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.

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.

Australopacifica atrata

(Steel, 1897)

Common names:
- English: /
- French: /
- German: /
- Dutch: /

Last update: March 2020

General information on *Australopacifica atrata*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Platyhelminthes	Rhabditophora	Tricladida	Geoplanidae	*Australopacifica*

Species in the same genus: N = 75 [2, 3]
Note: This species was included in the genus *Parakontikia* [2], however according to Jones [3] this species should be incorporated in the genus *Australopacifica* due to a lack of knowledge about its internal anatomy.

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

Native range: [4]
Australia.

Invasive range: [3]
Europe (geographical):
United Kingdom.

For more detailed locality information and the most recent distribution updates, please visit:
https://www.gbif.org/species/6476713
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R20014 *(Parakontikia atrata)*

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 *Australopacifica atrata* please see the references and online information listed at the end of this document.

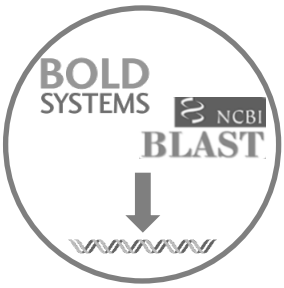
Species identification based on DNA barcodes

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.

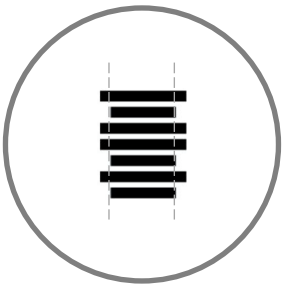
In this context, evaluated the inclusion of the invasive flatworm species and its close relatives in both publicly available reference libraries BOLD (www.boldsystems.org/) and GenBank (www.ncbi.nlm.nih.gov/nuccore/) to estimate the reliability with which a species identification can be obtained using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

Material and Methods [1]

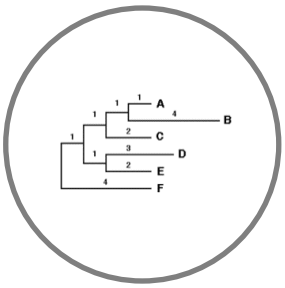


Download all sequence data available for the genus

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 gap in available sequence data, it is currently impossible to fully assess the reliability of these DNA markers.

Discussion

No DNA markers were found for which *Australopacifica* sequences were available (Table 1).

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, n/a: not applicable.

Markers analysed	**1**	**2**	**3**	**4**	**5**
COI	X	X	n/a	n/a	X
18S	X	X	n/a	n/a	X
28S	X	X	n/a	n/a	X
EF-1-alpha	X	X	n/a	n/a	X
ITS1	X	X	n/a	n/a	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, 3]**.**

Species in genus	**COI**	**18S**	**28S**	**EF-1-alpha**	**ITS1**
Australopacifica albolineata					
Australopacifica alfordensis					
Australopacifica antarctia					
Australopacifica atrata					
Australopacifica aurantia					
Australopacifica austiniana					
Australopacifica bimaculata					
Australopacifica blomefieldi					
Australopacifica buettneri					
Australopacifica castanea					
Australopacifica challengeri					
Australopacifica chamissoniana					
Australopacifica cooperi					
Australopacifica cucullata					
Australopacifica dendyi					
Australopacifica dietrichiana					
Australopacifica eschscholtziana					
Australopacifica fagicola					
Australopacifica fillii					
Australopacifica flavimarginata					
Australopacifica gamblei					
Australopacifica gelatinosa					
Australopacifica graminicola					
Australopacifica greeni					
Australopacifica gregoryana					
Australopacifica grubei					
Australopacifica guentheri					
Australopacifica hamiltoni					
Australopacifica himalayensis					
Australopacifica hoggii					
Australopacifica howitti					
Australopacifica humberti					
Australopacifica huttoni					
Australopacifica ijimai					
Australopacifica inflata					
Australopacifica iris					
Australopacifica jacksoniana					
Australopacifica korotneffi					
Australopacifica kotzebueana					
Australopacifica krausi					
Australopacifica laingii					
Australopacifica lapidicola					
Australopacifica lateropunctata					
Australopacifica leichhardtiana					

Species in genus	COI	18S	28S	EF-1-alpha	ITS1
Australopacifica leuckarti					
Australopacifica lucasi					
Australopacifica maculosa					
Australopacifica martensi					
Australopacifica meridionalis					
Australopacifica metschnikoffi					
Australopacifica moebiusi					
Australopacifica mortoni					
Australopacifica muelleriana					
Australopacifica nichollsi					
Australopacifica parva					
Australopacifica pulverulenta					
Australopacifica regina					
Australopacifica robusta					
Australopacifica rotunda					
Australopacifica rouxiana					
Australopacifica sarasiniana					
Australopacifica scaphoidea					
Australopacifica semoniana					
Australopacifica sowerbyi					
Australopacifica spectabilis					
Australopacifica splendens					
Australopacifica striata					
Australopacifica subpallida					
Australopacifica subviridis					
Australopacifica treubi					
Australopacifica trifasciata					
Australopacifica walhallae					
Australopacifica warragulensis					
Australopacifica willeyi					
Australopacifica zebra					
TOTAL species	1/75	0/75	1/75	1/75	0/75

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.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=1040604#null

Picture credits

Page 1: *Parakontikia atrata* By Reiner Richter [CC BY NC SA 4.0]
Page 2 (left): *Parakontikia atrata* 2 By Reiner Richter [CC BY NC SA 4.0]
Page 2 (right): *Australopacifica atrata* 2 By Hugh Jones

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.," 2019. [Online]. Available: https://bopco.be/output/iasfactsheets.

[2] S. Tyler, T. Artois, S. Schilling, M. Hooge, and L. Bush, "World List of turbellarian worms: Acoelomorpha, Catenulida, Rhabditophora. Australopacifica Ogren & Kawakatsu, 1991." [Online]. Available: http://www.marinespecies.org/turbellarians/aphia.php?p=taxdetails&id=479644. [Accessed: 21-Aug-2020].

[3] H. D. Jones, "Another alien terrestrial planarian in the United Kingdom: Australopacifica atrata (Steel, 1897) (Platyhelminthes: Tricladida: Continenticola)," *Zootaxa*, vol. 4604, no. 3, pp. 575–587, 2019.

[4] L. Winsor, "A provisional classification of Australian terrestrial geoplanid flatworms (Tricladida: Terricola: Geoplanidae).," *The Victorian Naturalist*, vol. 108, pp. 42–49, 1991.

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