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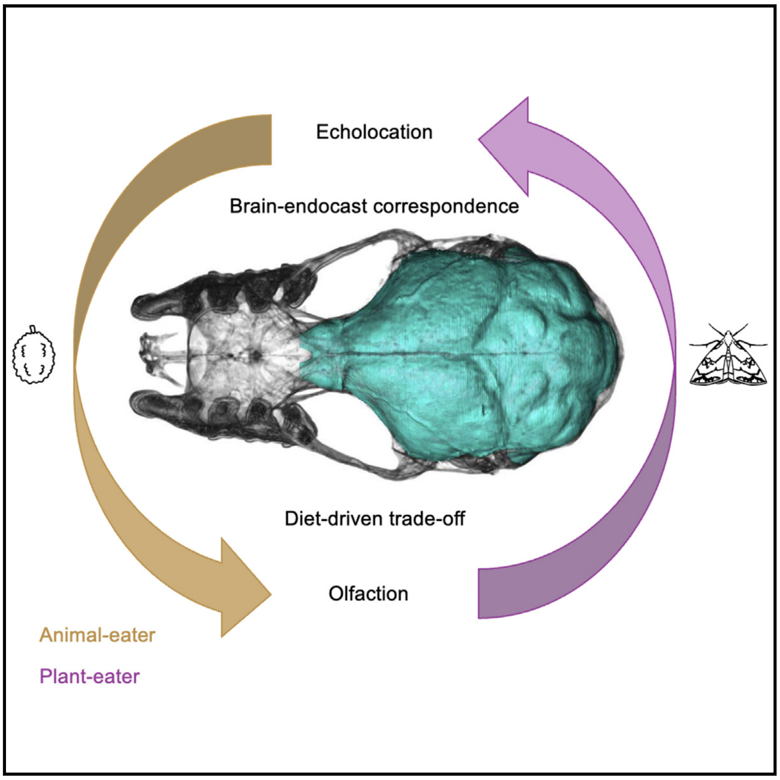
Echolocation and dietary adaptations mediate brain-endocast covariation in bats

Graphical abstract



Authors

Camilo López-Aguirre, Bushra Alam,
Muzna Mian, John M. Ratcliffe,
Mary T. Silcox

Correspondence

c.lopezaguirre@utoronto.ca

In brief

Natural sciences; Zoology; Evolutionary biology

Highlights

- Skull-brain complex is an under-utilized model to understand phenotypic evolution
- In bats, brain-endocast correspondence is mediated by diet and echolocation
- Variation in brain-endocast correspondence as a pathway of diversification
- Evolutionary trade-off between olfaction and echolocation

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Echolocation and dietary adaptations mediate brain-endocast covariation in bats

Camilo López-Aguirre,[1,2,3,4,8,9,*] Bushra Alam,[5] Muzna Mian,[6] John M. Ratcliffe,[2,7] and Mary T. Silcox[1,7]
[1]Department of Anthropology, University of Toronto Scarborough, Toronto, ON M1C 1A4, Canada
[2]Department of Biology, University of Toronto Mississauga, Toronto, ON L5L 1C6, Canada
[3]Museo de La Salle, Universidad de La Salle, Bogotá 111711, Colombia
[4]Escuela de Biología, Universidad Industrial de Santander, Bucaramanga 680002, Colombia
[5]Department of Biological Sciences, University of Toronto Scarborough, Toronto, ON M1C 1A4, Canada
[6]Faculty of Arts and Science, University of Toronto, Toronto, ON M1C 1A4, Canada
[7]These authors contributed equally
[8]Twitter: @cernestola
[9]Lead contact
*Correspondence: c.lopezaguirre@utoronto.ca
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SUMMARY

Bats exhibit remarkable ecological diversity, reflected in different species' sensory, cognitive and behavioral ecology. Bat brain volume has been linked to powered flight, laryngeal echolocation, and dietary transitions. Given the developmental trajectories and functional demands the skull and brain share, the bat skull-brain complex represents a unique means to better understand evolutionary processes and trade-offs. We assessed the brain-endocast correspondence across bats, considering whether changes in correspondence reflect ecological adaptations. We demonstrate that estimates of brain volume from both methods showed similar allometric trajectories, apart from those for cerebral cortex. Our results reveal a significant effect of diet and echolocation on brain-endocast correspondence of the cerebral cortex and olfactory bulbs, respectively. We suggest that shifts in the brain-endocast correspondence of the olfactory bulbs indicate an evolutionary trade-off between olfaction and echolocation. Our study offers a different perspective for future comparative neuroanatomical studies involving extinct and living species in museum collections.

INTRODUCTION

Bats are the second-most species-rich mammalian group, with an almost global distribution, and exhibit an extraordinary eco-morphological diversity linked to a wide range of sensory and foraging adaptations.[1–5] Two characteristics strongly associated with the diversification of bats are echolocation and diet, both of which are also tightly bound to their phenotypic evolution.[1,6,7] Indeed, the variety of craniodental phenotypes in bats showcases the interplay between biomechanical and sensory adaptations (e.g., in feeding, olfaction, vision, and hearing) in response to multiple selective pressures. What is more, powered flight and advanced laryngeal echolocation have evolved in tandem in bats, alongside cognitive specializations associated with these novel selective pressures on the mammalian brain.[8–10] Bat skulls have also evolved in ways linked to functional demands, making their evolutionary history informative with respect to how adaptive trade-offs shape the generation of phenotypic disparity.[1,11–13]

Given the many shared evolutionary pressures the brain and skull experience to support the sensory, cognitive, and behavioral capabilities of vertebrates, and their interconnected developmental processes,[14,15] the brain-skull complex represents an underappreciated model to understand covariation of tightly bound, but not entirely overlapping, suites of traits.[14,16] In the context of understanding morphological adaptations of brains and skulls of bats in relation to these traits and abilities, endocranial morphology has the potential to be extremely informative, reflecting the interface between hard and soft tissues.[17] Indeed, the analysis of endocranial morphology has revolutionized our general understanding of mammalian brain evolution,[18–20] allowing the study of extant and extinct taxa within a single comparative framework.[21]

