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.

Ludwigia grandiflora

(Michx.) Greuter & Burdet, 1987

Common names:
  English: water primrose, (perennial, large-flower, Uruguayan) primrose-willow
  French: jussie à grandes fleurs, ludwigie à grandes fleurs
  German: großblütiges Heusenkraut
  Dutch: grote waterteunisbloem

Last update: April 2020

General information on *Ludwigia grandiflora*

Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Eudicots	Myrtales	Onagraceae	*Ludwigia*

Species in the same genus: N = 85 [2]
Note: Zadini *et al*. [3] separated two 'chromosomal entities' within a variable species then called *Ludwigia uruguayensis*, elevating these to two closely related species: *L. grandiflora* and *L. hexapetala*, which was confirmed by morphological studies. The two species are known to hybridize.

Infra-species level: N = 0-2 [4]
Note: Some authors argue that the split between two species in the note above should be treated as subspecies instead.

Native range: [3]
South America; (northeast) Argentina, Bolivia, Paraguay, Uruguay.

Invasive range: [7, 8]
Europe (geographical):
Belgium, France, Germany, Ireland, Netherlands, Spain, Switzerland, United Kingdom.

For more detailed locality information and the most recent distribution updates, please visit:
https://www.gbif.org/species/5421039
https://gd.eppo.int/taxon/LUDUR/distribution
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R17663

Outside Europe (geographical):
Kenya, United States of America.

Morphology, biology, invasion, negative effects and remedies
For more information on *Ludwigia grandiflora* 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, no marker can reliably identify *Ludwigia grandiflora*.
Full ITS seem most promising to further investigate once additional sequence data becomes available.

Discussion

DNA markers for which *Ludwigia* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Ludwigia*. Four DNA markers were evaluated (Table 1). The now unaccepted species *L. uruguayensis* was not encountered as such in the online reference databases, the new, correct species names are already applied (above notes).

The **ITS** regions (full ITS, as well as ITS1 and ITS2 regions separately) are represented for over half of the *Ludwigia* species (Table 2). The full ITS marker appears to have potential to distinguish the *Ludwigia* species since the NJ-tree shows a high level of well-supported clustering. For *L. grandiflora* only two sequneces (both native regions) are available and clustering, separate from *L. peploides* and *L. hexapetala*. This is still insufficient to fully assess the ability of the full ITS region to identify *L. grandiflora*. Additional sequences for *L. grandiflora* (from the invasive region) and missing congeners would allow to better evaluate the potential of the ITS region to distinguish *L. grandiflora* from related species.

For both universal barcode markers **rbcL** and **matK,** as well as the **trnH-psbA** intergenic spacer many sequences are available for *L. grandiflora*, but the markers show little genetic variation among the few available species, resulting in non-clustering. The low genetic variation raises doubts about the taxonomic resolution of these markers for the genus *Ludwigia*.

For the **trnL-trnF** and **atpB-rbcL** intergenic spacers, **phyC gene** and **rpl32** no *L. grandiflora* sequence data is available and/or the markers show little genetic variation among the few represented species. Therefore, it is currently impossible to assess the ability of these markers to identify *L. grandiflora*.

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	X	X		X
full ITS	X	X			X
trnH-psbA		X	X		X

Table 2: Publicly available sequences downloaded (November 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 [7]. The list of species is limited to those members of *Ludwigia* for which at least one sequence was used in any of the NJ-trees. An 'X' indicates that at least one sequence was used in the final alignment, '(X)^2' indicates only ITS region 2 was available for analysis.

Species in genus	rbcL	matK	Full ITS	trnH-psbA
Ludwigia abyssinica			X	
Ludwigia adscendens	X	X	X	X
Ludwigia affinis			X	
Ludwigia africana				
Ludwigia alata			X	
Ludwigia albiflora				
Ludwigia alternifolia	X	X	X	X
Ludwigia anastomosans				
Ludwigia arcuata	X	X	X	
Ludwigia bonariensis			X	
Ludwigia brachyphylla				
Ludwigia brenanii				
Ludwigia brevipes			X	
Ludwigia bullata				
Ludwigia burchellii				
Ludwigia curtissii	X	X	X	
Ludwigia decurrens			X	
Ludwigia densiflora				
Ludwigia dodecandra				
Ludwigia elegans			X	
Ludwigia epilobioides				
Ludwigia erecta	X	X	X	
Ludwigia filiformis				
Ludwigia foliobracteolata				
Ludwigia glandulosa	X	X	X	X
Ludwigia grandiflora	**X**	**X**	**X**	**X**
Ludwigia hassleriana			X	
Ludwigia helminthorrhiza			X	
Ludwigia hexapetala	X	X	X	X
Ludwigia hirtella			X	
Ludwigia hookeri				
Ludwigia hyssopifolia	X		X	
Ludwigia inclinata	X		X	X
Ludwigia irwinii			X	
Ludwigia jussiaeoides			X	
Ludwigia lacustris				
Ludwigia lagunae			X	
Ludwigia lanceolata			X	
Ludwigia laruotteana				
Ludwigia latifolia				
Ludwigia leptocarpa	X	X	X	
Ludwigia linearis	X	X	X	
Ludwigia linifolia	X	X	X	

Species in genus	rbcL	matK	Full ITS	trnH-psbA
Ludwigia litoranea				
Ludwigia longifolia				
Ludwigia major			X	
Ludwigia maritima	X	X	X	
Ludwigia martii			X	
Ludwigia mexiae				
Ludwigia microcarpa	X	X	X	
Ludwigia multinervia				
Ludwigia myrtifolia			X	
Ludwigia neograndiflora			X	
Ludwigia nervosa	X	X	X	
Ludwigia octovalvis	X	X	X	X
Ludwigia ovalis	X	X	X	
Ludwigia palustris	X	X	X	X
Ludwigia peduncularis				
Ludwigia peploides	X	X	X	X
Ludwigia perennis			X	
Ludwigia perrium	X			
Ludwigia peruviana	X	X	X	X
Ludwigia pilosa	X	X	X	
Ludwigia polycarpa	X	X	X	
Ludwigia potamogeton				
Ludwigia prostrata	X	X	X	
Ludwigia pseudonarcissus			X	
Ludwigia quadrangularis			X	
Ludwigia ravenii			X	
Ludwigia repens	X	X	X	X
Ludwigia rigida			X	
Ludwigia sedoides	X		X	X
Ludwigia senegalensis				
Ludwigia sericea			X	
Ludwigia simpsonii			X	
Ludwigia spathulata			X	
Ludwigia speciosa				
Ludwigia sphaerocarpa			X	
Ludwigia stenorraphe			X	
Ludwigia suffruticosa	X	X	X	
Ludwigia tepicana				
Ludwigia tomentosa				
Ludwigia torulosa			X	
Ludwigia turbinata				
Ludwigia uruguayensis				
Ludwigia virgata	X	X	X	
TOTAL species	**29/86**	**25/86**	**57/86**	**12/86**

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
https://www.codeplantesenvahissantes.fr/fileadmin/PEE_Ressources/RTE/RE_1143_Ludwigia_grandiflora.pdf
http://www.q-bank.eu/Plants/Controlsheets/Ludwigia_grandiflora_office_guide.pdf
http://www.q-bank.eu/Plants/Factsheets/Ludwigia_grandiflora_EN.pdf
https://secure.fera.defra.gov.uk/nonnativespecies/downloadDocument.cfm?id=460

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