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Rodentolepis microstoma isolated from different species of Sigmodontinae rodents (Rodentia: Cricetidae) in the Cuenca del Plata, Argentina: Morphological aspects and molecular characterization

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ABSTRACT

The purpose of this paper was to study specimens of the genus *Rodentolepis* isolated from eight species of Sigmodontinae rodents (Rodentia: Cricetidae) from six provinces in the Cuenca del Plata, Argentina, based on morphological, morphometric and molecular characteristics (ITS1 rDNA and *cox*1 mtDNA). The genetic distances among studied specimens and other Hymenolepididae from rodents available in the GenBank were analyzed and phylogenetic inferences were provided. A total of 955 specimens of Sigmodontinae rodents were examined from seven localities of six provinces in the Cuenca del Plata region in Argentina. Tapeworms were removed from the rodents' small intestines. Conventional studies were used for the morphological and molecular analysis. Specimens of *R. microstoma* were identified. An amended diagnosis and detailed morphological description of this species is provided. The molecular analyses showed that the specimens studied form the same clade as that of *R. microstoma* previously studied from other hosts and regions. The genetic polymorphisms of *R. microstoma* observed corresponded to different groups of species hosts and regions. Moreover, eight species of sigmodontine rodents and 33 localities from the Cuenca del Plata region in Argentina constitute new host and geographical records. This study shows the importance of using integrative taxonomic approaches that combine morphological and molecular characters to understand biological diversity. Moreover, the discovery of *R. microstoma* in humans suggests the importance of further studies on this zoonotic cestode. This study provides important data on the taxonomy and distribution of *R. microstoma* to advance knowledge of the transmission dynamics of this parasite.

1. Introduction

The Hymenolepididae (Cyclophyllidea) are the family with the highest species richness recorded among the Cestoda, with more than 920 valid species (Czaplinski and Vaucher, 1994; Mariaux et al., 2017). This family includes tapeworms that parasitize mostly birds and mammals. Among mammals, most of the genera and species occur in Soricomorpha, Chiroptera, and Rodentia (e.g., Vaucher, 1971; Czaplinski and Vaucher, 1994; Georgiev et al., 2006; Mariaux et al., 2017). Among rodents, the families with the most frequently recorded host species of Hymenolepididae are Muridae, Geomyidae, Sciuridae, Cricetidae, and Spalacidae (Gardner and Schmidt, 1988; Makarikov and Tkach, 2013; Makarikov et al., 2013, 2015; Gardner et al., 2014). Phylogenetic hypotheses on Cyclophyllidea have been proposed based on partial genes of mitochondrial DNA (*cox*1) and on regions of nuclear ribosomal DNA (12S, 18S, and ITS2) (Von Nickisch-Rosenegk et al., 1999; Foronda et al., 2004; Tandon et al., 2011; Sharma et al., 2016). Also, morphological and molecular analyses have been provided for various genera and species of Hymenolepididae (e.g., Hoberg et al., 2001; Olson et al., 2001; Georgiev et al., 2006; Haukisalmi et al., 2010). In addition, a hypothesis about the relationship of mammalian hymenolepidids based on partial 28S rDNA sequencing showed a pronounced morphological variation

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among parasites infecting unrelated hosts which form a monophyletic group (Haukisalmi et al., 2010).

Some species of hymenolepidids are important for public health, particularly the species that parasitize rodents, such as *Hymenolepis diminuta* (Rudolphi, 1819), *Rodentolepis nana* (Von Siebold, 1852), and *Rodentolepis microstoma* (Dujardin, 1845), capable of infecting humans (Macnish et al., 2003; Marangi et al., 2003; Nkouawa et al., 2016). Around six hymenolepidid genera from rodents have been reported in North and South America (e.g., *Arostrilepis* Mas-Coma and Tenora, 1997, *Hobergia* Gardner, Dursahinhan, Campbell and Rácz, 2020, *Hymenandrya* Smith, 1954, *Hymenolepis* Weinland, 1858, *Monogynolepis* Czaplinski and Vaucher, 1994, *Rodentolepis* Spasskii, 1954), of which 17 species of the genus *Rodentolepis* have been reported parasitizing Cricetidae, two from South America, and only one from Argentina (Barker, 1915; Rider and Macy, 1947; Neiland and Senger, 1952; Schiller, 1952; Wardle and McLeod, 1952; Rêgo, 1967, 1970; Sutton, 1974; Cunningham and Olson, 2010; Guerreiro Martins et al., 2014; Panisse et al., 2017). The species with the widest geographic and host range are *R. nana* and *R. microstoma*, parasitizing several genera of Muridae and Cricetidae, such as *Apodemus*, *Arvicanthis*, *Mastomys*, *Mesocricetus*, *Microtus*, *Mus*, *Rattus*, and *Sigmodon* (e.g., Litchford, 1963; Cunningham and Olson, 2010; Gomez-Puerta and Valdivia-Carrera, 2018). *Rodentolepis akodontis* Rêgo (1967) and *R. srivastavai* Rêgo (1970) have also been recorded for different sigmodontine rodents (Cricetidae) in Brazil: *Akodon cursor* (Winge, 1887), *Akodon montensis* Thomas, 1913 and *Necromys lasiurus* (Lund, 1840) (e.g., Rêgo, 1967; Simões et al., 2011; Costa et al., 2019).

Sigmodontine rodents are endemic to the American continent, with a wide environmental and geographical distribution and a great diversity of diets (Redford and Eisenberg, 1992; Patton et al., 2015; Wilson et al., 2017). This group of rodents includes around 110 species grouped in 40 genera in Argentina (Galliari et al., 1996; Pardiñas et al., 2006; Teta et al., 2018). In this country, specimens of genus *Rodentolepis* sp. were found from *Oxymycterus rufus* (Fischer, 1814) and *Deltamys kempi* Thomas, 1917 in different locations of Buenos Aires province (Navone et al., 2009). Later, specimens assigned to *Rodentolepis* cf. *akodontis* of *O. rufus* and *A. montensis* were recorded in different locations of Buenos Aires, Corrientes, Entre Ríos, and Misiones provinces (Guerreiro Martins et al., 2014; Panisse et al., 2017).

The purpose of this paper was to study specimens of the genus *Rodentolepis* isolated from eight species of Sigmodontinae rodents (Rodentia: Cricetidae) from six provinces included in the Cuenca del Plata, Argentina, based on morphological and molecular characteristics. For the molecular characterization, nuclear Internal Transcribed Spacer 1 (ITS1) and mitochondrial partial gene cytochrome *c* oxidase 1 (*cox*1) sequences were used. The genetic distances among the specimens studied and other Hymenolepididae from rodents available in the GenBank were analyzed and phylogenetic inferences were provided.

2. Materials and methods

2.1. Study area and host sampling

A total of 955 Sigmodontinae rodents were examined: 251 specimens of *Akodon azarae* (Fischer, 1829) from 18 localities, 16 specimens of *Akodon dolores* Thomas, 1916 from one locality, 259 specimens of *Akodon montensis* from six localities, 15 specimens of *Akodon philipmiyersi* Pardiñas, D'Elía, Cirignoli y Suarez, 2005 from one locality, 112 specimens of *Necromys lasiurus* from nine localities, 10 specimens of *Oxymycterus misionalis* Sanborn, 1931 from two localities, 260 specimens of *Oxymycterus rufus* from 17 localities, and 32 specimens of *Thaptomys nigrita* (Lichtenstein, 1829) from seven localities, of six provinces in the Cuenca del Plata region in Argentina (Table 1). Rodents were obtained and identified by several collaborators between 1994 and 2018 (see Acknowledgments).

Table 1
List of new localities records for *Rodentolepis microstoma* from Cuenca del Plata region in Argentina.

