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Short Communication

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First Record of *Carios kelleyi* (Acari: Ixodida: Argasidae) in New Jersey, United States and Implications for Public Health

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Abstract

The soft tick *Carios kelleyi* (Cooley and Kohls), a parasite of bats known to occur in at least 29 of the 48 conterminous U.S. states, is here reported from New Jersey for the first time, based on larvae collected from big brown bats, *Eptesicus fuscus*. Although thought to be widespread in North America, the ecology of *C. kelleyi* is not well understood, despite reports of this species feeding on humans and its consequent potential as a disease vector. The association of *C. kelleyi* with bat species that regularly roost in human-made structures, such as attics and barns, and recent isolations from this tick of pathogens capable of infecting humans, companion animals, and livestock underscore the need for further studies of these bat ectoparasites.

Key words: *Ornithodoros*, big brown bat, *Eptesicus fuscus*, Vespertilionidae, vector borne diseases

Carios kelleyi (Cooley & Kohls, 1941), a so-called soft tick (Ixodida: Argasidae), that is almost exclusively a parasite of bats, has been reported from 29 of the conterminous U.S. states, as well as southern Alberta and Saskatchewan, Canada, Mexico, Costa Rica, and Cuba (Anderson et al. 1984, Vargas 1984, Bowles et al. 2013, Lindquist et al. 2016).

Unlike 'hard ticks' in the family Ixodidae, argasid nymphs and adults take multiple blood meals for short periods of time (minutes to hours); only argasid larvae remain attached and feed for several days (Sonenshine and Anastos 1960). *Carios kelleyi* is an endophilous species, which means they shelter in the cracks and crevices of bat roosts, including those in attics and barns, when not host-seeking or on-host and are therefore very unlikely to be collected during standard tick surveys from the environment (Eisen and Paddock 2020). In the northeastern United States, *C. kelleyi* is known to feed on bats in the genera *Eptesicus* and *Myotis* (Chiroptera: Vespertilionidae) (Dick et al. 2003) and has been collected in Connecticut, Maryland, New York, and Pennsylvania (Bowles et al. 2013). Here we report the first collections of *C. kelleyi* from New Jersey and review the potential epidemiological implications of this finding.

Materials and Methods

The Endangered and Nongame Species Program of the New Jersey Department of Environmental Protection, Division of Fish and Wildlife, has various ongoing efforts to monitor the population dynamics, habitat utilization, vulnerabilities, and conservation status of native bat species statewide. As part of regular summer mist-netting investigations, Division personnel surveyed for bats in Titusville, Mercer County, New Jersey (40.316459N, 74.881845W) on 12 June 2019, and in Sandyston Township, Sussex County (41.235515N, 74.746736W) on 26 June 2019. The surveys were performed using mist nets in standard 'triple-high' and 'double-high' pole set configurations (Bat Conservation and Management, Carlisle, PA), with poles supporting polyester 38 mm mesh nets specific for capturing bats (Avinet Research

Supplies, Portland, ME). At each location, three net arrays were deployed along a roughly 150-meter segment of stream corridor such that the width (9–12 m) and height (5–8 m) of the netting filled the flyway area between the stream's forested banks and the overhead canopy. Nets were raised into position at dusk and remained open for approximately 5 h. Bats captured during the survey were individually extracted from the nets and handled by experienced, rabies virus-vaccinated personnel. Standard data on bat species, age class, sex, reproductive status, weight, forearm measurement, and wing damage index (related to white-nose syndrome) were recorded for each bat but are not included in this report. When during normal handling and inspection of the bats with gloved hands, embedded ticks were noticed, which was noteworthy as an unusual event, they were carefully removed with forceps and placed alive in labeled vials. Ethanol (70%) was added on returning to the lab. Bats were given a unique, state-issued forearm band (Porzana Limited, East Sussex, United Kingdom) and released at the site of capture.

