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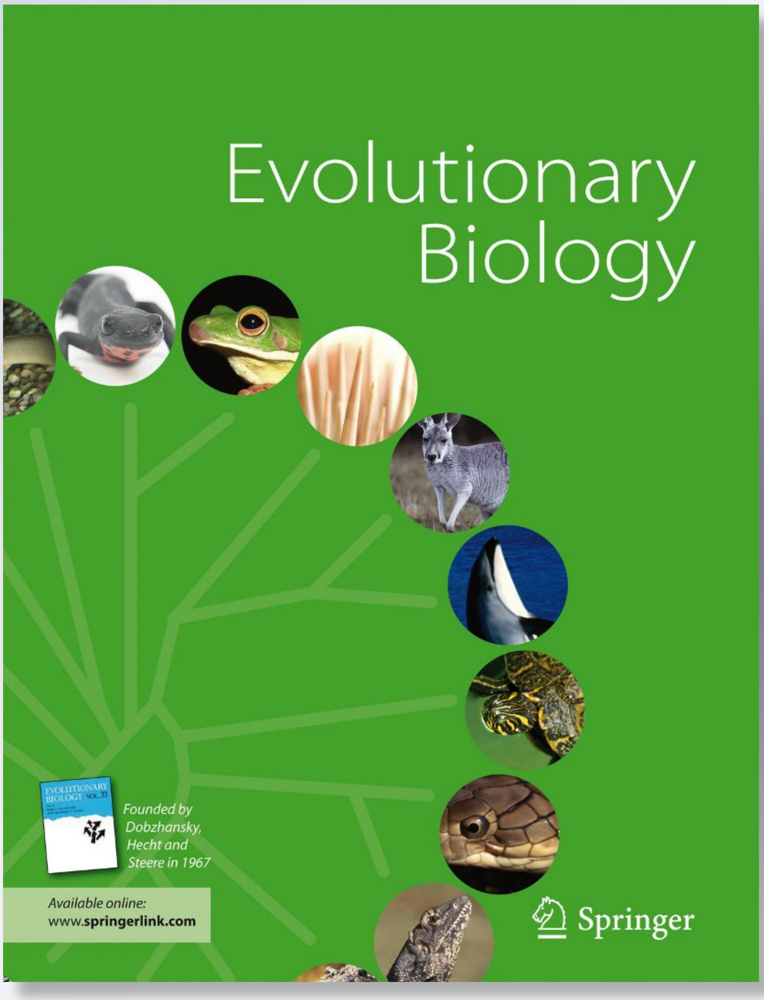

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RESEARCH ARTICLE

# The Evolution of Positive Allometry of Weaponry in Horned Lizards (*Phrynosoma*)

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**Abstract** The allometry of weapons and other conspicuous structures has long fascinated biologists. Recently, a debate has arisen about the roles of sexual and natural selection in driving the allometry of structures, with some authors suggesting that positive allometry is exclusively the result of sexual selection. Although some studies, often focusing on weapons, support this hypothesis, others have shown that many naturally selected structures also exhibit positive allometry. We study the allometry of the cranial horns in 14 species of horned lizards (*Phrynosoma*). These horns are purely defensive weapons and so are under natural, as opposed to sexual, selection. In almost all cases, the length of these horns is positively allometric through ontogeny (intraspecifically) and through evolution (interspecifically). Our findings demonstrate that positive allometry can be the product of natural selection, suggesting that the function of structures may dictate allometry and not the type of selection. For example, it is possible that weapons tend to be positively allometric. Our findings also suggest that longer horns may arise through the prolongation of horn growth, and that the horns that are most effective at defense from predators attacking a lizard from above are integrated with one another. Finally, we find that species with unusual horn morphologies have diverged from other species in their horn allometries, indicating that the evolution of morphological diversity can be mediated through the evolution of allometry.

**Keywords** Allometry · Evolution · Horns · Lizards · Morphometrics

## Introduction

The study of the allometry, or how the relative size or shape of structures changes through ontogeny, has long been of interest to biologists (Huxley 1932; Thompson 1945; Zelditch et al. 2004). Structures can be isometric, growing at the same rate as one another, or they can be positively or negatively allometric, where one structure grows faster or slower relative to another structure, respectively (Huxley 1932). Allometric structures are particularly interesting because they have disproportionate resources invested in them during ontogeny and form the basis of many adaptive hypotheses (e.g., Thompson 1945; Gould 1973; Kodric-Brown et al. 2006; Saporito et al. 2010). The realization that body size is an important correlate of morphology and ecology (Cooper and Purvis 2009) means that allometry influences many aspects of organismal biology.

Recently, interest in allometry has emphasized the quantitative study of the evolution of allometry and its bearing on diversification, and on multivariate approaches that allow simultaneous consideration of many traits. Differences in allometric relationships among closely related species and changes in allometries in response to selection indicate that allometries are heritable and can evolve (Emlen 1996; Okada and Miyatake 2009; Slater and

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Van Valkenburgh 2009; Klingenberg 2010). Heterochrony, or the study of the timing of developmental events, allows us to place allometry and development into a macroevolutionary framework and consider how allometries evolve (Alberch et al. 1979). Specifically, the size of a structure can be changed evolutionarily by changing the rate of growth, changing the timing of initiation of growth, or changing the length of the growth period (Alberch et al. 1979). The functional implications of such heterochronic changes mediate the evolution of the organismal performance traits on which natural selection acts (Arnold 1983). Ultimately, the adaptive evolution of allometry is responsible for the morphological diversification of organisms, where clades with more variable allometries tend to be more diverse phenotypically (Emlen et al. 2005, 2007; Gerber et al. 2008; Wilson and Sánchez-Villagra 2010).

A multivariate approach to studying allometry allows for the investigation of integration, or phenotypic covariation among traits (Hallgrimsson et al. 2009). Traits with uncorrelated allometries tend to be dissociated and free to evolve largely independently of one another (Polly et al. 2001; Revell and Collar 2009). In contrast, traits with correlated allometries tend to form modules that are internally integrated (Polly et al. 2001; Klingenberg 2008; Gonzalez-Voyer et al. 2009). High levels of integration can constrain evolution, thereby limiting morphological diversification (Gonzalez-Voyer et al. 2009; Wilson and Sánchez-Villagra 2010).

