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MORPHOLOGICAL AND BEHAVIORAL TRAITS ASSOCIATED WITH LOCOMOTION IN LIZARDS

by

CHASE KINSEY

(Under the Direction of Lance McBrayer)

ABSTRACT

Morphology, locomotion, and behavior are co-adapted to optimize performance and ultimately fitness. Successfully navigating a complex environment is dictated by an animal's locomotor behavior, and for some behaviors, its locomotor performance. The locomotor performance of an organism is directly related to the form and function of the structures involved in locomotion such that movement is efficient – that is, minimal loss of energy. The first chapter of this thesis focuses on the effects of obstacle placement and forelimb position on facultative bipedalism. Placing an obstacle beyond a lizard's acceleration threshold did not affect the frequency of bipedal posture. Furthermore, the forelimb position of streamlined species is stereotyped during bipedal running, whereas the forelimb positions are varied in short stocky species. The second chapter investigates shape variation in the scapula among Phrynosomatid lizards across a gradient of species that vary in the use of horizontal to vertical locomotor planes. I determined that while global scapula shape is relatively conserved among lizards, localized changes occur at the muscle attachment sites used in vertical vs. horizontal locomotion. Furthermore, scapular shape in relation to habitat use is phylogenetically conserved with the exception of some *Sceloporus* species which diverged independently towards terrestrial locomotion.

INDEX WORDS: Bipedal, Obstacle, Forelimb, Scrub lizard, *Sceloporus woodi*, Racerunner, *Aspidoscelis sexlineata*, Scapula, Morphology, Habitat, Phylogeny

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by

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B.S., Auburn University, 2015

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A Thesis Submitted to the Graduate Faculty of Georgia Southern University

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MASTER OF SCIENCE

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CHAPTER 1

FACULTATIVE BIPEDAL LOCOMOTION IN LIZARDS: THE ROLE OF OBSTACLE PLACEMENT AND THE FORELIMB

ABSTRACT

Many lizards are capable of bipedal locomotion via high acceleration and/or posterior shift in body center of mass (BCoM). Recent work indicates that bipedal posture is advantageous during obstacle negotiation (Parker and McBrayer, 2016). However, little is known about how bipedalism occurs beyond a lizard's acceleratory threshold. Furthermore, no study to date has examined the effects of forelimb position on the BCoM in the context of bipedal locomotion. This study quantified the frequency of bipedalism when sprinting with vs. without an obstacle at 0.8 meters from initiating a sprint. Forelimb positions were also quantified during bipedal running at the start of a sprint and when crossing an obstacle. Two species with contrasting body forms (and thus different BCoM) were studied (*Sceloporus woodi*, *Aspidoscelis sexlineata*) to assess potential variation due to body plan and obstacle crossing behavior. Lizards were coerced to sprint down a 1.4-meter track and filmed with high speed video. A subset of individuals were euthanized to quantify BCoM due to change in forelimb position. No significant difference in frequency of bipedalism was observed in *S. woodi* with or without an obstacle. However, *A. sexlineata* primarily used a bipedal posture when sprinting. Four commonly used forelimb positions were noted during bipedal locomotion: cranial extension, caudal extension, gait cycle, and cranial flexion and adduction. When using bipedal posture at an obstacle, *S. woodi* primarily used cranial flexion and adduction. Caudal extension of the forelimbs was used by *A. sexlineata* when using a bipedal posture. The BCoM of *Aspidoscelis sexlineata* is located more posterior (9.13mm ±0.78) than that of *S. woodi* (12.87mm ± 0.55). Caudal extension of the forelimbs shifted the BCoM posteriorly (8.47mm ±2.50). Caudal extension helped maintain a bipedal posture by shifting the BCoM, and these patterns appear to be stereotyped in *A. sexlineata*, but not *S. woodi*. This is the first study to show how lizards

respond to obstacles placed beyond their acceleration threshold, and the role of the forelimbs during bipedal locomotion.

INTRODUCTION

A terrestrial animal's ability to capture prey, avoid predation, and find mates is contingent on successfully navigating uneven terrain (Vanhooydonck et al., 2007). Physical substrates such as loose rock, thick vegetation, and woody debris provide challenges to terrestrial vertebrates (Pounds, 1988). Variation in substrate characteristics directly affects locomotor performance and behavior of terrestrial vertebrates during flight from predators (Collins et al., 2003; Cooper, 1999; Losos 1990). Bipedalism – which is displayed in some insects, mammals, and reptiles - is one mode of locomotion terrestrial vertebrates use to overcome obstacles (Tucker, 2012; Alexander, 2004). During predation events or social interaction, a terrestrial vertebrate's behavior, speed, and stability traversing obstacles may impinge upon their survivorship and/or fitness (Arnold, 1983; but see Garland and Losos, 1994).

Some terrestrial lizards alter their gait and/or posture while sprinting (Schuett et al., 2009). Stereotyped limb movement in quadrupedal locomotion is called a gait, and has predictable footfalls across various speeds (Snyder, 1952; Snyder, 1954; Snyder, 1962; Irschick and Jane 1999; Farley and Christine, 1997). Bipedalism occurs when only the hind limbs contact the ground, due to a posterior shift in the body center of mass (BCoM) (Snyder, 1954). The posterior shift in BCoM occurs in large part due to the production of high accelerative forces by the hindlimbs that would otherwise keep the forelimbs in contact with the ground (Aerts et al., 2003). Bipedalism is thought to have evolved independently in numerous lizard clades as a consequence of acceleration and changes in body center of mass (BCoM) (Aerts et al., 2003; Clemente, 2014). The placement of the BCoM varies depending on the length of a lizard's tail and trunk relative to the hip (Van Wassenbergh and Aerts, 2013). Lizards with an anteriorly placed BCoM are less likely to exhibit bipedalism compared to lizards with a posteriorly shift BCoM (Clemente, 2014). Thus, body shape is a key determinant in facultative bipedalism. Bipedal lizards can make small changes to their trunk and tail angle such that the BCoM is shifted over the hip (Van Wassenbergh and Aerts, 2013; Irschick and Jayne, 1999).

Kinematic data on the role of the hindlimb in bipedal locomotion suggest the hindlimb generates significant power, thereby effecting acceleration and maximal velocity (Wassenbergh and Aerts, 2013; Olberding et al., 2012; Snyder, 1954; Snyder 1962). Little attention has focused on the role of the forelimb during bipedal locomotion. Forelimb position may aid in obstacle navigation by shifting the BCoM posteriorly (Legrenuer et al., 2012). Snyder (1952) suggested there is no difference in limb movement between quadrupedal and bipedal locomotion. Yet, several species of lizards use varying forelimb positions while moving bipedally (Irschick and Jane 1999). Varying forelimb positions may be necessary for maintaining balance, touching or pushing off an obstacle, or elevating the center of mass for obstacle clearance (Kohlsdorf and Biewener, 2006). Certain forelimb positions during bipedal locomotion could shift the BCoM posteriorly to aid in the pitching motion caused by high starting accelerations (Aerts et al., 2003; McElroy and McBrayer, 2010). For example, caudal extension during obstacle navigation may 1) decrease contact with an obstacle by raising the distance of the limbs away from the obstacle (Self, 2012) and 2) shift the BCoM posteriorly to raise the hip height so that a lizard might clear an obstacle without losing forward speed (Olberding et al, 2012, Irschick and Jayne, 1999).

The objective of this study was to determine the role of obstacle placement and forelimb position during facultative bipedal locomotion in lizards. Two species, *Sceloporus woodi* and *Aspidoscelis sexlineata*, were selected based on their different body plans (and BCeoM), yet each often exihibits bipedal locomotion. *Sceloporus woodi* run bipedally more frequently when encountering an obstacle versus without an obstacle (Parker and McBrayer, 2016). Furthermore, *Sceloporus woodi* run bipedally when an obstacle is within their acceleration threshold (0.4m), but not when multiple obstacles are present in succession (Parker and McBrayer, 2016). *Aspidoscelis sexlineata*, however, employs a bipedal posture when crossing obstacles over long distances (Olberding et al., 2012). Although many species of lizards have been documented sprinting bipedally, no published studies have examined bipedalism with an obstacle placed beyond the initial acceleration threshold, i.e. after the initial two to five steps (0.4 – 0.5 m) of locomotion (McElroy and McBrayer, 2010). Transitioning to a bipedal posture at an obstacle when a lizard is already at maximal velocity suggests that bipedalism occurs as a behavior to maintain forward speed and is not dependent on initial acceleration. I predicted that (i) lizards will run bipedally more with an obstacle present than without and (ii) bipedal posture is used more at the obstacle than at the start of the trial. Furthermore, I predicted that (iii) caudal placement of the forelimbs shifts the BCoM posterior more than other forelimb positions and (iv) that forelimb positions are variable within the acceleration threshold but fixed when navigating an obstacle (beyond the acceleration threshold).

METHODS

Study Species and Field Site

The focus of this study was to address the frequency of bipedal posture during obstacle crossing, and the position of the forelimb during bipedal locomotion. Two facultative bipedal species with differing body plans were chosen as study species: the Florida Scrub Lizard (*Sceloporus woodi*) and the Racerunner (*Aspidoscelis sexlineata*). *Sceloporus woodi* is found in open sandy habitats in peninsular Florida (Jackson, 1973). *Aspidoscelis sexlineata* has an elongated trunk and a forward BCoM compared to *S. woodi* (Clemente, 2014*)*. *Aspidoscelis sexlineata* are found throughout the southeast and are found in sympatry with *S. woodi* in Ocala National Forest. *Aspidoscelis sexlineata* very commonly use bipedal locomotion which is attributed in part to a posteriorly placed body center of mass when sprinting bipedally (Clemente, 2014). The contrasting body plan yet similar mass and habitat use makes each species suitable to quantify both forelimb positions during bipedal running, and when traversing obstacles outside of their acceleration threshold.

Field Collections

Field collection occurred May to August 2016 and 2017. Eighty-eight adult male *S. woodi* and 35 *A. sexlineata* were noosed using a thin filament tied in a slipknot at the end of a fishing pole. Males were retained in cloth bags and transported to the animal facility at Georgia Southern University. Each lizard was kept in a separate 10-gallon tank with sandy substrate and a hide and fasted for 24 hours to ensure digestion did not affect locomotor performance. A 12/12-hour light cycle was used with misting every morning and crickets every three days. Lizards were released at point of capture. Recaptures on subsequent trips were avoided using toe clips and landmarks painted on individuals. Only males greater than 42 mm SVL were used in the analyses because females are more likely to be gravid which affect locomotor performance (Iraeta et al., 2010).

Sprint trials

Seventeen landmarks were placed externally on each lizard using non-toxic white paint (Appendix A) for tracking limb and tail movement in the video. A custom-built track was placed perpendicularly to two Mega Speed X4© high speed video cameras with RICOH lenses (50mm, F/1.4 VGA) mounted on tripods recorded sprint trials (300fps; resolution 1080 x 1024). The racetrack substrate was lined with cork to avoid slippage. A mirror placed at a 45-degree angle along the racetrack wall provided dorsal and lateral views of the lizard (Appendix B). Lizards were subjected to a trial with an obstacle at 0.8 meters, and a trial without an obstacle. Trials were assigned at random to each day. Obstacles were constructed of wooden blocks which spanned the width of the track to prevent lizards from maneuvering around the obstacle. Obstacle height and width was standardized to 35% of hind limb length for each lizard (Self, 2012). Broken or regenerated tails were noted and excluded from any analysis. Lizards were warmed to field active body temperature (~36ºC) in an incubator before each trial. Each lizard was held completely still at the start of the track, then released. Taps on the tail were used to coerce the individual down the racetrack to a hide. A sprint trial was captured for each lizard in each trial type. Only "successful" sprint trials were used for analysis. A successful sprint trial was defined as avoidance of side walls, pausing, or reversing direction. Bipedal trials were defined as completion of at least one full stride without the forelimbs touching the ground. Bipedalism at the obstacle was defined as the use of only the hind limbs for at least one full stride within four strides lengths preceding the obstacle. Bipedalism at the start of the trial was defined as using only the hind limbs for at least one full stride during the first four strides of a sprint. Whether a forelimb touched an obstacle when crossing was noted for each species. Videos were calibrated using a 30-point calibration cube, as well as a 10-centimeter ruler painted on the race track wall (Parker and McBrayer, 2016). Videos were loaded to the computer, spliced using Microsoft Movie Maker (compressed .AVI file), and digitized in MATlab using DLTdv5 software (Hedrick, 2008). A landmark placed at the junction of the frontal and parietal scale was used to calculate sprint velocity (m/sec) from each video.

