Find the Food First: An Omnivorous Sensory Morphotype Predates Biomechanical Specialization for Plant Based Diets in Phyllostomid Bats

Abstract

The role of mechanical morphologies in the exploitation of novel niche space is well characterized, however, the role of sensory structures in unlocking new niches is less clear. Here we investigate the relationship between the evolution of sensory structures and diet during the radiation of noctilionoid bats. With a broad range of foraging ecologies and a well-supported phylogeny, noctilionoids constitute an ideal group for studying this relationship. We used diffusible iodine-based contrast enhanced computed tomography (diceCT) scans of 44 noctilionoid species to analyze relationships between the relative volumes of three sensory structures (olfactory bulbs, orbits, and cochleae) and diet. We found a positive relationship between frugivory and both olfactory and orbit size. However, we also found a negative relationship between nectarivory and cochlea size. Ancestral state estimates suggest that larger orbits and olfactory bulbs were present in the common ancestor of family Phyllostomidae, but not in other noctilionoid. This constellation of traits indicates a shift toward omnivory at the base of Phyllostomidae, predating their radiation into an exceptionally broad range of dietary niches. This is consistent with a scenario in which changes in sensory systems associated with foraging and feeding set the stage for subsequent morphological modification and diversification.

Keywords: morphology, sensory, vision, olfaction, comparative analyses

Introduction

In the presence of ecological opportunity, novel morphological alterations can facilitate the exploration and exploitation of new niches. Divergence within these new niches may result in increased morphological disparity across lineages over time (Schluter 2000). Many studies of such disparity have focused on skeletal elements of morphology that enhance fitness by improving ecological performance (Herrel and Holanova 2008; Santana and Dumont 2009; Arnold 2015; Grant and Grant 1993; Schluter and Price 1997) and morphological divergence in response to diverse food types is a hallmark of well-studied radiations including those of Darwin's finches (Fyer and Iles 1972), cichlid fishes (Schluter 2000), and bats ((Freeman 2000; Baker et al. 2012; Dumont et al. 2012; Santana et al. 2012; Hedrick and Dumont 2018) In the limited cases in which sensory structures have been a focus of study within morphologically disparate lineages, that focus has been directed to the role of sensory structures in sexual selection, such as in sensory drive in cichlids (Seehausen et al. 2008), or in the context of environmental change, as in the effects of climate on echolocation frequency (Jacobs et al. 2017). Literature on insect sensory structures have made great strides in using these sensory systems to understand biochemical and neurological aspects of sensing (Beuhlmann et al. 2020, Martin et al. 2011). Researchers have even reached into the past to investigate the competitive advantage of sightedness in trilobites (Henze and Oakley, 2015). Nevertheless, sensory structures play key roles in foraging for food and the relationship between shifting diets, diversifying

45 foraging strategies, and associated morphological specializations has yet to be outlined. In this
46 study we examine a group of mammals – the neotropical leaf-nosed bats – that occupy a distinct
47 yet impressively broad morphospace among bats while exhibiting unusually broad dietary
48 diversity (Freeman 2000; Dumont et al. 2012, 2014; Shi and Rabosky 2015; Hedrick et al. 2019),
49 in an attempt to test a model of ecological radiation in which sensory structure changes occur
50 before subsequent mechanical specialization.

51

52 The size of sensory structures in vertebrates is directly related to their functionality. In
53 fishes, birds, and mammals, larger eyes are related to greater visual acuity (Müller and Peichl
54 2005; Müller et al. 2007; Land and Nilsson 2012; Eklöf et al. 2014; Veilleux and Kirk 2014;
55 Sadier et al. 2018). Larger olfactory bulbs in at least mammals and birds support more expansive
56 epithelia and therefore larger surface areas for odor detection (Barton et al. 1995; Corfield et al.
57 2015). Finally, cochlear volume correlates with aspects of cochlear morphology that impact
58 hearing performance (Kirk and Gosselin-Ildari 2009; Kössl and Vater 2011; Vater and Kössl
59 2011). Mammals with derived echolocation capabilities have enlarged cochleae relative to their
60 body size (Kössl and Vater 1985; Davies et al. 2013a,b) while those that lack such capabilities
61 have much smaller cochleae relative to their size (Vater and Kössl 2011). Despite the apparent
62 relationship between sensory structures and food procurement , the evolutionary history of
63 sensory structures is poorly understood.

64 An ideal group to study the evolution of foraging and the evolution of sensory structures
65 would consist of closely related lineages that display drastically different foraging strategies and
66 diets. Noctilionoid bats (Superfamily Noctilionoidea) are a well-studied example of such dietary
67 disparity, collectively exhibiting more dietary strategies than any single mammalian order,

68 despite being a clade of only five closely related families. Among the five noctilionoid families
69 the Mormoopidae (ghost-faced, naked-backed, and mustached bats), Furipteridae, and
70 Thyropteridae are insectivorous, the Noctilionidae (bulldog bats) are insectivorous and
71 piscivorous, and the Mystacinidae (short-tailed bats) are generalists that consumeinsects,
72 flowers, and fruit.. None of these families are particularly speciose though they contain notable
73 specializations such as *Mystacina tuberculata*'s crawling behavior and *Noctilio*'s ability to skim
74 fish out of water. Most of the dietary diversity within noctilionids lies within only one family,
75 Phyllostomidae (Neotropical leaf-nosed bats). The neotropical leaf nosed bats occupy the widest
76 diversity of dietary niches observed in vertebrates: nectar, pollen, fruit, foliage, birds, insects,
77 frogs, blood, and even other bats. Phyllostomids also are well known for their specialized skulls
78 and disparate hard-tissue morphologies (Dumont et al. 2014, Freeman 2000). As one family that
79 exhibits so many feeding strategies alongside well-known morphological disparity they are an
80 excellent starting place to trace the evolution of sensory structures related to foraging paralleling
81 mechanical adaptations to novel diets.