However, the underlying causes of integration among phenotypic traits are varied (Klingenberg 2008). Integration can be genetic, where common genes influence the expression of multiple traits (Hallgrimsson et al. 2009). Integration among traits is often developmental or epigenetic, whereby structures are derived from common developmental precursors or are subject to common interactions with developing structures or gradients to grow in a similar way (Polly et al. 2001; Klingenberg 2005; Jamniczky et al. 2010). Integration can also be functional, arising from constraints due to organismal performance demands (Young and Badyaev 2006; Schmidt and Fischer 2009). All of these causes of integration are interrelated, and so are difficult to tease apart, and all of them contribute to evolutionary integration—the co-evolution of traits (Klingenberg 2008). Allometry is an aspect of development that facilitates integration and is itself influenced by genetic, developmental, and functional commonalities and differences among traits (Gerber et al. 2008; Klingenberg 2008, 2010).

The allometry of physical weapons, such as horns, spines, and antlers provides an excellent avenue for the study of the evolution of allometry. In this regard, considerable work has focused on horns in bovid mammals and beetles (Emlen 1996; Moczek et al. 2002; Kodric-Brown et al. 2006). These structures are often sexually selected and primarily used in intra-sexual contests for mates (Moczek et al. 2002; Caro 2005; Stankowich and Caro 2009). For example, the size of male, but not female, bovid horns correlates negatively with territoriality, and positively with group size and male-biased sexual size dimorphism, all measures of the opportunity for male–male combat, and therefore, the intensity of intrasexual selection (Bro-Jorgensen 2007). Furthermore, male bovid horn shape correlates closely with intraspecific fighting style (Lundrigan 1996). In beetles, the presence or absence of horns is related to the reproductive strategy adopted by a male (Emlen et al. 2005). An expectation of positive allometry of horns and other sexually-selected structures has been hypothesized because of the importance of these weapons to reproductive success in males, and because large males tend to have more need to defend themselves from rivals (Packer 1983; Kodric-Brown et al. 2006). Interestingly, allometry has been used to differentiate between sexually and naturally selected structures, where the former have been hypothesized to be positively allometric, and the latter are expected to be isometric or negatively allometric (Kodric-Brown et al. 2006). Indeed, male horns are typically positively allometric, and the sexual-selection explanation for this pattern is strengthened by lower scaling coefficients for weapons possessed by females (Kodric-Brown et al. 2006). However, a simple association between sexually selected traits and positive allometry has since been shown to not be universal and has recently been strongly questioned (Bonduriansky 2007), suggesting a more complicated situation than previously thought.

Horns and other physical weapons have not been well studied from a defensive perspective in general (Caro 2005), despite the fact that many of these weapons, including bovid horns, are also used for this purpose (Lundrigan 1996; Stankowich and Caro 2009). The role of predation as a major force in evolution (Vermeij 1982; Lima and Dill 1990) is apparent when one considers that some weapons have evolved purely for defense (Edmunds 1974; Greene 1988; Caro 2005). Examples include the spines of porcupines, pufferfishes, and cordylid lizards, and the horns of horned lizards (Sherbrooke 1987; Sweitzer and Berger 1992; Wainwright and Turingan 1997; Losos et al. 2002; Sherbrooke 2003). In contrast to the positive allometry observed in many sexually selected weapons (Kodric-Brown et al. 2006), one might expect that purely defensive weapons scale with negative allometry with respect to body size for several reasons. First, small individuals are more vulnerable to predation, which is often illustrated by these individuals engaging in less risky behavior (Sweitzer and Berger 1992), and so would experience greater benefit from possessing relatively large weapons. Second, larger animals tend to be harder to subdue than smaller ones, and so large size in itself is a defense (Caro 2005). Finally, if weapons (whether defensive or sexually selected) are anteriorly located, as in the case of horned lizards (*Phrynosoma*), then negative allometry would be expected because vertebrate development typically proceeds anterior to posterior, meaning that anterior structures are more developed at any given time than posterior structures (Huxley 1932; Hopper and Hart 1985).

If the hypothesis that purely defensive weapons are negatively allometric is borne out, then it offers an interesting contrast to how many sexually selected weapons grow (Kodric-Brown et al. 2006), and provides a general framework for studying the growth and evolution of weapons in general. If, however, defensive horns grow with positive allometry, then this would add to the evidence that positive allometry is not strictly associated with sexual selection in the weapons of other animals (Kodric-Brown et al. 2006), and may suggest that weapons are simply less effective on small individuals (Pianka and Parker 1975; Bonduriansky 2007).

*Phrynosoma* lizards are highly variable in the number, size, and orientation of their horns (Sherbrooke 2003; Leache and McGuire 2006). Different species have between three and six horns on each side of the occipital region of the head, erupting from the parietal and squamosal bones, as well as a horn dorsal to each orbit, associated with the frontal bone (Presch 1969; Montanucci 1987; Powell et al. 2002). These horns vary in length by species, from small nubs to half the length of the head (Presch 1969; this study). The horns are purely defensive and not sexually selected; although male *Phrynosoma* sometimes use female horns as holds during copulation, they do not defend territories, and sexual size dimorphism tends to be female-biased, probably due to selection for male mobility (Fitch 1981; Powell and Russell 1985; Sherbrooke 1987; Zamudio 1998). In *P. mcallii*, individuals with relatively longer horns are less likely to be killed and eaten by loggerhead shrikes (Young et al. 2004). In these cases, lizards will assume a chin-down posture that positions the horns in a vertical orientation, with the points directed at predators approaching from above (Sherbrooke 1987). The horns also act to prevent ingestion by predators, sometimes leading to the puncturing of the esophagus and even death of the predator (Sherbrooke 2003). *Phrynosoma* also rely on a plethora of other anti-predator mechanisms in addition to their horns, including various forms of crypsis, the squirting of blood from their preocular sinuses, hissing, inflating their bodies, and fleeing, and these mechanisms are predator-specific (Middendorf et al. 2001; Sherbrooke and Middendorf 2001; Sherbrooke 2002, 2003; Sherbrooke and Mason 2005).

