Timing of Cranial Suture Closure in Placental Mammals: Phylogenetic Patterns, Intraspecific Variation, and Comparison With Marsupials

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ABSTRACT   Used as markers of postnatal growth closure sequences of 22 ectocranial sutures and synchondroses were recorded in a sample of 1161 skulls belonging to 38 species from all major placental clades: Afrotheria, Xenarthra, Laurasiatheria and Euarchontoglires (Boreoeutheria). The maximum closure level, which is not significantly correlated to body mass, is higher in Afrotheria and Xenarthra than in Boreoeutheria. Only the basioccipito-basisphenoid and the basioccipito-exoccipital synchondroses close in all species sampled, the supraoccipito-exoccipital and the inter-parietal sutures do in most species. Parsimov retrieved more heterochronic shifts for Afrotheria and Xenarthra than for Boreoeutheria. The amount of intraspecific variation differs among the species sampled being high among xenarthran species and low among afrotherians. Specimens (162) representing 12 marsupial genera were also analysed. Placentals exhibit a larger number of suture closures than marsupials and in both groups the sutures at the base of the skull are the first to fuse starting with the basioccipito-exoccipital. J. Morphol. 275:125–140, 2014.   © 2013 Wiley Periodicals, Inc.

KEY WORDS: evolution; heterochrony; phylogeny; growth; variation; skull

INTRODUCTION

Many mammalian skull features are formed around birth and during early postnatal growth skull bones start to fuse. This period is critical to recognise ossification centers and contacts among elements, thus serving for the recognition of homologous elements (Koyabu et al., 2012) and ultimately to address systematic issues. In this article, we present the most comprehensive examination of the timing of ectocranial suture closure in mammals. This study aims at understanding these patterns in terms of their systematic distribution, intraspecific variation and heterochrony. Sutures and synchondroses are important sites of interstitial growth of the cranium and can fuse when growth stops or slows down (Rice, 2008). As different parts of the skull grow at different rates over different time periods depending on the species (Weston, 2003), it is expected that heterochrony will be recorded among mammalian species.

Previous studies of timing of cranial suture closure in Placentalia dealt with primates (e.g., Krogman, 1930; Chopra, 1957; Wang et al., 2006; Cray et al., 2008; Flores and Barone, 2012), carnivorans (Schweiker, 1930; Brunner et al., 2004; Segura and Flores, 2009; Goswami et al., 2013; Segura, 2013), hystricognath rodents (Wilson and Sánchez-Villagra, 2009), a bat (Giannini et al., 2006), peccaries (Herring, 1974), deer (Sánchez-Villagra, 2010) and artiodactyls in general (Bärmann and Sánchez-Villagra, 2012); afrotherians have been barely studied (e.g., Hoson et al., 2009) and previous works did not examine cranial suture closure in xenarthrans. Marsupial clades have barely been studied in this regard (e.g., Gardner, 1973; Flores et al., 2006).

