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First records of *Stenochrus portoricensis* Chamberlin, 1922 (Schizomida: Hubbardiidae) from Meise Botanic Garden (Belgium), with morphological and molecular data

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Abstract. Several females and immature specimens of the short-tailed whipscorpion *Stenochrus portoricensis* Chamberlin, 1922 (Schizomida: Hubbardiidae) were collected in the "anthropogenic biome" glasshouse at the Meise Botanic Garden, constituting the first records of the species in Belgium. Specimens were identified based on both morphological and molecular characters. This facultatively parthenogenetic arachnid was described from Puerto Rico but is apparently very adaptable and easily spread, as it is now known from many South and North American countries in the wild, and from several European countries where it is mainly found in glasshouses. The risk of *S. portoricensis* becoming permanently established and its potential invasiveness are discussed.

Keywords: Arachnida, DNA barcoding, glasshouse, introduced species, short-tailed whipscorpions.

Introduction

The arachnid order Schizomida Petrunkevitch, 1945, commonly called short-tailed whipscorpions, currently comprises two families, 78 genera, and 389 extant species (World Schizomida Catalog 2025). Within the most diverse family, i.e., the Hubbardiidae Cook, 1899, the genus *Stenochrus* Chamberlin, 1922 was recently redefined (Monjaraz-Ruedas et al. 2019) and currently contains ten species, mainly occurring in North and Central America (World Schizomida Catalog 2025). Among schizomids, *Stenochrus portoricensis* Chamberlin, 1922**,** appears to be the most widespread species (Reddell & Cokendolpher 1995). This species is native to Central America and was first described from Coamo Springs, Puerto Rico (Giltay 1935, Monjaraz-Ruedas et al. 2022). Its Central American distribution comprises Panama, Costa Rica, Nicaragua, Honduras, Guatemala, Belize, Mexico, Cuba, Jamaica, the Dominican Republic, Puerto Rico, Saint-Barthélemy, and Dominica. The species also occurs in Florida (U.S.A.), the U.S. Virgin Islands, and the Bermuda Islands. Populations of *S. portoricensis* have also been reported from South American countries, including Venezuela, Colombia, Brazil, Ecuador, and the Galapagos Islands (Reddell & Cokendolpher 1995, Teruel & Questel 2019, Villarreal et al. 2023). In South America, it is considered non-native (de Armas 2010, Hernandes et al. 2023, Villarreal et al. 2023). Non-native populations also occur in Europe. Outdoor records have only been reported from the Canary Islands, Spain (Oromí & Martin 1992). In Spain, it has also been found inside an aqueduct (Barranco et al. 2014). In other European countries *S. portoricensis* has been reported from glasshouse environments in botanical gardens in the UK (Cloudsley-Thompson 1949, Reddell & Cokendolpher 1995), France (Ferrand & Noël 2024), the Netherlands (Noordijk & Heijerman 2022), Denmark (Craig et al. 2024), Germany (de Armas & Rehfeldt 2015, Krajčovičová et al. 2021), Switzerland (Lauterbach et al. 2020), Poland (Zawierucha et al. 2013), the Czech Republic (Korenko et al. 2009, Sentenská & Líznarová 2010), and Slovakia (Christophoryová et al. 2013).

