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But how does it smell? An investigation of olfactory bulb size among living and fossil primates and other euarchontoglirans

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Abstract

Primates are often considered to have a poor sense of smell. While all studies identify small olfactory bulbs (OB; the region of the brain responsible for processing scent) among haplorhines, whether or not strepsirrhines also possess small OBs is less clear, as is the evolutionary backdrop from which these patterns emerged. Here, we examine the relative size of the olfactory bulbs in cranial endocasts of living and fossil primates and their kin (Euarchontoglires [Primates, Dermoptera, Scandentia, Rodentia, Lagomorpha]), testing previous hypotheses. Regression analyses of OB volume and mass relative to endocranial volume (ECV) and body mass (BM), and ANOVAS of residuals, were performed on a dataset of 181 extant and 41 extinct species. Analyses show clear differences in the relative size of the OBs, with haplorhines possessing distinctly smaller OBs relative to all other clades. Pairwise tests indicate haplorhine OBs are significantly smaller than those of all other clades, including strepsirrhines; when the haplorhines are removed from analyses, strepsirrhines are significantly smaller than all other clades. This suggests that a reduction in OB size occurred at the crown primate node, a pattern also seen in ancestral state reconstruction (ASR) analyses. The ASR analyses suggest multiple

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iterations of olfactory bulb size decrease occurred in Haplorhini, reflecting large amounts of parallelism. These results likely differ from previous studies due to the inclusion of additional fossils and more appropriate outgroups based on up-to-date phylogenetic hypotheses.

KEYWORDS

brain, olfaction, paleoneurology, paleontology, primates

1 | INTRODUCTION

There is a long history of considering primates to be predominantly visual animals, less reliant on olfaction than many other mammals. Primates are often characterized as microsmatic (e.g., Negus, 1958; Smith, 1924; see discussion in Smith & Bhatnagar, 2004) on the premise that olfaction is less critical than vision in the arboreal milieu (LeGros-Clark, 1959). There is less consensus, however, on the degree to which this characterization applies generally across the order. Martin (1990), for example, suggested that there was independent evolution of reduced olfactory bulb size in diurnal lemurs and in haplorhines, while Baron et al. (1983) found that diurnal anthropoids contrasted with other primates (including nocturnal anthropoids) in having reductions to the main olfactory bulbs. Genetic evidence shows that primates generally have fewer olfactory receptor genes than other mammals tested, but there is also evidence for a contrast in the fraction of pseudogenes between strepsirrhines and haplorhines (Niimura et al., 2018), and among anthropoids. Moreover, there are disagreements about the pattern of olfactory loss and its potential relationship to visual changes (Dong et al., 2009; Matsui et al., 2010; Niimura et al., 2018). In light of these varying results, it seems fair to say that there is no clear consensus on the nature or pattern of evolution of the olfactory sense in primates.

There are many different approaches that can be taken to studying olfaction, including anatomical study of the size of the nasal fossa (e.g., Cave, 1967) or the area of the nasal turbinates (e.g., Lundeen & Kay, 2022; Lundeen & Kirk, 2019); histological study of the vomeronasal organ (e.g., Smith et al., 2011); and genetic studies on the number and functionality of olfactory genes (e.g., Dong et al., 2009; Matsui et al., 2010; Niimura et al., 2014, 2018). Although all of these different approaches bring valuable pieces to the puzzle of primate olfactory evolution, the study of the size of the olfactory bulbs (OB) of the brain offers certain advantages. The physical size of the main olfactory bulb (MOB) is correlated with the number of receptor neurons in the olfactory epithelium, which is related to olfactory sensitivity (Heritage, 2014), so the size of the MOB provides one way of approaching the question of how the sense of smell evolved in primate evolution (e.g., see Baron et al., 1983; Heritage, 2014; Martin, 1990). The olfactory fossa of the cranium provides an estimate of the size of the MOB. While it is certainly an overestimate, as the fossa also houses the accessory olfactory bulb (AOB) and an olfactory ventricle (Heritage, 2014), there is compelling evidence that the olfactory fossa represents a fairly good proxy of the MOB size in primates (Kay et al., 2004). This approach admittedly excludes important parts of the chemosensory system by failing to allow for the independent assessment of the relative size of the AOB (Stephan et al., 1982) and excluding the vomeronasal organ (Smith et al., 2007; Smith & Bhatnagar, 2004). However, it offers a powerful virtue over most other approaches as it can be quantified in the endocasts of many fossil taxa. Although nasal turbinates are also preserved in some fossils (e.g., see Kirk & Lundeen, 2020; Lundeen & Kay, 2022; Lundeen & Kirk, 2019), because the relevant structures are quite fragile, their intact preservation is a rarer phenomenon than the preservation of the rostral part of the neurocranial cavity. As such, the expanding body of data for virtual endocasts of fossil non-hominin primates (e.g., Bush, Simons, & Allman, 2004; Bush, Simons, Dubowitz, & Allman, 2004; Gingerich & Gunnell, 2005; Gonzales et al., 2015; Harrington et al., 2016, 2020; Kirk et al., 2014; Ni et al., 2019; Orliac et al., 2014; Ramdarshan & Orliac, 2016; Ryan et al., 2008; Silcox et al., 2007, 2009, 2010; Simons et al., 2007) allows for more places in the primate phylogeny for which data from extinct taxa can inform inferences about the pattern of olfactory evolution. This may be important given the growing evidence from the study of fossil endocasts that there was extensive parallelism in the evolution of overall brain size in primates (Allen, 2014; Gonzales et al., 2015; Kay et al., 2008; Ni et al., 2019; Silcox et al., 2022; Steiper & Seiffert, 2012). It is worth considering if olfactory bulb size evolution also shows similar degrees of parallel evolution.

The most comprehensive study of olfactory bulb evolution in Primates was performed by Heritage (2014). He applied ancestral state reconstruction (ASR) methods to study a dataset compiled from Stephan et al. (1981) and Pirlot and Kamiya (1982), combined with the fossil primate data available at the time (Bush, Simons, & Allman, 2004; Bush, Simons, Dubowitz, & Allman, 2004; Gurche, 1982;

Silcox et al., 2009, 2010; Simons et al., 2007), to map the evolution of OB size both in isolation and relative to overall brain size. His sample included 51 crown primates, 2 stem primates (i.e., plesiadapiforms), 1 dermopteran, 3 scandentians, and 26 "insectivorans" (i.e., a mix of eulipotyphlans and afrosoricidans) for a total of 83 species. The inclusion of "insectivorans" as the comparative backdrop by which to assess primate evolution reflects historical ideas that primates evolved from an ancestor that would be placed in this group (e.g., McKenna, 1966; Simpson, 1945; Szalay, 1968), as well as the view that these taxa form a good general model for what is primitive in mammals for the brain (Stephan, 1972). However, the modern consensus on primate relationships is that they are members of Euarchonta (Waddell et al., 1999) along with Scandentia and Dermoptera, with the latter order being the closest living relative to Primates (Mason et al., 2016; Zhang et al., 2019). The closest relatives to Euarchonta are not "insectivorans"; rather, the sister clade of this group is Glires (Rodentia + Lagomorpha) in the supraorder Euarchontoglires (Murphy et al., 2001). As such, the most appropriate comparative taxa for primates are not "insectivorans" but instead living and fossil rodents, rabbits, and pikas. Heritage (2014) also found that eulipotyphlans actually showed increases in both absolute and relative OB size compared with the inferred ancestral state (i.e., for Boreoeutheria). This conclusion would need to be reassessed in the context of an analysis with a more thorough sampling of Boreoeutheria and its outgroups, but it does nonetheless highlight an additional potential problem with using extant eulipotyphlans as a model for primitiveness (a point also made by Martin, 1968, 1990).

Heritage (2014) made a number of conclusions about the pattern of evolution of the OBs in primates: (1) the perception that OB size decreased at the crown primate node is mostly a result of an increase in total brain volume; (2) there was an *increase* in OB size at the strepsirrhine node; (3) OB size decreased at the haplorhine node, while apparent reductions at the anthropoid node are likely a product of increased total brain size; (4) there were reductions in OB size within Anthropoidea—in platyrrhines and convergently at the cercopithecoid node (and possibly otherwise within Catarrhini); (5) fossil crown primates were consistent with a haplorhine/strepsirrhine dichotomy in OB size. In light of these results, he argued that it is incorrect to characterize primates as an order as having evolved a decrease in olfactory sensitivity. However, a more recent study in which Primates were put into a broader context showed a different pattern. In an ASR analysis containing Mesozoic and Paleocene–Eocene of mainly placental mammals, a decrease in OB size in relation to both endocranial volume and body mass was observed at the base of crown primates (Bertrand et al., 2022; figs. S23 and S24).

The current analysis differs from that completed by Heritage (2014) in a number of key areas. First, primates are placed in the comparative context of Euarchontoglires, based on a rich sample of living and fossil members of Glires (Bertrand et al., 2016, 2017, 2018, 2021, 2024; Bertrand, Amador-Mughal, et al., 2019; Bertrand, San Martin-Flores, & Silcox, 2019; Bertrand & Silcox, 2016; Ferreira et al., 2022; Lang et al., 2022; López-Torres et al., 2020, 2023; Todorov et al., 2019; and the current study) and a more fulsome sample of treeshrews and dermopterans (Lang et al., 2022; San Martin-Flores et al., 2018; San Martin Flores et al., 2019; and the current study). Second, we have included an expanded sample of primates, and in particular now have access to data for a richer array of fossil primates (Gonzales et al., 2015; Harrington et al., 2016, 2020; Kirk et al., 2014; Long et al., 2015; Ni et al., 2019; Orliac et al., 2014; Ramdarshan & Orliac, 2016; Silcox et al., 2009, 2010; Simons et al., 2007; White et al., 2023; and the current study). Although there is some overlap in the fossil primate taxa included here and by Heritage (2014), access to CT data, or improved CT data, has allowed for reconsideration of the estimates of OB and/or endocranial volume (ECV) in some species (e.g., see Harrington et al., 2020; Kirk et al., 2014; and current study for *Simonsius* ["Parapithecus"] *grangeri*). The end result is a list comprising 222 species of which 41 are fossil or subfossil Euarchontoglires taxa.

