



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.

Lagarosiphon major

(Ridley) Moss, 1928

Common names:

English: (South African) oxygen weed, African elodea, curly waterweed

French: elodée à feuilles alternes, élodée crépue, grand lagarosiphon

German: große Wasserpest, Wechselblatt-Wasserpest, Schmalrohr, Wassergirlande

Dutch: verspreidbladige waterpest

Last update: March 2019



General information on *Lagarosiphon major*

Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Monocots	Alismatales	Hydrocharitaceae	<i>Lagarosiphon</i>

Species in the same genus: N = 9 [2, 3]

Infra-species level: N = 0

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



Native range: [2, 4]

southern Africa: Botswana, Lesotho, South Africa, Zambia, Zimbabwe.

Invasive range: [4–6]

Europe (geographical):

Austria, Belgium, France, Germany, Hungary, Ireland, Italy, Netherlands, Spain, Switzerland, United Kingdom.

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

<https://www.gbif.org/species/2865565?vnOffset=10>

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R08062>

Outside Europe (geographical):

Australia, New Zealand.

Morphology, biology, invasion, negative effects and remedies

For more information on *Lagarosiphon major* please see the references and online information listed at the end of this document.



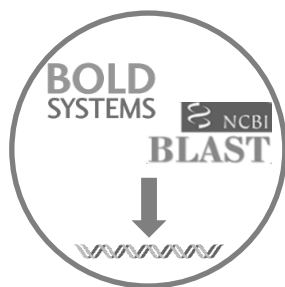
Species identification based on DNA barcodes

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.

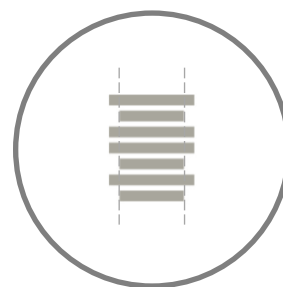
Material and Methods [1]



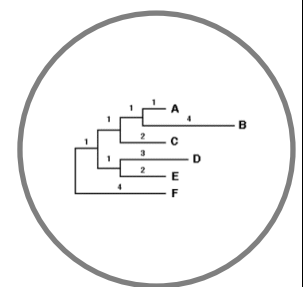
Download all sequence data available for the genus



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, *rbcl* is the most promising DNA marker for the identification of *Lagarosiphon major*. However, due to the large gap in available sequence data, it is currently impossible to fully evaluate the performance of this marker for species identification.

Discussion

DNA markers for which *Lagarosiphon* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Lagarosiphon*. Three DNA markers were evaluated (Table 1).

The *rbcl* sequences for *L. major*, from both native and invasive regions, cluster together, but only two other congeneric species are included in the analysis. The *rbcl* marker looks promising for the identification of *L. major*, however, due to the lack of sequence data for the congeneric species, it is currently impossible to fully assess the performance of this marker. The marker can currently only be used to distinguish *L. major* from two congeners, and adding sequences of missing species would allow for a better evaluation of the performance of *rbcl* for species identification.

The *trnH-psbA* intergenic spacer marker can only distinguish between *L. major* and *L. muscoides*, since only these two species are represented in the online reference databases for this marker (Table 2). To allow for a better evaluation of this marker, the missing species as well as additional sequences for the species now represented, should be added.

There is currently only one *matK* sequence available for *L. major*. The congener sequences of *L. muscoides* do not cluster, already indicating it is not advisable to apply this marker for species identification.



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 *L. major* sequence was available.

Markers analysed	1	2	3	4	5
rbcl					X
matK	X	X	1		X
trnH-psbA					X

Table 2: Publicly available sequences downloaded (March 2019) 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 [3]. An 'X' indicates that at least one sequence was used in the final alignment, a '1' indicates only one sequence was available for the final alignment.

Species in genus	rbcl	matK	trnH-psbA
<i>Lagarosiphon cordofanus</i>			
<i>Lagarosiphon hydrilloides</i>			
<i>Lagarosiphon ilicifolius</i>			
<i>Lagarosiphon madagascariensis</i>	1	1	
<i>Lagarosiphon major</i>	X	1	X
<i>Lagarosiphon muscoides</i>	X	X	X
<i>Lagarosiphon rubellus</i>			
<i>Lagarosiphon steudneri</i>			
<i>Lagarosiphon verticillifolius</i>			
TOTAL species	3/9	3/9	2/9

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://www.g-bank.eu/Plants/Factsheets/Lagarosiphon_major_EN.pdf
<https://www.environment.gov.au/biodiversity/invasive/weeds/publications/guidelines/alert/pubs/l-major.pdf>
http://www.nzpcn.org.nz/c/flora/factsheets/NZPCN_Species_3356.pdf
<http://www.nonnativespecies.org/downloadDocument.cfm?id=34>

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References

- [1] N. Smits, S. Gombeer, K. Meganck, A. Vanderheyden, Y. R. Van Bourgonie, T. Backeljau, and M. De Meyer, "Identifying IAS based on DNA barcoding using currently available sequence data: details on applied material and methods." 2019. [Online]. Available from: <https://bopco.be/output/iasfactsheets>.
- [2] J. J. Symoens and L. Triest, "Monograph of the African Genus *Lagarosiphon* Harvey (Hydrocharitaceae)" *Bull. du Jard. Bot. Natl. Belgique*, vol. 53, no. 3/4, pp. 441–4488, 1983.
- [3] "The Plant List. Version 1.1" *Published on the Internet*, 2013. [Online]. Available: <http://www.theplantlist.org/>. [Accessed: 15-Feb-2018].
- [4] Global Invasive Species Database, "Species profile: *Lagarosiphon major*" 2019.
- [5] J. van Valkenburg and R. Pot, "*Lagarosiphon major* (Ridl.) Moss (Verspreidbladige waterpest) nu ook gevestigd in de Groene Ruimte in Nederland" *Gorteria*, vol. 33, pp. 89–92, 2008.
- [6] R.-M. Lafontaine, R. C. Beudels-Jamar, T. Delsinne, and H. Robert, "Risk analysis of the Curly Waterweed *Lagarosiphon major* (Ridley) Moss. - Risk analysis report of non-native organisms in Belgium" Brussels, 2013.

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