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.

Heracleum sosnowskyi

Manden., 1944

Common names:
  English: Sosnowsky's hogweed
  French: berce de Sosnowskyi
  German: Sosnowskys Bärenklau
  Dutch: Sosnowsky's berenklauw

Last update: January 2019

General information on *Heracleum sosnowskyi*

Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Eudicots	Apiales	Apiaceae	*Heracleum*

Species in the same genus: N = 50-120 [2–7]

Note: The number of species in the genus depends on the taxonomic assessment; most authors define *Heracleum* as a taxonomically complex genus, with small morphological differences between species. Many species names are still unresolved, including *H. sosnowskyi*. This is related to several botanists still treating *H. sosnowskyi* as a subtaxon of *H. mantegazzianum* or *H. pubescens*, while it is considered a full species on the EU Regulation.

Hybridization of *H. sosnowskyi* with other species in genus *Heracleum* is reported and confuses the morphological species identification further.

Infra-species level: N = 0

Note: To our knowledge, no subspecies or varieties have been described. The species itself is seen as a subtaxon, see above.

Native range: [7–9]

Armenia, Azerbaijan, Georgia, (Asian part of) Turkey.

Invasive range: [7-8, 10]

Europe (geographical):

Belarus, Denmark, Estonia, Finland, Germany, Hungary, Latvia, Lithuania, Poland, Romania, (European part of) Russia, Serbia, Slovakia, Ukraine.

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

https://www.gbif.org/species/3642949
https://gd.eppo.int/taxon/HERSO/distribution
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R07252

Outside Europe (geographical):

(Southern part of) Russia.

Morphology, biology, invasion, negative effects and remedies

For more information on *Heracleum sosnowskyi* 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.
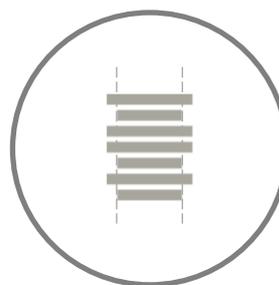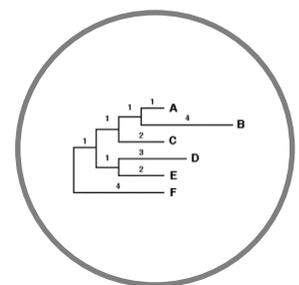
Material and Methods [1]
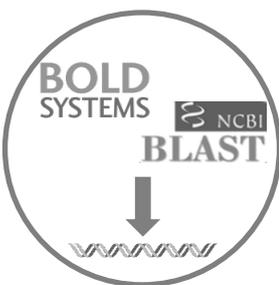
| Download all sequence data available for the genus | Filtering the data and selecting 'promising' markers | Aligning and trimming of the sequences | Building Neighbour-Joining tree with Bootstrap support |

Conclusion

Based on the present evaluation of the available sequence data, no marker can reliably identify *Heracleum sosnowskyi*. However, ETS and ITS seem promising to further investigate once new sequences of *H. persicum* and its congeners become available.

Discussion

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

In agreement with Logacheva *et al*. [12], the external transcribed spacer (**ETS**) displays more genetic variation than most other investigated markers. However, for 27 of the 31 represented species there is only one sequence available, while *H. sosnowskyi* is currently not represented. Because of this large number of species represented by a single sequence and because *H. sosnowskyi* and many congeners are missing from the analysis, it is currently impossible to assess the ability of ETS to identify the species. Adding sequences for *H. sosnowskyi* and for the congeners would allow to evaluate the potential of ETS to distinguish *H. sosnowskyi* from related species.

Several species form well-supported clusters in the **ITS2** and **full ITS** NJ-trees, and to lesser extend for **ITS1**. For the full ITS region, there is only one sequence available for *H. sosnowskyi* making it impossible to assess the usefulness of this marker. For both component regions, the three available *H. sosnowskyi* sequences do not cluster, hence it is not advisable to apply these markers for the identification of *H. sosnowskyi*. Since many *Heracleum* species are already represented in the ITS datasets, it might be interesting to further investigate the full ITS markers once additional *H. sosnowskyi* sequences become available.

For the universal barcode markers **rbcL** and **matK** no *H. sosnowskyi* sequences are available. In addition, these markers do not show enough genetic variation to distinguish the *Heracleum* species. Only a small number of species represented by more than two sequences form clusters, while the genus *Heracleum* is not retrieved as suggested by e.g. Downie *et al.* [4]. The low genetic variation raises doubts about the taxonomic resolution of these markers for the genus *Heracleum*.

The **psbA-trnH** intergenic spacer and **rps16** gene show little genetic variation and no *H. sosnowskyi* sequences are available, while for **trnT-trnL**, **rpl32-trnL**, **trnQ-rps16** and **rps16-trnK** intergenic spacer, **30S** ribosomal protein **S16** gene, **rpl16** intron and the **ycf2** gene only a few *Heracleum* species are represented. Therefore it is currently impossible to assess the ability of these markers to identify *H. sosnowskyi*.

Note: As an alternative to DNA barcoding, Jahodová *et al.* [13] concluded that *H. sosnowskyi* can be distinguished from the invasive congeners on the EU Regulation (i.e. *H. persicum* and *H. mantegazzianum*) using amplified fragment length polymorphisms (AFLP). This method is also PCR-based but uses differing enzyme-digested DNA lengths, rather than pairwise genetic distances to distinguish species. Visualizing the digested DNA on a gel differentiates these 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 *H. sosnowskyi* sequence was available, n/a: not applicable.

