Infection, Genetics and Evolution 58 (2018) 66–76

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Morphological-molecular characterization and phylogenetic relationships of a new *Trichuris* species (Nematoda: Trichuridae) parasitic on *Holochilus chacarius* (Cricetidae: Sigmodontinae) from the Chaco ecoregion (Argentina)

María del Rosario Robles[a,*], Cristina Cutillas[b], Rocío Callejón[b]

[a] Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CCT- CONICET- La Plata/Universidad Nacional de La Plata, Bv. 120 e/61 y 64, La Plata 1900, Buenos Aires, Argentina
[b] Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Spain

ARTICLE INFO

Keywords:
Trichuris massoiai n. sp.
Holochilus chacarius
Sigmodontinae rodents
Argentina
Molecular characterization

ABSTRACT

A new *Trichuris* species isolated from *Holochilus chacarius* (Cricetidae: Sigmodontinae) from the Chaco ecoregion in Argentina is described based on morphological characteristics and mitochondrial (*cox1*, *cob*) and nuclear (ITS2) markers. The new species is distinguished from 27 species of *Trichuris* from North and South American rodents based on morphological and biometrical features, such as the absence of a spicular tube, presence of a cylindrical spicular sheath, non-protrusive vulva, length of spicule, and proximal and distal cloacal tube. In addition, the results based on three molecular markers of the new species and three *Trichuris* species previously analysed from sigmodontine rodents: *Trichuris pardinasi*, *Trichuris navonae* and *Trichuris bainae* confirmed that the specimens here studied belong to a different species. Molecular data are further used to discuss the phylogenetic relationships among the *Trichuris* species of rodents from Argentina. The combined analysis of mitochondrial genes (*cox1* and *cob*) revealed four clades corresponding with four different species of *Trichuris*. *T. navonae* (Akodontini rodents) and *T. massoiai* n. sp. as a sister group related to *T. bainae* (Oryzomyini rodents) and separated of *T. pardinasi* (Phyllotini rodents).

1. Introduction

Species of *Trichuris* Roederer, 1761 (Nematoda: Trichuridae) have a cosmopolitan distribution and parasitize a broad range of mammalian hosts, such as ruminants, marsupials, rodents, and primates, including humans (Cafrune et al., 1999; Anderson, 2000). The genus *Trichuris* includes species of medical and veterinary importance (e.g. *T. trichiura* (Linnaeus, 1771), *T. suis* (Schrank, 1788) and *T. vulpis* (Froelich, 1789)). In many countries, human *Trichuris* has acquired from or are shared with domestic animals (Hall and Sonnenberg, 1956; Vazquez et al., 1997; Mirdha et al., 1998). The study of *Trichuris* species from different hosts is relevant to know the genetic distances between them, and their possible relation with epidemiological risks (e.g., Oliveros et al., 2000; Cutillas et al., 2002, 2007, 2009).

To date, 27 *Trichuris* species have been described from 11 families of North and South American rodents. Only one species of *Trichuris* has been recorded in each of the families Caviidae, Dasyproctidae, Echimyidae, Geomyidae, Muridae, Myocastoridae, and Octodontidae, while two species have been described in Sciuridae, and four each in Heteromyidae and Ctenomyidae. Cricetidae are parasitized by the most species, with four species in Neotominae of North America and six species in Sigmodontinae of South America, of which four have been recorded from Argentina (Suriano and Navone, 1994; Robles et al., 2006, 2014; Robles, 2011; see Table 1).

Trichuris species from Sigmodontinae rodents present an interesting evolutionary history since these hosts are endemic of America. These rodents have a wide variety of environmental and geographical distribution, and they have been included in diverse phylogenetic hypotheses (e.g., Steppan et al., 2004; Cox and Hautier, 2015). Five out of 6 species of *Trichuris* from sigmodontine rodents present similar morphological features, such as the absence of a spicular tube, spicular sheath with spines (mostly with a cylindrical shape), and a non-protrusive or slightly protruding vulva (e.g. Babero et al., 1976; Correa-Gomes et al., 1992; Robles et al., 2006, 2014; Robles, 2011). In addition, mainly morphometric characters with discriminatory value have been used to separate *Trichuris* species, although in many cases with a high degree of overlap in their measurements (i.e., Schwartz, 1926; Chandler, 1930; Knight, 1984; Babero and Murúa, 1987; Babero and Murúa, 1990). For this reason, some studies have used isoenzymatic patterns and molecular studies to identify these nematodes (Cutillas

* Corresponding author.
E-mail address: rosario@cepave.edu.ar (M.d.R. Robles).

https://doi.org/10.1016/j.meegid.2017.11.029
Received 22 August 2017; Received in revised form 13 November 2017; Accepted 30 November 2017
Available online 12 December 2017

Table 1
Species of *Trichuris* described from 11 families of North and South American rodents.

Species	Author	Reference	Type host	Other hosts	Host family	Localities
T. muris	Schrank, 1788		*Rattus* spp.	–	Muridae	Cosmopolita
T. gracilis	(Rudolphi, 1819)	Cameron and Reesal, 1951	*Dasyprocta leporina*	–	Dasyproctidae	(Trinidad) (Brazil)
T. opaca	Barker & Noyes, 1915	Tiner, 1950	*Ondatra zibethicus cinnamominus*	*Microtus p. pennsylvanicus Ondatra z. zibethicus*	Cricetidae	Nebraska, Wisconsin, Ohio, Wyoming, Maryland (USA)
T. fossor	Hall, 1916	Chandler, 1945; Kenneth and Leep, 1972	*Thomomys talpoides*	*Thomomys bottae bottae*	Geomyidae	California, Wyoming (USA)
T. myocastoris	Enigk, 1933	Lent and Freitas, 1936; Barus et al., 1975; Correa-Gomes et al., 1992	*Myocastor coypus*	–	Myocastoridae	San Pablo (Brazil)
T. citelli	Chandler, 1945	Chandler, 1945	*Spermophilus beecheyi*	–	Sciuridae	California (USA)
T. perognathi	Chandler, 1945	Chandler, 1945	*Perognathus californicus californicus*	*Perognathus pennicillata*	Heteromyidae	California (USA)
T. neotomae	Chandler, 1945	Chandler, 1945	*Neotoma fuscipes*	–	Cricetidae	California (USA)
T. peromysci	Chandler, 1946	Chandler, 1946	*Peromyscus californicus*	–	Cricetidae	California (USA)
T. madisonensis	Tiner, 1950	Tiner, 1950	*Tamias striatus*	–	*Sciuridae*	Wisconsin (USA)
T. dolichotis	Morini et al., 1955	Morini et al., 1955	*Dolichotis patagonum*	–	Caviidae	Argentina
T. dipodomys	(Read, 1956)	Read, 1956	*Dipodomys ordii*	–	Heteromyidae	New Mexico, Utah (USA)
T. stansburyi	Frandsen and Grundmann, 1961	Frandsen and Grundmann, 1961	*Peromyscus maniculatus*	–	Cricetidae	Utah (USA)
T. bradleyi	Babero et al., 1975	Babero et al., 1975	*Octodon degus*	–	Octodontidae	Santiago (Chile)
T. chilensis	Babero et al., 1976	Babero et al., 1976	*Abrothrix longipilis*	–	Cricetidae	Santiago (Chile)
T. fulvi	Babero and Murúa, 1987	Babero and Murúa, 1987	*Ctenomys fulvus phillipiensis*	–	Ctenomyidae	San Pedro de Atacama (Chile)
T. elatoris	Pfaffenberger and Best, 1989	Pfaffenberger and Best, 1989	*Dipodomys elator*	–	Heteromyidae	Texas (USA)
T. robusti	Babero and Murúa, 1990	Babero and Murúa, 1990	*Ctenomys robustus*	–	Ctenomyidae	La Hauyca (Chile)
T. travassosi	Correa-Gomes et al., 1992	Correa-Gomes et al., 1992	*Oligoryzomys nigripes*	–	Cricetidae	Arvorezinha (Brazil)
T. laevitestis	Suriano and Navone, 1994	Suriano and Navone, 1994; Robles and Navone, 2006	*Scapteromys aquaticus*	*Akodon azarae*	Cricetidae	Buenos Aires (Argentina)
T. bursacaudata	Suriano and Navone, 1994	Suriano and Navone, 1994	*Ctenomys talarum*	–	Ctenomyidae	Buenos Aires (Argentina)
T. pampeana	Suriano and Navone, 1994	Suriano and Navone, 1994; Rossin and Malizia, 2005	*Ctenomys azarae*	*Ctenomys talarum*	Ctenomyidae	La Pampa, Buenos Aires (Argentina)
T. pardinasi	Robles et al., 2006	Robles et al., 2006	*Phyllotis xanthopygus*	–	Cricetidae	Buenos Aires, Córdoba (Argentina)
T. navonae	Robles, 2011	Robles, 2011	*Akodon montensis*	–	Cricetidae	Misiones (Argentina)
T. thrichomysi	Torres et al., 2011	Torres et al., 2011	*Thrichomys apereoides*	–	Echimyidae	Minas Gerais (Brazil)
T. bainae	Robles et al., 2014	Robles et al., 2014	*Sooretamys angouya*	–	Cricetidae	Misiones (Argentina)
T. silviae	Panti May and Robles, 2016	Panti May and Robles, 2016	*Heteromys gaumeri*	–	Heteromyidae	Yucatán (Mexico)

USA: United States of America.

et al., 1996, 2002, 2004, 2007; Feliú et al., 2000).

