



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.

Cabomba caroliniana

A.Gray, 1837

Common names:

English: Carolina fanwort, Carolina water shield, grey fanwort, purple cabomba, green cabomba, Washington grass, fish grass

French: cabomba de Caroline, éventail de Caroline

German: grüne Cabomba, grüne Haarnixe, Haarnixenkraut

Dutch: waterwaaier

Last update: October 2018



General information on *Cabomba caroliniana*

Classification

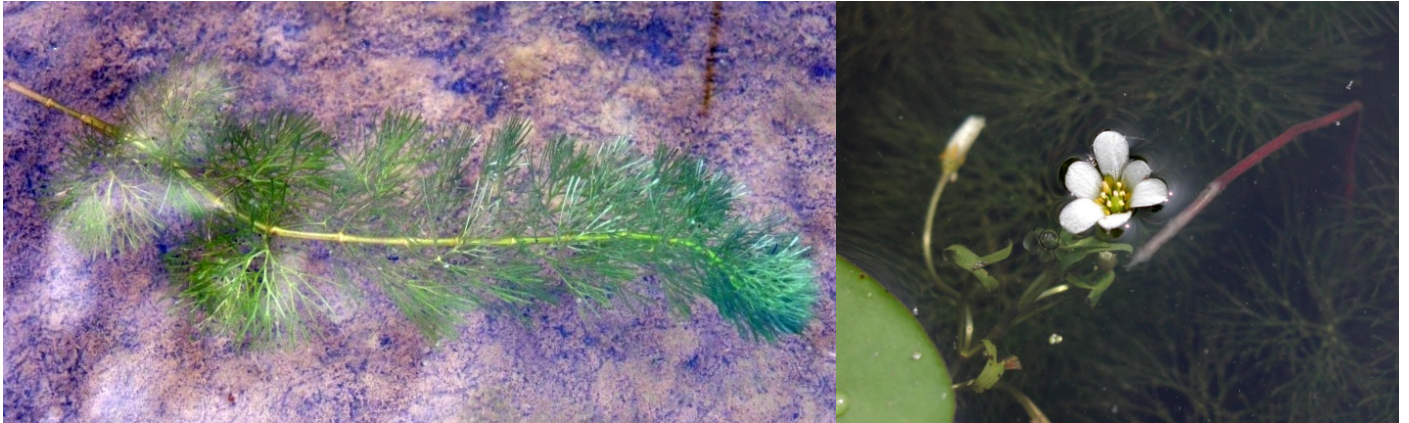
Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Ana-grade	Nymphaeales	Cabombaceae	<i>Cabomba</i>

Species in the same genus: N = 5 [2–4]

Note: In the family Cabombaceae, aside from *Cabomba*, there is only one other genus, with one species, *Brasenia schreberi*.

Infra-species level: N = 3 [2, 3, 5]

Note: Three varieties are listed with varying levels of recognition in literature: *C.c.* var. *flavida* with yellow flowers occurring in South America, *C.c.* var. *pulcherrima* with purple flowers occurring in parts of the southeast United States of America and *C.c.* var. *caroliniana* with white flowers occurring in the United States of America.



Native range: [6–9]

Argentina, Brazil, Paraguay, Uruguay, United States of America.

Invasive range: [6, 10]

Europe (geographical):

Austria, Belgium, France, Germany, Greece, Hungary, Netherlands, Serbia, Sweden, United Kingdom.

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

www.gbif.org/species/2882443

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R02476>

Outside Europe (geographical):

Australia, China, India, Japan, Malaysia, New Zealand.

