Patterns of ossification in southern vs. northern placental mammals

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Abstract

Consensus on placental mammal phylogeny is fairly recent compared to that for vertebrates as a whole. A stable phylogenetic hypothesis enables investigation into the possibility that placental clades differ from one another in terms of their development. Here, we focus on the sequence of skeletal ossification as a possible source of developmental distinctiveness in "northern" (Laurasiatheria and Euarchontoglires) vs. "southern" (Afrotheria and Xenarthra) placental clades. Previous analyses of mammalian ossification sequences have mainly focused on marsupials, monotremes, and northern placentals. We contribute data on cranial and postcranial ossification events during growth in Afrotheria, including elephants, hyraxes, golden moles, tenrecs, sengis, and aardvarks, and also draw on data for Xenarthra. We use three different techniques to quantify sequence heterochrony: continuous method, sequence-ANOVA and event-paring/Parsimov. By controlling for ties and taking into account results that all methods support, we show that afrotherians significantly differ from other placentals by an early ossification of the orbitosphenoid and caudal vertebrae. Our analysis suggests that xenarthrans are characterized by a late ossification of the sternum and an early ossification of the phalanges and pubis, while afrotherians exhibit early ossification of the sternum and late ossification of the phalanges and pubis. The latter observation is inconsistent with the Atlantogenata hypothesis in which afrotherians are considered as the sister clade of xenarthrans. Interestingly, ancestral nodes for Laurasiatheria and Euarchontoglires show very similar trends and our results suggest that developmental homogeneity in some ossification sequences may be restricted to northern placental mammals (Boreoeutheria).

Keywords: Afrotheria, development, evolution, heterochrony, ossification, phylogeny, skeletogenesis,.

Introduction

Until the close of the 20th century, many high-level nodes in the placental mammal Tree of Life were not well understood. The last 15 years have changed this state of affairs dramatically. While most individual orders and some inter-ordinal groups have been accurately recognized based on comparative anatomy for over a century, few zoologists would have predicted the now-stable phylogeny consisting of four major clades: Afrotheria, Xenarthra, Laurasiatheria, and Euarchontoglires (Murphy et al. 2001). Certain elements of these clades have a long history in comparative anatomy, including elephant-sea cow-hyrax as afrotherians; primate-tree shrew-dermopteran and rodent-rabbit as euarchontoglires; sloth-armadillo-anteater as xenarthrans; pangolin-carnivoran and artiodactyl-whale as laurasiatheres. Other groups are quite novel to morphologists (e.g., tenrec-golden mole-paenungulate; hippo-whale) and would not have been recognized without the analysis of molecular data that became widespread during the late 20th century (see review in Asher et al. 2009). Although there is some ambiguity concerning the position of the root, the most recent and broadly sampled analyses (e.g., Meredith et al. 2011) support a sister group relationship between afrotheres and xenarthrans in Atlantogenata and laurasiatheres and euarchontoglires in Boreoeutheria. Even with some ambiguity regarding the root position, a relatively well-resolved phylogeny for Placentalia offers the potential for a much improved understanding of mammalian character evolution.

Perhaps the best known dichotomy within Mammalia is that between marsupials and placentals. A number of authors (e.g., Smith 1997, 2001; Sánchez-

Villagra 2002; Sánchez-Villagra et al. 2008; Sears 2009; Keyte and Smith 2010) have shown differences in the sequence of ossification and soft-tissue events between these groups, particularly in regards to formation of the limbs and facial skeletons relative to development of the sense organs and brain. The phylogenetic distinction between marsupials and placentals is as old as theories of evolution themselves, and biologists have investigated patterns of marsupial and placental mammal development for just as long. Phylogenetic patterns within Placentalia have been deciphered more recently, but given that our confidence in intra-placental phylogenetics is now much stronger than it has ever been, it would be worth asking if and how placental mammal clades differ from one another in terms of development. Might there be additional developmental dichotomies within mammals, previously masked due to the lack of phylogenetic resolution among placental orders?

Based primarily on the distribution of their living representatives, Asher et al. (2009, 2011) informally referred to atlantogenatans and boreoeutherians as, respectively, "southern" and "northern" placental clades, and further raised the possibility that a developmental dichotomy might distinguish the two based on differences in the timing of permanent tooth eruption and variability of the axial skeleton. Most afrotherians and some xenarthrans show a late-erupting adult set of teeth (Asher and Lehmann 2008; Asher and Olbricht 2009; Ciancio et al. 2012) and more variation in vertebral formulae and anatomy than other mammals (Sánchez-Villagra et al. 2007; Buchholtz and Stepien 2009; Asher et al. 2011). In addition, the two southern groups frequently show non-descent of the male gonads (Werdelin and Nilsonne 1999; Kleisner et al. 2010) as well as distinctive features of placentation (Carter and Mess 2007).

Most previous studies of ossification sequences have focused on marsupials

(e.g., Nunn and Smith 1998), monotremes (Weisbecker 2011), and/or northern placentals (e.g., Sánchez-Villagra et al. 2008). These have revealed a surprising degree of developmental homogeneity in the skeleton of placental mammals (Bininda-Emonds et al. 2003; Goswami 2007; Weisbecker et al. 2008; Goswami et al. 2009; Wilson et al. 2010), possibly because most previous data on placental mammals tend to derive from northern clades. The few studies that have included southern placentals in evaluating ossification sequences (Hautier et al. 2010, 2011) show that heterochrony does play an important role in the skeletal development of xenarthrans. Similarly, the literature on mammalian sequence heterochrony has only recently included developmental data on afrotherians (Hautier et al. 2012; Werneburg et al. 2012). Werneberg et al. (2012) noted homogeneity in the prenatal development of *Tenrec, Echinops,* and *Dasypus* with each other and to sequences known for other mammals.

Here, we extend comparisons of ossification sequence between northern and southern placental groups, focusing on the sequences of cranial and postcranial ossification for elephants (*Loxodonta*), sengis (*Macroscelides*, *Elephantulus*), tenrecs (*Echinops*, *Tenrec*), golden moles (*Eremitalpa*), hyraxes (*Procavia*, *Heterohyrax*), and aardvarks (*Orycteropus*). We employ techniques for quantifying sequence heterochrony (Nunn and Smith 1998; Smith 2001; Keyte and Smith 2010; Jeffery et al. 2005; Germain and Laurin 2009) to test the hypothesis that southern and northern placental mammals are developmentally distinct in terms of their ossification sequences. We seek to provide a comparative basis upon which to measure if and how southern placental mammals depart from the conservative ossification patterns observed among other mammalian clades, and to ask if major developmental differences such as those evident between marsupials and placental mammals (Smith

2001) might also occur within placentals.

Material and Methods

Data collection - We sampled material from collections of the Museum für Naturkunde Berlin (ZMB), the Natural History Museum London (BMNH), the Muséum National d'Histoire Naturelle in Paris (MNHN), the Institut Royal des Sciences Naturelles de Belgiques in Brussels (IRSNB), the Paul Mellon Laboratory of Equine Reproduction in Newmarket UK (PMLR), the Smithsonian Institution in Washington (USNM), the Riksmuseet Stockholm (NRM), the Paläontologisches Institut und Museum Zürich (PIMUZ), the Laboratory of Artificial and Natural Evolution in Geneva (LANE), the Department of Zoology and Entomology at the University of Pretoria (UP), and the University Museum of Zoology Cambridge (UMZC). 119 afrotherian fetuses were studied representing 15 genera (Wilson and Reeder 2005): *Elephas*, *Loxodonta*, *Macroscelides*, *Elephantulus*, *Echinops*, *Tenrec*, *Amblysomus*, *Potamogale*, *Chrysochloris*, *Eremitalpa*, *Procavia*, *Heterohyrax*, *Dugong*, *Trichechus*, and *Orycteropus* (Figs. 1 and S1). The sample sizes of *Elephas*, *Potamogale*, *Amblysomus*, *Chrysochloris*, *Dugong*, and *Trichechus* were insufficient to provide ossification sequence for these species. In addition, and despite access to a substantial number of specimens of *Elephantulus* and *Macroscelides* at different sizes, ossification occurred within such a narrow size range for these species that we recovered no resolution for the cranial elements, all bones being tied at #1. Hence, they were not used to run the analyses but helped to check the accuracy of other observed sequences. Table 1 lists the sources for ossification sequences we obtained from the literature.

Table 1. Sources of data used in the analysis of ossification sequence with specimen and stage numbers.

	Specimen numbers/stages		
Species name	*Cranial*	*Postcranial*	References
Sauropsida			
Alligator mississippiensis	36/7	47/7	Rieppel 1993a
Lacerta vivpara	23/6	36/9	Rieppel 1993b
Coturnix coturnix	15/4		Nakane and Tsudzuki 1999
Afrotheria			
Loxodonta africana	17/6	17/13	Hautier et al. 2012
Tenrec ecaudatus	20/4	20/6	Present study
Echinops telfairi	5/4	5/5	Present study
Procavia capensis	32/2	32/6	Present study
Heterohyrax brucei	11/3	11/4	Present study
Elephantulus rozeti	10/1	10/3	Present study
Macroscelides proboscideus	14/1	14/5	Present study
Eremitalpa granti	11/3	11/8	Present study
Orycteropus afer	4/2	4/4	Present study
Dugong dugong	1/1	1/1	Present study
Trichechus manatus	1/1	1/1	Present study
Xenarthra			
Bradypus variegatus	4/2	4/4	Hautier et al. 2011
Choloepus didactylus	4/1	4/4	Hautier et al. 2011
Cyclopes didactylus	4/2	4/5	Hautier et al. 2011
Tamandua tetardactyla	4/4	4/4	Hautier et al. 2011
Dasypus novemcinctus	27/6	27/8	Hautier et al. 2011
Euarchontoglires			
Tupaiajavanica	24/6		Zeller 1987; Nunn and Smith 1998; Goswami 2007
Tarsius spectrum	21/6		Nunn and Smith 1998
Homo sapiens	60/	60/17	Mall1 906; Davies and Parsons 1927
Rattus norvegicus	N.a/6	N.a./14	Strong 1925
Mus musculus	N.a/7	41/5	Johnson 1933; Theiler 1972; Patton and Kaufman 1995; Kaufman 2008
Cavia porcellus	N.a/12	N.a./12	Petri 1935; Wilson et al. 2010
Mesocricetus auratus	168/7	168/8	Beyerlein et al. 1951; Kanazawa and Mochizuki 1974
Meriones unguiculatus	9/5	187/8	Yukawa et al. 1999; Sanchez-Villagra et al. 2008
Peromyscus melanophrys	13/5	7/4	Sanchez-Villagra et al. 2008; Weisbecker et al. 2008
Octodon degus		8/5	Wilson et al. 2010
Rhabdomys pumilio	61/12	61/11	Wilson et al. 2010
Laurasiatheria			
Myotis lucifugus		19/7	Adams 1992
Rousettus amplexicaudatus	11/7	12/10	Sanchez-Villagra et al. 2008; Weisbecker et al. 2008
Cryptotis parva	29/10		Sanchez-Villagra et al. 2008; Koyabu et al. 2011
Erinaceus amurensis	21/6		Koyabu et al. 2011
Talpa europaea	11/7	22/9	Prochel 2006; Goswami and Prochel 2007; Prochel et al 2008; Koyabu et al. 2011
Mogera wogura	16/7		Koyabu et al. 2011
Bos taurus		180/9	Lindsay 1969a,b
Sus scrofa	10/7	N.a./12	Stockli 1922; Nunn and Smith 1998
Felis catus	17/7		Nunn and Smith 1998
Manis javanica	12/4		Nunn and Smith 1998
Marsupiala			
Didelphis virginiana	16/6	16/9	de Oliveira et al. 1998
Trichosurus vulpecula	6/4	32/9	Weisbecker et al. 2008
Macropus eugenii	20/6	11/9	Nunn and Smith 1998; Weisbecker et al. 2008
Dasyurus viverrinus	18/7	19/10	Nunn and Smith 1998; Goswami 2007; Weisbecker et al. 2008
Sminthopsis macroura		11/8	Frigo and Wooley 1996
Antechinus stuartii		22/10	Weisbecker et al. 2008
Cercartetus concinnus		25/8	Weisbecker et al. 2008
Isoodon macrourus		15/10	Weisbecker et al. 2008
Fetaurus breviceps		22/6	Weisbecker et al. 2008
Vombatus ursinus		9/6	Weisbecker et al. 2008
Caluromys philander	9/6		Goswami 2007; Sanchez-Villagra et al. 2008
Perameles nasuta	10/9		Nunn and Smith 1998; Goswami 2007
Monodelphis domestica	28/8		Nunn and Smith 1998; Goswami 2007

