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Morphological Abnormalities in Blacklegged Tick, *Ixodes scapularis*, Initiated by Environmental Contaminant

John D Scott[1], Nguyen Nguyen[2], Jaclyn TA McKeown[3] and Evgeny V Zakharov[2,3,4]*

[1]*Upper Grand Tick Centre, Fergus, Ontario, Canada*

[2]*Canadian Centre for DNA Barcoding, Biodiversity of Ontario, University of Guelph, Ontario, Canada*

[3]*Centre for Biodiversity Genomics, University of Guelph, Ontario, Canada*

[4]*Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada*

Abstract

Environmental pollutants cause teratogenic abnormalities in ectoparasites and their hosts. These contaminants are consumed or absorbed, and often invisible causing deformities in arthropods, including ticks. Teratogen in arthropods arises as physiological and morphological change, and often halts proper metabolic function. During normal bird band operations, we collected a replete tick from an American Robin, *Turdus migratorius*, and the fully engorged larva molted to an unfed nymph. The larva was normal, but the subsequent nymph was deformed. Consequently, we could not identify the unusual specimen with standard taxonomy, so we took microphotographs and employed DNA barcoding by means of the mitochondrial cytochrome c oxidase subunit 1 gene. Based on molecular analysis, we confirmed the aberrant specimen was a blacklegged tick, *Ixodes scapularis*. Our finding collaborates that songbirds can pass teratogenic compounds to ticks during a blood meal, and cause environmental abnormalities in ticks.

Keywords: Blacklegged tick; *Ixodes scapularis*; Teratogen; Songbirds; American Robin; DNA barcoding; Molecular identificiation; Environmental contaminant; Canada

Introduction

Ticks are blood-sucking ectoparasites of medical and veterinary importance. They parasitize and transmit tick-borne zoonotic pathogens to vertebrates (avian, mammalian, reptilian), and can cause lasting and debilitating symptoms [1]. Prompt identification is important to get timely treatment and efficacious medical care. Tick identification is important to determine the tick species, and also ascertain potential zoonotic diseases. East of the Rocky Mountains, blacklegged ticks, *Ixodes scapularis* (Acari: Ixodidae), are vectors of at least nine tick-borne zoonotic pathogens, including *Borrelia burgdorferi* sensu lato (Bbsl) complex [2,3], *Borrelia miyamotoi* [4,5], *Bartonella* spp. [6,7], *Babesia* spp. [8,9], *Mycoplasma* spp. [10], *Anaplasma* spp. [11,12], *Ehrlichia muris* eauclairensis [13], hemolytic *Rickettsia* spp. [14], and Powassan Virus Disease [15]. Polymicrobial infections can occur in ticks and their hosts [16]. Taxonomically, there are at least 244 *Ixodes* species identified around the globe [1].

New-found pathogens in ixodid ticks are capturing special attention worldwide, especially *Babesia* spp. (Apicomplexa: Piroplasmida: Babesiidae) because they cause human babesiosis. These piroplasmids include *B. crassa*-like [17], *B. divergens* [18], *Babesia divergens*-like MO-1 [19], *B. duncani* [20], *B. microti* [21], *B. motasi* [22], *B. odocoilei* [9], *Babesia* sp. XXB/Hang Zhou [23], *Babesia* sp. TW1 [24], *Babesia* spp. CA1, CA3, and CA4 [25], and *B. venatorum* [26]. Pathologically, symptoms can range from subclinical to fatal. In North America, common symptoms of human babesiosis caused by *Babesia odocoilei* include profound fatigue, inflammation, impaired cognition, ischemia, and intolerance to physical exertion [9].

American Robins, *Turdus migratorius* (Passeriformes: Turdidae), are avian hosts of *I. scapularis* larvae and nymphs. Their diet consists of berries and small fruits, as well as invertebrates, including caterpillars and beetles. During frost-free, snow-free temperate months, robins eat earthworms. Since 2000, the population of robins has gradually declined in southwestern Quebec. Importantly, American Robins widely disperse songbird-transported ticks across North America [27].

