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Helminth communities of two populations of *Myotis chiloensis* (Chiroptera: Vespertilionidae) from Argentinean Patagonia

Antonella C. Falconaro*, Rocío M. Vega, Gustavo P. Viozzi

Laboratorio de Parasitología, INIBIOMA (CONICET-UNCo), Quintral 1250, 8400 Bariloche, Río Negro, Argentina

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ABSTRACT

Most of the studies on chiropteran endoparasites in Argentina come from the Central and Northeast regions of the country, and there is only one parasitological study of bats from the Argentinean Patagonia. The aim of this study is to describe the helminth fauna of 42 *Myotis chiloensis,* comparing the composition and the structure of the endoparasite communities between two populations, inhabiting different environments in Andean humid forest and the ecotone between forest and Patagonian steppe. A total of 697 helminths were recovered from 33 bats: five species of trematodes, *Ochoterenatrema* sp., *Paralecithodendrium* sp., *Parabascus limatulus*, *Parabascus* sp., and *Postorchigenes* cf. *joannae*, two species of cestodes, *Vampirolepis* sp. 1 and *Vampirolepis* sp. 2, and three species of nematodes, *Allintoshius baudi*, *Physaloptera* sp., and *Physocephalus* sp. All the helminths, but *Physocephalus* sp., were recovered from the small and large intestine. This is the first survey of *M. chiloensis*' helminth fauna. All the species, but *A. baudi*, represent new records of helminths in Patagonian bats. There were differences of parasite species richness between localities and both bat populations share almost half of the endoparasite species. Different preferences for intestinal regions were found for three species of trematodes in the bats from the site in the humid forest. *Myotis chiloensis* serves as both a definitive and intermediate host for endoparasites in the Patagonian ecosystem.

1. Introduction

Studies on chiropteran endoparasites are limited around the world, which means that the diversity of bat's parasites is probably underestimated (Lord et al., 2012; Portes Santos and Gibson, 2015). Most of the studies in Argentina come from the Central and Northeast regions of the country, where 17 species of nematodes, 12 of trematodes, and four of cestodes have been reported (Boero and Delpietro, 1970; Lunaschi, 2002a, 2002b, 2004, 2006; Lunaschi et al., 2003; Drago et al., 2007; Lunaschi and Drago, 2007; Ramallo et al., 2007; Lunaschi and Notarnicola, 2010; Oviedo et al., 2009, 2010, 2012, 2016; Fugassa, 2015; Portes Santos and Gibson, 2015; Milano, 2016) (Table 1). There is only one parasitological study of bats from Argentinean Patagonia, describing the trichostrongylid nematode, *Allintoshius baudi*, from *Myotis aelleni* collected in El Hoyo de Epuyén, Chubut province (Vaucher and Durette-Desset, 1980).

Myotis chiloensis, known as "Murcielaguito de Chile (Chilean Little bat)" is an endemic vespertilionid bat from the Argentinean and Chilean Patagonia. These bats have been collected in the Argentinean provinces of Neuquén, Río Negro, Chubut, and Tierra del Fuego (Barquez and Díaz, 2009; Barquez et al., 2013; Díaz et al., 2011). They are small, insectivorous bats which feed mainly on insects of the orders Lepidoptera, Diptera, Coleoptera, and Trichoptera (Giménez and Giannini, 2013; Ossa and Rodríguez San Pedro, 2015).

The aim of this study is to report for the first time the helminth fauna of *M. chiloensis* and to compare the composition and the structure of the endoparasite communities between two populations, inhabiting different environments in Andean Patagonia.

2. Materials and methods

A total of 42 specimens of *M. chiloensis* were captured in two rural localities in the Southwest region in the Patagonian province of Río Negro. The survey was part of the project "Estudio de fauna de pequeños vertebrados (anfibios, reptiles and mamíferos) en el Oeste de la provincia de Río Negro", directed by Dr. Richard Sage, and it did not require any additional approval by an animal ethics committee. The first sample (N = 16) was captured in the site "Manso" (41°35′45″ S, 71°45′58″ W), located in the humid forest, on December 2011. The second sample (N = 26), was captured in the site "Luis Ruiz" (41°54′40″ S, 71°25′23″ W), located in the ecotone between the forest and the steppe, on January 2014 (Fig. 1).

* Corresponding author.
E-mail addresses: anto.falconaro@gmail.com (A.C. Falconaro), rvega@comahue-conicet.gob.ar (R.M. Vega), gustavo.viozzi@crub.uncoma.edu.ar (G.P. Viozzi).

