Acta Parasitologica (2019) 64:316–330
https://doi.org/10.2478/s11686-019-00047-7

ORIGINAL PAPER

Prevalence, Morphological and Molecular Phylogenetic Analyses of the Rabbit Pinworm, *Passalurus ambiguus* Rudolphi 1819, in the Domestic Rabbits *Oryctolagus cuniculus*

Rewaida Abdel-Gaber[1,2] · Farid Ataya[3,4] · Dalia Fouad[1,5] · Mohamed Daoud[3] · Shatha Alzuhairy[6]

Received: 16 October 2018 / Accepted: 12 March 2019 / Published online: 2 April 2019

Abstract

Introduction *Passalurus ambiguus*, a pinworm nematode parasite, infects domestic and wild rabbits, hares, and rodents worldwide.

Materials and Methods The current parasitological study was performed during January–December 2016, to investigate helminth parasites infecting the domestic rabbit species *Oryctolagus cuniculus* at the Department of Animal Production, Faculty of Agriculture, Cairo University, Cairo, Egypt.

Results Of the twenty rabbit specimens examined for gastrointestinal nematodes, 75% were infected with adult oxyurid species, which were morphologically characterized using light and scanning electron microscopy studies. The oxyurid species had a triangular mouth opening surrounded by simple lips with four cephalic papillae and a pair of lateral amphidial pores with three teeth-like structures, an esophagus divided into a cylindrical corpus and globular bulb supported internally with tri-radiate valvular apparatus, and four caudal papillae distributed on the posterior end of males with a single short protruding spicule and ovijector apparatus opening ventrally by the vulva, surrounded by protruded lips in female worms. The species were compared morphometrically with other *Passalurus* species described previously; light differences were found in different body part sizes. Molecular characterization based on 18 small subunit (SSU) rDNA sequences showed ~ 85% similarity with other Chromadorea species. A preliminary genetic comparison between the 18S rDNA sequences of the isolated parasite and those of other oxyurid species suggested that it belonged to *Passalurus ambiguus*. The 18S rDNA sequence of the parasite was deposited in GenBank (accession no., MG310151.1).

Conclusion The 18S rDNA gene of *P. ambiguus* was shown to yield a unique genetic sequence that confirms its taxonomic position within the Oxyuridae family.

Keywords Laboratory animals · *Oryctolagus cuniculus* · *Passalurus* spp. · Morphological description · Molecular phylogenetic analysis

✉ Rewaida Abdel-Gaber
rewaida@sci.cu.edu.eg

[1] Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia

[2] Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt

[3] Department of Biochemistry, College of Science, King Saud University, Riyadh, Saudi Arabia

[4] Genetic Engineering Division, Molecular Biology Department, National Research Centre, Cairo, Egypt

[5] Department of Zoology and Entomology, Faculty of Science, Helwan University, Helwan, Egypt

[6] Electron Microscopy Unit, Central Laboratory, King Saud University, Riyadh, Saudi Arabia

Introduction

The domestic rabbit *Oryctolagus cuniculus* [39] can have commercialization potential in several avenues, including meat and skin [6, 29]. It presents advantages over other domestic species reared for human consumption, such as high level of prolificacy, easy husbandry, does not require grain feeding, and does not compete with humans for food [12, 52]. These animals have also been used in experimental model, and as domestic pets [21]. Rabbits have greater ability than other animal species to harbor many zoonotic agents [17, 37], and have been heavily parasitized both externally and internally [60]. Rabbits diseases are caused by known agents, including bacteria, parasites, protozoa, fungi, viruses,

genetics, and nutritional deficiencies [26]. However, miscellaneous causes comprised physical and chemical agents such as trauma, cold, heat and toxins have also been reported to cause diseases in rabbits [8, 43]. Parasitic diseases in animals used for human consumption are important due to the economic losses caused [50]. Verifying parasitic diseases is important among the commonly used laboratory mammals supplied by animal houses, some are heavily parasitized with helminths at the time of delivery, or become infected in destination laboratories, where they are occasionally harbored for long periods [27]. Nematodes comprise the largest group of endothermal laboratory animals helminth parasites [19]. Pinworms belonging to the Oxyuridae family [13] are the most common helminth parasites infecting laboratory animals in both medical and veterinary fields [3, 19, 35, 46, 70]. *Passalurus ambiguus* [58] is one of the most prevalent gastrointestinal nematodes infecting domestic and wild rabbits, hares, and rodents worldwide [20, 66, 16], which inhabits the cecum and colon of its hosts, and has a direct life cycle [54]. *P. ambiguus* has been assumed to be non-pathogenic, or considered less pathogenic than others [7, 48, 53, 54]. *Passalurus* species infecting rabbits are identified by examining male worms mamelons' location, number of cloacal papillae, number of spicules, and absence or presence of gubernaculum; however, male worms are rarely recovered because they die after mating, whereas female worms only differ in vulva location [4, 53]. Difficulty in determining these morphological differences emphasizes the need for more diagnostic features to differentiate these pinworm species [1, 6, 56]. Molecular tools have been recently used to identify morphologically similar species, and have effectively contributed to the taxonomical, phylogenetic, and epidemiological studies of different organisms [38, 47, 73]. In addition, these tools provide the most suitable genetic markers for studying genetic variation in oxyurid nematodes, especially *P. ambiguus* [59, 62].

The present study aimed to: (1) determine the natural occurrence of oxyurid nematodes in the domestic rabbit *Oryctolagus cuniculus*; (2) identify characteristic morphologies of isolated worms using light and scanning electron microscopy, which might contribute valuable information, to our knowledge, of oxyurids; and (3) clarify the taxonomic position of the present *Passalurus* species using molecular phylogenetic analysis.

Materials and Methods

Sample Collection and Parasitological Examinations

In total, 20 domestic rabbits *Oryctolagus cuniculus* (same age and body weight) were collected between January and December 2016 from the Department of Animal Production, Faculty of Agriculture, Cairo University, Cairo, Egypt. The animals were transported immediately to the Laboratory of Parasitology Research, Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt, for parasitological examination. All individuals were necropsied, and each gastrointestinal tract was isolated and dissected. Intestinal contents were washed and sieved to remove the finest particulates. Helminths were collected using a stereomicroscope by examining diluted aliquots of the intestinal content, as described by Georgi and Georgi [22]. Isolated worms were fixed in 70% ethanol and subsequently clarified with lactophenol for morphological identification, in accordance with the standard reference keys of Petter and Quentin [51]. *O. cuniculus* parasite prevalence was calculated according to Bush et al. [9]. Photomicrographs of the adult parasite specimens were obtained using Leica DM-2500 microscope (NIS ELEMENTS software, ver. 3.8) at the Laboratory of Parasitology Research in Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia. For scanning electron microscopy, specimens were fixed in 3% buffered glutaraldehyde (pH 7.2), dehydrated in a graded series of ethanol, infiltrated with amyl acetate, processed using a LEICA EM CPD300 dryer until the critical point, sputter-coated in an auto fine coater (JEOL, JEC-3000FC) with gold–palladium, and then photographed at 10-kV using an Etec Autoscan JEOL scanning electron microscope (JSM-6060LV) at the Central Laboratory, King Saud University, Riyadh, Saudi Arabia. Measurements of the recovered nematodes were performed on 20 adult worm specimens; data were obtained in millimeters, and presented as a range followed by the arithmetic mean ± SD in parentheses.

Molecular Analysis

DNA Extraction, Polymerase Chain Reaction (PCR) Amplification, and Sequencing

Genomic (g) DNA was extracted from ethanol-preserved samples using a Qiagen DNeasyTM tissue kit (Qiagen, Venlo, Netherlands), following manufacturer's instructions. The concentration and purification of gDNA were quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fischer Scientific, Inc., Wilmington, DE, USA), and 20 ng of genomic DNA was used for polymerase chain reaction (PCR). PCR was performed to amplify the target gDNA using Nem 18S F (5′-CGC GAA TRG CTC ATT ACA ACA GC-3′) and Nem 18S R (5′-GGG CGG TAT CTG ATC GCC-3′) designed by Floyd et al. [18]. PCRs were performed in 2 mM $MgCl_2$, 0.2 mM dNTPs mix., 2.5 µl of 10×rTaq DNA buffer, 2.5 µM of each primer, 1.25 U of rTaq polymerase buffer, and 1 µl of DNA sample, with the volume made to 25 µl with distilled H_2O in a thermocycler (BioRad), under the following conditions: 94 °C for 5 min (initial denaturation), followed by 35 cycles of 1 min at 94 °C (denaturation), 1 min at 50 °C (annealing), 1 min at 72 °C (extension), and post-PCR extension for 7 min

at 72 °C. Each amplicon was examined using electrophoresis on 1% agarose gel in 1 × Tris–acetate-EDTA (TAE) buffered gel stained with 1% ethidium bromide, visualized using a UV transilluminator. Bands with the predicted size were purified using a Pure LinkTM Quick Gel Extraction Kit (Invitrogen) following manufacturer's instructions. Amplicons were sequenced (in both directions) using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) with a 310 Automated DNA Sequencer (Applied Biosystems, USA) using the same primers as those used for annealing.

