Analysis of the Function and Evolution of Mite Pockets in Lizards

by

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Table of Contents

List of Tables

Chapter 3:

Chapter 4:

List of Figures

Chapter 1:

Chapter 2:

Abstract

More than 200 species of lizards possess mite pockets – small, pocket-like invaginations of the skin which are frequently inhabited by ectoparasitic mites, most notably mites in the families Trombiculidae and Leeuwenhoekiidae (Acari: Trombidiformes) known as "chiggers" in their parasitic larval stage. Pockets appear to shelter mites as they feed, and chiggers preferentially attach within mite pockets when available. However, the benefit the host receives from pockets and this association with ectoparasitic mites is unclear. Numerous hypotheses for the existence and function of mite pockets have been proposed by other researchers, but few of these hypotheses have been adequately tested. In the present study, the association between ectoparasitic mites, pockets, and hosts was examined broadly in the Phrynosomatidae, a diverse family of North American lizards, and specifically in the model species *Sceloporus jarrovi*. This study has three primary purposes: 1) to better understand the patterns of chigger mite infestation and the influence of host morphology and ecology on mite loads; 2) to experimentally test hypotheses for mite pocket function; and 3) to investigate the origin and evolutionary history of mite pockets in the Phrynosomatidae.

Patterns of chigger mite infestation in *Sceloporus jarrovi* were examined through the use of mite count data obtained from 339 specimens collected from four study sites in southeastern Arizona. Chigger mite loads varied considerably according to study site, host age, sex, and body size, but were highly concentrated within the nuchal mite pocket

for all study sites and host demographic groups. Mite pockets were found to possess an upper capacity limit largely determined by host body size, and once pockets were filled chigger larvae readily attached to secondary sites on the host. Chigger ectoparasitism was found to have no negative effects on adult body condition, but high mite loads significantly impair growth in juveniles.

Examination of 2425 museum specimens of 77 species of Phrynosomatidae revealed broadly similar patterns of chigger abundance and distribution as those observed in the field for *S. jarrovi*. Nuchal mite pockets were found to occur in 70 of the 77 species examined, with post-inguinal pockets present in 14 species. Chiggers throughout the Phrynosomatidae were consistently concentrated within the nuchal and/or post-inguinal mite pockets despite high variation host morphology, ecology, and mite loads. Phylogenetically-independent comparative methods revealed mite loads to be positively correlated with mite pocket size and host habitat, and negatively correlated with host midrange latitude. Nuchal mite pockets displayed considerable morphological diversity between taxa but were best developed in *Phrynosoma* and *Sceloporus*; in comparative analyses nuchal pocket size was positively associated with host body size and rugosity, and negatively associated with midrange latitude. In contrast, post-inguinal pockets were restricted to basal Sceloporinae groups, were small and poorly developed, and displayed no significant associations with host morphology or ecology.

Two hypotheses for mite pocket function were experimentally tested using *S. jarrovi* – damage amelioration and mate choice. The damage-amelioration hypothesis proposes that pockets function to concentrate mites into specialized structures that reduce and/or rapidly repair damage caused by mite feeding activities. Abundance and

distribution of chigger larvae on *S. jarrovi* indicates that mites preferentially attach within mite pockets when pockets are available, but that the upper-capacity limit may limit functional usefulness in locations or at times where mites are especially abundant. Histomorphometric analysis of pocket and non-pocket tissues found the epidermis and dermis of the pocket to be significantly thicker than non-pocket equivalents. As a result, although the feeding tubes (stylostomes) produced by chiggers were significantly longer in pocket tissues, chigger feeding cavities were located significantly more superficially in mite pocket tissues relative to non-pocket tissues. However, the amount of tissue destroyed by mites was the same between pocket and non-pocket tissues, and pockets did not display an unusual ability to rapidly repair mite-induced damage. Although pockets do not appear to function entirely as originally proposed, pockets are capable of successfully concentrating mites. Rather than directly reducing and repairing mite damage, pockets may instead reduce mite feeding efficiency, restrict mite damage to superficial tissues, and reduce the amount of irritation and/or pain felt by the host.

The mate choice hypothesis proposes that pockets function to concentrate and conceal brightly colored ectoparasites from conspecifics; in so doing, an individual would appear to possess few parasites and be perceived by potential mates as more desirable. This hypothesis was experimentally tested through the use of mate choice trials in *S. jarrovi*. To control for naturally occurring high variation in mite loads, males were cleaned of ectoparasites and assigned one of three treatment types – visible, hidden, and control. In visible and hidden groups, red-orange paint spots were used to simulate the presence of chiggers attached outside and inside the pocket, respectively. No simulated mites were placed on control males. Females were then allowed to view morphologically

similar pairs of males in mate choice arenas, with choice determined by the location of the female in relationship to each male. Although females displayed a significant preference towards one of the two possible males in the trials, female choice was not significantly influenced by male treatment type or prior ectoparasite load, and no evidence was found supporting the mate choice hypothesis for mite pocket function.

Ancestral state reconstruction estimated the hypothetical common ancestor of the Phrynosomatidae to have possessed modestly developed nuchal mite pockets, no post-inguinal pockets, and moderate chigger loads. This ancestor appears to have been semi-terrestrial, occurring at low elevation in a semi-arid habitat in present-day northern Mexico. Evolution of the Phrynosomatidae following divergence from this ancestor suggests that nuchal pockets were independently lost in the sand lizards (Callisaurini) and in some species of *Uta*, possibly as a result of specialization for arid-habitats. The phylogenetic distribution of post-inguinal pockets is more difficult to interpret; these pockets appear to have originated early in the Sceloporinae and were subsequently lost in some species of *Urosaurus* and derived *Sceloporus*. The expansion and diversification of derived *Sceloporus* into moist, low latitude, high elevation habitats with dense mite populations coincided with the enlargement of the nuchal pockets, thus suggesting a mite-related function.

Chapter 1

Abundance, Distribution, and Association of Chigger Mites (Acari: Trombiculidae) with Mite Pockets in the Lizard *Sceloporus jarrovi* (Sauria: Phrynosomatidae) in Southeastern Arizona

Introduction

Ectoparasitic mites are exceedingly common in most natural systems and feed upon a wide diversity of vertebrate hosts, including lizards (Wrenn and Loomis 1984; Sasa 1961; Wharton and Fuller 1952). Mite parasitism in lizards can result in a wide range of primary and secondary effects. Feeding activities of ectoparasitic mites can produce extensive local tissue damage and inflammation (Reardon and Norbury 2004; Goldberg and Holshuh 1993; Goldberg and Bursey 1991a; Chapter 2), with anemia or death in severe cases of parasitism (Sorci et al. 1994; Bull and Burzacott 1993; Goldberg and Holshuh 1993). Besides physical tissue damage and irritation resulting from feeding activities, ectoparasitic mites may also greatly impact host ecology and behavior by inhibiting growth (Klukowski and Nelson 2001; Foufopoulos 1999), decreasing lizard activity and home range (Main and Bull 2000), or by impairing vision and hearing

(Moritz et al. 1991; Melvin et al. 1943). Parasitic mites potentially also serve as vectors for endoparasites (Reardon and Norbury 2004; Newell and Ryckman 1964).

Of the wide diversity of mites known to parasitize lizards, chigger mites (Prostigmata: Trombiculidae and Leeuwenhoekiidae) are particularly widespread and abundant (Goldberg and Bursey 1993; Goldberg and Holshuh 1992; Arnold 1986; Wilkinson 1985; Bennett 1977). These mites have a biphasic life history alternating between obligate parasitic larvae (the chigger) and free-living predatory deutonymphs and adults inhabiting the soil. Chiggers vary greatly in their degree of host-specificity; although some chigger species are highly specialized to parasitize specific hosts, others will indiscriminately attack most vertebrates they encounter (Wrenn and Loomis 1984; Bennett 1977; Sasa 1961; Wharton and Fuller 1952). Upon locating a host, the larvae cement themselves to a suitable site, pierce the host epidermis, and begin secreting proteolytic saliva into the wound (Sasa 1961; Wharton and Fuller 1952; Jones 1950). Lysed cellular debris is then sucked up through the stylostome, a hollow keratinous tube which forms between the mite and feeding cavity from an interaction of host tissues and mite saliva. The larva alternates between secretion and suction feeding behaviors until engorged, a period which in lizards may vary between 8 to 50 days (Goldberg and Bursey 1993; Goldberg and Bursey 1991b; Melvin et al. 1943). Once engorged, the larva detaches from the host and continues development in the soil.

Numerous proximate factors appear to influence the prevalence and abundance of chiggers on lizards, most notably habitat, host body size, age, and sex. Chiggers tend to be most abundant in moist habitats with ample refugia and available soil (Clompton and Gold 1993; Bennett 1977; Sasa 1961), and consequently mite loads are typically greatest

on hosts that frequent similar microhabitats (Curtis and Baird 2008; Sclaepfer and Gavin 2001; Arnold 1986). Host body size is positively associated with mite loads in many lizard species, either intrinsically or due to interactions with other proximate factors such as age or sex (Ramirez-Morales et al. 2012; Carvalho et al. 2006; Cunha-Barros et al. 2003; Schlaepfer and Gavin 2001). Adults tend to possess higher mite loads than juveniles (Klukowski 2004; Foufopoulos 1999), and males commonly harbor higher mite loads than females (Delfino et al. 2011; Cox and John-Alder 2007; Klukowski and Nelson 2001; Smith 1996; Zippel et al. 1996); these differences in mite loads are frequently attributed to the interactions between host hormones and behavior (Fuxjager et al. 2011; Cox and John-Adler 2007; Klukowski 2004; Klukowski and Nelson 2001; Foufopoulos 1999; Smith 1996; Zippel et al. 1996).

In numerous lizard taxa, chiggers are often found in close association with mite pockets, small invaginations of the host integument frequently located in the nuchal, axial, or inguinal regions (Salvador et al. 1999; Bauer et al. 1990; Arnold 1986; Wilkinson 1985; Smith 1939). Despite being rather simple structures, mite pockets differ significantly in their morphology from the surrounding integument (Chapter 2), possessing a conspicuously thick, well-vascularized epidermis and dermis with little pigmentation, reduced scalation, and no associated underlying musculature. Although ectoparastic mites obtain food and most likely shelter from mite pockets, the benefits hosts may receive from pockets and the close association with mites are less clear. Pockets are not induced by mites, but instead are present on the lizard at birth and in individuals with no prior exposure to parasitic mites (Goldberg and Holshuh 1992; Bauer et al. 1990; Arnold 1986). Additionally, pockets occur in a wide range of lizard taxa and

appear to be linked with certain host morphologies and ecologies (Arnold 1986; Chapter 4). Although numerous hypotheses have been developed to explain the existence of mite pockets and the association with ectoparasitic mites (Salvador 1999; Bauer 1990; Arnold 1986, Wilkinson 1985; Appendix 1.I), the function of mite pockets remains unclear (Arnold 1993, 1986; Bauer 1993, 1990).

Many of the hypotheses developed to explain the presence and function of mite pockets are intimately tied to the abundance and distribution of ectoparasitic mites on the body of the host. Functional hypotheses, such as the damage-amelioration (Arnold 1986; Wilkinson 1985; Chapter 2), impairment-prevention (Salvador et al. 1999), and mate choice hypotheses (Chapter 3) all predict that pockets effectively modify the distribution of mites on the body of the host by concentrating them within the pocket for a specific purpose. However, analysis of mite pocket function is made difficult by the substantial variation in mite abundance and distribution between host species (de Carvalho et al. 2007; Garcia de la Pena et al. 2007; Cunha-Barros et al. 2003; Bennet 1977), as well as between and within populations (Delfino et al. 2011; Garcia de la Pena et al. 2007; Klukowski 2004; Schall et al. 2000; Foufopoulos 1999). As a result, prior to further examination of mite pocket function and experimental manipulation (Chapters 2 through 4), it is first necessary to collect and analyze baseline data on the abundance and distribution of ectoparasitic mites within a model system. The lizard *Sceloporus jarrovi* (Phrynosomatidae) is an ideal species for this purpose. All species of *Sceloporus* possess well-developed nuchal mite pockets (Smith 1939; Chapter 4), and members of this genus have been utilized in previous investigations of mite pocket function (Arnold 1986; Wilkinson 1985). *Sceloporus jarrovi* occur within the United States, are locally abundant

and easily collected; as a result, the biology of this species has been extensively studied (Ruby and Baird 1994; Beuchat 1989; Ruby 1986, 1981, 1978, 1977; Ballinger 1979, 1973; Simon and Middendorf 1976).

In the present study, the abundance and distribution of chiggers were studied in *S. jarrovi* through the use of mite count data obtained from lizards collected in the field. This study has three primary purposes: 1) to better understand general patterns of chigger infestation and the potential influence of host parameters (age, body size, and sex) and habitat on mite loads in a phrynosomatid lizard; 2) to determine chigger microhabitat preference via host attachment site specificity; and 3) to provide abundance and distribution data necessary for the evaluation of mite pocket hypotheses.

Methods

Study System

Sceloporus jarrovi is a saxicolous, montane iguanian that inhabits rocky canyons and woodlands between 1500 and 3300 meters above sea level in southwestern New Mexico, southeastern Arizona, and the north-central Mexican states of Chihuahua, Sonora, and Durango (Jones and Lovich 2009; Stebbins 1985; Ruby 1981; Ballinger 1973; Smith 1939). Although lizards at lower elevations may be active all year, winter activity is typically sporadic and restricted by weather conditions; in most populations individuals form non-territorial winter aggregations within refugia, most commonly rock crevices (Beauchat 1989; Ruby 1981, 1978, 1977; Ballinger 1973). Following spring

emergence, adults of both sexes establish and maintain territories against conspecifics. Peak territorial behavior occurs in September and October during the breeding season (Ruby 1981; 1978, 1977); after mating, territories degenerate as lizards return to communal winter refugia. Ovulation begins in November and the young are born alive the following May to June (Ruby 1981; Ballinger 1979, 1973).

Data on the abundance and distribution of ectoparasitic mites on *S. jarrovi* were obtained from a total of 339 individuals collected in 2010 and 2011 from four study sites in the Coronado National Forest of southeastern Arizona. In this region the predominant ectoparasites of *S. jarrovi* are the chiggers *Eutrombicula alfreddugesi* (Oudemans, 1910) and *E. lipovskyana* (Wolfenbarger, 1953) (Bennett 1977); because these closely related species could not be distinguished from each other in the field, they were treated together for the purposes of this study. In 2010, 154 lizards were collected between August 11 and September 17 from South Fork (16 adults: male n=9, female n=7; 19 juveniles: males n=8, female =11) and Barfoot (41 adults: male n=19, female n=22; 78 juveniles: males n=35, female n=43) study sites, both located in the Chiricahua Mountains (Cochise County). South Fork (1550 m elevation; 31.878 North, 109.180 West) is a riparian corridor of mixed oak woodland and rock talus that extends along the southern branch of Cave Creek Canyon. Few lizards were observed along the seasonal creek that runs through the middle of the site, and most individuals were encountered along either side of the steep, rocky canyon walls and rock outcrops. Barfoot (2550 m elevation; 31.920 North, 109.278 West) is a large, sparsely vegetated talus slope located approximately 150 m below the southern side of Barfoot Peak. A mixed pine/fir forest extends beneath the

talus and around the north side of the Peak. Lizard activity at Barfoot was primarily confined to the base of the exposed talus field within 30 m of the surrounding forest.

In 2011, the Horseshoe II wildfire caused extensive damage throughout the Chiricahua Mountains, including South Fork, and made Barfoot inaccessible for much of the year. As a result, most lizards captured in 2011 were obtained from the Pinaleno Mountains (Graham County), approximately 90 km northwest of the Chiricahuas. Of 185 total lizards collected in 2011 between August 12 and September 9, 101 were obtained from Soldier Creek (100 adults: male n=42, female n=58; 1 juvenile female), and 27 from Cluff Dairy (27 adults: male n=15, female n=12) campgrounds. The remaining 57 lizards examined that year were collected from South Fork (25 adult: male n=11, female n=14; 32 juvenile: male n=13, female n=19). Soldier Creek campground (2850 m elevation; 32.697 N, 109.921 W) consists of pine, fir, and aspen forest and grassy meadows, interspersed with isolated boulder clusters and rocky outcrops. Lizard activity at this site was concentrated on boulders, particularly those with relatively open canopies and high sun exposure. Cluff Dairy (2750 m elevation; 32.667 N, 109.873 W) is a predominantly pine forest with largely closed canopy featuring little understory growth and numerous rocky outcrops. Lizards at Cluff Dairy appeared to utilize tree trunks and rock outcrops with equal frequency.

Lizards were collected by hand-held noose, and data on snout-vent length (SVL, equivalent of body size), weight, age, and sex were obtained *in situ*. Snout-vent length was measured using digital calipers to the nearest 0.1 mm. Weight was measured using a Pesola spring scale to the nearest 0.1 g. Lizards were then given a unique toe clip combination for future identification and then placed individually in plastic bags for

transport back to camp for examination of ectoparasites. The body surface of each lizard was carefully examined through the use of a dissecting microscope or hand lens and the number of chiggers inhabiting each of the thirteen body regions of the host recorded (Figure 1.1). Following data collection all lizards were returned to the exact site of original capture and released.

Statistical analysis

All statistical analyses were performed using SPSS (SPSS Inc. 2011, version 20.0 for Windows). Mite load and attachment site preference data frequently displayed non-normal distributions during preliminary analyses; when necessary, square root transformations were used to normalize mite load data, followed by standard AN(C)OVA and regression analysis. When transformations were not successful in normalizing distributions, non-parametric Kruskall-Wallis tests were used to analyze mite prevalence, mite loads, and the distribution of mites on the host. Significant associations were then further examined through the use of Mann-Whitney U-tests for pairwise comparisons. With the exception of the nuchal pocket, relatively few mites were found to occur in many of the thirteen body regions examined (Figures 1.1, 1.2); as a result, particular emphasis was placed on total and nuchal pocket mite loads in most analyses. The only study site sampled in both years was South Fork; because total mite loads did not differ significantly between 2010 and 2011 (Mann Whitney $U=-1.042$, $p=0.298$), data from both years at South Fork were pooled together for all other analyses. Regression analysis and AN(C)OVAs were used to examine the relationships between regional mite loads and continuous host variables such as body size, weight, or collection date, as well as the

interaction of these with demographic variables (age class, sex, and site). Where necessary, residuals derived from regression analyses were used to eliminate the effects of body size on mite loads. In all analyses a two-tailed critical value of $\alpha=0.05$ was considered statistically significant.

Results

Chigger prevalence (the percentage of lizards with at least one attached mite) was high at all four study sites and demographic groups, ranging from 88.37 to 100% (Table 1.1). Pairwise comparisons revealed overall prevalence at Barfoot Peak (92.44%) to be significantly lower than that observed at South Fork (98.91%) (Mann-Whitney $U=-2.753$, $p=0.035$), Soldier Creek (100%) ($U=-3.299$, $p=0.006$), and Cluff Dairy (100%) ($U=-2.094$, $p=0.036$); no other significant differences in prevalence were observed between the remaining sites. When pooled between sites, prevalence in adults (207/209, 99.04%) was found to be significantly higher than that of juveniles (122/130, 93.85%) (Mann-Witney $U=2.773$, $p=0.006$). Host sex had no significant effect on chigger prevalence.

Both the intensity of infestation and distribution of mites on the body of the host varied considerably between study sites and host demographic groups (Tables 1.1 and 1.2; Figure 1.2). Total mite loads differed significantly between sites (Kruskall-Wallis $K=142.05$, $p<0.001$), with lizards from Barfoot Peak possessing significantly fewer mites than lizards collected from Cluff Dairy (Mann-Whitney $U=-6.656$, $p<0.001$), Soldier Creek ($U=-11.388$, $p<0.001$), and South Fork ($U=-6.049$, $p<0.001$). South Fork total

mite loads were in turn significantly lower than those from Cluff Dairy ($U=2.646$, $p=0.049$) and Soldier Creek ($U=4.864$, $p<0.001$), and no significant difference was observed in total loads between the latter two sites (Barfoot < [South Fork < (Cluff Dairy = Soldier Creek)]). Similar trends were observed when only adult lizards were analyzed.

At all four study sites the majority of trombidiform mites attached to the host were observed occupying the nuchal mite pockets (Figure 1.2, Table 1.2). Although the number of mites inhabiting the nuchal pockets differed significantly between sites (Kruskall-Wallis $K=46.258$, $p<0.001$), the trends observed for nuchal pocket load differed greatly from those of total mite load (above). Nuchal pocket mite loads were remarkably similar between sites, with the only notable exceptions occurring at South Fork. Lizards from South Fork had significantly higher nuchal pocket mite loads than either Barfoot (Mann-Whitney $U=6.186$, $p<0.001$) or Soldier Creek ($U=5.546$, $p<0.001$); pocket mite loads were otherwise similar across sites, with loads at Cluff Dairy intermediate between the extremes at Soldier Creek/Barfoot and South Fork and not significantly different from any site (effectively producing: [[Barfoot = Soldier Creek] < South Fork] = Cluff Dairy). Excluding juveniles from the analysis produced similar results. Nuchal pocket mite loads were not significantly correlated with non-pocket mite loads (the number of mites outside the pocket, effectively Total Load minus Nuchal Pocket Load), either for all individuals ($R=0.002$, $p=0.971$) or adults only ($R=0.060$, $p=0.386$). Taken together, these results suggest that although total mite loads vary significantly between sites, mite loads within the nuchal pockets remain largely stable between sites and demographic groups.

The proportion of total mite load occurring within the nuchal mite pockets (nuchal pocket proportion) varied significantly between sites (Kruskall-Wallis $K=80.916$, $p<0.001$; Figure 1.3). Trends in nuchal pocket proportion between sites differed greatly from those of total or nuchal pocket mite loads (described above); a high proportion of total mite load occurred within the mite pockets in lizards from Barfoot and South Fork (mean nuchal pocket proportion of 85.93 and 77.53%, respectively), while proportionally few mites occurred within pockets at Cluff Dairy (31.31%) and Soldier Creek (22.2%). With respect to overall nuchal pocket proportion, effectively [Barfoot = South Fork] > [Cluff Dairy = Soldier Creek] (Mann-Whitney $U=4.417$ to 7.335, all $p<0.001$). Sex had no effect on nuchal pocket proportion, and male nuchal pocket proportion was similar to that observed in females at all sites. At Barfoot and South Fork, the only sites with sufficiently high juvenile sample size, age class was also significantly associated with differences in nuchal pocket proportion. At both sites juveniles had a significantly greater proportion of their total mite load concentrated within the nuchal pockets than adults (Barfoot: $U=3.212$, $p=0.001$; South Fork: $U=4.095$, $p<0.001$).

The general pattern of mite abundance and distribution on the body of the host between study sites is presented in Figure 1.2. Although significant differences in total mite loads occurred between study sites in each of the thirteen body regions examined (Figure 1.2, Table 1.3), in general mites were most common within the nuchal pockets, relatively uncommon in the nuchal non-pocket, back, side, inguinal, and hindlimb regions, and typically only rarely occurred elsewhere on the body. Despite this general pattern, some variation in attachment site preference was noted between sites, with some body regions particularly preferred by chiggers at certain sites but not at others. Chiggers

were commonly observed around the eye sockets and eyelids at Cluff Dairy (mean load=14.96 ±19.90), occasionally producing dense clusters which appeared to interfere with occlusion of the eyes; in contrast, mites occurring on the head were very rare at all other sites (mean=0.05-0.74, ±0.26-1.64; Kruskall-Wallis K=76.192, $p<0.001$). At Soldier Creek, chiggers were relatively abundant on the side (mean=20.46 ±27.02), inguinal (24.62 ±27.13), and hindlimb (32.81 ±34.04) regions, and loads in these regions were frequently significantly higher than those observed at other study sites (Figure 1.2, Table 1.3). The distribution of mite loads among host body regions frequently displayed the same recurring pattern between sites as was observed for total and nuchal pocket loads (above); in gular, back, side, inguinal, hindlimb, post-inguinal, and tail regions, mite loads were significantly lower in Barfoot and South Fork lizards than those collected from Soldier Creek and Cluff Dairy ([Barfoot = South Fork] < [Soldier Creek = Cluff Dairy]). In summary, lizards from Barfoot and South Fork tended to possess low to moderate total chigger loads, with most of these mites concentrated within the pockets, particularly in juveniles; in contrast, mites were very abundant and more widely dispersed over the body in lizards from Cluff Dairy and Soldier Creek.

Besides study site and body region, host body size (snout-vent length or SVL), sex, and age class were found to have numerous direct and indirect effects on mite loads in *S. jarrovi*. Body size displays a significant positive correlation with both total mite load ($R=0.634$, $p<0.001$; Figure 1.4A) and to a lesser extent with nuchal pocket load ($R=0.111$, $p=0.040$; Figure 1.4B). Although variation occurs within and between study sites, similar significant associations were also obtained from subsamples of adults only. Adult lizard body size also varied significantly between study sites (AVOVA: F=28.407,

$p<0.001$; Figure 1.5). As adults, Barfoot lizards were significantly smaller than individuals from South Fork, and adults from both of these populations were in turn smaller than adults from either Soldier Creek or Cluff Dairy (note that this is the same pattern displayed by total mite load between sites). In all populations, adult males were significantly larger than females ($F=47.874$, $p<0.001$; Figure 1.5), and males also tended to have higher total mite loads ($F=4.827$, $p=0.029$; Figure 1.6), particularly at Cluff Dairy ($F=2.221$, $p=0.025$) and Soldier Creek ($F=2.301$, $p=0.021$). Unlike total load, nuchal pocket mite loads were remarkably similar between sexes, differing significantly only at Cluff Dairy ($F=1.953$, $p=0.053$; Table 1.2). Adults were found to have significantly higher total mite loads than juveniles at Barfoot (Mann-Whitney $U=3.176$, $p<0.001$) and South Fork ($U=3.170$, $p=0.002$; Table 1.1) (insufficient sample size prevented similar age class analysis of Soldier Creek and Cluff Dairy animals). Barfoot adults also had significantly more mites in the nuchal pocket than juveniles ($U=2.319$, $p=0.020$; Table 1.2). However, nuchal pocket proportion was significantly higher in juveniles than adults at both Barfoot ($U=3.212$, $p=0.001$) and South Fork ($U=4.095$, $p<0.001$; Table 1.2).

To exclude the interactive effects of body size, residuals obtained from the regressions of body size against total load and nuchal pocket load were used to reanalyze the relationships between mite loads, study sites, sexes, and age classes. Significant differences in total mite loads between study sites continue to persist in adults (ANOVA $F=13.326$, $p<0.001$; Figure 1.7), primarily between Soldier Creek and South Fork (Mann-Whitney $U=2.680$, $p=0.044$) and Soldier Creek/Barfoot ($U=5.251$, $p<0.001$). However the effects of sex on total load no longer remain significant after correcting for the effects of body size. Nuchal pocket loads remained significantly dissimilar between sites after

removing the effects of body size (ANOVA $F=7.282$, $p<0.001$), with loads at South Fork remaining significantly higher than Barfoot (Mann-Whitney $U=5.044$, $p<0.001$), Cluff Dairy ($U=2.853$, $p=0.026$), and Soldier Creek ($U=6.780$, $p<0.001$) (effectively: South Fork > [Barfoot = Cluff Dairy = Soldier Creek]); a nearly identical relationship was observed in adults when juveniles were excluded from the analysis. Although adults had significantly higher total mite loads than juveniles (above), when the effects of body size were removed this relationship became inverted at Barfoot, with juveniles having significantly more total mites than predicted based on their body size ($U=2.785$, $p=0.005$), while the difference between adults and juveniles became non-significant at South Fork.

Because weight in *S. jarrovi* is strongly correlated with body size (snout-vent length; $R=0.953$), which is in turn strongly correlated with mite loads (above), weight also initially displayed a strong correlation with total mite load ($R=0.610$, $p<0.001$) and nuchal pocket load ($R=0.145$, $p=0.008$). To determine if mites had any negative effect on lizard body condition, residuals produced from regression analyses were used to examine the relationship between mite load and weight exclusive of the effects of body size. When all animals are pooled, regression analysis of the resulting residuals produced a significant positive association between total mite load and weight ($R=0.138$, $p<0.011$; Figure 1.8); this relationship remained significant when adults were examined separately ($R=0.199$, $p=0.004$). Among adults, the relationship between total mite load and weight is significant in males ($R=0.274$, $p=0.007$) but not females ($R=0.094$, $p=0.320$). No significant relationship between nuchal pocket load and weight residuals was observed in adults when the effects of body size are removed. In contrast to adults, juveniles

displayed a significant negative relationship between the residuals of total mite load and weight (corrected for body size; $R=-0.249$, $p=0.004$) and nuchal pocket load and weight ($R=-0.412$, $p<0.001$; Figure 1.9). This negative relationship remained significant for both sexes of juveniles (males $R=-0.405$, $p=0.002$; females $R=-0.419$, $p<0.001$).

In comparison to site, body size, sex, and age class, collection date appeared to have a small but significant effect on mite loads. Regression analysis of total mite load residuals (removing the effects of body size) and Julian date produced a significant slightly negative relationship when all animals were pooled ($R=-0.130$, $p=0.017$; Figure 1.10). Reanalysis of this relationship in an ANCOVA model incorporating additional site, sex, and site by sex interactions resulted in a nearly significant effect for Julian date ($F=3.708$, $p=0.055$) with significant site effects ($F=9.058$, $p<0.001$). Examination of nuchal pocket mite loads produced similar results; simple regression analysis suggested that collection date had a significant negative effect on mite loads (R-0.203, $p<0.001$), but date became non-significant in the ANCOVA model (only site remained significant; $F=11.014$, $p<0.001$).

Discussion

Although mite loads and mite distributions varied considerably between sites and demographic groups, several trends are readily apparent. Chiggers were exceedingly common ectoparasites at all four study sites and for all host subgroups examined. Mite prevalence was high overall (Table 1.1), but prevalence at Barfoot was significantly

lower than at the other three sites; lower prevalence at Barfoot also coincides with significantly lower total and nuchal pocket mite loads (Table 1.1; Figure 1.6), suggesting that chiggers in general are less abundant and more widely dispersed in the environment at Barfoot. Because adult chiggers inhabit and tend to oviposit in moist soil (Clompton and Gold 1993; Sasa 1961; Wharton and Fuller 1952), the observed scarcity of mites is likely due to the predominant habitat at Barfoot – loose, exposed rock talus with little vegetative cover and soil. In contrast with Barfoot, mite loads were found to be considerably higher at South Fork and Cluff Dairy, both mesic, densely forested sites. Besides the availability of moisture and protective cover, habitat structure appears to play an important role by both affecting the abundance of mites as well as the distribution of the hosts. Although *S. jarrovi* will readily descend to the ground or up low vegetation, the species is primarily saxicolous, and lizards are most abundant on and around exposed rock (Jones and Lovich 2009; Stebbins 1985; Ruby 1981; Ballinger 1973; Smith 1939). Territories frequently encompass boulder piles, and rock crevices play an important role as refugia (Beauchat 1989; Ruby 1981, 1978, 1977; Ballinger 1973). The distribution of suitable rocky host habitats was spatially clumped at Cluff Dairy and Soldier Creek, and lizards were most commonly observed on the fractured boulder piles interspersed throughout both sites. High total mite loads at these two study sites is likely due to the dense concentration of available hosts within and around the boulders as well as the general availability of suitable crevices and soil for mite refugia. Although total mite loads displayed a significant positive correlation with lizard body size (Figure 1.4A) and adult body size differed significantly between study sites (Figure 1.5), the general trends observed in total mite loads between sites remained significant when the effects of body

size were removed (Figure 1.7). The apparent associations between mite load, habitat, and moisture observed here are largely in agreement with results published for other lizard species. In *Crotaphytus collaris* (Crotaphytidae), lizards occurring on exposed rock talus possessed significantly lower trombidiform mite loads than individuals inhabiting more mesic microhabitats with greater vegetative cover and moisture (Curtis and Baird 2008). *Norops polylepis* and *N. woodi* (Polychrotidae) collected within forest interiors possessed significantly greater chigger loads than individuals inhabiting forest edges (Sclaepfer and Gavin 2001); similar associations between chigger loads, host microhabitat usage, and moisture availability have also been reported for *Liolaemus* (Tropiduridae) (Rubio and Simonetti 2009), *Anolis* (Polychrotidae) (Zippel et al. 1996), *Oligosoma* (Scincidae) (Reardon and Norbury 2004), and *Uta* (Phrynosomatidae) (Spoecker 1967).

Besides collection site, chigger prevalence and total mite loads were also significantly influenced by host age and sex (Table 1.1). Overall prevalence was significantly higher in adults than in juveniles, and adults had significantly greater total mite loads than juveniles at both Barfoot and South Fork (the only sites where juvenile sample size was sufficiently high). Similar trends have been observed elsewhere (Klukowski 2004; Foufopoulos 1999) but do not appear to universally apply to all lizard species (Ramirez-Morales et al. 2012; Curtis and Baird 2008). Although the reasons are unclear, in some host-parasite systems mites may be more common and abundant on adults due to behavioral and hormonal differences between age classes. Adult *S. jarrovi* become increasingly territorial and active during the fall breeding season (Ruby 1981; 1978, 1977). In contrast, juveniles remain non-territorial until they reach sexual maturity,

typically late in the fall of their first year at low elevations (South Fork) or during their second year at higher elevations (Barfoot) (Ruby and Baird 1994; Ballinger 1979). As a result, mobile adults actively patrolling territories may come into contact with questing trombidiform larvae more frequently than juveniles (Garcia de la Pena et al. 2007; Talleklint-Eisen and Eisen 1999). Alternatively, simple differences in body size between adults and juveniles may explain much of the observed variation in mite loads between age classes. By virtue of larger body size, adults are likely easier targets for questing ectoparasites; additionally, because the number of suitable mite attachment sites on an individual presumably increases with host surface area, adults are expected to be able to house a greater number of mites than juveniles.

Differences in ectoparasite loads between males and females have been reported for a variety of lizard taxa, including *Sceloporus*. Males tend to have higher mite loads than females, and these differences are frequently attributed to sexual differences in behavior and circulating hormones, particularly testosterone (Delfino et al. 2011; Cox and John-Alder 2007; Garcia de la Pena et al. 2007, 2004; Klukowski and Nelson 2001; Foufopoulos 1999; Salvador et al. 1999; Smith 1996; Zippel et al. 1996). This pattern does not appear to hold for all species, however, and in some hosts mite loads are similar between sexes (de Carvalho et al. 2007; Garcia de la Pena et al. 2007; Reardon and Norbury 2004). In the present study adult males tended to have higher total mite loads than females, but these differences were significant only at Cluff Dairy and Soldier Creek (Table 1.1, Figure 1.6). As adults, male *S. jarrovi* are larger than females, and the pattern of adult body size between sites was similar to that of total mite load (compare Figure 1.5 to Figure 1.6); additionally, body size displayed significant positive associations with

total mite load (Figure 1.4A). The differences in total mite loads between sexes became non-significant once the effects of body size were removed, suggesting that size plays an important factor in influencing mite loads in *S. jarrovi* – adult males may generally have higher mite loads than females simply due to being physically larger. Although body size has been positively associated with total ectoparasite load in a wide variety of lizard taxa, including *Sceloporus* (Phrynosomatidae) (Foufopoulos 1999), *Tropidurus* (Tropiduridae) (Menezes et al. 2011; Carvalho et al. 2007), *Norops* (Polychrotidae) (Schlaepfer and Gavin 2001), and numerous species of Teiidae (Ramirez-Morales et al. 2012; Cunha-Barros et al. 2003), only rarely has host body size been accounted for in analyses of parasite load within and between populations (Rubio and Simonetti 2009). The interaction between host body size, sex, age, and mite load is frequently overlooked in studies of lizard ectoparasitism and is deserving of further investigation.

The distribution of chiggers attached to *S. jarrovi* was variable between sites and body regions but highly non-random, with the bulk of mites inhabiting the nuchal mite pockets at all study sites (Figure 1.2; Table 1.3). The consistent inhabitation of pockets regardless of habitat and individual host variation suggests some degree of attachment site specificity and preference for the mite pocket in the chiggers parasitizing *S. jarrovi* at these sites. Mite preference for pockets and/or skin folds as attachment sites has been reported for other populations of *S. jarrovi* (Bulte et al. 2009; Foufopoulos 1999; Bennett 1977), other species of Phrynosomatidae (Garcia-de la Pena 2004; Klukowski 2004; Smith 1996; Bennett 1977; Chapter 4), and numerous other lizard taxa (Delfino et al. 2011; Curtis and Baird 2008; de Carvalho et al. 2007; Cunha-Barros et al. 2003; Arnold 1986). The non-random distribution of chiggers and apparent predilection for the mite

pocket across a taxonomically wide range of hosts has been used as support for hypotheses of mite pocket function which rely on concentrating or concealing mites in certain regions on the body (Arnold 1986; Appendix 1.I). The preference that chiggers display towards mite pockets in the present study is largely supportive of those hypotheses that rely on the sequestration of ectoparasites for a particular function, such as damage-amelioration or impairment-prevention.

Nuchal pocket loads were relatively consistent across sites, age classes, and sexes (Figure 1.2; Table 1.2), despite highly significant variation in total mite loads (above). Like total mite load, nuchal pocket load displayed a weak but significant positive association with host body size (Figure 1.4B); when the effects of body size were removed, nuchal pocket mite loads remained significantly higher at South Fork but were otherwise similar between sites, sexes, and age classes. Nuchal pocket load also displayed no significant association with non-pocket mite loads across study sites or host demographic groups. These results suggest that nuchal pockets have an upper limit on mite capacity primarily determined by host body size (i.e. larger lizards possess larger pockets; Chapter 4), not by the total number of mites an individual possesses. Supporting this, nuchal pocket proportion (proportion of the total mite load which occurs within the nuchal pockets) was inversely related to total mite load across study sites. Additionally, mite load distributions in the gular, back, side, inguinal, hindlimb, post-inguinal, and tail regions across study sites were similar to the trends displayed in total mite loads – low at South Fork and Barfoot, high at Cluff Dairy and Soldier Creek (Table 1.3). Taken together, the results herein indicate that although chiggers prefer to utilize pockets, when pockets become saturated mites do not seek other hosts but will instead readily attach to

secondary sites elsewhere on the body, most commonly in the nuchal non-pocket, back, side, inguinal, or hindlimb regions (Figure 1.2).

In previous studies of mite ectoparasitism in *Sceloporus*, high mite loads have been found to have significant negative effects on host growth or body condition, particularly in males (Klukowski and Nelson 2001; Foufopoulos 1999; Smith 1996; but see Garcia-de la Pena et al. 2004). In this study mites were associated with several significant but contrasting effects on body condition dependent on host age and sex. When corrected for the effects of body size, a slight significant positive relationship was observed between total mite load and weight (Figure 1.8) when all animals were pooled; this relationship remained when juveniles were excluded from the analysis and was primarily driven by trends in adult males. These results appear to indicate that mites do not have a significant negative impact on body condition in adults, particularly in adult males. Instead, large males actually possessed significantly more mites than expected based on their body size and weight alone, suggesting high total mite loads in this demographic group are in part influenced by other endogenous factors (most likely hormonal and/or behavioral). In contrast, significant negative relationships were observed between weight and mite loads (both total and nuchal pocket loads) for juveniles, regardless of study site or sex (Figure 1.9). Juveniles tend to concentrate a significantly greater proportion of their total mite loads within the nuchal pocket than adults (Table 1.2), and high nuchal pocket mite loads in juveniles appear to retard weight gain equally in males and females. Similar results for juveniles have been reported for other populations of *S. jarrovi* (Foufopoulos 1999), differing primarily in that mite loads affected body condition in both sexes equally in the present study.

Mite loads tend to vary considerably throughout the year in most temperate lizard species. Ectoparasite burdens are generally low in winter and spring but build up slowly after hosts emerge from winter refugia, with peak loads occurring during the breeding season in summer or fall (Klukowski 2004; Foufopoulos 1999; Smith 1996; Goldberg and Bursey 1991a; Bennett 1977; Spoecker 1967). In the present study, mite loads displayed a slight overall decrease from late summer to fall (Figure 1.10) with considerable variation occurring between study sites. All study sites were located in a region of southeast Arizona which experiences yearly monsoon rains during June and July; in this geographic region, mite loads tend to reach their peak towards the end of the monsoon in July and into the early fall (August/September) (Foufopoulos 1999; Bennett 1977), potentially coinciding with the breeding season in *S. jarrovi* (Ruby and Baird 1994; Ballinger 1979, 1973). Because sampling in this study occurred primarily during August and early September, the observed slight decrease in mite loads appears to represent mite load abundance just following the yearly peak.

Conclusion

In summary, the results of this study have demonstrated: 1) chigger loads can vary considerably within the same host-parasite system according to study site, host age, and (to a lesser extent) sex; 2) host body size appears to play an underappreciated role in the determination of mite loads, and may be responsible for the majority of mite load variation observed between sites and host demographic groups; 3) consistent with many

hypotheses for mite pocket function, chiggers preferably concentrate their feeding activities within the nuchal mite pockets, particularly in circumstances where total environmental mite abundance is low to moderate; 4) nuchal pocket mite load is not correlated with the non-pocket load, suggesting that the very presence of pockets does not significantly affect the number of mites parasitizing an individual; 5) mite pockets have an upper capacity limit determined by host body size, and once pockets become filled chiggers will readily parasitize secondary sites; 6) high chigger loads can significantly impair growth rates in juveniles of both sexes, but appear to have no effect on adult body condition; and 7) total mite loads (and to a lesser extent nuchal pocket loads) decreased slightly in fall following the end of the summer monsoon season.

Besides providing evidence that chiggers preferentially utilize mite pockets when available, the results of this study suggest that if mite pockets do serve an adaptive, mite-related function, such as that predicted by the damage-amelioration (Arnold 1986; Chapter 2) or impairment-prevention (Salvador et al. 1999) hypotheses, then mite pockets would be predicted to be most useful in situations where total mite loads rarely exceed the capacity of the mite pocket. In the present study, such situations appear to occur most commonly at Barfoot and South Fork, where total chigger loads are relatively low and the bulk of the load occurs within the pocket. Similarly, because nuchal pocket proportion is consistently higher in juveniles than adults, mite pockets may be most functionally useful before sexual maturity. Additionally, because mite loads typically display considerable temporal variation in temperate regions (Klukowski 2004; Foufopoulos 1999; Smith 1996; Goldberg and Bursey 1991a; Bennett 1977; Spoecker 1967), pockets may likewise vary in usefulness to the host throughout the year. Although the abiotic and biotic factors

that influence ectoparasite loads in lizards are becoming increasingly well-known, it is unclear why these factors appear to vary in importance between species or within populations. Particularly little attention has thus far been paid to the systematics and biology of the chiggers involved in these host-parasite systems, and rarely are data available on the identity, host-specificity, and microhabitat preferences of these ectoparasites. With respect to the hypotheses for mite pocket function, the results presented in this study are suggestive and generally consistent with predictions of mite distribution made by functional hypotheses that invoke concentration or reallocation of mite loads. However, chigger distribution alone does not demonstrate pocket function (Bauer et al. 1993, 1990) and additional work is necessary to experimentally test and evaluate these hypotheses and better understand the relationships between mites and mite pockets (Chapters 2 through 4).

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References:

Arnold E.N. 1986. Mite pockets of lizards, a possible means of reducing damage by ectoparasites. *Biological Journal of the Linnean Society* 29:1-21.

Arnold E.N. 1993. Comment – Function of the mite pockets of lizards: an assessment of a recent attempted test. *Canadian Journal of Zoology* 71(4): 862-864.

Ballinger R.E. 1973. Comparative demography of two ovoviviparous iguanid lizards (*Sceloporus poinsetti* and *Sceloporus jarrovi*). *Ecology* 54(2): 269-283.

Ballinger R.E. 1979. Intraspecific variation in demography and life history of the lizard, *Sceloporus jarrovi* along an altitudinal gradient in southeastern Arizona. *Ecology* 60(5): 901-909.

Bauer A.M., A.P. Russell, and N.R. Dollahon. 1990. Skin folds in the gekkonid lizard genus *Rhacodactylus*: A natural test of the damage limitation hypothesis of mite pocket function. *Canadian Journal of Zoology* 68(6):1196-1201.

Bauer A.M., A.P. Russell, and N.R. Dollahon. 1993. Function of the mite pockets of lizards: a reply to E.N. Arnold. *Canadian Journal of Zoology* 71(4): 865-868.

Bennett S.G. 1977. Ecology and systematics of trombiculid mites on lizards from southern Arizona. M.S. Thesis. California State University, Long Beach. Long Beach, California. 192 pp.

Bertrand M. and D. Modry. 2004. The role of mite pocket-like structures on *Agama caudospinosa* (Agamidae) infested by *Pterygosoma livingstonei* sp. n. (Acari: Prostigmata: Pterygosomatidae). *Folia Parasitologica* 51: 61-66.

Beuchat C.A. 1989. Patterns and frequency of activity in a high altitude population of the iguanid lizard, *Sceloporus jarrovi*. *Journal of Herpetology* 23(2): 152-158.

Bull C.M. and D. Burzacott. 1993. The impact of tick load on the fitness of their lizard hosts. *Oecologia* 96: 415-419.

Bulte G., A.C. Plummer, A. Thibaudeau, and G. Blouin-Demers. 2009. Infestation of Yarrow's spiny lizards (*Sceloporus jarrovi*) by chiggers and malaria in the Chiricahua Mountains, Arizona. *The Southwestern Naturalist* 54(2): 204-207.

Clompton R.E. and R.E. Gold. 1993. Distribution and seasonal and diurnal activity patterns of *Eutrombicula alfreddugesi* (Acari: Trombiculidae) in a forest edge ecosystem. *Journal of Medical Entomology* 30: 47-53.

Cox R.M. and H.B. John-Alder. 2007. Increased mite parasitism as a cost of testosterone in male striped plateau lizards (*Sceloporus virgatus*). *Functional Ecology* 21(2): 327-334.

Cunha-Barros M., M. van Sluys, D. Vricibradic, C.A.B. Galdino, F.H. Hatano, and C.F.D. Rocha. 2003. Patterns of infestation by chigger mites in four diurnal lizard species from a restinga habitat (Jurubatiba) of southeastern Brazil. *Brazilian Journal of Biology* 63(3): 393-399.

Curtis J.L. and T.A. Baird. 2008. Within-population variation in free-living adult and ectoparasitic larval trombiculid mites on collared lizards. *Herpetologica* 64(2): 189-199.

De Carvalho A.L.G., A.F.B. de Araujo, and H.R. da Silva. 2006. Patterns of parasitism by *Eutrombicula alfreddugesi* (Oudemans) (Acari, Trombiculidae) in three species of *Tropidurus* Wied (Squamata, Tropiduridae) from Cerrado habitat of central Brazil. *Revista Brasileira de Zoologica* 23(4): 1010-1015.

Delfino M.M.S., S.C. Ribeiro, I.P. Furtado, L.A. Anjos, and W.O. Almeida. 2011. Pterygosomatidae and Trombiculidae mites infesting *Tropidurus hispidus* (Spix, 1825) (Tropiduridae) lizards in northeastern Brazil. *Brazilian Journal of Biology* 71(2): 549-555.

Garcia-de la Pena C., A. Conteras-Balderas, G. Castaneda, and D. Lazcano. 2004. Infestacion y distribucion corporal de la nigua *Eutrombicula alfreddugesi* (Acari : Trombiculidae) en el lacertilio de las rocas *Sceloporus couchii* (Sauria: Phrynosomatidae). *Acta Zoologica Mexicana* 20(2): 159-165.

Garcia-de la Pena C., G. Castaneda, and C.W. Barrows. 2007. Infestation by chigger mites in two lizard species from a dune habitat of northern Mexico. *Texas Journal of Science* 59(1): 23-32.

Goldberg S.R., and C.R. Bursey. 1991a. Integumental lesions caused by ectoparasites in a wild population of the side-blotched lizard (*Uta stansburiana*). *Journal of Wildlife Diseases* 27(1): 68-73.

Goldberg S.R. and C.R. Bursey. 1991b. Duration of attachment by mites and ticks on the iguanid lizards *Sceloporus graciosus* and *Uta stansburiana*. *Journal of Wildlife Diseases* 27(4): 719-722.

Goldberg S.R. and C.R. Bursey. 1993. Duration of attachment of the chigger, *Eutrombicula lipovskyana* (Trombiculidae) in mite pockets of Yarrow's spiny lizard, *Sceloporus jarrovi* (Phrynosomatidae) from Arizona. *Journal of Wildlife Diseases* 29(1): 142-144.

Goldberg S.R., and H.J. Holshuh. 1992. Ectoparasite-induced lesions in mite pockets of the Yarrow's spiny lizard, *Sceloporus jarrovii* (Phrynosomatidae). *Journal of Wildlife Diseases* 28(4): 537-541.

Goldberg S.R., and H.J. Holshuh. 1993. Histopathology in a captive Yarrow's spiny lizard, *Sceloporus jarrovii* (Phrynosomatidae), attributed to the mite *Hirstiella* sp. (Pterygosomatidae). *Transactions of the American Microscopical Society* 112(3): 234-237.

Foufopoulos J. 1999. Host-parasite interactions in the mountain spiny lizard *Sceloporus jarrovi*. Ph.D. Dissertation. University of Wisconsin – Madison. 215 pp.

Fuxjager M.J., J. Foufopoulos, R. Diaz-Uriarte, and C.A. Marler. 2011. Functionally opposing effects of testosterone on two different types of parasite: implications for the immunocompetence handicap hypothesis. *Functional Ecology* 25: 132-138.

Jones B.M. 1950. The penetration of the host tissue by the harvest mite, *Trombicula autumnalis* Shaw. *Parasitology* 40: 247-260.

Jones L.L.C. and R.E. Lovich. 2009. Lizards of the American Southwest: A Photographic Field Guide. Rio Nuevo Publishers. Tucson, Arizona.

Klukowski M. 2004. Seasonal changes in abundance of host-seeking chiggers (Acari: Trombicuilidae) and infestations on fence lizards, *Sceloporus undulatus*. *Journal of Herpetology* 38(1): 141-144.

Klukowski M. and C.E. Nelson. 2001. Ectoparasite loads in free-ranging northern fence lizards, *Sceloporus undulatus hyacinthinus*: effects of testosterone and sex. *Behavioral Ecology and Sociobiology* 49: 289-295.

Main A.R. and C.M. Bull. 2000. The impact of tick parasites on the behaviour of the lizard *Tiliqua rugosa*. *Oecologia* 122: 574-581.

Melvin R., C.L. Smith, O.H. Graham. 1943. Some observations on chiggers. *Journal of Economic Entomology* 36(6): 940.

Menezes V.A., A.F. Fontes, D. Gettinger, M. Van Sluys, and C.F.D. Rocha. 2011. A morphometric study of *Eutrombicula alfreddugesi* (Acari: Trombiculidae) infesting four sympatric species of *Tropidurus* (Squamata: Tropiduridae) in northeastern Brazil. *Phyllomedusa* 10(1): 79-84.

Moritz C., H. McCallum, S. Donnellan, and J.D. Roberts. 1991. Parasite loads in parthenogenetic and sexual lizards (*Heteronotia binoei)*: support for the Red Queen hypothesis. *Proceedings of the Royal Society of London B* 244: 145-149.

Newell I.M. and R.E. Ryckman. 1964. Hirstiella pyriformis sp. n. (Acari, Pterygosomidae), a new parasite of lizards from Baja California. *Journal of Parasitology* 50(1): 163-171.

Ramirez-Morales R., T. Lislevand, A. Retana-Salazar, T. Solhoy, and S. Roth. 2012. Ectoparasite loads of the Central American whiptail lizard *Ameiva festiva* (Squamata: Teiidae). *Herpetological Journal* 22(3): 151-155.

Reardon J.T and G. Norbury. 2004. Ectoparasite and hemoparasite infection in a diverse temperate lizard assemblage at Macraes Flat, South Island, New Zealand. *Journal of Parasitology* 90(6): 1274-1278.

Rubio A.V. and J.A. Simonetti. 2009. Ectoparasitism by *Eutrombicula alfreddugesi* larvae (Acari: Trombiculidae) on *Liolaemus tenuis* lizard in a Chilean fragmented temperate forest. *Journal of Parasitology* 95(1): 244-245.

Ruby D.E. 1977. Winter activity in Yarrow's spiny lizard, *Sceloporus jarrovi*. *Herpetologica* 33(3): 322-333.

Ruby D.E. 1978. Seasonal changes in the territorial behavior of the iguanid lizard *Sceloporus jarrovi*. *Copeia* 1978(3): 430-438.

Ruby D.E. 1981. Phenotypic correlates of male reproductive success in the lizard, *Sceloporus jarrovi*. *In* R.D. Alexander and D.W. Tinkle (eds.) Natural Selection and Social Behavior. Chiron Press, New York. Pp. 97-107.

Ruby D.E. 1986. Selection of home range site by females of the lizard, *Sceloporus jarrovi*. *Journal of Herpetology* 20(3): 466-469.

Ruby D.E. and D.I. Baird. 1994. Intraspecific variation in behavior: Comparisons between populations at different altitudes of the lizard *Sceloporus jarrovi*. *Journal of Herpetology* 28(1): 70-78.

Salvador A., J.P. Veiga, and E. Civantos. 1999. Do skin pockets of lizards reduce the deleterious effects of ectoparasites? An experimental study with *Psammodromus algirus*. *Herpetologica* 55(1): 1-7.

Sasa M. 1961. Biology of chiggers. *Annual Review of Entomology* 6: 221-244.

Schall J.J., H.R. Prendeville, and K.A. Hanley. 2000. Prevalence of the tick, *Ixodes pacificus*, on western fence lizards, *Sceloporus occidentalis*: trends by gender, size, season, site, and mite infestation. *Journal of Herpetology* 34(1): 160-163.

Schlaepfer M.A. and T.A. Gavin 2001. Edge effects on lizards and frogs in tropical forest fragments. *Conservation Biology* 15(4): 1079-1090.

Simon C.A. and G.A. Middendorf. 1976. Resource partitioning by an iguanid lizard: temporal and microhabitat aspects. *Ecology* 57(6): 1317-1320.

Smith H.M. 1939. The Mexican and Central American lizards of the genus *Sceloporus*. *Field Museum of Natural History, Zoological Series* 26: 1-397.

Sorci G., M. Massot, and J. Clobert. 1994. Maternal parasite load increases sprint speed and philopatry in female offspring of the common lizard. *American Naturalist* 144(1): 153-164.

Spoecker P.D. 1967. Ectoparasites of a Mojave Desert population of the lizard *Uta stansburiana stejnegeri* Schmidt. *American Midland Naturalist* 77(2): 539-542.

SPSS Inc. 2011. SPSS version 20.0 for Windows. SPSS Inc., Chicago IL.

Stebbins R.C. 1985. A Field Guide to Western Reptiles and Amphibians, 2nd ed. Houghton Mifflin Co. Boston, Mass.

Wharton G.W. and H.S. Fuller. 1952. A manual of the chiggers. *Memoirs of the Entomological Society of Washington* 4: 1-85.

Wilkinson M. 1985. Mite refugia in lizards. *Unpublished manuscript*.

Wrenn W.J. and R.B. Loomis. 1984. Host selectivity in the genus *Eutrombicula* (Acari: Trombiculidae). *In* D.A. Griffiths and C.E. Bowman (eds.), Acarology VI. Chichester, England: Ellis Horwood Limited. Pp. 160-165.

Zahavi A. 1975. Mate selection – a selection for a handicap. *Journal of Theoretical Biology* 53: 205-214.

Zahavi A. 1977. The cost of honesty – further remarks on the handicap principle. *Journal of Theoretical Biology* 67: 603-605.

Zippel K.C., R. Powell, J.S. Parmerlee Jr., S. Monks, A. Lathrop, and D.D. Smith. 1996. The distribution of larval *Eutrombicula alfreddugesi* (Acari: Trombiculidae) infesting *Anolis* lizards (Lacertilia: Polychrotidae) from different habitats on Hispaniola. *Caribbean Journal of Science* 32(1): 43-49.

Appendix 1.I: Summary of selected mite pocket hypotheses, sorted by function.

- *Nonfunctional:*
  - **Fortuitous Inhabitation** (Arnold 1986): Associations between mites and mite pockets are due to chance alone.
  - **Preservation Artifact** (Arnold 1986): Associations between mites and mite pockets are due to the unintentional detachment of mites outside mite pockets during preservation of lizards.
  - **Mite Inducement** (Wilkinson 1985): Mite pockets are induced by the feeding activity of parasitic mites.
  - **Phylogenetic Baggage** (Bauer et al. 1993; 1990): Mite pockets are the result of past adaptations or design parameters that have since lost utilitarian value.
  - **Spandrels of San Marco** (Bauer et al. 1993; 1990; Gould and Lewonton 1979): Mite pockets are the by-products of developmental processes involved in the development of skin folds.

- *Function unrelated to mites:*
  - **Physiological Function** (Arnold 1986): Mite pockets are involved in physiological functions such as water balance or the production of glandular secretions.
  - **Ecological Function** (Bauer et al. 1993; 1990): Mite pockets are utilized by the lizard for ecological functions such as crypsis, parachuting, defensive displays, or intraspecific identification.
  - **Bite Hold** (Reed, *unpublished*): Mite pockets serve as a bite hold for males during reproduction.

- *Function mite related:*
  - **Mutualistic Mites** (Arnold 1986): Mite pockets are inhabited by mites that form mutualistic associations with the lizard.
  - **Concentration/Impairment-Prevention** (Salvador et al. 1999): Pockets function to concentrate mites away from sensitive areas and prevent the impairment of vision, hearing, and motion.
  - **Concentration/Damage-Amelioration** (Arnold 1986; Chapter 2): Pockets serve to concentrate mites in specialized structures that quickly repair and contain damage caused by parasitic mites.
  - **Concentration/Handicap** (Zahavi 1977, 1975): Pockets serve to concentrate ectoparasites, which act as honest indicators of individual quality to conspecifics.
  - **Concealment – Mate Choice** (Reed, Chapter 3): Pockets serve to concentrate and conceal brightly colored mites from potential mates.
  - **Concealment – Defensive** (Reed, *unpublished*): Pockets serve to concentrate and conceal brightly colored mites to improve crypsis and avoid predation.
  - **Mite Removal** (Wilkinson 1985; Arnold 1986): Mite pockets concentrate harmful mites so they may later be removed or incapacitated.
  - **Biological Warfare** (Wilkinson 1985): Mite pockets may be used by lizard species resistant to parasitic mites to transport mites into the range of susceptible competitors, thereby giving the resistant species a competitive advantage.

Tables

Chiricahua Sites							
	Barfoot Peak						
		Adults			Juveniles		
	Total	Pooled	Male	Female	Pooled	Male	Female
N	119	41	19	22	78	35	43
Prevalence	**0.9244**[A, B, C]	0.9512	0.9474	0.9545	0.9103	0.9429	0.8837
Mean Total Load	**43.31**[D, E, F]	**64.85**[I]	41.37	85.14	**31.99**[I]	35.89	28.81
Std. Deviation	56.14	74.59	33.83	93.20	39.45	39.01	39.97
Range	0-388	0-388	0-130	0-388	0-221	0-154	0-221

	South Fork						
		Adults			Juveniles		
	Total	Pooled	Male	Female	Pooled	Male	Female
N	92	41	20	21	51	23	28
Prevalence	**0.9891**[A]	1.0000	1.0000	1.0000	0.9804	0.9565	1.0000
Mean Total Load	**92.42**[F, G, H]	**113.46**[J]	121.25	106.05	**75.51**[J]	69.52	80.43
Std. Deviation	55.76	64.65	49.74	76.75	40.75	42.29	39.52
Range	0-356	1-356	59-232	1-356	0-170	0-170	4-150

Pinaleno Sites							
	Cluff Dairy						
		Adults			Juveniles		
	Total	Pooled	Male	Female	Pooled	Male	Female
N	27	27	15	12	0	0	0
Prevalence	**1.0000**[B]	1.0000	1.0000	1.0000	n/a	n/a	n/a
Mean Total Load	**173.78**[D, G]	173.78	**224.87**[K]	**109.92**[K]	n/a	n/a	n/a
Std. Deviation	130.85	130.85	149.61	63.01	n/a	n/a	n/a
Range	17-651	17-651	17-651	25-254	n/a	n/a	n/a

	Solider Creek						
		Adults			Juveniles		
	Total	Pooled	Male	Female	Pooled	Male	Female
N	101	100	42	58	0	0	1
Prevalence	**1.0000**[C]	1.0000	1.0000	1.0000	1.0000	n/a	1.0000
Mean Total Load	**195.92**[E, H]	196.25	**250.45**[L]	**157.00**[L]	163.00	n/a	163.00
Std. Deviation	142.32	143.00	181.39	90.09	n/a	n/a	n/a
Range	18-714	18-714	18-714	28-458	n/a	n/a	n/a

C: -2.753, p=0.035
A: -2.094, p=0.036
B: -3.299, p=0.006
D: -6.656, p<0.001
E: -11.388, p<0.001
F: -6.059, p<0.001
G: -2.646, p=0.049
H: -4.864, p<0.001
I: 3.176, p=0.001
J: 3.170, p=0.002
K: 2.221, p=0.025
L: 2.301, p=0.021

Table 1.1: Chigger mite prevalence and total mite load descriptive statistics for *Sceloporus jarrovi* collected from four study sites in southeast Arizona, separated by age class and sex. Subscripts refer to significant Mann-Witney pairwise comparisons between sites, sexes, and age classes, see text for details.

Chiricahua Sites							
	Barfoot Peak						
		Adults			Juveniles		
	Total	Pooled	Male	Female	Pooled	Male	Female
N	119	41	19	22	78	35	43
Mean NP Load	**37.22**[A]	**49.68**[G]	32.16	64.82	**30.67**[G]	34.03	27.70
Std. Deviation	46.51	59.38	23.65	75.62	36.84	34.99	38.54
Range	0-322	0-322	0-77	0-322	0-207	0-117	0-207
NP Proportion	**0.8593**[C, D]	**0.7688**[H]	0.7774	0.7614	**0.9598**[H]	0.9483	0.9691

	South Fork						
		Adults			Juveniles		
	Total	Pooled	Male	Female	Pooled	Male	Female
N	92	41	20	21	51	23	28
Mean NP Load	**71.65**[A, B]	79.93	80.55	79.33	65.00	58.78	70.11
Std. Deviation	47.11	59.23	43.74	72.09	33.64	33.93	33.13
Range	0-344	1-334	23-188	1-334	0-149	0-149	3-133
NP Proportion	**0.7753**[E, F]	**0.7072**[I]	0.6643	0.7481	**0.8599**[I]	0.8455	0.8717

Pinaleno Sites							
	Cluff Dairy						
		Adults			Juveniles		
	Total	Pooled	Male	Female	Pooled	Male	Female
N	27	27	15	12	0	0	0
Mean NP Load	54.41	54.41	**68.33**[J]	**37.00**[J]	n/a	n/a	n/a
Std. Deviation	42.53	42.53	46.07	31.28	n/a	n/a	n/a
Range	0-155	0-155	0-83	3-155	n/a	n/a	n/a
NP Proportion	**0.3131**[C, E]	0.3131	0.3039	0.3366	n/a	n/a	n/a

	Solider Creek						
		Adults			Juveniles		
	Total	Pooled	Male	Female	Pooled	Male	Female
N	101	100	42	58	0	0	1
Mean NP Load	**43.50**[B]	43.92	51.57	38.38	1.00	n/a	1.00
Std. Deviation	54.11	54.21	69.13	39.91	n/a	n/a	n/a
Range	0-281	0-281	0-281	0-137	n/a	n/a	n/a
NP Proportion	**0.222**[D, F]	0.2283	0.2059	0.2445	0.0061	n/a	0.0061

A: -6.186, p<0.001
B: 5.546, p<0.001
C: 4.898, p<0.001
D: 7.307, p<0.001
E: 4.426, p<0.001
F: 6.677, p<0.001
G: 2.319, p=0.020
H: -3.212, p=0.001
I: 4.095, p<0.001
J: 1.953, p=0.053

Table 1.2: Nuchal pocket mite load descriptive statistics for *Sceloporus jarrovi* collected from four study sites in southeast Arizona, separated by age class and sex. Nuchal pocket (NP) proportion refers to the mean proportion of total mite load occurring in the mite pocket. Subscripts refer to significant Mann-Witney pairwise comparisons between sites, sexes, and age classes, see text for details.

	Head			Gular			Nuchal Pocket		
Pairwise Comparison	Test statistic	*p*	Relationship	Test statistic	*p*	Relationship	Test statistic	*p*	Relationship
Barfoot - South Fork	**-0.2816**	**0.029**	**BF < SF**	-1.426	0.923	=	**-2.95**	**0.019**	**BF < SF**
Barfoot - Soldier Creek	-0.1538	0.744	ns	**-4.955**	**<0.001**	**BF < SC**	0.987	1.000	ns
Barfoot - Cluff Dairy	**-6.636**	**<0.001**	**BF < CD**	**-4.244**	**<0.001**	**BF < CD**	-0.905	1.000	ns
South Fork - Cluff Dairy	**-3.847**	**0.001**	**SF < CD**	**-2.973**	**0.018**	**SF < CD**	1.724	0.508	ns
South Fork - Soldier Creek	1.816	0.416	ns	**-3.257**	**0.007**	**SF < SC**	**4.501**	**<0.001**	**SF > SC**
Soldier Creek - Cluff Dairy	**-5.949**	**<0.001**	**SC < CD**	0.613	1.000	ns	-1.878	0.362	ns

	Nuchal Non-Pocket			Back			Forelimb		
Pairwise Comparison	Test statistic	*p*	Relationship	Test statistic	*p*	Relationship	Test statistic	*p*	Relationship
Barfoot - South Fork	**-6.592**	**<0.001**	**BF < SF**	0.158	1.000	ns	**-2.555**	**0.064**	**BF < SF**
Barfoot - Soldier Creek	**-5.200**	**<0.001**	**BF < SC**	**-7.380**	**<0.001**	**BF < SC**	**-4.445**	**<0.001**	**BF < SC**
Barfoot - Cluff Dairy	**-5.800**	**<0.001**	**BF < CD**	**-6.726**	**<0.001**	**BF < CD**	**-7.14**	**<0.001**	**BF < CD**
South Fork - Cluff Dairy	-0.074	1.000	ns	**-6.586**	**<0.001**	**SF < CD**	**-4.863**	**<0.001**	**SF < CD**
South Fork - Soldier Creek	**2.651**	**0.048**	**SF > SC**	**-7.192**	**<0.001**	**SF < SC**	-1.402	0.966	ns
Soldier Creek - Cluff Dairy	-2.182	0.175	ns	1.376	1.000	ns	**-4.359**	**<0.001**	**SC < CD**

	Axial			Side			Inguinal		
Pairwise Comparison	Test statistic	*p*	Relationship	Test statistic	*p*	Relationship	Test statistic	*p*	Relationship
Barfoot - South Fork	-2.625	0.052	ns	-1.190	1.000	ns	0.026	1.000	ns
Barfoot - Soldier Creek	-2.536	0.067	ns	**-6.110**	**<0.001**	**BF < SC**	**-7.386**	**<0.001**	**BF < SC**
Barfoot - Cluff Dairy	**-2.858**	**0.026**	**BF < CD**	**-4.263**	**<0.001**	**BF < CD**	**-3.668**	**0.001**	**BF < CD**
South Fork - Cluff Dairy	-0.519	1.000	ns	**-3.202**	**0.008**	**SF < CD**	**-3.645**	**0.002**	**SF < CD**
South Fork - Soldier Creek	-0.590	1.000	ns	**-4.692**	**<0.001**	**SF < SC**	**-7.355**	**<0.001**	**SF < SC**
Soldier Creek - Cluff Dairy	-1.098	1.000	ns	0.353	1.000	ns	2.124	0.202	ns

	Hindlimb			Post-Inguinal			Tail		
Pairwise Comparison	Test statistic	*p*	Relationship	Test statistic	*p*	Relationship	Test statistic	*p*	Relationship
Barfoot - South Fork	-0.936	1.000	ns	-0.478	1.000	ns	-0.620	1.000	ns
Barfoot - Soldier Creek	**-8.826**	**<0.001**	**BF < SC**	**-7.180**	**<0.000**	**BF < SC**	**-9.455**	**<0.001**	**BF < SC**
Barfoot - Cluff Dairy	**-5.239**	**<0.001**	**BF < CD**	**-3.763**	**0.001**	**BF < CD**	**-6.117**	**<0.001**	**BF < CD**
South Fork - Cluff Dairy	**-4.405**	**<0.001**	**SF < CD**	**-3.337**	**0.005**	**SF < CD**	**-5.564**	**<0.001**	**SF < CD**
South Fork - Soldier Creek	**-7.712**	**<0.001**	**SF < SC**	**-6.611**	**<0.001**	**SF < SC**	**-8.716**	**<0.001**	**SF < SC**
Soldier Creek - Cluff Dairy	1.560	0.712	ns	1.840	0.395	ns	1.094	1.000	ns

Table 1.3: Mann-Witney pairwise comparisons of mite load in *Sceloporus jarrovi* for the twelve body regions examined between four study sites in southeastern Arizona. Significant differences in host body region mite loads between sites in bold. Very few mites were observed occurring on the Belly at any of the four sites, and as a result this region has been omitted here. Site abbreviations: BF = Barfoot; CD = Cluff Dairy; SC = Soldier Creek; SF = South Fork.

Figures

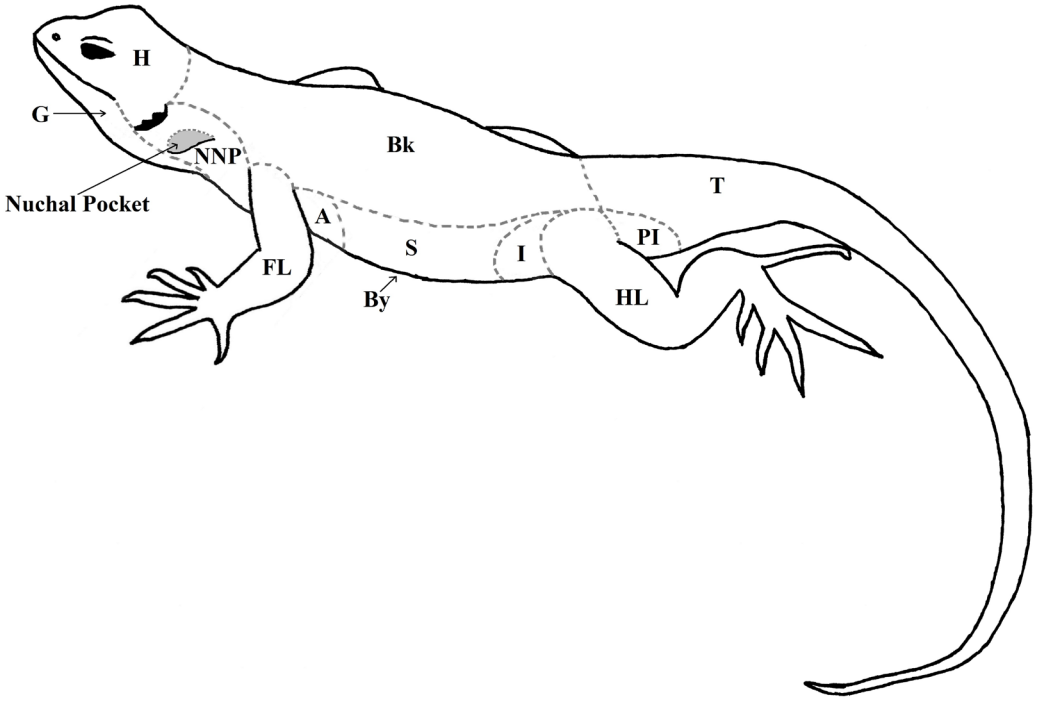

Figure 1.1: Division of lizard host into body regions for classification of mite attachment sites. Abbreviations: A – axial; Bk – back; By – belly; FL – forelimb; G – gular; H – head; HL – hindlimb; I – inguinal; NNP – nuchal non-pocket; PI – post-inguinal; S – side; T – tail. In all *Sceloporus* the nuchal pocket occupies the central nuchal region roughly midway between ear and shoulder (grey).

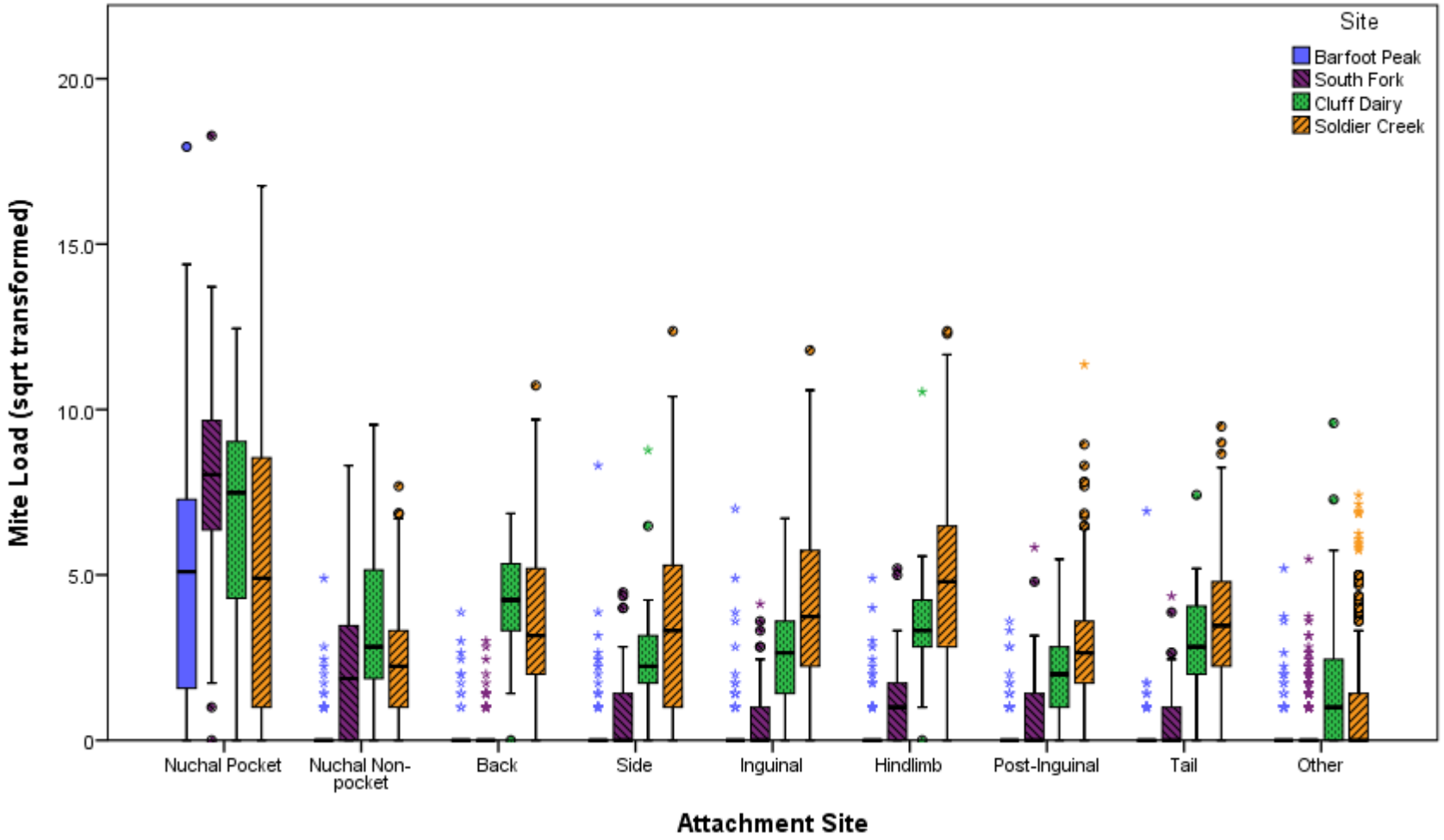

Figure 1.2: Distribution of chigger mites on the body of the host, *Sceloporus jarrovi*, grouped by study site. Of the original thirteen body regions examined (Figure 1.1), six regions (Head, Gular, Forelimb, Axial, Belly, and Post-Inguinal) had fewer than five percent of the total mite load and are here condensed in a single category ("Other") for readability.

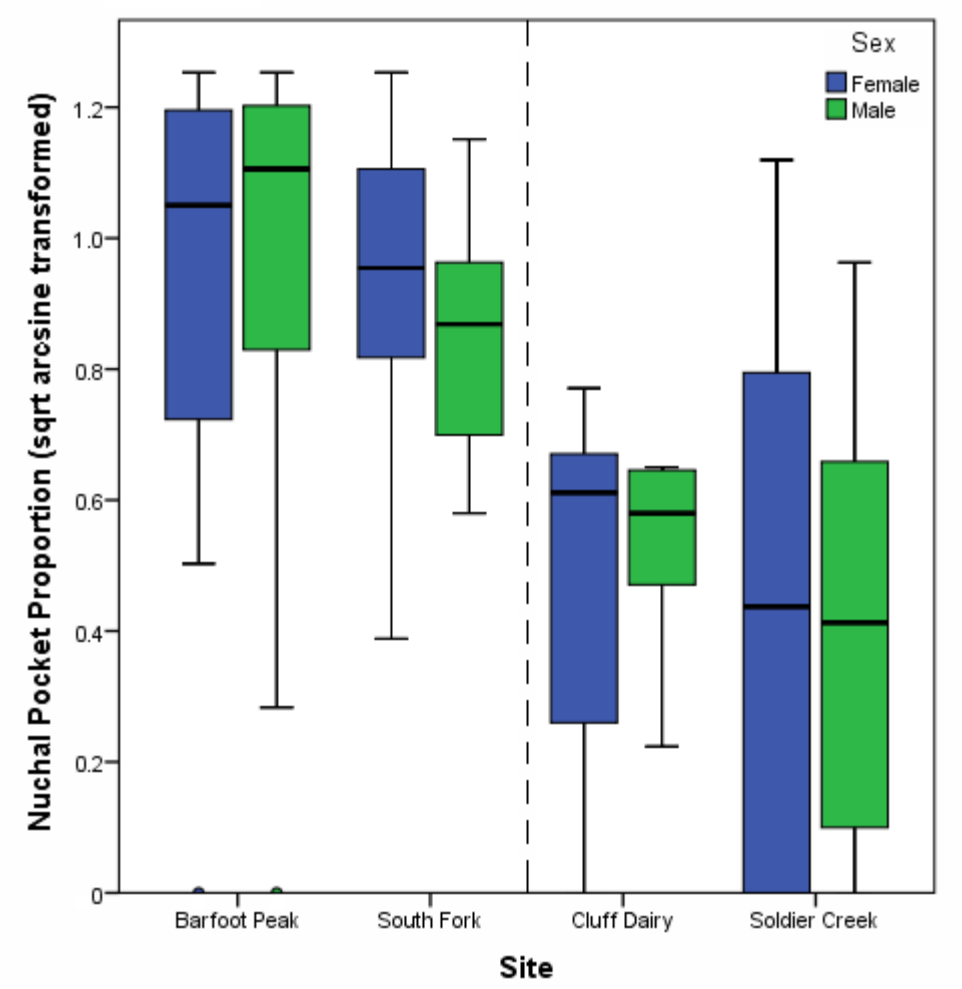

Figure 1.3: Proportion of total mite load occurring within the nuchal mite pocket (nuchal pocket proportion, square root arcsine transformation), separated by site and sex for adult *S. jarrovi*. Chiricahua study sites are left of the dotted line, Pinaleno sites to the right. Adults from Barfoot and South Fork have a significantly greater proportion of their total mite loads within their nuchal pockets than do Cluff Dairy and Soldier Creek lizards (Kruskall-Wallis $K=80.916$, $p<0.001$); pair-wise comparisons indicate (Barfoot = South Fork) > (Cluff Dairy = Soldier Creek).

