



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) aims at developing an expertise forum to facilitate the identification of biological samples of policy concern in Belgium and Europe. The project represents part of the Belgian federal contribution to the European Research Infrastructure Consortium LifeWatch.

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. In order to implement the proposed actions, however, 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 compiling 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 to the taxonomic level stated in the EU list using DNA barcoding. 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 <http://bopco.myspecies.info/> or contact us via bopco@naturalsciences.be.

More info on the EU Regulation on http://ec.europa.eu/environment/nature/invasivealien/index_en.htm.

Alternanthera philoxeroides

(Mart.) Griseb., 1879

Common names:

English: alligator weed, pig weed

French: herbe à alligator, alternanthere

German: Alligatorkraut

Dutch: alligatorkruid

Last update: August 2020



General information on *Alternanthera philoxeroides*

Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Eudicots	Caryophyllales	Amaranthaceae	<i>Alternanthera</i>

Species in the same genus: N = 122 [2]

Note: Hybridisation with other *Alternanthera* species has been reported.

Infra-species level: N = 2 [2, 3-4]

Note: Two forms are recognised, a northern range form called *A.p.* forma *angustifolia* and *A.p.* forma *philoxeroides* from the southern native range. Six subspecies names are now regarded as synonyms.



Native range: [3]

South America; Argentina, Brazil, Columbia, Paraguay, Peru, Uruguay.

Invasive range: [5-7]

Europe (geographical):

France, Italy, Netherlands, Spain.

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

<https://www.gbif.org/species/3084923>

<http://alien.jrc.ec.europa.eu/SpeciesMapper>

<http://www.europe-aliens.org/speciesFactsheet.do?speciesId=6584#>

Outside Europe (geographical):

Widespread all over Asia, Oceania and introduced into North and Central America, South Africa.

Morphology, biology, invasion, negative effects and remedies

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



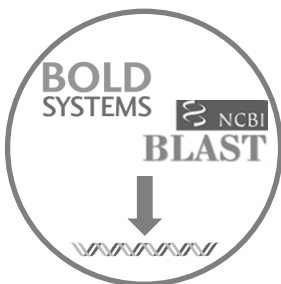
Species identification based on DNA barcodes

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. To assure correct species identifications, however, reference libraries need to include a sufficiently large number of sequences of (i) the IAS under investigation, in order to assess the intraspecific genetic divergence; (ii) the closely related species, in order to evaluate the interspecific genetic divergence; (iii) the different geographical areas covering the distribution range (native and invasive) of the IAS in order to detect potential population structure or local hybrids.

Against this background, 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.

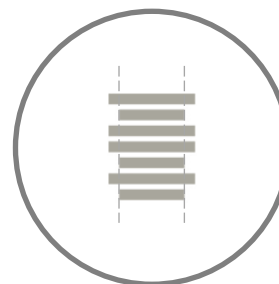
Material and Methods [1]



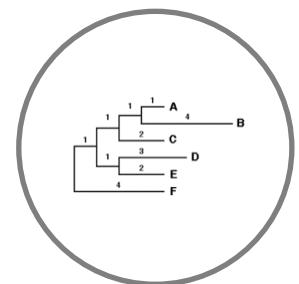
Download all sequence data available for the genus



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, the full ITS and ITS1 regions are the most promising DNA markers for the identification of *Alternanthera philoxeroides*. However, due to the large gap in available species coverage, it is currently impossible to fully evaluate the performance of the markers for species identification.

Discussion

DNA markers for which *Alternanthera* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Alternanthera*. Six DNA markers were evaluated (Table 1). The genus is poorly represented in the online reference libraries.

Concerning the full ITS or fragment regions, the *Alternanthera philoxeroides* sequences cluster together, except for two identical sequences (GenBank accession numbers KF493814 and KF493780). Both originate from of one unpublished study, and no location data could be retrieved. They are clustering with the similar looking species *A. pungens* and might involve misidentifications. If so, the full ITS and ITS1 represent promising markers for the identification of *A. philoxeroides*. To allow for a better evaluation of the performance of these markers for species identification, sequences for the missing congeners should be added to the analyses. For ITS2 two congeneric sequences cluster among *A. philoxeroides* making the marker less useful to differentiate *A. philoxeroides* from other *Alternanthera* species.

With the universal plant barcode region **matK**, the database might contain one potential misidentification (GenBank accession number MF159528). If so, the NJ-tree contains two clusters of *A. philoxeroides*. One cluster containing only South African sequences; the other cluster having sequences from China and the United States of America. Notwithstanding the fact that the *A. philoxeroides* sequences for two clusters, **matK** seems a promising DNA marker. Additional sequences for *A. philoxeroides* (from the native regions) and the missing congeners will better allow to evaluate the performance of the marker for species identification.

For the universal plant barcode region **rbcl** multiple *A. philoxeroides* sequences are available, but they do not form a cluster. In addition, the overall genetic variation between the species is very low. This low genetic variation raises doubts about the taxonomic resolution of this marker for the genus *Alternanthera*. For the **psbA-trnH** intergenic spacer few species are represented, while for the **rpl16** and **trnL** (gene and intergenic spacer) marker regions only one *A. philoxeroides* sequence is available. Therefore, it is currently impossible to assess the ability of these markers to identify the 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 unique *A. philoxeroides* sequence was available.

