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ABSTRACT: Testudinid turtles, comprising a single family with a fairly uniform shell morphology but a wide range of body sizes, present a unique opportunity for testing hypotheses regarding the evolution of shell size, shape, and functional performance. Here, we apply geometric morphometric methods and finite element models to test two hypotheses regarding the coevolution of size, shape, and mechanical performance (shell strength). First, we predicted that larger turtles will tend to have shell shapes distinct from those of smaller turtles. Second, we predicted that the shell shapes (independent of size) of smaller turtles will be stronger than those of larger turtles to compensate for the reduction in strength that accompanies a decrease in size. We used multivariate phylogenetic comparative methods to assess the significance of the relationship between shell size and shell shape, and used finite element analysis to assess the mechanical behavior of tortoise shells. We detected a relationship between shell shape and size—the shells of larger tortoise species had larger carapaces relative to plastrons, larger vertebral and pleural scutes relative to marginal scutes, and more vertically oriented marginal scutes. We did not detect a relationship between size-independent shell strength and size, however, and the direction of the trend was the opposite of what we predicted (larger tortoises tended to possess stronger shells than smaller tortoises). This is consistent with larger tortoise species having more highly domed carapaces than smaller species. Future studies should focus on the effect that additional functional factors (such as righting ability or heat exchange), and their interactions, might have on the selective pressures that affect the evolutionary path of tortoises.

Key words: Finite element analysis; Functional morphology; Mechanical stress; Testudinidae; Turtle shell

THE TORTOISES (Family Testudinidae) together comprise one of the most recognizable groups of turtles on the planet. Many factors contribute to the familiarity of tortoises among researchers and the public. The 60 living species of tortoise are distributed widely, being found on all turtle-occupied continents save Australia, as well as on numerous continental and oceanic islands. Tortoises show an impressive latitudinal range for terrestrial ectotherms, extending from the southern cone of South America and southernmost Africa, to southern Russia and central Kazakhstan. And throughout their range, tortoises are found in a variety of terrestrial environments, from coastal lowlands to deserts to tropical rainforests (Ernst and Barbour 1989; Bonin et al. 2006; Turtle Taxonomy Working Group 2014). Tortoises are well-known and easily recognized by their tall, rounded carapace, elephantine limbs, and herbivorous diets. Particularly well-known are certain oceanic island tortoises—primarily the Aldabra Giant Tortoises (*Aldabrachelys gigantea*) and the Galapagos Giant Tortoises (*Chelonoidis nigra* species complex)—whose massive size and long life span readily captures public attention.

Taken together, the uniform terrestrial habitats, herbivorous diets, and fairly conserved morphology of tortoises might suggest a lack of divergent evolution within the clade. Nevertheless, tortoises illustrate exceptional variation (for turtles) along one biological axis: body size. Although they are not the largest turtles (the sea turtles in Chelonioidea are larger), tortoises span the largest range of body sizes among living turtle families. Within the diminutive South African genera *Homopus* and *Psammobates,* the Speckled Padloper (*Homopus signatus*) is likely the smallest living turtle. Females of this species only grow to 100 mm in carapace length (CL); males are not much larger (Bonin et al. 2006). In contrast, the island giant species are the largest terrestrial turtles, with CLs exceeding 1000 mm and masses >400 kg in captivity or 300 kg in nature. Even outside of those unique environments, mainland tortoises can grow quite large as well—the continental *Centrochelys sulcata* can grow up to 1000 mm in CL and weigh up to 98 kg, and some extinct species were even larger (Falconer and Cautley 1844).

Within a single family having a fairly uniform shell morphology, the existence of such a wide range of body sizes presents a unique opportunity for testing hypotheses regarding size, shape, and functional performance. The turtle shell has been a consistently suitable model for studies in evolutionary morphology—the rigidity of the structure facilitates repeatable morphometric measurements, and the various scutes and bones of the shell provide a large number of potential measurements or landmarks, permitting detailed characterization of shell shape (e.g., Claude et al. 2003; Stayton 2011). In addition, a relationship between shell shape and functional performance has been demonstrated for numerous shell functions: mechanical strength (Stayton 2009), hydrodynamic efficiency (Rivera 2008), heat transfer (Boyer 1965), and stability when overturned (Domokos and Várkonyi 2008). The shell is simple enough that modelling functional performance is feasible, but sufficiently complex to permit many-to-one mapping of form onto function, where multiple different shell shapes can show the same level of functional performance. Tortoise shells are also remarkably uniform, relative to other turtle groups. Only one genus (*Kinixys*) shows any pattern of kinesis of the carapace. The only unusual taxon is *Malacochersus tornieri,* which has evolved a remarkably flat and flexible shell to better maneuver and hide in rock piles in its East African habitat (Ireland and Gans 1971). In short, turtle shells are generally ideal systems for functional evolutionary investigations and, specifically, the shell of testudinids is ideal for understanding the coordinated evolution of size, shape, and functional performance.

