



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.

Herpestes javanicus

(E. Geoffroy Saint-Hilaire, 1818)

Common names:

English: common mongoose, small Asian mongoose,
small Javan mongoose

French: mangouste, mangus, petite mangouste Indienne

German: kleiner Mungo

Dutch: Indische mangoest



Last update: October 2019

General information on *Herpestes javanicus*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Mammalia	Carnivora	Herpestidae	<i>Herpestes</i>

Species in the same genus: N = 10 [2-5]

Note: Given the invasive distribution pattern, the specific mongoose to consider in this factsheet should probably be the subspecies *Herpestes javanicus auropunctatus* as only this subspecies is considered to be invasive [5]. *Herpestes javanicus auropunctatus*, however, is considered by many authors as a separate species *Herpestes auropunctatus* [3,5]. We follow the classification of Wozencraft *et al.* [4] in which *Herpestes javanicus auropunctatus* is considered a subspecies of *Herpestes javanicus*, since this seems to be the classification used on the list of species of concern to the European Union. In addition, we maintain the Asian representatives of the genus *Herpestes* within this genus notwithstanding it has been proposed to assign them to the genus *Urva* [2].

Infra-species level: N = 12 [4]

Note: Twelve subspecies are recognized : *H.j. auropunctatus*, *H.j. exilis*, *H.j. javanicus*, *H.j. orientalis*, *H.j. pallipes*, *H.j. palustris*, *H.j. peninsulae*, *H.j. perakensis*, *H.j. rafflesii*, *H.j. rubrifrons*, *H.j. siamensis*, *H.j. tjerapai*.



Native range: [3-9]

Afghanistan, Bangladesh, Bhutan, Cambodia, southern China, India, Indonesia (Sumatra), Iran, Iraq, Java, Jordan, Laos, Malaysia, Myanmar, Nepal, Oman, Pakistan, Saudi-Arabia, Singapore, Thailand, Vietnam.

H.j. auropunctatus:

Afghanistan, Bangladesh, Bhutan, India, Iran, Iraq, Jordan, Myanmar, Nepal, Oman, Pakistan, Saudi-Arabia.

Invasive range: [3-8],

“The many introduced populations of *H. javanicus* around the world are all believed to be of *H. auropunctatus*, but further confirmation is desirable” [3]

Europe (geographical):

H.j. auropunctatus:

Bosnia and Herzegovina, Croatia and Montenegro.

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

<https://www.gbif.org/species/2434271>

<https://www.iucnredlist.org/species/70203940/45207619#text-fields>

<https://www.iucnredlist.org/species/70204120/70204139>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R07276>

Outside Europe (geographical):

H.j. auropunctatus:

Antigua, lesser Antilles, Bahamas, Barbados, Belize, Bermuda, China, Colombia, Comoros, Cuba, Dominican Republic, Fiji, French Guiana, Grenada, Guadeloupe, Guyana, Haiti, Honduras, Jamaica, Japan, Mauritius, Panama, Puerto Rico, Suriname, Tanzania, Tobago, Tonga, Trinidad, United States (Hawaiian islands), Virgin Islands.

Morphology, biology, invasion, negative effects and remedies

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



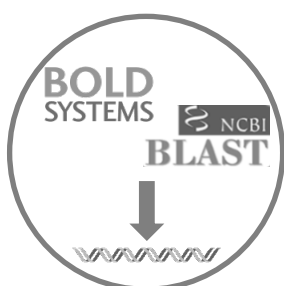
Species identification based on DNA barcodes

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.

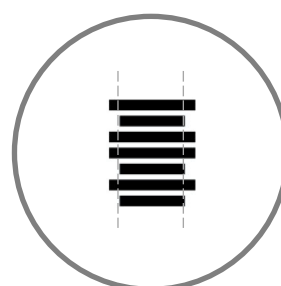
Material and Methods [1]



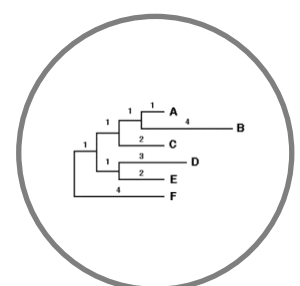
Download all sequence data available for the genus



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* is the most reliable DNA marker for the identification of *Herpestes javanicus auropunctatus*. To allow for a better evaluation of this marker, taxonomic confusion between *Herpestes javanicus auropunctatus* and *Herpestes javanicus javanicus* should be resolved in online databases.

