

Research Paper

*These authors contributed equally to the work.

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Corresponding author:

M.R. Robles;

E-mail: rosario@cepave.edu.ar

Morphological and molecular characterization of *Trichuris muris* (Nematoda: Trichuridae): studies from two commensal rodent species

J.A. Panti-May^{1,*}, M.Á. Gómez Muñoz^{2,*}, A.B. Yeh-Gorocica³,
S. Hernández-Betancourt³, F. Milano², C. Galliari⁴ and M. R. Robles⁴ 

¹Centro de Investigaciones Regionales 'Dr Hideyo Noguchi', Universidad Autónoma de Yucatán, Yucatán, México;;

²Laboratorio Biología de los Parásitos, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, Corrientes, Argentina;; ³Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Mérida, México and ⁴Centro de Estudios Parasitológicos y de Vectores CEPAVE (CCT-CONICET- La Plata), Universidad Nacional de La Plata (UNLP), Buenos Aires, Argentina

Abstract

In this paper we re-describe *Trichuris muris* based on morphological data following isolation from two commensal rodent species, *Mus musculus* from Mexico and *Rattus rattus* from Argentina. Furthermore, we provide a molecular characterization based on mitochondrial (cytochrome c oxidase subunit 1 mitochondrial gene) and nuclear (internal transcribed spacer 2 region) markers in order to support the taxonomic identification of the studied specimens of *T. muris* from *M. musculus*. We distinguished *T. muris* from 29 species of *Trichuris* found in American rodents based on morphological and biometrical features, such as the presence of a spicular tube, length of spicule, size of proximal and distal cloacal tube and non-protrusive vulva. We suggest that spicular tube patterns can be used to classify *Trichuris* species in three groups. Considering that the diagnosis among the species of this genus is mainly based on morphometry, this proposal represents a relevant contribution. We provide molecular studies on two markers, making this the first contribution for *T. muris* in the Americas. This study makes an important contribution to the integrative taxonomy of cosmopolitan nematode species, and its correct determination from the parasitological study of commensal rodents.

Introduction

The whipworm, *Trichuris muris* (Schrank, 1788) is a cosmopolitan nematode that has been extensively investigated and is commonly used as a model species in immunological, genetic, ecological and pharmacological research (e.g. Tomasovicova *et al.*, 1988; Wakelin, 1994; Callejón *et al.*, 2010; Hurst & Else, 2013). However, very few data exist on morphological and genetic variability of naturally occurring *T. muris*.

Trichuris muris was originally described by Schrank (1788) from an unspecified mouse species in Germany, but the host was most likely *Mus musculus* (Ribas *et al.*, 2020). Later, Hall (1916) compiled data on *T. muris* from *M. musculus*, *Rattus rattus* and other wild rodents (e.g. species of the genera *Apodemus* and *Microtus*) in France, Germany and Africa. Several decades later, Roman (1951) redescribed *T. muris* from *M. musculus* and *Apodemus silvaticus* in France. However, these morphological descriptions are incomplete and some features were not mentioned.

To date, few molecular studies have been carried out on *Trichuris* parasites of rodents and records mainly correspond to *T. muris* (e.g. Callejón *et al.*, 2010; Wasimuddin *et al.*, 2016; Ribas *et al.*, 2020). There are reports of at least 21 genes of this *Trichuris* species in seven rodent host species from Europe and Asia (e.g. Feliu *et al.*, 2000; Callejón *et al.*, 2010, 2013; Wasimuddin *et al.*, 2016).

At present, a total of 18 rodent genera from seven families, including Muridae, Arvicolidae, Cricetidae, Sciuridae, Echimyidae, Hystrichidae and Bathyergidae have been reported as hosts of *T. muris* (e.g. Roman, 1951; Skryabin *et al.*, 1957; Yamaguti, 1961; Feliu *et al.*, 1980, 2000; Fataliev, 1983; Genov, 1984; Ibrahim *et al.*, 1984; Prokopic & Genov, 1984; Boren *et al.*, 1993; Mafiana *et al.*, 1997; Gómez Muñoz *et al.*, 2018).

In Mexico, *T. muris* has been recorded mainly in commensal rodents such as *M. musculus*, *R. rattus* and *Rattus norvegicus* in the Mexican states of Hidalgo, Mexico City, Tabasco and Yucatan (Panti-May *et al.*, 2021), with the only record from the wild rodent *Heteromys irrotatus* found in the state of Morelos (Preisser & Falcón-Ordaz, 2019). Meanwhile, in Argentina *T. muris* has only been recorded in *R. rattus* found in one city, Corrientes (Gómez Muñoz *et al.*, 2018).

In this paper we re-describe *T. muris* based on morphological data following isolation from two commensal rodent species, *M. musculus* from Mexico and *R. rattus* from Argentina. Also, we provide a molecular characterization based on the cytochrome c oxidase subunit 1 mitochondrial gene (*cox1*) and nuclear internal transcribed spacer 2 region (ITS2) markers in order to support the taxonomic identification of the studied specimens of *T. muris* from *M. musculus*.

Materials and methods

Sample collection

In Mexico, 366 commensal rodents (313 *M. musculus* and 53 *R. rattus*) were collected in two rural localities in Yucatan: Molas in the municipality of Merida (October 2011 to March 2012); and Paraiso in the municipality of Maxcanu (May to September 2016). In Argentina, 206 *R. rattus* were collected (2013–2014) from Corrientes city, during both cold (May–July) and warm (September–November) seasons.

Mus musculus and *R. rattus* were trapped using Sherman traps placed inside houses and yards. Viscera were fixed in 96% ethanol (Mexico) or 10% formalin (Argentina) and examined in the laboratory. Nematodes were collected from the caeca and preserved in 70% ethanol.

Morphological analysis

Nineteen whipworms (11 males and eight females) from Mexico and 17 specimens (ten males and seven females) from Argentina were cleared in lactophenol and studied under light microscope. Drawings were made with the aid of a drawing tube. Some specimens were dried using the critical point method, examined under scanning electron microscope (Jeol 6360 LV, Jeol, Tokyo, Japan) and photographed. Morphological identification was conducted using characteristics listed by Robles *et al.* (2006) and Robles (2011). Measurements of *T. muris* are given in micrometres unless stated otherwise.

Host vouchers were deposited in the Mastozoological Collection (MC), Universidad Autonoma de Yucatan (CM 1077–1079, 1081, 1082, 1084, 1288, 1290, and 1294), Yucatan, Mexico, and Universidad Nacional del Nordeste, Ciudad de Corrientes, Argentina. Helminth specimens were deposited in the Colección Nacional de Helminthos (CNHE) of the Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico and the Helminthological Collection of the Museo de La Plata (MLP-He), Argentina.

Statistical analysis

Prevalence, mean abundance, mean intensity and range of infections were calculated (Bush *et al.*, 1997). Descriptive statistics (mean, standard deviation (SD) and range) for each morphological feature in male and female specimens of *T. muris* were calculated from each host species (table 1).

