



Identification of Invasive Alien Species using DNA barcodes

Royal Belgian Institute of Natural Sciences
Rue Vautier 29,
1000 Brussels, Belgium
+32 (0)2 627 41 23



Royal Museum for Central Africa
Leuvensesteenweg 13,
3080 Tervuren, Belgium
+32 (0)2 769 58 54



General introduction to this factsheet

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 in Belgium and Europe. The project represents part of the Belgian federal contribution to the European Research Infrastructure Consortium LifeWatch.

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. In order to implement the proposed actions, however, 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 compiling 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 to the taxonomic level stated in the EU list using DNA barcoding. For further information about the reasoning behind the applied approach and details on the materials and methods utilised, please see below and Smits *et al.* [1].

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

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

Procyon lotor

(Linnaeus, 1758)

Common names:

English: raccoon, common raccoon, northern raccoon

French: raton laveur

German: Waschbär

Dutch: wasbeer

Last update: October 2019



General information on *Procyon lotor*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Mammalia	Carnivora	Procyonidae	<i>Procyon</i>

Species in the same genus: N = 3 [2-6]

Infra-species level: N = 22 [5-6]

Note: Twenty-two subspecies have been recognised: *P.l. auspicatus*, *P.l. elucus*, *P.l. excelsus*, *P.l. fuscipes*, *P.l. gloveralleni*, *P.l. grinnelli*, *P.l. hernandezii*, *P.l. hirtus*, *P.l. incautus*, *P.l. inesperatus*, *P.l. insularis*, *P.l. litoreus*, *P.l. lotor*, *P.l. marinus*, *P.l. maynardi*, *P.l. megalodus*, *P.l. pacificus*, *P.l. pallidus*, *P.l. psora*, *P.l. pumilus*, *P.l. simus*, and *P.l. vancouverensis*.



Native range: [3], [7-10]

North and Central America.

Invasive range: [3], [7-12]

Europe (geographical):

Austria, Belarus, Belgium, Croatia, Czech Republic, Denmark, Estonia, France, Germany, Hungary, Italy, Ireland, Liechtenstein, Lithuania, Luxembourg, Netherlands, Norway, Poland, Romania, Russia (European part), Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine and United Kingdom.

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

<http://alien.jrc.ec.europa.eu/SpeciesMapper>

<https://www.gbif.org/species/5218786>

<https://www.iucnredlist.org/species/41686/45216638>

<https://www.cabi.org/isc/datasheet/67856>

Outside Europe (geographical):

Azerbaijan, Georgia, Guadeloupe, Japan, Martinique, Russia (Asian part) and Uzbekistan.

Morphology, biology, invasion, negative effects and remedies

For more information on *Procyon lotor* 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. To assure correct species identifications, however, reference libraries need to include a sufficiently large number of sequences of (i) the IAS under investigation, in order to assess the intraspecific genetic divergence; (ii) the closely related species, in order to evaluate the interspecific genetic divergence; (iii) the different geographical areas covering the distribution range (native and invasive) of the IAS in order to detect potential population structure or local hybrids.

Against this background, 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]



Conclusion

Based on the present evaluation of the available sequence data, *cytb* is the most reliable DNA marker for the identification of *Procyon lotor*. To allow for a better evaluation of the performance of this marker for species identification, the missing species, especially *P. pygmaeus*, should be added to the analyses.

Discussion

Since the genus *Procyon* comprises only three species, DNA markers for which *Procyon* sequences were available, were downloaded from GenBank and BOLD for all represented species of the family Procyonidae (as defined by Wozencraft [6]). In current databases no DNA sequences could be found for *Procyon pygmaeus*. Eight DNA markers were evaluated (Table 1).

In the NJ-tree for **cytb**, almost all Procyonidae species are represented and the *P. lotor* sequences cluster together with maximum support. However, this cluster also contains one *P. cancrivorus* sequence (GenBank accession number AB285333) which is not clustering with the other *P. cancrivorus* sequences. This sequence might involve a misidentification. If so, *cytb* currently represents the most promising DNA marker for the identification of *P. lotor*.

The *P. lotor* **COI** sequences cluster together, yet only eight out of the fourteen Procyonidae species are represented in the NJ-tree (Table 2). Adding sequences for the missing species will allow to better evaluate the performance of COI for the identification of *P. lotor*.

For **16S**, **ND5**, **ATP6**, **PNOC**, **Rag 1** and **Rag2** either: fewer *P. lotor* sequences are available, none cover the native region and/or less Procyonidae species are represented compared to other markers (Tables 1 & 2). Therefore it is currently not advisable to apply these markers for species identification.

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 species of the family are represented in the final NJ-tree. An 'X' indicates that the issue was encountered.



Markers analysed	1	2	3	4	5
COI					X
cytb			X	X	X
ND5		X			X
ATP6		X			X
PNOC	X	X			X
Rag 1	X	X	X		X
Rag 2	X	X			X
16S		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 [6]. An 'X' indicates that at least one sequence was used in the final alignment. A '1' indicates only one unique sequence was available. Species names with * are not mentioned in [6] but used on GenBank.

