Journal of Mammalogy, 99(5):1042–1054, 2018
DOI:10.1093/jmammal/gyy101

Putting the leaf-nosed bats in context: a geometric morphometric analysis of three of the largest families of bats

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Bats in the family Phyllostomidae exhibit the most diverse dietary ecology of any mammalian family and the link between their morphology and diet is well-studied. However, the morphology of phyllostomids has yet to be placed into a broader context by comparing them to bats from other families through the use of geometric morphometrics. Using geometric morphometrics, we examined shape trends, disparity, and links between shape and diet using the crania and dentaries of 176 species from 3 of the largest bat families: Vespertilionidae, Molossidae, and Phyllostomidae. Results indicate that cranium shape in insectivorous phyllostomids does not overlap with non-phyllostomid families, suggesting at least 2 insectivorous cranial morphotypes have evolved within Chiroptera and phyllostomid cranial shape had already diverged from other bat families prior to developing their broad dietary range. Further, phyllostomids have higher cranium shape (1.5×) and dentary shape (1.89×) disparity than molossids and vespertilionids, whose dentary and cranium shape disparity is roughly equal. Although the cranium is constrained in many ways (e.g., housing the brain, vision, olfaction), we suggest the fact that dentary shape is limited to food processing allowed it to expand into larger regions of morphospace. Finally, a preliminary dietary analysis based on prey hardness indicates substantial shape overlap within families except for those species with extreme diets (e.g., liquid, very-hard food). Many bats are dietary generalists that eat a wide variety of food types of varying hardness. The data from the 3 large families of bats presented here suggest that eating foods of intermediate hardness may not require substantial cranial reorganization.

Los murciélagos de la familia Phyllostomidae exhiben la dieta más diversa de cualquier familia de mamíferos y el vínculo entre su morfología y ecología trófica está bien estudiado. Sin embargo, hasta el momento, la morfometría geométrica no se ha utilizado para comparar la morfología de los filostómidos con murciélagos de otras familias. Utilizando la morfometría geométrica, examinamos las tendencias de forma, la disparidad y los vínculos entre la forma y la dieta utilizando los cráneos y mandíbulas de 176 especies en tres de las familias de murciélagos más diversas: Vespertilionidae, Molossidae y Phyllostomidae. Los resultados indican que la forma del cráneo en filostómidos insectívoros no se superpone con otras familias, sugiriendo que al menos 2 morfotipos craneales insectívoros han evolucionado dentro de Chiroptera, y que la forma craneal filostómida ya se había separado de otras familias de murciélagos antes de desarrollar su amplio rango dietético. Además, los filostómidos tienen mayor disparidad en forma del cráneo (1.5×) y forma dentaria (1.89×) que los molossidos y vespertilionidos, cuya disparidad entre la dentadura y el cráneo es más o menos igual. Aunque el cráneo está limitado de muchas maneras (por ejemplo, al acomodar el cerebro, la visión, el olfato), la forma dentaria se dedica al procesamiento de alimentos, lo que permitió expandirse a regiones más grandes del morfoespacio. Finalmente, un análisis dietético preliminar basado en la dureza de la presa indica una superposición sustancial de la forma intrafamiliar, excepto en aquellas especies con dietas extremas (por ejemplo, comida líquida, o muy dura). Muchos murciélagos son generalistas de dieta que consumen una gran variedad de tipos de alimentos de diferente dureza. Los datos de las tres familias grandes de murciélagos aquí presentados implican que comer alimentos de dureza intermedia no requiere una sustancial reorganización craneal.

Key words: Chiroptera, diet evolution, disparity, geometric morphometrics, Phyllostomidae

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Leaf-nosed bats (Phyllostomidae) are the most diverse mammalian family in terms of dietary ecology (Freeman 2000); diets include combinations of fruit, seeds, nectar, vertebrates, insects, and blood (Wetterer et al. 2000; Nogueira and Peracchi 2003; Simmons and Conway 2003; Nogueira et al. 2005; Monteiro and Nogueira 2009). Studies have examined the role of diet in shaping phyllostomid crania and dentaries (e.g., Monteiro and Nogueira 2009, 2011), their feeding behaviors (Vandoros and Dumont 2004), and their bite force (Aguirre et al. 2002, 2003; Dumont et al. 2009; Nogueira et al. 2009). These studies have demonstrated that phyllostomid cranium and dentary shapes are unquestionably linked to diet (Freeman 2000; Monteiro and Nogueira 2009) and more specifically, to food hardness, with fruits and insects providing a spectrum of hardness values (Freeman 1981; Aguirre et al. 2002; Giannini and Kalko 2005; Nogueira et al. 2009). There was a fundamental shift in cranium and dentary shape at the base of the Phyllostomidae as a result of the expansion into new dietary niches, which allowed phyllostomids to enter shape morphospace previously unoccupied by other bat groups (Freeman 2000). This led to significant increases in evolutionary rates within the phyllostomid clade (Jones et al. 2005; Dumont et al. 2011), which are evidently absent in all other bat clades (Shi and Rabosky 2015). Given their dietary diversity, phyllostomids have served as a model for understanding radiations of form resulting from evolutionary innovations in diet.

Although we know that phyllostomids have exceptional dietary diversity in comparison with other chiropteran families, only Freeman (1981, 2000) explicitly examined how cranial shape varies between phyllostomids and other families of bats, using traditional morphometrics. Geometric morphometrics recently was shown to be potentially more effective than traditional morphometrics in distinguishing among bat species (Schmieder et al. 2015) and geometric morphometrics also has the advantage that data retain the original shape of specimens making global shape trends easier to analyze (Corti 1993; Slice 2007; Zelditch et al. 2012). As such, geometric morphometrics is a powerful technique for analyzing shape and has been used a number of times to evaluate phyllostomids (e.g., Nogueira et al. 2009; Monteiro and Nogueira 2011), several clades within the Molossidae (Richards et al. 2012), Vespertilionidae (Evin et al. 2008; Ospina-Garces et al. 2016), and Rhinolophidae (Santana and Lofgren 2013). Despite these successes, there has not yet been a large-scale interfamily geometric morphometric study of bats. Putting phyllostomid cranium and dentary shape into a greater context with modern morphometric techniques is a necessary next step in understanding chiropteran diversity and the Phyllostomidae's place within that diversity.

Non-phyllostomid yangochiropteran bats specialize by feeding on insects with a range of hardnesses (Freeman 1981; Ghazali and Dzeverin 2013) and researchers have gone about assessing this relationship in different, sometimes noncomplementary ways. Given recent successes in evaluating diet in terms of food hardness values in the Phyllostomidae (Santana et al. 2012), it is of interest to know whether it is possible to distinguish differences in cranium and dentary shape based on food hardness in clades beyond phyllostomids. Previous studies have variably described bat head shape using only the dentary (Monteiro and Nogueira 2011), only the cranium (Richards et al. 2012; Santana and Lofgren 2013; Bolzan et al. 2015), or both the cranium and dentary (Noguiera et al. 2005, 2009; Sztencel-Jablonka et al. 2009). This issue is not unique to studies on Chiroptera and the difference in data among studies can make it difficult to compare data sets, especially across families. Studies of head shape often have different end goals and questions. For example, Santana and Lofgren (2013) examined rhinolophid skulls because they were interested in potential increased skull modularity relating to nasal echolocation. For studies examining the relationship between shape and diet, it is desirable to know whether the cranium or dentary contains more information for distinguishing between different diets considering the time investment involved in photographing and landmarking both cranium shape and dentary shape data sets.

