Morphological correlates of ant eating in horned lizards (*Phrynosoma*)

JAY J. MEYERS[1]*, ANTHONY HERREL[2] and KIISA C. NISHIKAWA[1]

[1]*Physiology and Functional Morphology Group, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, USA*
[2]*Laboratory of Functional Morphology, Department of Biology, University of Antwerp, Antwerp B-2610, Belgium*

The North American horned lizards (*Phrynosoma*) represent a morphologically specialized group of ant-eating lizards. Although variation in dietary fidelity is observed among the species, all appear to possess morphological specializations thought to be related to their ant-eating diets. Previous studies have examined morphological specialization in *Phrynosoma*, but they have not taken into account the phylogenetic relationships of its member species. In the present study, the morphological characteristics of the head, jaws and teeth that are thought to be important in prey capture and prey processing were examined to test whether variation in cranial morphology is associated with diet in lizards of the genus *Phrynosoma*. It is suggested that lizards of the genus *Phrynosoma* are indeed morphologically specialized and that ant-eating is associated with reduced dentition and an overall reduction in the robustness of morphological structures important in prey processing. Although this trend holds for the highly myrmecophagous species of *Phrynosoma*, a robust cranial morphology is apparent in the short-horned lizard clade (*Phrynosoma ditmarsi*, *Phrynosoma douglasii*, *Phrynosoma hernandesi*, *Phrynosoma orbiculare*), implying the ability to process a variety of dietary items. The present study suggests that additional feeding specializations exist within an already specialized clade (i.e. the short-horned lizard clade) and highlights the need for more detailed dietary and behavioural studies of feeding behaviour in this uniquely specialized group of lizards. © 2006 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2006, **89**, 13–24.

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INTRODUCTION

Lizards represent a speciose group of vertebrates, comprising over 4450 species that have radiated into a diversity of habitats (Zug, Vitt & Caldwell, 2001). Although lizards do fall into a limited number of broad categories with respect to food utilization (e.g. herbivores, omnivores and carnivores; Pough, 1973; Auffenberg, 1981; Shine, 1986; Cooper & Vitt, 2002; Herrel, Vanhooydonck & Van Damme, 2004), it is surprising that relatively few lizards appear to exhibit morphological specializations related to these dietary specializations (Greene, 1982; Schwenk, 2000). One group

that does appear to be morphologically specialized is the North American horned lizards of the genus *Phrynosoma* (Pianka & Parker, 1975; Montanucci, 1981; Powell & Russell, 1984). The 13 species of *Phrynosoma* (Hodges & Zamudio, 2004) are notable in appearance and possess a suite of distinct traits (including a round, spinous body and cryptic colouration) that have been suggested as adaptations to an ant-eating life-style (Pianka & Parker, 1975).

Although the traits described may be adaptations for these ant-eating lizards, they provide little information about the influence of ant eating on the morphology of the feeding apparatus (e.g. head, jaws, teeth, and tongue). Because the feeding system is responsible for acquisition and processing of prey, it is likely to be subjected to significant selective pressures. Consequently, morphological or behavioural special-

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*Corresponding author. Current address: Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA 70118, USA. E-mail: jmeyers1@tulane.edu

izations would be expected that ensure efficient utilization of ants as a food source. Alternatively, it could be predicted that ant-eating lizards will merely exhibit a loss of those morphological or behavioural traits related to the acquisition and processing of a wide variety of prey. Although not mutually exclusive, the two scenarios represent alternative strategies, one requiring adaptation and the other the loss of a generalized feeding morphology. Interestingly, both of these scenarios are apparent in many myrmecophagous mammals where, on the one hand, specialization of the tongue and hyobranchial system is seen and, on the other, a reduction in dentition, jaw adducting musculature and an associated reduction in the area of insertion for adductor musculature is observed (Reiss, 1997, 2000; Naples, 1999).

Although a reduction in the morphology of the feeding system may be adequate when feeding on ants, a reduced processing morphology might in turn affect the performance of the feeding system when attempting to feed on other prey types. Indeed, a review of the dietary ecology in *Phrynosoma* revealed that, among the 13 species, there is a gradation in the percentage of ants consumed (Pianka & Parker, 1975), with some species consuming large numbers of non-ant prey (Montanucci, 1981; Powell & Russell, 1984). The importance of non-ant prey in some *Phrynosoma* suggests that this group may exhibit significant diversity in the morphology of the feeding system.

Several lines of evidence hint that substantial morphological variation exists within *Phrynosoma*. Hotton (1956) already noted that *Phrynosoma* are differentiated from other iguanian lizards in having teeth which are blunt, less stout and lacking cusps. It was suggested that the reduced dental structure is symptomatic of a predominate diet of ants, which likely require minimal processing. In addition, other evidence suggests that the visible variation in head morphology in *Phrynosoma* may have important functional consequences regarding bite force. In a study examining the musculature of two species of *Phrynosoma*, distinct differences were noted in the relative proportions of the jaw adductor muscles (Jenkins & Tanner, 1968), implying potentially lower bite forces in the more myrmecophagous species. Bite force has important implications regarding dietary breadth, with poor biters potentially being restricted to a limited range of food items (Dumont, 1999; Herrel, De Grauw & Lemos-Espinal, 2001; Aguirre *et al.*, 2003). Because head shape serves as a good predictor of bite force in lizards (Herrel *et al.*, 2001), an examination of external morphometrics may provide insight into potential dietary constraints in *Phrynosoma*.

