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Eleanor Fausett April 20, 2023

First report of the Newly Introduced Asian Long-horned Tick, *Haemaphysalis longicornis,* in wildlife areas in the state of Georgia, USA.

By

Eleanor Fausett
MPH

Gangarosa Department of Environmental Health

Gonzalo Vazquez Prokopec
Committee Chair

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Eleanor Fausett

B.S. Environmental Science
Emory University
2022

Gonzalo Vazquez-Prokopec, Ph.D.

An abstract of
a thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health in Environmental Health
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Abstract

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Haemaphysalis longicornis (Acari: Ixodidae), the Asian longhorned tick, is invasive in the United States. Originally from east Asia, this tick rapidly expands its range to include most of the habitats where it is introduced. From initial detection in New Jersey in 2017, *H. longicornis* has invaded over 18 states and 195 counties. The goal of this study was to determine if *H. longicornis* is established in Georgia, USA. Existing research into habitat suitability and reports of existing collections informed the selection of field sites for this study. Sites selected changed over both field seasons based on new data available from the USDA about *H. longicornis* in the USA. Over 10,000 ticks collected over 2 years were dichotomously identified and then suspected samples of *H. longicornis* received molecular confirmation. The 2022 field season involved collecting ticks from 42 State parks and wildlife management areas throughout Georgia. From all sites surveyed, we collected 3 *H. longicornis* nymphs in Buck Shoals wildlife management area in White County, Georgia. This is the first collection of *H. longicornis* on public land. This difference in habitat indicates that the general habitat in Georgia could be suitable for *H. longicornis* establishment. Further research will occur at this location and the locations previously identified as suitable to understand the establishment of this species within Georgia.

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First report of the Newly Introduced Asian Long-horned Tick, *Haemaphysalis longicornis*, in wildlife areas in the state of Georgia, USA.

Ellie Fausett[1], Oscar David Kirstein[2], Alec Thompson[3], Stephanie Bellman[1], Gonzalo Vazquez-Prokopec[1]

[1]Department of Environmental Sciences, Emory University, Atlanta, GA, USA. [2]Entomology and Parasitology Laboratory, Ministry of Health Israel, Jerusalem, Israel. [3]University of Georgia, College of Veterinary Medicine

Introduction:

The Asian longhorned tick, *Haemaphysalis longicornis* (Neumann, 1901, Ixodida : Ixodidae), while endemic to East Asia, is an invasive species that has successfully colonized New Zealand, Australia, and other Pacific Islands [1]. *H. longicornis* was recently introduced to the United States in 2010 but was misidentified as the native species *Haemaphysalis leporispalustris* for a period of seven years, until genomic sequencing revealed that the species in question was *H. longicornis* [2-4]. Between 2010 and 2018, *H. longicorins* expanded to nine US states [5], with the most recent 2023 United States Department of Agriculture (USDA) report showing further spread into 18 states and 195 counties [6]. Niche models using *H. longicorins* original distribution and climatic variables predict that most of Eastern US and the Midwest are suitable for its establishment [7, 8], with the highest suitability found throughout the Appalachian Mountain range.

Haemaphysalis longicornis is a three-host tick known to feed on over 30 different hosts within the United States [6]. The most common hosts associated with *H. longicornis* are raccoons, opossums, and white-tailed deer [9]. Most *H. longicorins* larva in New York state were found were on mid-sized animals like raccoons, cats, and opossums [9]. *Amblyomma* *americanum* larva, the dominate tick in Georgia, feeds primarily on white tailed deer, racoons, and other mid-sized animals and was found cofeeding with *H. longicornis*[10]. *H. longicornis* reproduces parthenogenetically and sexually [11], and in the US there is evidence of both types of reproduction occurring [12]. Such efficient reproductive strategies lead *H. longicornis* to out-compete native tick species, as observed in New York state where the introduction of *H. longicornis* changed the species prevalence of native tick species, increasing the prevalence of the invasive tick [13]. The combination of a wide range of hosts and multiple efficient modes of reproduction are the main drivers explaining why *H. longicorins* species is spreading rapidly. Furthermore, the parthenogenetic reproduction of *H. longicornis* leads to high density of ticks infesting animals, with examples of livestock (primarily sheep and goats) having unusually large numbers of ticks [14] that in some cases may lead to animal death due to exsanguination. In New Zealand, *H. longicornis* has developed into an economically significant pest and disease vector, negatively influencing animal health and production [1]. It vectors *Theileria orientalis Ikeda* among dairy and beef cattle [15]. A cow in Virginia infected with *Theileria orientalis Ikeda* displayed signs, and researchers were able to connect this infection with a *H. longicornis* bite, illustrating the potential for disease transmission among cattle [15]. Therefore, *H. longicornis* is consider a major agricultural problem in the US and globally [16].