We evaluate interfamily shape trends in Chiroptera quantitatively, assessing cranium and dentary shape in 3 major bat families (Phyllostomidae, Vespertilionidae, Molossidae) using geometric morphometrics with phylogenetic comparative methods to evaluate the following questions: 1) Do non-insectivorous phyllostomids separate from a basal insectivore morphospace with insectivorous phyllostomids, molossids, and vespertilionids plotting on top of one another at the center of shape space or is the phyllostomids' insectivorous morphotype separate from other families? We hypothesize based on previous studies (Freeman 2000) that insectivorous phyllostomids will not differ substantially from non-phyllostomid insectivorous bats in either cranium or dentary shape. 2) Do phyllostomids have significantly more disparate cranium and dentary shapes than other bat families? Based on a long history of qualitative and quantitative studies, we hypothesize that phyllostomids will have much higher disparity in cranium and dentary shape than vespertilionids and molossids as a result of their dietary innovations, while the latter families will have similar levels of disparity (Shi and Rabosky, 2015). 3) Do bats separate in morphospace based on food hardness within all 3 families we examined or does the generalist approach maintained by many bats (Clare et al. 2009, 2014; Rex et al. 2010) complicate the relationship between shape and diet in the cranium and dentary? We expect based on previous studies examining food hardness and shape in phyllostomids (e.g., Santana et al. 2012) that bats specializing on different food hardnesses will separate clearly in morphospace regardless of family, with bats maintaining very-hard diets having a dorsoventrally taller cranium and larger muscle attachment sites on the caudal aspect of the dentary than bats specializing on softer diets to accommodate increased muscle area for the m. temporalis and m. masseter. However, based on previous success in discriminating diet with skull morphometric data, we are not sure how strong this relationship will be, especially given the paucity of adequate non-phyllostomid diet data. 4) Does dentary shape demonstrate a clearer relationship with diet than cranium shape? Considering that the function of the dentary is primarily for processing food, whereas the cranium has multiple functions, we predict that the dentary will discriminate food hardness better than the cranium in morphospace.

Materials and Methods

To explore shape diversity in our 3 families of interest, we collected geometric morphometric data from 326 bat dentaries and 323 crania representing 176 species (species by family: Phyllosomidae, *n* = 68; Molossidae, *n* = 32; Vespertilionidae, *n* = 76). We collected cranium and dentary data from the same specimens when possible, but in several cases the dentary or cranium was too damaged for both to be included (see Supplementary Data SD1). We also tried to balance the need for a large phylogenetic sample against the desire to document within-species variation. Therefore, we did not sample more than 6 specimens per species to attempt to account for individual variation where possible, but given the desire to have a large phylogenetic range, we were often unable to sample 6 individuals per taxon due to limitations in museum collections. The mean was 2 individuals per taxon. Further, to account for issues of sexual dimorphism present in some species in our data set, all specimens included were male.

To capture dentary and cranium shape, we employed 2-dimensional geometric morphometrics from images of crania and dentaries in lateral view. Since skulls are 3-dimensional structures, we note that using 2-dimensional geometric morphometrics and examining the crania solely in lateral view may have affected our results. We chose the lateral view because previous traditional morphometric-based studies have found that skull height is the most effective metric for distinguishing diet among phyllostomids (Dumont et al. 2009; Santana and Dumont 2009; Santana et al. 2010). Specimens were placed on grid paper with 1-cm squares and supported with clay such that the cranium and dentary midlines were parallel to the axis of the camera lens. Images were taken with a Canon EOS 70D with an EF 50 mm f-1.4 usm fixed focal length lens using an accessory close-up lens placed on a photo stand to ensure a static camera position. All images were imported into tpsDig2 (Rohlf 2006) for landmarking and semi-landmarking (Fig. 1). Landmarks represent homologous anatomical loci while semi-landmarks represent homologous curves (Zelditch et al. 2012). Seven landmarks and 4 curves with a total of 52 semi-landmarks were used to characterize dentary shape and 10 landmarks and 1 curve with 20 semi-landmarks were used to characterize cranium shape. The definitions and anatomical significance of landmarks are reported in the supplementary data (see Supplementary Data SD1).

Landmark data were imported into RStudio v. 0.99.902 (R Core Team 2017), opened in the R package *geomorph* (Adams and Otárola-Castillo 2013), and subjected to general Procrustes analysis (GPA), which translates, rotates, and rescales all specimens into the same orientation leaving only shape information (Zelditch et al. 2012). Semi-landmarks were slid such that bending energy was minimized along each curve (Bookstein

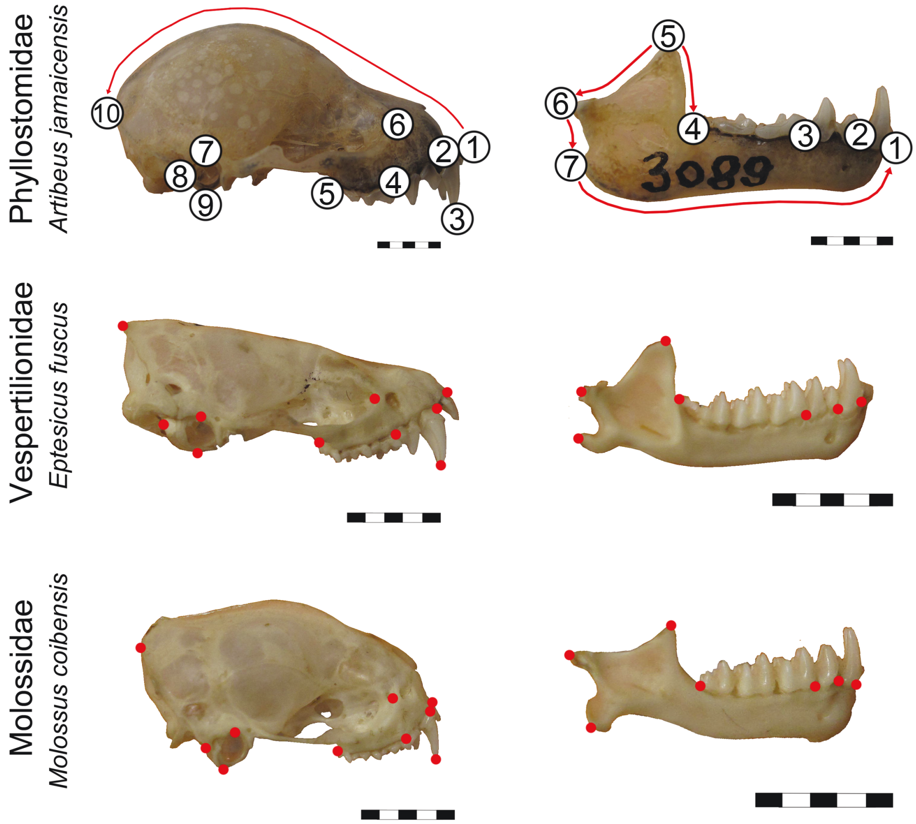

Fig. 1.—Landmarking scheme for the cranium (left) and dentary (right) with landmarks shown in order for *Artibeus jamaicensis* (representing Phyllostomidae), *Eptesicus fuscus* (Vespertilionidae), and *Molossus coibensis* (Molossidae). Curves represent semi-landmark curves. Scale bars = 5 mm.

1997; Perez et al. 2006). After GPA, we ran a principal components analysis (PCA) to evaluate general trends in shape space across all 3 families. We used a scree plot and the broken stick method to determine the number of principal components (PCs) to include in our analyses. Further, we did not consider PCs that included less than 10% of total variance or those PCs driven by singular taxa meaningful. After determining general shape trends, we examined whether allometry had a strong influence on our shape data using the common allometric coefficient (CAC) developed by Mitteroecker et al. (2004). When analyzing allometric data of a single group as was done in this analysis (all families were pooled), the CAC is equivalent to the regression score (Drake and Klingenberg 2008). The plot. allometry function in *geomorph* (Adams and Otárola-Castillo 2013) calculates shape scores based on a regression of shape against size, which is the allometric trend in the data (CAC), and plots the CAC against the log of centroid size. We found a significant relationship between shape and centroid size within all families examined, and all multivariate analyses of variance (MANOVAs) used the residuals of shape and size to mitigate the effects of allometry on shape.