Province	Locality	Latitude (S)	Longitude (W)
Buenos Aires	Arana	35°00′25.00″	57°54′34.00″
Buenos Aires	Laguna de Chascomús	35°32′38.52″	58°04′46.47″
Buenos Aires	Arroyo de las Brusquitas	38°14′05.97″	57°46′49.98″
Buenos Aires	Cerro de la Gloria	36°01′00.00″	57°26′00.00″
Buenos Aires	La Balandra	34°55′45.47″	57°42′58.39″
Buenos Aires	Olavarría	36°58′34.00″	60°14′13.00″
Buenos Aires	Parque Provincial Ernesto Tornquist, Sierra de la Ventana	38°04′44.55″	62°00′19.04″
Buenos Aires	Pereyra	34°50′14.00″	58°05′23.00″
Buenos Aires	Pergamino	33°52.9′39.56″	60°46.07′4.6″
Buenos Aires	Punta Indio	35°16′00.00″	57°15′00.00″
Buenos Aires	Reserva Natural de Hudson	34°44′00.00″	58°12′00.00″
Buenos Aires	Reserva Selva Marginal de Punta Lara	34°47′30.00″	58°00′05.00″
Corrientes	Estancia San Juan Poriahú	27°42′00.00″	57°12′14.00″
Corrientes	Estación Biológica Corrientes (ex Caprim)	27°33′00.62	58°40′52.33″
Corrientes	Finca La Adelita, Laguna Paiva	27°28′41.76″	58°44′41.14″
Corrientes	Reserva Santo Domingo 20 km al N de Paso de los Libres	29°36′14.27″	56°58′50.63″
Corrientes	Estancia El Cimarrón, RP 118, km 169	27°41′10.73″	57°12′41.91″
Entre Ríos	Arroyo Feliciano	30°58′21.00″	59°41′49.00″
Entre Ríos	Arroyo Caraballo	32°05′06.00″	58°10′30.00″
Entre Ríos	Estancia Santa Ana de Carpinchorí	30°47′39.25″	58°38′51.10″
Entre Ríos	Villa Elisa	32°09′14.73″	58°20′10.40″
Formosa	Estación de Animales Silvestres Guaycolec, Ruta Nacional 11, km 1201	25°58′57.80″	58°10′04.00″
Formosa	Reserva El Bagual	26°18′21.96″	58°49′53.34″
Formosa	Río Bermejo	26°19′45.00″	59°06′43.00″
Misiones	2 km aguas abajo desembocadura Parana-í Guazú	26°40′39.30″	54°50′08.20″
Misiones	Campo Anexo M. Belgrano, INTA, San Antonio	26°02′54.21″	53°46′32.40″
Misiones	Cuña Pirú	27°05′17.00″	54°57′09.00″
Misiones	Estancia Santa Inés	27°31′53.69″	55°52′30.48″
Misiones	Parque Provincial Piñalito	26°25′40.07″	53°50′38.26″
Misiones	Parque Provincial Urugua-í	25°51′25.58″	54°09′59.87″
Misiones	Refugio Moconá	27°08′29.04″	53°55′40.40″
Misiones	Reserva de Vida Silvestre Urugua-í, Fundación Vida Silvestre	25°58′32.29″	54°07′00.08″
Santa Fe	Oliveros	32°34′00.00″	60°51′00.00″

2.2. Ethics statement

The research was conducted according to Argentine laws. Sample collection was carried out during fieldwork under official permission. This study was conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. Rodent specimens were collected following the procedures and protocols approved by national laws (National Animal Protection law 14.346 and references in the provincial permits), and the ethical recommendations for Research on Laboratory Animals, Farm and Obtained from Nature of the National Council of Scientific and Technical Research (CONICET), and subsequently approved by the National Agency for the Promotion of Science and Technology of Argentina (ANPCYT). No endangered species were involved in this study.

Rodent specimens were deposited in the Mammalogy Collection of the Centro Nacional Patagónico (CNP), Puerto Madryn, Chubut, and in the Mammalogy Collection of the Museo de La Plata (MLP), La Plata, Buenos Aires.

2.3. Morphological analysis

Viscera were studied under a stereomicroscope (Olympus SZ61-TR). Cestode specimens were removed from the rodent's small intestines, fixed in 10% formalin and preserved in 70% ethanol. Some specimens were stained with hydrochloric carmine, dehydrated in a graded ethanol series, cleared in eugenol, and mounted in natural Canada balsam. In addition, serial histological sections of two specimens were made for a complete study of the internal morphology. One specimen was dried using the critical point method, examined and photographed by scanning electron microscopy (SEM) (JEOL, JSM 6360 LV). Specimens were studied and photographed using a polarized light microscope (Olympus BX51®), and drawings were made with the aid of a drawing tube.

Types of *Rodentolepis akodontis* (CHIOC 29.316a-b, 29.317, 29.318a-c, 29.319, 29.320) were studied with a Carl Zeiss Axiophot light microscope equipped with a Canon Power Shot S80 camera at the Coleção Helmintológica do Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.

The specimens identified as *Rodentolepis* cf. *akodontis* from previous surveys such as Guerreiro Martins et al. (2014) and Panisse et al. (2017) were restudied.

Table 2 shows measurements of specimens of *Rodentolepis microstoma* and other species of *Rodentolepis* recorded in Sigmodontinae rodents (*R. srivastavai* and *R. akodontis*) as follows: mean, standard deviation, and range in parentheses. All measurements are given in millimeters (mm) unless otherwise indicated. The scales of Figs. 1 and 2 are given in micrometers (μm).

Voucher specimens were deposited in the Helminthological Collection of Museo de La Plata (MLP-He), La Plata, Buenos Aires province.

2.4. Molecular analysis

2.4.1. DNA extraction, amplification, and sequencing

Fourteen specimens studied morphologically from six rodent species were stored in 96% ethanol until used for DNA extraction: *A. azarae* (n = 2), *A. montensis* (n = 4), *N. lasiurus* (n = 3), *O. misionalis* (n = 1), *O. rufus* (n = 3), and *T. nigrita* (n = 1).

Genomic DNA from individual Hymenolepididae was extracted and purified using the Wizard® Genomic DNA Purification Kit (Promega), according to the manufacturer's protocol for tissues. Quality of extractions was assessed using 0.8% agarose gel electrophoresis and ethidium bromide staining.

The ITS1 rDNA region was PCR-amplified using the forward F3 (5′ GCGGAAGGATCATTACACGTTC 3′) and the reverse R3 (5′ GCTCGACTCTTCATCGATCCACG 3′) (Macnish et al., 2002), and the cox1 mtDNA partial gene region was PCR-amplified using the forward pr-a (5′ TGGTTTTTTGTGCATCCTGAGGTTTA 3′) and the reverse pr-b (5′ AGAAAGAACGTAATGAAAATGAGCAAC 3') (Okamoto et al., 1997). The amplification conditions were: 95 °C for 15 min (initial denaturation), 35 amplification cycles (95 °C for 30 s, 57 °C for 25 s, 72 °C for 1 min), followed by final extension at 72 °C for 7 min for ITS1 region and 94 °C for 3 min (initial denaturation), 30 amplification cycles (94 °C for 50 s, 42 °C for 1 min 30 s, 72 °C for 1 min 30 s), followed by final extension at 72 °C for 7 min for cox1. The PCR was performed in a Multigene Labnet Internation, Inc. thermocycler and the products were checked on ethidium bromide-stained 1.5% Tris-Borate-EDTA (TBE) using 0.8% agarose gels electrophoresis and examined by UV transillumination. All PCR products were purified and sequenced in both directions using amplifying primers (Macrogen, Seoul, Korea).

2.4.2. Sequence alignment

Molecular analyses were performed on the rDNA (ITS1) and mtDNA (*cox*1) sequences and aligned using the MUSCLE alignment method included in MEGA, version 7.0 (Kumar et al., 2016). Additional Hymenolepididae species sequences from the National Centre for Biotechnology Information (NCBI) GenBank database were incorporated into the alignments (Table 3).

The nucleotide sequences of the protein-coding genes (*cox*1) were first translated into amino acids to confirm that they lacked internal stop codons and to predict cestode protein. To assess the similarity among the marker sequences of specimens analyzed in the present study and other Hymenolepididae species, the number of base differences per sequence with respect to those under investigation was assessed using the number of differences method of the MEGA 7 program version 7.0 (Kumar et al., 2016).

2.4.3. Comparative sequences analyses and phylogenetic inferences

Since we were looking to measure the diversity and conservancy between a set of sequences, considering that rDNA (ITS1) dataset sequences present a substantial length variation which compromises inferences of positional homology, an unrooted tree is proposed. Nevertheless, we propose a rooted tree for the mtDNA (*cox*1) dataset, using *Hymenolepis diminuta*, *Arostripelis horrida*, *Coronacanthus* spp. and *Staphylocystoides* spp. as outgroups (Table 3).