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Table 1
Previous and new records of endoparasites species infesting Argentinian bats. When available, the number of hosts investigated was detailed (N), indicated with a letter the corresponding study.

Helminth species	Host	Province	Author
Trematoda			
Anenterotrema liliputanium	*Molossops temminckii* (N = 2[a]), *Molossus rufus* (N = 20[b])	Misiones, Corrientes	[a]Lunaschi and Notarnicola, 2010; [b]Milano, 2016.
Anenterotrema eduardocaballeroi	*Molossops temminckii* (N = 5[b]), *Molossus rufus* (N = 20[b])	Corrientes	[b]Milano, 2016.
Gymnoacetabulum talaveraense	*Eumops pagatonicus* (N = 66[b]), *Molossus molossus, Myotis albescens* (N = 35[b]), *Myotis levis, Tadarida brasiliensis,*	Buenos Aires	Lunaschi, 2002; Drago et al., 2007, Lunaschi and Drago, 2007; [a]Lunaschi and Notarnicola, 2010; [b]Milano, 2016.
Limatulum umbilicatum	*Sturnira lilium*	Buenos Aires	Lunaschi et al., 2003; Drago et al., 2007.
Limatulum oklahomense	*Molossus rufus* (N = 20[b]), *Myotis* cf *nigricans* (N = 31[b]), *Myotis albescens* (N = 35[b])	Buenos Aires	[b]Milano, 2016.
Ochoterenatrema labda	*Eumops patagonicus* (N = 66[b]), *Molossops temminckii* (N = 5[b]), *Molossus rufus* (N = 20[b]), *Myotis albescens* (N = 35[b]), *Myotis cf nigricans* (N = 31[b]), *Myotis levis, Tadarida brasiliensis,*	Buenos Aires, Corrientes	Drago et al., 2007; [a]Lunaschi and Notarnicola, 2010; [b]Milano, 2016.
Ochoterenatrema sp.	*Myotis chiloensis*	Río Negro	This study.
Parabascus limatulus	*Tadarida brasiliensis* (N = 1[c]), *Myotis chiloensis*	Buenos Aires, Río Negro	[c]Lunaschi, 2004; This study.
Parabascus sp.	*Myotis chiloensis*	Río Negro	This study.
Paralecithodendrium conturbatum	*Tadarida brasiliensis*	Buenos Aires	Lunaschi and Drago, 2007.
Paralecithodendrium aranhai	*Eumops patagonicus* (N = 66[b]), *Myotis* cf *nigricans* (N = 31[b])	Buenos Aires	[b]Milano, 2016.
Paralecithodendrium sp.	*Myotis chiloensis*	Río Negro	This study.
Postorchigenes cf. *joannae*	*Myotis chiloensis*	Río Negro	This study.
Topsiturvitrema verticalia	*Myotis levis* (N = 15[d])	Buenos Aires	[d]Lunaschi, 2006; Lunaschi and Drago, 2007.
Urotrema scabridum	*Eumops bonaerensis, Eumops patagonicus* (N = 66[b]), *Molossops temminckii* (N = 5[b]), *Molossus molossus* (N = 6[b]), *Molossus rufus* (N = 20[b]), *Myotis albescens* (N = 35[b]), *Myotis levis, Myotis* cf *nigricans,* (N = 31[b]) *Phyllostomus* sp., *Tadarida brasiliensis*	Buenos Aires, Misiones, Corrientes	Drago et al., 2007; [a]Lunaschi and Notarnicola, 2010; [b]Milano, 2016.
Cestoda			
Vampirolepis decipiens	*Eumops abrasus*	Misiones	Boero and Delpietro, 1970.
Vampirolepis elongata	*Pygoderma bilabiatum*	Misiones	Boero and Delpietro, 1970.
Vampirolepis guarany	*Artibeus lituratus* (N = 10[b]), *Eptesicus patagonicus* (N = 66[b]), *Molossus rufus* (N = 20[b])	Corrientes, Misiones	[b]Milano, 2016.
Vampirolepis cf *macroti*	*Eptesicus furinalis* (N = 16[b])	Corrientes	[b]Milano, 2016.
Vampirolepis sp. 1	*Myotis chiloensis*	Río Negro	This study.
Vampirolepis sp. 2	*Myotis chiloensis*	Río Negro	This study.
Nematoda			
Allintoshius baudi	*Myotis aelleni, Myotis chiloensis*	Chubut, Río Negro	Vaucher and Durette-Desset, 1980; This study.
Allintoshius parallintoshius	*Myotis albescens* (N = 1[e])	Entre Ríos	[e]Oviedo et al., 2009.
Allintoshius sp.	*Eptesicus furinalis* (N = 7[e])	Entre Ríos	[e]Oviedo et al., 2009.
Aochotheca sp.	Chiroptera gen. sp., *Eptesicus furinalis* (N = 7[e]), *Myotis levis* (N = 14[e])	Entre Ríos	Ramallo et al., 2007; [e]Oviedo et al., 2009.
Anoplostrongylus sp.	*Eptesicus furinalis* (N = 16[b]), *Eumops bonaerensis* (N = 3[e]), *Eumops patagonicus* (N = 5e; N = 66[b]), *Eumops perotis* (N = 1[b]), *Molossus rufus* (N = 20[b])	Corrientes, Buenos Aires, Entre Ríos	[e]Oviedo et al., 2009; [b]Milano, 2016.
Biacantha normaliae	*Desmodus rotundus*	Jujuy, Salta, Tucumán	Oviedo et al., 2012.
Capillaria sp.	*Molossus rufus* (N = 20[b]), *Molossops temminckii* (N = 5[b]), *Sturnira lilium* (N = 9[b]), *Tadarida brasiliensis*	Buenos Aires, Corrientes	Drago et al., 2007; [b]Milano, 2016.
Cheiropteronema striatum	*Artibeus planirostris* (N = 64[f])	Jujuy, Salta, Tucumán	[f]Oviedo et al., 2010.
Litomosoides sp.	*Sturnira lilium*	Misiones	Boero and Delpietro, 1970.
Litomosoides molossi	*Molossus molossus*	Entre Ríos	Oviedo et al., 2016.
Litomosoides chandleri	*Artibeus planirostris, Eumops perotis, Sturnira erythromos, Sturnira lilium, Sturnira oporaphilum*	Entre Ríos	Oviedo et al., 2016.
Litomosoides saltensis	*Eptesicus furinalis*	Entre Ríos	Oviedo et al., 2016.
Molostrongylus acanthocolpos	*Myotis temminckii* (N = 14)	Entre Ríos	Oviedo et al., 2009.
Pterygodermatites sp.	*Eumops patagonicus* (N = 66[b])		[b]Milano, 2016.
Physaloptera sp.	Chiroptera gen. sp., *Eptesicus furinalis* (N = 7[e]), *Myotis chiloensis*	Entre Ríos, Río Negro	Ramallo et al., 2007; [e]Oviedo et al., 2009; This study.
Physocephalus sp.	*Myotis chiloensis*	Río Negro	This study
Rictularia sp.	*Molossops temminckii* (N = 9[e])	Entre Ríos	[e]Oviedo et al., 2009.