Sequence Alignment and Phylogenetic Analysis

Related sequences were identified by performing a BLAST search on the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/). DNA sequences were aligned using multiple sequence alignment software CLUSTAL-X [68] and compared with previously recorded data from GenBank, to analyze intra-specific differences. GenBank accession numbers of additional sequences utilized in the analyses were *Trypanoxyuris atelis* (gb| KU285460.1), *Wellcomia siamensis* (gb| EF180079.1), *Enterobius vermicularis* (gb| JF934731.1), *Setaria digitata* (gb| DQ094175.1), *Haemonchus contortus* (gb| EU086374.1), *Brugia malayi* (gb| AF036588.1), *Thelazia callipaeda* (gb| AB538282.1), *Baylisascaris procyonis* (gb| U94368.1), *Ascaris suum* (gb| U94367.1), *Syphacia obvelata* (gb| EF464554.1), *Dirofilaria immitis* (gb| AB973231.1), *Oxyuris equi* (gb| EF180062.1), *Brugia malayi* (gb| KP760120.1), *Aspiculuris tetraptera* (gb| EF464551.1), and *Passalurus ambiguus* (gb| EF464552.1). The alignment was corrected manually using the alignment editor of software MEGA 4.0 [67]. DNA sequence similarities were calculated with Sequence Identity Matrix of software BioEdit 4.8.9 [25]. Phylogenetic calculations were performed with PAUP 4.0b10 [65]. The data were analyzed with maximum parsimony (neighbor-interchange [CNI] level 3, random addition trees 100).

Results

In total, 265 specimens of adult oxyurid species were found in the ceca and large intestines of the examined *O. cuniculus* species. Of the 20 rabbit hosts, 15 were found to be naturally infected, with 75% prevalence rate of infection with this nematode parasite. The intensity of infection was 14–18 parasite specimens per infected rabbit. The parasitic infection rate was higher in male rabbits (90%, 9/10), and lowered in females (60%, 6/10).

Microscopic Examination

The recovered oxyurid species body was small, straight, and colorless to off-white with a narrow posterior extremity [Figs. 1(1–15), 2(16–29)]. The anterior extremity had a triangular mouth opening surrounded by simple lips with four cephalic papillae, a pair of lateral amphidial pores, and three teeth-like structures. The buccal cavity was followed by the esophagus, which was divided into an anterior cylindrical part, a corpus, and a globular bulb, which were supported internally by a tri-radiate valvular apparatus. The esophagus was followed by a long intestine that opened exteriorly, with an anal opening in females and cloacal opening in males. The excretory pore was located posterior to the esophagus–intestinal junction. Lateral cuticular wings were observed on the anterior, one-third the length of the body as it started from the buccal opening to about 0.354 mm in extension; they had three zones produced by two manifested plates, and the ends were covered by insensible cuticles.

Morphology of the Male Worm (Based on 10 Mature Specimens)

Male bodies measured 3.6–5.7 (4.4 ± 0.2) mm long and 1.2–0.17 (0.15 ± 0.01) mm wide. The esophagus (including the pharynx, corpus, and bulb) measured 0.52–0.64 (0.58 ± 0.01) mm long. The pharynx measured 0.063–0.076 (0.069 ± 0.001) mm long, the corpus was 0.29–0.37 (0.34 ± 0.01) mm long, and the bulb was 0.13–0.16 (0.14 ± 0.01) mm in diameter. The nerve ring and excretory pore were located at a distance of 0.13–0.15 (0.14 ± 0.01) and 1.14–1.16 (0.15 ± 0.01) mm from the anterior end, respectively. Ventral mamelons were difficult to identify. The posterior end was markedly coiled, with a single short protruded spicule measuring 0.09–0.18 (0.11 ± 0.01) mm long and located 0.24–0.31 (0.27 ± 0.01) mm from the caudal appendix. The gubernaculum was absent. The posterior end had a series of papillae on the sides of the anus arranged as follows: two large peri-cloacal papillae, one small pair seen in the post-cloacal position, and finally a pair of papillae on the sides of the tail, precisely at the beginning of the caudal appendix without marked striations. The body ended with a small coiled tail in the form of a comma and measured 0.46–0.62 (0.53 ± 0.01) mm long.

Morphology of Female Worm (Based on 10 Mature Specimens)

The body of female worms measured 9.6–12.8 (11.3 ± 0.3) mm long and 0.50–0.70 (0.63 ± 0.01) mm wide. The esophagus measured 0.57–0.68 (0.62 ± 0.01) mm long. The pharynx measured 0.089–0.095 (0.093 ± 0.001) mm long, the corpus was 0.34–0.49 (0.42 ± 0.01) mm long, and the bulb was 0.15–0.19 (0.18 ± 0.01) mm in diameter. The nerve ring, excretory pore, and vulval opening were located at a distance of 0.20–0.35 (0.40 ± 0.01), 0.86–1.12 (1.05 ± 0.01), and 0.98–1.32 (1.16 ± 0.11) mm from the

anterior end, respectively. An ovipositor and a uterus were present with complete independence. The uterus filled the body, and was packed with eggs that obscured the rest of the genitalia. The ovipositor terminated in a predetermined place, close to the vulva, which is surrounded by the muscular systems, at the time of oviposition when the ovipositor is exerted. The anal opening is observed in the ventral side, at the posterior end of the body, as a transverse slit

◂**Fig. 1** (1–15) Photomicrographs of the pinworm *P. ambiguus* infecting the domestic rabbit species *O. cuniculus* showing the following: (1) A whole mount of the female worm characterized by the anterior extremity with a triangular mouth opening surrounded with simple lips (LP), followed by the esophagus divided into the corpus (C) and bulb (B), leading to the large intestine (IN), and followed by the rectum (R) opening to the outside by an anal opening (AN). Note: the presence of the uterus (U) was filled with numerous eggs (EG), opened by the vulval opening (VU); and the body was covered by the transverse annulated cuticle (TA) and ending with a long tail (T). (2) A whole mount of the male worm was characterized by the anterior extremity with the mouth opening surrounded with simple lips (LP) followed by the esophagus divided into the corpus (C) and bulb (B), leading to the large intestine (IN), and followed by the rectum (R) opening to the outside by the cloacal opening (CO). Note the presence of a pair of testes (TE), a single spicule (SP), and the body covered by the transverse annulated cuticle (TA), and ending with a long tail (T). (3) The anterior extremity of the recovered worm showing the mouth opening surrounded with simple lips (LP), followed by the esophagus divided into the corpus (C) and bulb (B), leading to the large intestine (IN), the excretory bladder opening to the outside by the excretory pore (EP), and the body covered with transverse annulated cuticle (TA). (4–10) High magnification images of different body parts showing the following: (4) The anterior extremity with the mouth opening (MO) surrounded with simple lips (LP), cephalic papillae (P), and the buccal cavity followed by the corpus region of the esophagus (C). (5) Two portions of the esophagus were the corpus (C) and bulb (B), followed by the intestine (IN). (6) Transverse annulations (TA) of the cuticle. (7) The excretory bladder (EB) opened to the outside by the excretory pore (EP). (8) The vulval opening (VU) with eggs (EG) in the vagina. (9) The uterus (U) filled with numerous eggs (EG). (10) Eggs (EG). (11–15) The posterior ends of the recovered worms showing the following: (11) The anal opening (AN) of a female worm. (12) The posterior of a male ending with the cloacal opening (CO), a single spicule (SP), and two pairs of peri-cloacal papillae (PCP 1 and PCP 2), a pair of post-cloacal papillae (POCP), and a pair of caudal papillae (CP) before the tail region (T). (13, 14) The spicule (SP) and peri-cloacal papillae (PCP 1 and PCP 2) surrounding the cloacal opening (CO). (15) Caudal papillae (CP) before the tail region (T)

with a dome-like appearance and was characterized by the absence of cuticular striation at the anal region. The body ends with a long tapering straight tail in a moniliform prolongation with annular thickening in the cuticle, which measured 0.20–0.38 (0.34 ± 0.10) mm long. Eggs were elliptical with one side flattened, double thin-walled, and measured 0.06–0.096 (0.07 ± 0.001) mm long and 0.035–0.058 (0.047 ± 0.001) mm wide. The maximum and minimum values, as well as the mean values found of the different structures studied in this oxyurid species compared with those of previously described *Passalurus* species are shown in Tables 1 and 2.

