

Rapid Communication

Naturalized *Saururus*. What species are we actually talking about in the Netherlands and Belgium?

Johan L.C.H. van Valkenburg¹, Laurens F. Piet¹, Helena Duistermaat², Filip Verloove³, Jesse L. Beyer^{1,2}, Kevin Scheers⁴ and Wietse G.S.A. den Hartog¹

¹Netherlands Institute for Vectors, Invasive plants and Plant health (NIVIP), NVWA, Geertjesweg 15, 6706 EA Wageningen / Postbus 9102, 6700 HC Wageningen, The Netherlands

²Naturalis Biodiversity Center, Darwinweg 2, 2333 CR Leiden, The Netherlands

³Meise Botanic Garden, Nieuwelaan 38, B-1860 Meise, Belgium

⁴Research Institute for Nature and Forest (INBO), Herman Teirlinckgebouw, Havenlaan 88 bus 73, 1000 Brussel, Belgium

Corresponding author: Johan L.C.H. van Valkenburg (j.l.c.h.vanvalkenburg@nvwa.nl)

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Abstract

A known naturalized population of *Saururus* in (Flanders) Belgium was included in a molecular study and unexpectedly turned out to be of hybrid origin. This prompted a targeted survey of all known naturalized populations of *Saururus* in the Netherlands and Belgium. Plant material was analysed using both morphological and molecular methods, and results were compared with herbarium specimens from the native range and with DNA sequences available in GenBank. All naturalized populations proved to be hybrids between *Saururus cernuus* and *S. chinensis*, the only two species in the genus. A morphological key is provided to distinguish both parental species and the hybrid.

Key words: invasive, hybrid, ornamental, cryptic, mislabelling

Introduction

Saururus has long been known as an ornamental pond and aquarium plant by the vernacular name “Leids plantje”, or “Lizard's-tail”. Records and reports of naturalized *Saururus* for Belgium go back to 1977 (Verloove 2006) and for the Netherlands to 2015 (NDFF & FLORON 2025). No herbarium vouchers of these records from the Netherlands were available at Naturalis (L, U, AMD & WAG) and only one from Belgium at Meise Botanical Garden (BR). Until recently, they were assumed to be *S. cernuus* L., consistent with the labelling of all *Saururus* plants in horticultural trade in the Netherlands and Belgium (see e.g. Van den Neucker and Scheers 2022). During a joint fieldtrip in Flanders (Belgium) in August 2023, plants of a naturalized population at Sint-Lievens-Houtem were collected (coll. Valkenburg 4472). As standard practice for incorporation of a collection in the Q-bank plants database (Q-bank 2025), an additional sample for molecular analysis was collected. The resulting sequence data were subsequently uploaded to EPPO-Q-bank database (EPPO-Q-bank 2025).

Table 1. Collections (ID 1: *Saururus cernuus*; 2: *S. chinensis*; 3: *S. chinensis* × *cernuus*)

Collection	Initial ID	Final ID	Locality	Coordinates
Valkenburg 4472	1	3	Letterhoutem, Oost-Vlaanderen, Belgium	50.931733, 3.88475
Valkenburg 4509	1	3	Nieuwegein, Utrecht, Netherlands	52.00455, 5.09675
Valkenburg 4510	1	3	De Bilt, Utrecht, Netherlands	52.11047, 5.16917
Valkenburg 4511	1	3	Vlagheide, Schijndel, Noord-Brabant, Netherlands	51.60236, 5.48733
Valkenburg 4512	1	3	Maasdam, Zuid-Holland, Netherlands	51.78816, 4.55463
Valkenburg 4513	1	3	Amstelveen, Zuid-Holland, Netherlands	52.28031, 4.83049
Valkenburg 4514	1	3	Maarsen, Utrecht, Netherlands	52.15192, 5.07316
Valkenburg 4515	1	3	Amersfoort, Utrecht, Netherlands	52.17537, 5.38352
Valkenburg 4516	1	3	National Park de Meinweg, Vlodrop-station, Limburg, Netherlands	51.15692, 6.15906
Valkenburg 4517	1	3	Bedum, Groningen, Netherlands	53.28100, 6.62029
Valkenburg 4539	1	3	Culta	
Verloove 5876	1	3	Citadelle of Lille, Lille, département Nord, France	50.64111, 3.04444
Barendse s.n.	1	3	Lommel, Limburg, Belgium	51.1845, 5.2898
Verloove 6165	1	3	Aalst, Oost-Vlaanderen	50.9241, 4.0443
Packet s.n.	1	3	De Blankaart, Diksmuide, West-Vlaanderen, Belgium	50.9895, 2.8521
Van Landuyt s.n.	2	2	Plantentuin University Gent, Gent, Oost-Vlaanderen, Belgium	51.03540, 3.72272
Scheers 2024/2	1	1	Arboretum Bokrijk, Genk, Limburg, Belgium	50.9669, 5.4061
Scheers 2024/3	1	3	Heusden, Oost-Vlaanderen, Belgium	51.0452, 3.8225
Scheers 2024/1	1	3	Hingene, Antwerpen, Belgium	51.1097, 4.2697
Scheers 2024/4	1	3	Letterhoutem, Oost-Vlaanderen, Belgium	50.9317, 3.8850