Even with these advantages, relatively few studies have used tortoises, or other groups of turtles, to test hypotheses

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regarding functional evolutionary morphology (but, see Stayton 2011). Tortoise shells have been the subject of several morphometric studies (e.g., Germano 1992; Golubović et al. 2015), including those that utilize geometric morphometrics (Sacchi et al. 2007; Chiari et al. 2009; Chiari and Claude 2011; Macale et al. 2011). A few studies have even investigated the relationship between shape and righting performance in tortoises (e.g., Golubović et al. 2015), but connections between shell shape and shell strength have yet to be established in this group.

Here, we utilized the shells of tortoises to test two hypotheses regarding the coevolution of size, shape, and mechanical performance (shell strength). First, we investigated the relationship between size and size-independent shell strength (that is, the strength of shells that might differ in shape but which are scaled to the same size). All else being equal, the shells of larger turtles should be stronger than those of smaller turtles—they have larger cross-sectional areas and, consequently, a greater amount of material available to resist deformation and failure. Given these advantages, our second hypothesis was that larger turtles might be under less selective pressure to evolve particularly strong shell shapes. Instead, larger turtles might have optimized their shell shapes for other functions: heat exchange, or for ease of righting if overturned (Domokos and Várkonyi 2008). Conversely, smaller turtles might be under selective pressure to evolve stronger shell shapes, to compensate for the reduction in strength that accompanies an evolutionary decrease in size. Thus, we made two predictions: (1) that larger turtles tend to have shell shapes distinct from those of smaller turtles (i.e., there is interspecific allometry for tortoise shell shape); and, (2) that the shell shapes of smaller turtles are stronger than those of larger turtles (i.e., there is a negative relationship between actual shell size and size-independent shell strength). We tested the morphological hypothesis using geometric morphometric data and evolutionarily informed comparative methods. We then applied those same methods to a mechanical data set consisting of the results of finite element (FE) models of tortoise shells. Additionally, we described the quantitative patterns of shape variation observed among tortoise shells.

MATERIALS AND METHODS

Data Acquisition

We examined 448 specimens of 44 different testudinid species (92% of all extant species, assuming that the Galapagos Giant Tortoises constitute a single species; see File S1 in the Supplemental Material for a list of specimens and associated data). All tortoise genera were represented; the missing taxa included *Homopus solus,* and *Kinixys zombensis*. Data consisted of the three-dimensional coordinates of 53 landmarks, primarily located at scute triple junctions across all regions of the shell (Fig. 1). Most data were collected from the left side of the shell (unless that side was damaged) using a MicroScribe digitizer. However, some specimens were too large to be digitized using the Micro-Scribe; in those cases, morphological data were collected from photographs of museum specimens. Each specimen was photographed in dorsal, lateral, and ventral views. The camera was oriented perpendicularly to each specimen
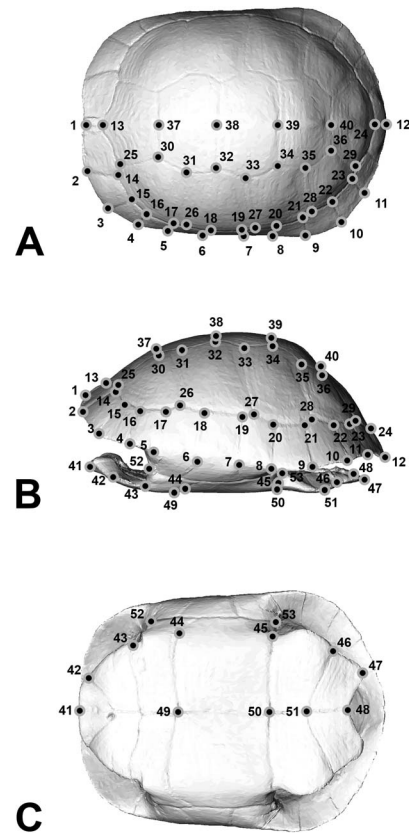

Fig. 1.—The 53 landmarks used to quantify shape variation among tortoises. The images are of a finite element model of *Glyptemys muhlenbergii* warped to match the average shape of *Testudo graeca*. Anterior is to the left. (A) Dorsal view. (B) Lateral view. (C) Ventral view.

during image capture. A piece of graph paper was used to check for distortion; this was found to be minimal in all views. The photographs were digitized using tpsDig v1.40 (Rohlf 2004), and the two-dimensional coordinates from all three views were combined into a single three-dimensional set using a custom MATLAB script (Stayton 2011). A maximum of five adult female and five adult males were digitized for each species. Whenever possible, wild-caught specimens were utilized; the captive specimens that were used in this study did not show the typical shell deformations associated with captivity. All specimens utilized were either adults or subadults; no hatchlings or juveniles were included in the study.

Morphometric and Preliminary Evolutionary Analysis

All landmark coordinates for all specimens were aligned using a Generalized Procrustes Analysis (GPA), which removes all nonshape information from the data (Zelditch et al. 2004). This includes information about size, as well as nonbiological information about shell orientation and position relative to the digitizer from the data. All subsequent shape analyses were conducted on these GPA-aligned specimens. Centroid size—the square root of the sum of all distances from each landmark to the centroid—was retained and used as a measure of size for all subsequent analyses. Centroid size, unlike more typical linear measures of shell size such as straight carapace length, is mathematically independent of shell shape (Zelditch et al. 2004) and is

thus an appropriate measure of size for these analyses. All morphometric computations were conducted with the gpagen command in the R package geomorph (Adams and Otárola-Castillo 2013).