Markers analysed	1	2	3	4	5
rbcl		X	X	X	X
matK		X	X	X	X
Full ITS			X	X	X
ITS1			X	X	X
ITS2			X	X	X
psbA-trnH	X	X			X
rpl16	1	X	1		X
trnL + IGS	1	X	1		X

Table 2: Publicly available sequences downloaded (February 2019) 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.

Species in genus	rbcl	matK	Full ITS	ITS1	ITS2	psbA-trnH	rpl16	trnL + IGS
<i>Alternanthera albotomentosa</i>			X	X	X			
<i>Alternanthera altacruzensis</i>		X	X	X	X		X	X
<i>Alternanthera bettzickiana</i>	X							
<i>Alternanthera brasiliana</i>	X	X	X	X	X		X	X
<i>Alternanthera caracasana</i>	X	X	X	X	X		X	X
<i>Alternanthera chacoensis</i>			X	X	X		X	X
<i>Alternanthera costaricensis</i>			X	X	X		X	X
<i>Alternanthera crucis</i>			X	X	X		X	X
<i>Alternanthera elongata</i>			X	X	X		X	X
<i>Alternanthera ficoidea</i>	X	X	X	X	X		X	X
<i>Alternanthera filifolia</i>			X	X	X		X	X
<i>Alternanthera flava</i>			X	X	X		X	X
<i>Alternanthera flavescens</i>	X	X	X	X	X		X	X
<i>Alternanthera flavicoma</i>							X	X
<i>Alternanthera galapagensis</i>			X	X	X		X	X
<i>Alternanthera geniculata</i>			X	X	X		X	X
<i>Alternanthera halimifolia</i>	X	X	X	X	X	X	X	X
<i>Alternanthera kurtzii</i>			X	X	X		X	X
<i>Alternanthera laguroides</i>	X		X	X	X		X	X
<i>Alternanthera lanceolata</i>	X		X	X	X		X	X
<i>Alternanthera littoralis</i>			X	X	X		X	X
<i>Alternanthera macbridei</i>			X	X	X		X	X
<i>Alternanthera microphylla</i>		X	X	X	X		X	X
<i>Alternanthera nesiotis</i>			X	X	X		X	X
<i>Alternanthera obovata</i>			X	X	X		X	X
<i>Alternanthera olivacea</i>							X	X
<i>Alternanthera paronychioides</i>	X	X	X	X	X		X	X
<i>Alternanthera philoxeroides</i>	X	X	X	X	X	X	X	X
<i>Alternanthera porrigens</i>			X	X	X		X	X
<i>Alternanthera pubiflora</i>	X	X				X	X	X
<i>Alternanthera pungens</i>	X	X	X	X	X	X	X	X
<i>Alternanthera ramosissima</i>	X							
<i>Alternanthera serpyllifolia</i>			X	X	X		X	X
<i>Alternanthera sessilis</i>	X	X	X	X	X	X	X	X
<i>Alternanthera snodgrassii</i>			X	X	X		X	X
<i>Alternanthera vestita</i>			X	X	X		X	X
TOTAL species	14/122	12/122	31/122	31/122	31/122	5/122	33/122	33/122

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.iucngisd.org/gisd/speciesname/Alternanthera+philoxeroides>
http://www.q-bank.eu/Plants/Factsheets/Alternanthera_philoxeroides_EN.pdf
<https://www.weedbusters.org.nz/weed-information/weed-list/alligator-weed/pdf/>
<http://www.msapms.org/factsheets/Alligatorweed.pdf>
https://www.daf.qld.gov.au/_data/assets/pdf_file/0007/59569/IPA-Alligator-Weed-PP4.pdf
https://keyserver.lucidcentral.org/weeds/data/media/Html/alternanthera_philoxeroides.pdf

Picture credits

Page 1: *Alternanthera philoxeroides* By Harry Rose [CC BY 2.0]

Page 2 (left insert): Fruit with attached perianth alligatorweed (*Alternanthera philoxeroides*) By Julia Scher, Federal Noxious Weeds Disseminules, USDA APHIS PPQ, Bugwood.org [CC BY-NC 3.0 US]

Page 2 (central): Terrestrial form *Alternanthera philoxeroides* By eyeweed [CC BY-NC-ND 2.0]

Page 2 (right insert): flowerhead *Alternanthera philoxeroides* By Harry Rose [CC BY 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: <http://bopco.myspecies.info/content/invasive-alien-species-ias-factsheets>.
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- [3] A. J. Sosa, M. H. Julien, and H. A. Cordo, "New research on *Alternanthera philoxeroides* (alligator weed) in its South American native range" in *Proceedings of the XI International Symposium on Biological Control of Weeds*, J. M. Cullen, D. T. Briese, D. J. Kriticos, W. M. Lonsdale, L. Morin, and J. K. Scott, Eds. Canberra, Australia: CSIRO Entomology, 2004, pp. 180–185.
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