

PATTERNS OF OSSIFICATION IN SOUTHERN VERSUS NORTHERN PLACENTAL MAMMALS

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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) versus “southern” (Afrotheria and Xenarthra) placental clades. We contribute data on cranial and postcranial ossification events during growth in Afrotheria, including elephants, hyraxes, golden moles, tenrecs, sengis, and armadillos. We use three different techniques to quantify sequence heterochrony: continuous method, sequence-ANOVA (analysis of variance) and event-pairing/Parsimov. We show that afrotherians significantly differ from other placentals by an early ossification of the orbitosphenoid and caudal vertebrae. Our analysis also suggests that both southern placental groups show a greater degree of developmental variability; however, they rarely seem to vary in the same direction, especially regarding the shifts that differ statistically. 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).

KEY WORDS: Afrotheria, development, evolution, heterochrony, ossification, phylogeny, skeletogenesis.

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. Although most individual orders and some interordinal 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: Afrothe-

ria, 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.

Marsupial and placental mammals are known to have consistent developmental differences in the ossification of their skulls and skeletons. 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 intraplacental 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?

Asher et al. (2009, 2011) noted potentially different patterns of dental eruption and high levels of intraspecific variation in presacral vertebral counts among atlantogenatans (or “southern” placentals) relative to boreoeutherians (or “northern” placentals), raising the possibility that a developmental dichotomy might distinguish the two. 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 Nilsson 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 *Dasybus* 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; Jeffery et al. 2005; Germain and Laurin 2009; Keyte and Smith 2010) 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.

Materials 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 Belgique 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). A total of 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