Taxonomic summary

Parasite: *Passalurus ambiguus* [58] (F: Oxyuridae [13]).
Type host: Domestic rabbits *Oryctolagus cuniculus* [39] (F: Leporidae).
Mode of transmission: Infection occurred by the ingestion of embryonated eggs in feces or in contaminated food, water, or bedding.
Morbidity and mortality: Infection in domestic rabbits was generally symptomless.
Site of infection: Cecum and large intestine of infected rabbit host.
Type locality: Department of Animal Production, Faculty of Agriculture, Cairo University, Cairo, Egypt.
Prevalence: 75.0% (15 out of 20).
Intensity: 14–18 specimens of adult oxyurid nematoda per infected rabbit.

Molecular analysis

A total of 1680 bps was deposited into GenBank under the accession no. MG310151.1 with 51.72% GC content, for 18 small subunit (SSU) rDNA gene sequences of the oxyurid species. Prior to phylogenetic analysis, only sites that could be unambiguously aligned among all chromadoreans were used. Pairwise comparative analysis of the isolated gDNA sequences from the present parasite species with a range of other Nematoda species and genotypes revealed a unique genetic sequence. The percentage identity calculations between these novel genetic sequences with others were retrieved from GenBank, revealing a high degree of similarity (up to 85%; Table 3). Nucleotide sequences and divergence comparisons showed that 18 SSU rDNA of the present species had the highest blast scores for other oxyurid species such as *P. ambiguus* (gb| EF464552.1), *Aspiculuris tetraptera* (gb| EF464551.1), *Oxyuris equi* (gb| EF180062.1), *Syphacia obvelata* (gb| EF464554.1), *Enterobius vermicularis* (gb| JF934731.1), *Wellcomia siamensis* (gb| EF180079.1), and *Trypanoxyuris atelis* (gb| KU285460.1) (Table 3; Fig. 3). In addition, the sequence divergence detected between the closely related oxyurid species ranging from a low value of 0.012 (*P. ambiguus* vs. previously described *P. ambiguus*) to a high value of 0.126 (*P. ambiguus* vs. *T. atelis*) (Table 4). The 18 SSU rDNA sequence data were used to divide the constructed dendrogram into two lineages (one major and one minor clade; Fig. 4). Sequence alignment revealed that the major clade showed clustering of all Chromadorea species, including the most related families Oxyuridae, Haemonchinae, Onchocercidae, Setariidae, Thelaziidae, and Ascarididae, with a sequence similarity ranging from 85 to 99%. The minor clade contained an out-group of SSU rDNA sequences for *Trichuris suis* (gb| GU070737.1), belonging to the family Trichuridae of class Enoplea, which showed lower similarity and higher divergence values. This sequence, in

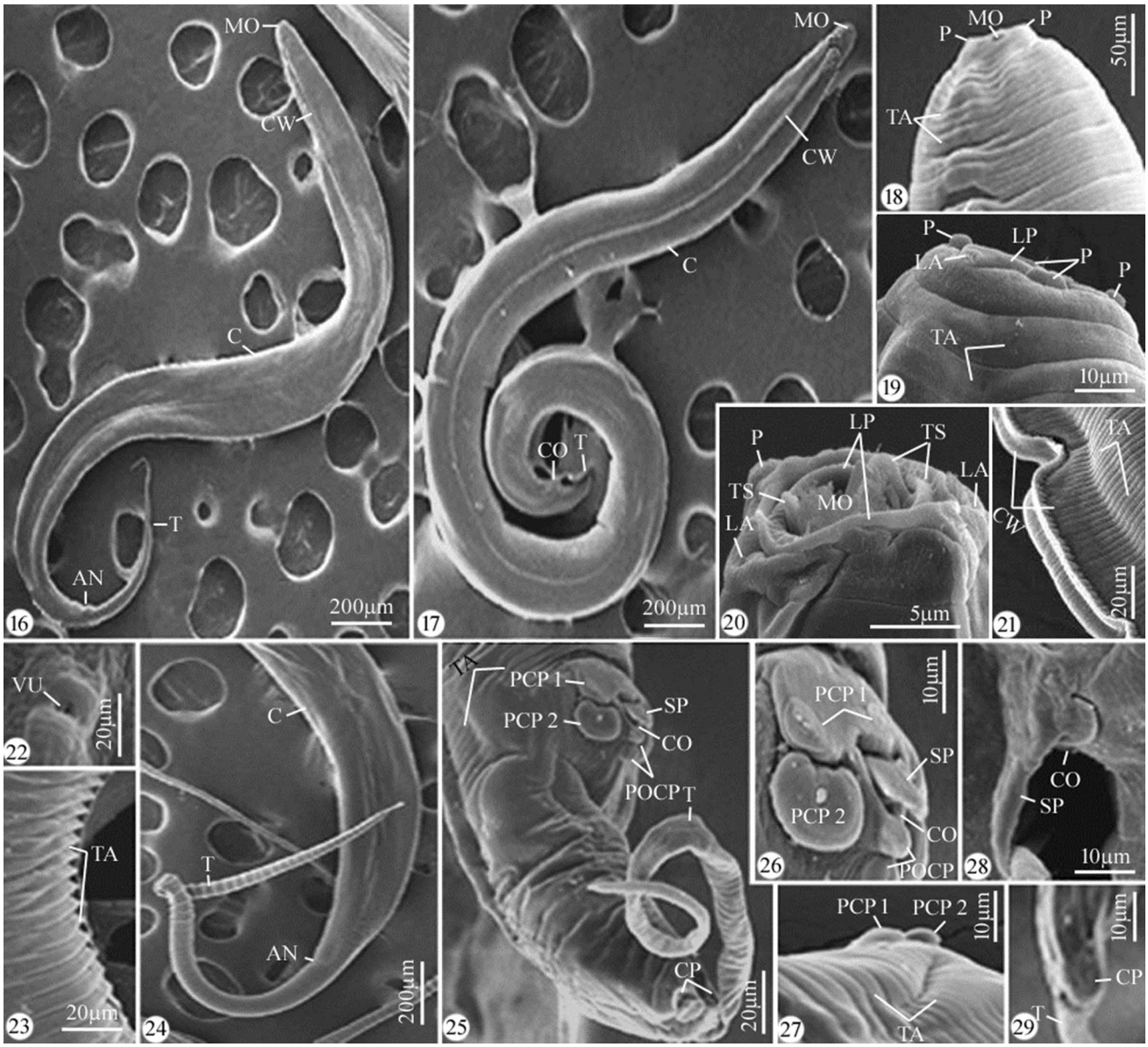

Fig. 2 (16–29) Scanning electron micrographs of *P. ambiguus* infecting *O. cuniculus* showing the following: (16) A whole mount of the female worm characterized by the anterior extremity with the mouth opening (MO) toward the outside by an anal opening (AN), and the body ended with a long tail (T), in addition to the presence of cuticular wings (CW). (17) A whole mount of the male worm characterized by anterior extremity with mouth opening (MO), the body ended with the cloacal opening (CO), and a long tail (T) with the presence of cuticular wings (CW). (18–29) High magnification images of different body parts showing the following: (18–20) The anterior extremity of the body showing the mouth opening (MO) with teeth-like structures (TS), surrounded with simple lips (LP), cephalic papillae (P), and lateral amphidial pores (LA) and the body covered with the transverse annulated (TA) cuticle. (21) Cuticular wings (CW). (22) The vulval opening (VU). (23) Transverse annulations (TA) of the cuticle. (24) The posterior end of a female worm ended with an anal opening (AN) and the tail region (T). (25–29) The posterior end of a male worm with the cloacal opening (CO), a single spicule (SP), and two pairs of peri-cloacal papillae (PCP 1 and PCP 2), one pair of post-cloacal papillae (POCP), and one pair of caudal papillae (CP) before the tail region (T)

conjunction with existing data, suggests placing this oxyurid species within the Oxyuridae family. The present species was shown to be deeply embedded in the genus *Passalurus*, in the same taxon as the previously deposited sequence of *P. ambiguus*.

