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Liolaemus lizards (Squamata: Liolaemidae) as hosts for the nymph of *Amblyomma parvitarsum* (Acari: Ixodidae), with notes on *Rickettsia* infection

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Abstract Adults of *Amblyomma parvitarsum* are common ectoparasites of South American camelids of the genera *Lama* and *Vicugna,* occuring in highlands of Argentina, Bolivia, Chile, Peru and also in Argentinean Patagonia. Whereas larval stages of this tick are known to feed on small lizards, host records for the nymphal instar have remained unreported. Supported by morphological and molecular analyses, herein we report *A. parvitarsum* nymphs parasitizing two *Liolaemus* species (Reptilia: Squamata) in the Andean Plateau of Argentina and Chile. Additionally, by a PCR screening targetting *gltA* and *ompA* genes, DNA of *Rickettsia* was detected in one of the collected nymphs. Obtained sequences of this agent were identical to a recent *Rickettsia* sp. described infecting adults of this tick species in Chile and Argentina.

Keywords *Liolaemus* · *Amblyomma parvitarsum* · Host-parasite association · *Rickettsia*

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Introduction

The tick *Amblyomma parvitarsum* Neumann is a high-altitude amblyommine with a distribution restricted to the Andean plateaus of Argentina, Bolivia, Chile and Peru and also present in the Argentinean Patagonia (Muñoz-Leal et al. 2014). Adults of this tick are common ectoparasites of South American wild and domestic camelids of the genera *Lama* and *Vicugna* (Mammalia: Artiodactyla), whereas the larval stage was reported parasitizing lizards of the genus *Liolaemus* (Squamata: Liolaemidae) (González-Acuña et al. 2004; Muñoz-Leal et al. 2014, Castillo et al. 2015). In turn, host records for nymphs of *A. parvitarsum* have remained unknown. Need et al. (1991) speculated that reptiles or small birds could be possible hosts for the nymph of *A. parvitarsum*; however, none of these possible host-parasite associations was ever documented. Recently, Martins et al. (2014) described the nymph of *A. parvitarsum* from a single laboratory reared specimen obtained from a reptile field-collected larva, a fact which still left the identity of possible hosts for the nymphal stage of this tick as uncertain.

As in other Neotropical Amblyomminae, adults of *A. parvitarsum* from Argentina and Chile have shown highly prevalent *Rickettsia* infections (Ogrzewalska et al. 2016). While this novel agent is phylogenetically closely related to the human pathogens *Rickettsia parkeri, Rickettsia sibirica* and *Rickettsia africae*, no human cases of rickettsiosis or human infestation by *A. parvitarsum* have been ever reported for the region of occurrence of this tick species (Ogrzewalska et al. 2016).

The aim of this study was to report lizards of the genus *Liolaemus* as hosts for the nymph of *A. parvitarsum* in Argentina and Chile to further understand the life cycle of this tick species, and to evaluate *Rickettsia* infection in this tick stage.

Materials and methods

Lizards of the genus *Liolaemus* were actively collected in three localities: Parinacota, Lauca National Park, in the Altitude Tropical ecoregion in Northern Chile, and Laguna Negra and Laguna Milena in Jujuy province, in the Altoandina Phytogeographic province in Northern Argentina (Cabrera 1994) (Table 1). In Chile, reptiles were captured according to the licenses given by the "Servicio Agrícola y Ganadero" (SAG) and the "Corporación Nacional Forestal" (CONAF) (documents 6007/2014 and 033/2014, respectively) and identified following Pincheira-Donoso and Núñez (2005). Lizards of Argentina were identified following Nuñez and Fox (1989) with the collaboration of C.S. Abdala and S. Quinteros (Instituto de Herpetología, Unidad Ejecutora Lillo-CONICET). Ticks were removed from lizards with tweezers and stored in vials with 70–90 % ethanol. In the laboratory, taxonomic identification of immature ticks was made by the observation of morphological characters using a stereomicroscope following the key of Martins et al. (2014) for nymphs of the genus *Amblyomma*. To confirm the morphological diagnosis, one nymph of each locality was submitted to DNA extraction by the guanidine isothiocyanate technique (Sangioni et al. 2005). PCR amplification targeting a ≈460-pb fragment of the tick mitochondrial 16S rRNA gene was performed using the primers 3′-CCGGTCTCAACTCAGATCAAGT-5′ (forward) and 3′-GCTCAATGATTTTT-TAAATTGCTGT-5′ (reverse) as described elsewhere (Mangold et al. 1998). Additionally, tick-extracted DNA was tested for *Rickettsia* bacteria by using primers CS-78 (forward) and CS-323 (reverse) targeting a relatively conserved fragment of the citrate synthase gene (*gltA*) that occurs in all *Rickettsia* species (Labruna et al. 2004). Positive samples were

