

Morphological Re-Description and 18S rDNA Sequence Confirmation of the Pinworm *Aspiculuris tetraptera* (Nematoda, Heteroxyneematidae) Infecting the Laboratory Mice *Mus musculus*

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This article was edited by Zafar Handoo.

Received for publication February 10, 2018.

Abstract

Aspiculuris tetraptera is a heteroxyneematid nematoda infecting most of the laboratory animals, occasionally mice which represent the mostly used animal for biological, medical, and pharmacological studies. The present study aimed to investigate the prevalence of nematode parasites infection in the laboratory mice *Mus musculus* in Egypt. Morphologically, this oxyurid possessed four distinct cephalic papillae on a cephalic plate, with three small rudimental lips carrying two sessile poorly developed labial papillae and one pair of amphidial pores. Esophagus divided into cylindrical corpus and globular bulb. Distinct cervical alae interrupted at the level of esophago-intestinal junction forming an acute angle. At the caudal end, twelve caudal papillae in male worms while an ovjector apparatus opening and a vulva surrounded by protruded lips in females were observed. The general morphological criteria include this nematode with other *Aspiculuris* species which were compared in the present study. Molecular characterization based on 18SSU rDNA sequencing performed to confirm the taxonomic position of this species and to documents the morphological data. Sequence alignment detects a percent of identity up to 88.0% with other Heteroxyneematidae species. Phylogenetic analysis showed that the present recorded is a putative sister taxon to *A. tetraptera* recorded in a previous study. The SSU rDNA sequence has been deposited in the GenBank under the accession no. MG019400.

Key words

Laboratory mice, Pinworms, *Aspiculuris* species, Morphological description, Molecular study

Pinworms are routinely found in animals from modern animal facilities, even in facilities free of viral and bacterial diseases that affect mice (Jacoby and Lindsey, 1998; Zenner and Regnault, 2000; Behnke et al., 2015). Oxyurids are also a common parasite of Muroidea (Rodentia) (Singleton et al., 1993; Pisanu et al., 2001). *Syphacia obvelata* and *Aspiculuris tetraptera* are oxyurid nematodes which are cosmopolitan monoxenous parasites that are transmitted through the ingestion of embryonated eggs (Stojcevic

et al., 2004; Robles and Navone, 2010; Khalil et al., 2014). Mice may be concurrently infected with both species of pinworms (Jacobson and Reed, 1974; Taffs, 1976; Nicklas et al., 1984; Gonçalves et al., 1998; Zenner, 1998; Agersborg et al., 2001). The common incidence of infection by both pinworm species can be explained by their preference for slightly different sites of the gastrointestinal tract. As such, these species do not compete directly for resources; they are able to maintain simultaneous infections (Pinto et al., 2001;

Bazzano et al., 2002). In concurrent infections, there may be higher numbers of *A. tetraptera* worms because their longer lifespan permits the accumulation of parasites in their hosts (Scott and Gibbs, 1986). The prevalence of pinworms in an infected rodent population depends on many factors, including gender, age, strain, immune status, and the concentration of parasite ova in the environment.

The genus *Aspiculuris* was established by Nitzsch (1821) and later re-described by Schulz (1924) from *Mus musculus*. Many species of *Aspiculuris* have been reported worldwide (Hugot, 1980; Inglis et al., 1990; Falcón-Ordaz et al., 2010). Species of *Aspiculuris* were separated by Quentin (1975) into two groups based on the shape of their cervical alae. Nematodes that display interrupted cervical alae with pointed posterior ends belong to the first group. Those in the second group possess a rounded posterior end to the cervical alae. *Aspicularis tetraptera* are common oxyurids belonged to the first group. This species has been described in the cecum and colon of *M. musculus* in different regions, such as Tunisia, Iran, Venezuela, Europe, Siberia, China, Japan, United States, and Egypt (Hugot, 1980; Neifer et al., 1991; Durden et al., 2000; Perec-Matysiak et al., 2006; Mahmoud et al., 2009; Abdel-Gaber, 2016), and to a lesser extent, it has been recorded in other hosts, such as *Cricetus*, *Rattus*, *Apodemus*, *Microtus*, *Arctomys*, *Jaculus*, *Clethrionomys*, and *Peromyscus* in the same regions (Mathies, 1959; Sasa et al., 1962). Quentin (1975) indicated this species in Central Africa in *Mastomys*, *Praomys*, and *Thamnomys*.

Species of *A. tetraptera* are characterized by their medium or small size, the presence of three lips, absence of buccal capsule, and presence of esophagus with a well-developed single bulb located at its posterior end (Bazzano et al., 2002; Perec-Matysiak et al., 2006; Malsawmtluangi and Tandon, 2009; Li et al., 2016). Recently, morphological identification requires the use of molecular characteristics for accurate identification and validation; these characteristics are common in nematoda systematics (Mc-Manus and Bowles, 1996; Semenova et al., 1996; Gasser, 2001; Jones et al., 2012; Chaudhary et al., 2016; Curtis et al., 2017).

Therefore, the present study reported the natural prevalence, morphological, and morphometric characteristics, in addition to molecular analysis of ribosomal DNA gene sequences of the recovered oxyurid pinworm infecting the laboratory mouse *M. musculus* to clarify the taxonomic status and phylogenetic position of this parasite species within Heteroxynematidae.

Materials and Methods

Animal collection and parasitological examination

Fifty specimens of adult laboratory mice (Muridae: *M. musculus*) reared at the Animal House at Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt; were randomly collected between December 2016 and September 2017. The collected mice were transported alive to the Laboratory of Parasitology Research for parasitological examination. Mice were anesthetized and killed according to the ethical rules for handling experimental animals. Mice were examined for any external signs of infection. After dissection, internal organs were removed from the rodent and examined for any parasitic infections. Isolated worms were fixed in 70% ethanol and subsequently clarified with lactophenol for morphological identification, in accordance with standard reference keys by Pinto et al. (2001). Prevalence of parasitic infection (number of infected mice/total number of mice hosts examined $\times 100$) of *M. musculus* was calculated according to Bush et al. (1997). Illustrations of adult specimens were prepared with the aid of a microscope Leica DM 2500, LAS software (3.8) and Corel Draw X4® software. Measurements were based on 20 adult worm species; data were taken in millimeters and are presented as a range followed by the arithmetic mean \pm SD in parentheses.

