Turkish Journal of Zoology
http://journals.tubitak.gov.tr/zoology/
Research Article
Turk J Zool
(2013) 37: 357-361

doi:10.3906/zoo-1210-9

First record of the schizomid *Stenochrus portoricensis* (Schizomida: Hubbardiidae) in Poland, with DNA barcode data

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Received: 11.10.2012 • **Accepted:** 19.01.2013 • **Published Online:** 29.04.2013 • **Printed:** 29.05.2013

Abstract: Several specimens of the tropical schizomid *Stenochrus portoricensis* Chamberlin, 1922 (family Hubbardiidae) were found in the Poznań Palm House, representing the first record of micro-whip scorpions (Schizomida) from Poland. *Stenochrus portoricensis* is an alien species in temperate climates, and has been accidentally introduced to greenhouses via potted plants. Its parthenogenetic lifestyle allows for the easy transfer of populations to new locations and the successful founding of new colonies. New DNA sequence data for *Stenochrus portoricensis* are presented, which comprise a fragment of the cytochrome c oxidase subunit I gene (DNA barcode sequence) from mitochondrial DNA, and a fragment of the 28S rRNA gene (D1–D3 regions) from nuclear DNA.

Key words: Micro-whip scorpions, alien species, artificial habitat, greenhouse, introduced animals, DNA barcode, COI, 28S rDNA

1. Introduction

Many different types of arachnids have successfully dispersed to new locations around the globe via passive associations with humans. The majority of these successful introductions result from their predatory lifestyle and their predominance in terrestrial habitats (Nedvěd et al., 2011). Some species are able to become acclimatized to their new situation and live in natural or seminatural environments. Others, however, cannot survive without environmental conditions that mimic their original habitat, of which greenhouses represent the most obvious example. Greenhouses create adequate conditions (temperature, light, moisture, and soil) and protected habitats for the acclimatization of alien species. Moreover, in the absence of natural enemies and pathogens, alien species are capable of developing and spreading successfully (Teodorescu and Matei, 2010). Among 3805 species of known arachnids inhabiting natural environments in Poland, only 10 are considered to be alien species (Głowaciński and Pawłowski, 2010). The first comprehensive studies of the alien arachnids (including only Acari) of greenhouses in Poland took place in upper Silesia. Skubała et al. (2001) revealed that only 13% of the detected mites (Oribatida) were alien species. In contrast, approximately 50% of the ptyctimous mites in the Poznań Palm House were alien (Niedbała, 2010).

The Palm House in Poznań was founded in 1910–1911. The plant collection contains more than 1000 species. At present, the Palm House is divided into 9 pavilions where representatives of different groups of vegetation are displayed (e.g., tropical, subtropical, temperate, aquatic). Specimens of *Stenochrus portoricensis* were found in pavilions with tropical rainforest undergrowth vegetation. The plants in the Palm House have been imported from other greenhouses located in Poland, as well as from the Netherlands, Germany, and from natural habitats elsewhere, e.g., *Copernicia macroglossa* H. Wendl. ex Becc. from Cuba. The first studies on the invertebrate fauna of the Poznań Palm House were performed in 1932 by Moszyński and Urbański, whose findings were extended by other researchers. The studies focused on fauna in general (Urbański, 1950; Brzezińska, 1952), on soil fauna (Michalak, 2006), and on individual taxa: Isopoda (Urbański, 1947), Formicidae (Pisarski, 1957), Araneae and Opiliones (Woźniczko, 1966), Turbellaria and Nemeritini (Kolasa, 1973), aquatic Oligochaeta (Legeżyński, 1974), and ptyctimous mites (Niedbała, 2010). Additionally, Wiśniewski and Hirschmann (1991a, 1991b) described 3 new species of mites. Although 12 papers on the invertebrate fauna of the Poznań Palm House have been published so far, our knowledge of the biodiversity of the greenhouses in general is still very poor.

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During further investigations into the invertebrate fauna of the Palm House, several specimens of schizomids were found amongst leaf litter and rotting wood. Schizomida, also known as micro-whip scorpions, are small arachnids mainly inhabiting the tropical and subtropical regions of the world (Reddell and Cokendolpher, 1995; Harvey, 2003; Beccaloni, 2009). Currently, the order contains 286 species in 53 genera (updated from Harvey, 2003, 2007), but only 3 species have been reported from Europe, and all 3 represent exotic introductions. *Schizomus crassicaudatus* (O.P. Cambridge, 1872) was originally described from Sri Lanka and later recorded from greenhouses in the Muséum national d'Histoire naturelle, Paris, France (Simon, 1896). *Zomus bagnallii* (Jackson, 1908) was originally described from greenhouses in the Royal Botanic Gardens, Kew, England (Jackson, 1908), and has since been found to be widespread throughout the Indo-Pacific region (e.g., Reddell and Cokendolpher, 1995; Harvey, 2001, 2010; Villarreal, 2010). *Stenochrus portoricensis* Chamberlin, 1922 is widespread throughout tropical North and South America, ranging from Florida to southeastern Brazil (e.g., Rowland and Reddell, 1980; Reddell and Cokendolpher, 1995; Harvey, 2003; Santos et al., 2008), with populations also recorded from the Canary Islands, United Kingdom, and the Czech Republic (Cloudsley-Thompson, 1949; Oromi and Martin, 1992; Reddell and Cokendolpher, 1995; Korenko et al., 2009; Sentenská and Líznarová, 2010). Jackson (1910) reported on an apparently new species of schizomid from a hothouse in Penrith, northern England, the identity of which has not yet been confirmed. It is likely to also be a population of *S. portoricensis*.

