



# Identification of Invasive Alien Species using DNA barcodes

Royal Belgian Institute of Natural Sciences  
Rue Vautier 29  
1000 Brussels, Belgium  
+32 (0)2 627 41 23



Royal Museum for Central Africa  
Leuvensesteenweg 13,  
3080 Tervuren, Belgium  
+32 (0)2 769 58 54



## 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](mailto:bopco@naturalsciences.be).

More info on the EU Regulation on [http://ec.europa.eu/environment/nature/invasivealien/index\\_en.htm](http://ec.europa.eu/environment/nature/invasivealien/index_en.htm).

## *Microstegium vimineum*

(Trin.) A.Camus, 1922

### Common names:

English: Japanese stilt grass, Chinese packing grass, Nepalese browntop, flexible sea grass

French: no vernacular name

German: Japanisches Stelzengras

Dutch: Japans steltgras

**Last update: February 2019**



## General information on *Microstegium vimineum*

### Classification

Kingdom	Phylum	Clade	Order	Family	Genus
Plantae	Magnoliophyta	Monocots	Poales	Poaceae	<i>Microstegium</i>

**Species in the same genus: N = 25** [2, 3]

**Infra-species level: N = 0** [2, 3]

Note: Four varieties and another subspecies encountered in literature are now seen as synonyms of the species proper.



**Native range:** [4–6]

Asia; Bhutan, China, India, Japan, Myanmar, Nepal, Philippines, (east) Russia, South Korea, Taiwan, Thailand, Vietnam.

**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:**

[www.gbif.org/species/5289808](http://www.gbif.org/species/5289808)

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R09459>

**Outside Europe (geographical):**

Azerbaijan, Costa Rica, Georgia, (western) Russia, Turkey, United States of America (majority of Eastern coastal to inland states and Puerto Rico).

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

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



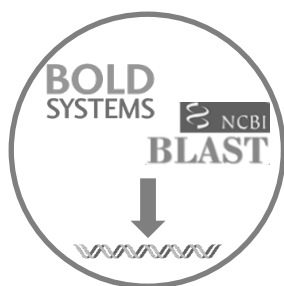
## Species identification based on DNA barcodes

### 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/](http://www.boldsystems.org/)) and GenBank ([www.ncbi.nlm.nih.gov/nuccore/](http://www.ncbi.nlm.nih.gov/nuccore/)) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

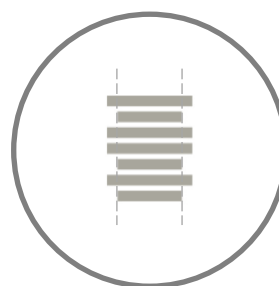
### Material and Methods [1]



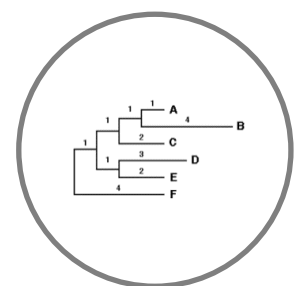
Download all sequence data available for the genus



Filtering the data and selecting 'promising'



Aligning and trimming of the sequences



Building Neighbour-Joining tree with Bootstrap support

### Conclusion

Based on the present evaluation of the available sequence data, the full ITS region is the most reliable DNA marker for the identification of *Microstegium vimineum*. To better allow the evaluation of the performance of this marker, the missing congeners and additional sequences for *M. vimineum* should be added to the analyses.

### Discussion

DNA markers for which *Microstegium* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Microstegium*. Three DNA markers were evaluated (Table 1). The genus is severely underrepresented on the online databases; most species have no sequence data available (Table 2).

The full ITS marker region and both components ITS1 and ITS2 have identical datasets to work with and result in similar NJ-trees, yet higher support values for the different clusters are found with the full marker region. The available *M. vimineum* sequences cluster with a single *M. japonicum* sequence (GenBank accession number KF163670). The latter is potentially a mislabelled *M. vimineum* sequence as there are two *M. japonicum* sequences clustering elsewhere. Additionally, one *M. vimineum* sequence (Genbank accession number EF211958) is found outside the previously mentioned *M. vimineum* cluster. This might involve a misidentification. If so, the full ITS region represents a promising DNA marker for the identification of *M. vimineum*. To allow for a better evaluation of the performance of the full ITS marker for species identification, the missing *Microstegium* species (Table 2) as well as additional vouchered sequences of *M. vimineum*, should be added.