The purpose of the present study was to discern the proper identification of an unusual-looking

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Correspondence:
John D Scott, Upper Grand Tick Centre, 365 St. David Street South, Fergus, Ontario, N1M 2L7, Canada, E-mail: jkscott@bserv.ca

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tick collected from an American Robin in southwestern Quebec, Canada.

Materials and Methods

Tick collection

The rare specimen in the present study was part of a larger tick-host-pathogen study conducted in eastern and central Canada. Bird banders collected ticks from songbirds during three activity periods (i.e., spring migration, fall migration, and nesting and fledgling). Ticks were collected using super-fine, stainless steel forceps, and then inserted in polypropylene tubes vented with tulle netting secured with a push cap (with a 7 mm hole) to prevent live ticks from escaping. With the exception of the target specimen, all ticks were identified using expertise of a professional acorologist. The unusual tick was compared to several specimens in our reference collection, in particular, *I. scapularis* (Figure 1).

When engorged ticks were held to molt, they were retained at high humidity (85% to 95%) in a self-sealing plastic bag with a slightly moistened section of paper towel. During the period encompassing fall migration, ixodid ticks were placed in a light chamber, and held at room temperature and high humidity (85% to 95%) with a photoperiod ratio of 16:8 (light: dark). After the larva-nymph molt, the special specimen was held in the lab for 10 d to make sure the body parts were sclerotized and fully mature. The exoskeleton from the larva-nymph molt was preserved in 99% isopropyl alcohol. Ventral and dorsal photos were taken of the nymph with a Keyence VHX-7000 Digital Microscope. In order to keep the entire nymph intact, the exoskeleton was initially DNA barcoded. For the second barcoding session, anatomical parts (i.e., pulvillus, tarsus, metatarsus, tibia) of two legs of the nymph were excised, and DNA barcoded. Similarly, for the third session, anatomical parts of two other legs were removed by excision, and processed by DNA barcoding.

PCR testing, DNA sequencing, and phylogenetic analysis

In order to decipher the identity of the tick species from the submitted samples (nymphal tick, larval exoskeleton), alcohol was removed, and the samples were lysed with 50 µL of invertebrate lysis buffer with 0.5 mg/mL of proteinase K (Promega, Madison, Wisconsin, USA). DNA was extracted from 50 µL of each lysate using a validated spin column protocol [28]. The target genetic marker (barcode region of the mitochondrial cytochrome c oxidase subunit 1 gene) was amplified using Polymerase Chain Reaction (PCR) employing the cocktail C_LepFolF/C_LepFolR [29]. This procedure was repeated twice using the outer segments of two different legs. Bi-directional cycle sequencing was performed using a standardized commercially available BigDye Terminator v3.1 kit. Sequencing reactions were analyzed by high-voltage capillary electrophoresis on an automated ABI 3730xL DNA Analyzer. The raw forward and reverse trace files were assembled into contigs, and the consensus sequences were edited in Codon Code Aligner v. 4.1.1. These DNA sequences were compared with the Barcode of Life Data (BOLD) systems identification database accessible at http://boldsystems.org/index.php/IDS_OpenIdEngine. The identity of the closest matching reference sequence was used to infer the species identity of the DNA contributor in the corresponding test sample. Additionally, a phylogenetic tree was generated for the queried sequence and its top matching reference sequences to provide additional support towards the identity of the queried sequence. Images, primers, sequences, and their associated trace files with quality scores were uploaded to the secure in-house BOLD project called "CCDB forensic sampling [ABCBF]."

The maximum likelihood method was used to compare sequences because it has the highest log likelihood (-797.29) [30]. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 18 nucleotide sequences. Codon positions included were 1^{st} + 2^{nd} + 3^{rd} + non-coding. All positions containing gaps and missing data were eliminated. There were a total of 81 positions in the dataset. Evolutionary analyses were conducted in MEGA7 [31].

