Evolution of body size in anteaters and sloths (Xenarthra, Pilosa): phylogeny, metabolism, diet and substrate preferences

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ABSTRACT: Pilosa include anteaters (Vermilingua) and sloths (Folivora). Modern tree sloths are represented by two genera, *Bradypus* and *Choloepus* (both around 4–6 kg), whereas the fossil record is very diverse, with approximately 90 genera ranging in age from the Oligocene to the early Holocene. Fossil sloths include four main clades, Megalonychidae, Megatheriidae, Nothrotheriidae, and Mylodontidae, ranging in size from tens of kilograms to several tons. Modern Vermilingua are represented by three genera, *Cyclopes*, *Tamandua* and *Myrmecophaga*, with a size range from 0.25 kg to about 30 kg, and their fossil record is scarce and fragmentary. The dependence of the body size on phylogenetic pattern of Pilosa is analysed here, according to current cladistic hypotheses. Orthonormal decomposition analysis and Abouheif C-mean were performed. Statistics were significantly different from the null-hypothesis, supporting the hypothesis that body size variation correlates with the phylogenetic pattern. Most of the correlation is concentrated within Vermilingua, and less within Mylodontidae, Megatheriidae, Nothrotheriidae and Megalonychidae. Influence of basal metabolic rate (BMR), dietary habits and substrate preference is discussed. In anteaters, specialised insectivory is proposed as the primary constraint on body size evolution. In the case of sloths, mylodontids, megatheriids and nothrotheriids show increasing body size through time; whereas megalonychids retain a wider diversity of sizes. Interplay between BMR and dietary habits appears to be the main factor in shaping evolution of sloth body size.

KEY WORDS: Body mass, Folivora, palaeobiology, phylogenetic signal, Vermilingua.

Body size is amongst the most interesting and meaningful of biological variables. It is correlated with basal metabolic rate (BMR), timing of activity, ontogeny, home range, diet, substrate preference, population density, trophic role and a plethora of other biological and ecological parameters (for a comprehensive background, see Smith & Savage 1955; Hildebrand 1988; Damuth & MacFadden 1990; Brown & West 2000). For instance, small animals require less food, which is advantageous in stressed conditions, whereas large animals are less vulnerable to predation and temperature fluctuations but require a comparatively larger home range. Especially for terrestrial environments, larger sizes are penalised because physical parameters impose strict limitations on physiology. Body size affects the structure and dynamics of trophic networks; for instance, ingestion rate scales at a $3/4$ ratio as body mass increases (Woodward *et al.* 2005), which often requires an increased home range to provide the necessary resources. The allometric relationship between body size and metabolism is also well known, and metabolic rate has been proposed as a fundamental biological trait in shaping ecological patterns (see Brown *et al.* 2004). For these reasons and others, body size estimation is a prerequisite

for most palaeobiological and palaeoecological studies. Furthermore, evolutionary changes in body size occur by means of a trade-off between ecological advantages (for instance, decreasing risk of predation) and anatomical constraints (for instance, limits for skeletal tissue strength). However, reconstructing and analysing body size in fossil taxa is challenging, because size has to be estimated from the physical dimensions of preserved remains (in vertebrates, this is usually teeth and bones).

Anteaters (Vermilingua) and sloths (Folivora) form Pilosa which, together with armoured Cingulata (armadillos, pampatheres and glyptodonts), constitute Xenarthra, one of the most remarkable clades of placental mammals, and also one of the most significant groups of Neotropical vertebrates. Today, anteaters comprise three genera (Rodrigues *et al.* 2008), including the small and fully arboreal silky anteater *Cyclopes* (about 0.250 kg), the mid-sized and semi-arboreal lesser anteater *Tamandua* (about 4.5 kg), and the terrestrial giant anteater *Myrmecophaga* (about 30 kg) (Nowak 1999). The fossil record of anteaters is scarce, and although they are recorded since the Miocene, little is known of the evolution of these animals (see Gaudin & Branham 1998; McDonald *et al.* 2008). Sloths are represented nowadays
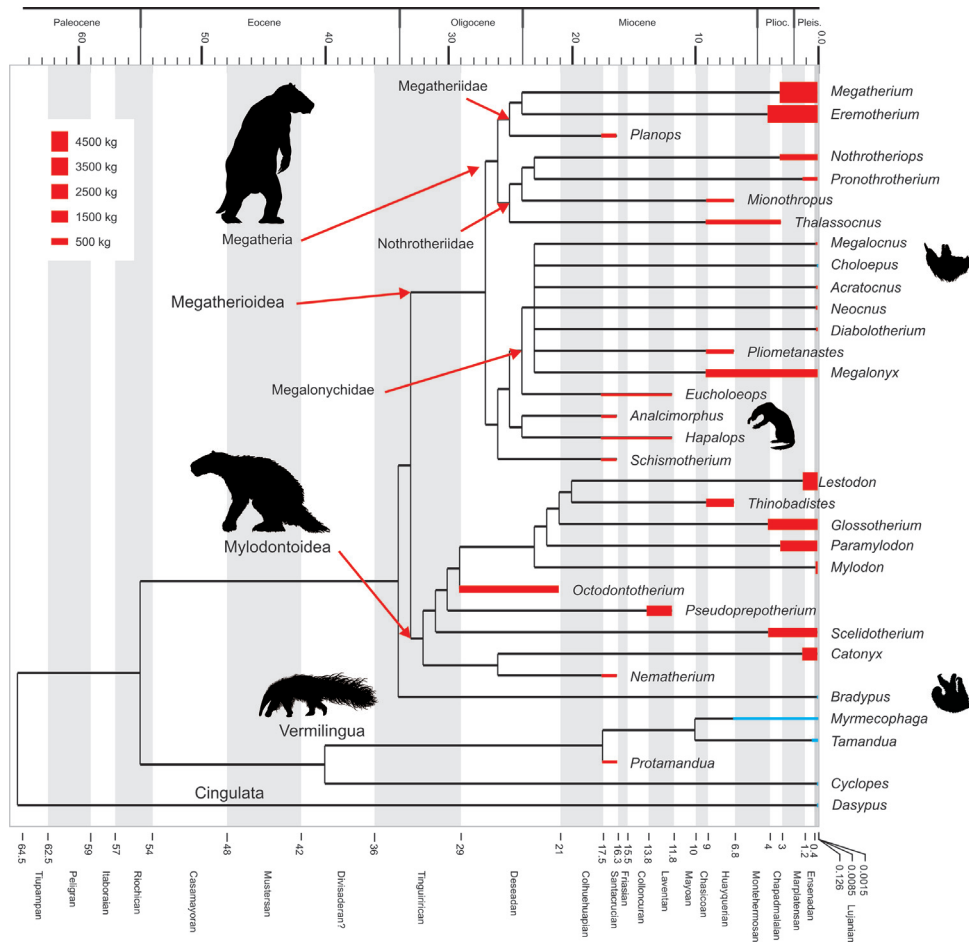
Figure 1 Cladogram depicting phylogenetic relationships within Pilosa, based on Pujos *et al.* (2012). Geological ages are represented on the horizontal axis and taxon bar length indicates stratigraphic range; based on McDonald & De Iuliis (2008). Height of taxon bars is proportional to body size in kg. Extant taxa depicted in blue; extinct ones in red. Clade names based on Gaudin (2004).

by two diphyletic genera: the two-toed sloth *Choloepus* (Megalonychidae) and the three-toed sloth *Bradypus* (monogeneric family Bradypodidae). They are nearly exclusively arboreal and folivorous mammals living in the dense tropical rainforests of Central and South America (Reid 1997; Chiarello 2008), and range from about 4 kg to 6 kg in body mass (Nowak 1999), which represents a remarkable instance of convergent evolution (Patterson & Pascual 1968; Webb 1985; Gaudin 2004; Nyakatura *et al.* 2010). However, in the past, sloths constituted a rich and diverse clade known since the early Oligocene (Gaudin & McDonald 2008; McDonald & De Iuliis 2008; Pujos *et al.* 2012). Some 90 genera (Pujos *et al.* 2012) have been named and are distributed amongst three extinct clades, Mylodontidae, Nothrotheriidae and Megatheriidae, as well as to Megalonychidae (which includes extinct taxa along with the extant genus *Choloepus*; Gaudin 2004). The clade including *Bradypus*, according to the phylogenetic hypotheses of Gaudin (2004) and Pujos *et al.* (2007) (see also McDonald & De Iuliis 2008), is currently only represented by the three-toed sloth, since no known or putative fossil bradypodids have been recorded. The diversity of fossil sloths encompassed arboreal, terrestrial, fossorial and aquatic or semiaquatic forms (McDonald & De Iuliis 2008, Pujos *et al.* 2012; Amson *et al.* 2014, 2015), with body masses ranging from dozens of kilograms to several tons (White 1993, 1997; Fariña *et al.* 1998; Bargo *et al.* 2000; De Esteban-Trivigno *et al.* 2008; Toledo *et al.* 2014) (Fig. 1). Sloths were geographically widespread, their collective range extending from southernmost Chile, Argentine Patagonia, and possibly Antarctica (Vizcaíno & Scillato-Yané 1995, but see MacPhee & Reguero 2010), in the south to the US State of

Alaska in the north (McDonald & De Iuliis 2008). Fossil sloth remains have been largely recovered from early-middle Miocene, Pliocene and late Pleistocene strata (Fig. 1), with less abundant records from the Oligocene (McDonald & De Iuliis 2008; Bargo *et al.* 2012; Pujos *et al.* 2016). During the megafaunal extinction of the terminal Pleistocene, practically all sloths (commonly referred to as ground sloths, although this term does not reflect the diversity of locomotor modes among extinct sloths) became extinct, with only the lineages leading to the modern tree sloths surviving.