H. longicornis is also considered a major public health concern [17]. In its native range, *H. longicornis* vectors bacterial, viral, and protozoal infections that cause sickness and mortality in humans [18]. For instance, in China *H. longicornis* vectors *Rickettsia japonica* and severe fever with thrombocytopenia syndrome (SFTSV, currently named Dabbie bandavirus), which raise concern that introducing the vector in the US could allow related pathogens to be locally transmitted [17]. Studies conducted within the United States prove that *Haemaphysalis* *longicornis* is a competent vector for *Anaplasma, Ehrlichia, Rickettsia,* and *Borrelia* species in some laboratory-based settings [19, 20]. The first reported *H. longicornis* bite on a human was in 2019, indicating that this tick identifies humans as a suitable host in the United States, as it does in Asia and New Zealand [21]. While the State of Georgia has habitat that is appropriate for *H. longicornis* establishment [7] yet the only findings of this species in the State have occurred on livestock pastures and private land but not on native habitat [7]. Studies on *H. longicornis'* ecological suitability show that this tick prefers grassland and edge habitats[4]. This is noteworthy because forest land comprises 66.2% of all land in the state of Georgia, with ranch and farmhouse property accounting for the remaining 13% [22]. This indicates that the invasive tick *H. longicornis* may have suitable habitat in Georgia. The movement of *H. longicornis* from agricultural land to natural habitats (state parks, wildlife management areas, local parks, hunting land) will increase the potential for human *H. longicornis* interactions. Public land such as wildlife management areas and state parks across Georgia have thousands of visitors each year and have the potential for increased human exposure to invasive ticks [23]. The previous locations were all private farms with limited opportunities for tick human interactions. It is probable that the invasive tick *H. longicornis* left farmland in Georgia and entered the general Georgia ecosystem. To assess the potential establishment of H. longicornis in natural habitats of Georgia, we conducted a tick survey in 42 state parks and wildlife management within all ecological zones of the State.

Material and Methods:

Study areas:

Surveillance was carried in different sites during 2021, 2022 and 2023 in areas selected based on existing information about the habitat suitability of *H. longicornis* [8]. In 2021, twelve locations were surveyed above the Coosa River in northwest Georgia and the Savannah River in the state's northeast. At the time of site selection, there were no confirmed reports of the tick within the state. These sites were selected due to their proximity to North Carolina, where the tick has been reported in the USDA report on *H. longicornis* prior to our survey. After the 2021 field season, *H. longicornis* was identified on farms in two counties in Georgia, USA (USDA, 2023). Due to the identification on farms, the hypothesis that the general Georgia environment is suitable for the tick was strengthen by the knowledge that the tick can survive within the state. The 2022 field season incorporated this finding into the preparation of locating new field sites. The *H. longicornis* farm locations were used to predict new locations of probable findings along with a habitat suitability mapping of potential *H. longicornis* habitats [7] where they used a MaxEnt modeling approach and most of the area north of Atlanta, Ga received a high predictability score from the model [7]. Fifteen unique locations were selected from Georgia state wildlife management areas within 50 miles of existing *H. longicornis* findings within Georgia and were surveyed twice over a four-month period (Figure 1). In addition to the area more likely to have H. longicornis, we expanded our collections to other State Parks and wildlife management areas within the State (Figure 2).

The following criteria were controlled for at all field sites to limit the interaction of outside variables: absence of livestock; suitable hosts like white-tailed deer and small mammals[6, 24]; an overall elevation less than 1000m above sea level; impervious surface cover is less than 10% of available land; no documented use of insecticides usage, and no open hunting season at the time of collection [24, 25]. In addition, all sites visited had no rain for the previous 16 hours to create favorable flagging conditions.