Molecular analysis

DNA extraction, polymerase chain reaction amplification, and sequencing

gDNA was extracted from ethanol-preserved samples using DNeasy tissue kit® (Qiagen, Hilden, Germany) following the manufacturer's instructions. The DNA was stored in 50 μ l of TE buffer at -20°C until further use. DNA concentration and purity were determined spectrophotometrically by measuring absorbance at wavelengths of 260 and 280 nm. PCR amplification was performed in a final volume of 25 μ l, containing 3 μ l of genomic DNA, 2.5 μ l of 10X Taq polymerase buffer, 10 pmol of each primer, 100 μ M of each dNTP (Finnzymes Products), and 1.5 U of Taq DNA polymerase (Finnzymes Products). The partial ribosomal 18S gene was amplified using the primer Nem 18SF (5'-CGC GAA TRG CTC ATT ACA ACA GC-3') and Nem 18SR (5'-GGG CGG TAT CTG ATC GCC-3') designed by Floyd et al. (2005). Polymerase chain reaction (PCR) consisted of an initial denaturation step at 94°C for 3 min, followed by 35 cycles of 1 min at 94°C ,

1 min at 50°C, followed by 1 min at 72°C, and finally, post-PCR extension was carried out for 7 min at 72°C. All PCR products were verified on 1% agarose gel in $\times 1$ Tris–acetate–EDTA (TAE) stained with 1% ethidium bromide visualized with UV transilluminator, and bands with predicted size were purified using Pure Link™ Quick Gel Extraction Kit (Invitrogen) following the manufacturer's instructions. Amplicons were sequenced (in both directions) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Thermo Fisher Scientific, Waltham, MA, USA) with the 310 Automated DNA Sequencer (Applied Biosystems, MA, USA) using the same primers for annealing.

Sequence alignment and phylogenetic analysis

BLAST search was carried out to identify related sequences on NCBI database. Sequences were aligned directly using CLUSTAL-X multiple sequence alignment (Thompson et al., 1997) and compared with previously recorded data from GenBank™ to analyze intra-specific differences. GenBank accession numbers of additional sequences utilized in the analyses were as follows: *Cosmocerca japonica* (LC052782), *Parascaris equorum* (JN617987), *Enterobius vermicularis* (HQ646164), *Cucullanus extraneus* (KT192060), *Pseudanisakis rajeae* (JN392470), *Skrjabinema kamosika* (AB699691), *Aspiculuris dinniki* (KT175736), *Aspiculuris tianjinensis* (KT175733), and *A. tetraptera* (KT175725, KT175728, KT175729, KJ143615, KJ143618, KJ143617, KJ143616, EF464551, and EU263107) as shown in Table (1). The alignment was corrected manually using the alignment editor of software BIOEDIT 4.8.9 (Hall 1999). Phylogenetic calculations were performed with PAUP 4.0b10 (Swofford, 2000). The data were analyzed with maximum parsimony (neighbor-interchange [CNI] level 3, random addition trees 100). Additionally, neighbor-joining was calculated by using MEGALIGN package (DNASTAR, Windows version 3.12e).

Results

A total of 28 out of 50 (56.0%) specimens of laboratory mice *M. musculus* were found to be naturally infected with an oxyurid nematode. The recovered parasite species were found in the cecum and upper colon of the infected host mice.

Description

In general, the body of the recovered worms was small, cylindrical in shape, and covered by a transversely stri-

ated cuticle. Head was bulb-like; mouth opening was surrounded by three less developed lips, one pair of lateral epaulettes, one pair of amphidial pores, and two pairs of large sub-median cephalic papillae. Mouth opening leads to the buccal cavity, followed by pharynx, esophagus, and long intestine opening exteriorly by an anal opening in females and cloacal opening in males. Anterior part of esophagus was club-shaped followed by well-developed bulb. Body in both sexes has distinct cervical alae, beginning immediately posterior to the anterior end of the cephalic vesicle. Cervical alae abruptly interrupted at the level of esophago-intestinal junction, forming an acute angle. Anterior end of the body has prominent, elaborate inflated region, forming cephalic vesicle (Fig. 1A–G, Tables 2,3).

Male worm (based on 10 mature specimens)

Body length was 2.23–3.29 (2.79 ± 0.1) mm with maximum width 0.16–0.20 (0.18 ± 0.1) mm. Cephalic vesicle was 0.06–0.09 (0.07 ± 0.001) mm long by 0.05–0.08 (0.06 ± 0.001) mm wide. Esophagus measured 0.32–0.40 (0.39 ± 0.1) mm long by 0.05–0.09 (0.07 ± 0.01) mm wide; while, the whole esophagus with bulb reached 0.13–0.17 (0.15 ± 0.1) mm long by 0.04–0.07 (0.05 ± 0.01) mm wide. Cervical alae began at 0.015–0.018 (0.017 ± 0.001) mm from the anterior end and measured about 0.21–0.29 (0.25 ± 0.001) mm long with recurved terminal ends by 0.029–0.038 (0.031 ± 0.001) mm wide. Nerve ring and excretory pore are located at 0.065–0.082 (0.078 ± 0.001) mm and 0.392–0.547 (0.491 ± 0.03) mm from the anterior end, respectively. Narrow lateral alae of the body end located at the beginning of the caudal alae are extended from the level of cloaca and surrounded the entire end of the body, bending ventrally at its tip as a vesicular swelling of the cuticle. Cloaca opening is located at 0.09–0.11 (0.10 ± 0.01) from the posterior extremity of the body. Testes are flexed over the anterior third of the intestine. Gubernaculum and spicules were absent. Posterior end with 12 caudal papillae included one pair precloacal, two pairs adcloacal, one pair postcloacal, two median papillae postcloacal, one behind the other, and a further posterior pair midway between cloaca and end of the tail. Tail, with blunt end, measured 0.11–0.14 (0.12 ± 0.1) mm long.

Female worm is larger than that of the male (based on 10 mature specimens)

Body length was 2.9–3.4 (3.1 ± 0.1) mm long with maximum width was 0.19–0.23 (0.20 ± 0.01) mm. Cephalic

Table 1. Nematoda species used in the phylogenetic analysis of *Aspiculuris tetraptera* in the present study.

