



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.

Ondatra zibethicus

(Linnaeus, 1766)

Common names:

English: muskrat, common (American) muskrat,
marsh hare, marsh rabbit, swamp rabbit

French: rat musqué

German: Bisam, Bisambiber, Bisamratte

Dutch: muskusrat



Last update: October 2019

General information on *Ondatra zibethicus*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Metazoa	Chordata	Mammalia	Rodentia	Cricetidae	<i>Ondatra</i>

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

Note: The genus *Ondatra* belongs to the subfamily Arvicolinae and includes one species: *Ondatra zibethicus*.

Infra-species level: N = 16 [3, 4]

Note: the species *Ondatra zibethicus* includes 16 subspecies: *O.z. albus*, *O.z. aquihnis*, *O.z. bemarki*, *O.z. cinnamominus*, *O.z. macromom*, *O.z. mergens*, *O.z. obscurus*, *O.z. occipitalis*, *O.z. osoyoosensis*, *O.z. pallidus*, *O.z. ripensis*, *O.z. rivalicus*, *O.z. roidmani*, *O.z. spatulatus*, *O.z. zalaphus* and *O.z. zibethicus*.



Native range: [4, 5]

Canada, Mexico and USA.

Invasive range: [4, 5]

Europe (geographical):

Albania, Austria, Belarus, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Sweden, Switzerland, Ukraine 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/R10336>

<https://www.cabi.org/ISC/datasheet/71816>

<https://www.gbif.org/species/5219858>

Outside Europe (geographical):

Argentina, Chile, China, Japan, Kazakhstan, Kyrgyzstan, Mongolia and Uzbekistan.

Morphology, biology, invasion, negative effects and remedies

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



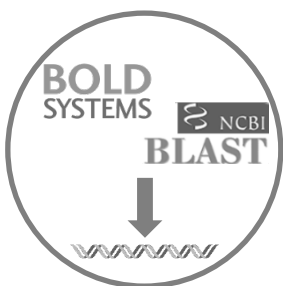
Species identification based on DNA barcodes

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.

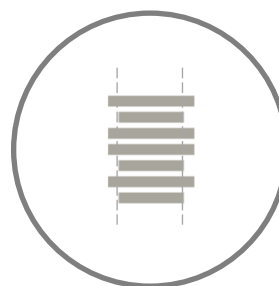
Material and Methods [1]



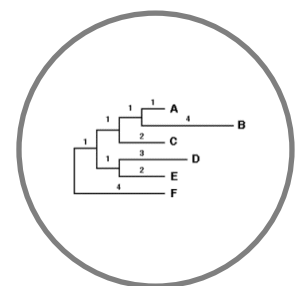
Download all sequence data available for the genus



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, *cytb* seems the most reliable DNA marker to identify *Ondatra zibethicus*. The missing genera should be added to the analysis to allow for a better evaluation of the ability of *cytb* to identify the *O. zibethicus* species.

Discussion

Since *Ondatra* is a monotypic genus, DNA markers for which *Ondatra zibethicus* sequences were available, were downloaded from GenBank and BOLD for all represented species of the subfamily Arvicolinae (according to Robovský *et al.* [6]). Due to the large amount of species in this subfamily (158 species), only genera are considered in Table 2. Three DNA markers were evaluated thoroughly and are discussed below (Table 1).

NJ-trees for **cytb** and **COI** currently give the best representation of the subfamily Arvicolinae, though missing genera should be added (Table 2) to allow for a better evaluation of both markers. For both NJ-trees *O. zibethicus* sequences cluster with maximum support. *Cytb* seems the best marker to confidently identify *O. zibethicus* due to a better sequence representation of the genera compared to COI (Table 2).

In the NJ-tree for **12S**, *O. zibethicus* sequences cluster together with high support. Sequences for *O. zibethicus* are well represented for this marker, however, few other genera in the subfamily Arvicolinae are available in online databases. Adding sequences for the missing species/genera, will allow for a better evaluation of the performance of 12S.

