International Journal for Parasitology: Parasites and Wildlife 17 (2022) 7–13

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Diversity of intestinal protozoa and clinical signs associated in wild-caught *Phoneutria nigriventer* kept in captivity for the anti-arachnid serum production

Thiago Mathias Chiariello [a,b], Ryan Emiliano da Silva [b], Jaciara de Oliveira Jorge Costa [b], Arlei Marcili [b,c,*]

[a] Biotério de Artrópodes, Instituto Butantan, Av. Vital Brasil, 1500, 05503-900, São Paulo, SP, Brazil
[b] Departamento de Medicina Veterinária Preventiva e Saúde Animal, Universidade de São Paulo (USP), Av. Prof. Dr. Orlando Marques de Paiva, 87, 05508-270, São Paulo, SP, Brazil
[c] Programa de Medicina e Bem-estar Animal e Saúde Única, Universidade Santo Amaro, R. Dr Enéas de Siqueira Neto, 340, 04829-300, São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Protozoa
Phoneutria nigriventer
Spider
Parasites
Molecular characterization

ABSTRACT

The phylum Arthropoda comprises approximately 85% of all described animal species. The class Arachnida includes some invertebrates of great importance as they are either involved in the transmission of diseases or poses a risk of human envenomation. Spiders belonging to the genus *Phoneutria* sp., are the arachnids exhibiting medical importance. These animals were quarantined and maintained in captivity at the Biotério de Artrópodes of the Instituto Butantan, São Paulo, Brazil, for the production of the anti-arachnid serum. A total 509 feces samples from different *Phoneutria nigriventer* were analyzed, and 131 (25.73%) samples were found to be positive for flagellates and ciliates. All positive samples were subjected to DNA extraction and amplification of 18S gene. A total of 16 sequences were obtained and analyzed using BLAST. Sequences were identified as *Colpoda steiini,* one as *Colpoda aspera,* one to *Colpoda* sp., and one as "ciliated". Four identified as *Parabodo caudatus,* two as *Urostipulosphaera* sp., one as *Helkesimastix* sp., and one as a Euglena-like. The presence of clinical signs was observed in 16 spiders. The intestinal protozoa that affect armed spiders were identified for the first time as an initial step for understanding the parasitic diseases in these organisms.

1. Introduction

Spiders are arachnids belonging to the phylum Arthropoda. More than 47,000 species have been described so far, with a great diversity concentrated in tropical and subtropical regions (Coddington and Levi, 1991; Pineda et al., 2014). Spiders possess two venom glands and a pair of chelicerae, which is used to perforate and inoculate the toxin; however, only a few species possess active venom against humans (Lucas, 1988).

Spiders from the genus *Phoneutria* belong to the family Ctenidae, suborder Labidognatha, order Araneidae. They are popularly known as armed spiders, which is attributed to the defensive display adopted when they sense threat, wherein the organism raises their first four legs, while resting on the hind legs, swinging the body from one side to another (Peigneur, 2018). Armed spiders are distributed in the southern region of Central America (Costa Rica) to South America (Bucaretchi et al., 2000; Peigneur, 2018). They are considered synanthropic animals and are frequently found in houses due to waste accumulation, which attract insects that are the main prey of these spiders (Ramos et al., 1998). *Phoneutria* sp. are very abundant in some areas, as they are adapted to different environments and possess toxic venoms against humans and animals. Therefore, they are considered as a public health concern in several countries of South America (Valério, 1982; Florez et al., 2003; Málaque and Antunes, 2004). They are responsible for most of the araneid accidents in Brazil and are considered as the main causative agents in several areas (Ministério Da Saúde, 1998; Málaque and Antunes, 2004). In 2020, 4763 spider envenomations were registered in São Paulo State, where approximately 60% were caused by *Phoneutria nigriventer* (Divisão De Zoonoses/CVE, 2020).

Due to the large number of araneid accidents in Brazil and the

* Corresponding author. Departamento de Medicina Veterinária Preventiva e Saúde Animal, Universidade de São Paulo (USP), Av. Prof. Dr. Orlando Marques de Paiva, 87, 05508-270, São Paulo, SP, Brazil.
E-mail address: amarcili@usp.br (A. Marcili).

https://doi.org/10.1016/j.ijppaw.2021.11.006
Received 22 October 2021; Received in revised form 23 November 2021; Accepted 23 November 2021
Available online 25 November 2021

severity of envenomation, anti-arachnid serum is made available for free by the Brazilian Ministry of Health for the individuals in public hospitals. The serum is polyvalent and is used for the treatment of envenomation caused by an armed spider (*Phoneutria* sp.), brown recluse spider (*Loxosceles* sp.), and yellow scorpion (*Tityus serrulatus*). Anti-arachnid serum is produced by the Instituto Butantan, São Paulo, Brazil, and hundreds of *Phoneutria nigriventer* are being captured to extract their venom for subsequent serum production.

Serum production requires a large amount of venom per year, and to meet this demand, it is extremely important to maintain healthy spiders with increased longevity, and consequently, there is more venom extracted per organism. The health status of these spiders, as well as the knowledge and understanding of several infectious and parasitic diseases of these organisms are of great importance to maintain them in captivity. Therefore, it is necessary to investigate the intestinal protozoans that affect these species.

