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

natural sciences .be

AFRICA museum

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.

In this factsheet we focus specifically on an invasive land planarian species which has already been detected in Europe (e.g. in gardens, orchards, warehouse, greenhouses). Due to the potential threat flatworms pose, the New Zealand flatworm *Arthurdendyus triangulatus* (Dendy, 1896), was the first flatworm to be added to the list of Invasive Alien Species of Union Concern in July 2019 (EU 2019/1262).

BopCo investigates and evaluates the usefulness of publicly available DNA sequence data to reliably identify invasive flatworm species recorded in Europe. The results are presented as factsheets (one per species) 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 invasive flatworm species 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 invasive flatworm species 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 https://bopco.be or contact us via bopco@naturalsciences.be.

Obama nungara

(Carbayo, Alvarez-Presas, Jones & Riutort, 2016)

Common names:
  English: Obama flatworm
  French: marron plate
  German: /
  Dutch: kastanjebruine landplatworm

Last update: March 2020

General information on *Obama nungara*

Classification

Kingdom	Phylum	Class/Clade	Order	Family	Genus
Animalia	Platyhelminthes	Rhabditophora	Tricladida	Geoplanidae	*Obama*

Species in the same genus: N = 40 [2 – 8]
Note: We follow the classification as presented by Carbayo *et al.* [8].

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

Native range: [2]
Argentina and Brazil.

Invasive range: [2, 8 – 10]
Europe (geographical):
Belgium, Corsica, France, Ireland, Italy, Netherlands, Portugal, Spain, Switzerland and United Kingdom.

For more detailed locality information and the most recent distribution updates, please visit:
https://www.gbif.org/species/8701771
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R19398

Outside Europe (geographical):
To our knowledge, the species has not been reported in other countries.

Morphology, biology, invasion, negative effects and remedies
For more information on *Obama nungara* 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 species, 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 species 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 species in order to detect potential population structure or local hybrids.

In this context, BopCo evaluated the inclusion of the invasive flatworm species and its 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.
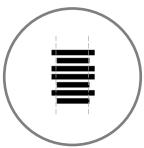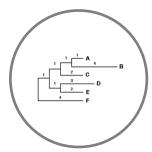
Material and Methods [1]

| 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 is the most promising DNA marker for the identification of *Obama nungara*. Additional sequences for *O. nungara* (especially from its native range) and for the missing congeners should be added to increase the accuracy of the analysis.

Discussion

DNA sequence of *Obama nungara* and other *Obama* species were downloaded from GenBank and BOLD. Five DNA markers were evaluated (Table 1).

In the NJ-trees for **18S, COI, EF-1-alpha** and **ITS1**, the *Obama nungara* sequences cluster together with high support. COI currently covers the widest taxonomic diversity in the genus *Obama* in GenBank and BOLD (Table 2). Additional sequences for *O. nungara* (especially from its native range) and for the missing congeners would allow for a better evaluation of the suitability of these DNA markers to identify *O. nungara*.

Sequences for **28S** do not form a supported cluster. The overall low observed genetic variation raises doubts about the taxonomic resolution power of this marker for the genus *Obama,* and the usefulness of 28S for the identification of *O. nungara*.

Table 1: Overview of the encountered issues concerning the DNA-based identification of the species [1]: (1) Insufficient publicly available DNA sequences of the species to capture the intra-species divergence; (2) Poor geographical coverage of the species sequences (native or invasive range missing); (3) The sequences do not form supported clusters; (4) Potential misidentification of a specimen which influences the clustering of the species sequences; and (5) Insufficient publicly available DNA sequences of the congeners to capture the inter-species divergence. An 'X' indicates that the issue was encountered.

Markers analysed	1	2	3	4	5
COI	X	X			X
18S	X	X			X
28S	X	X	X		X
EF-1-alpha	X	X			X
ITS1	X				X

Table 2: Publicly available sequences downloaded (March 2020) 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 – 8]. An 'X' signifies that at least one sequence was used in the final alignment. A '1' indicates only one unique sequence was available.

Species in genus	COI	18S	28S	EF-1-alpha	ITS1
Obama allandra	1				
Obama anthrophila	X	X	X	X	X
Obama apeva	X				
Obama applanata	1				
Obama argus	X				
Obama assu					
Obama baptistae	X				
Obama braunsi	X				
Obama burmeisteri	X	X	X	X	1
Obama carbayoi					
Obama carinata	X	X	X	X	
Obama carrierei					
Obama catharina					
Obama decidualis	X	X	X	X	
Obama dictyonata					
Obama divae					
Obama eudoxiae					
Obama eudoximariae	1				
Obama evelinae					
Obama ferussaci					
Obama ficki	1				
Obama fryi	X				
Obama glieschi					
Obama itatiayana	1				
Obama josefi	X	X	X	X	1
Obama ladislavii	X	X	X	X	X
Obama livia					
Obama maculipunctata	X				
Obama marmorata	X		X	X	
Obama metzi					
Obama nungara	**X**	**X**	**X**	**X**	**X**
Obama otavioi	X				
Obama poca					
Obama polyophthalma					
Obama riesteri					
Obama rufiventris					
Obama ruiva					
Obama schubarti					
Obama tribalis	1				
Obama trigueira					
TOTAL species	21/40	7/40	8/40	8/40	5/40

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.cabi.org/isc/datasheet/121981
http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=4405
https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=1037132#null

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Page 2: *Obama nungara* (Geoplanidae) By Jean-Lou Justine [CC BY SA 3.0]

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