



# 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. Non-native species causing such adverse effects are called Invasive Alien Species (IAS). In order to protect native biodiversity and ecosystems, and to mitigate the potential impact on human health and socio-economic activities, the issue of IAS is tackled in Europe by EU Regulation 1143/2014 of the European Parliament and Council. The IAS Regulation provides for a set of measures to be taken across all member states. The list of *Invasive Alien Species of Union Concern* is regularly updated. However, to implement the proposed actions, methods for accurate species identification are required when suspicious biological material is encountered.

Because morphology-based species identifications are not always possible (e.g. cryptic species, trace material, early life-stages), the purpose of the present work is to investigate and evaluate the usefulness of DNA sequence data to identify each of the IAS included in the EU Regulation. The results are presented as factsheets (one per IAS) 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 IAS, with 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 IAS using DNA barcoding to the taxonomic level stated in the EU list. 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).

More info on the EU Regulation on [http://ec.europa.eu/environment/nature/invasivealien/index\\_en.htm](http://ec.europa.eu/environment/nature/invasivealien/index_en.htm).

## *Myriophyllum heterophyllum*

Michx., 1803

Common names:

English: variable / broadleaved / two-leaved water-milfoil, changed-leaved parrotfeather

French: myriophylle à feuilles variées, myriophylle à feuilles de deux sortes

German: Verschiedenblättriges Tausendblatt

Dutch: ongelijkbladig vederkruid

Last update: May 2019



## General information on *Myriophyllum heterophyllum*

### Classification

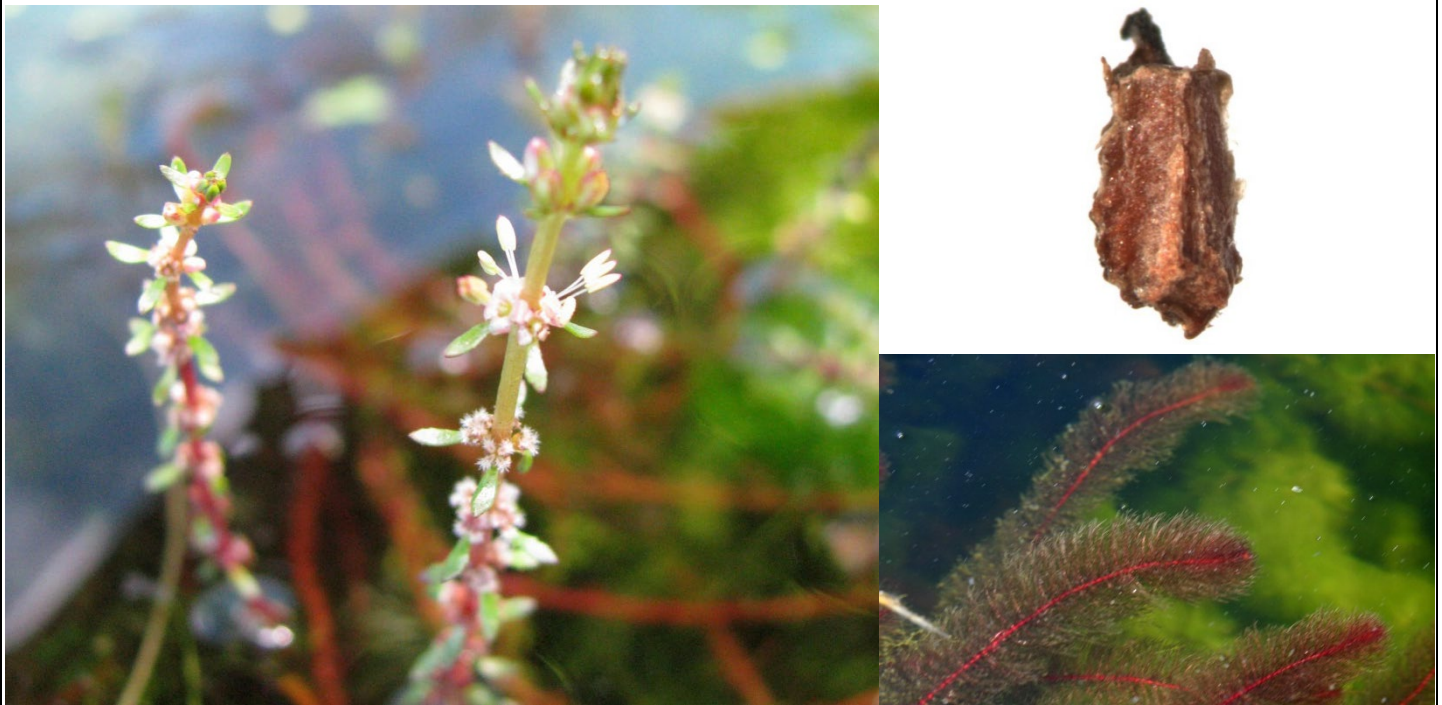
Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Eudicots	Saxifragales	Haloragaceae	<i>Myriophyllum</i>

