Atlas morphology, scaling and locomotor behaviour in primates, rodents and relatives (Mammalia: Euarchontoglires)

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The mammalian atlas, or first cervical vertebra, supports the weight of the head and enables complex movements of the head and neck via articulations with the cranium and axis (second cervical vertebra). Despite these ubiquitous functional roles, the morphology of the atlas varies widely among mammals, suggesting that its shape may be associated with allometry, ecology, behaviour or a combination of these factors. Here, we test the hypotheses that atlas shape is correlated with body size, head size and locomotor behaviour in Euarchontoglires, a diverse group of mammals including primates, rodents, lagomorphs, tree shrews and colugos. We use three-dimensional geometric morphometrics to quantify the shape of the atlas vertebra in 64 species, and use a phylogenetic comparative framework to examine trends in atlas shape across a broad swath of the mammalian tree. Our results indicate that body size is associated with atlas morphology across euarchontogliran mammals, whereas head size and locomotion are not. Strong phylogenetic signal in atlas shape among all groups reiterates the importance of considering evolutionary non-independence of traits, and we further discuss integration of phylogenetic and shape data.

ADDITIONAL KEYWORDS: functional morphology – cervical vertebrae – mammals – evolution – phylogenetic comparative methods.

INTRODUCTION

Mammalian cervical vertebrae support the head, allow flexion and extension of the neck and rotation of the head about the spine, house the spinal cord and anchor axial muscles (Evans, 1939). The mammalian atlas, or first cervical vertebra (C1), is morphologically and functionally distinct from the subsequent cervical vertebrae. Composed of ring-like arches and lacking a central vertebral body, the atlas articulates cranially with the occipital condyles of the skull to allow flexion and extension of the head; it also rotates about the odontoid process of the axis (C2) caudally, allowing head rotation (Evans, 1939). Despite the pronounced variation in atlas shape among mammals, our understanding of the comparative morphology of the atlas and its relationship to function has largely been limited to primates (e.g. Manfreda *et al.*, 2006; Nalley & Grider-Potter, 2015, 2017) and, to a lesser extent, tree shrews (Sargis, 2001) and carnivorans (Randau *et al.*, 2016a, b).

Quantitative studies of the primate atlas have demonstrated relationships between vertebra shape and locomotor behaviour. One three-dimensional (3D) geometric morphometric (GM) study of atlases from nine primate species, including humans, found that species with more erect postures during locomotion exhibited thinner arches, transverse processes that were more caudally and ventrally oriented, and articular facets that tended to be rounded and inclined (Manfreda *et al.*, 2006). This study also identified an allometric

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relationship in which increasing body size was associated with a more robust atlas with more caudally oriented transverse processes (Manfreda *et al.*, 2006). A recent study found a similar allometric relationship between linear measurements of atlas shape and body size in a sample of 20 primate species, as well as a positive correlation between craniocaudally longer posterior arches and more horizontal posture (Nalley & Grider-Potter, 2017). Research on the morphology of cervical vertebrae in primates frequently aims to understand the evolution of bipedal locomotion in hominins (Graf *et al.*, 1995; Johnson & Shapiro, 1998; Strait & Ross, 1999; Manfreda *et al.*, 2006) or to infer the posture and locomotion of fossil primates (Sanders, 1998; Patel *et al.*, 2007; Kikuchi *et al.*, 2012). However, the evolution of bipedalism is just one aspect of evolution in this clade. Extant primates display many modes of locomotion, including arboreal and terrestrial quadrupedalism, leaping, suspensory hanging and brachiation, and bipedalism (Napier, 1967; Gebo, 1987, 1996; Hunt *et al.*, 1996). This locomotor diversity is thought to have arisen via independent evolution of leaping, arboreal quadrupedalism and suspensory locomotor patterns in at least three separate primate radiations (Fleagle & Lieberman, 2015). Not only is such functional and behavioural diversity likely to be associated with changes in the axial skeleton, but the repeated evolution of locomotor modes in different clades of primates also provides a context in which to explore how variation in vertebral shape may be driven by both phylogeny and locomotion.

In contrast to primates, research on the relationship between the morphology of cervical vertebrae and their function in other mammals is limited. A qualitative comparison of the axial skeleton of tree shrews (Tupaiidae, Ptilocercidae) reported that the atlas of an arboreal species of *Ptilocercus* is craniocaudally expanded and may restrict mobility of the neck compared to the atlas of more terrestrial tupaiines (Sargis, 2001). The ancestor of the group that includes tree shrews, primates and colugos was likely arboreal and is hypothesized to have a craniocaudally expanded atlas (Sargis, 2001). Quantitative phylogenetic comparative analyses of the felid axial skeleton demonstrated conflicting allometric relationships between body size and several measures of atlas shape, but no relationship between atlas shape and locomotion or prey size (Randau *et al.*, 2016a,b). A series of broad comparative analyses have examined the morphology of the cranial base and found that orthograde and bipedal mammals from diverse taxonomic groups (including primates, rodents and marsupials) have shorter basioccipitals and more anteriorly positioned foramina magna than their non-orthograde or quadrupedal relatives (Russo & Kirk, 2013, 2017), but no observations have been extended to the atlas vertebrae that articulate with these structures. Finally, a 3D GM study found that facial anatomy correlated more strongly with basicranial anatomy in both primates and marsupials than with locomotor behaviour (Villamil, 2017; but see: Russo & Kirk, 2017).

We seek to gain a better understanding of the function of the atlas in mammal locomotion by expanding the taxonomic sample and exploring the relationship between morphology and locomotor behaviour in a broad group of mammals. In this study we quantify 3D atlas shape in 64 species of mammals from the superorder Euarchontoglires, including the orders Primates, Rodentia, Lagomorpha, Scandentia and Dermoptera (Meredith *et al.*, 2011). We harness previous work on atlas shape in primates to develop functional hypotheses that may underlie the variation in atlas shape observed in mammals, and extend these hypotheses to taxa that descended from the last common ancestor of primates and rodents but diversified into a wide range of body shapes, sizes and ecological roles. Using 3D geometric morphometrics, we quantify the shape of the cranial and caudal articular facets, the transverse processes, and the dorsal and ventral vertebral arches of the atlas.

We test two hypotheses about atlas function in euarchontogliran mammals. (1) We hypothesize that atlas shape is influenced by the demands of supporting the head. The atlas directly articulates with the skull, and head support may be maintained through biomechanical constraints imposed by specific shapes of articulations and bony processes on the cervical vertebrae (Graf *et al.*, 1995). X-ray studies of mammals and other vertebrates have demonstrated that the atlanto-occipital joint and atlanto-axial joint are generally flexed when animals are at rest (Vidal *et al.*, 1986; Graf *et al.*, 1995). If atlas shape is associated with supporting the head's weight, we expect to find a relationship between shape and relative head size such that species with relatively more massive heads for their body size have more robust vertebral arches and transverse processes. Specifically, we predict that relative increases in head size will correspond to craniocaudally elongated dorsal arches and increased attachment surfaces for atlanto-occipital muscles that flex and extend the atlanto-occipital joint (e.g. m. rectus capitis and m. longus capitis) (Fig. 1) (2) We hypothesize that atlas shape is influenced by locomotor behaviour. Different atlas morphologies allow varying ranges of neck rotation and flexibility among species (Graf *et al.*, 1995; Sargis, 2001), and the atlas has attachment sites for muscles that move the head, cervical spine and forelimbs. Although detailed cervical myology is not known for many species in our sample, we can extrapolate general areas of attachment on the atlas of the major muscles involved in locomotion from described primates and rodents (McEvoy, 1982; Bezuidenhout & Evans, 2005; Diogo, 2009; Diogo &

Wood, 2012; Kikuchi *et al.*, 2012). These muscles attach either to the transverse processes or the central tubercle of the dorsal arch, and generally belong to one of two groups: those that move the pectoral girdle, such as m. levator scapulae and m. trapezius, and those that connect the atlas to the skull, such as m. rectus capitis and m. longus capitis (Fig. 1). If atlas shape is influenced by locomotion, we expect to find differences in shape between species that have evolved different locomotor strategies. We predict that the atlas will exhibit craniocaudally elongated, more laterally extended transverse processes associated with the larger pectoral girdle muscles (e.g. m. levator scapulae) in species with specialized forelimbs, such as suspensory primates (Preuschoft *et al.*, 2010), and fossorial and semi-aquatic rodents (Samuels & Valkenburgh, 2008).