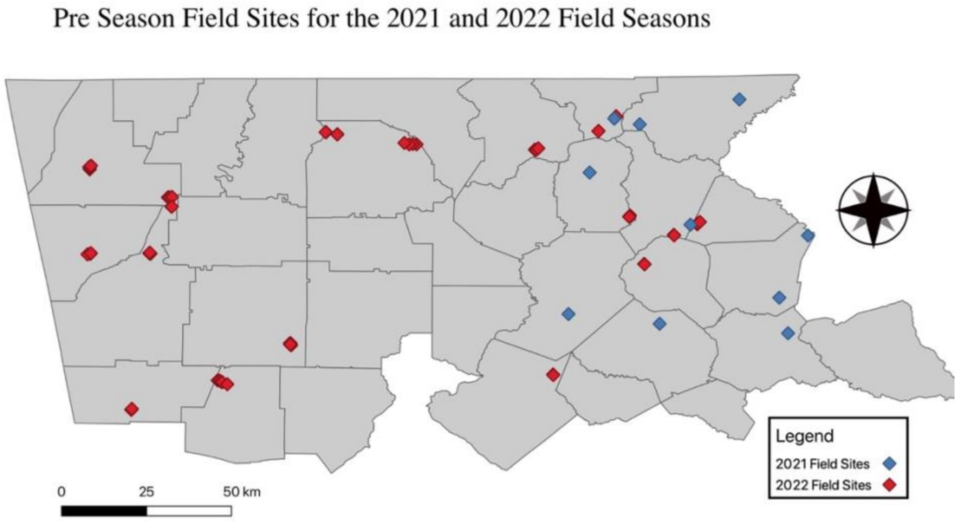


Figure 1: Habitat suitability for Georgia includes the counties above Atlanta, Ga. These counties are in gray while the 2021 field sites are in blue and the 2022 field sites are in red. These are the sites where in-depth flagging occurred for *H. longicornis* and not all the areas of tick collections.

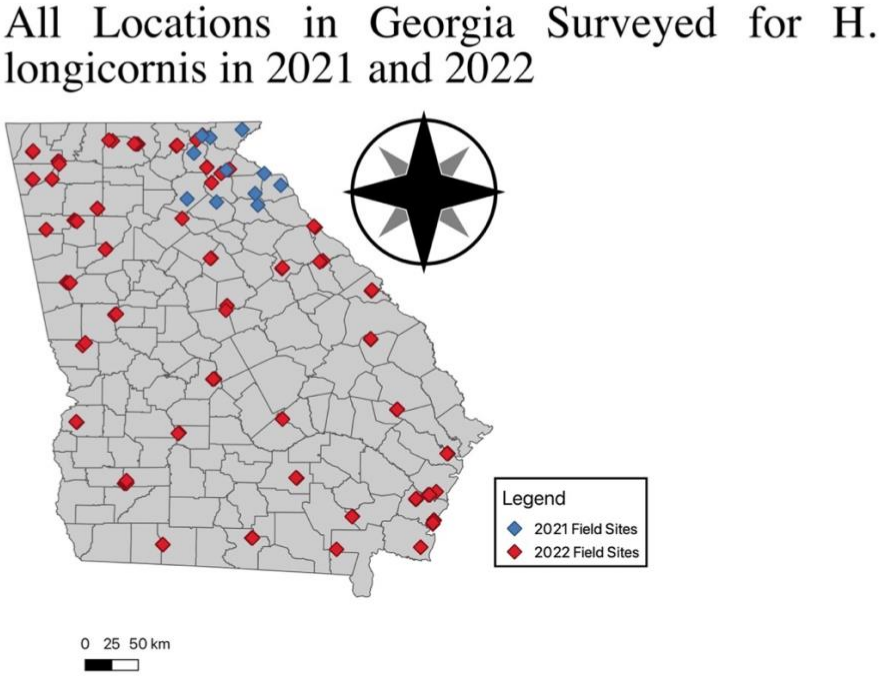


Figure 2: All flagged locations in Georgia, USA with 2021 field sites is in blue and the 2022 field sites are in red. There are 42 for the 2022 season and 12 for the 2021 season.

Field Methods

A team of field researchers preformed standard flagging protocol at all selected locations (Figure 2). Collections were carried out from May to August 2022 to align with existing trends in tick seasonality [24]. Ticks were collected using the flagging procedure and followed the standard flagging protocol [26] with one small modification. The nails holding the cloth to the pole were replaced with snaps to decrease cleaning time and prevent the unintentional introduction of invasive species to a new habitat (Figure 3). Each site had three miles of free flagging conducted by 3-5 lab members. This distance was divided into two explorations across two possible habitats at each location.