Evolutionary trade-offs in the cranial morphology of bats are thought to have happened in response to several interacting selective pressures.[6,22,23] For example, a potential trade-off in the masticatory apparatus of insectivorous bats has been proposed to optimize either bite force or biting speed,[22] while trade-offs between specializations for echolocation and olfaction have been linked to morphological adaptations in the bat rostrum.[23] For example, those laryngeal echolocating bats that emit their calls through their nose, rather than mouth (as do most bats) exhibit anatomical adaptations in the rostrum and nasal cavity that could have constrained the evolution of their olfactory capabilities.[6,23] In general, differences in sensory adaptations likely facilitated the morphological diversification of bats, reshaping allometric relationships and the modularity of the skull.[6]

With the advent of three-dimensional (3D) imaging techniques, the brain-endocast correspondence has been widely studied across tetrapods, mainly to assess how accurately endocranial morphology reflects brain morphology. With some degree of variation in some tetrapod groups,[17,24] endocranial morphology closely reflects brain morphology in mammals and birds.[25,26] However, beyond methodological implications of variability in brain-endocast correspondence, the assumption that ecological factors do not influence this correspondence remains mostly untested in mammals, including bats. Evidence of biological factors mediating brain-endocast correspondence has been presented in other tetrapods, indicating the presence of evolutionary processes shaping the brain-skull complex,[17,24] although largely in taxa that have braincases of endochondral origin (e.g., fishes and lizards[17,27]). It is therefore worth assessing whether this is true in taxa with crania that ossify intramembranously (e.g., mammals and birds) and we sought to do so in the work we present here.

Despite the increasing interest in endocranial morphological evolution, few studies have focused on the underlying assumptions of studying bat endocranial morphology.[28] For example, studies have interchangeably used endocranial volume and brain size in the same framework, without controlling for possible differences between both approaches.[29]

The majority of comparative studies on bat brain size and morphology have relied on the work of Baron et al.,[27] which is based on a combination of direct whole-brain measurements and dissection-based estimation of the volume of brain regions. Studies based on Baron et al.[27] have provided fascinating insights into the ecology[30] and evolution[31] of the bat brain. Considering the importance of this dataset, it seems critical to assess the degree to which comparable estimates of both overall brain volume, and the relative size of various brain components, can be gleaned from 3D imaging data, of the type also available for fossils. While recent comparative anatomical descriptions of bat endocranial morphology have provided information on the brain-endocranial correspondence,[32,33] this issue has not been considered through the lens of size or ecological regimes. Given the high cranial morphological variation in bats, and the role ecological correlates (e.g., diet and echolocation) have played in shaping its phenotypic diversification, those same ecological correlates could influence the brain-endocast correspondence, as has been shown in brain-skull covariation in humans and other primates.[34,35]

The study of the morphological evolution of the vertebrate brain also largely ignores brain-endocast correspondence, using endocranial morphology as proxy for brain morphology without qualification, in species extant and extinct. Moreover, those studies that do consider brain-endocast correspondence most often do so from a purely methodological perspective and do not consider phylogenetic or ecological correlates. Our purpose here was, therefore, (1) to explicitly consider brain-endocast correspondence in bats and (2) to do so within an evolutionary and ecological context. To these ends, we reconstructed the 3D endocasts of bats (Figure 1) and gathered dissection-based estimates of brain volume from the literature to (1) quantitively assess the dissection-endocranial correspondence and (2) explore differences in the brain-endocast correspondence by testing the effect of allometry, echolocation (i.e., constant frequency, dominant harmonic, multiharmonic, and no laryngeal echolocation), and diet (animalivorous versus phytophagous). We used phylogenetic comparative methods to account for the effect of phylogenetic relatedness on dissection-endocranial correspondence. We also investigated how the dissection-endocast correspondence varies across different brain regions, specifically, the olfactory bulb, cerebral cortex, and cerebellum. We predicted that there would be a strong relationship between the volumes derived from dissection and CT based methods, but that this relationship may vary based on the ease of identifying the boundaries for given areas in the X-ray computed tomography data. We also predicted that phylogenetic and ecological factors will have little effect on the nature of the brain-endocast relationship. We found a strong and significant correlation between brain and endocranial volume estimates. We uncovered an unexpectedly complex evolutionary scenario where phylogenetic relatedness, allometry, and ecological adaptations interact to shape variation brain-endocast correspondence in bats. We provide a new framework to understand the neurological evolution of mammals and other tetrapods, indicating that brain-endocast covariation is an unexplored evolutionary pathway of neuroecological diversification.

RESULTS

Dissection-endocast correspondence

Both our phylogenetic and non-phylogenetic regressions showed a statistically significant correlation between dissection-based brain volumes and endocranial volumes across all brain regions (Table 1). After correcting for phylogenetic relatedness, phylogenetic generalized least squares (PGLS) analyses revealed a stronger correlation ($R^2 = 0.888–0.982$) and weaker phylogenetic signal ($\lambda = 0.028–0.547$) in absolute brain and endocranial volume regressions (Figure 2 top row), and weaker correlation ($R^2 = 0.233–0.797$) and stronger phylogenetic signal ($\lambda = 0.000–0.848$) in relative brain and endocranial volume regressions (Figure 2, bottom row). Slopes of PGLS and standardized major axis (SMA) regressions were almost identical in models run with absolute volumes, whereas slopes of PGLS and SMA regressions differed when run with relative volumes, especially in cerebral cortex and cerebellum values.

Wilcoxon's signed-rank tests only revealed differences in absolute olfactory bulbs and cerebral cortex volumes between dissection-based and endocranial estimates (Figure S2; Table S2). Exploration of these differences in estimates of absolute cerebral cortex volumes revealed a pattern of decreasing percentage differences with increases in body mass: a 21.3% difference in small-sized species to a 10.69% in large-sized species (Figure S3).

