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Bone volume in the distal calcaneus correlates with body size but not leap frequency in galagids

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Abstract

Objectives: Primate leap performance varies with body size, where performance will be optimized in lightweight individuals due to the inverse relationship between force generation and body mass. With all other factors equal, it is less energetically costly to swing a light hindlimb than a heavier hindlimb. Previous work on the calcaneus of galagids hypothesized that bone volume in leaping galagids may be minimized to decrease overall hindlimb mass. We predict that (1) lighter taxa will exhibit relatively less calcaneal bone volume than heavier taxa, and (2) taxa that are high-frequency leapers will exhibit relatively less bone volume than lower frequency leapers.

Materials and Methods: Relationships among bone volume, body size, and leap frequency (high vs. low) were examined in a sample of 51 individuals from four genera of galagids (*Euoticus*, *Galago*, *Galagoides*, and *Otolemur*) that differ in the percentage of time engaged in leaping locomotion. Using µCT scans of calcanei, we quantified relative bone volume (BV/TV) of the distal calcaneal segment and predicted that it would vary with body size and frequency of leaping locomotion.

Results: Phylogenetic generalized least squares (PGLS) regression models indicate that body size, but not leaping frequency, affects BV/TV in the distal calcaneus. Relative bone volume increases with body size, supporting our first hypothesis.

Discussion: These results support previous work demonstrating a positive correlation between BV/TV and body size. With some exceptions, small galagids tend to have less BV/TV than larger galagids. Leaping frequency does not relate to BV/TV in this sample; larger taxonomic and/or behavioral sampling may provide additional insights.

KEYWORDS
ankle, bone volume, leaping, locomotion, primate

1 | INTRODUCTION

Galagids demonstrate elongation of the distal segment of the calcaneus between the astragalar and cuboid articulations (e.g., Boyer et al., 2013; Gebo, 1987, 1988; Hall-Craggs, 1965; Jouffroy & Günther, 1985; Jouffroy & Lessertisseur, 1979) that has been hypothesized to increase leap performance as a result of increasing hindlimb length. Hindlimb elongation increases performance in leaping and hopping mammals (e.g., Alexander, 1995; Emerson, 1985) because it increases the amount of time the extending hindlimb is in contact with the ground during the takeoff phase of the leap, thereby increasing the period over which takeoff forces can be produced (e.g., Günther et al., 1991; Peters & Preuschoft, 1984). Because velocity is the rate of change in distance per unit time, a longer hindlimb will maintain contact with the substrate for longer, allowing the extensor musculature to produce more force (Alexander, 1995), and increase the resulting leap velocity (and acceleration). This can ultimately increase an animal's leap height and distance (Peters & Preuschoft, 1984), two measures of leap performance likely necessary for more efficient travel, prey capture, and predator avoidance.

In addition to the leap performance benefits of hindlimb elongation, it is also beneficial for leapers to limit hindlimb mass, particularly in the distal segment of the limb, which decreases limb segment moment of inertia and consequently the energetic cost associated with swinging the limb. As leap acceleration is inversely proportional to mass, decreasing hindlimb mass will increase leap acceleration. However, leapers also need to have enough muscle mass to power the leap (Alexander, 1995), and as a consequence, leapers generally have large hindlimb muscles (Demes et al., 1998; Grand, 1977; Niemitz, 1979) which are situated more proximally in the limb (Niemitz, 1979). Previous comparative work has demonstrated that leapers exhibit reduced distal limb mass (Hildebrand, 1985), which could be accomplished by reducing bone mass and preserving muscle mass.

Given the importance of overall mass to the biomechanics of leap performance (i.e., all else being equal, a less massive animal is capable of leaping further and with greater velocity and acceleration than a more massive animal), it has been hypothesized that galagids may exhibit differences in calcaneal bone volume related to differences in body size and leap frequency within the clade (Lewton & Patel, 2020). Specifically, small-bodied, higher frequency leaping galagos should have reduced distal hindlimb bone mass to decrease the moment of inertia of the swinging hindlimb, thus decreasing energy requirements to move it (e.g., Howell, 1944; Preuschoft et al., 1996; Steudel, 1990). This hypothesis was formulated after an examination of distal calcaneal cross-sectional geometric properties in a subset of galagids that differ in locomotor behavior and body size (i.e., *Galago*, a relatively small-bodied, high frequency leaping taxon vs. *Otolemur*, a relatively large-bodied, low frequency leaping taxon) (Lewton & Patel, 2020). In that study, Lewton and Patel (2020) found, contrary to their expectations, that polar section modulus, a measure of bone strength, was not significantly stronger in *Galago* compared to *Otolemur*. Although bone strength did not differ between these genera, the ratio of

