Pennisetum setaceum or Pennisetum advena cultivars, what ornamental do we have in our garden

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Abstract
Pennisetum Rich. or following recent taxonomic insights Cenchrus L. is a genus with some 120 species worldwide, especially in warm areas. The genus includes some crops, some ornamentals but mostly species that are considered weedy. The name of one of the weedy species Pennisetum setaceum (Forssk.) Chiov. is also found on labels of ornamental grasses as P. setaceum "Rubrum." It has been debated to belong to a species on its own Pennisetum advena Wipff & Veldkamp or Cenchrus advena (Wipff & Veldkamp) Morrone, only known from cultivation, whereas others still adhere to a broader species concept of P. setaceum. The recent inclusion of P. setaceum on the EU List of Union concern has revitalized the discussion on this issue for commercial reasons. Based on a morphological and molecular comparison (ITS, rbcL, and the trnH-psbA intergenic spacer sequences) of the type specimen of P. advena, five of its "cultivars" in trade and collections of P. setaceum from different regions of the world we conclude that plants currently in trade in Western Europe belong to a separate species P. advena. A drooping inflorescence is consistent as is the difference in width of the leaf blade, the leaf blade being flat or involute, the central vein being swollen or not, and the length of the stipe being 0.3–1.1 mm in P. advena and 1.1–3.1 mm in P. setaceum. On the chloroplast markers rbcL and trnH-psbA, the species consistently differ in 2 and 4 base pairs, respectively. On the nuclear ITS sequence, there is only 90% overlap between the two species. This justifies these ornamentals to be excluded from the List of Union concern of EU regulation 1143/2014.

KEYWORDS
Cenchrus, EU regulation 1043/2014, fountain grass, invasive plant, NGS

1 | INTRODUCTION

Pennisetum Rich. or following recent taxonomic insights Cenchrus L. is a genus with some 120 species worldwide, especially in warm areas (Chemisquy et al., 2010; Kellogg, 2015). It includes some crops, some ornamentals but mostly species that are considered weedy. There has been or still is considerable confusion with respect to the proper identity of Pennisetum cultivars traded as, "Cherry Sparkler," "Fireworks," "Rubrum," "Sky Rocket," and "Summer Samba."
It has been debated that these cultivars belong to a species of its own, *Pennisetum advena* Wipff and Veldkamp (1999) or *Cenchrus advena* (Wipff & Veldkamp, 1999) Morrone (Chemisquy et al., 2010; Veldkamp, 2014), only known from cultivation. However, others still adhere to a broader species concept of *Pennisetum setaceum* (Groom et al., 2017). Finally, there are those that interpret a discussion on relatedness as a proxy for a hybrid status (Meyer, 2012; Padhye et al., 2008). The recent inclusion of *P. setaceum* (Figure 1) on the List of Union concern of EU regulation 1143/2014 has revitalized the discussion on this issue for commercial reasons (see, e.g., the recommendation by Val’hor, 2017). Listing of a species on the List of Union concern implies that all its lower taxa or hybrids are potentially subject to the same rules and prohibitions, thus resulting in an effective ban on sale of these popular cultivars traded under the name of either *P. setaceum* or *P. advena* (Figure 2).

Provisional molecular results pointing to a distinction in the aforementioned cultivars (Anonymous, 2017) have resulted in these taxa being provisionally excluded from the ban, while a more detailed morphological and molecular study is conducted. Results of this study are presented here.

**FIGURE 1**  *Pennisetum setaceum* as a weed in South Africa

**FIGURE 2**  *Pennisetum advena* with characteristic drooping inflorescences in an urban garden (photographer Edu Boer)

## 2 | METHODS

### 2.1 | Acquisitions of plants

For a study of *Pennisetum* species and cultivars in trade in the Netherlands, a large number of living plants was acquired from commercial growers and garden centers and subsequently grown in a quarantine glasshouse (Costerus, 2018; Costerus & van Valkenburg, 2018). In addition, a total of 168 herbarium specimen from the National Herbarium at Leiden belonging to 10 *Pennisetum* species were consulted for descriptive purposes.