In the laboratory, tick specimens were initially identified to genus using standard keys (Cooley and Kohls 1944). All ticks were found to have been feeding at length and were consequently blood-engorged (Fig. 1), but morphological species determination was facilitated by the presence in some specimens of an intact hypostome. After conclusively determining one complete specimen as *C. kelleyi*, we extracted DNA from two of its legs using the 'HotSHOT' (hot sodium hydroxide and Tris) method (Johnson et al. 2015) in a final volume of 20 ul. We then amplified the standard barcoding locus of the mitochondrial cytochrome oxidase 1 gene (*cox*1) with Amplitaq Gold Fast Master Mix (ThermoFisher Scientific, Waltham, MA) using a nested protocol using first primers LEP-F1 and LEP-R1 (Hebert et al. 2004) and then the internal primers LCO1490 and HCO2198 (Folmer et al. 1994) in two successive reactions. In both cases, we started with a 'touch-down' where the annealing temperature (T_a) was decreased from 53 to 49°C over 5 cycles, followed by 35 or 20 cycles using a T_a of 48°C, for the first and second polymerase chain reaction (PCR), respectively. The final PCR products were purified with ExoSAP-IT (ThermoFisher Scientific, Waltham, MA), and both strands were cycle-sequenced with each internal primer (Genscript, Piscataway, NJ). Consensus sequences were aligned using Sequencher (GeneCodes) and submitted to the National Center for Biotechnology Information BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to search for similar sequences.

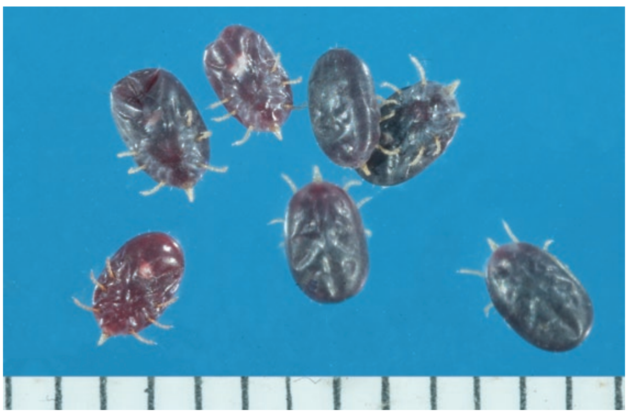

Fig. 1. Mix of dorsal and ventral views of a group of *Carios kelleyi* larvae obtained from New Jersey big brown bats. Notice all specimens are blood-engorged. The scale is in millimeters.

Results

A total of 15 larval argasid ticks, all of them *C. kelleyi*, were obtained from three big brown bats, *Eptesicus fuscus* (Palisot de Beauvois, 1796), in two New Jersey counties. At the Titusville site in Mercer County, seven *E. fuscus* were trapped, two of which were parasitized (nine larval ticks on one bat, three on the other). At the Sandyston site, in Sussex County, one *E. fuscus* had three larval ticks, while three other *E. fuscus* and one *Lasiurus borealis* (Müller, 1776) (eastern red bat (Chiroptera: Vespertilionidae)) yielded no ticks. Three of the tick specimens from Mercer County possessed a complete hypostome and were morphologically identified as *C. kelleyi*, a species that has also been classified in the genus *Alectorobius* and, originally, *Ornithodoros*. However, as discussed in depth by several specialists (Guglielmone et al. 2010, Guzmán-Cornejo et al. 2019, Mans et al. 2019), numerous competing genus-level classifications have been proposed for the Argasidae, based on both morphological and molecular analyses, yet there remains no agreement as to which genus most argasid species belong. For those investigators who recognize *Carios* as a valid genus, micromorphological genus characters are provided in the keys of Lindquist et al. (2016). Two voucher specimen of *C. kelleyi* were submitted to the Yale Peabody Museum; Accession#: YPM-ENT 311449 and YPM-ENT 311450 for specimen from Mercer Co. and Sussex Co., respectively.

