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Short communication

Anaplasmataceae presence in *Amblyomma calcaratum* associated with anteaters (*Tamandua tetradactyla*) in the rainforest ecoregion, Argentina

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ABSTRACT

Bacteria of the sister genera *Ehrlichia* and *Anaplasma* (Anaplasmataceae) are obligate intracellular Alphaproteobacteria that are transmitted mostly through arthropod vectors. These agents can infect different vertebrate cells, depending on the species involved, and can cause diseases in animals and humans. In this study, we evaluated the presence of Anaplasmataceae bacteria in *Amblyomma calcaratum* ticks collected from a road-killed *Tamandua tetradactyla* in the Rainforest ecoregion in Argentina. All samples were screened for Anaplasmataceae DNA using a real-time PCR assay targeting the 16S rRNA gene. Evidence of Anaplasmataceae DNA was detected in three out of thirty-nine *Am. calcaratum* ticks. Phylogenetic analysis of a portion of 16S rRNA gene positioned one sample (*Ehrlichia* sp. strain Ac124) with *Ehrlichia* sequences and the other two samples with *Anaplasma* sequences; *Anaplasma* sp. strain Ac145 close to *Anaplasma odocoilei* and *Anaplasma* sp. strain Ac152 in an ancestral position to most *Anaplasma* species. The *groEL* sequence obtained showed that *Ehrlichia* sp. strain Ac124 was phylogenetically related to *Ehrlichia* sp. strain Iberá reported infecting *Amblyomma tigrinum* from Iberá wetlands in Argentina. Phylogenetic analysis using the *rpoB* sequence positioned *Anaplasma* sp. strain Ac145 close to the canine pathogen *Anaplasma platys*, while *Anaplasma* sp. strain Ac152 was positioned close to the bovine pathogen *Anaplasma marginale*.

In this study, three Anaplasmataceae agents were detected in adults of *Am. calcaratum* associated with a *T. tetradactyla*. These results suggest that the number of Anaplasmataceae species, as well as their distribution, is largely unknown.

1. Introduction

Bacteria of the sister genera *Ehrlichia* and *Anaplasma* (Rickettsiales: Anaplasmataceae) are vector-borne, obligate intracellular Alphaproteobacteria. The genus *Ehrlichia* infects monocytes, neutrophils, and endothelial cells of vertebrates, depending on the species involved (Doyle et al., 2005). There are formally six recognized species that are tick-transmitted: *Ehrlichia canis, Ehrlichia chaffeensis, Ehrlichia ewingii, Ehrlichia muris, Ehrlichia minasensis* and *Ehrlichia ruminantium* (Dumler et al., 2001; Cabezas-Cruz et al., 2016). In Argentina, new strains of *Ehrlichia* have recently been detected to infect *Amblyomma* ticks (Cicuttin et al., 2017, 2020; Eberhardt et al., 2020; Fargnoli et al., 2020; Tarragona et al., 2022), suggesting that the number of *Ehrlichia* species may be underestimated. The genus *Anaplasma* infects the cytoplasm of erythrocytes, leukocytes, platelets, or endothelial cells of vertebrates, depending on the species involved (Tate et al., 2013; Vanstreels et al., 2018). Currently, there are nine formally recognized species that are vector-transmitted: *Anaplasma bovis, Anaplasma capra, Anaplasma caudatum, Anaplasma centrale, Anaplasma marginale, Anaplasma odocoilei, Anaplasma ovis, Anaplasma phagocytophilum* and *Anaplasma platys*, as

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Table 1
Sequences of primers used.

Gene	Primer	Sequence	Fragment size	Refs.
Tick 16S rRNA	16S+1	CCGGTCTGAACTCAGATCAAGT	451 bp	Mangold et al., 1998
	16S-1	GCTCAATGATTTTTTAAATTGCTGT		
Anaplasmataceae 16S rRNA real-time PCR	AE2 Fw	CGCAAGGYTKAGCTAATCCRTAAAAGT	177 bp	Monje et al., 2019
	AE-Rv	RCACCAGCTTCGAGTTAAGCCAAT		
Anaplasmataceae 16S rRNA	GE2F2	GTTAGTGGCAGACGGGTGAGT	1306 bp	Monje et al., 2019; Parola et al., 2000; Eberhardt et al., 2023
	AE-Rv	RCACCAGCTTCGAGTTAAGCCAAT		
	AE4-Fw	GTACCYAYAGAAGAAGTCCCGGCA	958 bp	
	16S ANA-F	CAGAGTTTGATCCTGGCTCAGAACG	468 bp	Stuen et al., 2003
	16S ANA-R	GAGTTTGCCGGGACTTCTTCTGTA		
rpoB	Ana-rpoBF	GCTGTTCCTAGGCTYTCTTACGCGA	525 bp	Dahmani et al., 2017
	Ana-rpoBR	AATCRAGCCAVGAGCCCCTRTAWGG		
Ehrlichia dsb	dsb330	GATGATGTCTGAAGATATGAAACAAAT	409 bp	Doyle et al., 2005.
	dsb728	CTGCTCGTCTATTTTACTTCTTAAAGT		
Ehrlichia groEL	EHRgro 2Fw	TTCTCCTATACARCTTCCTGA	1852 bp	This study
	EHRgro 5Rv	GCAAAATCAAATGCTATACG		
	EHRgro 3Fw	ATTGGCTCTTGCTATTGC	1224 bp	
	EHRgro 4Rv	AAATGAGCAATAACACATGG		

