



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).

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.

Callosciurus finlaysonii

(Horsfield, 1823)

Common names:

English: Finlayson's squirrel or variable squirrel

French : ecureuil de Finlayson

German: Finlayson-Schénhörnchen

Dutch: Finlaysonklappereekhoorn, Thaise eekhoorn

Last update: August 2023



General information on *Callosciurus finlaysonii*

Classification

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

Species in the same genus: N = 14 ²

Note: Because of their close phylogenetic relationship and their considerable color variation (see.g. Figure 1), *C. finlaysonii* and *C. erythraeus* have been referred to by Timmins & Duckworth ³ as the *C. erythraeus/finlaysonii* complex.

Infra-species level: N = 16 ²

Note: *C.f. albivexilli*, *C.f. annellatus*, *C.f. bocourti*, *C.f. boonsongi*, *C.f. cinnamomeus*, *C.f. ferrugineus*, *C.f. finlaysonii*, *C.f. folletti*, *C.f. frandseni*, *C.f. germani*, *C.f. harmandi*, *C.f. menamicus*, *C.f. nox*, *C.f. sinistralis*, *C.f. trotter*, *C.f. Williamsoni*.

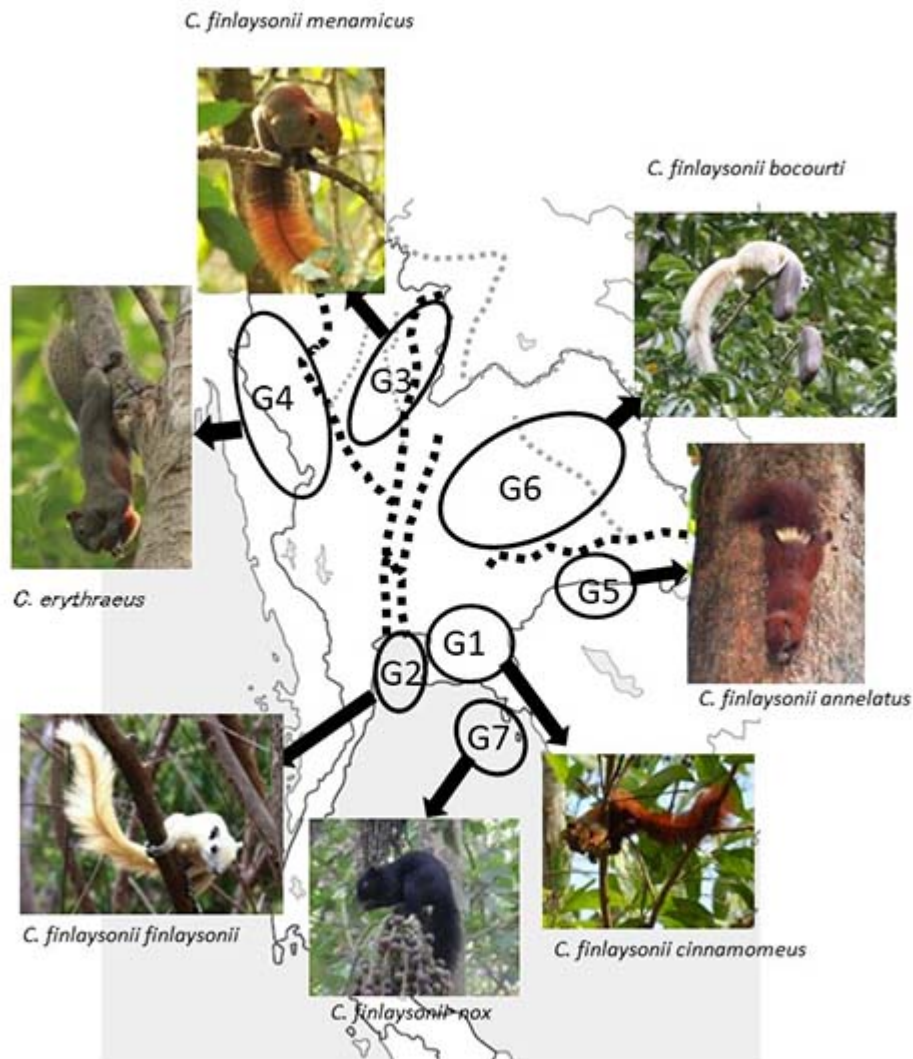


Figure 1: Fur color polymorphism and genetic groups among Finlayson's squirrels (*Callosciurus finlaysonii*) subspecies and Pallas's squirrels (*Callosciurus erythraeus*) in Thailand. Genetically divided into seven groups (G1-G7) based on rivers in dashed lines ⁴. G6 represents *C.f. bocourti*, which is the subspecies that was introduced in Italy in the 1980's (see section 'Invasive range').

