Additional U.S. collections of the Gulf Coast tick, *Amblyomma maculatum* (Acari: Ixodidae), from the State of Delaware, the first reported field collections of adult specimens from the State of Maryland, and data regarding this tick from surveillance of migratory songbirds in Maryland

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Abstract

This report describes collections of the Gulf Coast tick, *Amblyomma maculatum* Koch (Ixodida: Ixodidae), made during 2013 at the Bombay Hook National Wildlife Refuge (NWR), Delaware, and at two sites in Maryland: the Blackwater NWR and the Chester River Field Research Station (CRFRS). Ticks were collected via field drags, dry ice-baited traps, and/or from the human field researchers (collections of ticks crawling on clothing/boots) at Bombay Hook NWR and Blackwater NWR. A total of 21 *A. maculatum* were successfully collected at Bombay Hook NWR during May 28–30, 2013. Using a genus-specific quantitative real-time polymerase chain reaction (qPCR) assay and species-specific qPCR assays, a single male was found to be positive for the presence of *Rickettsia parkeri* DNA (a spotted fever group rickettsia). The repeated collection of this species in the adult stage at Bombay Hook NWR, the relatively large number collected (n=21), along with its continuity of presence pre- and post-winter, indicate that an established population may now exist at Bombay Hook NWR. A single adult female was collected from a field drag at Blackwater NWR on June 18, 2013; this specimen was negative for the presence of *Rickettsia* spp. DNA (including *R. parkeri* DNA). An adult male was collected on a researcher at CRFRS on August 8, 2013; this specimen was found to be positive for *R. parkeri* DNA. This report also summarizes data from 2008 to 2010 for *A. maculatum* collected during mist netting surveillance of migratory songbirds by the Foreman’s Branch Bird Observatory, located at CRFRS: a total of 104 immature *A. maculatum* were collected. The adult specimens of *A. maculatum* collected at Blackwater NWR and at CRFRS are regarded as representing the first documentation of adult field-collected *A. maculatum* within the state. Future sampling is needed at each location to determine if *A. maculatum* is firmly established, the prevalence of *R. parkeri* infection, and the epidemiological risk to humans.

Key words: *Amblyomma maculatum*, *Rickettsia parkeri*, Delaware, Maryland, U.S.A.
Introduction

The Nearctic and Neotropical Gulf Coast tick, *Amblyomma maculatum* Koch, and the associated human pathogen *Rickettsia parkeri* are increasingly being detected in the mid-Atlantic region of the United States (Sumner et al. 2007, Teel et al. 2010, Fornadel et al. 2011, Varela-Stokes et al. 2011, Wright et al. 2011, Jiang et al. 2012). Florin et al. (2013) reported the presence of *A. maculatum* and *R. parkeri* at the Bombay Hook National Wildlife Refuge (NWR), DE, after a 2-day collecting trip during May 2012. As a follow-up to that report, subsequent sampling events were conducted at the Bombay Hook NWR, the Blackwater NWR, MD, and the Chester River Field Research Station (CRFRS), Chestertown, MD. In addition, data from the Foreman’s Branch Bird Observatory (FBBO) on ticks collected during 2008–2010 bird banding operations at CRFRS are reported here.

Materials and methods

This report describes sampling events at Bombay Hook NWR (May 28–30, 2013), at Blackwater NWR (June 17–19, 2013), and at CRFRS (summer 2013), as well as tick collections from the annual surveillance of songbirds by FBBO at CRFRS (March through November, 2008–2010). Ticks were collected at Bombay Hook NWR and Blackwater NWR by three methods: field drags, dry ice-baited traps, and simply collecting all crawling ticks found on the clothing/boots of the field researchers. This last method was the sole collection technique used at CRFRS during the summer of 2013. All tick specimens were immediately preserved in 70% ethanol and later transported to the Naval Medical Research Center, Silver Spring, MD (collections from Bombay Hook NWR and Blackwater NWR) or to Old Dominion University, VA (collections from CRFRS) to test for the presence of *Rickettsia*. Only ticks morphologically identified as *A. maculatum* were subsequently tested for the presence of *Rickettsia* with a genus-specific quantitative real-time polymerase chain reaction (qPCR) assay and then, if positive, with *Rickettsia parkeri*-specific and *Candidatus Rickettsia andeanae*-specific qPCR assays, as described in Jiang et al. (2012).