Phylogenetic inferences were performed by Maximum Likelihood (ML) using the PHYML package from Guindon and Gascuel (2003) and Bayesian inferences (BI) were generated using MrBayes, version 3.2.6 (Ronquist and Huelsenbeck, 2003). Each dataset was analyzed separately, and both mitochondrial and ribosomal datasets were combined into a total evidence dataset. jModeltest was employed to compute the best partitioning scheme, as well as the best nucleotide substitution models for each partition (Posada, 2008). Models of evolution were chosen for subsequent analysis according to the Akaike Information Criterion (Posada and Buckley, 2004).

For the study of the dataset containing the concatenation of two markers (ITS1 and *cox*1), analyses based on BI were partitioned by gene, and models for individual genes within partitions were those selected by the jModelTest. For ML inference, best-fit nucleotide substitution models included the general time-reversible model with gamma-distributed rate GTR + G (ITS1), the general time-reversible model with gamma-distributed rate variation, and a proportion of invariable sites GTR + I + G (*cox*1 mtDNA) and general time-reversible models with gamma-distributed rate GTR + G (concatenated markers). Support for the topology was examined using bootstrapping (heuristic option) (Felsenstein, 1985) over 1000 replications to assess the relative reliability of clades. The commands used in MrBayes for BI were nst = 6 with gamma rates (ITS1), nst = 6 with invgamma rates (*cox*1) and nst = mixed (concatenated phylogenetic trees).

Bayesian posterior probabilities (BPP) comprise the percentage converted for BI. The standard deviation of split frequencies was used to determine whether the number of generations completed was sufficient. Each analysis was run for 10 million generations, and the tree was sampled every 500 generation. Trees from the first million generations were discarded based on an assessment of convergence. Burn-in was determined empirically by examination of the log likelihood values of the chains. After eliminating the first million trees as "burn-in", we constructed a 50% majority-rule consensus tree, with nodal values representing the probability (posterior probability) that the recovered clades exist, given the aligned sequence data. We accepted a clade in the Bayesian tree at around 70% posterior probability.

3. Results

3.1. Morphological analysis

Specimens of *R. microstoma* based on morphological characteristics were identified from the eight species of sigmodontine rodents, being the first records for these host species (number of recovery cestodes of

Table 2
Morphometrical characteristics of *Rodentolepis microstoma* and of the species of *Rodentolepis* recorded in Sigmodontinae rodents.

	Rodentolepis akodontis	Rodentolepis srivastavai	Rodentolepis microstoma							
Reference	Rêgo (1967)	Rêgo (1970)	Rêgo (1967)	Casanova et al. (2001)	Cunningham and Olson (2010)	Gómez Puerta and Valdivia Carrera, 2018	This paper[a]			
Host	*Akodon cursor* (=*A. arviculoides*)	*Necromys lasiurus* (=*Zygodontomys pixuna*)	–	*Mus musculus* (natural host)	*Mus musculus* (experimental)	*Mus musculus* (natural host)	*Akodon*	*Oxymycterus*	*Necromys*	*Thaptomys*
Countries	Brazil	Brazil	Brazil	Spain and France	England	Peru	Argentina	Argentina	Argentina	Argentina
Site of Infection	Small intestine	Small intestine	–	–	Bile duct and small intestine	Bile duct	Small intestine	Small intestine	Small intestine	Small intestine
Maximum width	0.82	0.98-1.40	1.42	–	0.45-1.42	0.83-1.38	0.68 (0.36–0.86)	0.77 (0.35–1.80)	1.11 (0.43–1.8)	0.80
Rostellum length	0.15	0.05	0.06	0.04-0.06	00.05-0.07	0.05-0.07	0.05 (0.05–0.06)	0.06 (0.04–0.10)	0.06 (0.06–0.07)	0.05
Rostellum width	0.07	0.05	0.05	0.03-0.06	0.02-0.05	0.03-0.05	0.04 (0.04–0.05)	0.07 (0.06–0.09)	0.05 (0.05–0.06)	0.04
Scolex length	0.30	0.15	0.25	0.14-0.20	0.11-0.15	–	0.14 (0.07–0.23)	0.13 (0.09–0.21)	0.15 (0.12–0.17)	0.15
Scolex width	0.36	0.21	–	0.18-0.29	0.20-0.28	0.21-0.26	0.19 (0.12–0.30)	0.18 (0.13–0.30)	0.18 (0.13–0.22)	0.16
Sucker maximum diameter	0.08	0.07	0.09	0.05-0.09	0.08-0.13	0.08-0.11	0.06 (0.05–0.11)	0.06 (0.05–0.08)	0.07 (0.06–0.08)	0.08
Sucker minimum diameter	0.08	0.07	–	–	0.07-0.11	–	0.05 (0.03–0.09)	0.06 (0.04–0.08)	0.06 (0.05–0.08)	0.06
Number of rostellar hooks	24	26–30	25–29	25 (23–29)	25 (22–26)	24–26	24 (23–29)	26 (22–31)	24 (24–27)	23
Rostellar hooks length	0.018	0.015-0.017	0.015	0.010-0.023	0.013	0.014-0.016	0.017 (0.015–0.020)	0.018 (0.011–0.023)	0.013 (0.013–0.016)	0.015 (0.014–0.016)
Length of testes	0.11	0.10	–	0.05-0.11	0.06-0.11	0.12-0.18	0.06 (0.04–0.12)	0.06 (0.04–0.12)	0.06 (0.04–0.09)	0.06
Width of testes	0.09	0.08	–	0.04-0.08	0.05-0.11	0.11-0.16	0.04 (0.02–0.06)	0.03 (0.02–0.06)	0.02 (0.02–0.04)	0.02
Length of cirrus sac	0.10	0.12	0.18	0.09-0.18	0.09-0.30	0.14-0.20	0.09 (0.05–0.17)	0.08 (0.04–0.17)	0.06 (0.04–0.09)	0.07
Width of cirrus sac	0.03	0.05	0.05	0.03-0.04	0.04-0.09	00.03-0.05	0.03 (0.02–0.05)	0.03 (0.02–0.06)	0.02 (0.02–0.04)	0.04
Eggs length	0.05	0.07	0.082-0.09	0.062-0.098	–	0.083-0.094	0.03 (0.02–0.04)	0.03 (0.02–0.04)	0.054 (0.052–0.057)	0.04
Eggs width	0.05	0.06	0.067	0.051-0.075	–	0.069-0.087	0.02 (0.02–0.03)	0.02 (0.02–0.03)	0.038 (0.036–0.041)	0.03
Oncosphera length	0.026	0.028	–	0.027-0.047	–	0.038-0.044	0.014 (0.014–0.016)	0.017 (0.016–0.018)	0.032 (0.029–0.036)	0.03
Oncosphera width	0.026	0.028	–	0.029-0.054	–	–	0.012 (0.012–0.013)	0.012 (0.012–0.013)	0.022 (0.020–0.025)	0.02

[a] Specimens previously studied from Guerreiro Martins et al. (2014) and Panisse et al. (2017) were included. Measurements are given in millimeters.