For the present detailed study, plants listed in Table 1 were used. Mother plants of the five major cultivars "Cherry Sparkler," "Fireworks," "Rubrum," "Sky Rocket," and "Summer Samba" were obtained from Henk de Jong (CNB Plants) (Valkenburg 3871, 3872, 3873, 3874, 3966). The type specimen of *P. advena* (Wipff 1723) and an African collection of *P. setaceum* (Mooney 9419) were consulted at Leiden (L, WAG). Naturalized *P. setaceum* plants were collected from the Canary Islands and in Catalonia, Spain (Simons 2006, Verloove 13345, 13647, 13650). Ornamental *P. setaceum* were collected in New Zealand (Valkenburg 3934) and cuttings of *P. advena* intercepted in a mislabeled commercial import from China (Valkenburg 4026).

### 2.2 | Macromorphological approach

Of all living material height of the plant including inflorescence was measured, color of the stem, width, length and color of leaves; color and length of inflorescence; length of spikelet; texture of axis of inflorescence; length of stipe; number of spikelets per fascicle; and color, length and presence of long hairs on bristles. For all herbarium specimens, macromorphological measurements were similar. All measurements are used to build an interactive image-driven key using LUCID software (Identic, Stafford Heights, Australia).

### 2.3 | DNA extractions

Genomic DNA was isolated from approximately 100 mg plant material with the DNeasy Plant Mini Kit (Qiagen, Venlo, the Netherlands) using the TissueLyser procedure and eluted with 50 μl prewarmed (65°C) AE buffer. DNA was stored at −20°C until use.

### 2.4 | PCR amplification and Sanger sequencing

PCRs for the chloroplast rbcL gene and trnH-psbA intergenic spacer and nuclear ITS (partial 18S, ITS1, 5.8S, ITS2, partial 28S) loci were performed in 25 μl reaction mixes containing 200 nmol/L of either primers rbcL-a F and rbcLa SI_Rev, trnH2, and psbAF or ITS5 and ITS4 (Table 2), respectively, 1 x MyFi™ Mix (Bio-line, Taunton, USA) and 2 μl genomic DNA. The cycle conditions for rbcL and trnH-psbA
loci were as follows: 5 min at 95°C, followed by 5 cycles of 30 s at 94°C, 30 s at 45°C, 30 s at 72°C and 35 cycles of 30 s at 94°C, 30 s at 50°C, 30 s at 72°C and a final extension for 10 min at 72°C. The cycle condition for ITS locus was as follows: 5 min at 95°C, followed by 40 cycles of 30 s at 94°C, 30 s at 52°C, 100 s at 72°C, and a final extension for 10 min at 72°C.

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Venlo, the Netherlands) preceding bidirectional cycle sequencing with the BigDye Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Bleiswijk, the Netherlands) using amplification primers as sequencing primers in separate reactions according to the manufacturer’s instructions. Cycle sequence products were purified with the DyeEx 2.0 Spin Kit (Qiagen, Venlo, the Netherlands) and sequenced using a 3500 Genetic Analyzer (Thermo Fisher Scientific, Bleiswijk, the Netherlands). Consensus sequences were generated from an assembly with trace files from both Sanger sequencing runs in Geneious R10 (Biomatters Auckland, New Zealand). Amplification primer sequences were trimmed in the assembly, and when needed, additional trimming was performed to obtain high-quality (PHRED >30) consensus sequences.

3 | RESULTS

3.1 | Macromorphological differences observed

The analysis of all living material and the herbarium collections of the wild samples and cultivars belonging to the 10 species can be found in Costerus (2018) and Costerus and van Valkenburg (2018). It has been reworked to an interactive image-driven identification key using LUCID software and can be found at https://keys.lucidcentral.org/keys/v3/pennisetum/en/index.html.

For this paper, we focus on the distinguishing characters between *P. advena* and *P. setaceum*.