Migratory and resident songbirds were sampled and checked for ticks during bird banding operations by FBBO (under appropriate state and federal bird collecting permits) at the CRFRS site; data regarding *A. maculatum* are presented here for the period March-November during 2008–2010. Up to 100 Japanese mist nets (6 m and 12 m) were set at this site in ecotone habitats between croplands and upland and wetland woodlots and were open between roughly 0600 hrs and 1300 hrs each day. Time permitting, each captured bird was thoroughly inspected for ticks, and data on bird species, sex, age, wing chord, and body condition were collected. Ticks from each bird were placed in uniquely numbered 2 ml screw-cap vials containing 70% ethanol and were sent to the University of Richmond for species identification (Durden and Keirans 1996, Keirans and Durden 1998).

Results and discussion

At Bombay Hook NWR, near Smyrna, DE, successful collection of *A. maculatum* occurred only at the site previously described in Florin et al. (2013), although several other sites within Bombay Hook NWR were sampled. The May 28–30, 2013 sampling event produced a total of 21 adult *A. maculatum* (7♂, 14♀), with approximately equal numbers collected by the drag and trap methods. Only a single male specimen was positive for *Rickettsia* genus-specific 17-kDa gene (*htrA*); this specimen subsequently tested positive for the *Rickettsia parkeri*-specific qPCR assay and negative
for the Candidatus Rickettsia andeanae-specific qPCR assay. The repeated collection of the adult stage at the same site, the relatively large number collected (n=21), and the continuity of pre- and post-winter presence indicates that an established population may now exist at Bombay Hook NWR. The mist netting operations by the FBBO produced a total of 4350 immature and 39 adult ticks from the surveyed birds, with 130 of these 4389 ticks identified as A. maculatum by light microscopy (125 larvae, 5 nymphs). Because of the challenges associated with morphological identification of immature ixodid ticks, especially those that are engorged or damaged when removed from hosts, we used sequence analysis of the 16S gene to confirm species identity on a subset of purported A. maculatum larvae and found that 4 of 5 ticks were identified correctly, with one tick confirmed as the congener A. americanum (Linnaeus). Thus, we can reasonably estimate that immature A. maculatum make up about 2.4% (104/4350) of immature ticks on passerine birds at FBBO, even accounting for errors in morphological species identification due to damaged samples. The birds from which these ticks were recovered represent both resident and migratory species (Table 1), many of which are among the bird species known to be most heavily parasitized by ticks in the eastern United States (Brinkerhoff et al. 2011). We failed to detect A. maculatum on birds before mid-July, in contrast to other tick species that occur on birds in spring and/or fall months. The temporal pattern of occurrence of immature A. maculatum on birds is reflective of the overall picture of tick parasitism, with a peak in immatures occurring in late summer when larval ixodid ticks are most active and abundant (Fig.1). We note that the areas under each of the two curves in Fig.1 are equivalent, and the higher peak of the A. maculatum curve represents more restricted seasonal activity rather than a large number of collected ticks of this species.

### TABLE 1. Species of songbirds sampled at the Chester River Field Research Station from which immature A. maculatum were recovered during 2008 through 2010. Numbers in parentheses indicate numbers of larvae and nymphs, respectively. No adult A. maculatum were collected from birds.