Next, we conducted a principal components (PC) analysis of the fitted coordinates for all specimens using the plotTangentSpace command in geomorph. We calculated mean values for each species on all PCs. After calculating species' means and determining the number of PCs to retain for individual analyses with a broken-stick test (Jackson 1993), we tested for phylogenetic signal on the full data set, as well as on individual PCs using the command physignal in geomorph. The phylogeny used was taken from a larger study of testudinoid relationships (McLaughlin and Stayton 2016). Finally, to visualize patterns of evolution in shape space, we used the phylomorphospace command in the R package phytools (Revell 2012) to project the tortoise phylogeny onto the first four PC axes.

Mechanical Analyses

The mechanical behavior of tortoise shells was assessed using FE analysis. FE analysis is an engineering technique that can be used to assess the behavior, mechanical or otherwise, of complex structures such as those used in comparative biology. The technique has been used with success in previous biomechanical studies (e.g., Dumont et al. 2009), including some on turtle shells (Stayton 2009, 2011; Rivera and Stayton 2011, 2013; Vega and Stayton 2011; Fish and Stayton 2013). FE models require three components for proper analysis: (1) information on the shape, or geometry, of the structure in question (these data consist of the x-, y-, and z-coordinates of a series of points known as "nodes," which define the vertices of a series of polyhedra known as "bricks"); (2) information on material properties (e.g., the elasticity of the material making up the model); and, (3) boundary conditions that define a set of parameters that allow the modeling software to solve equations associated with the analysis. Here, the boundary conditions consist of a series of 12 loads applied to various locations on the turtle shell, along with a series of four restraints that hold the shell in place while the loads are being applied (Fig. 2A,B).

We followed Stayton (2009) to generate FE models corresponding to the average shape of all testudinid species examined. Briefly, this method uses a thin-plate spline interpolation to warp an existing FE model into a shape corresponding to some other specimen. In this case, a model of a Bog Turtle (*Glyptemys muhlenbergii*) was used as the basis for all transformations (see Stayton [2009] for more details on model construction). Although *Glyptemys* is a member of Emydidae, all testudinoid turtles (Emydidae, Geoemydidae, and Testudinidae) possess shells with similar construction; thus, *Glyptemys* should make an acceptable basis for all subsequent modeling.

The original model consists of 41,610 tetrahedra, or bricks, defined by 10,628 points, or nodes, in three-dimensional space. Together, these nodes and bricks define the geometry of the *G. muhlenbergii* shell. All bricks in the original model were assigned the same material properties: elastic modulus of 22 GPa and a Poisson's ratio of 0.3. These values are characteristic of turtle shell bone (Erickson et al. 2002; Magwene and Socha 2013). We defined the locations
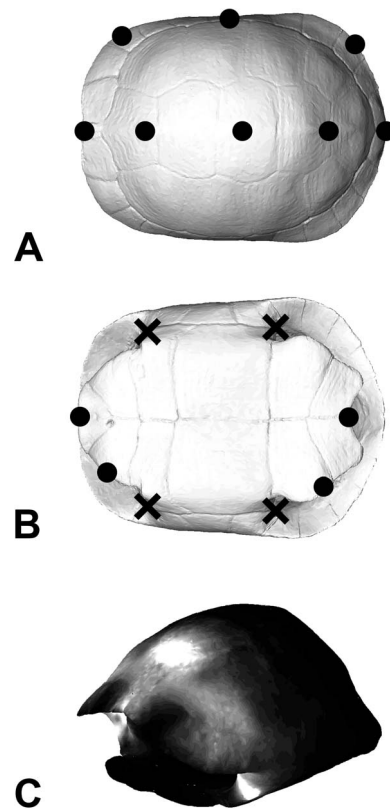

FIG. 2.—(A) and (B) Geometric parameters used in finite element analyses. Large dots represent the locations of individual loads. All loads were oriented normal to the surface of the shell model. Xs represent the locations of restraints used for analyses of each load. Restraints allowed rotation, but not translation. (C) Sample finite-element analysis output. Results are shown for an analysis of the second-most anterior load on the midline of the shell warped to reflect the geometry of *Testudo graeca*. Dark shades represent low stresses; light shades represent high stresses. Note the expected high stresses at the location of the load and at the anterior of the bridge (the latter are attributable to the rotation of the anterior of the carapace induced by the load).

of four restraints, permitting rotation but not translation, near the anterior and posterior margins of the bridge (Fig. 2B). Finally, we defined 12 separate load cases, distributed over the carapace of the model (Fig. 2A,B). Each load was oriented normal to the surface of the shell, and was assigned a force of 500 N (a strong bite from a coyote-sized predator; Christiansen and Wroe 2007). Little information is available on the ways in which predators attempt to break adult turtle shells (but, see Emmons 1989 for an example with tortoises), either by biting or through other methods. Thus, we chose to analyze a number of load cases, distributed widely over the turtle shell, to understand the overall shell responses to loading.