DNA extraction, amplification and sequencing

Whole genomic DNA from two individuals previously identified as *T. muris* from *M. musculus* was extracted and purified using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The *cox1* and the

ribosomal ITS2 were amplified by polymerase chain reaction (PCR). The following pairs of primers were used: the forward HC02198F and reverse CORA (Callejón *et al.*, 2016) for a segment of *cox1*; and the forward NC5F (Zhu *et al.*, 1998) and the reverse NC2R (Gasser *et al.*, 1993) for the ITS1–5.8S–ITS2 region. These fragments were amplified using PCR protocols and thermal profiles described previously by Callejón *et al.* (2016) for *cox1* and Robles *et al.* (2014) for ITS2. PCR-positive products were purified and subsequently sequenced (Macrogen Inc., Seoul, Korea). The consensus sequences were submitted to GenBank (<http://www.ncbi.nlm.nih.gov/>).

Sequence analysis

Two datasets of sequences were generated, one of *cox1* and the other of ITS2. The alignment of each dataset was performed with CLUSTAL W (Thompson *et al.*, 1994). The extremes of the alignment were trimmed to match the length of our sequences using MEGAX software (Kumar *et al.*, 2018).

The sequences generated in this study were aligned with *T. muris* sequences available in GenBank from different host species and geographical areas. Sequences of *Trichuris arvicolae* Feliu, Spakulová, Casanova, Renaud, Morand, Hugot, Santalla and Durand 2000 were used as out-groups (see online supplementary table S1).

Phylogenetic trees were produced using the statistical method maximum likelihood (ML) using MEGAX (Kumar *et al.*, 2018) and the Bayesian inference (BI) as implemented in MrBayes Ver. 3.2.6 software (Ronquist & Huelsenbeck, 2003). The nucleotide substitution model was estimated with the program jModelTest v2 (Darriba *et al.*, 2012) with the standard configuration. Akaike's information criteria were used to select the best-fit model of nucleotide substitution.

For ML inference, best-fit nucleotide substitution models included Hasegawa–Kishino–Yano (HKY) with invariant sites (HKY + I) for *cox1* and HKY + G with uniform rates for ITS2. Each one was performed with 1000 bootstrap repetitions for obtaining the best phylogenetic hypothesis of the *cox1* and ITS2 datasets. BI analysis was performed using Markov chain Monte Carlo (MCMC) chains for 1,000,000 generations with sample frequency set at 100. The first 25% of the trees sampled were discarded as 'burn-in'. This number of generations was considered sufficient because the SD dropped below 0.01.

Results

Infection status

Forty-two (13.4%) *M. musculus* from Mexico and 20 (9.7%) *R. rattus* from Argentina were found positive for whipworms. The prevalence, mean abundance, mean intensity and range of infections were: 13.4% (CI, 95% confidence intervals, 9.8–17.7%), 0.6 (CI 0.4–1.3), 4.7 (CI 3.1–9.7) and 1–54 in *M. musculus*, and 9.7% (CI 6.2–14.5%); 0.6 (CI 0.27–2.03); 7 (CI 3.25–19.95) and 1–70 in *R. rattus*.

In the following re-description, the ranges of measurements of *T. muris* are presented by combining the data from *M. musculus* and *R. rattus*. In addition, table 1 shows the main morphological features and measurements of *T. muris* with details of mean, SD and range in male and female specimens from each host species considered in the present study.

Table 1. Main morphological features and measurements (mean \pm standard deviation; range) of *Trichuris muris*.

Reference	Hall, 1916	Roman, 1951	Feliu <i>et al.</i> , 2000	Present study	Present study
Host	<i>Mus musculus</i> , <i>Rattus rattus</i> , <i>Arvicola amphibius</i> , <i>Apodemus sylvaticus</i> , <i>Mastomys natalensis</i> , <i>Microtus arvalis</i> , <i>Thrichomys apereoides</i> , <i>Holochilus physodes</i> , <i>Holochilus brasiliensis</i> , <i>Arvicanthis abyssinicus</i> , <i>Isothrix bistrata</i> , <i>Georychus capensis</i>	<i>A. sylvaticus</i> , <i>M. musculus</i>	<i>A. sylvaticus</i> , <i>Apodemus flavicollis</i> , <i>M. musculus</i>	<i>R. rattus</i>	<i>M. musculus</i>
Localities	France (Paris), Germany, Africa (Toro, Fort Portal)	France (Lyon)	Spain (Areu, Eugi, Montseny, Granollers, Py, Moulis)	Argentina (Corrientes)	Mexico (Yucatan)
Male (<i>n</i>)		Five <i>A. sylvaticus</i> ; five <i>M. musculus</i>	37	10	11
Body length (mm)	14–20	25.5 (19–33); 23.5 (19–28.5)	25.94 \pm 4.0 (18.4–33.6)	21.6 \pm 0.2 (16.6–24.4)	21.2 \pm 3.5 (16.4–26.6)
Anterior portion of body (mm)	12.5	16.5 (12.5–21.5); 14.5 (12–17.5)	16.9 \pm 2.7 (10.0–22.3)	13.5 \pm 1.2 (10.3–14.6)	13.3 \pm 1.8 (10.1–15.3)
Posterior portion of body (mm)	7.5	9 (6.5–11.5); 9 (8.5–11)	9.1 \pm 1.7 (5.8–11.8)	8.1 \pm 1 (6.3–9.8)	7.9 \pm 1.8 (5.4–11.3)
Anterior width	30	15.5 (13–19); 13.5 (13–14.5)	110 \pm 10 (9–113)	68 \pm 22 (50–100)	98 \pm 11 (80–123)
Oesophagus–intestine junction width		240 (230–255); 240 (195–285)	230 \pm 30 (118–290)	252 \pm 39 (200–300)	254 \pm 33 (200–300)
Maximum posterior width		415 (360–460); 420 (360–520)	350 \pm 50 (250–440)	399 \pm 40 (350–470)	412 \pm 31 (350–475)
Total oesophagus (mm)				13.5 \pm 1.2 (10.3–14.6)	13.3 \pm 1.8 (10.1–15.3)
Muscular portion				504 \pm 74 (370–600)	702 \pm 294 (475–1480)
Stichosoma portion (mm)				12.9 \pm 1.2 (9.8–14.0)	12.6 \pm 1.9 (9.6–14.8)
Spicule length	0.76	775 (600–860); 670 (480–835)	830 \pm 110 (580–990)	636 \pm 84 (540–760)	720 \pm 53 (570–775)
Spicule width at the base		22.5 (16–27); 18 (16–23.5)	30 \pm 1 (20–40)		31 \pm 7 (20–41)
Proximal cloacal tube			1380 \pm 280 (800–2180)	1100 \pm 300 (700–1700)	1100 \pm 200 (860–1600)
Distal cloacal tube			–	473 \pm 102 (300–600)	402 \pm 107 (263–580)
Body end–testis distance (mm)			1.4 \pm 0.40 (0.5–2.2)		1.5 \pm 0.4 (0.9–2.2)
Ratio anterior–posterior of body	05:03	1.9 (1.8–2.0); 1.6 (1.5–1.6)	1.9 \pm 0.3 (1.1–2.5)	1.7 \pm 0.1 (1.4–1.9)	1.7 \pm 0.2 (1.4–2.2)
Ratio total length–posterior of body				2.6 \pm 0.1 (2.4–2.9)	2.7 \pm 0.2 (2.4–3.2)
Ratio posterior of body–spicular length				13.3 \pm 2.3 (11.2–18.2)	10.9 \pm 2.0 (8.2–14.6)
Ratio proximal–distal–cloacal tube length				2.3 \pm 0.8 (1.3–4)	3.0 \pm 0.7 (2.1–4.7)
Female (<i>n</i>)		Five <i>A. sylvaticus</i> ; one <i>M. musculus</i>	16	7	8
Body length (mm)	23–31	33.5 (29–40); 27.5	35.4 \pm 6.1 (27.4–50.5)	28.2 \pm 3.6 (23.5–34.1)	24.1 \pm 2.5 (20.8–27.7)
Anterior portion of body (mm)	14–20	21 (16.5–23.5); 15.5	21.9 \pm 4.249 (15.495–31.9)	16.9 \pm 2.0 (13.9–18.9)	15.2 \pm 1.4 (13.4–17.9)
Posterior portion of body (mm)	8–11	13.5 (11–17); 12	13.5 \pm 2.2 (10.1–18.6)	11.2 \pm 2.0 (9.5–15.2)	8.8 \pm 1.4 (6.8–10.8)

