



OBSERVATION ARTICLE

REVISED *Amblyomma tapirellum* (Acari: Ixodidae) collected from tropical forest canopy [v2; ref status: indexed, <http://f1000r.es/2uy>]

Jose R Loaiza^{1,2}, Matthew J Miller³, Eldredge Bermingham³, Oris I Sanjur³, Patrick A Jansen³, Jose R Rovira³, Eric Alvarez³, Eric Rodriguez³, Philip Davis³, Larissa C Dutari^{2,4}, James Pecor⁵, Desmond Foley⁵, Meghan Radtke⁶, Montira J Pongsiri⁶

¹Programa Centroamericano de Maestría en Entomología, Universidad de Panamá, Ciudad de Panamá, Panama

²Centro de Biodiversidad y Descubrimiento de Drogas, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología, Ciudad de Panamá, Panama

³Smithsonian Tropical Research Institute, Panama City, Panama

⁴Department of Biotechnology, Acharya Nagarjuna University, Guntur, India

⁵Walter Reed Biosystematics Unit, Smithsonian Institution, Suitland MD, USA

⁶United States Environmental Protection Agency, Washington DC, USA

v2 First published: 23 Sep 2013, 2:194 (doi: [10.12688/f1000research.2-194.v1](https://doi.org/10.12688/f1000research.2-194.v1))
 Latest published: 28 Jan 2014, 2:194 (doi: [10.12688/f1000research.2-194.v2](https://doi.org/10.12688/f1000research.2-194.v2))

Abstract

Free-ranging ticks are widely known to be restricted to the ground level of vegetation. Here, we document the capture of the tick species *Amblyomma tapirellum* in light traps placed in the forest canopy of Barro Colorado Island, central Panama. A total of forty eight adults and three nymphs were removed from carbon dioxide–octenol baited CDC light traps suspended 20 meters above the ground during surveys for forest canopy mosquitoes. To our knowledge, this represents the first report of questing ticks from the canopy of tropical forests. Our finding suggests a novel ecological relationship between *A. tapirellum* and arboreal mammals, perhaps monkeys that come to the ground to drink or to feed on fallen fruits.

Article Status Summary

Referee Responses

Referees	1	2	3
v1 published 23 Sep 2013	 report 1	 report 1	 report 1
v2 published 28 Jan 2014 REVISED			 report 1

- 1 Brian Allan**, University of Illinois USA, **Erin Welsh**, University of Illinois Urbana-Champaign USA
- 2 Michael Levin**, Centers for Disease Control and Prevention USA
- 3 Matias Pablo Juan Szabó**, Federal University of Uberlândia Brazil

Latest Comments

No Comments Yet

Corresponding author: Jose R Loaiza (jloaiza@indicat.org.pa)

How to cite this article: Loaiza JR, Miller MJ, Bermingham E *et al.* (2014) ***Amblyomma tapirellum* (Acari: Ixodidae) collected from tropical forest canopy [v2; ref status: indexed, <http://f1000r.es/2uy>]** *F1000Research* 2014, 2:194 (doi: [10.12688/f1000research.2-194.v2](https://doi.org/10.12688/f1000research.2-194.v2))

Copyright: © 2014 Loaiza JR *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution Licence](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

Grant information: Financial support was provided by Smithsonian Tropical Research Institute – Environmental Protection Agency grant DW33-92296801-0 “Mosquito Species Diversity and Landscape Change” to MJM and OIS; and the Secretariat for Science, Technology, and Innovation of Panama (SENACYT) – National Research Investigator award (SNI) granted to JRL. Funds for DNA barcoding came from an award to MJM, JRL, OIS and EB from the Smithsonian Institution’s Consortium for the Barcode of Life (2011 COB award: “DNA barcodes of arthropod disease vectors in Panama” grant FED/CBOL-2011010014-009).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: No competing interests were disclosed.

First published: 23 Sep 2013, 2:194 (doi: [10.12688/f1000research.2-194.v1](https://doi.org/10.12688/f1000research.2-194.v1))

First indexed: 28 Feb 2014, 2:194 (doi: [10.12688/f1000research.2-194.v2](https://doi.org/10.12688/f1000research.2-194.v2))

REVISED Amendments from Version 1

We are grateful to the three reviewers who thoughtfully provided comments to our article. We believe that their comments are valuable and helpful for improving our manuscript. Please find detailed responses below; especially, we want to highlight that we have added additional detailed information about how CDC light traps (CDC-LTs) were installed, sampled, and re-installed following sampling on a daily basis. We believe these changes have improved our article and will also avoid reaching erroneous conclusions.

We want to emphasize that our primary aim with this article was to begin a discussion about whether or not ground-dwelling tick species could make it all the way up to the Neotropical forest canopy, and under what circumstances this phenomenon could occur. We are aware that there is a lot of speculation in our article, yet it is the product of what we observed in the field while conducting this study. In fact, speculation was the reason why we decided to submit this work under the *Observation Article* category rather than as a *Research Article* and, therefore we wanted to set the stage for other people to think about it, and perhaps, conduct more formal research on our observation

See referee reports

Introduction

Increasing interest in tick-borne diseases in the Neotropics and particularly in Panama during the last decade has fuelled studies on tick biology, behavior and distribution in this region¹⁻³. These studies have focused on tick species associated with humans and domesticated animals, likely due to their role as vectors of disease agents^{1,2,4}. However, basic knowledge about tick natural history still remains largely unexplored, especially for those taxa that thrive in tropical forests. The tick species *Amblyomma tapirellum*⁵ predominates over *Amblyomma cajennense* as the primary human tick parasite in lowland forest ecosystems of central Panama and Darien⁶.

