are CITES-listed

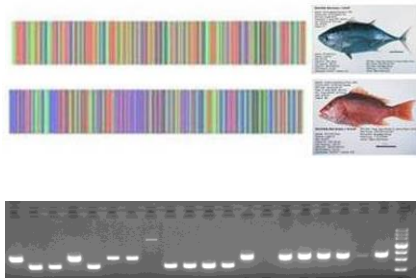
Strombus gigas



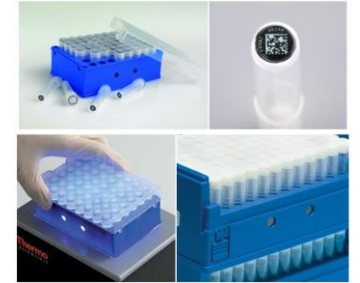
Confirmation of non CITES-listed species ID



DNA-based techniques



Access to laboratory facilities and sequence databases

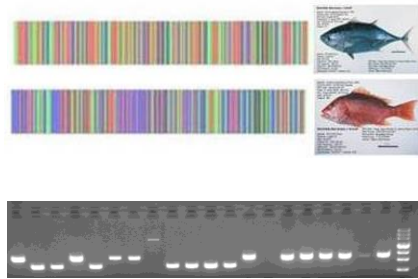


MORPHOLOGY??

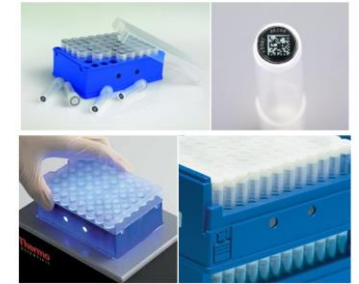
Early life stage, damaged specimen, processed sample, trace material,....

DNA Barcoding

DNA-based techniques



Access to laboratory facilities and sequence databases



MORPHOLOGY??

Early life stage, damaged specimen, processed sample, trace material,....

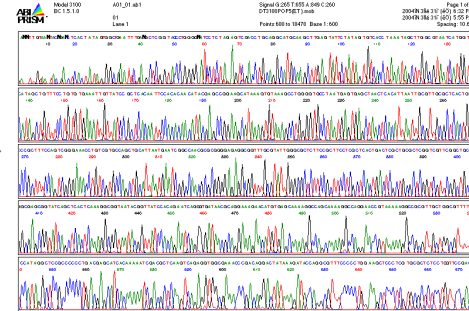
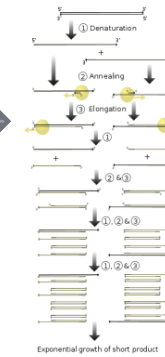


DNA Barcoding



DNA Extraction

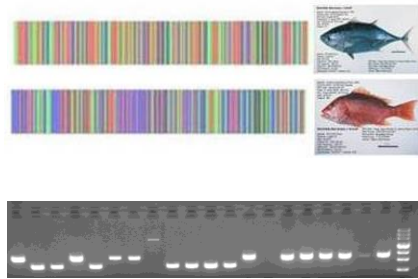
Amplification



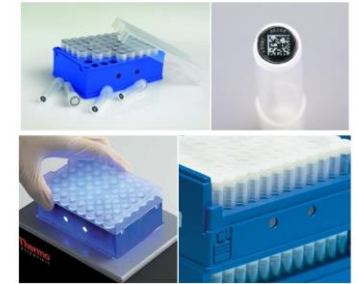
Sequencing

...TTGCCTTATGATCAGGAACC....

DNA-based techniques



Access to laboratory facilities and sequence databases



MORPHOLOGY??

Early life stage, damaged specimen, processed sample, trace material,....

