



First record of *Laelaps mazzai* (Mesostigmata: Laelapidae) in Peru associated with *Calomys sorellus* (Cricetidae: Sigmodontinae), an endemic rodent, with insights about its morphological variation

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ABSTRACT. *Laelaps mazzai* Fonseca (Mesostigmata: Laelapidae) is a parasitic mite associated with the genus *Calomys* Waterhouse (Rodentia: Cricetidae) that shows variations on the size of its dorsal shield. Although, there are six species of *Calomys* in Peru, there are no records of *L. mazzai* associated to those species and consequently, neither are records about its size variation. We report the first occurrence of *L. mazzai* in Peru, associated with *Calomys sorellus* Thomas and its variation in the dorsal shield size. Morphological and molecular analyses were used to identify the mites and explore potential phenotypic variations in this new host-parasite association. While the mites exhibited diagnostic characters consistent with *L. mazzai*, they displayed larger dorsal shields, longer distance between setae, and fewer setae in the medial podonotal region compared to mites from other *Calomys* species. However, molecular analysis of the 18S-ITS1-5.8S-ITS2-28S region showed minimal genetic divergence (0–0.2 %) between mites from *C. sorellus* and those from other *Calomys* species. The observed morphological variations are consistent with previous findings of phenotypic plasticity in *L. mazzai*, potentially influenced by the host and/or environmental factors.

KEYWORDS. Ectoparasite. Laelapinae. Mites. Phenotypic plasticity. Phylloini.

Primer registro de *Laelaps mazzai* (Mesostigmata: Laelapidae) en Perú asociado a *Calomys sorellus* (Cricetidae: Sigmodontinae), un roedor endémico, con notas sobre su variación morfológica

RESUMEN. *Laelaps mazzai* Fonseca (Mesostigmata: Laelapidae) es un ácaro parásito asociado al género *Calomys* Waterhouse (Rodentia: Cricetidae) y que presenta variaciones en el tamaño de su placa dorsal. Aunque, hay seis especies de *Calomys* en Perú, no hay registros de *L. mazzai* asociado a esas especies y por consecuencia, tampoco sobre su variación en el tamaño. Este estudio reporta el primer hallazgo de *L. mazzai* en Perú, asociado con *Calomys sorellus* Thomas, y la variación en el tamaño de su placa dorsal. Se utilizaron análisis morfológicos y moleculares para identificar a los ácaros y explorar potenciales variaciones fenotípicas en esta nueva asociación parásito-hospedador. Aunque, los ácaros exhibieron características diagnósticas consistentes con *L. mazzai*, también mostraron placas dorsales más grandes, con mayor distancia entre setas y con menor cantidad de setas en la región media podonotal comparado con otros ácaros de otras especies de *Calomys*. Sin embargo, los análisis moleculares de la región 18S-ITS1-5.8S-ITS2-28S mostraron diferencias genéticas mínimas (0–0,2 %) entre ácaros de *C. sorellus* y aquellos de otras especies de *Calomys*. La variación morfológica observada apoya la plasticidad fenotípica de *L. mazzai*, potencialmente influenciada por el hospedador y/o factores ambientales.

PALABRAS CLAVE. Ácaros. Ectoparásito. Laelapinae. Phylloini. Plasticidad fenotípica.

Laelaps mazzai Fonseca, 1939 (Mesostigmata: Laelapidae) is a parasitic mite mainly associated with rodents of the genus *Calomys* Waterhouse, 1837 (Sigmodontinae: Phyllotini) (Furman, 1972; Lareschi & Mauri, 1998; Espinoza-Carniglia et al., 2023). This mite was described based on one female and three males collected from a “wild rat” captured at an unspecified site in Salta Province, Argentina (Fonseca, 1939). Moreover, *L. mazzai* was reported associated with eight of the nineteen recognized *Calomys* species across central, northeastern and southeastern Argentina, south and southeastern Brazil, southeastern Bolivia, central Paraguay, and northeastern and northwestern Venezuela (Fonseca, 1959; Mauri, 1965, 1966, 1967; Furman, 1972; Alzuet & Mauri, 1973; Fernandez & Capri, 1978; Botelho & Williams, 1980; Botelho et al., 1981; Castro et al., 1987; Whitaker & Abrell, 1987; Whitaker & Dietz, 1987; Gettinger, 1992; Lareschi & Mauri, 1998; Lareschi et al., 2006; Nava & Lareschi, 2012; Sponchiado et al., 2015; Espinoza-Carniglia et al., 2023; Trejo & Berrizbeitia, 2024).