Adults of *A. tapirellum* have Baird's Tapir (*Tapirus bairdii*) as their primary host, but also opportunistically feed on other wildlife and domesticated mammals^{6,7}, and also humans (Table 1). *A. tapirellum* is one of the most common species collected with a cloth dragged through the understory vegetation, but it is not known to be found in arboreal mammals (Table 1), and in addition, a recent survey of tick occurrence on Panamanian birds found no evidence that this species feeds on birds (Miller *et al.*, *in prep.*). Here, we report *A. tapirellum* collected from mosquito light traps placed in the canopy of old-growth lowland tropical forest on Barro Colorado Island (BCI) in central Panama. To our knowledge, this is the first report of ticks being collected in the canopy of Neotropical forests and highlights the potentially complex ecological relationships of Neotropical ticks, which as a group, are potential vectors of zoonotic diseases in undisturbed forest habitats.

Methods

Centers for Disease Control and Prevention miniature light traps (CDC-LTs) baited with CO₂ (dry-ice) and 1-octen-3-ol were placed in areas of old-growth forest on BCI, in the Panama Canal (9.16457 N; -79.86347 W), which has served as a field station for studies of Neotropical flora and fauna for over 100 years. Six traps were placed in the forest canopy (20–30 meters off the ground) and six in the understory (1.5 meters off the ground) for seven consecutive days, every other month, from August 2009 to July 2010 (Figure 1).

When our field team first discovered the presence of ticks on the outside of CDC-LTs, recognizing that this was a novel occurrence, we carefully reviewed and modified our field protocol to ensure that our observance of host-seeking ticks in the forest canopy was not an artifact of our field methods. To wit, each morning when the traps were lowered, field members, including the senior author, first checked carefully for the presence of ticks on the exterior of each

Table 1. Reported hosts for *Amblyomma tapirellum* (Dunn, 1933) in Panama.

Order	Family	Species	References
Artiodactyla	Bovidae	<i>Bos taurus</i> Linnaeus 1758	Fairchild <i>et al.</i> 1966
Chiroptera	Phyllostomidae	<i>Carollia perspicillata</i> Linnaeus 1758*	Fairchild <i>et al.</i> 1966
Perissodactyla	Equidae	<i>Equus caballus</i> Linnaeus 1758	Fairchild 1943
Perissodactyla	Equidae	<i>Equus caballus</i>	Fairchild <i>et al.</i> 1966
Carnivora	Felidae	<i>Felis silvestris catus</i> Linnaeus 1758	Bermúdez <i>et al.</i> 2010
Primates	Hominidae	<i>Homo sapiens</i> Linnaeus 1758	Fairchild 1943
Primates	Hominidae	<i>Homo sapiens</i>	Fairchild <i>et al.</i> 1966
Primates	Hominidae	<i>Homo sapiens</i>	Bermúdez <i>et al.</i> 2012
Pilosa	Myrmecophagidae	<i>Myrmecophaga tridactyla</i> Linnaeus 1758	Fairchild <i>et al.</i> 1966
Artiodactyla	Cervidae	<i>Odocoileus virginianus</i> Zimmermann 1780	Bermúdez <i>et al.</i> 2010
Artiodactyla	Tayassuidae	<i>Pecari tajacu</i> Linnaeus 1758	Fairchild 1943
Artiodactyla	Tayassuidae	<i>Pecari tajacu</i>	Fairchild <i>et al.</i> 1966
Perissodactyla	Tapiridae	<i>Tapirus bairdii</i> Gill 1865	Fairchild 1943
Perissodactyla	Tapiridae	<i>Tapirus bairdii</i>	Fairchild <i>et al.</i> 1966
Perissodactyla	Tapiridae	<i>Tapirus bairdii</i>	Bermúdez <i>et al.</i> 2010

* This record is doubtful as the sample could have been pulled from the body of the collector