Stenochrus portoricensis is an adaptable species and occurs in a wide variety of habitats. It has been found under rocks, logs, tree bark, and amongst leaf litter and bromeliads in humid forests. The species also occurs in caves and endogean habitats, in burrows and crevices in soil, sometimes in association with ants and termites (Brach 1976, Reddell & Cokendolpher 1995, Hernandes et al. 2023, Villarreal et al. 2023). In South America, it has been found in dry tropical forest, including anthropized forest fragments, and in urban areas (Villarreal et al. 2023). *Stenochrus portoricensis* is a highly active carnivorous species. It prefers fresh dead or wounded invertebrates as prey, unless the prey items are very small. Small termites, Psocoptera, and Zoraptera were its favorite food items in captivity (Brach 1976). Natural enemies of *S. portoricensis* are unknown, except the amblypygid *Phrynus marginemaculatus* C.L. Koch, 1841 (de Armas 1989). *Stenochrus portoricensis* is believed to be facultatively parthenogenetic because males are very rare. Males have only been reported from Nicaragua, Guatemala, Mexico, Cuba, the Dominican Republic, and Puerto Rico. In all other countries throughout its range, only females have been found (Reddell & Cokendolpher 1995, Villarreal et al. 2023). Reproduction of *S. portoricensis* may be seasonal, as Brach (1976) first found females with embryos in August, followed by exceptionally large numbers of second-instar larvae. The embryos are attached to the underside of the abdomen of the female in a loosely compacted mass. Females of *S. portoricensis* may carry 1 to 16 eggs and 1 to 15 larvae (Giribet & Moreno-González 2021). *Stenochrus portoricensis* appears to produce few but relatively large larvae. In laboratory culture, the larvae were

found to be sticky on their ventral surfaces and were completely motionless. Attachment of the larvae to the parent is accomplished by means of the modified tarsi and the sticky ventral surface. There are five post-embryonic molts in *S. portoricensis*. The post-larvae closely resemble the parent, but are not as heavily sclerotized and are therefore much paler in color (Brach 1976). Because of its ability to attain high abundances (Brach 1976, Barranco et al. 2014, Monjaraz-Ruedas et al. 2022), its aforementioned adaptability, and its carnivorous lifestyle, it is important to monitor the presence and spread of *S. portoricensis* throughout Europe. Here, we report the first records of *S. portoricensis* in Belgium.

Materials and methods

The records of *Stenochrus portoricensis* reported here constitute incidental finds obtained during surveys for non-native land planarians and finds during targeted searches by the authors in Meise Botanic Garden. Schizomida observations posted on iNaturalist by citizen scientists were also included. Initial species identification was based on the descriptions of diagnostic features provided by Hernandes et al. (2023), using a Zeiss Discovery.V12 and an Olympus BX50 microscope. Diagnostic features were photographed using a BRESSER MikroCam II 20 MP (1″ sensor) microscope camera mounted on a motorized Leica MZ16A stereomicroscope. Images were captured and controlled using MikroCam Lab II software, and focus stacks were acquired for each specimen. Subsequently, these stacks were merged into fully focused composite images using Helicon Focus 8.3.0 (method B (depth map), settings: radius = 15; smoothing = 2). The female genitalia were removed from the abdomen and digested using half a tablet of Total Care Enzima product (Abbott Medical Optics, Santa Ana, CA) in a few milliliters of distilled water for three to four hours, then immersed back in 70 % ethanol to be photographed. Voucher specimens are deposited in the collection of the Royal Belgian Institute of Natural Sciences (RBINS IG N°34970/01 – 08).

Material examined

BELGIUM • 1 ♀; Meise Botanic Garden, "anthropogenic biome" glasshouse; 50°55'29.1"N 4°19'49.3"E; 5 February 2024; Tom Van den Neucker and Jan Soors leg.; in thick layer of dead leaves; GenBank no.: PV549431 (COI); PV549693 (28S); PV575025 (H3); RBINS IG N°34970/01 • 2 ♀♀; same data as for preceding; RBINS IG N°34970/02 • 1 ♀; same data as for preceding; 20 March 2024; Tom Van den Neucker leg.; GenBank no.: PV549432 (COI); PV549694 (28S); PV575026 (H3); RBINS IG N°34970/03 • 5 ♀♀; same data as for preceding; RBINS IG N°34970/04 • 1 ♀; same data as for preceding; 3 November 2024; Tom Van den Neucker and Jan Soors leg.; GenBank no.: PV549433 (COI); PV549695 (28S); RBINS IG N°34970/05 • 1 ♀; same data as for preceding; RBINS IG N°34970/06 • 1 ♀; same data as for preceding; 29 January 2025; Sonja Deneve leg.; GenBank no.: PV575027 (H3); RBINS IG N°34970/07 • 1 immature; same data as for preceding; RBINS IG N°34970/08 • 2 ♀♀; same data as for preceding; 25 July 2025; Arnaud Henrard leg.; RBINS IG N°34970/09.