Parasite species	Order/family	Host species	Source	Accession no.	Sequence length (bp)	Divergence	Percent identity (%)
<i>Cosmocerca japonica</i>	Ascaridida/ Cosmocercidae	<i>Rana ornativentris</i>	GenBank	LC052782	749	5.2	89.0%
<i>Parascaris equorum</i>	Ascaridida/ Ascarididae	Africa lion and wolf	GenBank	JN617987	814	5.2	89.0%
<i>Cucullanus extraneus</i>	Rhabditida/ Cucullanidae	<i>Pomacanthus maculosus</i>	GenBank	KT192060	1,150	4.9	90.0%
<i>Pseudanisakis rajae</i>	Rhabditida/ Acanthocheilidae	Elasmobranchs	GenBank	JN392470	903	4.9	90.0%
<i>Enterobius vermicularis</i>	Oxyurida/ Oxyuroidae	Human children	GenBank	HQ646164	2,867	5.2	91.0%
<i>Skrjabinema kamosika</i>	Oxyurida/ Oxyuroidae	<i>Capricornis crispus</i>	GenBank	AB699691	898	4.9	91.0%
<i>Aspiculuris tianjinensis</i>	Oxyurida/ Heteroxyneematidae	<i>Mus musculus domesticus</i>	GenBank	KT175733	967	3.2	92.0%
<i>Aspiculuris tetraptera</i>	Oxyurida/ Heteroxyneematidae	<i>M. musculus domesticus</i>	GenBank	KT175725	936	1.4	97.0%
<i>Aspiculuris dinniki</i>	Oxyurida/ Heteroxyneematidae	<i>Myodes glareolus</i>	GenBank	KT175736	995	0.6	98.0%
<i>A. tetraptera</i>	Oxyurida/ Heteroxyneematidae	<i>M. glareolus</i>	GenBank	KT175728	954	0.6	98.0%
<i>A. tetraptera</i>	Oxyurida/ Heteroxyneematidae	<i>M. glareolus</i>	GenBank	KT175729	964	0.6	98.0%
<i>A. tetraptera</i>	Oxyurida/ Heteroxyneematidae	<i>M. musculus musculus</i>	GenBank	KJ143615	419	0.1	99.0%
<i>A. tetraptera</i>	Oxyurida/ Heteroxyneematidae	<i>M. musculus musculus</i>	GenBank	KJ143618	419	0.1	99.0%
<i>A. tetraptera</i>	Oxyurida/ Heteroxyneematidae	<i>M. musculus musculus</i>	GenBank	KJ143617	419	0.1	99.0%
<i>A. tetraptera</i>	Oxyurida/ Heteroxyneematidae	<i>M. musculus musculus</i>	GenBank	KJ143616	419	0.1	99.0%
<i>A. tetraptera</i>	Oxyurida/ Heteroxyneematidae	<i>M. musculus musculus</i>	GenBank	EF464551	3,676	0.1	99.0%
<i>A. tetraptera</i>	Oxyurida/ Heteroxyneematidae	<i>M. musculus musculus</i>	GenBank	EU263107	1,032	0.1	99.0%

vesicle reached about 0.078–0.083 (0.082±0.001)mm long by 0.106–0.130 (0.123±0.01)mm wide. Esophagus measured 0.30–0.34 (0.32±0.01)mm long and 0.14–0.16 (0.15±0.01)mm wide; while, esophagus with bulb reached about 0.10–0.13 (0.11±0.01)mm long and 0.05–0.09 (0.07±0.01)mm wide. Nerve ring and excretory pore located at 0.078–0.090 (0.085±0.002)mm

and 0.564–0.780 (0.680±0.02)mm from the anterior end, respectively. Cervical alae with recurved terminal end was 0.27–0.29 (0.26±0.01)mm long. Distance from the anterior end to the beginning of cervical alae was 0.021–0.026 (0.024±0.001)mm. Vulva was preequatorial, surrounded by protruded lips, and situated at 1.112–1.630 (1.406±0.03)mm from the anterior extremity of