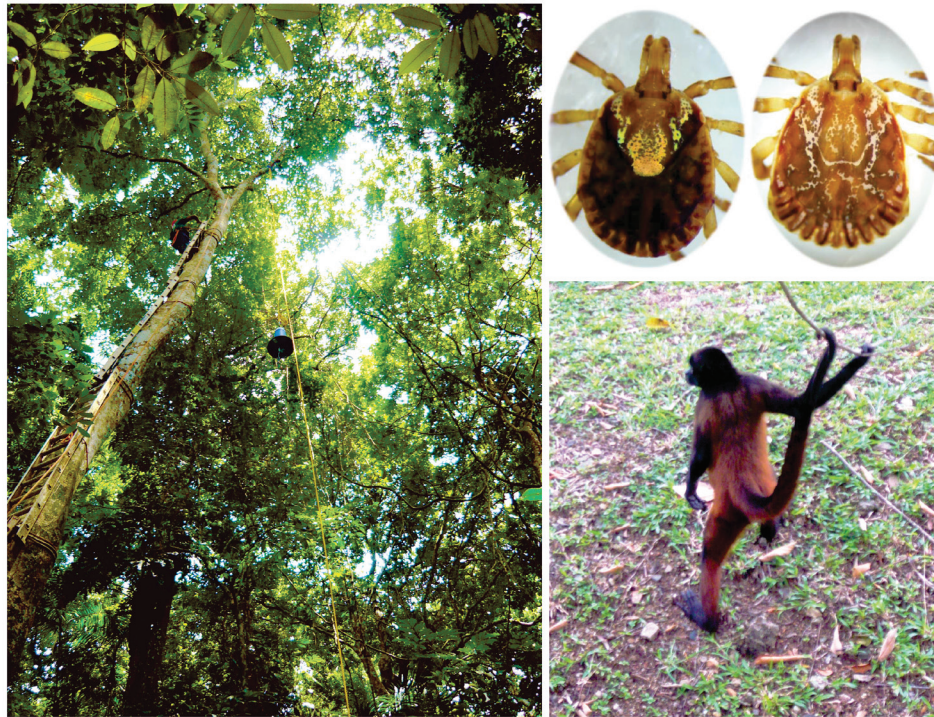


Figure 1. Left side: the set up of one Centers for Disease Control and Prevention miniature light trap in the forest canopy of Barro Colorado Island (BCI). Right upper side: the dorsal view of the scutum of one female (left) and one male (right) of *Amblyomma tapirellum* collected from the forest canopy at BCI. Right lower side: image of an adult of *Ateles geoffroyi panamensis* (Black Spider Monkey) walking freely around the Smithsonian Tropical Research Institute field station at BCI.

CDC-LT. This was done while the trap was suspended. Any ticks were immediately removed, placed in ethanol and labeled with appropriate metadata (date, trap number, etc.). Subsequently, the netting containing mosquitoes was secured in a plastic box for processing in the indoor laboratory space of BCI. The umbrella and the cylinder containing the fan mechanism of each trap were also taken back to the lab, but the igloo cooler was sealed in a white garbage bag and re-suspended in the mid-canopy (free from by-passers and foliage) during the day. In the evening, CDC-LTs were carried pre-assembled in Rubbermaid-style plastic boxes to the field and were quickly re-assembled in each field site, with loading of the solid CO₂ as the final step. At no time were either canopy or understory CDC-LTs placed on the ground while they were being serviced in the field. Ticks were counted by trap and preserved as vouchers as part of the ectoparasite - cryological collection of the Smithsonian Tropical Research Institute (STRI).

Observation

Forty eight adults and three nymphs of *A. tapirellum* were collected from CDC-LTs placed in the forest canopy at BCI (Table 2). All adults were identified using standard taxonomic keys⁶, while all three nymphs and one adult male and one adult female were confirmed as belonging to *A. tapirellum* based on a neighbor-joining tree generated from reference library of mitochondrial DNA barcoding (COI gene) sequences from Panamanian ticks (Miller *et al.*, *in prep.*). We selected four individuals of *A. geayi*, one of *A. longirostre*, five of

A. cajennense, three of *A. oblongoguttatum*, three of *A. tapirellum*, and two of *Haemaphysalis juxtakochi* (as an outgroup) to build the tree in MEGA4⁸ with group support evaluated via 500 bootstrap replicates (Figure 2). Mean Kimura 2 parameter (K2P) genetic distance between all five canopy collected ticks and the reference library specimens of *A. tapirellum* was 0.1% (maximum K2P distance 0.6%), well below the typical 2% threshold for interspecific distances for most barcoding studies⁹. Specimen data, sequences, and sequencing trace files for the five canopy-collected ticks and the 13 reference specimens are archived in the BOLD barcoding database (dx.doi.org/10.5883/DS-TICKSCAN) and are available on the online global database of DNA barcode sequences (<http://boldsystems.org>). Genbank accession numbers for the five canopy ticks generated in this study are: KF370887–KF370891, whereas the Genbank numbers for the adult reference library are: KF200081, KF200091, KF200097, KF200098, KF200101, KF200103, KF200105, KF200119, KF200124, KF200130, KF200133, KF200135, KF200159, KF200160, KF200171.

Interestingly, ticks were only extracted from CDC-LTs set up at the canopy level, no ticks were collected from traps at the understory, and only a few canopy traps were positive for ticks (Table 2). Our findings are unexpected because CDC-LTs are not commonly used to collect ticks, but rather blood-sucking insects such as mosquitoes, sand flies and biting midges. However, they reinforce the notion that ticks use CO₂ to locate their hosts¹⁰. Other *Amblyomma*

Table 2. Samples of *Amblyomma tapirellum* extracted from CO₂ – octenol baited Centers for Disease Control and Prevention (CDC) miniature light traps placed in the forest canopy of BCI, central Panama. Each row contains information about the number of specimens collected in one trap during one night. The number of tick positive CDC-LTs out of the total number of canopy traps per month are as following: August (5/42 = 0.119), October (4/42 = 0.095), January (1/42 = 0.023), March (1/42 = 0.023), May (18/42 = 0.418), and July (1/42 = 0.02).

