Parasitol Res (2016) 115:2933–2945
DOI 10.1007/s00436-016-5045-y

ORIGINAL PAPER

Molecular diversification of *Trichuris* spp. from Sigmodontinae (Cricetidae) rodents from Argentina based on mitochondrial DNA sequences

Rocío Callejón[1] · **María Del Rosario Robles**[2] · **Carlos Javier Panei**[3,4] · **Cristina Cutillas**[1]

Received: 10 February 2016 / Accepted: 7 April 2016 / Published online: 16 April 2016

Abstract A molecular phylogenetic hypothesis is presented for the genus *Trichuris* based on sequence data from mitochondrial *cytochrome c oxidase 1* (*cox1*) and *cytochrome b* (*cob*). The taxa consisted of nine populations of whipworm from five species of Sigmodontinae rodents from Argentina. Bayesian Inference, Maximum Parsimony, and Maximum Likelihood methods were used to infer phylogenies for each gene separately but also for the combined mitochondrial data and the combined mitochondrial and nuclear dataset. Phylogenetic results based on *cox1* and *cob* mitochondrial DNA (mtDNA) revealed three clades strongly resolved corresponding to three different species (*Trichuris navonae*, *Trichuris bainae*, and *Trichuris pardinasi*) showing phylogeographic variation, but relationships among *Trichuris* species were poorly resolved. 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. Thus, populations of *T. bainae* and *T. pardinasi* could be affected by geographical factors and co-divergence parasite-host.

Keywords Argentina · *cob* · *cox1* · ITS2 · Sigmodontinae · *Trichuris*

Rocío Callejón and María Del Rosario Robles contributed equally to this paper and should be considered as co-first authors

✉ Cristina Cutillas
cutillas@us.es

[1] Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, Prof. García González 2, Sevilla 41012, Spain

[2] Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CCT–CONICET–La Plata/Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina

[3] Virología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina

[4] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

Introduction

The Old World mice and rats (Murinae-Muridae) are the largest mammalian subfamily, comprising over 500 species (Musser and Carleton 2005). Possibly related to the Murinae are the Gerbillinae, hopping species that live in African and Asian deserts. The second-largest mammalian subfamily is the Sigmodontinae (Cricetidae), endemic from American continent. In the same family, they are often grouped with the Cricetinae (Old World hamsters) and sometimes with Arvicolinae (voles, lemmings, and muskrats). The Arvicolinae are a diverse Holarctic group (>125 species) (Steppan et al. 2004). The importance of sigmodontines cannot be underestimated. Some species are reservoirs of the etiological agents of a number of human and animal diseases. In South America, all hantaviruses known to cause Hantavirus Pulmonary Syndrome are associated with species of sigmodontine rodents, including *Oligoryzomys* spp., *Necromys benefactus* (Thomas, 1919), *Akodon azarae* (Fischer, 1829), *Calomys laucha* (Fischer, 1814), and *Sigmodon alstoni* (Thomas, 1881) (D'Elía 2003). In addition, sigmodontine rodents are reservoirs of protozoa (Jansen and Roque 2010; Lanos-Cuentas et al. 1999; Lallo et al. 2009) and helminthes (Maldonado et al. 2012; Robles et al. 2008, 2016; Cubillos et al. 1991; Miño et al. 2013; Kuhnen et al. 2012). In most of the cases, the infections in this host group may indicate the occurrence and level of environmental contamination

of different zoonotic parasites, affecting many domestic animals (Herrera et al. 2005; Reperant et al. 2009).

Trichuris Roederer, 1761 (Nematoda: Trichuridae) has a cosmopolitan distribution and comprises about 80 species that parasitize a broad spectrum of domestic and wild mammals (ruminants, marsupials, rodents, and primates, including humans) (Cafrune et al. 1999; Robles et al. 2006; Robles 2011). To date, 27 *Trichuris* species have been described from 10 families' rodents of North and South America. Four species are parasites of Cricetidae in North America: *Trichuris opaca* Barker and Noyes 1915 from Arvicolinae, *Trichuris neotomae* Chandler 1945, *Trichuris peromysci* Chandler 1946, and *Trichuris stansburyi* Frandsen and Grundmann 1961 from Neotominae; and six parasites of Cricetidae in South America: *Trichuris chilensis* Babero, Cattan and Cabello 1976; *Trichuris travassosi* Correa Gomes, Lanfredi, Pinto and Souza 1992; *Trichuris laevitestis* Suriano and Navone 1994; *Trichuris pardinasi* Robles, Navone and Notarnicola 2006; *Trichuris navonae* Robles 2011; and *Trichuris bainae* Robles, Cutillas, Panei and Callejón 2014, all from Sigmodontinae. The last four species are found from Argentina (Suriano and Navone 1994; Robles et al. 2006, 2014; Robles 2011).

Since 66 % of *Trichuris* spp. from Cricetidae share a similar morphological pattern (Tiner 1950; Babero et al. 1976; Correa-Gomes et al. 1992; Feliú et al. 2000; Robles et al. 2006; Robles 2011), some studies have used isoenzymatic patterns and molecular studies to differentiate these nematodes (Cutillas et al. 1996, 2002, 2004, 2007; Feliú et al. 2000). Gene sequences have proved useful for delimiting species with few detectable morphological differences (Cutillas et al. 2009; Liu et al. 2012), in sorting through misleading morphological variation to make an accurate species determination (Salaba et al. 2013), in discovering cryptic species of *Trichuris* in novel host species (Liu et al. 2013; Robles et al. 2014), or as independent corroborative data for species that are morphologically distinct (Cutillas et al. 2014).

Trichuris species from Sigmodontinae rodents present an interesting evolutive history since these hosts are endemic from American rodents. These rodents live in different environment and geographical distributions, and have been included in diverse phylogenetic hypotheses (e.g., Steppan et al. 2004; Cox and Hautier 2015). Five out of six species of *Trichuris* from sigmodontine rodents present similar morphological features, including the absence of a spicular tube, spicular sheath with spines (mostly with a cylindrical shape), and a non-protrusive vulva (Tiner 1950; Babero et al. 1976; Correa-Gomes et al. 1992; Feliú et al. 2000; Robles et al. 2006; Robles 2011). Therefore, it is probable finding several cryptic species. In this context, although most studies of *Trichuris* molecular systematics have focused on delimiting species, accurately inferring relationships among species is essential for testing macroevolutionary hypotheses including co-phylogeny and biogeography, both in support of the phylogenetic relationships proposed for their hosts and those linked to the geographic areas of distribution.

Molecular markers used for species-level questions in *Trichuris* include the Internal Transcribed Spacer 1 and 2 nuclear regions (ITS1 and ITS2) (Oliveros et al. 2000; Cutillas et al. 2002, 2004, 2007, 2009, 2015; Callejón et al. 2012b; Salaba et al. 2013; Robles et al. 2014; Doležalová et al. 2015), 18S nuclear ribosomal RNA gene (Callejón et al. 2013; Guardone et al. 2013; Doležalová et al. 2015), mitochondrial 16S ribosomal RNA gene (Callejón et al. 2012b), and protein-coding mitochondrial genes, including the 12 common genes obtained from mitochondrial genome sequences (Liu et al. 2012, 2013), *cytochrome c oxidase 1* (*cox1*) mitochondrial DNA partial gene (Callejón et al. 2013; Doležalová et al. 2015) and *cytochrome b* (*cob*) mitochondrial DNA partial gene (Cutillas et al. 2015; Callejón et al. 2015). These genes have different attributes and shortcomings for inferring *Trichuris* phylogeny, including substantially different rates and patterns of evolution. Studies combining more than one locus, such as nuclear and mitochondrial genes, together with morphological and ontogenetic comparisons, provide many advantages over single-locus studies, particularly for estimates of species-level relationships (Ballard and Rand 2005). Thus, different authors evaluated phylogenetic hypotheses for *Trichuris* based on two independent loci (Callejón et al. 2013, 2015; Liu et al. 2013; Doležalová et al. 2015).