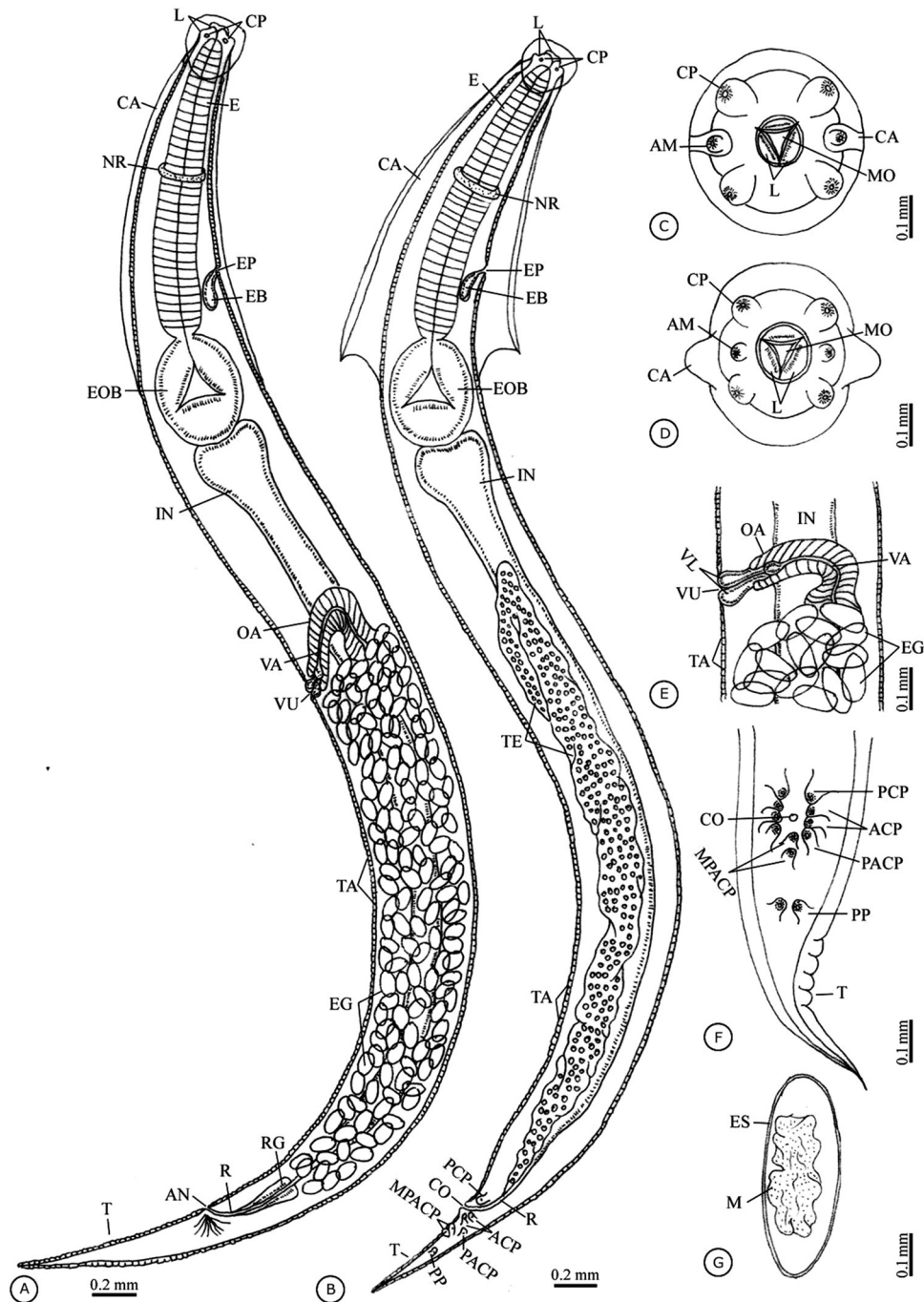


Figure 1: A–G, Line drawings of different body parts of *Aspicularis tetraptera*. A, Lateral view of female worm with mouth opening surrounded by three lips with cephalic papillae (CP) and amphids (AM), muscular esophagus (E), esophageal bulb region (EOB), intestine (IN), rectum (R) with rectal gland (RG), anal opening (AN) and ending with a long tapered tail (T). Note, transverse annulated (TA) cuticle, and the genital system characterized with a uterus filled with numerous eggs (EG), ovijector apparatus (OA), vagina (VA) and vulval opening (VU) surrounded by two fleshy vulval lips (VL). B, Lateral view of male worm with mouth opening surrounded by three lips with cephalic papillae (CP) and amphids (AM), followed by muscular esophagus (E), esophageal bulb region (EOB), intestine (IN), rectum (R) with rectal gland (RG), anal opening (AN), and ending with a long tapered tail (T). Note, transverse annulated (TA) cuticle, and the genital system with testes (TE), cloacal opening (CO) surrounded by precloacal papillae (PCP), adcloacal papillae

(ACP), postadcloacal papillae (PACP), median postadcloacal papillae (MPACP), and posterior papillae (PP). C–G, High magnifications of: C, Face view of anterior extremity of female worm mouth opening (MO) surrounded by three lips (L) with cephalic papillae (CP) and amphids (AM) with cervical alae (CA). D, Face view of anterior extremity of male worm mouth opening (MO) surrounded by three lips (L) with cephalic papillae (CP) and amphids (AM) with cervical alae (CA). E, Ovejector region (OA) of female showing vulva opening (VU), two fleshy vulval lips (VL), muscular vagina (VA), and eggs collected (EG) from uterus. F, Posterior end of male worm showing the cloacal opening (CO) with caudal papillae of precloacal papillae (PCP), adcloacal papillae (ACP), postadcloacal papillae (PACP), median postadcloacal papillae (MPACP), and posterior papillae (PP). G, Morula (M) surrounded by egg shell (ES).

the body. Ovejector apparatus measured about 0.29–0.38 (0.32±0.01) mm long. Muscular vagina proceeded forward for a short distance then turned backward joining uterus filled with eggs. Two ovaries flexed over the proximal part of the intestine. Anal pore located at 0.32–0.39 (0.37±0.01) mm from the posterior end of the body. Tail with blunt tip measured 0.30–0.42 (0.39±0.01) mm long. Eggs were unoperculated, smooth, filled by morula and measured 0.04–0.06 (0.05±0.01) mm long and 0.02–0.04 (0.03±0.01) mm wide.

Taxonomic summary

Parasite name: *Aspiculuris tetraptera* (Nitzsch, 1821; Family: Heteroxynematidae (Skrjabin and Schikhobalova, 1948)).

Host: Laboratory mice *M. musculus* (Linnaeus, 1758; Family: Muridae).

Mode of transmission: Ingestion of embryonated eggs in feces, or in contaminated food and water, or bedding.

Morbidity and mortality: Infected laboratory mice were generally symptomless externally.

Site of infection: Cecum and upper colon of infected host mice.

Prevalence and intensity: 28 out of 50 (56.0%) examined individuals were infected, with a total number of 120 nematodes.

Material deposition: Voucher specimens were deposited at museum in Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt.

Molecular analysis

A sequence of 840bp was deposited in GenBank under accession no. MG019400 with a GC content of 42.26%, for SSU rDNA gene sequences of the present oxyurid species. Pairwise comparison of the isolated gDNA sequence of the present parasite species with a range of other Spirurina species and genotypes revealed a unique sequence. The calculated identity between this novel sequence and those retrieved from

GenBank demonstrated a high degree of similarity, up to 88.0% (Table 1). Comparison of the nucleotide sequences and divergence showed that SSU rDNA of the present oxyurid species had the highest blast scores with a small number of nucleotide differences with other *A. tetraptera* species under the following accession numbers: EU263107, EF464551, KJ143616, KJ143617, KJ143618, KJ143615, KT175729, KT175728, KT175725, and KT175725; *A. dinniki* (acc. no. KT175736); *A. tianjinensis* (acc. no. KT175733); *E. vermicularis* (acc. no. HQ646164); and *S. kamosika* (acc. no. AB699691).

Phylogenetic analysis led to the construction of a neighbor-joining tree, constructed with partial sequences, which showed that Spirurina species consistently formed two major clades (Fig. 2). The first one represented the most related families in order Oxyurida, including families Heteroxynematidae and Oxyuridae with sequence similarity ranging between 99.0 and 91.0%. The second one was represented by four families Ascarididae (*P. equorum* JN617987), Anisakidae (*P. rajae* JN392470), Cucullanida (*C. extraneus* KT192060), and Cosmocercidae (*C. japonica* LC052782), belonging to the order Ascaridia with sequence similarity ranging between 90.0 and 89.0%. This sequence in conjunction with existing data, suggested the placement of this oxyurid species within family Heteroxynematidae. The present species was deeply embedded in the genus *Aspiculuris*, and is closely related to other *Aspiculuris* species, especially to other previously described *A. tetraptera* as a putative sister taxon.

Discussion

Laboratory animal models, especially rodents of the family Muridae, constitute important links in the food chains within the ecosystems they inhabit (Rosas, 1997, Gonçalves et al., 1998). These animals are often in contact with humans and domestic animals and can transmit various parasitic species (Bazzano et al., 2002). In conventional animal facilities, rodent colonies are frequently infected with helminth parasites, or become infected during the experimental

Table 2. Main morphological features and measurements of male *Aspiculuris tetraptera* compared with previous studies.