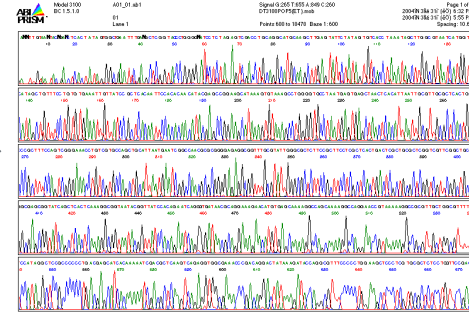
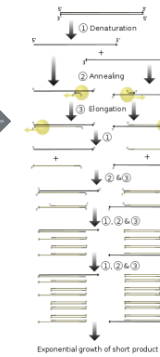


DNA Barcoding



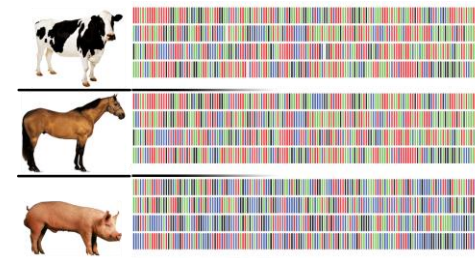
DNA Extraction

Amplification



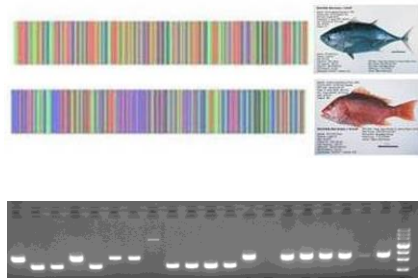
Sequencing
...TTGCCTTATGATCAGGAACC...

Comparison

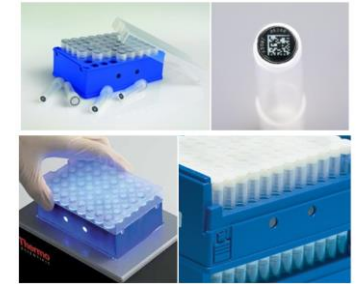


Online repositories

DNA-based techniques



Access to laboratory facilities and sequence databases



MORPHOLOGY??

Early life stage, damaged specimen, processed sample, trace material,....

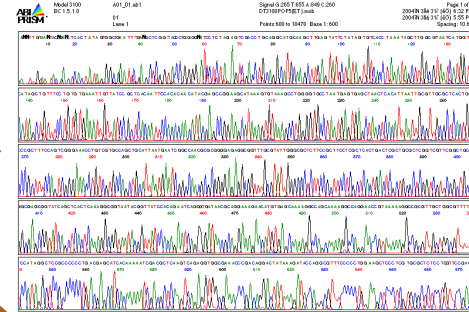
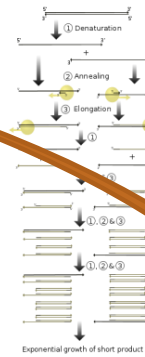


DNA Barcoding



DNA Extraction

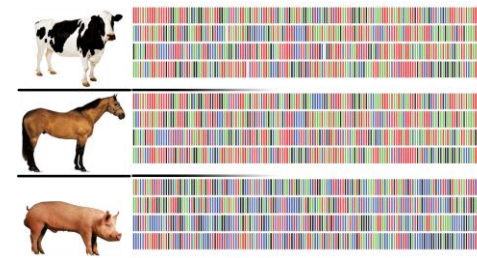
Amplification



Sequencing

TTGCCTTATGATCAGGAACC...

