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FIRST REPORT OF THE INTRODUCTION OF AN EXOTIC TICK, *AMBLYOMMA COELEBS* (ACARI: IXODIDAE), FEEDING ON A HUMAN TRAVELER RETURNING TO THE UNITED STATES FROM CENTRAL AMERICA

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KEY WORDS

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ABSTRACT

Introduction of ticks into the United States that can carry disease-causing pathogens to humans, companion animals, and wildlife has accelerated in recent years, mostly due to globalization, frequency of travel, and a rise in legal and illegal animal trades. We hereby report for the first time introduction of a live fully engorged *Amblyomma coelebs* feeding on a human into the United States from Central America. *Amblyomma coelebs* is geographically distributed in the Neotropical region and reaches the southern states of Mexico. This species is capable of transmitting a number of pathogens of public health and veterinary importance including spotted fever group rickettsiae, raising concern that *A. coelebs,* if it became established in the United States, might also be able to carry these pathogens. Considering the risks of exotic ticks as vectors of numerous pathogens and their potential to establish new populations under conducive climatic and habitat conditions, rigorous inspection practices of imported livestock and pet animals at ports of entry are vital. It is also important for travelers and practitioners to develop a heightened awareness of the public health risks associated with the unintended importation of exotic ticks and the potential such parasites have for breaching United States biosecurity defenses.

Introduction of ticks into the United States that can carry disease-causing pathogens to humans, companion animals, and wildlife has accelerated in recent years, mostly due to globalization, frequency of travel, and rise in legal and illegal animal trades (Keirans and Durden, 2001; Burridge, 2011). Ticks are vectors of a broad range of pathogens with global distributions, including bacteria, protozoa, filarial parasites, and arboviruses (Molaei et al., 2018), and are responsible for approximately 95% of the reported vector-borne diseases in the United States every year (Eisen et al., 2017).

Of the more than 140 exotic tick species introduced into the United States, 63 are reported to readily feed on humans and 23 are known to transmit pathogens of public health and veterinary importance (Keirans and Durden, 2001; Burridge, 2011). Exotic ticks have been reported to be infected with a number of human and veterinary disease-causing pathogens in the United States, including *Ehrlichia ruminantium* (formerly known as *Cowdria ruminantium*) infection (heartwater), a lethal disease of cattle, sheep, goats, and deer, in *Amblyomma sparsum* collected from tortoises imported into Florida from Africa (Burridge et al., 2000a, 2000b; Burridge, 2001), and *Rickettsia* sp. in *Amblyomma exornatum* in a reptile facility in Alabama (Reeves et al., 2006).

The recent introduction and establishment of the Asian longhorned tick, *Haemaphysalis longicornis,* into the United States in 2017 (Rainey et al., 2018) highlights the enduring risk and consequences associated with invasive tick species of medical and/or veterinary importance. Reports of established populations of the Asian longhorned tick are now increasing rapidly along the Eastern Seaboard and westward, with it being reported from Arkansas, Connecticut, Kentucky, Maryland, New Jersey, New York, North Carolina, Pennsylvania, Tennessee, Virginia, and West Virginia (Beard et al., 2018; https://www.aphis.usda.gov/animal_health/animal_diseases/tick/downloads/longhorned-tick-sitrep.pdf).

A 66-yr-old male resident of North Haven, Connecticut, with a history of recent leisure travel to Costa Rica and Panama discovered a live tick attached to his back (Fig. 1). The resident's Central American trip included visits to Coiba National Park in Panama and to the Osa Conservation area, Piedras Blancas National Park, Corcovado National Park, and Curú National Wildlife Refuge in Costa Rica. The tick was removed by a

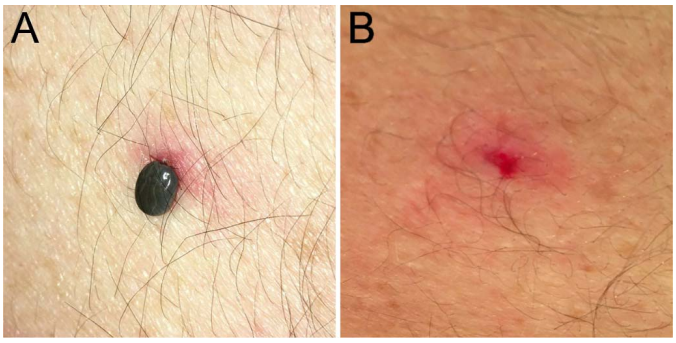

Figure 1. (**A**) *Amblyomma coelebs* engorged on the back of the patient. (**B**) Tick bite lesion on the patient's back after removing the tick. Color version available online.

dermatologist and submitted to the Connecticut Agricultural Experiment Station-Tick Testing Laboratory (CAES-TTL) on 23 March 2018 for species identification, engorgement status, and pathogen testing.

