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Flea and Louse Infestations of Cotton Rats (Sigmodon Hispidus) In The Southeastern United States

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FLEA AND LOUSE INFESTATIONS OF COTTON RATS (*Sigmodon hispidus*)

IN THE SOUTHEASTERN UNITED STATES.

by

ALENA E. AVILES

(Under the Direction of Lance A. Durden)

ABSTRACT

Ectoparasites were collected from cotton rats (*Sigmodon hispidus*) in 20 sites in the Southeastern United States (FL, GA, MS, NC and SC). Prevalence and mean intensity of parasitism by sucking lice (Anoplura) and fleas (Siphonaptera) of cotton rats were recorded at all sites. The geographical distribution of *S. hispidus* and its main louse and flea ectoparasites range from the neotropical region to the southeastern USA. It was hypothesized that the abundance of the cotton rat associated louse (*Hoplopleura hirsuta*) and flea (*Polygenis gwyni*) would increase the further south and closer to the distribution centers of each of these ectoparasite species. In addition, it was hypothesized that male cotton rats would exhibit higher infestations (mean intensities and prevalence) by ectoparasites than females. Because males of many ectoparasites are more mobile than females and may experience more periods off the host than females, I further hypothesized that sex ratios of both flea and louse populations would be female-biased. Data collected during this study supported the hypothesis that populations of *Polygenis* fleas on *S. hispidus* increased further south (closer to the center of distribution for this flea) and thus were dependent on site location. Conversely, there was not a significant trend in abundance noted for *Hoplopleura* lice on *S. hispidus*, which was unexpected given that this ectoparasite is a more permanent ectoparasite than *P. gwyni*. Male cotton

rats were not parasitized by statistically greater numbers of *H. hirsuta* or *P. gwyni* than were female cotton rats. Thus, the male host bias hypothesis was not supported for either ectoparasite species in this study. Populations of both *H. hirsuta* and *P. gwyni* were significantly female-biased, with about twice as many females as males on cotton rats. Overall, this study provides the first evidence for larger populations of an ectoparasite (*P. gwyni*) of a vertebrate towards the geographical center of distribution of the ectoparasite. Higher on-host populations of female versus male sucking lice and fleas in this study conform to similarly sex-biased data reported for several previous studies of ectoparasites on mammals. Conversely, the lack of significant differences for louse and flea infestations on male versus female cotton rats recorded during this study differs from some previous mammal-ectoparasite studies in which male hosts were more heavily infested.

INDEX WORDS: Infestation parameters, Ectoparasites, Rodents, Cotton rat, *Sigmodon hispidus*, Cotton rat flea, *Polygenis gwyni*, Cotton rat louse, *Hoplopleura hirsuta*, Georgia, Florida, North Carolina, South Carolina, Mississippi.

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IN THE SOUTHEASTERN UNITED STATES.

by

ALENA E. AVILES

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by

ALENA E. AVILES

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	Alan W. Harvey

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DEDICATION

    I would like to dedicate this thesis to my husband and children. To my husband,

Carlos, who never let me stop reaching for my dreams, thank-you for always being my

blessing in disguise and I will always love you. To my two beautiful children, Jerryd and

Abbee, you are the light of my life; if I could offer any advice it would be to follow your

dreams and never give up, remember everything happens for a reason and God closes

doors in our lives so that others may open our eyes to amazing opportunities.

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Dr. Patrick Abbot of Vanderbilt University collaborated on the *Bartonella* project that included ectoparasites from this thesis research.

Finally, I would like to thank my father and mother, Jerry and Cindy Edenfield. Their sacrifices allowed me to better my life and in turn the lives of my family and children. I would like to thank my father for his endless interest in the study of our natural world which has inspired and humbled me through all these years. He taught me to see the world from many perspectives and to notice the things no one else cares to see. Thank you. I would like to thank my mother whose amazing lifelong accomplishments have shown me that God really does open doors to amazing opportunities when all others seem closed. And for the strength that she helped me find within myself; giving up never equals success. Thank you. I love you both.

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CHAPTER 1

INTRODUCTION

Because some rodent ectoparasites serve as vectors of zoonotic pathogens and their rodent hosts may serve as reservoirs, it is important to record host-parasite interactions and infestation parameters for ectoparasites of rodents (Durden *et al*. 2000). Further, for individual species of ectoparasites, it is instructive to compare their abundance in different parts of their geographical range, to determine whether male and female hosts are differentially infested (i.e., are male hosts more heavily infested), and to determine if the on-host populations are numerically biased towards males or females.

Population densities of cotton rats can vary on a yearly basis, but in general *Sigmodon hispidus* (Rodentia: Cricetidae) is usually the most abundant small mammal species on farmlands and low scrubby habitats in the southern United States (Smith and Love 1958; Cameron and Spencer 1981). Cotton rats actively seek out food at dawn and dusk. Grassy plants including cultivated field crops are their primary source of food (Whitaker and Hamilton 1998). The distribution of *S. hispidus* (Figure 1) includes most of the Central American region, northward into the southeastern and south central United States (Cameron and Spencer 1981).

Prevalence is the number of rodent hosts infested with one or more ectoparasites of a given ectoparasite species divided by the number of cotton rats examined. This is the most commonly used descriptor of parasitic infestations because it provides a rapid and easily calculated parameter reflecting the proportion of a host population that is parasitized by a given parasite species (Bush *et al.* 1997). Mean intensity is the average number of a given ectoparasite among infested host species. Therefore, mean intensity is

the total number of a parasite species found at a particular sample site divided by the number of hosts infested (Bush *et al.* 1997). A number of factors can influence the prevalence (% of hosts infested) and mean intensity of infestation (mean number of parasites per infested host) by parasitic lice and/or fleas on a host including: environmental conditions, season, host body size, age, sex, activity level, and host body condition (Love and Smith 1958; Henry 1970; Poulin 1991; Kotiaho and Simmons 2001; Leung *et al.* 2001; Rolff 2001; Kelly 2005). Variations in infestation prevalence have been demonstrated for ectoparasites in previous rodent-ectoparasite surveys (e.g., Yourth *et al.* 2002a; Robb *et al.* 2003). It is important to consider differences in infestation prevalence as well as mean intensity, since together these two parameters give a reliable indication of overall parasite abundance in a host population (Rozsa *et al.* 2000) and, for ectoparasites this may have significance for vector-borne diseases. Although previously rarely used by parasitologists, the total number of ectoparasites of a given species on each individual host could also provide an important measure of parasite abundance. This is because just one easily plotted variable can be graphically portrayed against the other variable (geographical coordinates, etc.) as a regression and a more accurate reflection of ectoparasite populations in nature may be gained.

In this study I address three hypotheses. First, the abundance of two common species of ectoparasites of cotton rats, the flea *Polygenis gwyni* (Siphonaptera: Rhopalopsyllidae) (Figure 2), and the sucking louse, *Hoplopleura hirsuta* (Phthiraptera: Hoplopleuridae) (Figure 3), should increase the farther south (and closer to their respective centers of distribution) the study field site is sampled. Widely known as the "abundant center hypothesis", and a "general rule" of biogeography, the general

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consensus is that a species' abundance is greatest at the center of its geographical range

and lower toward the edges of its range due to environmental gradients (Brown 1984;

Sagarin and Gaines 2002; Alleaume-Benharira *et al.* 2006; Bell 2001; Sagarin *et al.*

2006). Abundance gradients with respect to the center of distribution have not previously

been evaluated for any species of ectoparasites associated with mammals. There are two

assumptions to consider here: 1) spatial variation in local abundance is related to the

likelihood of meeting a species' niche requirements; 2) these niche requirements are

geographically coordinated with the most desired conditions located near the center of the

species' distribution (Brown 1984; Kiflawi *et al.* 2000). Populations of both *H. hirsuta*

and *P. gwyni* are close to their northern range limit in northern Georgia with the

approximate centers of their ranges both being near Mexico (Figures 4 and 5). Therefore,

we expected both ectoparasites to be more common closer to their centers of distribution

(i.e., to the south). Both *P. gwyni* and *H. hirsuta* are very host specific and therefore

should not venture outside of the host range (Figure 1). The sucking louse *H. hirsuta* is a

specific ectoparasite associated with cotton rats and because of this specific host

interaction it will not go beyond the range of the rat (Pfaffenberger and DeBruin 1988).

The flea, *P. gwyni*, sometimes parasitizes the Virginia Opossum, *Didelphis virginiana*,

and various rodents, but it cannot become established in areas without its main host, the

cotton rat (Smit 1987). If both the flea and louse ectoparasites studied in this paper are

reaching their northernmost boundaries at the Georgia and South Carolina trap site

locations (Ferris 1921; Fox 1940; Morlan 1952; Pratt and Good 1954; Layne 1971;

Benton 1980; Kim *et al.* 1986; Smit 1987; Durden *et al.* 1994, 2000), then one might

predict that the population density of ectoparasites (abundance, prevalence and/or mean intensities) would be greater the more south in latitude that the trap sites are located.

The second hypothesis for this study is that male cotton rats will be more heavily infested (measured by prevalence and mean intensity) than females by both *H. hirsuta* and *P. gwyni*. Sexual differences in parasitism by ectoparasites can be the result of differences in the intensity and prevalence of infestation based on the sex of the host, with males typically being targeted more often than females (Zuk 1990, 1992; Sheridan *et al.* 2000). The most important variables to be considered as explanations related to host sex and infestation burdens are factors such as relative size and differences in the skin and its covering (male rodents are typically larger than females), difference in blood hormonal levels due to stress or reproductive condition, and behavioral factors such as differences in grooming, nesting and mobility (Marshall 1981a). Of these, the most commonly accepted reason is known as the immunocompetence hypothesis whereby testosterone enhances the expression of male secondary sexual characters while exerting a suppressive effect on the immune system thereby predisposing male hosts to higher intensities of parasite infestation**s** (Saino *et al.* 1995). Thus, if male rodents have a weaker immune response than do females, then males should have a greater prevalence and mean intensity of lice and fleas than females because testosterone-mediated sexual activity acts to decrease the amount of energy males can contribute to immunity.

The third hypothesis is that sex ratios of both *H. hirsuta* and *P. gwyni* collected from cotton rats during this study will be female-biased. Unequal, female-biased, parasite sex ratios have been noted in the literature for several ectoparasitic species (Marshall 1981a, b; Gorell and Schulte-Hostedde 2008). Male ectoparasites tend to have

a shorter lifespan and are smaller in average size than female ectoparasites and often less likely to stay attached to a single host. This is because males are usually more active on and off a given host, and thus more likely to be separated from the host's body or home, be more susceptible to host predation, or be killed by adverse environmental or nutritional conditions (Marshall 1981a). I further predicted that the female bias should be especially apparent in fleas because male fleas are generally more agile than females and may detach from their host (Marshall 1981a; Gorell and Schulte-Hostedde 2008) whereas sucking lice of both sexes are more heavily committed to permanent residence on their host (Durden and Loyd 2009).

In the present study, I attempted to relate flea and louse infestation parameters of cotton rats to host capture location and to the sex of the host, while also analyzing sex ratios of these two ectoparasites. This study provides statistical information concerning two species of ectoparasites and their abundance on their principal host, the cotton rat, in the southeastern Unites States. The two parasitic arthropods studied in this project, *Polygenis gwyni* (flea) and *Hoplopleura hirsuta* (sucking louse), are excellent subjects for this study because they are usually common, they are host specific, and they show little or no apparent seasonality (Morlan 1952; Smith and Love 1958; Henry 1970; Pfaffenberger and DeBuin 1988). The lack of a seasonal bias is a potentially important consideration because the ectoparasites analyzed during this study were not all collected at the same time of year.

The current study of infestation parameters by *P. gwyni* fleas and *H. hirsuta* lice on cotton rats of the southeastern United States was part of a long-term investigation in collaboration with Dr. Patrick Abbot of Vanderbilt University on the evolution and co-

infection of species of *Bartonella* within this particular rodent host and its ectoparasites (see Appendix A). This rodent is an excellent reservoir for a variety of strains of *Bartonella* (Kosoy *et al*. 1997, 2004 a, b). Some blood-feeding arthropods are known to be vectors of various species of *Bartonella* (Chomel *et al*. 1996; Maurin *et al*. 1997; Karem *et al*. 2000; Chang *et al*. 2001; La Scola *et al*. 2001, Durden *et al*. 2004) and, of these, *Polygenis gwyni* has been demonstrated to be an excellent source for mixed infections of various bartonellae in the Southeastern United States (Abbot *et al*. 2007).

CHAPTER 2

MATERIALS AND METHODS

Study Sites and Trapping

Rodents were live trapped at various locations throughout the southeastern United

States of America: Georgia (12 sites), Florida (4 sites), North Carolina (1 site), South

Carolina (1 site) and Mississippi (2 sites) (Table 1). Rodents, mainly cotton rats

(*Sigmodon hispidus*), were live trapped using Sherman live traps (H.B. Sherman Traps,

Inc., Tallahassee, FL). Each field site was determined based on landowner permission as

well as resources and available funding. State of Georgia county extension agents were

utilized to locate willing landowners possessing appropriate habitat conditions that are

associated with *S. hispidus*. Field sites used in the analysis of the current study include

the following counties: Bulloch Co. (32.444N, 81.783W), Bleckley Co. (32.397N,

83.347W), Columbia Co. (33.562N, 82.175W), Decatur Co. (30.909N, 84.583W),

McIntosh Co. (31.374N, 81.499W), Chatham Co. (31.942N, 81.035W), Screven Co.

(32.751N, 81.604W), Lowndes Co. (30.842N, 83.306W), Candler Co. (32.318N,

82.074W), Burke Co. (32.985N, 81.978W), Jenkins Co. (32.720N, 81.979W), and Glynn

Co. (31.170N, 81.499W) in Georgia; Brevard Co. (28.077N, 80.629W), Flagler Co.

(29.469N, 81.364W), Bay Co. (30.169N, 85.648W), and Leon Co. (30.444N, 84.258W)

in Florida; Charleston Co. (32.780N, 79.936W) in South Carolina; Jackson Co. (30.366N,

88.543W), Marion Co. (31.251N, 89.756W) in Mississippi; and Jackson Co. (35.372N,

83.199W) in North Carolina. On average, one site was sampled per county to trap

rodents and collect their ectoparasites (see Appendix B). The study sites offered an array

of rodent habitat including grassland, lightly grazed pasture, and cropland. At each study site, 25-50 live traps were placed around areas of suspected rodent activity. The traps were placed approximately 10 meters apart between 1200 and 1500 hours EST and left overnight. Traps were checked the next day between 0800 and 1200 hours EST. If there was no indication of rodent activity the traps were re-baited and left for another night. Each trap was baited with oatmeal mixed with a trace of peanut butter. Cotton nests were added during winter months to prevent rodent hypothermia.

