sciendo

J Vet Res 68, 363-371, 2024
DOI:10.2478/jvetres-2024-0045

Morphological and molecular characterisation of the nematode parasite *Graphidioides affinis* (Secernentea: Trichostrongylidae) in Patagonian maras, *Dolichotis patagonum*, kept in a zoo in Sofia, Bulgaria

Mariana Panayotova-Pencheva[1], Zdzisław Laskowski[2], Anna Maria Pyziel[2]✉

[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[2]Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences, 02-776 Warsaw, Poland
anna_pyziel@sggw.edu.pl

Received: January 8, 2024 *Accepted: August 1, 2024*

Abstract

Introduction: Patagonian maras, rodents endemic to South America, are classified as a near-threatened species. Various factors affect their health including parasitic diseases. The aim of this study was to perform morphometric, molecular and phylogenetic characterisation of one such parasitic disease agent, the nematode *Graphidioides affinis*, specimens of which were found in captive Patagonian maras. **Material and Methods:** In March 2023, 18 Patagonian maras kept at the Sofia Zoo in Bulgaria were investigated with the use of coprological methods. Following the investigation, the animals were dewormed with the use of albendazole. Dead adult nematodes found in the faeces of dewormed maras were collected and preserved in 70% ethanol, and morphometrically, molecularly and phylogenetically analysed. **Results:** The morphometric analyses confirmed the nematodes to be *Graphidioides affinis*. The partial nucleotide sequences of the small subunit ribosomal rDNA (*SSU*), the internal transcribe spacer 2 (*ITS2*) and the large subunit ribosomal DNA (*LSU*) of *G. affinis* were obtained. These are the first available nucleotide sequences of this parasite. The phylogenetic analyses of the species showed its distinctiveness in comparison to other gastrointestinal nematodes, as it was grouped separately. **Conclusion:** The Patagonian maras kept in a European zoo retained their original parasitofauna which are related to South America.

Keywords: *Dolichotis patagonum*, *Graphidioides affinis*, morphometry, molecular data, phylogenetic analysis.

Introduction

Patagonian maras, *Dolichotis patagonum* (Zimmermann, 1780) are relatively large rodents of the Caviidae family. They are endemic to South America, mainly Argentina, and distributed from 28°S to 50°S (3). They are kept as exotic mammals in zoological parks and gardens around the world, which helps in their conservation. This is desirable because Patagonian maras are considered to be a near-threatened species (17). There are diverse health hazards for these animals (19), and parasitoses are among the most common of them.

Various genera and species of parasites have been found in free or captive Patagonian maras to date. Among them are such nematodes as *Graphidioides affinis* (Megnin, 1895), *Trichostrongylus retortaeformis* (Zeder, 1800), *Strongyloides* sp., *Wellcomia dolichotis* and *Trichuris* spp. (2, 8, 14, 22, 23); the protozoa *Toxoplasma gondii*, *Besnoitia* sp. and *Giardia* spp. (7, 16, 23); and the arachnids *Otodectes cynotis* and *Sarcoptes scabiei* (4, 12). The aim of this study was to perform morphometric, molecular and phylogenetic characterisation of *G. affinis*, specimens of which were recently found in Patagonian maras kept at the Sofia Zoo in Bulgaria.

Material and Methods

Sample origin and area of the study. Parasitological monitoring of 18 Patagonian maras kept at the Sofia Zoo was performed with the use of coprological methods in

March 2023. The animals shared an enclosure with one kangaroo for more than 10 years. The enclosure consisted of an outdoor part (the larger area, covered by soil and vegetation) and an indoor part (covered by concrete).

Coprological study. Pooled faecal samples (1–2 g from each faecal pile) collected from the ground of the enclosure were processed using diagnostic techniques (9). Those were a common flotation technique with the use of sodium chloride as a flotation solution; a sedimentation technique to detect heavy eggs of flukes, including *Fasciola hepatica*; and the Baermann technique to detect lungworm larvae. After the samples were analysed, the animals were dewormed once orally with a 10% suspension of albendazole (Vermitan; Ceva Santé Animale, Libourne, France) at a dose of 8 mg/kg body weight. The medicine was mixed with a small amount of food after a 16-h fast. Dead adult nematode parasites were detected in the faeces of dewormed individuals. The specimens were collected in saline, washed in it for 30–60 min and then stored in 70% ethanol for further morphological and molecular analyses.

Morphological and morphometric study. Seven males and seven females of the species were cleared in lactophenol. Each of the cleared worms was placed in a drop of lactophenol between a microscope slide and coverslip and subjected to morphometric processing. The morphological structures of the helminths were measured after taking their microphotographs with a Motic Images Plus 3.0 camera (Motic, Xiamen, China) connected to an Amplival microscope (Carl Zeiss AG, Oberkochen, Germany), and subsequently analysed with the use of the camera's associated software. The morphometric data were processed using Excel (Microsoft, Redmond, WA, USA). The species determination was based on the morphometry of such features as the spicules, the rays of copulatory bursa, the ovijector and the vulva, as recommended previously (20). Some specimens, stored in 70% ethanol or embedded in glycerin-gelatin as permanent microscopic preparations, were deposited in the Institute of Experimental Morphology, Pathology and Anthropology with Museum comprising part of the Bulgarian Academy of Sciences in Sofia, Bulgaria.