Scaling effects frequently explain a large component of shape variation, particularly in geometric morphometric studies (Outomuro & Johansson, 2017). Allometric shape change associated with body size has been noted in primate atlases (Manfreda *et al.*, 2006), and an allometric relationship between body size and skull size is well documented in mammals generally (Gould, 1971; Emerson & Bramble, 1993; Fitch, 2000) and primates in particular (Jungers, 1984; Jungers *et al.*, 1995; Isler *et al.*, 2008). Further evidence suggests that body size allometry may influence locomotor performance and behaviour (Dial *et al.*, 2008). Therefore, we also examine the effect of allometry on atlas shape and include it as a covariate in analyses with head size and locomotor behaviour.

METHODS

SAMPLING AND DIGITAL MODEL GENERATION

We gathered data on atlas and axis shape from one individual from each of 64 species within the clade Euarchontoglires, focusing on the orders Primates ($N = 29$) and Rodentia ($N = 31$), as well as representatives from orders Lagomorpha ($N = 2$), Dermoptera ($N = 1$) and Scandentia ($N = 1$). Because our goal was to examine patterns of morphological variation across many species throughout deep evolutionary time, and because we observed substantially greater variation in atlas morphology across species than within species, we used a single representative of each species rather than a species mean. This approach has been implemented in many comparative studies using a broad taxonomic dataset (e.g. Kamilar & Bradley, 2011; Kamilar & Tecot, 2015; Boyer *et al.*, 2016).

Specimens included in this sample were obtained from the University of Washington Burke Museum of Natural History (UWBM), the University of Massachusetts Anthropological Primate Collection (UM-APC) and the Harvard Museum of Comparative Zoology (MCZ). Specimens were digitized using one of the following: (1) a Skyscan 1174 µCT scanner (Bruker
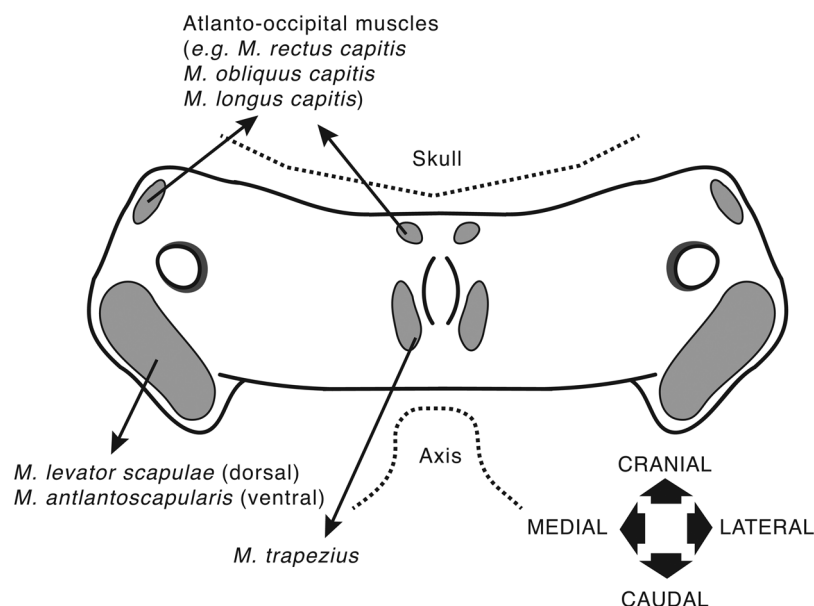

Figure 1. Diagram of an example atlas vertebra in dorsal view (based on the porcupine *Erethizon dorsatum*) showing general attachment areas for atlanto-occipital muscles (m. rectus capitis, m. obliquus capitis, m. longus capitis), which attach to the occiput, and pectoral girdle muscles (m. levator scapulae, m. atlantoscapularis, m. trapezius). This is an illustrative summary of muscle anatomy informed by more specific discussion found in other sources and is not exhaustive. (Based on McEvoy 1982 and Bezuidenhout and Evans 2005 for rodents, and Kikuchi *et al.* 2012 and Diogo & Wood 2012 for primates).

MicroCT, Kontich, Belgium, 2013), using a 0.25 mm aluminum filter with X-ray voltage of 50 kV, X-ray current of 800 μA and pixel size between 9 and 30 μm; (2) a Nikon X-Tek HMXST225 μCT scanner, with X-ray voltage of 65–75 kV, X-ray current of 65–75 μA and pixel size between 35 and 55 μm (Nikon Metrology, Inc., Brighton, Michigan, USA, 2015); (3) a NextEngine HD desktop laser scanner (NextEngine, Inc., Santa Monica, California, USA, 2013) using a point density of 40 000 points per square inch and 12 rotational positions per subscan. During laser scanning, specimens were oriented dorsoventrally in front of the scanner for the first subscan and then reoriented in the anterior–posterior direction for a second subscan using identical parameters. The two subscans were then aligned and merged using the NextEngine Scan Studio HD software to form one complete surface scan (NextEngine, Inc., 2013). Different scanners were employed according to the location and size of museum specimens; since we collected only surface data, both CT and laser scanning were adequate for our purposes. Specimen information is available in Supporting Information, Table S1.

CT scans from the Skyscan μCT were reconstructed as image stacks in NRecon v.1.6.9.18 (Bruker MicroCT, 2014) and μCT scans from the Nikon X-tek were reconstructed in VGStudioMax (Volume Graphics, Heidelberg, Germany, 2015). 3D models of the specimens were created using segmentation tools in Mimics Research 17.0 (Materialise NV, Leuven, Belgium, 2014) and exported as stereolithography (STL) files. Laser scans were exported as STL files using NextEngine Scan Studio HD. Polygon meshes from both types of scans were processed in Geomagic Studio 2014.1.0 (3D Systems, Rock Hill, South Carolina, USA, 2014). Small holes in the meshes were automatically filled to provide a continuous surface, and the interior polygons representing trabecular bone were removed from the models generated via μCT scanning in order to reduce the file size, resulting in the same surface-only mesh generated via laser scanning. All digital models were automatically remeshed to improve polygon mesh quality. The final models were exported as PLY files for landmarking.

BODY SIZE, HEAD SIZE AND LOCOMOTOR BEHAVIOUR

We used published accounts from the literature to compile body mass and locomotion data for all 64 species (Supporting Information, Table S1). For body mass data, we used the mean reported species values for adults (including both males and females) when multiple values were available, and $\log_{10}$-transformed these values for subsequent analyses. We explored two measures as proxies for head size: brain mass, compiled from the literature, and the geometric mean of skull length (distance from the most posterior projection of the cranium to the tip of the rostrum), width (distance at the maximum width) and height (distance at the tallest point). We collected skull measurements from the same specimens as the atlas data using digital calipers, and $\log_{10}$-transformed the skull geometric means for subsequent analyses (Supporting Information, Table S1). We compiled brain mass data from the literature and $\log_{10}$-transformed it prior to analysis. When brain mass was not available for a species but endocranial volume (ECV) was, we multiplied ECV × 1.036 g (the reported density of fresh brain tissue) to obtain a mass estimate following Isler *et al.* (2008). For our sample, $\log_{10}$ brain mass was strongly correlated with $\log_{10}$ skull geometric mean values, both across Euarchontoglires ($P < 0.001$, $R^2 = 0.93$) and within primates ($P < 0.001$, $R^2 = 0.98$) and rodents ($P < 0.001$, $R^2 = 0.97$) (linear regressions performed in R base library). None of the results presented here differed when head size and atlas shape were used. We recognize that the relative concentration of head weight (i.e. in the face or neurocranium) could have an effect on the function of the atlas in head support. Therefore, we also explored the effect of relative anterior and posterior head size on atlas shape using the ratio of anterior skull length (measured from the anterior edge of the foramen magnum to the anterior-most point on the skull) to posterior skull length (measured from the posterior edge of the foramen magnum to the posterior-most point on the skull) as a predictor variable. This analysis did not yield a significant result, and in the remainder of this paper we report only the results of the analyses performed using $\log_{10}$ skull geometric mean values as a proxy for head size.

The relationship between locomotion and atlas shape was analysed separately within primates and rodents, since some locomotor categories are unique to each group. For example, no primates are semi-aquatic and no rodents are suspensory. Lagomorpha, Dermoptera and Scandentia were excluded from these order-specific analyses due to low sampling. We defined four locomotor categories for primates: (1) terrestrial quadrupedal (species that primarily employ all four limbs to walk or run on the ground), (2) arboreal quadrupedal (species that primarily employ all four limbs to walk or climb above ground), (3) leaping (terrestrial and arboreal species that leap by propelling themselves primarily with the hindlimbs) and (4) suspensory (species that use quadrupedal suspension or bridging; or brachiation and forelimb suspension). While very fine-grain information on locomotor behaviour is available for many primate species (e.g. Gebo, 1987), we created these categories to capture the primary locomotor mode of divergent species and

link these behaviours to functional predictions about atlas shape, such as restricted range of motion in head or forelimb movements (Parks, 2012). References used to determine locomotor category are included in Supporting Information, Table S1.