The GenBank accession number is: Bankit2532828 gnl|uoguelph|ABCF682-21. COI-5P and accession is OL982522. The voucher specimen (BIO-21-112) is being held at the centre for Biodiversity Genomics, Guelph, Ontario.

Results

Tick collection

A fully engorged specimen (21-5A73) was collected from a juvenile American Robin on 02 August 2021 at Ste-Anne-de-Bellevue, Quebec, Canada (45.43 N, 73.94 W). Mist nets for songbird capture were erected at dawn and taken down at noon. After removal of the fully engorged larva from the American Robin, it was active for 13 d before it became quiescent. The time period from when the larva was collected from the American Robin until it emerged as a nymph was 36 d. At this point, the nymph seemed to be functioning normally. It was held for 10 days to sclerotisze, and then put in 70% isopropyl alcohol, and promptly photographed before discoloration occurred. Under microscopic observation, the hypostome and palps were missing, and the capitulum (anterior body segment) was distorted. Although *Ixodes* ticks vary in color, engorged larvae and nymphs normally have a gray color (Figure 2). High-magnification photos were taken of the dorsal and ventral sides of the nymph (Figures 3a-3c). It was noted that the anal groove curved anteriorly around the anus, which indicates that this nymph is a member of the genus *Ixodes*.

Molecular identification

A DNA sequence was not recovered for the larval exoskeleton; however, bi-directional forward and reverse traces were recovered

Figure 1: *Ixodes scapularis*, unfed nymph. This is a typical unfed nymph before taking a blood meal.

Figure 2: A juvenile American Robin parasitized by the fully engorged *I. scapularis* larva. The white arrow points to a brownish-mauve color (rather than gray) larva under the right eye (Photo: Ana Morales).

from the outer segments of two legs. A DNA barcode of 480 base pairs (bp) was generated from legs of the *I. scapularis* nymph. The recovered sequence had a 99.6% match to *Ixodes scapularis* [30]. One sequence of an *I. scapularis*, which was previously collected from a raptor in southwestern Quebec, was molecularly consistent [32]. Based on the inferred identity of the sequence, as well as the BOLD phylogenetic tree (Figure 4), we have established a species-level match to *I. scapularis*. The sequence of sample BIO-21-112-CCDBFR0655 is compared to known *I. scapularis*, *Ixodes pacificus* (western blacklegged tick), and *Ixodes muris* (mouse tick) reference sequences in BOLD using MEGA7.

Discussion

We report an aberrant *I. scapularis* in southwestern Quebec, and provide substantial evidence that a teratogenic contagion caused the tick malformations. When we examined the unfamiliar tick, we questioned its identification, and wondered what might be the actual cause of the unconventional abnormalities. Since the *I. scapularis* larva was morphologically normal, and the subsequent nymph was deformed, we provide substantive evidence that the juvenile American Robin consumed food containing an environmental contaminant. After assessing the events surrounding the larva-nymph molt, we concluded that an environmental teratogen had altered standard metabolic function and physiology of the tick, and induced the eccentric deformities.

Larva-nymph development

When a tick takes a blood meal, the contents go into its midgut and, during the molt, develop into the next life stage [33]. When the new life stage is about to emerge, it is directly dorsal to its former self. This source of new development clearly indicates that the midgut is the origin.

Initially, we considered that the abnormalities may have been of genetic origin; however, the scenario lends itself more closely to initiation by an environmental pollutant. We felt that if there were genetic abnormalities, they would have appeared in the larva. However, the malformations only appeared in the nymph. Furthermore, the DNA barcoding (99.6%) did not show any genetic abnormities. It was only after the larva-nymph molt that the nymph then manifested several abnormalities. Since the capitulum had not been handled or damaged with forceps, the morphologic abnormalities were most likely caused by a toxic chemical in the environment, and triggered malformation in the capitulum during the molt [34].

Source of nymphal abnormality

After assessing the timeline, and strange abnormalities, we assert that teratogens in the blood meal initiated an alteration in the embryonic development of the newly formed nymph [34]. In wildlife habitats, teratogenesis has been associated with amphibians, reptiles, birds, fish, mammals, and invertebrates [34]. During temperate months, the primary source of food for robins is earthworms. Although the source of chemical contamination was not probed in the present study, teratogens were most likely present in the blood meal from the American Robin, and resulted in the apparent causation of the aberrant *I. scapularis* nymph.