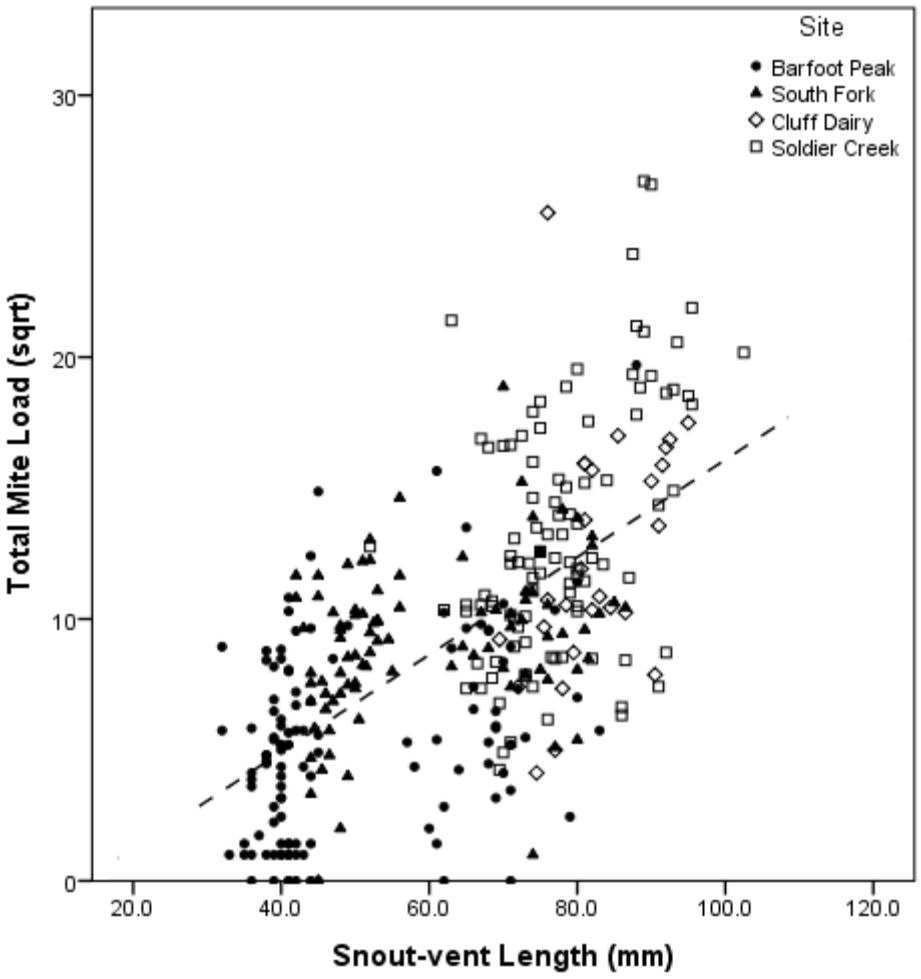

Figure 1.4A: Total mite load (square root transformed) as a function of host body size (snout-vent length). The dotted black line refers to the relationship for all lizards, pooled between sites (Pearson correlation coefficient $R=0.634$, $t=15.034$, $p<0.001$). Larger lizards have higher total mite loads than smaller lizards.

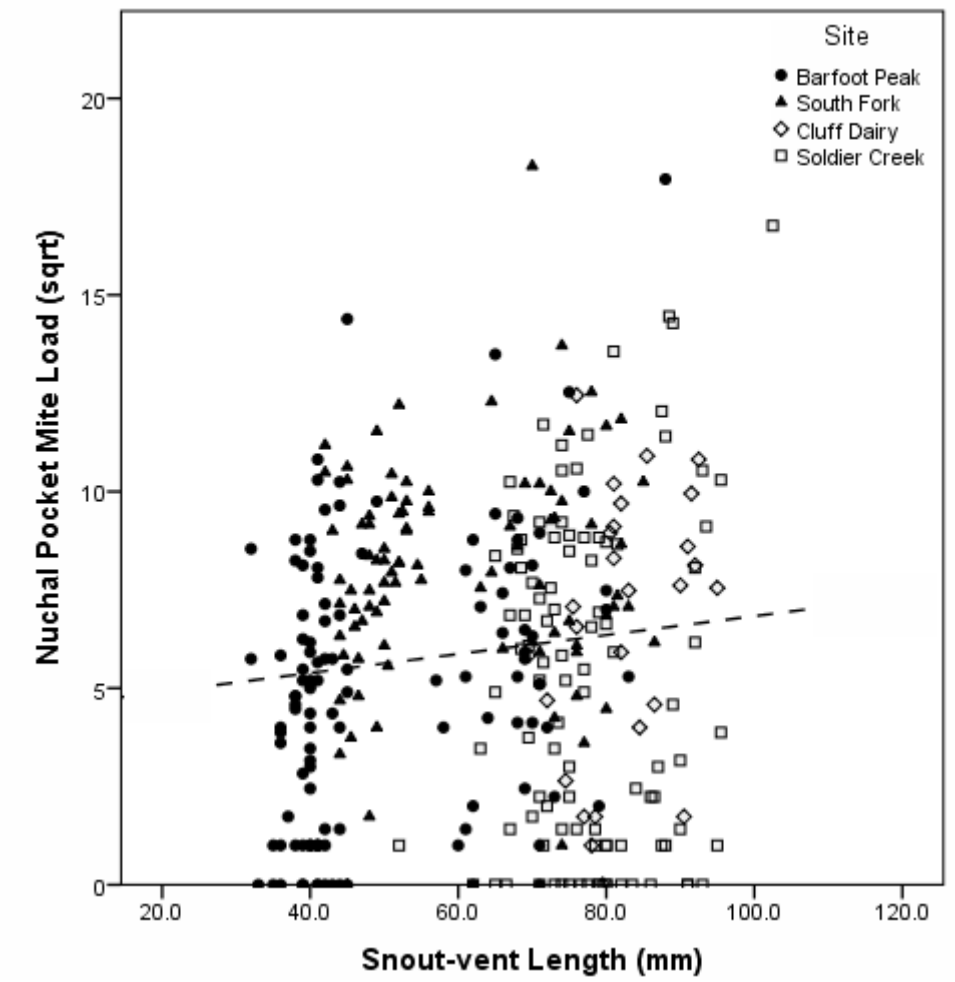

Figure 1.4B: Nuchal pocket mite load (square root transformed) as a function of host body size (snout-vent length). The dotted black line refers to the relationship for all lizards, pooled between sites (Pearson correlation coefficient R=0.111, t=2.057, p=0.040). As with total load (Figure 1.4A), larger lizards have higher nuchal pocket loads than smaller lizards.

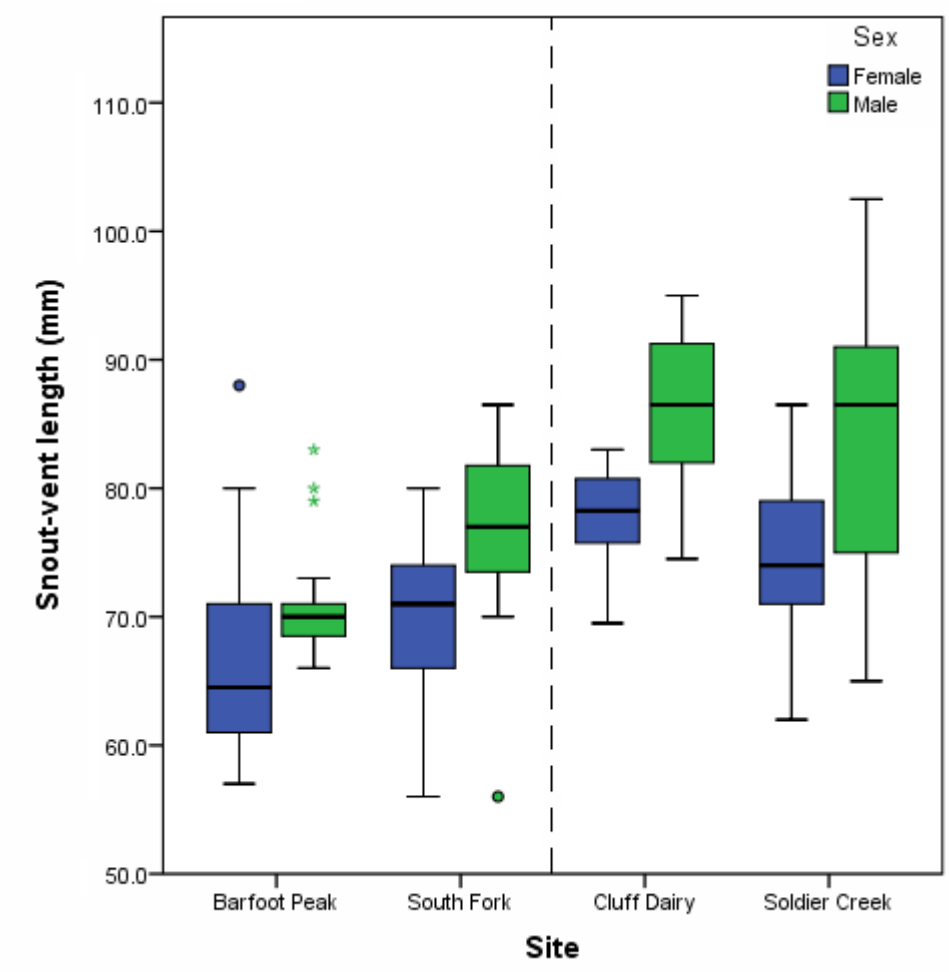

Figure 1.5: Distribution of adult body size (snout-vent length), separated by study site and sex. Chiricahua study sites are left of the dotted line, Pinaleno sites to the right. Differences in body size between sites (ANOVA $F=28.407$, $p<0.001$) and sexes ($F=47.874$, $p<0.001$) are both significant. Lizards collected from the Chiricahuas had significantly fewer mites than those from the Pinaleno study sites, and males had higher mite loads than females.

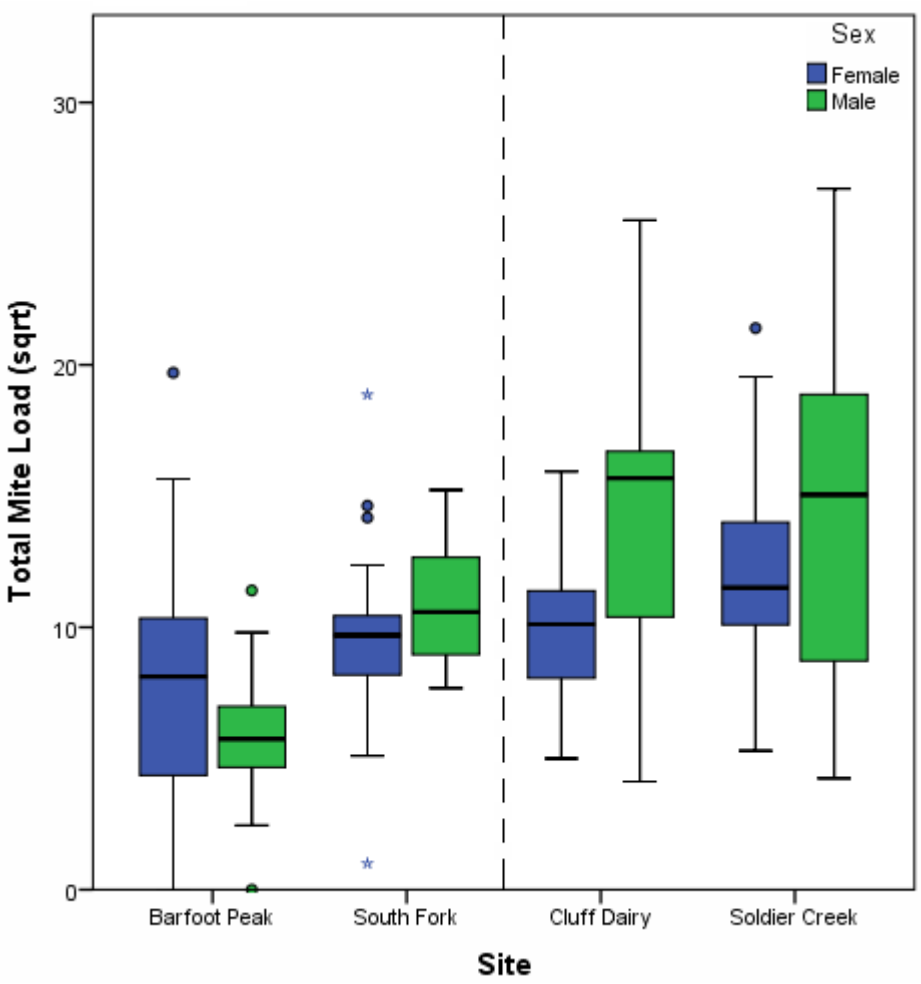

Figure 1.6: Distribution of total mite load (square root transformed) in adults, separated by study site and sex. Differences in total loads between sites (ANOVA $F=23.818$, $p<0.001$), sexes ($F=4.827$, $p=0.029$), and site by sex interaction ($F=3.795$, $p=0.011$) are all significant. Males are generally significantly larger than females, and lizards from Pinaleno sites are significantly larger than Chiricahua lizards. Note the close similarity between patterns in mite loads between sexes and sites (here) with the patterns in adult body size (Figure 1.5).

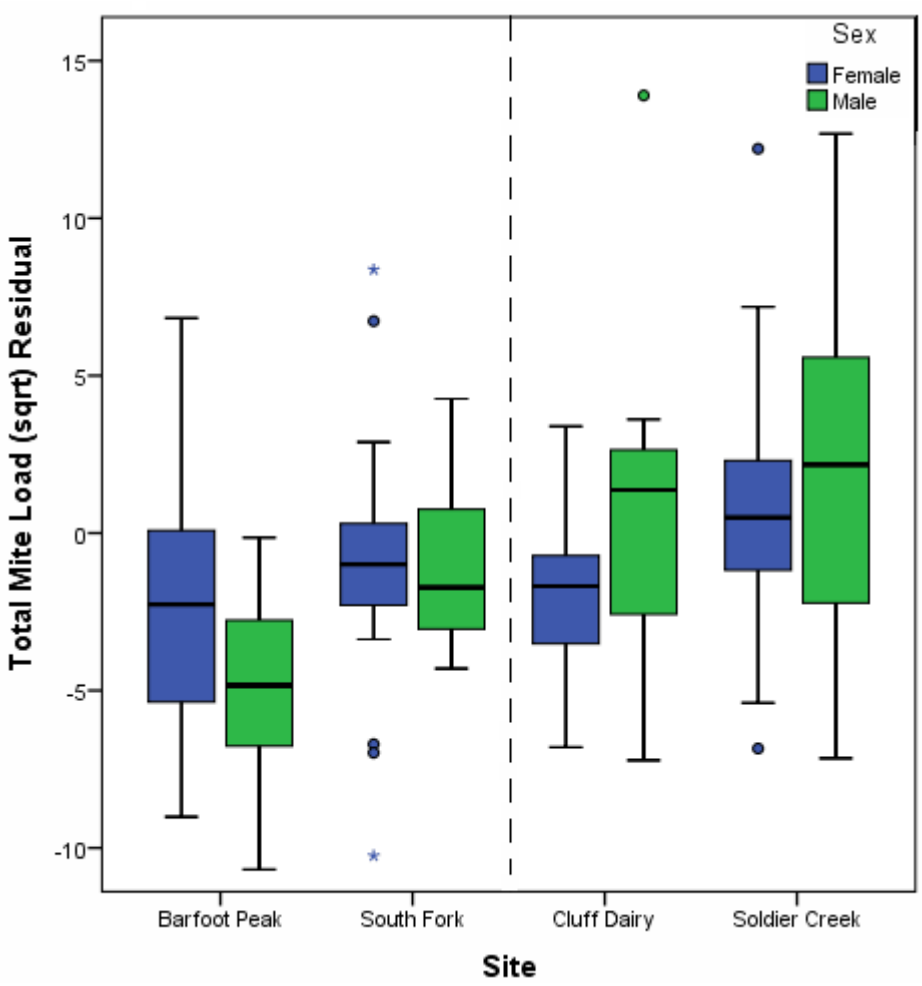

Figure 1.7: Distribution of the residuals of total mite load (square root transformed) in adults, separated by study site and sex, with the effects of body size on mite loads removed. Significant differences continue to exist in total mite loads between sites (ANOVA $F=13.326$, $p<0.001$) and for sex by site interactions ($F=2.914$, $p=0.035$), but no longer for sex ($F=0.013$, $p=0.919$). Compare to Figure 1.6.

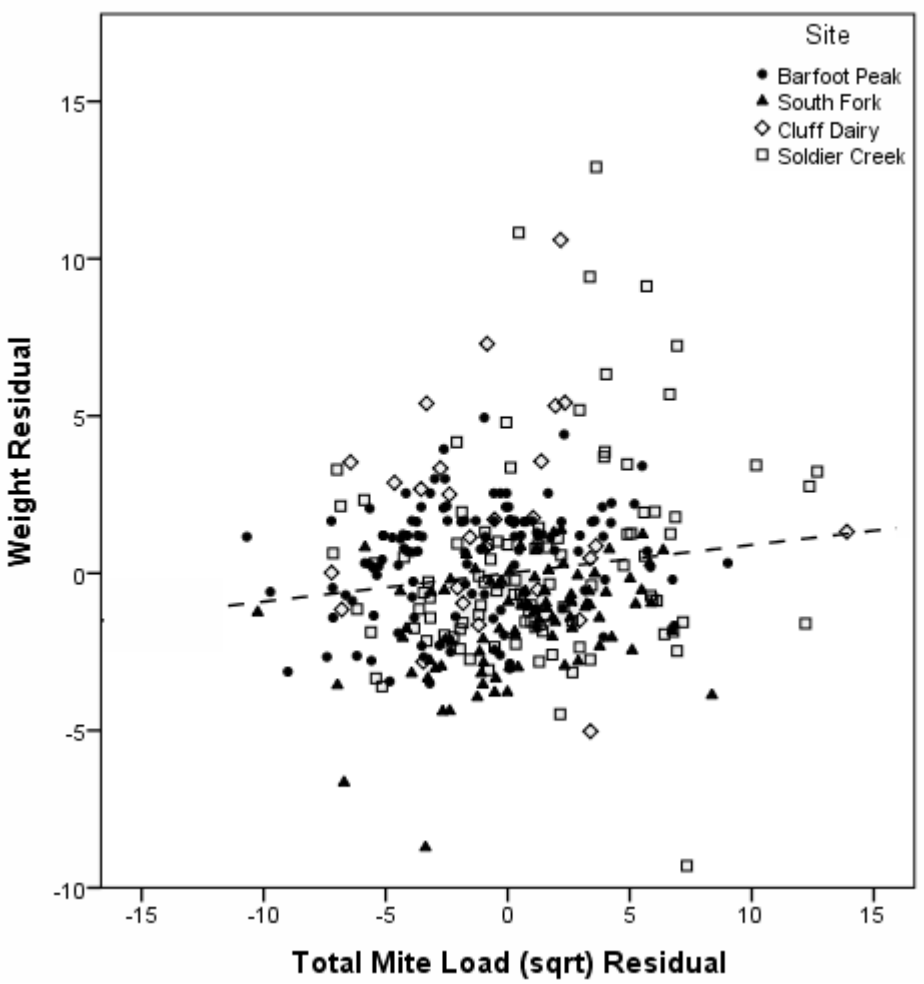

Figure 1.8: Relationship between total mite load (square root transformed) and body weight for all lizards, corrected for body size (SVL) through the use of residuals and separated by study site. The dotted black line refers to the relationship for all individuals, pooled between sites (Pearson correlation coefficient R=0.138, t=2.565, p=0.011). Lizards with high total mite loads are significantly heavier than lizards with lower mite loads, relative to body size (compare to Figure 1.9).

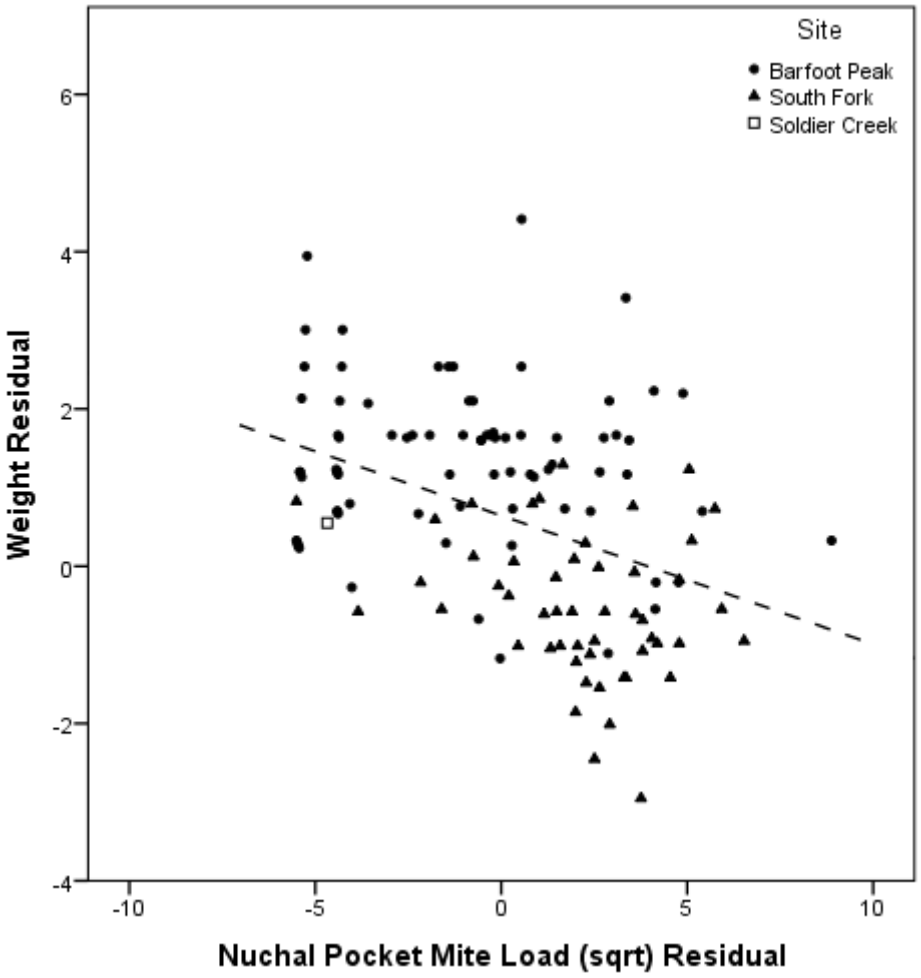

Figure 1.9: Relationship between nuchal pocket mite load (square root transformed) and body weight for juveniles, corrected for body size (SVL) through the use of residuals and separated by site. The dotted black line refers to the relationship for all juveniles (pooled between sites) (Pearson correlation coefficient $R=-0.412$, $t=-5.117$, $p<0.001$). Juveniles with higher nuchal pocket mite loads have lower body weights, relative to body size (compare to Figure 1.8).

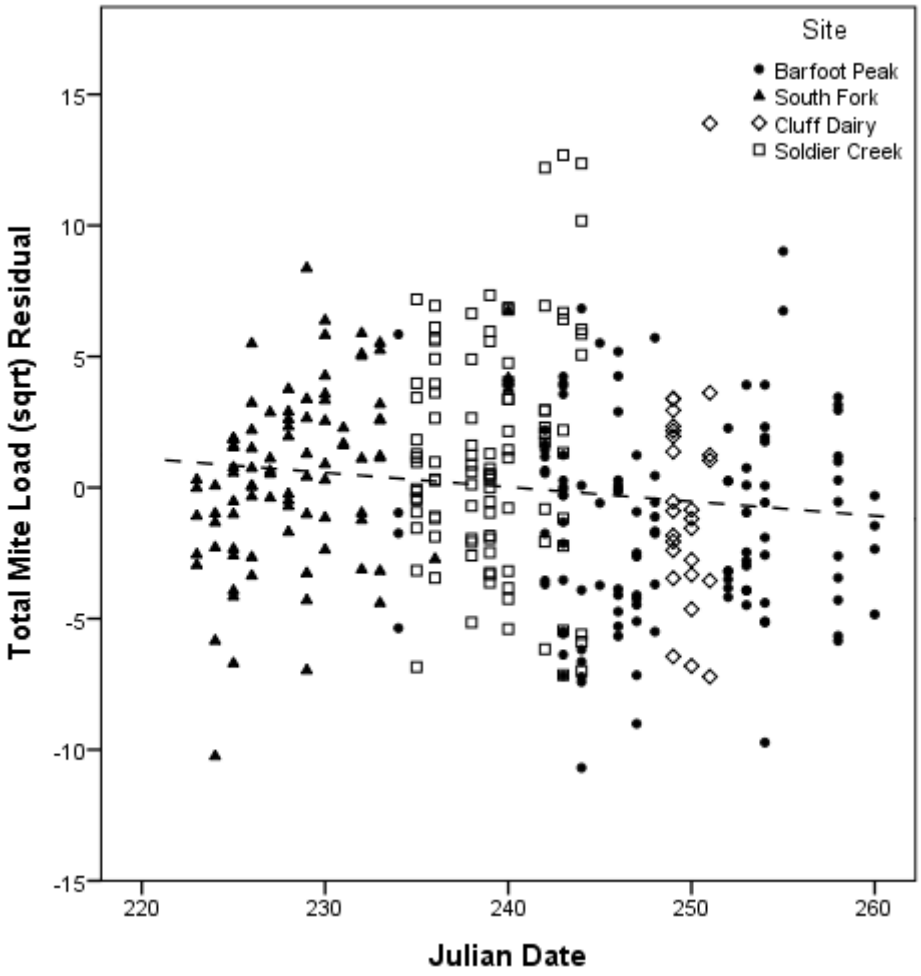

Figure 1.10: Relationship between collection date and total mite load (square root transformed), corrected for body size (SVL) through the use of residuals, for all individuals. The dotted black line refers to the relationship pooled between sites for all individuals (Pearson correlation coefficient R=-0.130, t=-2.401, p=0.017). In general, total mite loads decreased slightly over the course of the study, regardless of differences in body size and load between study sites. ANCOVA analysis suggests this relationship becomes nearly significant (F=3.708, p=0.055) once the effects of site and sex are incorporated into the model.

Chapter 2

Host Tissue Response and Repair to Ectoparasite Induced Damage – An Experimental Test of the Damage-Amelioration Hypothesis for Mite Pocket Function in *Sceloporus jarrovi* (Phrynosomatidae)

Introduction

Mite pockets are small, pocket-like invaginations of the integument known to occur in over 200 species of lizards from twelve families (Bertrand and Modry 2004; Frost et al. 2001; Leenders 2001; Salvador et al. 1999; Frost 1992; Bauer et al. 1990; Arnold 1986; Williams 1965; Smith 1939; Loveridge 1925). These structures typically have thick, well-vascularized skin with no associated musculature, and are frequently located in nuchal, axial, or inguinal regions in association with simple dermal folds (Arnold 1986; Wilkinson 1985). As may be expected given the name, mite pockets are frequently associated with mites. All inhabitants of mite pockets are parasitic, and the effects of mite parasitism on lizards are numerous. Mites may cause extensive local tissue damage, inflammation (Reardon and Norbury 2004; Goldberg and Holshuh 1993; Goldberg and Bursey 1991a) and skin lesions (Arnold 1986). In severe cases of mite ectoparasitism, anemia (Bull and Burzacott 1993; Goldberg and Holshuh 1993) or death

(Sorci et al. 1994; Goldberg and Holshuh 1993) may result. In addition to physical damage, mites can also greatly impact host ecology and behavior. Ectoparasitic mites can inhibit growth (Klukowski and Nelson 2001) and ecdysis (Walter and Shaw 2002), decrease lizard activity and home range (Main and Bull 2000), or impair vision and hearing (Moritz et al. 1991; Melvin et al. 1943). Mites are also known to serve as vectors for various endoparasites of lizards (Reardon and Norbury 2004; Newell and Ryckman 1964), and secondary infections may also potentially occur as a result of mite ectoparasitism.

Ectoparasitic mites frequently utilize mite pockets when available, and pockets likely provide mites protection from exposure and physical dislodgement (Cunha-Barros et al. 2003; Salvador et al. 1999). The most common inhabitants of mite pockets are chiggers, the obligate parasitic larvae of mites belonging to the families Trombiculidae and Leeuwenhoekiidae (Acari: Prostigmata) (Goldberg and Bursey 1993; Goldberg and Holshuh 1992; Arnold 1986; Wilkinson 1985; Bennett 1977); in addition to chiggers, scale mites (Prostigmata: Pterygosomatidae) (Bertand and Modry 2004) and ticks (Ixodida) (Schall et al. 2000; Salvador et al. 1999) may also occasionally occur in pockets. Although varying greatly in their degree of host-specificity, chiggers may parasitize a wide range of vertebrates including lizards (Wrenn and Loomis 1984; Bennett 1977; Sasa 1961; Wharton and Fuller 1952). The larvae feed by piercing the host epidermis with their chelicerae and secreting proteolytic saliva into the opening (Sasa 1961; Wharton and Fuller 1952; Jones 1950). Lysed tissues and cellular debris are then sucked up through the stylostome, a keratinous tube formed from the interaction of mite secretions and host tissues beneath the site of attachment (Shatrov and Stekolnikov

2011; Shatrov 2009; Hase et al. 1978; Jones 1950). While feeding, chiggers are immobile and incapable of relocating elsewhere on the host. The alternating process of secretion of digestive enzymes and suction of liquefied tissues is repeated until the mite reaches engorgement. In lizards, time to engorgement varies considerably by chigger species, host, and attachment site, ranging from an average of eight days for *Neotrombicula californica* (Trombiculidae) parasitizing *Uta stansburiana* (Phrynosomatidae) and *Sceloporus graciosus* (Phrynosomatidae) (Goldberg and Bursey 1991b) to over two months for *Eutrombicula lipovskyana* (Trombiculidae) parasitizing *Sceloporus jarrovi* (Phrynosomatidae) (Goldberg and Bursey 1993) and *Eutrombicula alfreddugesi* on *Phrynosoma* sp. (Phrynosomatidae) (Melvin et al. 1943). Upon engorging the mite detaches from the host and ultimately continues development in the soil as a free-living predator.

Although mites appear to receive some benefit from their association with mite pockets, the benefits pockets provide the lizard are less clear. Pockets are not induced by the feeding activities of the mites, but instead are present at birth and in individuals with no prior exposure to mites (Goldberg and Holshuh 1992; Bauer et al. 1990; Arnold 1986; pers. obs.). Pockets appear to have evolved independently in multiple lizard lineages and are often associated with certain host morphologies and ecologies (Arnold 1986; Chapter 4). Although exceptions are known, mite pockets appear to be most common in terrestrial species which frequent open canopied habitats in the tropics and sub-tropics (Arnold 1993; Bauer et al. 1993, 1990). Species occurring in cold or very dry habitats tend to lack pockets, and pockets rarely occur in very large or small species. Limbless lizards and snakes appear to lack pockets entirely, possibly due to the extensible

integument and large surface area these animals characteristically possess; if mites can easily locate suitable attachment sites on these hosts, the efficacy of pockets in directing or concentrating mite feeding activities would presumably be limited (Arnold 1986). Although numerous hypotheses for the existence and function of mite pockets have been proposed (Salvador et al. 1999; Bauer et al. 1990; Arnold 1986; Wilkinson 1985; summarized in Appendix 2.I), few of these hypotheses have been explicitly tested (but see Salvador et al. 1999) and the function of mite pockets remains controversial (Arnold 1993; Bauer et al. 1993, 1990).

In histological studies of four lizard species, Arnold (1986) found pocket tissue to be characterized by thickened epidermis and dermis, with dense concentrations of lymphocytes and collagen fiber bundles in the dermis. Pockets are not associated with underlying musculature, and connective tissue deep to the pocket is minimal. In the three species examined with pockets – *Prisurus carteri* (Gekkonidae), *Sceloporus variabilis* (Phrynosomatidae), and *Brookesia brevicaudatus* (Chamaeleonidae) – mite ectoparasitism resulted in epidermal keratinization in the area around the attachment site and concentrations of lymphocytes beneath the stylostome. Similar tissue responses to the feeding activities of mites have been observed in *Sceloporus jarrovi* (Goldberg and Holshuh 1992) and *Rhacodactylus auriculatus* (Carphodactylidae) (Bauer et al. 1990). Following mite engorgement and detachment, the epidermis reforms, lymphocytes disperse to normal concentrations, and the keratinous stylostome is sloughed off or absorbed. In the pocketless *Heliobolus lugubris* (Lacertidae), mite infestations were similar in effect but differed in magnitude; the epidermis became disfigured following gross keratinization, scale structure was lost, and lymphocytes formed very dense

concentrations in the underlying dermis (Arnold 1986). Based on the differences in tissue structure and reaction between these four species, Arnold proposed mite pockets function to ameliorate damage by concentrating mites and resisting or rapidly repairing damage caused by mite feeding activities. Under Arnold's damage-amelioration hypothesis, mite pockets make the best of a bad situation – if mite parasitism is largely unavoidable in certain habitats or circumstances, lizards could benefit by sequestering mites into specialized structures where the physical damage caused by the mites could be localized and ameliorated.

Despite offering a compelling explanation for the persistence of mite pockets in lizards, the damage-amelioration hypothesis has received little attention beyond Arnold's (1986) original investigations and several key tests have yet to be performed. Although Arnold examined lizard skin morphology and tissue response to mite damage in species with and without pockets, his comparisons were made between dissimilar, unrelated lizard species, which were very likely infested by different species of mites (chiggers were identified simply as 'trombiculid mites' in Arnold's (1986) paper). Chigger feeding behavior and resultant tissue damage can vary considerably between mite species (Shatrov 2009; Hase et al. 1978). Additionally, host response is greatly affected by the species of parasite involved (Goldberg and Holshuh 1993), as well as the number and degree of past exposure events (Goldberg and Holshuh 1992; Wright et al. 1988; Wikel 1982). As a result, a test of the damage-amelioration hypothesis would ideally be limited to comparisons of pocket and non-pocket tissue response to mite damage within the same host-parasite system, preferably between host individuals of similar age and with known or standardized mite infestation histories.

Sceloporus jarrovi (Phrynosomatidae) represents an ideal model for testing hypotheses of mite pocket function. Like all *Sceloporus*, nuchal pockets are present and well-developed in *S. jarrovi* (Smith 1939; Chapter 4). In large part due to the local abundance of *S. jarrovi* and the ease at which it is observed and captured, the biology of the species is well-known (Ruby and Baird 1994; Beuchat 1989; Ruby 1986, 1981, 1978, 1977; Ballinger 1979, 1973; Simon and Middendorf 1976). In addition, the abundance and diversity of parasites occurring on this species has also been extensively studied (Foufopoulos 1999; Goldberg and Bursey 1993; Goldberg and Holshuh 1992; Bennett 1977; Chapter 1).

In the present study, the damage-amelioration hypothesis of mite pocket function is experimentally tested in *S. jarrovi*. This hypothesis has two basic requirements: 1) chiggers preferentially concentrate their feeding activities within mite pockets, and 2) pockets reduce the impact of ectoparasitism by resisting and/or rapidly repairing damage caused by mites. To test the first requirement, the abundance and distribution of ectoparasitic mites was examined through the use of mite counts on lizards collected from the field. Comparative histological analyses of mite pocket and non-pocket tissue in response to mite damage was used to examine the second requirement.

Methods

Study System

Sceloporus jarrovi is a saxicolous, montane iguanian that inhabits rocky canyons and woodlands between 1500 and 3300 meters above sea level in southwestern New Mexico, southeastern Arizona, and central Mexico (Jones and Lovich 2009; Stebbins 1985; Ruby 1981; Ballinger 1973; Smith 1939). Although lizards may be active all year, winter activity tends to be limited, particularly in populations occurring at high elevations. During the winter individuals tend to form non-territorial aggregations in refugia, most commonly rock crevices (Beauchat 1989; Ruby 1981, 1978, 1977; Ballinger 1973). Both sexes establish territories shortly after emerging from refugia in the spring, with peak territorial behavior occurring during the September/October breeding season (Ruby 1981; 1978, 1977); after mating, territories break down as lizards return to winter hibernacula. Ovulation begins in November and the young are born alive the following May to June (Ruby 1981; Ballinger 1979, 1973; Carpenter 1960).

Data on the abundance and distribution of chiggers parasitizing *S. jarrovi* were obtained from 339 lizards collected in the fall of 2010 and 2011 from four study sites in the Coronado National Forest in southeast Arizona (Table 2.1). In this region, the predominant ectoparasites of *S. jarrovi* are the chiggers *Eutrombicula alfreddugesi* and *E. lipovskyana* (Bennett 1977); because these closely related species are morphologically indistinguishable in the field, their abundance and distribution data are presented together in the present study. Collection sites varied considerably in vegetation structure and availability of lizard refugia. South Fork (1550 m elevation; 31.878 North, 109.180 West) is a riparian corridor of mixed oak woodland and rock talus with steep rock canyon

walls. Barfoot (2550 m elevation; 31.920 North, 109.278 West) is a large, sparsely vegetated exposed talus slope located below the southern side of Barfoot Peak. Soldier Creek campground (2850 m elevation; 32.697 N, 109.921 W) consists of pine, fir, and aspen forest and grassy meadows, interspersed with isolated boulder clusters and rocky outcrops. Cluff Dairy (2750 m elevation; 32.667 N, 109.873 W) is a pine forest with largely closed canopy featuring little understory growth and numerous rocky outcrops. Refer to Chapter 1 for a full description of study sites, collection dates, and lizard microhabitat usage.

Lizards were captured using a hand-held noose, and data on mite abundance and distribution, snout-vent length, weight, approximate age, and sex were collected for all individuals shortly after capture. Snout-vent length was measured using digital calipers to the nearest 0.1 mm. Weight was measured using a Pesola spring scale to the nearest 0.1 g. Lizards were then given a unique toe clip combination for future identification and transported back to camp in individual plastic bags for examination of ectoparasites. To quantify mite abundance and distribution, the body of the host was divided into thirteen regions (Figure 2.1), and mite loads in each region were counted through the use of a stereomicroscope or hand lens at 4-10x magnification.

To standardize mite infestations between individuals for later histological analysis, mite loads were removed from all lizards following the collection of initial data. Mites were removed using a topical acaricide rinse (Reptile Spray, Natural Chemistry Inc.) containing dioctyle sodium sulfosuccinate and undecylentic acid in an aqueous solution. Similar topical acaricides have been shown elsewhere to eliminate or significantly reduce mite load with no observable effect on the lizard (Foufopoulos 1999;

Montanucci 1997; Sorci et al. 1994). Lizards were sprayed with the solution until thoroughly moist, avoiding the eyes and mouth, and allowed to sit for fifteen to thirty minutes. Following treatment, lizards were rinsed with warm tap water, allowed to dry, then released at their point of capture.

In 2011, twenty juveniles from South Fork (male n=9, female n=11) were recaptured approximately three weeks after initial collection with the aim of producing a standardized histological series of pocket and non-pocket tissue response to mite damage. Young animals were utilized to minimize the possible effects of prior infestation and augmented host sensitivity (Goldberg and Holshuh 1992). Since mites had been cleaned from these animals following initial capture and data collection, all mites occurring on these recaptured individuals were known to have attached recently, no more than three weeks prior to recapture. Data on mite abundance and distribution, snout-vent length, and weight were again collected, and the location of mites on the body photographed and marked using non-toxic paint to later locate previously infested tissues. Afterwards, mites were again cleaned off using an acaricide rinse, and lizards transferred to 4 m x 6 m outdoor pens at the American Museum of Natural History Southwestern Research Station near Portal, Arizona. Water and crickets were supplied *ad libitum*, and lizards were housed under natural conditions while recuperating. Three to six lizards were then euthanized at one week intervals over four weeks (n=20) through an intraperitoneal injection of chloral butanol, fixed in 10% neutral buffered formalin and preserved in 70% ethanol prior to tissue collection. To supplement this tissue series and examine tissue damage with mites *in situ*, an additional twenty pocket and non-pocket tissue samples were collected from twelve preserved specimens of *S. jarrovi* obtained from the

collections of the University of Michigan Museum of Zoology. These specimens were of approximately same age as the animals used in the tissue series, and were collected from the same county (Cochise) in late summer. A full list of specimens used in this project is presented in Appendix 2.II.

Pocket and non-pocket tissue previously infested with mites was removed and sent to the University of Michigan Histology Core Facility for tissue preparation and sectioning. Tissues were embedded in paraffin, serially sectioned at 5 micrometers, and stained with hematoxylin-eosin (Goldberg and Bursey 1991a; Goldberg and Holshuh 1993, 1992; Kiernan 1990; Humason 1962). Sections were initially examined and photographed prior to histomorphometry using an Olympus BX41 light optical microscope and QColor3 Olympus digital camera. To quantify the morphological differences between pocket and non-pocket tissues, as well as the response of host tissues to mite parasitism, computer-assisted histomorphometry was performed at the Histology Core Facility using a Nikon E800 light microscope equipped with a Nikon DS-Fi1 digital camera and NIS Imaging Elements software (Nikon, Version 4.0 for Windows). Measurements of epidermal thickness and dermal thickness in undamaged pocket and non-pocket tissue collected during the resting (quiescent) stage of the shedding cycle were taken to assess quantitative differences in attachment site morphology. Because the outermost keratinous layer (β-layer) of the skin was frequently lost in preparation, epidermal thickness was measured as the distance from the keratinous α-layer to the proximal limit of the *stratum germinativum*; measurements of dermal thickness included tissues of the superficial and deep dermis, measured as the distance between the proximal limits of the *stratum germinativum* to the deep dermis. Mite damage and host tissue

reaction was quantified using histomorphometric measurements of stylostome width and depth, the width of central feeding tube, and the size of the feeding cavity beneath the stylostome (measured as surface area in a two-dimensional plane) in parasitized pocket and non-pocket tissue.

Statistical analysis

All statistical analyses were done using SPSS (SPSS Inc. 2011, version 20.0 for Windows). Preliminary examination of mite load and distribution data revealed both to be highly skewed to the right; as a result, non-parametric Kruskall-Wallis tests were used to analyze the general distribution and attachment site preference of mites on the body of the host. Significantly non-random distributions were then further examined using Mann-Whitney U-tests for pair-wise comparisons between body regions (Figure 2.1). Quantitative differences in pocket and non-pocket morphology and response to parasite damage, measured as epidermal thickness, dermal thickness, stylostome depth and width, feeding tube width, and surface area of feeding cavity/tissue damage, were initially examined using Kruskall-Wallis analyses. If significant differences were recovered between pooled pocket and non-pocket tissues, Mann-Whitney U-tests were used to examine for pair-wise differences between tissue types (nuchal pocket, nuchal non-pocket, side, hindlimb, post-inguinal, and/or tail, depending on the type of samples available). In all analyses a two-tailed critical value of $\alpha=0.05$ was considered statistically significant.

Because epidermal thickness, dermal thickness, and the position of the feeding cavity within the host tissue appeared to differ greatly between pocket and non-pocket

samples in preliminary examination, *post hoc* analysis of stylostome depth relative to host *stratum germinativum* and proportional depth of stylostome penetration was performed using ANOVA. For each sample, measurement of stylostome length was subtracted from the average epidermal thickness for that individual to produce a standardized depth value centered around the *stratum germinativum*; negative values would indicate that the stylostome penetrated through the *stratum germinativum* into the dermis, while positive values would indicate that the stylostome remained within the epidermis, above the *stratum germinativum*. Degree of stylostome penetration into the dermis could not be examined using the raw data due to the substantial variation in dermal thickness between pocket and non-pocket tissues. To account for this, the position of the stylostome relative to the *stratum germinativum* (calculated above) was divided by the average dermal thickness for each individual, resulting in a value equivalent to the proportion of the total dermis penetrated by the stylostome.

Results

Chigger Distributions

Chiggers most frequently occurred within the nuchal pockets of *Sceloporus jarrovi*, but significant variation in mite loads and distributions were observed within and between study sites, age classes, and sexes (Figure 2.2, Table 2.1). Lizards from the Pinaleno Mountains (Soldier Creek and Cluff Dairy) had significantly higher total mite loads than lizards from the Chiricahua study sites (South Fork and Barfoot) (Kruskall-

Wallis K=142.052, df=3, p <0.001), and Mann-Whitney pair-wise comparisons revealed significant differences between all sites except Soldier Creek and Cluff Dairy. Despite this, nuchal pocket mite loads were remarkably similar between Barfoot, Soldier Creek, and Cluff Dairy; only at South Fork were pocket loads significantly different (higher than Barfoot-Soldier Creek-Cluff Dairy; K=46.258, df =3, p<0.001). Although mite loads were concentrated within the nuchal pocket at all sites (Figures 2.3 and 2.4), the proportion of total mites inhabiting the nuchal pockets differed significantly between study sites, from an average of 0.222 at Soldier Creek to 0.859 at Barfoot (K=80.916, p<0.001). In general, chiggers tend to concentrate within the pockets when overall loads are low but increasingly attach to other body regions as mite burden increases on an individual.

Adult lizards had significantly higher total mite loads than juveniles; adult load was also significantly greater than juvenile load for all body regions except the nuchal mite pockets (Mann-Whitney U=-0.922, df=1, p=0.357). Similar to the patterns observed in the study site comparisons (above), low total mite loads in juveniles corresponded with a high proportion of the mite load occurring within the nuchal pockets (mean proportion of total load occurring in the pocket=0.877); in contrast, mites in adults tended to be more evenly distributed across the body (mean proportion=0.353). Differences in nuchal pocket proportions between juveniles and adults were significant for both study sites at which juveniles were collected (Barfoot Peak: U=3.212, p=0.020; South Fork: U=4.095, p<0.001). Chigger loads in males and females were relatively similar for most body regions, including total load (Mann-Whitney U=0.695, df=1, p=0.487) and nuchal pocket load (0.942, df=1, p=0.346); males had significantly higher loads than females in just

four regions (head, gular, nuchal non-pocket, and forelimb). For a more detailed examination of mite loads and distribution, refer to Chapter 1.

Comparative Histology – undamaged tissues

Example sections of normal, undamaged mite pocket and non-pocket tissues are shown in Figure 2.3; for descriptive statistics of histomorphometric measurements of the epidermis and dermis, refer to Table 2.2. Terminology will largely follow that proposed by Maderson et al. (1998), and all descriptions are of tissues taken during the resting stage of the shedding cycle unless otherwise noted. Superficially, the interior of the pocket in *S. jarrovi* generally lacks pigmentation, and scalation is greatly reduced and irregular. The thick, outermost keratinous β-layer of the Oberhäutchen is easily sloughed off during tissue collection and preparation and was absent in most samples. As a result, the second keratinous layer, the α-layer, is typically the outermost layer visible. This layer is highly eosinophilic, appearing bright pink under hemotoxylin-eosin staining, and is typically more loosely organized within the pocket than in non-pocket tissues; in some regions of the nuchal pocket, this layer appears to have disassociated from the underlying lacunar/clear layer. The lacunar and clear layers are indistinguishable in most samples, and in mite pockets are frequently composed of three to six layers of ovoid, rather loosely organized cells above the *stratum germinativum*. In contrast, the cells in the lacunar/clear layers in the non-pocket tissue samples appear typical for squamates, composed of only one (rarely two) layer(s) of roughly rectangular cells oriented parallel with the exterior surface. The *stratum germinativum* is easily visible in both pocket and non-pocket samples. In pocket tissue, the cells of this layer are typically columnar and appear to be

undergoing replication in several samples; in non-pocket tissue, cells of the *stratum germinativum* tend to appear more cubical and were rarely observed in the process of replication. As a result of these differences, the epidermis within the pocket is significantly thicker and more variable in thickness (69.3±20.2 μm, n=45) than that of non-pocket tissue (22.7±7.7 μm, n=95) (Mann-Whitney U=9.484, $p<0.001$; Table 2.2; Figure 2.4A).

A moderate to dense layer of melanocytes was observed in the superficial dermis, deep to the *stratum germinativum*, in most non-pocket tissues. As would be expected, these cells are less common and more dispersed within tissues collected from the modestly pigmented belly and ventral body regions. Within the mite pocket, melanocytes are uncommon and frequently located deeper in the dermis between superficial and deep dermal layers. The remaining superficial dermis is relatively thin in most non-pocket tissues, appearing to contain two to four cell layers interspersed with some collagen fibers and spongy connective tissue, the latter of which is most prominent beneath the base of the scales. The superficial dermis in mite pocket tissues differs greatly from non-pocket tissues in thickness and consistency, frequently containing eight or more dense cell layers with little spongy connective tissue. Superficial dermis in non-pocket tissues is typically thin and difficult to distinguish from the deep dermis, with the exception of the regions beneath skin folds and scales. In these regions, the superficial dermis extends outwards into the interior of the fold or scale and frequently assumes a spongy appearance. Collagen fibers may be seen throughout the cell matrix but appear largely restricted to the interior portions of the superficial dermis and deep dermis. The deep dermis is relatively similar in pocket and non-pocket tissues, containing dense bundles of collagen fibers with

connective tissue and small blood vessels prominently appearing beneath. Taken together, these morphological differences result in a dermis that is significantly thicker in pocket tissue (196.4±47.8 μm, n=35) than in non-pocket tissue (86.2±22.9 μm, n=80) (Mann-Whitney U=8.467, $p<0.001$; Table 2.2; Figure 2.4B). In general, collagen bundles appear proportionally larger in non-pocket tissues than pocket tissues; additionally, blood vessels deep to the dermis appear to occur more frequently in pocket tissues than non-pocket tissues. Although dermal musculature was present in some non-pocket tissue samples, no underlying musculature was found associated with mite pockets in any of the samples examined.

Comparative Histology – damage caused by mites

Numerous chiggers in various states of engorgement were recovered *in situ* from tissues collected from UMMZ specimens; example sections of parasitized pocket and non-pocket tissues are presented in Figures 2.11 and 2.12, respectively. Mite feeding activities observed in the present study were largely similar regardless of attachment site and comparable to behaviors reported in the literature. As previously described (Shatrov and Stekolnikov 2011; Shatrov 2009; Hase et al. 1978; Jones 1950), the chigger first buries its chelicerae into the upper keratinous layer of the host epidermis and secretes an eosinophilic cone, which serves to cement the mite in place prior to feeding; although this cone often becomes indistinguishable from the developing stylostome as the mite feeds, impressions of the chelicerae are frequently still visible (Figure 2.6, top). Secretion of digestive enzymes through the center of the cone into the underlying epidermis, and subsequent interaction with the surrounding host tissues produces the keratinous, highly

eosinophilic stylostome with a distinct central feeding tube (best displayed in Figure 2.6, top). Although this basic morphology remained consistent regardless of attachment site, the dimensions of the stylostome varied significantly according to host tissue type (Table 2.2). Stylostomes formed within the mite pocket were nearly significantly longer than those in non-pocket tissues (Mann-Whitney U=1.884, p=0.060; Figure 2.7, top), and feeding tubes in pocket stylostomes were significantly wider than those in non-pocket stylostomes (Mann-Whitney U=2.573, p=0.010; Figure 2.7, bottom). Host tissue type had no significant effect on stylostome width.

Host tissue reactions to mite feeding activities appeared largely similar between tissue types. The epidermis lateral to the stylostome tended to exhibit mild hyperplasia and hyperkeratosis, with degeneration and necrosis occurring around the deeper edges of the stylostome. Lysis of host tissues by enzymes secreted by the mite resulted in a feeding cavity beneath the stylostome which contained a slurry of disassociated epithelial cells, cell contents, and mite saliva; this cavity was frequently surrounded by a region of necrosis and responding heterophils and macrophages. The position of the feeding cavity differed significantly between pocket and non-pocket tissues; stylostomes in non-pocket tissues penetrated significantly deeper into the host (ANOVA: $F= 17.763$, $p<0.001$; Figure 2.8) and proportionally deeper into the dermis ($F=68.242$, $p<0.001$; Figure 2.9) than those stylostomes located within the mite pocket. Feeding cavities within mite pockets were generally located between the *stratum germinativum* of the epidermis and the superficial dermis; these cavities appeared relatively compact and localized, with most of the tissue damage restricted to the uppermost layers of the superficial dermis (Figure 2.5). In non-pocket tissues, the location of the attachment site played a major

role in the position of the feeding cavity and type of host tissues damaged. Mites attached between scales or in regions where the host skin was relatively thin produced compact, ovoid-shaped feeding cavities in which the superficial dermis beneath the stylostome was completely lysed and the deep dermis penetrated (Figure 2.6, top); in contrast, mites located beneath scales commonly produced large, diffuse feeding cavities of variable size and shape within the spongy superficial dermis and only rarely reached the deep dermis (Figure 2.6, bottom). Despite these generalizations, the size and general shape of the feeding cavity were not significantly different between tissue types (Table 2.2). Inflammation and concentrations of heterophils in pocket tissue were variable, with foci commonly appearing beneath and lateral to feeding cavities; numerous heterophils were also present in the deep dermis near some of the larger blood vessels (Figure 2.5). An acute host immune response was less apparent in most non-pocket samples, with relatively few concentrations of heterophils or focal inflammation observed around the attachment site. Granulomas were uncommonly observed in both pocket and non-pocket tissues beneath the stylostome and feeding cavity. In nuchal pocket tissues, granulomas appeared to form from a combination of macrophage aggregations in the superficial dermis and epidermal extensions around and beneath the feeding cavity (Figure 2.10).

Comparative Histology – tissue repair

Sceloporus jarrovi with known parasite histories were euthanized at one week intervals following mite removal to examine the sequence and rate at which mite damage is repaired in pocket and non-pocket tissues. Although non-pocket tissues were photographed and marked *in situ* with paint prior to mite removal to aid in the

identification of prior attachment sites for tissue sampling, very few definitive instances of mite damage and subsequent repair could be successfully recovered in the non-pocket tissue repair series. As a result, this section will focus largely on repair within the mite pockets, with non-pocket tissue comparisons included when possible. For mite damage *in situ* (effectively Day 0 post-detachment and prior), refer to the previous section.

Ectoparasite damage within pockets remained considerable eight days after mites were killed with the acaricide treatment (Figure 2.11). Mite pocket stylostomes best correspond to the epidermal stylostome type as categorized by Hase et al. (1978). Pocket stylostomes generally remained imbedded in the epidermis, with the central feeding canal clearly visible. Most of these stylostomes appeared to have breached the epidermis, with the open feeding cavity positioned in the region between the *stratum germinativum* and superficial dermis. Deeper dermal stylostomes were rare and occurred most notably in regions where multiple mites fed in close physical association with one another; stylostomes would frequently fuse together in these instances, resulting in dense keratinous plugs. At this early stage in the repair process, disassociated cell contents and numerous heterophils had solidified and became part of the keratinous base of the stylostome, forming a plug that occluded the central feeding canal. Moderate to severe hyperplasia and hyperkeratosis of the epidermis was present around the lateral edges of the stylostome, resulting in a cup-like epidermal structure that remained evident long after the mite and stylostome had been sloughed. Regions of epidermal ulceration and caseous necrosis were present beneath the stylostome in some samples, particularly in tissues where multiple mites fed near one another. However, in most samples the epidermis had already begun to reform beneath the attachment site and was relatively

contiguous. Epidermis reforming beneath sites of mite attachment was thinner than unaffected pocket epidermis, and the *stratum germinativum* appeared to be actively undergoing cell division in many regions. Moderate to severe focal inflammation was present in many regions of the dermis, particularly in the superficial dermis just below the regions of mite damage and surrounding granulomas. Mature granulomas containing heterophils, macrophages, and foamy cytoplasm were also occasionally present in the superficial dermis. These granulomas formed beneath the attachment site but were not always closely associated with a stylostome or the cup-like epidermis described above. In a few samples, additional granulomas were observed just beneath or actually embedded in the epidermis, with no close association with recent mite activity, apparently in the process of being expelled. Blood vessels along the innermost lining of the pocket appeared slightly enlarged and were frequently engorged with erythrocytes.

By the second week following acaricide treatment (Figure 2.12), stylostomes had generally sloughed off from the initial attachment site but frequently remained within the pocket due to loose attachment to the surrounding α-layer. Both the central feeding canal of the stylostome and the heterophil-infiltrated base were evident in most samples. The cup-like structures produced by epidermal hyperplasia remained very distinct, making it easy to relocate recent mite attachment sites even in the absence of attached stylostomes. Epidermal reformation beneath the attachment site was largely complete at this stage, with few instances of epidermal ulceration and no underlying necrosis grossly visible. Epidermal thickness remained variable due to hyperplasia around previous attachment sites. Cell division in the *stratum germinativum* appeared to be most prevalent in areas just lateral to prior mite attachment. Inflammation was highly variable. In some

samples, heterophils and macrophages occurred only within the superficial dermis just beneath attachment sites; in other samples the host reaction was more severe, with dense concentrations of macrophages and inflammation occurring throughout the deep dermis, collagen bundles, and panniculus. Granulomas were absent in all samples.

Further tissue repair was evident by the third week following mite removal (Figure 2.13). Stylostomes were sloughed from the original attachment site and epidermal hyperplasia had subsided, and the associated cup-like structures were less distinct. No ulceration of the epidermis was present, and the epidermis had largely returned to normal state and thickness beneath prior attachment sites, with only a small region of remaining necrosis occurring in a few sections. Cell division in the *stratum germinativum* appeared more uniform and was no longer concentrated around prior attachment sites. Dermal response remained quite variable, with inflammation and dense clusters of heterophils occurring in some samples but diffuse in others; granulomas were once again absent.

Mite pocket tissues collected four weeks after treatment appeared very similar to those from the previous week (Figure 2.14). Stylostomes had been sloughed and were absent in most samples. Unequal hyperplasia of the epidermis was greatly reduced and the pocket epidermis appeared largely normal, but cup-like epidermal structures formed around prior attachment sites were still occasionally visible. No ulceration or necrosis was visible. Heterophil density within the dermis remained variable, but no granulomas were present.

Few definite instances of non-pocket tissue damage and subsequent repair could be recovered for comparison to pocket response in the field (experimental) series. One

sample, collected three weeks after treatment from the dorsal surface of the forelimb, displayed a keratinous stylostome plug retained beneath a scale (Figure 2.15, top). Numerous heterophils had infiltrated the base of the keratinous mass and connective tissue beneath the dermis, including the collagen bundles. No epidermal ulcer was present and the epidermis appeared to have redeveloped beneath the mass, although some necrosis of the underlying superficial dermis remained. Epidermal hyperplasia was absent, and the typical cup-like epidermal structures commonly observed in pocket samples were not present. In another sample, collected from the nuchal non-pocket region three weeks after treatment, the non-pocket tissue response very much resembled that of pocket tissue (Figure 2.15, bottom). Multiple mites were feeding beneath scales in this sample, and the fused stylostomes remained loosely attached. As was observed in pocket tissues, the bases of the stylostomes were invaded by heterophils and cell debris, and hyperplasia of the surrounding epidermis had resulted in cup-like structures around the prior attachment sites. The *stratum germinativum* appeared to be actively dividing in several regions around the attachment sites. Although slightly thicker than typical, the epidermis surrounding the attachment sites had regained normal appearance and no epidermal ulcerations or necrotic regions were visible. A few scattered heterophils were present in the superficial dermis in and around the attachment sites, but no obvious inflammation was evident and granulomas were absent.

Several instances of tissue repair were also noted in pocket and non-pocket tissue samples collected from preserved museum specimens with mites *in situ* (Figures 2.11 bottom, 2.20); although no data were available for past infestation history or time since detachment, tissue reactions in these samples appeared the same to those observed in the

experimental field series described above. Following mite detachment, stylostomes in both pocket and non-pocket tissues were invaded and occluded by heterophils and disassociated cell contents. Re-epithelization occurred via lateral extensions of the epidermis surrounding the stylostome, and these outgrowths frequently extended beneath both stylostome and feeding cavity in mite pocket tissues. In non-pocket tissues, re-epithelization occurred primarily between the stylostome and feeding cavity, with the resulting feeding cavity becoming surrounded by macrophage aggregations and forming a granuloma that persisted in the superficial dermis. Hyperplasia of the epidermis around the attachment site was observed in both pocket and non-pocket tissues, but distinct cup-like epidermal structures following stylostome detachment occurred in only a few samples (Figure 2.16, bottom). Inflammation and heterophil concentrations were most apparent in mite pocket tissues, but due to concurrent infestations could not be definitively be associated with the observed instances of tissue repair.

Discussion

Although the abundance and distribution of mites on *Sceloporus jarrovi* varied considerably by collection site, age, and sex, mites predominantly occurred within nuchal mite pockets when available, consistent with the first prediction of the damage-amelioration hypothesis (Table 2.1). Based on the inverse relationship between total mite load and the proportion of total load occurring within mite pockets, mites preferentially utilized pockets as attachment sites, only later attaching to secondary sites as pockets

become filled. Further supporting this, nuchal pocket loads remained remarkably constant between sites, age classes, and sexes despite high variation in total mite loads, suggesting that nuchal mite pockets have an upper capacity limit. If pockets do serve an adaptive, mite-related function, as suggested by the damage-amelioration hypothesis, these results also suggest that the value of pockets to the individual may vary greatly. Pockets would be expected to be most useful in environments where the bulk of the mite load could be accommodated within the limited space of the pocket (such as at South Fork and Barfoot), and less valuable in areas where mite loads far exceed pocket capacity (Cluff Dairy and Soldier Creek). Alternatively, because mite loads may fluctuate considerably throughout the year in temperate regions, particularly in late summer (Klukowski 2004; Foufopoulos 1997; Goldberg and Bursey 1991a; Spoecker 1967), mite pockets may be functionally most useful to lizards outside of peak seasons when mite abundance in the environment is relatively low to moderate.

As observed in this and other studies (Goldberg and Holshuh 1993; Goldberg and Bursey 1991a; Bauer et al. 1990; Arnold 1986), chiggers are capable of producing extensive tissue damage in lizards as a result of their feeding activities. A diagrammatic overview of chigger parasitism and mite pocket tissue response is shown in Figure 2.17A-F. Shortly after selecting an attachment site, the larva buries its chelicerae into the stratum corneum and secretes an eosinophilic cone, which functions to adhere the mite to the host (Shatrov 2009; Hase et al. 1978; Figure 2.17A). Afterwards, hydrolytic enzymes are secreted by the mite to dissolve host tissues beneath the attachment site for ingestion. This process, together with the interaction between mite saliva and keratin in the host

tissues, produces a keratinous stylostome with a central feeding canal (Shatrov 2009; Hase et al. 1978; Figure 2.17B).

Numerous factors likely influence the degree of damage chiggers cause to the host. The amount of time required to develop a mature stylostome varies by chigger and host species but this process can be rapid; in experimentally parasitized mice and chickens, stylostomes can be well-developed within 24 hours following attachment (Hase et al. 1978; Cross 1962a, b). Stylostome size and focal tissue damage increases as the mite feeds, peaking shortly before engorgement and detachment (Cross 1962a, b). Chigger engorgement in endothermic vertebrates is rapid, typically occurring within the first two to five days following attachment (Wright et al. 1988; Hase et al. 1978; Cross 1962a, b; Sasa 1961). For reasons that remain unclear, chiggers remain attached to lizards for considerably longer and more variable periods of time, and may require eight to more than 50 days before attaining engorgement (Goldberg and Bursey 1993; Goldberg and Bursey 1991; Melvin et al. 1943). If tissue damage in lizards increases with engorgement as it does in other animals (Cross 1962a, b), damage caused by mites would be expected to be greatest just prior to detachment. The location of mite attachment also appears to play a role in the type of stylostome produced, the amount and type of host tissue damaged, and the degree of host response, with deeper stylostomes resulting in greater tissue damage and a more acute host response (Shatrov 2009; Hase et al. 1978). In the present study, the stylostomes of *Eutrombicula alfredugesi/lipovskyana* in *S. jarrovi* mite pocket tissues generally correspond to the epidermal type described by Hase et al. (1978), penetrating the *stratum germinativum* with the resultant feeding cavity located between epidermis and superficial dermis (Figure 2.5, 2.21C). Stylostomes rarely

extended into the dermis of the mite pocket (mesenchymal type). Unlike the more typical epidermal type described above, mesenchymal stylostomes were characterized by extensive inflammation, dense concentrations of associated inflammatory cells, and a feeding cavity located beneath the *stratum germinativum*. Mesenchymal stylostomes were more commonly observed in non-pocket tissues (Figure 2.6). Similar non-pocket tissue responses were noted in *Uta stansburiana* (Phrynosomatidae) parasitized by the chigger *Neotrombicula californica* (Prostigmata: Trombiculidae) (Goldberg and Bursey 1991a); in this lizard, chiggers attached primarily to the eyelids and produced mesenchymal stylostomes that extended deep into the dermis.

The response of mite pockets to chigger-induced damage in the present study is largely consistent with that of the non-pocket tissue response reported in other vertebrates (Shatrov and Stekolnikov 2011; Shatrov 2009; Hase et al. 1978), including lizards (Goldberg and Holshuh 1992; Goldberg and Bursey 1991a; Bauer et al. 1990; Arnold 1986). Although non-pocket material was limited, the response of these tissues did not appear qualitatively different from that of mite pocket tissue. Chigger-induced damage initially generates a generalized host immune response involving focal inflammation and heterophil infiltration of the superficial and deep dermis (Figures 2.17B and C). This initial response is very similar to that which occurs in experimentally induced inflammation and wound repair, which in reptiles develops rapidly within the first 4-48 hours (Tucunduva et al. 2001; Smith and Barker 1988; Smith et al. 1988; Mateo et al. 1984). In mite pockets, heterophils were observed to migrate into the feeding cavities beneath stylostomes and invade the base of the stylostome within the first week following mite detachment; a similar sequence occurs in scab formation in response to epidermal

wounding (Smith and Barker 1988; Maderson and Roth 1978; Figure 2.17D). In the present study, focal inflammation beneath former attachment sites had largely subsided a week after mites were removed, replaced with a diffuse inflammatory reaction in the superficial and deep dermis that frequently persisted for the remainder of the study.

Lateral extensions of the epidermis developed beneath the stylostome within the first week following mite detachment, with all focal ulcerations repaired by the end of the second week (Figures 2.17D and E). The process and rate of re-epithelialization of the pocket in response to mite damage is approximately the same as that which occurs in response to other small wounds (Alibardi 2010; Smith et al. 1988; Maderson and Roth 1978). The stylostome is generally sloughed from the attachment site by the second week, shortly after re-epithelialization is complete. Focal necrosis of tissue surrounding the wound, which frequently occurs within the first few days following wounding (Smith et al. 1988; Smith and Barker 1988; Madersno and Roth 1978), was not observed during the repair of mite-induced damage.

Hyperplasia of the epidermis surrounding the site of mite attachment occurred within the first week following detachment but was most frequently noticeable following loss of the stylostome (Figure 2.17E). The resulting cup-like epidermal structures tended to degenerate over time as hyperplasia subsided and the epidermis became more uniform, making identification of prior attachment sites in older samples more difficult (Figure 2.17F). Although chiggers parasitizing *S. jarrovi* do not fully embed themselves in host tissue, a similar but more severe process of hyperplasia and invagination at the wound site can result in complete encapsulation of the mite in other hosts (Shatrov 2009; Grover et al. 1975).

The general absence of granulomas in the present study, particularly in pocket tissues collected two to four weeks following mite removal, is curious. In their examination of the chigger, *Eutrombicula lipovskyana*, infesting *S. jarrovi in situ*, Goldberg and Holshuh (1992) observed numerous granulomas forming beneath mite attachment sites in juveniles and adults (but not neonates), suggesting that lizards acquire increased sensitivity with subsequent infestations. Similarly, granulomas were observed forming beneath *Neotrombicula californica* chiggers feeding *in situ* on *Uta stansburiana* (Goldberg and Bursey 1991a). Because lizards used for histology in the present study were juveniles born earlier that spring, it is possible that many of these individuals were relatively immunologically naïve to ectoparasites and had yet to experience sufficient prior infestations to become sensitized. Alternatively, granuloma formation and persistence may be dependent upon host tissue type and the location of the tissue damage. In the present study, mites attaching to non-pocket tissues produced feeding cavities located in the superficial and deep dermis; host immune response in these tissues resulted in the formation of granulomas in the dermis that appeared to persist for at least several weeks following detachment. In contrast, feeding cavities in pocket tissues were located just deep to the *stratum germinativum*. Following the initial encapsulation by the host immune system, re-epithelization and regeneration of the epidermis appeared to expel these cavities along with the stylostome rapidly after mite detachment (Figure 2.11). This differential tissue response appears to result in distinct granulomas forming infrequently and being more transient in mite pocket tissues relative to non-pocket tissues. In mammals, granuloma formation and persistence appears to be highly variable, largely dependent upon the type of irritant and the length of time it remains in the skin;

granulomas may form within a few days or not for several weeks following irritation, and may persist for periods longer than a year (Murray 1999; Adams 1983; Adams 1976). Host tissues parasitized by chiggers are likely irritated by mite saliva, lysed and necrotic tissues, and possibly the stylostome itself. However, since stylostomes are lost shortly after mite detachment and most host tissue repair occurs within the first two weeks, the irritants typically encapsulated by granulomas may also be rapidly degenerated or expelled by the host. Finally, because chiggers vary in their feeding activities and resultant tissue damage, granulomas may not always be formed. In particular, granulomas appear uncommon in mites that produce short epidermal stylostomes with little damage to the dermis or in cases where inflammation is prolonged but of low intensity (Shatrov 2009; Hase et al. 1978); this scenario generally fits well with the pocket tissue reactions observed herein.

Conclusion

The results of the present study generally support predictions made by the damage-amelioration hypothesis; however, these data also suggest that some revisions are necessary to Arnold's (1986) original hypothesis, particularly regarding the mechanism(s) by which pockets may function to ameliorate ectoparasite damage. Consistent with the first requirement of the damage-amelioration hypothesis, chiggers largely concentrated their feeding activities within the nuchal mite pocket. This predisposition towards inhabiting the mite pocket occurred to varying degrees at each

study site and for all host demographic groups examined, suggesting this preference to be quite typical within this particular host-parasite system. The association between chiggers and mite pockets was particularly strong in situations where total mite loads were low to moderate, allowing most or all of the mites present on an individual to attach within the pocket. When mite loads were high, pockets became saturated and chiggers attached elsewhere on the body of the host, demonstrating that although pockets are the preferred attachment site, chiggers will attach and feed at secondary sites when pockets are unavailable rather than seek out other hosts.

The second condition of Arnold's (1986) damage-amelioration hypothesis – that pockets resist and/or rapidly repair ectoparasite-induced damage – was supported partially by the results of the present study. Pockets displayed numerous significant morphological differences when compared to non-pocket tissues, particularly in regard to the thickness of the epidermis and dermis. Chiggers feeding within mite pockets produced significantly deeper stylostomes with wider feeding tubes than larvae feeding outside the pocket. Due to the difference in epidermal thickness, longer stylostomes are likely required within pockets for mites to pass through the keratinous epidermis and reach the nutritionally rich living cells within the *stratum germinativum* and dermis. Chiggers respond in a similar manner when parasitizing mammals with a *stratum corneum* of variable thickness, and producing a long stylostome increases the amount of time the mite must feed on the host (Shatrov 2009); the potential consequences of this as an alternative mechanism for the damage-amelioration hypothesis is discussed below. Finally, granulomas were rarely encountered within pockets but appeared more common in non-pocket tissues. Although reasons for the apparent disparity in granuloma

formation and persistence between tissue types remain unclear, the relative absence of granulomas within pocket tissues suggests that mite parasitism within pockets may result in fewer long-term consequences to the host relative to non-pocket tissues.

Other data obtained in the present study do not support Arnold's (1986) second condition. The general response of mite pocket tissues to damage produced by chiggers did not differ from that observed in non-pocket tissues, and the amount of tissue damage (i.e. size of the feeding cavity) was the same in both host tissue types. Additionally, the rate at which mite damage was repaired in pockets is similar to that observed in non-pocket tissues; although the number of non-pocket tissue repair samples recovered in the present study was lower than expected, the rate at which mite damage was repaired within pockets was also similar to that of normal wound healing in non-pocket tissues (Alibardi 2010; Smith et al. 1988; Maderson and Roth 1978).

These data suggest that although pockets do not have an extraordinary ability to physically prevent damage caused by feeding mites, they may indirectly reduce mite damage by controlling placement of the feeding cavity and/or by reducing the rate at which mites can feed. Reptilian epidermis consists of multiple keratinous layers, and mite pocket epidermis and dermis are significantly thicker than non-pocket equivalents. Although stylostomes are significantly longer in pockets than in non-pocket tissues, the thick epidermis of the pocket appears to limit the ability of the mite to penetrate much deeper than the *stratum germinativum*. As a result, much of the damage done to the host remains restricted to the epidermis and upper dermis. In contrast, stylostomes in non-pocket tissues were comparatively short but extended through the epidermis and superficial dermis, with the feeding cavity located deep in the host dermis. Furthermore,

assuming that pain receptors (nociceptors) in lizards are most abundant in the dermis and deep epidermis as opposed to the superficial epidermis (as is the case with mammals; Zylka et al. 2005; Le Bars et al. 2001; Lumpkin and Caterina 2007; Weiss 1988), then mite parasitism in pockets may decrease the amount of irritation and/or pain to the host. Alternatively, innervation and sensitivity of the integument varies greatly by body region (Zylka et al. 2005; Weiss 1988; Iggo and Andres 1982; Quilliam 1980); mite pockets may reduce mite-induced irritation and pain by possessing relatively few sensory neurons relative to non-pocket integument. Studies of pain perception have overwhelmingly focused on mammals, particularly humans and certain model laboratory organisms, while remarkably little is currently known for reptiles. While determining the amount of pain or irritation mites produce in a lizard may not be immediately feasible, even simple histological examinations of neuron type and position within the integument would be beneficial in further exploring these alternative mechanisms for damage-amelioration.

Chiggers appear to remain attached inside mite pockets far longer than they do on non-pocket tissues (Goldberg and Bursey 1993; Goldberg and Bursey 1991b), suggesting the thick, keratinous epidermis within the pocket may act to reduce the rate at which chiggers can successfully feed. The amount of host tissue damage produced by an individual chigger is proportional to the rate of engorgement (Cross 1962a, b); by slowing mite feeding rate, pockets may be limiting the rate at which damage is produced while simultaneously providing more time for the host immune system to mount a successful defense. Lastly, time spent attached to the host is expected to be potentially hazardous for the mite. Available attachment sites within pockets are finite, and as pockets fill mites are forced into suboptimal sites on the host (Chapter 1) where they can

be injured, abraded, or desiccated. By lengthening engorgement time, mite pockets may also indirectly increase mite mortality due to exposure or physical dislodgement; mites may also be removed during host ecdysis, particularly within pockets where mites are more superficially attached. Many of these possibilities could be explored through the use of experimental studies conducted on captive animals; for example, lizards could be cleaned of all ectoparasites then exposed to a certain number of chiggers. Afterwards hosts could be housed in isolation and mite loads checked daily to monitor attachment duration and mite survivorship relative to initial attachment site (similar to Goldberg and Bursey 1993; 1991b).

Although the function of mite pockets remains unclear, evidence suggests that these structures effectively concentrate the activities of ectoparasitic mites and that pocket tissue is significantly different from non-pocket tissue. Although pockets do not appear to directly prevent or rapidly repair damage as predicted by Arnold (1986), pockets may still yet possess an alternative ameliorative function beneficial to the host.

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References

Adams D.O. 1976. The granulomatous inflammatory response – a review. *American Journal of Pathology* 84(1): 153-192.

Adams D.O. 1983. The biology of the granuloma. *In* Harry Ioachim (ed.) Pathology of Granulomas. Raven Press, New York. Pp. 1-20.

Alibardi L. 2010. Ultrastructural features of the process of wound healing after tail and limb amputation in lizards. *Acta Zoologica* 91: 306-318.

Arnold E.N. 1986. Mite pockets of lizards, a possible means of reducing damage by ectoparasites. *Biological Journal of the Linnean Society* 29:1-21.

Arnold E.N. 1993. Comment – Function of the mite pockets of lizards: an assessment of a recent attempted test. *Canadian Journal of Zoology* 71(4): 862-864.

Ballinger R.E. 1973. Comparative demography of two ovoviviparous iguanid lizards (*Sceloporus poinsetti* and *Sceloporus jarrovi*). *Ecology* 54(2): 269-283.

Ballinger R.E. 1979. Intraspecific variation in demography and life history of the lizard, *Sceloporus jarrovi* along an altitudinal gradient in southeastern Arizona. *Ecology* 60(5): 901-909.

Bauer A.M., A.P. Russell, and N.R. Dollahon. 1990. Skin folds in the gekkonid lizard genus *Rhacodactylus*: A natural test of the damage limitation hypothesis of mite pocket function. *Canadian Journal of Zoology* 68(6):1196-1201.

Bauer A.M., A.P. Russell, and N.R. Dollahon. 1993. Function of the mite pockets of lizards: a reply to E.N. Arnold. *Canadian Journal of Zoology* 71(4): 865-868.

Bennett S.G. 1977. Ecology and systematics of trombiculid mites on lizards from southern Arizona. M.S. Thesis. California State University, Long Beach. Long Beach, California. 192 pp.

Bertrand M. and D. Modry. 2004. The role of mite pocket-like structures on *Agama caudospinosa* (Agamidae) infested by *Pterygosoma livingstonei* sp. n. (Acari: Prostigmata: Pterygosomatidae). *Folia Parasitologica* 51: 61-66.

Beuchat C.A. 1989. Patterns and frequency of activity in a high altitude population of the iguanid lizard, *Sceloporus jarrovi*. *Journal of Herpetology* 23(2): 152-158.

Bull C.M. and D. Burzacott. 1993. The impact of tick load on the fitness of their lizard hosts. *Oecologia* 96: 415-419.

Carpenter C.C. 1960. Parturition and behavior at birth of Yarrow's spiny lizard (*Sceloporus jarrovi*). *Herpetologica* 16(2): 137-138.

Cross H.F. 1962a. In vitro studies of tissue reaching in chicks resulting from the feeding by larvae of *Trombicula splendens*. *Journal of Economic Entomology* 55(1): 22-26.

Cross H.F. 1962b. Dimensions of the clear areas in the skin of chicks that resulted from the feeding by larvae of two strains of *Trombicula splendens* at different periods. *Journal of Economic Entomology* 55(1): 27-33.

Cunha-Barros M., M. van Sluys, D. Vricibradic, C.A.B. Galdino, F.H. Hatano, and C.F.D. Rocha. 2003. Patterns of infestation by chigger mites in four diurnal lizard species from a restinga habitat (Jurubatiba) of southeastern Brazil. *Brazilian Journal of Biology* 63(3): 393-399.