Rodents occupy a broader range of locomotor categories than primates. Our sample includes gliding, swimming, burrowing and climbing rodents. While this diversity may be reflected in atlas shape, in order to maintain sufficient sample sizes among groups, we restricted locomotor categories for rodents to four broad primary behaviours: (1) climbing rodents are those that climb and/or glide and forage or travel in arboreal habitats, (2) semi-aquatic rodents are those that forage or travel in water, (3) burrowing rodents are those that forage or build burrows in the substrate and include tooth diggers and scratch diggers and (4) terrestrial rodents include those that move on the ground (including hopping) and either do not or rarely climb, dig or swim. Future work focusing on rodent morphology may be able to better parse rodent locomotion with a larger sample size but, given that we were interested in broad trends across Euarchontoglires rather than small-scale trends within Rodentia, such sampling was beyond the scope of our study. As in primates, our goal was to balance categories that relate to specific functional predictions of atlas shape (e.g. possible restriction of head or neck movements in species that climb or dig) with categories that can be applied broadly across a diverse clade.

3D SHAPE ANALYSIS

We used a combination of landmarks and semilandmarks applied along a curve to capture the 3D shape variation among atlas specimens (Fig. 2; Table 1). To avoid pseudoreplication of points, we landmarked only the right side of each specimen. We digitized landmarks and semilandmarks on PLY surface models of the vertebrae in Landmark 3.6 (Institute for Data Analysis and Visualization, University of California Davis, USA, 2007) (landmark coordinates are included in Supporting Information, File S2). After digitizing landmarks and semilandmarks, we performed generalized Procrustes analysis (GPA) on the atlas landmark coordinates using the R package *geomorph* (Adams & Otárola-Castillo, 2013). GPA superimposes all landmark configurations such that effects of scaling, translation and rotation are removed, leaving only differences in shape (Zelditch *et al.*, 2012). Semilandmarks were slid along the curve using the minimum bending energy criterion (Perez *et al.*, 2006) (sliding semilandmark curve positions are included in Supporting Information, File S3). We then performed a principal components analysis (PCA) of the Procrustes-aligned landmark coordinates in *geomorph*
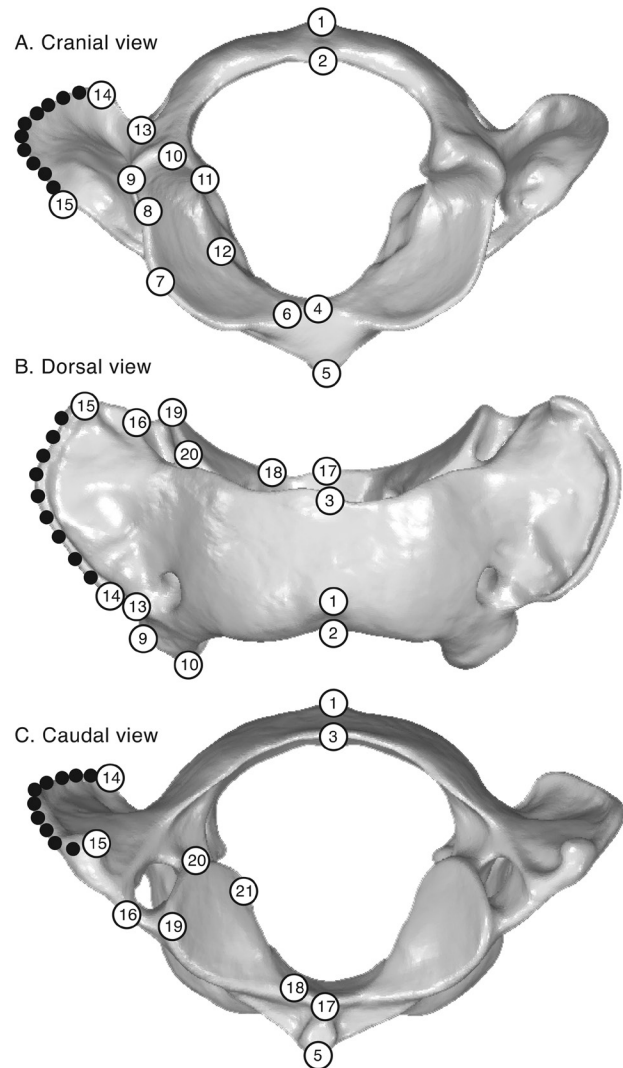

Figure 2. Landmark coordinate scheme for atlas specimens. White numbered circles are landmarks (descriptions in Table 1). Black circles are sliding semilandmarks.

to reduce the dimensionality of the shape data and determine the major axes of variation among taxa. We performed these analyses for our entire euarchontogliran dataset to test the relationship between shape and body and head size across the entire clade, and then repeated the GPA and PCA twice more for primate-only and rodent-only subsets to evaluate the relationship between shape and locomotor category within these groups.

COMPARATIVE ANALYSES

We performed phylogenetic comparative analyses using tree topology and branch-length estimates from the species level supertree of mammals published by Fritz *et al.* (2009) (Fig. 3). Polytomies were assigned

Table 1. Atlas landmark and semilandmark types and positions

Landmark	Type	Location
1	2	Dorsal tubercle of vertebral arch
2	2	Anterior-most point on edge of dorsal vertebral arch, centered
3	2	Posterior-most point on edge of dorsal vertebral arch, centered
4	2	Anterior-most projection of ridge between anterior articular facets
5	2	Ventral tubercle of vertebral arch
6	2	Convergence of ventral outline of anterior articular facet surface
7	2	Maximum curvature point of the anterior edge of the lower anterior articular facet
8	2	Inflection point of anterior edge between upper and lower anterior articular facets
9	2	Maximum curvature point of the lateral anterior edge of the upper anterior articular facet
10	2	Anterior-most point of the anterior articular facet edge
11	2	Maximum curvature point of the medial anterior edge of the upper anterior articular facet
12	2	Inflection point of the posterior edge of the lower anterior articular facet
13	2	Intersection of the anterior edge of the transverse process with the vertebral arch
14	2	Anterior-most point of the transverse process
15	2	Posterior-most point of the transverse process
16	2	Intersection of the posterior edge of the transverse process with the vertebral arch
17	2	Posterior-most point on edge of ventral vertebral arch, centered
18	2	Convergence of ventral outline of posterior articular facet
19	2	Lateral-most point on edge of posterior articular facet
20	2	Dorsal-most point on edge of posterior articular facet
21	2	Medial-most point on edge of posterior articular facet
Semi-landmarks	**Type**	**Location**
10	curve	Lateral edge of transverse process

branch lengths of zero. In order to determine the degree of phylogenetic non-independence in the Procrustes-aligned shape coordinates, we estimated the multivariate K-statistic in *geomorph* (K-mult; Adams, 2014a). K-mult is a multivariate generalization of Blomberg's K, which evaluates the degree of phylogenetic signal in trait data relative to that expected under a Brownian motion model of evolution (i.e. trait values have constant variance and divergence is proportional to branch). Statistical significance was determined using 1000 random permutations of the data.

Phylogeny can be a confounding factor in comparative analyses since closely related species often covary more strongly than distantly related species (Felsenstein, 1985). We used phylogenetic generalized least squares (PGLS) regression in order to account for phylogenetic covariance while testing relationships between shape and body size, head size and locomotion (Martins & Hansen, 1997; Pagel, 1999). To model high-dimensional Procrustes-aligned shape data as a response variable, we used D-PGLS, a distance-based method carried out by the procD.pgls function in *geomorph* (Adams, 2014b). D-PGLS transforms the independent and dependent variables of the model according to a phylogenetic variance–covariance matrix obtained under a Brownian motion, which in this case was generated from the Fritz *et al.* (2009) tree

pruned to our study taxa. With the D-PGLS approach, variation explained by the model is calculated using a distance matrix representing the phylogenetically corrected pairwise relationships among species in the multivariate data space (Adams, 2014b). The data were then randomly permuted across the tips of the phylogeny 1000 times. Estimates of the permuted data were compared to the observed values to assess significance using a residual randomization permutation procedure (RRPP) (Adams & Collyer, 2015).

