Biogeography of *Triatoma sanguisuga* (Hemiptera: Reduviidae) on Two Barrier Islands off the Coast of Georgia, United States

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ABSTRACT Thirty-three *Triatoma sanguisuga* (LeConte) adults and nymphs were collected during June and July 2009, at five sites on Cumberland Island and two sites on Sapelo Island, Georgia, to assess genetic diversity within and between sites. All but three specimens were found in a peridomestic habitat. The entire length (699 bp) of the cytochrome oxidase II mitochondrial gene was sequenced for each specimen. Twelve haplotypes were identified, nine from Cumberland Island and three from Sapelo Island. No haplotypes were shared between the two islands, indicating there is limited or no movement of gene flow between the islands. Phylogenetic relationships among the haplotypes were determined using both neighbor-joining and maximum parsimony analyses. The phylogenetic trees from both analyses were similar, with no distinct clades on either tree devoted to haplotypes from a single island. A haplotype network structure was determined using nested clade analysis, which produced two haplotype networks, one containing only specimens found on Cumberland Island. The second network included specimens from both islands, with the ancestral haplotype from Sapelo Island. This pilot study is the first to highlight triatomine populations in the southeastern United States using the cytochrome oxidase II mitochondrial gene, and indicates strong population structuring along the Georgia Coast.

KEY WORDS *Triatoma sanguisuga*, biogeography, Georgia, cytochrome oxidase II

Triatoma sanguisuga (LeConte) (Hemiptera, Reduviidae, Triatominae) is a haematophagous insect found throughout the southeastern United States, as far north as Pennsylvania and southwest to Texas (Lent and Wygodzinsky 1979). These insects, commonly known as bloodsucking conenoses, are potential vectors of *Trypanosoma cruzi*, the protozoan causal agent of Chagas disease (Miles et al. 2003). Autochthonous Chagas disease is rare in the United States, with only six cases since 1955 verified to have been transmitted by an insect vector (Dorn et al. 2007). *T. cruzi* has, however, been detected with a high frequency in wildlife populations in the United States (Pung et al. 1995). Areas impacted by Hurricane Katrina in the United States reported an increase in domestic *T. cruzi* infestations, as well as a human case in 2006 (Dorn et al. 2007).

Two triatomine species are known to inhabit Georgia. *T. sanguisuga* is frequently reported, whereas *Triatoma lecticularia* (Stål) is considered uncommon (Thurman et al. 1948, Beard et al. 1988, Pfeiler et al. 2006). We report in this work a pilot study to record the species of triatomine bugs on two barrier islands off the Georgia Coast using directed collections. In addition, we report on the phylogeographic relationships of the specimens based on mtDNA sequence data and collecting coordinates.

Materials and Methods

A directed sampling program was used to collect triatomine bugs from Cumberland Island and Sapelo Island between 21 June and 6 July 2009. These two barrier islands are separated by a distance of ≈52 km. Three adults and 11 nymphs were collected on Cumberland Island, whereas four adults and 15 nymphs were collected on Sapelo Island. The directed sampling technique involved identifying likely triatomine harborage, such as a sheltered position near a potential host nesting/resting location, and then conducting a visual search of that harborage. Sites examined included hollow trees, sheds, animal pens, abandoned buildings, and woodpiles.

Four primary sites were searched on Cumberland Island, and two primary sites were searched on Sapelo Island (Fig. 1). Primary sites included the area within a 50-m radius of where insects were collected. On Cumberland Island, donations from National Park Service (NPS) personnel and island residents resulted in four additional specimens. Adult specimens were identified to species using the key of Lent and Wygodzinsky (1979).

