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FLEAS (INSECTA: SIPHONAPTERA) ASSOCIATED TO THE ENDANGERED NEOTROPICAL MARSUPIAL MONITO DEL MONTE (Dromiciops gliroides MICROBIOTHERIA: MICROBIOTHERIIDAE) IN ITS SOUTHERNMOST POPULATION OF ARGENTINA

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ABSTRACT. Dromiciops gliroides is a nocturnal marsupial endemic to the temperate forests of southern South America and the only living representative of the Order Microbiotheria. Here we study the Siphonapteran fauna of the "monito del monte" from Los Alerces National Park, Chubut Province. We also present the southernmost record for Argentina of the association between D. gliroides and the fleas Plocopsylla (Schrammapsylla) diana and Chiliopsylla allophyla allophyla, recording for the first time both Siphonaptera in Chubut Province. Our result extends and adds to current parasite biodiversity for Patagonia and contributes new information about the ecology of D. gliroides in southern Argentina.

RESUMEN. Pulgas (Insecta: Siphonaptera) asociadas al marsupial neotropical amenazado "monito del monte" (Dromiciops gliroides Microbiotheria: Microbiotheriidae), en su población más austral de la Argentina. Dromiciops gliroides es un marsupial nocturno endémico de los bosques templados de Sudamérica y el único representante viviente del Orden Microbiotheria. En este trabajo estudiamos la fauna de sifonápteros del "monito del monte" del Parque Nacional Los Alerces, Chubut. Además, damos a conocer el registro más austral para la Argentina de la asociación entre D. gliroides y las pulgas Plocopsylla (Schrammapsylla) diana y Chiliopsylla allophyla allophyla, registrándose además por primera vez ambos sifonápteros en la provincia del Chubut. Nuestros resultados amplían el conocimiento sobre la biodiversidad de la región patagónica y contribuyen al conocimiento de la ecología de D. gliroides.

Key words: Argentinean Patagonia. Chubut Province. Ectoparasites. Flea-host associations.

Palabras clave: Asociación pulga-hospedador. Chubut. Ectoparásitos. Patagonia argentina.

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Dromiciops Thomas, 1894 ("monito del monte"), is the only living genus of the Order Microbiotheria and is an endangered, small, and nocturnal marsupial endemic to the temperate forests of southern Chile (between 35°50" S and 43°2" S) and adjacent areas of Argentina (between 39°07' S and 42°00' S) (Patterson & Rogers 2007; Celis-Diez et al. 2012; Gurovich et al. 2015). Dromiciops is easily distinguishable from other South American marsupials due to its external morphology and small size (30-40 grams). It is arboreal and the only South American marsupial that hibernates for long periods (Bozinovic et al. 2004); phylogenetically is more closely related to Australian marsupials due to its external morphology, chromosome structure, and pouch young anatomy (Reig 1955; Hershkovitz 1999; Schneider & Gurovich 2017). Until the beginning of 2016 Dromiciops was believed to be monotypic, with all its populations ascribed to Dromiciops gliroides Thomas, 1894 (Patterson & Rogers 2007). Recently, D'Elía et al. (2016), based on a morphological and molecular evidences, described two new species for the genus: D. mondaca D'Elia, Hurtado & D'Anatro, 2016, endemic of Chile, and D. bozinovici D'Elia, Hurtado & D'Anatro, 2016, distributed in Chile and Argentina, and delimited the distribution of *D. gliroides* to the southern portion of the distributional area of the genus, including the Island of Chiloé.

In Argentina *Dromiciops* commonly inhabits the temperate forests of the provinces of Río Negro and Neuquén (Birney et al. 1996; Martin 2010) with rare sightings further south in the province of Chubut (Martin 2003, 2010). Recently, Gurovich et al. (2015) confirmed the presence of *D. gliroides* in Los Alerces National Park, Chubut (42°50'40.3" S; 71°50'17.4" W), establishing the southernmost limit of the distribution of this marsupial in Argentina.

The ectoparasitic fauna for *D. gliroides* described in the literature for Argentina and Chile is composed of two species of siphonaptera, a tick species belonging to the suborder Ixodoidea and a mite species belonging to the Family Laelapidae (González-Acuña & Guglielmone 2005; Marin-Vial et al. 2007; Herrin & Sage 2012; Beaucournu et al. 2014; Lareschi et al. 2016). However, for the province of Chubut

at the moment there are no mentions of ectoparasites associated with this marsupial. With this background information, the objective of the present work is to present the first record of parasitic fleas of *D. gliroides* for the province of Chubut, the southernmost population of the monito del monte in Argentina.

