Arachnids (Arachnida: Araneae, Mesostigmata, Pseudoscorpiones) from tropical greenhouses at Rotterdam Zoo (the Netherlands), including a pholcid spider new to Europe

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Arachnids (Arachnida: Araneae, Mesostigmata, Pseudoscorpiones) from tropical greenhouses at Rotterdam Zoo (the Netherlands), including a pholcid spider new to Europe

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Abstract. An inventory of arachnids was conducted in three tropical greenhouses of Rotterdam Zoo, the Netherlands. This was part of a survey in which pitfall traps, banana baits, litter samples, hand searching and beating were used to map arthropod diversity. A total of twenty spider species, one mite taxon and one pseudoscorpion species were collected. Fourteen specimens were used for COI-barcoding, which yielded two of the listed species. Four spider species were recorded for the first time for the Netherlands: *Belisana ambengan* Huber, 2005, *Heteroonops spinimanus* (Simon, 1892), *Nesticella mogera* (Yaginuma, 1972) and *Triaeris stenaspis* Simon, 1891, the first of which is also a new record for Europe and appears to be an extremely rare pholcid species. From the species found, thirteen are non-native to the Netherlands and four of these are considered as expansive, since they have outdoor populations as well. Hand searching yielded the highest number of species, compared to the other four sampling techniques. A species saturation curve shows that in all likelihood not all species present in the surveyed greenhouses have been found during this survey.

Keywords: alien species, *Belisana ambengan*, faunistics, new records, Nesticidae, Oonopidae, Pholcidae

Zusammenfassung. Spinnentiere aus tropischen Gewächshäusern des Zoos in Rotterdam (Niederlande), mit einer Zitterspinne neu für Europa (Arachnida: Araneae, Mesostigmata, Pseudoscorpiones). In drei tropischen Gewächshäusern des Zoos in Rotterdam wurden Spinnentieruntersuchungen durchgeführt. Dies gehörte zu einem Programm in dem mit Bodenfallen, Bananenködern, Streuproben, Handfängen und Klopfproben die Arthropodenvielfalt kartiert wurde. Insgesamt wurden 20 Spinnenarten, ein Milbentaxon und eine Pseudoskorpionart erfasst. 14 Tiere wurde zum COI-Barcoding verwendet, was zwei der aufgeführten Arten ergab. Vier Spinnenarten wurden erstmals für die Niederlande nachgewiesen: *Belisana ambengan* Huber, 2005, *Heteroonops spinimanus* (Simon, 1892), *Nesticella mogera* (Yaginuma, 1972) und *Triaeris stenaspis* Simon, 1891, die erstgenannte ist auch ein Neunachweis für Europa und scheint eine sehr seltene Zitterspinnenart zu sein. Dreizehn der Arten sind nicht in den Niederlanden heimisch und vier davon werden als expansiv eingestuft, weil es von ihnen auch Freilandpopulationen gibt. Mit Handfängen wurden, im Vergleich zu den vier anderen Sammelmethoden, die meisten Arten erfasst. Eine Artensättingungskurve zeigt, dass wahrscheinlich noch nicht alle Arten erfasst wurden, die in den untersuchten Gewächshäusern vorkommen.

Greenhouses can provide a suitable environment for diverse groups of invertebrates. Each tropical greenhouse has its own characteristics caused by various factors, e.g. climate control, management, composition and origins of plant and soil material. Depending on these factors, complex artificial ecosystems can be formed to a certain extent. Greenhouses often import a large number of (tropical) plant species from remote sources, almost always with accompanying soil, resulting in the introduction of species living in or on these substrates. Some of these are able to settle in the greenhouse. Certain alien species that thrive in greenhouses might even become indoor pests or expansive if they manage to establish themselves outdoors (Wang et al. 2015). Additionally, synanthropic species may colonise or be introduced by transport from (nearby) manmade biotopes. Some native species living in natural habitats occasionally enter greenhouses as well (Kielhorn 2008).

A survey of arachnids was carried out in three tropical greenhouses in Rotterdam Zoo. This marks as the first extensive arachnid survey in Dutch tropical greenhouses since 1949 (van der Hammen 1949, 1969a). At that time, Leendert van der Hammen and fourteen other collectors surveyed eleven greenhouses in the midwest and centre of the Netherlands, including Rotterdam Zoo, by hand, searching over a seven-year period. Most greenhouses were, however, visited only once. Thirty-two arachnid species were collected, with only one species, *Zygiella x-notata* (Clerck, 1757), sampled at Rotterdam Zoo (van der Hammen 1949). Currently, only a handful of studies on arachnid faunas of European tropical greenhouses exist, e.g. the Eden Project in England (Snazell & Smithers 2007), several botanical and zoological gardens in the German states Berlin and Brandenburg (Kielhorn 2008, 2009, 2016), the Botanical Garden of the University of Debrecen in Hungary (Pfliegler 2014), the Botanical Garden of the PJ Šafárik University in Slovakia (Šestáková et al. 2017) and multiple zoological gardens in the Czech Republic (Hula & Pešan 2018).

