



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.

Bipalium kewense

Moseley, 1878

Common names:

English: hammerhead (flat)worm, shovel-headed garden worm

French: ver plat à tête de Marteau

German: Hammerhaiwurm

Dutch: hamerhoofdlandplatworm



Last update: March 2020

General information on *Bipalium kewense*

Classification

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

Species in the same genus: N = 47 [2 – 5]

Note: We follow the classification of Kawakatsu *et al.* [2], Stimpson [3], Tyler [4] and Jones & Sterrer [5].

Infra-species level: N = 2 [6]

Note: two subspecies are recognized: *B. k. kewense* and *B. k. viridis*.



Native range: [7]

Southeast Asia.

Invasive range:[2, 8 – 11]

Europe (geographical):

Austria, Azores, Belgium, Czech Republic, Finland, France, Germany, Ireland, Madeira, Monaco, Norway, Poland, Portugal, Spain and United Kingdom.

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

<https://www.gbif.org/species/2502938>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R02029>

https://inpn.mnhn.fr/espece/cd_nom/269178?lg=en

Outside Europe (geographical):

Argentina, Bermuda, Canada, Cape Verde, Colombia, Costa Rica, Cuba, China, France (Saint Martin island), Fiji, French Guiana, French Polynesia, Guadeloupe, Israel, Jamaica, Japan, La Réunion, Madagascar, Martinique, Montserrat, New Zealand, Panama, Peru, Saint Barthélemy, St. Helena, Taiwan, the Netherlands (Sint Maarten island), Uruguay and United States of America.

Morphology, biology, invasion, negative effects and remedies

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



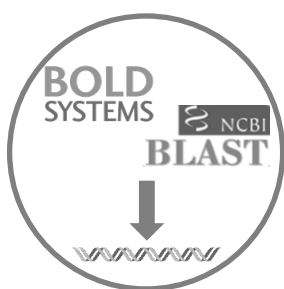
Species identification based on DNA barcodes

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.

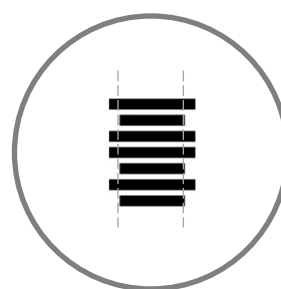
Material and Methods [1]



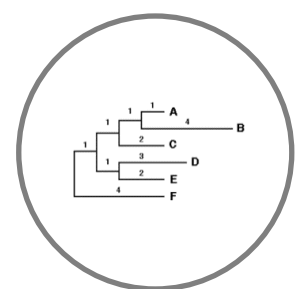
Download all sequence data available for the genus



Filtering the data and selecting 'promising' markers



Aligning and trimming of the sequences



Building Neighbour-Joining tree with Bootstrap support

Conclusion

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 *Bipalium kewense* and congeneric species were downloaded from GenBank and BOLD. Yet, only four out of the 47 currently recognized *Bipalium* 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 *B. kewense* sequences cluster together, though for both markers sequences from the native area of *B. kewense* and of the missing congeners should be added to the database to allow for a better evaluation of the DNA marker.

18S does not recover *B. kewense* as a cluster, though if this due to a misidentification is not clear as only two sequences for this species are currently available in online databases. Hence, it is premature to decide about the ability of this DNA marker to differentiate *B. kewense* from other *Bipalium* species.



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
18S	X	X	X	X	X
28S	X	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 – 5]. An 'X' signifies that at least one sequence was used in the final alignment. A '1' indicates only one unique sequence was available. Species names with * are not mentioned in [2 – 5] but used on GenBank.

