



# 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](mailto:bopco@naturalsciences.be).

More info on the EU Regulation on [http://ec.europa.eu/environment/nature/invasivealien/index\\_en.htm](http://ec.europa.eu/environment/nature/invasivealien/index_en.htm).

## *Lysichiton americanus*

Hultén & H.St.John, 1931

Common names:

English: (American, western, yellow) skunk cabbage

French: lysichite, faux-arum

German: Gelbe Scheinkalla, Amerikanischer Riesenaronstab, Amerikanischer Stinktierkohl

Dutch: moerasaronskelk, moeraslantaarn

Last update: November 2018



## General information on *Lysichiton americanus*

### Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Monocots	Alismatales	Araceae	<i>Lysichiton</i>

### Species in the same genus: N = 2 [2–5]

Note: Multiple misspellings exist for the genus name and epithet. The genus includes only one other species, namely *Lysichiton camtschatcensis* found in North-East Asia. Both species are used in ornamental cultivation. The two are morphologically distinct when flowering and hybridize in cultivation as *Lysichiton × hortensis*.

### Infra-species level: N = 0

Note: To our knowledge, no subspecies or varieties have been described.



### Native range: [6–8]

West coast of North America: Canada and United States of America (Alaska, California, Idaho, Montana, Oregon, Washington, Wyoming).

### Invasive range: [6, 7]

#### Europe (geographical):

Belgium, Denmark, Finland, France, Germany, Ireland, Netherlands, Norway, Sweden, Switzerland, United Kingdom.

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

<https://www.gbif.org/species/2869311>

<https://gd.eppo.int/taxon/LSYAM/distribution>

<http://bsbi.org/maps?taxonid=2cd4p9h.pex>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R08915>

#### Outside Europe (geographical):

To our knowledge, the species has not been reported in other countries.

### Morphology, biology, invasion, negative effects and remedies

For more information on *Lysichiton americanus* 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/](http://www.boldsystems.org/)) and GenBank ([www.ncbi.nlm.nih.gov/nuccore/](http://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, *trnL-trnF* is the most reliable DNA marker for the identification of *Lysichiton americanus*. To allow for a better evaluation of the performance of this marker for species identification, additional sequences for *L. americanus* from the invasive regions should be added to the analyses.

### Discussion

DNA markers for which *Lysichiton* sequences were available, were downloaded from GenBank and BOLD for all species of the genus *Lysichiton*. Four DNA markers were evaluated (Table 1). Additionally, all sequence data of the family Orontioideae members *Symplocarpus* and *Orontium* were downloaded (Table 2). No sequences from invasive populations of *L. americanus* are available. Additionally, the only congeneric species *L. camtschaticensis* is poorly represented, while sequences for the hybrids *Lysichiton × hortensis* are missing (Table 2).

The most interesting DNA marker is the **trnL** gene with **trnL-trnF** intergenic spacer. For this marker sequence data shows enough genetic variation whereby the *L. americanus* sequences cluster together. The sister species *L. camtschaticensis* is represented and clusters independently. Applying this marker in species identification should be adequate, but additional sequences for *L. americanus* from the invasive regions would allow for a better evaluation of the performance of *trnL-trnF* for species identification.

Using **ndhF** for NJ-tree analysis, the *L. americanus* sequences are found in a cluster, but with limited genetic variation, with only two sequences available per species. Additional sequences would allow for a better evaluation of the performance of this marker for identifications.

For the universal barcode markers **rbcl** and **matK** the sister species is not represented. Therefore, it is currently impossible to assess the ability of these markers to identify *L. americanus*.



**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, n/a: not applicable.

Markers analysed	1	2	3	4	5
<b>rbcl</b>		X			X
<b>matK</b>	X	X			X
<b>trnL-trnF</b>		X			
<b>ndhF</b>	X	X			

**Table 2:** Publicly available sequences downloaded (April 2020) 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.

Species in genus	rbcl	matK	trnL-trnF	ndhF
<b><i>Lysichiton americanus</i></b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
<i>Lysichiton camtschaticensis</i>			X	X
<i>Orontium aquaticum</i>	X	X	X	X
<i>Symplocarpus egorovii</i>			X	
<i>Symplocarpus foetidus</i>	X	X	X	X
<i>Symplocarpus nabekuraensis</i>			X	
<i>Symplocarpus nipponicus</i>			X	X
<i>Symplocarpus renifolius</i>			X	X
<b>TOTAL species</b>	<b>1/2</b>	<b>1/2</b>	<b>2/2</b>	<b>2/2</b>

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.g-bank.eu/Plants/Factsheets/Lysichiton\\_americanus\\_EN.pdf](http://www.g-bank.eu/Plants/Factsheets/Lysichiton_americanus_EN.pdf)  
<http://ias.biodiversity.be/species/show/13>  
<https://www.nobanis.org/globalassets/speciesinfo/l/lysichiton-americanus/lysichiton-americanus.pdf>  
<https://www.cabi.org/ISC/datasheet/31580>  
<https://waarnemingen.be/exo/be/nl/18775.pdf>

### Picture credits

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### References

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