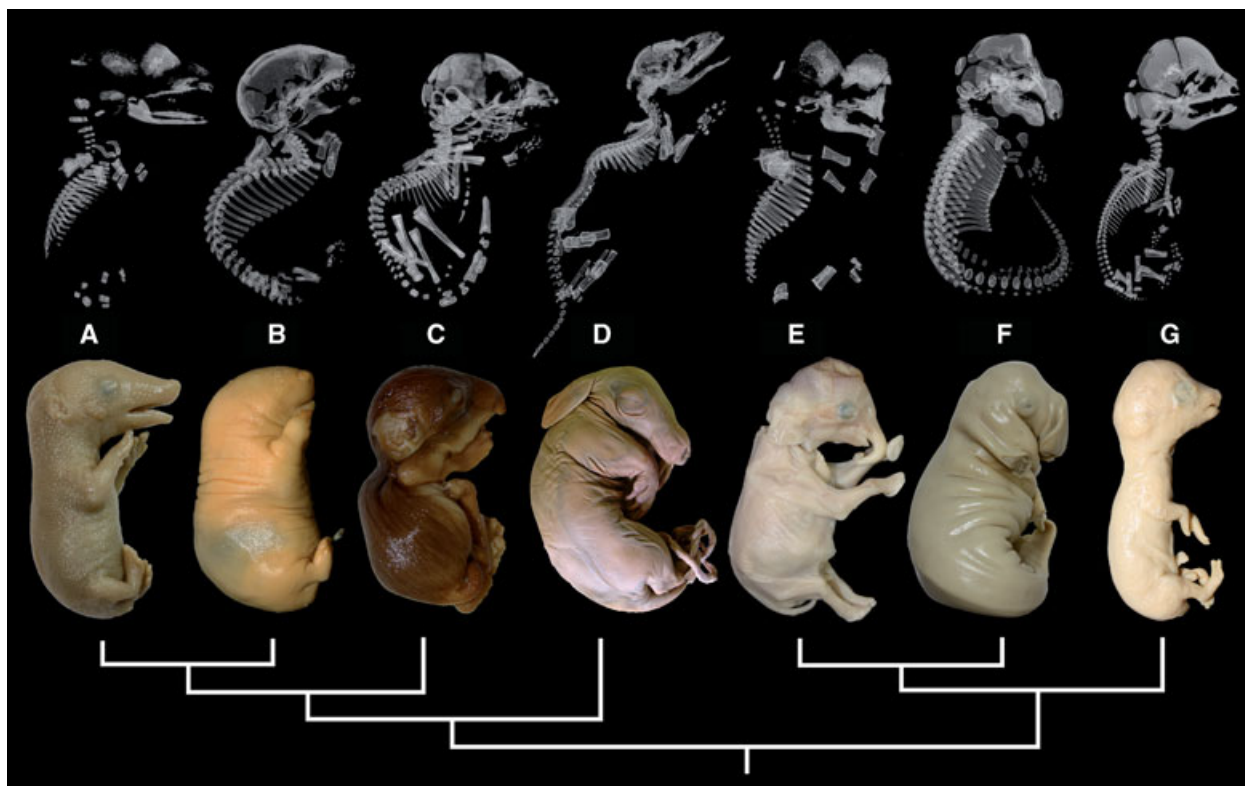


Figure 1. Representative ontogenetic stages of afrotherians and their phylogenetic relationships following Asher (2007). Lateral view of specimens (left) in (A) *Tenrec ecaudatus* PIMUZ MSV-Tec 11 CRL = 24 mm; (B) *Eremitalpa granti* NRM 538503 CRL = 25.5 mm; (C) *Elephantulus rozeti* ZMB28 CRL = 33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

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.

THREE-DIMENSIONAL (3D) DATA ACQUISITION

Skeletons were imaged using high-resolution X-ray microtomography (μ CT; 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. 3D rendering and visualization were performed using Drishti v.1.0 (Drishti Paint and Render; Limaye 2006). All the results obtained from 3D 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 centers 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 S1 and S2. 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 (analysis of variance) 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

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

Species name	Specimen numbers/stages		References
	Cranial	Postcranial	
Sauropsida			
<i>Alligator mississippiensis</i>	36/7	47/7	Rieppel 1993a
<i>Lacerta vivipara</i>	23/6	36/9	Rieppel 1993b
<i>Coturnix coturnix</i>	15/4		Nakane and Tsudzuki 1999
Afrotheria			
<i>Loxodonta africana</i>	17/6	17/13	Hautier et al. 2012
<i>Tenrec ecaudatus</i>	20/4	20/6	Present study
<i>Echinops telfairi</i>	5/4	5/5	Present study
<i>Procapra capensis</i>	32/2	32/6	Present study
<i>Heterohyrax brucei</i>	11/3	11/4	Present study
<i>Elephantulus rozeti</i>	10/1	10/3	Present study
<i>Macroselides proboscideus</i>	14/1	14/5	Present study
<i>Eremitalpa granti</i>	11/3	11/8	Present study
<i>Orycteropus afer</i>	4/2	4/4	Present study
<i>Dugong dugong</i>	1/1	1/1	Present study
<i>Trichechus manatus</i>	1/1	1/1	Present study
Xenarthra			
<i>Bradypus variegatus</i>	4/2	4/4	Hautier et al. 2011
<i>Choloepus didactylus</i>	4/1	4/4	Hautier et al. 2011
<i>Cyclopes didactylus</i>	4/2	4/5	Hautier et al. 2011
<i>Tamandua tetradactyla</i>	4/4	4/4	Hautier et al. 2011
<i>Dasybus novemcinctus</i>	27/6	27/8	Hautier et al. 2011
Euarchontoglires			
<i>Tupaia javanica</i>	24/6		Zeller 1987; Nunn and Smith 1998; Goswami 2007