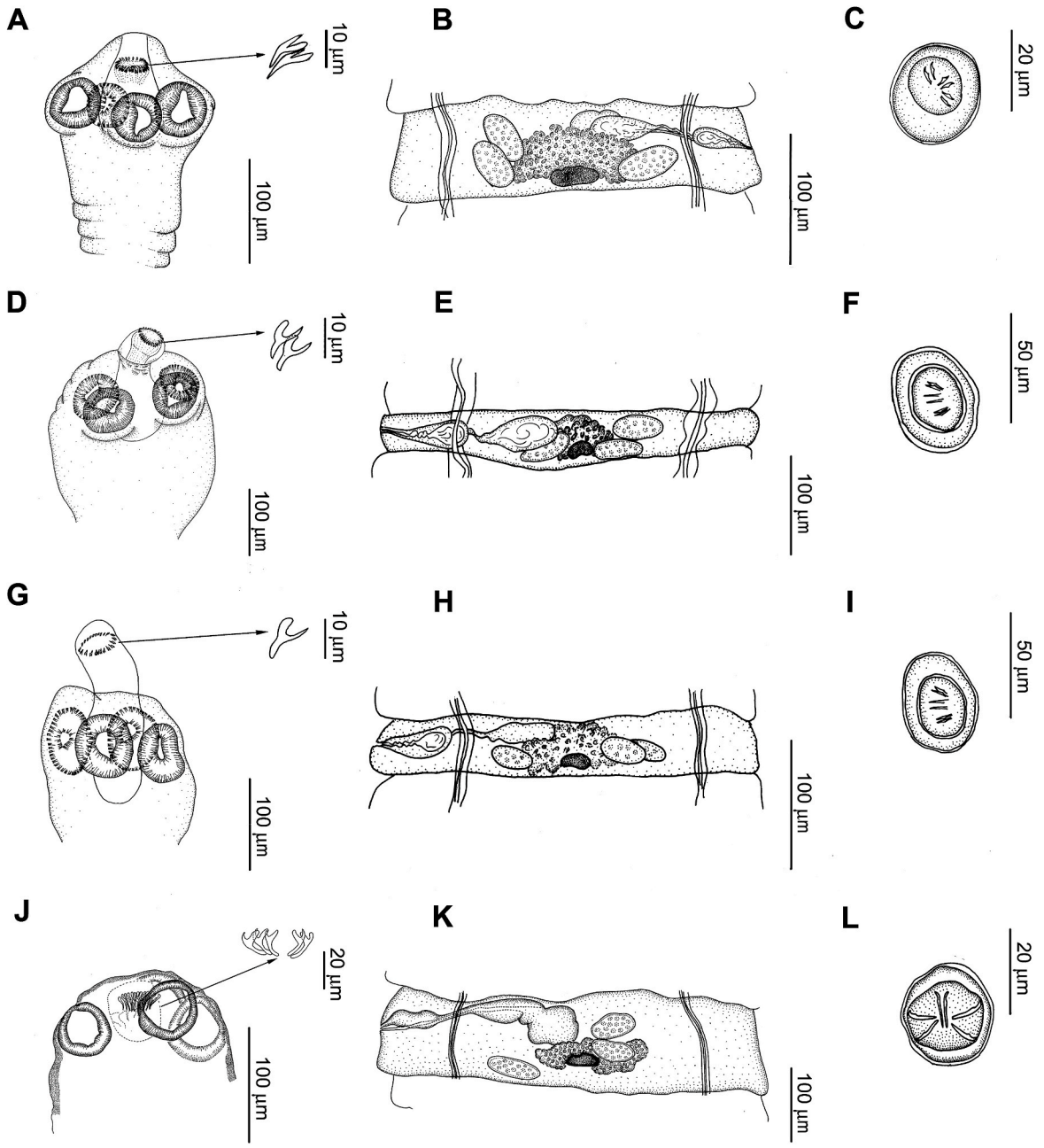

Fig. 1. Morphological features of *Rodentolepis microstoma:* (A, D, G, J) scolex and rostellar hooks; (B, E, H, K) mature proglottids; (C, F, I, L) egg from different host species, (A–C) *Akodon*; (D–F) *Necromys*; (G–I) *Thaptomys*; (J–L) *Oxymycterus*.

each species host: *A. azarae*, n = 63; *A. dolores*, n = 3; *A. montensis*, n = 171; *A. philipmyersi*, n = 8; *N. lasiurus*, n = 62; *T. nigrita*, n = 1, *O. misionalis*, n = 1; *O. rufus*, n = 173). Moreover, 33 localities from the Cuenca del Plata region in Argentina constitute new geographical records for the species (see Table 1).

3.1.1. *Rodentolepis microstoma* (Figs. 1 and 2)

Amended diagnosis: Cyclophyllidea, Hymenolepididae. Strobila long, craspedote, with proglottids wider than long, and in gradual maturation. Scolex with four muscular suckers. Rostellum armed with a single crown of hooks of the cricetoid type (Nº = 22–31), retractable into contractile rostellar pouch. Entire worm covered with acicular filitriches (sensu Chervy, 2009). Young proglottids contain only primordia of testes. Mature proglottids containing three spherical to oval testes, arranged one poral and two aporal, are disposed aligned or in a triangle; vas deferens form an external seminal vesicle; cirrus sac ovoid, does not touch or extends beyond osmoregulatory canals, enclosing cirrus smooth and internal seminal vesicle; lobated ovary, located in the medial zone; vitelline gland compact, lobated, median; vagina located below the cirrus sac; well-developed seminal receptacle, visible even in gravid proglottids; common genital pore unilateral, dextral, near the midpoint of the margin. In gravid segments, the uterus occupies almost the entire proglottid, and contains numerous eggs. Eggs are ovoid, enclosing embryophore with polar filaments (difficult to observe) and oncosphere with embryonic hooks.

3.1.2. Taxonomic summary

Rodent hosts: *Mus musculus* Linnaeus, 1758, *Apodemus* spp. Kaup, 1829, *Mastomys* spp. Thomas, 1915, *Mus musculus* Linnaeus, 1758, *Rattus norvegicus*, *R. rattus*, *Meriones* spp. Illiger, 1811 (Muridae); *Dendromus* spp. Smith, 1829 (Nesomyidae); *Mesocricetus auratus* (Waterhouse, 1839), *Microtus* spp. Schrank, 1798, *Akodon azarae*, *A. dolores*,

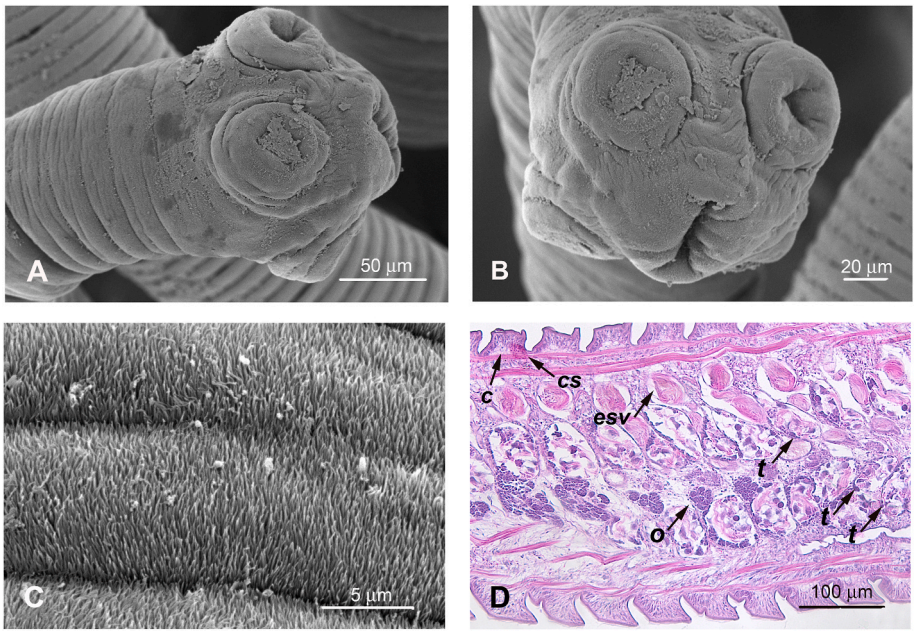

Fig. 2. Scanning electron micrographs of *Rodentolepis microstoma:* (A) scolex with invaginated rostellum, lateral view; (B) scolex with invaginated rostellum, apical view; (C) acicular filitriches (mature proglottids). Histological section of *Rodentolepis microstoma*: (D) testes (t), ovary (o), external seminal vesicle (esv), cirrus sac (cs) and cirrus (c) in mature proglottid.

A. montensis, *A. philipmyersi, Necromys lasiurus*, *Thaptomys nigrita, Oxymycterus misionalis, O. rufus.* (Cricetidae) (Hughes, 1940; Dvorak et al., 1961; Litchford, 1963; Cunningham and Olson, 2010; Guerreiro Martins et al., 2014; Panisse et al., 2017; Panti-May et al., 2018).

New localities: see Table 1.

Voucher specimens: MLP-He 6804, 6806, 6810, 6811, 7592-7622.

Remarks: The specimens here studied show diagnostic morphological features of genus *Rodentolepis* and the parasitic species of American rodents are compared below (Czaplinski and Vaucher, 1994).