Table 1 Comparative measurements of the male *Passalurus ambiguus* with previously described species

Parasite species Parameters	*Passalurus ambiguus* [58]	*Passalurus nonanulatus* [61]	*Passalurus ambiguus* [57]	*Passalurus ambiguus* (present study)
Host	*Oryctolagus cuniculus*	*Sylvilagus floridanus*	*Oryctolagus cuniculus*	*Oryctolagus cuniculus*
Host-locality	Tunis, Tunisia	Carimagua, Colombie	Granada, Spain	Cairo, Egypt
Body length	4.500	4.100	2.5–6.4 (4.37)	3.6–5.7 (4.4 ± 0.2)
Body width	0.150	0.250	0.2–0.5 (0.32)	1.2–0.17 (0.15 ± 0.01)
Distance of nerve ring from the anterior extremity	0.140	0.150	0.150–0.170	0.13–0.15 (0.14 ± 0.01)
Distance of excretory pore from the anterior extremity	1.150	1.170	–	1.14–1.16 (0.15 ± 0.01)
Total esophagus length	0.560	0.500	0.370–0.412	0.52–0.64 (0.58 ± 0.01)
Esophagus corpus length	–	–	–	0.29–0.37 (0.34 ± 0.01)
Diameter of esophagus bulb	0.150	0.150	0.120	0.13–0.16 (0.14 ± 0.01)
Spicule length	0.125	0.105	0.090–0.120	0.09–0.18 (0.11 ± 0.01)
Distance of spicule from the caudal appendix	–	–	0.300–0.400	0.24–0.31 (0.27 ± 0.01)
Tail length	0.500	0.400	–	0.46–0.62 (0.53 ± 0.01)

Table 2 Comparative measurements of the female *Passalurus ambiguus* with previously described species

Parasite species Parameters	*Passalurus ambiguus* [58]	*Passalurus nonanulatus* [61]	*Passalurus ambiguus* [57]	*Passalurus ambiguus* (present study)
Host	*Oryctolagus cuniculus*	*Sylvilagus floridanus*	*Oryctolagus cuniculus*	*Oryctolagus cuniculus*
Host-locality	Tunis, Tunisia	Carimagua, Colombie	Granada, Spain	Cairo, Egypt
Body length	8.200	8.000	8.10–12 (9.86)	9.6–12.8 (11.3 ± 0.3)
Body width	0.550	0.500	0.32–0.62 (0.45)	0.50–0.70 (0.63 ± 0.01)
Distance of nerve ring from the anterior extremity	0.210	0.150	0.190–0.210	0.20–0.35 (0.40 ± 0.01)
Distance of excretory pore from the anterior extremity	1.000	0.950	–	0.86–1.12 (1.05 ± 0.01)
Distance of vulval opening from the anterior extremity	1.350	1.250	1.5–1.9	0.98–1.32 (1.16 ± 0.11)
Total esophagus length	0.600	0.600	0.495–0.535	0.57–0.68 (0.62 ± 0.01)
Esophagus corpus length	–	–	–	0.34–0.49 (0.42 ± 0.01)
Diameter of esophagus bulb	0.130	0.150	0.155	0.15–0.19 (0.18 ± 0.01)
Tail length	2.200	1.450	–	0.20–0.38 (0.34 ± 0.10)
Egg length	0.080–0.110	0.085–0.170	0.090–0.103	0.06–0.096 (0.07 ± 0.001)
Egg width	0.040–0.050	0.030–0.070	–	0.035–0.058 (0.047 ± 0.001)

Discussion

Oxyurids are cosmopolitan nematode parasites of public health-related importance [2, 33]. Species of the genus *Passalurus* (Oxyuridae) have been found worldwide in rabbits, hares and rodents, and only a few studies have specifically focused on these nematode parasites [44, 55, 63]. This genus thus far include five species: *P. ambiguus* Rudolphi [58], *P. nonanulatus* [61], *P. abditus* [10], *P. parvus* [30], and *P. assimitis* [71]. *P. ambiguus* is considered the typical species of the genus *Passalurus*; it was first described in *Oryctolagus cuniculus* and *Lepus europeus* inhabiting the Palearctic region [24]. This was followed by the observation of *P. nonanulatus* infecting *Sylvilagus jloridanus* in the Great Lakes region in Colombia and Ontario [14, 23]. In Egypt, *P. ambiguus* is the most prevalent helminths found in domestic rabbits. The morphological characteristics of the oxyurid species isolated in this study are similar to those of genus *Passalurus*,

Table 3 Oxyurid species used in the phylogenetic analysis of the present *Passalurus ambiguus*

Parasite species	Order/family	Host species (Country)	Source	Accession no.	Sequence length (bp)	Percent identity (%)
Trypanoxyuris atelis	Oxyurida/Oxyuridae	*Ateles geoffroyi* (Mexico)	GenBank	KU285460.1	1729	85
Wellcomia siamensis	Oxyurida/Oxyuridae	*Hystrix brachyuran* (Thailand)	GenBank	EF180079.1	1834	90
Enterobius vermicularis	Oxyurida/Oxyuridae	*Homo sapiens* (South Korea)	GenBank	JF934731.1	1825	91
Setaria digitata	Spirurida/Setariidae	*Bos Taurus* (not mentioned)	GenBank	DQ094175.1	1684	91
Haemonchus contortus	Rhabditida/Haemonchidae	*Giraffa camelopardalis* (USA)	GenBank	EU086374.1	1679	92
Brugia malayi	Spirurida/Onchocercidae	*Anopheles* spp. (not mentioned)	GenBank	AF036588.1	1776	92
Thelazia callipaeda	Spirurida/Thelaziidae	*Homo sapiens* (Japan)	GenBank	AB538282.1	1532	92
Baylisascaris procyonis	Ascaridida/Ascarididae	*Procyon lotor* (not mentioned)	GenBank	U94368.1	1754	92
Ascaris suum	Ascaridida/Ascarididae	*Sus scrofa* (not mentioned)	GenBank	U94367.1	1754	92
Syphacia obvelata	Oxyurida/Oxyuridae	*Mus musculus* (not mentioned)	GenBank	EF464554.1	3003	93
Dirofilaria immitis	Spirurida/Onchocercidae	*Canis lupus familiaris* (Japan)	GenBank	AB973231.1	2252	93
Oxyuris equi	Oxyurida/Oxyuridae	*Equus caballus* (USA)	GenBank	EF180062.1	1735	94
Brugia malayi	Spirurida/Onchocercidae	*Meriones unguiculatus* (not mentioned)	GenBank	KP760120.1	669	94
Aspiculuris tetraptera	Oxyurida/Heteroxynematidae	*Mus musculus* (not mentioned)	GenBank	EF464551.1	3676	95
Passalurus ambiguus	Oxyurida/Oxyuridae	*Oryctolagus cuniculus* (not mentioned)	GenBank	EF464552.1	2779	99

such as a triangular mouth opening surrounded with simple lips, cephalic papillae, amphidial pores, and three ventrally subdivided dents or teeth-like structures; the shape and size of the spicule; the presence or absence of the gubernaculum; the number and form of mamelons; and the distribution of the cloacal papillae in males [1, 28, 51, 64, 70, 72]. According to these characteristics, the present species was identified as *P. ambiguus* and showed with 75% prevalence in domestic rabbit *O. cuniculus*. This result was in agreement with the data obtained by Ashmawy et al. [5], who stated that the percentage of infection in domestic rabbits infected with *P. ambiguus* was likely to be higher in young animals that are more susceptible to infection than in older animals. Furthermore, recorded values of individual prevalence are higher in males (90%) than in females (60%). A higher level of invasiveness in male hosts was also reported by Klimpel et al. [36], Kataranovski et al. [32], and Abdel-Gaber [2], who reported a suitable hypothesis for the varying levels of parasitic infection; they suggested that females resist infection more effectively than males. The present pinworm parasite species was compared morphologically and morphometrically with other species; it showed remarkable similarity to *P. ambiguus*. Similar reports have been found in other studies by Rudolphi [58], Skinker [61], and Romero et al. [57], with few differences in body part measurements. SEM allowed the clear observation of the cloacal area topography in males. Three pairs of papillae (the first two pairs were peri-cloacal and larger than the last pair, which was small, sessile, and positioned just post-cloacally). This finding agrees with the description of male *P. ambiguus* provided by Hugot et al. [28]. In addition, another pair of small papillae were identified, situated at the point where the tail narrows and the caudal appendix begins, which is also a feature of *P. ambiguus* males described by Skinker [61]. Estimating the number and position of papillae in this area are known to vary from one species to another, and their nature might be attributed to the nature of the insemination process in *Passalurus*, as confirmed by Skinker [61] and then by Hugot et al. [28]; these features were later used by researchers to differentiate *P. ambiguus* from other described *Passalurus* species. Further, the present *P. ambiguus* differs from other *Passalurus* species such as *P. nonanulatus* [61] isolated from *Sylvilagus floridanus* (which can be differentiated by the absence of the typical transverse cuticular striations known as the moniliform appearance), *P. abditus* [10] isolated from *Spermophilus variegatus* (which can be differentiated by the presence of moniliform formations, the distance from the vulva to the front extremity (2.5–2.9 mm) and different sized eggs (0.098 × 0.031 mm), *P. parvus* [30] isolated from *Schoinobates volans* (which can be distinguished by the tail length of the female (0.42 mm and macro rings), and *P. assimitis* [71] isolated from *Lepus sinensis* (differentiable by the absence of caudal papillae). Molecular biology tools in association with traditional morphological techniques have effectively contributed to reliable unequivocal identification, and differentiation of closely related species [11, 31, 34, 41, 46, 49, 55, 69]. In the present study, a nuclear rDNA region of the recovered species was amplified using