The relationship of diet and morphology in *Phrynosoma* was first addressed by Montanucci (1989), who found that several components of the skull and dentary are indeed correlated with the percentage of ants in the diet. However, the analysis was hampered by species variation in body size being corrected for by expressing all morphological variables as ratios, which may be problematic (Atchley, Gaskins & Anders, 1976). Additionally, the analysis did not take into account the phylogenetic relationships of the species of concern.

The present study measured the variation in morphology of the head and dentary of *Phrynosoma* and analysed the data in a strict phylogenetic context to test for any adaptive variation in cranial morphology that is associated with diet in the group.

MATERIAL AND METHODS

MORPHOLOGICAL MEASUREMENTS

To examine potential morphological patterns associated with ant-eating in *Phrynosoma*, morphological measurements were made on 604 specimens, representing all 13 species of horned lizards and two species used for outgroup analysis (*Uma notata* and *Callisaurus draconoides*). External morphological measurements were taken to the nearest 0.01 mm using digital calipers (Mitutoyo CD-15DC; Mitutoyo Ltd), including the following external measurements: snout-vent length (svl), measured from the snout tip to the vent; head length (hl), measured from the snout tip to the posterior edge of the parietal; head height (hh), measured at the highest point posterior to the orbit, excluding horns on the parietal and dentary; head width (hw), widest part of the skull excluding the horns; and mandible length (ml), measured from the posterior tip of the retro-articular process to the tip of the dentary (Fig. 1A).

Additionally, for a subset of animals (one to five individuals of each species; Table 1), the following characteristics of the dentary were measured: the length from posterior articular to the midline of the coronoid (ac), height of the dentary at the first (adh) and last (pdh) tooth, coronoid height (ch), length of the tooth row (ltr), number of teeth (tn) (Fig. 1B), and the height, width and angle of the four largest anterior (ath, atw, ata) and posterior teeth (pth, ptw, pta) (Fig. 1C). The largest anterior and posterior teeth were determined visually based on the height and width of each tooth. Mandibles were mounted in putty perpendicular to the camera to allow for a medial view of the dentary. A scale bar was placed next to the specimen and high resolution digital photographs were made using a Nikon Coolpix camera (type 4500). Images were then imported into Image J and the aforementioned angles and distances were measured

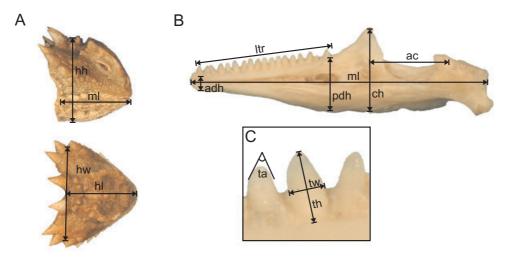The percentage of ants in the diet of the 13 species of *Phrynosoma* was obtained from a review of horned

Figure 1. Morphological variables measured. A, head height (hh), mandible length (ml), head width (hw), and head length (hl). B, characters measured on the dentary include length of the tooth row (ltr), length from posterior articular to the centre of the coronoid (ac), height of the dentary at first tooth (adh), height of dentary at the last tooth (pdh), coronoid height (ch), tooth angle (ta), tooth width (tw), and tooth height (th). Tooth measures represent the height, width, and angle of the four largest anterior (ath, atw, ata) and posterior (pth, ptw, pta) teeth of each individual.

lizard diets (Pianka & Parker, 1975). In accordance with Montanucci (1989), dietary data for *Phrynosoma boucardi* and *Phrynosoma orbiculare* were combined because these are considered as one species. Unfortunately, the diet of *Phrynosoma douglasii* is currently unknown because this species was only recently recognized as being separate from *Phrynosoma hernandesi* (Zamudio, Jones & Ward, 1997) and earlier dietary studies did not distinguish between them. Thus, although external morphological measurements were made for *P. douglasii*, this species was excluded from the analyses. Along with *Phrynosoma ditmarsi* and *P. orbiculare*, the aforementioned species belong to a subgroup referred to as the short-horned lizard clade. Because no data for *P. douglasii* are presented here, reference to this group throughout the text is with the exclusion of this species. Additionally, because the dietary data for *P. hernandesi* presented by Pianka & Parker (1972) likely combines data from both species, dietary data for *P. hernandesi* were taken from Montanucci (1981). Data for the percentage of ants in the diet of our outgroup species, *C. draconoides* and *U. notata*, were extracted from Pianka & Parker (1972) and Turner (1998), respectively.

STATISTICAL ANALYSES

Two sets of analyses were performed. The first used traditional statistical methods and the second comprised a phylogenetically informed analysis taking into account the relationships among the species. All analyses were performed on logarithmically-transformed species means (Table 1). Because neither percentage (% ants) nor count (number of teeth) data tended to meet assumptions of normality, the percentage of ants in the diet was arcsine-transformed and the number of teeth was square root-transformed before further analyses (Sokal & Rohlf, 1995). To remove the effects of body size in the analysis, residuals were calculated by regressing each variable against snout-vent length. A principal components analysis (PCA) was then performed on the residual data of all morphological variables to visualize shape differences between species. Because factor loadings are derived values calculated from variables measured in multiple dimensions, they fundamentally represent an estimate of differences in shape and can be used to compare global differences among the species. The broken stick method (Jackson, 1993) was used to determine which components explained a significant amount of the variation in morphological traits. The residual values of those variables with high component loadings were then used in subsequent regression analysis. Because the aim of the study was to understand morphological changes in relation to dietary specialization, linear regressions were performed on all morphological variables with the percentage of ants as the dependent variable. Bonferroni correction was used to adjust significance levels for multiple testing (Hochberg, 1988). To determine which morphological variables were the best predictors of diet, a stepwise multiple regression analysis was also performed using significant variables from the PCA