Species in family	COI	Cytb	ND5	ATP6	PNOC	Rag 1	Rag 2	16S
<i>Bassaricyon alleni</i>	1	X	1	1	X	1	X	1
<i>Bassaricyon beddardi</i>	1	1						
<i>Bassaricyon gabbii</i>	1	X	X		1	1	1	X
<i>Bassaricyon lasius</i>								
<i>Bassaricyon pauli</i>								
<i>Bassaricyon neblina*</i>		X						
<i>Bassariscus astutus</i>	1	1	1	1	X	1	X	
<i>Bassariscus sumichrasti</i>		1	1		1	1		
<i>Nasua narica</i>	X	X	X		1	1	1	X
<i>Nasua nasua</i>	X	X	X	X	X	1	X	X
<i>Nasua meridensis*</i>		1						
<i>Nasua olivacea</i>		X						
<i>Potos flavus</i>	X	X	1		X	1	X	
<i>Procyon cancrivorus</i>	X	X	1	X	1	X	1	
<i>Procyon lotor</i>	X	X	X	X	X	X	X	X
<i>Procyon pygmaeus</i>								
TOTAL species	9/14	11/14	9/14	5/14	9/14	9/14	8/14	5/14

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.iucnredlist.org/species/41686/45216638>
<https://www.cabi.org/isc/datasheet/67856>
<https://www.pbs.org/wnet/nature/raccoon-nation-raccoon-fact-sheet/7553/>
<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=2839>
https://www.nobanis.org/globalassets/speciesinfo/p/procyon-lotor/procyon_lotor.pdf
<https://ias.biodiversity.be/species/show/29>

Picture credits

Page 1: *Procyon lotor* By Arthur Chapman [CC BY-NC-SA 2.0]
Page 2 (left): *Procyon lotor*_qt13 By Quartl [CC-BY-SA-3.0]
Page 2 (right): *Procyon lotor*_(Linnaeus_1758) By Michael Gäbler [CC-BY-3.0]

References

- [1] N. Smits, 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: <http://bopco.myspecies.info/content/invasive-alien-species-ias-factsheets>.
- [2] K. M. Helgen and D. E. Wilson, *A systematic and zoogeographic overview of the raccoons of Mexico and Central America*. Mexico City, Mexico: Instituto de Biología e Instituto de Ecología, UNAM, 2005.
- [3] K. M. Helgen and D. E. Wilson, "Taxonomic status and conservation relevance of the raccoons (*Procyon spp.*) of the West Indies," *Journal of Zoology*, vol. 259, no. 1, pp. 69–76, 2003.
- [4] A. de Villa-Meza, R. Avila-Flores, A. D. Cuarón, and D. Valenzuela-Galván, "*Procyon pygmaeus* (Carnivora: Procyonidae)," *Mammalian Species*, vol. 43, no. 877, pp. 87–93, 2011.
- [5] ITIS, "*Procyon lotor*," *Integrated Taxonomic Information System*. [Online]. Available: https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=180575#null. [Accessed: 16-Oct-2019].
- [6] W. Wozencraft, "Order Carnivora", in *Mammal Species of the World: A taxonomic and geographic reference*, 3rd ed., D. E. Wilson and D. M. Reeder, Eds. Baltimore, Maryland: John Hopkins University Press, 2005, pp. 532–628.
- [7] R. Timm, A. D. Cuarón, K. Helgen, and J. F. González-Maya, "*Procyon lotor*," *The IUCN Red List of Threatened Species*, 2016. [Online]. Available: <https://www.iucnredlist.org/species/41686/45216638>. [Accessed: 16-Oct-2019].
- [8] K. Helgen, J. Maldonado, D. Wilson, and S. Buckner, "Molecular Confirmation of the Origin and Invasive Status of West Indian Raccoons," *Journal of Mammalogy*, vol. 89, no. 2, pp. 282–291, 2008.
- [9] K. Kauhala, "Introduced carnivores in Europe with special reference to central and northern Europe," *Wildlife Biology*, vol. 2, no. 1, pp. 197–204, 1996.
- [10] CABI, "*Procyon lotor*," *Invasive Species Compendium*. Wallingford, UK: CAB International., 2019. [Online]. Available: <https://www.cabi.org/isc/datasheet/67856#tonotesOnTaxonomyAndNomenclature>. [Accessed: 16-Oct-2019].
- [11] J. T. García *et al.*, "Recent invasion and status of the raccoon (*Procyon lotor*) in Spain," *Biological Invasions*, vol. 14, no. 7, pp. 1305–1310, 2012.
- [12] A. C. Frantz, P. Cyriacks, and L. Schley, "Spatial behaviour of a female raccoon (*Procyon lotor*) at the edge of the species' European distribution range," *European Journal of Wildlife Research*, vol. 51, no. 2, pp. 126–130, 2005.

To cite this factsheet, please use

Barcoding Facility for Organisms and Tissues of Policy Concern, 2019. Factsheet on *Procyon lotor*; October 2019. In: Identification of Invasive Alien Species using DNA barcodes. BopCo, Belgium. Available from: www.bopco.myspecies.info/content/invasive-alien-species-ias-factsheets, accessed on DD-MM-YYYY.



DISCLAIMER: The information represented in this factsheet has been compiled from many different sources. Every reasonable effort has been made to ensure that the material presented is accurate and reflects the current (see date last update) scientific knowledge. However, recent changes in e.g. taxonomy and distribution, or the publication of additional reference sequences may not be implemented. The views which are expressed in the “Conclusion” are those of the author(s) and have not been peer-reviewed. BopCo does not guarantee the accuracy of the data included in this factsheet. The content of the factsheet is for information only and is not intended as legal advice. BopCo may not be held responsible for the use which may be made of the information contained therein. If you should notice any issues considering the content of this factsheet, or if you would like to contribute any additional information to it, please contact us through bopco@naturalsciences.be.