Molecular analysis conducted in 2024 revealed the plant to be a hybrid, with *S. chinensis* (Lour.) Baill. as the maternal parent. This finding triggered us to sample all known naturalized occurrences of *Saururus* in the Netherlands and Belgium, as well as material from botanical gardens. As both species have a disjunct distribution and a true revision at genus level is not available, their identification is not straightforward. European populations of *Saururus* were not known to the authors of the treatment of Saururaceae in Species Plantarum Flora of the World (Brach and Xia 2005) and the generic treatment in the European Garden Flora (Cullen et al. 2011) did not provide a satisfactory diagnostic key or description. Therefore, dissimilar descriptions in the Flora of China (Xia 1999) and Flora of North America (Buddell and Thieret 1997) had to be compared with the European plants in order to tell the species apart. This was complemented by a detailed analysis of herbarium collections available at Naturalis, Leiden. We present here a key to the morphological identification of the two species and the hybrid as well as the molecular data to tell the taxa apart.

Materials and methods

Plant material of naturalized populations and from botanical gardens in the Netherlands and Belgium was collected from August till October 2024, following the initial collection in August 2023 in Belgium. In addition, material from a mother plant that has supplied the horticultural market for the last 65 years was sampled in November 2024. All collections are vouchered at WAGPD and can be consulted on Q-bankplants.eu. Finally, material was sampled from earlier herbarium material available at Meise Botanic Garden (BR), including a sample from Lille in France (Table 1). For molecular analysis, fresh leaf material from Valkenburg 4472 and all collections from

the Netherlands was used, whereas for the collections from Belgium silica dried leaf material was used. For the phylogenetic analysis a further 10 sequences from GenBank were used. The viability of the pollen was tested on fresh anthers of Valkenburg 4509 and 4514.

DNA extraction

DNA extraction was performed on 200 mg of plant material, which was added to 300 µL lysis buffer and 20 µL proteinase K and subsequently crushed using a pestle. 125 µL of the crushed plant extract was used for subsequent isolation on the KingFisher platform using the QuickPick Plant DNA Kit (Bio-Nobile) according to the manufacturer's protocol. Obtained DNA extract was purified using a poly(vinyl)polypyrrolidone spin column and stored at –20 °C until sequencing.

Illumina sequencing

The “Illumina DNA Prep, (M) Tagmentation” kit was used according to the manufacturer's specifications to generate 150PE Illumina-ready libraries. Illumina Unique Dual Indexes were used for indexing. Five to twelve amplification cycles were performed during the library generation step depending on input DNA concentration. Library yields were quality assessed by fluorescence on a Qubit Flex fluorometer (Thermo Fisher Scientific, MA, USA), and correct insert sizes were verified using D1000 ScreenTape on a TapeStation 4200 system (Agilent Technologies, CA, USA). *Saururus* libraries were equimolarly pooled targeting at least 2 Gb per sample, and 150PE sequencing was performed using a 300-cycle P1 cartridge and flow cell combination on a NextSeq 2000 system (Illumina, CA, USA).

De novo assembly and extraction of chloroplast loci

Within CLC genomics workbench v21.0.3 a specialized workflow was developed to extract the three most commonly used loci from Illumina data. The workflow employs a de novo assembly strategy and uses a BLAST-based sorting to extract all chloroplast contigs. From the chloroplast contigs the barcodes trnH-psbA and rbcL were extracted using an in-silico primer-based approach with the corresponding primers (Table 2). The barcode matK was retrieved using a BLAST search with an extensive matK sequence database based on the Refseq database of NCBI.