Here, we study the evolution of *Phrynosoma* horn allometry in the context of the defensive function of the horns. First, we formulate an explicit hypothesis of homology of the horns among species, one that must be rigorously tested in future studies. Although some comparative studies have considered the number of horns possessed by different species and the bones that they erupt from (Presch 1969; Montanucci 1987), they have not attempted to homologize each horn among all species, a necessary first step for any comparative study of the horns. Second, we test the hypothesis that the horns scale with negative allometry both intraspecifically, in the context of ontogeny, and interspecifically, in the context of the evolution of species size. In both cases, we predict that smaller lizards will have relatively larger horns to enhance their defensive utility. Third, we consider how the allometry of horns has evolved and quantify the degree of integration among horns. Integration among horns may be complex due to multiple, potentially interacting underlying causes. If developmental integration is at play, then we would predict that horns that erupt from the same bone will be more closely integrated than horns that erupt from different (i.e., parietal, squamosal, and frontal) bones. However, if functional integration is the dominant factor influencing horn morphology and scaling, then larger horns that are closer to the midline may be more integrated if the dominant use of horns is to deter over-head predators, or lateral horns may be more integrated if the dominant use of horns is to deter gape-limited predators. Developmental and both functional integration possibilities may be at play and the developmental and lateral horn functional integration hypotheses may yield the same patterns, making the underlying cause of integration difficult to pinpoint. Finally, we test whether species with unusual horn morphologies also have unusual horn allometries. We would expect this to be the case if the evolution of allometry were driving the evolution of horn morphology.

## Methods

### Establishing Horn Homology

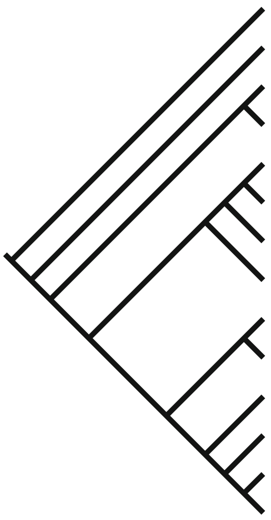
To establish an explicit hypothesis of horn homology among the species of *Phrynosoma*, we compiled numbers of horns extending from the parietal and squamosal bones from the literature (Presch 1969; Montanucci 1987) in addition to our own observations of skeletal material available at the University of Arizona Museum of Natural History and images available on the Digimorph website (http://www.digimorph.org, University of Texas, Austin). We examined specimen UAZ-35511 of *P. ditmarsi*, specimen UAZ-56768 of *P. modestum*, and one unnumbered specimen of each of *P. platyrhinos* and *P. solare*. We used horn position and bone of attachment as the primary clues to hypothesize horn homology. We use the horn nomenclature of Powell et al. (2002), where horns are abbreviated with the first letter of the bone they extend from, followed by a numerical subscript, indicating their order from medial to lateral. In addition to parietal and squamosal horns, all *Phrynosoma* species have a frontal horn, $F_0$, dorsal to the eye. Although there is considerable variation in the size of horns, there is very little intraspecific variation in the number of horns (Montanucci 1987; this study). We excluded specimens that had aberrant horn numbers (<1% of those sampled) from analysis (Powell et al. 2002).

## Sampling and Data Collection

We examined 934 specimens, belonging to 14 of the 16–17 extant species of *Phrynosoma*, including *P. asio*, *P. blainvilli*, *P. braconieri*, *P. cornutum*, *P. coronatum*, *P. ditmarsi*, *P. douglasii*, *P. hernandesi*, *P. mcallii*, *P. modestum*, *P. orbiculare*, *P. platyrhinos*, *P. solare*, and *P. taurus* (Table 1). Specimens that we examined were from the University of Arizona Museum of Natural History, the University of Texas at Arlington, and the US Museum of Natural History (Appendix S1). When possible, we measured 100 specimens from each species to ensure robust sample sizes and attempted to sample specimens along the complete ontogenetic range (histograms of SVL are plotted in Appendix S2). Species for which we sampled fewer than 100 specimens are rare in museums and/or nature (Table 1).

For each specimen, we measured snout-vent length (SVL), body width (BW), and head length (HL), as well as the lengths of horns $P_2$, $S_1$, $S_2$ and $F_0$ (Fig. 1). These are the longest horns that are present in all or most of the species examined. We also measured horns $P_1$ and $S_3$, but we did not analyze these data because many species lacked those horns. We took each measurement three times to 0.01 mm using a Mitutoyo digital caliper and used the log-transformed mean in all subsequent analyses. We measured all bilaterally symmetrical structures on the right side when possible. We measured SVL as the distance from the anterior tip of the snout to the cloaca, BW as the widest distance between the limbs and transverse to the spine, and HL from the occiput of the skull to the tip of the rostral. We then calculated body length (BL) as SVL minus HL for use in studying the scaling of the head. We measured all horn lengths as the distance from the base of the keratinized horn sheath, where scalation begins, to the horn tip, following Powell et al. (2002).

**Table 1** Phylogeny, species, RMA regression slopes for each listed variable against SVL (against BL for HL), numbers of horns, and sample sizes for species of *Phrynosoma*



| | Variable | | | | | | # Horns | | Sample Size | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **HL** | **BW** | **$P_2$** | **$S_1$** | **$S_2$** | **$F_0$** | **P** | **S** | **M** | **F** | **Total** |
| *P. asio* | **0.76** | **1.10** | **1.20** | **1.49** | **1.56** | **1.16** | 1 | 3 | 19 | 31 | 50 |
| *P. cornutum* | **0.63** | **1.07** | **1.22** | **1.20** | **1.24** | 1.03 | 1 | 3 | 48 | 52 | 100 |
| *P. braconieri* | **0.51** | 0.97 | 1.23 | 1.33 | **1.62** | 1.45 | 1 | 3 | 5 | 10 | 15 |
| *P. taurus* | **0.78** | **1.09** | 1.19 | **1.52** | **1.74** | 0.94 | 1 | 2 | 8 | 16 | 24 |
| *P. coronatum* | **0.72** | 1.25 | **1.30** | **1.34** | **1.46** | 1.17 | 1.5 | 3 | 16 | 11 | 27 |
| *P. blainvilli* | **0.68** | 0.93 | **1.69** | **1.65** | **1.49** | **1.25** | 1.5 | 3 | 46 | 31 | 77 |
| *P. mcallii* | **0.55** | 1.05 | 1.03 | **1.24** | **1.22** | 1.13 | 1.5 | 3 | 56 | 46 | 102 |
| *P. solare* | **0.69** | **1.15** | **1.30** | **1.32** | **1.41** | **0.85** | 2 | 3-4 | 38 | 62 | 100 |
| *P. platyrhinos* | **0.55** | **1.11** | **1.39** | **1.24** | **1.31** | 0.99 | 1 | 3 | 51 | 49 | 100 |
| *P. modestum* | **0.60** | **1.14** | **1.41** | **1.44** | | 1.09 | 1 | 3 | 48 | 52 | 100 |
| *P. orbiculare* | **0.68** | 0.98 | **1.40** | **1.72** | **1.73** | 1.00 | 1 | 3 | 29 | 32 | 61 |
| *P. douglasii* | **0.69** | **2.15** | **1.75** | **1.50** | **1.36** | 1.02 | 1 | 3 | 20 | 29 | 49 |
| *P. hernandesi* | **0.66** | 1.12 | **1.23** | **1.41** | **1.41** | 1.00 | 1 | 3 | 31 | 56 | 87 |
| *P. ditmarsi* | **0.73** | **1.16** | 0.93 | **1.34** | | 1.13 | 1 | 3 | 16 | 26 | 42 |
| PI Contrasts | 1.03 | 1.06 | 2.89 | **2.21** | **2.88** | **2.17** | | | | | |