Comparison

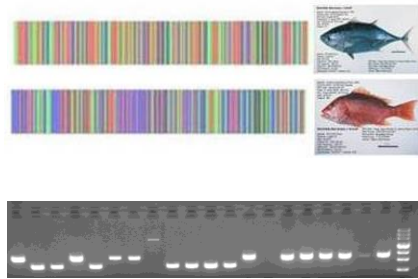


Online repositories

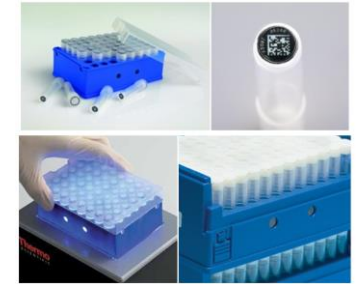


Species identification

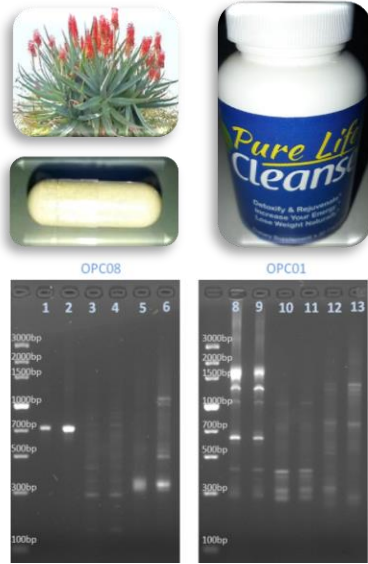
DNA-based techniques



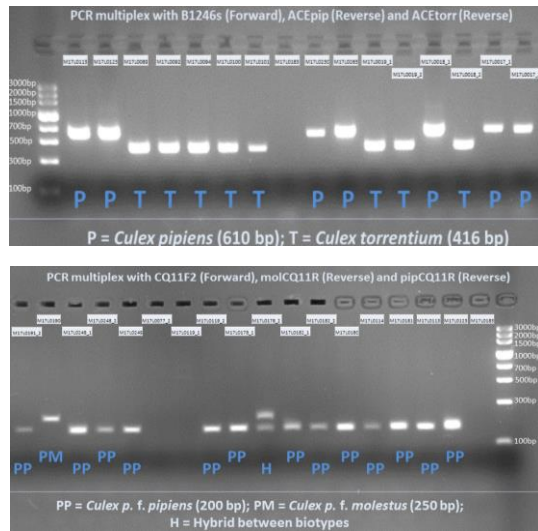
Access to laboratory facilities and sequence databases



Random Amplified Polymorphic DNA CITES species identification



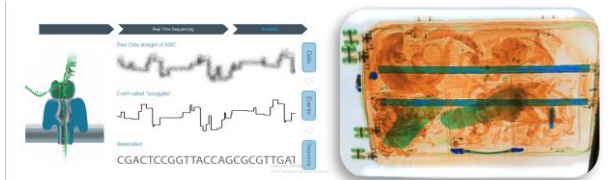
Size fragment analyses Mosquito species & biotype identifications



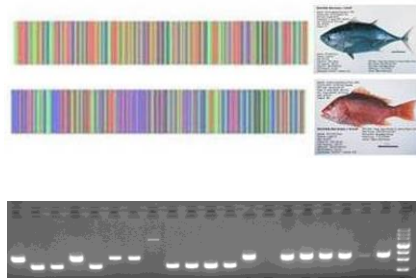
eDNA - qPCR Detection of the invasive *Xenopus laevis* using environmental DNA (eDNA) from aquatic samples



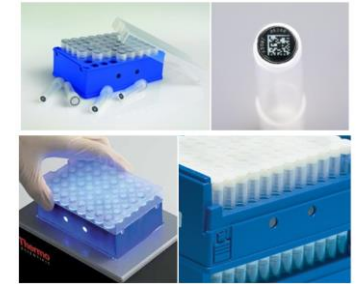
MinION Understanding illegal wild meat markets and associated health risks in target EU countries



DNA-based techniques



Access to laboratory facilities and sequence databases



Disease organisms and their vectors: ID of parasitic worm found in a cod fillet → *Pseudoterranova decipiens* = sealworm



Agricultural pest: ID of morphologically undistinguishable fruit fly pests from orchards in South Africa



Pest species: ID and tracing origin of insect larva and pupa found in drum of chemical product → *Plodia interpunctella* = a world-wide pest of stored products



Exotic species: ID of a new alien invasive terrestrial flatworm for Belgium → *Caenoplana bicolor* which is native to Australia