The main objective of this manuscript is to develop a molecular phylogenetic hypothesis for whipworm species isolated from five species of Sigmodontinae rodents from Argentina using mitochondrial genes, including partial sequences of *cox1* and *cob* mtDNA. A phylogenetic reconstruction based on combined analysis of mitochondrial genes (*cox1* and *cob*) and mitochondrial and nuclear markers (*cox1*, *cob*, and ITS2) is also evaluated.

Materials and methods

Sampling

Twenty adult *Trichuris* specimens were collected from different cricetid (Sigmodontinae) rodents and geographical origins (Argentina) (Table 1): *T. navonae*—seven from *Akodon montensis* Thomas, 1913 and three from *Thaptomys nigrita* Lichtenstein 1829; *T. bainae*—four from *Sooretamys angouya* Fischer 1814; and *T. pardinasi*—three from *Phyllotis bonariensis* Crespo 1964 and three from *Phyllotis xanthopygus* Waterhouse 1837.

Akodon montensis is widespread from eastern Paraguay, northeastern Argentina in gallery forests along rivers or wetlands in the provinces of Formosa, Chaco, Corrientes, and Misiones, and along the southern coast of Brazil. Moreover,

Table 1 *Trichuris* specimens collected in different rodent species from Argentina

Trichuris spp.	Number of studied specimens		Host species	Locality/province (code)	Geographical point
	Cox1	*Cob*			
T. navonae	2	2	*Akodon montensis*	Refugio Moconá, Departamento San Pedro, Misiones province (RM)	27°8′ S, 53°55′ W
	3	3		Reserva de Vida Silvestre Urugua-í, Fundación Vida Silvestre, Departamento General Manuel Belgrano, Misiones province (UR)	25°59′08.19″ S, 54°06′36.15″ W
	2	2		Campo Anexo M. Belgrano, INTA, San Antonio, Departamento General Manuel Belgrano, Misiones province (SA)	26°02′52.60″ S, 53°46′21″ W
	1	2	*Thaptomys nigrita*	Refugio Moconá, Departamento San Pedro, Misiones province (SA)	26°02′54.21″ S, 53°46′32.40″ W
	–	1		Campo Anexo M. Belgrano, INTA, San Antonio, Departamento General Manuel Belgrano, Misiones province (RM)	27°8′ S, 53°55′ W
T. bainae	2	2	*Sooretamys angouya*	Refugio Moconá, Departamento San Pedro, Misiones province (RM)	27°8′ S, 53°55′ W
	2	2		Estación de Animales Silvestres Guaycolec, Departamento Formosa, Formosa province (GU)	25°58′51″ S, 58°9′52″ W
T. pardinasi	3	3	*Phyllotis bonariensis*	Cerro Bahía Blanca, Parque Provincial Ernesto Tornquist, Sierra de la Ventana, Partido de Tornquist, Buenos Aires province (SV)	38°04′47.99″ S, 62°00′22.48″ W
	3	2	*Phyllotis xanthopygus*	Cerro Los Linderos, Departamento Calamuchita, Córdoba province (SC)	32°00′17.82″ S, 64°56′ 01.51″ W

this species is the dominant member of the small mammal assemblage in both secondary forest and other anthropogenic disturbed habitats in Argentina. *Thaptomys nigrita* is restricted to moist tropical forest and second-growth forest in southeastern Brazil, eastern Paraguay, and northeastern Argentina. *Sooretamys angouya* is widely distributed in eastern South America, from the Brazilian Atlantic rainforest to the humid forests of eastern Argentina and Paraguay. *Phyllotis bonariensis* lives in rocky places at about 400 m elevation in a range of hills north of Bahia Blanca, Buenos Aires province. *Phyllotis xanthopygus* lives in rocky microhabitats on arid Andean slopes from Junín in Peru to Magallanes in Chile and Santa Cruz in Argentina, where boulder fields, rock slides, cliffs, small shale outcroppings, stone walls, and stone huts are all satisfactory habitations. Also, this species is found at higher elevations in the Sierras of San Luis and Córdoba provinces in Argentina (D'Elia and Pardiñas 2015).

Species identification was performed according to previous studies (Robles et al. 2006, 2014; Robles 2011). Worms were washed extensively in 0.9 % saline solution and stored in 70 % ethanol until required for DNA extraction, PCR amplification, and posterior sequencing.

Sequences

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.

Mitochondrial *cox1* partial gene was amplified by PCR using an Eppendorf AG thermocycler and conditions specified for *Trichinella* isolates by Nagano et al. (1999) and sequenced using the following primers: The forward PCR primer was modified from the reverse primer of Folmer et al. (1994): HC02198F 5′-TGATTTTTTGGTCACCCTGAAGTTTA-3′, and the reverse PCR primer was modified from Nagano et al. (1999) to correspond to more broadly conserved *cox1* sequence: CORA 5′-ACYACATAGTAGGTRTCATG-3′. These two primers were used successfully for *cox1* PCR amplification of all species of *Trichuris* studied. Amplification reactions consisted of 5 μl 10× PCR buffer, 2 μl 10 mM dNTP mixture (0.4 mM each), 3 μl 50 mM $MgCl_2$, 5 μl primer mix (1 mM each), 5 μl template DNA, 0.5 μl Taq DNA polymerase (2.5 U), and autoclaved distilled water to 50 μl. The following PCR conditions were applied: 94 °C for 5 min (denaturing), 40 cycles at 94 °C for 1 min (denaturing), 48 °C for 1 min (annealing), 72 °C for 1 min (primer extension), followed by a post-amplification extension at 72 °C for 7 min.

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*

trichiura Linnaeus 1771 (NC_017750). The forward PCR primer D769 5′-GAGTAATTTTTATAATRCGRGAAGT-3′ and the reverse PCR primer D770 5′-AATTTTCAGGRTCTCTRC TTCAATA-3′ were used successfully for *cob* PCR amplification of all species of *Trichuris* analyzed. Amplification reactions consisted of 0.5 mM of each primer, 200 mM dNTP, 3 mM $MgCl_2$, and 1 U of AmpliTaq® polymerase in a volume of 25 μl. PCR cycling parameters included 94 °C for 5 min (denaturing), 36 cycles of 94 °C for 30 s (denaturing), 50 °C for 30 s (annealing), and 72 °C for 30 s (primer extension), followed by a post-amplification extension at 72 °C for 7 min.

The PCR products were checked on ethidium bromide-stained 2 % Tris-Borate-EDTA (TBE) agarose gels. Bands were eluted from the 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.

Analyses

Sequences based on mtDNA (*cox1* and *cob*) were aligned using CLUSTAL X (Larkin et al. 2007) as described by Callejón et al. (2013). The nucleotide sequences of the protein-coding genes (*cox1* and *cob*) were first translated "in silico" to confirm that they lacked internal stop codons and to verify (by BLAST match) that inferred amino acid sequences were characteristic of the predicted nematode protein.

ITS2 sequences based on nuclear ribosomal DNA (rDNA) obtained from GenBank (Robles et al. 2014) were incorporated to a comparative analysis (Table 2). Nucleotide sequence data reported in this paper are available in the GenBank™ database (accession numbers in Table 3).

Sequences from two outgroup taxa (Table 2) were included in each analysis to root the phylogenetic trees: *Trichuris muris* Schrank, 1788 from *Mus domesticus* Schwarz and Schwarz, 1943 (Murinae) and *Trichuris arvicolae* Feliú, Spakulová, Casanova, Renauld, Morand, Hugot et al. 2000 from *Myodes glareolus* Schreber 1780 (Arvicolinae) from Spain.