<i>Tarsius spectrum</i>	21/6		Nunn and Smith 1998
<i>Homo sapiens</i>	60/	60/17	Mall 1906; Davies and Parsons 1927
<i>Rattus norvegicus</i>	N.a./6	N.a./14	Strong 1925
<i>Mus musculus</i>	N.a./7	41/5	Johnson 1933; Theiler 1972; Patton and Kaufman 1995; Kaufman 2008
<i>Cavia porcellus</i>	N.a./12	N.a./12	Petri 1935; Wilson et al. 2010
<i>Mesocricetus auratus</i>	168/7	168/8	Beyerlein et al. 1951; Kanazawa and Mochizuki 1974
<i>Meriones unguiculatus</i>	9/5	187/8	Yukawa et al. 1999; Sanchez-Villagra et al. 2008
<i>Peromyscus melanophrys</i>	13/5	7/4	Sanchez-Villagra et al. 2008; Weisbecker et al. 2008
<i>Octodon degus</i>		8/5	Wilson et al. 2010
<i>Rhabdomys pumilio</i>	61/12	61/11	Wilson et al. 2010
Laurasiatheria			
<i>Myotis lucifugus</i>		19/7	Adams 1992
<i>Rousettus amplexicaudatus</i>	11/7	12/10	Sanchez-Villagra et al. 2008; Weisbecker et al. 2008
<i>Cryptotis parva</i>	29/10		Sanchez-Villagra et al. 2008; Koyabu et al. 2011
<i>Erinaceus amurensis</i>	21/6		Koyabu et al. 2011
<i>Talpa europaea</i>	11/7	22/9	Prochel 2006; Goswami and Prochel 2007; Prochel et al. 2008; Koyabu et al. 2011
<i>Mogera wogura</i>	16/7		Koyabu et al. 2011
<i>Bos taurus</i>		180/9	Lindsay 1969a,b
<i>Sus scrofa</i>	10/7	N.a./12	Stockli 1922; Nunn and Smith 1998
<i>Felis catus</i>	17/7		Nunn and Smith 1998
<i>Manis javanica</i>	12/4		Nunn and Smith 1998
Marsupialia			
<i>Didelphis virginiana</i>	16/6	16/9	de Oliveira et al. 1998
<i>Trichosurus vulpecula</i>	6/4	32/9	Weisbecker et al. 2008
<i>Macropus eugenii</i>	20/6	11/9	Nunn and Smith 1998; Weisbecker et al. 2008
<i>Dasyurus viverrinus</i>	18/7	19/10	Nunn and Smith 1998; Goswami 2007; Weisbecker et al. 2008
<i>Sminthopsis macroura</i>		11/8	Frigo and Wooley 1996
<i>Antechinus stuartii</i>		22/10	Weisbecker et al. 2008
<i>Cercartetus concinnus</i>		25/8	Weisbecker et al. 2008
<i>Isodon macrourus</i>		15/10	Weisbecker et al. 2008
<i>Fetaurus breviceps</i>		22/6	Weisbecker et al. 2008
<i>Vombatus ursinus</i>		9/6	Weisbecker et al. 2008
<i>Caluromys philander</i>	9/6		Goswami 2007; Sanchez-Villagra et al. 2008
<i>Perameles nasuta</i>	10/9		Nunn and Smith 1998; Goswami 2007
<i>Monodelphis domestica</i>	28/8		Nunn and Smith 1998; Goswami 2007

