



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.

Persicaria perfoliata

(L.) H.Gross, 1919

Common names:

English: Asiatic tearthumb, giant climbing tearthumb, devil's tail tearthumb, mile-a-minute weed

French: renouée perfoliée

German: durchwachsener Knöterich

Dutch: gestekelde duizendknoop

Last update: March 2019



General information on *Persicaria perfoliata*

Classification

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

Species in the same genus: N = 60-150 [2–5]

Note: The earlier valid scientific name for this species is *Polygonum perfoliatum*, which is still often encountered and used as the accepted name in many sources.

The species number is uncertain due to incomplete reclassification of species from *Polygonum*, a high phenotypic plasticity and hybridisation between the *Persicaria* species.

Infra-species level: N = 0

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



Native range: [6, 7]

Bangladesh, Bhutan, China, India, Indonesia, Japan, Korea, Malaysia, Nepal, Papua New Guinea, Philippines, Russia, Taiwan, Thailand, Vietnam.

Invasive range: [6, 8]

Europe (geographical):

Netherlands (one occurrence in 2018), Turkey.

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

<https://www.gbif.org/species/4033648>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R19287>

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

Outside Europe (geographical):

Canada, New Zealand (reported as eradicated), United States of America.

Morphology, biology, invasion, negative effects and remedies

For more information on *Persicaria perfoliata* 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]



Conclusion

Based on the present evaluation of the available sequence data, *trnL-trnF*, *ITS2* and *matK* are the most reliable DNA markers for the identification of *Persicaria perfoliata*. In order to better evaluate the performance of these markers for species identification, additional sequences for *P. perfoliata* and the missing congeners should be added.

Discussion

DNA markers for which *Persicaria* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Persicaria*. Six DNA markers were evaluated (Table 1). In the present analysis all sequence data for the old genus name *Polygonum* was included to cover potential synonyms.

A marker region including the *trnL* gene, the *trnL-trnF* intergenic spacer and a minor part of the *trnF* gene currently provides the highest species coverage of *Persicaria* (Table 2). The available *P. perfoliata* sequences form a highly supported clustering. Similar results were found when using the universal barcode marker *matK*, the full *ITS* region or the component *ITS2*, of which the *ITS2* database is more elaborate concerning species coverage (Table 2) as well as the number of available sequences per species. For all databases the number of *P. perfoliata* sequences is limited and restricted to the native region (except for *matK*). These markers can all be used for the identification of *P. perfoliata*, but additional sequences for *P. perfoliata* (from the invasive regions) and for the missing congeners would allow for a better evaluation of the performance of these markers.

With the universal barcode marker *rbcl*, the resulting NJ-tree shows little genetic variation and the available *P. perfoliata* sequences do not cluster. The same is true for the *atpB-rbcl* intergenic spacer, for which less sequence data is available. The low genetic variation raises doubts about the taxonomic resolution of these markers for the genus *Persicaria*.

For the *trnH-psbA* and *rpl32-trnL* intergenic spacer and the *LFY* gene fewer sequences are available and/or the markers show little genetic variation among the different species. Therefore it is currently impossible to assess the ability of these markers to identify *P. perfoliata*.

Mosafari *et al.* [5] and Yasmin *et al.* [9] propose alternative methods to distinguish a limited number of *Persicaria* species, but *P. perfoliata* was not included in either publication.



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 *Persicaria perfoliata* sequence was available.

Markers analysed	1	2	3	4	5
rbcl	X		X		X
matK	X				X
atpB-rbcl	X	X	X		X
trnH-psbA	X	X	1		X
ITS2		X			X
trnL-trnF	X	X			X