DNA extraction and PCR amplification. Genomic DNA was extracted individually from four ethanol-preserved nematode specimens with the use of a NucleoSpin Tissue DNA extraction kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. The complete sequence of the internal transcribed spacer 2 (*ITS2*) and a partial region of the large subunit ribosomal DNA (*LSU*) were amplified by PCR using the following set of primers: forward-ITS2F (5′-ACG TCT GGT TCA GGG TTG TT-3′) and reverse-BD3R (5′-TAT GCT TAA GTT CAG CGG GT-3′) (15). Additionally, a set of primers was designed for the purpose of this study to amplify a partial region of the small subunit ribosomal rDNA (*SSU*): forward-SSU_07 (5′-AAA GAT TAA GCC ATG CAT G-3′) and reverse-N1070R (5′-TTG CAA CCA TAC TAC CCC AGG AAC CGA A-3′). The PCR was performed in a T100 thermal cycler (Bio-Rad, Hercules, CA, USA) in a volume of 50 µL. Each 50 µL PCR reaction mix contained 20 µL of Molecular Biology Reagent Water (Sigma-Aldrich, St. Louis, MO, USA), 25 µL of AccuStart II PCR ToughMix (×2 concentration) (Quantabio, Beverly, MA, USA), 1 µL of GelTrack Loading Dye (×50 concentration) (Quantabio), 1 µL of forward primer (20 mM), 1 µL of reverse primer (20 mM) and 2 µL of template DNA. The conditions for PCR were as follows: 94°C for 2 min to denature the DNA; 35 cycles at 94°C for 30 s, 62°C for 45 s (for *ITS2*) or 55°C for 90 s (for *SSU*), and 72°C for 30 s (for *ITS2*) or for 2 min (for *SSU*); and a final extension at 72°C for 5 min (for *ITS2*) or 10 min (for *SSU*) to ensure complete amplification. The PCR products were purified with the use of the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel), eluted with 30 µL of Molecular Biology Reagent Water (Sigma-Aldrich) and sequenced in both directions by Genomed S.A. (Warsaw, Poland) using the primers used for amplification (5 mM). The sequences were then assembled into contigs using CodonCode Aligner version 8.0 (CodonCode Corporation, Centerville, MA, USA). Obtained nucleotide sequences were compared to the NCBI database of sequences using the basic local alignment search tool (BLAST) (1).

Phylogenetic analyses. Phylogenetic analysis was performed based on partial nucleotide sequences of the *SSU* and *ITS2* genes using the newly generated sequences and matching sequences available in GenBank (Tables 1 and 2). Forward and reverse sequences were assembled using CodonCode Aligner version 8.0 software. Contiguous sequences were submitted to GenBank, those of the *SSU* gaining accession nos OR611714.1 and OR611716.1 and the *ITS2* sequence being logged as OR353426.1. Both sets of sequences were aligned using AlignX implemented in Vector NTI Advance 11 (Invitrogen/ Life Technologies, Carlsbad, CA, USA). The alignment was trimmed to the length of the shortest sequence. Phylogenetic trees were constructed using Bayesian inference as implemented in MrBayes version 3.2.0 software (18). The general time-reversible model with estimates of invariant sites and gamma-distributed among-site variation for *SSU* and gamma distributed among-site variation for *ITS2* was chosen as the best-fitting nucleotide substitution model for the dataset. Model selection was made with JModelTest version 2.1.4 software (5, 11). In the *SSU* analysis the sequence of *Caenorhabditis elegans* (GenBank accession no. AY268117.1) was used as the outgroup. The Bayesian inference analysis was performed as follows: a Monte Carlo Markov chain was run for 2,000,000 generations, log-likelihood scores were plotted and the final 75% of trees were used to produce the consensus tree.

Table 1. List of taxa included in the molecular analysis of *Graphidioides affinis* using sequence data of the *SSU* rDNA

GenBank accession no.	Species	Host	Location
AY268117.1	*Caenorhabditis elegans*	Not applicable	USA
AJ920341.1	*Chabertia ovina*	*Ovis aries*	Canada
AJ920350.1	*Trichostrongylus colubriformis*	*Ovis aries*	Canada
AJ920359.1	*Tetrabothriostrongylus mackerrasae*	*Antechinus stuartii*	Canada
EU086374.1	*Haemonchus contortus*	*Giraffa camelopardalis*	USA/Lion Country Safari/region of origin: Africa
L04152.1	*H. similis*	Not provided	USA
OP288108.1	*Globocephalus urosubulatus*	*Sus scrofa*	Brazil
AY295811.1	*Necator americanus*	*Homo sapiens*	Guatemala
DQ094176.1	*Strongylus equinus*	Not provided	Great Britan
AJ920342.1	*Cylicocyclus insignis*	*Equus caballus*	Canada
LC415112.1	*Oesophagostomum muntiacum*	*Muntiacus reevesi*	Japan
AJ920340.1	*Cyclodontostomum purvisi*	*Rattus sordidus*	Canada
OP288110.1	*Stephanurus dentatus*	*Sus scrofa*	Brazil
MN218457.1	*Uncinaria stenocephala*	*Canis latrans*	Canada
AJ920351.1	*Ostertagia leptospicularis*	*Bos taurus*	Canada
AJ920352.1	*Ostertagia ostertagi*	*Bos taurus*	Canada
AY295820.1	*Troglostrongylus wilsoni*	*Lynx rufus*	USA
KC771250.1	*Dictyocaulus viviparus bisontis*	*Bison bonasus*	Poland
KM374671.1	*Dictyocaulus cervi*	*Cervus elaphus*	Poland
MH756629.1	*Dictyocaulus skrjabini*	*Cervus elaphus*	Poland
MH688454.1	*Lamanema chavezi*	*Lama lama*	Germany/region of origin: South America
JX877671.1	*Travassostrongylus orloffi*	*Didelphis marsupialis*	Mexico
JX877677.1	*Travassostrongylus callis*	*Didelphis marsupialis*	Panama
AJ920355.1	*Heligmosomoides polygyrus*	*Mus musculus*	Canada
JX877678.1	*Carolinensis perezponcedeleoni*	*Nyctomys sumichrasti*	USA
JX877672.1	*Vexillata convoluta*	*Cratogeomys merriami*	Mexico
AJ920360.1	*Nematodirus battus*	*Ovis aries*	Canada
OR611714.1	***Graphidioides affinis***	*Dolichotis patagonum*	Bulgaria/Sofia Zoo/region of origin: South America
OR611716.1	***Graphidioides affinis***	*Dolichotis patagonum*	Bulgaria/Sofia Zoo/region of origin: south America