(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 dataset is then converted into transformed ranks (presented in Table S3 and S4 for our samples). The ranked dataset 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 nonparametric 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 this 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 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 artifactual heterochronies. In our sample, this occurs primarily due to lack of coverage of the earliest ossification events, that is, leading to a number of events tied at #1. We sought to overcome such methodological noise by discounting the “sig-

nificance” 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 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 (CI) of its hypothetical ancestor. Here, we compared the placental ancestral sequence with the value of the nodes representing the four major placental clades, that is 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 CI 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.

Event-pair analyses were performed in the phylogenetic context shown in Fig. 2. Following previous studies (e.g., Sánchez-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 $(17^2 - 17)/2 = 136$ events for cranial elements and the other with $(25^2 - 25)/2 = 300$ 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) 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 use the PGI heterochrony search algorithm by Harrison and Larsson (2008) as it is currently

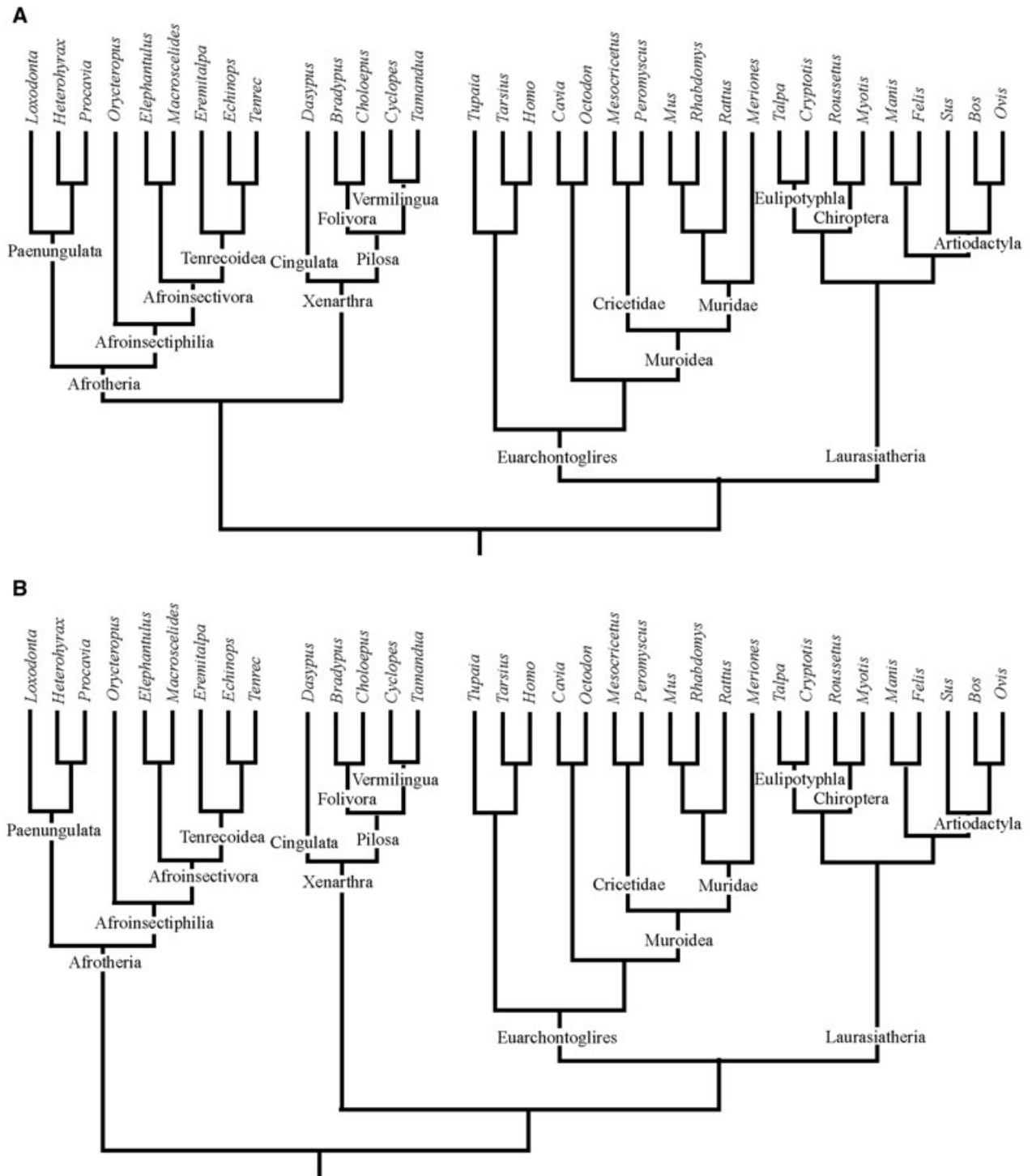


Figure 2. Two alternative hypotheses of phylogenetic relationships among the species studied in this analysis (Phillips et al. 2006; Asher 2007; Möller-Krull et al. 2007; Prasad et al. 2008; Meredith et al. 2011). (A) Atlantogenata, that is, sister-group status between afrotherians and xenarthrans; (B)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

not programmed to analyze 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 ACCTAN and DELTRAN event shifts in the onset of ossification of cranial and postcranial elements are presented in Table S5. 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 artifactual “significance” of heterochronic shifts that are not directly observable. To take into account this methodological artifact, 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; following Sánchez-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 (2004a,b, 2005), Lecompte (2008), and Rowe (2008) for rodents, and Douady et al. (2003, 2004) and 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. To avoid an artifactual 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 S1). 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) 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 tenrecids (Hautier et al. 2012; Werneburg et al. 2012). In our series of other afrotherians, 13 of 24 elements of the postcranial skeleton ossify first (rank 1, Table S2). A similar concentration of relative simultaneity for the earliest events was also found in other mammals (Table S2). 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 S2). In the yellow-spotted hyrax, *Heterohyrax*, the ischium and sternum ossify second, followed by the pubis and tarsals, and then the carpals. In the armadillo, *Orycteropus*, the pubis ossifies second, followed by the sternum and tarsals, and then the carpals (Table S2). 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 S2). 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 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), and plotted the developmental trajectory of Xenarthra 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, 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 14 (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, $F = 8.12 \times 10^{-5}$, $P = 0.9929$; 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 (Figs. 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 Supporting Information (Figs. 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% CI 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 exoccipital, basisphenoid, and orbitosphenoid, whereas ancestral xenarthrans clearly 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 onset of ossification of the parietal, jugal, palatine, squamosal, pterygoid, nasal, and basioccipital. However, it is worth mentioning here again that, with the exception of the jugal, most of these shifts may be due to artifacts 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.