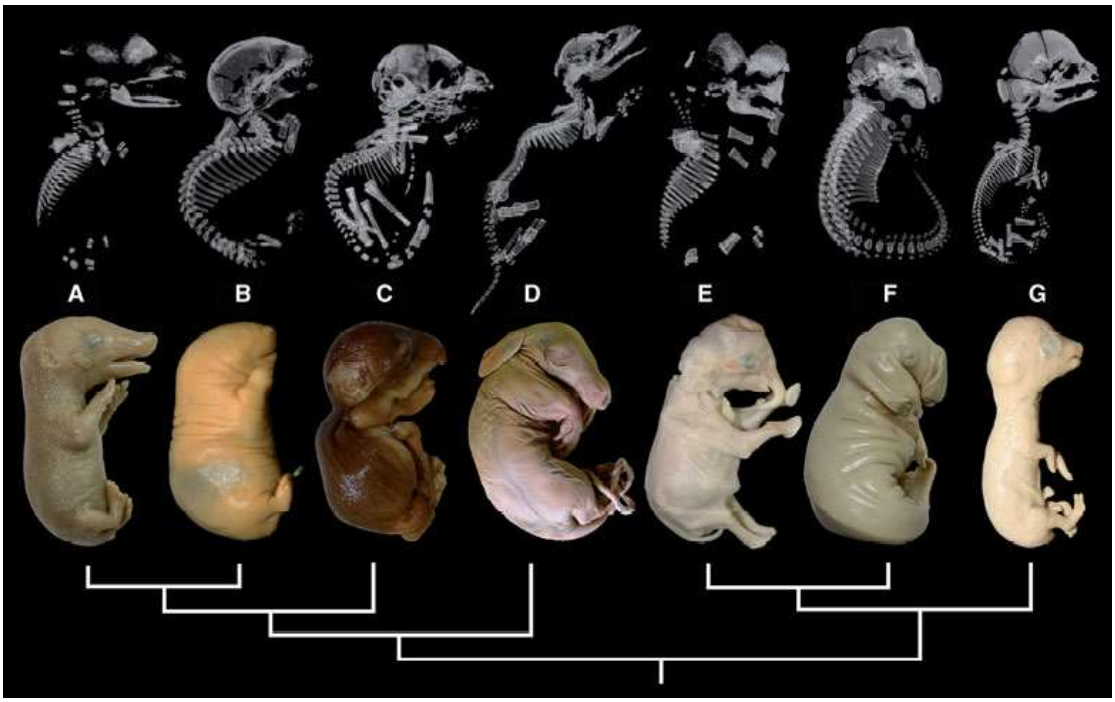

Figure 1. Representative ontogenetic stages of afrotherians and their phylogenetic relationships following Asher (2007). Lateral view of specimens (left) in A) *Tenrec ecaudatus* PIMUZ MSV-Tec 11 CRL=24mm; B) *Eremitalpa granti* NRM 538503 CRL=25.5mm; C) *Elephantulus rozeti* ZMB28 CRL=33mm; D) *Orycteropus afer* UP Aardvark2 CRL=210mm; E) *Loxodonta africana* UMZC 2011-10-01 CRL=34.7mm; F) *Dugong australis* IRSNB 5386 CRL=212mm; *Heterohyrax brucei* USNM 184769 CRL=57.4.

3-D data acquisition - Skeletons were imaged using high-resolution X-ray microtomography (μCT - Fig. 1) at the Helmholtz Zentrum (Berlin, Germany), the engineering department of the University of Cambridge (Cambridge, UK), the Natural History Museum (London, UK), and VISCOM SARL (Saint Ouen l'Aumône, France). Threshold values between ossified parts and soft tissues were substantial and easily allowed osteological reconstructions. 3-D rendering and visualization were performed using Drishti v.1.0 (Drishti Paint and Render, Limaye 2006). All the results obtained from three-dimensional reconstructions were checked through the acquisition of shadow images, comparable to a conventional high-resolution x-ray as

described in Weisbecker et al. (2008). Ossification centres were readily apparent in both 3D reconstructions and shadow X-rays. Following Weisbecker (2011), we distinguished clearly ossified bones from elements displaying barely detectable ossification. Pooled elements (e.g. carpals, metacarpals, phalanges) were considered ossified when at least one of the constituent elements had started its ossification.

Quantification of developmental trajectories - The sequence of ossification of a number of specific elements is given in Tables 2 and 3. To maximize compatibility with previous studies (e.g. Sanchez-Villagra et al. 2008; Weisbecker et al. 2008; Hautier et al. 2011), cranial and postcranial elements of the skeleton were treated separately in the analyses. Following Hautier et al. (2011, 2012), we used two methods to quantify sequence heterochrony: the sequence method (Nunn and Smith 1998; Smith 2001; Keyte and Smith 2010) and Parsimov (Jeffery et al. 2005). In addition, we also applied the continuous method recently developed by Germain and Laurin (2009).

The sequence-ANOVA method used by Nunn and Smith (1998), Smith (2001) and Keyte and Smith (2010) requires that every species be sampled for the same series of elements. Thus, several species could not be included (postcranial: *Meriones*, *Ovis*, *Bos* and *Sus*; cranial: *Tarsius*, *Rattus*, *Meriones*, *Mesocricetus*, *Felis*, *Sus*, *Ovis*, *Bos* and *Manis*). The clavicle and the jugal, absent in some of the studied species, were not considered in the analyses. The dataset for sequence ANOVA method thus comprised 21 taxa for the analysis of 16 cranial elements, and 28 taxa for the analysis of 23 postcranial elements. For our remaining sample, the first step consists of constructing the developmental sequence by ordering the events by their relative stage for each taxon. The sequence method is less explicitly phylogenetic than Parsimov and the continuous method. Nevertheless, it can effectively illuminate the pattern of

change of different skeletal elements of afrotherians relative to the mean developmental trajectory of other placental mammals. In the case of ties, we used the average rank for the tied events (Siegel and Castellan 1988). For instance, if three ossification events occur simultaneously at fifth in the overall series, each would receive a rank of 6 (*i.e.* [5+6+7]/3). If in the same series the next two characters occur simultaneously at sixth place, their rank would be 8.5 (*i.e.* [8+9]/2). The data set is then converted into transformed ranks (presented in Table S6 and S7 for our samples). The ranked data set is then plotted graphically, illustrating the major differences across species and enabling statistical scrutiny.

Smith (2001) used ANOVA to recognize characters that show significantly more differences in rank position between than within groups (see also Nunn and Smith 1998). Our results given by ANOVA were confirmed using a non-parametric Mann-Whitney U test. These methods provide a quantitative approach to detect events that are advanced or delayed in one group relative to another. Sequence-ANOVA allows only the determination of the existence of a heterochronic shift. It does not convey absolute information on the direction of a shift, but these can be compared to an explicit reference taxon. Shifts identified by sequence-ANOVA are therefore discussed here in terms of "earlier" and "later" relative to this reference taxon.

In the present study, sequence-ANOVA illuminates the pattern of change of different cranial and postcranial elements of afrotherians and xenarthrans relative to the developmental trajectory of other placental mammals (i.e. Euarchontoglires and Laurasiatheria). The variability in the mean placental ranks was graphically displayed with error bars of ±1 standard deviation in order to show the extent to which afrotherian and xenarthran sequences depart from the range of variation observed in other mammals. However, the ranking procedure presented some disadvantages.

When ties accumulate within the ontogeny of any single taxon (exacerbated in species sampled by relatively few ontogenetic stages), it will tend to increase considerably the value of the transformed rank and to create artefactual heterochronies. In our sample, this occurs primarily due to lack of coverage of the earliest ossification events, i.e., leading to a number of events tied at #1. We sought to overcome such methodological noise by discounting the "significance" of heterochronies resulting from the accumulation of events tied at the beginning of a given ontogenetic series.

In the continuous analysis approach of Germain and Laurin (2009), the sequence intervals are standardized between 0 and 1, with 0 corresponding to the first and 1 to the last rank position. In the case of a sequence of *n* ranks, the number of intervals corresponds to $n - 1$. Thus, sequence data are normalized by the highest rank, which is potentially a major drawback of the method. Indeed, normalized data become highly biased by the resolution of the sequence, especially for bones that ossify early and are coded as #1 or #2. The next step consists in applying squared-change parsimony (Maddison 1991) and Felsenstein's (1985) independent contrasts to characterize sequence heterochrony by inferring ancestral conditions. This part of the analysis is conducted with the PDAP module of Mesquite (Maddison and Maddison 2002; Midford et al. 2003). Continuous analysis helps to characterize the ancestral ossification sequence, but also enables detection of heterochronies by comparing a nodal (or tip) value with the nodal value and confidence interval of its hypothetical ancestor. Here, we compared the placental ancestral sequence with the value of the nodes representing the four major placental clades, i.e. Afrotheria, Xenarthra, Laurasiatheria, and Euarchontoglires. As noted by Germain and Laurin (2009), the continuous approach is not strictly statistical, but if the best estimate of a daughter-node falls outside the confidence interval of the ancestral node, it shows that a

significant heterochronic event took place. The ancestral ossification sequence could not be calculated for bones showing the same rank in all taxa (i.e., dentary, clavicle, and carpals). The dataset for the continuous method comprised 24 taxa for the analyses of 16 cranial elements, and 25 taxa for the analyses of 22 postcranial elements.

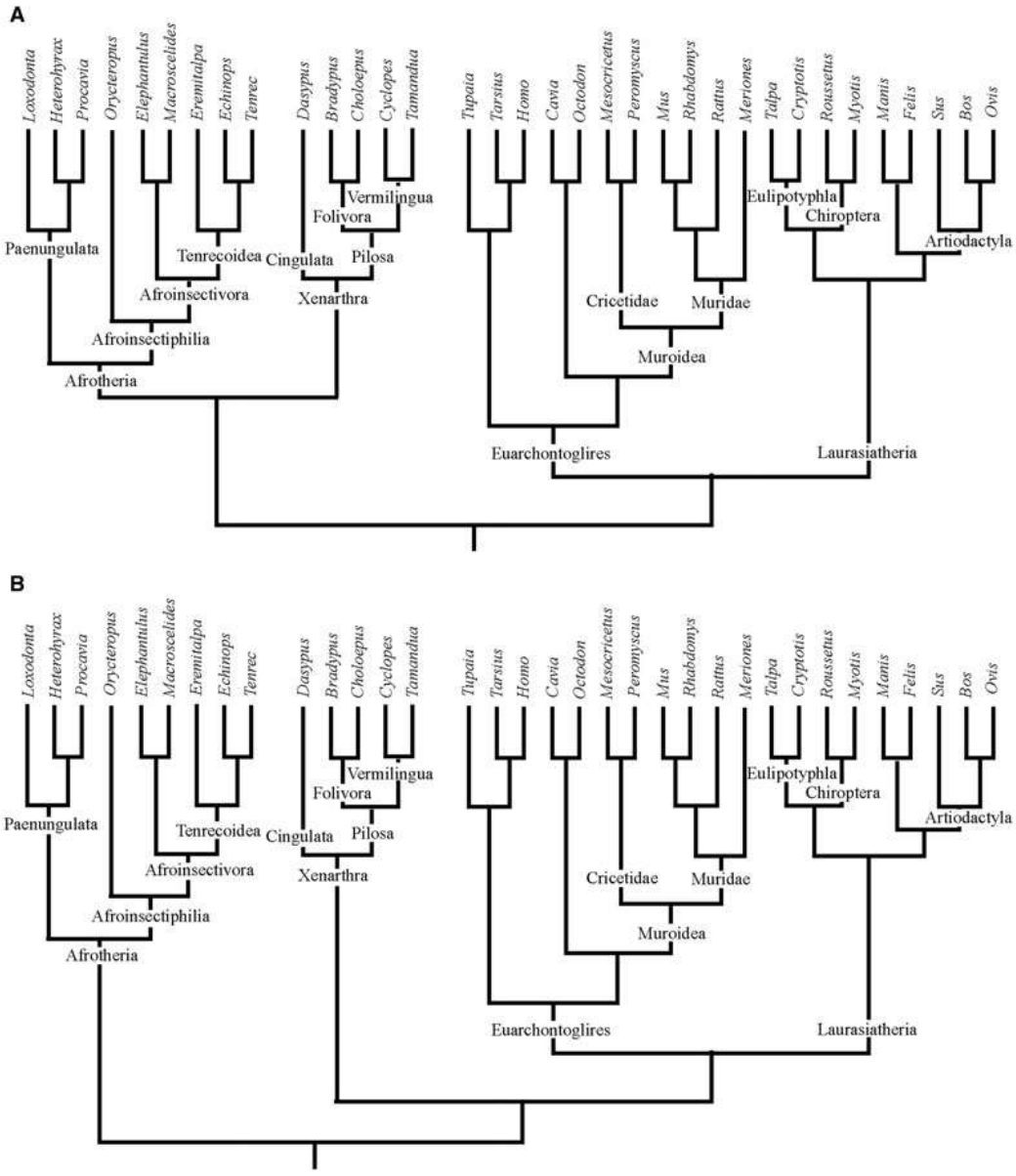

Figure 2. Two alternative hypotheses of phylogenetic relationships among the species studied in this analysis (Phillips et al. 2006, Asher 2007, Möller-Krull et al. 2007, Prasad et al. 2008, Meredith et al. 2011). A) Atlantogenata, i.e., sister-group status between afrotherians and xenarthrans; B) Exafroplacentalia, i.e., Xenarthra at the base of (Euarchontoglires+Laurasiatheria).