The aim of the present paper is to give insight into the arachnid fauna of the tropical greenhouses at Rotterdam Zoo and document species found in the Netherlands for the first time. Additionally, a graph is given to illustrate which method proved most effective in collecting arachnids in tropical greenhouses.

Material and methods

Locations

This study was carried out in three tropical greenhouses located at Rotterdam Zoo: Amazonica, Victoria Serre and the Kwekerij.

Amazonica (51.9280°N, 4.4473°E, -1 m alt., Fig. 1) is a large circular greenhouse designed as a butterfly garden, opened in 2013. It has a diameter of 60 m and its highest point is 12 m. A total of 318 plant species are growing in open soil in Amazonica, mostly of South American origin. The average temperature is 25–28°C during daytime and goes down to 18–23°C at night. During warm summer days, the average temperature can be as high as 32–34°C.

The Victoria Serre (51.9260°N, 4.4529°E, -1 m alt., Fig. 2) is a large aviary built in 1945. It consists of three distinct but connected buildings, each hosting a different array of

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Asian bird species. In the first and second part, the vegetation is grown in patches of soil alongside a footpath. In the third part, the vegetation grows around a 10 m diameter pool containing giant water lilies. The diameter of the circular-shaped third part is 24 m and its highest point is circa 8 m. A total of 61 plant species are growing in open soil. The average temperature is 25°C during daytime and 20°C at night.

The Kwekerij ('nursery') (51.9243°N, 4.4544°E, -1 m alt., Fig. 3) is a complex consisting of thirteen small greenhouses built in 1993, which function as nurseries for tropical plants. In contrast to the other greenhouses, it is not open to the public. The arthropod survey was carried out in four greenhouses with heights ranging from 9.8 to 11.6 m. The majority of the planting consists of a mixture of potted plants that can also be found in Amazonica and Victoria Serre. In one of the sampled greenhouses, several plants are (permanently) grown in an elongated patch of soil. Most of the greenhouses have the same temperature fluctuations as in Amazonica.

Figs 1-3: Impression of the three sampling locations at Rotterdam Zoo. **1.** Amazonica; **2.** Victoria Serre; **3.** the Kwekerij

Sampling methods

The arachnids were surveyed in two separate periods: 4. Jun. – 10. Jul. (week 23–28) and 27. Aug. – 19. Sep. (week 35–38) 2018. Samples were collected each week using five methods. (i) Pitfall traps, composed of a 500 ml yoghurt can filled with circa 50 ml ethylene glycol solution. A total of five pitfall traps were placed per greenhouse, in the most dissimilar habitats possible. The traps were protected by a plastic lid supported by four clout nails. The pitfalls were emptied once a week. (ii) Banana baits, which were obviously primarily used to attract arthropods other than Arachnida, e.g. Drosophilidae and certain Coleoptera. A half peeled ripening banana was placed inside a plastic container on the ground, with 10 mm mesh covering the entrance, at two sites per greenhouse complex. The bananas were sampled once a week. (iii) Leaf litter samples, which consisted of sieving the top soil layer (i.e. 2 cm) within an area of 50 × 39 cm. The samples were sifted through a beetle sieve with a mesh size of 10 mm. The samples were taken weekly at different locations, once per greenhouse complex. In the Kwekerij, the samples were always taken at the only location with permanent vegetation. (iv) Hand searching, which included manually looking for arachnids and moving objects such as rocks, wood and litter. (v) Beating, consisted of shaking branches by hand over a sweep net. A few web-building spider species (i.e. *Parasteatoda tepidariorum* (C. L. Koch, 1841), *Pholcus phalangioides* (Fuesslin, 1775) and *Uloborus plumipes* Lucas, 1846) were counted instead of sampled, because of their unmistakable identification and common occurrence. At the start of the inventory, specimens of these species were collected to verify the identification. The exact sampling locations of hand searching and beating were randomised, with the aim of ultimately surveying as many locations as possible. Hand searching and beating were performed once a week, within a fixed period of time (i.e. 60 minutes). All samples and traps were evenly spread across each greenhouse.

