



# 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).

## *Ludwigia peploides*

(Kunth) P.H.Raven, 1964

### Common names:

English: floating primrose-willow, (creeping, California) water primrose, marsh purslane, clovestrip

French: jussie rampante, jussie d'Orx, ludwigie faux-péplis

German: flutende Heusenkraut

Dutch: kleine waterteunisbloem

**Last update: November 2018**



## General information on *Ludwigia peploides*

### Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Eudicots	Myrtales	Onagraceae	<i>Ludwigia</i>

### Species in the same genus: N = 86 [2]

Note: Hybrids are encountered with other *Ludwigia* species.

### Infra-species level: N = 4 [3]

Note: Four subspecies are reported: *L.p. glabrescens* (Kuntze) Raven, *L.p. montevidensis* (Sprengel) Raven, *L.p. stipulacea* (Ohwi) Raven, and *L.p. peploides* (Kunth) Raven.



### Native range: [4]

Widespread in Central, South and North America; incl. most of southern United States of America.

### Invasive range: [5–7]

#### Europe (geographical):

Belgium, France, Germany, Greece, Italy, Netherlands, Portugal, Spain, Switzerland, United Kingdom.

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

<https://www.gbif.org/species/5420991>

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R08830>

#### Outside Europe (geographical):

Asia (Cambodia, Indonesia, Japan, Malaysia, Taiwan, Thailand, Turkey), Africa (Burkina Faso, Madagascar, Mali), Oceania (Australia, New Zealand), United States of America (New York, Oregon, Washington).

### Morphology, biology, invasion, negative effects and remedies

For more information on *Ludwigia peploides* 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 reliably identify *Ludwigia peploides*. *matK* and full ITS seem most promising to further investigate once new 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). For each of them, only up to one third of *Ludwigia* species were represented in the databases (Table 2).

For universal barcode marker *matK*, there is sequence clustering for some *Ludwigia* species, including the sequences of *L. peploides*. However, all *L. peploides* sequences are from a small area in its native region in the USA, obscuring potential intra-specific variation. Additional sequences for *L. peploides* (from the invasive and other native regions) and especially from the many missing congeners would allow to better evaluate the potential of *matK* to distinguish *L. peploides* from related species.

When using the full ITS or ITS2 region a supported clustering is observed for *Ludwigia* congeners. However, too few sequences are available for the full ITS region for *L. peploides* and a potential misidentification complicates the clustering. For the shorter ITS2 fragment region, with more *L. peploides* there no clustering. Additional *L. peploides* sequences for the full ITS region, as well as sequences from the missing congeners would allow to better evaluate the potential of the ITS regions for identifications.

For universal barcode marker *rbcl* there are many sequences for *L. peploides*, both from native and multiple invasive regions, but the marker shows little genetic variation, resulting in non-clustering of *Ludwigia* species. It is not advisable to apply this marker for species identification

For the *trnH-psbA*, *trnL-trnF* and *atpB-rbcl* intergenic spacers, as well as the *phyC* and *rpl32* genes, few species are represented and/or the marker shows little genetic variation for *L. peploides* and the congeneric species. Therefore it is currently impossible to assess the ability of these markers to identify *L. peploides*.



**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, a '1' indicates only one target sequence was available.

Markers analysed	1	2	3	4	5
rbcl			X		X
matK	X	X			X
full ITS	X	X		X	X
ITS2		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 [2]. An 'X' indicates that at least one sequence was used in the final alignment.

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



Species in genus	rbcl	matK	Full ITS	trnH-psbA
<i>Ludwigia longifolia</i>				
<i>Ludwigia major</i>			X	
<i>Ludwigia maritima</i>	X	X	X	
<i>Ludwigia martii</i>			X	
<i>Ludwigia mexiae</i>				
<i>Ludwigia microcarpa</i>	X	X	X	
<i>Ludwigia multinervia</i>				
<i>Ludwigia myrtifolia</i>			X	
<i>Ludwigia neograndiflora</i>			X	
<i>Ludwigia nervosa</i>	X	X	X	
<i>Ludwigia octovalvis</i>	X	X	X	X
<i>Ludwigia ovalis</i>	X	X	X	
<i>Ludwigia palustris</i>	X	X	X	X
<i>Ludwigia peduncularis</i>				
<b><i>Ludwigia peploides</i></b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
<i>Ludwigia perennis</i>			X	
<i>Ludwigia perrium</i>	X			
<i>Ludwigia peruviana</i>	X	X	X	X
<i>Ludwigia pilosa</i>	X	X	X	
<i>Ludwigia polycarpa</i>	X	X	X	
<i>Ludwigia potamogeton</i>				
<i>Ludwigia prostrata</i>	X	X	X	
<i>Ludwigia pseudonarcissus</i>			X	
<i>Ludwigia quadrangularis</i>			X	
<i>Ludwigia ravenii</i>			X	
<i>Ludwigia repens</i>	X	X	X	X
<i>Ludwigia rigida</i>			X	
<i>Ludwigia sedoides</i>	X		X	X
<i>Ludwigia senegalensis</i>				
<i>Ludwigia sericea</i>			X	
<i>Ludwigia simpsonii</i>			X	
<i>Ludwigia spathulata</i>			X	
<i>Ludwigia speciosa</i>				
<i>Ludwigia sphaerocarpa</i>			X	
<i>Ludwigia stenorraphe</i>			X	
<i>Ludwigia suffruticosa</i>	X	X	X	
<i>Ludwigia tepicana</i>				
<i>Ludwigia tomentosa</i>				
<i>Ludwigia torulosa</i>			X	
<i>Ludwigia turbinata</i>				
<i>Ludwigia uruguayensis</i>				
<i>Ludwigia virgata</i>	X	X	X	
<b>TOTAL species</b>	<b>29/86</b>	<b>25/86</b>	<b>57/86</b>	<b>12/86</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://www.g-bank.eu/Plants/Factsheets/Ludwigia\\_peploides\\_EN.pdf](http://www.g-bank.eu/Plants/Factsheets/Ludwigia_peploides_EN.pdf)

<https://www.nvwa.nl/binaries/nvwa/documenten/plant/planten-in-de-natuur/exoten/risicobeoordelingen/factsheet-kleine-waterteunisbloem/factsheet-kleine-waterteunisbloem-nvwa-20170307.pdf>

### Picture credits

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### References

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