Among the molecular markers, the internal transcribed spacers 1 and 2 nuclear regions (ITS1 and ITS2 rDNA) (Oliveros et al., 2000; Cutillas et al., 2002, 2004, 2007, 2009, 2015; Callejón et al., 2012, 2016; Salaba et al., 2013; Robles et al., 2014; Doležalová et al., 2015), the nuclear 18S ribosomal RNA gene (Callejón et al., 2013; Guardone et al., 2013; Doležalová et al., 2015), mitochondrial 16S ribosomal RNA gene (Callejón et al., 2012) and protein-coding mitochondrial genes, including the 12 common genes obtained from mitochondrial genome sequences (Liu et al., 2012, 2013), and partial cytochrome *c* oxidase subunit I (*cox1*) (Callejón et al., 2013, 2016; Doležalová et al., 2015) and cytochrome *b* (*cob*) encoding genes (Cutillas et al., 2015; Callejón et al., 2015, 2016). These genes have different attributes and shortcomings for inferring *Trichuris* phylogeny, including substantially different rates of evolution.

Holochilus chacarius Thomas, 1906 inhabits swamps, flooded grasslands, and cultivated fields in open, mostly non-forested habitats. This rodent swims, dives, and climbs well and also uses subterranean galleries (Massoia, 1971, 1976; D'Elía and Pardiñas, 2015). Also, this is strictly herbivorous and considered of commercial importance, since it can cause extensive damage to rice, banana, sugarcane, and other crops (Massoia, 1974, 1976; D'Elía and Pardiñas, 2015). *Holochilus chacarius* occupies lowlands of the Chaco ecoregion from western Brazil and South Paraguay into Argentina. The range in Argentina includes a narrow band in the provinces of Jujuy, Salta, Santiago del Estero, and Tucuman, and a second, wider region in the Humid Chaco from south Formosa province to northeastern Buenos Aires (D'Elía and Pardiñas, 2015). Different species of nematodes have been recorded in *H. chacarius* from different provinces in Argentina (Salta, Chaco and Formosa) (Notarnicola, 2005; Notarnicola et al., 2010; Digiani et al., 2013, 2015). Nevertheless, any survey of *Trichuris* species has been recorded in this host species.

The purpose of this paper is to describe a new *Trichuris* species isolated from *H. chacarius* from the Formosa province (Chaco ecoregion), Argentina, based on morphological characteristics and mitochondrial (*cox1*, *cob*) and nuclear (ITS2) markers. Molecular data of mitochondrial markers (*cox1* and *cob*) are also used to analyse and discuss the phylogenetic relationships among the *Trichuris* species of rodents from Argentina.

2. Material and methods

2.1. Material examined

The nematodes were collected in the laboratory from the caecum fixed in alcohol of dead rodents and posteriorly, these were preserved. Twenty adult *Trichuris* specimens were collected from three individuals of *H. chacarius chacarius* (subspecies name according to Massoia, 1976; see Voglino et al., 2004) (Sigmodontinae) from Instituto de Investigación para la Pequeña Agricultura Familiar del Noreste Argentino (IPAF-NEA), dependent of Instituto Nacional de Tecnología Agropecuaria (INTA), Laguna Blanca (25°12′ S 58°7′ W), Formosa province (Argentina).

2.2. Ethics statement

The research was conducted according to Argentine laws. Sample collection was carried out during fieldwork under official permits granted by the Ministerio de Producción y Ambiente de la Provincia de Formosa (authorization s/n; Guía de Tránsito: 001504). This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. Specimens collected in live traps were humanely sacrificed (euthanasia by thoracic compression under ether anaesthesia), following the procedures and protocols approved by national laws (Animal Protection National law 14.346 and references in the provincial permits), and Ethics Committee for Research on Laboratory Animals, Farm and Obtained from Nature of National Council of Scientific and Technical Research (CONICET), and subsequently by the National Agency for the Promotion of Science and Technology of Argentina (ANPCYT) (PICT 2010-0924 and 2015-1348). No endangered species were involved in this study.

2.3. Morphological analysis

Nematodes were preserved in 70% ethanol, cleared in lactophenol, and studied using a light microscope. Morphological identification was conducted using characteristics listed by Chandler (1930), Robles et al. (2006) and Robles (2011). Drawings of specimens were made with the aid of a drawing tube. Three specimens of this population were dehydrated in an ethanol series, dried using the critical point method, and examined with scanning electron microscope (Jeol 6360 LVLV, Tokyo, Japan). Measurements are given as follows: holotype male or allotype female, and paratypes with mean, standard deviations, and range in parentheses. All measurements are given in millimetres (mm). Scale bars of figures are given in micrometres (μm).

The nomenclatural acts have been registered in ZooBank, the online registration system for the ICZN. Specimens of nematodes were deposited in the Helminthological Collection of the Museo de La Plata (MLP-He), La Plata, Buenos Aires, and hosts in the Mastozoological Collections of the Centro Nacional Patagónico (CNP), Puerto Madryn, Chubut, Argentina.

2.4. DNA extraction, amplification, and sequencing

A total of 3 adult specimens of *Trichuris* from *H. c. chacarius* were studied and compared with 17 adult specimens collected from 5 host species from Sigmodontinae rodents previously analysed (Robles et al., 2014; Callejón et al., 2016).

The complete specimens previously identified were washed extensively in 0.9% saline solution and stored in 70% ethanol until used for DNA extraction. Genomic DNA from individual worms was extracted using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's protocol. Quality of extractions was assessed using 0.8% agarose gel electrophoresis and ethidium bromide staining.

The ITS2 rDNA region was PCR-amplified using a Perkin Elmer thermocycler and the PCR mix and PCR conditions are cited by Callejón et al. (2015). DNA sequences of the primers were mentioned by Gasser et al. (1996) and Robles et al. (2014). The mitochondrial *cox1* partial gene was PCR-amplified using an Eppendorf AG thermocycler and conditions specified for *Trichinella* isolates by Nagano et al. (1999) and sequenced using the primers cited by Folmer et al. (1994) and Callejón et al. (2016). The mitochondrial *cob* partial gene was amplified and sequenced using primers designed from comparisons of complete mtDNA genome sequences of *Trichuris discolor* Linstow, 1906 (NC_018596), *Trichuris ovis* Abildgaard, 1795 (NC_018597), *Trichuris suis* Schrank, 1788 (NC_017747) and *Trichuris colobae* Cutillas, De Rojas, Zurita, Oliveros and Callejón, 2015 (NC_017750) mentioned by Callejón et al. (2015).

The PCR products were checked on ethidium bromide-stained 2% Tris-Borate-EDTA (TBE) agarose gels. Bands were eluted from agarose by using the Wizard® SV Gel and PCR Clean-Up System (Promega). The purified PCR products were concentrated, and directly sequenced by Stab Vida (Portugal). All sequences were completely double-stranded for verification using reactions primed from the PCR primers.

2.5. Sequence alignment and phylogenetic analysis

Molecular analyses were performed on the rDNA and mtDNA datasets, based on our sequences of ITS2, *cox1* and *cob* and those obtained from GenBank database (Table 2).

To obtain a nucleotide sequence alignment file, we used MUSCLE

Table 2
Sequences of *Trichuris* and outgroups species obtained from GenBank and used for molecular analyses.

Species	Host species/geographical origin	Gene/region	Accession numbers
Trichuris pardinasi	*P. xanthopygus*/Sierra de la Ventana (Buenos Aires)	ITS2	HG934448
			HG934445
			HG934449
	P. xanthopygus/Sierra de Córdoba (Córdoba)		HG934447
			HG934446
Trichuris navonae	*A. montensis*/Refugio Moconá (Misiones)		HG934435
			HG934436
			HG934437
			HG934438
	A. montensis/Urugua-í (Misiones)		HG934443
			HG934444
			HG934441
	A. montensis/San Antonio (Misiones)		HG934434
	T. nigrita/San Antonio (Misiones)		HG934440
			HG934442
	T. nigrita/Urugua-í (Misiones)		HG934439
Trichuris bainae	*S. angouya*/Refugio Moconá (Misiones)		HG934431
			HG934432
	S. angouya/Guaycolec (Formosa)		HG934433
			LT549472
Trichuris pardinasi	*P. xanthopygus*/Sierra de la Ventana (Buenos Aires)	*Cox1*	HG934451
			HG934452
			HG934453
	P. xanthopygus/Sierra de Córdoba (Córdoba)		HG934454
			HG934455
			HG934456
Trichuris navonae	*A. montensis*/Refugio Moconá (Misiones)		HG934459
			HG934462
	A. montensis/Urugua-í (Misiones)		HG934458
			HG934460
			HG934464
	A. montensis/San Antonio (Misiones)		HG934461
			HG934463
	T. nigrita/San Antonio (Misiones)		HG934457
Trichuris bainae	*S. angouya*/Refugio Moconá (Misiones)		HG934466
			HG934465
	S. angouya/Guaycolec (Formosa)		HG934467
			LN899586
Trichuris pardinasi	*P. xanthopygus*/Sierra de la Ventana (Buenos Aires)	*Cob*	LN899578
			LN899579
			LN899577
	P. xanthopygus/Sierra de Córdoba (Córdoba)		LN899580
			LN899581
Trichuris navonae	*A. montensis*/Refugio Moconá (Misiones)		LN899565
			LN899566
	A. montensis/Urugua-í (Misiones)		LN899567
			LN899568
			LN899569
	A. montensis/San Antonio (Misiones)		LN899571
			LN899570
	T. nigrita/San Antonio (Misiones)		LN899572
			LN899573
	T. nigrita/Urugua-í (Misiones)		LN899584
Trichuris bainae	*S. angouya*/Refugio Moconá (Misiones)		LN899574
			LN899575
	S. angouya/Guaycolec (Formosa)		LN899576
			LN899582
Trichuris muris	*Mus domesticus*/Spain	*Cox1*	HE653130
		Cob	LM994701
Trichuris arvicolae	*Myodes glareolus*/Spain	*Cox1*	FR851284
		Cob	LM994698
Trichuris vulpis	*Canis lupus familiaris*/Spain	*Cox1*	HE653135.1
		Cob	LM994699.1

Outgroup	Host species/geographical origin	Gene/region	Accession numbers
Trichinella spiralis	–/–	*Cox1*	NC_002681.1
		Cob	NC_002681.1

alignment method (Edgar, 2004) by the MEGA program version 5.2 (Tamura et al., 2011). The nucleotide sequences of the protein-coding genes (*cox1* and *cob*) were first translated into amino acids to confirm that they lacked internal stop codons and to verify (by BLAST match) that inferred amino acid characteristic sequences of the predicted nematode protein. In order to assess the similarity among all marker sequences obtained in this study, with other *Trichuris* species obtained from GenBank, we analysed the number of base differences between sequences studied using number of differences method of MEGA 5 program version 5.2 (Tamura et al., 2011).

