

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 <u>http://bopco.myspecies.info/</u> or contact us via <u>bopco@naturalsciences.be</u> More info on the EU Regulation on <u>http://ec.europa.eu/environment/nature/invasivealien/index_en.htm</u>

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: August 2020

General information on Cabomba caroliniana

Classification

Classification								
Kingdom	Phylum	Clade	Order	Family	Genus			
Plantae	Magnoliophyta	Ana-grade	Nymphaeales	Cabombaceae	Cabomba			

Species in the same genus: N = 6 [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 http://www.europe-aliens.org/speciesFactsheet.do?speciesId=535#

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





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			Х		Х
matK					Х
trnH-psbA					Х
Full ITS	Х	Х			Х



ITS1	Х	Х				Х	
ITS2	Х	Х				Х	
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							
unique sequence was available.							
Species in genus	rbcl	. matK	trnH-psbA	Full ITS	ITS1	ITS2	
Cabomba aquatica	1		Х				
Cabomba caroliniana	Х	X	Х	Х	Х	Х	
Cabomba furcata	Х	1	Х	Х	Х	Х	
Cabomba haynesii							
Cabomba palaeformis							
Cabomba schwartzii							
TOTAL species	3/6	2/6	3/6	2/6	2/6	2/6	
For a more elaborate discussion of the available databases, the sequence selection process, the outcome of the NJ-tree analyses,							

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]

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To cite this factsheet, please use

Barcoding Facility for Organisms and Tissues of Policy Concern, 2019. Factsheet on *Cabomba caroliniana;* August 2020. In: Identification of Invasive Alien Species using DNA barcodes. BopCo, Belgium. Available from: <u>www.bopco.myspecies.info/content/invasive-alien-species-ias-factsheets</u>, accessed on DD-MM-YYYY.

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