Related species	<i>Aspiculuris ackerti</i>	<i>Aspiculuris artigasi</i>	<i>Aspiculuris versterae</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris tetraptera</i>	<i>A. tetraptera</i>	<i>A. tetraptera</i>	<i>Aspiculuris huascaensis</i>	<i>Aspiculuris tianjinensis</i>	<i>A. tetraptera</i>	<i>A. tetraptera</i>	<i>A. tetraptera</i>	
Parameters	Kruidenier and Mehra (1959)	Araujo (1965)	Hugot (1980)	Hugot (1980)	Hugot (1980)	Miller and Schmidt (1982)	Kohn and Macedo (1984)	Pinto et al. (1994)	Landaeta-Aqueveque et al. (2007)	Falcón-Ordaz et al. (2010)	Liu et al. (2012)	Khalil et al. (2014)	Abdel-Gaber and Fol (2015)
Host species	<i>Neotoma albigda</i>	<i>M. musculus</i>	<i>Mastomys natalensis</i>	<i>Mastomys natalensis</i>	<i>Mastomys natalensis</i>	<i>Neotoma cinerea</i>	<i>Haemulon sciurus</i>	<i>M. musculus</i>	<i>M. musculus</i>	<i>M. musculus</i>	<i>Clethrionomys rufocanus</i>	<i>M. musculus</i>	<i>M. musculus musculus</i>
Host locality	Coconimo, Arizona	Sao Paulo, Brazil	Onderstepoort, South Africa	Maracay, Venezuela	Colorado, Idaho, USA	Colorado, Idaho, USA	Rio de Janeiro, Brazil	Rio de Janeiro, Brazil	Santiago, Chile	Hidalgo, Mexico	Tianjin, China	Cairo, Egypt	Cairo, Egypt
Site of infection	Intestine	Intestine	Caecum	Caecum	Caecum	Caecum	Intestine	Intestine	Caecum and upper colon	Intestine	Intestine	Caecum	Caecum and upper colon
Body length	4.41–4.87 (4.64)	2.945–3.472	1.59	2.7	3.01–6.35 (5.03)	2.90–3.60 (3.22)	2.4–3.1	2.65–3.95 (3.393±0.427)	2.16–2.69 (2.48±0.172)	3.69–5.12 (4.69)	2.55–2.57 (2.56±0.014)	2.73–3.87 (3.21±0.1)	2.23–3.29 (2.79±0.1)
Body width	0.162–0.174 (0.168)	0.156–0.180	0.10	0.11	0.137–0.293 (0.236)	0.15–0.20 (0.17)	0.17–0.21	0.17–0.25 (0.202±0.2)	0.078–0.114 (0.096±0.01)	0.204–0.388 (0.307)	0.088 (0.088±0)	0.18–0.20 (0.20±0.1)	0.16–0.20 (0.18±0.1)
Esophageal length	0.264–0.271 (0.267)	0.213–0.243	0.310	0.38	0.260–0.480 (0.343)	0.18–0.27 (0.23)	0.288–0.360	0.320–0.390 (0.375±0.02)	0.291–0.294 (0.272±0.013)	0.343–0.397 (0.384)	0.439–0.450 (0.44±0.007)	0.38–0.45 (0.41±0.1)	0.32–0.40 (0.39±0.1)
Esophageal width	0.039–0.040 (0.039)	0.036–0.054	–	–	0.033–0.060 (0.051)	–	–	–	–	–	–	0.06–0.11 (0.08±0.01)	0.05–0.09 (0.07±0.01)
Esophageal bulb length	0.145–0.155 (0.150)	0.105–0.135	0.10	0.14	0.113–0.183 (0.157)	0.10–0.12 (0.11)	–	0.110–0.140 (0.133±0.01)	0.090–0.110 (0.097±0.006)	0.074–0.142 (0.122)	0.244 (0.24±0)	0.14–0.19 (0.16±0.1)	0.13–0.17 (0.15±0.1)

(Continued)

Table 2. Main morphological features and measurements of male *Aspiculuris tetraptera* compared with previous studies. (Continued)

Related species	<i>Aspiculuris ackerti</i>	<i>Aspiculuris artigasi</i>	<i>Aspiculuris versterae</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris A. ackerti tetraptera</i>	<i>A. tetraptera</i>	<i>A. tetraptera</i>	<i>Aspiculuris huascaensis</i>	<i>Aspiculuris tianjinensis</i>	<i>A. tetraptera</i>	<i>A. tetraptera</i>	<i>A. tetraptera</i>		
Parameters	Kruidenier and Mehra (1959)	Araujo (1965)	Hugot (1980)	Hugot (1980)	Hugot (1980)	Miller and Schmidt (1982)	Kohn and Macedo (1984)	Pinto et al. (1994)	Landaeta-Aqueveque et al. (2007)	Falc3n-Ordaz et al. (2010)	Liu et al. (2012)	Khalil et al. (2014)	Abdel-Gaber and Fol (2015)	A. tetraptera (Present study)
Esophageal bulb width	0.071–0.079 (0.075)	0.075–0.096	0.08	0.09	0.065–0.120 (0.090)	0.06–0.08 (0.07)	0.11–0.13 (0.12)	0.115–0.140	–	0.040–0.063 (0.051± 0.006)	0.059–0.123 (0.098)	0.077–0.088 (0.08± 0.007)	0.07–0.09 (0.08± 0.01)	0.04–0.07 (0.05± 0.01)
Distance from anterior extremity	0.158	0.108–0.144	0.09	0.10	0.150–0.213 (0.186)	0.11–0.13 (0.12)	0.115–0.140	0.115–0.145 (0.129± 0.013)	0.115–0.145 (0.129± 0.013)	0.072–0.093 (0.080± 0.008)	0.116–0.133 (0.123)	0.088 (0.088±0)	–	0.065–0.082 (0.078± 0.001)
Excretory pore	0.600–0.876	0.325	0.78	0.69–1.21 (0.96)	0.69–0.78 (0.73)	0.619	0.750–0.910 (0.854± 0.056)	0.495–0.579 (0.545± 0.035)	0.495–0.579 (0.545± 0.035)	0.93–1.03 (1.01)	0.144–0.147 (0.145± 0.002)	–	0.392–0.547 (0.491± 0.03)	
No. of Caudal papillae	11	5	5	5	10	12	12	12	12	12	12	12	12	12
Tail length	0.238–0.280 (0.259)	0.147–0.183	0.12	0.17	0.049–0.117 (0.092)	0.10–0.12 (0.11)	0.144–0.172	0.162–0.187 (0.178± 0.009)	0.162–0.187 (0.178± 0.009)	0.075–0.114 (0.102± 0.013)	0.181–0.245 (0.220)	0.12–0.144 (0.132± 0.01)	0.13–0.15 (0.14± 0.1)	0.11–0.14 (0.12± 0.1)

Table 3. Main morphological features and measurements of female *Aspiculuris tetraptera* compared with previous studies.