Referring to
network



INSTITUTE
OF TROPICAL
MEDICINE
ANTWERP



Referring to network



INSTITUTE OF TROPICAL MEDICINE ANTWERP



Network of scientific institutes and collaborators



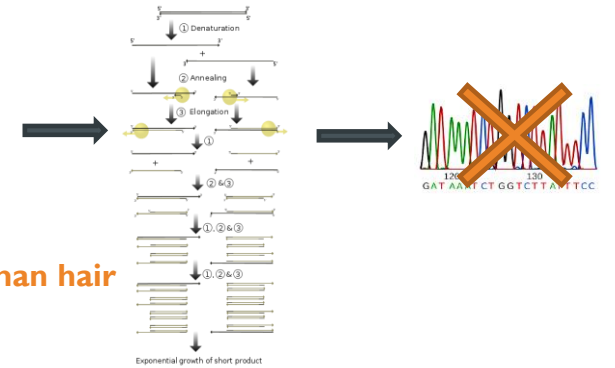
ID of fungi for notary office from Brussels



no in-house taxonomic expertise or technical facilities



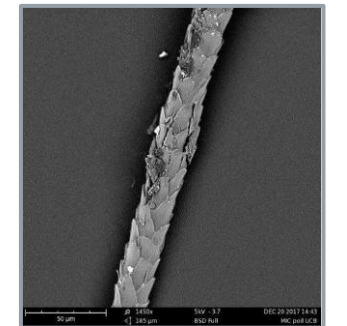
ID of a non-human hair



shrew or harvest mouse or dormouse



Botanic Garden Meise

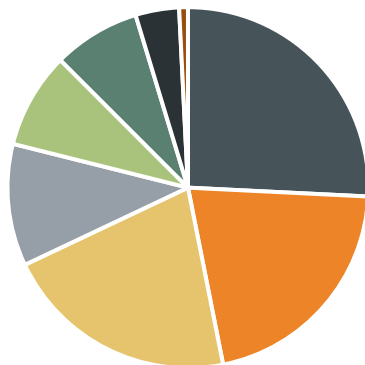


PUNCTUAL IDENTIFICATION REQUESTS



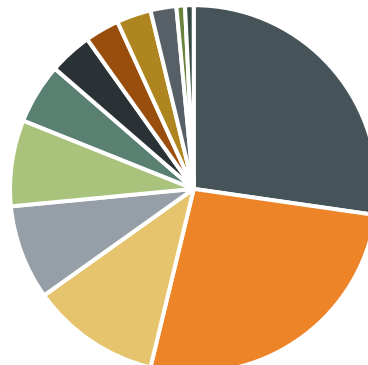
OVERVIEW IDENTIFICATION REQUESTS UNTIL OCTOBER 2021 (N=128)

Policy Concern



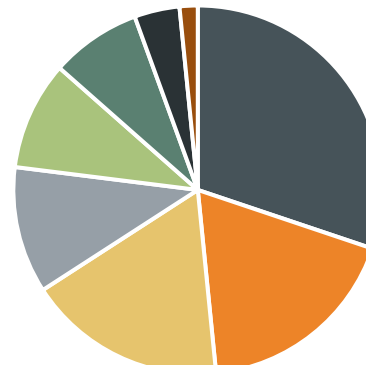
- Pest species
- Agricultural pest
- Protected species
- Biodiversity
- Public health
- Exotic species
- Food safety
- Museum validation

Taxon diversity



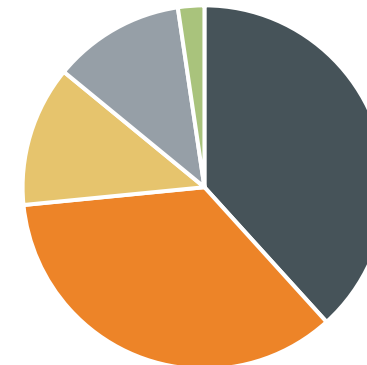
- Diptera
- Other Insecta
- Arachnida
- Mollusca
- Mammalia
- Other invertebrates
- Aves
- Plantae
- Pisces
- Reptilia
- Fungi
- Virus

