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First morphological and genetic report of the hard tick, *Amblyomma tigrinum* (Acari: Ixodidae) in the Andean cat, *Leopardus jacobita*

Primer registro morfológico y genético de la garrapata dura, *Amblyomma tigrinum* (Acari: Ixodidae) en el gato andino, *Leopardus jacobita*

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The Andean cat (*Leopardus jacobita* Cornalia 1865) is one of the rarest feline species in the world and one of the most threatened in America, where no ixodofauna are known to parasitize these small cats. Here describe the morphological and genetic findings of hard tick specimens in an Andean cat. Four hard tick specimens (2 females and 2 males) were collected from an Andean cat in the locality of Patacamaya, Department of La Paz, Bolivia. DNA was extracted using 1 or 2 of the tick legs, causing minimal damage to the specimens. Morphological and genetic characteristics corresponded to *Amblyomma trigrinum*, with an identity percentage of 99.43 %. This research is the first morphological and genetic report of adult hard ticks of the species *A. tigrinum* parasitizing an Andean cat, extending the distribution of this tick to the Bolivian biogeographic region of high mountains and the Altiplano in the La Paz department, and emphasizes the circulation of this zoonotic parasite in the country.

Key words: Andean region; Feline; Ixodid.

El gato andino (*Leopardus jacobita* Cornalia 1865) es una de las especies felinas más raras del mundo y de las más amenazadas de América, además de no conocerse la ixodofauna que parasita a estos pequeños felinos. Aquí se describen morfológica y genéticamente ejemplares de garrapatas duras en un gato andino. Se recolectaron 4 especímenes de garrapatas duras (2 hembras y 2 machos) de 1 gato andino de la localidad de Patacamaya en el departamento de La Paz, Bolivia. Los ejemplares fueron identificados por sus características morfológicas y genéticas. El ADN se extrajo utilizando 1 o 2 patas de las garrapatas con un daño mínimo a los ejemplares. Las características morfológicas y genéticas correspondieron a *Amblyomma trigrinum*, con un porcentaje de identidad del 99.43 %. Esta investigación constituye el primer reporte morfológico y genético de garrapatas duras en estado adulto de la especie *A. tigrinum* parasitando a un gato andino, ampliando la distribución de esta garrapata a la región biogeográfica boliviana de alta montaña y altiplano en el departamento de La Paz, y enfatiza la circulación de este parásito zoonótico en el país.

Palabras clave: Felino; Ixodido; región andina.

Hard ticks (Acari: Ixodidae), so named due to the hardness of their integument and the presence of a dorsal shield, are obligate blood-sucking ectoparasites with worldwide importance as vectors of diseases (viruses, bacteria, protozoa, and nematodes), which affect wild and domestic animals and humans (Sonenshine and Roe 2014; Guglielmone and Robbins 2018). The Ixodidae family include the primary number of species reported worldwide (n = 752), where *Amblyomma* is the third genus with the highest species richness. One hundred thirty-six taxa have been described within this group (Mastropaolo *et al.* 2014; Guglielmone *et al.* 2020), of which 58 have been reported in South America (Nava *et al.* 2017; Dantas-Torres *et al.* 2019; Martins *et al.* 2019), and in Bolivia, 29 hard ticks are known, of which 22 belong to the genus *Amblyomma* (Mastropaolo *et al.* 2014; Rodríguez *et al.* 2019).

Amblyomma tigrinum (Koch 1844) is a species with a wide range of distribution in South America (Argentina, Bolivia, Brazil, Chile, French Guyana, Paraguay, Perú, Uruguay, and Venezuela), where adult stages frequently

parasitize canids, felids (domestic cat, *Panthera onca* and *Puma concolor*), procyonids, bovids, cervids, suids and larval or nymphal stages of canids, felids, cavids, cricetids and murids, as well as many families of birds (Nava *et al.* 2017). It presents a cycle of 3 parasitic stages, with larvae and nymphs developing better in birds and adults in canids; larvae feed for 5 to 6 days, nymphs feed for 6 to 8 days, and females lay viable eggs after feeding on canids (Labruna *et al.* 2002). This life cycle is probably regulated by temperature, without diapause, allowing it to be found in habitats with contrasting climates (Nava *et al.* 2017). Thus, in Bolivia, this species was reported in the Cochabamba, Chuquisaca, and Santa Cruz departments, observing that in adult stages, parasitize domestic dogs (*Canis lupus familiaris*), crab-eating foxes (*Cerdocyon thous* Linnaeus 1766), "borochis" (*Chrysocyon brachyurus* Illiger 1815) and "aguarachays" (*Lycalopex gymnocercus* Fischer 1814), whereas in immature stages parasitize rodents of genus *Galea* (guinea pigs), and probably *G. leucoblephara* (Burmeister 1861) species (Mastropaolo *et al.* 2014).