Brain and endocranial allometry

Our phylogenetic allometric regressions indicated similar allometric scaling of brain and endocranial volumes with body mass (Table 2; Figure 3). All allometric regressions had a significant phylogenetic signal, and were stronger for dissection-based volumes ($\lambda = 0.928–1.000$) than for endocranial volumes

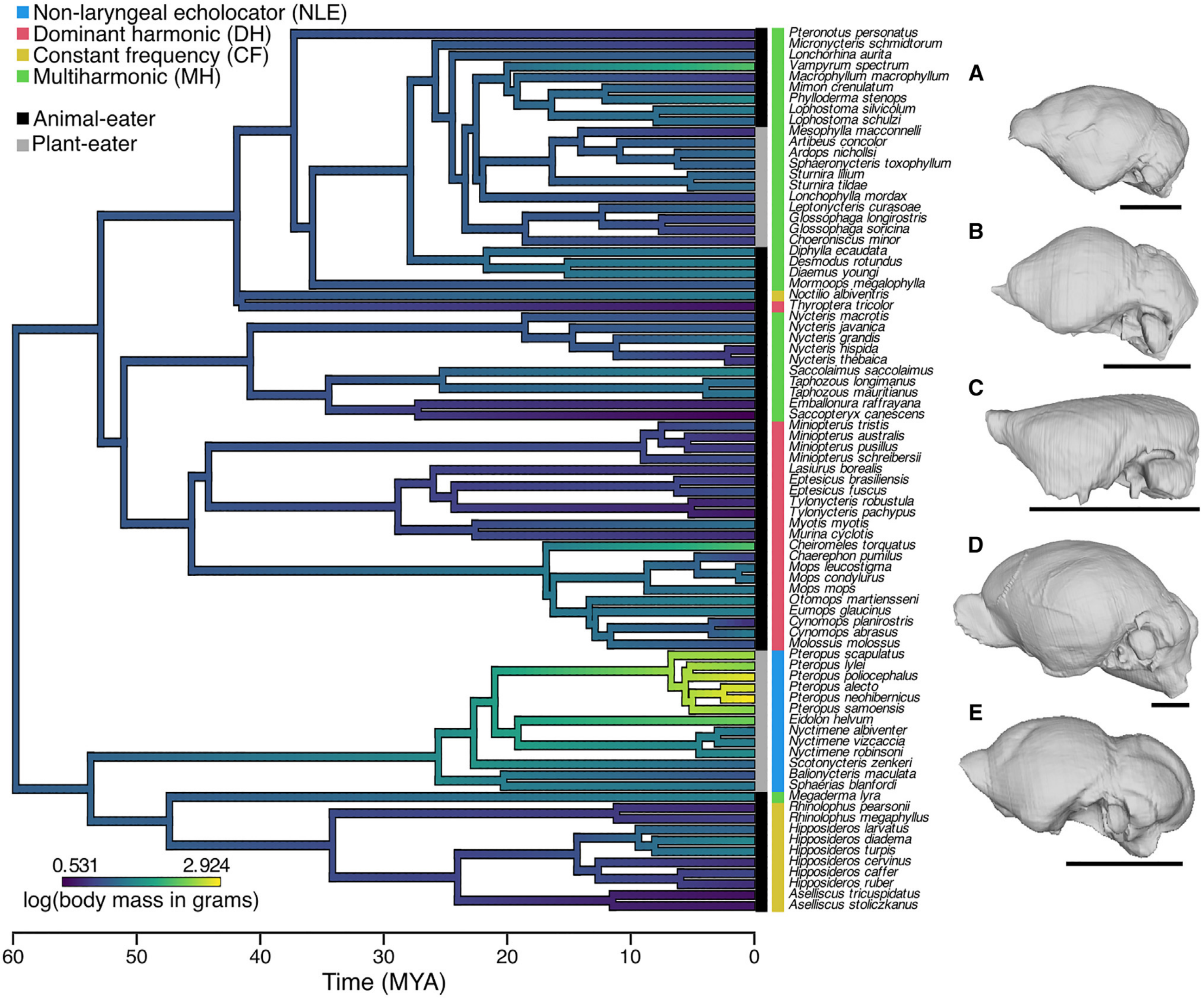

Figure 1. Phylogeny of 83 species of bats analyzed in this study, depicting body size, diet and call type

Diet was categorized as either animal-eating or plant-eating and call type was categorized as either multiharmonic (MH), dominant harmonic (DH), constant frequency (CF), or non-laryngeal echolocators (NLE). Timescale depicts millions of years before the present. Endocasts in lateral view of *Artibeus concolor* (A), *Nycteris javanica* (B), *Tylonycteris robustula* (C), *Pteropus lylei* (D), and *Hipposideros caffer* (E), with black bars representing a 5 mm scale.

Phylogeny based on Upham et al.[36] and body size, diet and call type based on Thiagavel et al.[31]

($\lambda = 0.667–0.854$). Similarly, coefficients of determination of allometric regressions were higher for dissection-based volumes ($R^2 = 0.722–0.923$) than for endocranial volumes ($R^2 = 0.612–0.884$), indicating more linear allometric scaling of dissection-based estimates. Allometric slopes were very similar between dissection and endocranial volumes across all brain regions (differing on average by 1%), except for the cerebral cortex where the difference in allometric slopes between dissection and endocranial volumes was of 6.91%.