The specimens collected in Poznań represent the first record of the order Schizomida from Poland. Apart from recording their presence, we also report on newly obtained mitochondrial and nuclear molecular sequence data of *S. portoricensis*.

2. Materials and methods

2.1. Materials

Twenty-four specimens of *S. portoricensis* were collected from soil, wood, and leaf litter by handpicking, sieving, and Barber pitfall sampling in 3 pavilions containing tropical vegetation in the Poznań Palm House. All specimens were preserved in 75% ethanol, and are now deposited in the Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Poznań, Poland (AMUP), and the Western Australian Museum, Perth, Australia (WAM).

2.2. Molecular methods

We selected the 3 best preserved specimens from which to obtain sequence data. Total genomic DNA was extracted from the specimens using a nondestructive method, as described by Dabert et al. (2008). A 681-bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified with primers bcdF01 (5'-CATTTTCHACTAAYCATAARGATATTGG-3') and bcdR04 (5'-TATAAACYTCDGGATGNCCAAAAAA-3') (Dabert et al., 2010); an 850-bp fragment of the nuclear 28S rDNA was amplified with primers 28SF0001 (5'-ACCCVCYNAATTTAAGCATAT-3') and 28SR0990 (5'-CCTTGGTCCGTGTTTCAAGAC-3') (Mironov et al., 2012). PCRs were carried out in 5-µL reaction volumes containing 2.5 µL Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany), 0.25 µM of each primer, and 1 µL of DNA template, using a thermocycling profile of 1 cycle of 5 min at 95 °C followed by 35 steps of 30 s at 95 °C, 90 s at 50 °C, 1 min at 72 °C, with a final step of 5 min at 72 °C. After amplification, the PCRs were diluted with 10 µL of water, and 5 µL of the diluted PCR reaction was analyzed by electrophoresis on a 1% agarose gel. The amplicons were directly sequenced in the forward direction by using 1 µL of the PCR reaction and 50 pmol of sequencing primer. Sequencing was performed with a BigDye Terminator (v. 3.1) on an ABI Prism 3130XL Analyzer (Applied Biosystems). Contigs were aligned and manually assembled in ChromasPro v. 1.32 (Technelysium Pty, Ltd.), and converted to amino acids in GeneDoc v. 2.7.000 (Nicholas and Nicholas, 1997). The resulting sequences were compared with the GenBank database (blast.ncbi.nlm.nih.gov) using the blastn and discontiguous megablast programs (Zhang et al., 2000).

3. Results

***Stenochrus portoricensis* Chamberlin, 1922**

(Figures 1, 2, 3)

Stenochrus portoricensis Chamberlin, 1922: 11–12.

Schizomus antilus Hilton, 1933: 91–92 (synonymized by Rowland and Reddell, 1980: 14).

Schizomus cavernicolens Chamberlin and Ivie, 1938: 102, figures 4–7 (synonymized by Rowland and Reddell, 1977: 87).

Figure 1. *Stenochrus portoricensis* dorsal view.

Figure 2. *Stenochrus portoricensis* lateral view.

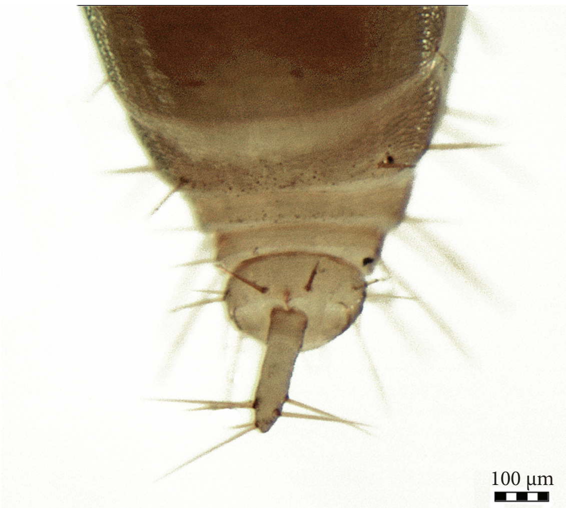

Figure 3. Flagellum of *Stenochrus portoricensis*.

Schizomus floridanus Muma, 1967: 18–20, figures 13–15 (synonymized by Rowland and Reddell, 1977: 87).