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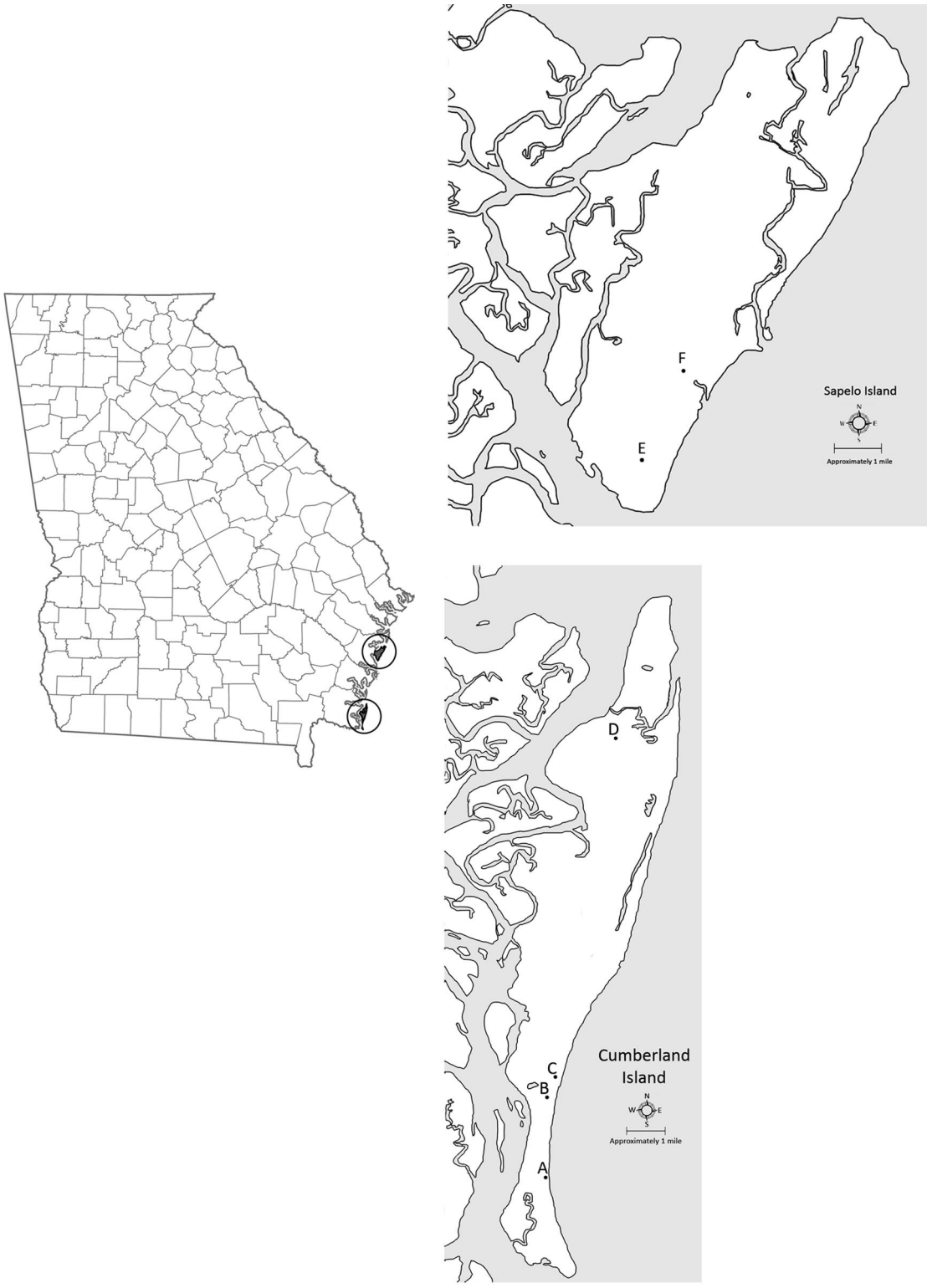

Fig. 1. Top left: map of Georgia, with the locations of Cumberland Island (bottom) and Sapelo Island (top) marked. Bottom right: map of Cumberland Island with the four primary collection sites marked. Top right: map of Sapelo Island with the two primary collection sites marked.