2.1. Host and parasitological procedures

The bats were sacrificed by an overdose of sodic pentobarbital injectable and preserved and fixed in a 10% formaldehyde neutral solution. The bats' abdominal and thoracic cavities and organs were examined with a stereoscopic microscope. The intestine was divided in three sections: 1) duodenum, 2) small intestine (jejunum and ileum), and 3) large intestine and rectum. The site of infection and the number of parasites found were registered and the specimens which were found detached from the host during the necropsy were not assigned to any particular intestinal section. Permanent and transitory slides were made in order to identify the helminths. The trematodes were dehydrated gradually in ethylic alcohol (70%, 96%), stained with Grenacher's carmin, and mounted in Canada balsam. The cestodes and nematodes were cleared with Aman's lactophenol. All parasites were photographed and measured using a Zeiss Primo Star compound microscope equipped with a digital camera (Zeiss Axiocam ERc 5s). One specimen of each species was deposited in the Colección Nacional de Parasitología, Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina (MACN-Pa).

Fig. 1. Location of the sampling sites in the province of Río Negro, Argentina.

2.2. Statistical procedures

The prevalence (P) and mean intensity (MI) was calculated for each species of endoparasite in order to characterize quantitatively the infections (Bush et al., 1997; Villarreal et al., 2006). The infracommunities species richness was quantified and a Mann-Whitney *U* test was used for comparing them between the two localities. In order to estimate the undetected species, the species richness was estimated using Chao and Jackknife ($Chao_{Locality}$ ± SE, $Jack_{Locality}$ ± SE) (these analyses were performed in R 3.4.2 using the vegan package). The Jaccard Index was used for comparing the similarity of presence/absence of species between the two localities (Krebs, 1989) and the Simpson's Diversity Index ($D_{Locality}$) and the Pielou's Evenness Index ($J'_{Locality}$) were used for evaluating the diversity and dominance of species in the two localities (Bush et al., 1997; Villarreal et al., 2006). The Fager's Affinity Index was used for establishing the association rate between two species (Fager, 1957). In order to determine the preference for an intestinal region, a Kruskall-Wallis test was used, comparing the abundance of species among the sites of infection (Conover, 1980).

