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.

Diversibipalium multilineatum

(Makino & Shirasawa, 1983)

Common names:
English: /
French: /
German: /
Dutch: meerlijnige landplatworm

Last update: November 2025

General information on *Diversibipalium multilineatum*

Classification

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

Species in the same genus: N = 97 [2, 3]

Note: We follow the classification of Kawakatsu *et al.* [2] and Justine *et al.* [3]. *Diversibipalium* is a genus, created to house different species which have not yet been assigned to a specific genus based on morphological characteristics.

Infra-species level: N = 0

Note: To our knowledge, no subspecies have been described

Native range: [2, 4]

Japan.

Invasive range: [3 – 7]

Europe (geographical):

France, Italy, Netherlands and Switzerland.

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

https://www.gbif.org/species/8462585
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R19503
http://alien.jrc.ec.europa.eu/SpeciesMapper
https://inpn.mnhn.fr/espece/cd_nom/815842 (France)

Outside Europe (geographical):

South Korea.

Morphology, biology, invasion, negative effects and remedies

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

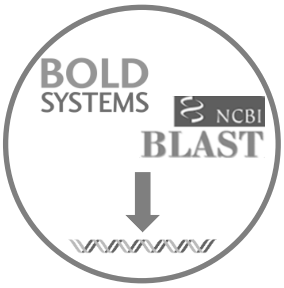
Species identification based on DNA barcodes

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.

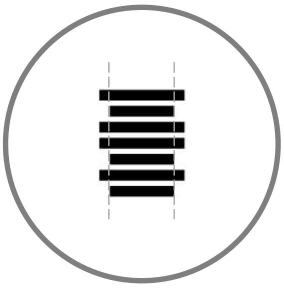
Against this background, BopCo evaluated the inclusion of the invasive flatworm species and its close relatives in both publicly available reference libraries BOLD (www.boldsystems.org/) and GenBank (www.ncbi.nlm.nih.gov/nuccore/) to estimate the reliability with which a species identification can be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

Material and Methods [1]

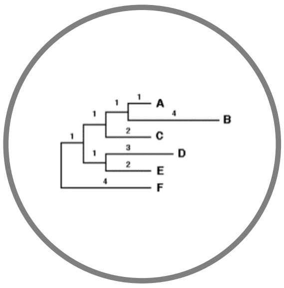


Download all sequence data available for the genus

Filtering the data and selecting ‘promising’ markers



Aligning and trimming of the sequences



Building Neighbour-Joining tree with Bootstrap support

Conclusion

Due to a large gap in available sequence data, it is currently impossible to fully assess the reliability of this DNA marker. Markers **COI** and **28S** are potentially good markers to identify *Diversibipalium multilineatum,* however more sequence data should be added to the analysis for a more reliable conclusion.

Discussion

Relevant DNA sequences for *Diversibipalium multilineatum* and congeneric species were downloaded from GenBank and BOLD.

In the NJ-trees for **COI** and **28S**, the *D. multilineatum* sequences cluster together, except for one (COI, HM346600) which is placed with *Bipalium adventitium*. This latter sequence may be a misidentification [8]. Additional sequences for *D. multilineatum*, especially from its native range would allow for a better evaluation of both markers.

EF-1-alpha does not recover *D. multilineatum* as a cluster. Additional sequences for *D. multilineatum* are needed to allow for a better evaluation of this marker.

For marker **18S** only three sequences and for marker of which one is does not cluster. Therefore it is currently impossible to assess the ability of this marker to identify *D. multilineatum*.

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 *Diversibipalium multilineatum* sequence was available.

Markers analysed	**1**	**2**	**3**	**4**	**5**
COI		X		X	X
18S	X	X	X	X	X
28S	X	X			X
EF-1-alpha	X	X	X	X	X

Table 2: Publicly available sequences downloaded (November 2025) 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]. 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**
Diversibipalium andrewsi				
Diversibipalium bimaculatum				
Diversibipalium bleekeri				
Diversibipalium boehmigi				
Diversibipalium brauni				
Diversibipalium brunneum				
*Diversibipalium castaneum**	X		X	
Diversibipalium catenatum		X		
Diversibipalium claparedei				
Diversibipalium claviforme				
*Diversibipalium contortolineatum**		X	X	
Diversibipalium delicatum				
Diversibipalium dendrophilum				
Diversibipalium dihangense				
*Diversibipalium duplalaticlavium**			X	
Diversibipalium ellioti				
Diversibipalium engeli				
Diversibipalium everetti				
Diversibipalium expeditionis				
Diversibipalium falcatum				
Diversibipalium fenestratum				
Diversibipalium ferudpoorense				
Diversibipalium flowei				
Diversibipalium fuligineum				
Diversibipalium fulvum				
Diversibipalium fuscocephalum				
Diversibipalium gebai				
Diversibipalium giganteum				
Diversibipalium grandidieri				
Diversibipalium grayi				
Diversibipalium gulliveri				
Diversibipalium haasei				
Diversibipalium hasseltii				
Diversibipalium hildebrandi				
Diversibipalium houghtoni				
Diversibipalium indicum				
Diversibipalium isabellinum				
Diversibipalium jalorense				
Diversibipalium jansei				
Diversibipalium keshavi				
Diversibipalium kirkaptricki				
Diversibipalium koreense				

