



# 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](mailto:bopco@naturalsciences.be).

## *Arthurdendyus triangulatus*

(Dendy, 1896)

Common names:

- English: New Zealand flatworm
- French: ver plat de Nouvelle-Zélande
- German: Neuseelandplattwurm
- Dutch: Nieuw-Zeelandse platworm

**Last update: March 2020**



## General information on *Arthurdendyus triangulatus*

### Classification

Kingdom	Phylum	Class/Clade	Order	Family	Genus
Animalia	Platyhelminthes	Rhabditophora	Tricladida	Geoplanidae	<i>Arthurdendyus</i>

**Species in the same genus: N = 6** [2, 3]

Note: We follow the classification of Winsor & Stevens [3].

**Infra-species level: N = 0**

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



**Native range:** [4, 5]

New Zealand.

**Invasive range:** [4, 5]

#### Europe (geographical):

Denmark (Faroe Islands), Iceland, Ireland, Switzerland and United Kingdom.

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

<https://www.gbif.org/species/2502792>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R01506>

[https://www.inaturalist.org/taxa/475834-Arthurdendyus-triangulatus#Invasive\\_species](https://www.inaturalist.org/taxa/475834-Arthurdendyus-triangulatus#Invasive_species)

<https://invasivespeciesireland.com/species-accounts/established/terrestrial/new-zealand-flatworm> (Ireland)

#### 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 *Arthurdendyus triangulatus* please see the references and online information listed at the end of this document.



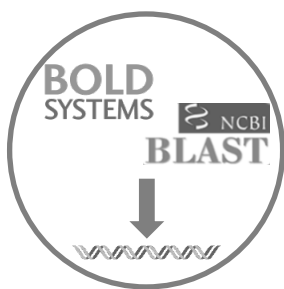
## Species identification based on DNA barcodes

### 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/](http://www.boldsystems.org/)) and GenBank ([www.ncbi.nlm.nih.gov/nucleotide/](http://www.ncbi.nlm.nih.gov/nucleotide/)) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

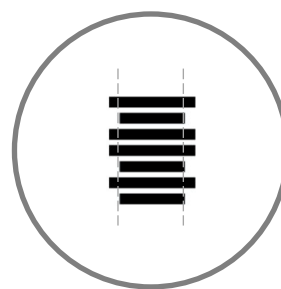
### Material and Methods [1]



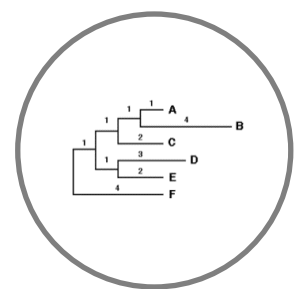
Download all sequence data available for the genus



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

Relevant DNA sequences for *Arthurdendyus triangulatus* and congeneric species were downloaded from GenBank and BOLD. Yet, only two out of the five currently recognized *Arthurdendyuys* 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-trees for **COI** and **ITS1** the *A. triangulatus* sequences cluster with high support, additional sequences for *A. triangulatus* sequences (from the native region) and for the missing congeners would allow for a better evaluation of COI and ITS1.

In the NJ-tree for **18S** the *A. triangulatus* sequences cluster together, except for one (AF033038) which is placed separately. This latter sequence might involve a misidentification or a second type of 18S [6].

For **28S** and **EF-1-alpha** only one unique sequence for *A. triangulatus* is available from online databases.



**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 *Arthurdendyus triangulatus* sequence was available.

Markers analysed	1	2	3	4	5
<b>COI</b>		X			<b>X</b>
<b>18S</b>	X	X		X	X
<b>28S</b>	1	X	1		X
<b>EF-1-alpha</b>	1	X	1		X
<b>ITS1</b>		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 [3]. 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
<i>Arthurdendyus albidus</i>					
<i>Arthurdendyus australis</i>					
<i>Arthurdendyus latissimus</i>					
<i>Arthurdendyus testaceus</i>	1	1	1	1	1
<b><i>Arthurdendyus triangulatus</i></b>	<b>X</b>	<b>1</b>	<b>X</b>	<b>1</b>	<b>X</b>
<i>Arthurdendyus vegrandis</i>					
<b>TOTAL species</b>	<b>2/6</b>	<b>2/6</b>	<b>2/6</b>	<b>2/6</b>	<b>2/6</b>

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/109121>

<https://www.biodiversityireland.ie/wordpress/wp-content/uploads/New-Zealand-Flatworm.pdf>

<https://www.gardenorganic.org.uk/new-zealand-flatworms>

### Picture credits

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Page 2 (down): New Zealand Flatworm (*Arthurdendyus triangulatus*) By S. Rae [CC-BY-2.0]

### 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: <https://bopco.be/output/iasfactsheets>.
- [2] H. D. Jones and B. M. Gerard, "A new genus and species of terrestrial planarian (Platyhelminthes; Tricladida; Terricola) from Scotland, and an emendation of the genus *Artioposthia*," *Journal of Natural History*, vol. 33, no. 3, pp. 387–394, 1999.
- [3] L. Winsor and M. Stevens, "Terrestrial Flatworms (Platyhelminthes: Tricladida: Terricola) from sub-Antarctic Macquarie Island," *Kanunna*, vol. 1, pp. 17–32, 2005.
- [4] H. Jones and B. Boag, "The distribution of New Zealand and Australian terrestrial flatworms (Platyhelminthes: Turbellaria: Tricladida: Terricola) in the British Isles - the Scottish survey and MEGALAB WORMS," *Journal of Natural History*, vol. 30, pp. 955–975, 1996.
- [5] B. Boag, L. Palmer, R. Neilson, and S. Chambers, "Distribution and prevalence of the predatory planarian *Artioposthia triangulata* (Dendy) (Tricladida: Terricola) in Scotland," *Annals of Applied Biology*, vol. 124, no. 1, pp. 165–171, 1994.
- [6] S. Carranza, G. Giribet, C. Ribera, J. Bagueña, and M. Riutort, "Evidence that two types of 18S rDNA coexist in the genome of *Dugesia* (*Schmidtea*) *mediterranea* (Platyhelminthes, Turbellaria, Tricladida)," *Molecular Biology and Evolution*, vol. 13, no. 6, pp. 824–832, 1996.

### To cite this factsheet, please use

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