



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).

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.

Channa argus

(Cantor, 1842)

Common names:

English: amur snakehead, northern snakehead, ocellated snakehead

French: poisson à tête de serpent du Nord

German: Amur-Schlängenkopf

Dutch: noordelijke slangekopvis

Last update: April 2023



General information on *Channa argus*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Teleostei	Anabantiformes	Channidae	<i>Channa</i>

Species in the same genus: N = 51²⁻⁴

Note: We follow the classification of Betancur *et al.*² where the family Channidae is placed within the order Anabantiformes.

Hybridization between *C. argus* and *C. maculata* is reported and very common in aquaculture.

The number of species varies from one reference website to another, even those following the same classification.

Infra-species level: N = 0

Note: To our knowledge, no subspecies are currently recognized.



Native range:^{5,6}

China, North Korea, South Korea, Russia (South-West).

Invasive range:⁶⁻⁹

Europe (geographical):

Czech Republic

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R03330>

<https://www.gbif.org/species/4284921>

<https://fishbase.mnhn.fr/country/CountryList.php?ID=4799&GenusName=Channa&SpeciesName=argus>

Outside Europe (geographical):

Aral sea basin, Japan, Kazakhstan, Turkmenistan, Russia, USA (California, Florida, and Maryland) and Uzbekistan.

Morphology, biology, invasion, negative effects and remedies

For more information on *Channa argus* please see the references and online information listed at the end of this document.



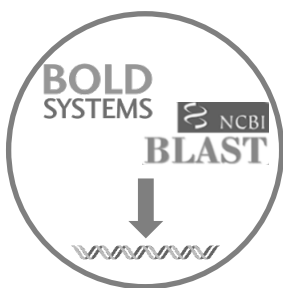
Species identification based on DNA barcodes

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.

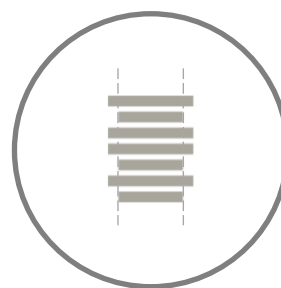
Material and Methods ¹



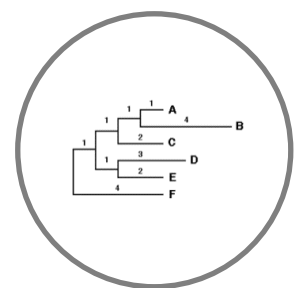
Download all sequence data available for the genus



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, cytb and ND2 are the most reliable DNA marker for the identification of *Channa argus*. To allow for a better evaluation of the performance of this marker, additional sequences for *C. argus* from the invasive regions should be added to the analyses.

Discussion

DNA markers for which *Channa* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Channa*. Seven DNA markers were evaluated (Table 1). In accordance with WoRMS ¹⁰ all *C. argus kimurai* sequences were treated as *C. argus* (Table 2).

Hybrids between *C. argus* and *C. maculata* have been reported in aquaculture ⁴ (*Channa argus x maculata*), this poses a potential problem for identification based on morphology. Therefore, making distinction between these two species is difficult and the results should be interpreted with caution ¹¹⁻¹³.

Channa argus sequences form the best supported clusters with the markers **COI**, **cytb**, **ND2** and **D-Loop**. NJ-trees for COI, cytb and ND2 currently give a the best illustration of the genus *Channa* (Table 2). Sequences for the *C. argus* are recovered in a supported cluster and have sufficient geographical coverage. Therefore, COI and cytb are best suited to identify *C. argus* from other *Channa* congeneric species. The sequence *C. lucius* (accession number KT358957) clustering with *C. argus* in the cytb tree is due to a misidentification and should be re-labeled as *C. argus*.

However, for the marker **D-Loop**, the genus *Channa* is under-represented with only 14 species (out of 51, Table 1). Moreover, the sequences of *Channa argus* lack geographical coverage. The same conclusion applies to the **ATP6** marker, with only 16 species represented (out of 51). Additional sequences from missing *Channa* species would allow for a better evaluation of the markers CR and ATP6.



For both DNA markers **16S** and **12S**, *Channa argus* forms a supported cluster. For **16S** one potential misidentified sequence of *C. striata* (MH428669) is present in the *C. argus* cluster. Therefore, 16S and 12S are not suitable markers.

The marker **RAG1** only has three unique sequences representing *C. argus*, which is not a sufficient representation of the genetic diversity within the species. Therefore, the ability of this marker to identify *C. argus* cannot be evaluated at this time.

Remark: In all mitochondrial markers COI, 16S, 12S, cytb, ATP6 and D-Loop, one *Channa argus* sequence (Accession number: GU937112) occurs with the *C. maculata* cluster. However, this sequence refers to tissue collected from a fish market (no geographical origin) and might be a misidentification¹⁴.