Table 1. Mean and standard deviation of all head, dentary and tooth measurements

	Uma notata	*Callisaurus draconoides*	*Phrynosoma asio*	*Phrynosoma braconnieri*	*Phrynosoma cornutum*	*Phrynosoma coronatum*	*Phrynosoma ditmarsi*
% ants	20	20	31	49	61	45	11
N	30	37	53	17	47	38	32
svl	61.35 ± 15.57	74.72 ± 9.81	92.78 ± 20.2	59.03 ± 10.7	76.31 ± 19.13	72.15 ± 17.86	62.13 ± 13.34
hl	13.78 ± 1.84	14.64 ± 1.35	20.11 ± 3.36	14.24 ± 1.53	15.77 ± 2.66	17.15 ± 2.96	16.05 ± 2.47
hh	7.62 ± 1.70	8.34 ± 1.02	15.27 ± 2.75	11.65 ± 1.26	13.35 ± 2.28	14.08 ± 2.98	14.69 ± 3.08
hw	11.25 ± 2.04	11.94 ± 1.19	21.51 ± 3.76	17.02 ± 2.32	18.74 ± 3.27	18.87 ± 3.98	20.91 ± 4.04
ml	14.69 ± 2.61	15.98 ± 1.58	20.12 ± 3.78	13.48 ± 1.54	15.18 ± 2.86	16.27 ± 3.47	16.79 ± 3.03
ac*	6.65 ± 0.64	4.83 ± 0.16	6.75 ± 1.72	4.58	6.86 ± 0.42	4.86 ± 0.30	7.34
adh*	0.54 ± 0.01	0.36 ± 0.04	0.83 ± 0.30	0.41	0.75 ± 0.13	0.59 ± 0.15	0.91
pdh*	2.56 ± 0.23	1.94 ± 0.09	3.28 ± 0.61	2.07	3.08 ± 0.17	2.17 ± 0.27	4.62
ch*	3.70 ± 0.53	2.71 ± 0.10	4.01 ± 0.99	1.66	3.16 ± 0.10	2.67 ± 0.30	4.70
ltr*	9.45 ± 1.01	7.47 ± 0.45	8.81 ± 1.66	5.77	8.25 ± 0.47	6.92 ± 1.05	8.91
tn*	19.33 ± 0.58	21.67 ± 1.53	19.50 ± 0.71	20.00	19.00 ± 1.00	20.00 ± 1.73	24.00
ath*	0.67 ± 0.04	0.72 ± 0.10	0.66 ± 0.09	0.61 ± 0.01	0.56 ± 0.09	0.55 ± 0.09	0.97 ± 0.79
atw*	0.28 ± 0.03	0.22 ± 0.01	0.23 ± 0.02	0.26 ± 0.05	0.25 ± 0.05	0.29 ± 0.08	0.29 ± 0.08
ata*	94.61 ± 5.16	49.78 ± 16.81	57.19 ± 12.47	55.47 ± 10.23	70.68 ± 16.55	70.75 ± 16.47	72.53 ± 15.58
pth*	0.74 ± 0.05	0.82 ± 0.10	0.75 ± 0.12	0.72 ± 0.07	0.65 ± 0.15	0.65 ± 0.15	1.03 ± 0.79
ptw*	0.28 ± 0.04	0.23 ± 0.01	0.24 ± 0.02	0.28 ± 0.06	0.27 ± 0.07	0.32 ± 0.10	0.31 ± 0.09
pta*	100.77 ± 6.87	59.17 ± 16.07	65.15 ± 10.36	64.26 ± 8.90	74.13 ± 11.55	72.85 ± 13.48	74.25 ± 13.85

*Variables were measured on the dentaries of one to five individuals of each species. Percent ants represent mean values taken from the literature.

N, sample size; svl, snout vent length; hl, head length; hh, head height; hw, head width; ml, mandible length; ac, articular-coronoid; adh, anterior dentary height; pdh, posterior dentary height; ch, coronoid height; tn, tooth number; ath, atw, ata, pth, ptw, pta, anterior and posterior tooth height, width and angle. Tooth measurements represent the mean ± standard deviation of the four largest anterior and posterior teeth.

with the percentage of ants as the dependent variable and the morphological traits as independent variables.

Because closely-related species share a part of their evolutionary history, they cannot be considered as independent data points (Felsenstein, 1985). Therefore, to take into account species relatedness, independent contrasts of the morphological data were calculated using three separate phylogenies. Although the phylogenetic relationships of horned lizards have received extensive attention (Reeve, 1952; Presch, 1969; Montanucci, 1987; Reeder & Montanucci, 2001; Hodges & Zamudio, 2004), only phylogenies from the two most recent studies were used here. These phylogenies are reasonably well resolved, but they differ in the position of the short-horned lizard group, as basal or derived in the two studies (Fig. 2). Reeder & Montanucci (2001) did not provide estimates of divergence times so branch lengths were set equal to one (Diaz-Uriarte & Garland, 1998). Phylogenetic analyses with and without outgroup clades were performed. Because it is reasonable to assume that divergence at the generic level occurs over greater time periods then that of species, the nodes contrasting generic groups (i.e. the sand lizards *Uma* vs. *Callisaurus*, and the sand lizards vs. *Phrynosoma*) were set to two (Van Damme & Vanhooydonck, 2001). Branch length estimates for the *Phrynosoma* phylogenies (no outgroup) of Hodges & Zamudio (2004) were available (Hodges, 2004) and these estimates were used when the outgroup clade was not included in the analyses.

