

Assessing concurrent patterns of environmental niche and morphological evolution among species of horned lizards (*Phrynosoma*)

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Abstract

The prediction that variation in species morphology is related to environmental features has long been of interest to ecologists and evolutionary biologists. Many studies have demonstrated strong associations between morphological traits and local habitat characteristics, but few have considered the extent to which morphological traits may be associated with environmental features across broad geographic areas. Here, we use morphological, environmental and phylogenetic data compiled from *Phrynosoma* species to examine morphological and climatic variation across the geographic ranges of these species in an evolutionary context. We find significant phylogenetic signal in species' environmental niches, but not in morphological traits. Furthermore, we demonstrate a significant correlation between species' environmental niches and morphological traits when phylogenetic history is accounted for in the analysis. Our results suggest the importance of climatic variables in influencing morphological variation among species, and have implications for understanding how species distributions are constrained by environmental variation.

Introduction

Ecologists and evolutionary biologists have long been interested in how variability in species morphology may be related to variation in climate (Scholander, 1955, 1956; Mayr, 1956; Brown & Lee, 1969). One approach used to identify patterns in these variables has been the application of ecogeographic 'rules' that describe predictable relationships between morphology and climate across a variety of taxonomic groups. For example, Bergmann's rule predicts that body size will increase with increasing latitude (i.e. decreasing temperature) (Bergmann, 1847; James, 1970; Blackburn *et al.*, 1999). Similarly, Allen's rule predicts that relative limb length will decrease with increasing latitude (Allen, 1877). These 'rules' were initially formulated based on data from endotherms, but additional research has found apparent support in ectothermic taxa (Ray, 1960; Lind-

sey, 1966; Cushman *et al.*, 1993; Ashton, 2002; Ashton & Feldman, 2003; Morrison & Hero, 2003; Angilletta *et al.*, 2004; Knouft, 2004; Cruz *et al.*, 2005). Although the consistency and taxonomic applicability of ecogeographic rules is debatable (Geist, 1987; Mousseau, 1997; Bernardo & Reagan-Wallin, 2002; Ashton & Feldman, 2003; Meiri & Dayan, 2003; Adams & Church, 2008), the fundamental principle that some component of species' morphological variation is related to climatic variability is important for understanding species distributions, mechanisms underlying diversification and potential responses to changes in the Earth's climate (Millien *et al.*, 2006).

Another approach used to understand how morphology relates to the environment comes from the discipline of ecomorphology (Van der Klaauw, 1948; Wainwright, 1991). Ecomorphological studies typically focus on relationships between an organism's morphology and its environment at fine spatial scales (Wainwright & Reilly, 1994). For example, strong associations between morphological traits and local environmental characteristics have been documented in bats (Norberg, 1994; Hodgkison *et al.*, 2004), birds (Zeffler *et al.*, 2003), ray-finned

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fishes (Kerfoot & Schaefer, 2006), and scincid (Melville & Swain, 2000) and iguanid lizards (Williams, 1972, 1983; Losos, 1994; Roughgarden, 1995; Losos *et al.*, 1997; Herrel *et al.*, 2001). These associations have been important in understanding factors regulating local community structure as well as mechanisms responsible for evolutionary diversification, particularly adaptive radiations (Schluter, 2000), but the local spatial scale of these studies limits inferences that can be made about broad-scale spatial patterns of variation (e.g. across the geographic range of species).

Considering recent applications of geographic information systems (GIS) data to the study of ecological and evolutionary patterns, the opportunity now exists to integrate the concept of ecomorphology with the fundamental basis for ecogeographic rules to assess whether relationships between species' morphology and climate can be detected across broad spatial scales. This approach involves characterizing species' environmental niches using regional GIS-derived environmental data extracted from individual locality points (Kozak *et al.*, 2008). In the context of GIS, the term 'environmental niche' refers to the integration of climate and species distribution data to characterize species' abiotic requirements at landscape (and larger) scales, following niche theory (Austin & Meyers, 1996; Austin, 2007). If this approach also accounts for the influence of phylogenetic history on relationships between phenotypic and climatic traits, inferences about the lability of these relationships can be made. Calsbeek *et al.* (2006) applied such a phylogenetic approach to demonstrate that scale size, which may be associated with osmoregulation in reptiles, is correlated with general climatic measures of temperature and precipitation both within and among species of Caribbean *Anolis* lizards. This univariate analysis afforded insights into broad-scale relationships between a phenotypic trait and climate, but a multivariate examination of the morphological and climatic features that vary across a species' range holds promise to elucidate general patterns of species distributions that have yet to be realized.

