



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.

Gunnera tinctoria

(Molina) Mirb., 1805

Common names:

English: giant rhubarb, Chilean gunnera, Chilean rhubarb, nalca, panque

French: rhubarbe géante du Chili

German: Mammutblatt, Chilensische Gunnera

Dutch: mammoetblad, gewone gunnera

Last update: April 2019



General information on *Gunnera tinctoria*

Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Eudicots	Gunnerales	Gunneraceae	<i>Gunnera</i>

Species in the same genus: N = 50-70 [2, 3]

Note: The species is unrelated to actual rhubarb, but the plant looks similar.

Gunnera is the only genus in the family. Before, multiple genus *Panke* names were used to refer to *G. tinctoria*.

Infra-species level: N = 2 [2]

Note: At least two varieties are accepted, but seldom mentioned: *G.t.* var. *meyeri* and *G.t.* var. *valdiviensis*.



Native range: [3, 4]

South America; Argentina, Bolivia, Chile, Colombia, Ecuador, Peru, Venezuela.

Invasive range: [3, 4]

Europe (geographical):

Belgium, France, Germany, Ireland, Portugal (Azores), Spain, Sweden, United Kingdom.

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

<https://www.gbif.org/species/2984306>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R06923>

Outside Europe (geographical):

New Zealand, United States of America (California).

Morphology, biology, invasion, negative effects and remedies

For more information on *Gunnera tinctoria* 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, the full ITS region is the most reliable DNA marker for the identification of *Gunnera tinctoria*. To allow for a better evaluation of the performance of this marker, the missing congeners should be added to the analyses.

Discussion

DNA markers for which *Gunnera* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Gunnera*. Five DNA markers were evaluated (Table 1). Data labelled with the previous genus name *Panke* was searched for, but not encountered. A synonym for *G. tinctoria* still found in literature and on the online databases is *G. chilensis*. Sequence files with this identification were considered the same species.

For the **full ITS** region there is well-supported clustering of the represented *Gunnera* species, including *G. tinctoria*. Yet for the latter only two sequences are available. The component **ITS1** region has the highest species coverage, but overall species clustering has lower support values than for the full ITS. The available *G. tinctoria* ITS1 sequences cluster with *G. masafuerae*, for which no full ITS sequences are available for comparison. For **ITS2**, *G. tinctoria* clusters together with many other species, due to lower genetic variation in this region. Additional sequences for *G. tinctoria*, *G. masafuerae* and the missing congeners to the full ITS region and the ITS1 dataset would allow for a better evaluation of the performance of this marker for identifications and of the influence of *G. masafuerae* on the clustering of *G. tinctoria* full ITS sequences.

For both universal barcode markers **rbcl** and **matK** few species are represented and the markers show little genetic variation among the species. Therefore, it is premature to decide about the ability of these DNA markers to differentiate *G. tinctoria* from other *Gunnera* species.

For the **rpoC1** and **cytochrome C biogenesis protein-like** genes, **trnH-psbA** and **atpB-rbcl** intergenic spacers, the **rpl16** and **trnL** gene fewer sequences are available (especially for *G. tinctoria*) and/or the markers shows little genetic variation among the different species. Therefore it is not advisable to apply these markers 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.

Markers analysed	1	2	3	4	5
rbcl	X	X	X		X
matK	X	X	X		X
full ITS	X	X			X

Table 2: Publicly available sequences downloaded (April 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 [2]. An 'X' indicates that at least one sequence was used in the final alignment, an '(X)¹' indicates only ITS region 1 was available for analysis.

Species in genus	rbcl	matK	ITS(1)
<i>Gunnera arenaria</i>			X
<i>Gunnera atropurpurea</i>			X
<i>Gunnera bolivari</i>			X
<i>Gunnera boliviana</i>			X
<i>Gunnera bracteata</i>			(X) ¹
<i>Gunnera brephogea</i>			X
<i>Gunnera cordifolia</i>	X		X
<i>Gunnera densiflora</i>			X
<i>Gunnera dentata</i>	X		X
<i>Gunnera hamiltonii</i>	X		X
<i>Gunnera herteri</i>	X		X
<i>Gunnera insignis</i>	X		X
<i>Gunnera kauaiensis</i>			X
<i>Gunnera lobata</i>	X		X
<i>Gunnera macrophylla</i>	X		X
<i>Gunnera magellanica</i>	X		X
<i>Gunnera manicata</i>	X	X	X
<i>Gunnera masafuerae</i>			(X) ¹
<i>Gunnera mexicana</i>			X
<i>Gunnera monoica</i>	X		X
<i>Gunnera morae</i>			X
<i>Gunnera peltata</i>			X
<i>Gunnera perpensa</i>	X	X	X
<i>Gunnera petaloidea</i>	X		X
<i>Gunnera pilosa</i>	X		X
<i>Gunnera prorepens</i>			X
<i>Gunnera talamancana</i>			X
<i>Gunnera tinctoria</i>	X	X	X
TOTAL species	14/ 50-70	3/ 50-70	26(28)¹/ 50-70

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

www.q-bank.eu/Plants/Factsheets/Gunnera_tinctoria_EN.pdf
<http://www.nonnativespecies.org/factsheet/downloadFactsheet.cfm?speciesId=1647>
<http://invasoras.pt/en/gallery/gunnera-tinctoria-en/>
<https://www.es.govt.nz/Document%20Library/Factsheets/Pest%20plant%20factsheets/gunnera-on-stewart-island.pdf>
[https://www.waikatoregion.govt.nz/assets/PageFiles/12732/Chilean%20Rhubarb%20\(Giant%20gunnera\)%20factsheet.pdf](https://www.waikatoregion.govt.nz/assets/PageFiles/12732/Chilean%20Rhubarb%20(Giant%20gunnera)%20factsheet.pdf)
http://www.q-bank.eu/Plants/Factsheets/Gunnera_tinctoria_EN.pdf

Picture credits

Page 1: *Gunnera tinctoria* Specimen in cultivation at the Botanical Garden, University of Copenhagen, Denmark By Dryas [CC BY-SA 3.0]
Page 2 (left): *Gunnera tinctoria* (Chilean rhubarb) Botanischer Garten Linz Austria By Isiwal [CC BY-SA 3.0 AT]
Page 2 (middle): showing young growth of *Gunnera tinctoria* in Zurich University botanical garden By kafka4prez [CC BY-SA 2.0]
Page 2 (right): Giant-rhubarb, fruits and seeds By Roger Culos [CC BY-SA 3.0]

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] "The Plant List. Version 1.1" *Published on the Internet*, 2013. [Online]. Available: www.theplantlist.org/. [Accessed: 15-Feb-2018].
- [3] C. Riches, "*Gunnera tinctoria* (giant rhubarb)" *CABI Invasive Species Compendium*, 2018. [Online]. Available: <https://www.cabi.org/isc/datasheet/107826>. [Accessed: 24-Apr-2019].
- [4] M. Gioria and B. A. Osborne, "Biological Flora of the British Isles: *Gunnera tinctoria*" *J. Ecol.*, vol. 101, pp. 243–264, 2013.

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