Identification and COI-barcoding

All specimens were collected by transferring them into vials containing 96% ethanol. Identification of spiders was primarily done following Nentwig et al. (2019). The pseudoscorpions were identified with Legg & Farr-Cox (2016). Some specimens were photographed using a Zeiss Axioskop microscope equipped with an AxioCamMRc5 digital camera, creating stacked images with a great depth of field. Male specimens of *Belisana ambengan* were sent to Bernhard Huber (Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany) for identification.

From the collected specimens, fourteen were subsampled for molecular analysis of the cytochrome c oxidase subunit I gene (COI) (Tab. 1). DNA extraction and amplification were performed at the Naturalis DNA Barcoding Facility. DNA primers LCO1490 and HCO2198 were used to amplify a 658-bp region of the mitochondrial COI gene. The resulting PCR products were sequenced at BaseClear using the Sanger sequencing method (for standardised protocols on extraction, amplification and sequencing, see Ivanova et al. 2009). The COI-sequences were matched against the BOLD database with the basic local alignment search tool (BLAST). Sequences with at least 99.0% similarity were declared a match (Hebert et al. 2003). The voucher specimens and parts of the collected material were deposited in the spider collection (RMNH.ARA) of Naturalis Biodiversity Center. Another part of the material is stored in the private collection of the first author.

Data analysis

In order to evaluate the course of sampling, a growth curve illustrating the cumulative percentage of additional arachnid and arthropod species was used. To compare the differences in species richness between the sampling methods, a univariate analysis of variance was performed. Differences were considered to be significant at $p<0.05$ values.

Tab. 1: List of arachnids recorded in the greenhouses at Rotterdam Zoo. Abbreviations: * – species recorded in the Netherlands for the first time; A – alien species (established) in the Netherlands; BB – banana baits; BE – beating; E – expansive alien species in the Netherlands; HS – hand searching; j – juvenile; LS – litter sieving; N – native species in the Netherlands; PT – pitfall traps; s – subadult

Order/Family/Species			Status	Methods of collection				
				BB	BE	HS	LS	PT
Araneae								
Agelenidae								
1		*Eratigena atrica* (C. L. Koch, 1843)	N			1 ♂		
Araneidae								
2		*Araneus diadematus* Clerck, 1757	N			1 j		
3		*Nuctenea umbratica* (Clerck, 1757)	N			1 j		
Dysderidae								
4		*Dysdera* cf. *crocata* C. L. Koch, 1838						1 s♀, 3 jj
Linyphiidae								
5		*Erigone atra* Blackwall, 1833	N				2 ♀♀	
6		*Mermessus trilobatus* (Emerton, 1882)	E	1 ♀				2 s♀♀
7		*Ostearius melanopygius* (O. Pickard-Cambridge, 1880)	E			1 ♀		
Nesticidae								
8	*	*Nesticella mogera* (Yaginuma, 1972)	A					2 ♂♂, 3 jj
Oonopidae								
9	*	*Heteroonops spinimanus* (Simon, 1892)	A				2 ♀	
10	*	*Triaeris stenaspis* Simon, 1891	A					7 ♀♀, 1 j
Pholcidae								
11	*	*Belisana ambengan* Huber, 2005	A		2 ♂♂, 1 s♀, 4 jj			
12		*Pholcus phalangioides* (Fuesslin, 1775)	A			119 (observations), 1 ♂		
13		*Psilochorus simoni* (Berland, 1911)	A			1 ♂	1 s♀	1 ♀
Salticidae								
14		*Hasarius adansoni* (Audouin, 1826)	A	1 ♂, 1 ♀, 1 j		1 ♂, 1 ♀, 4 s♀♀, 2 jj	1 ♂, 2 s♂♂, 3 s♀♀, 12 jj	2 ♂♂, 5 s♂♂, 1 ♀, 9 s♀♀, 21 jj
Segestriidae								
15		*Segestria bavarica* C. L. Koch, 1843	N			1 ♀		
Theridiidae								
16		*Coleosoma floridanum* Banks, 1900	A			1 ♂, 1 s♂, 1 ♀, 10 s♀♀, 1 j	6 ♂♂, 1 s♂, 6 ♀♀, 14 s♀♀, 1 j	4 s♀♀, 2 jj
17		*Parasteatoda tepidariorum* (C. L. Koch, 1841)	E		1 ♀	42 (observations), 2 ♀	1 j	1 ♂
18		*Steatoda grossa* (C. L. Koch, 1838)	E		1 s♀	1 ♀, 1 j		2 ♂♂
Uloboridae								
19		*Uloborus plumipes* Lucas, 1846	A		3 jj	407 (observations)		
Zodariidae								
20		*Zodarion italicum* (Canestrini, 1868)	N				2 jj	3 jj
Mesostigmata								
Phytoseiidae								
1		Typhlodromini gen. sp.					3 ex.	3 ex.
Pseudoscorpiones								
Chthoniidae								
1		*Ephippiochthonius tetrachelatus* (Preyssler, 1790)	N				2 ex.	1 ex.
Total number of different taxa				**2**	**4**	**12**	**10**	**12**
Total number of specimens				**4**	**11**	**601**	**60**	**74**