The Andean cat (*Leopardus jacobita* Cornalia 1865) is one of the rarest feline species in the world and one of the most threatened in America (Andean Cat Alliance 2011). It is classified as "Endangered (EN)" with decreasing populations in its area of distribution, mainly includes the Andes of Argentina, Bolivia, Chile and Perú, and Argentine Patagonia (Reppucci *et al.* 2024), and as "Critically Endangered (CR)" in Bolivia (Villalba *et al.* 2009), where no ixodofauna or other parasite fauna are known to parasitize these small cats. This case report describes the morphological and genetic findings of *A. tigrinum* in an Andean cat from La Paz, Bolivia, constituting the first record of the parasite-host association of an ixodid species in *L. jacobita* in South America.

A male, sub-adult Andean cat (*L. jacobita*) was delivered on March 15, 2016, to the "Vesty Pakos Wildlife Custody Center and Municipal Biopark" from the Patacamaya town in the La Paz department (17° 14' 16.98" S; 67° 54' 51.98" W). The animal was quarantined, and its health status was assessed (Beltrán-Saavedra *et al.* 2020). Four ticks were observed on the upper ear edges, 3 specimens were collected and preserved in 96 % ethanol during a superficial inspection, and 1 specimen was separated for another unpublished study. These 3 specimens were sent to the laboratory of the Bioparque Municipal "Vesty Pakos" in La Paz city, Bolivia, for identification.

Morphological identification was carried out following the dichotomous keys and descriptions based on Nava *et al.* (2017) and making comparisons with specimens deposited in the Bolivian Fauna Collection (CBF) of the Institute of Ecology of the Universidad Mayor de San Andrés. The morphometry was performed using an optical microscope (Olympus, Model CX31RBSFA) and a stereo-microscope (Motic, Model DM143FBGG).

The genetic identification was made from 1 leg of each of the ticks (1 female and 1 male), sent to the Center for Genetic Research (CINGEN) of the Institute for Scientific Technical Research (IITCUP) of the Police University in La Paz city, Bolivia, using the commercial system Wizard® Genomic DNA Purification Kit (Promega), adding 100 mM DTT (Sigma) and 10 mg/ml proteinase K (Sigma). The extracts were quantified using the Qubit 2.0 fluorometer (Invitrogen) according to the manufacturer's specifications. The identification of the specimens was performed by characterizing the cytochrome oxidase I (COI) gene according to the protocol described by Hebert *et al.* (2003) using the commercial GoTaq® Colorless Master Mix kit (Promega), with a final primer concentration of 2 µM at an annealing temperature of 53 °C. The amplified products were subjected to alcoholic purification according to the protocol established at CINGEN, followed by unbalanced PCR with the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's recommendations. Next, a second alcoholic purification was performed by adding 10 µl of HI - Di Formamide (Applied Biosystems) to the final product. According to the manufacturer's specifications, this product was sequenced on an ABI3500 Genetic Analyzer (Applied Biosystems). The 2 sequences obtained were analyzed with the program Sequencing Analysis v.6.5. (Applied Biosystems), considering data with an optimal quality value as valid. Once the sequences were verified, they were aligned with the MUSCLE algorithm using the Mega 7.0 program (Kumar *et al.* 2016). These sequences were analyzed in the GenBank database (NIH genetic sequence database; Benson *et al.* 2005) using the MEGABLAST algorithm. The obtained sequences, being identical, were deposited in GenBank under the accession number MW193726. On the other hand, partial and complete sequences of the cytochrome oxidase I (COI) gene were downloaded from this database. The phylogenetic analysis was performed using the sequences analyzed with the maximum likelihood (ML) method (Cavalli-Sforza and Edwards 1967; Felsenstein 1981; Kishino and Hasegawa 2001) and Generalized Reversible Time (GRT; Tavaré 1986) substitution models, considering 1000 bootstraps, using *Amblyomma javanense* (Supino 1897; MK165451.1) as an external group.

The collected and analyzed tick specimens (with morphological and genetic identification) were sent to the Colección Boliviana de Fauna (CBF) for access and deposit according to the cataloging: CBF-Ixo-00036.

