



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) 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.

Callosciurus erythraeus

(Pallas, 1779)

Common names:

English: Pallas's squirrel, red-bellied tree squirrel, belly-banded squirrel

French: ecureuil à ventre rouge

German: Pallashörnchen, Rotbauchhörnchen

Dutch: Pallas eekhoorn

Last update: November 2018



General information on *Callosciurus erythraeus*

Classification

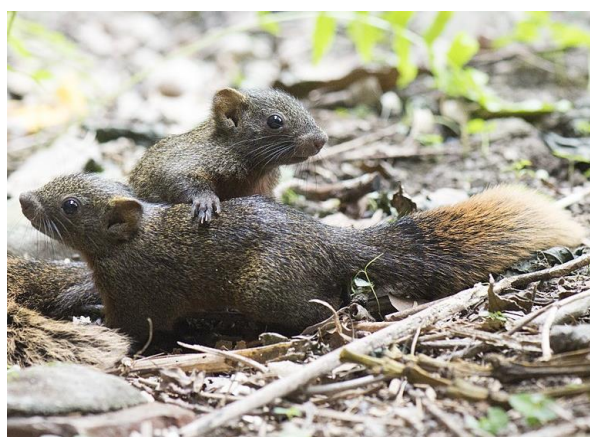
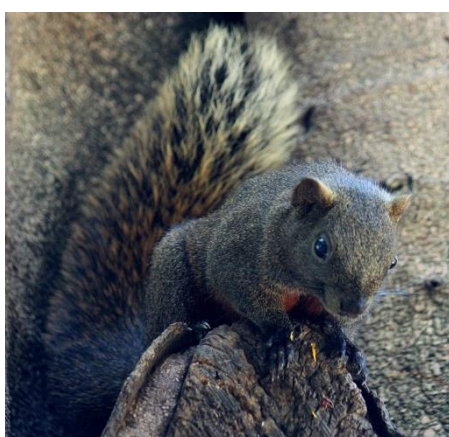
Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Mammalia	Rodentia	Sciuridae	<i>Callosciurus</i>

Species in the same genus: N = 14 [2–4]

Note: We follow the classification as presented by Thorington *et al.* [3]. Geographical variation in colour forms is considerable.

Infra-species level: N = 26 [2,5]

Note: *C.e. atrodorsalis*, *C.e. bartoni*, *C.e. bhutanensis*, *C.e. bonhotei*, *C.e. castaneiventris*, *C.e. erythraeus*, *C.e. erythrogaster*, *C.e. flavimanus*, *C.e. gloveri*, *C.e. gordonii*, *C.e. griseimanus*, *C.e. harringtoni*, *C.e. hendeei*, *C.e. hyperythrus*, *C.e. intermedius*, *C.e. michianus*, *C.e. ningpoensis*, *C.e. pranis*, *C.e. rubeculus*, *C.e. shanicus*, *C.e. siamensis*, *C.e. sladeni*, *C.e. styani*, *C.e. thai*, *C.e. haiwanensis* and *C.e. zimmeensis*.



Native range: [3,6,7]

Bangladesh, Bhutan, Cambodia, China, India, Laos, Malaysia, Myanmar, Taiwan, Thailand, Tibet and Vietnam.

Invasive range: [3,7,8]

Europe (geographical):

Belgium, France, Italy and Netherlands.

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/2437394>

<https://www.cabi.org/isc/datasheet/91200#toDistributionMaps>

https://fauna-eu.org/cdm_dataportal/taxon/51984315-18f3-4a8d-8e40-065bcc0bf410

Outside Europe (geographical):

Argentina, Hong Kong and Japan.

Morphology, biology, invasion, negative effects and remedies

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



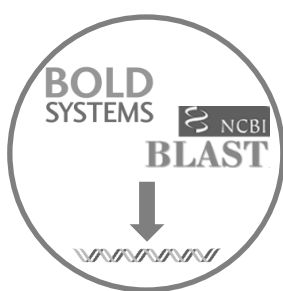
Species identification based on DNA barcodes

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.