(Continued)

Table 1. (Continued.)

Reference	Hall, 1916	Roman, 1951	Feliu <i>et al.</i> , 2000	Present study	Present study
Anterior width			120 ± 10 (100–130)	51 ± 7 (40–60)	105 ± 13 (94–128)
Oesophagus–intestine junction width		275 (255–295); 210	240 ± 20 (210–290)	215 ± 48 (150–290)	228 ± 21 (195–252)
Maximum posterior width	–400	530 (195–585); 540	490 ± 60 (390–580)	446 ± 10 (300–600)	488 ± 36 (426–540)
Total oesophagus (mm)				16.9 ± 2.0 (13.9–18.9)	15.2 ± 1.4 (13.4–17.9)
Muscular portion				602.8 ± 68 (530–740)	850 ± 197 (600–1175)
Stichosoma portion (mm)				16.3 ± 2.0 (13.3–18.3)	14.6 ± 1.5 (12.5–17.0)
Distance between oesophagus–intestine and vulva	-	100 (50–140); 35	10 ± 3 (40–150)		8.5 ± 19 (0–15)
Vagina length	-		780 ± 180 (450–1110)		
Body end–seminal receptacle distance			320 ± 240 (120–990)		
Body end–anus distance			30 ± 5 (10–30)		
Eggs length	57–62	56.5 (46.5–65); 64.5 (56.5–71)	65 ± 2 (62–68)	68.3 ± 6.8 (60–80)	60 ± 2 (57–63)
Eggs width		27.5 (24.5–30.5); 31 (29–35)	31 ± 1 (28–32)	34 ± 3.7 (30–40)	30 ± 1 (28–32)
Ratio anterior–posterior of body	07:04		1.6 ± 0.2 (1.3–1.9)	1.5 ± 0.2 (1.2–1.8)	1.8 ± 0.2 (1.4–2.1)
Ratio total length–posterior of body				2.5 ± 0.2 (2.2–2.8)	2.8 ± 0.2 (2.4–3.1)

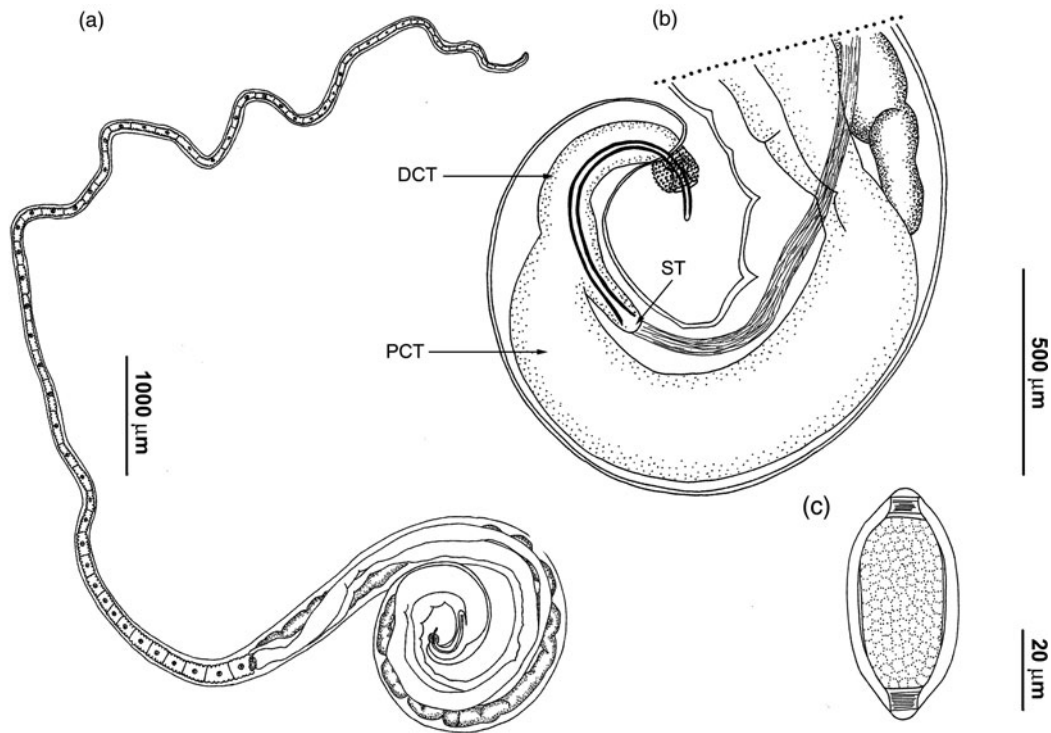


Figure 1. Drawings of *Trichuris muris*. (a) Complete male specimen; (b) male, posterior end, spicular tube (ST), spicule and proximal (PCT) and distal cloacal tube (DCT), spiny spicular sheath, lateral view; (c) egg.

Re-description: morphological and biometrical results

Diagnosis of *T. muris* (Schrank, 1788) Hall, 1916 (figs 1 and 2)

Anterior part of body long, narrow, tapered and whip-like; posterior part of body broad and handle-like (fig. 1a). Cuticle with fine transverse striations. Bacillary band located laterally in anterior portion of body. These structures are limited laterally to abundant and visible bacillary glands with conspicuous pores (fig. 2a). Stichosome with one row of stichocytes and one pair of conspicuous cells at oesophagus–intestine junction level (fig. 2d). Male with spicular tube. Proximal cloacal tube united laterally to distal cloacal tube (fig. 1a, b). Proximal cloacal tube is two to three times wider than the distal cloacal tube (fig. 1b). Sperm can usually be seen. Spicular sheath is spinous cylindrical forming a distal bulb. Spines evenly distributed, more scattered on the bulb portion (figs 1b and 2b, c). Testis ends near final third of distal cloacal tube, showing different degree of convolutions (fig. 1b). Cloaca subterminal with one pair of paracloacal papillae not ornamented. Female with non-protrusive vulva located at oesophagus–intestine junction level (fig. 2d). Anus subterminal with long caudal end.