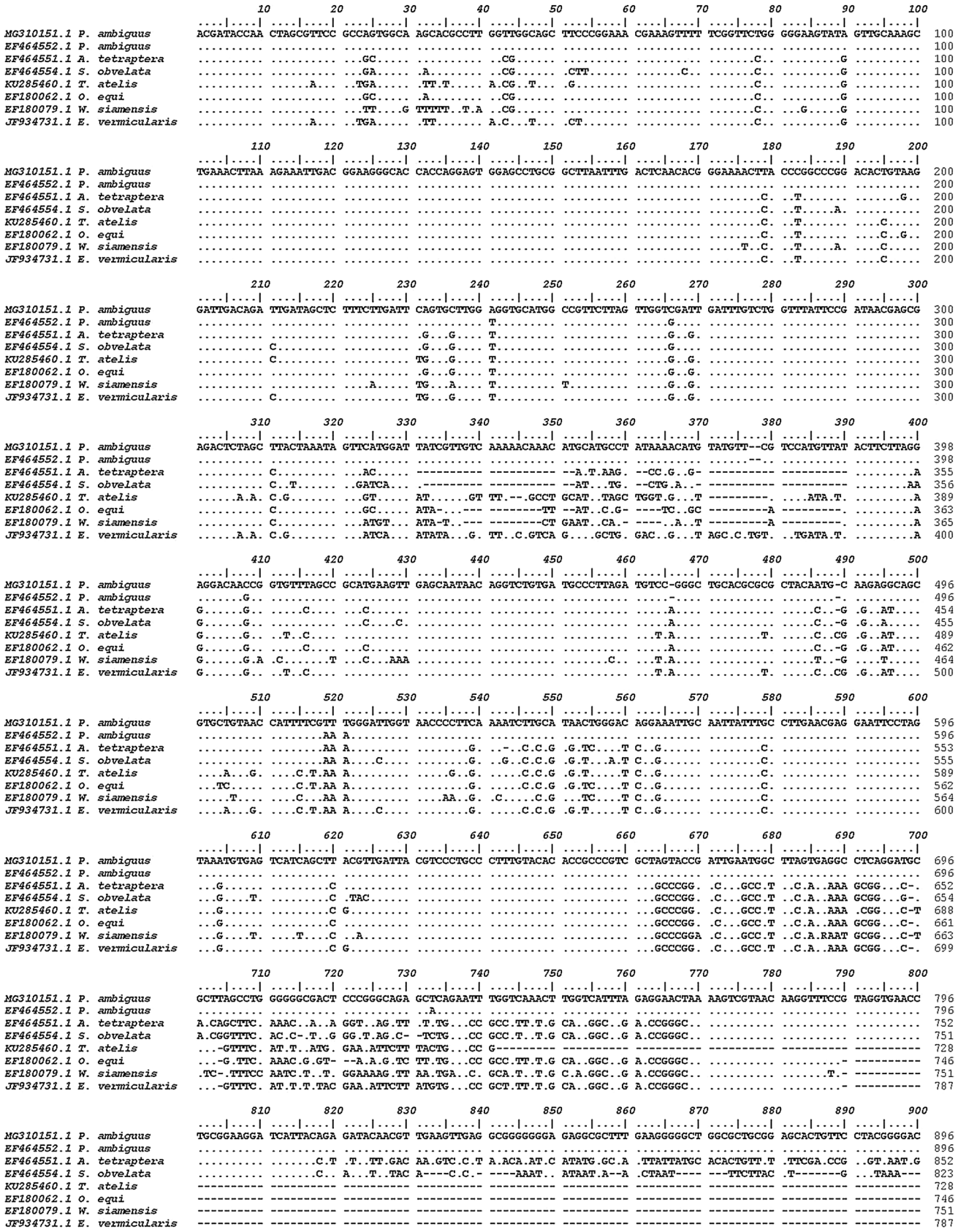

Fig. 3 Sequence alignment of 18S rDNA of *Passalurus ambiguus* with the most related oxyurid species (Only variable sites are shown). Dots represent bases identical to those of the first sequences, and dashes indicate gaps

Table 4 Estimates of evolutionary divergence between sequences

Parasite species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. MG310151.1 *Passalurus ambiguus*		0.012	0.101	0.113	5.203	0.125	0.110	0.146	0.110	0.125	0.128	0.128	0.116	0.117	0.107	0.126
2. EF464552.1 *Passalurus ambiguus*	0.012		0.087	0.099	5.113	0.111	0.097	0.131	0.097	0.110	0.114	0.114	0.102	0.103	0.094	0.112
3. EF464551.1 *Aspiculuris tetraptera*	0.101	0.087		0.029	6.413	0.067	0.029	0.088	0.029	0.044	0.037	0.037	0.057	0.037	0.023	0.055
4. KP760120.1 *Brugia malayi*	0.113	0.099	0.029		6.459	0.074	0.008	0.083	0.006	0.021	0.031	0.031	0.072	0.019	0.037	0.066
5. EF180079.1 *Wellcomia siamensis*	5.203	5.113	6.413	6.459		6.381	6.474	6.617	6.459	6.692	6.529	6.529	6.407	6.718	6.537	6.566
6. JF934731.1 *Enterobius vermicularis*	0.125	0.111	0.067	0.074	6.381		0.076	0.118	0.074	0.081	0.078	0.078	0.068	0.077	0.061	0.025
7. DQ094175.1 *Setaria digitate*	0.110	0.097	0.029	0.008	6.474	0.076		0.090	0.004	0.021	0.031	0.031	0.070	0.021	0.033	0.070
8. EU086374.1 *Haemonchus contortus*	0.146	0.131	0.088	0.083	6.617	0.118	0.090		0.086	0.090	0.099	0.099	0.119	0.097	0.084	0.098
9. AF036588.1 *Brugia malayi*	0.110	0.097	0.029	0.006	6.459	0.074	0.004	0.086		0.019	0.033	0.033	0.070	0.017	0.037	0.068
10. AB538282.1 *Thelazia callipaeda*	0.125	0.110	0.044	0.021	6.692	0.081	0.021	0.090	0.019		0.039	0.039	0.077	0.031	0.035	0.075
11. U94368.1 *Baylisascaris procyonis*	0.128	0.114	0.037	0.031	6.529	0.078	0.031	0.099	0.033	0.039		0.000	0.064	0.041	0.047	0.070
12. U94367.1 *Ascaris suum*	0.128	0.114	0.037	0.031	6.529	0.078	0.031	0.099	0.033	0.039	0.000		0.064	0.041	0.047	0.070
13. EF464554.1 *Syphacia obvelata*	0.116	0.102	0.057	0.072	6.407	0.068	0.070	0.119	0.070	0.077	0.064	0.064		0.074	0.061	0.083
14. AB973231.1 *Dirofilaria immitis*	0.117	0.103	0.037	0.019	6.718	0.077	0.021	0.097	0.017	0.031	0.041	0.041	0.074		0.041	0.066
15. EF180062.1 *Oxyuris equi*	0.107	0.094	0.023	0.037	6.537	0.061	0.033	0.084	0.037	0.035	0.047	0.047	0.061	0.041		0.053
16. KU285460.1 *Trypanoxyuris atelis*	0.126	0.112	0.055	0.066	6.566	0.025	0.070	0.098	0.068	0.075	0.070	0.070	0.083	0.066	0.053	