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For this paper, we focus on the distinguishing characters between *P. advena* and *P. setaceum*.
Most obvious morphological characters when judging its ornamental value are the culm of *P. setaceum* being rarely branched as opposed to *P. advena* and the inflorescence of the latter being flexuous and drooping as opposed to rigid in *P. setaceum*. Both characters are added to the attractiveness of the species. Leaf characters in addition to the color of *P. setaceum* never being purplish are the consistently larger width and flatness of the blade in *P. advena* as well as the more prominent thickened central vein and involute leaf in *P. setaceum*. These character differences also apply to the ornamental *P. setaceum* collected in New Zealand. More detailed measurements on the inflorescence and leaves of *P. setaceum*, *P. advena* and cultivars are given in Table 3 with the values as published for both species (Veldkamp, 2014) for comparison.

### 3.2 Sequence analysis

The results of the sequence analysis for the chloroplast rbcL gene and trnH-psbA intergenic spacer as well as for the nuclear ITS (partial 18S, ITS1, 5.8S, ITS2, partial 28S) sequence are summarized in Table 4. Based on the rbcL and trnH-psbA sequences, *P. advena* and *P. setaceum* can be distinguished from each other. For rbcL, there are two nucleotide (nt) differences, and for trnH-psbA region, there are four nt differences between *P. advena* and *P. setaceum*. All five cultivars could be matched to the type specimen of *P. advena*. Interestingly, the trnH-psbA region of *P. setaceum* sample “Simons 2006” could not be unambiguously sequenced by Sanger technology. Illumina sequence data revealed that this problem was caused by the presence of two different SNPs in both *P. advena* (n = 3) and *P. setaceum* (n = 2) sequences. Sanger sequencing of the ITS region revealed the presence of SNPs in both *P. advena* (n = 3) and *P. setaceum* (n = 2) sequences. For *P. advena*, the ratio between the ITS sequences of the cultivars “Cherry Sparkler” and “Sky Rocket” could not be obtained by Sanger sequencing. Illumina sequencing of these cultivars revealed that both cultivars contained variants of the ITS sequences. Based on all three loci (rbcL, trnH-psbA, and ITS), these cultivars could therefore be matched to *P. advena*.

### Table 3

|               | *P. advena* | *Cherry Sparkler* | *Fireworks* | *Rubrum* | *Sky Rocket* | *Summer Samba* | *P. setaceum* (Veldkamp) | *P. advena* (Veldkamp) | *P. setaceum* (Veldkamp) |
|---------------|-------------|-------------------|-------------|-----------|--------------|------------------|-------------------------|------------------------|-------------------------|
| **Leaf width (mm)** | 7-9 | 4-6.5 | 3-8.5 | 2-4 | 3-5.5 | 4-6 | 1-2 | 6-11 | 1-3.7 |
| **Leaf length (cm)** | 50 | 36-42 | 28-49 | 20-46 | 20-41 | 31-44 | 15-27 | 22-52 | 30-100 |
| **Leaf color** | Green, purple | Green, purple, white | Green, purple, white | Green, purple | Green, purple, white | Green, purple, white | Green | Green, purple, white |
| **Length of inflorescence (cm)** | 21-27 | 21 | 16-23 | 15-22 | 17-18 | 18-22 | 17 | 22-32 | 10-26 |
| **Length of fascicle (mm)** | 5.2-5.8 | 4-5 | 4-6 | 3-5 | 4-6.5 | 4-5 | 5.5-6.2 | 5.2-6.5 | 4.7-6.8 |
| **Length of stipe (mm)** | 0.44-0.82 | 0.6-0.9 | 0.3-0.6 | 0.4-0.5 | 0.7-1.0 | 0.4-0.5 | 1.7-1.8 | 0.5-1.1 | 1-3 |
| **Length of longest bristle (mm)** | 30-37 | 23-32 | 22-29 | 25-33 | 22-34 | 25-33 | 28-32 | 21-33.5 | 28-32 |
4 | DISCUSSION

From a scientific point of view, the above-mentioned Pennisetum
advena and P. setaceum should be addressed as Cenchrus species,
following recent molecular and phylogenetic studies on the
genera Cenchrus and Pennisetum (Chemisquy et al., 2010; Donadio
et al., 2009). Numerous publications have already made the new
necessary combinations for the American (Chemisquy et al., 2010),
European (Verloove, 2012; Verloove et al., 2014), Southeast Asian
(Veldkamp, 2014), Australian (Symon, 2010), and Pacific species
(Tornabene & Wagner, 2013). However, as the ornamental plant
trade is rather conservative, we have retained the old Pennisetum
names for P. advena and P. setaceum and the other common orna-
mentals P. orientale and especially P. alopecuroides both represented
by numerous cultivars in trade. Likewise, the name Pennisetum
has been retained on the EU List of Union concern based on the risk
assessment by Danas and Verloove (2015).