Males (based on 21 specimens): body length 16.4–26.6 mm, anterior portion of body 10.1–15.3 mm long, posterior portion of body 5.4–11.3 mm long. Anterior body width 50–123, maximum posterior body width 350–475, width at oesophagus–intestine junction 200–300. Total length of oesophagus 10.1–15.3 mm, muscular portion 370–1480 long; stichosoma portion 9.6–14.8 mm long. Spicular length 540–775. Proximal cloacal tube 700–1700 long; distal cloacal tube 263–600 long. Ratio between anterior–posterior portion 1.4–2.2. Ratio between total body length and posterior portion length 2.4–3.2. Ratio between

posterior portion length and spicular length 8.2–18.2. Ratio between proximal cloacal tube length and distal cloacal tube length 1.3–4.7.

Females (based on 15 specimens): body length 20.8–34.1 mm, anterior portion of body 13.4–18.9 mm long, posterior portion of body 6.8–15.2 mm long. Anterior body width 40–128, maximum posterior body width 300–600, width at oesophagus–intestine junction 150–290. Total length of oesophagus 13.4–18.9 mm, muscular portion 530–1175 long; stichosoma portion 12.5–18.3 mm long. Distance between oesophagus–intestine junction and vulva 0–15. Eggs oval with bipolar plugs; 57–80 length by 28–40 width (fig. 1c). Ratio between anterior portion and posterior portion of body 1.2–2.1. Ratio between total body and posterior portion length 2.2–3.1.

Taxonomic summary

Hosts: *M. musculus* Linnaeus, 1758 and *R. rattus* Linnaeus, 1758.

Localities: Molas (20°48'55"N and 89°37'55"W) and Paraíso (20°40'34.4"N and 90°06'542"W), Yucatan, Mexico; (27°28'04.2"S and 58°50'03.8"W) Corrientes, Argentina.

Site of infection: caecum.

Voucher specimens: MLP-He 7427 and CNHE 10703, Mexico; UNNEPHEL 166, Argentina.

Differential diagnosis

Trichuris muris was compared with 29 congeners described from North and South American rodents (Barker, 1915; Chandler, 1945, 1946; Tiner, 1950; Cameron & Reesal, 1951; Morini *et al.*,

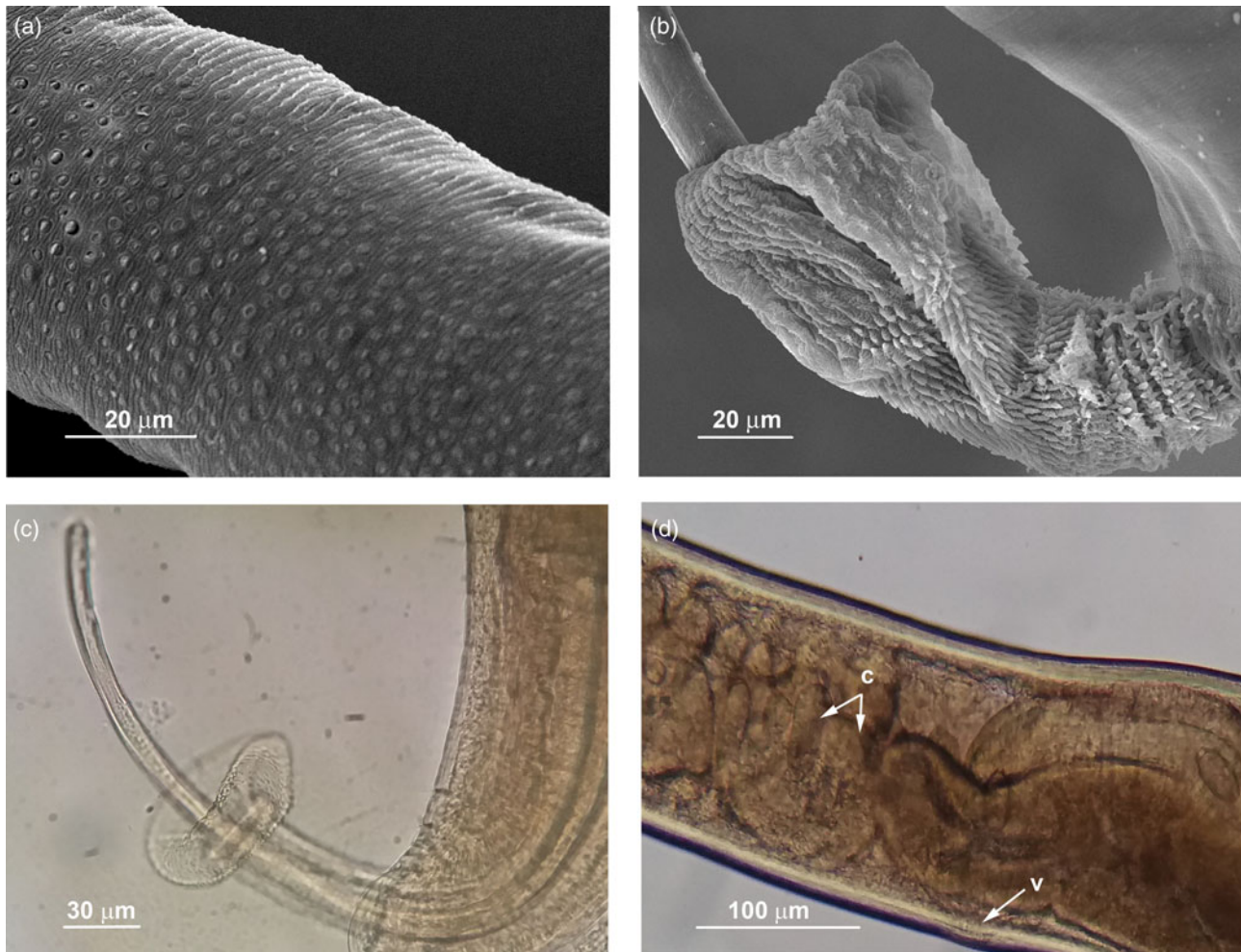


Figure 2. Scanning electron micrographs of *Trichuris muris*: (a) bacillary band, with detail of bacillary glands; and (b) male, detail of spiny distribution on distal portion of spicular sheath (collapsed bulb). Optical microscope micrographs of *T. muris*: (c) male, detail of the distal portion of spiny spicular sheath, forming a distal bulb; and (d) female, oesophagus-intestine junction, one pair of cells (c) and vulva (v), lateral view.