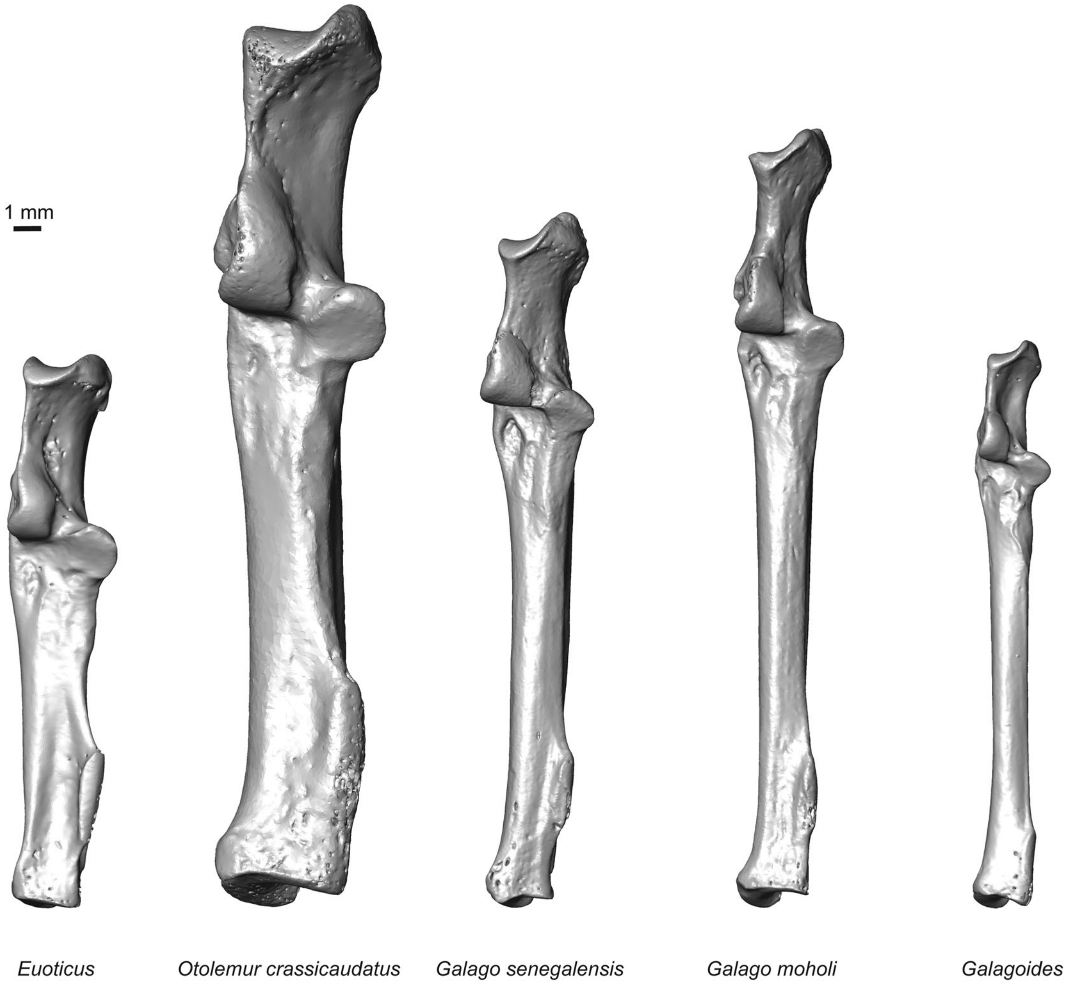

FIGURE 1 Three-dimensional surface models of representative calcanei in dorsal view of *Euoticus elegantulus* (PCM NH.MER32.459), *Otolemur crassicaudatus* (AMNH 216241), *Galago senegalensis* (AMNH 35445), *Galago moholi* (AMNH 86503) and *Galagoides demidoff* (PCM NH. MER32.70). All bones are from the right foot and the scale bar is 1 mm

maximum to the minimum second moment of area (a representation of bone distribution within a cross-section, ranging from circular to elliptical) indicated adaptation to more stereotyped loading patterns in *Galago* compared to *Otolemur* (Lewton & Patel, 2020). Given the smaller body mass of high frequency leaping galagos, it was then proposed that a tradeoff may exist in which leap performance is optimized over bone strength, aided by reduced calcaneal bone mass.

Extant species of galagids vary in body mass, distal calcaneus elongation, and frequency and type of leaping behaviors. Broadly, small-bodied galagids in the genera *Galago* (i.e., lesser galagos) and *Galagoides* (i.e., dwarf galagos) exhibit more distally elongated calcanei (Figure 1) and use a higher frequency of leaping in their locomotor repertoires than large-bodied galagids of genus *Otolemur* (i.e., greater galagos), which exhibit less distally elongated calcanei and a lower frequency of leaping behaviors (Boyer et al., 2013; Charles-Dominique & Bearder, 1979; Gebo, 1987, 1989; Hall-Craggs, 1965; Jouffroy & Lessertisseur, 1979). Specifically, the lesser galagos (i.e., *Galago senegalensis* and *Galago moholi*, Nash et al., 1989) range in average body mass from 0.18 to 0.28 kg (Smith & Jungers, 1997), have relatively long distal calcanei (Boyer et al., 2013), and leap 50%–60% of the time (Gebo, 1988; Jouffroy & Günther, 1985). In comparison, the greater galagos (i.e., *Otolemur* spp.) range in average body mass from 0.76 to 1.32 kg (Kappeler, 1991; Smith & Jungers, 1997), have relatively short distal calcanei (Boyer et al., 2013), and leap only ~20% of the time (Crompton, 1984).

The dwarf forest galagos (e.g., *Galagoides* spp.) and the needle-clawed galagos (*Euoticus elegantulus*) are not well-studied, and there is little quantitative (or qualitative, for that matter) data on locomotor behaviors. *Galagoides demidoff* is the smallest known species, averaging 0.06 kg (Smith & Jungers, 1997), and exhibits relatively long distal calcanei (Boyer et al., 2013). Moreover, *G. demidoff* has been characterized as using a fine-branch habitat and moving by both branch-running and leaping (characterized as “scurrying” by Oxnard et al. (1990)), which has been reported to account for 40%–60% of its locomotor activity (Charles-Dominique, 1977; Gebo, 1988; Walker, 1979). Unfortunately, there exist no quantitative data on the locomotor profile of *Euoticus*, which averages 0.30 kg in body mass (Smith & Jungers, 1997), has relatively short distal calcanei (Boyer et al., 2013), and has been described as moving primarily by branch-running and leaping (Charles-Dominique, 1977).[1]

The aim of this study is to examine distal calcaneal bone volume, a proxy for calcaneal mass, in four genera of galagids, with an emphasis on the effects of body size and leap frequency on calcaneal bone volume. This study expands upon the work by Lewton and Patel (2020) that was limited to species of *Galago* and *Otolemur* by including a more phylogenetically diverse sample, which allows for a more thorough investigation of the effects of variation in body size and leap frequency across extant members of the galagid clade. We hypothesize that (1) small-bodied galagids will exhibit less relative bone volume in the distal calcaneus compared to larger-bodied galagids, and (2) galagids that leap more frequently will exhibit less relative bone volume in the distal calcaneus than galagids that leap less frequently.