Protozoa are the most abundant eukaryotic form of life and are found in all habitats, including the body cavities of animals and humans, for instance in the intestinal lumen, blood, and several cells and their nuclei (Levine, 1988). Protozoan parasites are largely documented to be present in insects; however, they are poorly documented in arachnids, with no reports in spiders. Veterinary medicine in terrestrial invertebrates is in its initial phase; therefore, the lack of knowledge regarding the different clinical aspects and on the infectious and parasitic diseases of spiders poses an urgent need of a quarantine period to collect live animals, with both inflow and outflow of individuals. Therefore, due to the lack of related studies, it is necessary to identify the parasites that affect the spiders of the genus *Phoneutria* sp., as well as their relationship with the host, with an aim to optimize these for the production of venom and greater longevity when kept in captivity.

In this study, we aimed to investigate, for the first time, the protozoa diversity found in *P. nigriventer* feces through molecular characterization using SSUrDNA, and describe the clinical signs observed in the parasitized spiders.

2. Materials and methods

2.1. Phoneutria nigriventer

A total of 509 spiders used in this study were collected from several natural regions of São Paulo state and Resende city (Rio de Janeiro) by the Biotério de Artrópodes, Instituto Butantan staff, or received at the reception of Venomous Animals of Instituto Butantan, which were brought by either the citizens or animal control services from different municipalities of São Paulo state. All spiders were quarantined for 7–14 days for observation of their behavior, their general health status, and the presence of ectoparasites, such as mites, and then, the feces samples were collected. During this period, the spiders were placed in glass containers with a humidified cotton ball and a cardboard substrate (Fig. 1) maintained at a constant room temperature of 24 °C (±0.5 °C). They were fed with cockroaches (*Phoetalia palida* or *Nauphoeta cineria*) bred in captivity. To avoid the presence of parasites, the offered cockroaches were subjected to routine health control, including parasitological examination of feces, and the water used in the cotton balls was also tested to guarantee its quality.

2.2. Sample collection and storage

Feces samples were collected (<10 mg) with a disposable Pasteur pipette directly from the bottom of the glass containers in which each spiders were placed. Screening for the presence or absence of parasites was conducted directly on smears and through observation under a microscope (10X) (Nikon® YS100). The samples were considered positive for protozoans when organisms presenting movement were seen and were morphologically distinct from worm-like parasites. The positive samples were stored at constant temperature (24 °C ± 0.5 °C) in 0.5 mL Eppendorf® tubes containing PBS 1X.

Fig. 1. *Phoneutria nigriventer* kept in glass containers with a humidified cotton ball and a cardboard substrate.

2.3. DNA extraction and amplification reactions

DNA extraction and purification were performed using a commercial kit (Genomic Purelink, Thermofisher). Three sets primers were used to 18S amplification: 18SFU (ATGCTTGTCTCAAAGGRYTAAGCCATGC) and 18RFU (CWGGTTCACCWACGGAAACCTTGTTACG) amplifies a fragment of 1000 pb (Tikhonenkov et al., 2016), d3 (TGGAGGGCAAGTCTGGTG), and r7 (GGGCGGTGTGTACAAA) amplifies a fragment of 1000 pb (Milyutina et al., 2001; Yubuki et al., 2016), along with the barcodes V7–V8 (Maia Da Silva et al., 2004) amplifies a fragment of 800 pb.

For PCR, the following reaction mixture was prepared: 100 ng of genomic DNA, 100 ng of each primer, and 20 µL of GreenTaq (Sinapse®) mix solution. The same amplification cycles and annealing temperatures were used for the three set primers with repeated 30 times as follows: denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min, and initial denaturation at 94 °C for 5 min and final extension at 72 °C for 10 min. The reactions were performed in two different equipments, Gradient Thermocycler (Thermo Fisher) and Vapo.Protected Mastercycler (Eppendorf).

To extraction control, DNA samples were submitted to cytochrome *c* oxidase subunit I gene amplification (Folmer et al., 1994).

The amplification products were subjected to electrophoresis using an agarose gel (1.5%) prepared in TAE buffer at 50 V/100 mA, stained with Syber Safe according the manufacturer (Thermo Fisher®), and imaged using a UV light transilluminator (Thermo Fisher®).

2.4. Purification, sequencing, and In silico analysis

DNA fragments amplified through were separated using electrophoresis on a 2% agarose gel and stained with Gel-Red (Biotium). The fragments were purified using the Exosap-IT Kit (Thermo Fisher®). The purified products were directly subjected to the sequencing reaction using the Big Dye Terminator kit (PerkinElmer®), according to the manufacturer's specifications, and the reaction was conducted in the ABI PRISM 310 Genetic Analyzer (PerkinElmer®) in VPS FMVZ-USP. The reactions were subjected to 30 cycles of reaction as follows: 15 s at 96 °C, 15 s at 50 °C, and 4 min at 60 °C, with an initial cycle of 1 min at 96 °C.