Regressions include all individuals. Interspecific RMA slopes of PICs through the origin are also presented. Shaded cells indicate significant positive allometry, boxed cells indicate significant negative allometry, unembellished cells are not significantly different from isometry. Empty cells indicate absence of a horn for a given species. Horn numbers indicate the number of horns on each side; 0.5 indicates a median parietal horn that is unpaired

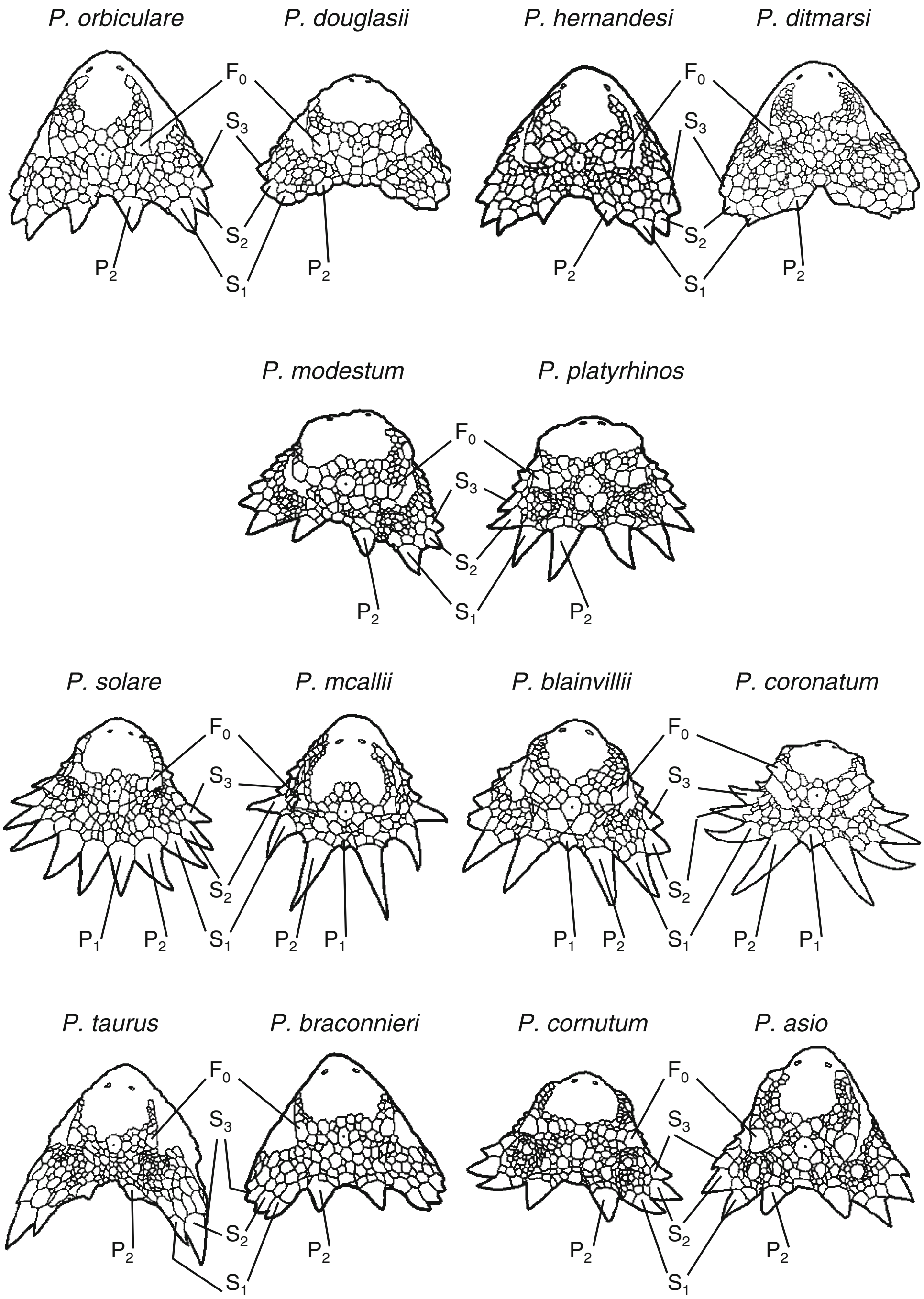


**Fig. 1** Line drawings of cranial scalation of species of *Phrynosoma* examined. Horns $P_1$, $P_2$, $S_1$, $S_2$, $S_3$ and $F_0$ are labeled on each species to illustrate the pattern of horn homology hypothesized in this study. Heads are scaled to be of comparable size so that relative horn sizes can be compared visually

## Statistical Analysis

For each species we regressed log-transformed BW, $P_2$, $S_1$, $S_2$, and $F_0$ on log-transformed SVL with reduced major axis (RMA) regression using R (R Core Development Team 2010). To study the allometry of the head, we also regressed HL on BL instead of SVL, because HL is a subset of SVL. We tested the assumption of normality of the residuals, which was met, for each regression using a Kolmogorov–Smirnov test (results in Appendix S3) using a script written in R and available from PJB. We then tested for isometry of the slope using a one-sample *t* test (Ricker 1984). To test for sexual differences in scaling, we computed RMA slopes for each sex separately using R and compared them using the *t* test of Clarke (1980). We found no significant differences between the sexes in scaling relationships (results in Appendix S4), so we only present results for analyses of all individuals for each species.