We considered that a pathogenic microorganism may have instigated the malformations in the nymph. For example, *B. odocoilei*, a parasite of red blood cells, may have been implicated. However, it is not known to alter physical structure of an ixodid tick or its hosts (i.e., cervids, birds, humans) [9]. Over millions of years, pathogens have adapted to their vectors and their hosts.

Species trends and potential teratogenesis

Anatomy-altering findings in the nymph align with teratogens in the ecosystem where the American Robin had been foraging for food. These birds feed on plant vegetation and organic matter both in the topsoil and the soil surface. At night, earthworms come to soil surface to feed on organic matter and, in early morning, are eaten by ground-frequenting songbirds, especially American Robins. Plant vegetation, and subsequent organic matter, is easily contaminated by organic chemical in the air. Of note, an established population of *I. scapularis* is present in the locality where the nymph was collected, and the American Robin banded [2].

The estimated population trends in the Montreal area for American Robins over the past 10 year (2008 to 2018) has been a

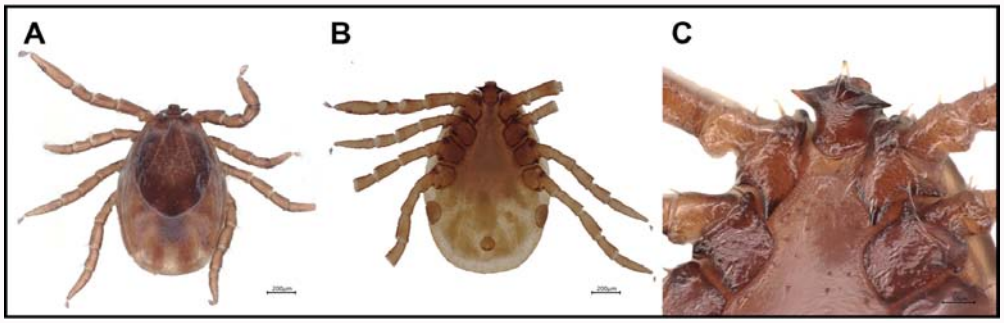

Figure 3: *Ixodes scapularis* nymph showing abnormal morphological characteristics: A. dorsal view, entire body, B. ventral view, entire body, and C. ventral view, anterior section.

