



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 Smits *et al.* [1].

More info about BopCo on <http://bopco.myspecies.info/> or contact us via bopco@naturalsciences.be.

More info on the EU Regulation on http://ec.europa.eu/environment/nature/invasivealien/index_en.htm.

Eichhornia crassipes

(Mart.) Solms, 1883

Common names:

English: common water hyacinth

French: eichhornie commune, jacinthe d'eau

German: Wasserhyazinthe

Dutch: gewone waterhyacint

Last update: October 2018



General information on *Eichhornia crassipes*

Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Monocots	Commelinales	Pontederiaceae	<i>Eichhornia</i>

Species in the same genus: N = 6-9 [2-4]

Note: The family Pontederiaceae is taxonomically problematic and traditionally put in the order Liliales, but more recently moved to the order Commelinales. At the moment, six genera are included in the family, all predominantly neotropical aquatics, but their relationships are still unresolved and the current genera are not monophyletic. The genus *Eichhornia* appears to be paraphyletic and needs to be revised [5, 6].

There is no conclusion whether *E. natans*, *E. meyeri* and *E. venezuelensis* are separate species or synonyms of *E. crassipes*. [7, 8]

Infra-species level: N = 0

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



Native range: [2, 3, 9]

Brazil.

Invasive range: [10-12]

Europe (geographical):

Belgium, Czech Republic, France, Hungary, Italy, Portugal, Romania, Russia, Spain.

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

www.gbif.org/species/2765940

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

www.europe-aliens.org/speciesFactsheet.do?speciesId=5380#

Outside Europe (geographical):

Widespread in Africa, Central and North America (incl. Caribbean), Asia (incl. Middle-East) and Oceania.

Morphology, biology, invasion, negative effects and remedies

For more information on *Eichhornia crassipes* 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/nucleotide/) 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, no marker can be used to identify *Eichhornia crassipes*. *ndhF* seems most promising to further investigate once new sequence data becomes available.

Discussion

DNA markers for which *Eichhornia* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Eichhornia*. Three DNA markers were evaluated (Table 1). The online repositories have a bias in available sequence data towards *E. crassipes*, while few other congeners are represented, except for markers *rbcl* and *ndhF* (Table 2). Due to the taxonomically problematic situation multiple genera were included in the analyses as outgroups.

For *ndhF* only one sequence is available for each of the represented *Eichhornia* species, except for *E. crassipes*. For the latter two sequences are available, yet they show no genetic variation. The complex situation of *Eichhornia* is evident from the NJ-tree; representatives of the genus *Eichhornia* do not cluster, but are spread among clusters of other genera in the family. Taking into account the limited dataset and subsequent small amount of information on inter- and intra-species variation, it is currently impossible to assess the ability of this marker to identify *E. crassipes*. However, compared to *rbcl*, the NJ-tree of *ndhF* shows more genetic variation. Additional sequences for *E. crassipes*, the congeners and other closely related species of the Pontederiaceae family would allow a better evaluation of the performance of *ndhF* to distinguish *E. crassipes* from related species.

For the universal plant barcode marker *rbcl*, ten *E. crassipes* sequences were withheld in the final analysis. As is the case for most congeneric species represented by two or more sequences, the *E. crassipes* sequences do not cluster together in the NJ-tree. This, in combination with the overall low genetic variation displayed in the NJ-tree, raises doubts about the resolution of *rbcl* for this genus. Hence, it is not advisable to apply this marker to differentiate *E. crassipes* from other *Eichhornia* species.

For *matK*, ATP synthase subunits, *coll*, *trnH-psbA* intergenic spacer, multiple ribosomal proteins and NADH dehydrogenase subunits fewer species are represented. Therefore it is currently impossible to evaluate the performance of these markers to identify *E. crassipes*. Of those, only *matK* is reported in Tables 1 & 2, since it is a universal barcode marker.



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
ndhF	X	X			X

Table 2: Publicly available sequences downloaded (April 2018) 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 [4]. Since there is a discussion to lump all the genera of family Pontederiaceae, all available species in the family were included in the analysis. Grouped by genus, with the number in brackets indicating the number of species following [4]. An 'X' indicates that at least one sequence was used in the final alignment, species names with '*' are not accepted in [4] but used on GenBank.

Species in genus	rbcl	matK	ndhF
<i>Eichhornia azurea</i>	X		X
<i>Eichhornia crassipes</i>	X	X	X
<i>Eichhornia diversifolia</i>	X		X
<i>Eichhornia heterosperma</i>	X		X
<i>Eichhornia meyeri</i> *	X		X
<i>Eichhornia paniculata</i>	X		X
<i>Eichhornia paradoxa</i>	X		X
<i>Eichhornia natans</i> *			
<i>Eichhornia venezuelensis</i> *			
<i>Heteranthera</i> (12)	X		X
<i>Hydrothrix</i> (1)	X	X	X
<i>Monochoria</i> (7)	X	X	X
<i>Pontederia</i> (6)	X	X	X
<i>Scholleropsis</i> (1)			
TOTAL species	6 (7)* /6-9	1 /6-9	6 (7)* /6-9

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://archives.eppo.int/MEETINGS/2008_conferences/eichhornia_files/poster_tellez_systematics.pdf
<http://www.cabi.org/isc/datasheet/20544>
http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=200027394
<http://www.iucngisd.org/gisd/speciesname/Eichhornia+crassipes>
http://www.q-bank.eu/Plants/lookalikes/Floating_aquatics/Floating_aquatics.HTML
<https://waarnemingen.be/exo/be/nl/18820.pdf>
<http://www.msapms.org/factsheets/Waterhyacinth.pdf>

Picture credits

Page 1: Huge swamp field being populated by *Eichhornia crassipes* By Nick Lubushko [CC BY-SA 4.0]
Page 2 (left): *Eichhornia crassipes* flower By H. Zell, Botanical Garden KIT, Karlsruhe, Germany [GFDL or CC BY-SA 3.0]
Page 2 (middle): close-up of seeds By Photo Steve Hurst, USDA PLANTS Database [Public Domain]
Page 2 (right): *Eichhornia crassipes* By Amada44 [GFDL or CC BY 3.0]

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