well as six candidate species and numerous unclassified species transmitted by unidentified vectors (Vanstreels et al., 2018; Rar et al., 2021). In Argentina, due to its importance in veterinary health, most studies have focused on the presence of *An. marginale* and *An. platys* in livestock and domestic animals (Ruybal et al., 2009; Eiras et al., 2013; Cicuttin et al., 2014; Mazzucco Panizza et al., 2022). Recently, we reported the presence of an *Anaplasma* species closely related to *An. odocoilei* infecting capybaras and their ticks in the Iberá wetlands (Monje et al., 2020; Eberhardt et al., 2023), suggesting that the number and distribution of *Anaplasma* species in this region may also be underrated.

The collared anteater (*Tamandua tetradactyla*) has a distribution that extends all along the eastern side of the Andes from Colombia to Uruguay, and northern Argentina (Hayssen, 2011). *Amblyomma calcaratum* Neumann, 1899 is a hard tick species (Ixodidae) distributed from Argentina to Mexico (Guglielmone et al., 2021). Adults of *Am. calcaratum* typically feed on anteaters (Pilosa: Myrmecophagidae), while the immature stages mostly feed on passerine birds (Nava et al., 2017). *Amblyomma calcaratum* has been listed as a very rare parasite of humans, and this tick is not involved as a vector of human or animal pathogens (Guglielmone et al., 2021).

As the diversity of *Anaplasma* and *Ehrlichia* species may be underestimated, and to increase the limited information available on bacteria of sanitary importance infecting *Am. calcaratum*, this study aimed to assess the presence of Anaplasmataceae bacteria in specimens of this tick found parasitizing a road-killed anteater in the Rainforest ecoregion of northeastern Argentina.

2. Materials and methods

Ticks were collected in 2021 from a road-killed *T. tetradactyla* specimen found on the side of Provincial Route 2 (−27.214580, −54.030876) in Misiones province, Argentina. The anteater carcass was thoroughly examined *in situ* for ticks, and the samples found were conserved in 96% ethanol. The morphological identification of nymphs and adults was made following Nava et al. (2017). DNA extraction was made from individual ticks using standard phenol-chloroform methods. All samples were screened for Anaplasmataceae DNA using a real-time PCR assay targeting the 16S rRNA gene, as previously described (Monje et al., 2019; Eberhardt et al., 2023) (see Table 1). To confirm the presence of Anaplasmataceae DNA, positive samples were further amplified with two overlapping PCRs that amplify a region of approximately 1400 bp of the 16S rRNA gene. The first PCR used primers 16SANA-F and 16SANA-R, as described by Stuen et al. (2003). The second PCR was semi-nested and used the following primers: GE2F2 (Parola et al., 2000), AE-Rv and AE4-Fw (Table 1), as described by Eberhardt et al. (2023). These samples were then amplified using primers specifically targeting the disulfide oxidoreductase (*dsb*) and the heat-shock protein (*groEL*) gene for the genus *Ehrlichia*, and specific primers targeting the RNA polymerase subunit beta (*rpoB*) gene for the genera *Anaplasma*, as described by Doyle et al. (2005) and Dahmani et al. (2017). For the *groEL* gene, a nested PCR was used with the following primers: EHRgro2Fw/EHRgro5Rv and EHRgro3Fw/EHRgro4Rv (Table 1). Both rounds consisted of 3 min at 94 °C for initial denaturation, followed by 40 cycles of 30 s at 94 °C, 30 s at 52 °C and 40 s at 65 °C. For the second round, 0.4 µl of the first round were used as DNA template. To confirm the morphological identification of ticks, we determined the respective haplotype of Anaplasmataceae-positive specimens by sequencing a portion of the tick 16S rRNA gene, as described by Mangold et al. (1998). Real-time PCR assays were performed on a StepOne system (Applied Biosystems) using HOT FIREPol EvaGreen qPCR Mix Plus (Solis Biodyne), and conventional PCRs were performed in an Ivema T-18 thermocycler (Ivema Desarrollos, Argentina) using EasyTaq DNA Polymerase (TransGen Biotech), under the conditions suggested by the manufacturers. All PCRs included a positive control (*E. canis* from an infected dog, *An. centrale* from an infected calf or *Amblyomma triste*, as applicable) and a negative control using ultrapure water. Positive samples were purified and sequenced using amplifying primers. Phylogenetic trees were constructed by using MEGA 7.0 (https://www.megasoftware.net), and best-fitting substitution models were determined with the Akaike Information Criterion, using the maximum-likelihood model test.