The study area is the Parque Nacional Los Alerces (PNLA) (42° 50′ 40.3° S; 71° 50′ 17.4° W) situated in Chubut province, Argentina (**Fig. 1**). This national park lies in the Subantarctic region (Cabrera 1971) and incorporates 263 000 ha (APN 1997). PNLA contains the largest portion of Valdivian forest in Argentine soiland; has a mean annual temperature of 8 °C and has an annual precipitation between 800 and 3000 mm, mainly falling from April to October. Snow falls from June to September but can fall as late as October (APN 1997; Gurovich et al. 2015).

Trapping was conducted using custom-made wire cage traps $(26 \times 13 \times 13 \text{ cm})$ during October, November and early December 2014, and March 2015. Traps were baited with banana, apple, a combination of apple and banana, or bait balls (oats, honey and peanut butter) and set at dusk. All traps were placed 0.5-2 meters above the ground on horizontal branches and were opened and checked within 2 hours of sunrise the following day (see Gurovich et al. 2015). Four individuals of D. gliroides were trapped in 2014 and 5 individuals in 2015. In both opportunities the trapped D. gliroides were checked for fleas and other ectoparasites. The authors wish to add that all specimens of Dromiciops caught in PNLA were trapped and released. YG who was involved in the trapping did not have permission to kill and keep the specimens as voucher material. It would be necessary in the future to try to keep specimens for more studies in order to see if indeed this is D. gliroides or another species as described by D'Elía et al. (2016). External anatomical observations of the animals trapped (see Gurovich et al. 2015) suggest they are similar to the D. gliroides found in the province of Río Negro (Bariloche); however further in-depth studies are needed to confirm this.

Fleas were collected in the field, by examining the pelage of the marsupials, and stored

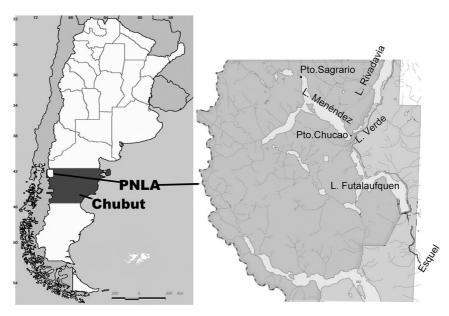


Fig. 1. Study area: Parque Nacional Los Alerces (PNLA) (42°50'40.3" S; 71°50'17.4" W), Chubut Province, Argentina.

in 96% ethanol. After field collection, in the lab, fleas were cleared and softened in 10% KOH, dehydrated in an increasing series of ethanol (80% to 100%); the fleas were then further diaphanized in eugenol, and mounted in Canadian balsam in order to be observed with a microscope Zeiss Axio Lab A1. Voucher specimens will be deposited in the Colección de Entomología del Museo de La Plata (MLP, La Plata, Buenos Aires province, Argentina). For taxonomic identification we used originals descriptions of the species. Flea specimens studied are listed below as well as information including taxonomy summary, sex, field number, host, and locality. A brief report with their main morphological characteristics, known host species and geographical distribution is also included.

The following two species and subspecies of fleas were collected from *D. gliroides* specimens from PNLA.

Family Ctenophthalmidae: Subfamily Ctenophthalminae Chiliopsylla allophyla allophyla Rothschild, 1915

Material examined.— $1 \supseteq (MLP-D.g.1)$, *D. gliroides* from PNLA, Chubut province.

Taxonomic summary

Type host and locality.—*Dromiciops gliroides*; Temuco, Chile.

Other known hosts.— Mammalia: Rodentia: Cricetidae - Abrothrix hirta, Abrothrix olivacea, Chelemys macronyx, Irenomys tarsalis, Loxodontomys micropus, Oligoryzomys longicaudatus. Octodontidae - Aconaemys sp. (Beaucournu et al. 2014; Lareschi et al. 2016).

Known geographical distribution.— Chile. Argentina: Río Negro (Beaucournu et al. 2014; Lareschi et al. 2016).