In this contribution, the correlation between body mass and phylogenetic structure is analysed and discussed. In addition to this, the relationships between body size and other biological traits, such as metabolism, diet and substrate preference, are considered, with the aim of formulating hypotheses on body size evolution within Pilosa.

1. Methods

The main study subject is the phylogenetic tree proposed by Pujos *et al.* (2012), complemented by those of Pujos *et al.* (2007) and Gaudin (2004). While the current report was in review, Amson *et al.* (2016) provided a new phylogenetic hypothesis, in which thalassocnines were removed from Nothrotheriinae and presented as closely related to Megatheriinae. The effects of this hypothesis are not considered here. Body mass estimates were recovered from the literature, except as noted below. Means were calculated from estimations in the literature for each genus. Genera for which estimates could not be obtained were not considered in the analyses (Table 1).

Table 1 Body mass estimations of taxa considered in the three cladograms analysed, with respective sources of data. Extant taxa in bold type.

Taxa	Body mass estimation (kg)	Source of data
Acratocnus	23.450	White 1993
Analcimorphus	66.908	Toledo *et al*. 2014
Bradypus	**4.230**	**Nowak 1999**
Catonyx	1591.000	De Esteban-Trivigno *et al*. 2008
Choloepus	**6.250**	**Nowak 1999**
Cyclopes	**0.280**	**Nowak 1999**
Dasypus	**1.510**	**Nowak 1999**
Diabolotherium	28.045	this work (see Appendix)
Eremotherium	3232.358	Stuart 1991; Smith *et al*. 2003; McDonald 2005
Eucholoeops	46.449	White 1993; Croft 2000; Vizcaino *et al*. 2006; Toledo *et al* 2014
Glossotherium	1205.324	Fariña *et al*. 1998; Stuart 1991; Smith *et al*. 2003; Vizcaino *et al*. 2006
Hapalops	38.400	White 1993; Toledo *et al*. 2014
Lestodon	3435.563	Smith *et al*. 2003; Vizcaino *et al*. 2006; De Esteban-Trivigno *et al*. 2008
Megalocnus	50.150	White 1993
Megalonyx	623.472	Smith *et al*. 2003; McDonald 2005; Fields 2010
Megatherium	4586.524	Fariña *et al*. 1998; Smith *et al*. 2003; Vizcaino *et al*. 2006
Mionothropus	91.203	this work (see Appendix)
Mylodon	1593.000	Fariña *et al*. 1998; Smith *et al*. 2003; Vizcaino *et al*. 2006
Myrmecophaga	**28.500**	**Nowak 1999**
Nematherium	89.329	Toledo *et al*. 2014
Neocnus	20.600	White 1993
Nothropus	N/A	not considered in this work
Nothrotheriops	362.498	Smith *et al*. 2003; McDonald 2005
Pronothrotherium	93.777	this work (see Appendix)
Octodontotherium	485.000	Shockey & Anaya 2011; Vizcaino *et al*. 2012
Octomylodon	N/A	not considered in this work
Paramylodon	1153.640	McDonald 2005
Parocnus	N/A	not considered in this work
Pelecyodon	N/A	not considered in this work
Planops	163.071	this work (see Appendix); White 1993
Pleurolestodon	N/A	not considered in this work
Pliometanastes	185.050	McDonald 2005
Pliomorphus	NA	not considered in this work
Protamandua	8.298	this work (see Appendix)
Pseudoprepotherium	1024.962	Croft 2000
Scelidodon	1546.510	Croft 2000; Smith *et al*. 2003
Scelidotherium	899.605	Fariña *et al*. 1998; De Esteban-Trivigno *et al*. 2008; Smith *et al*. 2003
Schismotherium	43.722	Toledo *et al*. 2014
Tamandua	**4.500**	**Nowak 1999**
Thalassocnus	203.200	De Esteban-Trivigno *et al*. 2008
Thinobadistes	645.890	McDonald 2005

In some genera for which body size estimations are not published, but postcranial measurements are available (*Mionothropus*, *Diabolotherium*, *Pronothrotherium*, *Planops* and *Protamandua*), published allometric predictive equations or *ad hoc* regressions were performed to obtain body size estimations (see Appendix).

The outgroup in all the cladograms used for the analyses is the armadillo *Dasypus hybridus* (Cingulata, Dasypodidae), following Pujos *et al*. (2007). For analytical purposes, vermilinguan relationships from Gaudin (2004) were included in the cladogram of Pujos *et al*. (2012). For simplicity, the cladograms are referred to as A for Gaudin (2004), B for Pujos *et al*. (2007), and C for Pujos *et al*. (2012), as in Figures 2, 3 and 4.

Most statistical algorithms (for example, phylogenetically independent contrasts (PIC), phylogenetic autoregression (PA) and generalised estimating equation (GEE); see Paradis 2006) for analysing the correlation between the phylogenetic pattern and a given continuous or discrete trait require *a priori* data for branch lengths and evolutionary models. For extant taxa, branch lengths are determined using molecular information for modelling diversification times. For fossil taxa, however, branch lengths must be reconstructed from first and last appearance dates and then diversification times estimated by modelling evolutionary processes and rates. Nevertheless, estimation of diversification times by modelling algorithms based on the fossil record carries the burden of multiple statistical and evolutionary assumptions (see Münkemüller *et al*. 2012 for a review of methods).

Therefore, in contrast to Raj Pant *et al*. (2014), methods that require estimates of diversification times are not used in the present study. Instead, methods are employed that analyse only the topology of the cladogram, such as the orthonormal decomposition (OD) method proposed by Ollier *et al*. (2006) and the Abouheif C-mean test (Abouheif 1999; Pavoine *et al*. 2008; see also Münkemüller *et al*. 2012), described below.

