Identification of Invasive Alien Species using DNA barcodes

Royal Belgian Institute of Natural Sciences
Rue Vautier 29
1000 Brussels, Belgium
+32 (0)2 627 41 23

Royal Museum for Central Africa
Leuvensesteenweg 13,
3080 Tervuren, Belgium
+32 (0)2 769 58 54

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.

Asclepias syriaca

L., 1762

Common names:
English: (common, broadleaf, silky) milkweed, (Virginia) silkweed, butterfly flower, wild cotton weed, silky swallow-wort
French: herbe à coton, herbe à (la) ouate, petits cochons, asclépiade commune, asclépiade de cornut, cochons de lait, asclépiade de Syrie
German: (Syrische, gewöhnliche, gehörnte) Seidenpflanze
Dutch: zijdeplant

Last update: April 2019

General information on *Asclepias syriaca*

Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Eudicots	Gentianales	Apocynaceae	*Asclepias*

Species in the same genus: N = 100-200 [2–6]
Note: The range in estimated species number indicates many unresolved names and the need for a global revision of *Asclepias*. Hybrids between the similar looking *A. syriaca* and *A. speciosa*, and other *Asclepias* have been reported.

Infra-species level: N = 0
Note: The different subspecies and forms encountered in literature are considered synonyms.




Native range: [7, 8]
Canada and United States of America.

Invasive range: [3, 8, 9]
Europe (geographical):
Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, France, Georgia, Germany, Hungary, Italy, Lithuania, Moldova, Netherlands, Poland, Romania, Serbia, Slovenia, Spain, Sweden, Switzerland, Ukraine.

For more detailed locality information and the most recent distribution updates, please visit:
www.gbif.org/species/3170247
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R01529

Outside Europe (geographical):
Japan.

Morphology, biology, invasion, negative effects and remedies
For more information on *Asclepias syriaca* please see the references and online information listed at the end of this document.

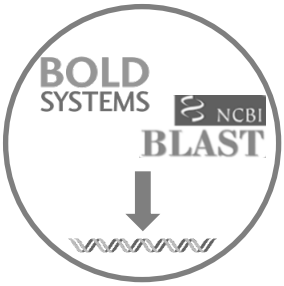
Species identification based on DNA barcodes

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.

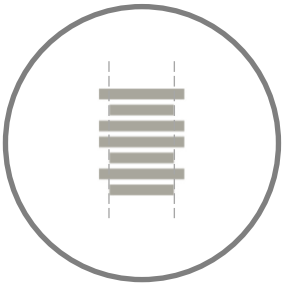
In this context, BopCo evaluated the inclusion of the IAS and their close relatives in both publicly available reference libraries BOLD (www.boldsystems.org/) and GenBank (www.ncbi.nlm.nih.gov/nuccore/) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

Material and Methods [1]

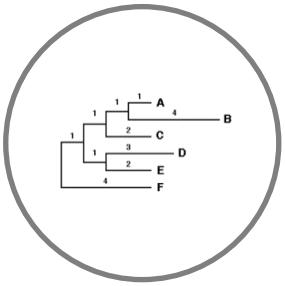


Download all sequence data available for the genus

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, the full ITS region is the most promising DNA marker for the identification of *Asclepias syriaca*. However, due to the large gap(s) in available sequence data, it is currently impossible to fully evaluate the performance of the marker for species identification.

Discussion

DNA markers for which *Asclepias* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Asclepias*. Seven DNA markers were evaluated (Table 1). Although the species number of the genus is still unresolved, a large number of species are represented and available in the analysis for most markers (Table 2), except for the ITS markers.

For the full ITS and the fragment regions, only a small number of congeneric species is represented and only few *A. syriaca* sequences are available. In the case of ITS2 there seems to be a potential misidentified sequence (GenBank accession number MG218918). If correcting for this, *A. syriaca* sequences form clusters using both the **full ITS** region, and the **ITS1** or **ITS2** fragments. However, due to the limited amount of available sequence data, it is premature to decide about the ability of these DNA markers to differentiate *A. syriaca* from other *Asclepias* species

Both universal barcode markers **rbcL** and **matK**, as well as the **trnG**, **rpl16**, and **trnL** genes, and the **trnH-psbA** intergenic spacer all show little genetic variation among the different *Asclepias* species. This low resolution in the genus is also concluded by Goyder *et al*. [10] from a subset of markers. This low genetic variation raises doubts about the taxonomic resolution of these markers for the genus *Asclepias* and their usefulness for the identification of *A. syriaca.*

For the **trnL-trnF** and **trnT-trnL** intergenic spacers few species are represented, while for **trnC-rpoB** most species (including *A. syriaca*) are represented by one sequence only. Therefore it is currently impossible to assess the ability of these markers to identify *A. syriaca.*

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.