Tab. 2: List of the sequenced specimens that could be assigned to a Barcode Index Number (BIN) in the BOLD database, which verified the identification

Order/Family/Species	Sequenced specimens	Voucher ID	Assigned BIN	% Match with BIN	GenBank Accession
Araneae					
Nesticidae					
Nesticella mogera	1 ♂, 2 jj	RMNH.5105512, 5105515, 5105516	BOLD:ACQ6894	99.02–99.35	MW664906, MW664907, MW664908
Oonopidae					
Heteroonops spinimanus	1 ♀	RMNH.5105513	BOLD:AAN6310	99.81	MW664913
Salticidae					
Hasarius adansoni	1 ♂	RMNH.5105521	BOLD:AAW0165	100	MW664912
Theridiidae					
Coleosoma floridanum	1 ♂, 1 ♀	RMNH.5105519, 5105520	BOLD:AAV1727	99.82–100	MW664909, MW664910
Zodariidae					
Zodarion italicum	1 j	RMNH.5105524	BOLD:ACX0947	100	MW664911
Pseudoscorpiones					
Chthoniidae					
Ephippiochthonius tetrachelatus	1 ex	RMNH.5105525	BOLD:AAJ0945	100	MW664914

Results

Twenty-two taxa were identified: twenty spider species, one mite taxon and one pseudoscorpion species (Tab. 1). Nine specimens could be identified by matching their COI-sequence against the BOLD database (Tab. 2). The majority of the species recorded in the greenhouses are alien to the Netherlands, the remaining ones are (semi)synanthropic.

Spiders (Araneae)

In total, twenty taxa (734 ex.) from twelve families were recorded (Tab. 1). *Uloborus plumipes* was the most dominant species overall, but completely absent from the Victoria Serre. Other very common species included *Pholcus phalangioides*, *Hasarius adansoni* (Audouin, 1826), *Parasteatoda tepidariorum* and *Coleosoma floridanum* Banks, 1900. Four species, *Nesticella mogera, Heteroonops spinimanus*, *Triaeris stenaspis* and *Belisana ambengan*, represent new records for the Netherlands. These and several other remarkable species are discussed in detail below.

Fig. 4: Habitus of adult male *Nesticella mogera* from the Amazonica greenhouse, 10.–18. Sep. 2018; body length: 2.1 mm

Nesticidae

Nesticella mogera (Fig. 4)

Material examined. 4.–11. Jun. 2018 – 1 ♂, pitfall trap, Kwekerij; 25. Jun. – 2. Jul. 2018 – 3 jj, pitfall trap, Kwekerij (RMNH.5105515, 5105516, 5105517); 10.–18. Sep. 2018 – 1 ♂, pitfall trap, Amazonica (RMNH.5105512).

Identification. Nentwig et al. (2019). The identification of two out of three identical juvenile specimens found in Kwekerij was confirmed by COI barcoding.

The specimens sampled at Rotterdam Zoo represent the first record of this species in the Netherlands. In Europe, *N. mogera* was previously found in several heated buildings in Great Britain (Snazell & Smithers 2007), Denmark (Lissner & Scharff 2021), Germany (Kielhorn 2009, Reiser 2013), Poland (Bielak-Bielecki & Rozwałka 2011), Finland (Koponen et al. 2016), Italy (Pantini et al. 2020), Hungary (Pfliegler 2014) and the Czech Republic (Růžička 2016). The presence of this East-Asian troglophile species was expected, because the specimen collected in 2009 in Poland was found at a building-garden centre in Lublin in potted plants deriving from the Netherlands (Bielak-Bielecki & Rozwałka 2011). This suggests that *N. mogera* probably has been present in the Netherlands since at least 2009.

Oonopidae

Heteroonops spinimanus (Fig. 5)

Material examined. 11. Sep. 2018 – 1 ♀, litter sieving, Amazonica; 19. Sep. 2018 – 1 ♀, litter sieving, Amazonica (RMNH.5105513).