DNA-based species identification

To validate the morphological species identification of the specimens collected at Meise Botanic Garden, the individual DNA of four specimens was extracted from either two legs or the entire body, excluding the abdomen (to preserve species descriptive characteristics), using the NucleoSpin DNA Insect kit (Macherey-Nagel) and following the manufacturer's instructions (elution volume: 50 µL). The tissue was homogenized beforehand using a BeadBeater.

Three DNA fragments were targeted: the cytochrome *c* oxidase subunit I (COI), the 28S ribosomal RNA (28S), and the histone 3 (H3), amplified using different primer pairs and PCR cycling conditions (Appendix). All amplifications were performed in 20 µL reaction mixtures, including 2 µL of DNA template, 2 µL of 10X buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.4 µM of each primer (or 0.8 µM if containing degenerated nucleotides), and 0.03 units/µL of Platinum™ Taq DNA Polymerase (Invitrogen™). Amplicons and negative controls were checked on a 2% agarose gel using a UV transilluminator and the MidoriGreen™ Direct (NIPPON Genetics Europe) method. Positive amplifications were subsequently purified using the ExoSAP-IT™ protocol (following manufacturer's instructions) and sequenced in both directions using the BigDye® chemistry (outsourced to Macrogen™ Europe).

Afterwards, the DNA extracts were dried using the GenTegra technology and stored at room temperature at the Royal Belgian Institute of Natural Sciences (RBINS).

Assembled bidirectional nucleotide strands were trimmed, corrected, and translated into amino acids to check for stop codons (if coding region) using Geneious Prime® 2019.2.3 (Biomatters Ltd.). A consensus sequence was generated for each DNA fragment and each specimen. Subsequently, consensus sequences were compared using the BOLD Identification System for COI, with the Species Level Barcode Records option (www.boldsystems.org), and the Basic Local Alignment Search Tool (BLAST) of GenBank for 28S and H3 (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Subsequently, a Neighbour-Joining (NJ) tree (Tamura-Nei distance model, 1000 bootstrap replicates) was constructed to examine the clustering support of available and generated sequences of each species of the *Stenochrus* genus. The tree was constructed based on an alignment of COI sequences downloaded from BOLD (publicly available) and GenBank. These were aligned with the generated consensus sequences and two outgroup sequences using MUSCLE in Geneious Prime®. Sequences of less than 400 base pairs (bp) and duplicate sequences per species and per country of origin were discarded, except for the generated sequences. The final alignment included 105 COI sequences and was trimmed to retain an 875 bp overlapping region.

Results

Observations

On 5 February 2024, three specimens of *Stenochrus portoricensis* were collected in the "anthropogenic biome" glasshouse at Meise Botanic Garden. Subsequent collections included six specimens on 20 March 2024, two on 3 November 2024, two on 29 January 2025, and two more on 25 July 2025. Some additional Schizomida were observed, i.e. on 12 December 2024 (posted on iNaturalist on 13 December 2024), and on 19 December 2024 (posted on iNaturalist on 19 December 2024 and 27 May 2025 respectively); one immature specimen was observed and posted on iNaturalist on 29 January 2025, one adult female was observed and sampled on 29 January 2025 (posted on iNaturalist same date) and finally an immature specimen was observed and sampled on 29 January 2025 (posted on iNaturalist on 31 January 2025) (Table 1). The "anthropogenic biome" glasshouse in Meise has a tropical regime, with humidity kept above 70 %. On each sampling occasion conducted by the authors, one or more specimens were observed amongst a thick layer of dead leaves in a border with several plants (Fig. 1A-B), including *Spathiphyllum wallisii*, *Coffea arabica*, *C. liberica,* and *Tamarindus indica*.