We assessed differences in shape among families in an evolutionary context using phylogenetic comparative methods because phylogeny can create a confounding effect since taxa that are more closely related typically covary at a higher rate than taxa that are more distantly related (Felsenstein 1985). We created a pruned phylogenetic tree with only the species included in our study in the R package *APE* (Paradis et al. 2004) based on the time-calibrated Chiroptera tree published by Shi and Rabosky (2015; Fig. 2). To apply phylogenetic corrections, each tip must be represented by a single specimen. Therefore, we computed the mean shape for all species for use in all phylogenetic analyses following Sherratt et al. (2014). To determine the extent which shape was influenced by phylogeny, we calculated the multi-*K* statistic developed by Adams (2014). This statistic is a multivariate extension of Blomberg's *K* statistic and estimates whether phylogeny influences shape where $K_{\text{mult}} = 0$ shows no phylogenetic structure in the data and $K_{\text{mult}} = 1$ shows phylogenetic structure perfectly in line with a Brownian motion model of evolution (Blomberg et al. 2003). We ran phylogenetic generalized least squares analyses to test whether the size-adjusted residuals of cranium shape and dentary shape for all pooled PCs differed significantly among molossids, vespertilonids, and phyllostomids using the procD. pgls function in *geomorph* (Zelditch et al. 2012; Adams and Otárola-Castillo 2013). Unfortunately, it is not yet possible to run pairwise comparisons using phylogenetic generalized least squares analysis with high-dimensional shape data (Collyer et al. 2015), so we ran 3 separate MANOVAs using size-adjusted residuals comparing the 3 groups to determine the presence of significant differences among them.

Next, we sought to investigate the relationship between food hardness and shape in morphospace within each family using the full complement of our data rather than species means. We ran family-level PCAs and allometric analyses classifying as many species as possible into the 5 food hardness groups defined by Freeman (1981) for non-phyllostomids and Santana et al. (2010) for phyllostomids (1—liquid, 2—soft, 3—medium, 4—hard, 5—very-hard; see Supplementary Data SD2). We defined diet categories on mechanical demand (food hardness) rather than traditional diet categories (e.g., frugivory, sanguinivory), since previous studies have found that hardness rather than food type more effectively separates species (Santana et al. 2010). Different studies calculate hardness and diet in different, often conflicting ways producing unreliable results (Dumont et al. 2011). Therefore, we limited our hardness data to that provided by Freeman (1981) for molossids and vespertilionids and to Santana et al. (2010) for phyllostomids, who published the most complete hardness data for the families that we studied.

We then ran a disparity analysis to evaluate the presence of significant differences in shape among families using the morphol.disparity function in *geomorph*. Specifically, we estimated disparity by generating the Procrustes variance for each family and then evaluated the significance of the Procrustes variances between groups through 999 permutations (Zelditch et al. 2012).

RESULTS

Comparing dentaries among families.—PC1 and PC2 summarized 55.6% of the total shape variation in dentary shape among all families (Fig. 3A). PC3 summarized an additional 15% of total shape variation (see Supplementary Data SD3 and SD4). The remaining PCs individually summarized less than 10% of total variation and were driven by singular taxa (e.g., *Desmodus*) and were not included in subsequent analyses (see Supplementary Data SD5).

PC1 (36.8%) demonstrated a gradation from phyllostomids that specialize on hard canopy fruits (subfamily Stenodermatinae—negative PC1 axis), to phyllostomids, through molossids, and finally to vespertilionids (positive PC1 axis in Fig. 3A). Taxa on the positive end of PC1 had a generally gracile dentary with well-defined mandibular processes that were subequal in size relative to one another. The ventral border of the dentary for these taxa had a gentle curve unlike the strong curve in taxa such as *Myotis blythii* (lesser mouse-eared myotis) or the absence of a curve as in *Platalina genovensium* (long-snouted bat). In contrast, taxa at the negative end of PC1 had a very robust coronoid, large areas for muscle attachment on all the mandibular processes, and the ventral border of the dentary lacked a curve. This region of morphospace was populated by stenodermatine species including *Ardops nichollsi* (tree bat) and *Artibeus jamaicensis* (Jamaican fruit bat). PC2 (18.8%) was defined largely by a trend of bats with a diet of hard insects such as *Antrozous pallidus* (pallid bat—positive PC2) to bats with a liquid diet such as *P. genovensium* (negative PC2). The dentaries of taxa at the negative end of PC2 had no ventral curve, a very reduced angular process, and subequally sized, dorsally inclined coronoid and condyloid processes. The dentaries of taxa at the positive end of PC2 had an expanded coronoid process, a strong ventral curve, and a pronounced

