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

natural sciences .be

AFRICA museum

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

Gymnocoronis spilanthoides

DC., 1838

Common names: water snowball
  English: Senegal teaplant, temple plant, costata, spade-leaf plant, waterball, water snowball
  French: faux hygrophile
  German: falsche Wasserfreund
  Dutch: smalle theeplant

Last update: January 2022

General information on *Gymnocoronis spilanthoides*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Plantae	Magnoliophyta	Magnoliopsida	Asterales	Asteraceae	*Gymnocoronis*

Species in the same genus: N = 2 [2–5]
Note: We follow the classification of Tippery *et al.* (2014) and Ardenghi *et al.* (2016) where most species names still encountered on The Plant List and other outdated sources are considered synonyms of either *Gymnocoronis spilanthoides* or *G. latifolia*.

Infra-species level: N = 3 [4, 6, 7]
Note: Three varieties are encountered in literature; *G.* var. *spilanthoides*, *G.* var. *attenuata* and *G.* var. *subcordata*.

Native range: [7, 8]
Argentina, Bolivia, Brazil, Mexico, Paraguay, Peru, Uruguay.

Invasive range: [3, 4, 6, 9]
Europe (geographical):
Italy, Hungary, Netherlands.

For more detailed locality information and the most recent distribution updates, please visit:
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R06930
https://www.gbif.org/species/6063677
https://gd.eppo.int/taxon/GYNSP/distribution
https://www.verspreidingsatlas.nl/10419

Outside Europe (geographical):
Australia, China, India, Japan, New Zealand, Taiwan.

Morphology, biology, invasion, negative effects and remedies
For more information on *Gymnocoronis spilanthoides* 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.

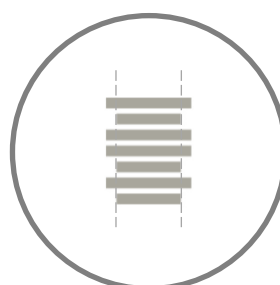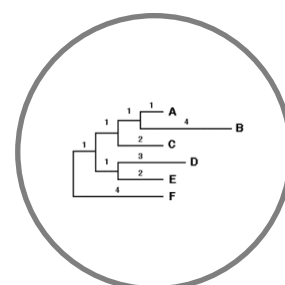**Material and Methods [1]**
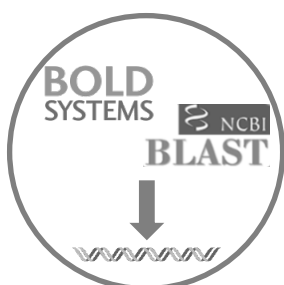
Download all sequence data available for the genus

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, no marker can reliably identify *Gymnocoronis spilanthoides* from the one other species in the genus.
MatK seems most promising to further investigate once new sequence data of the missing species becomes available.

Discussion

DNA markers for which *Gymnocoronis* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Gymnocoronis* (as defined by [4, 5]). Three DNA markers were evaluated (Table 1). Species of the closest genus *Adenostemma* are also included in the analyses as *G. spilanthoides* has synonyms with this genus name. No sequence data could be retrieved from the online databases for the only other species in the genus. Variety names, as often used in horticulture, were not found in sequence data description.

In the NJ-tree for **matK**, *G. spilanthoides* sequences (one native and two invasive locations) cluster together. Additional sequences and sequences from the missing congener would allow for a conclusive evaluation.

In the NJ-tree for **rbcL**, the only two sequences for *G. spilanthoides* do not cluster together, but rather with the added *Adenostemma* sequences. The genetic variation appears to be too low in this DNA marker to distinguish between the species of the two genera.

In the NJ-tree for **ITS**, the *G. spilanthoides* sequences (one native and one invasive location) cluster together, except for one (GenBank accession number MH050167) which is placed with *Adenostemma* species. This latter sequence might involve a misidentification or mislabelling. If so, ITS represents a promising DNA marker for the identification once sequence data is available for the missing 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, an '(X)' indicates the non-clustering was due solely to (a) potential misidentified or mislabelled sequence(s).

Markers analysed	1	2	3	4	5
matK	X				X
rbcL	X	X	X		X
ITS	X		(X)	X	X

Table 2: Publicly available sequences downloaded (January 2022) 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 [4, 5]. An 'X' indicates that at least one sequence was used in the final alignment.

Species in genus	matK	rbcL	ITS
G. latifolia			
G. spilanthoides	X	X	X
TOTAL species	1/2	1/2	1/2

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://gd.eppo.int/download/doc/1127_minids_GYNSP.pdf
https://www.pir.sa.gov.au/__data/assets/pdf_file/0003/232419/G_spilanthoides_June2011.pdf
http://wssa.net/wp-content/uploads/Gymnocoronis-spilanthoides.pdf
http://www.nzflora.info/factsheet/Taxon/Gymnocoronis-spilanthoides.html
https://sydneyweeds.org.au/wp-content/uploads/2016/12/Weed-Fact-Sheet-Senegal-Tea.pdf
https://www.waikatoregion.govt.nz/assets/PageFiles/3826/4283%20-%20Senegal%20Tea%20factsheet%202015_web.pdf
http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0003/345567/Senegal-tea.pdf

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Seed pictures available at https://keys.lucidcentral.org/keys/v3/daisy_fruit/key/daisy_fruit/Media/Html/Gynocoronis_spilanthoides.htm

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

[3]   "Data sheets on pests recommended for regulation: *Gymnocoronis spilanthoides*" 2017. EPPO Bulletin (2017) 0 (0), 1–5, doi: 10.1111/epp.12430

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[5]   N. P. Tippery, E. E. Schilling, J. L. Panero, D. H. Les, and C. S. Williams, "Independent Origins of Aquatic Eupatorieae (Asteraceae)" *Syst. Bot.*, vol. 39, no. 4, pp. 1217–1225, 2014.

[6]   L. Gunasekera, "*Gymnocoronis spilanthoides* (Senegal tea plant)" *CABI Invasive Species Compendium*. [Online]. Available: https://www.cabi.org/isc/datasheet/26246.

[7]   A. N. Schmidt-Lebuhn and G. Norton, "An Online Multi-Access Identification Key to the Propagules of Selected Biosecurity-Relevant Asteraceae (Daisy or Sunflower Family)" *Invasive Plant Sci. Manag.*, vol. 10, no. 2, pp. 210–211, Jun. 2017.

[8]   U.S. Fish & Wildlife Service, "Senegal Tea Plant (*Gymnocoronis spilanthoides*) Ecological Risk Screening Summary" 2019.

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