



Identification of Invasive Alien Species using DNA barcodes

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General introduction to this factsheet

The Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo) provides an expertise forum to facilitate the identification of biological samples of policy concern in Belgium and Europe. BopCo is funded by the Belgian Science Policy Office (Belspo), and it represented part of the Belgian federal contribution to the European Research Infrastructure Consortium LifeWatch (November 2015 – February 2022).

Non-native species which are being introduced into Europe, whether by accident or deliberately, can be of policy concern since some of them can reproduce and disperse rapidly in a new territory, establish viable populations and even outcompete native species. As a consequence of their presence, natural and managed ecosystems can be disrupted, crops and livestock affected, and vector-borne diseases or parasites might be introduced, impacting human health and socio-economic activities. Non-native species causing such adverse effects are called Invasive Alien Species (IAS). In order to protect native biodiversity and ecosystems, and to mitigate the potential impact on human health and socio-economic activities, the issue of IAS is tackled in Europe by EU Regulation 1143/2014 of the European Parliament and Council. The IAS Regulation provides for a set of measures to be taken across all member states. The list of *Invasive Alien Species of Union Concern* is regularly updated. However, to implement the proposed actions, methods for accurate species identification are required when suspicious biological material is encountered.

Because morphology-based species identifications are not always possible (e.g. cryptic species, trace material, early life-stages), the purpose of the present work is to investigate and evaluate the usefulness of DNA sequence data to identify each of the IAS included in the EU Regulation. The results are presented as factsheets (one per IAS) compiled using publicly available DNA sequence data and information aggregated from various sources. Each factsheet consists of two major parts: (i) a short introduction to the specific IAS, with information on its taxonomy and current occurrence/distribution in Europe, (ii) an investigation with respect to the usefulness of publicly available DNA sequences to identify this IAS using DNA barcoding to the taxonomic level stated in the EU list. For further information about the reasoning behind the applied approach and details on the materials and methods utilised, please see below and Smitz *et al.* [1].

More info about BopCo on <https://bopco.be> or contact us via bopco@naturalsciences.be.

More info on the EU Regulation on http://ec.europa.eu/environment/nature/invasivealien/index_en.htm.

Myocastor coypus

(Molina, 1782)

Common names:

English: coypu, nutria

French: ragondin, rat gondin

German: Nutria, Sumpfbiber, Schweifbiber, Schweifratte, Coypu

Dutch: beverrat, nutria

Last update: October 2019



General information on *Myocastor coypus*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Mammalia	Rodentia	Myocastoridae	<i>Myocastor</i>

Species in the same genus: N = 1 [2, 3]

Note: We follow the classification as presented by Patton *et al.* [2]. *Myocastor coypus* is the only extant representative for the genus *Myocastor* and the family Myocastoridae.

Infra-species level: N = 4 [2, 4 - 6]

Note: Four subspecies are recognised: *M.c. bonariensis*, *M.c. coypus*, *M.c. melanops* and *M.c. sanctaecruzae*.



Native range: [2, 5, 7]

Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay.

Invasive range: [5, 7, 8]

Europe (geographical):

Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Romania, Russia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

For more detailed locality information and the most recent distribution updates, please visit:

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R09720>

<https://www.gbif.org/species/3240573>

<https://www.iucnredlist.org/species/14085/121734257>

<https://eol.org/pages/328471>

Outside Europe (geographical):

Armenia, Azerbaijan, Botswana, Canada, China, Georgia, Israel, Japan, Jordan, Kazakhstan, Kenya, Korea, Tajikistan, Tanzania, Thailand, Turkey, Turkmenistan, United States of America, Zambia, Zimbabwe.

Morphology, biology, invasion, negative effects and remedies

For more information on *Myocastor coypus* please see the references and online information listed at the end of this document.



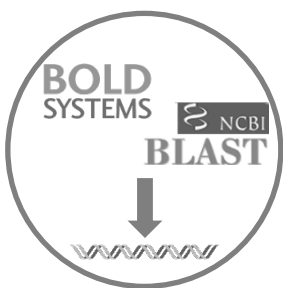
Species identification based on DNA barcodes

Introduction

DNA barcoding is a species identification method that uses a short genetic sequence (DNA barcode) to compare an unknown sample to a database of reference sequences with known species affiliations. The underlying rationale is that the divergence of nucleotide sequences among different species is larger than the nucleotide divergence between sequences within a species. DNA barcoding can facilitate the identification of IAS samples, especially when morphological characteristics are absent or useless. However, to assure correct species identifications, reference libraries need to include a sufficiently large number of sequences of (i) the IAS under investigation to assess the intraspecific genetic divergence, (ii) the closely related species to evaluate the interspecific genetic divergence, and (iii) the different geographical areas covering the distribution range (native and invasive) of the IAS to detect potential population structure or local hybrids.

In this context, BopCo evaluated the inclusion of the IAS and their close relatives in both publicly available reference libraries BOLD (www.boldsystems.org/) and GenBank (www.ncbi.nlm.nih.gov/nucleotide/) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

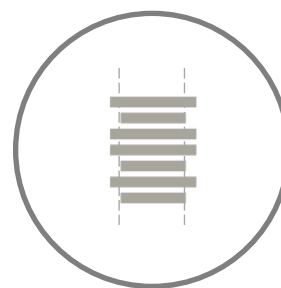
Material and Methods [1]



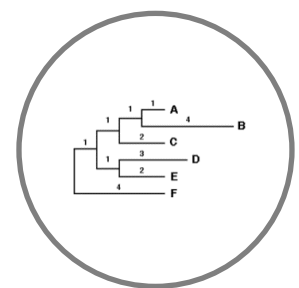
Download all sequence data available for the genus



Filtering the data and selecting 'promising' markers



Aligning and trimming of the sequences



Building Neighbour-Joining tree with Bootstrap support

Conclusion

Based on the present evaluation of the available sequence data, 12S and cytb are the most reliable DNA markers to identify *Myocastor coypus*. The missing species should be added to the analysis to allow for a better evaluation of the ability of 12S and cytb to identify *Myocastor coypus*.