We applied a multivariate approach integrating morphological, climatic and phylogenetic data to examine associations between morphological and environmental traits among species in the genus *Phrynosoma*. This genus includes 17 species (Montanucci, 2004; Leaché & McGuire, 2006) that occur across a wide range of elevations and habitats in western North America and Mexico (Sherbrooke, 2003). All *Phrynosoma* species share a similar body form, but vary in body size, tail length and number, length and arrangement of horns. Some species are broadly distributed (e.g. *Phrynosoma hernandesi*) whereas others are known to occur in only a few localities (e.g. *Phrynosoma ditmarsii*; Fig. S1). The range of environmental parameters that characterize each species' environmental niche has not been formally assessed, but is presumably variable within and among species based on differences in geographic distributions. Because recent

work has largely resolved the evolutionary relationships within the genus (Montanucci, 1987; Zamudio *et al.*, 1997; Reeder & Montanucci, 2001; Hodges & Zamudio, 2004; Leaché & McGuire, 2006; Mulcahy *et al.*, 2006), *Phrynosoma* presents an appropriate system to investigate variation in morphological traits and climatic features characterizing species distributions in a phylogenetic context.

In this study, we examine patterns of environmental niche evolution and morphological evolution among *Phrynosoma* species. First, we use GIS data to characterize the broad-scale environmental attributes of each species' niche. We analyze these environmental data with information on the phylogenetic relationships among *Phrynosoma* species to examine patterns of niche evolution within this genus. We predict to find phylogenetic signal in species' environmental niches, supporting a pattern of niche conservatism (Wiens & Graham, 2005). Second, we use morphological data collected from museum specimens to characterize the morphological attributes of each *Phrynosoma* species. We analyze these data in the context of phylogenetic relationships to assess broad-scale patterns of body size and morphological trait evolution within the genus. We predict to find phylogenetic signal in morphological traits, such that closely related species share more similar body forms than distantly related species. Finally, we integrate data from the previous two analyses to determine the relationship between environmental and morphological traits in a phylogenetic context. We predict that environmental niche and morphological traits will be correlated even after we account for the influence of evolutionary history.

Methods

Associations between phylogeny and environmental niche

We compiled locality data from across the geographic ranges of the 17 species of *Phrynosoma* (Montanucci, 2004; Leaché & McGuire, 2006; Fig. S1) from natural history museum collection records accessed through the Global Biodiversity Information Facility (<http://www.gbif.org>) and HerpNet (<http://www.herpNet.org>) databases, from published data (Montanucci, 2004), and from field-collected data (J. Lemos Espinal, personal communication). We imported locality data for each species into DIVA-GIS (version 5.2; Hijmans *et al.*, 2001) and extracted environmental data at each locality from 20 GIS data layers in the WorldClim Global Climate database (30-s resolution; Hijmans *et al.*, 2005). These layers represent annual trends, seasonality and extreme or limiting environmental factors, and include elevation (m), annual mean temperature (°C), mean diurnal range (°C), isothermality, temperature seasonality, maximum temperature of warmest month (°C), minimum temperature of coldest month (°C), temperature annual range

(°C), mean temperature of wettest quarter (°C), mean temperature of driest quarter (°C), mean temperature of warmest quarter (°C), mean temperature of coldest quarter (°C), annual precipitation (mm), precipitation of wettest month (mm), precipitation of driest month (mm), precipitation seasonality, precipitation of wettest quarter (mm), precipitation of driest quarter (mm), precipitation of warmest quarter (mm) and precipitation of coldest quarter (mm). We converted temperature data to Kelvin to eliminate negative values and \log_{10} -transformed all environmental data. We conducted a principal components analysis (PCA; SYSTAT version 11.0) on the environmental data to characterize the environmental space (i.e. the environmental niche) occupied by each species (Knouft *et al.*, 2006). We calculated the mean scores of all principal components (PCs) for each species and used these mean scores in subsequent analyses.

We tested for phylogenetic conservatism in the environmental niche using likelihood ratio tests of each PC axis. These tests were based on λ , a quantitative measure of the contribution of phylogeny to trait evolution (Pagel, 1999). This parameter indicates whether a given phylogeny correctly predicts covariance among species with respect to a given trait, and ranges from a value of 0 to value of 1. If a trait is evolving independently, λ takes a value of 0, corresponding to a star phylogeny; if a trait is evolving as expected under a random-walk model given a tree topology, λ takes a value of 1. If a trait is evolving such that the influence of phylogeny is less than under a random-walk model, λ takes an intermediate value ($0 < \lambda < 1$; Freckleton *et al.*, 2002).