Bold indicates isolates examined in the present investigation

Table 2. List of taxa included in the molecular analysis of *Graphidioides affinis* using sequence data of *ITS2* rDNA

GenBank accession no.	Species	Host	Location
KF765464.1	*Heligmosomoides polygyrus*	*Apodemus sylvaticus*	Norway
MH688454.1	*Lamanema chavezi*	*Lama lama*	Germany/region of origin: South America
Y10789.1	*Chabertia ovina*	*Ovis aries*	Australia
LC415112.1	*Oesophagostomum muntiacum*	*Muntiacus reevesi*	Japan
OQ773550.1	*Uncinaria stenocephala*	*Ursus arctos*	Turkey
OR353426.1	***Graphidioides affinis***	*Dolichotis patagonum*	Bulgaria/Sofia Zoo/region of origin: South America
AF194138.1	*Nematodirus battus*	Not provided	UK
KX358861.1	*Cooperia oncophora*	*Bison bonasus caucasicus*	Sweden/Avesta Visentpark
KX358862.1	*Ostertagia ostertagi*	*Bison bonasus caucasicus*	Sweden/Avesta Visentpark
X86025.1	*O. leptospicularis*	*Bos taurus*	
KX358860.1	*Haemonchus contortus*	*Bison bonasus caucasicus*	Sweden/Avesta Visentpark
AJ577460.1	*Spiculopteragia boehmi*	Not provided	France

Bold indicates isolates examined in the present investigation

Results

Coprological findings. Examination of the fresh faeces of *D. patagonum* by the direct flotation technique revealed large nematode eggs. They were oval, symmetrical and double-shelled, containing numerous small, hard-to-distinguish blastomeres which were 127–140 µm long and 68–75 µm wide (Fig. 1). Neither eggs nor larvae were found with the sedimentation or Baermann techniques.

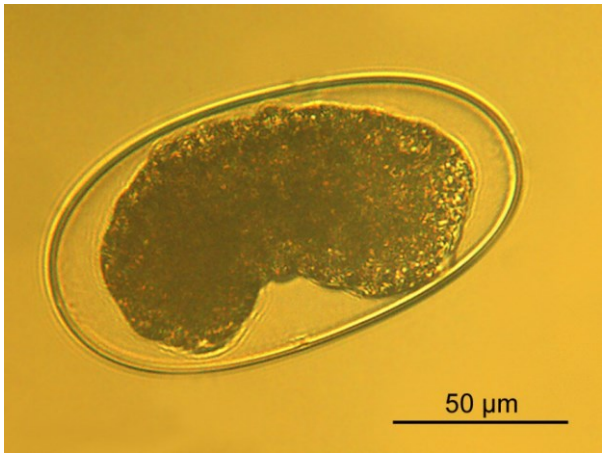

Fig. 1. An egg of *Graphidioides affinis* found in the faeces of *Dolichotis patagonum* from the Sofia Zoo, Bulgaria

Additionally, 20 small, thin, red nematodes, which were dead, were found in the faeces of the animals on the second and third day after deworming.

Morphometric characterisation. Spindle-shaped nematodes were observed, with a cylindrical anterior end and barely perceptible papillae around the oral opening (Fig. 2a). The nematodes' bodies were covered with transverse and longitudinal cuticular striations (Fig. 2b). The oesophagi were seen to be cylindrical, with a bulbous dilated distal part (Fig. 2c). **Male specimens.** Male *G. affinis* nematodes had bodies 1–2 cm long, 130–155 µm wide at the end of the oesophagus, and maximally 250–340 µm wide. The length of their oesophagi was 505–558 µm, and the maximum width of them was 82–95 µm. The copulatory bursae were well developed and cup-shaped, with one dorsal lobe and two symmetrical left and right lobes (Fig. 3a). A pair of weakly pronounced pre-bursal papillae was present (Fig. 3b). The anteroventral rays of the bursae were short, thin and directed forward, and the posteroventral rays were long, massive and directed backwards (Fig. 3b). The lateral rays were equally wide, the medio- and posterolateral rays extended to the bursal edge, but the anterolateral rays terminated a short distance before it. The exterodorsal rays did not reach the edge of the bursae (Fig. 3c). The dorsal rays were distally cleft, with each of the two branches slightly apically cleft (Fig. 3d). The spicules were equal in size, 1,930–2,700 µm long and filiform, with a tubular structure, pointed distal ends and minimal wings extending distally, through which they touched each other (Fig. 4). The gubernacula were 159–172 µm long and broad based, with raised lateral parts directed anteriorly which gave them a shape like an anchor (Fig. 3c). **Female specimens.** Female *G. affinis* nematodes had bodies 1.4–2.4 cm long, 185–244 µm wide at the end of the oesophagus, and maximally 260–440 µm wide. The length of their oesophagi was 609–714 µm, and the maximum width of them was 97–124 µm. The ovijectors' total length was 1,110–1,448 µm, and their anterior part was several times longer than the posterior part (Fig. 5a). The vulvae were transverse slits located 4.8–6.0 mm from the tail tip (Fig. 5b and c).