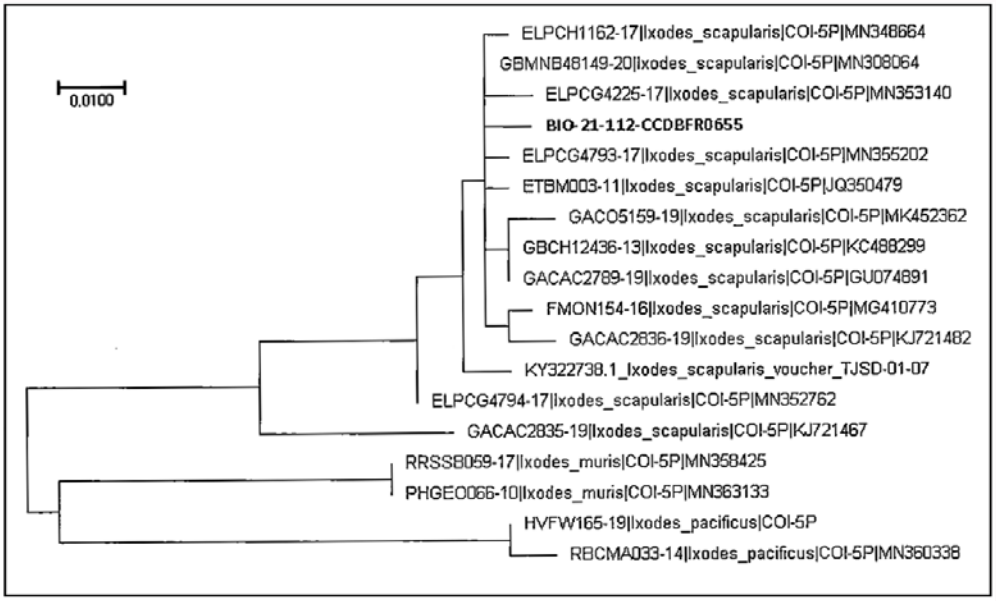

Figure 4: Molecular phylogenetic tree of sample BIO-21-112-CCDBFR0655 is compared to known *Ixodes scapularis*, *Ixodes pacificus*, and *Ixodes muris* reference sequences from BOLD analysis by using the Maximum Likelihood method. The corrected distance is 10%.

gradual decrease (-3.3% per yr) [35]. We show plausible evidence that environmental contaminants may be a contributing factor in species trends and, in this particular situation, population decline of American Robins [35]. Based on our findings, it appears that teratogens in the environment are playing a role in this decrease.

Several organic chemicals can have teratological effects on *I. scapularis* ticks. The lead author of the present study has observed extra legs on *I. scapularis* adults collected in 1997 at Point Pelee National Park (PPNP) located south of Leamington, Ontario. From 1931 to 1973, the central part of the park was an apple orchard that was actively farmed, and chlorinated hydrocarbons were used for insect pest and disease control [36]. Not only did DDT have long-lasting teratogenic effect, it acted as a borreliacidal agent at PPNP until 2000 to 2005. During the highly contaminated period, the Bbsl was nil [37-39]. Based on the findings of independent ecologists, the expiration of five amphibian and six snake species was attributed to heavy use of chlorinated hydrocarbons in the apple orchards, These pesticides persisted for over 30 yr following the agricultural period [40].

Conclusion

Initially, we attempted to use in-house taxonomic expertise to identify the nymphal tick that exhibited malformations. Since we could not find a suitable match, we employed molecular identification to determine the basic nomenclature of an *Ixodes* tick. Based on DNA sequencing and phylogenetic analysis, we authenticate that the unusual nymph was a 99.6% match to archetypal *I. scapularis*. When morphological abnormalities form in ticks, DNA barcoding provides an authentic methodology to confirm the identification.

Author Contributions

Conceptualization, J.D.S.; methodology, N.N. and J.T.A.M; software, N.N.; validation, J.D.S. and E.V.Z.; formal analysis, N.N.; investigation, J.D.S. and J.T.A.M.; resources, J.D.S., N.N., J.T.A.M.; data curation, N.N.; writing-original draft preparation, J.D.S., N.N.; writing-review and editing, J.D.S. and E.V.Z.; supervision, J.D.S. and E.V.Z.; project administration, J.D.S. All authors have read and agreed to the published version of the manuscript.

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References

1. Nicholson WA, Sonenshine DE, Noden BH. Ticks (*Ixodida*). In Medical and Veterinary Entomology, 3rd Ed; Mullen GR, Durden LA, editors. Academic Press. London, UK. 2019;603-672.
2. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease a tick-borne spirochetosis? Science. 1982;216(4552):1317-9.
3. Wilske B, Preac-Mursic V, Göbel UB, Graf B, Jauris S, Soutschek E, et al. An OspA serotyping system for *Borrelia burgdorferi* based on reactivity of monoclonal antibodies and OspA sequence analysis. J Clin Microbiol. 1993;31(2):340-50.
4. Jobe DA, Lovrich SD, Oldenburg DG, Kowalski TJ, Callister SM. *Borrelia miyamotoi* infection in patients from upper Midwestern United States, 2014-2015. Emerg Infect Dis. 2016;22(8):1471-3.
5. Wagemakers A, Oei A, Fikrig MM, Miellet WR, Hovius JW. The relapsing fever spirochete *Borrelia miyamotoi* is cultivable in a modified Kelly-Pettenkofer medium, and is resistant to human complement. Parasit Vectors. 2014;7:418.
6. Adelson ME, Rao RV, Tilton RC, Cabets K, Eskow E, Fein L, et al. Prevalence of *Borrelia burgdorferi, Bartonella* spp., *Babesia microti*, and *Anaplasma phagocytophila in Ixodes scapularis* ticks collected in northern New Jersey. J Clin Microbiol. 2004;42:2799-2801.
7. Breitschwerdt EB, Bradley JM, Maggi RG, Lashnits E, Reicherter P. *Bartonella* Associated Cutaneous Lesions (BACL) in people with neuropsychiatric symptoms. Pathogens. 2020;9(12):1023.
8. Narurkar R, Mamonska-Dyga A, Nelson JC, Liu D. Autoimmune hemolytic anemic associated with babesiosis. Biomark Res. 2017;5:14.