The main diagnostic character of *L. mazzai* is the hypertrichous dorsal shield in both sexes and the deutonymphs (Fonseca, 1939; Espinoza-Carniglia & Lareschi, 2023). Previous studies supported the phenotypic plasticity only in the dorsal shield size of female mites, while none in other ventral characteri, as females of *L. mazzai* showed variations corresponding to different *Calomys* species from Argentina. However, these variations were not

linked with genetic differences (Espinoza-Carniglia et al., 2023).

In Peru, six *Calomys* species were reported, five of which are endemic, and any of them are reported in association with *L. mazzai*, until now. One of those endemic Peruvian rodent species is *Calomys sorellus* (Thomas, 1900) that occurs in highlands above 2000 m (Zeballos et al., 2014).

The aim of this study is to report for the first time *L. mazzai* associated with *C. sorellus* in Peru, by using both morphological and molecular identification methods, to explore potential phenotypic variations in this new host-parasite association. In addition, *L. mazzai* is compared at a molecular level with other sequences of *Laelaps* species associated with South American cricetid rodents available in Genbank.

Mites were collected from two specimens identified as *C. sorellus*, captured in two localities in Ancash, Peru: L1, Comunidad 24 de Junio, Distrito Independencia, Provincia Huaraz, Ancash, Peru (9°34'10.0" S 77°35'03.0" W, 3922 masl), and L2, Tambillo, Distrito Huachis, Provincia Huari, Ancash, Peru (9°40'22.0" S 77°12'45.0" W, 3954 masl) (Fig. 1). Collected mites were preserved in 96 % ethanol until studied. The morphological identification of the rodents was carried out following the criteria of Herskovitz (1962). The host specimens were deposited at the Natural History Museum of the National University of San Marcos, Peru (MUSM), under catalog numbers: MUSM 49753 (Locality L1), MUSM 50042 (Locality L2).

