



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.

More info on the EU Regulation on http://ec.europa.eu/environment/nature/invasivealien/index_en.htm.

Pennisetum setaceum

(Forssk.) Chiov., 1923

Common names:

English: (African, crimson, tender) fountain grass

French: herbe aux écouvillons pourpres / rouge

German: Lampenputzergras (Afrikanisches, einjähriges)

Dutch: fraai lampenpoetsgras

Last update: November 2018



General information on *Pennisetum setaceum*

Classification

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

Species in the same genus: N = 83-160 [2-4]

Note: In literature and online reference sequence libraries, the species is often labelled as *Cenchrus setaceus* (Forssk.) Morrone. Recent molecular studies indeed argue that *Pennisetum* and *Cenchrus* should be fused, along with other genera, into one genus *Cenchrus*. Due to this proposed unifying, new naming and unresolved synonyms, the exact number of species is unspecified.

Infra-species level: N = 3 [2,5]

Note: At least three subspecies or varieties are found in the literature, but now regarded as synonyms.

Various other cultivars are grown, e.g., the red-leaved varieties of *P. setaceum* and traded under this well-known name, but are in fact the species *P. advena*.



Native range: [6]

Northern and Northeast Africa (Algeria, Egypt, Eritrea, Ethiopia, Kenya, Libya, Morocco, Somalia, Sudan, Tunisia) to the Middle East (Israel, Jordan, Lebanon, Oman, Qatar, Saudi Arabia, Syria, Yemen).

Invasive range: [6]

Europe (geographical):

France, Italy, Malta, Portugal, Spain, Sweden.

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

<https://www.gbif.org/species/5828232>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R03000>

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

Outside Europe (geographical):

Australia, Caribbean, Namibia, New Zealand, South Africa, United States of America, Venezuela.

Morphology, biology, invasion, negative effects and remedies

For more information on *Pennisetum setaceum* 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, ITS2 is the most reliable DNA marker for the identification of *Pennisetum setaceum*. To allow for a better evaluation of the performance of this marker for species identification, the missing congeners should be added to the analyses.

Discussion

DNA markers for which *Pennisetum* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Pennisetum*. Eight DNA markers were evaluated (Table 1). For most markers *P. setaceum* has only few sequences available. Considering the proposed inclusion of *Pennisetum* into genus *Cenchrus*, all sequence data labelled as the latter was added to the analyses and the new nomenclature was followed (Table 2).

For the full ITS region as well as its composing regions ITS1 and ITS2, available *P. setaceum* sequences are clustering. The **full ITS** region has the smallest dataset, and a limited amount of *P. setaceum* sequences. For the **ITS2** region, more sequence data is available and the *P. setaceum* sequences cluster together with high support, yet only the invasive range is represented. In the case of **ITS1**, the cluster of *P. setaceum* sequences includes a mislabelled sequence of *Cenchrus polystachios* (GenBank accession number AY628108). All three ITS fragments represent promising DNA markers for the identification of *P. setaceum*. Currently, the use of ITS2 is most promising, however, increased species representation for all three fragments would allow for a better evaluation of the performance of these markers.

Sequences of the trnL gene and the trnL-trnF intergenic spacers were pooled and trimmed to retain the **trnL** region. In this way more species could be included (Table 2), but this DNA marker shows little genetic variation, resulting in non-clustering of the sequences. *Pennisetum setaceum* could not be differentiated from its congeners and the low genetic variation raises doubts about the taxonomic resolution of this marker for the genera *Pennisetum* and *Cenchrus*.

The two universal barcode markers **rbcl** and **matK** showed little genetic variation and have low species coverage (Table 2). Neither of them clusters the available *P. setaceum* sequences and hence it is not advisable to apply these markers for the identification of *P. setaceum*.



The **kn1**, **ndhF** and **rpl16** gene and **trnH-psbA** intergenic spacer show little genetic variation among the different species. This extreme low variation, of multiple markers, is described by Roux *et al.* [7], who called it “the species’ global super-genotype a selected trait for optimal establishment and persistence in non-native areas”.

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 *P. setaceum* sequence was available.

Markers analysed	1	2	3	4	5
rbcl	X		X	X	X
matK	X	X	X		X
Full ITS	X	X			X
ITS1	X		X	X	X
ITS2					X
trnL	X	X	X		X
kn1		X	X	X	X
ndhF	X	X	X	X	X
rpl16	X	X			X
trnH-psbA	1	X	1		X

Table 2: Publicly available sequences downloaded (October 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 are adapted to the unification and follow [8], in which *Pennisetum* has been merged with *Cenchrus*. An ‘X’ indicates that at least one sequence was used in the final alignment, an ‘(X)¹’/ ‘(X)²’ indicates only ITS region 1/2 was available for analysis.

