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.

Perccottus glenii

Dybowski, 1877

Common names:
   English: Chinese sleeper, Amur sleeper
   French: goujon de l'amour
   German: Chinesische Grundel, Schlafergrundel, Amurgründling
   Dutch: Amurgrondel, Amoergrondel

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General information on *Perccottus glenii*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Actinopterygii	Gobiiformes	Odontobutidae	*Perccottus*

Note: This classification follows Wiley and Johnson [2], who consider the Gobiiformes as an order. This is the classification adopted by GenBank, BOLD and the Encyclopedia of Life. Fishbase and several authors [e.g. 3,4], however, consider the Gobioidei as a suborder within the order Perciformes.

Species in the same genus: N = 1 [5,6]
Note: There are 6 genera in the Odontobutidae: *Micropercops* (5 species), *Odontobutis* (8), *Sineleotris* (3), *Terateleotris* (1), *Neodontobutis* (5) and *Perccottus* (1).

Infra-species level: N = 0 [5]
Note: To our knowledge, no subspecies have been described.

Native range: [7]
North-eastern China, the northern part of North Korea and in the far East of Russia.

Invasive range: [7–10]
Europe (geographical):
Belarus, Bulgaria, Croatia, Estonia, Germany, Hungary, Latvia, Lithuania, Moldova, Poland, Romania, Russia (European part), Serbia, Slovakia, Ukraine.

For more detailed locality information and the most recent distribution updates please visit:
https://www.gbif.org/species/2390064
https://www.fishbase.de/Country/CountryList.php?ID=4696&GenusName=Perccottus&SpeciesName=glenii
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R11034

Outside Europe (geographical):
Kazakhstan, Mongolia, Russia (Asian part), Turkmenistan, and formerly present in Uzbekistan (but now declared eradicated).

Morphology, biology, invasion, negative effects and remedies
For more information on *Perccottus glenii* 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 *Perccottus glenii*. To allow for a better evaluation of the performance of these markers for species identification, the missing species and genera of the family Odontobutidae should be added to the analyses.

Discussion

Since *Perccottus* is a monotypic genus, DNA markers for which *Perccottus glenii* sequences were available, were downloaded from GenBank and BOLD for all represented species of the family Odontobutidae. Seven DNA markers were evaluated (Table 1). For all investigated markers, the number of represented species and genera in the analyses were comparable (Table 2). The markers, however, differed in the number of sequences available per species, especially for *Perccottus glenii*.

At first glance, all DNA markers (Table 1) look promising for identifying *Perccottus glenii* since for each, except for D-loop which is discussed later, *P. glenii* forms a cluster with maximum support. **COI** and **cytb** are the preferred markers at the moment since *P. glenii* is represented by multiple sequences from both the native and invasive range, accounting for more intra-specific variation coverage [11,12]. To allow for a better evaluation of these markers the missing species and genera of the family Odontobutidae (Table 2) should be added to the analyses.

For **16S, 12S, NADH1** and **NADH2** gene fewer *P. glenii* sequences are available and they originate only from the native region. Additional sequences for *P. glenii*, especially from the invasive regions, as well as the missing species and genera should be added to the analyses to fully assess the ability of these markers to identify *P. glenii*.

The non-clustering of the *Perccottus glenii* sequences for **D-loop** is due to one sequence (GenBank accession number KM657956) that does not cluster with the other two sequences, but rather clusters with *Odontobutis* sequences. The respective D-loop sequence was derived from a mitochondrial genome. All other sequences derived from this genome (all investigated markers are mitochondrial in origin) cluster with *P. glenii* sequences with maximum support, making it doubtful that this non-clustering concerns a misidentification.

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, a '1' indicates only one target sequence was available, n/a: not applicable.

Markers analysed	1	2	3	4	5
COI					X
cytb					X
D-loop	X	X	X		X
16S	X	X			X
12S	X	X			X
NADH1		X			X
NADH2		X			X

Table 2: Publicly available sequences downloaded (August 2018) 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 [5]. An 'X' indicates that at least one sequence was used in the final alignment, a '1' indicates only one sequence was used in the final alignment.