Table 1 Localities and hosts of the nymphs of *Amblyomma parvitarsum* collected in Argentina and Chile

Country/province	Locality	Coordinates	Altitude (m)	Host (n)	Stage	Date of collection	References
Argentina							
Jujuy	Laguna Negra	22°31′S; 66°41′W	4579	*Liolaemus puritamensis* (1♂)	5 N	7 Feb. 2015	INTA-2322
	Laguna Milena	22°43′S; 67°04′W	4567	*Liolaemus puritamensis* (1♀)	1 N	4 Feb. 2015	INTA-2321
Chile							
Arica and Parinacota	Paranicota	18°10′S; 69°17′W	4440	*Liolaemus pleopholis* (6♂, 4♀)	5 N	13 Dec. 2014	CNC-3303

INTA Tick collection of the Instituto Nacional de Tecnología Agropecuaria (Rafaela, Santa Fe, Argentina)

CNC Tick collection "Coleção Nacional de Carrapatos" of the University of São Paulo, Brazil

N nymph

subsequently submitted to a second PCR protocol using the primers Rr190.70 (forward) and Rr190.701 (reverse), targeting a fragment of the 190-kDa outer membrane protein gene (*ompA*) from only some *Rickettsia* species belonging to the spotted fever group (Regnery et al. 1991). Obtained sequences were submitted to a BLAST analysis (Altschul et al. 1990) in order to infer similarities with other *Amblyomma* spp. available in GenBank.

Results

A total of 11 ticks were collected from 4 infested lizards belonging to the following two species: *Liolaemus puritamensis* Núñez & Fox (1 male, 1 female) and *Liolaemus pleopholis* Laurent (2 males) (Fig. 2; Table 1) in Argentina and Chile (Fig. 1). Two of these parasitized specimens were caught outside their burrows in the middle of camelid manure heaps. All ticks were morphologically identified as nymphs of *A. parvitarsum* according to the following characters: scutum with surface slightly shagreened, few punctations, and cervical grooves long, reaching the scutal median third, deeper at the anterior third; eyes

Fig. 1 Map of South America showing the localities in Argentina and Chile where nymphs of *Amblyomma parvitarsum* were collected

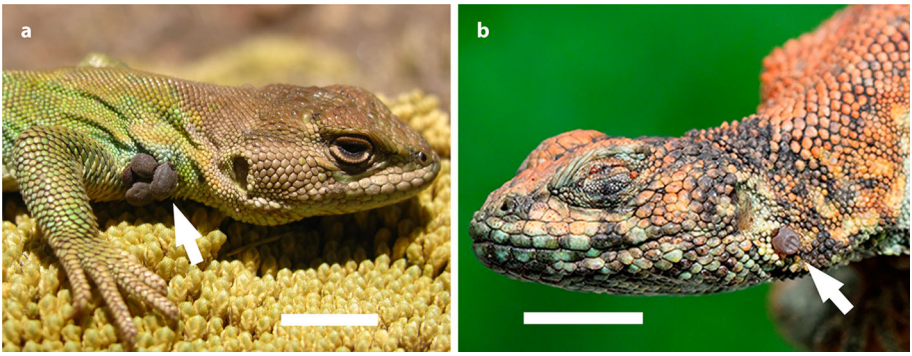

Fig. 2 Nymphs of *Amblyomma parvitarsum* (*white arrow*) parasitizing *Liolaemus pleopholis* (**a**) and *Liolaemus puritamensis* (**b**). The *scale bar* is equivalent to 1 cm

flat at lateral scutal angles at the level of scutal midlength; basis capituli dorsally rectangular, hypostome rounded apically with dentition 2/2, palpal article IV apically projecting from article III; capitulum without cornua or auricula; chitinous tubercles at posterior body margin absent; coxa I with 2 short sub-equal rounded triangular spurs, the external slightly stouter, and coxae II–IV with a small triangular spur (Martins et al. 2014). This taxonomic diagnosis was confirmed by molecular tools for two specimens collected in Argentina and one collected in Chile. All 16S rDNA sequences belonging to two nymphs from Chile (Genbank accession numbers: KX230481 and KX230482) and to a nymph from Argentina (Genbank accession number: KX230479) were identical among each other and with the sequence of *A. parvitarsum* available in GenBank (AY498561). The remaining sequence of a nymph from Argentina (Genbank accession number: KX230480) differed to the other four sequences in just one base. Nymphs collected in Chile were deposited at the tick collection "Coleção Nacional de Carrapatos" (CNC) of the Faculty of Veterinary Medicine of the University of São Paulo, Brazil (CNC 3303) and specimens collected in Argentina were deposited at the Tick Collection of the Instituto Nacional de Tecnología Agropecuaria (INTA), Rafaela, Argentina (INTA 2321, INTA 2322) (Table 1). Both *L. puritamensis* lizards collected in Argentina were deposited at the National Collection of the Fundación Miguel Lillo, Tucuman, Argentina.