82 This study addresses the link between diet and sensory structures within the evolution of
83 noctilionoid bats using a well-supported phylogeny of the superfamily and a rich data set of
84 diceCT (diffusible iodine-based contrast-enhanced computed tomography) (Gignac and Kley
85 2014; Gignac et al. 2016) scans of bat specimens representing all dietary categories. However,
86 we removed the common vampire bat, *Desmodus rotundus,* from analyses as a dietary class with
87 a sample size of one would prevent statistical analyses. We use reconstructed soft tissue volumes
88 to examine variation in the relative sizes of sensory structures and to estimate ancestral states
89 throughout Noctilionoidea. Based on published studies of how these bats use olfactory, visual,
90 and auditory cues during foraging, we predict that, relative to their size, frugivores and

nectarivores evolved larger eyes and olfactory bulbs than animalivorous bats (piscivores, insectivores, and carnivores), while animalivorous bats evolved larger cochlea than plant feeding bats. We also expect that animalivorous bats evolved larger cochleae relative to the other sensory organs, and that phytophagous bats evolved larger orbit and olfactory bulb volumes relative to their cochleae. Because the phyllostomid ancestor was likely an insectivore that incorporated plant matter into its diet (Freeman 2000; Baker et al. 2012), we expect to find enlarged orbits and olfactory bulbs in the common ancestor of phyllostomids, but not in the common ancestors of their sister-groups. Finally, given the novel cranial shape and corresponding dietary shift to specialized frugivory within the phyllostomid subfamily stenodermatinae (Dumont et al. 2012; Shi and Rabosky 2015), we expect corresponding shifts in the rates of evolution of eye and olfactory bulb size at the base of this clade.

Materials and Methods

Species representation and scan collection

We measured the volumes of sensory tissues for 79 specimens representing 44 species of the Neotropical Noctilionoideae and one outgroup species (*Mystacina tuberculata*) from the mammal collections of the American Museum of Natural History and the University of Massachusetts, Amherst. These species represent all of the major clades and dietary ecologies except for the vampire bats. The specimens were fixed in 70% formalin and stored in ethanol for varying time periods. All specimens were stained in a solution of Lugol's iodine (I_2KI) for two weeks following Hedrick et al. (2018) before scanning them with a Nikon Metrology (X-Tek)

HTH 225 ST MicroCT scanner (Nikon Metrology Inc., Tokyo, Japan) at the Center for Nanoscale Systems at Harvard University. Bats were scanned against a molybdenum target at approximately 40 microns with voltage and current optimized for each scan (typically 70uA and 70kV). The resulting scans were then aligned in the proprietary CTPro software (CT Pro, Nikon Metrology Inc., Tokyo, Japan) and slices were reconstructed in VG Studio Max (Volume Graphics Inc., Germany).

Soft Tissue Reconstruction and Adjusting for Head Size

We imported the aligned scans into Mimics (Materialise, Leuven, Belgium – version 20) and masked them to segment out the olfactory bulbs, eye orbits, and cochleae. For the olfactory bulb, we masked the cavity demarcated by the cribriform plate and the imprint of the olfactory bulb on the internal surface of the cranium. We were unable to separate the accessory olfactory bulb from the primary olfactory bulb. For cochleae, we masked the hollow interior of the complete bony labyrinth and we removed the vestibular system in GeoMagic Studio 2014 (3Dsystems, SC, USA). The preservation of museum specimens almost invariably shrinks the eye itself (Hedrick et al., 2018). Therefore, we used the orbital space as defined by its muscular boundaries and eyelid as a proxy for orbit size (see Hedrick et al., 2018 for details). We used Mimics to measure volumes of the left and right orbits, olfactory bulbs, and cochleae for each specimen, shown in figure 1. We averaged the volumes of tissues from the left and right of each specimen and calculated species means for species for which we had more than one specimen. As our goal is to understand the spatial relationships among the sensory organs within the context of the skull, we used cranial centroid size as a proxy for head volume and used it to adjust for head size. Centroid size is the sum of squared distances from the center of a cloud of

landmarks to each landmark and is proportional to the volume enclosed by the landmarks. To ensure our results were not unjustly biased by skull length, centroid size was assessed in relation to other metrics of skull size (skull length, width) as well (supporting materials). Centroid size values were taken from Hedrick et al. (2019), which had cranial centroid sizes for all specimens included in this analysis except *Dermanura phaeotis.* The centroid size of *D. phaeotis* was substituted with the centroid size of *D. watsoni*, which is closely related and so similar in size and morphology that they are difficult to distinguish

Dietary Categories

We grouped bat species into dietary categories following Rojas et al. (2018), who scored the degree to which bat species rely on fruit, nectar, and insects/animals on an ordinal scale from zero to three (no reliance to total reliance). We placed most species into the single dietary category with the highest score. We categorized species with high scores in multiple food sources as generalists (supporting materials).

Comparative Analyses

Comparative analyses were conducted using the tree generated by Rojas et al. (2016), pruned to reflect the taxa represented in our sample. Phylogenetic regressions of $\log_{10}$ sensory structure volumes against $\log_{10}$ centroid size were performed with the *phytools* function phyl.resid in R (Revell 2012). These residuals represent the variance in sensory structure volume that is not explained by centroid size and are treated as representative of sensory structure volume for all subsequent analyses. Maximum likelihood estimates of lambda are built into the regression function to account for phylogenetic signal of trait values. We used one-way

158 ANOVAs to determine whether the volumes of sensory structures varied among species within
159 different dietary categories, and Games-Howell *post hoc* tests to pinpoint differences between
160 categories. Spearman rank-order correlations were used to identify trends in size corresponding
161 to increased or decreased reliance on foods from each of the dietary categories.

162 Relative sensory investment for each specimen was quantified as the percentage of total
163 sensory volume represented by each sensory structure, and we compared sensory investment
164 across dietary categories using ANOVAs with Games-Howell *post hoc* tests. Because the bulk of
165 large-eyed frugivores are nested within one clade (Stenodermatinae), this clade might override
166 the signal coming from other frugivorous groups. For that reason, we first considered
167 stenodermatines grouped with the non-stenodermatine frugivores, then as a distinct group
168 separate from the non-stenodermatine frugivores.

169 *Ancestral Reconstructions and Shifts in Evolutionary Rates*

170 Ancestral states were inferred for $\log_{10}$ size-adjusted volumes (volume/centroid) and $\log_{10}$
171 centroid size using the function fastAnc in the R package *phytools* (Revell 2012). We reverse log
172 transformed the resulting estimates and 95% confidence intervals following Thiagavel et al.
173 (2018) and then compared ancestral estimates and confidence intervals among the ancestors of
174 Phyllostomidae, Mormoopidae, Noctilionidae, and their most recent common ancestors. These
175 ancestral estimates were then compared with calculated averages and confidence intervals of
176 modern bats grouped by diet and family.