Biological drivers of brain-endocast correspondence

Phylogenetic regressions assessing the effect of biological factors on the brain-endocast correspondence indicated that diet and call type were the only factors that had a significant effect on brain-endocast correspondence, whereas echolocation category and duty cycle did not have a significant effect (Table 3; Figure 4). The model including diet as a covariate was significant for the brain-endocast correlation of cerebral cortex volume. To further explore the effect of diet on the cerebral cortex brain-endocast correspondence, we ran a PGLS for each dietary group, independently. No phylogenetic signal was found in the model for animalivorous bats ($\lambda = 0$, $p = 1$), and a strong phylogenetic signal was found in the model for phytophagous bats ($\lambda = 0.788$, $p = 0.041$). Based on PGLS models, dissection-endocast cerebral cortex correspondence is statistically significant for both animal-eating ($R^2 = 0.353$, $F = 27.765$, $p < 0.001$, slope $= 0.738$) and plant-eating bats ($R^2 = 0.358$, $F = 13.435$, $p = 0.001$, slope $= 1.318$), each group

Table 1. Phylogenetic generalized least squares (PGLS) and standardized major axis (SMA) regressions testing the correlation between dissection and endocranial volume estimates of whole-brain, olfactory bulbs, cerebellum, and cerebral cortex size. Regressions were run with both absolute and relative volumes

			n	λ	*P*	R^2	Slope	F	*p*
PGLS	Absolute	Brain volume	83	0.028	0.540	0.979	1.012	3623	<0.001
		Olfactory bulb	75	0.144	0.077	0.888	0.937	576.4	<0.001
		Cerebellum	75	0.547	<0.001	0.914	0.999	786.5	<0.001
		Cerebral cortex	75	0.000	1.000	0.982	0.941	3695	<0.001
	Relative	Brain volume	83	0.000	1.000	0.797	1.056	314.5	<0.001
		Olfactory bulb	75	0.254	0.017	0.493	0.788	72.56	<0.001
		Cerebellum	75	0.530	<0.001	0.361	0.929	42.21	<0.001
		Cerebral cortex	75	0.848	<0.001	0.233	0.821	23.2	<0.001
SMA	Absolute	Brain volume	83			0.980	1.023		<0.001
		Olfactory bulb	75			0.923	0.987		<0.001
		Cerebellum	75			0.938	1.051		<0.001
		Cerebral cortex	75			0.982	0.951		<0.001
	Relative	Brain volume	83			0.769	1.591		<0.001
		Olfactory bulb	75			0.688	1.007		<0.001
		Cerebellum	75			0.769	1.591		<0.001
		Cerebral cortex	75			0.857	1.052		<0.001

showing a different slope and relatively low coefficients of determination.

The model including call type was within 0.4% of error of falling within our significance threshold of $\alpha = 0.05$ for the brain-endocast correlation of olfactory bulb volume. Given this, we further explored the effect of call type, fitting a phylogenetic regression for each call type category separately, revealing a significant correlation between brain and endocranial estimates of olfactory bulb volume and no phylogenetic signal across all categories (Tables S5 and S6). MH and CF bats showed the most different slopes (flattest and steepest, respectively), along with the weakest correlations ($R^2 = 0.232$ and 0.641, respectively). MH bats showed more dispersion from the line of best-fit, possibly explaining their weak correlation. Due to the unique echolocating flexibility of *Noctilio albiventris*, we repeated our PGLS for CF bats without it, obtaining similar results ($R^2 = 0.496$, $F = 8.871$, $p = 0.021$, slope = 1.073).

Since MH bats include both animal and plant eating species, we decided to explore the olfactory bulb brain-endocranial relative volume correspondence on each dietary group within MH bats. Phylogenetic regressions revealed a lack of phylogenetic signal in the models of both dietary groups ($\lambda = 0$, $p = 1$), and a statistically significant correlation between olfactory bulb brain-endocranial volume estimates only in animal-eating MH bats (animal-eating: $R^2 = 0.468$, $F = 18.55$, $p < 0.001$; plant-eating: $R^2 = -0.093$, $F = 0.145$ $p = 0.709$).

All our results from our phylogenetic analyses were consistent when run using different phylogenies, signaling low sensitivity to phylogenetic uncertainty (Tables S4–S6).

DISCUSSION

Our comparison of dissection- and endocranial-based estimates of brain size in bats revealed a strong correspondence between these methods. However, we found statistically significant differences in absolute estimates of cerebral cortex volume. This part of the bat endocast is difficult to unambiguously differentiate from other regions based on presence and/or morphology of the rhinal sulcus and unclear boundaries between neocortex and other brain regions.[32] Contrary to our predictions, we found strong evidence in support of ecological factors mediating the brain-endocast correspondence, signaling a level of evolvability in the skull-brain complex previously underexplored in bats, and which we might also expect to see in other ecologically diverse mammal groups. Moreover, our results indicate that different ecological factors shape the brain-endocast correlation across brain regions and over evolutionary time, and that this is reflected in the modular structure of the brain.[37,38]

We also identified a significant effect of diet and type of echolocation call on brain-endocast covariation, even after accounting for the effect of phylogenetic relatedness and allometry. A significant effect of diet on brain-endocast cerebral cortex absolute volume correlation could indicate that changes in brain-endocast covariation might have facilitated evolution of greater brain sizes in plant-eating bats.[39] An interplay between diet and echolocation ability and call type shaping the brain-endocast correspondence of the olfactory bulbs in our results signals a potential evolutionary trade-off between olfaction and echolocation, as has been previously suggested.[6,11,12,23] We hypothesize that shifts in the brain-endocast correlation might have facilitated sensory diversification in bats and in turn the evolution of novel dietary and echolocating strategies.

Correspondence between brain size estimates

The strong correlation between dissection-based brain size estimates and endocranial volumes in our sample shows that both