One of the nymphs collected in Chile was positive to *Rickettsia* infection. Obtained sequences for *gltA* (Genbank accession number: KX258877) and *ompA* (Genbank accession number: KX258878) genes were identical to sequences of *Rickettsia* sp. (Genbank accession numbers KR296943, KR296944) isolated from adults of *A. parvitarsum* from Argentina and Chile (Ogrzewalska et al. 2016).

Discussion

In the Neotropical Zoogeographic Region at least 15 tick species feed mostly on reptiles to accomplish their life cycle in nature (i.e., *Amblyomma albopictum* Neumann, *Amblyomma antillorum* Kohls, *Amblyomma argentinae* Neumann, *Amblyomma dissimile* Koch, *Amblyomma fuscum* Neumann, *Amblyomma humerale* Koch, *Amblyomma macfarlandi* Keirans, Hoogstraal & Clifford, *Amblyomma pilosum* Neumann, *Amblyomma quadricavum* Schulze, *Amblyomma sabanarae* Stoll, *Amblyomma scutatum* Neumann, *Amblyomma torrei* Pérez Vigueras, *Amblyomma rotundatum* Koch, *Amblyomma usingeri* Keirans,

Hoogstraal & Clifford, *Amblyomma williamsi* Banks) (Guglielmone et al. 2004). To date, *A. parvitarsum* has been known to parasitize reptiles, specifically *Liolaemus* lizards (González-Acuña et al. 2004), only during its larval stage. By this study, we confirm that nymphs of this hard tick exploit *Liolaemus* lizards as well. Considering life stages of Neotropical *Amblyomma* and their associated hosts, the biological cycle of *A. parvitarsum* seems to have unique features, such as larvae and nymphs parasitizing small sized reptiles and adults feeding on South American camelids.

Two *Liolaemus* species were infested with nymphs of *A. parvitarsum* in highlands of Argentina and Chile. Lizards of this genus are present in all the extension of the Andean plateau of these countries (Nuñez and Fox 1989; Quinteros and Abdala 2007); therefore it is likely that other sympatric and parapatric congeneric species could also serve as hosts for nymphal stages of *A. parvitarsum*. However, according to the apparent reptile-mammal feeding preferences of this tick species, parasitized lizards should only occur in areas within the distributional range of South American camelids of the genera *Lama* and *Vicugna*.

Liolaemus pleopholis is a burrow dweller lizard (Pincheira-Donoso and Núñez 2005), and parasitized specimens of this species were caught outside their hideouts located in the middle of camelid manure heaps. Once ended its feeding period, engorged nymphs might molt between the excrements, and this could be one of the reasons explaining the occurrence of adults of this tick in camelid dung, as documented in literature (Muñoz-Leal et al. 2014). In turn, this would also facilitate the encountering of the mammal hosts at the moment they visit the place to defecate. However, this situation was noticed in 2/3 of parasitized lizards in Chile, so more observations among the distribution of this tick and its associated hosts are needed to support this statement.

Considering that adults of *A. parvitarsum* feed almost exclusively on South American camelids, the detection of *Rickettsia* sp. DNA in one of the examined nymphs, identical with the recently reported *Rickettsia* sp. infecting adult stages of this tick in Andean highlands from Chile and Argentina (Ogrzewalska et al. 2016), suggests that this microorganism has transstadial transmission. Although experimental evidence might confirm this association between *A. parvitarsum* and this *Rickettsia* sp., phylogenetically closely related *R. parkeri, R. sibirica* and *R. africae* are maintained by transstadial and transovarial transmission in their natural tick vectors (Podboronov and Pchelkina 1989; Socolovschi et al. 2009; Nieri-Bastos et al. 2013).

Finally, by this study new ecological evidence is brought to light in order to understand the life cycle of *A. parvitarsum* and its associated rickettsial agent in the extreme conditions of the South American Andean plateau.

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