Morphological identification

The external morphology of the *S. portoricensis* specimens

found in Belgium (see Figs 1C-E, 2A-C) is in accordance with the diagnostic features listed by Hernandes et al. (2023): the anterior process of the propeltidium bears one pair of setae medially (longitudinally) aligned and two setal pairs; a short spur is present on the prolateral face of the pedipalps' trochanter (Fig. 2D); the cheliceral movable fangs are smooth, provided without teeth (Fig. 2E-F); the flagellum is short with three annuli (Fig. 2G-H); the metapeltidium is entire; the spermathecae have two pairs of elongated, wrinkled lobes, the outer lobes shorter, and laterals about 4-5 times shorter than the median ones (Fig. 3A-D).

Remark: Although Ferrand & Noël (2024) described the presence of a short spur on the prolateral face of the pedipalps' trochanter in the text, the arrow in their fig. 5b is misplaced and appears to point to an artifact on the palpal femora. However, the trochanter spur is clearly visible in their photograph.

DNA-based species identification

The generated sequences (COI: 1,009 bp; 28S: 680 - 797 bp; H3: 303 - 328 bp) were identical across the processed specimens. They were deposited in GenBank with accession numbers: COI: PV549431-PV549433; 28S: PV549693-PV549695, and H3: PV575025-PV575027. To note: the Sanger sequencing of the amplicons generated using the universal primer combination LCO1490–HCO2198 systematically resulted in low-quality sequences, especially in the region located between 160 and 310 bp, while the alternative COI primer combination C1–J–1718–spider / C1–N–2776–spider provided high-quality strands.

Using the Identification System of BOLD for COI, and the BLAST tool on GenBank for 28S, high matches (99.53 - 100%) were obtained with representatives of the species *Stenochrus portoricensis* (Table 2). The low similarity matches obtained in GenBank for H3 result from gaps in the reference database; viz., there are presently no H3 sequences published online for the *Stenochrus* genus.

The NJ tree construction further supports these results, with all generated COI sequences clustering with maximal support with other *Stenochrus portoricensis* sequences downloaded from the online DNA repositories (BOLD and GenBank) (Fig. 4). The DNA-based investigation validates the morphological identifications of the specimens collected at Meise Botanic Garden.

Table 1. List of Schizomida observations and collected samples posted on iNaturalist (https://www.inaturalist.org).

Date	iNaturalist observations	RBINS IG N°
12 December 2024	https://www.inaturalist.org/observations/254938744	not collected
19 December 2024	https://www.inaturalist.org/observations/255601250	not collected
19 December 2024	https://www.inaturalist.org/observations/284544511	not collected
29 January 2025	https://www.inaturalist.org/observations/259993417	not collected
29 January 2025	https://www.inaturalist.org/observations/259994672	34970/07
29 January 2025	https://www.inaturalist.org/observations/260167321	34970/08