Animal Collection

Procedures for the collection and handling of captured rodents were approved by the Institutional Animal Care and Use Committee (IACUC) at Georgia Southern University (research protocol number I06003) and a Georgia State scientific collection permit (29-WCH-07-160). Trapped animals were lightly anesthetized through intramuscular administration of ketamine hydrochloride and then moved to a white tray, where they were carefully examined for ectoparasites and sexed (male rodents identified by descended testes); all procedures were done at the field site. Captured rodents were marked with a unique number using permanent ink on their dorsal surface where the fur was light colored, allowing quick identification of recaptured animals. Collected ectoparasites were placed in individually labeled vials containing 95% ethanol. Following recovery from anesthesia, all rodents were released at their capture site. Based on previously published standards, a sample size of at least 20 host rodents were collected at each field site, when possible to insure accurate host-ectoparasite interactions (Schwan 1984).

Ectoparasite Collection and Identification

Ectoparasites were collected from anesthetized rodents by combing each animal with a flea comb over a large white pan. The entire pelage was then systematically searched to collect sucking lice by the use of small forceps (Dumoxel no.5); Ectoparasites were then placed in labeled vials containing 95% ethanol, RNALater, or frozen, depending on the exact protocol needed to screen them for *Bartonella* spp. bacteria used for the pathogen genetics portion of this study. Collected ectoparasites were then transferred to a research laboratory at Georgia Southern University, identified to species, sex, and/or stage using a high power binocular microscope, then packaged and sent via FedEx to Vanderbilt University for DNA extraction and further analysis for the bartonellosis study.

Data Analysis

Rodents were characterized according to the state of their infestation. Infested rodents had one or more of the species of ectoparasites being studied (*P. gwyni* or *H. hirsuta)* while uninfested rodents had none of these particular ectoparasites. Prevalence was defined as the proportion (%) of infested individuals for each ectoparasite species. Mean intensity was defined as the mean number of an ectoparasite species (either *P. gwyni* or *H. hirsuta*) per infested rodent (Bush *et al.* 1997). Eighteen of the 20 sites sampled were considered in statistical analyses for mean intensity and prevalence's, with Flagler Co. Florida and Jackson Co. North Carolina being excluded from analyses because only one rat per site was captured and neither rodent was infested by ectoparasites belonging to either of the species of interest in the current study.

For all 20 sampling sites, a linear regression was performed to determine if rodent infestations with fleas or lice increased further south in latitude. For this analysis raw numbers of flea and louse counts per rodent were used including rodents that had zero counts for fleas and lice (meaning these ectoparasites were absent from the host during field examination).

To compare infestation of males versus female cotton rats, I used a one way analysis of variance (ANOVA) to test whether the amount of infestation was dependent on sex of the rodent (male or female), based on mean intensity and prevalence data collected at eighteen of the 20 sample sites. To normalize the distribution of prevalence and intensity data, I performed a square root transformation (Sokal & Rohlf 1995).

Sex ratios are often expressed as the count of females per one male in the ectoparasite literature (Marshall 1981a). However, to test sex ratios of ectoparasites, I used the raw numbers of male versus female lice and male versus female fleas in a Pearson's Chi-Square analysis. Raw data are presented in Appendix B. All statistical analyses were performed using JMP 7.0 for Windows XP.

CHAPTER 3

RESULTS

Overall, the results of this study showed that the flea *P. gwyni* was significantly more abundant with decreasing latitude (i.e., further south). There was no statistical difference between male versus female cotton rats in either louse or flea infestations. Sex ratios of both *H. hirsuta* and *P. gwyni* were significantly female-biased.

A total of 271 cotton rats were examined from 20 sites (12 in GA, 4 in FL, 2 in MS, 1 in SC and 1 in NC). One species of sucking louse (*Hoplopleura hirsuta*) and six species of fleas (*Ctenophthalmus pseudagyrtes*, *Orchopeas howardi*, *Peromyscopsylla hamifer*, *Peromyscopsylla scotti*, *Polygenis gwyni* and *Stenoponia americana*) were collected from cotton rats (Table 1). Of these flea species, only *P. gwyni* was recorded in sufficiently large numbers to warrant further analysis.

Effect of site location on ectoparasite infestation

The regression analysis revealed that the number of fleas (*P. gwyni*) on cotton rats was dependent on site location ($R^2 = 0.03$, df = 1, $p = 0.0040$, Figure 6) with significantly higher infestations recorded in more southern sites.

The abundance of the louse (*H. hirsuta*) on cotton rats did not show a comparable trend in the regression analysis to that of the flea. The noted trend actually seemed to show greater numbers of lice the higher in latitude that the trap site was located, but this was not a significant difference ($R^2 = 0.0016$, df = 1, $p = 0.5039$, Figure 7).

Effect of rodent sex on ectoparasite infestation

Male and female cotton rats did not differ in the mean intensity of either louse populations ($F_{1,38} = 1.4621$, $p = 0.2341$, Figure 8) or flea populations ($F_{1,38} = 0.4617$, $p = 0.5009$, Figure 8). Likewise, there was no difference in prevalence for either lice ($F_{1,38} = 0.0628$, $p = 0.8034$, Figure 9) or fleas ($F_{1,38} = 0.0478$, $p = 0.8281$, Figure 9) between male and female cotton rats.

Ectoparasite sex ratios

The sex ratio of the louse (*H. hirsuta*) averaged 2.6 females per male ($n = 482$) whereas the sex ratio of the flea (*P. gwyni*) averaged 1.4 females per male ($n = 471$), for all trap locations combined. The total number of female lice (349) was significantly greater than the total number if male lice (133) at the 0.05 alpha level ($x^2 = 96.796$, df $= 1$, $p = 0.001$, Table 2). Similarly, the total number of female fleas (271) was significantly greater than the total number of male fleas (200) collected ($x^2 = 10.7026$, df $= 1$, $p = 0.001$, Table 2).

CHAPTER 4

DISCUSSION

The relationships between ectoparasite infestation abundance, site location and host sex is complicated. The main finding of this study was that the likelihood of a cotton rat being parasitized by *Polygenis* fleas was dependent on the particular location in which it was sampled. However, it was interesting to see that this was not the case for *Hoplopleura* lice. Infestation was independent of rodent sex; therefore, the hypothesis predicting male biased prevalence and mean intensity of lice and fleas was not supported. Sex ratios of both fleas and lice were biased with almost 3 times as many female versus male lice and almost 1.5 times as many female versus male fleas recorded.

The prediction that the abundance of the flea studied in this project increased further south based on latitude was statistically supported. This corroborates other studies that describe the distribution of *Polygenis gwyni* as reaching its northern most boundary close to several of the trap locations stated in this study (such as Columbia County Georgia and Charleston County South Carolina) (Ferris 1921; Fox 1940; Morlan 1952; Pratt and Good 1954; Layne 1971; Benton 1980; Kim *et al*. 1986; Smit 1987; Durden *et al*. 1994, 2000). However, it is interesting to note that this was not the case for *Hoplopleura hirsuta*. This difference between the two ectoparasite species is intriguing and could be related to the fact that *H. hirsuta* is a permanent ectoparasite of cotton rats in all stages of its life cycle but that the life cycle of *P. gwyni* includes significant off-host stages (egg, larva and pupa) (Durden and Loyd 2009; Durden and Hinkle 2009). It seems plausible that the off-host stages of *P. gwyni* are influenced by some habitat gradient(s) that do not affect, or have little effect, on *H. hirsuta.* Gradients in ambient temperature

either throughout the year or during the winter are a possible cause for this phenomenon with off-host stages of *P. gwyni* showing increased survival or shorter generation times under conditions of warmer temperatures which would have occurred in the more southern locations sampled during this study. However, other factors such as humidity or precipitation gradients, soil/vegetation types, predators or competing arthropods are also feasible explanations for the observed gradient in *P. gwyni* populations. Conversely, all stages of *H. hirsuta* would presumably be buffered against these off-host factors by their permanent location on the host.

There was no difference in the infestation (as measured by prevalence and mean intensity) of the two ectoparasites studied on male versus female cotton rats. This does not corroborate some previous studies that attribute high levels of testosterone in male hosts with an increase in parasite load (Saino *et al.* 1995; Hughes and Randolph 2001). Conversely, parasite loads on some rodent hosts may depend more on the quality of the individual rodent than on rodents of different sexes (Thompson 1990); individual rodents could be affected by environmental conditions and foraging habits. Male hosts are often parasitized by greater numbers of ectoparasites of a given species than are female conspecific hosts (Marshall 1981b) for several potential reasons. In addition to the aforementioned effect of testosterone on host immunosuppression, male hosts often have larger home ranges than females and tend to accumulate more ectoparasites such as ticks, chiggers and (sometimes) fleas that can quest for hosts from vegetation or leaf litter (Mohr 1961). Male hosts also tend to have more aggressive or sexual physical encounters with other conspecfic hosts which present increased opportunities for ectoparasite transfer and accumulation (Gorell and Schulte-Hostedde 2008). The fact that

neither *H. hirsuta* nor *P. gwyni* were significantly more abundant on male hosts compared to female hosts in this study, suggests that the behavior of cotton rats does not differ widely by host sex. Alternatively, some factor(s) may dictate that populations of both *H. hirsuta* and *P. gwyni* are more homogeneous within their cotton rat populations than are the populations of some other ectoparasites on other host species.

Data from this study agree with the hypothesis that female fleas and lice are more common than males on cotton rats. Marshall (1981a) evaluated this phenomenon for ectoparasites in general and suggested that there are usually two main reasons to explain this outcome. While ectoparasites emerge in approximately equal numbers, an unequal trend thereafter is nearly always found in favor of female ectoparasitic arthropods in natural populations; the result being either inadequate sampling methods or the tendency for male ectoparasites to be shorter lived than their female counterparts (Marshall 1981a). Also, male fleas and lice are more vagile and could become detached from the host (Gorell and Schulte-Hostedde 2008). Marshall (1981a) noted that solely looking at the host for ectoparasites is an adequate method for permanent ectoparasites such as lice, but that it may be necessary to also sample the nest site of the rodent in order to obtain an accurate count for certain flea species. Cotton rat nests were not examined during this study for logistical reasons of locating nests that could unequivocally be ascribed to cotton rats and not to other species of rodents. Nevertheless, the female bias for both *H. hirsuta* and *P. gwyni* on cotton rats was strongly supported for this study.

Overall, data from this study revealed significantly larger on-host populations of the flea *P. gwyni* further south and closer to the center of distribution for this flea, no significant difference between louse and flea infestations on male versus female cotton

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rat hosts, and significantly female-biased on-host populations for both *P. gwyni* and the

louse *H. hirsuta*. A related study (see Appendix A) assessed *Bartonella* infections of *P.*

gwyni collected from the same cotton rats.

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Table 1. Ectoparasites recovered from Cotton Rats, *Sigmodon hispidus*, at each trap site.

State and County	Ectoparasites*
Georgia:	

Bulloch (32.444N, 81.783W)
Sigmodon hispidus
n=47 (27M, 20F)
	Sucking Louse:
	    Hoplopleura hirsuta (25M, 46F, 150N)
	Fleas:
	    Polygenis gwyni (43M, 50F)
	    Ctenophthalmus pseudagyrtes (1M)

Bleckley (32.397N, 83.347W)
Sigmodon hispidus
n=14 (5M, 9F)
	Sucking Louse:
	    Hoplopleura hirsuta (24M, 49F, 56N)
	Fleas:
	    Polygenis gwyni (2M, 1F)

Columbia (33.562N, 82.175W)
Sigmodon hispidus
n=15 (3M, 12F)
	Sucking Louse:
	    Hoplopleura hirsuta (2M, 5F, 4N)
	Fleas:
	    Polygenis gwyni (6M, 18F)
	    Ctenophthalmus pseudagyrtes (1M)
	    Peromyscopsylla scotti (1F)

Decatur (30.909N, 84.5833W)
Sigmodon hispidus
n=2 (1M, 1F)
	Sucking Louse:
	    Hoplopleura hirsuta (5F, 6N)
	Fleas:
	    Polygenis gwyni (1M)

Screven (32.751N, 81.605W)
Sigmodon hispidus
n=4 (1M, 3F)
	Sucking Louse:
	    Hoplopleura hirsuta (3M, 6F, 7N)
	Fleas:
	    Polygenis gwyni (16M, 16F)

Burke (32.985N, 81.978W)
Sigmodon hispidus
n=7 (3M, 4F)
	Sucking Louse:
	    Hoplopleura hirsuta (6M, 7F, 17N)
	Fleas:
	    Polygenis gwyni (1M)

McIntosh (31.374N, 81.499W)
Sigmodon hispidus
n=6 (3M, 3F)
	Sucking Louse:
	    Hoplopleura hirsuta (8M, 13F, 38N)
	Fleas:
	    Polygenis gwyni (3F)

Lowndes (30.842N, 83.306W)
Sigmodon hispidus
n=4 (2M, 2F)
	Sucking Louse:
	    Hoplopleura hirsuta (1M, 1F, 1N)
	Fleas:
	    Polygenis gwyni (2M, 2F)

Table 1. Continued

Jenkins (32.720N, 81.979W)
Sigmodon hispidus
n=10 (5M,5F)

Sucking Louse:
    Hoplopleura hirsuta (8M, 16F, 63N)
Fleas:
    Polygenis gwyni (1M, 2F)

Glynn (31.170N, 81.499W)
Sigmodon hispidus
n=5 (2M, 3F)

Sucking Louse:
    Hoplopleura hirsuta (1M, 1F, 1N)
Fleas:
    Polygenis gwyni (8M, 11F)

Chatham (31.942N, 81.035W)
Sigmodon hispidus
n=10 (6M, 4F)

Sucking Louse:
    Hoplopleura hirsuta (6M, 29F, 17N)

Candler (32.318N, 82.074W)
Sigmodon hispidus
n=14 (6M, 8F)

Sucking Louse:
    Hoplopleura hirsuta (5M, 17F, 6N)
Fleas:
    Polygenis gwyni (9M, 13F)

Florida:

Brevard (28.077N, 80.629W)
Sigmodon hispidus
n=28 (12M, 16F)

Sucking Louse:
    Hoplopleura hirsuta (1M, 5F, 17N)
Fleas:
    Polygenis gwyni (47M, 57F)

Flagler (29.469N, 81.364W)
Sigmodon hispidus
n=1 (1M)

N/A

Bay (30.169N, 85.648W)
Sigmodon hispidus
n=40 (18M, 22F)

Sucking Louse:
    Hoplopleura hirsuta (25M, 88F, 172N)
Fleas:
    Polygenis gwyni (7M, 17F)

Leon
Sigmodon hispidus (30.444N, 84.258W)
n=23 (10M,13F)

Sucking Louse:
    Hoplopleura hirsuta (7M, 33F, 18N)
Fleas:
    Polygenis gwyni (32M, 52F)
    Orchopeas howardi (1M)

Table 1. Continued

South Carolina:

Charleston (32.780N, 79.936W)
Sigmodon hispidus
n=18 (9M, 9F)

Sucking Louse:
    Hoplopleura hirsuta (6M, 19F, 2N)
Fleas:
    Polygenis gwyni (14M, 11F)
    Orchopeas howardi (1F)
    Stenoponia americana (3M, 2F)

North Carolina:

Jackson (35.372N, 83.199W)
Sigmodon hispidus
n=1 (1F)

Flea:
    Peromyscopsylla hamifer (1F)

Mississippi:

Jackson (30.366N, 88.543W)
Sigmodon hispidus
n=1 (1M)

Fleas:
    Polygenis gwyni (1M, 4F)

Marion (31.251N, 89.756W)
Sigmodon hispidus
n=17 (10M, 7F)

Sucking Louse:
    Hoplopleura hirsuta (3F)
Fleas:
    Polygenis gwyni (11M, 17F)

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*For each ectoparasite species, the numbers of different life stages recovered are listed (key: M, Male(s); F, Females(s), N, Nymph(s))

Table 2. Sex Ratios* of *Hoplopleura hirsuta* and *Polygenis gwyni* on Cotton Rats in the southeastern United States.