To examine interspecific scaling relationships, we took the mean of the ten individuals with the largest SVL for each species to get an estimate of adult morphology and used the log-transformed variables in analyses. We also calculated relative HL, BW, and horn lengths by taking the ratio of each variable to either BL (for HL) or SVL (for the other variables). Although the use of ratios is sometimes problematic, if they are normally distributed, they offer the advantages of an intuitively understandable measure (Sokal and Rohlf 1995) whose meaning does not change between studies (Smith 1999). All calculated ratios were normally distributed, as indicated by Kolmogorov–Smirnov tests ($P > 0.2$ in all cases). Finally, to analyze the evolution of scaling relationships, we also considered growth rates and time of growth onset for each species. We used the RMA slope as an estimate of growth rate and the intercept an estimate of growth onset; the larger the intercept, the larger a horn is at birth (Alberch et al. 1979). To study the relationships among these variables we calculated phylogenetically independent contrasts (PICs; Felsenstein 1985) using Mesquite v.2.73 (Maddison and Maddison 2010) and the total evidence phylogenetic tree of *Phrynosoma* presented in Fig. 4 of Leache and McGuire (2006), shown in our Table 1. We tested for adequate standardization of PICs by calculating Pearson correlation coefficients between PICs and their standard deviations (Garland et al. 1992) and between PICs and their node heights (Freckleton and Harvey 2006). We then used RMA regression of PICs, as implemented in Mesquite, to examine interspecific scaling relationships between each variable and SVL. To consider integration among horns, we calculated pairwise correlation coefficients among relative horn lengths, intraspecific slopes, and intraspecific intercepts. We accounted for phylogeny by calculating the correlations from evolutionary variance–covariance (eVCV) matrices, which we computed in R (R Core Development Team 2010) using the package Geiger. The eVCV matrices gave us covariances among variables, and the variances for each variable. The variance for each variable is a measure of its rate of evolution (Felsenstein 1985; Clobert et al. 1998).

To test whether species with unusual horn morphologies also had unusual allometries, we created morphological and allometric spaces using principal component analysis (PCA). For these analyses, we included BL, HL, BW, and lengths of the horns $P_2$, $S_1$, $S_2$ and $F_0$, but excluded *P. ditmarsi* and *P. modestum* from analyses of horn $S_2$ because that horn was difficult to measure on many specimens. We created a morphospace using an evolutionary PCA using a covariance matrix (Revell 2009) in R to analyze our mean adult data for the variables listed above. We also created an allometric space using all of our morphometric data following the approach of Gerber et al. (2008). First, we ran a PCA using a covariance matrix for each species separately. We then compiled a dataset including the PC-1 allometric loadings for each variable and each species. Finally, we ran an evolutionary PCA using a covariance matrix (Revell 2009) in R on this dataset. Importantly, this approach differs from that of Gerber et al. (2008) in that it takes phylogeny into account during the interspecific analysis. We identified species with unusual horn morphologies and allometries by calculating the standard deviation of PC-1 and PC-2 for each evolutionary PCA, and using the standard deviation to calculated 95% confidence intervals. We considered species with factor scores outside the 95% confidence intervals to be unusual. We visualized both the morphospace and the allometric space by plotting species factor scores for PC-1 and PC-2 from each evolutionary PCA and mapped the phylogeny on these plots using Mesquite's Plot Tree 2D function. Finally, we tested whether the morphospace and the allometric space were concordant using Pearson correlations of PICs of the evolutionary PCA factor scores for each space. For all analyses where we tested significance, we corrected for multiple comparisons in each table using a method that takes not only multiple comparisons into account, but also false discovery rate (Benjamini and Hochberg 1995; Williams et al. 1999), setting table-wide alpha = 0.05.

## Results

### Horn Homology

We formulated our hypotheses of horn homology primarily by considering horn position, and conducted our analyses based on these hypotheses. It is important to note that we have not tested these hypotheses, which should be done using a developmental approach. Most species of *Phrynosoma* have one parietal horn ($P_2$) on each side, with the exception of the clade containing *P. coronatum*, *P. blainvilli*, *P. mcallii* and *P. solare* (Table 1). In this clade, *P. solare* has two parietal horns on either side, while the other species have one on each side ($P_2$), and a single medial horn ($P_1$) (Fig. 1). In *P. mcallii*, the medial horn is much reduced and is expressed as a slight tuberosity. We hypothesize that the more medial paired horn of *P. solare* is homologous to the medial horn of the other species due to their similar position (similar to the hypothesis of Montanucci 1987). Presch (1969) observed that *P. braconieri* also has a small medial parietal horn, but we saw no evidence of this, in agreement with Montanucci (1987).

Most species of *Phrynosoma* have three squamosal horns, although *P. solare* sometimes has four (Presch 1969; Montanucci 1987). We again homologize the squamosal horns based on position from medial to lateral (Fig. 1) because in all species, the medial horn clearly arises from the squamosal bone, lateral to the squamosal-parietal suture. Most of the species lack any small protuberances between the horns we measured that might obscure patterns of homology of the focal horns. However, *P.braconnieri*, *P. coronatum*, *P. modestum* and *P. platyrhinos* do have small protuberances between $P_2$ and $S_1$ horns, and the influence of these on homologizing the horns remains to be investigated. $S_1$ is highly reduced and $S_2$ is highly enlarged in *P. taurus*, which is unique among *Phrynosoma*. $S_2$ is highly reduced in *P. ditmarsi*, *P. douglasii* and *P. modestum*, often to the point of not being easily measurable.

## Horn Allomtery

With few exceptions, horns $P_2$, $S_1$ and $S_2$ grew with significant positive allometry, while $F_0$ tended to be isometric (Table 1). Only horn $F_0$ in *P. solare* was significantly negatively allometric. Body width tended to scale isometrically or positively allometrically to SVL, and head length was universally significantly negatively allometric relative to BL (Table 1). Interspecific scaling relationships using PICs showed even more pronounced positive allometry of the horns and isometry between HL and BL, and between BW and SVL (Table 1). Detailed statistics of these regressions are available in Appendix S5.

## Horn Heterochrony and Integration

We found no significant relationship between PICs of SVL and either the rate of growth (slope) of the horns or the onset of growth (intercept) (Table 2). Despite this, we found evidence for morphological and allometric integration among horns. Specifically, the relative lengths of horns $P_2$ and $S_1$ were significantly integrated (Table 3). Horns $P_2$ and $S_1$ and horns $S_1$ and $S_2$ also had integrated growth rates (Table 4) and growth onsets (Table 5). Hence, the relative size, rate of growth, and growth onset have co-evolved for these pairs of horns.

