



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.

Muntiacus reevesi

(Ogilby, 1839)

Common names:

English: Reeves' muntjac, Formosan Reeves' muntjac, Chinese muntjac

French: muntjac

German: Chinesischer Muntjak

Dutch: muntjak

Last update: November 2018



General information on *Muntiacus reevesi*

Classification

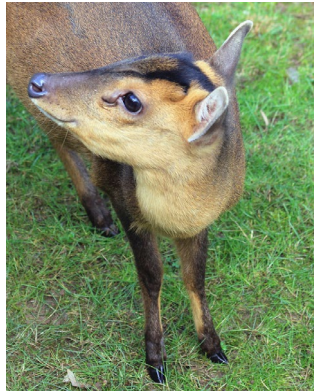
Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Mammalia	Artiodactyla	Cervidae	<i>Muntiacus</i>

Species in the same genus: N = 11 [2-3]

Note: We follow the classification as presented by Wilson & Reeder [2]. The genus *Muntiacus* belongs to the Cervinae subfamily.

Infra-species level: N = 3 [2-4]

Note: Three subspecies have been described: *M.r. jiangkouensis*, *M.r. micrurus*, *M.r. reevesi*.



Native range: [5]

China and Taiwan.

Invasive range: [6-10]

Europe (geographical):

Belgium, France, Ireland, Netherlands 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/R09640>

<https://www.gbif.org/species/2440946>

<https://www.cabi.org/ISC/datasheet/74281>

https://inpn.mnhn.fr/espece/cd_nom/61043

Outside Europe (geographical):

Japan.

Morphology, biology, invasion, negative effects and remedies

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



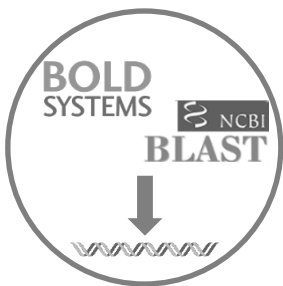
Species identification based on DNA barcodes

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.

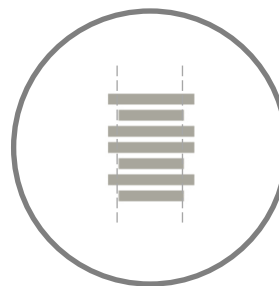
Material and Methods [1]



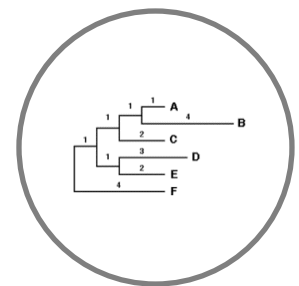
Download all sequence data available for the genus



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, ND4 and *cytb* are the most reliable DNA markers for the identification of *Muntiacus reevesi*. Adding sequences of the missing congeners, as well as of species now represented by one sequence only, will allow to better evaluate the performance of these markers for species identification.

Discussion

DNA markers for which *Muntiacus reevesi* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Muntiacus* (as defined by Wilson & Reeder [2]). Six DNA markers were evaluated (Table 1).

In the NJ-trees for **ND4** and ***cytb***, the *Muntiacus reevesi* sequences cluster together, yet the species is represented by a limited number of sequences for both markers. The overall support for the different *Muntiacus* species is higher in the ND4 NJ-tree, including *M. muntjak*. Additional sequences for *M. reevesi*, especially from its invasive regions, and of the (missing) congeners would allow to better evaluate the performance of the markers for species identification.

For **COI**, **16S**, **12S** and **D-Loop**, fewer sequences are available for both *M. reevesi* and its congeners. Also, low support for the represented congeners is observed when analysing the 16S and 12S markers. Therefore it is currently impossible to fully assess the ability of these markers to identify *M. reevesi*.



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
12S	X	X			X
16S		X			X
cytb		X			X
D-Loop	X	X			X
ND4		X			X

Table 2: Publicly available sequences downloaded (November 2018) 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 genus	COI	12S	16S	cytb	D-Loop	ND4
<i>Muntiacus atherodes</i>	1	1	1	X	1	1
<i>Muntiacus crinifrons</i>	X	1	X	X	X	X
<i>Muntiacus feae</i>			1	1		1
<i>Muntiacus gongshanensis</i>						1
<i>Muntiacus muntjak</i>	X	X	X	X	X	X
<i>Muntiacus puhoatensis</i>						
<i>Muntiacus putaoensis</i>	1	1	X	X	1	X
<i>Muntiacus reevesi</i>	X	X	X	X	X	X
<i>Muntiacus rooseveltorum</i>			X	X		X
<i>Muntiacus truongsoneensis</i>			1	X		X
<i>Muntiacus vuquangensis</i>	1	1	X	X	1	X
TOTAL species	6/11	6/11	9/11	9/11	6/11	10/11

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/42191/22166608>
<http://eol.org/pages/308477/overview>
<https://www.cabi.org/ISC/datasheet/74281>
https://animaldiversity.org/accounts/Muntiacus_reevesi/
<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=2263>
http://www.gt-ibma.eu/wp-content/uploads/2018/03/terlin_2017_oncfs_bilan-muntjac.pdf
http://www.mammal.org.uk/sites/default/files/factsheets/muntjac_deer_complete.pdf
<http://www.biodiversityireland.ie/record-biodiversity/invasive-species-union-concern/muntiacus-reevesi-gb-nnss-taxidermy-specimen/>
<http://especies-exotiques-envahissantes.fr/espece/muntiacus-reevesi/>
https://inpn.mnhn.fr/espece/cd_nom/61043?lg=en

Picture credits

Page 1: Male muntjac at Dumbleton Hall (UK) By Nilfanion [CC BY SA 3.0]
Page 2 (left): Reeves' muntjac *M. reevesi* from Malvern, UK By gailhampshire [CC BY 2.0]
Page 2 (middle): *M. reevesi* at the Zoo of Praha By Karelj [CC BY SA 3.0]
Page 2 (right): Chinese male muntjac *M. reevesi* at the Zoo of Augsburg By Rufus46 [CC BY SA 3.0]

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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- [7] CABI Invasive Species Compendium, "Datasheet *Muntiacus reevesi* (Reeves' muntjac)." [Online]. Available: <http://doi.wiley.com/10.1111/j.1365-2907.2010.00170.x>. [Accessed: 07-Nov-2018].
- [8] INPN- Inventaire National du Patrimoine Naturel, "*Muntiacus reevesi* (Ogilby, 1839) - Muntjac de Chine, Muntjac de Reeves - Présentation." [Online]. Available: https://inpn.mnhn.fr/espece/cd_nom/61043. [Accessed: 21-Nov-2019].
- [9] M. Terlin, "Une population de Muntjac de Reeves en région Centre-Val de Loire : Bilan des connaissances (2017). Délégation interrégionale Centre-Val de Loire, Ile-de-France.," 2017.
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