Image 1: Standard flannel flag with added snaps.

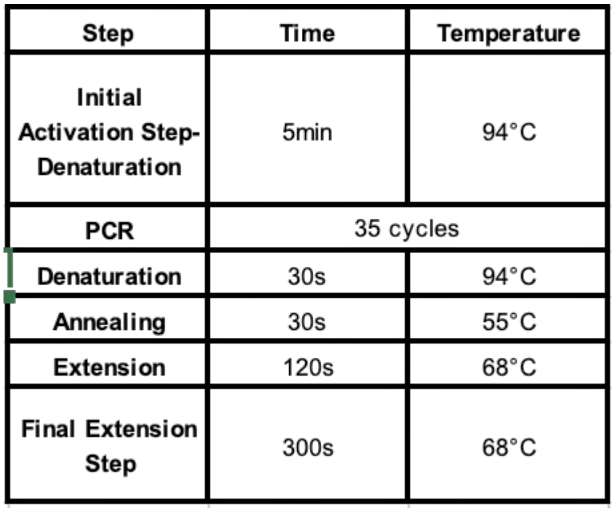
Image 2: Researchers flagging in the field.

Ticks were collected alive and brought back to the laboratory setting for processing. In the laboratory, they were cleaned with a 3% bleach solution, a 3% hydrogen peroxide solution, and then three distilled water washes. After cleaning, ticks were identified using a compound microscope and a dichotomous tick identification key. Samples were then stored in ethanol. To confirm the species identified with the dichotomous key, molecular confirmation was performed. Ticks were collected and stored in 90% ethanol in a -80 freezer while awaiting molecular confirmation. For molecular confirmation, samples were first bisected laterally and left out to evaporate off excess ethanol for 12 hours before DNA extraction. DNA extraction occurred with a commercial DNeasy Blood and Tissue Qiagen Kit and followed the manufactures protocol for insects.

After DNA was extracted, traditional PCR used the 16S rRNA and cytochrome c oxidase subunit 1 (COI) genes for amplification (Table 1 and Table 2). The PCR result were visualized on 2% agarose gels with GelRed (Biotium, Hayward, California). Amplification occurred with the QIAuick gel extraction kit. Bands were extracted from the gel and sent for Sanger Sequencing.

Primer (F and R)	Gene	Length (bp)	Reference
Cox1-F (5′GGAACAATATATTTAATTTTTGG-3′) Cox1-R (5′ATCTATCCCTACTGTAAATATATG-3′)	*COI*	849	[12]

Table 1: Primer used to amplify the target sequence in the PCR for molecular confirmation of *H. longicornis.*



Step	Time	Temperature
Initial Activation Step-Denaturation	5min	94°C
PCR	35 cycles	
Denaturation	30s	94°C
Annealing	30s	55°C
Extension	120s	68°C
Final Extension Step	300s	68°C

Table 2: Thermal profile for Polymerase Chain Reaction (PCR) to detect *H. longicornis*.