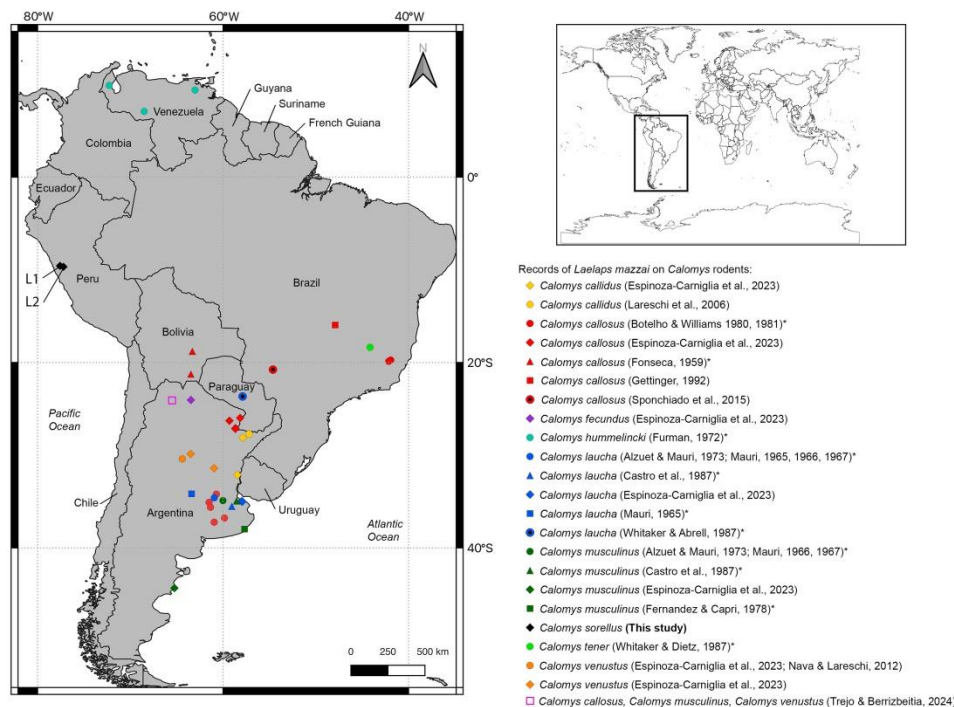


Figure 1. Localities of *Calomys sorellus* in Peru. L1, Comunidad 24 de Junio, Distrito Independencia, Provincia Huaraz, Ancash, Peru (9°34'10.0" S 77°35'03.0" W, 3922 m), and L2, Tambillo, Distrito Huachis, Provincia Huari (9°40'22.0" S 77°12'45.0" W, 3954 m). Previous records of *Laelaps mazzai* from *Calomys* species are indicated in different colors. Asterisks (*) indicate an approximate point since the specific coordinates were not available in the literature.

Twenty-nine mites were collected from the two *C. sorellus* examined (23 mites from L1 and six mites from L2). Out of them, 26 were females, one male and two deutonymphs. Protonymphs and larvae were not collected. A subsample of four female mites (two from each individual host) were processed for DNA extraction. DNA was isolated individually from each mite using Chelex®100 following a non-destructive method for mites described in Savchenko & Lareschi (2019). A PCR was performed to amplify the nuclear region 18S-ITS1-5.8S-ITS2-28S (De Rojas et al., 2002). The PCR protocol consisted of an initial denaturation of 10 min to 95 °C, then 35 cycles of denaturation for 20 s to 95 °C, annealing for 30 s to 51 °C, extension for 90 s to 72 °C and a final extension of 10 min to 72 °C. The final products of PCR were sequenced in Macrogen (Seoul, Korea). Sequence obtained in this study (deposited in Genbank under the accession number PQ276488), as well as others available in Genbank (see in Savchenko & Lareschi, 2022; Espinoza-Carniglia et al., 2023) were compared with a Bayesian probabilities tree constructed using MrBayes 3.2.6. after 10 million of generations. The final tree was visualized using Figtree v1.4.4. An analysis of genetic distances (number of base substitutions per site) was performed in MEGA X (Kumar et al., 2018) to evaluate genetic divergence between species; genetic distances were transformed into percentage.

After DNA extraction, mite exoskeletons, and the remaining mites collected, were prepared for inspection under light microscope. All mites were cleared in lactophenol and mounted individually in Hoyer's medium (Walter & Krantz, 2009) to be identified using a light microscope. Identification of females and males were based on the original description (Fonseca, 1939) as well as on Furman (1971, 1972) and Tipton (1960), and comparison with the lectotype (IBSP604c) of *L. mazzai* (Coleção Acarológica do Instituto Butantan, SP, Brazil). The identification of deutonymphs followed the description of Espinoza-Carniglia & Lareschi (2023). Evans & Till (1965, 1979) were followed for chaetotaxy and shields nomenclature of the mites. For measurements, a subsample of six female mites from L1 and four females from L2 were considered. Only the dorsal shield of female mites was used since a previous study showed that differences in sizes are presented in that structure (Espinoza-Carniglia et al., 2023). To analyze images, the Leica Application Suite software (V.4.12) was used, with measurements provided in micrometers (µm) of the length (LDS) and width of the dorsal shield (WDS), the distance between z5 setae (z5-z5), between J2 setae (J2-J2), between J5 setae (J5-J5), between Z5 setae (Z5-Z5) and the length of the Z5 (Z5L) (Fig. 2a). The results were presented as the average measurement followed by minimum and maximum values between brackets. Voucher mites are kept at División

