Article DOI: https://doi.org/10.3201/eid3010.240821

Migration of *Amblyomma maculatum* Ticks and *Rickettsia parkeri* in the Northeastern United States

Appendix

Additional Methods

Initial examination of the tick at the Connecticut Agricultural Experiment Station Tick Testing Laboratory using a dissecting microscope and a standard taxonomic key (*1*) revealed that the tick was a female *Amblyomma maculatum*. To corroborate morphological identification, DNA was extracted from the tick specimen using DNAzol BD (Molecular Research Center, https://www.mrcgene.com) as previously described (*2*). PCR assay was conducted to amplify a portion of the ribosomal internal transcribed spacer 2 (*ITS2*) region using the following primer pair: 5′–CGAGACTTGGTGTGAATTGCA–3′ (forward) and 5′–TCCCATACACCACATTTCCCG–3′ (reverse) (*3*). A Taq PCR Core Kit (QIAGEN, https://www.qiagen.com) and Applied Biosystems Veriti Thermal Cycler (ThermoFisher Scientific, https://www.thermofisher.com) were used to conduct PCR assays consistent with the manufacturer's instructions. PCR amplicons were purified using the QIAquick PCR purification kit (QIAGEN), and double stranded sequencing was performed at the Keck DNA Sequencing Facility, Yale University (New Haven, Connecticut, USA). Sequences were subsequently annotated using ChromasPro version 2.1.8 (Technelysium, https://technelysium.com.au) and submitted to the NCBI GenBank to compare with available sequences in the database. The specimen was determined to be *A. maculatum* based on 100% pairwise identity with several sequences of that tick species in GenBank. The sequence was later submitted to GenBank (accession no. OR625121.1).

A PCR assay was performed to amplify a 645-basepair portion of a rickettsial outer membrane protein gene (*omp*A) using the following primer pair: 5′–

ATGGCGAATATTTCTCCAAAA–3′ (forward) and 5′–ATTACCTATTGTTCCGTTAATGGCA–3′ (reverse) (*4*). PCR and sequencing of the purified amplicons were performed as described above. The annotated sequence was identified as *Rickettsia parkeri* based on >99% percent identity with several sequences belonging to this species in the GenBank database. The sequence was subsequently submitted to GenBank (accession no. OR517310.1).

An indirect immunofluorescence antibody (IFA) assay to detect IgG antibodies reactive with *Rickettsia parkeri* antigens was performed at the Centers for Disease Control and Prevention, as described previously (*5*).

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