Table 2: Publicly available sequences downloaded (March 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] and [3]. The list of species is limited to those members of *Persicaria* 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	atpB-rbcl	trnH-psbA	ITS2	trnL-trnF
<i>Persicaria acuminata</i>	X	X		X	X	X
<i>Persicaria amphibia</i>	X	X	X	X	X	X
<i>Persicaria angustifolia</i>	X					
<i>Persicaria arifolia</i>	X	X		X	X	X
<i>Persicaria assamica</i>					X	
<i>Persicaria barbata</i>	X	X		X	X	X
<i>Persicaria bicornis</i>		X		X	X	X
<i>Persicaria bistorta</i>	X	X				
<i>Persicaria bungeana</i>	X		X		X	X
<i>Persicaria capitata</i>	X	X	X	X	X	X
<i>Persicaria careyi</i>	X	X		X	X	X
<i>Persicaria chinensis</i>	X	X	X	X	X	X
<i>Persicaria decipiens</i>	X	X		X		X
<i>Persicaria dichotoma</i>	X			X	X	
<i>Persicaria dissitiflora</i>			X		X	X
<i>Persicaria dubia</i>	X					
<i>Persicaria ferruginea</i>		X		X	X	X
<i>Persicaria filiformis</i>	X	X	X	X	X	X
<i>Persicaria foliosa</i>	X	X		X	X	X
<i>Persicaria glabra</i>	X	X		X	X	X
<i>Persicaria glacialis</i>	X	X	X	X	X	X
<i>Persicaria hastatosagittata</i>			X		X	X
<i>Persicaria hirsuta</i>	X	X		X	X	X
<i>Persicaria hispida</i>		X		X	X	X
<i>Persicaria hydropiper</i>	X	X	X	X	X	X
<i>Persicaria hydropiperoides</i>	X	X		X	X	X
<i>Persicaria japonica</i>		X	X	X	X	X
<i>Persicaria jucunda</i>		X	X		X	X
<i>Persicaria kawagoeana</i>		X		X	X	X
<i>Persicaria lanigera</i>	X	X				X
<i>Persicaria lapathifolia</i>	X	X	X	X	X	X
<i>Persicaria limbata</i>		X		X	X	X
<i>Persicaria longiseta</i>	X	X	X	X	X	X
<i>Persicaria maackiana</i>	X	X		X	X	X
<i>Persicaria macrantha</i>		X		X	X	X
<i>Persicaria maculata</i>						X
<i>Persicaria maculosa</i>	X	X	X	X	X	X
<i>Persicaria meisneriana</i>	X	X		X	X	X
<i>Persicaria microcephala</i>	X	X			X	X
<i>Persicaria minor</i>	X	X		X	X	X
<i>Persicaria mitis</i>	X	X			X	



Species in genus	rbcl	matK	atpB-rbcl	trnH-psbA	ITS2	trnL-trnF
<i>Persicaria neofiliformis</i>			X		X	X
<i>Persicaria nepalensis</i>	X	X	X	X	X	X
<i>Persicaria odorata</i>	X	X				
<i>Persicaria orientalis</i>		X	X	X	X	X
<i>Persicaria pennsylvanica</i>	X	X		X	X	X
<i>Persicaria perfoliata</i>	X	X	X	X	X	X
<i>Persicaria posumbu</i>	X	X	X	X	X	X
<i>Persicaria pubescens</i>	X	X	X	X	X	X
<i>Persicaria pulchra</i>	X	X		X		X
<i>Persicaria punctata</i>	X	X		X	X	X
<i>Persicaria puritanorum</i>		X		X	X	X
<i>Persicaria robustior</i>	X	X		X	X	X
<i>Persicaria runcinata</i>	X	X	X	X	X	X
<i>Persicaria sagittata</i>	X	X	X	X	X	X
<i>Persicaria segetum</i>		X		X	X	X
<i>Persicaria senegalensis</i>		X		X	X	X
<i>Persicaria senticosa</i>	X		X		X	X
<i>Persicaria setacea</i>		X		X	X	X
<i>Persicaria setosula</i>	X	X		X		X
<i>Persicaria sphaerocephala</i>			X		X	X
<i>Persicaria strigosa</i>	X	X				X
<i>Persicaria taquetii</i>		X		X	X	X
<i>Persicaria tenella</i>	X	X				X
<i>Persicaria thunbergii</i>	X	X	X		X	X
<i>Persicaria tinctoria</i>	X	X	X	X	X	X
<i>Persicaria virginiana</i>	X	X		X	X	X
<i>Persicaria viscofera</i>		X	X	X	X	X
<i>Persicaria viscosa</i>	X	X	X	X	X	X
<i>Persicaria vivipara</i>	X	X				
<i>Persicaria wallichii</i>	X				X	
<i>Persicaria weyrichii</i>	X					
TOTAL species	51 /60-150	59 /60-150	28 /60-150	50 /60-150	59 /60-150	62 /60-150

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/Persicaria_perfoliata_EN.pdf
<https://www.extension.purdue.edu/extmedia/fnr/fnr-481-w.pdf>
<https://www.agriculture.nh.gov/publications-forms/documents/mile-a-minute.pdf>
https://massnrc.org/pests/linkedddocuments/mamflyer_mass.pdf

Picture credits

Page 1: distinctive blue fruit *Persicaria perfoliata* (mile-a-minute weed) By Tom Potterfield [CC BY-NC-SA 2.0]
Page 2 (left): *Persicaria perfoliata* in Incheon, Korea By Dalgial [CC BY-SA 3.0]
Page 2 (right): "*Polygonum perfoliata*" By Qwert1234 [CC BY-SA 3.0]

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