Native range : ⁵

Central Indochina from central Myanmar southeast through much of Thailand, Laos and Cambodia to the Mekong Delta in South Vietnam where 16 subspecies are described (also see figure 1).



Invasive range: ⁶

Europe (geographical) :

Italy (possibly eradicated)

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R02574>

<https://www.gbif.org/species/2437399>

https://animaldiversity.org/accounts/Callosciurus_finlaysonii/#62706019-A41F-11E3-B562-002500F14F28

<https://www.thainationalparks.com/species/finlaysons-squirrel>

Outside Europe (geographical):

Japan and Singapore

Morphology, biology, invasion, negative effects and remedies

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



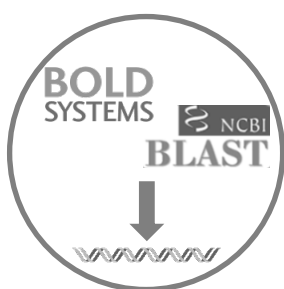
Species identification based on DNA barcodes

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.

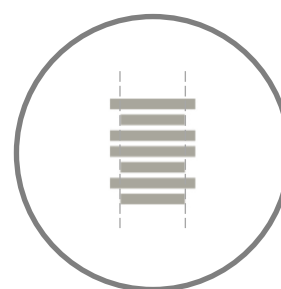
Material and Methods¹



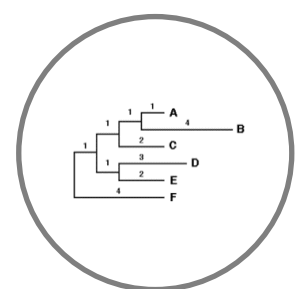
Download all sequence data available for the genus



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 finlaysonii*. However, based on the present evaluation of the available sequence data, *cytb* is the most reliable DNA markers for the identification of the *C. erythraeus/finlaysonii* complex.

Discussion

DNA markers for which *Callosciurus finlaysonii* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Callosciurus*. Three DNA markers were evaluated (Table 1).

In all cases, the *C. finlaysonii* sequences did not group in a unique cluster in the NJ-trees while supported clusters are observed for the *C. erythraeus/finlaysonii* complex, for all evaluated DNA markers. The occurrence of hybrids between *C. finlaysonii* and *C. erythraeus* have been reported by Timmins & Duckworth³ (*C. erythraeus* x *finlaysonii*), this poses a potential problem for identification. Therefore, making distinction between these two species is difficult and the results should be interpreted with caution.

Nevertheless the fact that distinction between *C. finlaysonii* and *C. erythraeus* is not possible with any of the markers, based on the current representation for DNA marker *cytb* in online databases (Table 2), however *C. finlaysonii* and *C. erythraeus* sequences do form a supported cluster. One separate *C. finlaysonii* sequence (Accession number: KP126037) is not included in this cluster and could be a potential misidentification. If so, *cytb* appears to be a potential marker for the identification of the *C. erythraeus/finlaysonii* complex.

For DNA markers **COI** and **D-loop** the online representation of these markers is limited (Table 2), therefore it is currently difficult to assess the ability of these markers to identify the *C. erythraeus/finlaysonii* complex. To allow for a better evaluation of the performance of this marker for species identification, additional sequences of the missing *Callosciurus* species 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 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	X	X
cytb			X	X	X
D-Loop			X	X	X

Table 2: Publicly available sequences downloaded from BOLD and GenBank (March 2023) 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.

Species in genus	COI	cytb	D-Loop
<i>Callosciurus adamsi</i>		X	
<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>	X	X	
<i>Callosciurus melanogaster</i>			
<i>Callosciurus nigrovittatus</i>		X	
<i>Callosciurus notatus</i>	X	X	
<i>Callosciurus orestes</i>	X	X	X
<i>Callosciurus phayrei</i>		X	
<i>Callosciurus prevostii</i>	X	X	
<i>Callosciurus pygerythrus</i>		X	
<i>Callosciurus quinquestriatus</i>			
TOTAL species	6/14	11/14	4/14

For further details on 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 (<https://bopco.myspecies.info/content/contact>).



References and online information

Online information

<https://ias.biodiversity.be/species/show/127>

<https://www.gbif.org/species/2437399>

<https://www.iucnredlist.org/species/3596/22254494>

<https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-thaise-eekhoorn>

Picture credits

Page 1: *Callosciurus finlaysonii* By Marcellinlay [CC BY NC]

Page 2: Distribution of *C. finlaysonii* and subspecies in Thailand ⁷

References

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