1.1 Orthonormal decomposition of variance

This method, proposed and developed by Ollier *et al*. (2006), performs an orthonormal transformation on a matrix obtained from the tree's topology, to construct a new mathematical structure function called an orthogram (see also Paradis 2006; Münkemüller *et al*. 2012) by computing vectors (orthobases) that describe the tree's topology without relying on
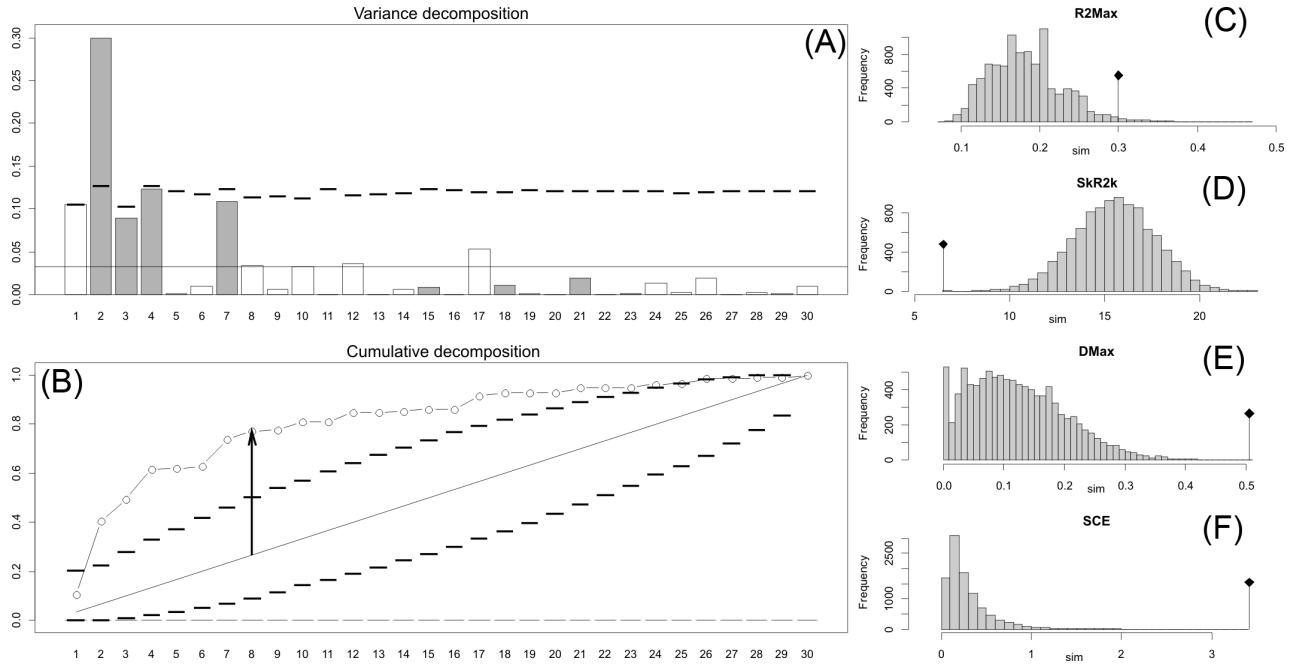
Figure 2 Orthonormal decomposition results for Cladogram A. (A) Orthogram plot: height of bars is proportional to the squared coefficients (white and grey bars represents positive and negative coefficients); dashed line is the upper confidence limit at 5 %, built from Monte Carlo permutations; horizontal solid line is the mean value; (B) Cumulative orthogram plot: circles represent observed values of cumulated squared coefficients (vertical axis); the expected values under H_0 are disposed on the straight line; dashed lines represent the bilateral confidence interval; (C–F) Histograms of observed values of the four statistic tests: black dot depicts the observed parameter value.
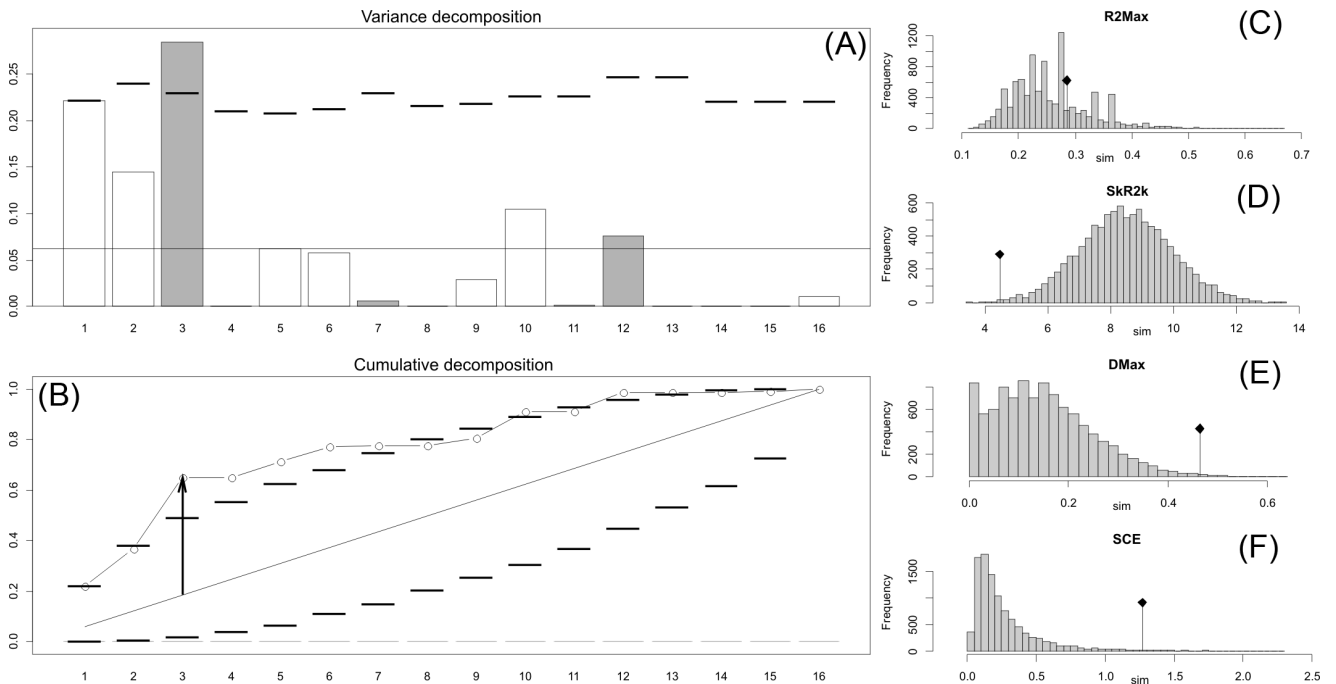

Figure 3 Orthonormal decomposition results for Cladogram B. See Figure 2 for explanation.

estimated branch lengths and diversification times. The correlation between the variance of the trait and this suite of ortho-bases (after a number of Monte Carlo permutations of the trait values of tips), and therefore the proportion of the variance of the trait explained by the topology of the tree (Paradis 2006), is tested by four statistics with a confidence limit of 0.05 and absence of correlation (observed tip values are exchangeable) considered as the null hypothesis (see Ollier *et al.* 2006). These statistics are the following: R2Max (maximal R^2), the values of which peak when a significant share in variance dependence occurs at a single node (otherwise variance dependence is distributed along several nodes); Dmax (maximal deviation), which corresponds to the Kolmogorov-Smirnov statistic and tests whether the studied variable may be part of a random sample from a uniform distribution; SkR2k (sum of k-nth R^2), which describes whether the variance distribution is skewed toward the tree's tips or root; and SCE (sum of cumulative errors), which describes the averaged variation in the values
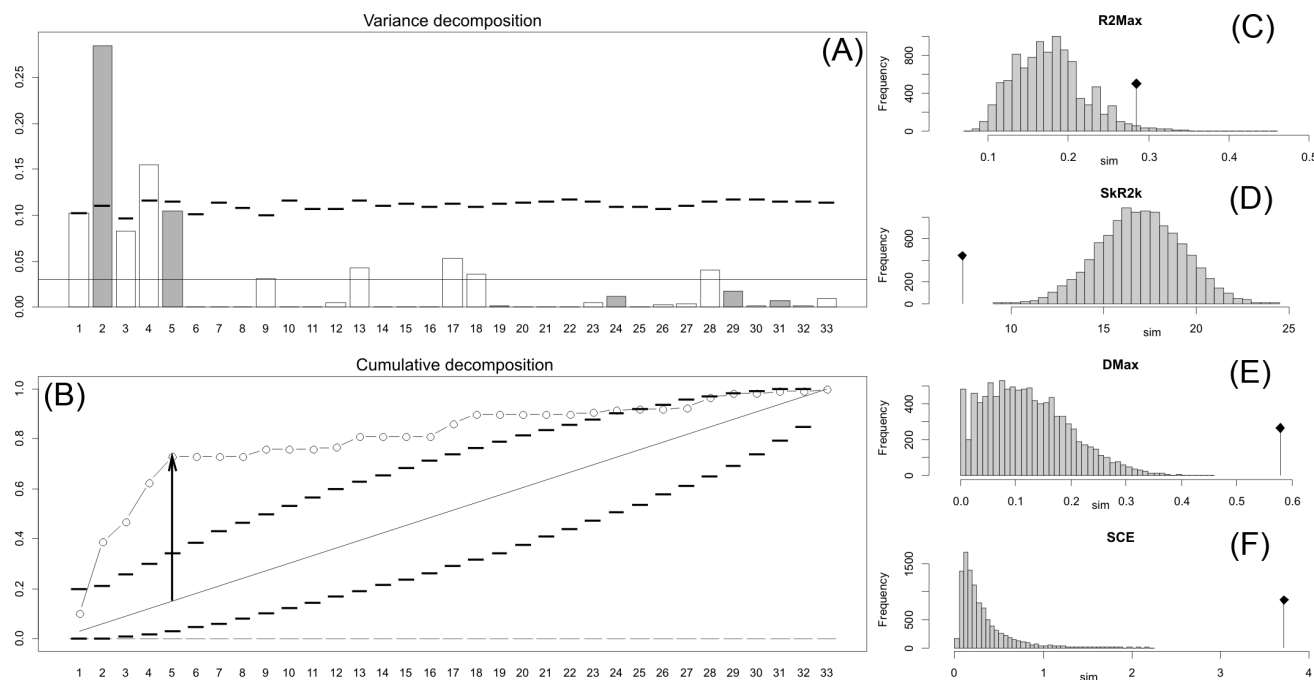
Figure 4 Orthonormal decomposition results for Cladogram C. See Figure 2 for explanation.