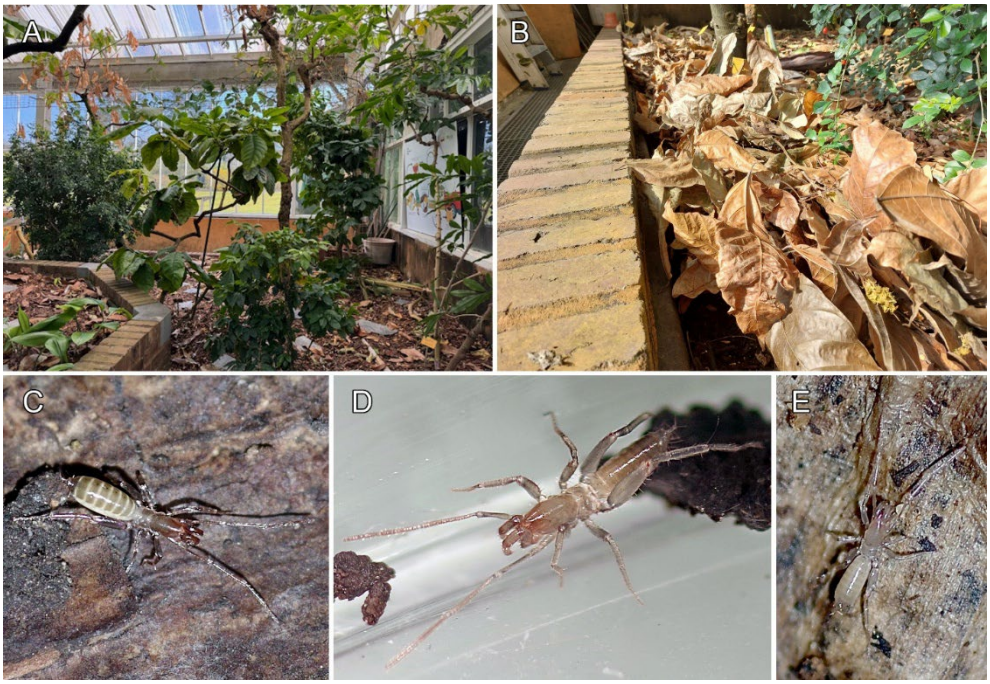

Figure 1. *Stenochrus portoricensis* Chamberlin, 1922, living specimens observed in the "anthropogenic biome" glasshouse of Meise Botanic Garden, Belgium. A-B. Habitat. C-D. Female specimens. E. Immature specimen - not collected. Photos by Sonja Deneve.

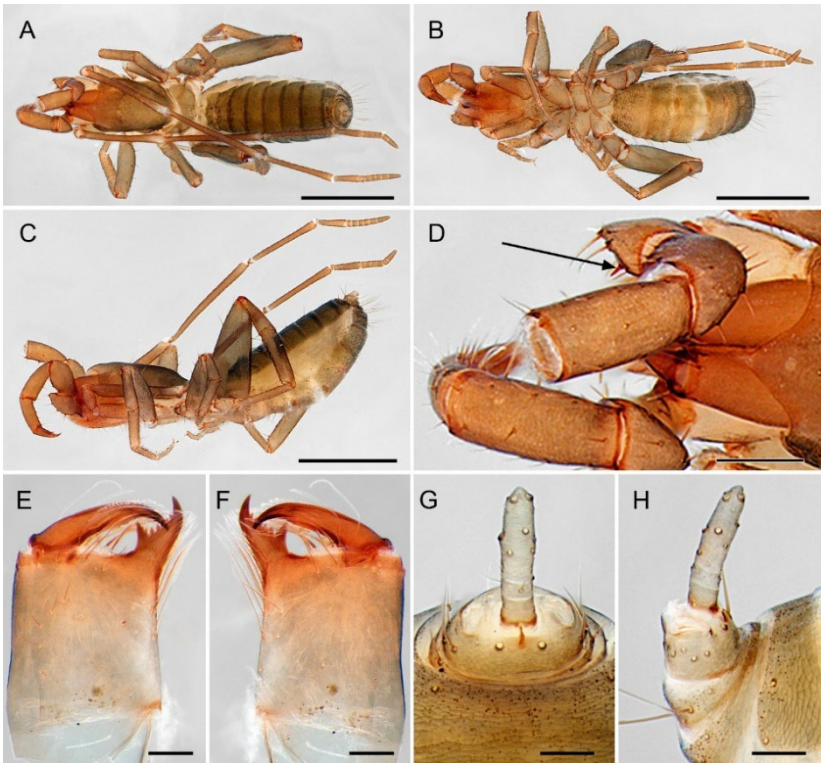

Figure 2. *Stenochrus portoricensis* Chamberlin, 1922, morphological characters of the female (A-D: RBINS IG N°34970/07; E-F: RBINS IG N°34970/04; G-H: RBINS IG N°34970/06). A. Habitus, dorsal view. B. Idem, ventral view. C. Idem, lateral view. D. Detail view of the pedipalp, the arrow pointing to the prolateral spur of the trochanter. E. Right chelicerae, retrolateral view. F. Idem, prolateral view. G. Flagellum, dorsal view. F. Idem, lateral view. Scale bars: A-C = 1 mm; D = 0.2 mm; E-H = 0.1 mm.