Event-pair analyses were performed in the phylogenetic context shown in Fig 2. Following previous studies (e.g. Sànchez-Villagra et al. 2008; Weisbecker et al. 2008), we constructed two separate data matrices for the postcranial and cranial datasets. For all species, an event-pair matrix was produced based on the ossification sequences in which the ossification onset in the 17 cranial elements and 25 postcranial elements was compared with every other event. Two separate data matrices were obtained: one with ½ (17^2 - 17) = 136 events for cranial elements and the other with ½ (25^2 - 25) = 300 event pairs for the postcranial elements. Three character states were used to represent the relative timing of one event relative to another: 0, 1, and 2, corresponding to prior, simultaneous, or subsequent ossification of one element relative to another (respectively). We used Parsimov (Jeffery et al. 2005) in order to document the patterns of change in event pairs. This program employs a parsimony approach to search for the minimal amount of heterochrony required to explain sequence differences between species (Jeffery et al. 2005). We did not to use the PGi heterochrony search algorithm by Harrison and Larsson (2008) as it is currently not programmed to analyse datasets with ties excluded. We ran the analyses using both ACCTRAN and DELTRAN optimizations as recommended by Jeffery et al. (2005). The ACCTRAN option assumes accelerated transformations (favoring reversals); the DELTRAN option provides delayed transformations (favoring convergences; Maddison and Maddison 1992). Only the events that were reported using both approaches were interpreted as heterochronies, although we also examined the extent to which results from one or the other reflected results from sequence-ANOVA. The consensus results of ACCTRAN and DELTRAN event shifts in the onset of ossification of cranial and postcranial elements are presented in Table S8. Due to its focus on minimum heterochrony, Parsimov is highly conservative. As for the

sequence-ANOVA method, the accumulation of ties increases the probability of artefactual "significance" of heterochronic shifts that are not directly observable. In order to take into account this methodological artefact, we ran two Parsimov analyses, one with the original data and a second with all ties converted to missing data (i.e., coded as "?" for unknown timing; see Sànchez-Villagra et al. 2009).

Because of the uncertain phylogenetic position of the placental root (Hallström and Janke 2010), the event-pair analyses and the continuous analyses were performed in two phylogenetic contexts. The afrotherians were considered either as the sister clade of xenarthrans (i.e., Atlantogenata, Meredith et al. 2011), or as the sister clade of all other placental mammals (i.e., Exafroplacentalia, Hallström and Janke 2010; alternate phylogenetic topologies are given in Fig. 2). Branch lengths and divergence times used in the continuous analyses derived from Meredith et al. (2011, all calibrations). Taxa that were absent from this study were added following Steppan (2004 a and b, 2005), Lecompte (2008), and Rowe (2008) for rodents, and Douady et al. (2003, 2004) Douady and Douzery (2003) for other mammals.

The sequence ANOVA and the continuous method require a fairly dense series of developmental stages (Hautier et al. 2011), both methods being subject to type II errors due to the accumulation of ties at early events. In order to avoid an artefactual elevation of the "significance" of early shifts due to low resolution of the earliest developmental events, we avoided species with poorly resolved sequences: *Macroscelides*, *Elephantulus*, *Orycteropus*, *Procavia*, *Cyclopes*, and *Choloepus* for the cranial elements; *Macroscelides*, *Orycteropus*, and *Heterohyrax*, and *Choloepus* for the postcranial elements. Marsupials were only considered in Parsimov analyses; following Weisbecker et al. (2010) we scored the epipubic bone as the last to ossify in placental mammals.

Results

Ossification patterns of the skull in Afrotheria - Due either to rapid ossification (e.g., *Procavia*) and/or small sample size (e.g., *Orycteropus*), our afrotherian sample for cranial events shows many ties. Nevertheless, afrotherian species display a similar cranial ossification sequence to that of other placental mammals (Table S4). Our growth series exhibits a concentration of ossification events within the first few stages with 11 (i.e. premaxilla, maxilla, palatine, dentary, frontal, parietal, squamosal, basioccipital, nasal, pterygoid, exoccipital) out of 17 elements (65%) ossifying first in all afrotherians but *Loxodonta* and *Echinops*. With 6 ranks, the elephant ossification sequence is the most complete (Hautier et al. 2012). Considering the low resolution of our cranial ossification sequences, we describe here only common patterns observed in all afrotherians. Bones of the oral, zygomatic, orbital (with the exception of the lacrimal) and vault regions ossify before those of the basicranium and posterior skull, similar to the pattern observed in most other placental mammals considered here. Following the bones tied at rank #1, ossification occurs in the basiphenoid, alisphenoid, orbitosphenoid and lacrimal. The basicranium is the last region to start its ossification; the periotic is the last bone to ossify (or is tied for last in *Eremitalpa* and *Orycteropus*).

Ossification patterns in the postcranial skeleton of Afrotheria – Previous studies already described the sequences of ossification in the African elephant and tenrecs (Hautier et al. 2012; Werneburg et al. 2012). In our series of other afrotherians, 13 out of 24 elements of the postcranial skeleton ossify first (rank 1, Table S5). A similar concentration of relative simultaneity for the earliest events was also found in other mammals (see Table S5). Specifically, the initial ossifications reported here involve the clavicle, humerus, ribs, femur, radius, ulna, scapula, tibia,

fibula, cervical, thoracic lumbar and sacral vertebrae. Then, the timing of ossification varies slightly depending on the group. In the rock hyrax *Procavia*, both manual and pedal phalanges ossify second. They are followed by sternum, just before the ossification of the pubis. The ossification of the tarsals is next, followed by the carpals (Table S5). In the yellow-spotted hyrax, *Heterohyrax*, the ischium and sternum ossify second, followed by the pubis and tarsals, and then the carpals. In the aardvark, *Orycteropus*, the pubis ossifies second, followed by the sternum and tarsals, and then the carpals (Table S5). In Grant's golden mole, *Eremitalpa*, the ischium ossifies second, followed by the pedal phalanges, and then the metatarsals. The autopod is the last region to start its ossification, the metacarpals being the penultimate bones to ossify, just before the tarsals and the carpals that start their ossification simultaneously (Table S5). The sequence of the short-eared elephant shrew *Macroscelides* appears poorly resolved with the tarsals ossifying at rank #2 and the carpals at rank #3. In contrast, in the North African elephant shrew, *Elephantulus*, the ischium and the metacarpals ossify second. They are followed by the pubis, metatarsals, tarsals, and caudal vertebrae that all start their ossification simultaneously, just before the ossification of the pedal and manual phalanges. Once again, the carpals are the last bone to ossify, which is a widespread pattern among placentals (Weisbecker et al. 2008; Wilson et al. 2010).

Interestingly, although our resolution for some taxa is low, some of these (e.g., *Eremitalpa*) are among our best-sampled species in terms of the number of differently-sized stages. Thus, such lack of resolution may actually comprise positive evidence for relatively fast ossification.

Sequence heterochrony in southern placentals as determined by sequence

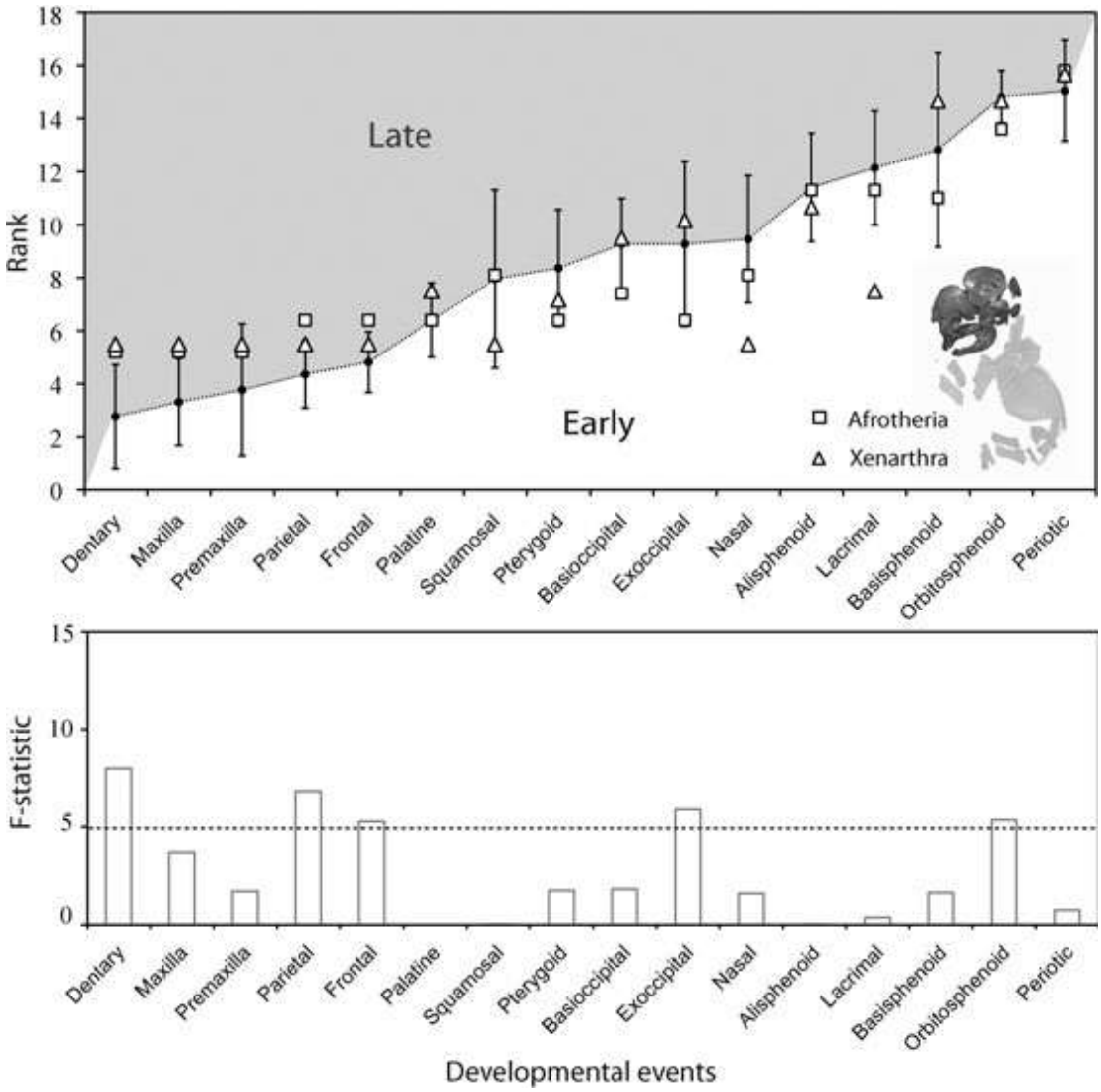

Figure 3. Ossification sequence of cranial elements in afrotherians relative to the mean ranks of northern placentals (Euarchontoglires and Laurasiatheria, solid circles); error bars of ±1 standard deviation. Upper panel: the mean rank of *Loxodonta*, *Echinops*, *Tenrec*, *Eremitalpa*, and *Heterohyrax* are represented by solid squares. The mean rank of xenarthrans (open triangles) was added for comparisons (also see Hautier et al. 2011). Lower panel: results of the ANOVAs between afrotherians

ANOVA - ANOVA can only be used to compare the ossification between two groups. Thus, we focused on statistical differences between afrotherians and northern placentals (i.e., Boreoeutheria; Fig. 3, lower part), but plotted the developmental trajectory of Xenarthra in order to compare their trajectory to that of Afrotheria. Relative to northern placentals, onset of ossification in five of the 16 cranial elements

is significantly distinct in afrotherians (Fig. 3). Specifically, we find a late ossification of the dentary, parietal, and frontal and an early ossification of the exoccipital and orbitosphenoid. Apart from the shift involving the orbitosphenoid, the low resolution of our cranial ossification sequences prevented detection of any unambiguous heterochronic shift for cranial elements between afrotherians and other mammals. Because the late ossification of the dentary, parietal, and frontal and the early ossification of the exoccipital are among several events tied at #1, their apparent shifts are likely a methodological artefact. These ties occurred only in the earliest stages, and will hopefully be resolved with future studies using more complete sequences. Alternatively, if the fast ossification apparent in species such as *Eremitalpa* proves more widespread (e.g., also in macroscelidids), near-simultaneous ossifications detected here may in fact prove to be a genuine feature of some mammals.

Relative to northern placentals, xenarthrans showed three of 16 cranial elements that differ statistically: a late ossification of the dentary and early ossifications of the nasal and lacrimal (Hautier et al. 2011). Because of the accumulation of ties in the xenarthran cranial sequence, the significance of their heterochronic shifts was previously considered to be ambiguous (Hautier et al. 2011). After discounting the "significance" of heterochronies involving events tied at #1 (i.e. dentary, maxilla, premaxilla, frontal, parietal, palatine, squamosal, pterygoid, basioccipital, exoccipital), we found that three bones ---the nasal, lacrimal, and periotic--- out of seven (i.e. 42%) showed similar shifts in both Xenarthra and Afrotheria.

