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.

Ameiurus melas

(Rafinesque, 1820)

Common names:
  English: black bullhead
  French: poisson-chat
  German: Schwarzer Katzenwels, Schwarze Zwergwels
  Dutch: zwarte (Amerikaanse) dwergmeerval

Last update: August 2023

General information on *Ameiurus melas*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Actinopterygii	Siluriformes	Ictaluridae	*Ameiurus*

Species in the same genus: N = 7 [2]

Infra-species level: N = 0

Note: To our knowledge, no subspecies have been described.

Native range: [3]

Canada (Manitoba, Saskatchewan), USA (central United States: from Great Lakes in the north to Mexican border), Mexico

Invasive range: [4–10]

Europe (geographical):

Albania, Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, France, Germany, Hungary, Ireland, Italy, Luxemburg, Netherlands, Norway, Poland, Portugal, Republic of Macedonia, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzerland, Ukraine and United Kingdom

Outside Europe (geographical):

Canada (British Columbia), Chile, China, Puerto Rico, USA (East and West coast).

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

https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R00826
https://www.gbif.org/species/2340977
https://fishbase.mnhn.fr/summary/Ameiurus-melas.html [11]
https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.94466 [3]
https://laji.fi/en/taxon/MX.5084366
https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=730

Morphology, biology, invasion, negative effects and remedies

For more information on *Ameiurus melas* 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.
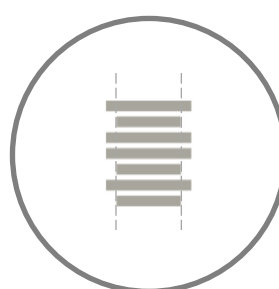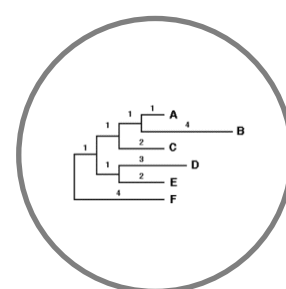
Material and Methods [1]
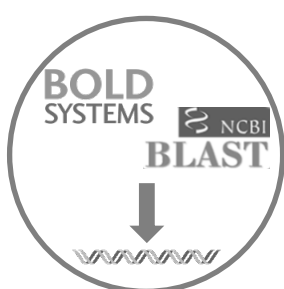
Download all sequence data available for the genus

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 and cytb are the most promising DNA markers for the identification of *Ameiurus melas*. However, due to appearance of some *A. nebulosus* in the *A. melas* cluster and vice versa, it is currently impossible to fully assess the reliability of this marker.

Discussion

DNA markers for which *Ameiurus* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Ameiurus*. Five DNA markers were evaluated (Table 1). In Padhi [12] the existence of two distinct lineages of *A. melas* is described, based on the phylogenetic analyses of mitochondrial DNA markers 12S, 16S and cytb.

The occurrence of hybrids between *A. melas* and *A. nebulosus* have been reported by Hunnicutt *et al.* [13] (*A. melas x nebulosus*), this poses a potential problem for identification. Therefore, making distinction between these two species is difficult and the results should be interpreted with caution.

Markers **COI** and **cytb** represent all known *Ameiurus* species and unique sequences for *A. melas* appear in a supported cluster. However, in the NJ-tree the *A. melas* cluster includes some *A. nebulosus* sequences and one *A. melas* (MK439913) is placed with *A. nebulosus* for the COI marker. These sequences might involve a misidentification or can be the result of hybridisation as described by Hunnicutt *et al.* [13]. Therefore, it is currently unclear whether we can rely on these markers to identify *A. melas*.

The **D-loop** marker groups *A. melas* in two supported clusters. This existence of two distinct lineages of *A. melas* is described by Padhi [12] and needs further investigation.

For **12S** the sequences for *A. melas* form a supported cluster which also includes a *A. nebulosus*. The low genetic variation raises doubts about the usefulness of this marker for the identification of *A. melas*.

Marker **16S** does not recover *A. melas* as a supported cluster.