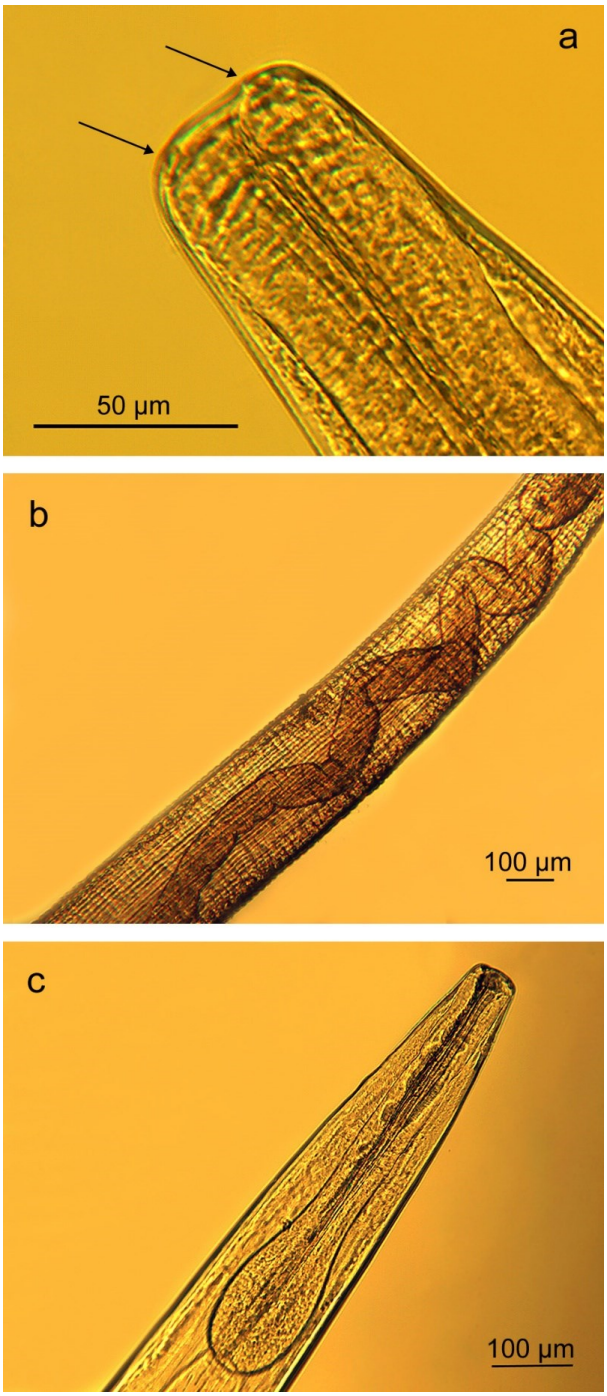

Fig. 2. *Graphidioides affinis* adult worm found in the faeces of *Dolichotis patagonum* from the Sofia Zoo, Bulgaria. a – anterior end, papillae around the oral opening (arrows); b – cuticular striations on the body; c – oesophagus

The tips of the tails were rounded, and at a point 321–442 from them were the anuses, after which the bodies sharply narrowed (Fig. 5d). Eggs in the uteri were 107–171 µm long and 60–94 µm wide. In all cases and for male and female specimens alike, the morphometry of the worms corresponded to *G. affinis*.

DNA sequences. Products of the PCR which were partial *SSU*, *ITS2* and *LSU* sequences were obtained from all four ethanol-preserved worm specimens. In all cases the presence of the DNA of *G. affinis* was detected. The nucleotide sequence of the partial *SSU* was 871 bp in length for the first isolate of the species (GenBank accession no. OR611714.1), whereas three other isolates were 100% homologous sequences of 945 bp in length (GenBank accession no. OR611716.1). Four obtained partial nucleotide sequences of the *ITS2* and the partial *LSU* of 269 bp in length were 100% homologous and were submitted to the NCBI as a single sequence (GenBank accession no. OR353426.1).

Phylogenetic analysis. Bayesian analysis of the *SSU* sequence data with *Caenorhabditis elegans* (GenBank accession no. AY268117.1) as an outgroup revealed nine strongly supported clades (Fig. 6). Four individual clades of *Trichostrongylus colubriformis*, *Tetrabothriostrongylus mackerrasae*, *Nematodirus battus* and *C. elegans* were created.