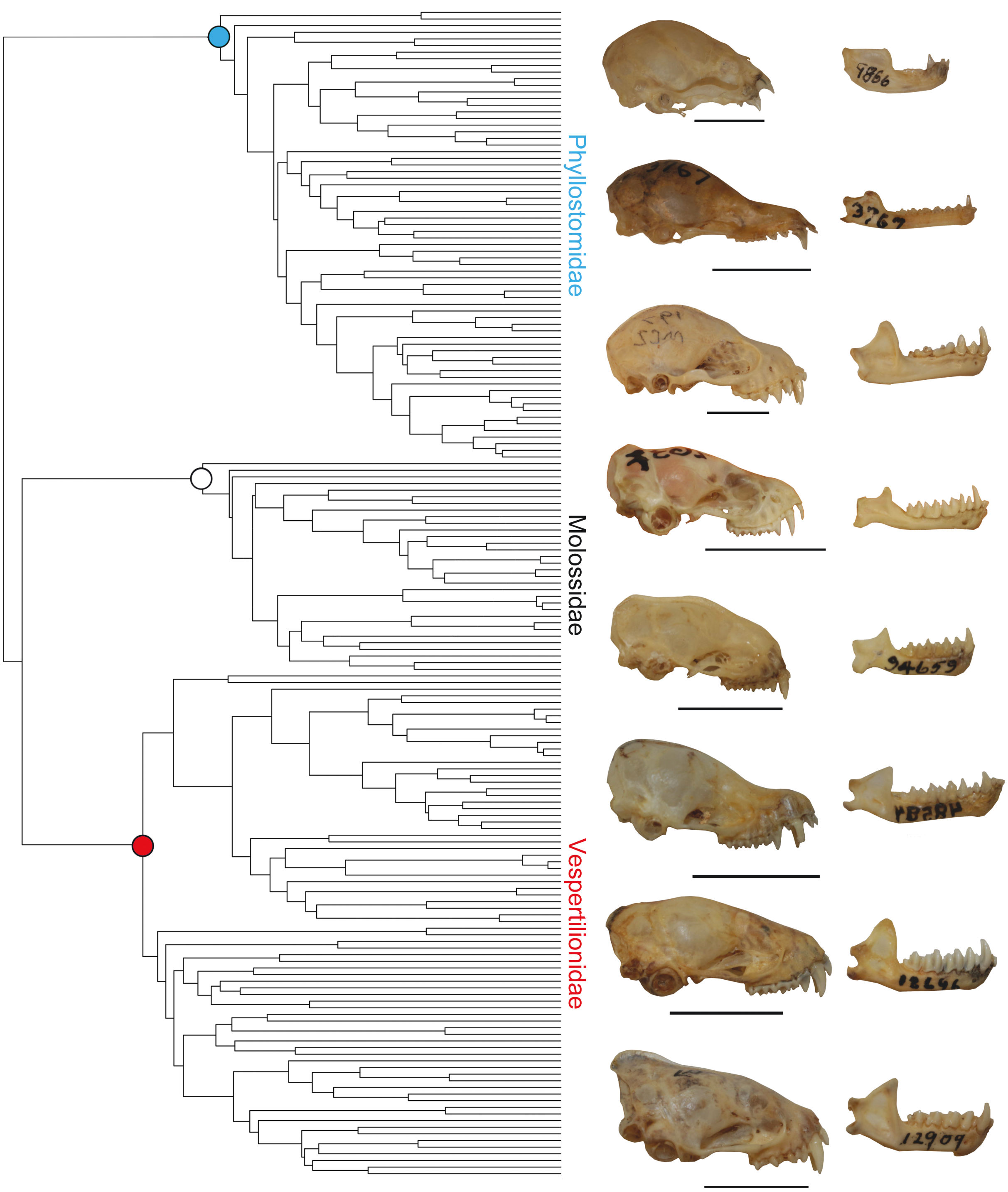

Fig. 2.—Pruned tree from Shi and Rabosky (2015) incorporating all species included in our analyses. Crania and dentaries of representative taxa (from top to bottom): *Desmodus rotundus*, *Glossophaga soricina*, *Artibeus lituratus*, *Tadarida brasiliensis*, *Molossus molossus*, *Myotis velifer*, *Antrozous pallidus*, and *Pipistrellus kuhlii*. Scale bars = 10 mm.

chin. PC3 (15%) primarily separated sanguinivorous bats from other bat taxa with all non-sanguinivorous bats plotting on the negative end of PC3 and *Desmodus rotundus* (common vampire bat), *Diphylla ecaudata* (hairy-legged vampire bat), and *Diaemus youngi* (white-winged vampire bat) plotting on the positive end of PC3 (see Supplementary Data SD5). The consensus shape for the positive end of PC3 was similar in form to the shape of *D. rotundus* with a massive reduction in the molar tooth row and expansion of the muscle attachment sites for jaw-closing muscles (e.g., m. masseter, m. temporalis) in comparison with the gracile jaws found in taxa on the negative end of PC3.

After examining shape trends in morphospace, we analyzed allometry, phylogenetic signal, and levels of disparity. Allometry

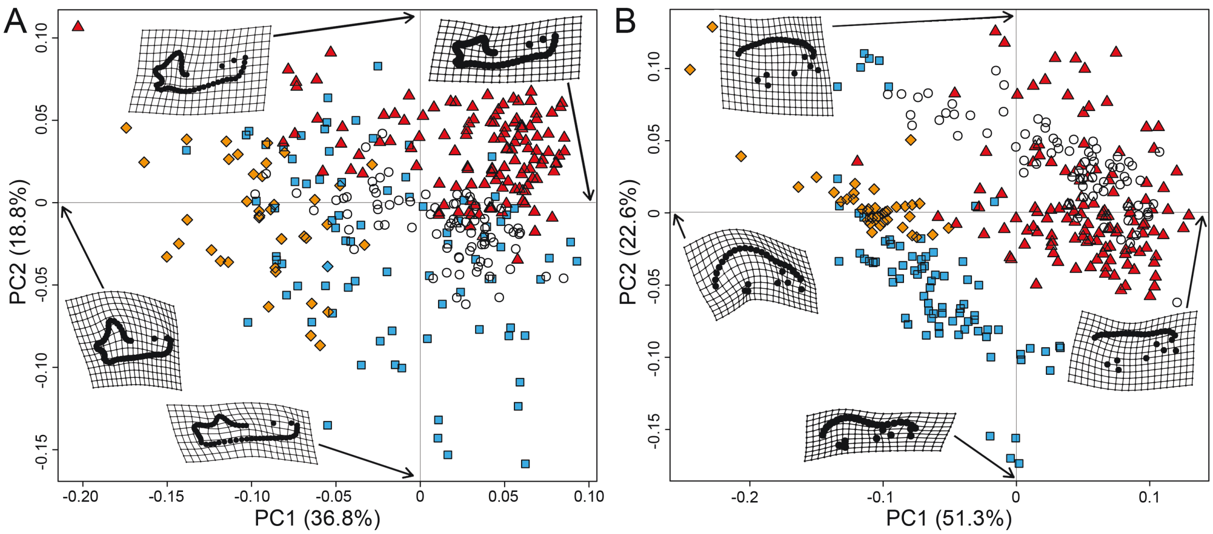

Fig. 3.—Principal components analysis of A) bat dentary shape and B) cranium shape for all specimens included in our analysis. TPS (thin plate spline) grids are inset showing shape change across morphospace with each TPS grid symbolizing the consensus shape at positive and negative principal component extremes. Arrows correspond to their adjacent TPS grid. Vespertilionidae (triangles), Molossidae (circles), non-stenodermatine Phyllostomidae (squares), and Stenodermatinae (diamonds).

had a significant impact on dentary shape ($P < 0.001$), but it did not explain a large proportion of variation in the data ($R^2 = 0.101$). There was a significant effect of phylogeny on the data ($K_{\text{mult}} = 0.604$; $P = 0.01$), which is lower than expected under a Brownian motion model of evolution ($K_{\text{mult}} = 1$). It is not surprising that this is significant given that we examined dentary shape at the family level (Table 1). A phylogenetic generalized least squares analysis of allometry-adjusted residuals including all families was also significant ($P < 0.001$). All MANOVAs comparing families demonstrated significant differences in dentary shape (Table 2). In terms of disparity in dentary shape, phyllostomids had significantly larger Procrustes variance than the other 2 families, while the other groups were not significantly different in dentary shape disparity (Table 3).

Comparing crania among families.—A PCA of bat crania among families revealed that PC1 and PC2 summarize 73.9% of total shape variation, with additional PCs being driven largely by single taxa (e.g., *Desmodus*, Fig. 3B; see Supplementary Data SD6 and SD7). There was more separation between phyllostomids and non-phyllostomids in cranium shape than in dentary shape, but little separation between molossids and vespertilonids in cranium shape. Stenodermatines were distinct relative to non-stenodermatine phyllostomids and other families.

A combination of PC1 and PC2 separated the Phyllostomidae from the other families such that phyllostomids predominantly occupy the lower left quadrant of morphospace. PC1 (51.3%) connoted a shift from crania with a strong dome shape and a short face (e.g., *Ametrida centurio* [little white-shouldered bat], *Centurio senex* [wrinkle-faced bat]) on the negative end to crania with a flat skull roof on the positive end (e.g., *Lasionycteris noctivigans* [silver-haired bat]). There was a slight shift in tooth

Table 1.—Summary of phylogenetic signal (*K*-multiple statistic, see Adams 2014 for details) for all families as a unit and each individual family for cranium and dentary shape.

	K-multiple statistic	*P*-value
Jaw shape		
All families	0.6040	0.01
Phyllostomids only	0.8072	0.01
Vespertilionoids only	0.7010	0.01
Molossids only	0.5727	0.01
Skull shape		
All families	0.5964	0.01
Phyllostomids only	0.6369	0.01
Vespertilionoids only	0.6099	0.01
Molossids only	0.4969	0.02

Table 2.—Overall phylogenetic generalized least squares analysis and interfamily (Mo = Molossidae, Ph = Phyllostomidae, and Ve = Vespertilionidae) phylogenetic generalized least squares analysis comparisons for dentary shape and cranium shape. *P*-values in bold are statistically significant.