Hybrid analysis

The CLC genomics workbench v21.0.3 was used to perform a reference assembly on references for *Saururus chinensis* (AF215921) and *Saururus cernuus* (MH493067) for 18S ribosomal RNA gene, partial sequence, internal

Table 2. Primers used in this study. The first primer mentioned is the forward primer.

Locus	Primer	Sequence	Reference
<i>trnH-psbA</i>	trnH2	5'-CGC GCA TGG TGG ATT CAC AAT CC-3'	Tate (2002)
<i>trnH-psbA</i>	psbAF	5'-GTT ATG CAT GAA CGT AAT GCT C-3'	Sang et al. (1997)
<i>rbcL</i>	<i>rbcL</i> -a_f	5'-ATG TCA CCA CAA ACA GAG ACT AAA GC-3'	Kress and Erickson (2007) Kress et al. (2009)
<i>rbcL</i>	<i>rbcLa</i> SI_Rev	5'-GTA AAA TCA AGT CCA CCR CG-3'	Kress et al. (2009)

transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence. The references were aligned and trimmed to the same length. The average coverage for each mapping was recorded and the sum of both coverages is compared to the coverage of *S. chinensis*. The obtained ratio was used to determine the hybrid status for each sample individually, A ratio close to 0 or 1 was interpreted as non-hybrid whereas a ratio close to 0.5 was interpreted as hybrid status. The choice to use coverage instead of read count is to account for the difference in length between the two references.

Phylogenetic analysis

Saururus sequences were aligned using MUSCLE version 3.8.425 (Edgar 2004) within Geneious Prime® 2021.1.1 (Biomatters Auckland, New Zealand) with standard settings. Phylogenetic likelihood trees with bootstrap values were generated with FastTree 2.2.11 and *Houttuynia cordata* Thunb. NC047437 was used as an outgroup. For *Saururus chinensis* MZ636548, MK134898, MK134899, MK134900, MN263891, NC050853, and PV608438 were used. As there are no complete chloroplast sequences available for *Saururus cernuus*, a chimeric sequence was generated, matK was used from DQ882200, rbcL from KP402668, and trnH-psbA from KP402490. Loci matK, rbcL, and trnH-psbA were concatenated and the resulting sequence was used for phylogenetic analysis.

Pollen viability

Pollen is tested on viability with Cotton blue-lactophenol (Saarela 2012; solution of 200 mg cotton blue, 250 ml water, 250 ml glycerol, 250 ml phenol, 250 ml lactic acid). Several (3–7) anthers of a single inflorescence are brought on an object glass with a small drop of the dye. The anthers are opened with a sharp needle and squashed to release the pollen, and subsequently covered with a cover glass. After ten minutes the pollen content will colour blue if it is viable, non-viable pollen will stay colourless. Pollen is scored under a compound microscope (63× magnification) for percentage of viable pollen.

Data accessibility

Sequences will be made available at <https://qbank.eppo.int/plants/>.