2 | METHODS

The comparative sample (Table S1) includes calcanei of 55 individuals of galagids that differ in body size and leap frequency (*Euoticus* [$n = 16$], *Galago* [$n = 14$], *Galagoides* [$n = 12$], and *Otolemur* [$n = 13$]; Figure 1). *Galagoides* and *Galago* are the smallest taxa and are frequent leapers, while *Euoticus* and, to a greater extent, *Otolemur* are larger and less frequent leapers. Specimens derive from the American Museum of Natural History (AMNH, $n = 30$), Powell-Cotton Museum (PCM, $n = 21$), and Duke Lemur Center (DLC, $n = 4$). Right calcanei were chosen; when only the left calcaneus was available the resulting scan data were digitally mirror-imaged (see following paragraph).

The AMNH specimens were μCT scanned at the Molecular Imaging Center at the University of Southern California on a SCANCO μCT 50 scanner using 90 kV and 200 μA energy settings, and voxel resolution of 6–14 μm. The PCM specimens were μCT scanned at the Cambridge Biotomography Centre at the University of Cambridge on a Nikon XTH 225 ST scanner using 125–145 kV and 170–200 μA energy settings, and voxel resolution of 12–23 μm. The DLC specimens were scanned using a Nikon XTH 225 ST scanner with 25–43 μm resolution; these specimens (DLC:2061m doi:10.17602/M2/M14846, DLC:3141f doi:10.17602/M2/M15187, DLC:NN_Og01 doi:10.17602/M2/M15701, and DLC:NN_Og02 doi:10.17602/M2/M33652) were obtained from MorphoSource (Project 170: Duke Lemur Center Cadaveric Specimens; Yapuncich et al., 2019). All raw scans were saved in either 16-bit DICOM or TIFF format. Following Lewton and Patel (2020), and prior to any subsequent analyses, μCT image stacks of isolated calcanei were digitally mirror-imaged (in the case of left calcanei) and reoriented in a standard anatomical position using Avizo Lite v. 9 or v. 2019 software (Thermo Fisher Scientific). Calcanei were oriented such that the distally elongated segment was oriented vertically, with the proximal end of the bone-facing superiorly, and the sustentacular facet facing the plane of the viewer. The calcaneal distal segment was defined as the region between the sustentacular facet and the distal navicular articulation (Figure 2a). Two three-dimensional volumetric models were generated of the distal calcaneal segment: (1) total bone volume (BV, Figure 2c) and (2) total filled volume (TV, Figure 2d). The Surface Area Volume module in Avizo was used to quantify the volume of each model, and relative bone volume was calculated as total bone volume/total filled volume (BV/TV).

The effects of sexual dimorphism and rearing environment (i.e., captive vs. wild) on BV/TV were assessed separately using Kruskal–Wallis tests for each taxon and for the sample as a whole. Kruskal–Wallis tests were used because there were three levels per factor (sex: male, female, unknown sex, and rearing: wild, captive, unknown rearing). No statistical difference in BV/TV was discerned between males and females (Table 1), and all further analyses were conducted using a combined-sex sample. BV/TV did, however, differ significantly between captive- and wild-reared individuals of *Otolemur crassicaudatus* and *G. moholi*, and as a result, the two captive individuals of each of these taxa were removed (*O. crassicaudatus*: AMNH 80238, AMNH 245093, *G. moholi*: DLC-2061m, DLC-3141f), thereby yielding a final sample size of 51 individuals (Table 2).

