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RESEARCH NOTE

Amblyomma scutatum Neumann, 1899 (Ixodida: Ixodidae) parasitizing *Ctenosaura similis* (Gray, 1831) (Squamata: Iguanidae) in Costa Rica

Ana JIMÉNEZ-ROCHA[1,4], Sergio BERMUDEZ CASTILLERO[2,4], Antony SOLÓRZANO-MORALES[3], Ernesto ROJAS SANCHEZ[1], César PÉREZ[1], Gaby DOLZ[3]

[1]*Parasitology Laboratory, School of Veterinary Medicine, National University, Heredia, Costa Rica*
[2]*Department of Research in Medical Entomology, Gorgas Memorial Institute for Health Research, Panama*
[3]*Zoonosis and Entomology Laboratory, Population Medicine Program, School of Veterinary Medicine, National University, Heredia, Costa Rica*
[4]Corresponding authors: ana.jimenez.rocha@una.ac.cr; sbermudez@gorgas.gob.pa

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ASBTRACT: A total of 38 ticks were collected on black spiny-tailed iguana, *Ctenosaura similis*, in a secondary dry forest from northern lowlands of Guanacaste, Costa Rica. At the time of finding, the animal has not mobility of the hind legs and tail. Ticks were identified morphological and molecularly as *Amblyomma scutatum*. PCR amplification tests to *Rickettsia*, Anaplasmataceae and *Borrelia* were negative. Possible caused of the lack of mobility is discussed.

Keywords: *Ctenosaura similis, Amblyomma scutatum*, lowland dry forest, Costa Rica.
Zoobank: https://zoobank.org/C5A85DFD-4743-4244-BF7D-CCA9CD5FDEE7

Amblyomma scutatum Neumann, 1899 is a tick species that maintains a distribution through dry areas in northern Mexico, Guatemala, Honduras, El Salvador, Nicaragua, and Costa Rica (Guglielmone et al., 2021). All stages of this species are parasites of Iguanidae (*Iguana* and *Ctenosaura*) (Guglielmone et al., 2021). Even so, there is one report of a female parasitizing a Cane toad, *Rhinella marina* L. 1758 (Romero et al., 2021). The identification of this species is difficult, since there are morphological differences found in ticks from different countries, mainly in the shape of the scutum, the ornamentation patterns, the presence and form of spurs in the coxae, and the dentition of the hypostoma (Guglielmone et al., 2021). Therefore, molecular tools are being used for more accurate taxonomic identification. Romero et al. (2021) reported *Candidatus* Rickettsia colombianensi in *A. scutatum* in El Salvador. However, limited information about distribution and host data presents a challenge to this tick records.

In Costa Rica, *A. scutatum* has been previously reported on the black spiny-tailed iguana *Ctenosaura similis* (Gray, 1831) and *Ctenosaura* sp. under the name garrobos in 10 sites of the Puntarenas and Guanacaste provinces (Álvarez-Calderón et al., 2005; U.S. National Tick Collection (USNTC) records). The aim of this paper is to present new information about *A. scutatum* in Costa Rica, based on morphological and molecular identification.

Host locality and observation: In June 2021 a *C. similis* female was observed without mobility in its pelvic limbs and flaccid tail in Daria (10.231453 N, -85.546432 W), Santa Cruz, Guanacaste province, Costa Rica (Fig. 1). This region is in a secondary lowland dry forest at 75 meters above sea level. During the observation, the iguana only moved using its front limbs, with no movement at the pelvic limbs and tail. Despite the efforts, the animal could not be trapped for veterinary examination. Ninety days later the animal was found dead with severe injuries due to the bites and scratches caused by other animals; in addition, many ticks were observed on its body (Fig. 2). The ticks were collected, placed in 70% alcohol, and transported to the Parasitology Laboratory of the Veterinary School, Universidad Nacional de Costa Rica (VS-UNA-CR).