Foufopoulos J. 1999. Host-parasite interactions in the mountain spiny lizard *Sceloporus jarrovi*. Ph.D. Dissertation. University of Wisconsin – Madison. 215 pp.

Frost D.R. 1992. Phylogenetic analysis and taxonomy of the *Tropidurus* group of lizards (Iguania: Tropiduridae). *American Museum Novitates* 3033: 1-68.

Frost D.R., M.T. Rodrigues, T. Grant, and T.A. Titus. 2001. Phylogenetics of the lizard genus *Tropidurus* (Squamata: Tropiduridae: Tropidurinae): Direct optimization, descriptive efficiency, and sensitivity analysis of congruence between molecular data and morphology. *Molecular Phylogenetics and Evolution* 21(3):352-371.

Goldberg S.R., and C.R. Bursey. 1991a. Integumental lesions caused by ectoparasites in a wild population of the side-blotched lizard (*Uta stansburiana*). *Journal of Wildlife Diseases* 27(1): 68-73.

Goldberg S.R. and C.R. Bursey. 1991b. Duration of attachment by mites and ticks on the iguanid lizards *Sceloporus graciosus* and *Uta stansburiana*. *Journal of Wildlife Diseases* 27(4): 719-722.

Goldberg S.R. and C.R. Bursey. 1993. Duration of attachment of the chigger, *Eutrombicula lipovskyana* (Trombiculidae) in mite pockets of Yarrow's spiny lizard, *Sceloporus jarrovi* (Phrynosomatidae) from Arizona. *Journal of Wildlife Diseases* 29(1): 142-144.

Goldberg S.R., and H.J. Holshuh. 1992. Ectoparasite-induced lesions in mite pockets of the Yarrow's spiny lizard, *Sceloporus jarrovii* (Phrynosomatidae). *Journal of Wildlife Diseases* 28(4): 537-541.

Goldberg S.R., and H.J. Holshuh. 1993. Histopathology in a captive Yarrow's spiny lizard, *Sceloporus jarrovii* (Phrynosomatidae), attributed to the mite *Hirstiella* sp. (Pterygosomatidae). *Transactions of the American Microscopical Society* 112(3): 234-237.

Grover J.J., D.W. Duszynski, and B.C. Bogan. 1975. Histochemistry of the tissue capsule surrounding intradermal mites, *Hannemania* spp. (Acarina: Trombiculidae). *Journal of Parasitology* 61(2): 382-384.

Hase T., L.W. Roberts, P.K. Hildebrandt, and D.C. Cavanaugh. 1978. Stylostome formation by *Leptotrombidium* mites (Acari: Trombiculidae). *Journal of Parasitology* 64(4): 712-718.

Humason G.L. 1962. Animal Tissue Techniques. W.H. Freeman and Company. San Francisco, California.

Iggo A. and K.H. Andres. 1982. Morphology of cutaneous receptors. *Annual Review of Neuroscience* 5: 1-31.

Jones B.M. 1950. The penetration of the host tissue by the harvest mite, *Trombicula autumnalis* Shaw. *Parasitology* 40: 247-260.

Jones L.L.C. and R.E. Lovich. 2009. Lizards of the American Southwest: a Photographic Field Guide. Rio Nuevo Publishers. Tucson, Arizona.

Kiernan J.A. 1990. Histological and Histochemical Methods: Theory and Practice, 2nd Ed.. Pergamon Press, Inc. Oxford, England.

Klukowski M. 2004. Seasonal changes in abundance of host-seeking chiggers (Acari: Trombicuilidae) and infestations on fence lizards, *Sceloporus undulatus*. *Journal of Herpetology* 38(1): 141-144.

Klukowski M. and C.E. Nelson. 2001. Ectoparasite loads in free-ranging northern fence lizards, *Sceloporus undulatus hyacinthinus*: effects of testosterone and sex. *Behavioral Ecology and Sociobiology* 49: 289-295.

Le Bars D., M. Gozariu, and S.W. Cadden. 2001. Animal models of nociception. *Pharmacological Reviews* 53: 597-652.
Leenders T. 2001. A Guide to Amphibians and Reptiles of Costa Rica. Distribuidores Zona Tropical, S.A.: Miami, Florida. Pp. 184-185.
Loveridge A. 1925. A mite pocket in the gecko, *Gymnodactylus lawderanus* Stoliczka. *Proceedings of the Zoological Society of London* 1925: 1431.
Lumpkin E.A. and M.J. Caterina. 2007. Mechanisms of sensory transduction in the skin. *Nature* 445: 858-865.
Maderson P.F.A. and S.I. Roth. 1978. A histological study of the early stages of cutaneous wound healing in lizards *in vivo* and *in vitro*. *Journal of Experimental Zoology* 180: 175-186.
Main A.R. and C.M. Bull. 2000. The impact of tick parasites on the behaviour of the lizard *Tiliqua rugosa*. *Oecologia* 122: 574-581.
Mateo M.R., E.D. Roberts, and F.N. Enright. 1984. Inflammation induced by subcutaneous turpentine inoculation of young American alligators (*Alligator mississippiensis*). *American Journal of Veterinary Research* 45: 1870-1875.
Melvin R., C.L. Smith, O.H. Graham. 1943. Some observations on chiggers. *Journal of Economic Entomology* 36(6): 940.
Montanucci R.R. 1997. A safe procedure for mite eradication. *Herpetological Review* 28(1): 38-39.
Moritz C., H. McCallum, S. Donnellan, and J.D. Roberts. 1991. Parasite loads in parthenogenetic and sexual lizards (*Heteronotia binoei)*: support for the Red Queen hypothesis. *Proceedings of the Royal Society of London B* 244: 145-149.
Murray H.W. 1999. Granulomatous inflammation: host antimicrobial defense in the tisssues in visceral leishmaniasis. *In* J.I. Gallin and R. Snyderman (eds.) Inflammation: Basic Principles and Clinical Correlates, 3rd ed. Lippincott Williams and Wilkins, Philadelphia. Pp. 979-994.
Newell I.M. and R.E. Ryckman. 1964. Hirstiella pyriformis sp. n. (Acari, Pterygosomidae), a new parasite of lizards from Baja California. *Journal of Parasitology* 50(1): 163-171.
Quilliam T.A. 1980. Nerve fibers and free nerve endings in the epidermis of seven mammalian and one reptilian species. *In* R.I.C. Spearman and P.A. Riley (eds.) The Skin of Vertebrates. Dorset Press, Dorchester, England. Pp. 293-301.
Reardon J.T and G. Norbury. 2004. Ectoparasite and hemoparasite infection in a diverse temperate lizard assemblage at Macraes Flat, South Island, New Zealand. *Journal of Parasitology* 90(6): 1274-1278.
Ruby D.E. 1977. Winter activity in Yarrow's spiny lizard, *Sceloporus jarrovi*. *Herpetologica* 33(3): 322-333.
Ruby D.E. 1978. Seasonal changes in the territorial behavior of the iguanid lizard *Sceloporus jarrovi*. *Copeia* 1978(3): 430-438.
Ruby D.E. 1981. Phenotypic correlates of male reproductive success in the lizard, *Sceloporus jarrovi*. *In* R.D. Alexander and D.W. Tinkle (eds.) Natural Selection and Social Behavior. Chiron Press, New York. Pp. 97-107.
Ruby D.E. 1986. Selection of home range site by females of the lizard, *Sceloporus jarrovi*. *Journal of Herpetology* 20(3): 466-469.

Ruby D.E. and D.I. Baird. 1994. Intraspecific variation in behavior: Comparisons between populations at different altitudes of the lizard *Sceloporus jarrovi*. *Journal of Herpetology* 28(1): 70-78.
Salvador A., J.P. Veiga, and E. Civantos. 1999. Do skin pockets of lizards reduce the deleterious effects of ectoparasites? An experimental study with *Psammodromus algirus*. *Herpetologica* 55(1): 1-7.
Sasa M. 1961. Biology of chiggers. *Annual Review of Entomology* 6: 221-244.
Schall J.J., H.R. Prendeville, and K.A. Hanley. 2000. Prevalence of the tick, *Ixodes pacificus*, on western fence lizards, *Sceloporus occidentalis*: trends by gender, size, season, site, and mite infestation. *Journal of Herpetology* 34(1): 160-163.
Shatrov A.B. 2009. Stylostome formation in trombiculid mites (Acariformes: Trombiculidae). *Experimental and Applied Acarology* 49: 261-280.
Shatrov A.B. and A.A. Stekolnikov. 2011. Redescription of a human-infesting European trombiculid mite *Kepkatrombicula desaleri* (Acari: Trombiculidae) with data on its mouthparts and stylostome. *International Journal of Acarology* 37(1): 176-193.
Simon C.A. and G.A. Middendorf. 1976. Resource partitioning by an iguanid lizard: temporal and microhabitat aspects. *Ecology* 57(6): 1317-1320.
Smith D.A. and I.K. Barker. 1988. Healing of cutaneous wounds in the common garter snake (*Thamnophis sirtalis*). *Canadian Journal of Veterinary Research* 52: 111-119.
Smith D.A., I.K. Barker, and O.B. Allen. 1988. The effect of ambient temperature and type of wound on healing of cutaneous wounds in the common garter snake (*Thamnophis sirtalis*). *Canadian Journal of Veterinary Research* 52: 120-128.
Smith H.M. 1939. The Mexican and Central American lizards of the genus *Sceloporus*. *Field Museum of Natural History, Zoological Series* 26: 1-397.
Sorci G., M. Massot, and J. Clobert. 1994. Maternal parasite load increases sprint speed and philopatry in female offspring of the common lizard. *American Naturalist* 144(1): 153-164.
Spoecker P.D. 1967. Ectoparasites of a Mojave Desert population of the lizard *Uta stansburiana stejnegeri* Schmidt. *American Midland Naturalist* 77(2): 539-542.
SPSS Inc. 2011. SPSS version 20.0 for Windows. SPSS Inc., Chicago IL.
Stebbins R.C. 1985. Field Guide to Western Reptiles and Amphibians, 2nd ed. Houghton Mifflin Co. Boston, Mass.
Tucunduva M., P. Borelli, and J.R.M.C. Silva. 2001. Experimental study of induced inflammation in the Brazilian boa (*Boa constrictor constrictor*). *Journal of Comparative Pathology* 125: 174-181.
Walter D.E. and M. Shaw. 2002. First record of the mite *Hirstiella diolii* Baker (Prostigmata: Pterygosomatidae) from Australia, with a review of mties found on Australian lizards. *Australian Journal of Entomology* 41: 30-34.
Weiss L. 1988. Cell and Tissue Biology: a Textbook of Histology, 6th ed. Urban and Schwarzenberg, Baltimore.
Wharton G.W. and H.S. Fuller. 1952. A manual of the chiggers. *Memoirs of the Entomological Society of Washington* 4: 1-85.
Wikel S.K. 1982. Immune responses to arthropods and their products. *Annual Review of Entomology* 27: 21-48.
Wilkinson M. 1985. Mite refugia in lizards. *Unpublished manuscript*.

Williams E.E. 1965. The species of Hispaniolan green anoles (Sauria: Iguanidae). *Breviora* 227:1-16

Wrenn W.J. and R.B. Loomis. 1984. Host selectivity in the genus *Eutrombicula* (Acari: Trombiculidae). *In* D.A. Griffiths and C.E. Bowman (eds.), Acarology VI. Chichester, England: Ellis Horwood Limited. Pp. 160-165.

Wright S.M., S.K. Wikel, and W.J. Wrenn. 1988. Host immune responsiveness to the chigger, *Eutrombicula cinnabaris*. *Annals of Tropical Medicine and Parasitology* 82: 283-293.

Zahavi A. 1975. Mate selection – a selection for a handicap. *Journal of Theoretical Biology* 53: 205-214.

Zahavi A. 1977. The cost of honesty – further remarks on the handicap principle. *Journal of Theoretical Biology* 67: 603-605.

Zylka M.J., F.L. Rice, and D.J. Anderson. 2005. Topographically distinct epidermal nociceptive circuits revealed by axonal tracers targeted to *Mrgprd*. *Neuron* 45: 17-25.

Appendix 2.I: Summary of selected mite pocket hypotheses, sorted by function.

- *Nonfunctional:*
  - **Fortuitous Inhabitation** (Arnold 1986): Associations between mites and mite pockets are due to chance alone.
  - **Preservation Artifact** (Arnold 1986): Associations between mites and mite pockets are due to the unintentional detachment of mites outside mite pockets during preservation of lizards.
  - **Mite Inducement** (Wilkinson 1985): Mite pockets are induced by the feeding activity of parasitic mites.
  - **Phylogenetic Baggage** (Bauer et al. 1993; 1990): Mite pockets are the result of past adaptations or design parameters that have since lost utilitarian value.
  - **Spandrels of San Marco** (Bauer et al. 1993; 1990; Gould and Lewonton 1979): Mite pockets are the by-products of developmental processes involved in the development of skin folds.

- *Function unrelated to mites:*
  - **Physiological Function** (Arnold 1986): Mite pockets are involved in physiological functions such as water balance or the production of glandular secretions.
  - **Ecological Function** (Bauer et al. 1993; 1990): Mite pockets are utilized by the lizard for ecological functions such as crypsis, parachuting, defensive displays, or intraspecific identification.
  - **Bite Hold** (Reed, *unpublished*): Mite pockets serve as a bite hold for males during reproduction.

- *Function mite related:*
  - **Mutualistic Mites** (Arnold 1986): Mite pockets are inhabited by mites that form mutualistic associations with the lizard.
  - **Concentration/Impairment-Prevention** (Salvador et al. 1999): Pockets function to concentrate mites away from sensitive areas and prevent the impairment of vision, hearing, and motion.
  - **Concentration/Damage-Amelioration** (Arnold 1986; Chapter 2): Pockets serve to concentrate mites in specialized structures that quickly repair and contain damage caused by parasitic mites.
  - **Concentration/Handicap** (Zahavi 1977, 1975): Pockets serve to concentrate ectoparasites, which act as honest indicators of individual quality to conspecifics.
  - **Concealment – Mate Choice** (Reed, Chapter 3): Pockets serve to concentrate and conceal brightly colored mites from potential mates.
  - **Concealment – Defensive** (Reed, *unpublished*): Pockets serve to concentrate and conceal brightly colored mites to improve crypsis and avoid predation.
  - **Mite Removal** (Wilkinson 1985; Arnold 1986): Mite pockets concentrate harmful mites so they may later be removed or incapacitated.
  - **Biological Warfare** (Wilkinson 1985): Mite pockets may be used by lizard species resistant to parasitic mites to transport mites into the range of susceptible competitors, thereby giving the resistant species a competitive advantage.

Appendix 2.II: Specimens used for tissue collection for testing the damage-amelioration hypothesis. All whole specimens, tissue blocks, and microslides are housed at the University of Michigan Museum of Zoology (UMMZ), Ann Arbor.

Mites *in situ* collection (*Sceloporus jarrovi*, n=12): UMMZ 69894 (386), 69894 (387), 69894 (388), 69894 (389), 69894 (390), 69894 (393), 69894 (394), 69894 (395), 71118 (A), 71118 (C), 71119 (G), and 71119 (H).

Experimental field series (*Sceloporus jarrovi*, n=20) UMMZ 242006 (UMFS 14257) to UMMZ 242025 (UMFS 14276).

Tables

		Chiricahua sites		Pinaleno Sites	
		Barfoot	**South Fork**	**Soldier Creek**	**Cluff Dairy**
	N	119	92	101	27
Total Load	Mean	**43.311**$^{A, B, C}$	**92.424**$^{A, D, E}$	**195.921**$^{C, D}$	**173.778**$^{B, E}$
	Std. deviation	56.140	55.761	142.318	130.847
	Range	0-388	0-356	18-714	17-651
Nuchal	Mean	**37.219**^{F}	**71.652**^{F,G}	**43.496**^{G}	54.407
Pocket	Std. deviation	46.512	47.109	54.106	42.527
Load	Range	0-322	0-344	0-281	0-155
	NP Proportion	0.859	0.775	0.222	0.313

		Adults	**Juveniles**	**Males**	**Females**
	N	209	130	154	185
Total Load	Mean	**151.330**^{H}	**50.069**^{H}	129.597	98.265
	Std. deviation	120.601	44.097	142.579	85.436
	Range	0-714	0-221	0-714	0-458
Nuchal	Mean	53.469	43.907	51.662	48.251
Pocket	Std. deviation	56.223	39.306	49.779	51.301
Load	Range	0-334	0-207	0-281	0-334
	NP Proportion	0.353	0.876	0.398	0.491

A: -6.019, p<0.001
B: -6.656, p<0.001
C: -11.388, p<0.001
D: -4.864, p<0.001
E: -2.646, p=0.049
F: -6.186, p<0.001
G: 5.546, p<0.001
H: 9.244, p<0.001

Table 2.1: Descriptive statistics for mite loads observed on *S. jarrovi* in 2010 and 2011, separated by field site, age class, and sex. NP proportion refers to the mean proportion of total mite load occurring within the nuchal pockets. Subscripts refer to significant Mann-Witney pairwise comparisons between sites and age classes, with significant differences in **bold**. See text for details.

	Pocket Tissue				
Trait	N	Mean	Std. Deviation	Minimum	Maximum
Epidermis Thickness	45	**69.287**^{A}	20.214	36.45	120.19
Dermis Thickness	35	**196.438**^{B}	47.794	119.01	319.01
Stylostome Depth	27	**70.486**^{C}	27.667	22.97	113.43
Stylostome Width	29	100.933	16.677	65.89	129.76
Feeding Tube Width	27	**10.831**^{D}	2.283	7.09	15.70
Tissue Damage - Area (μm^2)	12	12089.580	2986.338	5342.65	16245.70
Tissue Damage - Perimeter	12	499.107	80.975	368.90	680.12

	Non-Pocket Tissue				
Trait	N	Mean	Std. Deviation	Minimum	Maximum
Epidermis Thickness	95	**22.718**^{A}	7.717	10.19	43.56
Dermis Thickness	80	**86.201**^{B}	22.931	43.11	129.00
Stylostome Depth	16	**57.068**^{C}	14.117	30.20	85.40
Stylostome Width	34	93.143	17.974	52.91	124.79
Feeding Tube Width	25	**8.88**^{D}	2.329	4.99	12.68
Tissue Damage - Area (μm^2)	11	12192.138	4423.219	5638.00	20579.57
Tissue Damage - Perimeter	11	515.116	99.129	371.10	632.04

A: 9.484, p<0.001
B: 8.467, p<0.001
C: 1.884 p=0.060
D: 2.573 p=0.010

Table 2.2: Descriptive statistics for histomorphometric measurements of pocket and non-pocket morphology, stylostome characteristics, and tissue reaction, divided by host tissue type. All measurements in units of micrometers unless otherwise noted. Superscripts refer to significant Mann-Witney pairwise comparisons, with significant differences in **bold**. See text for details.

Figures

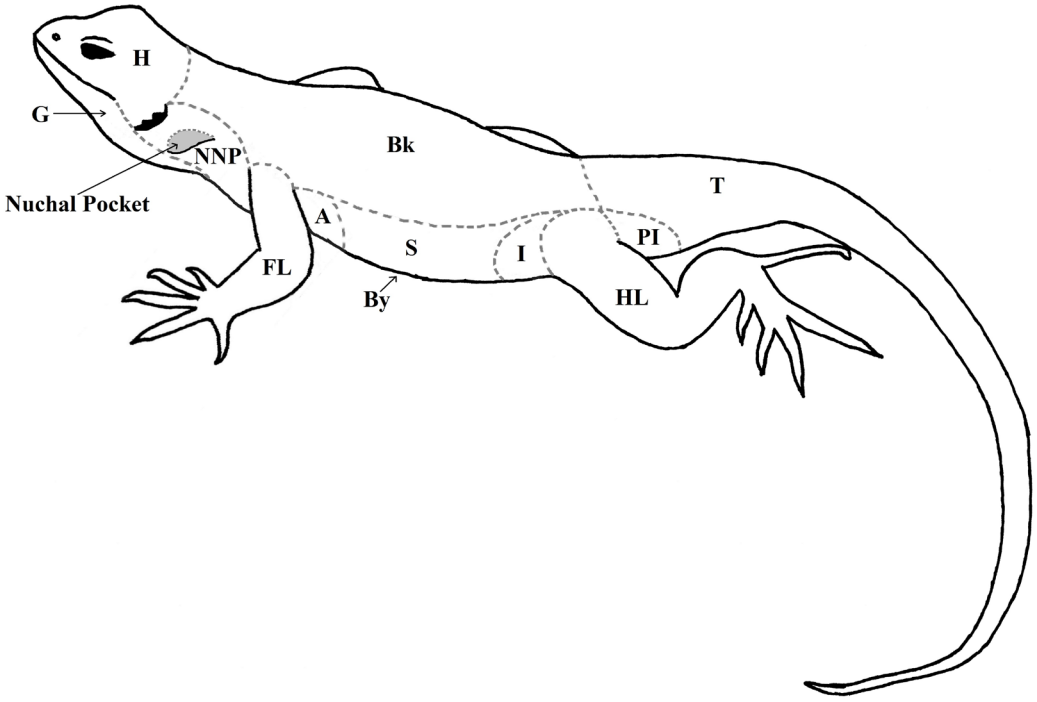

Figure 2.1: Division of lizard host into body regions for classification of mite attachment sites. Abbreviations: A – axial; Bk – back; By – belly; FL – forelimb; G – gular; H – head; HL – hindlimb; I – inguinal; NNP – nuchal non-pocket; PI – post-inguinal; S – side; T – tail. In all *Sceloporus* the nuchal pocket occupies the central nuchal region roughly midway between ear and shoulder (grey).

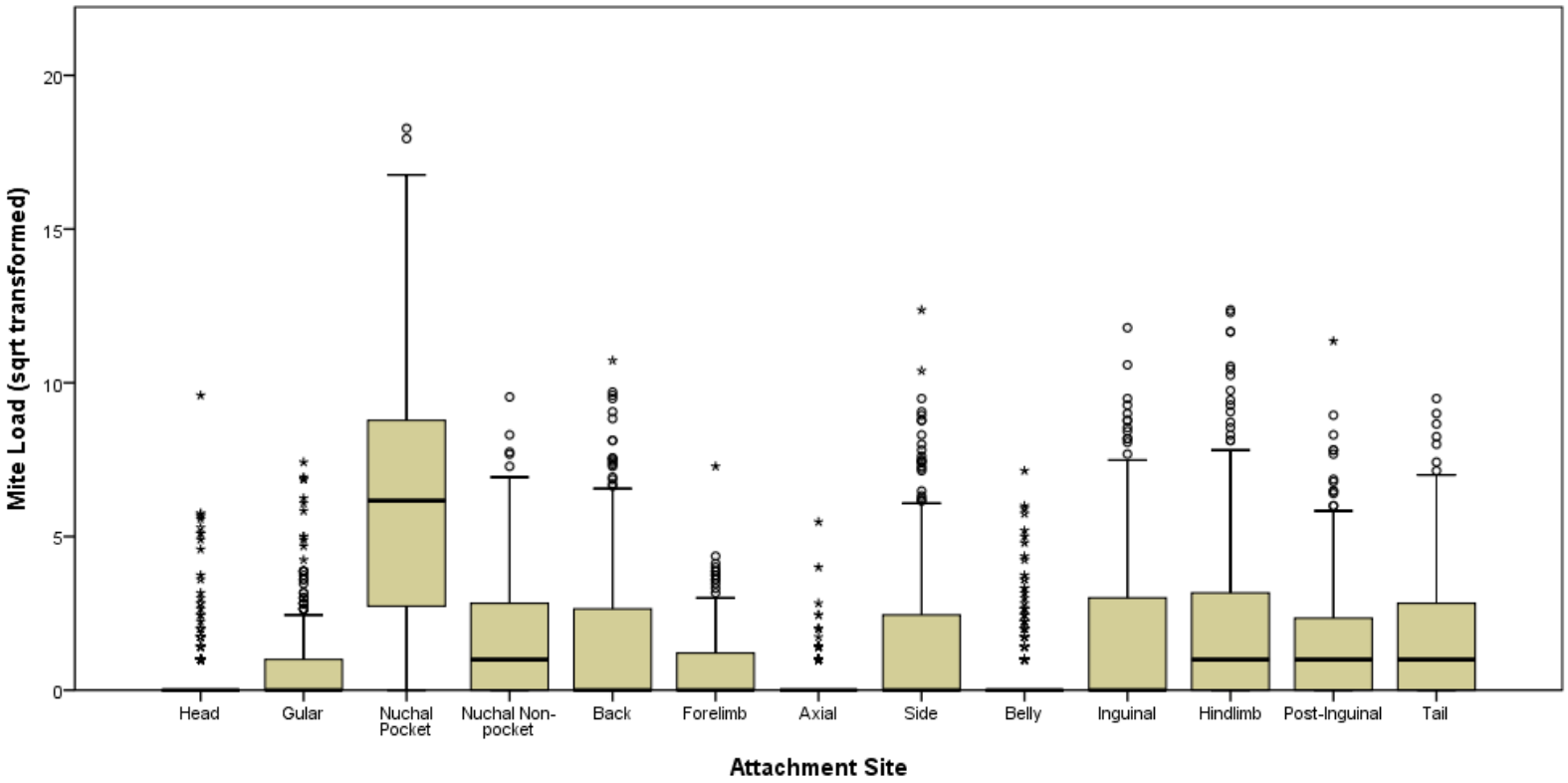

Figure 2.2: Distribution of chigger mite loads (square root transformed) on the body of *Sceloporus jarrovi* (n=339) collected from southeastern Arizona, separated by body region and pooled across years, study sites, age classes, and sexes. Although the bulk of the mite load occurred within the nuchal mite pocket, variation in mite loads between individuals was considerable, as evidenced by the number of outliers observed.

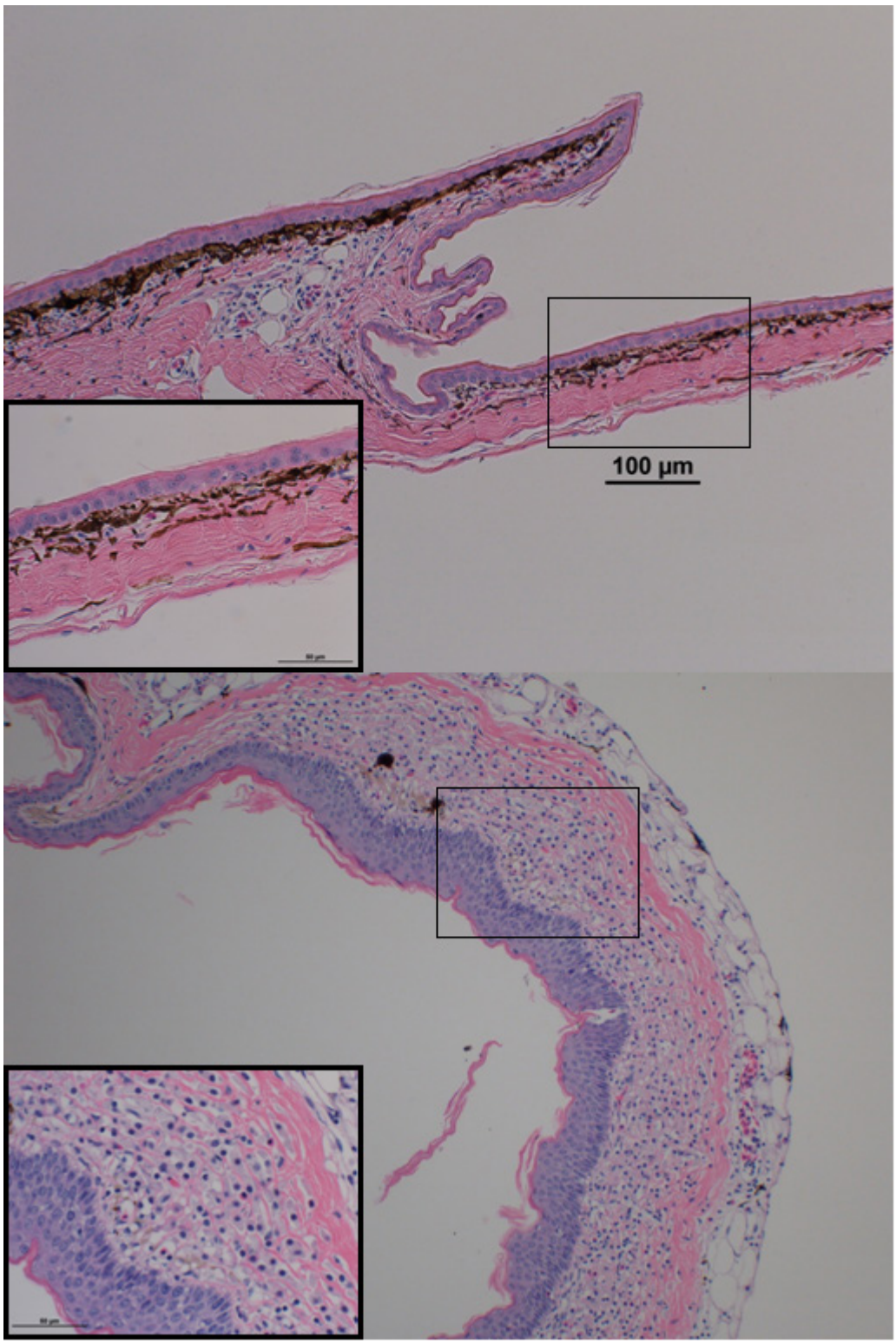

Figure 2.3: Representative undamaged non-pocket tissue (UMFS 14265 tail, **top**) and mite pocket tissue (UMFS 14267, **bottom**), with detail in inset. In particular, note the difference in thickness of the epidermis and dermis and the increased vascularization in pocket tissue. H-E, 10x with 40x inset.

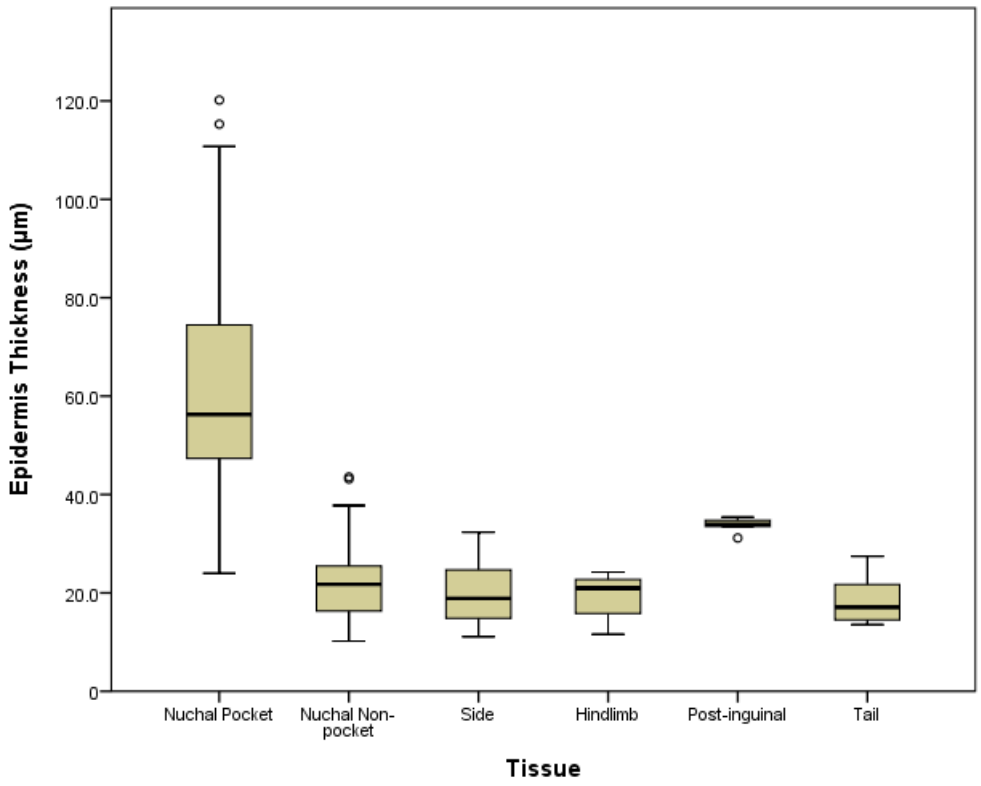

Figure 2.4A: Thickness of epidermis in pocket and non-pocket tissues. Pocket epidermis (n=45) is significantly thicker than non-pocket epidermis (n=95) (Mann-Witney $U=9.484$, $p<0.001$).

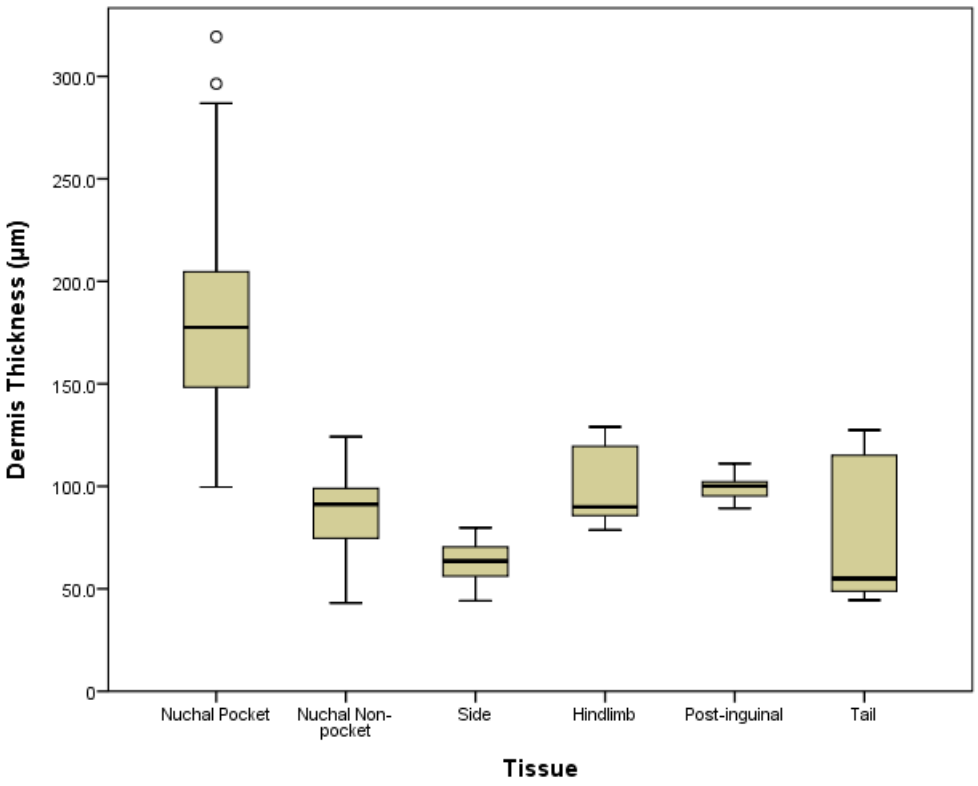

Figure 2.4B: Thickness of dermis in pocket and non-pocket tissues. Pocket dermis (n=35) is significantly thicker than non-pocket dermis (n=80) (Mann-Withney U=8.484, $p<0.001$).

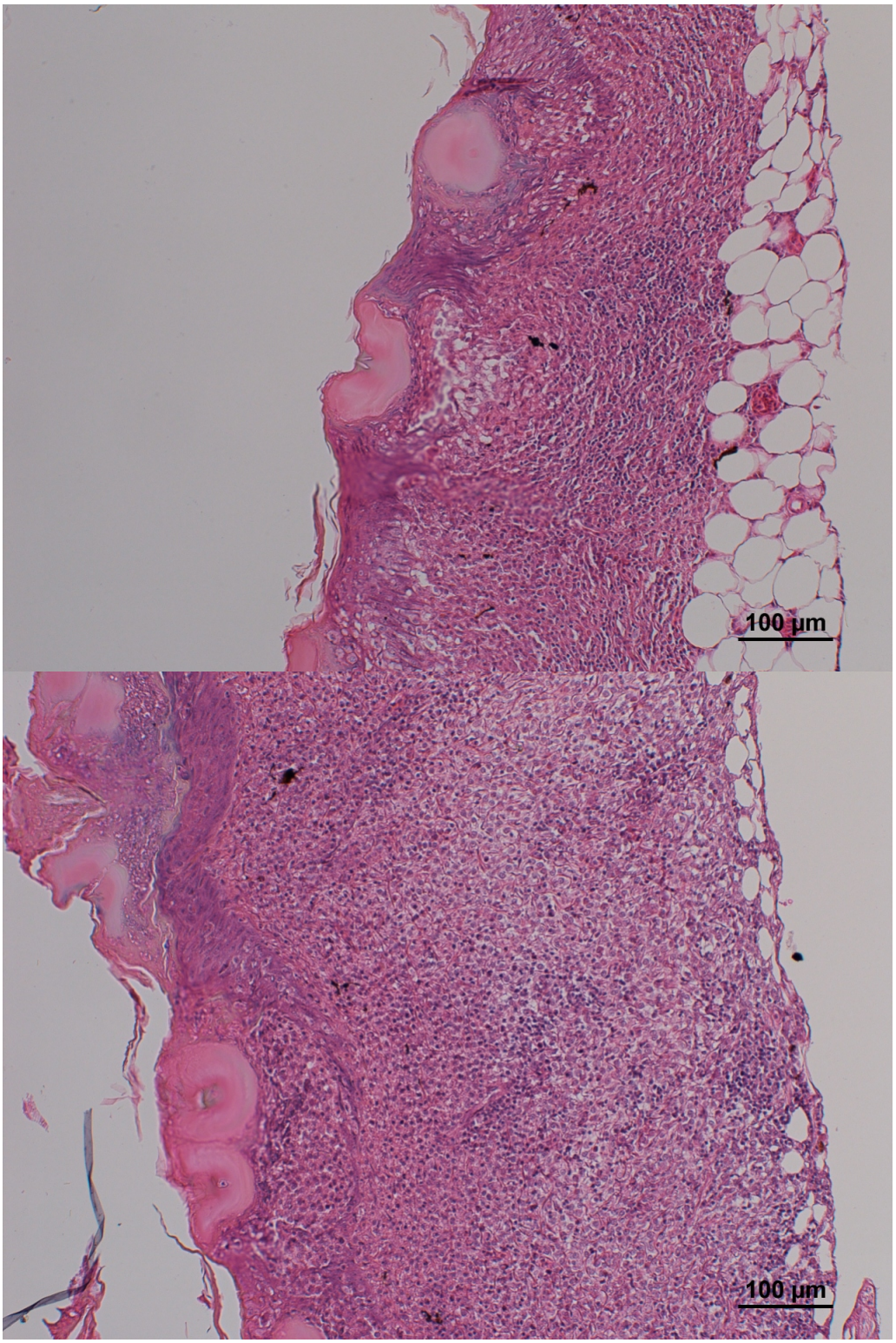

Figure 2.5: Mite pocket tissues collected with mites initially *in situ*. **Top:** UMMZ 69894 (387); **Bottom:** UMMZ 69894 (386). Stylostomes similar to those in non-pockets, but with tissue damage restricted to the superficial dermis. Note old stylostomes and re-epithelized epidermis in bottom, upper left, indicative of prior mite parasitism. H-E 10x.

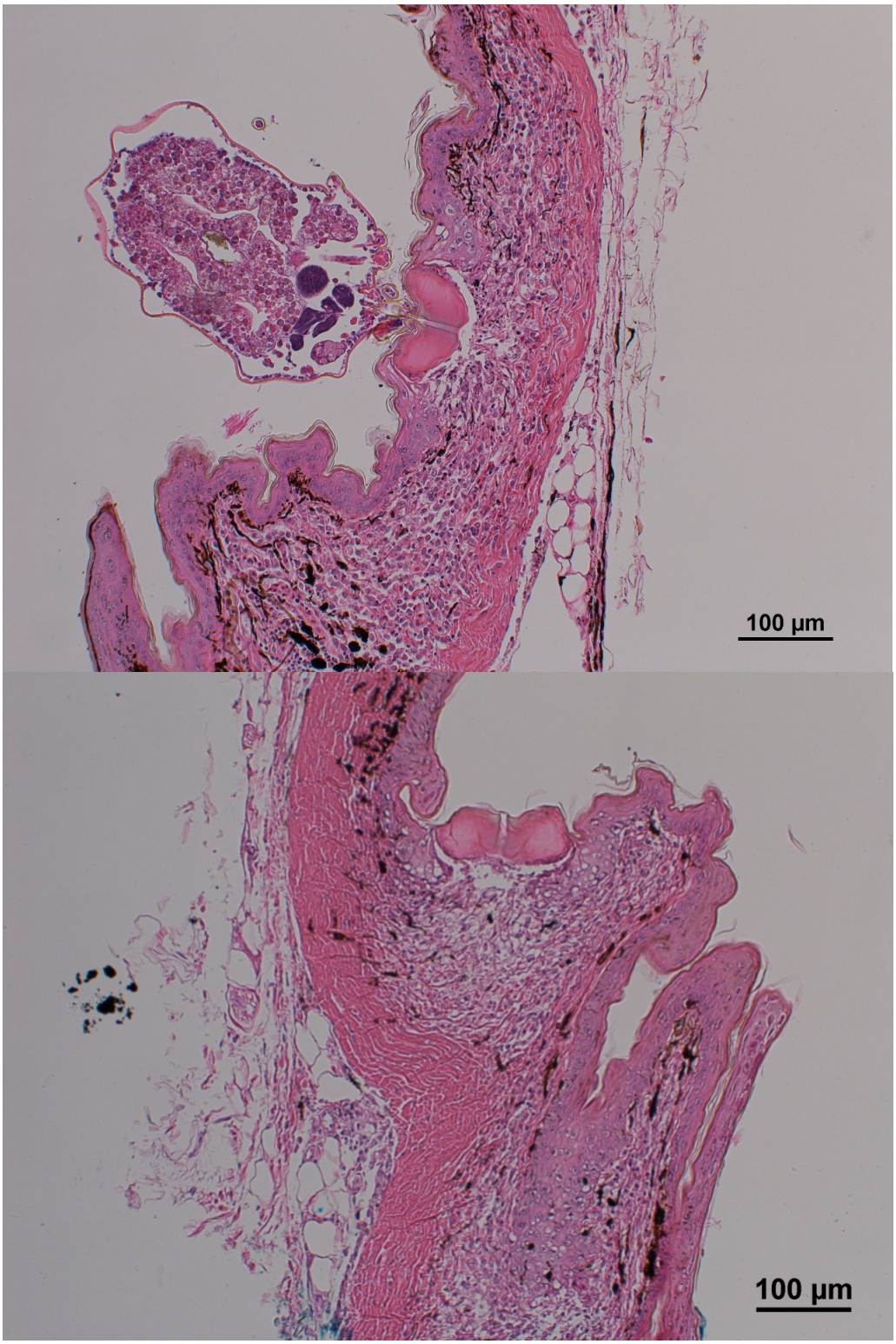

Figure 2.6: Non-pocket tissues collected with mites initially *in situ*. **Top:** Nuchal non-pocket tissue with mite still attached (UMMZ 69894 (395)). **Bottom:** Post-inguinal tissue with mite formerly attached beneath the scale. Note stylostomes, feeding tubes, and deep tissue disruption beneath attachment sites **(**UMMZ 69894(395)). H-E 10x.

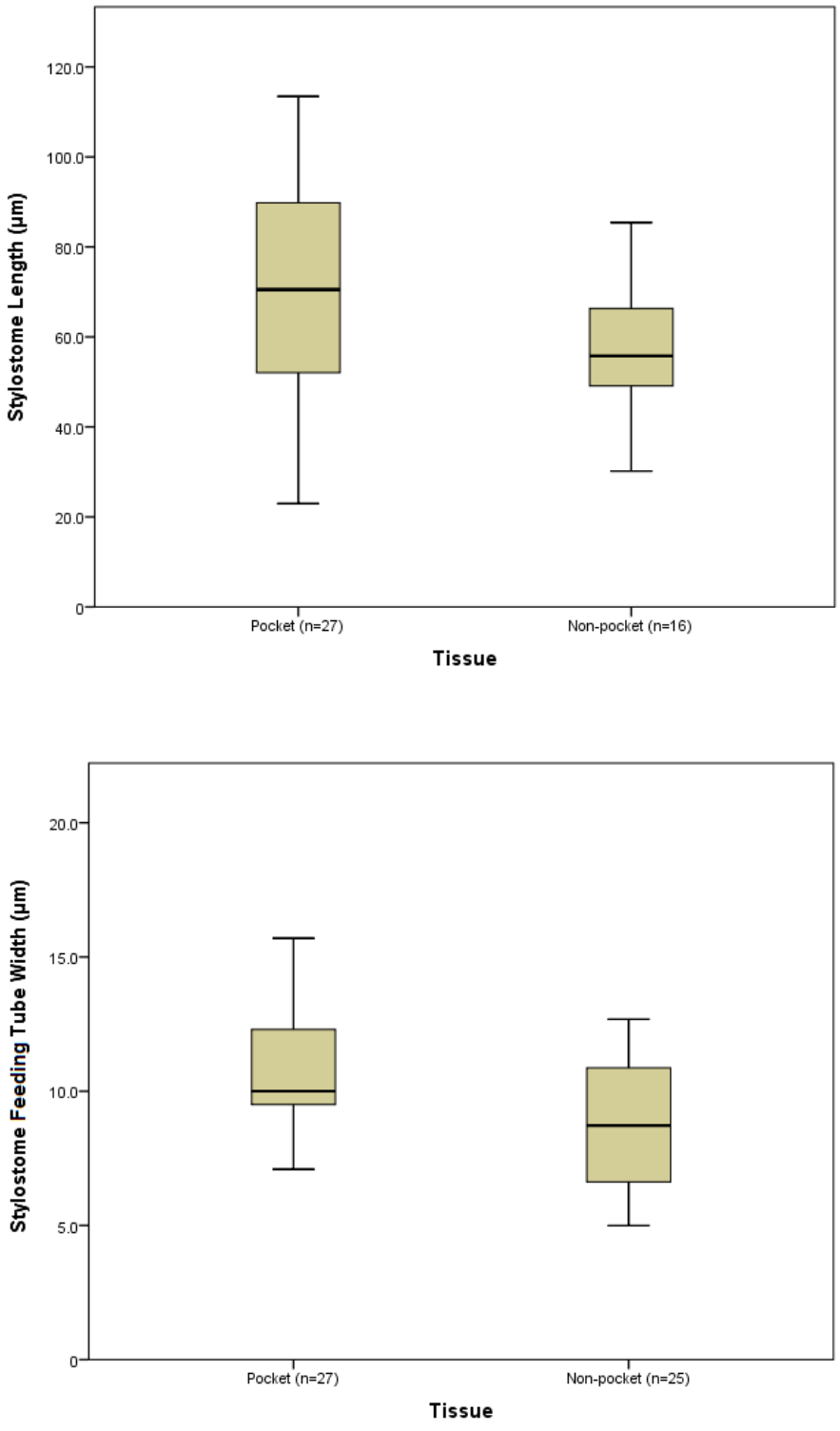

Figure 2.7: Effect of attachment site on stylostome development. **Top:** Stylostomes are nearly significantly longer in pocket than non-pocket tissues (Mann-Whitney U=1.884, p=0.060). **Bottom:** Feeding tubes are significantly wider in pockets (U=2.573, p=0.010).

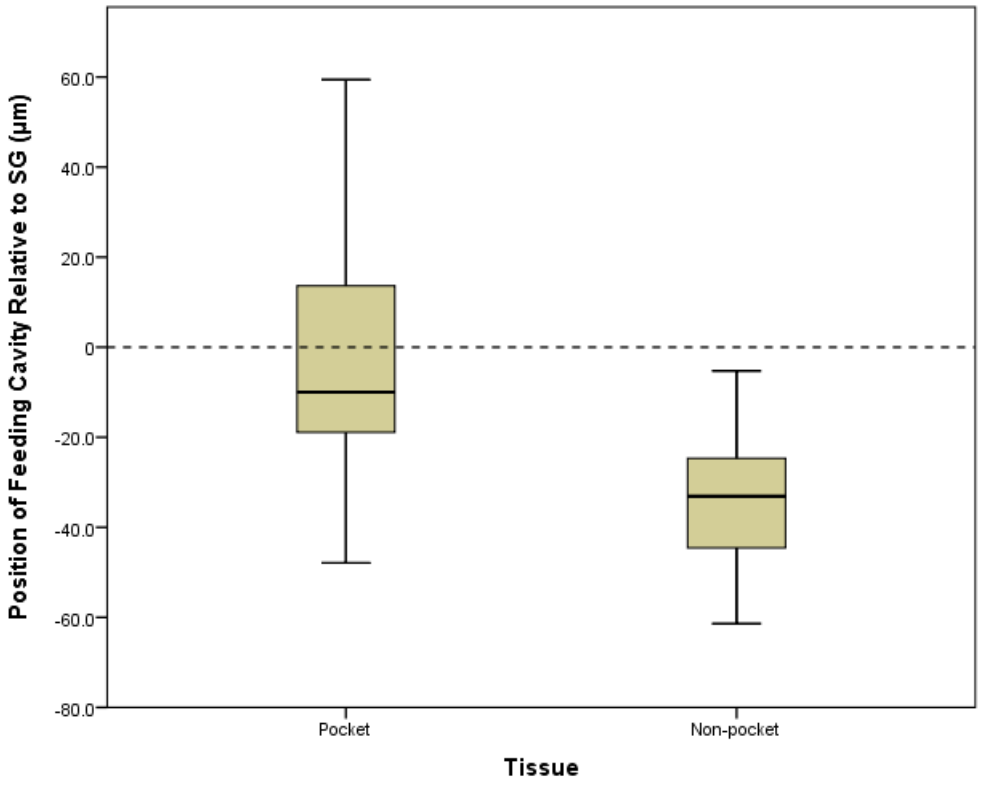

Figure 2.8: Position of the mite feeding cavity within host tissues, relative to the *stratum germinativum* (SG, dotted line). Positive numbers refer to a feeding cavity within the epidermis, above the SG; negative numbers refer to a feeding cavity within the dermis. The difference in feeding cavity position is significantly different between pocket (n=27) and non-pocket tissues (n=16) (ANOVA $F=17.763$, $p<0.001$).

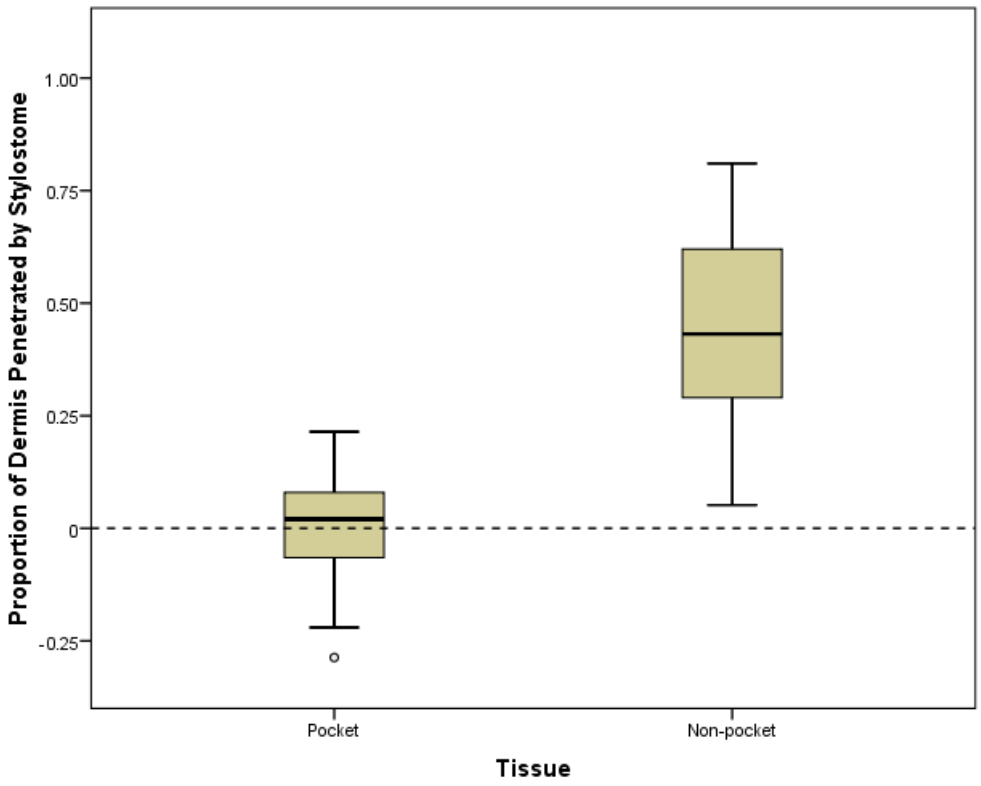

Figure 2.9: Proportion of the total host dermis penetrated by the stylostome, separated by tissue type. Location of *stratum germinativum* represented by dotted line. Negative values represent stylostomes which remained within the epidermis. Dermal penetration was significantly deeper in non-pocket tissues (n=15) relative to pocket tissues (n=27) (ANOVA $F=68.242$, $p<0.001$).

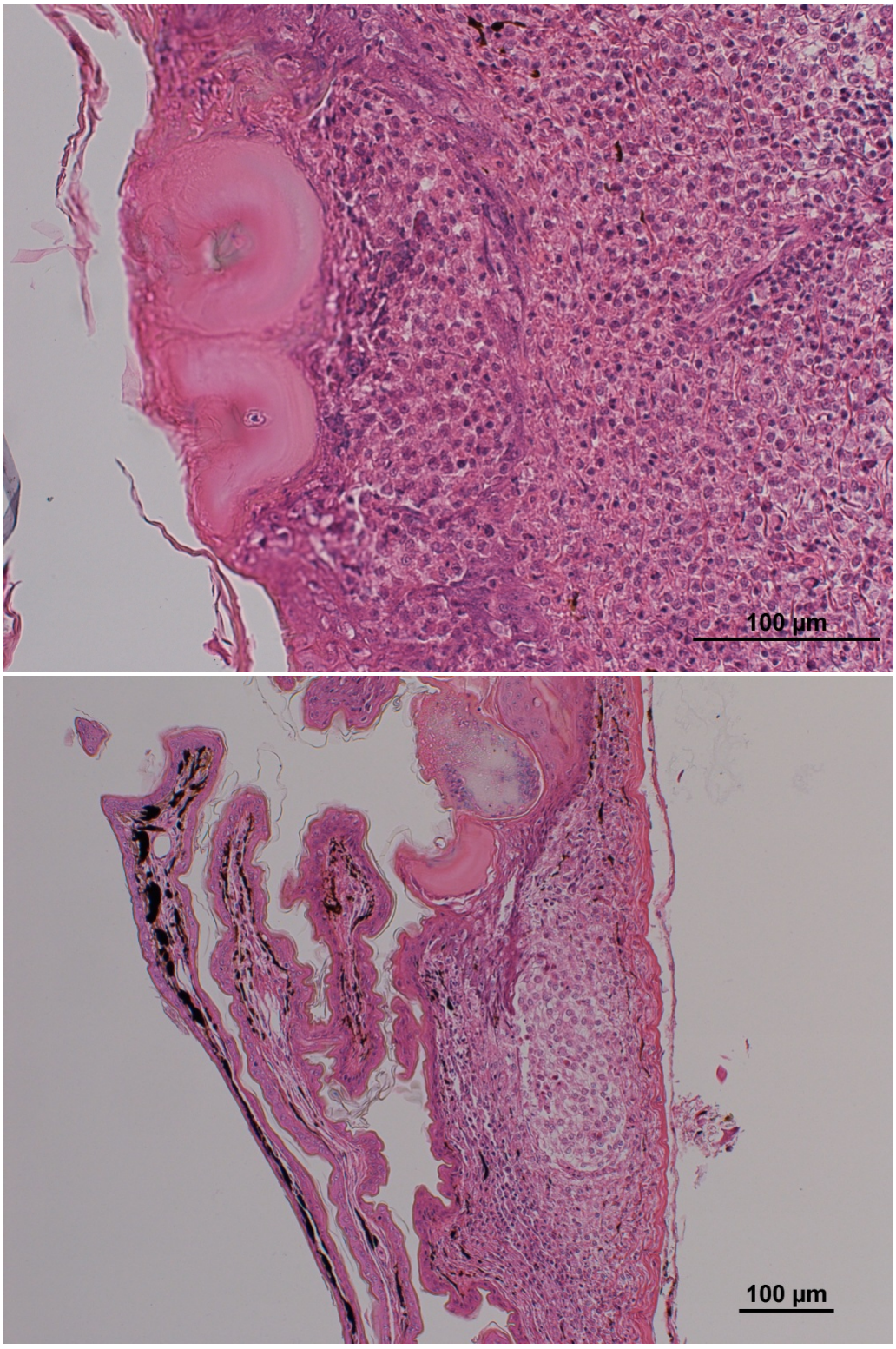

Figure 2.10: Granulomas in tissue collected with mites *in situ*. **Top:** Nuchal pocket tissue granuloma with epithelial extensions (UMMZ 69894 (386), 20x). **Bottom:** Side tissue granuloma composed of macrophages with foamy cytoplasm; also note re-epithelization beneath old stylostome near top (UMMZ 69894 (394), 10x). H-E.

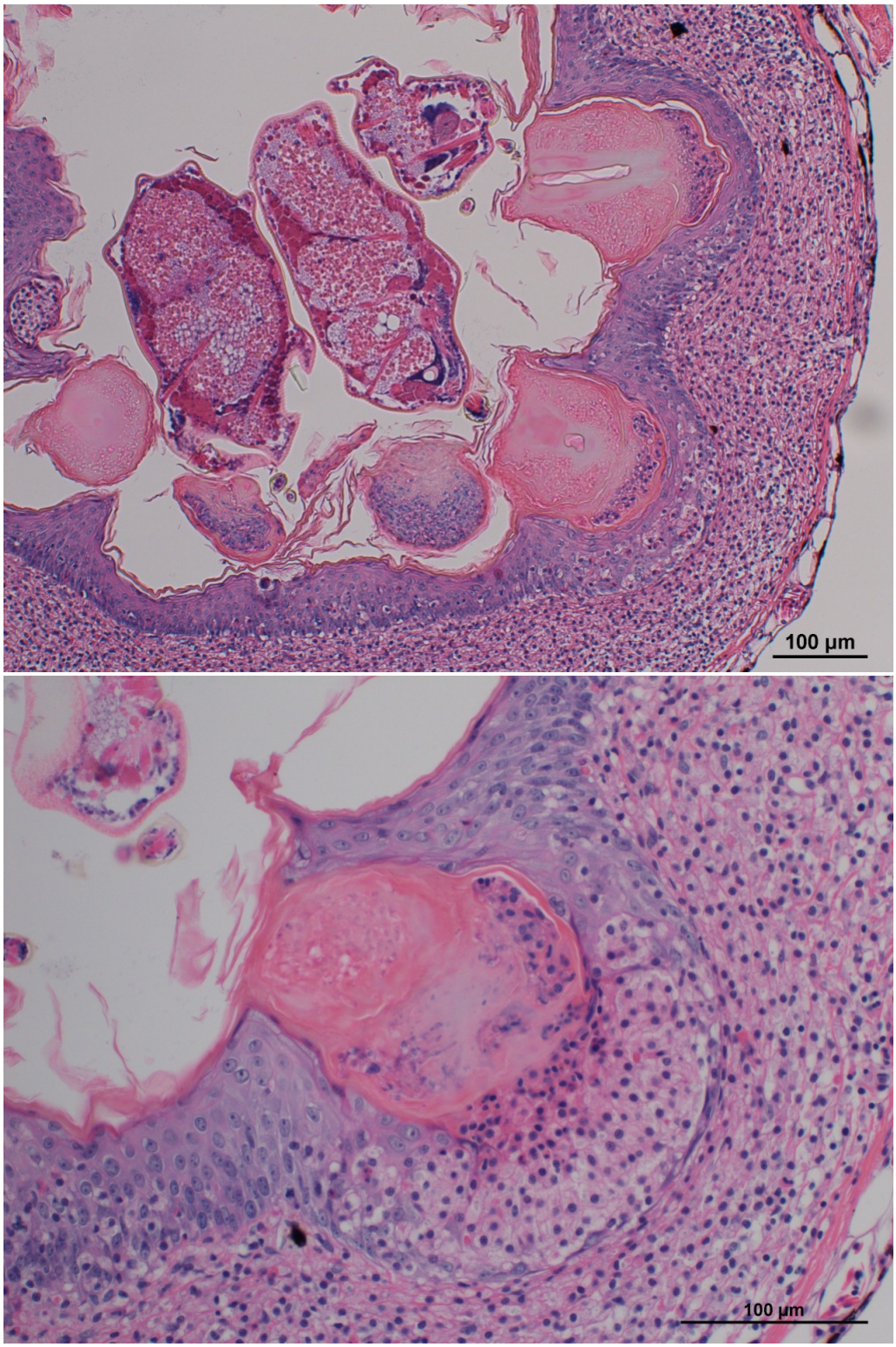

Figure 2.11: Mite pocket tissue recovery, eight days after mites were killed (both UMFS 14258). **Top:** Stylostomes still attached but invaded by heterophils, with epidermal hyperplasia and initial reforming of epidermis beneath mite attachment (10x). **Bottom:** Inflammation around site of attachment and granulomas also present (20x). H-E.

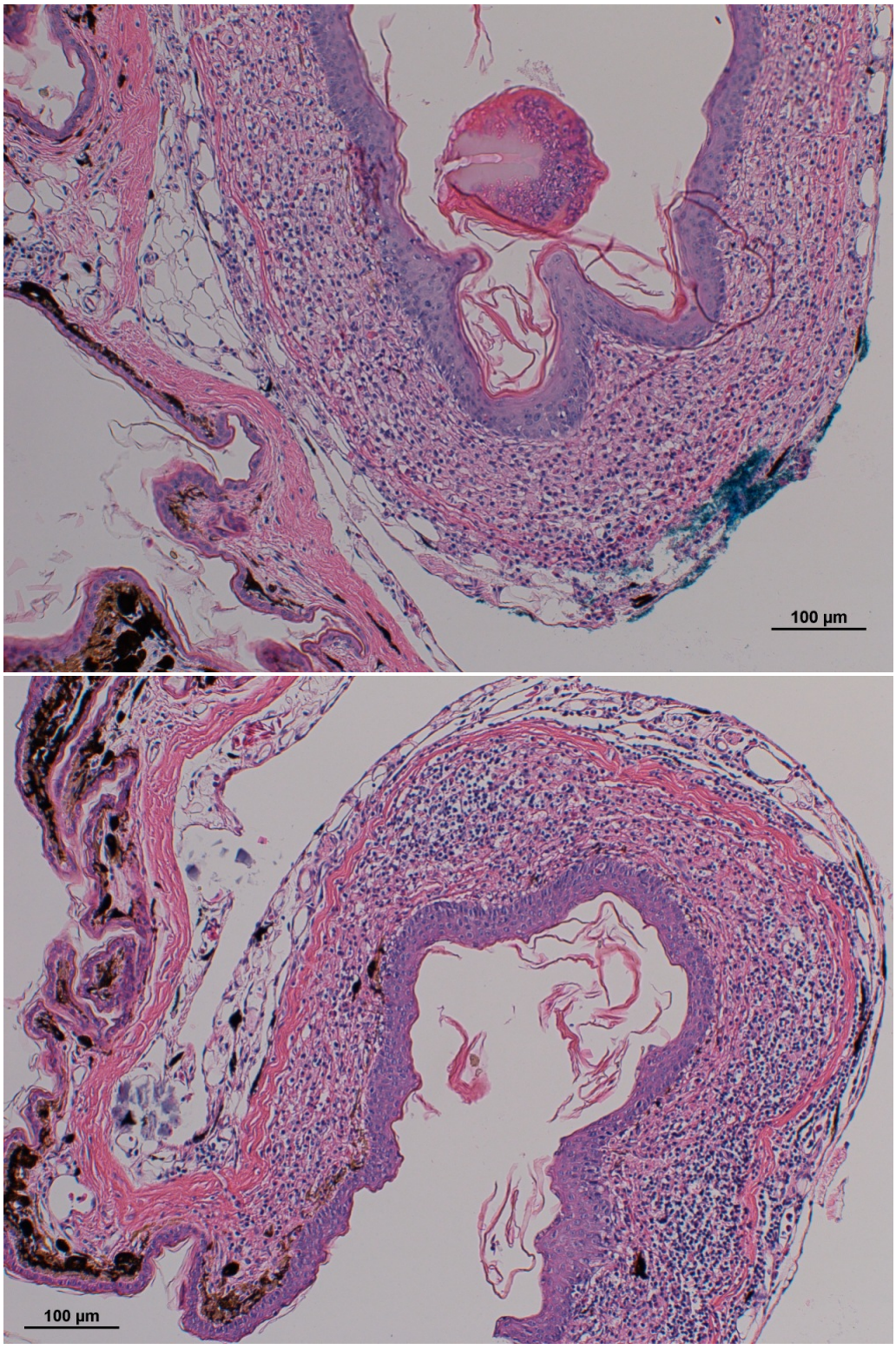

Figure 2.12: Mite pocket tissue recovery, two weeks after mites were killed. **Top:** Note stylostomes detached but prior attachment sites still evident **(**UMFS 14271). **Bottom:** Inflammation is variable and granulomas are absent (UMFS 14257). H-E 10x.

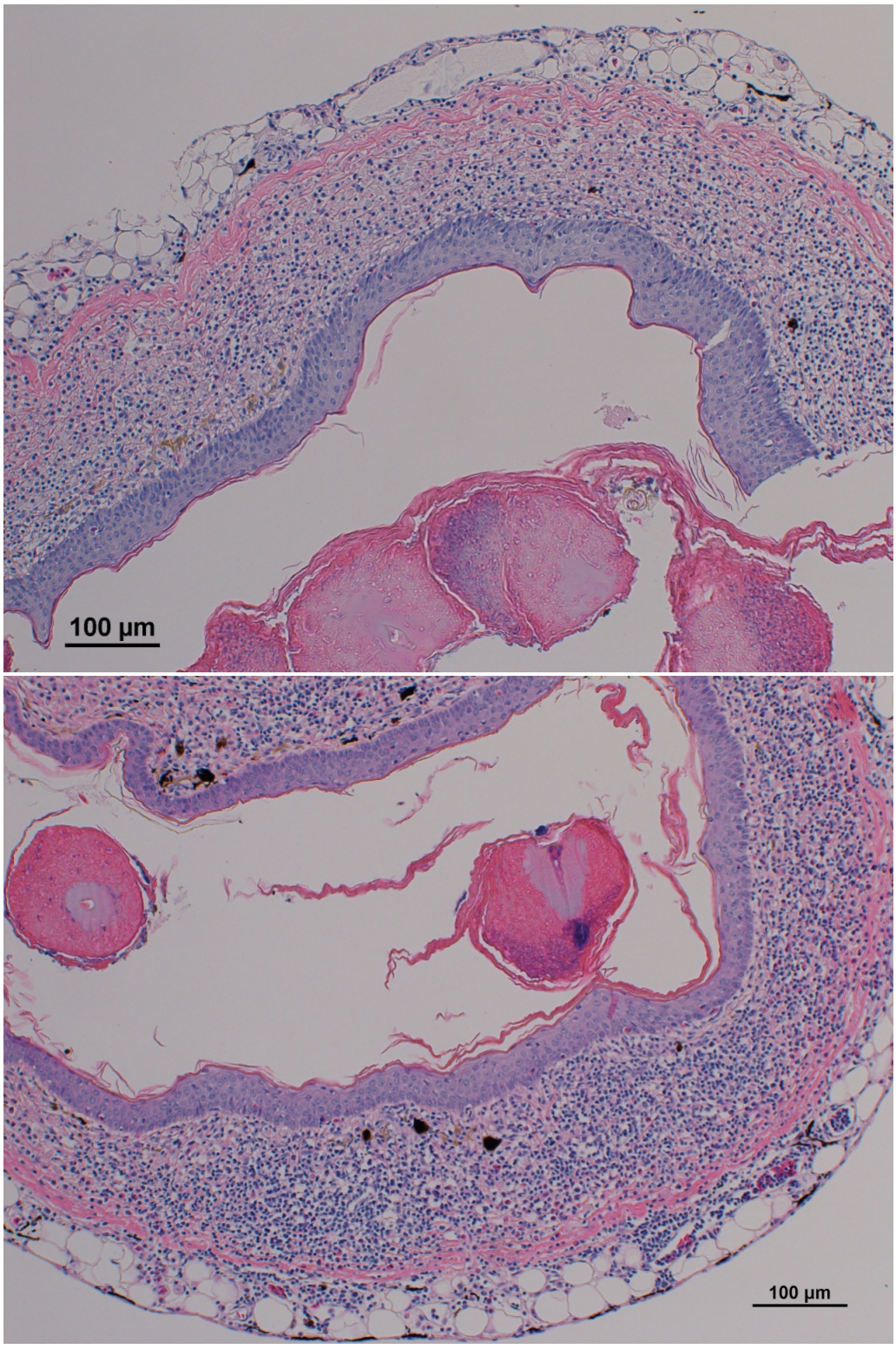

Figure 2.13: Mite pocket tissue recovery, three weeks after treatment. Stylostomes have detached and prior attachment sites are now less clear while inflammation remains variable between samples **Top:** UMFS 14267. **Bottom:** UMFS 14260. H-E 10x.

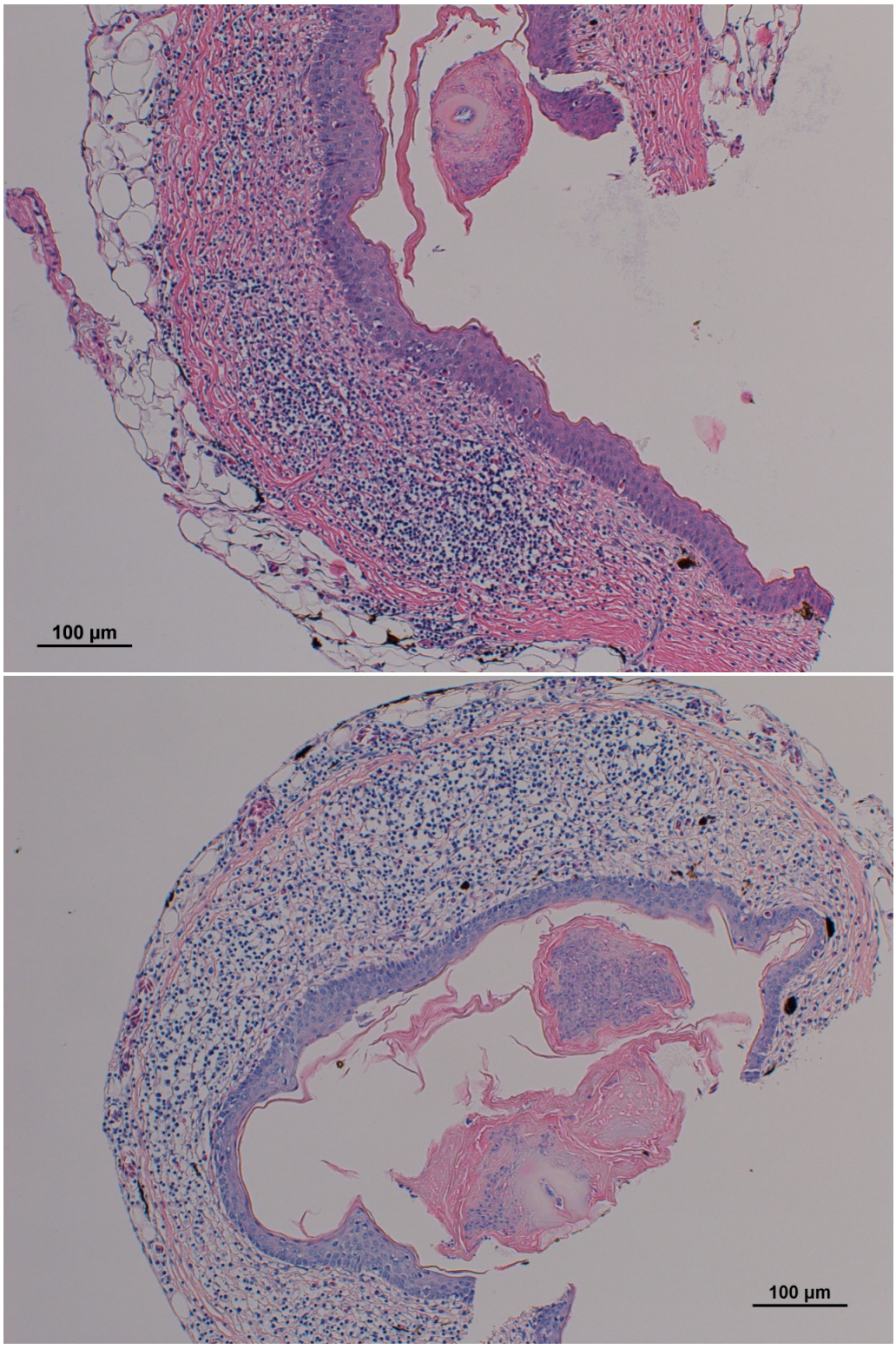

Figure 2.14: Mite pocket tissue recovery at four weeks. Stylostomes have been sloughed and epidermis largely normal; inflammation remains locally variable **Top:** UMFS 14259. **Bottom:** UMFS 14262. H-E 10x.

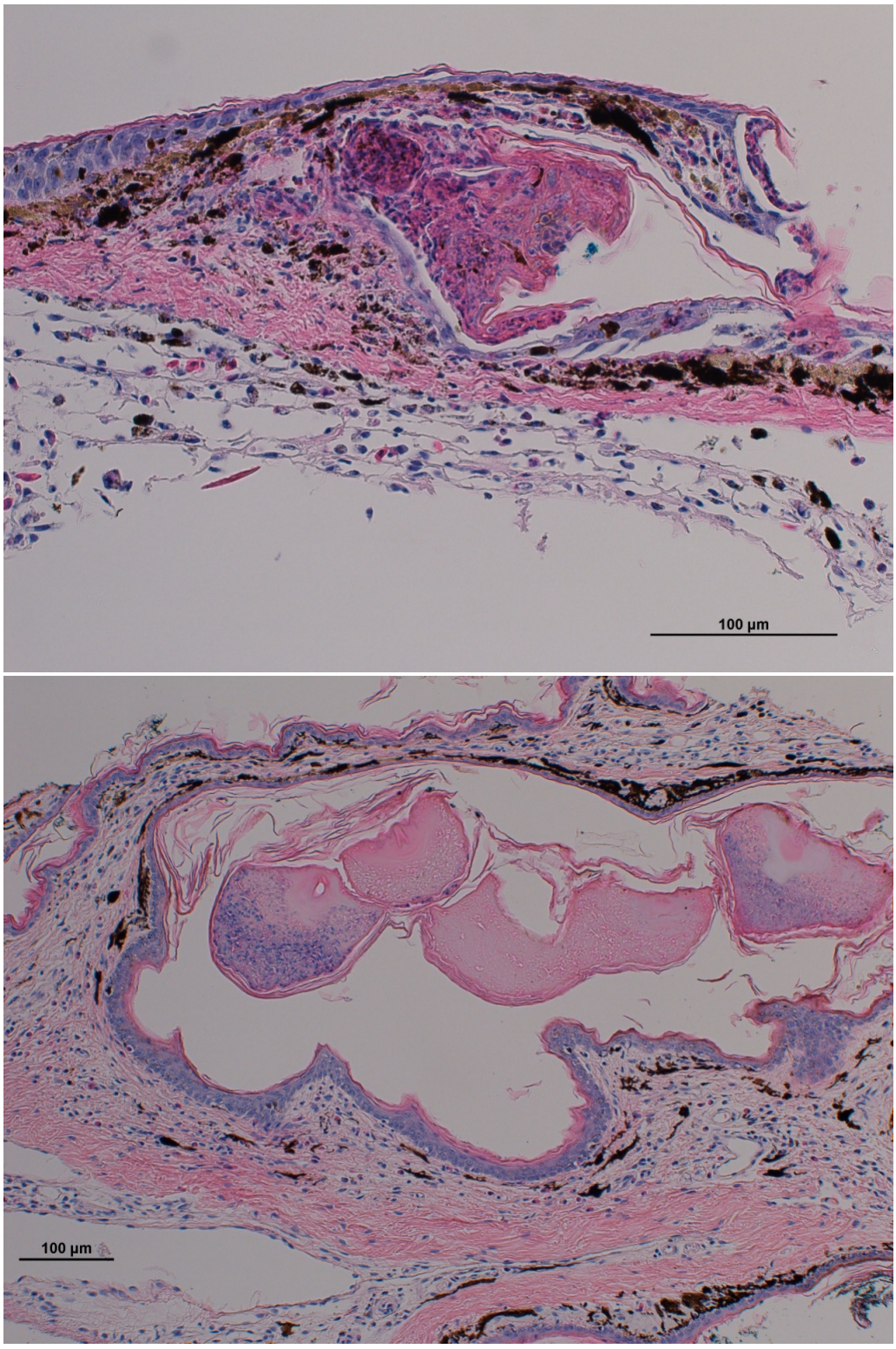

Figure 2.15: Non-pocket tissue recovery. **Top:** Forelimb (UMFS 14267, 20x), three weeks post-removal; compare to Figure 2.3. **Bottom:** Nuchal non-pocket tissue (UMFS 14264, 10x), three weeks post treatment; attachment sites are still visible in the epidermis, otherwise tissue is largely normal. H-E.