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) Insufficient publicly available DNA sequences of the subfamily to capture the inter-species divergence. An 'X' indicates that the issue was encountered. A '1' indicates only one *O. zibethicus* sequence was available.

Markers analysed	1	2	3	4	5
COI					X
cytb					X
12S	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. Species were grouped by genus, with the number in brackets indicating the number of species following [6]. An 'X' indicates that at least one sequence was used in the final alignment. A '1' indicates only one sequence was available.

Genera in subfamily	COI	cytb	12S
<i>Alticola</i> (12)	1	X	
<i>Arborimus</i> (3)	X	X	
<i>Arvicola</i> (3)	X	X	
<i>Blanfordimys</i> (2)		X	
<i>Caryomys</i> (2)	1	X	X
<i>Chionomys</i> (3)		X	1
<i>Dicrostonyx</i> (8)	X	X	
<i>Dinaromys</i> (1)		X	
<i>Ellobius</i> (5)	X	X	1
<i>Eolagurus</i> (2)	X	X	
<i>Eothenomys</i> (10)	X	X	X
<i>Hyperacrius</i> (2)		1	
<i>Lagurus</i> (1)	X	X	
<i>Lasiopodomys</i> (3)	X	X	X
<i>Lemmiscus</i> (1)	X		
<i>Lemmus</i> (5)	X	X	
<i>Microtus</i> (63)	X	X	X
<i>Myodes</i> (12)	X	X	X
<i>Myopus</i> (1)	X		
<i>Neodon</i> (7)	X	X	X
<i>Neofiber</i> (1)		1	
<i>Ondatra</i> (1)	X	X	X
<i>Phaiomys</i> (1)			
<i>Phenacomys</i> (2)	X	X	
<i>Proedromys</i> (2)	X	X	1
<i>Prometheomys</i> (1)		X	
<i>Synaptomys</i> (2)	1	X	
<i>Volemys</i> (2)	X	X	
TOTAL species	80/158	134/158	30/158

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

https://www.nobanis.org/globalassets/speciesinfo/o/ondatra-zibethicus/ondatra_zibethicus.pdf

<https://ias.biodiversity.be/species/show/28>

<https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-muskusrat>

<https://circabc.europa.eu/sd/a/7cf3a0bb-8ac3-48be-8e12-12c7783caa3e/TSSR-2016-003%20Ondatra%20zibethicus.pdf>

<https://www.iucnredlist.org/species/15324/22344525>

https://animaldiversity.org/accounts/Ondatra_zibethicus/

Picture credits

Page 1: *Ondatra zibethicus* eating a carrot at Steinbrücker Teich, Darmstadt, Germany By Alexander Klink [CC BY 4.0]

Page 2 (left): *Ondatra_zibethicus_CT* By Cephas [CC BY-SA 3.0]

Page 2 (right): *Ondatra zibethicus* By David Menke [Public domain]

References

- [1] N. Smitz, S. Gombeer, K. Meganck, A. Vanderheyden, Y. R. Van Bourgonie, T. Backeljau, and M. De Meyer, "Identifying IAS based on DNA barcoding using currently available sequence data: details on applied material and methods." 2019. [Online]. Available from: <https://bopco.be/output/iasfactsheets>.
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- [3] S. Laurence, D. W. Coltman, J. C. Gorrell, and A. I. Schulte-Hostedde, "Genetic structure of muskrat (*Ondatra zibethicus*) and its concordance with taxonomy in North America" *Journal of Heredity*, vol. 102, no. 6, pp. 688–696, 2011.
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- [6] J. Robovský, V. Řičánková, and J. Zrzavý, "Phylogeny of Arvicolinae (Mammalia, Cricetidae): Utility of morphological and molecular data sets in a recently radiating clade" *Zoologica Scripta*, vol. 37, no. 6, pp. 571–590, 2008.

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