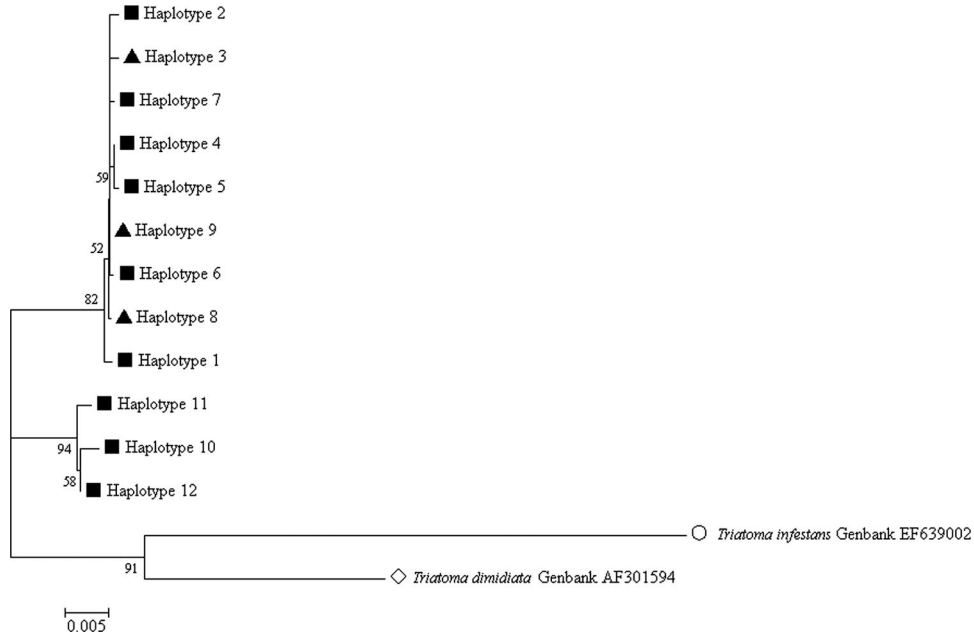

Fig. 2. Neighbor-joining tree composed of 12 different haplotypes for *T. sanguisuga* using the cytochrome oxidase II mitochondrial gene. The bootstrap value was 1,000. Haplotypes that have a square by them were found on Cumberland Island, whereas haplotypes with a triangle by them were found on Sapelo Island.

All triatomine specimens were placed in ethanol and stored at −20°C. Tissue samples used for DNA extraction were obtained from each specimen by either removing two to three legs or the midgut. DNA was extracted using a modified protocol for insects employing the Wizard Genomic DNA purification kit (Promega, Madison, WI) or Qiagen DNeasy DNA extraction kit (Qiagen, Valencia, CA). Primers for polymerase chain reaction used to isolate the cytochrome oxidase II mitochondrial gene (*coxII*) were designed from previously created primers TL2-J3043 and TK-N3796 and altered to more closely match the sequence of the *coxII* gene of *Triatoma dimidiata* (Dotson and Beard 2001, Simon et al. 2006). The forward primer was a modified TL2-J3043, 5′-GGCAGAAATTATATGYAATGRATTTAA-3′. The reverse primer was a modified TK-N3796, 5′-ACCATATACTGGTTTAAGAG-3′ (Dotson and Beard 2001, Simon et al. 2006). Typical polymerase chain reaction techniques were followed. Products were viewed on a 1.5% agarose gel stained with ethidium bromide.

Sequencing for both the forward and reverse strands was done using fluorescent dye terminator chemistry and analyzed with ABI 3730XL sequencers. The resulting sequences were edited and curated using Sequencher 4.1.4 (Gene Codes, Ann Arbor, MI), aligned using MUSCLE (Edgar 2004), and compared with BLAST (Altschul et al. 1990). Returned BLAST results were for *T. dimidiata* and *Triatoma infestans*, indicating that the correct gene had been sequenced. GenBank accession numbers can be found in Table 1.

Phylogenetic analyses were performed using neighbor-joining and maximum parsimony in MEGA 4.1 (Tamura et al. 2007, Kumar et al. 2008). A trimmed sequence of 672 bp was used for the analyses. When a group of insects had the same sequence, a single haplotype was used in the analysis. A bootstrap value of 1,000 was used in creating each tree. Outgroups were *coxII* sequences from GenBank, one from *T. dimidiata* (GenBank AF301594) and one from *T. infestans* (GenBank EF639002).