Morphology, biology, invasion, negative effects and remedies

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



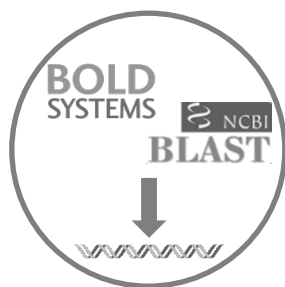
Species identification based on DNA barcodes

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/) and GenBank (www.ncbi.nlm.nih.gov/nuccore/) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

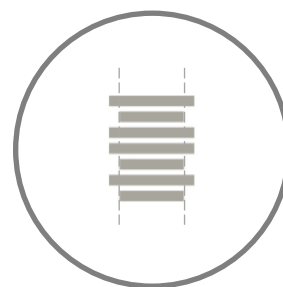
Material and Methods [1]



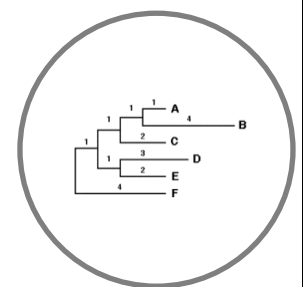
Download all sequence data available for the genus



Filtering the data and selecting 'promising' markers



Aligning and trimming of the sequences



Building Neighbour-Joining tree with Bootstrap support

Conclusion

Based on the present evaluation of the available sequence data, the *trnH-psbA* intergenic spacer is the most reliable DNA marker for the identification of *Cabomba caroliniana*. To allow for a better evaluation of the performance of this marker for species identification, the two missing congeners should be added to the analyses.

Discussion

DNA markers for which *Cabomba* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Cabomba*. Four DNA markers were evaluated (Table 1). Neither *C. palaeformis* nor *C. haynesii* are represented for any of the investigated markers (Table 2). Like *C. caroliniana* these species are native to South America, but have not yet shown to be invasive. However, *C. haynesii* is used as an aquarium plant, which was the main introduction pathway for *C. caroliniana*.

The *trnH-psbA* intergenic spacer region, can be used to distinguish between the represented *Cabomba* species, since they cluster with high support. Both native and invasive regions are covered for *C. caroliniana*. According to Ghahramanzadeh *et al.* [11] this marker has the highest success rate in lab procedures (i.e. PCR and Sanger Sequencing) compared to the other markers. Because not all congeners are represented, adding sequences of the missing species might alter the clustering.

For the universal barcode markers *rbcl* and *matK* and the *ITS* regions fewer sequences are available or, in the case of *rbcl* there is little genetic variation among the represented species. In the current state of the online reference libraries it is not advisable to apply these markers for species identification.



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
matK					X
trnH-psbA					X
Full ITS	X				X

Table 2: Publicly available sequences downloaded (March 2018) from BOLD and GenBank (including sequences extracted from plastid genomes) which were withheld as reliable and informative in the final alignment that was used for building the NJ-trees. The species names follow [3]. An 'X' indicates that at least one sequence was used in the final alignment, a '1' indicates only one sequence was available.

Species in genus	rbcl	matK	trnH-psbA	Full ITS
<i>Cabomba aquatica</i>	1		X	
<i>Cabomba caroliniana</i>	X	X	X	X
<i>Cabomba furcata</i>	X	1	X	X
<i>Cabomba haynesii</i>				
<i>Cabomba palaeformis</i>				
TOTAL species	3/5	2/5	3/5	2/5

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

<http://eol.org/pages/596430/overview>
<http://www.cabi.org/isc/datasheet/107743>
<https://gd.eppo.int/taxon/CABCA>
http://www.q-bank.eu/Plants/Factsheets/Cabomba_caroliniana_EN.pdf
<http://www.iucngisd.org/gisd/speciesname/Cabomba+caroliniana>

Picture credits

Page 1: Carolina fanwort - *Cabomba caroliniana* A. Gray - infestation - US By Leslie J. Mehrhoff, University of Connecticut [CC BY 3.0]

Page 2 (left): Carolina fanwort (*Cabomba caroliniana* A. Gray) By Leslie J. Mehrhoff, University of Connecticut [CC BY 3.0]

Page 2 (right): *Cabomba caroliniana* - Carolina fanwort By Show ryu [CC BY-SA 3.0]

References

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