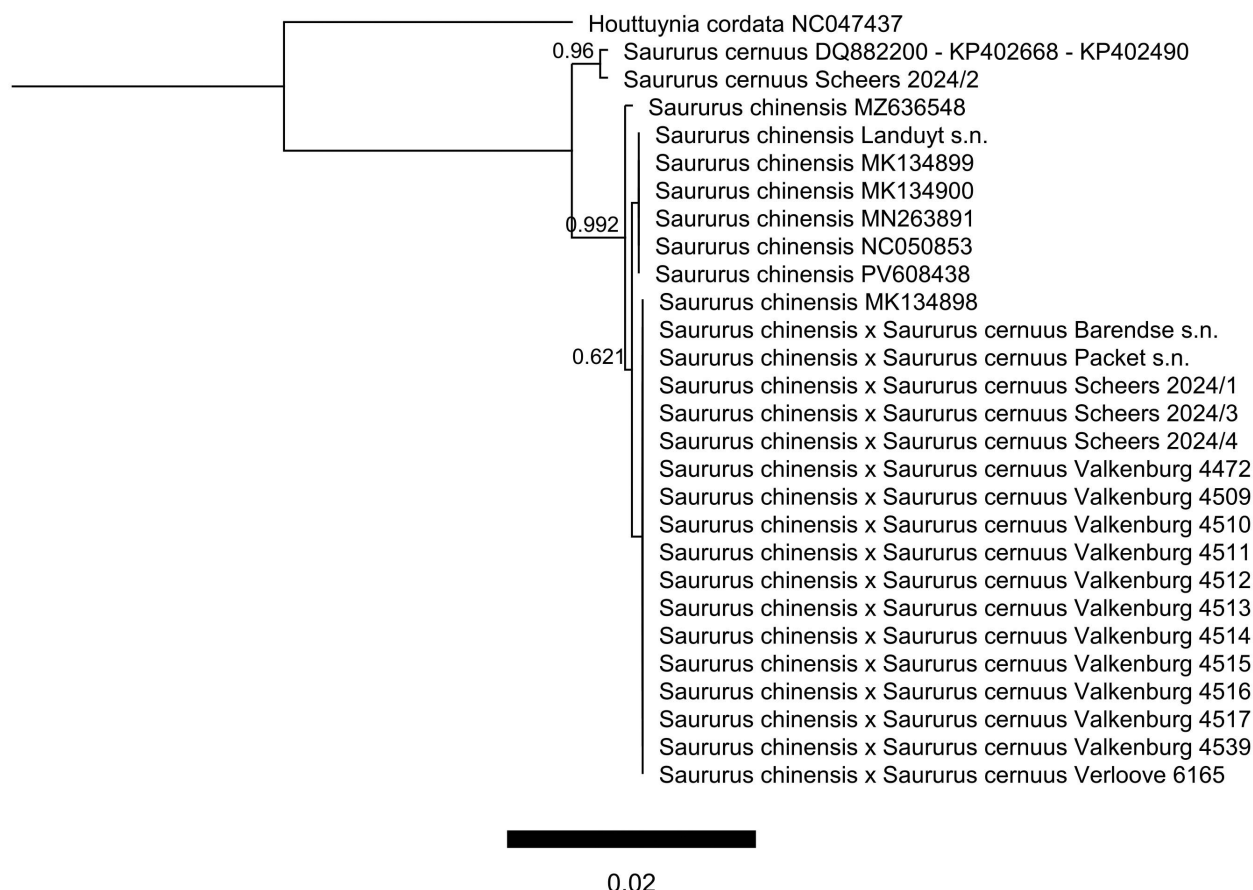


Figure 1. Phylogenetic tree based on the concatenated chloroplast loci matK, rbcL, and trnH-psbA.

Results

Chloroplast DNA

Molecular chloroplast data was obtained for 19 specimens from silica dried material and from fresh material. Unfortunately, the herbarium voucher from Lille (Verloove 5876) did not yield sufficient good quality DNA for analysis. In total, three different genotypes were obtained clustering in two distinct groups, representing the two *Saururus* species (Figure 1). All but one sample showed a clustering with *S. chinensis* references, with 17 of 18 *S. chinensis* (hybrid/nonhybrid) samples being identical. A similarity score between 98.93–99.01% was observed between *S. chinensis* and *S. cernuus* and only a 3 nt difference was observed within the *S. chinensis* cluster.

Hybrid status

The obtained ratios of *S. chinensis*/*S. cernuus* coverage range from 0.004 to 0.956. The majority of the samples showed an intermediate ratio with an average of 0.48 (95% CI: 0.42–0.54) and are deemed of hybrid status, while one sample is found on both ends of the scale (0.004 and 0.956) and is classified as non-hybrid. This means that one “pure” *Saururus chinensis*, one “pure” *Saururus cernuus*, and 17 hybrids were identified (Table 3). The

Table 3. Ratio of *Saururus chinensis* / *Saururus cernuus* coverage of 19 specimens. 17 of 19 samples showed a ratio close to 0.5, meaning they are of hybrid status.

Collection	Ratio	Identified as
Barendse s.n.	0.490	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Van Landuyt s.n.	0.958	<i>Saururus chinensis</i>
Packet s.n.	0.473	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Scheers 2024/1	0.483	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Scheers 2024/2	0.004	<i>Saururus cernuus</i>
Scheers 2024/3	0.518	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Scheers 2024/4	0.420	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Valkenburg 4472	0.466	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Valkenburg 4509	0.461	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Valkenburg 4510	0.480	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Valkenburg 4511	0.476	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Valkenburg 4512	0.504	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Valkenburg 4513	0.458	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Valkenburg 4514	0.438	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Valkenburg 4515	0.462	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Valkenburg 4516	0.477	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Valkenburg 4517	0.473	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Valkenburg 4539	0.470	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>
Verloove 6165	0.537	<i>Saururus chinensis</i> × <i>Saururus cernuus</i>

sample Scheers 2024/2, identified as *Saururus cernuus* based on chloroplast markers, with a ratio of 0.004, was identified as “pure” *Saururus cernuus*.