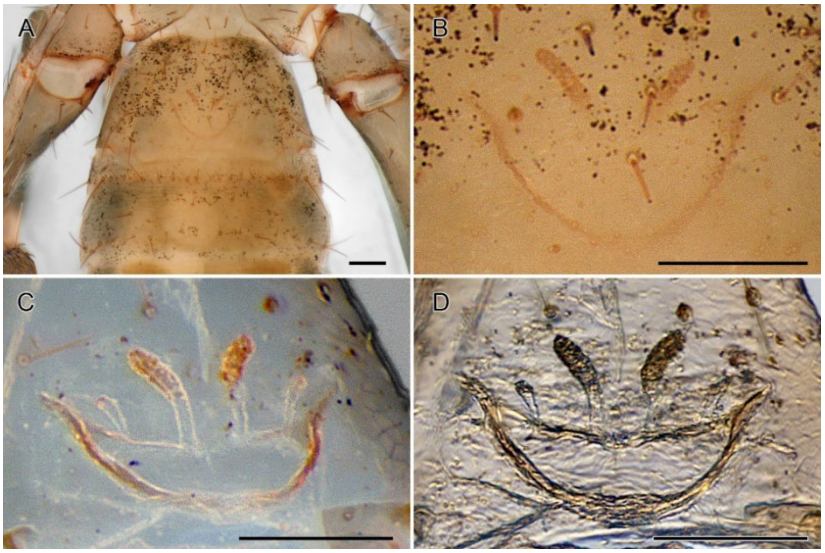

Figure 3. *Stenochrus portoricensis* Chamberlin, 1922, female genitalia (RBINS IG N°34970/07). A. anterior part of abdomen, ventral view. B. Idem, close view of the genitalia visible through the tegument. C. Cleared vulva, reflected light. D. Idem, transmitted light. Scale bars: 0.1mm.

Table 2. Results of the match in BOLD (closest similarity expressed in percentages) for COI, and using GenBank blast (pairwise identity expressed in percentages) for 28S and H3 DNA fragments, with GenBank accession numbers of the closest matches. na: no generated sequence.

	COI BOLD similarity (%)	28S GenBank similarity (%)	H3 GenBank similarity (%)
RBINS IG N°34970/01	*Stenochrus portoricensis* 99.53%; OK641861	*Stenochrus portoricensis* 100%; MN020555	*Baalrog sbordonii* 93.7%; KY018263
RBINS IG N°34970/03	*Stenochrus portoricensis* 99.53%; OK641861	*Stenochrus portoricensis* 100%; MN020555	*Baalrog sbordonii* 93.4%; KY018263
RBINS IG N°34970/05	*Stenochrus portoricensis* 99.68%; OK641861	*Stenochrus portoricensis* 100%; MN020555	na
RBINS IG N°34970/07	na	na	*Baalrog sbordonii* 93.7%; KY018263

Figure 4. Neighbour-Joining tree including eight species belonging to the genus *Stenochrus* based on the cytochrome *c* oxidase subunit I (COI), with four sequences of *Stenochrus portoricensis* renamed as *Stenochrus longimanus* (Rowland, 1971), misidentified, and five sequences as *Stenochrus cavernicolens* (Chamberlin & Ivie, 1938), misidentified, following the results of the study of Monjaraz-Ruedas et al. (2022) (Tamura-Nei distance model; 875 bp fragment; 1000 bootstrap replicates). The bootstrap values are shown at the branch points (COI sequences of query specimens highlighted in blue), and two outgroup sequences of *Bamazomus* were included to root the tree (GenBank accession numbers OR859584 and KY573419). The minimum branch support displayed is 50; other branches were collapsed.