Species in genus	COI	18S	28S
<i>Bipalium adensameri</i>			
<i>Bipalium admarginatum</i>			
<i>Bipalium adventitium</i>	X	1	X
<i>Bipalium alternans</i>			
<i>Bipalium bergendali</i>			
<i>Bipalium cantori</i>			
<i>Bipalium choristosperma</i>			
<i>Bipalium crassatrium</i>			
<i>Bipalium distinguendum</i>			
<i>Bipalium ephippium</i>			
<i>Bipalium everetti</i>			
<i>Bipalium fuscatum</i>			
<i>Bipalium fuscolineatum</i>			
<i>Bipalium gestroi</i>			
<i>Bipalium glaucum</i>			
<i>Bipalium graffi</i>			
<i>Bipalium haberlandti</i>			
<i>Bipalium hilgendorfi</i>			
<i>Bipalium interruptum</i>			
<i>Bipalium javanum</i>			
<i>Bipalium kewense</i>	X	X	X
<i>Bipalium kisoensis</i>			
<i>Bipalium kraepelini</i>			
<i>Bipalium marginatum</i>			
<i>Bipalium mjobergi</i>			
<i>Bipalium monolineatum</i>			
<i>Bipalium moseleyi</i>			
<i>Bipalium myadenosium</i>			
<i>Bipalium nigrum</i>			
<i>Bipalium nobile</i>	1	1	X
<i>Bipalium ochroleucum</i>			
<i>Bipalium pennsylvanicum</i>			
<i>Bipalium penrissenicum</i>			
<i>Bipalium penzigi</i>			
<i>Bipalium persephone</i>			
<i>Bipalium poiense</i>			
<i>Bipalium rigaudi</i>			
<i>Bipalium robiginosum</i>			
<i>Bipalium semperi</i>			
<i>Bipalium simrothi</i>			
<i>Bipalium strubelli</i>			
<i>Bipalium sudzukii</i>			



Species in genus	COI	18S	28S
<i>Bipalium trilineatum</i> *		1	
<i>Bipalium univittatum</i>			
<i>Bipalium vagum</i>	X		
<i>Bipalium virile</i>			
<i>Bipalium weismanni</i>			
<i>Bipalium wiesneri</i>			
TOTAL species	4/47	3/47	3/47

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=204236#null

<https://www.cabi.org/isc/datasheet/112705>

<https://australianmuseum.net.au/learn/animals/worms/shovel-headed-garden-worm/>

Picture credits

Page 1: *Bipalium kewense* By Pierre Gros in Justine *et al.* [9] DOI:10.7717/peerj.4672/fig-3

Page 2: *Bipalium kewense* By Scott Loarie [CC 00]

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] M. Kawakatsu, R. E. Ogren, E. Froehlich, and G.-Y. Sasaki, "Additions and corrections of the previous land planarian indices of the world (Turbellaria, Seriata, Tricladida, Terricola).," *Bulletin of Fiji Women's College (series 2)*, vol. 40, pp. 157–177, 2002.
- [3] W. Stimpson, "Prodomus descriptiones animalium evertibratum quae in Expeditione ad Oceanum, Pacificum Septentrionalem a Republica Federata missa, Jognne Rodgers Duce, observavit et descripsit.," *Proceedings of the Academy of Natural Sciences of Philadelphia*, vol. 9, pp. 19–31, 1857.
- [4] S. Tyler, S. Schilling, M. Hooge, and L. Busch, "Turbellarian taxonomic database. Version 1.8." [Online]. Available: <http://turbellaria.umaine.edu/>. [Accessed: 23-Mar-2020].
- [5] H.D. Jones and W. Sterrer, "Terrestrial planarians (Platyhelminthes, with three new species) and nemertines of Bermuda," *Zootaxa*, vol. 1001, pp. 31-58, 2005.
- [6] H. Moseley, "Notes on the structure of several forms of land planarians, with a description of two new genera and several new species, and a list of all species at present known.," *The Quarterly Journal of Microscopical Science*, vol. 17, pp. 273–292, 1877.
- [7] 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.
- [8] 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.
- [9] J.-L. Justine, L. Winsor, D. Gey, P. Gros, and J. Thévenot, "Giant worms chez moi! Hammerhead flatworms (Platyhelminthes, Geoplanidae, Bipalium spp., Diversibipalium spp.) in metropolitan France and overseas French territories," *PeerJ*, vol. 6:e4672, pp. 1–45, 2018.
- [10] 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.
- [11] 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.

To cite this factsheet, please use

Barcoding Facility for Organisms and Tissues of Policy Concern, 2020. Factsheet on *Bipalium kewense*; March 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.

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