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 niger

Linnaeus, 1758

Common names:
   English: Bryant's fox squirrel, eastern fox squirrel
   French: écureuil fauve, écureuil renard
   German: Schwarzhörnchen
   Dutch: Amerikaanse voseekhoorn, zwarte eekhoorn

Last update: November 2018

General information on *Sciurus niger*

Classification

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

Species in the same genus: N = 28 [2–6]
Note: We follow the classification as presented by Thorington *et al.* [4].

Infra-species level: N = 10 [4, 5, 7–9]
Note: Ten subspecies have been described: *S.n. avicennia, S.n. bachmani, S.n. cinereus, S.n. limitis, S.n. ludovicianus, S.n. niger, S.n. rufiventer, S.n. shermani, S.n. subauratus, S.n. vulpinus*.

Native range: [4,10]
Canada (Manitoba and Saskatchewan), Mexico (Coahuila) and United States of America (Alabama, Arkansas, Colorado, Delaware, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New York, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Virginia, West Virginia and Wisconsin).

Invasive range: [4,9–11]
Europe (geographical):
Not established in Europe, but individuals were spotted in Germany, Netherlands and United Kingdom.

For more detailed locality information and the most recent distribution updates, please visit:
https://www.gbif.org/species/5219683
https://www.cabi.org/isc/datasheet/64742
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R19317

Outside Europe (geographical):
Canada (British Columbia and Ontario), United States of America (Arizona, California, Idaho, Oregon, Montana, New Mexico, Washington and Wyoming).

Morphology, biology, invasion, negative effects and remedies
For more information on *Sciurus niger* 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.
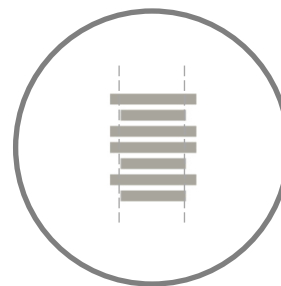
Material and Methods [1]
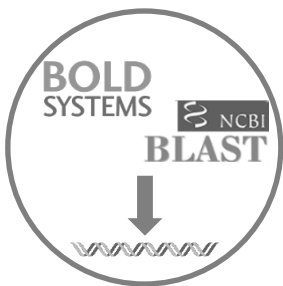
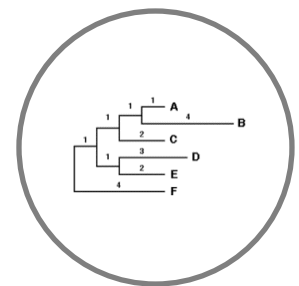| Download all sequence data available for the genus | Filtering the data and selecting 'promising' markers | Aligning and trimming of the sequences | Building Neighbour-Joining tree with Bootstrap support |

Conclusion
Based on the present evaluation of the available sequence data, cytb is the most reliable DNA marker for the identification of *Sciurus niger species*. 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 niger* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Sciurus* (as defined by Thorington *et al.* [5]). Five DNA markers were evaluated (Table 1).

In the NJ-tree for **cytb**, the *S. niger* sequences cluster together, yet the geographical coverage of the sequences is limited to the species' native range. *S. vulgaris*, *S. anomalus* (both native to Europe, the former widespread in Eurasia [6, 12, 13], the latter found in eastern Europe [6]), and *S. carolinensis* (a species with a high potential for invasiveness, which is also listed on the EU Regulation [14]) are all well represented and form supported clusters in the cytb NJ-tree. However, adding sequences from the missing congener species (Table 2; 15 of the 28 *Sciurus* species represented), and from the invasive range of *S. niger*, will allow to better evaluate the performance of the marker for species identification.

For **12S**, *S. niger* is only represented by two sequences, which do not cluster in the NJ-tree. Also, 13 congeneric species are represented by one sequence only (Table 2). Due to this lack of sequences and the absence of clustering, it is currently impossible to assess the ability of this marker to identify *S. niger*.

For **16S**, **COI** and **D-Loop**, *S. niger* 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 reliable evaluation about the usefulness of these markers in the frame of a 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 *S. niger* sequence was available.

Markers analysed	1	2	3	4	5
COI	X	X	1		X
12S	X	X	X		X
cytb		X			X
D-Loop		X			X
16S	1	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 [5]. 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
Sciurus aberti		1	X		
Sciurus aestuans	X	X	X		1
Sciurus alleni		1			
Sciurus anomalus			X		
Sciurus arizonensis					
Sciurus aureogaster	1		1		
Sciurus carolinensis	X	X	X	X	1
Sciurus colliaei		1			
Sciurus deppei		1	X		1
Sciurus flammifer		1			
Sciurus gilvigularis		1			1
Sciurus granatensis		1	X		1
Sciurus griseus					
Sciurus ignitus					
Sciurus igniventris		1	1		
Sciurus lis		1	X	X	
Sciurus nayaritensis					
Sciurus niger	**1**	**X**	**X**	**X**	**1**
Sciurus oculatus		1	X		
Sciurus pucheranii					
Sciurus pyrrhinus		1			1
Sciurus richmondi					
Sciurus sanborni					
Sciurus spadiceus		1	1		1
Sciurus stramineus			X		
Sciurus variegatoides		1	X		1
Sciurus vulgaris	X	X	X	X	X
Sciurus yucatanensis					
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
http://biodiversite.wallonie.be/servlet/Repository/?ID=33515
https://www.cabi.org/isc/datasheet/64742
https://animaldiversity.org/accounts/Sciurus_niger/
https://www.iucnredlist.org/species/20016/115155257
http://eol.org/pages/347428/overview
http://www.nhptv.org/natureworks/easternfoxsquirrel.htm
http://www.gt-ibma.eu/espece/sciurus-niger/
http://www.nonnativespecies.org/factsheet/downloadFactsheet.cfm?speciesId=4362

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Page 2 (right): *Sciurus niger* baby By Travis Witt, Liveon001 [CC BY SA 3.0]

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]   R. W. Jr. Thorington, J. L. Koprowski, M. A. Steele, and J. F. Whatton, *Squirrels of the World*, Johns Hopkins Baltimore, Maryland, USA, 2012.

[5]   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.

[6]   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].

[7]   ITIS Report, "ITIS Standard Report Page: *Sciurus niger*." [Online]. Available: https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=180172#null. [Accessed: 07-Nov-2018].

[8]   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.

[9]   GBIF, "*Sciurus niger* Linnaeus, 1758 in GBIF Secretariat (2017). GBIF Backbone Taxonomy." [Online]. Available: https://www.gbif.org/species/5219683. [Accessed: 07-Nov-2018].

[10]   A. V. Linzey, R. Timm, L. Emmons, and F. Reid, "*Sciurus niger* (errata version published in 2017). The IUCN Red List of Threatened Species," 2016. [Online]. Available: https://www.iucnredlist.org/species/20016/115155257. [Accessed: 07-Nov-2018].

[11]   E. Baiwy and V. Schockert, "Risk analysis of the Fox squirrel, *Sciurus niger*, Risk analysis report of non-native organisms in Belgium," 2013.

[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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