Species in genus	rbcl	matK	ITS	trnL	kn1	ndhF	rpl16	trnH-psbA
<i>Cenchrus abyssinicus</i>				X		X		
<i>Cenchrus agrimonioides</i>								
<i>Cenchrus alopecuroides</i>	X	X	X	X	X	X	X	X
<i>Cenchrus americanus</i>	X	X	X	X	X	X	X	X
<i>Cenchrus annuus</i>								
<i>Cenchrus arnhemicus</i>								
<i>Cenchrus articularis</i>								
<i>Cenchrus basedowii</i>				X	X	X		
<i>Cenchrus beckeroides</i>								
<i>Cenchrus biflorus</i>	X							
<i>Cenchrus brevisetosus</i>								
<i>Cenchrus brownii</i>				X	X	X	X	
<i>Cenchrus cafer</i>	X	X				X		
<i>Cenchrus caliculatus</i>								
<i>Cenchrus caudatus</i>		X		X	X	X		
<i>Cenchrus chilensis</i>				X	X	X	X	
<i>Cenchrus ciliaris</i>	X	X		X			X	X
<i>Cenchrus clandestinus</i>	X	X		X	X	X	X	
<i>Cenchrus complanatus</i>								
<i>Cenchrus distachyus</i>								
<i>Cenchrus distichophyllus</i>								
<i>Cenchrus divisus</i>	X		X					
<i>Cenchrus domingensis</i>								
<i>Cenchrus durus</i>								
<i>Cenchrus echinatus</i>		X		X			X	
<i>Cenchrus elegans</i>								
<i>Cenchrus elymoides</i>								
<i>Cenchrus exiguus</i>								
<i>Cenchrus flaccidus</i>	X	X	(X) ²	X	X	X	X	X
<i>Cenchrus flexilis</i>								
<i>Cenchrus foermerianus</i>				X		X		



Species in genus	rbcl	matK	ITS	trnL	kn1	ndhF	rpl16	trnH-psbA
<i>Cenchrus geniculatus</i>				X	X	X		
<i>Cenchrus glaucifolius</i>				X				
<i>Cenchrus gracilescens</i>								
<i>Cenchrus gracillimus</i>								
<i>Cenchrus henryanus</i>								
<i>Cenchrus hohenackeri</i>	X	X	(X) ¹ (X) ²	X				X
<i>Cenchrus hordeoides</i>				X	X	X		
<i>Cenchrus intectus</i>				X	X	X		
<i>Cenchrus lanatus</i>				X	X	X	X	
<i>Cenchrus latifolius</i>				X	X	X	X	
<i>Cenchrus laxius</i>								
<i>Cenchrus ledermannii</i>								
<i>Cenchrus longisetus</i>	X	X	(X) ¹ (X) ²	X	X	X	X	
<i>Cenchrus longispinus</i>	X							
<i>Cenchrus longissimus</i>		X				X		
<i>Cenchrus massaicus</i>				X		X		
<i>Cenchrus megianus</i>	X	X	(X) ¹ (X) ²	X	X	X	X	X
<i>Cenchrus michoacanus</i>								
<i>Cenchrus mitis</i>								
<i>Cenchrus monostigma</i>								
<i>Cenchrus multiflorus</i>								
<i>Cenchrus mutilatus</i>				X	X	X	X	
<i>Cenchrus myosuroides</i>				X			X	
<i>Cenchrus nanus</i>								
<i>Cenchrus nervosus</i>				X		X	X	
<i>Cenchrus nodiflorus</i>								
<i>Cenchrus nubicus</i>								
<i>Cenchrus occidentalis</i>								
<i>Cenchrus orientalis</i>			(X) ¹ (X) ²	X	X	X		
<i>Cenchrus palmeri</i>								
<i>Cenchrus pauper</i>								
<i>Cenchrus pedicellatus</i>	X		(X) ²	X	X	X		
<i>Cenchrus pennisetiformis</i>	X							
<i>Cenchrus peruvianus</i>								
<i>Cenchrus petiolaris</i>								
<i>Cenchrus pilcomayensis</i>				X	X	X	X	
<i>Cenchrus pilosus</i>				X			X	
<i>Cenchrus pirottae</i>								
<i>Cenchrus platyacanthus</i>								
<i>Cenchrus preslii</i>								
<i>Cenchrus prieurii</i>								
<i>Cenchrus procerus</i>								
<i>Cenchrus prolificus</i>								
<i>Cenchrus pseudotriticoides</i>	X	X				X		
<i>Cenchrus pumilus</i>								
<i>Cenchrus purpureus</i>	X	X	X	X		X	X	X
<i>Cenchrus qianningensis</i>								
<i>Cenchrus ramosus</i>			(X) ¹ (X) ²	X	X	X	X	
<i>Cenchrus rigidus</i>								
<i>Cenchrus riparius</i>								
<i>Cenchrus robustus</i>								
<i>Cenchrus rupestris</i>								
<i>Cenchrus sagittatus</i>								
<i>Cenchrus schweinfurthii</i>			(X) ¹ (X) ²	X		X		
<i>Cenchrus setaceus</i>	X	X	X	X	X	X	X	X
<i>Cenchrus setiger</i>	X	X		X				
<i>Cenchrus setosus</i>								
<i>Cenchrus shaanxiensis</i>								
<i>Cenchrus sichuanensis</i>								
<i>Cenchrus sieberianus</i>				X	X	X	X	
<i>Cenchrus somalensis</i>								
<i>Cenchrus sphacelatus</i>						X		
<i>Cenchrus spinifex</i>	X						X	