Site Location	Lice* (*H. hirsuta*)	n**	Fleas* (*P. gwyni*)	n**
Bulloch Co. – GA	1.8	46F;25M;150N	1.2	50F;43M
Bleckley Co. – GA	2.0	49F;24M;56N	0.5	1F;2M
Columbia Co. – GA	2.6	8F;3M;3N	3.2	16F;5M
Decatur Co. – GA	--	5F;0M;6N	0.0	0F;1M
McIntosh Co. – GA	1.6	13F;8M;22N	--	3F;0M
Chatham Co. – GA	4.8	29F;6M;17N	0.0	0F;0M
Screven Co. - GA	2.0	6F;3M;7N	1.0	16F;16M
Lowndes Co. – GA	1.0	1F;1M;1N	1.0	2F;2M
Candler Co.- GA	3.4	17F;5M;6N	1.4	13F;9M
Burke Co. – GA	1.2	7F;6M;17N	0.0	0F;1M
Jenkins Co. – GA	2.0	16F;8M;63N	2.0	2F;1M
Glynn Co. – GA	1.0	4F;4M;8N	1.3	10F;8M
Brevard Co. – FL	5.0	5F;1M;17N	1.2	57F;47M
Flagler Co. – FL	--	0F;0M;0N	--	0F;0M
Bay Co. – FL	3.5	88F;25M;176N	2.4	17F;7M
Leon Co. – FL	4.7	33F;7M;18N	1.6	52F;32M
Charleston Co. – SC	2.7	19F;7M;2N	0.8	11F;14M
Jackson Co. – MS	--	0F;0M;0N	4.0	4F;1M
Marion Co. – MS	--	3F;0M;0N	1.5	17F;11M
Jackson Co. – NC	--	0F;0M;0N	--	0F;0M
Total Sites Combined	2.6	349F;133M;428N	1.4	271F;200M

*expressed as number of females per one male
**F=females
   M=males
   N=nymphs
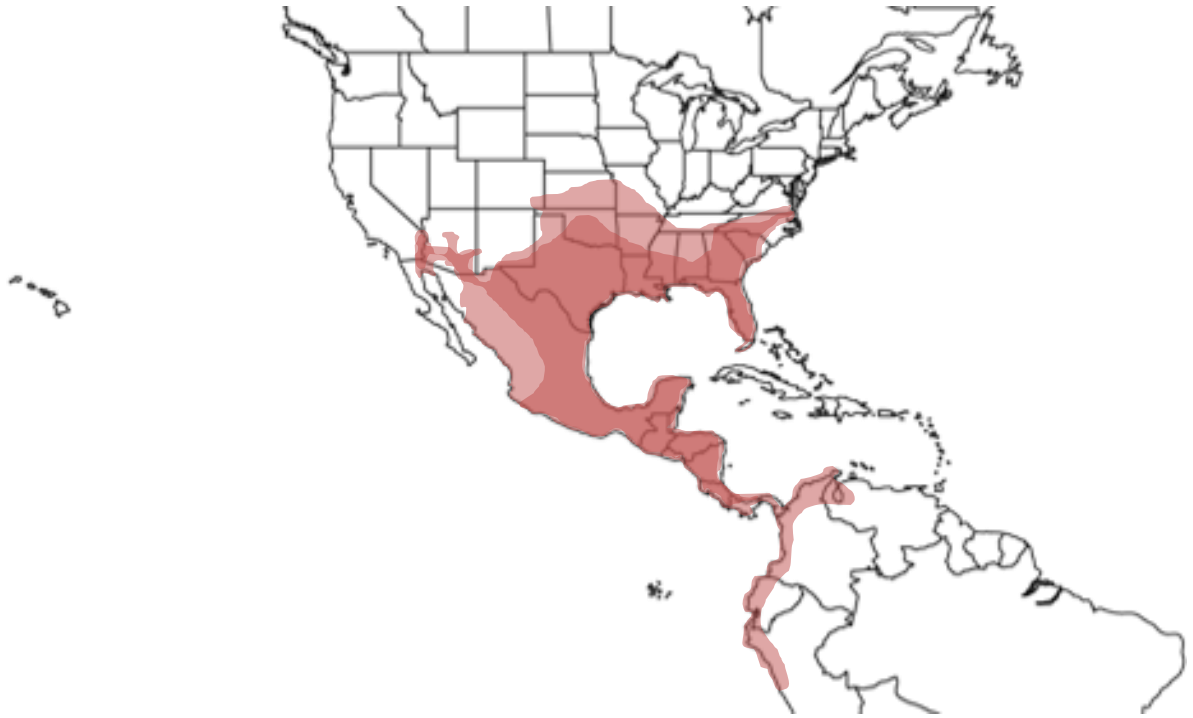
Figure 1. Approximate geographical distribution of *Sigmodon hispidus* (Cotton Rat), shaded in red-modified from Hall & Kelson (1959), Cameron and Spencer (1981) and Whitaker and Hamilton (1998).

Figure 2. Male (left) and female (right) *Polygenis gwyni* flea. (Specimens cleared in Potassium hydroxide).

Figure 3. Male (left) and female (right) *Hoplopleura hirsuta* sucking louse. (Specimens cleared in Potassium hydroxide).

Figure 4. Approximate geographical distribution of *Hoplopleura hirsuta* (sucking louse) shaded in blue. Data compiled from Ferris (1921), Morlan (1952), Smith and Love (1958), Henry (1970), Kim et al. (1986), Pfaffenberger and DeBrian (1988), Durden *et al.* (1993, 2000) and Durden and Musser (1994).
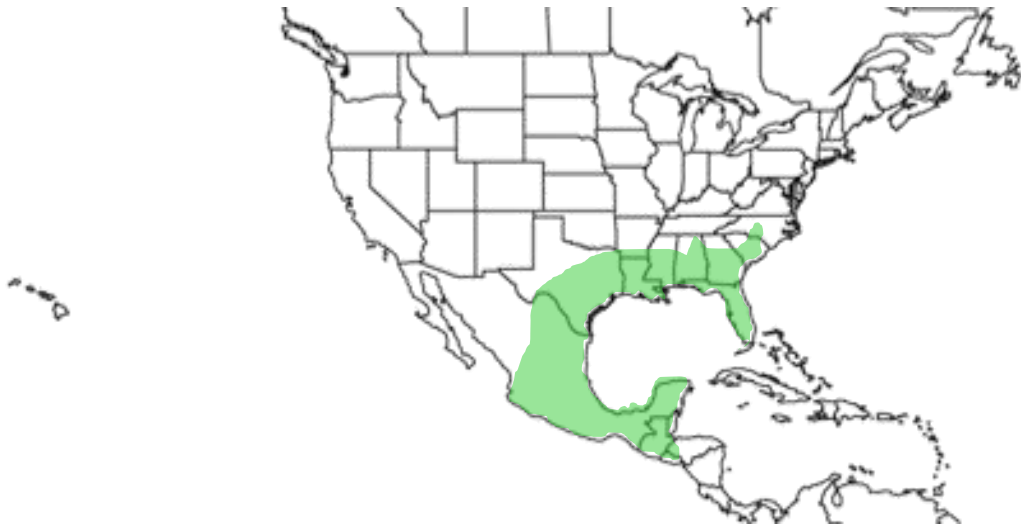
Figure 5. Approximate geographical distribution of *Polygenis gwyni* (flea) shaded in green. Data compiled from Fox (1940), Morlan (1952), Pratt and Good (1954), Smith and Love (1958), Henry (1970), Layne (1971), Benton (1980), Smit (1987), Pfaffenberger and DeBrian (1988) and Durden *et al*. (1993, 2000).

Figure 6. Regression analysis of the number of fleas on cotton rats for each trap site given by latitude. The abundance of fleas per trap site was analyzed using raw numbers of fleas per rodent at each trap site including individual rodents with zero fleas recorded.
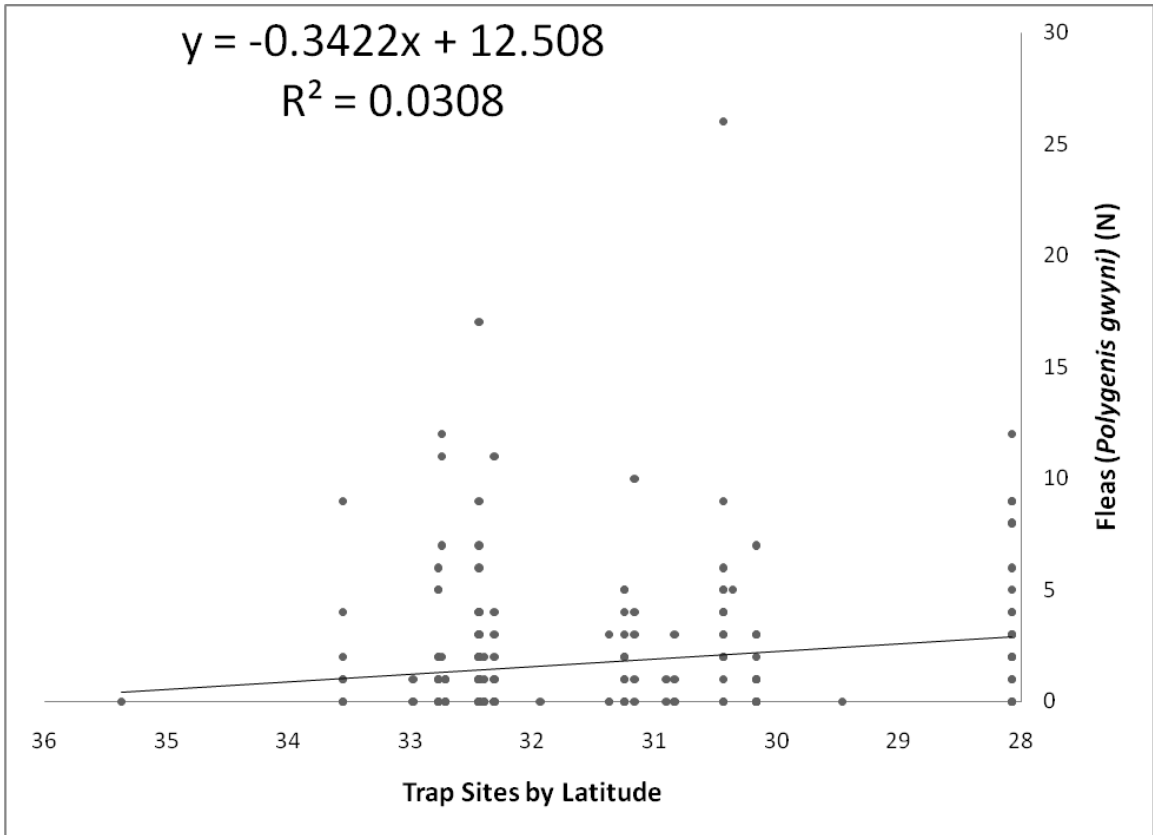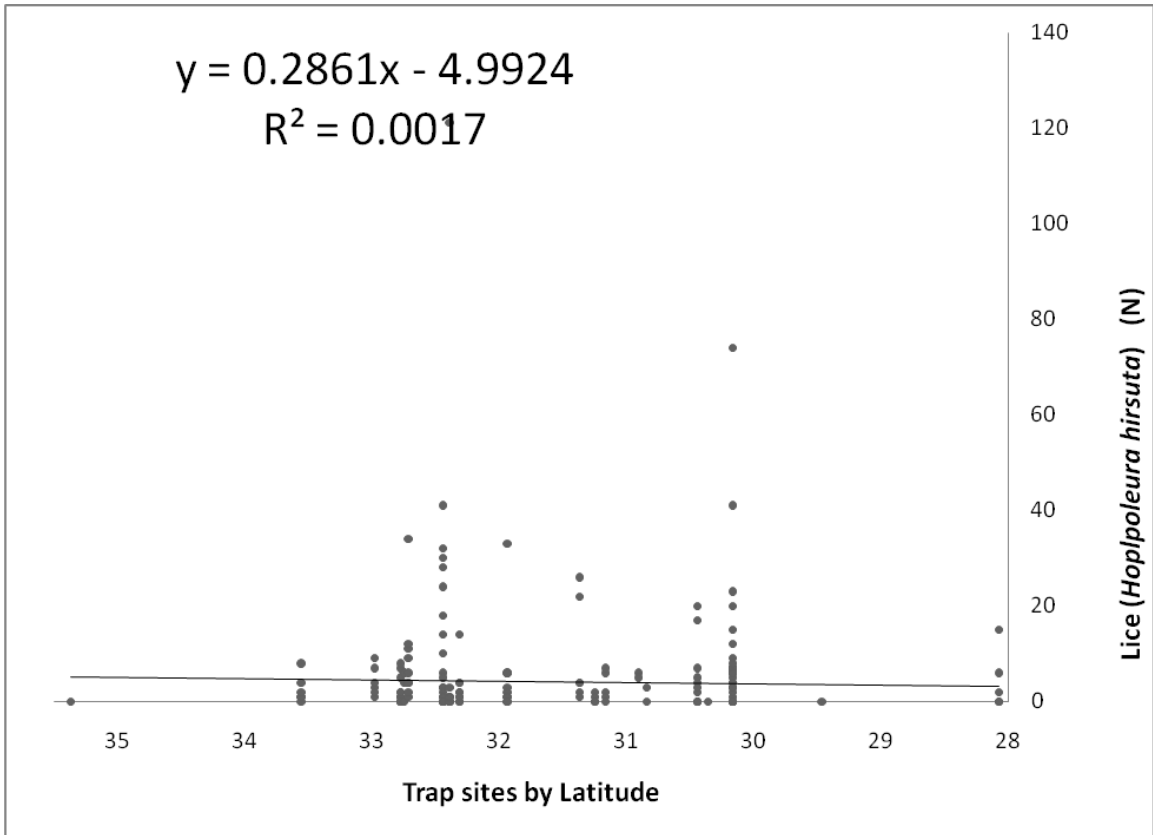
Figure 7. Regression analysis of the number of lice on cotton rats for each trap site given by latitude. The abundance of lice per trap site was analyzed using raw numbers of lice per rodent at each trap site including individual rodents with zero lice recorded.
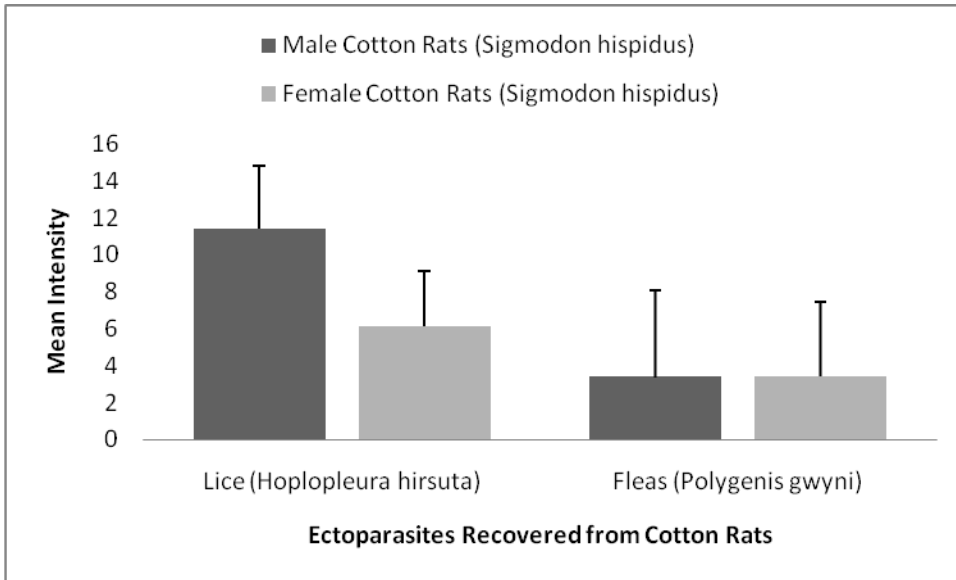
Figure 8. Mean intensity of *Hoplopleura hirsuta* and *Polygenis gwyni* (only hosts