The postcranial sequences are much more resolved than those for the cranium. Specifically, four of 23 postcranial elements differ statistically in afrotherians and boreoeutherians (Fig. 4, lower part): afrotherians exhibit a late ossification of the ulna,

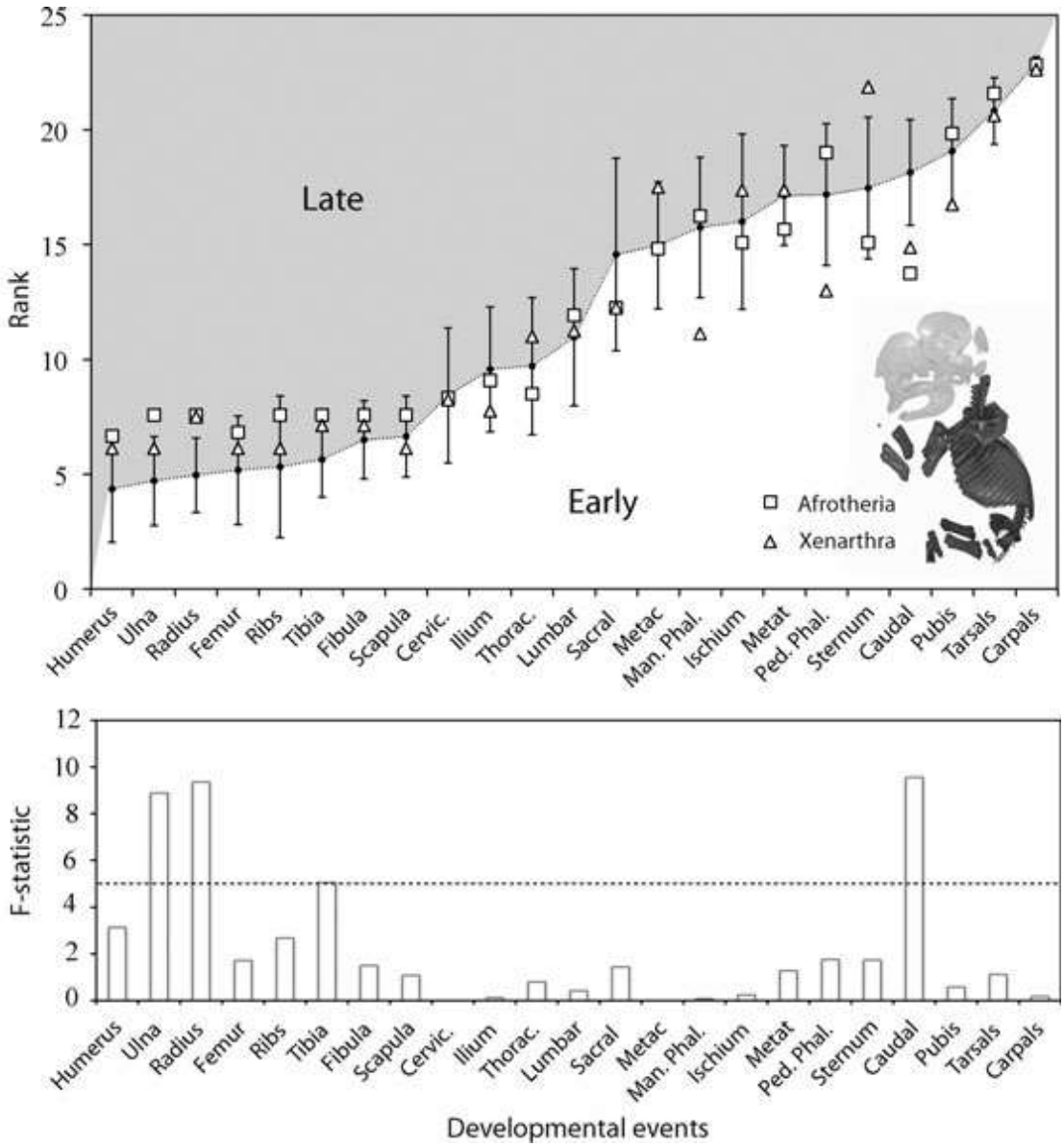

Figure 4. Ossification sequence of postcranial elements in afrotherians relative to the mean ranks of northern placentals (solid circles). Upper panel: mean ranks are represented by six afrotherians (*Loxodonta*, *Echinops*, *Tenrec*, *Eremitalpa*, *Elephantulus*, and *Procavia;* open squares), fourteen northern placentals (solid circles) with error bars of ±1 standard deviation. The mean rank of xenarthrans (open triangles) was added for comparisons (also see Hautier et al. 2011). Results of the ANOVAs between afrotherians (open squares) and placentals (solid circles) with F-statistics are shown in the lower panel, as described in the caption of Fig. 3.

radius and tibia, and an early ossification of the caudal vertebrae (Fig. 4). As shown for the cranial elements, the shifts involving the ulna, radius and tibia are most likely

to be explained by the concentration of ties in early ranks due to an artefact of sampling in the earliest stages. These elements ossify first across all sampled afrotherians, but an increased density of ontogenetic stages would surely break up some or all of these ties. Xenarthrans were shown to differ from other placentals by a late ossification of the sternum and clavicle, and an early ossification of pubis, pedal and manual phalanges; again the shift involving the clavicle is most likely to be explained by an artefact of sampling in the earliest stages (Hautier et al. 2011). By weeding out heterochronies that occur among a series of early tied events (i.e. humerus, ulna, radius, femur, ribs, tibia, fibula, scapula, cervicals), we found that four bones ---the ilium, metatarsals, sacral and caudal vertebrae--- out of fourteen (i.e. 28%) showed similar shifts in both Xenarthra and Afrotheria.

Sequence heterochrony in southern placentals as determined by the continuous method – We observed no significant difference (cranial, $F=8.12*10^{-5}$, P=0.9929; postcranial, F=0.00298, P=0.9863) for the reconstruction of the ancestral ossification sequence of Placentalia by considering alternatively both phylogenetic positions of the placental root (Exafroplacentalia *vs* Atlantogenata). The results presented here (Fig. 5 and 6) are based on the analysis in which Afrotheria is the basal-most placental clade (i.e., Exafroplacentalia Fig. 2), but the alternative hypothesis (Atlantogenata, Fig. 2) is also presented in the supplementary data (Figure S2 and S3).

No genuine heterochronic shifts for cranial elements were retrieved for either afrotherian or xenarthran ancestral nodes using the continuous method (Fig. 5). All the bones clearly fall inside the 95% confidence interval of the distribution of placental ancestral sequences. The node representing the common ancestor of Afrotheria (Fig. 5) is mainly characterized by an early timing of ossification of the

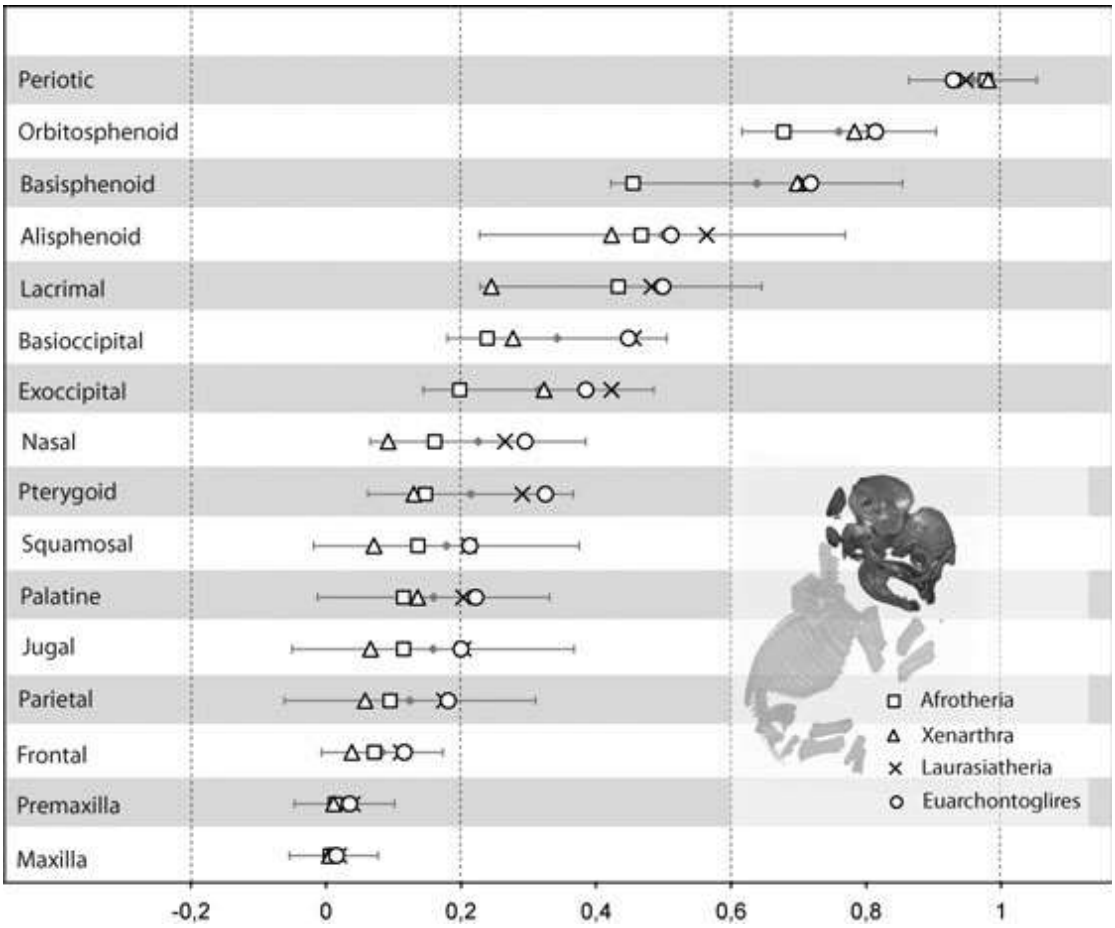

Figure 5. Optimisation of the ancestral cranial sequence of Placentalia obtained by squared change analysis using parsimony by considering the Exafroplacentalia hypothesis and including the results obtained for the ancestral nodes of the four major clades of placental mammals, i.e. Afrotheria, Xenarthra, Laurasiatheria, and Euarchontoglires. Bars correspond to 95% confidence intervals. The width of the confidence interval is directly proportional to evolutionary rates and inversely proportional to the amount of available character data.

exoccipital, basisphenoid and orbitosphenoid, whereas ancestral xenarthrans clear ly depart from other placentals by an early ossification of the lacrimal. Both southern placental groups seem to depart from boreoeutherians by an early shift in the onset of ossification of the parietal, palatine, nasal, pterygoid, jugal, squamosal, and basioccipital. However, it is worth mentioning here again that, with the exception of the jugal, most of these shifts may be due to artefacts of sampling and the

accumulation of ties in the earliest stages. By removing ties in the dataset (i.e. maxilla, premaxilla, frontal, parietal, palatine, squamosal, pterygoid, basioccipital, exoccipital), two bones ---the nasal and jugal--- out of seven (i.e. 28%) showed similar trends in both Xenarthra and Afrotheria.

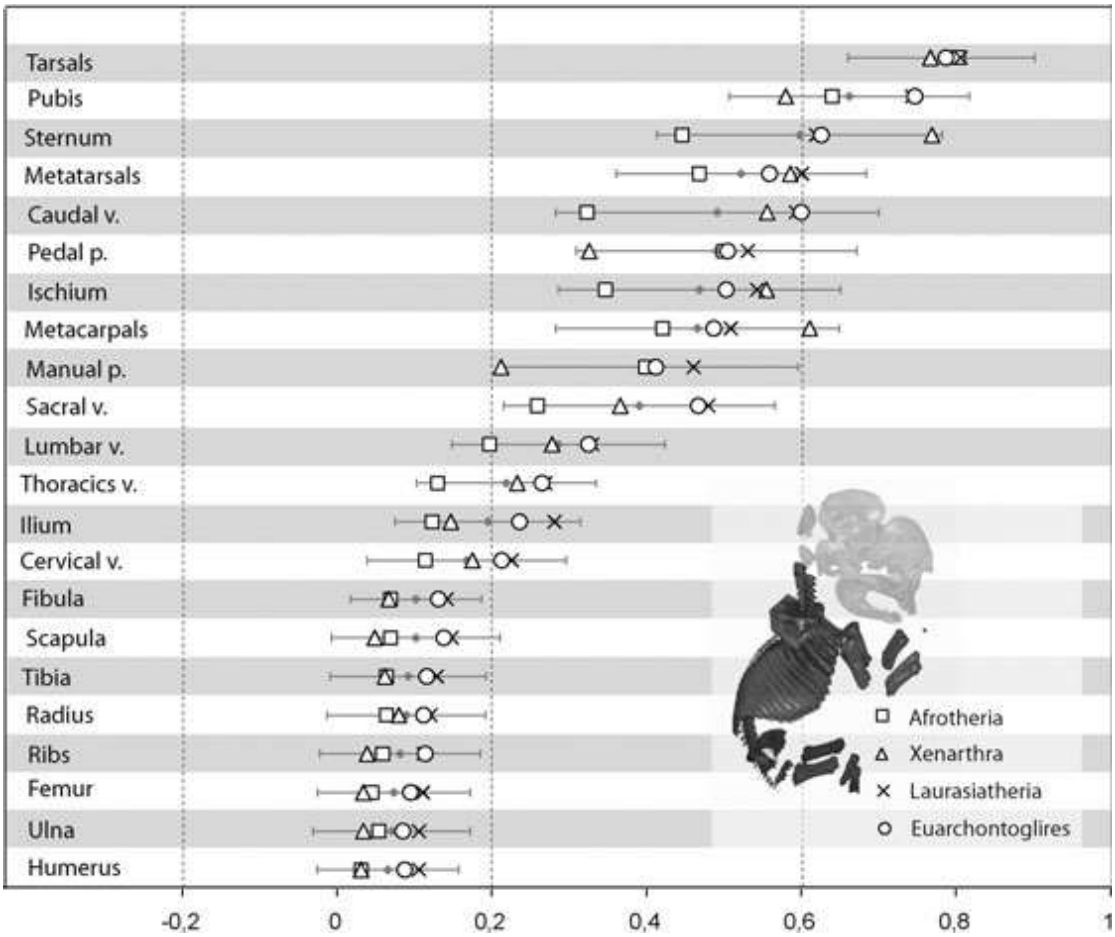

Figure 6. Optimisation of the ancestral postcranial sequence of Placentalia obtained by squared change analysis using parsimony by considering the Exafroplacentalia hypothesis and including the results obtained for the ancestral nodes of the four major clades of placental mammals, as described in the caption to Fig. 5.