Jaw shape	*d.f.*	R^2	*F*-statistic	*P*-value
All families	2	0.0032	0.2752	**0.001**
Residuals	173			
Total	175			

Jaw shape	Ph	Ve	Mo
Ph		**0.009**	**0.023**
Ve			**0.001**
Mo			

Skull shape	*d.f.*	R^2	*F*-statistic	*P*-value
All families	2	0.0043	0.3711	**0.001**
Residuals	173			
Total	175			

Skull shape	Ph	Ve	Mo
Ph		0.072	**0.002**
Ve			**0.002**
Mo			

Table 3.—Disparity analyses for the crania and dentaries with all families separated. Procrustes variance listed followed by pairwise *P*-values. Mo = Molossidae, Ph = Phyllostomidae, and Ve = Vespertilionidae. *P*-values in bold are statistically significant.

Jaw shape	Procrustes variance		Mo	Ph	Ve
Mo	0.0070	Mo		**0.001**	0.775
Ph	0.0138	Ph			**0.001**
Ve	0.0073	Ve			
Skull shape	**Procrustes variance**		**Mo**	**Ph**	**Ve**
Mo	0.0096	Mo		**0.001**	0.653
Ph	0.0147	Ph			**0.001**
Ve	0.0090	Ve			

and orbit positioning along PC1 to accommodate the shift in the angulation of the dorsal aspect of the cranium. PC2 (22.6%) did not strongly separate the 3 bat families, although non-stenodermatine phyllostomids tended to plot on the negative end of PC2 with the exception of vampires, which had strongly positive PC2 scores. Nectivorous bats (e.g., *P. genovensium*, *Leptonycteris yerbabuenae* [lesser long-nosed bat], *Lonchophylla thomasi* [Thomas' nectar bat]) with elongate crania and well-defined rostral and caudal cranium regions dominated the negative end of PC2. In contrast, the positive end of PC2 was driven by moth-eating bats (e.g., *Lasiurus cinereus* [hoary bat]) and outliers such as *D. rotundus*. The consensus shape was defined as a shortened cranium with a high profile, but lacking the dome shape that characterizes highly negative PC1 taxa (e.g., *A. centurio*).

Beyond general shape trends, we examined allometry, phylogenetic signal, and disparity, which had similar trends to what we found for the interfamily dentary data. Similar to bat dentaries, crania had significant allometry ($P < 0.001$), although the crania had a higher percent of variation explained by allometry than dentaries ($R^2 = 0.232$). Phylogeny was also significant for bat crania ($K_{\mathrm{mult}} = 0.596$; $P < 0.001$; Table 1) although lower than would be expected under a Brownian motion model of evolution. The phylogenetic generalized least squares analysis comparing all groups was significant ($P < 0.001$). Phyllostomids differed significantly in cranium shape from molossids ($P = 0.002$), but not vespertilionids ($P = 0.072$). This is likely due to *Harpiocephalus harpia* (lesser hairy-winged bat) plotting within the phyllostomid morphospace. Vespertilionids and molossids were significantly different ($P < 0.002$; Table 2). Also similar to the bat dentary data set, the phyllostomids had a significantly larger Procrustes variance than the Molossidae and Vespertilionidae, while the other families were not significantly different from one another (Table 3).

Within-clade analyses.—Due to their dominance in both cranium and dentary morphospaces at the interfamily level, the phyllostomid-only PCA demonstrated similar shape trends to the overall interfamily morphospace (Fig. 4). For the dentary, PC1 (41.3%) separated liquid-eating bats from all other diet categories. Bats on the positive end of PC1 had an elongate ramus with reduced mandibular processes, while bats on the negative end of PC1 had well-developed mandibular processes, especially the coronoid process. There was minimal separation in morphospace between non-liquid-eating bats with all 4 groups plotting directly on top of one another. There was less separation among diet categories in the cranium morphospace than the dentary morphospace. However, there was marked expansion of the very-hard diet category in the cranium morphospace due to *C. senex*, *Sphaeronycteris toxophyllum* (visored bat), and *Trachops cirrhosus* (fringe-lipped bat) plotting outside of the other diet category morphospaces (Fig. 4B). Nectivorous bats plotted on the positive end of PC1 to the right of the other diet categories and *D. rotundus* plotted on the negative end of PC1 showing some separation between liquid-eating bats and other diets. Phyllostomid dentaries had a significant allometric component ($P = 0.041$) with allometry accounting for a very small percent of variation ($R^2 = 0.020$). Similarly, allometry was a significant contributor to cranium shape ($P = 0.02$) and accounted for a small percent of overall variation ($R^2 = 0.032$). Phylogeny had a significant impact on shape in phyllostomids for both the cranium and dentary (Table 1).

The molossid dentary and cranium shape morphospaces illustrated more separation among species in different dietary hardness categories (Fig. 5). PC1 (40.6%) for the dentary drew a dichotomy between dentaries with a more robust mandibular process region (e.g., *Molossus* spp.) and dentaries with reduced mandibular processes (e.g., *Nyctinomops* spp.). There was also a strong dichotomy between medium-hardness diets (e.g., *Nyctinomops*, *Eumops*, *Tadarida*) and very-hard diets (e.g., *Molossus*), although the sample sizes are too small to be definitive. PC2 (13.9%) accounted for minimal differences in overall dentary shape (13.9%). Taxa at the positive end of PC2 had slightly more robust dentaries with less defined mandibular processes relative to taxa at the negative end of PC2. The molossid cranium shape morphospace was driven by PC1, which accounted for 68.7% of total shape variation, with PC2 only accounting for 7% of total shape variation. Species at the positive end of PC1 had convex crania dorsally (e.g., *Molossus* spp.) and eat very-hard insects, while taxa at the negative end of PC1 had flattened crania with no skull dome (e.g., *Nyctinomops*) and had medium-hardness diets. Allometry played a significant role in molossid dentary ($P = 0.006$) and cranium ($P = 0.028$) shape. As in phyllostomids, allometry played a relatively small role in the shape of dentaries ($R^2 = 0.047$) and crania ($R^2 = 0.059$). Phylogenetic signal was a significant predictor of shape for both crania and dentaries (Table 1).

Like the molossids, the vespertilionids made up a small portion of the interfamily morphospace for both dentary and cranium shape. Within the vespertilionid dentary shape space, PC1 (45.9%) was driven by a contrast between species that consume very-hard insects and other diet categories (Fig. 6). *Harpiocephalus harpia* plotted at the extreme positive end of PC1 with robust mandibular processes, especially the coronoid process. Bats that consume soft, medium, and hard foods overlapped at the negative end of PC1. Consumers with moderately hard diets occupied a larger morphospace than other diet groups. There was limited separation and shape variation along PC2 (22.8%). Similar to the situation among phyllostomids, the cranium morphospace clouded the apparent diet-based separation seen in dentaries with all 4 diet hardness categories plotting on top of one another. PC1 (38.9%) and PC2 (25.1%) were both related to differences