1955; Read, 1956; Frandsen & Grundmann, 1961; Todd & Lepp, 1972; Babero et al., 1975, 1976; Barus et al., 1975; Babero & Murua, 1987, 1990; Pfaffenberger & Best, 1989; Correa-Gomes et al., 1992; Suriano & Navone, 1994; Robles et al., 2006, 2014, 2018; Robles, 2011; Torres et al., 2011; Panti-May & Robles, 2016; Eberhardt et al., 2019; Falcón-Ordaz et al., 2020).

Trichuris muris differs from *Trichuris bradleyi* Babero, Cattán & Cabello, 1975; *Trichuris chilensis* Babero, Cattán & Cabello, 1976; *Trichuris elatoris* Pfaffenberger & Best, 1989; *Trichuris robusti* Babero & Murua, 1990; *Trichuris travassosi* Correa-Gomes, Lanfredi, Pinto & Souza, 1992; *Trichuris bursa-caudata* Suriano & Navone, 1994; *Trichuris pampeana* Suriano & Navone, 1994; *Trichuris pardinasi* Robles, Navone & Notarnicola, 2006; *Trichuris navonae* Robles & Navone, 2006; *Trichuris bainaie* Robles, Cutillas, Panei & Callejon, 2014; and *Trichuris massoi* Robles, Cutillas & Callejon, 2018 due to the presence of a spicular tube (in these 11 species, the spicule lies entirely within the distal cloacal tube).

Among the 16 species with a spicular tube (*Trichuris opaca* Barker & Noyes, 1915; *Trichuris fossor* Hall, 1916; *Trichuris myocastoris* Enigk, 1933; *Trichuris citelli* Chandler, 1945; *Trichuris neotomae* Chandler, 1945; *Trichuris perognathi* Chandler, 1945; *Trichuris peromysci* Chandler, 1946; *Trichuris madisonensis*

Tiner, 1950; *Trichuris dipodomis* Read, 1956; *Trichuris stansburyi* Frandsen & Grundmann, 1961; *Trichuris fulvi* Babero & Murua, 1987; *Trichuris laevitesti* Suriano & Navone, 1994; *Trichuris thrichomysi* Torres, Nascimento, Menezes, Garcia, dos Santos, Maldonado Jr, Miranda, Lanfredi & de Souza, 2011; *Trichuris silviae* Panti-May & Robles, 2016; *Trichuris cutillasae* Eberhardt, Robles, Monje, Beldomenico & Callejón, 2019; and *Trichuris guanacastei* Falcón-Ordaz, Monzalvo-López & García-Prieto, 2020) different patterns can be observed.

The spicular tube is a pouch containing the proximal part of the spicule, while the last portion of spicule is included in the distal cloacal tube. Three types of spicular tube can be interpreted: (a) inconspicuous contains more than a 70% of the spicule in species such as *T. fulvi* and *T. laevitesti*; (b) conspicuous short and thick, containing less or equal 40% of the spicule in species such as *T. fossor*, *T. citelli*, *T. madisonensis*, *T. cutillasae* and *T. muris*; and (c) conspicuous, containing about 40–60% of the spicule such as *T. peromysci*, *T. stansburyi* and *T. thrichomysi*.

With this considered, *T. muris* can be distinguished from other *Trichuris* species for having the shortest spicule (540–775). Also, *T. muris* can be separated from *T. cutillasae*, *T. stansburyi*, *T. thrichomysi*, *T. madisonensis*, *T. fulvi*, *T. fossor*, *T. citelli*, *T. laevitesti*, *T. perognathi*, *T. neotomae*, *T. peromysci* and *T. dipodomis*, by

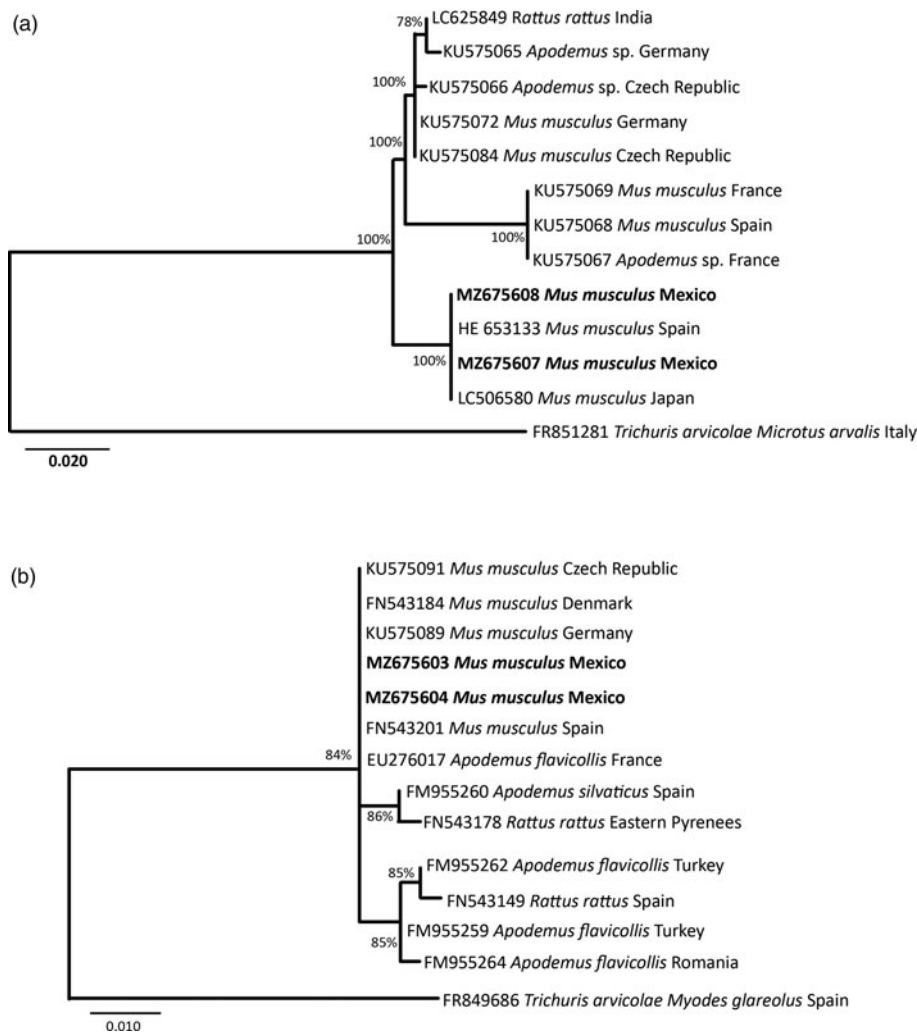


Figure 3. Phylogenetic tree of *Trichuris muris* from rodents of different geographical areas inferred using maximum likelihood based on: (a) the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene; and (b) the ribosomal internal transcribed spacer 2 (ITS2) regions (maximum composite likelihood). The newly generated sequences are highlighted in boldface type.

having a spicular sheath with a spiny distal spherical bulge. *Trichuris muris* has a shorter distal cloacal tube than *T. cutillasae*, *T. thrichomyisi*, *T. silviae*, *T. myocastoris*, *T. fossor*, *T. citelli* and *T. laevitesticis*. Moreover, *T. muris* has a non-protrusive vulva similar to most of the species of *Trichuris* mentioned, while other species present a slightly protruding vulva, with a cuticular evagination or lips protruding as *T. stansburyi*, *T. bainaie*, *T. chilensis* and *Trichuris gracilis*. The males of *T. gracilis* and *Trichuris dolichotis* have not been described and the females of these species can be distinguished from *T. muris* by the dimensions of the body length, as well as the lengths of the anterior and posterior portions of the body.