3. Results

Thirty-nine ticks were collected (1 nymph, 34 males and 4 females) parasitizing the road-killed *T. tetradactyla*. All ticks were morphologically identified as *Am. calcaratum* based on the absence of a marginal groove, scutum ornate with irregular pale spots in a Y-shape in the antero-lateral fields, absence of carena, presence of cornua, palps with postero-dorsal projection in article II and one spur on coxae II-IV in males; the presence of a short cornua, oval porous areas, coxa I with two distinct spurs of equal size, coxae II-IV with a short triangular spur, spur on coxa IV larger than the spurs on coxae II-III, absence of chitinous tubercles on the rear margin of the body, shield adorned with an irregular pale patch in the rear field in females; and by the base capituli dorsally subtriangular, without cornua, hypostome spatulate with a dental formula of 2/2, scutum with a rough surface and deep punctuations more numerous in lateral and posterior fields, absence of tubercles,

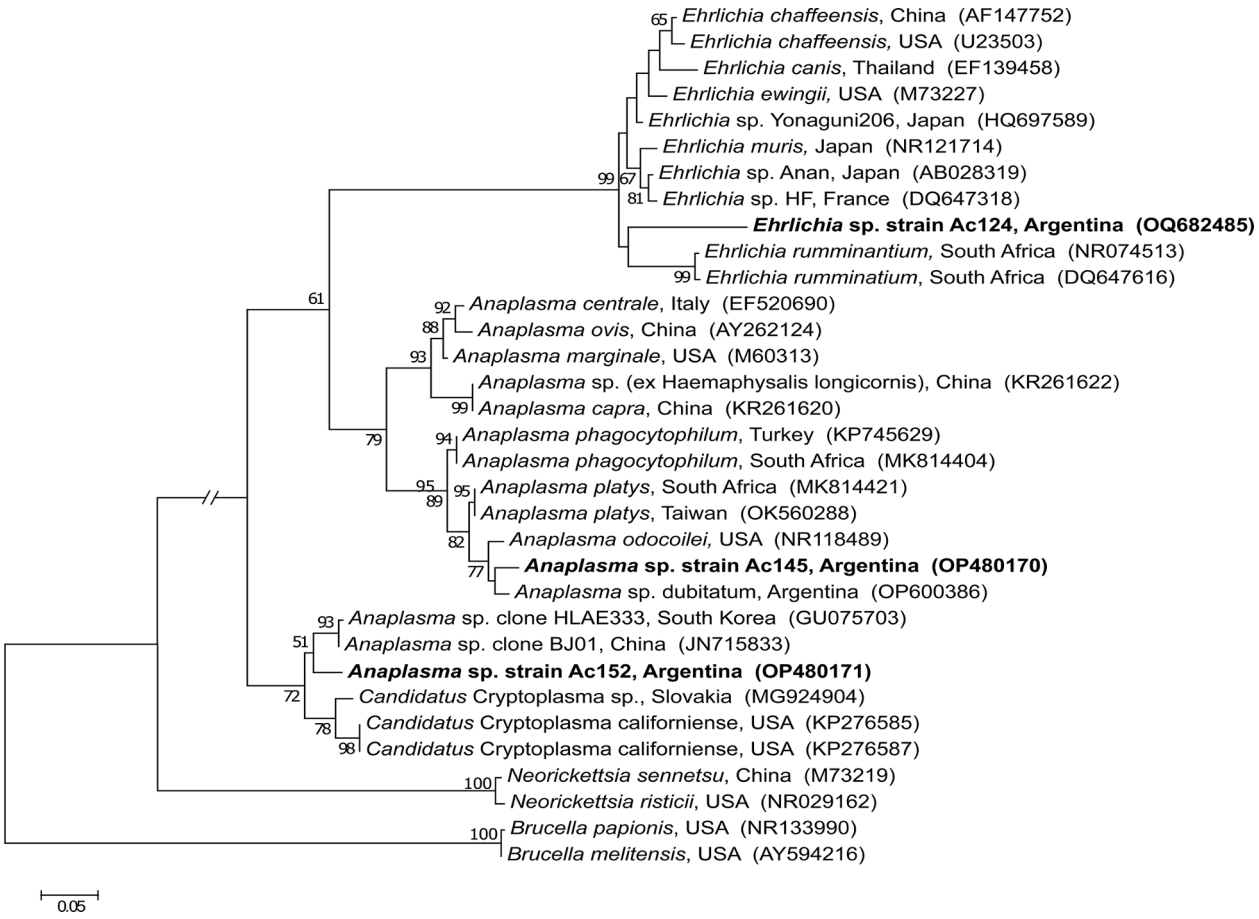

Fig. 1. Maximum Likelihood tree constructed from 16S rRNA gene sequences of Anaplasmataceae (1205 positions included in the final dataset). The Kimura 2-parameter model was selected as the best model based on Akaike's information criteria. A discrete Gamma distribution (+*G*) was used to model evolutionary rate differences among sites. The numbers represent bootstrap support generated from 1000 repetitions, and values lower than 50% are not shown. Sequences generated in this study are shown in bold. GenBank access codes are shown in parentheses.