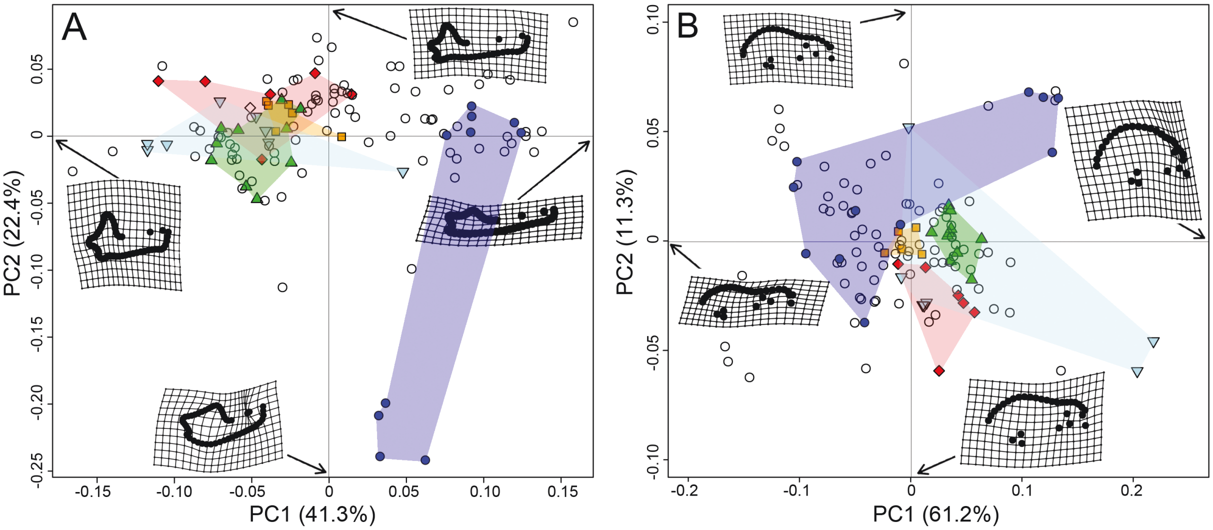

Fig. 4.—A) Phyllostomid dentary and B) cranium shape. TPS (thin plate spline) grids are inset showing shape change across morphospace with each TPS grid symbolizing the consensus shape at positive and negative principal component extremes. Arrows correspond to their adjacent TPS grid. Dietary hardness: filled circles = liquid, squares = soft, diamonds = medium, triangles = hard, inverted triangle = very-hard. Taxa not examined by Santana et al. (2010) are white.

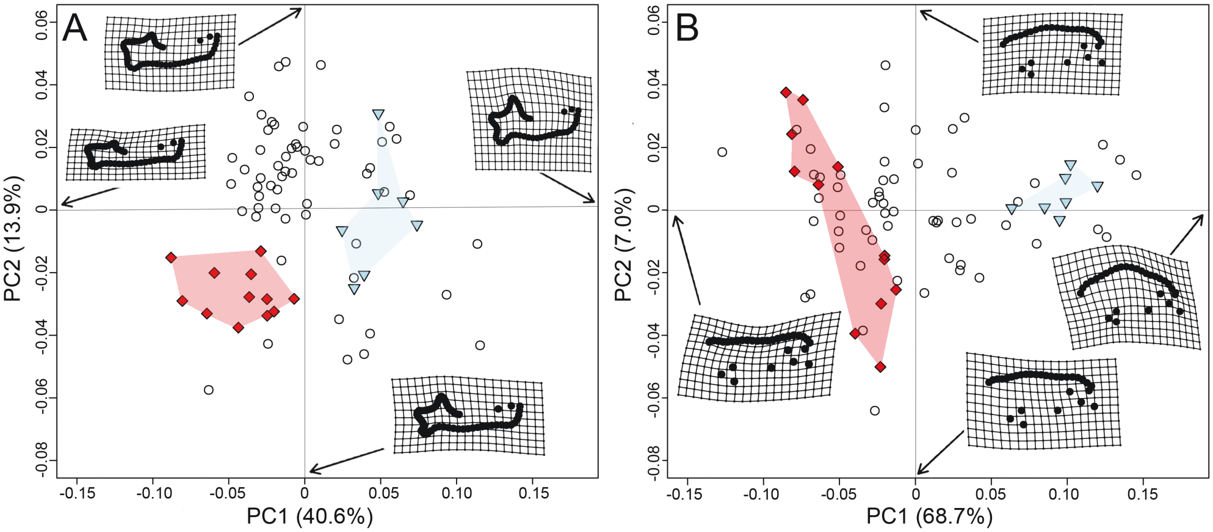

Fig. 5.—A) Molossid dentary and B) cranium shape. TPS (thin plate spline) grids are inset showing shape change across morphospace with each TPS grid symbolizing the consensus shape at positive and negative principal component extremes. Arrows correspond to their adjacent TPS grid. Dietary hardness: squares = soft, diamonds = medium, triangles = hard, inverted triangle = very-hard. Taxa not examined by Freeman (1981) are white.

cranium profile from flatter crania to more dome-shaped crania. Furthermore, taxa with medium-hard diets occupied the majority of cranium morphospace, whereas they were more localized in the dentary. There was a significant role of allometry in the dentary ($P < 0.001$) and cranium ($P < 0.001$) and it played a slightly greater, but still small role in shape variation (dentary: $R^2 = 0.195$; cranium: $R^2 = 0.0714$) relative to phyllostomids and molossids. As in the other 2 main families, phylogeny for both cranium and dentary shape were significantly correlated (Table 1).

Discussion

Our study evaluated interfamily shape trends in chiropteran crania and dentaries using geometric morphometrics and phylogenetic comparative methods based on a large sample of species, which allowed us to better characterize the distinctiveness of the Phyllostomidae relative to other chiropterans. Although we found substantial overlap between phyllostomids and the other clades in dentary shape, there was limited overlap between phyllostomids and other clades in cranium shape. Whereas phyllostomids have diverged into a wide array of dietary niches, the lack of overlap between phyllostomids and other bats suggests the development of 2 insectivore cranial morphotypes, a result not found by Freeman's (2000) traditional morphometric analysis. This may be related to a fundamental shift in cranium morphology at the base of the phyllostomids, leading to the radiation of phyllostomids into a wide array of dietary niches.

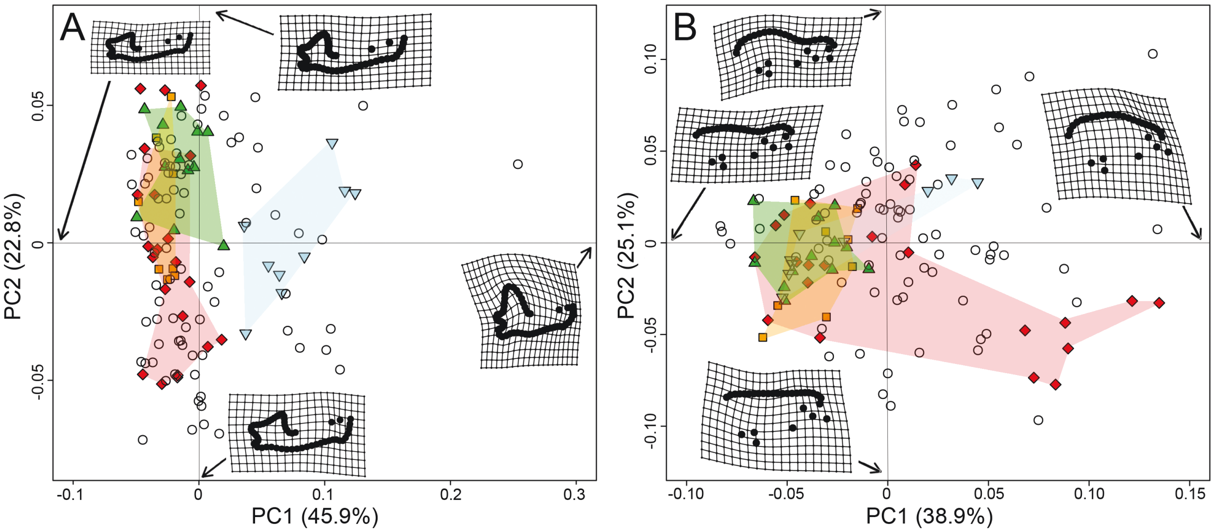

Fig. 6.—A) Vespertilionid dentary and B) cranium shape. TPS (thin plate spline) grids are inset showing shape change across morphospace with each TPS grid symbolizing the consensus shape at positive and negative principal component extremes. Arrows correspond to their adjacent TPS grid. Dietary hardness: squares = soft, diamonds = medium, triangles = hard, inverted triangle = very-hard. Taxa not examined by Freeman (1981) are white.