Ethogram and BCoM analysis

To understand forelimb function during bipedalism, an ethogram was constructed by reviewing a subset of sprint trials of both *S. woodi* (Parker and McBrayer, 2016) and *A. sexlineata* (collected summer, 2016) (Fig 1). Images from Irschick and Jayne (1999) were also used to determine variation in forelimb positions. After sprint trials were completed, 12 *A. sexlineata* and 20 *S. woodi* were euthanized with MS-222 to assess the change in positional BCoM due to forelimb position. Only lizards which ran bipedally in sprint trials were euthanized. The BCoM of a subset of euthanized lizards were measured using two scales (described in Clemente 2014). Two scales (0.0001g accuracy) were set parallel to each other with a wooden beam placed across each scale. The scales were tared to the mass of the beam. Each lizard was placed on the beam and BCoM calculated using methods from Clemente (2014). The BCoM was calculated later on frozen, then slightly thawed lizards with forelimbs placed in both cranial, caudal, and alternating (gait cycle) positions to quantify the effects of the forelimb on BCoM. Cranial and caudal positions were averaged together to obtain the flexed/ adducted position.

Statistical analysis

One-hundred trials of *S. woodi*, and thirty-six trials for *A. sexlineata* were retained for analysis. Chi-squared tests were used to test the frequency of bipedal posture in each species with or without an obstacle. Sprint trials containing bipedal posture were retained for forelimb positional analysis. Chi-squared tests were used to test the frequency of forelimb positions at the start of the trial with and without and obstacle, and at the obstacle. Body center of mass from the hip was calculated using the methods from Clemente (2014). A one-way ANOVA was used to analyze variation in BCoM between caudal and cranial forelimb positions for each species. All analyses were conducted using JMP (v. 12.1.0 SAS institute) and figures created in SigmaPlot (v. 12.0 Systat Software). Alpha was set to $p < 0.05$.

RESULTS

Frequency of bipedal posture with and without an obstacle

The presence or absence of an obstacle on the frequency of bipedal posture was not different in either *S. woodi* or *A. sexlineata* (Table 1; Fig 2). Furthermore, whether species ran bipedally more at the start of a sprint as opposed to the obstacle was examined. The presence or absence of an obstacle does not affect the frequency of bipedal posture in *S. woodi* ($p = 0.64$, $\chi^2 = 0.219$, df = 1, n = 100). Also, frequency of bipedal posture is not different at the start of a trial vs. at the obstacle in *S. woodi* ($p = 0.088$, $\chi^2 = 2.905$, df = 1, n = 40). Regardless of obstacle presence, *S. woodi* primarily ran quadrupedally (Table 1; Fig 2). The frequency of bipedal posture in *A. sexlineata* was not affected by the presence or absence of an obstacle ($p = 0.95$, $\chi^2 = 0.004$, df = 1, n = 35). Furthermore, the frequency of bipedal posture is not different at the start of a trial vs. at the obstacle for *A. sexlineata* ($p = 0.13$, $\chi^2 = 2.288$, df = 1, n = 30). *Aspidoscelis sexlineata* primarily used a bipedal posture regardless of obstacle presence (Table 1; Fig 2).

Effects of Forelimb Position on BCoM

Four forelimb positions were common during bipedal locomotion: limbs adducted and extended posteriorly (caudal extension), limbs abducted and extended anteriorly (cranial extension), limbs adducted and flexed proximally (cranial flexion and adduction), and a gait cycle where limbs rotate around the shoulder axis (Fig 1). In *A. sexlineata*, cranial extension placed the BCoM anteriorly at 9.8 (± 2.25) mm from the hip while caudal extension moved the BCoM posteriorly to 8.47 (± 2.50) mm from the hip (Fig 4) ($p = 0.006$, $t = 2.03$, $n = 36$). In *S. woodi* cranial extension shifted the BCoM anteriorly to 13.506 (±0.56) mm from the hip while caudal extension moved the BCoM posterior 12.25 (± 0.56) mm from the hip (Fig 4) ($p = 0.04$, $t = 2.02$, $n = 46$).

Forelimb positions for S. woodi

The frequency of the four forelimb positions used during bipedal posture at the start of a trial and 0.8 meters from the start without an obstacle was quantified for *S. woodi.* (Figs 3A, 3B). The frequency of forelimb position is not different at the start of a trial and at 0.8 meters without an obstacle ($p = 0.4513$, $\chi^2 = 1.591$, df = 1, n = 23). When running bipedally at the start of a sprint trial, *S. woodi* kept its forelimbs in a gait cycle motion in 47.1% of the trials, while flexion and adduction was observed in 41.2%, and cranial extension was observed in 11.7% of trials ($p = 0.0028$, df = 3, n = 17). During bipedal locomotion at 0.8 meters from the start of the trial, 66.7% of forelimb positions were a gait cycle motion and 33.3% were observed as flexion and adduction ($p = 0.03$, df = 3, n = 6).

With an obstacle present, the frequency of forelimb position is variable at the start of a trial and at 0.8 meters ($p = 0.0074$, $\chi^2 = 9.811$, df = 1, n = 28). When using a bipedal posture at the start of the trial, *A. sexlineata* kept its forelimbs in a gait cycle motion in 56.2% of the trials and flexion and adduction was observed in 43.8% of the trials ($p < 0.0001$, df = 3, n = 16). When running bipedally at 0.8 meters over the obstacle, flexion and adduction was used in75% of the trials, cranial extension was used in 16.7% of the trials, and a gait cycle motion was used in 8.3% of the trials ($p = 0.001$, df = 3, n = 12). *Sceloporus woodi* touched the obstacle with their forelimbs 19 out of 51 trials, and all instances were with a quadrupedal posture (Table 1; Fig 5) ($p = 0.07$, $\chi^2 = 3.35$, df = 1, n = 51).

Forelimb positions for A. sexlineata

The frequency of forelimb position during bipedal locomotion at the start of a trial and at 0.8 meters without an obstacle was quantified for *A. sexlineata* (Figs 3C, 3D). The frequency of forelimb position is similar at the start of a trial and at 0.8 meters for *A. sexlineata* ($p = 0.2450$, $\chi^2 = 1.352$, df = 1, n = 29). During bipedal locomotion at the start of the trial, caudal extension was used in 93.3% of the trials while gait cycle was used in 6.7% of the trials ($p < 0.0001$, df = 3, n = 15). While running bipedally at 0.8 meters, caudal extension was used 100% of the time ($p < 0.0001$, df = 3, n = 12).

The frequency of forelimb position is similar at the start of a trial and at the obstacle for *A. sexlineata* (p = 0.2721, χ^2 = 12.206, df = 1, n = 27). When running bipedally at the start of a sprint trial, caudal extension 100% of the time (p <0.0001, df = 3, n = 14). Only 1 out of 17 *A. sexlineata* touched the obstacle while sprinting bipedally, and this individual immediately transitioned to a quadrupedal posture after contact.

DISCUSSION

The goal of this study was to understand the mechanisms and tradeoffs associated with facultative bipedal locomotion. It is clearly established that bipedalism involves a shift in the BCoM (Van Wassenbergh and Aerts, 2013; Aerts et al., 2003; Clemente, 2014), and that the presence of an obstacle often elicits the facultative use of the posture in lizards (Parker and McBrayer, 2016; Tucker and McBrayer, 2012). Here the obstacle's placement beyond a lizard's acceleration threshold was quantified, but had little effect of the frequency of bipedal posture. Furthermore, the forelimbs had predictable patterns of use that should aid the posterior movement of the BCoM. *Sceloporus woodi* rarely maintains a bipedal posture during a sprint (Parker and McBrayer, 2016). Regardless of obstacle presence, *S. woodi* infrequently used bipedal posture in comparison to *A. sexlineata*. When running bipedally, the forelimbs of *S. woodi* were generally flexed and adducted. This position does not significantly shift the BCoM posterior. Thus, using flexion and adduction provides clearance over an obstacle but does not aid in maintaining a bipedal posture. *Aspidoscelis sexlineata*, which ran bipedally in 88% of all trials, primarily used caudal extension both when crossing the obstacle and at the start of a trial. The posterior shift in BCoM from caudal extension and a long tail relative to the trunk is likely beneficial as *A. sexlineata* frequently maintains a bipedal posture over long distances (Olberding, 2015). Given that the degree of facultative bipedalism is highly variable among taxa (cite), the choice of species' with highly contrasting body forms enable the establishment of the range of strategies, and uses, of this posture. Here, I show the frequency of bipedalism differs regardless of obstacle presence. Furthermore, forelimb position during bipedal locomotion is variable in *S. woodi* and stereotyped in *A. sexlineata*, suggesting that forelimb position plays a role in shifting the BCoM posterior during bipedal locomotion.

Locomotor frequency with and without an obstacle

Sceloporus woodi exhibits facultative bipedalism (Tucker et al., 2012). The use of a bipedal posture increases when an obstacle is placed within the acceleration threshold of 0.4 - 0.5 m (Parker and McBrayer, 2016). However, an obstacle placed beyond this (0.8 meters) from the start of a sprint had little effect on the frequency of bipedal posture (Fig 2). *Sceloporus woodi* has a short tail relative to their trunk which makes sustained bipedalism over long distances difficult. Furthermore, the lack of bipedalism in *S. woodi* during the strides crossing an obstacle suggests that bipedalism is primarily an effect of initial acceleration (Wassenbergh and Aerts, 2013).

In contrast, *Aspidoscelis sexlineata* has a longer tail relative to the trunk and can maintain a bipedal posture over long distances (Olberding, 2015). Regardless of obstacle placement, *A. sexlineata* primarily ran bipedally (Fig 2). Continual bipedal locomotion with and without an obstacle suggests that that bipedalism is a common form of locomotion in this species. Thus, the streamlined body plan of *A. sexlineata* seems well suited for bipedalism (Clemente, 2014, Aerts et al., 2003).

