



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) aims at developing an expertise forum to facilitate the identification of biological samples of policy concern in Belgium and Europe. The project represents part of the Belgian federal contribution to the European Research Infrastructure Consortium LifeWatch.

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. In order to implement the proposed actions, however, 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 compiling 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 to the taxonomic level stated in the EU list using DNA barcoding. 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 <http://bopco.myspecies.info/> or contact us via bopco@naturalsciences.be.

More info on the EU Regulation on http://ec.europa.eu/environment/nature/invasivealien/index_en.htm.

Percottus glenii

Dybowski, 1877

Common names:

English: Chinese sleeper, Amur sleeper

French: goujon de l'amour

German: Chinesische Grundel, Schlafgrundel, Amurgründling

Dutch: Amurgrondel, Amoergrondel

Last update: October 2018



General information on *Perccottus glenii*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Actinopterygii	Gobiiformes	Odontobutidae	<i>Perccottus</i>

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 Gobioidae 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>

<http://alien.jrc.ec.europa.eu/SpeciesMapper>

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. To assure correct species identifications, however, reference libraries need to include a sufficiently large number of sequences of (i) the IAS under investigation, in order to assess the intraspecific genetic divergence; (ii) the closely related species, in order to evaluate the interspecific genetic divergence; (iii) the different geographical areas covering the distribution range (native and invasive) of the IAS in order to detect potential population structure or local hybrids.

Against this background, 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, 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 (as defined by Froese and Pauly [5]). 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 **CR** 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.

Markers analysed	1	2	3	4	5
COI					X
cytb					X
CR	X	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.

Species in family	COI	cytb	CR	16S	12S	NADH1	NADH2
<i>Micropercops borealis</i>							
<i>Micropercops cinctus</i>							
<i>Micropercops dabryi</i>							
<i>Micropercops hotayensis</i>							
<i>Micropercops swinhonis</i>	X	X	X	X	X	X	X
<i>Odontobutis haifengensis</i>	X	X	X	X	X	X	X
<i>Odontobutis hikimius</i>				X	X		
<i>Odontobutis interrupta</i>	X	X	X		X	X	X
<i>Odontobutis obscura</i>	X	X		X	X		X
<i>Odontobutis platycephala</i>	X	X	X	X	X	X	X
<i>Odontobutis potamophila</i>	X	X	X	X	X	X	X
<i>Odontobutis sinensis</i>	X	X	X	X	X	X	X
<i>Odontobutis yaluensis</i>	X	X	X	X	X	X	X
<i>Percottus glenii</i>	X	X	X	X	X	X	X
<i>Neodontobutis aurarmus</i>							
<i>Neodontobutis hainanensis</i>							
<i>Neodontobutis macropectoralis</i>							
<i>Neodontobutis ngheanensis</i>							
<i>Neodontobutis tonkinensis</i>							
<i>Sineleotris chalmersi</i>							
<i>Sineleotris namxamensis</i>							
<i>Sineleotris saccharae</i>							
<i>Terateleotris aspro</i>							
TOTAL species	9/23	9/23	8/23	9/23	10/23	8/23	9/23

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
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<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/>
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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]

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