Transformation proceeded as follows for each species' mean shape (for more details see Stayton 2009): x-, y-, and z-coordinates of all tortoise landmarks were obtained for homologous points on the *Glyptemys* FE model. A thin-plate spline interpolation was fit between the two shapes, using the original *Glyptemys* model as the original data and the tortoise species' mean shape as the target. This thin-plate spline interpolation function was then applied to the x-, y-, and z-coordinates of all nodes in the model, producing a new model that approximates the shape of the target tortoise

species. In addition, because the original model already had material properties, loads, and restraints assigned to its bricks and certain nodes, these parameters were carried over to the transformed models. Thus, all models had the same material properties, and loads and restraints in corresponding locations.

Once built, all models were cleaned (to eliminate extremely skewed elements that might produce high stresses as artifacts) and scaled to a common size, because our hypothesis was only concerned with relative shell strength attributable to differences in shell shape and not with absolute strength. This meant scaling all models to a common surface area (Dumont et al. 2009). We then analyzed all 12 load cases, treating the shell as a linear elastic material. After analysis, the maximum von Mises stress (a combined value of stress that predicts failure well; Dumont et al. 2009) observed for any element in the turtle shell, and the mean stress for all elements, were calculated for each of the load cases of each specimen (see Fig. 2C for a visual example of these results). Maximum stresses are the most biologically relevant aspect of shell mechanics, given that they determine failure initiation on account of a load. Maximum stresses can sometimes be elevated by artifacts introduced by the transformation, however, so we used the set of all 12 maximum stresses, as well as 12 mean stresses, as data for hypothesis testing.

Hypothesis Testing

We tested our hypotheses through a series of multivariate regressions of size onto either shell shape or shell strength. To assess the relationship between shell size and shell shape, we conducted a multivariate regression of centroid size versus all shape axes using the plotAllometry command in geomorph. This method calculates the common allometric component (CAC; Mitteroecker et al. 2004) of the shape data, and assesses the magnitude of this component using a resampling procedure. This method also produces shapes characteristic of the maximum and minimum CAC scores, which we inspected to determine the common morphological characteristics of both small and large shells. However, this method does not take the phylogenetic relationships among tortoise species into account. Thus, we also conducted a phylogenetic multivariate analysis of variance (pMANOVA) on our data, using the aov.phylo command in the geiger package in Program R (Harmon et al. 2008), with centroid size as a factor. Given its unusual ecology, and the fact that its shell is unlikely to be used for the same functions as other tortoises, *Malacochersus tornieri* was not included in any analyses. We conducted each analysis twice: once including the island giant species (*Aldabrachelys gigantea* and *Chelonoidis nigra*), and once without those species. Our rationale for this exclusion was that the unique environments where island giant species occur (including no natural predators for adults) might place different selection pressures on their shell shapes than on mainland species (Jaffee et al. 2011).

For assessing the relationship between shell size and shell strength, we conducted a MANOVA on either the set of maximum stresses for each load case for each species, or on the mean stresses for each load case for each species. Again, *M. tornieri* was not included in these analyses, and again, we conducted each set of analyses twice: once including the

island giant species, and once without. Where our tests indicated a phylogenetic signal in the data, we assessed the significance of the MANOVA using evolutionary simulations (i.e., we conducted a pMANOVA when appropriate).

Results

The PC analysis of landmark data for all tortoises yielded 10 significant PC axes. The first two of these axis accounted for approximately 20.50% of the variation found in the data set. High values on the first PC axis corresponded to a shell shape that had a very flat carapace and plastron. The vertebral, marginal, and pleural scutes of individuals with high PC1 scores were relatively small. The bridge of the shell was anteroposteriorly long in such individuals, and the gap between the carapace and plastron was large. *Malacochersus tornieri* showed the highest PC1 scores in the data set. The shell shape corresponding to low PC1 scores had a tall carapace that curved downward at the posterior end and a bridge that was relatively small. The vertebral, pleural, and marginal scutes were also relatively large. Members of the genus *Astrochelys* were representative of shapes characteristic of low PC1 scores (Fig. 3A, 4A).

PC2 accounted for 10.27% percent of the variation in the data set. High values on the axis were associated with a shell shape that had an anteriorly raised carapace that produced a large anterior aperture between carapace and plastron. The bridge on this shell shape was relatively long along the anterior–posterior axis, while the vertebral scutes were relatively small. Species within *Manouria* had some of the highest PC2 scores in the data set and were separated from other turtles along that axis. In contrast, low PC2 values were associated with a shell that had a carapace that curved downward anteriorly, which produced a smaller anterior aperture to the shell. Shells with low PC2 scores also possessed a carapace that extended ventrally posterior to the hindlimbs, which produced a vertical shelf at the posterior end of the shell. Species of *Kinixys* were associated with this shell shape (Fig. 3A, 4A).

PC3 accounted for 9.33% of the variation in the data set. Shell shapes associated with high and low values on this axis showed differences in scute size, height of the gap between the carapace and plastron, and bridge length. High values on this axis were associated with a shell that had relatively small vertebral and pleural scutes. The carapace was anteriorly and posteriorly sloped downward, which decreased the height of the gap between the carapace and plastron. In contrast, low values on the PC3 axis were associated with shells that had a large gap between the carapace and plastron along with large vertebral and pleural scutes. The island giant species *Aldabrachelys* and *Chelonoidis nigra,* as well as *Astrochelys yniphora,* were distinguishable from the rest of the data set along this axis by their low PC3 scores (Fig. 3B, 4B).