As at the time there were no DNA sequences in GenBank under the names *Carios kelleyi* or *Ornithodoros kelleyi*, the best match we obtained to the 534 bp cytochrome oxidase sequence we submitted was accession KY678235.1 (84.5%; 100% query cover) for *Ornithodoros faccini* (*O. faccini* identified by Barros-Battesti et al. 2015). The next best match was accession KX712088.1 (82.3%; 100% query cover) for *Nothoaspis amazoniensis* (*N. amazoniensis* identified by Nava et al. 2010). Once the morphological identification was confirmed, we submitted the *cox*1 barcode for *C. kelleyi* to GenBank (accession# MT780277).

Discussion

We report the first collections of *C. kelleyi* in New Jersey from big brown bats, *E. fuscus*, in Mercer and Sussex counties. The infestation rate of 0–9 larval ticks per bat matches those reported elsewhere (Mitchell and Hitchcock 1965, Steinlein et al. 2001, Dick et al. 2003, Lausen 2005). Technically, this is not the first species of *Carios* collected in New Jersey since, in 2001, a 90–94 million-year-old specimen described as *Carios jerseyi* Klompen and Grimaldi was found in amber from Middlesex County (Klompen and Grimaldi 2001).

The detection of *C. kelleyi* in New Jersey is not altogether surprising because this species has been reported from neighboring states (Bowles et al. 2013), and because collections of this tick appear to be primarily opportunistic. In this case, the NJ Division of Fish and Wildlife personnel handling the bats were not specifically surveying for ticks. Instead, they were surprised by the unusual finding of ticks on *E. fuscus* in New Jersey. While anecdoctal, this raises the possibility of a recent increase in prevalence. Therefore, it is worth assessing and summarizing what is known about the epidemiological risk of *C. kelleyi* and similar soft ticks as disease vectors.

Soft ticks in the genus *Ornithodoros* that primarily feed on small rodents are well-known vectors of relapsing fever *Borrelia*, a group of pathogens needing additional study (Talagrand-Reboul et al. 2018). In the western United States, exposure to relapsing fever *Borrelia* chiefly occurs during the spring and summer months, when mountain cabins are cleaned of rodents in advance of visitors, and the loss of rodents drives argasid ticks to seek blood meals from humans (Trevejo et al. 1998, Schwan et al. 2009). Similarly, while the risk of human bites from *C. kelleyi* is thought to be low, there are reports of *C. kelleyi* feeding on humans when their preferred hosts (bats) are removed by property owners, either out of fear, health concerns, or perceived nuisance (Sonenshine and Roe 2013). For example, a large infestation (potentially hundreds) of *C. kelleyi* was reported in a home in Iowa following the removal of a bat colony. One engorged *C. kelleyi* nymph containing human blood was retrieved from the home (Gill et al. 2004).

Significantly, *C. kelleyi* has been found infected with a novel spotted fever *Rickettsia*, a novel relapsing fever-related *Borrelia*, and *Bartonella henselae* (Loftis et al. 2005). Also, a novel relapsing fever spirochete, identified as *Borrelia johnsonii*, was found in *C. kelleyi* in Iowa (Schwan et al. 2009), and a recent survey of over 7,000 blood samples from Wisconsin patients thought to have been suffering from tick-borne diseases found one positive sample for *B. johnsonii* by quantitative PCR (Kingry et al. 2018).

In conclusion, while there is currently limited evidence that *C. kelleyi* is an important vector of pathogens (Reeves et al. 2006), their prevalence on synanthropic *E. fuscus* may be increasing in New Jersey, creating the potential for pathogen transmission to humans, companion animals, and livestock. Specific monitoring of their occurrence and association with New Jersey bats as well as of any pathogens they may harbor is warranted to assess the possibility of *C. kelleyi* becoming a disease vector of zoonotic or veterinary concern (Gill et al. 2004).

Ethics

The New Jersey Division of Fish and Wildlife is not considered a research 'facility' and, as such, is not subject to Institutional Animal Care and Use Committee (IACUC) requirements; however, all the Division's federally funded objectives and methods are reviewed and approved by the U.S. Fish and Wildlife Service, including the activities described herein.

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