



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. 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.

More info on the EU Regulation on 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: May 2019



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.

Infra-species level: N = 1 [7]

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



Native range: [7, 8]

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

Invasive range: [7-9]

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>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R18934>

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. 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/) 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]



Conclusion

Based on the present evaluation of the available sequence data, 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). For three markers, a large number of *Myriophyllum* species were represented (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. However, 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 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 and the resulting NJ-tree is well supported. The *M. aquaticum* sequences cluster, but only represent the invasive region. Ghahramanzadeh *et al.* [12] designate 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, only about one third of the genus is represented (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
rbcl		X	X		X
matK		X		X	X
psbA-trnH		X			X
ITS		X			X
trnK	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
<i>Myriophyllum alpinum</i>		X		X	X
<i>Myriophyllum alterniflorum</i>	X	X	X	X	X
<i>Myriophyllum amphibium</i>				X	
<i>Myriophyllum aquaticum</i>	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 dicocum</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
<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 mezianum</i>					
<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 porcatum</i>					
<i>Myriophyllum quitense</i>	X	X		X	X



Species in genus	rbcl	matK	psbA-trnH	ITS	trnK
<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 sparsiflorum</i>					
<i>Myriophyllum spicatum</i>	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
<i>Myriophyllum votschii</i>		X		X	X
TOTAL species	16/70	43/70	8/70	43/70	41/70

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

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

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