Table 1. Primary and secondary locations on Cumberland Island and Sapelo Island where specimens were found

Cumberland Island			
Primary location	Secondary location	Adults/ Nymphs found	Haplotypes found
A	First shed	0/1	7
	Second shed	0/6	7, 11, 12
	Outdoor woodpile	0/1	10
B	Donated; found inside house	0/2	1, 2
	Donated; found inside barn	1/0	5
C	Donated; found entering house	1/0	4
D	In spider web	1/0	5
	Behind drywall	0/1	6
Total found		14	
Sapelo Island			
E	Outdoor woodpile	4/8	9
F	Chicken coop	0/2	3, 9
	Open shed	0/5	8
Total found		19	

Primary location refers to the areas that were searched (see Fig. 1). Secondary location refers to points within the primary location.

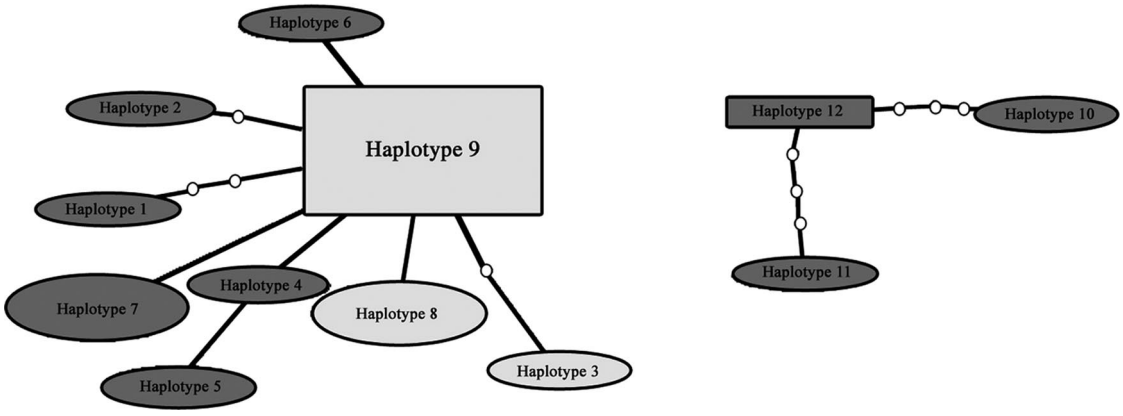

Fig. 3. Haplotype networks created for *T. sanguisuga* haplotypes from the cytochrome oxidase II mitochondrial gene found on Cumberland Island and Sapelo Island. The area of the shapes represents the number of consensus sequences for a haplotype. The squares represent the ancestral haplotypes, whereas the circles represent more derived haplotypes. The uncolored circles represent hypothetical intermediate haplotypes.

All sequences were used in a nested clade analysis with the program TCS to create a haplotype tree (Clement et al. 2000). The area of the shapes in the network corresponds to the number of sequences of that haplotype (Fig. 3).

Results

Thirty of the 33 specimens collected from the islands were found in peridomestic habitats, such as sheds, chicken coops, or woodpiles. Three donated specimens had been found in a domestic setting. Overall, specimens were only collected from sheltered locations and displayed a clumped distribution in that more than one insect was often collected at any single harborage (Table 1).

Specimens collected by directed search on Cumberland Island were found in an outdoor woodpile, spider web, and behind a stack of drywall panels leaning against a wall inside an outbuilding (Table 1). Four specimens from Cumberland Island were provided by collaborators, including two found inside one house, one entering another house, and one inside a barn. The greatest number of specimens ($n = 8$) collected on Cumberland Island was found in two neighboring outbuildings that contained plywood boards leaning against an inside wall (Table 1).

Twelve of the specimens on Sapelo Island were collected from a stack of cypress boards stored outdoors, whereas two were found in a single crack between wallboards in a chicken coop and five were collected in an open shed between plywood boards leaning up against a wall (Table 1).

Twelve haplotypes were identified from the 33 specimens, as follows: nine from Cumberland Island and three from Sapelo Island. These haplotypes included 52 variable sites, all of which were polymorphic with two variants (Table 2). Sequences had an average nucleotide percentage of 31.1% for T, 19.4% for C, 35.7% for A, and 13.8% for G. Table 3 lists percentage differences among haplotypes, which ranged from 0.1% up to 6.1%. Neighbor-joining and maximum parsimony trees both had similar topography with two major clades; one contained haplotypes from both islands, and the other comprised three haplotypes from Cumberland Island (Fig. 2).