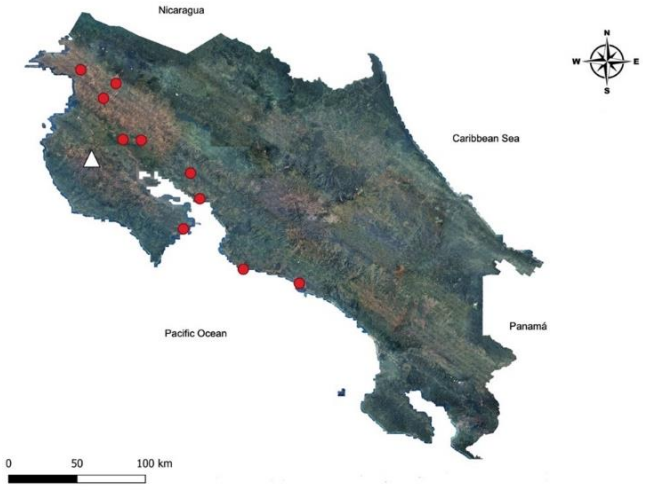

Figure 1. Distribution of *Amblyomma scutatum* in Costa Rica. Past collection sites in descending order: Santa Rosa, Hacienda la Norma, Liberia, Parque Nacional Palo Verde, Bebedero, Monteverde, Puntarenas, Curú, Jacó, Quepos (red circles), and current collection site: Diriá (white triangle).

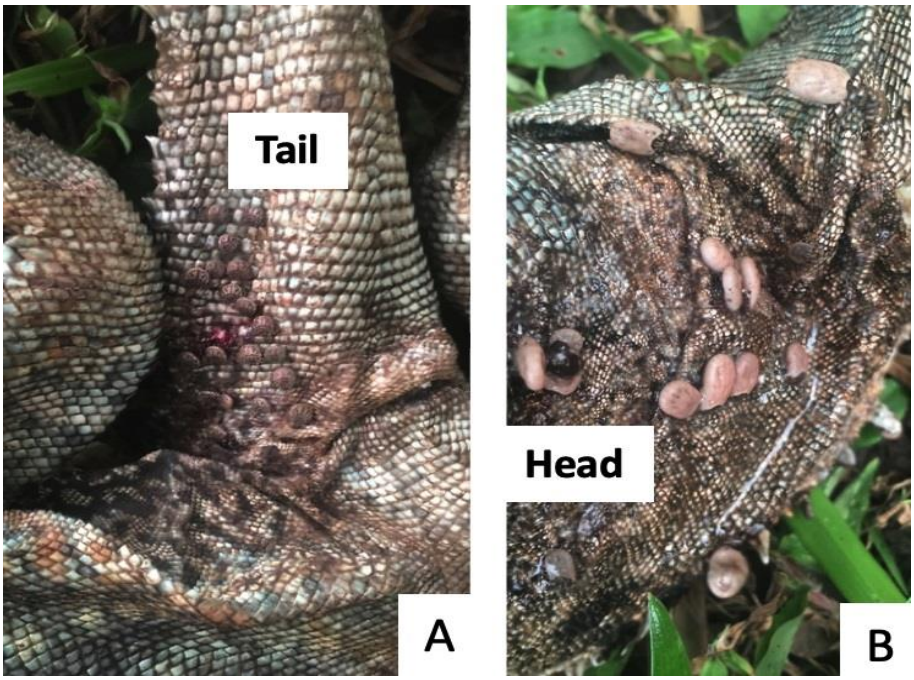

Figure 2. *Ctenosaura similis* parasitized by *Amblyomma scutatum*. **A.** Group of ticks at the ventral base of the tail, **B.** Partially and fully engorged females on the left side of the neck.

Morphological and molecular identification: The ticks was carried out using the taxonomic key of Guzmán-Cornejo et al. (2011), using a Nikon SMZ 800 stereo microscope and photographed with a 12 MP camera. DNA was extracted from three male and one female ticks using the Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer`s instructions, and molecular identification was performed through PCR amplification of a ~460 base pair (bp) fragment of the tick mitochondrial 16S rRNA gene using CCGGTCTGAACTCAGATCAAGT and GCTCAATGATTTTTTAAATTGCTGTGG sequences proposed by Norris et al. (1996). In addition, DNA from ticks were subjected to PCR assays targeting bacteria of the genera *Rickettsia*, *Borrelia* and the family Anaplasmataceae (Table 1). Amplifications were visualized in 1% agarose gels stained with GelRed™ Nucleic Acid Gel Stain (Biotium, 5 µg/ml). Positive samples were sent to Macrogen (Seoul, Korea) for sequencing. The sequences were edited and aligned using the Biological Sequence Alignment Editor (BioEdit version 7.2.5) (Hall 1999) and compared with sequences of the NCBI (National Center for Biotechnology Information) database using the BLASTn algorithm.

