



# 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) aims at developing an expertise forum to facilitate the identification of biological samples of policy concern in Belgium and Europe. The project represents part of the Belgian federal contribution to the European Research Infrastructure Consortium LifeWatch.

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. In order to implement the proposed actions, however, 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 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 IAS to the taxonomic level stated in the EU list 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 <http://bopco.myspecies.info/> 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 aquaticum*

(Vell.) Verdc., 1973

Common names:

English: Brazilian water-milfoil, parrot feather, water-feather

French: myriophylle du Brésil, myriophylle aquatique

German: Brasilianisches Tausendblatt

Dutch: parelvederkruid

Last update: August 2020



## General information on *Myriophyllum aquaticum*

### 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. We follow the classification of WCVP for the purpose of this factsheet.

### Infra-species level: N = 1 [7]

Note: One variety is encountered in an aquatic plant hobbyist publication; *M.a.* var. *santacatarinense*.



### Native range: [7, 8]

South America; Argentina, Brazil, Chile, Paraguay, Peru, Uruguay.

### Invasive range: [7-10]

#### Europe (geographical):

Austria, Belgium, France, Germany, Ireland, Italy, Netherlands, Portugal, Romania, Spain, United Kingdom.

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

<https://www.gbif.org/species/5361785>

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

<http://www.europe-aliens.org/speciesFactsheet.do?speciesId=9151#>

<http://alien.jrc.ec.europa.eu/SpeciesMapper>

#### Outside Europe (geographical):

Widespread in many parts of the world; Central and North America, Australia and New Zealand, South-Eastern Asia, Southern Africa up to Kenya.

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

For more information on *Myriophyllum aquaticum* see the references and online information listed at the end of this document.



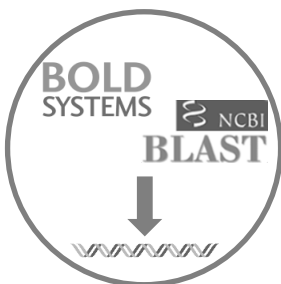
## Species identification based on DNA barcodes

### 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. To assure correct species identifications, however, reference libraries need to include a sufficiently large number of sequences of (i) the IAS 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 IAS in order to detect potential population structure or local hybrids.

Against this background, 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.

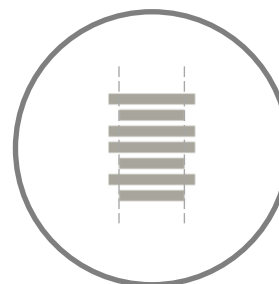
### Material and Methods [1]



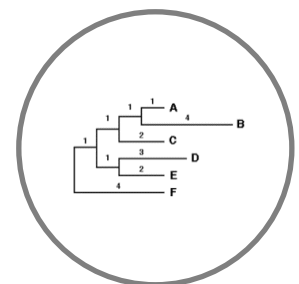
Download all sequence data available for the genus



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, full ITS and possibly matK are the most reliable DNA markers for the identification of *Myriophyllum aquaticum*.**

### 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). Representation of the *Myriophyllum* species is low to very low. (Table 2).

The use of the **full ITS** region provides a supported clustering to delineate *M. aquaticum* from the available congeners. The use of this marker has already been advised by Moody & Les [5] and Thum *et al.* [11]. Using the full region rather than only the component ITS1 or ITS2 regions gives higher support with the same species representation. For now, the available sequences of *M. aquaticum* all come from the invasive range. Additional sequences for *M. aquaticum* from the native regions as well as for the missing congeners, would allow to better evaluate the performance of the full ITS region for species identification.

For the universal barcode marker **matK**, all *M. aquaticum* sequences cluster together, but one *M. verticillatum* (GenBank accession number KC737256) is included. Considering the position of other *M. verticillatum* sequences, it possibly involves a mislabelling. If so, matK can be used alongside full ITS for the identification of *M. aquaticum*.

