



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.

Sciurus carolinensis

Gmelin, 1788

Common names:

English: eastern gray squirrel, gray squirrel

French: écureuil gris

German: Grauhörnchen

Dutch: grijze eekhoorn

Last update: October 2018



General information on *Sciurus carolinensis*

Classification

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

Species in the same genus: N = 28 [2–8]

Note: We follow the classification as presented by Thorington *et al.* [8].

Infra-species level: N = 5 [5–9]

Note: Five subspecies have been described: *S.c. carolinensis*, *S.c. extimus*, *S.c. fuliginosus*, *S.c. hypophaeus*, *S.c. pennsylvanicus*.



Native range: [4, 8-10]

North-eastern USA and south-eastern Canada.

Invasive range: [4, 5, 8–11]

Europe (geographical):

Ireland, Italy and United Kingdom.

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

<https://www.gbif.org/species/5219681>

<https://www.cabi.org/isc/datasheet/49075>

https://fauna-eu.org/cdm_dataportal/taxon/08210056-6c6e-4778-89a0-4e9a88164c7a

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R13698>

Outside Europe (geographical):

Localized introduced populations in Canada (Alberta, British Columbia, Manitoba, New Brunswick, Nova Scotia, Quebec, Saskatchewan), in Mexico (Baja California), on the island of Pitcairn (British Overseas Territory), in South Africa, and in the United States of America (California, Idaho, Louisiana, Massachusetts, Montana, North Dakota, Oregon, Texas, Washington, Wisconsin).

Morphology, biology, invasion, negative effects and remedies

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



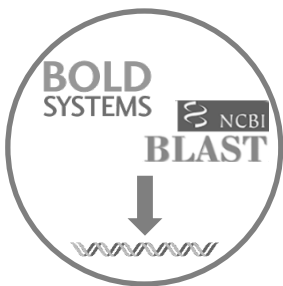
Species identification based on DNA barcodes

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.

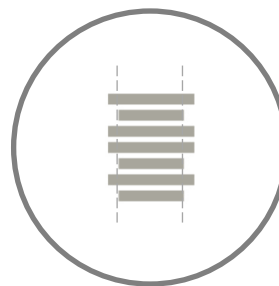
Material and Methods [1]



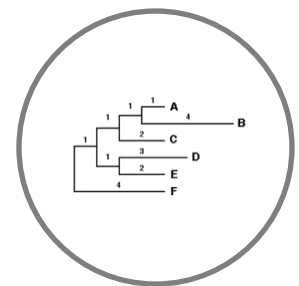
Download all sequence data available for the genus



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, *cytb* is the most reliable DNA marker for the identification of *Sciurus carolinensis*. Adding sequences of the missing congeners will allow to better evaluate the performance of the marker for species identification.

Discussion

DNA markers for which *Sciurus carolinensis* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Sciurus* (as defined by Thorington *et al.* [6]). Five DNA markers were evaluated (Table 1).

In the NJ-trees for **cytb** and **12S**, 15 and 17 out of 28 congeneric species are represented, respectively (Table 2). In both, the *S. carolinensis* sequences cluster together, yet a larger number of *Sciurus* sequences are available for *cytb*. In addition, *S. vulgaris*, *S. anomalus* (both native to Europe, the former widespread in Eurasia [7, 12, 13], the latter found in eastern Europe [7]), and *S. niger* (a species with a high potential for invasiveness, which is also listed on the EU Regulation [14]) form supported clusters in the *cytb* NJ-tree. Adding sequences from the missing congeners will allow to better evaluate the performance of the markers for species identification.

For **16S**, **COI** and **D-Loop**, *S. carolinensis* is supported as a cluster whenever multiple sequences are available. However, there are not enough congeneric species represented in the public databases to make a full evaluation of the usefulness of these markers for molecular species identification.



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 *Sciurus carolinensis* sequence was available.

Markers analysed	1	2	3	4	5
COI	X	X			X
12S	X	X			X
cytb		X			X
D-Loop	X	X			X
16S	X	X	1		X

