



# 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](mailto:bopco@naturalsciences.be).

More info on the EU Regulation on [http://ec.europa.eu/environment/nature/invasivealien/index\\_en.htm](http://ec.europa.eu/environment/nature/invasivealien/index_en.htm).

## *Fundulus heteroclitus*

(Linnaeus, 1766)

Common names:

English: Mummichog

French: Choquemort

German: -

Dutch: -

Last update: November 2023



## General information on *Fundulus heteroclitus*

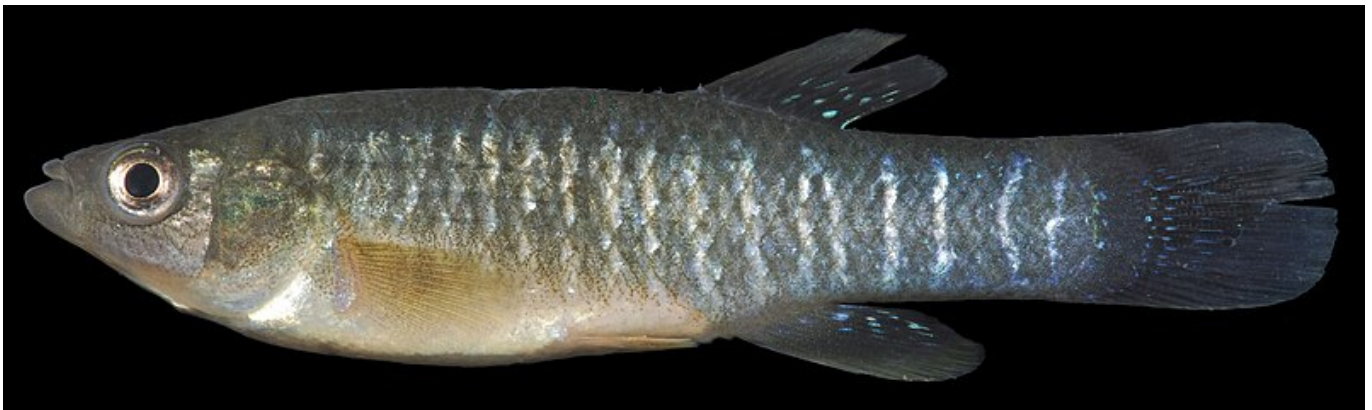
### Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Actinopterygii	Cyprinodontiformes	Fundulidae	<i>Fundulus</i>

Species in the same genus: N = 40<sup>2</sup>

Infra-species level: N = 0<sup>2</sup>

Note: To our knowledge, no subspecies are currently recognized.



**Native range:**<sup>2</sup>

North American Atlantic coast; from Gulf of St. Lawrence (Canada) to northeast Florida, USA

**Invasive range:**<sup>3</sup>

**Europe (geographical):**

Portugal and Spain.

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R06466>

<https://www.gbif.org/species/5712056>

<https://fishbase.mnhn.fr/summary/Fundulus-heteroclitus.html>

**Outside Europe (geographical):**

To our knowledge, the species has not been reported in other countries.

### **Morphology, biology, invasion, negative effects and remedies**

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



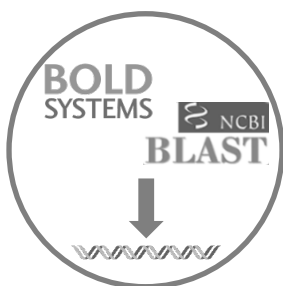
## Species identification based on DNA barcodes

### 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/](http://www.boldsystems.org/)) and GenBank ([www.ncbi.nlm.nih.gov/nuccore/](http://www.ncbi.nlm.nih.gov/nuccore/)) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

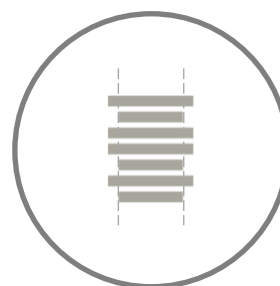
### Material and Methods <sup>1</sup>



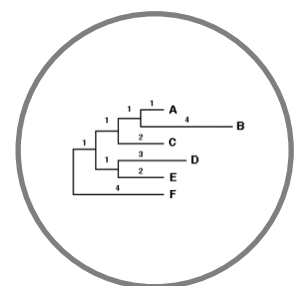
Download all sequence data available for the genus



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 D-loop are the most promising DNA markers for the identification of *Fundulus heteroclitus*. To increase the accuracy of this identification, the missing species should be added to the analyses as well as sequences from the invasive regions.

### Discussion

DNA markers for which *Fundulus* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Fundulus*. DNA markers that are represented in at least 10 out of the 40 *Fundulus* species were selected (Table 2). This resulted in six markers that were evaluated (Table 1).