coxa I with two triangular spurs, the outer one longer and more pointed, coxae II-IV with a small triangular spur each, and width/length ratio of the scutum less than 1.3 in nymphs. The arthropod 16S rRNA sequences obtained from Anaplasmacetaceae-infected ticks showed 100% identity with the corresponding sequence of *Am. calcaratum* haplotype I (KU953951).

Three males (samples Ac124, Ac145 and Ac152) were positive for the presence of Anaplasmataceae DNA, from which sequences of about 1200 bp of the 16S rRNA gene were obtained. The phylogenetic analysis positioned the 16S rRNA sequence obtained from sample Ac124, herein named *Ehrlichia* sp. strain Ac124, in a monophyletic clade close to *E. ruminantium* (NR074155, 16S rRNA identity 96.41%) (1205 positions

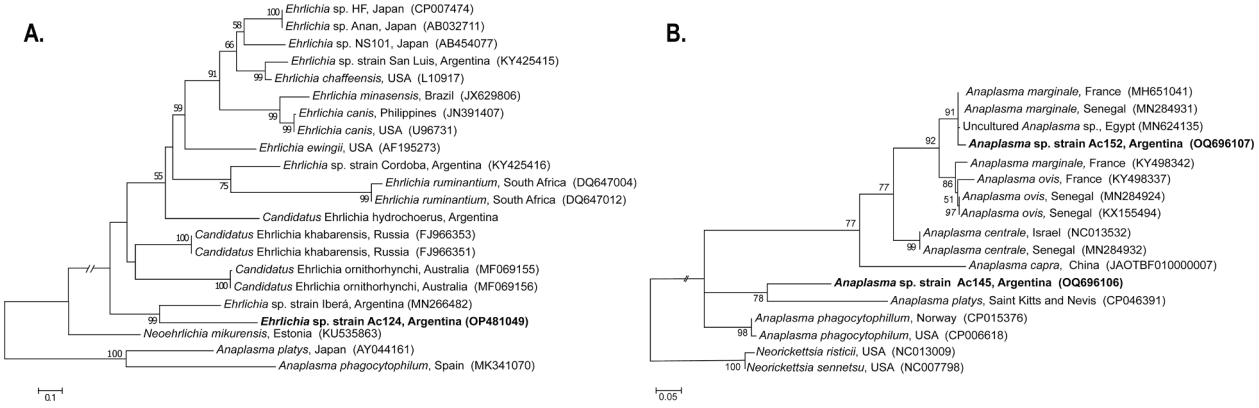

Fig. 2. Maximum Likelihood trees constructed from (A) *groEL* sequences of *Ehrlichia* spp. and (B) *rpoB* sequences of *Anaplasma* spp. Model selection was based on Akaike's information criteria. The Hasegawa-Kishino-Yano model with a discrete Gamma distribution (+*G*) was selected as the best model for *groEL* sequences (873 positions included in the final dataset), while the Kimura 2-parameter model with a discrete Gamma distribution and Invariable sites (+*G* + *I*) was selected as the best model for *rpoB* sequences (357 positions included in the final dataset). The numbers represent bootstrap support generated from 1000 repetitions, and values lower than 50% are not shown. Sequences generated in this study are shown in bold. GenBank access codes are shown in parentheses.

