



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.

Plotosus lineatus

(Thunberg, 1787)

Common names:

English: striped eel catfish

French: poisson-chat rayé, balibot rayé

German: Gestreifte Korallenwels

Dutch: gestreepte koraalmeerval

Last update: January 2022



General information on *Plotosus lineatus*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Actinopterygii	Siluriformes	Plotosidae	<i>Plotosus</i>

Species in the same genus: N = 9 [2,3]

Infra-species level: N = 0 [3]

Note: To our knowledge, no subspecies have been described.



Native range: [3-5]

Indo-Pacific: Red Sea and East Africa to Samoa, north to southern Japan, southern Korea, and the Ogasawara Islands, south to Australia and Lord Howe Island. Palau and Yap in Micronesia. Sometimes enters freshwaters of East Africa (Lake Malawi) and Madagascar.

Invasive range: [4,5]

Europe (geographical):

Turkey.

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

<https://www.gbif.org/species/7965247>

<https://easin.irc.ec.europa.eu/spexplorer/species/factsheet/R11839>

Outside Europe (geographical):

Egypt, Israel, Lebanon, Syria, Tunisia.

Morphology, biology, invasion, negative effects and remedies

For more information on *Plotosus lineatus* 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]



Conclusion

Based on the present evaluation of the available sequence data, no DNA marker can reliably identify *Plotosus lineatus*. COI and *cytb* seem the most promising markers to further investigate once new sequence data becomes available.

Discussion

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

None of the analysed DNA markers recovers *Plotosus lineatus* as (a) supported cluster(s). Compared to the other markers, the COI dataset is the most elaborate. Many COI sequences are available for *P. lineatus*, both from the native and the invasive range, and the highest number of congeners are represented by at least one sequence. One *P. canius* sequence (GenBank accession number MN747967) clusters with *P. lineatus*, however, this sequence is a potential misidentification. All *P. japonicus* and *P. limbatus* sequences end up within the same cluster as the *P. lineatus* sequences. This is in line with the findings of Kundu *et al.* [2], except that in our analysis, containing more *P. lineatus* and *P. japonicus* sequences, all *P. japonicus* sequences end up among the *P. lineatus* sequences and not in a separate cluster. *P. lineatus* can thus not be separated from *P. japonicus* and *P. limbatus*. To allow for a better evaluation of the performance of COI for species identification, the missing species (Table 2) as well as more sequences of *P. japonicus* and *P. limbatus* should be added to the analysis.

For *cytb*, fewer sequences are available for both *P. lineatus* and the congeners. Again the *P. japonicus* and *P. limbatus* sequences cluster together with *P. lineatus*. Again, *P. lineatus* cannot be separated from *P. japonicus* and *P. limbatus*. Additional sequences for *P. lineatus*, especially from the invasive regions, as well as more sequences for the (missing) congeners should be added to the analysis to fully assess the ability of this marker to identify *P. lineatus*.

For **12S**, **16S**, **ND4** and **rag-2** only a few species are represented by one or more sequences. Therefore, it is currently impossible to assess the ability of these markers to identify *P. lineatus*.



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	X	X
cytb		X	X		X
12S	X	X	X		X
16S	X	X	X		X
ND4	X	X	X		X
rag-2	X	X	X		X

Table 2: Publicly available sequences downloaded (January 2022) 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 [3]. 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	12S	16S	ND4	rag-2
<i>Plotosus abbreviatus</i>						
<i>Plotosus canius</i>	X	X	X		1	X
<i>Plotosus fisadoha</i>						
<i>Plotosus japonicus</i>	X	X	X	X	X	
<i>Plotosus limbatus</i>	X	1				1
<i>Plotosus lineatus</i>	X	X	X	X	X	X
<i>Plotosus nhatrangensis</i>						
<i>Plotosus nkunga</i>	X					
<i>Plotosus papuensis</i>						
TOTAL species	5/9	4/9	3/9	2/9	3/9	3/9

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://www.cabi.org/isc/datasheet/116398>

<https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-gestreepte-koraalmeerval> [NL]

Picture credits

Page 1: Striped catfish (*Plotosus lineatus*) By Rickard Zerpe [CC BY 2.0]

Page 2 (left): Striped Catfish - *Plotosus lineatus* By François Libert [CC BY-NC-SA 2.0]

Page 2 (right): *Plotosus lineatus* By Fernando Losada Rodríguez [CC BY-SA 4.0]

References

- [1] N. Smits, 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>.
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