3. Results

3.1. Species composition

A total of 697 helminths was recovered from 33 bats: five species of trematodes, *Ochoterenatrema* sp. (Lecithodendriidae), *Paralecithodendrium* sp. (Lecithodendriidae), *Parabascus limatulus* (Braun, 1900) (Phaneropsolidae), *Parabascus* sp. (Phaneropsolidae), and *Postorchigenes* cf. *joannae* (Zdzitowiecki, 1967) (Phaneropsolidae), two species of cestodes, *Vampirolepis* sp. 1 and *Vampirolepis* sp. 2 (Hymenolepididae), and three species of nematodes, *A. baudi* (Trichostrongyloidea), *Physocephalus* sp. (Spiruroidea), and *Physaloptera* sp. (Physalopteroidea) (Fig. 2). All the species of helminths, but the larvae of *Physocephalus* sp., which was found encysted in the peritoneum, were recovered from the small and large intestine (Fig. 3). A total of 103 parasites were found detached from the host, and the site of infection could not be specified. The total number of worms, the abundance, the prevalence, and the mean intensity of each helminth species in both component communities are shown in Table 2.

Only two associations were significant: the association between the trematodes *Parabascus limatulus* and *Parabascus* sp., which showed the highest value of association (0.90, $t = 3.05$) in Manso, and the association between *Ochoterenatrema* sp. and *P. limatulus* (0.84, $t = 2.45$).

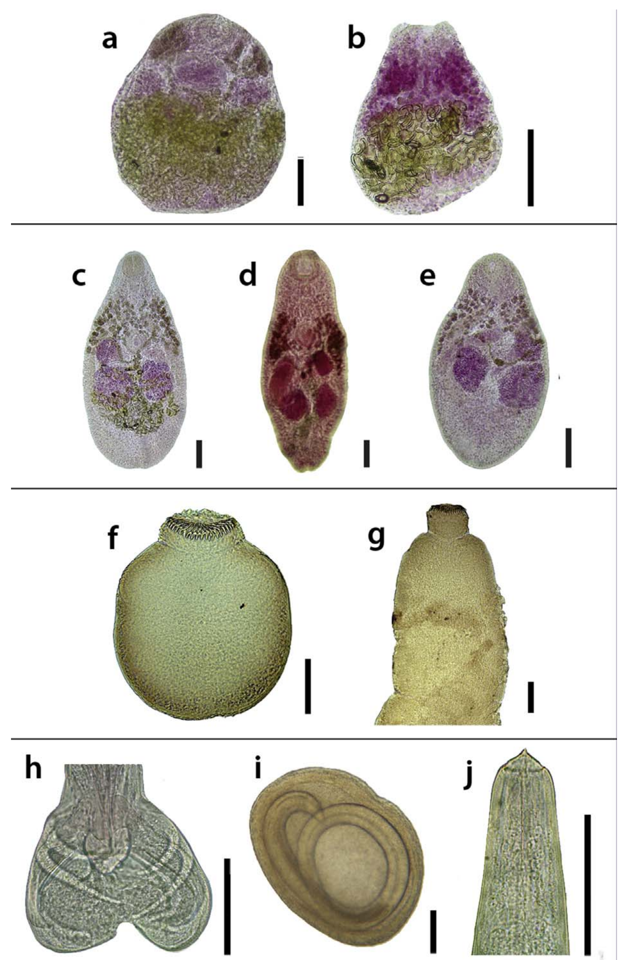

Fig. 2. Endoparasites of *Myotis chiloensis*. a. *Ochoterenatrema* sp. (ventral view), b. *Paralecithodendrium* sp. (ventral view), c. *Parabascus limatulus* (ventral view), d. *Parabascus* sp. (dorsal view), e. *Postorchigenes* cf. *joannae* (dorsal view), f. *Vampirolepis* sp. 1 (scolex), g. *Vampirolepis* sp. 2 (scolex), h. *Allintoshius baudi* (male's bursa), i. *Physocephalus* sp. (encysted larvae), j. *Physaloptera* sp. (anterior region). Scale bar = 100 μm.