Contingency of Forelimb Position based on Body Plan

Aspidoscelis sexlineata have a long trunk and can reach maximum forward speed around 4 m/s when navigating obstacles (Olberding et al., 2012). The BCoM of *A. sexlineata* is shifted posteriorly by their long tail and vertically elevated trunk during bipedalism (Aerts et al, 2003; Clemente, 2014). In conjunction with tail and trunk elevation *A. sexlineata* uses caudal extension during bipedal locomotion (Figs 3C, 3D). This position aids in posteriorly shifting the body center of mass (BCoM) when maintaining a bipedal posture over long distances. *Aspidoscelis sexlineata* do not modify their hindlimb kinematics when approaching an obstacle but instead adjust the elevation of the hindlimb during obstacle negotiation (Olberding et al, 2012). Likewise, caudal extension was used both at the start of the trial and when crossing an obstacle (Figs 3C, 3D). This suggests that forelimb position may not only be a behavioral adjustment for navigating obstacles, but also a mechanism to adjust BCoM. Shifting the BCoM posteriorly aids in maintaining bipedal postures over long distances (Aerts et al, 2003). The forelimbs act as support in lizards during quadrupedal locomotion (Snyder, 1952). However, *A. sexlineata* touched the obstacle with their forelimbs only three out of 18 trials when sprinting bipedally and immediately reverted to a quadrupedal posture when they did (Fig 5). Extending the forelimb toward the obstacle leads to a forward shift in the BCoM, potentially leading to quadrupedal locomotion. Maintaining a bipedal posture helps the lizards navigate obstacles while maintaining forward velocity (Self, 2012; Olberding et al., 2012).

When sprinting bipedally at the start of a trial, *S. woodi* showed behavioral adjustments in the forelimbs from a quadrupedal posture which does not posteriorly shift the BCoM (Figs 3A, 3D). The continuing gait cycle in the forelimbs at the start of a trial and lack of sustained bipedalism suggests that bipedalism is a result of high acceleration (Van Wassenbergh and Aerts, 2013), and that motor control of the forelimbs is likely the same as during quadrupedal locomotion. Yet, the forelimbs are primarily flexed and adducted when bipedally crossing an obstacle (Figs 3A, 3D). To avoid collision with an obstacle, lizards must raise both hip height and forelimbs to avoid touching the obstacle (Irschick and Jayne, 1991). The hips and forelimbs are raised as a product of bipedalism, which enhances obstacle avoidance (Van Wassenbergh and Aerts, 2013). As bipedalism is less frequent, keeping the forelimbs flexed and adducted allows obstacle clearance without shifting the BCoM. *Sceloporus woodi* have a short tail relative to their trunk and reach velocities around 2.4 m/s when crossing an obstacle (Parker and McBrayer, 2016). *Sceloporus woodi* did not touch the obstacle with their forelimbs in 100% of the bipedal trials (Fig 5). As bipedalism is not a posture for sustained locomotion, *S. woodi* need only hold the forelimbs up against the trunk to avoid contacting the obstacle which could disrupt forward speed (Self, 2012; Kohlsdorf and Biewener, 2006).

Conclusion

Aspidoscelis sexlineata, which has a long tail relative to the trunk, and *S. woodi*, which has a short tail relative to the trunk, were used to understand how bipedal posture and forelimb position varies when faced with a distantly placed obstacle. An obstacle placed beyond their acceleration threshold had no significant effect on the frequency of locomotion. Furthermore, forelimb position was stereotyped in *A. sexlineata*, which primarily uses a bipedal posture, and variable in *S. woodi*, which primarily uses a quadrupedal posture. While bipedalism aids in obstacle negotiation, its occurrence is primarily an effect of a high starting acceleration. However, lizards which primarily use a bipedal posture adjust their forelimbs such that the BCoM is shifted posterior. Thus, lizards with body plans better suited for bipedal locomotion are likely to employ behavioral adjustments to aid in maintaining a bipedal posture, regardless of obstacle presence. Future studies on this topic should quantify the shift of BCoM in videos from the forelimbs, and the variable frequency of bipedalism when navigating obstacles. Furthermore, future work should expand to other bipedal species so that phylogenetic inferences can be made.

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TABLES AND FIGURES

Table 1.1. Summary statistics of locomotor behavior in sprint trials with and without an obstacle. Numbers are the frequency of occurrence for each behavior among species and trials. Bipedalism at the start of a trial was quantified within the first four strides of a sprint. Bipedalism at 0.8 meters was quantified as four strides preceding 0.8 meters. Pauses before and after an obstacle were quantified in the four preceding strides of the obstacle. (*n* = number observed).

Frequency of locomotor behaviors in *Sceloporus woodi* and *Aspidoscelis sexlineata*

	S. woodi Obstacle Presence		*A. sexlineata* Obstacle Presence	
Variable *(Sample Size)*	Obstacle *(n = 51)*	No Obstacle *(n = 49)*	Obstacle *(n = 18)*	No Obstacle *(n = 17)*
Number of bipedal runs	20	17	16	15
Number of quadrupedal runs	31	32	2	2
Bipedal at start of trial	16	17	12	15
Bipedal at 0.8 meters	11	6	14	14
Forelimbs touch obstacle	19	---	3	---
Pause on obstacle	6	---	1	---
Pause before obstacle	10	---	0	---
Pause after obstacle	26	---	2	---

Figure 1.1. Ethogram of common forelimb positions observed during bipedalism in lizards. Lateral and dorsal views are shown.

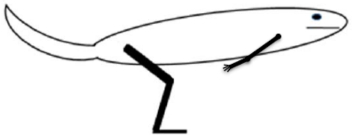
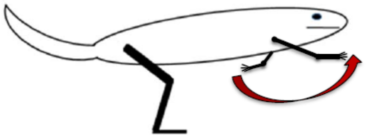
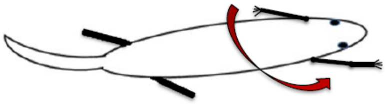
NAME	**LATERAL VIEW**	**DORSAL VIEW**
Cranial Extension Limbs Anteriorly Abducted and Flexed		
Caudal Extension Limbs Adducted and Extended Posteriorly		
Gait Cycle the cranial-caudal protraction and retraction of the forelimbs during a gait cycle		
Cranial Flexion and Adduction Limbs Proximally Adducted and Flexed		

Figure 1.2. Frequency of bipedal posture with vs. without an obstacle. **A)** Bipedal posture was used significantly more than quadrupedal posture with and without an obstacle for *A. sexlineata*. **B)** Quadrupedal posture was used significantly more than bipedal posture without an obstacle for *S. woodi*. Differing letters indicate p-values are ≤ 0.05 from X^2 analysis.

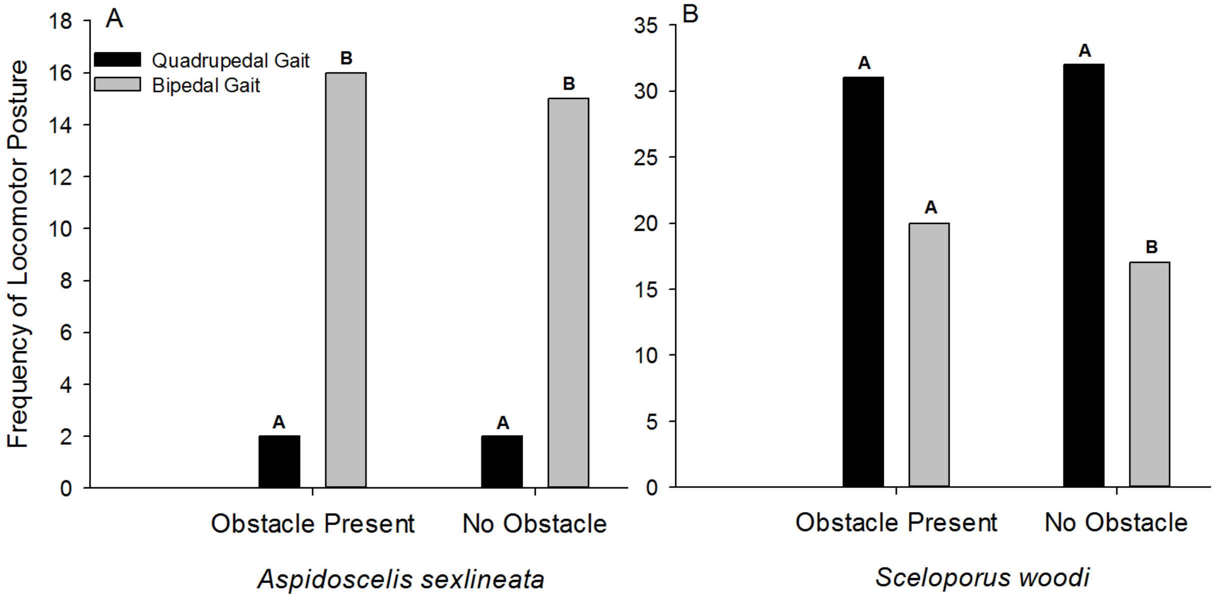


Figure 1.3. The frequency of forelimb positions during bipedal locomotion at the start of a sprint trial and at 0.8 meters with and without an obstacle for *S. woodi* and *A. sexlineata*. **(A)** Without an obstacle, *S. woodi* used flexion adduction and gait cycle significantly more than other forelimb positions at both the start of the sprint and 0.8 meters. **(B)** In trials with an obstacle *S. woodi* used both flexion adduction and gait cycle at the start of the sprint trial, but used flexion addduction when crossing an obstacle. In trials without an obstacle **(C)** and trials with an obstacle **(D)** *A. sexlineata* used caudal extension when running bipedally. Comparisons are made across trials with an without obstacles , not across species.

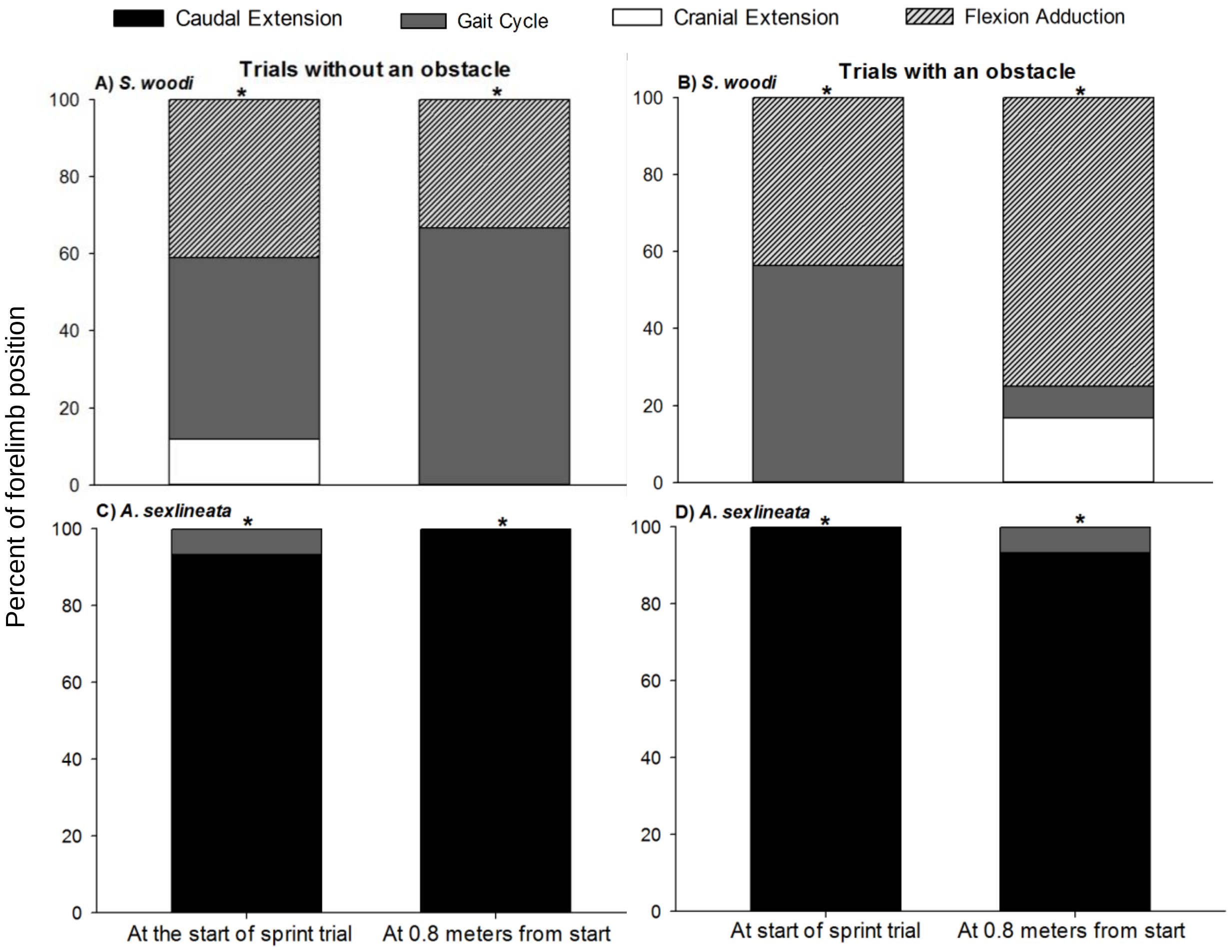


Figure 1.4. The BCoM with forelimbs in caudal extension (8.47 ± 2.50mm) was significantly different from cranial extension (9.8 ± 2.25mm), but not gait cycle in *A. sexlineata*. In *S. woodi* cranial extension shifted the BCoM anteriorly (13.506 ± 0.56mm) while caudal extension moved the BCoM posterior (12.25 ± 0.56mm). Standard error is represented by bars. Differing letters indicate p-values are ≤ 0.05.