As predicted, we found that phyllostomids have the largest disparity in dentary and cranium shape among the families we examined, but also that their relative dentary disparity is much larger than their cranium disparity. This could be because the dentary primarily functions in food processing while the cranium performs a series of functions, thus placing more constraints on its evolution. When diet is divided based on hardness as suggested by Santana et al. (2010) rather than traditional diet categories (e.g., nectivory, insectivory), soft, medium, and hard diets cluster on top of each other. However, very-hard diets and liquid diets typically separate out in morphospace, suggesting that dietary extremes require a major reworking of cranium morphology (Dumont et al. 2009).

Crania versus dentaries: more than 1 way to be an insectivore?—Phyllostomids were hypothesized to have diverged from other bat clades in shape as a result of the innovation of new diets from ancestral insectivory (e.g., Wetterer et al. 2000; Dumont et al. 2011), especially in relation to the evolution of obligate frugivory in stenodermatines (Jones et al. 2005; Dumont et al. 2011; Rojas et al. 2012; Shi and Rabosky 2015). If morphological innovations were driven by the expansion of dietary ecospace, morphological changes in the dentary and cranium between insectivorous phyllostomids and non-phyllostomids would be relatively minor, as previously suggested by Freeman (2000). When viewed in dentary morphospace, insectivorous phyllostomids plot closely with molossids and the majority of vespertilionids (Fig. 3A). The insectivorous dentary morphotype is typified by a relatively elongate form with well-developed, but not exaggerated mandibular processes across all 3 families. Phyllostomids like *Carollia* that consume both insects and more-specialized foods are outside of the molossid and vespertilionid morphospace, but closely appressed to it. Phyllostomids relying less on insectivory (e.g., nectivory, strict frugivory) were further from the ancestral condition found at the center of morphospace, similar to what Freeman (2000) uncovered for crania. For example, *P. genovensium* is a cactus specialist (Sahley and Baraybar 1996) and plots strongly positive on PC2, and stenodermatine bats, which are highly specialized frugivores, plot strongly positive on PC1, both away from the insectivore morphospace.

In contrast, phyllostomids have minimal overlap with molossids and vespertilionids in cranium morphospace, including the primarily insectivorous basal phyllostomids *Macrotus* and *Micronycteris* (Gardner 1977; Kalka and Kalko 2006), while non-phyllostomids families closely overlap with one another. The vast majority of phyllostomids have a curve to the dorsal aspect of their crania with a distinct rostrum and a dome-shaped caudal aspect of their skull, whereas the majority of non-phyllostomids in our study had a very flat skull roof with a minor and gradual curve from the front of the rostrum to the occiput. *Desmodus*, *Diaemus*, and *Diphylla* are the only phyllostomids to have a sloped skull with no curve, and they both plot away from the Phyllostomidae and closer to the other families. This may be related to the basal position of the vampires (Jones et al. 2005), but the Desmodontidae are highly specialized for sanguinivory and thus it is difficult to make assertions about their morphology due to the paucity of intermediate forms.

Given the complete separation of phyllostomids from the other families in cranium shape, we suggest that there are at least 2 cranial morphotypes for insectivores in Chiroptera, but a single insectivore dentary morphotype. One possible reason for this is echolocation (Pedersen 1998). Phyllostomids are nasal echolocators, whereas vespertilionids and molossids both echolocate orally. Freeman (2000) suggested that insectivory was achieved differently based on mechanisms of generating echolocation calls. She found that nasal-echolocating rhinolophids have longer, narrower faces and taller sagittal crests than their oral-echolocating relatives. Pedersen (1998) found differences in the organization of phyllostomid and rhinolophid cranial morphology and shape, in which rhinolophids may sacrifice olfaction for a louder call, whereas phyllostomids retain olfaction. However, the relationship between morphology and the loudness of calls has yet to be tested. In spite of these trends, facial measurements do not appear to strongly correlate with echolocation parameters, even when comparing nasal versus oral echolocators (Goudy-Trainor and Freeman 2002). Further, molossids do not follow the general oral-echolocating trend and have longer heads than expected (Goudy-Trainor and Freeman 2002). Finally, the use of oral and nasal echolocation varies within families. *Desmodus* and *Carollia* are known to echolocate orally in spite of being phyllostomids (Griffin and Novick 1955; Schmidt 1988) and *Corynorhinus*, a vespertilionid, is known to echolocate nasally (Griffin 1958). Therefore, the morphological trends we have presented here are not unequivocally related to differences in echolocation. Future clade-wide studies including pteropodids, which developed frugivory independently from phyllostomids; rhinolophoids, which developed nasal echolocation independently from phyllostomids; and the Nycteridae, which appear to locate prey through detecting sound rather than by echolocation (Fenton et al. 1983), will help to resolve the potential confounding echolocation variable. Additionally, even basal, primarily insectivorous phyllostomids eat fruit (Freeman 2000), and the change in morphology may be related to a shift away from insectivory rather than the development of a second insectivorous morphotype, which could be clarified by analyzing the immediate insectivorous outgroups of the phyllostomids.

Based on our data, we suggest that the main difference in the phyllostomid morphotype from other families is a decoupling of the rostrum and the braincase. Future studies on integration similar to the study done on phyllostomid dentaries by Monteiro and Nogueira (2009) at the family level in Chiroptera will help to clarify this trend.

Differences in dentary and cranium disparity among families.—Based on the dietary diversity of phyllostomids and their adaptive radiation (Freeman 2000; Van Cakenberghe et al. 2002; Dumont 2007; Rex et al. 2010), we predicted that dietary diversity would be associated with increased disparity in the shape of the cranium and dentary in phyllostomids relative to other families. However, we did not expect the dentary to have a greater degree of shape disparity than the cranium. Procrustes variance for phyllostomid crania was 1.5 times higher than vespertilionids and molossids, whereas for dentary Procrustes variance, phyllostomids were 2 times higher than molossids and 1.9 times higher than vespertilionids (Table 3). One primary task of the head is to break apart and consume food. The cranium has a number of additional tasks (e.g., housing the brain, hearing, vision, olfaction—Hanken and Hall 1993; Marroig and Cheverud 2001, 2004). On the other hand, the dentary is specialized specifically for food processing. Originally, we posited that although cranium shape and dentary shape are necessarily integrated so that they can function as a unit, the discrepancy in relative disparity might have been related to the dentary having a stronger dietary signal than the cranium.

When we separated the families into family-specific data sets, we found that the dentary data set had somewhat more dietary clustering in the Phyllostomidae and the Vespertilionidae than the cranium data set, and that the Molossidae had similar levels of clustering for both data sets (Figs. 4–6). Neither phyllostomids nor vespertilionids exhibited unmistakable clustering of diet, especially for intermediate diet types (soft, medium, hard food—see below). Nogueira et al. (2009) found that cranium and dentary shapes were significantly correlated with size-adjusted bite force but that cranium shape had greater explanatory power for bite force than did dentary shape. Their data were based only on phyllostomids, and since they used data on bite force, their sample size was limited to 14 species. Therefore, although the difference in disparity is a somewhat tantalizing result, its cause is currently unclear and more studies, such as studies of bite force, need to be performed on taxa outside of the Phyllostomidae. We were limited in our ability to effectively analyze clustering quantitatively. We relied on PCA rather than canonical variates analysis because we did not want to artificially assign specimens to groups that would impact their clustering, given the lack of clear data on diet or food hardness for the majority of the species included in our analysis.