The hard ticks analyzed, 1 female and 2 males, were morphologically identified as *Amblyomma tigrinum* (Figures 1 and 2). The female specimen presented overall length 5.75 mm and total width 3.05 mm; the male specimens presented overall length 4 mm and total width 1.95 mm, and an oval body more elongated and with a narrower portion in the males. The scapula appeared rounded with deep cervical grooves in the anterior portion (female = 4.53 mm; male = 2.88-3.18 mm) and less deep in the posterior portion, forming a sigmoid (female = 4.90 mm; male = 3.08-3.38 mm). The marginal groove was complete in males, delimiting the posterior region by scallops with absent carinae.

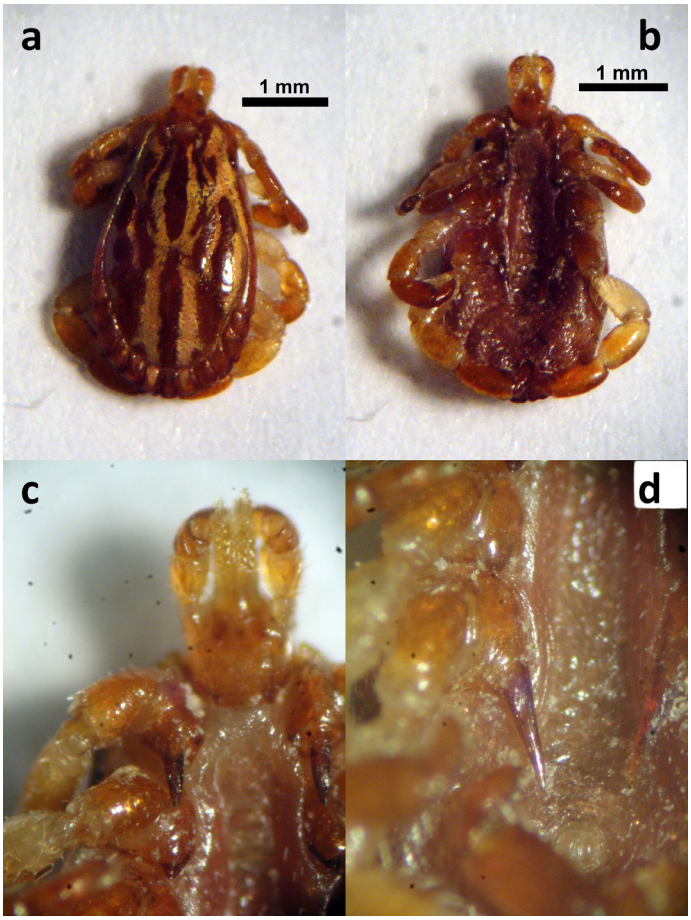

Figure 1. Male *Amblyomma trigrinum* collected from *Leopardus jacobita*: a) dorsal view; b) ventral view; c) ventral view of capitulum and coxa I with an external spine longer than internal spine; d) ventral view of coxa IV with an elongated spine that does not reach the level of the anus.

In females, there was an absence of chitinous tubercles on the posterior margin of the body. The eyes were flattened, and an ornate shield was observed, with pale yellowish spots predominating in the outline and narrow, divergent reddish-brown spots on the posterior cervical part. An extended narrow central area and a glabrous notum were observed in females. The base of the head was dorsally subrectangular (female = 0.60 mm; male = 0.43 mm), without cornua and with oval porous areas in females and with cornua in males. The hypostome was spatulated, and the dental formula 3/3. The genital opening was located at the level of coxa II, in the shape of a "U". In coxa I, 2 differentiated spines were observed; the external spine was long, narrow, and pointed, and the internal spine was small. In coxa II - IV, in females, and II - III, in males, there was a short, blunt, triangular spine. The male also presented a long, narrow, and sharp spine but did not reach the level of the anus on coxa IV, spineless trochanters. A small dorsal spine was presented on the II - IV tibia. Comma-shaped spiral plates were observed.

Sequence analysis with the MEGABLAST algorithm from the GenBank database allowed the identification of tick specimens within the *A. tigrinum* species with an identity of 99.43 % concerning the *A. tigrinum* isolate BRA5A2 (GenBank accession number KU302511.1). The phylogenetic relationships (Figure 3) revealed the grouping of the samples analyzed with individuals of the species *A. tigrinum* considering 31 species of the genus *Amblyomma*, where *A. maculatum* (Koch 1844) and *A. triste* (Koch 1844) were presented as sister groups.