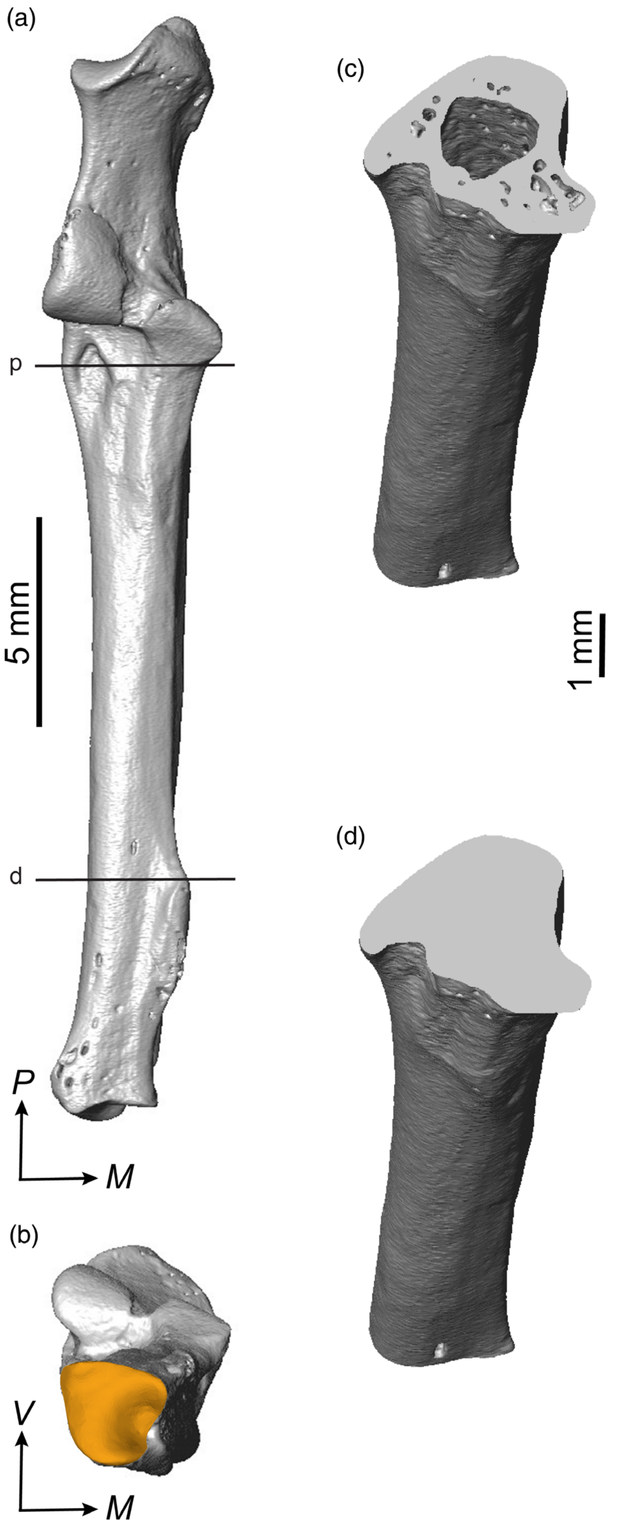

FIGURE 2 Calcaneus surface model of a representative *Galago senegalensis* specimen (AMNH 35445) demonstrating the location of its distal segment (a, dorsal view), which is between the sustentacular facet proximally (p) and navicular articulation distally (d). The calcaneal cuboid facet is highlighted in orange (b, distal view). Relative bone volume (BV/TV) was calculated as the ratio of segment bone volume (c) to total filled segment volume (d). Note that the segments in (c) and (d) are oriented such that the internal aspect of the segments can be viewed

Variation among species was explored using boxplots and Kruskal–Wallis tests with Steel-Dwass All Pairs multiple comparisons, which are the nonparametric equivalent of Tukey HSD tests and control for the overall error rate associated with performing multiple statistical tests (Critchlow & Fligner, 1991). All Kruskal-Wallis tests were performed in JMP Pro v. 15 (SAS Institute, 2019).

To assess the effects of locomotor mode and body size on BV/TV in the pooled-sex sample while accounting for the potential effects of phylogeny, PGLS regressions were constructed with model formulae of BV/TV ~ ln-size + locomotion. 'Locomotion' was a categorical variable with two levels representing the percentage of time a species spends leaping, with levels 'low' (i.e., more quadrupedal behaviors) and 'high' (i.e., more leaping behaviors, Table 2). For species in which there are no quantitative data on leaping activity (such as *E. elegantulus*), qualitative descriptions of locomotor profiles were used to assign locomotor category (see references in Table 2). PGLS regressions were implemented using the 'caper' package for R (Orme et al., 2018; R Core Team, 2019). We pruned the phylogeny of Springer et al. (2012) to represent the eight taxa in our sample. These PGLS models used a maximum likelihood branch length optimization to estimate Pagel's lambda, a parameter indicative of phylogenetic correlation structure. A lambda value of 1 indicates phylogenetic signal in the model, while a lambda value of 0 indicates phylogenetic independence (i.e., lack of phylogenetic signal in the model).

For our methods to be broadly applicable to other morphological datasets, we ran two sets of regressions with different variables for body size: (1) size based on published species mean body mass from Smith and Jungers (1997) and Kappeler (1991); and (2) a measure of calcaneal cuboid facet area (CCFA) following Yapuncich et al. (2015), who showed that CCFA is a strong predictor of body mass in primates. The advantage of using species mean body mass is that it allows the use of samples in which CCFA is not calculable (e.g., fragmentary specimens including potential fossils). The disadvantage of using species mean body mass is that it does not provide unique size estimates for individual specimens. CCFA was calculated from a whole-bone calcaneal surface model using Geomagic Wrap 2017 software (3D Systems, Inc.). The surface mesh triangles corresponding to the calcaneal cuboid facet were manually selected using the lasso tool and the "compute area" tool was used to calculate its total area (Figure 2b). Thus, our two full regression models were (1) BV/TV ~ ln-body mass + locomotion, and (2) BV/TV ~ ln-CCFA + locomotion.

To investigate how BV/TV scales with body size, we also conducted PGLS regressions of BV/TV on size alone (again, either ln-body mass or ln-CCFA) and examined the slopes of the body size parameter estimates. The expected isometric slope for the relationship between BV/TV (a dimensionless measure) and size is zero (see Ryan & Shaw, 2013). Because our sample size is small ($n = 8$), the slope 95% confidence intervals were generated using the t-distribution and $n - 1$ degrees of freedom.

The four PGLS models were compared using an Akaike information criterion correction for small sample sizes (AICc, Burnham & Anderson, 2002; Garamszegi & Mundry, 2014).

TABLE 1 Kruskal-Wallis test results for sex and rearing differences on the whole sample ($n = 55$) and by taxon[a]

		Sex			Rearing		
	n	χ^2	*df*	*p*	χ^2	*df*	*p*
Whole sample	55	0.29	2	0.86	5.98	2	0.05
Euoticus elegantulus	16	5.32	2	0.07	–	–	–
Galago gallarum	1	–	–	–	–	–	–
Galago moholi	8	2.33	2	0.31	4.00	1	0.046
Galago senegalensis	5	3.00	1	0.08	0.50	1	0.48
Galagoides demidoff	12	0.26	2	0.88	0	1	1
Otolemur crassicaudatus	9	4.20	2	0.12	4.20	1	0.04
Otolemur garnettii	2	–	–	–	–	–	–
Otolemur monteiri	2	–	–	–	–	–	–

[a] No data when sample size ≤2.