Number of ticks	Life stage and sex	Collection date
2	1 male, 1 female	August, 2009
1	Female	August, 2009
2	1 male, 1 female	August, 2009
1	Male	August, 2009
1	Male	August, 2009
2	1 male, 1 female	October, 2009
1	Female	October, 2009
1	Female	October, 2009
2	Females	October, 2009
1	Female	January, 2010
2	1 male, 1 female	March, 2010
2	1 male, 1 female	May, 2010
2	1 male, 1 female	May, 2010
2	Females	May, 2010
1	Male	May, 2010
2	Females	May, 2010
1	Male	May, 2010
3	1 male, 1 female, 1 nymph	May, 2010
3	2 males, 1 female	May, 2010
2	2 nymphs	May, 2010
1	Female	May, 2010
2	1 male, 1 female	May, 2010
2	1 male, 1 female	May, 2010
2	1 male, 1 female	May, 2010
3	2 males, 1 female	May, 2010
2	1 male, 1 female	May, 2010
1	Female	May, 2010
1	Male	May, 2010
2	1 male, 1 female	May, 2010
1	Female	July, 2010

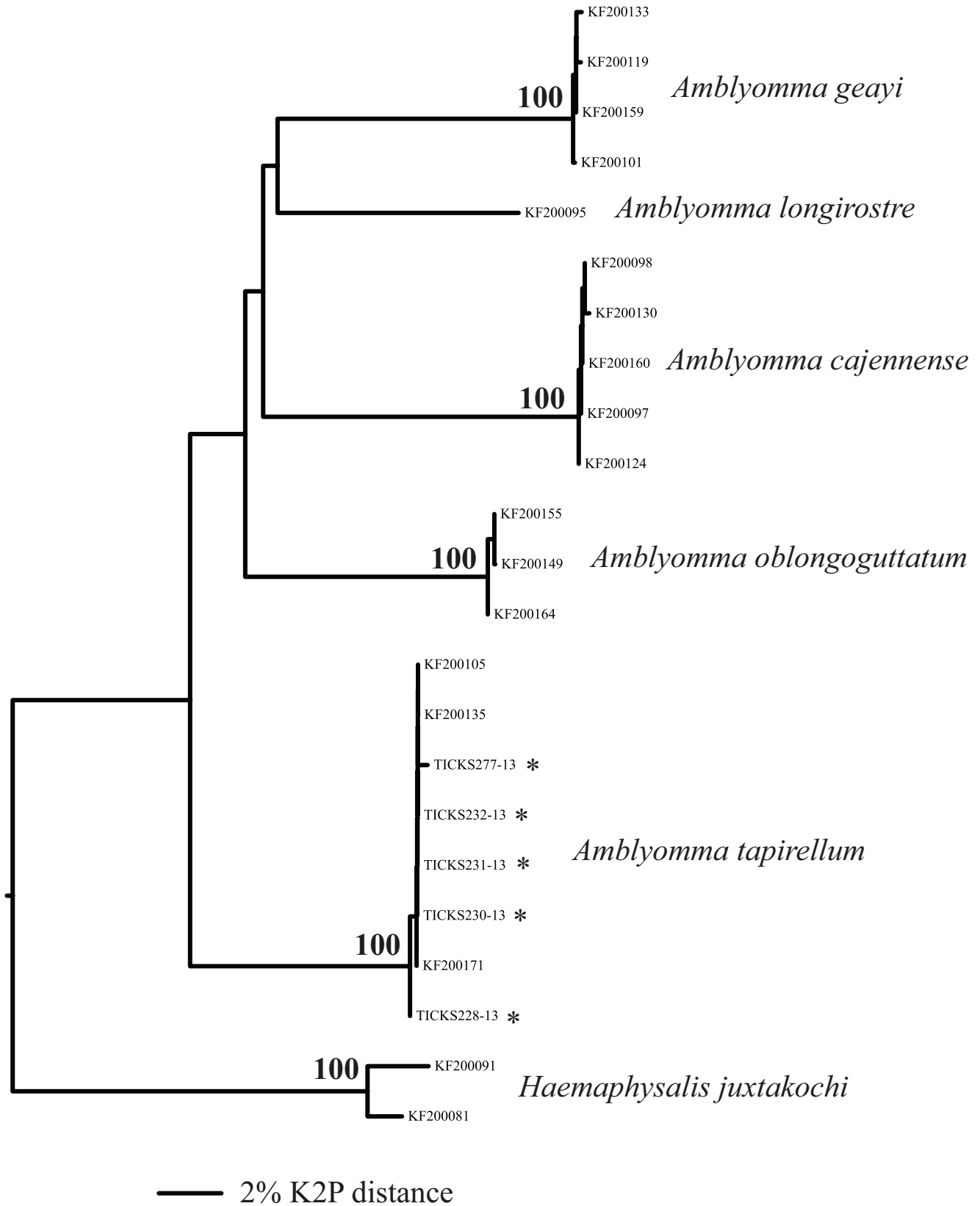


Figure 2. Neighbor-joining tree generated in MEGA 4. Node support (as a percentage) was estimated from 500 bootstrap replicates. Taxa indicated with asterisks (*) represent canopy collected ticks from this study; otherwise tip labels refer to Genbank accession numbers.