This research constitutes the first report of *A. tigrinum* parasitizing *L. jacobita*, expanding the parasite distribution to the Bolivian biogeographic Andean region in the La Paz department (Mastropaolo *et al.* 2014), although the pathological significance of this tick in Andean cats remains unknown. To our knowledge, no previous studies on macroparasites exist for this host species. Regarding microparasites, Napolitano *et al.* (2019) conducted serological and molecular studies on 17 pathogens in *L. jacobita*, including those transmitted by hard ticks, and obtaining negative results in all cases. On the other hand, Rojas-Barón *et al.* (2022) mention *L. jacobita* as a potential definitive host of *Gurltia paralysans* together with *L. colocolo*. However, there are no records of this. The latter agrees with previous records of this tick in other hosts made in the Argentine Andean-Patagonian Domain (Guglielmone *et al.* 2000). Likewise, it represents the first record in a reference DNA barcode library of ticks identified in Bolivia.

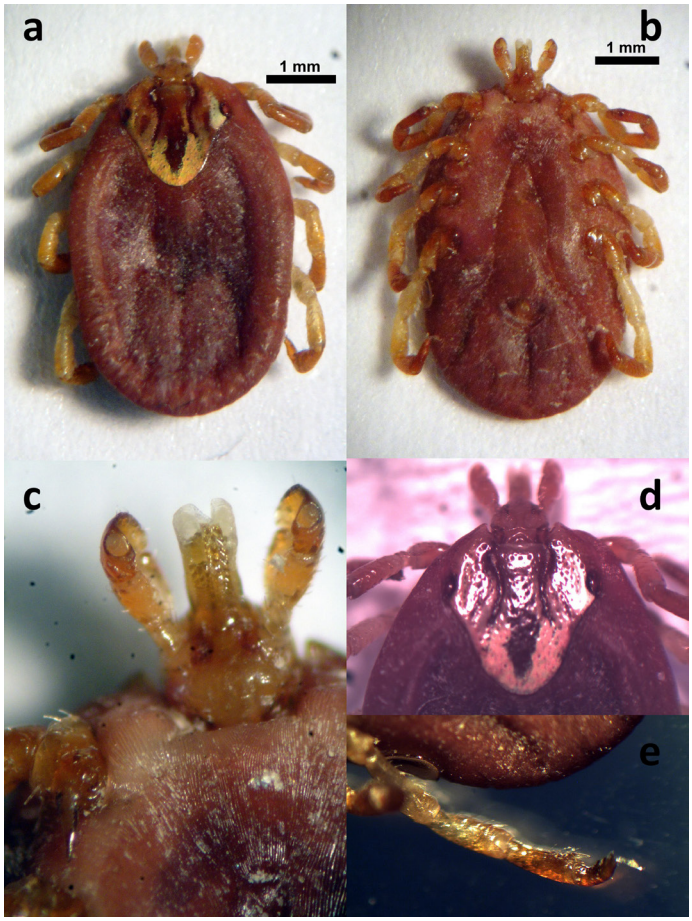

Figure 2. Female *Amblyomma trigrinum* collected from *Leopardus jacobita*: a) dorsal view; b) ventral view; c) ventral view of capitulum and coxa I with an external spine longer than internal spine; d) dorsal view of shield and porous areas at the base of head; e) dorsal spine in right leg IV tibia.