PC4 accounted for 7.54% of the variation in the data set. Shapes with high and low values on this axis differed mainly in the length of the gap between the carapace and plastron, as well as marginal scute size. High values on this axis corresponded to a shell shape having a small gap between carapace and plastron, and a short bridge. The marginal scutes were wide and long, especially toward the posterior. The carapace extended downward on the posterior edge to produce a hinge. Low scores on PC4 indicated shells with
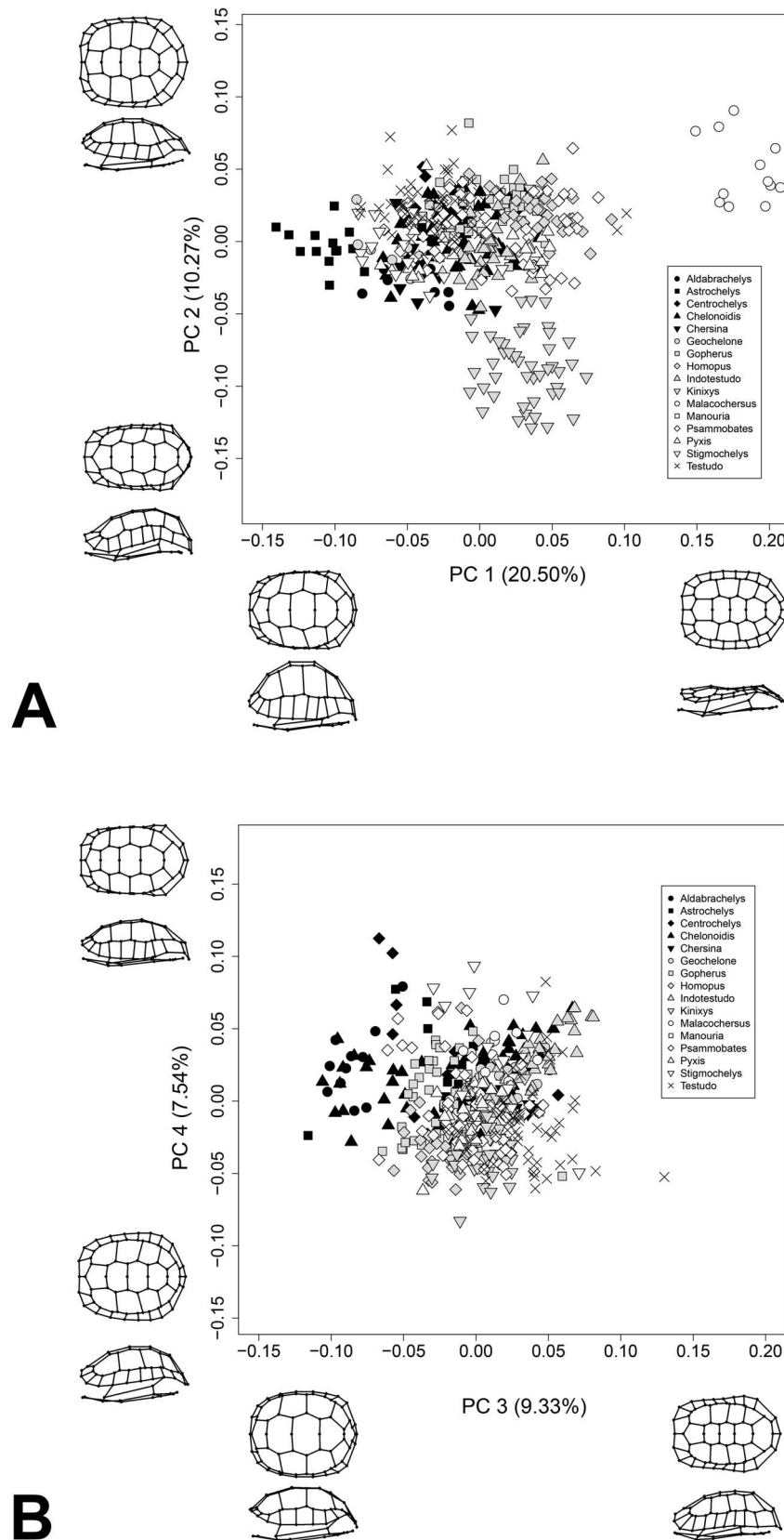
FIG. 3.—Morphospace of all specimens examined within Family Testudinidae, categorized by genus. (A) Principal components (PCs) 1 and 2; and, (B) PCs 3 and 4. Images at the corners show shapes representative of maximum and minimum scores on each PC axis, with all other PC scores set to 0.
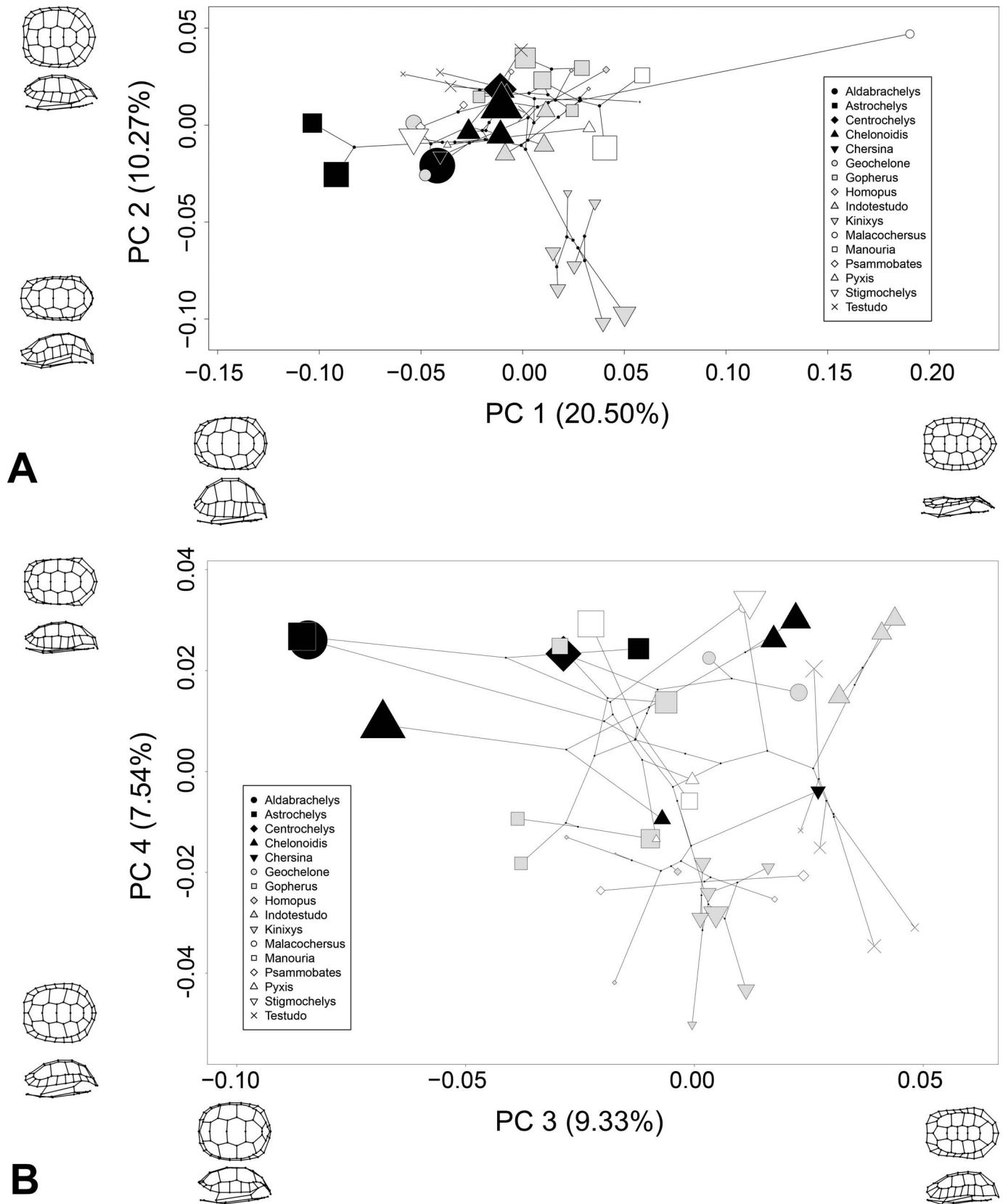
FIG. 4.—Phylomorphospace of all species within Family Testudinidae, categorized by genus. (A) Principal components (PCs) 1 and 2; and, (B) PCs 3 and 4. Images at the corners show shapes representative of maximum and minimum scores on each PC axis, with all other PC scores set to 0. Symbol size is proportional to log (centroid size).