Phylogenetic trees were inferred using nucleotide data and produced using two methods: Bayesian inference (BI) and maximum likelihood (ML) using MrBayes version 3.2.6 (Ronquist and Huelsenbeck, 2003) and PhyML (Guindon and Gascuel, 2003), respectively.

ITS2 rDNA sequences were not included for phylogenetic analyses due to the substantial length variation of *Trichuris* spp. which compromise inferences of positional homology. JMODELTEST (Posada, 2008) program was used to determinate the best-fit substitution model for the parasite data (*cox1* and *cob*). Models of evolution were chosen for subsequent analyses according to the Akaike Information Criterion (Huelsenbeck and Rannala, 1997; Posada and Buckley, 2004). For the study of the dataset containing the concatenation of two markers (*cox1* and *cob*), analyses based on BI were partitioned by gene and models for individual genes within partitions were those selected by jModeltest. For ML inference, best-fit nucleotide substitution models included GTR + G dataset for *cox1* and *cob* datasets. Support for the topology was examined using bootstrapping (heuristic option) (Felsenstein, 1985) over 1000 replications. The commands used in MrBayes-3.2.6 for BI was nst = 6 gamma rates (*cox1* and *cob*). For BI, the standard deviation of split frequencies was used to assess if the number of generations completed was sufficient; the chain was sampled every 500 generations and each dataset was run for 10 million generations. Adequacy of sampling and run convergence was assessed using the effective sample size diagnostic in TRACER program version 1.6 (Rambaut and Drummond, 2007). Trees from the first million generations were discarded based on an assessment of convergence. The Bayesian Posterior Probabilities (BPP) is percentage converted.

Sequences from different *Trichuris* spp. from different host species available in GenBank were included in each analysis: *Trichuris muris* Schrank, 1788 from *Mus domesticus* Schwarz and Schwarz, 1943 (Murinae), *Trichuris arvicolae* Feliú, Spakulová, Casanova, Renauld, Morand, Hugot et al. 2000 from *Myodes glareolus* Schreber, 1780 (Arvicolinae) from Spain and *Trichuris vulpis* Roederer, 1761 from *Canis lupus familiaris* (Canidae). Sequences from *Trichinella spiralis* Owen, 1835 as outgroup were included in each analysis to root the phylogenetic trees.

3. Results

3.1. Morphological and biometrical results

Trichuris massoiai n. sp. (Figs. 1 and 2).

Diagnosis: anterior part of body long, narrow, tapered, and whip-like; posterior part of body broad, and handle-like (Fig. 1A). Cuticle with fine transversal striation. Bacillary band located laterally in anterior portion of body (Figs. 1B, C, 2A–C). Bacillary band 0.07–0.09 from anterior end of body, and extends to body width region of 0.19–0.28. Cuticular inflations appear bordering bacillary band from 0.2–0.35 to 1.25–1.55 in the anterior end of body (Fig. 1B). These structures limit laterally to abundant and visible bacillary glands with conspicuous pore. Cuticle around vulvar aperture with transversally striated pattern (Fig. 2H). Stichosome with 1 row of stichocytes, and 1 pair of conspicuous cells at oesophagus-intestinal junction level (Fig. 1D, F). Male without spicular tube. Proximal cloacal tube, united laterally to distal cloacal tube (Fig. 1E). Spicular sheath cylindrical, with spines distributed from proximal to distal portion (Figs. 1E, 2D); proximal spines with rounded point and distal spines with sharpened point (Fig. 2E–G). Testis ends near final third of distal cloacal tube, showing different degree of convolutions (Fig. 1E). Cloaca subterminal with 1 pair of paracloacal papillae not ornamented (Fig. 2E). Female with non-protrusive vulva located at oesophagus-intestinal junction level (Figs. 1F, 2H). Anus subterminal with long caudal end (Fig. 1G).

Male (*7 specimens*): body length 20.7, 19.7 ± 1.8 (17.2–21.4). Anterior portion of body 12.8, 12.5 ± 1.5 (10.6–14.0) long and thick portion of body 7.9, 7.3 ± 0.47 (6.6–7.9) long (Fig. 1A). Anterior body width 0.08, 0.06 ± 0.01 (0.05–0.07), maximum posterior body width 0.50, 0.37 ± 0.038 (0.33–0.43), width at oesophagus-intestinal junction level 0.15, 0.16 ± 0.014 (0.14–0.17) (Fig. 1D). Total length of oesophagus 12.82, 12.51 ± 1.55 (10.6–13.98), muscular portion 0.60, 0.56 ± 0.67 (0.50–0.67) long, stichosome portion 12.2, 11.94 ± 1.56 (10.05–13.31) long. Spicule length 3.8, 3.44 ± 0.22 (3.07–3.65) (Fig. 1E). Spicular sheath densely spinose 3.5, 3.1 ± 1.2 (2.8–3.5) long (Figs. 1E, 2D–G). Proximal cloacal tube 2.4, 2.2 ± 0.16 (1.97–2.37) long, distal cloacal tube 2.8, 1.36 ± 0.31 (2.43–2.9) long (Fig. 1E). Ratio between anterior and posterior body length is 1:1.7. Ratio between total body length and posterior portion length 2.62, 2.74 ± 0.2 (2.5–3.05). Ratio between total body length and spicule length 5.34, 5.87 ± 0.25 (5.6–6.1). Ratio between posterior portion length and spicule length 2.03, 2.13 ± 0.13 (1.96–2.3). Ratio between proximal cloacal tube length and distal cloacal tube length 0.83, 0.81 ± 0.07 (0.72–0.88). Ratio between maximum posterior body width and posterior portion length 0.06, 0.052 ± 0.013 (0.04–0.06).

Female (*7 specimens*): body length 30.55, 32.70 ± 1.7 (30.57–35.48). Anterior portion of body 18.86, 21.08 ± 1.4 (19.6–23.6) long and thick portion of body 11.68, 11.62 ± 0.5 (10.8–12.36) long. Anterior body width 0.075, 0.075 ± 0.02 (0.05–0.1); maximum posterior body width 0.46, 0.44 ± 0.06 (0.36–0.55); width at oesophagus-intestinal junction 0.17, 0.18 ± 0.03 (0.13–0.22) (Fig. 1F). Total length of oesophagus 18.86, 21.08 ± 1.4 (19.5–23.6), muscular portion 0.77, 0.65 ± 0.09 (0.47–0.77) long, stichosome portion 18.09, 20.43 ± 1.5 (19.0–23.13) long. Distance between oesophagus-intestinal junction and vulva 0.12, 0.14 ± 0.01 (0.12–0.16). Eggs oval, with bipolar plugs, (n = 10) 0.018–0.020 × 0.040–0.042 (Fig. 1H). Ratio between anterior and posterior body length is 1:1.8. Ratio between total body length and posterior portion length 2.61, 2.81 ± 0.01 (2.6–2.9). Ratio between maximum posterior body width and posterior portion length 0.04, 0.038 ± 0.006 (0.03–0.04).

Type material: holotype male MLP-He 7153, allotype female MLP-He 7154, and 12 additional paratypes MLP-He 7155 deposited at the Helminthological Collection of the Museo de La Plata. urn: lsid:zoobank.org:pub:05205E11–3138-4CEF-A05E-B31299DAC34D.

Type host: *Holochilus chacarius* Thomas, 1906 (Sigmodontinae: Oryzomyini). *Symbiotype*: female CNP 3939. *Other hosts housed*: CNP 1894 and CNP 1895.

Type locality: IPAF-NEA (INTA), Laguna Blanca (25°12′ S 58°7′ W), Formosa province (Argentina).

Site of infection: caecum.

Etymology: dedicated to the memory of Elio Massoia (1936–2001), author of numerous contributions that significantly expanded the knowledge of the diversity of South American mammals, including several on the genus *Holochilus*.

Differential diagnosis: twenty seven *Trichuris* species from North and South American rodents (Table 1) were compared with the new species. *Trichuris massoiai* n. sp. can be separated from 13 of the species that parasitize American rodents, i.e., *T. citelli*, *T. dipodomys*, *T. fossor*, *T. fulvi*, *T. laevitestis*, *T. perognathi*, *T. peromysci*, *T. madisonensis*, *T. muris*, *T. neotomae*, *T. silviae*, *T. stansburyi* and *T. thrichomysi* by the absence of a spicular tube (the spicule lies entirely within the distal cloacal tube).

Trichuris massoiai n. sp. has a shorter spicule than *T. bradleyi*, *T. bursacaudata*, *T. pampeana* and *T. pardinasi* and a longer spicule than *T. bainae*, *T. chilensis*, *T. elatoris*, *T. opaca*, *T. navonae*, and *T. travassosi*.