We first tested the null hypothesis that environmental niches are not phylogenetically conserved among *Phrynosoma* species by estimating the log-likelihood of the data when λ was constrained to zero (indicating that the environmental niche is evolving independently among species, i.e. no phylogenetic signal; Smith *et al.*, 2005). Then we tested the alternate hypothesis that environmental niches are phylogenetically conserved by estimating the log-likelihood of the data when λ was allowed to take its maximum likelihood value. We used the likelihood ratio test statistic ($-2\log_e[H_0/H_1]$) to assess the difference between these models, where H_0 represents the null model ($\lambda = 0$) and H_1 represents the alternative model ($\lambda = \text{maximum likelihood}$). If the estimated maximum likelihood is significantly different than zero, then the data do not support the null model and we can conclude that there is phylogenetic signal in the environmental niche. Analyses were performed with Continuous (version 1.0; Pagel, 1997, 1999), a program that implements generalized least squares models to analyze comparative data across species. We used branch lengths estimated from ND4 mitochondrial DNA data (Fig. 1; Leaché & McGuire, 2006) as a measure of phylogenetic relatedness in this analysis and in subsequent analyses. Although there is some uncertainty relative to the placement of *Phrynosoma mcallii* and *Phrynosoma mode-*

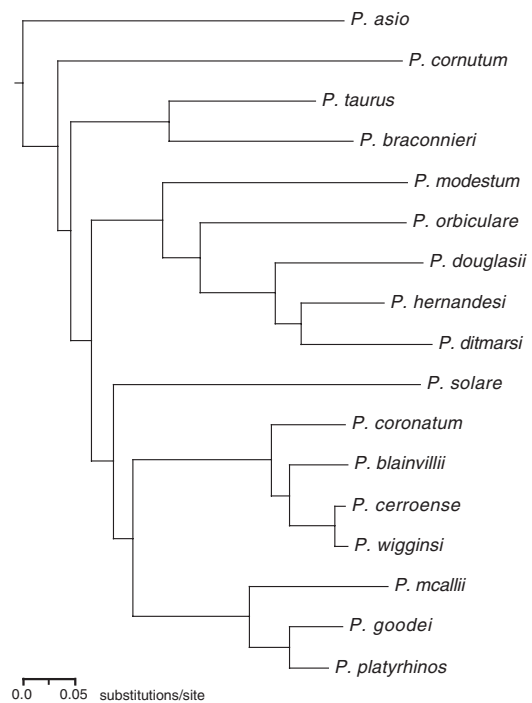


Fig. 1 Phylogenetic hypothesis for *Phrynosoma* species based on ND4 mitochondrial DNA sequence data (Leaché & McGuire, 2006). The phylogeny is rooted with *Callisaurus draconoides*, *Cophosaurus texanus*, *Holbrookia maculata*, *Uma notata* and *Uta stansburiana* (not shown).

stum in the phylogeny inferred from the ND4 locus (Leaché & McGuire, 2006), we used it as our phylogenetic hypothesis because ND4 is the only locus from which sequence data are currently available for all 17 *Phrynosoma* species.

For this analysis and for subsequent likelihood ratio tests, we employed a false discovery rate procedure to account for performing separate likelihood ratio tests on each trait (PC axis). This procedure controls for the expected proportion of incorrectly rejected null hypotheses (i.e. the false discovery rate) and operates by adjusting significance levels (*P*-values) based on their distribution and the number of statistical tests that are performed (Benjamini & Hochberg, 1995; Curran-Everett, 2000).

Associations between phylogeny and morphological traits

We measured morphological characters from specimens of 14 *Phrynosoma* species in natural history museum collections (University of Arizona Museum of Natural History, University of Colorado Museum of Natural History, University of Texas at Arlington Amphibian and Reptile Biodiversity Research Center). Three species from the previous analysis (*Phrynosoma cerroense*, *P. coronatum* and *P. douglasii*) were not included because specimens were not available (Table 1). We measured

Table 1 Sample sizes used in the three analyses conducted in this study.