TABLE 1.—Results of tests for phylogenetic signal within Family Testudinidae. PC refers to the principal components axis, or axes, used in the test; K/Kmult provides the value of Blomberg's K, or its multivariate extension. Statistically significant results are shown in bold.

PC	K/Kmult	P-value
All	0.491896	**<0.001**
1	0.900177	**<0.001**
2	0.843414	**<0.001**
3	0.445849	0.008
4	0.845337	**<0.001**
5	0.431959	0.004
6	0.307094	0.152
7	0.612265	**<0.001**
8	0.272930	0.288
9	0.423768	0.007
10	0.303524	0.153
11	0.458143	**0.004**
12	0.501070	**<0.001**
1–12	0.614644	**<0.001**

TABLE 2.—Tests for allometry within testudinid species. PC refers to the principal components axis, or axes, used in the test. Slope refers the slope of the univariate regression of the PC scores against centroid size.

PC	Slope	P-value
1	−8.5778e−05	0.473
2	−6.1969e−05	0.138
3	6.9958e−05	0.369
4	−4.1812e−05	0.672
5	4.3692e−05	0.064
6	−4.832e−05	0.089
7	−3.6903e−05	0.359
8	4.557e−05	0.016
9	3.1918e−05	0.391
10	3.7336e−05	0.363
11	−2.2624e−05	0.477
12	−2.4591e−05	0.429

long bridges, small gaps for the apertures in the shell, and relatively small marginal scutes (Fig. 3B, 4B).