Phylogenetic trees were inferred using nucleotide data and produced using three methods: Bayesian Inference (BI), Maximum Parsimony (MP), and Maximum Likelihood (ML) using the PhyML package (Guindon and Gascuel 2003), MEGA 5.0 program (Tamura et al. 2011), and MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003), respectively. jModelTest version 0.1.1 (Posada 2008) was used to compare the fit of nucleotide substitution models using the Akaike information criterion. For the study of the three concatenated datasets (*cox1*, *cob*, and ITS2), analyses were partitioned by gene. For Bayesian analysis, models for individual genes within partitions were those selected by jModelTest. For ML inference using PhyML, the rapid bootstrap algorithm (with GTRCAT) was used (1000 replicates) to assess the relative reliability of clades, whereas the best ML tree was found using the GTRGAMMA model. Models selected by jModelTest for BI included nst = 6 with gamma rates (*cox1* and *cob*), nst = 6 with invgamma rates (ITS2), and nst = mixed for concatenated analysis. For the Bayesian analysis, we ran three independent runs of four Markov chains for 10 million generations, sampling every 500 generations.

Results

Cox1 mtDNA partial gene

A single *cox1* PCR product was amplified from each *Trichuris* species included in this study. The partial sequences were 401 and 405 base pairs (bp) in length. The G + C content of the *cox1* partial gene of *Trichuris* species was 35–38 %, the maximum values corresponding to three individuals of *T. bainae* and the minimum values to six individuals of *T. navonae* from Misiones (Table 3).

The multiple alignment of 20 *cox1* nucleotide sequences (including outgroups) yielded a dataset of 341 characters. jModelTest determined that the best-fit model for *cox1* mtDNA datasets was GTR + G, which was used for Maximum Likelihood and Bayesian analysis.

The range of intra-population similarity of *Trichuris* spp. based on *cox1* mtDNA sequences was 97.7–100 % with the maximum value corresponding to *T. bainae* from Formosa and the minimum value corresponding to *T. pardinasi* from Buenos Aires. On the other hand, the inter-population similarity was analyzed in *T. bainae* isolated from Misiones and Formosa with the maximum value (99.6 %) and *T. pardinasi* isolated from Córdoba and Buenos Aires with the minimum value (97.0 %).

The comparative study between different *cox1* mtDNA sequences obtained for each species (alignment not shown) revealed the highest similarity (90.4 %) between *T. pardinasi* from Córdoba and *T. bainae* from Misiones, whereas the lowest similarity (86.7 %) was observed between *T. navonae* from Misiones and *T. pardinasi* from Córdoba. The *cox1* datasets provided moderate phylogenetic resolution among most *Trichuris* taxa regardless of inference method. The consensus tree showed three clear clades corresponding with three different *Trichuris* spp. with good resolution; nevertheless, within the *Trichuris* spp. group from Argentina, all relationships among clades were not resolved by the three methods (BI, MP, and ML) (Table 4). The topology showed all species of *Trichuris* from South America (Argentina) separated from those belonging to *Trichuris* spp. from Europe (Spain) with absolute support (100 % Bayesian Posterior Probabilities (BPP) and 100 % ML Bootstrap Value (BV)). Furthermore, *T. arvicolae* from Europe appeared clustered together and

Table 2 Sequences of *Trichuris* spp. and outgroup species obtained from GenBank and used for phylogenetic analyses

Species	Host species/geographical origin	Marker	Accession number
Trichuris navonae	*A. montensis*/Refugio Moconá (Misiones)	ITS2	HG934435
			HG934436
			HG934437
			HG934438
	A. montensis/Urugua-í (Misiones)	ITS2	HG934443
			HG934444
			HG934441
	A. montensis/San Antonio (Misiones)	ITS2	HG934434
	T. nigrita/San Antonio (Misiones)	ITS2	HG934440
	T. nigrita/Urugua-í (Misiones)	ITS2	HG934439
Trichuris bainae	*S. angouya*/Refugio Moconá (Misiones)	ITS2	HG934431
			HG934432
	S. angouya/Guaycolec (Formosa)	ITS2	HG934433
Trichuris pardinasi	*P. xanthopygus*/Sierra de la Ventana (Buenos Aires)	ITS2	HG934448
			HG934445
			HG934449
	P. xanthopygus/Sierra de Cordoba (Cordoba)	ITS2	HG934447
			HG934446
Outgroup			
Trichuris muris	*Mus domesticus*/Spain	*Cox1*	HE653130
		Cob	LM994701
		ITS2	FN543175
Trichuris arvicolae	*Myodes glareolus*/Spain	*Cox1*	FR851284
		Cob	LM994698
		ITS2	FR849660

separated of *T. muris* from Europe with high support (100 % BPP, 99 % MP, and 92 % ML BV) (unpublished data).

Trichuris spp. from Argentina showed three main clades by BI, MP, and ML methods (Table 4). Clade 1 clustered *T. navonae* from different hosts and localities from Misiones. Clade 2 clustered *T. bainae* from Misiones and Formosa. Finally, Clade 3 clustered *T. pardinasi* populations from Buenos Aires and Córdoba. In addition, a subclade 2a including *T. bainae* from Formosa was observed by the three methods with robust support (Table 4) separated of *T. bainae* from Misiones and a subclade 3a including *T. pardinasi* from Buenos Aires with moderate support which appeared separated from *T. pardinasi* from Córdoba.

Cob mtDNA partial gene

A single *cob* PCR product was amplified from each *Trichuris* species included in this study. The partial sequences were 505 bp in length. The G+C content of the *cob* partial gene of *Trichuris* species ranged from 28 to 32 %, the maximum values corresponding to one individual of *T. pardinasi* from Córdoba and one individual of *T. pardinasi* from Buenos Aires and the minimum values to seven individuals of *T. navonae* from Misiones (Table 3).

The multiple alignment of 21 *cob* nucleotide sequences (including outgroups) yielded a dataset of 494 characters. jModelTest determined that the best-fit model for *cob* mtDNA datasets was GTR+G, which was used for Maximum Likelihood and Bayesian analysis.

The range of intra-population similarity of *Trichuris* spp. based on *cob* mtDNA sequences was 98.4–99.8 % with the maximum value corresponding to *T. bainae* from Formosa and Misiones and the minimum value corresponding to *T. pardinasi* from Córdoba. On the other hand, the inter-population similarity was analyzed in *T. bainae* isolated from Buenos Aires and Formosa with the maximum value (99.6 %) and *T. pardinasi* isolated from Córdoba and Buenos Aires with the minimum value (98.0 %).

When the *cob* mtDNA sequences of the different species and host isolates of the genus *Trichuris* were compared, the highest similarity (88.9 %) was obtained between *T. navonae* from Misiones and *T. bainae* from Misiones and Formosa, respectively, whereas the lowest similarity (84.8 %) was observed between *T. pardinasi* compared with *T. navonae* and *T. bainae* populations. The *cob* datasets provided moderate

Table 3 GenBank accession number of *cox1* and *cob* partial gene sequences of 19 individuals of *Trichuris* spp. isolated from five rodent species from Argentina