The number of base substitutions per site between sequences is shown. Analyses were conducted using the Maximum Composite Likelihood model. The analysis involved 16 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA4.0

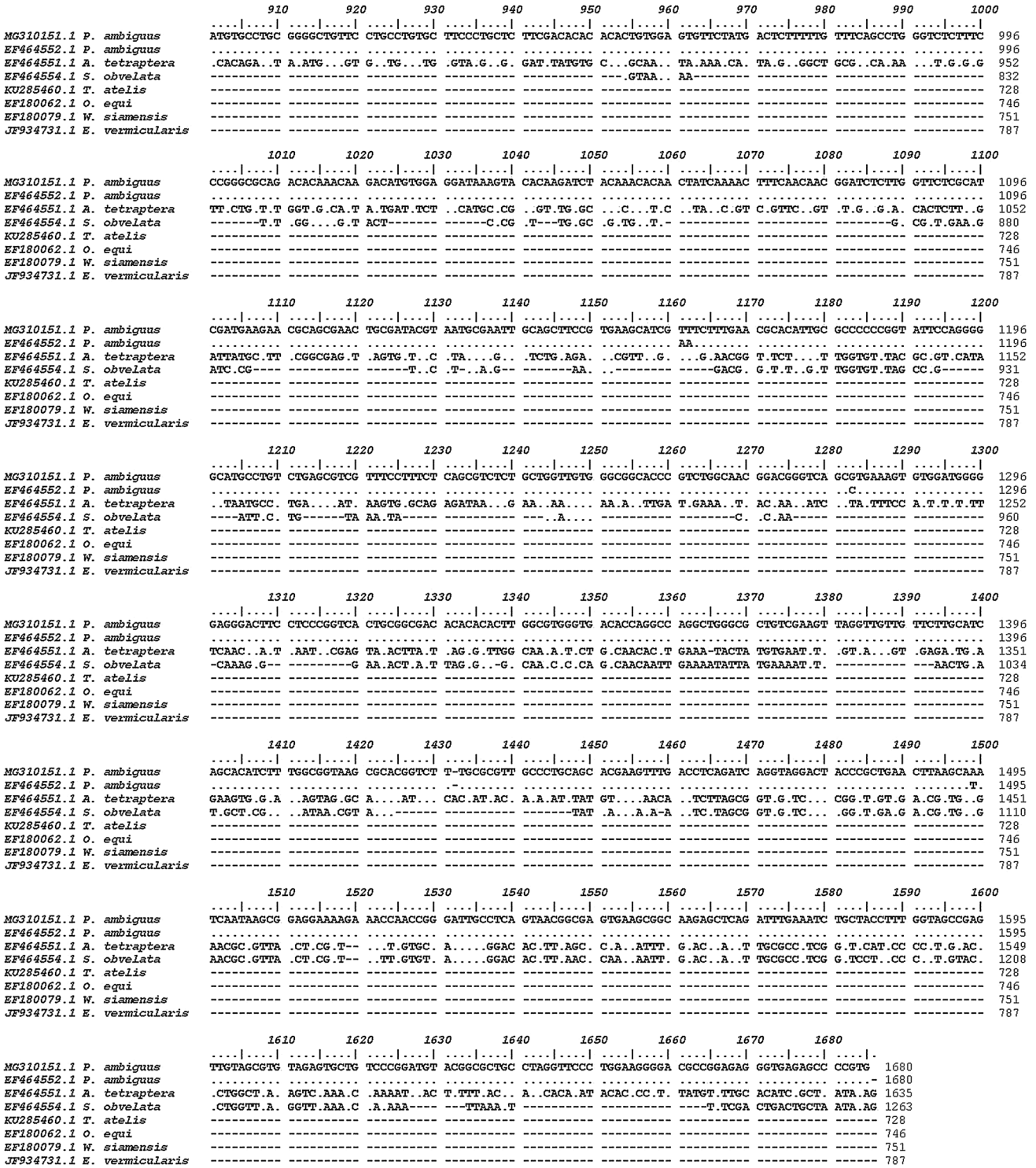

Fig. 4 Molecular phylogenetic analysis performed using Maximum Likelihood method. The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (− 2506.5752) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then the topology with superior log likelihood value was selected. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 16 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA4.0

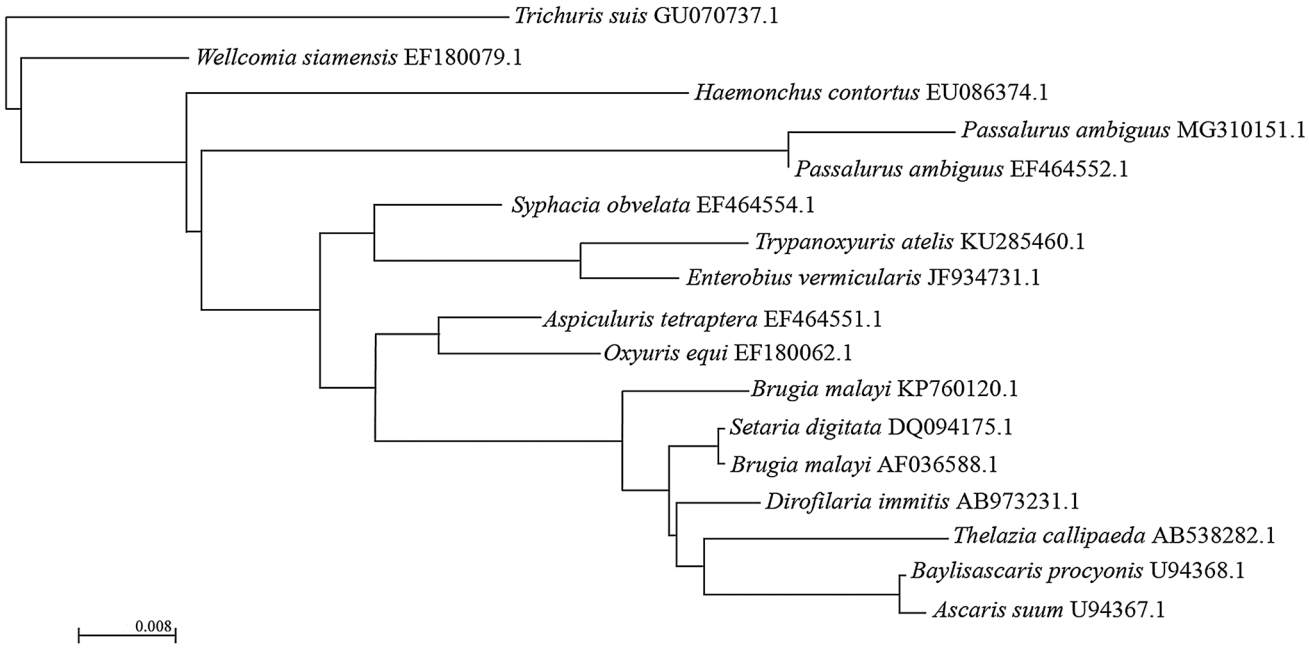

Fig. 4 (continued)

Nem 18S F/Nem 18S R primers described previously by Floyd et al. [18]. Molecular genetic sequences might provide reliable genetic markers for examining the taxonomic status of nematodes [31, 34, 41, 49]. The possible explanation for using this gene region in our study is that 18S rDNA sequence data have revolutionized the phylogenetic analysis. This is a powerful tool for resolving taxonomic issues, which allows the discrimination of genera and species across various organisms because of the occurrence of some intraspecific variations, and differences might exist between rDNA copies within an individual. This hypothesis is in accordance with the findings of Liu et al. [41] who stated that 18S rDNA represented more variable and valuable informative than for other parts of the rDNA locus. The 18S rDNA gene region, representing the oxyurid species identified in this study, had sequences similar to homologous regions within the nuclear ribosomal sequence of other described Chromadorea species. The phylogenetic tree estimated in this study was performed using Maximum Likelihood (ML) and strongly supported all Chromadorea nematodes, including the most related families Oxyuridae, Haemonchinae, Onchocercidae, Setariidae, Thelaziidae, and Ascarididae; these data are in agreement with those of De Ley and Blaxter [15] and Khalil et al. [33], who reported seven families belonging to the infraorder Oxyuridomorpha. Our results indicated that the present *P. ambiguus* was more closely related to the previously described *P. ambiguus* and *W. siamensis* than to *E. vermicularis*. These results were consistent with those of recent studies by Sheng et al. [59] and Liu et al. [40]. The complete genomes for only three species from the infraorder Oxyuridomorpha [31, 4049] have been reported. Therefore, in this study,the genomic sequences of *P. ambiguus* were characterized to stimulate a reassessment of the systematic relationships of oxyurid nematodes using available genomic datasets. In addition, the genomic sequences of nuclear SSU rDNA gene for the present *P. ambiguus* revealed the monophyly of the infraorder Oxyuridomorpha, and supported the taxonomic position of the present *Passalurus* species, which is deeply embedded in the genus *Passalurus* with a close relationship to the previously described *P. ambiguus*. These data are concomitant with previous studies by Park et al. [49], Kim et al. [34], De Ley and Blaxter [15], Nadler et al. [45], and Liu et al. [41, 42].