Related species	<i>Aspiculuris ackerti</i>	<i>Aspiculuris artigasi</i>	<i>Aspiculuris versterae</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris ackerti</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris huascaensis</i>	<i>Aspiculuris tianjinensis</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris tetraptera</i>
Parameters	Kruidenier and Mehra (1959)	Araujo (1965)	Hugot (1980)	Hugot (1980)	Miller and Schmidt (1982)	Kohn and Macedo (1984)	Pinto et al. (1994)	Landaeta-Aqueveque et al. (2007)	Falcón-Ordaz et al. (2010)	Liu et al. (2012)	Khalil et al. (2014)	Abdel-Gaber and Fol (2015)
Host species	<i>Neotoma albigda</i>	<i>Mus musculus</i>	<i>Mastomys natalensis</i>	<i>M. natalensis</i>	<i>Neotoma cinerea</i>	<i>Haemulon sciurus</i>	<i>M. musculus</i>	<i>M. musculus</i>	<i>M. musculus</i>	<i>Clethrionomys rufocanus</i>	<i>M. musculus</i>	<i>M. musculus</i>
Host locality	Coconimo, Arizona	Sao Paulo, Brazil	Onderstepoort, South Africa	Maracay, Venezuela	Colorado, Idaho, USA	Rio de Janeiro, Brazil	Río de Janeiro, Brazil	Santiago, Chile	Hidalgo, Mexico	Tianjin, China	Cairo, Egypt	Cairo, Egypt
Site of infection	Intestine	Intestine	Caecum	Caecum	Caecum	Intestine	Intestine	Caecum and upper colon	Intestine	Intestine	Caecum	Caecum and upper colon
Body length	5.5–6.8 (6.2)	3.534–5.394	1.95	1.2	5.46–8.97 (7.41)	3.60–4.61 (4.18)	3.1–3.6	3.53–4.51 (3.53–4.51)	3.024–3.528 (3.219±0.150)	5.38–7.00 (6.46)	3.55–4.12 (3.83±0.403)	3.2–3.6 (3.5±0.1)
Body width	0.197–0.232 (0.210)	0.153–0.234	0.15	0.08	0.225–0.391 (0.330)	0.20–0.30 (0.27)	0.175–0.245	0.230–0.270 (0.260±0.01)	0.099–0.153 (0.120±0.016)	0.282–0.447 (0.352)	0.199–0.288 (0.244±0.062)	0.21–0.26 (0.24±0.01)
Esophageal length	0.290–0.348 (0.317)	0.252–0.336	0.49	0.43	0.305–0.465 (0.387)	0.25–0.29 (0.27)	0.331–0.433	0.400–0.450 (0.425±0.01)	0.312–0.351 (0.322±0.012)	0.417–0.480 (0.461)	0.328–0.382 (0.35±0.038)	0.33–0.39 (0.27±0.01)
Esophageal width	0.042–0.052 (0.044)	0.048–0.057	–	–	0.045–0.080 (0.061)	–	–	–	–	–	–	0.17–0.19 (0.18±0.01)
Esophageal bulb length	0.145–0.171 (0.158)	0.129–0.159	0.14	0.16	0.150–0.225 (0.177)	0.12–0.15 (0.13)	–	0.140–0.160 (0.152±0.006)	0.090–0.111 (0.103±0.005)	0.123–0.167 (0.152)	0.108 (0.108±0)	0.12–0.15 (0.140±0.01)

(Continued)

Table 3. Main morphological features and measurements of female *Aspiculuris tetraptera* compared with previous studies. (Continued)

Related species	<i>Aspiculuris ackerti</i>	<i>Aspiculuris artigasi</i>	<i>Aspiculuris versterae</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris ackerti</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris huascaensis</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris tetraptera</i>	<i>Aspiculuris tetraptera</i>		
Parameters	Kruidenier and Mehra (1959)	Araujo (1965)	Hugot (1980)	Hugot (1980)	Miller and Schmidt (1982)	Kohn and Macedo (1984)	Pinto et al. (1994)	Landaeta-Aqueveque et al. (2007)	Falcón-Ordaz et al. (2010)	Liu et al. (2012)	Khail et al. (2014)	Abdel-Gaber and Fol (2015)	A. (Present study)
Esophageal bulb width	0.068–0–0.087 (0.080)	0.087–0.111	0.095	0.13	0.080–0.150 (0.107)	0.08–0.12 (0.10)	–	–	0.054–0.087 (0.063±0.008)	0.088–0.137 (0.118)	0.081 (0.081±0)	0.07–0.11 (0.09±0.01)	0.05–0.09 (0.07±0.01)
Distance from anterior extremity	0.155–0.185 (0.168)	0.120–0.168	0.14	0.13	0.162–0.238 (0.202)	0.15	0.158–0.216	0.12–0.162 (0.140±0.013)	0.081–0.105 (0.093±0.007)	0.121–0.157 (0.134)	0.144–0.147 (0.145±0.002)	–	0.078–0.090 (0.085±0.002)
Excretory pore	–	0.756–1.056	0.53	0.9	0.82–1.67 (1.10)	0.81–0.89 (0.86)	0.734–0.936	0.860–0.950 (0.888±0.032)	0.555–0.675 (0.590±0.038)	1.09–1.23 (1.17)	–	–	0.564–0.780 (0.680±0.02)
Vulval opening	2.1–2.5 (2.3)	1.457–2.015	0.85	1.65	1.52–2.89 (2.49)	1.54–2.36 (1.76)	1.1–1.4	1.61±0.038 (1.55–1.67)	1.204–1.428 (1.269±0.066)	2.07–2.43 (2.27)	1.56–1.86 (1.71±0.21)	–	1.112–1.630 (1.406±0.03)
Tail length	0.232–0.690 (0.445)	0.444–0.660	0.435	0.55	0.088–0.110 (0.101)	0.36–0.40 (0.39)	0.360–0.486	0.347–0.537 (0.477±0.06)	0.330–0.372 (0.358±0.016)	0.92–1.14 (1.05)	0.382–0.405 (0.393±0.021)	0.34–0.48 (0.42±0.01)	0.30–0.42 (0.39±0.01)
Eggs length	0.097–0.106 (0.101)	0.084–0.090	0.09	0.095	0.093–0.110 (0.102)	0.075–0.090 (0.084)	0.72–0.90	0.084–0.092 (0.087±0.005)	0.054–0.069 (0.061±0.003)	0.092–0.096 (0.094)	0.115–0.129 (0.122±0.008)	0.05–0.07 (0.06±0.01)	0.04–0.06 (0.05±0.01)
Eggs width	0.0390.047 (0.044)	0.036–0.039	0.04	0.045	0.038–0.055 (0.045)	0.038–0.051 (0.045)	0.036–0.053	0.043–0.052 (0.046±0.005)	0.018–0.030 (0.022±0.003)	0.051–0.058 (0.054)	0.054–0.072 (0.06±0.008)	0.03–0.05 (0.04±0.01)	0.02–0.04 (0.03±0.01)