Table 2 Orthogram decomposition results for each of the three cladograms analysed, based on 10,000 Monte Carlo permutations. Observed values, standard deviation (St. Dev), type of alternative hypothesis test and p-values provided for each statistic.

	Observed	St. Dev.	Test	p-value at 0.05
Gaudin 2004 (A)				
R2Max	0.300	2.505	greater	0.0161
SkR2k	6.521	−4.300	less	0.0001
Dmax	0.505	4.791	two-sided	0.0001
SCE	3.417	12.390	greater	0.0001
Pujos *et al.* 2007 (B)				
R2Max	0.285	0.409	greater	0.2591
SkR2k	4.477	−2.787	less	0.0013
Dmax	0.464	3.156	two-sided	0.0048
SCE	1.270	4.436	greater	0.0054
Pujos *et al.* 2012 (C)				
R2Max	0.285	2.446	greater	0.0162
SkR2k	7.353	−4.338	less	0.0001
Dmax	0.578	6.028	two-sided	0.0001
SCE	3.723	13.384	greater	0.0001

calculated for the orthogram. Orthonormal decomposition was computed for the three cladograms studied using the R package *ade4* (Dray & Dufour 2007).

Abouheif C-mean

Based on the Moran I index, this statistic computes the correlation between the variable under study and a matrix of phylogenetic proximity among taxa (branch tips), following the method by Abouheif (1999). The absence of phylogenetic correlation is considered the null hypothesis. The observed and expected values of the C-mean, its standard deviation and p-values were computed for cladograms A, B, and C using the R package *adephylo* (Dray & Jombart 2008). If observed C-mean is greater than expected (greater than 0), positive autocorrelation is detected, whereas a negative autocorrelation is indicated by a lower-than-expected C-mean.

2. Results

2.1. Orthonormal decomposition

The results obtained are consistent overall, despite some differences in details (Figs 2, 3, 4). R2max is significantly different from the predicted for the null hypothesis for cladograms A and C, indicating that a greater share of variance of log-body mass dependence is concentrated in a few nodes (Figs 2, 3, 4). On the other hand, it was not significantly different from the predicted for cladogram B, indicating that there is no single node where the variance of log-transformed body mass is concentrated; rather, the variance dependence is spread across the tree. The other three statistics are significantly different from those predicted (Table 2), indicating that the variance of the trait is not part of a uniform distribution and is not concentrated at the root. According to the cumulative decomposition
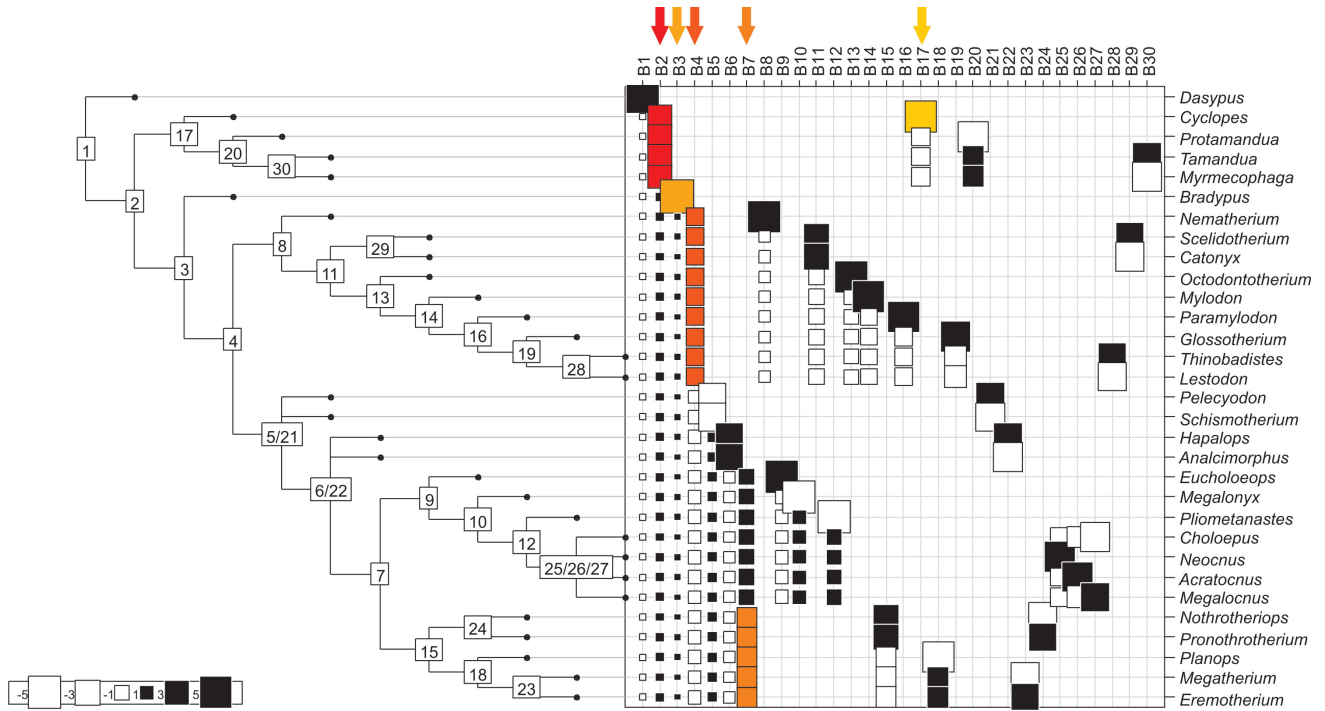
Figure 5 Cladogram A (Gaudin 2004), as analysed in the orthonormal decomposition test, showing the observed matrix of orthonormal vectors (orthobases) ordered from left to right by decreasing value of explained tree complexity. Labels in the tree indicate vectors describing variance dependence of node's descendants; size of squares represents the value of orthonormality (against which the variance is decomposed; observed variance from values predicted by null model is showed in Supplementary Information File 1). The first ten most significant vectors are shaded in red (decreasing from bright red to yellow).
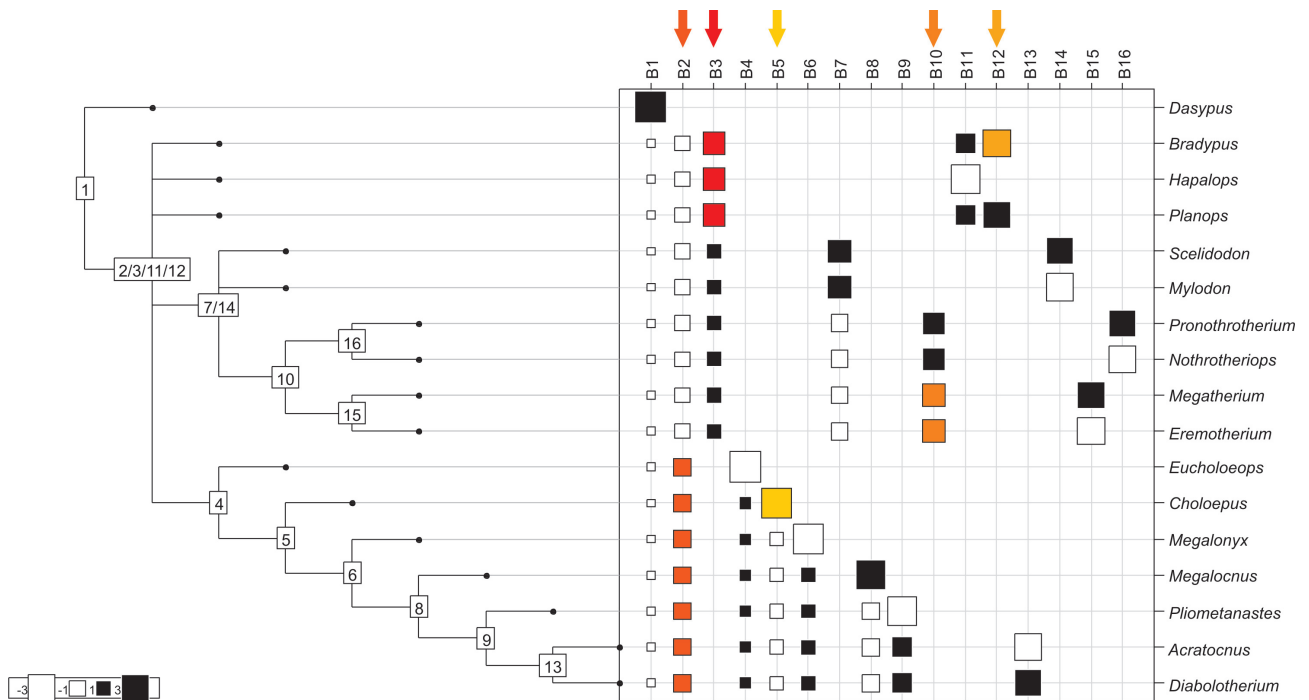

Figure 6 Cladogram B (Pujos *et al.* 2007), as analysed in the orthonormal decomposition test. The first ten most significant vectors are shaded in red (see Figure 5 for explanation; and Supplementary Information File 2).

plots, in all cases, several nodes show values extending beyond the confidence limits built by the Monte Carlo permutations (especially for A and C trees – see Figures 5, 6 and 7). Ollier *et al.* (2006) named this pattern diffuse phylogenetic dependence.