177 We used Bayesian Analysis of Macroevolutionary Mixtures (BAMM) v2.5 (Mitchell and
178 Rabosky 2017) in conjunction with the *BAMMtools* (Rabosky et al. 2014) and *coda* packages
179 (Plummer et al. 2006) in R to determine the plausibility of rate shifts in the evolution of centroid

size and size-adjusted volumes of sensory structures. For each analysis, the priors generated by the package were used with 'expectedNumberOfShifts = 1' as recommended for small trees. The Markov Chain Monte Carlo chains (MCMC) were run for ten million generations and sampled every one thousand generations. MCMCs were checked for convergence after removing the first 10% as burn-in. Credible shift sets were generated for centroid size and each of the size adjusted sensory volumes using a marginal odds ratio equal to 5.

Results

Phylogenetic Regressions and Residuals

The $\log_{10}$ adjusted volumes of each of the three sensory structures correlated positively with skull volume (olfactory $r_{(43)}=0.844$, $p<0.001$; orbit $r_{(43)}=0.799$, $p<0.001$; cochlea $r_{(43)}=0.624$, $p<0.001$). Analysis of size-adjusted volumes (residuals from phylogenetic regressions of sensory volumes against centroid size of the skull) reveal distinct trends in olfactory bulb, orbit, and cochlea size among dietary categories. ANOVAs and *post hoc* tests indicate that frugivores have the largest eyes and olfactory bulbs while nectarivores tend to have small cochlea (Table 1). Frugivores occupy a distinct morphospace in orbit versus olfactory volume residuals (Figure 2 a). All frugivores possess positive olfactory and orbit residuals while most other bats have lower residual values on one or both axes. Nectarivores occupy a distinct morphospace because of small cochlea volume residuals (Figure 2 b and c), though they have moderate olfactory and orbit volume residuals.

We expected to find large eyes and olfactory bulbs among nectarivores but we did not. Finding small cochlea among nectarivores was unexpected as well. To determine whether this

result (smaller than expected sensory sizes for all structures in nectarivores) was due to centroid size overestimating skull size, we explored the relationship between centroid size and traditional linear skull measurements. Centroid size of the skull correlates very well with skull length, but overestimates skull width in nectarivorous bats. A phylogenetic regression of cochlea volume on skull width yields the same result as the centroid size regression - nectarivorous bats have low cochlea residuals. Because this finding holds even for the skull metric least represented by centroid size, the result is unlikely to be caused by length-bias in centroid measurements artificially distorting skull volume estimates in these species.

Spearman rank correlations indicate strong relationships between scores for dietary reliance and sensory volume residuals. (Figure 3). The degree of animalivory is correlated negatively with orbit ($rs_{(43)}$= -0.540, $p<0.001$) and olfactory residuals ($rs_{(43)}$= -0.509, $p<0.001$), while degree of frugivory is positively correlated with both orbit ($rs_{(43)}$= 0.613, $p<0.001$) and olfactory residuals ($rs_{(43)}$= 0.588, $p<0.001$). The degree of nectarivory is negatively correlated with cochlea size ($rs_{(43)}$= -0.582, $p<0.001$). There is no evidence of correlation between frugivory and cochlea size, animalivory and cochlea size, nectarivory and olfactory size, or nectarivory and orbit size.

Sensory Proportions

Overall, phyllostomid eyes and olfactory bulbs account for larger proportions of total sensory volume compared to the other noctilionoid families, which feature larger proportions of cochlear volume. ANOVAs comparing the relative proportions of sensory organs between phyllostomids and outgroups (Figure 4) indicated that phyllostomid orbits account for a larger

proportion of their total sensory volume ($F_{(1,43)}$=18.41, p<0.001), cochleae account for a smaller proportion ($F_{(1,43)}$=138.46, p<0.001), and olfactory bulbs account for a larger proportion ($F_{(1,43)}$=5.03, p=0.03). Comparisons of sensory proportions among dietary groups within Phyllostomidae showed moderate variation in the relative proportion of the cochlea ($F_{(3,31)}$=3.45, p=0.028), more variation in the proportion of orbit ($F_{(3,31)}$=4.15, p=0.014), and little variation in olfactory bulb proportion ($F_{(3,31)}$=2.32, p=0.095). Games-Howell *post hoc* tests indicate that the significance in orbit proportion is accounted for primarily by the difference between animalivores and nectarivores (p=0.024, t=3.74) and animalivores and frugivores (p=0.013, t=3.59). When the stenodermatine bats are counted as their own dietary class within Phyllostomidae, ANOVA and Games-Howell *post hoc* tests point to significant differences in olfactory bulb proportion ($F_{(4,30)}$=3.72, p=0.014), with the stenodermatine frugivores having a lower olfactory proportion than the other frugivores (p=0.001, t=5.82). In terms of proportion accounted for by the orbit, variation among groups ($F_{(4,30)}$=5.96, p=0.001) is accounted for by the difference between stenodermatines and other frugivores (p=0.001, t=5.53) and animalivores (p=0.005, t=4.38), as well as the difference between nectarivores and non-stenodermatine frugivores (p=0.036, t=5.51), and nectarivores and animalivores (p=0.034, t=3.74). ANOVAs for cochlear volume proportions find significance when stenodermatine bats are treated alone ($F_{(4,30)}$=2.88, p=0.039), but post-hoc tests do not detect notable variation among groups. Overall, stenodermatines exhibit higher orbit and lower olfactory proportions than non-stenodermatine frugivores. Non-stenodermatine frugivores have a lower proportion of orbit volume than nectarivores in part because their olfactory bulbs are larger, not because their eyes are smaller.

Ancestral State Estimation

Ancestral state estimation yields an inferred ancestor for phyllostomids that is considerably different from the ancestors of other noctilionoid families. In terms of centroid size, the ancestor of modern phyllostomids had already begun to diverge towards a larger overall skull volume (Figure 5 a). The phyllostomid ancestor also had relatively larger olfactory bulbs and orbits than other ancestral nodes in the tree (Figure 5 b and c). The preservation of these ancestral changes is maintained across all dietary groups within Phyllostomidae - even the animalivores have larger olfactory bulbs, orbits, and centroid sizes than extant non-phyllostomid noctilionoids. Frugivores have much larger eyes and olfactory bulbs than the predicted phyllostomid ancestor. Modern nectarivores have diminished cochleae relative to the phyllostomid ancestor and other dietary classes within Phyllostomidae (Figure 5 d). Frugivores, generalists, and animalivores in the family have not diverged from each other substantially, or from the phyllostomid ancestor in terms of cochlea size.

