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), and it represented part of the Belgian federal contribution to the European Research Infrastructure Consortium LifeWatch (November 2015 – February 2022).

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.

Lepomis gibbosus

(Linnaeus, 1758)

Common names:
  English: pumpkinseed, pond perch
  French: perche soleil, perche japonaise, Crapet-soleil
  German: Gemeine Sonnenbarsch, Kürbiskernbarsch
  Dutch: zonnebaars

Last update: January 2022

General information on *Lepomis gibbosus*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Actinopterygii	Perciformes	Centrarchidae	*Lepomis*

Species in the same genus: N = 13 [2-4]

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

Native range: [5]
Canada (southern Ontario and Quebec) and the northeast of the United States of America.

Invasive range: [5-7]
Europe (geographical):

Austria, Belarus, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Luxembourg, Macedonia, Moldova, Montenegro, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom, Sweden.

For more detailed locality information and the most recent distribution updates, please visit:
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R08376
https://www.gbif.org/species/2394486
https://www.nobanis.org/globalassets/speciesinfo/l/lepomis-gibbosus/lepomis_gibbosus.pdf

Outside Europe (geographical):
Algeria, Brazil, Canada (British Columbia), Chile, Cuba, Democratic Republic of the Congo, Georgia, Guatemala, Morocco, United States of America, Venezuela.

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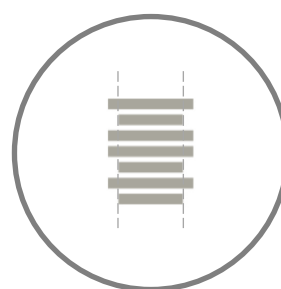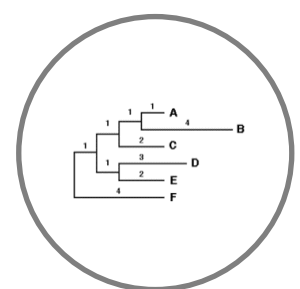
Material and Methods [1]
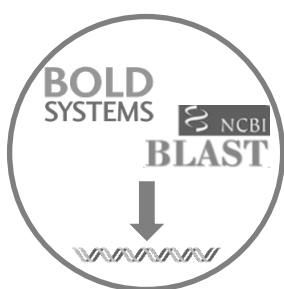
| Download all sequence data available for the genus | Filtering the data and selecting 'promising' markers | Aligning and trimming of the sequences | Building Neighbour-Joining tree with Bootstrap support |

Conclusion
Based on the present evaluation of the available sequence data, COI is the most reliable DNA marker for the identification of *Lepomis gibbosus*. To allow for a better evaluation of the performance of this marker, sequences of *L. peltastes* and *L. symmetricus* should be added to the analysis.

Discussion
DNA markers for which *Lepomis* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Lepomis*. Eight DNA markers were evaluated (Table 1).

The DNA marker **COI** is the most promising marker for identifying *Lepomis gibbosus*. All COI sequences for *L. gibbosus* form one cluster containing the many native and non-native sequences which are available for the species. In addition, almost all other species in the genus are represented by multiple sequences. To fine-tune the evaluation of this marker, sequences of *L. peltastes* and *L. symmetricus* (Table 2) should be added to the analysis.

For Cytb and **16S** fewer sequences are available for both *L. gibbosus* and its congeners, in comparison to COI. In the case of cytb, two *L. auritus* sequences end up within the *L. gibbosus* cluster, yet these might involve hybrids between both species [8].

The sequences of *L. gibbosus* also cluster together in the NJ-trees based on the **12S, D-loop, ND1, ND2** and **Rho** DNA markers. However, additional sequences of the congeners and from the invasive region of *L. gibbosus* should be added to the analyses to allow for a better evaluation of the performance of these markers to identify *L. gibbosus*.