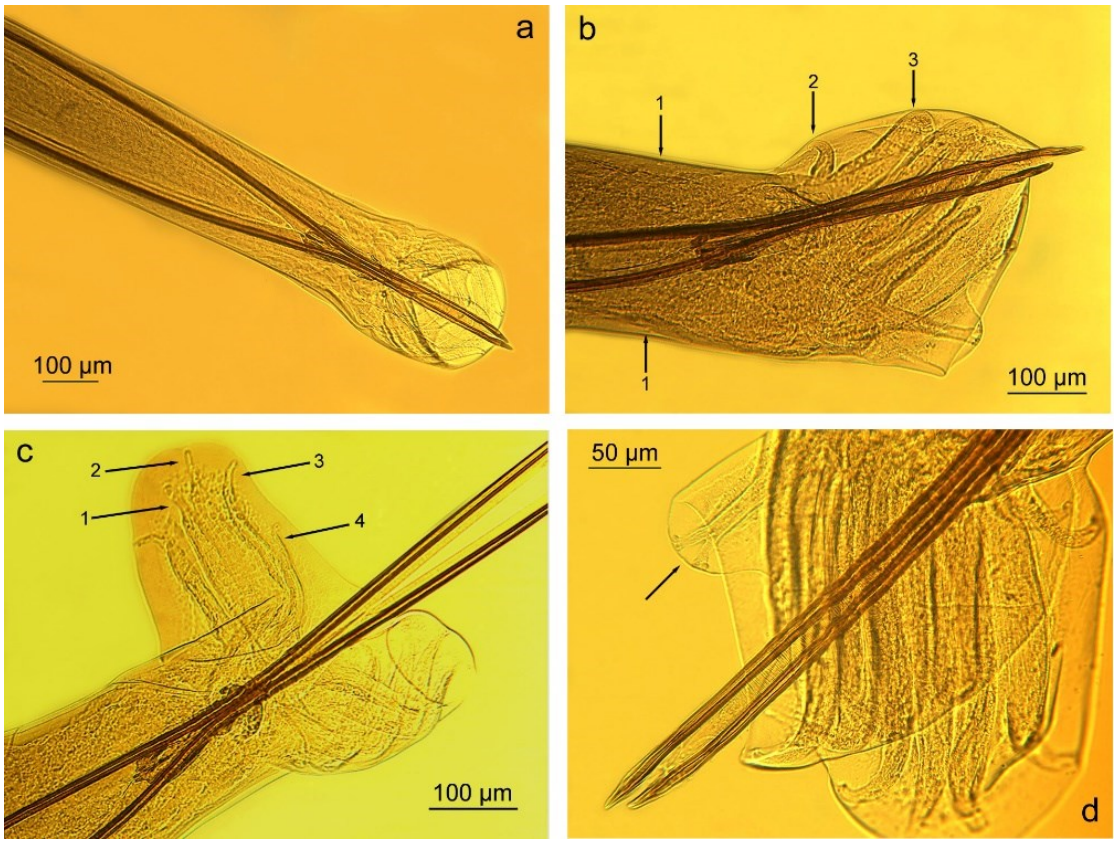

Fig. 3. Posterior body end of male *Graphidioides affinis* found in the faeces of *Dolichotis patagonum* from the Sofia Zoo, Bulgaria. a – common view; b – 1- pre-bursal papillae, 2 - anteroventral ray of copulatory bursa, 3 - posteroventral ray of copulatory bursa; c – copulatory bursa, 1 – anterolateral ray, 2 – mediolateral ray, 3 – posterolateral ray, 4 – exterodorsal ray; d – dorsal ray of copulatory bursa (arrow)

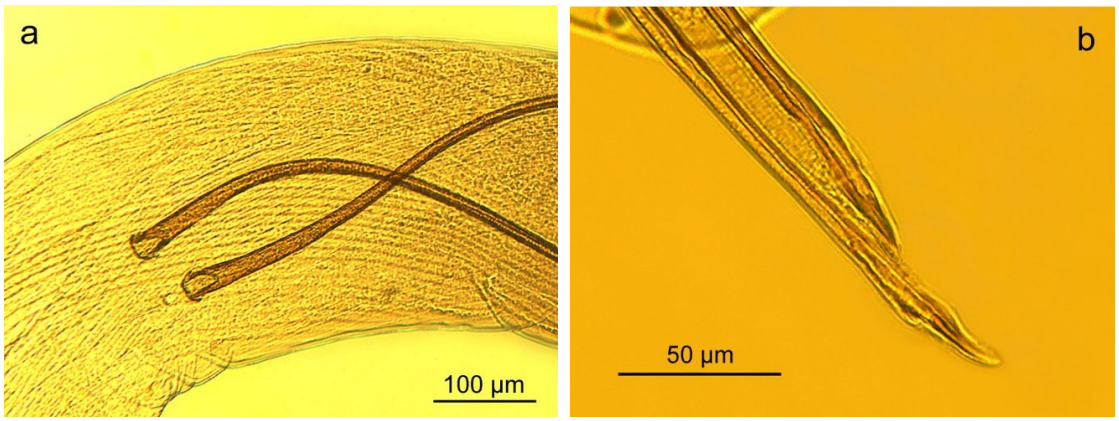

Fig. 4. Spicules of *Graphidioides affinis* found in the faeces of *Dolichotis patagonum* from the Sofia Zoo, Bulgaria. a – proximal parts; b – distal parts

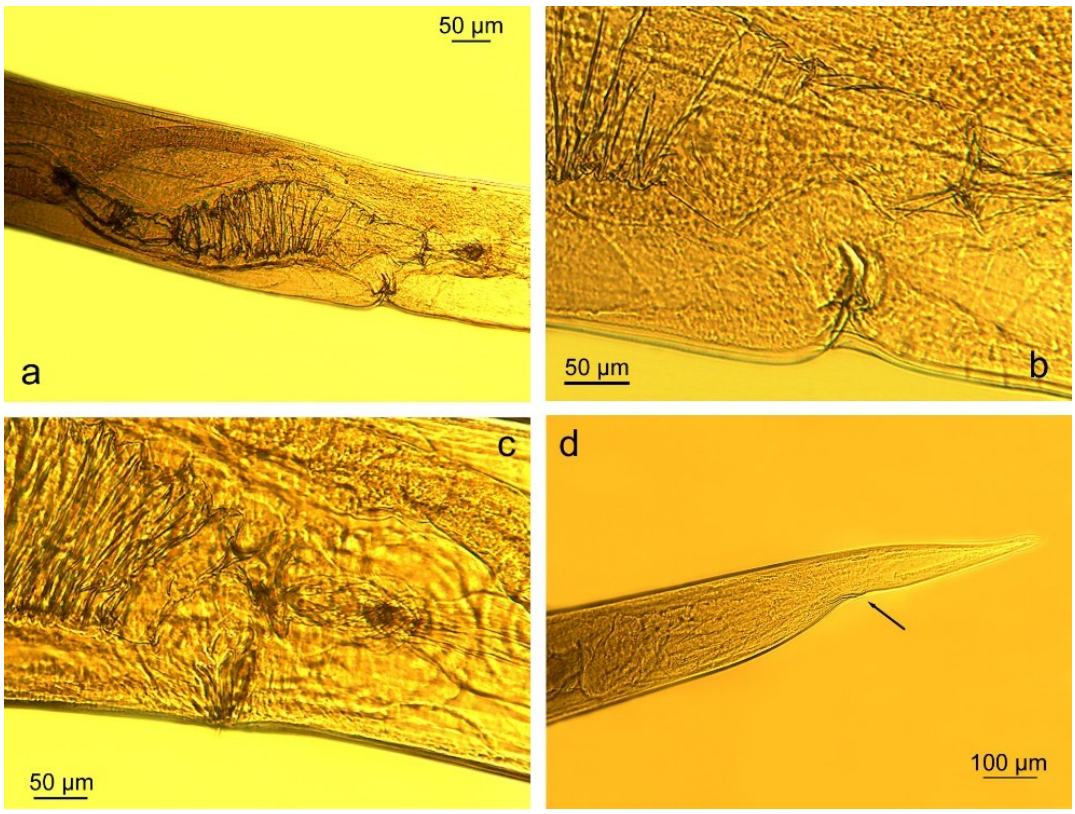

Fig. 5. Female *Graphidioides affinis* found in the faeces of *Dolichotis patagonum* from the Sofia Zoo, Bulgaria. a – ovijector; b and c – vulva; d – posterior end; anus (arrow)