BAMM and Evolution Rate Shifts

BAMM analysis of centroid size and the volumes of sensory structures revealed an unexpected suite of rate changes among noctilionoids and notable heterogeneity in the rate of orbit evolution (Figure 6). The centroid posterior distribution has 31 configurations in its 95% credible shift set. A one-shift configuration was most sampled at 37%, with the shift occurring in the *Artibeus* lineage with a marginal odds ratio of 120.1. The zero-shift configuration was 4.9% of the posterior. The 95% credible shift set for olfactory bulb volume contains four configurations, and 86% of the posterior configurations have zero shifts. In contrast, the 95% credible shift set for orbit contains 185 shift configurations, none of which predict a zero-shift configuration. The two most sampled sets are a two-shift configuration and three-shift

configuration, each of which is only 12% of the posterior distribution. Both configurations feature a shift at the base of the stenodermatine clade (marginal odds 205.5). The first configuration has an additional shift at the base of the phyllostomine clade (marginal odds 138.3), while the second has two additional separate shifts within phyllostomines, one in the lineage of *Chrotopterus auritus* (marginal odds 22.82) and the other in the lineage of *Phyllostomus discolor* (marginal odds 30.08). Prior and posterior probabilities along with marginal odds trees for each sensory structure and centroid size are included as supporting materials. Zero-shift configurations have the most support for both cochlea and olfactory bulb volumes while rate shifts are supported for orbit volume and centroid size. The 95% credible set for the cochlear volume contains only three configurations, and 65% of the posterior configurations have zero shifts.

Discussion

Estimating ancestral sensory structure volumes allows insight into the evolutionary history of foraging and the transition into novel dietary niches. We found evidence that the enlargement of visual and olfactory structures preceded the evolution of increased morphological disparity associated with dietary diversification. This suggests that a step towards omnivory could have been necessary before selection could act on morphological aspects of the skull required for specialization for new food sources. In essence, animals need to be able to find new foods for selective forces to be imposed by the new food resources. Our results also suggest that sensory and mechanical systems have some degree of evolutionary autonomy. Although analyses of the vertebrate skull are crucial for understanding the morphological underpinnings and correlates of ecological diversification, they have focused predominantly on the links between

293 dietary ecology and skeletal traits (Felice et al. 2019; Soria-Barreto et al. 2019). Although food
294 consumption is a central role for the vertebrate skull, it is important to consider that the skull
295 harbors sensory components whose functions extend beyond mechanical demands to include
296 foraging (Conith et al. 2019), mating (Baum and Kelliher 2009), and communication (Brennan
297 and Zufall 2006, Arch and Narins 2008, Fleishman 1992).

298 We expected a shift in the rates of trait evolution at the base of the stenodermatine
299 subfamily coincident with the well-known shift in speciation rate within the Neotropical
300 noctilionoids (Dumont et al. 2012; Rojas et al. 2012; Shi and Rabosky 2015). Due to these bats'
301 reliance on figs, which signal ripeness with olfactory cues and possibly visual cues (Thies et al.
302 1998; Hodgkison et al. 2013), we expected shifts in the rate of orbit and olfactory bulb evolution
303 but no shifts in the rate of cochlea evolution. While a rate-shift in olfactory size is highly
304 improbable, a shift in the rate of orbit bulb evolution is likely. We expected only one shift in the
305 rate of evolution of orbit size at the base of stenodermatines but found support for several
306 possible shifts across frugivorous phyllostomids. However, certainty around any particular
307 configuration is low due to the heterogeneity in the rates of orbit evolution. Much like the
308 olfactory bulb, there are no detectable shifts in rate of evolution of cochlea size. The variability
309 in the rate of orbit evolution suggests that orbit size can respond quickly to dietary changes. In
310 contrast, the lack of rate shifts in olfactory size suggests that the olfactory bulb may be more
311 constrained and unable to evolve as rapidly (Yohe et al. 2020). It is also possible that radical
312 alterations in the size of the olfactory bulb from the ancestor were not needed to enter
313 phytophagous niches.

314 A frugivorous sensory morphotype notable for relatively large olfactory bulbs and orbits,
315 developed in parallel across multiple phyllostomid lineages that rely totally or partially on fruit.

316 The size of these sensory structures increases with the proportion of a bat's diet composed of
317 fruit and large orbits and olfactory bulbs evolved independently in at least three lineages where
318 fruit is an important resource. Bats that eat insects exclusively have markedly smaller olfactory
319 bulbs and orbits both within and outside of phyllostomids, indicating that the development of
320 enlarged structures used for visual and olfactory foraging is linked with diet and not just
321 ancestry. The presence of the frugivorous-like sensory morphotype – larger than expected
322 olfactory bulbs and orbits – in the ancestor of the phyllostomids indicates a shift toward
323 omnivory requiring foraging for fruits had already occurred at the base of the family.

324 Our results provide independent support for the proposition that the phyllostomid
325 ancestor incorporated plant matter into its diet (Freeman 2000; Santana and Dumont 2009; Baker
326 et al. 2012, Yohe et al. 2015) . In terms of sensory morphology, the divergence of the ancestor of
327 modern phyllostomids from its ancestors was characterized by increasing olfactory and orbit
328 volume, both of which are strongly correlated with increased frugivory (Figures 2, 4). The
329 ancestral shift to omnivory at the base of the phyllostomids left a lasting sensory imprint: the
330 pattern of relative sensory volume in animalivorous phyllostomids is more similar to that of
331 plant-eating phyllostomids than it is to those of other animalivorous noctilionoids, though the
332 absolute volumes are smaller. In addition, many predominantly animalivorous phyllostomids in
333 our data set occasionally eat fruit, which indicates their capacity to find and forage on plants.
334 Taken together, the phylogenetic data, comparative analyses of hard tissue, and comparative
335 analysis of soft tissue all provide strong evidence that the phyllostomid ancestor was
336 omnivorous, a flexible morphotype from which many dietary specializations evolved. From an
337 original state of specialization for aerial insectivory, ecological generalization by way of a shift

toward omnivory preceded rapid re-specialization into a myriad of dietary niches associated with incredible morphological disparity.

