



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.

Trachemys scripta

(Thunberg *in* Schoepff, 1792)

Common names:

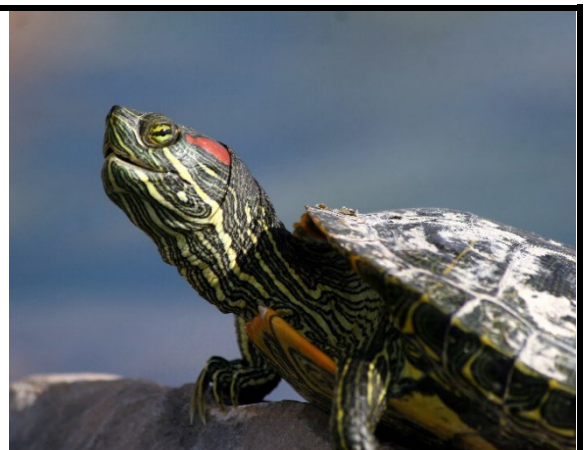
English: pond slider: yellow-bellied slider (*T.s. scripta*), red-eared slider (*T.s. elegans*), Cumberland slider (*T.s. troostii*)

French: tortue de Floride (*T.s. elegans*)

German: Nordamerikanische Buchstaben-Schmuckschildkröte: Rotwangen- (*T.s. elegans*), Gelbwangen- (*T.s. scripta*), Cumberland-Schmuckschildkröte (*T.s. troostii*)

Dutch: lettersierschildpad, roodwangschildpad (*T.s. elegans*), geelbuikschildpad (*T.s. scripta*), geelwangschildpad (*T.s. troostii*)

Last update: January 2019



General information on *Trachemys scripta*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Reptilia	Testudines	Emydidae	<i>Trachemys</i>

Species in the same genus: N = 15 [2]

Infra-species level: N = 3 [2]

Note: Three subspecies are recognised; *Trachemys scripta scripta*, *T.s. troostii* and *T.s. elegans*.



Native range: [2, 3]

Northeast Mexico and eastern part of the United States of America.

Invasive range: [4-6]

Europe (geographical):

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, United Kingdom.

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R18154>

<https://www.gbif.org/species/2443002>

<http://reptile-database.reptarium.cz/species?genus=Trachemys&species=scripta>

Outside Europe (geographical):

Africa (La Réunion, South Africa), Asia, Bermuda, Canada, Central America (including Caribbean), Oceania, South America.

Morphology, biology, invasion, negative effects and remedies

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



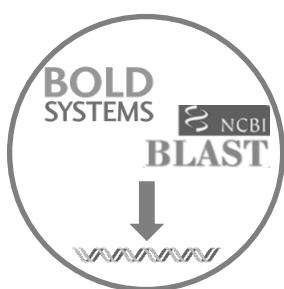
Species identification based on DNA barcodes

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.

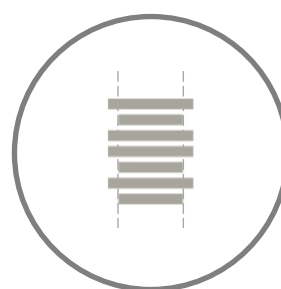
Material and Methods [1]



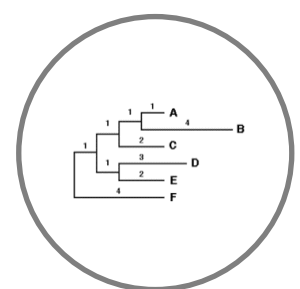
Download all sequence data available for the genus



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, ND4 is the most reliable DNA markers for the identification of *Trachemys scripta*. To better allow the evaluation of the performance of this marker for species identification, sequences of *T. callirostris* should be added to the analyses.

Discussion

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

ND4 appears to be the most reliable DNA marker for the identification of *Trachemys scripta* since the available *T. scripta* sequences form a well-supported cluster. In addition, 15 out of the 16 *Trachemys* species are represented in the NJ-tree and for most species multiple sequences are available. To better allow to evaluate this marker sequences of *T. callirostris* (Table 2) should be added to the analyses.

For **cytb**, *T. scripta* is recovered with high support, while for **COI**, the *T. scripta* sequences cluster together, except for one (GenBank accession number MH274718). The latter sequence might involve a misidentification. If so, both DNA markers look promising to differentiate *T. scripta* from other *Trachemys* species, yet fewer species are represented than is the case for ND4.

None of the other evaluated DNA markers (**12S**, **HNFL**, **R35**, **ODC**, **RAG-1**, **RAG-2**, **cmos**, and **reln**) recovered *T. scripta* as a supported cluster, a result that was also obtained by Spinks *et al.* [7]. Hence, these DNA markers do not look promising for species identification of *T. scripta*.