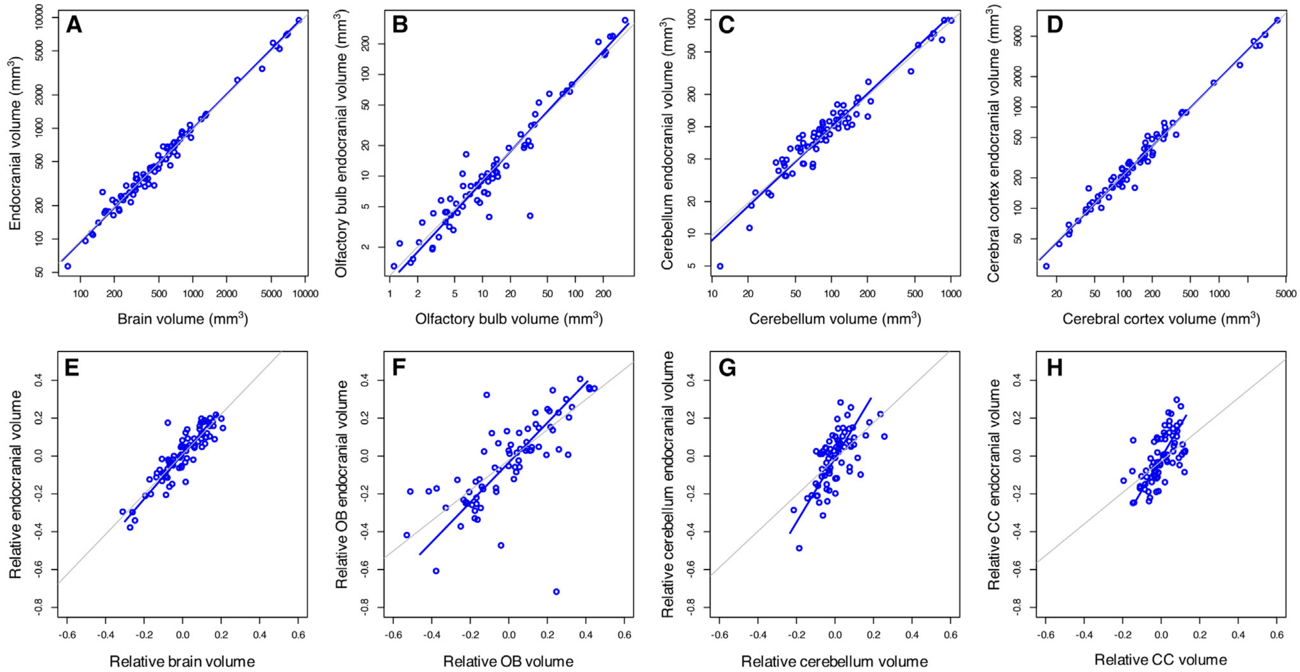

Figure 2. Correspondence between dissection and endocranial volumes

Dissection-endocast correspondence for the whole brain (A and E), olfactory bulbs (OB; B and F), cerebellum (C and G) and cerebral cortex (CC; D and H). Dissection-endocast correspondence was assessed both in absolute values (top row, A–D) and relative values (bottom row, E–H). All analyses were performed with (gray line) and without (blue line) phylogenetic correction.

approaches approximate chiropteran brain size variation patterns similarly, as demonstrated by all slopes having values close to 1 (0.94–1.05, see Table 1). Slopes of the correlation between dissection-based and endocranial absolute volumes remained constant across SMA and PGLS regressions, whereas slopes of regressions of relative dissection and endocranial volumes changed when controlling for phylogenetic relatedness. However, significant differences in absolute values of cerebral cortex volumes reveal that mixing brain size estimates might introduce unwanted sources of variation and that caution should thus be taken.[17,32]

The pattern of decreasing differences in cerebral cortex absolute volume estimates with increasing body size also suggests that differences between dissection-based and endocranial brain size estimates might impact the study of bats and other small mammals disproportionally. Given the anatomy of the bat endocast and the difficulty to unambiguously differentiate the cerebral cortex from other brain regions it is possible that differences in endocranial- and dissection-based volume estimates are partly methodological. One study used a mix of dissection-based brain size estimates from a study by Baron et al.[27] and endocranial volumes to reconstruct the evolution of brain size in bats,[29] illustrating the potential utility of using endocranial morphology to understand macroevolutionary trajectories. Based on our results, we emphasize the importance of considering methodological differences when combining different lines of evidence to draw conclusions on historical evolutionary processes.

Table 2. Phylogenetic generalized least squares regressions testing how dissection and endocranial volume absolute estimates of whole-brain, olfactory bulbs, cerebellum and cerebral cortex volume correlate with body mass

		n	λ	P	R^2	Slope	F	p
Brain volume	Dissection	83	0.918	<0.001	0.923	0.685	983.4	<0.001
	Endocast	83	0.810	<0.001	0.8883	0.694	653.4	<0.001
Olfactory bulb	Dissection	75	0.931	<0.001	0.724	0.758	198.1	<0.001
	Endocast	75	0.622	<0.001	0.621	0.765	135.1	<0.001
Cerebellum	Dissection	75	1.000	<0.001	0.875	0.650	526	<0.001
	Endocast	75	0.804	<0.001	0.760	0.650	260.7	<0.001
Cerebral cortex	Dissection	75	0.934	<0.001	0.910	0.769	760.7	<0.001
	Endocast	75	0.855	<0.001	0.844	0.715	443.7	<0.001

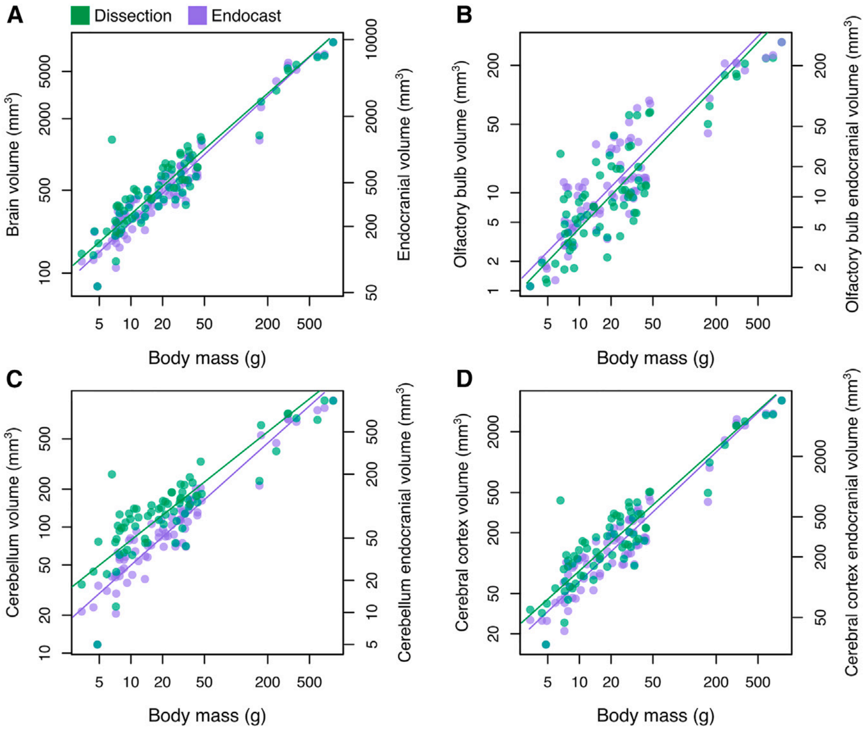

Figure 3. Allometric regressions of dissection and endocranial absolute estimates of brain volumes

Whole brain (A), olfactory bulb (B), cerebellum (C), and cerebral cortex (D).