<table>
<thead>
<tr>
<th>Bird species, number of individuals from which ticks were collected</th>
<th>Resident/ migratory species</th>
<th>Number of immature A. maculatum collected (larvae, nymphs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grasshopper Sparrow (Ammodramus savannarum), 3</td>
<td>Migrant</td>
<td>24 (23, 1)</td>
</tr>
<tr>
<td>Common Yellowthroat (Geothlypis trichas), 7</td>
<td>Migrant</td>
<td>21 (larvae only)</td>
</tr>
<tr>
<td>Song Sparrow (Melospiza melodia), 2</td>
<td>Resident</td>
<td>20 (larvae only)</td>
</tr>
<tr>
<td>Wood Thrush (Hylocichla mustelina), 3</td>
<td>Migrant</td>
<td>13 (larvae only)</td>
</tr>
<tr>
<td>Carolina Wren (Thryothorus ludovicianus), 6</td>
<td>Resident</td>
<td>12 (11, 1)</td>
</tr>
<tr>
<td>Indigo Bunting (Passerina cyanea), 3</td>
<td>Migrant</td>
<td>11 (larvae only)</td>
</tr>
<tr>
<td>Field Sparrow (Spizella pusilla), 5</td>
<td>Resident</td>
<td>9 (larvae only)</td>
</tr>
<tr>
<td>Brown Thrasher (Toxostoma rufum), 2</td>
<td>Resident</td>
<td>6 (larvae only)</td>
</tr>
<tr>
<td>House Wren (Troglodytes aedon), 3</td>
<td>Migrant</td>
<td>5 (larvae only)</td>
</tr>
<tr>
<td>Blue Grosbeak (Passerina caerula), 1</td>
<td>Migrant</td>
<td>1 (nymph)</td>
</tr>
<tr>
<td>Gray Catbird (Dumetella carolinensis), 1</td>
<td>Resident</td>
<td>1 (larva)</td>
</tr>
<tr>
<td>Northern Cardinal (Cardinalis cardinalis), 1</td>
<td>Resident</td>
<td>1 (nymph)</td>
</tr>
<tr>
<td>Yellow-breasted Chat (Icteria virens), 1</td>
<td>Migrant</td>
<td>1 (nymph)</td>
</tr>
</tbody>
</table>

*Resident species are those that are known to overwinter in eastern Maryland, USA; however, individual birds may or may not spend the winter at this field site.
Although several disparate sites were sampled within Blackwater NWR, only one site resulted in collection of *A. maculatum*: a single adult female was collected via a field drag on June 18, 2013 in highly disturbed, secondary growth habitat (GPS coordinates N38° 26’ 40.6”, W076° 05’ 45.3”) located approximately 90 m from the Visitors Center/Gift Shop. This specimen was tested for the presence of *Rickettsia* and found to be negative.

On August 8, 2013, a single adult male *A. maculatum* was found crawling on a human at CRFRS and was subsequently submitted to Old Dominion University for analysis. This tick was found to be positive for *R. parkeri* DNA. The presence of *A. maculatum* in Maryland has been documented by passive surveillance (Stromdahl *et al.* 2011, Jiang *et al.* 2012), yet to our knowledge these specimens from Blackwater NWR and from CRFRS represent the first documented reports of adult *A. maculatum* collected in the field within Maryland and also the first report of an *R. parkeri* infection from a field-collected tick within Maryland (the CRFRS specimen). The occurrence of immature ticks on birds during breeding season is suggestive of an established population of *A. maculatum* at CRFRS even though adults are rarely encountered at this site. Furthermore, the occurrence of this tick on migratory bird species may account for its establishment at this site. Although we did not detect *A. maculatum* on birds during spring migration in our three years of sampling, this tick species has been recovered from migratory birds in the southern and southeastern U.S.A. in spring, fall, and winter (Kinsey *et al.* 2000, Rainwater *et al.* 2007, Robbins *et al.* 2010), and migration could conceivably move enough ticks northward to establish new populations (Scott *et al.* 2001, Ogden *et al.* 2008).
Future sampling is needed at each location to determine whether *A. maculatum* is firmly established, as well as the prevalence of *R. parkeri* infection, the epidemiological risk to humans of Tidewater spotted fever.

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