with ectoparasites) infesting male and female cotton rats.
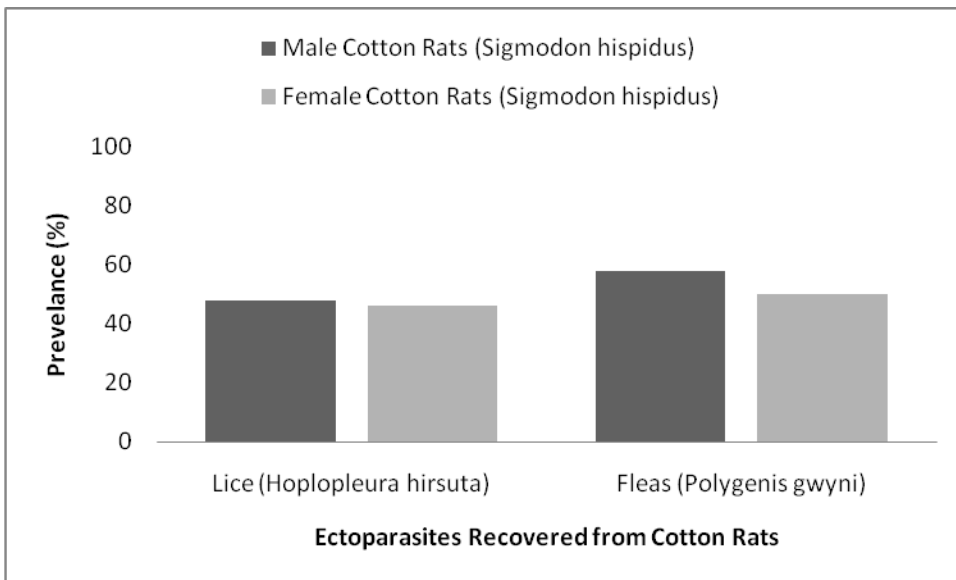
Figure 9. Prevalence of *Hoplopleura hirsuta* and *Polygenis gwyni* (number of hosts

infested divided by the total number of hosts examined) of male and female cotton

rats.

APPENDIX A

MIXED INFECTIONS, CRYPTIC DIVERSITY, AND VECTOR-BORNE

PATHOGENS: EVIDENCE FROM *POLYGENIS* FLEAS

AND *BARTONELLA* SPECIES

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Mixed Infections, Cryptic Diversity, and Vector-Borne Pathogens: Evidence from *Polygenis* Fleas and *Bartonella* Species[∇]

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Coinfections within hosts present opportunities for horizontal gene transfer between strains and competitive interactions between genotypes and thus can be a critical element of the lifestyles of pathogens. *Bartonella* spp. are *Alphaproteobacteria* that parasitize mammalian erythrocytes and endothelial cells. Their vectors are thought to be various biting arthropods, such as fleas, ticks, mites, and lice, and they are commonly cited as agents of various emerging diseases. Coinfections by different *Bartonella* strains and species can be common in mammals, but little is known about specificity and coinfections in arthropod vectors. We surveyed the rate of mixed infections of *Bartonella* in flea vectors (*Polygenis gwyni*) parasitizing cotton rats (*Sigmodon hispidus*) in which previous surveys indicated high rates of infection. We found that nearly all fleas (20 of 21) harbored one or more strains of *Bartonella*, with rates of coinfection approaching 90%. A strain previously identified as common in cotton rats was also common in their fleas. However, another common strain in cotton rats was absent from *P. gwyni*, while a rare cotton rat strain was quite common in *P. gwyni*. Surprisingly, some samples were also coinfected with a strain phylogenetically related to *Bartonella clarridgeiae*, which is typically associated with felids and ruminants. Finally, a locus (*pap31*) that is characteristically borne on phage in *Bartonella* was successfully sequenced from most samples. However, sequence diversity in *pap31* was novel in the *P. gwyni* samples, relative to other *Bartonella* previously typed with *pap31*, emphasizing the likelihood of large reservoirs of cryptic diversity in natural populations of the pathogen.

Most host populations harbor more than one pathogen strain at a given time, leading to mixed infections or "coinfections" in individual hosts (10, 26, 48). Unfortunately, there are gaps in our understanding of within-host pathogen interactions. The problem is particularly acute in vector-borne diseases, where little is known regarding mixed infection interactions in natural populations of the vectors themselves. Rather, with only a few notable exceptions (e.g., reference 25), most population-level or clinical data on mixed infections derive from human studies or other mammalian models. The distinction is crucial because of the role that vectors play in pathogen transmission.

The bacterial pathogen *Bartonella* sp. has become one of a few model organisms for studying the evolution and ecology of vector-borne diseases (28). This is due to diverse efforts to describe *Bartonella* biology at multiple levels, from cells and immune systems (12, 13, 14, 30), to populations and communities (31, 32), to species and clades (36, 44). The recent publication of full genome sequences is obviously key (2). *Bartonella* sp. is a short, gram-negative, fastidious bacterium belonging to the *Alphaproteobacteria* (1). Closely related to *Brucella* spp., *Bartonella* organisms are parasites of mammalian erythrocytes and endothelial cells (12, 13, 14) and are transmitted by blood-feeding insects, such as ticks, fleas, lice, and flies (9, 19, 20, 21, 23, 28). Infection of a host causes chronic bacteremia and creates a reservoir for vectors that can transmit the bacteria to new susceptible hosts. While prolonged bacteremia is normally associated with severe sickness in a susceptible host, *Bartonella*-caused bacteremia typically remains asymptomatic in the reservoir host. Some bartonellae are known to be transmitted by the bite (anterior station transmission) or in the feces (posterior station transmission) of insect vectors. For example, in humans, *Bartonella bacilliformis*, which causes Oroya fever (verruga peruana, or Carrion's disease) in Andean South America is transmitted by the bites of infectious sandflies (5), and *Bartonella quintana*, which causes trench fever in many parts of the world, is transmitted via the feces of infected body lice (21). Fleas infected with *Bartonella henselae* (the causative agent of cat scratch disease and of related conditions such as bacillary angiomatosis [30]) and other bartonellae appear to transmit these agents via their infectious feces (9, 19, 20). Current phylogenetic information indicates six distinct groups worldwide, of which all but one are found in the United States (44). Host and vector affiliations are complex, and the evidence is against strict one-to-one host specificity (28, 32, 33). A consistent trend is that groups of *Bartonella* species tend to be restricted to natural groups of mammalian hosts (rodents, cats, dogs, humans, etc.), indicating a diffuse but long-term coevolutionary history.

We surveyed the incidence of mixed *Bartonella* infections in natural populations of the flea *Polygenis gwyni* parasitizing the Eastern woodrat (*Neotoma floridana*) and the hispid cotton rat (*Sigmodon hispidus*). Previous surveys of mammalian hosts indicated that mixed infections of *Bartonella* can be common (22). An intensive survey of *S. hispidus* in the southeastern United States, for example, revealed that this host exhibits a particularly high infection prevalence overall, as well as non-

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TABLE 1. Loci and GenBank accession numbers used in the present study to reconstruct *Bartonella* phylogenetic relationships and to identify the species relationships of cloned *gltA* amplicons from *Polygenis* fleas

Species (strain)	GenBank accession no. for amplicon				
	GltA	RpoB	GroEL	FtsZ	RibC
B. alsatica (IBS382)	AF204273	AF165987	AF299357	AF467763	AY116630
B. bacilliformis (KC584T)	U28076	AF165988	Z15160	AF007266	AJ236918
B. birtlesii (IBS 325T)	AF204272		AF355773	AF467762	AY116632
B. bovis (91-4T)	AF293394	DQ356078			AY116637
B. clarridgeiae (Houston-2T)	U84386	AF165990	AF014831	AF141018	BCL236916
B. doshiae (R18T)	AF207827	AF165991	AF014832	AF467754	AY116627
B. elizabethae (F9251T)	U28072	AF165992	AF014834	AF467760	AY116633
B. grahamii (V2T)	Z70016	AF165993		AF467753	AY166583
B. henselae (Houston-1T)	L38987	AF171070	AF014829	AF061746	AJ132928
B. koehlerae (C-29T)	AF176091	AY166580	AY116641	AF467755	AY116634
B. quintana (FullerT)	Z70014	AF165994	AF014830	AF061747	AJ236917
B. schoenbuchensis (R1T)	AJ278183	AY167409b	AY116642	AF467765	AY116628
B. taylorii (M6T)	AF191502	AF165995	AF304017	AF467756	AY116635
B. tribocorum (IBS 506T)	AJ005494	AF165996	AF304018	AF467759	
B. vinsonii subsp. *arupensis* (OK 94-513	AF214557	AY166582b	AF304016	AF467758	AY116631
B. vinsonii subsp. *berkhoffii* (93-CO1T)	U28075	AF165989	AF014836	AF467764	AY116629
B. vinsonii subsp. *vinsonii* (BakerT)	Z70015	AF165997	AF014835	AF467757	AY116636
B. bovis (FC7049UT)	AF071190		AF071194	AF467761	
Brucella sp.	AE014291	DQ086137	AE014292	AE014291	

negligible rates of coinfection (33). There is little comparable information on mixed infection rates in *Bartonella* vectors (49). However, with mammalian host populations multiply infected with strains that are vectored by insects with generalist host affiliations (e.g., ticks and fleas), the expectation is that rates of mixed infections in competent vectors should be quite high. The relevance of whether or not this is the case not only bears on the basic natural history and disease dynamics of *Bartonella* but also on the pattern and tempo of disease emergence (5). *Bartonella* has been described as the consummate "versatile pathogen" (27) for the breadth of its host affiliations and plasticity of its lifestyles (2). Population-level data are necessary supplements to evolutionary inferences about the genus, because retrospective analyses of such events as lateral gene transfer (4) can become forward-looking and predictive when accompanied by real-time data on the ecological context for such events (15, 24, 34, 47, 55).

MATERIALS AND METHODS

Trapping and collection methods. Cotton rats and Eastern woodrats were trapped in Bulloch and Screven Counties (one site in each county) in southeastern Georgia, using Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, FL) baited with rolled oats and a trace of peanut butter and set near areas of rodent activity. Trapped animals were lightly anesthetized via intramuscular administration of ketamine hydrochloride and then transferred to a white tray, where they were carefully examined for ectoparasites. Retrieved ectoparasites were transferred to individually labeled cryovials containing 95% ethanol. Fleas were later identified using the methods of Smit (52) and Lewis and Lewis (38). All fleas collected were *Polygenis gwyni*, which is the species that typically parasitizes the cotton rat in the southern United States (38, 52). This flea species has also been reported previously from the Eastern woodrat (16). Following recovery from anesthesia, all cotton rats and woodrats were released at their capture site. Mammals were live trapped under permit 9172 issued by the Georgia Department of Natural Resources, and animal procedures were approved by the IACUC committee at Georgia Southern University (research protocol no. I06003). Voucher flea specimens have been deposited in the Ectoparasite Collection at Georgia Southern University under accession numbers L-358 and L-1102.