A third difference lies in our approach to considering OB size. One of the challenges of studying change through time in any brain region is the problem of accounting for proportional changes in other regions, such as the neocortex, which has massively increased in primate evolution (Frahm et al., 1982; Jerison, 2012; Long et al., 2015). Martin (1990, fig. 8.16) considered olfactory bulb volume relative to overall body mass as a way of trying to decouple changes in OB size from overall changes in brain size. This approach was critiqued by Smith and Bhatnagar (2004) who suggested that scaling by body mass may distort comparisons within taxa, which is certainly a fair point since body mass also evolves. Thus, Heritage (2014) chose to look at olfactory bulb volume by itself. However, in light of the strong relationship between OB mass and body mass in primates (see below), and an even stronger relationship in the rest of the sample, it is challenging to separate overall changes in body mass from changes in OB size. We find some of Heritage's (2014) unscaled OB volume results to be basically uninterpretable, since apparent contrasts with the scaled results appear to mostly reflect major differences in body mass (e.g., larger absolute OBs in Lemuroidea vs. Lorisoidea and in Hominoidea vs. Cercopithecoidea). As such, we have returned to the practice of scaling OB size to body mass, with the understanding that neither ECV nor body mass provides a perfect method of considering change in relative size since both of those variables also evolve.

Finally, we have chosen to adopt a wider set of methods than Heritage (2014) to analyze our data. Although we agree that ASR is a critical component of studying evolution through time in OB size, we probe the assumptions of the models underlying such reconstructions by comparing the results from four different methods. We also include some traditional and phylogenetically informed regression and ANOVA analyses to allow for data exploration and significance testing of some of our key inferences.

The goals of the current article are to consider the following set of questions:

i. To what degree are OB volume (OBV) or mass (OBM) and BM vs. ECV correlated? Is it necessary/appropriate, or informative to study olfactory bulb size in relation to both? To what degree does phylogeny impact these considerations?
ii. Can a reduction in OB size be traced down to the basal primate node or crown primate node (as suggested by Bertrand et al., 2022)? As such, can primates as an order be characterized as having evolved a decrease in olfactory sensitivity?
iii. Was there an increase in OB size in living strepsirrhines (as suggested by Heritage, 2014)?
iv. Were there decreases in OB size at either the haplorhine or anthropoid nodes (or both)?
v. Is there evidence for further changes in OB size within strepsirrhine or anthropoid clades?
vi. Do scandentians show reductions in olfactory sensitivity as mediated by OB size (i.e., as suggested by LeGros-Clark, 1959; but see Martin, 1990) that are either shared with Primates or evolved independently? What are the implications of these results for using treeshrews as behavioral models for living primates?
vii. Are there changes in OB size in other euarchontogliran groups (e.g., Rodentia, Lagomorpha) that parallel the changes occurring in Primates?
viii. To what degree do fossils impact the results of these analyses?

2 | MATERIALS AND METHODS

The dataset used for these analyses included 181 extant and 41 extinct species of Euarchontoglires, representing all of the major clades (crown Haplorhini [$n = 40$]; crown Strepsirrhini [$n = 23$]; Dermoptera [$n = 2$]; Scandentia [$n = 14$]; Rodentia [$n = 93$]; Lagomorpha [$n = 28$]; stem haplorhines and strepsirrhines [$n = 9$]; stem Primates (i.e., plesiadapiforms) [$n = 5$]; stem Glires [$n = 2$]). Olfactory bulb volume (OBV), olfactory bulb mass (OBM), total endocranial volume (ECV), and body mass (BM) data were either acquired from previous sources or measured from the endocasts directly (see Table S1 for information on provenance). For newly generated data, the olfactory bulbs were measured from the endocast using the "Volren" module in AVIZO 9.1.1 or 2023.1.1 software (Visualization and Sciences Group, 1995–2020), to separate the olfactory bulbs from the rest of the endocast along the circular fissure (Figure 1). For previously published specimens, other methods were used such as using the "Volume edit" tool in AVIZO (see Bertrand, Amador-Mughal, et al., 2019), which provides comparable results. Volumes were then quantified using the "Surface Area Volume Module." This sample also includes the endocasts of several new fossil specimens for which body mass estimates have not been published, including three species of subfossil lemur and a stem lorisid, *Mioeuoticus shipmani*. For these taxa, virtual endocasts were generated from X-ray computed tomographic data in Avizo 9.1.1 using a combination of manual segmentation and interpolation as presented in Lang et al. (2022). Any holes in the endocranial cavity were closed by a straight line between preserved pieces of bone. Their body mass was estimated using the cranial length equation published in Silcox et al. (2009) for primates with habitual horizontal body positions (body mass $= 1000 \times 10^{((\log CL * 3.937013) - 7.222246)}$). All volume data are presented in cubic millimeters (mm^3) and all mass data are presented in grams (g). Olfactory bulb volume was converted to mass (g) using the following formula: grams $= (mm^3 * 1.036)/1000$, as this conversion best reflects brain tissue density (Stephan, 1960). These data were log-transformed for all analyses. Due to the incomplete preservation of the cranium of *Plesiadapis cookei* (lacking the petrosal lobules and much of the ventral surface; Orliac et al., 2014: Figure S2), the endocast of this taxon was removed from all volume analyses but included in body mass analyses. Conversely, *Plesiadapis tricuspidens* was included in the ECV analyses but not in the BM analyses. Orliac et al. (2014) provided an estimate of the olfactory bulbs only from the flattened version of the endocast of *P. tricuspidens*. Assuming that the entire endocast was deformed to a similar degree, their size relative to ECV is likely to be reasonably accurate, but their size relative to body mass would inevitably be artificially low.

Regression analyses were performed to evaluate the relationship between OBV versus ECV and OBM versus BM. Both ordinary least squares (OLS) and phylogenetic generalized least squares (PGLS) regressions were performed to allow for an assessment of the degree to which any results from the OLS analyses are

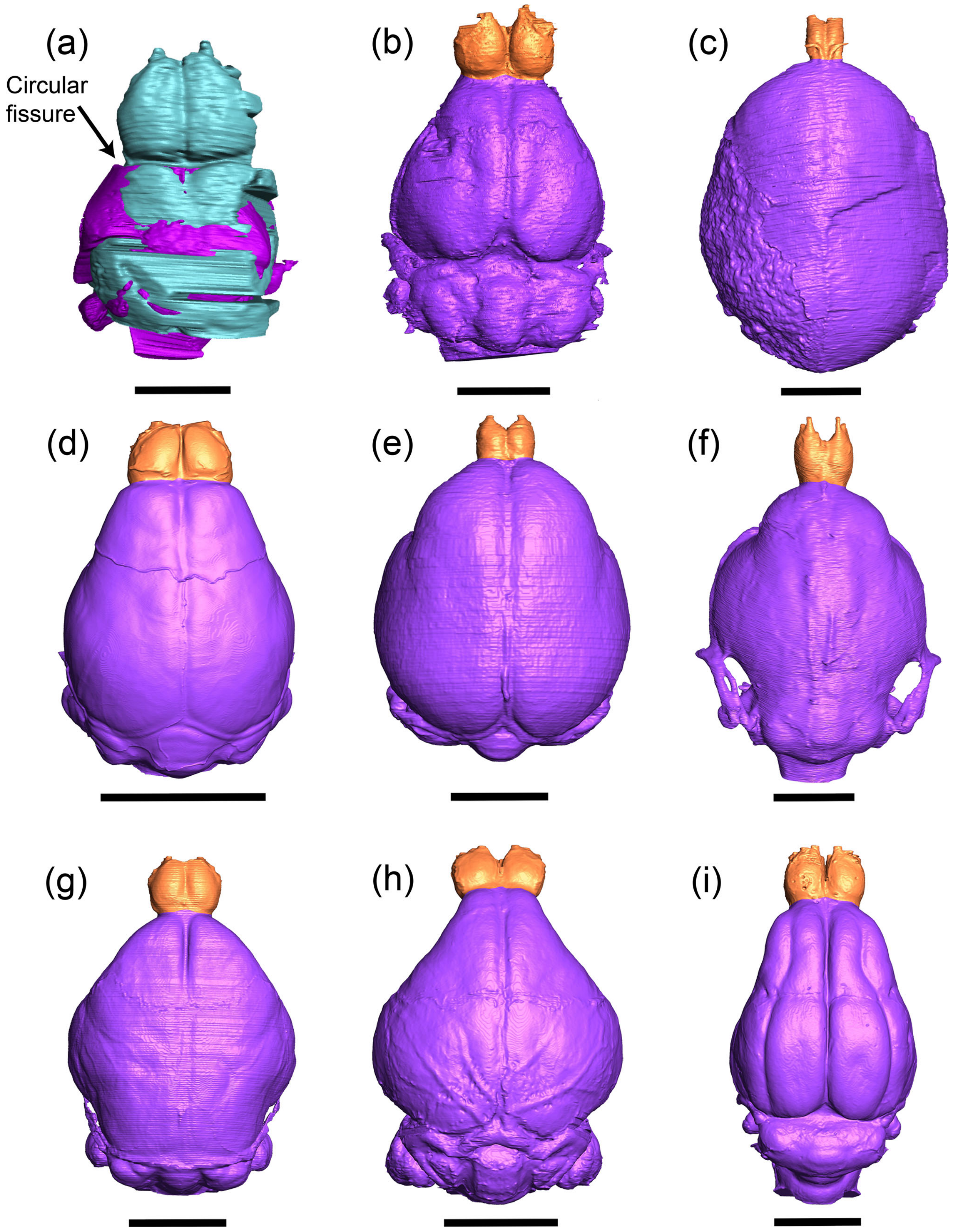

FIGURE 1 Endocasts of Euarchontoglires: (a) composite endocast of *Labidolemur kayi* (USNM 530208 and USNM 530221) showing circular fissure; (b) *Microsyops annectens* (UW 12362); (c) *Simonsius grangeri* (DPC 18651); (d) *Ptilocercus lowii* (USNM 481107); (e) *Rooneyia viejaensis* (TMM 40688-7); (f) *Adapis parisiensis* (NHM M 1345); (g) *Xerus rutilus* (AMNH 179092); (h) *Brachylagus idahoensis* (AMNH 92869); (i) *Cynocephalus volans* (AMNH 16697). Olfactory bulbs shown in orange for all taxa except *Labidolemur*.

aligned with the phylogenetic tree, as reflected by any changes in the pattern of significant results in the PGLS analysis.