Though the typical trend of generalists becoming specialists is supported by ecological and evolutionary theory (Futuyma and Morena 1988, Schluter 2000), further research should be directed at investigating transitions from reliance on a specific food resource to generalist ecologies. Overall, our findings strengthen the case for coupling traditional studies of bony structures with analyses of soft tissues and serve to highlight the role of sensory evolution in ecological diversification. Our analysis of sensory systems in an evolutionary context offers new, more nuanced insights into the evolutionary history of diet and foraging suggesting sensory adaptations should precede biomechanical adaptations in cases where organisms would have to be able to find new food resources in order to begin specializing for consumption of those food resources.

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Tables

Table 1 a): Olfactory Residual Post Hoc Table

OB P values	Frugivore	Animalivore	Nectarivore	Generalist
Frugivore	XXX XXX	**p=0.001, t=4.70**	**p=0.019, t=6.67**	**p=0.026, t=3.22**
Animalivore		XXX XXX	p=0.975, t=0.41	p=0.213, t=2.01
Nectarivore			XXX XXX	p=0.093, t=2.91
Generalist				XXX XXX

ANOVA: $F_{(3,41)}$=9.146, p<0.001

Games-Howell: η^2=.4, [.17;.52], F=9.15;

Table 1 b): Orbit Residual Post Hoc Table

Orb P values	Frugivore	Animalivore	Nectarivore	Generalist
Frugivore	XXX XXX	**p<0.001, t=7.48**	**p=<0.001, t=6.01**	**p=0.001, t=4.56**
Animalivore		XXX XXX	p=0.016, t=3.43	p=0.045, t=2.81
Nectarivore			XXX XXX	p=0.996, t=0.23
Generalist				XXX XXX

ANOVA: $F_{(3,41)}$=9.146, p<0.001

Games-Howell: η^2=.61, [.41;.69], F=21.39

Table 1 c) Cochlea Residual Post Hoc Table

Cochlea P values	Frugivore	Animalivore	Nectarivore	Generalist
Frugivore	XXX XXX	p=0.993, t=0.27	**p=0.011, t=7.98**	p=0.003, t=4.19
Animalivore		XXX XXX	**p=0.002, t=5.22**	p=0.094, t=2.45
Nectarivore			XXX XXX	**p=0.049, t=3.52**
Generalist				XXX XXX

ANOVA: $F_{(3,41)}$=7.41, p<0.001

Games-Howell: η^2=.35, [.12;.47], F=7.41

Table 1. Results of ANOVAs and subsequent post hoc tests performed on residuals of sensory volumes regressed against centroid size. Values in bold text indicate that frugivores differ from the other dietary classes in terms of orbit and olfactory size while nectarivores are unique with respect to cochlea size.

Figures

Figure 1

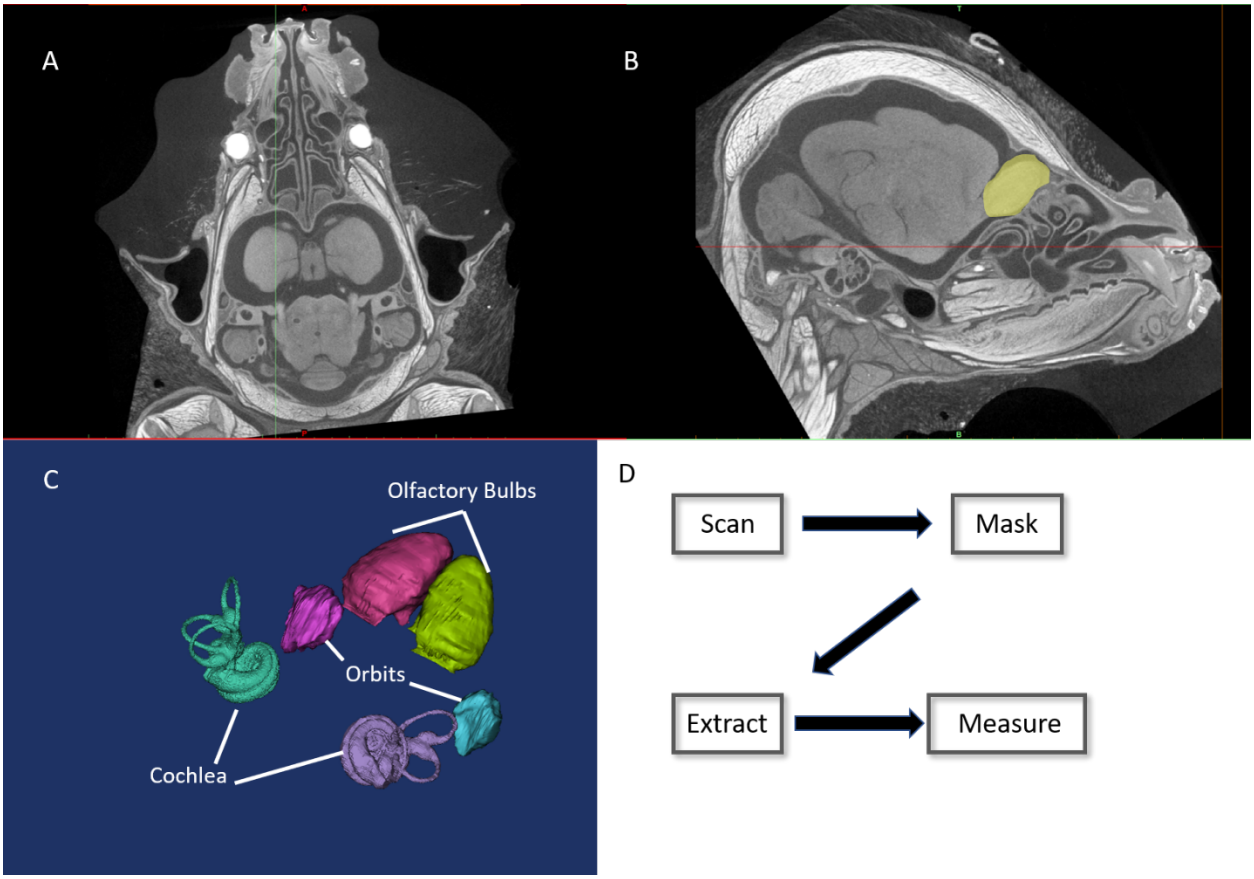
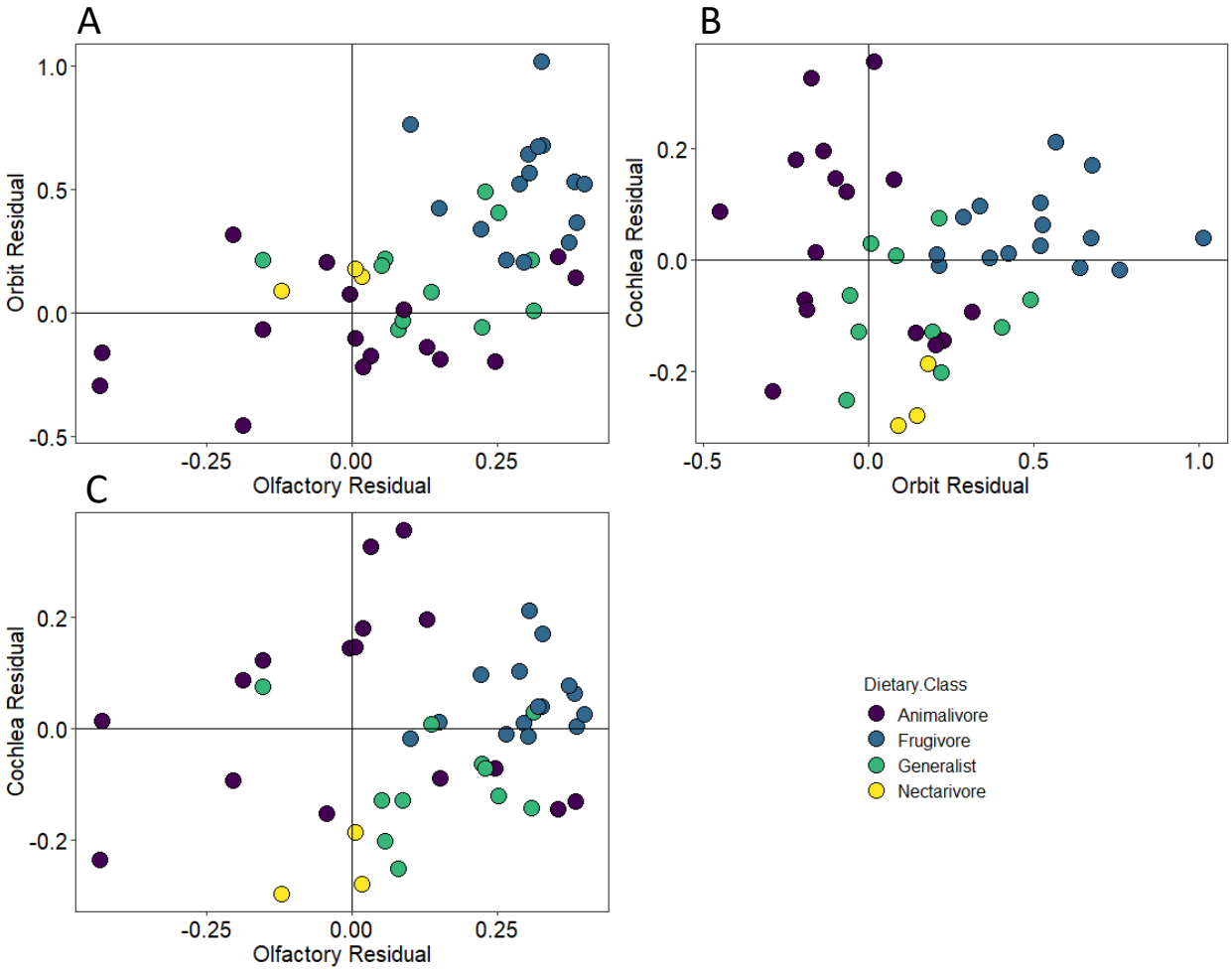