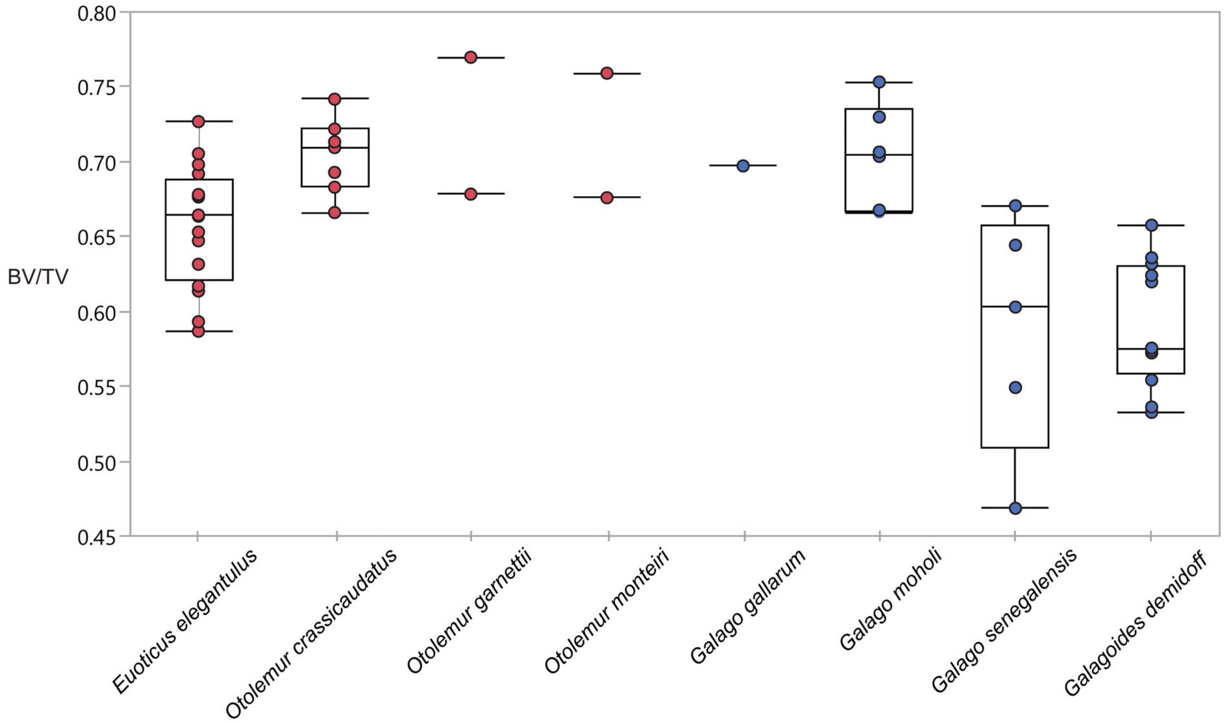

FIGURE 3 Boxplots of distal calcaneus relative bone volume (BV/TV). Horizontal lines within each box illustrate the sample median. Boxes envelop the inter-quartile range of the sample distribution and whiskers encompass the range excluding outliers. Red symbols indicate species with low leaping frequencies, blue symbols indicate species with high leaping frequencies. *Galagoides demidoff* significantly differs from *Galago moholi* ($p = 0.02$), *Otolemur crassicaudatus* ($p = 0.01$), and *Euoticus elegantulus* ($p = 0.02$)

3 | RESULTS

A Kruskal-Wallis test reveals differences in BV/TV across taxa (χ^2: 31.4, df: 7, $p < 0.0001$). The Steel-Dwass All Pairs multiple comparisons test indicates that *G. demidoff* has significantly lower BV/TV than *Euoticus* ($p = 0.02$), *O. crassicaudatus* ($p = 0.01$), and *G. moholi* ($p = 0.02$). No other pairwise comparisons were statistically significant (Figure 3). Qualitatively, *G. demidoff* and *G. senegalensis* have the lowest calcaneal BV/TV, whereas *Otolemur* spp. and *G. moholi*, have the highest BV/TV, and *Euoticus* is intermediate (Figure 3). Although not statistically significant, a boxplot of BV/TV by taxon suggests there may be interspecific differences between *G. moholi* and *G. senegalensis* in BV/TV, as their ranges of variation only overlap minimally (Figure 3).

The PGLS regressions demonstrate that body size, but not locomotion, has a significant effect on BV/TV (Table 3). The two regression models that included locomotion as a covariate were poorer fits to the data than the two models that only included a size predictor, with AICc values of −19 and −20 for the full models and −24 and −25 for the reduced models. The slopes of both body size parameter

TABLE 2 Sample characteristics ($n = 51$) and summary statistics for body mass, calcaneal cuboid facet area (CCFA), and relative bone volume (BV/TV)

										CCFA (mm^2)		BV/TV	
Genus	Species	Female	Male	Unknown sex	Wild	Captive	Leap %[a]	Leaping locomotion	Mass (kg)[b]	Mean	SD	Mean	SD
Euoticus	*elegantulus*	7	7	2	16	0	nd	Low	0.300	6.56	0.48	0.66	0.04
Galago	*gallarum*	0	1	0	1	0	nd	High	0.200	5.95		0.70	
Galago	*moholi*	4	0	2	6	0	53	High	0.180	5.32	0.40	0.70	0.03
Galago	*senegalensis*	0	2	3	1	4	53–63	High	0.213	5.47	2.10	0.59	0.08
Galagoides	*demidoff*	3	5	4	8	4	40	High	0.062	2.76	0.34	0.59	0.04
Otolemur	*crassicaudatus*	2	5	0	7	0	22	Low	1.150	17.78	2.41	0.70	0.03
Otolemur	*garnettii*	0	0	2	0	2	nd	Low	0.764	21.00	3.85	0.72	0.06
Otolemur	*monteiri*	0	1	1	2	0	nd	Low	1.320	21.80	0.67	0.72	0.06

[a] Percentage of locomotor bouts engaged in leaping behaviors; *Galago moholi*: Jouffroy and Günther (1985); *Galago senegalensis*: Crompton (1984); Gebo (1987); *Galagoides*: Charles-Dominique (1977); Walker (1979); Gebo (1987); *Otolemur*: Crompton (1984); nd, no data.