Identification. The identity of this species was verified by analysis of the COI gene of one female. Alternatively, females are distinguished from other nonscutate oonopids by the characteristic spination of the palp (Saaristo 2001).

The current survey produced the first record of this species for the Netherlands. *Heteroonops spinimanus* has been sampled from greenhouses in Germany (Kielhorn 2008) and the

Fig. 5: Adult female *Heteroonops spinimanus* from the Amazonica greenhouse, 19. Sep. 2018; body length: 1.8 mm

Czech Republic (Hula & Pešan 2018) as well. It is a Central-American litter-dwelling species, hence its presence in the litter sieving samples at Rotterdam Zoo (Pfeiffer 1996, Kielhorn 2008). Males are either rare or extremely short-lived, and were initially assigned to a different species (Platnick & Dupérré 2009). It is nevertheless possible that at least some of the introduced populations consist of parthenogenetic individuals (Platnick & Dupérré 2009).

Triaeris stenaspis (Fig. 6)
Material examined. 25. Jun. – 2. Jul. 2018 – 1 ♀, 1 j, pitfall trap, Kwekerij (RMNH.5105518); 2.–9. Jul. 2018 – 1 ♀, pitfall trap, Kwekerij; 27. Aug. – 3. Sep. 2018 – 1 ♀, pitfall trap, Victoria Serre; 3.–10. Sep. 2018 – 1 ♀, pitfall trap, Kwekerij; 3.–10. Sep. 2018 – 3 ♀♀, pitfall trap, Victoria Serre (RMNH.5105514).
Identification. Nentwig et al. (2019). *Triaeris stenaspis*, easily recognizable by the characteristic shape of its large dorsal scutum and the relatively small ventral scutum on the opisthosoma, is a parthenogenetic species (Korenko et al. 2009).

The current arachnid survey marks the first time that specimens of *T. stenaspis* were collected from the Netherlands. This oonopid spider of African origin was to be expected, as it was collected from several other greenhouses in Europe (Korenko et al. 2007, Pfliegler 2014, Rozwałka et al. 2016, Šestáková et al. 2017, Rembold et al. 2020, Lissner & Scharff 2021). Furthermore, *T. stenaspis* was recently recorded at a tropical butterfly house (in Whipsnade Zoo, England) in which the trees and shrubs all originate from the Netherlands (Telfer 2020). Because of its synanthropic and parthenogenetic nature, it can easily invade greenhouses.

Fig. 6: Habitus of adult female *Triaeris stenaspis* from the Victoria Serre greenhouse, 27. Aug. – 3. Sep. 2018; body length: 1.7 mm

Pholcidae
Belisana ambengan (Figs 7-8)
Material examined. 9. Jul. 2018 – 1 j, beating, Kwekerij; 11. Sep. 2018 – 2 ♂♂, 1 s♀, 1 j, beating, Amazonica; 19. Sep. 2018 – 2 jj, beating, Victoria Serre (RMNH.ARA.18236).
Identification. These specimens were identified by Bernhard Huber as *Belisana ambengan*, based on his own first description (Huber 2005).

This find is surprising, because it represents the first record of a *Belisana* species in Europe and the species was solely known from its holotype (a male) and a prosoma of a female. Even more surprisingly, one male and one female were also found by the second author in a tropical greenhouse of Wildlands Adventure Zoo (Emmen, province of Drenthe) on 2. Jul. 2019 and an additional female was collected a few days later on 6. Jul. 2019 in the same greenhouse by D. Sies (all coll. Naturalis Biodiversity Center). The male from Emmen was used to make Figs 6-7, since the specimens from Rotterdam were already in the collection of B. Huber (Bonn, Germany). The type material of *B. ambengan* was collected in a secondary forest in Ambengan, Bali, Indonesia (Huber 2005). The record of this species at Rotterdam Zoo suggests that it might be more widely distributed in Indonesia than its type locality, since the chance of an isolated population to translocate to the Netherlands is negligibly small. A large part of the tropical plants at Rotterdam Zoo is imported from Indonesia.