DNA methods. Whole genomic DNA from *P. gwyni* was extracted using a DNeasy tissue kit (QIAGEN, Inc.). Each sample was tested for the presence or absence of *Bartonella* by PCR amplification of an approximately 400-bp amplicon from the citrate synthase *gltA* gene, using the universal oligonucleotide primers BhCS781.p and BhCs1137.n (33). *gltA* was chosen because of its high discriminating power for *Bartonella* (36), the existing coverage in GenBank of the genus using this gene, and its prior use in identifying *Bartonella* in the flea host, *S. hispidus* (32). PCR products were visualized by electrophoresis and ethidium bromide staining under UV light on 1.5% agarose gels. Samples yielding successful *gltA* amplicons were then retested with oligonucleotides papn1 and papn2, designed from the bacteriophage-associated gene *pap31* in *B. henselae*. Both PCR amplifications were carried out at 10-μl volumes, containing 1× Invitrogen 10× buffer, 2.0 mM MgCl₂, 100 μM of each deoxynucleoside triphosphate, 5 pmol of each primer, 1 U of Invitrogen DNA *Taq* polymerase, sterile PCR-grade water, and approximately 5 to 10 ng of whole genomic DNA. Reaction conditions were 1 cycle at 94°C for 2 min and 30 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 60 s, followed by 1 cycle at 72°C for 15 min. Products from both reactions were cloned via a pCR2.1-TOPO vector (Invitrogen Life Technologies, Carlsbad, CA) and TOPO TA cloning kit and Top10 competent cells, according to the manufacturer's instructions. Positive clones for both genes were PCR amplified at 50-μl volumes, as above, purified with a QIAGEN PCR purification kit (QIAGEN, Inc.) following the manufacturer's instructions, and sequenced at the Vanderbilt University Medical Center Sequencing Core Facility and the University of Arizona Genomic Analysis and Technology Core Facility, with either Invitrogen vector primer *IVT7* or *M13R*. Resulting sequences were then compared against known *Bartonella* sequences in GenBank, using default parameters in BLAST.

Phylogenetic methods. We determined the phylogenetic affinities of the *gltA* amplicons by first constructing a backbone phylogeny of 18 *Bartonella* species, isolated from a wide range of mammalian hosts from each of the five recognized host clusters (Table 1). Initially, species were selected by the availability of sequences in public databases from seven housekeeping genes commonly used in *Bartonella* species delineation (*16S*, *ITS*, *ftsZ*, *gltA*, *groEL*, *ribC*, and *rpoB*; not all gene sequences were available for all species). However, *16S* and *ITS* were not used because of strong phylogenetic incongruence and alignment uncertainty in these loci. The remaining genes were first aligned using a partial order alignment algorithm (implemented in the software package POA v.2, using default parameters [37]) and then checked by eye for obvious discrepancies. Individual alignments were then concatenated, yielding a global ca. 4.6-kb alignment.

Maximum parsimony trees were constructed in PAUP 4.0b10 (53), using simple sequence addition, the TBR swapping algorithm, and 10 random addition replicates for each search iteration. Parsimony trees were compared to those generated by a partitioned Bayesian analysis in the software package MrBayes v.3.1.2 (50), under models and parameters separately estimated via Modeltest v.3.6 (45), each conditioned on the same starting tree estimated by maximum likelihood on the entire data set using a general time-reversible model of evo-

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TABLE 2. Identification of *Bartonella* spp. isolates cloned from each flea, based on reconstruction of *gltA* or *pap31* phylogenies using GenBank sequences and those derived from the present study[a]

P. gwynii sample no.	Host	Mammal ID	Site	Distinct *gltA* genogroup[b]				n[d]	*gltA* GenBank accession no(s)	Distinct pap31 clade[c]			n[d]	*pap31* GenBank accession no(s)
				A1/A5	B2/B3	B4	*B. clarridgeiae*			I	II	III		
1	S. hispidus	AEA 10-17	A	+	+		+	12	EF616644/655		+	+	8	EF625688/695
2	S. hispidus	AEA 10-17	A	+		+		5	EF616656/660				-	
3	S. hispidus	AEA 11-20	A					-					-	
4	S. hispidus	AEA 11-20	A	+	+			8	EF616661/668	+			3	EF625696/698
5	N. floridana	AEA 01-13	B	+		+		12	EF616669/680		+		1	EF625699
6	N. floridana	AEA 01-14	B	+				11	EF616681/691				-	
7	S. hispidus	LAD-3331	B	+	+			2	EF616692/693	+	+		9	EF625700/708
8	S. hispidus	LAD-3331	B	+				10	EF616694/703			+	2	EF625709/710
9	S. hispidus	LAD-3331	B					-		+	+		6	EF625711/716
10	S. hispidus	LAD-3332	B	+	+			7	EF616704/710	+	+		8	EF625717/724
11	S. hispidus	LAD-3332	B	+		+	+	7	EF616711/717	+	+	+	5	EF625725/729
12	S. hispidus	LAD-3333	B	+	+			2	EF616718/719	+	+		7	EF625730/736
13	S. hispidus	LAD-3333	B	+				7	EF616720/726		+		1	EF625737
14	S. hispidus	LAD-3333	B	+				8	EF616727/734	+	+		3	EF625738/740
15	S. hispidus	LAD-3333	B	+			+	9	EF616735/742	+	+		2	EF625741/742
16	S. hispidus	LAD-3334	B	+				1	EF616643	+	+		7	EF625743/749
17	S. hispidus	LAD-3334	B	+	+			7	EF616744/750	+	+		10	EF625750/759
18	S. hispidus	LAD-3334	B	+	+		+	15	EF616751/765	+	+	+	15	EF625760/774
19	S. hispidus	LAD-3335	B	+			+	6	EF616766/771	+			11	EF625775/785
20	S. hispidus	LAD-3335	B	+	+		+	29	EF616772/800	+	+	+	16	EF625786/801
21	S. hispidus	LAD-3335	B	+	+			19	EF616801/819	+	+	+	15	EF625802/816

[a] The two collection sites were Candler County, GA (A) and Bulloch County, GA (B). The *gltA* columns represent the full diversity of positive matches based on phylogenetic reconstruction. Most matched previously undescribed "genogroups" from *Sigmodon hispidus*, as shown in Fig. 1 and Fig. 3. The plus signs indicate a positive match. The *pap31* clades are provisional designations based on the topology depicted in Fig. 3.
[b] See Fig. 1.
[c] See Fig. 3.
[d] Number of clones sequenced.

lution (6). Priors were not changed from default values. We ran four simultaneous Metropolis-coupled Monte Carlo Markov chains for 1,000,000 generations, with a heating parameter of 0.1. We sampled every 100 generations and calculated a consensus topology after a "burn-in" of 2,500 trees. The consensus tree was then used as a backbone constraint, and cloned *gltA* amplicons from the *Polygenis* samples of *Bartonella* were grafted onto the tree using a simple distance-based neighbor-joining algorithm. For most positive *Polygenis* samples, this involved replicates of >5 *Bartonella* clones per individual flea. A newly described species, *Bartonella rochalimae*, which is closely related to *B. clarridgeiae* (18), was also grafted onto the tree using a *gltA* sequence from GenBank (accession no. DQ683195).

pap31 sample size was smaller, and there are generally fewer data available on the gene in *Bartonella*. Positive sequences were simply aligned with known *Bartonella* orthologs from GenBank, and both Bayesian and maximum likelihood trees were constructed using the Bayesian methodology described above and for the maximum likelihood tree using a general time-reversible model of nucleotide evolution with parameters estimated from the data. The maximum likelihood analysis was performed with the software program Garli v0.95 (www.bio.utexas.edu/faculty/antisense/garli/Garli.html) (57).

Nucleotide sequence accession numbers. The *gltA* and *pap31* sequences have been deposited in GenBank under the accession numbers EF616644 to EF616819 and EF625688 to EF625816, respectively.

RESULTS

Eight *S. hispidus* and two *N. floridana* rats were trapped from two sites, from which 21 *P. gwyni* fleas were collected. Either *gltA* or *pap31* amplicons of the expected size were detected in 20 of 21 fleas, and replicate sequences were obtained from most samples, such that any *Taq* error or PCR recombination could be identified and not included in the diversity estimates (Table 2). In 17 fleas, we sequenced multiple and divergent *gltA* or *pap31* clones, permitting us to survey the frequency of single or multiple infections. Cloning efficiency varied between individual fleas, resulting in an unequal number of sequences per gene per flea (Table 2). There was some, but not perfect, overlap between *gltA* and *pap31* as positive evidence for mixed infections. Using *gltA* only, mixed *Bartonella* infections were detected in 12 of 16 fleas positive for *Bartonella* and for which five or more sequence replicates were obtained (Table 2). Most *gltA* amplicons exhibited greater than 94% sequence similarity to undescribed *Bartonella vinsonii*-like genogroups previously cultured from *S. hispidus* in the southeastern United States (Fig. 1 and 2) (33). However, in six fleas collected from five different *S. hispidus* isolates, *gltA* amplicons were detected with closest similarity to *B. clarridgeiae*, a species nominally associated with felines and ruminants (44) but which may be closely related to species with broader host ranges (18, 39). BLAST searches with the *pap31* sequences resulted in highest sequence similarity scores with either *B. quintana* or *B. henselae*, although neither of these species was greater than 94% similar to any of the *pap31* sequences (Fig. 3 and 4), reflecting the still-limited survey of *pap31* diversity in *Bartonella* available in GenBank. However, the *pap31* phylogeny revealed three distinct clades of *P. gwyni*-associated *Bartonella*, perhaps mirroring the divergence between strains detected by *gltA*. One clade was characterized by a 1-bp deletion near the boundary between a putative conserved transmembrane domain and an extracellular loop sequence (Fig. 4) (42), producing a UAA stop codon downstream and thus presumably a truncated protein. This deletion was perfectly matched by an alanine-to-valine replacement downstream in a putative inner membrane loop sequence.
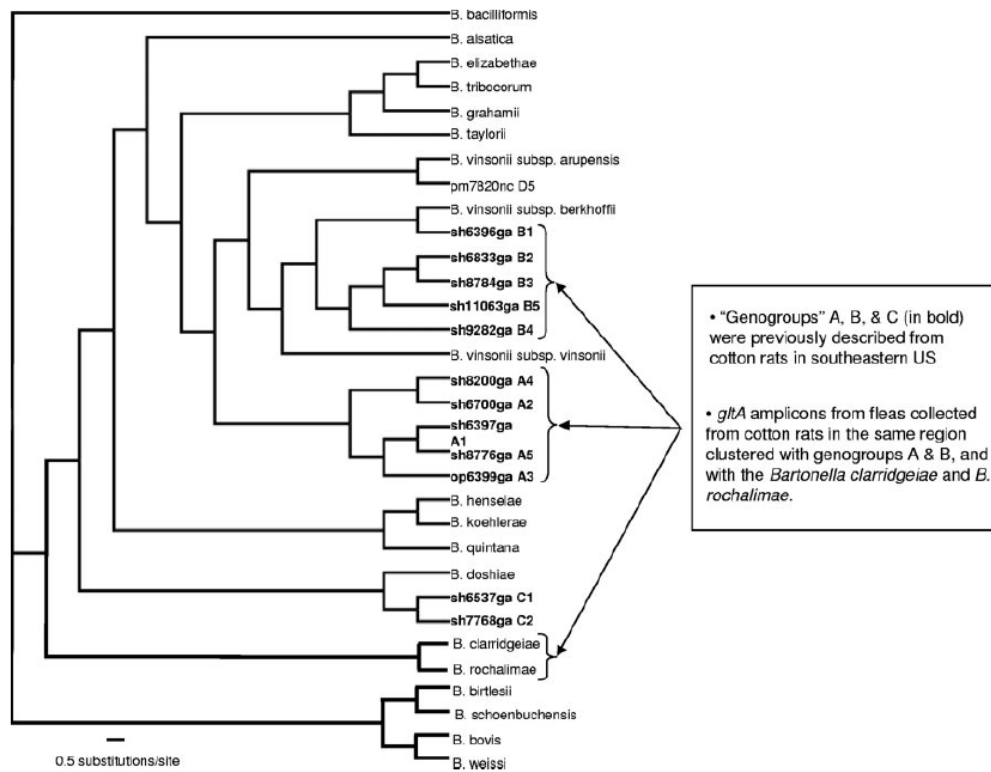
FIG. 1. Bayesian phylogeny of the genus *Bartonella*, including many of the described species. The tree is rooted with *B. bacilliformis* and is based on partial sequences from five concatenated loci, with the exception of those shown in bold (see Table 1 for GenBank accession numbers). The bold taxa represent type isolates of the genogroups (designated A thru D) discovered in previous surveys of cotton rats in the southeastern United States (32, 33). Only *gltA* sequences are available for these. All nonterminal resolved nodes had clade credibility values of >98, based on the Bayesian analysis. The overall topology was supported by parsimony analysis. Arrows indicate the phylogenetic placement of the different *P. gwyni*-derived isolates on the constrained *Bartonella* phylogeny, based on neighbor-joining placement of the amplicons on the tree. With the exception of the isolates similar to *B. clarridgeiae* and *B. rochalimae*, most were >99% similar to the designated A or B genogroup. Most amplicons were confirmed by redundant sequencing of multiple cloned products.

DISCUSSION

We surveyed the prevalence of *Bartonella* in a population of rodent fleas, collected from a general locale in which small mammals had been previously intensively surveyed (31, 32, 33). Because we surveyed in a manner that discriminated between single and mixed infections in fleas, we also estimated the fraction of fleas harboring more than one *Bartonella* isolate and the phylogenetic affinities of coinfecting isolates. We found four noteworthy results.

First, the prevalence of *Bartonella* was surprisingly high, exceeding characteristic records from various putative arthropod vectors (35, 49, 54). Estimating prevalence requires population-level sampling, and only in recent years have such surveys of *Bartonella* in presumed vectors begun to emerge. Cat fleas (*Ctenocephalides felis*) are important agents of zoonotic *Bartonella* transmission and have been examined in a number of studies sufficient to yield population-level data (41, 49). Estimates of cat-associated *Bartonella* prevalence (e.g., *B. henselae*, *B. quintana*, *B. koehlerae*, and *B. clarridgeiae*) have ranged from 20 to 30%, although some *C. felis* populations may

exhibit higher rates (35). Less is known about other flea or arthropod vectors from natural populations of mammals. Studies reporting nonnegligible rates of infection in fleas from various small mammals typically have ranged from 10 to 40% (41, 54). Not surprisingly, there are still few studies that report simultaneous estimates of prevalence in vectors and their mammalian hosts (54).

