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RESEARCH ARTICLE

# Migratory Songbirds Transport *Amblyomma longirostre* and *Amblyomma maculatum* Ticks to Canada

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## ABSTRACT

Birds transport ticks into Canada during northward spring migration, and some of these ticks are infected with tick-borne zoonotic pathogens. Some *Amblyomma* species harbour pathogens that cause debilitating diseases that can be fatal to humans, and domestic and wildlife animals. At least 65 *Amblyomma* spp. are indigenous in the Western Hemisphere, and approximately half bite humans. *Amblyomma longirostre* carries *Rickettsia amblyommatis* which causes spotted fever group rickettsiosis, a febrile disease in humans. Additionally, *Amblyomma maculatum* harbors and transmits *Rickettsia parkeri*, a spotted fever group rickettsiosis, and this tick bites humans. In the present study, we use two technologies to identify ticks. To confirm identification, we took microphotographs followed by DNA barcoding of the cytochrome c oxidase I gene. Based on molecular analysis, we confirmed that the two *Amblyomma* spp. were *Amblyomma longirostre*, a neotropical tick and *Amblyomma maculatum*, the Gulf Coast tick. Based on our tick-bird findings, we confirm that migratory songbirds transport *Amblyomma* ticks into Canada, and have the potential, either directly or indirectly, to transmit tick-borne zoonotic pathogens to humans.

## Introduction

Migratory birds transport ticks into Canada from as far south as the Neotropics. Because of the large number of *Amblyomma* spp. (Acari: Ixodidae), confirmatory identification can be a challenge. Ticks consist of 14 genera, and *Amblyomma* is the third largest species [1]. The distribution of *Amblyomma* spp. is worldwide, and are mainly indigenous in humid, tropical or subtropical regions [2]. Globally, there are at least 138 *Amblyomma* spp., and 63 (46%) of these species bite humans [1]. Virtually all terrestrial vertebrate species serve as hosts, and at least 65 *Amblyomma* spp. have been reported in the Western Hemisphere. In Brazil, nymphs of 27 *Amblyomma* species have been collected and described [3]. In Mexico, 26 *Amblyomma* spp. have been reported, including *A. maculatum* and *A. longirostre* [4]. In Venezuela, 25 *Amblyomma* spp. are native [5]. *Amblyomma longirostre* and *A. maculatum* were previously reported in migrating passerines entering Canada during spring migration [6–11].

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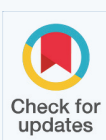
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Proper identification of *Amblyomma* spp., which infest northbound migratory songbirds (Order: Passeriformes), is a formidable task.

*Amblyomma* larvae and nymphs parasitize passerines, whereas adults infest non-avifauna. In order to identify these juvenile ticks to the species level requires two algorithms: (i) rear larvae and nymphs to the adult stage, and use taxonomic keys, or (ii) use molecular characterization [12]. Based on the native area of *Amblyomma* ticks, southern temperate and neotropical songbirds transport *Amblyomma* spp. from as far south as Central America, the Caribbean Islands, and the northern part of South America.

Notably, acarologists reported that *Amblyomma americanum*, the lone star tick, does not overwinter in the southernmost region of Canada [10]. During the fall, researchers set out unfed *A. americanum* ticks (larvae, nymphs, and adults) in vented vials, inserted in a vented plastic canister that was wrapped with hardware cloth for mouse exclusion. At the deciduous forest site, the canister was placed in a hoof-proof crate, and placed in a well drained area among fresh leaf litter to remain in a quiescent state overwinter [13]. Since all larvae died during the winter, the researchers concluded that neotropical *Amblyomma* spp. are not winter hardy in Canada and, thus, were not able to maintain an established population.

Pathologically, *Amblyomma* ticks can cause insidious diseases that inflict pain and suffering on people and domestic animals. In fact, fully engorged females may cause tick paralysis [14].

The present study reports two *Amblyomma* spp. infesting songbirds during northbound spring migration, and both taxonomic keys and molecular identification were employed to confirm their identification.