Fig. 7: Habitus of adult male *Belisana ambengan* from Wildlands Adventure Zoo Emmen, 2. Jul. 2019; body length: 1.3 mm

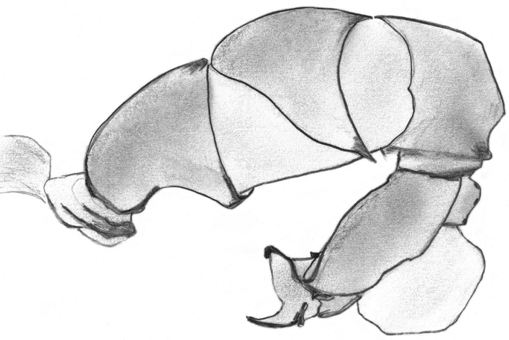

Fig. 8: Right male palp in retrolateral view of *Belisana ambengan* from Wildlands Adventure Zoo Emmen, 2. Jul. 2019. Hairs not drawn and bulbal apophysis is bent inwards and not visible, see Huber (2005) for its morphology

Theridiidae
Coleosoma floridanum
Material examined. 4.–11. Jun. 2018 – 1 s♀, pitfall trap, Victoria Serre; 11.–18. Jun. 2018 – 2 s♀, pitfall trap, Victoria Serre; 2.–9. Jul. 2018 – 1 s♀, 2 jj; 3. Jul. 2018 – 1 ♀, 1 ♂, litter sieving, Victoria Serre (RMNH.5105519, 5105520); 19. Sep. 2018 – 1 ♀, 1 ♂, litter sieving, Victoria Serre (RMNH.ARA.18233). The remaining specimens mentioned in Tab. 1 were collected at both Amazonica and Victoria Serre in nearly all sampling weeks, but not stored in a collection.
Identification. Nentwig et al. (2019) and COI barcoding.

Females of this Pantropical species carrying egg sacs were a common sight in the greenhouses at Rotterdam Zoo. Despite its common occurrence at the Rotterdam greenhouses, the presence of *C. floridanum* was unnoticed in the Netherlands since their first and only previous record by van Helsdingen (1995), probably due to their small size. In 2019 it was also found by the second author in a tropical greenhouse of Wildlands Adventure Zoo (Emmen, province of Drenthe) and it is suggested that this spider is more common than previously suspected.

Uloboridae
Uloborus plumipes
Material examined. 26. Jun. 2018 – 3 jj, beating, Kwekerij. Due to their high visibility and easily recognizable traits, specimens belonging to this species were counted instead of sampled during hand searching.
Identification. Nentwig et al. (2019). Their high visibility obviously accounted for the relatively high numbers of records.

Uloborus plumipes was the most abundant arachnid species from Amazonica and Kwekerij. In fact, it is a very widely distributed species in tropical and commercial greenhouses and garden centres in the Netherlands (Noordijk et al. 2018). It was previously stated that this species might be parthenogenetic in urban areas, but Oxford (2011) convincingly contradicted this statement. Its notable absence from Victoria Serre might be attributed to limiting, site-specific factors, e.g. use or avoidance of certain pesticides (Suvák 2013).

Zodariidae
Zodarion italicum
Material examined. 11. Jun. 2018 – 1 j, litter sieving, Kwekerij (RMNH.5105524); 11.–18. Jun. 2018 – 1 j, pitfall trap, Victoria Serre; 19. Jun. 2018 – 1 j, litter sieving, Kwekerij; 3.–10. Sep. 2018 – 1 j, pitfall trap, Kwekerij; 10.–18. Sep. 2018 – 1 j, pitfall trap, Amazonica.
Identification. As no adult specimens were collected, the identity of *Z. italicum* was determined by analysing the COI gene of one juvenile.

The presence of this native myrmecophagous species seemed unusual, because it was previously solely known from a natural site in the far south of the country (province of Limburg) (van Helsdingen 2018). *Zodarion italicum* is a South European species extending its range to the north, and has been recorded in greenhouses at the Botanic Garden Berlin-Dahlem and other places with anthropogenic habitats like railway stations and airports in Western and Central Europe (Malten et al. 2005, Pekár et al. 2005, Kielhorn 2008). It is therefore highly likely that the population at Rotterdam Zoo originates from other greenhouses or urban transport areas rather than the surrounding natural habitat.

Mesostigmata
Phytoseiidae
Typhlodrominae
Typhlodromini
Material examined. 26. Jun. 2018 – 2 ex., litter sieving, Victoria Serre; 25. Jun. – 2. Jul. 2018 – 2 ex., pitfall trap, Victoria Serre; 17. Sep. 2018 – 1 ex., litter sieving, Victoria Serre; 10.–18. Sep. 2018 – 1 ex., pitfall trap, Victoria Serre.
Identification. Six morphologically identical mites were sampled at Victoria Serre. Two of these specimens were sent to Henk Siepel (Radboud University, Nijmegen), who identified them as the tribus Typhlodromini (Tab. 1). No decisive answer could be given about what genus or species the specimens belong to, but they are likely the same species.

