



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



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.

Caenoplana coerulea

Moseley, 1877

Common names:

English: (Australian) blue garden flatworm, blue (land) planarian

French: /

German: /

Dutch: blauwe tuinplatworm

Last update: November 2020



General information on *Caenoplana coerulea*

Classification

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

Species in the same genus: N = 19 [2 - 4]

Note: We follow the classification of Ogren *et al.* [2], Jones *et al.* [3] and Mateos *et al.* [4].

Infra-species level: N = 2 [5]

Note: two subspecies have been described: *C. c. coerulea* and *C. c. vaga*.



Native range: [6 – 8]

Australia and New Zealand.

Invasive range: [6 – 9]

Europe (geographical):

France, Spain (Gran Canaria island, Menorca island and mainland) and United Kingdom.

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

<https://www.gbif.org/species/2502810>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R20020>

<https://bie.ala.org.au/species/urn:lsid:biodiversity.org.au:afd.taxon:fbe074ac-277a-4964-a709-4a79424124ea> (Australia)

Outside Europe (geographical):

Argentina, Israel and USA.

Morphology, biology, invasion, negative effects and remedies

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



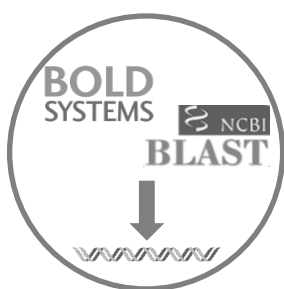
Species identification based on DNA barcodes

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/nucleotide/) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

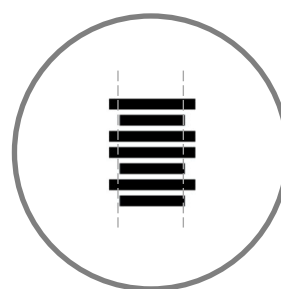
Material and Methods [1]



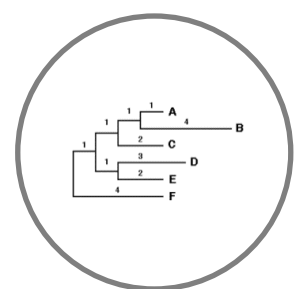
Download all sequence data available for the genus



Filtering the data and selecting 'promising' markers



Aligning and trimming of the sequences



Building Neighbour-Joining tree with Bootstrap support

Conclusion

Based on the present evaluation of the available sequence data, no marker can reliably identify *Caenoplana coerulea*.

Discussion

Relevant DNA sequences for *Caenoplana coerulea* and congeneric species were downloaded from GenBank and BOLD. Yet, only two out of the 19 currently recognized *Caenoplana* 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** most sequences seem to be misidentified or confused with *Artioposthia lucasi*. Only two sequences (DQ666030 and JQ514564), which were collected from invasive specimens, are most likely to belong to *C. coerulea*. More sequences of the genus *Caenoplana* as well as *C. coerulea* are needed to assess the potential of this DNA marker.

18S recovers *C. coerulea* as two supported clusters, which coincide with two different types of 18S gene [10]. Currently, the genus *Caenoplana* is only represented by *C. coerulea* in the online database GenBank for this DNA marker, all collected in Menorca except one. Hence, it is premature to decide about the ability of this DNA marker to differentiate *C. coerulea* from other *Caenoplana* species.

For **28S** only one sequence is available for *C. coerulea*.



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 *Caenoplana coerulea* sequence was available.

Markers analysed	1	2	3	4	5
COI	X		X	X	X
18S	X	X	X		X
28S	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 - 4]. 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
<i>Caenoplana albolineata</i>			
<i>Caenoplana barringtonensis</i>			
<i>Caenoplana citrina</i>			
<i>Caenoplana coerulea</i>	X	X	1
<i>Caenoplana daemeli</i>			
<i>Caenoplana decolorata</i>	X		
<i>Caenoplana dendyi</i>			
<i>Caenoplana dubia</i>			
<i>Caenoplana hillii</i>			
<i>Caenoplana hoggii</i>			
<i>Caenoplana ponderosa</i>			
<i>Caenoplana purpurea</i>			
<i>Caenoplana spenceri</i>			
<i>Caenoplana subviridis</i>			
<i>Caenoplana sulphurea</i>			
<i>Caenoplana tenuis</i>			
<i>Caenoplana variegata</i>	X		1
<i>Caenoplana viridis</i>			
<i>Caenoplana walhallae</i>			
TOTAL species	3/19	1/19	2/19