Conclusion

The present study provides useful tools for the rapid identification, and systematic and phylogenetic analyses of pinworms infecting animals and humans. In addition, the 18S rDNA gene of *P. ambiguus* was shown to yield a unique genetic sequence that confirms its taxonomic position of a species within the Oxyuridae family. Moreover, *O. cuniculus* should be considered a potential natural reservoir of different parasite species, and further research into the diagnosis, prevention, and control of passaluriasis in rabbits and other animals is required.

Acknowledgements The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding this Research Group No. RGP-VPP- 173.

Compliance with Ethical Standards

Conflicts of Interest The authors declare no conflicts of interest.

Ethical Approval 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 the Institutional Animal Care and Use Committee (IACUC) in Faculty of Science, Cairo University, Egypt [CU/I/S/18-16].

References

1. Abade Dos Santos FA, Carvalho C, Nuno O, Correia JJ, Henriques M, Peleteiro MC, Fevereiro M, Duarte MD (2017) Detection of rabbit Haemorrhagic disease virus 2 during the wild rabbit (*Oryctolagus cuniculus*) eradication from the Berlengas archipelago, Portugal. BMC Vet Res 13(1):336
2. Abdel-Gaber R (2016) Syphacia obvelata (Nematode, Oxyuridae) infecting laboratory mice *Mus musculus* (Rodentia, Muridae): phylogeny and host–parasite relationship. Parasitol Res 115:975–985
3. Abdel-Gaber R, Fol M (2015) Aspicularis tetraptera (Nematode, Heteroxynematidae) of laboratory mice *Mus musculus* (Rodentia, Muridae): a potential risk of zoonotic infection for researchers. Ciência e Técnica Vitivinícola 30(8):125–136
4. Arkoulis N, Zerbinis H, Simatos G, Nisiotis A (2012) Enterobius vermicularis (pinworm) infection of the liver mimicking malignancy: presentation of a new case and review of current literature. Int J Surg Case Rep 3:6–9
5. Ashmawy KI, El-Sokkary MY, Abu-Akkada SS, Dewair AW (2010) Incidence of *Passalurus ambiguus* in domestic rabbits in Behera Province. Alex J Vet Sci 30(1):115–120
6. Baker DG (2007) Flynn's parasites of laboratory animals, 2nd edn. Blackwell Publishing, Hoboken, p 813
7. Bin Z, Chunsheng B (1987) Scanning electron microscopic observations of the integumental surface of adult *Passalurus ambiguus*. Acta Zool Sin 33(4):383–384
8. Boag B, Lello J, Fenton A, Tompkins DM, Hudson PJ (2001) Patterns of parasite aggregation in the wild European rabbit (*Oryctolagus cuniculus*). Int J Parasitol 31:1421–1428
9. Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revised. J Parasitol 83(4):575–583
10. Caballero E (1937) *Passalurus abditus* nouvelle espèce de nématode parasite d'un Rongeur mexicain. Annales de Parasitologie Humaine et comparée 15:504–506
11. Chang TK, Liao CW, Haung YC (2009) Prevalence of Enterobius vermicularis infection among preschool children in kinder gardens of Taipei City, Taiwan in 2008. Korean J Parasitol 47:185–187
12. Cheeke PR (1986) Potentials of rabbit production in tropical and subtropical agricultural systems. J Anim Sci 63:1581–1856
13. Cobbold TS (1864) Entozoa: an introduction to the study of helminthology, more particularly to the internal parasites of man. p 508. Quoted from Petter and Quentin (1976)
14. Danheim BL, Ackert JE (1929) On the anatomy of the nematode *Passalurus ambiguus* (Rudolphi). Trans Am Microsc Soc 48:80–85
15. De Ley P, Blaxter M (2002) Systematic position and phylogeny. In: Lee DL (ed) The biology of nematodes. Taylor and Francis, London and New York, pp 1–30
16. Dufour B, Hugot JP, Lepetz S, Le Bailly M (2015) The horse pinworm (Oxyuris equi) in archaeology during the Holocene: review of past records and new data. Infect Genet Evol 33:77–83
17. Durden LA, Hu R, Oliver JH, Cilek JE (2000) Rodent ectoparasites from two locations in northwestern Florida. J Vector Ecol 25:222–228
18. Floyd R, Rogers A, Lambshead P, Smith C (2005) Nematode-specific PCR primers for the 18S small subunit rRNA gene. Mol Ecol Notes 5:611–612
19. Flynn RJ (1973) Parasites of laboratory animals. The Iowa State University Press, Ames, p 882
20. Frank R, Kuhn T, Mehlhorn H, Rueckert S, Pham D, Klimpel S (2013) Parasites of wild rabbits (*Oryctolagus cuniculi*) from an urban area in Germany, in relation to worldwide results. Parasitol Res 112:4255–4266
21. Fuentes GC, Newgren J (2008) Physiology and clinical pathology of laboratory New Zealand white rabbits housed individually and in groups. J Am Assoc Lab Anim Sci 47(2):35–38
22. Georgi JR, Georgi ME (1990) Parasitology for veterinarians. Saunders, Philadelphia
23. Hall ER, Kelson KR (1959) The mammals of North America. The Ronald Press Company, New York, p 1083
24. Hall MC (1916) Nematode parasites of mammals of the orders Rodentia, Lagomorpha and Hyracoidea. Proc USA Natl Mus 50:1–258
25. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
26. Hallal-Calleros C, Morales-Montor J, Vázquez-Montiel JA, Hoffman KL, Nieto-Rodríguez A, Flores-Pérez AI (2013) Hormonal and behavioral changes induced by acute and chronic experimental infestation with Psoroptes cuniculi in the domestic rabbit *Oryctolagus cuniculus*. Parasites Vectors 6:341–361
27. Hugot JP (1980) Sur le genre Aspiculuris Schulz, 1924 (Nematoda, Heteroxynematidae), Oxyures parasites de Rongeurs Muroidea. Bulletin du Museum National d'Histoire Naturelle 2:723–735
28. Hugot JP, Bain O, Cassone J (1982) Insemination traumatique et tube de ponte chex l'Oxyure parasite du lapin domestique. Compte Rendu Hebdomadaire Seances de l'Academie des Sciences t. 294(19 avril 1982) Ser. III:707–710
29. Jobet E, Bougnoux ME, Morand S, Rivault C, Cloarec A, Hugot JP (1998) Use of random amplified polymorphic DNA (RAPD) for generating specific DNA probes for oxyuroid species (Nematoda). Parasite 5:47–50
30. Johnston TH, Mawson PM (1938) Some nematodes from Australian marsupials. Rec S Aust Mus 6:187–198
31. Kang S, Sultana T, Eom KS, Park YC, Soonthornpong N, Nadler SA, Park JK (2009) The mitochondrial genome sequence of *Enterobius vermicularis* (Nematoda: Oxyurida)—an idiosyncratic gene order and phylogenetic information for Chromadorean nematodes. Gene 429:87–97
32. Kataranovski D, Vukićević-Radić OD, Kataranovski M, Radović DL, Mirkov II (2008) Helminth fauna of *Mus musculus* Linnaeus 1758 from the suburban area of Belgrade, Serbia. Arch Biol Sci 60(4):609–617
33. Khalil AI, Lashein GH, Morsy GH, Abd El-Mottaleb DI (2014) Oxyurids of wild and laboratory rodents from Egypt. Life Sci J 11(3):94–107
34. Kim T, Kim J, Cho S, Min GS, Park C, Carreno RA, Nadler SA, Park JK (2014) Phylogeny of Rhigonematomorpha based on the complete mitochondrial genome of *Rhigonema thysanophora* (Nematoda: Chromadorea). Zoolog Scr 43:289–303