Species	Host species/geographical origin	Number of base pairs	G + C%	Accession numbers
Cox1				
Trichuris navonae	*A. montensis*/Refugio Moconá (Misiones)	405	35	HG934459
		405	35	HG934462
	A. montensis/Urugua-í (Misiones)	405	36	HG934458
		405	36	HG934460
		405	35	HG934464
	A. montensis/San Antonio (Misiones)	405	35	HG934461
		405	35	HG934463
	T. nigrita/San Antonio (Misiones)	405	35	HG934457
Trichuris bainae	*S. angouya*/Refugio Moconá (Misiones)	405	38	HG934466
		405	37	HG934465
	S. angouya/Guaycolec (Formosa)	405	38	HG934467
		401	38	LN899586
Trichuris pardinasi	*P. bonariensis*/Sierra de la Ventana (Buenos Aires)	405	37	HG934451
		405	37	HG934453
		405	36	HG934452
	P. xanthopygus/Sierra de Córdoba (Córdoba)	405	37	HG934455
		405	36	HG934454
		405	37	HG934456
Cob				
Trichuris navonae	*A. montensis*/Refugio Moconá (Misiones)	505	28	LN899565
		505	28	LN899566
	A. montensis/Urugua-í (Misiones)	505	29	LN899567
		505	28	LN899568
		505	28	LN899569
	A. montensis/San Antonio (Misiones)	505	28	LN899571
		505	28	LN899570
	T. nigrita/San Antonio (Misiones)	505	28	LN899572
		505	29	LN899573
	T. nigrita/Refugio Moconá (Misiones)	505	29	LN899584
Trichuris bainae	*S. angouya*/Refugio Moconá (Misiones)	505	30	LN899574
		505	30	LN899575
	S. angouya/Guaycolec (Formosa)	505	30	LN899576
		505	30	LN899582
Trichuris pardinasi	*P. bonariensis*/Sierra de la Ventana (Buenos Aires)	505	31	LN899578
		505	31	LN899579
		505	32	LN899577
	P. xanthopygus/Sierra de Córdoba (Córdoba)	505	32	LN899580
		505	31	LN899581

phylogenetic resolution among most *Trichuris* taxa regardless of inference method. The consensus trees showed good resolution within the *Trichuris* spp. group from Argentina; however, all relationships among clades were not resolved by the three methods (BI, MP, and ML) (Table 4). The topology showed all species of *Trichuris* from South America (Argentina) separated from those belonging to *Trichuris* spp. from Europe (Spain) with absolute support (100 % BPP and 100 % ML BV) (Table 4).

Overall, in the *Trichuris* populations from Argentina, there were three clades (Table 4). Clade 1 clustered *T. navonae* from different hosts from different geographical localities from Misiones region, Clade 2 clustered *T. bainae* from Misiones and Formosa, and Clade 3 clustered *T. pardinasi* populations from Buenos Aires and Córdoba (Table 4). The topology showed that Clade 1 (*T. navonae*) related with Clade 2 (*T. bainae*). In addition, a subclade 2b clustered *T. bainae* from Misiones separated of *T. bainae* from Formosa. A subclade 3a including *T. pardinasi* from Buenos Aires with moderate support was recovered in *cob* data. In contrast to the *cox1* mtDNA, a subclade 2a corresponding to *T. bainae* from Formosa was not revealed by *cob* mtDNA sequences (Table 4).

Table 4 Monophyly of mitochondrial and ribosomal partial sequences of selected groups based on several combinations of datasets and inference methods

	Cox1 mtDNA BPP/MP/ML	*Cob* mtDNA BPP/MP/ML	Mitochondrial genes (*cox1* + *cob*) BPP/MP/ML	Mitochondrial (*cox1*, *cob*) and nuclear (ITS2, Robles et al. 2013) markers BPP/MP/ML
Trichuris populations from Argentina	100/–/100	100/–/100	100/–/100	100/–/100
Clade 1	69/98/100	100/100/100	100/100/100	100/100/99
Clade 2	90/99/100	99/100/100	100/100/100	100/100/99
Clade 3	95/99/100	–/100/–	100/100/–	100/100/95
Clade 1 clustered with Clade 2	–/68/–	96/–/60	100/68/80	100/75/–
Subclade 2a	98/89/82	–/–/–	93/91/88	One sequence of subclade 2a included in the phylogenetic analysis
Subclade 2b	–/–/–	82/–/–	–/–/–	–/–/–
Subclade 3a	90/60/100	61/84/80	100/85/70	100/85/86
Subclade 3b	–/–/–	–/–/–	–/–/–	–/–/–

BPP Bayesian Posterior Probability, *MP* Maximum Parsimony, *ML* Maximum Likelihood bootstrap. Clade 1: *T. navonae*; Clade 2: *T. bainae*, Subclade 2a: *T. bainae* from Formosa, Subclade 2b: *T. bainae* from Misiones; Clade 3: *T. pardinasi*, Subclade 3a: *T. pardinasi* from Buenos Aires, Subclade 3b: *T. pardinasi* from Córdoba

Phylogenetic relationship based on concatenated *cox1* and *cob* mtDNA sequence datasets

The combined analysis of mitochondrial genes (*cox1* and *cob*) revealed a similar topology than those obtained by separate analysis of the two genes (Fig. 1). Thus, three clades were observed according to three different species of *Trichuris*. The concatenated analysis of the mitochondrial genes showed that Clade 1 (*T. navonae*) related with Clade 2 (*T. bainae*) with moderate support (100 % BPP, 68 % MP, and 80 % ML BV).

In concordance with *cox1* and *cob* data, a subclade 2a and subclade 3a were recovered (Fig. 1, Table 4).

Phylogenetic relationship based on concatenated mtDNA (*cox1* and *cob*) and rDNA (ITS2) sequence datasets

BI, MP, and ML analysis of the combined mtDNA (*cox1* and *cob*) and rDNA (ITS2) datasets yielded a similar tree topology to that observed by partitioned mitochondrial and nuclear markers (Fig. 2). Phylogenetic trees revealed a strong support for Clade 1, Clade 2, and Clade 3 (>95 %). The combined analysis of the mtDNA and rDNA sequences revealed the sister-group relationship among Clade 1 and 2. Furthermore, in concordance with *cox1* and *cob* data, a subclade 3a including *T. pardinasi* from Buenos Aires with high support was recovered (Fig. 2 and Table 4).

Discussion

Comparative analyses of coding and non-coding regions of ribosomal DNA (rDNA) have become a useful tool for the construction of the phylogenetic trees of many organisms including nematodes (Subbotin et al. 2001). Internal Transcribed Spacer regions (ITS1, ITS2) of the rDNA are often useful for differentiating closely related nematode species (Xie et al. 1994). The ribosomal DNA segments ITS1 and ITS2 have been shown to be two of the best molecular markers for analyzing genetic relationships at species level in *Trichuris*. For example, sequences obtained by amplification of the ITSs of seven different *Trichuris* species (*Trichuris leporis* Frolich 1789, *T. ovis*, *T. muris*, *T. arvicolae*, *T. suis*, *T. discolor*, and *T. trichiura*) permit reliable diagnosis (Oliveros et al. 2000; Cutillas et al. 2002, 2004, 2007, 2009; Callejón et al. 2010, 2012a). Furthermore, a comparative phylogenetic study of ITS1 and ITS2 sequences from *Trichuris* species from different hosts revealed three different genetic lineages corresponding with host groups (Callejón et al. 2012a). Similarly, analyses of nuclear ribosomal RNA, including SSU (Callejón et al. 2013) and ITS sequences (Ravasi et al. 2012; Nissen et al. 2012), support the specific characterization of *T. suis* and the determination of a new species: *Trichuris colobae* Cutillas, De Rojas, Zurita, Oliveros and Callejón 2014, a parasite of the primate *Colobus guereza kikuyensis* Rúppell 1835 (Cutillas et al. 2014).

Nevertheless, previous studies have mainly relied on sequence analysis of the ITS regions (Cavallero et al. 2015; Ravasi et al. 2012; Ghai et al. 2014; Cutillas et al. 2009; Nissen et al. 2012), and as this region contains multiple repeats, the alignment of sequences even between closely related worms will include a number of gaps. This makes inference of the phylogenetic relationship between *Trichuris* species problematic, and it is therefore highly recommended to supplement such analysis with other genetic markers such as mitochondrial DNA genes (Callejón et al. 2013).