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=1039860#null

<https://www.cabi.org/isc/abstract/20113125993>

Picture credits

Page 1: *Caenoplana coerulea* By Doug Beckers [CC BY SA 2.0]

Page 2 (left): *Caenoplana coerulea*_Chatswood_West By Peter Woodard [public domain]

Page 2 (right): Blue garden flatworm By John Tann [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] R. E. Ogren, M. Kawakatsu, and E. M. Froehlich, "Additions and Corrections of the previous land Planarian Indices of the World (Turbellaria, Tricladida, Terricola)," *Bulletin of Fuji Women's College*, vol. 31, no. 2, pp. 61–86, 1993.
- [3] H. D. Jones, E. Mateos, M. Riutort, and M. Álvarez-Presas, "The identity of the invasive yellow-striped terrestrial planarian found recently in Europe: *Caenoplana variegata* (Fletcher & Hamilton, 1888) or *Caenoplana bicolor* (Graff, 1899)?," *Zootaxa*, vol. 4731, no. 2, pp. 193–222, 2020.
- [4] Mateos, E., Jones, H., J., Riutort, M. and Álvarez-Presas, M., "A new species of alien terrestrial planarian in Spain: *Caenoplana decolorata*." *PeerJ*, vol. 8:e10013, 2020 DOI 10.7717/peerj.10013
- [5] L. H. Luis-Negrete, F. Brusa, and L. Winsor, "The blue land planarian *Caenoplana coerulea*, an invader in Argentina," *Revista Mexicana de Biodiversidad*, vol. 82, no. 1, pp. 287–291, 2011.
- [6] 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.
- [7] D. Suárez, S. Martín, and M. Naranjo, "First report of the invasive alien species *Caenoplana coerulea* Moseley, 1877 (Platyhelminthes, Tricladida, Geoplanidae) in the subterranean environment of the Canary Islands," *Subterranean Biology*, vol. 26, pp. 67–74, 2018.
- [8] K. Breugelmans, J. Q. Cardona, T. Artois, K. Jordaens, and T. Backeljau, "First report of the exotic blue land planarian, *Caenoplana coerulea* (Platyhelminthes, Geoplanidae), on Menorca (Balearic Islands, Spain)," *Zookeys*, vol. 199, pp. 91–105, 2012.
- [9] G. I. Sánchez, "Cuatro planarias terrestres exóticas nuevas para Andalucía," *Revista Sociedad Gaditana de Historia Natural*, vol. 8, pp. 42–55, 2014.
- [10] S. Carranza, G. Giribet, C. Ribera, J. Baguña, 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

Barcoding Facility for Organisms and Tissues of Policy Concern, 2020. Factsheet on *Caenoplana coerulea*; November 2020. In: Identification of Invasive Flatworm Species using DNA barcodes. BopCo, Belgium. Available from: <https://bopco.be/projects/flatworm-factsheets>, accessed on DD-MM-YYYY.

DISCLAIMER: The information represented in this factsheet has been compiled from many different sources. Every reasonable effort has been made to ensure that the material presented is accurate and reflects the current (see date last update) scientific knowledge. However, recent changes in e.g. taxonomy and distribution, or the publication of additional reference sequences may not be implemented. The views which are expressed in the "Conclusion" are those of the author(s) and have not been peer-reviewed. BopCo does not guarantee the accuracy of the data included in this factsheet. The content of the factsheet is for information only and is not intended as legal advice. BopCo may not be held responsible for the use which may be made of the information contained therein. If you should notice any issues considering the content of this factsheet, or if you would like to contribute any additional information to it, please contact us through bopco@naturalsciences.be.