All data used in the phylogenetic analyses comprise log-transformed species means, or the alternative transformation for percentage and count data (see above). Morphological variables were input into the PDTREE program (Garland, Harvey & Ives, 1992), allowing phylogenetic independent contrasts to be calculated for each variable. Inspection of the diagnostics (Garland, Midford & Ives, 1999) in the PDTREE program allowed verification that branch lengths were adequate for the analyses. The standardized contrasts of morphological variables were then regressed against the contrast of snout-vent length (forced through the origin; Garland *et al.*, 1992) and the residuals calculated. To determine which morphological variables were correlated with the percentage of ants in the diet, linear regressions were performed using the residual contrasts of the morphological variables

Table 1. *Continued*

	Phrynosoma hernandesi	*Phrynosoma mcallii*	*Phrynosoma modestum*	*Phrynosoma orbiculare*	*Phrynosoma platyrhinos*	*Phrynosoma solare*	*Phrynosoma taurus*
% ants	41	78	66	41	56	89	56
N	26	83	43	30	74	65	29
svl	69.19 ± 14.62	67.20 ± 8.26	52.15 ± 7.49	68.39 ± 13.73	70.16 ± 8.74	79.40 ± 16.77	69.45 ± 9.51
hl	15.78 ± 2.29	14.99 ± 1.20	12.21 ± 1.31	16.90 ± 2.13	15.26 ± 1.20	19.81 ± 3.64	15.37 ± 1.39
hh	12.39 ± 1.94	10.28 ± 1.00	8.63 ± 1.30	14.12 ± 2.21	11.28 ± 1.11	13.89 ± 2.25	12.53 ± 1.31
hw	20.59 ± 3.71	16.00 ± 1.53	13.16 ± 2.72	20.00 ± 3.19	16.36 ± 2.06	20.72 ± 3.82	21.50 ± 2.59
ml	17.44 ± 3.74	12.12 ± 1.42	11.51 ± 1.48	16.72 ± 2.60	14.36 ± 1.45	15.29 ± 2.69	15.39 ± 1.68
ac*	5.24 ± 0.62	3.55 ± 0.08	3.55 ± 0.30	5.98 ± 0.49	4.47 ± 0.20	5.57 ± 0.28	6.03
adh*	0.52 ± 0.07	0.64 ± 0.13	0.48 ± 0.05	0.54 ± 0.05	0.67 ± 0.01	0.58 ± 0.05	0.58
pdh*	2.59 ± 0.62	1.52 ± 0.14	1.19 ± 0.11	2.90 ± 0.23	1.83 ± 0.16	1.85 ± 0.10	2.62
ch*	3.31 ± 0.45	1.64 ± 0.14	1.54 ± 0.10	3.23 ± 0.08	2.09 ± 0.33	2.32 ± 0.09	3.08
ltr*	7.88 ± 1.18	5.29 ± 0.19	4.96 ± 0.71	8.18 ± 0.59	6.33 ± 0.34	7.46 ± 0.39	7.83
tn*	16.33 ± 1.53	14.67 ± 2.08	18.00 ± 2.65	19.00 ± 1.00	17.00 ± 1.41	16.00 ± 2.00	19.00
ath*	1.09 ± 0.70	1.14 ± 0.65	0.75 ± 0.04	0.75 ± 0.04	0.75 ± 0.04	0.76 ± 0.02	0.72 ± 0.05
atw*	0.34 ± 0.03	0.31 ± 0.04	0.30 ± 0.04	0.27 ± 0.02	0.28 ± 0.02	0.28 ± 0.02	0.30 ± 0.01
ata*	71.76 ± 14.31	84.50 ± 14.42	81.58 ± 19.23	74.34 ± 20.09	67.48 ± 8.48	71.95 ± 5.78	83.21 ± 14.06
pth*	1.12 ± 0.71	1.17 ± 0.66	0.79 ± 0.02	0.82 ± 0.03	0.83 ± 0.03	0.85 ± 0.00	0.83 ± 0.04
ptw*	0.36 ± 0.04	0.32 ± 0.03	0.28 ± 0.06	0.26 ± 0.03	0.28 ± 0.05	0.31 ± 0.02	0.32 ± 0.01
pta*	73.63 ± 13.09	87.08 ± 10.97	82.27 ± 17.91	76.89 ± 19.51	72.47 ± 11.94	78.92 ± 9.76	92.01 ± 13.13

and the percentage of ants as the dependent variable. A stepwise regression of the residual contrasts of all the variables vs. the percentage of ants in the diet (again forced through the origin) allowed determination of the variables that were the best predictors of dietary specialization in *Phrynosoma*. Because the main study goal was to address the evolution of ant-eating within *Phrynosoma*, the results of these analyses were performed without the outgroup species. However, because the morphological shift from non-ant-specialized to ant-specialized species might also be informative, the analyses were also conducted using two members from the sand lizard group, *U. notata* and *C. draconoides*, as the outgroup clade (Reeder & Wiens, 1996). Because the phylogenetic analyses differed little with the inclusion of the outgroup clade, these findings are discussed only briefly in the results.