The specimen measured 5 mm in length and 4 mm in width and was identified as a fully engorged *Amblyomma* nymph upon initial morphological identification. This was followed by capturing dorsal and ventral images of the specimen (Fig. 2) using an AXIO Scope.A1 (Zeiss, Göttingen, Germany) with an attached RT3 camera system (SPOT Imaging, Sterling Heights, Michigan). Capturing higher resolution scanning electron microscopy (SEM) images was not feasible due to the risk of exposing the fully engorged tick specimen to the SEM vacuum, sample surface charging, and beam damage.

For genetic identification, DNA was extracted from the tarsi and tibiae of the specimen using the DNeasy Blood & Tissue extraction kit (Qiagen, Valencia, California) according to the

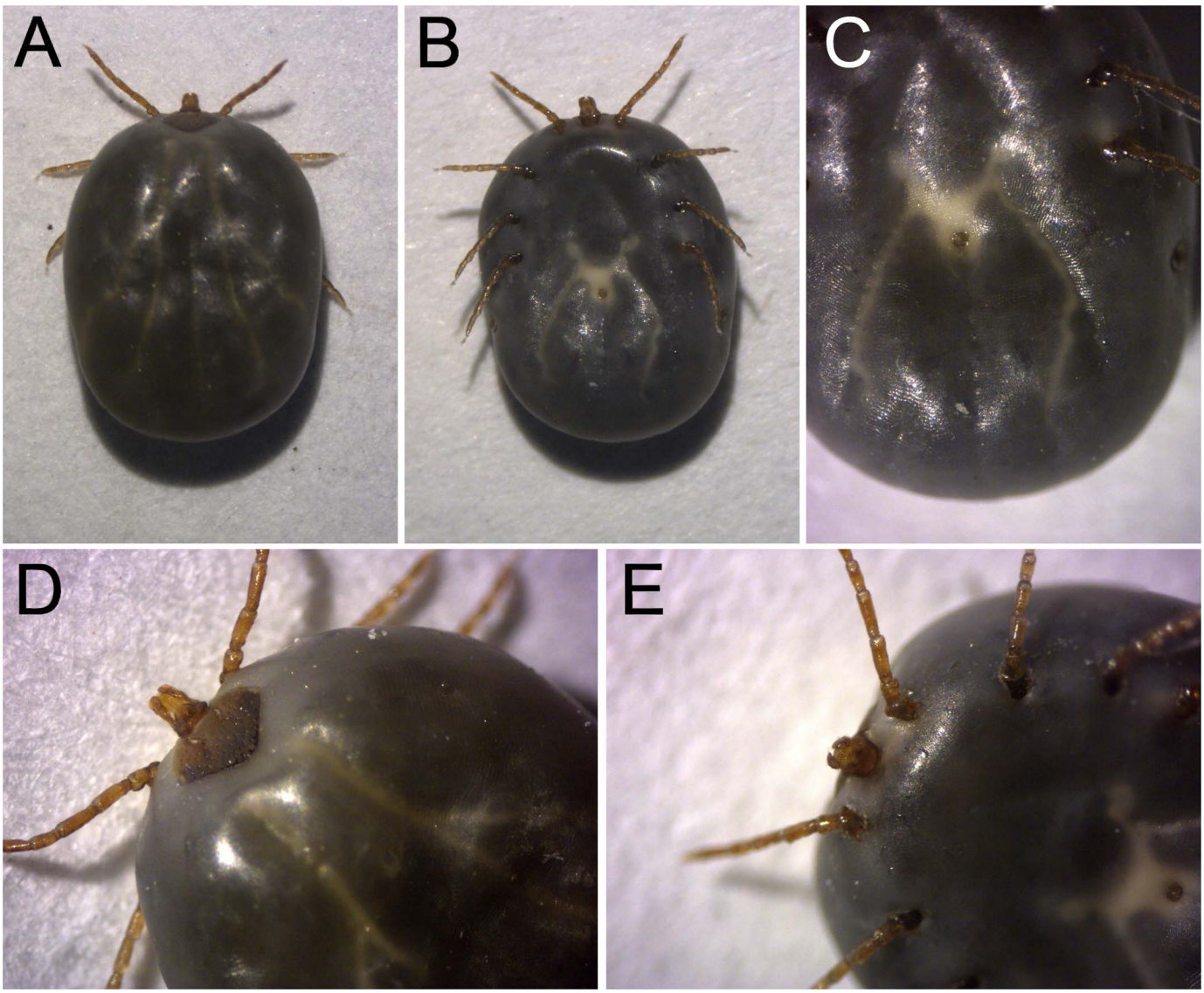

Figure 2. Light microscopy images of the engorged *Amblyomma coelebs*. (**A**) Dorsal, (**B**) ventral, (**C**) ventral close-up of anus, (**D**) dorsal close-up of capitulum, and (**E**) ventral close-up of capitulum. Color version available online.

manufacturer's recommendations. The mitochondrial 16S region of the extracted DNA was amplified by PCR using the method described by Ushijima et al. (2003). The PCR product was then purified using a QIAquick PCR Purification Kit (Qiagen) and sequenced in both directions directly in cycle-sequencing reactions (Keck Sequencing Facility, New Haven, Connecticut) with a 3730xl DNA Analyzer (Applied Biosystems, Foster City, California). Sequences were annotated with ChromasPro (Technelysium Pty Ltd., Tewantin, Queensland, Australia) and identified by comparison to the GenBank DNA sequence database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). The annotated sequence was deposited into the NCBI GenBank (GenBank accession MN065775). The identity of the specimen was determined by morphological features and genetic analysis as *Amblyomma coelebs* Neumann.