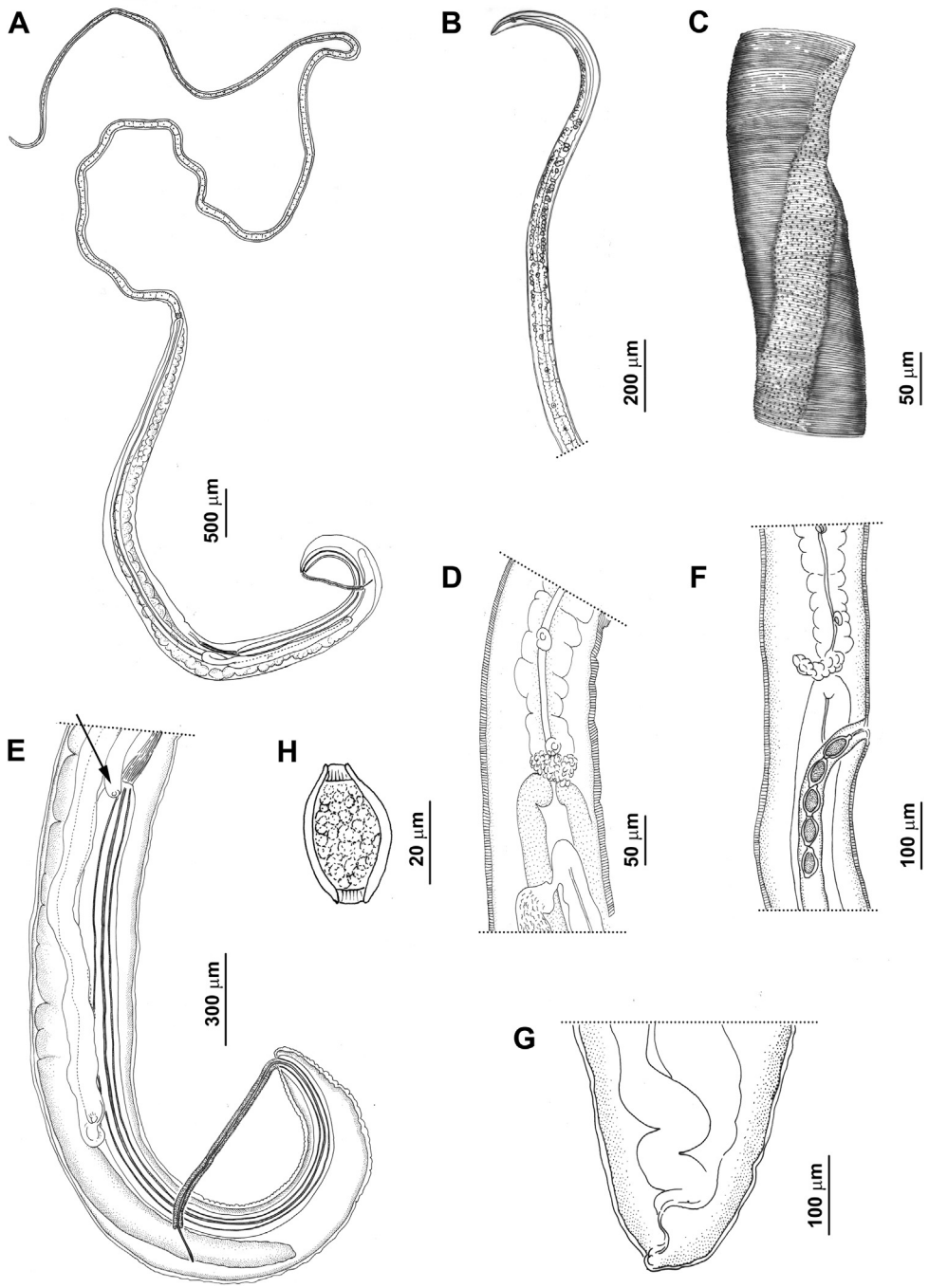

Fig. 1. Drawings of *Trichuris massoiai* n. sp. (A) Complete male specimen. (B) Anterior extremity, with bacillary band and view of cuticular inflations. (C) Anterior extremity, with cuticle transversally striated and bacillary band. (D) Male, oesophagus-intestine junction and proximal portion of testis, with bacillary band view. (E) Male, posterior end, spiny spicular sheath, spicule and proximal and distal cloacal tube (arrow show the tubes junction), lateral view. (F) Female, oesophagus-intestine junction and vulva, lateral view. (G) Female, posterior end, lateral view. (H) Egg.

The new species differs from *T. bursacaudata*, *T. myocastoris* and *T. opaca* by lacking a spicular sheath with a spiny distal spherical bulge or a spiny campanuliform shape. Among those species with a cylindrical spicular sheath, the new species can be separated from *T. travassosi* and *T. pampeana* by the distribution of the spines (both species with a dense distribution of spines in proximal portion and dispersed in distal portion).

Moreover, the new species has a shorter distal cloacal tube than *T. bradleyi*, *T. bursacaudata* and *T. pardinasi* and a longer distal tube than *T. bainae*, *T. navonae*, *T. robusti*, and *T. travassosi*. In addition, *T. massoiai* n. sp. presents a shorter proximal cloacal tube than *T. bradleyi*, *T. chilensis* and *T. pardinasi*.

The new species has a non-protrusive vulva, as do most of the species of *Trichuris* mentioned. However, some species present a slightly protruding vulva, with a cuticular evagination or lips protruding as *T. bainae*, *T. chilensis*, and *T. gracilis*.

Although, the males of *T. gracilis* and *T. dolichotis* have not been described, these species can be separated from the new species by their lengths of the anterior (21.52–27.44 and 17 vs. 19.6–23.6) and posterior (15.1–18.4 and 13 vs. 10.8–12.36) portions of the body of the females. In this way, the ratios between anterior and posterior body length of both species are smaller than that ratio observed in the new species (about 1:1.4–1.5 and 1: 1.3 vs. 1:1.8).

The most similar biometrical and morphological features were found between *T. massoiai* n. sp. and *T. robusti* from *Ctenomys robustus* (from Chile), which share the absence of a spicular tube, the spicular and proximal cloacal tube length and a non-protrusive vulva. However, these species can be distinguished by the distal cloacal tube length (2.43–2.9 vs. 2.3) and the ratio between anterior and posterior body length in males and females (1:1.7 and 1:1.8 vs. about 1:1 and 1:0.8–09).

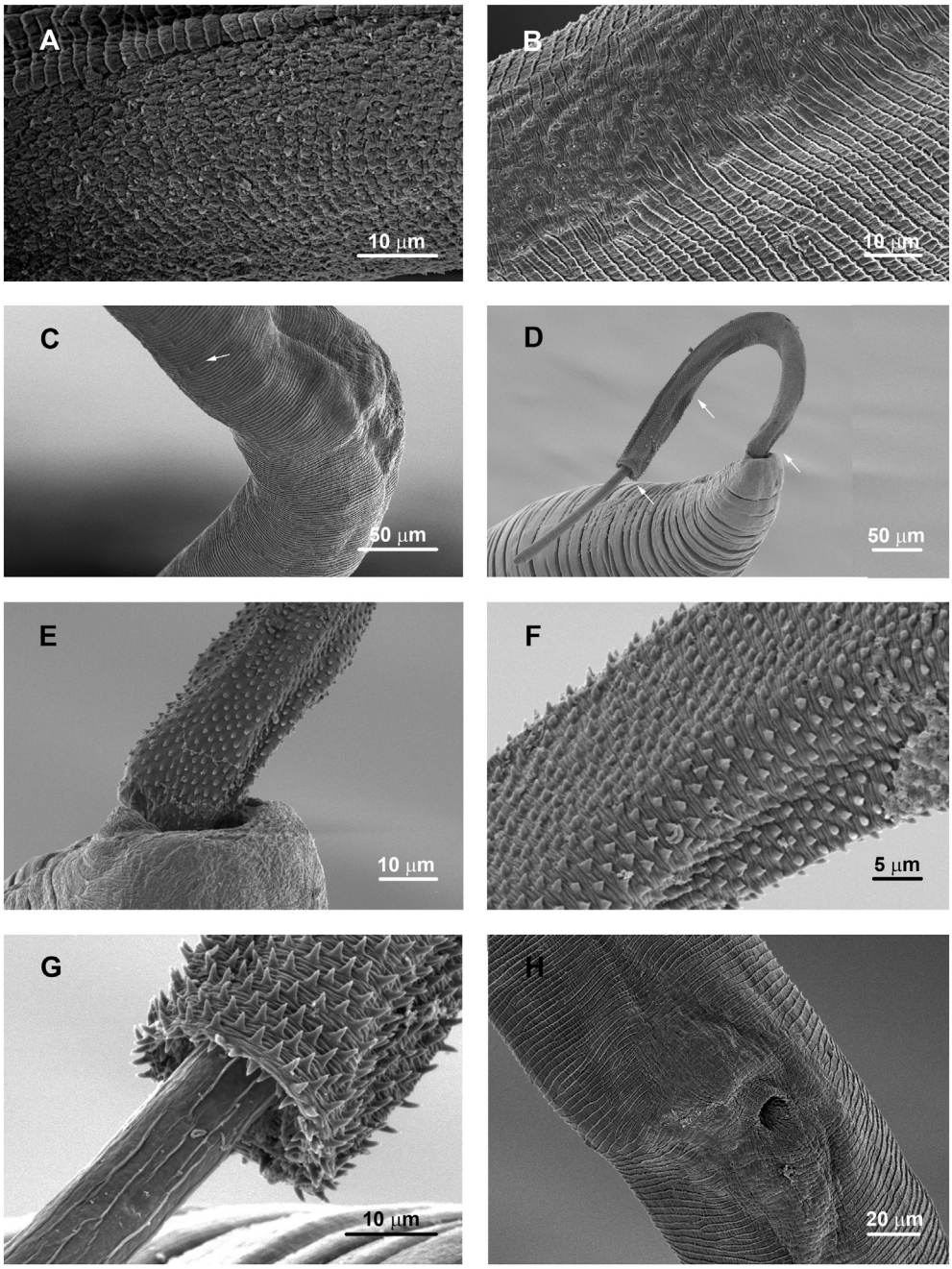

Fig. 2. Scanning electron micrographs of *Trichuris massoiai* n. sp.- SEM. (A) Bacillary band, with detail of bacillary glands of middle part of anterior extremity. (B) Bacillary band, with detail of bacillary glands of distal part of anterior extremity. (C) Female, the arrow shows the bacillary band located laterally. (D) Male, posterior end, spiny spicular sheath. Each arrow shows the detail observed in the follow three figures (E, F, G), respectively. (E) Male, detail of the proximal portion of spiny spicular sheath. (F) Male, detail of the middle portion of spiny spicular sheath. (G) Male, detail of the distal portion of spiny spicular sheath. (H) Female, vulvar aperture with transversally striated pattern, ventral view.