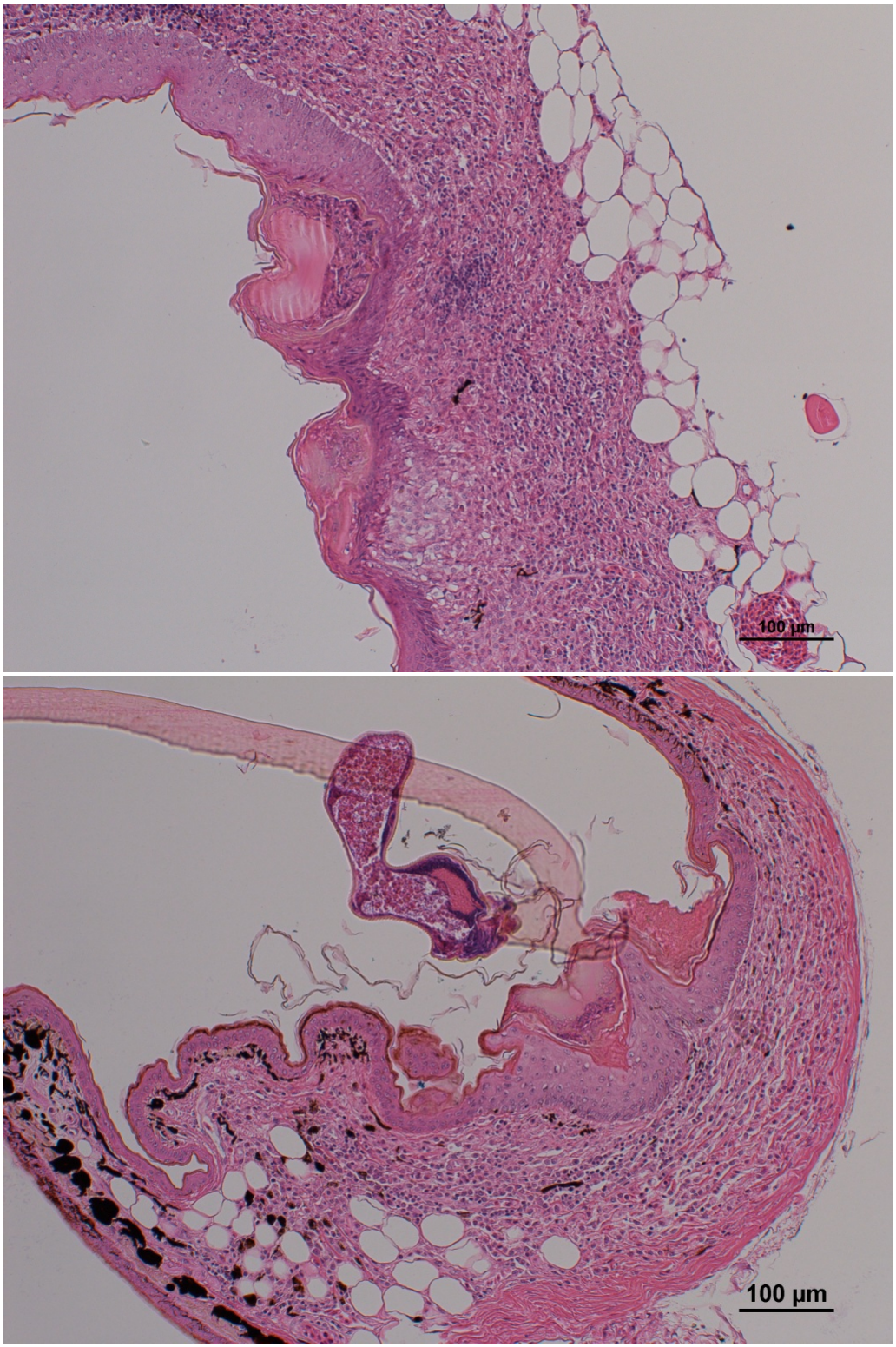

Figure 2.16: Recovery in museum specimens with unknown parasite histories follows the same sequence as that observed in the experimental group. **Top:** Nuchal pocket (UMMZ 69894 (387). **Bottom:** Nuchal non-pocket tissue (UMMZ 71118C). H-E, 10x.

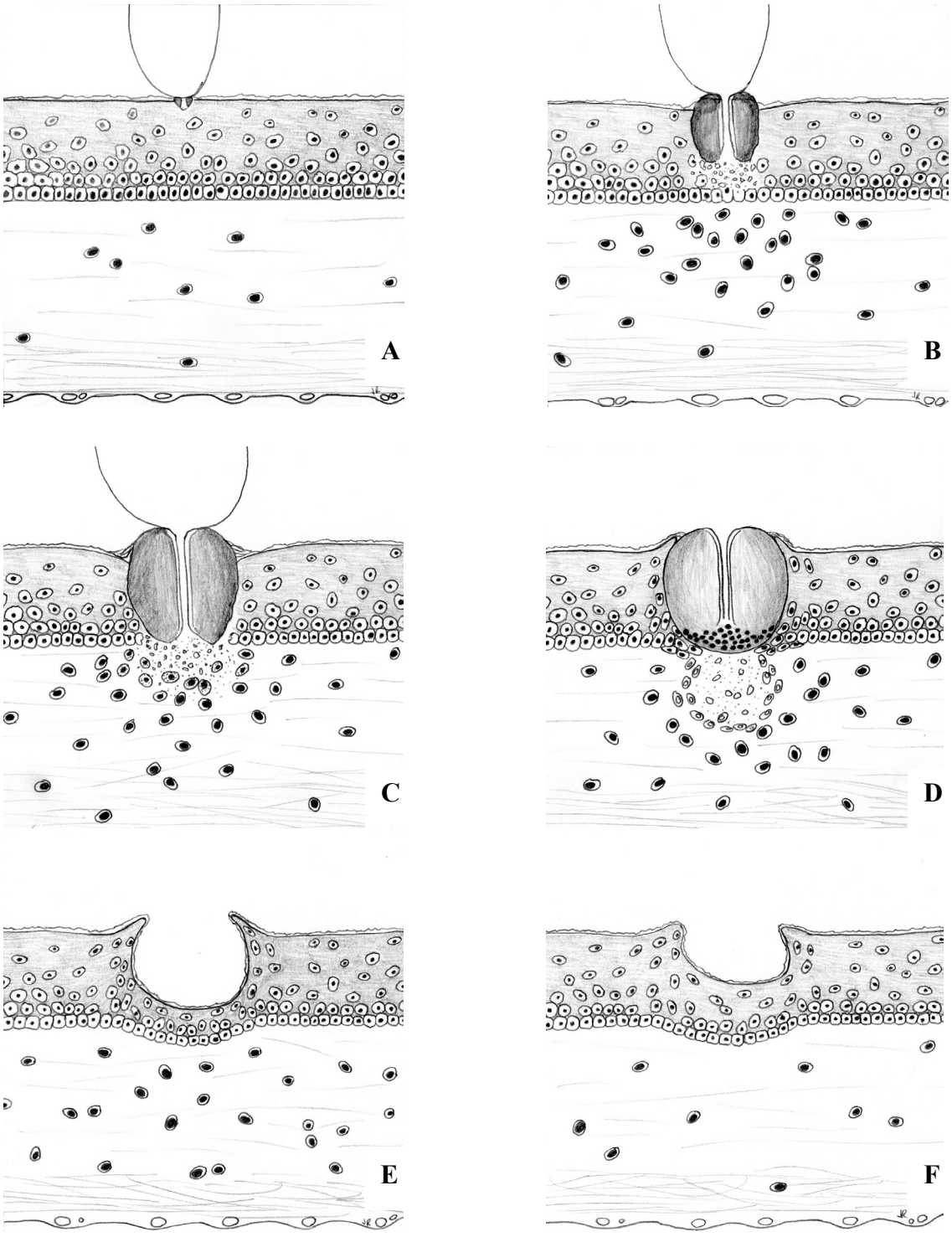

Figure 2.17A-F: Diagrammatic overview of chigger parasitism and host tissue response. Mite attaches and secretes eosinophilic cone (**a**); stylostome develops and host immune response (**b**); epithelial stylostome complete, focal host response (**c**); mite detaches, stylostome invaded, and possible granuloma formation (**d**); stylostome sloughed, re-epithelialization, subsiding inflammatory response, redevelopment of epidermis (**e, f**).

Chapter 3

Do Ectoparasites affect Female Mate Choice in Lizards?
An Experimental Test of the Mate Choice Hypothesis for Mite Pocket Function using *Sceloporus jarrovi* (Phrynosomatidae)

Introduction

The mite pockets of lizards are small integumental structures which are known to occur in over 200 species from twelve families, including the Agamidae (Bertrand and Modry 2004), Chamaeleonidae (Arnold 1986), Gekkonidae (Bauer et al. 1990; Arnold 1986; Loveridge 1925), Lacertidae (Salvador et al. 1999), Phrynosomatidae (Arnold 1986; Smith 1939), Opluridae (pers. obs.), Polychrotidae (Leenders 2001; Williams 1965), and Tropiduridae (Frost et al. 2001; Frost 1992). Characterized by thick, well-vascularized skin with no associated musculature (Arnold 1986; Wilkinson 1985), mite pockets are typically located in the nuchal, axial or inguinal regions in close association with nearby dermal folds. Mite pockets are frequently inhabited by ectoparasitic mites, most commonly chiggers (Prostigmata: Trombiculidae and Leeuwenhoekiidae) (Goldberg and Bursey 1993; Goldberg and Holshuh 1992; Arnold 1986; Wilkinson 1985; Bennett 1977; Chapter 1), although scale mites (Prostigmata: Pterygosomatidae) (Bertand

and Modry 2004) and ticks (Ixodida) (Schall et al. 2000; Salvador et al. 1999) may also occasionally occur. All inhabitants of mite pockets are parasitic, and mites appear to preferentially attach and feed within these structures when possible (Klukowski 2004; Cunha-Barros et al. 2003; Salvador et al. 1999; Arnold 1986; Bennett 1977; Chapter 1). While the reasons for such site-specificity remain poorly understood, mite pockets likely offer ectoparasitic mites ideal attachment sites and protection from exposure and physical dislodgement (Cunha-Barros et al. 2003; Salvador et al. 1999).

The possible benefits that hosts receive from mite pockets and their associated ectoparasites are less clear. Pockets are present at birth and are not induced by the feeding activities of the mites (Goldberg and Holshuh 1992; Bauer et al. 1990; Arnold 1986; pers. obs.). Mite pockets appear to have evolved independently in multiple lineages and are often associated with certain host morphologies and ecologies (Arnold 1986; Chapter 4). Pockets appear most common in species occurring in open canopied habitats at semi-tropical and tropical latitudes; terrestrial species tend to possess pockets more frequently than arboreal species, although exceptions are known (Arnold 1993; Bauer et al. 1993; 1990). Pockets rarely occur in species inhabiting very arid habitats or high latitudes. Very large or small species typically lack mite pockets, and pockets do not occur in limbless lizards or snakes. The peculiar associations between lizards, mites, and mite pockets have led to the development of numerous hypotheses for mite pocket existence and function (Salvador et al. 1999; Bauer et al. 1990; Arnold 1986; Wilkinson 1985; summarized in Appendix 3.I). However, few studies have explicitly tested these hypotheses (but see Salvador et al. 1999), and the function of mite pockets remains unclear and controversial (Arnold 1993; Bauer et al. 1993; 1990).

First developed by the author following observations of mite abundance and distribution on iguanian (Phrynosomatidae, Tropiduridae, and Opluridae) lizards, the mate choice hypothesis proposes that pockets function to concentrate and conceal brightly colored ectoparasites from potential mates. Ectoparasites, particularly chigger mites, predominantly congregate within pockets when pockets are available (Klukowski 2004; Cunha-Barros et al. 2003; Salvador et al. 1999; Chilton et al. 1992; Arnold 1986; Bennett 1977; Chapters 1, 4). These mites are frequently bright orange or red in coloration, and patches of feeding mites can be quite conspicuous, particularly in exposed regions (pers. obs.). Acute color vision is known to occur in many lizards (Leal and Fleishman 2004; Fleishman and Persons 2001), and numerous taxa, including *Sceloporus*, utilize patches of bright coloration for intraspecific communication (LeBas and Marshall 2000; Martin and Forsman 1999; Cooper and Burns 1987). Phylogenetically, mite pockets tend to be best developed in diurnal and visually oriented lizard clades, such as the Phrynosomatidae (Arnold 1986; Smith 1939) and Tropiduridae (Frost et al. 2001; Frost 1992). Species reliant on vision may obtain several benefits from concealing their brightly colored ectoparasites. By concealing mites, an individual would appear to a prospective mate to possess fewer ectoparasites and be perceived as more fit than they actually are. In lizards, males with low ectoparasite loads may directly signal their capability to avoid or resist parasites, and if heritable this ability may be passed to their offspring; alternatively, females may prefer males with low parasite loads to avoid becoming parasitized themselves (as reviewed by Moller et al. 1999; Tokarz 1995; Clayton 1991; Maynard Smith 1991; Read 1988). Similarly, because orange or red color patches signal female non-receptivity in many species of lizards, including *Sceloporus*

(Jones and Lovich 2009; Olsson 1995; Ferguson 1976; Vinegar 1972; Clark 1965), females could potentially benefit from concealing brightly colored mites from males.

Compared to other groups of organisms, female mate choice in lizards appears to be relatively uncommon (Olsson and Madsen 1995; Tokarz 1995). Females may choose males based on territory quality (Hews 1993; 1990), behavioral display (Crews 1975; Jenssen 1970), male size (Martin and Forsman 1999; Censky 1997; Cooper and Vitt 1993), body symmetry (Martin and Lopez 2000), or coloration (Kwiatkowski and Sullivan 2002; Baird et al. 1997; Sigmund 1983). Although parasite load negatively influences female mate choice in numerous vertebrate taxa (Kavaliers et al. 2003; Rosenqvist and Johansson 1995; Clayton 1990; Hillgarth 1990; Moller 1990; Kennedy et al. 1987; Read 1988; Edwards and Barnard 1987), very few workers have examined the effects parasites may have on mating behavior in lizards. In *Sceloporus occidentalis*, the endoparasite *Plasmodium mexicanum* decreases male social behavior (Schall and Sarni 1987), decreases dominance (Schall and Dearing 1987), and increases the proportion of black ventral coloration in males (Ressel and Schall 1989). Although ectoparasites are likely visible to conspecifics and may play a more direct role in mate choice, no study appears to have examined their potential influence in lizards.

Sceloporus jarrovi (Phrynosomatidae) is an ideal species for testing hypotheses of mite pocket function in lizards. Like all *Sceloporus* species, *S. jarrovi* possesses well-developed nuchal mite pockets (Smith 1939; Chapter 4). The natural history of this species is well-known, particularly in southeastern Arizona (Ruby and Baird 1994; Beuchat 1989; Ruby 1986, 1981, 1978, 1977; Ballinger 1979, 1973; Simon and Middendorf 1976). Additionally, data for the abundance, distribution, and diversity of

ectoparasites occurring on this species are also available (Foufopoulos 1999; Goldberg and Bursey 1993; Goldberg and Holshuh 1992; Bennett 1977; Chapter 1).

Sceloporus jarrovi is a viviparous, montane iguanian that occurs between 1500 and 3300 meters above sea level in southwestern New Mexico, southeastern Arizona, and central Mexico (Jones and Lovich 2009; Stebbins 1985; Ruby 1981; Ballinger 1973; Smith 1939). This species is primarily saxicolous and locally abundant, inhabiting rocky canyons and woodlands throughout its range. Non-territorial aggregations are formed between November and April in winter refugia, most commonly rock crevices (Ruby 1981; 1978); although individuals may be active all year at low elevations, activity during the winter tends to be minimal and sporadic (Beauchat 1989; Ruby 1981, 1977b; Ballinger 1973). Shortly after emerging from refugia in April and May, adults of both sexes begin establishing and maintaining territories. The territorial behavior displayed by this species is typical for most iguanians, consisting of push-ups, head-bobs, and lateral compressions that make the bright blue throat and belly coloration conspicuous to conspecifics and observers. Territorial behavior and aggression is most intense during the breeding season in September and October, particularly in males; females appear to mate only once per season, but males are promiscuous (Ruby 1981). Territories break down afterwards as lizards return to winter aggregations (Ruby 1978, 1977b). Ovulation begins in November and young are born alive the following May to June (Ruby 1981; Ballinger 1979, 1973; Carpenter 1960).

Mate choice in *S. jarrovi* has not been explicitly studied. Females display a high degree of site-fidelity from year to year and males tend to establish their territories to encompass as many females as possible (Ruby 1981, 1978). Territories of both sexes

frequently overlap, and individuals utilize spatial and temporal partitioning to minimize contact (Ruby and Baird 1994; Ruby 1978; Simon and Middendorf 1976; pers. obs.). The number of female territories overlapping male territories can vary considerably (3.75 ± 2.4 SD individual females at one high elevation site; Ruby and Baird 1994). During the breeding season females remain relatively inconspicuous and inactive, and males must spend considerable amounts of time searching their territories for receptive females; as a result male reproductive success is positively correlated with male activity and movement (Ruby 1981). Although Ruby did not observe females directly comparing males, approximately 25% of females in his study were visibly courted by more than one male. Additionally, subordinate males within the territories of dominant males were observed to court females, but females always rejected such attempts (Ruby 1981). Because females appear to mate only once per breeding season (Ruby 1981), it would benefit females to be choosy when multiple males are available. These data suggests that the opportunities to compare males are limited and females may actively choose when possible.

Trombiculid mites are abundant in the environment and commonly parasitize *S. jarrovi*, occurring most frequently within the nuchal mite pockets (Goldberg and Bursey 1993; Goldberg and Holshuh 1992; Bennett 1977; Chapter 1). Peak parasitism coincides with the breeding season of the host, with loads typically significantly higher in males than females (Foufopolous 1999; Bennett 1977; Chapter 1). Although it is not known if *S. jarrovi* detects ectoparasites on conspecifics and uses this in determining mate choice, such information would be potentially valuable in evaluating prospective mates.

In the present study, the mate choice hypothesis for mite pocket function is experimentally tested in *Sceloporus jarrovi* through the use of female mate choice trials.

If pockets do function to concentrate and conceal mites from prospective mates, males with visible ectoparasites are predicted to be preferred by females less frequently than males with hidden or no ectoparasites. Additionally, males with mites concealed in the pockets are predicted to be chosen as frequently as males with no mites.

Methods

Study System

A total of 128 adult *S. jarrovi* were collected using a hand-held noose in 2010 and 2011 from three study sites in southeastern Arizona and transported to the American Museum of Natural History Southwestern Research Station (SWRS) near Portal, Arizona, for mate choice trials. In 2010, 43 adult *S. jarovi* were collected between September 13 and September 25 from South Fork (male n=8, female n=4) and Barfoot (male n=20, female n=11) study sites, both part of the Coronado National Forest. South Fork (1550 m elevation; 31.878 North, 109.180 West) is a riparian corridor of mixed oak woodland and rock talus that extends along the southern branch of Cave Creek Canyon, approximately 2.5 km southeast of SWRS; in contrast, Barfoot (2550 m elevation; 31.920 North, 109.278 West) is a large, sparsely vegetated talus slope located approximately 150 m below the southern side of Barfoot Peak, approximately 8.5 km northwest of SWRS.

Mate choice trials were intended to be continued in 2011 using animals collected from South Fork and Barfoot study sites, but due to damage caused by the Horseshoe II wildfire, lizards used in mate choice trials in 2011 were obtained primarily from the

Soldier Creek campground in the Pinaleno Mountains, approximately 110 km northwest of SWRS. Like the Chiricahuas, the Pinalenos is a sky island mountain range in southeast Arizona predominantly encompassed by the Coronado National Forest. Soldier Creek campground (2850 m elevation; 32.697 N, 109.921 W) consists of pine, fir, and aspen forest and grassy meadows, interspersed with isolated boulder clusters and rocky outcrops. Eighty-five adult *S. jarrovi* were collected between September 11 to September 20, 2011 for mate choice trials; 73 from Soldier Creek (male n=36, female n=37) and the remainder from South Fork (male n=10, female n=2). For a full description of study sites and lizard microhabitat usage, refer to Chapter 1.

Snout-vent length, weight, mite load and distribution data were collected for all lizards shortly after capture. Snout-vent length was measured using digital calipers to the nearest 0.1 mm. Weight was measured using a Pesola spring scale to the nearest 0.1 g at initial capture and approximately every week the individual was held in captivity. Data on mite load and distribution were collected through the use of mite counts. Because the bulk of the ectoparasite load in *S. jarrovi* is concentrated in the nuchal mite pockets (Goldberg and Bursey 1993; Goldberg and Holshuh 1992; Bennett 1977; Chapter 1), mite count data was collected for the nuchal pockets as well as total body load for each individual after capture. A digital camera (Canon Rebel X SLR) was used to take photographs of the dorsum, throat, and belly of each male used in the mate choice trials. Photographs were taken in partial shade under natural lighting in late morning, after lizards had time to bask; these photographs were then ranked in a linear scale (lightest to darkest) for males of each year and used to quantify relative throat and belly coloration.

When not participating in mate choice trials, lizards were segregated by sex and housed in small groups in 4m x 6m outdoor pens under natural conditions at the SWRS Animal Behavior Observatory. Numerous basking perches and refugia were provided for each pen, and crickets and water supplied *ad libitum*. Lizards were allowed to acclimate to these conditions for at least a week before they were used in trials, and most individuals were kept in captivity for no more than two weeks total. Upon conclusion of the experiment, all lizards were returned to their site of collection and released.

Mate Choice Trials

Mate choice trials were conducted using a balanced incomplete block design in which consistent male pairs were set against a random female from the available female pool. To minimize the confounding effects of male size or coloration on female choice during the trials, males were paired as closely as possible by snout-vent length, weight, and overall coloration to produce fifteen male pairings (n=6 in 2010, n=9 in 2011). Trombiculid loads vary considerably in *S. jarrovi*, particularly within males (Foufopoulos 1999; Bennett 1977; Chapter 1); because the distribution and abundance of chiggers on the host could not be experimentally controlled, mite burdens were standardized in males through removal and subsequent replacement with artificial 'mites' in the form of paint spots. Mites were removed using a topical acaracide rinse (Reptile Spray, Natural Chemistry Inc.) containing dioctyle sodium sulfosuccinate and undecylentic acid in an aqueous solution. Similar topical acaricides have been demonstrated elsewhere to eliminate or significantly reduce ectoparasitic mite load with no observable effect on the lizard (Foufopoulos 1999; Montanucci 1997; Sorci et al. 1994). Lizards were sprayed

with the solution until thoroughly moist, avoiding the eyes and mouth, and allowed to sit for fifteen to thirty minutes. Lizards were then rinsed with warm tap water and allowed to dry. A soft brush or cloth was used to remove any remaining dead mites afterwards.

Following mite removal, male pairs were randomly assigned to one of three possible treatment pairings: Control-Hidden, Control-Visible, and Hidden-Visible. Treatment was then randomly assigned to each male within the pair. In Hidden and Visible males, standardized artificial mite loads were simulated using nontoxic red-orange acrylic paint spots placed inside or just outside the nuchal mite pocket, respectively. Paint spot coloration and size were matched as closely as possible to actual trombiculid mites (Figure 3.1), and simulated loads were analogous to nuchal loads observed during the collection of mite load data (approximately 50-60 mites per Hidden and Visible treatment male, the average nuchal load for adult males at these study sites; Chapter 1). Control group males served as a negative control and had no artificial mites added. Male pairs and treatment assignments remained constant throughout the duration of the experiment for each year.

Mate choice trials were performed in a partitioned arena enclosure similar to that described in LeBas and Marshall (2000) (Figure 3.2). Males were placed individually in compartments separated from the main female chamber via a transparent acrylic barrier. Males could not view or interact with each other, but were initially simultaneously visible to a single female placed in the central portion of the enclosure. Two choice regions were located within the female chamber directly opposite of each male chamber. Females could move about freely in the central chamber of the arena, but upon entering one of the choice regions a partition prevented females from viewing the opposing male.

Females entering a choice region adjacent to a male were considered to have made a choice for that male. For trials in which females were observed in both choice regions the region with the greatest number of observations was considered as choice for that male. Females that remained in the large central chamber throughout the trial without entering either choice region were classified as having made no choice.

Mate choice trials (n=138) were performed between September 27 and October 4, 2010 (n=66), and September 25 to September 30th, 2011 (n=72), at the height of the breeding season (Ruby 1981, 1977). All treatment pairings (Control-Hidden, Control-Visible, and Hidden-Visible) were equally represented in each year. All trials were conducted outdoors in partial shade on sunny to mostly sunny days between 900 and 1500 hours, when *S. jarrovi* are most active (Beuchat 1989; Simon and Middendorf 1976). Individuals were allowed to bask for at least an hour to warm up prior to trials. Females and male pairs were randomly selected from the available pool of lizards for each trial. No female or male pair participated in more than two trials in a row or three trials total in a single day. Females encountered each male pair no more than once during the course of the experiment. At the start of each trial, the female was placed at the lower center of the neutral region where both males could be viewed simultaneously; males from each pair were randomly placed in left and right male chambers. The orientation and position of lizards within the arena was recorded initially and every three minutes afterwards for 45 minutes, resulting in sixteen spot observations per trial. Trials conducted during 2011 (n=72) were also digitally recorded through the use of a video recorder (Samsung SMX-F50) for later examination. All observations and recordings were conducted behind a blind to minimize disturbance to the lizards. At the conclusion

of each trial, lizards were returned to their respective pens and the arena cleaned to remove any scent markings.

Statistical analysis

Statistical analysis of mate choice data was performed in SPSS (SPSS Inc. 2011, version 20.0 for Windows). Binomial tests were used to determine if any choice bias existed due to the orientation of the arena or male starting position. Fischer's exact test was used to determine if females are actively making a choice based on the criteria described above. Male traits that could potentially influence female choice, including body size (snout-vent length and/or weight), time of day, coloration (throat and/or belly), pre-existing mite load prior to removal, and experimental treatment, were examined through the use of chi-square goodness of fit tests, ANOVA, and simple regressions. Finally, female choice bias towards certain males within treatment pairings (contextual bias) was analyzed using binomial tests. In all analyses a two-tailed critical value of $\alpha=0.05$ was considered significant.

Results

1) Are females making a choice?

During mate choice trials lizards often remained motionless but alert within the arena for the first five to fifteen minutes before moving about and exploring their surroundings. Females displayed choice for a particular male in 107 of the 138 total trials

conducted (Table 3.1, top); this result was significant for 2011 (Fischer's exact $t_{2, 72}$=7.211, p=0.019) and nearly significant for 2010 ($t_{2, 66}$=3.464, p=0.074). Choice was also significant if data is pooled for both years ($t_{2, 138}$=14.363, p=0.005). No bias in female choice was due to arena orientation or male starting side for either year (binomial test: 2010 p=0.551; 2011 p=0.252). In multiple trials males were observed to orient and move towards the female and occasionally perform head bobs and/or substrate licks. Females generally appeared to notice behaviors performed by males and frequently oriented and moved towards either in the arena, often contacting the clear acrylic divider or moving back and forth in front of it. Once entering a choice region, females rarely exited the region or equivocated between the two males; in only eleven instances were females observed in both male choice regions (2010 n=1, 2011 n=10). Females who ended up making a choice did so relatively quickly (mean=12.42±11.27 minutes), although females in 2011 (9.53±9.00) chose significantly more quickly than in 2010 (16.40±9.88) (ANOVA $F_{1, 107}$=10.548, p=0.002) (Figure 3.3). Additionally, larger male pairs elicited female choice significantly more rapidly than smaller male pairings ($F_{18, 106}$=1.839, p=0.033), regardless of year. In the remaining 31 trials in which no choice was made, females remained largely stationary or moved about the central region of the arena without showing any noticeable interest in either male.

2) What are females basing their choice on?

Within the subset of females who made a choice during trials (n=107 of 138 total trials), male treatment appears to have had inconsistent effects on female choice (Table 3.2, Figure 3.4). Females in 2010 chose Control males nearly significantly less

frequently than expected if choice was made randomly (χ^2=3.125, p=0.077), while Visible males were chosen more frequently (χ^2=3.571, p=0.059). In 2011, choice for Hidden treatment males was nearly significantly higher than predicted (χ^2=2.814, p=0.093). When pooled, no significant bias was displayed towards any of the three male treatments (Table 3.2, bottom). Post-hoc analysis of choice frequency between years by male treatments revealed a significant treatment by year interaction (ANOVA $F_{2, 30}$=4.492, p=0.022), but no significant effect for either treatment or year alone (Figure 3.4).

Male snout-vent length, weight, original mite load (prior to removal), and coloration appeared to have little effect on female choice during the trials (Table 3.3). The only male traits to significantly affect female choice were total mite load (ANOVA $F_{1, 90}$=4.289, p=0.041) and nuchal pocket load (ANOVA $F_{1, 90}$=5.090, p=0.027) in 2010. For both associations, males chosen by females possessed significantly higher mite loads prior to removal than males not chosen. Neither of these associations was significant in 2011 or when data was pooled between years. Post-hoc analysis of male throat and belly coloration found females displayed no significant preference for lighter or darker colored males within male pairings in the pooled dataset (throat χ^2=0.234, p=0.629; belly χ^2=1.579, p=0.209). Snout-vent length, weight, and coloration were all found to have no effect on female choice.

Males used in mate choice trials in 2011 were significantly larger (ANOVA $F_{1, 30}$=43.896, p≤0.001), heavier ($F_{1, 30}$=32.536, p≤0.001), and had higher prior total mite loads ($F_{1, 30}$=4.791, p=0.039) than 2010 males (Table 3.4, top); these differences between 2010 and 2011 males are due to the change in the source populations, from South Fork

and Barfoot in 2010 to Soldier Creek in 2011, as described above (Chapter 1). Although male pools differed significantly between years, within years males utilized for mate choice trials were paired together by size and coloration and displayed no significant differences in body length, weight, or mite load (Table 3.4). Because females were obtained from the same source populations as the males each year and these male traits had little to no effect on female choice (Table 3.3), the differences between male pools in 2010 and 2011 are largely negligible.

Female snout-vent length, weight, and mite load were found to have no significant effect on whether a particular female made a choice or not during the trials (Table 3.4, bottom). Like males, female snout-vent length, weight, and total mite loads were significantly higher in 2011 than in 2010, but did not differ significantly within years between females who chose and those who did not. Additionally, female choice was not significantly affected by the time the mate choice trial started.

3) Is there any female bias towards particular males within male treatment pairings?

Binomial tests of female choice within the three possible male treatment pairings (Control-Hidden, Control-Visible, and Hidden-Visible) detected a significant female bias towards Visible treatment males when paired with Control males in 2010 ($p=0.035$) (Table 3.5). In Control-Visible male pairings, females exhibited significant preference for the male with the darker belly coloration ($\chi^2=4.571$, $p=0.033$); throat coloration had no significant effect (Table 3.6). No significant within-pair treatment bias was observed in 2011 or in the pooled dataset, although females exhibited a similar significant preference for males with darker bellies in Hidden-Visible male pairings in 2011

(χ^2=5.261, p=0.022); a similar nearly significant preference was also observed in the pooled dataset (χ^2=2.778, p=0.096).

Discussion

Although female *S. jarrovi* appear to orient towards and actively choose males relatively quickly when given the opportunity to do so (Tables 3.1 and 3.3), it remains unclear what females may be basing their decisions on. Although some slight female bias was observed for certain male treatments – towards Visible males and away from Control males in 2010, and towards Hidden males in 2011 – none of these biases were statistically significant (Table 3.2). Additionally, the distribution of female choice by male treatment as predicted by the mate choice hypothesis (Control males = Hidden males > Visible males) was not found (Figure 3.4). Based on these results the mate choice hypothesis – that mite pockets function to conceal brightly colored ectoparasites from potential mates – was not supported. Contrary to the findings in numerous other taxa (Kavaliers et al. 2003; Rosenqvist and Johansson 1995; Clayton 1990; Hillgarth 1990; Moller 1990; Kennedy et al. 1987; Read 1988; Edwards and Barnard 1987), ectoparasite load, either simulated or prior to removal, does not appear to significantly influence the likelihood of a particular male in being chosen by a female in *S. jarrovi*. In the social lizard *Lacerta vivipara* (Lacertidae), individuals were found not to specifically avoid parasitized conspecifics, but instead were attracted to conspecifics regardless of parasite load (Sorci et al. 1997). Because preferred microhabitats for *Sceloporus jarrovi*

tend to be isolated and spatially clumped at all of the field sites examined (with the possible exception of Barfoot), perception and avoidance of parasitized individuals may be secondary to locating and securing an adequate territory within a scarce microhabitat. Alternatively, the lack of evidence for female choice based on ectoparasite load may be due to the reliance on artificial 'mites' in lieu of actual ectoparasites in mate choice trials. Experimentally manipulating or otherwise standardizing ectoparasite burdens in *S. jarrovi* is made challenging largely due to difficulties in handling and manipulating the mites. Mite loads in *S. jarrovi* are highly variable, particularly during the breeding season in the late fall (Foufopolous 1999; Bennett 1977; Chapter 1). Trombiculid mites are the most common ectoparasites of *S. jarrovi* (Goldberg and Holshuh 1992; Bennett 1977), but these mites are difficult to experimentally manipulate due to their small size, alternation of life stages (from parasitic larva to free-living nymph and adult), and feeding habits. Although it was not possible to rear trombiculid mites for experimental application to lizards in the present study, these mites may be raised with some difficulty in captivity (Tanskul et al. 1988; Sasa 1961). While certainly more difficult, the use of actual mites in future studies of mate choice is thus not necessarily impossible.

Female *S. jarrovi* displayed no significant preference for male body length, weight, or coloration in the mate choice trials (Table 3.3), and no aspects of female morphology were found significantly associated with the likelihood of making a choice (Table 3.4, bottom). Additionally, the response females displayed was similar despite significant variation in male traits between source populations (Tabled 3.3 and 3.4). While overt female choice appears uncommon in lizards (as reviewed in Olsson and Madsen 1995; Tokarz 1995), the complete lack of influence of male or female

morphology on female choice in *S. jarrovi* is still surprising. Unlike previous studies of female choice in lizards in which larger males were found to be more desirable to females (Martin and Forsman 1999; Censky 1997; Cooper and Vitt 1993; Andrews 1985), male body size had no significant affect on female choice in *S. jarrovi*. Although males were largely matched by size within male pairings to minimize the effects of body size in treatment choice, no preference was displayed towards larger male pairings in the present study, and small males were chosen by females as frequently as large males. Although male coloration is significantly associated with female choice in other lizard species (Kwiatkowski and Sullivan 2002; Baird et al. 1997; Sigmund 1983), male throat and belly coloration had little effect on female choice in *S. jarrovi*, apparently influential only under certain circumstances (Table 3.6). If not utilized for courtship, these sexually dimorphic colorations in *Sceloporus* may instead function primarily for intraspecific communication and sex recognition (Cooper and Burns 1987).

Conclusion

The results of the present study do not support the mate choice hypothesis for mite pocket function. Although females appear to be displaying a significant preference towards one of the two possible males presented during the mate choice trials, female choice was not based on male treatment or prior ectoparasite load. If pockets do possess a specific function (Appendix 3.I), it is not to conceal brightly colored ectoparasites from conspecifics. Additionally, no measured aspect of male morphology had a significant

effect on female choice, and female morphology did not influence the likelihood of a female in making a choice. Given these results, what might female *Sceloporus jarrovi* be basing their mate choice decisions on? One possible explanation is that female mate choice in lizards could be subtle and dependent on a variety of cues and context, which may be easily and unintentionally overlooked in experimental studies of mate choice. In *S. jarrovi*, choice may be based on traits not measured or accounted for in the present study, such as male behavior, olfactory cues, or differences in male coloration outside the range of normal human perception. Many male iguanids, including *Sceloporus*, perform stereotypical behaviors during intraspecific interactions and courtship (Cooper and Burns 1987; Ruby 1977a; Ferner 1976; Carpenter 1967; Greenberg 1945); however, few studies have explicitly examined the effects of these male behaviors on female mate choice. In *Anolis* (Polychrotidae), males capable of frequently performing courtship displays are generally preferred by females (Sigmund 1983; Crews 1975; Jenson 1970; Greenberg and Noble 1944), and such displays may indicate male vigor or endurance to the female. In some species of *Anolis*, male courtship displays also appear to stimulate female receptivity and follicular development prior to copulation (Crews 1975). Inclusion of stereotypical male behaviors, such as the number or frequency of push-ups, head-bobs, or lateral presentation displays into mate choice trials could be fruitful.

Alternatively, it is possible female *S. jarrovi* rely primarily on chemosensory cues instead of vision to perceive conspecific ectoparasite load, as has been demonstrated in other organisms (Maksimowich and Mathis 2001; Kavaliers and Colwell 1995), including lizards (Martin et al. 2007a). In *Psammodromus algirus* (Lacertidae), sexually receptive females exhibit significantly more tongue-flick responses to femoral pore

secretions from males with few ticks than heavily infested males (Martin et al. 2007a). In addition to parasite burden, female lacertids rely on olfaction to assess other male traits potentially important in mate choice, including male immune response (Lopez and Martin 2005), circulating hormone levels (Martin et al. 2007a, b; Martin and Lopez 2006), age (Lopez et al. 2003), and degree of morphological symmetry (Martin and Lopez 2006, Martin and Lopez 2000). Although iguanids utilize olfaction for numerous aspects associated with social behavior, such as conspecific recognition and territory maintenance (Alberts 1993; Duvall 1981, 1979), no study appears to have explicitly examined the role of chemoreception in female choice within this group. Chemosensory information could easily be incorporated into female mate choice trials, however; for example, swabs of male femoral pore and/or cloacal secretions could be presented to female *S. jarrovi*, with female choice determined by the number of tongue-flicks directed towards the swabs.

If females lack the ability to discriminate between males, or if females are unable to reliably assess male quality through external cues, females may appear to prefer the first suitable male they encounter. If so, female choice may be displayed (i.e. choice for a male versus choice for no male), but males would appear to be chosen at random with respect to morphology, behavior, or experimental treatment. In some populations of *Anolis carolinensis*, females appear to display little significant preference for certain males and instead select mates seemingly at random, regardless of male body size, throat coloration, or ectoparasite load (MacDonald and Echternacht 1991; Andrews 1985). In the sand lizard *Lacerta agilis*, females mate with multiple males, chosen seemingly at random without regard to male size or nuptial coloration, during the short period in which

females are receptive (Olsson and Madsen 1995). Although larger, older males are capable of siring offspring with enhanced survivability, they are not preferred by females over smaller, younger males. Additionally, male nuptial patch size or coloration is not positively correlated with ectoparasite (tick) burdens, and females do not reject males on the basis of tick loads. Based on patterns of territoriality in *S. jarrovi* (Ruby and Baird 1994; Ruby 1981, 1978; Simon and Middendorf 1976), females would be expected to be exposed to multiple males during the course of the breeding season. However, due to differences in individual activity patterns, females may not have the opportunity to actively compare males as they did in the trials, and female choice may instead be largely influenced by male activity patterns and male-male competition for territories and females. This is not to say that female *S. jarrovi* display no mate choice, but that choice in this species may be expressed in a less obvious manner than that tested in the experiment. For example, females could conceivably change daily activity patterns or spatial activity within their territory to encounter or avoid certain males and thus indirectly choose who they mate with. Because adults of both sexes are territorial and philopatric (Ruby 1981; 1978; 1977), this possibility could be examined through the use of field work to analyze the effects of dominant male removal or novel introduction has on female diel behavior in natural populations.

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References

Alberts A.C. 1993. Chemical and behavioral studies of femoral gland secretions in iguanid lizards. *Brain, Behavior, and Ecology* 41: 255-260.

Andrews R.M. 1985. Mate choice by females of the lizard, *Anolis carolinensis*. *Journal of Herpetology* 19: 284-289.

Arnold E.N. 1986. Mite pockets of lizards, a possible means of reducing damage by ectoparasites. *Biological Journal of the Linnean Society* 29:1-21.

Arnold E.N. 1993. Comment – Function of the mite pockets of lizards: an assessment of a recent attempted test. *Canadian Journal of Zoology* 71(4): 862-864.

Baird T.A., S.F. Fox, and J.K. McCoy. 1997. Population differences in the roles of size and coloration in intra- and intersexual selection in the collard lizard, *Crotaphytus collaris*: influence of habitat and social organization. *Behavioral Ecology* 8(5): 506-517.

Ballinger R.E. 1973. Comparative demography of two ovoviviparous iguanid lizards (*Sceloporus poinsetti* and *Sceloporus jarrovi*). *Ecology* 54(2): 269-283.

Ballinger R.E. 1979. Intraspecific variation in demography and life history of the lizard, *Sceloporus jarrovi* along an altitudinal gradient in southeastern Arizona. *Ecology* 60(5): 901-909.

Bauer A.M., A.P. Russell, and N.R. Dollahon. 1990. Skin folds in the gekkonid lizard genus *Rhacodactylus*: A natural test of the damage limitation hypothesis of mite pocket function. *Canadian Journal of Zoology* 68(6):1196-1201.

Bauer A.M., A.P. Russell, and N.R. Dollahon. 1993. Function of the mite pockets of lizards: a reply to E.N. Arnold. *Canadian Journal of Zoology* 71(4): 865-868.

Bennett S.G. 1977. Ecology and systematics of trombiculid mites on lizards from southern Arizona. M.S. Thesis. California State University, Long Beach. Long Beach, California. 192 pp.

Bertrand M. and D. Modry. 2004. The role of mite pocket-like structures on *Agama caudospinosa* (Agamidae) infested by *Pterygosoma livingstonei* sp. n. (Acari: Prostigmata: Pterygosomatidae). *Folia Parasitologica* 51: 61-66.

Beuchat C.A. 1989. Patterns and frequency of activity in a high altitude population of the iguanid lizard, *Sceloporus jarrovi*. *Journal of Herpetology* 23(2): 152-158.

Carpenter C.C. 1960. Parturition and behavior at birth of Yarrow's spiny lizard (*Sceloporus jarrovi*). *Herpetologica* 16(2): 137-138.

Carpenter C.C. 1967. Aggression and social structure in iguanid lizards. *In* W.W. Milstead (ed.), Lizard Ecology: A Symposium. University of Missouri Press, Columbia. Pp. 87-105.

Censky E.J. 1997. Female mate choice in the non-territorial lizard *Ameiva plei* (Teiidae). *Behavioral Ecology and Sociobiology* 40(4): 221-225.

Chilton N.B., C.M. Bull, and R.H. Andrews. 1992. Niche segregation in reptile ticks: attachment sites and reproductive success of females. *Oecologica* 90: 255-259.

Clark R.F. 1965. An ethological study of the iguanid lizard genera *Callisaurus*, *Ciphosaurus*, and *Holbrookia*. *Emporia State Research Studies* 13: 1-66.

Clayton D.H. 1990. Mate choice in experimentally parasitized rock doves: lousy males lose. *American Zoologist* 30: 251-262.

Clayton D.H. 1991. The influence of parasites on host sexual selection. *Parasitology Today* 7(12): 329-334.
Cooper W.E. Jr. and L.J. Vitt. 1993. Female mate choice of large male broad-headed skinks. *Animal Behaviour* 45: 683-693.
Cooper W.E. Jr. and N. Burns. 1987. Social significance of ventrolateral coloration in the fence lizard, *Sceloporus undulatus*. *Animal Behaviour* 35: 526-532.
Crews D. 1975. Effects of different components of male courtship behaviour on environmentally induced ovarian recrudescence and mating preferences in the lizard, *Anolis carolinensis*. *Animal Behaviour* 23: 349-356.
Cunha-Barros M., M. van Sluys, D. Vricibradic, C.A.B. Galdino, F.H. Hatano, and C.F.D. Rocha. 2003. Patterns of infestation by chigger mites in four diurnal lizard species from a restinga habitat (Jurubatiba) of southeastern Brazil. *Brazilian Journal of Biology* 63(3): 393-399.
Duvall D. 1979. Western fence lizard (*Sceloporus occidentalis*) chemical signals I: conspecific discriminations and release of a species-typical visual display. *Journal of Experimental Zoology* 210: 321-326.
Duvall D. 1981. Western fence lizard (*Sceloporus occidentalis*) chemical signals II: a replication with naturally breeding adults and a test of the Cowles and Phelan hypothesis of rattlesnake olfaction. *Journal of Experimental Zoology* 218: 351-361.
Edwards J.C. and C.J. Barnard. 1987. The effects of *Trichinella* infection on intersexual interactions between mice. *Animal Behaviour* 35: 533-540.
Ferner J.W. 1976. Notes on natural history and behavior of *Sceloporus undulatus erythrocheilus* in Colorado. *American Midland Natrualist* 96(2): 291-302.
Ferguson G.W. 1976. Color change and reproductive cycling in female collared lizards (*Crotaphytus collaris*). *Copeia* 1976(3): 491-494.
Fleishman L.J. and M. Persons. 2001. The influence of stimulus and background colour on signal visibility in the lizard *Anolis cristatellus*. *Journal of Experimental Biology* 204: 1559-1575.
Foufopoulos J. 1999. Host-parasite interactions in the mountain spiny lizard *Sceloporus jarrovi*. Ph.D. Dissertation. University of Wisconsin – Madison. 215 pp.
Frost D.R. 1992. Phylogenetic analysis and taxonomy of the *Tropidurus* group of lizards (Iguania: Tropiduridae). *American Museum Novitates* 3033: 1-68.
Frost D.R., M.T. Rodrigues, T. Grant, and T.A. Titus. 2001. Phylogenetics of the lizard genus *Tropidurus* (Squamata: Tropiduridae: Tropidurinae): Direct optimization, descriptive efficiency, and sensitivity analysis of congruence between molecular data and morphology. *Molecular Phylogenetics and Evolution* 21(3):352-371.
Goldberg S.R. and C.R. Bursey. 1993. Duration of attachment of the chigger, *Eutrombicula lipovskyana* (Trombiculidae) in mite pockets of Yarrow's spiny lizard, *Sceloporus jarrovi* (Phrynosomatidae) from Arizona. *Journal of Wildlife Diseases* 29(1): 142-144.
Goldberg S.R., and H.J. Holshuh. 1992. Ectoparasite-induced lesions in mite pockets of the Yarrow's spiny lizard, *Sceloporus jarrovii* (Phrynosomatidae). *Journal of Wildlife Diseases* 28(4): 537-541.
Greenberg B. 1945. Notes on the social behavior of the collared lizard. *Copeia* 1945(4): 225-230.

Greenberg B. and G.K. Noble. 1944. Social behavior of the American chameleon (*Anolis carolinensis* Voigt). *Physiological Zoology* 17(4): 392-439.
Hews D.K. 1990. Examining hypotheses generated by field measures of sexual selection on male lizards, *Uta palmeri*. *Evolution* 44(8): 1956-1966.
Hews D.K. 1993. Food resources affect female distribution and male mating opportunities in the iguanian lizard *Uta palmeri*. *Animal Behaviour* 46: 279-291.
Hillgarth N. 1990. Parasites and female choice in the ring-necked pheasant. *American Zoologists* 30: 227-233.
Jenssen T.A. 1970. Female response to filmed displays of *Anolis nebulosus* (Sauria, Iguanidae). *Animal Behavior* 18: 640-647.
Jones L.L.C. and R.E. Lovich. 2009. Lizards of the American Southwest: A Photographic Field Guide. Rio Nuevo Publishers. Tucson, Arizona.
Kavaliers M., M.A. Fudge, D.D. Colwell, and E. Choleris. 2003. Aversive and avoidance responses of female mice to the odors of males infected with an ectoparasite and the effects of prior familiarity. *Behavioral Ecology and Sociobiology* 54(5): 423-430.
Kennedy C.E.J. J.A. Endler, S.L. Poynton, and H. McMinn. 1987. Parasite load predicts mate choice in guppies. *Behavioral Ecology and Sociobiology* 21(5): 291-295.
Klukowski M. 2004. Seasonal changes in abundance of host-seeking chiggers (Acari: Trombicuilidae) and infestations on fence lizards, *Sceloporus undulatus*. *Journal of Herpetology* 38(1): 141-144.
Kwiatkowski M.A. and B.K. Sullivan. 2002. Geographic variation in sexual selection among populations of an iguanid lizard, *Sauromalus obesus* (=*ater*). *Evolution* 56(10): 2039-2051.
Leal M. and L.J. Fleishman. 2004. Differences in visual signal design and detectability between allopatric populations of *Anolis* lizards. *The American Naturalist* 163(1): 26-39.
LeBas N.R. and N.J. Marshall. 2000. The role of signaling and mate choice in the agamid lizard *Ctenophorus ornatus*. *Proceedings of the Royal Society, London* 267: 445-452.
Leenders T. 2001. A Guide to Amphibians and Reptiles of Costa Rica. Distribuidores Zona Tropical, S.A.: Miami, Florida. Pp. 184-185.
Lopez P. and J. Martin. 2005. Female Iberian wall lizards prefer male scents that signal a better cell-mediated immune system. *Biology Letters* 1: 404-406.
Lopez P., P. Aragon, and J. Martin 2003. Responses of female lizards, *Lacerta monticola*, to males' chemical cues reflect their mating preference for older males. *Ecology and Sociobiology* 55(1): 73-79.
Loveridge A. 1925. A mite pocket in the gecko, *Gymnodactylus lawderanus* Stoliczka. *Proceedings of the Zoological Society of London* 1925: 1431.
MacDondald D. and A.C. Echternacht. 1991. Red-throated and gray-throated *Anolis carolinensis*: do females know the difference? *Anolis Newsletter* 4: 92-100.
Maksimowich D.S. and A. Mathis. 2001. Pheromonal markers as indicators of parasite load: parasite-mediated behavior in salamanders (*Plethodon angusticlavius*). *Acta Ethologica* 3: 83-87.

Martin J. and A. Forsman. 1999. Social costs and development of nuptial coloration in male *Psammodromus algirus* lizards: an experiment. *Behavioral Ecology* 10(4): 396-400.
Martin J. and P. Lopez. 2000. Chemoreception, symmetry, and mate choice in lizards. *Proceedings of the Royal Society of London, B* 267: 1265-1269.
Martin J. and P. Lopez 2006. Links between male quality, male chemical signals, and female mate choice in Iberian Rock Lizards. *Functional Ecology* 20: 1087-1096.
Martin J., E. Civantos, L. Amo, and P. Lopez. 2007a. Chemical ornaments of male lizards *Psammodromus algirus* may reveal their parasite load and health state to females. *Behavioral Ecology and Sociobiology* 62: 173-179.
Martin J., P. Lopez, M. Gabirot, and K.M. Pilz. 2007b. Effects of testosterone supplementation on chemical signals of male Iberian wall lizards: consequences for female mate choice. *Behavioral Ecology and Sociobiology* 61: 1275-1282.
Maynard Smith J. 1991. Theories of sexual selection. *Trends in Ecology and Evolution* 6(5): 146-151.
Moller A.P. 1990. Parasites, sexual ornaments, and mate choice in the barn swallow *Hirundo rustica*. *In* J.E. Loye and M. Zuk (eds.), Ecology, Behavior, and Evolution of Bird-Parasite Interactions. Oxford University Press, Oxford. Pp. 328-343.
Moller A.P., P. Christe, and E. Lux. 1999. Parasitism, host immune function, and sexual selection. *Quarterly Review of Biology* 74(1): 3-20.
Montanucci R.R. 1997. A safe procedure for mite eradication. *Herpetological Review* 28(1): 38-39.
Olsson M. 1995. Forced copulations and costly female resistance behavior in the Lake Eyre dragon, *Ctenosaurus maculosus*. *Herpetologica* 51(1): 19-24.
Olsson M. and T. Madsen. 1995. Female choice on male quantitative traits in lizards – why is it so rare? *Behavioral Ecology and Sociobiology* 36: 179-184.
Read A.F. 1988. Sexual selection and the role of parasites. *Trends in Ecology and Evolution* 3(5): 97-102.
Ressel S. and J.J. Schall. 1989. Parasites and showy males: malarial infection and color variation in fence lizards. *Oecologia* 78: 158-164.
Rosenqvist G. and K. Johansson. 1995. Male avoidance of parasitized females explained by direct benefits in a pipefish. *Animal Behavior* 49: 1039-1045.
Ruby D.E. 1977a. The function of shudder displays in the lizard *Sceloporus jarrovi*. *Copeia* 1977(1): 110-114.
Ruby D.E. 1977b. Winter activity in Yarrow's spiny lizard, *Sceloporus jarrovi*. *Herpetologica* 33(3): 322-333.
Ruby D.E. 1978. Seasonal changes in the territorial behavior of the iguanid lizard *Sceloporus jarrovi*. *Copeia* 1978(3): 430-438.
Ruby D.E. 1981. Phenotypic correlates of male reproductive success in the lizard, *Sceloporus jarrovi*. *In* R.D. Alexander and D.W. Tinkle (eds.) Natural Selection and Social Behavior. Chiron Press, New York. Pp. 97-107.
Ruby D.E. 1986. Selection of home range site by females of the lizard, *Sceloporus jarrovi*. *Journal of Herpetology* 20(3): 466-469.
Ruby D.E. and D.I. Baird. 1994. Intraspecific variation in behavior: Comparisons between populations at different altitudes of the lizard *Sceloporus jarrovi*. *Journal of Herpetology* 28(1): 70-78.

Salvador A., J.P. Veiga, and E. Civantos. 1999. Do skin pockets of lizards reduce the deleterious effects of ectoparasites? An experimental study with *Psammodromus algirus*. *Herpetologica* 55(1): 1-7.
Sasa M. 1961. Biology of chiggers. *Annual Reviews of Entomology* 6: 221-244.
Schall J.J. and G.A. Sarni. 1987. Malarial parasitism and the behavior of the lizard, *Sceloporus occidentalis*. *Copeia* 1987(1): 84-93.
Schall J.J. and M.D. Dearing. 1987. Malarial parasitism and male competition for mates in the western fence lizard, *Sceloporus occidentalis*. *Oecologia* 73: 389-392.
Schall J.J., H.R. Prendeville, and K.A. Hanley. 2000. Prevalence of the tick, *Ixodes pacificus*, on western fence lizards, *Sceloporus occidentalis*: trends by gender, size, season, site, and mite infestation. *Journal of Herpetology* 34(1): 160-163.
Sigmund W.R. 1983. Female preference for *Anolis carolinensis* males as a function of dewlap color and background coloration. *Journal of Herpetology* 17(2): 137-143.
Simon C.A. and G.A. Middendorf. 1976. Resource partitioning by an iguanid lizard: temporal and microhabitat aspects. *Ecology* 57(6): 1317-1320.
Smith H.M. 1939. The Mexican and Central American lizards of the genus *Sceloporus*. *Field Museum of Natural History, Zoological Series* 26: 1-397.
Sorci G., M. Massot, and G. Clobert. 1994. Maternal parasite load increases sprint speed and philopatry in female offspring of the common lizard. *The American Naturalist* 144(1): 153-164.
SPSS Inc. 2011. SPSS version 20.0 for Windows. SPSS Inc., Chicago IL.
Stebbins R.C. 1985. A Field Guide to Western Reptiles and Amphibians, 2nd ed. Houghton Mifflin Co. Boston, Mass.
Tanskul P., K.J. Linthicum, P. Watcharapichat, D. Phulsuksombati, S. Mungviriya, S. Ratanatham, N. Suwanabun, J. Sattabongkot, and G. Watt. 1988. A new ecology of scrub typhus associated with a foci of antibiotic resistance in rice farmers in Thailand. *Journal of Medical Entomology* 35: 551-555.
Tokarz R.R. 1995. Mate choice in lizards: A review. *Herpetological Monographs* 9: 17-40.
Vinegar M.B. 1972. The function of breeding coloration in the lizard, *Sceloporus virgatus*. *Copeia* 1972(4): 660-664.
Wilkinson M. 1985. Mite refugia in lizards. *Unpublished manuscript*.
Williams E.E. 1965. The species of Hispaniolan green anoles (Sauria: Iguanidae). *Breviora* 227:1-16
Zahavi A. 1975. Mate selection – a selection for a handicap. *Journal of Theoretical Biology* 53: 205-214.
Zahavi A. 1977. The cost of honesty – further remarks on the handicap principle. *Journal of Theoretical Biology* 67: 603-605.

Appendix 3.I: Summary of selected mite pocket hypotheses, sorted by function.

- *Nonfunctional:*
  - **Fortuitous Inhabitation** (Arnold 1986): Associations between mites and mite pockets are due to chance alone.
  - **Preservation Artifact** (Arnold 1986): Associations between mites and mite pockets are due to the unintentional detachment of mites outside mite pockets during preservation of lizards.
  - **Mite Inducement** (Wilkinson 1985): Mite pockets are induced by the feeding activity of parasitic mites.
  - **Phylogenetic Baggage** (Bauer et al. 1993; 1990): Mite pockets are the result of past adaptations or design parameters that have since lost utilitarian value.
  - **Spandrels of San Marco** (Bauer et al. 1993; 1990; Gould and Lewonton 1979): Mite pockets are the by-products of developmental processes involved in the development of skin folds.

- *Function unrelated to mites:*
  - **Physiological Function** (Arnold 1986): Mite pockets are involved in physiological functions such as water balance or the production of glandular secretions.
  - **Ecological Function** (Bauer et al. 1993; 1990): Mite pockets are utilized by the lizard for ecological functions such as crypsis, parachuting, defensive displays, or intraspecific identification.
  - **Bite Hold** (Reed, *unpublished*): Mite pockets serve as a bite hold for males during reproduction.

- *Function mite related:*
  - **Mutualistic Mites** (Arnold 1986): Mite pockets are inhabited by mites that form mutualistic associations with the lizard.
  - **Concentration/Impairment-Prevention** (Salvador et al. 1999): Pockets function to concentrate mites away from sensitive areas and prevent the impairment of vision, hearing, and motion.
  - **Concentration/Damage-Amelioration** (Arnold 1986; Chapter 2): Pockets serve to concentrate mites in specialized structures that quickly repair and contain damage caused by parasitic mites.
  - **Concentration/Handicap** (Zahavi 1977, 1975): Pockets serve to concentrate ectoparasites, which act as honest indicators of individual quality to conspecifics.
  - **Concealment – Mate Choice** (Present study): Pockets serve to concentrate and conceal brightly colored mites from potential mates.
  - **Concealment – Defensive** (Reed, *unpublished*): Pockets serve to concentrate and conceal brightly colored mites to improve crypsis and avoid predation.
  - **Mite Removal** (Wilkinson 1985; Arnold 1986): Mite pockets concentrate harmful mites so they may later be removed or incapacitated.
  - **Biological Warfare** (Wilkinson 1985): Mite pockets may be used by lizard species resistant to parasitic mites to transport mites into the range of susceptible competitors, thereby giving the resistant species a competitive advantage.

Tables

	Choice			No Choice		
Male pair	2010	2011	**Total**	2010	2011	**Total**
C-H	17	20	**37**	5	4	**9**
C-V	15	19	**34**	7	5	**12**
H-V	13	23	**36**	9	1	**10**
			107			**31**

	Choice		
Treatment	2010	2011	**Total**
Control	11	18	**29**
Hidden	15	27	**42**
Visible	19	17	**36**

	Choice within Male Pairings					
Year	C	H	C	V	H	V
2010	8	9	3	12	6	7
2011	7	13	11	8	14	9
Total	15	22	14	20	20	16

Table 3.1: Overview of results of mate choice trials from 2010 and 2011, separated into female choice versus no choice (**top**), female choice by male treatment (**center**), and female choice within male treatment pairings (**bottom**). Abbreviations: C (Control males); H (Hidden males); V (Visible males). See text for details.

2010 (choice in 45 of 66 trials)				
Treatment	Observed	Expected	χ^2	*P*
Control	11	16	3.125	0.077
Hidden	15	15	0.000	1.000
Visible	19	14	3.571	0.059

2011 (choice in 62 of 72 trials)				
Treatment	Observed	Expected	χ^2	*P*
Control	18	19.5	0.231	0.631
Hidden	27	21.5	2.814	0.093
Visible	17	21	1.524	0.217

Pooled 2010-11 (choice in 107 of 128 trials)				
Treatment	Observed	Expected	χ^2	*P*
Control	29	35.5	2.380	0.123
Hidden	42	36.5	1.658	0.198
Visible	36	35	0.057	0.811

Table 3.2: Female choice by male treatment by year (**top**, **center**) and pooled (**bottom**). Values in expected column reflect null model of females choosing males randomly based on treatment in the subset of trials in which choice was made. Chi-square goodness of fit and p-values are also provided. Control treatment males were chosen nearly significantly less frequently than expected in 2010. Visible and Hidden males were chosen nearly significantly more frequently than expected in 2010 and 2011, respectively. See Figure 3.4 and text for more detail.

	Effects of Male Traits on Female Choice					
	2010		2011		Pooled 2010-11	
Male Trait	$F_{1,90}$	*P*	$F_{1,124}$	*P*	$F_{1,214}$	*P*
Snout-vent length	0.013	0.910	0.022	0.883	0.016	0.901
Weight	0.254	0.615	0.014	0.906	0.059	0.808
Total mite load	**4.289**	**0.041**	0.321	0.572	0.464	0.497
Nuchal pocket load	**5.090**	**0.027**	0.019	0.890	1.283	0.259
Throat coloration	0.042	0.839	0.640	0.425	0.474	0.492
Belly coloration	0.604	0.439	0.163	0.687	0.744	0.389

Table 3.3: ANOVA analysis of male traits potentially influencing female choice for any particular male. Significant associations are in **bold**. Males with higher prior total and nuchal pocket mite loads were chosen significantly more frequently by females than males with lower loads in 2010. No other male traits had a significant effect on female choice. See text for details.

	Analysis of Male Traits Within and Between Years					
	Within 2010		Within 2011		Between 2010-2011	
Male Trait	$F_{2,12}$	*P*	$F_{2,18}$	*P*	$F_{2,30}$	*P*
Snout-vent length	0.021	0.979	0.003	0.997	**43.896**	**≤0.001**
Weight	0.034	0.967	0.097	0.908	**32.536**	**≤0.001**
Total mite load	1.413	0.293	0.350	0.710	**4.791**	**0.039**
Nuchal pocket load	1.304	0.318	0.761	0.484	0.064	0.802

	Analysis of Female Traits Within and Between Years					
	2010		2011		Between 2010-2011	
Female Trait	$F_{1,66}$	*P*	$F_{1,72}$	*P*	$F_{1,138}$	*P*
Snout-vent length	0.019	0.892	0.266	0.608	**54.483**	**≤0.001**
Weight	0.068	0.795	0.188	0.666	**68.722**	**≤0.001**
Total mite load	0.001	0.979	0.201	0.655	**6.886**	**0.010**
Nuchal pocket load	0.814	0.370	0.015	0.904	0.605	0.438
Time trial started	2.379	0.128	0.180	0.673	0.129	0.720

Table 3.4: ANOVA analyses of differences in morphology and parasite load within male (**top**) and female (**bottom**) pools used in mate choice trials. Significant associations are in **bold**.

Males in 2011 were significantly larger, heavier, and had higher total mite loads than males in 2010 due to the change in study sites. However, within years males did not differ significantly between each other or treatment types.

Similarly, females in 2011 were significantly larger, heavier, and had higher total mite loads than females in 2010 due to the change in study sites. However, female choice was not significantly influenced by female body size, mite load, or trail time within either study year. See text for more details.

		2010		
		Choice (N)	Observed Proportion	*P*
C-H pairing	Control	8	0.47	1.000
	Hidden	9	0.53	
C-V pairing	Control	12	**0.80**	**0.035**
	Visible	3	**0.20**	
H-V pairing	Hidden	6	0.46	1.000
	Visible	7	0.54	

		2011		
		Choice (N)	Observed Proportion	*P*
C-H pairing	Control	7	0.35	0.263
	Hidden	13	0.65	
C-V pairing	Control	11	0.58	0.648
	Visible	8	0.42	
H-V pairing	Hidden	14	0.61	0.405
	Visible	9	0.39	

		Pooled 2010-2011		
		Choice (N)	Observed Proportion	*P*
C-H pairing	Control	15	0.41	0.324
	Hidden	22	0.59	
C-V pairing	Control	14	0.41	0.392
	Visible	20	0.59	
H-V pairing	Hidden	20	0.56	0.618
	Visible	16	0.44	

Table 3.5: Binomial tests for bias of female choice within male treatment pairings, separated by year. Expected proportion = 0.5 if no bias was present. Females displayed significant bias towards Visible treatment males in Control-Visible pairings in 2010, but otherwise exhibited no other contextual bias.

	2010			
	Throat		Belly	
	χ^2	*P*	χ^2	*P*
C-H pairing	0.222	0.637	0.222	0.637
C-V Pairing	0.000	1.000	**4.571**	**0.033**
H-V Pairing	0.077	0.782	0.077	0.782

	2011			
	Throat		Belly	
	χ^2	*P*	χ^2	*P*
C-H pairing	1.800	0.180	0.000	1.000
C-V Pairing	1.316	0.251	0.053	0.819
H-V Pairing	0.391	0.532	**5.261**	**0.022**

	Pooled 2010-11			
	Throat		Belly	
	χ^2	*P*	χ^2	*P*
C-H pairing	1.684	0.194	0.105	0.746
C-V Pairing	0.758	0.384	1.485	0.223
H-V Pairing	0.111	0.739	2.778	0.096

Table 3.6: Chi-square goodness of fit of female choice based on male throat and belly coloration within male treatment pairings. Females significantly preferred males with darker bellies in 2010 within Control-Visible pairings and in 2011 within Hidden-Visible pairings. Nearly significant bias was exhibited by females towards males with darker bellies in the pooled Hidden-Visible pairings.

Figures

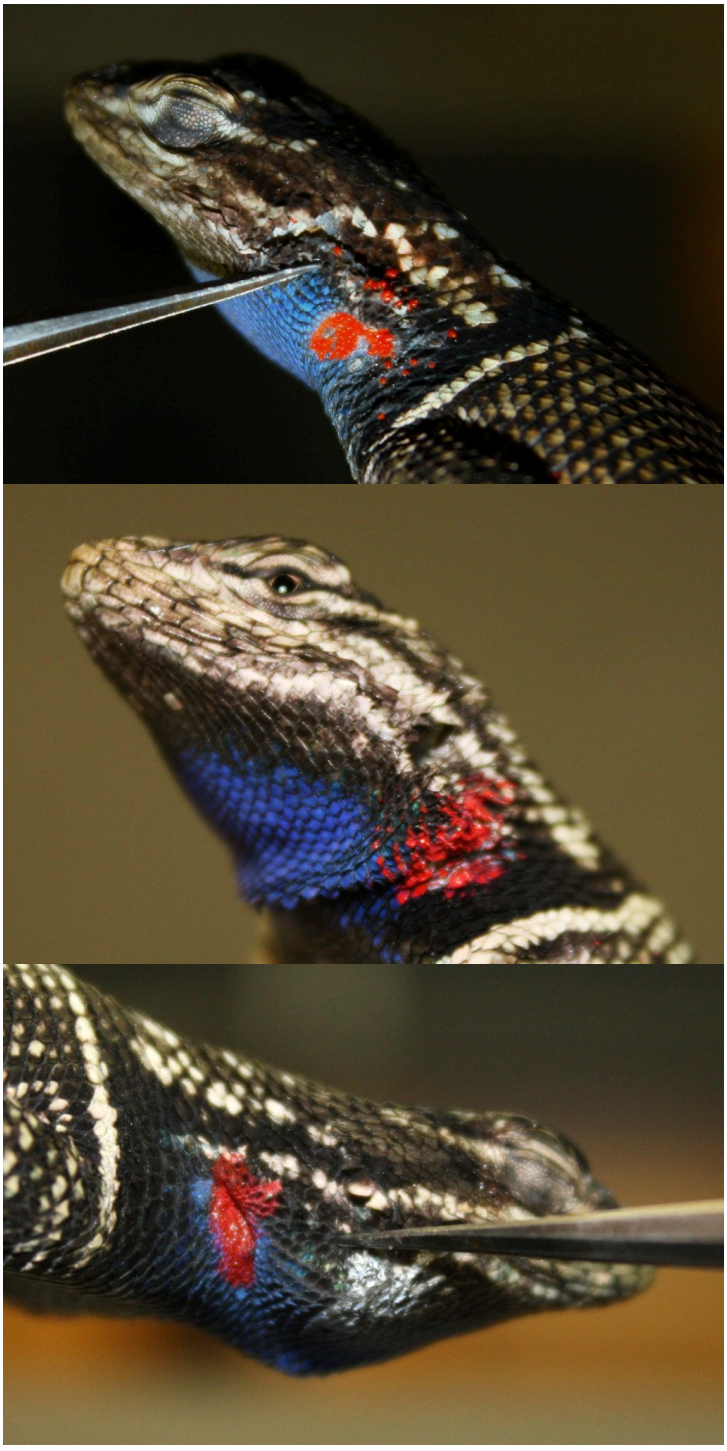
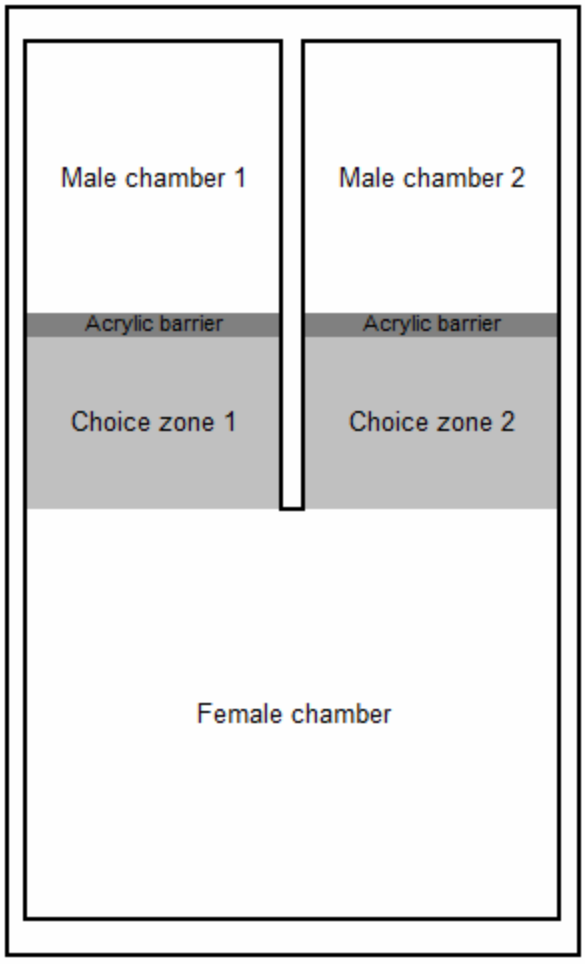


Figure 3.1: Photographs of trombiculid mites *in situ* within the pocket of an adult male (top), an adult male with visible treatment (paint spots outside the pocket to simulate mites, center), and an adult male with hidden treatment (paint spots inside the pocket, bottom). See text for details.



Arena dimensions (cm)

Total outside length = 124.46
Total outside width = 60.96
Total outside depth = 15.24

Total inside length = 120.65
Total inside width = 56.83
Total inside depth = 13.97

Female chamber length = 44.45
Female chamber width = 56.83

Male chamber length = 46.36
Male chamber width = 27.94

Choice zone length = 29.85
Choice zone width = 27.94

Acrylic thickness = 0.48

Figure 3.2: Diagram of mate choice arena with dimensions. See text for details.

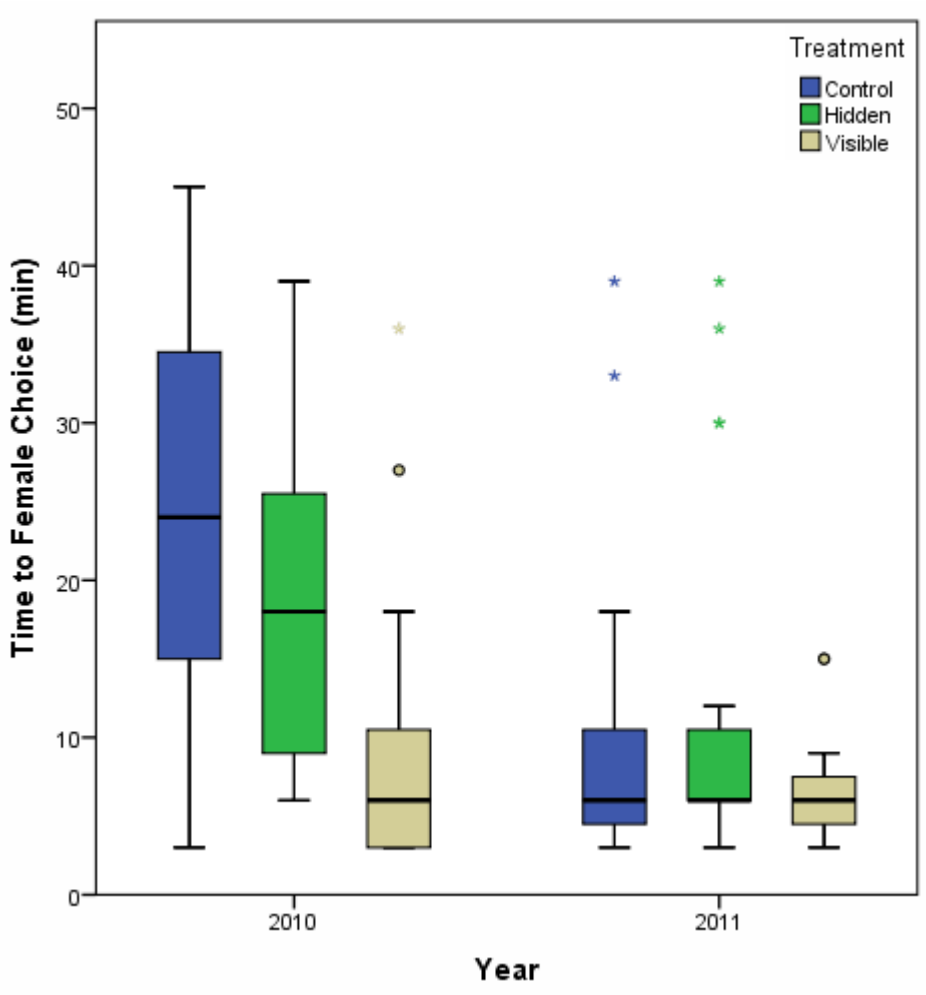


Figure 3.3: Time required for females to make a choice in mate choice trials, separated by year and male treatment. Choice was obtained significantly faster in 2011 than in 2010 (ANOVA $F_{1, 107}$=10.548, p=0.002). In 2010 trials, visible treatment males were chosen significantly faster than hidden or control males ($F_{2, 45}$=7.446, p=0.002); no significant difference occurred between male treatment groups in 2011 ($F_{2, 62}$=1.033, p=0.362).

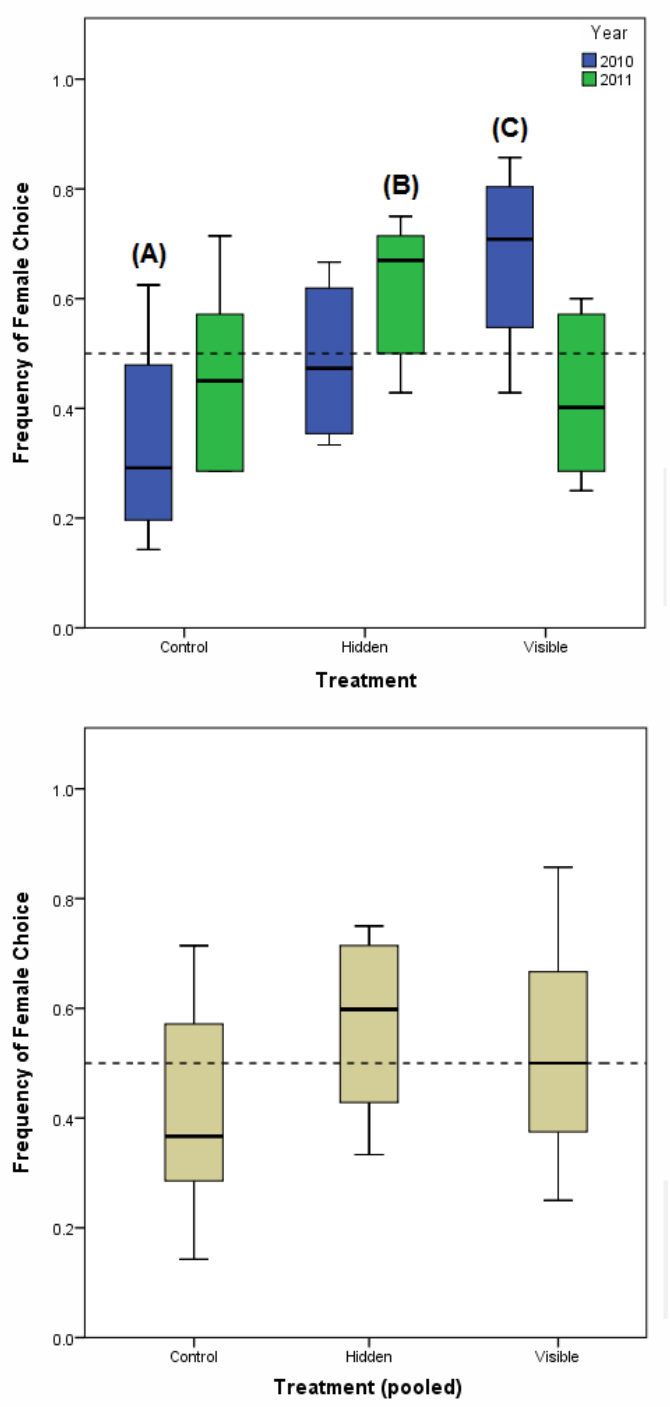


Figure 3.4: Frequency of female choice by male treatment, shown separated by year (**top**) and pooled (**bottom**). Dashed line represents the frequency of female choice by male treatment if females were choosing males randomly (null hypothesis, frequency =0.5). Nearly significant deviations from the expected frequency were observed for Control (**A**: χ^2=3.125, p=0.077) and Visible males (**C**: χ^2=3.571, p=0.059) in 2010, and for Hidden males (**B**: χ^2=2.814, p=0.093) in 2011. No significant deviations were observed when data was pooled between years. See Table 3.2 and text for more detail.

Chapter 4

Effects of Host Morphological and Ecological Variation on Ectoparastic Mite Loads and the Evolution of Mite Pockets in Phrynosomatidae (Sauria: Iguania)

Introduction

Numerous lizard species have small dermal pockets frequently associated with ectoparasitic mites. Commonly referred to as mite pockets in the literature, these structures have been described in over 120 species of lizards from twelve families, including the Phrynosomatidae (Arnold 1986; Smith 1939), Tropiduridae (Frost et al. 2001; Frost 1992), Agamidae (Bertrand and Modry 2004), Lacertidae (Salvador et al. 1999), Gekkonidae (Bauer et al. 1990; Arnold 1986; Loveridge 1925), Chamaeleonidae (Arnold 1986), Polychrotidae (Leenders 2001; Williams 1965), and Opluridae (pers. obs.). Typically occurring in the nuchal region or at the base of the limbs, pockets are characterized by thick, well-vascularlized skin with no associated musculature (Arnold 1986; Wilkinson 1985). All inhabitants of mite pockets are parasitic. Pockets are most commonly inhabited by chigger mites (Prostigmata: Trombiculidae, Leeuwenhoekiidae) (Goldberg and Bursey 1993; Goldberg and Holshuh 1992; Arnold 1986; Wilkinson 1985; Bennett 1977; pers. obs.), although scale mites (Prostigmata: Pterygosomatidae) (Bertand

and Modry 2004) and ticks (Ixodida) (Schall et al. 2000; Salvador et al. 1999) may also utilize them in some species. Mites appear to preferentially attach and feed within pockets when present (Klukowski 2004; Cunha-Barros et al. 2003; Salvador et al. 1999; Arnold 1986; Bennett 1977; Chapter 1); although the reasons for such site-specificity remain poorly understood, pockets likely offer mites ideal attachment sites and protection from exposure and physical dislodgement (Cunha-Barros et al. 2003; Salvador et al. 1999).

Lizards do not appear to receive any obvious benefit from mite pockets or the association with ectoparasites. Pockets are not induced by the feeding activities of mites, but instead are present at birth and also occur in individuals with no prior ectoparasite infestations (Goldberg and Holshuh 1992; Bauer et al. 1990; Arnold 1986; pers. obs.). The phylogenetic distribution and diversity in mite pocket location and development suggest these structures have evolved independently multiple times in unrelated lineages (Arnold 1986). Mite pockets appear to be loosely associated with certain host morphologies and ecologies (Arnold 1986; pers. obs.). Thus, very large or very small species appear to lack pockets, and pockets are absent from limbless lizards and all snakes, possibly because pockets cannot attract and concentrate mites on an animal with a large surface area (Arnold 1986). Pockets appear to occur most frequently in species inhabiting open canopied habitats in semi-tropical and tropical areas, but are rare in species inhabiting very arid habitats or high latitudes (Arnold 1986; Wilkinson 1985). Terrestrial species also appear more likely to have pockets than arboreal species. The peculiar associations between lizards, mites, and mite pockets have led the development of numerous hypotheses for mite pocket existence and function (Salvador et al. 1999;

Bauer et al. 1990; Arnold 1986; Wilkinson 1985; Appendix 4.I). However, few studies have explicitly tested these hypotheses (but see Salvador et al. 1999; Chapter 2 and 3), and the distribution and function of mite pockets remains a mystery.

