



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.

Gambusia holbrooki

Girard, 1859

Common names:

English: eastern mosquitofish

French: gambusie de l'est

German: Östliche Moskitofisch

Dutch: oostelijk muskietenvisje

Last update: November 2023



General information on *Gambusia holbrooki*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Teleostei	Cyprinodontiformes	Poeciliidae	<i>Gambusia</i>

Species in the same genus: N = 43²

Note: This classification follows the latest version of Eschmeyer's Catalog of Fishes². *Gambusia affinis* and *Gambusia holbrooki* were previously considered to be one species *G. affinis*. Phylogenetic analysis confirmed both *G. affinis* and *G. holbrooki* are indeed two distinct species^{3,4}. Due to this taxonomic history older records might refer to *G. holbrooki* as *G. affinis*.

Infra-species level: N = 0

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



Native range: ^{5,6}

Central and North America, Mexico

Invasive range: ⁶⁻⁸

Europe (geographical):

Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, France, Greece, Hungary, Italy, Portugal, Romania, Slovenia, Spain, Republic of Macedonia, Russia, Turkey

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R06574>

<https://www.gbif.org/fr/species/2350570?vnOffset=20>

<https://www.cabidigitallibrary.org/doi/10.1079/cabicompndium.82089>

Outside Europe (geographical):

Afghanistan, Armenia, Australia, Azerbaijan, Chile, Egypt, Eritrea, Ethiopia, Georgia, India, Islamic Republic of Iran, Iraq, Kazakhstan, Kenya, Kyrgyzstan, Lebanon, Madagascar, Malaysia, Mauritius, Morocco, Papua New Guinea, Saudi Arabia, Sudan, Syrian Arab Republic, Tajikistan, Tunisia, Turkmenistan, Ukraine, Uzbekistan, United Arab Emirates, Viet Nam, and Yemen. Uncertain species but likely *G. holbrooki* for Libya and Somalia.

Morphology, biology, invasion, negative effects and remedies

For more information on *Gambusia holbrooki* 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



Conclusion

Based on the present evaluation of the available sequence data *cytb*, *COI* and *ND2* markers are the most promising DNA markers for the identification of *Gambusia holbrooki*. However, due to the large lack in available sequence data, and because hybridizations occurs with other species of the genus *Gambusia*, it is currently impossible to fully assess the reliability of these markers.

Discussion

DNA markers for which *Gambusia* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Gambusia*. Seven DNA markers were selected and evaluated (Table 1). Hybridization between *Gambusia holbrooki* and *G. affinis*, *G. georgei*, *G. heterochir* and *G. nobilis* is possible and occurs often. According to Purcell ⁹ separating *G. holbrooki* from *G. affinis* is only possible with additional microsatellite markers. Therefore the markers suggested below should be considered with care.

In the NJ-trees for **cytb** and **COI**, the *Gambusia holbrooki* sequences cluster together, with a significant high level support. However, a few sequences of *G. affinis* (one for CytB and five for COI) are also present in the cluster formed by *G. holbrooki*. Because *G. holbrooki* and *G. affinis* are morphologically very similar ¹⁰ it is possible that some sequences were derived from misidentified specimens. Under this assumption, both markers are promising to differentiate *G. holbrooki* from other *Gambusia* species.

The marker **ND2** looks promising, too. However, there are no *G. holbrooki* sequences from the invasive range. In order to better evaluate the performance of ND2 for species identification, more ND2 sequences from the invasive range of *G. holbrooki* and other *Gambusia* species should be added to the analysis.

The **16S** marker should be investigated more comprehensively: the cluster obtained for *G. holbrooki* is supported but we found only a few sequences with the same portions of the 16S gene fragment to obtain a performing alignment. Hence, more *G. holbrooki* and other *Gambusia* 16S sequences are needed to assess the reliability of his marker to identify *G. holbrooki*.



None of the other evaluated DNA markers (**12S**, **D-Loop** and **RPS7**) recovered *G. holbrooki* as a supported cluster. Hence, it is premature to decide about the ability of these DNA markers to differentiate *G. holbrooki* from other *Gambusia* species.

