Journal of Helminthology

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

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Research Paper

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Cite this article: Panti-May JA, Gómez Muñoz MÁ, Yeh-Gorocica AB, Hernández-Betancourt S, Milano F, Galliari C, Robles MR (2023). Morphological and molecular characterization of *Trichuris muris* (Nematoda: Trichuridae): studies from two commensal rodent species. *Journal of Helminthology* **97**, e38, 1–10. https://doi.org/10.1017/S0022149X23000160

Received: 4 January 2023
Revised: 27 February 2023
Accepted: 13 March 2023

Keywords:
integrative taxonomy; North America; rodents; South America; whipworms

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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 (*cox*1) 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 Helmintos (CNHE) of the Instituto de Biologia, Universidad Nacional Autonoma de Mexico, 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 *cox*1 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 *cox*1; 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 *cox*1 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 *cox*1 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 *cox*1 and HKY + G with uniform rates for ITS2. Each one was performed with 1000 bootstrap repetitions for obtaining the best phylogenetic hypothesis of the *cox*1 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.

https://doi.org/10.1017/S0022149X23000160 Published online by Cambridge University Press

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

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

(*Continued*)

Table 1. (*Continued.*)

Reference	Hall, 1916	Roman, 1951	Feliu *et al.*, 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)

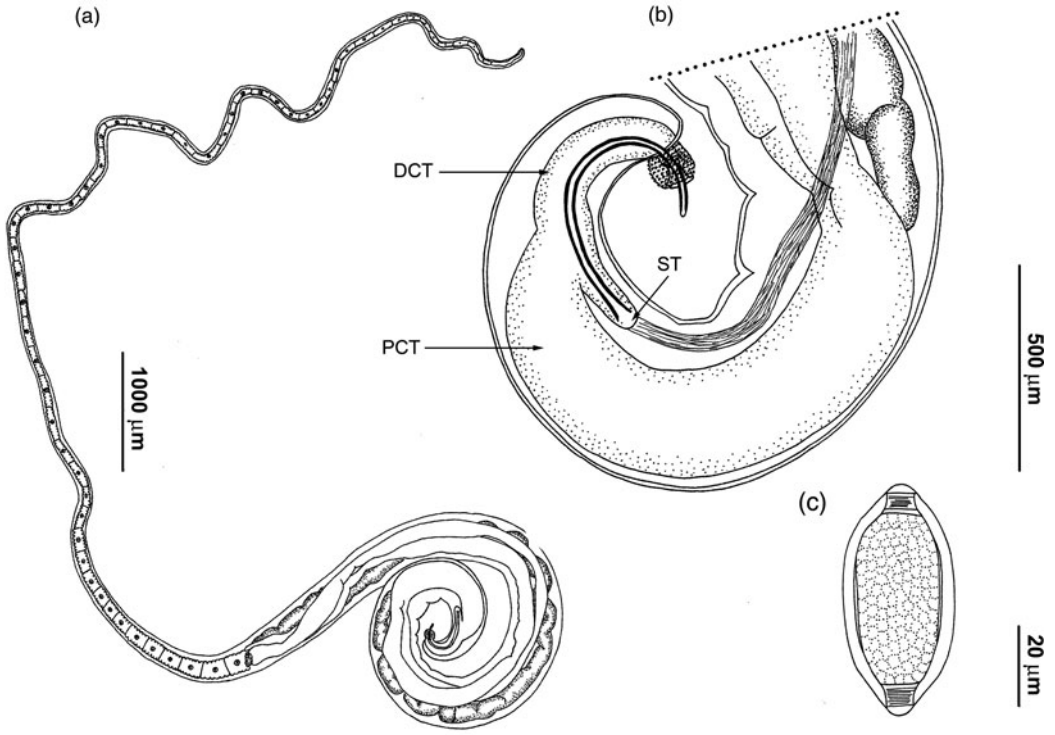

Figure 1. Drawings of *Trichuris muris*. (a) Complete male specimen; (b) male, posterior end, spicular tube (ST), spicule and proximal (PCT) and distal cloacal tube (DCT), spiny spicular sheath, lateral view; (c) egg.

