



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.

Lampropeltis getula

(Linnaeus, 1766)

Common names:

English: eastern kingsnake, common kingsnake, chain kingsnake

French: serpent roi

German: Gewöhnliche Königsnatter

Dutch: gewone koningsslang

Last update: March 2023



General information on *Lampropeltis getula*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Reptilia	Squamata	Colubridae	<i>Lampropeltis</i>

Species in the same genus: **N = 21** ^{2,3}

Infra-species level: **N = 0** ^{2,3}

Note: subspecies *L. g. getula*, *L. g. californiae*, *L. g. holbrooki*, *L. g. nigra*, and *L. g. splendida* have been revalidated to species status by Pyron & Burbrink (2009) ⁴. Pyron & Burbrink (2009) consider *L. g. floridana*, *L. g. meansi* and *L. g. "sticticeps"* as synonyms of *L. g. getula* while Krysko et al. (2017) ⁶ concluded from genetic, ecological and morphological data *L. floridana* and *L. meansi* as distinct species.

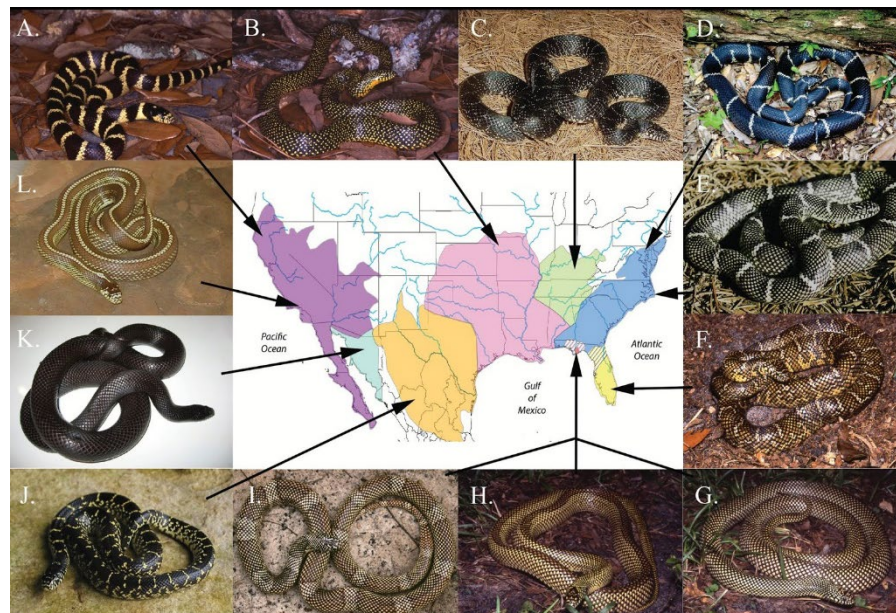


Figure 1: Distribution of kingsnakes in the *Lampropeltis getula* complex in North America. ⁵ See complete caption in section 'Reference and online information'

Native range: ⁶

Pacific to Atlantic coasts of the southern USA and Baja California, northern Mexico.

Invasive range: ^{6,7}

Europe (geographical):

Lampropeltis g. californiae banded (see **A** in Figure 1) has been established in the Canary Islands since 2007 ⁷. Subspecies *L. g. californiae* was revalidated in 2009 to species status *L. californiae* (also see section 'Infra-species level' above).

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R08113>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R20061>

<https://www.gbif.org/species/9799308>

<https://www.naturalsciences.be/fr/news/item/21990>

<https://herpetomania.fi/wp-content/uploads/2022/04/Official-response-to-Lampropeltis-getula-IAS-case.pdf>

https://www.dght.de/files/web/news/stellungnahme_isa_listing/jointResponseLampropeltisgetula_Final.pdf

[https://eur-lex.europa.eu/legal-](https://eur-lex.europa.eu/legal-content/DE/TXT/HTML/?fbclid=IwAR3iSyCxGy8U7z7EES2gPKuDTH5FKFmHkly8hjRSWjaiLS7VgMu4Ys0x8fI&uri=CELEX%3A32022R1203)

[content/DE/TXT/HTML/?fbclid=IwAR3iSyCxGy8U7z7EES2gPKuDTH5FKFmHkly8hjRSWjaiLS7VgMu4Ys0x8fI&uri=CELEX%3A32022R1203](https://eur-lex.europa.eu/legal-content/DE/TXT/HTML/?fbclid=IwAR3iSyCxGy8U7z7EES2gPKuDTH5FKFmHkly8hjRSWjaiLS7VgMu4Ys0x8fI&uri=CELEX%3A32022R1203)

Outside Europe (geographical):

To our knowledge, *L. californiae* has only been reported in the Canary Islands.

Morphology, biology, invasion, negative effects and remedies

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



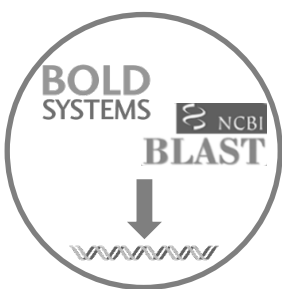
Species identification based on DNA barcodes

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.