included in the final dataset, Fig. 1). The 16S rRNA sequence obtained from sample Ac145, herein named *Anaplasma* sp. strain Ac145, was positioned close to *Anaplasma* sp. from *Amblyomma dubitatum* (OP600386, 16S rRNA identity 98.85%), *An. odocoilei* (NR118489, 16S rRNA identity 98.55%) and *An. platys* (MK814421, 16S rRNA identity 98.17%), while sample Ac152, herein named *Anaplasma* sp. strain Ac152, was phylogenetically positioned in a separated clade with *Anaplasma* sp. clone BJ01 (JN71583316S rRNA identity 98.68%) and *Anaplasma* sp. clone HLAE333 (GU075703, 16S rRNA identity 98.29%). Recent studies conducted in South America reported finding novel Anaplasmataceae agents infecting wild mammals such as *T. tetradactyla, Myrmecophaga tridactyla, Bradypus variegatus, Nasua nasua, Leopardus pardalis* (de Souza et al., 2017; Calchi et al., 2020). Unfortunately, only short 16S rRNA sequences are available for those *Anaplasma* from South America. Phylogenetic analysis including these sequences (436 positions included in the final dataset) placed *Anaplasma* sp. strain Ac 145 in the same clade with *Candidatus* Anaplasma brasiliensis and the *Anaplasma* sp. from *N. nasua* and *L. pardalis* (Supplementary Figure), while *Anaplasma* sp. strain Ac152 and *Ehrlichia* sp. strain Ac124 were not related to any of these sequences.

A fragment of approximately 1100 bp of the ehrlichial *groEL* gene was amplified from the *Ehrlichia* sp. strain Ac124, whereas the ehrlichial *dsb* gene could not be amplified from the same sample. Phylogenetic analysis using *groEL* sequences revealed that *Ehrlichia* sp. strain Ac124 is phylogenetically related to *Ehrlichia* sp. strain Iberá (MN266482, *groEL* identity: 89.50%) (Fig. 2A). Both *Anaplasma*-positive samples (Ac145 and Ac152) yielded amplified fragments of approximately 500 bp of the *rpoB* gene. Phylogenetic analysis using the *rpoB* sequence positioned *Anaplasma* sp. strain Ac145 in the same clade as the canine pathogen *An. platys* (CP046391, *rpoB* identity 82.37%) (Fig. 2B), consistent with the positioning of the 16S rRNA sequence. Additionally, the *rpoB* sequence positioned *Anaplasma* sp. strain Ac152 close to the bovine pathogen *An. marginale* (CP023731, *rpoB* identity 98.43%) (Fig. 2B), in a distant position from that observed in 16S rRNA analysis.

All the sequences generated in this study have been deposited in GenBank (OQ564493-OQ564495, OQ682485, OP481049, OP480170-OP480171, OQ696106-OQ696107).

4. Discussion

To the best of our knowledge, the three Anaplasmataceae agents here reported (*Ehrlichia* sp. strain Ac124, *Anaplasma* sp. strain Ac145 and *Anaplasma* sp. strain Ac152) are novel. This study also constitutes the first report of Anaplasmataceae infecting *Am. calcaratum*. *Ehrlichia* sp. strain Ac124 expands the currently growing list of *Ehrlichia* sp. reported in southern South America. The phylogenetic analysis, using the *groEL* sequence obtained herein, consistently positioned *Ehrlichia* sp. strain Ac124 close to *Ehrlichia* sp. strain Iberá, previously reported to infect *Am. tigrinum* associated with pampas foxes in the Iberá wetlands ecoregion of Argentina (Eberhardt et al., 2020). In this analysis, both ehrlichiae were placed at a distant position from the rest of South American *Ehrlichia* but close to '*Candidatus* Ehrlichia ornithorhynchi' reported to infect platypus in Australia (Gofton et al., 2018) and '*Candidatus* Ehrlichia khabarensis' reported to infect voles from eastern Russia (Rar et al., 2010). In this study, we were not able to amplify the *dsb* gene of *Ehrlichia* sp. strain Ac124, similar to the findings reported by Eberhardt et al. (2020). In addition, we report the presence of *Anaplasma* species in *Am. calcaratum*, named here as *Anaplasma* sp. strain Ac145 and *Anaplasma* sp. strain Ac152. The phylogenetic analysis using 16S rRNA and *rpoB* sequences revealed that *Anaplasma* sp. strain Ac145 is closely related to *Anaplasma* sp. found in *Am. dubitatum, An. odocoilei* and *An. platys*, which all share the same tropism for thrombocytes and can infect capybaras (Monje et al., 2020), white-tailed deer (Tate et al., 2013), dogs (Inokuma 2007) and humans (Arraga-Alvarado et al., 2014). However, unexpectedly, the phylogenetic analysis showed that *Anaplasma* sp. strain Ac152 was positioned differently in the 16S rRNA and *rpoB* sequence-derived trees. The 16S rRNA sequence placed this agent in a clade ancestral to most formally recognized *Anaplasma* species, as it greatly diverges from them, showing 95.31% identity to *An. platys*, 95.19% to *An. centrale* and 95.02% to *An. marginale*. On the other hand, the *rpoB* analysis positioned *Anaplasma* sp. strain Ac152 in the same clade as the widely distributed bovine pathogen *An. marginale*. This unexpected result is consistent with the loss of phylogenetic information caused by horizontal transfer of 16S rRNA gene segments, which has been observed in other Alphaproteobacteria (van Berkum et al., 2003) and members of *Helicobacter* genus (Dewhirst et al., 2005).