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 congeneric species are represented in the final NJ-tree. An 'X' indicates that the issue was encountered, an '(X)' indicates the non-clustering was due solely to (a) potential misidentified or mislabelled sequence(s).

Markers analysed	1	2	3	4	5
COI					X
cytb			(X)	X	X
16S					X
12S	X				X
D-loop	X				X
ND1					X
ND2	X				X
Rho	X	X			X

Table 2: Publicly available sequences downloaded (December 2021) 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 [2-4]. An 'X' indicates that at least one sequence was used in the final alignment, a '1' indicates only one sequence was available.

Species in genus	COI	cytb	16S	12S	D-loop	ND1	ND2	Rho
Lepomis auritus	X	X	X	X	X	X	X	1
Lepomis cyanellus	X	X	X	X	X	X	X	X
Lepomis gibbosus	X	X	X	X	X	X	X	X
Lepomis gulosus	X	X	X	X	X	X	X	X
Lepomis humilis	X	X	X	1			X	X
Lepomis macrochirus	X	X	X	X	X	X	X	X
Lepomis marginatus	X	X	1				1	1
Lepomis megalotis	X	X	X	1	X	1	X	X
Lepomis microlophus	X	X	X			1	X	X
Lepomis miniatus	X	X	X	1			X	X
Lepomis peltastes								
Lepomis punctatus	X	X	X	X	X	X	X	1
Lepomis symmetricus	1	X	1				1	1
TOTAL species	**12/13**	**12/13**	**12/13**	**9/13**	**7/13**	**8/13**	**12/13**	**12/13**

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.cabi.org/isc/datasheet/77080
https://www.nobanis.org/globalassets/speciesinfo/l/lepomis-gibbosus/lepomis_gibbosus.pdf
https://animaldiversity.org/accounts/Lepomis_gibbosus/
https://ias.biodiversity.be/species/show/3
https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=382

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Page 2 (right): Zonnebaars (*Lepomis gibbosus*) By Jelle Wissink [CC BY-SA 4.0]

References
[1]   N. Smitz, S. Gombeer, K. Meganck, A. Vanderheyden, Y. R. Van Bourgonie, T. Backeljau, and M. De Meyer, "Identifying IAS based on DNA barcoding using currently available sequence data: details on applied material and methods." 2019. [Online]. Available from: https://bopco.be/output/iasfactsheets.

[2]   Near, T. J., & Koppelman, J. B. (2009). Species diversity, phylogeny and phylogeography of Centrarchidae. Centrarchid Fishes: Diversity, Biology, and Conservation. Blackwell Science, London, 1-38.

[3]   Near, T. J., & Kim, D. (2021). Phylogeny and time scale of diversification in the fossil-rich sunfishes and black basses (Teleostei: Percomorpha: Centrarchidae). Molecular Phylogenetics and Evolution, 161, 107156.

[4]   R. Froese and D. Pauly, "Fishbase" 2021. [Online]. Available: www.fishbase.org. [Accessed: 28-Jan-2022].

[5]   Yavno, S., Gobin, J., Wilson, C. C., Vila-Gispert, A., Copp, G. H., & Fox, M. G. (2020). New and Old World phylogeography of pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758): the North American origin of introduced populations in Europe. Hydrobiologia, 847(2), 345-364.

[6]   Deputy Direction of Nature (Ministry of Agriculture, Fish, Food and Environment of Spain), "EU non-native organism risk assessment scheme", 2018.

[7]   CABI, "*Lepomis gibbosus* [original text by Gordon Copp and Michael Godard]." Invasive Species Compendium. Wallingford, UK: CAB International., 2021. [Online]. Available: https://www. https://www.cabi.org/isc/datasheet/77080.

[8]   Harris, P. M., Roe, K. J., & Mayden, R. L. (2005). A mitochondrial DNA perspective on the molecular systematics of the sunfish genus *Lepomis* (Actinopterygii: Centrarchidae). Copeia, 2005(2), 340-346.

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