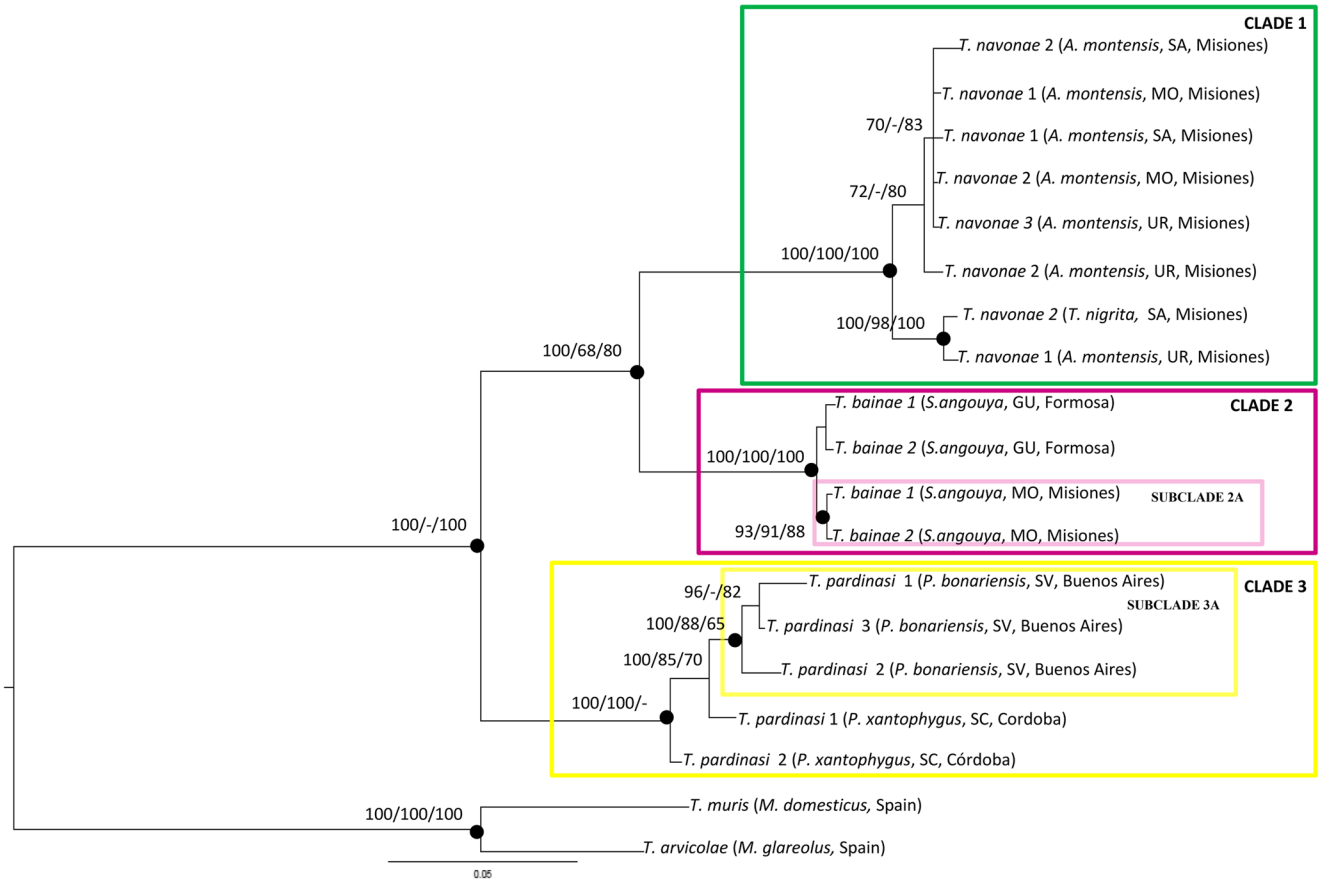

Fig. 1 Phylogenetic tree of *Trichuris* species based on combined analysis of *cytochrome c oxidase 1* and *cytochrome b* mitochondrial DNA sequences. Tree shown was inferred using Bayesian Inference. Bayesian Posterior Probabilities (BPP) of clades are listed first, followed by Maximum Parsimony (MP) and Maximum Likelihood (ML) bootstrap values, respectively, for clade frequencies exceeding 60 %

MtDNA has proved useful in molecular phylogeny due to its maternal inheritance, rapid rate of divergence, and lack of recombination (Arrivillaga et al. 2002). Mitochondrial genes data have been used for the characterization of *Trichuris* species and their relationships (Cutillas et al. 2009; 2012b, 2015; Liu et al. 2012; Hawash et al. 2015; Doležalová et al. 2015).

Phylogenetic investigations that include multiple loci have advantages over single-locus studies not only for estimates of species-level relationships (Ballard and Rand 2005) but for testing hypotheses of species or species delimitation (Pérez-Ponce de León and Nadler 2010; Nadler and Pérez-Ponce de León 2011). Recent progress has been made in understanding the phylogeny of the *Trichuris* genus. There seem to be host-specific patterns in infection with particular *Trichuris* species or subspecies (Betson et al. 2015). Future research priorities should include multiple genetic marker analysis of *Trichuris* sampled from different hosts and diverse geographical locations to provide insights into parasite transmission within and between host species.

In the present paper, we carried out a molecular study, based on multiple genetic marker analysis, of populations of *Trichuris* spp. isolated from five Sigmodontinae rodents' species from Argentina. Three species were identified: *T. navonae*, *T. bainae*, and *T. pardinasi*. The overall A+T content of the *cox1* and *cob* sequences in these species is consistent with mitochondrial genomes of *T. trichiura* (68.1 %) and *T. suis* (71.5 %) (Liu et al. 2012).

The percentage of inter-specific similarity observed along the three species based on *cox1* and *cob* mtDNA partial genes far exceeded the intra-population and inter-population similarity. The highest values of inter-specific similarity based on *cox1* partial gene (90.4 %) were observed between *T. bainae* from Misiones and *T. pardinasi* from Córdoba, while the highest values of inter-specific similarity based on *cob* partial gene (88.9 %) was observed between *T. navonae* (Misiones) and *T. bainae* (Formosa). Although the clades were strongly supported, in the case of subclades they were moderate, suggesting that these individuals may represent the same species, although they could be on the way to diverge. Attending to the inter-specific similarity observed in the genus *Trichuris* based