Discussion

Since Myocastoridae is a monotypic family, DNA markers for which *Myocastor coypus* sequences were available, were downloaded from GenBank and BOLD for all represented species of the tribe Myocastorini (as defined by Fabre [3, 9]). Five DNA markers were evaluated thoroughly and are discussed below (Table 1).

In the NJ-tree for **12S**, all genera of the tribe Myocastorini cluster separately with high support. *Myocastor coypus* and other species in the tribe Myocastorini are currently better represented by cytb in online databases (Table 2). The NJ-tree for **cytb** shows high support for the cluster of *Myocastor coypus* sequences, but doesn't represent a clear distinction for the genus *Proechimys*. Therefore, a combination of markers 12S and cytb is currently the best choice for a reliable identification of *M. coypus*.

The **CR** NJ-tree shows a better support for different genera compared to other markers. However, the genus *Hopломys* does not represent a separate cluster. The NJ-tree for **COI** has a similar result compared to NJ-tree of cytb. For both markers COI and CR only two sequences for *M. coypus* are available. Adding sequences for *M. coypus* will allow for a better evaluation of the performance of markers COI and CR.

In the NJ-tree for **16S**, sequences of *M. coypus* cluster with high support, except for one (GenBank accession number AF422886). This sequence might involve a misidentification. If so, 16S represents a promising DNA marker for the identification of *M. coypus*.

Due to the large number of mitochondrial genomes in GenBank, many genes were available for evaluation. However as most of them give a similar result (NJ-tree) to markers listed above they are not individually mentioned here.



Table 1: Overview of the encountered issues concerning the DNA-based identification of the IAS [1]: (1) Insufficient publicly available DNA sequences of the IAS to capture the intra-species divergence; (2) Poor geographical coverage of the IAS sequences (native or invasive range missing); (3) The IAS sequences do not form supported clusters; (4) Potential misidentification of a specimen which influences the clustering of the IAS sequences; and (5) Not all congeneric species are represented in the final NJ-tree. An 'X' indicates that the issue was encountered.

Markers analysed	1	2	3	4	5
COI	X				X
CR	X				X
cytb	X				X
12S	X				X
16S	X		X	X	X

Table 2: Publicly available sequences downloaded (October 2019) from BOLD and GenBank (including sequences extracted from mitochondrial genomes) which were withheld as reliable and informative in the final alignment that was used for building the NJ-trees. The species names follow [2]. An 'X' indicates that at least one sequence was used in the final alignment. A '1' indicates only one sequence was available.

Species in tribe	COI	CR	cytb	12S	16S
<i>Callistomys pictus</i>	1	1	X	X	1
<i>Hoplomys gymnurus</i>	1	1	X	X	X
<i>Myocastor coypus</i>	X	X	X	X	X
<i>Proechimys brevicauda</i>	1	X	1	X	X
<i>Proechimys canicollis</i>					
<i>Proechimys chrysaecolus</i>					
<i>Proechimys cuvieri</i>	X	X	X	X	X
<i>Proechimys decumanus</i>					
<i>Proechimys echinothrix</i>	X	X	X	X	X
<i>Proechimys gardneri</i>	X	X	X	X	X
<i>Proechimys goeldii</i>	X	X	X	X	X
<i>Proechimys guairae</i>	X	X	X	X	X
<i>Proechimys guyannensis</i>	X	X	X	X	X
<i>Proechimys hoplomysoides</i>	X	X	1	X	1
<i>Proechimys kulinae</i>	X	X	X	X	X
<i>Proechimys longicaudatus</i>	X	X	X	X	X
<i>Proechimys mincae</i>	X	X	X	X	X
<i>Proechimys oconnelli</i>					
<i>Proechimys pattoni</i>	X	X	X	X	X
<i>Proechimys quadruplicatus</i>	X	X	X	X	X
<i>Proechimys roberti</i>	X	X	X	X	X
<i>Proechimys semispinosus</i>	X	X	X	X	X
<i>Proechimys simonsi</i>	X	X	X	X	X
<i>Proechimys steerei</i>	X	X	X	X	X
<i>Proechimys trinitatis</i>	X	X	X	X	X
<i>Thrichomys apereoides</i>	1	1	X	X	1
<i>Thrichomys inermis</i>			X		
<i>Thrichomys laurentius</i>			X		
<i>Thrichomys pachyurus</i>			X		
TOTAL species	22/29	22/29	25/29	22/29	22/29

For a more elaborate discussion of the available databases, the sequence selection process, the outcome of the NJ-tree analyses, the usefulness of the investigated DNA sequences for species identification, as well as information on how to send samples for analyses please contact BopCo directly.



References and online information

Online information

<http://www.iucngisd.org/gisd/species.php?sc=99>

<https://www.cabi.org/isc/datasheet/73537>

<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=2282> (UK)

<https://ias.biodiversity.be/species/show/27> (Belgium)

<https://www.fws.gov/fisheries/ANS/erss/highrisk/Myocastor-coypus-ERSS-FINAL-Sept-2017.pdf> (USA)

Picture credits

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Page 2 (middle): *Myocastor coypus* Biberratte By Mike Krüger [CC BY-SA 3.0]

Page 2 (right): Ragondin *Myocastor coypus* By Lucas Fernandez [CC BY-SA 4.0]

References

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