



Research

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Evolutionary biology

Bayesian hierarchical models suggest oldest known plant-visiting bat was omnivorous

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The earliest record of plant visiting in bats dates to the Middle Miocene of La Venta, the world's most diverse tropical palaeocommunity. *Palynephyllum antimaster* is known from molars that indicate nectarivory. Skull length, an important indicator of key traits such as body size, bite force and trophic specialization, remains unknown. We developed Bayesian models to infer skull length based on dental measurements. These models account for variation within and between species, variation between clades, and phylogenetic error structure. Models relating skull length to trophic level for nectarivorous bats were then used to infer the diet of the fossil. The skull length estimate for *Palynephyllum* places it among the larger lonchophylline bats. The inferred diet suggests *Palynephyllum* fed on nectar and insects, similar to its living relatives. Omnivory has persisted since the mid-Miocene. This is the first study to corroborate with fossil data that highly specialized nectarivory in bats requires an omnivorous transition.

1. Introduction

Mammals have evolved complex dentition that enables both shearing and grinding occlusion, and these features are key traits in mammalian diversification [1]. The evolution of cusp morphology in response to feeding ecology makes mammalian molars indicators of diet [2,3]. Teeth are so abundant in the fossil record that a substantial portion of extinct mammals are known only from their molars [4]. One mammalian molar can provide enough characters to infer both the phylogeny and diet of the species it represents [5,6].

The oldest known plant-visiting fossil bat, *Palynephyllum antimaster* (Chiroptera: Phyllostomidae), is represented by two molars from the Miocene of La Venta, Colombia [7]. Similar to those of extant nectarivorous phyllostomids, these molars are narrow, and their cusps are reduced lingually and flattened labially [1,8]. The two primarily nectarivorous phyllostomid subfamilies, Glossophaginae and Lonchophyllinae, share adaptations for acquiring nectar including an elongated rostrum and palate, reduced dentition and elongated tongues [8–10]. These phyllostomids compose a phenotypic optimum that prevents eating hard foods such as beetles or figs [11–13]. Behavioural and dietary analyses show these bats vary in nectar specialization. The generalist *Glossophaga soricina* actively seeks both flower resources and insects [14], while the specialized *Leptonycteris yerbabuena* primarily feeds on nectar from columnar cacti and insects are a minor and coincidental component of its diet [15].

Table 1. Parameters estimated from the relationship between skull lengths and molar dimensions, and between trophic level and skull length. Each covariate corresponds to a mean measurement per species *i*. For multi-level regression formulae, parameter estimates vary by subfamily *j*, for either Glossophaginae or Lonchophyllinae. HPD, highest posterior density interval; GLS, greatest length of skull; M2L, tooth length; M2W, tooth width (both of complete second upper molar).

formula	parameter	mean (95% HPD)
$GLS_i \sim \alpha + \beta_1 \cdot M2L_i + \beta_2 \cdot M2W_i + \epsilon_i + \Sigma$	α	2.94 (2.86, 3.03)
	β_1	1.01 (0.64, 1.37)
	β_2	-0.43 (-0.64, -0.21)
	τ_{ind}	401.94 (75.03, 1602)
	median (GLS)	25.63 (22.19, 29.42)
$trophic_i \sim \alpha_{j[i]} + \beta \cdot GLS_i + \epsilon_i + \Sigma$	α_{Gloss}	-0.046 (-0.42, 0.19)
	α_{Lonch}	-0.006 (-0.37, 0.24)
	β	-0.093 (-0.17, 0.03)
	τ_{ind}	74.76 (31.41, 160.34)
	$\tau_{subfamily}$	352.84 (1.55, 1861.6)
	median (trophic)	-0.317 (-0.64, 0.01)

The nectarivorous morphology shared by extant bats obscures other aspects of the evolution from ancestral insectivory [16]. Switching diets from protein-rich insects to carbohydrate-rich nectar requires adaptations in sugar metabolism and kidney function, as well as body-size-dependent strategies for finding enough nectar [17,18]. Several studies have proposed the evolution of diet specialization requires a transition through omnivory [19,20], and we hypothesize that early nectarivores fed on both nectar and insects. Inferring proxies for the body size and dietary composition of *Palynephyllum* can thus illuminate key aspects of the evolutionary transition to a specialized nectar diet.

