



# Identification of Invasive Alien Species using DNA barcodes

Royal Belgian Institute of Natural Sciences  
Rue Vautier 29  
1000 Brussels, Belgium  
+32 (0)2 627 41 23



Royal Museum for Central Africa  
Leuvensesteenweg 13,  
3080 Tervuren, Belgium  
+32 (0)2 769 58 54



## 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).

## *Gambusia affinis*

(Baird and Girard, 1853)

Common names:

English: western mosquitofish

French: gambusie de l'ouest

German: Westlicher Moskitofisch

Dutch: muskietenvisje

Last update: November 2023



## General information on *Gambusia affinis*

### Classification

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

### Species in the same genus: N = 43<sup>2</sup>

Note: This classification follows the latest version of Eschmeyer's Catalog of Fishes<sup>2</sup>. *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<sup>3,4</sup>. 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:<sup>5,6</sup>

Central and North America.

### Invasive range:<sup>5-8</sup>

#### Europe (geographical):

Albania, Bulgaria, France, Greece, Hungary, Italy\*, Portugal, Romania, Russia, Slovenia, Spain and Ukraine.

\*A recent review<sup>9</sup> assumes that all introductions to Europe before 1927 were of *G. holbrooki*, therefore most likely Italy is the only European country in which *G. affinis* is present.

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R06573>

<https://www.gbif.org/fr/species/2350580?vnOffset=20>

<https://www.fishbase.org.au/v4/summary/3215>

#### Outside Europe (geographical):

Argentina, Bahamas, Bangladesh, Bolivia, Botswana, Cambodia, Canada, Cape Verde, Chile, China, Democratic Republic of Congo, Dominican Republic, East Timor, Ecuador, Federated States of Micronesia, Fiji, Haiti, India, Israel, Japan, Jordan, Laos PDR, Marshall Islands, Mozambique, Myanmar, Nauru, Nepal, New Zealand, Pakistan, Palau, Peru, Philippines, Samoa, Singapore, Solomon Islands, South Africa, Sri Lanka, State of Palestine, Syrian Arab Republic, Taiwan, Tanzania, Thailand, Vanuatu, Vietnam, Zambia, and Zimbabwe.

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

For more information on *Gambusia affinis* 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>



### 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* species. However, due to the large lack in available sequence data, and because hybridisations 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 <sup>10</sup> 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 affinis* sequences cluster together, with a significant high level support. One potential misidentified sequence of *G. speciosa* (accession number: JX679670) is present in the *G. affinis* cluster for *cytb*. 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. affinis* and *G. holbrooki* are morphologically very similar <sup>7</sup>, it is possible that some sequences were derived from misidentified specimens. Hence, assuming that these sequences were from misidentified specimens, both markers are promising to differentiate *G. affinis* from other *Gambusia* species.

The marker **ND2** looks promising. However, there are no *G. affinis* 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. affinis* and other *Gambusia* species should be added to the analysis.

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



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

**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
<b>COI</b>				X	X
<b>cytb</b>				X	X
<b>ND2</b>		X			X
<b>D-Loop</b>		X	X		X
<b>16S</b>	X	X			X
<b>12S</b>		X	X		X
<b>RPS7</b>	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 <sup>2</sup>. An 'X' indicates that at least one sequence was used in the final alignment.

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



Species in genus	COI	cytb	ND2	D-Loop	12S	16S	RPS7
<i>Gambusia senilis</i>							
<i>Gambusia sexradiata</i>	X	X					
<i>Gambusia speciosa</i>		X	X				X
<i>Gambusia vittata</i>	X	X	X		X	X	
<i>Gambusia wrayi</i>		X	X		X	X	X
<i>Gambusia xanthosoma</i>							
<i>Gambusia yucatana</i>	X	X			X		
<i>Gambusia zarskei</i>							
<b>TOTAL species</b>	<b>14/43</b>	<b>26(29)*/43</b>	<b>17(18)*/43</b>	<b>5/43</b>	<b>9/43</b>	<b>8/43</b>	<b>15(16)*/43</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://www.b-aqua.com/Pages/Fiche.aspx?Id=1656>  
<http://www.iucngisd.org/gisd/speciesname/Gambusia+affinis>  
<https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.82079>  
<https://en.wikipedia.org/wiki/Mosquitofish>

### Picture credits

Page 1: An adult female *Gambusia affinis* (western mosquitofish) at the University of Mississippi Field Station; by Fredlyfish4 [CC BY-SA 4.0]  
Page 2 (left) : *Gambusia affinis* (kadayashi) male; by Nozo [CC BY-SA 3.0]  
Page 2 (right) : A western mosquitofish (*Gambusia affinis*) in Tyson Research Center, Missouri; By Fredlyfish4 [CC BY-SA 4.0]

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