For the universal barcode markers **rbcl** and **matK** there is few data available (Table 2) displaying little genetic variation. Therefore it is currently impossible to assess the ability of these markers to identify *M. vimineum*.

For **trnL-trnT** intergenetic spacer, **psbA-trnH** intergenetic spacer, **ndhF** gene, **rps16** gene, **trnC-petN** intergenetic spacer, **trnL** and **trnG** gene, fewer sequences or lower species representation are available. Hence, it is premature to decide about the ability of these DNA markers to differentiate *M. vimineum* from other *Microstegium* species.



**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
<b>rbcl</b>			X		X
<b>matK</b>			X		X
<b>Full ITS</b>	X	X	X	X	X

**Table 2:** Publicly available sequences downloaded (November 2018) 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]. An 'X' indicates that at least one sequence was used in the final alignment.

Species in genus	rbcl	matK	Full ITS
<i>Microstegium batangense</i>			
<i>Microstegium biaristatum</i>			
<i>Microstegium borianum</i>			X
<i>Microstegium brandisii</i>			
<i>Microstegium butuoense</i>			
<i>Microstegium delicatulum</i>			
<i>Microstegium dispar</i>			
<i>Microstegium eucnemis</i>			
<i>Microstegium falconeri</i>			
<i>Microstegium fasciculatum</i>			X
<i>Microstegium fauriei</i>			X
<i>Microstegium geniculatum</i>			X
<i>Microstegium glabratum</i>			X
<i>Microstegium japonicum</i>	X	X	X
<i>Microstegium lanceolatum</i>			
<i>Microstegium monoracemum</i>			
<i>Microstegium nudum</i>	X	X	X
<i>Microstegium petiolare</i>			
<i>Microstegium rufispicum</i>			
<i>Microstegium somae</i>			X
<i>Microstegium spectabile</i>			
<i>Microstegium stapfii</i>			
<i>Microstegium steenisii</i>			
<i>Microstegium tenue</i>			
<b><i>Microstegium vimineum</i></b>	<b>X</b>	<b>X</b>	<b>X</b>
<b>TOTAL species</b>	<b>3/25</b>	<b>3/25</b>	<b>9/25</b>

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://www.agriculture.nh.gov/publications-forms/documents/japanese-stiltgrass.pdf>

<http://www.iucngisd.org/gisd/pdf.php?sc=686>

[http://www.docs.dcnr.pa.gov/cs/groups/public/documents/document/dcnr\\_010258.pdf](http://www.docs.dcnr.pa.gov/cs/groups/public/documents/document/dcnr_010258.pdf)

[http://www.q-bank.eu/Plants/Factsheets/Microstegium\\_vimineum\\_NL.pdf](http://www.q-bank.eu/Plants/Factsheets/Microstegium_vimineum_NL.pdf) [NL]

### Picture credits

Page 1: immature Japanese stiltgrass in June By James H. Miller & Ted Bodner, Southern Weed Science Society, Bugwood.org [CC BY 3.0]

Page 2 (left): Japanese stiltgrass inflorescence By NY State IPM Program at Cornell University [CC BY 2.0]

Page 2 (right): Seeds of Japanese stiltgrass, *Microstegium vimineum* By Steve Hurst, USDA NRCS PLANTS Database [CC BY 3.0]

Page 2 (central): Japanese stiltgrass, infesting woodland, USA By Chris Evans, Illinois Wildlife Action Plan, Bugwood.org [CC BY 3.0]

### References

- [1] N. Smits, 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] E. Bisby FA, Roskov YR, Orrell TM, Nicolson D, Paglinawan LE, Bailly N, Kirk PM, Bourgoin T, Baillargeon G., "Species 2000 & ITIS Catalogue of Life: 2010 Annual Checklist" 2010. [Online]. Available: <http://www.catalogueoflife.org/annual-checklist/2010/info/cite>. [Accessed: 20-Feb-2018].
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- [5] J. P. Thompson, "*Microstegium vimineum* (Nepalese browntop)" *CABI Invasive Species Compendium*, 2018. [Online]. Available: <https://www.cabi.org/isc/datasheet/115603>. [Accessed: 18-Dec-2018].
- [6] D. E. Redman, "Distribution and habitat types for Nepal *Microstegium* [*Microstegium vimineum* (Trin.) Camus] in Maryland and the District of Columbia" *Castanea*, vol. 60, no. 3, pp. 270–275, 1995.

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