Nested clade analysis produced two separate networks (Fig. 3). One network, in contrast to our findings of no shared haplotypes between the two islands, contained haplotypes from both islands with the ancestral haplotype arising from Sapelo. The other network contained three haplotypes from collections made at the southern end of Cumberland Island in primary site A. This area of the island contains NPS workshops, dwellings, and the island garbage dump.

Three of the four primary sites on Cumberland Island contained more than one haplotype (Table 1). Primary site A contained four different haplotypes. Three of these haplotypes, represented by one specimen each, were segregated on both the neighbor-joining and maximum parsimony trees. All of these specimens were nymphs, and two were found in the same shed, whereas one was found ≈27 m away in an outdoor woodpile. Another (haplotype 5) was found in two different locations, at the north end of the island in primary site D and in primary site B, ≈15.25 km apart, suggesting that this maternal line dispersed a considerable distance on the island.

Specimens on Sapelo Island were all found on the southern end of the island at two primary sites (E and F). Haplotype 9 was found at both primary sites, ≈3.5 km apart (Fig. 1). Primary site E contained a pile of cypress boards that provided 12 specimens, all of which showed the same haplotype. This haplotype was also found in one nymph collected in a chicken coop at primary site F, again indicating maternal line dispersal within the island.

Discussion

The majority of triatomines, on both islands, were found in peridomestic locations, despite similar di-

Table 2. Variable sites for the 699-bp cytochrome oxidase II mitochondrial gene of Triatomine found on Cumberland and Sapelo Islands

Haplotypes	Variable sites																																																				Location		GenBank
	13	18	31	38	67	94	100	109	119	125	126	157	158	169	182	184	196	242	253	268	307	310	313	322	331	337	349	355	367	374	391	397	406	409	412	418	422	472	475	517	520	523	526	547	562	566	568	589	604	610	646	670	C	S	
Haplotype 1	T	T	C	T	T	T	A	G	G	A	T	C	T	A	A	T	C	C	A	T	C	G	C	C	T	T	T	C	T	C	T	T	A	C	C	T	T	C	T	C	T	A	T	C	C	T	A	C	T	T	A	C	*		JF500880
Haplotype 2	.	.	.	C	.	C	.	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*		JF500881
Haplotype 3	.	.	.	C	.	.	.	.	.	G	.	.	.	.	G	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		*	JF500885
Haplotype 4	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	*		JF500875
Haplotype 5	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	*		JF500879
Haplotype 6	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	*		JF500883
Haplotype 7	.	.	.	C	.	.	.	.	.	G	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*		JF500876
Haplotype 8	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		*	JF500886
Haplotype 9	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		*	JF500884
Haplotype 10	C	C	T	.	C	.	G	A	A	G	.	T	C	G	.	C	A	T	G	C	T	A	T	T	C	C	C	T	C	.	C	C	G	T	T	C	.	.	.	T	C	.	C	T	T	C	G	T	.	C	.	.	*		JF500878
Haplotype 11	C	C	T	.	C	.	G	A	A	G	.	T	C	G	.	C	A	T	G	C	T	A	T	T	C	C	C	T	.	.	C	C	G	T	T	.	.	.	.	T	.	G	C	T	.	C	.	T	C	C	G	.	*		JF500882
Haplotype 12	C	C	T	.	C	.	G	A	A	G	.	T	C	G	.	C	A	T	G	C	T	A	T	T	C	C	C	T	.	.	C	C	G	T	T	C	.	.	.	T	.	.	C	T	.	C	.	T	.	C	.	.	*		JF500877

Haplotypes found on Cumberland Island are designated by a *C* under location, whereas those found of Sapelo Island are designated by an *S*.

Table 3. Percentage of base pair differences among haplotypes found on Cumberland and Sapelo Islands