Ordinary least squares (package: RRPP, version: 2.0.0, function: lm.rrpp; Collyer & Adams, 2018) regressions were performed on both volume and mass variables with 10,000 iterations using a randomized residual permutation procedure for significance testing. Using these same parameters, separate OLS regressions were also performed on specific clades (Haplorhini, Strepsirrhini, Scandentia, Rodentia, and Lagomorpha) to examine the relationship between these variables within each of these groups. Scatter plots for the entire dataset showing the relationship between OBV versus ECV and OBM versus BM were presented with linear models. Boxplots of residuals from the OLS regressions of mass and volume were plotted to show variation in relative olfactory bulb size between clades. An analysis of variance (ANOVA) using a randomized residual permutation procedure (iterations = 10,000) was performed on the residuals from the OLS regressions to examine the impact of clade on the relationship between these variables and to test for the significance of differences among clades. Clades with small sample sizes (i.e., Dermoptera, stem Primates, stem Glires) were excluded from the ANOVAs. Subsequent pairwise tests were performed to identify significant differences between clades. We have chosen to use linear models as the most intuitive way of allowing for data exploration and because ANOVAs tend to be quite robust, even to violations of normality (e.g., Blanca Mena et al., 2017).

Additionally, phylogenetic generalized least-squares (package: phylolm, function: phylolm, version: 2.6.2; Ho et al., 2016) regressions were performed on these same data to examine the scaling relationships without the influence of phylogeny. For the PGLS regression, Pagel's lambda was used as the covariance model as this model was determined to have the best fit based on calculations of the Akaike Information Criterion from multiple models (Table S2). The phylogenetic tree used for these analyses was generated from vertlife.org. Ten thousand birth-death node-dated trees were obtained for the entire extant sample. A single tree was generated from this set of trees using maximum clade credibility (package: phangorn, version: 2.11.1, function: maxCladeCred; Schliep, 2011). Fossils were added to this tree in Mesquite v. 3.70 based on hypotheses of relationships from Gunnell (1989), Bloch et al. (2007), Harrison (2010), Silcox et al. (2010), Gonzales et al. (2015), Kistler et al. (2015), Ni et al. (2016, 2019), Seiffert et al. (2018, 2020), Arnaudo et al. (2020), López-Torres et al. (2020, 2023), Bertrand et al. (2021, 2024), and Marciniak et al. (2021). This tree includes plesiadapiforms (Silcox et al., 2017) as stem primates. The complete tree (Figure S1) was then exported as a nexus file and branch lengths were standardized in R using the compute.brlen function (package: ape, version: 5.7-1; Paradis et al., 2019) based on Grafen's (1989) computation. Phylogenetic signal was assessed using Pagel's lambda for several variables including: logOBV, logECV, logBM, percent volume of OBV relative to ECV (%OBV), percent mass of OBM relative to BM (%OBM), and the volume and mass residuals of the OLS regression.

Ancestral character states for two traits (%OBV relative to ECV and %OBM relative to BM) were reconstructed using four methods: (1) by estimating states at internal nodes using maximum likelihood with Grafen branch lengths (package: phytools, version: 2.1-1, function: fastAnc; Revell, 2012); and by estimating internal nodes under parsimony in Mesquite using (2) Grafen branch lengths; (3) branch lengths = 1; and (4) branch lengths based on fossil and molecular calibrations. The time-calibrated tree was produced using the cal3TimePaleoPhy function (package: paleotree; version: 4.2.3; Bapst, 2012). This function requires two matrices to create a time-calibrated tree, one of time bin ages and another specifying which time bin each taxon is placed in. Ten time bins, with durations ranging from 1.25 to 4.15 million years, were designated. For fossils that are associated with exact dates, these dates were used to determine what time bin they were placed in (see Table S7). Fossil ages for taxa with less precise age estimates were determined from the average of their estimated occurrence dates (FAD and LAD), and based on this number, they were placed in 1 of the 10 bins. All extant taxa were placed in a Pleistocene time bin ranging from 2.59 million years to the present (year 0). As node ages can be supplied to this function and used in the resulting time-calibrated tree, ages for all nodes older than 10 million years were included, except for nodes that occur immediately before or after the fossils to allow for more latitude in calculating the fossil node ages. These node ages were obtained from the extant phylogeny taken from vertlife.org. With these parameters, 100 time-calibrated trees were produced and a single tree was extracted using maximum clade credibility (package: phangorn, version: 2.11.1, function: maxCladeCred; Schliep, 2011).

Using these four methods allows for an assessment of the differing contributions of branch lengths and reconstruction algorithms on the results. Nodal values for critical clades are included in Tables 3 and Table S8. There are two phylogenetic hypotheses about which there is broad enough debate that we felt it was important to consider alternative views. *Rooneyia viejaensis* has traditionally been considered an omomyoid, on the haplorhine side of the tree (e.g., Ni et al., 2016). However, some recent analyses have instead found it to lie among the adapoids, and as such, it would be a stem strepsirrhine rather than a haplorhine (e.g., Seiffert et al., 2020). Although we treated it as an omomyoid in the main analysis, we also reconstructed relevant nodes with it in the position of an adapoid using Parsimony (BL = 1 analysis) in Mesquite, as this is the least assumption-laden analysis. Second, there have been disagreements (e.g., Asher et al., 2019; Meng, 1990; Meng et al., 2003) about the relationships of Reithroparamyinae (represented in this analysis by *Reithroparamys* and *Rapamys*). In the current study, based on Meng et al. (2003), Reithroparamyinae was placed at the base of rodents, but closer to the crown clade than Paramyinae in the main analysis. However, we also considered the effects on the Parsimony (BL = 1) analysis of placing Paramyinae and Reithroparamyinae in an unresolved polytomy at the base of Rodentia (following Asher et al., 2019), and in moving Reithroparamyinae to be the sister clade of the Squirrel Related Clade (following Meng, 1990).

3 | RESULTS

The results of the OLS analyses indicate a significant but moderate impact of ECV on OBV ($r^2 = 0.169$; $p < 0.001$; Table 1) with BM having a comparatively greater impact on OBM ($r^2 = 0.367$; $p < 0.001$; Table 1). The patterns of variation shown in the regression analyses are similar for volume and mass data, with olfactory bulb size having a positive linear relationship with ECV and BM (Figure 2a,b). Additionally, both regression plots show a clear phylogenetic pattern in the relative scale of the olfactory bulbs. For instance, scandentians possess larger olfactory bulbs relative to both volume and mass than all other clades (except for stem Glires), anthropoids and Tarsiiformes possess smaller olfactory bulbs than all other clades, and both orders in Glires (rodents and lagomorphs) show similar scaling relationships to one another. In both volume and mass regressions, the slope of the relationships is also similar among all non-primate clades, with strepsirrhines and anthropoids possessing relatively shallower slopes (Table S3). While strepsirrhines largely plot near to, and share similar scaling relationships with, other Euarchontoglires, anthropoids are distinct in both volume and mass regressions relative to all clades except Tarsiiformes. The observation about the distinctly small olfactory bulbs of the haplorhines is further supported in the ANOVAs of residuals in which the relative size of the olfactory bulbs is significantly different between haplorhines and all other clades (Table S4.1). Despite the fact that the scandentians appear large relative to other groups, they were not found to be significantly different from any other clade, aside from the haplorhines. To examine whether the small OB sizes identified in the haplorhines are being driven by the anthropoids or characterize tarsiiforms as well, ANOVAs of residuals were performed with haplorhines separated into Anthropoidea and Tarsiiformes (Table S4.2). The results indicate significant differences between tarsiiforms and anthropoids and all other clades but not between these two clades (Table S4.2).

The boxplots of residuals from the volume and mass OLS regressions (Figure 2c,d) show differences among clades. Of all clades, the stem Glires (*Anagale gobiensis*, *Carcinella sigei*, and *Labidolemur kayi*) and the lagomorphs appear to have the largest median relative olfactory bulb sizes. However, this observation likely results from the distance between the whole group regression line and the tail end of the lagomorph distribution. Indeed, the slope of the whole group regression is being reduced by the anthropoids (Figure 2a). When assessed relative to mass, Scandentia has the largest OBs, excluding the stem members of Glires (Figure 2d; Table S1). The clades that possess the smallest relative olfactory bulbs are the anthropoids and tarsiiforms, as haplorhines that are significantly different from all other clades (Table S4.1).

Clade-specific regressions show differences in the extent to which ECV and BM impact relative olfactory bulb size (Table 1). For scandentians, lagomorphs, and rodents, r^2s range between 0.88 and 0.971 for OBV versus ECV and between 0.834 and 0.953 for OBM versus BM, suggesting that olfactory bulb size is strongly associated with ECV and BM. In contrast, the r^2s for strepsirrhines and haplorhines are lower, especially for the haplorhines. With regard to strepsirrhines, r^2s for volume and mass analyses are 0.782 and 0.757, respectively, while for haplorhines, ECV and BM have the smallest impact on relative olfactory bulb size, with r^2s of 0.364 and 0.471, respectively.

Given the extremely small olfactory bulbs of the haplorhines and their strong impact on the slope calculated for the total sample, the same OLS analyses of OBV versus ECV and OBM versus BM, as well as the ANOVAs of residuals from these analyses, were rerun without haplorhines. Without haplorhines, the r^2s for volume and mass are significantly higher in the total sample ($r^2 = 0.794$; $p < 0.001$, and $r^2 = 0.759$; $p < 0.001$ respectively; Table S5). The same phylogenetic patterns are reproduced in the regressions of volume and mass. Here, however, the strepsirrhines possess the smallest olfactory bulbs and scandentians possess the largest relative to both metrics, excepting only the stem members of Glires (Figure S2). The ANOVA of residuals from the non-haplorhine OLS regression of OBV and ECV indicates significant

TABLE 1 Results of OLS regression of logOBV~logECV (volume) and logOBM~logBM (mass) analyses for the entire sample of Euarchontoglires (Overall) and each clade individually.