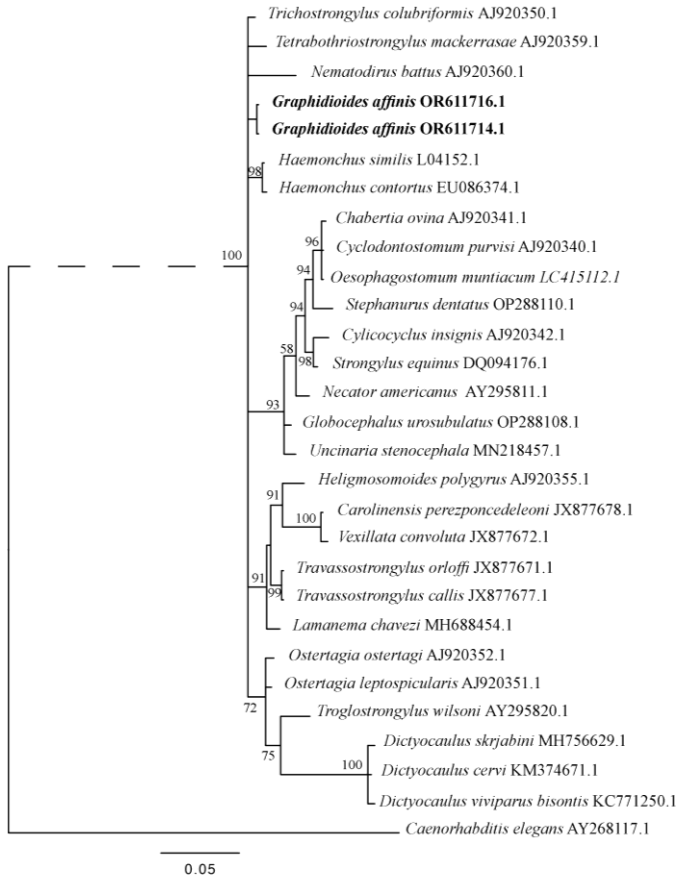

Fig. 6. Phylogenetic tree of parasitic nematodes based on *SSU* rDNA partial sequences, constructed with the use of Bayesian inference analysis using MrBayes version 3.2.0. The general time-reversible model with estimates of invariant sites and gamma-distributed among-site variation model was chosen based on jModelTest version 2.1.4 using the Akaike information criterion. The analysis was run for 2,000,000 generations

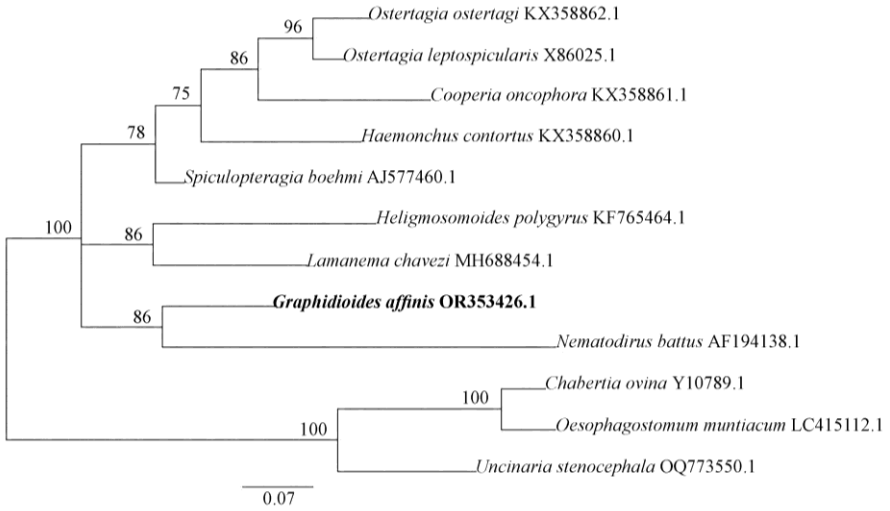

Fig. 7. Phylogenetic tree of parasitic nematodes based on *ITS2* partial sequences, constructed with the use of Bayesian inference analysis using MrBayes version 3.2.0. The gamma-distributed among-site variation model was chosen based on jModelTest version 2.1.4 using the Akaike information criterion. The analysis was run for 2,000,000 generations. Bold indicates isolates examined in the present investigation

Table 3. Comparative morphometry of *Graphidioides affinis* nematodes in materials from Patagonian maras (*Dolichotis patagonum*) investigated in different studies