Although the exact origin of the ornamental plants commonly
referred as P. setaceum “Rubrum” is unclear, apart from it being Old
World, its introduction in the United States in 1916 is well docu-
mented as well as the quest for a valid name for the species. For an
elaborate discussion, see Wipff and Veldkamp (1999).

Confusion on hybrid status goes back to Padhye et al. (2008) who
somehow misinterpreted the Wipff and Veldkamp (1999) discussion
on the origins of P. advena. This misinterpretation was later repeated
by Meyer (2012) and has been adopted by numerous people who
apparently failed to verify this in the original publication by Wipff and
Veldkamp (1999). This view has been expressed already by Melanie
Schori from USDA (pers. comm. 2017) in response to questions
raised by European umbrella organizations of plant growers to the
Community Plant Variety Office, following listing of P. setaceum
on the List of Union concern.

Descriptions of some of the patented cultivars such as
“Fireworks,” “Cherry Sparkler,” and “Sky Rocket” can be found online
as it applies to the United States. Within a European context, as a
different legislation applies reference can be made to the website
of the Community Plant Variety Office (https://cpvoextranet.cpvo.
europa.eu/mypvr/#/en/publicsearch). Descriptive aspects and a
comprehensive bibliography of relevant cultivars are already dealt
with in the recommendation for Pennisetum by Val’Hor (2017) and
by Costerus (2018).

Distinguishing morphological characters for P. advena have
been elaborately discussed by Wipff and Veldkamp (1999). Some
refinements resulting from our study can be added. Branching in
aerial nodes does sometimes occur in P. setaceum as observed in
cultivated plant in New Zealand (Valkenburg 3934) and wild plants
in Ethiopia (P.C.M. Jansen 3946). Drooping inflorescence is consis-
tent as is the difference in width of the leaf blade, the leaf blade
being flat or involute, the central vein being swollen or not, and the
length of the stipe being 0.3–1.1 mm in P. advena and 1.1–3.1 mm
in P. setaceum.

From an European perspective, a difference in potential risk of
invasiveness of P. advena compared with P. setaceum would be a
major criterion to justify it not to be regulated. Simpson and Bashaw
(1969) showed that P. advena (purple P. setaceum) rarely sets seed
under field conditions and does not behave as an apomictic species

| Species          | collection no. | Sequencing | rbCL  | trnH-psbA | ITS  |
|-----------------|----------------|------------|-------|-----------|------|
| P. advena       | Valkenburg 4026 | S, I  | a 3   | a         | aI 7, aI 8 |
| P. advena       | Wipff 1723     | S, I  | a 3   | a         | aI 7, aI 8 |
| “Cherry Sparkler” | Warkenburg 3874 | S, I  | a 3   | a         | aI 7, aI 8 |
| “Fireworks”     | Warkenburg 3873 | S, I  | a 3   | a         | aI 7, aI 8 |
| “Rubrum”        | Warkenburg 3874 | S, I  | a 3   | a         | aI 7, aI 8 |
| “Sky Rocket”    | Warkenburg 3872 | S, I  | a 3   | a         | aI 7, aI 8 |
| “Summer Samba”  | Warkenburg 3871 | S, I  | a 3   | a         | aI 7, aI 8 |
| P. setaceum     | Mooney 9419    | I     | s 4   | s         | sI, sII |
| P. setaceum     | Simons 2006    | S, I  | s 4   | s         | sI, sII |
| P. setaceum     | Valkenburg 3934| S     | s 4   | s         | sI, sII |
| P. setaceum     | Verloove 13345 | S     | s 4   | s         | sI, sII |
| P. setaceum     | Verloove 13647 | S     | s 4   | s         | sI, sII |
| P. setaceum     | Verloove 13650 | S     | s 4   | s         | sI, sII |

TABLE 4 Sequencing results of the rbCL, trnH-psbA, and ITS loci for the Pennisetum specimen