RESULTS

A PCA resulted in four axes explaining morphological variation, with only the first two axes being significant using the broken stick method (Jackson, 1993). All of the morphological variables exhibited high loadings on one or the other of the two axes (Table 2). A plot of the PCA scores distinguishes *Phrynosoma* from both outgroup species (*C. draconoides* and *U. notata*) and also suggests a division within *Phrynosoma* between the short-horned lizard clade and the other species of *Phrynosoma* (Fig. 3). A multiple analysis of variance of the first two principal components indicates signifi-

Table 2. Eigenvalues, percent variation explained and factor loadings of the morphological variables

	Factor 1	Factor 2
Eigenvalue	6.191	3.751
Percent variation explained	38.694	23.447
Head length	−0.212	0.725*
Head height	0.199	0.877*
Head width	−0.075	0.858*
Mandible length	0.742*	−0.028
Area adductor insertion	0.834*	0.213
Anterior dentary height	0.157	0.635*
Posterior dentary height	0.892*	0.308
Coronoid height	0.915*	0.050
Length tooth row	0.866*	−0.009
Tooth number	0.474*	−0.259
Anterior tooth height	0.735*	−0.266
Anterior tooth width	0.701*	0.112
Anterior tooth angle	−0.162	0.704*
Posterior tooth height	0.779*	−0.301
Posterior tooth width	0.746*	0.120
Posterior tooth angle	−0.040	0.649*

Only the first two factors where significant using the broken stick method. Asterisks denote variables with high factor loadings that were used in further analyses.

cant differences between these groups (Wilks's lambda $F = 17.14$, $P < 0.0001$). A Bonferroni post-hoc test revealed morphological differences between the short-horned lizard clade and other *Phrynosoma* in the first
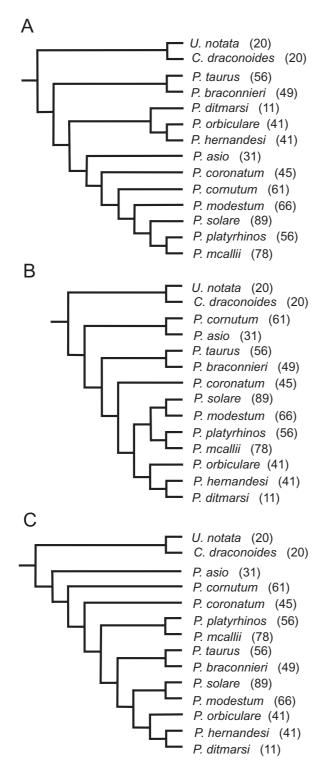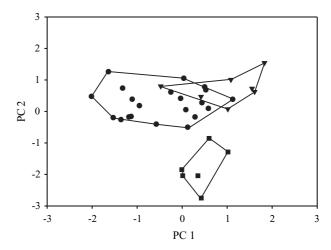
Figure 3. Plot of the two significant principal components from a principal components analysis of all morphological variables. Note that *Phrynosoma* are distinctly different from the outgroup species and that, within *Phrynosoma*, the short-horned lizard clade (*Phrynosoma ditmarsi*, *Phrynosoma hernandesi*, *Phrynosoma orbiculare*) appears to show a divergent morphology. ●, *Phrynosoma*, excluding short-horned lizards; ▼, short-horned lizards; ■, outgroup species (*Callisaurus draconoides* and *Uma notata*)

Figure 2. Independent contrasts of the morphological variables were calculated using three current phylogenies. A, most parsimonious phylogeny of Reeder & Montanucci (2001). B, first parsimonious phylogeny of Hodges & Zamudio (2004: 967). C, the equally parsimonious second phylogeny of Hodges & Zamudio (2004: 967). The percentage of ants in the diet is noted in parentheses.

principal component ($P < 0.001$). Although the short-horned lizard clade is not significantly different from the outgroup on PC1 ($P = 0.580$), other *Phrynosoma* appear to differ more substantially ($P = 0.097$). All *Phrynosoma* differ significantly from the outgroup species on PC2 ($P < 0.0001$).

The results of regression analyses of the percentage of ants against morphological variables revealed that several variables are highly correlated with an ant eating diet. These include head height, mandible length, distance between the articular and coronoid, posterior dentary height, coronoid height, length of the tooth row, and tooth number (Table 3). All significant variables were negatively correlated with an increasing percentage of ants in the diet, suggesting a general reduction of relative skull size and processing morphology in the more myrmecophagous species. The results of the stepwise multiple regression ($R = 0.930$, $F_{2,9} = 28.94$, $P < 0.001$) indicated a model with mandible length and anterior dentary height being retained. If mandible length is removed from the analysis, then another model is retained with posterior dentary height as the only predictor ($R = 0.839$, $F_{1,10} = 23.74$, $P = 0.001$). All three of these variables were negatively correlated with the percentage of ants in the diet, indicating that the mandible is becoming shorter and more slender.

Regression analysis of the residual contrasts of the morphological data using the phylogeny of Reeder & Montanucci (2001) revealed several variables that

Table 3. Results of linear regression analysis of morphological variables regressed against the percentage of ants in the diet

