# 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 invicta*

Buren, 1972

Common names:
English: Red imported fire ant
French: Fourmi de feu
German: Rote Feuerameise
Dutch: Rode vuurmier

**Last update: February 2023**

## General information on *Solenopsis invicta*

**Classification**

| Kingdom | Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|---|
| Animalia | Arthropoda | Insecta | Hymenoptera | Formicidae | *Solenopsis* |

**Species in the same genus: N = 191** [2,3]

Note: *Solenopsis invicta* is part of the *S. saevissima* subcomplex [2] and therefore difficult to distinguish morphologically and by DNA from *S. interrupta, S. macdonaghi, S. megergates, S. pythia, S. quinquecuspis, S. richteri, S. saevissima* and *S. weyrauchi.*

**Infra-species level: N = 0**

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


**Native range:** [4]

South America

**Invasive range:** [4,5]

**Europe (geographical):**

Italy

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

https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R20064
https://www.gbif.org/species/5035230
https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.50569

**Outside Europe (geographical)**:

North and Central America, Australia, Hong Kong, Macau, Malaysia, Singapore, Taiwan, New Zealand, Japan, China and South Korea

**Morphology, biology, invasion, negative effects and remedies**

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

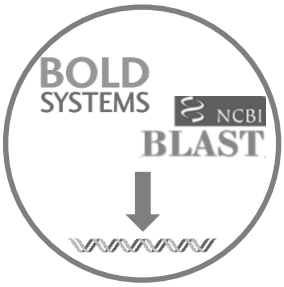
## Species identification based on DNA barcodes

### 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.

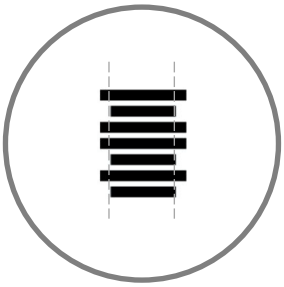
In this context, BopCo evaluated the inclusion of the IAS and their close relatives in both publicly available reference libraries BOLD (www.boldsystems.org/) and GenBank (www.ncbi.nlm.nih.gov/nuccore/) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

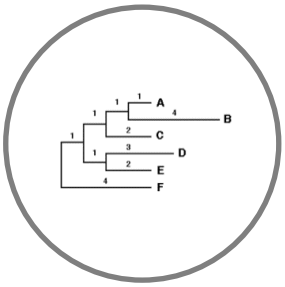
### Material and Methods [1]



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 is the most reliable DNA marker for the identification of *Solenopsis invicta*. To allow for a better evaluation of the performance of this marker for species identification, additional sequences for the missing *Solenopsis* congeners should be added.

### Discussion

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

The occurrence of hybrids between *S. invicta* and *S. richteri* have been reported by Seltzer *et al.* [6] (*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.

There are many **COI** sequences of *Solenopsis invicta* available from different areas. In the NJ tree they form two well-supported clusters. However, these two clusters cluster together with a sequence of *S. richteri* (Accession number: MF926001) with high support. Based on the potential for hybridization of *S. Invicta* and *S. richteri* [6] this could be cause for concern as distinction between the two species could be potentially problematic. One separate *S. invicta* sequence (Accession number: KP730067) is not included in this cluster and could be a potential misidentification. Overall, COI appears to be a potential marker for the identification of *S. invicta.*

Two unique **18S** sequences are available for *S. invicta*, both from the same location. They cluster together with strong support. In order to better evaluate the performance of 18S for species identification, more 18S sequences of *S. invicta* and other *Solenopsis* species should be added to the analysis.

The **Gp-9** sequences of *S. invicta* form a relatively well supported cluster. However (1) most sequences are from the same area (USA), (2) the cluster contains sequences of other species, and (3) it also compizes sequences of interspecific *S. invicta* x *S. richteri* hybrids. Similar observations hold for the *S. invicta* clustering of DNA Marker **Odorant,** although here the situation is even more complex since *S. invicta* forms three clusters, instead of one. All *S. invicta* Odorant sequences were generated by Gotzek *et al.* [7]. Gp-9 and Odorant were unable to distinguish between *S. invicta* and other members of the subcomplex *saevissima* [2] (*S. quinquecuspis, S. saevissima, S. macdonaghi, S. megergates* and *S. richteri*). Therefore, these two markers are not appropriate for species identification.

For markers **28S, Elongation Factor** (eEF-1 and eEF-2) 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. invicta.*

**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.

| Markers analysed | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| **COI** | | | | X | X |
| **18S** | X | X | | | X |
| **Gp-9** | 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 [3], An 'X' indicates that at least one sequence was used in the final alignment.

| Species in genus | **COI** | **18S** | **Gp-9** | **Odorant** |
|---|---|---|---|---|
| *Solenopsis altinodis* | X | | | |
| *Solenopsis amblychila* | | | X | |
| *Solenopsis aurea* | X | | X | |
| *Solenopsis carolinensis* | X | X | | |
| *Solenopsis daguerrei* | | | X | X |
| *Solenopsis elhawagryi* | X | | | |
| *Solenopsis fugax* | X | X | | |
| *Solenopsis geminata* | X | X | X | |
| *Solenopsis globularia* | X | | | |
| *Solenopsis gnoma* | X | | | |
| *Solenopsis helena* | X | | | |
| *Solenopsis iheringi* | X | | | |
| *Solenopsis indet* | X | | | |
| *Solenopsis interrupta* | | | X | X |
| ***Solenopsis invicta*** | **X** | **X** | **X** | **X** |
| *Solenopsis japonica* | X | | | |
| *Solenopsis krockowi* | X | | | |
| *Solenopsis latro* | X | | | |
| *Solenopsis macdonaghi* | | | X | X |
| *Solenopsis mameti* | X | X | | |
| *Solenopsis megergates* | | | X | X |
| *Solenopsis molesta* | X | X | | |
| *Solenopsis papuana* | | X | | |
| *Solenopsos pergandei* | X | | | |
| *Solenopsis picta* | X | | | |
| *Solenopsis punctaticeps* | X | | | |
| *Solenopsis pusillignis* | | | X | X |
| *Solenopsis quinquecuspis* | | | X | X |
| *Solenopsis richteri* | X | | X | X |
| *Solenopsis saevissima* | X | | X | X |
| *Solenopsis seychellensis* | X | | | |
| *Solenopsis subterranea* | X | | | |
| *Solenopsis succinea* | X | X | | |
| *Solenopsis terricola* | X | | | |
| *Solenopsis xyloni* | X | X | X | |
| **TOTAL species** | **27/191** | **9/191** | **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

https://entnemdept.ufl.edu/creatures/urban/ants/red_imported_fire_ant.htm
https://pictureinsect.com/nl/wiki/Solenopsis_invicta.html
https://www.efsa.europa.eu/en/efsajournal/pub/7998

### Picture credits

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Page 2 (left): *Solenopsis_invicta*_casent0178134_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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7. Gotzek, D., Shoemaker, D. D. W. & Ross, K. G. Molecular variation at a candidate gene implicated in the regulation of fire ant social behavior. *PLoS One* **2**, (2007).


### To cite this factsheet, please use

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