### Species in the same genus: N = 60-70 [2]–[6]

Note: The genus' members are notoriously difficult to identify morphologically and are often described regionally while having a cosmopolitan distribution. A hybrid with *M. laxum* is described from the native range and hybridisation occurs with *M. hippuroides* in introduced regions.

### Infra-species level: N = 0

Note: To our knowledge, no subspecies or varieties are described.



### Native range: [6, 7]

North America; eastern coasts of Canada and United States of America.

### Invasive range: [8–10]

#### Europe (geographical):

Austria, Belgium, Croatia, France, Germany, Netherlands, Spain, Switzerland, United Kingdom.

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

<https://www.gbif.org/species/5361762>

<https://gd.eppo.int/taxon/MYPHE/distribution>

<https://easin.irc.ec.europa.eu/spexplorer/species/factsheet/R09747>

#### Outside Europe (geographical):

China, Guatemala, western coast of Canada.

### Morphology, biology, invasion, negative effects and remedies

For more information on *Myriophyllum heterophyllum* 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 IAS samples, especially when morphological characteristics are absent or useless. However, to assure correct species identifications, reference libraries need to include a sufficiently large number of sequences of (i) the IAS under investigation to assess the intraspecific genetic divergence, (ii) the closely related species to evaluate the interspecific genetic divergence, and (iii) the different geographical areas covering the distribution range (native and invasive) of the IAS to detect potential population structure or local hybrids.

In this context, BopCo evaluated the inclusion of the IAS and their 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

Based on the present evaluation of the available sequence data, the full ITS region is the most reliable DNA marker for the identification of *Myriophyllum heterophyllum*.

### Discussion

DNA markers for which *Myriophyllum* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Myriophyllum*. Six DNA markers were evaluated (Table 1). For only three markers, a large number of *Myriophyllum* species were represented (Table 2).

For the **full ITS** region the sequence data analysis method described by Moody & Les [4] was used instead of the method applied to the other DNA markers evaluated and described in [1]. The *M. heterophyllum* sequences are found in two or three clusters with in-between a distinct cluster of *M. hippuroides* sequences. One sequence of *M. heterophyllum* (GenBank accession number JF717417) should be considered as a misidentification. This marker provides the highest species representation at the moment (Table 2) and can be used for species identification, as described by Thum *et al.* [10]. With ITS1 the *M. hippuroides* sequences do not cluster together, and many closely related species cannot be distinguished. The ITS2 marker displays little genetic variation and the sequences of *M. heterophyllum* do not cluster together.

The **psbA-trnH** intergenic spacer dataset contains only *M. heterophyllum* sequences from the European invasive range. These form a well-supported cluster, but only few congeners are represented and *M. hippuroides*, which clusters with *M. heterophyllum* sequences for ITS is not represented (Table 2). Ghahramanzadeh *et al.* [11] designate psbA-trnH the "best performing barcode". However, to allow for a better evaluation of the performance of this marker for identifications, missing congeners and *M. heterophyllum* sequences from the native region should be included.

For the universal barcode marker **matK**, the three *M. heterophyllum* sequences do not cluster together due to one sequence (GenBank accession number MK520342) clustering with similar looking species. This sequence is possibly mislabelled. This marker can already be used to exclude a large number of *Myriophyllum* congeners as the tree is well-supported, but the marker is not able to distinguish *M. heterophyllum* from the closely related *M. pinnatum*. Additional sequences for *M. heterophyllum* would allow for a better evaluation of this marker.



A dataset of **trnL** sequences (with the trnL-trnF intergenic spacer) provides a NJ-tree with clustering of *M. heterophyllum* sequences, all lacking range distribution info, with *M. hippuroides* included in the cluster. Many congeners are not yet represented (Table 2). In the current state of the online reference libraries it is not advisable to apply this marker for species identification.

The universal barcode marker **rbcl** displays little genetic variation and the sequences of *M. heterophyllum* do not cluster together. In contrast, in Ghahramanzadeh *et al.* [11] they do cluster together, though with a more limited dataset. Additional sequences for the missing congeners would allow to better evaluate this marker.