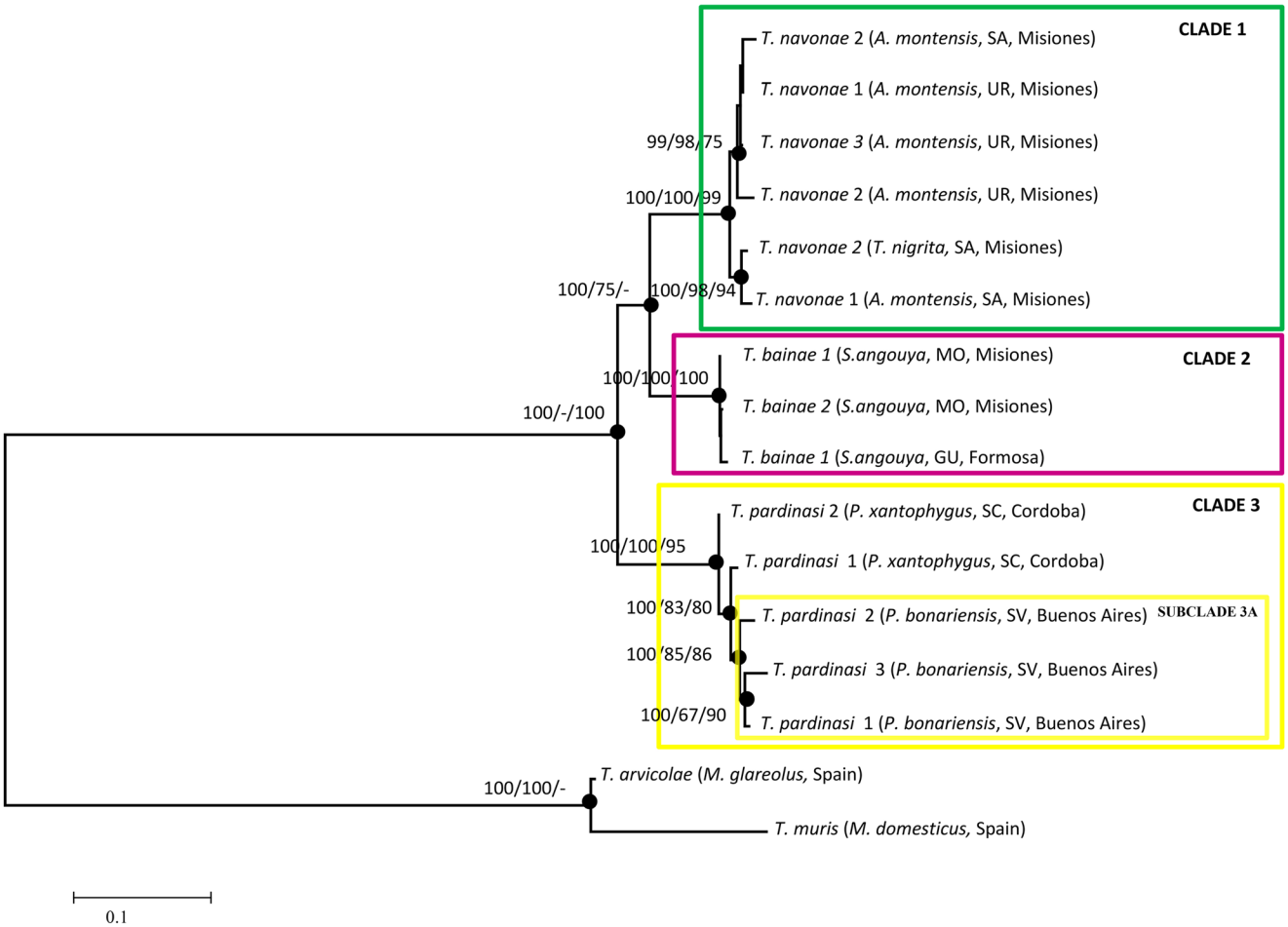

Fig. 2 Phylogenetic tree of *Trichuris* species based on combined analysis of mitochondrial DNA (*cytochrome c oxidase 1* and *cytochrome b*) and nuclear ribosomal DNA (Internal Transcribed Spacer 2) inferred using Maximum Likelihood analysis. Bayesian Posterior Probabilities of clades are listed first, followed by Maximum Parsimony and Maximum Likelihood bootstrap values, respectively, for clade frequencies exceeding 60 %

on *cox1* partial gene (68.7–84.3 %, Callejón et al. 2013) and *cob* partial gene (69.2–97.1 %, unpublished data), the present results confirm that *T. pardinasi*, *T. navonae*, and *T. bainae* are different species.

The phylogenetic analysis of the concatenated sequences (*cox1* and *cob*) revealed three different clades: Clade 1 and Clade 2 (*T. navonae* and *T. bainae*, respectively) which appeared related but separated and a Clade 3 clustering both populations of *T. pardinasi* (Buenos Aires and Córdoba). These results are in agreement with those cited by Robles et al. (2014). The concatenation of these mitochondrial genes with nuclear sequences (ITS2) revealed phylogenetic results similar to those observed by individual gene analysis. Thus, these results suggest that resolving relationships representing the deepest nodes in the *Trichuris* phylogeny will require analysis of additional independent loci represented by nuclear genes.

Since the survival of *Trichuris* individuals in a host depends on many factors as host immunologic status (mainly associated with the taxonomy) or/and ecology aspects, the characteristics of its life cycle and environmental factors have been the main aspects used by different authors to explain the geographical distribution of *Trichuris* species (Bundy et al. 1988; Bundy and Cooper 1989; Grencis et al. 1993; Anderson 2000). Thus, the question is if the species of *Trichuris* could act as markers of their hosts and/or areas and if this could be observed as a co-divergence or captured process in co-phylogeny. In this context, the geographical and host distributions of *Trichuris* spp. have been poorly analyzed.

Rodent hosts could be used to understand the ecological and evolutionary factors that affect the geographical and host distribution of *Trichuris* spp. The *Trichuris* species from sigmodontine rodents clustered together and separated from *Trichuris* species isolated from murine and arvicoline rodents. The host distribution of *T. navonae*, *T. bainae*, and *T. pardinasi* showed correspondence with different tribes included in Sigmodontinae rodents such as Clade 1—Akodontini, Clade 2—Oryzomyini, and Clade 3—Phyllotini. *T. navonae* (Clade 1) is a parasite of *A. montensis*,

one of the most abundant host species present in a wide geographical distribution from the Atlantic forest and Cerrado to Brazil, Paraguay, and Argentina (Pardiñas et al. 2006, 2008; Cirignoli et al. 2011; De la Sancha 2014). Also, *T. navonae* is present in another sympatric host species, *T. nigrita*, that is not easy to capture and is less abundant (Bonvicino et al. 2002; Patton et al. 2008). Both rodents occur in the same microhabitat, living in primary and secondary forests (Pardiñas et al. 2005).

T. bainae (Clade 2) is a parasite of *S. angouya*, a rodent with sympatric distribution with the two akodontines mentioned above, although this species lives more frequently in trees and ground (Bonvicino et al. 2002; Cirignoli et al. 2005; Teta et al. 2007; Percequillo et al. 2008). This rodent is considered common (easy to capture) but not abundant (Bonvicino et al. 2002).

In this study, the clades showed different levels of host specificity. Clade 1 and 2 indicated the presence of different species of *Trichuris* in the same biome, but with each species associated with a different host tribe and ecological habits. In addition, these species of whipworm follow their hosts along their geographical distribution, such as *T. bainae* (Clade 2 and subclade 2a) in *S. angouya* from Misiones and Formosa provinces. Although the humid Chaco (Formosa) and tropical forests (Misiones) are very different in floristic composition, getting the Argentina area, these biomes are geographically closed (Mayle 2004; Pennington et al. 2000).

On the other hand, *T. pardinasi* (Clade 3) is a parasite of *P. bonariensis* and *P. xanthopygus*, both abundant species which are found in a wide variety of habitats, but these are restricted mainly to rocky outcrops (Polop 1989; Kramer et al. 1999). *P. bonariensis* is distributed only in Sierra de la Ventana, southeast of Buenos Aires province (Argentina) (Crespo 1964; Pardiñas and Jayat 2008), while *P. xanthopygus* has a wide distribution, along the Andes from west central Peru to Santa Cruz Province (Argentina) and the adjacent Magellan Region of Chile (Musser and Carleton 2005). The population of *P. bonariensis* from Sierra de la Ventana was originally cited as an endemic species by Crespo (1964), Reig (1987), Galliari et al. (1996), and Musser and Carleton (2005). Later, this population was considered as *P. xanthopygus* as stated by Pardiñas et al. (2004). Currently, there is no solid evidence available to justify this second proposal and specific status (Steppan et al. 2007); for this reason, we follow the first taxonomic proposal. Clade 3 indicated the presence of the same species of *Trichuris* in two congener host species in two disjoint areas suggesting specificity at the host generic level (or specific level if futures studies confirm that *P. bonariensis* (subclade 3a) and *P. xanthopygus* are conspecific). Notably, the Sierras de Córdoba and Sierra de la Ventana are areas considered faunistic islands that share a considerable number of species and subspecies, i.e., mollusks, insects, and amphibians (Ringuelet 1961), as well as *T. pardinasi*.

Interestingly, *Trichuris* from different host origin (Muridae and Cricetidae) from the Old World generated a monophyletic group separated from *Trichuris* of Sigmodontinae (Cricetidae) from the New World (Figs. 1 and 2). The question leads again to the lack of knowledge on the behavior of distribution of *Trichuris*: Are the geographical factors and processes that affect the distribution of *Trichuris* greater than those of phylogenetic origin?

Conclusion

Mitochondrial (*cox1* and *cob*) markers, used in the present work, are useful tools to discriminate *Trichuris* spp. (*T. navonae*, *T. bainae*, and *T. pardinasi*) and different populations of *T. bainae* and *T. pardinasi* with different geographical origins and rodent hosts. The phylogenetic tree based on combined mitochondrial and nuclear sequences of *Trichuris* spp. from different rodent hosts from Argentina revealed three clades corresponding to these three *Trichuris* spp. showing different levels of host specificity. More comprehensive understanding of the co-divergence parasite-host will require increased taxa sampling of *Trichuris* species and the increased resolution provided by multigene molecular phylogenies.