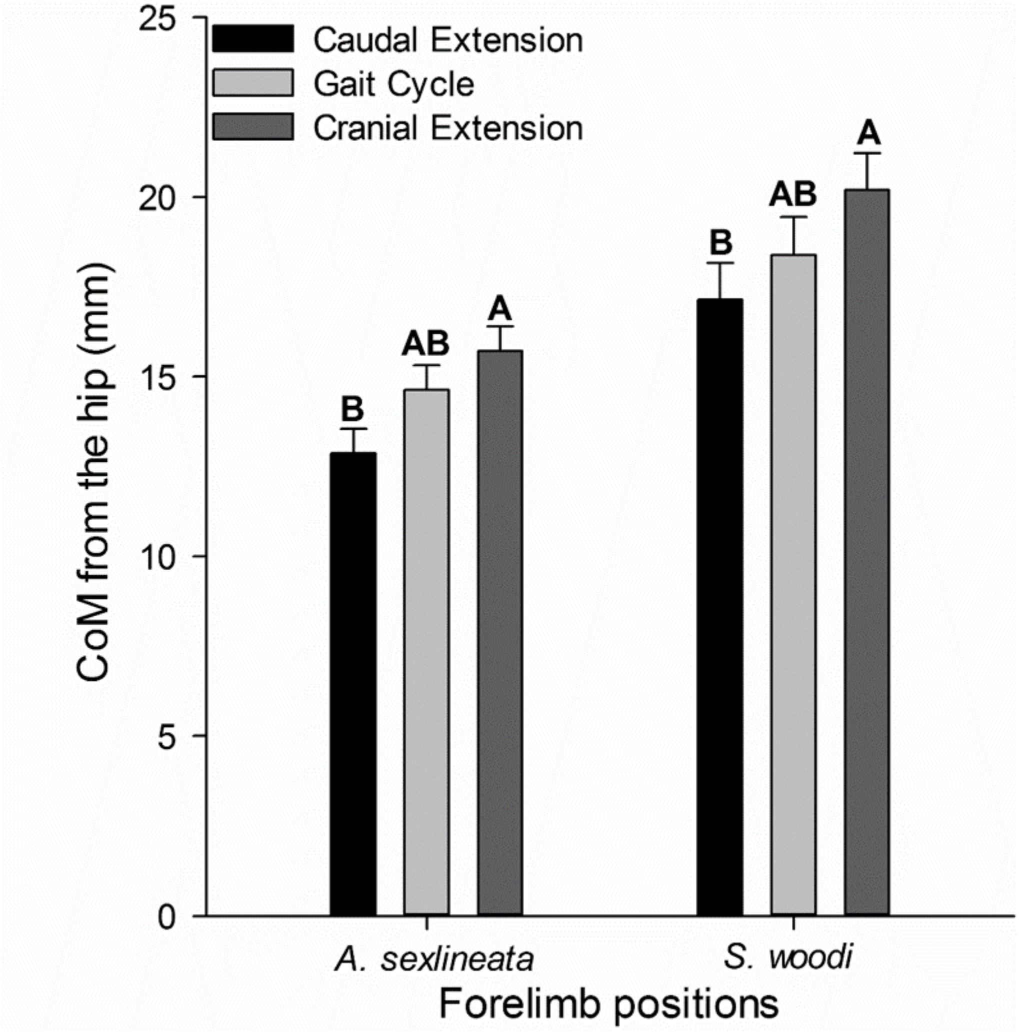
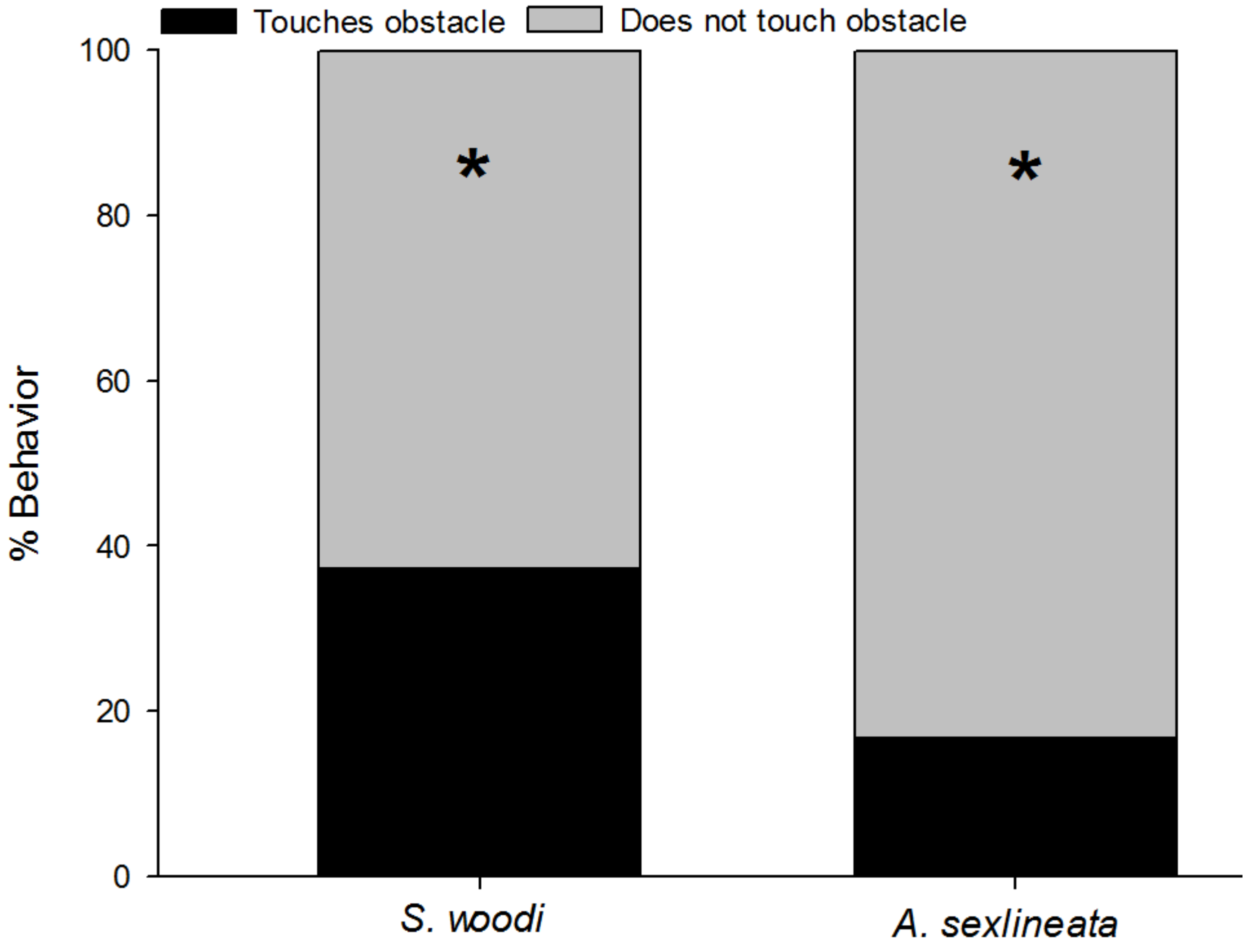


Figure 1.5. Sprint trials for each species where forelimbs touch the obstacle. When crossing an obstacle, *S. woodi* touched the obstacle in 37% of the trials, regardless of locomotor posture. When crossing an obstacle, *A. sexlineata* touched the obstacle in 18% of the trials, regardless of locomotor posture. Overall, *S. woodi* are more likely to touch the obstacle than *A. sexlineata.* Differing letters indicate p-values were ≤ 0.05 via X^2 analysis.



CHAPTER 2

THE MORPHOLOGICAL VARIATION OF THE SHOULDER GIRDLE IN LIZARDS WITH REGARDS TO HABITAT PREFRENCE

ABSTRACT

Often, a predictable relationship exists between an organism's habitat and its locomotor biomechanics. Lizards primarily use vertical or horizontal habitats structures (i.e. arboreal or open terrestrial habitats) where selection is expected to optimize morphological and functional performance on their dominate substrate type. Thus, studying the functional evolution of the appendicular skeleton aids our understanding of the degree of coupling between phenotypic variation and various habitats or locomotor modes. This study quantified the variation of scapular shape across 26 species of lizard that vary across 4 substrate types. A lateral view of the scapula was photographed from skeletal specimens from various museums (AMNH, USNM, CMNH, and UTEP). Pictures were digitized and imported into MorphoJ along with a pruned phylogeny for analysis. Specimens were sorted along an environmental gradient (terrestrial, arboreal, saxicolous, or generalist). A principal component analysis and canonical variate analysis were performed on scapular shape. Then, the resulting scores were mapped to the phylogeny. Variation in the width and height of the suprascapular junction and width of the coracoid explains most of the variation among scapula shape. The scapula shape of terrestrial lizards is significantly distinct. Arboreal and generalist lizards were more similar in scapular shape, with saxicolous as intermediate in the morphospace. An ancestral state reconstruction using Brownian motion suggests that scapula shape associated with terrestrial lizards is ancestral with the *Sceloporus* clade shifting towards more vertical habitat structures. Yet, some species within *Sceloporus* have diverged independently towards terrestrial locomotion. Thus, the appendicular skeleton is both constrained by phylogenetic history, yet molded by selection during lineage diversification along habitat gradients.

INTRODUCTION

Selection optimizes phenotypes for performance such that a predictable relationship exists between an organism’s morphology and its habitat (Herrel et al, 2002). Habitat-matrix models suggest that habitat specialists are adapted to perform optimally within their specific habitat (Pounds, 1988). Changes in locomotor performance and muscular function occur across a variety of taxa which experience variable environmental conditions. Muscle activation, and ultimately power generation, increase when running on an incline (Beiwener and Gillis, 1999). Specialized climbers such as geckoes have strong retractor muscles and flexion moments at the elbow that aid in movement on vertical perches (Zaaf et al, 1999). Ducks and eels also experience shifts in muscle activation such that power generation changes when transitioning between land and water (Beiwener and Gillis, 1999). Animals utilizing similar habitat with similar locomotor styles are expected to experience morphological convergence (Losos, 1990). For example, convergence in axial skeletal morphology occurs in small cursorial mammals with specialized locomotion (Seckel and Janis, 2008). Also, several clades of lizard have variation in limb morphology and muscle mass distribution in relation to habitat preference (Gifford et al., 2008; Herrel et al., 2008; Kaliontzopoulou et al, 2010). Thus, morphological variation can significantly affect the function and performance of an organism within a particular habitat (Melville and Swain, 2000).

