



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.

Solenopsis richteri

Forel, 1909

Common names:

English: black imported fire ant

French: fourmi de feu noir

German: Schwarze Feuerameise

Dutch: zwarte vuurmier

Last update: February 2023



General information on *Solenopsis richteri*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	<i>Solenopsis</i>

Species in the same genus: N = 191^{2,3}

Note: *Solenopsis richteri* is part of the *S. saevissima* species complex and therefore difficult to distinguish morphologically and by DNA from *S. interrupta*, *S. invicta*, *S. macdonaghi*, *S. megergates*, *S. pythia*, *S. quinquecupis*, *S. saevissima* and *S. weyrauchi*.

Infra-species level: N = 0

Note: To our knowledge, no subspecies are currently recognized.



Native range:^{2,3}

Argentina, Brazil and Uruguay.

Invasive range:⁴

Europe (geographical):

To our knowledge, the species has not yet been reported in Europe.

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R20063>

<https://www.gbif.org/species/5035017>

<https://www.cabidigitallibrary.org/doi/10.1079/cabicompium.50571>

Outside Europe (geographical):

United States of America

Morphology, biology, invasion, negative effects and remedies

For more information on *Solenopsis richteri* please see the references and online information listed at the end of this document.



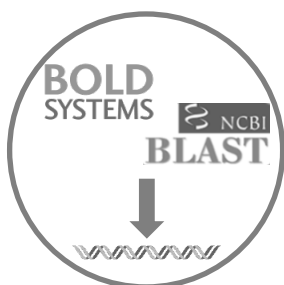
Species identification based on DNA barcodes

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.

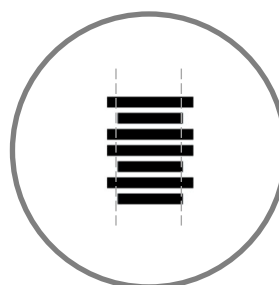
Material and Methods [1]



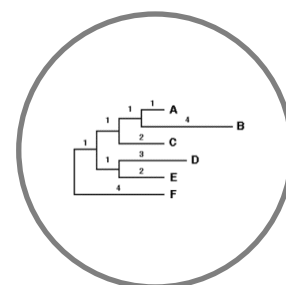
Download all sequence data available for the genus



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, COI, Gp-9 and Odorant are the most promising DNA markers for the identification of *Solenopsis richteri*. However, due to the large gaps in available sequence data, it is currently impossible to fully assess the reliability of these markers.

Discussion

DNA markers of the genus *Solenopsis* for which *S. richteri* sequences were available, were downloaded from GenBank and BOLD. Three DNA markers were evaluated (Table 1).

The occurrence of hybrids between *S. invicta* and *S. richteri* have been reported by Seltzer *et al.*⁵ (*Solenopsis invicta* x *richteri*), this poses a potential problem for identification. Therefore, making distinction between these two species is difficult and the results should be interpreted with caution.

The online database (GenBank and BOLD) contains only one **COI** sequence of *S. richteri*. Therefore it is impossible to assess the ability of this marker to identify *S. richteri*.

There are only three unique **Gp-9** sequences available for *S. richteri*, two of which form a supported cluster in the NJ tree. Both sequences originate from specimens collected in the native distribution of *S. richteri*. Hence, more *S. richteri* and other *Solenopsis* Gp-9 sequences (including those of the *S. invicta* x *S. richteri* hybrids) are needed to assess the reliability of his marker to identify *S. richteri*.



The **Odorant** sequences were generated by Gotzek *et al.* ⁶, who only dealt with *S. richteri* from its native area. This is hence a biased and incomplete *S. richteri* sample. However, these *S. richteri* sequences form a weakly supported tetratomy with *S. quinquecupis*. *S. richteri* and other *Solenopsis* sequences (including those of the *S. invicta* x *S. richteri* hybrids) are needed to assess the reliability of this marker to identify *S. richteri*.

For **28S, Elongation Factor** (eEF-1 and eEF-2), **Gp-9** and **Wnt** only few sequences are available 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 *S. richteri*.



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 species of the family are represented in the final NJ-tree. An 'X' indicates that the issue was encountered, a '1' indicates only one unique target sequence was available.

Markers analysed	1	2	3	4	5
COI	X	X	1		X
Gp-9		X	X	X	X
Odorant	X	X			X

Table 2: Publicly available sequences downloaded (February 2023) from BOLD and GenBank (including sequences extracted from mitochondrial 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, An 'X' indicates that at least one sequence was used in the final alignment. A '1' indicates only one unique target sequence was available.

Species in genus	COI	Gp-9	Odorant
<i>Solenopsis altinodis</i>	X		
<i>Solenopsis amblychila</i>		X	
<i>Solenopsis aurea</i>	X	X	
<i>Solenopsis carolinensis</i>	X		
<i>Solenopsis daguerrei</i>		X	X
<i>Solenopsis elhawagryi</i>	X		
<i>Solenopsis fugax</i>	X		
<i>Solenopsis geminata</i>	X	X	
<i>Solenopsis globularia</i>	X		
<i>Solenopsis gnoma</i>	X		
<i>Solenopsis helena</i>	X		
<i>Solenopsis iheringi</i>	X		
<i>Solenopsis indet</i>	X		
<i>Solenopsis interrupta</i>		X	X
<i>Solenopsis invicta</i>	X	X	X
<i>Solenopsis japonica</i>	X		
<i>Solenopsis krockowi</i>	X		
<i>Solenopsis latro</i>	X		
<i>Solenopsis macdonaghi</i>		X	X
<i>Solenopsis mameti</i>	X		
<i>Solenopsis megergates</i>		X	X
<i>Solenopsis molesta</i>	X		
<i>Solenopsos pergandei</i>	X		
<i>Solenopsis picta</i>	X		
<i>Solenopsis punctaticeps</i>	X		
<i>Solenopsis pusillignis</i>		X	X
<i>Solenopsis quinquecuspis</i>		X	X
<i>Solenopsis richteri</i>	1	X	X
<i>Solenopsis saevissima</i>	X	X	X
<i>Solenopsis seychellensis</i>	X		
<i>Solenopsis subterranea</i>	X		
<i>Solenopsis succinea</i>	X		
<i>Solenopsis terricola</i>	X		
<i>Solenopsis xyloni</i>	X	X	
TOTAL species	27/191	13/191	9/191

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.iucngisd.org/gisd/species.php?sc=784>

<https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-solenopsis-richteri>

https://animaldiversity.org/accounts/Solenopsis_richteri/

https://wiki.bugwood.org/Solenopsis_richteri

Picture credits

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Page 2 (right): *Solenopsis richteri*_casent0103101_Caudal View By April Nobile [CC BY-SA 3.0]

Page 2 (left): *Solenopsis richteri*_casent0103101_Dorsal View By April Nobile [CC BY-SA 3.0]

References

1. Smitz, N., Gombeer, S., Meganck, K., Vanderheyden, A., Van Bourgonie, Y.R., Backeljau, T. and De Meyer, M. Identifying IAS based on DNA barcoding using currently available sequence data: details on applied material and methods. *BopCo* (2019). [Online]. Available: <https://bopco.be/output/iasfactsheets>.
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