However, we collected fleas from *S. hispidus* in an area that, because of extensive prior work (31, 32, 33), corresponds to an intensively scrutinized regional population (the coastal plain and piedmont of Georgia). In one study, Kosoy et al. (32) found rates of *Bartonella* infection in *S. hispidus* in central and southern Georgia approaching 80%. Thus, the degree of *Bartonella* infection in the *P. gwyni* population we surveyed is consistent with more extensive surveys of *S. hispidus* and lends confidence that these small sample estimates are representative.

Second, we found substantial rates of mixed *Bartonella* infections. More than half of the fleas we surveyed were infected by more than one *Bartonella gltA* genotype (Table 2). If the

	Bartonella genogroups described from the cotton rat, *Sigmodon hispidus* (Kosoy et al. 1997)										B. clarr.	B. rochal.	Bartonella gltA amplicons from fleas, *Polygenis gwyni* (this study)										
	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5			#1	#2	#2	#4	#4	#6	#10	#11	#11	#13	#20
S. hispidus A1	-																						
S. hispidus A2	0.970	-																					
S. hispidus A3	0.978	0.970	-																				
S. hispidus A4	0.964	0.988	0.966	-																			
S. hispidus A5	0.997	0.970	0.981	0.964	-																		
S. hispidus B1	0.926	0.920	0.922	0.920	0.926	-																	
S. hispidus B2	0.932	0.926	0.929	0.926	0.932	0.979	-																
S. hispidus B3	0.932	0.926	0.929	0.926	0.932	0.979	0.997	-															
S. hispidus B4	0.938	0.932	0.933	0.926	0.938	0.982	0.991	0.991	-														
S. hispidus B5	0.938	0.932	0.933	0.926	0.938	0.979	0.994	0.994	0.997	-													
B. clarridgeiae	0.889	0.875	0.884	0.872	0.869	0.878	0.878	0.878	0.887	0.884	-												
B. rochalimae	0.887	0.883	0.903	0.880	0.887	0.890	0.890	0.890	0.899	0.896	0.960	-											
P. gwyni #1	**0.991**	0.961	0.966	0.955	**0.988**	0.917	0.923	0.923	0.929	0.929	0.866	0.880	-										
P. gwyni #2a	0.938	0.932	0.933	0.926	0.938	0.982	0.991	0.991	**1.000**	0.997	0.887	0.898	0.929	-									
P. gwyni #2b	0.970	**1.000**	0.970	0.988	0.970	0.920	0.926	0.926	0.932	0.932	0.875	0.883	0.961	0.932	-								
P. gwyni #4a	**0.997**	0.967	0.974	0.961	0.964	0.923	0.929	0.929	0.935	0.935	0.866	0.893	0.998	0.935	0.967	-							
P. gwyni #4b	**0.997**	0.967	0.974	0.961	0.964	0.923	0.929	0.929	0.935	0.935	0.866	0.893	0.998	0.935	0.967	0.994	-						
P. gwyni #6	**1.000**	0.970	0.978	0.964	0.967	0.926	0.932	0.932	0.938	0.938	0.869	0.887	0.991	0.938	0.970	0.967	0.997	-					
P. gwyni #10	0.929	0.923	0.925	0.923	0.929	0.976	0.994	**0.997**	0.988	0.991	0.875	0.887	0.920	0.988	0.923	0.926	0.926	0.929	-				
P. gwyni #11a	0.938	0.932	0.933	0.926	0.938	0.979	0.994	0.994	0.997	**1.000**	0.884	0.896	0.929	0.997	0.932	0.935	0.935	0.938	0.991	-			
P. gwyni #11b	0.872	0.872	0.888	0.869	0.872	0.876	0.872	0.872	0.881	0.878	**0.947**	**0.942**	0.869	0.881	0.872	0.869	0.869	0.872	0.869	0.878	-		
P. gwyni #13	**0.991**	0.961	0.970	0.955	**0.988**	0.917	0.923	0.923	0.929	0.929	0.861	0.877	0.982	0.929	0.961	0.988	0.988	0.991	0.920	0.929	0.864	-	
P. gwyni #20	**0.991**	0.961	0.966	0.955	**0.988**	0.917	0.923	0.923	0.929	0.929	0.861	0.877	0.982	0.929	0.961	0.988	0.988	0.991	0.920	0.929	0.869	0.982	-

FIG. 2. Matrix of genetic similarity (fraction of identical sites) in a 337-bp fragment of *gltA* cloned from *Polygenis gwyni* fleas collected from cotton rats (*Sigmodon hispidus*). Only representative flea-*Bartonella* samples are shown. Many fleas contained mixed infections, and examples are shown (numbers 2, 4, and 11). The grey shading highlights the values that compare the *P. gwyni* strains to the corresponding strains A1 through A5 and B1 through B5 previously cultured from *S. hispidus* (33). The bold values represent the highest similarity values for each flea-associated *Bartonella* sample to the various A or B genogroups described from cotton rats. *B. clarridgeiae* and the newly described *B. rochalimae* (18) are flagellated species distantly related to *Bartonella* species described from cotton rats but nevertheless are genetically similar to amplicons from the fleas of cotton rats.

pap31 screens are included, the rate is even higher. Kosoy et al. (33) originally described four broad genotypic clusters associated with various small rodents from the southeastern United States, designated A through D. Type sequences originally used to define groups A and B together form a diverse but monophyletic group of *B. vinsonii*-like isolates from *S. hispidus*, as originally indicated in the neighbor-joining distance *gltA* tree of Kosoy et al. (33). We detected isolates similar to A and B in the surveyed fleas and, unsurprisingly, did not detect the *Peromyscus*-associated D group. Surprisingly, we did not detect genogroup C, previously cultured from regional samples of *S. hispidus* (31, 32). Cluster A is, by far, the most common *Bartonella* genogroup isolated from cotton rats in the region (31, 32). However, C is more prevalent than B (31, 32), a pattern opposite of what we found in *P. gwyni* from cotton rats (Table 2; Fig. 1). This pattern may simply be an artifact of small sample sizes and may not hold up to more-extensive surveys. However, one possibility is that the different *P. gwyni/S. hispidus* isolates exhibit either unequal resident times in the vectors and hosts and/or transmission biases, potentially presenting an opportunity to uncover differential adaptation and specificity in *Bartonella* (M. Kosoy, personal communication).

Third, we successfully amplified a fragment similar to the heme-binding *pap31* in fleas evidently infected with *B. vinsonii*-like isolates (as determined by *gltA*). This is notable, because in both *B. quintana* and *B. henselae*, *pap31* is generally known to be phage-borne and orthologous to a large family of heparin-binding protein-coding genes (*hbp*) critical to heme acquisition, cellular adhesion, and possibly pathogenesis (8, 11, 56). Recent work has described a *pap31* homolog from bacteriophages in *B. vinsonii* subsp. *berkhoffii* (40), a species that was previously thought to lack bacteriophages and *hbpA* protein homologs (8). Assuming the *gltA* results are a reliable guide, we found *pap31*-like sequences in fleas infected by Kosoy et al. genogroups A and B; *pap31* amplicons were cloned in fleas apparently lacking coinfections and harboring either the A or B *gltA* genogroup alone (Table 2). Although there is not yet sufficient coverage of the genus with *pap31* to identify the

isolates we detected, three distinct genogroups are evident (Fig. 3). Possibly, the two derived genogroups within the clade correspond to Kosoy et al.'s (33) genogroups A and B.

In *B. quintana*, *pap31* is a member of a five-gene family, composed of three tandemly arrayed paralogs and two other homologs (42). A possible complication is the uncertain copy number of *pap31* homologs across the genus. However, the clade that includes the *P. gwyni* samples is rooted by *hbpA* from *B. quintana*, to the exclusion of other members of the gene family, and includes orthologous sequences from *B. henselae* and *B. vinsonii* subsp. *berkhoffi* (Fig. 3). Moreover, the *pap31* transmembrane protein includes outer membrane loops with the potential to incorporate nearly random in-frame chromosomal sequences. In *B. quintana*, the five homologs are difficult to align at these sites (42) (data not shown). With the exception of some isolates exhibiting distinct similarity to *B. henselae* (Fig. 4), the loop sequences from *P. gwyni* isolates exhibit very little amino acid polymorphism between the conserved transmembrane domains. It is thus likely that the *pap31* topology reflects orthologous sequence variation in the *Bartonella* isolates we surveyed. Because of the sampling design and the high rate of coinfection, it is not possible to determine the significance of the truncated *hbpA* pseudogene. However, possibilities include that some isolates harbor an antigenic variant of the full *hbpA* protein, similar to the *msp2* locus in *Anaplasma* sp. (3, 7), or that the *pap31* pseudogene is a loss-of-function mutant derived from a cryptic strain that has undergone a change in lifestyle (23, 43).

In this vein, the fourth and perhaps most surprising result was the presence in two fleas of an isolate sharing >94% *gltA* similarity to *B. clarridgeiae* and the newly described species *B. rochalimae* (the next closest relative is *Bartonella bovis*, at >87% sequence similarity) (Fig. 1). *B. clarridgeiae* itself has not been described from rodents; rather, felids or canids are the primary reservoirs (49). Species near *B. clarridgeiae* have been reported in various mammalian hosts, however, and recently, a *B. clarridgeiae*-like isolate was identified in rat fleas from Egypt (39). Among the highest BLAST scores for the *B.*
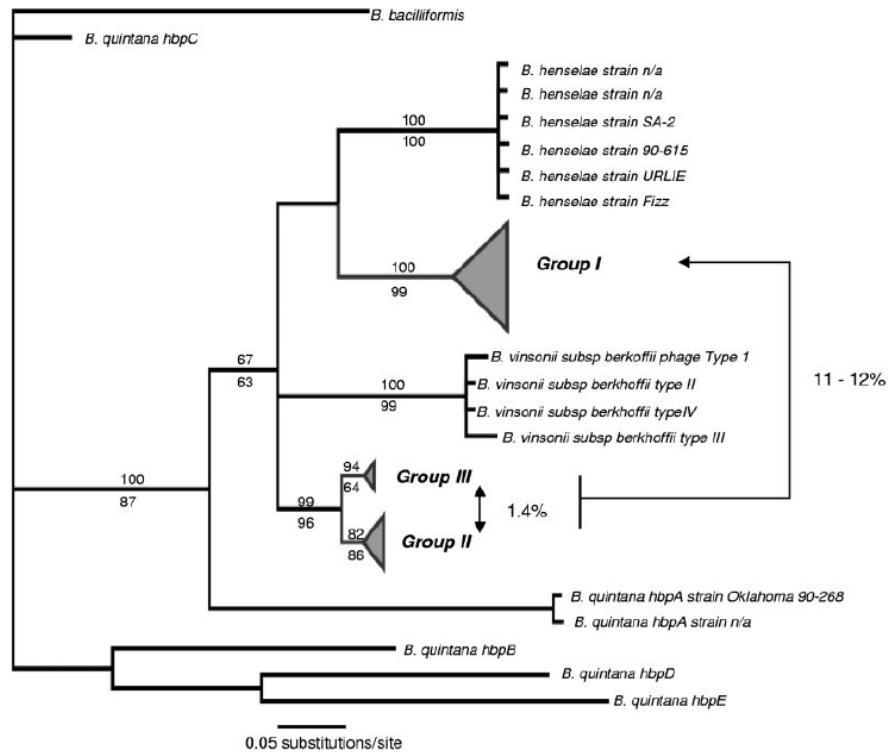
38

FIG. 3. Consensus tree of *Bartonella* taxa based on partial *pap31* sequences. Numbers above interior branches represent clade credibility values from the Bayesian analysis (above) and 100 maximum likelihood bootstrap replicates (below). Most amplicons were confirmed by redundant sequencing of multiple cloned products. Three distinct genotypic groups are evident from the fleas of cotton rats, as shown. The genetic distance between groups II and III, based on 152 bp of alignable transmembrane domain sequences, was approximately 1.4% (uncorrected *p* distance). Group I differed from both by approximately 11 to 12%. The *Bartonella* isolates in these groups have no clear identity based on BLAST searches of *pap31* sequences. Highest BLAST scores were returned for *B. henselae* or *B. quintana*, but this probably reflects the limited taxonomic sampling of *pap31* across the genus.

clarridgeiae-like isolates in *Polygenis* were uncultured species from rodents and other small mammals (17, 25, 46). Both fleas were coinfected with Kosoy genogroup A or B. In the case of one flea, all three principle *gltA* variants were detected. The

significance of a *B. clarridgeiae* species in *Sigmodon* is unknown; because of the size of the survey and the absence of simultaneous information on the competence of *Polygenis* as a *B. clarridgeiae* vector and *B. clarridgeiae* bacteremia in cotton
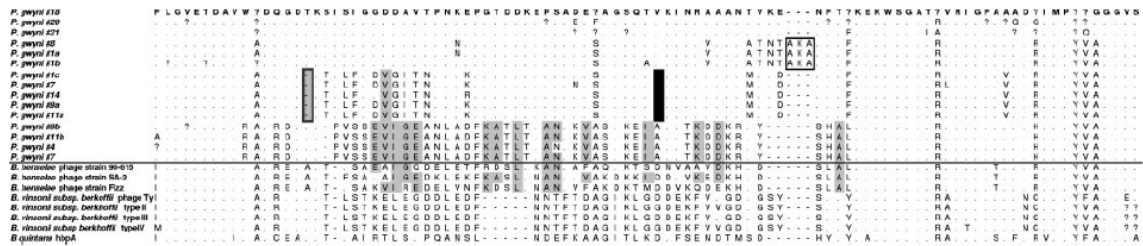

FIG. 4. Amino acid fragments of *pap31* homologs from various taxa and a subset amplified from representative flea samples in the present study. The fragment corresponds to an outer membrane loop sequence between conserved transmembrane domains 3 and 4, as described in reference 42. These loops may be composed of nearly random host chromosomal sequences (56), as evident in the lack of conservation between the related *B. henselae* and *B. quintana*. Seven of the nine *P. gwyni* isolates exhibit strong conservation, likely indicating a recent common ancestor. Some samples align more closely to *B. henselae* strains, as indicated by gray shading. In one group (clade II from Fig. 3), a 1-bp deletion (grey hatched box) causes a UAA stop codon downstream. A gap has been inserted to maintain the alignment in these samples. The black box highlights an insertion of three residues. Dots indicate matches with the topmost sequence, dashes indicate gaps, and question marks indicate uncertainties due to unresolvable ambiguities in the nucleotide sequences. The black line separates the *pap31* amplicons from the present study and those from known species. Dissimilar amplicons were cloned from the same flea samples, as illustrated by *P. gwyni* numbers 1, 9, and 11.

rats, the biological significance is difficult to judge. However, like *B. bacilliformis*, the etiological agent of bartonellosis (Carrion's disease) in humans, and *B. bovis*, *B. clarridgeiae* is one of the few flagellated bartonellae (51) and has long been a problematic species because of its uncertain phylogenetic placement and the odd host range that it shares with *B. bovis* (44). It may not be a coincidence that an isolate resembling these hyper-generalist species has been discovered in *Polygenis*. Efforts to understand the molecular basis of variation in host specificity in the genus (2) would benefit from closer examination of *B. clarridgeiae* and its relatives (18).