Molecular analysis

The *cox1* mtDNA and the ITS2 rDNA encoding gene revealed two haplotypes, each one of *T. muris* from Mexico, which were submitted to GenBank under accession numbers MZ675607 and MZ675608 (*cox1*) and MZ675603 and MZ675604 (ITS2).

The aligned data set of the *cox1* gene had a length of 324 base pairs (bp) and included 12 *T. muris* sequences from different parts of the world. Although the *T. muris* haplotypes from Mexico showed different relationships between them in each phylogenetic analysis (ML and BI), in both cases the haplotypes obtained in this survey from *M. musculus* can be grouped together

with a *M. musculus* haplotype from Japan and another from Spain (figs 3a and 4a).

The aligned data set of the ITS2 region had a length of 386 bp and included 13 *T. muris* sequences from different parts of the world. In each phylogenetic analysis (ML and BI), the relationships between the haplotypes did not indicate relevant differences and in both cases those of *T. muris* from Mexico were observed as branches of a polytomy, together with haplotypes from other host species and areas (figs 3b and 4b).

Discussion

The identification of the cosmopolitan nematode *T. muris* that parasitizes commensal and wild rodents required an integrative taxonomy approach (morphological features and molecular data). This study provides a relevant base for further taxonomic research related to other *Trichuris* species, especially for detecting possible differences in diagnostic characters. An example of such differences is how the morphometric features of *T. muris* were wider in the re-description. Although *T. muris* had been mentioned as parasitizing *Rattus* species in some studies (e.g. Mafiana *et al.*, 1997; Smales, 2016), until now only Hall (1916) had provided some measurements but without mentioning whether *R. rattus* had been the symbiotype of the specimen studied.

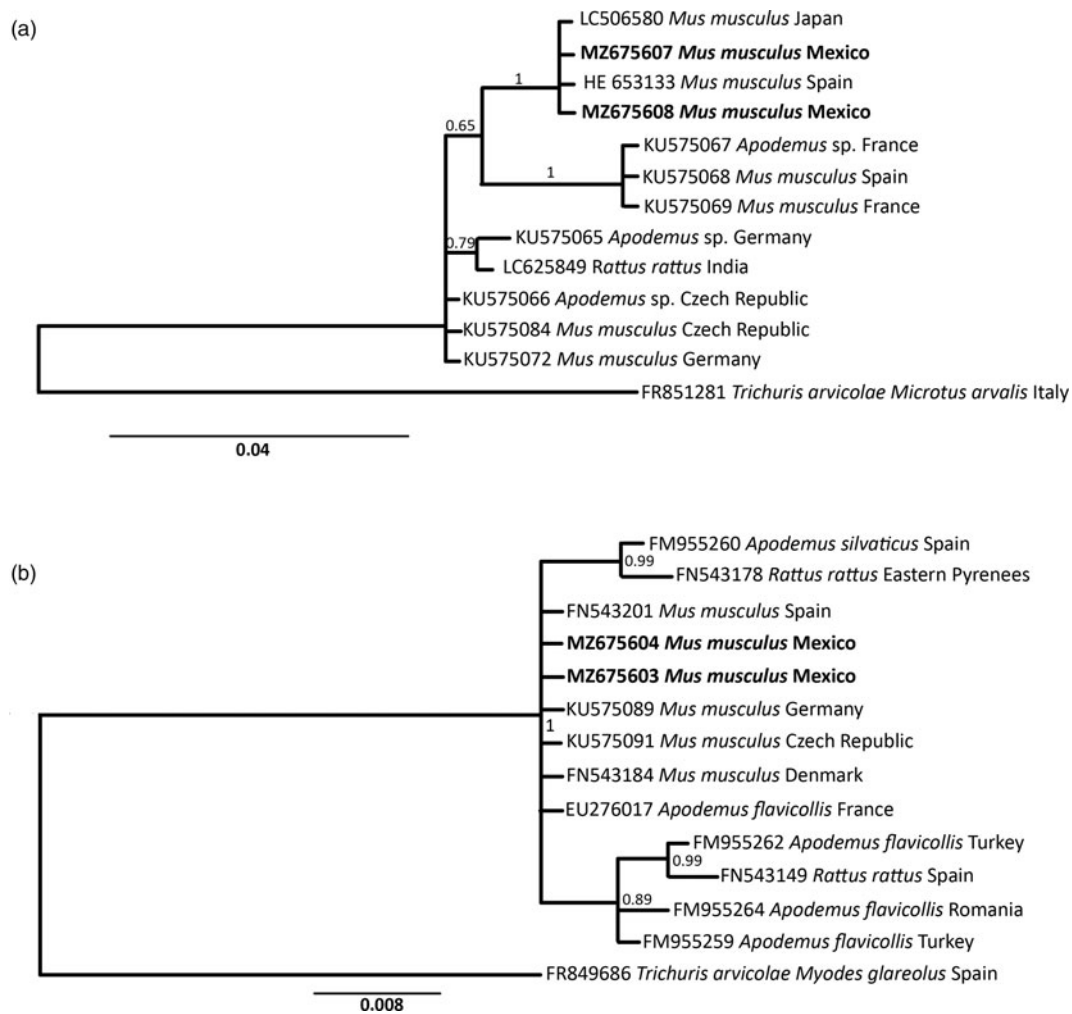


Figure 4. Phylogenetic tree of *Trichuris muris* from rodents of different geographical areas inferred using Bayesian inference based on: (a) the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene; and (b) the ribosomal internal transcribed spacer 2 (ITS2) regions. The newly generated sequences are highlighted in boldface type.

In this paper a characterization of different patterns of spicular tube is provided, which will facilitate contributions to the separation of species of this genus in further surveys. This feature is a structure that is often mentioned and described in several studies of *Trichuris*, although not in all. In order to suggest the corresponding structural pattern, the measurements and drawings of the original papers were considered (e.g. Chandler, 1945; Tiner, 1950; Babero & Murua, 1987). Using this information, three types of spicular tube patterns can be interpreted and serve to classify *Trichuris* species into three groups. Considering that the diagnosis among the species of this genus is mainly based on morphometry, this proposal represents an important contribution to the field.

Despite being one of the most recognized commensal rodent nematodes, genetic data for *T. muris* is lacking in some regions of the world. Here we provide molecular studies on two markers, the results of which were added to the available sequences of the species and represent the first sequences of *T. muris* from the American continent. Unfortunately, since the parasite specimens of *T. muris* from *R. rattus* found in Argentina were conserved in formol they could not be studied molecularly.