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.

Markers analysed	1	2	3	4	5
COI			X	X	X
cytb					X
12S			X		X
ND4					X
HNFAL		X	X		X
R35			X		X
ODC	X	X	X		X
RAG-1		X	X		X
RAG-2	X		X		X
cmos	X		X		X
reln		X	X		X

Table 2: Publicly available sequences downloaded (January 2019) 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 [2]. An 'X' indicates that at least one sequence was used in the final alignment. Species name with * are not mentioned in [2], but used on GenBank and listed in [7].

Species in genus	COI	cytb	12S	ND4	HNFAL	R35	ODC	RAG-1	RAG-2	cmos	reln
<i>Trachemys adiutrix</i>		X	X	X		X	X	X	X	X	
<i>Trachemys callirostris</i>											
<i>Trachemys decorata</i>	X			X	X	X					X
<i>Trachemys decussata</i>		X	X	X	X	X	X	X	X	X	X
<i>Trachemys dorbigni</i>		X	X	X		X	X	X	X	X	
<i>Trachemys emolli*</i>	X	X	X	X	X	X	X	X	X	X	X
<i>Trachemys gaigeae</i>	X	X	X	X	X	X	X	X	X	X	
<i>Trachemys grayi</i>		X	X	X	X	X	X	X	X	X	X
<i>Trachemys nebulosa</i>		X		X		X	X				
<i>Trachemys ornata</i>	X	X	X	X		X	X	X	X	X	
<i>Trachemys scripta</i>	X	X	X	X	X	X	X	X	X	X	X
<i>Trachemys stejnegeri</i>	X	X		X	X	X		X			X
<i>Trachemys taylori</i>	X	X		X	X	X		X			
<i>Trachemys terrapen</i>	X			X	X	X					X
<i>Trachemys venusta</i>	X	X	X	X	X	X	X	X	X	X	X
<i>Trachemys yaquia</i>	X			X		X					
TOTAL species	10/16	12/16	9/16	15/16	10/16	15/16	10/16	11/16	9/16	9/16	8/16

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://www.cabi.org/isc/datasheet/61560>
<http://www.iucngisd.org/gisd/species.php?sc=71>
<https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=1261>
<https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=1262>
<https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=1263>
<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=3566>
[\[NL\] https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-geelbuikschildpad](https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-geelbuikschildpad)
[\[NL\] https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-roodwangschildpad](https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-roodwangschildpad)
[\[NL\] https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-geelwangschildpad](https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-geelwangschildpad)
<http://species.biodiversityireland.ie/profile.php?taxonId=186868>
https://www.pestsmart.org.au/wp-content/uploads/2010/11/Pond_slider_DAFWA_220410.pdf

Picture credits

Page 1: red-eared slider turtle (*Trachemys scripta elegans*) By Brent Myers [CC BY 2.0]

Page 2 (left): *Trachemys scripta scripta* By ekenitr [CC BY-NC 2.0]

Page 2 (right): *Trachemys scripta troostii* By Quartl [CC BY-SA 3.0]

References

- [1] N. Smits, 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] Turtle Taxonomy Working Group *et al.*, "Turtles of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status (8th Ed.)" in *Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group*, Chelonian Research Monographs. A. G. J. Rhodin, J. B. Iverson, P. P. van Dijk, R. A. Saumure, K. A. Buhmann, P. C. H. Pritchard, and R. A. Mittermeier, Eds. 2017, pp. 1–292.
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- [4] K. Tsiamis, E. Gervasini, I. Deriu, A.C. Cardoso. "Updates on the baseline distribution of Invasive Alien Species of Union concern (2019)", EUR 29726 EN, Publications Office of the European Union, Luxembourg, 2019, ISBN 978-92-79-02062-2, doi:10.2760/28412, JRC116322.
- [5] European Alien Species Information Network - EASIN, "Species Search and Mapping." [Online]. Available: <https://easin.jrc.ec.europa.eu/spexplorer/>.
- [6] CAB, "*Trachemys scripta elegans* [original text by IUCN SSC Invasive Species Specialist Group]." *Invasive Species Compendium*. Wallingford, UK: CAB International., 2019. [Online]. Available: <https://www.cabi.org/isc/datasheet/61560>.
- [7] P. Q. Spinks, R. C. Thomson, G. A. Lovely, and H. B. Shaffer, "Assessing what is needed to resolve a molecular phylogeny: simulations and empirical data from emydid turtles" *BMC Evol. Biol.*, vol. 9, no. 1, p. 56, Mar. 2009.

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