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
cytb				X	X
ND2		X			X
D-Loop		X	X		X
16S	X	X			X
12S		X	X		X
RPS7	X	X	X		X

Table 2: Publicly available sequences downloaded (November 2023) from BOLD and GenBank (including sequences extracted from mitochondrial genome) which were withheld as reliable and informative in the final alignment that was used for building the NJ-trees. The species names follow Eschmeyer's Catalog ². An 'X' indicates that at least one sequence was used in the final alignment, a '1' indicates only one *Gambusia* sequence was available. Species names with * are not mentioned in ² but used on GenBank.

Species in genus	COI	cytb	ND2	D-Loop	12S	16S	RPS7
<i>Gambusia affinis</i>	X	X	X	X	X	X	X
<i>Gambusia alvarezi</i>							
<i>Gambusia amistadensis</i>							
<i>Gambusia atrora</i>		1	1		1	1	
<i>Gambusia aurata</i>	X	X	X				X
<i>Gambusia baracoana</i>							
<i>Gambusia beebei</i>							
<i>Gambusia bucheri</i>							
<i>Gambusia caymanensis</i> *		1					
<i>Gambusia clarkhubbsi</i>		1	1				1
<i>Gambusia dominicensis</i>							
<i>Gambusia eurystoma</i>		1					
<i>Gambusia gaigei</i>			1				1
<i>Gambusia geiseri</i>		X	1				X
<i>Gambusia georgei</i>							
<i>Gambusia heterochir</i>		X	1				1
<i>Gambusia hispaniolae</i>		1	1				1
<i>Gambusia holbrooki</i>	X	X	X	X	X	X	X
<i>Gambusia hubbsi</i> *		X	X				X
<i>Gambusia hurtadoi</i>		X	1				1
<i>Gambusia krumholzi</i>		X					
<i>Gambusia lemaitrei</i>		1	1				1
<i>Gambusia longispinis</i>							
<i>Gambusia luma</i>		X					
<i>Gambusia manni</i>	X	X	X				X
<i>Gambusia marshi</i>	X	1					
<i>Gambusia melapleura</i>		1					
<i>Gambusia monticola</i>							
<i>Gambusia nicaraguensis</i>	X	X	1				1
<i>Gambusia nobilis</i>							
<i>Gambusia oligosticta</i> *		1					
<i>Gambusia panuco</i>	X	1					
<i>Gambusia pseudopunctata</i>							
<i>Gambusia punctata</i>	X	X		X	X	X	
<i>Gambusia puncticulata</i>	X	1		1	1	1	
<i>Gambusia quadruncus</i>							
<i>Gambusia regani</i>	X						



Species in genus	COI	cytb	ND2	D-Loop	12S	16S	RPS7
<i>Gambusia rhizophorae</i>	X	X	1	X	X	X	1
<i>Gambusia senilis</i>							
<i>Gambusia sexradiata</i>	X	X					
<i>Gambusia speciosa</i>		X	1				1
<i>Gambusia vittata</i>	X	X	1		1	1	
<i>Gambusia wrayi</i>		X	X		1	1	1
<i>Gambusia xanthosoma</i>							
<i>Gambusia yucatana</i>	X	1			1		
<i>Gambusia zarskei</i>							
TOTAL species	14/43	26(29)*/43	17(18)*/43	5/43	9/43	8/43	15(16)*/43

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

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<https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.82089>
https://en.wikipedia.org/wiki/Eastern_mosquitofish
<https://www.fishbase.org.au/v4/summary/4521>

Picture credits

Page 1: A female eastern mosquitofish in a central Floridian pond, by Marsh Bunny. [CC BY-SA 4.0]
Page 2: A female Eastern Mosquitofish, *Gambusia holbrooki*. Muddy Creek, Rhode River, SERC, Edgewater, Anne Arundel County, MD - 02/17/16.
Photo by Robert Aguilar, by Smithsonian Environmental Research Center. [CC-BY-2.0]

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