The sequences obtained through PCR of different set primers were analyzed using BLAST (Basic Local Alignment Search Tool). The sequences were deposited in GenBank (MZ233408-MZ233423).

2.5. Description of the clinical signs associated with the presence of intestinal protozoans

The animals were evaluated during the quarantine period. The following observational parameters were evaluated: the quality of feces, weight of the animal at the point of entry and exit from the quarantine, behavior, food intake, and other concomitant events related to the spiders positivity for the presence of protozoan parasites, such as the presence of other parasites in the feces, ootheca production, and ecdysis.

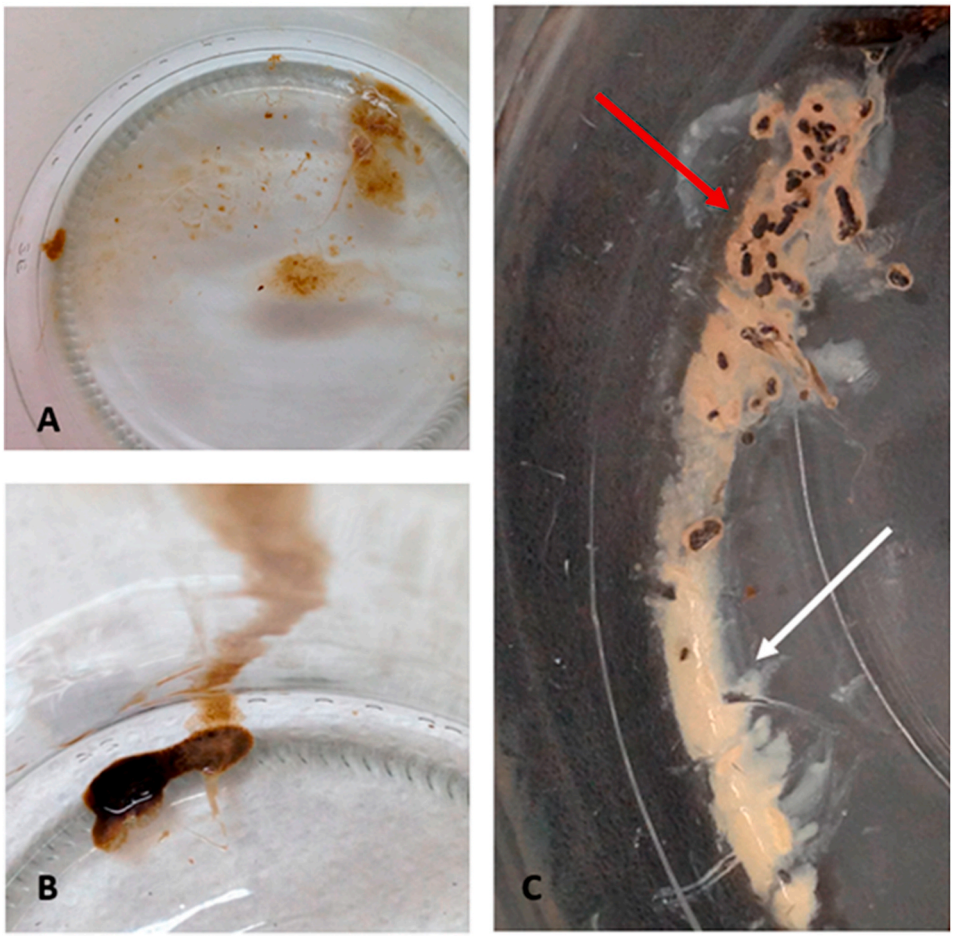

Fig. 2. – A and B, Diarrheal stools, without differentiation of solid and liquid portion. C, Normal stools of *Phoneutria nigriventer* (red arrow). The white arrow indicates the urine portion, white in color due to urate. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3. Results

Feces samples from 509 *Phoneutria nigriventer* were analyzed under optical microscope, and 131 (25.73%) were found to be positive for the presence of protozoans (data not shown). A total of 45 (34.35%) spiders that were positive in the parasitological examination were females, 35 (26.71%) were males, and 46 (35.11%) were juveniles, and for five (3.81%) spiders, we could not obtain the information on their sex.

Parasitological examination using light microscope was related to the clinical signs in 42.2% (19/45). Fourteen (10.6%) out of the total positive spiders exhibited diarrhea (Fig. 2) associated with weight loss and anorexia, and two of them exhibited infection by unidentified helminths, which was also associated with the infection by protozoa, thereby making not possible to identify the exact cause of the observed clinical signs. However, two spiders (1.53%) only showed weight loss. Out of the 14 animals that exhibited clinical signs, eight (57.14%) died during the quarantine period.

Other events also occurred concomitantly with the infection, for instance, two spiders produced ootheca, and one underwent ecdysis. There was no signs of ectoparasites or external lesions in any spider that was positive for parasitological examination. Table 1 lists the spiders that showed any clinical signs or events observed during the quarantine period, and their association with the identified or unidentified organisms.