<i>Phrynosoma</i> species	<i>N</i> [*]	<i>N</i> [†]	<i>N</i> [‡]
<i>P. asio</i>	8	19	3
<i>P. blainvillii</i>	278	11	6
<i>P. braconnieri</i>	5	7	3
<i>P. cerroense</i>	22	0	0
<i>P. cornutum</i>	425	230	128
<i>P. coronatum</i>	16	0	0
<i>P. ditmarsii</i>	6	17	5
<i>P. douglassii</i>	19	0	0
<i>P. goodei</i>	17	7	7
<i>P. hermandesi</i>	291	80	48
<i>P. mcallii</i>	46	51	6
<i>P. modestum</i>	174	78	49
<i>P. orbiculare</i>	30	6	5
<i>P. platyrhinos</i>	457	40	12
<i>P. solare</i>	82	27	14
<i>P. taurus</i>	6	11	0
<i>P. wigginsi</i>	6	3	0

*Associations between phylogeny and environmental niche, †Associations between phylogeny and morphological traits, ‡Associations between environmental niche and morphological traits.

the following morphological traits from each specimen: snout-vent length (SVL), tail length, head length, head height, head width, occipital horn length, femur length, tibia length, length of longest toe on hindfoot (always the fourth toe), humerus length, radius length and length of longest toe on forefoot (always the third toe). All measurements were recorded to the nearest 0.01 mm using digital calipers (Mitutoyo 500–151; Mitutoyo Ltd., Telford, UK). Measurements of paired traits were taken on the left side of the specimen, and analyses were limited to include only adult specimens with all measured traits intact. All measurements were normalized by \log_{10} -transformation before analysis.

Because the aim of this study was to understand general patterns of morphological trait conservatism, we analyzed the combined data from both sexes to increase sample sizes. We ran linear regressions of the morphological trait measurements against SVL to generate body size-independent residual values for each trait (Meyers *et al.*, 2006). Then we conducted a PCA on these residual values to characterize morphological differences among species. Mean scores of all PCs for each species were calculated and used in subsequent analyses.

We tested for phylogenetic signal in body size and in body-size adjusted morphological traits using likelihood ratio tests. These tests were performed as described above, except that morphological trait data were used instead of environmental data. We ran one likelihood ratio test on mean SVLs to infer the effect of phylogeny on the body size distribution of *Phrynosoma* species. We ran separate tests for each PC axis of the morphological data to determine the influence of phylogenetic relatedness on species' morphological traits.

Associations between environmental niche and morphological traits

We used a subset of localities from the morphological trait dataset to examine the relationship between species' environmental niches and morphology. This analysis was limited to include only the measured specimens with reliable latitude and longitude data for their collection localities, and restricted to species with at least three reliable localities. Five species (*P. cerroense*, *P. coronatum*, *P. douglassii*, *P. taurus* and *P. wigginsi*) did not meet these criteria and were not included (Table 1).

First, we performed PCAs on the restricted 12-species datasets. Then we ran likelihood ratio tests (as described above) to test for phylogenetic signal in the environmental and morphological trait data in the restricted dataset. Finally, we ran additional likelihood ratio tests to assess the relationship between climatic features and morphological traits while controlling for the influence of phylogeny. For the latter analyses, we tested the null hypothesis of no correlation by estimating the log-likelihood of the data when the covariances among pairs of traits were constrained to zero. Then we tested the alternate hypothesis that climate and morphology are correlated by estimating the log-likelihood of the data when the covariance matrix was allowed to take its maximum likelihood value. We used the likelihood ratio test statistic ($-2\log_e[H_0/H_1]$) to assess the difference between these models, where H_0 represents the null model (covariances = 0) and H_1 represents the alternative model (covariances = maximum likelihood). Mean scores of the first two PCs for environmental niche and morphological trait data for each species were used in the analysis.

Results

Associations between phylogeny and environmental niche

The climate-based PCA resulted in five axes explaining the variance in environmental attributes among *Phrynosoma* species (Table 2). Likelihood ratio tests indicated significant phylogenetic signal in only the second PC axis (Table 3). This axis displayed high positive factor loadings for annual precipitation, precipitation of wettest month and precipitation of wettest quarter, and high negative loadings for temperature seasonality, maximum temperature of warmest month and temperature annual range (Table 2).

Associations between phylogeny and morphological traits

The morphology-based PCA resulted in three axes explaining the variation in morphological traits among the 14 species considered in this analysis (Table 4). We

Table 2 Eigenvalues, percentage of total variance explained, and principal component loadings from analysis of environmental data extracted from localities of 17 *Phrynosoma* species.