A total of 38 ticks (13 males and 25 females, out of which 22 engorged) were morphologically identified as *A. scutatum*, with few different morphological characteristics as compared to those reported by of Guzman-Cornejo et al. (2011). Previously, several authors have highlighted the morphological differences reported throughout the distribution of *A. scutatum* (Guglielmone et al., 2021). In our specimens, we found slight differences in the shape of the ornamentation of the scutum and size of the spurs of the coxa I (Fig. 3). According to some authors, these differences may be due to variations in the population or their distribution (see Guglielmone et al., 2021); however this fact should be better evaluated. In this sense, there are few sequences to compare this species, and include only the 16s rRNA gene, thus it is necessary to develop new analysis that allow a better morphological and molecular comparison that helps to define this species. In the present study the molecular analysis showed a 99.02% (403/407 bp) of similarity to *A. scutatum* from El Salvador (MW369633). The present partial sequence of 16S rRNA gene was deposited in GenkBank under the accession number GM OM691677, which correspond the first sequence of *A. scutatum* in Costa Rica. Voucher specimens of the ticks were deposited in VS-UNA-CR (PA-127-121). The DNA of *Rickettsia*, *Borrelia* or those of the Anaplasmataceae family could not be detected in our samples.

Although the lack of mobility of the *C. similis* female was not analyzed in this work, some hypotheses could be associated with lesions seen on the first day of observation of this animal, as such injury, pathology, climate, or a case of tick paralysis. This last assumption is supported by the literature, and in our case, the number of ticks found on the host. Tick paralysis is triggered by neurotoxins from the saliva of the ticks, particularly engorged females. Exposure to these neurotoxins can affect the host mobility and even cause the death of the hosts (Mans et al., 2004; Hanson et al., 2007). Because paralysis is associated with the presence of ticks on the host, the signs are reversible by removal of the ticks (Baeza, 1979; Hanson et al., 2007). This type of paralysis has been reported both in cold and warm-blooded animals, including humans (Dunn, 1918; Baeza, 1979; Mans, 2004; Hanson et al., 2007). Of the more than 70 species of ticks that have been associated with tick paralysis, *Amblyomma dissimile* Koch, 1844, *Amblyomma rotundatum* Koch, 1844, and *Robertsicus elaphensis* (Price 1959) (cit. as *Amblyomma elaphense*) have been implicated in paralysis in reptiles and amphibians in the Americas (Gothe and Neitz, 1991; Mans et al., 2004). Future studies are required to clarify the role of *A. scutatum* on *C. similis* in natural conditions.

Table 1. Details of the PCR protocols used for amplifying selected tick-borne agents.

Agent	Gene (PCR method)	Primers (Sequence 5'-3')	Fragment length (bp)	References
Anaplas-mataceae	16S rRNA (real time PCR)	**ECHSYBR-F** (AACACATGCAAGTCGAACGG) **ECHSYBR-R** (CCCCCGCAGGGATTATACA)	145-153	Li et al. (2001)
Borrelia spp.	Spacer region between the 5S and 23S rRNA (nested PCR)	**23SN1** (ACCATAGACTCTTATTACTTTGAC) **23SC1** (TAAGCTGACTAATACTAATTACCC) **23SN2** (ACCATAGACTCTTATTACTTTGACCA) **5SCB** (GAGAGTAGGTTATTGCCAGGG)	226	Rijpkema et al. (1995)
Rickettsia spp.	*gltA* (PCR)	**CS78** (GCAAGTATCGGTGAGGATGTAAT) **CS323** (GCTTCCTTAAAATTCAATAAATCAGGAT)	401	Labruna et al. (2004)

Figure 3. *Amblyomma scutatum* **A.** Dorsal view, female, **B.** Ventral view, female. **C.** Dorsal view, male, **D.** Ventral view, male.

Authors' contributions

Ana Jiménez-Rocha: Conceptualization, formal analysis, data curation (lead), writing (supporting); **Sergio Bermúdez Castillero:** Original draft, data curation (supporting), writing - review & editing (lead), visualization (lead). **Antony Solórzano-Morales:** Methodology (lead), formal analysis (supporting), software; **Ernesto Rojas**

Sánchez: Data curation (supporting), resources (supporting), visualization (supporting). **César Pérez:** Resources (supporting), investigation, methodology (supporting). **Gaby Dolz:** Project administration, resources (lead), supervision, validation. All authors participated in the writing of the last draft of the manuscript.