3.2. Molecular data analysis

Nucleotide sequence data from *T. massoiai* n. sp. are reported and are available in GenBank (Table 3).

3.2.1. ITS2 rDNA region

These sequences were 319 base pairs (bp) (exclusive of the primers) and their G + C content ranged from 60.2% to 60.5% (Table 3). The multiple alignments of 20 ITS2 sequences of *Trichuris* species from South America yield a dataset of 363 characters.

The maximum value of intra-population similarity of *Trichuris* species based on ITS2 corresponded to *T. bainae* from Misiones and the minimum value corresponded to *T. navonae* from Misiones (Table 4). On the other hand, the maximum and minimum values of inter-population similarity were observed in *T. bainae* isolated from Misiones and Formosa and *T. pardinasi* isolated from Córdoba and Buenos Aires, respectively. The comparative study between different ITS2 sequences

Table 3
GenBank accession numbers of ITS2, *cox1* and *cob* partial sequences of 3 individuals of *Trichuris massoiai* n. sp. isolated from a rodent species from Argentina.

Host species/ geographical origin	Marker	Number of base pair	G + C%	Accession numbers
H. chacarius/IPAF NEA (Formosa)	ITS2	319	60.2	LT221880
		319	60.5	LT221881
		319	60.2	LT221882
	Cox1	359	36.8	LT221884
		359	36.8	LT221885
		359	36.8	LT221886
	Cob	505	30.8	LT221888
		505	30.8	LT221889
		505	30.8	LT221890

Table 4
Intra-population (*), inter-population (●) and inter-specific similarity observed in ITS2 partial sequences in *Trichuris* populations isolated from different rodent species.

Species	*T. pardinasi* (Buenos Aires)	*T. pardinasi* (Córdoba)	*T. navonae* (Misiones)	*T. bainae* (Misiones)	*T. bainae* (Formosa)	*T. massoiai* n. sp. (Formosa)
T. pardinasi (Buenos Aires)	96.1–97.8%*					
T. pardinasi (Córdoba)	97.0–99.2%●	99.7%*				
T. navonae (Misiones)	88.2–91.5%	89.8–92.3%	95.6–99.7%*			
T. bainae (Misiones)	87.9–90.1%	90.4–90.6%	93.1–95.6%	100%*		
T. bainae (Formosa)	87.9–90.6%	90.4–91.2%	93.7–95.6%	98.9–99.4%●	98.6%*	
T. massoiai n. sp. (Formosa)	89.8–92.3%	92.2–93.1%	86.0–89.2%	87.6–88.9%	87.6–88.9%	99.1–99.8%*

Table 5
Intra-population (*), inter-population (●) and inter-specific similarity observed in *cox1* partial sequences in *Trichuris* populations isolated from different rodent species.

Species	*T. pardinasi* (Buenos Aires)	*T. pardinasi* (Córdoba)	*T. navonae* (Misiones)	*T. bainae* (Misiones)	*T. bainae* (Formosa)	*T. massoiai* n. sp. (Formosa)
T. pardinasi (Buenos Aires)	97.4–98.2%*					
T. pardinasi (Córdoba)	95.6–97.3%●	98.2–98.8%*				
T. navonae (Misiones)	85.1–88.3%	85.1–87.7%	96.5–100%*			
T. bainae (Misiones)	88.3–89.8%	90.1–90.3%	89.2–90.4%	99.7%*		
T. bainae (Formosa)	88.3–89.5%	90.1–90.6%	88.9–89.8%	99.4–99.6%●	100%*	
T. massoiai n. sp. (Formosa)	84.8–85.7%	85.7–86.3%	89.2–90.4%	89.2–89.5%	88.9%	100%*

obtained for each species revealed the highest similarity between *T. navonae* and the two populations of *T. bainae* from Misiones and Formosa, whereas the lowest similarity was observed between *T. massoiai* n. sp. from Formosa and *T. navonae* from Misiones (Table 4).

3.2.2. Cox1 mtDNA encoding gene

The *cox1* partial sequences were 359 base pairs (bp) in length. The G + C content of the *cox1* partial gene of *Trichuris* species was 36.8% (Table 3). The multiple sequence alignment of 22 *cox1* nucleotide sequences (including the outgroup) of *Trichuris* species from South America and Europe yielded a dataset of 342 characters. jModelTest determined that the best-fit model for *cox1* mtDNA datasets was GTR + G, which was used for Bayesian analyses and maximum likelihood.

The maximum value of intra-population similarity of *Trichuris* species based on *cox1* corresponded to *T. navonae* from Misiones, *T. bainae* from Formosa and *T. massoiai* n. sp. from Formosa, and the minimum value corresponded to *T. navonae* from Misiones (Table 5). In addition, the maximum and minimum values of inter-population similarity were observed in *T. bainae* isolated from Misiones and Formosa and *T. pardinasi* isolated from Córdoba and Buenos Aires, respectively. The comparative study between different *cox1* sequences obtained for each species revealed the highest similarity between *T. pardinasi* from Córdoba and *T. bainae* from Formosa, whereas the lowest similarity was observed between *T. massoiai* n. sp. from Formosa and *T. pardinasi* from Buenos Aires (Table 5).

The consensus tree showed four phylogenetic groups corresponding with four different *Trichuris* species (*T. navonae* (Clade 1), *T. massoiai* n. sp. (Clade 2), *T. bainae* (Clade 3) and *T. pardinasi* (Clade 4)) with good resolution, nevertheless, within the *Trichuris* species group from Argentina, not all relationships among the different species of *Trichuris* were resolved with robust support by the two methods (BI and ML). In addition, a subclade 3a including *T. bainae* from Formosa was observed separated from *T. bainae* from Misiones by the two methods with strong support, and a subclade 4a including *T. pardinasi* from Buenos Aires appeared separated from *T. pardinasi* from Córdoba with strong support (Table 7).

Furthermore, *Trichuris* spp. from rodents of South America (Argentina) form a solid group separated of *Trichuris* spp. from Europe (Spain) (*T. muris*, *T. arvicolae* and *T. vulpis*) (Table 7).

3.2.3. Cob mtDNA partial gene

The partial sequences were 505 bp in length. The G + C content of the *cob* partial gene of *Trichuris* species was 30.8% (Table 3). The multiple alignment of 23 *cob* nucleotide sequences (including the outgroup) of *Trichuris* species from South American and European yielded a dataset of 495 characters. jModelTest determined that the best-fit model for the *cob* datasets was GTR + G, which was used for Bayesian analyses and maximum likelihood.

The maximum and minimum value of intra-population similarity of *Trichuris* species based on *cob* datasets corresponded to *T. navonae* from Misiones (Table 6). On the other hand, the maximum and minimum values of inter-population similarity were observed in *T. bainae* isolated from Buenos Aires and Formosa and *T. pardinasi* isolated from Córdoba and Buenos Aires, respectively. When the *cob* sequences of the different species and host isolates of the genus *Trichuris* were compared, the highest similarity was obtained between *T. navonae* from Misiones and *T. massoiai* n. sp. from Formosa, whereas the lowest similarity was observed between *T. pardinasi* from Cordoba and *T. massoiai* n. sp. from

Table 6
Intra-population (*), inter-population (●) and inter-specific similarity observed in *cob* partial sequences in *Trichuris* populations isolated from different rodent species.

Species	*T. pardinasi* (Buenos Aires)	*T. pardinasi* (Córdoba)	*T. navonae* (Misiones)	*T. bainae* (Misiones)	*T. bainae* (Formosa)	*T. massoiai* n. sp. (Formosa)
T. pardinasi (Buenos Aires)	98.6–99.8%*					
T. pardinasi (Córdoba)	89.2–98.4%●	98.4%*				
T. navonae (Misiones)	84.0–85.5%	84.8–86.1%	97.2–100%*			
T. bainae (Misiones)	84.4–84.8%	84.8–86.3%	87.7–89.5%	99.8%*		
T. bainae (Formosa)	84.6–84.8%	84.8–86.3%	87.9–89.5%	99.1–99.6%●	99.8%*	
T. massoiai n. sp. (Formosa)	83.2–83.9%	83.0–84.0%	86.9–89.8%	86.7–87.1%	86.0–87.0%	99.2–99.8%*

Table 7
Monophyly of mitochondrial and ribosomal partial sequences of selected groups based on several combinations of datasets and inference methods. BPP = Bayesian Posterior Probability; ML = Maximum Likelihood bootstrap. Clade 1: *T. navonae*; Clade 2: *T. massoiai* n. sp.; Clade 3: *T. bainae*; Subclade 3a: *T. bainae* from Formosa; Clade 4: *T. pardinasi*; Subclade 4a: *T. pardinasi* isolated from *P. bonariensis* from Buenos Aires.