Phylogenetic and non-phylogenetic comparative methods are ideal to examine the relationships between mites and mite pockets in lizards. Although non-phylogenetic comparative methods have long been used to study adaptation and the evolution of organismal traits, the inclusion of phylogenetic data is necessary to distinguish between traits shared due to common ancestry versus traits shared due to common environment (Felsenstein 1985). Numerous phylogenetic comparative methods have been developed to address this issue and tease apart the influence of phylogeny from environment (as reviewed by Martins 2000; Garland et al. 1999; Gittleman and Luh 1992; Cheverud et al. 1985). In reptiles, phylogenetic comparative methods have been used frequently to study the evolution of life-history traits (Oufiero et al. 2011, Niewiarowski et al. 2004; Miles and Dunham 1992; Dunham and Miles 1985; Stearns 1984), morphology (Oufiero et al. 2011; Bergmann and Irschick 2010; Cox et al. 2003; Butler et al. 2003; Dunham and Miles 1985), and behavior (Miles et al. 2007; Martins 1993). No study appears to have utilized phylogenetically-informed comparative methods in the analysis of parasite-host interaction in lizards.

In the present study, conventional and phylogenetically-informed comparative methods were used to examine the relationships between mites, mite pockets, and host ecology and morphology in 77 species of the Phrynosomatidae (Sauria: Iguania). Additionally, ancestral state reconstruction and phylogenetic character mapping were used to infer the evolution and diversification of mite pockets and associated host traits

within this family. Specifically, this study has four primary goals: (1) to determine what biotic and abiotic factors affect ectoparasite loads in phrynosomatid lizards; (2) to determine what factors are associated with the present occurrence and morphology of mite pockets in extant species of Phrynosomatidae; (3) to explore the origin and evolution of mite pockets in this group; and (4) to provide additional data for use in generating and testing hypotheses for mite pocket function.

Study system

The family Phrynosomatidae is an exceptional group for examining the effects of host morphology and ecology on ectoparasite loads and the evolution of mite pockets in lizards. The family is ecologically, morphologically, and behaviorally diverse, containing approximately 130 species in nine or ten genera (Wiens et al. 2010; Jones and Lovich 2009; Frost and Etheridge 1989; Smith 1939). Members of this family occur throughout North and Central America, from southern Canada to Panama, with the highest diversity in the southwestern United States and Mexico. Like many other iguanians, phrynosomatid species tend to be diurnal and terrestrial, although numerous ecological specialists are known as well. Mite pockets are present and well-developed in many members of the family (Wiens and Reeder 1997; Reeder and Wiens 1996; Arnold 1986; Wilkinson 1985; Smith 1939). Ectoparasitism in several phyrnosomatid species has been thoroughly investigated, particularly within the genera *Sceloporus* (Klukowski 2004; Klukowski and Nelson 2001; Schall et al. 2000; Foufopoulos 1999; Goldberg and Bursey 1993; Goldberg and Holshuh 1993, 1992; Goldberg and Bursey 1991b; Bennett 1977; Chapter 1) and *Uta* (Goldberg and Bursey 1991a, b; Bennett 1977; Spoecker

1967). Chiggers (larvae of mites belonging to the families Trombiculidae and Leeuwenhoekiidae) are the predominant ectoparsites of phrynosomatids, although pterygosomatid mites and ixodid ticks may also occasionally occur. Finally, the phylogenetic relationships within the Phrynosomatidae have been extensively studied, particularly for *Phrynosoma* (Hodges and Zamudio 2004; Reeder and Montanucci 2001), *Sceloporus* (Leache 2010; Leache and Mulcahy 2007; Martinez-Mendez and Mendez de la Cruz 2007; Flores-Villelea et al. 2000; Sites et al. 1992; Larsen and Tanner 1975, 1974; Smith 1939), *Urosaurus* (Feldman et al. 2011; Wiens 1993b), and *Uta* (Upton and Murphy 1997; Ballinger and Tinkle 1972).

Predicted relationships between mites, mite pockets, and hosts

Although mites are common ectoparasites of lizards, mite loads frequently vary considerably between individuals and species (Klukowski 2004; Cunha-Barros et al. 2003; Foufopoulos 1999; Werner 1983; Bennett 1977; Chapter 1). Numerous aspects of lizard morphology, ecology, and behavior are expected to affect the probability of an individual coming into contact with ectoparasites and serving as a suitable host. In the present study I focus on nine major aspects of host morphology and ecology likely to affect ectoparasitism: body size (snout-vent length), dorsal scale count, rugosity, pocket size and morphology, habitat, microhabitat, and geographic distribution measured as mid-point latitude and mean elevation. Previous studies of lizard ectoparasitism have shown that larger animals frequently have higher parasite loads (Ramirez-Morales et al. 2012; Carvalho et al. 2006; Cunha-Barros et al. 2003; Schlaepfer and Gavin 2001; Foufopoulos 1999; Bull and Burzacott 1993; Chapter 1; but see Delfino et al. 2011; Werman 1983).

Due to their greater surface area, large host species are expected to offer mites a greater number of suitable attachment sites than smaller species and are also likely easier targets for foraging ectoparasites prior to attachment. In addition to body size, ectoparasite burdens are also likely affected by scalation of the host (Cunha-Barros et al. 2003). Mite loads are predicted to be negatively correlated with dorsal scale count, a metric of body scale size in phrynosomatids (Smith 1939; see methods). Species with high dorsal counts tend to have small juxtaposed scales which fit together closely, resulting in a superficially smooth and uniform external appearance; these species present relatively little exposed skin, limiting the number and size of potential attachment sites to parasites. In contrast, species with low dorsal counts tend to have large imbricate scales with comparatively high amounts of exposed skin at the base, resulting in a very spiny appearance overall. Similarly, mite loads are predicted to increase with lizard rugosity, a metric of lizard roughness corrected for body size. Finally, because pockets tend to be the preferred attachment site when available (Klukowski 2004; Cunha-Barros et al. 2003; Salvador et al. 1999; Arnold 1986; Bennett 1977; Chapter 1), pocket size is expected to be positively correlated with mite loads.

Besides morphology, host ecology is expected to influence ectoparasite loads in lizards. Parasitic trombiculid larvae tend to prefer cool, moist microhabitats with ample refugia (Clompton and Gold 1993; Bennett 1977; Sasa 1961; Wharton 1952), and hosts which occupy these favored microhabitats generally have high mite loads (Curtis and Baird 2008; Sclaepfer and Gavin 2001; Arnold 1986). Due to differences in moisture and available soil refugia, mite loads are predicted to be lowest in lizard species occurring in arid habitats and greatest in the tropics (Arnold 1986). Positive relationships between

habitat moisture and ectoparasitic mite loads have been observed for several lizard species to date (Curtis and Baird 2008; Sclaepfer and Gavin 2001; Rubio and Simonetti 2009; Zippel et al. 1996; Spoecker 1967), but no researcher has yet examined this relationship on a larger scale (i.e. greater than five species). Based upon a similar rationale, mite loads are predicted to be positively correlated with latitude and negatively correlated with elevation. Finally, because trombiculid mites frequently inhabit and oviposit in the soil or other moist, sheltered microhabitats (Clompton and Gold 1993; Sasa 1961; Wharton and Fuller 1952), mite loads are predicted to be greatest in terrestrial lizards and least in arboreal species (Bauer et al. 1993, 1990; Arnold 1986).

Mite pockets are morphologically simple structures, often superficially similar to the lateral, nuchal, and gular skin folds common to many lizard groups. Pockets commonly occur in the same body regions and often in close association with nearby dermal folds, and are not induced by the activities of ectoparasites (Arnold 1986). As such, pockets are likely to have evolved from two basic processes: (1) from the gradual invagination and elaboration of existing shallow skin folds to form deeper, more distinct pocket-like structures; or (2) from the reduction and loss of existing folds. Lateral, nuchal, and gular skin folds have frequently been used as phylogenetic characters within the Iguania (Montanucci 1996, Frost 1992; Frost and Etheridge 1989; Etheridge and de Queiroz 1988). The gular fold is ancestrally complete in the Phrynosomatidae, meeting in the midline of the throat and merging with the lateral nuchal folds on either side of the neck. This condition is present in most genera with the exception of all species of *Sceloporus* (Frost and Etheridge 1989) and some *Phrynosoma* (Montanucci 1996). In *Sceloporus*, the gular fold is absent entirely and the ventral edges of the lateral nuchal

folds merge instead with the body wall, producing well-defined nuchal mite pockets in most species. Gular folds are present in some degree in all *Phrynosoma*, but are interrupted medially in some species. Lateral nuchal folds are present in all *Phrynosoma* and merge with the lateral aspect of the gular fold, but in this genus the boundaries of the nuchal pocket are frequently indistinct as a result.

Methods

Data Collection

Data for 77 species of Phrynosomatidae were compiled from the literature and through examination of 2425 preserved specimens from the University of Michigan Museum of Zoology and the Field Museum of Chicago. Representatives from all nine recognized genera were included: *Sceloporus* (53 species, n=1740), *Phrynosoma* (8, n=246), *Urosaurus* (6, n=164), *Uta* (4, n=130), *Uma* (2, n=34), *Callisaurus* (1, n=33), *Cophosaurus* (1, n=30), *Holbrookia* (1, n=32), and *Petrosaurus* (1, n=16). A full list of museum specimens used and literature reviewed can be found in Appendices 4.I and 4.II, respectively.

Data on trombiculid mite abundance and distribution were collected through the use of mite counts on preserved museum specimens (Appendix 4.II). The body of the lizard was subdivided into thirteen regions (Figure 4.1) and mite loads in each counted and recorded. Mite counts were used to calculate mean values for total mite load, pocket load (load within the nuchal and/or post-inguinal pockets, depending on which were

present), and non-pocket load for each species. To control for potential differences in ectoparasite loads between adults and juveniles (Salvador et al. 1999, Chapter 1), only data collected from adult individuals were incorporated into the analysis. Additionally, mite loads tend to vary by time of year, peaking in late summer and early fall in temperate species (Klukowski 2004; Foufopoulos 1999; Goldberg and Bursey 1991a; Spoecker 1967; Chapter 1); as a result, specimens collected during the summer and fall were preferred whenever possible. Specimens collected from the same general geographic region were preferentially sampled to minimize possible differences between populations of the same species. Whenever possible I focused my sampling on specimens collected recently (within the past thirty years) in good to pristine condition to minimize the probability of accidental mite detachment. Finally, because mites could potentially be lost inadvertently by previous researchers handling the same specimens, damaged, dissected, or otherwise obviously manipulated specimens were excluded from this study.

In addition to mite load and distribution, data for snout-vent length (SVL), dorsal scale count, and pocket depth and width was collected from museum specimens. Snout-vent length was measured for each specimen to the nearest 0.1 mm and mean values calculated for adult individuals for each species. In all cases, mean species SVL obtained from museum specimens was very similar to values reported in the literature. Dorsal scale counts refer to the number of scales counted in a straight line down the center of the back of the lizard, from the interparietal at the base of the head to the posterior insertion of the hindlimbs. Dorsal scale counts were first used extensively by Smith (1939, 1936) to diagnose and characterize groups of *Sceloporus*. Because dorsal counts generally

display little variation within a species regardless of age or sex, Smith argued these counts could be viewed as standardized indices of scale size relative to body size. Besides their use in systematics (Wiens 1993a, b; Frost 1992; Larson and Tanner 1975, 1974; Smith 1939, 1936), scale size and morphology also affect a species ability to thermoregulate and appear to function as adaptations to minimize water loss in many genera (Ouiferro et al. 2011, Soule and Kerfoot 1972; Soule 1966).

In addition to mean dorsal counts and body size, rugosity scores were calculated from museum specimens to correct for body size and quantify species roughness. Rugosity is simply the mean dorsal count divided by the mean SVL for the species. Species with high rugosity scores have large scales relative to body size and are very rough in general appearance, with much exposed skin between the imbricate scales; typical examples include most members of the *Sceloporus* spinosus (mean=2.92), magister (2.63), and torquatus (2.12) species groups. In contrast, species with low rugosity scores are relatively smooth in appearance, frequently with small tightly juxtaposed scales; for example the *Sceloporus* gadovae (0.69) and megalepidurus (0.79) groups, or the sand lizards *Holbrookia* (0.41) and *Uma* (0.32).

Measurements for the depth and width of the nuchal and post-inguinal pockets were collected from museum specimens. Depth was defined as the distance from the center of the opening to the deepest portion of the pocket, measured in a straight line. Width was measured as the length of the opening of the pocket, in an approximately straight line from where the pocket merges with the body wall anteriorly and posteriorly. All measurements were made to the nearest 0.01 mm with the use of a digital caliper. To better quantify the diversity within pockets in the Phrynosomatidae, pocket depth and

width were used to generate two indices – pocket type and pocket surface area. Pocket type refers to the general shape of the pocket when viewed in cross-section and was determined by the mean depth to width ratio for the species. Pockets were categorized broadly as three general types based on the mean depth to width ratio. Fold type pockets were shallow with large openings and depth to width ratios less than 0.33; these pockets tended to be simple and often resembled modified skin folds. Ovoid pockets had depth to width ratios between 0.33 and 0.66; ovoid pockets were better developed than the fold type, often larger and more visible externally. Pit pockets were those pockets with depth to width ratios greater than 0.66. In this type the opening was frequently very small, with the exterior skin flap tightly adpressed against the body wall. At the extreme, pit type pockets can appear nearly tube-like, as exemplified by some individuals of *Sceloporus horridus* and *S. nelsoni*.

Pocket depth and width were also used to estimate the surface area of the pocket. Pockets in the Phrynosomatidae tended to be roughly triangular in cross-section, with both the proximal and distal surfaces of the pocket interior available to parasites. As a result, the surface area of the pocket could be estimated as twice the area of a triangle, or simply the product of the depth times the width of the pocket. The surface area of the pocket was determined for each specimen examined and used to calculate mean pocket surface areas for the species.

Habitat and microhabitat data for each species were obtained from the literature (Appendix 4.III). Eight general habitat categories were recognized, ordered roughly by prevailing precipitation: desert, arid scrub, semi-arid scrub/grassland, dry forest, temperate evergreen forest, temperate mixed forest, temperate broadleaf forest, and

tropical evergreen forest. Microhabitat was categorized as primarily terrestrial, saxicolous, or arboreal. Specific habitat and microhabitat preferences were collected for each species and incorporated into the coding scheme above. Species found in multiple habitats or microhabitats were assigned to the category in which they are most commonly reported from.

Latitudinal and elevational data were collected from the literature and museum specimen records. Maximum and minimum latitude was obtained primarily from geographic distribution maps, with mean latitude calculated as the midpoint of the range of a species. Mean elevation for most species was calculated from minimum and maximum ranges reported in the source literature and checked against museum specimen records to ensure accuracy. Elevational data were unavailable for a small number of species; for these species, mean elevation was calculated wholly from museum specimen records.

Phylogenetic Information

The phylogenetic tree used in the analyses (Figure 4.2) was based on the combined mitochondrial and nuclear DNA phylogeny of Wiens et al. (2010; their Figure 5). This tree was chosen for multiple reasons. It represents the most comprehensive phylogeny of the Phrynosomatidae to date, including 122 of the approximately 136 recognized species. The topology of the tree was largely congruent with previously published trees using both genomic and morphological data (Wiens and Reeder 1997, Reeder 1995, Reeder and Wiens 1996, Etheridge and de Queiroz 1988, Frost and Etheridge 1989). None of the characters analyzed in the present study were used to

construct the Wiens et al. (2010) tree, thus avoiding circularity problems. Additionally, Wiens et al. (2010) included branch length data (in units of inferred nucleotide substitutions per site) in their analysis, data necessary to perform the phylogenetically independent contrast and ancestral state reconstruction analyses used this project.

The tree in Wiens et al. (2010) was modified by removing those species in which sufficient museum specimens and/or data were unavailable (n=53). Eight species which did not appear in the original tree but for which adequate morphological, ecological, and parasitological data were available were added (*Sceloporus acanthinus*, *S. asper*, *S. internasalis*, *S. teapensis*, *Urosaurus clarionensis*, *Urosaurus gadovi*, *Uta antiqua*, and *Uta nolascensis*). The relationships of each of these species with those currently in the Wiens et al. (2010) tree were inferred using other published phylogenies for *Sceloporus* (Wiens and Reeder 1997), *Urosaurus* (Feldman et al. 2011; Wiens 1993b), and *Uta* (Upton and Murphy 1997; Ballinger and Tinkle 1972). These species were added using a procedure similar to that outlined by Oufierro et al. (2011); briefly, species added to terminal branches (n=6) were added half way up the existing branch to create a sister pair of taxa with equal branch lengths. The sister species *S. asper* and *S. heterolepis* (Wiens and Reeder 1997) were most closely related to *S. malachiticus* in the Wiens et al. (2010) tree. This pair was arbitrarily set to originate one-third of the distance up the original *S. malachiticus* branch, then speciate in the middle of the remaining branch, resulting in equal branch lengths for these three species. All tree and branch manipulations were carried out in Mesquite (Maddison and Maddison 2007; version 2.60 for Windows).

Phylogenetically Independent Contrasts

Independent contrasts were generated using the PDAP module (Midford et al. 2005, version 1.15) in Mesquite (Maddison and Maddison, 2007, version 2.6 for Windows). All traits were entered as continuous variables; categorical characters such as habitat and microhabitat were coded as continuous characters through the use of dummy values in ascending order of precipitation and degree of arboreality, respectively. Although latitude, elevation, habitat, and microhabitat are not organismal characters inherited in a strict genetic sense, they can be inherited in the sense that organisms are born in and typically experience conditions similar to those of their parents (Oufierro et al. 2011; Garland et al. 1992). This assumption is reasonable for organisms with poor mobility and dispersal, such as phrynosomatid lizards.

To the 23 character traits obtained from museum specimens and the source literature, an additional 13 characters were added through transformations of the original data. Because mite load data are frequently highly variable and skewed to the right (Schall et al. 2000; Foufopoulos 1999; Werman 1983; Chapter 1), mite load indices were log 10 transformed to improve normality and meet parametric test assumptions. Additionally, because ectoparasite loads have been found to positively correlate with host body size in some species (Gutsche et al. 2012; Ramirez-Morales et al. 2012; Rubio and Simonetti 2009; Chapter 1) but not others (Delfino et al. 2011), mite load indices were also divided by mean SVL to account for differences in body size between species.

Developed by Felsenstein (1985) and as outlined in Garland et al. (2005, 1992), independent contrasts is a phylogenetically based statistical method which converts non-independent measurements taken from related taxa into independent contrasts which can

be utilized for standard statistical analyses. Raw contrasts between pairs of data from terminal sister taxa are generated through subtraction; these raw contrasts are then divided by the standard deviation, equal to the square roots of the sums of the branch lengths, to generate phylogenetically independent standardized contrasts for the terminal taxa. Contrasts deeper in the tree are generated similarly by first estimating the phenotypes of the hypothetical ancestors and applying an internal branch length correction to these estimated phenotypes before dividing by the square root of the sums of the branch lengths (Garland et al. 2005; Felsenstein 1985). The resulting interior nodes are essentially weighted averages inversely proportional to the lengths of the branches leading to the daughter nodes. To ensure contrasts are weighted equally in subsequent analyses (necessary for the use of ordinary probability tables), absolute values of standardized contrasts were plotted against their standard deviations (the square roots of the sums of the branch lengths) (PDAP Screen 1-2; Garland et al. 1992). Ten of the 36 characters were found to be adequately standardized using the original branch length data provided by Wiens et al. (2010) (Table 4.8). The remaining 26 characters were sufficiently standardized through the use of branch length transformation as described by Garland et al. (1992): square root transformation (n=20), natural log transformation (n=1), and branch length set to 1 transformation (n=4). One character (maximum elevation) could not be standardized through any of the transformations and was discarded from further analyses.

Following standardization, independent contrasts were generated for each of the remaining 35 characters using the output function of the PDAP module (Midford et al. 2005; version 1.15) and exported into SPSS for standard statistical analysis.

Ancestral State Reconstruction

In addition to producing estimates of the phenotypes of internal nodes within the phylogeny, independent contrasts can also be used to generate estimates of the root node of a phylogeny. This estimate can be viewed both as the phylogenetically correct mean value for the trait among all species in the phylogeny as well as the estimated phenotype of the hypothetical ancestor (Garland et al. 2005; Garland and Ives 2000). Computing the estimated phenotype of the root node is similar to generating standardized contrasts and is described in depth in Garland et al. (1999). Additionally, by rerooting the phylogeny and computing the calculation for the root node phenotype, it is possible to generate phenotypic estimates for any point within the tree (Garland et al. 2005, 1999).

Two methods of ancestral state reconstruction were utilized to examine the evolution of mite pockets and the characters potentially associated with mite loads. First, ancestral state reconstruction was estimated using maximum parsimony and the resulting character states traced over the phylogeny within Mesquite (Feldman et al. 2011; Maddison and Maddison 2007). Secondly, ancestral states were estimated for 29 interior nodes within the phylogeny by using Mesquite to reroot the tree at the node of interest (Garland et al. 1999); then in a novel approach, the estimated state values for these internal nodes were plotted against the distance from the root node while retaining the original tree topography. Both methods have advantages and disadvantages; character mapping is easily performed for small clades with relatively few potential character states, and is most commonly used to trace the evolution of categorical characters (see Feldman et al. 2011 for an excellent example using *Urosaurus*). However, character mapping becomes far more complex in large trees with many potential character states,

and trends in the data become less discernable. Character changes in large trees can be more easily visualized by plotting the tree topology over estimated nodal values, but this method is rather large-grained and results in loss of detail. Because nodal values represent phylogenetically correct mean estimates for numerous species (Garland et al. 2005; Garland and Ives 2000), plots using nodal estimates are also less affected by outliers in the data than simple traces over the phylogeny. Direction, relative rate of change, and divergence can be easily visualized and interpreted using nodal estimate plots, and is particularly useful for the phylogenetic analysis of trends occurring in large and complex clades.

Ancestral state reconstruction of 35 characters was performed for fifteen species groups and fourteen internal nodes in the phylogeny, including the root node (Figure 4.3). Character states at nodes were estimated using the PDAP module (Midford et al. 2005) in Mesquite after rerooting the tree at the node of interest (Garland et al. 1999). Species group nodes were based on those identified by Wiens et al. (2010) in their treatment of the Phrynosomatidae. Sixteen of the twenty *Sceloporus* groups recognized by Wiens et al. (2010) were included in this anaylsis (Table 4.1). Small groups containing only one or two species were combined with their sister group to produce eleven *Sceloporus* species group nodes containing 50 species in total: variabilis (n=6), utiformis/siniferus (n=4), pyrocephalus/gadovae/jalapae (n=5), spinosus (n=3), formosus (n=7), melanorhinus/magister (n=3), scalaris (n=3), undulatus (n=5), grammicus (n=3), megalepidurus/torquatus (n=4), and poinsetti groups (n=7). In addition, ancestral states for the Callisaurini (containing the genera *Callisaurus*, *Cophosaurus*, *Holbrookia*, and *Uma*; n=5), *Phrynosoma* (n=8), *Uta* (n=4), and *Urosaurus* (n=6) species groups were

also computed. Fourteen internal nodes representing common ancestors to these groups were also calculated, including the common ancestor (root node) to all Phrynosomatidae. Following the estimation of hypothesized ancestral character states in Mesquite, these characters were exported to SPSS and graphed as scatterplots. In all nodal plots, the ancestral character state was plotted as the dependent variable against the distance of the node from the root using Wiens et al.'s (2010) original branch length units (inferred nuclear and mitochondrial nucleotide substitutions per site) as the independent variable. The topography of the Wiens et al (2010) phylogeny was then mapped over the nodes to produce diagrams of estimated character evolution over time within different clades.

Statistical Analyses

Both conventional and phylogenetically informed statistical analyses were used. Conventional statistics are equivalent to a star phylogeny (i.e. no phylogenetic information) and are often less conservative than analyses using datasets containing phylogenetic information (Pafilis et al. 2009; Garland et al. 2005; 1999; 1992). Phylogenetically independent contrasts were generated in Mesquite and exported to SPSS for analysis. All analyses were performed in SPSS (SPSS Inc. 2011, version 20.0 for Windows) using a two-tailed critical value of $\alpha=0.05$.

Simple linear regressions and general linear models were used to test for the effects of host morphology and ecology on mite loads. Because mite loads were generally rare in many of the thirteen body categories initially examined, non-pocket body categories were combined into a single variable (Non-pocket load) in the analyses. The resulting five mite load metrics (total load, pocket load, nuchal pocket load, post-

inguinal pocket load, and non-pocket load) were log-transformed to improve normality and satisfy standard statistical assumptions for the model. In the general linear models, habitat, microhabitat, mean latitude, mean elevation, mean SVL, mean dorsal count, nuchal pocket surface area, and post-inguinal pocket surface area were used as predictor variables, with one of the five mite load indices as the response variable. Preliminary models including rugosity as a compound variable instead of snout-vent length and dorsal count tended to result in poorer fits; as a result, rugosity was excluded from the final analyses. Regressions using data generated from phylogenetically independent contrasts were performed through the origin, as specified by Garland et al. (2005, 1992).

General linear models and multiple regressions were used to test the association between mite pockets and host morphology and ecology. In preliminary analyses, nuchal pocket size (measured as surface area) was found to be significantly positively correlated with body size (SVL) in both non-phylogenetic ($p<0.001$) and phylogenetic ($p<0.001$) data sets (Figure 4.11); post-inguinal pocket size was significant for non-phylogenetic ($p=0.010$) but not phylogenetic ($p=0.422$) data sets (Table 4.6). To standardize for the effects of body size on these traits in subsequent multivariate analyses, the residuals for nuchal pocket (non-phylogenetic and phylogenetic) and post-inguinal (non-phylogenetic only) pocket size were obtained from regressions of pocket surface area on body size; these residuals were then used as response variables in lieu of the raw data. Habitat, microhabitat, mean latitude, mean elevation, and mean dorsal count were used as predictor variables in these analyses. As with the mite load data, pocket regressions using phylogenetically independent contrasts were performed through the origin (Garland et al. 2005; 1992).

Results

Factors affecting mite loads

Of the five mite load indices examined, total load, pocket load, nuchal pocket load, and non-pocket load were significantly positively correlated with each other in both phylogenetic and non-phylogenetic analyses (Pearson's correlation coefficient ranging from R=0.313 to 0.949, p=0.006 to <0.001, n=77; Table 4.2). In contrast, few significant correlations were found for post-inguinal pocket mite load and other mite load indices, occurring only for pocket load and nuchal pocket load (R=0.281, p=0.013 and R=0.299, p=0.008, respectively) in the phylogenetically-informed analysis, and for total load (R=0.313, p=0.006) in the non-phylogenetic analyses. Despite high variation in mite loads between species, the distribution of chigger mites on the body of the host was non-random and concentrated within the mite pockets (mean proportion of total load within the mite pockets=0.791 ± 0.274, n=77), particularly the nuchal pocket (0.706 ± 0.334). Correspondingly, total mite load displayed strong correlations for both mite pocket load (phylogenetic: R=0.947, p<0.001, n=76; non-phylogenetic: R=0.949, p<0.001, n=77; Figure 4.4) and nuchal pocket load (phylogenetic: R=0.889, p<0.001; non-phylogenetic: R=0.900, p<0.001; Figure 4.5). Post-inguinal pockets significantly contributed to total mite load (R=0.313, p=0.006; Figure 4.6) and total pocket load (R=0.281, p=0.013) only in the non-phylogenetic analyses. Interestingly, mite loads inside the pockets were also found to be significantly positively correlated with non-pocket mite load (i.e. the mite load occurring outside the pocket) (phylogenetic: R=0.548, p<0.001; non-phylogenetic: R=0.509, p<0.001; Figure 4.7).

Numerous host morphological and ecological variables were found to significantly affect mite loads in lizards in the univariate analyses (Table 4.3). In the phylogenetic data set, a significant negative correlation was found between all five mite load indices and mean latitude (R= -0.305 to -0.445, p= 0.007 to <0.001; Table 4.3, Figure 4.8). Significant positive associations were found between nuchal pocket surface area and all four relevant mite load indices (R= 0.383 to 0.574, p= 0.001 to <0.001; Table 4.3, Figure 4.9). Smaller but significant positive correlations were also found for post-inguinal pocket surface area and three mite load indices (R= 0.271 to 0.291, p= 0.01 to 0.016; Table 4.3). Significant positive relationships were observed for habitat and the three pocket load indices (R= 0.255 to 0.957, p= 0.025 to <0.001; Table 4.3, Figure 4.10), but not with total load (R=0.192, p=0.094) or non-pocket load (R=0.051, p=0.657). The number of significant correlations for each mite load index ranged from two (log post-inguinal mite load: latitude and habitat) to four (log pocket load: latitude, nuchal pocket surface area, post-inguinal surface area, and habitat). Mean snout-vent length, mean dorsal count, rugosity, mean elevation, and microhabitat had no significant effect on host mite loads in the phylogenetically-informed dataset.

Both phylogenetic and non-phylogenetic analyses produced qualitatively similar results though significance levels were generally higher and significant correlations more frequently found when phylogenetic data was not taken into account (Table 4.3). Non-phylogenetic results for mean latitude (R= -0.281 to -0.530, p= 0.013 to <0.001; Figure 4.8), nuchal pocket surface area (R= -0.238 to 0.621, p= 0.037 to <0.001; Figure 4.9), and habitat (R= 0.286 to 0.368, p= 0.012 to 0.001; Figure 4.10) were similar to those obtained from the phylogenetically-informed analysis. Additional significant associations in the

non-phylogenetic analyses were observed for mean snout-vent length, mean dorsal count, rugosity, and elevation. Mean elevation returned just one significant association (log nuchal pocket load, $R=0.261$, $p=0.022$). Of all variables examined, only microhabitat had no significant association with mite loads in the conventional analysis.

Multiple regressions and general linear models incorporating all eight morphological and ecological variables tended to return similar results as those found in the univariate analyses (Table 4.4). Nuchal pocket surface area ($R=0.511$, $p<0.001$), post-inguinal pocket surface area ($R=0.294$, $p=0.002$), and mean latitude ($R=-0.263$, $p=0.012$) were all found significantly correlated with log total mite load; results for log pocket load were comparable. Log nuchal pocket load was found to be significantly associated with nuchal pocket surface area ($R=0.604$, $p<0.001$) and habitat ($R=0.195$, $p=0.047$); for log post-inguinal load, a highly significant correlation was found for habitat ($R=0.951$, $p<0.001$), but no other variables were found to greatly influence load. Like total and nuchal loads, log non-pocket load returned significant correlation with nuchal pocket surface area and post-inguinal surface area ($R=0.305$, $p=0.031$ and $R=0.272$, $p=0.011$, respectively), but also displayed a near-significant association with elevation ($R=0.223$, $p=0.051$). As in the univariate analysis, mean snout-vent length, mean dorsal scale count, and microhabitat returned no significant associations with host mite loads in general linear models incorporating phylogenetically-informed data.

Similar results were obtained in models utilizing data without phylogenetic information (Table 4.4). As before, log total mite load and log pocket load were found significantly correlated with nuchal pocket surface area ($R=0.454$, $p=0.001$ and $R=0.546$, $p<0.001$, respectively), post-inguinal pocket surface area ($R=0.224$, $p=0.021$ and

R=0.204, p=0.024), and latitude (R=-0.344, p=0.003 and R=-0.271, p=0.010). Latitude was also significantly correlated with log nuchal pocket load (R=-0.274, p=0.007) and log non-pocket load (R=-0.275, p=0.049) in this model but not in the phylogenetically-informed analyses (R=-0.151, p=0.141 and R=-0.217 and p=0.063, respectively). Post-inguinal surface area and mean elevation were significant factors affecting log post-inguinal pocket loads (R=0.779, p<0.001 and R=0.216, p=0.018, respectively), but habitat had no effect (R=0.003, p=0.973). No significant relationships were found for habitat, microhabitat, mean snout-vent length, and mean dorsal count.

Factors associated with mite pockets

Pocket morphology varied considerably in both depth/width (D/W) ratio and surface area (SA) among the 77 species of Phrynosomatidae examined (Table 4.5, Figures 4.18 to 4.21). Distinct nuchal pockets were found in *Petrosaurus* (n=1), *Phrynosoma* (n=8), *Sceloporus* (n=53), *Urosaurus* (n=6), and two of the four species of *Uta* examined. Fold and shallow ovoid type nuchal pockets were characteristic of *Petrosaurus* (mean D/W=0.376), *Urosaurus* (mean D/W=0.395 ± 0.095, range=0.249-0.514), and *Uta* (mean D/W=0.314 ± 0.032, range=0.292-0.337); pockets in these genera were also small, rarely exceeding mean surface areas of 4.0 mm^2. In contrast, nuchal pockets were generally deeper, larger, and more variable in *Phrynosoma* (mean D/W=0.471 ± 0.091, range=0.294-0.582) and *Sceloporus* (mean D/W=0.483 ± 0.152, range=0.274-0.933); although ovoid type pockets were most prevalent in these genera, fold and pit forms were not uncommon, particularly in *Sceloporus*. Pocket size also varied considerably in both groups (*Phrynosoma* mean SA=19.030 ± 14.130 mm^2;

Sceloporus mean SA=9.714 ± 5.113 mm^2), reflective of the greater development and diversity of pocket morphology in these clades. Distinct post-inguinal pockets were present in just fourteen of the 77 species examined, occurring in *Holbrookia* (n=1), *Petrosaurus* (n=1), *Sceloporus* (n=6), *Urosaurus* (n=2), and *Uta* (n=4). Post-inguinal pockets were typically shallow (mean D/W=0.222-0.414, range=0.191-0.585) and very small (mean SA=1.019 ± 0.237 to 1.978 ± 0.570 mm^2) relative to nuchal pockets. Both nuchal and post-inguinal pockets were present in species of *Uta* (n=2), *Petrosaurus* (n=1), *Urosaurus* (n=2), and *Sceloporus* (n=6).

Numerous aspects of host morphology and ecology were found to be significantly associated with pocket size (surface area) in both univariate (Table 4.6) and general linear model (Table 4.7) analyses. In the phylogenetically-informed univariate analysis, nuchal pocket surface area was significantly correlated with mean snout-vent length (R=0.543, p<0.001; Figure 4.11), rugosity (R=0.365, p=0.001), and mean latitude (R=-0.349, p=0.002; Figure 4.12). Snout-vent length and rugosity were also found significantly associated with nuchal pocket size in the non-phylogenetic data set (R=0.605, p<0.001, Figure 4.11; and R=0.401, p<0.001, respectively), in addition to mean dorsal count (R=-0.229, p=0.045), mean elevation (R=0.237, p=0.038), and post-inguinal surface area (R=-0.260, p=0.022). A nearly significant negative association was also found between nuchal pocket size and mean latitude when data were analyzed in a non-phylogenetic context (R=-0.220, p=0.054; Figure 4.12).

Post-inguinal pocket surface area was found significantly negatively correlated with mean snout-vent length (R=-0.294, p=0.01), rugosity (R=-0.300, p=0.008), mean elevation (R=-0.275, p=0.015), and nuchal pocket surface area (R=-0.260, p=0.022) in

the non-phylogenetic contrast analysis; however, none of the eight morphological and ecological variables were found significantly associated with post-inguinal pocket size when phylogenetic information is included. No association was found for habitat and microhabitat for nuchal or post-inguinal pocket surface area in either analysis.

With the exception of post-inguinal pockets in the phylogenetically-informed analysis, a significant positive correlation was found for snout-vent length and pocket surface area. To correct for this association, the residuals of nuchal pocket surface area and snout-vent length were calculated and used in lieu of raw snout-vent length data in the general linear models (Table 4.7). A significant negative correlation was found for mean dorsal count and corrected nuchal pocket size in both phylogenetic (R=-0.227, p=0.030) and non-phylogenetic (R=-0.263, p=0.044) models; mean latitude and elevation were both found significant using phylogenetically independent contrast data (R=-0.368, p=0.001 and R=0.264, p=0.013, respectively) but were only marginally significant in the uninformed data set (R=-0.223, p=0.083 and R=0.210, p=0.069). Microhabitat was not significant in the phylogenetic analysis but highly significant in the non-phylogenetic model (R=-0.364, p=0.002). Analyses for post-inguinal pocket surface area recovered a single significant negative association for mean elevation (R=-0.280, p=0.030) in the non-phylogenetic data set; no significant associations were found for post-inguinal pocket surface area using phylogenetically-informed data.

Ancestral state reconstructions – common ancestor of Phrynosomatidae

Ancestral state reconstruction of twenty-one morphological, ecological, and parasitological traits of the hypothesized root node of the Phrynosomatidae is presented

in Table 4.8. This ancestor is hypothesized to have been a moderately-sized lizard (mean snout-vent length=63.55 mm) inhabiting a semi-arid scrub habitat in lowland northern Mexico. The microhabitat score (1.62) is intermediate between fully terrestrial (1) and saxicolous (2). The dorsal count (mean dorsal=118.32) is relatively high for a lizard of this body size, suggesting a smooth-bodied animal with low rugosity. A small fold-type nuchal pocket (NP depth/width=0.23, mean surface area=4.40 mm^2) was present, but post-inguinal pockets were likely absent. Mite loads appear to have been modest (log total load=1.29), with much of the load concentrated in the pocket (log nuchal pocket load=1.08), but high variance in the mite load data makes precise estimation of the ancestral mite loads difficult. For all traits the estimated upper and lower confidence intervals are well within the range of values expressed by extant species. Taken together, these character estimates suggest an ancestor morphologically and ecologically similar to some of the larger extant species of *Uta* and *Urosaurus*.

Ancestral state reconstruction – character plots

Ancestral state reconstructions of five mite load indices and ten host traits were calculated through the use of maximum parsiomony (square changed) and plotted over the phylogeny of Phrynosomatidae (Figures 4.13 to 4.25). Definite trends in these plots are often difficult to detect due to the number of taxa included and range of possible character states. Log total mite loads (Figure 4.13) were typically higher throughout the Sceloporinae (the lower dichotomy in the tree containing *Uta*, *Urosaurus*, *Petrosaurus*, and *Sceloporus*) than the Phrynosomatinae (upper dichotomy containing *Phrynosoma* and the sand lizards, Callisaurini). Within the Sceloporinae, loads remain relatively low in

the basal groups (*Uta*, *Petrosaurus*, and most *Urosaurus*) and in certain *Sceloporus* species groups, particularly formosus, magister, and grammicus groups. Total loads are comparatively high in the basal pyrocephalus, gadovae, and jalapae groups and in the derived poinsetti group. With the exception of *Cophosaurus*, mite ectoparasitism appears rare in the Callisaurini. Log pocket and log nuchal pocket mite load traces display trends very similar to those for log total load, as might be expected given the propensity of mites to occupy the pockets. In contrast, mites were rarely observed within the post-inguinal region (Figure 4.14), occurring with frequency only within *Uta*, *Urosaurus*, and the basal *Sceloporus* variabilis group (all lineages with post-inguinal pockets). However, even in these lineages post-inguinal mite loads tend to constitute a small proportion of the total mite load. Mite loads outside the pockets (Figure 4.15) were generally low in most lineages, particularly in most *Phrynosoma* (with the exception of *P. asio*), *Urosaurus*, and derived *Sceloporus* groups. Within *Sceloporus*, only the basal pyrocephalus, gadovae, and jalapae groups possessed moderately high non-pocket mite loads.

More distinct evolutionary trends were frequently obtained from reconstructions of host morphology. Body size (mean snout-vent length; Figure 4.16) was ancestrally low but increased independently in both Phrynosomatinae and Sceloporinae clades, most prominently in *Phrynosoma* and in the derived *Sceloporus* spinosus, magister, and poinsetti species groups. Body size remained small and similar to the common ancestor in *Uta*, *Urosaurus*, and in the basal *Sceloporus* species groups (variabilis, utiformis, siniferus, merriami, pyrocephalus, gadovae, and jalapae). A remarkably consistent trend was displayed for log dorsal scale count (Figure 4.17). Dorsal scale count was moderately high ancestrally, with little to no change in the Phrynosomatinae and only

increasing substantially in *Uma*. In contrast, the number of dorsal scales decreased gradually throughout the evolution of the Sceloporinae, resulting in low dorsal scale counts and high rugosity scores for nearly all *Sceloporus* groups.

Mite pockets varied considerably in size, shape, and phylogenetic distribution within the Phrynosomatidae. Based on the morphological criteria of Arnold (1986), distinct nuchal pockets occurred in all genera except *Callisaurus*, *Cophosaurus*, *Holbrookia*, and *Uma*, with questionable nuchal pockets occurring in some *Uta* (Figures 4.18 and 4.19). Small fold type pockets appear to be present in the common ancestor of the family and to have remained relatively unchanged in the *Urosaurus*, *Petrosaurus*, and *Uta*. Ovoid pockets arose independently in *Phrynosoma* and *Sceloporus*; in the latter, nuchal pockets gradually increased in size, particularly in the more derived species groups. Pit pockets occur in just seven species of *Sceloporus* and are restricted to the basal species groups (variabilis n=3; siniferus n=2; pyrocephalus n=1, and spinosus n=1).

Definitive post-inguinal pockets are uncommon in Phrynosomatidae, present only in *Uta*, *Petrosaurus*, and the basal *Sceloporus* variabilis and gadovae groups (Figures 4.20 and 4.21). Although post-inguinal pockets do not appear to have occurred in the common ancestor the Phrynosomatidae, the subsequent evolution of these pockets in the basal Sceloporinae is unclear. Post-inguinal pockets may have originated very early in this clade, and were subsequently lost independently in *Urosaurus* and derived *Sceloporus* lineages; alternatively, these pockets may have originated independently in *Uta*, *Petrosaurus*, and the *Sceloporus* variabilis species group (the presence of post-inguinal pockets in *Sceloporus gadovae* is unquestionably an independent origin).

The latitudinal and elevational distribution of the Phrynosomatidae has changed greatly since their estimated origin in northern Mexico (mean latitude=28.59; mean elevation=706.69 m) (Figures 4.22 and 4.23). The distribution of the Callisaurini has remained largely unchanged since this ancestor, occurring today primarily in the lowlands of northern Mexico and southwestern United States. In contrast, *Phrynosoma* has since dispersed throughout North America, from extreme southern Canada to Guatemala. In general, the Sceloporinae display a gradual trend toward decreasing latitude and increasing elevation, particularly in the derived species groups. Certain *Sceloporus* groups display distinct regional affinities; variabilis, utiformis, siniferus, spinosus, and formosus all appear to have originated and diversified at low latitudes near the Isthmus of Tehuantepec and Yucatan Peninsula. The undulatus group is primarily temperate in current distribution, yet appears to have arisen in central Mexico. Phrynosomatinae and the basal Sceloporinae are relatively conservative in their elevational distribution, apparently changing little since their lowland origin; in contrast, the evolution of *Sceloporus* coincides with an increasingly montane distribution, particularly in the derived scalaris, torquatus, and poinsetti groups.

In both habitat and microhabitat, members of Phrynosomatinae are very similar to the hypothesized common ancestor (Figures 4.24 and 4.25). Much greater ecological diversity is displayed in the Sceloporinae, particularly *Sceloporus*. With the exception of the pyrocephalus group and *S. teapensis* (variabilis group), basal groups tend to occur in more arid habitats while derived groups become increasingly more mesic. Microhabitat use in the Sceloporinae is equally diverse but without an overarching evolutionary trend; instead, microhabitat preferences appear to become relatively fixed early within certain

clades, leading to primarily terrestrial (scalaris, undulatus), saxicolous (pyrocephalus, torquatus), and arboreal (formosus, grammicus) groups.

Ancestral state reconstruction – species group plots

Scatterplot traces for the estimated ancestral nodes of fifteen species groups and fourteen internal nodes are presented in Figures 4.26 to 4.39. Five indices of mite load and ten host morphological and ecological traits were reconstructed and plotted against the distance from the root node in units of inferred nucleotide substitutions per site. Three rough morphological and ecological clusters appear for many of the traits examined:

1) The subfamily Phrynosomatinae, containing *Phrynosoma* (P1) and the sand lizards (*Callisaurus*, *Cophosaurus*, *Holbrookia*, and *Uma* – collectively Wiens et al.'s (2010) tribe Callisaurini, P2).

2) *Uta* (S1), *Urosaurus* (S2), *Petrosaurus*, and the basal *Sceloporus* species groups: variabilis (S3), utiformis-siniferus (S4), and pyrocephalus-gadovae-jalapae (S5).

3) The derived *Sceloporus* groups: spinosus (S6), formosus (S7), melanorhinus-magister (S8), scalaris (S9), undulatus (S10), grammicus (S11), megalepidurus-torquatus (S12), and poinsettii (S13).

These three clusters are particularly discrete for mean snout-vent length (Figure 4.26), mean dorsal scale count (Figure 4.27), habitat (Figure 4.34), and microhabitat (Figure 4.35).

Nodal plots of species groups display many of the same patterns as seen in the ancestral character traces (above), offering greater clarity of major trends at the expense

of species-level detail and potential outliers. From a modest ancestral snout-vent length (mean=63.55), of body size increased independently in the Phrynosomatinae (P1 and P2) and in the derived *Sceloporus*, most notably spinosus (S6), formosus (S7), and poinsetti (S13) groups (Figure 4.26). Body size decreased early in the evolution of the Sceloporinae, resulting in the small body size displayed in extant *Uta*, *Urosaurus*, and basal *Sceloporus* variabilis (S3), utiformis-siniferus (S4), and pyrocephalus-gadovae-jalapae (S5) groups. The number of dorsal body scales tends to gradually decrease throughout the evolution of the Sceloporinae, particularly at the transition from basal to derived *Sceloporus* groups (S1 to S5 versus S6 and onward; Figure 4.27). In contrast, the number of dorsal scales increased in the Phrynosomatinae, particularly in the sand lizards (Callisaurini – P1).

Ancestral state reconstruction of the root node indicate that the common ancestor of the Phrynosomatidae likely possessed a small fold type nuchal pocket (mean surface area=4.40 mm^2, mean depth/width=0.23) but lacked a post-inguinal pocket. Evolution of nuchal pocket type (Figure 4.28) and surface area (Figure 4.29) suggest an independent transition from fold to ovoid type coinciding with an increase in pocket surface area in both *Phrynosoma* and most Sceloporinae lineages, particularly *Sceloporus*. Nuchal pockets appear to have been secondarily and independently lost during the evolution of Callisaurini (P1) and *Uta* (S1). Within extant Phrynosomatidae, distinct post-inguinal pockets are found only in *Uta*, *Petrosaurus*, and the *Sceloporus* variabilis and gadovae groups; character reconstruction suggests post-inguinal pockets originated early in the evolution of the Sceloporinae but were lost independently in *Urosaurus* and during the transition from basal to derived *Sceloporus* groups (Figures 4.30 and 4.31). The presence

of post-inguinal pockets in *Sceloporus gadovae* (combined pyrocephalus/gadovae/jalapae group, S5) is difficult to interpret; given the phylogenetic position of this species, post-inguinal pockets may have arisen independently or may potentially be relictural.

In the Sceloporinae, latitudinal and elevational reconstructions indicate a gradual trend toward a decrease in latitude and increase in elevation throughout the evolution of the group (Figures 4.32 and 4.33). Following the early divergence of *Uta* (S1), the remaining Sceloporinae diversity appears to have originated principally in northern and central Mexico (roughly 24-26 degrees latitude). This latitudinal trend coincided with an increase in elevation, particularly in the derived *Sceloporus* groups (S6-S13). In contrast to the Sceloporinae, little distributional change occurred during the evolution of the Phrynosomatinae outside of a slight northward shift, particularly in the Callisaurini (P1).

Changes in the geographic distribution during the evolution of Sceloporinae coincided with a shift towards more mesic habitats (Figure 4.34) and increased microhabitat diversity (Figure 4.35). Once again, *Uta* (S1), *Urosaurus* (S2), and basal *Sceloporus* groups (variabilis (S3), utiformis-siniferus (S4), and pyrocephalus-gadoave-jalapae (S5) appear to diverge early to form an ecologically similar cluster of semi-arid, saxicolous/arboreal specialists. Derived *Sceloporus* appear to have originated under more mesic habitats than the basal Sceloporinae, later diversifying into saxicolous and arboreal clades. Unlike the Sceloporinae, both *Phrynosoma* and Callisaurini evolved towards an increasingly arid and terrestrial lifestyle.

Relatively few clear patterns occur in mite loads throughout the evolution of the Phrynosomatidae. With the exception of *Uta*, total and nuchal pocket loads (Figure 4.36 and 37, respectively) in the Sceloporinae have remained modest and largely unchanged

since the common ancestor (log mean total load=1.29; log mean nuchal pocket load=1.08). A slight increase in mite loads appear to occur late in the evolution of *Sceloporus*, particularly within the nuchal pocket, but high variance in data within groups prevents a definitive conclusion. Total and nuchal mite loads appear to have generally increased early in the Phrynosomatinae before returning to ancestral levels in the Callisaurini; only in *Phrynosoma* do total and nuchal loads appear to have increased greatly since the common ancestor. Results for log pocket load (combined nuchal and post-inguinal loads) are largely similar.

Mite loads in the post-inguinal region appear to have remained low throughout the evolution of the Phrynosomatidae (Figure 4.38). Post-inguinal loads were highest shortly after the divergence of the Sceloporinae and within those basal lineages (*Uta* and *Urosaurus*), but decreased independently both *Sceloporus* and Phrynosomatinae clades. Mite loads outside the pockets have increased in the Phrynosomatinae, particularly in *Phrynosoma*; in contrast, non-pocket loads decreased early in the Sceloporinae and have generally remained low throughout the group (Figure 4.39).

Discussion

(1) Factors affecting mite loads

Significant positive associations between pocket mite loads and total load (Table 4.2) are evidence that mite loads are highly concentrated within the pockets (Figure 4.4), particularly within the nuchal pockets (Figure 4.5). That this general pattern occurred in

most of the species examined, despite differences in morphology and ecology (in hosts and presumably also ectoparasites), is further evidence that pockets serve as preferred attachment sites for chigger mites. This non-random distribution of mites on the body of the host has been reported for a wide variety of other vertebrates (Garben et al. 1978; Sasa 1961; Wharton and Fuller 1952), including lizards (Klukowski 2004; Cunha-Barros et al. 2003; Salvador et al. 1999; Chilton et al. 1992; Arnold 1986; Bennett 1977; Chapter 1). The potential implications of this distribution for mite pocket function are discussed below in section (4).

Numerous aspects of host ecology and morphology were found significantly associated with mite loads in phrynosomatid lizards (Tables 4.3 and 4.4). The results obtained from conventional contrasts were typically less conservative but otherwise similar to those obtained from the phylogenetically-informed dataset. Results from univariate regressions and general linear models were also frequently comparable. In general, lizard species inhabiting preferred chigger mite microhabitats were most heavily parasitized, and those species occurring in sub-optimal mite microhabitats the least parasitized. Based on the known habitat preferences of trombiculid larvae (Clompton and Gold 1993; Bennett 1977; Sasa 1961; Wharton 1952), mite loads were predicted to be positively associated with host body size, rugosity, pocket size, and habitat; negative correlations were predicted for host dorsal scale count, latitude, elevation, and microhabitat (see Introduction). Of the eight variables examined, mean latitude was most frequently found to be significantly negatively associated with mite loads (Figure 4.8), with correlation coefficients ranging from -0.205 to -0.530. These results are consistent with the hypothesis that mite loads would increase with decreasing latitude. This

association may be due to a number of potentially interrelated factors, most importantly moisture and temperature stability. Because parasitic trombiculid larvae possess thin cuticles and readily desiccate, they are generally most abundant in shaded or sheltered microhabitats which provide ample moisture (Clompton and Gold 1993; Garben et al. 1978; Bennett 1977; Sasa 1961; Wharton 1952). Due to higher rainfall and humidity, such optimal microhabitats would be expected to be more commonly available to chiggers in the tropics than at higher latitudes. Additionally, temperature stability in the tropics presumably allows mites to breed throughout the year, potentially building up to larger and more stable populations. In contrast, populations of chigger larvae in temperate regions tend to be highly cyclic, frequently high in the late summer and early fall, and very low during winter and spring (Klukowski 2004; Foufopoulos 1999; Goldberg and Bursey 1991a; Spoecker 1967; Jones 1950).

Closely related to the trends observed in latitude, numerous significant positive associations were uncovered for habitat and mite loads (R=0.195 to 0.957), particularly for those loads occurring within the mite pockets (Tables 4.3 and 4.4; Figure 4.10). Normally reported as a categorical variable in the literature, in this study habitat was coded as a continuous variable and ordered by precipitation from most arid (desert) to most mesic (tropical rain forest). As a result, this variable possibly reflects regional moisture levels even better than latitude. As with latitude, the observed positive association between habitat and mite load is likely due to the ecological preferences of parasitic trombiculid larvae, as described above. In more arid habitats suitable mite refugia are presumably rare, likely occurring primarily in moist microhabitats near sources of water, in shaded vegetated regions, or within rock crevices (Werman 1983;

Bennett 1977). Similar associations between habitat, moisture, and mite loads have been previously reported elsewhere. In *Crotaphytus collaris* (Crotaphytidae), individuals occurring in more mesic habitats also possessed higher chigger mite loads (Curtis and Baird 2008). In both *Liolaemus tenuis* (Tropiduridae) and *Norops polylepis* (Polychrotidae), individuals inhabiting forest interiors possessed higher mite loads than those occurring at forest edges; in both species, higher mite loads in forest interiors were attributed to higher humidity and moisture (Rubio and Simonetti 2009; Schlaepfer and Gavin 2001). Similarly, subtle differences in moisture and habitat were used to explain differences in mite loads observed in the arid specialist species *Uta stejnegeri* (Phrynosomatidae) and *Uma exsul* (Phrynosomatidae) (Garcia-de la Pena et al. 2007).

Very few significant correlations were recovered for mean elevation and mite loads (Tables 4.3 and 4.4). Although the recovered associations were relatively weak (R=0.216-0.261), these results are consistent with those reported in the literature. Similar positive associations between mite load and elevation have been found in *Anolis coelestinus* and *A. cybotes* (Polychrotidae), with higher mite loads occurring in montane populations (Zippel et al. 1996); however, in these species the difference in mite loads between populations was attributed to availability of moisture in suitable habitats and not elevation *per se*. Analogous results were obtained for mite loads and elevation in *Uta stansburiana* (Phrynosomatidae), with moisture and not elevation again attributed for the observed difference (Spoecker 1967). Based on these results and those obtained in the current study, elevation appears to play a relatively minor role in affecting mite loads in lizards.

No significant associations were found between mite load and microhabitat in the present study. Similar to habitat, microhabitat was transformed into a continuous variable in the analyses, arranged in order of increasing arboreality. Because parasitic trombiculid larvae hatch from eggs laid in the soil (Garben et al. 1978; Sasa 1961), mite loads were predicted to be inversely associated with the degree of arboreality of the host. As described by Arnold (1993, 1986), mites and mite pockets appear to occur most frequently in lizard species which spend a considerable amount of time in the terrestrial environment. The presence of pockets in predominantly arboreal *Rhacodactylus* geckos (Gekkonidae) has been used as an argument against mite pocket function (Bauer et al. 1993, 1990), based on the assumption that pockets would be most functionally useful and beneficial to a terrestrial host. In *Rhacodactylus*, chigger mite loads tend to be highest in species which are largely or partially terrestrial, and lizards presumably encounter these parasites when they venture to the forest floor (Bauer et al. 1993, 1990). In *Crotaphytus collaris*, mite loads were higher in lizards occurring in terrestrial grassland microhabitats than in saxicolous individuals inhabiting boulder fields (Curtis and Baird 2008). Similarly, differences in mite loads among three species of Hispanolian *Anolis* (Polychrotidae) were attributed to variation in host microhabitat use and degree of terrestriality, with the highest mite loads occurring in the species which most frequently visited the ground (Zippel et al. 1996). Mite infestation patterns in Brazilian *Tropidurus* lizards (Tropiduridae) appear to follow similar trends (Menezes et al. 2011). Given the results of previous studies, it is unclear why no significant associations between microhabitat and mite loads were obtained in the present study. Microhabitat was recorded as simply one of three possible categories – terrestrial, saxicolous, and arboreal.

If the association between host microhabitat use and mite loads is subtle, it may be necessary to include a more sophisticated measurement of microhabitat than that used in this project. Alternatively, strict arboreality is relatively rare in the Phrynosomatidae, occurring primarily in *Urosaurus* and some *Sceloporus*, and even these species may occasionally venture to the ground. Duplicating this study on a lizard group containing a wider range of microhabitat specialists, such as the Tropiduridae, Gekkonidae, or Polychrotidae, may be necessary to discern the possible effects host microhabitat usage has on ectoparasitic mite burden.

In addition to ecological traits, numerous host morphological characters were found to be significantly associated with mite load. Positive correlations were recovered for most indices of mite load and pocket size, measured as surface area (Table 4.3 and 4.4; Figure 4.9). In general, species with larger pockets also possessed higher mite loads. These findings are consistent with the overall trend in mite loads observed in this study – the vast majority of mites on the host occurred within the pocket, most commonly the nuchal pocket (Chapter 1). As may be expected, increasing mite load specificity led to tighter relationships; this can easily be observed for the association between nuchal pocket surface area and total load, pocket load, and nuchal pocket load (R=0.511, 0.588, and 0.604, respectively, in the phylogenetically-informed general linear model). Although pocket size, number, and location have been casually implicated in affecting mite loads by previous authors (Menezes et al. 2011; Garcia-de la Pena et al. 2007; de Carvalho et al. 2006), no other study appears to have explicitly tested this association.

Of the two possible pocket types that occur in the Phrynosomatidae, a greater number of significant associations (and frequently tighter relationships) occurred between

mite load and nuchal pocket surface area. This difference in relative importance is likely a reflection of the scarcity of post-inguinal pockets and the greater importance of the nuchal pocket in contributing to mite loads. Post-inguinal pockets occurred only in *Uta*, *Petrosaurus*, and *Sceloporus* variabilis and gadovae species groups, whereas definitive nuchal pockets were found in all species examined with the exception of members of the Callisaurini and two species of *Uta*. In addition, nuchal pockets were consistently larger than post-inguinal pockets, and presumably could house an greater number of mites as a result. Nonetheless, post-inguinal pockets were found to contribute significantly to total and pocket mite loads, particularly in the phylogenetically-informed data set. As expected, post-inguinal pocket size was significantly correlated with post-inguinal mite loads in the conventional analysis; surprisingly, similar associations were not recovered using phylogenetically-informed data. These data suggest that the correlation obtained from conventional contrasts is simply a result of the phylogenetic clustering of post-inguinal pockets and that the association is not applicable to the Phyrnosomatidae as a whole. These results illustrate the importance of including phylogenetic data in analyzing character contrasts between taxa.

Several unexpected significant positive associations were observed between pocket size and non-pocket mite load (Tables 4.3 and 4.4). This relationship occurred in conventional and phylogenetically-informed analyses, particularly for the nuchal pocket ($R=0.288$-0.383, $p=0.001$-0.031). This association would be predicted to occur if mite pockets increased total ectoparasite burdens as a result of providing ideal microhabitats to the mites. Alternatively, if pockets possessed a mite-related function, then larger pockets would be expected to occur in those species with naturally high parasite loads. To

distinguish between these two possibilities, a *post hoc* regression analysis of the relationship between pocket size and the relative proportion of non-pocket mite load was performed. If pockets increase mite loads, the proportion of non-pocket mite load would be predicted to be positively correlated with mite pocket size. A standardized value of non-pocket load proportion was used in lieu of raw mite counts to control for the high amount of variability in mite loads between species; this proportion was determined simply as the log mean non-pocket mite load divided by the log mean total load for each species. This proportion of relative non-pocket mite load was then regressed onto the standardized residuals of nuchal pocket surface area and snout-vent length to control for the effects of body size. This analysis was performed using both conventional and phylogenetically-independent contrast data (Figure 4.40). In the conventional contrasts, a significant negative correlation was found between the proportion of non-pocket mite load and pocket size ($R=-0.235$, $p=0.039$); mites tend to become more concentrated within pockets as pockets increase in size. This relationship became non-significant once phylogenetic data was included ($R=0.023$, $p=0.843$), suggesting that the proportion of total mite load occurring outside the pockets has remained largely unchanged despite variation in host morphology and ecology. Nonetheless, the lack of a significant positive association between the proportion of non-pocket mite loads and pocket size suggests that pockets do not significantly contribute to total mite loads in the Phrynosomatidae.

Relatively few significant associations were recovered for the remaining host morphological characters examined. Host body size (snout-vent length) was found significantly correlated with mite loads only in simple regressions of conventional data; no relationships were found in phylogenetically-informed data or in the general linear

models (Tables 4.3 and 4.4). Snout-vent length has been found positively correlated with mite loads in numerous studies (Ramirez-Morales et al. 2012; Carvalho et al. 2006; Cunha-Barros et al. 2003; Schlaepfer and Gavin 2001; Foufopoulos 1999; Bull and Burzacott 1993; Chapter 1). Larger hosts may be easier targets for questing chigger larvae, be more likely to encounter mites due to increased mobility and higher energetic requirements, and presumably offer additional suitable attachment sites by virtue of larger surface area. However, body size does not influence mite loads in some species of lizards (Delfino et al. 2011; Garcia-de la Pena et al. 2007; Cunha-Barros et al. 2003; Werman 1983), and in other species the association appears dependent on host sex (Gutsche et al. 2012; Fuxjager et al. 2011; Garcia-de la Pena et al. 2007), habitat (Ramirez-Morales et al. 2012), or other factors (Cunha-Barros et al. 2003). The lack of congruence between results in the present analysis and those of previously published studies suggest that numerous variables potentially interact with body size to influence mite loads in lizards. Exclusive of extrinsic factors, body size is frequently closely associated with age and sex, both of which may also potentially affect parasitism in lizards. With the notable exception of *Phrynosoma*, males frequently attain larger body sizes than females in most species of Phrynosomatidae. Behavioral and hormonal differences between age classes and sexes may also greatly influence parasite loads (Fuxjager et al. 2011; Klukowski and Nelson 2001; Olsson et al. 2000; Foufopoulos 1999; Salvador et al. 1996; Garben et al. 1978). Excluding the potential interactive effects of age and sex to explicitly examine the effects of body size on mite load would be difficult but potentially rewarding (see Chapter 1).

The number of dorsal scales and host rugosity appear to have little influence on mite loads, with the only significant associations found in univariate analyses of conventional contrast data (Tables 4.3 and 4.4). These results are consistent with the prediction that mite loads would increase with host rugosity due to the greater availability of suitable attachment sites. Similar explanations have been proposed in the literature for the differences in observed mite loads between other lizard species (Delfino et al. 2011; Menezes et al. 2011; Cunha-Barros et al. 2003), but no author appears to have explicitly tested this association or attempted to quantify rugosity, as in the present study. These associations become non-significant once phylogenetic data is included in the analyses; additionally, no significant relationships are observed between rugosity and non-pocket mite loads, where differences in host body scalation would be expected to have the greatest influence. Rugosity or scalation thus appears to have little effect on mite loads in the Phrynosomatidae once the influence of phylogeny are removed, and any observed differences in mite loads between taxa are likely instead due to other morphological, ecological, and parasitological factors.

Taken in total, chigger mite loads in the Phrynosomatidae appear to be primarily affected by host latitude, habitat, and mite pocket size. Ecological associations between host latitude, habitat, and mite load appear to be mediated primarily by the preferences and constraints of the parasites, which tend to occur in cool, moist microhabitats (Clompton and Gold 1993; Garben et al. 1978; Bennett 1977; Sasa 1961; Wharton 1952). The absence of associations between mite loads and most aspects of host morphology suggests a general lack of host specificity in the ectoparasites. These results are largely consistent with those previously published elsewhere for chigger mites (Trombiculidae),

the predominant inhabitants of mite pockets (Goldberg and Bursey 1993; Goldberg and Holshuh 1992; Arnold 1986; Wilkinson 1985; Bennett 1977). Trombiculid mites are known to feed on a wide variety of vertebrate hosts (Sasa 1961; Wharton and Fuller 1952), and this lack of host specificity is presumably a result of a combination of chigger life history and simple nutritional requirements. Chiggers hatch from eggs laid in the soil into obligately parasitic larvae which actively quest for vertebrate hosts; after feeding on liquefied host tissues, larvae drop off the host and develop into free-living predators in the soil. Because they spend relatively little time on the host and are capable of effectively feeding on a wide range of taxa, in general chigger larvae appear to behave opportunistically, parasitizing the first suitable hosts encountered after hatching. Given this lack of host-specificity, mite pockets do not appear to have been the result of co-evolution between parasite and host. Instead, opportunistic mites utilize pockets whenever present and preferentially concentrate their feeding activities within them. Such behavior by the parasite does not necessarily preclude a function for mite pockets – for instance, by taking advantage of the proclivities of the mite in seeking out sheltered, enclosed microhabitats, pockets may effectively concentrate mites in specific areas on the host for various possible functions (see section (4), below).

(2) Factors associated with mite pockets

Numerous morphological and ecological factors were found to be significantly associated with mite pocket size (Tables 4.6 and 4.7); these results tended to be similar but more variable than those obtained for mite loads (section (1), above). As might be expected, snout-vent length was positively correlated with nuchal pocket size

(phylogenetic: R=0.543, p≤0.001; conventional: R=0.605, p≤0.001); larger species possess larger nuchal pockets. Mite loads were not significantly associated with body size, and as such appear unrelated to the positive association between body size and pocket surface area. Oddly, this trend did not hold for post-inguinal pockets; without phylogenetic data, post-inguinal pocket size was negatively associated with body size (R=-0.294, p=0.010), and no association was found in the phylogenetically-informed contrasts (R=0.093, p=0.422). These results are likely due to the phylogenetic distribution of nuchal and post-inguinal pockets in the Phyrnosomatidae. Nuchal pockets are common in this family and were present in most taxa examined (n=70 of 77 species). In contrast, post-inguinal pockets are primarily restricted to the basal Sceloporinae (n=14), all of which exhibit relatively small body size (Figures 4.16 and 4.26). Besides small body size and the presence of post-inguinal pockets, the basal Sceloporinae display a suite of similar character states which tend to unify them as a morphologically and ecologically homogeneous group, as described above in the ancestral state reconstruction section. As a result, it is not entirely unsurprising that the few significant associations recovered for post-inguinal pockets become insignificant once the effects of phylogeny are accounted for in the analysis. Because of this, much of the following discussion will focus primarily on factors associated with nuchal pockets.

Dorsal scale count and rugosity were significantly associated with nuchal pocket size in several of the analyses. In general, as dorsal scale count decreases, lizards become more rugose and nuchal pocket size increases. Because rugosity was a compound variable which included a body size component, the associations between rugosity and nuchal pocket size were frequently tighter and more significant than dorsal count;

however, dorsal scale count remained significant even after the effects of body size were removed through the use of residuals (Table 4.7; phylogenetic: R=-0.227, p=0.030; conventional: R=-0.263, p=0.044). These relationships are likely driven by the general phylogenetic trend within the Sceloporinae, and particularly *Sceloporus*, towards an increase in nuchal pocket size and decrease in number of dorsal scales (most easily seen in Figures 4.27 and 4.29). These results are largely supportive of those hypotheses for mite pocket function which include some element of ectoparasite concentration. If pockets function to concentrate mites, they would be most beneficial to rugose species which possess large areas of exposed skin between scales potentially available to ectoparasites. As described above, rugosity and dorsal scale count rarely significantly affected mite loads (Tables 4.3 and 4.4); however, by increasing nuchal pocket size, rugose species may effectively redirect mites into the pockets and prevent attachment elsewhere.

Nuchal pocket size was significantly associated with latitude and elevation in the analyses, particularly in the phylogenetically-independent contrasts. Nuchal pockets tended to increase in size with decreasing latitude (Figure 4.12), similar to the relationship also observed between latitude and mite load (Tables 4.3 and 4.4; Figure 4.8). These results provide additional support for hypotheses of mite pocket function which involve some aspect of mite concentration or concealment (see Chapter 1); if pockets function to concentrate mites, larger pockets would be predicted to occur in species which also inhabit regions with higher mite loads. These associations are also largely congruent with general trends in the Sceloporinae, in which the derived groups typically possess large pockets and originated or presently occur at relatively low

latitudes (Figures 4.29 and 4.32, respectively). The relationships observed between nuchal pocket size and elevation are less discernable and more difficult to interpret. Although both nuchal pocket size and elevation tend to increase in the derived Sceloporinae relative to more basal groups, there were few significant associations recovered for elevation and mite load. Mite loads do not vary significantly by elevation, and yet species occurring at higher elevations tend to possess larger nuchal pockets. The presence of this association in both conventional and phylogenetically-informed contrasts indicates this relationship is not due to the effects of phylogeny, but rather is a more general trend inherent to the Phrynosomatidae. The reasons for this trend, however, remain unclear.

Very few significant associations were observed between nuchal pocket size and habitat or microhabitat. The lack of associations, particularly for habitat, is rather surprising given the results obtained for mite loads in the present study and the predictions made elsewhere for the ecological distribution of mite pockets (Arnold 1993, 1986; Bauer et al. 1993, 1990). If pockets serve a mite-related function, pockets would be predicted to be significantly associated with the same habitats and microhabitats in which the ectoparasites predominantly occur. Mite loads, particularly those in the pockets, frequently displayed significant positive associations with habitat in both phylogenetically-informed and conventional analyses. Because habitat categories were ordered roughly by moisture content, these relationships indicate that mite loads increased with moisture, as expected based on the ecological preferences of the mites (Clompton and Gould 1993; Bennett 1977; Sasa 1961; Wharton and Fuller 1952; Figure 4.10). Despite the significant trends in mite load data, habitat appeared be unconnected

with nuchal or post-inguinal pocket size (Tables 4.6 and 4.7). Alternatively, the effects of habitat are possibly being overshadowed by other ecological variables associated with moisture, such as latitude and elevation. As may be expected, univariate regression of habitat on latitude returned a significant negative correlation for both phylogenetically-informed ($R=-0.305$, $p=0.007$) and conventional ($R=-0.514$, $p\leq0.001$) data, lending support to this alternative explanation.

Microhabitat returned just one significant association with pocket size in the conventional data (nuchal pocket: R=-0364, p=0.002); this relationship became non-significant once phylogenetic data was included. Degree of arboreality appeared to have little to no effect on the size of pockets in the Phrynosomatidae. Similarly, no associations were observed between mite loads and microhabitat (Tables 4.3 and 4.4). This finding appears contrary to the predictions that pockets would be larger and most useful in terrestrial lizards which presumably encounter more mites than arboreal species (Arnold 1993, 1986; Bauer et al. 1993, 1990). If pockets serve a definite function, these results suggest that arboreal as well as saxicolous and terrestrial species may equally benefit from the presence of pockets. Arboreal species may pick up questing mites while descending to the ground to forage, escape predators, or disperse. Additionally, unfed chigger larvae frequently display a tendancy to climb upwards from terrestrial refugia while searching for hosts (Garben et al. 1978; Jones 1950); it is possible that in doing so these mites may ascend some distance off the ground and come into contact with moderately arboreal and saxicolous lizard species. Alternatively, since degree of arboreality is in reality a continuous character, some important subtlety may be missed by defining it simply as a three state categorical character, as in the present study. Finally,

the bulk of the Phrynosomatidae are terrestrial or saxicolous, and few species are primarily arboreal; replicating this analysis on a different lizard group with more diverse and specialized microhabitat preferences, such as the Polychrotidae or Gekkonidae, may be very fruitful.

(3) Origin and Evolution of Pockets in Phrynosomatidae

Distinct nuchal pockets were present in all species examined with the exception of two species of *Uta* and all of the Callisaurini (*Callisaurus*, *Cophosaurus*, *Holbrookia*, and *Uma*). Ancestral state reconstruction indicates that small, simple nuchal pockets appear to have been present in the common ancestor of the Phrynosomtadae (Table 4.8). Based on the currently accepted higher-order relationships within this family, these data suggest that nuchal pockets were independently lost shortly after the divergence of these two lineages. In contrast, nuchal pockets in *Phrynosoma* and the remaining Sceloporinae tended to increase in size and complexity, particularly in the derived *Sceloporus* groups. Based on observations of nuchal and gular fold morphology, nuchal pockets in Phrynosomatidae appear to have originated primarily through the reduction and loss of the gular fold. In the Sceloporinae, and particularly within *Sceloporus*, gradual reduction and loss of the gular fold have resulted in the ventral edges of the lateral nuchal fold merging with the body wall, producing a well-defined nuchal pocket. In contrast, post-inguinal pockets in Phrynosomatidae likely originated through the elaboration and invagination of pre-existing folds at the junction of the hindlimb and body. Although the data in the present study are insufficient to test specific hypotheses regarding pocket origin, these hypotheses could be examined through the separate incorporation of pocket

depth and width into the independent contrast and ancestral state reconstruction analyses. Under the scenario proposed for the origination of the nuchal pocket, reduction of the gular fold is expected to correspond to a reduction in pocket width relative to depth; ancestral state reconstruction of pocket width could be used to examine the evolution of nuchal pockets in the Sceloporinae. Similarly, reconstruction of pocket depth could be used to test the invagination and elaboration hypothesis of post-inguinal pocket formation in basal Sceloporinae.

Independent elaboration and enlargement of pockets in Phrynosomatidae is congruent with multiple morphological and ecological character changes evident in the ancestral state traces (Figures 4.13 to 4.25) and nodal scatterplots (Figures 4.26 to 4.39). Evolution in nuchal pocket surface area appears to closely follow reconstructed ancestral nuchal mite loads, particularly in *Sceloporus*; likewise, trends in the evolution in post-inguinal pockets and post-inguinal load are also similar. These results suggest that historically pockets appear to have been the preferred attachment site for mites in this group of lizards. In the derived *Sceloporus* groups, an increase in nuchal pocket size also coincides with a general decrease in reconstructed non-pocket mite loads. Of particular note, non-pocket loads appear to have increased early in the evolution of *Sceloporus* shortly before divergence of the derived groups (S6-S13), while total mite loads remained unchanged. This period coincides with the loss of the post-inguinal pocket and enlargement of the nuchal pocket in *Sceloporus*. As post-inguinal pockets were lost, mites appear to have relocated outside the pockets. As nuchal pockets became larger and more developed during the evolution of the derived groups, mite attachment site preference changed accordingly, resulting in higher nuchal pocket and lower non-pocket

mite loads. Mites appear to display attachment site preference in lizards (Klukowski 2004; Cunha-Barros et al. 2003; Salvador et al. 1999; Chilton et al. 1992; Arnold 1986; Bennett 1977; Chapter 1), but underlying plasticity allows these parasites to attach in sub-optimal locations when preferred sites are unavailable; in this case, the loss of the post-inguinal pocket appears to have led to a temporary increase in mite loads outside the pocket. The resulting shift in mite load coinciding with the evolution of larger nuchal pockets could be the result of two possible non-mutually exclusive explanations. Mites may have behaved opportunistically, relocating inside the nuchal pocket as it increased in size. Alternatively, enlargement of the nuchal pocket could have occurred as a host response to increasing mite loads, indicative of a specific mite-related function for pockets. These possibilities are discussed in further detail in section (4).

In both univariate regressions and general linear models (Tables 4.6 and 4.7; Figures 4.11 and 4.12), nuchal pocket size was frequently significantly associated with body size, dorsal scale count/rugosity, latitude, and elevation. These associations are largely congruent with the evolutionary trends depicted by the ancestral state traces and scatterplots, particularly in *Sceloporus* and *Phrynosoma*. In the former group, a shift in geographic distribution towards lower latitudes and higher elevations coincided with a general increase in nuchal pocket size, body size and a decrease in dorsal scale count (i.e. increased rugosity). These data are largely consistent with the biogeographical hypotheses proposed for the origin and evolution of *Sceloporus* by Sites et al. (1992) and Leache and Mulcahy (2007).

(4) Hypotheses for Mite Pocket Function

Chigger mite loads were highly concentrated within the mite pockets in most of the phrynosomatid species examined (Table 4.2, Figures 4.4 to 4.6), and similar mite attachment site preferences have been reported for other species of lizards (Klukowski 2004; Cunha-Barros et al. 2003; Salvador et al. 1999; Arnold 1986; Bennet 1977). Pockets appear to be ideal microhabitats for parasitic mites, offering exposed skin for attachment and shelter from the host and environment. The sequestration of mites within the pocket is a crucial aspect of several of the proposed hypotheses for mite pocket function (Appendix 4.I). Mite ectoparasitism may be largely unavoidable in many circumstances, and pockets have been proposed to serve an adaptive function in making the best of a bad situation. Pockets have been suggested to contain specialized tissues which reduce and repair the damage caused by the feeding activities of mites (damage-amelioration hypothesis of Arnold 1986; Wilkinson 1985; see Chapter 2). Alternatively, pockets may concentrate mites away from more sensitive regions, such as the eyes, ears, or toes (impairment-prevention hypothesis of Salvador et al. 1999). The data presented in this study indicate that mite attachment site preference remains largely stable throughout the evolution of the Phrynosomatidae, despite variation in mite loads, pocket location and host morphology. The ease and frequency at which pockets appear to arise, attract, and successfully contain mites across a wide variety of taxa is also significant. Finally, ancestral state reconstruction suggests that the evolution of larger nuchal pockets in *Sceloporus* (Figure 4.29) coincided with the geographic expansion of the genus into moist habitats (Figure 4.34) at low latitudes (Figure 4.32), regions which were significantly associated with high trombiculid mite loads in phylogenetically-informed

and conventional contrast analyses (Tables 4.3, 4.4). These results lend support to those mite-related functional hypotheses which require sequestration of ectoparasites.

When pockets are not present, filled, or otherwise unavailable (see Chapter 1), chiggers tend to form dense feeding aggregations in other regions on the host. Four of the 77 phrynosomatid species included in the present study lacked mite pockets entirely (*Callisaurus draconoides*, *Cophosaurus texanus*, *Uma inornata*, and *U. notata*). Although no ectoparasites were observed on specimens of *Uma*, chigger larvae were moderably abundant on specimens of *C. draconoides* and *C. texanus*, occurring predominantly around skin folds in the nuchal, hindlimb, and post-inguinal regions. Chigger mites display similar attachment site behaviors in other phrynosomatid species lacking pockets, congregating around the nuchal folds and eyelids in *Holbrookia maculata*, *Uta stegnegeri*, *Uta stansburiana*, and *Uma exsul* (Garcia-de la Pena et al. 2007; Goldberg and Bursey 1991a; Bennett 1977). Similar aggregations have been reported for other lizard clades in which mite pockets are absent. Chiggers parasitizing the skinks *Tiliqua rugosa* and *Mabuya agilis* (Scincidae) frequently concentrate within the axillae (Cunha-Barros et al. 2003; Chilton et al. 1992), while in *Cnemidophoris* (Teiidae) the lateral skin folds are preferred (Bennett 1977).