Samples positive for parasitological examination were subjected to three amplification reactions, each reaction using a set primer, thereby resulting in a total of 393 reactions. Of the 131 positive samples for the parasitological examination, 80 (61%) were amplified using one or more set primers, while 51 (38.9%) were not amplified. Of the 131 reactions performed with the 18SFU-18SRU set primer, 58 (44.27%) were amplified, and 73 (55.72%) were not amplified. When using the d3-r7 set primer, 65 (49.6%) samples were amplified, and 66 (50.3%) were not amplified. Moreover, only two samples (1.52%) were amplified using the barcode v7-v8.

Using the 80 amplified samples, 16 sequences were obtained (Table 2). These sequences belonged to 14 spiders, since samples 2201a and 2201b belonged to the same spider, and samples 3317a and 3317b belonged to different spiders. After BLAST sequencing analysis, eight organisms (47%) were identified as ciliate, eight (47%) as flagellates, and one (6%) as an algal specie. Among the eight samples sequenced as flagellated protozoa, five (62.5%) were identified as *Colpoda steinii*, one (12.5%) as *Colpoda aspera*, one (12.5%) belonged to the genus *Colpoda* sp., and one (12.5%) was identified as "ciliated organism". Among the eight flagellates, four (50%) were identified as *Parabodus caudatus*, two (25%) as *Urostipulosphaera* sp., one (12.5%) as *Helkesimastix* sp., and one (12.5%) as a euglena-like protozoan.

Table 1
Clinical signs and events associated with the protozoan infection in *Phoneutria nigriventer*.

SPIDER	CLINICAL SIGN/EVENT	ORGANISM	DEATH
2127	Diarrhea, weight loss and anorexia	*Urostipulosphaera* sp.	N
2132	Diarrhea, weight loss and anorexia	UN	Y
2133	Diarrhea, weight loss and anorexia	Ciliated	Y
2182	Weight-loss	UN	N
2176	Weight loss	UN	N
2196	Diarrhea, weight loss and anorexia	*Colpoda steinii*	Y
2435	Oothecae production	UN	N
2664	Oothecae production	*Parabodo caudatus*	N
3031	Ecdysis	UN	N
3036	Diarrhea, associated helminth infestation	Helminths	N
3039	Diarrhea, weight loss and anorexia	UN	N
3051	Diarrhea, weight loss and anorexia	UN	Y
3126	Diarrhea, associated helminth infestation	Helminths	N
3318	Diarrhea, weight loss and anorexia	UN	Y
3388	Diarrhea, weight loss and anorexia	UN	Y
3403	Diarrhea, weight loss and anorexia	*Urostipulosphaera* sp.	Y
3413	Diarrhea, weight loss and anorexia	UN	N
3414	Diarrhea, weight loss and anorexia	Uroglena-like	Y
3431	Diarrhea, weight loss and anorexia	UN	N

UN – Unidentified.
N – No.
Y – Yes.

4. Discussion

Parasitological studies on spiders, with an emphasis on infection by the cavitary nematodes belonging to the family Mermithidae are diverse. Poinar Jr. (1987) demonstrated that under laboratory conditions, spiders are susceptible to infection by nematodes belonging to the order Rhabditida, which causes death of the host. There are also studies demonstrating the relationship between several species of spiders and parasitoid insects, such as flies and wasps. However, studies on the identification of intestinal protozoa infecting captive or free-living armed spider as well as description of the course of their infection have never been conducted.

The sequencing and identification of intestinal protozoa obtained from the feces of *Phoneutria nigriventer* have been demonstrated in the present study for the first time to the best of our knowledge, and we additionally described the clinical signs and events that were directly or indirectly related to the infection. Despite that the spiders were kept in captivity, the fecal samples used in this study were from the spiders that were captured from their natural environment; therefore, the identified protozoa reflected the infections caused in the spiders while they were in the wild. Subsequently, the spiders were kept in captivity for inclusion in the breeding stock of spiders that are used for extracting the venom in the Biotério de Artrópodes, Instituto Butantan, which made it possible for us to monitor the evolution of the parasitized animals. During the quarantine period, the spiders were fed with cockroaches obtained from the insect livestock of the Biotério de Artrópodes. The insect live stock is maintained exclusively to feed the arachnids kept for venom extraction and undergo routine sanitary control, including parasitological examination of feces, which rules out the possibility that the protozoan infection of the spiders may have been acquired when kept in captivity. The water offered through humidified cotton was also tested by internal protocols of Instituto Butantan to eliminate the risk of contamination during the quarantine period.

Due to the lack of previous studies, three different sets of primers were used to amplify SSUrDNA. The set primers proposed by Tikhonenkov et al. (2016) (18SSFU/18SSRU) were standardized to amplify the 18SrRNA gene, which is the most common of the three sets used. The set primers proposed by Milyutina et al. (2001) (r3/d7), and Yubuki et al. (2016) were standardized to amplify the samples related to the Excavata supergroup, which encompasses protozoa from the Kinetoplastida subgroup (Maslov et al., 2001). This subgroup is divided into the suborders Bodonina and Trypanosomatina, which contain free-living species, pathogens of invertebrates, vertebrates, and even plants (Maslov et al., 2001; Simpson et al., 2006). The barcode region V7–V8 (Maia Da Silva et al., 2004) (609F/706R) was used to amplify a specific region for the identification of protozoans from the family of mandatory parasites Trypanosomatidae (Simpson et al., 2006).

