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.

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 http://bopco.myspecies.info/ or contact us via bopco@naturalsciences.be.

Dolichoplana striata

Moseley, 1877

Common names:
English: /
French: /
German: /
Dutch: crème tweestreep, Kleine geelstreep

Last update: March 2020

General information on *Dolichoplana striata*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Platyhelminthes	Rhabditophora	Tricladida	Rhynchodemidae	*Dolichoplana*

Species in the same genus: N = 13 [2]

Note: We follow the classification as presented by Ogren *et al.* [2].

Infra-species level: N = 0

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

Native range: [3, 4]

Sri Lanka and Indonesia.

Invasive range: [5, 6]

Europe (geographical):

Austria, Belgium, Czech Republic, Finland, Germany, Ireland, Norway, Poland and Portugal.

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

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

https://www.gbif.org/species/4416161

Outside Europe (geographical):

Barbados, Bermuda, Brazil, Guyana, Palau Island, Philippines, Singapore, and United States of America.

Morphology, biology, invasion, negative effects and remedies

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

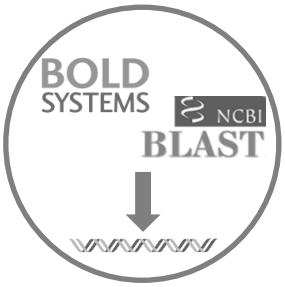
Species identification based on DNA barcodes

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.

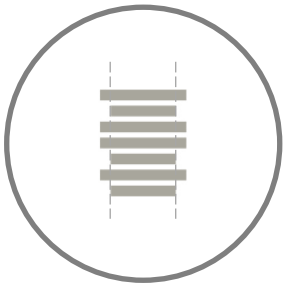
Against this background, BopCo evaluated the inclusion of the invasive flatworm species and its close relatives in both publicly available reference libraries BOLD (www.boldsystems.org/) and GenBank (www.ncbi.nlm.nih.gov/nuccore/) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

Material and Methods [1]

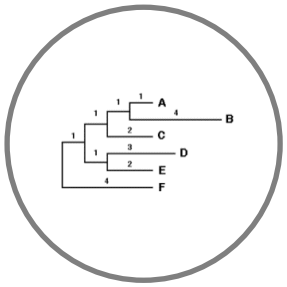


Download all sequence data available for the genus

Filtering the data and selecting 'promising' markers



Aligning and trimming of the sequences



Building Neighbour-Joining tree with Bootstrap support

Conclusion

Due to the large gaps in available sequence data, it is currently impossible to fully assess the reliability of these markers.

Discussion

Relevant DNA sequences for *Dolichoplana striata* and congeneric species were downloaded from GenBank and BOLD. Yet, only one out of the 13 currently recognized *Dolichoplana* species are represented in the DNA reference databases (Table 1), therefore it is impossible to assess the usefulness of DNA markers to identify the species.

In the NJ-tree for **COI** and **28S**, the *D. striata* sequences cluster together, for both markers only a few sequences of *D. striata* available. Additional sequences for *D. striata* (from the native region) and for the missing congeners would allow for a better evaluation of COI and 28S.

For **18S** and **EF-1-alpha** only one sequence is available for *D. striata*. Therefore it is currently impossible to assess the ability of these markers to identify *D. striata*.

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, a '1' indicates only one unique *Dolichoplana striata* sequence was available.

Markers analysed	1	2	3	4	5
COI	X	X			X
18S	1	X	1		X
28S	X	X			X
EF-1-alpha	1	X	1		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]. 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**
Dolichoplana bosci				
Dolichoplana carvalhoi				
Dolichoplana conradti				
Dolichoplana feildeni				
Dolichoplana joubini				
Dolichoplana mertoni				
Dolichoplana nietneri				
Dolichoplana picta				
Dolichoplana procera				
Dolichoplana signata				
Dolichoplana striata	**X**	**1**	**X**	**1**
Dolichoplana tristis				
Dolichoplana vircata				
TOTAL species	1/13	1/13	1/13	1/13

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

Picture credits

Page 1: *Dolichoplana striata* By Hugh Jones
Page 2 (right): *Dolichoplana striata* Dorsal view Fig.8A By Alvarez-Presas *et al.* [4]
Page 2 (left): *Dolichoplana striata* Lateral view showing eyespot Fig.8C By Alvarez-Presas *et al.* [4]

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.," 2018. [Online]. Available: http://bopco.myspecies.info/content/ias-factsheets-and-accompanying-information.

[2] R. E. Ogren, M. Kawakatsu, and E. M. Froehlich, "Additions and corrections of the previous land planarian indices of the world (Turbellaria, Seriata, Tricladida, Terricola). Addendum IV. Geographic Locus Index: Bipaliidae; Rhynchodemidae (Rhynchodeminae; Microplaninae); Geoplanidae (Geoplaninae; Caenopl," *Bulletin of Fuji Women's College*, vol. 35, no. 2, pp. 63–103, 1997.

[3] B. Boag and G. W. Yeates, "The potential impact of the New Zealand flatworm, a predator of earthworms, in western Europe," *Ecological Applications*, vol. 11, no. 5, pp. 1276–1286, 2001.

[4] M. Álvarez-Presas, E. Mateos, À. Tudó, H. Jones, and M. Riutort, "Diversity of introduced terrestrial flatworms in the Iberian Peninsula: A cautionary tale," *PeerJ*, vol. 2:e430, pp. 1–35, 2014.

[5] J.-L. Justine, L. Winsor, D. Gey, P. Gros, and J. Thévenot, "The invasive new guinea flatworm *Platydemus manokwari* in France, the first record for Europe: Time for action is now," *PeerJ*, vol. 2:e297, pp. 1–22, 2014.

[6] F. Carbayo, M. Álvarez-Presas, C. T. Olivares, F. P. L. Marques, E. Froehlich, and M. Riutort, "Molecular phylogeny of Geoplaninae (Platyheminthes) challenges current classification: proposal of taxonomic actions.," *Zoologica Scripta*, vol. 42, pp. 508–528, 2013.

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