



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.

Nyctereutes procyonoides

(Gray, 1834)

Common names:

English: raccoon dog

French: chien viverrin, chien martre

German: Marderhund, Tanuki, Enok, Obstfuchs

Dutch: wasbeerhond, marterhond



Last update: August 2019

General information on *Nyctereutes procyonoides*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Mammalia	Carnivora	Canidae	<i>Nyctereutes</i>

Species in the same genus: N = 1 [2-4]

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

Note: Six subspecies are usually distinguished: *N.p. albus*, *N.p. koreensis*, *N.p. orestes*, *N.p. procyonoides*, *N.p. ussuriensis* and *N.p. viverrinus*.



Native range: [5-7]

Southern China, Japan, North and South Korea, eastern Mongolia, (Asian part) Russia and northern Vietnam.

Invasive range: [5-10]

Europe (geographical):

Austria, Belarus, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Lithuania, Macedonia, Moldova, Netherlands, Norway, Poland, Romania, (European part) Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine and United Kingdom (only two confirmed sightings).

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R10085>

<https://www.gbif.org/species/2434552>

Outside Europe (geographical):

Armenia, Azerbaijan, Georgia and Kazakhstan.

Morphology, biology, invasion, negative effects and remedies

For more information on *Nyctereutes procyonoides* 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/nuccore/) 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, D-loop and *cytb* are the most reliable DNA markers for the identification of *Nyctereutes procyonoides*.

Discussion

Since *Nyctereutes* is a monotypic genus, markers for which *N. procyonoides* sequences were available were downloaded from GenBank and BOLD for all species of the family Canidae. Seven DNA markers were evaluated (Table 1).

For each DNA marker, the *N. procyonoides* sequences form a well-supported cluster in the NJ-tree. Considering the number of represented Canidae species, **D-loop** followed by ***cytb*** are the preferred markers to identify *N. procyonoides*. Some of the species which are not yet represented for these markers (Table 2) belong to the Vulpini, the fox-like canids to which all *Nyctereutes*, *Otocyon*, *Urocyon* and *Vulpes* species belong [9]. Adding sequences for these species would allow to better evaluate the performance of these markers for the identification of *N. procyonoides*.

Fewer Canidae species are represented in the **COI**, **COII** and **12S** NJ-trees, yet these NJ-trees also recover *N. procyonoides* as a well-supported cluster. These DNA markers are promising alternatives for D-loop and *cytb*, and additional sequences for the missing species would allow for a better evaluation of these markers.

For **16S** and the **TRSP** gene fewer *N. procyonoides* sequences are available and they originate only from the native region. Additional sequences for *N. procyonoides*, especially from the invasive regions, should be added to the analyses to fully assess the ability of these markers to identify *N. procyonoides*.



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
cytb					X
D-loop					X
COII					X
16S		X			X
12S					X
TRSP	X	X			X

Table 2: Publicly available sequences downloaded (August 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 [4]. An 'X' indicates that at least one sequence was used in the final alignment.

Species in family	COI	cytb	D-loop	COII	16S	12S	TRSP
<i>Atelocynus microtis</i>		X		X			X
<i>Canis anthus</i>	X	X	X	X	X	X	
<i>Canis aureus</i>	X	X	X	X	X	X	X
<i>Canis himalayensis</i>		X	X				
<i>Canis indica</i>		X	X				
<i>Canis latrans</i>	X	X	X	X	X	X	X
<i>Canis lupus</i>	X	X	X	X	X	X	X
<i>Canis rufus</i>			X				
<i>Canis simensis</i>	X	X	X	X			X
<i>Cerdocyon thous</i>	X	X	X	X			X
<i>Chrysocyon brachyurus</i>	X	X	X	X	X	X	X
<i>Cuon alpinus</i>	X	X	X	X	X	X	X
<i>Lupulella adusta</i>	X	X	X	X	X	X	X
<i>Lupulella mesomelas</i>	X	X	X	X	X	X	X
<i>Lycalopex culpaeus</i>	X	X	X	X			X
<i>Lycalopex fulvipes</i>			X				X
<i>Lycalopex griseus</i>	X	X	X	X			X
<i>Lycalopex gymnocercus</i>	X	X	X	X			X
<i>Lycalopex sechurae</i>	X	X	X	X	X	X	X
<i>Lycalopex vetulus</i>		X	X	X			X
<i>Lycaon pictus</i>	X	X	X	X	X	X	X
<i>Nyctereutes procyonoides</i>	X	X	X	X	X	X	X
<i>Otocyon megalotis</i>	X	X	X	X	X	X	X
<i>Speothos venaticus</i>	X	X	X	X	X	X	X
<i>Urocyon cinereoargenteus</i>	X	X	X	X	X	X	X
<i>Urocyon littoralis</i>	X	X	X	X	X	X	X
<i>Vulpes bengalensis</i>							
<i>Vulpes cana</i>		X	X				X
<i>Vulpes chama</i>	X						X
<i>Vulpes corsac</i>	X	X	X	X	X	X	X
<i>Vulpes ferrilata</i>	X	X	X	X	X	X	
<i>Vulpes lagopus</i>	X	X	X	X	X	X	X
<i>Vulpes macrotis</i>	X	X	X	X			X
<i>Vulpes pallida</i>			X				
<i>Vulpes rueppellii</i>		X	X				X
<i>Vulpes velox</i>	X		X				
<i>Vulpes vulpes</i>	X	X	X	X	X	X	X
<i>Vulpes zerda</i>	X	X	X	X	X	X	X
TOTAL species	28/38	32/38	35/38	28/38	20/38	20/38	30/38

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.cabi.org/isc/datasheet/72656>

https://www.nobanis.org/globalassets/speciesinfo/n/nyctereutes-procyonoides/nyctereutes_procyonoides-final.pdf

www.nonnativespecies.org/downloadDocument.cfm?id=1411

<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=2377>

<https://ias.biodiversity.be/species/show/78>

<https://www.provincedeliege.be/sites/default/files/media/10915/Chien%20viverrin%20%28Nyctereutes%20procyonoides%29.pdf> [FR]

Picture credits

Page 1: Raccoon dog By Max Pixel [CC0]

Page 2 (left): *Nyctereutes procyonoides* By Piotr Kuczynski [CC BY-SA 3.0]

Page 2 (right): Tanuki, Raccoon Dog (*Nyctereutes procyonoides*) By Josh More [CC BY-NC-ND 2.0]

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