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.

Pueraria montana var. *lobata*

(Willd.) Maesen & S.M.Almeida ex Sanjappa & Predeep, 1992

Common names:
English: kudzu (bean, hemp or vine), nepalem, Japanese arrowroot
French: puéraire hirsute, koedzoe du Japon
German: Kudzu-Bohne
Dutch: kudzu, Japanse klimop

Last update: May 2019

General information on *Pueraria montana* var. *lobata*

Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plants	Magnoliophyta	Eudicots	Fabales	Fabaceae	*Pueraria*

Species in the same genus: N = 20 [2-5]
Note: *Pueraria* is seen as an 'unnatural grouping', with many species and several varieties difficult to place.
The EU Regulation on IAS refers to *Pueraria lobata*, however, the accepted designation is *Pueraria montana* var. *lobata*.

Infra-species level: N = 3 [4, 5]
Note: Apart from *P.m.* var. *lobata,* also *P.m.* var. *thomsoni* and *P.m.* var. *chinensis* are recognized within the species. The varieties differ in measurements but overlap in distribution.

Native range: [5, 6]
Southeast Asia to Australia; Bangladesh, Bhutan, Cambodia, China, India, Indonesia, Japan, Laos, Malaysia, Myanmar, New Zealand, North and South Korea, Philippines, Taiwan, Thailand, Vietnam.

Invasive range: [4, 7]
Europe (geographical):
Italy, Portugal (Azores), Russia, Switzerland, Ukraine.

For more detailed locality information and the most recent distribution updates, please visit:
https://www.gbif.org/species/2977647
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R12644
https://gd.eppo.int/taxon/PUELO/distribution

Outside Europe (geographical):
Bermuda, Brazil, Dominican Republic, Fiji, French Polynesia, Jamaica, Kiribati, Mexico, Micronesia, Niue, Palau, Panama, Paraguay, Samoa, Sierra Leone, Solomon Islands, South Africa, Tonga, United States of America.

Morphology, biology, invasion, negative effects and remedies
For more information on *Pueraria montana* var. *lobata* 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.
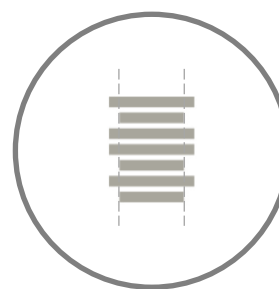
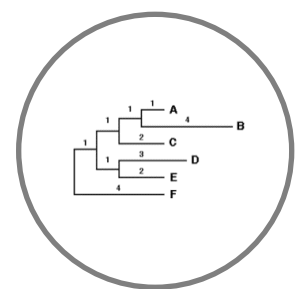### Material and Methods [1]
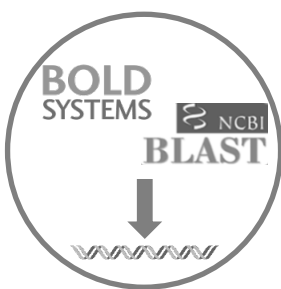
Download all sequence data available for the genus

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, a combination of rps16, trnH-psbA and 5S is the most promising tool for the identification of the variety *Pueraria montana* var. *lobata*. However, due to the large gaps in available sequence data, it is currently impossible to fully assess the performance of these markers.

Discussion

DNA markers for which *Pueraria* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Pueraria*. Seven DNA markers were evaluated (Table 1). Since the name provided on the EU list actually concerned a variety within the species *Pueraria montana*, markers should be selected that can distinguish at this taxonomic level. One variety (*P.m. chinensis*), however, is not yet represented in GenBank and BOLD.

The **5S ribosomal RNA gene** dataset only contains *P. montana* sequences, which are not all identified to the variety level. Notwithstanding the lack of sequence data for other species in the genus and the limited geographical origin of the sequences, the well supported clustering distinguishes among the varieties *P.m.* var. *lobata*, *P.m.* var. *thomsonii* and two sequences labelled just *P. montana.* This feature was also described by Sun *et al*. [8]. Because not all congeneric species are represented, additional sequences of missing species or varieties should be added to allow to fully assess the performance of this DNA marker to differentiate *P.m.* var. *lobata* from other *Pueraria* species and *P. montana* varieties.

Using sequences of **rps16** gene and **trnH-psbA** intergenic spacer, a well-supported clustering of *P.m.* var. *lobata* sequences is found, but few congeners are available (Table 2). To be able to fully evaluate the performance of these markers for identifications the missing varieties and congeners should be added to the analyses.

The **ITS2** marker can be used to distinguish *P. montana* and its varieties from *P. candollei*, *P. tuberosa* and *P. peduncularis*. With the currently available data, *P.m.* var. *lobata* cannot be distinguished from *P.m.* var. *thomsonii* or from those sequences labeled just *P. montana*. To allow for a better evaluation of this marker, the missing species (Table 2) as well as additional sequences for the species now represented, should be added.