[b] Species mean body masses from Smith and Jungers (1997) except for *O. monteiri*, which is from Kappeler (1991).

TABLE 3 Results of phylogenetic generalized least squares (PGLS) analysis of the effects of size (calcaneal cuboid facet area [CCFA] and body mass) and locomotion on relative bone volume (BV/TV)

Model	Parameter estimate	Standard error	Slope 95% confidence interval[a]	Scaling[b]	*t*-value	*p*	Lambda estimate	Lambda 95% confidence interval	Adjusted R^2	AICc
BV/TV ~ ln(CCFA) + locomotion						0.118	0	0–0.63	0.410	−19.860
Intercept	0.544	0.059			9.150	0.0003				
ln CCFA	0.066	0.036	−0.020 to 0.151	Isometry	1.818	0.129				
locomotion	−0.022	0.052			−0.431	0.685				
BV/TV ~ ln(body mass) + locomotion						0.163	0	0–0.62	0.320	−18.816
Intercept	0.729	0.060			12.200	<0.0001				
ln body mass	0.044	0.029	−0.025 to 0.112	Isometry	1.513	0.191				
locomotion	−0.017	0.057			−0.302	0.775				
BV/TV ~ ln(CCFA)						0.033	0	0–0.64	0.486	−25.169
Intercept	0.560	0.043			13.010	<0.0001				
ln CCFA	0.053	0.019	0.008–0.098	+ Allometry	2.760	0.033				
BV/TV ~ ln(body mass)						0.048	0	0–0.63	0.425	−24.271
Intercept	0.712	0.022			32.955	<0.0001				
ln body mass	0.036	0.015	0.002–0.071	+ Allometry	2.485	0.048				

Abbreviation: AICc, Akaike information criterion correction.

[a] Derived from *t*-distribution and $n - 1$ degrees of freedom.

[b] Scaling expectation from isometry is $\beta = 0$.

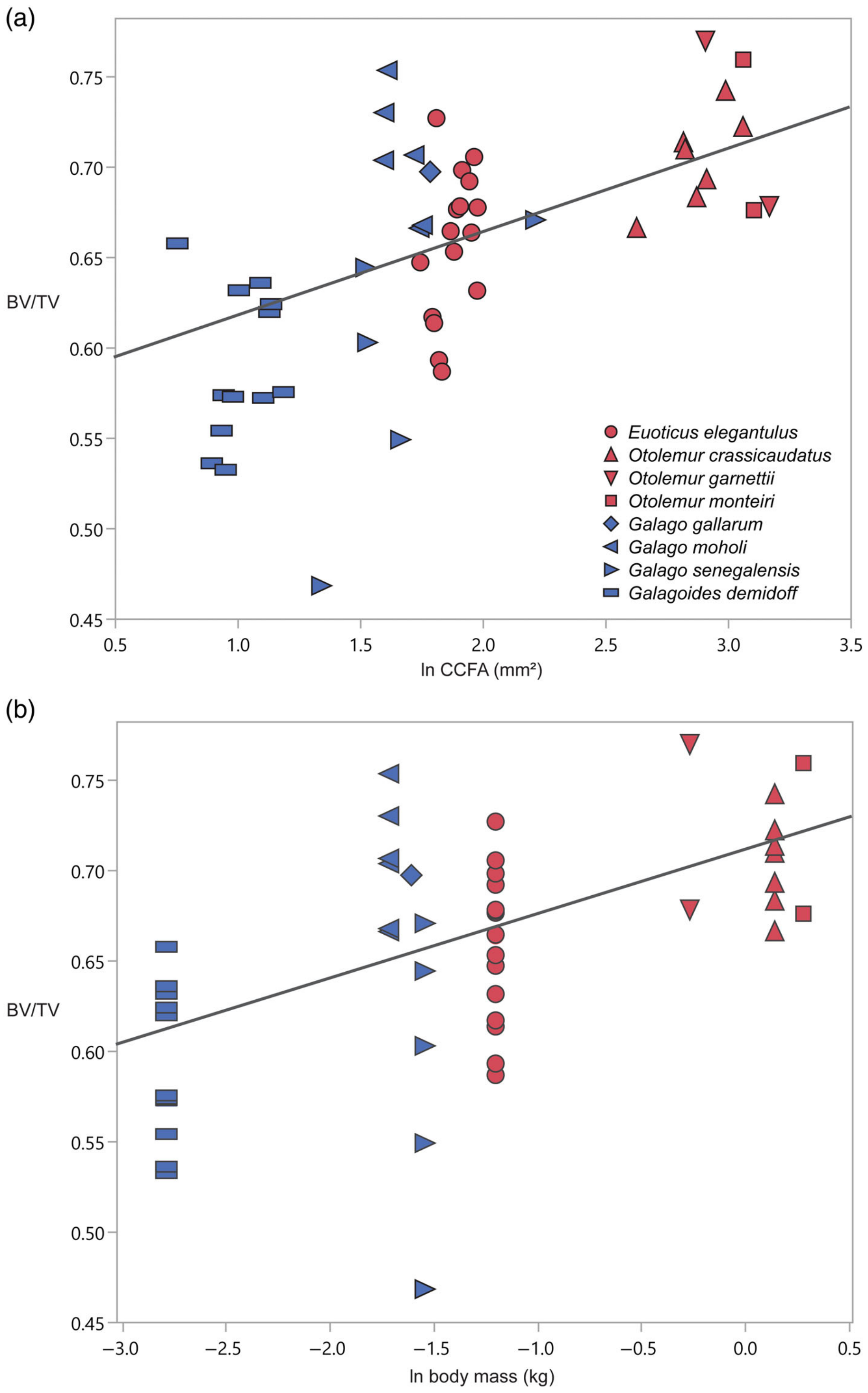

FIGURE 4 Bivariate plots of relative bone volume (BV/TV) on ln CCFA (a) and ln body mass (b). Distal calcaneus BV/TV is positively correlated with estimates of body size. Red symbols indicate species with low leaping frequencies, blue symbols indicate species with high leaping frequencies. Legend in (b) as in (a). Lines represent the phylogenetic generalized least squares fits for models containing only BV/TV and size (BV/TV ~ ln(CCFA) and BV/TV ~ ln(mass)) on species means (slopes and intercepts in Table 3)

estimates in the reduced models (ln-CCFA $\beta = 0.05$, $p = 0.03$, ln-body mass $\beta = 0.04$, $p = 0.05$) indicate that BV/TV scales with slight positive allometry (Table 3). The relationships between BV/TV and size estimates are shown in Figure 4.