Acknowledgments We thank Carlos Galliari, Ulyses Pardiñas, Marcela Lareschi, Guillermo Panisse, Natalia Guerreiro Martins, and Juliana Notarnicola for their help during host collections; Carlos Galliari and Ulyses Pardiñas for the identification of the hosts; and Graciela T. Navone for her support in the laboratory. The present work was supported by a grant from the V Plan Propio de Investigación of the University of Sevilla, Spain, the Agencia Nacional de Promoción Científica y Tecnológica (PICT 2010–0924), and Proyecto de incentivos de la Universidad Nacional de La Plata (UNLP N 627 and travel grant).

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Anderson RC (2000) The Superfamily Trichinelloidea. In: Nematode parasites of vertebrates: their development and transmission, 2nth edn. CAB International, Wallingford, pp 605–621

Arrivillaga JC, Norris DE, Feliciangeli MD, Lanzaro GC (2002) Phylogeography of the neotropical sand fly *Lutzomyia longipalpis* inferred from mitochondrial DNA sequences. Infect Genet Evol 2: 83–95

Babero BB, Cattan PE, Cabello C (1976) A new species of whipworm from the rodent *Akodon longipilis* in Chile. Trans Am Microsc Soc 95:232–235

Ballard JWO, Rand DM (2005) The population biology of mitochondrial DNA and its phylogenetic implications. Annu Rev Ecol Evol Syst 36:621–642

Betson M, Søe MJ, Nejsum P (2015) Human trichuriasis: whipworm genetics, phylogeny, transmission and future research directions. Curr Trop Med Rep 2:209–217

Bonvicino CR, Lindbergh SM, Maroja LS (2002) Small nonflying mammals from conserved and altered areas of Atlantic Forest and Cerrado: comments on their potential use for monitoring environment. Braz J Med Biol 62:1–12

Bundy DAP, Cooper ES (1989) *Trichuris* and trichuriasis in humans. Adv Parasitol 28:107–173

Bundy DAP, Kan SP, Rose R (1988) Age-related prevalence, intensity and frequency distribution of gastrointestinal helminth infection in urban slum children from Kuala Lumpur, Malaysia. Trans R Soc Trop Med Hyg 82:289–294

Cafrune MM, Aguirre DH, Rickard LG (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, Nieberding C, Foronda P, Feliu 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, De Rojas M, Feliu C, Balao F, Marrugal A, Henttonen H, Cutillas C (2012a) Phylogeography of *Trichuris* populations isolated from different Cricetidae rodents. Parasitology 139:1795–1812

Callejón R, Halajian A, De Rojas M, Marrugal A, Guevara DC, Cutillas C (2012b) 16S partial gene DNA and internal transcribes 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 SA (2015) Nuclear and mitochondrial genes for inferring *Trichuris* phylogeny. Parasitol Res 114:4591–4599

Cavallero S, De Liberato C, Friedrich KG, Di Cave D, Masella V, D'Amelio S, Berrilli F (2015) Genetic heterogeneity and phylogeny of *Trichuris* spp. from captive non-human primates based on ribosomal DNA sequence data. Infect Genet Evol 34:450–456

Cirignoli S, Teta P, Pardiñas UFJ, D'Elía G (2005) Oryzomyini Vorontsov 1959 (sensu Voss and Carleton 1993). In: Bárquez RM, Díaz MM, Ojeda RA (eds) Los Mamíferos de la Argentina. Sociedad Argentina para el Estudio de los Mamíferos, Argentina, pp 166–175

Cirignoli S, Galliari C, Pardiñas UFJ, Podestá D, Abramson R (2011) Mamíferos de la Reserva Valle del Cuña Pirú, Misiones, Argentina. Mastozool Neotrop 18:25–43

Correa-Gomes D, Lanfredi RM, Pinto RM, 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 PG, Hautier L (2015) Evolution of the rodents. Advances in phylogeny, functional morphology, and development (eds) Cambridge University Press, 611 pp

Cubillos V, Torres P, Gallardo M (1991) Aspectos histopatológicos en un nuevo hospedador de *Taenia taeniformis*. Arch Med Vet 23:77–79

Cutillas C, German P, Arias P, Guevara DC (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 DC (2002) Determination of *Trichuris muris* from murid hosts and *T. 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 DC (2004) Determination of *Trichuris skrjabini* by sequentiation 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 JM, Guevara D (2007) Molecular identification of *Trichuris vulpis* and *T. suis* isolated from different hosts. Parasitol Res 100:383–389

Cutillas C, Callejón R, De Rojas M, Tewes B, Úbeda JM, Ariza C, Guevara DC (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 (2014) *Trichuris colobae* n. sp. (Nematoda: Trichuridae), a new species of *Trichuris* from *Colobus guereza kikuyensis*. Parasitol Res 113:2725–2732

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 kikuyensis*. Parasitol Res 113:2725–2732

D'Elia G, Pardiñas UFJ (2015) Subfamilia Sigmodontinae Wagner, 1843. In: Patton J, Pardiñas UFJ, D'Elia G (eds) Mammals of South America, 2nd edn. The University of Chicago Press, Rodents, pp 63–685

D'Elía G (2003) Rats, mice, and relatives IV: Sigmodontinae. In: Hutchins M, Geist V, Kleiman D, McDade M (eds) Grzimek's animal life encyclopedia. Thomson-Gale, Farmington Hills, pp n263–n279

De la Sancha NU (2014) Patterns of small mammal diversity in fragments of subtropical Interior Atlantic Forest in eastern Paraguay. Mammalia. doi:10.1515/mammalia-2013-0100

Doležalová J, Oborník M, Hajdušková E, Jirků M, Petrželková KJ, 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. doi:10.14411/fp.2015.063

Feliú C, Spakulová M, Casanova JC, Renaud F, Morand S, Hugot JP, 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

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

Galliari C, Pardiñas UFJ, Goin F (1996) Lista comentada de los mamíferos argentinos. Mastozool Neotrop 3:39–61

Ghai RR, Simons ND, Chapman CA, Omeja PA, Davies TJ, Ting N, Goldberg TL (2014) Hidden population structure and cross-species transmission of whipworms (*Trichuris* sp.) in humans and non-human primates in Uganda. PLoS Negl Trop Dis 8:e3256. doi:10.1371/journal.pntd.0003256

Grencis RK, Else KJ, Bancroft AJ, Bundy AP (1993) *Trichuris* Update '93. Parasitol Today 9:309–310

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 197:364–36

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704

Hawash MB, Andersen LO, Gasser RB, Stensvold CR, Nejsum P (2015) Mitochondrial genome analyses suggest multiple *Trichuris* species in humans, baboons, and pigs from different geographical regions. PLoS Negl Trop Dis 9:e0004059

Herrera HM, Norek A, Freitas TPT, Rademaker V, Fernandes O, Jansen AM (2005) Domestic and wild mammals infection by *Trypanosoma evansi* in a pristine area of the Brazilian Pantanal region. Parasitol Res 96:12–126

Jansen AM, Roque ALR (2010) Domestic and wild mammalian reservoirs. In: Telleria J, Tibayrenc M (eds) American trypanosomiasis: Chagas disease one hundred years of research. Elsevier, London, pp 249–276

Kramer KM, Monjeau JA, Birney EC, Sikes RS (1999) *Phyllotis xanthopygus*. Mamm Species 617:17

Kuhnen VV, Graipel ME, Pinto CJC (2012) Differences in richness and composition of gastrointestinal parasites of small rodents (Cricetidae, Rodentia) in a continental and insular area of the Atlantic forest in Santa Catarina state. Brazil Braz J Biol 72:563–567

Lallo MA, Pereira A, Araújo R, Favorito SE, Bertolla P, Bondan Fernandes E (2009) Ocorrência de *Giardia*, *Cryptosporidium* e microsporídios em animais silvestres em área de desmatamento no Estado de São Paulo, Brasil. Ciência Rural 39:1465–1470