	PCA axes				
	1	2	3	4	5
Eigenvalue	7.505	4.650	3.042	1.474	1.053
% total variance explained	37.520	23.250	15.210	7.370	5.260
Elevation	0.630	0.168	0.110	-0.341	0.325
Annual mean temperature	-0.900	-0.030	0.367	0.154	0.105
Mean diurnal range	0.128	-0.300	0.345	-0.715	0.331
Isothermality	-0.618	0.468	-0.078	-0.598	0.080
Temperature seasonality	0.528	-0.628	0.255	0.264	0.114
Max. temp. of warmest month	-0.570	-0.562	0.440	0.215	0.289
Min. temp. of coldest month	-0.951	0.201	0.009	0.110	0.092
Temperature annual range	0.659	-0.621	0.299	0.029	0.112
Mean temp. of wettest quarter	-0.297	0.117	0.845	-0.018	-0.191
Mean temp. of driest quarter	-0.753	-0.086	-0.275	-0.018	0.510
Mean temp. of warmest quarter	-0.683	-0.366	0.517	0.320	0.137
Mean temp. of coldest quarter	-0.951	0.183	0.151	0.012	0.094
Annual precipitation	0.404	0.840	0.127	0.250	0.084
Precipitation of wettest month	0.157	0.916	0.266	0.133	0.053
Precipitation of driest month	0.731	0.178	0.262	0.064	0.336
Precipitation seasonality	-0.566	0.521	0.311	-0.102	-0.051
Precipitation of wettest quarter	0.193	0.911	0.233	0.125	0.057
Precipitation of driest quarter	0.791	0.134	0.210	0.159	0.277
Precipitation of warmest quarter	0.407	0.325	0.752	-0.091	-0.076
Precipitation of coldest quarter	0.004	0.432	-0.651	0.298	0.410

found no evidence of phylogenetic signal in body size (Table 3). After correcting for multiple tests using the false discovery rate procedure, likelihood ratio tests of body-size adjusted morphological traits did not indicate

Table 3 Results of likelihood ratio tests for three analyses.

Analysis (no. species)	Trait	λ , maximum likelihood	Log-likelihood, $\lambda = 0$	Log-likelihood, λ estimated	Likelihood ratio test statistic (<i>P</i> -value)
Phylogeny and Environmental Niche (17)	PC1	0.584	-42.420	-41.053	1.367 (0.098)
	PC2	0.982	-43.224	-37.968	5.257 (0.001)
	PC3	0	-28.774	-28.774	0 (1)
	PC4	0	-21.589	-21.589	0 (1)
	PC5	0.153	-22.185	-22.112	0.074 (0.700)
Phylogeny and Morphological Traits (14)	SVL	0.976	16.959	18.170	1.211 (0.120)
	PC1	0.789	-27.250	-25.703	1.548 (0.079)
	PC2	0.934	-22.631	-20.066	2.565 (0.024)
Environmental Niche and Morphological Traits (12)	PC3	0	-18.177	-18.177	0 (1)
	PC1*	0.475	-30.010	-29.638	0.461 (0.337)
	PC2*	1	-34.614	-32.955	1.659 (0.069)
	PC3*	1	-25.170	-25.082	0.088 (0.675)
	PC4*	0	-20.974	-20.974	0 (1)
	SVL	1	15.942	17.267	1.325 (0.104)
	PC1†	0.839	-15.608	-14.835	0.773 (0.214)
PC2†	0.919	-24.403	-23.024	1.379 (0.097)	
PC3†	0	-16.505	-16.505	0 (1)	

Values in bold indicate trait exhibiting significant phylogenetic signal. *Environmental niche, †Morphology.

significant phylogenetic signal in any of three PC axes (Table 3).

Associations between environmental niche and morphological traits

The PCA of the environmental data resulted in four axes explaining variance in environmental attributes among the 12 species of *Phrynosoma* considered in this analysis (Table 5). The PCA of the morphological trait data resulted in three axes explaining the variance in morphological traits among species (Table 6). Likelihood ratio tests indicated a lack of phylogenetic signal in all traits, including the second PC axis of environmental niche data (PC2 niche: $-2\log_e[H_0/H_1] = 1.659$, $P = 0.069$), which was statistically significant in the first analysis. We attribute the lack of significant phylogenetic signal in this axis to the reduced sample size in this analysis, in terms of both number of specimens and number of species analysed (Table 1; Blomberg *et al.*, 2003).

Results of the likelihood ratio tests that incorporated covariance with phylogenetic history indicate that morphological traits and environmental attributes are significantly correlated among species of *Phrynosoma*. Specifically, we found significant associations between the first two (PC1 morphology vs. PC1 environment) and second two (PC2 morphology vs. PC2 environment) PC scores (PC1 morphology vs. PC1 environment: $-2\log_e[H_0/H_1] = 4.380$, $P = 0.003$; PC1 morphology vs. PC2 environment: $-2\log_e[H_0/H_1] = 0.130$, $P = 0.611$; PC2 morphology vs. PC1 environment: $-2\log_e[H_0/H_1] = 0.081$, $P = 0.688$; PC2 morphology vs. PC2 environment:

Table 4 Eigenvalues, percentage of total variance explained and principal component loadings from analysis of morphological character residuals of 14 *Phrynosoma* species.