## Materials and Methods

### Tick collection

Twenty-one ticks were collected from naturally infested, migratory songbirds captured by mist nets at Oriskany Banding Station, 42° 56' 48" N, 79° 56' 45" W located 4 km west of Cayuga, Ontario. Attached ticks were removed with super-fine pointed stainless steel forceps, and inserted into 8.5 mL, 15 x 75 mm polypropylene, round-bottom tubes, containing a slightly moistened piece of paper towel. After inserting the ticks, a piece of tulle netting was placed over the mouth of the tube, and a customized push-

cap with a 7 mm hole was pressed into the opening of the tube to prevent ticks from escaping and to provide ventilation. This tube was placed in a self-sealing plastic bag containing a slightly moistened section of paper towel. Bird banders provided background information for each bird. Live ticks were sent directly to the lab (J.D.S) for identification. Engorged ixodid ticks were held to molt to the next developmental life stage using a photoperiod of 16:8 h (light: dark) with ambient temperature and 90–95% humidity. As the daylength shortened in August, a 12W, full-spectrum LED daylight bulb (LifeEnergy Systems, Canada) was used, and plugged into an electric timer. Complete tick records (i.e., bird species, collection date, geographic location, tick species, and developmental life stage) were recorded in a tick log for each bird parasitism.

A taxonomic tick key was used to tentatively identify the *Amblyomma* nymph that later molted to an adult male [5].

Ventral and dorsal microphotographs were taken of each *Amblyomma* tick in the barcoding laboratory with a Keyence VHX-700 Digital Microscope (KEYENCE Canada Inc., Mississauga, Ontario, Canada).

### Molecular analysis

The barcode region of the mitochondrial cytochrome c oxidase I (COI-5P) gene was sequenced at the Canadian Centre for DNA Barcoding ([www.ccdb.ca](http://www.ccdb.ca)) following protocols outlined in deWaard JR, et al. [15]. A primer cocktail of the Folmer [16], and LepF1 and LepR1 [17], MLepF1 and MLepR2 [18] was used to amplify the target region. The total PCR reaction volume was 12.5 µL: 6.25 µL of 10% D-(+)-trehalose dihydrate for microbiology (≥ 99.0%, Fluka Analytical), 0.125 µL of ultra-pure water (Hyclone, Thermo Scientific), 2.5 µL of 25 mM MgCl<sub>2</sub> (Invitrogen), 0.125 µL of each primer, 0.0625 µL of 10 mM dNTP (KAPA Biosystems), 0.0625 µL of 5 U/µL KAPA Taq Hotstart DNA Polymerase (KAPA biosystems), and 2 µL of DNA template.

PCR products were bidirectionally Sanger sequenced on ABI 3730XL. The total sequencing reaction volume was 11 µL: 0.25 µL of BigDye terminator v3.1 (Applied Biosystems), 1.875 µL of 5X sequencing buffer (400 mM Tris-HCl pH 9.0 + 10 mM MgCl<sub>2</sub> (Invitrogen), 5 µL of 10% D-(+)-trehalose dihydrate from *Saccharomyces cerevisiae* (≥ 99%; Sigma-Aldrich), 0.875 µL of ultra-pure water (Hyclone, Thermo Scientific), 1 µL of diluted PCR template of primer and 2 µL of diluted PCR template.

Two shorter, overlapping COI amplicons (307 bp and 407 bp) were targeted, and contigs were assembled, and were aligned using codoncode aligner v8.0.2 (CodonCode Corporation). The barcode sequences, as well as photographs and metadata for specimens, were uploaded to the Barcode of Life Data System (BOLD) [19,20]. For species identification, BOLD used the BLAST algorithm [21] to identify the single base indel before aligning the protein translation through profile to a hidden Markov model of the COI protein [22].

## Results

A live, fully engorged nymph was collected from a migrating White-throated Sparrow, *Zonotrichia albicollis*, band number 2981-37767, on 02 May 2022 (Figure 1A). In the lab, the nymph likely failed to molt to due to an environmental contaminant in the food supply and/or drinking water. When the brownish nymph appeared to be dead, it was put in 94% ethyl alcohol for preservation.

Also, a live, fully engorged, ixodid nymph was collected from a migrating Magnolia Warbler, *Dendroica magnolia*, band number 2950-30680, on 19 May 2022 (Figure 1B). In the lab, the nymph successfully molted to a male in 31 d and, after the molt, this tick was held to sclerotized, and a microphotograph was taken.