Allometry

Dissection- and endocranial-based absolute estimates of brain volume showed similar allometric trajectories across brain regions, even after controlling for phylogenetic signal, except for the cerebral cortex. Differences in allometric slopes of dissection-based and endocranial cerebral cortex volumes reveal different scaling patterns with body size, suggesting a decoupling in the phenotypic evolution of the brain and the endocranium in this region.[40,41] Shifts in allometric trajectories of the brain have been linked to morphofunctional diversification of the brain in primates and birds.[40,42] Also, differences in the allometric slopes of the cerebral cortex volumes reflect our results that showed body size-driven differences in absolute cerebral cortex volume estimates.

Changes in allometric relationships can facilitate morphological variability, promoting phenotypic evolvability and diversification.[43] Unique allometric slopes across brain regions might also reveal that the evolution of the modular structure of the brain might have been linked to lability of allometric relationships.[37,38,40]

Effect of diet on the cerebral cortex

Diet-mediated modifications in the brain-endocast correspondence of the cerebral cortex support our hypothesis that ecological adaptations correlate with variations in the skull-brain complex throughout bat evolution. The steeper slope found in plant-eating bats, compared to animal-eating bats, may be related to the enlarged brains of frugivorous bats,[29,39,44,45] indicating that a shift in the brain-endocast covariation was part of the neuroecological diversification of frugivorous bats. Bigger brains in plant-eating bats support the absence of an evolutionary trade-off between the size of the brain and other expensive tissues (e.g., intestine[46]), as has been found in small mammals and counter to the pattern seen in larger mammals like primates.[47] However, given the repeated evolution of phytophagy in bats (i.e., phyllostomid and pteropodid bats, perhaps more than once in the former group[31]), further studies are necessary to assess whether the same pattern is shared across all phytophagous clades. Stemming from an insectivorous ancestor, sensory adaptations have been identified as functional novelties important for the evolution of plant-eating bats.[6,11,12]

Moreover, greater phenotypic disparity and higher rates of phenotypic evolution have been found in plant-based diets,[1,3,48] revealing the critical role of diet for the diversification of bats.[49] An effect of diet on the brain-endocast correspondence has been described in other tetrapods, possibly as a result of bone enforcement for the attachment of masticatory musculature constraining morphological variation.[24] It remains to be tested whether the brain-endocast disparity of the cerebral cortex can be correlated to sensory specializations for flight and echolocation,[8] or if it could be a product of richer phytophagous diets facilitating brain enlargement.[50]

Effect of echolocation on the olfactory bulbs

Across groups and even between species, the olfactory bulbs are one of the most morphologically disparate regions of the mammalian brain, showing some of the highest rates of evolution among brain regions.[51,52] In bats, increased size of the olfactory bulbs has been interpreted as a sensory adaptation to frugivorous diets, linked to selective pressures for increased olfactory capabilities.[12] Stemming from a presumed insect-eating ancestor, sensory adaptations to detect new food sources (e.g., fruits and nectar) have been correlated with the ecomorphological and taxonomic diversification of bats.[12,53,54] Generalist dietary ecologies might have helped the transition from an insectivorous ancestor to other specialized diets, like nectarivory, frugivory, and sanguivory.[12] Additionally, crucial morphological adaptations linked to the laryngeal echolocation of bats are present in the same region of the skull where the olfactory bulbs sit.[6,23]

The interaction between selective pressures for olfactory and echolocating capabilities might have acted as an evolutionary trade-off, where the morphology of structures linked to olfaction and echolocation covaried and coevolved.[6,23] The structure of the nasal chambers and the turbinal bones inside them have

Table 3. Phylogenetic generalized least squares regressions testing competing models of covariation between dissection and endocranial volume estimates of whole-brain, olfactory bulbs, cerebellum, and cerebral cortex volumes

		n	λ	*P*	SS	MS	F	*p*
Diet	Brain volume	83	0	1	0.000	0.000	1.407	0.239
	Olfactory bulb	75	0.154	0.055	0.000	0.000	0.043	0.836
	Cerebellum	75	0.53	<0.001	0.000	0.000	0.038	0.846
	Cerebral cortex	75	0	1	0.001	0.001	4.930	0.030[a]
Echolocation	Brain volume	83	0	1	0.000	0.000	0.215	0.644
	Olfactory bulb	75	0.175	0.039	0.001	0.001	1.722	0.194
	Cerebellum	75	0.515	<0.001	0.000	0.000	0.235	0.629
	Cerebral cortex	75	0.79	0.001	0.000	0.000	1.998	0.162
Duty cycle	Brain volume	83	0	1	0.000	0.000	0.118	0.889
	Olfactory bulb	75	0.18	0.041	0.001	0.001	1.115	0.334
	Cerebellum	75	0.451	<0.001	0.000	0.000	0.738	0.482
	Cerebral cortex	75	0.79	0.002	0.000	0.000	0.974	0.383
Call type	Brain volume	83	0	1	0.000	0.000	0.203	0.894
	Olfactory bulb	75	0	1	0.003	0.001	2.568	0.054[a]
	Cerebellum	75	0	1	0.000	0.000	0.708	0.550
	Cerebral cortex	75	0.79	0.004	0.000	0.000	0.663	0.578