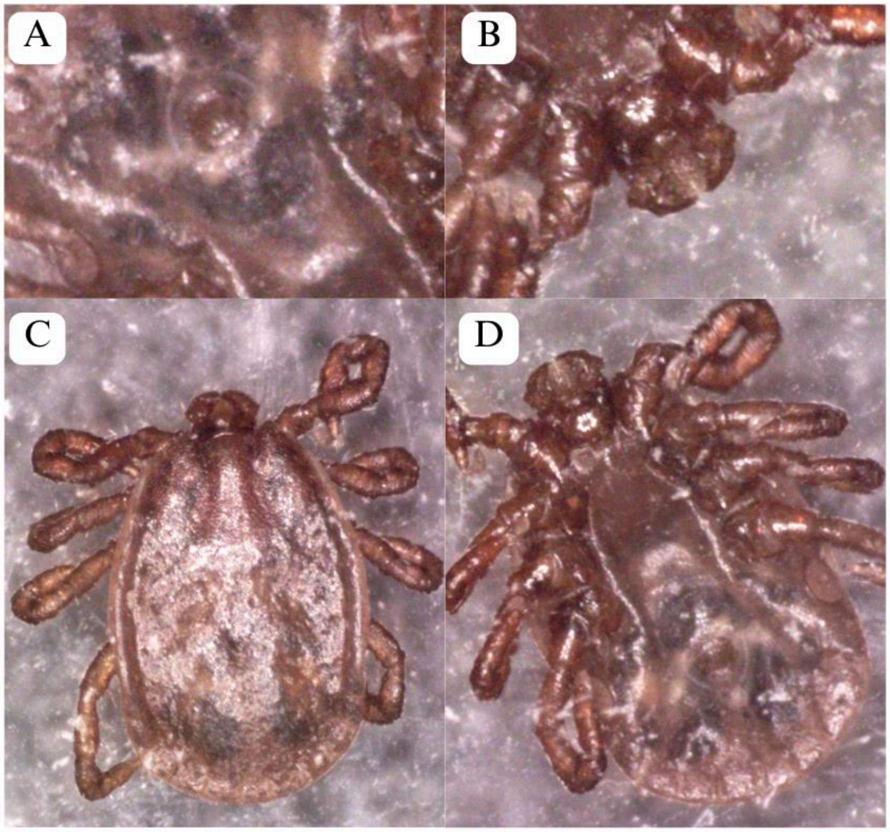


Image 3: High resolution photos from samples from Buck Shoals wildlife management area. Image A is of the tick's anus. Image B is of the tick mouth parts which are commonly used to identify *Haemaphysalis longicornis.* Image C is a dorsal view of the Nymph. Image D is a ventral view of the *Haemaphysalis longicornis.*

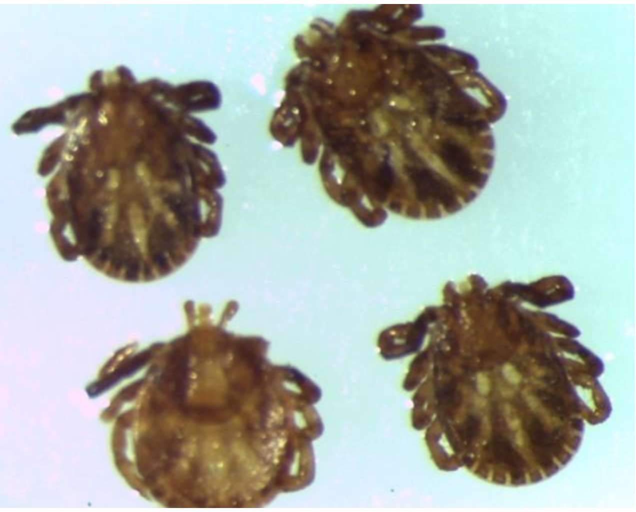


Image 4: Nymphs collected from field work on March 19, 2023. The bottom left tick is an *Amblyomma americanum* nymph used to visually compare with the *H. longicornis* nymphs.

Results:

The 2021 field season collected 7,687 ticks. *Amblyomma americanum* was the dominant species collected with 7,550 samples collected (98.2%) (Table 3). Most of these samples came from two main field sites in Haddock County, GA. The 2022 field season yielded 3,373 ticks collected from 24 state parks and wildlife management areas (Table 4). These ticks were sampled from all ecoregions in the state and sampling occurred at each location twice throughout the summer.

2021 Field Collections Total	
Species	**Number Collected**
Amblyomma maculatum Adult	19
Dermacentor variabilis Adult	100
Amblyomma americanum Female	1072
Amblyomma americanum Male	895
Amblyomma americanum Nymph	5583
TOTAL	7669

Table 3: Species of ticks collected in the 2021 Field season.

2022 Field Collections Total	
Species	**Number Collected**
Amblyomma maculatum Adult	8
Dermacentor variabilis Adult	30
Ixodes Scapularis Adult	24
Haemaphysalis longicornis Nymph	3
Amblyomma americanum Adult	600
Amblyomma americanum Nymph	2708
TOTAL	3373

Table 4: Species of ticks collected in the 2022 Field season.

Date	Species	Other Ticks Collected	Location	Elevation	Humidity	Temperature
06/24/2022	*Haemaphysalis longicornis*	*Amblyomma americanum* Nymph =19	BS WMA	350 m	84%	93 F
07/24/2022	*Haemaphysalis longicornis*	*Amblyomma americanum* Nymph=14	BS WMA	352 m	80%	88 F
07/24/2022	*Haemaphysalis longicornis*	*Amblyomma americanum* Nymph= 14	BS WMA	352 m	80%	88 F

Table 5: Site specific information about the *Haemaphysalis longicornis* ticks found at Buck Shoals Wildlife Management area in White County, Georgia.