	PCA axes		
	1	2	3
Eigenvalue	2.680	2.456	1.482
% total variance explained	24.370	22.320	13.470
Residual tail length	-0.213	0.458	0.114
Residual head length	0.677	-0.459	-0.323
Residual head height	0.875	-0.067	-0.035
Residual head width	0.642	-0.285	0.304
Residual occipital horn length	-0.424	0.743	0.220
Residual femur length	0.572	0.511	0.035
Residual tibia length	0.401	0.551	0.048
Residual longest toe length (hindfoot)	0.059	0.517	-0.659
Residual humerus length	0.290	0.363	0.293
Residual radius length	0.503	0.638	0.071
Residual longest toe length (forefoot)	-0.057	0.161	-0.833

Table 5 Eigenvalues, percentage of total variance explained and principal component loadings from analysis of environmental data extracted from localities of 12 *Phrynosoma* species.

	PCA axes			
	1	2	3	4
Eigenvalue	8.474	5.239	2.584	1.393
% total variance explained	42.370	26.200	12.920	6.970
Elevation	0.811	0.134	0.399	-0.039
Annual mean temperature	-0.940	0.229	0.111	0.038
Mean diurnal range	0.115	-0.047	0.897	0.077
Isothermality	-0.379	0.797	0.179	0.035
Temperature seasonality	0.289	-0.791	0.415	0.098
Max. temp. of warmest month	-0.822	-0.231	0.425	0.086
Min. temp. of coldest month	-0.886	0.393	-0.146	0.066
Temperature annual range	0.449	-0.689	0.543	-0.003
Mean temp. of wettest quarter	-0.640	0.118	0.538	-0.170
Mean temp. of driest quarter	-0.791	0.208	0.307	0.402
Mean temp. of warmest quarter	-0.901	-0.116	0.282	0.060
Mean temp. of coldest quarter	-0.890	0.423	-0.008	0.041
Annual precipitation	0.590	0.749	-0.113	0.104
Precipitation of wettest month	0.398	0.885	0.063	-0.007
Precipitation of driest month	0.762	0.334	0.246	0.195
Precipitation seasonality	-0.332	0.646	0.327	-0.343
Precipitation of wettest quarter	0.455	0.864	0.055	-0.031
Precipitation of driest quarter	0.851	0.118	0.159	0.256
Precipitation of warmest quarter	0.504	0.544	0.478	-0.261
Precipitation of coldest quarter	0.085	0.205	-0.019	0.930

$-2\log_e[H_0/H_1] = 2.968$, $P = 0.015$) (Fig. 2a, b). The first PC for environmental data displayed high positive loadings for elevation, annual precipitation, precipitation of driest month and precipitation of driest quarter, and high negative loadings for mean annual temperature, mean temperature of warmest and coldest quarters, mean

Table 6 Eigenvalues, percentage of total variance explained, and principal component loadings from analysis of morphological character residuals of 12 *Phrynosoma* species.

	PCA axes		
	1	2	3
Eigenvalue	2.735	2.520	1.529
% total variance explained	24.870	22.900	13.900
Residual tail length	0.454	-0.429	0.058
Residual head length	-0.239	0.844	-0.258
Residual head height	0.298	0.827	0.001
Residual head width	0.022	0.641	0.386
Residual occipital horn length	0.636	-0.597	0.112
Residual femur length	0.714	0.328	0.043
Residual tibia length	0.627	0.167	0.048
Residual longest toe length (hindfoot)	0.463	0.009	-0.706
Residual humerus length	0.430	0.122	0.267
Residual radius length	0.818	0.147	0.068
Residual longest toe length (forefoot)	0.085	-0.004	-0.848

temperature of wettest and driest quarters, minimum temperature of coldest month and maximum temperature of warmest month. The first PC of morphological trait data exhibited high positive factor loadings for occipital horn length, femur length, tibia length and radius length. The second PC of environmental data displayed high positive factor loadings for isothermality, annual precipitation, precipitation of wettest month and precipitation of wettest quarter, and high negative loadings for temperature seasonality and temperature annual range. The second PC of morphological trait data displayed high positive factor loadings for variables describing head shape.