Lanos-Cuentas EA, Roncal N, Villaseca P, Paz L, Ogusuku E, Perez JE (1999) Natural infections of *Leishmania peruviana* in animals in the Peruvian Andes. Trans R Soc Trop Med Hyg 93:15–20

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948

Liu GH, Gasser RB, Su A, Nejsum P, Peng L, Lin RQ, Li MW, Xu MJ, Zhu XQ (2012) Clear genetic distinctiveness between human- and pig-derived *Trichuris* based on analysis of mitochondrial datasets. PLoS Negl Trop Dis 6:e1539

Liu GH, Gasser RB, Nejsum P, Wang Y, Chen Q, Song HQ, Zhu XQ (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. doi:10.1371/journal.pone.0066249

Maldonado A, Simões R, Thiengo SC (2012) Angiostrongyliasis in the Americas. In: Lorenzo-Morales J (Eds.). Zoonosis Rijeka: In Tech 303–320

Mayle FE (2004) Assessment of the Neotropical dry forest refugia hypothesis in the light of palaeoecological data and vegetation model simulations. J Quat Sci 19:713–720

Miño MH, Rojas Herrera E, Notarnicola J (2013) The wild rodent *Akodon azarae* (Cricetidae: Sigmodontinae) as intermediate host of *Taenia taeniaeformis* (Cestoda: Cyclophyllidea) on poultry farms of central Argentina. Mastozool Neotrop 20:406–412

Musser GG, Carleton MD (2005) Superfamily Muroidea. In: Wilson DE, Reeder DA (eds) Mammal species of the world: a taxonomic and geographic reference. Johns Hopkins University Press, Baltimore, pp 894–1531

Nadler SA, Pérez-Ponce de León GPP (2011) Integrating molecular and morphological approaches for characterizing parasite cryptic species: implications for parasitology. Parasitology 138:1688–1709

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

Nissen S, Al-Jubury A, Hansen TV, Olsen A, Christensen H (2012) Genetic analysis of *Trichuris suis* and *T. trichiura* recovered from humans and pigs in a sympatric setting in Uganda. Vet Parasitol 188:68–77

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

Pardiñas UFJ, Jayat JP (2008) *Phyllotis bonariensis*. The IUCN Red List of Threatened Species. Version 2014.2. <www.iucnredlist.org>. Downloaded on 29 July 2014

Pardiñas UF, Abba AM, Merino ML (2004) Micromamíferos (Didelphimorphia y Rodentia) del sudoeste de la provincia de Buenos Aires (Argentina): taxonomía y distribución. Mastozool Neotro 11:211–232

Pardiñas UF, D'Elia G, Cirignoli S, Suarez P (2005) A new species of *Akodon* (Rodentia, Cricetidae) from the Northern Campos grasslands of Argentina. J Mammal 86:462–474

Pardiñas UFJ, D'Elía G, Teta P, Ortiz PE, Jayat PJ, Cirignoli S (2006) Subfamilia Sigmodontini, Tribu Akodontini. In: Barquez RM, Díaz MM, Ojeda RA (eds) Mamíferos de Argentina, sistemática y distribución. Tucumán, Argentina, pp 146–202

Pardiñas UFJ, D'Elía G, Fagundes V, Christoff A, Geise L (2008) *Akodon montensis*. The IUCN Red List of Threatened Species. Version 2014.2. <www.iucnredlist.org>. Downloaded on 28 July 2014

Patton J, Catzeflis F, Weksler M, Percequillo A, D'Elia G, Pardiñas UFJ (2008) *Thaptomys nigrita*. The IUCN Red List of Threatened Species. Version 2014.2. <www.iucnredlist.org>. Downloaded on 31 July 2014

Pennington RT, Prado DE, Pendry CA (2000) Neotropical seasonally dry forests and Quaternary vegetation changes. J Biogeogr 27:261–273

Percequillo C, Weksler M, Pardiñas UFJ, D'Elía G, Teta P, Patterson B (2008) *Sooretamys angouya*. The IUCN Red List of Threatened Species. Version 2014.2. www.iucnredlist.org. Downloaded on 28 July 2014

Pérez-Ponce de León G, Nadler SA (2010) Critical Comment. What we don't recognize can hurt us: a plea for awareness about cryptic species. J Parasitol 96:453–464

Polop J (1989) Distribution and ecological observations of wild rodents in Pampa de Achala, Córdoba, Argentina. Stud Neotrop Fauna Environ 24:53–59

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Ravasi DF, O'Riain MJ, Davids F, Illing N (2012) Phylogenetic evidence that two distinct *Trichuris* genotypes infect both humans and non-human primates. PLoS One 7:e44187

Reig OA (1987) An assessment of the systematics and evolution of the Akodontini with the description of new fossil species of *Akodon* (Cricetidae: Sigmodontinae). Fieldiana Zool 39:347–399

Reperant LA, Hegglin D, Tanner I, Fischer C, Deplazes P (2009) Rodents as shared indicators for zoonotic parasites of carnivores in urban environments. Parasitology 136:329–337

Ringuelet RA (1961) Rasgos fundamentales de la zoogeografia de la Argentina. Physis 22:151–170

Robles MR (2011) New species of *Trichuris* (Nematoda: Trichuridae) from *Akodon montensis* Thomas, 1913 of the Paranaense forest in Argentina. J Parasitol 97:319–327

Robles MR, Navone GT, Notarnicola J (2006) A new species of *Trichuris* (Nematoda: Trichuriidae) from Phyllotini rodents in Argentina. J Parasitol 92:100–104

Robles MR, Carballo MC, Navone GT (2008) A new species of *Liniscus* (Nematoda: Trichuridae) from *Oxymycterus rufus* and *Akodon azarae* (Cricetidae: Sigmodontinae) in Buenos Aires Province, Argentina. J Parasitol 94:909–917

Robles MR, Cutillas C, Panei CJ, 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. doi:10.1371/journal.pone.0112069

Robles MR, Kinsella JM, Galliari C, Navone GT (2016) New host, geographic records, and histopathologic studies of *Angiostrongylus* spp. (Nematoda: Angiostrongylidae) in rodents from Argentina, with updated summary of records from rodent hosts and host specificity assessment. Mem Inst Oswaldo Cruz 111:181–191

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

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

Steppan SJ, Adkins RM, Anderson J (2004) Phylogeny and divergence-date estimates of rapid radiations in muroid rodents based on multiple nuclear genes. Syst Biol 53:533–553

Steppan SJ, Ramirez O, Banbury J, Huchon D, Pacheco V, Walker L, Spotorno AO (2007) A molecular reappraisal of the systematics of the leaf-eared mice *Phyllotis* and their relatives. In: Kelt DA, Lessa E, Salazar-Bravo J, Patton JL (eds) The quintessential naturalist: honoring the life and legacy of Oliver P. Pearson. University of California Press, Berkeley, pp 799–826

Subbotin SA, Vierstraete A, De Ley P, Rowe J, Waeyenberge L, Moens M, Vanfleteren JR (2001) Phylogenetic relationships within the cyst-forming nematodes (Nematoda, Heteroderidae) based on analysis of sequences from the ITS regions of ribosomal DNA. Mol Phylogenet Evol 21:1–16

Suriano DM, Navone GT (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

Teta P, Pardiñas UFJ, Andrade A, Cirignoli S (2007) Distribución de los géneros *Euryoryzomys* y *Sooretamys* (Rodentia, Cricetidae) en Argentina. Mastozool Neotrop 14:279–284

Tiner JD (1950) Two new species of *Trichuris* from North America with description of *Trichuris leporis* (Nematoda: Aphasmidia). J Parasitol 36:350–354

Xie H, Bain O, Williams SA (1994) Molecular phylogenetic studies on filarial parasites based on 5S ribosomal spacer sequences. Parasite 1: 141–151