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Article

First report of *Amblyomma tapirellum* Dunn, 1933 (Ixodida: Ixodidae) in Costa Rica

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Abstract

A total of six *Amblyomma tapirellum* ticks were found for the first time in rainforest from the lowland to the southern Pacific of Costa Rica. Tick identification was carried out by morphology and afterward confirmed by molecular analysis, using polymerase chain reaction (PCR) and DNA barcoding. Further studies are required to determine the distribution of *A. tapirellum* and this species' potential as a vector of bacterial agents to humans and wild hosts.

Key words: *Amblyomma tapirellum*, lowland rainforest, Costa Rica

Introduction

Ticks are a group of hematophagous mites that parasitize all classes of terrestrial vertebrates (Labruna *et al.* 2005). They cause serious problems to animal husbandry and human health. To date, most studies of Neotropical ticks have been conducted in rural and urban environments, although most species can be found on wildlife. Consequently, species living in natural environments are little known (Szabó *et al.* 2003). This is particularly true in Central America, where only a few studies have reported the diversity of ticks in wild environments, with the result that our knowledge of this region's tick fauna is limited.

Amblyomma tapirellum Dunn, 1933 was first described from Panama and afterward reported from Belize, Nicaragua and Colombia (Dunn 1933, Fairchild *et al*. 1966, Varma, 1973). Adults of this species are considered parasites of ungulates, although they have been found parasitizing other mammals, such as Carnivora, Chiroptera, Pilosa, and Rodentia (Fairchild *et al*. 1966, Bermúdez *et al*. 2010, García *et al*. 2014, Bermúdez *et al*. 2015). In addition, there are reports of humans parasitized by adults of *A. tapirellum* (Fairchild *et al*. 1966, Bermúdez *et al*. 2012). In the case of the immature stages, only wild carnivores have been confirmed as hosts to date (Bermúdez *et al*. 2015), although other groups of mammals would appear to be suitable hosts. Many aspects of the biology and distribution of *A. tapirellum* are unknown. In this paper, we provide additional information concerning the identification and distribution of *A. tapirellum* and report its presence in the tropical rainforest of Costa Rica.

Materials and methods

From 2011 to 2012, we examined ticks deposited in the collections of Universidad Nacional de Costa Rica (UNA-CR) and Instituto Nacional de Biodiversidad de Costa Rica (INBio). Collected ticks were preserved in 70% alcohol, pending their morphological identification and molecular analysis. All specimens were identified using the taxonomic keys of Fairchild *et al*. (1966). Subsequently, to confirm their identity as *A. tapirellum*, specimens were examined for characteristics described by Dunn (1933), and compared with specimens deposited in the "Dr. Eustorgio Méndez" Zoological Collection of the Gorgas Memorial Institute for Health Studies, including one female of the Dunn´s Collections which is labeled as T-1. Voucher specimens were deposited in UNA-CR and INBio.

Material examined from UNA-CR (Fig.1): 1 ♂ Costa Rica, Puntarenas, Golfito, Golfo Dulce, Parque Nacional Corcovado, sendero Río Claro. 10 m. January 6, 2012. V.M. Montenegro, R. Quesada.Collected by blanket dragging in secondary forest. 8°28'54.4" N, 83°35'31.3" W. 1♀ Costa Rica, Puntarenas, Golfito, Golfo Dulce, Parque Nacional Corcovado, Sendero Sirena-Cruce-Guanacaste, 20 m. August 4, 2012. V.M. Montenegro, R. Quesada. Collected by blanket dragging in secondary forest. 8°29'08.37" N, 83°35'32.94" W. 1♀ Costa Rica, Puntarenas, Golfito, Golfo Dulce, Parque Nacional Corcovado, sendero Espaveles, 40 m. May 3, 2012. V.M. Montenegro, R. Quesada. Collected by blanket dragging in primary forest. 8°28'55,76" N, 83°35'18.05" W.1♀ Costa Rica, Puntarenas, Golfito, Golfo Dulce, Parque Nacional Corcovado, Sendero Culebra, 10 m. May 4 2012. V.M. Montenegro, R. Quesada. Collected by blanket dragging, in primary forest. 8°29'13.35" N, 83°34'51.84"W.

Revised material from INBIO (Fig. 1): 1 ♀ Costa Rica, Puntarenas, Golfito, Golfo Dulce, Parque Nacional Corcovado, Est. (Estación) Agujas, 300 m. January 12, 2012. J. A. Azofeifa, D. Azofeifa. Collected on bromeliads. 8°32'11.79" N, -83°25'31.8" W #103426. 1 ♂ Costa Rica, Puntarenas, Humedal Sierpe, 100 m (Fig. 1). January 5, 2011. Collected on vegetation. 8°51'57.8" N, -84°11'43.6" W.