Without light-level geolocators, we were not able to track the movement (i.e., geographic location, flight pace) of the White-throated Sparrow and the Magnolia Warbler from the start of their flight path to their bird banding stopover.

## Molecular identification

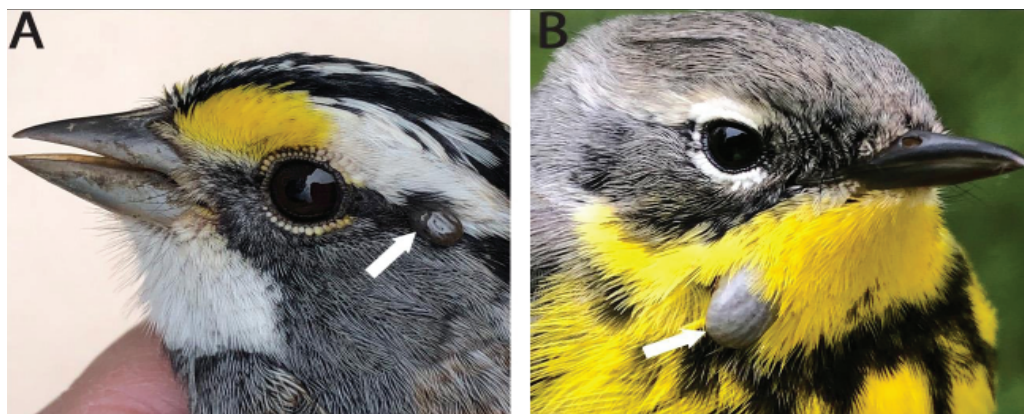
A full COI database search on the BOLD ID Engine returned a list of closest matches to the queries. BIOUG85238-H10 (*Amblyomma maculatum*) generated a sequence of 658 bp, and matched 100% with the *A. maculatum* amplicons stored in BOLD. Also, BIOUG85238-H11 generated a 407 bp sequence, and matched 100% with *A. longirostre* amplicons stored in BOLD. Voucher specimens of ticks collected in the present study have been deposited in the Centre for Biodiversity Genomics collection. Since we kept the *A. longirostre* and the *A. maculatum* ticks as voucher specimens, we did not test these ticks for pathogens.

## Discussion

In this study, we reiterated the fact that migratory songbirds transport ticks from southern latitudes into Canada during northbound spring migration. We successfully reared an *Amblyomma maculatum* nymph to an adult. Both *Amblyomma* ticks in the present study are known to carry pathogens that cause febrile illnesses in humans. In the tick identification laboratories, we used two techniques to identify ticks, namely allowing ticks to molt to adults and, likewise, using genomic molecular identification. We also wanted to assess what impact *Amblyomma* ticks might have on Canadian residents, especially those ticks transported by passerine migrants.

## Flight path of host birds

*Amblyomma maculatum* has a distributional range encompassing the Caribbean Islands, countries and U.S. states bordering the Gulf of Mexico [23]. Together, both larvae and nymphs of *A. maculatum* parasitize



**Figure 1** A. White-throated Sparrow parasitized by a fully engorged *Amblyomma* species nymph. B. Magnolia Warbler parasitized by a *Amblyomma* species nymph. White arrows point to nymphal ticks. Photos: Nancy Furber.



southern temperate and neotropical songbirds during northward spring migration. These songbirds commonly head to the boreal forest (primary breeding area) that spans central Canada countrywide and, onward, into Alaska (Figure 2A). Biogeographically, the Magnolia Warbler, a ground-feeding passerine, has wintering grounds encompassing the Caribbean Islands, and southern Mexico to Panama.

*Amblyomma longirostre* is a neotropical tick that is indigenous from Panama to southern Brazil and Argentina (Figure 2B) [5,12,24]. However, this tick species has been collected from birds throughout Mexico [4]. It is possible that the *A. longirostre* nymph in the present study was transported directly from the Mexico-United States border area by a White-throated Sparrow. However, it is more likely that the migratory flight path was a 2-step journey. First, a passerine migrant transported an *A. longirostre* larva from South America to the southern U.S. states, and molted to a nymph via transstadial passage and, the following year, a White-throated Sparrow transported the nymph to Cayuga, Ontario.

