



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).

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.

Koenigia polystachya

(Wall. Ex Meisn.) T. M. Schust. & Reveal

Common names:

English: Himalayan knotweed, cultivated knotweed

French: renouée de l'Himalaya, renouée à nombreux épis

German: Himalaja-Knöterich, Himalaja-Bergknöterich, Himalaya-Knöterich, Vielähriger Knöterich

Dutch: Afghaanse duizendknoop



Last update: June 2023

General information on *Koenigia polystachya*

Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Eudicots	Caryophyllales	Polygonaceae	<i>Koenigia</i>

Species in the same genus: N = 35 ²

Note: According to Schuster *et al.* ³ the following species were recently declared as synonyms of *Koenigia polystachya* and therefore are frequently (mis)used to refer to *Koenigia polystachya*: *Aconogonon polystachyum*, *Persicaria polystachya*, *Peutalis polystachyum*, *Pleuropteryrum polystachyum*, *Polygonum polystachyum*, *Reynoutria polystachya* and *Rubrivena polystachya*. CABI ⁴ refers to *Koenigia polystachya* (Himalayan knotweed) as *Persicaria wallichii*.

Infra-species level: N = 0

Note: To our knowledge no subspecies have been described.



Native range: ^{4,5}

Afghanistan, Assam, South-Central China, East Himalaya, Nepal, Pakistan, Tibet, West Himalaya

Invasive range: ⁴

Europe (geographical):

Austria, Belgium, Czechia, Denmark, France, Germany, Ireland, Italy, Liechtenstein, Netherlands, Norway, Poland, Spain, Sweden, Switzerland and United Kingdom

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

<https://www.gbif.org/species/8848208>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R11145>

<https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.120210>

Outside Europe (geographical):

Canada (British Columbia, Newfoundland), Korea, Myanmar, New Zealand and USA (Alaska, California, Massachusetts, Montana, Oregon, Washington).

Morphology, biology, invasion, negative effects and remedies

For more information on *Koenigia polystachya* 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]



Conclusion

Based on the present evaluation of the available sequence data, ITS marker is the most reliable DNA marker for the identification of *Koenigia polystachya*. To increase the accuracy of this identification, the missing congeners should be added to the analyses.

Discussion

DNA markers for which *Koenigia polystachya* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Koenigia*. Six DNA markers were evaluated (Table 1), each of which with a low species coverage (Table 2).

In NJ-trees for ITS (= ITS1 and/or ITS2) and ITS2 *Koenigia polystachya* sequences are recovered in supported clusters. Additionally, the online database (GenBank and BOLD) includes sequences from both native and invasive areas. ITS1 has only four unique sequences for *K. polystachya* and only includes the invasive area. For both ITS1 and ITS2 the missing congeners should be added to the analyses and one *K. polystachya* sequence (Accession number: GQ206266) should be considered as a potential misidentification.

Although there are **trnL** sequences for many *Koenigia* species, (Table 2), there are only two unique **trnL** sequences for *K. polystachya*, and these do not cluster together: one *K. polystachya* sequence (Accession number: JN235040) may be a misidentification as it is identical to a *K. cathayana* sequence (Accession number: JN235039). Hence, for **trnL** there is only one reliable unique sequence for *Koenigia polystachya*. Similarly, there is only one unique **MatK** sequence of *K. polystachya*. Therefore it is currently impossible to assess the ability of **trnL** and **MatK** to identify *K. polystachya*.

In the NJ-tree for **rbcl** the sequences of *Koenigia polystachya* do not form a well supported cluster. Therefore, it is seems that **rbcl** is not an appropriate marker to identify *K. polystachya*.



Table 1: Overview of the encountered issues concerning the DNA-based identification of the IAS¹: (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) Insufficient publicly available DNA sequences of the congeners to capture the inter-species divergence. An 'X' indicates that the issue was encountered, a '1' indicates only one *Koenigia polystachya* sequence was available.

Markers analysed	1	2	3	4	5
rbcl			X		X
matK	X	X	1		X
trnL	X	X	1	X	X
ITS (full)					X
ITS1	X	X			X
ITS2					X

Table 2: Publicly available sequences downloaded (June 2023) 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 follow². An 'X' indicates that at least one sequence was used in the final alignment. *Sequence present in online database but the species was not included in species list according to².

Species in genus	rbcl	MatK	trnL	ITS	ITS1	ITS2
<i>Koenigia alaskana</i>	X		X	X		X
<i>Koenigia alpina</i>	X	X	X			
<i>Koenigia campanulata</i>	X	X	X	X	X	X
<i>Koenigia cathayana</i>			X	X	X	X
<i>Koenigia chuanzangensis</i>			X	X	X	X
<i>Koenigia coriaria</i>			X			
<i>Koenigia cyanandra</i>	X	X	X	X	X	X
<i>Koenigia davisiae</i>			X			
<i>Koenigia delicatula</i>	X		X	X	X	X
<i>Koenigia divaricata</i>	X	X	X	X	X	X
<i>Koenigia fennica</i>						
<i>Koenigia fertilis</i>	X		X	X	X	X
<i>Koenigia filicaulis</i>						
<i>Koenigia forrestii</i>	X	X	X	X	X	X
<i>Koenigia hedbergii</i>						
<i>Koenigia hookeri</i>	X	X	X	X	X	X
<i>Koenigia hubertii</i> *			X	X	X	X
<i>Koenigia islandica</i>	X	X	X	X	X	X
<i>Koenigia jurii</i>				X	X	X
<i>Koenigia lichiangensis</i>	X	X	X	X	X	X
<i>Koenigia limosa</i>						
<i>Koenigia mollis</i>	X	X	X	X	X	X
<i>Koenigia monandra</i>						
<i>Koenigia nepalensis</i>	X	X	X	X	X	X
<i>Koenigia nummularifolia</i>	X	X	X	X	X	X
<i>Koenigia ocreata</i>			X			
<i>Koenigia panjutinii</i>				X	X	X
<i>Koenigia phytolaccifolia</i>			X			
<i>Koenigia pilosa</i>			X	X	X	X
<i>Koenigia polystachya</i>	X	1	1	X	X	X
<i>Koenigia relicta</i>				X	X	X
<i>Koenigia rumicifolia</i>			X			
<i>Koenigia songarica</i>	X	X	X	X	X	X
<i>Koenigia tortuosa</i>	X	X	X	X	X	X
<i>Koenigia tripterocarpa</i>			X			
<i>Koenigia weyrichii</i>						
<i>Koenigia yatagaiana</i>						
TOTAL species	17/35	14/35	25/35	21/35	20/35	21/35



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://ir.library.oregonstate.edu/concern/defaults/b27742008>

<https://gd.eppo.int/taxon/POLPS>

Picture credits

Page 1: *Koenigia polystachia* by Vinayaraj [CC BY SA 4.0]

Page 2: *Koenigia polystachya*_-Himalayan_Knotweed_ By Vinayaraj [CC BY SA 4.0]

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