Zoología de Invertebrados, Museo de La Plata (MLP, La Plata, Buenos Aires, Argentina). The field number of each mite is provided (females: VSO534-1, VSO534-2, VSO534-3, VSO534-4, VSO534-5, VSO534-6, JPP035-1, JPP035-2, JPP035-3, JPP035-4, male: VSO534-7, deutonymphs: VSO534-8, VSO534-9).

Characters of all 29 mites obtained from both hosts were consistent with the original descriptions for both sexes and deutonymphs of *L. mazzai*. Female mites were recognized by a combination of the following diagnostic characters: hyperthricha in the dorsal shield, coxa I with strong setiform proximal seta and stout setiform distal seta, coxa II with a strong setiform posterior seta and coxa III with spiniform posterior seta. The male was recognized because of a denser hyperthricha in the dorsal shield at the level of the second pair of legs and the anal shield separated from the sternoventral shield. Deutonymphs were recognized by the dorsal hyperthricha.

Although the general morphology of female *L. mazzai* mites herein studied was similar to female mites associated with other species of *Calomys*, the measurements of female mites from Peru were larger than those from the other countries. Meanwhile, Venezuelan females were medium sized, and the Argentinean ones were the smallest, except mites from *C. musculus* (Table I). Moreover, the number of setae in the medial podonotal region in female mites from Peru is lower than those from other countries (Fig. 2a-b).

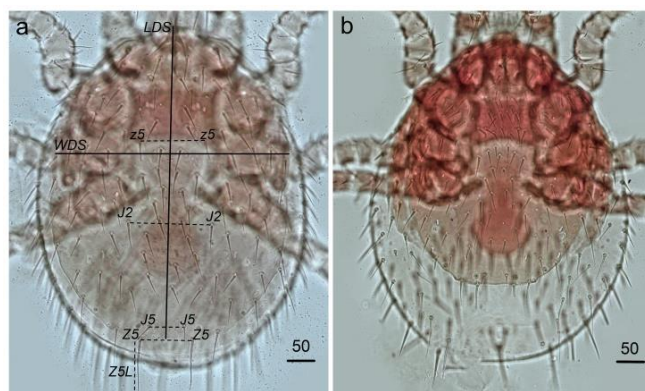


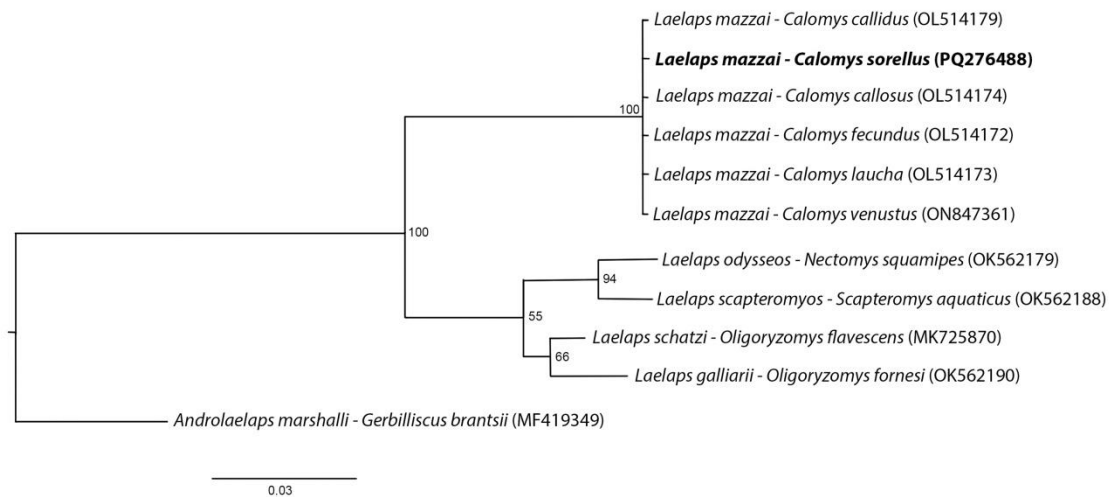
Figure 2. Comparison of the dorsal view of females of *Laelaps mazzai*. a. Mite collected from *Calomys sorellus* in Comunidad 24 de Junio, Distrito Independencia, Provincia Huaraz, Peru; b. Mite collected from *Calomys fecundus* 32 km SW from la Union, Salta Province, Argentina. References= Continuous line: Length (LDS) and width of the dorsal shield (WDS), dashed line: distance between z5 setae (z5-z5), between J2 setae (J2-J2), between J5 setae (J5-J5), between Z5 setae (Z5-Z5), and length of the Z5 (Z5L). Scale bars are in micrometers (µm).