For the full Euarchontoglires dataset, we used a D-PGLS model, including log body mass and log geometric mean of skull size as covariates to model the relationship between atlas shape, body size and head size. D-PGLS in *geomorph* performs a type 1 sequential ANOVA in which terms are added to the model in a user-defined sequence. We included body mass as the first factor in the model to account for the correlation between body mass and head size in mammals (Isler *et al.*, 2008; Boddy *et al.*, 2012). Because stepwise comparison of models is not possible in *geomorph*, we also performed single-factor analyses of atlas shape and body mass to isolate the allometric component of shape variation in a phylogenetic context. Significance for all models was evaluated at $\alpha = 0.05$.

In the primate and rodent subsets, we used two separate models to test the functional hypotheses that
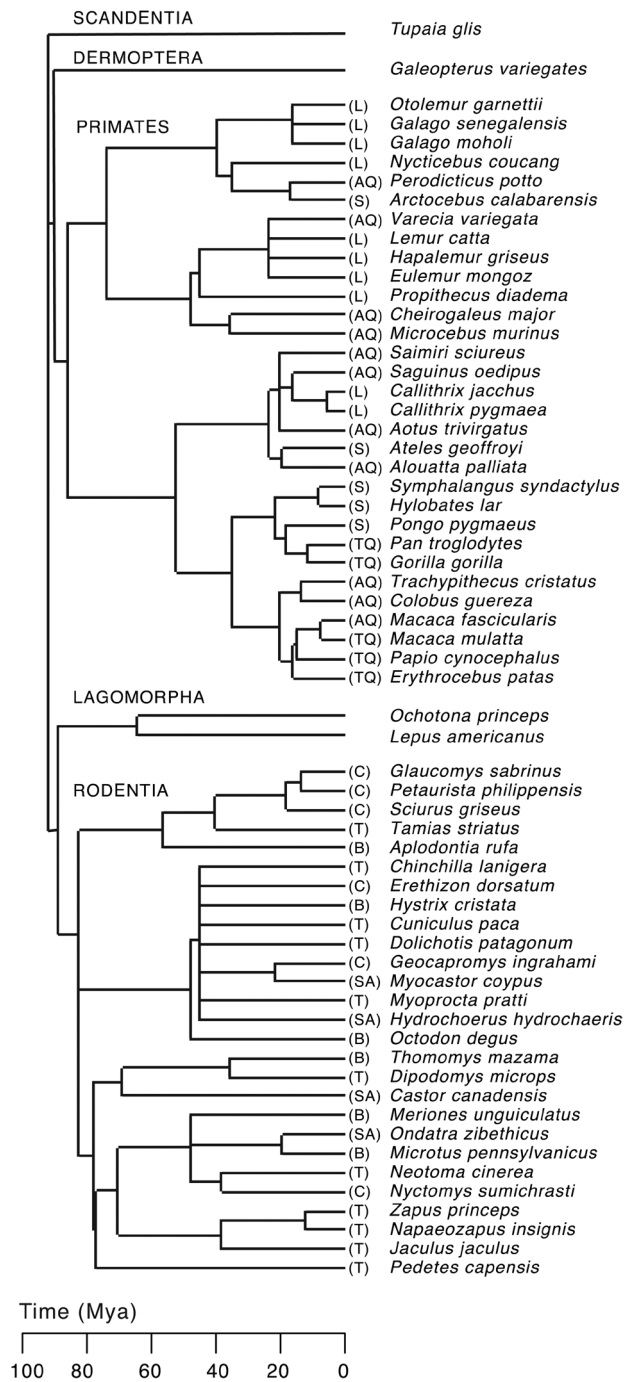
Figure 3. Phylogeny of euarchontogliran species included in this study, pruned from a species-level supertree of mammals (Fritz *et al.*, 2009). Scale represents time in millions of years. Locomotor groups for primates: (L) leaping, (AQ) arboreal quadrupedal, (TQ) terrestrial quadrupedal, (S) suspensory. Locomotor groups for rodents: (C) climbing, (T) terrestrial, (B) burrowing, (SA) semi-aquatic.

atlas shape is related to head size and to locomotor category. In both models, body mass was included as the first factor to correct for its allometric effects on head

size and locomotion. As in the larger Euarchontoglires analyses, we also performed single-factor analyses of shape and body mass to determine the effects of allometry on atlas shape. We used the procD.allometry function in *geomorph* to generate plots showing the non-phylogenetically corrected relationship between body mass and the common allometric component of shape (Mitteroecker *et al.*, 2004). Although these plots do not contain information on phylogenetic body size trends, they illustrate patterns in atlas shape variation among large and small taxa.

RESULTS

PRINCIPAL COMPONENTS ANALYSIS AND ATLAS MORPHOSPACE

The PCA performed with the 64 specimen GPA-aligned euarchontogliran atlases revealed that 66.6% of the total variance in shape is explained by the first four principal component (PC) axes (PC1 = 32.6%, PC2 = 17.7%, PC3 = 9.5%, PC4 = 6.9%, all subsequent PCs <5%). PC1 describes variation in the craniocaudal length of the vertebral body and transverse processes (Fig. 4). The atlas of the colugo (*Galeopterus variegates*) falls at the extreme negative end of PC1 and is by far the most craniocaudally expanded, while primates such as the chimpanzee *Pan troglodytes* occupy the positive extreme of PC1 with relatively craniocaudally compressed vertebral arches and narrow transverse processes. PC2 primarily describes variation in the lateral extension of the transverse processes. Species with very negative PC2 scores, such as the kangaroo rat (*Dipodomys microps*), have very mediolaterally short transverse processes, while species with positive PC2 scores, such as the snowshoe hare (*Lepus americanus*), have relatively extended, mediolaterally long transverse processes (Fig. 4). Although they overlap, rodents and primates largely occupy different regions of morphospace, with primates generally having craniocaudally shorter vertebrae with more laterally extended transverse processes than rodents. Both lagomorph species have atlases that are of average craniocaudal width but have very laterally extended transverse processes. The tree shrew *Tupaia glis* falls within the rodent-occupied morphospace, while *G. variegates* has by far the craniocaudally longest atlas shape and is morphologically distant from all other species.

The first five principal components of the primate-only morphospace explain 66.5% of the total variation in the primate atlas (PC1 = 25.8%, PC2 = 15.2%, PC3 = 11.9%, PC4 = 7.9%, PC5 = 6.1%, all subsequent PCs <5%). The primate-only morphospace displays less clear trends in craniocaudal length versus transverse process width than the euarchontoglirian
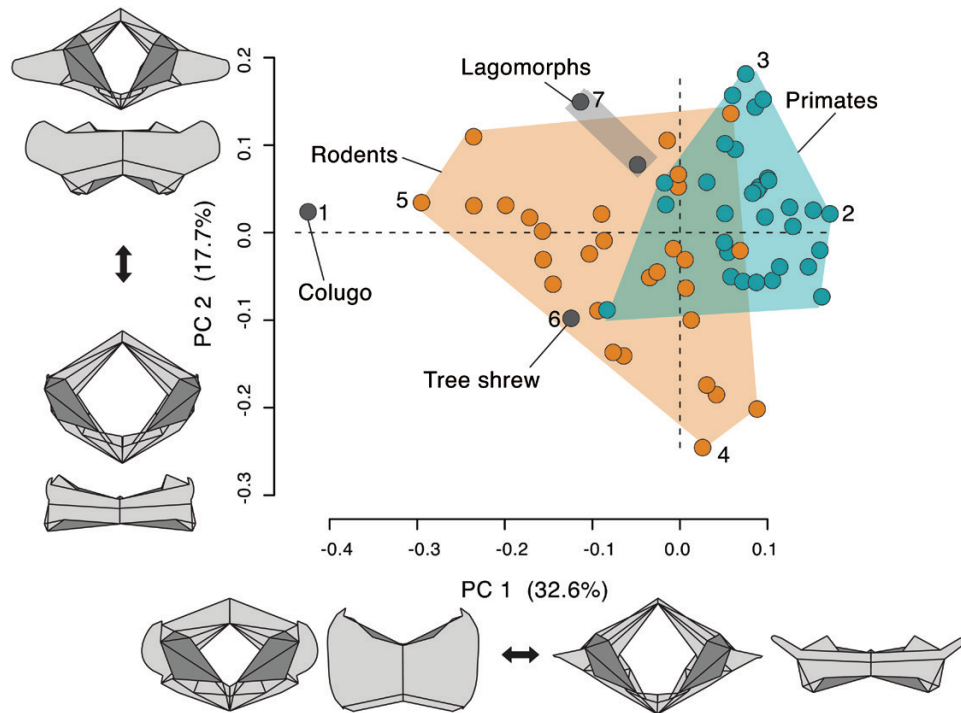
Figure 4. Euarchontoglires atlas morphospace, with convex hulls displaying morphospace occupation of different mammal orders. Atlas shapes at the positive and negative extremes of PC1 and PC2 are illustrated by cranial view and dorsal view wireframes generated from landmark coordinate data. 1, *Galeopterus variegatus*; 2, *Symphalangus syndactylus*; 3, *Otolemur garnettii*; 4, *Zapus princeps*; 5, *Dolichotis patagonum*; 6, *Tupaia glis*; 7, *Lepus americanus*.