		Residual *df*	SS	Residual SS	r^2	*F*	*Z* (from *F*)	*p*
Volume	Overall	219	7.83	38.50	0.169	44.54	4.42	0.00
	Haplorhini	43	6.73	11.76	0.364	24.61	3.51	0.00
	Strepsirrhini	25	4.25	1.11	0.792	95.39	5.34	0.00
	Rodentia	96	19.84	2.70	0.880	706.22	9.91	0.00
	Lagomorpha	26	2.53	0.14	0.948	471.89	8.40	0.00
	Scandentia	12	0.33	0.01	0.971	398.15	7.08	0.00
Mass	Overall	219	16.73	28.90	0.367	126.80	6.12	0.00
	Haplorhini	43	2.97	3.34	0.471	38.28	3.98	0.00
	Strepsirrhini	25	2.29	0.74	0.757	77.78	4.98	0.00
	Rodentia	96	17.91	3.56	0.834	482.74	9.08	0.00
	Lagomorpha	26	2.53	0.39	0.866	168.58	6.31	0.00
	Scandentia	12	0.49	0.02	0.953	243.19	6.23	0.00

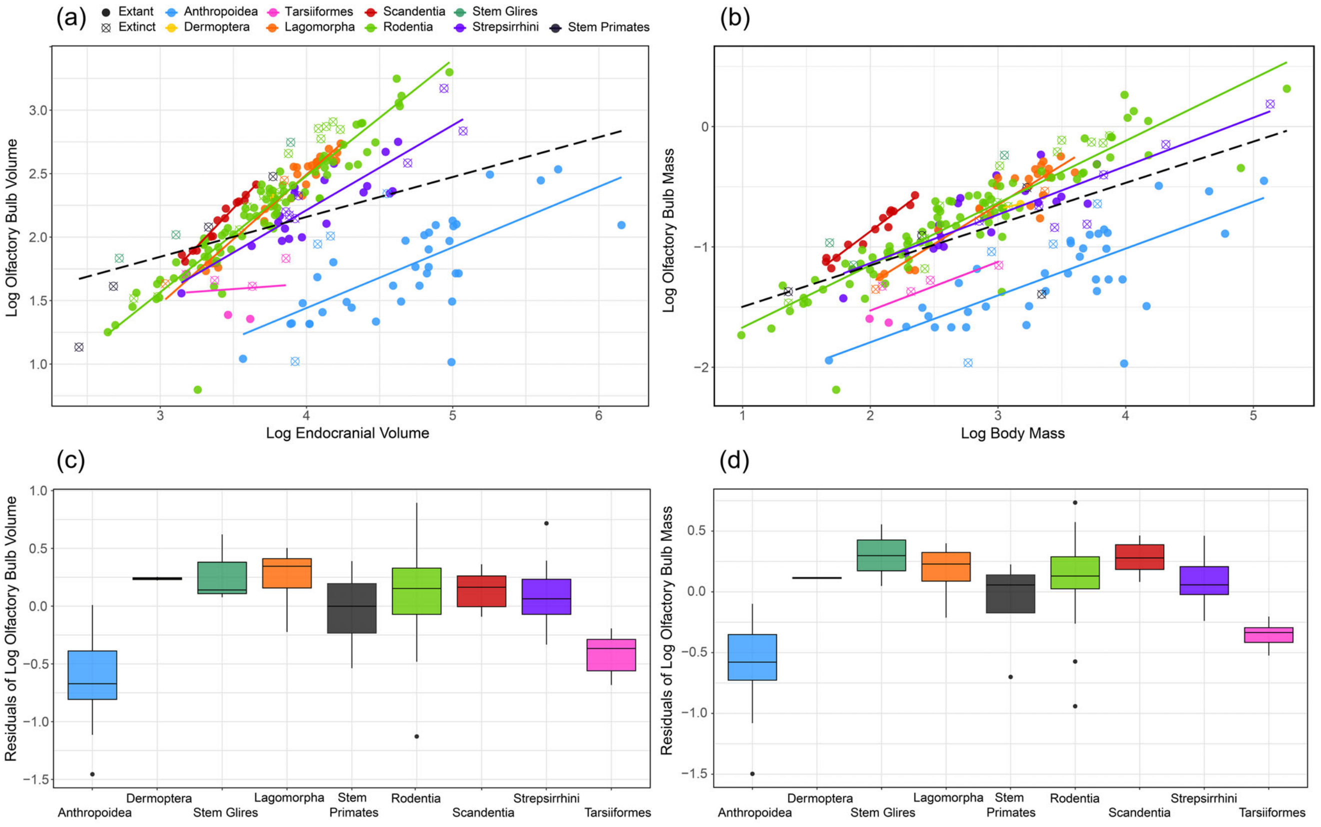

FIGURE 2 Regression analyses of logOBV and logECV (a) and logOBM and logBM (b) for the entire sample of Euachontoglires. Boxplots of residuals from OLS regressions of logOBV and logECV (c) and logOBM and logBM (d). Regression lines for clades where $n \geq 4$.

differences between strepsirrhines and all other clades, as well as between rodents and scandentians (Table S4.2). Similarly, the ANOVA of OBM and BM residuals also indicates significant differences between strepsirrhines and all other clades, as well as between scandentians and lagomorphs, but not scandentians and rodents, as in the volume ANOVA (Table S4.2).

To summarize, crown primates are distinct from other members of Euarchontoglires in possessing small olfactory bulbs relative to both endocranial volume and body mass. This is most evident and extreme for the anthropoids and tarsiiforms, which also show a reduced correlation between olfactory bulb size and endocranial volume and body mass, indicating that, in addition to having small OBs, there is more variation in relative OB size in haplorhines. While strepsirrhines are not as distinct from the rest of Euarchontoglires, when haplorhines are removed from the analyses, the strepsirrhines still possess significantly smaller relative olfactory bulb sizes compared with their close relatives, except with respect to Lagomorpha when assessed against mass.

When PGLS analyses are performed on these same data, the relationship between OBV/OBM and ECV/BM is no longer significant, suggesting that variation in these traits is occurring between phylogenetic lineages (Table S6). Correspondingly, the phylogenetic signal in these variables (logOBV, logECV, logBM, %OBV, % OBM, and residuals from the OLS analyses of volume) and mass is high (lambda ≥ 0.76; Table 2). However, within each clade, there are differences in how these traits scale with phylogeny. By far, the clade that shows the clearest phylogenetic signal in olfactory bulbs scaling is the haplorhines, with significant lambda values of 0.99 for %OBV and 0.70 for %OBM, suggesting that these scaling relationships largely follow a Brownian motion model of evolution and that closely related species are more similar to one another in their relative olfactory bulb sizes than they are to more distantly related species (Münkemüller et al., 2012). The strepsirrhines show the second strongest phylogenetic signal, but the phylogenetic signal in OBV and BM is not statistically significant (*p*-value >0.05; Table 2). Rodents show the weakest phylogenetic signal across all measured variables, ranging from lambda = 0.25 for %OBM to 0.69 for %OBV. Lagomorpha shows relatively high phylogenetic signals in OBV, ECV, and BM, but a lower phylogenetic signal in %OBM and a non-significant phylogenetic relationship for %OBV (*p*-value >0.05; Table 2). There is no statistically significant relationship identified between phylogeny and any of the measured variables for Scandentia.

TABLE 2 Phylogenetic signal (lambda) in log olfactory bulb volume (OBV), log endocranial volume (ECV), log body mass (BM), percent OBV of ECV (%OBV), and percent OBM of BM (%OBM).

	Variable	Lambda	*p*
Overall	OBV	0.86	0.00
	ECV	0.94	0.00
	BM	0.88	0.00
	%OBV	0.93	0.00
	%OBM	0.76	0.00
Haplorhini	OBV	0.86	0.00
	ECV	0.99	0.00
	BM	0.95	0.00
	%OBV	0.99	0.00
	%OBM	0.70	0.00
Strepsirrhini	OBV	0.00	1.00
	ECV	0.78	0.01
	BM	0.74	0.05
	%OBV	0.98	0.00
	%OBM	0.67	0.01
Rodentia	OBV	0.66	0.00
	ECV	0.66	0.00
	BM	0.56	0.00
	%OBV	0.69	0.00
	%OBM	0.25	0.00
Lagomorpha	OBV	0.87	0.00
	ECV	0.96	0.00
	BM	0.94	0.00
	%OBV	0.00	1.00
	%OBM	0.61	0.05
Scandentia	OBV	0.00	1.00
	ECV	0.74	0.32
	BM	0.41	0.73
	%OBV	0.00	1.00
	%OBM	0.65	0.19

In the ASR analyses, the results for nodal values (representing %OBV and %OBM) are nearly identical between the two analyses (Likelihood and Parsimony) that included Grafen branch lengths (Table S8). The BL = 1 Parsimony analysis produced values that are quite similar to those from the Grafen branch length analyses for clades not known from fossil taxa (e.g., Scandentia, Mouse Related Clade) but show fairly marked differences in many cases for clades that do include fossils. Typically, the nodal reconstructions in the Grafen Branch length analyses show a stronger effect from the modern values so that deeper nodes in the tree have values closer to those for the extant taxa. As a result, the values for the versions of clades including stem taxa are generally more similar to the values for the crown clade in the Grafen Branch length analyses than in the Parsimony BL = 1 analysis. Consequently, in general, the Grafen branch length analyses produced values that accelerated major transitions (i.e., reconstructing reductions in OB size as happening at deeper nodes in the tree). So for example, the values reconstructed for Primates (including stem taxa) are much lower relative to both %OBV and %OBM in the Grafen branch length (% OBV = 3.38; %OBM = 0.038) analyses than in the

BL = 1 parsimony analysis (%OBV = 5.49; %OBM = 0.069), but this difference shrinks for the crown clade (Grafen branch length: %OBV = 3.38; BL = 1: %OBV = 2.89; Grafen branch length: %OBM = 0.38; BL = 1: %OBM = 0.18). In the time-calibrated analysis, many nodes are reconstructed as having higher (less reduced) values for %OBV than the other two analyses, so that some of the early transitions in relative size are inferred to be less extreme. For example, the %OBV for crown primates is inferred to be 4.58%, suggesting a subtler reduction than the other two analyses compared with the stem primate node at 5.27%. This pattern is less apparent for %OBM, where the nodal values for the time-calibrated analysis vary from being higher, lower, and intermediate relative to the other two analyses.

For the parsimony ASR using the time-calibrated branch lengths, %OBV at the base of the Euarchontoglires lineage is reconstructed to be 5.68% with a subsequent decrease in the euarchontan lineage to 5.32% and a small increase in the Glires lineage to 5.88% (Tables 3 and S8; Figure 3). Within these two superorders, %OBV continues to decrease, with particularly marked decreases for the crown clades except for Scandentia, which maintains a relatively high %OBV (5.16%). In only the lineage containing *Labiolemur kayi* does %OBV increase markedly. Regarding the parsimony ASR with the time-calibrated tree, %OBM at the base of Euarchontoglires is reconstructed to be 0.060% with both the Euarchonta (0.063%) and Glires (0.059%) nodes being reconstructed as similar. Scandentia is reconstructed as having a sharp increase in %OBM (0.10%), while the %OBM at the Primatomorpha node is similar to that inferred for Euarchonta generally (0.062%). Among members of Glires, there is a decrease in %OBM from the Glires node (0.059%) for all clades, with the reduction being most marked with respect to Lagomorpha (0.031%). There are subsequent small increases inferred for the Mouse Related Clade to 0.056% and the Squirrel Related Clade to 0.053%, up from 0.051% at the crown Rodentia node. Increases are also identified in the stem Glires node relating to *Carcinella sigei* and *L. kayi*, as well as at the *Niptomomys* lineage within Primates (Figure 3b). Figure 4 shows ASR of %OBV and %OBM at specific nodes based on the parsimony time-calibrated branch length analysis. The major patterns are similar in the Grafen and BL = 1 branch length analyses, with all analyses showing notable decreases in the %OBV and %OBM at the Lagomorpha, crown Primates, Anthropoidea, Catarrhini, Platyrrhini, crown Cercopithecoidea, and Hominoidea nodes.