Stakeholders



- Government
- Research Institute
- Private person
- University
- Company
- Museum
- Small business
- NGO

Outcome



- Morphology-based ID
- DNA-based ID
- Literature-based information
- Morphology-based + DNA-based ID
- Referral to an external expert

RECURRENT IDENTIFICATION REQUESTS EXAMPLES



Bird strikes: ID bird remains for Belgian Air Force and Brussels Airport



Disease organisms and their vectors: ID of mosquitoes from Belgium and countries of deployment for the Belgian Army



Disease organisms and their vectors: Identifying all life stages of (exotic) mosquitoes within the framework of the Monitoring of Exotic MOsquitoes in Belgium (MEMO) project



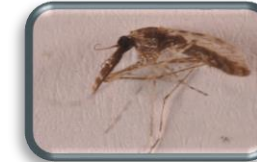
Aedes albopictus



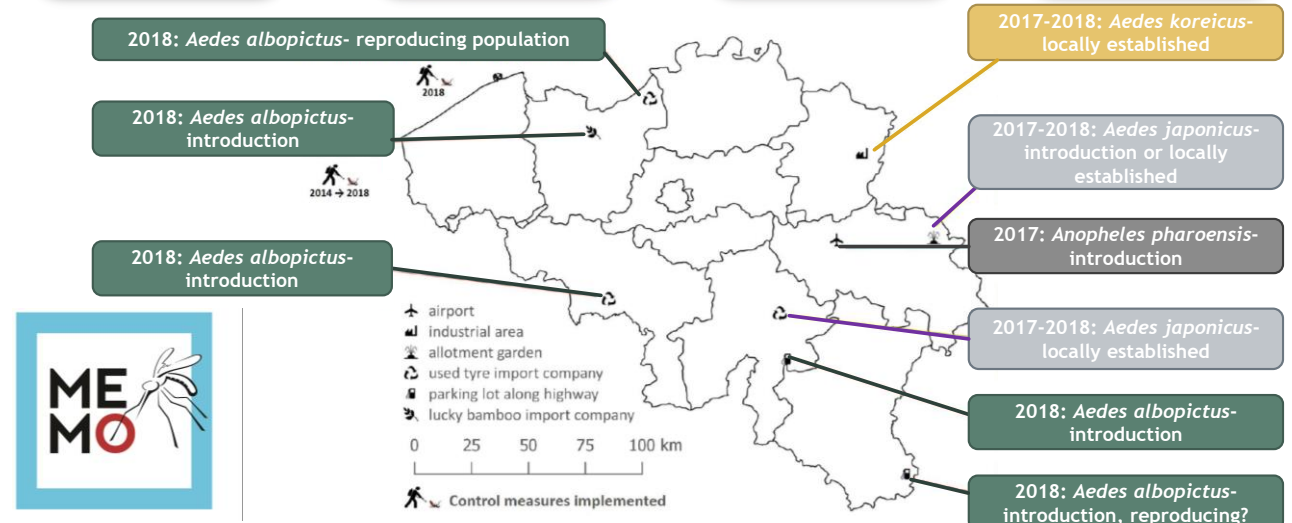
Aedes koreicus



Anopheles pharoensis



Aedes japonicus

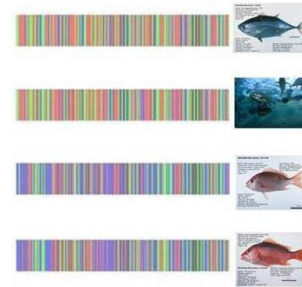


Map of Belgium indicating the PoEs where EMS were detected in 2017 and 2018 (data ITM)

Limitations of DNA-based technologies

- Reference database: success species identification depends on available sequence data
 - comprehensive, otherwise identification only to higher taxonomic level
 - reliable, if mistakes in the databases no confident identification can be made

