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), 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.

Pseudorasbora parva

(Temminck and Schlegel, 1846)

Common names:
English: false razbora, stone moroco, stone moroko, stone morokos, topmouth gudeon, topmouth minnow
French: goujon asiatique
German: Blaubandbärbling, Blaubandgründling, Keilfleckbarbe, Amurbärbling, Pseudokeilfleckbarbe, Asiatischer Gründling
Dutch: blauwband

Last update: January 2019

General information on *Pseudorasbora parva*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Pseudorasbora*

Species in the same genus: N = 5 [2-8]
Note: Hybrids between *Pseudorasbora parva* and *P. pumila*, as well as between *P. parva* and *Pungtungia herzi* have been reported [e.g. 5–7].

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



Native range: [9]
Asia; including the north-eastern part of China, Japan, Korea, Russia (Amur basin) and Taiwan.

Invasive range: [9,10]
Europe (geographical):
Albania, Austria, Belarus, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, France, Germany, Greece, Hungary, Italy, Lithuania, Luxembourg, Moldova, Montenegro, Netherlands, North Macedonia, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom.

For more detailed locality information and the most recent distribution updates, please visit:
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R12451
https://www.gbif.org/species/2362868

Outside Europe (geographical):
Afghanistan, Algeria, Armenia, Fiji, Iran, Kazakhstan, Laos, Uzbekistan.

Morphology, biology, invasion, negative effects and remedies
For more information on *Pseudorasbora parva* please see the references and online information listed at the end of this document.

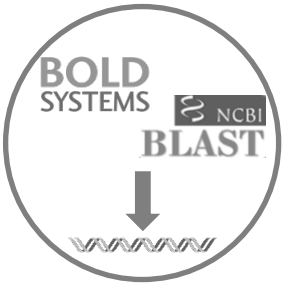
Species identification based on DNA barcodes

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.

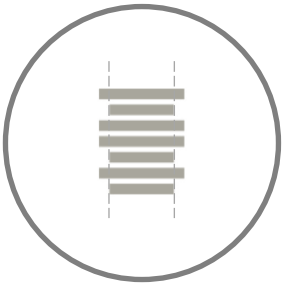
In this context, BopCo evaluated the inclusion of the IAS and their close relatives in both publicly available reference libraries BOLD (www.boldsystems.org/) and GenBank (www.ncbi.nlm.nih.gov/nuccore/) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

Material and Methods [1]

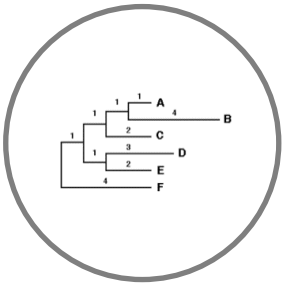


Download all sequence data available for the genus

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 reliable DNA markers for the identification of *Pseudorasbora parva*. To allow for a better evaluation of the performance of these markers for species identification, sequences for *P. interrupta* should be added.

Discussion

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

At first glance, **COI, cytb, 16S, 12S, D-loop** and **ND5** look promising to identify *Pseudorasbora parva,* since for each of them *P. parva* forms a highly supported cluster. However, only for COI and cytb multiple sequences from both native and invasive regions are available. Therefore, these are the preferential DNA markers to be used for the identification of *P. parva*, although sequence information of *P. interrupta* is missing for both markers (Table 2). To allow for a better evaluation of the performance of these markers, this missing species should be added since in all analyses including *P. interrupta* (i.e. **RAG-1**, **RAG-2** and **egr1**), the available *P. interrupta* sequence clusters together with *P. parva*. However, *P. interrupta* is endemic to streams on Fenghuang Mountain in Guangdong (China) [2] and thus little chance exists of it being encountered in Europe.

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.

Markers analysed	**1**	**2**	**3**	**4**	**5**
COI					X
cytb					X
16S		X			X
12S	X	X			X
D-loop		X			X
ND5		X			X
RAG-1			X		X
RAG-2	X	X	X		X
egr1	X	X	X		X

Table 2: Publicly available sequences downloaded (January 2019) 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]. An 'X' indicates that at least one sequence was used in the final alignment, a '1' indicates only one sequences was available for the final alignment.

Species in genus	**COI**	**cytb**	**16S**	**12S**	**D-loop**	**ND5**	**RAG-1**	**RAG-2**	**egr1**
Pseudorasbora elongata	X	X	X	X	X	X	X	X	X
Pseudorasbora interrupta							1	1	1
Pseudorasbora parva	X	X	X	X	X	X	X	X	X
Pseudorasbora pugnax	X	X	X		X	X			
Pseudorasbora pumila	X	X	X	X	X	X	X	X	X
TOTAL species	4/5	4/5	4/5	3/5	4/5	4/5	4/5	4/5	4/5

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

http://www.cabi.org/isc/datasheet/67983
http://ias.biodiversity.be/species/show/5
https://www.nobanis.org/globalassets/speciesinfo/p/pseudorasbora-parva/pseudorasbora_parva.pdf
https://nas.er.usgs.gov/queries/greatlakes/FactSheet.aspx?SpeciesID=64&Potential=Y&Type=2&HUCNumber=
http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=2876
https://www.fws.gov/injuriouswildlife/pdf_files/Pseudorasbora_parva_WEB_9-15-2014.pdf
http://species.biodiversityireland.ie/profile.php?taxonId=14244&taxonName=parva
https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-blauwband [NL]

Picture credits

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Page 2 (right): *Pseudorasbora parva*, Dniester Estuary By Yuriy Kvach [CC BY-SA 3.0]

References

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