This work provides morphological and molecular data of *T. muris* from commensal rodents in North and South America, contributing to its integrative taxonomic characterization.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0022149X23000160>.

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Competing interests. None.

Ethical standards. All applicable institutional national and international guidelines for the care and use of animals were followed. The bioethics

committees for the use of animals from the Campus de Ciencias Biológicas y Agropecuarias, Yucatan, Mexico (protocol number CB-CCBA D-2016-002) and National Agency for the Promotion of Science and Technology and National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina approved the protocols used in this study.

References

- Babero BB and Murua RB** (1987) The helminth fauna of Chile. X. A new species of whipworm from a Chilean rodent. *Transactions of the American Microscopical Society* **106**(2), 190–193.
- Babero BB and Murua RB** (1990) A new species of whipworm from a South American hystricomorph rodent. *Memórias do Instituto Oswaldo Cruz* **85** (2), 211–213.
- Babero BB, Cattán PE and Cabello C** (1975) *Trichuris bradleyi* sp. n., a whipworm from *Octodon degus* in Chile. *Journal of Parasitology* **61**(6), 198–206.
- Babero BB, Cattán PE and Cabello C** (1976) A new species of whipworm from the rodent *Akodon longipilis* in Chile. *Transactions of the American Microscopical Society* **95**(2), 232–235.
- Barker FD** (1915) Parasites of the American muskrat (*Fiber zibethicus*). *Journal of Parasitology* **1**(4), 184–197.
- Barus V, Madjumdar G and Mikailov TK** (1975) Morphology and taxonomy of *Trichocephalus myocastoris* (Enigk, 1933). *Folia Parasitologica* **22**(3), 207–213.
- Boren JC, Lochmiller RL, Boggs JE and Leslie DM** (1993) Gastrointestinal helminths of eastern woodrat populations in Central Oklahoma. *Southwestern Naturalist* **38**(2), 146–149.
- Bush A, Lafferty KD, Lotz JM and Shostak W** (1997) Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**(4), 575–583.
- Callejón R, De Rojas M, Nieberding C, Foronda P, Feliú C, Guevara D and Cutillas C** (2010) Molecular evolution of *Trichuris muris* isolated from different Muridae hosts in Europe. *Parasitology Research* **107**(3), 631–641.
- Callejón R, Nadler S, De Rojas M, Zurita A, Petrášová J and Cutillas C** (2013) Molecular characterization and phylogeny of whipworm nematodes inferred from DNA sequences of *cox1* mtDNA and 18S rDNA. *Parasitology Research* **112**(11), 3933–3949.
- Callejón R, Robles MR, Panei CJ and Cutillas C** (2016) Molecular diversification of *Trichuris* spp. from Sigmodontinae (Cricetidae) rodents from Argentina based on mitochondrial DNA sequences. *Parasitology Research* **115**(8), 2933–2945.
- Cameron TWM and Reesal MR** (1951) Studies on the endoparasitic fauna of Trinidad mammals. *Canadian Journal of Zoology* **29**(4), 276–289.
- Chandler AC** (1945) *Trichuris* species from California rodents. *Journal of Parasitology* **31**(4), 284–287.
- Chandler AC** (1946) *Trichuris peromysci* n. sp. from *Peromyscus californicus*, and further notes on *T. perognathi* Chandler, 1945. *Journal of Parasitology* **32**(1), 208.
- Correa-Gomes D, Lanfredi RM, Pinto RM and De Souza W** (1992) Description of *Trichuris travassosi* n. sp. (Nematoda: Trichurinae) from a Brazilian rodent, by light and scanning electron microscopy. *Memórias do Instituto Oswaldo Cruz* **87**(Suppl 1), 1–10.
- Darriba D, Taboada GL, Doallo R and Posada D** (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**(8), 772.
- Eberhardt AT, Robles MR, Monje LD, Beldomenico PM and Callejón R** (2019) A new *Trichuris* species (Nematoda: Trichuridae) from capybaras: morphological–molecular characterization and phylogenetic relationships. *Acta Tropica* **190**(1), 244–252.
- Falcón-Ordaz J, Monzalvo-López RJ and García-Prieto L** (2020) New species of *Trichuris* (Nematoda: Trichuridae) parasitizing *Heteromys salvini* (Rodentia: Heteromyidae) from Costa Rica, with a key to *Trichuris* species described from Heteromyidae. *Revista Brasileira de Parasitologia Veterinária* **29**(2), e022019.
- Fataliev G** (1983) *Trichocephalus* (*Trichuris*) species of commercial fur-bearing animals in the Small Caucasus and the adjoining Mil-sko-Karabakhsk steppe of the Azerbaijan SSR and their ecological characteristics. *Izvestiya Akademii Nauk Azerbaidzhanskoi SSR Biologicheskie Nauki* **3**(1), 57–61.
- Feliú C, Mas-Coma S and Gallego J** (1980) Contribución al conocimiento de la helmintofauna de micromamíferos ibéricos. VII. Parásitos de *Mus musculus* Linnaeus, 1758 y *Mus spretus* Lataste, 1883 (Rodentia: Muridae) [Contribution to the knowledge of the helminthofauna of Iberian small mammals. VII. Parasites of *Mus musculus* Linnaeus, 1758 and *Mus spretus* Lataste, 1883 (Rodentia: Muridae)]. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* **38**(2), 295–309. [In Spanish.]
- Feliú C, Spakulová M, Casanova JC, Renaud F, Morand S, Hugot JP, Santalla F and Durand P** (2000) Genetic and morphological heterogeneity in small rodent whipworms in southwestern Europe: characterization of *Trichuris muris* and description of *Trichuris arvicolae* n. sp. (Nematoda: Trichuridae). *Journal of Parasitology* **86**(3), 442–449.
- Frandsen JC and Grundmann AW** (1961) *Trichuris stansburyi* and *Gongylonema mysciphilia*, two new species of nematodes from the deer mouse in Utah. *Proceedings of the Helminthological Society of Washington* **28**(1), 91–94.
- Gasser RB, Chilton NB, Hoste H and Beveridge I** (1993) Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic Acids Research* **21**(10), 2525–2526.
- Genov T** (1984) *Helminths of insectivorous mammals and rodents in Bulgaria*. Sofia, Publication House of the Bulgarian Academy of Sciences.
- Gómez Muñoz MA, Robles MR, Milano AMF and Navone GT** (2018) Helminth infection levels on *Rattus rattus* (Rodentia: Muridae) from Corrientes city, Argentina. *Mastozoología Neotropical* **25**(1), 221–227.
- Hall MC** (1916) Nematode parasites of mammals of the orders Rodentia, Lagomorpha, and Hyracoidea. *Proceedings of the United States National Museum* **50**(2131), 1–258.
- Hurst RJ and Else KJ** (2013) *Trichuris muris* research revisited: a journey through time. *Parasitology* **140**(11), 1325–1339.
- Ibrahim MA, Ogunsusi RA, Nwude N and Aliu Y** (1984) Helminths of the African giant rat (*Cricetomys gambianus* Waterhouse) in Zaria, Nigeria. *Revue d'Élevage et de Médecine Vétérinaire des pays Tropicaux* **37**(3), 304–307.
- Kumar S, Stecher G, Li M, Knyaz C and Tamura K** (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**(6), 1547–1549.
- Mafiana CF, Osho MB and Sam-Wobo S** (1997) Gastrointestinal helminth parasites of the black rat (*Rattus rattus*) in Abeokuta, southwest Nigeria. *Journal of Helminthology* **71**(3), 217–220.
- Morini EG, Boero J and Rodriguez A** (1955) Parásitos intestinales en el 'Marra' (*Dolichotis patagonum patagonum*) [Intestinal parasites in the 'Marra' (*Dolichotis patagonum patagonum*)]. *Publicación Mision de Estudios de Patología Regional* **26**(1), 83–89. [In Spanish.]
- Panti-May JA and Robles MR** (2016) A new species of *Trichuris* Roederer, 1761 (Nematoda: Trichuridae) from *Heteromys gaumeri* Allen and Chapman (Rodentia: Heteromyidae) in Yucatan, Mexico. *Systematic Parasitology* **93**(7), 721–730.
- Panti-May JA, Duarte-Jiménez A, Hernández-Betancourt SF and Rodríguez-Vivas RI** (2021) A checklist of the helminth parasites of invasive murid rodents in Mexico. *Therya* **12**(1), 85–93.
- Pfaffenberger GS and Best T** (1989) *Trichuris elatoris* sp. n. (Nematoda: Trichuridae) from the Texas kangaroo rat (*Dipodomys elator*). *Proceedings of the Helminthological Society of Washington* **56**(1), 76–81.
- Preisser WC and Falcon-Ordaz J** (2019) A checklist of the parasitic helminths of cricetid and heteromyid rodents in Mexico. *Therya* **10**(3), 329–334.
- Prokopic J and Genov T** (1984) Distribution of helminths in micro-mammals (Insectivora and Rodentia) under different ecological and geographical conditions. *Academia Praha* **9**(1), 1–159.
- Read CP** (1956) *Trichuris dipodomis* n. sp., from Ord's kangaroo rat. *Proceedings of the Helminthological Society of Washington* **23**(1), 119.
- Ribas A, Wells K, Morand S, Chaisiri K, Agatsuma T, Lakim MB, Yuh Tuh FY and Saijuntha W** (2020) Whipworms of south-east Asian rodents are distinct from *Trichuris muris*. *Parasitology International* **77**(1), 102–128.
- Robles MR** (2011) New species of *Trichuris* (Nematoda: Trichuridae) from *Akodon montensis* Thomas, 1913 of the Paranaense forest in Argentina. *Journal of Parasitology* **97**(2), 319–327.

