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.

Salvinia molesta

D.S. Mitch., 1972

Common names:
  English: African payal, African pyle, Australian azolla, Kariba weed, giant azolla, water fern, giant salvinia, aquarium watermoss, salvinia moss
  French: salvinia géante, fougère d'eau
  German: Bueschelfarn, Lästiger Schwimmfarn
  Dutch: grote vlotvaren

Last update: January 2022

General information on *Salvinia molesta*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Plantae	Pteridophyta	Polypodiopsida	Salviniales	Salviniaceae	*Salvinia*

Species in the same genus: N = 10-15 [2–4]
Note: *Salvinia molesta* and three other species are part of a complex, the other species are *S. auriculata, S. biloba* and *S. herzogii*. Morphological uniformity and hybrid origins of these and other species create confusion about the number of extant species. Hybridisation between species of the genus occurs; *S. molesta* itself has intermediate characteristics that would be expected between *S. biloba* and *S. herzogii*.

Infra-species level: N = 0
Note: To our knowledge, no subspecies have been described.

Native range: [5]
South-eastern Brazil.

Invasive range: [6, 7]
Europe (geographical):
Austria, Belgium, Denmark, France, Germany, Italy, Netherlands, Portugal, Romania, Spain, Switzerland.

For more detailed locality information and the most recent distribution updates, please visit:
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R13499
https://www.gbif.org/species/5274863
https://gd.eppo.int/taxon/SAVMO/distribution

Outside Europe (geographical):
Globally widespread.

Morphology, biology, invasion, negative effects and remedies
For more information on *Salvinia molesta* 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.
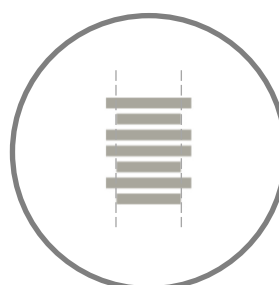
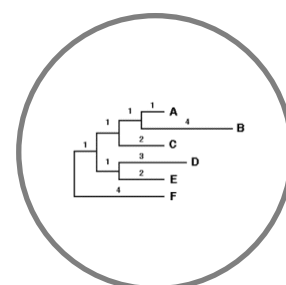**Material and Methods [1]**
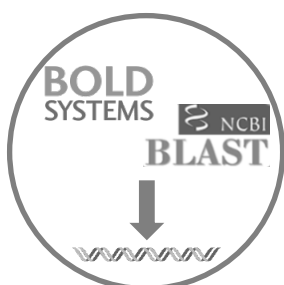
Download all sequence data available for the genus

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, atpB, rps4 and trnL are the most promising DNA markers for the identification of *S. molesta*. However, due to the large gaps in available sequence data, it is currently impossible to fully assess the reliability of these markers.

Discussion

DNA markers for which *Salvinia* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Salvinia* (as defined by [2]). Eight DNA markers were evaluated (Table 1). The species of priority to add in the dataset, since they belong a species complex, are: *S. auriculata, S. biloba, S. herzogii*. However, these were not represented in the online reference databases.

For markers **atpB**, **rps4** and **trnL**, the represented species (Table 2) have at least two sequences and clustering is supported for all. However, many and especially the most closely related species are not yet represented. Additional sequence data from *S. molesta*, covering both invasive and native range is needed as well. Hence, it is premature to decide about the ability of these DNA markers to differentiate *S. molesta* from other *Salvinia* species

For DNA marker **rbcL**, not enough sequence data is available. Two sequences of *Salviania molesta* cluster, but with little variation from a potentially mislabelled *S. cucullate*. Other species have only one sequence available.

For the intergenic spacer **psbA-trnH**, five sequences from the same study do cluster together, but two other sequence of *S. molesta* are not related and instead clustered with *S. minima*. Few congener are represented.

The genus is entirely underrepresented for markers **matK**, **ITS** and **trnG**. Therefore, it is currently impossible to assess the ability of these markers to identify *S. molesta.*

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, an '(X)' indicates the non-clustering was due solely to (a) potential misidentified or mislabelled sequence(s), a '1' indicates only one *S. molesta* sequence was available for the NJ-tree, n/a: not applicable.

Markers analysed	1	2	3	4	5
rbcL	X	X		X	X
matK	X	X	n/a		X
psbA-trnH	X	X	(X)	X	X
ITS	X	X	1		X
atpB	X	X			X
rps4	X	X			X
trnL	X	X			X
trnG	X	X			X

Table 2: Publicly available sequences downloaded (January 2022) 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 [2]. An 'X' indicates that at least one sequence was used in the final alignment, a '1' indicates only one sequences was available for the final alignment.

Species in genus	rbcL	matK	psbA-trnH	ITS	atpB	rps4	trnL	trnG
S. auriculata								
S. biloba								
S. cucullata	X	X			X	X		
S. hastata								
S. herzogii								
S. martynii								
S. minima	X		X	X	X	X	X	X
S. molesta	**X**	**X**	**X**	**1**	**X**	**X**	**X**	**X**
S. natans	X		X	X	X	X	X	
S. nuriana								
S. nymphellula								
S. oblongifolia	X			X	X	X	X	X
S. sprucei								
TOTAL species	**5 /10-15**	**2 /10-15**	**3 /10-15**	**4 /10-15**	**5 /10-15**	**5 /10-15**	**4 /10-15**	**3 /10-15**

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://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=298
https://www.daf.qld.gov.au/__data/assets/pdf_file/0003/65964/Salvinia-PP12.pdf
http://www.gri.msstate.edu/research/invspec/factsheets/4P/Giant_salvinia.pdf
https://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/gsalvinia.pdf
http://www.iucngisd.org/gisd/species.php?sc=569
https://www.fws.gov/fisheries/ans/erss/highrisk/ERSS-Salvinia-molesta-FINAL.pdf
https://keys.lucidcentral.org/keys/v3/eafrinet/weeds/key/weeds/Media/Html/Salvinia_molesta_(Kariba_Weed).htm
http://especes-exotiques-envahissantes.fr/espece/salvinia-molesta/ [FR]

Picture credits
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Page 2 (right): *Salvinia molesta* By John Forlonge [CC BY-NC-ND 2.0]

References
[1]   N. Smitz, 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]   Y. Roskov *et al.*, "Species 2000 & ITIS Catalogue of Life, 2019 Annual Checklist" *Species 2000*, 2019. [Online]. Available: www.catalogueoflife.org/annual-checklist/2019.

[3]   N. S. Nagalingum, M. D. Nowak, and K. M. Pryer, "Assessing phylogenetic relationships in extant heterosporous ferns (Salviniales), with a focus on *Pilularia* and *Salvinia*" *Bot. J. Linn. Soc.*, vol. 157, no. 4, pp. 673–685, 2008.

[4]   L. W. May, "The Genus *Salvinia*: Its Biology, Control and Economic Utilization" Fordham University, 1986.

[5]   I. W. Forno, "Native distribution of the *Salvinia auriculata* complex and keys to species identification" *Aquat. Bot.*, vol. 17, pp. 71–83, 1983.

[6]   K. Parys and A. Mikulyuk, "*Salvinia molesta* (kariba weed)" *CABI Invasive Species Compendium*, 2019. [Online]. Available: https://www.cabi.org/isc/datasheet/48447. [Accessed: 26-Mar-2020].

[7]   EPPO, "Pest Risk Analysis for *Salvinia molesta*," Paris, 2017.

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