The amplification reactions using 131 positive samples for the parasitological examination were performed with each of the above-described primers, thereby resulting in a total of 393 reactions. Herein, 51 samples were not amplified using any of the three primers, which was expected owing to the nature of this work, reflecting the difficulty in choosing specific primers due to the lack of previous studies concerning the intestinal microbiota of arachnids, specifically protozoans. The non-amplification of the samples can also be attributed to the insufficient amount of DNA or presence of reaction inhibitors.

The genus *Colpoda* sp. was identified in most of the sequenced

Table 2
BLAST analysis of SSUrDNA sequences amplified from intestinal protozoan in *Phoneutria nigriventer*.

Sample		Sequence Size (bp)	% Similarity	Organism	Query Cover (%)	e-value	Accession number GenBank
2127	**MZ233408**	975	93,67%	*Urostipulosphaera* sp.	99	0.0	MK153246
2133	**MZ233409**	514	98%	Ciliated	98	0.0	KF995027
2196	**MZ233410**	707	100%	*Colpoda steinii*	100	0.0	KY454454
2201a	**MZ233411**	549	97,64%	*Colpoda steinii*	99	0.0	KJ607915
2201b	**MZ233412**	453	90,09%	*Colpoda steinii*	96	2e-158	KJ607915
2209	**MZ233413**	423	94,27%	*Colpoda steinii*	98	0.0	KJ607915
2664	**MZ233414**	379	96,32%	*Parabodo caudatus*	100	3e-175	AY028450
2727	**MZ233415**	353	92,68%	*Colpoda steinii*	99	1e-143	MH715344
2737	**MZ233416**	373	81,43%	*Helkesimastix* sp.	80	1e-59	FJ410915
3129	**MZ233417**	178	94,89%	*Parabodo caudatus*	98	4e-71	DQ207590
3317a	**MZ233418**	676	97,05%	*Parabodo caudatus*	100	0.0	X53910
3317b	**MZ233419**	436	98,86%	*Parabodo caudatus*	100	0.0	DQ207590
3347	**MZ233420**	318	98,74	*Colpoda aspera*	100	1e-157	KF111344
3403	**MZ233421**	635	95,59%	*Urostipulosphaera* sp.	99	0.0	MK153247
3414	**MZ233422**	228	99,12%	Uroglena-like	100	1e-111	MK834582
3415	**MZ233423**	248	97,58%	*Colpoda* sp.	100	6e-116	MH715344

Samples 2201a and 2201b correspond to the same animal, but collected at different periods.
Samples 3317a and 3317b correspond to the same animal, but collected at different periods.
Sequences in bold and italic were obtained in this study.

samples. In a sample identified as *Colpoda steinii*, the host presented signs, such as diarrhea, weight loss, and anorexia, thereby leading to death during the quarantine period. This genus belongs to the phylum Ciliophora, and class Colpodea (Warren, 2011). They are adaptable and widely distributed, often found in wet soil, decaying vegetation, still water, and bodies of fresh or slightly salty water (Lynn, 2008; Costache et al., 2011). This protozoan can divide and take an active form at temperatures between 8 and 35 °C, with an optimum temperature between 25 and 28 °C. They also exhibit high adaptability to different conditions, such as pH, osmolarity, and anaerobic environments (Costache et al., 2011). *Colpoda steinii* is described as a facultative parasite of invertebrate organisms, such as slugs, but there are only a few reports on this species as symbionts, probably commensal, of other organisms, such as reptiles, amphibians, and mammals (Reynolds, 1936; Lynn, 2008; Costache et al., 2011). The ciliate *Colpoda aspera* has also been described previously as a parasite of terrestrial gastropods. Despite the lack of information, they are believed to be facultative commensals and not as serious pathogens (Van As and Basson, 2004).

Reynolds (1936) demonstrated that gastropods can be easily infected by *Colpoda steinii* under the laboratory conditions after consuming contaminated lettuce, suggesting that they might acquire the infection in the same way in the wild. The infection of *Phoneutria nigriventer* by these protozoans can be attributed to the consumption of infected prey, because the animals preyed by spiders can also be infected upon feeding plants or other contaminated food. Upon observing armed spiders under laboratory conditions revealed that these organisms consume water directly from the provided sources (water recipients, drops on the enclosure, etc.), suggesting that they can also ingest *Colpoda* sp. directly during water consumption in the natural environment. Although only one sample belonging to a spider that died during the quarantine was identified as *Colpoda steinii*, Reynolds (1936) described that laboratory-infected slugs died because of infection after one month.

Parabodo caudatus (Stein) Moreira et al. binomial: *Bodo caudatus* Stein, belongs to the phylum Euglenozoa, family Bodonidae. None of the animals infected with these protozoa presented any clinical signs or adverse events. *Parabodo caudatus* is a biflagellate bacterivore found in fresh or salt water, still water with little or no oxygen, sewage, and as intestinal parasites. It is the closest free-living clade to Trypanosomatids (Skalický et al., 2017). This species is poorly documented, but studies have indicated its presence in the urine of a dog presented with hematuria and in gorilla feces (Vandersea et al., 2015; Votýpka et al., 2018).