Variable	Non-phylogenetic analyses			Phylogeny of Reeder & Montanucci (2001)			Phylogeny 1 of Hodges & Zamudio (2004)			Phylogeny 2 of Hodges & Zamudio (2004)		
	Slope	R	P	Slope	R	P	Slope	R	P	Slope	R	P
Head length	-112.934	0.230	0.472	-5.866	0.017	0.959	2.701	0.046	0.887	10.729	0.234	0.465
Head height	-175.807*	0.687*	0.014*	-85.242	0.368	0.239	-157.926*	0.625*	0.030*	-145.292*	0.642*	0.024*
Head width	-144.201	0.538	0.071	-15.057	0.051	0.874	-81.222	0.254	0.425	-139.027	0.447	0.145
Mandible length	-239.710*	0.872*	< 0.001*	-256.625*	0.734*	0.007*	-248.686*	0.728*	0.007*	-269.265*	0.857*	< 0.001*
Area adductor insertion	-97.435*	0.657*	0.020*	-54.880	0.414	0.181	-85.122	0.572	0.052	-82.377*	0.591*	0.043*
Anterior dentary height	-74.236	0.476	0.118	-72.552*	0.648*	0.023*	-74.146*	0.598*	0.040*	-55.264	0.454	0.138
Posterior dentary height	-74.798*	0.839*	0.001*	-59.123*	0.658*	0.020*	-75.313*	0.745*	0.005*	-68.099*	0.753*	0.005*
Coronoid height	-79.334*	0.823*	0.001*	-59.006*	0.612*	0.034*	-75.580*	0.689*	0.013*	-75.567*	-0.738*	0.006*
Length tooth row	-136.959*	0.704*	0.011*	-81.515	0.418	0.176	-119.854	0.547	0.066	-133.134*	0.637*	0.026*
Tooth number	-38.348*	0.806*	< 0.002*	-31.233*	0.741*	0.006*	-16.168*	0.695*	0.012*	-31.751*	0.789*	0.002*
Anterior tooth height	-1.669	0.013	0.969	7.630	0.072	0.823	-1.054	0.012	0.970	32.825	0.254	0.425
Anterior tooth width	52.422	0.153	0.635	57.199	0.259	0.416	151.929	0.519	0.084	109.736	0.393	0.206
Anterior tooth angle	93.948	0.382	0.220	46.640	0.273	0.390	63.380	0.337	0.285	65.730	0.364	0.245
Posterior tooth height	-2.388	0.015	0.964	10.132	0.078	0.809	16.697	0.299	0.771	39.909	0.250	0.434
Posterior tooth width	34.306	0.117	0.716	44.013	0.245	0.443	-10.609*	0.636*	0.026*	88.850	0.364	0.245
Posterior tooth angle	145.018	0.487	0.108	73.423	0.399	0.199	82.260	0.367	0.241	81.350	0.367	0.240

*Highly correlated with ant-eating. No outgroup was used in these analyses. Phylogenies for Hodges & Zamudio (2004) included branch lengths extracted from Hodges (2004).

were significantly correlated with the percentage of ants in the diet. These included mandible length, anterior and posterior dentary heights, coronoid height, and the number of teeth (Table 3). All the significant variables were negatively correlated with the percentage of ants in the diet, suggesting a general reduction in overall robustness of the feeding apparatus in *Phrynosoma*. To determine which of these variables best predicts a myrmecophagous diet, a multiple stepwise regression was performed with the contrasts calculated using the phylogeny of Reeder & Montanucci (2001) (Figs 2A, 4). A single model was retained with tooth number as the only predictor ($R = 0.741$, $F_{1,10} = 12.185$, $P = 0.006$). If tooth number is removed, another model containing mandible length, anterior dentary height, and head width is created ($R = 0.741$, $F_{3,8} = 17.66$, $P < 0.006$).

When the morphological variables are analysed using the first phylogeny (Fig. 2B) of Hodges & Zamudio (2004), both head height and posterior tooth width are correlated with ant eating (Table 3), in addition to the variables that were significant in the previous analyses (mandible length, anterior and posterior dentary heights, coronoid height, and the number of teeth). Stepwise regression revealed a model that retained only posterior dentary height ($R = 0.745$, $F_{1,10} = 12.469$, $P = 0.005$). If posterior dentary height is removed, then a model containing mandible length, anterior dentary height, and coronoid height is produced ($R = 0.940$, $F_{3,8} = 20.06$, $P < 0.001$). All four of these variables were negatively correlated with the percentage of ants in the diet and indicate a mandible that is getting shorter, thinner anteriorly, and has a less developed coronoid process (for general shape differences, see Fig. 4).

The results of regression analyses on the second phylogeny proposed by Hodges & Zamudio (2004) (Fig. 2C) differed only slightly from the two previous phylogenetic analyses. Again, head height, mandible length, posterior dentary height, coronoid height, and tooth number all showed significant correlations with diet. As in the nonphylogenetic analysis, the distance between the articular and coronoid and the length of the tooth row were also significantly correlated (Fig. 5). Stepwise regression revealed two variables that were retained in previous models, both mandible length and posterior dentary height ($R = 0.930$, $F_{2,9} = 28.78$, $P < 0.001$). If mandible length is removed from the analysis, then another model containing tooth number and head length is retained ($R = 0.884$, $F_{2,9} = 16.08$, $P < 0.001$).

DISCUSSION

It has been suggested that lizards exhibit little or no phenotypic specialization in relation to diet (Greene,

1982; Schwenk, 2000). However, the results of the present study suggest that, at least within the ant-eating genus *Phrynosoma*, there is a strong evolutionary trend toward reduction in the development of the feeding system as the percentage of ants in the diet increases. Although phylogenetic analyses tended to be more conservative than traditional analyses, it was found that a consistent set of characteristic variables were significantly correlated with ant eating irrespective of the analysis used. A total of four variables (ml, pdh, ch, tn) exhibiting significant correlations with ants in the diet were common to all analyses. Additionally, several variables were common to at least two analyses (hh, adh, ltr, ac; Table 3). Myrmecophagy in *Phrynosoma* thus appears to be associated with shortening of the length of the mandible (ml, ltr), a reduction in the total number of teeth (tn), an increasingly slender mandible (as demonstrated by reduced adh and pdh), a decrease in head height, and a reduction in the area of insertion of the jaw adductor musculature (ac, ch).