As noted in the Methods section, we considered the effect of three modifications to the pattern of phylogenetic relationships using the framework of the Parsimony, BL = 1 analysis (Table S6). When *Rooneyia* was treated as an adapoid, it had the effect of decreasing reconstructed nodal values (i.e., smaller %OBV and %OBM) in closely related clades (e.g., Strepirrhini, crown Strepsirrhini, Lemuroidea, Lorisoidea) and increasing values (i.e., higher %OBV and %OBM) for clades near the base of Haplorhini (i.e., stem Haplorhini, Tarsiiformes). These small shifts do not substantively impact the main patterns discussed in Section 4. Similarly, moving Reithroparamyinae had small impacts on reconstructed nodal values for adjacent nodes (i.e., Rodentia, the Squirrel Related Clade [SRC]), but does not alter any of the main conclusions.

4 | DISCUSSION

In the Introduction, we listed a series of questions about the evolution of the olfactory bulbs. This discussion is framed around our attempts to answer those questions.

I. **To what degree are OB volume (OBV) or mass (OBM) and BM versus ECV correlated? Is it necessary/appropriate/informative to study olfactory bulb volume in relation to both? To what degree does phylogeny impact these considerations?**

These questions are related to the framework around how OB evolution is studied. As noted in the Introduction, some authors have argued (e.g., Heritage, 2014; Smith & Bhatnagar, 2004), for example, that it makes sense to study olfactory bulb volume in isolation, and in particular that scaling by body mass is ill-advised. As BM and ECV are themselves highly correlated (r^2 for log BM∼log ECV = 0.756 in the total dataset here), it may also seem redundant to consider olfactory bulb size in relation to both variables.

The overall correlations of OBM with BM ($r^2 = 0.367$) and OBV with ECV ($r^2 = 0.169$) for this dataset are statistically significant ($p < 0.001$), with body mass actually being more highly correlated with olfactory bulb size than endocranial volume is. If only non-haplorhines are considered, the relationship of OBM with log body mass is even higher ($r^2 = 0.795$). In Figure 2b, it is clear that the overall regression line for the relationship between OBM and BM does a fairly good job of describing variation in other groups, but that haplorhines (who all fall below that line) are less tightly clustered. When considered within major clades (Table 1), the r^2 values for OBM∼BM are above 0.75 for all clades considered except Haplorhini ($r^2 = 0.471$). This means that analyses that

TABLE 3 Ancestral state reconstructions of percent OBV of ECV (%OBV), and percent OBM of BM (%OBM) at each node according to the three estimation methods: (1) likelihood reconstruction using a tree with Grafen computed branch lengths (BL), (2) parsimony reconstruction using a tree with Grafen computed branch lengths, and (3) parsimony reconstruction using a tree with branch lengths set to 1, and (4) parsimony reconstruction using a tree with time calibrated branch lengths (for groups represented by both the crown and stem clade, the lower of the two is in bold).

Percent OBV relative to ECV					Percent OBM relative to BM				
	Likelihood	Parsimony	Parsimony	Parsimony		Likelihood	Parsimony	Parsimony	Parsimony
Node	Grafen BL	Grafen BL	BL = 1	Time calib. BL	Node	Grafen BL	Grafen BL	BL = 1	Time calib. BL
Euarchontoglires	4.84	4.84	6.23	5.68	Euarchontoglires	0.0612	0.061	0.092	0.060
Glires	5.4	5.4	7.04	5.88	Glires	0.0613	0.061	0.084	0.059
Glires (crown)	**5.26**	**5.26**	**5.38**	**5.77**	Glires (crown)	**0.0592**	**0.059**	**0.056**	**0.058**
Lagomorpha	3.34	3.34	4.23	4.26	Lagomorpha	0.0288	0.029	0.041	0.031
Ochotonidae	2.68	2.68	3.32	2.73	Ochotonidae	0.0379	0.038	0.04	0.035
Leporidae	3.37	3.37	3.99	3.97	Leporidae	0.0261	0.026	0.028	**0.015**
Leporidae (crown)	**3.31**	**3.35**	**3.33**	**3.23**	Leporidae (crown)	**0.0252**	**0.025**	**0.024**	0.026
Rodentia	4.45	4.45	4.87	4.69	Rodentia	0.0482	0.048	**0.043**	0.051
Rodentia (crown)	**4.17**	**4.17**	**3.78**	**4.53**	Rodentia (crown)	**0.0477**	0.048	0.045	0.051
Squirrel related clade	3.23	3.74	3.52	3.86	Squirrel related clade	0.0518	0.052	0.05	0.053
Sciuroidea	3.15	3.15	3.34	3.80	Sciuroidea	**0.0491**	0.049	**0.04**	**0.044**
Sciuroidea (crown)	**3.12**	**3.12**	**3.15**	**3.23**	Sciuroidea (crown)	0.0493	0.049	0.051	0.061
Aplodontiidae	3.19	3.19	3.24	3.53	Aplodontiidae	0.0664	0.066	0.07	0.115
Sciuridae	3.01	3	2.87	**2.98**	Sciuridae	**0.0478**	0.048	0.043	**0.043**
Sciuridae (crown)	**3**	**2.98**	**2.5**	3.08	Sciuridae (crown)	0.0487	0.048	**0.037**	0.045
Ctenohystrica + SRC	3.74	3.74	3.78	4.29	Ctenohystrica + SRC	0.0441	0.044	0.044	0.049
Ctenohystrica	3.7	3.7	4.03	4.27	Ctenohystrica	0.032	0.032	0.037	0.048
Mouse Related Clade	3.44	3.43	3.49	4.35	Mouse related clade	0.0654	0.065	0.052	0.056
Euarchonta	4.05	4.05	5.42	5.32	Euarchonta	0.061	0.061	0.101	0.063
Scandentia	4.97	4.97	5.04	5.16	Scandentia	0.1464	0.15	0.143	0.101
Primatomorpha	3.8	3.8	4.99	5.30	Primatomorpha	0.0459	0.046	0.068	0.062
Primates	3.8	3.77	5.49	5.27	Primates	0.0445	0.044	0.069	0.061
Primates (crown)	**3.38**	**3.38**	**2.89**	**4.58**	Primates (crown)	**0.0378**	**0.038**	**0.018**	**0.046**
Strepsirrhini	1.98	1.98	2.36	2.82	Strepsirrhini	**0.0241**	**0.024**	**0.018**	0.020
Strepsirrhini (crown)	**1.87**	**1.87**	**1.95**	**2.81**	Strepsirrhini (crown)	0.0252	0.025	0.024	0.020
Lemuroidea	1.46	1.46	1.53	1.87	Lemuroidea	0.0213	0.021	0.023	0.021

(Continues)

TABLE 3 (Continued)

Percent OBV relative to ECV					Percent OBM relative to BM				
	Likelihood	Parsimony	Parsimony	Parsimony		Likelihood	Parsimony	Parsimony	Parsimony
Node	Grafen BL	Grafen BL	BL = 1	Time calib. BL	Node	Grafen BL	Grafen BL	BL = 1	Time calib. BL
Lorisoidea	2.04	2.04	1.97	2.11	Lorisoidea	0.0376	0.037	0.031	0.033
Haplorhini	1.53	1.53	2.16	4.21	Haplorhini	0.0175	0.017	0.015	0.038
Tarsiiformes	1.79	1.79	2.36	3.89	Tarsiiformes	**0.0184**	**0.018**	**0.016**	**0.018**
Tarsiiformes (crown)	**0.82**	**0.82**	**0.88**	**0.92**	Tarsiiformes (crown)	0.0213	0.021	0.021	0.022
Anthropoidea	1.08	1.08	1.21	0.76	Anthropoidea	**0.0129**	0.012	0.01	0.0098
Anthropoidea (crown)	**1.01**	**1.01**	**0.73**	**0.71**	Anthropoidea (crown)	0.0122	**0.011**	**0.0063**	**0.0083**
Catarrhini	0.42	0.42	0.61	0.70	Catarrhini	0.0052	0.0035	0.0044	0.0040
Catarrhini (crown)	0.42	**0.37**	**0.41**	0.55	Catarrhini (crown)	0.0049	0.0031	**0.0029**	**0.0033**
Platyrrhini	0.38	0.38	0.38	**0.13**	Platyrrhini	0.006	0.0059	0.0041	**0.0019**
Platyrrhini (crown)	**0.35**	**0.35**	**0.27**	0.16	Platyrrhini (crown)	**0.0058**	**0.0057**	0.0041	0.0034
Cercopithecoidea	0.3	0.3	0.42	0.61	Cercopithecoidea	0.0054	0.0025	0.0029	0.0038
Cercopithecoidea (crown)	**0.25**	**0.25**	**0.23**	**0.35**	Cercopithecoidea (crown)	**0.0044**	**0.0022**	**0.0019**	**0.0033**
Hominoidea	0.08	0.08	0.19	0.25	Hominoidea	0.001	0.00081	0.0015	0.0017