In order to confirm the morphological identification, DNA from four specimens Corcovado National Park (UNA-CR) was extracted using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer´s instructions. These samples were amplified by polymerase chain reaction (PCR) with the primers LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' described by Folmer *et al*. (1994), to obtain a 658-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Conditions used for this reaction were reported by Hebert *et al.* (2003). Amplified fragments were sent for purification and sequencing to Macrogen (Seoul, Korea). Sequences were edited using the Biological Sequence Alignment Editor (BioEdit version 7.2.5) (Hall, 1999), aligned with the Clustal W algorithm (Thompson *et al.* 1994), and compared with sequences of the NCBI (National Center for Biotechnology Information) database using the BLASTn algorithm (Altschul *et al*. 1990). Subsequently, a phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis software (MEGA version 5) (Tamura *et al*. 2011) by the Neighbor-Joining method (Saitou & Nei, 1987), and corrected with p-distance, yielding a substitution model to analyze evolutionary divergence between species (Nei & Kumar, 2000; Srivathsan & Meier, 2012). COI gene sequences from other *Amblyomma* species, available in GenBank, were included in the analysis: *Amblyomma auricularium* (KF200137), *Amblyomma calcaratum* (KF200144), *Amblyomma dissimile* (KF200168), *Amblyomma geayi* (KF200159), *Amblyomma longirostre* (KF200095), *Amblyomma nodosum* (KF200131), *Amblyomma oblongoguttatum* (KF200165), *Amblyomma ovale* (KF200158), *Amblyomma pecarium* (KF200153), *Amblyomma sabanerae* (KF200152), *A. tapirellum* (KF200171 and KF370891), *Amblyomma varium* (KF200157) and *Ixodes affinis* (KF200161) as an outgroup.

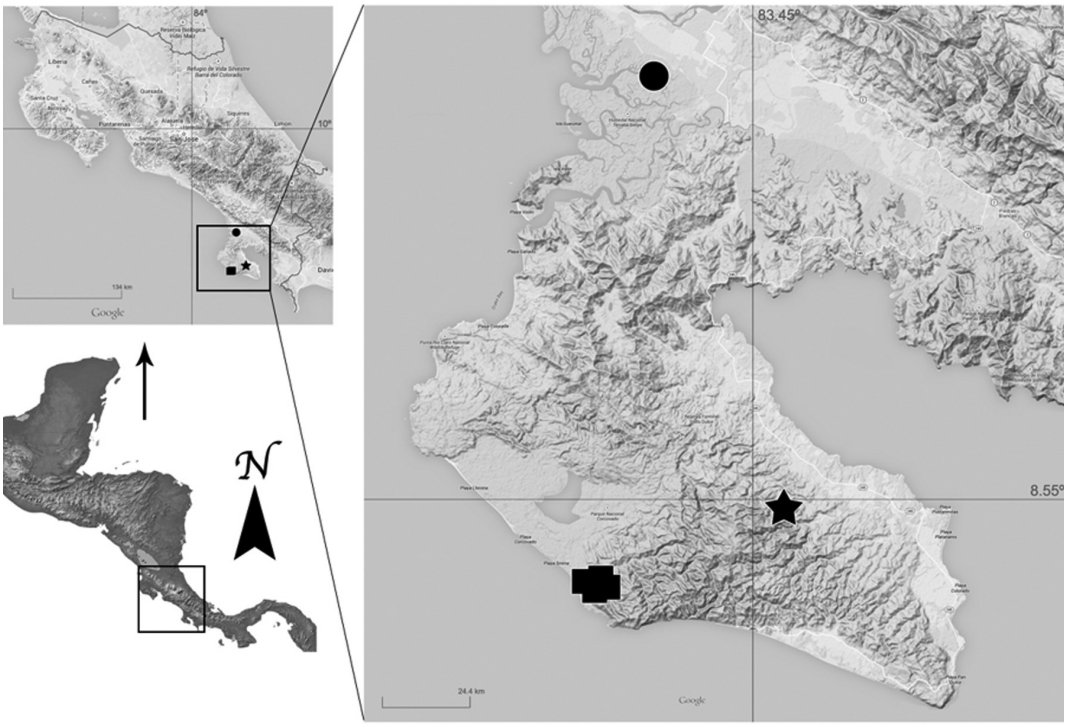

FIGURE 1. Distribution of *Amblyomma tapirellum* in Costa Rica. Circle: Humedal Sierpe (Sierpe wetland), Star: Estación Agujas (Agujas station), Squares: Sendero Río Claro (Río Claro trail), Sendero Sirena-Cruce-Guanacaste (Sirena-Cruce Guanacaste trail), Sendero Espaveles (Espaveles trail), Sendero Culebra (Culebra trail).