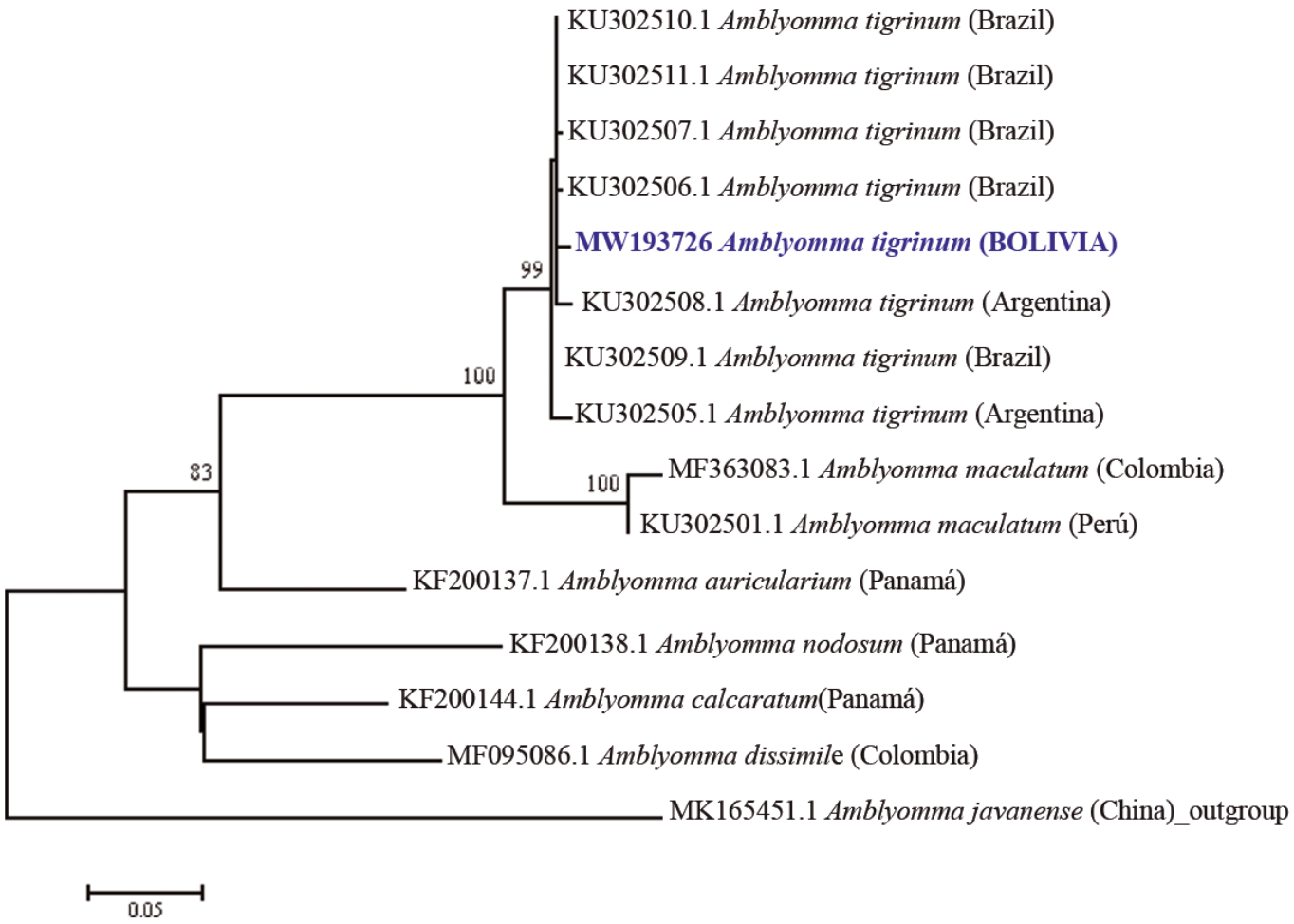

Figure 3. Maximum likelihood tree of COI gene of *Amblyomma* species. In parentheses is the sequence of origin country. The scale bar indicates the number of substitutions per site. Numbers on branches correspond to support values. Specimens reported in this study are shown in bold and blue.

The genetic identification of hard ticks from 1 or 2 legs enables rapid and accurate diagnosis of this parasitism, which is essential for determining potential sanitary risks (Ondrejicka *et al.* 2016). Furthermore, ticks belonging to the *A. maculatum* species complex, including *A. tigrinum* ticks are recognized as vectors of *Rickettsia parkeri*, a zoonotic pathogen (Romer *et al.* 2014). In a previous study conducted in Cochabamba, Bolivia, molecular analyses confirmed the presence of *R. parkeri* in ticks, while serological tests revealed antibodies against this microparasite in dogs (Tomassone *et al.* 2010). In summary, it is crucial to consider the diseases caused by ticks of the *A. maculatum* species complex, which includes *A. tigrinum*, since *A. maculatum*, for example, is the primary causative agent of spotted fever in humans in the New World (Estrada-Peña *et al.* 2005; Nieri-Bastos *et al.* 2018). Thus, it is advisable to expand the morphological and genetic records of this hard tick and its intermediate and definitive hosts in Bolivian territory.

The phylogenetic tree clusters the samples with others from Brazil and Argentina, although with a different haplotype from those previously reported. It also shows that *A. triste* and *A. maculatum* are sister species of *A. tigrinum*.

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