With few exceptions, nodes concentrating greater share of the body mass variance dependence vary among the cladograms analysed (compare Figs 5–7), demonstrating that differences among phylogenetic hypotheses are a factor in orthonormal decomposition results. However, some overall patterns are apparent. In cladograms A and C, higher trait variance dependence was detected at the basal nodes, whilst in cladogram B, some crown nodes are also important (see its R2Max coefficient). In both A and C cladograms, the clades that concentrated a greater phylogenetic dependence are Vermilingua (cladogram
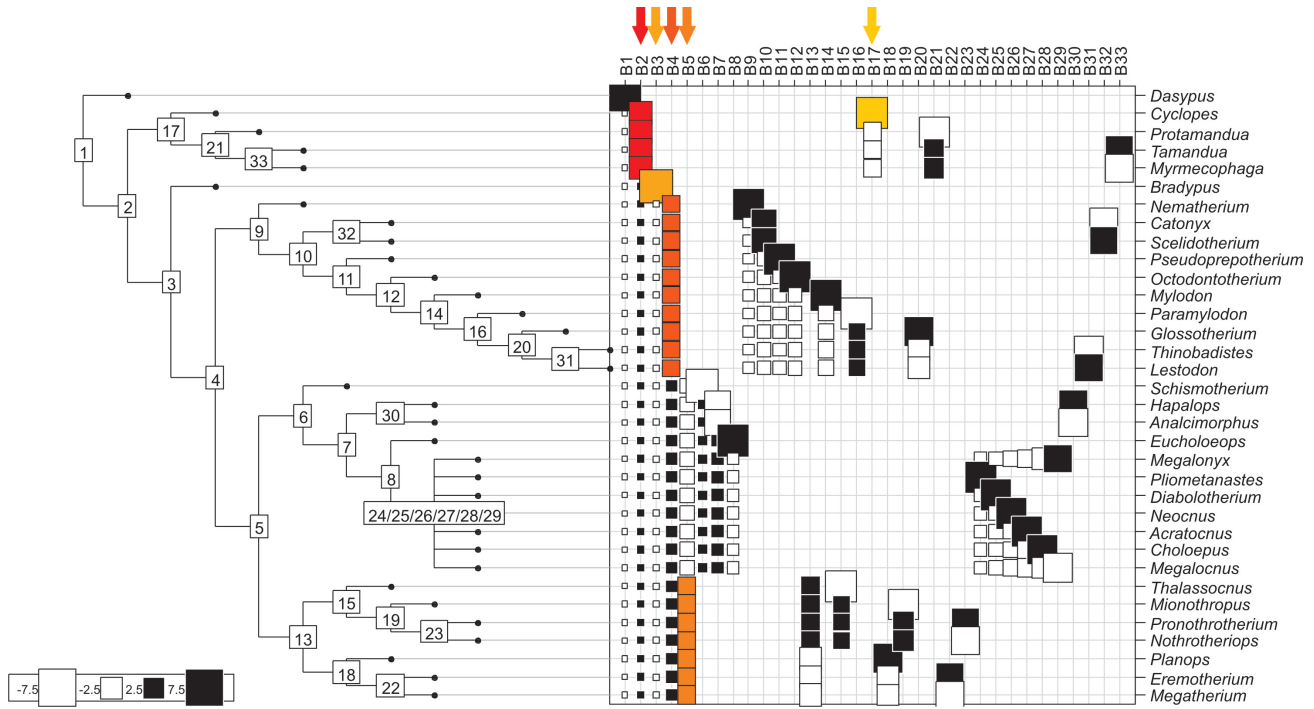
Figure 7 Cladogram C (Pujos *et al.* 2012), as analysed in the orthonormal decomposition test. The first ten most significant vectors are shaded in red (see Figure 5 for explanation; and Supplementary Information File 3).

Table 3 Abouheif C-mean results for each of the three cladograms analysed, based on 10,000 randomisations. Observed values, standard deviation (St. Dev), type of alternative hypothesis test and p-values provided for the statistic.

Abouheif Cmean	Observed	St. Dev.	Test	p-value
Gaudin 2004 (A)	0.594	5.278	greater	0.0010
Pujos *et al.* 2007 (B)	0.421	3.056	greater	0.0060
Pujos *et al.* 2012 (C)	0.639	6.435	greater	0.0010

B does not include anteaters), Mylodontoidea (cladogram B does not recover mylodonts as a natural group) and Megatheria (Megatheriidae + Nothrotheriidae *sensu* Gaudin 2004). In cladogram B, along with Megatheriidae, Megalonychidae is detected as one of the clades with higher phylogenetic dependence. Some trees' tips are also detected, highlighting taxa where body size is significantly lesser or greater than expected from the null model (*Cyclopes* and *Bradypus*; but also some megalonychids such as

Choloepus, *Megalonyx*, *Pliometanastes* and *Megalocnus*). In summary, the body mass–phylogeny relationship is strongest in Vermilingua (cladograms A and C; i.e., this group shows the greatest phylogenetic dependence), followed by Mylodontoidea (cladograms A and C), then Megatheria *sensu* Gaudin (2004: Nothrotheriidae and Megatheriidae) in cladograms A and C, Megatheriidae in cladogram B and, finally, Megalonychidae in cladograms A, B and C.

2.2. Abouheif C-mean
The observed position of the C-mean statistic is significantly different from the expected sampling distribution of the null hypothesis developed by randomisation of the tips at a 0.05 alpha (Table 3; Fig. 8). A statistically significant autocorrelation is therefore detected for all the trees, suggesting that phylogeny is a significant factor for body mass. In other words, closely related taxa are more similar in body mass than expected by the null model.
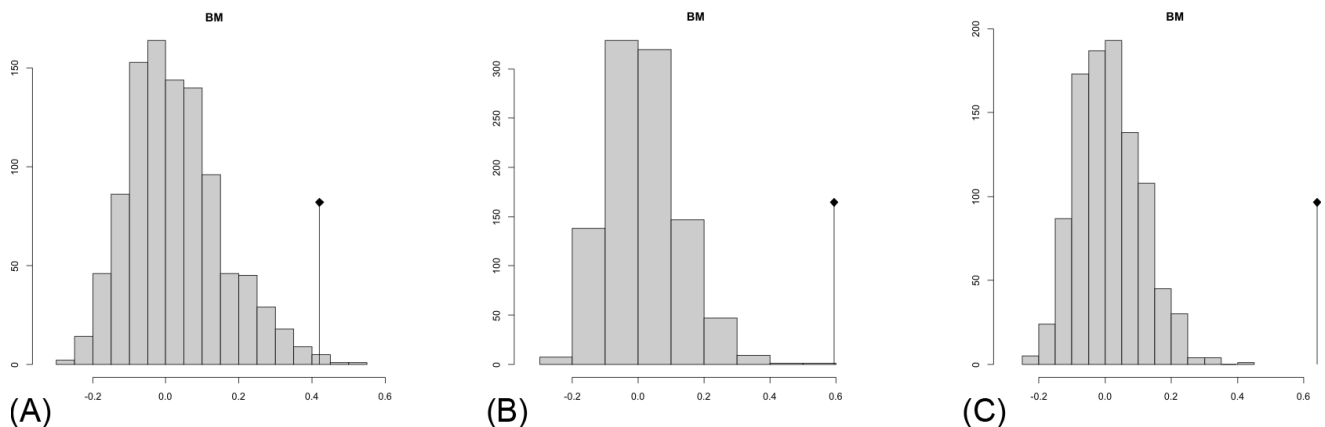

Figure 8 Abouheif C-mean results from the three cladograms. Black dots indicate the position of the observed C-mean statistic relative to the H_0 hypothesis by randomisations along the tips of the phylogeny. The frequency distribution (vertical axis) represents the mean C-statistics (horizontal axis) calculated from the body mass data (tips).