	Parameter	Origin / References				
		Sofia Zoo, Bulgaria (Present data)		Argentina (1)	London Zoo, UK (7)	Barcelona Zoo Spain (5)
		Range	Mean ± SD			
Adult male nematodes (n = 7)	Body length (cm)	1–2	1.5 ± 0.35	0.9–1.7	1.1	1.1–1.3
	Body width at the end of the oesophagus (µm)	130–155	144 ± 8.97	-	-	-
	Maximum body width (µm)	250–340	291.67 ± 32.5	300	350	350
	Oesophagus length (µm)	505–558	537.6 ± 21.18	-	650	-
	Maximum oesophagus width (µm)	82–95	89 ± 5.79	-	-	-
	Spicule length (µm)	1,930–2,700	2365 ± 266.08	2,800	3,400	2,400–2,700
	Gubernaculum length (µm)	159–172	167.29 ± 4.61	150	120	60
	Gubernaculum width (µm)	48–57	52.29 ± 3.86	80	75	68
Adult female nematodes (n = 7)	Body length (cm)	1.4–2.4	1.7 ± 0.33	1.6–2.1	2.1	2–2.5
	Body width at the end of the oesophagus (µm)	185–244	207 ± 19.86	-	-	-
	Maximum body width (µm)	260–440	360 ± 72.34	500	700	750
	Oesophagus length	609–714	642 ± 37.22	-	650	-
	Maximum oesophagus width (µm)	97-124	114 ± 10.46	-	-	-
	Ovijector total length (µm)	1110–1448	1,230 ± 117.41	1,200	1,525	-
	Vulva-tail tip distance (mm)	4.8–6.0	5 ± 0.48	6	5.5	-
	Anus-tail tip distance (µm)	321–442	355 ± 40.54	500	-	-
	Length of eggs in the uterus (µm)	107–171	133 ± 26.02	140	135	-
	Width of eggs in the uterus (µm)	60–94	75 ± 11.25	75	75	-

SD – standard deviation

The taxons of *G. affinis* (GenBank accession nos: OR611714.1 and OR611716.1) obtained in this study were placed in separate clades, as were *Haemonchus similis* and *H. contortus*. Another cluster comprised six subclades: one including taxa of *Chabertia ovina*, *Cyclodontostomum purvisi* and *Oesophagostomum muntiacum* with *Stephanurus dentatus* as a sister taxon; another with *Cylicocyclus insignis* and *Strongylus equinus* with *Necator americanus* as a sister taxon; and one with *Globocephalus urosubulatus* and *Uncinaria stenocephala* as the sister taxa of those mentioned above. Another cluster comprised four subclades: one

including *Carolinensis perezponcedeleoni* and *Vexillata convolute* with *Heligmosomoides polygyrus* as their sister taxon; and another including *Travassostrongylus orloffi* and *T. callis* with *Lamanema chavezi* placed as a sister taxon to the whole group. The last cluster comprised *Ostertagia ostertagi* and *O. leptospicularis* with a subclade including *Troglostrongylus wilsoni* and *Dictyocaulus skrjabini*, *D. cervi* and *D. viviparus* subs. *bisontis* as sister taxa.

Bayesian inference analysis of the *ITS2* sequence data revealed seven strongly supported clades (Fig. 7). The taxon of *G. affinis* (GenBank accession no. OR353426.1) obtained in this study created an individual clade, as were formed by *Spiculopteragia boehmi*, *H. polygyrus*, *L. chavezi* and *N. battus*. Other clades comprised *H. contortus* with *Cooperia oncophora* and *O. ostertagi* with *O. leptospicularis* as sister taxa. Another clade comprised *U. stenocephala* and a subclade including *Ch. ovina* and *Oe. muntiacum*.

Discussion

According to the obtained morphometric data, the nematodes detected in the faeces of Patagonian maras were identified as *G. affinis*. The species was originally described in Patagonian maras from South America (2). To the best of our knowledge, since then, there have only been two studies providing data on the morphometry of *G. affinis* (6, 8). The morphometric data presented in this study are consistent with those of the previous ones (Table 3).

Dolichotis patagonum, the host of *G. affinis*, is a rodent of the Caviomorpha parvorder and Caviidae family. According to a previous study, six out of eight species of *Graphidioides* are parasites of Caviomorpha families, *i.e.* the Caviidae, Chinchillidae, Octodontidae and Myocastoridae (13), and the distribution of these trichostrongyloid nematodes is closely related to the distribution of their host families (21). Therefore, it is not surprising that *G. affinis* was found in *D. patagonum*, although the particular animals investigated did not originate from their natural habitat in South America, and lived in captivity in a zoo in Europe. In fact, this finding is not exceptional, as *Graphidioides affinis* was previously recorded in captive Patagonian maras in London Zoo (8) and Barcelona Zoo (6), as well as in a free-ranging population in the Whipsnade Wild Animal Park in the UK (14). Besides infecting *D. patagonum,* the nematode was also found in *Lagostomus maximus* (Desmarest, 1817), a host in the Chinchillidae family (10). These data lead us to hypothesise that a parasite–host specificity has arisen between *G. affinis* and *D. patagonum* over the course of their long co-evolution and that *G. affinis* is a highly host-specific parasite. In support of this theory is the failure to find any *G. affinis* infestation in the kangaroo that lived for years with this group of Patagonian maras.

Conclusion

The study acquired the first nucleotide sequences of *G. affinis* which were included in the performed phylogenetic analyses. In both analyses the species emphasised its distinctiveness in comparison to other gastrointestinal nematodes, as it was grouped separately.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The study was financed by the Institute of Veterinary Medicine of the Warsaw University of Life Sciences.

Animal Rights Statement: None required.

Acknowledgements: The researchers are grateful to all who brought about and sustain the Agreement for Collaboration between the Institute of Experimental Morphology, Pathology and Anthropology with Museum of the Bulgarian Academy of Sciences and the Sofia Zoo. The publication was co-financed by Science development fund of the Warsaw University of Life Sciences.