Species in family	COI	cytb	D-loop	16S	12S	NADH1	NADH2
Micropercops borealis							
Micropercops cinctus							
Micropercops dabryi							
Micropercops hotayensis							
Micropercops swinhonis	X	X	1	X	X	1	
Odontobutis haifengensis	X	X	1	1	X	1	1
Odontobutis hikimius				1	X		
Odontobutis interrupta	X	1	1	1	X	1	1
Odontobutis obscura	X	X	X	X	X	1	X
Odontobutis platycephala	X	X	X	X	X	X	X
Odontobutis potamophila	X	X	X	X	X	X	X
Odontobutis sinensis	X	X	X	X	X	X	X
Odontobutis yaluensis	1	X	X	X	X	X	X
Perccottus glenii	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Neodontobutis aurarmus							
Neodontobutis hainanensis	1	1	1			1	1
Neodontobutis macropectoralis							
Neodontobutis ngheanensis							
Neodontobutis tonkinensis							
Sineleotris chalmersi	1	1	1	1	1	1	1
Sineleotris namxamensis							
Sineleotris saccharae							
Terateleotris aspro							
TOTAL species	**11/23**	**11/23**	**11/23**	**11/23**	**11/23**	**11/23**	**10/23**

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 markers for species identification, as well as for more information on how to send samples for analyses please contact BopCo directly.

References and online information

Online information
https://data.inbo.be/purews/files/762889/Verreycken_2013_RiskAnalysisOfTheAmurSleeperPerccottusGlenii
http://ias.biodiversity.be/species/show/82#
http://www.cabi.org/isc/datasheet/110577
https://nas.er.usgs.gov/queries/greatlakes/FactSheet.aspx?SpeciesID=44&Potential=Y&Type=2&HUCNumber=
https://www.nobanis.org/species-alerts/perccottus-glenii/
https://www.fws.gov/injuriouswildlife/pdf_files/Perccottus_glenii_WEB_9-15-2014.pdf
https://www.zin.ru/Animalia/Pisces/eng/taxbase_e/species_e/perccottus/perccottus_glenii_eng.pdf

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Page 2 (right): Chinese sleeper (*Perccottus glenii*) - detail of the head, freshwater fish of Odontobutidae family By Petrtyl [CC BY-SA 3.0]

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] E. O. Wiley and G. D. Johnson, "A teleost classification based on monophyletic groups" in *Origin and Phylogenetic Interrelationships of Teleosts*, J. S. Nelson, H. P. Schultze, and M. V. H. Wilson, Eds. München, Germany: Verlag Dr. Friedrich Pfeil, 2010, pp. 123–182.

[3] A. Agorreta, D. San Mauro, U. Schliewen, J.L. Van Tassell, M. Kovačić, R. Zardoya and L. Rüber, "Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies" *Mol. Phylogenet. Evol.*, vol. 69, no. 3, pp. 619--633, 2013.

[4] C. E. Thacker, "Phylogeny of Gobioidei and Placement within Acanthomorpha, with a New Classification and Investigation of Diversification and Character Evolution" *Copeia*, vol. 2009, no. 1, pp. 93–104, Feb. 2009.

[5] R. Froese and D. Pauly, "Fishbase" 2018. [Online]. Available: www.fishbase.org. [Accessed: 29-Aug-2018].

[6] A. Iwata, "Systematics of Odontobutidae" in *The biology of gobies.*, R. A. Patzner, J. L. Van Tassell, M. Kovacic, and B. G. Kapoor, Eds. CRC Press, 2011, pp. 61–77.

[7] CABI, "*Perccottus glenii* [original text by Joanna Grabowska]." *Invasive Species Compendium. Wallingford, UK: CAB International.*, 2018. [Online]. Available: https://www.cabi.org/isc/datasheet/110577. [Accessed: 29-Aug-2018].

[8] H. Verreycken, "Risk analysis of the Amur sleeper *Perccottus glenii*, risk analysis report of non-native organisms in Belgium. Rapporten van het Instituut voor Natuur- en Bosonderzoek (INBO.R.2013.40)." 2013.

[9] A. N. Reshetnikov, "The introduced fish, rotan (*Perccottus glenii*), depresses populations of aquatic animals (macroinvertebrates, amphibians, and a fish)" *Hydrobiologia*, vol. 510, no. 1–3, pp. 83–90, Dec. 2003.

[10] A. N. Reshetnikov and G. F. Ficetola, "Potential range of the invasive fish rotan (*Perccottus glenii*) in the Holarctic" *Biol. Invasions*, vol. 13, no. 12, pp. 2967–2980, Dec. 2011.

[11] M. Luca, D. Ureche, D. Nicuță, G. Ghiorghiță, R. C. Druică, and L. D. Gorgan, "The genetic variability of the invasive *Perccottus glenii* from Siret River, using the cytochrome b gene" *Rom. Soc. Cell Biol. ©*, vol. XIX, no. 1, pp. 11–20, 2014.

[12] A. N. Reshetnikov, "Spatio-temporal dynamics of the expansion of rotan *Perccottus glenii* from West-Ukrainian centre of distribution and consequences for European freshwater ecosystems" *Aquat. Invasions*, vol. 8, no. 2, pp. 193–206, 2013.

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