Helkesimastix sp. belongs to the phylum Cercozoa, family Sainouridae (Cavalier-Smith et al., 2009). It was identified in one sample and was not associated with any clinical signs. Three species were described: *Helkesimastix faecicola*, *Helkesimastix major*, and *Helkesimastix marina*. *Helkesimastix* sp. is flagellated, small, and cylindrical, with unknown life cycle stages (Bass et al., 2016). The first two species were discovered in sheep and goat manure and was described as protozoa that are carried passively through the digestive tract in the form of cysts. Later, both were also found in soil and fresh water (Woodcock and Lapage, 1915; Woodcock, 1921; Tikhonenkov et al., 2012). Although not very abundant, *Helkesimastix* sp. is one of the most widely recorded zooflagellates in soil (Cavalier-Smith et al., 2009).

The genus *Urostipulosphaera* sp. was identified in two sequenced samples, while a *Uroglena*-like genus was identified in one sample. The morphotype similar to that of *Uroglena* sp. is represented by free-living colonial flagellates present in fresh water (Pusztai and Pavel, 2019). *Urostipulosphaera* sp. are colonial flagellates that belong to the phylum Ochrophyta, family Ochyromonadaceae. They were isolated from freshwater samples collected from several European countries (Pusztai and Pavel, 2019). There are no reports in the literature regarding the isolation of this genus from animals or their excreta. All spiders with samples associated with *Urostipulosphaera* sp. and *Uroglena*-like protozoa presented diarrhea, weight loss, and anorexia. One spider positive for *Urostipulosphaera* sp. and one for *Uroglena*-like died during the quarantine period. The presence of clinical signs and high lethality indicated that these protozoans might exhibit high pathogenicity in *Phoneutria nigriventer* under captivity as indicated in this study.

Similar to the genus *Colpoda* sp., the genera *Helkesimastix* sp. and *Urostipulosphaera* sp., *Parabodo caudatus*, and *Euglena*-like parasites are characterized as the widely distributed free-living protozoans found in soil and water. Armed spiders are habitual of wandering and feed on various insects, which may explain the acquisition of protozoan infection identified in the present study. Through correlating the information related to parasitism in vertebrates under captive conditions, some aspects can be suggested. In general, the impact of the parasite on its host depends on several factors, including parasitic load, parasite species, and the host immune system, among others. Due to their habits, armed spiders are constantly on the move, and similar to many free-living animals, they rarely remain in contact with their excreta or with the same environment for a prolonged period. Even if they remain in contact, the environment is altered by rain, wind, and other factors, often making the parasitic infections self-limiting. When kept in captivity, the organism is forced to live in a limited area, significantly increasing the likelihood of continuous exposure to the parasites. For this reason, the parasites identified in this study may exhibit different importance to the host when infecting captive or wild spiders.

Although little is known about the spiders, stress has been shown to

exert negative and positive impacts on invertebrates, thereby triggering a series of physiological changes in response. Chronic stress has been shown to negatively affect the immune response of animals and can lead to weight loss and other effects (Adamo, 2021). Parasitism is one of the causal factors of chronic stress, among others, such as the capture of the animal, manipulation to which they are subjected during the quarantine and the captivity routine, increasing the animal's exposure to stress and its effects. Studies conducted on different animals have shown that the nutritional stress in young animals can affect sexual maturity in several ways, including adult animal size, reproductive success, behavior, life span, among others (Metcalfe and Monaghan, 2001; Jespersen and Toft, 2003). The type of relationship that exists between the protozoans identified in the present study and the host is unknown, as well as the impact caused; however, the intestinal protozoans can lead to nutrient malabsorption, poor digestion, abnormalities in the intestinal mucosa, and imbalance in the intestinal microbiota (Solomons, 1982; Hoste, 2001). The stress due to captivity associated with pathogenicity caused by parasitic infection can be directly and indirectly related to the longevity of parasitized spiders, as well as their development and other physiological functions.

All clinical signs observed in this study were relevant to the digestive tract, including diarrhea, weight loss, and anorexia. Two animals showed signs of helminth infestation concomitant with protozoan infection, with the same gastrointestinal conditions that were being observed. The signs presented by most of these animals exhibited a possible correlation with the protozoan infection; however, there are no studies in the literature aimed at investigating the pathogenicity of intestinal parasites in spiders, thereby reinforcing the need for further research to better understand the results and observations described in the present study. Two spiders produced viable oothecaes and a third one underwent ecdysis without complications during the quarantine period, and it was not possible to associate these events with the intestinal parasite infection.

5. Conclusion

In general, a large number of *Phoneutria nigriventer* received at the Biotério de Artrópodes exhibited intestinal protozoa infection. Moreover, some of the spiders presented crucial clinical signs and symptoms. The virulence and pathogenicity of intestinal protozoa in spiders are unknown; therefore, under captivity, the parasites were easily be carried by the insects or fomites, which exerts a great impact on the herd.

