



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

Elodea nuttallii

(Planch.) H.St.John, 1920

Common names:

English: (Esthwaite, free-flowered, western, Nuttall's) waterweed

French: elodée de Nuttall, élodée à feuilles étroites

German: Nuttalls Wasserpest, St. John-Wasserpest

Dutch: smalle waterpest

Last update: November 2018



General information on *Elodea nuttallii*

Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Monocots	Alismatales	Hydrocharitaceae	<i>Elodea</i>

Species in the same genus: N = 9 [2–5]

Note: We follow the classification of the WCVF [6].

The congener *Elodea canadensis* is also encountered as an invasive species in Europe and hybrids between *E. canadensis* and *E. nuttallii* occur naturally.

Infra-species level: N = 0

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



Native range: [5, 6]

Canada, United States of America.

Invasive range: [7, 8]

Europe (geographical):

Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Hungary, Italy, Ireland, Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, Sweden, Switzerland, United Kingdom.

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

<https://www.gbif.org/species/5329212>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R05501>

Outside Europe (geographical):

China, Japan.

Morphology, biology, invasion, negative effects and remedies

For more information on *Elodea nuttallii* 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]



Conclusion

Based on the present evaluation of the available sequence data *matK*, *trnH-psbA* and *trnL-trnF* are the most promising DNA marker(s) for the identification of *Elodea nuttallii*. However, due to the large gaps in available sequence data, it is currently impossible to fully evaluate the performance of these markers for species identification.

Discussion

DNA markers for which *Elodea* sequences were available, were downloaded from GenBank and BOLD for all species of the genus *Elodea*. Six genetic markers were evaluated (Table 1). Sequences still labelled under the synonym genus *Egeria* were also downloaded.

For *matK*, *ndhD* and the *trnH-psbA* and *trnL-trnF* intergenic spacer regions the *Elodea nuttallii* sequences form well-supported clusters. However, only two to three out of the nine species in genus *Elodea* are represented in the NJ-trees (Table 2). Additional sequences for *E. nuttallii*, *E. canadensis* (since it is another *Elodea* species known to be invasive in Europe) and for the other congeners would allow to better evaluate the potential of these DNA markers to distinguish *E. nuttallii* from related species.

For *rbcl*, the full ITS region, as well as the component ITS1 and ITS2 regions, multiple unique *E. nuttallii* sequences are available and four out of the nine *Elodea* species are represented, but they do not cluster. Hence it is not advisable to apply these markers to differentiate *E. nuttallii* from other *Elodea* species.



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				X
ITS			X	X	X
trnH-psbA	X				X
trnL-trnF	X	X			X
ndhD	X	X			X

Table 2: Publicly available sequences downloaded (November 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 [6]. An 'X' indicates that at least one sequence was used in the final alignment.

Species in genus	rbcl	matK	ITS	trnH-psbA	trnL-trnF	ndhD
<i>Elodea bifoliata</i>	X		X			
<i>Elodea callitrichoides</i>						
<i>Elodea canadensis</i>	X	X	X	X	X	X
<i>Elodea densa</i>						
<i>Elodea granatensis</i>	X	X	X			
<i>Elodea heterostemon</i>						
<i>Elodea najas</i>			X			
<i>Elodea nuttallii</i>	X	X	X	X	X	X
<i>Elodea potamogeton</i>						
TOTAL species	4/9	3/9	5/9	2/9	2/9	2/9

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://www.nonnativespecies.org/factsheet/downloadFactsheet.cfm?speciesId=1304>
<https://www.nobanis.org/globalassets/speciesinfo/e/elodea-canadensis/elodea.pdf>
http://www.q-bank.eu/Plants/Factsheets/Elodea_nuttallii_EN.pdf
<http://www.q-bank.eu/Plants/lookalikes/Hydrocharitaceae/Hydrocharitaceae.HTML>

Picture credits

Page 1: Parts of the western waterweed (*Elodea nuttallii*). Note the highly curved, twisted and pointed leaves By Christian Fischer [CC BY-SA 3.0]
Page 2 (left): Aspect of flowering western waterweed (*Elodea nuttallii*) in a loamy pond By Christian Fischer [CC BY-SA 3.0]
Page 2 (right): Female flower of *Elodea nuttalli* in Germany By R a mueller [CC BY-SA 3.0]

References

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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.