Schizomus longimanus Rowland, 1971: 119–120, figures 4–6, 17 (synonymized by Rowland and Reddell, 1977: 87).

Schizomus loreto Armas, 1977: 5–7, figures 3, 4a–d (synonymized by Armas, 1989: 23).

Full synonymy to 2002 can be found in Harvey, 2003: 125.

Specimens examined: POLAND: Poznań Palm House (pavilions: V, VI, and VIII), January–April 2012, 18 specimens (11 females and 7 juveniles) in AMUP and 6 specimens (1 female and 5 juveniles) in WAM.

Diagnosis: *Stenochrus portoricensis* differs from all other species of the genus, except *S. davisi* (Gertsch, 1940), *S. guatemalensis* (Chamberlin, 1922), *S. leon* Armas, 1995, *S. mexicanus* (Rowland, 1971), *S. mulaiki* (Gertsch, 1940), *S. palaciosi* (Reddell and Cokendolpher, 1986), *S. pecki* (Rowland, 1973), and *S. reddelli* (Rowland, 1971), by the presence of only 2 pairs of dorsal propeltidial setae. Females can be distinguished from those of these species by the heavily sclerotized median spermathecae, which are visible through the cuticle of sternite II, and males by seta dm4 of the flagellum being situated on approximately the same level as dl1 (Rowland and Reddell, 1980; Reddell and Cokendolpher, 1986; Armas, 1995).

Sequence data: We sequenced a 681-bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene (DNA barcode region chosen by the Consortium for the Barcode of Life, http://barcoding.si.edu) and an 850-bp fragment of the nuclear 28S rDNA, including the hypervariable D1–D3 regions of 3 females of *S. portoricensis* (GenBank Acc. numbers JX280413–15, JX280416, respectively). No intraspecific variation was found in the COI nucleotide sequences, and no frame shift was observed after conversion of these sequences into amino acids. Sequence data for *S. portoricensis* have been previously reported for several gene regions (e.g., Giribet and Ribera, 2000; Giribet et al., 2002; Regier et al., 2010), but our data represent the first records of DNA barcodes for this species: a fragment of COI from mitochondrial DNA, and hypervariable regions D1–D3 from 28S ribosomal RNA gene. The sequences generated for *S. portoricensis* showed nucleotide identities higher than 80% (COI) and 90% (28S rDNA) compared to the other schizomid sequences published in GenBank. One or both of these genes have been obtained for other schizomids including *Hubbardia pentapeltis* Cook, 1899 (Giribet et al., 2002), *Bamazomus* sp., *Brignolizomus woodwardi* (Harvey, 1992), *Draculoides* spp. and *Paradraculoides* spp. (Harvey et al., 2008), and an unidentified schizomid (Arabi et al., 2012).

4. Discussion

The specimens of *S. portoricensis* recorded from the Poznań Palm House represent the first record of this species from Poland, and one of the few records of Schizomida from Europe. Previous records include populations from the Canary Islands (Oromi and Martin, 1992), United Kingdom (Cloudsley-Thompson, 1949; Reddell and Cokendolpher, 1995), and the Czech Republic (Korenko et al., 2009; Sentenská and Líznarová, 2010). While the Canary Island population seems to be free-living in natural ecosystems, the other records are confined to hothouses and other artificial habitats.

While many populations of *S. portoricensis* consist only of females and juveniles, populations with adult males have been reported from southern Mexico (Campeche, Chiapas, and Yucatán states), Guatemala, Cuba, Dominican

Republic, and Puerto Rico (Armas, 1977; Rowland and Reddell, 1980; Reddell and Cokendolpher, 1995). Like the European and Brazilian populations, it seems likely that many other populations represent accidental introductions, presumably originally stemming from a bisexual Central American source. The lack of males from most populations led Reddell and Cokendolpher (1995) to suggest that *S. portoricensis* is a facultative parthenogen. A parthenogenetic lifestyle would also allow for the easy transfer of populations to new locations as only single female individuals, either adult or immature, would be needed to found new colonies.

The occurrence of *S. portoricensis* in Poznań is likely to have begun with soil and litter of plants introduced into the Palm House from external sources, similar to the hypothesis suggested by Tourinho and Kury (1999) and Kobelt and Nentwig (2008), who claimed that alien arachnids are most probably introduced with garden plants and container shipments. Greenhouses constitute isolated, inland islands for introduced local populations, and a study using molecular data focusing on migration routes of this species within Europe may prove beneficial. The molecular data presented in this paper of *S. portoricensis* is a first step in this process.

Acknowledgments
We wish to express our sincere thanks to members of the Student Naturalist Society (Invertebrate Section) of Adam Mickiewicz University for their assistance in the collection of specimens. We also thank Michał Śmiłowski and the staff of the Poznań Palm House for their kindness and support during the collection of the samples. The present study was partially supported by the Polish MSHE grant No. N N303 017937.

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