Transovarial transmission of *Anaplasma* and *Ehrlichia* has not been demonstrated (Brouqui and Matsumoto, 2007; Mazzucco Panizza et al., 2022). Therefore, it is assumed that immature stages of *Am. calcaratum* acquire the bacteria while feeding on an infected host. *Amblyomma calcaratum* primarily feeds on anteaters and several orders of Aves (Nava et al., 2017), making these vertebrates possible reservoir hosts of these Anaplasmataceae. Others previously reported the presence of Anaplasmataceae in anteaters (Calchi et al., 2020), wild geese (Werther et al., 2017) and carnivorous birds (Machado et al., 2012). However, further research is needed to determine the ecological role of anteaters and birds in the transmission and maintenance of the Anaplasmataceae species detected in this study, as well as their potential for zoonotic transmission.

CRediT authorship contribution statement

Paula J. Vaschalde: Methodology, Investigation, Formal analysis, Writing – original draft. **Fernando S. Flores:** Conceptualization, Resources, Funding acquisition, Writing – review & editing. **M. Celeste Facelli Fernández:** Investigation, Writing – original draft. **Johann Barolin:** Investigation, Writing – original draft. **Laura B. Tauro:** Resources, Funding acquisition, Writing – original draft. **Lucas D. Monje:** Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2023.102222.

References

Arraga-Alvarado, C.M., Qurollo, B.A., Parra, O.C., Berrueta, M.A., Hegarty, B.C., Breitschwerdt, E.B., 2014. Case report: molecular evidence of *Anaplasma platys* infection in two women from Venezuela. Am. J. Trop. Med. Hyg. 91 (6), 1161–1165. https://doi.org/10.4269/ajtmh.14-0372.

Brouqui, P., Matsumoto, K., 2007. Bacteriology and Phylogeny of Anaplasmataceae. Raoult, D., Parola, P. Rickettsial Diseases, Infectious Disease and Therapy. Informa Healthcare, pp. 179–198. https://doi.org/10.3109/9781420019971.013.

Cabezas-Cruz, A., Zweygarth, E., Vancová, M., Broniszewska, M., Grubhoffer, L., Passos, L.M.F., Ribeiro, M.F.B., Alberdi, P., de la Fuente, J., 2016. *Ehrlichia minasensis* sp. nov., isolated from the tick *Rhipicephalus microplus*. Int. J. Syst. Evol. Microbiol. 66, 1426–1430. https://doi.org/10.1099/ijsem.0.000895.

Calchi, A.C., Vultão, J.G., Alves, M.H., Yogui, D.R., Desbiez, A.L.J., De Santi, M., Santana, M.S., da Silva, T.M.V., Werther, K., Teixeira, M.M.G., Machado, R.Z., André, M.R., 2020. *Ehrlichia* spp. and *Anaplasma* spp. in Xenarthra mammals from Brazil, with evidence of novel *'Candidatus* Anaplasma spp.'. Sci. Rep. 10 (1), 12615. https://doi.org/10.1038/s41598-020-69263-w.

Cicuttin, G.L., De Salvo, M.N., Nava, S., 2017. Two novel *Ehrlichia* strains detected in *Amblyomma tigrinum* ticks associated to dogs in peri-urban areas of Argentina. Comp. Immunol. Microbiol. Infect. Dis. 53, 40–44. https://doi.org/10.1016/j.cimid.2017.07.001.

Cicuttin, G.L., De Salvo, M.N., Pérez, P.D., Silva, D., Félix, M.L., Venzal, J.M., Nava, S., 2020. A novel *Ehrlichia* strain (Rickettsiales: anaplasmataceae) detected in *Amblyomma triste* (Acari: ixodidae), a tick species of public health importance in the Southern Cone of America. Pathog. Glob. Health 114, 318–322. https://doi.org/10.1080/20477724.2020.1795579.

Cicuttin, G.L., Vidal, P., Nazarena De Salvo, M., Beltrán, F.J., Gury Dohmen, F.E., 2014. Detección molecular de *Rickettsia massiliae* y *Anaplasma platys* en garrapatas *Rhipicephalus sanguineus* y caninos domésticos del municipio de Bahía Blanca (Argentina). Rev. Chil. Infectol. 31, 563–568. https://doi.org/10.4067/S0716-10182014000500008.

Dahmani, M., Davoust, B., Rousseau, F., Raoult, D., Fenollar, F., Mediannikov, O., 2017. Natural Anaplasmataceae infection in *Rhipicephalus bursa* ticks collected from sheep in the French Basque Country. Ticks Tick Borne Dis. 8 (1), 18–24. https://doi.org/10.1016/j.ttbdis.2016.09.009.