	Cox1	*Cob*	Mitochondrial genes (*Cox1* + *Cob*)
BPP/ML			
Clade 1 (*T. navonae*)	−/75	100/98	100/99
Clade 2 (*T. massoiai* n. sp.)	100/99	100/100	100/100
Clade 3 (*T. bainae*)	98/87	92/86	100/98
Clade 4 (*T. pardinasi*)	99/99	82/−	78/−
Clade 1 clustered with Clade 2	−/−	86/84	99/93
Clade 1 grouped with Clade 2 and 3	−/−	90/100	100/95
Trichuris populations from Argentina	75/98	100/98	100/99
Subclade 3a	85/89	92/86	82/75
Subclade 4a	95/94	95/92	90/−

Formosa (Table 6).

The consensus trees were in congruence with those obtained based on *cox1* datases revealing four clades with good resolution by the two methods (BI and ML) corresponding with four different *Trichuris* species from Argentina (Table 7). The topology showed the Clade 1 (*T. navonae*) related to Clade 2 (*T. massoiai*). In addition, a subclade 3a clustered *T. bainae* from Formosa with moderate support separated from *T. bainae* from Misiones. A subclade 4a including *T. pardinasi* from Buenos Aires with moderate support was observed (Table 7).

In congruence with *cox1* phylogenetic results, *Trichuris* spp. from rodents of South America (Argentina) form a solid group separated of *Trichuris* spp. from Europe (Spain) (*T. muris*, *T. arvicolae* and *T. vulpis*) (Table 7).

3.2.4. Phylogenetic relationship based on concatenated cox1 and cob mtDNA sequence datasets

The combined analysis of mitochondrial enconding genes (*cox1* and *cob*) revealed a similar topology that those obtained by separate analysis of the 2 genes providing much greater phylogenetic resolution among *Trichuris* taxa (Fig. 3). Thus, four clades were observed corresponding to four different species of *Trichuris* (*T. navonae*, *T. massoiai* n. sp., *T. bainae* and *T. pardinasi*). The concatenated analysis of the mitochondrial genes showed the Clade 1 (*T. navonae*) related to Clade 2 (*T. massoiai* n. sp.) with strong support (BPP and ML). In addition, *T. navonae* and *T. massoiai* n. sp. appeared as sister group and both species related to Clade 3 (*T. bainae*) and separated from Clade 4 (*T. pardinasi*) highly supported (Fig. 3, Table 7). Furthermore, some strongly supported subclades correspond to the geographic origin of the sample. Thus, the species *T. bainae* and *T. pardinasi* appeared with two different subclades each one (Fig. 3, Table 7). In addition, *T. navonae*, *T. massoiai* n. sp., *T. bainae* and *T. pardinasi* from Argentine appeared as a solid group separated of *T. muris*, *T. arvicolae* and *T. vulpis* from Spain.

4. Discussion

In this paper, *T. massoiai* n. sp. was distinguished from 27 *Trichuris* species from North and South American rodents by morphological, including morphometric features, such as the absence of a spicular tube, presence of a cylindrical spicular sheath, non-protrusive vulva, length of spicule, and proximal and distal cloacal tube. In addition, the morphometric characteristics of each species of *Trichuris* compared, allowed the separation of close species (see differential diagnosis and Robles, 2011). The phylogenetic and geographic distance of the hosts supports the separation of different species. Mainly, the morphologically closest species parasitize host groups of a different suborder (Hystricomorpha - e.g. *T. robusti*, *T. gracilis* and *T. dolichotis*).

The most markers studied among *Trichuris* species are the ribosomal DNA regions (ITS1 and ITS2) because they have been shown to be

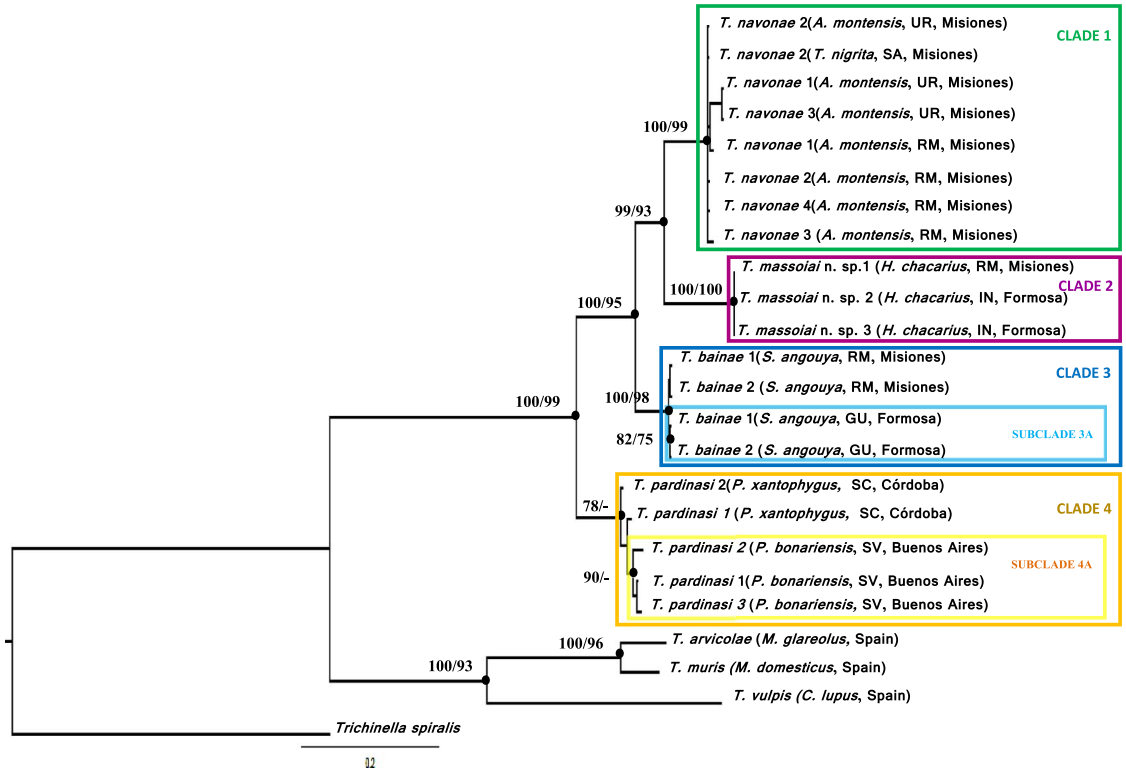

Fig. 3. Phylogenetic tree of *Trichuris* species from rodents of Sigmodontinae of different geographical origins based on concatenated mitochondrial genes (*cox1* and *cob*) inferred using Maximum Composite Likelihood. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown on the branches (Bayesian Inference/Maximum Composite Likelihood). Bootstrap values lower than 65% are not shown.

among the best molecular markers for diagnostic studies in *Trichuris* spp. (Oliveros et al., 2000; Cutillas et al., 2002, 2004, 2007, 2009; Callejón et al., 2010, 2012; Robles et al., 2014). On the other hand, both mitochondrial genes (*cox1* and *cob*) and complete genome data have been used for characterization of nematodes and their relationships (Azevedo and Hyman, 1993; Hugall et al., 1994; Blouin et al., 1997; Keddie et al., 1998; Lavrov and Brown, 2001). Recently, different authors have carried out a phylogenetic study for genus *Trichuris* based on mitochondrial genes in order to clarify the relationships between different species (Callejón et al., 2013, 2015; Doležalová et al., 2015).

In the case of *Trichuris* species from Cricetidae in South America, all the records are recent. Robles et al. (2014) based on ITS2 rDNA region, and Callejón et al. (2016) based on the mitochondrial partial enconding genes *cox1* and *cob*, reported different genetic lineages among *Trichuris* species from sigmodontine rodents that supported the proposal of a new species (*T. bainae*).

This paper presents a molecular study based on three molecular markers of four *Trichuris* species. *T. massoiai* n. sp. isolated from *H. chacarius* was compared with *T. navonae*, *T. bainae* and *T. pardinasi* of different Sigmodontinae rodent species from Argentina. The percentages of inter-specific similarity observed between *T. massoiai* n. sp. and these species were in agreement with the range of inter-specific similarity described in other species of *Trichuris* (Cutillas et al., 2002; Callejón et al., 2013, 2015, 2016; Robles et al., 2014). Thus, according to the levels of similarity between described species of *Trichuris* based on ITS2 rDNA region (37.7%, Callejón et al., 2013–94.5% Robles et al., 2014), *cox1* mtDNA (68.7–90.4%, Callejón et al., 2013, 2016) and *cob* mtDNA (69.2–97.1%, Callejón et al., 2016) enconding genes, is supported that the specimens studied in the present paper, belong to a new species.

The combined analysis of mtDNA encoding genes (*cox1* and *cob*) revealed four clades corresponding with four different species of *Trichuris*: Clade 1 (*T. navonae*) was related to Clade 2 (*T. massoiai* n. sp.) and, these two clades were related to Clade 3 (*T. bainae*) and separated from Clade 4 (*T. pardinasi*). Relationships among the most comprehensive clades of *Trichuris* species have been resolved in the same way by molecular sequence data in this study. Thus, phylogenetic analysis of the individual markers (*cox1* and *cob* mtDNA) and combined analysis of mtDNA showed strong resolution grouping *T. navonae* and *T. massoiai* n. sp. and both species related to *T. bainae* as a sister group separated to *T. pardinasi*. Phylogenetic reconstruction based on concatenated sequences had greater phylogenetic resolution for delimiting species and populations intra-specific of *Trichuris* than those based on partitioned genes. These results were observed in previous studies where populations of *T. bainae* and *T. pardinasi* could be affected by geographical factors and co-divergence parasite-host (Callejón et al., 2016).