9. Scott JD, Sajid MS, Pascoe EL, Foley JE. Detection of *Babesia odocoilei* in humans with babesiosis symptoms. Diagnostics. 2021;11(6):947.

10. Eskow E, Adelson ME, Rao RVS, Mordechai E. Evidence for disseminated *Mycoplasma fermentans* in New Jersey residents with antecedent tick attachment and subsequent musculoskeletal symptoms. J Clin Rheumatol. 2003;9(2):77-87.

11. Dumler JS, Choi KS, Garcia-Garcia JC, Barat NS, Scorpio DG, Garyu JW, et al. Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*. Emerg Infect Dis. 2005;11(12):1828-34.

12. Kandhi S, Ghazanfar H, Qureshi ZA, Kalangi H, Jyala A, Perez ESA. An atypical presentation of a severe case of *Anaplasma phagocytophilum*. Cureus. 2022;14(3):e23224.

13. Johnson TI, Graham CB, Maes SE, Hojgaard A, Fleshman A, Boegler KA, et al. Prevalence and distribution of seven human pathogens in host-seeking *Ixodes scapularis* (Acari: Ixodidae) nymphs in Minnesota, USA. Ticks Tick Borne Dis. 2018;9(6):1499-507.

14. Swanson KI, Norris DE. Co-circulating microorganisms in questing *Ixodes scapularis* nymphs in Maryland. J Vector Ecol. 2007;32:243-51.

15. Anderson JF, Armstrong PM. Prevalence and genetic characterization of Powassan virus strains infecting *Ixodes scapularis* in Connecticut. An J Trop Med Hyg. 2012;87(4):754-9.

16. Sanchez-Vincente S, Tagliafierro T, Coleman JL, Benach JL, Tokarz R. Polymicrobial nature of tick-borne diseases. M Bio. 2019;10(5):e02055-19.

17. Jia N, Zheng YC, Jiang JF, Jiang RR, Jiang BG, Wei R, et al. Human babesiosis caused by a *Babesia crassa*-like pathogen: A case series. Clin Infect Dis. 2018;67(7):1100-19.

18. Zintl A, Mulcahy G, Skerrett HE, Taylor SM, Gray JS. *Babesia divergens*: A bovine blood parasite of veterinary and zoonotic importance. Clin Microbiol Rev. 2003;16(4):622-36.

19. Herwaldt B, Persing DH, Precigout EA, Goff WL, Mathiesen DA, Taylor PW, et al. A fatal case of babesiosis in Missouri: Identification of another piroplasm that infects humans. Ann Intern Med. 1996;124(7):643-50.

20. Conrad PA, Kjemtrup AM, Carreno RA, Thomford J, Wainwright K, Eberhard M, et al. Description of *Babesia duncani* n. sp. (Apicomplexa: Babesiidae) from humans and its differentiation from other piroplasms. Int J Parasitol. 2006;36(7):779-89.

21. Gray JS, Weiss LM. *Babesia microti*. In: Emerging Protozoan Pathogens, Chan N, editor. Taylor and Francis, Abingdon, UK. 2008;303-49.