Habitats are complex and exist along a gradient of multidimensional space (Fig 1). Depending on the degree of habitat specialization, unique skeletal specializations might evolve such that the body plan is better suited for certain habitats. For instance, unique morphological variation occurs in sticklebacks living in either saltmarsh or freshwater environments (Seebacher et al., 2016). Locomotion on land occurs along a gradient between horizontal and vertical planes. Habitat specialization along this gradient may lead to predictable variation in structures used for locomotion. Morphology of limb elements in carnivorans moving through similar habitats converge despite distant evolutionary histories (Samuels et al., 2012).

Lizards can move along horizontal or vertical planes (i.e. open terrestrial vs arboreal habitats) where selection is expected to optimize morphological and functional performance on the dominate habitat type (Anzai et al, 2014). Species which primarily move in the horizontal plane are terrestrial while species primarily moving in the vertical plane are arboreal (or saxicolous) (Fig 1). Species which live primarily on rocks and boulders (saxicolous) and generalists are considered intermediate and move to some degree across multiple dimensions of the habitat gradient. Some species are specialized to efficiently move in horizontal or vertical planes, or both. For instance, the forelimbs of terrestrial *Sceloporus* lizards are relatively shorter than the hindlimb when compared to saxicolous or arboreal species (Herrel, 2002). Enlarged muscle attachment sites are also expected in the scapulacoracoid as it is the link between the axial skeleton and the forelimbs interacting with the substrate. An expanded suprascapula is noted in an arboreal anole species compared to a trunk-ground species (Herrel et al., 2008). Dorsal expansion of the suprascapula may be related to the attachment sites of the retractor muscles, which aid in vertical climbing (Herrel, 2008). Tree-ground anoles have longer anteroposterior scapula than tree-crown anoles suggesting that the longer scapula may aid in terrestrial locomotion (Tinius and Russell, 2014).

The pectoral girdle, consisting of the scapula, clavicle, and connected limb elements is distinct and sensitive to selective pressures such as environmental constraint and locomotor convergence (Sears et al., 2015). Bony elements connecting the forelimbs to the axial skeleton are collectively called the scapulacoracoid. The scapulacoracoid can be divided into four distinct faces (suprascapula, scapula, coracoid, and epicoracoid) based on muscle attachment sites (Fig. 2) (Tinius and Russell, 2014). These four distinct faces may evolve as a whole structure, or undergo individual shape changes, and are thus structurally complex (Sears et al., 2015). Cursorial mammals using similar locomotor gaits share similar scapular anatomy primarily along the metacromion process on the scapula (Seckel and Janis, 2008). Likewise, the scapula of squirrels evolves as single functional units in some regards but as distinct units in others (Swiderski, 1993). Examining shape data for smaller sections of the pectoral girdle, such as the scapulacoracoid, will aid in quantifying local and global shape changes in relation to habitat and phylogeny (Sears et al., 2015; Morgan, 2009).

The scapulacoracoid has been shown to vary with habitat in many terrestrial vertebrates (Tinius and Russell, 2014; Herrel et al., 2008; Seckel and Janis, 2008; Swiderski, 1993). Yet, little is known about how the scapulacoracoid might vary for taxa in lineages evolving among sand, rock, and forested habitats. Phrynosomatid lizards are an excellent study system to address scapular variation as the clade consists of related species which are specialists among horizontal or vertical planes, or generalists operating across an environmental gradient. Scapulae must allow for free movement of the proximal limb element by forming the connection between the muscles of the humerus and the trunk (Eaton Jr., 1944). Running vertically on trees versus horizontally on a slippery granular medium like sand utilize muscles differently (Herrel et al, 2008; Tinius and Russell, 2014). For example, lizards moving on an incline experience greater limb flexion and greater muscle recruitment (Foster and Higham, 2012). As the protractors and retractors in the forelimbs originate on the scapula, evolutionary transitions in habitat use may lead to scapular shape variation across species. By using geometric morphometrics, small scale morphological changes related to muscle function can be quantified. In turn, these data can provide insight into how species adapt to novel habitats during lineage diversification.

The objective of this study is to determine how scapula shape changes across 26 species of Phrynosomatid lizards that occupy a gradient of habitat types spanning horizontal to vertical habitats. I hypothesize that scapula shape varies across species in differing locomotor planes due to changes in the gravitational forces acting on the scapula and it associated musculature. Thus, morphological variation is likely an adaptive response to (e.g.) shifting from a predominantly terrestrial habit, to an increasingly vertical one. I predict that (i) terrestrial lizards have a narrower and shorter scapulacoracoid as the forelimbs produce little force during terrestrial locomotion, thus muscular function is reduced (Snyder, 1954; Snyder 1962). Furthermore, I predict that (ii) arboreal lizards have wider attachment sites for the scapulodeltoideus near the junction of the suprascapula and scapula and that (iii) generalist and saxicolous species using both horizontal and vertical planes have an intermediate shape between arboreal and terrestrial lizards. Finally, I predict that (iv) variation in the scapula are correlated with habitat preference, but constrained by evolutionary history as lizards invade novel habitat types.

METHODS

Collection from Museums

Phrynosomatidae is an excellent study system to address the coupling of morphology and phylogeny as the family contains over 136 species in nine genera across a large range throughout North America. The genus *Sceloporus* alone contains over 80 species which utilize various habitats across a wide spatial scale (Uetz, 2009; Wiens et al., 2010). Although the geographic range of certain species can be large, most species have preferred habitats such as prairies, deserts, or coastal plains forests (Leaché and Reeder, 2002). Most members of the family can be categorized as being terrestrial, saxicolous, arboreal, or generalist in their habitus (Table 1.) (Herrel et al, 2002). Each of these four classifications include locomotion on a horizontal plane (i.e. terrestrial), vertical plane (i.e. arboreal), and the intermediate plane (saxicolous and generalist).

Scapulae of 26 skeletonized lizard species were photographed from the American Museum of Natural History (AMNH), the National Museum of Natural History (USNM), the Carnegie Museum of Natural History (CM), and the University of Texas at El Paso Biodiversity Collections (UTEP) (Table 1). Lateral views of the scapula complex were photographed using a Microsoft 950 camera with ProShot© software. The camera was directly above each specimen, with the lens at a 90 degree angle to the specimen. Each scapula was placed on a white background with grid lines and a scale bar. Scapulae were placed beneath the camera such that morphological structures were in the same orientation for each scapula. A .TPS file was created using tpsUtil (version 1.74) and built using .JPG images. The .TPS file was loaded into tpsDig2 (version 2.30)(Rohlf, 2010). Thirteen landmarks were digitized on the scapula in consecutive order on each image (Fig 2). Skeletal elements with missing landmarks were noted in software. Out-of-focus images and/or bones improperly orientated were discarded from digitization and analysis.

Analysis

Shape variation from the 13 landmarks was extracted using a Procrustes superimposition and were aligned by principal axes using MorphoJ (version 1.06d) (Klingenburg, 2011). Procrustes superimposition removes the effect of isometric size, position, and orientation (Bookstein, 1999; Dryden and Mardia, 1998). Shape variables were regressed along centroid size and the residuals retained for data analysis. Regressing shape along centroid size removes the effect of allometry as a method of size correction (Klingenberg, 2016; Klingenberg and Marugán-Lobón, 2013). Cumulative frequency of landmark data across specimens was regressed along the squared Procrustes distance and any extraordinary deviations in landmark data was removed from the analysis. The covariance matrix was calculated directly from the Procrustes coordinates.

Principal Component Analysis (PCA) was performed on the covariance matrix to reduce the number of major axes to visualize major patterns of shape variation across species. Changes associated with PC1 and PC2 were phylogenetically corrected and mapped onto the phylogeny in morphospace. Three PC axes were retained for further analysis using the scree plot method as the slope was significantly different than the other PC axes (Jackson 1993). PC3 was only included to avoid under estimating shape variance and is not included in the morphospace (Jackson, 1993). Canonical Variate Analysis (CVA) and Discriminant Function Analysis (DFA) were performed to examine how well species could be classified to their preferred habitat. Leave-one-out cross-validation was used to assess how reliably the test separated the groups as DFA over-estimates the classification rates with small sample sizes (Lachenbruch, 1967). The null hypothesis for the DFA states that groups are similar and thus not correctly classified. All analyses were run on phylogenetically informed data. Distinctiveness between the groups were visualized by plotting CV1 and CV2 scores. In cases where multiple individuals per species were presented, individuals were averaged within a species. Shape variables were mapped onto the pruned phylogeny using squared-change parsimony via the Brownian motion model of evolution (Maddison 1991). The phylogeny from Pyron et al (2016) was pruned using Mesquite (ver. 3.4; Maddison and Maddison, 2018). Permutation tests (10000) of the independent contrasts measured the strength of the phylogenetic signal in the data. Four models of evolution (Brownian Motion (BM), BM with equal constraint, BM with diagonal constraint, and Ornstein-Uhlenbock (OU)) were simulated on 1000 trees using mvMorph statistical package in R (Clavel et al., 2015). Optimized parameters were simulated under BM and compared to BM1 with equal constraint where there is evolutionary covariance, BM2 with diagonal constraint where there is no evolutionary covariance, and OU where evolutionary rates are pulled towards some optima (Clavel et al., 2015; Revell et al., 2008; Hanson, 1997). Ancestral state reconstruction of species' habitat use was performed using a Brownian Motion model with equal constraint, as this model best fit the optimized parameters.

RESULTS

Principal Components Analysis

The first three PC axes account for 59.7% of the total variation in shape. Remaining individual PC axes are each less than 8% and are not discussed further. Shape variation along PC1 (28.7% of the total variation) reflects a dorsal shift in the suprascapular junction (Landmarks 9 and 10). Furthermore, landmarks outlining the scapula and anterior coracoid undergo a mediolateral shift. Principal Component 2 (18.7% of the total variation) describes a ventral shift of the suprascapular junction (landmark 9 but not 10), with a posterior shift in the ventral aspect of the coracoid (Landmarks 2 and 3) (Fig 3). Principal component 3 (12.3% of the total variation) shows an anterior shift in the coracoid (Landmark 1) with a slight dorsal shift of the suprascapular junction (Landmark 10). There is minimal clustering of closely related species in the morphospace, as indicated by a weak phylogenetic signal ($p = 0.0506$, $k = 0.223$, $\lambda = 0.00006$) (Fig 3).

Canonical Variate Analysis

The first two CV axes account for 87.4% of the total variation. Shape variation associated with CV1 (55.8% of the total variation) reflects a dorsal shift in the suprascapular junction (Landmarks 9 and 10) as well as a narrower coracoid (Landmark 1 and 3). Shape variation in CV2 (31.6% of the total variation) reflects a ventral shift in the suprascapular junction (Landmarks 9 and 10) as well as a wider coracoid (Landmarks 1, 3, and 13). Terrestrial species are significantly different from arboreal ($p = 0.0105$), generalist ($p = 0.0311$) and saxicolous ($p = 0.0082$) species (Fig 4). Saxicolous species are not significantly different from either arboreal ($p = 0.3325$) nor generalist ($p = 0.9262$) species. Likewise, generalist species are not significantly separated from the arboreal species ($p = 0.7467$). The canonical variate analysis was repeated using phylogenetically corrected data and mapped on to the phylogeny. Phylogenetically informed analysis reveals consistent groupings of lizard species by habitat preference, yet a strong phylogenetic signal is observed where trait variation occurs within clades ($p = 0.0066$, $k = 0.428$, $\lambda = 0.47$) (Fig 4).