- Different markers
- Data publically available
- No requirements for sequences
- No curation of data



Reference library
Online repositories



- Barcodes, e.g. COI (animals)
- Not all publically available
- Strict requirements:
 - vouchers
 - metadata
 - quality & length



BOLD



DESIGNED TO SUPPORT THE GENERATION & APPLICATION OF DNA BARCODE DATA

BOLD is a cloud-based data storage and analysis platform developed at the Centre for Biodiversity Genomics in Canada. It consists of four main modules, a data portal, an educational portal, a registry of BIVs (putative species), and a data collection and analysis workbench.

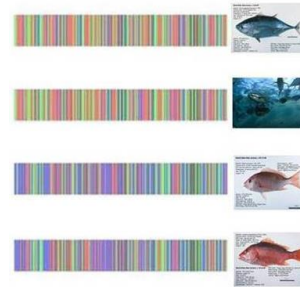
Please note that this version of BOLD is in beta and will contain bugs. Users can help address these bugs by testing the system and reporting issues to support@boldsystems.org. This version is very different from the prior one but has access to all the same data.



Limitations of DNA-based technologies

- Reference database: success species identification depends on available sequence data
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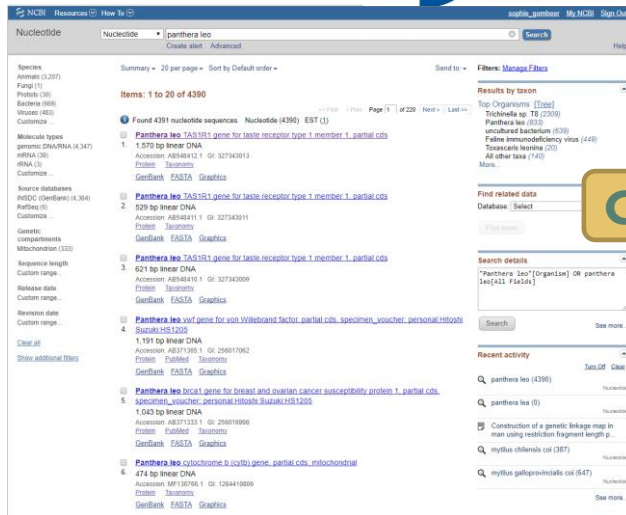
- Different markers
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Reference library
Online repositories

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BOLD
SYSTEMS



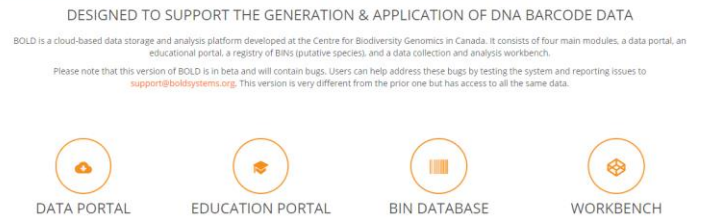
Comprehensive

BOLD



Reliable

Use both databases in combination to compare and interpret the results, taking in to account the strengths and weaknesses of each



IDENTIFICATION PROJECTS & FILLING THE GAPS EXAMPLES



Invasive Alien Species:

in silico testing of available sequence data

Evaluate ID reliability of 49 IAS on EU list based on publicly available DNA sequences to identify gaps in the online databases

49 species on Regulation
1143/2014 issued by the
European Commission



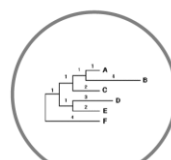
Download all sequence data available for the genus