morphospace. Primate taxa with negative PC1 scores have craniocaudally narrow, dorsoventrally compressed vertebral arches, and craniocaudally narrow, mediolaterally short transverse processes that angle dorsally from the midline of the vertebra (Fig. 5). The orangutan (*Pongo pygmaeus*) sits at the negative extreme of PC1. Species with positive PC1 scores, like the bush baby (*Otolemur garnettii*), have robust, dorsoventrally thicker, craniocaudally longer vertebral arches, and laterally extended, craniocaudally longer transverse processes that project at a ventral angle from the vertebra midline (Fig. 5). PC2 describes variation in lateral extension of the transverse processes, as well as some variation in the dorsoventral position of the transverse processes and articular facets. Primate species with negative PC2 scores, such as the siamang (*Symphalangus syndactylus*) at the negative extreme, have atlases with more laterally extended transverse processes that are positioned, along with the articular facets, below the midline of the vertebra and closer to the ventral arch. Conversely, species with positive PC2 scores tend to exhibit transverse processes with little lateral extension, which project at a more dorsal angle and are positioned, along with the articular facets, above the midline and closer to the dorsal arch. The

mouse lemur (*Microcebus murinus*) represents the positive extreme of PC2 in the primate morphospace, and is morphologically distant from most other primates (Fig. 5). Arboreal quadruped primates occupy the largest region of morphospace and tend to have less laterally extended, dorsally angled transverse processes. Terrestrial quadruped taxa fall mostly on the negative PC1 and PC2 axes and exhibit dorsoventrally thinner, more craniocaudally compressed vertebral arches and ventrally positioned articular facets. Suspensory taxa display craniocaudally compressed vertebrae with a range of transverse process extension lengths, and leaping primates have among the craniocaudally longest and dorsoventrally most robust atlases.

The rodent-only atlas morphospace displays similar trends in shape to the Euarchontoglires morphospace, with 75.4% of the total variation described by the first five principal component axes (PC1 = 34.0%, PC2 = 16.4%, PC3 = 12.6%, PC4 = 6.9%, PC5 = 5.4%, all subsequent PCs <5%). PC1 describes variation in craniocaudal length of the atlas within rodents (Fig. 6). The kangaroo rat (*D. microps*) occupies the negative extreme of PC1, which represents species with gracile, craniocaudally compressed vertebral arches and very mediolaterally short transverse
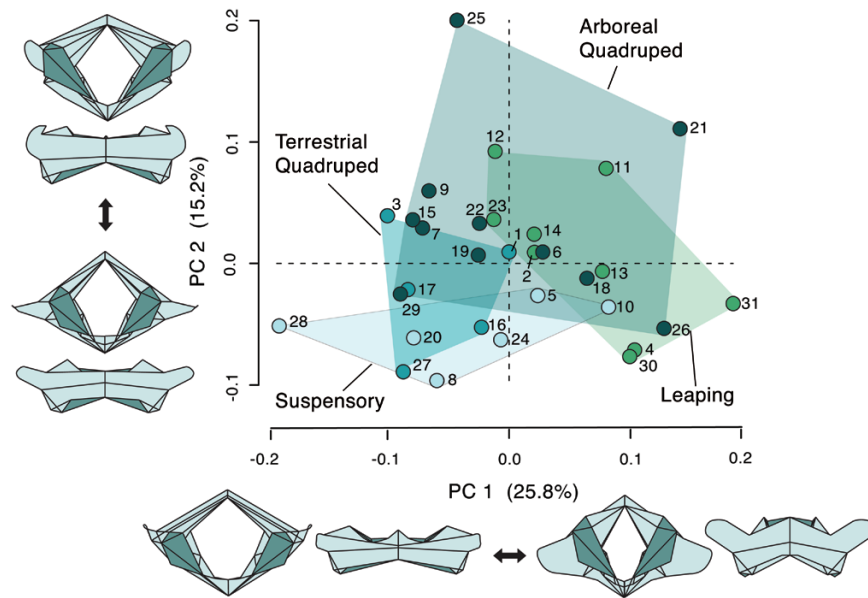
Figure 5. Primate atlas morphospace, with convex hulls displaying morphospace occupation of different primate locomotor groups. Atlas shapes at the positive and negative extremes of PC1 and PC2 are illustrated by cranial view and dorsal view wireframes generated from landmark coordinate data. 1, *Erythrocebus patas*; 2, *Galago senegalensis*; 3, *Gorilla gorilla*; 4, *Lemur catta*; 5, *Nycticebus coucang*; 6, *Saguinus oedipus*; 7, *Saimiri sciureus*; 8, *Symphalangus syndactylus*; 9, *Aotus trivirgatus*; 10, *Arctocebus calabarensis*; 11, *Callithrix jacchus*; 12, *Callithrix pygmaea*; 13, *Eulemur mongoz*; 14, *Galago moholi*; 15, *Macaca fascicularis*; 16, *Macaca mulatta*; 17, *Papio cynocephalus*; 18, *Perodicticus potto*; 19, *Alouatta palliata*; 20, *Ateles geoffroyi*; 21, *Cheirogaleus major*; 22, *Colobus guereza*; 23, *Hapalemur griseus*; 24, *Hylobates lar*; 25, *Microcebus murinus*; 26, *Varecia variegata*; 27, *Pan troglodytes*; 28, *Pongo pygmaeus*; 29, *Trachypithecus cristatus*; 30, *Propithecus diadema*; 31, *Otolemur garnettii*.

processes. Species with positive PC1 scores have robust, craniocaudally longer vertebral arches and craniocaudally expanded but mediolaterally short transverse processes, such as in the crested porcupine (*Hystrix cristata*), which represents the positive extreme of PC1 (Fig. 6). PC2 of the rodent atlas morphospace describes variation in the lateral extension of the transverse processes. Species with extremely negative PC2 scores, such as the muskrat (*Ondatra zibethicus*), have transverse processes that extend laterally and caudally, while species with high positive PC2 scores, such as the chipmunk (*Tamias striatus*), have craniocaudally short, less laterally expanded transverse processes (Fig. 6). Terrestrial taxa span the range of morphospace lacking only the strongly developed transverse processes characteristic of the highly negative PC2 axis and semi-aquatic rodents. Climbing taxa plot almost entirely within the span of terrestrial taxa near the centre of the morphospace. Burrowing rodents overlap extensively with climbing rodents and a portion of terrestrial rodents. Semi-aquatic taxa border the terrestrial, climbing, and burrowing groups. They have relatively craniocaudally long atlases with well developed, caudally projecting transverse processes.

PHYLOGENETIC SIGNAL

K-mult values significantly greater than zero for all groups indicate the presence of phylogenetic signal in atlas shape (Table 2). While atlas shape data are phylogenetically structured, closely related species are less similar in atlas vertebra shape than would be expected under pure Brownian motion (i.e. K-mult = 1).