In lizards in general, relatively few studies have examined the relationship between dentition and diet. Most studies have focused broadly on dietary groups and have noted some differentiation from the general insectivorous pattern in omnivores, herbivores, myrmecophages, and carnivores (Hotton, 1955; Montanucci, 1968, 1989; Shine, 1986; Herrel *et al*., 2004; for a review, see Schwenk, 2000). The present study demonstrated that, within the specialized clade of ant-eating lizards, there is a distinct trend toward a reduction in the number of teeth with an increasing percentage of ants in the diet. Tooth number decreases from a high of 24 in the *P. ditmarsi* to as few as 16 in the highly myrmecophagous *Phrynosoma solare* (Table 1). Hotton (1956), who made the most comprehensive attempt to address the role of diet in shaping dentition, found that among iguanian lizards, *Phrynosoma* are differentiated in having teeth which are blunt, less stout, and lack cusps. It was suggested that because ants are characterized by small size and an integument of intermediate hardness, that they can be consumed whole and require little or no processing using the teeth.

Teeth in lizards are thought to primarily serve in grasping, cropping, restraining, and puncturing of a food item (Schwenk, 2000). Unlike mammals, lizards do not masticate and prey processing relies on simpler processing and reduction movements. If ant-eating lizards can immobilize and digest ant prey without first reducing them mechanically, then even these simple processing movements may not be utilized and the loss of associated processing morphology may indeed be predictive of a myrmecophagous diet. Although difficult to test empirically, this explanation is supported by convergence of reduced (or even lost) dentition in diverse mammalian taxa. Many mammalian myrmecophages tend to show a range of tooth development, from peg-like homodont dentition to the complete loss of all dentary and maxillary teeth (Naples, 1999; Reiss, 2000; Sacco & Van Valkenburgh, 2004). Character loss does not necessarily imply any functional advantage, yet the repeated loss of dentition in myrmecophagous mammals, and in *Phrynosoma* in the present study, suggests that tooth reduction may be a response to disuse related to the lack of prey-processing behaviours.

In addition to loss of dentition, the significant regression analyses included many characters that allude to possible differences in bite force. Because the ability to bite harder can influence processing abilities, it may have important implications regarding dietary breadth (Dumont, 1999; Herrel *et al*., 2001; Aguirre *et al*., 2003). Head shape has often been used to estimate differences in the robustness of the head and skull and is often thought to be related to bite force potential. Indeed, several recent studies have found that both head width and head height are good predictors of bite force in lizards (Herrel *et al*., 1999, 2001, 2004; Lappin, 1999). Taller and wider heads likely provide a greater volume for the jaw adductor muscles that are used to generate bite forces. Interestingly, there was a trend in the present study toward a reduction in head height, suggesting lower bite forces in the more myrmecophagous species. Reduced bite force is also supported by two more direct measures of jaw adductor size, the height of the coronoid process (ch) and area between the coronoid process and the articular (ac). Both ch and ac estimate the area of insertion for the jaw adducting musculature and hence a reduction of these areas suggests reduced jaw musculature with increasing myrmecophagy (Fig. 5).

Is bite force functionally important to ant eating animals? The repeated loss of processing morphology in ant-eating animals suggests that ants may generally be viewed as food items requiring minimal mechanical reduction. Meyers & Herrel (2005) found that, in contrast to the dietary generalist *U. notata*, the specialized *Phrynosoma platyrhinos* does not process ant prey upon ingestion, but instead swallows ants whole. These findings imply that neither high bite forces nor well-developed dentition are necessary to process ant prey in lizards of the genus *Phrynosoma*. Yet, as prey are generally more easily digested when mechanically reduced prior to swallowing (Prinz *et al*., 2003), it is likely that horned lizards exhibit other specializations that allow them to successfully immobilize (Schwenk & Sherbrooke, 2003; Sherbrooke, 2003) and digest ant prey.