Two opposing ecological and evolutionary processes seem to be at work in *Bartonella*. The cryptic diversity in the vectors of *Bartonella*, and the absence of strains common in mammalian hosts, may reflect an evolutionary trend towards differential adaptation to host-specific niches, in either vectors or reservoirs. If so, it seems *Bartonella* possesses a tendency towards fine-scale adaptation, ecological specialization, and divergence between essentially syntopic populations, despite mixed infections, close physical proximity, and generalist lifestyles. The mechanisms by which *Bartonella* genomes are protected during the process of specialization to host-associated niches, while maintaining broad host affiliations and thus mixed infections (29), are presently unknown.

ACKNOWLEDGMENTS

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APPENDIX B: INDIVIDUAL MAMMAL DATA

Example entry with explanations:

1.1**a**		**a** Site by State and County
Sigmodon hispidus **b**	F, J **c**	**b** Mammal species
Hoplopleura hirsuta **d**	3F, 1N **e**	**c** Mammal gender, Life Stage
Polygenis gwyni	1M	**d** Arthropod species
Stenoponia americana	1F	**e** Number of arthropods collected
Peromyscopsylla hamifer	2F	**f** Accession number
LAD 210 **f**		

Legend:

sites: *symbols:*
1.1 – Georgia, Bulloch   M - Male
1.2 – Georgia, Bleckley   F - Female
1.3 – Georgia, Columbia   N - Nymph
1.4 – Georgia, Decatur   J - Juvenile
1.5 – Georgia, Screven   A - Adult
1.6 – Georgia, Burke
1.7 – Georgia, McIntosh
1.8 – Georgia, Lowndes
1.9 – Georgia, Jenkins
1.10 – Georgia, Glynn
1.11 – Georgia, Chatham
1.12 – Georgia, Candler
2.1 – Florida, Brevard
2.2 – Florida, Flagler
2.3 – Florida, Bay
2.4 – Florida, Leon
3.1 – South Carolina, Charleston
4.1 – North Carolina, Jackson
5.1 – Mississippi, Jackson
5.2 – Mississippi, Marion

APPENDIX B. Continued

Data:

1.1
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1F, 2N
LAD 210

1.1
Sigmodon hispidus   F, J
Hoplopleura hirsuta   3M,8F, 7N
LAD 1332

1.1
Sigmodon hispidus   M, A
LAD 2354

1.1
Sigmodon hispidus   F, A
Polygenis gwyni   1M,1F
LAD 2355

1.1
Sigmodon hispidus   F, A
Hoplopleura hirsuta   6N
Polygenis gwyni   2F
LAD 2356

1.1
Sigmodon hispidus   F, A
Polygenis gwyni   1F
LAD 2370

1.1
Sigmodon hispidus   M, A
LAD 2737

1.1
Sigmodon hispidus   M, A
Polygenis gwyni   2M,1F
LAD 2738

1.1
Sigmodon hispidus   M, A
Polygenis gwyni   1F
LAD 2815

1.1
Sigmodon hispidus   F, J
LAD 2844

1.1
Sigmodon hispidus   M, A
Polygenis gwyni   6M,3F
LAD 2848

1.1
Sigmodon hispidus   F, A
Polygenis gwyni   2M,2F
LAD 2849

1.1
Sigmodon hispidus   M, A
Polygenis gwyni   2M,5F
LAD 2850

1.1
Sigmodon hispidus   M, J
Polygenis gwyni   2M,2F
LAD 2851

1.1
Sigmodon hispidus   F, J
Polygenis gwyni   1M,1F
LAD 2903

1.1
Sigmodon hispidus   F, A
LAD 3258

1.1
Sigmodon hispidus   M, A
Polygenis gwyni   2M,5F
LAD 2850

1.1
Sigmodon hispidus   M, J
Polygenis gwyni   2M,2F
LAD 2851

1.1
Sigmodon hispidus   F, J
Polygenis gwyni   1M,1F
LAD 2903

1.1
Sigmodon hispidus   F, A
LAD 3258

1.1
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1F,2N
LAD 3259

1.1
Sigmodon hispidus   F, A
Polygenis gwyni   2F
LAD 3260

1.1
Sigmodon hispidus   F, A
Hoplopleura hirsuta   2M,4F,26N
LAD 3261

1.1
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1M,2F,11N
LAD 3262

1.1
Sigmodon hispidus   F, A
Polygenis gwyni   1M
LAD 3263

1.1
Sigmodon hispidus   F, A
Hoplopleura hirsuta   6M,4F,18N
LAD 3265

APPENDIX B. Continued

1.1
Sigmodon hispidus F, A
Hoplopleura hirsuta 1M,2F,27N
Polygenis gwyni 1M
LAD 3266

1.1
Sigmodon hispidus M, A
Hoplopleura hirsuta 1M,1F,8N
LAD 3267

1.1
Sigmodon hispidus M, A
Hoplopleura hirsuta 1M,1F,3N
LAD 3268

1.1
Sigmodon hispidus M, A
Hoplopleura hirsuta 1M,3F,20N
Polygenis gwyni 1M
LAD 3269

1.1
Sigmodon hispidus M, A
LAD 3270

1.1
Sigmodon hispidus M, A
Polygenis gwyni 1M,2F
LAD 3331

1.1
Sigmodon hispidus M, A
Polygenis gwyni 2F
LAD 3332

1.1
Sigmodon hispidus M, A
Polygenis gwyni 1M,3F
LAD 3333

1.1
Sigmodon hispidus F, A
Polygenis gwyni 2M,2F
LAD 3334

1.1
Sigmodon hispidus F, A
Polygenis gwyni 2M,1F
LAD 3335

1.1
Sigmodon hispidus M, A
Hoplopleura hirsuta 1M,2F
Polygenis gwyni 2M
LAD 3384

1.1
Sigmodon hispidus M, A
Polygenis gwyni 1F
LAD 3385

1.1
Sigmodon hispidus F, A
Ctenophthalmus pseudagyrtes 1M
LAD 3386

1.1
Sigmodon hispidus M, J
Polygenis gwyni 1F
LAD 3394

1.1
Sigmodon hispidus M, J
Polygenis gwyni 1M,1F
LAD 3395

1.1
Sigmodon hispidus M, J
Polygenis gwyni 2F
LAD 3396

1.1
Sigmodon hispidus M, J
Hoplopleura hirsuta 1F,1N
LAD 3397

1.1
Sigmodon hispidus F, J
Hoplopleura hirsuta 8M,15F,18N
Polygenis gwyni 13M,4F
LAD 3402

1.1
Sigmodon hispidus F, J
Hoplopleura hirsuta 1F
Polygenis gwyni 2F
LAD 3412

1.1
Sigmodon hispidus M, A
Polygenis gwyni 1M,5F
LAD 3413

1.1
Sigmodon hispidus M, J
Hoplopleura hirsuta 1N
Polygenis gwyni 1F
LAD 3414

1.1
Sigmodon hispidus M, A
Polygenis gwyni 1F
LAD 3415

1.1
Sigmodon hispidus   F, A
LAD 3416

1.1
Sigmodon hispidus   M, A
Polygenis gwyni   1F
AEA 0114

1.2
Sigmodon hispidus   F, A
Polygenis gwyni   1M,1F
LAD 471

1.2
Sigmodon hispidus   F, A
LAD 479

1.2
Sigmodon hispidus   M, J
LAD 481

1.2
Sigmodon hispidus   F, A
LAD 483

1.2
Sigmodon hispidus   F, J
Hoplopleura hirsuta   1F
LAD 485

1.2
Sigmodon hispidus   F, J
LAD 487

1.2
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M
LAD 489

1.3
Sigmodon hispidus   F, A
Polygenis gwyni   2M
Peromyscopsylla scotti   1F
LAD 1620

1.1
Sigmodon hispidus   F, A
Polygenis gwyni   1M
AEA 0113

1.2
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1M,1F,1N
LAD 470

1.2
Sigmodon hispidus   M, A
LAD 478

1.2
Sigmodon hispidus   F, J
Hoplopleura hirsuta   1N
LAD 480

1.2
Sigmodon hispidus   M, A
Hoplopleura hirsuta   21M,47F,53N
Polygenis gwyni   1M
LAD 482

1.2
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M
LAD 484

1.2
Sigmodon hispidus   M, J
LAD 486

1.2
Sigmodon hispidus   F, J
Hoplopleura hirsuta   1N
LAD 488

1.3
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1M,4F,3N
LAD 490

1.3
Sigmodon hispidus   M, A
Polygenis gwyni   1M
LAD 1627

1.3

Sigmodon hispidus   F, J
Polygenis gwyni   1M
LAD 2233

1.3

Sigmodon hispidus   F, J
LAD 2234

1.3

Sigmodon hispidus   F, J
Polygenis gwyni   2F
LAD 2235

1.3

Sigmodon hispidus   F, J
Polygenis gwyni   1F
LAD 2236

1.3

Sigmodon hispidus   F, A
Polygenis gwyni   1F
LAD 2237

1.3

Sigmodon hispidus   F, A
Polygenis gwyni   1F
LAD 2238

1.3

Sigmodon hispidus   F, A
LAD 2239

1.3

Sigmodon hispidus   F, A
LAD 2247

1.3

Sigmodon hispidus   M, J
Hoplopleura hirsuta   1F
LAD 2248

1.3

Sigmodon hispidus   F, A
Hoplopleura hirsuta   1F
Polygenis gwyni   2M,2F
Ctenophthalmus pseudagyrtes   1M
LAD 2249

1.3
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M,1N
Polygenis gwyni   1F
LAD 2250

1.3
Sigmodon hispidus   F, A
Polygenis gwyni   2F
LAD 2280

1.4
Sigmodon hispidus   M, A
Hoplopleura hirsuta   6N
Polygenis gwyni   1F
LAD 1343

1.4
Sigmodon hispidus   F, A
Hoplopleura hirsuta   5F
Polygenis gwyni   1M
LAD 1344

1.5
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M,3F,2N
Polygenis gwyni   5M,7F
LAD 2328

1.5
Sigmodon hispidus   F, A
Hoplopleura hirsuta   2M,2F
Polygenis gwyni   2F
LAD 2336

1.5
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1F,5N
Polygenis gwyni   8M,3F
LAD 2337

1.5
Sigmodon hispidus   F, A
Polygenis gwyni   3M,4F
LAD 2338

1.6
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1F
AEA 0819

1.6
Sigmodon hispidus   F, A
Hoplopleura hirsuta   2M,1F,4N
AEA0818

1.6
Sigmodon hispidus   M, A
Hoplopleura hirsuta   3N
AEA 0806

1.6
Sigmodon hispidus   F, J
Hoplopleura hirsuta   1M,3N
Polygenis gwyni   1M
AEA 0804

1.6
Sigmodon hispidus   M, J
Hoplopleura hirsuta   1M,3F
AEA 0212

1.6
Sigmodon hispidus   F, J
Hoplopleura hirsuta   2N
AEA 0215

1.6
Sigmodon hispidus   F, A
Hoplopleura hirsuta   2M,2F,5N
AEA 0217

1.7
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1M,3F
LAD 1577A

1.7
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M,1F
LAD 1577B

1.7
Sigmodon hispidus   F, J
Hoplopleura hirsuta   1M
AEA 0301

1.7
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M,2F,1N
AEA 0306

1.7
Sigmodon hispidus   M, A
Hoplopleura hirsuta   4M,7F,15N
Polygenis gwyni   3F
AEA 0315

1.7
Sigmodon hispidus   M, A
Hoplopleura hirsuta   22N
AEA 0322

1.8
Sigmodon hispidus   F, J
Hoplopleura hirsuta   1M,1F,1N
LAD 3398

1.8
Sigmodon hispidus   M, A
Polygenis gwyni   1F
LAD 3399

1.8
Sigmodon hispidus   F, A
Polygenis gwyni   2M,1F
LAD 3400

1.8
Sigmodon hispidus   M, J
LAD 3401

1.9
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1N
AEA 0813

1.9
Sigmodon hispidus   M, A
Hoplopleura hirsuta   2N
Polygenis gwyni   1F
AEA 0815

1.9
Sigmodon hispidus   M, A
Hoplopleura hirsuta   11N
AEA 0817

1.9
Sigmodon hispidus   F, A
Hoplopleura hirsuta   12N
AEA 0907

1.9
Sigmodon hispidus   M, A
Hoplopleura hirsuta   2F,32N
Polygenis gwyni   1F
AEA 0919

1.9
Sigmodon hispidus   F, A
Hoplopleura hirsuta   3F,1N
AEA 0103

1.9
Sigmodon hispidus   M, A
Hoplopleura hirsuta   4M,2F
AEA 0107

1.9
Sigmodon hispidus   F, A
Hoplopleura hirsuta   3M,5F,1N
Polygenis gwyni   1M
AEA 0111

1.9
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M,3F
AEA 0121

APPENDIX B. Continued

1.9
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1F,3N
AEA 0125

1.10
Sigmodon hispidus   M, A
Hoplopleura hirsuta   2N
Polygenis gwyni   3M,1F
AEA 0302

1.10
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1N
AEA 0303

1.10
Sigmodon hispidus   F, J
Hoplopleura hirsuta   2M,3F,1N
Polygenis gwyni   1M
AEA 0304

1.10
Sigmodon hispidus   F, J
Polygenis gwyni   1M,2F
AEA 0307

1.10
Sigmodon hispidus   M, A
Hoplopleura hirsuta   2M,1F,4N
Polygenis gwyni   3M,7F
AEA 0318

1.11
Sigmodon hispidus   M, J
Hoplopleura hirsuta   3M,23F,7N
LAD 2284

1.11
Sigmodon hispidus   F, A
Hoplopleura hirsuta   2F,1N
LAD 2285

1.11
Sigmodon hispidus   F, J
Hoplopleura hirsuta   6N
LAD 2286

1.11
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1F
AEA 0906

1.11
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M,1F
AEA 0910

1.11
Sigmodon hispidus   M, A
AEA 0914

1.11
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1N
AEA 0920

1.11
Sigmodon hispidus   M, A
Hoplopleura hirsuta   2M,2F,2N
AEA 1002

1.11
Sigmodon hispidus   M, A
AEA 0402

1.11
Sigmodon hispidus   M, A
AEA 0911

1.12
Sigmodon hispidus   F, A
AEA 1001

1.12
Sigmodon hispidus   M, J
Hoplopleura hirsuta   1F,1N
AEA 1106

1.12
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1F,3N
Polygenis gwyni   1M,1F
AEA 1120