Filtering the data and selecting 'promising' markers

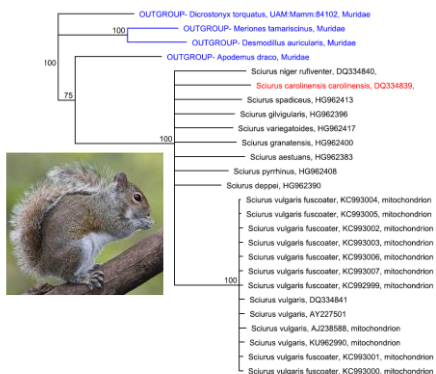


Aligning and trimming of the sequences

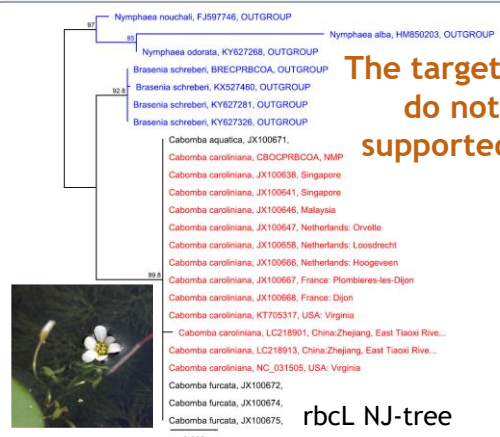


Building Neighbour-joining tree with Bootstrap support

Insufficient publicly available DNA sequences of the target species to capture the intra-species divergence



Insufficient publicly available DNA sequences of the congeners to capture the inter-species divergence



The target sequences do not form a supported cluster

IDENTIFICATION PROJECTS & FILLING THE GAPS EXAMPLES



Invasive Alien Species:

in silico testing of available sequence data

Evaluate ID reliability of 49 IAS on EU list based on publicly available DNA sequences to identify gaps in the online databases

49 species on Regulation 1143/2014 issued by the European Commission



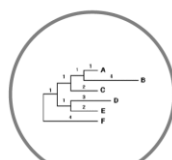
Download all sequence data available for the genus



Filtering the data and selecting 'promising' markers

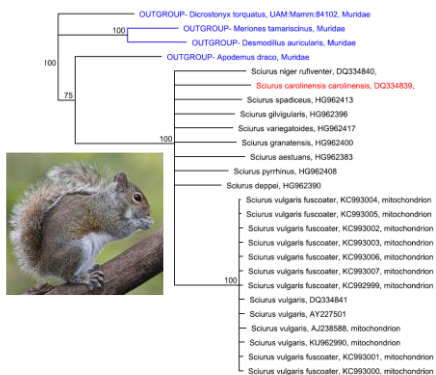


Aligning and trimming of the sequences



Building Neighbour-Joining tree with Bootstrap support

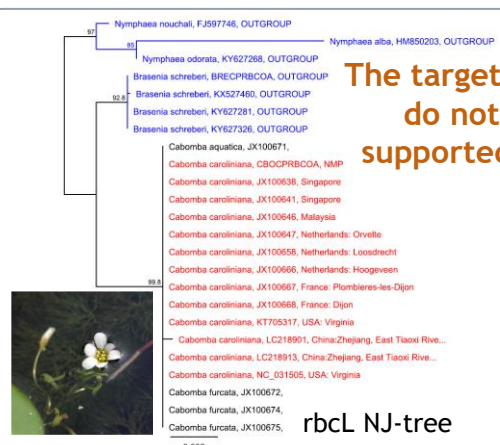
Insufficient publicly available DNA sequences of the target species to capture the intra-species divergence



16S NJ-tree

Eastern Gray squirrel (*Sciurus carolinensis*)