3. Discussion

The results indicate a significant correlation between body size and phylogeny in all the cladograms considered, which is consistent with previous proposals (Vizcaíno *et al.* 2012; Raj Pant *et al.* 2014). Former attempts to investigate the evolution of body size in sloths and anteaters have been approached in a qualitative manner by McNab (1985), who proposed that xenarthran evolutionary patterns link body size to a low rate of metabolism and poor temperature regulation. One consequence of the reduced present diversity of sloths is that they are not fully reliable biological models for actualistic reconstruction of extinct sloth biology (Vizcaíno *et al.* 2008).

The application of quantitative methods to the investigation of body size evolution in xenarthrans is relatively recent. Vizcaíno *et al.* (2012) addressed the evolution of body size in sloths, within a wider analysis of the evolution of large body size in South American herbivores. These authors noted a clear trend, manifested mainly by mylodontoids, megatheriids and nothrotheriids, of increasing body size, approaching gigantism, from the Miocene/Pliocene to the Pleistocene, a proposal that is supported by the analyses and results of the current work.

As mentioned above, Raj Pant *et al.* (2014) analysed the evolution of body size in sloths, using Akaike information criterion for testing phylogenetical models based on Brownian motion, evolutionary stasis, driven-active trend and trended-random walks. These authors reconstructed diversification times and evolutionary rates and concluded that body size evolution in sloths was complex, but dominated by a trend toward giant size. Trended-walk evolutionary models were reconstructed for Mylodontidae and Megatheriidae + Nothrotheriidae, and a stasis model for Megalonychidae was obtained in one of their simulations. Their results are also consistent with those from the present contribution. However, the accuracy of estimating diversification times from the fossil record, for which actual branch lengths are unavailable, has been questioned (see Abouheif 1999; Ollier *et al.* 2006). Thus, methodologies that rely exclusively on the topological structure of a phylogenetic tree (i.e., are independent of inferred branch lengths and/or evolutionary models) would seem to provide a more robust analysis of the evolution of a biological trait. In addition, such methodologies provide a test for those employing branch length estimates and evolutionary models.

Different phylogenetic hypotheses can lead to different results in the application of autocorrelation or phylogenetic dependence. Whilst a truism, this is not a minor issue and must be taken into account carefully when applying statistical techniques that analyse relationships of biological traits and phylogenies. However, results obtained here are similar in overall pattern and resemble those of previous studies. The methods applied here, especially orthonormal decomposition, seem to be efficient in detecting phylogenetic autocorrelation and providing heuristic and valuable information, without the need for branch length estimations and *a priori* evolutionary assumptions on diversification times.

Effectively, the results presented here indicate that as a group, anteaters display the greatest correlation between body size variance and the topology of the tree (and, hence, between size variation and phylogenetic relationships). This suggests that body size diversity in anteaters has been strongly influenced by their phylogenetic history. Based on the same results, similar hypotheses can also be proposed for major clades amongst sloths.

The following sections will discuss the relationship between the evolution of body size, as reconstructed from our results and from previous works, and biological variables, with the aim of proposing hypotheses regarding evolutionary patterns in anteaters and sloths.

One of the most meaningful biological variables related to body size is basal metabolic rate (BMR). Following the seminal work of McNab (1985), extant xenarthrans are characterised by low body temperatures, and low BMRs are also expected following Kleiber's (1932) model for placental mammals. McNab (1985) argued that xenarthran evolution shows a pattern related to low BMR (linked also to poor temperature regulation and low reproductive potential), so here, low BMR may be proposed as a primary factor shaping body size evolution.

3.1. Anteaters: BMR, dietary specialisations and substrate preferences

Of interest is the relative importance of the phylogenetic signal detected for anteaters, despite their limited extant and fossil diversity. Anteaters show a relatively wide disparity in body size, but no vermilinguan reached the large (hundreds of kilograms) and gigantic (tons) body sizes attained by extinct sloths. The results obtained here (a significant relationship between body mass variation and phylogeny) may be reformulated as the working hypothesis that the evolution of body size in anteaters is constrained by their phylogeny. It is necessary to consider which aspects of the evolving biology of anteaters may have constrained the evolution of their body size. Extant anteaters are specialised myrmecophagous mammals (see Naples 1999) and this dietary habit has also been inferred for their fossil relatives (McDonald *et al.* 2008), based on their conservative morphology. Myrmecophagy imposes harsh restrictions on several biological traits, including body size. Numerous aspects of preying on social insects (e.g., their defensive strategies and low nutritional value, low predator/prey size ratio; see Reiss 2000) are key in understanding the evolution of vermilinguan body size. Low nutritional value (social insects have almost no fat, except for larvae and winged reproductive females) and small prey size force anteaters to increase prey intake rates (Naples 1999) in order to meet their nutritional requirements. This is compensated for partly by their low BMR, which also occurs in other mammals that feed on ants and termites, such as the aardvark *Orycteropus* and the pangolins *Manis, Phataginus* and *Smutsia* (McNab 1984, 1985; Reiss 2000). Moreover, detoxification rates for social insect defence chemicals may be enhanced by a lower BMR (McNab 1985). Nevertheless, anteaters diminish the pressures imposed by high rates of prey consumption and insect defensive strategies by not concentrating their efforts on a single nest, but rather by conducting only brief feeding bouts at any given nest (Reiss 2000; Rodrigues *et al.* 2008). Thus, feeding requires a great investment of energy for moving and gathering prey over a large foraging area (Montgomery 1985). As body size increases, the required foraging area increases exponentially, a potential factor in constraining body size evolution in anteaters. Additionally, the pantropical distribution of social insects such as termites that form large colonies could have constrained the geographical distribution of anteaters to warm or warm-temperate areas (McNab 1985; Reiss 2000). In this regard, the presence of *Protamandua* in Miocene deposits of Patagonia has been considered as indicative of subtropical and warm-temperate environments for the Santa Cruz Formation (Kay *et al.* 2012).

Substrate preference and locomotor habits have also varied during the evolution of the group. *Cyclopes* is fully arboreal, *Tamandua* is semiarboreal and *Protamandua* is inferred as having arboreal habits (Hirschfeld 1976; Gaudin & Branham 1998; Bargo *et al.* 2012); whereas *Myrmecophaga* is ground-dwelling. Arboreal habits, or at least climbing locomotor adaptations, have been suggested as the primitive condition for anteaters (Gaudin & Branham 1998; Gaudin & Croft 2015), so *Myrmecophaga* may be considered as derived with regard to substrate preference (see Young *et al.* 2003 for an

account of occasional climbing habits of giant anteaters), whilst *Cyclopes* could be proposed as showing extreme adaptations from a long phylogenetic history of arboreality. Because large body size is a major impediment to arboreality (Cartmill 1985), it is not surprising that the fully terrestrial *Myrmecophaga* is the largest anteater, the semiarboreal *Tamandua* is mid-sized, whilst the fully arboreal *Cyclopes* is the smallest one. The evolution of the giant anteater has been apparently closely related to the colonisation of open habitats by termites since the Miocene (McDonald *et al.* 2008).

3.2. Sloths

Mylodontoids and megatheriid + nothrotheriid sloths experienced convergent increases in body mass since the Miocene, reaching gigantic sizes during the Pleistocene (Figs 2, 3, 4). Vizcaíno *et al.* (2012) described the tendency for increasing maximum body size through evolution as a common feature in Cenozoic South American mammalian herbivore lineages. This is especially true for xenarthrans, mainly sloths and glyptodonts, which reached a climax during the Pleistocene, with a diversity of forms having masses of several tons.

Proposing explanatory hypotheses for sloth body size evolution is not as straightforward as for anteaters. In sharp contrast with extant sloths, extinct sloths were quite diverse in body size and morphology, as well as in dietary habits, substrate preference and locomotor modes (see McDonald & De Iuliis 2008 for a synopsis). However, the same working hypothesis as for anteaters may be proposed: the evolution of body size in sloths (mainly mylodontoids, megatheriids and nothrotheriids) is constrained by their phylogenetic history, and the evolutionary panorama of sloth body size may also be analyzed with respect to the relationships among BMR, diet, and substrate preference.