Discriminant Function Analysis

Discriminant function analysis (DFA) tested how well species were correctly classified (Table 2). Terrestrial lizards are correctly separated from arboreal lizards ($n = 10,12$; $p = 0.009$). Terrestrial lizards are correctly separated from generalist lizards ($n = 6,12$; $p = 0.031$). Terrestrial lizards are appropriately classified from saxicolous lizards ($n = 9,12$; $p = 0.012$). Thus, terrestrial lizards were correctly classified as terrestrial and well separated from other habitat groupings. Arboreal, generalist, and saxicolous are all misclassified among each other and were poorly separated into their appropriate habitat groupings.

Ancestral State Reconstruction

Four models of evolution were used to examine ancestral state reconstructions (Table 3). The best fit model for ancestral state reconstruction is Brownian motion model with equal constraints ($AIC = -78.3$). Species which primarily move along a terrestrial or saxicolous substrate represent the ancestral character state (Fig 5). A shift towards the vertical plane occurs in the *Sceloporus* clade. Despite being nested with a general and arboreal clade, *Sceloporus woodi* reverts to a terrestrial habitat, while the small clade consisting of *Sceloporus poinsettii, S. mucronatus,* and *S. jarrovii* are saxicolous and thus intermediate.

DISCUSSION

The goal of this study is to examine scapula shape in Phrynosomatid lizards with regards to their habitat preference. Selection often operates to couple form and function in organisms such that organismal traits are optimized along habitat gradients (Anzai et al, 2014; Depecker et al., 2006). Inherent shape variation occurs between lizard species, yet this variation is enhanced along gradients of dominant habitat use. Furthermore, global shape change is relatively conserved compared to local changes in the coracoid and suprascapular junction. Local changes in the coracoid and suprascapular junction separate the horizontal and vertical planes of locomotion. This study shows that morphological variation in scapula shape is related to both habitat preference and phylogeny and is driven by localized morphological changes. Thus, locomotor function in a particular habitat type plays a role in the morphological evolution of the appendicular skeleton (Arnold 1983; Herrel et al., 2002).

Muscular function associated with scapular variation

Morphological variation is expected to occur in species moving over horizontal or vertical planes as differing inclines can affect muscular function (Herrel et al., 2008; Losos, 1990; Collar et al., 2011). Most of the variation in scapula shape is explained by CV1 and CV2. The dorsal shift of the scapula indicated by CV1 is prevalent in terrestrial species (Fig. 4). Lizard scapular shape undergoes a dorsal and lateral expansion along the junction of the scapula and suprascapula and a narrowing of the coracoid. Changes occurring in the scapula are primarily localized. The M. scapulodeltoideus anterior and M. scapulodeltoideus posterior originate on the suprascapula and are responsible for humeral abduction (Herrel et al., 2008). Abduction of the forelimbs lead to a sprawling gait which is characteristic of terrestrial species when sprinting. The M. corocahumeralis posterior and anterior originate along the ventral surface of the coracoid and aid in humeral adduction and protraction, respectively (Herrel et al., 2008). Adduction and protraction primarily aid in vertical locomotion. As such, narrowing of the coracoid occurs in the specialized scapulae of terrestrial species where pulling movements aren't as crucial to locomotion.

Recruitment of muscle fibers in arboreal lizards differs from that of more terrestrial lizards (Herrel et al., 2008). Increased muscular strain during vertical locomotion potentially changes the morphology of the skeleton (Daley and Biewener, 2003; Herrel et al., 2008). The M. coracohumeralis posterior and the M. suprocoracoideus are associated with the coracoid. These two muscles aid in humeral adduction and shoulder stabilization respectively (Herrel et al., 2008). The scapulacoracoid undergoes an anterior shift in the scapula with both and anterior and dorsal shifts in the position of the coracoid (Fig. 4). Enlarged attachment sites associated with the supracoracoideus confers a strong mechanical advantage in the adduction and retraction of limbs during climbing (Depecker et al., 2006). Thus, muscular functions associated with the coracoid suggest a shape optimized for traversing vertical surfaces, as expected of arboreal species.

Phylogeny versus habitat

Phylogenetic signal of shape data estimates how closely related species resemble each other (Bloomberg and Garland, 2002). Furthermore, phylogeny and ecomorphology are not independent from one another (Stayton, 2005; Blomberg et al., 2003). A weak phylogenetic signal in the PCA and strong phylogenetic signal in the CVA suggest shape variation is correlated with evolutionary relatedness while the degree of separation in terrestrial lizards suggests shape variation is correlated with habitat. Given that it is a large and complex structure, the scapula is evolutionarily conserved, yet also experiences subtle, localized shape changes in particular regions when species invade novel habitat structures.

The ancestral state for the species studied was terrestrial, with saxicolous, arboreal, and generalist being derived conditions among the *Sceloporus* species. With the genus *Sceloporus*, the ancestral state was estimated to be arboreal. Clearly, arboreal lizards experience different skeletal stress from gravitational forces which may alter their morphology (Herrel et al., 2002). Thus, the scapula of arboreal lizards is derived such that locomotion is efficient in the vertical plane. Despite the shift towards vertical habitat structures (i.e. arboreal and generalist) in *Sceloporus* lizards, *S. woodi, S. poinsettii,* and *S. mucronatus* diverge independently toward terrestrial habitats (Fig 5). Selective pressures may occur along the phylogeny such that evolutionarily conserved skeletal elements undergo morphological changes (Openshaw and Keogh, 2014). The invasion of terrestrial habitats within *Sceloporus* species imposes novel selective pressures which lead to novel changes in scapular shape among these closely related species (Melville et al., 2006).

Conclusion

Both habitat and phylogeny play a role in understanding how phenotypic variation occurs across species. Lizards which traverse three dimensional habitats undergo specialized muscular recruitment to efficiently move along an environmental gradient (Herrel et al., 2002). Shape changes in the scapula should occur such that specialized muscular functions are optimized for an organism's dominant habitat and/or substrate type. In *Sceloporus* species, these shape changes occur locally at the suprascapular junction and the coracoid. However, global scapular shape is phylogenetically conserved. Thus, the appendicular skeletal is both constrained by phylogenetic history yet molded by selection during lineage diversification along an environmental gradient. Future work should perform *in vivo* studies in lizards across the habitat gradient to compare morphological changes with locomotor performance.

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Table 2.1. Species included in this study, as well as catalog number, preferred habitat, and the citation used to classify dominant habitat type. Thirteen genera are included (*Cophosaurus, Gambelia, Dipsosaurus, Sauromalus, Callisaurus, Crotaphytus, Holbrookia, Petrosaurus, Phrynosoma, Sceloporus, Urosaurus, Aspidoscelis, Tropidurus),* as well as seven outgroup taxa (denoted by the (*) asterisks).

Family	Specimen	Catalog ID	Habitat	References
Crotaphytidae	*C. texanus**	UTEP290	Saxicoly	Degenhardt *et al*. 1996
Crotaphytidae	*G. wislizenii**	NMNH220226	Terrestrial	Nussbaum *et al*. 1983
Iguanidae	*D. dorsalis**	NMNH12266	Terrestrial	Grismer 2002; Stebbins 2003
Iguanidae	*S. ater**	CM53850	Saxicoly	Stebbins 2003; Grismer 2002
Phrynosomatidae	*C. draconoides*	CM37482	Terrestrial	Grismer 2002; Stebbins 2003
Phrynosomatidae	*C. collaris*	NMNH217271	Saxicoly	Degenhardt *et al*. 1996; McGuire 1996
Phrynosomatidae	*H. maculata*	CM313427	Terrestrial	Collins 1993; Hammerson 1999; Stebbins 2003
Phrynosomatidae	*P. mearnsi*	AMNH154854	Saxicoly	Grismer 2002; Stebbins 2003
Phrynosomatidae	*P. cornutum*	UTEP45 UTEP385	Terrestrial	Degenhardt *et al*. 1996; Bartlett and Bartlett 1999
Phrynosomatidae	*S. angustus*	AMNH154821	Terrestrial	Frost 2007
Phrynosomatidae	*S. clarkii*	NMNH525725	Arboreal	Degenhardt *et al*. 1996; Stebbins 2003
Phrynosomatidae	*S. grammicus*	AMNH96245	Arboreal	Bartlett and Bartlett 1999
Phrynosomatidae	*S. jarrovii*	CM49006	Saxicoly	Mendoza-Quijano, 2007
Phrynosomatidae	*S. magister*	CM43007	Arboreal	Degenhardt *et al*. 1996; Hammerson 1999; Stebbins 2003
Phrynosomatidae	*S. mucronatus*	AMNH92271	Saxicoly	Canseco-Márquez et al. 2007
Phrynosomatidae	*S. olivaceus*	NMNH220251 NMNH220252 NMNH313439 AMNH92885 AMNH155061 AMNH92887	Arboreal	Smith 1946; Kennedy 1973; Bartlett and Bartlett 1999
Phrynosomatidae	*S. poinsetti*	CM38707	Saxicoly	Degenhardt et al. 1996, Stebbins 2003
Phrynosomatidae	*S. spinosus*	NMNH47035 NMNH220254	Generalist	Hernández-Ibarra *et al*. 2001; Vazquez and Quintero 2007
Phrynosomatidae	*S. undulatus*	NMNH313443 NMNH220257	Generalist	Hammerson et al. 2007
Phrynosomatidae	*S. vandenbugianus*	AMNH155013	Generalist	Hollingsworth et al. 2007
Phrynosomatidae	*S. woodi*	NMNH541640	Terrestrial	De Marco 1992
Phrynosomatidae	*U. graciosus*	CM145046	Terrestrial	Grismer 2002; Stebbins 2003
Phrynosomatidae	*U. ornatus*	CM53756	Generalist	Hammerson 1999
Teiidae	*A. tigris**	UTEP479 UTEP604	Terrestrial	Hammerson et al., 2007
Teiidae	*A. sexlineata**	CM70569	Terrestrial	Degenhardt et al. 1996
Tropiduridae	*T. itambere**	NMNH148772	Saxicoly	Frost et al., 2001

Table 2.2. The classification matrix returned from the discriminant function analysis, and the number of specimens allocated to each cell (numerator) out of the total (demoninator). P-values less than 0.05 state the habitat type was correctly classified and well seperated from other habitat types. Terrestrial lizards were appropriately classified and well seperated from other habitat types.

Type	**Species Classification**				
	P-value	**Arboreal**	**Generalist**	**Saxicolous**	**Terrestrial**
Arboreal--Generalist	0.739				
Arboreal		7/10	3/10	--	--
Generalist		3/6	3/6	--	--
Arboreal–Saxicolous	0.347				
Arboreal		6/10	--	4/10	--
Saxicolous		6/9	--	3/9	--
Arboreal–Terrestrial	0.009				
Arboreal		6/10	--	--	4/10
Terrestrial		4/12	--	--	8/12
Generalist–Saxicolous	0.934				
Generalist		--	1/6	5/6	--
Saxicolous		--	6/9	3/9	--
Generalist–Terrestrial	0.031				
Generalist		--	6/6	0/6	--
Terrestrial		--	6/12	6/12	--
Saxicolous–Terrestrial	0.012				
Saxicolous		--	--	5/9	4/9
Terrestrial		--	--	6/12	6/12

Table 2.3. Four models of evolution were compared during ancestral state reconstruction on scapula shape. Brownian Motion 1 (BM1) represents a simulated Brownian motion model with optimized rates of evolution. The model most closely matching BM1 is Brownian Motion 2 (BM2) with equal constraint. Thus, BM2 is chosen for the ancestral reconstruction of habitat preference on the phylogeny. Brownian Motion 3 (BM3) with diagonal constraint and the Ornstein-Uhlenbeck (OU1) model were significantly different from the simulation.