The number of mites is remarkably low. Phytophagous species were absent in the samples. In most greenhouses the numbers of phytophagous mites are high, regularly at such levels that it urges for pest control (Gerson & Weintraub 2012). Since herbivorous mites are absent, the predatory mites found at Rotterdam Zoo probably prey upon collembolans (Petrova et al. 2004).

Pseudoscorpiones
Only one, native pseudoscorpion species (3 ex.) was found inside the greenhouses (Tab. 1).

Chthoniidae
Ephippiochthonius tetrachelatus
Material examined. 4.–11. Jun. 2018 – 1 ♀, pitfall trap, Victoria Serre; 19. Jun. 2018 – 1 ♀, litter sieving, Victoria Serre (RMNH.5105525); 25. Jun. – 2. Jul. 2018 – 1 ♀, pitfall trap, Victoria Serre.
Identification. Legg & Farr-Cox (2016) and COI barcoding.

This pseudoscorpion species is native to Europe and hemisynanthropic, although its association with anthropogenic habitats differs mainly by latitude (Šťáhlavský 2001). In the Netherlands, it can be found at a variety of dry and weakly humid locations and is also common in greenhouses, foraging under stones, logs and in leaf-litter (van der Hammen 1969b). In Scandinavia and northeast Poland, however, it lives almost exclusively in heated buildings or other synanthropic habitats, while in the Mediterranean it occurs widely independent of soil humidity (Šťáhlavský 2001). *Ephippiochthonius tetrache-*

latus is frequently translocated to remote localities far from its native Western Palearctic and Nearctic range (Muchmore 2000). It is therefore suggested that the population of this species in Rotterdam Zoo, like *Zodarion italicum*, originates from other (European) greenhouses rather than the outside surroundings. Recently, Kaňuchová et al. (2016) also found it in many compost heaps, which could possibly serve as a means of transport between the greenhouses.

General evaluation

The growth of the cumulative percentage of additional species slightly decreased over time in both arachnid and arthropod species (Fig. 9). Although the last survey week did not yield any additional arachnid species, the number of additional arthropod species (e.g. Diptera, Isopoda, Myriapoda) slightly increased. The arachnid species curve seems saturated, but the presumed asymptote is reached only in the last survey week. However, the arthropod species curve does not reach a clear saturation point.

The mean species richness per sample was found to significantly differ between the sampling methods. Out of the five collecting methods, hand searching yielded the significantly highest mean number of arachnid species per sample, followed by both pitfall trapping and litter sieving, while both beating and the banana baits had the lowest mean species richness (Fig. 10). Hand searching yielded four species not caught by the other methods, while the pitfall traps and the litter sieving resulted both in two unique and beating in one unique species.

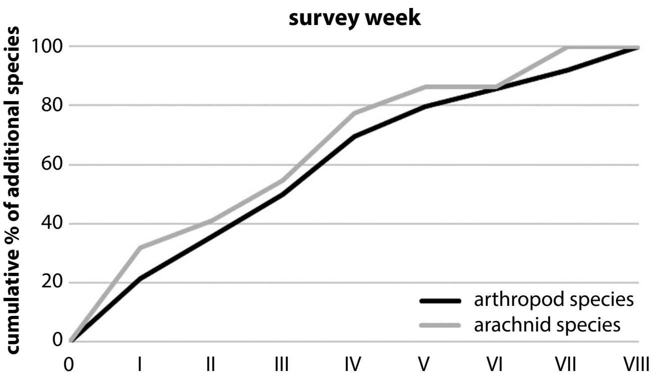

Fig. 9: The growth of the cumulative percentage of additional species over time.

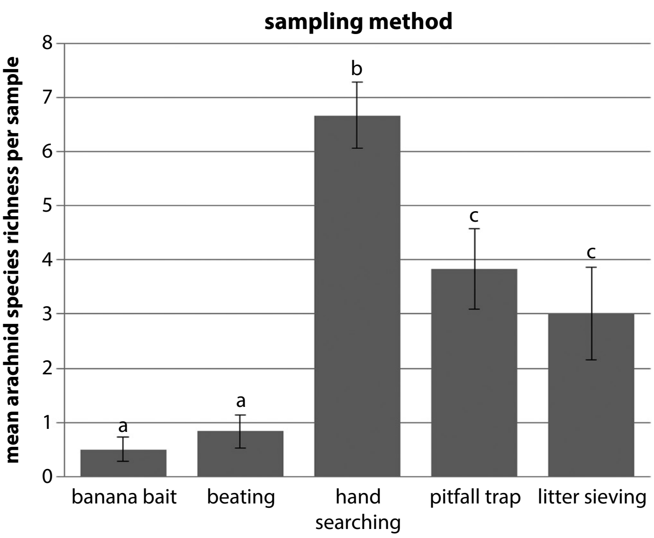

Fig. 10: The mean arachnid species richness per sample per sampling method, including SE-bars and statistical significance markings (with α level set at 0.05)