For the **trnK** gene there is high species coverage (Table 2), yet fewer sequences per species are available. The tree is not well resolved and support values are low. The sequences of *M. heterophyllum* do not cluster together. In the current state of the online reference libraries it is not advisable to apply this marker for species identification.

For the **atpB-rbcl** and **rpl32-trnL** intergenic spacer, **small subunit rRNA**, and the **GAPDH** gene fewer sequences are available and *M. heterophyllum* sequences are even lacking. Therefore, it is currently impossible to assess the ability of these markers to identify *M. heterophyllum*.

**Table 1:** Overview of the encountered issues concerning the DNA-based identification of the IAS [1]: (1) Insufficient publicly available DNA sequences of the IAS to capture the intra-species divergence; (2) Poor geographical coverage of the IAS sequences (native or invasive range missing); (3) The IAS sequences do not form supported clusters; (4) Potential misidentification of a specimen which influences the clustering of the IAS sequences; and (5) Not all congeneric species are represented in the final NJ-tree. An 'X' indicates that the issue was encountered.

Markers analysed	1	2	3	4	5
<b>rbcl</b>			X	X	X
<b>matK</b>	X	X	X	X	X
<b>psbA-trnH</b>		X			X
<b>ITS</b>		X	X	X	X
<b>trnK</b>	X	X	X		X
<b>trnL</b>		X	X		X

**Table 2:** Publicly available sequences downloaded (May 2019) from BOLD and GenBank which were withheld as reliable and informative in the final alignment that was used for building the NJ-trees. The species names follow [6]. An 'X' indicates that at least one sequence was used in the final alignment.

Species in genus	rbcl	matK	psbA-trnH	ITS	trnK	trnL
<i>Myriophyllum alpinum</i>		X		X	X	
<i>Myriophyllum alterniflorum</i>	X	X	X	X	X	X
<i>Myriophyllum amphibium</i>				X		
<i>Myriophyllum aquaticum</i>	X	X	X	X	X	X
<i>Myriophyllum axilliflorum</i>						
<i>Myriophyllum balladoniense</i>		X		X	X	
<i>Myriophyllum caput-medusae</i>		X		X	X	
<i>Myriophyllum coronatum</i>		X		X	X	
<i>Myriophyllum crispatum</i>		X		X		
<i>Myriophyllum decussatum</i>		X		X	X	
<i>Myriophyllum dicoccum</i>		X			X	
<i>Myriophyllum drummondii</i>		X		X	X	
<i>Myriophyllum echinatum</i>		X		X		
<i>Myriophyllum exasperatum</i>						
<i>Myriophyllum farwellii</i>	X	X		X	X	
<i>Myriophyllum filiforme</i>		X		X	X	
<b><i>Myriophyllum heterophyllum</i></b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
<i>Myriophyllum hippuroides</i>	X	X		X	X	X
<i>Myriophyllum humile</i>	X	X		X	X	
<i>Myriophyllum lapidicola</i>		X		X	X	
<i>Myriophyllum latifolium</i>		X		X	X	
<i>Myriophyllum laxum</i>		X		X	X	X
<i>Myriophyllum limnophilum</i>		X		X	X	
<i>Myriophyllum lophatum</i>		X		X	X	



Species in genus	rbcl	matK	psbA-trnH	ITS	trnK	trnL
<i>Myriophyllum mattogrossensis</i>		X		X	X	
<i>Myriophyllum mezianum</i>						
<i>Myriophyllum muricatum</i>		X		X	X	
<i>Myriophyllum oguraense</i>		X		X	X	X
<i>Myriophyllum papillosum</i>		X		X	X	
<i>Myriophyllum pedunculatum</i>		X		X	X	
<i>Myriophyllum petraeum</i>		X		X	X	
<i>Myriophyllum pinnatum</i>	X	X		X	X	
<i>Myriophyllum porcatum</i>						
<i>Myriophyllum quitense</i>	X	X		X	X	
<i>Myriophyllum robustum</i>	X	X	X	X	X	
<i>Myriophyllum salsugineum</i>		X		X	X	
<i>Myriophyllum sibiricum</i>	X	X		X	X	X
<i>Myriophyllum simulans</i>	X	X	X	X	X	
<i>Myriophyllum sparsiflorum</i>						
<i>Myriophyllum spicatum</i>	X	X	X	X	X	X
<i>Myriophyllum tenellum</i>	X	X		X	X	
<i>Myriophyllum tetrandrum</i>						
<i>Myriophyllum tillaeoides</i>		X		X	X	
<i>Myriophyllum trachycarpum</i>		X		X	X	
<i>Myriophyllum triphyllum</i>		X		X	X	
<i>Myriophyllum tuberculatum</i>	X		X			
<i>Myriophyllum ussuriense</i>	X	X		X	X	
<i>Myriophyllum variifolium</i>		X		X	X	
<i>Myriophyllum verrucosum</i>		X		X	X	
<i>Myriophyllum verticillatum</i>	X	X	X	X	X	X
<i>Myriophyllum votschii</i>		X		X	X	
<b>TOTAL species</b>	<b>16/70</b>	<b>43/70</b>	<b>8/70</b>	<b>43/70</b>	<b>41/70</b>	<b>9/70</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.des.nh.gov/organization/commissioner/pip/factsheets/bb/documents/bb-23.pdf>