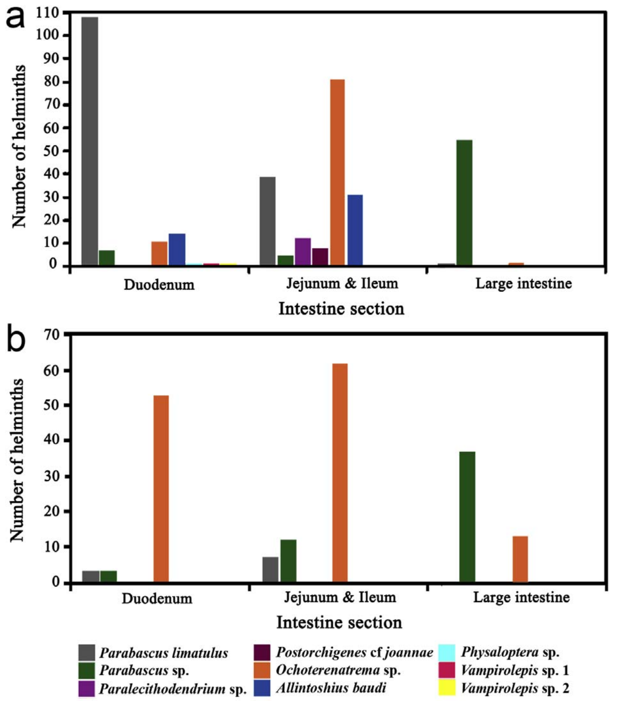

Fig. 3. Intestinal location of the endoparasites of *Myotis chiloensis*, represented by the number of helminths found infecting each intestinal region from the bats from a. Manso, b. Luis Ruiz.

The nematode *A. baudi* also presented significant associations when paired with trematodes: with *Ochoterenatrema* sp. the Fager's index value was 0.76 ($t = 2.05$), with *P. limatulus* was 0.82 ($t = 2.56$) and with *Parabascus* sp. was 0.73 ($t = 1.72$).

3.2. Comparison of the component communities

The richness of parasite species was higher in Manso (nine species) than in Luis Ruiz (five species), and was significantly different between localities ($U = 74$, $p = .0005$). The expected species richness in Manso was slightly higher than the observed in Manso ($\text{Chao}_{\text{Manso}} = 10.88 \pm 3.52$, $\text{Jackk}_{\text{Luis Ruiz}} = 10.88 \pm 1.33$). Meanwhile, the number of observed species richness and the expected were similar in Luis Ruiz ($\text{Chao}_{\text{Manso}} = 5.00 \pm 0.43$, $\text{Jackk}_{\text{Luis Ruiz}} = 5.96 \pm 0.96$). The Jaccard index showed that both bat populations share almost half of the endoparasite species (0.45). The diversity indexes indicated that none of the component communities was dominated by a particular species ($D_{\text{Manso}} = 0.19$, $J'_{\text{Manso}} = 0.52$; $D_{\text{Luis Ruiz}} = 0.35$, $J'_{\text{Luis Ruiz}} = 0.47$).

3.3. Distribution of the helminths within the hosts

Differences in the abundances among the intestinal regions were found in some species of trematodes in the bats from Manso. Three species of trematodes showed significantly different abundances: *P. limatulus* (H[2] = 15.8315, $p = .0004$), which infected mainly the duodenum (Fig. 3a), *Ochoterenatrema* sp. (H[2] = 7.6357, $p = .0220$) located mainly in the middle intestine (Fig. 3a), and *Parabascus* sp. (H[2] = 10.7617, $p = .0046$) in the large intestine. There were no differences in the abundances among the intestinal sections found for these species of trematodes in Luis Ruiz.

4. Discussion

4.1. Species composition

This is the first survey of *M. chiloensis*' helminth fauna. All the species, but *A. baudi*, represent new records of helminths in Patagonian bats.