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 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
COI				X	X
RAG 1	X	X		X	X
cytb				X	X
16S		X		X	X
ND2					X
12S		X		X	X
D-Loop		X		X	X
ATP6		X		X	X

Table 2: Publicly available sequences downloaded (April 2023) 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. Species names with * are not mentioned in ² but used on GenBank.

Species in genus	COI	RAG 1	cytb	16S	ND2	12S	D-Loop	ATP6
<i>Channa amphibeus</i>								
<i>Channa andrao</i>	X	X	X	X	X	X	X	X
<i>Channa ara</i> *	X			X	X			
<i>Channa argus</i>	X	X	X	X	X	X	X	X
<i>Channa argus kimurai</i> *			X	X	X	X	X	X
<i>Channa aristonei</i>	X							
<i>Channa asiatica</i>	X	X	X	X	X	X	X	X
<i>Channa aurantimaculata</i>	X	X	X	X	X	X	X	X
<i>Channa aurantipectoralis</i>	X							
<i>Channa auroflammea</i>	X	X	X	X				
<i>Channa aurolineata</i> *	X	X	X	X				
<i>Channa bankanensis</i>	X	X	X	X	X			
<i>Channa baramensis</i>	X	X	X	X	X			
<i>Channa barca</i>	X	X	X	X	X			
<i>Channa bipuli</i>	X	X	X	X				
<i>Channa bleheri</i>	X	X	X	X	X			
<i>Channa brahmacharyi</i>								
<i>Channa brunnea</i>	X							
<i>Channa burmanica</i>	X	X	X	X	X	X	X	X
<i>Channa cocnhayia</i>								
<i>Channa cyanospilos</i>		X		X	X			
<i>Channa diplogramma</i>	X	X	X	X	X	X	X	X
<i>Channa gachua</i>	X	X	X	X	X	X	X	X
<i>Channa harcourtbutleri</i>	X	X	X	X	X			
<i>Channa hoaluensis</i>								
<i>Channa kelaartii</i>	X	X	X	X				
<i>Channa limbata</i> *	X	X	X	X	X			
<i>Channa lipor</i>	X							
<i>Channa longistomata</i>								
<i>Channa lucius</i>	X	X	X	X	X	X	X	X
<i>Channa maculata</i>	X	X	X	X	X	X	X	X
<i>Channa marulia</i> *		X						
<i>Channa marulioides</i>	X	X	X	X	X			
<i>Channa marulius</i>	X	X	X	X	X	X	X	X
<i>Channa melanoptera</i>								
<i>Channa melanostigma</i>	X							
<i>Channa melasoma</i>	X	X	X	X	X			
<i>Channa micropeltes</i>	X	X	X	X	X	X		X
<i>Channa ninhbinhensis</i>								



Species in genus	COI	RAG 1	cytb	16S	ND2	12S	D-Loop	ATP6
<i>Channa nox</i>		X	X	X	X			
<i>Channa orientalis</i>	X	X	X	X	X	X		
<i>Channa ornatipinnis</i>	X	X	X	X	X	X	X	
<i>Channa panaw</i>	X	X	X	X	X			
<i>Channa pardalis</i>	X	X	X	X				
<i>Channa pleurophthalma</i>	X	X	X	X	X			
<i>Channa pomanensis</i>	X							
<i>Channa pseudomarulius</i>	X	X	X	X				
<i>Channa pulchra</i>	X	X	X	X	X	X	X	X
<i>Channa punctata</i>	X	X	X	X	X	X		X
<i>Channa quinquefasciata</i>	X	X	X	X		X		
<i>Channa rara</i>	X							
<i>Channa shingon</i>								
<i>Channa stewartii</i>	X	X	X	X	X	X	X	X
<i>Channa stiktos</i>	X							
<i>Channa striata</i>	X	X	X	X	X	X	X	X
<i>Channa torsaensis</i>								
TOTAL species	40(43)*/51	34(37)*/51	33(36)*/51	34(38)*/51	28(31)*/51	18(19)*/51	14(15)*/51	15(16)*/51

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.health.belgium.be/nl/node/30309>
<https://www.iasregulation.be/fr/350/>
<https://eunis.eea.europa.eu/species/126924>
<http://www.iucngisd.org/gisd/speciesname/Channa+argus>
<https://www.b-aqua.com/Pages/Fiche.aspx?Id=975>

Picture credits

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Page 2 (left): *Channa argus* with fry, by Drennack-inaturalist via U.S. Fish & Wildlife Service [CC-BY-NC 4.0]
Page 2 (right): thern Snakehead (*Channa argus*) at the Tokyo Tower Aquarium in Japan, by George Berninger Jr. [CC BY-SA 4.0]

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