	Haplotype 1	Haplotype 2	Haplotype 3	Haplotype 4	Haplotype 5	Haplotype 6	Haplotype 7	Haplotype 8	Haplotype 9	Haplotype 10	Haplotype 11	Haplotype 12
Haplotype 1	0.0	0.7	0.7	0.6	0.7	0.6	0.6	0.6	0.4	5.9	5.6	5.3
Haplotype 2	0.7	0.0	0.6	0.4	0.6	0.4	0.4	0.4	0.3	6.1	5.8	5.5
Haplotype 3	0.7	0.6	0.0	0.4	0.6	0.4	0.4	0.4	0.3	6.1	5.8	5.5
Haplotype 4	0.6	0.4	0.4	0.0	0.1	0.3	0.3	0.3	0.1	5.9	5.6	5.3
Haplotype 5	0.7	0.6	0.6	0.1	0.0	0.4	0.4	0.4	0.3	5.8	5.8	5.5
Haplotype 6	0.6	0.4	0.4	0.3	0.4	0.0	0.3	0.3	0.1	5.9	5.6	5.3
Haplotype 7	0.6	0.4	0.4	0.3	0.4	0.3	0.0	0.3	0.1	5.9	5.6	5.3
Haplotype 8	0.6	0.4	0.4	0.3	0.4	0.3	0.3	0.0	0.1	5.9	5.6	5.3
Haplotype 9	0.4	0.3	0.3	0.1	0.3	0.1	0.1	0.1	0.0	5.8	5.5	5.2
Haplotype 10	5.9	6.1	6.1	5.9	5.8	5.9	5.9	5.9	5.8	0.0	1.1	0.6
Haplotype 11	5.6	5.8	5.8	5.6	5.8	5.9	5.6	5.6	5.5	1.2	0.0	0.6
Haplotype 12	5.3	5.5	5.5	5.3	5.5	5.3	5.3	5.3	5.2	0.6	0.6	0.0

Haplotypes in bold were from Sapelo Island and the rest were originated from Cumberland Island.

rected search in sylvatic areas. Directed search discovered more insects in protected harborage locations, under buildings with a roof ($n = 8$) versus exposed ($n = 2$) in close proximity to small rodent populations, presumably mice. A search of tree holes provided no triatomines even at Cumberland Island primary site A, which is known to harbor a large raccoon population because of its proximity to a dumpster. The number of secondary locations that provided one specimen ($n = 6$) was similar to locations that provided more than one specimen ($n = 5$). Adults were found at three of eight secondary locations using directed search, whereas two of four donated specimens were adults.

Haplotype distribution on the islands indicates that the insects do move around a single island, but the absence of a common haplotype between islands suggests that there is limited long-distance, >50-km, movement. The farthest distance between two (identical) haplotype collection points was 15.25 km. *T. sanguisuga* females lay eggs individually (Hays 1965), so it may be that the same female could lay eggs in different places. The lack of a food source, along with temperature, is known to contribute to the movement of some species of triatomine to a new habitat (Ekkens 1981, Lehane et al. 1992).

Some heavily populated primary sites on each island contained more than one haplotype. In primary site E on Sapelo Island, only one haplotype was found of 12 specimens. However, at primary site A on Cumberland Island, five haplotypes were found from eight specimens. The difference in the number of haplotypes could be the result of the amount of traffic in those respective areas. Site E on Sapelo Island is a private residence and not subject to public traffic. In contrast, the Cumberland Island site included a dormitory for NPS volunteers and visiting scientists, the main shop for NPS facilities workers, and the island's only dumpsters. Site A on Cumberland Island was certainly subject to more off-island human visitors and had a large resident raccoon population, increasing the likelihood of an insect being transported to the area or finding a host.

The neighbor-joining, maximum parsimony, and the haplotype networks found three haplotypes (10, 11, and 12 all found at primary site A on Cumberland Island) that separate into a distinct clade. These data hint at speciation because our experience with this gene fragment indicates that it traditionally separates haplotypes at the species level (Sillam-Dussès and Forschler 2010). However, all these specimens were nymphs and could not be identified to species using published morphological characters, so the haplotype separation may indicate another species, *T. lecticularia*, which has been reported from Georgia (Lent and Wygodzinsky 1979), although there are no records from either island.

Although our conclusions are constrained by a limited sample size, it is interesting that no haplotypes were shared between Cumberland and Sapelo Islands, and the within-island haplotype distribution is suggestive of limited dispersal. These insects are presumed to vector *T. cruzi*, and movement of vectors is an important influence on dispersal of parasites, and gene flow within and between parasite populations. Our study suggests that a broader-scale analysis of haplotype distributions will be a profitable approach to examining dispersal, gene flow, and population biology of *T. sanguisuga* and their heterospecifics.

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