4.1.1. Trematodes

Two of the species of trematodes belong to the Lecithodendriidae family and three to the Phaneropsolidae family. Specimens of the first family belong to the genus *Ochoterenatrema* and *Paralecithodendrium*. There are five species of the genus *Ochoterenatrema* parasitizing bats all around the world (Cain, 1966; Odening, 1969; Castiblanco and Vélez, 1982; Lotz and Font, 1983; Lunaschi, 2002a, 2002b; Milano, 2016) and *Ochoterenatrema labda* was the only species registered previously in Argentina (Lunaschi, 2002a, 2002b; Milano, 2016). The specimens found during this study were placed in this genus due to their ventral sucker slightly smaller than the oral sucker, the pseudogonotyl to the left of the ventral sucker, the testes at the level of the ventral sucker and the pseudocirrus-sac between the intestinal bifurcation and the ventral sucker (Lotz and Font, 2008a). However, the trematodes found in *M. chiloensis* have morphological differences with the other species of the genus. The main differences between *Ochoterenatrema* sp. from *M. chiloensis* and *O. labda* is the spinous tegument and the ovary, which is entire in our specimens. The combination of these characteristics suggests that *Ochoterenatrema* sp. would be a new species. There are two previous records of *Paralecithodendrium* species in Argentina: *Paralecithodendrium conturbatum* and *Paralecithodendrium aranhai*, parasitizing bats from Buenos Aires province (Lunaschi and Drago, 2007;

Table 2
Comparison of the population parameters between the two sampling localities. IH: Infested hosts; N: Total of bats; A = Abundance; P%: Prevalence; CI: Confidence interval for the prevalence; MI: Mean intensity.

	Manso					Luis Ruiz				
	IH/N	A	P%	CI	MI	IH/N	A	P%	CI	MI
Trematoda										
Ochoterenatrema sp.	9/16	103	56%	0.81	11.4	8/26	154	32%	0.34	19.3
Paralecithodendrium sp.	3/16	12	19%	0.38	4.0	–	–	–	–	–
Parabascus limatulus	10/16	159	63%	0.86	15.9	6/26	10	24%	0.40	1.7
Parabascus sp.	10/16	101	63%	0.86	10.1	8/26	69	32%	0.50	8.6
Postorchigenes cf. *joannae*	2/16	8	13%	0.29	4.0	–	–	–	–	–
Cestoda										
Vampirolepis sp. 1[a]	1/16	1	6%		1	–	–	–	–	–
Vampirolepis sp. 2[a]	1/16	1	6%		1	–	–	–	–	–
Nematoda										
Allintoshius baudi	12/16	48	75%	0.96	4.0	–	–	–	–	–
Physaloptera sp.	–	–	–	–	–	1/26	1	4%	0.12	1
Physocephalus sp.	7/16	16	44%	0.68	2.3	5/26	14	19%	0.34	2.8

[a] Immature proglottids were found in the host with the scolex.

Milano, 2016). The specimens found in our survey were assigned to this genus due to the position of their testes, which is at the same level of the ventral sucker (Lotz and Font, 2008a). Nevertheless, a further identification was impossible due to the bad condition of the specimens. Both the records of *Ochoterenatrema* sp. and *Paralecithodendrium* sp. extend the genus distribution to the South and they are recorded for the first time in bats of the genus *Myotis* in Argentina.

Regarding the phaneropsolid trematodes, two species were assigned to the genus *Parabascus* due to their long caeca, which extend posterior to the testes, the cirrus-sac oriented anteriorly, the submedian genital pore and the vitellarium covering the region from the pharynx to the ventral sucker (Lotz and Font, 2008b). As far as we know, there are six previously described species of the genus (Kochseder, 1968; Khotenovsky, 1972; Marshall and Miller, 1979; Lunaschi, 2004; Kirillov et al., 2012). Some specimens were identified as *Parabascus limatulus* (Braun, 1900). This species was previously re-described based on one specimen found parasitizing *Tadarida brasiliensis* in the province of Buenos Aires by Lunaschi (2004), who described the caeca as short. Some of the specimens found in this study resemble that of Lunaschi (2004), except in the length of the caeca, which is why we adopt the diagnosis of the *Parabascus* genus sensu Lotz and Font (2008b), who characterize the caeca as long. The rest of the specimens fit in the genus *Parabascus* sensu Lotz and Font (2008b), but have a unique cirrus-sac. This feature and the combination of other features such as the relation between the diameters of the suckers, the ovary and testes' position and the vitellarium's distribution suggest that *Parabascus* sp. found in this study would be a new species. *Posthorchigenes* cf. *joannae* specimens resemble the species *Posthorchigenes joannae* sensu Zdzitowiecki (1967), recorded for the first time parasitizing the bat *Myotis daubentoni* in Poland (Zdzitowiecki, 1967). The specimens found in our survey were assigned to this genus due to the length of their caeca, posterior to the testes; the cirrus-sac oriented posteriorly and near to the ventral sucker, the submedian genital pore, the submedian ovary at the same level than the ventral sucker, and the vitellarium extending through the anterior region (Lotz and Font, 2008b). *Posthorchigenes* cf. *joannae* shows a cirrus-sac elongated, mostly posterior to the ventral sucker, and the genital pore is at the end of the cirrus-sac. The taxonomic results of this work represent the first record of *Parabascus* species infecting Patagonian bats, the first record of *P. limatulus* parasitizing a *Myotis* bat in Argentina, and the first record of a species of the genus *Postorchigenes* in Argentina.