Rodentolepis microstoma can be separated from *R. evaginata* (Barker and Andrews, 1915), *R. johnsoni* (Schiler, 1952), *R. octocoronata* (Von Linstow, 1879) and *R. oregonensis* (Neiland and Senger, 1952) by the number of rostellar hooks (22–31 vs. 10, 10, 8, 10, respectively). The specimens of *R. microstoma* studied have scolex smaller than *R. nana* (Von Siebold, 1852) and *R. octocoronata* (0.07–0.23 vs. 0.30–0.40, 0.39, respectively). Moreover, *R. microstoma* has the cirrus sac longer than *R. nana* and *R. johnsoni* (0.04–0.17 x 0.02–0.05 vs. 0.05–0.07 x 0.02–0.025, 0.11 x 0.04, respectively), and is smaller than *R. octocoronata* (0.04–0.17 x 0.02–0.05 vs. 0.30 x 0.37). *Rodentolepis microstoma* can be separated from *R. evaginata*, *R. nana, R. octocoronata*, *R. oregonensis* and *R. srivastavai* (Rêgo, 1970) by the size of the eggs (Barker, 1915; Neiland and Senger, 1952; Schiller, 1952; Wardle and McLeod, 1952; Rêgo, 1970; Sutton, 1974).

Although some features, e.g., cirrus sac, size of eggs, length and shape of hooks, may separate *R. microstoma* and *R. srivastavai*, these should be reviewed in detail, (Rêgo, 1970). In addition, *R. microstoma* and *R. akodontis* show a similar morphology of scolex, length and shape of hooks, number of rostellar hooks, size of suckers, size of cirrus sac and eggs (e.g., Rêgo, 1967; Casanova et al., 2001; Cunningham and Olson, 2010; Gomez-Puerta and Valdivia-Carrera, 2018).

3.2. Molecular analysis

Nucleotide sequence data of the ITS1 rDNA fragment and *cox*1 partial sequences of mtDNA from *R. microstoma* are reported and are available in GenBank (GenBank accession number) (Table 3).

The ITS1 rDNA region revealed 13 haplotypes (ON000402-ON000414): these sequences were 517 base pairs (bp) (exclusive of the primers) and their G + C content was 50.9–52% The multiple alignment of 38 ITS1 sequences (including sequences of species representing members of the genus *Rodentolepis* from rodents and *H. sapiens*, and *H. diminuta* from *Rattus* spp. and *Lemur catta* available in GenBank, Table 3) shows a dataset of 559 characters.

The intra-specific similarity observed in ITS1 sequences of *R. microstoma* from Argentina ranged from 94.91 to 99.74% (Table 4). Similar values of intra-specificity are found among other species of hymenolepidids. For the genus *Rodentolepis*, the minimum values of inter-specific similarity were observed between *R. microstoma* (*T. nigrita*) and *R. nana* (80.28%) and the maximum between *R. microstoma* (*O. rufus*) and *R. nana* (81.44%). Between different genera, the minimum and maximum values of inter-specific similarity were observed in *H. diminuta* vs. *R. microstoma* (*N. temchuki*) (53.59%) and *H. diminuta* vs. *R. microstoma* (*A. azarae*) (55.20%), respectively (Table 4).

*Cox*1 mtDNA encoding gene revealed 12 haplotypes (ON005424–ON005435): these sequences were 350 base pairs (bp) (exclusive of the primers) and their G + C content was 32–33.5%. The multiple alignment of 43 *cox*1 partial sequences (including sequences of species representing members of the genus *Rodentolepis* and *H. diminuta* from *Rattus* spp. and *H. sapiens* and other outgroups available in GenBank [Table 3]) yields a dataset of 350 characters.

The intra-specific similarity observed in *cox*1 sequences of *R. microstoma* from Argentina ranged from 88 to 99.74% (Table 5). Similar values of intra-specificity are found among other species of hymenolepidids. For the genus *Rodentolepis*, the minimum values of inter-specific similarity were observed between *R. microstoma* (*A. montensis*) and *R. nana* (81.10%) and the maximum between *R. microstoma* (Australia, Portugal, Spain) and *R. nana* (85.45%). Between different genera, the minimum and maximum values of inter-specific similarity were observed in *H. diminuta* vs. *R. microstoma* (*A. montensis*) (77.97%) and *H. diminuta* vs. *R. nana* (82.41%), respectively (Table 5).

Phylogenetic trees based on ITS1 rDNA provided robust phylogenetic resolution among Hymenolepididae taxa studied, regardless of the inference method (ML and BPP). The topology showed the existence of two main clades within genus *Rodentolepis*: Clade 1 including *R. microstoma*, with three subclades, and Clade 2 including *R. nana* and

Table 3
Sequences of *Rodentolepis* from rodents and other Hymenolepididae species used for phylogenetic analyses (GenBank Accession numbers).

Species	Host species/ Geographical origin	Code	Gene/ Region	GenBank Accession numbers
***Rodentolepis microstoma* This paper**	***Akodon montensis*/ Misiones**	**RmC18**	**ITS1**	ON000414
		RmC19		ON000413
		RmC24		ON000412
	***Thaptomys nigrita*/Misiones**	**RmC41**		ON000411
	***Oxymycterus misionalis*/ Misiones**	**RmC57**		ON000410
	***Necromys lasiurus*/ Misiones**	**RmC70**		ON000407
		RmC73		ON000408
		RmC74		ON000409
	***Akodon azarae*/ Buenos Aires**	**RmC76**		ON000405
		RmC77		ON000406
	***Oxymycterus rufus*/Buenos Aires**	**RmC79**		ON000402
		RmC80		ON000403
		RmC82		ON000404
Rodentolepis microstoma	*Homo sapiens*/ Australia	Rm1		AY221156
		Rm2		AY221158
		Rm3		AY221160
		Rm4		AY221161
		Rm5		AY221162
		Rm6		AY221163
		Rm7		AY221164
		Rm8		AY221167
		Rm10		AY221155
	Mus musculus/ Canary Islands	Rm9		JN258040
		Rm11		JN258040
	Mus spretus/ Portugal	Rm12		AY221165
Rodentolepis nana	*Homo sapiens*/ Asia	Rn1		MH629970
		Rn2		MH629973
		Rn3		AF461124
	Rattus rattus/Iran	Rn4		KJ917784
	Mus musculus/ México	Rn5		HM447238
	Homo sapiens/ Asia	Rn6		MH629972
		Rn7		MH629969
		Rn8		MH829968
		Rn9		MH629967
Rodentolepis fraterna	*Rattus rattus*/ Spain	Rf		JN258041
***Rodentolepis microstoma* This paper**	***Akodon montensis*/ Misiones**	**RmC18**	***Cox*1**	ON005434
		RmC34		ON005433
	***Thaptomys nigrita*/Misiones**	**RmC41**		ON005432
	***Oxymycterus misionalis*/ Misiones**	**RmC57**		ON005431
	***Necromys lasiurus*/ Misiones**	**RmC70**		ON005430
		RmC73		ON005429
		RmC74		ON005425
	***Akodon azarae*/ Buenos Aires**	**RmC76**		ON005424
		RmC77		ON005435
	***Oxymycterus rufus*/Buenos Aires**	**RmC79**		ON005428
		RmC80		ON005426
		RmC82		ON005427
Rodentolepis microstoma	*Mus musculus*/ Peru	Rm1		MG570384
	Mus musculus/ China	Rm2		LC063188
	Mus musculus/ Japan	Rm3		AB494473
Rodentolepis nana	*Mus musculus*/ Japan	Rn1		AB494471
	Mesocricetus auratus/Uruguay	Rn2		AB494472
	Homo sapiens/ Mexico	Rn3		HM447234
		Rn4		HM447235
	Homo sapiens/ Mexico	Rn5		HM447238
	Mus sp./Mexico	Rn6		LC063187
	Mus musculus/ China	Rn7		KU821727
	Rattus rattus/ India	Rn8		KY079336
	Rattus norvergicus/China	Rn9		GU433102
	Rattus sp./Egypt	Rn10		GU433103
	Rattus sp./Egypt	Rn11		GU433104
Rodentolepis fraternal	*Homo sapiens*/ Egypt	Rf		JN258053
	Rattus rattus, Mus musculus/Spain			
Outgroups				
Hymenolepis diminuta	*Rattus norvergicus*/South Africa	Hd1	ITS1	MG322245
	Rattus rattus/ South Africa	Hd2		MG322244
	Lemur catta/ China	Hd3		KP317833
Hymenolepis diminuta	*Rattus norvergicus*/ United State	Hd1	*Cox*1	MH472979
		Hd2		MH472980
	Rattus rattus/ United State	Hd3		MH472981
	Homo sapiens/ United State	Hd4		MH472982
	Rattus norvergicus/ United State	Hd5		MH472983
	Rattus rattus/ United State	Hd6		MH472986
		Hd7		KF689687
	Rattus norvergicus/China	Hd8		LC063185
	Rattus norvergicus/ Poland	Hd9		KF689686
Arostripelis horrida	*Cletrhionomys gloereolus*/ Lithuania	Ah1		DQ340976
		Ah2		DQ340977
Coronacanthus magnihamatus	*Clethrionomys glareolus*/Belarus	Cm		KJ710327
Coronacanthus vassilevi	*Neomys fodiens*/ Bulgary	Cv		KJ710328
Coronacathus integrus		Ci		KJ710329
Staphylocystoides gulyaevi		Sg		KC789837
Staphylocystoides parissima	*Sorex monticolus*/ USA	Sp		KC789840