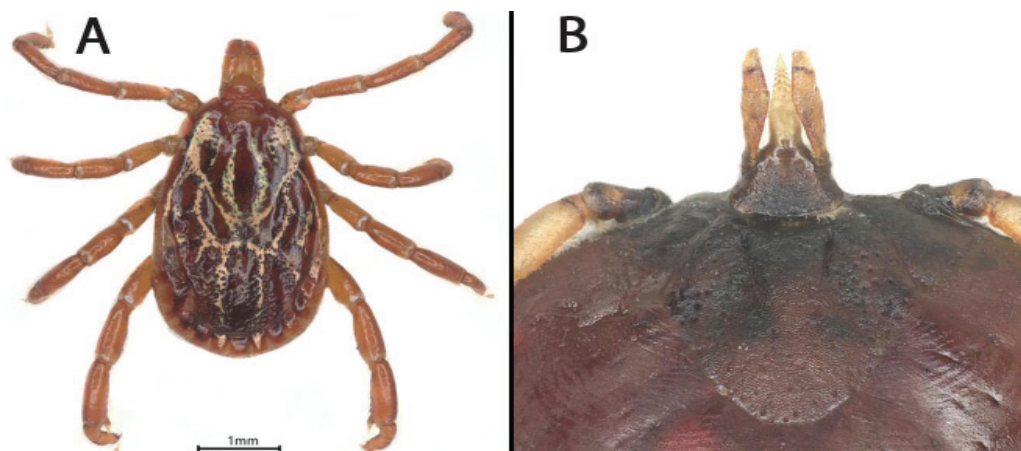
### Potential pathogens of *Amblyomma* species

*Amblyomma longirostre* larvae and nymphs parasitize passerines, but this tick species is not known to bite humans. Adults of this tick species feed primarily on porcupines (*Coendou* spp.) [12]. Although *A. longirostre* does not parasitize humans, *Rickettsia amblyommatis* (formerly *R. amblyommii* [25], a human pathogen, has been detected in *A. longirostre* [26]. *Rickettsia amblyommatis*, a gram-negative bacterium, which causes spotted fever group rickettsiosis, has been detected in several tick species.

As an interconnecting link, other tick species (i.e., lone star tick) bite humans, and could transmit *R. amblyommatis* to humans.

*Amblyomma maculatum* larvae and nymphs bite humans and parasitize a diversity of passerines and small mammals. Adults commonly infest ruminants, mainly on the head and ears [2]. These adults can cause severe injury to the hide, often rendering the hides unusable. Secondary infections leave cattle predisposed to screw worm and severe dermatophilosis [2]. Notably, *A. maculatum* ticks harbor and transmit *R. parkeri*, a gram-negative, obligate, intracellular bacterium that causes *Rickettsia parkeri* rickettsiosis (a spotted fever group rickettsia disease). Historically, the first human case was reported in 2004 in Virginia, and the patient exhibited a fever, mild headache, malaise, diffuse myalgias and arthralgias, and multiple eschars on the lower extremities [27]. Both *R. parkeri* and *R. rickettsii*, which causes Rocky Mountain spotted fever, have different vectors, but have similar clinical symptoms, namely febrile, eschar-associated illness. In contrast, *Rickettsia parkeri* typically has an eschar (a crusty lesion), whereas *R. rickettsii* does not [28]. Also, since migratory songbirds transport *A. americanum* larvae and nymphs into Canada, and *R. parkeri* has been detected in *A. americanum* [29]. This tick species has the potential to transmit *R. parkeri* to vertebrates, including humans [30].

Case reports have indicated occurrence of eschars in patients with Rocky Mountain spotted fever [31,32]. These early reports likely represent infections caused by *R. parkeri* or some other spotted fever group rickettsioses, and masqueraded as Rocky Mountain spotted fever. *Rickettsia parkeri* was most likely



**Figure 2** A. *Amblyomma maculatum*, male, dorsal view, following complete molt from nymph. B. *Amblyomma longirostre*, nymph, dorsal view of gnathosoma. Photos: Jaclyn McKeown.

isolated from patients with what appeared to be Rocky Mountain spotted fever, only to be misidentified as *Rickettsia rickettsii* by available diagnostic methods. Moreover, *A. maculatum* and, to a lesser extent, *A. americanum* can both cause tick paralysis in humans and other larger animals [33].