22. Kim JY, Cho SH, Joo HN, Tsuji M, Cho SR, Park IJ, et al. First case of human babesiosis in Korea: Detection and characterization of a novel type of *Babesia* sp. (K01) similar to ovine *Babesia*. J Clin Microbiol. 2007;45(6):2084-7.

23. Man SQ, Qiao K, Cui J, Feng M, Fu YF, Cheng XJ. A case of human infection with a novel *Babesia* species in China. Infect Dis Poverty. 2016;5:28.

24. Shih CM, Liu LP, Chung WC, Ong SJ, Wang CC. Human babesiosis in Taiwan: Asymptomatic infection with a *Babesia microti*-like organism in a Taiwanese woman. J Clin Microbiol. 1997;35(2):450-4.

25. Kjemtrup AM, Conrad PA. Human babesiosis: An emerging tick-borne disease. Int J Parasitol. 2000;30(12-13):1323-37.

26. Herwaldt BL, Cacciò S, Gherlinzoni F, Aspöck H, Slemenda SB, Piccaluga P, et al. Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe. Emerg Infect Dis. 2003;9(8):942-8.

27. Scott JD, Pesapane RR. Detection of *Anaplasma phagocytophilum*, *Babesia odocoilei*, *Babesia* sp., *Borrelia burgdorferi* sensu lato, and *Hepatozoon canis* in *Ixodes scapularis* ticks collected in eastern Canada. Pathogens. 2012;10(10):1265.

28. Ivanova NV, Dewaard JR, Hebert PDN. An inexpensive, automation-friendly protocol for recovering high quality DNA. Mol Ecol Notes. 2006;6:998-1002.

29. Hernández-Triana LM, Prosser SW, Rodríguez-Perex MA, Charerri LG, Hebert PDN, Gregory TR. Recovery of DNA barcodes from black fly museum specimens (Diptera: Simuliidae) using primer sets that target a variety of sequence lengths. Mol Ecol Resour. 2014;14(3):508-18.

30. TamuraK, Nei M. Estimation of the number of nucleotide substitutions in the region of the mitochondrial DNA in humans and chimpanzees. Mol Biol Evol. 1993;10(3):512-26.

31. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetic analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870-4.

32. Scott JD, Foley JE, Young MR, Durden LA. First report of a blacklegged tick, *Ixodes scapularis* Say (Acari: Ixodidae), parasitizing a raptor in Canada. Syst Appl Acarol. 2017;22(2):208-16.

33. Kaufman R W. Integument and ecdysis. In Biology of ticks. 2nd Ed. Sonenshine DE, Roe RM, editors. Oxford University Press. Oxford, UK. 2013;1:99-196.

34. Jørgensen SE, Fath BD. Teratogenesis. In Encyclopedia of Ecology, 2nd Ed. Conley JM, Richard SM, editors. Elsevier. Amsterdam, Netherlands. 2008;3528-3536.

35. Birds Canada. NatureCounts. 2022.

36. Metcalf CL, Flint WP, Metcalf PL. Destructive and useful insects their habits and control, 4th Ed. McGraw-Hill Book Company, New York. 1962;684-778.

37. Banerjee SN, Morshed MG, Scott JD. Epizootiology of the Lyme disease spirochete, *Borrelia burgdorferi* in blacklegged tick, *Ixodes scapularis* and small mammals at Point Pelee National Park. 2000.

38. Banerjee SN, Banerjee M, Fernando K, Scott JD, Mann R, Morshed MG. Presence of spirochete causing Lyme disease, *Borrelia burgdorferi*, in the blacklegged tick, *Ixodes scapularis*, in southern Ontario. CMAJ. 2000;162(11):1567-9.

39. Thorndyke, R. Blacklegged tick (*Ixodes scapularis*) collection for testing for the Lyme disease bacteria (*Borrelia burgdorferi*) at Point Pelee National Park of Canada, 2012. Parks Canada. 2013.

40. Markle CE, Chow-Fraser G, Chow-Fraser P. Long-term habitat changes in a protected area: Implications for herpetofauna habitat management and restoration. PLoS One. 2018;13(2):e0192134.