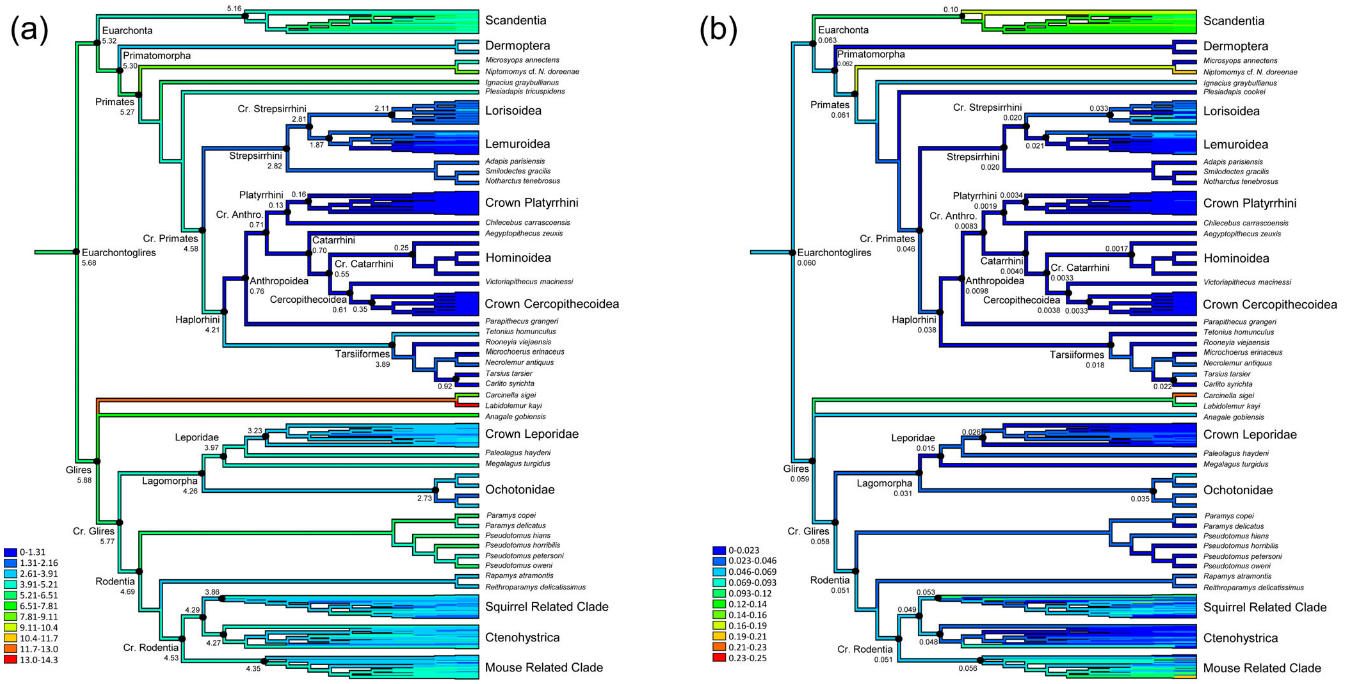

FIGURE 3 Parsimony ancestral state reconstructions of percent OBV relative to ECV (a) and percent OBM relative to BM (b) using the tree with time-calibrated branch lengths.

look at unscaled olfactory bulb size in groups other than haplorhines (including strepsirrhines) will be difficult to interpret because larger animals generally have larger olfactory bulbs; the same is also true of haplorhines but to a lesser degree. Significantly, regarding primates, this shows that there is more variation in the scaling of the olfactory bulbs with respect to body size in haplorhines alone than there is in most non-primates *and* in strepsirrhines.

With respect to the question of whether it is necessary to consider scaling in relation to both ECV and BM, although in most cases the results are similar, the places in which they are different highlight why considering both is important. In the ancestral state reconstruction, when the reconstructed nodal values (Figure 3; Table 3) for clades that include stem members are compared with the reconstructions for the crown clades (i.e., excluding fossils that fall on the stem) it is nearly universally true that the reconstructed values for OBV relative to ECV for the crown clades are lower than for the clades that include the stem taxa. One way to interpret this would be huge amounts of parallelism, with every crown clade showing independent reductions in the size of the olfactory bulbs. However, an alternative explanation could be that the pattern is being created by widespread increases in absolute brain size occurring independently of olfactory bulb size. It has long been suggested (Jerison, 1961; also see Bertrand et al., 2022) that there is a temporal effect on relative brain size in mammals, and Bertrand, Amador-Mughal, et al. (2019) upheld this view based on the then-available data for fossil rodents (albeit weakly) and primates (more strongly). Such a trend could explain the pattern in the nodal values, as well as the generally leftward inclination of the evolutionary trajectories in Figure 4a toward smaller OBs relative to ECV. Although it is true that when the stem and crown clades are compared for the reconstructed nodal values scaled by body mass (Table 3) there is a similar pattern in many cases, this is not true for Strepsirrhini, Sciuroidea, or Tarsiiformes. In all three of these cases, there are fossil taxa on the stem for these clades that have olfactory bulbs that seem large when considered relative to ECV but not relative to BM, suggesting that the results scaled by ECV may reflect increases in brain size or other brain regions in the crown clades and not reductions in the olfactory bulbs specifically. Previous authors have hypothesized increases in the neocortex for Eocene mammals generally (Bertrand et al., 2022) and for Sciuroidea specifically (Bertrand et al., 2021). Therefore, considering the proportional changes of different brain regions is crucial to improving our understanding of brain evolution as a whole.

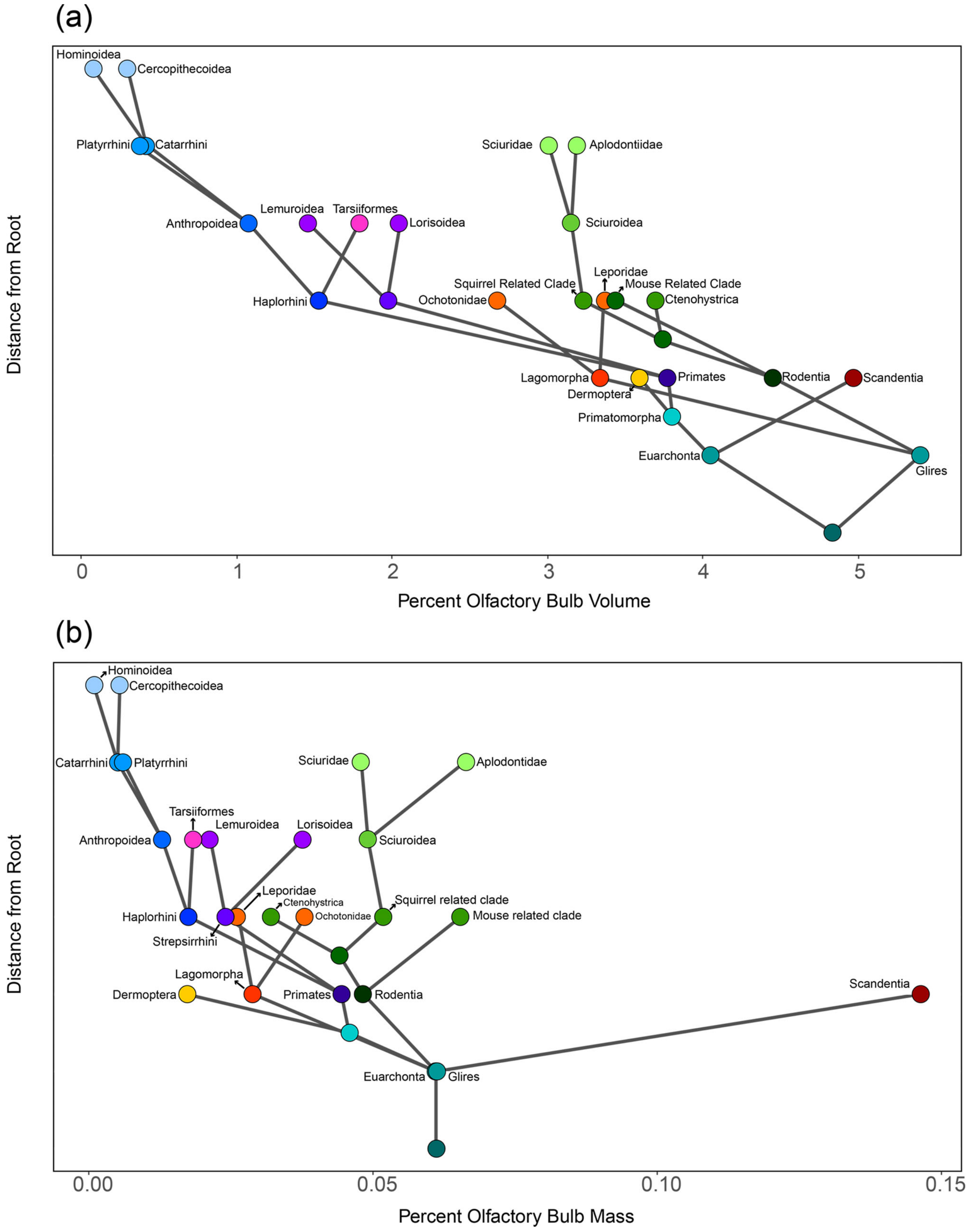

FIGURE 4 Ancestral state reconstructions of percent OBV relative to ECV (a) and percent OBM relative to BM (b) at specific nodes based on parsimony reconstructions using the tree with time-calibrated branch lengths . Y axes represent distance from root.

It is clear that there is generally a very strong phylogenetic effect on the relationship between olfactory bulb size and both ECV and BM. In comparing the OLS and PGLS results (Tables 1 and S6), all of the significant results from the OLS analyses are not significant in the PGLS analysis. In other words, the pattern of variation generally matches the pattern of phylogenetic relationships. Heritage (2014) found no evidence of an effect of phylogeny on the relationship between OBV and ECV in haplorhines. Our results differ in finding a significant phylogenetic effect for all variables considered for Haplorhini and for most variables in other clades (Table 2). There are a few interesting exceptions. Strepsirrhini shows a strong phylogenetic signal for relative olfactory bulb size but not absolute size. This may reflect the large absolute size of the bulbs in the subfossil lemurs, which do not form a clade but are instead interspersed at various points in the strepsirrhine tree (following Kistler et al., 2015; Marciniak et al., 2021). This difference in findings again highlights the importance of considering olfactory bulb size in a relative framework. The weak phylogenetic effect on olfactory bulb size relative to body mass in Rodentia may reflect the fact that there is a lot of variation within particular rodent clades in terms of body mass. Perhaps olfactory bulb size is less labile in this clade. Alternatively, ecological factors specific to particular taxa may have a high impact in shaping their size. For instance, locomotion has been shown to have a strong impact on the brain and its components in Sciuroidea (Bertrand et al., 2021). In contrast, in Lagomorpha, there is a significant phylogenetic effect on olfactory bulb size relative to BM but not ECV, which would suggest that brain size varies to some degree without changes in olfactory bulb size. Finally, there is no significant relationship between OB size and phylogeny within Scandentia. This might reflect the fact that this clade is generally less variable than the other clades being considered (i.e., see Figure 2).