PC5 separated shells with short bridges and carapaces that extended ventrally in the posterior from those with long, tall bridges and no large-scale changes in the slope of the posterior carapace. PC6 separated shells with a narrow carapace, little gap between the anterior carapace and plastron, and large pleural scutes, from those with extensive gaps, smaller pleural scutes, and a generally wide carapace. Tortoises of the genera *Psammobates* and *Homopus* had the highest PC6 scores while *Malacochersus* has the lowest PC6 score.

Scores on PC7 were determined by the relative sizes of the pleural and vertebral scutes, whereas PC8 contrasted the relative sizes of the vertebral and marginal scutes. High scores on PC9 were associated with shells with carapaces taller and wider than those with low PC9 scores. Finally, shell shapes with high and low values on PC10 differed mainly in the size of their vertebral and pleural scutes, with higher scores indicating smaller vertebral, but larger pleural, scutes.

Our evolutionary analyses revealed evidence of strong phylogenetic signal in shell size, overall shell shape, and in aspects of shell shape captured individually on all significant PCs (Table 1; Fig. 4). Significant signal was found on individual PCs 1, 2, 4, 7, 11, and 12. We also found evidence of phylogenetic signal in mean shell stress ($K = 0.50$, $P <$ 0.001), but not maximum shell stress ($K = 0.24$, $P = 0.33$) for all species. The same pattern was found for the data excluding the island giant species: we found signal in mean stress ($K = 0.49$, $P < 0.001$), but not maximum shell stress ($K = 0.23$, $P = 0.43$).

Hypothesis Testing

We found a relationship between shell shape and size, whether or not island giant tortoises were included ($F = 3.49$ or 3.58 with or without island giant species, respectively; both $P < 0.002$). In general, large tortoises tended to have larger carapaces relative to their plastrons, with larger vertebral and pleural scutes and more vertically oriented marginal scutes. Smaller tortoises had wider plastrons, somewhat longer plastrons (especially toward the posterior of the shell) and longer bridges. The carapaces of larger

tortoises were also more highly domed than those of small turtles. A significant relationship between size and shape was only found along PC8 among the individual PCs (Table 2).

We found no evolutionary relationship between shell size and strength, as measured by mean or maximum stress for 12 load cases. This held whether or not island giant tortoises were included for both maximum stress ($F = 1.23$ or 2.00 with or without the island giant species, respectively; both $P > 0.05$) and mean stress ($F = 2.65$ or 1.61 with or without the island giant species, respectively; both $P > 0.05$). Additionally, the direction of the (nonsignificant) trends in these regressions was always opposite of that predicted by our hypotheses. For example, a regression of the independent contrasts for centroid size versus the independent contrasts for the mean of all 12 maximum stresses for each species produced a negative slope. In other words, evolutionary increases in size were associated with evolutionary decreases in size-independent stress for a given load, and thus increases in strength.

DISCUSSION

Our study presents the first multivariate analysis of shell shape across the Family Testudinidae. Some of the morphological patterns that we detected were unsurprising: *Malacochersus tornieri* and the genus *Kinixys* showed the most distinctive shapes along the major axes of shell shape variation among tortoises. These shapes are readily explained by unusual aspects of these species' ecology or physiology. *Malacochersus tornieri* was the most distinct species in the data set, differentiating itself with extremely high scores on the axes of PCs 1 and 2. Pancake Tortoises have evolved a novel strategy among confamilial taxa for avoiding predators rather than withstanding their attacks. This species lives in habitats associated with large rock piles and climbs with great quickness and agility so that, upon detecting a predator, it can retreat to a crevice between the rocks to avoid capture (Bonin et al. 2006). Our FE analysis confirmed that this species possesses a particularly weak shell. Even when assuming it has continuous bone throughout the carapace and plastron (a trait not observed in actual specimens), a given load would produce stresses up to 200% higher in this species than in any other species.

Kinixys was separated out from the other taxa on the basis of their low PC2 scores, high PC3 scores, as well as their relatively low PC4 scores. This diversity and variability most

likely comes from the distinctive hinge that forms from posterior extension of their carapace. Although there are a few other genera that exhibit unique PC scores (such as *Astrochelys*), the majority of genera maintained their position around the average values on most PC axes. This is especially true for the genera *Testudo, Indotestudo,* and *Pyxis,* whose values are consistently average for all 10 significant PC axes.

It is notable that the first several PC axes summarized relatively little variation in shell shape (between approx. 21% for PC1 and 8% for PC4). Diversification in turtle shell shape has proceeded along multiple independent axes of variation, emphasizing the importance of the multivariate tests for all of hypotheses investigated here. Such a pattern has been observed in previous studies of shell shape in specific groups of turtles, and in turtles as a whole (Claude et al. 2003; Stayton 2011; McLaughlin and Stayton 2016). The pattern might be attributable to the length of time during which these species have evolved, or to a more specific property of turtle shells for which many axes of variation are available for selection to act.