Concerning DNA analysis, DNA was extracted only from one female obtained from L1. The sequence of 18S-ITS1-5.8S-ITS2-28S fragment obtained of *L. mazzai* from *C. sorellus* was not significantly different compared to *L. mazzai* obtained from the other *Calomys* species (genetic distances: 0-0.2 %, Fig. 3).

Table I. Measurements taken from *Laelaps mazzai* females collected from different host species and countries. Results are presented as average followed by minimum and maximum values between brackets.

Host rodent species	n	LDS	WDS	z5-z5	J2-J2	J5-J5	Z5-Z5	Z5L	Country (Reference)
<i>Calomys sorellus</i> (Thomas, 1900)	11	740 [717–765]	547 [532–564]	156 [149–165]	206 [199–217]	86 [78–102]	127 [122–131]	121 [117–130]	Peru (This study)
<i>Calomys hummelincki</i> (Husson, 1960)	15	725 [697–752]	475 [440–510]	nd	nd	nd	nd	114 [107–121]	Venezuela (Furman, 1972)
<i>Calomys callidus</i> (Thomas, 1916)	10	640 [632–645]	466 [454–479]	119 [108–125]	162 [147–171]	56 [51–60]	93 [89–97]	95 [90–106]	Argentina (Espinoza-Carniglia et al., 2023)
<i>Calomys callosus</i> (Rengger, 1830)	13	623 [611–638]	452 [441–470]	117 [111–124]	160 [151–175]	57 [51–67]	92 [84–106]	95 [86–100]	Argentina (Espinoza-Carniglia et al., 2023)
<i>Calomys fecundus</i> (Thomas, 1926)	6	597 [590–610]	439 [426–450]	119 [112–126]	156 [151–166]	57 [55–59]	93 [90–97]	91 [81–96]	Argentina (Espinoza-Carniglia et al., 2023)
<i>Calomys laucha</i> (Fischer, 1814)	12	608 [597–616]	432 [422–445]	117 [114–125]	155 [146–163]	56 [53–60]	93 [89–101]	93 [89–98]	Argentina (Espinoza-Carniglia et al., 2023)
<i>Calomys musculus</i> (Thomas, 1913)	7	659 [645–686]	467 [450–485]	125 [121–130]	162 [147–172]	56 [54–60]	96 [89–102]	104 [101–108]	Argentina (Espinoza-Carniglia et al., 2023)
<i>Calomys venustus</i> (Thomas, 1894)	21	629 [616–645]	464 [448–478]	122 [114–137]	165 [154–173]	59 [54–64]	96 [90–104]	96 [91–99]	Argentina (Espinoza-Carniglia et al., 2023)

n= number of measured mites, LDS= length of the dorsal shield, WDS= width of the dorsal shield, z5-z5= distance between z5 setae, J2-J2= distance between J2 setae, J5-J5= distance between J5 setae, Z5-Z5= distance between Z5 setae, Z5L= length of the Z5 setae, nd= no data available

