# 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.

# *Parthenium hysterophorus*

L., 1753

Common names:
English: Santa Maria feverfew, whitetop weed, bitterweed, carrot grass, false ragweed
French: fausse camomille, parthenium matricaire, absinthe marron
German: Karottenkraut
Dutch: schijnambrosia

**Last update: February 2019**

## General information on *Parthenium hysterophorus*

**Classification**

| Kingdom | Phylum | Clade | Order | Family | Genus |
|---|---|---|---|---|---|
| Plantae | Magnoliophyta | Eudicots | Asterales | Asteraceae | *Parthenium* |

**Species in the same genus: N = 14-16** [2–4]

**Infra-species level: N = 0** [2, 5]
Note: The in literature encountered *P. hysterophorus* var. *lyratum* is now considered as a synonym.
There is mention of a North American race that could be distinguished from a South American race, which can be elevated to specific level. These are however not further described in morphology and invasiveness.



**Native range:** [5, 6]
North to South America around the Gulf; Mexico and Caribbean islands.

**Invasive range:** [4–8]
**Europe (geographical):**
Belgium, Poland.

**For more detailed locality information and the most recent distribution updates, please visit:**
www.gbif.org/species/3086784
https://gd.eppo.int/taxon/PTNHY/distribution
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R10890

**Outside Europe (geographical)**:
Widespread across Africa, Asia and Oceania and into the northern United States of America.

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

## Species identification based on DNA barcodes

### Introduction

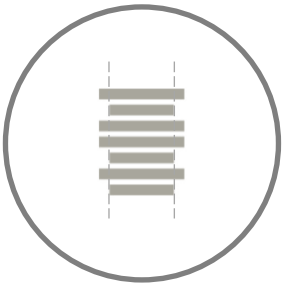
DNA barcoding is a species identification method that uses a short genetic sequence (DNA barcode) to compare an unknown sample to a database of reference sequences with known species affiliations. The underlying rationale is that the divergence of nucleotide sequences among different species is larger 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.

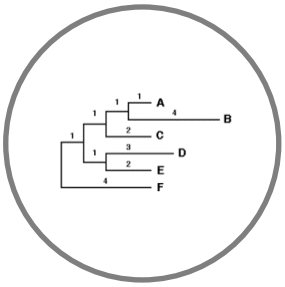
### Material and Methods [1]


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, ITS2 is the most promising DNA marker for the identification of *Parthenium hysterophorus*, followed by D35. However, additional sequences should be added to allow for a better evaluation of the performance of these markers for species identification.

### Discussion

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

For **ITS2** marker sequences are available for eight out of fourteen species (Table 2). The cluster of *P. hysterophorus* is highly supported, but only includes sequences from invasive regions. Additional sequences for *P. hysterophorus* from the native regions, as well as for the missing congeners would allow for a better evaluation of the performance of this marker.

Marker **D35** also looks promising, as the available *P. hysterophorus* sequences form a supported cluster. However, less sequences are available than for ITS2. To better allow to evaluate the performance of D35 for species identification, the missing species as well as additional sequences for the species now represented by one sequence only should be added to the analyses.

For the universal barcode marker **matK** the available *P. hysterophorus* sequences cluster together with high support, but only few *Parthenium* species are represented in the analysis. To allow for a better evaluation of the performance of this marker the missing congeners should be added to the analyses.

For the universal barcode marker **rbcL** only few species are represented and the genetic variation between the species seems low. Hence, it is not advisable to apply this marker for species identification.