35. Klimpel S, Abdel-Ghaffar F, Al-Rasheid KA, Aksu G, Fischer K, Strassen B, Mehlhorn H (2011) The effects of different plant extracts on nematodes. Parasitol Res 108(4):1047–1054
36. Klimpel S, Förster M, Günter S (2007) Parasite fauna of the bank vole *Clethrionomys glareolus* in an urban region of Germany: reservoir of zoonotic metazoan parasites? Parasitol Res 102:69–75
37. Kylie J, McEwen SA, Boerlin P, Reid-Smith RJ, Weese JS, Turner PV (2017) Prevalence of antimicrobial resistance in fecal *Escherichia coli* and *Salmonella enterica* in Canadian commercial meat, companion, laboratory, and shelter rabbits (*Oryctolagus cuniculus*) and its association with routine antimicrobial use in commercial meat rabbits. Prev Vet Med 147:53–57
38. Li H, Shao R, Song N, Song F, Jiang P, Li Z, Cai W (2015) Higher–level phylogeny of paraneopteran insects inferred from mitochondrial genome sequences. Sci Rep 5:8527
39. Linnaeus C (1758) Systema Naturae, Ed. X. (Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata). Holmiae. Sys Nat 1:1–824
40. Liu GH, Li S, Zou FC, Wang CR, Zhu XQ (2016) The complete mitochondrial genome of rabbit pinworm *Passalurus ambiguus*: genome characterization and phylogenetic analysis. Parasitol Res 115:423–429
41. Liu GH, Shao R, Cai XQ, Li WW, Zhu XQ (2015) Gnathostoma spinigerum mitochondrial genome sequence: a novel gene arrangement and its phylogenetic position within the class chromadorea. Sci Rep 5:12691
42. Liu GH, Shao R, Li JY, Zhou DH, Li H, Zhu XQ (2013) The complete mitochondrial genomes of three parasitic nematodes of birds: a unique gene order and insights into nematode phylogeny. BMC Genomics 14:414
43. Martino PA, Luzi F (2008) Bacterial infections in rabbit as companion animal: a survey of diagnostic samples in Italy. 9th World Rabbit Congress, Verona, Italy, pp 1013–1014
44. Millazzo C, Ribasa A, Casanova JC (2010) Helminths of the brown rat (*Rattus norvegicus*) (Berkenhout, 1769) in the city of Palermo, Italy. Helminthology 47(4):238–240
45. Nadler SA, D'Amelio S, Dailey MD, Paggi L, Siu S, Sakanari JA (2005) Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova*, and *Contracaecum* from northern Pacific marine mammals. J Parasitol 91:1413–1429
46. Neimanis AS, Ahola H, Zohari S, Larsson Pettersson U, Brojer C, Capucci L, Gavier-Widen D (2018) Arrival of rabbit haemorrhagic disease virus 2 to northern Europe: emergence and outbreaks in wild and domestic rabbits (*Oryctolagus cuniculus*) in Sweden. Transbound Emerg Dis. 65(1):213–220
47. Ogedengbe ME, El-Sherry S, Whale J, Barta JR (2014) Complete mitochondrial genome sequences from five Eimeria species (Apicomplexa; Coccidia; Eimeriidae) infecting domestic turkeys. Parasites Vectors 7:335
48. Okerman L (1994) Diseases of domestic rabbits, 2nd edn. Blackwell Scientific Publications, Oxford (England), p 160
49. Park JK, Sultana T, Lee SH, Kang S, Kim HK, Min GS, Eom KS, Nadler SA (2011) Monophyly of clade III nematodes is not supported by phylogenetic analysis of complete mitochondrial genome sequences. BMC Genomics 12:392
50. Percy DH, Barthold SW (1993) Pathology of laboratory rodents and rabbits. Iowa State University Press, Iowa
51. Petter AJ, Quentin JC (2009) Oxyuroidea. In: Anderson RC, Chabaud AG, Willmott S (eds) Keys to the nematode parasites of vertebrates (Archival Volume). CAB Int, London, pp 218–247
52. Pinto RM, Gomez DC, Menezes RC, Gomez CT, Noronha D (2004) Helminths of rabbits (Lagomorpha, Leporidae) deposited in the Helminthological Collection of the Oswaldo Cruz Institute. Revista Brasileira de Zoologia 21(3):599–604
53. Pritt S, Cohen K, Sedlacek H (2012) The laboratory rabbit, guinea pig, hamster, and other rodents. Chapter 15 Parasitic Diseases, pp 415–446
54. Rinaldi L, Russo T, Schioppi M, Pennacchio S, Cringoli G (2007) *Passalurus ambiguus*: new insights into copromicroscopic diagnosis and circadian rhythm of egg excretion. Parasitol Res 101:557–561
55. Robles MR, Navone GT (2007) A new species of *Syphacia* (Nematoda: Oxyuridae) from *Oligoryzomys nigripes* (Rodentia: Cricetidae) in Argentina. Parasitol Res 101(4):1069–1075
56. Robles MR, Navone GT (2010) Redescription of Syphacia venteli Travassos 1937 (Nematoda: Oxyuridae) from *Nectomys squamipes* in Argentina and Brazil and description of a new species of *Syphacia* from *Melanomys caliginosus* in Colombia. Parasitol Res 106:1117–1126
57. Romero Rodriguez J, Pozo DG, Herrera JL (1973) Estudios sobre el genero *Passalurus* Dujardin, 1845, parasitando al *Oryctolagus cuniculus* domesticus (L.) y *Lepus granatensis* R. Revista Iberica de Parasitologia 33:315–329
58. Rudolphi CA (1819) Entozoarum synopsis cui accedunt mantissa duplex et indices locupletissimi. Berlin 8:811
59. Sheng L, Cui P, Fang SF, Lin RQ, Zou FC, Zhu XQ (2015) Sequence variability in four mitochondrial genes among rabbit pinworm (*Passalurus ambiguus*) isolates from different localities in China. Mitochondrial DNA 26:501–504
60. Singla LD, Singla N, Parshad VR, Juyal PD, Naresh KS (2008) Rodents as reservoirs of parasites in India. Integr Zool 3(1):21–26
61. Skinker MS (1931) Three new parasitic nematode worms. Proc US Natl Mus 79:1–9
62. Solórzano-García B, Nadler SA, Ponce Perez, de León G (2015) *Trypanoxyuris atelis* and *T. atelophora* (Nematoda: Oxyuridae) in wild spider monkeys (Abeles geoffroyi) in tropical rain forest in Mexico: morphological and molecular evidence. Parasitol Int 64:229–235
63. Sotillo J, Trelis M, Cortés A, Luz Valero M, Sánchez del Pino M, Esteban JG, Marcilla A, Toledo R (2012) Proteomic analysis of the pinworm *Syphacia muris* (Nematoda: Oxyuridae), a parasite of laboratory rats. Parasitol Res 61(4):561–564
64. Sultan K, Elhawary NM, Sorour SG, Sharaf HM (2015) Observations of the rabbit pinworm *Passalurus ambiguus* (Rudolphi, 1819) in domestic rabbits (*Oryctolagus cuniculus*) in Egypt using a scanning electron microscope. Trop Biomed 32(4):745–752
65. Swofford DL (2000) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, MA
66. Szkucik K, Pyz-Łukasik R, Szczepaniak KO, Paszkiewicz W (2014) Occurrence of gastrointestinal parasites in slaughter rabbits. Parasitol Res 113:59–64
67. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
68. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882
69. Vermund SH, Wilson CM (2000) Pinworm (*Enterobius vermicularis*). Semin Pediatr Infect Dis 11:252–256
70. Vicente JJ, Rodrigues HO, Gomes DC, Pinto RM (1997) Nematóides Do Brasil. Parte V: Nematóides De Mamíferos. Revista Brasileira de Zoologia 14(1):1–452
71. Wu HW (1933) Helminthological notes I. Sinensia 4:51–59

72. Yamaguti S (1961) Systema Helminthum. Volume I. The nematodes of vertebrates. Part I and II. Interscience Publisher, New York, pp 535–560
73. Yang X, Gasser RB, Koehler AV, Wang L, Zhu K, Chen L, Feng H, Hu M, Fang R (2015) Mitochondrial genome of *Hypoderaeum conoideum*–comparison with selected trematodes. Parasites Vectors 8:97