## Evolution of Horn Morphology and Allometry

**Table 3** Relationships among PICs for relative lengths of various horns in species of *Phrynosoma*

| | $P_2$ | $S_1$ | $S_2$ | $F_0$ |
|---|---|---|---|---|
| $P_2$ | *0.007* | **0.005** | 0.949 | 0.560 |
| $S_1$ | **0.776** | *0.002* | 0.057 | 0.756 |
| $S_2$ | 0.022 | 0.589 | *0.003* | 0.429 |
| $F_0$ | −0.198 | 0.106 | 0.266 | *0.000* |

Pearson correlations are below the diagonal, *P* values are above. Significant values are in bold. Rates of evolution are in the italicized diagonal

**Table 4** Relationships among PICs for the scaling relationships (RMA slopes) of *Phrynosoma* horns

| | $P_2$ | $S_1$ | $S_2$ | $F_0$ |
|---|---|---|---|---|
| $P_2$ | *0.695* | **0.039** | 0.754 | 0.656 |
| $S_1$ | **0.626** | *0.418* | **0.019** | 0.965 |
| $S_2$ | 0.107 | **0.688** | *0.291* | 0.988 |
| $F_0$ | 0.152 | 0.015 | −0.005 | *0.333* |

Pearson correlations are below the diagonal, *P* values are above. Significant values are in bold. Rates of evolution are in the italicized diagonal

**Table 5** Relationships among PICs for the RMA intercepts for *Phrynosoma* horns

| | $P_2$ | $S_1$ | $S_2$ | $F_0$ |
|---|---|---|---|---|
| $P_2$ | *3.153* | **0.002** | 0.094 | 0.643 |
| $S_1$ | **0.813** | *1.884* | **0.004** | 0.734 |
| $S_2$ | 0.530 | **0.791** | *0.995* | 0.673 |
| $F_0$ | 0.158 | 0.116 | 0.144 | *1.731* |

Pearson correlations are below the diagonal, *P* values are above. Significant values are in bold. Rates of evolution are in the italicized diagonal

The first two morphological PCs explained ~92% of variance, and all high magnitude loadings were for horns, as opposed to body proportions (Table 6). PC-1 explained variance associated with size and indicated that all horns were positively allometric to all body dimensions. PC-2 explained size-associated variance and represented an inverse relationship between the relative length of horn $P_2$

**Table 2** Interspecific RMA regressions of PICs through the origin, with SVL as the x variable and either slopes (rate) or intercepts (onset) as y variables

| Variable | n | $R^2$ | RMA | T | *P* |
|---|---|---|---|---|---|
| Rate of $P_2$ | 13 | 0.0131 | −3.73 | −0.40 | 0.697 |
| Rate of $S_1$ | 13 | 0.0027 | 2.48 | 0.18 | 0.861 |
| Rate of $S_2$ | 11 | 0.0251 | 2.33 | 0.51 | 0.623 |
| Rate of $F_0$ | 13 | 0.1926 | −2.22 | −1.69 | 0.116 |
| Onset of $P_2$ | 13 | 0.0095 | 7.47 | −0.51 | 0.617 |
| Onset of $S_1$ | 13 | 0.0001 | −5.20 | −0.64 | 0.533 |
| Onset of $S_2$ | 11 | 0.0001 | −4.32 | 0.29 | 0.779 |
| Onset of $F_0$ | 13 | 0.3693 | 5.13 | 0.78 | 0.452 |

**Table 6** Standardized loadings for evolutionary PCAs on morphological and allometric (species PC-1 value) data

| Variable | Morphological PCA | | Allometric PCA | | |
|---|---|---|---|---|---|
| | PC-1 | PC-2 | PC-1 | PC-2 | PC-3 |
| HL | 0.184 | −0.104 | −0.134 | −0.239 | −0.026 |
| BL | 0.162 | −0.137 | −0.037 | −0.048 | **−0.445** |
| BW | 0.162 | −0.233 | **−0.873** | 0.140 | 0.003 |
| $P_2$ | **0.528** | **0.778** | 0.259 | **0.717** | **0.273** |
| $S_1$ | **0.514** | 0.010 | 0.217 | −0.235 | **0.495** |
| $S_2$ | **0.523** | **−0.441** | 0.185 | **−0.553** | −0.072 |
| $F_0$ | **0.312** | **−0.341** | **0.263** | 0.213 | **−0.690** |
| Eigenvalue | 1.232 | 0.378 | 0.101 | 0.066 | 0.042 |
| % Explained | 70.24 | 21.54 | 44.83 | 29.57 | 18.50 |

Morphological PC-3 explained 5.6% of total variance, and allometric PC-4 explained only 3.3% of total variance, so are not included. Loadings >0.25 in magnitude are in bold

**Table 7** Pearson correlations among PICs of species factor scores for PC-1 and PC-2 from the morphological and allometric PCAs

| | Morphology | | Allometry | |
|---|---|---|---|---|
| | PC-1 | PC-2 | PC-1 | PC-2 |
| Morphology PC-1 | | 1.000 | 0.105 | 0.512 |
| Morphology PC-2 | 0.000 | | 0.570 | **0.026** |
| Allometry PC-1 | 0.492 | 0.183 | | 1.000 |
| Allometry PC-2 | −0.210 | **0.636** | 0.000 | |

Correlations are below the diagonal and *P* values are above the diagonal. Significant *P* values are in bold

and horns $S_2$ and $F_0$ (Table 6). The first two allometric PCs explained ~74% of variance. PC-1 suggested that as all horns, particularly $F_0$, evolved to grow more quickly, body width evolved to grow more slowly (Table 6). PC-2 primarily represented an inverse relationship between the scaling of horns $P_2$ and $S_2$ (Table 6).

Examination of both morphological (Fig. 2a) and allometric (Fig. 2b) spaces shows little phylogenetic structure to where species are positioned. There is some degree of coincidence between horn morphological and allometric space. Morphological PC-1 correlates with $R > -0.4$ with allometric PC-1, and morphological PC-2 correlates with $R > 0.6$ with allometric PC-2, although only the latter relationship is significant (Table 7). *P. douglassi* was identified as an outlier for both morphological and allometric PC-1 factor scores, while *P. taurus* was identified as an outlier for both morphological and allometric PC-2 factor scores (Fig. 2). Indeed, these two species have the most unusual horn morphologies, with *P. douglasii* having the smallest horns of any *Phrynosoma*, and *P. taurus* having reduced $P_2$ and large $S_2$ horns relative to other horns (Fig. 1).