We developed models to estimate the skull length of *Palynephyllum*, which we then used to infer the trophic level of the fossil. Our analyses take advantage of the flexibility of Bayesian methods to simultaneously model variation among individuals, between species and among clades [21].

2. Material and methods

(a) Estimating skull length

We measured tooth length (M2L) and tooth width (M2W) of the complete second upper molar (M2) of *Palynephyllum* and 31 nectarivorous phyllostomid bat species (25 Glossophaginae; 6 Lonchophyllinae), and the greatest length of skull (GLS) for extant taxa (electronic supplementary material, figure S1). Multiple individuals were measured and measurements were averaged for most species (electronic supplementary material, table S1). All data were log-transformed.

To estimate GLS, we used the mean M2L and M2W of nectarivorous species as covariates in four regression models. The first model fits a single intercept (α) and slope (β) for all the data. The second estimates a single intercept allowing slopes to differ (β_j), where *j* is either the subfamily Glossophaginae or Lonchophyllinae. The third allows the intercepts (α_j) to vary by subfamily and fits a single slope. The fourth allows both slopes and intercepts to differ for subfamilies.

Hierarchical Bayesian models have several advantages compared with standard regressions. First, different sources of

error are estimated separately (summarized as ϵ in table 1). The model accounts for within-species error, error of residual estimation and variation among different subfamilies. Second, the posterior distribution of each parameter can be sampled to estimate the GLS of *Palynephyllum*. Third, by including the variance-covariance matrix from a published phylogeny of Phyllostomidae that unambiguously resolves *Palynephyllum* as a lonchophylline [5,22], we could account for the phylogenetic structure of the covariates (Σ). Few recent studies have implemented phylogenetic Bayesian regressions [21,23]. Here we extend this approach to include both individual and subfamily variation.

The fit of models to observations was compared using posterior predictive checks. Models were selected to minimize differences between predicted and observed data. The posterior distribution of parameters for the best-fit model and the M2L and M2W measurements of *Palynephyllum* were used to estimate GLS for this fossil. We tested differences between predicted and observed posterior distributions.

(b) Estimating palaeodiet

A similar modelling approach was used to infer trophic level from the estimated *Palynephyllum* GLS. We used new and published GLS measurements to fit four phylogenetic regressions to infer trophic level (electronic supplementary material, table S2). The continuous value for trophic level is a quantitative summary of the dietary niche of the species. Values close to zero correspond to omnivorous diets. Highly specialized plant-visiting bats have more negative values, while highly animalivorous bats have more positive values. The trophic level of *Palynephyllum* was estimated by sampling the parameters of the best-fit model. The supplementary methods describe model implementation and validation in detail.

3. Results

The Bayesian hierarchical models show craniodental allometry is similar in the two subfamilies (table 1; electronic supplementary material, figures S2 and S3) [24]. Including phylogenetic relatedness improved GLS model fit (electronic supplementary material, tables S3 and S4). GLS estimates of