species have been previously collected with CO₂ baited traps¹¹, but no study has ever reported host-seeking ticks collected in this fashion from the canopy of a tropical forest. This finding indicates that *A. tapirellum* is not restricted to the ground, but uses both vertical strata (e.g., canopy and ground) to seek hosts. The fact that adults of both sexes as well as nymphs were recovered from canopy traps suggests that *A. tapirellum* can complete its life cycle in the canopy, but this is most likely the result of foresia – the passive movement of one organism by another – by hosts moving vertically. Candidate vectors for movement into the canopy include two monkey species: Mantled Howler Monkey (*Alouatta palliata*) and Black Spider Monkey (*Ateles geoffroyi panamensis*). These two monkey species were often seen near CDC-LTs, and on one occasion, destroyed a trap (Figure 1). Yet, at present there are no records of *A. tapirellum* collected from these monkeys or any other arboreal mammals in Panama (Table 1). In addition, the majority of ticks were collected at the beginning of the dry-wet transition period in May 2010 (Table 2), when ground populations of *A. tapirellum* are quite abundant and monkeys may come to the ground to feed on ripe and over-ripe fruits¹². This possibility suggests that an association between arboreal monkeys and ticks is opportunistic, perhaps occurring principally at the peak of the fruiting season¹³. However, ticks were also collected during August and October of 2009, and so, tick-monkey ground interactions could also be the result of monkey behaviors such as drinking from terrestrial sources or chasing games¹⁴. However, we cannot be sure that monkeys are responsible for transporting *A. tapirellum* into the canopy, nor can we explain why ticks were only found on canopy traps and not understory traps; additional studies will be required. Fairchild and collaborators⁶ noted that *A. geayi* and *A. varium* are practically confined to arboreal sloths. Sloths descend to the ground every three to eight days, dig a hole, defecate, and climb back up into the trees, a behavior that puts the animal at risk if predators are nearby¹⁵, and it may also increase the odds of getting ground ticks. Our findings highlight the lack of information on the basic ecology of some species of Neotropical ticks, and argue for an expanded vision of wildlife-tick relationships when planning and conducting disease ecology studies in the Neotropics. Future Neotropical tick surveys in forest areas should include canopy sampling to better understand the bionomics of *A. tapirellum* and its role in pathogen transmission to wildlife.

Author contributions

JRL, MJM, EB, OIS, JP, and MJP conceived the study. JRL, JP, and MJP designed the project.

JRL, JRR, EA, ER, PD, LCD, JP, DF and MR carried out the research. MJM, PAJ, and DF contributed to the design of experiments and provided expertise in the field. JRL, MJM, PAJ, and DF prepared the first draft of the manuscript. EB, OIS, JP, MR, and MJP contributed to the experimental design and preparation of the manuscript. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

Competing interests

No competing interests were disclosed.

Grant information

Financial support was provided by Smithsonian Tropical Research Institute – Environmental Protection Agency grant DW33-92296801-0 “Mosquito Species Diversity and Landscape Change” to MJP and OIS; and the Secretariat for Science, Technology, and Innovation of Panama (SENACYT) – National Research Investigator award (SNI) granted to JRL. Funds for DNA barcoding came from an award to MJM, JRL, OIS and EB from the Smithsonian Institution’s Consortium for the Barcode of Life (2011 COB award: “DNA barcodes of arthropod disease vectors in Panama” grant FED/CBOL-2011010014-009).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We are grateful to Oris Acevedo, Belkis Jimenez and Hilda Castañeda for logistical support at BCI; Celestino Aguilar for providing the DNA barcoding labwork; Eyda Gomez for helping managing the project logistics and for technical guidance; Mauricio Quintero, Anel J. Duncan, Denis Lezcano, Gaspar Ho and Apolonio Valdez for field assistance. The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the supporting agencies.

References

- Bermúdez SE, Miranda R: **Distribution of ectoparasites of *Canis lupus familiaris* L. (Carnivora: Canidae) from Panama.** *Revista MVZ Córdoba.* 2011; **16**: 2274–2282.
[Reference Source](#)
- Bermúdez C SE, Castro A, Esser H, et al.: **Ticks (Ixodida) on humans from central Panama, Panama (2010–2011).** *Exp Appl Acarol.* 2012; **58**(1): 81–88.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Murgas IL, Castro AM, Bermúdez SE: **Current status of *Amblyomma ovale* (Acari: Ixodidae) in Panama.** *Ticks Tick Borne Dis.* 2013; **4**(1–2): 164–166.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Bermúdez SE, Ereemeeva ME, Karpathy SE, et al.: **Detection and identification of rickettsial agents in ticks from domestic mammals in eastern Panama.** *J Med Entomol.* 2009; **46**(4): 856–861.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Dunn LH: **Two New Species of Ticks from Panama (*Amblyomma tapirellum* and *A. pecaricum*).** *Parasitology.* 1933; **25**(3): 353–358.
[Publisher Full Text](#)
- Fairchild G, Kohls G, Tipton V: **The Ticks of Panama (Acarina: Ixodidae).** In: Wenzel R.L., Tipton V.J. (Eds.), *Ectoparasites of Panama.* Field Museum of Natural History Chicago, Illinois, 1966; pp. 167–219.
[Reference Source](#)
- Fairchild GB: **An annotated list of bloodsucking insects, ticks and mites known from Panama.** *Am J Trop Med Hygiene.* 1943; **23**(8): 569–591.
[Reference Source](#)
- Kumar S, Tamura K, Nei M: **MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment.** *Brief Bioinform.* 2004; **5**(2): 150–163.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hebert PD, Cywinska A, Ball SL, et al.: **Biological identifications through DNA barcodes.** *Proc Biol Sci.* 2003; **270**(1512): 313–321.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sonenshine DE: **Pheromones and other semiochemicals of ticks and their use in tick control.** *Parasitology.* 2004; **129**(suppl): S405–S425.
[PubMed Abstract](#) | [Publisher Full Text](#)