Remarks of the morphology.— Chiliopsylla Rothschild, 1908 is easily distinguished from all other known genera of Ctenophthalmidae by the genal comb which is vertical, composed of 5 spines several of which are lanceolate and one is placed immediately below and touching the vestigial eye. Tergite II with vestigial comb composed of pseudosetae. Chiliopsylla allophyla allophyla is characterized by the genal comb with the most dorsal spine being extremely short and broad. Pronotal comb is made of approximately 20 spines. Males with fixed process of clasper with 2 unusually long bristles, acetabular bristle has a movable process long and slender, forming a strongly sigmoid curve. Distal arm of sternite IX with a lateral horizontal projection near the base, the projection bearing a dense tuft of slender bristles and a stouter bristle, main part of distal arm with

3 twisted, 2 are little and are extended beyond the middle of ventral margin. In female, the margin of sternite VII has a shallow rounded sinus, the lobe above the sinus somewhat variable, rounded or triangular. Spermatheca with hilla is a little longer than pyriform bulga (Rothschild 1915; Hopkins & Rothschild 1966).

Family Stephanocircidae: Subfamily Craneopsyllinae Plocopsylla (Schrammapsylla) diana Beaucournu, Gallardo &Launay, 1986

Material examined.— 1 $\stackrel{\bigcirc}{\sim}$ (MLP-D.g.2), *D. gliroides* from PNLA, Chubut province.

Taxonomic summary

Type host and locality.— *Rhyncholestes raphanrus* Osgood, 1924; Palomar, Chiloe, Chile.

Other known hosts.— Mammalia: Microbiotheria: Microbiotheriidae - *Dromiciops gliroides*. Rodentia: Cricetidae - *A. hirta, A. olivacea*. Octodontidae - *Aconaemys porteri* (Beaucournu et al. 2014; Lareschi et al. 2016).

Known geographical distribution.— Chile. Argentina: Neuquén: Cerro Chapelco, Hua Hum, Lago Curruhue, Lago Huechulafquen, Lago Lolog, Laguna Verde, Ruca Malen (Beaucournu et al. 2014; Lareschi et al. 2016).

Remarks of the morphology.— Plocopsylla Jordan, 1931 has the largest number of species within the Craneopsyllinae, including 31 species distributed in the sub-Andean region, from Colombia to Argentina and Chile (Schramm & Lewis 1988; Sanchez et al. 2015). Schramm & Lewis (1988) revised the genus Plocopsylla on the basis of male characters and separated the species into two groups A (including four subgroups) and B (including two subgroups). Beaucournu et al. (2004) called group B subgenus Schrammapsylla Beaucournu & Ménier, 2004, defined by the shape of the sternite IX, which is gently curved. Plocopsylla (Schrammapsylla) diana is characterized by prectenidial width of the helmet which is less than the length of longest helmet spine. Both sexes with 18 to 20 spines on the helmet. Genal comb with five spines, the tapered genal spine is slightly shorter than its lower neighbour and separated by a gap that is much wider than the basal width of the genal spine. Male with sternite IX with thick proximal arm with a blunt apex. Fringe on fixed process of 14 long bristles, usually with a gap between bristles five and six and with smaller marginal and lateral bristles near the fringe. Fixed process with a broad mesal lobe projecting above the dorsoapical margin. Movable process elongated. Female with dorsal anal lobe with one tiny and several long bristles, the lowest of which arises at the apex of the elongated ventrolateral extension. Spermatheca with no penetration of hilla into the bulga. Anal stylet abruptly tapered, twice as long as basal width (Schramm & Lewis 1988; Beaucournu et al. 2004).

The flea fauna associated with *D. gliroides* of the PNLA shows a low specific richness, characterized by only two taxa: *P.* (*Schrammapsylla*) *diana* and *C. allophyla allophyla*. According to the literature, both siphonaptera are present in the temperate rainforest areas of the southern part of the continent and are distributed in both Chile and Argentina (Beaucournu et al. 2014; Lareschi et al. 2016).

In particular, *C. allophyla allophyla* has also been found to parasitize murine, cricetid and caviid rodents both in Argentina and Chile, although in a small number (Beaucournu et al. 2014; Lareschi et al 2016). However, Hopkins & Rothschild (1966) have recorded *C. allophyla allophyla* in *D. glirodes* in Valdivia, Chile, with high infestation numbers, suggesting a possible case of parasite-host co-evolution. This hypothesis is supported by Hastriter (2001), who defines *D. gliroides* as the natural host of *C. allophyla allophyla*, as both flea and marsupial species have a very similar distributional range.