Discussion

DNA markers for which *Herpestes* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus (as defined by Wozencraft *et al.* [4]). Five DNA markers were evaluated (Table 1). Since only *H.j. auropunctatus* is considered the invasive form [3], suitable DNA markers should be able to distinguish the subspecies. We therefore evaluate the reliability of the DNA markers to identify both at the species and the subspecies level.

In the NJ-tree based on **cytb** sequence data, *H.j. auropunctatus* and *H.j. javanicus* form two separate well-supported clusters. In addition, all *Herpestes* species are represented in the *cytb* dataset (Table 2). *Cytb* therefore seems reliable to identify *H. javanicus* from other *Herpestes* species and to distinguish the sequences belonging to the invasive subspecies *H.j. auropunctatus* from (non-invasive) sequences belonging to *H.j. javanicus*.

For **ND2** *H.j. javanicus* forms a separate cluster. However there are two distinct clusters for *H.j. auropunctatus*. Adding sequences for *H.j. auropunctatus* will allow for a better evaluation of the performance of ND2.

For **COI** only *H.j. auropunctatus* and few other *Herpestes* sequences are available. Therefore it is currently impossible to assess the ability of COI to distinguish *H.j. auropunctatus* from other *H.j. javanicus* sequences. **CR** and **FGB** do not separately cluster *H.j. javanicus* and *H.j. auropunctatus* sequences. The family Herpestidae is also poorly represented for CR (Table 2). Therefore it is not advisable to apply these markers for the identification of *H.j. auropunctatus*.



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' signifies that the issue was encountered, a 'J' signifies the issue was encountered specifically for *Herpestes javanicus javanicus* sequences an 'A' signifies the issue was encountered specifically for *Herpestes javanicus auropunctatus* sequences, while a '1' signifies only one target sequence was available for analysis.

Markers analysed	1	2	3	4	5
COI	J	J,A			X
cytb					
ND2			X		X
CR			X	X	X
FGB	J,A	A			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' signifies that at least one sequence was used in the final alignment. A '1' indicates only one sequence was available.

Species in genus	COI	cytb	ND2	CR	FGB
<i>Herpestes brachyurus</i>	X	X	X	1	X
<i>Herpestes edwardsi</i>		X	X	X	X
<i>Herpestes fuscus</i>		1	1		
<i>Herpestes icheumon</i>	X	X	1	X	1
<i>Herpestes javanicus javanicus</i>		X	X	X	X
<i>Herpestes javanicus auropunctatus</i>	X	X	X	X	X
<i>Herpestes naso</i>		1	1		1
<i>Herpestes semitorquatus</i>	1	1	X		1
<i>Herpestes smithii</i>		1			
<i>Herpestes urva</i>		1	X		1
<i>Herpestes vitticollis</i>		X			
TOTAL species	4/10	10/10	8/10	4/10	7/10

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/70203940/45207619>
<https://www.cabi.org/isc/datasheet/80508> (*H. auropunctatus*)
https://animaldiversity.org/accounts/Herpestes_javanicus/
<https://laji.fi/en/taxon/MX.47123/biology>
https://sta.uwi.edu/fst/lifesciences/sites/default/files/lifesciences/documents/ogatt/Herpestes_auropunctatus%20-%20Small%20Indian%20Mongoose.pdf
<http://www.iucngisd.org/gisd/species.php?sc=86>
<http://www.tsusinvasives.org/home/database/herpestes-javanicus>
<https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-indische-mangoeste> [NL]

Picture credits

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Page 2 (right): Small Asian Mongoose (*Herpestes javanicus*), Big Island Hawaii_By Peter Prokosch [CC BY-NC-SA 2.0]

References

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