De Sousa, K.C.M., Calchi, A.C., Herrera, H.M., Dumler, J.S., Barros-Battesti, D.M., Machado, R.Z., André, M.R., 2017. Anaplasmataceae agents among wild mammals and ectoparasites in Brazil. Epidemiol. Infect. 145, 3424–3437. https://doi.org/10.1017/S095026881700245X.

Dewhirst, F.E., Shen, Z., Scimeca, M.S., Stokes, L.N., Boumenna, T., Chen, T., Paster, B.J., Fox, J.G., 2005. Discordant 16S and 23S rRNA gene phylogenies for the genus *Helicobacter*: implications for phylogenetic inference and systematics. J. Bacteriol. 187 (17), 6106–6118. https://doi.org/10.1128/JB.187.17.6106-6118.2005.

Doyle, C.K., Labruna, M.B., Breitschwerdt, E.B., Tang, Y.W., Corstvet, R.E., Hegarty, B.C., Bloch, K.C., Li, P., Walker, D.H., McBride, J.W., 2005. Detection of medically important *Ehrlichia* by quantitative multicolor TaqMan real-time polymerase chain reaction of the *dsb* gene. J. Mol. Diagn. 7, 504–510. https://doi.org/10.1016/S1525-1578(10)60581-8.

Dumler, J.S., Barbet, A.F., Bekker, C.P., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma, Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and "HGE agent" as subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evol. Microbiol. 51, 2145–2165. https://doi.org/10.1099/00207713-51-6-2145.

Eberhardt, A.T., Manzoli, D.E., Fernandez, C., Zurvera, D., Monje, L.D., 2023. *Anaplasma* species infecting questing ticks in the Iberá wetlands ecoregion. Argentina. Exp. App. Acarol. https://doi.org/10.1007/s10493-023-00788-1.

Eberhardt, A.T., Fernandez, C., Fargnoli, L., Beldomenico, P.M., Monje, L.D., 2020. A putative novel strain of *Ehrlichia* infecting *Amblyomma tigrinum* associated with Pampas fox (*Lycalopex gymnocercus*) in Esteros del Iberá ecoregion, Argentina. Ticks Tick-Borne Dis. 11, 101318 https://doi.org/10.1016/j.ttbdis.2019.101318.

Eiras, D.F., Craviotto, M.B., Vezzani, D., Eyal, O., Baneth, G., 2013. First description of natural *Ehrlichia canis* and *Anaplasma platys* infections in dogs from Argentina. Comp. Immunol. Microbiol. Infect. Dis. 36, 169–173. https://doi.org/10.1016/j.cimid.2012.11.008.

Fargnoli, L., Fernandez, C., Monje, L.D., 2020. Novel *Ehrlichia* strain infecting cattle tick *Amblyomma neumanni*, Argentina, 2018. Emerg. Infect. Dis. 26, 1027–1030. https://doi.org/10.3201/eid2605.190940.

Gofton, A.W., Loh, S.M., Barbosa, A.D., Paparini, A., Gillett, A., Macgregor, J., Oskam, C. L., Ryan, U.M., Irwin, P.J., 2018. A novel *Ehrlichia* species in blood and *Ixodes ornithorhynchi* ticks from platypuses (*Ornithorhynchus anatinus*) in Queensland and Tasmania, Australia. Ticks Tick-Borne Dis. 9, 435–442. https://doi.org/10.1016/j.ttbdis.2017.12.011.

Guglielmone, A.A., Nava, S., Robbins, R.G., 2021. Neotropical Hard Ticks (Acari: Ixodida: Ixodidae): A critical Analysis of Their taxonomy, distribution, and Host Relationships. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-030-72353-8.

Hayssen, V., 2011. *Tamandua tetradactyla* (Pilosa: myrmecophagidae). Mamm. Species 43, 64–74. https://doi.org/10.1644/875.1.

Inokuma, H., 2007. Vectors and reservoir hosts of Anaplasmataceae. Raoult, D., Parola, P. Rickettsial Diseases. CRC Press, pp. 199–212.

Machado, R.Z., André, M.R., Werther, K., de Sousa, E., Gavioli, F.A., Alves Junior, J.R., 2012. Migratory and carnivorous birds in Brazil: reservoirs for *Anaplasma* and *Ehrlichia* species? Vector Borne Zoonotic Dis. 12 (8), 705–708. https://doi.org/10.1089/vbz.2011.0803.

Mangold, A.J., Bargues, M.D., Mas-Coma, S., 1998. Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: ixodidae). Parasitol. Res. 84, 478–484. https://doi.org/10.1007/s004360050433.

Mazzucco Panizza, M., Cutullé, C., Primo, M.E., Morel, N., Sebastian, P.S., Nava, S., 2022. Assays to evaluate the transovarial transmission of *Anaplasma marginale* by *Rhipicephalus microplus*. Vet. Parasitol. 311, 109808 https://doi.org/10.1016/j.vetpar.2022.109808.