Veterinary medicine in spiders is poorly developed; therefore, the lack of information regarding the intestinal microbiota and clinical signs associated with parasite infections has become a major challenge for the diagnosis and need to implement a directed treatment. In this study, the intestinal protozoa that affect armed spiders were identified for the first time as an initial step for understanding the parasitic diseases in these organisms, and for the adaptation of health management protocols for *Phoneutria nigriventer* that are cultured in captivity for various applications.

Declaration of competing interest

None.

Acknowledgements

This work was financially supported by FAPESP, the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES; funding code 001), and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

Adamo, S.A., 2021. The effects of the stress response on immune function in invertebrates: an evolutionary perspective on an ancient connection. Horm. Behav. 62, 324–330. https://doi.org/10.1016/j.yhbeh.2012.02.012.

Bass, D., Silberman, J.D., Brown, M.W., Pearce, R.A., Tice, A.K., Jousset, A., Geisen, S., Hartikainen, H., 2016. Coprophilic amoebae and flagellates, including *Guttulinopsis, Rosculus* and *Helkesimastix*, characterise a divergent and diverse rhizarian radiation and contribute to a large diversity of faecal-associated protists. Environ. Microbiol. 18 https://doi.org/10.1111/1462-2920.13235, 12064-1619.

Bucaretchi, F., De Deus, R.C.R., Hyslop, S., Madureira, P.R., De Capitani, E.M., Vieira, R. J., 2000. A clinico-epidemiological study of bites by spiders of the genus *Phoneutria*. Rev. Inst. Med. Trop. 42, 17–21. https://doi.org/10.1590/S0036-46652000000100003.

Cavalier-Smith, T., Lewis, R., Chao, E.E., Oates, B., Bass, D., 2009. *Helkesimastix marina* n. sp. (Cercozoa: Sainouroidea superfam. N.) a gliding zooflagellate of novel ultrastructure and unusual ciliary. Behav. Protist 160, 452–479. https://doi.org/10.1016/j.protis.2009.03.003.

Costache, C., Bursaşiu, S., Filipaş, C., Colosi, I., 2011. A case of ciliate Protozoa *Colpoda* Spp. (Ciliata: Colpodidae) detected in human urine. Iran. J. Parasitol. 6, 99–104.

Ministério Da Saúde, 1998. Acidentes por animais peçonhentos [cited 2020 Jul 10]. Avaiable from: http://www.saude.gov.br/saude-de-a-z/acidentes-por-animais-peconhentos/13682-situacao-epidemiologica-dados.

Divisão De Zoonoses/CVE, 2020. Distribuição dos Acidentes por Aranhas Segundo Coeficiente de Incidência, Óbitos e Letalidade, Segundo Ano de Ocorrência. Estado de São Paulo, Período de 1988 a 2020 [cited 2020 Dec 2020]. Avaiable from: http://www.saude.sp.gov.br/resources/cve-centro-de-vigilancia-epidemiologica/areas-de-vigilancia/doencas-de-transmissao-por-vetores-e-zoonoses/dados/peconhentos/peco_aranhas.pdf.

Florez, E.D., Ortiz, A., Montoya, M., 2003. Accidentes pòr mordedura de la araña de las bananeras Phoneutria boliviensis (Araneae, Ctenidae) en la región de Uraba, Colombia. ENTOMOLOGO 96, 1–4.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3 (5), 294–299.

Jespersen, L.B., Toft, S., 2003. Compensatory growth following early nutritional stress in the Wolf spider Pardosa prativaga. Funct. Ecol. 17, 737–746. https://doi.org/10.1111/j.1365-2435.2003.00788.x.

Levine, N.D., 1988. Introduction In: Levine ND. *The Protozoan Phylum Apicomplexa*, 1 ed., I.. CRC Press.

Lucas, S., 1988. Spiders in Brazil. Toxicon 26, 759–772. https://doi.org/10.1016/0041-0101(88)90317-0.

Lynn, D.H., 2008. The Ciliated Protozoa: Characterization, Classification, and Guide to the Literature, 3 ed. Pergamon Press.

Maia Da Silva, F., Rodrigues, A.C., Campaner, M., Takata, C.S.A., Brigido, M.C., Junqueira, A.C.V., Coura, J.R., Takefa, G.F., Shaw, J.J., Teixeira, M.M.G., 2004. Randomly amplified polymorphic DNA analysis of *Trypanosoma rangeli* and allied species from human, monkeys and other sylvatic mammals of the Brazilian Amazon disclosed a new group and a species-specific marker. Parasitology 128 (3), 283–294. https://doi.org/10.1017/S0031182003004554.

Málaque, C.M.S., Antunes, E., 2004. Mecanismo de Ação do veneno de *Phoneutria* e aspectos clínicos do foneutrismo. In: Cardoso, J.L.C., De França, F.O., Wen, F.H., Málaque, C.M.S., Haddad, J.R.V. (Eds.), Animais Peçonhentos no Brasil: Biologia, clínica e terapêutica dos Acidentes.