A preliminary review of the evolutionary changes in substrate preference and diet was performed by Pujos *et al.* (2012), and later expanded by Gaudin & Croft (2015). Pujos *et al.* (2012) reviewed published data and concluded that among pre-Miocene sloths, only the skeletal remains of the Deseadan (late Oligocene) *Octodontotherium* are known well enough to permit the proposal of palaeobiological hypotheses. Most of the available palaeobiological information is, therefore, restricted to Miocene and Pleistocene sloths. It is worth reiterating that, in contrast to anteaters, extant sloths are not suitable models for understanding fossil sloth biology and evolution; or, at least, that inferences based on the extant forms must be made with considerable caution.

3.2.1. Substrate preference. McNab (1985) related the low BMR of extant sloths to their relatively low percentage of skeletal musculature. Grand (1978) analysed the mechanical advantages of the suspensory mode of life of extant sloths in relation to optimisation of muscle needed for ensuring stability and movement. This optimisation, resulting in a reduction of muscular mass in modern sloths, would not apply to most fossil sloths, as they were almost certainly more muscular (Bargo *et al.* 2000; Vizcaíno *et al.* 2006; Toledo *et al.* 2013, 2015).

Pujos *et al.* (2012) optimised substrate preference categories of sloths in their cladogram, which supported previous proposals that the suspensory habits of extant sloths are convergent (Patterson & Pascual 1968; Webb 1985; Gaudin 2004; McDonald & De Iuliis 2008; Nyakatura *et al.* 2010). These authors considered a terrestrial habit basal for all sloths, and reconstructed a basal semiarboreal substrate preference for megalonychids, megatheriids and nothrotheriids, as proposed previously by Webb (1985). Mylodontoids, a clade that shows a clear trend towards the evolution of giant body size, retained their ancestral terrestrial habit. In accordance with McNab

(1985), and based on the well-developed digging abilities and potential fossorial habits of the mylodontids *Scelidotherium* and *Glossotherium* (see Bargo *et al.* 2000; Vizcaíno *et al.* 2001), Vizcaíno *et al.* (2006) proposed that these ground sloths could have had a lower BMR than expected from their body size and dietary habits. Fossorial habits (including both the digging of a shelter and occupation of an existing cave or burrow) implies living in an environment with a more constant and perhaps elevated temperature, thus saving thermoregulatory energy costs by reducing BMR and reducing the heat storage when the animals were moving or resting inside the burrow (McNab 1985; T. J. Gaudin pers. comm. 2016). Following Pujos *et al.* (2012), digging abilities might be a basal feature for mylodontids, and possibly of even more basal mylodontoids such as the Miocene *Nematherium* (see Bargo *et al.* 2012; Toledo *et al.* 2013).

Basal megatherioids analysed here (*Hapalops*, *Schismotherium* and *Analcimorphus*) were arboreal or semiarboreal. This is also true for early Miocene megalonychids (*Eucholoeops*), whilst the Miocene megatheriid *Prepotherium* was essentially terrestrial (White 1993, 1997; Bargo *et al.* 2012; Toledo *et al.* 2013, 2015). In this context, it can be argued that one of the consequences of the evolutionary tendency of mylodontoids, nothrotheriids and megatheriids towards large (and gigantic) body sizes from the Oligocene/Miocene to the Pleistocene was the shift from a diversity of substrate preference and locomotor modes, that included arboreal, semiarboreal and terrestrial forms, to almost exclusively terrestrial forms. One of the exceptions to this trend is the large-sized nothrotheriid (but see Amson *et al.* 2016) *Thalassocnus*, for which a semiaquatic habit has been proposed (Muizon & McDonald 1995; Muizon *et al.* 2004; Amson *et al.* 2014, 2015). In this regard, an aquatic lifestyle imposes fewer constraints to large body size as compared to terrestrial and arboreal habits. Among megalonychids, their less marked evolutionary tendency toward large body sizes may have been influenced by the fact that some of them evolved in insular environments (H. G. McDonald pers. comm. 2016; e.g., Pleistocene megalonychids recovered from Cuba, Hispaniola and Puerto Rico islands; see Pujos *et al.* 2016), and may have been subjected to selective pressures against larger body size related to their home range extent (see Burness *et al.* 2001). Other genera evolving in continental environments (e.g., *Megalonyx*) experienced less geographic constraint on body sizes. Nevertheless, maintenance of a greater diversity of body sizes and substrate uses and locomotor habits in megalonychids, including arboreal and terrestrial forms (Pujos *et al.* 2007, 2012), requires further analysis.

3.2.2. BMR and dietary habits. As stated above, the primary relationship between body size evolution and BMR was proposed by McNab (1985). Extant sloths can regulate their energetic expenditure by varying body temperature, as a consequence of their low BMR (McNab 1978). Based on the concept that relatively large body sizes could help to improve temperature regulation of xenarthrans through thermal inertia, McNab (1985) proposed a relationship between the inferred low BMR of extinct sloths (mainly Pleistocene ground sloths) and their large to giant body sizes.

As in most mammals, features of the masticatory apparatus of sloths are related to diet and digestive physiology and, hence, to BMR and body size. All sloths are hypselodont (see Vizcaíno 2009, for a review of xenarthran dental features) and, in general, are reconstructed as herbivorous animals (but see Fariña 1996; Fariña & Blanco 1996; Bargo 2001; Vizcaíno 2009). Extant sloths are folivorous (*Bradypus*) and frugivorous-folivorous (*Choloepus*) browsers (Chiarello 2008). They carry out foregut fermentation in complex chambered stomachs (Montgomery & Sunquist 1975) and food undergoes a long

transit time, not only in the stomach but also in the intestines (Gilmore *et al.* 2008). Pujos *et al.* (2012) optimised reconstructed foraging modes on their cladogram, and proposed an ancestral browsing foraging mode (i.e., selective feeding) as ancestral for the entire clade.

Analyses on available dental area for food processing (see Janis 1990) have shed light on the relationship between diet and BMR. Vizcaíno *et al.* (2006) showed that the relatively low values of occlusal surface area (OSA) of xenarthrans could be related to their low BMR, implying that their lower energetic requirements suggest a comparatively lower food intake than other mammalian herbivores of similar body mass. This could have facilitated the evolutionary increase of body size in sloths, permitting them to dominate the large herbivore guild during the Pleistocene (see Vizcaíno *et al.* 2012). Mylodontids have the lowest OSA values. This is possibly related to limited oral food processing, which may have been compensated by an increased digestive efficiency (perhaps involving foregut fermentation; see Vizcaíno *et al.* 2006). Based on morpho-functional and biomechanical aspects of dietary apparatus, a similar relationship between limited oral food processing and putative caecal fermentation was proposed by Naples & McAfee (2012) for the megatheriid *Eremotherium* (but modern sloths show only foregut fermentation; see Gilmore *et al.* 2008). The megatheriid *Megatherium*, the largest sloth known, had much higher OSA values than mylodontids (Vizcaíno *et al.* 2006). This is related to increased oral processing and, perhaps, also feeding on more nutritional food. Fariña (1996) and Fariña & Blanco (1996) suggested that *Megatherium* could incorporate animal tissues in its diet. Bargo (2001) proposed that *Megatherium* was a browser (a selective feeder according to Bargo *et al.* 2006) of fruits and other moderate to soft food items, although Green & Kalthoff (2015), using microwear analysis of orthodentine, proposed that it could eat plants with low to moderate intrinsic toughness. Finally, Vizcaíno *et al.* (2006) proposed that a combination of high OSA values and relatively higher nutritional food intake could indicate that *Megatherium* had a BMR similar to that of other herbivorous placentals of similar body size.

Studies on jaw biomechanics have suggested that megatherioid sloths combined browsing habits with prominent orthal and/or anteromedial masticatory movements (Naples 1982; Bargo *et al.* 2009; Vizcaíno 2009; but see Naples & McAfee 2014). In nothrotheriids and megatheriids, shearing/cutting orthal movements have been reconstructed (Bargo 2001; Muizon *et al.* 2004; Vizcaíno 2009), suggesting a folivorous diet. Pujos *et al.* (2012) considered a grazing foraging mode (i.e., bulk feeding) as characteristic of mylodontines since the early Miocene (*Pseudoprepotherium*), whereas more basal mylodontoids and scelidotheriines were considered browsers. Grazers usually feed on vegetation with lower nutritional value (including a high percentage of grasses, for instance) than that eaten by browsers (which ingest mainly dicotyledonous leaves and buds). Analyses of mylodontoids teeth and mandibular morphology indicate that grinding mesiolingual components were the main masticatory movements present in both Miocene and Pleistocene mylodontids (Bargo & Vizcaíno 2008; Bargo *et al.* 2009, 2012), which suggests an habitual processing of fibrous and turgid items such as roughage, roots and tubers.