Species in genus	rbcl	matK	ITS	trnL	kn1	ndhF	rpl16	trnH-psbA
<i>Cenchrus squamulatus</i>			(X) ¹ (X) ²	X	X	X	X	
<i>Cenchrus stramineus</i>	X		(X) ¹	X			X	X
<i>Cenchrus tempisquensis</i>								
<i>Cenchrus thulinii</i>								
<i>Cenchrus trachyphyllus</i>				X		X		
<i>Cenchrus tribuloides</i>								
<i>Cenchrus trisetus</i>								
<i>Cenchrus tristachyus</i>				X		X	X	
<i>Cenchrus uliginosus</i>								
<i>Cenchrus unisetus</i>				X		X	X	
<i>Cenchrus violaceus</i>		X	(X) ¹	X	X	X		
<i>Cenchrus weberbaueri</i>								
<i>Cenchrus yemensis</i>								
TOTAL species	20/107	17/107	5 (14) ¹ (14) ² /107	41/107	24/107	37/107	26/107	9/107

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.q-bank.eu/Plants/lookalikes/Pennisetum/Pennisetum.html>
http://www.q-bank.eu/Plants/Factsheets/Pennisetum_setaceum_EN.pdf
<https://www.unce.unr.edu/publications/files/ho/2014/fs1411.pdf>
https://www.hicattle.org/Media/HICattle/Docs/pennisetum_setaceum.pdf
http://www.pir.sa.gov.au/_data/assets/pdf_file/0012/240042/fountain_grass_fsheets.pdf
<https://www.nvwa.nl/onderwerpen/invasieve-exoten/documenten/plant/planten-in-de-natuur/exoten/risicobeoordelingen/factsheet-fraai-lampenpoetsersgras>
<https://keys.lucidcentral.org/keys/v3/pennisetum/>

Picture credits

Page 1: *Pennisetum setaceum* growing at Canary Island, Spain By Frank Vincentz [GFDL or CC BY-SA 3.0]
Page 2 (left): Seeds of Fountain Grass in Como, Australia By John Tann [CC BY 2.0]
Page 2 (right): *Pennisetum setaceum* kz1 at Canary Island, Spain By Krzysztof Ziarnik, Kenraiz [CC BY-SA 4.0]

References

- [1] N. Smits, S. Gombeer, K. Meganck, 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: <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] M. A. Chemisquy, L. M. Giussani, M. A. Scataglini, E. A. Kellogg, and O. Morrone, "Phylogenetic studies favour the unification of *Pennisetum*, *Cenchrus* and *Odontelytrum* (Poaceae): a combined nuclear, plastid and morphological analysis, and nomenclatural combinations in *Cenchrus*" *Ann. Bot.*, no. 106, pp. 107–130, Jul. 2010.
- [4] S. Donadio, L. M. Giussani, E. A. Kellogg, F. O. Zuolaga, and O. Morrone, "A preliminary molecular phylogeny of *Pennisetum* and *Cenchrus* (Poaceae-Panicaceae) based on the trnL-F, rpl16 chloroplast markers" *Taxon*, vol. 58, no. 2, pp. 392–404, 2009.
- [5] Q. Groom, I. Hoste, and F. Verloove, "*Pennisetum setaceum* (Forssk.) Chiov." 2017.
- [6] S. Kaufman, "*Pennisetum setaceum* (fountain grass)" *CABI Invasive Species Compendium*, 2018. [Online]. Available: <https://www.cabi.org/ISC/datasheet/116202>. [Accessed: 30-Oct-2018].
- [7] J. J. Le Roux, A. M. Wicczorek, M. G. Wright, and C. T. Tran, "Super-Genotype : Global Monoclonality Defies the Odds of Nature" *PLoS One*, vol. 2, no. 7, p. e590, 2007.
- [8] WCVP, "World Checklist of Vascular Plants, version 2.0" 2020. [Online]. Available: <http://wcvp.science.kew.org/>. [Accessed: 15-Apr-2020].

To cite this factsheet, please use

Barcoding Facility for Organisms and Tissues of Policy Concern, 2019. Factsheet on *Pennisetum setaceum*; October 2018. In: Identification of Invasive Alien Species using DNA barcodes. BopCo, Belgium. Available from: <https://bopco.be/output/iasfactsheets>, accessed on DD-MM-YYYY.

DISCLAIMER: The information represented in this factsheet has been compiled from many different sources. Every reasonable effort has been made to ensure that the material presented is accurate and reflects the current (see date last update) scientific knowledge. However, recent changes in e.g. taxonomy and distribution, or the publication of additional reference sequences may not be implemented. The views which are expressed in the "Conclusion" are those of the author(s) and have not been peer-reviewed. BopCo does not guarantee the accuracy of the data included in this factsheet. The content of the factsheet is for information only and is not intended as legal advice. BopCo may not be held responsible for the use which may be made of the information contained therein. If you should notice any issues considering the content of this factsheet, or if you would like to contribute any additional information to it, please contact us through bopco@naturalsciences.be.