**Figure 3. Phylogenetic tree obtained from Bayesian inference analysis of 18S-ITS1-5.8S-ITS2-28S region of *Laelaps* species from South America. Sequences of *Laelaps* from other sigmodontine rodents were incorporated from Genbank (accession number indicated in paranthesis). Bayesian posterior probabilities are indicated at nodes. Labels indicate mite and host species.**

The morphological and molecular results confirmed the presence of *L. mazzai* on *C. sorellus* in two localities from Peru. These results increased to nine the *Calomys* species from South America associated to *L. mazzai*, in addition to broadening its geographical distribution, by approximately 1500 km to the northwest of Bolivia and 2000 km to the southwest of Venezuela (see Fig. 1). This record is relevant because it demonstrates the strong association between *L.*

mazzai *Calomys* species, even across the Cordillera de Los Andes barrier. These results were expected, since previous studies indicate that *L. mazzai* is associated with *Calomys* further to the north (Venezuela) and further to the south in Argentina, Bolivia and Brazil (Fonseca, 1959; Mauri, 1965, 1966, 1967; Furman, 1972; Alzuet & Mauri, 1973; Fernandez & Capri, 1978; Botelho & Williams, 1980; Botelho et al., 1981; Castro et al., 1987; Whitaker & Abrell,

1987; Whitaker & Dietz, 1987; Gettinger, 1992; Lareschi & Mauri, 1998; Lareschi et al., 2006; Nava & Lareschi, 2012; Sponchiado et al., 2015; Espinoza-Carniglia et al., 2023; Trejo & Berrizbeitia, 2024).

Although this study confirmed that all diagnostic characters were consistent with *L. mazzai*, we found variations in the size of female dorsal shields. These morphological variations are consistent with previous studies of *L. mazzai* from *Calomys* that suggest that females of this mite species could present high levels of phenotypic plasticity between hosts and geographic locations in Argentina (Espinoza-Carniglia et al., 2023). Morphotypes of female *L. mazzai* from *C. fecundus* in Salta Province, Argentina had small dorsal shields, similar to the lectotype of *L. mazzai*. However, female mites of *C. sorellus* from Peru had larger dorsal shields than the rest of the *Calomys*, similar to *C. hummelincki* females from Venezuela and *C. musculinus* from Argentina (Furman, 1972; Espinoza-Carniglia et al., 2023), although with fewer hypertrichae. These phenotypic variations could be primarily enhanced by the host traits, because they represent the direct resources that at least laelapid females use for feeding and living (Seeman & Nahrung, 2018). In this sense, the morphological differences observed in *L. mazzai* were consistent with three different groups of *Calomys*: the highland species (*C. lepidus* Thomas, *C. musculinus* Thomas, *C. sorellus* Thomas), the lowland ones (*C. callidus* Thomas, *C. callosus* Rengger, *C. fecundus* (Thomas), *C. laucha* Fischer, *C. venustus* Thomas), and a last intermediate group that only includes *C. hummelincki* Husson (Haag et al., 2007; Zeballos et al., 2014; Salazar-Bravo, 2015). These groups of *Calomys* have different morphology and phylogenetic stories, which could drive size differences in mites. Furthermore, each group is subjected to different environmental conditions in relation to altitude, which could indirectly affect the laelapidae (Seeman & Nahrung, 2018; Poulin, 2021).

The results obtained in this study contribute to the knowledge of *L. mazzai* phenotypic plasticity, while opening new prospects for future investigations that could look into the associations of *L. mazzai* with other Peruvian rodents that are also endemic and have different distributions.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS CONTRIBUTIONS

MEC: methodology, conceptualization, data curation, writing – original draft preparation, research, redaction: review and editing.

CY: methodology, conceptualization, writing: review and editing.

EMR: methodology, funding, writing: review and editing.

ML: methodology, funding, resources, conceptualization, writing: review and editing.

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