Although evidence here and from other studies suggest that ectoparasitic mites display specific attachment site preferences which differ between hosts, opportunistic feeding behavior cannot be completely excluded based on the data available. Differentiating between attachment site specificity and opportunism in ectoparasitic mites is very difficult, and few studies have explicitly or experimentally examined attachment site preferences. Despite this potential ambiguity, it is worth noting that opportunistic

behavior by ectoparasites does not necessarily preclude the functional significance of pockets. Even if mites acted purely opportunistically in determining host attachment site, pockets would still offer ideal microhabitats and could function to concentrate mites into specific body regions. Attachment site plasticity and opportunism may also partially explain the morphological and phylogenetic diversity in mite pockets – if pockets do provide ideal microhabitats for mites and are structurally simple to produce (as the data suggests), then morphologically variable pockets could arise independently at various locations on the body in different lineages and still successfully concentrate mites.

Providing ideal microhabitat for your parasites in the form of mite pockets is potentially problematic for the host if pockets attract additional parasites and add to total mite burden. Alternatively, by successfully concentrating mites, pockets may free up sub-optimal attachment sites elsewhere on the body for later parasitism and potentially lead to higher mite loads in species with pockets (Bauer et al. 1993, 1990). Little work has been performed examining these possibilities. In *Psammodromus algiris* (Lacertidae), sealing nuchal pockets with glue prevented ticks from attaching within (Salvador et al. 1999); in response, ticks redistributed to the ears and axillae in lizards with blocked pockets, but loads between blocked and unblocked control groups were not significantly different. In a similar unpublished study conducted by the author, blocking pockets in *Sceloporus jarrovi* (Phrynosomatidae) resulted in shift in mite distribution as mites relocated from the nuchal pocket (open animals) to the gular, nuchal non-pocket, inguinal, and hindlimb regions instead (sealed animals) (pers. obs.). In general, mite loads were not significantly different between open and sealed pocket groups; only in adult males with open pockets did mite loads significantly increase relative to sealed

treatment. Although the bulk of the mite load in nearly all species examined in the present study occurred within the mite pockets, mite loads were also generally positively correlated with mite pocket size, as would be expected if the possession of pockets contributed to higher mite loads. Additionally, a significant positive correlation was found between pocket surface area and non-pocket mite load. However, *post hoc* analyses of the relative proportion of non-pocket mite load and pocket size suggest that pockets do not increase overall mite load, but instead appear to affect the distribution of mites on the body of the host (Figure 4.40). Taken together, these data appear to indicate that pockets do not significantly contribute to mite loads in lizards, and the association observed between non-pocket mite load, pocket mite load, and pocket size is largely a result of a naturally higher number of mites occurring on some species relative to others. Although mite pockets are generally capable of concentrating mites and house the bulk of the total ectoparasite burden (Chapter 1), pockets are unable to sequester all mites that occur on the host, and the relative proportion of non-pocket mite load has remained largely stable throughout the Phrynosomatidae. The relatively low but stable proportion of mites occurring outside the pockets may result from overflow as pockets become filled and mites attach elsewhere on the body. Alternatively, non-pocket mite loads may reflect subtle differences in mite attachment site preference between parasite demographic groups, similar to that observed in ticks (Chilton et al. 1992). If pockets serve a definite function to concentrate mites, then the generally consistent presence of non-pocket mites may result from lag between mite pocket evolution and mite load; under this scenario pockets would appear to represent imperfect but largely successful adaptations.

Conclusion

In summary, examination of the abundance and distribution of chigger mites in 77 species of Phrynosomatidae revealed that the propensity of trombiculid mites to concentrate their feeding behaviors within the mite pocket is a phylogenetically widespread phenomenon, generally occurring wherever pockets are present regardless of host morphology or ecology. In most species, mite pockets housed the vast majority of the total mite load present on the host. Mite loads were found to be positively correlated with mite pocket size and host habitat, and negatively correlated with host latitude, in both phylogenetically-informed and conventional analyses. Host body size, rugosity, and microhabitat had little to no effect on ectoparasitic mite load. Mite pocket morphology varied considerably between species and tended to follow distinct phylogenetic trends. Nuchal mite pockets were found in 70 of the 77 species examined and displayed a wide range of morphological diversity, particularly within the genera *Phrynosoma* and *Sceloporus*. Nuchal pocket size was positively correlated with host body size and rugosity, and negatively with latitude in phylogenetically-informed and conventional analyses. In contrast, post-inguinal pockets were present in only 14 of 77 species, tended to be small, morphologically homogeneous, and were predominantly restricted to the basal Sceloporinae genera. Ancestral state reconstruction estimated the hypothetical common ancestor of the Phyrnosomatidae to have been a semi-terrestrial, low elevation species which occurred in a semi-arid habitat in present-day northern Mexico. This ancestral species is estimated to have possessed modestly developed nuchal mite pockets, no post-inguinal pockets, and moderate mite loads. Estimated evolution in phrynosomatid lizards following divergence from this common ancestor suggests

numerous evolutionary trends in host morphology, ecology, and parasite burdens, roughly corresponding to three major phylogenetic clusters – Phrynosomatinae, the basal Sceloporinae, and the derived *Sceloporus* species groups. Expansion and diversification of *Sceloporus* into trombiculid mite-rich habitats at low latitudes and high elevations also coincided with the evolution of larger nuchal pockets, suggesting that mite pockets in this group may function to concentrate and contain ectoparasites as proposed by various mite pocket hypotheses (see Appendix 4.1).

Prior to the present study, remarkably little study has been undertaken to examine the relationships between mites, mite pockets, and their hosts beyond casual descriptions and relatively simple examinations. Although these associations are becoming better understood through the use of phylogenetically independent contrasts and ancestral state reconstruction, this peculiar but widespread parasite-host phenomenon remains a fruitful topic for future study.

The inclusion of additional species would be useful in further examining the trends recovered in the present study. The Phrynosomatidae include approximately 136 species (Wiens et al. 2010), of which only 77 were available in sufficient numbers for study. Although the present investigation included representatives of all genera and *Sceloporus* species groups (sensu Wiens et al. 2010), the inclusion of additional species in an analysis of phylogenetically independent contrasts or ancestral state reconstruction would improve the robustness of the results obtained herein, as well as potentially resolve some of the difficulties encountered in the present study. Most notably, additional focus should be placed on the basal Sceloporinae, where the transistion from post-inguinal to nuchal pockets appears to coincide with numerous changes in host morphology, ecology,

and parasite loads. Much of the emphasis in this study was placed on *Sceloporus*, but parallel evolution of nuchal pockets appears to have occurred within *Phrynosoma* shortly after their divergence from the Callisaurini. A similar examination of the Phrynosomatinae and subsequent comparison to the trends observed in the Sceloporinae would be very informative.

Data from outside the Phrynosomatidae would be useful to analyze how mite loads and pockets may interact with host morphology and ecology in other lizard systems. Although the higher-order relationships within the Igaunia remain uncertain (Schulte et al. 2003; Frost and Etheridge 1989), several of the proposed sister groups to Phrynosomatidae also possess mite pockets, including the Tropiduridae (Frost et al. 2001; Frost 1992), Opluridae (pers. obs.), and Polychrotidae (Leenders 2001; Williams 1965). Although very high species diversity and difficulties in phylogenetic resolution may make a thorough examination of mites and pockets in the Polychrotidae difficult, both Tropiduridae and Opluridae are comparatively small groups with well-resolved phylogenies. Furthermore, many of the species in the latter two families are ecologically very similar to members of Phrynosomatidae, particularly *Sceloporus*. Inclusion of non-phrynosomatids as outgroups in the phylogenetic analysis would also be useful in better understanding the origin and early evolution of pockets within the Phrynosomatidae. The distribution of pockets in these sister families suggest that pockets have had a long evolutionary history within the Iguania, but the details of this history remain largely unknown.

Additional variables could be included in a similar analysis to explore other relationships between mites and their hosts or to examine alternative hypotheses for mite

pocket function. For example, mite loads in males tend to be higher than females in some lizard species (Gutsche et al. 2012; Garcia-de la Pena et al. 2004; Klukowski and Nelson 2001; Olsson et al. 2000; Foufopoulos 1999; Salvador et al. 1996; Zippel et al. 1996) but not in other species (Raimrez-Morales et al. 2012, Delfino et al. 2011; Curtis and Baird 2008; Schlaepfer and Gavin 2001); to better control for the potential effects of sex on mite loads, males and females could be analysed separately, rather than pooled (as in the present study). Additional information, such as reproductive strategy or degree of sexual dimorphism, could also be included in the model to better understand how mite loads may differ between sexes as well as species. Other aspects of host morphology or ecology which potentially affect mite loads, such as foraging behavior or diel activity, could be included to better refine existing models. Phylogenetically independent comparative methods could be used to test the bite hold hypothesis for mite pocket function (Appendix 4.I) in a wide range of lizard taxa if data on reproductive behavior and pocket location were included in the analysis. Similarly, the mate choice hypothesis for pocket function is reliant on mites being physically observable by conspecifics; if pockets conceal mites, they would be predicted to occur primarily in diurnal, visually-oriented species. Comparative methods could compliment experimental studies in testing these and other hypotheses for mite pocket function.

Ancestral state reconstruction and mapping would benefit greatly from the inclusion of fossil and biogeographic data. Such data could be used to better estimate the origin and subsequent evolution of traits, the rate at which traits and associations evolve, and in timing the divergence of taxa. Fossil and biogeographic data have been previously applied in a phylogenetic context to explain the present diversity and distribution of

Sceloporus (Leache and Mulcahy 2007; Sites et al. 1992), and these data could be similarly applied to better reconstruct ancestral character states. Although phrynosomatids are relatively rare in the fossil record and appear to have garnered little attention, representatives of anatomically modern *Sceloporus*, *Holbrookia*, and *Phrynosoma* were known to be present by the early Miocene, approximately 25 million years ago (Yatkola 1976; Robinson and van Devender 1973; Holman 1970). Molecular data and biogeography can also be used to estimate divergence times (Leache and Mulcahy 2007), and these data could easily be incorporated into ancestral state reconstruction analyses to set divergence time for taxa. Biogeographic data in both Sites et al. (1992) and Leache and Mulcahy (2007) are largely congruent with the ancestral state models for the ecological traits examined in the present study, but additional information would be useful in further testing the predictions made by the model.

Finally, in focusing primarily on host morphology and ecology, the present study has explored only half of the story of mites and mite pockets. Parasitological variables, such as host-specificity, ecology, and diversity where largely beyond the scope of this study, yet play an undoubtedly important role in this parasite-host interaction. Relatively few studies have examined the mites associated with mite pockets, with nearly all of the focus directed towards species which commonly parasitize humans or domesticated animals. The diversity of mites parasitizing lizards remains little known, but limited evidence suggests this diversity may be extensive; for example, in his survey of trombiculid mite diversity occurring on lizards from southern Arizona, Bennett (1977) recognized fourteen species of trombiculid mites from seven genera parasitizing 24 lizard species. All but one of these trombiculid species displayed little to no host specificity,

but were instead primarily associated with specific geographic distributions and habitats. Several of the trombiculid mite species described by Bennett (1977) appeared to display some degree of attachment site specificity, and similar results have also been reported for ticks (Chilton et al. 1992; Petney and Al-Yaman 1985; Andrews et al. 1982; Andrews and Petney 1981) and pterygosomatid mites (Bertand 2000). Attachment site preference may commonly occur in other ectoparasitic mites, but unfortunately mite taxonomy and diversity is rarely a focus in the mite pocket literature, and most trombiculid mite infestations in North American lizards are simply attributed (possibly erroneously) to the widespread generalist *Eutrombicula alfreddugesi*. However, additional study of attachment site preference could be beneficial in potentially explaining the current phylogenetic distribution, location, and morphological diversity of mite pockets. If chigger species vary in their attachment site preferences, perhaps the development and subsequent loss of post-inguinal pockets observed in the basal Sceloporinae coincided with taxonomic changes in parasite burden. Such a scenario could be used as evidence for adaptation and mite pocket function.

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References

Andrews R.H. and T.N. Petney. 1981. Competition for sites of attachment to hosts in three parapatric species of reptile tick. *Oecologica* 51: 227-232.

Andrews R.H., T.N. Petney, and C.M. Bull. 1982. Niche changes between parasite populations: an example from ticks on reptiles. *Oecologica* 55: 77-80.

Arnold E.N. 1986. Mite pockets of lizards, a possible means of reducing damage by ectoparasites. *Biological Journal of the Linnean Society* 29:1-21.

Arnold E.N. 1993. Comment – Function of the mite pockets of lizards: an assessment of a recent attempted test. *Canadian Journal of Zoology* 71(4): 862-864.

Ballinger R.E. and D.W. Tinkle. 1972. Systematics and evolution of the genus *Uta* (Sauria: Iguanidae). *Miscellaneous Publications of the Museum of Zoology, University of Michigan*: 145: 1-88.

Bauer A.M., A.P. Russell, and N.R. Dollahon. 1990. Skin folds in the gekkonid lizard genus *Rhacodactylus*: A natural test of the damage limitation hypothesis of mite pocket function. *Canadian Journal of Zoology* 68(6):1196-1201.

Bauer A.M., A.P. Russell, and N.R. Dollahon. 1993. Function of the mite pockets of lizards: a reply to E.N. Arnold. *Canadian Journal of Zoology* 71(4): 865-868.

Bennett S.G. 1977. Ecology and systematics of trombiculid mites on lizards from southern Arizona. M.S. Thesis. California State University, Long Beach. Long Beach, California. 192 pp.

Bergmann P.J. and D.J. Irschick. 2010. Alternate pathways of body shape evolution translate into common patterns of locomotor evolution in two clades of lizards. *Evolution* 64(6): 1569-1582.

Bertrand M. 2000. Morphologic adaptations to parasitism on reptiles: Pterygosomatidae (Prostigmata: Raphignathina). *In* F. Bernini, R. Nannelli, G. Nuzzacci, and E. De Lillo (eds.), Acarid Phylogeny and Evolution: Adaptations in Mites and Ticks. Proceedings of the Fourth Symposium of the European Association of Acarologists. Kluwer Academic Publishers, Dordrecht, Netherlands. Pp. 235-240.

Bertrand M. and D. Modry. 2004. The role of mite pocket-like structures on *Agama caudospinosa* (Agamidae) infested by *Pterygosoma livingstonei* sp. n. (Acari: Prostigmata: Pterygosomatidae). *Folia Parasitologica* 51: 61-66.

Butler M.A., T.W. Schoener, and J.B. Losos. 2003. The relationship between sexual size dimorphism and habitat use in Greater Antillean *Anolis* lizards. *Evolution* 54(1): 259-272.

Cheverud J.M., M.M. Dow, and W. Leutenegger. 1985. The quantitative assessment of phylogenetic constraints in comparative analyses: Sexual dimorphism in body weight among primates. *Evolution* 36(6): 1335-1351.

Chilton N.B., C.M. Bull, and R.H. Andrews. 1992. Niche segregation in reptile ticks: attachment sites and reproductive success of females. *Oecologica* 90: 255-259.

Clompton R.E. and R.E. Gold. 1993. Distribution and seasonal and diurnal activity patterns of *Eutrombicula alfreddugesi* (Acari: Trombiculidae) in a forest edge ecosystem. *Journal of Medical Entomology* 30: 47-53.

Cox R.M., S.L. Skelly, and H.B. John-Alder. 2003. A comparative test of adaptive hypotheses for sexual size dimorphism in lizards. *Evolution* 57(7): 1653-1669.

Cunha-Barros M., M. van Sluys, D. Vricibradic, C.A.B. Galdino, F.H. Hatano, and C.F.D. Rocha. 2003. Patterns of infestation by chigger mites in four diurnal lizard species from a restinga habitat (Jurubatiba) of southeastern Brazil. *Brazilian Journal of Biology* 63(3): 393-399.

Curtis J.L. and T.A. Baird. 2008. Within-population variation in free-living adult and ectoparasitic larval trombiculid mites on collared lizards. *Herpetologica* 64(2): 189-199.

De Carvalho A.L.G., A.F.B. de Araujo, and H.R. da Silva. 2006. Patterns of parasitism by *Eutrombicula alfreddugesi* (Oudemans) (Acari, Trombiculidae) in three species of *Tropidurus* Wied (Squamata, Tropiduridae) from Cerrado habitat of central Brazil. *Revista Brasileira de Zoologica* 23(4): 1010-1015.

Delfino M.M.S., S.C. Ribeiro, I.P. Furtado, L.A. Anjos, and W.O. Almeida. 2011. Pterygosomatidae and Trombiculidae mites infesting *Tropidurus hispidus* (Spix, 1825) (Tropiduridae) lizards in northeastern Brazil. *Brazilian Journal of Biology* 71(2): 549-555.

Dunham A.E. and D.B. Miles. 1985. Patterns of covariation in life history traits of squamate reptiles: the effects of size and phylogeny reconsidered. *American Naturalist* 126(2): 231-257.

Etheridge R. and K. de Queiroz. 1988. A phylogeny of the Iguanidae. *In* R. Estes and G. Pregill (eds.), Phylogenetic Relationships of the Lizard Families: Essays Commemorating Charles L. Camp. Stanford University Press, Standford, California. Pp. 283-367.

Feldman C.R., O. Flores-Villela, T.J. Papenfuss. 2011. Phylogeny, biogeography, and display evolution in the tree and brush lizard genus *Urosaurus* (Squamata: Phrynosomatidae). *Molecular Phylogenetics and Evolution* 61: 714-725.

Felsenstein J. 1985. Phylogenies and the comparative method. *American Naturalist* 125(1): 1-15.

Flores-Villela O., K.M. Kjer, M. Benabib, and J.W. Sites. 2000. Multiple data sets, congruence, and hypothesis testing for the phylogeny of basal groups of the lizard genus *Sceloporus* (Squamata, Phrynosomatidae). *Systematic Biology* 49(4): 713-739.

Foufopoulos J. 1999. Host-parasite interactions in the mountain spiny lizard *Sceloporus jarrovi*. Ph.D. Dissertation. University of Wisconsin – Madison. 215 pp.

Fuxjager M.J., J. Foufopoulos, R. Diaz-Uriarte, and C.A. Marler. 2011. Functionally opposing effects of testosterone on two different types of parasite: implications for the immunocompetence handicap hypothesis. *Functional Ecology* 25: 132-138.

Frost D.R. 1992. Phylogenetic analysis and taxonomy of the *Tropidurus* group of lizards (Iguania: Tropiduridae). American Museum Novitates 3033: 1-68.

Frost D.R. and R. Etheridge. 1989. A phylogenetic analysis and taxonomy of iguanian lizards (Reptilia: Squamata). *Miscellaneous Publications of the Museum of Natural History, University of Kansas* 81:1-65.

Frost D.R., M.T. Rodrigues, T. Grant, and T.A. Titus. 2001. Phylogenetics of the lizard genus *Tropidurus* (Squamata: Tropiduridae: Tropidurinae): Direct optimization, descriptive efficiency, and sensitivity analysis of congruence between molecular data and morphology. *Molecular Phylogenetics and Evolution* 21(3):352-371.

Garben A.F.M., J.E.M.H. van Bronswijk, and T. van Ebbenhorst Tengbergen. 1978. Distribution and dispersal of the chigger *Neotrombicula autumnalis* (Shaw, 1790) (Trombiculidae, Acari). Part I: The behavior of the unfed and feeding larvae. *Netherlands Journal of Zoology* 28(2): 193-205.

Garcia-de la Pena C., G. Castaneda, and C.W. Barrows. 2007. Infestation by chigger mites in two lizard species from a dune habitat of northern Mexico. *Texas Journal of Science* 59(1): 23-32.

Garland T.E. and A. Ives. 2000. Using the past to predict the present: Confidence intervals for regression equations in phylogenetic comparative methods. *The American Naturalist* 155(3): 346-364.

Garland T.E., A.W. Dickerman, C.M. Janis, and J.A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Systematic Biology* 42(3): 265-292.

Garland T.E., P.E. Midford, and A.R. Ives. 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. *American Zoologist* 39(2): 374-388.

Garland T.E., A.F. Bennett, and E.L. Rezende. 2005. Phylogenetic approaches in comparative physiology. *Journal of Experimental Biology* 208: 3015-3035.

Gittleman J.L. and H.-K. Luh. 1992. On comparing comparative methods. *Annual Review of Ecology and Systematics* 23: 383-404.

Goldberg S.R., and C.R. Bursey. 1991a. Integumental lesions caused by ectoparasites in a wild population of the side-blotched lizard (*Uta stansburiana*). *Journal of Wildlife Diseases* 27(1): 68-73.

Goldberg S.R. and C.R. Bursey. 1991b. Duration of attachment by mites and ticks on the iguanid lizards *Sceloporus graciosus* and *Uta stansburiana*. *Journal of Wildlife Diseases* 27(4): 719-722.

Goldberg S.R. and C.R. Bursey. 1993. Duration of attachment of the chigger, *Eutrombicula lipovskyana* (Trombiculidae) in mite pockets of Yarrow's spiny lizard, *Sceloporus jarrovi* (Phrynosomatidae) from Arizona. *Journal of Wildlife Diseases* 29(1): 142-144.

Goldberg S.R., and H.J. Holshuh. 1992. Ectoparasite-induced lesions in mite pockets of the Yarrow's spiny lizard, *Sceloporus jarrovii* (Phrynosomatidae). *Journal of Wildlife Diseases* 28(4): 537-541.

Goldberg S.R., and H.J. Holshuh. 1993. Histopathology in a captive Yarrow's spiny lizard, *Sceloporus jarrovii* (Phrynosomatidae), attributed to the mite *Hirstiella* sp. (Pterygosomatidae). *Transactions of the American Microscopical Society* 112(3): 234-237.

Gutsche A., F. Mutschmann, W.J. Streich, and H. Kampen. 2012. Ectoparasites in the endangered Utila spiny-tailed iguana (*Ctenosaura bakeri*). *Herpetological Journal* 22(3): 157-161.

Hodges W.L. and K.R. Zamudio. 2004. Horned lizard (*Phrynosoma*) phylogeny inferred from mitochondrial genes and morphological characters: understanding conflicts using multiple approaches. *Molecular Phylogenetics and Evolution*: 31: 961-971.

Holman J.A. 1970. Herpetofauna of the Wood Mountain Formation (Upper Miocene) of Saskatchewan. *Canadian Journal of Earth Sciences* 7(5): 1317-1325.

Jones B.M. 1950. The sensory physiology of the harvest mite *Trombicula autumnalis* Shaw. *Journal of Experimental Biology* 27: 461-494.

Klukowski M. 2004. Seasonal changes in abundance of host-seeking chiggers (Acari: Trombicuilidae) and infestations on fence lizards, *Sceloporus undulatus*. *Journal of Herpetology* 38(1): 141-144.
Klukowski M. and C.E. Nelson. 2001. Ectoparasite loads in free-ranging northern fence lizards, *Sceloporus undulatus hyacinthinus*: effects of testosterone and sex. *Behavioral Ecology and Sociobiology* 49: 289-295.
Larsen K.R. and W.W. Tanner. 1974. Numeric analysis of the lizard genus *Sceloporus* with special reference to cranial osteology. *Great Basin Naturalist* 34: 1-41.
Larsen K.R. and W.W. Tanner. 1975. Evolution of the sceloporine lizards (Iguanidae). *Great Basin Naturalist* 35: 1-20.
Leache A.D. 2010. Species trees for spiny lizards (Genus *Sceloporus*): Identifying points of concordance and conflict between nuclear and mitochondrial data. *Molecular Phylogenetics and Evolution* 54:162-171.
Leache A.D. and D.G. Mulcahy. 2007. Phylogeny, divergence times, and species limits of spiny lizards (*Sceloporus magister* species group) in western North American deserts and Baja California. *Molecular Ecology* 16:5216-5233.
Leenders T. 2001. A Guide to Amphibians and Reptiles of Costa Rica. Distribuidores Zona Tropical, S.A.: Miami, Florida. Pp. 184-185.
Loveridge A. 1925. A mite pocket in the gecko, *Gymnodactylus lawderanus* Stoliczka. *Proceedings of the Zoological Society, London* 1925: 1431.
Maddison W.P. and D.R. Maddison. 2007. Mesquite: A Modular System for Evolutionary Analysis. Version 2.6. http://mesquiteproject.org.
Martinez-Mendez N. and F.R. Mendez-de la Cruz. 2007. Molecular phylogeny of the *Sceloporus torquatus* species-group (Squamata: Phrynosomatidae). *Zootaxa* 1609: 53-68.
Martins E.P. 1993. A comparative study of the evolution of *Sceloporus* push-up displays. *American Naturalist* 142(6): 994-1018.
Martins E.P. 2000. Adaptation and the comparative method. *Trends in Ecology and Evolution* 15(7): 296-299.
Menezes V.A., A.F. Fontes, D. Gettinger, M. van Sluys, and C.F.D. Rocha. 2011. A morphometric study of *Eutrombicula alfreddugesi* (Acari: Trombiculidae) infesting four sympatric species of *Tropidurus* (Squamata: Tropidurudiae) in northeastern Brazil. *Phyllomedusa* 10(1): 79-84.
Midford P.E., T. Garland Jr., and W.P. Maddison. 2005. PDAP Package of Mesquite. Version 1.07. http://mesquiteproject.org/pdap_mesquite/index.html.
Miles D.B. and A.E. Dunham. 1992. Comparative analysis of phylogenetic effects in the life-history patterns of iguanid reptiles. *American Naturalist* 139(4): 848-869.
Miles D.B., J.B. Losos, and D.J. Irshick. 2007. Morphology, performance, and foraging mode. In Lizard Ecology: The Evolutionary Consquences of Foraging Mode. Eds. S.M. Reilly, L.D. McBrayer, and D.B. Miles. Cambridge University Press, Cambridge. Pp. 49-93.
Montanucci R.R. 1996. Morphological variation in the gular fold in the horned lizard genus, *Phrynosoma* (Iguania: Phrynosomatidae). *Herpetologica* 52(1): 46-55.
Niewiarowski P.H., M.J. Angilletta, and A.D. Leache. 2004. Phylogenetic comparative analysis of life-history variation among populations of the lizard *Sceloporus undulatus*: an example and prognosis. *Evolution* 58(3): 619-633.

Olsson M., E. Wapstra, T. Madsen, and B. Silverin. 2000. Testosterone, ticks, and travels: A test of the immunocompetence-hanidcap hypothesis in free-ranging male sand lizards. *Proceedings of the Royal Society, London B* 267: 2339-2343.

Oufiero C.E., Gartner G.E., Adolph S.C., and T. Garland. 2011. Latitudinal and climatic variation in body size and dorsal scale counts in *Sceloporus* lizards: a phylogenetic perspective. *Evolution* 65(12): 3590-3607.

Pafilis P., J. Foufopoulos, N. Poulakakis, P. Lymberakis, and E.D. Valakos. 2009. Tail shedding in island lizards (Lacertidae, Reptilia): Decline of antipredator defenses in relaxed predation environments. *Evolution* 63(5): 1262-1278.

Pagel M. 1999. The maximum likelihood approach to reconstruction ancestral character states of discrete characters on phylogenies. *Systematic Biology* 48(3): 612-622.

Petney T.N. and F. Al-Yaman. 1985. Attachment sites of the tortoise tick *Hyalomma aegyptium* in relation to tick density and physical condition of the host. *Journal of Parasitology* 71: 287-289.

Ramirez-Morales R., T. Lislevand, A. Retana-Salazar, T. Solhoy, and S. Roth. 2012. Ectoparasite loads of the Central American whiptail lizard *Ameiva festiva* (Squamata: Teiidae). *Herpetological Journal* 22(3): 151-155.

Reeder T.W. and R.R. Montanucci. 2001. Phylogenetic analysis of the horned lizards (Phrynosomatidae: *Phrynosoma*): evidence from mitochondrial DNA and morphology. *Copeia* 2001(2): 309-323.

Reeder T.W. and J.J. Wiens. 1996. Evolution of the lizard family Phrynosomatidae as inferred from diverse types of data. *Herpetological Monographs* 10: 43-84.

Robinson M.D. and T. R. van Devender 1973. Miocene lizards from Wyoming and Nebraska. *Copeia* 1973(4): 698-704.

Rohde K. 1979. A critical evaluation of intrinsic and extrinsic factors responsible for niche restriction in parasites. *American Naturalist* 114: 648-671.

Rubio A.V. and J.A. Simonetti. 2009. Ectoparasitism by *Eutrombicula alfreddugesi* larvae (Acari: Trombiculidae) on *Liolaemus tenuis* lizard in a Chilean fragmented temperate forest. *Journal of Parasitology* 95(1): 244-245.

Salvador A., J.P. Veiga, J. Martin, P. Lopez, M. Abelenda, and M. Puerta. 1996. The cost of producing a sexual signal: Testosterone increases the susceptibility of male lizards to ectoparasite infestation. *Behavioral Ecology* 7(2): 145-150.

Salvador A., J.P. Veiga, and E. Civantos. 1999. Do skin pockets of lizards reduce the deleterious effects of ectoparasites? An experimental study with *Psammodromus algirus*. *Herpetologica* 55(1): 1-7.

Sasa M. 1961. Biology of Chiggers. *Annual Review of Entomology* 6: 221-244.

Schall J.J., H.R. Prendeville, and K.A. Hanley. 2000. Prevalence of the tick, *Ixodes pacificus*, on western fence lizards, *Sceloporus occidentalis*: trends by gender, size, season, site, and mite infestation. *Journal of Herpetology* 34(1): 160-163.

Schlaepfer M.A. and T.A. Gavin 2001. Edge effects on lizards and frogs in tropical forest fragments. *Conservation Biology* 15(4): 1079-1090.

Schulte J.A., J.P. Valladares, and A. Larson. Phylogenetic relationships within Iguanidae inferred using molecular and morphological data and a phylogenetic taxonomy of iguanian lizards. *Herpetologica* 59: 399-419.

Sites J.W., J.W. Archie, C.J. Cole, and O.F. Villela. 1992. A review of phylogenetic hypotheses for lizards of the genus *Sceloporus* (Phrynosomatidae): Implications for

ecological and evolutionary studies. *Bulletin of the American Museum of Natural History* 213: 1-110.
Smith H.M. 1936. Lizards of the Torquatus group of the genus *Sceloporus* Wiegmann, 1828. *University of Kansas Science Bulletin* 24(21): 539-693.
Smith H.M. 1939. The Mexican and Central American Lizards of the Genus *Sceloporus*. Zoological Series, Field Museum of Natural History, Vol. 26.
Soule M. 1966. Trends in the insular radiation of a lizard. *American Naturalist* 100: 47-64.
Soule M. and W. Kerfoot. 1972. On the climatic determination of scale size in a lizard. *Systematic Zoology* 21: 97-105.
Spoecker P.D. 1967. Ectoparasites of a Mojave Desert population of the lizard *Uta stansburiana stejnegeri* Schmidt. *American Midland Naturalist* 77(2): 539-542.
SPSS Inc. 2011. SPSS version 20.0 for Windows. SPSS Inc., Chicago IL.
Stearns S.C. 1984. The effects of size and phylogeny on patterns of covariation in the life history traits of lizards and snakes. *American Naturalist* 123(1): 56-72.
Upton D.E. and R.W. Murphy. 1997. Phylogeny of the side-blotched lizards (Phrynosomatidae: *Uta*) based on mtDNA sequences: Support for a midpeninsular seaway in Baja California. *Molecular Phylogenetics and Evolution* 8(1): 104-113.
Werman S.D. 1983. Population dynamics of the lizard mite, *Hirstiella pyriformis* (Acari: Pterygosomatidae) on the chuckwalla, *Sauromalus obesus* (Sauria: Iguanidae). *Journal of Parasitology* 69(2): 381-386.
Wharton G.W. and H.S. Fuller. 1952. A manual of the chiggers. *Memoirs of the Entomological Society of Washington* 4: 1-185.
Wiens J.J. 1993a. Phylogenetic relationships of the phrynosomatid lizards and monophyly of the *Sceloporus* group. *Copeia* 1993(2): 287-299.
Wiens J.J. 1993b. Phylogenetic systematics of the tree lizards (genus *Urosaurus*). *Herpetologica* 49(4): 399-420.
Wiens J.J. and T.W. Reeder. 1997. Phylogeny of the spiny lizards (Sceloporus) based on molecular and morphological evidence. *Herpetological Monographs* 11: 1-101.
Wiens J.J., C.A. Kuczynski, S. Arif, and T.W. Reeder. 2010. Phylogenetic relationships of phrynosomatid lizards based on nuclear and mitochondrial data, and a revised phylogeny for *Sceloporus*. *Molecular Phylogenetics and Evolution* 54: 150-161.
Wilkinson M. 1985. Mite refugia in lizards. *Unpublished manuscript*.
Williams E.E. 1965. The species of Hispaniolan green anoles (Sauria: Iguanidae). *Breviora* 227:1-16
Yatkola D.A. 1976. Mid-Miocene lizards from Western Nebraska. *Copeia* 1976(4): 645-654.
Zippel K.C., R. Powell, J.S. Parmerlee Jr., S. Monks, A. Lathrop, and D.D. Smith. 1996. The distribution of larval *Eutrombicula alfreddugesi* (Acari: Trombiculidae) infesting *Anolis* lizards (Lacertilia: Polychrotidae) from different habitats on Hispaniola. *Caribbean Journal of Science* 32(1): 43-49.

Appendix 4.I: Summary of selected mite pocket hypotheses, sorted by function.

- *Nonfunctional:*
  - **Fortuitous Inhabitation** (Arnold 1986): Associations between mites and mite pockets are due to chance alone.
  - **Preservation Artifact** (Arnold 1986): Associations between mites and mite pockets are due to the unintentional detachment of mites outside mite pockets during preservation of lizards.
  - **Mite Inducement** (Wilkinson 1985): Mite pockets are induced by the feeding activity of parasitic mites.
  - **Phylogenetic Baggage** (Bauer et al. 1993; 1990): Mite pockets are the result of past adaptations or design parameters that have since lost utilitarian value.
  - **Spandrels of San Marco** (Bauer et al. 1993; 1990; Gould and Lewonton 1979): Mite pockets are the by-products of developmental processes involved in the development of skin folds.

- *Function unrelated to mites:*
  - **Physiological Function** (Arnold 1986): Mite pockets are involved in physiological functions such as water balance or the production of glandular secretions.
  - **Ecological Function** (Bauer et al. 1993; 1990): Mite pockets are utilized by the lizard for ecological functions such as crypsis, parachuting, defensive displays, or intraspecific identification.
  - **Bite Hold** (Reed, *unpublished*): Mite pockets serve as a bite hold for males during reproduction.

- *Function mite related:*
  - **Mutualistic Mites** (Arnold 1986): Mite pockets are inhabited by mites that form mutualistic associations with the lizard.
  - **Concentration/Impairment-Prevention** (Salvador et al. 1999): Pockets function to concentrate mites away from sensitive areas and prevent the impairment of vision, hearing, and motion.
  - **Concentration/Damage-Amelioration** (Arnold 1986; Chapter 2): Pockets serve to concentrate mites in specialized structures that quickly repair and contain damage caused by parasitic mites.
  - **Concentration/Handicap** (Zahavi 1977, 1975): Pockets serve to concentrate ectoparasites, which act as honest indicators of individual quality to conspecifics.
  - **Concealment – Mate Choice** (Reed, Chapter 3): Pockets serve to concentrate and conceal brightly colored mites from potential mates.
  - **Concealment – Defensive** (Reed, *unpublished*): Pockets serve to concentrate and conceal brightly colored mites to improve crypsis and avoid predation.
  - **Mite Removal** (Wilkinson 1985; Arnold 1986): Mite pockets concentrate harmful mites so they may later be removed or incapacitated.
  - **Biological Warfare** (Wilkinson 1985): Mite pockets may be used by lizard species resistant to parasitic mites to transport mites into the range of susceptible competitors, thereby giving the resistant species a competitive advantage.

Appendix 4.II: Museum specimens

Locality, number of specimens used, and catalog numbers are provided for each species used in the phylogenetic contrasts and ancestral state reconstruction analyses. Numbers in parentheses refer to specimen lots (i.e. multiple specimens under the same catalog number). FMNH: Field Museum of Natural History, Chicago; UMMZ: University of Michigan Museum of Zoology, Ann Arbor.

Callisaurus draconoides (Arizona; n=5): 69814(2), 71069(3); (California; n=11): UMMZ 51786, 67283, 69817(2), 70094, 105875, 127983, 132040, 133809, 223224, 229736; (Sonora; n=17): UMMZ 72120, 114999(3), 115000(5), 134018(6), 136158.

Holbrookia maculata (Arizona; n=5): UMMZ 91588, 91602(3), 91603; (Texas; n=27): UMMZ 52809-11, 52813, 52820-1, 52837-9, 52842, 52847-8, 52850-1, 52853-4, 52856-8, 52860, 52864-5, 69060, 69063, 81992, 114232, 126919.

Holbrookia texana (Arizona; n=30): UMMZ 69791(2), 69798, 69802(6), 105652, 105689(3), 105692(2), 105724, 105735, 105807, 105893(11), 135310.

Petrosaurus mearnsi (Baja California; n=4): UMMZ 105848(3), 105853; (Baja California Sur; n=4): UMMZ 76478(4); (California; n=8): UMMZ 69822(6), 71049(2).

Phrynosoma asio (Oaxaca; n=31): UMMZ 82398(3), 82399(6), 82400-1, 82402(2), 82403(2), 82404(6), 82410-4, 82415(2), 114974, 119573, 124769.

Phrynosoma cornutum (Arizona; n=3): UMMZ 114161, 114973, 175892; (Kansas; n=12): UMMZ 66897, 91531(3), 96056-8, 97492, 101329, 107975, 122295, 122297; (Oklahoma; n=17): UMMZ 64223, 64225, 71448, 77571, 81367-9, 81370(3), 81371(3), 81937(2), 86091, 86534.

Phrynosoma douglassii (Oregon; n=29): UMMZ 137433, 137434(2), 137435(3), 137436-7, 174152-7, 174159-62, 174164, 174166, 174173, 174183, 174192, 174194-5, 174199, 174212-4.

Phrynosoma hernandesi (Arizona; n=10): UMMZ 79194-6, 85015, 85018, 105766, 105788, 124601, 218778, 218924; (Nevada; n=17): UMMZ 43849-62, 43868-9, 85016; (New Mexico; n=3): UMMZ 123547, 127847, 133206.

Phrynosoma modestum (New Mexico; n=5): 72236, 121706, 123541-2, 124077; (Texas; n=29): UMMZ 46986-9, 51546-8, 51550, 51552, 51555-6, 51558, 51561-2, 66143, 67362, 69027, 70799-801, 71035, 77446, 91488, 91491, 91413, 114249, 121707, 123544, 142554.

Phrynosoma orbiculare (Distrito Federale; n=4): UMMZ 95191, 99921(2), 123115; (Puebla; n=3): UMMZ 63935, 105000, 117660; (St. Luis Potosi; n=19): UMMZ 77335(9), 77336(3), 77337(4), 77340, 77343, 128383; (Veracruz; n=6): UMMZ 104999, 112978(4), 112979.

Phrynosoma platyrhinos (Arizona; n=3): UMMZ 71037(3); (Utah; n=26): UMMZ 59552, 60175(3), 69471-2, 69473(6), 70640-1, 70643(3), 70644(3), 70645(2), 73411, 91832(2), 91861.

Phrynosoma solare (Arizona; n=28): UMMZ 60089(3), 65084(2), 65085, 67328, 69753-6, 72240, 72622, 79199, 91607(4), 91608(6), 91609(2), 102660, 230428.

Sceloporus acanthinus (Guatemala; n=25): UMMZ 84067(15), 107530-9.

Sceloporus aeneus (Michoacan; n=31): UMMZ 92341, 94 350, 94352, 94355-6, 94361, 94363-4, 94369, 94371-2, 94374-5, 94377-9, 94381-4, 94386-8, 94390-4, 98988, 99777, 12639.

Sceloporus asper (Michoacan; n=14): UMMZ 81959, 85399(2), 112573, 114889(3), 121608; FMNH 32038-40, 32042-3, 83876.

Sceloporus bicanthalis (Hildago; n=13): UMMZ 71440(10), 106391(2), 123019; (Vera Cruz; n=18): UMMZ 89291(3), 101936(6), 101937-9, 101940(3), 101941, 105020, 123019.

Sceloporus carinatus (Guatemala; n=57): UMMZ 98164, 109672(5), 120126(5), 120127(9), 120128(4), 120129(3), 120130, 120131(2), 120132(2), 120133(6), 120134(4), 120135, 120137-8, 120139(2), 126460-1, 126462(7), 126463.

Sceloporus chrysostictus (Yucatan; n=30): UMMZ 68204, 68208, 68209(2), 68211, 68213, 72902, 72904, 72913, 72920, 72927, 78567(2), 80866(9), 80873, 80874(3), 83117, 83118(3).

Sceloporus clarkii (Arizona; n=29): UMMZ 69878(2), 85640, 91589(9), 91604(3), 91605(3), 102681(2), 114876(3), 114877(2), 114878, 116720, 130995, 148387.

Sceloporus couchii (Coahilla; n=3): FMNH 46114, 47181, 47183; (Nuevo Leon; n=26): FMNH 25421-2, 38619-20, 116114-8, 116120, 116122, 116124, 112127, 116130-1, 116134-5, 116137-9, 179158-9, 179169; UMMZ 81890(2).

Sceloporus cozumelae (Yucatan; n=26): UMMZ 71763(3), 72891-3, 72895, 78569(4), 78570, 78571(2), 78572(2), 78573(9), 79470.

Sceloporus cyanogenys (Tamaulipas; n=25): UMMZ 102873(20), 102874-5, 102966-7, 104312.

Sceloporus dugesi (Guanajato; n=6): UMMZ 119089(5), 143720; (Michoacan; n=24): UMMZ 85406(2), 114859(6), 114860(13), 118717, 119090(2).

Sceloporus edwardtaylori (Oaxaca; n=31): UMMZ 81822-3, 81827(6), 81828(5), 81830(3), 81831-2, 81883(2), 81834, 81837, 81838(5), 81839(2), 113776, 124775.

Sceloporus formosus (Guerrero; n=3): UMMZ 118793(3); (Oaxaca; n=10): UMMZ 114922, 118793, 124092, 126218, 126220(4), 130926-7; (Veracruz; n=17): UMMZ 85378, 85379(2), 99930(2), 105021, 105022(2), 114924, 120377(8).

Sceloporus gadovae (Guerrero; n=25): UMMZ 104649(3), 114915(10), 121633(12).

S. graciosus (New Mexico; n=33): UMMZ 127849(18), 127852-3, 132008(8), 132009(5).

Sceloporus grammicus (Veracruz; n=32): UMMZ 112967, 120375(18), 120381(13).

Sceloporus heterolepis (Michoacan; n=20): UMMZ 94396, 112574(5), 119044(3), 119045(4), 121503, 121504(2), 121505(2), 121506-7.

Sceloporus horridus (Michoacan; n=28): UMMZ 104712, 104713(7), 104716(4), 114828(5), 119093-4, 119096, 121614(2), 121615(2), 121616-8, 229896.

Sceloporus insignis (Michoacan; n=34): UMMZ 119099(5), 119101(11), 119102, 121609(15), 121610-11.

Sceloporus internasalis (Guatemala; n=29): UMMZ 126475(3), 126476, 126477(7), 129772, 129773(5), 129774(8), 129776(4).

Sceloporus jalapae (Oaxaca; n=7): UMMZ 114883(3), 121643, 126887, 134026-7; (Puebla; n=10): FMNH 110355-6, 110359, 110361-2, 113936; UMMZ 88603(2), 88607, 126533; (Veracruz; n=10): FMNH 110357-8, 113937-8, 113939-40, 113943-4, 113949, 113954; (no locality; n=6): FMNH 110350-3, 113941, 113956.

Sceloporus jarrovi (Arizona; n=37): UMMZ 75762(2), 75763, 75764(2), 75765, 80897, 85622, 85623(6), 85624(8), 85645, 102682(4), 102683(3), 105698(7); (Sonora; n=12): UMMZ 78396(10), 114143(2).
Sceloporus lundelli (Belize; n=1): UMMZ 80675; (Campeche; n=2): UMMZ 81906, 81908; (Yucatan; n= 30): FMNH 36470-80, 40690-703; UMMZ 72880, 83113-6.
Sceloporus magister (Arizona; n=23): UMMZ 223800-4, 223807-15, 223818-24, 223826, 223828, 223830.
Sceloporus malachiticus (Guatemala; n=29): UMMZ 67690(4), 71764(2), 71766(4), 98154(19).
Sceloporus megalepidurus (Puebla; n=44): UMMZ 88596(13), 88597(3), 88598(14), 105023(4), 116873(10).
Sceloporus melanorhinus (Michoacan; n=33): UMMZ 114830(13), 114831, 114832(4), 114833(3), 114834, 114835(2), 114836, 114837(3), 114838, 114839(3), 114840.
Sceloporus merriami (Texas; n=33): UMMZ 66218, 173109, 182310, 182316, 182318-20, 182324, 182327, 182330-1, 182333-4, 182337, 182342, 182344, 182370, 182372, 182382, 182386-90, 182392-7, 182399-400, 183600.
Sceloporus minor (St. Luis Potosi; n=12): UMMZ 77277(12); (Tamaulipas; n=24): UMMZ 101380(24).
Sceloporus mucronatus (Hildago; n=11): UMMZ 106384(8), 126232(3); (Puebla; n=18): UMMZ 88600, 88636(2), 88637(5), 88638(2), 89312(8); (Veracruz; n=3): UMMZ 119796, 120376(2).
Sceloporus nelsoni (Jalisco; n=4): FMNH 33477-9, 106439; (Nayarit; n=16): FMNH 33484, 33485(2); UMMZ 101970(5), 101971(5), 114885(2), 118567; (Sinaloa; n=10): FMNH 33477-9, 71490-2; UMMZ 81958(2), 102585, 115116; (Unknown, likely Jalisco; n=3): FMNH 106436-8.
Sceloporus occidentalis (California; n=16): UMMZ 132031(16); (Oregon; n=25): UMMZ 71509(2), 71510(2), 113189(5), 133779, 134488-90; 134991(2), 134492(2), 134493(7), 134494.
Sceloporus ochoternai (Guerrero; n=19): FMNH 33399-404, 33406-7, 33409-10, 102125-6, 102128; UMMZ 72148(4), 121644, 229899; (Morelos; n=2): FMNH 33398; UMMZ 114884; (Puebla; n=1): UMMZ 112578; (Unknown; n=4): FMNH 102111, 102114, 102129, 102133.
Sceloporus olivaceus (Tamaulipas; n=17): UMMZ 69243(6), 69245(2), 90174, 95223-5, 101423, 110803-4, 111176, 126212; (Texas; n=17): UMMZ 42325, 42328(2), 53982, 55310, 66734, 70501, 70791, 71008, 71143, 74746-8, 74750, 98896, 113127, 116804.
Sceloporus orcutti (Baja California; n=4): UMMZ 113054(4); (Baja California Sur; n=2): UMMZ 76482(2); (California; n=7): UMMZ 57500, 69890, 70787, 71150, 72655, 80900, 133805.
Sceloporus parvus (Tamaulipas; n=46): UMMZ 101405-14, 105498(13), 110808(2), 110809(5), 110810(3), 111178-9, 111180(10), 120241.
Sceloporus poinsettii (Texas; n=40): UMMZ 55722, 55726-9, 66093-5, 66097-101, 66103-6, 66108, 66110-5, 66117-22, 66125-9, 66134, 66138, 66140-1, 175874.
Sceloporus prezygus (Chiapas; n=20): UMMZ 94659(20); (Guatemala; n=15): UMMZ 120155(3), 120156(6), 120157(2), 120185(3), 127345.

Sceloporus pyrocephalus (Michoacan; n=29): UMMZ 104581(2), 104582-3, 104584(3), 104585(2), 104586(2), 104587, 104588(2), 104589(4), 104590(2), 104734(7), 105242(2).
Sceloporus scalaris (Jalisco; n=7): UMMZ 101948(3), 101949(4); (Tamaulipas; n=22): UMMZ 101415-20, 105499(5), 107141-4, 111182(7).
Sceloporus serrifer (Chiapas; n=11): UMMZ 94655(11); (Puebla; n=3): UMMZ 89313(3); (Tamaulipas; n=14): UMMZ 111183, 111188(4), 111189(3), 111190(6).
Sceloporus siniferus (Oaxaca; n=44): UMMZ 78852(7), 81840(9), 81843(4), 81844(5), 81846(2), 81847(8), 818848(2),114869(2), 114871, 119842(3), 119972.
Sceloporus smaragdinus (Guatemala; n=24): UMMZ 100492-3, 100494(9), 100495(6), 100496(3), 100497(2), 100498(2).
Sceloporus spinosus (Oaxaca; n=14): UMMZ 105411(6), 114775, 114853(4), 114854, 114855-6; (Puebla; n=18): UMMZ 88617-8, 88619(2), 88620, 88622(3), 88625(10).
Sceloporus squamosus (Guatemala; n=34): UMMZ 106886(2), 106887-90, 106891(2), 106892(6), 106893, 106895, 106896(6), 106897(2), 106898(2), 106900, 107503-4, 107509, 107513, 107514(2), 107516.
Sceloporus taeniocnemis (Guatemala; n=46): UMMZ 89183(7), 89184(3), 89185(3), 89186-8, 91237, 91238(3), 91239(4), 91240-1, 91242(4), 91244(5), 91245(4), 91246, 91247(2), 91248, 91249(2), 91250.
Sceloporus teapensis (Veracruz; n=30): UMMZ 121172-3, 126420(9), 127361, 127364, 128238, 128265(2), 128266, 128268, 128270(2), 128271, 128273(3), 128274(3), 128277-8.
Sceloporus torquatus (Michoacan; n=17): UMMZ 85373(2), 85374-6, 94336-40, 94343, 99202-4, 99207-8, 129745; (San Luis Potosi; n=6): UMMZ 77271(3), 119091(2), 126234; (Tamaulipas; n=13): UMMZ 102970-2, 111244, 111246(3), 111247(2), 111248-51.
Sceloporus tristictus (formerly *S. undulatus*; Arizona; n=35): UMMZ 73301, 193423, 214422, 221223-4, 223832-45, 223931, 223933-4, 223937, 223940-2, 223946, 223949-51, 223953-7.
Sceloporus utiformis (Colima; n=32): UMMZ 80081, 80082(4), 80083(6), 80084-6, 80087(2), 80088(2), 80089-90, 80091(4), 80092(6), 80093(2); (Michoacan; n=27): UMMZ 94295, 94395, 104573(2), 104574(2), 104575-9, 104722-4, 114916(5), 114917(2), 114918, 119086(2), 119087, 121635(2).
Sceloporus variabilis (Costa Rica; n=3): UMMZ 131804, 238811-2; (Guatemala; n=20): UMMZ 91259(2), 91260(2), 91261(2), 91262(2), 98162, 98163(4), 107072, 120178, 120180(3), 120184(3); (Tamaulipas; n=7): UMMZ 101512(7).
Sceloporus virgatus (Arizona; n=35): UMMZ 148401, 148409, 148423, 148575, 148580, 148583, 148585-6, 148588, 148590, 148593-617.
Sceloporus woodi (Florida; n=46): UMMZ 54087(5), 56168, 79590(3), 95570(8), 97550(2), 100676, 100849, 102761(2), 103737-8, 108384(4), 109282(3), 109283(13).
Uma inornata (California; n=3): FMNH 210163-4; UMMZ 75926.
Uma notata (Arizona; n=8): FMNH 37730; UMMZ 67367, 200766-7, 203573-6; (California; n=23): FMNH 11506, 26171, 26173-5, 26179, 26181-2, 26184, 26186, 26227, 34349, 34350-1; UMMZ 68794, 71031(2), 113145, 121674, 133808(2), 134166(2).

Urosaurus bicarinatus (Oaxaca; n=32): UMMZ 82379(2), 82380, 82381(4), 82382(2), 82386, 82387(4), 82388-90, 82391(3), 82392(3), 82393, 82394(2), 82396(2), 112626, 113778(2), 115033.
Urosaurus clarionensis (Colima; n=24): UMMZ 84223(8), 84224(16).
Urosaurus gadovi (Michoacan; n=32): UMMZ 112614-5, 112616(10), 112617, 115005, 115006(2), 115007(6), 115008, 115010, 115013, 115016(3), 115019, 115020, 121647(2).
Urosaurus graciosus (Arizona; n=7): UMMZ 67339-41, 67343-5, 69852; (California; n=7): FMNH 26188-9, 26127, 26218, 26226, 37723, 213912; (Utah; n=1): FMNH 167061.
Urosaurus nigricaudus (Baja California; n=2): FMNH 161608-9; (Baja California Sur; n=16): FMNH 25850(8), 25851-4; UMMZ 56053-5, 84226; (California; n=2): FMNH 37721-2.
Urosaurus ornatus (Arizona; n=34): UMMZ 148435-6, 148474, 148499-505, 148507-9, 148511-2, 148514-5, 148517-24, 148526-33, 148573; (Utah; n=7): 68576(7).
Uta antiqua (Baja California; n=34): UMMZ 127449(33), 128907.
Uta nolascensis (Sonora; n=26): UMMZ 128905(26).
Uta palmeri (Sonora; n=33): UMMZ 127198(10), 127200(14), 127201(9).
Uta stansburiana (California; n=31): UMMZ 127972(31); (Nevada; n=6): UMMZ 127996(6).

Appendix 4.III: Literature reviewed for ecological, morphological, and distributional data

Anderson J.D. and W.Z. Lidicker Jr. 1963. A contribution of our knowledge of the herpetofauna of the Mexican state of Aguascalientes. *Herpetologica* 19(1): 40-51.

Andrews R.M., F.R. Mendez-de la Cruz, M. Villagran-Santa Cruz, and F. Rodriquez-Romero. 1999. Field and selected body temperatures of the lizards *Sceloporus aeneus* and *Sceloporus bicanthalis*. *Journal of Herpetology* 33(1): 93-100.

Ballinger R.E. and D.W. Tinkle. 1972. Systematics and evolution of the genus *Uta* (Sauria: Iguanidae). *Miscellaneous Publications Museum of Zoology, University of Michigan* 145: 1-88.

Bell E.L. and A.H. Price. 1990. *Sceloporus occidentalis*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp. 631.1-631.17.

Bell E.L., H.M. Smith, and D. Chiszar. 2003. An annotated list of the species-group names applied to the lizard genus *Sceloporus*. *Acta Zoologica Mexicana* 90: 103-174.

Brattstrom B.H. 1955. Notes on the herpetology of the Revillagigedo Islands, Mexico. *American Midland Naturalist* 54(1): 219-229.

Brooks G.R. and J.C. Mitchell. 1989. Predator-prey size relationships in three species of lizards from Sonora, Mexico. *The Southwestern Naturalist* 34(4): 541-546.

Calisi R.M. and D.K. Hews. 2007. Steroid correlates of multiple color traits in the spiny lizard *Sceloporus pyrocephalus*. *Journal of Comparative Physiology B* 177: 641-654.

Campbell J.A. and A. Munoz-Alonso. 2007. *Sceloporus smaragdinus*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. www.iucnredlist.org. Downloaded on 18 March 2012.

Campbell J.A. and A. Munoz-Alonso. 2007. *Sceloporus taeniocnemis*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. www.iucnredlist.org. Downloaded on 18 March 2012.

Canseco-Marquez L., J.A. Campbell, P. Ponce-Campos, A. Munoz-Alonso, and A. Garcia Aguayo. 2007. *Sceloporus gadoviae*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.

Canseco-Marquez L., F. Mendoza-Quijano, and P. Ponce-Campos. 2007. *Sceloporus aeneus*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.

Canseco-Marquez L., F. Mendoza-Quijano, and P. Ponce-Campos. 2007. *Sceloporus asper*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.

Canseco-Marquez L., F. Mendoza-Quijano, and P. Ponce-Campos. 2007. *Sceloporus formosus*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.

Canseco-Marquez L., F. Mendoza-Quijano, and P. Ponce-Campos. 2007. *Sceloporus jalapae*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.

Canseco-Marquez L., F. Mendoza-Quijano, and P. Ponce-Campos. 2007. *Sceloporus ochoterenae*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.

Canseco-Marquez L., F. Mendoza-Quijano, and P. Ponce-Campos. 2007. *Sceloporus torquatus*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.
Censky E.J. 1986. *Sceloporus graciosus*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp. 386.1-386.4.
Cole C.J. 1963. Variation, distribution, and taxonomic status of the lizard, *Sceloporus undulates virgatus* Smith. *Copeia* 1963(2): 413-425.
Conant R. and J.T. Collins. A Field Guide to Reptiles and Amphibians of Eastern and Central North America, 3rd ed. Houghton Mifflin Company; Boston.
Crenshaw Jr. J.W. 1955. The life history of the southern spiny lizard, *Sceloporus undulatus undulatus* Latreille. *American Midland Naturalist* 54(2): 257-298.
Davis W.B. and H.M. Smith. 1953. Lizards and turtles of the Mexican state of Morelos. *Herpetologica* 9(2): 100-108.
Davis J. and N.A.M. Verbeek. 1972. Habitat preferences and the distribution of *Uta stansburiana* and *Sceloporus occidentalis* in coastal California. *Copeia* 1972(4): 643-649.
Dixon J.R., C.A. Ketchersid, and C.S. Lieb. 1972. The herpetofauna of Queretaro, Mexico, with remarks on taxonomic problems. *The Southwestern Naturalist* 16(3/4): 225-237.
Duellman W.E. 1961. Amphibians and reptiles of Michoacan. *University of Kansas Publications, Museum of Natural History* 15:1-148.
Duellman W.E. 1965. Amphibians and Reptiles of the Yucatan Peninsula, Mexico. *University of Kansas Publications of the Museum of Natural History* 15(12): 577-614.
Dunham A.E., D.W. Tinkle, and J.W. Gibbons. 1978. Body size in island lizards: A cautionary tale. *Ecology* 59(6): 1230-1238.
Feldman C.R., O. Flores-Villela, and T.J. Papenfuss. 2011. Phylogeny, biogeography, and display evolution in the tree and brush lizard genus *Urosaurus* (Squamata: Phrynosomatidae). *Molecular Phylogenetics and Evolution* 61: 714-725.
Frost D.R. 2007. *Uta nolascensis*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.
Frost D.R., G.A. Hammerson, P. Ponce-Campos, and H. Gadsden. 2007. *Sceloporus nelsoni*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.
Fugler C.M. and R.G. Webb. 1957. Some noteworthy reptiles and amphibians from the states of Oaxaca and Veracruz. *Herpetologica* 13(2): 103-108.
Grant B.W. and A.E. Dunham. 1990. Elevational covariation in environmental constraints and life histories of the desert lizard *Sceloporus merriami*. *Ecology* 71(5): 1765-1776.
Grismer L.L. 2002. Amphibians and Reptiles of Baja California. University of California Press, Berkeley.
Grismer L.L. and J.A. McGuire. 1996. Taxonomy and biogeography of the *Sceloporus magister* complex (Squamata: Phyrnosomatidae) in Baja California, Mexico. *Herpetologica* 52(3): 416-427.
Guillette Jr. L.J. 1982. The evolution of viviparity and placentation in the high elevation, Mexican lizard *Sceloporus aeneus*. *Herpetologica* 38(1): 94-103.

Guillette Jr. L.J., R.E. Jones, K.T. Fitzgerald, and H.M. Smith. 1980. Evolution of viviparity in the lizard genus *Sceloporus*. *Herpetologica* 36(3): 201-215.
Hall W.P. and H.M. Smith. 1979. Lizards of the *Sceloporus orcutti* complex of the cape region of Baja California. *Breviora* 452: 1-26.
Hardy L.M. and R.W. McDiarmid. 1969. Amphibians and reptiles of Sinaloa, Mexico. *University of Kansas Publications, Museum of Natural History* 18: 39-252.
Hodges W.L. 2004. Evolution of viviparity in horned lizards (*Phrynosoma*): testing the cold-climate hypothesis. *Journal of Evolutionary Biology* 17(6): 1230-1237.
Jackson J.F. 1973. Distribution and population phenetics of the Florida scrub lizard, *Sceloporus woodi*. *Copeia* 1973(4): 746-761.
Jackson J.F. and S.R. Telford, Jr. 1974. Reproductive ecology of the Florida scrub lizard, *Sceloporus woodi*. *Copeia* 1974(3): 689-694.
Jennings, M.R. 1990. *Petrosaurus mearnsi*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp. 495.1-495.3.
Jones L.L.C. and R.E. Lovich (eds.). 2009. Lizards of the American Southwest. Rio Nuevo Publishers; Tuscon, Arizona.
Kennedy J.P. 1973. *Sceloporus olivaceus*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp. 143.1-143.4.
Köhler G. 2003. Reptiles of Central America. Herpeton; Offenbach, Germany.
Lavin P. and F. Menoza Quijano. 2007. *Sceloporus couchi*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.
Lee D.S. and J.B. Funderburg. 1977. *Sceloporus woodi*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp. 196.1-196.2.
Lee J.C. 1996. The Amphibians and Reptiles of the Yucatan Peninsula. Cornell University Press; Ithaca, New York.
Lemos-Espinal J.A., Smith G.R., and R.E. Ballinger. 1999. Reproduction in Gadow's spiny lizard, *Sceloporus gadovae* (Phrynosomatidae), from arid tropical Mexico. *The Southwestern Naturalist* 44(1): 57-63.
Lewis T.H. and M.L. Johnson. 1955. Observations on the herpetofauna of the Mexican state of Nayarit. *Herpetologica* 11(3): 177-181.
Linsdale J.M. 1940. Amphibians and reptiles in Nevada. *Proceedings of the American Academy of Arts and Sciences* 73(8): 197-257.
Lynch J.D. and H.M. Smith. 1965. New or unusual amphibians and reptiles from Oaxaca, Mexico. *Herpetologica* 21(3): 168-177.
Marcellini D. and J.P. MacKey. 1970. Habitat preferences of the lizards, *Sceloporus occidentalis* and *S. graciosus* (Lacertilia, Iguanidae). *Herpetologica* 26(1): 51-56.
Martinez-Mendez N. and F.R. Mendez-de la Cruz. 2007. Molecular phylogeny of the *Sceloporus torquatus* species-group (Squamata: Phrynosomatidae). *Zootaxa* 1609: 53-68.
Mayhew W.W. 1963. Biology of the granite spiny lizard *Sceloporus orcutti*. *American Midland Naturalist* 69(2): 310-327.
Mather C.M. and J.W. Sites Jr. 1985. *Sceloporus variabilis*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp. 373.1-373.3.

Mendez-de la Cruz F.R., M. Villagran-Santa Cruz, and R.M. Andrews. 1998. Evolution of viviparity in the lizard genus *Sceloporus*. *Herpetologica* 54(4): 521-532.
Mendoza-Quijano F. and O. Florese-Villela. 2007. *Sceloporus megalepidurus*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.
Mendoza-Quijano F., J. Vazquez Diaz, and G.E. Quintero Diaz. 2007. *Phyrnosoma orbiculare*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded on 17 March 2012.
Mendoza-Quijano F., J. Vazquez Diaz, and G.E. Quintero Diaz. 2007. *Sceloporus minor*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.
Milstead W.W. 1969. Studies on beach lizards in Veracruz, Mexico. *Herpetologica* 25(2): 140-146.
Milstead W.W. 1970. Late summer behavior of the lizards *Sceloporus merriami* and *Urosaurus ornatus* in the field. *Herpetologica* 26(3): 343-354.
Montanucci R.R. 1981. Habitat separation between *Phyrnosoma douglassi* and *P. orbiculare* (Lacertilia: Iguanidae) in Mexico. *Copeia* 1981(1): 147-153.
Olson R.E. 1973. Variation in the canyon lizard, *Sceloporus merriami* Stejneger. *Herpetologica* 29(2): 116-127.
Olson R.E. 1987. Taxonomic revision of the lizards *Sceloporus serrifer* and *cyanogenys* of the Gulf Coastal Plain. *Bulletin of the Maryland Herpetological Society* 23(4): 158-167.
Parker W.S. 1974. *Phrynosoma solare*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp. 162.1-162.2.
Parker W.S. 1982. *Sceloporus magister*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp. 290.1-290.4.
Parker W.S. and E.R. Pianka. 1973. Notes on the ecology of the iguanid lizard *Sceloporus magister*. *Herpetologica* 29(2): 143-152.
Ponce-Campos P. and A. Garcia Aguayo. 2007. *Sceloporus heterolepis*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.
Ponce-Campos P. and A. Garcia Aguayo. 2007. *Sceloporus insignis*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.
Ponce-Campos P. and A. Garcia Aguayo. 2007. *Sceloporus utiformis*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded 15 September 2011.
Ponce-Campos P. and A. Garcia Aguayo. 2007. *Urosaurus clarionensis*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Downloaded on 15 September 2011.
Pough F.H. 1973. *Uma inornata*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp. 126.1-126.2.
Powell R., J.T. Collins, and E.D. Hooper Jr. 2012. A Key to the Herpetofauna of the Continental United States and Canada. University of Kansas Press; Lawrence, Kansas.

Price, A.H. 1990. *Phrynosoma cornutum*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp. 469.1-469.7.
Ramirez-Bautista A. and E.G. Davila-Ulloa. 2009. Reproductive characteristics of a population of *Sceloporus dugesii* (Squamata: Phrynosomatidae) from Michoacan, Mexico. *The Southwestern Naturalist* 54(4): 400-408.
Ramirez-Bautista A. and G. Gutierrez-Mayen. 2003. Reproductive ecology of *Sceloporus utiformis* (Sauria: Phrynosomatidae) from a tropical dry forest of Mexico. *Journal of Herpetology* 37(1): 1-10.
Ramirez-Bautista A. and V. Olvera-Bercerril. 2004. Reproduction in the boulder spiny lizard, *Sceloporus pyrocephalus* (Sauria: Phrynosomatidae), from a tropical dry forest of Mexico. *Journal of Herpetology* 38(2): 225-231.
Ramierez-Bautista A. and L.J. Vitt. 1998. Reproductive biology of *Urosaurus bicarinatus* (Sauria: Phrynosomatidae) from a tropical dry forest of Mexico. *The Southwestern Naturalist* 43(3): 381-390.
Ramirez-Bautista A., A.L. Ortiz-Cruz, M. Del Coro Arizmendi, and J. Campos. 2005. Reproductive characteristics of two syntopic lizard species, *Sceloporus gadoviae* and *Sceloporus jalapae* (Squamata: Phrynosomatidae), from Tehuacan Valley, Puebla, Mexico. *Western North American Naturalist* 65(2): 202-209.
Ramirez-Bautista A., Z. Uribe-Pena, and L.J. Guillette Jr. 1995. Reproductive biology of the lizard *Urosaurus bicarinatus bicarinatus* (Reptilia: Phrynosomatidae) from Rio Balsas Basin, Mexico. *Herpetologica* 51(1): 24-33.
Rau C.S. and R.B. Loomis. 1977. A new species of *Urosaurus* (Reptilia, Lacertilia, Iguanidae) from Baja California, Mexico. *Journal of Herpetology* 11(1): 25-29.
Rose B.R. 1976. Habitat and prey selection of *Sceloporus occidentalis* and *Sceloporous graciosus*. *Ecology* 57(3): 531-541.
Serrano-Cardozo V.H., J.A. Lemos-Espinal, and G.R. Smith. 2008. Comparative diet of three sympatric *Sceloporus* in the semiarid Zapotitlan Valley, Mexico. *Revista Mexicana de Biodiversidad* 79: 427-434.
Sites J.W. Jr. and J.R. Dixon. 1982. Geographic variation in *Sceloporus variabilis* and its relationship to *S. teapensis*. *Copeia* 1982(1): 14-27.
Smith H.M. 1934. Descriptions of new lizards of the genus *Sceloporus* from Mexico and Southern United States. *Transactions of the Kansas Academy of Science* 37: 263-285.
Smith H.M. 1934. Notes on some lizards of the genus *Phrynosoma* from Mexico. *Transactions of the Kansas Academy of Science* 37: 287-297.
Smith H.M. 1936. Description of a new Mexican subspecies of *Sceloporus spinosus* Wiegmann (Lacertilia). *University of Kansas Science Bulletin* 24(18): 469-473.
Smith H.M. 1936. Lizards of the Torquatus group of the genus *Sceloporus* Wiegmann, 1828. *University of Kansas Science Bulletin* 24(21): 539-693.
Smith H.M. 1939. The Mexican and Central American Lizards of the Genus *Sceloporus*. Zoological Series, Field Museum of Natural History, vol 26.
Smith H.M. 1949. Miscellaneous notes on Mexican lizards. *Journal of the Washington Academy of Science* 39: 34-43.
Smith H.S. and C.B. Bumzahem. 1955. The identity of the trans-isthmic Mexican populations of the malachite tree lizards (*Sceloporus malachiticus* Cope). *Herpetologica* 11(3):118-120.

Smith H.M. and K.R. Larsen. 1975. A new species of the formosus group of the lizard genus *Sceloporus*. *Copeia* 1: 47-50.
Smith H.M. and J.D. Lynch. 1967. A new cryptic lizard (Iguanidae: *Sceloporus*) with comments on other reptiles from Oaxaca, Mexico. *Herpetologica* 23(1): 18-29.
Smith H.M. and A.H. Savitzky. 1974. Another cryptic associate of the lizard *Sceloporus formosus* in Guerrero, Mexico. *Journal of Herpetology* 8(4): 297-303.
Smith H.M. and E.H. Taylor. 1945. An annotated checklist and key to the reptiles of Mexico, exclusive of the snakes. *US. Natl. Mus. Bull.* 187: 1-239.
Stebbins R.C. 1985. A Field Guide to Western Reptiles and Amphibians, 2nd ed. Houghton Mifflin Company; Boston.
Stuart L.C. 1963. Checklist of herpetofauna of Guatemala. *Miscellaneous Publications of the Museum of Zoology, University of Michigan* 122: 1-150.
Stuart L.C. 1970. A brief review of the races of *Sceloporus serrifer* Cope with special reference to *S. serrifer prezygus* Smith. *Herpetologica* 26(1): 141-149.
Stuart L.C. 1971. Comments on the malachite *Sceloporus* (Reptilia: Sauria: Iguanidae) of southern Mexico and Guatemala. *Herpetologica* 27(3): 235-259.
Taylor E.H. 1936. Notes on the herpetological fauna of the Mexican state of Sinaloa. *University of Kansas Science Bulletin* 24(20): 505-537.
Taylor E.H. 1936. Notes on the herpetological fauna of the Mexican state of Sonora. *University of Kansas Science Bulletin* 24(19): 475-503.
Vinegar M.B. 1975. Demography of the striped plateau lizard, *Sceloporus virgatus*. *Ecology* 56(1): 172-182.
Vitt L.J. and N.A. Dickson. 1988. *Urosaurus graciosus*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp. 448.1-448.3.
Webb R.G. 1967. Variation and distribution of the iguanid lizard *Sceloporus bulleri*, and the description of a related new species. *Copeia* 1967(1): 202-213.
Webb R.G. 1969. Variation, status, and relationship of the iguanid lizard *Sceloporus shannonorum*. *Herpetologica* 25(4): 300-307.
Webb R.G. and R.H. Baker. 1962. Terrestrial vertebrates of the Pueblo Nuevo area of southwestern Durango, Mexico. *American Midland Naturalist* 68(2): 325-333.
Whitford W.G. and F.M. Creusere. 1977. Seasonal and yearly fluctuations in Chihuahuan Desert lizard communities. *Herpetologica* 33(1): 54-65.
Whiting M.J. and J.R. Dixon. 1996. *Phrynosoma modestum*. In: Catalog of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. Pp.630.1-630.6.
Wiens J.J. 1993. Phylogenetic systematics of the tree lizards (genus *Urosaurus*). *Herpetologica* 49: 399-420.
Wiens J.J. and T.A. Penkrot. 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Systematic Biology* 51(1): 69-91.
Wiens J.J., T.W. Reeder, and A.N.M. de Oca. 1999. Molecular phylogenetics and evolution of sexual dichromatism among populations of the Yarrow's spiny lizard (*Sceloporus jarrovii*). *Evolution* 53(6): 1884-1897.

Wilson L.D. and J.R. McCranie. 1979. Notes on the herpetofauna of two mountain ranges in Mexico (Sierra Fria, Aguascalientes, and Sierra Morones, Zacatecas). *Journal of Herpetology* 13(3): 271-278.

Tables

Group	ASR	Species
variabilis	S3	**chrysostictus**, **couchii**, **cozumelae**, **parvus,** smithi, **teapensis**, **variabilis**
angustus		angustus, grandaevus
siniferus	S4	**carinatus**, **siniferus**, **squamosus**
utiformis	S4	**utiformis**
merriami		**merriami**
pyrocephalus	S5	**nelsoni**, **pyrocephalus**
gadovae	S5	**gadovae**, maculosus
jalapae	S5	**jalapae**, **ochoterenae**
graciosus		arenicolous, **graciosus**, vandenburgianus
spinosus	S6	**edwardtaylori**, **horridus**, **spinosus**
formosus	S7	**acanthinus**, adleri, cryptus, **formosus**, **internasalis**, lunaei, **lundelli**, **malachiticus**, salvini, **smaragdinus**, stejenegeri, subpictus, **taeniocnemis**, tanneri
melanorhinus	S8	**melanorhinus**
magister	S8	hunsakeri, licki, lineatulus, **magister**, **orcutti**, zosteromus
clarkii		**clarkii**
scalaris	S9	**aeneus**, **bicanthalis**, chaneyi, goldmani, **scalaris**, subniger
undulatus	S10	cautus, consobrinus, cowlesi, exsul, **occidentalis**, **olivaceus**, **tristichus**, undulatus, **virgatus**, **woodi**
grammicus	S11	anahuacus, **asper**, **grammicus**, **heterolepis**, palaciosi, shannonorum
megalepidurus	S12	halli, **megalepidurus**, pictus
torquatus	S12	bulleri, **insignis**, **jarrovi**, lineolateralis, **torquatus**
poinsettii	S13	**cyanogenys**, cyanostictus, **dugesii**, macdougalli, **minor**, **mucronatus**, oberon, ornatus, **poinsettii**, **prezygus**, **serrifer** sugilatus

Table 4.1: *Sceloporus* species groups as defined by Wiens et al. (2010), arranged phylogenetically from most basal to derived. Species included in this project are in **bold**. Abbreiviations for species groups used in the ancestral state reconstruction (ASR) are provided. See text for details.

	Log Total Load	Log Pocket Load	Log Nuchal Pocket Load	Log Post-inguinal Load	Log Non-pocket Load
Log Total Load	-	**≤0.001**	**≤0.001**	0.071	**≤0.001**
	-	**0.947**	**0.889**	0.207	**0.716**
Log Pocket Load	**≤0.001**	-	**≤0.001**	**0.013**	**≤0.001**
	0.949	-	**0.947**	**0.281**	**0.548**
Log Nuchal Pocket Load	**≤0.001**	**≤0.001**	-	**0.008**	**≤0.001**
	0.9	**0.961**	-	**0.299**	**0.484**
Log Post-inguinal Load	**0.006**	0.067	0.873	-	0.625
	0.313	0.21	0.018	-	0.057
Log Non-pocket Load	**≤0.001**	**≤0.001**	**≤0.001**	**≤0.001**	-
	0.688	**0.509**	**0.436**	**0.503**	-

Table 4.2: Two-tailed p-values and Pearson's correlation coefficients for mite load metrics produced through univariate linear regressions. Phylogenetically informed contrasts are presented above the diagonal; non-phylogenetic contrasts below the diagonal. The top number in each cell denotes p-value and the bottom number the associated correlation coefficient. Post-inguinal load refers the the mite load within the post-inguinal pocket, if present. Statistically significant relationships are in **bold**.

Phylogenetically independent contrasts

	Mean SVL	Mean Dorsal	Rugosity	Mean Latitude	Mean Elevation	NP Surface Area	PI Surface Area	Habitat	Microhabitat
Log Total Load	0.148 0.167	0.862 -0.02	0.427 0.092	**≤0.001** **-0.445**	0.407 0.096	**≤0.001** **0.524**	**0.01** **0.291**	0.094 0.192	0.871 0.019
Log Pocket Load	0.11 0.184	0.582 -0.064	0.269 0.128	**≤0.001** **-0.434**	0.678 0.048	**≤0.001** **0.572**	**0.017** **0.271**	**0.025** **0.255**	0.954 0.007
Log Nuchal Pocket Load	0.101 0.189	0.963 -0.005	0.288 0.123	**≤0.001** **-0.406**	0.659 0.051	**≤0.001** **0.574**	0.318 0.115	**0.007** **0.307**	0.865 -0.02
Log Post-inguinal Load	0.716 -0.042	0.839 -0.024	0.494 0.079	**0.003** **-0.329**	0.366 0.105	0.35 0.108	0.762 0.035	**≤0.001** **0.957**	0.284 0.124
Log Non-pocket Load	0.209 0.145	0.776 -0.033	0.218 0.142	**0.007** **-0.305**	0.058 0.217	**0.001** **0.383**	**0.016** **0.274**	0.657 0.051	0.67 0.049

Non-phylogenetic contrasts

	Mean SVL	Mean Dorsal	Rugosity	Mean Latitude	Mean Elevation	NP Surface Area	PI Surface Area	Habitat	Microhabitat
Log Total Load	0.067 0.21	**0.001** **-0.382**	**0.004** **0.327**	**≤0.001** **-0.512**	0.055 0.22	**≤0.001** **0.52**	0.538 0.071	**0.012** **0.286**	0.809 0.028
Log Pocket Load	0.083 0.199	**≤0.001** **-0.444**	**≤0.001** **0.362**	**≤0.001** **-0.514**	0.066 0.211	**≤0.001** **0.564**	0.639 0.054	**0.002** **0.346**	0.817 0.027
Log Nuchal Pocket Load	**0.022** **0.26**	**≤0.001** **-0.488**	**≤0.001** **0.432**	**≤0.001** **-0.53**	**0.022** **0.261**	**≤0.001** **0.621**	0.293 -0.121	**0.001** **0.368**	0.679 0.048
Log Post-inguinal Load	**0.027** **-0.253**	0.437 0.09	0.054 -0.22	0.874 -0.018	0.746 -0.038	**0.037** **-0.238**	**≤0.001** **0.737**	0.904 0.014	0.807 0.028
Log Non-pocket Load	0.18 0.154	0.383 -0.101	0.251 0.132	**0.013** **-0.281**	0.138 0.171	**0.011** **0.288**	0.209 0.145	0.523 0.074	0.828 0.025

Table 4.3: Univariate linear regressions for mite loads on host morphology and ecology. Top number in each cell denotes two-tailed p-value, bottom number the associated Pearson's correlation coefficient. 'NP' refers to nuchal pocket, 'PI' to post-inguinal. Statistically significant relationships are in **bold**. Regressions for phylogenetically independent contrasts are drawn through the origin (as per Garland et al. 1992).