Results and discussion

The morphological identification (Fig. 2) was consistent with *A. tapirellum* for all specimens examined. The partial region of the COI gene of the four UNA-CR tick samples was sequenced and included in GenBank (accession numbers: KP247501, KP247502, KP247503, KP247504); they shared between 99.6% and 100% of nucleotide identity, 99.8% to 100% when compared with sequences of *A. tapirellum* from Panama (GenBank accession numbers KF200120 and KF370891). All *A. tapirellum* sequences were grouped into the same cluster (Fig. 3). The species *A. oblongoguttatum* (accession number KF200165) shared the greatest sequence similarity (84.7% to 84.9%) with the *A. tapirellum* group.

This is the first report of *A. tapirellum* from Costa Rica and increases to 24 the number of *Amblyomma* species known from this country (Álvarez *et al*. 2005). However, the presence of *A. tapirellum* was not unexpected, since it was previously reported in Nicaragua (Fairchild *et al*. 1966) and has been described from Chiriquí, Panama (Fairchild *et al*. 1966), a city relatively close to the Costa Rican province of Puntarenas. In Costa Rica, *Amblyomma mixtum* and *A. oblongoguttatum* share similar patterns of scutal ornamentation with *A. tapirellum*, but they can be differentiated by characters discussed in Dunn (1933) and Fairchild *et al*. (1966). Ecologically, there are also differences between these species. *Amblyomma mixtum* is associated with open habitats and chiefly parasitizes cows and horses (Alvarez *et al*. 2000, Alvarez & Bonilla, 2007), while *A. oblongoguttatum* seems to inhabit both open environments and wooded areas and is not specific to any host group (Voltzit, 2007, Dolz, 2014). In contrast, *A. tapirellum* is usually found in less

disturbed environments and the tapir (*Tapirus bairdii* Gill, 1865) is its main host (Fairchild *et al*. 1966).

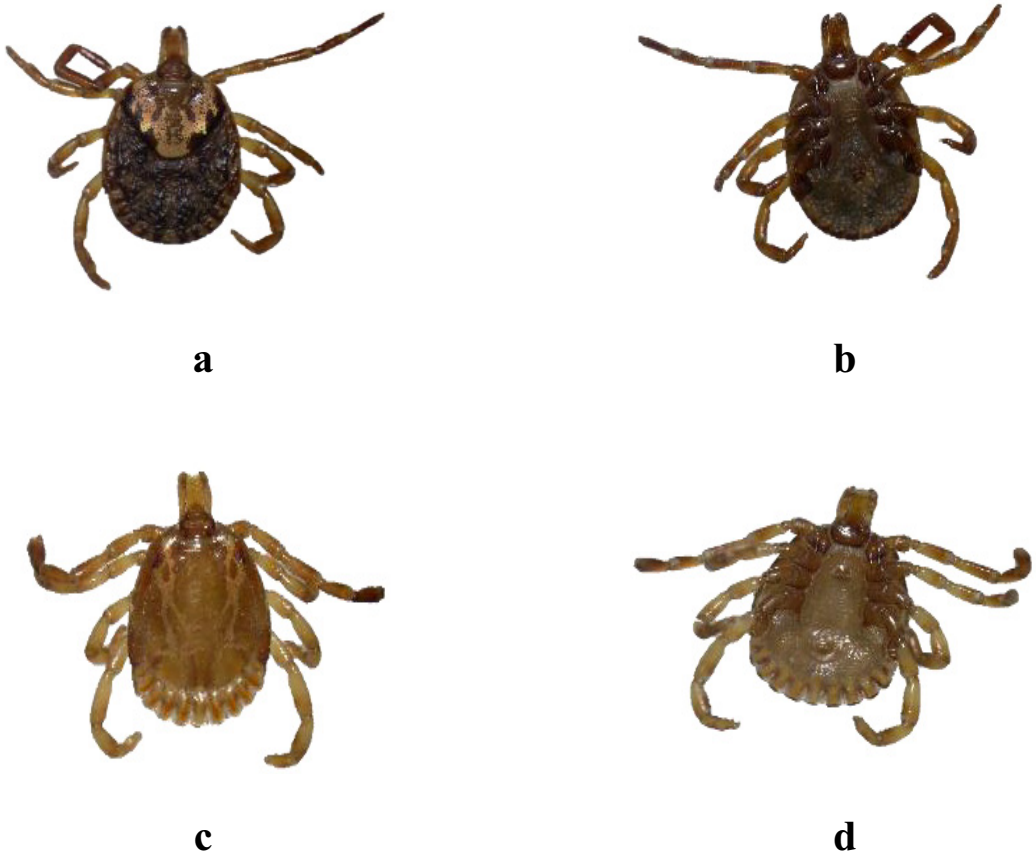

FIGURE 2: General images of *Amblyomma tapirellum*. Female: a. dorsal, b. ventral. Male: c. dorsal, d. ventral.