D-PGLS MODELS

We assessed the relationship between atlas shape and morphological and behavioural variables using D-PGLS analysis in *geomorph*, which applies a constant-variance (Brownian motion) phylogenetic correction to account for non-independence of related species. Across the full Euarchontoglires dataset, body mass is significantly associated with atlas shape in a single factor model ($P = 0.001$; Table 3A). Body mass is also significantly associated with shape when included in a two-factor model with skull size ($P = 0.001$), but head size approximated by skull size is not ($P = 0.229$) (Table 3B). Within rodents and primates, we performed a single factor D-PGLS analysis of atlas shape and body mass to determine the allometric component of atlas shape variation. We also performed two separate
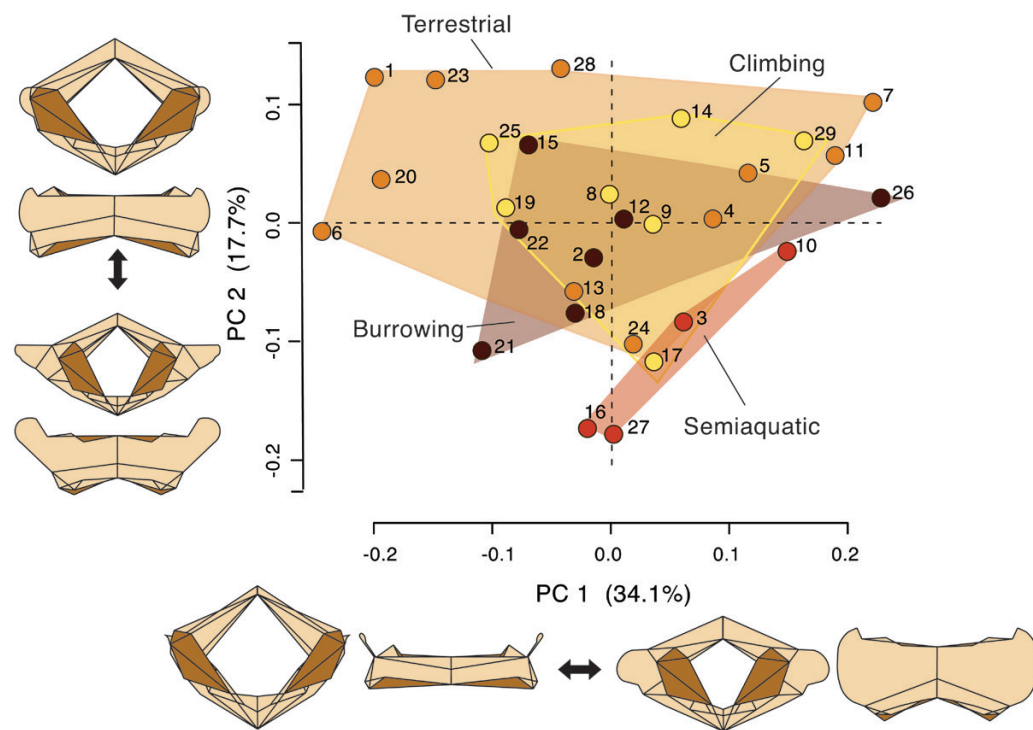
Figure 6. Rodent atlas morphospace, with convex hulls displaying morphospace occupation of different rodent locomotor groups. Atlas shapes at the positive and negative extremes of PC1 and PC2 are illustrated by cranial view and dorsal view wireframes generated from landmark coordinate data. 1, *Zapus princeps*; 2, *Aplodontia rufa*; 3, *Castor canadensis*; 4, *Chinchilla lanigera*; 5, *Cuniculus paca*; 6, *Dipodomys microps*; 7, *Dolichotis patagonum*; 8, *Erethizon dorsatum*; 9, *Geocapromys ingrahami*; 10, *Hydrochoerus hydrochaeris*; 11, *Myoprocta pratti*; 12, *Octodon degus*; 13, *Pedetes capensis*; 14, *Sciurus griseus*; 15, *Thomomys mazama*; 16, *Myocastor coypus*; 17, *Anomalurus derbianus*; 18, *Cannomys badius*; 19, *Glaucomys sabrinus*; 20, *Jaculus jaculus*; 21, *Meriones unguiculatus*; 22, *Microtus pennsylvanicus*; 23, *Napaeozapus insignis*; 24, *Neotoma cinerea*; 25, *Nyctomys sumichrasti*; 26, *Hystrix cristata*; 27, *Ondatra zibethicus*; 28, *Tamias striatus*; 29, *Petaurista philippensis*.

Table 2. Phylogenetic signal of Procrustes-aligned shape data as calculated by K-mult (Adams, 2014a). Significance determined by 1000 random permutations of the data

Phylogenetic signal of shape:

Groups	K-mult	p-rand
Euarchontoglires	0.6187	0.001*
Primates	0.4159	0.001*
Rodents	0.6864	0.001*

two-factor analyses to test the functional hypotheses that atlas shape is related to head size and locomotion, with body mass included as a covariate. In primates, body mass is significantly correlated with atlas shape in the single-factor model ($P = 0.039$) (Table 3C). However, in the two-factor head-size model, body mass is weakly associated with shape ($P = 0.065$) and skull size is not associated with shape ($P = 0.366$) (Table 3D).

In the analysis of primate locomotion, neither body mass nor locomotor category is associated with atlas shape ($P = 0.073$, $P = 0.658$, respectively) (Table 3E). In rodents, body mass is significantly related to atlas shape in a single-factor analysis ($P = 0.003$) (Table 3F). Likewise, rodent body mass is significantly associated with atlas shape ($P = 0.009$) in the two-factor head-size model but skull size is not ($P = 0.283$) (Table 3G). Finally, in the rodent two-factor locomotion model, once again body mass is significantly associated with atlas shape ($P = 0.008$) but locomotion is not ($P = 0.073$) (Table 3H).

ALLOMETRIC COMPONENT OF SHAPE

Plots depicting the relationship between the CAC of shape (Mitteroecker *et al.*, 2004) and $\log_{10}$ body mass suggest some common trends in atlas morphology among groups (Fig. 7). Across Euarchontoglires, as well as within primates and rodents, larger-bodied mammals have more robust vertebral arches and more

Table 3. Summaries of D-PGLS models assessing the relationship between atlas shape and body size, relative head size (approximated by the geometric mean of skull length, width, and height), and locomotor behaviour for Euarchontoglires (A–B), Primates (C–E) and Glires (F–H) (procD.pgls function, *geomorph*, Adams, 2014b). Significance of terms determined by comparison of observed values to 1000 random permutations of the data

Euarchontoglires:

A.   procD.pgls(shape coordinates ~ $\log_{10}$ body mass)

	DF	SS	MS	R^2	F	Z	p
$\log_{10}$ **body mass**	1	0.003820	0.0038197	0.066039	4.3839	3.8242	0.001*
residuals	62	0.054019	0.0008713				
total	63	0.057839					

B.   procD.pgls(shape coordinates ~ $\log_{10}$ body mass + $\log_{10}$ skull geometric mean)

	DF	SS	MS	R^2	F	Z	p
$\log_{10}$ **body mass**	1	0.003820	0.0038197	0.066039	4.4351	3.5921	0.001*
$\log_{10}$ **skull g-mean**	1	0.001418	0.0014842	0.025662	1.7234	1.1066	0.229
residuals	61	0.052535	0.0008612				
total	63	0.056673					

Primates:

C.   procD.pgls(shape coordinates ~ $\log_{10}$ body mass)

	DF	SS	MS	R^2	F	Z	p
$\log_{10}$ **body mass**	1	0.0015877	0.0015877	0.053629	1.6434	1.7543	0.039*
residuals	29	0.0280179	0.0009661				
total	30	0.0296056					

D.   procD.pgls(shape coordinates ~ $\log_{10}$ body mass + $\log_{10}$ skull geometric mean)

	DF	SS	MS	R^2	F	Z	p
$\log_{10}$ **body mass**	1	0.0015877	0.0015877	0.053629	1.6730	1.5278	0.065
$\log_{10}$ **skull g-mean**	1	0.0014451	0.0014452	0.048813	1.5228	0.9747	0.366
residuals	28	0.0265727	0.0009490				
total	30	0.0296056					

E.   procD.pgls(shape coordinates ~ $\log_{10}$ body mass + locomotion)

	DF	SS	MS	R^2	F	Z	p
$\log_{10}$ **body mass**	1	0.0015877	0.0015877	0.053629	1.6401	1.5064	0.073
locomotion	3	0.0028489	0.0009496	0.096229	0.9810	0.8322	0.658
residuals	26	0.0251690	0.0009680				
total	30	0.0296056					

Rodents:

F.   procD.pgls(shape coordinates ~ $\log_{10}$ body mass)

	DF	SS	MS	R^2	F	Z	p
$\log_{10}$ **body mass**	1	0.0035857	0.0035857	0.14859	4.7121	3.1999	0.003*
residuals	27	0.0205457	0.0007610				
total	28	0.0241314					

G.   procD.pgls(shape coordinates ~ $\log_{10}$ body mass + $\log_{10}$ skull geometric mean)

	DF	SS	MS	R^2	F	Z	p
$\log_{10}$ **body mass**	1	0.0035857	0.0035857	0.14859	4.8444	2.5282	0.009*
$\log_{10}$ **skull g-mean**	1	0.0013014	0.0013014	0.053929	1.7582	1.0757	0.283
residuals	26	0.0192443	0.0007402				
total	28	0.0241314					