The specimen was submitted on 30 March 2018 at ambient temperature to the Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, to screen for *Rickettsia* spp. pathogens including those common to Central and South America. The tick was macerated using a sterile scalpel and DNA extracted using a DNeasy Blood & Tissue extraction kit (Qiagen) according to the manufacturer's instructions. The DNA was examined using a pan-rickettsia qPCR assay, PanR8, as previously published (Kato et al., 2013), and the results did not indicate the presence of rickettsial pathogens. Both a positive control and a no template negative control were run along with the sample.

We hereby report for the first time introduction of a live fully engorged *A. coelebs* feeding on a human in the United States. Like other *Amblyomma* ticks, *A. coelebs* is a 3-host parasite (M. L. Levin, available at https://www.merckvetmanual.com/integumentary-system/ticks/amblyomma-spp.) and is geographically distributed in the Neotropics (Onofrio et al., 2006). Adults of this species frequently feed on tapirs (*Tapirus terrestris*) as their usual hosts (Labruna and Guglielmone, 2009), but they have also been reported parasitizing a wide range of other hosts including humans, equines (Beldomenico et al., 2003), cattle, peccaries, South American foxes, wild felids, opossums, armadillos, ringtailed cats, and various large rodents, and larvae and nymphs may also attach to birds (Ogrzewalska et al., 2010; Lopes et al., 2016; Nava et al., 2017). Reports also indicate that nymphs might be more generalists and exploit a larger host range that includes carnivores, marsupials, rodents, birds, and occasionally humans (Beldomenico et al., 2003; Ogrzewalska et al., 2009, 2010; Garcia et al., 2015; Sponchiado et al., 2015; Aguirre et al., 2018; Ito et al., 2018).

Given the wide host range and geographical distribution, it is surprising there has been no documented introduction of *A. coelebs* into the United States. The National Veterinary Services Laboratories, United States Department of Agriculture, has no record of an introduction, nor do any published reports exist of this species being introduced into the country. However, Keirens and Durden (2001) have reported on introduction of this species on seed imported into Louisiana, and Burridge (2011) has also made reference to this introduction.

Infection of *A. coelebs* with spotted fever group rickettsiae (SFGR), *Rickettsia amblyommatis* (formerly known as *'Rickettsia amblyommii'* and later *'Candidatus* Rickettsia amblyommii'), has been reported from French Guyana and from the southeastern, midwestern, and western Amazon, Brazil (Parola et al., 2007; Martins et al., 2014; Silveira et al., 2015; Witter et al., 2016). As likely the most prevalent and widely distributed SFGR species in the Americas (Karpathy et al., 2016), *R. amblyommatis* has also been reported from several other tick species of the genus *Amblyomma*, most commonly from *Amblyomma americanum* (Labruna et al., 2004, 2007; Fitak et al., 2014; Hermance et al., 2014; Castro et al., 2015). In laboratory analysis, the WB-8-2^{T} strain of *R. amblyommatis* has been shown to be non-pathogenic to guinea pigs (*Cavia porcellus*) and has produced mild and transient infections in the tunica vaginalis of male meadow voles (*Microtus pennsylvanicus*) when inoculated with densely infected cell culture suspensions (Burgdorfer et al., 1981). Serological evidence suggests that humans develop a robust immune response to this organism (A. Medina and colleagues, unpubl. data; Apperson et al., 2008), and it may be associated with disease manifestations in some patients (Delisle et al., 2016). The inability of *R. amblyommatis* to cause disease in guinea pigs (Burgdorfer et al., 1981), coupled with a lack of epidemiological evidence of human infections, suggest that this rickettsia species is not pathogenic to humans. However, its high prevalence in ticks may complicate the diagnosis and surveillance of other SFGR infections in humans (Karpathy et al., 2016).

Considering the risks of exotic ticks as vectors of numerous pathogens and their greater potential to rapidly establish new populations under conducive climatic condition and habitats and the availability of competent hosts (Ogden et al., 2008; Wu et al., 2016), rigorous inspection practices of imported livestock and pet animals at ports of entry are vital. Continuous monitoring of the infection status of parasitizing ticks and evaluating their competency for transmission of the local tick-borne pathogens could also prove important to protecting human and veterinary health. It is also important for travelers and practitioners to develop a heightened awareness of the public health risks associated with the unintended importation of exotic ticks and the potential such parasites have for breaching United States biosecurity defenses.

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