On the other hand, P. (Schrammapsylla) diana is observed parasitizing cricetid and octodontid rodents, and to a greater extent small marsupials of the genus Dromiciops and Rhyncholestes, which appear to be the main hosts of this species of flea (Beaucournu et al. 2014). This is interesting as the type host for *P.* (*Schrammapsylla*) diana is Rhyncholestes raphanurus (the poorly known Chilean shrew opossum), and this marsupial is only known from one site in Argentina, Puerto Blest at Parque Nacional Nahuel Huapi, which is situated further north from PNLA in the province of Río Negro, Argentina (e.g. Martin 2011). However the latter author does note use potential distribution models generated for Rhyncholestes raphanurus for all known localities, that Rhyncholestes may be found in isolated areas in temperate Valdivian forests in Chubut, Argentina.

In Argentina, the previous records of P. (Schrammapsylla) diana and C. allophyla allophyla correspond to the provinces of Neuquén and Río Negro and are found parasitizing D. gliroides and typical forest rodents that are registered in sympatry with D. gliroides (Gardner 2007; Patton et al. 2015). However, there are no records of these fleas for the province of Chubut, so our information expands the knowledge related to this region's biodiversity and updates current information about the relationships between these fleas and their host mammal. Likewise, the presence of P. (Schrammapsylla) diana and C. allophyla allophyla in the D. gliroides population of the PNLA constitute the southern limit of both parasite-host associations for Argentina. This finding supports the hypothesis that D. gliroides is the main host of P. (Schrammapsylla) diana and C. allophyla allophyla, and these flea species follow its host in its geographical distribution. Considering the low population density that D. gliroides usually presents, the presence of both siphonaptera in forest rodents, sympatric to the monito del monte, suggests that these rodents act as alternative hosts for these species of fleas.

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LITERATURE CITED

- APN. 1997. Plan preliminar de manejo del Parque Nacional Los Alerces. Administración de Parques Nacionales, Bariloche, Argentina.
- Beaucournu, J. C., L. Moreno, & D. Gonzalez-Acuña. 2014. Fleas (Insecta-Siphonaptera) of Chile: a review. Zootaxa 3900:151-203.

- BEAUCOURNU, J. C., M. H. GALLARDO, & K. MÉNIER. 2004. Deux puces nouvelles du Chili et d'Argentine (Insecta-Siphonaptera: Stephanocircidae et Rhopalopsyllidae) et érection doun sous-genre chez *Plocopsylla* Jordan1931. Parasite 11:249-252.
- BIRNEY, E. C., R. S. SIKES, J. A. MONJEAU, N. GUTHMAN, & C. J. PHILLIPS. 1996 Comments on Patagonian marsupials from Argentina. Contributions in Mammalogy: A Memorial Volume Honoring Dr. J. Knox Jones. Jr. (H. H. Genoways, R. J. Baker, eds.). Museum of Texas Tech University, Lubbock, Texas.
- BOZINOVIC, F., G. RUIZ, & M. ROSENMANN. 2004. Energetics and torpor of a South American "living fossil", the microbiotheriid *Dromiciops gliroides*. Journal of Comparative Physiology, B. Biochemical, Systematic, and Environmental Physiology 174:293-297.
- Cabrera, L. A. 1971 Fitogeografía de la República Argentina. Boletín de la Sociedad Argentina de Botánica 1-42.
- Celis-Diez, J. L. et al. 2012. Population abundance, natural history, and habitat use by the arboreal marsupial *Dromiciops gliroides* in rural Chiloé Island, Chile. Journal of Mammalogy 93:134-148.
- D'ELÍA, G., N. HURTADO, & A. D'ANATRO. 2016. Alpha taxonomy of *Dromiciops* (Microbiotheriidae) with the description of two new species of monito del monte. Journal of Mammalogy 97:1136-1152.
- Gardner, A. L. 2007. Mammals of South America, Volume 1, Marsupials, Xenarthrans, Shrews, and Bats. University of Chicago Press Chicago, Illinois.
- GONZÁLEZ-ACUÑA, D., & A. A. GUGLIELMONE. 2005. Ticks (Acari: Ixodoidea: Argasidae, Ixodidae) of Chile. Experimental and Applied Acarology 35:147-163.
- GUROVICH Y., H. J. STANNARD, & J. M. OLD. 2015. The presence of the marsupial *Dromiciops gliroides* in Parque Nacional Los Alerces, Chubut, Southern Argentina, after the synchronous maturation and flowering of native bamboo and subsequent rodent irruption. Revista Chilena de Historia Natural 88:1-12.
- HASTRITER, M. W. 2001. Fleas (Siphonaptera: Ctenophthalmidae and Rhopalopsyllidae) from Argentina and Chile with two new species from the rock rat, *Aconaemys fuscus*, in Chile. Annals-Carnegie Museum Pittsburgh 70:169-178.
- Herrin, C. S., & R. D. Sage. 2012. Description of a new species of *Haemogamasus* (Mesostigmata, Laelapidae, Haemogamasinae) from Chubut, Río Negro and Neuquén provinces, Argentina. ZooKeys 173:51-77.
- HERSHKOVITZ, P. 1999. *Dromiciops gliroides* Thomas, 1894, last of the Microbiotheria (Marsupialia), with a review of the family Microbiotheriidae. Fieldiana Zoology 93:1-60.
- HOPKINS, G. H. E., & M. ROTHSCHILD. 1966. An illustrated catalogue of the Rothschild Collection of fleas (Siphonaptera) in the British Museum (Natural History). Vol. IV: Hystrichopsyllidae (Ctenophthalminae, Dinopsyllinae, Doratopsyllinae and Listropsyllinae). British Museum, London.
- Lareschi, M., J. Sanchez, & A. Autino. 2016. A review of the fleas (Insecta: Siphonaptera) from Argentina. Zootaxa 4103:239-258.