The incorporation of the new species of *Trichuris* from Sigmodontinae rodents with known phylogenetic relationships, no longer exhibit an exact congruence between parasite species and host tribes. Since *T. navonae* (parasite from Akodontini) and *T. massoiai* n. sp. or *T. bainae* (parasites from Oryzomyini) are sister groups, and these three clades are separated from *T. pardinasi* (parasite from Phyllotini). In addition, the phylogenetic results showed *Trichuris* spp. from Argentine form a solid group separated of *Trichuris* spp. from Spain revealing, possibly, a greater influence of geographic distribution than of parasite-host co-divergence, considering mainly, the position of *T. vulpis*.

Although, at present, the geographical and host distributions of *Trichuris* species have been poorly studied, future studies may address whether the species of *Trichuris* could act as markers of their hosts and/or geographic distribution.

5. Conclusions

A *Trichuris* species is isolated from *Holochilus chacarius* (Cricetidae: Sigmodontinae) for first time of the Chaco ecoregion in Argentina, and this is described as a new species based on morphological characteristics and mitochondrial (*cox1*, *cob*) and nuclear (ITS2) markers. Phylogenetic relationships among the *Trichuris* species of rodents from Argentina have been analysed in this study, and the combined analysis of mitochondrial genes (*cox1* and *cob*) revealed four clades corresponding with four different species. More comprehensive understanding of the co-divergence of parasites and hosts will require increased taxa sampling of *Trichuris* species and the resolution provided by multigene molecular phylogenies.

Acknowledgements

We thank Graciela T. Navone for her support in the laboratory and important advice with nematodes; Ulyses Pardiñas, Carlos Galliari, Marcela Lareschi, Guillermo Panisse, Natalia Guerreiro Martins, and Jualiana Notarnicola for their help during host collections; Ulyses Pardiñas and Carlos Galliari for identification of the hosts; Mike Kinsella for the valuable critical reading of the manuscript and for the revision into English; María Cristina Estivariz for the drawings; Patricia Sarmiento for the SEM photographs. The present work was supported by a grant of the V Plan Propio de Investigación of the University of Sevilla, Spain, and Agencia Nacional de Promoción Científica y Tecnológica (PICT 2010-0924; 2015-1348); Proyecto de incentivos de la Universidad Nacional de La Plata (UNLP N 627 and travel grant).

References

Anderson, R.C., 2000. Nematode Parasites of Vertebrates. Their Development and Transmission, 2nd ed. CAB International ed, Wallingford Oxon UK.
Azevedo, J.L.B., Hyman, B.C., 1993. Molecular characterization of lengthy mitochondrial DNA duplications from the parasitic nematode *Romanomermis culicivorax*. Genetics 133, 933–942.
Babero, B.B., Murúa, R.B., 1987. The helminth fauna of Chile. X. A new species of whipworm from a Chilean rodent. Trans. Am. Microsc. Soc. 106, 190–193.
Babero, B.B., Murúa, R.B., 1990. A new species of whipworm from a South American hystricomorph rodent. Mem. Inst. Oswaldo Cruz 85, 211–213.
Babero, B.B., Cattan, P.E., Cabello, C., 1975. *Trichuris bradleyi* sp. n. a whipworm from *Octodon degus* in Chile. J. Parasitol. 61, 198–206.
Babero, B.B., Cattan, P.E., Cabello, C., 1976. A new species of whipworm from the rodent *Akodon longipilis* in Chile. Trans. Am. Microsc. Soc. 95, 232–235.
Barus, V., Madjumdar, G., Mikailov, T.K., 1975. Morphology and taxonomy of *Trichocephalus myocastoris* (Enigk, 1933). Folia Parasitol. 22, 207–213.
Blouin, M.S., Yowell, C.A., Courtney, C.H., Dame, J.B., 1997. *Haemonchus placei* and *Haemonchus contortus* are distinct species based on mtDNA evidence. Int. J. Parasitol. 27, 1383–1387.
Cafrune, M.M., Aguirre, D.H., Rickard, L.G., 1999. Recovery of *Trichuris tenuis* Chandler, 1930, from camelids (*Lama glama* and *Vicugna vicugna*) in Argentina. J. Parasitol. 85, 961–962.
Callejón, R., de Rojas, M., Foronda, P., Feliú, C., Guevara, D., Cutillas, C., 2010. Molecular evolution of *Trichuris muris* isolated from different Muridae hosts in Europe. Parasitol. Res. 107, 631–641.
Callejón, R., Halajian, A., De Rojas, M., Marrugal, A., Guevara, D.C., Cutillas, C., 2012. 16S partial gene DNA and internal transcribed spacers ribosomal DNA as differential markers of *Trichuris discolor* populations. Vet. Parasitol. 186, 350–363.
Callejón, R., Nadler, S., De Rojas, M., Zurita, A., Petrášová, J., Cutillas, C., 2013. Molecular characterization and phylogeny of whipworm nematodes inferred from DNA sequences of *cox1* mtDNA and 18S rDNA. Parasitol. Res. 112, 3933–3949.
Callejón, R., Cutillas, C., Nadler, S.A., 2015. Nuclear and mitochondrial genes for inferring *Trichuris* phylogeny. Parasitol. Res. 114, 4591–4599.
Callejón, R., Robles, M.d.R., Panei, C.J., Cutillas, C., 2016. Molecular diversification of *Trichuris* spp. from Sigmodontinae (Cricetidae) rodents from Argentina based on mitochondrial DNA sequences. Parasitol. Res. 115, 2933–2945.
Cameron, T.W.M., Reesal, M.R., 1951. Studies on the endoparasitic fauna of Trinidad mammals. Can. J. Zool. 29, 276–289.
Chandler, A.C., 1930. Specific characters in the genus *Trichuris*, with description of a new species *Trichuris tenuis*, from a camel. J. Parasitol. 16, 198–206.
Chandler, A.C., 1945. *Trichuris* species from California. J. Parasitol. 31, 284–287.
Correa-Gomes, D., Lanfredi, R.M., Pinto, R.M., De Souza, W., 1992. Description of *Trichuris travassosi* n. sp. (Nematoda:Trichurinae) from a Brazilian rodent, by light and scanning electron microscopy. Mem. Inst. Oswaldo Cruz 87, 1–10.
Cox, P.G., Hautier, L., 2015. Evolution of the rodents. In: Advances in Phylogeny, Functional Morphology, and Development. Cambridge University Press, Cambridge, UK.
Cutillas, C., German, P., Arias, P., Guevara, D.C., 1996. Characterization of *Trichuris skrjabini* by isoenzyme gel electrophoresis: comparative study with *Trichuris ovis*. Acta Trop. 62, 63–69.
Cutillas, C., Oliveros, R., De Rojas, M., Guevara, D.C., 2002. Determination of *Trichuris*