Discussion

Impact on the natural environment

A subset of the alien species might be able to thrive outdoors. *Mermessus trilobatus* (Emerton, 1882) established successful populations in a wide variety of open natural habitats in the Netherlands and other European countries (van Helsdingen 2009, Holec et al. 2012). *Ostearius melanopygius* (O. Pickard-Cambridge, 1880) is frequently found in manure heaps, and also in natural areas (Roberts 1998, Nentwig et al. 2019). *Parasteatoda tepidariorum* and *Steatoda grossa* (C. L. Koch, 1838) are regularly found on outer walls of artificial structures in the Netherlands (pers. obs.). Apparently, these species are able to survive outside of buildings in the European temperate zone. All other alien species from Tab. 1 remain very strongly associated with heated buildings in the current Dutch climate, although in 2005 one stray individual of *Hasarius adansoni* was found in debris in a riverine flood area (van Helsdingen 2009). Their impact on natural habitats is therefore now insignificant, but it remains to be seen which species will protrude to the outdoors due to the increasing warming of the climate.

Species to be expected

The sampling stopped at the point when no further species were collected (Fig. 9): the presumed asymptote. However, Gotelli & Colwell (2011) state that the asymptote of a growth curve can be reached only when at least 20 samples are taken. Moreover, future imports of plants and accompanying soil can introduce additional arachnid species to Rotterdam Zoo. Therefore, it is expected that a subsequent survey will confirm the presence of additional species.

The composition of species found at Rotterdam Zoo shows overlap with other surveys of arachnid fauna in greenhouses (Snazell & Smithers 2007, Kielhorn 2008, Pfliegler 2014, Šestáková et al. 2017, Hula & Pešan 2018). In most studies, the dominant species is either *Parasteatoda tepidariorum* or *Pholcus phalangioides*, while *Uloborus plumipes* is the most ubiquitous species at Rotterdam Zoo. However, the unexpected discovery of a new pholcid spider for Europe at two zoos in the Netherlands is remarkable. Another similar example is the recent discovery of a small pholcid in a Dutch tropical greenhouse, *Spermophora kerinci* Huber, 2005 (Noordijk 2020); this species is further only known from Indonesian rainforests and a tropical greenhouse in England and Germany (Snazell & Smithers 2007, Kielhorn 2009). It is therefore suggested that arachnid communities in European tropical greenhouses are interconnected, probably due to similar importing routes for the planting material or due to exchange of plants or other products. For this reason, it is expected that some of the additional alien species sampled at other tropical greenhouses in Europe will be recorded in Dutch tropical greenhouses in the near future as well, e.g. *Nesticodes rufipes* (Lucas, 1846), *Theotima minutissima* (Petrunkevitch, 1929), *Eukoenenia florenciae* (Rucker, 1903) and *Pseudanapis aloha* Forster, 1959 (Snazell & Smithers 2007, Kielhorn 2008, Šestáková et al. 2017, Hula & Pešan 2018).

Comparison of collecting methods

The results of the current study are similar to other studies of arachnofauna in greenhouses involving comparable methods, e.g. Šestáková (2017), where hand collection yielded the highest total number of species and unique species. These results were to be expected, since hand searching covers most strata of the vegetation. Except for the banana baits, each method however yielded unique species (Tab. 1), and they are therefore complementary. The most surprising find, i.e. *Belisana ambengan*, was collected only by beating.

In future arachnid surveys in greenhouses, it is advised to use at least a combination of methods that sample the vegetation (hand searching or beating) and that yield epigeic arachnids (pitfall trapping or litter sieving) (for similar comparisons in the field, see Churchill & Arthur 1999, Jiménez-Valverde & Lobo 2005, Cardoso et al. 2008). Although the number of species did not differ significantly, pitfall trapping is favoured above litter sieving due to its passive and continuous nature. In order to gain more knowledge about arachnid communities in greenhouses, and their potential source function for invasive alien species, it is recommended to carry out more arachnid surveys in this relatively understudied habitat.

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