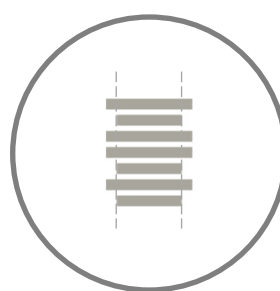
Material and Methods [1]



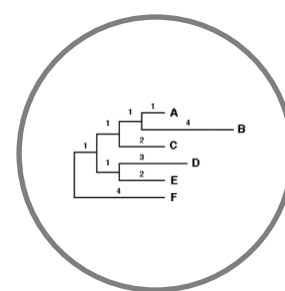
Download all sequence data available for the genus



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, no marker can reliably identify *Callosciurus erythraeus*. Cytb seems most promising, yet there is a need for a thorough systematic revision of the genus, and especially of the *C. erythraeus*-*C. finlaysonii* complex.

Discussion

DNA markers for which *Callosciurus erythraeus* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Callosciurus* (as defined by Thorington *et al.* [3]). Three DNA markers were evaluated (Table 1).

In all cases, the *C. erythraeus* sequences did not group in a single-species cluster in the NJ-trees. Multiple supported clusters are observed, but for all three DNA markers (**COI**, **cytb** and **CR**), one or two sequences of *Callosciurus finlaysonii* are placed within a *C. erythraeus* sequence cluster. Misidentifications cannot be eliminated since both species belong to the *C. erythraeus*-*C. finlaysonii* complex [9], which means that both species are hardly distinguishable based on morphological characteristics. Other publications raised this issue, and suggested that genetic and morphological data published in the last decades should therefore be thoroughly revised [10, 11]. Assuming that the mixed in *C. finlaysonii* sequences are indeed all misidentifications, *cytb* seems to be the most promising DNA marker for the identification of *C. erythraeus*. However, to better allow for the evaluation of the performance of *cytb* for species identification, sequences of the missing and less well represented congeners should be added.

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	X
cytb			X	X	X
CR			X	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 [3]. An 'X' indicates that at least one sequence was used in the final alignment, a '1' indicates only one unique sequence was available for the final alignment.

Species in genus	COI	cytb	CR
<i>Callosciurus adamsi</i>	X	1	
<i>Callosciurus baluensis</i>			
<i>Callosciurus caniceps</i>		X	X
<i>Callosciurus erythraeus</i>	X	X	X
<i>Callosciurus finlaysonii</i>	X	X	X
<i>Callosciurus inornatus</i>	1	X	
<i>Callosciurus melanogaster</i>			
<i>Callosciurus nigrovittatus</i>		X	
<i>Callosciurus notatus</i>	X	X	
<i>Callosciurus orestes</i>	1	1	
<i>Callosciurus phayrei</i>		1	
<i>Callosciurus prevostii</i>	X	X	X
<i>Callosciurus pygerythrus</i>			
<i>Callosciurus quinquestriatus</i>			
TOTAL species	7/14	10/14	4/14

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

<http://ias.biodiversity.be/species/show/126>
<https://www.iucnredlist.org/species/3595/22254356>
<https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-pallas-eekhoorn>
<https://species.biodiversityireland.ie/profile.php?taxonId=187074&taxonName=pallas>
<https://circabc.europa.eu/sd/a/59ccbe20-4953-4305-a486-c732e5c0e108/Identification%20guide.pdf>
<http://www.nonnativespecies.org/factsheet/downloadFactsheet.cfm?speciesId=4363>

Picture credits

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Page 2 (middle): Écureuil à ventre rouge à Taiwan By J. Patrick Fischer [CC BY-SA 3.0]
Page 2 (right): Deux jeunes *Callosciurus erythraeus* By LiCheng Shih [CC BY-SA 2.0]

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- [8] INPN, "*Callosciurus erythraeus* (Pallas, 1779) - Écureuil à ventre rouge - Présentation," *Inventaire National du Patrimoine Naturel*. [Online]. Available: https://inpn.mnhn.fr/espece/cd_nom/61174. [Accessed: 09-Nov-2018].
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