Markers analysed	1	2	3	4	5
rbcL		X	X		X
matK		X	X		X
Full ITS	X	X			X
trnH-psbA	X	X			X
rpl16	X	X	X		X
trnG	X	X			X
trnL	X		X		X

Table 2: Publicly available sequences downloaded (April 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, an '$(X)^2$' indicates only ITS region 2 was available for analysis.

Species in genus	rbcL	matK	ITS(2)	trnH-psbA	rpl16	trnG	trnL
Asclepias albens			X				X
Asclepias albicans	X	X	X	X	X	X	X
Asclepias ameliae					X	X	
Asclepias amplexicaulis	X	X		X	X	X	X
Asclepias arenaria	X	X		X	X	X	X
Asclepias asperula	X	X		X	X	X	X
Asclepias atroviolacea	X	X		X	X	X	X
Asclepias aurea					X	X	
Asclepias auriculata	X	X		X	X	X	X
Asclepias barjoniifolia					X	X	
Asclepias boliviensis	X	X			X	X	X
Asclepias brachystephana	X	X		X	X	X	X
Asclepias brevipes			X				X
Asclepias californica	X	X	$(X)^2$		X	X	X
Asclepias candida					X	X	
Asclepias cinerea					X	X	
Asclepias circinalis	X	X		X	X	X	X
Asclepias connivens	X	X		X	X	X	X
Asclepias contrayerba	X	X			X	X	X
Asclepias cordifolia	X	X			X	X	X
Asclepias coulteri	X	X	X	X	X	X	X
Asclepias crispa			X		X	X	X
Asclepias cryptoceras	X	X		X	X	X	X
Asclepias cucullata			X		X	X	X
Asclepias cultriformis					X	X	
Asclepias curassavica	X	X	X	X	X	X	X
Asclepias curtissii	X	X		X	X	X	X
Asclepias cutleri	X	X	X	X	X	X	X
Asclepias densiflora					X	X	
Asclepias elegantula					X	X	
Asclepias emoryi	X	X		X	X	X	X
Asclepias engelmanniana	X	X		X	X	X	X
Asclepias eriocarpa	X	X	$(X)^2$	X	X	X	X
Asclepias erosa	X	X	$(X)^2$	X	X	X	X
Asclepias exaltata	X	X		X	X	X	X
Asclepias fascicularis	X	X	X	X	X	X	X
Asclepias feayi	X	X		X	X	X	X
Asclepias foliosa					X	X	
Asclepias fournieri	X	X		X	X	X	X
Asclepias fulva					X	X	
Asclepias gentryi	X	X		X	X	X	X
Asclepias gibba			X		X	X	X
Asclepias glaucescens	X	X		X	X	X	X
Asclepias grandirandii					X	X	
Asclepias hallii	X	X		X	X	X	X
Asclepias humilis					X	X	

Species in genus	rbcL	matK	ITS	trnH-psbA	rpl16	trnG	trnL
Asclepias humistrata	X	X		X	X	X	X
Asclepias hypoleuca	X	X		X	X	X	X
Asclepias inaequalis			X			X	X
Asclepias incarnata	X	X	X	X	X	X	X
Asclepias involucrata	X	X		X	X	X	X
Asclepias jorgeana	X	X		X	X	X	X
Asclepias labriformis	X	X		X	X	X	X
Asclepias lanceolata	X	X		X	X	X	X
Asclepias lanuginosa	X	X	(X)[2]	X	X	X	X
Asclepias latifolia	X	X		X	X	X	X
Asclepias lemmonii	X	X		X	X	X	X
Asclepias leptopus	X	X	X	X	X	X	X
Asclepias linaria	X	X	X	X		X	X
Asclepias linearis						X	
Asclepias longifolia	X	X	X	X	X	X	X
Asclepias lynchiana	X	X		X	X	X	X
Asclepias macropus					X	X	
Asclepias macrotis	X	X	X	X	X	X	X
Asclepias macroura	X	X			X	X	X
Asclepias macvaughii						X	
Asclepias masonii	X	X	X	X	X	X	X
Asclepias mcvaughii					X	X	
Asclepias meadii					X	X	
Asclepias melantha	X	X		X	X	X	X
Asclepias mellodora	X	X		X	X	X	X
Asclepias michauxii	X	X		X	X	X	X
Asclepias mirifica					X	X	
Asclepias multicaulis					X	X	
Asclepias nivea	X	X		X	X	X	X
Asclepias notha	X	X		X	X	X	X
Asclepias nummularia	X	X		X	X	X	X
Asclepias nyctaginifolia	X	X		X	X	X	X
Asclepias obovata	X	X		X	X	X	X
Asclepias oenotheroides	X	X		X	X	X	X
Asclepias otarioides	X	X		X	X	X	X
Asclepias ovalifolia	X	X	(X)[2]	X	X	X	X
Asclepias ovata	X	X		X	X	X	X
Asclepias pedicellata	X				X	X	
Asclepias pellucida	X	X		X	X	X	X
Asclepias perennis	X	X		X	X	X	X
Asclepias pilgeriana	X	X		X	X	X	X
Asclepias praemorsa			X		X	X	X
Asclepias pratensis	X	X		X	X	X	X
Asclepias pringlei	X	X		X	X	X	X
Asclepias prostrata	X	X		X	X	X	X
Asclepias puberula					X	X	
Asclepias pumila					X	X	
Asclepias purpurascens	X	X	(X)[2]	X	X	X	X
Asclepias quadrifolia	X	X	(X)[2]	X	X	X	X
Asclepias quinquedentata	X	X		X	X	X	X
Asclepias randii					X	X	
Asclepias rosea	X	X		X	X	X	X
Asclepias rubra	X	X		X	X	X	X
Asclepias scaposa	X	X		X	X	X	X
Asclepias schaffneri	X	X		X	X	X	X
Asclepias scheryi	X	X		X	X	X	X
Asclepias similis	X	X		X	X	X	X
Asclepias solanoana	X	X		X	X	X	X
Asclepias solstitialis					X	X	
Asclepias speciosa	X	X	(X)[2]	X	X	X	X
Asclepias standleyi					X	X	
Asclepias stathmostelmoides					X	X	
Asclepias stellifera			X				X