## Discussion

### Allometry of *Phrynosoma* Horns

We reject our original hypothesis that *Phrynosoma* horns grow with negative allometry, finding instead that virtually all horns that we studied in all species, with the exception of the diminutive $F_0$, grew with positive allometry (Table 1). Further, we found that, in general, the squamosal horns grew faster relative to the body than parietal or frontal horns. Our results coincide closely with those for *P. orbiculare*, the only species for which horn ontogenetic allometry was previously examined (Powell et al. 2002). This pattern of positive allometry of horns is even more pronounced interspecifically, and so larger individuals within species as well as larger species themselves have disproportionately large horns. Our findings are surprising in the context of our original hypothesis because horns in *Phrynosoma* are (1) anteriorly positioned structures (explained below), (2) are not sexually selected (Zamudio 1998; Sherbrooke 2003), and because (3) young or small

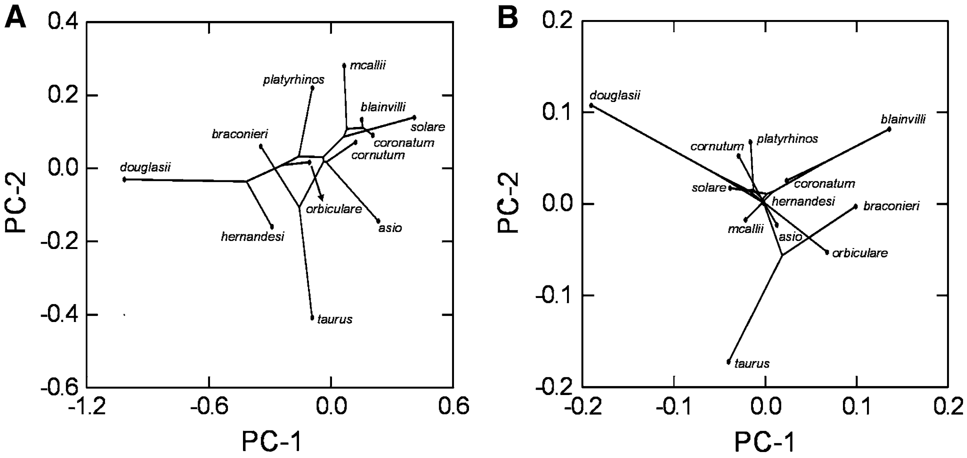


**Fig. 2** Plots of morphological (**a**) and allometric (**b**) space for *Phrynosoma* defined by the first two PCs in each case, derived from evolutionary PCAs of species values. Specific epithets are supplied for each plot and the phylogeny used is mapped onto each plot. See text for details

individuals tend to be more vulnerable to predation and so may benefit more from defenses than large individuals (Caro 2005).

Since vertebrate development proceeds anterior to posterior, one would expect that anteriorly positioned structures such as the head would be negatively allometric and more posterior structures positively allometric as they "catch up" on account of them being less developed (Huxley 1932; Hopper and Hart 1985; Bénazéraf et al. 2010). Indeed, head length in all species of *Phrynosoma* scales significantly negatively allometrically relative to the body, as expected, but the horns are, almost without exception positively allometric (Table 1). Therefore, only the horns, and not all cranial structures, are growing disproportionately quickly. This suggests that functional considerations may dictate the scaling of the horns. In fin whales (*Balaenoptera physalus*) head scaling is also driven by functional considerations, showing dramatic positive allometry due strong selection for increased buccal volume to facilitate feeding on plankton (Goldbogen et al. 2010). Many mammalian sexually selected horns also grow positively allometrically relative to the head and body (Gould 1973; Bro-Jorgensen 2007).

Increasing numbers of recent studies have cast doubt on the hypothesis that only sexually selected structures are positively allometric and its corollary that structures that are not sexually selected scale isometrically or negatively allometrically with respect to body size (Kodric-Brown et al. 2006). For example, in a number of frog species, both sexually dimorphic and monomorphic structures are positively allometric, but many exceptions occur (Schulte-Hostedde et al. 2011). In a variety of animals, including birds, guppies, flies, and water striders, it appears that positive allometry of sexually dimorphic structures is the exception rather than the rule (Bonduriansky 2006, 2007). Our research adds to the growing body of evidence rejecting a exclusive relationship between sexual selection and positive allometry.

Bonduriansky (2007) noted that an association between positive allometry and sexual selection was at least partly due to sampling bias, with researchers focusing on exaggerated structures. We note that many of these structures are, in fact, weapons that must be of a certain size to be effective (Emlen 1996; Lundrigan 1996; Caro et al. 2003; Caro 2005; Bro-Jorgensen 2007). A reasonable alternative hypothesis is that weapons tend to be positively allometric so that their effectiveness is enhanced with the size of the animal. Our *Phrynosoma* horn data, as well as a number of other studies lend credence to such a hypothesis. Many sexually selected weapons are also used for defense and so are also under natural selection (Lundrigan 1996; Caro et al. 2003). The poison glands of dendrobatid (poison arrow) frogs also grow with positive allometry and are purely defensive (Saporito et al. 2010). Other defensive weapons, such as porcupine quills and pufferfish spines (Sweitzer and Berger 1992; Wainwright and Turingan 1997) exist, but their scaling has not been examined.

Although general laws of scaling are attractive because of their explanatory power (Gillooly et al. 2001), our suggestion that weapons are expected to be positively allometric comes with some caveats. First, although we would expect weapons to be positively allometric, we would not expect all structures that are positively allometric to be weapons. Antlers of the extinct Irish Elk (*Megaloceros giganteus*) were used in display and were positively allometric (Gould 1973, 1974), as are many ornaments (Kodric-Brown et al. 2006; Baldauf et al. 2010). Second, the allometry of a structure is the product of the effect and relative strength of both sexual and natural selection on both the structure and body size, as well as resource allocation trade-offs (Gould 1974; Bonduriansky 2007). For example, strong selection for large body size may change a previously positively allometric structure into one that is isometric or negatively allometric. Related to this issue is the selection of body size measures. Using a single linear measurement as a proxy for body size can give different results from using a multivariate proxy or body mass (e.g., Bickel and Losos 2002).

Our finding that *Phrynosoma* horns are positively allometric has the implication that small animals may be particularly vulnerable to predation because they are not only small, but also lack effective weapons. A lack of effective defenses increases perceived risk in many animals, leading them to adopt less risky behaviors (Stankowich and Blumstein 2005), such as foraging in suboptimal but safer habitats (Sweitzer and Berger 1992). Indeed, the effectiveness of horns on small *Phrynosoma* has been previously questioned (Powell et al. 2002). It is possible that young individuals or small species are too small to wield weapons large enough to be effective, in which case the observed positive allometry would be expected (Bonduriansky 2007). However, to suggest that young or small *Phrynosoma* are defenseless is incorrect because lizards of this genus employ a range of defensive strategies, including crypsis (Sherbrooke and Middendorf 2001; Sherbrooke 2002, 2003). Indeed, some of the smaller species (e.g., *P. douglasii*, *P. braconnieri*) have very short horns and are not known to use them actively in defense (Sherbrooke 1987). One explanation for the observed positive allometry of these reduced horns is phylogenetic conservatism of allometry, whereby all species of *Phrynosoma* have descended from an ancestor with positively allometric horns.