Discussion

In this study, we examined patterns of environmental niche and morphological trait variation among *Phrynosoma* species in a phylogenetic context. Results from our first analysis testing for associations between phylogeny and species' environmental niches indicated significant phylogenetic signal in the environmental variables associated with the second PC axis. This axis displayed positive factor loadings for precipitation variables and negative loadings for temperature variables. These results indicate that closely related species (e.g. *P. coronatum*–*P. wigginsi*, *P. douglasii*–*P. hernandesi*) occupy more similar environmental niches than distantly related species (e.g. *Phrynosoma asio*–*P. ditmarsii*, *P. cornutum*–*P. hernandesi*), and support a general pattern of niche conservatism among *Phrynosoma* species. Niche conservatism has been debated in the literature with some studies demonstrating evidence of the phenomenon (Peterson *et al.*, 1999; Peterson & Holt, 2003) and other studies reporting equivocal results (Rice *et al.*, 2003; Knouft *et al.*, 2006).

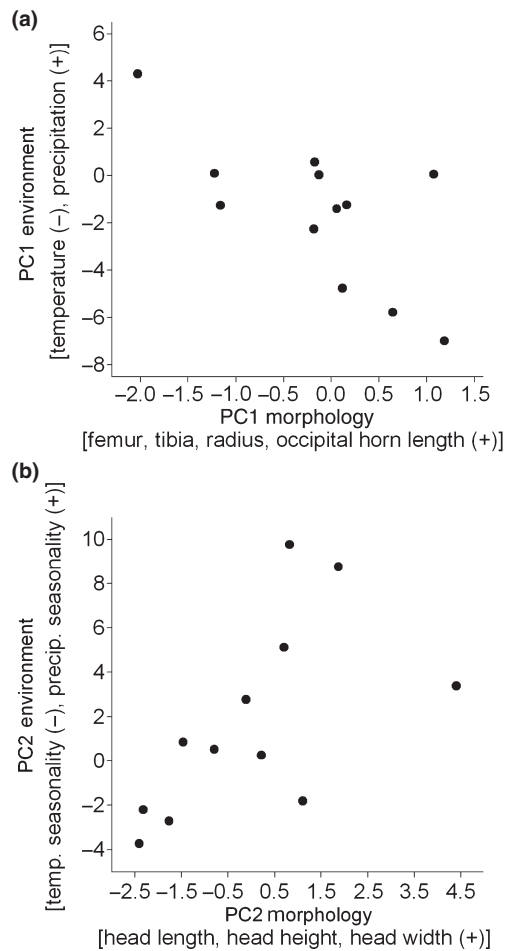


Fig. 2 (a) Relationship between mean PC1 morphology scores and mean PC1 environmental scores; and (b) mean PC2 morphology scores and mean PC2 environmental scores among species of *Phrynosoma*.

Understanding the extent to which niches are conserved can reveal how climatic tolerances limit species' geographic ranges (Wiens & Graham, 2005) and aid in predicting future responses of species to climate change (Parmesan & Yohe, 2003). Our results suggest that because climatic conditions associated with *Phrynosoma* species occurrences are constrained by evolutionary history, climate change may result in geographic range shifts among species in the genus if they respond by tracking climatic conditions to which they are adapted (Coope, 1994; Parmesan, 2006). Interestingly, even though closely related species occupy similar environmental niches, most currently do not overlap extensively in geographic ranges (Sherbrooke, 2003; Leaché & McGuire, 2006). These observations suggest the importance of ecological factors in addition to abiotic conditions associated with available habitats in determining current geographic range limits (Adams, 2004; Kozak &

Wiens, 2006) and potential future climate change-driven range shifts (Peterson *et al.*, 2002).

Results of our second analysis suggest no discernable pattern in the evolution of body size or form among *Phrynosoma* species. We found a lack of phylogenetic signal in the three PC axes of body-size adjusted morphological traits, and no evidence of phylogenetic signal in body size. This lack of phylogenetic signal in body size may seem surprising because this trait has been shown to be conserved among taxa across longer temporal scales (e.g. among stegocephalians; Laurin, 2004) than the scale considered in this study (see below). It is possible that the lack of phylogenetic signal may be due to decreased statistical power as this analysis was limited to 14 species. Blomberg *et al.* (2003) demonstrated that statistical power for detecting phylogenetic signal is high when 17 or more species are included in an analysis, but decreases with 16 or fewer species. Alternatively, the lack of phylogenetic signal in body size of *Phrynosoma* may not be surprising considering that this trait can be relatively variable among lizards. For example, body size appears to evolve readily in response to selective forces in West Indian *Anolis* lizards (Schoener, 1969) and is highly variable depending on geography both within and among *Sceloporus* lizard species (Sears & Angilletta, 2004).