1S = Sanger sequencing.
2I = Illumina sequencing.
3a = Pennisetum advena sequence.
4s = Pennisetum setaceum sequence.
5sI = Pennisetum setaceum variant I sequence.
6sII = Pennisetum setaceum variant II sequence.
7aI = Pennisetum advena variant I sequence.
8aII = Pennisetum advena variant II sequence.
nor is the plant capable of selfing as opposed to \textit{P. setaceum}. Chilling injury studies point to hardness issues for \textit{P. advena} when temperatures drop below 5°C for 2 weeks (Padhye et al., 2008). In addition, there are no records of invasive behavior of “purple” \textit{P. setaceum}.

Sequences from the chloroplast loci \textit{rbcL} and \textit{trnH-psbA} as well as the nuclear loci ITS showed enough resolution to distinguish \textit{P. advena} from \textit{P. setaceum}. However, Blast searches in the NCBI GenBank with the \textit{rbcL} and \textit{trnH-psbA} sequences revealed very high identities (up to 100%) with other \textit{Pennisetum}, \textit{Cenchrus}, and \textit{Setaria} species, which makes identification based on these two loci alone difficult. Furthermore, GenBank contains a few accessions for \textit{P. setaceum} (or \textit{Cenchrus setaceus}), but none for \textit{P. advena}. In addition, the \textit{rbcL} and \textit{trnH-psbA} sequences (GenBank acc. GU135184 and GU135350) for the \textit{Cenchrus setaceus} voucher J.R. Abbott 24732 (FLAS) are identical to the \textit{rbcL} and \textit{trnH-psbA} sequences of \textit{P. advena} from this study, suggesting a misidentification.

To obtain more resolution for molecular identification, the sequence of the nuclear ITS locus was used. Interestingly, both \textit{P. advena} and \textit{P. setaceum} each contained two variants of the ITS sequences with 2 and 3 SNP, respectively. Most likely these variants are caused by their polyploid nature, as \textit{P. setaceum} for instance is known to be hexaploid (Martel et al., 2004), although plants in general may contain multiple copies of ITS (Feliner & Rosselló, 2007). The ratio between the two variants was not the same in each of the specimens, but this could be caused by a sequence artifact or an uneven distribution of the variants among the different chromosome copies.

Using the ITS sequence as a proxy for species hybridization has been successfully used for the identification of \textit{Impatiens} hybrids (van Valkenburg et al., 2019). The cultivars “Cherry Sparkler” and “Sky Rocket” contained the ITS sequences from both \textit{P. advena} and \textit{P. setaceum}, suggesting that these specimens have a hybrid status. Interestingly, they contain both \textit{P. advena} ITS variants, but only one of the \textit{P. setaceum} variants. This points to a hybrid origin, with \textit{P. advena} being the seed plant.

## 5 | CONCLUSION

Based on molecular and morphological characters, both species can be clearly separated and the popular ornamentals in trade in Europe belong to \textit{P. advena}. This justifies these ornamentals to be excluded from the List of Union concern of EU regulation 1143/2014.

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## CONFLICT OF INTEREST

The authors have no conflict of interest to report.

## AUTHOR CONTRIBUTIONS

Johannes Leonnardus Cornelis Hendrikus van Valkenburg: Conceptualization (lead); Data curation (equal); Formal analysis (supporting); Investigation (equal); Supervision (lead); Writing-original draft (equal); Writing-review & editing (equal). Maarten Costerus: Conceptualization (equal); Data curation (equal); Formal analysis (lead); Writing-original draft (equal); Writing-review & editing (equal). Marcel Westenberg: Data curation (equal); Formal analysis (equal); Writing-original draft (equal); Writing-review & editing (equal).

## DATA AVAILABILITY STATEMENT

DNA sequences: ITS, \textit{rbcL}, and the \textit{trnH-psbA} intergenic spacer sequences were deposited in NCBI GenBank under the following accession numbers: MW177954-MW178003. Sequences will also be made available at https://qbank.eppo.int/plants/. Herbarium vouchers are available at BR, L, WAG, WAG-PD, and online at https://www.q-bankplants.eu/.

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