



# 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](mailto:bopco@naturalsciences.be).

More info on the EU Regulation on [http://ec.europa.eu/environment/nature/invasivealien/index\\_en.htm](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: April 2020



## 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 = 25 [2, 3]

Note: The family Pontederiaceae is taxonomically problematic and traditionally put in the order Liliales, but more recently moved to the order Commelinales. [4]

Recently, the family Pontederiaceae has been re-circumscribed to have two genera, *Heteranthera* and *Pontederia*. The latter is now including the previous genera *Pontederia s.s.*, *Monochoria* and the previously polyphyletic *Eichhornia* which was in need of revision [5, 6]. The valid name of this IAS is now *Pontederia crassipes*.

Where previously there was no conclusion on the acceptance of *E. natans*, *E. meyeri* and *E. venezuelensis* [6–8] and the species number of *Eichhornia* was six up to nine, the newly described *Pontederia* has 25 accepted species.

### 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](http://www.gbif.org/species/2765940)

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R05440>

#### 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. 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/](http://www.boldsystems.org/)) and GenBank ([www.ncbi.nlm.nih.gov/nuccore/](http://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, 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). Due to the taxonomic re-designation, the genera *Heteranthera*, *Hydrothrix*, *Monochoria*, *Scholleropsis* and *Pontederia* were included in the analyses as well.

For *ndhF* only one sequence is available for each of the represented (former) *Eichhornia* species, except for *E. crassipes*. For the latter three sequences are available and they cluster together. 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. Considering the limited dataset, 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* and the missing congeners of genus *Pontederia* would allow a better evaluation of the performance of *ndhF* to distinguish *E. crassipes* from related species.

For the universal plant barcode marker *rbcl*, 11 *E. crassipes* sequences were withheld in the final analysis and these 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 genus *Pontederia*. Hence, it is not advisable to apply this marker to differentiate *E. crassipes* from other species.

For *matK*, *ATP* synthase subunits, *coll*, *psbA-trnH* 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
<b>rbcl</b>		X	X		X
<b>matK</b>		X	X		X
<b>ndhF</b>	X	X			X

**Table 2:** Publicly available sequences downloaded (April 2020) 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 [2, 3] whereby a number of genera were lumped in genus *Pontederia*. An 'X' indicates that at least one sequence was used in the final alignment.

Species in genus	rbcl	matK	ndhF
<i>Pontederia africana</i>			
<i>Pontederia australasica</i>			
<i>Pontederia azurea</i>	X		X
<i>Pontederia brevipetiolata</i>			
<i>Pontederia cordata</i>	X	X	X
<b><i>Pontederia crassipes</i></b>	<b>X</b>	<b>X</b>	<b>X</b>
<i>Pontederia cyanea</i>	X		X
<i>Pontederia diversifolia</i>	X		X
<i>Pontederia elata</i>			
<i>Pontederia hastata</i>	X		X
<i>Pontederia heterosperma</i>	X		X
<i>Pontederia korsakowii</i>	X	X	X
<i>Pontederia meyeri</i>	X		X
<i>Pontederia natans</i>			
<i>Pontederia ovalis</i>			
<i>Pontederia paniculata</i>	X		X
<i>Pontederia paradoxa</i>	X		X
<i>Pontederia parviflora</i>			
<i>Pontederia plantaginea</i>			
<i>Pontederia rotundifolia</i>	X		X
<i>Pontederia sagittata</i>	X		X
<i>Pontederia subovata</i>			
<i>Pontederia triflora</i>			
<i>Pontederia vaginalis</i>	X	X	X
<i>Pontederia valida</i>			
<b>TOTAL species</b>	<b>14/25</b>	<b>4/25</b>	<b>14/25</b>

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://archives.eppo.int/MEETINGS/2008_conferences/eichhornia_files/poster_tellez_systematics.pdf)  
[http://www.efloras.org/florataxon.aspx?flora\\_id=1&taxon\\_id=200027394](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](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

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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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