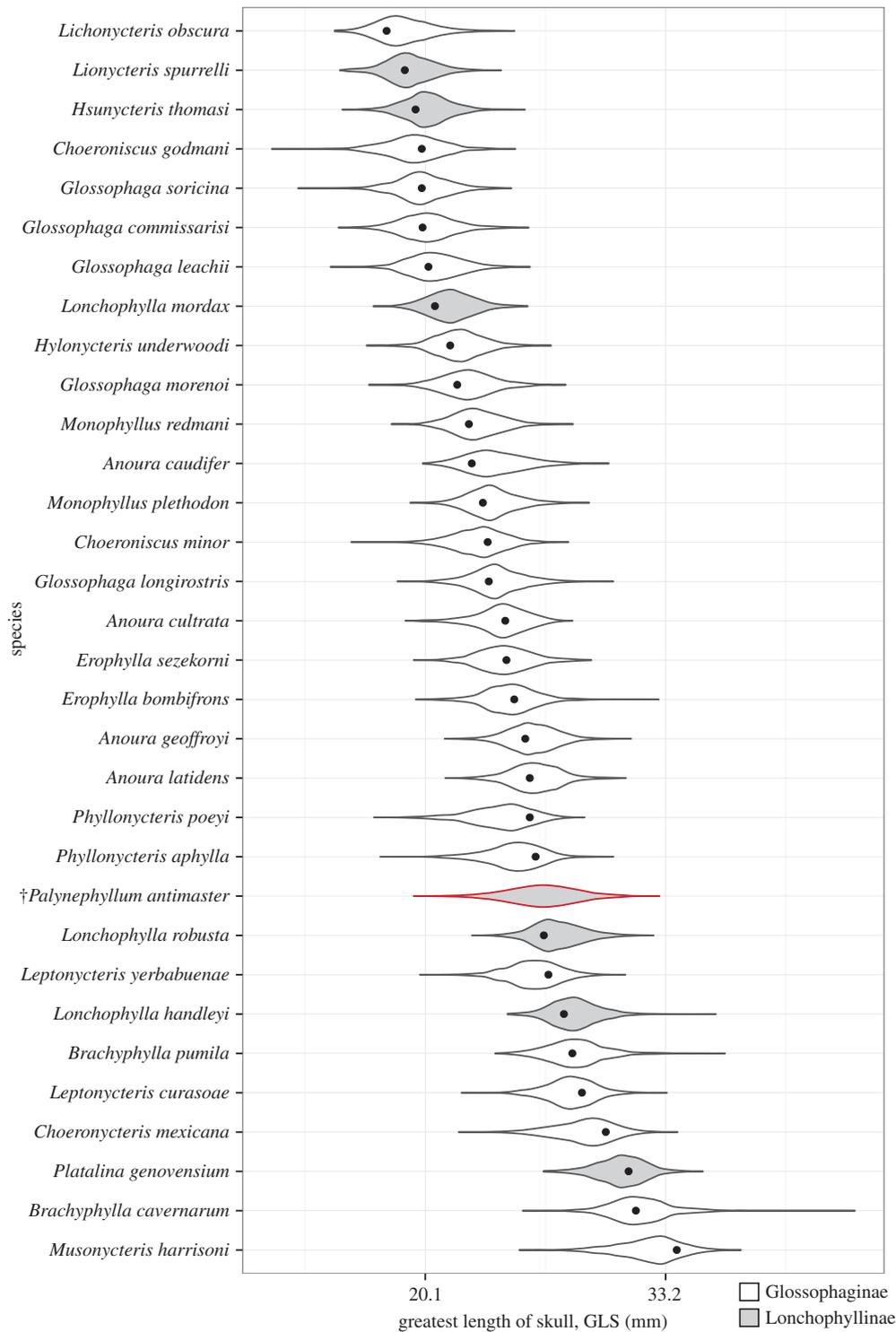


Figure 1. Posterior predicted distributions of the greatest length of skull (GLS) estimates for species of nectar-feeding phyllostomids. Black dots indicate the observed mean value of GLS measurements from the skulls of specimens used in this study. (Online version in colour.)

Palynephyllum from models that account for relatedness have narrower credible intervals than models that do not. The inferred *Palynephyllum* skull length (approx. 26 mm) places this bat among the larger lonchophyllines (figure 1).

The best-fit model of trophic level had a single slope and different intercepts for the two subfamilies (electronic supplementary material, figure S4). The baseline trophic level was distinctly more omnivorous for lonchophyllines than for glossophagines, as indicated by the higher intercept (median $\alpha_{\text{Gloss}} - 0.046$; $\alpha_{\text{Lonch}} - 0.006$). The slope coefficient suggested a negative relationship for both groups between trophic position and GLS; however, the slope posterior distribution

included zero (table 1). The trophic level inferred for the fossil (approx. -0.32) suggests *Palynephyllum* included insects and fruit in its diet. Given the fair predictive power of the model (electronic supplementary material, figure S5), exclusive nectarivory can be rejected (figure 2).