H.   procD.pgls(shape coordinates ~ $\log_{10}$ body mass + locomotion)

	DF	SS	MS	R^2	F	Z	p
$\log_{10}$ **body mass**	1	0.0035857	0.0035857	0.14859	5.0088	2.6352	0.008*
locomotion	3	0.0033754	0.0011251	0.13988	1.5727	1.3942	0.074
residuals	24	0.0171703	0.0007154				
total	28	0.0241314					

DF, degrees of freedom; F, F-ratio; MS, mean squares; p, p-value; R^2, coefficient of determination; SS, sums of squares; Z, test statistic.
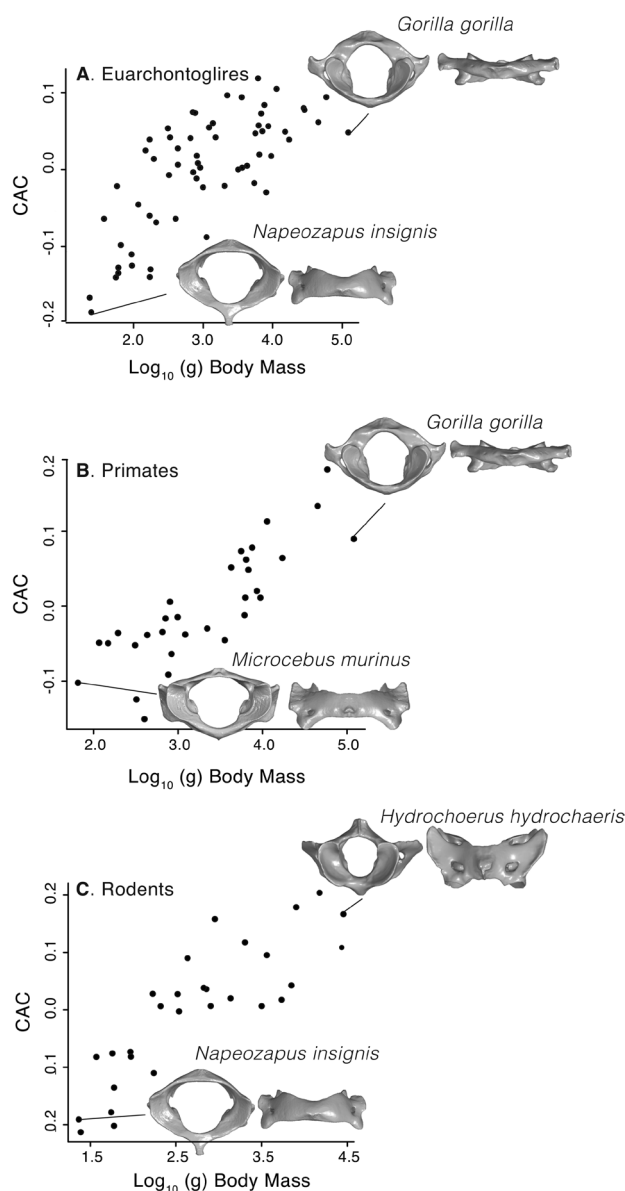
Figure 7. The relationship between the common allometric component (CAC) of atlas shape and $\log_{10}$ transformed body mass in: A, the full euarchontogliran dataset, including primates, rodents, lagomorphs, tree shrew and colugo; B, the primates-only dataset; C, the rodents-only dataset. These plots illustrate body-size allometric trends in atlas shape but do not contain phylogenetic information or the results of the PGLS regressions. Anterior and dorsal views of the atlas vertebra for the smallest- and largest-bodied species in each dataset are included.

laterally extended transverse processes (Fig. 6A–C). These plots do not contain phylogenetic information, but they do support the significant effect of body size allometry recovered in all three single-factor D-PGLS models.

DISCUSSION

Our study illustrates similar trends of overall variation in atlas length and width within two groups of mammals, although shape varies widely between different orders. Quantifying 3D atlas morphology in a taxonomically and ecologically diverse sample of mammals allowed us to extend functional hypotheses that have previously only been tested in primates to their relatives within Euarchontoglires (Manfreda *et al.*, 2006; Nalley & Grider-Potter, 2017). We found that phylogeny is a major predictor of atlas shape among Euarchontoglires as a whole, as well as within Primates and Rodentia. We also found allometric changes in atlas shape across all groups even when accounting for phylogenetic relationships. However, we found no support in either group for our hypothesis that atlas shape is related to the size of the head or to locomotor behaviour.

ATLAS MORPHOLOGY AND EVOLUTIONARY ALLOMETRY IN EUARCHONTOGLIRES

The dominant axes of shape variation in the euarchontogliran atlas are related to the craniocaudal length of the vertebral arches and transverse processes, and the lateral extension of the transverse processes. This echoes the results of comparative studies of the morphology of the axial skeleton in other mammals (e.g. Sargis, 2001; Pierce *et al.*, 2011). We found that body size is significantly associated with atlas shape both across euarchontogliran mammals and within primates and rodents, even with a conservative phylogenetic correction (i.e. strict Brownian motion). Evolutionary allometry, or non-linear scaling of morphology with size across species (Gould, 1966), has been reported in the axial skeletons of primates and other mammals (Shapiro & Simons, 2002; Manfreda *et al.*, 2006; Jones, 2015; Nalley & Grider-Potter, 2015, 2017; Randau et al., 2016a, b). Our results agree with those from the 3D GM study by Manfreda *et al.* (2006), which found that larger bodied species of primates had increasingly robust atlases. This result extends to the larger euarchontogliran clade and rodents, where atlas shape tends to be more robust in larger species, with craniocaudally longer vertebral arches and craniocaudally longer, laterally extended transverse processes (Fig. 6). One explanation for this interspecies allometry in atlas shape could be that as body size increases, a more robust dorsal arch and expanded muscle attachment surfaces on the transverse processes accommodates relatively larger muscle mass, larger nuchal ligaments, and a more massive cranium (Manfreda *et al.*, 2006).

ATLAS SHAPE AND HEAD SIZE

Our hypothesis that atlas shape is influenced by relative head size was not supported across Euarchontoglires,

within primates or within rodents. X-ray studies of mammals and other vertebrates have demonstrated that the lower cervical vertebral column is generally maintained in a vertical orientation while the animal is at rest, while the atlanto-occipital articulation and upper cervical joints are flexed (Vidal *et al.*, 1986; Graf *et al.*, 1995). In this case, we might expect that supporting the weight of the head would require different atlas shapes for larger heads than smaller heads; for example, craniocaudally longer vertebral arches with increased attachment surfaces for atlanto-occipital muscles. This expectation was not borne out by our data. The geometric mean of the skull has previously been linked to some 2D measurements of cervical vertebra morphology in primates, such as vertebral arch craniocaudal length and transverse process mediolateral length (Nalley & Grider-Potter, 2017). In that study, the best-fit models explaining many 2D vertebra shape variables also include body mass and neck inclination angle (a proxy for posture) in addition to skull geometric mean (Nalley & Grider-Potter, 2017). We may have seen no effect of head size in our study because we examined the complete 3D shape of the atlas, or because neck inclination mediates the influence of head size on atlas shape. Although postural behaviour data could be difficult to collect for many species in our sample, future work should explore the link between atlas shape, posture and head size further using skeletal proxies of head and neck posture (Russo & Kirk, 2013; Nalley & Grider-Potter, 2017).

ATLAS SHAPE AND LOCOMOTOR BEHAVIOUR

We expected that atlas shape in mammals could be influenced by locomotion because many muscle groups involved in the movement of the head, vertebral column and forelimbs (such as m. rectus capitis, m. longus capitis, m. levator scapulae or m. trapezius) attach to the transverse processes, dorsal arches and dorsal tubercles on the atlas (McEvoy, 1982; Bezuidenhout & Evans, 2005; Diogo, 2009; Diogo & Wood, 2012), and because cervical vertebra shape may influence flexibility of the head and neck during different kinds of movement (Graf *et al.*, 1995). However, locomotor category was not significantly associated with atlas shape in either primates or rodents.