Results from our final analysis of associations between the environmental niche and morphology indicate that morphological traits (excluding body size) are correlated with climatic features among *Phrynosoma* species. We found significant correlations between the first two (PC1 morphology vs. PC1 environment) and second two (PC2 morphology vs. PC2 environment) PCs. Because the influence of phylogeny was controlled for in this analysis, these correlations are not attributed to the evolutionary relatedness of species. They suggest that climate, in conjunction with evolutionary history, is an important constraint on morphological character traits in *Phrynosoma* species, and that climatic conditions were potentially an important factor in formation of morphological variation (Gvozdk *et al.*, 2008).

A date for the origin of the *Phrynosoma* clade has not been formally assessed, but we can offer a rough estimate of the age of this radiation using the molecular clock estimates of 1.3–2.0% pairwise sequence divergence per million years suggested for iguanid mtDNA (Macey *et al.*, 1998, 1999). Average pairwise ND4 sequence divergence suggests the earliest split in *Phrynosoma* (between *P. asio* and the common ancestor of the other 16 species) to be between 9.4 and 11.5 million years ago (12.25–15.01% uncorrected *P*-values) under the 1.3% rate, or between 6.1 and 7.5 million years ago under 2% rate. These estimates should be interpreted with caution because the molecular clock estimates of sequence divergence are not based on ND4 and do not account for potential differences in the rate of molecular evolution among lineages (Hillis *et al.*, 1996). Nevertheless, they suggest that *Phrynosoma* originated during the middle to late Miocene.

North America and Mexico have endured numerous geologic, oceanographic and climatic changes since this time (Chapin, 2008) that influenced the evolution of *Phrynosoma* species and offered many opportunities for morphological diversification under different environmental conditions.

The methods used in this study do not allow us to discern whether morphological character traits vary directly in response to climate or indirectly in response to vegetation, substrate composition, other habitat features associated with climate, or with biotic interactions associated with climate, such as prey availability. Hence, these results do not indicate a causal relationship between bioclimatic features and morphology. However, we can identify some general patterns between morphology and climate based on the correlations between the first and second PCs. For example, the correlation between the first PCs for morphology and climate suggests that *Phrynosoma* species with long limbs occur in dry lowland habitats with relatively high temperatures. This pattern holds for *P. mcallii*, which occurs in the lower Colorado River basin of southwestern United States and Mexico (Mulcahy *et al.*, 2006); the reverse pattern holds for *P. hernandesi*, a short-limbed species that occurs at high elevations and cooler temperatures across the western United States into Sonora, Chihuahua and Durango, Mexico. The correlation between the second PCs suggests that species with large heads occur in wet environments with high temperature seasonality (i.e. wide annual fluctuations in temperature). This pattern is also supported by empirical data. One example is *P. ditmarsii*, a broad-headed species that occurs in evergreen woodlands and deciduous forests in the state of Sonora, Mexico (Sherbrooke, 2003).

The results presented here support one of the fundamental predictions of ecogeographic rules: morphological traits are related to climatic features; however, the mechanisms driving this relationship remain unclear. Species morphology may be regulated through physiological processes related to temperature and/or precipitation (Porter & Tracy, 1983; Qualls & Shine, 1998; Yom-Tov & Geffen, 2006). Alternatively, climate may be driving the characteristics of the physical habitat, thus creating the classic ecomorphological relationship among species. In either case (or a combination of both), the broad-scale correlation between climate and morphology suggests a potentially important relationship that may regulate species distributions and constrain the ability of species to respond to variation in climate. On one hand, studies suggest that many species will respond to climate change as predicted by niche conservatism by shifting their ranges to track climatic changes (Parmesan & Yohe, 2003; Hickling *et al.*, 2006; Devictor *et al.*, 2008). On the other hand, morphology may be one of the fastest biological responses to climatic changes (Barnosky *et al.*, 2003; Millien *et al.*, 2006; Wolf *et al.*, 2009), in which case shifts in morphology may occur prior to geographic range shifts. Because correlations between morphology

and climate remain after accounting for the effect of phylogeny in this study, morphological changes in *Phrynosoma* may occur rapidly and complicate predicted responses based on niche conservatism alone. In general, species responses to climate change will likely be complex, and approaches that integrate ecological, distributional and phylogenetic data are necessary to fully understand the relationship between climate and species distributions.

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Supporting information

Additional supporting information may be found in the online version of this article:

Figure S1 Locality data for the 17 *Phrynosoma* species.

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