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.*,

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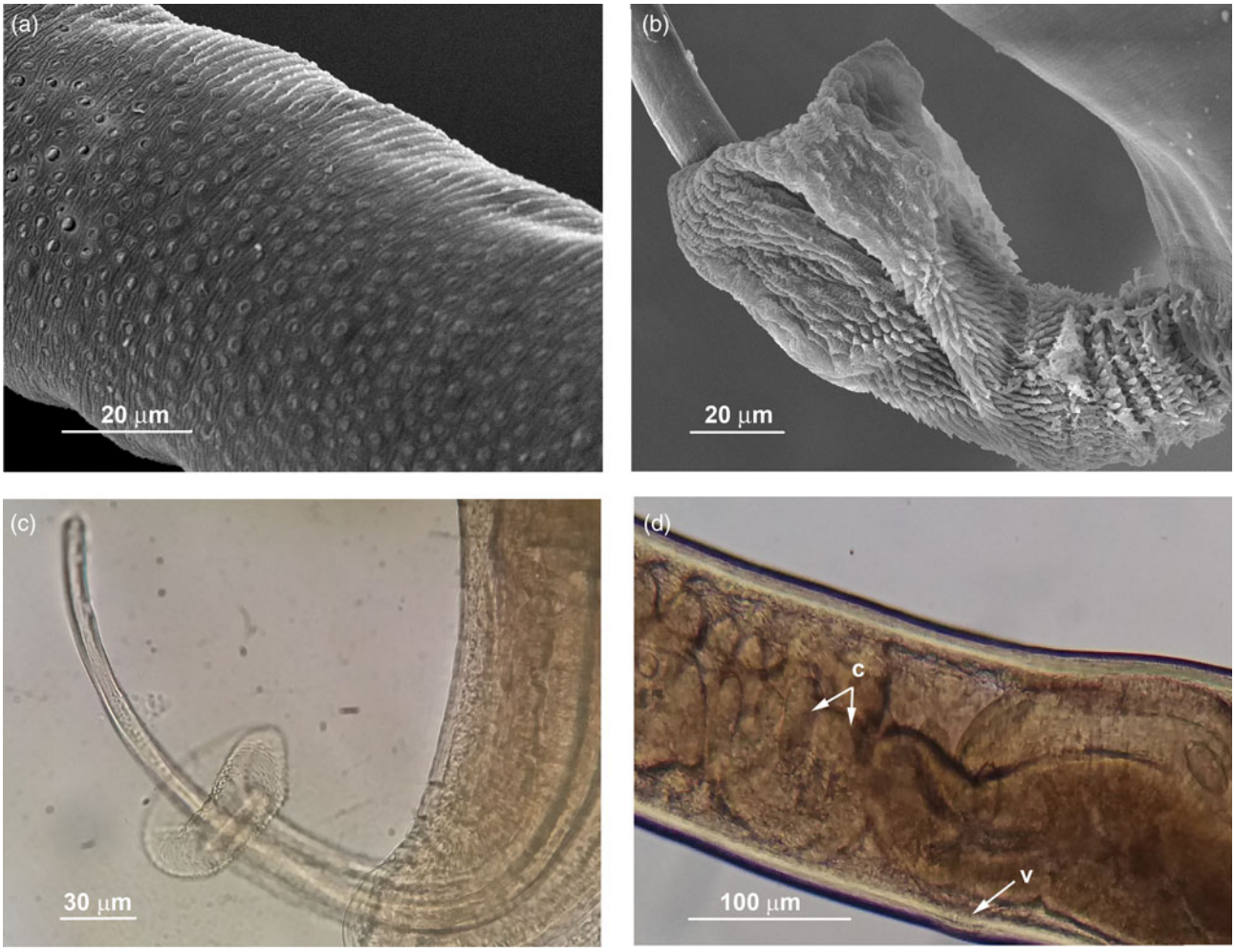

Figure 2. Scanning electron micrographs of *Trichuris muris*: (a) bacillary band, with detail of bacillary glands; and (b) male, detail of spiny distribution on distal portion of spicular sheath (collapsed bulb). Optical microscope micrographs of *T. muris*: (c) male, detail of the distal portion of 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, Cattan & Cabello, 1975; *Trichuris chilensis* Babero, Cattan & Cabello, 1976; *Trichuris elatoris* Pfaffenberger & Best, 1989; *Trichuris robusti* Babero & Murua, 1990; *Trichuris travassosi* Correa-Gomes, Lanfredi, Pinto & Souza, 1992; *Trichuris bursacaudata* Suriano & Navone, 1994; *Trichuris pampeana* Suriano & Navone, 1994; *Trichuris pardinasi* Robles, Navone & Notarnicola, 2006; *Trichuris navonae* Robles & Navone, 2006; *Trichuris bainae* Robles, Cutillas, Panei & Callejon, 2014; and *Trichuris massoiai* 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 laevitestis* 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. laevitestis*; (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. laevitestis*, *T. perognathi*, *T. neotomae*, *T. peromysci* and *T. dipodomis*, by