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

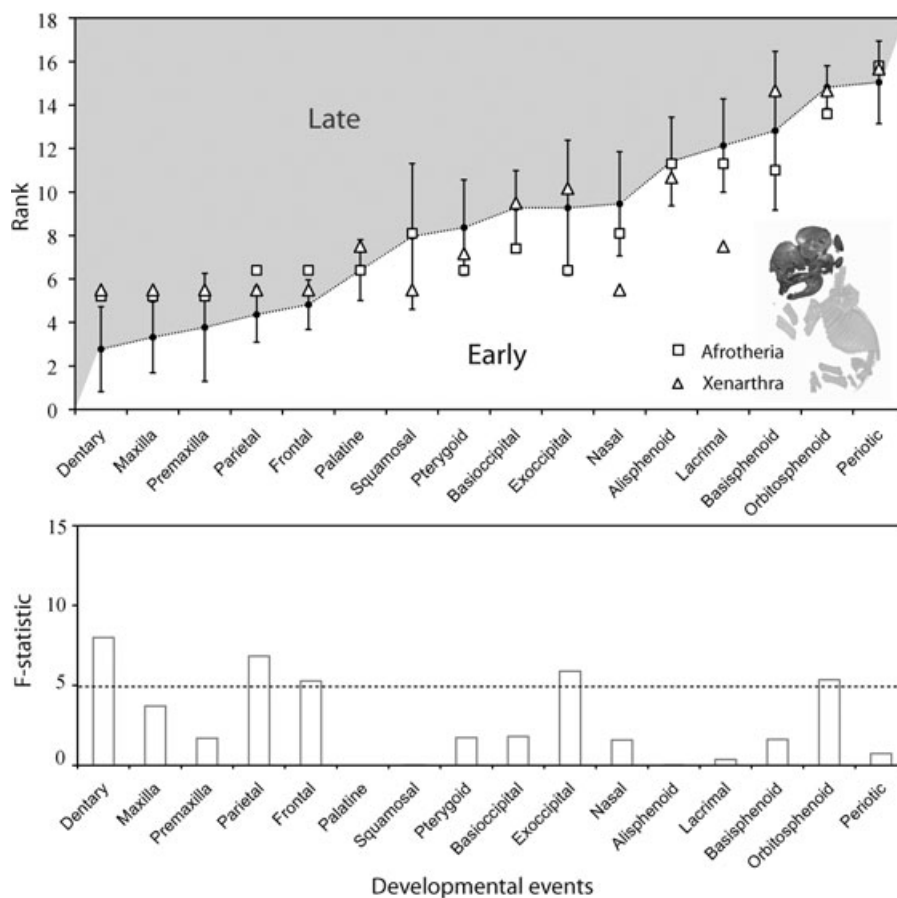


Figure 3. Ossification sequence of cranial elements in afrotherians relative to the mean ranks of northern placentals (Euarchontoglires and Laurasiatheria, solid circles); error bars of ± 1 standard deviation. Upper panel: the mean rank of *Loxodonta*, *Echinops*, *Tenrec*, *Eremitalpa*, and *Heterohyrax* 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 analysis of variances between afrotherians (open squares) and placentals (solid circles) with *F*-statistics. The dotted line denotes statistical significance at $P < 0.05$. Shifts can be observed between the two groups and are statistically significant when they exceed the dotted line (early ossification of orbitosphenoid and exoccipital; late ossification of dentary, parietal, and frontal). However, the significance of events tied at rank #1 is potentially artefactual (see text), and shifts can be observed between the two groups without being statistically significant (e.g., nasal, basioccipital, basisphenoid).