R. fraterna with strong support of branches (100% ML bootstrap values BV and Bayesian posterior probability BPP). Furthermore, within Clade 1 were observed: subclade 1a including 12 haplotypes from *H. sapiens* from Australia, *Mus musculus* from Canary Islands and *Mus spretus* from Portugal (100% ML BV and BPP), subclade 1b including haplotypes of *O. rufus* from Buenos Aires province and *O. misionalis* from Misiones province (99% ML BV and BPP) and subclade 1c with a polytomy of three branches (100% ML BV and 100% BPP) including haplotypes of *A. montensis* from Misiones province (100% ML BV and 73% BPP), haplotypes of *N. lasiurus* and *T. nigrita* from Misiones province (100% ML BV and BPP), and haplotypes of *A. azarae* from Buenos Aires province (99% ML BV and 85% BPP). Subclades including *R. microstoma* from Argentina (b and c) form a group separate from subclade 1a including those of other geographical origin (100% ML BV and 84% BPP). In addition, a separate group is observed formed by *H. diminuta* as an outgroup (100% ML BV and 100% BPP) (Fig. 3).

Table 4
Intra-specific and inter-specific similarity observed in ITS1 partial sequences in *Rodentolepis* and *Hymenolepis* species isolated from different host species and geographical origin.

	Rodentolepis microstoma (*Oxymycterus rufus*)	*Rodentolepis microstoma* (*Oxymycterus misionalis*)	*Rodentolepis microstoma* (*Akodon azarae*)	*Rodentolepis microstoma* (*Akodon montensis*)	*Rodentolepis microstoma* (*Necromys temchuki*)	*Rodentolepis microstoma* (*Thaptomys nigrita*)	*Rodentolepis microstoma*	*Rodentolepis nana*	*Hymenolepis diminuta*
Rodentolepis microstoma (*Oxymycterus rufus*)	98.71%								
Rodentolepis microstoma (*Oxymycterus misionalis*)	98.77%	–							
Rodentolepis microstoma (*Akodon azarae*)	94.91%	95.09%	97.58%						
Rodentolepis microstoma (*Akodon montensis*)	96.89%	97.34%	97.02%	99.61%					
Rodentolepis microstoma (*Necromys temchuki*)	96.05%	96.37%	96.89%	97.93%	99.74%				
Rodentolepis microstoma (*Thaptomys nigrita*)	95.40%%	95.73%	95.05%	97.09%	98.89%	–			
Rodentolepis microstoma	94.81%	95.129%	94.09%	95.97%	94.61%	93.77%	99.59%		
Rodentolepis nana	81.44%	80.86%	80.77%	80.47%	80.54%	80.28%	80.58%	99.78%	
Hymenolepis diminuta	53.63%	53.98%	55.20%	54.20%	53.59%	54.56%	54.05%	54.75%	98.95%

Table 5
Intra-specific and inter-specific similarity observed in *cox*1 partial sequences in *Rodentolepis* and *Hymenolepis* species isolated from different host species.

	Rodentolepis microstoma (*Oxymycterus rufus*)	*Rodentolepis microstoma* (*Oxymycterus misionalis*)	*Rodentolepis microstoma* (*Akodon azarae*)	*Rodentolepis microstoma* (*Akodon montensis*)	*Rodentolepis microstoma* (*Necromys temchuki*)	*Rodentolepis microstoma* (*Thaptomys nigrita*)	*Rodentolepis microstoma*	*Rodentolepis nana*	*Hymenolepis diminuta*
Rodentolepis microstoma (*Oxymycterus rufus*)	98.71%								
Rodentolepis microstgoma (*Oxymycterus misionalis*)	94.29%	–							
Rodentolepis microstoma (*Akodon azarae*)	89.61%	90.29%	97.58%						
Rodentolepis microstoma (*Akodon montensis*)	88.29%	89.86%	88%	99.61%					
Rodentolepis microstoma (*Necromys temchuki*)	92.57%	91.33%	89.05%	88%	99.74%				
Rodentolepis microstoma (*Thaptomys nigrita*)	92.47%	92.86%	89.43%	88.29%	96.86%	–			
Rodentolepis microstoma	89.81%	88.86%	89.23%	86.67%	90.35%	91.43%	99.62%		
Rodentolepis nana	82.36%	82.60%	83.48%	81.10%	83.65%	84.10%	85.45%	98.65%	
Hymenolepis diminuta	81.41%	80.25%	81.11%	77.97%	81.78%	82.83%	81.94.7%	83.413%	99.70%

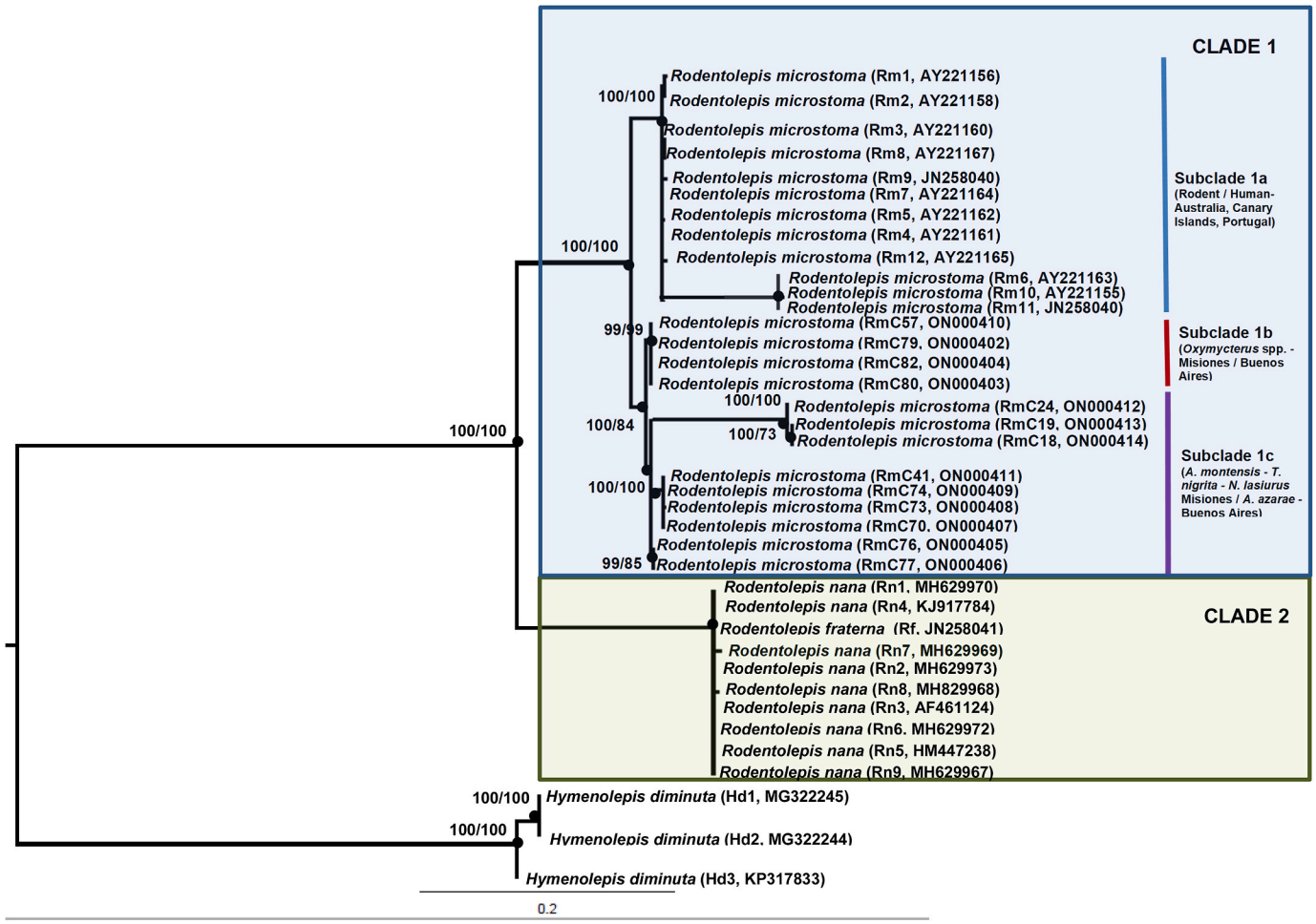

Fig. 3. Phylogenetic tree of *Rodentolepis* spp. (Hymenolepididae: Cestoda) based on ITS1 mitochondrial DNA. Phylogenetic tree inferred using Bayesian method. Maximum Likelihood bootstrap values of clades are listed first, followed by Bayesian Posterior Probabilities respectively, for clade frequencies exceeding 65%.