Table 2: Publicly available sequences downloaded (August 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 [6]. 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	cytb	D-Loop	16S
<i>Sciurus aberti</i>		1	X		
<i>Sciurus aestuans</i>	X	X	X		1
<i>Sciurus alleni</i>		1			
<i>Sciurus anomalus</i>			X		
<i>Sciurus arizonensis</i>					
<i>Sciurus aureogaster</i>	1		1		
<i>Sciurus carolinensis</i>	X	X	X	X	1
<i>Sciurus colliae</i>		1			
<i>Sciurus deppei</i>		1	X		1
<i>Sciurus flammifer</i>		1			
<i>Sciurus gilvularis</i>		1			1
<i>Sciurus granatensis</i>		1	X		1
<i>Sciurus griseus</i>					
<i>Sciurus ignitus</i>					
<i>Sciurus igniventris</i>		1	1		
<i>Sciurus lis</i>		1	X	X	
<i>Sciurus nayaritensis</i>					
<i>Sciurus niger</i>	1	X	X	X	1
<i>Sciurus oculatus</i>		1	X		
<i>Sciurus pucheranii</i>					
<i>Sciurus pyrrhinus</i>		1			1
<i>Sciurus richmondi</i>					
<i>Sciurus sanborni</i>					
<i>Sciurus spadiceus</i>		1	1		1
<i>Sciurus stramineus</i>			X		
<i>Sciurus variegatoides</i>		1	X		1
<i>Sciurus vulgaris</i>	X	X	X	X	X
<i>Sciurus yucatanensis</i>					
TOTAL species	5/28	17/28	15/28	4/28	10/28

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/30>
<http://share.bebif.be/data/ias/Risk%20analyses/Sciurus%20carolinensis.pdf>
<https://www.naturaldevon.org.uk/wp-content/uploads/2017/10/Grey-Squirrel-DISI-Species-Factsheet.pdf>
http://www.mammal.org.uk/sites/default/files/factsheets/grey_squirrel_complete.pdf
<http://www.biodiversityireland.ie/wordpress/wp-content/uploads/Grey-Squirrel.pdf>
<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=3175>
<http://dialapest.co.uk/images/squirrels-spec-sheet.pdf>

Picture credits

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Page 2 (middle): Jeune écureuil gris By Luxboyer [CC BY SA 3.0]
Page 2 (right): *Sciurus carolinensis* By Jim Ferguson [CC BY 2.0]

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>.
- [2] P. Myers, R. Espinosa, C. S. Parr, T. Jones, G. S. Hammond, and T. A. Dewey, "The Animal Diversity Web (online)," 2018. [Online]. Available: <https://animaldiversity.org>.
- [3] R. W. Crosby, "Phylogeny of the Squirrels (Rodentia, Sciuridae)," University College Station, Texas, 2012.
- [4] "*Sciurus carolinensis* Gmelin, 1788 in GBIF Secretariat," *GBIF Backbone Taxonomy*, 2017. [Online]. Available: <https://www.gbif.org/species/5219681>.
- [5] ITIS taxonomy, "ITIS Standard Report Page: *Sciurus carolinensis*." [Online]. Available: https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=180175#null.
- [6] R. W. Jr. Thorington and R. S. Hoffman, "Family Sciuridae," in *Mammal Species of the World: A Taxonomic and Geographic Reference* (D. E. Wilson and D. M. Reeder, eds.), 3rd ed., 2005, pp. 754–818.
- [7] ITIS Report, "ITIS Standard Report Page: *Sciurus*." [Online]. Available: https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=180171#null. [Accessed: 07-Nov-2018].
- [8] R. W. Jr. Thorington, J. L. Koprowski, M. A. Steele, and J. F. Whatton, *Squirrels of the World*, Johns Hopkins Baltimore, Maryland, USA, 2012.
- [9] R. Banks, R. McDiarmid, and A. Gardner, *Checklist of Vertebrates of the United States, the U.S. Territories, and Canada*, Resource P. Washington DC, USA: United States Department of the Interior Fish and Wildlife Service, 1987.
- [10] J. Koprowski, K. E. Munroe, and A. Edelman, "Gray not grey: the ecology of *Sciurus carolinensis* in their native range in North America | Request PDF," in *The Grey Squirrel: Ecology & Management of an Invasive Species in Europe*, C. Shuttleworth, P. Lurz, and J. Gurnell, Eds. European Squirrel Initiative, 2016.
- [11] ISSG, "Global Invasive Species Database (GISD). *Sciurus carolinensis*," 2011. [Online]. Available: <https://www.cabi.org/isc/datasheet/49075>.
- [12] S. Bertolino, N. C. di Montezemolo, D. G. Preatoni, L. A. Wauters, and A. Martinoli, "A grey future for Europe: *Sciurus carolinensis* is replacing native red squirrels in Italy," *Biol. Invasions*, vol. 16, no. 1, pp. 53–62, 2014.
- [13] A. Grill *et al.*, "Molecular phylogeography of European *Sciurus vulgaris*: refuge within refugia?," *Mol. Ecol.*, vol. 18, no. 12, pp. 2687–2699, 2009.
- [14] Eu Regulation 1143/2014, "List of Invasive Alien Species of Union concern - Environment - European Commission." [Online]. Available: http://ec.europa.eu/environment/nature/invasivealien/list/index_en.htm.

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