Most of the trends seen in the phylomorphospaces are consistent with expected patterns; however, there are a few surprises that may warrant further investigation. As expected based on morphospace data, *Astrochelys radiata* and *M. tornieri* have evolved in opposite directions on PC1. Whereas *A. radiata* has evolved a tall and rounded shell, that of *M. tornieri* is short and flexible. Some members of *Geochelone* and *Manouria* (e.g., *G. elegans* and *G. platynota,* and *M. emys* and *M. impressa*) have evolved in opposite directions on PC2, while both genera as a whole have evolved in opposite directions on PC1. All members of *Kinixys* evolved low values on PC2 except for one species, *K. natalensis*. This unique pattern might be attributable to *K. natalensis* having a very rudimentary carapacial hinge relative to all other *Kinixys*—in this species, the hinge appears to be restricted to the marginal scutes (Bonin et al. 2006).

Among the more interesting observations were the pattern of parallel evolution between *Aldabrachelys gigantea* and *Chelonoidis nigra,* and the apparent convergence of *Astrochelys yniphora* with those taxa, on PC axes 3 and 4. It does not appear that these taxa have evolved similarly low PC3 scores as a sole result of similar size. *Astrochelys yniphora* is not an exceptionally large tortoise, and the next largest tortoise, *Centrochelys sulcata,* does not possess the next lowest score on PC3. The taxa with the smallest sizes (species within *Homopus* and *Psammobates*) do not have the highest scores on this axis. More quantitatively, a phylogenetic regression of PC3 scores onto size, with the island giant species included or excluded, is nonsignificant ($P > 0.05$ for both cases). The trend indicated by that regression is opposite that which might be expected: larger turtles showing a trend toward higher PC3 values when the island giant species are excluded.

One possible explanation for this pattern is that shell shapes associated with low scores on PC3 are particularly appropriate for older (past the juvenile stage) individuals of the large island turtles, which have no natural predators. When defending themselves against predators, most tortoises withdraw the head, tail, and limbs into the shell (like most other turtles), leaving relatively thick skin (sometimes containing osteoderms) and claws of the limbs exposed. Such a strategy works best with a relatively narrow aperture between the carapace and plastron; otherwise, the limbs would not be able to cover all of the softer tissue around the neck, tail, shoulders, or hips. The island giant species are free to evolve shells with extremely large gaps between the carapace and plastron, however, which might allow for longer necks to access food sources that would be otherwise inaccessible, or to more effectively compete with conspecifics (especially among males). This would be the case for any turtles that lack predators as subadults or adults. Such an explanation applies equally well to *A. yniphora* (which, as an adult, has no natural predators), and the extinct *Cylindraspis* of the Mascarene Islands. It would be interesting to determine whether or not these species also showed evolutionary trends toward low PC3 scores after splitting from their closest relatives.

More generally, a pattern of overall allometry in shell shape was detected regardless of whether or not the island giant species were excluded. We predicted that the shapes associated with smaller turtles would be stronger than those associated with larger turtles. The shapes of the carapaces of the smaller turtles were less domed than those of larger species, however, and the bridges were not much larger, which would indicate less mechanical strength (all else being equal). This impression was confirmed by the results of our mechanical analyses: when shells were scaled to the same size, the shell shapes of smaller tortoises generated slightly higher stresses than those of larger tortoises. The lack of statistical significance in pattern is sufficient for us to reject our hypothesis.

One interesting aspect of our mechanical results was the evidence of phylogenetic signal in mean values, but not maximum values, of shell stress for all species. Maximum stresses can be more prone to artifacts than mean stresses (Dumont et al. 2009), so it is possible that our maximum stress data might not capture a biologically meaningful signal and are instead reflecting some error in the FE analysis. However, maximum stresses can also be more strongly affected by slight changes in shape than values for mean stress. Thus, given that a phylogenetic signal was detected for shell shape, mean stresses would be expected to more reliably track phylogeny than the more sensitive maximum stresses.

The lack of an evolutionary relationship between shell size and strength could be caused by a number of factors. Developmental constraints on shell shape are one possibility, but this seems unlikely for tortoises; despite their perceived uniformity, tortoises actually show a great deal of variation in shell shape compared with other turtle families (Stayton 2011). The difference in shape between *M. tornieri* and *A. yniphora* is probably greater than between any two species in any other turtle family. We prefer the hypothesis that shell shape in tortoises is governed by a variety of factors, some of which might disrupt a simple relationship between shell size and strength. Smaller turtles might rely on crypsis or refugia to avoid predator attacks, and shell shapes that would be most effective for those strategies are not well-suited to resisting forces (Keswick and Hofmeyr 2015). Burrowing species such as *Testudo horsfieldii* or members of *Gopherus* are likely to be under selective pressure to decrease shell height (Bonnet et al. 2001). Species living in different

thermal environments might be under divergent selection to change their surface area:volume ratios, and thus change the rate at which they exchange heat with the environment. Finally, male–male competition occurs more frequently in larger species of tortoise, which would lead to the evolution of a taller, more domed shell shape that provides better righting ability (Domokos and Várkonyi 2008). Such shapes would also be more effective at resisting forces. In future studies, we intend to examine the relationships between functional factors such as righting ability or heat exchange and shell shape, to obtain a more nuanced view of the selective pressures that have shaped the ecomorphological evolution of tortoises.

SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at http://dx.doi.org/10.1655/Herpetologica-D-15-00074.S1

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