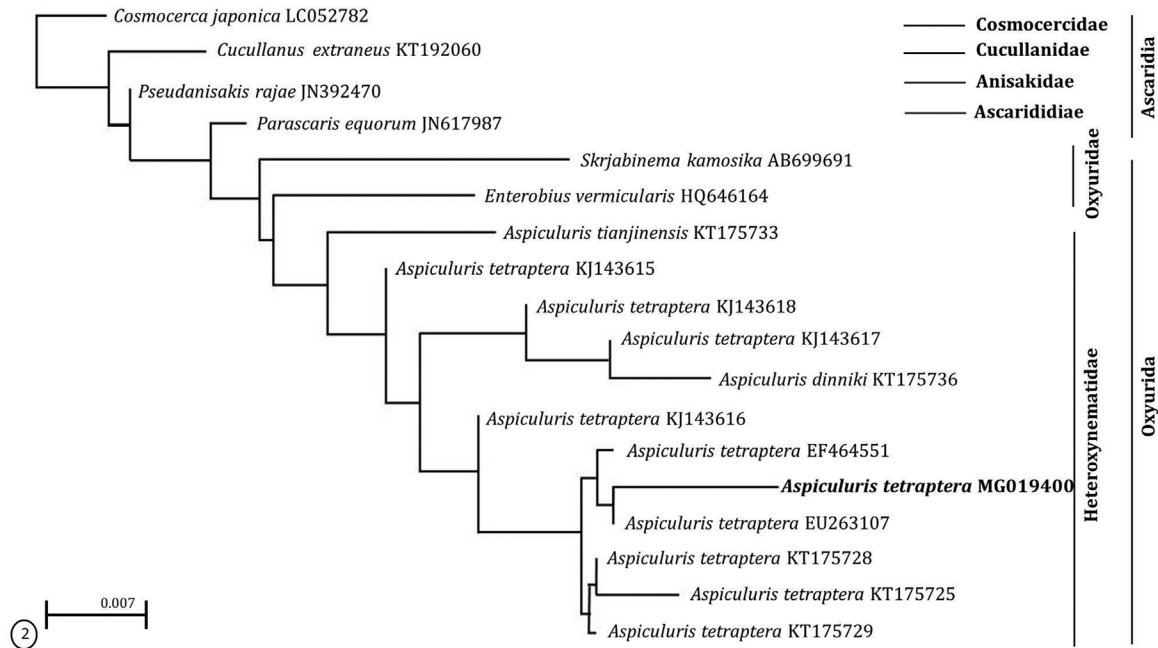


Figure 2: Phylogenetic tree generated by neighbor-joining analyses of the partial SSU rDNA sequence of *Aspiculuris tetraptera* and oxyurid species with the strongest BLAST matches and in part with some ascarid species. GenBank accession numbers are given after the species names. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Oxyurid parasite examined in the present study is bolded.

period (Sato et al., 1995, Reh binder et al., 1996). Oxyurids are cosmopolitan nematoda parasites of public health importance (Khalil et al., 2014). The order Oxyurida includes three families namely Oxyuridae (Cobbold, 1864); Pharyngodonidae (Travassos, 1919); and Heteroxynematidae (Skrjabin and Schikhobalova, 1948). Nematodes from the genera *Syphacia* and *Aspiculuris* are common parasitic pinworms of rodents all over the world (Adamson, 1994; Robles and Navone, 2007; Millazzo et al., 2010; Sotillo et al., 2012; Verma et al., 2013; Weyand et al., 2016; Zarei et al., 2016; Stewart et al., 2017).

Based on morphological characters, the oxyurid species described here showed the characteristic features of the genus *Aspiculuris*, including the presence of four distinct cephalic papillae lying on the cephalic plate, and three small rudimental lips that carry two sessile poorly developed labial papillae. According to the present results, *A. tetraptera* naturally infect the laboratory mice *M. musculus*, which is consistent with data reported by Hugot (1988), Mohd Zain et al. (2012) Pakdel et al. (2013), and Panti-May et al. (2015) who stated that species of the genus *Aspiculuris* belonging to the family Heteroxynematidae are characterized as parasites of Muroidea. Based on the stated characters herein, the present species was identified as *A. tetraptera* and recorded in 56.0% of the examined specimens represent-

ing a higher prevalence. This is in agreement with data obtained by Bluszcz et al. (1987), Bazzano et al. (2002), Izdebska and Rolbiecki (2006), Klimpel et al. (2007), Kataranovski et al. (2008), and Baird et al. (2012), who reported that mice are naturally infected with different oxyurid species, with 9.0 to 75.0% infection range.