- Marín-Vial, P., D. González-Acuña, J. L.Celis-Diez, P. E. Cattan, & A. A. Guglielmone. 2007. Presence of *Ixodes neuquenensis* Ringuelet, 1947 (Acari: Ixodidae) on the endangered Neotropical marsupial monito del monte (*Dromiciops gliroides* Thomas, 1894, Microbiotheria: Microbiotheriidae) at Chiloé Island, Chile. European Journal of Wildlife Research 53:73-75.
- Martin, G. M. 2003. Nuevas localidades para marsupiales patagónicos (Didelphimorphia y Microbiotheria) en el noroeste de la provincia del Chubut, Argentina. Mastozoología Neotropical 10:148-153.
- Martin, G. M. 2010. Geographic distribution and historical occurrence of *Dromiciops gliroides* Thomas (Marsupialia, Microbiotheria). Journal of Mammalogy 91:1025-1035.
- MARTIN, G. M. 2011. Geographic distribution of *Rhyncholestes raphanurus* Osgood, 1924 (Paucituberculata: Caenolestidae), an endemic marsupial of the Valdivian Temperate Rainforest. Australian Journal of Zoology 59:118-26.
- Patterson, B., & M. Rogers. 2007. Order Microbiotheria Ameghino, 1889. Mammals of South America, Vol. 1: Marsupials, Xenarthrans, Shrews, and Bats (A. Gardner, ed.). University of Chicago Press, Chicago, Illinois.

- PATTON J. L., U. F. J. PARDIÑAS, & G. D'ELÍA. 2015. Mammals of South America, Volume 2 - Rodents. The University of Chicago Press, Chicago, Illinois.
- REIG, O. A. 1955. Noticia preliminar sobre la presencia de micro-biotherinos vivientes en la fauna sudamericana. Investigaciones Zoológicas Chilenas 2:121-130.
- ROTHSCHILD, N. C. 1915. A synopsis of the British Siphonaptera. Entomologist's Monthly Magazine 49-112.
- SANCHEZ, J., J. C. BEAUCOURNU, & M. LARESCHI. 2015. Revision of fleas of the genus *Plocopsylla* belonging to the 'angusticeps-lewisi' complex in the Andean biogeographic region, with the description of a new species. Medical and Veterinary Entomology 29:147-158.
- Schneider, N. Y., & Y. Gurovich. 2017. Morphology and evolution of the oral shield in marsupial neonates including the newborn monito del monte (*Dromiciops gliroides*, Marsupialia, Microbiotheria) pouch young. Journal of Anatomy 231:59-83.
- SCHRAMM, B. A., & R. E. LEWIS. 1988. Revision of *Plocopsylla* (Siphonaptera: Stephanocircidae). Koelts Scientific Bookes, Koenigstein.