It would appear that bite force is of little functional relevance when feeding on ants. However, detailed dietary information of *Phrynosoma* lizards reveals a
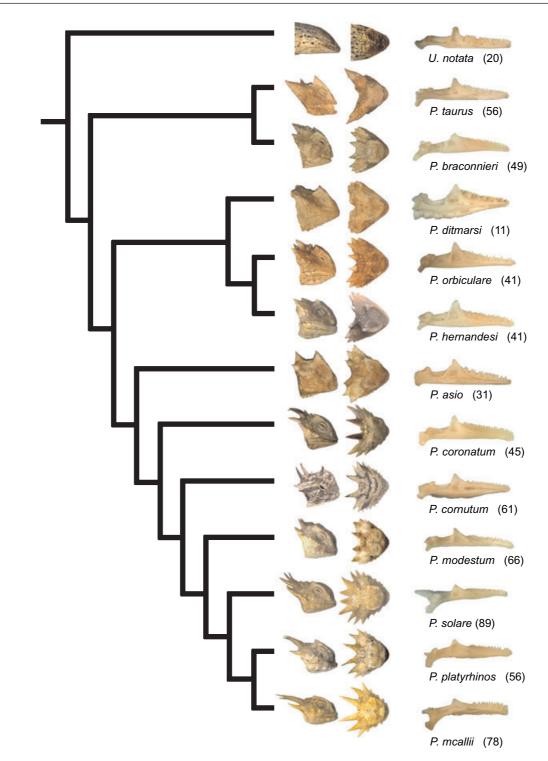
Figure 4. Phylogeny (Reeder & Montanucci, 2001) of *Phrynosoma* and an outgroup species *Uma notata*. Lateral and dorsal views of the head and a medial view of the dentary are plotted onto the phylogeny, displaying the morphological variation among the different species. Species means of the percentage of ants in the diet are noted in parentheses. The heads of *Phrynosoma* exhibit notable shape differences, with the short-horned lizard clade (*Phrynosoma ditmarsi*, *Phrynosoma orbiculare*, *Phrynosoma hernandesi*) exhibiting stout heads and jaws with a reduction of spines on the skull. By contrast, the highly myrmecophagous species are characterized by more slender jaws and well-developed occipital spines (*Phrynosoma solare* and *Phrynosoma mcallii*). Dentaries appear to vary in overall robustness, but also with respect to coronoid height and development of the retro-articular process.
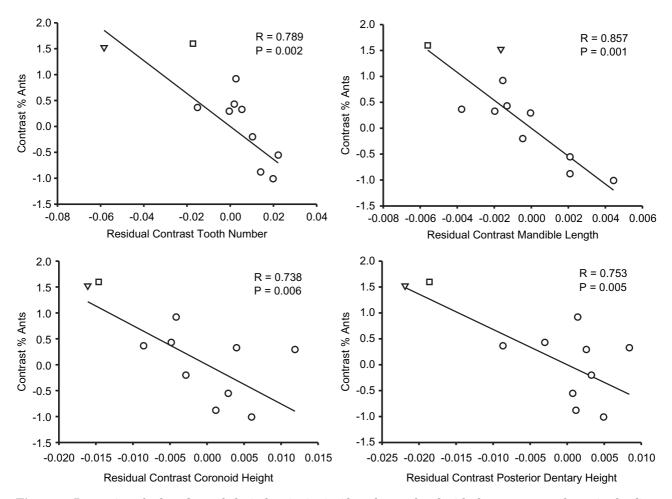
Figure 5. Regression of selected morphological traits is significantly correlated with the percentage of ants in the diet. All variables represent residual contrasts calculated using the second phylogeny of Hodges & Zamudio (2004). ▽, contrast between *Phrynosoma ditmarsi* and *Phrynosoma hernandesi*; □, contrast between the short-horned lizard clade (*P. ditmarsi*, *P. hernandesi*, *Phrynosoma orbiculare*) and *Phrynosoma solare* and *Phrynosoma modestum*; ○, the contrasts between other clades reveal a reduction in feeding morphology with increasing ants in the diet, however, in the short-horned lizard clade, a robust cranial morphology is present.

significant percentage of non-ant prey in the diet. Alternative dietary items consist of a wide range of insects and, in some species, may be dominated by hard prey items such as beetles (Pianka & Parker, 1975; Montanucci, 1981, 1989; Powell & Russell, 1984; J. J. Meyers pers. observ.). Because prey can differ drastically in speed, size, palatability, and hardness, it is clear that the functional significance of this dietary variation cannot be disregarded. For an array of food items to be utilized, the morphological structures and behavioural repertoire necessary to capture, process, and digest diverse prey types must be present. The inability to immobilize prey before swallowing can have serious consequences. The diet of many species contains ants that can inflict a painful sting and some prey may be able to inflict fatal wounds on their horned lizard predators (Sherbrooke, 2002)

Interestingly, although there is a trend toward reduced processing morphology with increasing ant-eating in *Phrynosoma*, it appears that some species exhibit the opposite trend. Notably, *P. ditmarsi* has a very robust skull with the absolute largest coronoid process and area for jaw adductor muscle insertion (Table 1). This robustness is surprising considering that *P. ditmarsi* is one of the smaller species of *Phrynosoma*. Montanucci (1989) also noted the hypertrophy of these skeletal elements and suggested that this species is built for crushing prey. He supported this contention with observations of extensive prey processing in *P. ditmarsi* when feeding on non-ant prey. Observations of *P. hernandesi* feeding on beetles also revealed extensive prey-crushing behaviour (J. J. Meyers pers. observ.). The findings of the present study suggest that, in addition to *P. ditmarsi*, the

other members of short-horned lizard clade (*P. hernandesi* and *P. orbiculare*) also exhibit a more robust processing morphology.

The differences in the short-horned lizard clade are apparent when examining the results of the PCA of morphological traits, which displayed high factor loadings for variables describing characteristics of the dentary (Table 2). The first factor, which indicates the development of the prey-processing machinery (teeth, dentary size, jaw musculature insertion), appears to separate the short-horned clade from other *Phrynosoma* and places the generalist species as morphologically intermediate between the two groups (Fig. 3). These divergent morphologies within *Phrynosoma* suggest that, as far as processing machinery is concerned, morphological divergence occurs in two trajectories with respect to dietary generalists. Although the majority of *Phrynosoma* appear to exhibit a loss or reduction in the development of the mandible, some members of the short-horned lizard clade display morphologies that are even more robust than that of dietary generalists. The regression analyses of the morphological variable vs. the percentage of ants in the diet strongly suggest that the development of a robust morphology is associated with reduced ant-eating behaviour. Thus, it appears that, within this specialized clade of ant-eating lizards, there are divergent forms displaying alternative feeding strategies: one group appears to be specialized on eating ants and another also appears to be adapted to eating harder prey.

The findings of the present study demonstrate that the myrmecophagous diet of horned lizards has resulted in significant morphological adaptive modification of the feeding system. The phenotypic variation present among *Phrynosoma* species suggests that some species may be better equipped to capture and process a diversity of prey; however, is this at the expense of being less adept at capturing ant prey? It is expected that the feeding performance of ant-eating *Phrynosoma* will likely differ from the short-horned lizard clade; otherwise, the robust morphology might be considered as the optimal feeding morphology to allow these species to expand their dietary niche. Clearly, the present study points to the need for a more detailed analysis of diet in all the species to determine not only the type of prey consumed, but also their functional properties. In addition, comparative studies of feeding behaviour are needed to determine the extent to which the different species of *Phrynosoma* are adapted to an ant-eating diet.

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