11. Kesinger BJ, Allan BF: **Efficacy of dry ice-baited traps for sampling *Amblyomma americanum* (Acari: Ixodidae) varies with life stage but not habitat.** *J Med Entomol.* 2011; **48**(3): 708–711.
[PubMed Abstract](#) | [Publisher Full Text](#)
12. Leigh EG: **Tropical Forest Ecology: A View from Barro Colorado Island.** Oxford and New York: Oxford University Press. ISBN 0–19–509602–9/OCLC 3678102. 1999.
[Reference Source](#)
13. Foster RB: **The seasonal rhythm of fruitfall on Barro Colorado Island.** In Leigh, E.G., Ran A.S. & Windsor, D. (eds). *The ecology of a tropical forest: seasonal rhythms and long-term changes.* Smithsonian Institution Press, Washington. 1982; 151–172.
[Reference Source](#)
14. Campbell C, Aureli F, Chapman C, *et al.*: **Terrestrial Behavior of *Ateles* spp.** *Int J Primatol.* 2005; **26**(5): 1039–1051.
[Publisher Full Text](#)
15. Voirin JB, Kays R, Lowman MD, *et al.*: **Evidence for Three-Toed Sloth (*Bradypus variegatus*) predation by spectacled owl (*Pulsatrix perspicillata*).** *Edentata* no. 8–10 2009.
[Reference Source](#)

Current Referee Status:

Referee Responses for Version 2



Matias Pablo Juan Szabó

Institute of Biomedical Sciences and School of Veterinary Medicine, Federal University of Uberlândia, Uberlândia, Brazil

Approved: 28 February 2014

Referee Report: 28 February 2014

In this new version of the manuscript, authors detailed handling procedures and care taken to avoid accidental trap infestation after the first tick encounter on the mosquito trap. Nonetheless, the phenomenon presented by Jose Loaiza and collaborators is uncommon and deserves further investigation.

However, I suggest removing from the final version the following phrases from the manuscript: “*This possibility suggests that an association between arboreal monkeys and ticks is opportunistic, perhaps occurring principally at the peak of the fruiting season. However, ticks were also collected during August and October of 2009, and so, tick-monkey ground interactions could also be the result of monkey behaviors such as drinking from terrestrial sources or chasing games.*” Monkeys as tick carriers up to the canopy is very speculative by itself, explaining how it occurs is excessive speculation. Should this phenomenon occur, many other possibilities should be explored.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Responses for Version 1



Matias Pablo Juan Szabó

Institute of Biomedical Sciences and School of Veterinary Medicine, Federal University of Uberlândia, Uberlândia, Brazil

Approved with reservations: 08 November 2013

Referee Report: 08 November 2013

The phenomenon presented by Jose Loaiza and collaborators is astonishing by several criteria and thus should be carefully evaluated to dispel any doubt. The mobility described for *Amblyomma tapirellum* is atypical and the explanations rather speculative (which is acceptable considering the absolute lack of knowledge on the matter, as clearly stated by authors). At the same time, finding 48 adult ticks (I will focus on the stage found in the highest numbers) on different occasions in different light traps suspended 20m above the ground indicates a standard.

One has to consider that for regular access to traps, adult ticks either climbed trees by themselves, fed on an arboreal host and moulted to adults on trees, were taken to the trees as adults by hosts (monkeys) and detached from them or had access to the traps during handling. Apart from the last possibility all others are worthwhile considering and deserve further research. In fact, movement from tree to suspended mosquito trap is, by itself, a challenge for ticks. I am not acquainted with such traps but it seems from the picture that it is held by a string or something similar and thus ticks would have to crawl on a variable length of string to reach the trap. Regardless, before further speculation, it is essential to dismiss trap infestation during handling through review, and careful description of field procedures. Considering the uncommon finding and its ecological importance such information is of paramount importance for the article.

According to the authors, this tick species is quite prevalent on the ground or on ground vegetation. For this reason, I strongly recommend describing the handling of traps in detail. Were they mounted /examined/maintained on the ground at any moment? By personal experience, it is common to find ticks crawling on boxes used to carry dry ice (CO₂) for tick traps. After finding the first specimens in the traps was any specific care taken to avoid accidental access of ticks to the traps? Were there any differences in the handling of traps in the canopy in comparison to those closer to the ground?

Should these doubts be eliminated in the text, the manuscript will be acceptable for further discussion and publication.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

1 Comment

Author Response

Jose Loaiza, Instituto de Investigaciones Cientificas y Servicios de Alta Tecnologia, Panama
Posted: 20 Jan 2014

- *"According to the authors, this tick species is quite prevalent on the ground or on ground vegetation. For this reason, I strongly recommend describing the handling of traps in detail. Were they mounted/examined/maintained on the ground at any moment? By personal experience, it is common to find ticks crawling on boxes used to carry dry ice (CO₂) for tick traps. After finding the first specimens in the traps was any specific care taken to avoid accidental access of ticks to the traps? Were there any differences in the handling of traps in the canopy in comparison to those closer to the ground?"*

We appreciate Dr. Szabó's concerns, and as we mentioned above, we apologize for not providing greater detail in our description of our field methods. We hope that the added text will sufficiently describe our field techniques. As we point out, independent confirmation of our surprising finding is on-going in Panama.

We have taken advantage during this revision to fix spelling and grammatical errors in the text and figures:

- In Figure 2, *Amblyomma gaeyi* was replaced by *Amblyomma geayi*

Competing Interests: No competing interests were disclosed.