Most of the differences between the postcranial sequence of southern placentals and the ancestral placental sequence obtained by squared-change parsimony are not statistically significant (Fig. 6). Reflecting the results of sequence-ANOVA, Afrotheria and Xenarthra show similar trends for the bones that ossify first

in the sequence (i.e. humerus, ulna, femur, ribs, radius, tibia, scapula, fibula, ilium), but because these early ossifications involved several events tied at #1, their apparent shift is likely a methodological artefact. By discounting the "significance" of heterochronies resulting from the accumulation of events tied at the beginning of the ontogenetic series, the early ossification of the sternum, ischium, thoracic, lumbar sacral and caudal vertebrae distinguish afrotherian ancestral node from other mammals (Fig. 6). Xenarthrans differ statistically from the ancestral sequence of placentals by a late ossification of the sternum and metacarpals, and an early ossification of the pedal and manual phalanges (Fig. 6); these four bones fall just inside the 95% confidence interval of the placental ancestral sequence. The timing of the onset of ossification in the pubis of Xenarthra is not significant, but suggestive, and clearly distinct from that of other placentals, reflecting the results of the sequence ANOVA. By weeding out heterochronies that occur among a series of early tied events (i.e. humerus, ulna, radius, femur, ribs, tibia, fibula, scapula, cervicals), we found that four bones ---the ilium and sacral vertebrae--- out of fourteen (i.e. 14%) showed similar shifts in both Xenarthra and Afrotheria.

Sequence heterochrony in southern placentals as determined by Parsimov - A simple mapping of the state of the characters of the event-pairing analysis confirmed the previous observations, and reveals that few of them are phylogenetically informative as they involved shifts toward or away from simultaneity (i.e., accumulated ties). By using this visual method, we found that Afrotheria is characterized by a late ossification of the pedal phalanges compared to the caudal vertebra; the Afroinsectivora (Macroscelididae + Chrysolochloridae + Tenrecidae) is characterized by an early ossification of the sternum compared to the ischium and a late ossification of the pedal phalanges compared to the sternum; and the Tenrecoidea

(Chrysolochloridae + Tenrecidae) is characterized by a late ossification of the lacrimal compared to the palatine, frontal parietal, basioccipital, pterygoid, and exoccipital.

With ties included (i.e., not treated as missing data), Parsimov analysis does not identify any heterochronic shift for cranial or postcranial elements for the nodes representing the Afrotheria, Laurasiatheria, and Euarchontoglires (Table S8), regardless of the position of the placental root (i.e., Atlantogenata *vs* Exafroplacentalia). Only few heterochronic shifts were detected for Xenarthra, which appear different depending on the position of the placental root (Table 6). However, most of these shifts occur at a single developmental stage and should be considered as a methodological artefact that becomes evident by running the analyses with ties coded as missing data (Table S8). When events coded as ties are included, the Atlantogenata clade is characterized by an early heterochronic shift of the scapula with respect to radius and humerus, and an early ossification of the ribs in relation to fibula, tibia, ulna, radius and humerus. None of these shifts were retrieved with ties coded as missing data (Table S8).

Discussion

Comparison of the methods - While running Parsimov with missing data for ties successfully removes likely artefacts, it fails to retrieve any convincing heterochronic shift. As noted in previous work (Harrison and Larsson 2008; Sánchez-Villagra et al. 2008; Weisbecker et al. 2008; Werneburg and Sánchez-Villagra 2009; Wilson et al. 2010; Hautier et al. 2011, 2012), Parsimov analysis is overly conservative and suffers from a preponderance of type I errors (rejecting a valid null hypothesis). Compared to Parsimov, the continuous (Germain and Laurin 2009) and

sequence-ANOVA (Nunn and Smith 1998; Smith 2001; Keyte and Smith 2010) methods suffer less from this deficiency, and are more consistent with one another. The sequence-ANOVA and the continuous methods are typically used to compare taxa that show similar number of stages. Given the variable resolution across species in our dataset, we acknowledge that the results obtained using these methods should be treated carefully, especially regarding the cranial elements. While both methods are subject to type II errors (accepting an invalid null hypothesis) due to the accumulation of ties at early events, it is possible to control for such artefacts by weeding out such early ties. Moreover, they can be controlled simply by observing heterochronies occurring at higher ranks.

Each method has its own strengths, amenable to scrutinizing different but complementary questions: the continuous method compared the placental ancestral sequence with the value of the nodes representing Afrotheria and Xenarthra, whereas the sequence-ANOVA directly compared the ossification sequence of afrotherians and xenarthrans with the developmental trajectory of other placental mammals (i.e. Boreoeutherians). With these qualifications in mind, we showed that all bones that showed significant shifts for afrotherians with sequence-ANOVA (i.e. early ossification of the orbitosphenoid and caudal vertebrae) also showed similar shifts using the continuous method, even if they fall inside the 95% confidence interval of the placental ancestral sequence. Otherwise, a great majority of cranial and postcranial elements display similar direction of heterochronic shifts using one method or the other. Moreover, the results of the continuous method confirmed previous results (Hautier et al. 2011) showing that xenarthrans significantly differ from other placentals by a late ossification of the sternum and an early ossification of the phalanges and pubis. Thus, combining methods represents a reasonable strategy to

detect consistent ossification heterochronies in southern placentals relative to other mammals.

Southern vs northern placentals – Until now, the examination of ossification in the developmental series of a tenrec (Werneburg et al. 2012) and elephant (Hautier et al. 2012) revealed neither major deviations from the patterns observed among boreoeutherians nor any similarity with heterochronic shifts observed in xenarthrans. Comparing the development of soft tissues (which we have not examined in this study), Werneberg et al. (2012) found that the armadillo shows fewer heterochronic differences relative to the tenrecids than to the mouse. They concluded that this relative absence of heterochronic shifts between the two southern placentals could indicate either support for Atlantogenata or a preponderance of symplesiomorphies. Although afrotherians have much in common with the developmental trajectory of other placentals, the sequence-ANOVA showed that they differ from northern placentals by an early ossification of the orbitosphenoid and caudal vertebrae (Figs. 3 and 4). The most conspicuous difference of afrotherians relative to other placental groups concerns the advanced ossification of the caudal vertebrae. Given that the vertebral elements of the skeleton always ossify in the first three ranks in afrotherians, we cannot exclude that the significance of this early ossification is due to an artefact of sampling in early stages; but it likely represents a characteristic heterochronic shift of afrotherians, the adaptive significance of which remains unclear regarding the morphological diversity of the group.

Afrotherians resemble xenarthrans in terms of the timing of ossification of the periotic, sacral and caudal vertebrae (Figs. 3 and 4). Although they are characterized by an early shift in the onset of ossification of the sacral and caudal vertebrae, not a single significant heterochronic shift involved the vertebral column at the node

joining xenarthrans to other mammals (Hautier et al. 2011). In contrast, xenarthrans significantly differ from other placentals by a late ossification of the sternum and an early ossification of the phalanges and pubis (Hautier et al. 2011), while afrotherians display an opposite direction of shift for these bones, showing late ossification of the phalanges and pubis and an early ossification of the sternum. These heterochronic shifts therefore appear to clearly distinguish xenarthrans from afrotherians.

In concordance with the results of the sequence-ANOVA, the continuous method showed that afrotherians generally match the ancestral placental sequence, with all bones falling inside the 95% confidence interval (Fig. 5 and 6). However, it is also worth noting that none of the four major placental groups seemed to have diverged much from the placental ancestral sequence. On the one hand, an early ossification of the exoccipital, basisphenoid, orbitosphenoid, sternum, ischium, thoracic, lumbar, sacral and caudal vertebrae distinguish the afrotherian ancestral node from those of other mammals; on the other hand, xenarthrans are characterized by a late ossification of the sternum and metacarpals and an early ossification of the phalanges and pubis. As such, no genuine similarities were retrieved between afrotherian and xenarthran ancestral nodes using the continuous method.

Interestingly, both northern placental ancestral nodes show very similar trends (Fig. 5 and 6). Using sequence-ANOVA on postcranial sequences of all mammals but afrotherians and xenarthrans (Fig. 7), we detected only one significant difference between the two northern groups: the late ossification of the ilium in laurasiatheres relative to euarchontoglires. Previous comparative analyses of ossification sequence (Goswami 2007; Weisbecker et al. 2008, Sánchez-Villagra et al. 2008; Goswami et al. 2009; Wilson et al. 2010; Weisbecker 2011) have not identified significant heterochronic shifts in ossification timing, consistent with a surprisingly high level of

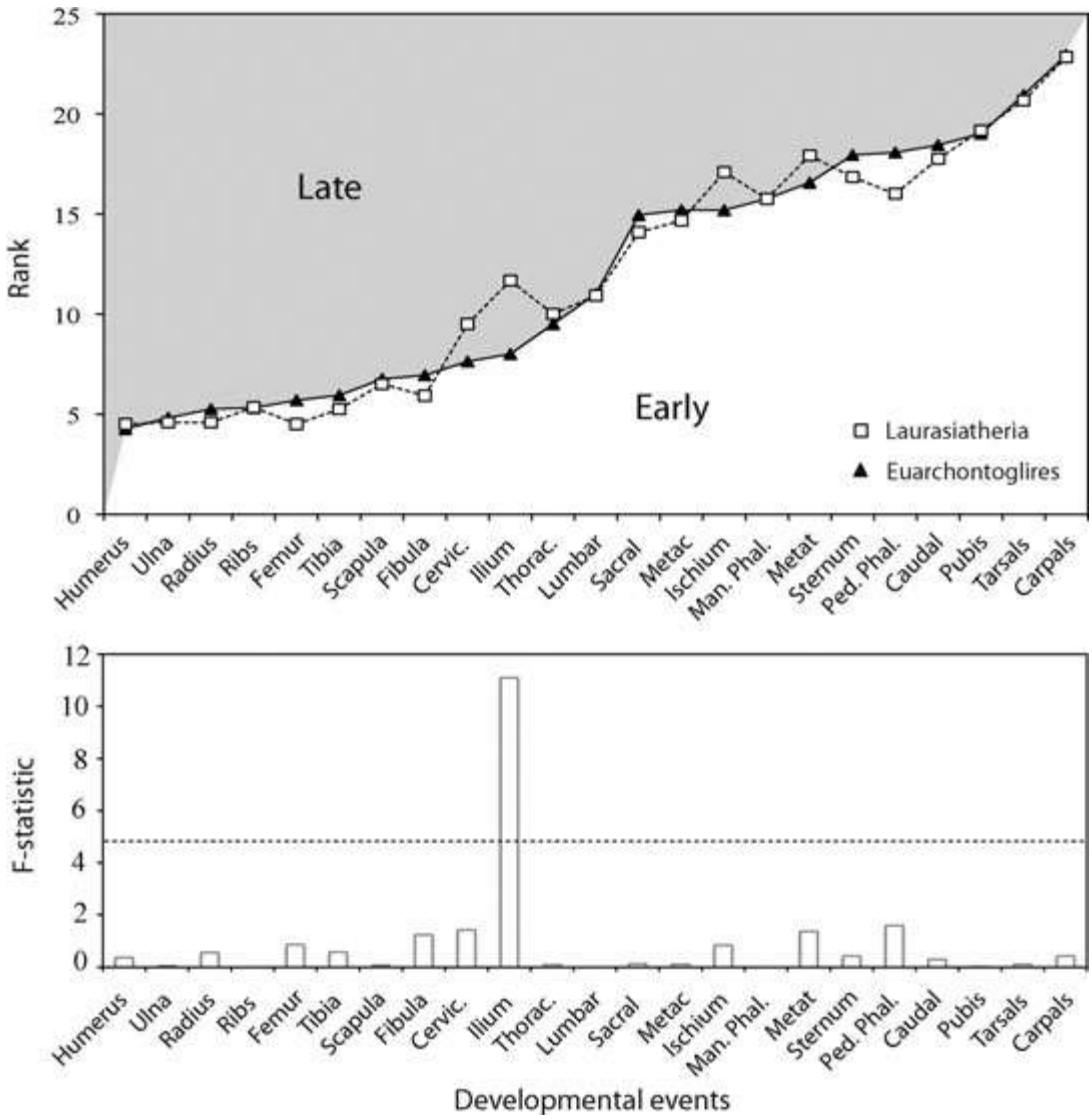

Figure 7. Ossification sequence of postcranial elements in Laurasiatheria (open squares) relative to the mean ranks of Euarchontoglires (solid triangles). Upper panel: mean ranks are represented eight Euarchontoglires (solid triangles), and six Laurasiatheria (open squares). Results of the ANOVAs with F-statistics are shown in the lower panel. The dotted line represents $P<0.05$. Heterochronic shifts are statistically significant at $P<0.05$ when they exceed the dotted line.

conservatism among these northern placental clades (but see Koyabu et al. 2011). While sequence resolution was greater for many of the taxa included in those previous studies (see Tables S4 and S5), no afrotherian or xenarthran placentals were included. The results of the analysis presented here suggest that this developmental homogeneity may be restricted to the Boreoeutheria, even if we cannot yet completely

discard the influence of potential sampling artefacts. Both southern placental groups showed a greater degree of developmental variability, in contrast to the pattern observed in boreoeutherians; however, they rarely seem to vary in the same direction, especially regarding the shifts that differ statistically (Figs 3-6). This apparent developmental homogeneity of boreoeutherians deserves further scrutiny as a possible shared, derived feature distinguishing them from afrotherians and xenarthrans.

