



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.

Hydrocotyle ranunculoides

L.f., 1782

Common names:

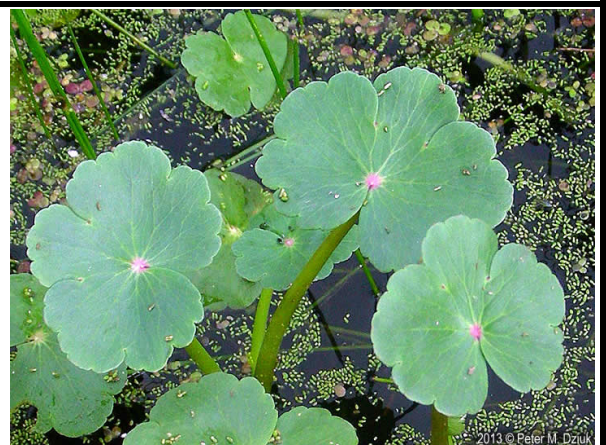
English: water-pennywort, floating (marsh)pennywort

French: hydrocotyle flottante, hydrocotyle fausse-renoncule

German: großer Wassernabel

Dutch: grote waternavel, braziliaantje, Braziliaanse of Amerikaanse waternavel

Last update: August 2018



General information on *Hydrocotyle ranunculoides*

Classification

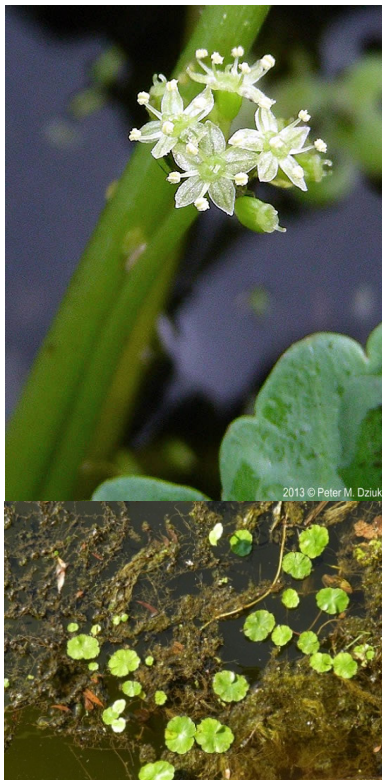
Kingdom	Phylum	Clade	Order	Family	Subfamily	Genus
Plantae	Magnoliophyta	Eudicots	Apiales	Araliaceae	Hydrocotyloideae	<i>Hydrocotyle</i>

Species in the same genus: N = 100-169 [2–6]

Note: The genus *Hydrocotyle* was formerly placed in family Apiaceae, but currently they are placed in the family Araliaceae. *Hydrocotyle* and its sister genus *Trachymene* (45 species) form a sister clade to the rest of the Araliaceae. The World Checklist of Vascular Plants accepts 169 species but is still under review.

Infra-species level: N = 2 [7]

Note: There are two varieties that are often regarded as synonyms: *H.r.* var. *genuina* and *H.r.* var. *natans*.



Native range: [8, 9]

North to South America.

Invasive range: [8, 9]

Europe (geographical):

France, Germany, Ireland, Italy, Netherlands, Poland, Spain, United Kingdom.

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

www.gbif.org/species/7978544

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R07537>

Outside Europe (geographical):

Africa (Ethiopia, Malawi, Uganda, Zambia), Australia, Israel, Japan and Yemen.

Morphology, biology, invasion, negative effects and remedies

For more information on *Hydrocotyle ranunculoides* 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* is the most promising DNA marker for the identification of *Hydrocotyle ranunculoides*. However, due to the large gap in available sequence data, it is currently impossible to fully assess the performance of this marker. In addition, *ETS* and *trnL* seem promising markers to further investigate once new sequence data becomes available.

Discussion

DNA markers for which *Hydrocotyle* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Hydrocotyle*. Five DNA markers were evaluated (Table 1). For all investigated markers, low species coverage is available in the analyses (Table 2).

For the universal plant barcode marker **matK**, some species, including *H. ranunculoides* (for which only two unique sequences are available) cluster together. However, the dataset is limited in distribution ranges, number of represented species and available sequences per species. To allow a better evaluation of the performance of this marker for species identification, the missing species (Table 2) as well as additional sequences for *H. ranunculoides* (from invasive regions) and the species now represented by one sequence only should be added.