4.1.2. Cestodes

There are previous records of species of cestodes of the genus *Vampirolepis* infecting chiropterans belonging to the Molossidae, Phyllostomidae and Vespertilionidae families (Boero and Delpietro, 1970; Milano, 2016). The two species found in this study fit in *Vampirolepis* due to their armed scolex with fraternoid hooks (Vaucher, 1992). Despite the fact that the anatomy of the mature proglottids are needed in order to identify the species, a preliminary differentiation between other Argentinean species and the ones found in this study was carried out using the number of the rostellum's hooks. *Vampirolepis* sp. 1 has the same number of hooks as *Vampirolepis* sp. 2, however, they differ in the shape and measurements of the scolex, the sizes of the suckers and the hook's morphology. This study reports two species of the genus parasitizing bats of the genus *Myotis* in Argentina for the first time, increasing the host range and the distribution range of the *Vampirolepis* species.

4.1.3. Nematodes

This study reports three species of nematodes: *A. baudi* (Ornithostrongylidae), *Physaloptera* sp. (Physalopteridae), and *Physocephalus* sp. (Spirocercidae). There are species of nematodes belonging to 12 genera recorded in Argentinean bats from the provinces of Misiones, Corrientes, Entre Ríos, Buenos Aires, Jujuy, Salta, Tucumán, and Chubut. Only three are found infecting *Myotis* bats (Vaucher and Durette-Desset, 1980; Ramallo et al., 2007; Oviedo et al., 2009). Our specimens of *A. baudi* resemble the specimens found by Vaucher and Durette-Desset (1980) parasitizing *M. aelleni*. They belong to the genus due to the shape of the rays six and eight of the male's bursa and the size of the synlophe, being the ventral ridges bigger than the dorsal ones (Rossi and Vaucher, 2002). The genus *Physaloptera* was registered previously infecting the bat *Eptesicus furinalis* in the province of Entre Ríos (Oviedo et al., 2009). The specimen found in *M. chiloensis* was assigned to this genus due to the presence of a cephalic collarette. This is the second record of a juvenile stage of a *Physaloptera* nematode infecting Argentinean bats. The nematode larvae found encysted in the peritoneum resembles the species of the genus *Physocephalus* (Diesing, 1861). The L3 of this genus has an esophagus divided in a muscular and glandular region, which extends beyond the middle of the body and in the posterior end they present two concentric rings with digitiform processes (Alicata, 1935). Up to date, there were no records of larval stages of nematodes from bats in Argentina. The presence of *Physocephalus* L3 encysted in the peritoneum indicates that *M. chiloensis* can play the role of an intermediate host for this species. Owls (*Tyto alba*) and domestic cats were observed attacking and feeding on *T. brasiliensis* bats in the city of Rosario, Argentina (Romano et al., 1999). The life cycle of the *Physocephalus* L3 might continue in any of these animals in the Patagonia.

4.2. Community structure

4.2.1. Associations between species

The highest association value was between the two species belonging to the *Parabascus* genus. Despite the fact that the association values of *Ochoterenatrema* sp. with *P. limatulus* and *Parabascus* sp. were high in the bats from both localities, only the association between *Ochoterenatrema* sp. and *P. limatulus* was statistically significant. These values might indicate that these trematodes share a common intermediate host. The associations between gastrointestinal helminths from a definitive host could be reflecting the interactions between the species infecting the intermediate host (Poulin, 2001). Metacercariae (Mariluan et al., 2012) and larvae of cestode (Pers. Obs.) have been found in nymphs (Order Ephemeroptera, Plecoptera, Diptera, and Trichoptera) from Patagonian streams. In contrast to the trematodes, which have heteroxenous cycles, trychostrongyloid nematodes have monoxenous cycles, and the infectation happens when the bat ingests the eggs (Anderson, 1988, 2000). Therefore, the high association values between *A. baudi* and the three species of trematodes could be explained by two factors. On one hand, the Fager's Index is sensitive to the number of infected hosts by the endoparasite species. *Allintoshius baudi* was found infecting most of the bats from Manso, which might explain the high association values with the trematodes. On the other hand, it might be due to facilitation processes. *Allintoshius baudi* could be inducing a bat's immunosuppression, benefiting the infectation by other species of endoparasites (Poulin, 2001).