(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% CI 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 14 (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

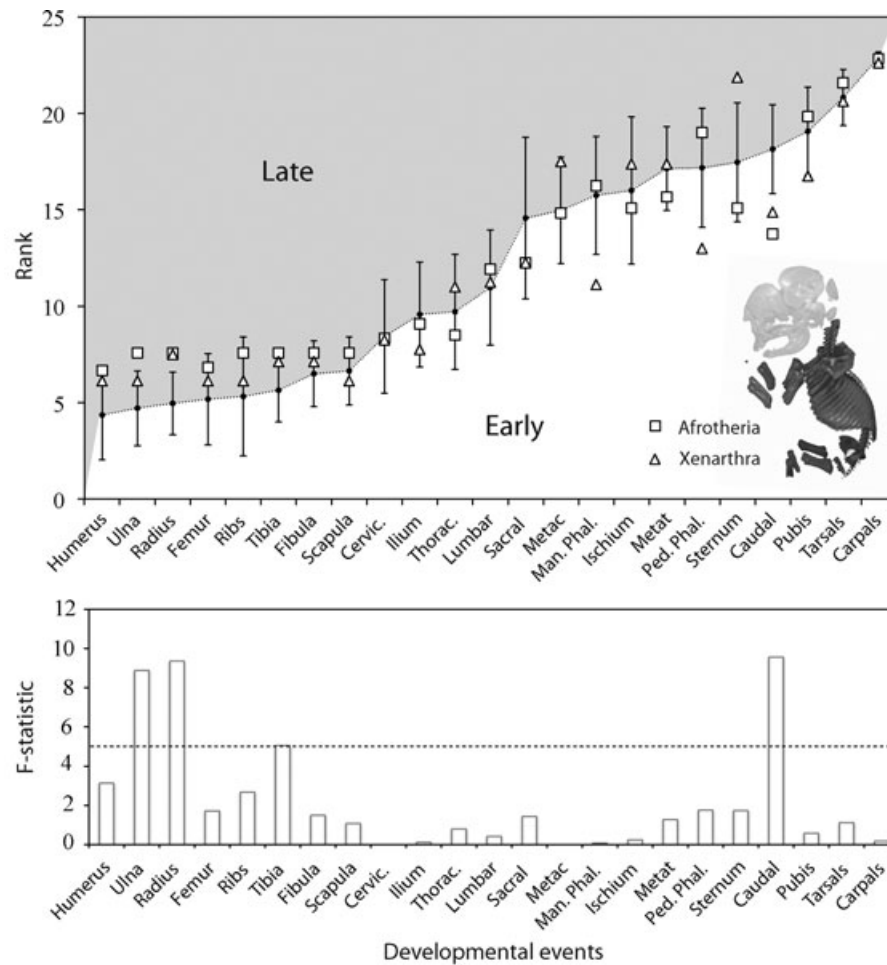


Figure 4. Ossification sequence of postcranial elements in afrotherians relative to the mean ranks of northern placentals (solid circles). Upper panel: mean ranks are represented by six afrotherians (*Loxodonta*, *Echinops*, *Tenrec*, *Eremitalpa*, *Elephantulus*, and *Procvavia*; open squares), 14 northern 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 analysis of variances 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.

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 S5), 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 S5). 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 S5). 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 S5).

Discussion

COMPARISON OF THE METHODS

While running Parsimov with missing data for ties successfully removes likely artifacts, 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