Phylogenetic signal in the regression models is estimated to be 0, but the 95% confidence intervals for the lambda estimates are quite large (approximately 0–0.6). Examination of the likelihood profile plot for lambda demonstrates a relatively flat surface, indicating equally likely estimates of lambda within the confidence interval.

4 | DISCUSSION

We tested the hypotheses that (1) small-bodied galagids exhibit lower relative bone volume (BV/TV) in the calcaneal distal segment compared to larger-bodied taxa, and (2) galagid taxa that leap more frequently have lower distal calcaneus BV/TV than galagids that leap with lower frequency. Our results support the first hypothesis as there is a significant, positive correlation between body size and BV/TV. The smallest species studied here, *G. demidoff*, which averages 0.06 kg in body mass (Smith &

Jungers, 1997), indeed exhibited less BV/TV than the other species examined, while the largest taxon studied here, *Otolemur* spp., which averages 0.76–1.32 kg (Kappeler, 1991; Smith & Jungers, 1997), exhibited the highest levels of BV/TV.

There are some interesting and unexpected exceptions to these observations within the genus *Galago*. Despite *G. moholi* having an average body mass less than *G. senegalensis* (0.18 vs. 0.21 kg, respectively, Smith & Jungers, 1997), it was *G. moholi* that exhibited greater BV/TV in the distal calcaneus (0.70 for *G. moholi* vs. 0.59 for *G. senegalensis*), although this comparison was not statistically significant. The direction of difference between these species is opposite from predicted based on body mass alone. Perhaps behavioral differences between these species may explain differences in relative distal calcaneal bone volume, but the primatological literature does not document clear interspecific differences in leaping behavior, substrate preference, diet, or ecological niche among these species of lesser galagos. Both *G. moholi* and *G. senegalensis* are characterized as vertical clingers and leapers, are found in woodland habitats (e.g., Nash et al., 1989), and consume invertebrates and gums (Bearder & Martin, 1980; Harcourt & Bearder, 1989; Nash, 1986; Nash & Whitten, 1989). Another possibility for this unexpected difference may relate to the composition of the *G. senegalensis* sample, which relies on captive-reared specimens due to a paucity of wild-reared specimens in museum collections (Table 2). It is feasible that captive-reared animals might exhibit differences in BV/TV from their wild-reared counterparts due to potential differences in diet, activity level, and availability of naturalistic substrates, all factors that have been suggested to impact skeletal morphology (e.g., Antonelli et al., 2016; Hartstone-Rose et al., 2014; Lewton, 2017; O'Regan & Kitchener, 2005). However, we are not aware of any published studies documenting such potential differences. Furthermore, the one wild-reared *G. senegalensis* specimen in our sample falls within the BV/TV range of the captive-reared *G. senegalensis* individuals, suggesting that the captive-reared sample may be representative for this taxon.

Our results pertaining to the effects of body size on relative distal calcaneal bone volume in galagids accord well with previous research investigating relationships between body size and relative bone volume in other skeletal elements and taxa. Previous work has demonstrated a positive correlation between body size and trabecular bone volume fraction (and weak positive allometry) in primate humeri and femora (Ryan & Shaw, 2013) as well as sciuromorph rodent femora (Mielke et al., 2018). However, studies conducted at a phylogenetically broader level that incorporate several orders of mammals have found either very slight positive correlations between trabecular BV/TV and body mass (e.g., Doube et al., 2011) or no correlation at all (Barak et al., 2013). The scaling exponents for trabecular bone retrieved from these previous studies range from 0.17 (Mielke et al., 2018) to 0.25 (Ryan & Shaw, 2013), while Barak et al.'s (2013) meta-analysis on a broad sample of mammals demonstrated a slope of 0.05. In this study, our slopes for the relationship of cortical BV/TV on size were qualitatively lower than these previous studies which sampled trabecular BV/TV (our slope estimates: mass: 0.04, CCFA: 0.05). Differences between our results and those of previous studies may reflect differences in trabecular versus cortical bone, differences in sampling locations (i.e., small regions of interest in the case of trabecular studies, versus the entire distal calcaneal elongation in our study), or the effects of our small sample sizes. Although our regressions of cortical BV/TV on size were statistically significant, the biological relevance of these slopes is difficult to interpret. While our current results indicate weak positive allometry in calcaneal bone volume, future work with larger samples of leaping prosimians (i.e., strepsirrhines and tarsiers) should further assist in discerning the significance of scaling in calcaneal bone volume.

Although this previous work focused on trabecular bone volume in long bone epiphyses, and our work focuses on diaphyseal cortical bone volume (as it is the dominant type of bone found in the distal calcaneus [Lewton & Patel, 2020]), taken together, these findings suggest that relative bone volume (or bone volume fraction) in general scales with weak positive allometry with body size within phylogenetically narrow clades, regardless of the limb segment or bone tissue type under investigation. These results accord well with research on long bone mass allometry, in which both large and small mammals were found to demonstrate positive allometry of appendicular bone mass (and this pattern of positive allometry of long bone mass was exaggerated in small compared to large mammals, Christiansen [2002]). It would be interesting to investigate patterns of relative cortical bone volume in the diaphysis of other hindlimb elements (e.g., tibia, femur) in a more taxonomically diverse sample of primates that leap frequently. For example, indriids and cheirogaleids would be especially informative comparative clades, as species within them vary in body mass and leap frequency.