In Costa Rica, studies of tick ecology have been largely carried out in urban and rural areas, so there is little data of the ecology of ticks associated with wildlife (Alvarez *et al*. 2005, Troyo *et al*. 2012, Jiménez *et al*. 2013, Dolz *et al*. 2013, Troyo *et al*. 2014). It is possible that additional research in forests will confirm the presence of other tick species in Costa Rica.

Four specimens of *A. tapirellum* were found in the forests of Corcovado National Park. Various potential hosts, such as peccaries and tapirs, have been reported in this park, which probably means that *A. tapirellum* is established there (Almeida *et al*. 2009, Altrichter & Almeida, 2009). However, collared peccaries (*Tayassu tajacu*, Linnaeus, 1978), white-lipped peccaries (*Tayassu pecari* Link, 1795), and Baird´s tapir (*Tapirus bairdii* Gill, 1865) are all suffering illegal hunting pressure, and their numbers have declined in several parts of Corcovado National Park (Carrillo & Sáenz, 2011; Bustamante *et al*. 2013). For this reason, ecological studies are needed to determine which vertebrates are crucial to the life cycle of *A. tapirellum*, especially its immature stages.

Finally, records of *A. tapirellum* feeding on humans (Fairchild *et al*. 1966, Bermúdez *et al*. 2012) could represent a risk to people, who frequent the trails in Corcovado National Park, if populations of the principal natural hosts decrease because of poaching. We recommend further research on the ecology of this tick, especially investigations of pathogens that can be transmitted by *A. tapirellum* and that may affect humans and wild animals.

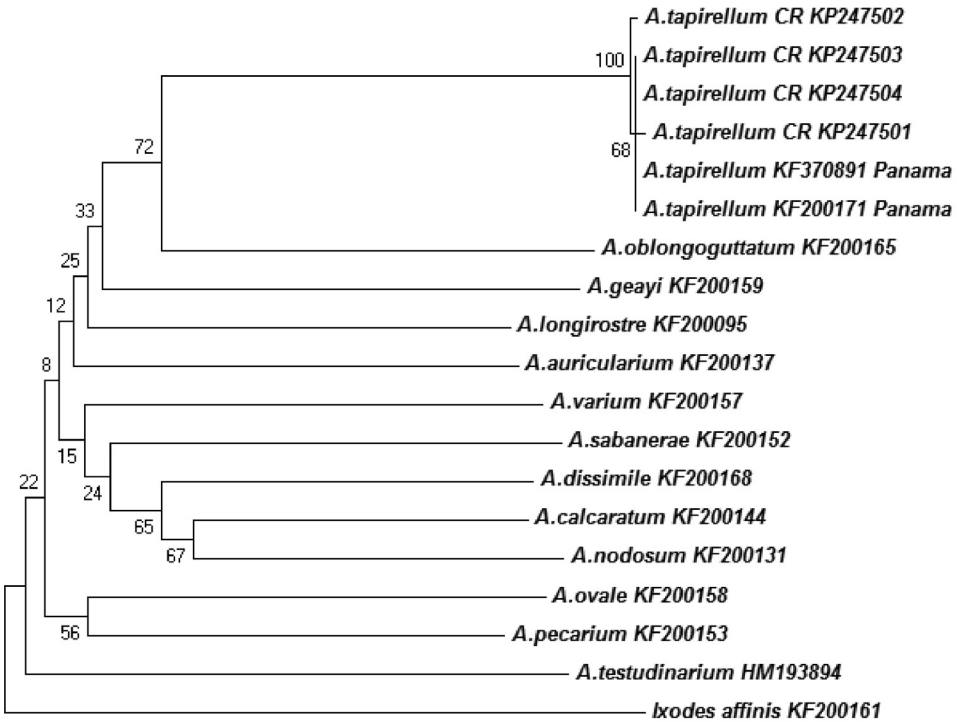

FIGURE 3. Neighbor-joining tree based on partial sequences of COI gen (~630 pb) of some *Amblyomma* species. Horizontal line length is proportional to the number of nucleotide differences of each branch. Bootstrap values for 500 replicates were applied and *Ixodes affinis* was used as the outgroup.

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