References

1. Camacho C., Coulouris G., Avagyan V., Ma N., Papadopoulos J., Bealer K., Madden T.L.: BLAST+: architecture and applications. BMC Bioinform 2009, 10, 421.
2. Cameron T.W.: Studies on the two new genera and some little known species of the nematode family Trichostrongylidae Leiper. J Helminthol 1923, 1, 71–96, doi: 10.1017/S0022149X00002765.
3. Campos C.M., Tognelli M.F., Ojeda R.A.: *Dolichotis patagonum*. Mamm Species 2001, 652, 1–5, doi: 10.1644/1545-1410(2001)652<0001:DP>2.0.CO;2.
4. da Cruz C.L., Alpino T., Kottwitz J.: Recurrent ear mite (*Otodectes cynotis*) infestation in three related groups of Patagonian cavies (*Dolichotis patagonum*). J Zoo Wildl Med 2017, 48, 484–490, doi: 10.1638/2016-0140R1.1.
5. Darriba D., Taboada G.L., Doallo R., Posada D.: JModelTest 2: more models, new heuristics and parallel computing. Nat Methods 2012, 9, 772.
6. Diakou A., Almagro V., Tahas S.A., Llorens M.T.: Severe gastric parasitosis in a mara (*Dolichotis patagonum*) kept in a zoo. Poster at the 11th European Wildlife Diseases Association Conference, 25–29 August 2014, Edinburgh, UK, doi: 10.13140/RG.2.2.35280.84484.
7. Díaz-Ayala N., Hidalgo-Hermoso E., Cabello-Araya C., Carvallo-Chaigneau F.: Infection with *Toxoplasma gondii* in a red kangaroo (*Macropus rufus*) and a Patagonian mara (*Dolichotis patagonum*) in captivity. Rev Bras Parasitol Vet 2016, 25, 523–526, doi: 10.1590/S1984-29612016076.
8. Durette-Desset M.C., Denke M.: Description de nouveaux Nématodes parasites d'un Lièvre africain et compléments à l'étude morphologique de quelques Trichostrongylidae (Description of new parasitic nematodes of an African hare and additions to the morphological study of some Trichostrongylidae – in French). Bull Mus Natn Hist Nat 1978, 3 ser., 515, Zoologie 354, 331–347.
9. Foreyt W.J.: Diagnostic Parasitology. In: *Veterinary Parasitology Reference Manual*, Fifth Edition, edited by W.J. Foreyt, Blackwell Publishing, Iowa State University Press, Ames, IA, USA, 2001, pp. 3–9.

10. Foster G.W., Branch L.C., Machicote M., Kinsella J.M., Villarreal D., Forrester D.J.: Gastrointestinal helminths of the plains vizcacha (*Lagostomus maximus*) from Argentina, with observations on interspecific interactions between nematodes and cestodes. Comp Parasitol 2002, 69, 26–32, doi: 10.1654/1525-2647(2002)069[0026:GHOTPV]2.0.CO;2.
11. Guindon S., Gascuel O.A.: Simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 2003, 52, 696–704, doi: 10.1080/10635150390235520.
12. Kim K.T., Lee S.H., Kwak D.: Sarcoptic mange in captive maras: the first known outbreak and complete recovery with colony-wide acaricide treatment. J Vet Med Sci 2015, 77, 593–595, doi: 10.1292/jvms.14-0560.
13. Krüger C.P.: Artrópodes e Helmintos parasitos de *Cavia aperea* Exerleben, 1777 (Rodentia: Caviidae) no sul do Brasil (Arthropods and parasitic helminths from *Cavia aperea* Exerleben, 1777 (Rodentia: Caviidae) in southern Brazil – in Portuguese). Dissertation 2006, Universidade Federal de Pelotas, Pelotas, p. 68.
14. Porteous I., Pankhurst S.: Social structure of the mara (*Dolichotis patagonum*) as a determinant of gastro-intestinal parasitism. Parasitology 1998, 116, 269–275, doi: 10.1017/S0031182097002205.
15. Pyziel A.M., Laskowski Z., Demiaszkiewicz A.W., Höglund J.: Interrelationships of *Dictyocaulus* spp. in wild ruminants with morphological description of *Dictyocaulus cervi* sp. (Nematoda: Trichostrongyloidea) from red deer, *Cervus elaphus*. J Parasitol 2017, 103, 506–518, doi: 10.1645/16-75.
16. Rico-Hernandez G., Juan-Salles C., Garner M.M., Barr B.C.: Pulmonary besnoitiasis in captive maras (*Dolichotis patagonum*) associated with interstitial pneumonia. Vet Pathol 2004, 41, 408–411, doi: 10.1354/vp.41-4-408.
17. Roach N.: *Dolichotis patagonum*. The IUCN Red List of Threatened Species 2016: e.T6785A22190337, https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T6785A22190337.en.
18. Ronquist F., Huelsenbeck J.P.: MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 2003, 19, 1572–1574, doi: 10.1093/bioinformatics/btg180.
19. Rosas-Rosas A.G., Juan-Sallés C., Garner M.M.: Pathological findings in a captive colony of maras (*Dolichotis patagonum*). Vet Rec 2006, 158, 727–731, doi: 10.1136/vr.158.21.727.
20. Skryabin K.I., Shihobalova N.P., Shults R.S.: Trichostrongilidy jivotnih i cheloveka. Osnovy Nematodologii (Volume 3, Trichostrongylides of animals and humans. In: *Essentials of Nematodology*, edited by K.I. Skrjabin – in Russian), Izdatelstvo Akademii Nauk SSSR, Moscow, 1954.
21. Sutton C.A., Durette-Desset M.C.: A description of *Graphidioides kravetzi* n. sp. and the revision of *Graphidioides* Cameron, 1923 (Nematoda Trichostrongyloidea), parasites of Neotropical rodents. Syst Parasitol 1995, 31, 133–145.
22. Sutton C.A., Hugot J.: Contribution à la connaissance de la faune parasitaire d'Argentine XVIII. Étude morphologique de *Wellcomia dolichotis* n. sp. (Oxyuridae, Nematoda), parasite de *Dolichotis patagonum*. Syst Parasitol 1987, 10, 85–93.
23. Tahas S.A., Diakou A.: Persistent *Giardia* spp. and *Trichuris* spp. infection in maras (*Dolichotis patagonum*) at a zoo in Greece. J Zoo Wildl Med 2013, 44, 389–394, doi: 10.1638/2012-0191R.1.