	BM1 (Simulation)	**BM2 (Equal Constraint)**	**BM3 (Diagonal Constraint)**	**OU1**
Log Likelihood	43.6101	43.1555	34.0353	42.8751
AIC	-77.2203	-78.31104	-60.07063	-65.75033
Rank	2	1	4	3

Figure 2.1 Habitats exists as gradients that span multiple dimensions. Locomotion is generally two dimensional yet may occur in multiple planes (i.e. the angle of locomotion may occur in more than one plane with respect to gravitational forces). Some species have specialized morphology for efficient locomotion in a particular plane (e.g. horizontal), while others may be adept in traversing many planes (e.g. saxicolous or generalist species). Such species may spend equal proportions of time traversing all planes (e.g. an intermediate species using boulders). While the potential angles of all habitat structures associated with locomotion is infinite, yet discrete classifications can be assigned based on the literature (terrestrial, saxicolous, arboreal, and generalist) and knowledge of species.

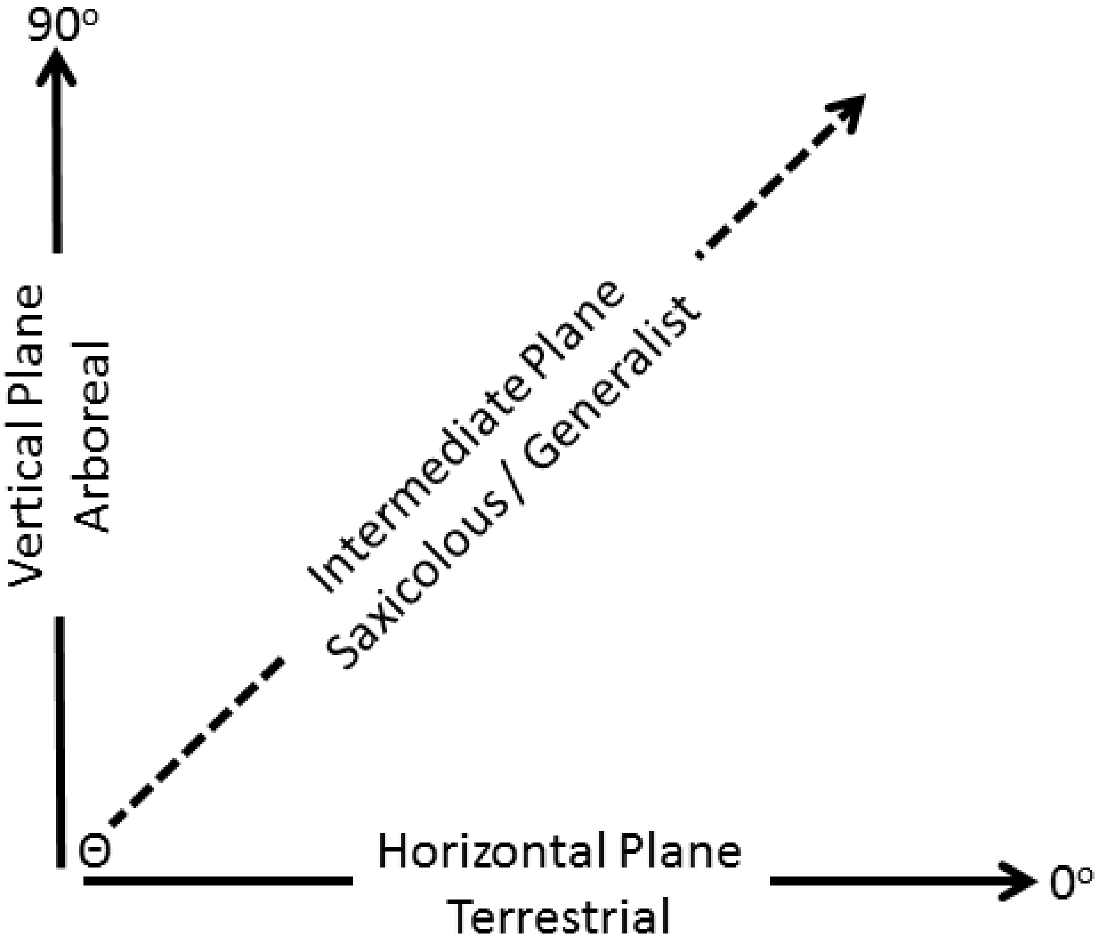


Figure 2.2. The outline of the left scapula with 13 landmarks. Each landmark was chosen based on homologous features and classified as either type I, type II, or type III. Landmarks 9 and 10 outline the suprascapular junction. The ventral aspect of the coracoid is outlined by landmarks one, two, three, and 13. Landmarks four through eight outline the fenestrae. Landmarks 11 through 13 outline the glenoid fossa. Dashed boxes represent the action produced by muscles attached to that area of the scapula.

#	Type	Description
1	II	Posterior-most extremity of the epicoracoid*
2	II	Medial extremity of the epicoracoid at medial contact with coracosternal groove*
3	II	Anterodorsal extremity of the epicoracoid
4	III	Posterior extremity of the primary coracoid fenestra*
5	II	Anteromedial extremity of the epicoracoid*
6	I	Dorsal anterior extremity of the first coracoid ray at contact with epicoracoid*
7	II	Posterior extremity of the scapulocoracoid fenestra*
8	II	Ventroanterior extremity of the scapular ray at contact with epicoracoid*
9	II	Anterior most extremity of the suprascapula*
10	II	Most posterior extremity of the suprascapula*
11	II	Dorsal extremity of the superior glenoid buttress*
12	II	Anterior extremity of the glenoid fossa at contact between scapula and coracoid*
13	II	Posterior extremity of the coracoid at point of contact with epicoracoid*

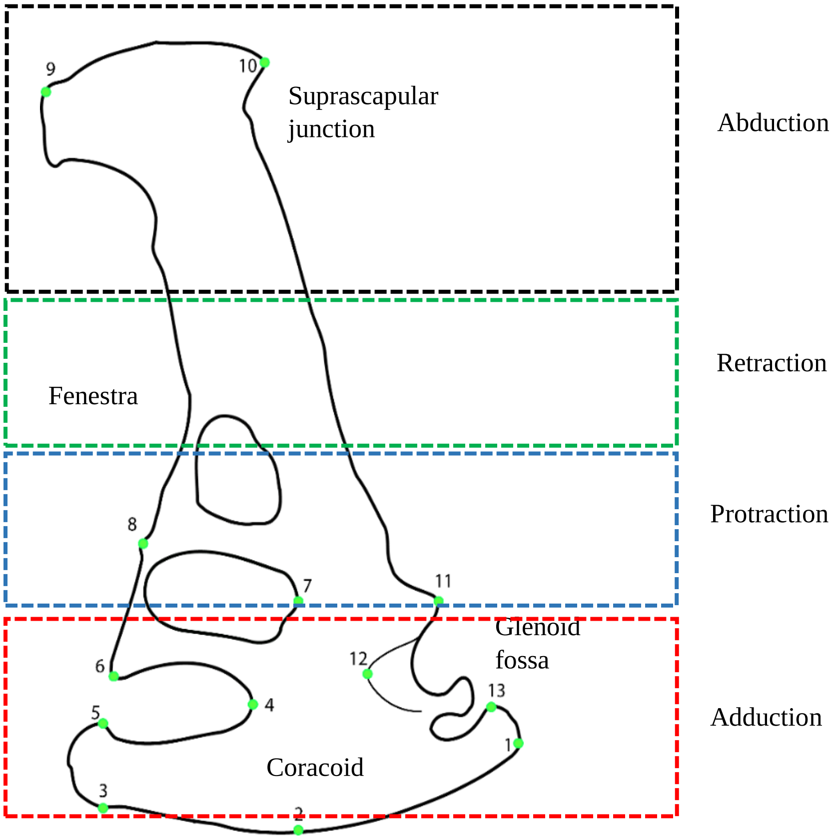


Figure 2.3. Plot of principal component one (PC1) and two (PC2) scores mapped on the phylogeny. Data points are color coded by family, with the exception of the genus *Sceloporus*. There is little separation among families as shown by multiple crossing branches in the morphospace. SSJ = suprascapular junction.

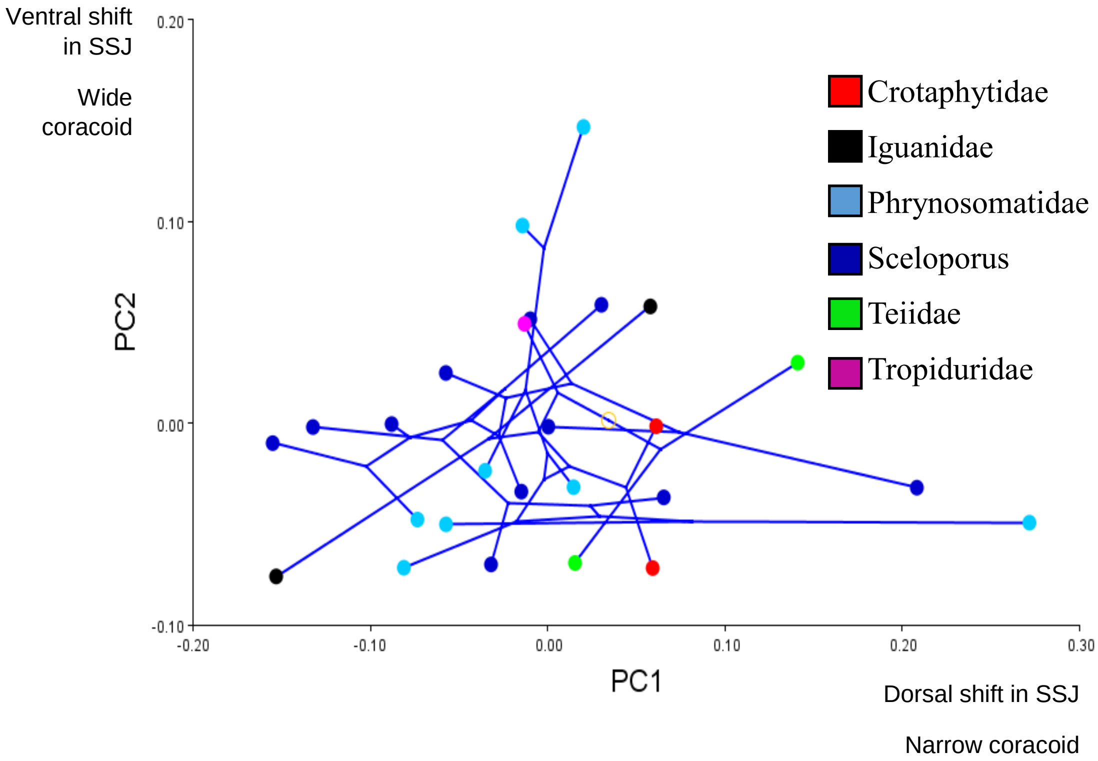


Figure 2.4. Scatterplot of canonical variate scores among terrestrial, arboreal, generalist, and saxicolous lizard species. Canonical variate one (CV1) indicates a dorsal shift in the suprascapular junction (SSJ), and narrowed coracoid width. CV2 indicates a ventral shift in the SSJ with a wider coracoid. **A)** A high degree of separation occurs in terrestrial lizards despite the number of crossing branches. Generalists and arboreal lizards are clumped. Saxicolous lizards show variation mostly along CV2. **B)** The terrestrial ellipse (C.I. = 95%) is significantly different from the others. Other ellipses are not different from one another. Ellipses centroids are marked by a red "X".