Maslov, D.A., Podlipaev, A.S., Luke, J., 2001. Phylogeny of the Kinetoplastida: taxonomic problems and insights into the evolution of parasitism. Mem. Inst. Oswaldo Cruz 96, 397–402. https://doi.org/10.1590/S0074-02762001000300021.

Metcalfe, N.B., Monaghan, P., 2001. Compensation for bad start: grow now, pay later? Trends. Ecol. Evol. 16, 254–260. https://doi.org/10.1016/s0169-5347(01)02124-3.

Milyutina, I.A., Aleshin, V.V., Mikrjukov, K.A., Kedrova, O.S., Petrov, N.B., 2001. The unusually long small subunit ribosomal RNA gene found in amitochondriate amoeboflagellate Pelomyxa palustris: its rRNA predicted secondary structure and phylogenetic implication. Gene 272, 131–139. https://doi.org/10.1016/s0378-1119(01)00556-x.

Pineda, S.S., Undhein, E.A., Rupasinghe, D.B., Ikonomopoulou, M.P., King, G.F., 2014. Spider venomics: implications for drug discovery. Future Med. Chem. 6, 1629–1643. https://doi.org/10.4155/fmc.14.103.

Poinar, J.R.G., 1987. Nematode parasites of spiders. Ecophysiol. Spiders 299–308. https://doi.org/10.1007/978-3-642-71552-5_22.

Pusztai, M., Pavel, S., 2019. Elucidating the evolution and diversity of Uroglena-like colonial flagellates Crysophyceae): polyphyletic origin of the morphotype. Eur. J. Phycol. 54, 1–13. https://doi.org/10.1080/09670262.2019.1574030.

Ramos, E.F., Almeida, C.E., Gouvea, E., Carmo-Silva, M.D., 1998. Considerações sobre a atividade de locomoção, preferência por ecótopos e aspectos territoriais de *Phoneutria nigriventer* (Keyserling, 1891). Rev. Bras. Biol. 58, 71–78.

Reynolds, B.D., 1936. *Colpoda steini*, a facultative parasite of the land slug, Agriolimax agrestics. J. Parasitol. 22, 48–53.

Simpson, A.G.B., Stevens, J.R., Lukes, J., 2006. The evolution and diversity of kinetoplastid flagellates. Trends Parasitol. 22, 168–174. https://doi.org/10.1016/j.pt.2006.02.006.

Solomons, N.W., 1982. Giardiasis: nutritional implications. Rev. Infect. Dis. 4, 859–869. https://doi.org/10.1093/4.4.859.

Tikhonenkov, D.V., Mylnikov, A.P., Gong, Y.C., Feng, W.S., Mazei, Y., 2012. Heterotrophic flagellates from freshwater and soil habitats in subtropical China (Wuhan area, Hubei province). Acta Protozool. 51, 65–79. https://doi.org/10.4467/16890027AP.12.006.0389.

Tikhonenkov, D.V., Janouškovec, J., Keeling, P.J., Mylnikov, A.P., 2016. The morphology, ultrastructure and SSU rRNA gene sequence of a new freshwater flagellate, *Neobodo borokensis* n. sp. (Kinetoplastea, Excavata). J. Eukaryot. Microbiol. 63, 220–232. https://doi.org/10.1111/jeu.12271.

Van As, J., Basson, L., 2004. Ciliophoran (Ciliophora) parasites of Terrestrial Gastropods. In: Barker, G.M. (Ed.), Natural Enemies of Terrestrial Molluscs. CABI Publishing.

Vandersea, M.W., Birkenheuer, A.J., Litaker, R.W., Vaden, S.L., Renschler, J.S., Gookin, J.L., 2015. Identification of P*arabodo caudatus (*class Kinetoplastea) in urine voided from a dog with hematuria. *JVDI* 27, 117–120. https://doi.org/10.1177/1040638714562827.

Votýpka, J., Paflco, B., Modrý, D., Mbohli, D., Tagg, N., Petrželková, K.J., 2018. An unexpected diversity of trypanosomatids in fecal samples of great apes. Paras. Wildl. 7, 322–325. https://doi.org/10.1016/j.ijppaw.2018.09.003.

Warren, A., 2011. Colpoda Steini in: World Ciliophora Database [cited 2020 Dec 15]. Avaiable from. http://www.marinespecies.org/aphia.php?p=taxdetails&id=426727.

Woodcock, H.M., 1921. Notes on coprozoic flagellates: 1. On the presence of an accessory flagellum in the genus *Helkesimastix*, Woodcock and Lapage. Arch. Zool. Exp. Gen. 60, 11–15.

Woodcock, H.M., Lapage, G., 1915. Observations on the life-cycle of a new flagellate, *Helkesimastix faecicola*, n. g., n. sp.: together with remarks on the question of syngamy in the trypanosomes. Proc. Roy. Soc. 88, 353–370.

Yubuki, N., Zadrobílkova, E., Čepička, I., 2016. Ultrastructure and Molecular Phylogeny of *Iotanema spirale* gen. nov. et sp. nov., a New Lineage of Endobiotic Fornicata with Strikingly Simplified Ultrastructure. J. Eukaryot. Microbiol. 64, 422–433. https://doi.org/10.1111/jeu.12376.