Michael Levin

Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

Not Approved: 05 November 2013

Referee Report: 05 November 2013

The authors speculate that questing *A. tapirellum* ticks, which are normally feeding primarily on tapirs, could be transported and, moreover, dropped into carbon dioxide baited mosquito traps by primates. (Yes, “Questing” is the appropriate term, which describes a stage in the tick life cycle when it is ready to take a blood-meal, whether it is searching for food actively or passively.) This “delivery of questing ticks” is a tenuous conjecture at best.

Unlike mosquitoes, ixodid ticks feed only once per life stage; they rarely attach to and get transported by unsuitable hosts, on which ticks would not feed. Ticks, that “by mistake” get onto a wrong target – be it a flagging device or an inappropriate host, get off of it within minutes unless they are frozen in dry ice. Ticks which do find a suitable host and begin feeding do not just suddenly drop off, but continue taking a blood-meal for several days until full engorgement (or until the host’s death). *A. tapirellum* has a 3-host life cycle; it means that at each stage engorged (blood-fed) ticks fall off the host into the substrate in order to molt into the next life stage or to lay eggs (unlike in mosquitoes, female ixodid ticks die after just one oviposition). It takes several weeks for an engorged tick to molt and start questing again. Therefore, it is highly implausible that unfed (questing) ticks would be delivered en masse into the traps by any hosts. Engorged ticks, on the other hand, might have been delivered in this way but only if an infested animal crawled into a trap for a rest and fed ticks had time to fall off of it. However, neither spider nor howler monkeys could possibly fit into miniature light traps, not to mention that neither species is known to serve as a host for *A. tapirellum*.

It is much more likely that the appearance of questing *Amblyomma* ticks in mosquito traps resulted from a procedural (trap-handling) artifact. Authors placed their carbon dioxide baited traps either 1.5 meters above the ground (not really “at the ground level”), or 20-30 meters up in the trees. Obviously, traps hanging 1.5 meters above the ground could be serviced right where they were, without contact with the tick-infested ground cover. However, those traps placed 20 meters high would have had to be lowered to the ground daily for checking and replacement of the dry ice, and that would give ticks a chance to crawl onto the traps out of their own volition. Ticks are attracted to carbon dioxide and fast crawling ticks like *Amblyomma* spp. can be collected in large numbers quite quickly. Unfed questing ticks that crawled onto and eventually into dry ice container(s) would be discovered the next day to cause a “sensational discovery”. Uneven distribution and seasonal dynamics of ticks explain why some traps had ticks and others did not. Differences in placement of traps explain why only high-hanging traps caught ticks.

The suppositions advanced in this account can only stem from ignorance regarding ticks, their biology, behavior, and even the standard terminology. It is usually advisable to consider simple explanations of unexpected results prior to making “astounding” claims.

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Competing Interests: No competing interests were disclosed.

1 Comment

Author Response

Jose Loaiza, Instituto de Investigaciones Cientificas y Servicios de Alta Tecnologia, Panama

Posted: 20 Jan 2014

- *"Authors placed their carbon dioxide baited traps either 1.5 meters above the ground (not really "at the ground level")."*

To avoid confusion we have changed the text to read "understory."

- *"Obviously, traps hanging 1.5 meters above the ground could be serviced right where they were, without contact with the tick-infested ground cover. However, those traps placed 20 meters high would have had to be lowered to the ground daily for checking and replacement of the dry ice, and that would give ticks a chance to crawl onto the traps out of their own volition. Ticks are attracted to carbon dioxide and fast crawling ticks like *Amblyomma* spp. can be collected in large numbers quite quickly. Unfed questing ticks that crawled onto and eventually into dry ice container(s) would be discovered the next day to cause a "sensational discovery". Uneven distribution and seasonal dynamics of ticks explain why some traps had ticks and others did not. Differences in placement of traps explain why only high-hanging traps caught ticks."*

We appreciate the reviewer calling attention to our lack of detail about our field sampling practice. It is clear that the original draft of our manuscript was without sufficient detail to demonstrate that our finding is not likely to be the consequence of trap-handling artifacts. In fact, when we first found ticks on our traps, our team recognized the novelty of our finding and we took critical steps to ensure that the ticks originated in the canopy and were not accidentally sampled from the ground. We have added the following text to our manuscript:

"When our field team first discovered the presence of ticks on the outside of CDC-LTs, recognizing that this was a novel occurrence, we carefully reviewed and modified our field protocol to ensure that our observance of host-seeking ticks in the forest canopy was not an artifact of our field methods. To wit, each morning when the traps were lowered, field members, including the senior author, first checked carefully for the presence of ticks on the exterior of each CDC-LT. This was done while the trap was suspended. Any ticks were immediately removed and placed in ethanol and labeled with appropriate metadata (date, trap number, etc.). Subsequently, the netting containing mosquitoes was secured in a plastic box for processing in the indoor laboratory space of Barro Colorado Island. The umbrella and the cylinder containing the fan mechanism of each trap were also taken back to the lab, but the igloo cooler was sealed in a white garbage bag and re-suspended in the mid-canopy (free from by-passers and foliage) during the day. In the evening, CDC-LTs

were carried pre-assembled in Rubbermaid-style plastic boxes to the field and were quickly re-assembled in each field site, with loading of the solid CO₂ as the final step. At no time were either canopy or understory CDC-LTs placed on the ground while they were being serviced in the field."