With the **psbA-trnH** intergenic spacer and the smaller datasets of **trnL-trnF** intergenic spacer and **rps16-trnQ** intergenic spacers low genetic variation and a different species clustering is observed. For all three DNA markers *Parthenium confertum* clusters with *P. hysterophorus*, making it not advisable to apply these markers to identify *P. hysterophorus.*

**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 |
| **matK** | X | X | | | X |
| **ITS2** | | X | | | X |
| **D35** | X | X | | | X |
| **psbA-trnH** | | | X | | X |
| **trnL-trnF** | X | | X | | X |
| **rps16-trnQ** | X | X | X | | 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** | **ITS2** | **D35** | **psbA- trnH** | **trnL-trnF** | **rps16-trnQ** |
|---|---|---|---|---|---|---|---|
| *Parthenium alpinum* | | | X | X | X | X | X |
| *Parthenium argentatum* | X | X | X | X | X | X | X |
| *Parthenium bipinnatifidum* | | | | | | | |
| *Parthenium cineraceum* | | | X | X | X | X | X |
| *Parthenium confertum* | | | X | X | X | X | X |
| *Parthenium fruticosum* | | | | | | | |
| ***Parthenium hysterophorus*** | **X** | **X** | **X** | **X** | **X** | **X** | **X** |
| *Parthenium incanum* | | X | X | X | X | X | X |
| *Parthenium integrifolium* | X | X | X | X | X | X | X |
| *Parthenium ligulatum* | | | X | X | X | X | X |
| *Parthenium parviceps* | | | | | | | |
| *Parthenium rollinsianum* | | | | | | | |
| *Parthenium schottii* | | | | | | | |
| *Parthenium tomentosum* | | | | | | | |
| **TOTAL species** | **3/14** | **4/14** | **8/14** | **8/14** | **8/14** | **8/14** | **8/14** |

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.q-bank.eu/Plants/Factsheets/Parthenium_hysterophorus_EN.pdf
http://www.q-bank.eu/Plants/lookalikes/Parthenium/Parthenium.HTML
http://www.iucngisd.org/gisd/speciesname/Parthenium+hysterophorus
https://denr.nt.gov.au/data/assets/pdf_file/0017/404306/Parthenium-ID-sheet-2017.pdf
https://www.environment.gov.au/biodiversity/invasive/weeds/publications/guidelines/wons/pubs/p-hysterophorus.pdf
http://keys.lucidcentral.org/keys/v3/eafrinet/weeds/key/weeds/Media/Html/Parthenium_hysterophorus_(Parthenium_Weed).htm#Similar%20species

### Picture credits

Page 1: Santa Maria Feverfew (*Parthenium hysterophorus*) By Mary Keim [CC BY-NC-SA 2.0]
Page 2 (left): *Parthenium hysterophorus* plant with flowers (Central Queensland, Australia) By Ethel Aardvark [CC BY 3.0]
Page 2 (central): *Parthenium hysterophorus* By Jason Sharp [CC BY-NC-SA 2.0]
Page 2 (right): *Parthenium hysterophorus*, roadside India By Yercaud Elango [CC BY-SA 4.0]

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

[3] J. L. Strother, "*Parthenium*" in *Flora of North America*, Published., Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA: eFloras, 2006.

[4] C. Lakshmi and C. Srinivas, "*Parthenium*: A wide angle" *Indian J Dermatol Venereol Leprol*, vol. 73, no. 5, pp. 296–306, 2007.

[5] S. Adkins and A. Shabbir, "Biology, ecology and management of the invasive parthenium weed (*Parthenium hysterophorus* L.)" *Pest Manag. Sci.*, vol. 70, no. 7, pp. 1023–1029, 2014.

[6] S. Brunel, D. Panetta, G. Fried, D. Kriticos, R. Prasad, A. O. Lansink, A. Shabbir, and T. Yaacoby, "Preventing a new invasive alien plant from entering and spreading in the Euro-Mediterranean region: the case study of *Parthenium hysterophorus*" *EPPO Bull.*, vol. 44, no. 3, pp. 479–489, Dec. 2014.

[7] A. A. Bajwa, B. S. Chauhan, M. Farooq, A. Shabbir, and S. W. Adkins, "What do we really know about alien plant invasion? A review of the invasion mechanism of one of the world's worst weeds" *Planta*, vol. 244, no. 1, pp. 39–57, Jul. 2016.

[8] C. Parker, A. Shabbir, and J. Vélez-Gavilán, "*Parthenium hysterophorus* (parthenium weed)" *CABI Invasive Species Compendium*, 2018. [Online]. Available: https://www.cabi.org/ISC/datasheet/45573. [Accessed: 30-Jan-2019].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