The analysis of the dataset based on *cox*1 mtDNA showed partial congruence with respect to the phylogenetic relationships between *Rodentolepis* spp. based on dataset ITS1. Phylogenetic analysis provided robust phylogenetic resolution among Hymenolepididae taxa, regardless of the inference method (ML and BPP). The topology showed the existence of two main clades within genus *Rodentolepis*: Clade 1 including *R. microstoma*, with five subclades, and Clade 2 including *R. nana* with strong support of branches (100% ML bootstrap values BV and Bayesian posterior probability BPP). Furthermore, within Clade 1 were observed: subclade 1a including 3 haplotypes from *M. musculus* from Peru, China, and Japan (100% ML BV and 95% BPP); subclade 1b including haplotypes of *O. rufus* from Buenos Aires province and *O. misionalis* from Misiones province (80% ML BV), and 1c with a polytomy of three branches including a haplotype of *A. montensis* from the province of Misiones (100% ML BV and BPP); haplotypes of *A. azarae* from the province of Buenos Aires and *A. montensis* from the province of Misiones (100% ML BV and BPP); and haplotypes of *N. lasiurus* and *T. nigrita* from the province of Misiones. Subclades including *R. microstoma* from Argentina (b y c) form a separate group of subclade 1a including those of other geographical origin (100% ML BV and BPP). In addition, a separate grouped is formed including *H. diminuta* and other Hymenolepidids as an outgroup (100% ML BV and BPP) (Fig. 4).

The concatenated dataset of ribosomal (ITS1) and mitochondrial (*cox*1) gene sequences included 931 aligned sites and only 22 taxa (outgroups not included for phylogenetic analysis). Phylogenetic analyses of this dataset yielded a tree with branches that were strongly supported (100% ML BV and 92–100% BPP). Phylogenetic inferences match the phylogenetic results based on separate markers. Thus, two main clades were observed: Clade 1 including *R. microstoma* and Clade 2 including *R. nana*. Clade 1 includes the same clades as shown in ITS1 and the *cox*1 trees, but the relation among them is slightly different, since subclade 1b forms a sister group with a part of subclade 1c (100% ML bootstrap values BV and BPP) (Fig. 5).

4. Discussion

Rodentolepis microstoma was first described as *Taenia microstoma* by Dujardin (1845) from bile ducts of mice, and was later transferred to the genus *Hymenolepis* Weinland, 1858 (Blanchard, 1891). Later, Spasskii (1954) in a revision of the Hymenolepididae family, transferred *H. microstoma* again to the genus *Rodentolepis*. Since Schmidt (1986) considered *Rodentolepis* as synonymous with *Vampirolepis* Spasskii, 1954 genus, a new combination, *Vampirolepis microstoma*, was proposed. In the last taxonomic revision of the Hymenolepididae family, Czaplinski and Vaucher (1994) considered the genus *Rodentolepis* as valid.

Rodentolepis microstoma is recorded in America, Africa, Europe, and Asia from a wide range of rodent genera (e.g., *Apodemus*, *Arvicanthis*, *Dendromys*, *Leggada*, *Mastomys*, *Merionis*, *Mesocricetus*, *Microtus*, *Mus*, *Promomys*, *Rattus*, and *Sigmodon*) (Dvorak et al., 1961; Litchford, 1963; Hickman, 1964; Casanova et al., 2001). *Rodentolepis microstoma* infection in humans feces in Australia suggests the possibility of a potential zoonosis (Macnish et al., 2003).

Mature tapeworms occur in the small intestine; however,

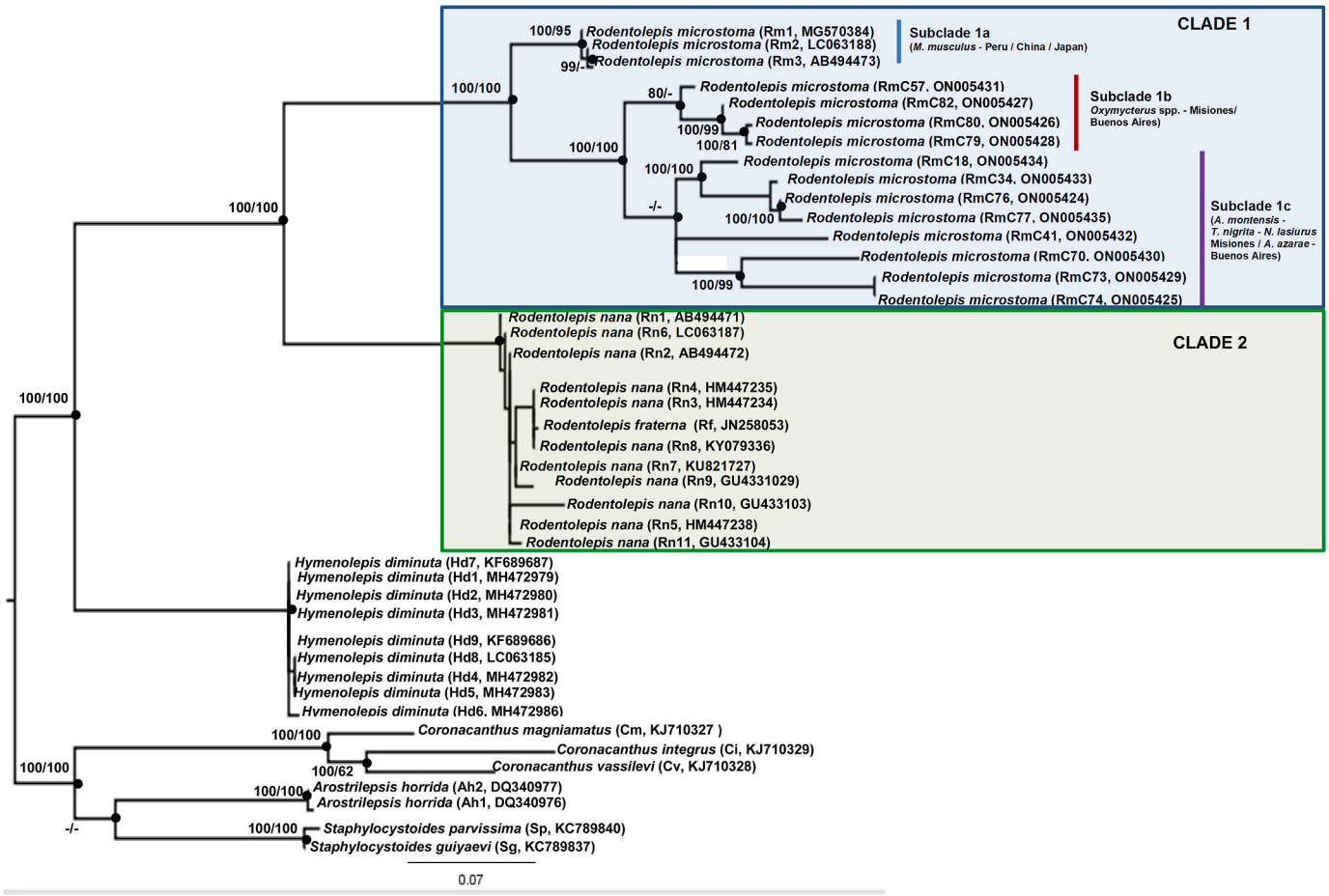

Fig. 4. Phylogenetic tree of *Rodentolepis* spp. (Hymenolepididae: Cestoda) based on *cox*1 mitochondrial DNA. Phylogenetic tree inferred using Bayesian method. Maximum Likelihood bootstrap values of clades are listed first, followed by Bayesian Posterior Probabilities respectively, for clade frequencies exceeding 65%.