Phylogenetically independent contrasts

	Log Total Load		Log Pocket Load		Log Nuchal Pocket Load		Log Post-Inguinal Load		Log Non-Pocket Load	
	coefficient	*p*	coefficient	*p*	coefficient	*p*	coefficient	*p*	coefficient	*p*
NP Surface Area	**0.511**	**0.000**	**0.588**	**0.000**	**0.604**	**0.000**	-0.032	0.496	**0.305**	**0.031**
PI Surface Area	**0.294**	**0.002**	**0.274**	**0.003**	0.111	0.235	-0.030	0.397	**0.272**	**0.011**
Habitat	0.033	0.732	0.117	0.209	**0.195**	**0.047**	**0.951**	**0.000**	-0.093	0.393
Microhabitat	-0.048	0.615	-0.070	0.446	-0.066	0.488	-0.002	0.964	0.016	0.885
Mean SVL	-0.143	0.236	-0.180	0.124	-0.156	0.197	0.026	0.564	-0.004	0.979
Mean Latitude	**-0.263**	**0.012**	**-0.205**	**0.040**	-0.151	0.141	-0.049	0.205	-0.217	0.063
Mean Elevation	0.023	0.814	-0.053	0.583	-0.061	0.541	0.001	0.970	**0.223**	**0.051**
Mean Dorsal	0.056	0.544	0.022	0.805	0.085	0.363	-0.062	0.082	0.020	0.850
$F_{7, 69}$		**7.048**		**8.234**		**6.938**		**99.623**		**3.663**
R^2		**0.453**		**0.492**		**0.449**		**0.921**		**0.301**

Non-phylogenetic contrasts

	Log Total Load		Log Pocket Load		Log Nuchal Pocket Load		Log Post-Inguinal Load		Log Non-Pocket Load	
	coefficient	*p*	coefficient	*p*	coefficient	*p*	coefficient	*p*	coefficient	*p*
(Constant)		0.002		0.003		0.003		0.108		0.664
NP Surface Area	**0.454**	**0.001**	**0.546**	**0.000**	**0.546**	**0.000**	-0.052	0.653	0.234	0.146
PI Surface Area	**0.224**	**0.021**	**0.204**	**0.024**	0.033	0.694	**0.779**	**0.000**	**0.273**	**0.024**
Habitat	0.041	0.714	0.122	0.242	0.122	0.217	0.003	0.973	-0.088	0.524
Microhabitat	-0.061	0.557	-0.067	0.488	-0.072	0.434	0.123	0.190	0.032	0.806
Mean SVL	-0.003	0.979	-0.068	0.557	-0.056	0.615	-0.012	0.918	0.073	0.640
Mean Latitude	**-0.344**	**0.003**	**-0.271**	**0.010**	**-0.274**	**0.007**	-0.017	0.864	**-0.275**	**0.049**
Mean Elevation	0.073	0.463	0.006	0.950	0.002	0.982	**0.216**	**0.018**	0.199	0.111
Mean Dorsal	-0.111	0.324	-0.169	0.111	-0.193	0.057	0.096	0.343	0.083	0.553
$F_{8, 68}$		**8.367**		**10.871**		**12.841**		**12.440**		**2.333**
R^2		**0.496**		**0.561**		**0.602**		**0.594**		**0.215**

Table 4.4: Multiple regressions examining the effects of multiple biotic and abiotic host variables on log mite load indices. 'NP' and 'PI' refer to nuchal pocket and post-inguinal pocket, respectively. Statistically significant relationships are in **bold**. Phylogenetically independent regressions lack a constant due to regression through the origin (Garland et al. 1992).

Nuchal Pocket								
	Species	N	Depth/width Ratio			Surface Area (mm^2)		
Genus	Present / Examined	Specimens	Mean	s	Range	Mean	s	Range
Callisaurus	0 / 1	33	-	-	-	-	-	-
Cophosaurus	0 / 1	30	-	-	-	-	-	-
Holbrookia	0 / 1	32	-	-	-	-	-	-
Petrosaurus	1 / 1	16	0.376	n/a	n/a	3.335	n/a	n/a
Phrynosoma	8 / 8	246	0.471	0.091	0.294 - 0.582	19.030	14.129	2.534 - 44.923
Sceloporus	53 / 53	1740	0.483	0.152	0.274 - 0.933	9.714	5.113	2.218 - 25.866
Uma	0 / 2	34	-	-	-	-	-	-
Urosaurus	6 / 6	164	0.395	0.095	0.249 - 0.514	2.4905	1.1926	1.145 - 4.377
Uta	2 / 4	130	0.314	0.032	0.292 - 0.337	3.5716	0.5658	3.172 - 3.972

Post-inguinal Pocket								
	Species	N	Depth/width Ratio			Surface Area (mm^2)		
Genus	Present / Examined	Specimens	Mean	s	Range	Mean	s	Range
Callisaurus	0 / 1	33	-	-	-	-	-	-
Cophosaurus	0 / 1	30	-	-	-	-	-	-
Holbrookia	1 / 1	32	0.222	n/a	n/a	1.406	n/a	n/a
Petrosaurus	1 / 1	16	0.314	n/a	n/a	1.028	n/a	n/a
Phrynosoma	0 / 8	246	-	-	-	-	-	-
Sceloporus	6 / 53	1740	0.414	0.128	0.245 - 0.585	1.978	0.570	1.298 - 2.899
Uma	0 / 2	34	-	-	-	-	-	-
Urosaurus	2 / 6	164	0.341	0.007	0.336 - 0.346	1.019	0.237	0.851 - 1.186
Uta	4 / 4	130	0.235	0.039	0.191 - 0.285	1.162	0.585	0.347 - 1.718

Table 4.5: Descriptive statistics for mite pocket morphology in the Phrynosomatidae, separated by genus. Of the 77 phrynosomatid species examined in this project, 70 species possessed nuchal pockets while only 14 possessed post-inguinal pockets.

Phylogenetically independent contrasts

	Mean SVL	Mean Dorsal	Rugosity	Mean Latitude	Mean Elevation	NP Surface Area	PI Surface Area	Habitat	Microhabitat
Nuchal Pocket	**≤0.001**	0.099	**0.001**	**0.002**	0.616	-	0.878	0.377	0.98
Surface Area	**0.543**	-0.19	**0.365**	**-0.349**	0.058	-	-0.018	0.102	0.003
Post-inguinal	0.422	0.926	0.986	0.744	0.724	0.878	-	0.572	0.073
Surface Area	0.093	0.011	-0.002	-0.038	-0.041	-0.018	-	0.065	0.206

Non-phylogenetic contrasts

	Mean SVL	Mean Dorsal	Rugosity	Mean Latitude	Mean Elevation	NP Surface Area	PI Surface Area	Habitat	Microhabitat
Nuchal Pocket	**≤0.001**	**0.045**	**≤0.001**	0.054	**0.038**	-	**0.022**	0.628	0.488
Surface Area	**0.605**	**-0.229**	**0.401**	-0.22	**0.237**	-	**-0.26**	0.056	-0.08
Post-inguinal	**0.01**	0.299	**0.008**	0.911	**0.015**	**0.022**	-	0.569	0.456
Surface Area	**-0.294**	0.12	**-0.3**	0.013	**-0.275**	**-0.26**	-	-0.066	-0.086

Table 4.6: Univariate linear regressions for nuchal pocket (NP) and post-inguinal pocket (PI) surface area on host morphology and ecology. Top number in each cell denotes the two-tailed p-value, bottom number the associated Pearson's correlation coefficient. Statistically significant relationships are in **bold**.

Phylogenetically independent contrasts

	Nuchal Pocket SA Residuals			Post-inguinal Surface Area	
	coefficient	*p*		coefficient	*p*
PI Surface Area	-0.007	0.947	**NP Surface Area**	-0.042	0.792
Habitat	0.034	0.758	**Habitat**	0.046	0.714
Microhabitat	-0.117	0.277	**Microhabitat**	0.191	0.120
Mean Latitude	**-0.368**	**0.001**	**Mean Latitude**	-0.011	0.932
Mean Elevation	**0.264**	**0.013**	**Mean Elevation**	0.012	0.928
Mean Dorsal	**-0.227**	**0.030**	**Mean Dorsal**	0.024	0.842
			Mean SVL	0.101	0.520
$F_{5, 72}$		**4.347**	$F_{6, 71}$		0.808
R^2		**0.271**	R^2		0.051

Non-phylogenetic contrasts

	Nuchal Pocket SA Residuals			Post-inguinal SA Residuals	
	coefficient	*p*		coefficient	*p*
(Constant)		0.009	**(Constant)**		0.279
PI residual	-0.034	0.753	**NP residual**	-0.042	0.753
Habitat	-0.090	0.490	**Habitat**	-0.002	0.991
Microhabitat	**-0.364**	**0.002**	**Microhabitat**	-0.058	0.665
Mean Latitude	-0.223	0.083	**Mean Latitude**	-0.107	0.462
Mean Elevation	0.210	0.069	**Mean Elevation**	**-0.280**	**0.030**
Mean Dorsal	**-0.263**	**0.044**	**Mean Dorsal**	0.064	0.664
$F_{5, 72}$		**4.451**	$F_{5, 72}$		1.255
R^2		**0.276**	R^2		0.097

Table 4.7: Multiple regression analysis examining the effects of host morphology and ecology on nuchal mite pocket (NP) and post-inguinal pocket (PI) size, measured as surface area (SA), using phylogenetically informed (top) and conventional contrasts (bottom). Statistically significant relationships are in **bold**. Residuals were used where host body size (SVL) was found to be significantly correlated with pocket surface area. See text for details. Phylogenetically independent regressions lack a constant due to regression through the origin (Garland et al. 1992).

Trait	Branch Transformation	Estimated Root Node	SE	Lower 95% CE	Upper 95% CE
Max SVL	none	87.034	14.265	58.623	115.444
Mean SVL	none	63.554	8.796	46.036	81.073
Mean Dorsal	none	118.322	15.239	87.972	148.673
Rugosity	square root	0.605	0.273	0.062	1.148
Minimum Latitude	square root	25.034	2.463	20.129	29.939
Maximum Latitude	square root	32.168	4.271	23.662	40.674
Mean Latitude	square root	28.598	2.926	22.770	34.426
Minimum Elevation	branch=1	40.172	384.844	-726.477	806.821
Maximum Elevation	not applicable[a]	-	-	-	-
Mean Elevation	branch=1	706.690	462.696	-215.048	1628.427
NP Depth/Width	square root	0.234	0.079	0.077	0.391
NP Surface Area	square root	4.402	3.594	0[b]	11.559
PI Depth/Width	none	0.099	0.067	0[b]	0.232
PI Surface Area	none	0.444	0.343	0[b]	1.126
Habitat	none	2.406	1.365	1[b]	5.124
Microhabitat	square root	1.617	0.355	1[b]	2.323
Log Mean Total Load	square root	1.294	1.391	0[b]	1.837
Log Mean Nuchal Pocket Load	square root	1.080	1.269	0[b]	1.690
Log Mean Post-Inguinal Pocket Load	square root	0.394	0.363	0[b]	0.850
Log Mean Pocket Load	none	1.257	1.424	0[b]	1.850
Log Mean Non-Pocket Load	none	0.801	0.976	0[b]	1.401

[a]: Maximum elevation could not be adequately standardized for PIC and was excluded from the analysis; see text for details.

[b]: Lower bound set to one (Habitat, Microhabitat) or zero (pocket surface area, pocket depth/width ratio, and mite loads).

Table 4.8: Estimated reconstructed character states for the hypothetical ancestor of Phrynosomatidae. See text for details.

Figures

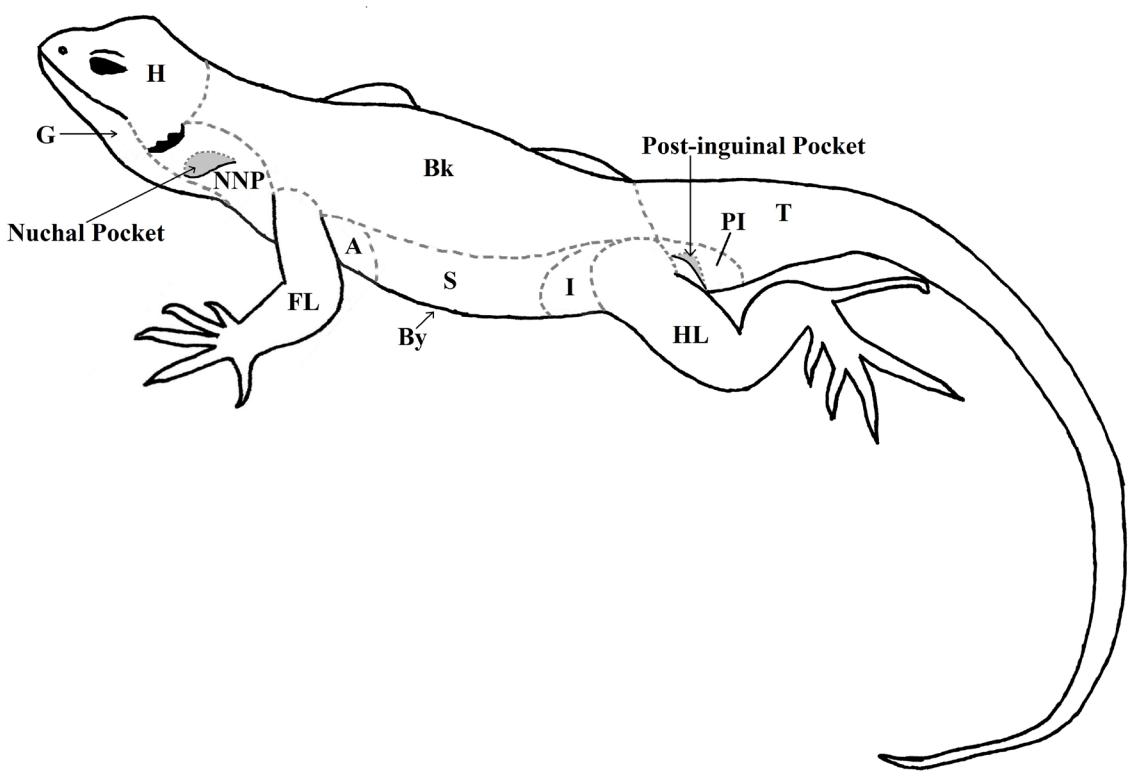

Figure 4.1: Division of a generalized lizard into body regions for classification of mite attachment sites. Two forms of mite pockets occur in the Phrynosomatidae – a nuchal pocket, occurring in the central nuchal region roughly midway between ear and shoulder; and/or a post-inguinal pocket, located just posterior to the insertion of the hindlimb.

Abbreviations: A – axial; Bk – back; By – belly; FL – forelimb; G – gular; H – head; HL – hindlimb; I – inguinal; NNP – nuchal non-pocket; PI – post-inguinal; S – side; T – tail.

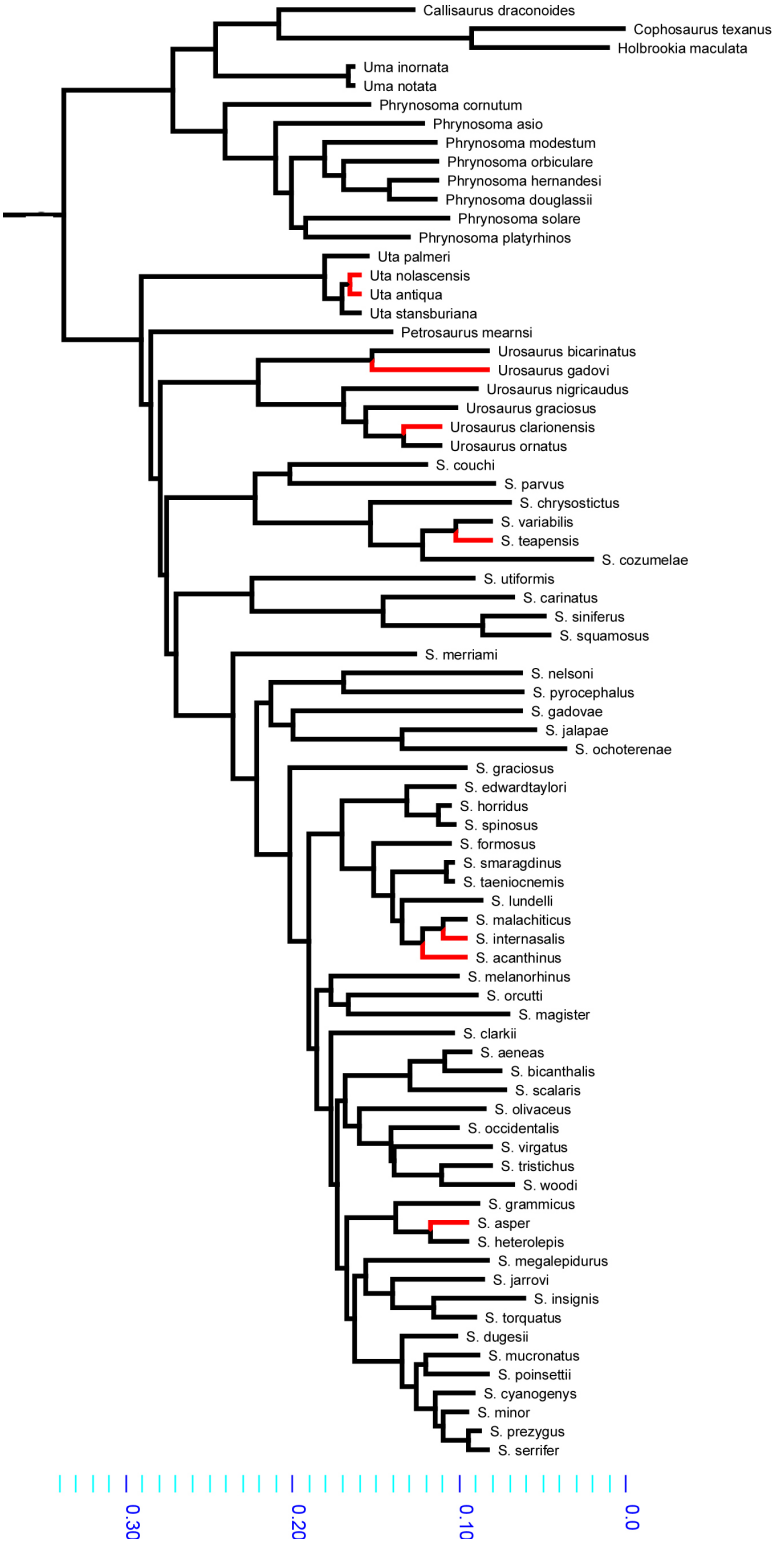

Figure 4.2: Phylogenetic relationships of the 77 Phrynosomatidae species analyzed in this study with branch lengths, modified from Wiens et al. (2010). Taxa added to the original phylogeny in red. See text for details.

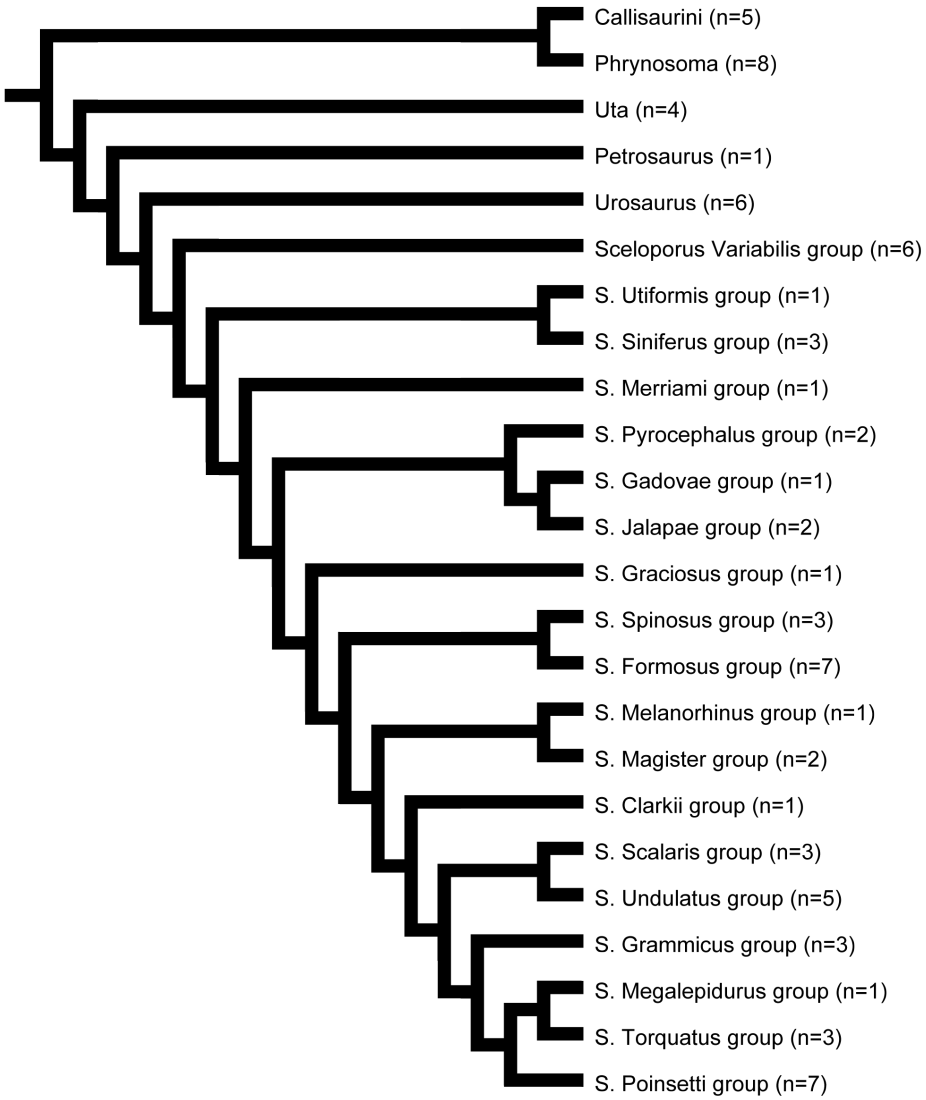

Figure 4.3: Hypothesized phylogenetic relationships of the 77 Phrynosomatidae species analyzed in this study, shown here as species groups *sensu* Wiens et al. (2010) for ease of viewing. See Table 4.1 for specific contents of *Sceloporus* groups; numbers of species examined included in parentheses. Topology from Wiens et al. (2010).

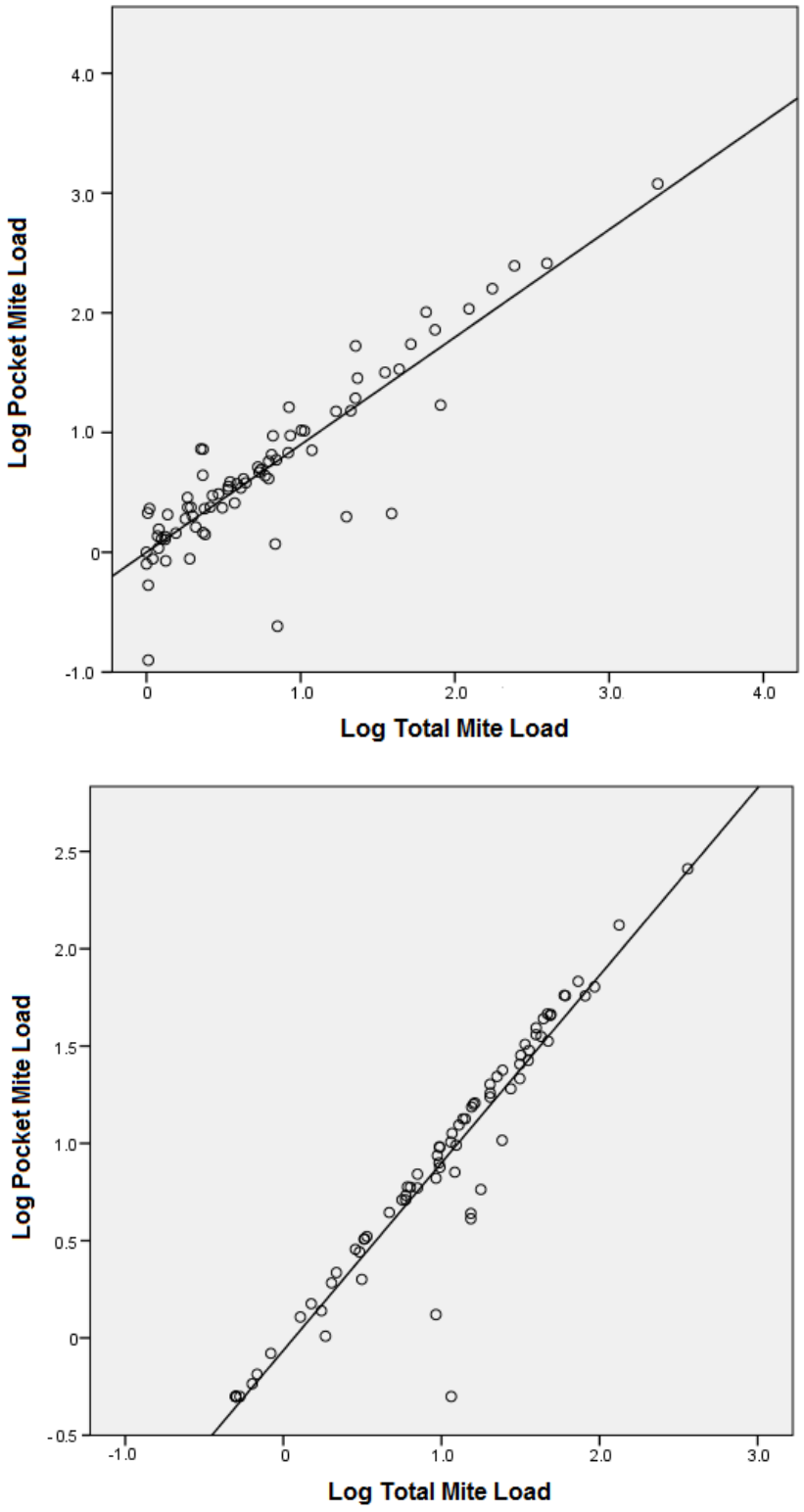

Figure 4.4: Bivariate scatterplot of the relationship between log mean total mite load and log pocket load for phylogenetically independent contrasts (**top**: R=0.947, t=25.483, p<0.001) and conventional contrasts (**bottom**: R=0.949, t=26.205, p<0.001).

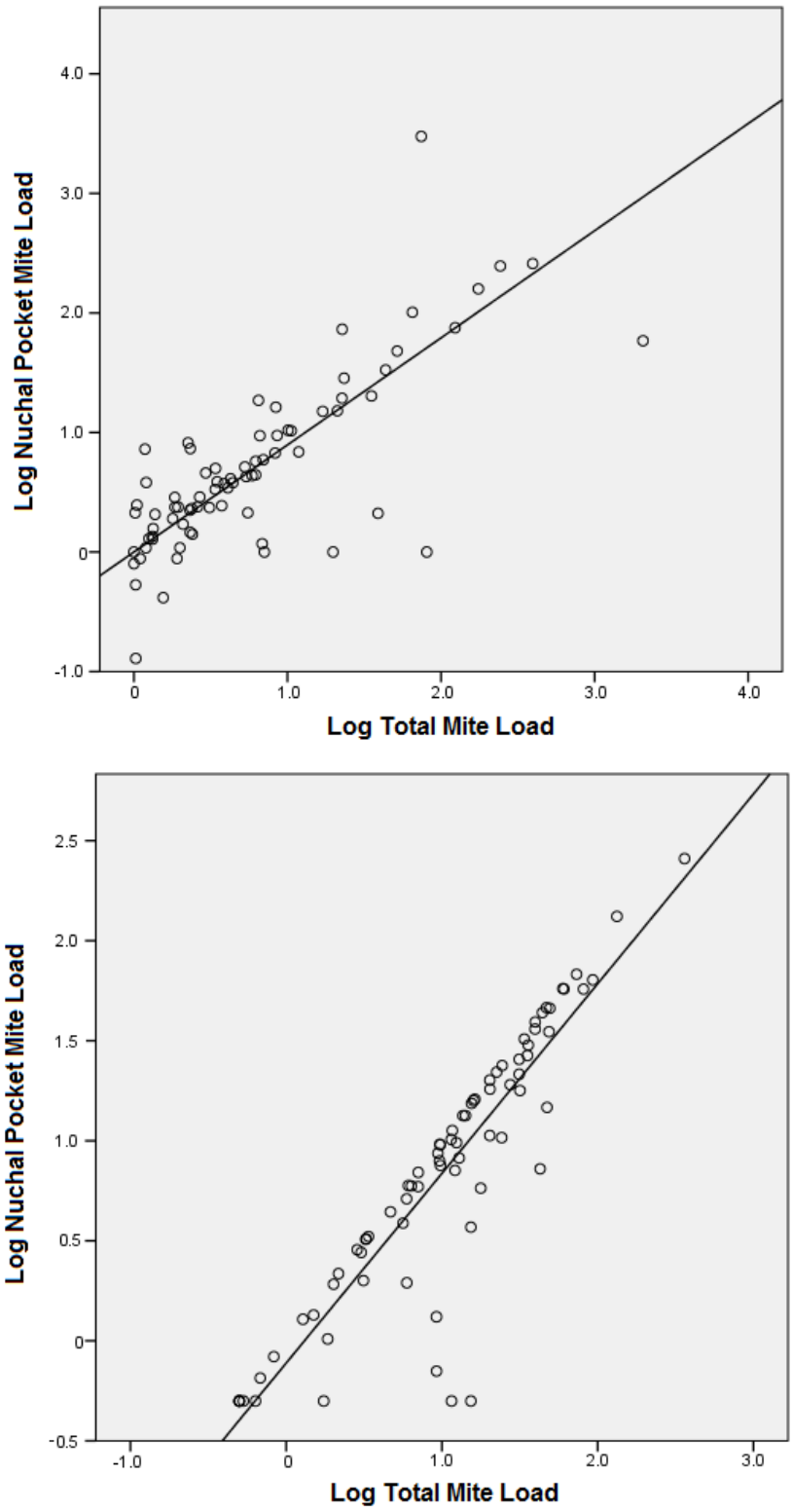

Figure 4.5: Bivariate scatterplot of the relationship between log mean total mite load and log nuchal pocket mite load for phylogenetically independent contrasts (**top**: R=0.889, t=16.775, p<0.001) and conventional contrasts (**bottom**: R=0.900, t=17.884, p<0.001).

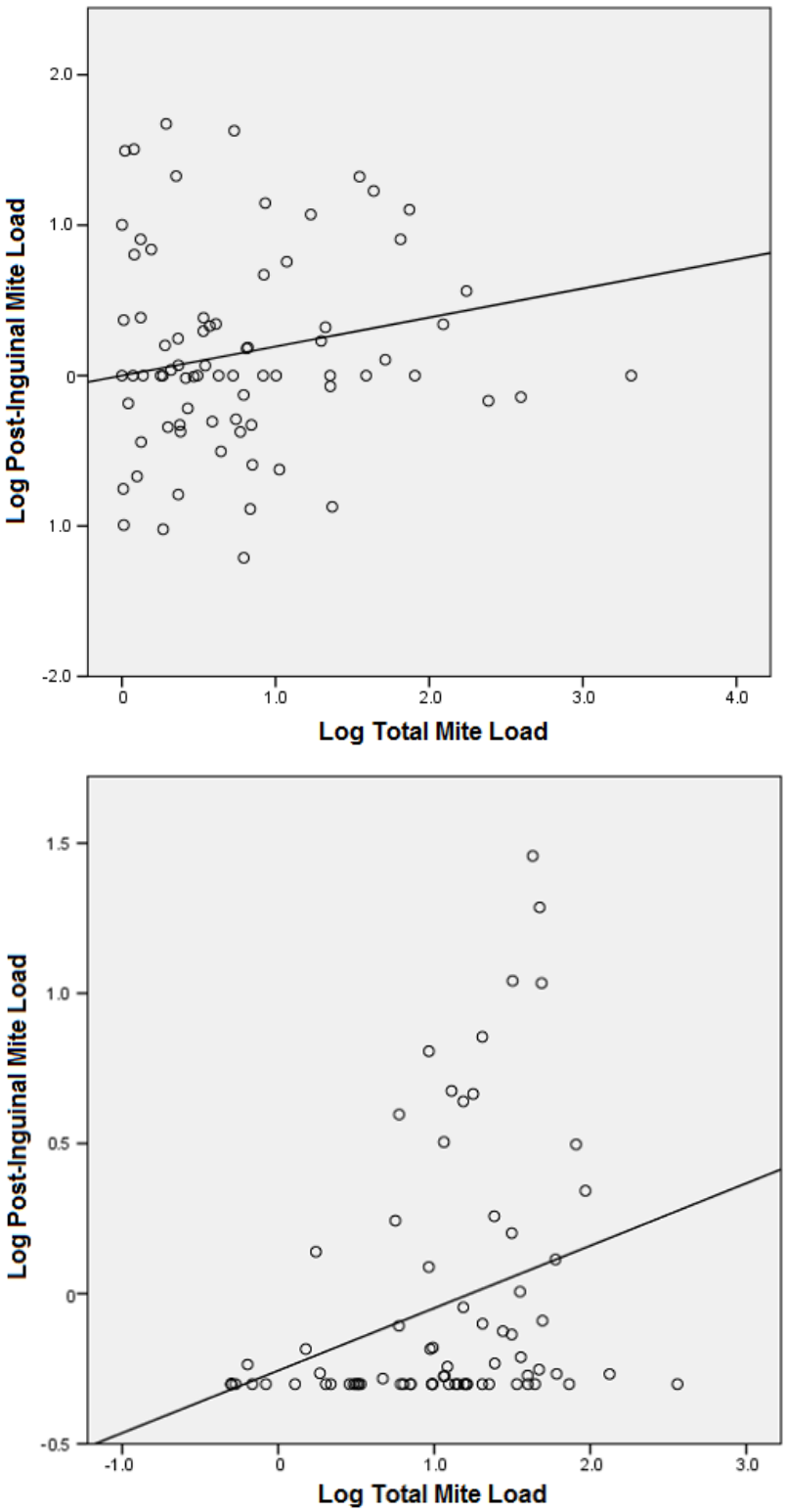

Figure 4.6: Bivariate scatterplot of the relationship between log mean total mite load and log post-inguinal mite load for phylogenetically independent contrasts (**top**: R=0.207, t=1.833, p=0.071) and conventional contrasts (**bottom**: R=0.313, t=2.856, p=0.006).

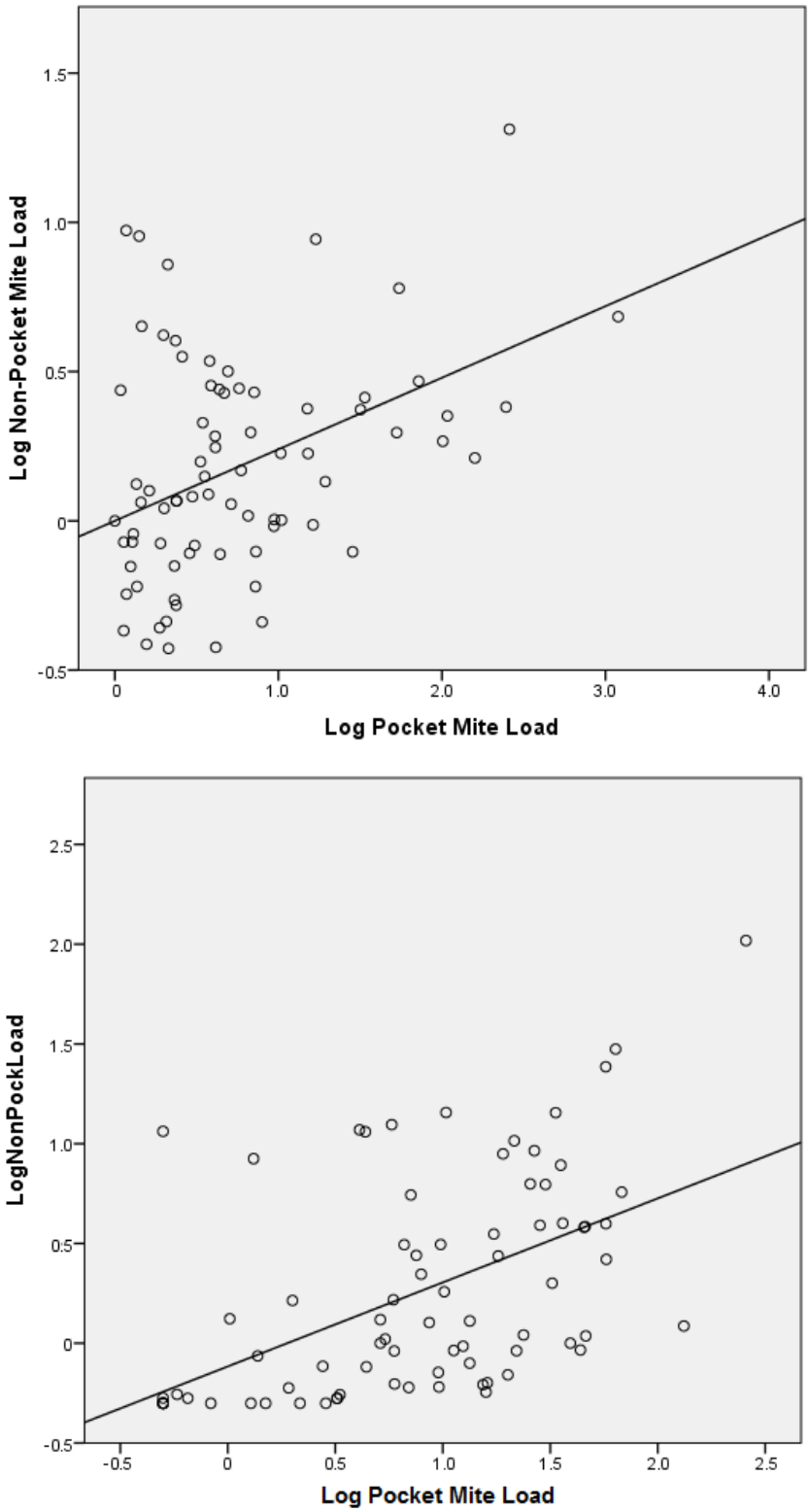

Figure 4.7: Bivariate scatterplot of relationship between log mean pocket mite load and log non-pocket load for phylogenetically independent (**top**: R=0.548, t=5.668, p<0.001) and conventional contrasts (**bottom**: R=0.509, t=5.123, p<0.001).

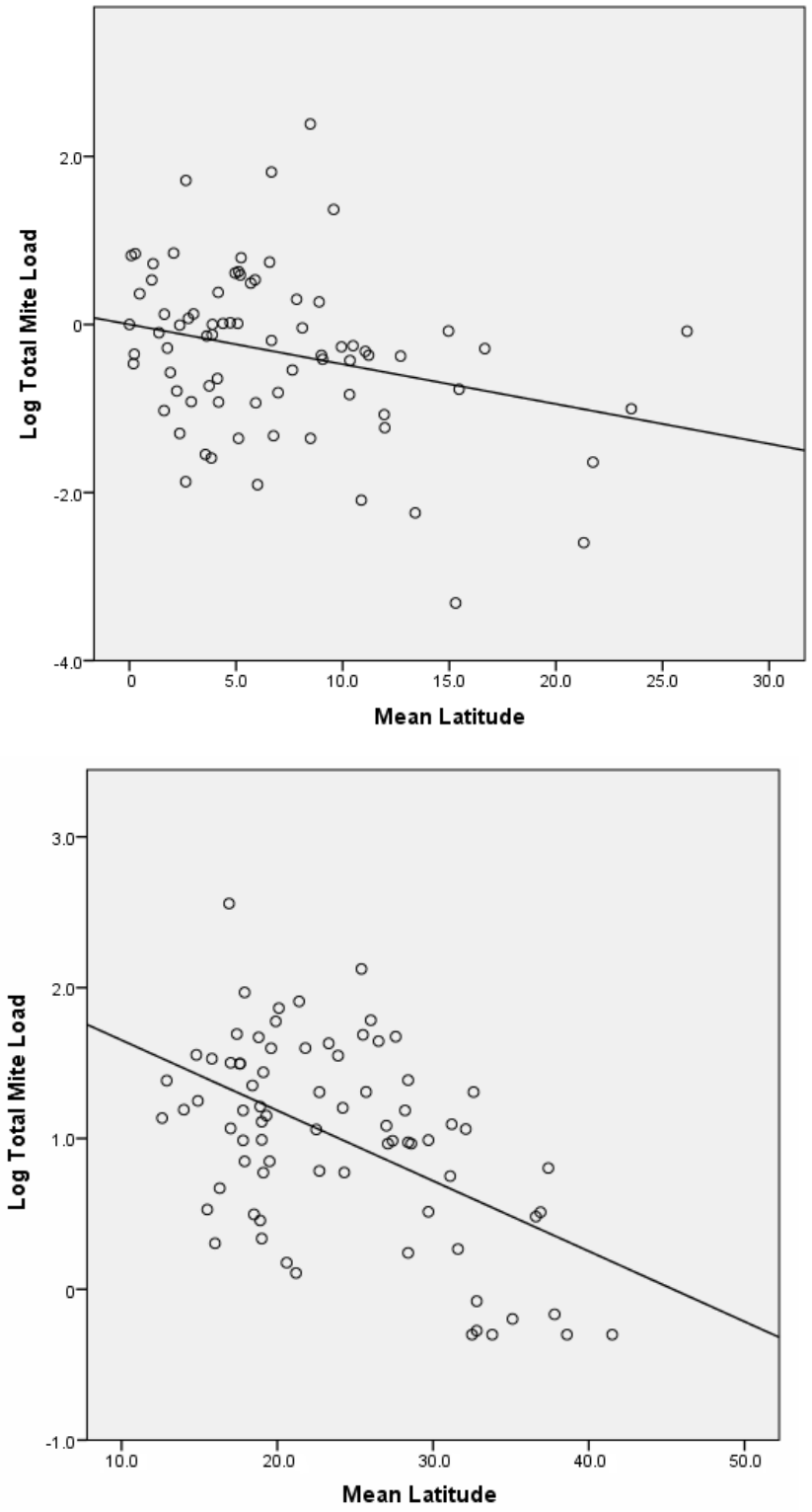

Figure 4.8: Bivariate scatterplot of relationship between log mean total mite load and mean latitude for phylogenetically independent (**top**: R=-0.445, t=-4.302, p<0.001) and conventional contrasts (**bottom**: R=-0.512, t=-5.159, p<0.001).

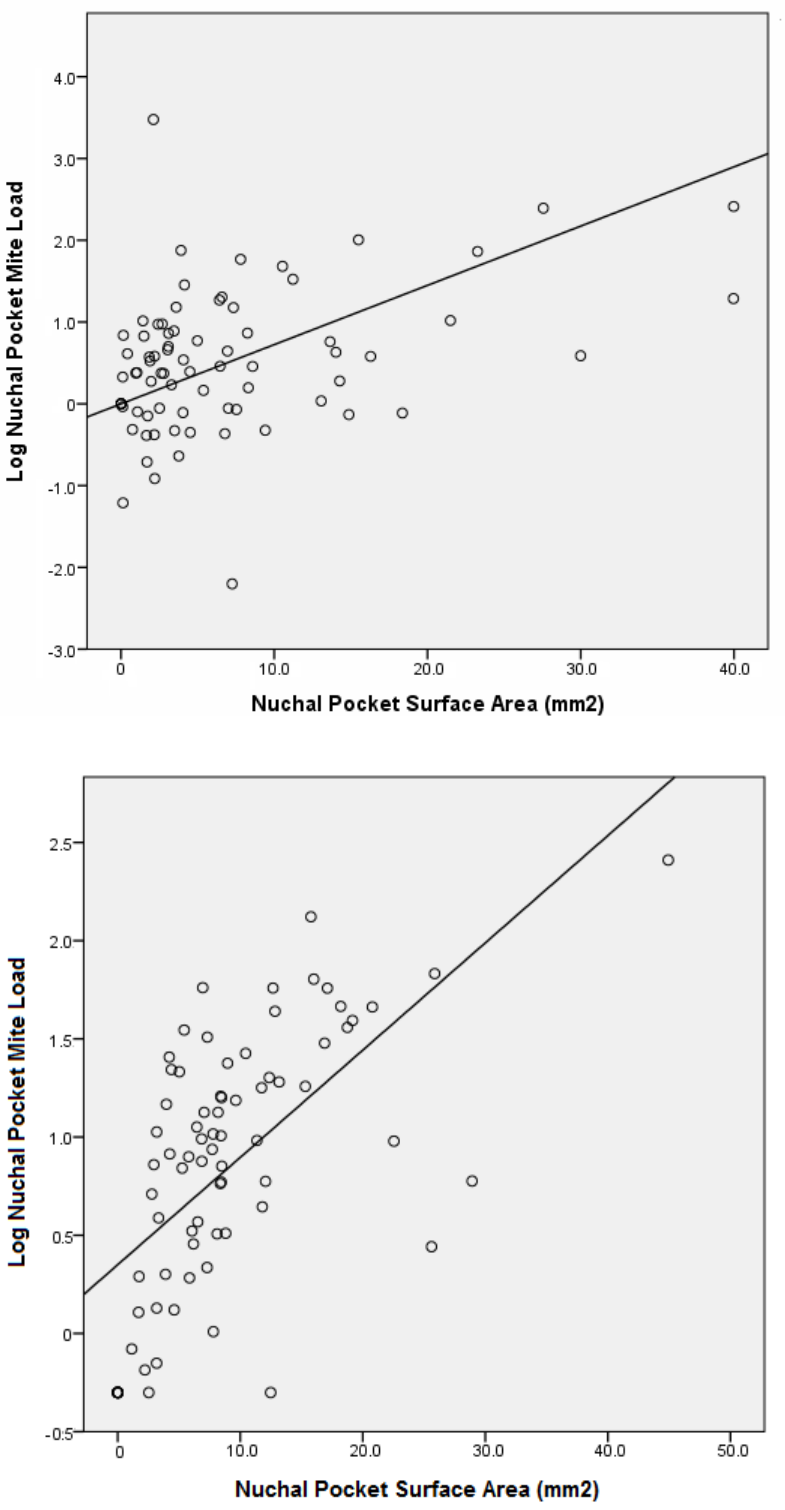

Figure 4.9: Bivariate scatterplot of relationship between log mean nuchal pocket mite load and mean nuchal pocket surface area (mm^2) for phylogenetically independent (**top**: R=0.574, t=6.075, p<0.001) and conventional contrasts (**bottom**: R=0.621, t=6.858, p<0.001).

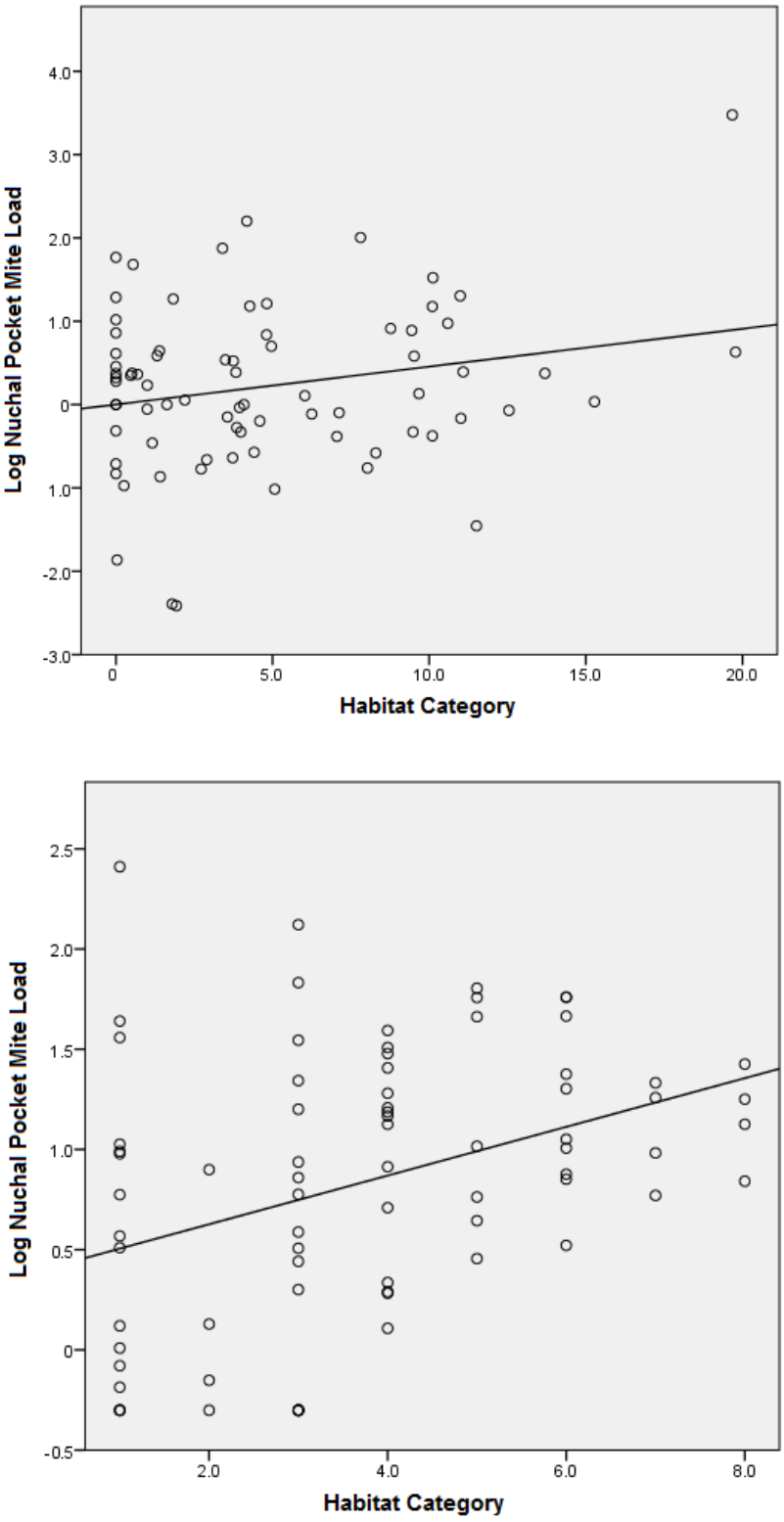

Figure 4.10: Bivariate scatterplot of relationship between log mean nuchal pocket mite load and habitat for phylogenetically independent (**top**: R=0.307, t=2794, p=0.007) and conventional contrasts (**bottom**: R=0.368, t=3.424, p=0.001).

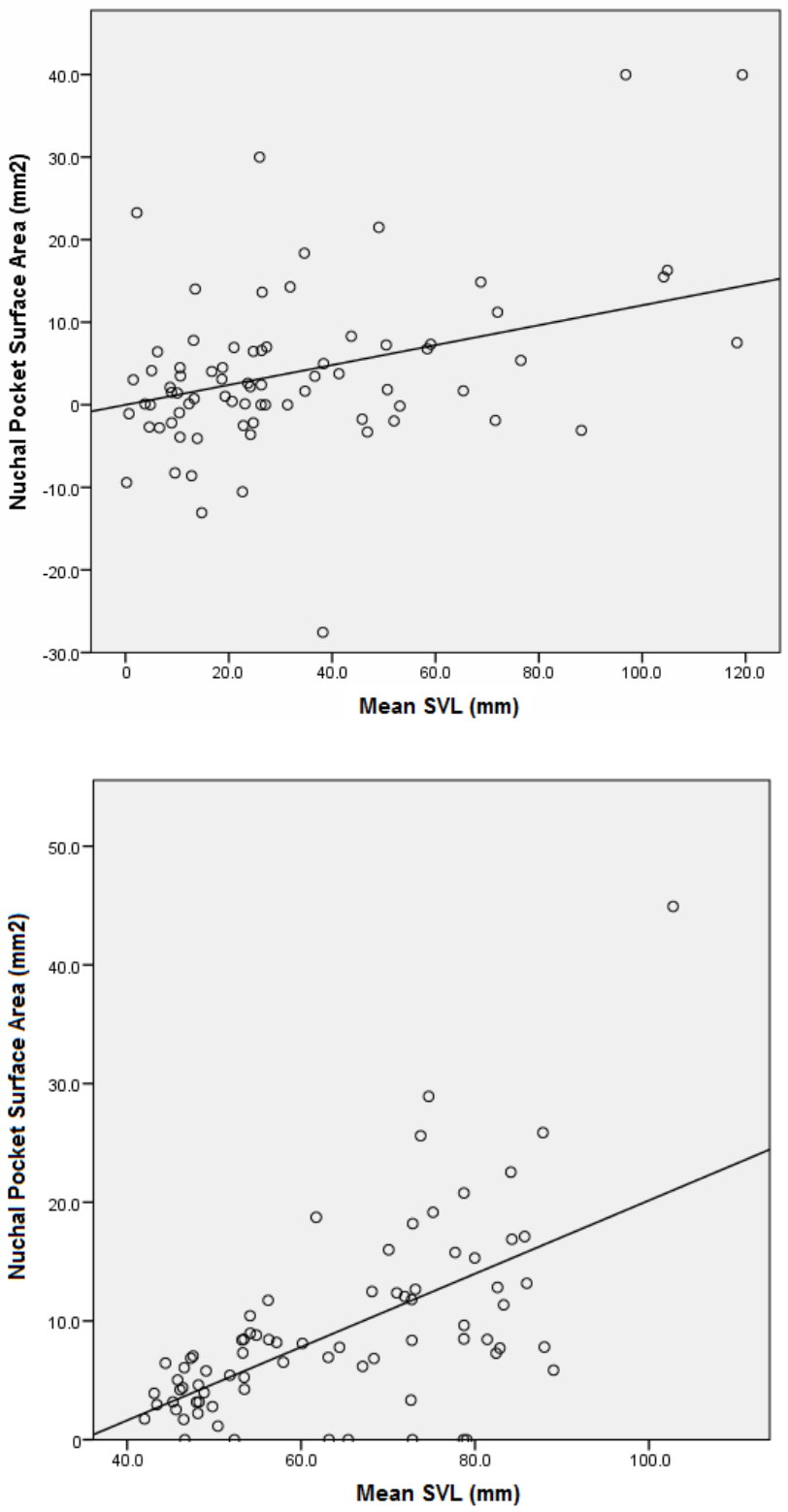

Figure 4.11: Bivariate scatterplot of relationship between mean nuchal pocket surface area and mean snout-vent length for phylogenetically independent (**top**: R=0.543, t=5.606, p<0.001) and conventional contrasts (**bottom**: R=0.605, t=6.584, p<0.001).

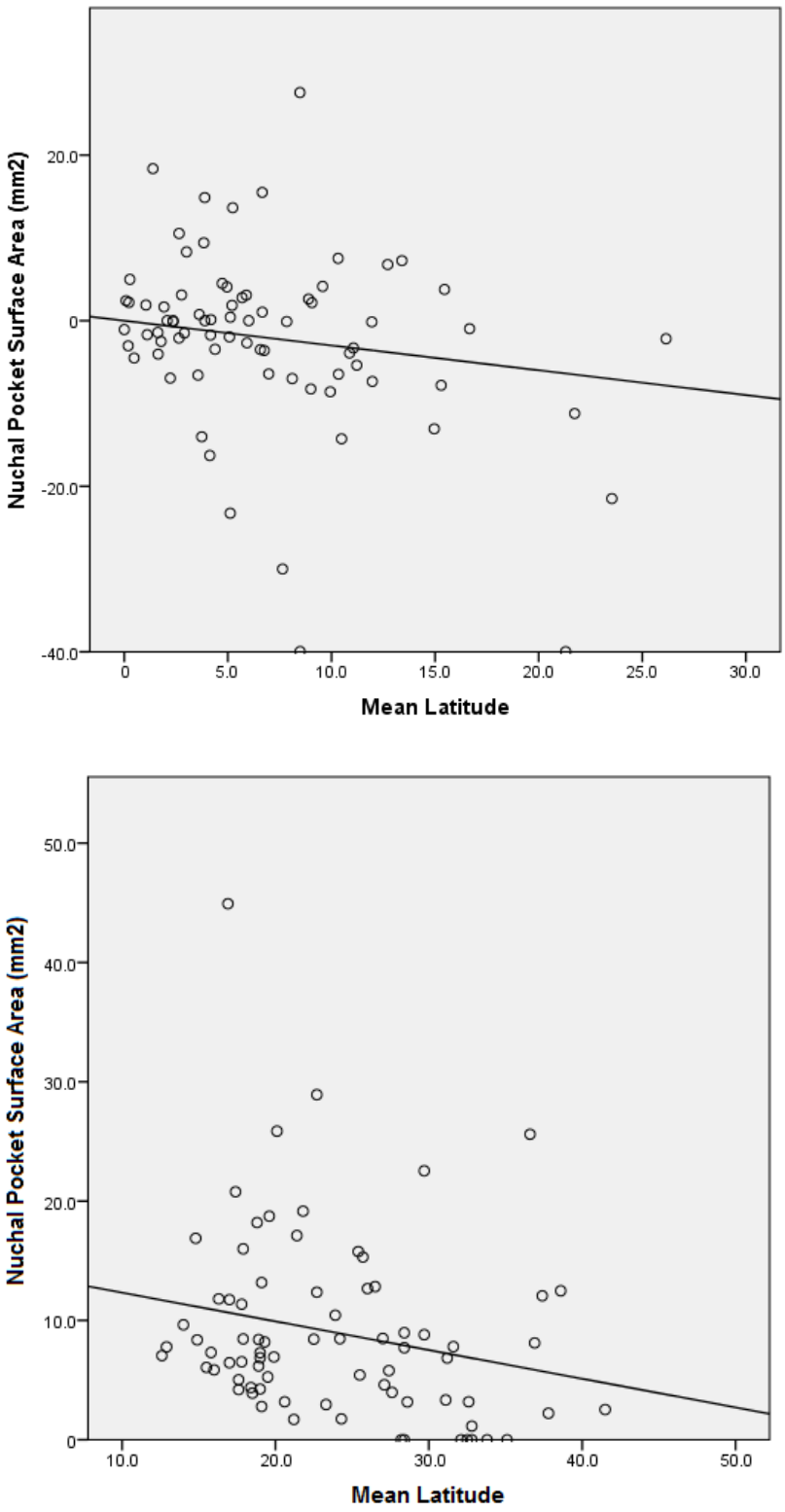

Figure 4.12: Bivariate scatterplot of relationship between mean nuchal pocket surface area and mean latitude for phylogenetically independent (**top**: R=-0.349, t=-3.229, p=0.002) and conventional contrasts (**bottom**: R=-0.220, t=-1.956, p=0.054).

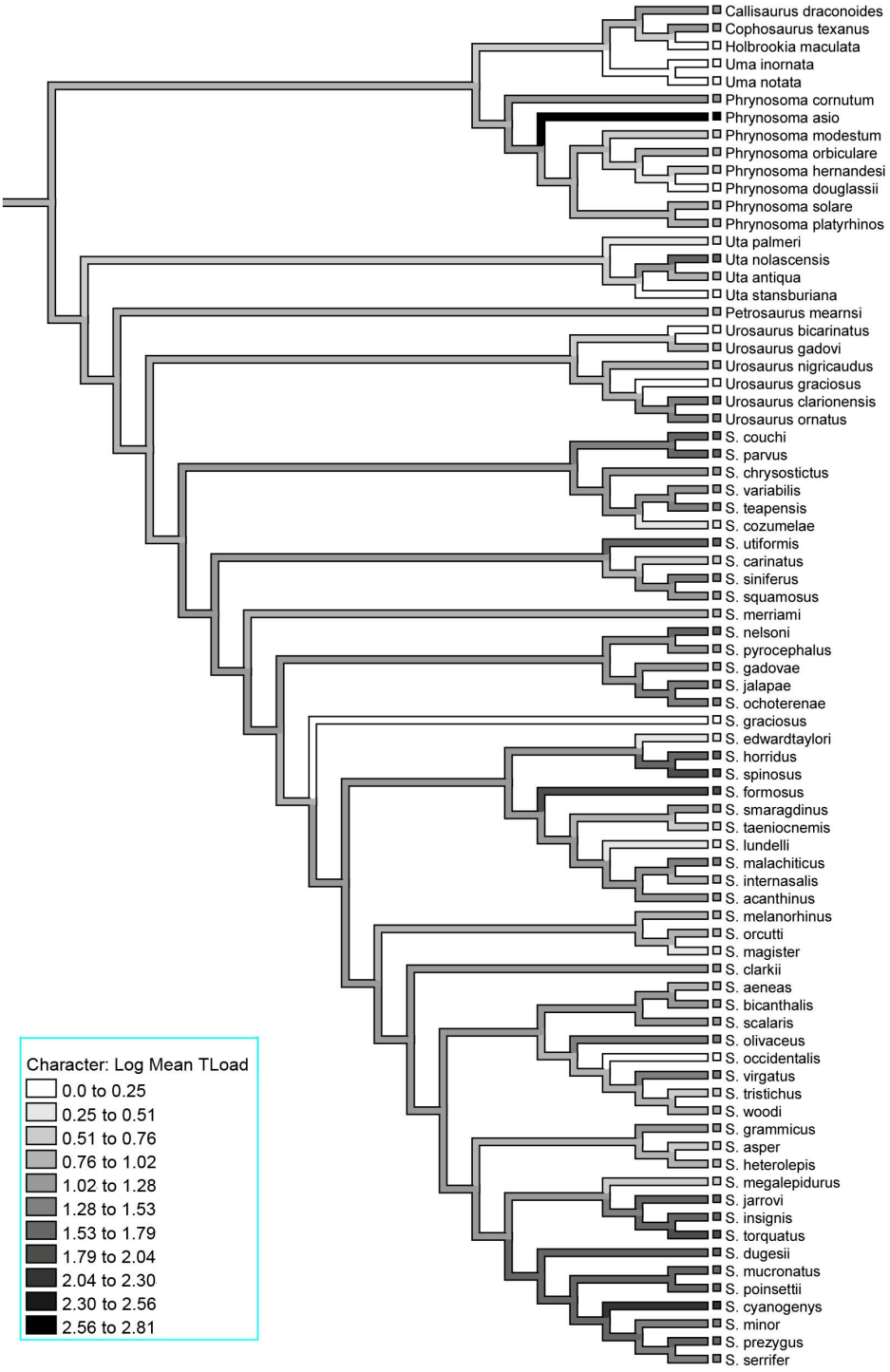

Figure 4.13: Ancestral state reconstruction for log mean total mite load, plotted over the topology of the Phrynosomatidae.

Figure 4.14: Ancestral state reconstruction for log post-inguinal mite load, plotted over the topology of the Phrynosomatidae.

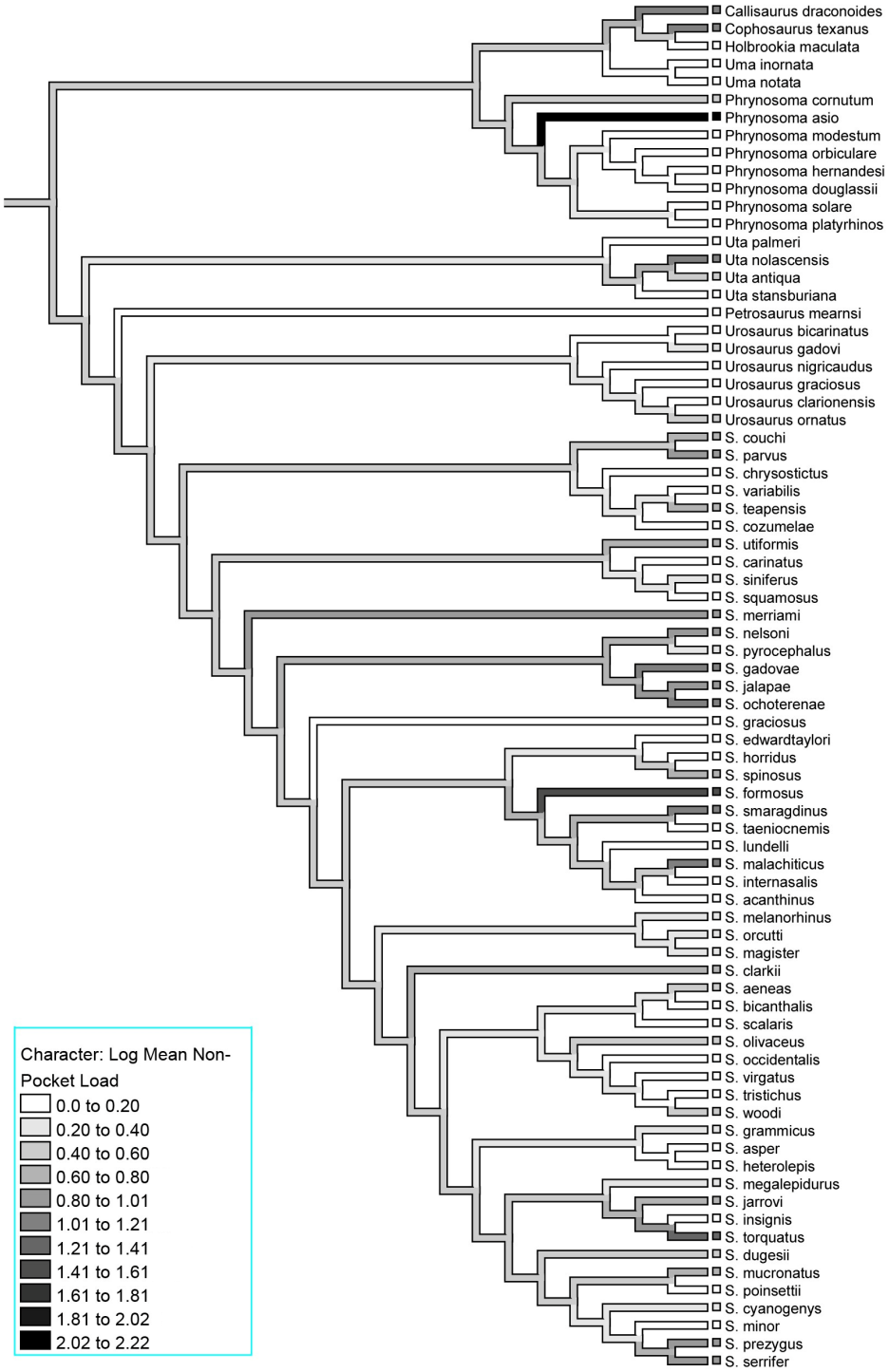

Figure 4.15: Ancestral state reconstruction for log non-pocket mite load (total mite load excluding nuchal and post-inguinal pocket load), plotted over the topology of the Phrynosomatidae.

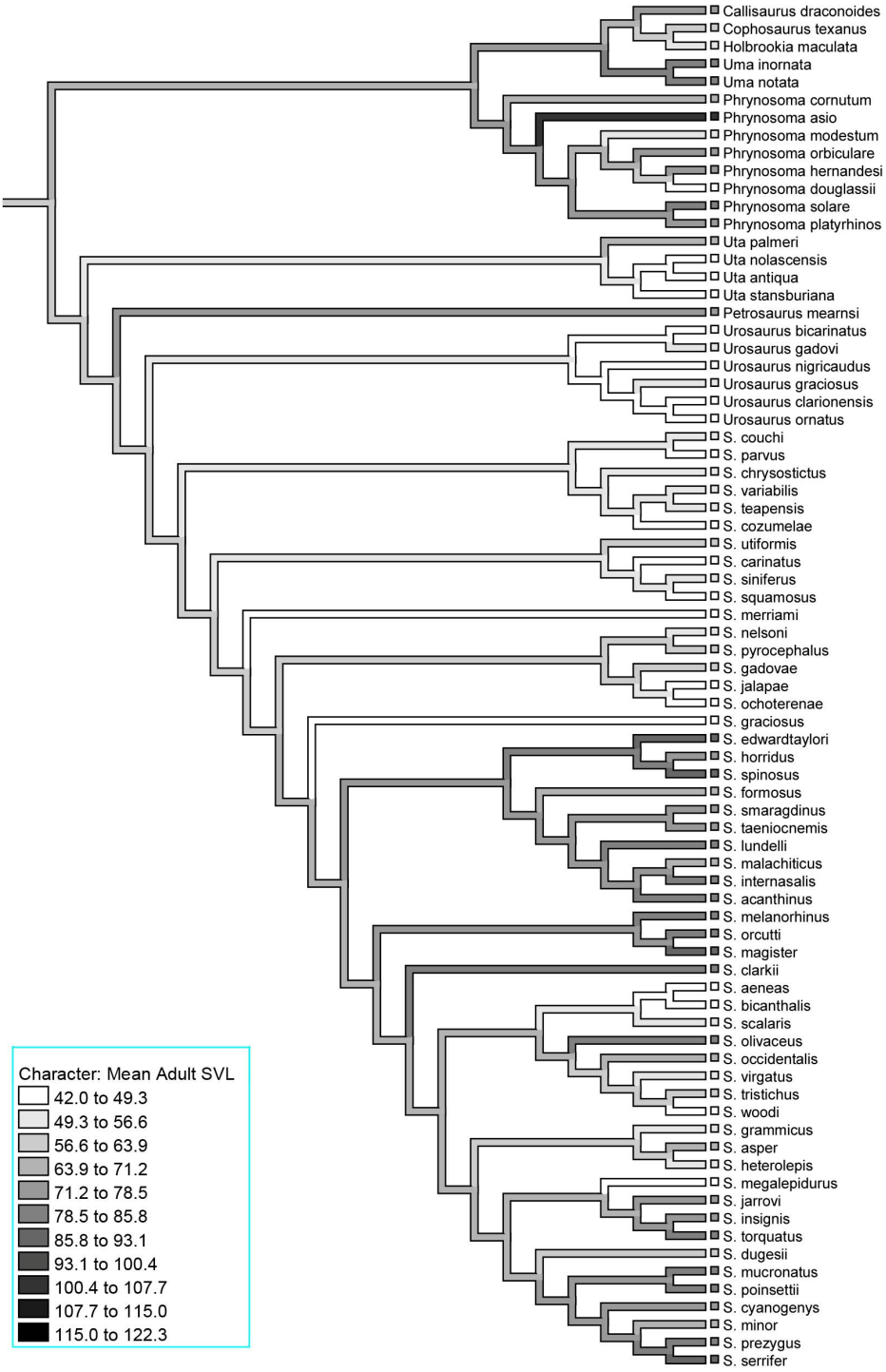

Figure 4.16: Ancestral state reconstruction for mean adult snout-vent length (mm), plotted over the topology of the Phrynosomatidae.

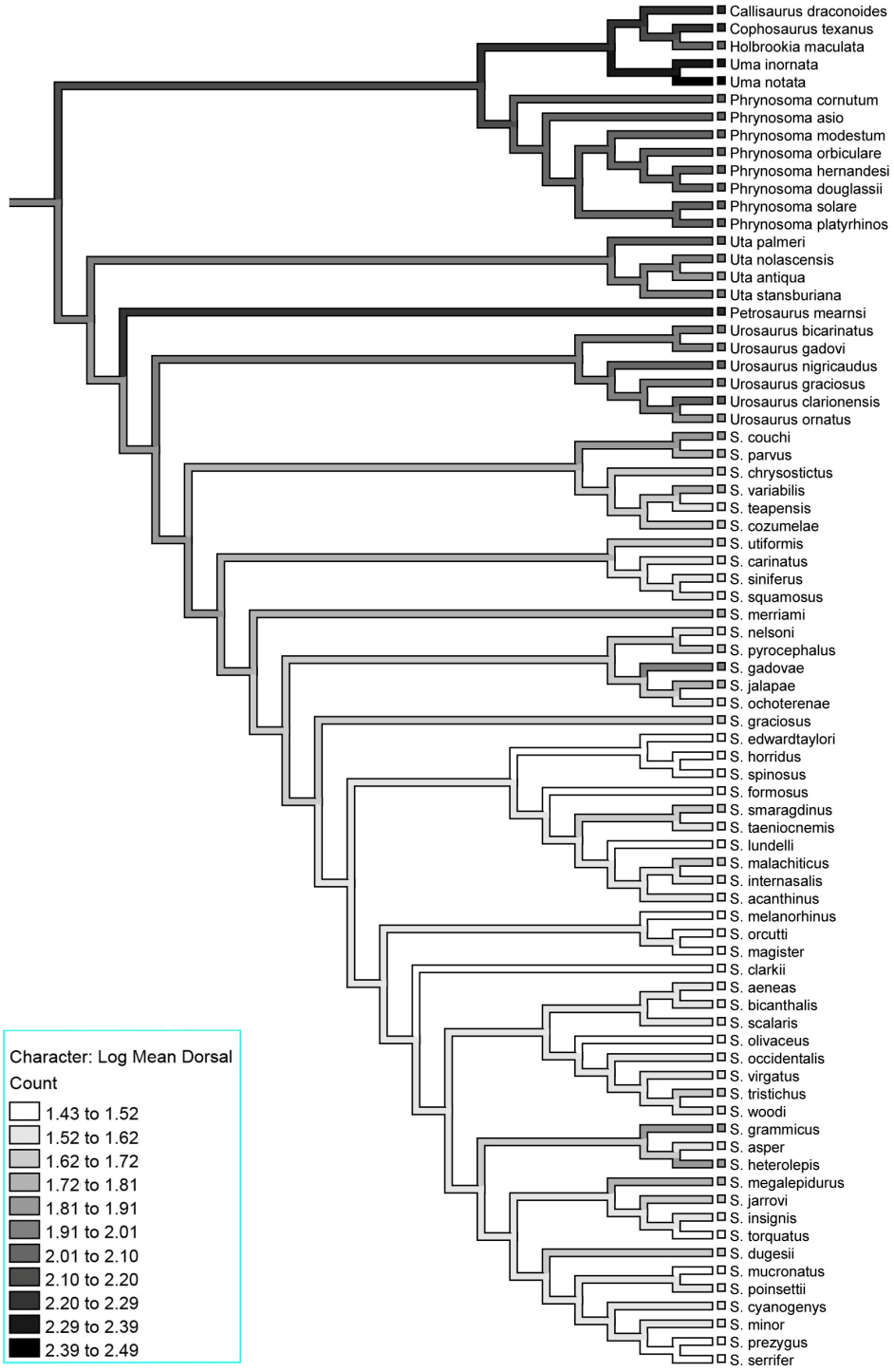

Figure 4.17: Ancestral state reconstruction for log dorsal scale count, plotted over the topology of the Phrynosomatidae. Dorsal count is used to quantify species rugosity; relatively smooth species tend to possess high dorsal scale counts (dark shading), while rugose species tend to possess low dorsal scale counts (light shading).

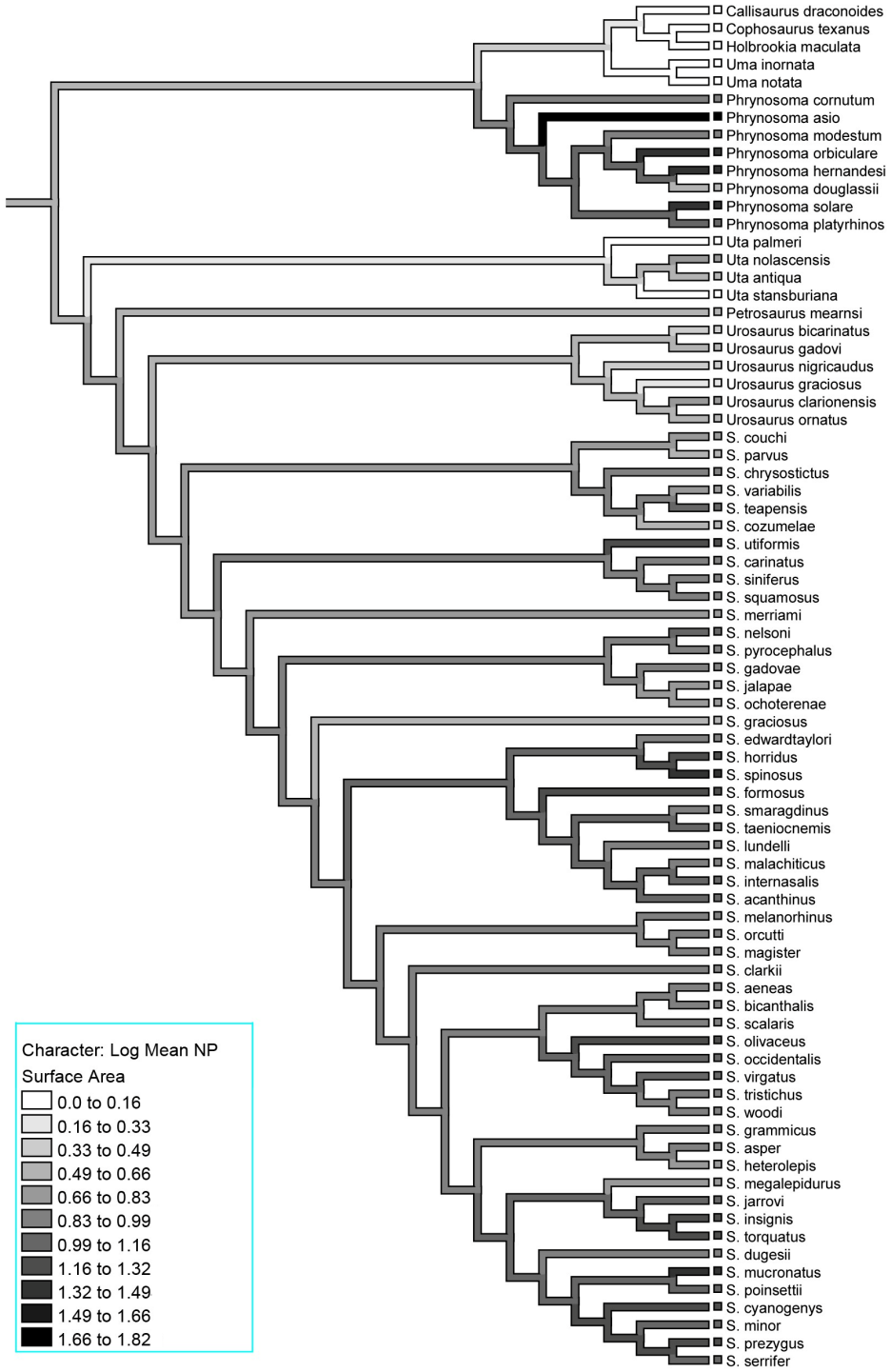

Figure 4.18: Ancestral state reconstruction for nuchal pocket size, measured as log nuchal pocket surface area (mm^2), plotted over the topology of the Phrynosomatidae.