4.3. Comparison between component communities

The differences in the species richness between Manso and Luis Ruiz could be due to differences in the environments, which imply differences in the bat's diet. The environments studied show differences in the humidity, vegetation, abundance, and type of waterbodies, reflecting the differences between forest and ecotone (Raffaele et al., 2014). The site Manso is located in the Andean forest, near the Manso Inferior river and the numerous flood areas around it, whereas the Luis Ruiz site is located in the ecotone between the forest and the steppe, near a small, shallow, bog lake. In general, lakes with the latter characteristics, as also lentic environments, support lower species richness of insects than lakes from forests or lotic environments (Heino, 2009; Hanson et al., 2010). The small shallow lake located in Luis Ruiz probably have a lower diversity of insects with naiads in their life cycle.

Insects intervene as intermediate hosts in the life cycles of many species of endoparasite infecting chiropterans (Hilton and Best, 2000). Due to the fact that Manso shows higher quantity of waterbodies than Luis Ruiz, and presents an heterogeneous bottom of inorganic and organic material, and therefore, a higher insect diversity, it is expected that the bats from this locality would have high values of species richness. Both species richness estimators suggest that the expected number of species is approximately 11 in Manso and five in Luis Ruiz. Two of the species found parasitizing the bats in Manso were rare species (*Vampirolepis* sp. 1 and sp. 2), represented by one individual each. It could be expected that the 11th species in this locality would be a rare species, too.

Both of the endoparasite communities from the *M. chiloensis* populations share approximately half of the species, being trematodes the majority. This could be explained by the presence of common intermediate hosts in both sites. On the other hand, the ecological indexes calculated suggest that there is no dominant endoparasite species. However, it should be noted that the highest abundance and prevalence values belong to three species of trematodes (*Ochoterenatrema* sp., *P. limatulus,* and *Parabascus* sp.), in contrast to the other helminths.

4.4. Preferences for intestinal regions

Intestinal helminths may show a preference for the site of attachment in the host's gut. However, interactions among species may result in an alteration of the site of attachment (Poulin, 2001). *Ochoterenatrema* sp, *P. limatulus,* and *Parabascus* sp. were found parasitizing the three intestinal regions in Manso. Nevertheless, different preferences appeared for each trematode: *Ochoterenatrema* sp. occupied preferably the small intestine, *P. limatulus* the duodenum, and *Parabascus* sp. the large intestine. There are different factors that could be affecting the distribution of the endoparasites along the intestine, such as specialization, reproductive efficiency and competition. Due to negative interactions between species, one species of parasite may alter their distribution in order to minimize the spatial overlap with the others (Poulin, 2001). Interspecific competition is one of the main factors delimiting the fundamental niche of one parasite species (Holmes, 1990). On the other hand, none of the trematodes species showed a preference for the intestinal regions in Luis Ruiz, probably because the trematodes' abundance and intensity were lower than in Manso. Interespecific competition depends on the intensities of the species that are interacting, which means that in small endoparasite infrapopulations this kind of competition would not be significant (Dobson, 1985). It should be noted that *Ochoterenatrema* sp. was the most abundant species in both the duodenum and the small intestine, unlike in Manso. This could be explained by the low quantity of *P. limatulus* in the duodenum. When the numbers are high for *P. limatulus*, these two species could be interacting negatively, as seen in Manso.

The role of insectivorous bats in the ecosystem is highly important, due to their role as both definitive and, more rarely, intermediate host, seen in the large number of parasite species found infecting bat populations (Boero and Delpietro, 1970; Saoud and Ramadan, 1976; Esteban et al., 2001; Lunaschi and Drago, 2007; Fugassa, 2015; Portes Santos and Gibson, 2015; Milano, 2016; Clarke-Crespo et al., 2017; Esteban et al., 1999, 2001; Shimalov et al., 2002), and *M. chiloensis* is not the exception to this. Due to their endoparasites, these bats could be acting as a link between the aquatic and terrestrial environments, allowing the exchange of matter and energy. Nevertheless, little is known about the diversity of endoparasites in Neotropical bats, and about a third of the known bats have been studied in this matter in South America (Portes Santos and Gibson, 2015). The species reported in this study provide useful information and contribute to the actual knowledge on bat's helminth fauna, by reporting for the first time the parasite diversity in a poorly studied bat species, *M. chiloensis*, and extending the distributions of several known endoparasite genus to the South of the continent.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijppaw.2017.12.004.

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