For the ITS region(s) few sequence data is available. For the **full ITS** and **ITS1** there is only one sequence available for *H. ranunculoides*, while for **ITS2** the three available sequences do not cluster together, likely due to a misidentification. Due to the limited amount of available *H. ranunculoides* sequences, it is currently impossible to fully assess these markers.

The **ETS** region and the **trnL** gene show potential as markers to delineate between the *Hydrocotyle* species, but *H. ranunculoides* is currently not represented. Adding sequences for *H. ranunculoides* (from the invasive and native regions) and for the missing congeners would allow to better evaluate the potential of *ETS* and *trnL* to distinguish *H. ranunculoides* from related species.

The other universal plant barcode marker **rbcl** does not display enough genetic variation to distinguish among the *Hydrocotyle* species and the *H. ranunculoides* sequences do not for a cluster. This is also the case for the **psbA-trnH** intergenic spacer dataset,



which has the highest species representation and a high number of available *H. ranunculoides* sequences. This marker is reported as informative and useful by van de Wiel *et al.* [10], but their results are not recovered when including all publicly available sequence data. The low genetic structure raises doubts about the taxonomic resolution of both *rbcl* and *psbA-trnH* for this genus. Hence, it is not advisable to apply these markers to differentiate *H. ranunculoides* from other *Hydrocotyle* 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, a '1' indicates only one *H. ranunculoides* sequence was available, n/a: not applicable.

Markers analysed	1	2	3	4	5
rbcl	X	X	X	X	X
matK	X	X			X
psbA-trnH			X		X
Full ITS & ITS1	X	X	1		X
ITS2	X	X	X	X	X
trnL	X	n/a	n/a		X
ETS	X	n/a	n/a		X

Table 2: Publicly available sequences downloaded (August 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 [7]. An 'X' indicates that at least one sequence was used in the final alignment, a '1' indicates only one sequences was available.

Species in genus	rbcl	matK	psbA-trnH	full ITS & ITS1	ITS2	trnL	ETS
<i>H. alata</i>			X			X	X
<i>H. americana</i>	X	X			X		
<i>H. asterocarpa</i>						X	X
<i>H. blepharocarpa</i>			X			X	X
<i>H. bonariensis</i>	X	X	X	X	X	X	X
<i>H. bowlesioides</i>	X	X				X	
<i>H. callicarpa</i>						X	X
<i>H. conferta</i>				X	X		
<i>H. decorata</i>						X	X
<i>H. diantha</i>			X			X	X
<i>H. dimorphocarpa</i>						X	X
<i>H. hirta</i>							X
<i>H. hispidula</i>							X
<i>H. hydrophila</i>				X	X		
<i>H. javanica</i>			X	X	X		
<i>H. lemnoides</i>			X				X
<i>H. leucocephala</i>	X	X	X		X		
<i>H. maritima</i>			X	X	X		
<i>H. medicaginoides</i>						X	X
<i>H. mexicana</i>				X	X		
<i>H. modesta</i>	X	X					
<i>H. muriculata</i>							X
<i>H. nepalensis</i>	X	X	X	X	X		
<i>H. novae-zealandiae</i>			X	X	X	X	
<i>H. pedicellosa</i>		X	X			X	X
<i>H. pilifera</i>							X
<i>H. plebeya</i>			X			X	X
<i>H. ramiflora</i>			X	X	X		
<i>H. ranunculoides</i>	X	X	X	1	X		
<i>H. robusta</i>	X						
<i>H. rugulosa</i>							X
<i>H. scutellifera</i>			X			X	X
<i>H. spinulifera</i>						X	X
<i>H. setulosa</i>	X	X					
<i>H. sibthorpioides</i>	X	X	X	X	X	X	
<i>H. tetragonocarpa</i>			X			X	X
<i>H. umbellata</i>	X	X	X	X	X		
<i>H. verticillata</i>	X	X	X	X	X	X	X
<i>H. vulgaris</i>	X	X	X	X	X		
<i>H. wilfordii</i>			X		X		
<i>H. yabei</i>			X	X	X		
TOTAL species	13 /100-169	13 /100-169	22 /100-169	15 /100-169	18 /100-169	18 /100-169	21 /100-169



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

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