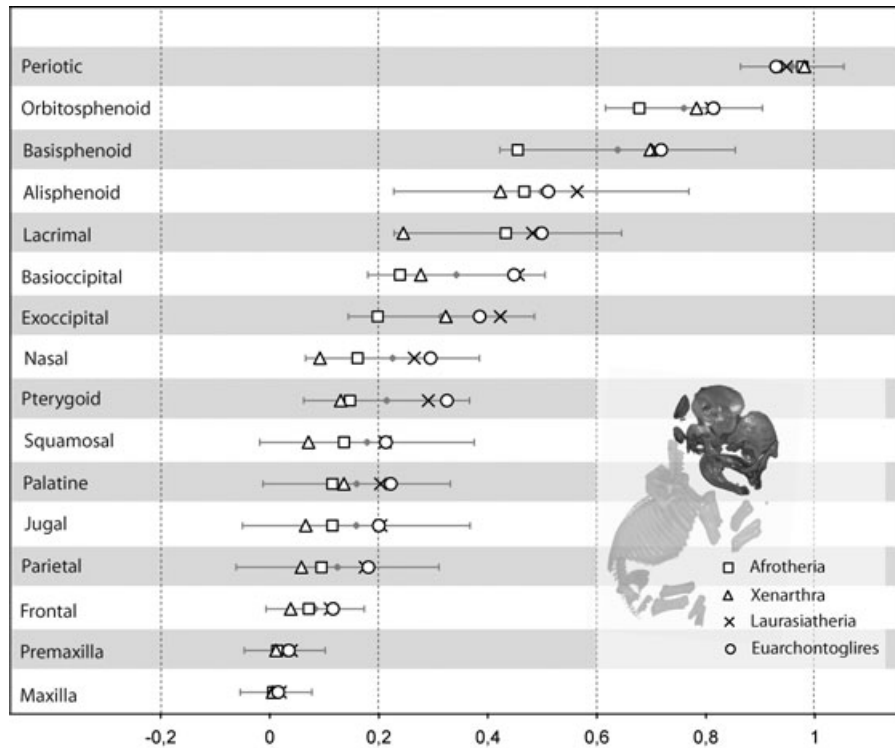


Figure 5. Optimization of the ancestral cranial sequence of Placentalia obtained by squared change analysis using parsimony by considering the Exafroplacentalia hypothesis and including the results obtained for the ancestral nodes of the four major clades of placental mammals, that is Afrotheria, Xenarthra,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

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 a 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. Although 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 artifacts 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% CI 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 VERSUS 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), Werneburg 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

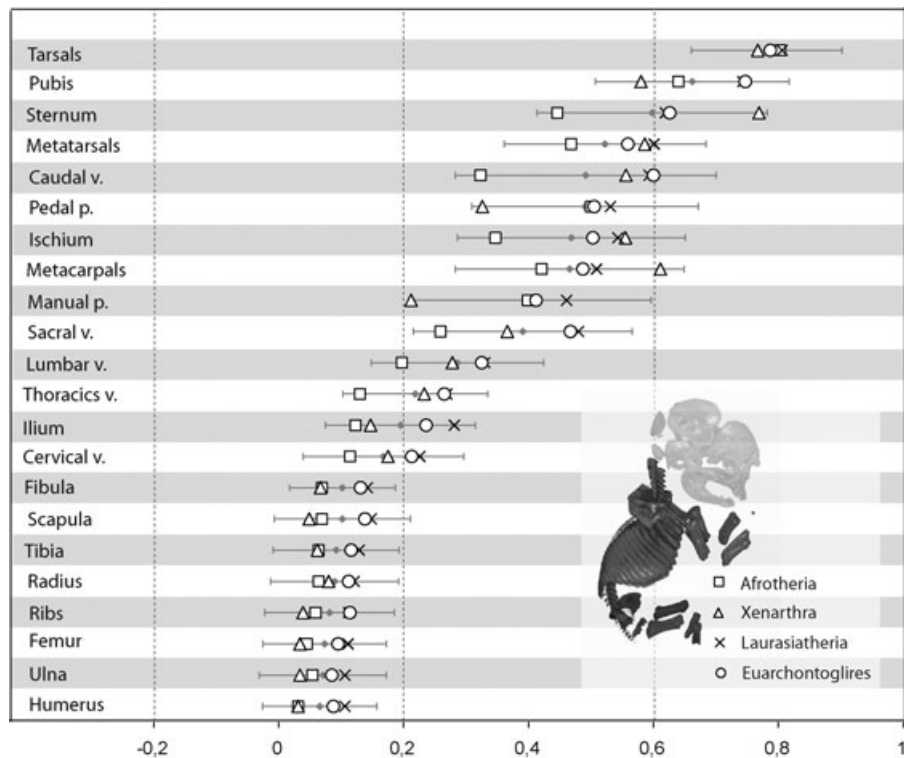


Figure 6. Optimization of the ancestral postcranial sequence of Placentalia obtained by squared change analysis using parsimony by considering the Exafroplacentalia hypothesis and including the results obtained for the ancestral nodes of the four major clades of placental mammals, as described in the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

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 considering 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), whereas 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% CI (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 (Figs. 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