- *"The suppositions advanced in this account can only stem from ignorance regarding ticks, their biology, behavior, and even the standard terminology. It is usually advisable to consider simple explanations of unexpected results prior to making "astounding" claims."*

We would like to emphasize that the entire point of F1000Research observational articles is that they are precisely designed to document unexpected results that may improve our understanding of the natural world:

"Science's most important breakthroughs, from the discovery of microorganisms to the theory of evolution, have come about through observation. As part of the scientific method, observations are made to record a fact or an occurrence to help either prove or disprove a hypothesis. However, we all know the unpredictable nature of science and the curve balls it can throw" (See more at:

<http://blog.f1000research.com/2013/09/12/the-observation-article-recording-phenomena-in-science>)

Our observations are robust and repeatable. As a research team working at one of the world's oldest tropical research institutions, we are acutely aware of just how much remains to be discovered in our tropical forests, especially in the extremely inaccessible tropical forest canopy. Given that nymphs and larvae of *A. tapirellum* typically cannot be identified to species in the field (e.g. Bermúdez et al. 2010), and that a Google Scholar search retrieves only 27 articles (including ours) when searching for "*Amblyomma tapirellum*", certainly Dr. Levin is not suggesting that our scientific knowledge of the life history of this and other Neotropical ticks species is complete. Fortunately, third party teams are currently actively working in Panama to more fully document the frequency that host-seeking ticks exploit the tropical canopy and confirm our finding. We are grateful for the opportunity given to us by F1000Research to publish our observation that has encouraged those efforts.

Competing Interests: No competing interests were disclosed.



Brian Allan¹, **Erin Welsh**²

¹ Department of Entomology, University of Illinois, Urbana, IL, USA

² University of Illinois Urbana-Champaign, Champaign, IL, USA

Approved: 17 October 2013

Referee Report: 17 October 2013

In addition to being notable for describing a unique approach for sampling ticks in Neotropical forests, this article presents the first reported instance of the *Amblyomma tapirellum* tick found in the forest canopy. The authors suggest that this may indicate host transportation of these normally ground-dwelling ticks

vertically through the canopy by an unreported host. Though the authors mention that the vertical movement of sloths may lead to ticks being acquired while on the ground, they primarily focus on two candidate host species for the vertical transport of ticks: the Mantled Howler Monkey and the Black Spider Monkey. They hypothesize that the monkeys acquire the ticks on the ground during the peak of fruiting season, and then transport the ticks vertically as they return to the canopy. This is a thoughtful insight that prompts additional questions regarding the natural history of this tick and differences in host acquisition between ground-dwelling and canopy-dwelling populations. One suggestion is to highlight the interesting contradiction that arose from finding no ticks in the ground CDC light traps (CDC-LTs) despite the statement that “ground populations of *A. tapirellum* are quite abundant” during the sampling period. The lack of ticks in ground traps compared to canopy traps could suggest differences in host acquisition strategies between the two environments. In addition, the capture of these ticks in the canopy may indicate that an arboreal life strategy in ticks is a more common phenomenon than previously thought. However, the authors take a different approach regarding stating the article’s significance. Instead of highlighting potentially interesting differences in host acquisition in ticks, the authors focus on implications for disease ecology. Though in this paper these ticks are suggested to be vectors for zoonotic disease, no mention is made of pathogens known to be associated with *A. tapirellum*, which leaves the implications for disease ecology or public health vague and ill-defined. Despite this, the findings in this paper are ecologically interesting and could be framed in a behavioral ecology context to emphasize the importance of finding ground-dwelling ticks actively seeking for hosts in the canopy.

Additional, minor revisions could be incorporated to improve the clarity of the paper’s findings. In several instances, *A. tapirellum* is referred to as a vector yet the authors offer no evidence that these ticks transmit a known illness. By definition, a vector is an agent that transmits disease. Additionally, ticks captured by the CDC-LTs are described here as “questing”, but the correct term to describe this behavior is “hunting” or “host seeking”, since “questing” refers to a specific, relatively passive, host acquisition strategy. The authors state that the presence of ticks in the canopy traps “reinforce[s] the notion that ticks use CO₂ to locate their hosts”, a characteristic which is true of many species of ticks. However, this raises the question of why ground traps did not capture ticks, a finding for which no explanations were proposed.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: While we also have initiated research on the ecology of ticks and tick-borne pathogens in Panama, we can identify no competing interests that might influence the objectivity of our review.

1 Comment

Author Response

Jose Loaiza, Instituto de Investigaciones Cientificas y Servicios de Alta Tecnologia, Panama
Posted: 20 Jan 2014

- *"In several instances, A. tapirellum is referred to as a vector yet the authors offer no evidence that these ticks transmit a known illness."*

The sentence in the Introduction now reads: "...highlights the potentially complex ecological relationships of Neotropical ticks, which as a group, are potential vectors of zoonotic disease in undisrupted forest habitats."

- *"Additionally, ticks captured by the CDC-LTs are described here as "questing", but the correct term to describe this behavior is "hunting" or "host seeking", since "questing" refers to a specific, relatively passive, host acquisition strategy"*

References to "questing" were changed to "host-seeking".

- *"The authors state that the presence of ticks in the canopy traps "reinforce[s] the notion that ticks use CO-2 to locate their hosts", a characteristic which is true of many species of ticks. However, this raises the question of why ground traps did not capture ticks, a finding for which no explanations were proposed"*

We agree that it is curious that we did not find ticks on the understory traps. However, as we don't have a plausible explanation, we defer from additional speculation, as it is apparent that simply reporting our finding has created enough controversy without additional conjecture.

Competing Interests: No competing interests were disclosed.