- Robles MR, Navone GT and Notarnicola J** (2006) A new species of *Trichuris* (Nematoda: Trichuridae) from Phyllotini Rodents in Argentina. *Journal of Parasitology* **92**(1), 100–104.
- Robles MR, Cutillas C, Panei CJ and Callejón R** (2014) Morphological and molecular characterization of a new *Trichuris* species (Nematoda-Trichuridae), and phylogenetic relationships of *Trichuris* species of cricetid rodents from Argentina. *PLoS One* **9**(11), e112069.
- Robles MR, Cutillas C and Callejón R** (2018) Morphological–molecular characterization and phylogenetic relationships of a new *Trichuris* species (Nematoda: Trichuridae) parasitic on *Holochilus chacarius* (Cricetidae: Sigmodontinae) from the Chaco ecoregion (Argentina). *Infection, Genetics and Evolution* **58**(1), 66–76.
- Roman E** (1951) Etude écologique et morphologique sur les Acanthocéphales et les Nématodes parasites des rats de la région Lyonnaise [Ecological and morphological study on Acanthocephala and parasitic Nematodes of rats in the Lyon region]. *Mémoires du Muséum National d'Histoire Naturelle Série A Zoologie* **2**(2), 49–270. [In French.]
- Ronquist F and Huelsenbeck JP** (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**(12), 1572–1574.
- Schrank FP** (1788) Verzeichniß der bisher hinlänglich bekannten Eingeweidewürmer, nebst einer Abhandlung über ihre Anverwandtschaften, pp. [1–12], 1–116, 1 Tabelle. München. (Strobl). [In German.]
- Skryabin KI, Shikhobalova NP and Orlov YV** (1957) Trichocephalidae and Capillariidae of animals and man and the diseases caused by them. *Osnovy Nematologii* **6**(1), 496. [In Russian.]
- Smales LR** (2016) The gastrointestinal helminths of *Rattus niobe* (Rodentia: Muridae) with descriptions of two new genera and three new species (Nematoda) from Papua New Guinea and Papua Indonesia. *Zootaxa* **4117**(2), 183–197.
- Suriano DM and Navone GT** (1994) Three new species of the genus *Trichuris* Roederer, 1761 (Nematoda: Trichuridae) from Caviomorph and Cricetid rodents in Argentina. *Research and Reviews in Parasitology* **54**(1), 39–46.
- Thompson JD, Higgins DG and Gibson TJ** (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**(22), 4673–4680.
- Tiner JD** (1950) Two new species of *Trichuris* from North America with description of *Trichuris leporis* (Nematoda: Aphasmodia). *Journal of Parasitology* **36**(4), 350–354.
- Todd SK and Lepp LD** (1972) Redescription of *Trichuris fossor* Hall, 1916 (Nematoda: Trichuridae) from the Northern Pocket Gopher, *Thomomys talpoides*. *Proceedings of the Helminthological Society of Washington* **39**(2), 203–205.
- Tomasovicova O, Corba J, Spaldonova R and Bondy R** (1988) The susceptibility of different inbred strains of mice to *Trichocephalus muris* infection. *Helminthologia* **25**(1), 273–277.
- Torres E JL, Nascimento APF, Menezes AO, Garcia J, dos Santos MAJ, Maldonado A, Miranda K, Lanfredi RM and de Souza W** (2011) A new species of *Trichuris* from *Thrichomys apereoides* (Rodentia: Echimyidae) in Brazil: Morphological and histological studies. *Veterinary Parasitology* **176**(2–3), 226–235.
- Wakelin D** (1994) Genetic control of immunity to helminth infections. pp. 335–359. In Chowdhury N and Tada IJ (Eds) *Helminthology*. Berlin, Springer-Verlag.
- Wasimuddin JB, Ribas A, Baird SJE, Piálek J and De Bellocq JG** (2016) Testing parasite ‘intimacy’: The whipworm *Trichuris muris* in the European house mouse hybrid zone. *Ecology and Evolution* **6**(9), 2688–2701.
- Yamaguti S** (1961) *Systema helminthum: Volume II*. New York, Interscience Publishers Inc.
- Zhu XQ, Jacobs DE, Chilton NB, Sani RA, Cheng NABY and Gasser RB** (1998) Molecular characterization of a *Toxocara* variant from cats in Kuala Lumpur, Malaysia. *Parasitology* **117**(2), 155–164.