Discussion

The observations of *Stenochrus portoricensis* from Meise Botanic Garden reported here constitute the first records of the species in Belgium. Multiple records throughout 2024 and 2025 suggest that the species is firmly established in the "anthropogenic biome" glasshouse in Meise. It is highly likely that *S. portoricensis* reached Belgium via plant exchange with other European botanical gardens. Its widespread occurrence in European botanical gardens suggests that it is easily transferred with potted plants or potting soil (Cloudsley-Thompson 1949, Korenko et al. 2009, Christophoryová et al. 2013, Zawierucha et al. 2013, de Armas & Rehfeldt 2015, Lauterbach et al. 2020, Krajčovičová et al. 2021, Noordijk & Heijerman 2022, Craig et al. 2024, Ferrand & Noël 2024). This is probably also the case with other non-native schizomids

recorded in European glasshouses. These include *Bamazomus serendipitus* Craig, 2024, which has recently been newly described from specimens found in a glasshouse in Denmark (Craig et al. 2024), *Schizomus crassicaudatus* (O. Pickard-Cambridge, 1872) observed in France (Reddell & Cokendolpher 1995), and *Zomus bagnallii* (Jackson, 1908), which is known to occur in glasshouses in the Netherlands, Germany, and the United Kingdom (Reddell & Cokendolpher 1995, de Armas & Rehfeldt 2015, Noordijk & Groothuis 2023).

Although *Stenochrus portoricensis* is an adaptable species, capable of surviving in a variety of habitats (Brach 1976, Reddell & Cokendolpher 1995, Hernandes et al. 2023, Villarreal et al. 2023), its invasive potential in outdoor environments in Belgium is likely low, primarily limited by cold winter temperatures, which contrast with its native tropical ranges. Belgium has a temperate oceanic climate with warm summers but no dry season (Köppen-Geiger climate zone Cfb), whereas in Central and South America, *S. portoricensis* predominantly occurs in tropical rainforest, monsoon, and savannah climates (Köppen-Geiger climate zones Af, Am, and Aw) (Beck et al. 2023). However, its transfer via plants or soil to warmer regions of Europe poses a risk. Since it is a hardy and long-lived (in captivity at least 15 months) carnivorous species, and since it can be very abundant in nature (Brach 1976), the precautionary principle justifies preventive measures. These should include inspection and quarantine of plants and potting soil from botanical gardens known to harbour non-native schizomids.

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Appendix

Table reporting the primers involved for the amplification of the three DNA fragments, i.e. Cytochrome *c* oxidase subunit I (COI), 28S ribosomal RNA and Histone 3 (H3), as well as the applied PCR cycling conditions.

Primer name	Primer sequence	Target DNA fragment	Reference	PCR cycling conditions					
				Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
LCO1490	GGTCAACAAATCATAAAGATATTGG	COI	Folmer et al. 1994	94 °C 3 min	94 °C 30 sec	45 °C 30 sec	72 °C 1 min	/	5
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA			/	94 °C 30 sec	51 °C 1 min	72 °C 1 min	72 °C 10 min	35
C1-J-1718-spider	GGNGGATTTGGAAATTGRTTRGTTCC		Vink et al. 2005	94 °C 5 min	94 °C 45 sec	48 °C 45 sec	72 °C 1 min	72 °C 10 min	30
C1-N-2776-spider	GGATAATCAGAATANCGNCGAGG								
28SF0001	ACCCVCYNAATTTAAGCATAT	28S	Mironov et al. 2012	94 °C 5 min	94 °C 45 sec	50 °C 45 sec	72 °C 1 min	72 °C 10 min	30
28SR0990	CCTTGGTCCGTGTTTCAAGAC								
H3aF	ATGGCTCGTACCAAGCAGACVGC	H3	Colgan et al. 1998	94 °C 5 min	94 °C 40 sec	54 °C 50 sec	72 °C 1 min	72 °C 10 min	40
H3aR	ATATCCTTRGGCATRATRGTGAC								