Reconciling cranium and dentary shape with food hardness.––Although bats are the second largest mammalian order in terms of species diversity, recent studies have found that their diversification rates are "remarkably homogenous" (Shi and Rabosky 2015) with 1 notable exception, the stenodermatines (Dumont et al. 2011). Dumont et al. (2011) found a clear association among diet, morphology, and bite performance in phyllostomids based on an analysis of 86 species with 2 major radiations: 1 at the base of Phyllostomidae and 1 with stenodermatines, with the trophic level evolving 3.5 times faster in stenodermatines than other phyllostomids. This disparity likely is due to the evolution of obligate frugivory. Dumont et al. (2011) further showed that the relatively shorter skulls of stenodermatines generated a higher mechanical advantage than other phyllostomids, allowing even small stenodermatines to have the ability to eat very-hard fruit. This trend is apparent not only in the skull of stenodermatines, but also in the expansion of processes of the dentary. Bats with high bite force have a high coronoid process, expanded angular process, and a tall dentary, among other characters (Noguiera et al. 2009). Even molar complexity has been shown to be highest in obligate frugivores in comparison with insectivorous and omnivorous bats (Santana et al. 2011).

Stenodermatines are the primary group that expand outside of the basal phyllostomid morphospace in both dentary and cranium shape (Fig. 3). The shorter skull of stenodermatines has been considered a "key innovation" allowing for the relatively high diversification of phyllostomids in comparison with other bats (Dumont et al. 2011). In the Phyllostomidae-only cranium and dentary morphospace, the story is somewhat more clouded (Fig. 4). *Centurio* and *Sphaeronycteris* clearly are separated from other phyllostomids, but other bats that consume very-hard foods (*Phyllostomus hastatus*, [greater spear-nosed bat]) overlap with bats that consume softer food items (*Phylloderma stenops* [pale-faced bat], *Tonatia saurophila* [stripe-headed round-eared bat], *Micronycteris minuta* [tiny big-eared bat]; Fig. 4). Dentary morphospace for phyllostomids is driven by bats that have a liquid diet (nectivores, sanguivores) rather than very-hard diets. In contrast, within vespertilionid dentary morphospace the very-hard diet morphotype separates clearly from other dietary groups (Fig. 6). This also appears to be true within the Molossidae. However, the molossids in our data set encompass only 2 dietary groups, medium and very-hard, and there likely is a substantial phylogenetic component since molossids with a very-hard diet all are members of the genus *Molossus* (Fig. 5).

Although the Phyllostomidae contains a number of dietary specialists, it is becoming increasingly clear that many phyllostomids are more generalists than previously thought. For example, bats that eat primarily soft fruits such as *Carollia* also eat harder fruits (Giannini and Kalko 2004). In addition, *Glossophaga soricina*, *Glossophaga commissarisi*, and *Anoura*, which are often classified as nectivorous, also consume fruits and insects (Howell 1974; Heithaus et al. 1975; Willig 1986). This suggests that although specialization may make new niches available, maintaining the capacity for generalist behavior increases niche flexibility (Clare et al. 2009, 2014; Rex et al. 2010). Even bats that eat very-hard fruits, such as *Centurio*, may only do so for part of the year (Dumont et al. 2009; Santana et al. 2012), and biting behavior may allow bats to eat harder fruits than otherwise expected regardless of shape (Dumont 1999; Dumont et al. 2005; Santana et al. 2012). Morphology may not tell the entire story for diet since differences in behavior (Santana and Dumont 2009) and multiple morphologies have the potential to serve the same function (Wainwright et al. 2005) as we demonstrate for insectivorous phyllostomid crania in comparison with insectivorous species in other bat families.

Conclusions.—By examining cranium and dentary shape in 3 of the largest chiropteran families, we were able to evaluate skull shape of phyllostomids in a broader phylogenetic context (Freeman 1981) and reexamine shape trends identified by previous studies (Nogueira et al. 2009; Dumont et al. 2011). We found that while there is substantial overlap between phyllostomids and non-phyllostomids in dentary shape, especially among insectivorous phyllostomids, there is limited overlap between phyllostomids and other families in cranium shape. This suggests that 2 insectivorous cranium morphotypes are present in Chiroptera, with 1 evolving early in the evolution of the Phyllostomidae (contra Freeman 2000). We also found that phyllostomids had much higher disparity in their cranial and dentary shape than vespertilionids and molossids. Surprisingly, we were not able to demonstrate a clear relationship between food hardness and cranium or dentary shape in the 3 families in spite of previous studies demonstrating that it exists among phyllostomids (Noguiera et al. 2009; Santana and Dumont 2009). Even specialist bats often exhibit generalist behavior and eat a range of food hardnesses (Dumont et al. 2009; Clare et al. 2009, 2014), which is perhaps obscuring trends. Additionally, diet data are quite hard to collect given seasonal changes, locality differences, and differences in methods for collecting data (e.g., feces, stomach contents, etc.). Cranium and dentary shape in stenodermatines are not only separate from those of other phyllostomids, but also from molossids and vespertilionids. Previous work has shown a significant shift in diversification rates at the base of Stenodermatinae (Jones et al. 2005; Dumont et al. 2011; Shi and Rabosky 2015), which we confirm is reflected in morphology from a geometric morphometric-based perspective. Finally, we found that dentary disparity in phyllostomids is higher relative to non-phyllostomid clades than cranium disparity. We suggest that this may be because shape constraints are higher in the cranium than the dentary because the skull has a variety of functions while the dentary evolved specifically for food processing. However, due to a lack of diet data in many clades, these trends are not yet fully clear.

ACKNOWLEDGMENTS

We thank V. D. Munteanu, A. Vander Linden, G. Mutumi, S. Cordero, A. Conith, and D. Pulaski for discussion and L. M. Dávalos for translating our abstract into Spanish. We also thank J. Chupasko (MCZ), M. Omura (MCZ), E. Hoeger (AMNH), B. O'Toole (AMNH), E. Westwig (AMNH), K. Doyle (UMA), K. Zyskowski (YPM), and D. Lunde (NMNH) for specimen access and help in museums. Finally, we thank D. S. Rogers (editor) and 2 anonymous reviewers for comments greatly improving the quality of this manuscript. Research was carried out on NSF 1612211 (BPH) and NSF DEB 1442278 (ERD).

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Landmark and semi-landmarks (shown in Fig. 1 of the main text) with definitions as well as a brief description of their importance in characterizing overall cranium and dentary shape.

Supplementary Data SD2.—Dietary categories for all taxa analyzed based on Santana et al. (2010) for phyllostomids and Freeman (1981) for molossids and vespertilionids. Taxa were ranked 0–5 (0 = no data, 1 = liquid diet, 2 = soft diet, 3 = medium-hardness diet, 4 = hard diet, 5 = very-hard diet). Categories in Freeman (1981) were continuous 1–4, so our categories were simplified so that all categories in Freeman (1981) were rounded up.

Supplementary Data SD3.—Principal components analysis of data from bat dentaries, including relative contributions of each PC (1–10) and PC scores for all specimens for PC 1–4, centroid size, and allometry scores.

Supplementary Data SD4.—Principal components analysis of data from bat dentaries, where means were taken for each taxon represented by multiple specimens. This includes relative contributions of each PC (1–10) and PC scores for all taxon means for PC 1–4.

Supplementary Data SD5.—Principal components analysis of bat dentary shape (PC1 × PC3) showing the separation of *Desmodus* from other taxa. PC3 and all subsequent PCs are driven by singleton taxa. However, the shape difference between *Desmodus* and all other bats in dentary shape (PC3) still accounted for a relatively large percentage of overall shape variation (15%).

Supplementary Data SD6.— Principal components analysis of data from bat crania, including relative contributions of each PC (1–10) and PC scores for all specimens for PC 1–4, centroid size, and allometry scores.

Supplementary Data SD7.— Principal components analysis of data from bat crania, in which means were taken for each taxon represented by multiple specimens. This includes relative contributions of each PC (1–10) and PC scores for all taxon means for PC 1–4.

Supplementary Data SD8.—Complete cladogram for all taxa included in the study. Pruned from the complete tree presented by Shi and Rabosky (2015).

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Submitted 8 December 2017. Accepted 1 August 2018.

Associate Editor was Duke Rogers.