Three *H. longicornis* ticks were found during the 2022 field season (Table 5). They were all found at Buck Shoals Wildlife Management area in White County, Georgia. These ticks were found in two separate months at virtually the exact location. Multiple infected hosts would cross through this area to deposit these ticks. The area was intensely surveyed after each initial sample was collected because preliminary field identification marked these ticks for further review based on noticeable differences in hypostome definition [24]. All three ticks were found in their nymph stage. After collection, the ticks were molecular confirmed and genetically sequenced to confirm they were indeed *H. longicornis* (Figure 3).

Discussion:

We report the finding of three *H. longicornis* ticks in a new county and habitat type from the existing findings within the State of Georgia [6], representing the first evidence of *H. longicornis* in the general Georgia ecosystem. The criteria followed by USDA for determining tick establishment is at least six individuals, or at least two of the three life stages that seek hosts, found within one calendar year [23]. While six new ticks were not located in the 2022 field season, a further visit to Buck Shoals on early 2023 led to the detection of 60 specimens visually identified as *H. longicornis* that are awaiting molecular confirmation.

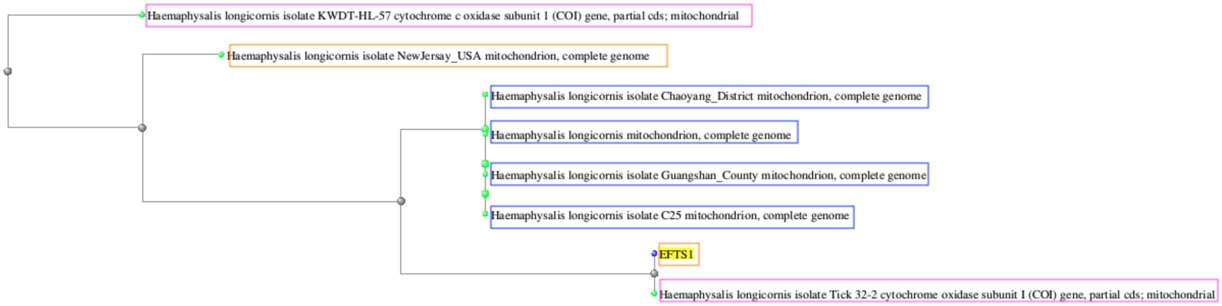


Figure 3: Phylogenetic tree for H. longicornis species with a max score greater than 1245. Specimens outlined in blue are from China, outlined in pink from Australia, and outlined in orange from USA. EFTS1 is a combination of cleaned consensus sequences found at Buck Shoals wildlife management area.

A phylogenetic tree created the opportunity to compare the sequences of *H. longicornis* found in Georgia with existing samples from other locations. Samples were selected from China, Australia, and the United States. Samples were selected based on BLAST Max Score meaning that the ticks selected had the highest alignment score calculated from the sum of the matched nucleotides and mismatched data was subtracted from the overall score.

The ticks found in Georgia had a 100% match with a set of mitochondrial DNA from Australia. The other US species from New Jersey had larger genetic differences. This supports the theory of multiple invasions associated with *H. longicornis*[27]. There were at least three female *H. longicornis* adults introduced to the US to account for this genetic difference. It could be that the genetic difference between the sample found in New Jersey and the ones found by this study are due to a different introduced female. These genetic differences are likely due to multiple *H. longicornis* invasions in the US.

When *H. longicornis* nymphs were collected they displayed interesting behavior in relation to *A. americanum* nymphs. All *H. longicornis* nymphs were collected in clusters of *A. americanum* nymphs. Two of the three *H. longicornis* species were found ventral side to ventral side with *A. americanum* nymphs. Separation of these ticks occurred by researchers removing one nymph from the other and noticing the differences in hypostome definition. This unusual nymph behavior was noted during collection.

In addition, all three nymphs were found in a new county. Existing *H. longicornis* individuals found within Georgia were found on farmland in Hall, Pickens, and Habersham County. The new county identified is White County and is adjacent to Hall and Habersham counties. This means that a host probably picked up the tick in or around a farm and brought it to this new area. The habitat suitability of *H. longicornis* in Georgia could be impacted by the prevalence of reliable hosts. To understand possible nonagricultural host behavior, data from wildlife management area hunting collections was overlayed with the map of tick collections to understand host abundance at these locations (Figure 4). White tailed deer are the most common host of *H. longicornis* within the United States[6]. The Buck Shoals wildlife management area where the ticks were collected hosts a large white tailed deer population and has an average of 5 deer per square mile in the county[28].

