



# 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).

## *Diversibipalium multilineatum*

(Makino & Shirasawa, 1983)

Common names:

English: /

French: /

German: /

Dutch: /

Last update: March 2020



## General information on *Diversibipalium multilineatum*

### Classification

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

### 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 – 6]

#### Europe (geographical):

France, Italy 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](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/](http://www.boldsystems.org/)) and GenBank ([www.ncbi.nlm.nih.gov/nuccore/](http://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]



### Conclusion

Due to a large gap in available sequence data, it is currently impossible to fully assess the reliability of this DNA marker.

### Discussion

Relevant DNA sequences for *Diversibipalium multilineatum* and congeneric species were downloaded from GenBank and BOLD. Yet, only three out of the 97 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**, the *D. multilineatum* sequences cluster together, except for one (HM346600) which is placed with *Bipalium adventitium*. This latter sequence may be a misidentification [7]. Additional sequences for *D. multilineatum*, especially from its native range would allow for a better evaluation of COI.

**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 one sequence and for marker **28S** only two clustering sequences are available for *D. multilineatum*. Therefore it is currently impossible to assess the ability of these markers 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	X
18S	1	X	1		X
28S	X	X			X
EF-1-alpha	X	X	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, 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
<i>Diversibipalium andrewsi</i>				
<i>Diversibipalium bimaculatum</i>				
<i>Diversibipalium bleekeri</i>				
<i>Diversibipalium boehmigi</i>				
<i>Diversibipalium brauni</i>				
<i>Diversibipalium brunneum</i>				
<i>Diversibipalium catenatum</i>				
<i>Diversibipalium claparedei</i>				
<i>Diversibipalium claviforme</i>				
<i>Diversibipalium delicatum</i>				
<i>Diversibipalium dendrophilum</i>				
<i>Diversibipalium dihangense</i>				
<i>Diversibipalium ellioti</i>				
<i>Diversibipalium engeli</i>				
<i>Diversibipalium everetti</i>				
<i>Diversibipalium expeditionis</i>				
<i>Diversibipalium falcatum</i>				
<i>Diversibipalium fenestratum</i>				
<i>Diversibipalium ferudpoorensis</i>				
<i>Diversibipalium flowei</i>				
<i>Diversibipalium fulgineum</i>				
<i>Diversibipalium fulvum</i>				
<i>Diversibipalium fuscocephalum</i>				
<i>Diversibipalium gebai</i>				
<i>Diversibipalium giganteum</i>				
<i>Diversibipalium grandidieri</i>				
<i>Diversibipalium grayi</i>				
<i>Diversibipalium gulliveri</i>				
<i>Diversibipalium haasei</i>				
<i>Diversibipalium hasseltii</i>				
<i>Diversibipalium hildebrandi</i>				
<i>Diversibipalium houghtoni</i>				
<i>Diversibipalium indicum</i>				
<i>Diversibipalium isabellinum</i>				
<i>Diversibipalium jalorense</i>				
<i>Diversibipalium janseni</i>				
<i>Diversibipalium keshavi</i>				
<i>Diversibipalium kirkaptricki</i>				
<i>Diversibipalium koreense</i>				
<i>Diversibipalium kuhlii</i>				



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

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