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 Smitz *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.

Tamias sibiricus

(Laxmann, 1769)

Common names:
  English: Asian chipmunk, Siberian chipmunk
  French: tamia de Sibérie, écureuil de Corée
  German: Burunduk, Streifenhörnchen
  Dutch: Aziatische (gestreepte) grondeekhoorn, Siberische
          grondeekhoorn, boeroendoek, Koreaanse grondeekhoorn

Last update: November 2018

General information on *Tamias sibiricus*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Mammalia	Rodentia	Sciuridae	*Tamias*

Species in the same genus: N = 25 [2–4]

Note: Chipmunks are commonly considered to be either a single genus, *Tamias*, or three genera: *Tamias*, *Eutamias*, and *Neotamias*. In the latter case, *Tamias* would contain the eastern chipmunk, *Eutamias* would consist of the Siberian chipmunk, and *Neotamias* would include the remaining 23, mostly western, species. We follow the classification as presented by Thorington *et al*. [3] recognising one single genus *Tamias* including all 25 species

Infra-species level: N = 9 [3]

Note: Nine subspecies are recognised: *T.s. altaicus, T.s. lineatus*, *T.s. okadae*, *T.s. ordinalis*, *T.s. orientalis*, *T.s. pallasi*, *T.s. senescens*, *T.s. sibiricus* and *T.s. umbrosus.*

Native range: [5, 7]

China, Japan (Hokkaido), Kazakhstan, North and South Korea, Mongolia, Russian Federation (eastern and western Siberia, northern Russia, Russian Far East).

Invasive range: [3,5–8]

Europe (geographical):

Austria, Belgium, Denmark, France, Germany, Italy, Netherlands and Switzerland. Presence reported in Denmark and United Kingdom.

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/2437450
https://ias.biodiversity.be/species/show/31
https://www.cabi.org/ISC/datasheet/62788
https://www.iucnredlist.org/species/21360/115161465

Outside Europe (geographical):

Hong Kong, Japan (Honshu, Shikoku, and Kyushu islands), Russian Federation (central Russia).

Morphology, biology, invasion, negative effects and remedies

For more information on *Tamias sibiricus* 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/nuccore/) to estimate the reliability with which a species identification can be obtained using DNA barcoding.
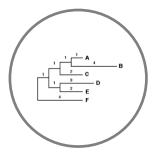
Material and Methods [1]

| 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 *Tamias sibiricus*.

Discussion

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

In the NJ-trees for **COII** and **cytb**, the *T. sibiricus* sequences cluster together and both markers appear to be useful in differentiating *T. sibiricus* from the other *Tamias* species. Also, *T. striatus*, the only other species commercialised in Europe [9], forms well supported clusters in both NJ-trees. *T. sibiricus* is better represented in the cytb compared to COII datasets, regarding the number of sequences as well as their geographical coverage (native and invasive). Also, the congeneric species are found to be better represented in the cytb dataset, in terms of number of species (Table 2) and number of sequences per species. Therefore, cytb is the most reliable DNA marker for differentiating *Tamias sibiricus* from other *Tamias* species.

For **COI**, **CR**, **16S** and **RAG1**, fewer sequences are available for *T. sibiricus* and its congeners, or the marker shows little genetic variation. Therefore it is currently impossible to assess the ability of these markers to identify *T. sibiricus*.

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, a '1' indicates only one unique *Tamias sibiricus* sequence was available.

Markers analysed	1	2	3	4	5
COI		X			X
COII		X			X
cytb					
CR	X	X			X

16S	X	X	X	X	X
RAG1	1	X	1		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.

Species in genus	COI	COII	cytb	CR	16S	RAG1
Tamias alpinus			X			
Tamias amoenus	X	X	X	X	X	X
Tamias bulleri		X	X		X	
Tamias canipes	X	X	X	X	X	
Tamias cinereicollis	X	X	X	X	X	
Tamias dorsalis	X	X	X	X	X	
Tamias durangae		X	X		X	
Tamias merriami		X	X	X	X	
Tamias minimus		X	X	X	X	
Tamias obscurus		X	X	X		
Tamias ochrogenys			X		X	
Tamias palmeri		X	X		X	
Tamias panamintinus		X	X	X	X	
Tamias quadrimaculatus		X	X	X	X	
Tamias quadrivittatus	X	X	X	X	X	
Tamias ruficaudus	X	X	X	X	X	X
Tamias rufus	X	X	X	X	X	
Tamias senex		X	X	X	X	X
Tamias sibiricus	**X**	**X**	**X**	**X**	**X**	**X**
Tamias siskiyou			X			
Tamias sonomae		X	X	X	X	
Tamias speciosus			X	X		
Tamias striatus	X	X	X	X	X	
Tamias townsendii		X	X	X	X	
Tamias umbrinus	X	X	X	X	X	
TOTAL species	**10/25**	**21/25**	**25/25**	**19/25**	**21/25**	**4/25**

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://ias.biodiversity.be/species/show/31
http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=3472
https://animaldiversity.org/accounts/Tamias_sibiricus/
http://www.biodiversityireland.ie/wordpress/wp-content/uploads/Siberian-Chipmunk.pdf
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http://nonnativespecies.ie/wp-content/uploads/2014/03/Tamias-sibiricus-Siberian-chipmunk.pdf
http://www.biodiversity.de/sites/default/files/products/factsheets/nefo-faktenblatt-invasive-streifenhoernchen.pdf

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Page 2 (right): *Tamias sibiricus* en cage sur un marché chinois By Vmenkov [CC BY 3.0]

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