Figure 1: A) an axial cross section of a specimen, B) a sagittal cross section of the same bat showing a slice of the olfactory mask, C) the structures extracted, D) the workflow resulting in volumetric measurements. The software directly calculates the volumes of the shapes with no additional manual measurements needing to be taken.

560 Figure 2



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562 Figure 2. Plots of sensory structure residuals colored by diet: A) orbit residuals versus olfactory
563 residuals, all of the frugivores cluster in quadrant I; B) cochlea residuals against orbit residuals;
564 and C) cochlea residuals versus olfactory residuals.

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Figure 3

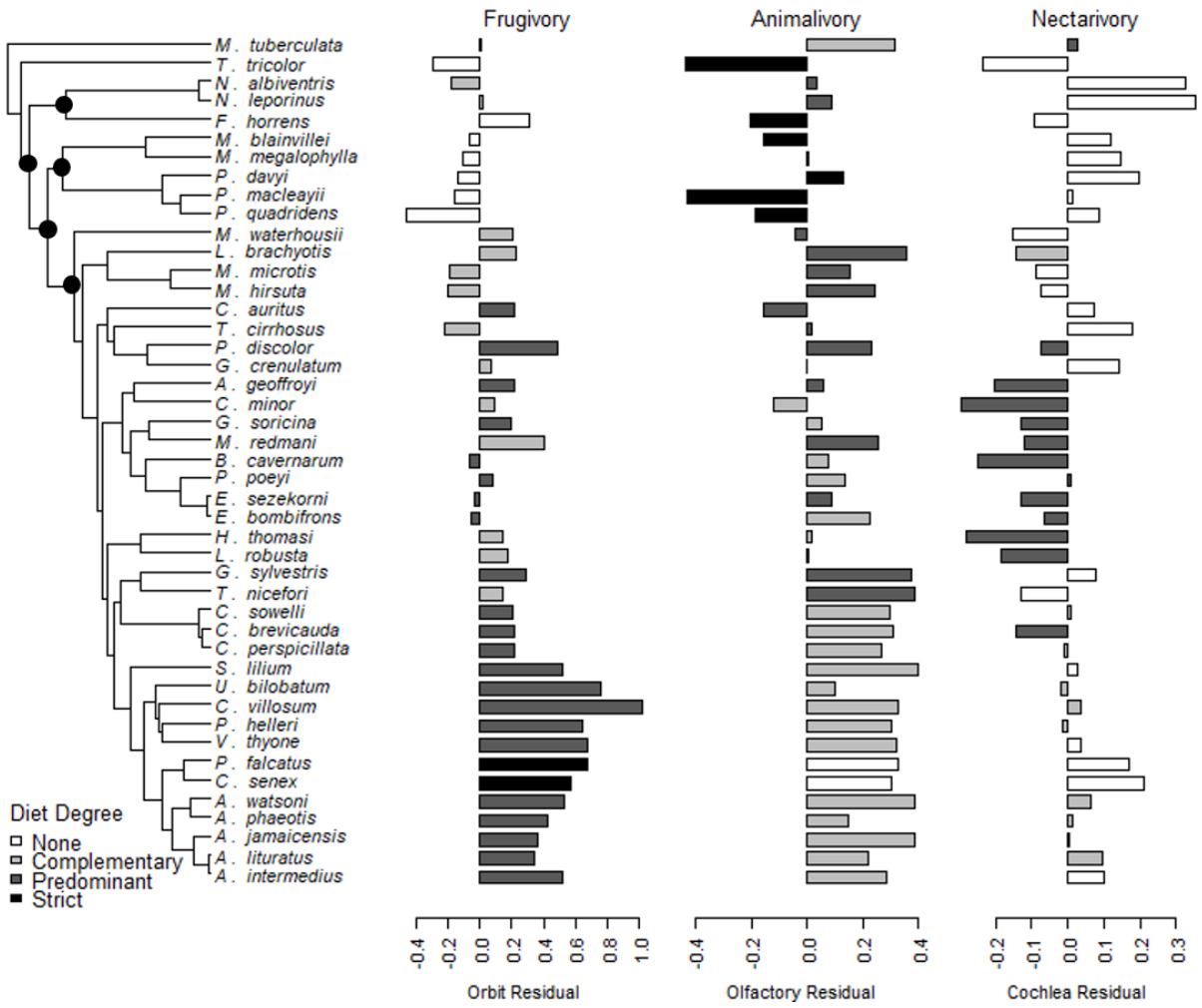

Figure 3. Residual values of orbit, olfactory bulb, and cochlea size for each species colored by whether the reliance on a specific diet is absent (none), complementary, predominant, or strict. Orbit residuals illustrate degree of frugivory, olfactory residuals are colored based on degree of animalivory, and cochlea is colored based on degree of nectarivory. Ancestral state reconstructions are provided for nodes highlighted in the tree in Fig 4.

589 Figure 4

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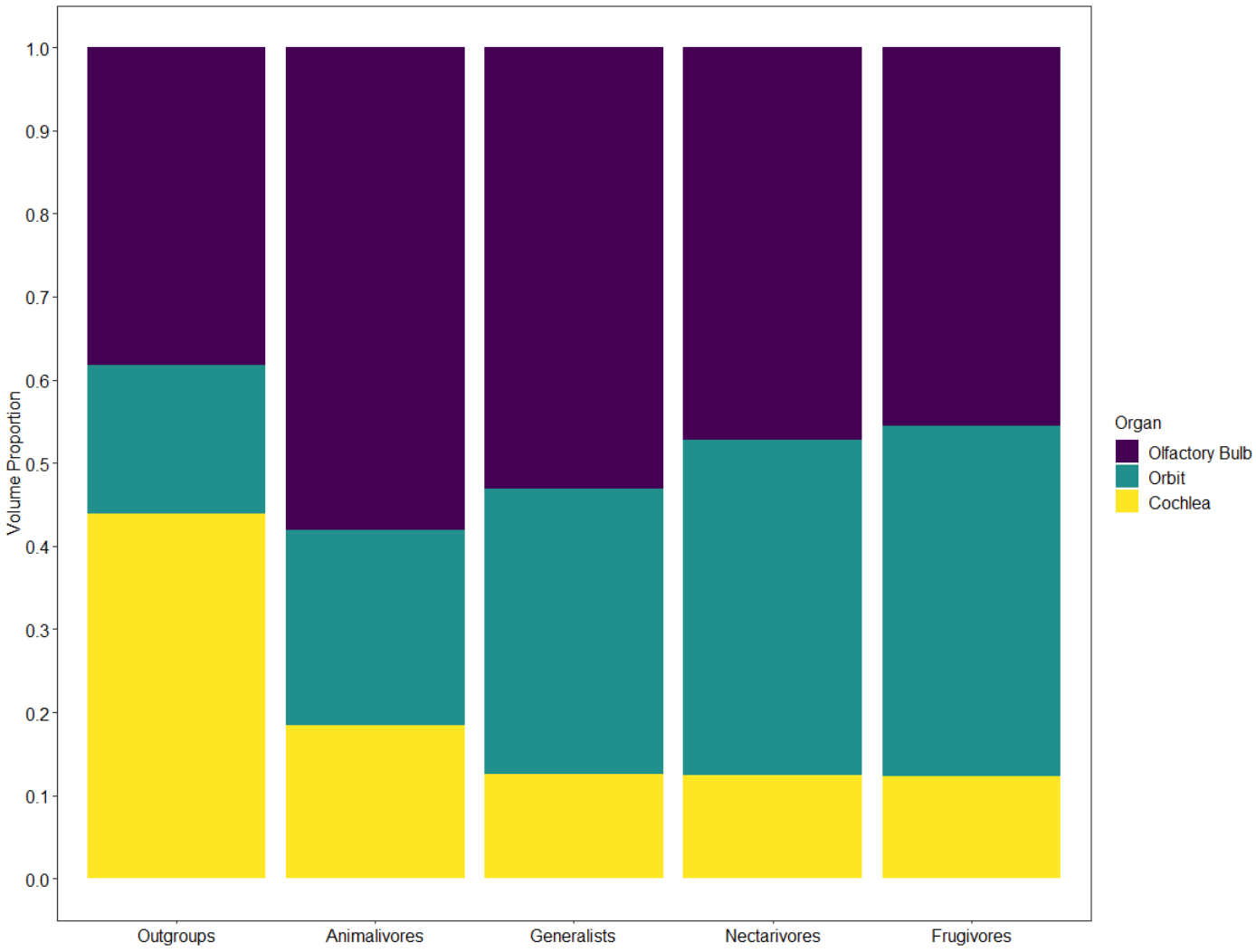

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593 Figure 4. Average proportions of the olfactory bulb, orbit, and cochlea for non-phyllostomids
594 (Outgroups) compared with the different dietary classes among phyllostomids.