Even though some of the other DNA markers tested (**rbcL, matK, ITS2, trnE-trnT** and AS2) had a better species representation in the final dataset (Table 2), none of them recovered *P.m.* var. *lobata* as a supported cluster and they often displayed little genetic variation. Hence, it is not advisable to apply these markers for species identification.

For the **trnT-trnL**, **trnL-trnF** and **petB-petD** intergenic spacers few species are represented and/or the marker shows little genetic variation among the different species. Therefore it is currently impossible to assess the ability of these markers to identify *P.m.* var. *lobata*.

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		X
matK		X	X		X
ITS2		X	X		X
rps16	X	X			X
trnH-psbA					X
trnE-trnT	X	X	X	X	X
AS2	X	X	X		X
5S		X			X

Table 2: Publicly available sequences downloaded (April 2019) 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 [3]. An 'X' indicates that at least one sequence was used in the final alignment.

Species in genus	rbcL	matK	ITS2	trnH-psbA	rps16	trnE-trnT	AS2	5S
Pueraria alopecuroides		X				X	X	
Pueraria bella								
Pueraria bouffordii								
Pueraria calycina		X				X	X	
Pueraria candollei	X	X	X	X	X	X	X	
Pueraria edulis						X	X	
Pueraria garhwalensis								
Pueraria grandiflora								
Pueraria imbricata		X				X	X	
Pueraria lacei							X	
Pueraria maesenii								
Pueraria montana	X	X	X	X		X	X	X
Pueraria mirifica								
Pueraria montana var. *chinensis*								
Pueraria montana* var. *lobata	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Pueraria montana var. *thomsonii*	X	X	X	X	X	X	X	X
Pueraria neocaledonica								
Pueraria pulcherrima		X			X	X	X	
Pueraria sikkimensis		X				X	X	
Pueraria tuberosa	X		X	X			X	
Pueraria xyzhuii								
TOTAL species	**4/18**	**8/18**	**4/18**	**4/18**	**3/18**	**9/18**	**11/18**	**2/18**

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.nonnativespecies.org/factsheet/downloadFactsheet.cfm?speciesId=4379
https://npgsweb.ars-grin.gov/gringlobal/taxonomydetail.aspx?314966
http://www.inspection.gc.ca/plants/seeds/testing-grading/seeds-identification/pueraria-montana/eng/1397755917428/1397755957679
https://bugwoodcloud.org/mura/mipn/assets/File/Midwest%20Invasives%20Fact%20Sheets/PDF/kudzu.pdf
https://invasivespecies.wa.gov/documents/priorities/KudzuFactSheet.pdf
https://www.invasive.org/weedcd/pdfs/wgw/kudzu.pdf
https://www.eddmaps.org/ipane/ipanespecies/vines/pueraria_montana_var_lobata.htm

Picture credits
Page 1: *Pueraria montana* var. *lobata* (leaves and flowers) By Forest Starr & Kim Starr [CC BY 3.0]
Page 2 (left): *Pueraria montana* var. *lobata* (flowers habit with ridge) By Forest Starr & Kim Starr [CC BY 3.0]
Page 2 (right, top): kudzu seeds (*Pueraria montana* var. *lobata*) By Steve Hurst, USDA NRCS PLANT Database via Bugwood.org [CC BY-NC 3.0 US]
Page 2 (right, bottom): kudzu (*Pueraria lobata*) seed pods By Robert P Collins [Public Domain]

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: http://www.theplantlist.org/. [Accessed: 15-Feb-2018].

[3]   A. N. Egan, M. Vatanparast, and W. Cagle, "Parsing polyphyletic *Pueraria*: Delimiting distinct evolutionary lineages through phylogeny" *Mol. Phylogenet. Evol.*, vol. 104, pp. 44–59, Nov. 2016.

[4]   L. J. G. Van Der Maesen, "Revision of the genus *Pueraria* DC. with some notes on *Teyleria* Backer" 1985.

[5]   W. Cage, "Parsing polyphyletic *Pueraria*: deliminiting distinct evolutionary lineages through phylogeny" East Carolina University, 2013.

[6]   "103. *Pueraria* Candolle, Ann. Sci. Nat. (Paris) 4: 97. 1825" in *Flora of China*, 2010, pp. 244–248.

[7]   N. Pasiecznik, "*Pueraria montana* var. *lobata* (kudzu)" *CABI Invasive Species Compendium*, 2018. [Online]. Available: https://www.cabi.org/isc/datasheet/45903. [Accessed: 13-May-2019].

[8]   Y. Sun, P.-C. Shaw, and K.-P. Fung, "Molecular authentication of radix *Puerariae lobatae* and radix *Puerariae thomsonii* by ITS and 5S rRNA spacer sequencing" *Biol. Pharm. Bull.*, vol. 30, no. 1, pp. 173–175, 2007.

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