muris from murid hosts and *Trichuris arvicolae* (Nematoda) from arvicolid rodents by amplification and sequentiation of the ITS1-5.8S-ITS2 segment of the ribosomal DNA. Parasitol. Res. 88, 574–582.
Cutillas, C., Oliveros, R., De Rojas, M., Guevara, D.C., 2004. Determination of *Trichuris skrjabini* by sequencing of the ITS1-5.8S-ITS2 segment of the ribosomal DNA: comparative molecular study of different species of trichurids. J. Parasitol. 90, 648–652.
Cutillas, C., De Rojas, M., Ariza, C., Úbeda, J.M., Guevara, D.C., 2007. Molecular identification of *Trichuris vulpis* and *Trichuris suis* isolated from different hosts. Parasitol. Res. 100, 383–389.
Cutillas, C., Callejón, R., De Rojas, M., Tewes, B., Úbeda, J.M., Ariza, C., Guevara, D.C., 2009. *Trichuris suis* and *Trichuris trichiura* are different nematode species. Acta Trop. 11, 299–307.
Cutillas, C., De Rojas, M., Zurita, A., Oliveros, R., Callejón, R., 2015. *Trichuris colobae* n. sp. (Nematoda: Trichuridae), a new species of *Trichuris* from *Colobus guereza kikuyuensis*. Parasitol. Res. 113, 2725–2732.
D'Elía, G., Pardiñas, U.F.J., 2015. Subfamily Sigmodontinae Wagner, 1843. In: Patton, J.L., Pardiñas, U.F.J., D'Elía, G. (Eds.), Mammals of South America, Volume 2 – Rodents. The University of Chicago Press, Chicago, pp. 63–70.
Digiani, M.C., Notarnicola, J., Paulos, M.S., 2013. *Mazzanema* n. gen. and *Mazzanema fortuita* n. comb. for *Longistriata fortuita* Freitas, lent and Almeida, 1937 (Nematoda, Heligmonellidae), a parasite of the marsh rat *Holochilus chacarius* (Rodentia, Cricetidae) from northern Argentina. J. Parasitol. 99, 816–820.
Digiani, M.C., Notarnicola, J., Navone, G.T., 2015. Rediscovery and new morphological data on two *Hassalstrongylus* (Nematoda: Heligmonellidae) coparasitic in the marsh rat *Holochilus chacarius* (Rodentia: Cricetidae) from Argentina. J. Parasitol. 101, 556–564.
Doležalová, J., Oborník, M., Hajdušková, E., Jirků, M., Petrželková, K.J., Bolechová, P., Cutillas, C., Callejón, R., Jaroš, J., Beránková, Z., Modrý, D., 2015. How many species of whipworms do we share? Whipworms from man and other primates form two phylogenetic lineages. Folia Parasitol. 62, 063. http://dx.doi.org/10.14411/fp.2015.063.
Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinf. 5, 113.
Feliú, C., Spakulová, M., Casanova, J.C., Renaud, F., Morand, S., Hugot, J.P., Santalla, F., 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). J. Parasitol. 86, 442–449.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial *cytochrome* c *oxidase* subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.
Frandsen, J.C., Grundmann, A.W., 1961. *Trichuris stansburyi* and *Gongylonema mysciphilia*, two new species of nematodes from the deer mouse in Utah. Proc. Helminthol. Soc. Wash. 28, 91–94.
Gasser, R.B., Nansen, P., Guldberg, P., 1996. Fingerprinting sequence variation in ribosomal DNA of parasites by DGGE. Mol. Cell. Probes 10, 99–105.
Guardone, L., Deplazes, P., Macchioni, F., Magi, M., Mathis, A., 2013. Ribosomal and mitochondrial DNA analysis of *Trichuridae nematodes* of carnivores and small mammals. Vet. Parasitol. 197, 364–369.
Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.
Hall, J.E., Sonnenberg, B., 1956. An apparent case of human infection with the whipworm of dogs, *Trichuris vulpis* (Froelich, 1789). J. Parasitol. 42, 197–199.
Huelsenbeck, J.P., Rannala, B., 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. Science 276, 227–232.
Hugall, A.F.C., Moritz, J.S., Wolstenholme, D.R., 1994. Low, but strongly structured mitochondrial DNA diversity in root-knot nematodes (Meloidogyne). Genetics 136, 903–912.
Keddie, E.M., Higazi, T., Unnasch, T.R., 1998. The mitochondrial genome of Onchocerca volvulus: sequence, structure, and phylogenetic analysis. Mol. Biochem. Parasitol. 95, 111–127.
Kenneth, S.T., Leep, D.L., 1972. Redescription of *Trichuris fossor* Hall, 1916 (Nematoda: Trichuridae) from the northern pocket gopher, *Thomomys talpoides*. Proc. Helminthol. Soc. Wash. 39, 203–205.
Knight, R.A., 1984. Morphological differences in *Trichuris ovis* associated with different host species. J. Parasitol. 70, 842–843.
Lavrov, D.V., Brown, W.M., 2001. *Trichinella spiralis* mtDNA: a nematode mitochondrial genome that encodes a putative ATP8 and normally structured tRNAs and has gene arrangement relatable to those of coelomate metazoans. Genetics 157, 621–637.
Lent, H., Freitas, J.F.T., 1936. Sobre o *Trichuris* da nutria. An. Acad. Bras. Cienc. 8, 319–322.
Liu, G.H., Gasser, R.B., Su, A., Nejsum, P., Peng, L., Lin, R.Q., Li, M.W., Xu, M.J., Zhu, X.Q., 2012. Clear genetic distinctiveness between human- and pig-derived *Trichuris* based on analysis of mitochondrial datasets. PLoS Negl. Trop. Dis. 6, e1539. http://dx.doi.org/10.1371/journal.pntd.0001539.
Liu, G.H., Gasser, R.B., Nejsum, P., Wang, Y., Chen, Q., Song, H.Q., Zhu, X.Q., 2013. Mitochondrial and nuclear ribosomal DNA evidence supports the existence of a new *Trichuris* species in the endangered François' leaf-monkey. PLoS One 8, e66249.
Massoia, E., 1971. Caracteres y rasgos bioecológicos de *Holochilus brasiliensis chacarius* Thomas ("rata nutria") de la provincia de Formosa y comparaciones con *Holochilus brasiliensis vulpinus* (Brants) (Mammalia, Rodentia, Cricetidae). Revista de Investigaciones Agropecuarias, INTA, ser. 1. Biología y Producción Animal 8, 13–40.
Massoia, E., 1974. Ataques graves de *Holochilus* y otros roedores a cultivos de caña de azúcar. Instituto Nacional de Tecnología Agropecuaria, Revista de Información sobre Investigación y Desarrollo Agropecuario. IDIA, pp. 321–324.
Massoia, E., 1976. Mammalia. In: Ringuelet, R.A. (Ed.), Fauna de agua dulce de la República Argentina. Fundación Editorial Ciencia y Cultura, Buenos Aires, Argentina, pp. 1–128.
Mirdha, B.R., Singh, Y.G., Samantray, J.C., Mishra, B., 1998. *Trichuris vulpis* infection in slum children. Indian J. Gastroenterol. 17, 154.
Morini, E.G., Boero, J., Rodriguez, A., 1955. Parásitos intestinales en el «Marra» (*Dolichotis patagonum patagonum*). In: Publicación Misión de Estudios de Patología Regional Argentina. 26. pp. 83–89.
Nagano, I., Wu, Z., Matsuo, A., Pozio, E., Takahashi, Y., 1999. Identification of *Trichinella* isolates by polymerase chain reaction-restriction fragment length polymorphism of the mitochondrial cytochrome c-oxidase subunit I gene. Int. J. Parasitol. 29, 1113–1120.
Notarnicola, J., 2005. Description of adult and fourth-stage larva of *Litomosoides navonae* n. sp. (Nemadoda: Onchocercidae), a parasite of five species of sigmodontine rodents from northeastern Argentina. Syst. Parasitol. 62, 171–183.
Notarnicola, J., Digiani, M.C., López, P., 2010. Redescription of the nematodes *Litomosoides patersoni* (Mazza, 1928) (Onchocercidae) and *Stilestrongylus stilesi* Freitas, Lent and Almeida, 1937 (Heligmonellidae) parasites of *Holochilus chacarius* (Rodentia, Cricetidae) from Salta, Argentina. J. Parasitol. 96, 993–1001.
Oliveros, R., Cutillas, C., De Rojas, M., Arias, P., 2000. Characterization of four species of *Trichuris* (Nematoda: Enoplida) by their second internal transcribed spacer ribosomal DNA sequence. Parasitol. Res. 86, 1008–1013.
Panti May, A., Robles, M.d.R., 2016. A new species of *Trichuris* (Nematoda: Trichuridae) from *Heteromys gaumeri* (Rodentia: Heteromyidae) in Yucatan, Mexico. Syst. Parasitol. 93, 721–730.
Pfaffenberger, G.S., Best, T., 1989. *Trichuris elatoris* sp. n. (Nematoda: Trichuridae) from the Texas kangaroo rat (*Dipodomys elator*). Proc. Helminthol. Soc. Wash. 56, 76–81.
Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.
Rambaut, A., Drummond, A., 2007. Tracer v1.6. Available from. http://beast.bio.ed.ac.uk/.
Read, C.P., 1956. *Trichuris dipodomis* n. sp., from Ord's kangaroo rat. Proc. Helminthol. Soc. Wash. 23, 119.
Robles, M.d.R., 2011. New species of *Trichuris* (Nematoda: Trichuridae) from *Akodon montensis* Thomas, 1913 of the Paranaense forest in Argentina. J. Parasitol. 97, 319–327.
Robles, M.d.R., Navone, G.T., 2006. Redescription of *Trichuris laevitestis* (Nematoda: Trichuridae) from *Akodon azarae* and *Scapteromys aquaticus* (Sigmodontinae: Muridae) in Buenos Aires province, Argentina. J. Parasitol. 92, 1053–1057.
Robles, M.d.R., Navone, G.T., Notarnicola, J., 2006. A new species of *Trichuris* (Nematoda: Trichuriidae) from Phyllotini rodents in Argentina. J. Parasitol. 92, 100–104.
Robles, M.d.R., Cutillas, C., Panei, C.J., 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, e112069. http://dx.doi.org/10.1371/journal.pone.0112069.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Rossin, M.A., Malizia, A.I., 2005. Redescription of Trichuris pampeana (Nematoda: Trichuridae) from South American subterranean rodent *Ctenomys talarum* Thomas, 1898 (Rodentia: Octodontidae). J. Parasitol. 91, 127–130.
Salaba, O., Rylková, K., Vadlejch, J., Petrtýl, M., Scháňková, Š., Brožová, A., Jankovská, I., Jebavý, L., Langrová, I., 2013. The first determination of *Trichuris* sp. from roe deer by amplification and sequentiation of the ITS1-5.8S-ITS2 segment of ribosomal DNA. Parasitol. Res. 112, 955–960.
Schwartz, B., 1926. A possible new source of infection of man with *Trichuris*, with a consideration of the question of physiological varieties among helminths. Archiv. fur Schiffs- und Tropen-Hygiene 9, 544–577.
Steppan, S.J., Adkins, R.M., Anderson, J., 2004. Phylogeny and divergence-date estimates of rapid radiations in muroid rodents based on multiple nuclear genes. Syst. Biol. 53, 533–553.
Suriano, D.M., Navone, G.T., 1994. Three new species of the genus *Trichuris* Roederer, 1761 (Nematoda-Trichuridae) from Caviomorph and Cricetid rodents in Argentina. Res. Rev. Parasitol. 54, 39–46.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.
Tiner, J.D., 1950. Two new species of *Trichuris* from North America with description of *Trichuris leporis* (Nematoda: Aphasmidia). J. Parasitol. 36, 350–354.
Torres, E.J.L., Nascimento, A.P.F., Menezes, A.O., Garcia, J., dos Santos, M.A.J., Maldonado, A., Miranda, K., Lanfredi, R.M., de Souza, W., 2011. A new species of *Trichuris* from *Thrichomys apereoides* (Rodentia: Echimyidae) in Brazil: morphological and histological studies. Vet. Parasitol. 176, 226–235.
Vazquez, T.O., Martínez, B.I.I., Romero, C.R., Valencia, R.S., Tay, Z.J., 1997. Mixed infection by *Trichuris trichiura* and *Trichuris vulpis*. Rev. Gastroenterol. Peru 17, 255–258.
Voglino, D., Pardiñas, U.F.J., Teta, P., 2004. *Holochilus chacarius chacarius* (Rodentia, Cricetidae) en la provincia de Buenos Aires, Argentina. Mastozool. Neotrop. 11, 243–247.