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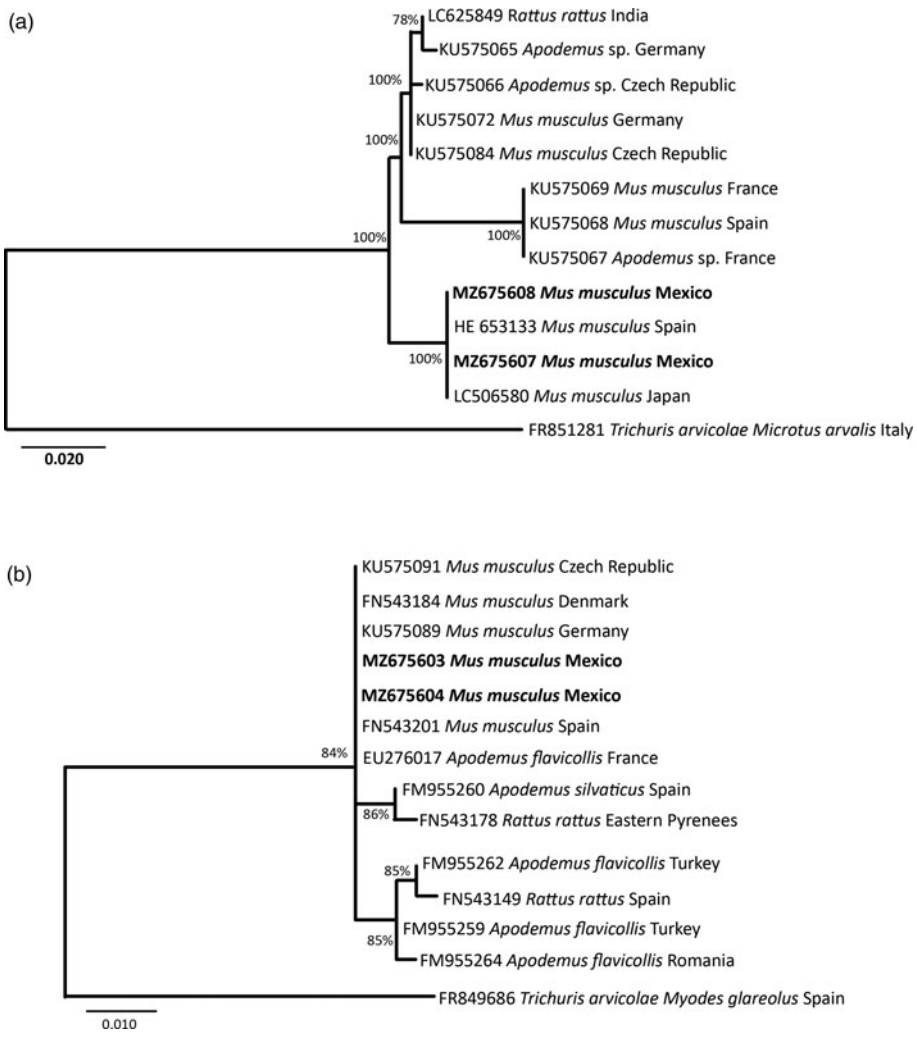

Figure 3. Phylogenetic tree of *Trichuris muris* from rodents of different geographical areas inferred using maximum likelihood based on: (a) the mitochondrial cytochrome c oxidase subunit 1 (*cox*1) gene; and (b) the ribosomal internal transcribed spacer 2 (ITS2) regions (maximum composite likelihood). 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. thrichomysi*, *T. silviae*, *T. myocastoris*, *T. fossor*, *T. citelli* and *T. laevitestis*. 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. bainae*, *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 (*cox*1) and MZ675603 and MZ675604 (ITS2).