Analyses of modularity in developmental timing have identified differences between placentals and marsupials based overwhelmingly on data from northern placentals (Goswami 2007; Goswami et al. 2009; Bennett and Goswami 2011). Those studies suggested that, while little evidence exists for modular shifts in timing of cranial ossification, postcranial ossification in placentals is characterized by alignment of anterior and posterior axial and/or appendicular elements. In contrast, marsupials show significant dissociation of anterior and posterior postcranial elements. The results provided here suggest that there may be significant anterior-posterior modularity in the postcranial skeleton of southern placentals as well. As detailed above and shown in Fig. 6, the majority, although not all, of shifts characterising the southern clades are concentrated in the posterior postcranial skeleton. Similarly, although more limited, the majority of cranial shifts appear to involve the basicranial region, rather than being distributed across the skull. An explicit quantitative analysis is required to test these patterns, but the data presented here suggest that shifts in developmental modularity may not only distinguish marsupials from placentals, but also northern from southern placentals.

Foetal age vs ossification - By combining our data with reliable estimates of gestational age (Hildebrandt et al. 2007), we have recently been able to define major ossification events in light of the absolute timing of development in elephants

(Hautier et al. 2012). We showed that elephants, in which pregnancy lasts about two years, display a number of features of their ossification patterns that differ from those of other placental mammals, especially regarding the absolute timing of ossification. As a proportion of overall pregnancy, ossification in elephants starts very early and progresses rapidly. Specifically, the elephant exhibits the same percentage of bones showing an ossification centre at the end of the first third of its gestation period as the mouse and hamster have close to birth. In this regard, they resemble humans and cows. This shows that the formation of the skeleton is not delayed in animals with a long gestation time. This correlation remained tentative considering the limited taxon sample at our disposal (Hautier et al. 2012), and new comparisons are still needed in order to estimate which life-history traits are linked to the timing of ossification in placental mammals. Tenrecs are not characterized by a short gestation (Eisenberg and Gould 1970), but Werneberg et al. (2012) found that they show a rodent-like, late onset of ossification.

Collections of non-model organisms such as afrotherians often include specimens collected decades ago and the specimens studied here generally lack data on individual age. However, it is still worth noticing that, in groups characterized by a long gestation period such as hyraxes, elephants, and sea cows, most of the cranial and postcranial elements have already started to ossify in the youngest specimens, even in the ones that appear very small compared to adults or stillborns. In contrast, the prenatal growth of small species such as sengis or golden moles appears "explosive", despite our wide range of sampled foetal sizes. That is, most bones ossify in a brief time towards the end of gestation, mirroring the pattern of ossification observed in rodents (Hautier al. 2012). As mentioned previously, to the extent that they do represent the real phenomenon of fast ossification and are not artefacts of

poor sampling, these observations would help explain the low sequence resolution and the accumulation of ties in the earliest stages of development of groups characterized by a relatively short gestation time.

Conclusion

Our results show that heterochrony has occurred in the early skeletal development of afrotherians as well as southern placentals as a whole. The results are significant in spite of the uncertain phylogenetic position of the placental root (Hallström et al. 2007; Wildman et al. 2007, Hallström and Janke 2010). Some species within both southern groups ossify their skeleton very quickly, making it difficult to obtain a highly resolved sequence of events. Nevertheless, afrotherians and xenarthrans differ from other groups in terms of their ossification sequences (e.g., in the sternum and caudal vertebrae). Moreover, data from these groups highlights a previously overlooked heterochrony among northern placentals: the late ossification of the ilium among laurasiatheres. Finally, if afrotherians and xenarthrans comprise the first branches diverging from the placental mammal Tree of Life, we showed that they do not vary in the same direction, inconsistent with the Atlantogenata hypothesis in which afrotherians are the sister clade of xenarthrans. This observation need not detract from the Atlantogenata hypothesis, for example when interpreted as evidence for increased constraint in northern placentals, as opposed to phenotypic homogeneity in southern placentals. Further analyses of sequence heterochrony incorporating new material, including data from new breeding programmes of unconventional model species (e.g. Milinkovitch and Tzika 2007; Tzika & Milinkovitch 2008, Werneburg et al. 2012), are needed to further understand the extent to which northern and southern placental mammals show distinctive patterns of ontogeny, and whether or not

northern placental mammals exhibit an elevated degree of developmental conservatism compared to southern placentals.

Acknowledgments

We are grateful to Peter Giere and Frieder Mayer (Museum für Naturkunde, Berlin), M. Herbin, C. Bens, F. Renoult, C. Denys and J. Cuisin (Museum National d'Histoire Naturelle, Paris), Paula Jenkins, Louise Tomsett, and Roberto Portela Miguez (Natural History Museum, London), Georges Lenglet (Institut Royal des Sciences Naturelles de Belgiques, Brussels) and their colleagues for access to comparative material. For access to material and facilities, we thank Richard Truman (LSU School of Veterinary Medicine, Baton Rouge, Louisiana), the Laboratory of Paleontology and the Institut des Sciences de l'Evolution de Montpellier. A. Heaver (University of Cambridge), Nikolai Kardjilov and all the staff of the Helmholtz Zentrum (Berlin), R. Abel and R. Garwood (Natural History Museum London), R. Lebrun (Institut des Sciences de l'Evolution de Montpellier), Ross MacPhee, Nicole Edmison and James Thostenson (American Museum of Natural History, New York), F. Landru, C. Morlier, G. Guillemain and all the staff from Viscom SARL (St Ouen l'Aumône, France) provided generous help and advice with acquisition of CT scans. We thank Eudes Thouand for providing living accommodation in Paris. We thank Damien Germain for interesting discussion on the continuous method. We are grateful to Pierre-Henri Fabre for his help with phylogenetics. Access to the Helmholtz Zentrum Berlin was facilitated by the European Commission under the 7th Framework Programme through the "Research Infrastructures" action of the "Capacities" Programme, Contract No CP-CSA_INFRA-2008-1.1.1, number ZZ6507-NMI3. For support of the project as a whole, we acknowledge a research grant from the

Leverhulme Trust, UK. Michel C. Milinkovitch and Athanasia C. Tzika acknowledge support from the University of Geneva and the Swiss National Science Foundation (FNSNF, grant 31003A_125060). Lionel Hautier also acknowledges support from Sidney Sussex College.

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Supplementary information

S1. List of studied specimens.

Abbreviations: ZMB, Museum für Naturkunde Berlin; BMNH, Natural History Museum London; MNHN, Muséum National d'Histoire Naturelle in Paris; IRSNB, Institut Royal des Sciences Naturelles de Belgiques in Brussels; PMLR, Paul Mellon Laboratory of Equine Reproduction in Newmarket UK; USNM, Smithsonian Institution in Washington; NRM, Riksmuseet Stockholm; PIMUZ, Paläontologisches Institut und Museum Zürich; LANE Laboratory of Artificial and Natural Evolution in Geneva; UP, Department of Zoology and Entomology at the University of Pretoria; NRM, Swedish Museum of Natural History; UMZC, University Museum of Zoology Cambridge.

- *Loxodonta africana*: PMLER 1, PMLER 2, PMLER 3, PMLER 4, PMLER 5, PMLER 6, PMLER 7, PMLER 8, MNHN 1918-52, BMNH 1983-553, IRSNB 31804, UMZC2011.10.1, UMZC2011.10.2, UMZC2011.10.3, UMZC2011.10.4, UP 1, UP 2.

- *Elephas maximus*: BMNH 83554.

- *Macroscelides proboscideus*: UMZC 2011.1.1, UMZC 2011.1.2, UMZC 2011.1.3, UMZC 2011.1.4, UMZC 2011.1.5, UMZC 2011.1.6, UMZC 2011.1.7, UMZC 2011.1.8, UMZC 2011.1.9, UMZC 2011.1.10, UMZC 2011.1.11, UMZC 2011.1.12.

- *Elephantulus rozeti*: ZMB 26, ZMB 27, ZMB 28, ZMB 29, ZMB 30, ZMB 31, ZMB 32, ZMB 34b, ZMB 35, ZMB 39.

- *Potamogale velop*: ZMB 10.3.33.

- *Tenrec ecaudatus*: AMNH 1, AMNH 2, AMNH 3, AMNH 4, AMNH 8, AMNH9, UMZC 6, ZMB 44579, MNHN 1890-2750, PIMUZ Tenrec HSY-TEC-11, ZMB 2b, ZMB 3A, ZMB 4A, ZMB 1880a, ZMB 1880b, ZMB 1880c, ZMB 1880d, ZMB 1880e, ZMB 1880f.

- *Echinops telfairi*: PIMUZ ET 2095K, PIMUZ ET 2095M, PIMUZ ET 2103, LANE MCM 15a, LANE MCM 17a.

- *Chrysochloris stuhlmanni*: BMNH 74-672, BMNH 74-671, BMNH 74-667.

- *Amblysomus hottentatus*: ZMB 8.

- *Eremitalpa granti*: NRM 538503, NRM 538500, NRM 538502, NRM 538501B, NRM 538500F, NRM 538500E, NRM 538500D, NRM 538500C, NRM 538501A, NRM 538500B, NRM 538500A.

- *Procavia capensis*: ZMB A4403a, ZMB A4403b, ZMB Un27a, ZMB Un27b, ZMB Un30a, ZMB Un30b, ZMB 12, ZMB 5, ZMB 9A, ZMB 10A, MNHN 1936-180, MNHN 1901-322, MNHN 1901-685a, MNHN 1901-685c, MNHN 1901-685d, MNHN 1901-685e, MNHN 1901-685f, MNHN 1886-320, MNHN 2, UP Dassie 1, UP Dassie 2a, UP Dassie 2b.

- *Heterohyrax brucei*: USNM 184771, UNSM 184769, USNM 161902, USNM 161902b, USNM 163935, USNM 576172, USNM 576173, USNM 181606, USNM 181604, USNM 181601.

- *Dugong australis*: IRSNB5386.

- *Trichechus manatus*: BMNH 1865-4-28-9.

- *Orycteropus afer*: UP Aardvark1, UP Aardvark2, BMNH 84-9-5-15, ZMB no number.