[a] Significant *p* values.

been linked to both echolocation and olfaction in bats, signaling a trade-off between both senses.[54,55] Externally, the morphological diversification of the nose (and its associated nose leaf in phyllostomid and most rhinolophoid bats) might have had an important role in the evolution of cerebral adaptations for olfaction.[23]

A steeper slope in the brain-endocast correlation of the relative size of the olfactory bulbs in CF bats could be evidence of this echolocation-olfaction evolutionary trade-off.[23] Almost all CF bats are likely nasal emitters, presenting morphological adaptations in the nasal region of the skull (i.e., complete nostril separation) for echolocation,[23,56] which in turn could have shaped modifications of olfactory bulbs tissue over evolutionary time. The differences in the presence and direction of a significant brain-endocast correlation between animal-eating and plant-eating MH bats indicate an interplay between dietary and echolocating strategies shaping the evolution of the olfactory bulbs. Most phytophagous MH bats exhibit some flexibility in how they emit their echolocation calls.[57,58] Species' diet characterization into only two groups was due to uneven and small sample sizes across finer categories (e.g., carnivorous, piscivorous, and nectarivorous), but future studies should explore this pattern at more refined ecological scales. The decoupling of the brain and endocranial variation of the olfactory bulbs in MH phytophagous bats might signal a connection between skull-brain covariation and behavioral flexibility. However, given the remarkable diversity of MH phytophagous bats, our results should be further tested in detail for this group of bats. Combined, we hypothesize that our results provide evidence of two different instances of evolutionary trade-offs between olfactory and echolocating structures, where changes in skull-brain covariation facilitate the evolution of ecological innovations.[14,23,24]

Conclusions

In summary, we show that dissection-based and endocranial estimates of brain size are highly correlated and follow similar variation patterns. Our results also support the idea that both are accurate proxies for the study of bat brain ecology and evolution. However, differences in absolute values of the cerebral cortex indicate that caution should be taken when combining methods within a single framework. Our results also suggest that the brain-endocast correspondence changes in response to different selective pressures, revealing an understudied evolutionary pathway of ecomorphological diversification in bats. Region-specific patterns of brain-endocast covariation reveal idiosyncratic evolutionary pathways, in line with the mosaic brain evolution hypothesis.[37,38] A significant effect of diet on the brain-endocast correspondence of the cerebral cortex could be evidence of different evolutionary pathways of brain size in phytophagous and animalivorous bats.[39,44,59] Specifically, we found differences in the brain-endocast correlation of the olfactory bulbs in two bat groups, signaling two independent evolutionary shifts. Since our data come from different sources (i.e., brain and endocranial measurements do not come from the same individuals), the effect of inter-individual variability in brain-endocranial correlations should be further explored in future studies. We hypothesize that these shifts represent unique cases of functional trade-offs between olfaction and echolocation shaping the evolution of the brain and skull of bats. Further studies are needed to explore the evolvability of the brain-endocast correspondence in other mammals and tetrapods.

Limitations of the study

While our dataset represents a comprehensive sample of bat species, eight bat families are not included in this study.

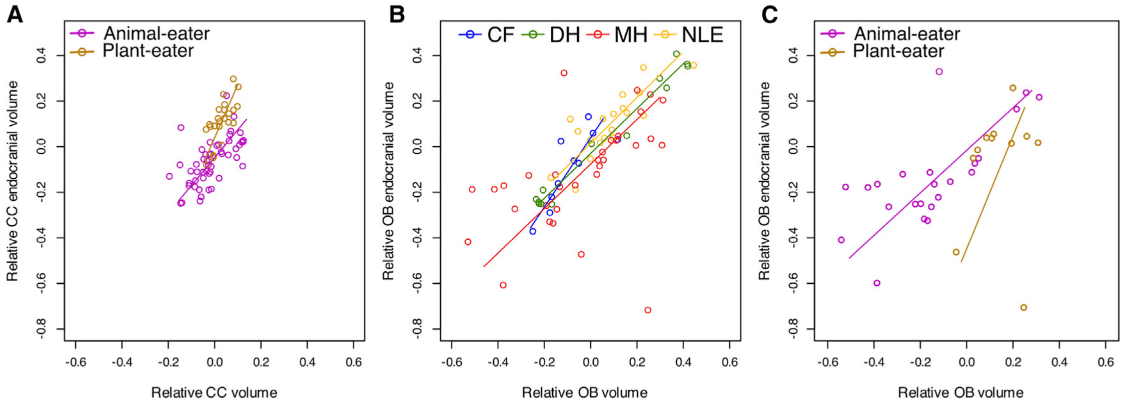

Figure 4. Phylogenetic regressions testing the correlation between dissection and endocranial-based estimates

Cerebral cortex (CC, A) and olfactory bulbs (OB) volumes and the effect of call type (B) and diet (C).

Similarly, the representation of phytophagous bats is limited to 24 taxa, which limits our capacity to assess dietary patterns at a finer ecological scale. Additionally, the different sources of dissection-based and endocranial-based estimates of brain volume used in this study could under- or overestimate intraspecific and interindividual variation. Hence, we are not able to provide correction factors or equations to estimate brain size based on endocranial or dissection estimates.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Camilo López-Aguirre (c.lopezaguirre@utoronto.ca).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All the raw data have been deposited at the Dryad Digital Repository and can be accessed using the following link: https://doi.org/10.5061/dryad.905qfttsr.
- All original R code has been deposited at the Dryad Digital Repository and can be accessed using the following link: https://doi.org/10.5061/dryad.905qfttsr.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, C.L.-A., J.M.R., and M.T.S.; methodology, C.L.-A., B.A., and M.M.; investigation, C.L.-A.; writing – original draft, C.L.-A.; writing – review & editing, C.L.-A., B.A., M.M., J.M.R., and M.T.S.; funding acquisition, C.L.-A., J.M.R., and M.T.S.; resources, C.L.-A., J.M.R., and M.T.S.; supervision, C.L.-A., J.M.R., and M.T.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw data	This paper	https://doi.org/10.5061/dryad.905qfttsr
Phylogeny	Upham et al.[36]	https://doi.org/10.1371/journal.pbio.3000494
Software and algorithms		
R (version 4.3.0)	R Core Team	https://cran.r-project.org/bin/macosx/
AVIZO (version 9.1.1)	Thermo Fisher Scientific	https://www.thermofisher.com/co/en/home/electron-microscopy/products/software-em-3d-vis/avizo-software.html

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

All samples used in this study were taken from pre-existing digital sources, all experiments were purely computational, and no physical subjects, living or dead, were used in this work.