<https://waarnemingen.be/pda/shellphp/exo/be/nl/17153.pdf>

<https://waarnemingen.be/pda/shellphp/exo/be/fr/17153.pdf>

<http://www.iucngisd.org/gisd/speciesname/Myriophyllum+heterophyllum>

<http://www.nonnativespecies.org/factsheet/downloadFactsheet.cfm?speciesId=2286>

### Picture credits

Page 1: twoleaf watermilfoil By Leslie J. Mehrhoff [CC BY 3.0]

Page 2 (left): twoleaf watermilfoil By Leslie J. Mehrhoff [CC BY 3.0]

Page 2 (right, top): *Myriophyllum heterophyllum* 10889, 4-partite schizocarp By The Groningen Institute of Archaeology, Digital Seeds Atlas of the Netherlands [All Rights Reserved] Permission sought directly from the copyright holders.

Page 2 (right, bottom): twoleaf watermilfoil By Leslie J. Mehrhoff [CC BY 3.0]

### References

- [1] N. Smits, S. Gombeer, K. Meganck, 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] P. F. Stevens, "Angiosperm Phylogeny Website, Version 14" 2001. [Online]. Available: <http://www.mobot.org/MOBOT/research/APweb>. [Accessed: 14-Mar-2018].
- [3] M. L. Moody and D. H. Les, "Systematics of the Aquatic Angiosperm Genus *Myriophyllum* (Haloragaceae)" *Syst. Bot.*, vol. 35, no. 1, pp. 121–139, 2010.
- [4] M. L. Moody and D. H. Les, "Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations" *Proc. Natl. Acad. Sci.*, vol. 99, no. 23, pp. 14867–14871, 2002.
- [5] R. A. Thum, M. P. Zuellig, R. L. Johnson, M. L. Moody, and C. Vossbrinck, "Molecular markers reconstruct the invasion history of variable leaf watermilfoil (*Myriophyllum heterophyllum*) and distinguish it from closely related species" *Biol. Invasions*, vol. 13, no. 7, pp. 1687–1709, 2011.
- [6] WCVP, "World Checklist of Vascular Plants, version 2.0." 2020. [Online]. Available: <http://wcvp.science.kew.org/>. [Accessed: 15-Apr-2020].
- [7] L. W. J. Anderson, Ed., "1st International Symposium On Watermilfoil (*Myriophyllum spicatum*) And Related Haloragaceae Species" 1985, p. 223 p.
- [8] N. Jasprica, A. Lasić, D. Hafner, and A. Bratoš Cetinić, "European invasion in progress: *Myriophyllum heterophyllum* Michx. (Haloragaceae) in Croatia" *Nat. Croat.*, vol. 26, no. 1, pp. 99–103, 2017.
- [9] R. A. Thum and M. Zuelig, "*Myriophyllum heterophyllum* (broadleaf watermilfoil)" *CABI Invasive Species Compendium*, 2018. [Online]. Available: <https://www.cabi.org/isc/datasheet/34940>. [Accessed: 24-May-2019].
- [10] R. A. Thum, A. T. Mercer, and D. J. Wcisel, "Loopholes in the regulation of invasive species: genetic identifications identify mislabeling of prohibited aquarium plants" *Biol. Invasions*, vol. 14, no. 5, pp. 929–937, 2012.
- [11] R. Ghahramanzadeh *et al.*, "Efficient distinction of invasive aquatic plant species from non-invasive related species using DNA barcoding" *Mol. Ecol. Resour.*, vol. 13, no. 1, pp. 21–31, 2013.

### To cite this factsheet, please use

Barcoding Facility for Organisms and Tissues of Policy Concern, 2019. Factsheet on *Myriophyllum heterophyllum*; May 2019. In: Identification of Invasive Alien Species using DNA barcodes. BopCo, Belgium. Available from: <https://bopco.be/output/iasfactsheets>, 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](mailto:bopco@naturalsciences.be).