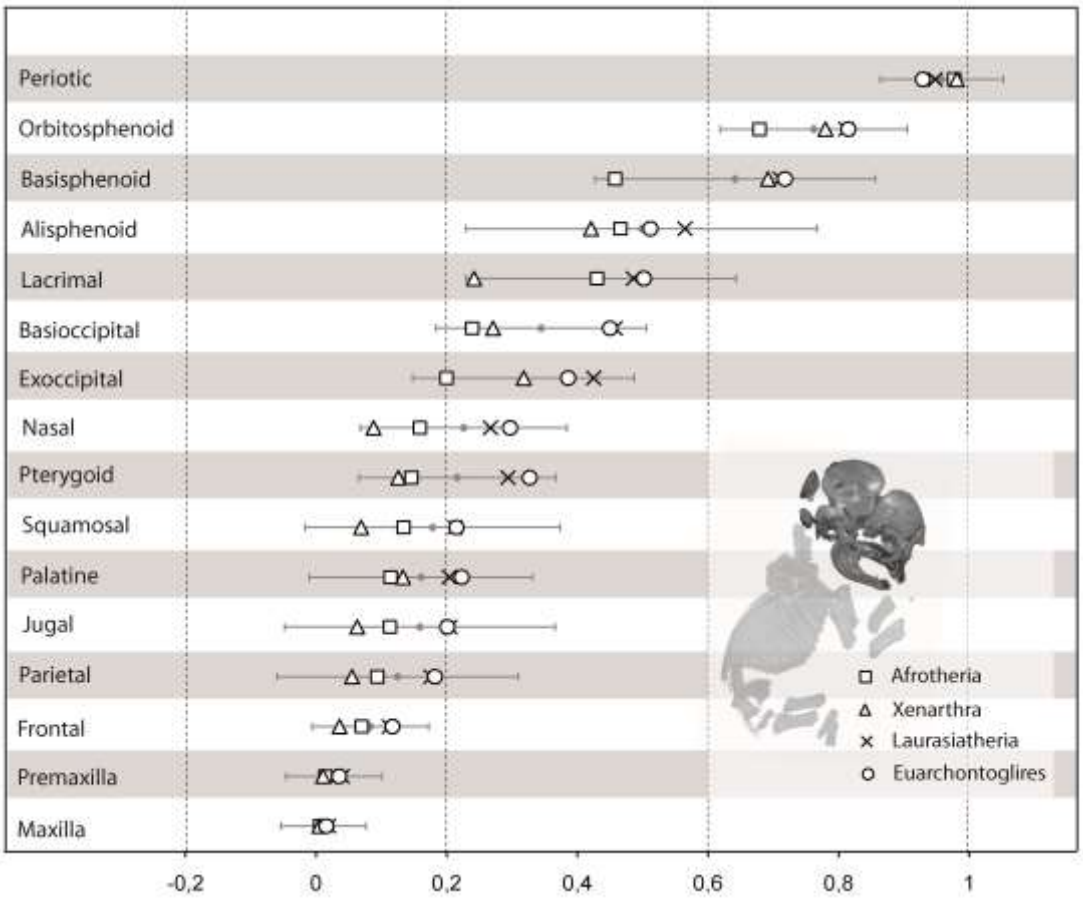

Figure S2. Optimisation of the ancestral cranial sequence of Placentalia for obtained by squared change analysis using parsimony by considering the Atlantogenata hypothesis and including the results obtained for the ancestral nodes of the four major clades of placental mammals, i.e. Afrotheria, Xenarthra, Laurasiatheria, and Euarchontoglires. Bars correspond to 95% confidence intervals. The width of the confidence interval is directly proportional to evolutionary rates and inversely proportional to the amount of available character data.

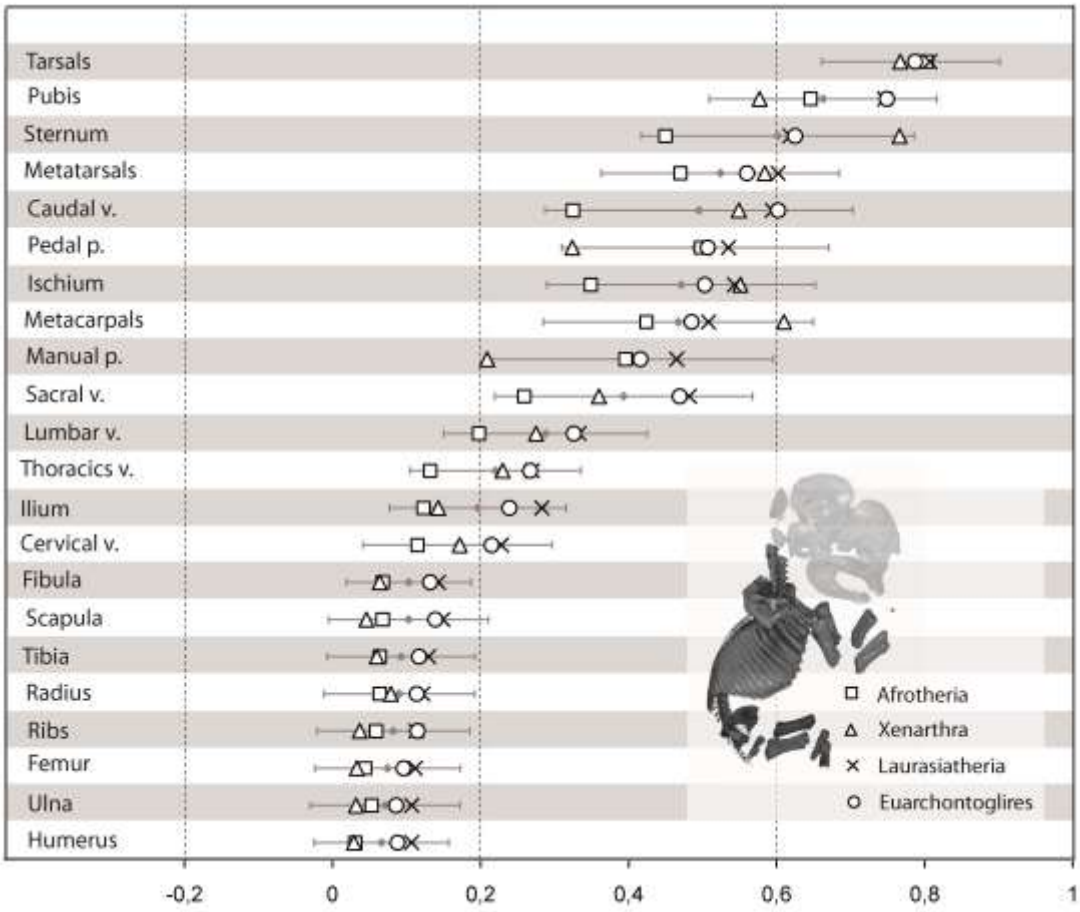

Figure S3. Optimisation of the ancestral postcranial sequence of Placentalia obtained by squared change analysis using parsimony by considering the Atlantogenata hypothesis and including the results obtained for the ancestral nodes of the four major clades of placental mammals, as described in the caption to Fig. 5.

Table S4. Relative timing of onset of ossification (ranks) in the cranial elements for all species examined and compiled from the literature.

	Bradypus	*Choloepus*	*Dasypus*	*Cyclopes*	*Tamandua*	*Tupaia*	*Tarsius*	*Homo*	*Rattus*	*Mus*	*Peromyscus*	*Meriones*	*Mesocricetus*	*Rhabdomys*	*Cavia*	*Talpa*	*Mogera*	*Cryptotis*	*Erinaceus*	*Rousettus*	*Felis*	*Sus*	*Manis*	***Loxodonta***	***Tenrec***	***Echinops***	***Procavia***	***Heterohyrax***	***Macroscelides***	***Elephantulus***	***Eremitalpa***	***Orycteropus***	*Monodelphis*	*Caluromys*	*Didelphis*	*Perameles*	*Dasyurus*	*Macropus*	*Trichosurus*	*Alligator*	*Lacerta*	*Caiman*
Premaxilla	1	1	1	1	1	1	1	2	2	1	1	1	2	2	1	1	1	1	1	3	1	1	1	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	1	1	1	1	1	1	1	1	2
Maxilla	1	1	1	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	1	2	1	1	1	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	1	1	1	1	1	1	1	1	2
Palatine	2	1	1	1	1	4	2	4	2	1	2	3	2	1	1	1	2	2	3	3	?	?	?	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	1	3	3	2	3	3		3	3	1	2
Dentary	1	1	1	1	1	1	?	1	1	1	1	1	1	1	1	1	1	1	1	1	?	?	?	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	1	1	2	?	?	?	?	1	1	1
Frontal	1	1	1	1	1	2	2	3	1	1	2	1	2	1	1	1	2	2	2	2	2	2	1	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	2	2	3	3	2	2	2	2	2	2
Parietal	1	1	1	1	1	2	3	3	2	1	2	4	2	1	1	1	1	3	1	2	3	3	2	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	4	2	3	5	4	3	4	5	2	3
Squamosal	1	1	1	1	1	3	2	3	2	3	4	4	2	6	1	2	2	5	3	1	4	1	2	**2**	**1**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	3	3	6	4	4	2	2	2	2	1
Basioccipital	2	1	3	1	1	4	6	6	2	1	2	2	3	3	2	2	3	6	3	4	5	5	3	**3**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	4	4	3	6	5	4	4	6	4	3
Nasal	1	1	1	1	1	4	?	4	3	3	4	3	3	5	2	2	2	4	3	3	?	?	?	**2**	**1**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	4	3	5	4	5	?	3	4	2	1
Pterygoid	1	1	2	?	1	5	?	4	2	1	2	2	?	1	3	2	2	3	3	4	?	?	?	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	7	5	3	5	5	?	3	1	1	1
Exoccipital	2	1	5	1	1	3	4	3	2	1	3	3	2	4	3	3	4	6	3	4	5	3	3	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	2	3	3	3	3	2	3	3	3	3
Basisphenoid	4	1	7	?	2	6	6	8	4	3	3	4	2	7	4	4	6	7	3	5	6	6	4	**5**	**2**	**2**	**1**	**1**	**1**	**1**	**1**	**2**	6	4	3	8	6	5	4	3	4	3
Jugal	1	1	1	?	1	2	2	3	3	2	4	4	5	8	1	?	?	?	?	2	3	4	1	**2**	**?**	**?**	**1**	**1**	**1**	**1**	**1**	**1**	3	2	3	2	3	2	2	1	2	1
Lacrimal	2	1	1	1	1	4	3	7	3	4	4	4	6	9	3	4	4	6	5	3	?	?	?	**2**	**2**	**2**	**1**	**1**	**1**	**1**	**3**	**1**	3	4	4	6	5	?	3	2	5	?
Alisphenoid	3	1	4	2	1	4	5	5	5	1	4	4	4	10	1	6	7	8	3	5	5	3	3	**2**	**2**	**2**	**1**	**1**	**1**	**1**	**3**	**1**	5	3	?	5	4	3	3	?	3	?
Orbitosphenoid	4	1	6	2	3	5	?	7	6	5	5	5	7	11	5	5	5	9	4	7	?	?	?	**4**	**2**	**2**	**1**	**2**	**1**	**1**	**2**	**1**	7	5	?	7	6	?	4	7	6	4
Periotic	4	1	8	2	4	7	7	4	?	6	5	?	?	12	5	6	7	10	6	6	7	7	4	**6**	**3**	**3**	**2**	**3**	**1**	**1**	**3**	**2**	8	6	?	9	7	6	4	6	4	4

Table S5. Relative timing of onset of ossification (ranks) in the postcranial elements for all species examined and compiled from the literature.