METHOD DETAILS

Endocranial reconstruction and measurement

Virtual endocasts of 83 bat species representing all 5 extant bat superfamilies (Table S1) were reconstructed using published CT scans of bat crania made available on Morphosource.[60] Our sample comprised 13 of 21 bat families, a broad body mass range (3.4–840g) and all major dietary adaptations (carnivory, frugivory, insectivory, nectarivory, sanguivory; Figure 1). Only adult specimens (fully fused cranial sutures and complete dental eruption) with complete endocranial preservation (no cracks or deformation of bones forming the braincase) were selected. Reconstruction of endocasts was performed using the software AVIZO v. 9.1.1 using the magic wand tool to select the endocranially space based on a set threshold, and manually tracing a line to close any openings between the endocranial cavity and any other external cavity. All virtual endocasts were segmented in frontal view.[61] The interpolate tool was used to automatically segment the endocranial cavity between two individually segmented slices (separated only by one slice). This approach reduces CT scan data processing times without compromising resolution of the final virtual model.

Following anatomical descriptions of endocranial correlates of brain morphology in bats,[32] we sub-segmented partial virtual endocasts to isolate the following neuroanatomical regions: the olfactory bulbs, cerebral cortex and cerebellum (Figure S1). Olfactory bulbs (involved in processing of olfactory stimuli[62]) were segmented at the narrowest point of the circular fissure on lateral view. The cerebral cortex (involved in multiple cognitive functions[38]) here is defined as the sum of the neocortex and paleocortex, and was measured as a unit in light of the variable presence and morphology of the rhinal sulcus in bat endocasts[32] that limits our capacity to accurately differentiate the neocortex and paleocortex from endocranial data. Cerebral cortices were segmented anteriorly along the circular fissure and posteriorly along the transverse and temporal sinuses. We removed the hypophysis from the ventral side of the endocast on frontal view.

The cerebellum (involved in motor control[63]) was segmented anteriorly along the groove marking the posterior edge of the mesencephalon, posteriorly along the groove marking the bottom of the vermis and laterally along the grooves marking the edges of the cerebellar hemispheres. Volumes of whole endocasts and each neuroanatomical region were measured in mm^3 using the surface area volume module in AVIZO v. 9.1.1.

Brain volume and body mass data acquisition

Absolute whole-brain mass and brain region volumes for the olfactory bulbs, cerebellum and cerebral cortex were retrieved from.[27] Brain region volumes were estimated based on the area occupied by each region on a sample of slices performed by.[27] Absolute brain mass values were converted to volume values (mm^3) by dividing by density of fresh brain tissue (1.036 g/mL), following.[64] Cerebral cortex volumes were calculated by summing neocortex and paleocortex values reported in.[27] Body mass data were retrieved from.[27,65] Species were pooled into three body mass categories; small (3.4–10.3g), medium-sized (10.31–27.2g) and large (27.21–840g). These categories were defined by classifying the body mass values into three groups of equal frequencies, ensuring each category has enough samples for robust statistical comparisons.

We estimated relative whole-brain volume by extracting the residuals of a SMAz regression of whole-brain volume against body mass. Relative brain region-specific volumes were estimated by extracting the residuals of an SMA regression of each brain region's volume against the remainder brain volume (e.g., whole-brain volume minus the volume of the region of interest). All SMA regressions were performed in R, using the package smatr.[66]

Biological characterization of species

Information of species' diet, echolocation category, call type and duty cycle were gathered from the literature.[1,31,56,67] Species were classified as either animal-eating or plant-eating, based on their diets' compositions. Species were assigned to one of two echolocation categories: laryngeal echolocator or non-laryngeal echolocator (NLE). For laryngeal echolocating species, call type was categorized following[31] as multi-harmonic (MH), downward sweeping DH, and CF. Duty cycle was categorized as low (LDC) or high (HDC), following.[56]

Statistical analyses

We applied phylogenetically informed and non-phylogenetic statistical analyses to assess brain-endocast correspondence, the former with PGLSz regressions, the latter with SMA regressions as described above. We used Shi & Rabosky's 2015 bat phylogeny for all phylogenetic analyses.[68] Log_{10}-transformed values of absolute brain volumes and body masses were used for all statistical analyses.

First, we assessed brain-endocast correspondence, both in absolute and relative values, regressing endocranial volumes against brain volumes. This was performed for the whole brain and for each neuroanatomical region separately. We applied Wilcoxon's signed-rank tests to examine whether absolute brain and endocast volumes are significantly different across neuroanatomical regions. Second, we assessed whether the brain volume-body mass allometric relationship differs between brain and endocranial estimates. Finally, we assessed whether biological factors might influence the brain-endocast association, regressing endocranial volume against brain volume, including one biological factor (dietary group, echolocation capabilities, call type or duty cycle) as a covariate. This was repeated for whole-brain and each neuroanatomical region. The phylogenetic signal in PGLS regressions was tested using Pagel's λ.

We tested the sensitivity of our results to phylogenetic uncertainty by extracting a sample of 100 pruned phylogenies for the 83 species in our sample from the phylogenetic framework developed in Upham et al.,[36] and a maximum clade credibility tree was retrieved using the function maxCladeCred function in phangorn and used to rerun our phylogenetic analyses.[69] A statistical significance threshold of $\alpha = 0.05$ was used for all our analyses.