II. **Can a reduction in OB size be traced down to the basal primate or crown primate nodes? As such, can primates as an order be characterized as having evolved a decrease in olfactory sensitivity?**

In the OLS analysis, Primates differ significantly in olfactory bulb size relative to both ECV and BM compared with all other clades and all anthropoids and tarsiiforms and many strepsirrhines (including fossils) lie below the general regression line for the relationship between OBM and BM. The same pattern is seen for OBV and ECV though one fossil anthropoid occurs just above the regression line (*Victoriapithecus macinessi*). However, all the various ancestral state reconstruction (ASR) analyses show very little difference between the reconstructed nodal values for the Primatomorpha (=dermopterans, stem and crown primates) and Primates nodes (Figure 3; Tables 3 and 8), which reflects the relatively large olfactory bulbs in the stem primates (plesiadapiforms). In contrast, there is a notable reduction in relative olfactory bulb size at the crown primate node in all ASR analyses. The magnitude of the change depends on the branch length estimation method. There is a larger inferred change in the parsimony analysis with branch lengths set to 1 than in either the analyses that include the Grafen standardized branch lengths or in the time-calibrated analysis. In light of the fairly marked contrast between stem and crown primates, the nodal reconstruction that shows the larger magnitude change (as suggested in the $BL = 1$ analysis) is better supported with the current dataset.

In either case, the fact that there is a crown primate effect in both the ECV and BM analyses under all assumption sets indicates that there is a change at that node that is not simply attributable to a shift in overall brain size, which is a difference from Heritage's (2014) results. Interestingly, though, this finding is consistent with genetic results that all living primates have fewer olfactory receptor genes than other euarchontans (Niimura et al., 2018) and is also consistent with the results from Bertrand et al. (2022) that found a decrease in olfaction at the base of crown Primates when put into a broader context of Paleocene and Eocene placental mammals.

III. **Was there an increase in OB size in living strepsirrhines (as suggested by Heritage, 2014)?**

There is no consistent finding of an increase in OB size in living strepsirrhines in our results—this is a clear difference from Heritage's (2014) results. When haplorhines are included in the OLS analysis, OB size in strepsirrhines is only significantly different from the haplorhines, but not any other clade, suggesting that the reduction in OB size is primarily characteristic of Haplorhini (Table 1; Figure 2). However, when the haplorhines are removed, the differences between the strepsirrhines and other Euarchontoglires become more apparent, as OB size in Strepsirrhini is then identified as significantly smaller than all other clades. While strepsirrhines are not as derived in relative

OB size as the haplorhines they are still significantly smaller than their non-primate relatives. This is also supported by the ASR analyses, which all show reductions in the relative size of the olfactory bulbs from the crown primate to strepsirrhine node, with the ECV analyses showing a further reduction in the transition from stem to crown strepsirrhines (although of a tiny magnitude in the time-calibrated analysis). However, this latter effect reverses, or no change is reconstructed, in the BM analyses, suggesting that it is a product of changing overall brain size (or of expansions to particular regions such as the neocortex) rather than olfactory bulb size. What is more, the BL = 1 parsimony analysis for body mass shows no difference in the inferred nodal value between crown primates and stem strepsirrhines (0.018%), with a subsequent increase in crown strepsirrhines (0.024%), while the other analyses infer a reduction as happening earlier, in the transition from crown Primates to Strepsirrhini.

One likely reason for these ambiguities is that we only have data for one fossil crown strepsirrhine (*Mioeuoticus*), so the inferences about nodal values at the base of the crown clade are largely driven by variation in modern strepsirrhines. This variation is reflective of the diversity that evolved in the adaptive radiation of lemuroids on Madagascar, millions of years after the events we are trying to make inferences about. There is also clearly some effect of activity period on olfactory bulb size (see below), which is additionally complicating these inferences about what is primitive.

Suffice it to say, there is no compelling evidence for a clade-wise increase or decrease in olfactory bulb size for strepsirrhines relative to the common ancestor of crown Primates occurring independently of other changes in the brain, although there may have been clade-specific changes for groups within Strepsirrhini (see below).

IV. **Were there decreases in OB size at either the haplorhine or anthropoid nodes (or both)?**

The OLS analysis found a significant difference between haplorhines and all other clades considered, including strepsirrhines, while in the ASR analyses the haplorhine node is consistently reconstructed with lower values for relative OB size than the crown primate node. However, there is quite a bit of variability in relative olfactory bulb size among the various omomyoids, with some having larger olfactory bulbs than adapoids. Of these, the values for *Tetonius* stand out, as that taxon has the largest olfactory bulbs relative to ECV of any crown primate. It is worth noting, however, that the estimates for that taxon date back to Gurche (1982), and as such are based on external measurements rather than CT data. The relevant specimen (AMNH 4194) should probably be reassessed with more modern methods. The same critique does not apply to *Necrolemur antiquus*, however, which has already been reassessed (Harrington et al., 2020), and which had olfactory bulbs similar to notharctine adapoids in size relative to ECV, and larger in size relative to body mass. Also, one of the two crown tarsiiforms (*Tarsius tarsier*) included in the analysis has olfactory bulbs whose relative sizes fall within the range of variation of living strepsirrhines. It is nonetheless worth noting that tarsiiforms are found to be significantly different from all clades other than anthropoids in the OLS analyses. As such, there is some evidence for a reduction in olfactory bulb size at the haplorhine node, although the variation among living and fossil tarsiiforms makes this somewhat ambiguous.

Anthropoids were found to be significantly different from all other clades except tarsiiforms in the OLS analysis, and the ASR analyses consistently show a marked reduction in relative size (compared with both ECV and BM) at that node, with additional reductions in both the ECV and BM analyses at the crown anthropoid node. The one stem anthropoid for which we have data (*Simonsius grangeri*) has relatively smaller olfactory bulbs than any of the omomyoids (with the exception of the ambiguous taxon *Rooneyia* when its OBs are considered relative to body mass). *Simonsius grangeri* has larger olfactory bulbs relative to ECV than any living anthropoid, but not relative to BM. These various indications suggest that there were reductions in olfactory bulb sizes in the common ancestor of all anthropoids relative to primitive haplorhines, but that it is more ambiguous whether there was a subsequent shift that affected all crown anthropoids independent of changes in total brain size and other regions of the brain more specifically.

V. **Is there evidence for further changes in OB size within strepsirrhine or anthropoid clades?**

There is a fair amount of interesting variation in olfactory bulb size among strepsirrhines, which may be related to the broad diversity of ecological regimes present in that group. So, for example, *Archaeolemur* has unusually small olfactory bulbs compared with most strepsirrhines, suggesting that its monkey-like biology might have extended to its sensory systems (as also suggested based on its semicircular canals; Walker et al., 2008). As previous authors have noted (e.g., Barton, 2006; Barton et al., 1995; Martin, 1990), there is some relationship between nocturnality (and also diet; e.g., see Barton, 2006) and larger olfactory bulb size, with the relatively largest olfactory bulbs among crown strepsirrhines being observed in nocturnal cheirogaleids and lorisoids. However, there is clearly also a phylogenetic element to this pattern, since the relative size of the OBs is very similar in diurnal *Lemur catta* and its cathemeral kin (*Hapalemur*, *Eulemur*). It is also interesting to note that the olfactory bulbs are relatively smaller in *Lepilemur* than in its cheirogaleid sister taxa, in spite of it also being nocturnal. This may be related to its folivorous diet, suggesting that the effect of activity pattern is mediated by other ecological variables (as also found by Barton et al., 1995).

With respect to anthropoids, there is an offset between the olfactory bulb size inferred for the common ancestor of crown Anthropoidea and for the ancestors of both Platyrrhini and Catarrhini. This could suggest that there were parallel and independent reductions in the size of the olfactory bulbs in those two clades. This inference is supported by the contrast between *Chilecebus* (a stem platyrrhine), which has a relative olfactory bulb size (for both ECV and BM) within the modern platyrrhine range, and the notably larger olfactory bulbs of *Aegyptopithecus* (a stem catarrhine), which are relatively large for catarrhines no matter how scaled. Interestingly, the largest olfactory bulbs relative to ECV for any living anthropoid are in *Aotus*, suggesting that in that clade there is also some effect of nocturnality, as primates with relatively large OBs tend to be nocturnal (Barton et al., 1995). Because *Aotus* is well nested within the platyrrhine tree (all of which are otherwise diurnal) their relatively larger olfactory bulbs would represent a reversal, rather than retention of a primitive state, that could be related to the impact of ecology on its olfactory bulb size.

Higher in the catarrhine tree, *Victoriapithecus* has notably large olfactory bulbs in relation to both endocranial volume and body mass for a cercopithecoid (see also Gonzales et al., 2015), which suggests that there may have been additional reductions in relative size in cercopithecoid evolution. The ASR analyses also universally reconstruct a marked reduction in olfactory bulb size in hominoid evolution; data from fossils to assess that inference are hard to come by since the olfactory bulbs are so overgrown by the cerebrum in hominoids that it is not possible to isolate them from the rest of the endocast. In any case, these various lines of evidence suggest that there were several instances of decreases in the relative size of the olfactory bulbs among catarrhines that cannot be attributed to increases in overall brain size because they are also apparent in the body mass analysis. As such, the relatively low correlation between olfactory bulb size and brain size in haplorhines discussed above is at least partly a product of successive decreases among anthropoids occurring in association with evolutionary branching events, as supported by the finding of a significant effect of phylogeny within this clade.

VI. **Do scandentians show reductions in olfactory sensitivity as mediated by OB size (i.e., as suggested by LeGros-Clark, 1959; but see Martin, 1990) that are either shared with Primates, or evolved independently? What are the implications of these results for using treeshrews as behavioral models for living primates?**

Contrary to LeGros-Clark's (1959) suggestion, Scandentia is inferred to have large olfactory bulbs for a modern member of Euarchonta when considered relative to ECV (Figure 4a; as also found by Heritage, 2014), but really quite extraordinarily large OBs for any kind of euarchontogliran when considered relative to body mass (Figure 4b). This finding is in keeping with the fact that treeshews have by far the most functional olfactory receptor genes among euarchontans (Niimura et al., 2018). In comparison to the stem primates (i.e., plesiadapiforms), the olfactory bulb sizes are similar when assessed relative to ECV, but very much larger in scandentians when considered relative to body mass. This suggests that overall brain size may have increased in scandentians through evolutionary time from the common ancestor with other euarchontans, as inferred for primates, but unlike in primates this overall increase in brain size was also matched with increases in the size of the olfactory bulbs. In other words, in primate evolution the rest of the brain increased in size while the olfactory bulbs stayed the same or got smaller across various nodes, but in scandentians brain size increase happened both in the total brain and in the olfactory bulbs. What's interesting is that diurnal treeshrews also exhibit improvements to the visual system that occurred convergently with those of primates (Kaas, 2002). So diurnal treeshrews have a very different set of sensory specializations than primates, with strengths in both vision *and* smell. This difference in pattern makes treeshrews problematic models for the ancestral primate, and to an even greater degree as models for any crown primates, with respect to the sensory system.