Our second hypothesis, that galagids that leap more frequently have less relative distal calcaneal bone volume than those that leap less frequently, was not supported. Some, but not all, of the high-frequency leapers exhibit less bone volume than the low-frequency leapers. For example, *Galagoides*, a high-frequency leaper, has significantly lower BV/TV than the low-frequency leapers *Euoticus* and *O. crassicaudatus*. However, *G. senegalensis*, a high-frequency leaper, does not differ from taxa that do not leap frequently (Table 2, Figure 3). Therefore, distal calcaneus cortical BV/TV does not clearly relate to leaping behavior in galagids.

Our hypotheses for body size and locomotor differences in cortical BV/TV within galagids were based on general principles of the mechanics of leaping, and in particular, how leaping performance parameters such as velocity and acceleration may be optimized by decreasing hindlimb mass. Our results suggest that cortical BV/TV is lower in small-bodied galagids, but this is not consistent with leap frequency, as some high-frequency leapers (e.g., *G. moholi*) exhibit qualitatively higher BV/TV than other high-frequency leapers (see the previous discussion). Several factors may influence these results: there may be other musculoskeletal features that reduce in size in leapers (e.g., overall muscle mass or body mass); relative bone volume in general may be relatively constant across body sizes (Barak et al., 2013); other behaviors within the locomotor repertoire of these species may have a greater effect on calcaneal BV/TV; or the characterization of galagid locomotor behavior into two categories (i.e., high- vs. low-frequency leapers) is too coarse to parse any BV/TV variation among taxa. The latter issue could be resolved in the future with the use of continuous variables for locomotion (e.g., using the percentage of time spent leaping as a continuous variable), but this is currently hindered by a lack of quantitative behavioral data on taxa such as *E. elegantulus*.

Examining patterns of variation related to locomotion within the galagid clade, as we have done here, is useful as a "narrow phylogeny" approach to identifying adaptation (sensu Ross et al., 2002). Increasing taxon representation of other taxa that leap frequently (i.e., a "convergence approach," Coddington, 1994) would also be useful to understand the biomechanical adaptations of distal calcaneal bone volume. In particular, complementing this study with data from tarsiers (*Carlito*, *Cephalopachus*, and *Tarsius*), which leap ~60% of the time (Crompton & Andau, 1986; MacKinnon & MacKinnon, 1980) and are most comparable in behavior to *G. senegalensis*, could ascertain whether the bone volume condition observed in *G. senegalensis* is related to leaping behavior or not. Tarsiers and lesser galagos have similar functional demands during vertical clinging and leaping (e.g., generation of sufficient propulsive force, tarsal rotational capabilities to maintain pedal grasping), and thus it would be reasonable to predict that tarsier and lesser galago distal calcaneal BV/TV would be comparable due to their overall similarity in positional behavior. The addition of cheirogaleids to the sample would likely further our understanding of the relationship of bone volume to leaping behavior, as they are small-bodied leapers, but do not use specialized vertical clinging and leaping behaviors (Oxnard et al., 1990).

A limitation of our study is the small number of taxa ($n = 8$) sampled. This small sample likely weakens the strength of the PGLS results on the effects of size and locomotion on BV/TV and impacts the estimates of phylogenetic signal in these models (Mundry, 2014; Münkemüller et al., 2012), as estimates of lambda are unreliable when samples are small (Freckleton et al., 2002). Our results suggest that this is the case here as well, with wide confidence intervals on the lambda estimates (Table 3) and relatively flat lambda likelihood surfaces. However, small samples not-withstanding, including phylogenetic information in these analyses should provide a better model fit than non-phylogenetic models (Freckleton et al., 2002).

In summary, leaping locomotor performance can be optimized by decreasing hindlimb mass to increase leap velocity and acceleration. We investigated the effects of body size and leap frequency on relative distal calcaneal bone volume in four genera of galagids. We hypothesized that small-bodied galagids would exhibit less relative distal calcaneal bone volume than large-bodied galagids, and that, to increase leap performance, galagids that leap more frequently would exhibit less relative distal calcaneal bone volume than galagids that leap less often. Our results demonstrate that body size, but not leap frequency, has a significant effect on relative calcaneal bone volume as quantified here: with some exceptions, as body size increases, relative calcaneal bone volume increases.

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CONFLICT OF INTEREST

The authors do not have any conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Kristi L. Lewton: Conceptualization (equal); data curation (lead); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (equal); project administration (lead); resources (lead); supervision (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Emily-Elizabeth Cardenas:** Investigation (supporting); writing – review and editing (supporting). **Daniela Cruz:** Investigation (supporting); writing – review and editing (supporting). **Jocelyn Morales:** Investigation (supporting); writing – review and editing (supporting). **Biren A. Patel:** Conceptualization (equal); funding acquisition (supporting); methodology (equal); writing – review and editing (equal).

DATA AVAILABILITY STATEMENT

The µCT data that form the basis of this study are available by request in MorphoSource Project P530: "Primate Leaping and Bone Functional Adaptation" (www.morphosource.org). All raw data analyzed here and DOIs for each dataset are listed in Data S1.

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ENDNOTE

[1] Anecdotal reports by Charles-Dominique (1977) note that *Euoticus* may be a capable leaper, although its leaps are not always clearly propulsive. When moving between trees, *Euoticus* leaps "always in a downward direction and involving a loss in height" (Charles-Dominique, 1977, p. 113), which Oxnard et al. (1990) refer to as "falling leaps" (p. 54).

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