1.12
Sigmodon hispidus   M, A
AEA 0509

1.12
Sigmodon hispidus   F, A
Polygenis gwyni   5M,6F
AEA 0518

1.12
Sigmodon hispidus   F, A
Hoplopleura hirsuta   4F
AEA 0606

1.12
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1N
Polygenis gwyni   2M,2F
AEA 0608

1.12
Sigmodon hispidus   M, J
Hoplopleura hirsuta   1M,1F
AEA 0612

APPENDIX B. Continued

1.12
Sigmodon hispidus   M, J
AEA 0918

1.12
Sigmodon hispidus   F, A
AEA 0920

1.12
Sigmodon hispidus   F, J
AEA 0909

1.12
Sigmodon hispidus   F, A
Polygenis gwyni   1M
AEA 1005

1.12
Sigmodon hispidus   M, A
Hoplopleura hirsuta   4M,9F,1N
Polygenis gwyni   3F
AEA 1017

1.12
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1F
Polygenis gwyni   1F
AEA 0508

2.1
Sigmodon hispidus   F, A
Hoplopleura hirsuta   2F
LAD 433

2.1
Sigmodon hispidus   M, A
Polygenis gwyni   6M,6F
LAD 436

2.1
Sigmodon hispidus   M, A
Polygenis gwyni   2F
LAD 437

2.1
Sigmodon hispidus   F, A
Polygenis gwyni   3M,3F
LAD 438

2.1
Sigmodon hispidus   M, A
Polygenis gwyni   2M,2F
LAD 439

2.1
Sigmodon hispidus   F, A
Polygenis gwyni   4M,5F
LAD 440

2.1
Sigmodon hispidus   M, A
Polygenis gwyni   5M,3F
LAD 441

2.1
Sigmodon hispidus   F, A
Polygenis gwyni   2M,1F
LAD 442

2.1
Sigmodon hispidus   M, A
Polygenis gwyni   7M,1F
LAD 444

2.1
Sigmodon hispidus   F, A
Polygenis gwyni   2F
LAD 445

2.1
Sigmodon hispidus   F, J
Polygenis gwyni   3M,6F
LAD 446

2.1
Sigmodon hispidus   F, A
LAD 447

2.1
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M,3F,2N
Polygenis gwyni   1M,4F
LAD 448

2.1
Sigmodon hispidus   F, A
Polygenis gwyni   1M,4F
LAD 449

2.1
Sigmodon hispidus   M, A
Polygenis gwyni   1M
LAD 450

2.1
Sigmodon hispidus   M, A
Polygenis gwyni   1M,2F
LAD 451

2.1
Sigmodon hispidus   F, A
Polygenis gwyni   5M,4F
LAD 452

2.1
Sigmodon hispidus   F, A
LAD 453

2.1
Sigmodon hispidus   F, A
Polygenis gwyni   1M,2F
LAD 454

2.1
Sigmodon hispidus   M, A
Polygenis gwyni   1M,3F
LAD 455

2.1
Sigmodon hispidus   M, A
Polygenis gwyni   2F
LAD 456

2.1
Sigmodon hispidus   M, J
LAD 457

2.1
Sigmodon hispidus   F, J
Hoplopleura hirsuta   15N
Polygenis gwyni   1M
LAD 458

2.1
Sigmodon hispidus   M, A
Polygenis gwyni   1M,2F
LAD 459

2.1
Sigmodon hispidus   M, A
Polygenis gwyni   1M,1F
LAD 460

2.1
Sigmodon hispidus   F, A
LAD 461

2.1
Sigmodon hispidus   F, J
LAD 462

2.1
Sigmodon hispidus   F, A
Polygenis gwyni   1M,2F
LAD 463

2.2
Sigmodon hispidus   M, A
LAD 477

2.3
Sigmodon hispidus   F, A
LAD 2304

2.3
Sigmodon hispidus   F, J
LAD 2305

2.3
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M,2F
LAD 2306

2.3
Sigmodon hispidus   F, A
Hoplopleura hirsuta   3N
LAD 2307

2.3
Sigmodon hispidus   F, A
LAD 2308

2.3
Sigmodon hispidus   M, J
LAD 2309

2.3
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1M,2F,6N
LAD 2310

2.3
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M,1F,4N
Polygenis gwyni   1F
LAD 2311

2.3
Sigmodon hispidus   M, J
LAD 2312

2.3
Sigmodon hispidus   F, A
LAD 2313

2.3
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M,2F,4N
LAD 2314

2.3
Sigmodon hispidus   F, A
Hoplopleura hirsuta   6N
LAD 2315

2.3
Sigmodon hispidus   F, A
LAD 2316

2.3
Sigmodon hispidus   M, A
Hoplopleura hirsuta   2M,8F,13N
LAD 2317

2.3
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1F
Polygenis gwyni   1F
LAD 2318

2.3
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1M,1F
LAD 2319

2.3
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1F,11N
LAD 2320

2.3
Sigmodon hispidus F, A
Hoplopleura hirsuta 2F,5N
LAD 2321

2.3
Sigmodon hispidus F, A
LAD 2322

2.3
Sigmodon hispidus M, A
Hoplopleura hirsuta 1F,2N
Polygenis gwyni 2M,1F
LAD 2500

2.3
Sigmodon hispidus M, A
Hoplopleura hirsuta 1M,4F,2N
Polygenis gwyni 1F
LAD 2501

2.3
Sigmodon hispidus M, A
LAD 2502

2.3
Sigmodon hispidus F, A
Hoplopleura hirsuta 1M,3F,1N
LAD 2503

2.3
Sigmodon hispidus F, A
Polygenis gwyni 2F
LAD 2504

2.3
Sigmodon hispidus F, A
Hoplopleura hirsuta 1F,2N
LAD 2505

2.3
Sigmodon hispidus M, A
Hoplopleura hirsuta 14N
Polygenis gwyni 3F
LAD 2506

2.3
Sigmodon hispidus F, A
LAD 2507

2.3
Sigmodon hispidus F, A
Hoplopleura hirsuta 1M,6F,8N
LAD 2508

2.3
Sigmodon hispidus M, A
Hoplopleura hirsuta 1M,3F
Polygenis gwyni 1M,2F
LAD 2509

2.3
Sigmodon hispidus M, A
LAD 2510

2.3
Sigmodon hispidus M, A
LAD 2511

2.3
Sigmodon hispidus M, A
Hoplopleura hirsuta 1F,5N
LAD 2512

2.3
Sigmodon hispidus M, A
Hoplopleura hirsuta 2F,5N
LAD 2513

2.3
Sigmodon hispidus M, A
Hoplopleura hirsuta 1M,1F,4N
Polygenis gwyni 1F
LAD 2514

2.3
Sigmodon hispidus M, A
Hoplopleura hirsuta 2M,3F,15N
Polygenis gwyni 3M,4F
LAD 2515

2.3
Sigmodon hispidus F, A
Hoplopleura hirsuta 2F,6N
LAD 2516

2.3
Sigmodon hispidus F, A
Polygenis gwyni 1F
LAD 2517

2.3
Sigmodon hispidus M, A
Hoplopleura hirsuta 6M,17F,51N
Polygenis gwyni 1M
LAD 2518

2.3
Sigmodon hispidus F, A
Hoplopleura hirsuta 1F,3N
LAD 2519

2.3
Sigmodon hispidus F, A
Hoplopleura hirsuta 1M,2N
LAD 2520

2.4
Sigmodon hispidus F, A
Hoplopleura hirsuta 2F,2N
Polygenis gwyni 4M
LAD 2382

APPENDIX B. Continued

2.4
Sigmodon hispidus   F, A
Polygenis gwyni   2M,1F
LAD 2383

2.4
Sigmodon hispidus   F, A
Polygenis gwyni   8M,18F
LAD 2384

2.4
Sigmodon hispidus   F, A
Orchopeas howardi   1M
LAD 2400

2.4
Sigmodon hispidus   M, A
Polygenis gwyni   3M,6F
LAD 2421

2.4
Sigmodon hispidus   M, A
Hoplopleura hirsuta   2F
Polygenis gwyni   1M,1F
LAD 2422

2.4
Sigmodon hispidus   F, A
Polygenis gwyni   2M,2F
LAD 2423

2.4
Sigmodon hispidus   M, A
Hoplopleura hirsuta   3F
Polygenis gwyni   4F
LAD 2424

2.4
Sigmodon hispidus   M, A
Polygenis gwyni   3M,3F
LAD 2425

2.4
Sigmodon hispidus   F, A
Polygenis gwyni   2M,3F
LAD 2468

2.4
Sigmodon hispidus   M, A
Hoplopleura hirsuta   4M,10F,6N
Polygenis gwyni   2M,2F
LAD 2469

2.4
Sigmodon hispidus   F, A
Polygenis gwyni   2F
LAD 2470

2.4
Sigmodon hispidus   F, A
Polygenis gwyni   1F
LAD 2471

2.4
Sigmodon hispidus   M, A
Polygenis gwyni   1F
LAD 2472

2.4
Sigmodon hispidus   F, A
Polygenis gwyni   1F
LAD 2473

2.4
Sigmodon hispidus   F, J
Polygenis gwyni   1F
LAD 2474

2.4
Sigmodon hispidus   M, A
Polygenis gwyni   1F
LAD 2475

2.4
Sigmodon hispidus   F, A
Polygenis gwyni   1M,2F
LAD 2476

2.4
Sigmodon hispidus   F, A
LAD 2477

2.4
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1M,1F,5N
Polygenis gwyni   3M,2F
LAD 2478

2.4
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M,11F,5N
LAD 2479

2.4
Sigmodon hispidus   M, J
Hoplopleura hirsuta   1M,4F
LAD 2480

2.4
Sigmodon hispidus   M, A
Polygenis gwyni   1M
LAD 2481

3.1
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M
LAD 998

3.1
Sigmodon hispidus   M, A
Polygenis gwyni   3M,2F
Stenoponia americana   1M,1F
LAD 999

3.1
Sigmodon hispidus   F, A
Polygenis gwyni   1M
LAD 1000

3.1
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1F
LAD 1001

3.1
Sigmodon hispidus   F, A
Polygenis gwyni   3M,3F
LAD 1002

3.1
Sigmodon hispidus   M, A
Hoplopleura hirsuta   2M,5F
Polygenis gwyni   2F
LAD 1003

3.1
Sigmodon hispidus   F, A
Polygenis gwyni   1M
LAD 1004

3.1
Sigmodon hispidus   F, A
Stenoponia americana   1F
LAD 1013

3.1
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1F
Stenoponia americana   1M
LAD 1014

3.1
Sigmodon hispidus   F, A
Hoplopleura hirsuta   1M
Stenoponia americana   1M
LAD 1015

3.1
Sigmodon hispidus   F, A
Hoplopleura hirsuta   2F
LAD 1016

3.1
Sigmodon hispidus   F, A
Polygenis gwyni   1F
LAD 1133

3.1
Sigmodon hispidus   M, A
Hoplopleura hirsuta   1M,7F
Polygenis gwyni   1M
LAD 1134

3.1
Sigmodon hispidus   F, A
Polygenis gwyni   1M,1F
LAD 1135

3.1
Sigmodon hispidus   M, A
Hoplopleura hirsuta   2N
LAD 1138

3.1
Sigmodon hispidus   M, A
Polygenis gwyni   1M
LAD 1309

3.1
Sigmodon hispidus   M, A
Polygenis gwyni   1M
Orchopeas howardi   1F
LAD 1314

3.1
Sigmodon hispidus   M, A
Hoplopleura hirsuta   2M,3M
Polygenis gwyni   3M,2F
LAD 1315

4.1
Sigmodon hispidus   F, J
Peromyscopsylla hamifer   1F
LAD 1078

5.1
Sigmodon hispidus   M, J
Polygenis gwyni   1M,4F
LAD 358

5.2
Sigmodon hispidus   M, A
Polygenis gwyni   2M,3F
LAD

5.2
Sigmodon hispidus   M, A
LAD

5.2
Sigmodon hispidus   F, A
Polygenis gwyni   3M,1F
LAD

5.2
Sigmodon hispidus   M, A
Hoplopleura hirsuta   2F
LAD

5.2
Sigmodon hispidus   M, A
LAD

5.2
Sigmodon hispidus   M, A
LAD

5.2
Sigmodon hispidus   F, A
Polygenis gwyni   1M,2F
LAD

5.2
Sigmodon hispidus   M, J
LAD

APPENDIX B. Continued

5.2
Sigmodon hispidus M, A
Polygenis gwyni 1M
LAD

5.2
Sigmodon hispidus M, A
Polygenis gwyni 1M,1F
LAD

5.2
Sigmodon hispidus M, J
Polygenis gwyni 1F
LAD

5.2
Sigmodon hispidus F, J
Polygenis gwyni 1F
LAD

5.2
Sigmodon hispidus M, J
Polygenis gwyni 1M,1F
LAD

5.2
Sigmodon hispidus M, J
Polygenis gwyni 1M,1F
LAD

5.2
Sigmodon hispidus F, A
Hoplopleura hirsuta 1F
LAD

5.2
Sigmodon hispidus F, A
Polygenis gwyni 5F
LAD

5.2
Sigmodon hispidus F, A
Polygenis gwyni 1M,1F
LAD