4. Discussion

Similar to robust extant lonchophyllines known for their strong bites relative to specialized nectarivores [25], the diet of the oldest known nectarivorous bat fossil probably included

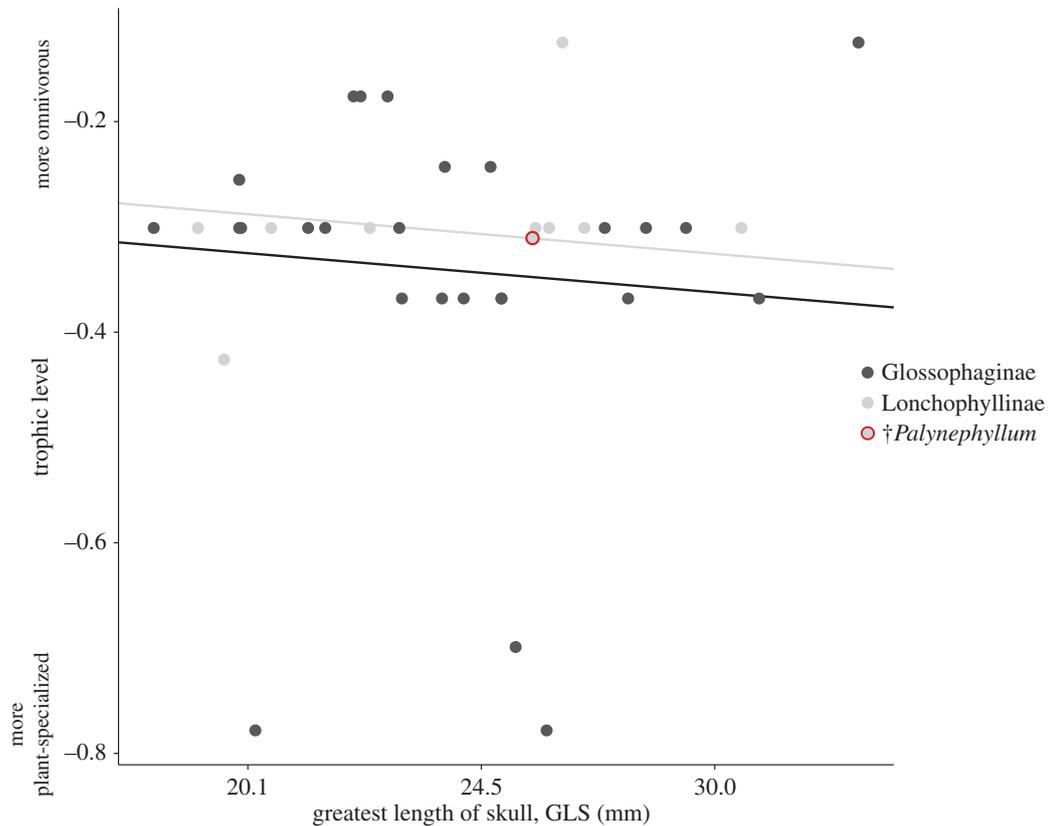


Figure 2. Observed mean values of the greatest length of skull (GLS) and the observed trophic level values. Plotted in the red circle is the predicted GLS of *Palynephyllum antimaster* with the trophic level inferred from the best-fit model. (Online version in colour.)

insects, fruit and nectar. This is also consistent with dental similarities between *Palynephyllum* and the known omnivore *Hylonycteris* [17]. Modest molar crests are present in other nectarivorous bats that feed on insects [6,7]. The models suggest the ecological niche for this large, omnivorous nectarivore has persisted since at least the mid-Miocene.