## Heterochrony and Integration

The evolution of morphological proportions is generally thought of as arising through changes in developmental timing (Alberch et al. 1979). Structures can evolve to be larger by starting growth earlier, prolonging growth for longer, or growing more quickly (Alberch et al. 1979). The attractiveness of this concept is that it bridges the gap between intraspecific development and the evolution of morphology by considering interspecific changes in development. We were able to evaluate heterochrony of horns in *Phrynosoma* by comparing intraspecific allometries among species. Using SVL as a proxy for age (Gerber et al. 2008), we found no significant relationship between the evolution of SVL and the rate of growth of horns or their growth onset (Table 2), which is surprising because larger species tended to have relatively longer horns (Table 1). This is exemplified by the finding that horns grew with positive allometry even in species with extremely short horns (e.g., *P. ditmarsi*, *P. douglassi*, and *P. hernandesi*) and suggests that growth in larger species with longer horns is prolonged. However, given that we lacked actual age data, it is difficult to rigorously test whether and how the time of cessation of horn growth has evolved in *Phrynosoma*. Nevertheless, it is likely that the horn growth period was foreshortened with the evolution of the short horned clade, consisting of the three species mentioned above and *P. orbiculare* (Table 1; Leache and McGuire 2006). Modification of the time of growth offset has played a role in the evolution of female-biased sexual size dimorphism in this clade, where males simply stop growing earlier than females (Powell and Russell 1985; Zamudio 1998).

We found some evidence for functional integration among horns because only the most medial and largest horns (those involved in defense from predators that attack from above) were morphologically integrated (horns $P_2$ and $S_1$—Table 3). The functional integration hypothesis is further strengthened because these horns erupt from different bones. Such functional integration is likely due to similar external selective pressures acting on these horns (Young and Badyaev 2006; Schmidt and Fischer 2009), and this selection may be acting to lengthen horns in species that use them for defense, such as *P. mcallii* (Young et al. 2004). Integration among traits can result from similar allometric patterns among those traits (Polly et al. 2001), and we found evidence for this as well because growth rate and onset were significantly correlated for horns $P_2$ and $S_1$ as well (Tables 4, 5). However, we also found that growth rates and onsets for horns $S_1$ and $S_2$ were significantly correlated, a pattern also seen in *P. orbiculare* (Powell et al. 2002). This could either be due to some degree of developmental integration because both horns erupt from the squamosal bone, or functional integration due to selection on lateral horns used in deterring gape-limited predators, such as snakes (e.g., Sherbrooke 2003).

It is further difficult to tease apart horn length covariation due to functional integration from that due to developmental integration (Klingenberg 2008; Jamniczky et al. 2010) because the most functional horns are also closest together, and so may be subject to the same epigenetic signals. As indicated above, the strongest evidence for functional integration in this case is strong covariation between horns $P_2$ and $S_1$, which are outgrowths of different bones in the skull. Interestingly, the allometries of horns in *Phrynosoma* have evolved in concert with one another, but not with body size (Table 2).

Looking more broadly at the biology of *Phrynosoma*, other traits have co-evolved with horn length, including body shape, sprint speed, and diet (Pianka and Parker 1975; Montanucci 1989; Meyers et al. 2006; Bergmann et al. 2009). More heavily defended species may, in general, be stockier in body shape and move more slowly than less defended counterparts, as this pattern has also been documented in cordylid lizards, stickleback fishes, and pufferfishes (Brainerd and Patek 1998; Bergstrom 2002; Losos et al. 2002). Horn size and allometry in *Phrynosoma* may indeed be shaped by many interacting factors, including climate. For example, it has been found that species with longer horns tend to live in warmer and drier geographic areas (Luxbacher and Knouft 2009).

## The Evolution of Morphological Diversity

The realization that allometries are heritable and evolve has placed the study of intraspecific development in the context of macroevolution (Emlen 1996; Klingenberg 2010; Wilson and Sánchez-Villagra 2011). The evolution of allometry changes morphological constraints, facilitating changes in organismal performance, and is a mechanism for the diversification of morphology (Gerber et al. 2008; Gonzalez-Voyer et al. 2009; Slater and Van Valkenburgh 2009; Wilson and Sánchez-Villagra 2010). We used a recently-developed approach to mapping species onto an allometric space (Gerber et al. 2008) to show that species of *Phrynosoma* fall in similar places in both allometric and morphometric spaces (Table 7). Our approach builds on that of Gerber et al. (2008) by taking phylogeny into account and quantitatively testing for correspondence between allometric and morphometric spaces. We showed that the correspondence between morphology and allometry is primarily driven by morphological and allometric PC-2s, both of which are primarily defined by an inverse relationship between the horns $P_2$ and $S_2$ (Table 6). In both morphospace and allometric space *P. douglassi* and *P. taurus* are outliers from other species (Fig. 2) and are characterized by unusual horn morphologies. *P. douglassi* has the smallest horns of any species (Fig. 1), to the point that they are difficult to distinguish from adjacent scales. *P. taurus* (Fig. 1) is unique in that its $P_2$ and $S_1$ horns are highly reduced, and its $S_2$ horn is the largest. Underlying similarly unique horn allometries of these two species suggest that the evolution of allometry has led to the diversification of horn morphology.

## Horn Homology

We have shown that the cranial horns of *Phrynosoma* are positively allometric, matching findings of other studies of the scaling of weaponry, whether sexually or naturally selected (Kodric-Brown et al. 2006; Saporito et al. 2010). We have also shown how the allometry of horns has evolved in an integrated way to yield the diversity of horn lengths found in *Phrynosoma*. Both of these key findings rely on our hypothesis of horn homology (Fig. 1). Other studies have either described the number of horns erupting from each bone (Presch 1969; Montanucci 1987), or have only considered the relative length of the longest horn in their analyses (Leache and McGuire 2006; Bergmann et al. 2009). Explicitly homologizing horns among species was necessary to conduct our analyses but was not the focus of our study, and so our approach to homologizing horns was relatively simple. Due to the conservatism in the number and position of horns in *Phrynosoma*, we are relatively confident in most of our hypothesized homologies. We have provided a hypothesis (Fig. 1) that can now be tested using a developmental approach that examines patterns of horn chondrification and ossification, as well as the genes expressed in horn development.

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