Morphology

Based on herbarium collections from the native range of *S. cernuus* and *S. chinensis* held at Naturalis, as well as on collections of the hybrid from the Netherlands and Belgium, a comparative description is given along with a key for flowering material.

Saururus cernuus:

Emerse **stem** pubescent (check multiple stem sections). Emerse young **leaves**: petiole pubescent, glabrescent; lamina herbaceous, the young and immature ones at the base on both sides variably densely hairy (especially on veins), always green (not visible in herbarium collections). **Peduncle** below the inflorescence pubescent. **Bract beneath each flower** boat shaped or spatulate, pubescent (abaxial and fringes), upper half 1–2× as wide as the lower half adnate to the peduncle, green (not visible in herbarium collections). **Stamens, filaments** 3–4 mm long.

Saururus chinensis:

Emerse **stem** glabrous. Emerse young **leaves**: petiole glabrous or fimbriate at the base; lamina slightly leathery, sometimes upper leaves reported whitish (not visible nor mentioned in herbarium collections), the young and immature ones glabrous or on the upper side at the base on the veins with a few hairs. **Peduncle** below the inflorescence glabrous or rarely at the adaxial base with a tuft of hairs. **Bract beneath each flower** spatulate,



Figure 2. Habit showing interconnected shoots of previous year and current growth season *S. chinensis* × *cernuus* (Valkenburg 4472).

fimbriate, upper half ca. 3–4× as wide as the lower half adnate to the peduncle, white (not visible in herbarium collections). **Stamens, filaments** ca. 1 mm long.

Saururus chinensis × *cernuus*:

Emerse **stem** glabrous to minutely hairy. Emerse young **leaves**: petiole glabrous to minutely hairy; lamina slightly leathery, upper leaves green, the young and immature ones glabrous or on the upper side at the base on the veins with a few hairs. **Peduncle** below the inflorescence glabrous to sparsely pubescent. **Bract beneath each flower** spatulate, fimbriate, upper half ca. 3× as wide as the lower half adnate to the peduncle, white. **Stamens, filaments** 1.5–2 mm long (Figures 2, 3)

Pollen viability

Inflorescences of Valkenburg 4509 and 4514 were obtained on September 3rd 2025, approximately a year after collecting the voucher specimens for the molecular work. Valkenburg 4509 was still flowering, showing many white, unopened anthers (as in Figure 3 upper part of inflorescence). The anthers of this specimen tested 50–70% viability (Figure 4). Valkenburg 4514



Figure 3. Detail of inflorescence *S. chinensis* × *cernuus* (Valkenburg 4472).

was beyond flowering, showing only brown (as in Figure 3 lower part of inflorescence) and opened anthers. However, some pollen was still present, and these tested partly as viable as well.

Discussion

All plants naturalized so far have proven to be of hybrid status, linking their occurrence to the ornamental plant trade.

Potential to become invasive

Rooted at the margins of waterbodies, the hybrid species can establish large floating mats in the absence of active management, especially in ponds and slow flowing waters as observed in Belgium (Patinet et al. 2023). This has so