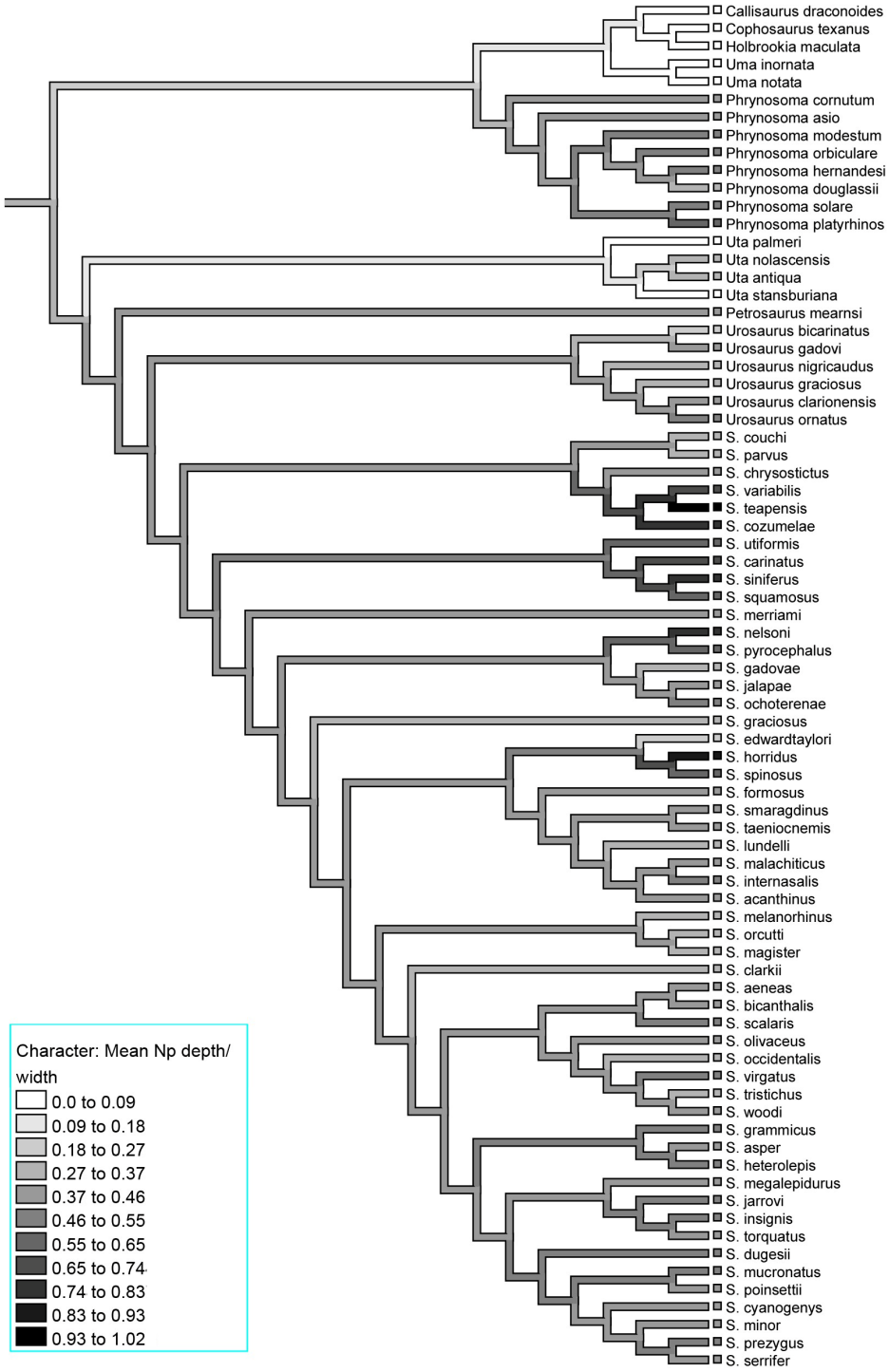

Figure 4.19: Ancestral state reconstruction for nuchal pocket shape, measured as the depth/width ratio of the nuchal pocket, plotted over the topology of the Phrynosomatidae. Light shading indicates species with shallow, fold-type pockets; dark shading indicates species with deeper, ovoid- or pit-type pockets. See text for details.

Figure 4.20: Ancestral state reconstruction for post-inguinal pocket size, measured as post-inguinal pocket surface area (mm^2), plotted over the topology of the Phrynosomatidae.

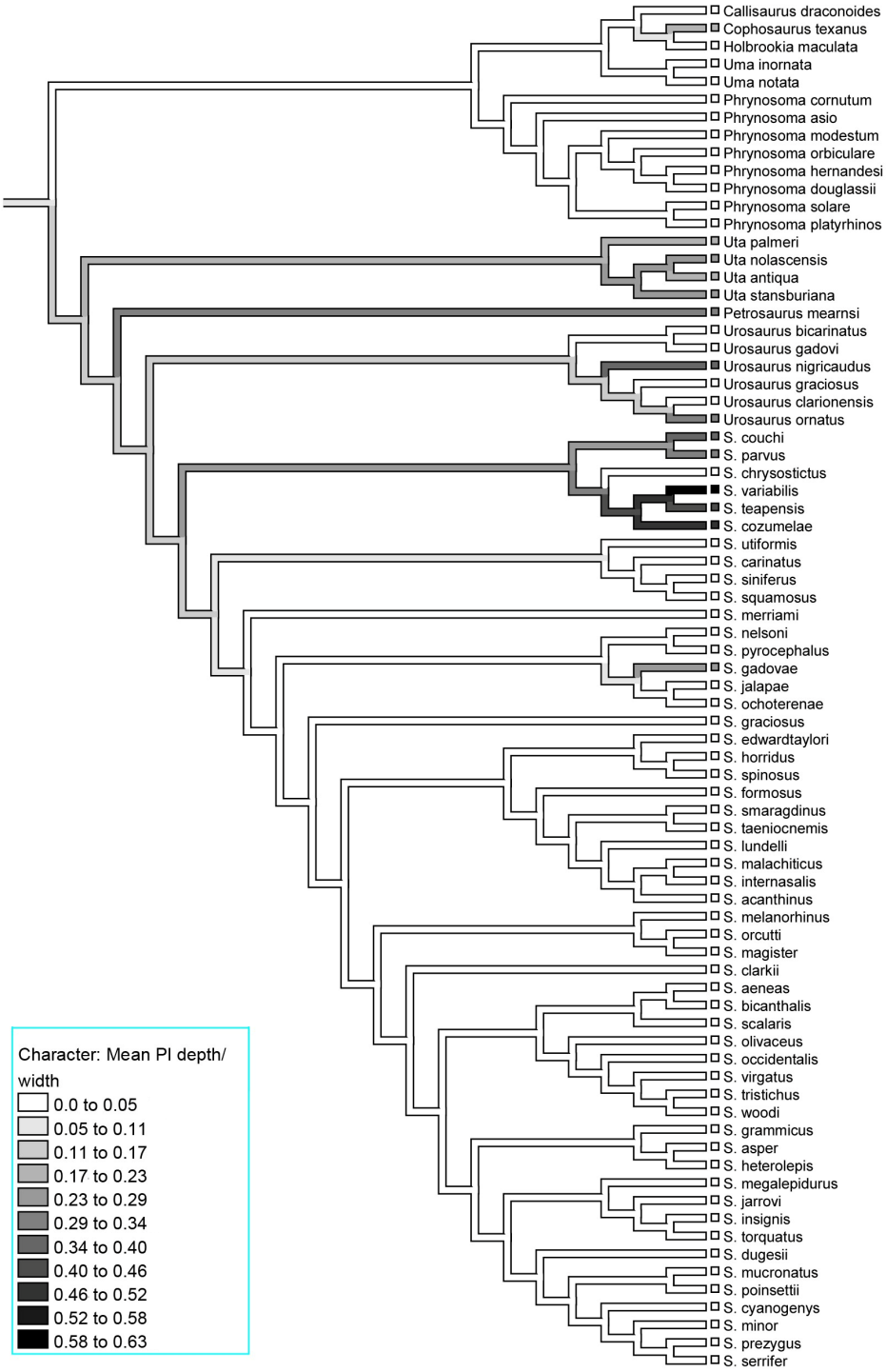

Figure 4.21: Ancestral state reconstruction for post-inguinal pocket shape, measured as the depth/width ratio of the post-inguinal pocket, plotted over the topology of the Phrynosomatidae. See text for details.

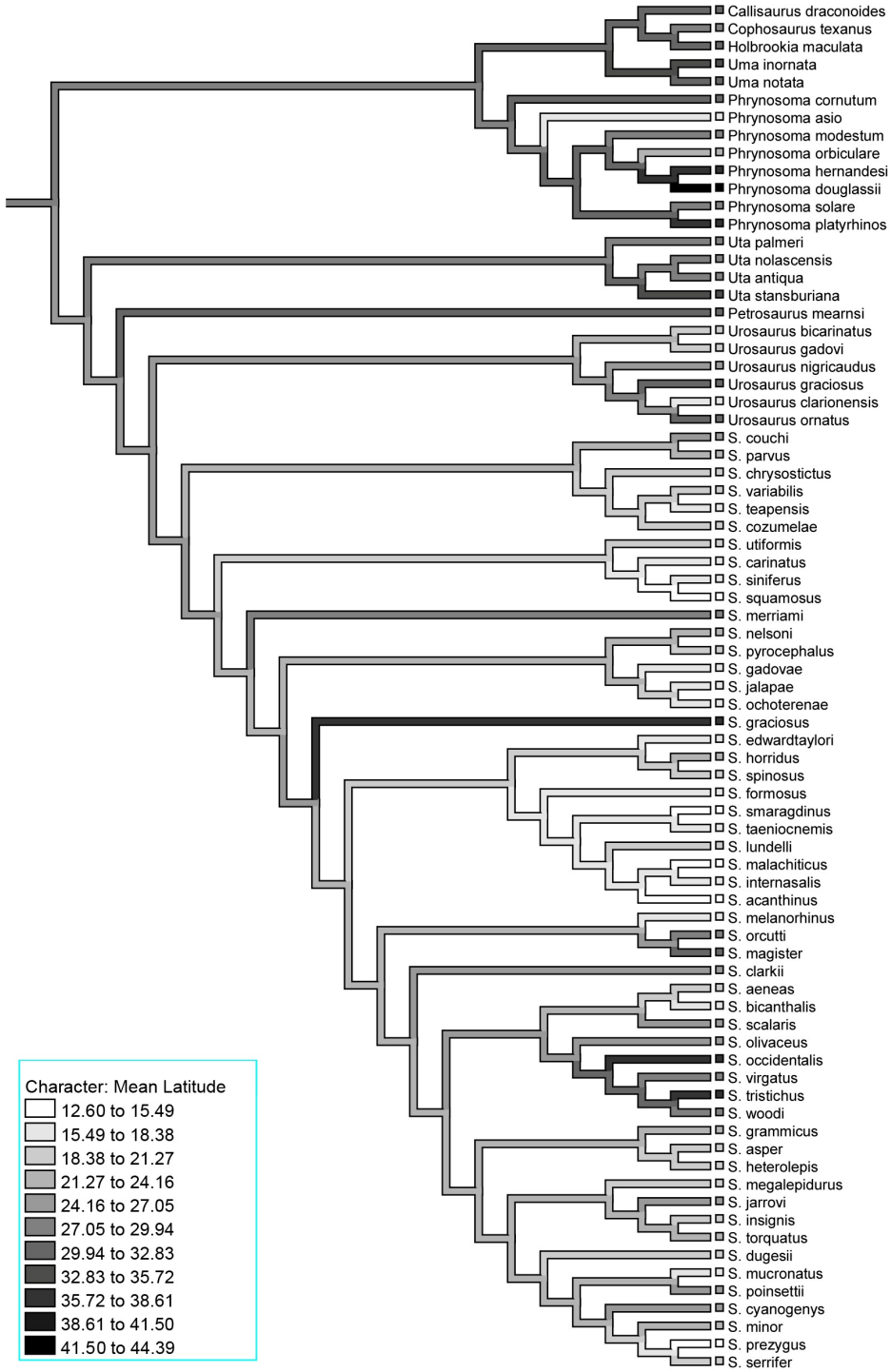

Figure 4.22: Ancestral state reconstruction for mean latitude, plotted over the topology of the Phrynosomatidae.

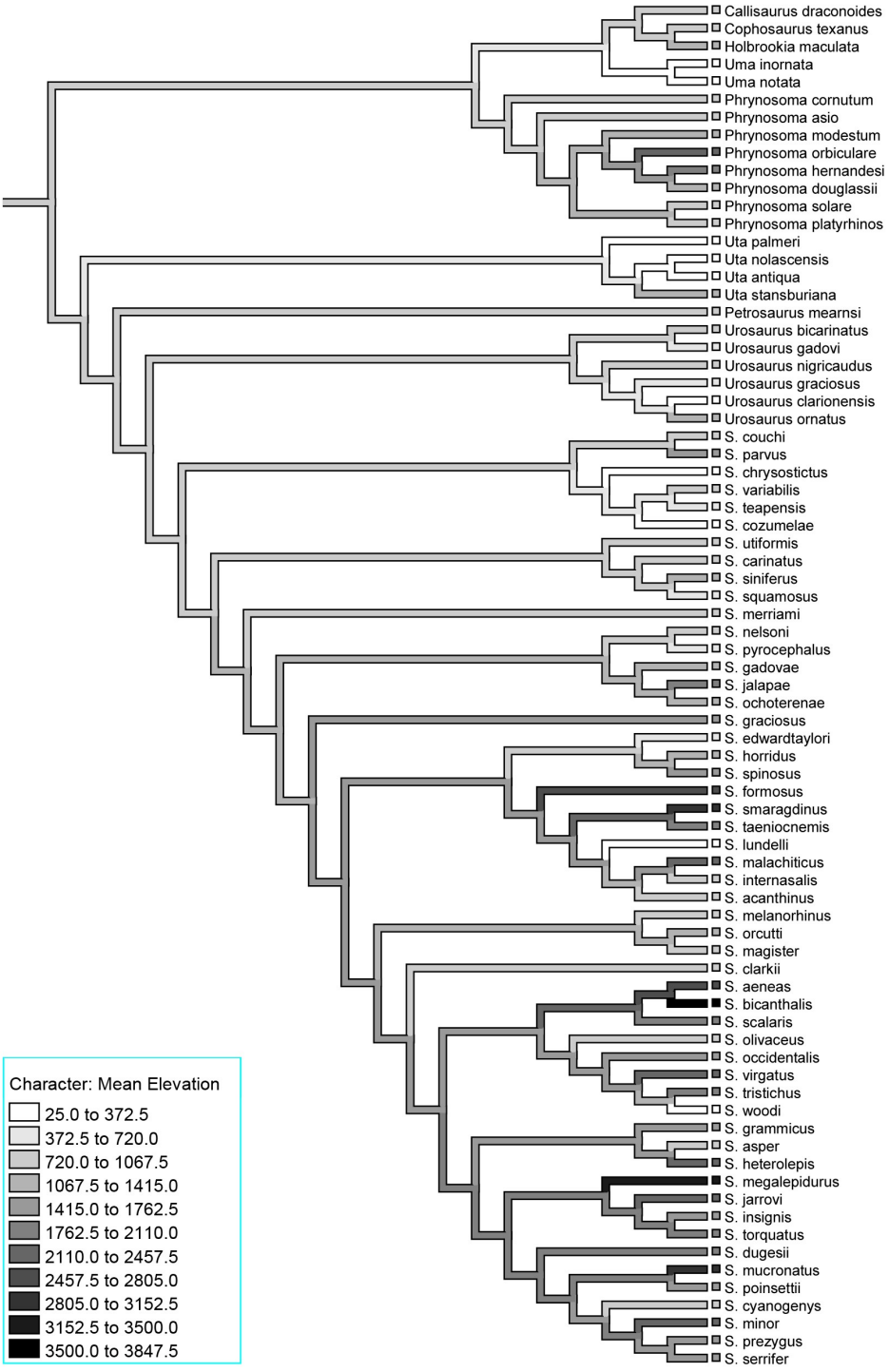

Figure 4.23: Ancestral state reconstruction for mean elevation, plotted over the topology of the Phrynosomatidae.

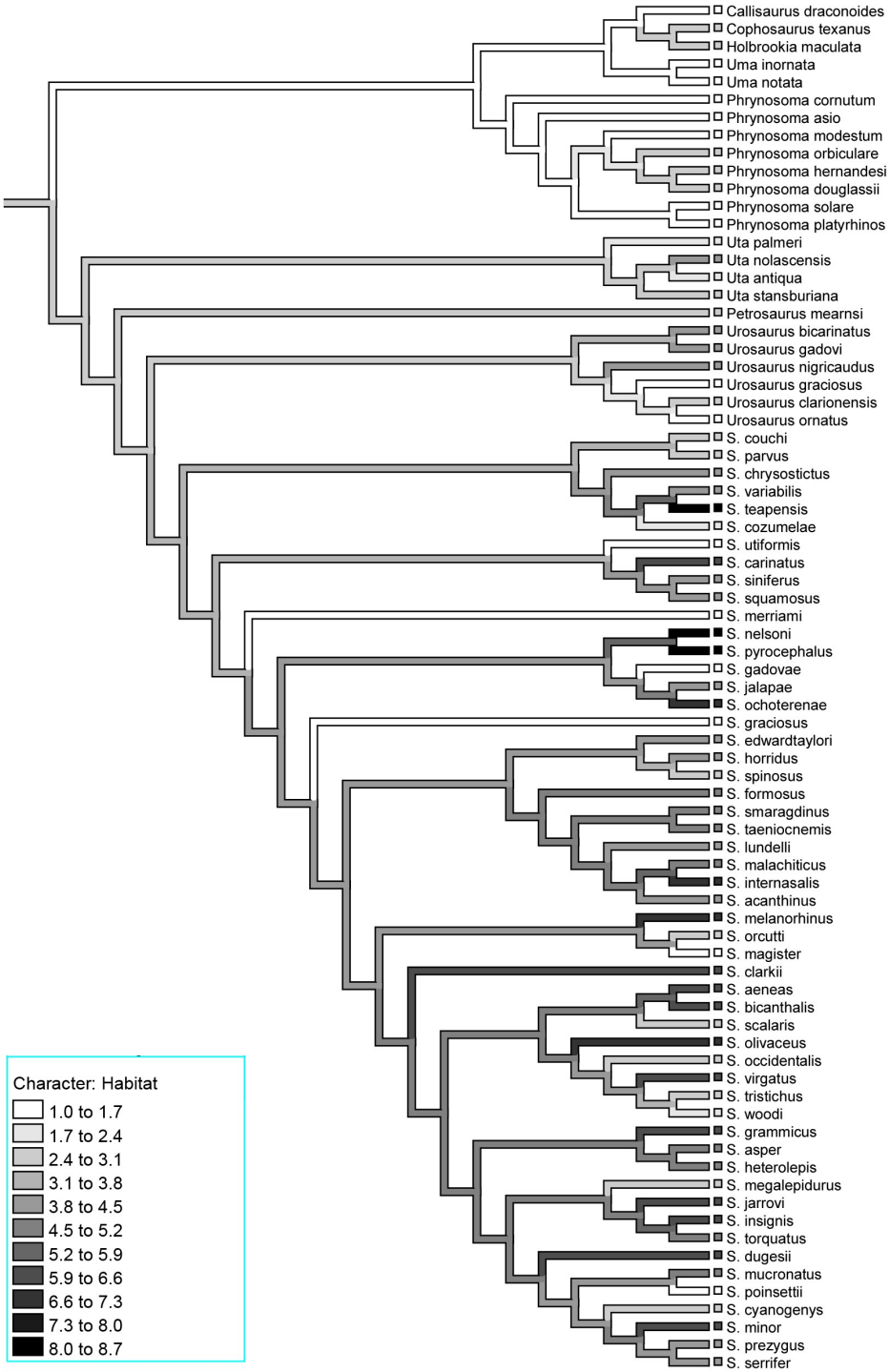

Figure 4.24: Ancestral state reconstruction for habitat, ranked in order from most (1.0) to least arid (8.0), and plotted over the topology of the Phrynosomatidae. See text for details.

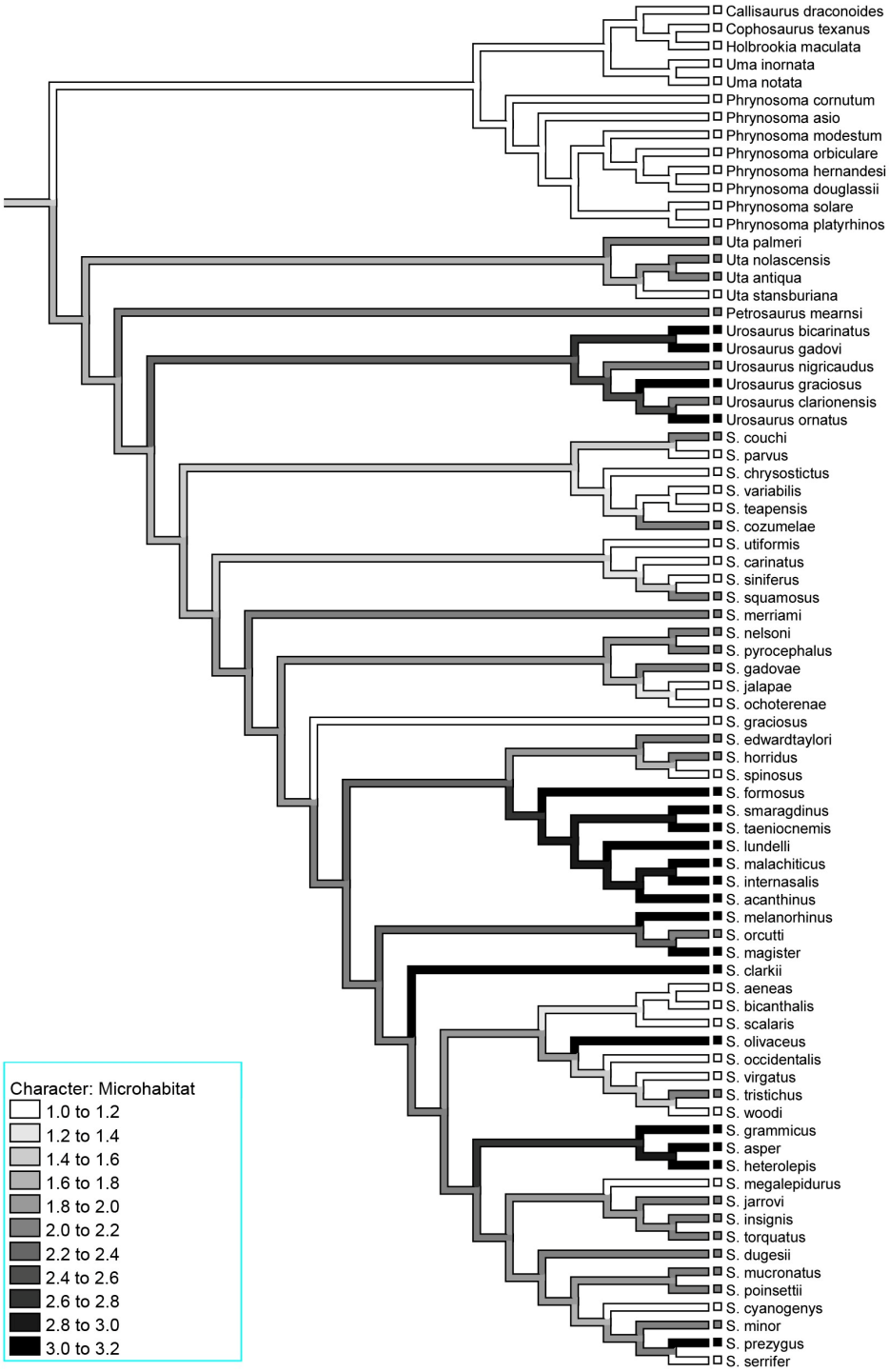

Figure 4.25: Ancestral state reconstruction for microhabitat, ranked in order from most (1.0) to least terrestrial (3.0), and plotted over the topology of the Phrynosomatidae. See text for details.

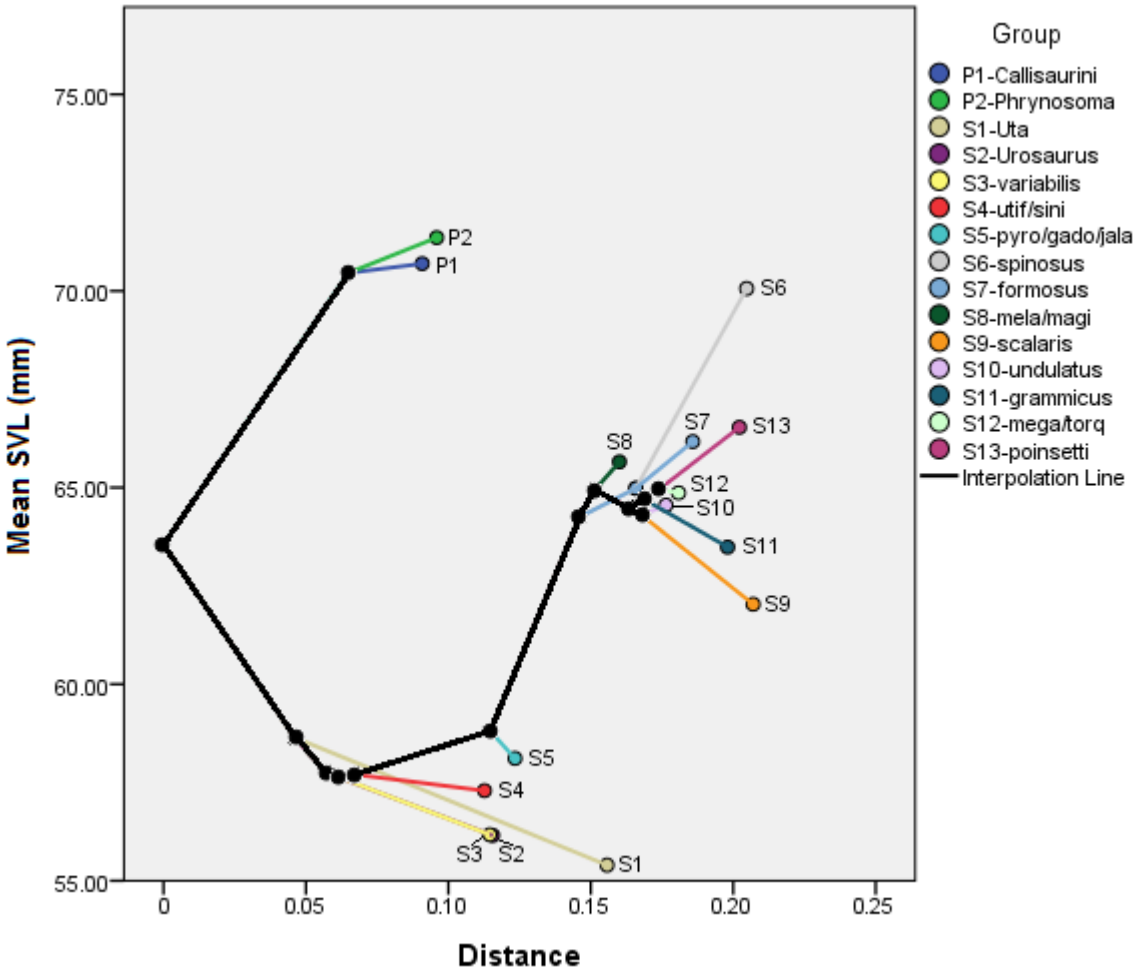

Figure 4.26: Ancestral state reconstruction for internodal and species group values for mean snout-vent length (mm), plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

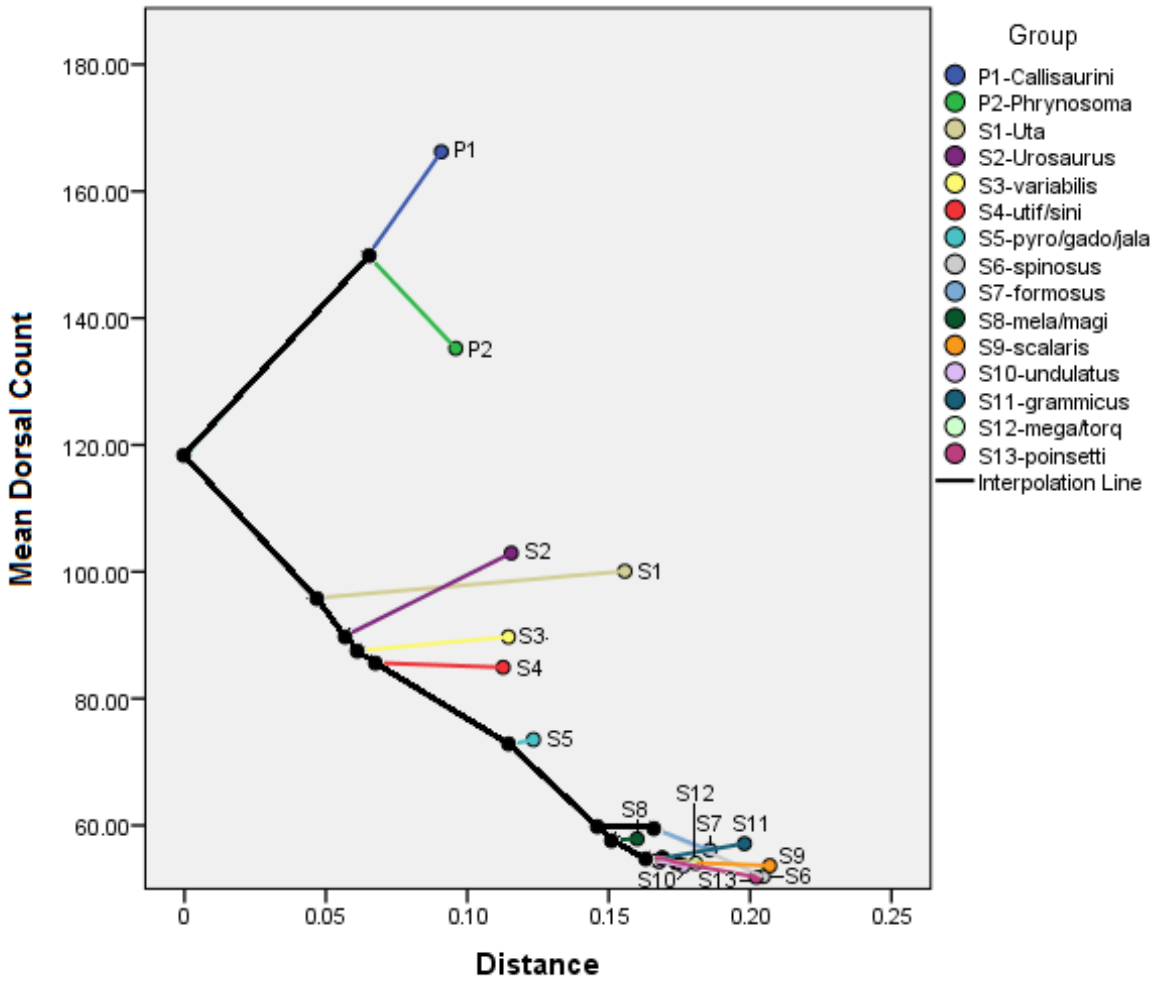

Figure 4.27: Ancestral state reconstruction for internodal and species group values for mean dorsal scale count, plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

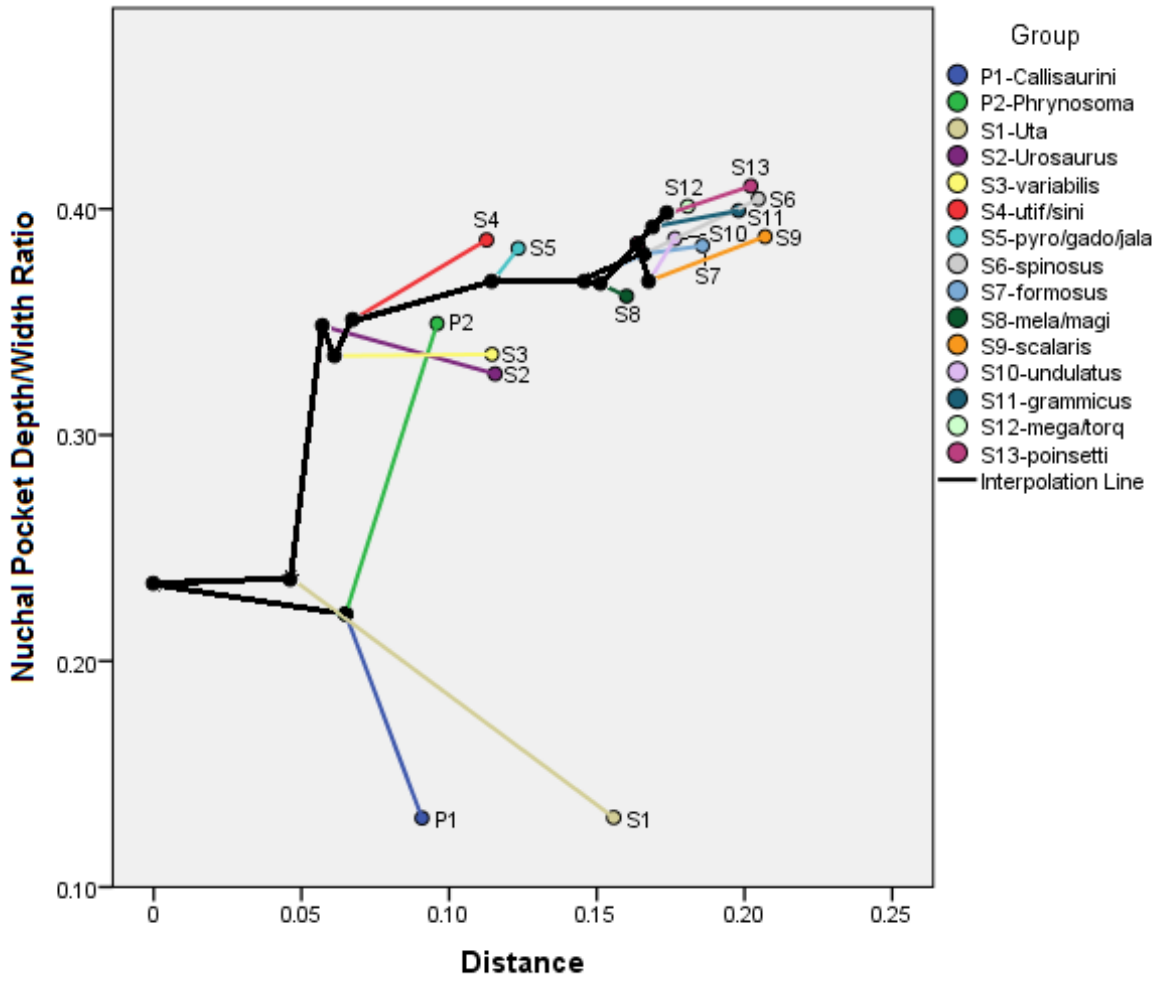

Figure 4.28: Ancestral state reconstruction for internodal and species group values for mean nuchal pocket depth/width, plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

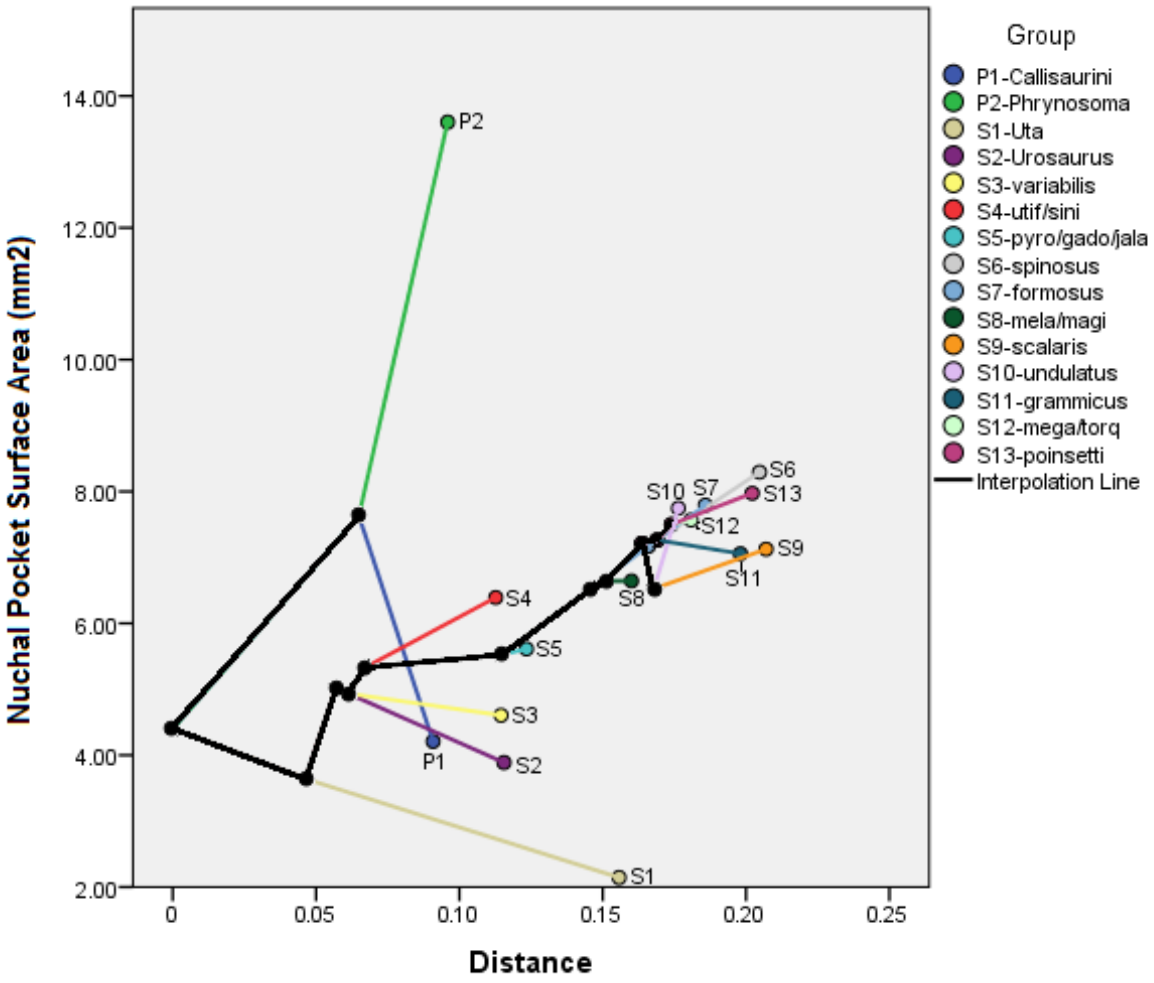

Figure 4.29: Ancestral state reconstruction for internodal and species group values for mean nuchal pocket surface area (mm^2), plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

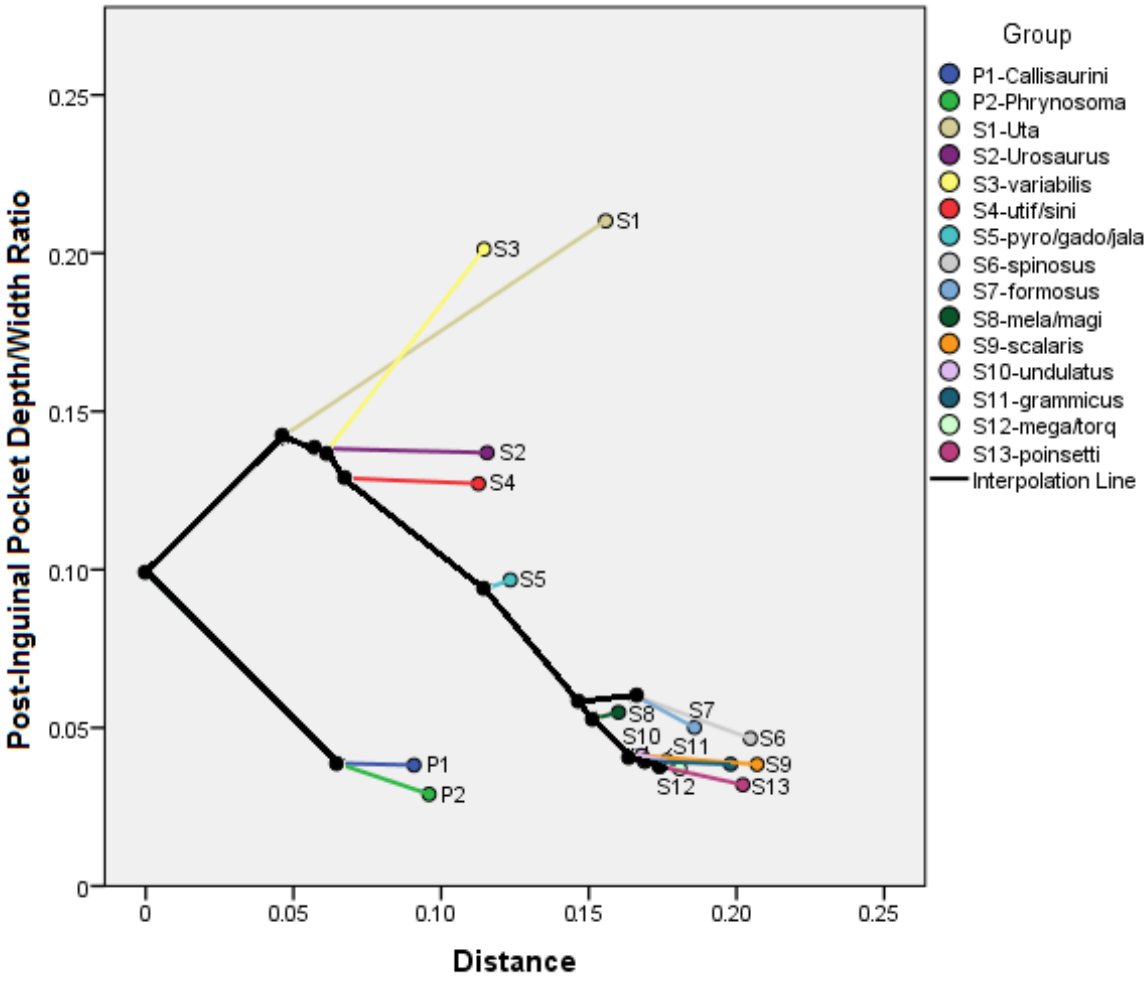

Figure 4.30: Ancestral state reconstruction for internodal and species group values for mean post-inguinal pocket depth/width, plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

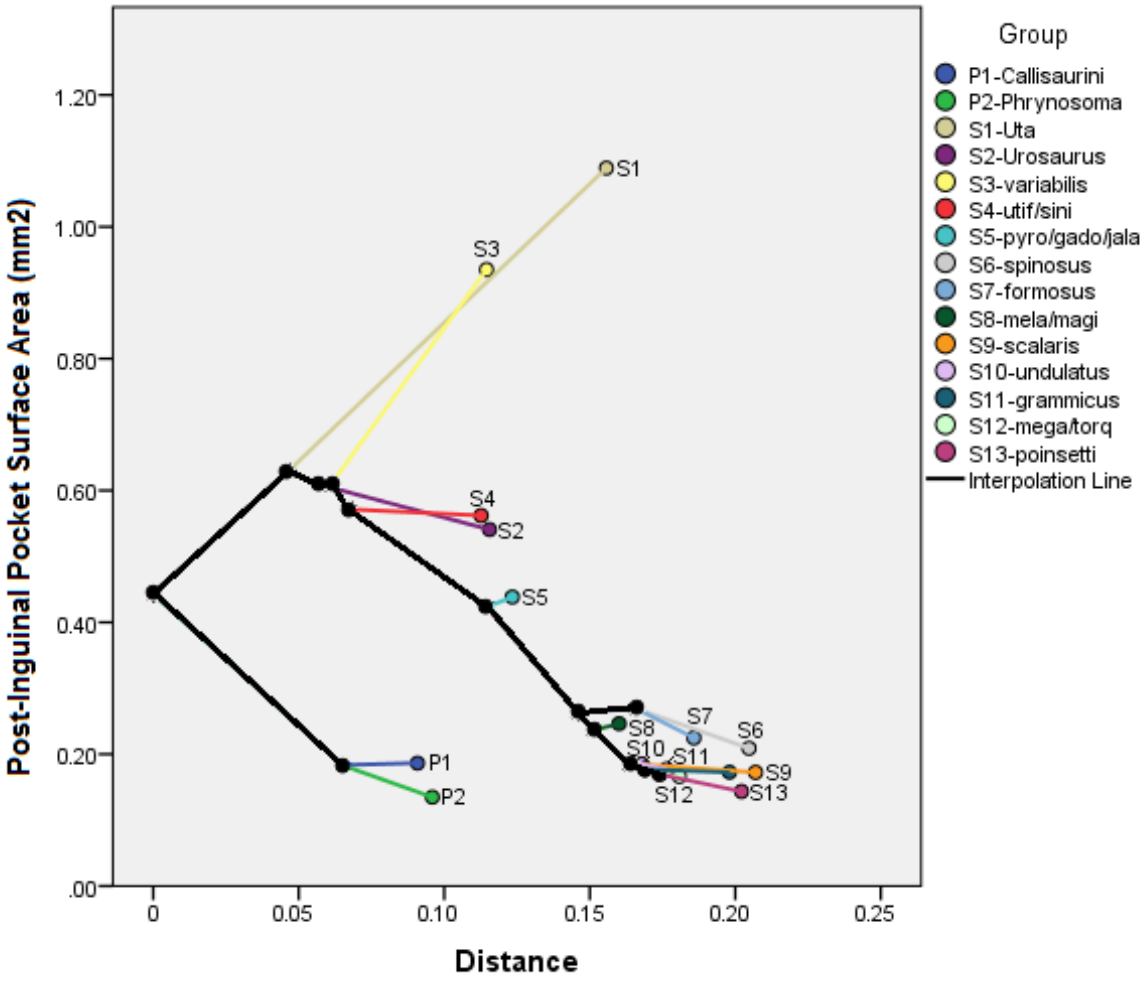

Figure 4.31: Ancestral state reconstruction for internodal and species group values for mean post-inguinal pocket surface area (mm^2), plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

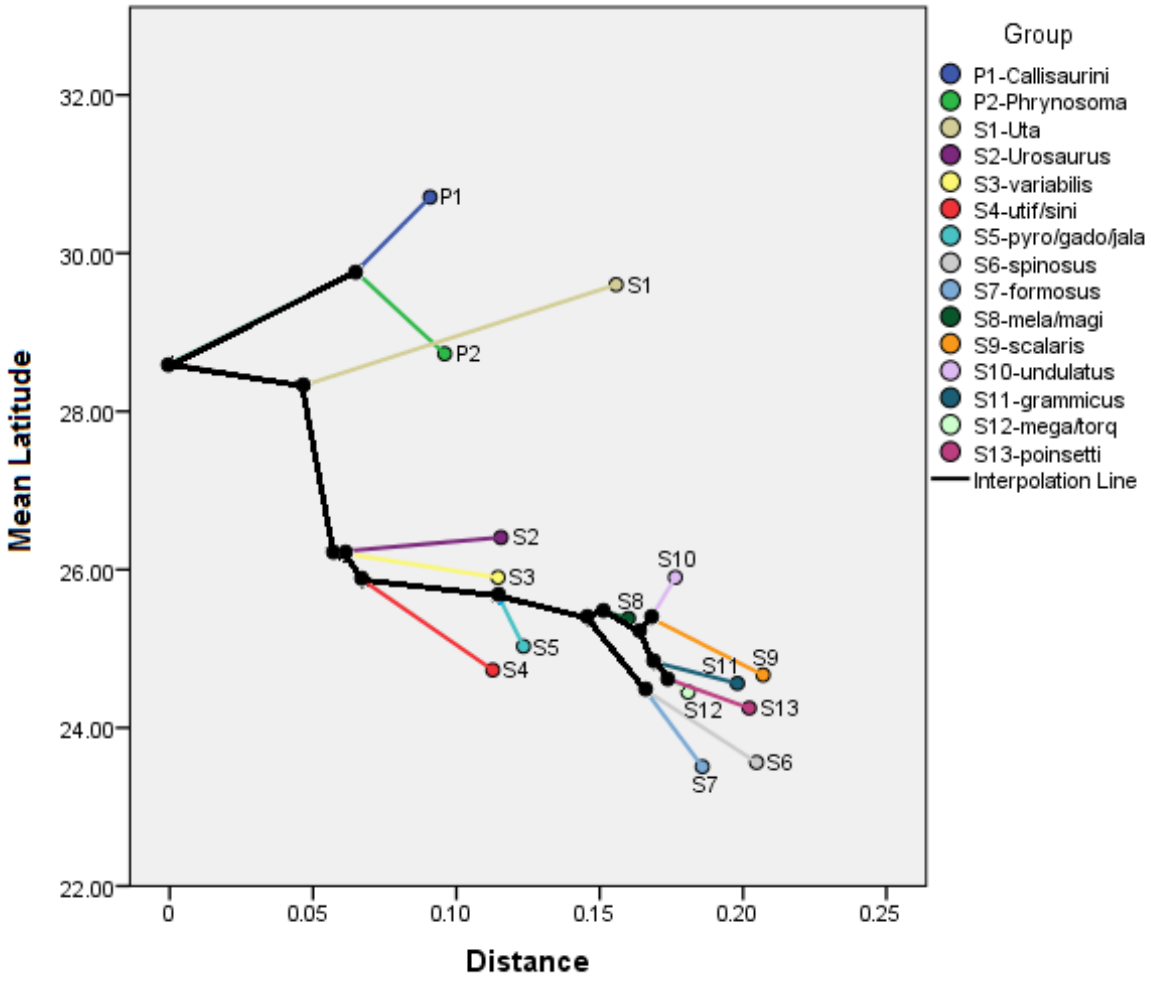

Figure 4.32: Ancestral state reconstruction for internodal and species group values for mean latitude, plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

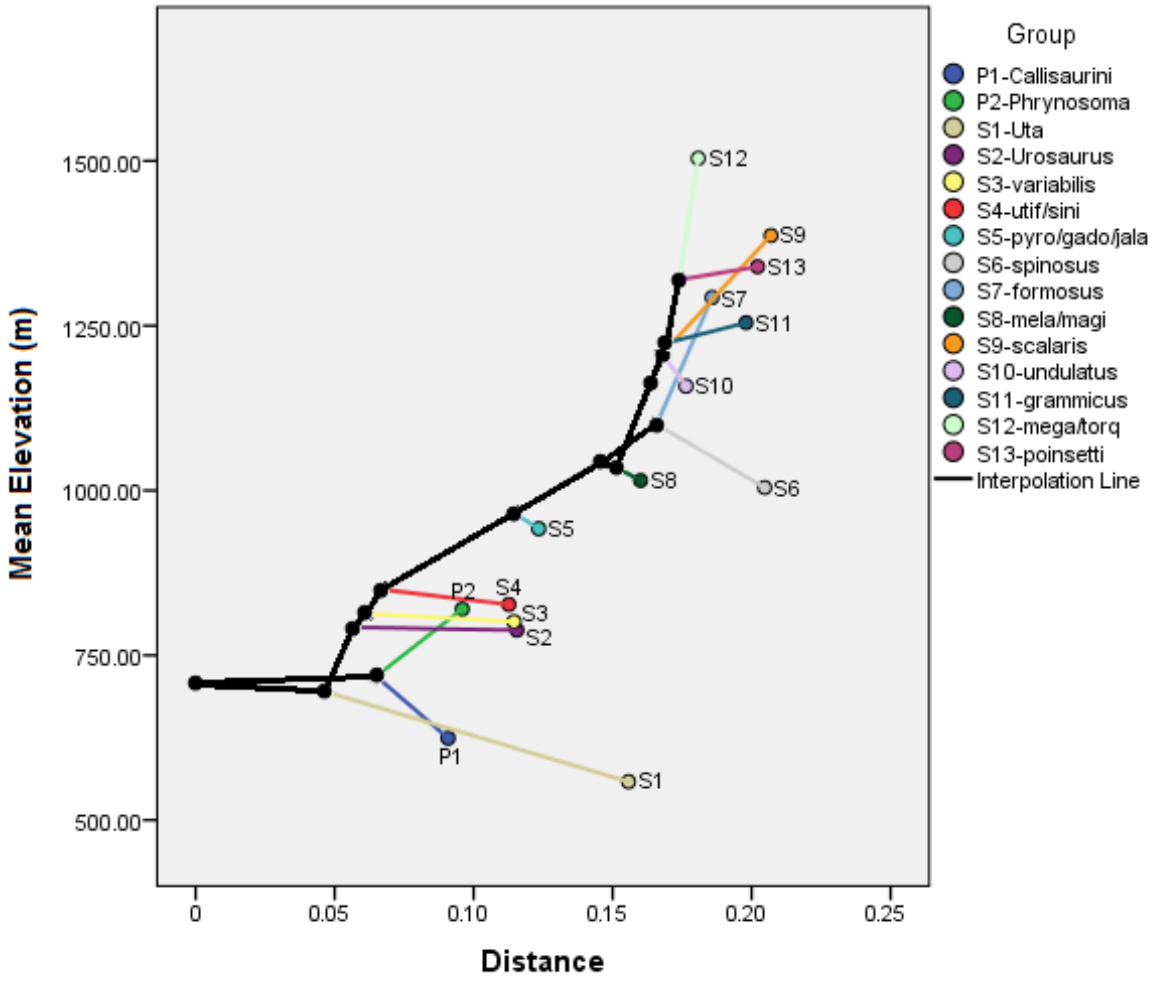

Figure 4.33: Ancestral state reconstruction for internodal and species group values for mean elevation, plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

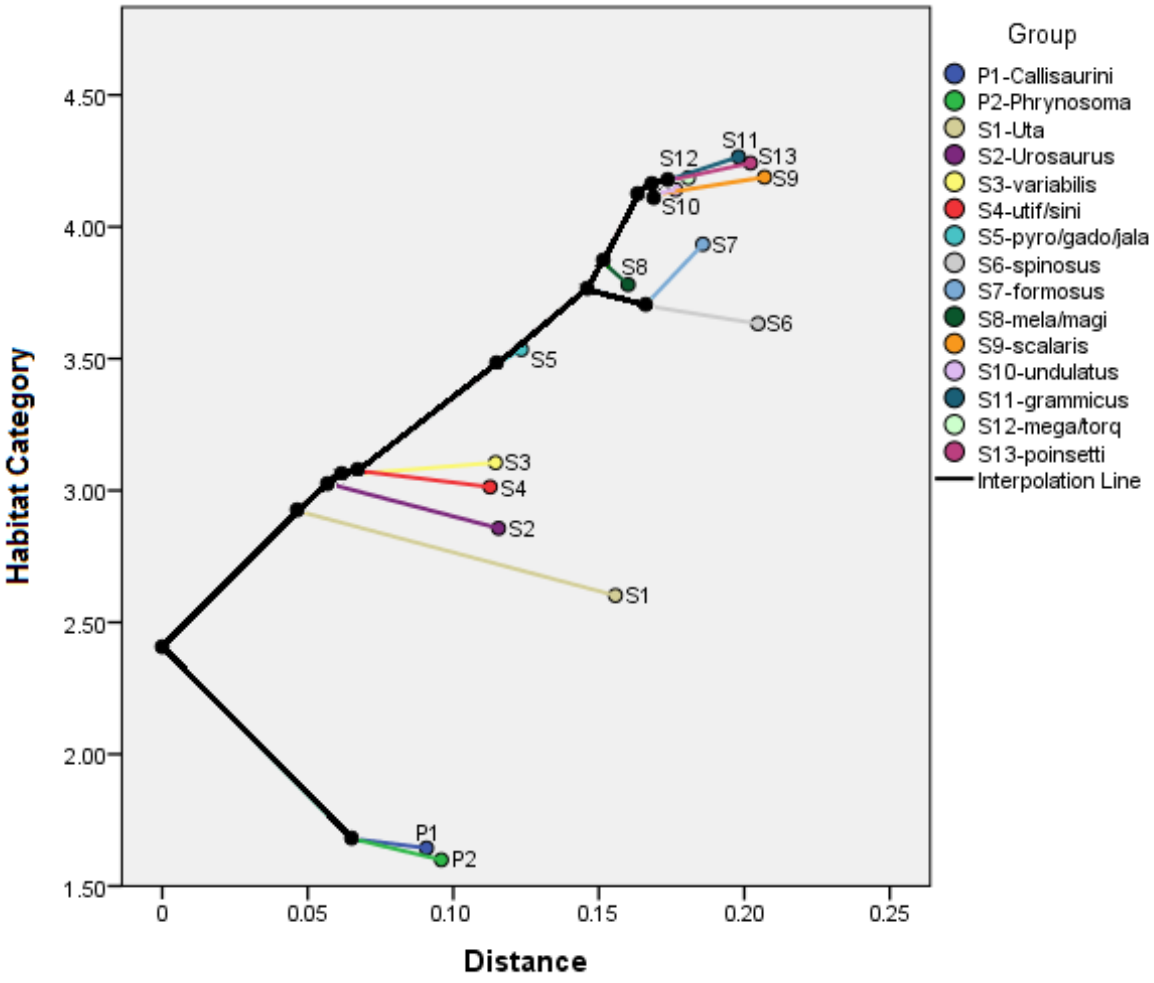

Figure 4.34: Ancestral state reconstruction for internodal and species group values for habitat, plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

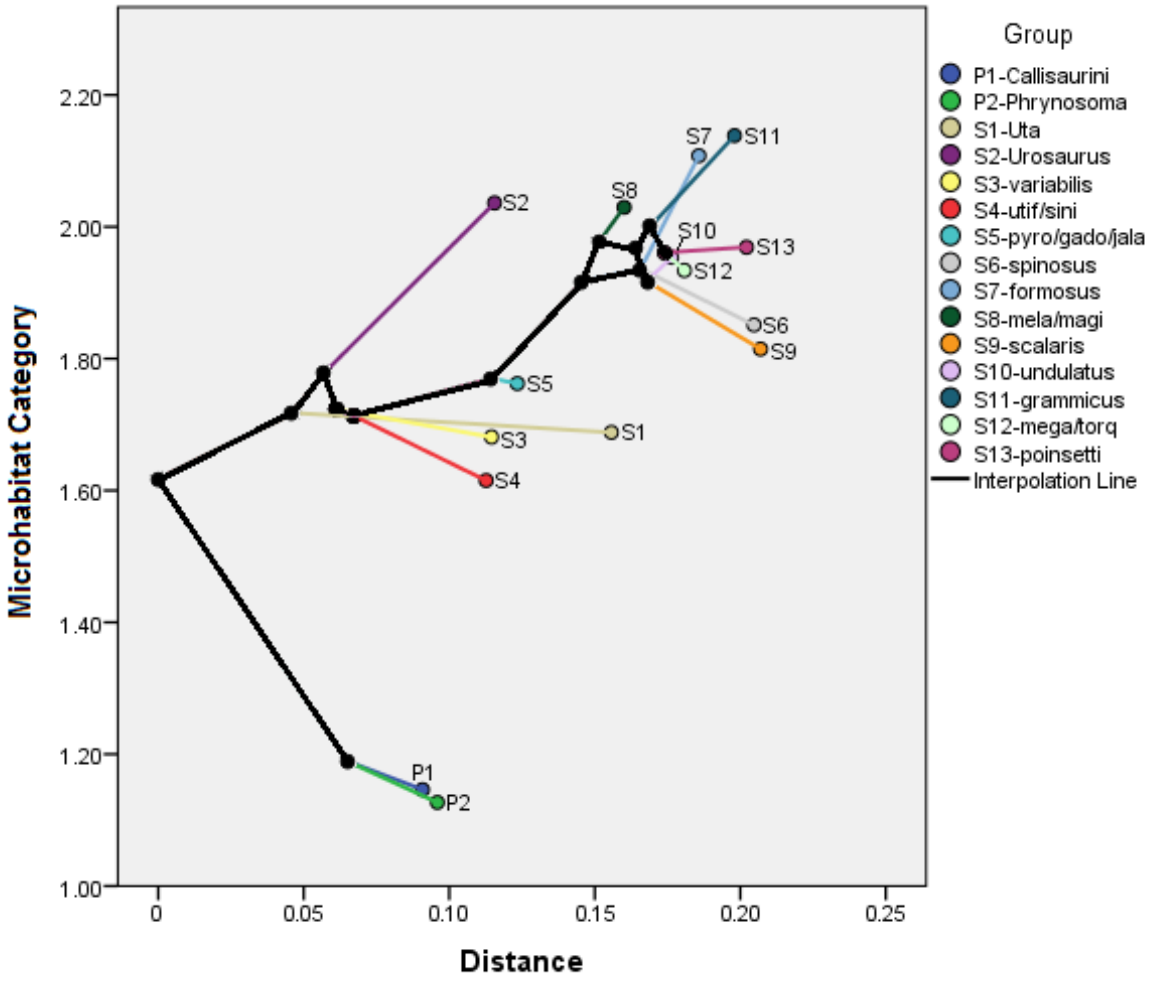

Figure 4.35: Ancestral state reconstruction for internodal and species group values for microhabitat, plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

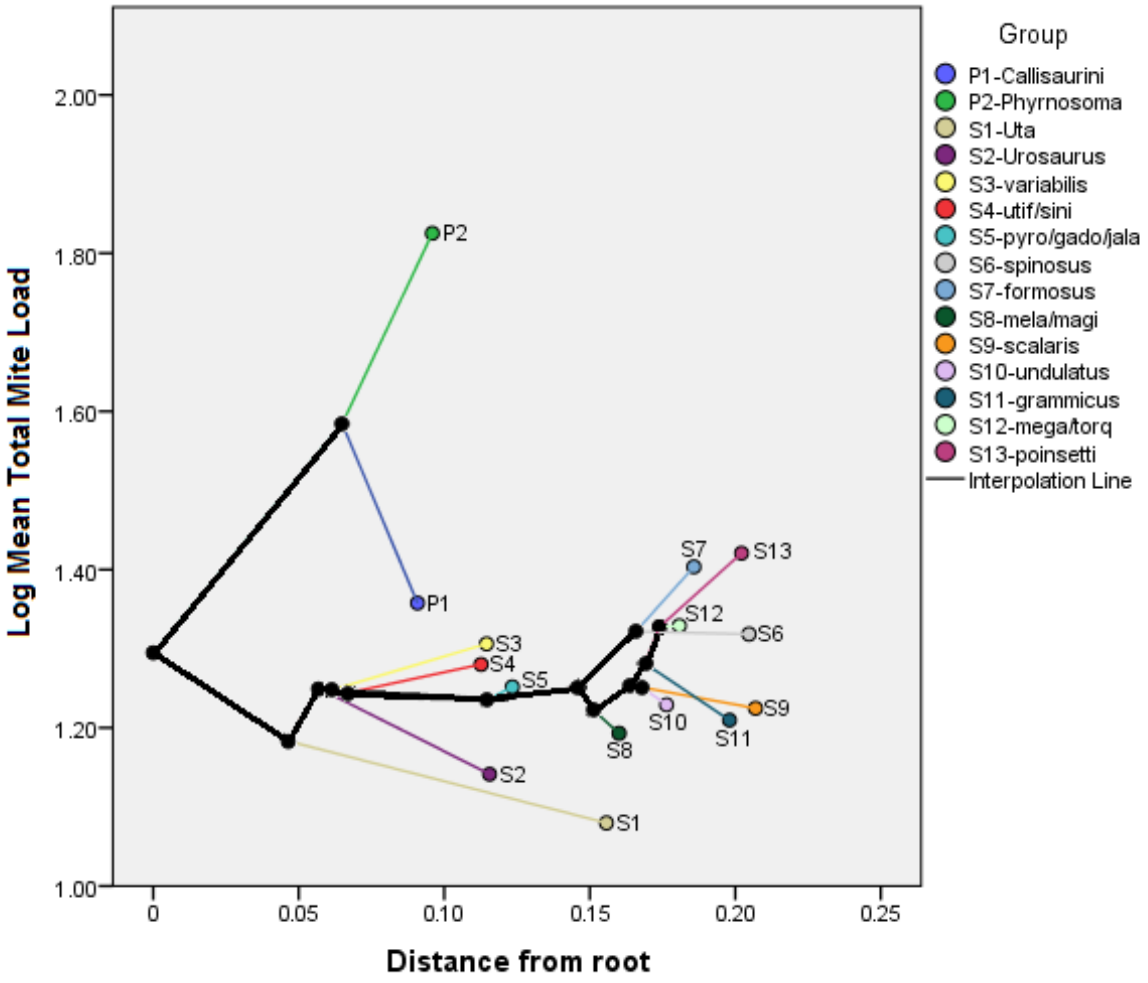

Figure 4.36: Ancestral state reconstruction for internodal and species group values for log total mite load, plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

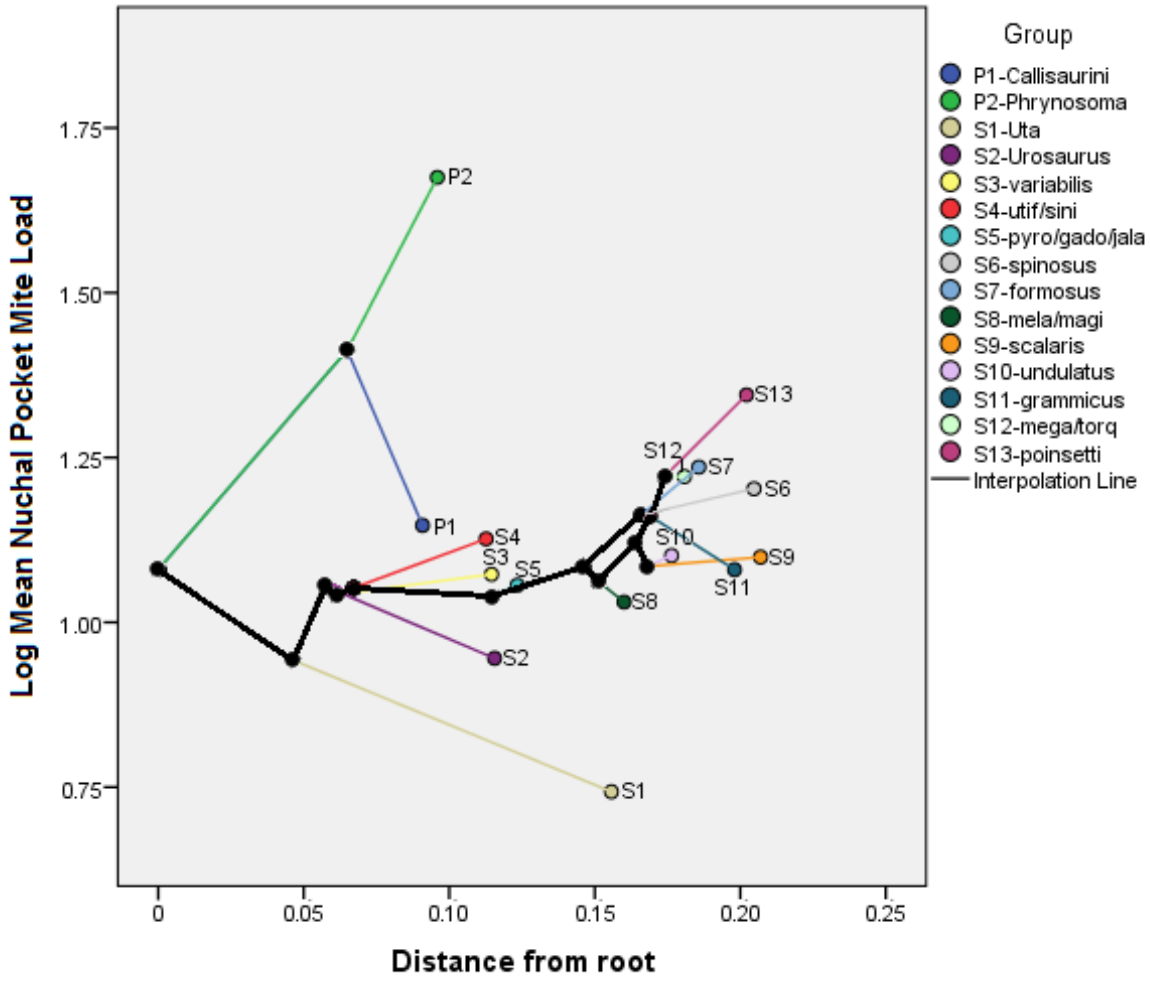

Figure 4.37: Ancestral state reconstruction for internodal and species group values for log nuchal pocket mite load, plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

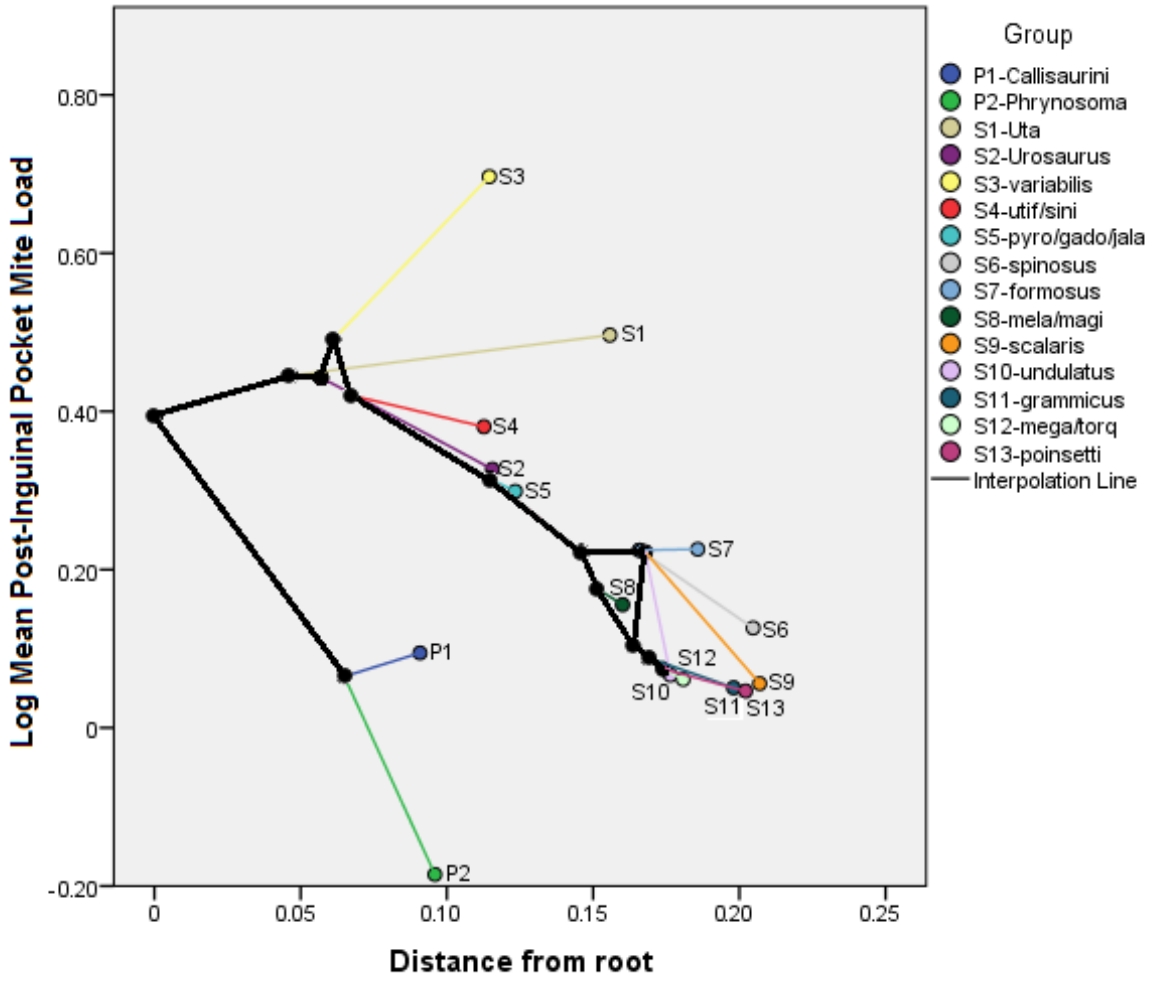

Figure 4.38: Ancestral state reconstruction for internodal and species group values for log post-inguinal pocket mite load, plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

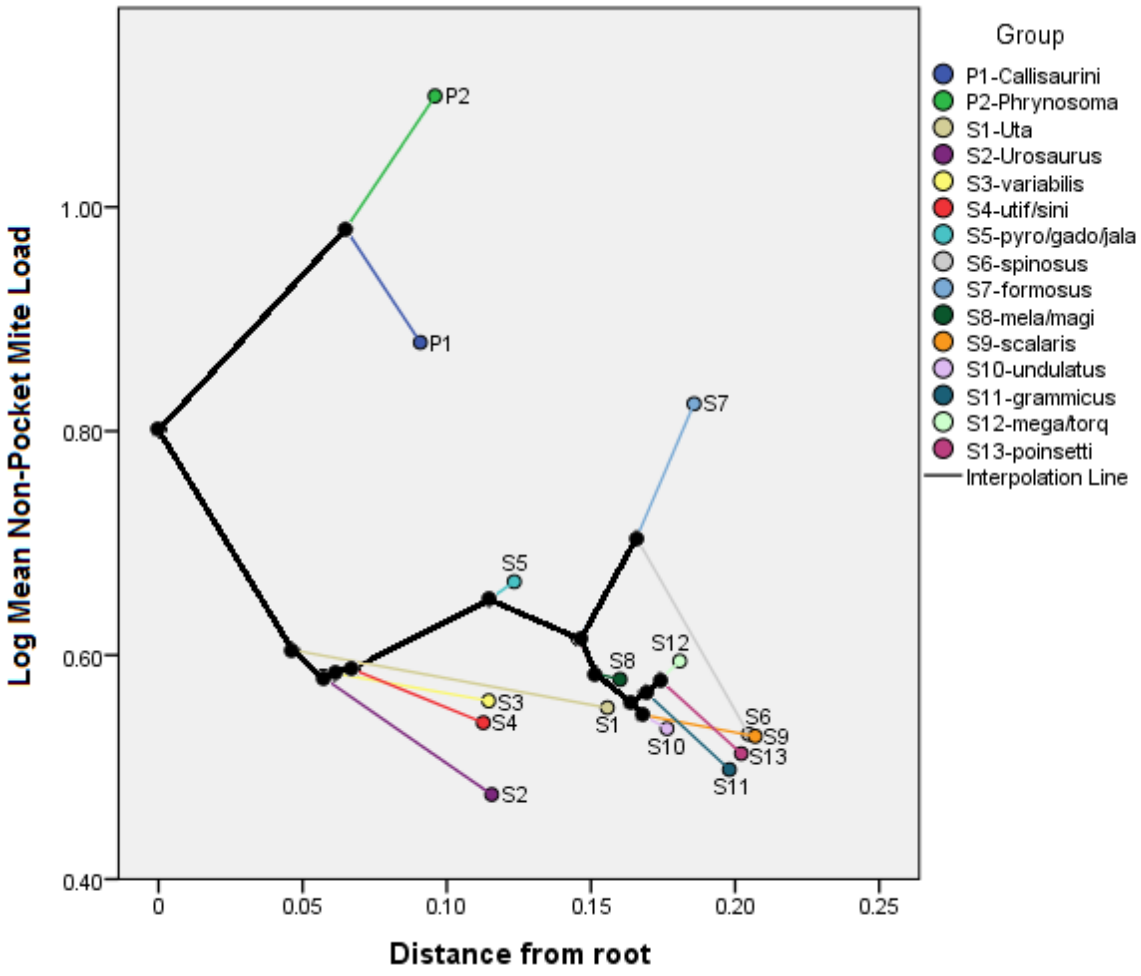

Figure 4.39: Ancestral state reconstruction for internodal and species group values for log non-pocket mite load, plotted over the topology of the Phrynosomatidae. *Sceloporus* species groups and branch lengths *sensu* Wiens et al. 2010 (Table 4.1). Distance refers to distance from root node in units of inferred nucleotide substitutions.

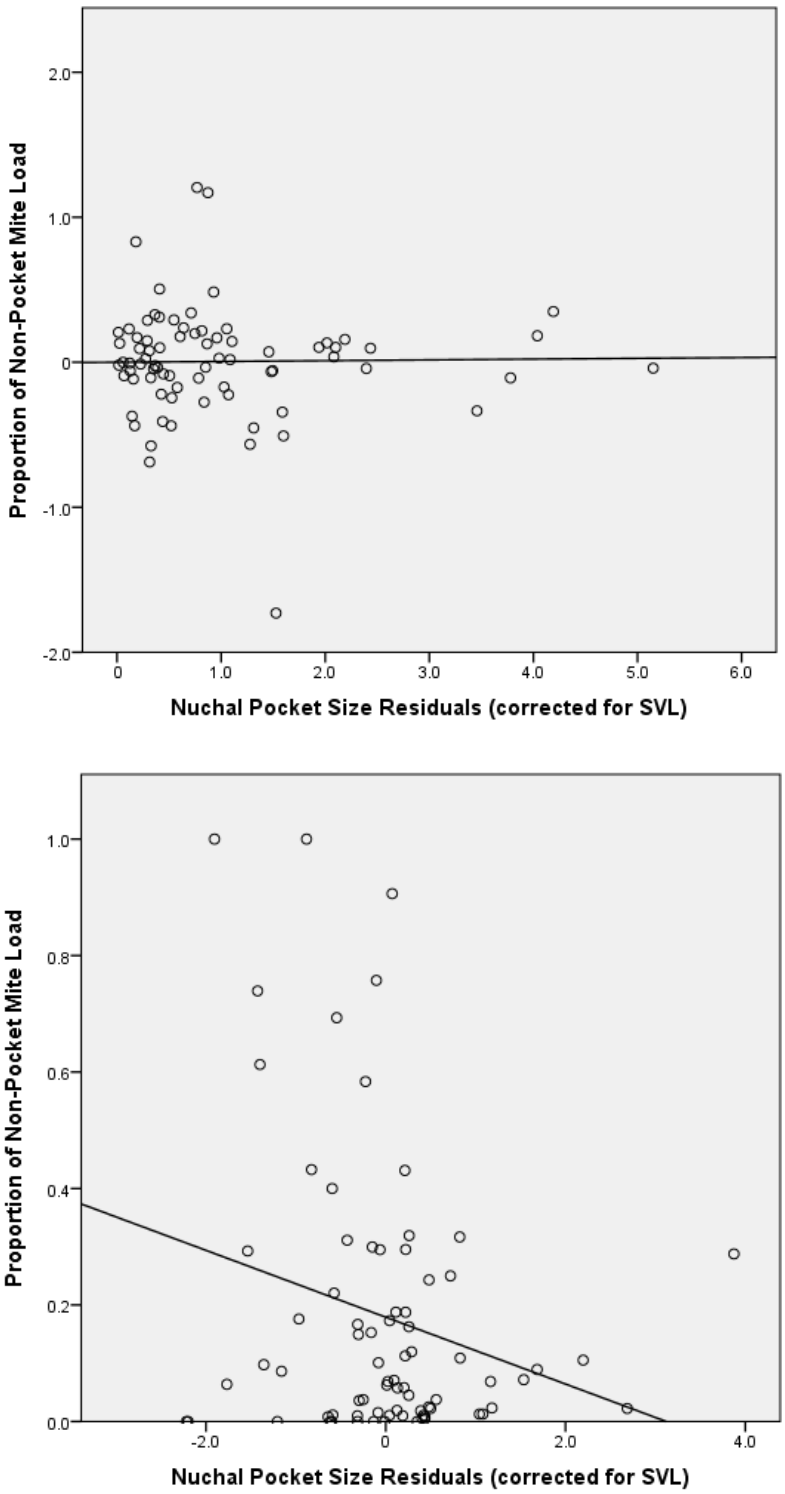

Figure 4.40: Bivariate scatter plots of the relationship between the proportion of non-pocket mite load and mite pocket size, displayed here as residuals of nuchal pocket surface area regressed on body size (SVL). Phylogenetic contrasts on **top** (R=0.023, t=-0.199, p=0.843), conventional **below** (R=-0.235, t=-2.096, p=0.039).