The aligned data set of the *cox*1 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.

https://doi.org/10.1017/S0022149X23000160 Published online by Cambridge University Press

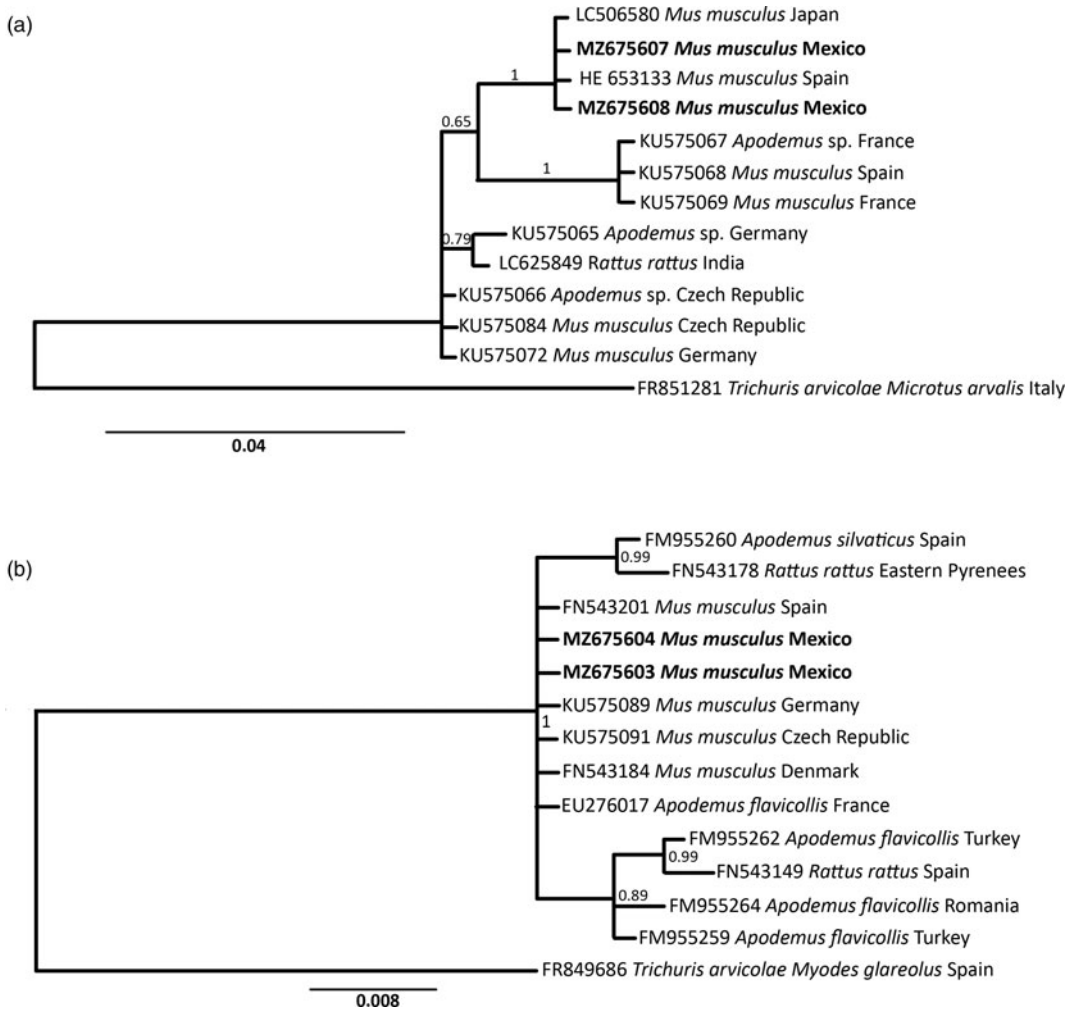

Figure 4. Phylogenetic tree of *Trichuris muris* from rodents of different geographical areas inferred using Bayesian inference based on: (a) the mitochondrial cytochrome c oxidase subunit 1 (*cox*1) gene; and (b) the ribosomal internal transcribed spacer 2 (ITS2) regions. The newly generated sequences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

Acknowledgements. We thank all of the students at the Facultad de Medicina Veterinaria y Zootecnia and Laboratorio Biología de los Parásitos for their support in the fieldwork in Yucatan, Mexico and Corrientes, Argentina, respectively. We also thank Andrea Servian for her assistance with the Bayesian analysis and Josh Taylor for checking the English writing of the manuscript.

Financial support. This project was partially funded by PROMEP-México-Proyecto 103.5/09/1258 (Red epidemiológica de enfermedades zoonóticas y transmitidas por vectores de importancia en salud pública) and by the General Secretary of Science and Technology of the Universidad Nacional del Nordeste (PI 16F/006) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

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 Biologicas 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.

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https://doi.org/10.1017/S0022149X23000160 Published online by Cambridge University Press

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