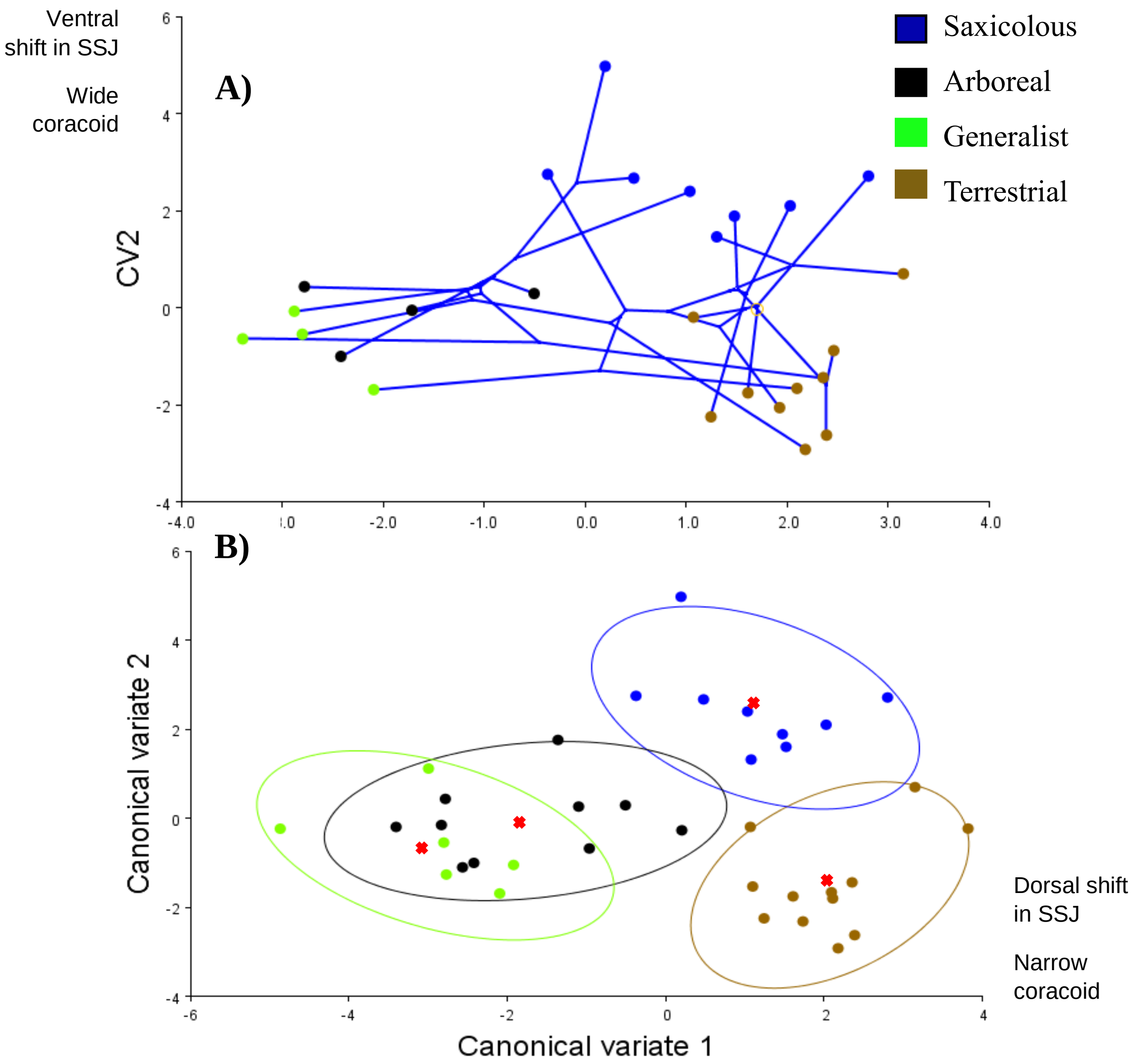
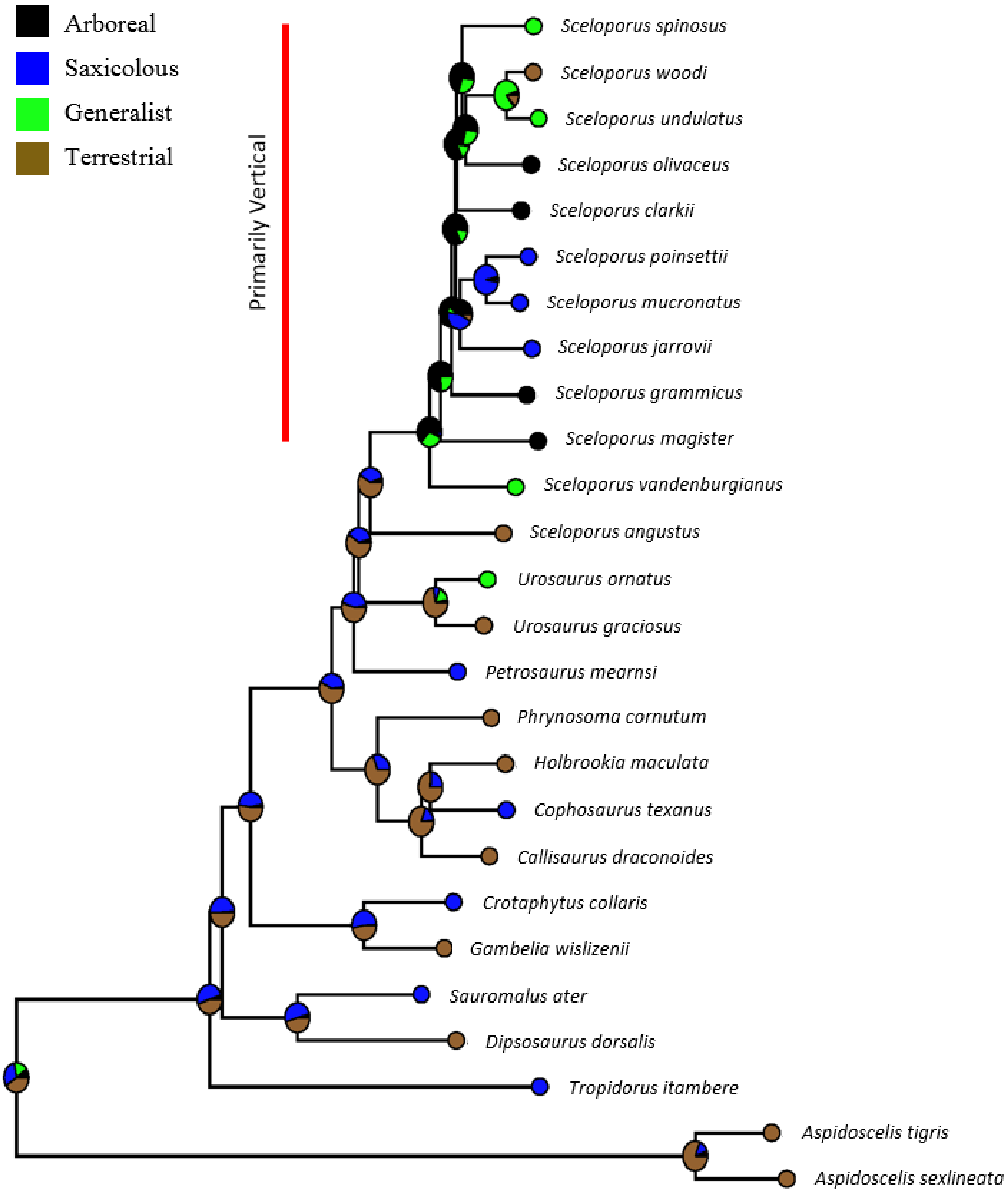


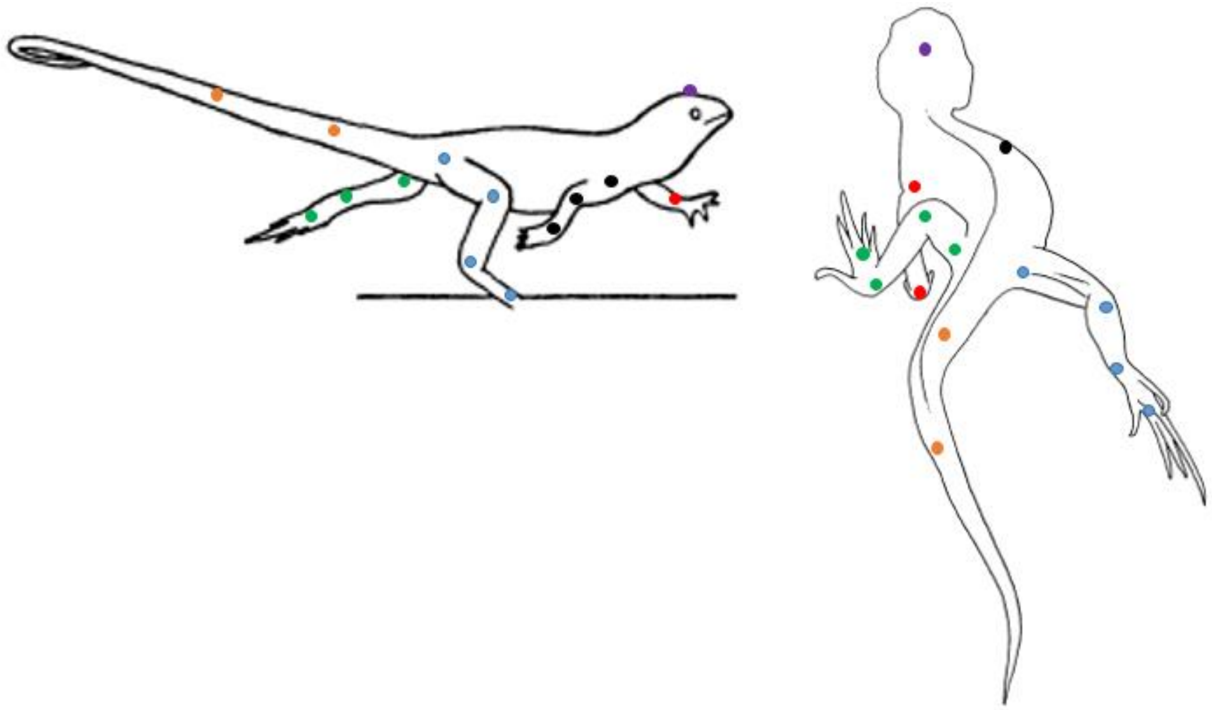
Figure 2.5. Character states mapped to the phylogeny of 26 species of Phrynosomatid lizard. The *Sceloporus* clade primarily uses vertical habitats yet shows numerous evolutionary shifts. Colored pie charts indicate the likelihood of each habitat type present at that node.



APPENDICES

Appendix A:

Kinematic landmarks placed on lizard. Purple: head landmark on scale posterior to the pineal eye; Black and Red: Joint landmarks on forelimb; Green and Blue: joint landmarks on hind limb; Orange: landmark at base of the tail.



Appendix B:

Cross section of racetrack setup. Two high speed cameras were set in parallel vertically above the racetrack on tripods. A mirror as set at the most interior wall at 45 degree angle. The base of made of cork material to allow an easier grip.