The La Venta fauna of Colombia (11.6–13.5 Ma) is one of the most diverse Cenozoic vertebrate fossil biotas [6]. Eight animalivorous bat species have been discovered [7], implying a diversity of dietary niches were already exploited. Phylogenies show some level of nectarivory evolved before dedicated frugivory [12]. How insectivorous bats first included nectar in their diets remains poorly understood. An abrupt transition to nectar feeding is unlikely because high metabolic rates require adaptations beyond nectar acquisition [26,27]. Nectarivores survive at the upper limit of the mammalian energy budget, with physiological adaptations that allow them to rapidly convert sugar into energy [28,29]. A mixed diet of nectar and insects, as inferred for *Palynephyllum*, mitigates

the dramatic shift in metabolic rates and corroborates previous hypotheses of an omnivorous transition prior to nectar specialization [19,20]. By inferring the skull length and trophic level for *Palynephyllum*, we introduce, we believe, the first fossil evidence supporting this prediction.

Data accessibility. Data, R code for models and figures are available on Dryad (<http://dx.doi.org/10.5061/dryad.1168f>).

Authors' contributions. L.R.Y. developed all models. L.M.D. and L.R.Y. designed the study. P.M.V., N.B.S. and B.E.G. collected data. D.R. contributed trophic data. L.R.Y., D.R. and L.M.D. wrote the manuscript and all authors contributed to revisions. All authors interpreted data.

Competing interests. We declare we have no competing interests.

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References

- Ungar PS. 2010 *Mammal teeth: origin, evolution, and diversity*. Baltimore, MD: JHU Press.
- Price SA, Hopkins SSB, Smith KK, Roth VL. 2012 Tempo of trophic evolution and its impact on mammalian diversification. *Proc. Natl Acad. Sci. USA* **109**, 7008–7012. (doi:10.1073/pnas.1117133109)
- Hunter JP, Jernvall J. 1995 The hypocone as a key innovation in mammalian evolution. *Proc. Natl Acad. Sci. USA* **92**, 10 718–10 722. (doi:10.1073/pnas.92.23.10718)
- Luo Z-X. 2007 Transformation and diversification in early mammal evolution. *Nature* **450**, 1011–1019. (doi:10.1038/nature06277)
- Dávalos LM, Velasco PM, Warsi OM, Smits PD, Simmons NB. 2014 Integrating incomplete fossils by isolating conflicting signal in saturated and non-independent morphological characters. *Syst. Biol.* **63**, 582–600. (doi:10.1093/sysbio/syu022)
- Santana SE, Strait S, Dumont ER. 2011 The better to eat you with: functional correlates of tooth structure in bats. *Funct. Ecol.* **25**, 839–847. (doi:10.1111/j.1365-2435.2011.01832.x)
- Czaplewski NJ, Takai M, Naeher TM, Shigehara N, Setoguchi T. 2003 Additional bats from the middle Miocene La Venta fauna of Colombia. *Rev. Acad. Colomb. Cienc. Exact. Fis. Nat.* **27**, 263–282.
- Freeman PW. 1995 Nectarivorous feeding mechanisms in bats. *Biol. J. Linn. Soc.* **56**, 439–463. (doi:10.1111/j.1095-8312.1995.tb01104.x)