The present parasite species was compared morphologically and morphometrically with other *Aspiculuris* species as shown in Tables 2 and 3, and exhibited strong similarities to those reported in other studies by Yamaguti (1935), Hugot (1980), Pinto et al. (1994), Landaeta-Aqueveque et al. (2007), Khalil et al. (2014), and Abdel-Gaber and Fol (2015). Only a few differences in measurements of the different body parts were observed. However, this species differs from other *Aspiculuris* species in the structure of the cephalic region, length of esophagus, position of nerve ring, excretory pore and vulva opening, number and arrangement of cloacal papillae in males, and size of eggs in females. The presently described parasite species resembled *A. tetraptera* and *Aspiculuris huascaensis* by having cervical alae ending at the mid-length of the esophageal bulb with the same number of caudal papillae. However, it can be distinguished from *A. huascaensis* by having a single sessile pre-cloacal papilla located between two cuticular folds and slightly anteriorly to the cloaca, whereas *A. tetraptera* lack both the sessile

pre-cloacal papilla and two cuticular folds. In addition, males of *A. tetraptera* have a double pedunculate papilla immediately posterior to the cloaca, and the anterior most papilla located between the caudal folds of the tail is double, while, those of *A. huascaensis* lack a doubled medial papilla associated with the cloaca and a simple anterior most papillae was recorded. These data were consistent with a previous report by Falcón-Ordaz et al. (2010). In agreement with Ashour (1980), Pinto et al. (1994), and Abdel-Gaber and Fol (2015), 12 papillae were present in *A. tetraptera*; conversely, Schulz (1924) reported 10 and Yamaguti (1935) and Falcón-Ordaz et al. (2010) reported 14 papillae. In addition, the division of the dorsal and both subventral lips of *A. tetraptera* males are unique. Chitwood and Chitwood (1950) noted a similar division of the dorsal lip only of *A. ackerti* with sexual dimorphism of this character in male worms and no tendency for separation in the female specimens.

Aspiculuris is one of five subgenera listed by Akhtar (1955) in which the cephalic bulb and lateral alae are present, the cervical alae end in a sickle shaped margins, but the cervical and lateral alae are not continuous, as stated by Petter and Quentin (2009). The same results were obtained in the present study on the recovered worms, which have well-developed cervical alae extending into cephalic vesicle, and poorly marked cuticular striations. Furthermore, the present described parasite species were similar to other species of *Aspiculuris*, such as *Aspiculuris dinnicki* (Schulz, 1924); *Aspiculuris schulzi* (Popov and Nasarova, 1930); *Aspiculuris azerbaijanica* (Tarzhimanova, 1969); *Aspiculuris ariana* (Erhadová-Kotrlá and Daniel, 1970); and *Aspiculuris witenbergi* (Quentin, 1975); with cervical alae that are abruptly interrupted with the pointed posterior ends and forming an acute angle toward the anterior. However, it differs from *Aspiculuris kazakstanica* (Nasarova and Sweschikova, 1930); *Aspiculuris americana* (Erickson, 1938); *Aspiculuris lahorica* (Akhtar, 1955); *Aspiculuris pakistanica* (Akhtar, 1955); *Aspiculuris africana* (Quentin, 1966); *Aspiculuris tschertkowi* (Tarzhimanova, 1969); *Aspiculuris rysavyi* (Kotrla and Daniel, 1970); and *Aspiculuris versterae* (Hugot, 1980), since the posterior end of the cervical alae of those species does not form an acute angle, and the caudal alae of males is not close to the tip. In addition, with the exception of *A. tschertkowi*, which has 16 caudal papillae, the remaining species have a smaller number of papillae than *A. huascaensis*, varying from 4 to 11 versus 12 papillae.

Due to close morphological similarities, molecular phylogenetic approaches have been used extensively in association with traditional morphological techniques as reliable methods for confirmation of accurate identification, and differentiation between pinworms infect-

ing laboratory rodents (Jacobs et al., 1997; Zhu et al., 1998; Vermund and Wilson, 2000; Morales-Hojas et al., 2001; Nakano et al., 2006; Li et al., 2007; Zhu et al., 2007; Chang et al., 2009). In the present study, a nuclear rDNA region of the recovered parasite species was amplified using the species-specific primers Nem 18SF/Nem 18SR, designed by Floyd et al. (2005). It is apparent that, the phylogenetic tree based on nuclear SSU rDNA sequences estimated in this study supported strongly the higher taxonomic groups of both orders: Oxyurida (representing the two main families Oxyuridae and Heteroxynematidae) and Ascaridia (representing four families, namely Cosmocercidae, Cucullanidae, Anisakidae, and Ascaridiidae). These results are in agreement with data obtained by Blaxter et al. (1998) who reported that clade III of the full dataset of the nematoda phylogeny was represented by all members of the suborder spirurina and clustered into four classical orders of Ascaridia, Oxyurida, Rhigonematida, and Spirurida. Anderson (2000) proposed that Ascaridia and Spirurida were sister groups, which in turn, were more closely related to Strongylida than a group consisting of Oxyurida plus Rhigonematida. Subsequent analyses of SSU rDNA sequences strongly supported the monophyly of clade III taxa with bootstrap values for the clade exceeding 95.0% (De Ley and Blaxter, 2002; Bert et al., 2006; Holterman et al., 2006; Wjová et al., 2006; Qiu et al., 2016; Ribas et al., 2017).

Khalil et al. (2014) reported that the order Oxyurida incorporates three main families, Oxyuridae (Cobbold, 1864); Pharyngodonidae (Travassos, 1919); and Heteroxynematidae (Skryabin and Schikhobalova, 1948); which was consistent with the results of the current study. In addition, Petter and Quentin (2009) included *Syphacia obvelata* and *Syphacia muris* of the genus *Syphacia* with 22 genera in the family Oxyuridae, and included the genus *Aspiculuris* together with seven further genera in the subfamily Heteroxynematinae belonging to the family Heteroxynematidae. This was consistent with the present findings indicating that Oxyuridae species, represented by the genus *Aspiculuris*, is monophyletic in origin, supporting the taxonomic position of the present *Aspiculuris* species, which is deeply embedded in the genus *Aspiculuris* with a close relationship with other described species of *A. tetraptera* as a more related sister taxon.

Conclusion

Recent field studies have provided useful tools for the rapid identification and phylogenetic analysis of pinworms infecting laboratory rodents. The 18S rDNA gene of *A. tetraptera* yielded a unique sequence that confirms the taxonomic position within the family Heteroxynematidae.

Compliance with Ethical Standards

All procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and have been approved and authorized by Institutional Animal Care and Use Committee (IACUC) in Faculty of Science, Cairo University, Egypt (No. CU/I/S/19/16).

Conflict of Interest

The authors have declared that they have no conflict of interest regarding the content of this article.

Acknowledgments

The authors are thankful to Faculty of Science, Cairo University, Cairo, Egypt, and extend their appreciation to The Deanship of Scientific Research at King Saud University for funding this work through research group no (RG-002).

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