For markers 12S, 16S and D-loop additional sequences for *A. melas* (also from the invasive regions) and the missing congeners should be added. Therefore, it is premature to decide about the ability of these DNA markers to identify *A. melas*.

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		X	X	X	
cytB		X	X	X	
D-loop		X	X	X	X
16S		X	X		X
12S		X	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]. 'X' indicates that at least one sequence was used in the final alignment.

Species in genus	COI	cytb	D-loop	16S	12S
Ameiurus brunneus	X	X	X	X	X
Ameiurus catus	X	X	X	X	X
Ameiurus melas	**X**	**X**	**X**	**X**	**X**
Ameiurus natalis	X	X	X	X	X
Ameiurus nebulosus	X	X	X	X	X
Ameiurus platycephalus	X	X			
Ameiurus serracanthus	X	X			
TOTAL species	**7/7**	**7/7**	**5/7**	**5/7**	**5/7**

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

References
1. Smitz N, Gombeer S, Meganck K, Vanderheyden A, Van Bourgonie YR, Backeljau T, De Meyer M. 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
2. Ferraris CJ. *Checklist of Catfishes, Recent and Fossil (Osteichthyes: Siluriformes), and Catalogue of Siluriform Primary Types*. Vol 1418.; 2007. doi:10.11646/zootaxa.1418.1.1
3. Godard M, Sabapathy Allen U. *Ameiurus melas* (black bullhead). CABI Compendium. Published 2022. Accessed August 24, 2023. https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.94466
4. Vancheva N, Bobeva A, Pehlivanov L, Stefanov T, Georgiev BB. Alien parasites on an alien fish species: monogeneans from the black bullhead *Ameiurus melas* (Siluriformes) in the Lake Srebarna Biosphere Reserve, Bulgaria, with the first record of *Gyrodactylus nebulosus* in the Palaearctic. *Parasitol Res*. 2020;119(7). doi:10.1007/s00436-020-06699-z
5. Copp GH, Tarkan AS, Masson G, Godard MJ, Koščo J, Kováč V, Novomeská A, Miranda R, Cucherousset J, Pedicillo G, Blackwell BG. A review of growth and life-history traits of native and non-native European populations of black bullhead *Ameiurus melas*. *Rev Fish Biol Fish*. 2016;26(3):441-469. doi:10.1007/s11160-016-9436-z
6. Musil J, Drozd B, Bĺaha M, Gallardo JM, Randak T. First records of the black bullhead, *Ameiurus melas* in the Czech Republic freshwaters. *Cybium*. 2008;32(4).
7. Movchan Y V., Talabishka EM, Velikopolskiy IJ. Fishes of the genus *Ameiurus* (Ictaluridae,Siluriformes) in the Transcarpathian water bodies. *Vestn Zool*. 2014;48(2):149-156. doi:10.2478/vzoo-2014-0015
8. Minchin D. A checklist of alien and cryptogenic aquatic species in Ireland. *Aquat Invasions*. 2007;2(4). doi:10.3391/ai.2007.2.4.4
9. Elvira B. Identification of non-native freshwater fishes established in Europe and assessment of their potential threats to the biological diversity. *Animal Biology*. 2001;(December 2000).
10. Yu F, Yu J, Zhou Y, Yan J, Fang Y, Wang W, Yang Z. Phylogenetic study of *Ameiurus melas* based on complete mitochondrial DNA sequence. *Mitochondrial DNA Part A*. 2016;27(6):4706-4707. doi:10.3109/19401736.2015.1106511
11. Froese R, Pauly D. www.fishbase.org. FishBase.
12. Padhi A. Mitochondrial DNA sequence revealed contrasting demographic history between the black bullhead (*Ameiurus melas*) and its cryptic lineage in North America. *Aquat Living Resour*. 2010;23(3):325-333. doi:10.1051/alr/2010029
13. Hunnicutt DW, Cingolani J, Voss MA. Use of mtDNA to identify genetic introgression among related species of catfish. *Journal of Great Lakes Research*. 2005;31:482-491.