



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



General introduction to this factsheet

The Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo) aims at developing an expertise forum to facilitate the identification of biological samples of policy concern in Belgium and Europe. The project represents part of the Belgian federal contribution to the European Research Infrastructure Consortium LifeWatch.

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. In order to implement the proposed actions, however, 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 compiling 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 to the taxonomic level stated in the EU list using DNA barcoding. For further information about the reasoning behind the applied approach and details on the materials and methods utilised, please see below and Smits *et al.* [1].

More info about BopCo on <http://bopco.myspecies.info/> or contact us via bopco@naturalsciences.be.

More info on the EU Regulation on http://ec.europa.eu/environment/nature/invasivealien/index_en.htm.

Lysichiton americanus

Hultén & H.St.John, 1931

Common names:

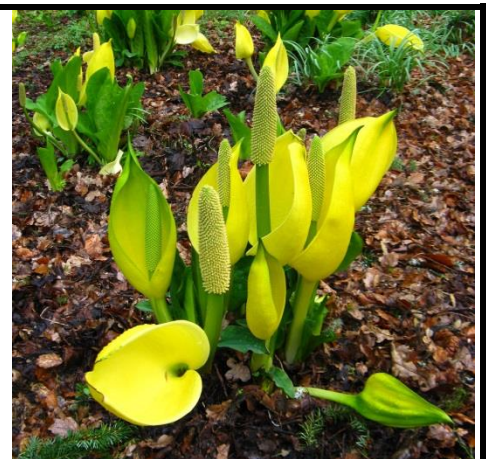
English: (American, western, yellow) skunk cabbage

French: lysichite, faux-arum

German: Gelbe Scheinkalla, Amerikanischer Riesenaronstab, Amerikanischer Stinktierkohl

Dutch: moerasaronskelk, moeraslantaarn

Last update: August 2020



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>

<http://alien.jrc.ec.europa.eu/SpeciesMapper>

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. To assure correct species identifications, however, reference libraries need to include a sufficiently large number of sequences of (i) the IAS under investigation, in order to assess the intraspecific genetic divergence; (ii) the closely related species, in order to evaluate the interspecific genetic divergence; (iii) the different geographical areas covering the distribution range (native and invasive) of the IAS in order to detect potential population structure or local hybrids.

Against this background, 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, trnL with intergenic spacer 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 represented species of the subfamily Orontioideae (as defined by Stevens [9]). Four DNA markers were evaluated (Table 1). No sequences from invasive populations of *L. americanus* are available. Additionally, the only other 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.

Markers analysed	1	2	3	4	5
rbcl		X			X
matK	X	X			X
trnL + IGS	X	X			
ndhF	X	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 subfamily	rbcl	matK	trnL + IGS	ndhF
<i>Lysichiton americanus</i>	X	X	X	X
<i>Lysichiton camtschatcensis</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
TOTAL species	3/8	3/8	8/8	6/8

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/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

Page 1: *Lysichiton americanus* (Yellow Skunk Cabbage) By S. Rae [CC BY 2.0]
Page 2 (left): *Lysichiton americanus* in botanical garden in Bydgoszcz By Krzysztof Ziarnik, Kenraiz [CC BY-SA 4.0]
Page 2 (middle): American skunk cabbage, blossom detail By Walter Siegmund [CC BY-SA 3.0]
Page 2 (top): *Lysichiton americanus*, leaves By Albert Bridge [CC BY-SA 2.0]
Page 2 (bottom): *Lysichiton americanus*, seed from berry By The Groningen Institute of Archaeology, Digital Seeds Atlas of the Netherlands [All Rights Reserved] Permission sought directly from the copyright holders.

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: <http://bopco.myspecies.info/content/invasive-alien-species-ias-factsheets>.
- [2] WCV, "World Checklist of Vascular Plants, version 2.0.," 2020. [Online]. Available: <http://wcvp.science.kew.org/>. [Accessed: 15-Apr-2020].
- [3] Y. Roskov *et al.*, "Species 2000 & ITIS Catalogue of Life, 2019 Annual Checklist," *Species 2000*, 2019. [Online]. Available: www.catalogueoflife.org/annual-checklist/2019.
- [4] T. Rotteveel, "*Lysichiton americanus* (American skunk cabbage)," *CABI Invasive Species Compendium*, 2018. [Online]. Available: <https://www.cabi.org/ISC/datasheet/31580>. [Accessed: 30-Oct-2018].
- [5] J. Armitage and B. Phillips, "A hybrid swamp lantern," *The Plantsman*, vol. 10, no. 3, pp. 155–157, 2011.
- [6] EPPO, "Data sheets on quarantine pests: *Lysichiton americanus*" *EPPO Bull.*, vol. 36, pp. 7–9, 2006.
- [7] F. Klingenstein and B. Alberternst, "NOBANIS – Invasive Alien Species Fact Sheet – *Lysichiton americanus*" *Online Database of the European Network on Invasive Alien Species - NOBANIS*, 2010. [Online]. Available: <https://www.nobanis.org/globalassets/speciesinfo/l/lysichiton-americanus/lysichiton-americanus.pdf>. [Accessed: 30-Oct-2018].
- [8] "*Lysichiton americanus*" *Flora of North America*, 2008. [Online]. Available: http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=222000246. [Accessed: 30-Oct-2018].
- [9] P. F. Stevens, "Angiosperm Phylogeny Website, Version 14," 2001. [Online]. Available: <http://www.mobot.org/MOBOT/research/APweb>. [Accessed: 14-Mar-2018].

To cite this factsheet, please use

Barcoding Facility for Organisms and Tissues of Policy Concern, 2019. Factsheet on *Lysichiton americanus*; August 2020. In: Identification of Invasive Alien Species using DNA barcodes. BopCo, Belgium. Available from: www.bopco.myspecies.info/content/invasive-alien-species-ias-factsheets, accessed on DD-MM-YYYY.

DISCLAIMER: The information represented in this factsheet has been compiled from many different sources. Every reasonable effort has been made to ensure that the material presented is accurate and reflects the current (see date last update) scientific knowledge. However, recent changes in e.g. taxonomy and distribution, or the publication of additional reference sequences may not be implemented. The views which are expressed in the "Conclusion" are those of the author(s) and have not been peer-reviewed. BopCo does not guarantee the accuracy of the data included in this factsheet. The content of the factsheet is for information only and is not intended as legal advice. BopCo may not be held responsible for the use which may be made of the information contained therein. If you should notice any issues considering the content of this factsheet, or if you would like to contribute any additional information to it, please contact us through bopco@naturalsciences.be.