Monje, L., Eberhardt, A.T., Ruiz, M.F., Beldomenico, P.M., 2020. Natural infection of wild capybaras (*Hydrochoerus hydrochaeris*) with novel *Anaplasma* spp. and *Ehrlichia* spp. Int. J. Infect. Dis. 101, 545. https://doi.org/10.1016/j.ijid.2020.09.1414.

Monje, L.D., Fernandez, C., Percara, A., 2019. Detection of *Ehrlichia* sp. strain San Luis and *Candidatus* Rickettsia andeanae in *Amblyomma parvum* ticks. Ticks Tick-Borne Dis. 10, 111–114. https://doi.org/10.1016/j.ttbdis.2018.09.008.

Nava, S., Venzal, J.M., Martins, T.F., Guglielmone, A.A., González Acuña, D., 2017. Ticks of the Southern Cone of America: Diagnosis, Distribution and Hosts With Taxonomy, Ecology and Sanitary Importance. Academic Press, an impint of Elsevier, London, San Diego, CA.

Parola, P., Roux, V., Camicas, J.L., Baradji, I., Brouqui, P., Raoult, D., 2000. Detection of ehrlichiae in African ticks by polymerase chain reaction. Trans. R. Soc. Trop. Med. Hyg. 94 (6), 707–708. https://doi.org/10.1016/s0035-9203(00)90243-8.

Rar, V., Tkachev, S., Tikunova, N., 2021. Genetic diversity of *Anaplasma* bacteria: twenty years later. Infect. Genet. Evol. 91, 104833 https://doi.org/10.1016/j.meegid.2021.104833.

Rar, V.A., Livanova, N.N., Panov, V.V., Doroschenko, E.K., Pukhovskaya, N.M., Vysochina, N.P., Ivanov, L.I., 2010. Genetic diversity of *Anaplasma* and *Ehrlichia* in the Asian part of Russia. Ticks Tick-Borne Dis. 1, 57–65. https://doi.org/10.1016/j.ttbdis.2010.01.002.

Ruybal, P., Moretta, R., Perez, A., Petrigh, R., Zimmer, P., Alcaraz, E., Echaide, I., Torioni de Echaide, S., Kocan, K.M., de la Fuente, J., Farber, M., 2009. Genetic diversity of *Anaplasma marginale* in Argentina. Vet. Parasitol. 162, 176–180. https://doi.org/10.1016/j.vetpar.2009.02.006.

Stuen, S., Nevland, S., Moum, T., 2003. Fatal cases of tick-borne fever (TBF) in sheep caused by several 16S rRNA gene variants of *Anaplasma phagocytophilum*. Ann. N. Y. Acad. Sci. 990, 433–434. https://doi.org/10.1111/j.1749-6632.2003.tb07407.x.

Tarragona, E.L., Flores, F.S., Lamattina, D., Torrents, J., Sebastian, P.S., Nava, S., 2022. Two novel *Ehrlichia* (Rickettsiales: anaplasmataceae) strains detected in ticks (Ixodida, Ixodidae) and opossums (Didelphimorphia: didelphidae) in Argentina. Ticks Tick-Borne Dis. 13, 102043 https://doi.org/10.1016/j.ttbdis.2022.102043.

Tate, C.M., Howerth, E.W., Mead, D.G., Dugan, V.G., Luttrell, M.P., Sahora, A.I., Munderloh, U.G., Davidson, W.R., Yabsley, M.J., 2013. *Anaplasma odocoilei* sp. nov. (family Anaplasmataceae) from white-tailed deer (*Odocoileus virginianus*). Ticks Tick-Borne Dis. 4, 110–119. https://doi.org/10.1016/j.ttbdis.2012.09.005.

van Berkum, P., Terefework, Z., Paulin, L., Suomalainen, S., Lindström, K., Eardly, B.D., 2003. Discordant phylogenies within the rrn loci of Rhizobia. J. Bacteriol. 185 (10), 2988–2998. https://doi.org/10.1128/JB.185.10.2988-2998.2003.

Vanstreels, R.E.T., Yabsley, M.J., Parsons, N.J., Swanepoel, L., Pistorius, P.A., 2018. A novel candidate species of *Anaplasma* that infects avian erythrocytes. Parasit. Vectors 11, 525. https://doi.org/10.1186/s13071-018-3089-9.

Werther, K., Luzzi, M.C., Gonçalves, L.R., de Oliveira, J.P., Alves Junior, J.R.F., Machado, R.Z., André, M.R., 2017. Arthropod-borne agents in wild Orinoco geese (*Neochen jubata*) in Brazil. Comp. Immunol. Microbiol. Infect. Dis. 55, 30–41. https://doi.org/10.1016/j.cimid.2017.09.003.