The DNA markers **COI** and **D-loop** are the most promising markers for identifying *Fundulus heteroclitus*. To improve the evaluation of these markers, sequences of missing species (Table 2) should be added to the analysis, especially for D-loop. *F. bermudae* and *F. relictus* are two important missing species; according to Rabosky *et al.* <sup>4</sup> these species are closely related to *F. heteroclitus*. No non-native sequences are available for COI and D-loop.

For the markers **RAG1** and **GYLT**, the *F. heteroclitus* sequences are grouped in a well-supported cluster together with *F. bermudae* and *F. relictus*. Therefore, these markers are not suitable to identify *F. heteroclitus*.

DNA marker **cytb** is the only marker that includes *F. heteroclitus* sequences from a non-native region. However, this marker does not recover *F. heteroclitus* as a supported cluster and is therefore not a suitable marker to identify *F. heteroclitus*.

For marker **Ldh-B** the available sequences are situated in different parts of the gene. This makes it currently impossible to use this marker to identify *F. heteroclitus*.



**Table 1:** Overview of the encountered issues concerning the DNA-based identification of the IAS <sup>1</sup>: (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
cytb			X		X
RAG1	X	X	X		X
GYLT	X	X	X		X
D-loop		X			X
Ldh-B		X	X		X

**Table 2:** Publicly available sequences downloaded (October 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 <sup>5</sup>. An 'X' indicates that at least one sequence was used in the final alignment, '1' indicates only one *Fundulus* sequence was available.

Species in genus	COI	cytb	RAG1	GYLT	D-loop	Ldh-B
<i>Fundulus albolineatus</i>						
<i>Fundulus bermudae</i>		X	1	X		
<i>Fundulus bifax</i>		X				
<i>Fundulus blairae</i>	X	X	X	X		1
<i>Fundulus catenatus</i>	X	X	X	X		1
<i>Fundulus chrysotus</i>	X	X	X	X		1
<i>Fundulus cingulatus</i>	X	X		1		
<i>Fundulus confluentus</i>	X	X	X	X		
<i>Fundulus diaphanus</i>	X	X	X	X	X	1
<i>Fundulus dispar</i>	X	X	X	2	1	
<i>Fundulus escambiae</i>	X	X	X	X		
<i>Fundulus eurizonus</i>	X	X		X		
<i>Fundulus grandis</i>	X	X	X	X	X	X
<i>Fundulus grandissimus</i>	X					
<b><i>Fundulus heteroclitus</i></b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
<i>Fundulus jenkinsi</i>	X	X		X		
<i>Fundulus julisia</i>		X	1	1	X	
<i>Fundulus kansae</i>	X	X	X	X		
<i>Fundulus lima</i>	X	X	X	X	X	
<i>Fundulus lineolatus</i>	X	X	X	X		1
<i>Fundulus luciae</i>	X	1	1	1		
<i>Fundulus majalis</i>	X	X	X	X		1
<i>Fundulus notatus</i>	X	X	X	X	1	
<i>Fundulus notti</i>	X	X	X	X		1
<i>Fundulus olivaceus</i>	X	X	X	X	1	1
<i>Fundulus parvipinnis</i>	X	X	X	X	X	1
<i>Fundulus persimilis</i>						
<i>Fundulus philpisteri</i>	X					
<i>Fundulus pulvereus</i>		X	X	X		1
<i>Fundulus rathbuni</i>		X	X	X		
<i>Fundulus relictus</i>		X		X		
<i>Fundulus rubrifrons</i>	X	X	X	X		
<i>Fundulus saguanus</i>						
<i>Fundulus sciadicus</i>	X	X	1	1	X	1
<i>Fundulus seminolis</i>		X	X	X		



Species in genus	COI	cytb	RAG1	GYLT	D-loop	Ldh-B
<i>Fundulus similis</i>	X	X	X	X		1
<i>Fundulus stellifer</i>	X	X	X	X		
<i>Fundulus waccamensis</i>	1					
<i>Fundulus xenicus</i>	X					
<i>Fundulus zebrinus</i>	X	X	X	X	X	
<b>TOTAL species</b>	<b>30/40</b>	<b>33/40</b>	<b>28/40</b>	<b>32/40</b>	<b>11/40</b>	<b>14/40</b>

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/Fundulus\\_heteroclitus/](https://animaldiversity.org/accounts/Fundulus_heteroclitus/)

<https://www.gbif.org/species/144100564>

### Picture credits

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Page 2 (below): *Fundulus heteroclitus* RR 08-11-19 0580 By Robert Aguilar, Smithsonian Environmental research Center [CC-BY-2.0]

### References

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