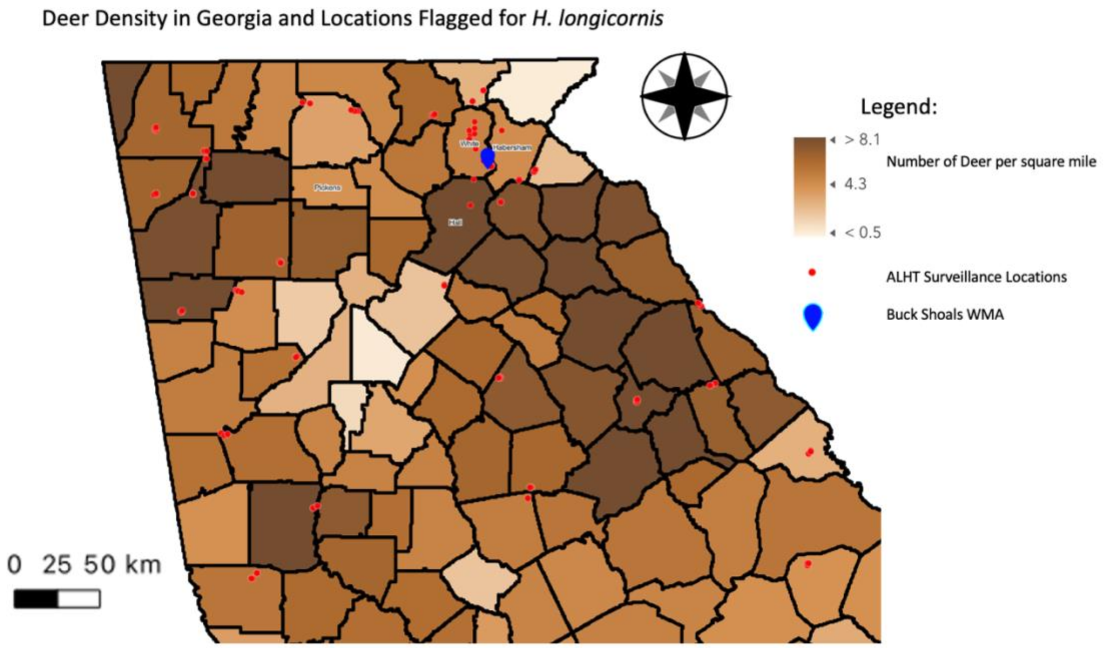


Figure 4: Deer density data from the department of wildlife resources as number of deer per mile in county is laid under surveillance locations for *H. longicornis* [28].

Understanding the movements of these deer and the prevalence of deer communities could provide relevant information for the suitable habitat of *H. longicornis.* The white-tailed deer's range includes most of the state of Georgia and factors besides host availability will most likely limit its spread through the state.

Significance:

The establishment of *H. longicornis* in the Georgia environment could have large-scale implications for the existing tick species and could impact the overall ecology of the region. While there are only 3 confirmed nymphs of *H. longicornis* in the Georgia nonagricultural ecosystem, continued surveillance has detected more samples that are awaiting molecular confirmation. If these samples receive molecular confirmation for *H. longicornis,* there will be enough evidence to prove the establishment of the species in Georgia. *H. longicornis* has the potential to be a significant agricultural and public health threat within the state of Georgia. The first implication of the introduction of the tick will be in tick species composition across Georgia. *A.americanum* is the dominate tick in Georgia and existing research proves that *H. longicornis* will occupy the same niche [22]. In parts of New York state, it took 4 years for *H. longicornis* to reach the same prevalence in areas as *A. americanum [13].* The implication is that *H. longicornis* can outcompete native ticks and establish itself quickly. Native tick species all decreased in New York with the introduction of *H. longicornis [13].* Ticks contribute to the overall ecosystem by acting as a food source for birds, reptiles, and mammals [29]. Invasive ticks are not identified as a suitable food source, which limits the natural control of ticks in the environment [29]. Due to a lack of predators, *H. longicornis* could rapidly establish itself within the environment and outcompete native ticks due to lack of predation. Without known predators, *H. longicornis* could increase its prevalence while decreasing native species and thus negatively impact the natural food web within communities.