Statement of ethics approval

No ethical approval was necessary according to the Costa Rican laws.

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Conflict of interest

None.

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REFERENCES

Alvarez-Calderón, V., Hernández-Fonseca, V. and Hernández-Gamboa, J. 2005. Catálogo de garrapatas suaves (Acari: Argasidae) y duras (Acari: Ixodidae) de Costa Rica. Brenesia, 63 (64): 81-88. [In Spanish]

Baeza, CR. 1979. Tick paralysis-Canal Zone, Panama. Morbility and Mortality Weekly Reports, 14: 428-33.
doi: 10.2307/23298680

Dunn, L. 1918. Studies on the iguana tick, *Amblyomma dissimile*, in Panama. Journal of Parasitology, 5: 1-10.
doi: 10.2307/3271172

Gothe, R. and Neitz, A. 1991. Tick paralysis: pathogenesis and etiology, In: Advances in disease vector research, Vol. 8. Harris, K.F. (Ed.). Springer, New York, USA, 177-204.

Guglielmone, A.A., Nava, S. and Robbins, R.G. 2021. Neotropical hard ticks (Acari: Ixodida: Ixodidae). A critical analysis of their taxonomy, distribution, and host relationships. Springer, Switzerland, 486 pp.
doi: 10.1007/978-3-030-72353-8

Guzman-Cornejo, C., Robbins, R.G., Guglielmone, A.A., Montiel-Parra, G. and Pérez, T.M. 2011. The *Amblyomma* (Acari: Ixodida: Ixodidae) of Mexico: identification keys, distribution and hosts. Zootaxa, 2998 (1): 16-38.
doi:10.11646/zootaxa.2998.1.2

Hall, T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95-98.
doi: 10.14601/phytopathol_mediterr-14998u1.29

Hanson, B.A., Frank, P.A., Mertins, J. and Corn, J.L. 2007. Tick paralysis of a snake caused by *Amblyomma rotundatum* (Acari: Ixodidae). Journal of Medical Entomology, 44: 155-157.
doi: 10.1093/jmedent/41.5.155

Labruna, M.B., Whitworth, T., Horta, M.C., Bouyer, D.H., Mc Bride, J.W., Pinter, A., Popov, V., Gennari, S.M. and Walker, D.H. 2004. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an endemic area for Brazilian spotted fever in the state of São Paulo, Brazil, Journal of Clinical Microbiology, 42: 90-98.
doi: 10.1128/JCM.42.1.90-98.2004

Li, J.S.-Y, Yager, E., Reilly, M., Freeman, C., Reddy, G.R., Reilly, A.A., Chu, F.K. and Winslow, G.M. 2001. Outer membrane protein-specific monoclonal antibodies protect SCID mice from fatal infection by the obligate intracellular bacterial pathogen *Ehrlichia chaffeensis*. The Journal of Immunology, 166 (3): 1855-1862.
doi:10.4049/jimmunol.166.3.1855

Mans, B.J., Gothe, R. and Neitz, A.W.H. 2004. Biochemical perspectives on paralysis and other forms of toxicoses caused by ticks. Parasitology, 129: S95-S111.
doi: 10.1017/s0031182003004670

Norris, D.E., Klompen, J.S.H., Keirans, J.E. and Black IV, W.C. 1996. Population genetics of *Ixodes scapularis* (Acari: Ixodidae) based on mitochondrial 16 S and 12 S genes. Journal of Medical Entomology, 33: 78-89.
doi: 10.1093/jmedent/33.1.78

Rijpkema, S., Molkenboer, M., Schouls, L., Jongejan, F. and Schellekens, J. 1995. Simultaneous detection and genotyping of three genomic groups of *Borrelia burgdorferi* sensu lato in Dutch *Ixodes ricinus* ticks by characterization of the amplified intergenic spacer region between 5S and 23S rRNA genes. Journal of Clinical Microbiology, 33: 3091-3095.
doi: 10.1128/jcm.33.12.3091-3095.1995

Romero, L., Costa, F. and Labruna, M. 2021. Ticks and tick-borne *Rickettsia* in El Salvador. Experimental and Applied Acarology, 83 (4): 545-554.
doi:10.1007/s10493-021-00610-w

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