For the **trnK** gene there is a similar number of species represented as the above markers (Table 2), but with fewer sequences per species. Overall, the NJ-tree is not well resolved and support values for clusters are low, but the available of *M. aquaticum* sequences cluster together. Additional sequences from the native region, as well as sequences of the missing congeners would allow to better evaluate the performance of this marker for species identification.

The **psbA-trnH** intergenic spacer dataset contains multiple *M. aquaticum* sequences, but only represent the invasive region. Ghahramanzadeh *et al.* [12] designated psbA-trnH as the "best performing barcode". However, only few species are represented (Table 2) and additional sequences for the missing congeners should be added to the analyses to allow for a better evaluation of the performance of this marker.

The universal barcode marker **rbcl** does not display much genetic variation and the available sequences of *M. aquaticum* do not cluster together. Additionally, the genus is underrepresented (Table 2). The low genetic variation raises doubts about the



taxonomic resolution of this marker for the genus *Myriophyllum*.

For **atpB-rbcl** and **rpl32-trnL** intergenic spacer, **small subunit rRNA**, **GAPDH** gene and the **trnL** gene combined with trnL-trnF intergenic spacer, fewer sequences are available and/or the marker shows little genetic variation among the different species. Therefore it is currently impossible to assess the ability of these markers to identify *M. aquaticum*.

**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
<b>psbA-trnH</b>		X			X
<b>Full ITS</b>		X			X
<b>ITS1</b>		X			X
<b>ITS2</b>		X			X
<b>trnK</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]. The list of species is limited to those members of *Myriophyllum* for which at least one sequence was used in any of the NJ-trees. Species name with \* is not mentioned in [6] but used on GenBank. An 'X' indicates that at least one sequence was used in the final alignment, an '(X)<sup>2</sup>' indicates only ITS region 2 was available for analysis.

Species in genus	rbcl	matK	psbA-trnH	Full ITS & ITS1 (ITS2)	trnK
<i>Myriophyllum alpinum</i>		X		X	X
<i>Myriophyllum alterniflorum</i>	X	X	X	X	X
<i>Myriophyllum amphibium</i>				X	
<b><i>Myriophyllum aquaticum</i></b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
<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 dicocum</i>		X		(X) <sup>2</sup>	X
<i>Myriophyllum drummondii</i>		X		X	X
<i>Myriophyllum echinatum</i>		X		X	
<i>Myriophyllum farwellii</i>	X	X		X	X
<i>Myriophyllum filiforme</i>		X		X	X
<i>Myriophyllum heterophyllum</i>	X	X	X	X	X
<i>Myriophyllum hippuroides</i>	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
<i>Myriophyllum limnophilum</i>		X		X	X
<i>Myriophyllum lophatum</i>		X		X	X
<i>Myriophyllum mattogrossensis</i>		X		X	X
<i>Myriophyllum muricatum</i>		X		X	X
<i>Myriophyllum oguraense</i>		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 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
<i>Myriophyllum simulans</i>	X	X	X	X	X
<i>Myriophyllum spicatum</i>	X	X	X	X	X
<i>Myriophyllum tenellum</i>	X	X		X	X
<i>Myriophyllum tillaeoides</i>		X		X	X



Species in genus	rbcl	matK	psbA-trnH	Full ITS & ITS1 (ITS2)	trnK
<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
<i>Myriophyllum votschii</i>		X		X	X
<b>TOTAL species</b>	<b>16/70</b>	<b>42/70</b>	<b>8/70</b>	<b>42 (43)<sup>2</sup>/70</b>	<b>40/70</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

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<http://www.fisheriesireland.ie/invasive-species-1/350-myriophyllum-aquaticum-id-sheet-1/file>

### Picture credits

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Page 2 (right): flowers from *Myriophyllum aquaticum* By C. Haden via Nonindigenous Aquatic Species Database [Public Domain]

### References

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