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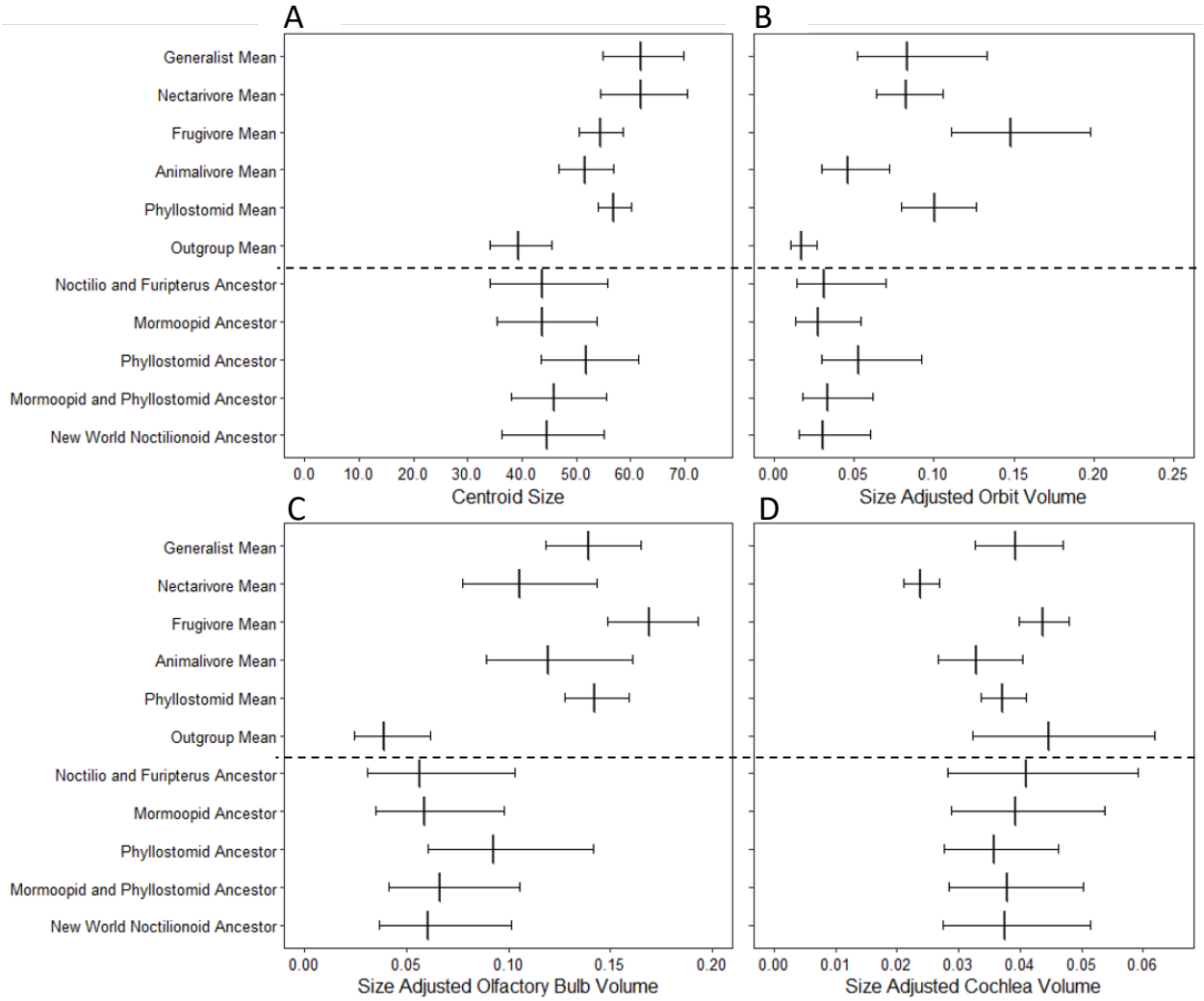
601 Figure 5



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603 Figure 5. Ancestral state reconstructions for centroid size (A) and size-adjusted volumes of the
604 orbit (B), olfactory bulb (C), and cochlea volumes (D). Ancestral nodes are marked in Figure 3.

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606 Figure 6

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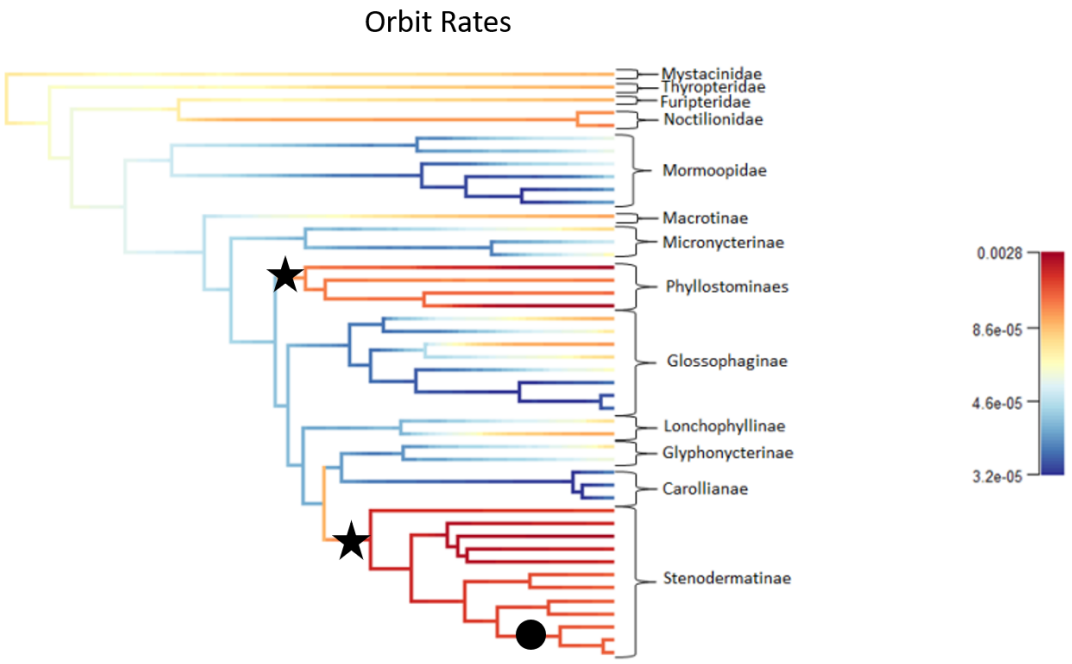

608

609 Figure 6. Rate of evolution in orbit volume. Warm colors indicate faster rates and cool colors
610 indicate slower rates. Stars indicate locations of orbit shifts in the most probable rate-shift
611 configuration. The circle indicates the location of the centroid shift in the most probable rate-
612 shift configuration. Note that the difference between red and pale orange is two orders of
613 magnitude.