R. microstoma was recorded on several occasions in the bile duct of the mammalian host (e.g., Cunningham and Olson, 2010; Gomez-Puerta and Valdivia-Carrera, 2018). Litchford (1963) showed that this species can also parasitize the mouse, hamster, and rat duodenum. In the present study, specimens of host species were found in the small intestine, mainly in the duodenum.

The morphometric characters of *R. microstoma* provided by different studies agree with the specimens examined in this study (e.g., scolex size, suckers, proglottids, testes, cirrus sac, eggs). The range of number of rostellar hooks was wider in the present survey at 22–31 than the ranges recorded previously of 23–29 by Casanova et al. (2001), 22–26 by Cunningham et al. (2010), 24–26 by Gomez-Puerta and Valdivia-Carrera (2018). On the other hand, some morphologically close species, such as *R. srivastavai* and *R. akodontis*, show overlapping hook number ranges (26–30 and 24, respectively) (Rêgo, 1967, 1970). This data must be reviewed due to the frequent loss of hooks during recovery and study of the specimens, which may indicate a greater or complete overlap of those ranges.

In addition, some minimum variations in size and arrangement of testes were observed among the specimens of *R. microstoma* from different host genera, as well as the size of the eggs and cirrus sac (crossing or not crossing excretory canals). Thus, the disposition of the testes can be presented as a polymorphic character, as is suggested in the present paper.

These observations show phenotype plasticity since *R. microstoma* occurs in a wide range of host species and areas. Particularly, in this study, morphological variations are observed in *R. microstoma* among the eight species of Sigmodontinae rodents and 33 localities of Argentina, expanding their host and geographic distribution, and morphometrical features from previous studies (Table 2).

Originally, the surveys of Guerreiro Martins et al. (2014) and Panisse et al. (2017) assigned the same specimens studied in the present paper to *Rodentolepis* cf. *akodontis*. However, the morphological revision of these specimens and several others indicated the presence of phenotypic variability and their identification as *R. microstoma*. In addition, the type specimens of *R. akodontis* were also reviewed and these could not be separated from *R. microstoma* either (see Rêgo, 1967; Casanova et al., 2001; Cunningham and Olson, 2010; Gomez-Puerta and Valdivia-Carrera, 2018). Consequently, the validity of *R. akodontis* as a full species is questioned, and a full review should be made. Nevertheless, considering the poor state of conservation of type specimens, neotypes should be designed, and the species *R. akodontis* treated as inquirenda.

The molecular analyses showed that the specimens studied form a same clade with *R. microstoma* previously studied from other hosts and regions (ITS: *H. sapiens* form Australia, *Mus* spp. from Canary Islands and Portugal; *cox*1: *Mus* spp. from Peru, China, and Japan). The specimens form 3 subclades (Clade 1a-c) which correspond to different group of species hosts and regions. Specimens of *R. microstoma* from humans and Muridae distributed outside American continent form a subclade 1a, separate from the rest, and subclade 1b is the sister group of subclade 1c (ITS and *cox*1). Specimens of subclade 1b are parasites of *Oxymycterus* spp., and are a clade separate from the rest of Akodontini, such as the genera *Akodon*, *Necromys* and *Thaptomys*. Therefore, the genetic polymorphism observed of *R. microstoma* corresponds with some of the phylogenetic proposals of the hosts (D'Elía, 2003; Salazar-Bravo et al.,

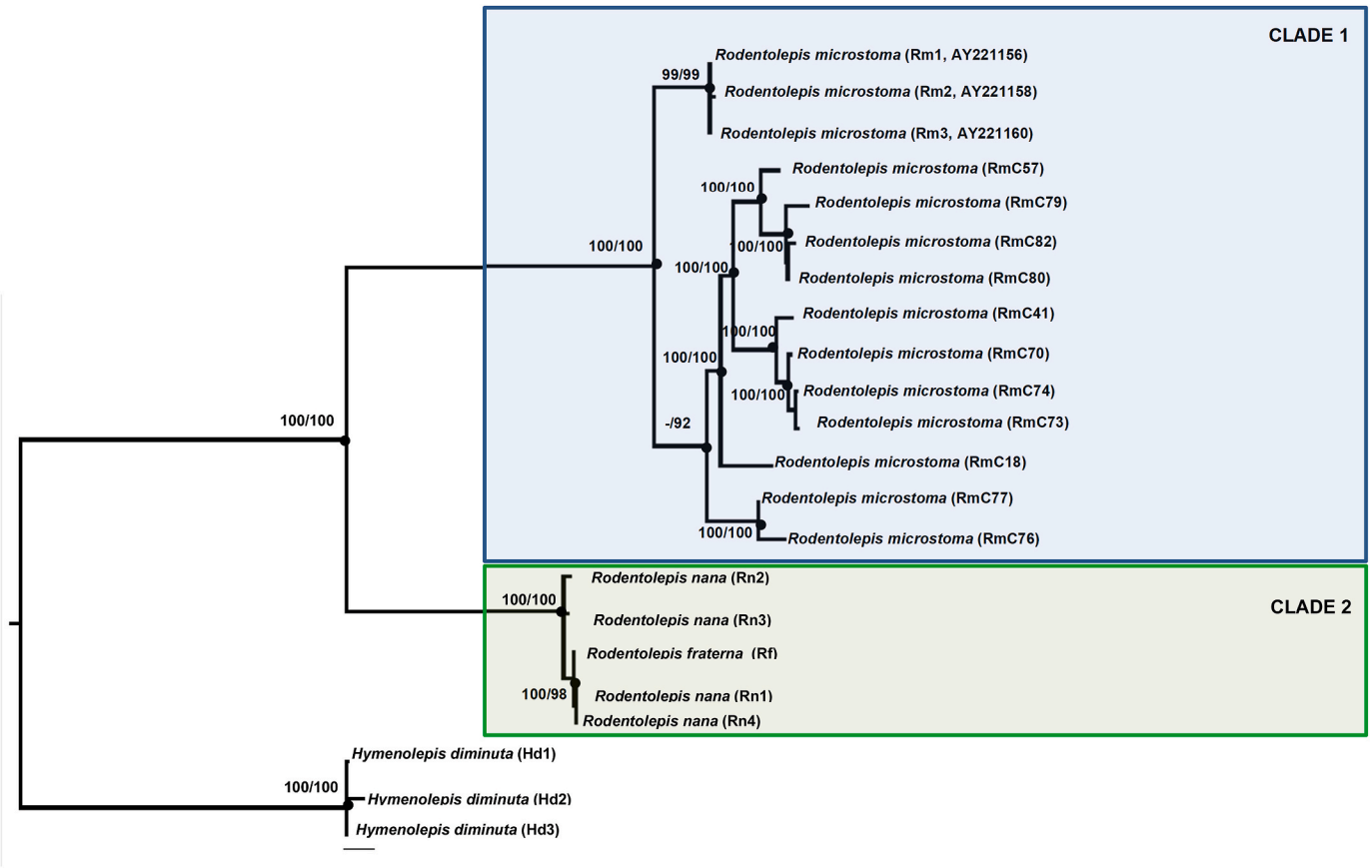

Fig. 5. Phylogenetic tree of *Rodentolepis* spp. (Hymenolepididae: Cestoda) based on concatenated *cox*1 mitochondrial and ITS1 ribosomal DNA. Phylogenetic tree inferred using Bayesian method. Maximum Likelihood bootstrap values of clades are listed first, followed by Bayesian Posterior Probabilities respectively, for clade frequencies exceeding 65%.

2013). Each subclade from the ITS1 and *cox*1 phylogenetic trees shows percentages of robustness. The phylogenetic tree based on concatenated ITS1 and *cox*1 shows different relationships among some of the subclades from Argentinian specimens.

This study shows the importance of using integrative taxonomic approaches that combine morphological and molecular characters to understand biological diversity. Thus, when it comes to morphologically very similar specimens, where subtle differences are observed, it can be defined whether they are part of the intraspecific variability of the species or indicate different taxa.

In addition, the discovery of *R. microstoma* in humans (Macnish et al., 2003) suggests the importance of further studies on this zoonotic cestode. This study provides significant data on the taxonomy and distribution of *R. microstoma* to advance knowledge of the transmission dynamics of this parasite.

Declaration of competing interest

The authors declare that they have no competing interests.

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