Our results suggest that atlas shape in primates is more similar in closely related species, regardless of locomotor behaviour. Atlas vertebra shape in felids is also associated with phylogenetic relatedness rather than ecological similarity, whereas the shape of the lumbar vertebrae differs significantly between arboreal, scansorial and terrestrial felids (Randau *et al.*, 2016a). Differences in thoracolumbar vertebral morphology and spinal column configuration have also been reported to confer varying degrees of spinal flexibility

and stability in primates (Shapiro & Simons, 2002; Russo, 2010; Williams, 2012; Granatosky *et al.*, 2014). The lack of association between atlas vertebra shape and locomotion suggests that other regions of the vertebral column may be more influenced than the cervical vertebrae by major locomotor behaviours. While our primate atlas morphospace displays little clear separation between locomotor groups, a few qualitative trends are apparent. Groupings of terrestrial quadrupeds and leapers are almost entirely separate and fall on opposite ends of the PC1 axis, corresponding to craniocaudally shorter and craniocaudally longer vertebral arches, respectively. Likewise, suspensory primate species are grouped in a portion of the morphospace that corresponds to laterally extended transverse processes, which echoes our prediction that species with forelimb-intensive suspensory locomotion might show increased attachment surfaces for shoulder muscles such as m. levator scapulae.

When phylogeny is taken into account, we similarly find no indication that atlas shape is associated with locomotion in rodents, and species with divergent locomotor behaviours and habitat use occupy similar regions of morphospace (e.g. the fossorial mountain beaver *Aplodontia rufa* and the flying squirrel *Glaucomys sabrinus* both fall near the centre of morphospace) (Fig. 6). Rodents that are specialized for semi-aquatic locomotion are an exception. Semi-aquatic rodents in general display a variety of adaptations for swimming, including robust forelimb bones with large muscle attachment surfaces (Stein, 1988; Samuels & Van Valkenburgh, 2008), which may necessitate increased attachment surfaces on the atlas for muscles such as m. levator scapulae and m. trapezius. Semi-aquatic rodents cluster in the region of our morphospace indicating a more robust atlas shape with broad, expanded transverse processes, which could accommodate the attachment of larger muscles associated with swimming strength or efficiency. Burrowing rodents also possess morphological specializations for digging (e.g. Hopkins, 2005; Samuels & Van Valkenburgh, 2008). We know that the skulls of head-lift digging rodents exhibit a modified occipital region and expanded nuchal crest for attachment of muscles involved in lifting and stabilizing the head and neck, such as m. splenius and various capitis muscles (Samuels & Van Valkenburgh, 2009); this may translate to a deeper atlas with a more robust dorsal arch or increased transverse process surface area. Identifying specific adaptations of the cervical vertebrae to the functional demands of digging in rodents, especially comparisons among head-lift diggers, scratch-diggers and tooth-diggers, is a rich area for future research.

Within species and across individuals, mammals employ a flexible array of locomotor and postural behaviours, depending on age, substrate and habitat, among

other factors (Jenkins, 1971; Preuschoft *et al.*, 2010; Fleagle & Lieberman, 2015). While detailed positional behaviour is documented in some primates, it is lacking for many other primate species and most other mammals. For that reason, we defined broad locomotor categories for this study that captured documented variation in behaviour. Although these categories are less precise than quantitative measures of positional repertoire, they are more appropriate when comparing among distantly related and sparsely documented species, and more readily available in the literature. One possible way to address the lack of behavioural data is to use skeletal proxies of posture. For example, the size-adjusted craniocaudal length of the atlas vertebral arches in primates increases with more pronograde neck posture, as measured by the angle of the neck (Nalley & Grider-Potter, 2017). Unlike primates, rodents retain a primarily pronograde posture, even bipedal species (Bartholomew & Caswell, 1951). The features of the axial skeleton shared by two bipedal, richochetal species in our sample, the kangaroo rat (*Dipodomys microps*) and the jerboa (*Jaculus jaculus*), are therefore directly associated with locomotor behaviour. These include highly craniocaudally compressed vertebral arches, mediolaterally short transverse processes (Fig. 6) and the location and orientation of the foramen magnum (Russo & Kirk (2013, 2017). However, a third bipedal richochetal species, the springhare (*Pedetes capensis*), does not fall near *D. microps* or *J. jaculus* in our morphospace (Fig. 6). Future work exploring the link between behaviour, skeletal indicators of posture and morphology in species with documented positional repertoires will help further elucidate the relationship between positional repertoire and cervical vertebral morphology.

ATLAS SHAPE AND PHYLOGENY

Atlas shape exhibits phylogenetic signal across Euarchontoglires, as well as within Primates and Rodentia. Strong phylogenetic signal has been reported for body mass and brain mass in primates, while phylogenetic signal for ecological and behavioural traits is generally lower than in morphological traits (Kamilar & Cooper, 2013). With regard to the axial skeleton in felids, cervical vertebra shape is more phylogenetically conserved than thoracic and lumbar vertebra shape (Randau *et al.*, 2016a). The phylogenetic signal observed in our measures of atlas shape is in line with this result, although further studies of the full axial skeleton across a broader range of mammals are needed to confirm that the thoracic and lumbar regions are either less constrained or under greater selection than the cervical vertebrae. Mammalian cervical number is strongly developmentally constrained, with very few species possessing more, or fewer, than seven cervical vertebrae (Asher *et al.*, 2011). This

constraint has been linked to the muscularization of the diaphragm during mammalian development (Buchholtz *et al.*, 2012), and congenital abnormalities, neural problems and neonatal cancer are strongly associated with homeotic transformations of cervical vertebral number in humans (Galis, 1999; Galis *et al.*, 2006). Although little is known about possible constraints on cervical vertebra shape in addition to cervical number, the configuration of the brainstem and upper spinal cord may contribute to the conservation of atlas morphology across mammal groups.

The importance of appropriately dealing with phylogenetic non-independence of trait data is well understood (Felsenstein, 1985; Blomberg, Garland, & Ives, 2003). In many implementations of PGLS regression, it is possible to adjust the phylogenetic signal of the residual errors by specifying intermediate values of lambda, or estimating the maximum likelihood estimate of lambda for a particular model (Revell, 2012). However, existing methods for PGLS are unable to accommodate multivariate traits such as shape, which consist of many variables (landmark coordinates) that are meaningless individually (Zelditch *et al.*, 2012; Adams, 2014b). Reducing the number of shape variables via PCA of the landmark coordinates, and using scores from the first or first of several PC axes in individual PGLS regression analyses, is not an optimal approach because it treats a biased sample of a multivariate pattern as univariate traits, and assumes that axes of evolutionary covariation between multivariate phenotypes align with the principal components axes of variation (Monteiro, 2013; Uyeda, Caetano & Pennell, 2015). In order to use the total information about atlas shape contained in our landmark data, we used the distance-based D-PGLS approach in *geomorph* for PGLS regression of high-dimensional data, even though this approach assumes Brownian motion evolution in the regression model's residuals (Adams, 2014b). Because this method is limited to Brownian motion, we are unable to determine whether another model of evolution better fits the regression residuals without abandoning our multivariate landmark data. Likewise, it would be inaccurate to disregard the underlying phylogenetic structure in our regression models. The incorporation of phylogeny into our analyses is a possible reason that our results contradict work that has previously shown a relationship between primate atlas shape and locomotion (Manfreda *et al.*, 2006), as trait differences between species are now scaled by divergence time in our regressions.

CONCLUSIONS

Comparative studies of morphology allow us to explore the evolution of structures across diverse groups. This study begins to address the deficit of knowledge about

cervical vertebrae morphology in mammals by rigorously quantifying the 3D shape of the atlas (C1) in a taxonomically and ecologically broad sample, including primates, rodents and their close relatives. We found that shape variation in the atlas contains phylogenetic signal and is linked to body size but not relative head size or locomotion when shared evolutionary history is taken into account. Across Euarchontoglires and within rodents, increased body mass is significantly correlated with more robust vertebral arches and laterally extended transverse processes, perhaps to accommodate shoulder and neck muscle mass that increases with body size. Overall this study provides new, quantitative information on the morphological evolution of the atlas by extending functional hypotheses previously studied only in primates. Our results indicate that the morphology of the cervical vertebrae may be shaped by several factors, including the demands of supporting a relatively larger body and accommodating larger muscles, and provides a framework for exploring influences on atlas shape in more specialized groups. Finally, this work reiterates the importance of analysing morphology in a phylogenetic framework in which species are statistically independent data points.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Specimen information, skull measurements, body mass data, and locomotor categories.
File S1. Full citations for references used to determine locomotor categories in Table S1.
File S2. 3D Landmark coordinates for all specimens as a Morphologika file.
File S3. Sliding semi-landmark csv file used to perform Procrustes alignment in geomorph.