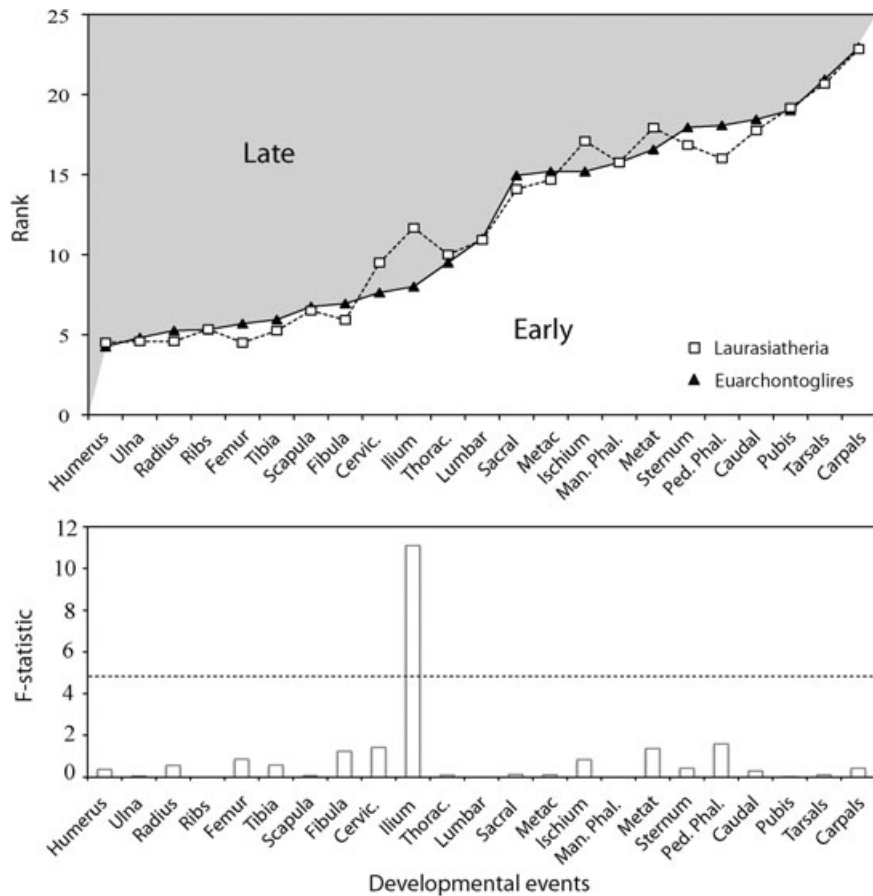


Figure 7. Ossification sequence of postcranial elements in Laurasiatheria (open squares) relative to the mean ranks of Euarchontoglires (solid triangles). Upper panel: mean ranks are represented eight Euarchontoglires (solid triangles), and six Laurasiatheria (open squares). Results of the analysis of variances 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.

comparative analyses of ossification sequence (Goswami 2007; Sánchez-Villagra et al. 2008; Weisbecker 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 conservatism among these northern placental clades (but see Koyabu et al. 2011). Although sequence resolution was greater for many of the taxa included in those previous studies (see Tables S1 and S2), 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 artifacts. 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, although 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 earlier and shown in Fig. 6, the majority, although not all, of shifts characterizing 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.

FETAL AGE VERSUS 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 2 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 center 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 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 nonmodel 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 fetal sizes. That is, most bones ossify in a brief time toward the end of gestation, mirroring the pattern of ossification observed in rodents (Hautier et al. 2012). As mentioned previously, to the extent that they do represent the real phenomenon of fast ossification and are not artifacts 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.

Conclusions

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, but perhaps indicative of a relaxed level of developmental constraint in the two groups.

Another interesting observation that may prove fruitful in evaluating phenotype among southern placental mammals concerns body temperature. Compared to northern placentals, Afrotheria and Xenarthra both contain a large number of species that exhibit substantial variability (McKenchie and Mzilikazi 2011) and/or relatively low body temperatures (Clarke and Rothery 2008). As temperature is an important factor in skeletal development (e.g., Lindsey and Moodie 1967), it would be reasonable to conclude that systematic differences in phenotype may arise if/when gestational temperatures also vary systematically across species.

Further analyses of sequence heterochrony, combined with a reassessment of metabolic rates, mammalian phylogenetics, and data from new breeding programmes of unconventional model species (e.g., Milinkovitch and Tzika 2007; Tzika & Milinkovitch 2008; Werneberg 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.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. List of studied specimens.

Figure S2. Optimization 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, that is 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. Optimization 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 S1. Relative timing of onset of ossification (ranks) in the cranial elements for all species examined and compiled from the literature.

Table S2. Relative timing of onset of ossification (ranks) in the postcranial elements for all species examined and compiled from the literature.

Table S3. Ranked data for timing of cranial events.

Table S4. Ranked data for timing of postcranial events.

Table S5. Detailed heterochronies in the onset of ossification in cranial elements in afrotherians and major clades of mammals using ACCTAN and DELTRAN consensus obtained from Parsimov analyses.