	Bradypus	*Choloepus*	*Dasypus*	*Cyclopes*	*Tamandua*	*Homo*	*Rattus*	*Mus*	*Peromyscus*	*Meriones*	*Mesocricetus*	*Rhabdomys*	*Cavia*	*Octodon*	*Talpa*	*Cryptotis*	*Myotis*	*Rousettus*	*Sus*	*Bos*	***Loxodonta***	***Tenrec***	***Echinops***	***Procavia***	***Heterohyrax***	***Macroscelides***	***Elephantulus***	***Eremitalpa***	***Orycteropus***	*Monodelphis*	*Didelphis*	*Dasyurus*	*Macropus*	*Trichosurus*	*Wombatus*	*Antechinus*	*Isoodon*	*Petaurus*	*Cercartetus*	*Sminthopsis*	*Alligator*	*Lacerta*
Clavicle	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	?	1	**?**	**1**	**1**	**?**	**?**	**1**	**1**	**?**	**1**	1	1	1	1	2	1	1	1	1	1	1	?	1
Humerus	1	1	1	1	1	2	2	2	2	2	2	1	2	1	3	3	2	1	1	2	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	2	2	1	1	2	1	1	1	1	1	1	1	1
Ribs	1	1	1	1	1	5	2	2	2	?	2	1	3	1	1	2	3	1	3	3/4	**3**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	2	2	1	1	2	1	1	1	1	1	2	4	3
Femur	1	1	1	1	1	2	3	2	2	2	3	1	2	1	3	3	2	1	1	2	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	5	5	2	2	3	2	2	3	1	2	3	1	1
Radius	1	1	3	1	1	3	2	2	2	2	3	1	2	1	2	3	2	1	1	3	**3**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	2	2	1	1	2	1	1	1	1	1	1	1	1
Ulna	1	1	1	1	1	1	2	2	2	2	3	1	2	1	2	3	2	1	1	3	**3**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	2	2	1	1	2	1	1	1	1	1	1	1	1
Scapula	1	1	1	1	1	5	3	2	2	2	3	1	3	1	2	3	3	1	2	3	**3**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	2	2	1	1	2	1	1	1	1	1	1	4	3
Cervic.	1	1	6	1	1	7	3	2	2	2	3	1	4	1	2	3	3	3	4	4	**4**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	2	2	1	1	2	1	1	1	1	1	2	3	3
Thorac.	1	1	7	1	2	7	3	2	2	3	3	2	5	2	2	3	3	5	4	4	**3**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	3	2	1	1	2	1	1	1	1	1	2	4	5
Tibia	1	1	2	1	1	3	3	2	2	2	3	1	2	1	2	3	2	2	1	3	**3**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	5	5	2	2	3	2	2	2	1	2	3	1	1
Fibula	1	1	2	1	1	5	3	2	2	2	3	2	2	1	2	3	2	2	2	3	**3**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	5	5	2	2	3	2	2	2	1	2	3	1	1
Lumbar	1	1	8	1	2	9	4	2	2	3	3	3	5	2	4	4	3	5	4	3/4	**5**	**3**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	4	3	2	2	3	2	2	2	1	2	3	4	5
Sacral	1	1	10	1	2	11	4	3	2	4	4	5	8	3	6	4	6	7	4	3/4	**6**	**3**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	5	5	2	4	4	2	3	2	1	2	3	5	5
Caudal	3	2	10	1	2	14	4	3	3	6	6	10	10	3	7	5	6	7	6	5	**7**	**3**	**2**	**1**	**1**	**1**	**3**	**1**	**1**	6	5	3	4	4	3	3	3	2	3	4	5	6
Ilium	1	1	4	1	1	6	3	2	2	3	3	2	4	1	5	4	3	5	2	4	**3**	**1**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	5	5	2	2	4	2	2	2	1	2	3	6	3
Man. Phal.	1	1	1	2	2	6	7	3	3	5	4	9	6	2	8	4	5	6	5	4	**9**	**3**	**2**	**2**	**1**	**1**	**4**	**1**	**1**	2	5	3	1	1	1	3	2	2	2	3	7	3
Ped. Phal.	1	1	5	2	2	8	7	4	3	5	4	10	7	3	8	6	3	6	7	4	**10**	**3**	**3**	**2**	**1**	**1**	**4**	**3**	**1**	8	6	5	3	3	2	7	2	2	2	5	7	2
Ischium	3	1	10	2	2	12	5	3	3	5	6	4	5	1	8	4	5	7	8	4	**5**	**3**	**3**	**1**	**2**	**1**	**2**	**2**	**1**	7	6	4	2	4	3	3	4	2	4	3	6	7
Pubis	2	1	10	2	2	15	5	3	3	8	6	7	10	4	8	6	5	6	11	8	**9**	**4**	**3**	**4**	**3**	**1**	**3**	**5**	**2**	8	7	8	7	8	5	8	7	3	7	6	7	4
Metac	4	1	9	2	2	7	4	3	3	4	4	6	6	3	8	6	3	4	5	4	**5**	**3**	**2**	**1**	**1**	**1**	**2**	**6**	**1**	6	4	3	3	4	2	4	4	1	4	3	5	3
Metat	3	1	10	2	2	8	4	3	3	5	6	7	7	3	8	7	4	7	7	4	**8**	**3**	**2**	**1**	**1**	**1**	**3**	**4**	**1**	8	6	5	6	6	4	6	5	3	5	4	2	2
Tarsals	3	3	12	3	3	10	6	4	4	7	7	10	11	4	8	7	6	8	9	7	**12**	**5**	**4**	**5**	**3**	**2**	**3**	**7**	**3**	9	8	9	8	7	4	9	6	4	6	7	9	6
Carpals	4	4	13	5	4	16	8	5	4	8	8	11	12	5	9	7	7	10	12	9	**12**	**6**	**5**	**6**	**4**	**3**	**5**	**7**	**4**	10	8	10	9	9	6	10	7	5	7	8	8	8
Sternum	4	4	11	4	4	13	5	3	3	?	5	8	9	3	6	4	3	9	10	6	**11**	**3**	**2**	**3**	**2**	**1**	**1**	**1**	**3**	8	6	6	6	6	4	5	5	3	5	5	10	9
Epipubics	5	5	14	6	5	17	9	6	5	9	9	12	13	6	10	8	8	11	13	10	**13**	**7**	**6**	**7**	**5**	**4**	**6**	**8**	**5**	5	9	4	5	5	4	6	8	6	8	4	10	9

Table S6. Ranked data for timing of cranial events.

	Bradypus	*Dasypus*	*Tamandua*	*Tupaia*	*Homo*	*Mus*	*Peromyscus*	*Rhabdomys*	*Cavia*	*Talpa*	*Cryptotis*	*Mogera*	*Erinaceus*	*Rousetus*	***Loxodonta***	***Temrec***	***Echinops***	***Procavia***	***Heterohyrax***	***Eremitalpa***	***Orycteropus***
Premaxilla	4,5	5	7	2	3	5,5	2	6,5	4,5	3,5	2	2,5	2,5	7,5	**2**	**6**	**4**	**8**	**7,5**	**6,5**	**7,5**
Maxilla	4,5	5	7	2	1,5	5,5	2	6,5	4,5	3,5	2	2,5	2,5	4	**2**	**6**	**4**	**8**	**7,5**	**6,5**	**7,5**
Palatine	10,5	5	7	10	9,5	5,5	6	3	4,5	3,5	4,5	7	9,5	7,5	**8**	**6**	**4**	**8**	**7,5**	**6,5**	**7,5**
Dentary	4,5	5	7	2	1,5	5,5	2	3	4,5	3,5	2	2,5	2,5	1,5	**2**	**6**	**4**	**8**	**7,5**	**6,5**	**7,5**
Frontal	4,5	5	7	4,5	5,5	5,5	6	3	4,5	3,5	4,5	7	5	4	**8**	**6**	**4**	**8**	**7,5**	**6,5**	**7,5**
Parietal	4,5	5	7	4,5	5,5	5,5	6	3	4,5	3,5	6,5	2,5	2,5	4	**8**	**6**	**4**	**8**	**7,5**	**6,5**	**7,5**
Squamosal	4,5	5	7	6,5	5,5	12	12,5	11	4,5	8,5	9	7	9,5	1,5	**8**	**6**	**12,5**	**8**	**7,5**	**6,5**	**7,5**
Basioccipital	10,5	11	7	10	13	5,5	6	8	9,5	8,5	11	10	9,5	11	**13**	**6**	**4**	**8**	**7,5**	**6,5**	**7,5**
Nasal	4,5	5	7	10	9,5	12	12,5	10	9,5	8,5	8	7	9,5	7,5	**8**	**6**	**12,5**	**8**	**7,5**	**6,5**	**7,5**
Pterygoid	4,5	10	7	13,5	9,5	5,5	6	3	12	8,5	6,5	7	9,5	11	**8**	**6**	**4**	**8**	**7,5**	**6,5**	**7,5**
Exoccipital	10,5	13	7	6,5	5,5	5,5	9,5	9	12	11	11	11,5	9,5	11	**8**	**6**	**4**	**8**	**7,5**	**6,5**	**7,5**
Basisphenoid	15	15	14	15	16	12	9,5	12	14	12,5	13	14	9,5	13,5	**15**	**13,5**	**12,5**	**8**	**7,5**	**6,5**	**15,5**
Lacrimal	10,5	5	7	10	14,5	14	12,5	13	12	12,5	11	11,5	15	7,5	**8**	**13,5**	**12,5**	**8**	**7,5**	**15**	**7,5**
Alisphenoid	13	12	7	10	12	5,5	12,5	14	4,5	15,5	14	15,5	9,5	13,5	**8**	**13,5**	**12,5**	**8**	**7,5**	**15**	**7,5**
Orbitosphenoid	15	14	15	13,5	14,5	15	15,5	15	15,5	14	15	13	14	16	**14**	**13,5**	**12,5**	**8**	**15**	**13**	**7,5**
Periotic	15	16	16	16	9,5	16	15,5	16	15,5	15,5	16	15,5	16	15	**16**	**16**	**16**	**16**	**16**	**15**	**15,5**

Table S7. Ranked data for timing of postcranial events.

	Bradypus	*Choloepus*	*Dasypus*	*Cyclopes*	*Tamandua*	*Homo*	*Rattus*	*Mus*	*Peromyscus*	*Mesocricetus*	*Rhabdomys*	*Cavia*	*Octodon*	*Talpa*	*Cryptotis*	*Myotis*	*Rousettus*	*Bos*	*Sus*	***Loxodonta***	***Tenrec***	***Echinops***	***Procavia***	***Heterohyrax***	***Macroscelides***	***Elephantulus***	***Oryctéropus***	***Eremitalpa***
Humerus	8	10	3.5	7.5	5.5	2.5	2.5	6.5	7	1.5	4.5	3.5	6	9.5	6	3.5	3.5	1.5	3	**1**	**5.5**	**5.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Ribs	8	10	3.5	7.5	5.5	7	2.5	6.5	7	1.5	4.5	7.5	6	1	1	11	3.5	6.5	9	**6.5**	**5.5**	**5.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Femur	8	10	3.5	7.5	5.5	2.5	8	6.5	7	7.5	4.5	3.5	6	9.5	6	3.5	3.5	1.5	3	**2**	**5.5**	**5.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Radius	8	10	9	7.5	5.5	4.5	2.5	6.5	7	7.5	4.5	3.5	6	5	6	3.5	3.5	6.5	3	**6.5**	**5.5**	**5.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Ulna	8	10	3.5	7.5	5.5	1	2.5	6.5	7	7.5	4.5	3.5	6	5	6	3.5	3.5	6.5	3	**6.5**	**5.5**	**5.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Scapula	8	10	3.5	7.5	5.5	7	8	6.5	7	7.5	4.5	7.5	6	5	6	11	3.5	6.5	7	**6.5**	**5.5**	**5.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Cervic.	8	10	12	7.5	5.5	12	8	6.5	7	7.5	4.5	9.5	6	5	6	11	9	14.5	11.5	**11**	**5.5**	**5.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Thorac.	8	10	13	7.5	15.5	12	8	6.5	7	7.5	10	12	13	5	6	11	12	14.5	11.5	**6.5**	**11**	**5.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Tibia	8	10	7.5	7.5	5.5	4.5	8	6.5	7	7.5	4.5	3.5	6	5	6	3.5	7.5	6.5	3	**6.5**	**5.5**	**5.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Fibula	8	10	7.5	7.5	5.5	7	8	6.5	7	7.5	10	3.5	6	5	6	3.5	7.5	6.5	7	**6.5**	**5.5**	**5.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Lumbar	8	10	14	7.5	15.5	16	14	6.5	7	7.5	12	12	13	11	13.5	11	12	6.5	11.5	**13**	**16**	**14.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Sacral	8	10	18	7.5	15.5	18	14	16.5	7	14.5	14	18	17.5	13.5	13.5	21	18.5	6.5	11.5	**15**	**16**	**14.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Caudal	18.5	20	18	7.5	15.5	21	14	16.5	17.5	19.5	21	20.5	17.5	15	17	21	18.5	19	16	**16**	**16**	**14.5**	**9**	**9.5**	**11**	**18.5**	**10**	**8.5**
Ilium	8	10	10	7.5	5.5	9.5	8	6.5	7	7.5	10	9.5	6	12	13.5	11	12	14.5	7	**6.5**	**5.5**	**14.5**	**9**	**9.5**	**11**	**10.5**	**10**	**8.5**
Man. Phal.	8	10	3.5	17.5	15.5	9.5	21.5	16.5	17.5	14.5	19	14.5	13	19	13.5	18	15	14.5	14.5	**18.5**	**16**	**14.5**	**18.5**	**9.5**	**11**	**21.5**	**10**	**8.5**
Ped. Phal.	8	10	11	17.5	15.5	14.5	21.5	21.5	17.5	14.5	21	16.5	17.5	19	19	11	15	14.5	17.5	**20**	**16**	**20**	**18.5**	**9.5**	**11**	**21.5**	**10**	**18**
Ischium	18.5	10	18	17.5	15.5	19	18	16.5	17.5	19.5	13	12	6	19	13.5	18	18.5	14.5	19	**13**	**16**	**20**	**9**	**19.5**	**11**	**15.5**	**10**	**17**
Pubis	16	10	18	17.5	15.5	22	18	16.5	17.5	19.5	16.5	20.5	21.5	19	19	18	15	22	22	**18.5**	**21**	**20**	**21**	**21**	**11**	**18.5**	**20**	**20**
Metac	22	10	15	17.5	15.5	12	14	16.5	17.5	14.5	15	14.5	17.5	19	19	11	10	14.5	14.5	**13**	**16**	**14.5**	**9**	**9.5**	**11**	**15.5**	**10**	**21**
Metat	18.5	10	18	17.5	15.5	14.5	14	16.5	17.5	19.5	16.5	16.5	17.5	19	22	16	18.5	14.5	17.5	**17**	**16**	**14.5**	**9**	**9.5**	**11**	**18.5**	**10**	**19**
Tarsals	18.5	21	22	21	21	17	20	21.5	22.5	22	21	22	21.5	19	22	21	21	21	20	**22.5**	**22**	**22**	**22**	**22**	**22**	**18.5**	**21.5**	**22.5**
Carpals	22	22.5	23	23	22.5	23	23	23	22.5	23	23	23	23	23	22	23	23	23	23	**22.5**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**22.5**
Sternum	22	22.5	21	22	22.5	20	18	16.5	17.5	17	18	19	17.5	13.5	13.5	11	22	20	21	**21**	**16**	**14.5**	**20**	**19.5**	**11**	**10.5**	**21.5**	**8.5**

Table S8. Detailed heterochronies in the onset of ossification in cranial elements in afrotherians and major clades of mammals using ACCTRAN and DELTRAN consensus obtained from Parsimov analyses.