Species in genus	rbcL	matK	ITS	trnH-psbA	rpl16	trnG	trnL
Asclepias stenophylla	X	X		X	X	X	X
Asclepias subaphylla		X	X	X	X	X	X
Asclepias subulata	X	X	X	X	X	X	X
Asclepias subverticillata	X	X			X	X	X
Asclepias sullivantii	X	X	(X)[2]	X	X	X	X
Asclepias syriaca	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Asclepias texana					X	X	
Asclepias tomentosa	X	X		X	X	X	X
Asclepias tuberosa	X	X	X	X	X	X	X
Asclepias uncialis	X	X		X	X	X	X
Asclepias variegata	X	X		X	X	X	X
Asclepias verticillata	X	X			X	X	
Asclepias vestita	X	X			X	X	X
Asclepias vinosa					X	X	
Asclepias viridiflora	X	X	(X)[2]		X	X	X
Asclepias viridis	X	X		X	X	X	X
Asclepias viridula		X			X	X	
Asclepias virletii	X	X		X	X	X	X
Asclepias welshii	X	X		X	X	X	X
Asclepias woodii			X				X
Asclepias woodsoniana	X	X		X	X	X	X
Asclepias zanthodacryon	X	X		X	X	X	X
TOTAL species	**93 /100-200**	**94 /100-200**	**24 (34)[2] /100-200**	**84 /100-200**	**123 /100-200**	**127 /100-200**	**101 /100-200**

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://plants.ces.ncsu.edu/plants/all/asclepias-syriaca/
https://plants.usda.gov/plantguide/pdf/cs_assy.pdf
http://www.q-bank.eu/Plants/Factsheets/Asclepias_syriaca_EN.pdf
https://monarchwatch.org/bring-back-the-monarchs/milkweed/milkweed-profiles/asclepias-syriaca/

Picture credits

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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] "The Plant List. Version 1.1" *Published on the Internet*, 2013. [Online]. Available: http://www.theplantlist.org/. [Accessed: 15-Feb-2018].

[3] P. C. Bhowmik and J. D. Bandeen, "The Biology of Canadian Weeds 19. *Asclepias syriaca* L." *Can. J. Plant Sci.*, vol. 56, no. 3, pp. 579–589, Jul. 1976.

[4] J. P. Riser II, S. L. Emel, and E. H. Roalson, "Genetics and Ecological Niche Define Species Boundaries in the Dwarf Milkweed Clade (*Asclepias*: Asclepiadoideae: Apocynaceae)" *Int. J. Plant Sci.*, vol. 180, no. 2, pp. 160–177, 2018.

[5] R. E. Woodson, "The North American Species of *Asclepias* L." *Ann. Missouri Bot. Gard.*, vol. 41, no. 1, pp. 1–211, 1954.

[6] S. B. Broyles, "Hybrid Bridges To Gene Flow: A Case Study In Milkweeds (*Asclepias*)" *Evolution (N. Y).*, vol. 56, no. 10, pp. 1943–1953, 2002.

[7] K. D. Frank, "Common Milkweed" in *Ecology of Center City, Philadelphia*, Philadelphia: Fitler Square Press, 2015, pp. 173–184.

[8] C. Teeling, "*Asclepias syriaca* (Common Milkweed)" *CABI Invasive Species Compendium*, 2010. [Online]. Available: https://www.cabi.org/isc/datasheet/7249. [Accessed: 21-Feb-2018].

[9] B. Tokarska-Guzik and E. Pisarczyk, "Risk Assessment of *Asclepias syriaca*" 2001.

[10] D. Goyder, A. Nicholas, and S. Liede-Schumann, "Phylogenetic relationships in subtribe Asclepiadinae (Apocynaceae: Asclepiadoideae)" *Ann. Missouri Bot. Gard.*, vol. 94, no. 2, pp. 423–434, 2007.

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