### Co-infestation of *Amblyomma* and *Ixodes* ticks and presence of pathogens

Co-infestation of juvenile *Amblyomma* ticks and *Ixodes scapularis* can co-occur in nature [34]. For instance, *A. americanum* can harbor several *Babesia* species including *B. sp. bigemina*, *B. sp. Coco*, and *B. ovata* [35]. These tick species have the potential to transmit tick-borne zoonotic pathogens. Recently, researchers detected three pathogens (i.e., *Anaplasma phagocytophilum*, *Babesia odocoilei*, and *Borrelia burgdorferi sensu lato*) in *I. scapularis* simultaneously [36]. These same pathogens were also detected in the blood of songbirds [37]. In particular, *B. odocoilei* is a single-cell parasite that infects red blood cells causing human babesiosis [38]. Of epidemiological significance, this intraerythrocytic pathogen is being diagnosed and treated clinically in febrile patients [38]. Human patients have symptoms that vary from asymptomatic to severe, and exhibit variable circulatory, gastrointestinal, rheumatological and neurological manifestations. Advanced human babesiosis cases can be fatal. Symptoms of human babesiosis caused by *B. odocoilei* include pronounced inflammation, lingering fatigue, marked thirst, insomnia, exertional intolerance, ischemia, chills, cold intolerance, digital numbness, tissue/organ dysfunction, muscle aches (especially legs), sweats (especially at night), loss of balance, impaired cognition, anxiety, tearfulness, and mood alteration [38,39]. Because *B. odocoilei* sequesters in the capillaries of the brain, this zoonosis can produce cerebral pathophysiology, coma-related symptomology, intolerance to mental exertion, and brain fog. Self-perpetuating, fibrin-bonded entanglements cause occlusions in multiple capillaries and post-capillary venules, and hinder blood flow and circulation [40,41]. Sequestering *Babesia* spp. can complete their life cycle within these self-perpetuating entanglements (local proliferation), and cause febrile symptoms. Additionally, these babesial piroplasmids are stealth pathogens, and remain isolated from the circulating immune system and spleen [41]. These entanglements induce capillary blockage and, therefore, impede the transfer of oxygen and nutrients throughout the body. As a result, mitochondria are forced to produce ATP anaerobically

and, consequently, these minute cell organelles operate inefficiently producing undue lactic acid. Once this deep-seated, covert-like infection develops to the advanced stage, it is recalcitrant to treat with current anti-*Babesia* regimens and, thus, becomes chronic [38,39]. Sequestration has been documented in other *Babesia* spp. including *Babesia canis* in dogs [42] and *Babesia lengau* in domestic cats [43]. *Babesia microti*, which is a non-sequestering *Babesia* sp., is scarce in eastern Canada; the ratio of *B. odocoilei* to *B. microti* is 41 to 1 [36].

### Positive identification of *Amblyomma* species

Molecular characterization is indispensable in distinguishing between *Amblyomma* species, especially larvae and nymphs. However, we do not know of a database that has all *Amblyomma* spp. Whenever there are malformations in morphological characteristics due to environmental contamination, taxonomic keys may lose their applicability [44]. Because of the large number of *Amblyomma* spp. in the Western Hemisphere, any juvenile *Amblyomma* sp. infesting a passerine migrant in Canada during northward spring migration requires careful taxonomic examination plus rearing or, preferably, molecular identification to have credulity.

## Conclusion

Migratory songbirds transport songbird-transported, *Amblyomma* ticks hundreds of kilometres during migration, and these ixodid ectoparasites can carry a wide variation of tick-borne zoonotic pathogens. The most trustworthy method of identifying juvenile *Amblyomma* species is by rearing them to adults or by molecular characterization using Sanger sequencing that has tacit genomic markers, such as the cytochrome c oxidase subunit 1 gene (COI-5P) region.

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### Ethical consideration

Ethical approval is not required to removing ticks from songbirds.

### Authors' contributions

Conceptualization and design: JDS. Collection and methodology: JDS, and JTAM. Formal analysis: JDS. Drafting of manuscript: JDS. Accuracy of data JDS, JTAM and CMS. All authors read and approved the final version of the manuscript.

## Competing financial and investment interests

The authors declare that they have no competing financial or investment interests related to this tick-borne zoonotic study.

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