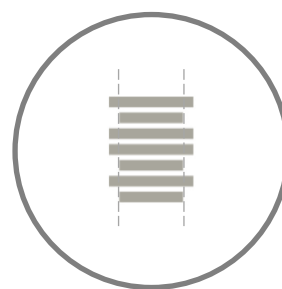
Material and Methods¹



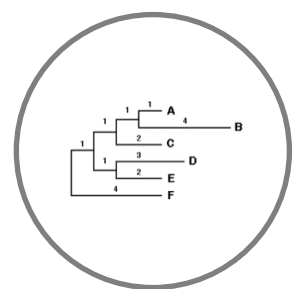
Download all sequence data available for the genus



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, DNA markers *cyt b*, COI and NADH are the most reliable for the identification of the complex *Lampropeltis getula* (incl. *L. getula*, *L. californiae* = *L. nigrita*, *L. holbrooki*, *L. nigra*, *L. splendida* and *L. floridana*, *L. meansi*). DNA sequences from *L. getula* complex cluster together for all markers.

However, to improve the performance of those markers for species identification within the *L. getula* complex, additional sequences for the congeners, especially *L. californiae*, which is the invasive subspecies in the Canary Islands, should be added to the analyses.

Discussion

12 DNA markers that were available for the *L. getula* complex (incl. *L. getula*, *L. californiae* (= *L. nigrita*), *L. holbrooki*, *L. nigra*, *L. splendida*, *L. floridana*, and *L. meansi*) were downloaded from GenBank and BOLD and for the 21 species of the genus *Lampropeltis*^{2,3}

The 12 DNA markers were evaluated but only the NJ-trees obtained for **COI**, **16S**, **12S**, **cyt b** and **NADH** clustered the members of the *L. getula* complex together and were then further analysed (Table 1). For the six other markers, viz. **CI8**, **CI2**, **GAD2**, **LA TCL**, **SPTBN1**, **Vimentin**, they did not cluster together, neither did the representatives of the other species of the same genus. For **CI3**, no representative of the *L. getula* complex was available.

The 21 *Lampropeltis* species were represented (see Table 2). DNA markers **cyt b**, **COI** and **NADH** were the markers for which the highest number of species of the *L. getula* complex were represented (18 species for **cyt b**, 16 species for **NADH**, 13 species for **COI**; Table 2). However, none of these genetic markers was able to identify the different species members of the *L. getula* complex. To allow for a better evaluation of the performance of the selected genetic markers for species identification, *L. getula* sequences from invasive areas, especially *L. californiae* established in the Canary Islands, should be added to the analysis.



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	X
16S	X	X		X	X
12S	X	X		X	X
cyt b	X	X		X	X
NADH	X	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, a '1' indicates only one sequence was available for *Lampropeltis californiae*.

Species in genus	COI	16S	12S	cyt b	NADH
<i>Lampropeltis mexicana</i>	X		X	X	X
<i>Lampropeltis elapsoides</i>	X	X		X	
<i>Lampropeltis zonata</i>	X			X	
<i>Lampropeltis micropholis</i>				X	
<i>Lampropeltis ruthveni</i>	X		X		X
<i>Lampropeltis triangulum</i>	X		X	X	X
<i>Lampropeltis alterna</i>	X			X	X
<i>Lampropeltis gentilis</i>	X			X	X
<i>Lampropeltis annulata</i>				X	
<i>Lampropeltis extenuata</i>	X			X	
<i>Lampropeltis splendida</i>					X
<i>Lampropeltis getula</i>	X	X	X	X	X
<i>Lampropeltis nigra</i>	X			X	X
<i>Lampropeltis holbrooki</i>	X			X	X
<i>Lampropeltis floridana</i>				X	X
<i>Lampropeltis meansi</i>				X	X
<i>Lampropeltis californiae</i> = <i>L. nigrita</i>					X
<i>Lampropeltis abnorma</i>				X	
<i>Lampropeltis webbi</i>				X	X
<i>Lampropeltis pyromelana</i>	X	X		X	X
<i>Lampropeltis polyzona</i>				X	
<i>Lampropeltis calligaster</i>	X	X		X	X
<i>Lampropeltis knoblochi</i>					X
TOTAL species	13/21	4/21	4/21	19/21	16/21

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://easin.jrc.ec.europa.eu/easin/News/DetailNews/d8ad8966-d260-4e25-a4df-ae8f05f4e407>

http://www.lifelampropeltis.com/images/pdf/Summary_Project.pdf

<https://bibdigital.rjb.csic.es/records/item/10960-redirectio>

https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.1006761/Lampropeltis_californiae

Picture credits

Page 1: *Lampropeltis getula*_By_Tsreptilien [CC BY NC 2.0]

Page 2: Distribution of Kingsnakes in the *Lampropeltis getula* complex in North America ⁴. *Lampropeltis californiae* (banded, **A**), *L. holbrooki* (**B**), *L. nigra* (**C**), *L. g. getula* (**D**), *L. g. sticticeps* (considered as a synonym of *L. getula*; see Pyron & Burbrink 2009)⁵ (**E**), *L. g. floridana* (**F**), *L. g. meansi* (**G-I**), *L. splendida* (**J**), *L. g. nigrita* (**K**), *L. californiae* (striped, **L**). Note 1: *L. getula*, *L. californiae*, *L. holbrooki*, *L. nigra*, and *L. splendida* have been revalidated to species status by Pyron & Burbrink (2009).

References

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