Summing up, a relationship between low nutritional food, grazing habits and low BMR can be proposed as an influential factor for mylodontoid evolution towards large body sizes. On the contrary, megatherioids appeared to maintain the ancestral sloth browsing foraging mode (Pujos *et al.* 2012). Green & Kalthoff (2015) suggest that microwear patterns in fossil sloths are important descriptors of environmental grit amount along with food type.

3.2.3. Body temperature and palaeoenvironment.

Lastly, the relationships among body size evolution, body temperature and environment are addressed. Amongst the placental mammals, extant sloths have the poorest ability to regulate body temperature (McNab 1985). The two-toed sloth *Choloepus* has a low BMR and moderate thermal conductance, having a high temperature differential derived from its long hairy coat (McNab 1985). *Bradypus* has a similar low BMR, but has a lower minimal conductance due to its dense coat of guard hairs, which is not present in *Choloepus*. McNab (1985) proposed that the poor thermal regulation of extant sloths is a consequence of their slow metabolism, which is linked, in turn, to small relative muscle masses (which was related by Grand (1978) to mechanical advantages of suspensory habits), but also to their strictly folivorous dietary habits.

Environments inhabited by extinct sloths in the past showed a wider range of temperatures than the tropical conditions where extant sloths live today. During the Eocene–Oligocene, warm and tropical to subtropical conditions prevailed for most of South America. From the Oligocene to the early Miocene, colder temperatures that resulted in glaciations caused the replacement of tropical and subtropical environments by temperate–cold environments. By the middle Miocene, global warming induced the return of subtropical conditions (Mid Miocene Climatic Optimum; see Zachos *et al.* 2001), although xeric palaeofloras also developed in southern South America. From the middle Miocene to the Pliocene, the establishment of the Circumpolar and Humboldt oceanic currents produced an increase in temperate conditions, as well as in aridity. The rise of the Andes further increased arid conditions to the east by the Plio-Pleistocene. Finally, oscillation between glacial and warm periods during the last half of the Pleistocene produced a concomitant retraction of humid, temperate environments and an expansion of drier and cooler conditions (see Hinojosa (2005) for a synopsis of climatic and palaeofloristic changes in South America during the Cenozoic).

Taking into account the fossil record, it appears that most clades of sloths (despite their putative low BMR and poor body temperature regulation) were able to successfully cope with environmental fluctuations until the very end of the Pleistocene. As noted above, extinct sloths (especially giant ground sloths) were considerably more muscular than extant sloths. This feature, combined with a hairy covering and large body mass, may have functioned to produce a higher body temperature and greater thermal inertia due to a smaller ratio of surface to volume, resulting in greater thermal tolerance (McNab 1985). Thus, a thick furry body covering and greater body mass may have compensated for low BMR. McNab (1985) suggested that mylodontids may, thus, have had low BMRs, but their thick body covering would have allowed them to withstand the seasonally cold environments developed from the Miocene–Pliocene to the Pleistocene (McNab 1985), and permitted *Mylodon* to extend its range to the southern tip of South America. Fossorial habits were proposed for some genera (see above), which could further facilitate thermal adaptation, according to ideas presented by McNab (1985). In addition, megalonychids, proposed to be cave-dwelling forms (e.g., *Megalonyx*; see McDonald 2003) could have benefitted in a similar way. However, a reduction or lack of a hairy covering has been suggested for some giant megatheriids, such as *Eremotherium* (McNab 1985) and *Megatherium* (Fariña 2002), which may have been related to their greater thermal inertia due to their enormous body size.

Thus, considering their relative success in cold and open environments in the last part of the Cenozoic (see Cione *et al.* 2003), and as Vizcaíno *et al.* (2012) remarked, sloths (as well as cingulates) do not appear to have undergone a decrease

in diversity during the Plio-Pleistocene, in contrast to other South American mammalian lineages. Indeed, not only did xenarthrans overcome competition with holarctic lineages entering South America after the Pliocene, but some groups also successfully expanded into, and became integral parts of, North American habitats (see McDonald 2005; Vizcaíno *et al.* 2012; Fariña *et al.* 2013; Cione *et al.* 2015). Although several clades of mammalian herbivores included members with large body size, the ecological success of xenarthrans (mainly mylodontids, nothrotheriids and megatheriids, amongst sloths) is indicated, since they dominated the megaherbivore guild in South America until the megafaunal extinction of the terminal Pleistocene (Cione *et al.* 2003; Vizcaíno *et al.* 2012; see Steadman *et al.* 2005 for a discussion of factors involved in giant sloth extinctions). Megalonychids, as mentioned above, maintained a greater diversity of body size than mylodontoids, megatheriids or nothrotheriids until the Pleistocene in most of the Americas. In the Caribbean region, megalonychids survived into the Holocene (McDonald & De Iuliis 2008), and included several arboreal forms (see White 1993, 1997). This may indicate that environmental changes toward more open and dry habitats were not as drastic in the Caribbean region as in the continental areas of South and North America, and selective pressure towards increased terrestriality and body size was attenuated for these sloths (see Pujos *et al.* 2016 for a discussion about evolution of suspensory habits). However, the paucity of palaeobiological information from this region requires further study in order to gain better insight into this possibility.

4. Concluding remarks

Low BMR (and hence lower energetic requirements than for a placental mammal of similar size) and hypselodonty may have provided sloths with the evolutionary capacity to increase body size, which was key for their ecological success during the Plio-Pleistocene (Vizcaíno *et al.* 2012). The following remarks can be highlighted:

1. Methods applied in this work, especially orthonormal decomposition, have been demonstrated to provide heuristic information about evolutionary patterns.
2. In the three cladograms analysed, a significant relationship between body mass and phylogeny was found, especially for anteaters and the more inclusive sloth clades (Mylodontoidea, Nothrotheriidae, Megatheriidae and Megalonychidae).
3. For anteaters, constraints imposed by diet on the evolution of body size can be proposed. For sloths, additional support to previous studies is provided, indicating that megatheriids, nothrotheriids and mylodontoids showed a clear evolutionary trend towards large body sizes, probably linked to environmental changes since the Miocene.

4. A relationship between BMR and dietary habits can be proposed as the main factor influencing the evolution of body size, with substrate preferences playing a secondary role. Further studies investigating BMR and diet, based on an increased sample of fossil pilosans, are needed.
5. The stratigraphic pattern of the fossil record is also recognised as a bias (i.e., the dominance of Miocene and Plio-Pleistocene taxa). Hence, further fossil remains and palaeobiological studies, especially on pre-Miocene forms, are needed to elucidate a more complete understanding of body size evolution in anteaters and sloths.

5. Acknowledgements

6. Appendix. Estimation equations developed in this work

Follows morphometric and statistical procedures and extant mammal database provided in Toledo *et al.* (2014). FMNH = Field Museum of Natural History, Chicago. Abbreviations correspond to linear measurements used in Toledo *et al.* (2014), as follows: AMFL = length of medial facet of astragalus; AHW = width of astragalar head; APDF = anteroposterior diameter of femur at midshaft; APDH = anteposterior diameter of humerus at midshaft; ASL = length of astragalus; AEFL = length of ectal facet of astragalus; ATMW = maximum width of astragalar trochlea; CL = total length of calcaneus; CTW = width of calcaneal tuber; FCL = functional length of calcaneus; FFL = femoral functional length; HDEW = humeral distal epiphysis width; HHW = width of humeral head; HL = humerus total length; ItuW = width between greater and lesser humeral tuberosities; OTL = length of olecranal tubercle; PGW = width of patellar groove; RDEW = radial distal epiphysis width; RH = radial height at midshaft; RHL = length of radial head; RHW = width of radial head; RL = radius total length; TDF = transverse diameter of femur at midshaft; TDH = transverse diameter of humerus at midshaft; TDU = transverse diameter of ulna at midshaft; SL = scapular length.

Taxa	Formula	RE	Source of data
Diabolotherium	Formula = −1,345 + SL + HL + HHW + ItuW + TDH + APDH + HDEW + TDU + RL + RHW + RDEW + ATMW	0.972	Pujos *et al.* 2007
Mionothropus	Formula = 3,006 + HL + HHL + HDASW + ItuW + HHW + OTL + TDU + RH + RHL + TDF + FFL + APDF + PGW	0.958	De Iuliis *et al.* 2011
Protamandua (calcaneus) FMNH 368	Formula = −1,932 + CL + FCL + CTW	1.038	this work
Protamandua (astragalus) FMNH 366, FMNH 367	Formula = −1,345 + ATMW + ASL + AEFL + AMFL + AHW	1.265	this work

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