In white county, Georgia, over 60% of the land is suitable for *H. longicornis*. Suitable land was defined as any land without impervious surfaces, water, or an elevation greater than 1000m. Raster pixel land use data from the US Geological Survey and uploaded into QGIS for analysis. All suitable land was selected and placed under surveillance locations within the county.

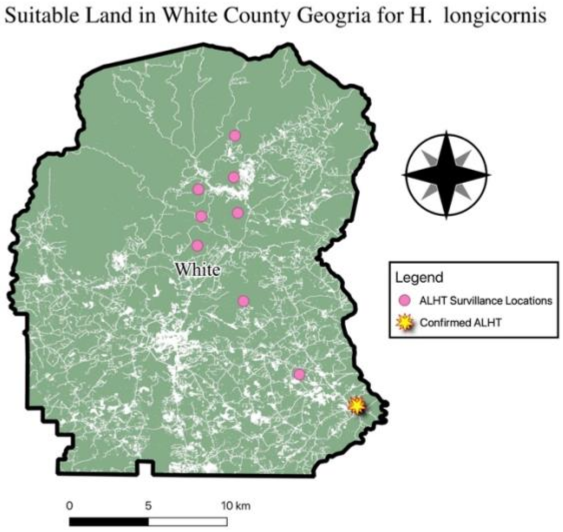


Figure 5: Suitable land (any land without impervious surfaces, water, or an elevation greater than 1000m) in White County, Georgia under locations for surveillance within the county.

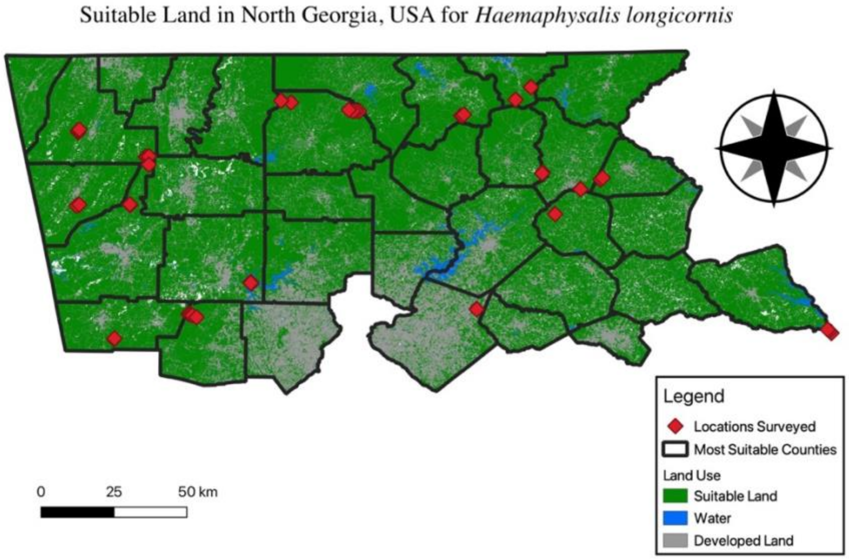


Figure 6: All suitable land in North Georgia as defined as forest, grasslands, pasture lands, and contained the criteria mentioned earlier.

Figures 5 and 6 are significant since they illustrate the suitability of most counties for the establishment of *H. longicornis*. There are no large barriers to movement like rivers or major cities that would prevent hosts from spreading the ticks to new counties. Existing habitat suitability studies indicate that this land contains all environmental factors for *H. longicornis* [7] and the analysis of the land use type indicates that most of the land in north Georgia has suitable habitat for *H. longicornis.* Figure 4 details white tailed deer density and indicates that adequate hosts are available within the land marked suitable by modeling [7, 8]and by figure 6.

Finally, the *H. longicornis* ticks were found in locations with no livestock, unlike *H. longicornis* ticks previously found in Georgia [6]. This difference indicates that the general habitat in Georgia could be suitable for *H. longicornis* establishment. This is significant since *H. longicornis* has more suitable land. Further research will occur at this location and the locations previously identified as suitable to understand the establishment of this species within Georgia. This study collected data from these initial locations and further research will establish how species composition is changing with the introduction of *H. longicornis*.

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