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.

Morone americana

(Gmelin, 1789)

Common names:
English: white perch, narrow-mouthed bass, sea perch, silver perch, wreckfish
French: baret, perche blanche, bar-perche ou petit bar, bar blanc d'Amerique, cernier atlantique, perche blanche
German: Seebarsch
Dutch: Amerikaanse zeebaars

Last update: June 2023

General information on *Morone americana*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Actinopterygii	Moroniformes	Moronidae	*Morone*

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

Infra-species level: N = 0
Note: To our knowledge, no subspecies have been described.

Native range: [3]
Canada (New Brunswick, Nova Scotia and Quebec) and United States of America (Connecticut, Maryland, New Jersey, Rhode Island, South Carolina)

Invasive range: [3]
Europe (geographical):
To our knowledge, the species has not yet been reported in Europe.

For more detailed locality information and the most recent distribution updates, please visit:
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R09615
https://www.gbif.org/species/2394604
https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.74160

Outside Europe (geographical):
Canada (Ontario) and United States of America (Colorado, Illinois, Indiana, Iowa, Kansas, Kentucky, Maine, Massachusetts, Michigan, Minnesota, Missouri, Nebraska, New Hampshire, New York, Ohio, Oklahoma, Pennsylvania, Vermont, Virginia, West Virginia, Wisconsin)

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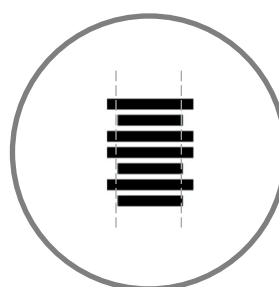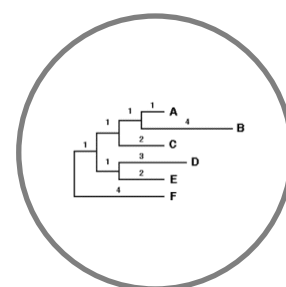
Material and Methods [1]
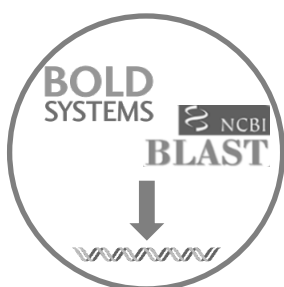
| Download all sequence data available for the genus | Filtering the data and selecting 'promising' markers | Aligning and trimming of the sequences | Building Neighbour-Joining tree with Bootstrap support |

Conclusion

Based on the present evaluation of the available sequence data, COI is a reliable DNA marker for the identification of *Morone americana*. To allow for a better evaluation of the performance of this marker for species identification, additional sequences for the genus *Morone* should be added.

Discussion

DNA markers of the genus *Morone* for which *Morone americana* sequences were available, were downloaded from GenBank and BOLD. Five DNA markers were evaluated (Table 1).

In the NJ-tree for **COI**, all sequences form clusters according to the respective species of the genus *Morone* with the exception of one BOLD sequence of *M. americana* (EFA168-16). However, according to GenBank this sequence was misidentified and belongs to *Sander vitreus*. Hence COI is useful in differentiating *M. americana* from the other *Morone* species.

For DNA markers **12S** and **16S** *M. americana* sequences form supported clusters, however for both markers *M. americana* only invasive areas are represented. Adding sequences from native areas should increase the reliability of these markers to identify *M. americana.*

For DNA markers **Rho** and **cytb** only two unique sequences for *M. americana* are available in the online databases (GenBank and BOLD). Therefore, it is currently impossible to assess the ability of these markers to identify *M. americana*.

For **18S** and **28S** fewer sequences are available and the markers show little genetic variation for *M. americana* and the congeneric species. Therefore it is currently impossible to assess the ability of these markers to identify *M. americana*.

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 genus are represented in the final NJ-tree. An 'X' indicates that the issue was encountered.

Markers analysed	1	2	3	4	5
COI					
12S		X			X
16S		X			X
cyt b	X	X			
Rho	X	X			X

Table 2: Publicly available sequences downloaded (June 2023) 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 in genus	COI	12S	16S	cytb	Rho
Morone americana	X	X	X	X	X
Morone chrysops	**X**	**x**	**X**	**X**	**X**
Morone mississippiensis	X	X		X	
Morone saxatilis	x	X	X	X	X
TOTAL species	**4/4**	**4/4**	**3/4**	**4/4**	**3/4**

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://animaldiversity.org/accounts/Morone_americana/
https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=777

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Page 2: *Morone americana* By Smithsonian Environmental Research Centre [CC BY SA 2.0]

References
1.   Smitz N, Gombeer S, Meganck K, et al. Identifying IAS based on DNA barcoding using currently available sequence data: details on applied material and methods. *BopCo*. Published online 2019. Accessed August 25, 2023. https://bopco.be/output/iasfactsheets
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3.   Siriwardena S. Morone americana (white perch). CABI Invasive Species Compendium. Published 2008. Accessed June 27, 2023. https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.74160#REF-DDB-111231

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