9. Harper CJ, Swartz SM, Brainerd EL. 2013 Specialized bat tongue is a hemodynamic nectar mop. *Proc. Natl Acad. Sci. USA* **110**, 8852–8857. (doi:10.1073/pnas.1222726110)
10. Datzmann T, von Helversen O, Mayer F. 2010 Evolution of nectarivory in phyllostomid bats (Phyllostomidae Gray, 1825, Chiroptera: Mammalia). *BMC Evol. Biol.* **10**, 165. (doi:10.1186/1471-2148-10-165)
11. Dumont ER, Samadevam K, Grosse I, Warsi OM, Baird B, Davalos LM. 2014 Selection for mechanical advantage underlies multiple cranial optima in New World leaf-nosed bats. *Evolution* **68**, 1436–1449. (doi:10.1111/evo.12358)
12. Dumont ER, Dávalos LM, Goldberg A, Santana SE, Rex K, Voigt CC. 2012 Morphological innovation, diversification and invasion of a new adaptive zone. *Proc. R. Soc. B* **279**, 1797–1805. (doi:10.1098/rspb.2011.2005)
13. Monteiro LR, Nogueira MR. 2011 Evolutionary patterns and processes in the radiation of phyllostomid bats. *BMC Evol. Biol.* **11**, 137. (doi:10.1186/1471-2148-11-137)
14. Clare EL, Goerlitz HR, Drapeau VA, Holderied MW, Adams AM, Nagel J, Dumont ER, Hebert PDN, Brock Fenton M. 2014 Trophic niche flexibility in *Glossophaga soricina*: how a nectar seeker sneaks an insect snack. *Funct. Ecol.* **28**, 632–641. (doi:10.1111/1365-2435.12192)
15. Cole FR, Wilson DE. 2006 *Leptonycteris yerbabuena*. *Mamm. Species* **797**, 1–7. (doi:10.1644/797.1)
16. Rojas D, Vale Á, Ferrero V, Navarro L. 2011 When did plants become important to leaf-nosed bats? Diversification of feeding habits in the family Phyllostomidae. *Mol. Ecol.* **20**, 2217–2228. (doi:10.1111/j.1365-294X.2011.05082.x)
17. Tschapka M. 2004 Energy density patterns of nectar resources permit coexistence within a guild of Neotropical flower-visiting bats. *J. Zool.* **263**, 7–21. (doi:10.1017/S0952836903004734)
18. Fleming TH, Geiselman C, Kress WJ. 2009 The evolution of bat pollination: a phylogenetic perspective. *Ann. Bot.* **104**, 1017–1043. (doi:10.1093/aob/mcp197)
19. Gillette DD. 1975 Evolution of feeding strategies in bats. *Tebuiwa* **18**, 39–48.
20. Ferrarezzi H, Gimenez EA. 1996 Systematic patterns and the evolution of feeding habits in Chiroptera (Archonta: Mammalia). *J. Comp. Biol.* **1**, 75–94.
21. De Villemereuil P, Wells JA, Edwards RD, Blomberg SP. 2012 Bayesian models for comparative analysis integrating phylogenetic uncertainty. *BMC Evol. Biol.* **12**, 102. (doi:10.1186/1471-2148-12-102)
22. Paradis E, Claude J, Strimmer K. 2004 APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290. (doi:10.1093/bioinformatics/btg412)
23. Jacquemin SJ, Doll JC. 2014 Body size and geographic range do not explain long term variation in fish populations: a Bayesian phylogenetic approach to testing assembly processes in stream fish assemblages. *PLoS ONE* **9**, e93522. (doi:10.1371/journal.pone.0093522)
24. Griffiths TA. 1982 Systematics of the New World nectar-feeding bats (Mammalia, Phyllostomidae), based on the morphology of the hyoid and lingual regions. *Am. Mus. Novit.* **2742**, 1–45. (doi:10.1097/01.MPA.0000020657.86805.1B)
25. Fleming TH, Hooper ET, Wilson DE, Hooper T. 1972 Three Central American bat communities: structure, reproductive cycles, and movement patterns. *Ecology* **53**, 556–569. (doi:10.2307/1934771)
26. McNab BK. 2003 Standard energetics of phyllostomid bats: the inadequacies of phylogenetic-contrast analyses. *Comp. Biochem. Physiol. A* **135**, 357–368. (doi:10.1016/S1095-6433(03)00090-4)
27. Anderson KJ, Jetz W. 2005 The broad-scale ecology of energy expenditure of endotherms. *Ecol. Lett.* **8**, 310–318. (doi:10.1111/j.1461-0248.2005.00723.x)
28. Voigt CC, Speakman JR. 2007 Nectar-feeding bats fuel their high metabolism directly with exogenous carbohydrates. *Funct. Ecol.* **21**, 913–921. (doi:10.1111/j.1365-2435.2007.01321.x)
29. Ayala-Berdon J, Schondube JE. 2015 A physiological perspective on nectar-feeding adaptation in phyllostomid bats. *Physiol. Biochem. Zool.* **84**, 458–466. (doi:10.1086/661541)