VII. **Are there changes in OB size in other euarchontogliran groups (e.g., Rodentia, Lagomorpha) that parallel the changes occurring in Primates?**

One of the challenges in interpreting the data for Glires is that it is unclear to what degree the fossil taxa treated as stem members of the group here represent what is really primitive. Apatemyids were behaviorally specialized in a manner similar to a living aye-aye (i.e., they were extractive foragers; Von-Koenigswald, 1990), and anagalids may have been specialized for fossoriality (Bohlin, 1951; López-Torres et al., 2023). It is worth noting that *Anagale* is from the late Eocene and is potentially quite derived compared with what the ancestor of Glires, which probably originated 30 Mya earlier, would have looked like. As such, the relatively large olfactory bulbs in both of those groups may represent specializations associated with behavioral adaptations. Only additional material from early fossil members of Glires will allow that hypothesis to be tested. Most of the material for early Glires is composed of teeth and incomplete cranial elements (Fostowicz-Frelik, 2020). One well-preserved taxon, the early Glires and Eurymylidae *Rhombomylus* published in (Meng et al., 2003 by Meng and colleagues, appears to display relatively large olfactory bulbs when compared with early rodents (i.e., Ischyromyidae), which could suggest a reduction in the relative proportion of the OBs at the base of rodents. Furthermore, Bertrand et al. (2022) found a reduction in relative OB size but an increase in absolute OB size at the base of rodents, which would imply that some other regions of the brain such as the neocortex may have increased in size and the olfactory bulbs increased in size as well, which mirrors the results found for scandentians. With respect to lagomorphs, the ECV and BM analyses provide somewhat different pictures of the timing of reductions in the clade. The ECV analysis suggests a reduction in relative OB size in ochotonids, with it being ambiguous whether any change occurred in leporids (i.e., the results differ based on which ASR analysis is considered; Table 3), while the BM analyses suggest a reduction in OB size in leporids and an increase in ochotonids. Among crown lagomorphs all the smallest taxa are ochotonids, so this may be a case where body size evolution is muddying the picture of whether any shifts occurred in this group. Alternatively, the complex scent-marking behavior of ochotonids (Meaney, 1986) may contribute to their larger OB relative mass, and perhaps the discrepancy with the ECV analysis reflects that lack of fossil ochotonids in the analysis to accurately reconstruct what is primitive for brain size in that group.

For rodents, there is a lot of variation in relative OB size, as one would expect from the behavioral diversity in the group. It is interesting to note that the Mouse Related Clade is similar to ochotonids in showing an opposite pattern of OB evolution in the ECV and BM analyses—again, this may reflect the challenge of untangling body mass evolution from the evolution of the brain or could represent a behavioral adaptation. We observe a decrease in the size of the olfactory bulbs relative to ECV at the crown Rodentia node, and this decrease continues in the SRC, while in relation to body mass, the size of the OB increases in the SRC. This would suggest that other regions of the brain, such as the neocortex, increase in proportion, but that also the olfactory bulbs increased as well in the lineage leading to the SRC. This result is also similar to those found for scandentians and early rodents (see above). One interesting result that is consistent in the ECV and BM analyses is that both reconstruct Aplodontiidae (i.e., mountain beavers who are fossorial) as showing increases in the relative size of the olfactory bulbs relative to its sciuroid ancestor (which is inferred to have been arboreal; Bertrand et al., 2018). Aplodontiidae had previously (Bertrand et al., 2018) been found to have undergone a reduction in overall brain size. This analysis suggests that such a reduction did not apply to the olfactory bulbs, which may have actually increased in relative size (as the result is found in the BM analysis as well as the ECV analysis). These results are consistent with previous ancestral state reconstructions that focused on Sciuroidea and Ischyromyidae (Bertrand et al., 2021). An increase in the relative size of the olfactory bulbs was also recovered from the Sciuroidea to the Aplodontiidae node.

VIII. **To what degree do fossils impact the results of these analyses?**

As noted above, the fact that crown clades are nearly universally reconstructed as having smaller olfactory bulbs than the versions of those clades that include stem members (see Tables 3 and S8) may reflect a general temporal effect in brain size (Bertrand et al., 2022; Bertrand, Amador-Mughal, et al., 2019; Jerison, 1961). This likely pattern means that including fossils in analyses with modern taxa is going to require careful thinking about the meaning of relative measures of the brain. In a sense, working with only modern taxa is simpler, since they all represent the theoretical endpoint of that trend. Also, as discussed for the primitive Glires taxa included, it can be misleading to treat fossil taxa as though they themselves are not specialized. Fossil forms are not simply ancestors (or representatives of ancestral clades)—they were functioning animals with their own peculiarities. On the other hand, morphological convergence is ubiquitous in organisms, and therefore including fossils might, in some cases, provide a better reconstruction of a common ancestor, as is the case for Aplodontiidae, which have been inaccurately considered to represent the ancestral condition for squirrels. Throughout their evolution, Aplodontiidae specialized toward a fossorial lifestyle from an arboreal ancestor that it shares with squirrels. This conclusion can only be reached because of the fossil record (Hopkins, 2008).

Therefore, we would argue that including fossils in these analyses is absolutely critical to pinpointing when changes actually happened in euarchontogliran evolution. As noted above, making inferences about early strepsirrhine evolution is challenging from a sample of modern taxa that includes the effects of the adaptive radiation on Madagascar. The same argument could be made about other primate groups (e.g., platyrrhines, cercopithecoids, hominoids, etc.). With respect to changes in overall brain size, including fossils has shown that the pattern is much more complex (and non-parsimonious) than would have been expected from modern taxa, with a lot of parallelism (Allen, 2014; Gonzales et al., 2015; Kay et al., 2008; Ni et al., 2019; Silcox et al., 2022; Steiper & Seiffert, 2012). This analysis suggests that the same is also true for olfactory bulb size, albeit to a lesser degree than would be suggested by the ECV analysis alone.

5 | CONCLUSIONS

Here, we investigated the scaling relationships of OB size across Primates and their close relatives to test previous hypotheses and assumptions about the nature of olfaction within these groups. From these analyses, there are a few key messages that are relevant to the questions about the evolution of olfaction and about sensory evolution more generally. The results here suggest that there were many changes over the course of primate evolution in the relative size of the olfactory bulbs. Some of these shifts impact large clades (e.g., decreases in crown Primates, Platyrrhini, Cercopithecoidea, etc.), while others are specific to particular smaller clades (e.g., increases in cheirogaleids, *Aotus*). Nevertheless, at least in regard to the amount of brain tissue dedicated to the sense of smell, crown Primates can be described as microsmatic, having reduced relative olfactory bulb sizes across all lineages relative to the stem primate and euarchontan nodes (Figures 3 and 4). While reductions in relative OB size occur in all other orders, none of these reductions are as drastic as in the crown primates, especially the haplorhines. Additionally, and contrary to previous analysis (i.e., Heritage, 2014), we identify no increase in relative OB for Strepsirrhini. Although the strepsirrhine scaling relationship between OB size, endocranial volume, and body mass is similar to the rest of Euarchontoglires (Figure 2a,b) and distinct from the haplorhines, they are still significantly smaller than all non-haplorhines clades tested. Outside of Primates, OB size in Scandentia was previously thought to be reduced, as is the case with Primates (LeGros-Clark, 1959); however, the current analyses find a marked increase in the relative size of OB for this order (as also found by Heritage, 2014). Unlike primates, Scandentia appears to have had an increase in total brain size that included a corresponding increase in OB size.

Although the regression and ANOVA results point to some of these larger patterns, Ancestral State Reconstruction was critical to helping to unravel the meaning of these results and to identify contrasts that were not predicted a priori. Having said that, ASR analyses themselves depend on various assumptions, as is clear from the contrasts between the analyses with branch lengths calculated in varying ways, so it seems key to examine varying ASR models as well, with the understanding that results that appear across methods are likely to be the most robust. It would also likely be valuable to incorporate analyses that allow for varying evolutionary rates. Nonetheless, a critical aspect of the current results is that, no matter what models are used, it is not possible to understand the full complexity of the pattern in OB evolution without including fossil and subfossil data. Olfaction represents a special case in the study of sensory evolution because the relevant part of the brain is often distinguishable in endocasts and, therefore, can be effectively measured in fossils.

As highlighted by Smith et al. (2007) studying the evolution of olfaction (or more generally chemoreception) requires many lines of evidence. As such, the current work should be viewed as one thread in a complex tapestry, useful as a source of hypotheses to test using other methods rather than representing any kind of definitive word on the evolution of olfaction in primates. The contrasts between the findings here and those of Heritage (2014) show that even analyses using similar types of data and similar methods can reach very different conclusions. We would argue that the more relevant comparative framework of the current analysis, paired with the larger samples of fossils, means that our results are more likely to be correct. However, many of the inferences made here are dependent on the signal coming from one or a few fossils (e.g., a single-stem anthropoid, a single-stem catarrhine, etc.). To take one example from this dataset, if the only omomyoid known from endocranial data was *Microchoerus erinaceus* (which has quite small olfactory bulbs, similar in relative size to *Tarsius tarsier*) then it would seem clear and unambiguous that there was a transition to smaller OB size at the base of Haplorhini. However, the diversity of data among omomyoids makes this finding more ambiguous. Time will tell whether additional stem taxa at various other critical nodes in the primate tree make some of the patterns identified here more or less compelling.

AUTHOR CONTRIBUTIONS

Madlen Maryanna Lang: Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing – original draft; writing – review and editing. **Mary Teresa Silcox:** Conceptualization; formal analysis; funding acquisition; project administration; supervision; visualization; writing – original draft; writing – review and editing. **Łucja Fostowicz-Frelik:** Data curation; writing – review and editing. **Adam Lis:** Data curation; writing – review and editing. **Sergi López-Torres:** Data curation; funding acquisition; supervision; writing – review and editing. **Gabriela San Martin-Flores:** Data curation; writing – review and editing. **Ornella C. Bertrand:** Data curation; funding acquisition; investigation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

We declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data and code necessary to reproduce the analyses can be found in the Supplementary Information or at the following link: https://github.com/madlenlang/Olfactory-Bulb—AR—2024.git. All of the endocast files are available from Morphosource.

PATIENT CONSENT STATEMENT

No patients were involved in this research.

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