Markers analysed	1	2	3	4	5
rbcL	X	X	n/a	n/a	X
matK	X	X	n/a	n/a	X
Full ITS	X	X	1	X	X
ITS1	X	X	X		X
ITS2	X	X	X		X
ETS	X	X	n/a	n/a	X

Table 2: Publicly available sequences downloaded (January 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]. The list of species is limited to those members of *Heracleum* for which at least one sequence was used in any of the NJ-trees. An 'X' indicates that at least one sequence was used in the final alignment.

Species in genus	rbcL	matK	ETS	Full ITS	ITS1	ITS2
Heracleum aconitifolium			X		X	X
Heracleum afghanicum				X	X	X
Heracleum alpinum				X	X	X
Heracleum amanum				X	X	X
Heracleum anisactis					X	X
Heracleum antasiaticum				X	X	X
Heracleum apiifolium			X	X	X	X
Heracleum argaeum				X	X	X
Heracleum asperum				X	X	X
Heracleum austriacum			X	X	X	X
Heracleum bhutanicum				X	X	X
Heracleum bivittatum	X	X	X	X	X	X
Heracleum cachemiricum				X	X	X
Heracleum candicans	X	X	X	X	X	X
Heracleum carpaticum				X	X	X
Heracleum chorodanum			X	X	X	X
Heracleum crenatifolium				X	X	X
Heracleum cyclocarpum					X	X
Heracleum dissectifolium	X	X	X	X	X	X
Heracleum dissectum	X	X	X	X	X	X
Heracleum dulce	X					
Heracleum elgonense				X	X	X
Heracleum fargesii	X	X	X	X	X	X
Heracleum forrestii	X	X	X	X	X	X
Heracleum franchetii	X	X	X	X	X	X

	rbcL	matK	ETS	Full ITS	ITS1	ITS2
Heracleum freynianum				X	X	X
Heracleum grandiflorum				X	X	X
Heracleum grossheimii				X	X	X
Heracleum hemsleyanum	X	X	X	X	X	X
Heracleum henryi	X	X	X	X	X	X
Heracleum hookerianum				X	X	X
Heracleum humile					X	X
Heracleum inexpectatum				X	X	X
Heracleum kingdonii	X	X	X	X	X	X
Heracleum lasiopetalum			X		X	X
Heracleum lehmannianum				X	X	X
Heracleum leskovii				X	X	X
Heracleum ligusticifolium			X	X	X	X
Heracleum mantegazzianum	X	X	X	X	X	X
Heracleum marashicum					X	X
Heracleum maximum	X	X	X	X	X	X
Heracleum millefolium	X	X	X	X	X	X
Heracleum minimum			X	X	X	X
Heracleum moellendorffii	X	X	X	X	X	X
Heracleum nyalamense	X	X	X	X	X	X
Heracleum obtusifolium		X	X	X	X	X
Heracleum olgae			X	X	X	X
Heracleum oreocharis	X	X		X	X	X
Heracleum orphanidis				X	X	X
Heracleum osseticum					X	X
Heracleum paphlagonicum			X		X	X
Heracleum pastinaca					X	X
Heracleum pastinacifolium			X	X	X	X
Heracleum persicum	X	X		X	X	X
Heracleum peshmenianum					X	X
Heracleum platytaenium				X	X	X
Heracleum ponticum				X	X	X
Heracleum pubescens				X	X	X
Heracleum rapula	X	X		X	X	X
Heracleum rawianum					X	X
Heracleum rechingeri				X	X	X
Heracleum rigens			X	X	X	X
Heracleum roseum				X	X	X
Heracleum scabridum	X	X		X	X	X
Heracleum scabrum				X	X	X
Heracleum schelkovnikovii			X	X	X	X
Heracleum sibiricum	X	X				X
Heracleum sosnowskyi				**X**	**X**	**X**
Heracleum souliei	X	X	X	X	X	X
Heracleum sphondylium	X	X	X	X	X	X
Heracleum stenopteroides			X	X	X	X
Heracleum stenopterum	X	X		X	X	X
Heracleum stevenii				X	X	X
Heracleum subtomentellum	X	X		X	X	X
Heracleum tiliifolium	X	X	X	X	X	X
Heracleum trachyloma				X	X	X
Heracleum transcaucasicum				X	X	X
Heracleum vicinum	X	X	X	X	X	X
Heracleum wallichii				X	X	X
Heracleum wenchuanense	X	X	X	X	X	X
Heracleum wolongense	X	X	X	X	X	X
Heracleum woodii				X	X	X
Heracleum xiaojinense	X	X	X	X	X	X
Heracleum yungningense	X	X	X	X	X	X
Heracleum yunnanense				X	X	X
TOTAL species	**31** /50-120	**31** /50-120	**37** /50-120	**72** /50-120	**83** /50-120	**84** /50-120

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

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Picture credits

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Page 2 (left): *Heracleum sosnowskyi* in Szczecin Podjuchy (NW Poland) By Krzysztof Ziarnek [CC BY-SA 3.0]
Page 2 (right): *Heracleum sosnowskyi* seeds By Krzysztof Ziarnek [CC BY-SA 4.0]

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