Species in genus	**COI**	**18S**	**28S**	**EF-1-alpha**
Diversibipalium kuhlii				
Diversibipalium layardi				
Diversibipalium lehnerti				
Diversibipalium lomani				
Diversibipalium longitudinalis				
Diversibipalium lunatum				
Diversibipalium maculatum				
Diversibipalium madagascarense				
Diversibipalium marenzelleri				
*Diversibipalium marginatanigrum**	X	X	X	
Diversibipalium mayottensis		X	X	
Diversibipalium megacephalum				
Diversibipalium modiglianii				
Diversibipalium molle				
Diversibipalium multilineatum	**X**	**X**	**X**	**X**
Diversibipalium murinum				
Diversibipalium natunense				
Diversibipalium negritorum				
Diversibipalium nigrilumbe				
Diversibipalium ocellatum				
Diversibipalium olivaceps				
Diversibipalium piceum				
Diversibipalium pictum				
Diversibipalium quadricinctum				
Diversibipalium rauchi				
Diversibipalium richtersi				
Diversibipalium ridleyi				
Diversibipalium roonwali				
Diversibipalium rotungense				
Diversibipalium ruteofulvum				
Diversibipalium salvini				
Diversibipalium sarasini				
Diversibipalium sexcinctum				
Diversibipalium shipleyi				
Diversibipalium simplex				
Diversibipalium smithi				
Diversibipalium solmsi				
Diversibipalium sordidum				
Diversibipalium sp. ‘black’*	X			
Diversibipalium sp. ‘blue’*	X			
Diversibipalium splendens				
Diversibipalium steindachneri				
Diversibipalium stimpsoni				
Diversibipalium sumatrense				
Diversibipalium superbum				
Diversibipalium sylvestre				
Diversibipalium tamatavense				
Diversibipalium tau				
Diversibipalium tennenti				
Diversibipalium transversefasciatum				
Diversibipalium trilineatum				
Diversibipalium tripartitum				
Diversibipalium unicolor				
Diversibipalium vinosum				
Diversibipalium virchowi				
Diversibipalium virgatum				
Diversibipalium vittatum				
Diversibipalium weberi				
Diversibipalium whitehousei				
Diversibipalium wrighti				
TOTAL species	1(5)*/97	3(5)*/97	2(6)*/97	1/97

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=1039311#null

Picture credits

Page 1: *Diversibipalium_multilineatum* By L. Cavigioli in G. Mazza *et al.* [3] [CC BY SA 3.0]
Page 2: *Diversibipalium_multilineatum*_from_Italy By L. Cavigioli in G. Mazza *et al.* [3] [CC BY SA 3.0]

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] 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] 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.

[4] G. Mazza *et al.*, "First report of the land planarian *Diversibipalium multilineatum* (Makino & Shirasawa, 1983) (Platyhelminthes, Tricladida, Continenticola) in Europe," *Zootaxa*, vol. 4067, no. 5, pp. 577–580, 2016.

[5] 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.

[6] M. Kawakatsu, E. Froehlich, H. D. Jones, M. Kawakatsu, and T. Kawakatsu, "Additions and corrections of the previous land planarian indices of the world (Platyhelminthes, Seriata, Tricladida, Continenticola, Geoplanoidae)," 2014. [Online]. Available: http://www.riverwin.jp/pl/m14_2/K14II.pdf. [Accessed: 23-Mar-2020].

[7] de Waart SA, Vanhove MPM, Justine J-L, Kmentová N, "Going Dutch: European distribution of non-native land flatworm species belonging to Geoplaninae and Bipaliinae with focus on the Netherlands." *NeoBiota* 99: 285-321. 2025 https://doi.org/10.3897/neobiota.99.145703

[8] J. Lou Justine, T. Théry, D. Gey, and L. Winsor, "First record of the invasive land flatworm *Bipalium adventitium* (Platyhelminthes, Geoplanidae) in Canada," *Zootaxa*, vol. 4656, no. 3, pp. 591–595, 2019.

To cite this factsheet, please use

Barcoding Facility for Organisms and Tissues of Policy Concern, 2020. Factsheet on *Diversibipalium multilineatum; November 2025*. In: Identification of Invasive Flatworm Species using DNA barcodes. BopCo, Belgium. Available from: https://bopco.be/projects/flatworm-factsheets, accessed on DD-MM-YYYY.