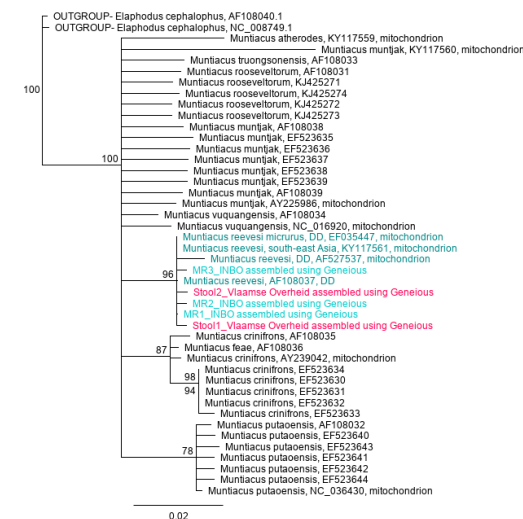
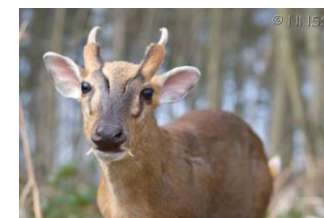
Insufficient publicly available DNA sequences of the congeners to capture the inter-species divergence



The target sequences do not form a supported cluster

rbcl NJ-tree

Carolina fanwort (*Cabomba caroliniana*)



0.02

IDENTIFICATION PROJECTS & FILLING THE GAPS EXAMPLES



Forensic species:

Building a barcode reference library for the Belgian rove beetle species (Staphylinidae) of forensic importance in collaboration with the NICC



National Institute of
Criminalistics and Criminology



48 species of rove beetles
of forensic interest



Estimate post mortem interval



IDENTIFICATION PROJECTS & FILLING THE GAPS EXAMPLES

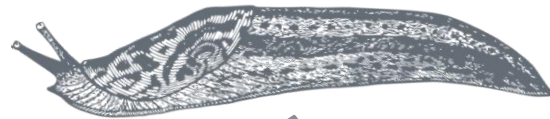


Disease vectors:

ID of terrestrial snails and slugs acting as intermediate hosts of nematode parasites, e.g. metastrongyloid lungworms that infect canids, felids and humans

Institute of Parasitology
JLU Giessen

Aristotle University
of Thessaloniki



Ambigolimax valentianus
(N = 1)

Deroceras sp.
(N = 1)

Limax cf. *conemenosi*
(N = 1)

Tandonia cf. *sowerbyi*
(N = 1)

Cornu aspersum
(N = 3)

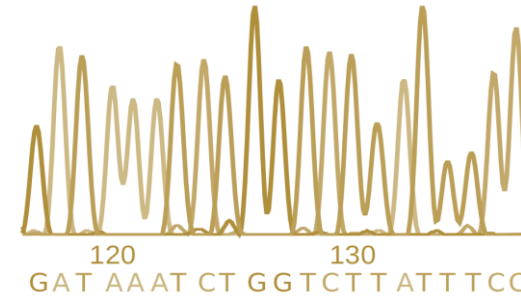
Eobania vermiculata
(N = 6)

Helix lucorum
(N = 1)

BOPCO SEQUENCE DATABASE



- Sanger sequencing of \neq marker regions:
COI, 16S rRNA, NADH4, 28S, EF1-alpha, wg, LW
Rh, ITS, cytb, rbcL, matK, trnH-psbA, COII, ...



- Generated sequences:
 - Deposition in GenBank (including BioProjects)
 - Voucher specimen barcodes → BOLD



A BARCODING FACILITY FOR ORGANISMS & TISSUES OF POLICY CONCERN



bopco@naturalsciences.be

HTTP://BOPCO.MYSPECIES.INFO/

Welcome to BopCo

The **Barcoding Facility for Organisms and Tissues of Policy Concern** (BopCo) aims at developing an expertise forum to facilitate the identification of biological samples of policy concern. Such identifications can rely on traditional morphology-based approaches requiring taxonomic expertise and/or DNA-based techniques demanding specific skills and access to a fully equipped molecular laboratory.

The intent of BopCo therefore is (1) to act as a focal point for identifying biological materials upon request, using both morphological and DNA-based techniques, (2) to produce well-documented DNA barcodes of relevant taxa, (3) to maintain reference collections of barcoded organisms and the corresponding DNA barcode databases, and (4) to explore and implement new tools and techniques for species identification and DNA barcoding.



Download Folder
 Request Now



Request Now

Request species identification

Download Folder