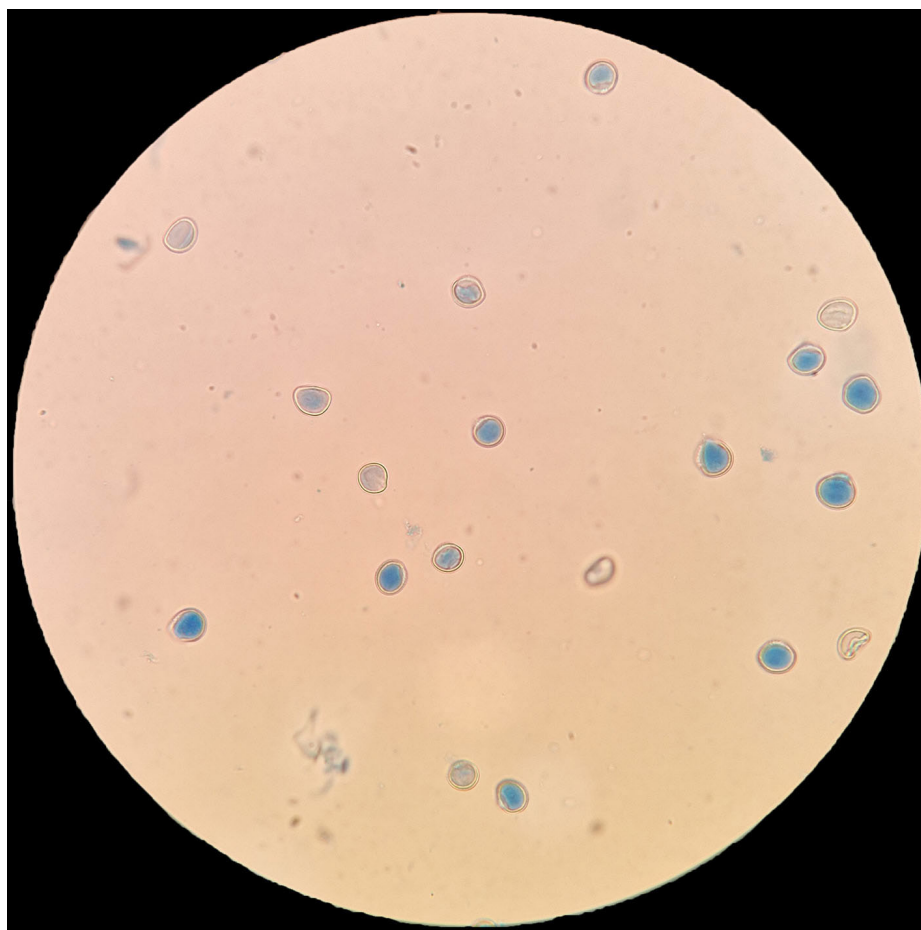


Figure 4. Pollen of Valkenburg 4509 stained with lactophenol cotton blue, showing ca. 50% viable pollen (blue coloured).

far not been observed in the Netherlands, where all locations were subject to periodic management, consisting of mechanical removal of excess plant growth obstructing waterflow. The reports from France of the removal of a patch of approximately 70 square meters of *Saururus cernuus* on the banks of the Loire River (Sarat et al. 2018) may also refer to the hybrid taxon.

Hybrid status preventing effective fruit set?

Vegetative reproduction by extension of the rhizome and fragmentation appear to be the principal means of potential spread in Belgium (Patinet et al. 2023). No seedlings have been observed so far. Whether the plants produce potentially viable seed has not been studied. However, pollen is 50–70% viable, and ovaries seem to be growing which could imply that fertilization has been successful. Reproduction in horticulture at present is exclusively by vegetative propagation, although at some stage the hybrid must have evolved in horticulture. The native range of the parental species does not overlap, with *S. cernuus* confined to North America and *S. chinensis* confined to eastern Asia. At some point they must have been grown together and somehow a resulting seed must have produced a more desirable healthy plant that was selected for further production.

Hybrid vigour

A comparative study of the growth of both parental species and their hybrid could help determine whether hybrid vigour contributes to the successful establishment of these plants in the Netherlands and Belgium. Likewise, this potential hybrid vigour may explain why the plants found in horticulture are hybrids. One public record of *Saururus chinensis* (GenBank accession MK134898) shows 100% similarity in cpDNA sequences to the hybrids identified in this study, raising the question of whether this record represents a true non-hybrid individual or potentially the maternal ancestor of the hybrid lineage.

Observations on naturalised *Saururus* populations, using the morphological key, in other European countries would be helpful to further clarify the proper identity of naturalised *Saururus* in Europe. If it would turn out to be not just the hybrid, then comparisons could potentially be made within and between different taxa, in various sites, concerning invasiveness.

Authors' contribution

Research conceptualization: JV; sample design and methodology: JV, LP, FV; investigation and data collection: JV, LD, FV, KS, WH; data analysis and interpretation: LP, LD, JB, JV; ethics approval: NVT; funding provision: NVT; roles/writing – original draft: JV, LP; writing – review and editing: LD, FV, JB.

Authors' ORCIDs

Johan L.C.H. van Valkenburg: [0000-0001-7281-7819](https://orcid.org/0000-0001-7281-7819); Laurens F. Piet: [0000-0002-2195-1150](https://orcid.org/0000-0002-2195-1150); Helena Duistermaat: [0000-0002-0931-030X](https://orcid.org/0000-0002-0931-030X); Filip Verloove: [0000-0003-4144-2422](https://orcid.org/0000-0003-4144-2422); Jesse L. Beyer: [0000-0002-6685-6421](https://orcid.org/0000-0002-6685-6421); Kevin Scheers: [0000-0002-4756-4247](https://orcid.org/0000-0002-4756-4247).

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