As in any other feature considered, the phylogenetic context is fundamental. The solid knowledge
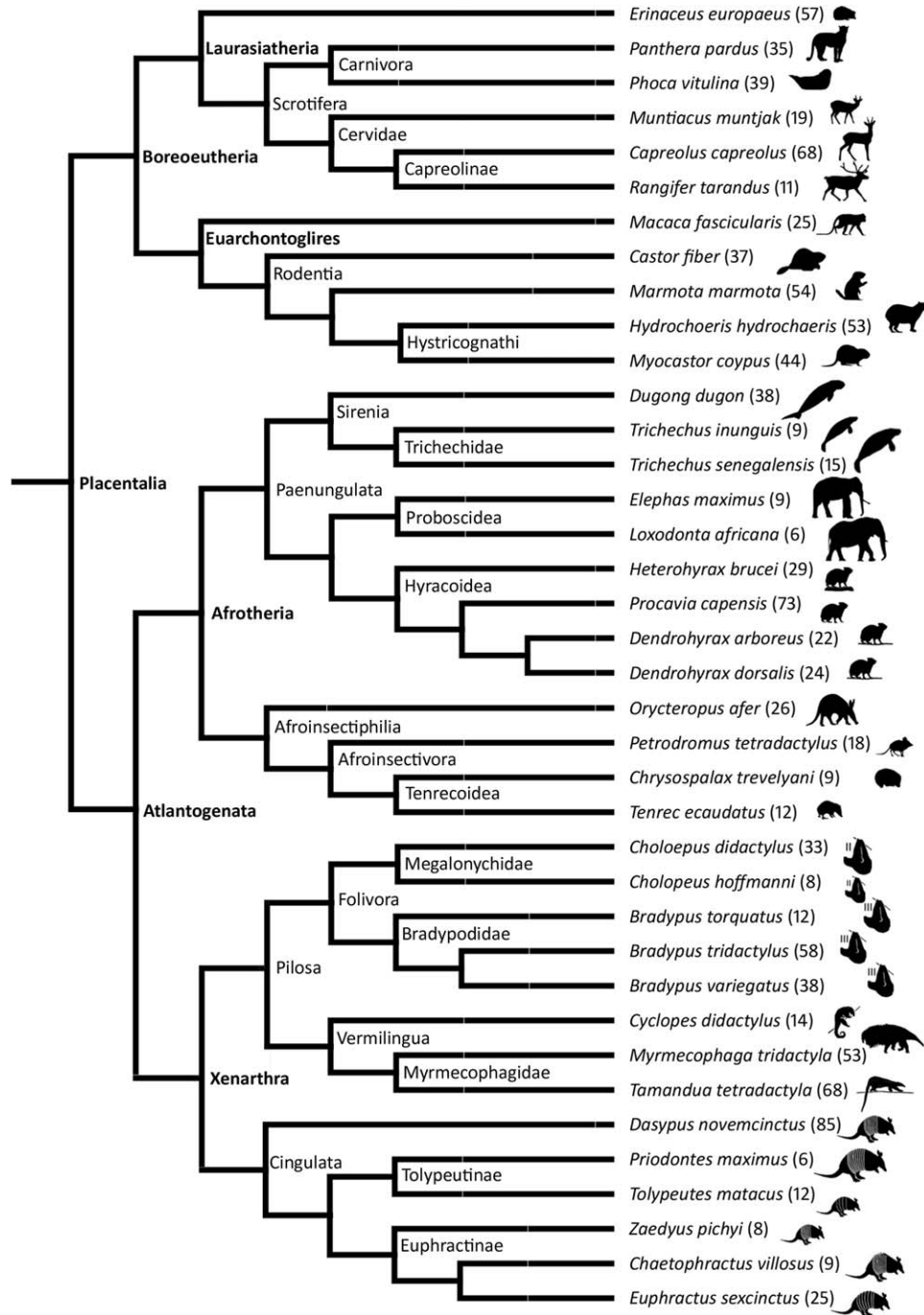

Fig. 1. Phylogenetic tree of major placental clades according to Meredith et al. (2011), with details on relations among 'families' and genera based on several references (Gaudin and Branham, 1998; Gaudin, 2004, Gaudin et al., 2006; Gilbert et al., 2006; Kuntner et al., 2011). Clade names according to Asher and Helgen (2010), taxonomy according to Wilson and Reeder (2005). In parentheses, number of specimens studied for each taxon.

of the major pattern of mammalian phylogeny (Meredith et al., 2011) makes a study of patterns of sutural fusion in these animals timely. There is broad consensus about the monophyly of four major placental clades: Xenarthra (armadillos, anteaters and sloths), Afrotheria (e.g., elephants, hyraxes, manatees, tenrecs, golden moles and others), Laurasiatheria (e.g., 'ungulates', bats and carnivorans), and Euarchontoglires (e.g., primates and rodents) with the former two perhaps grouped together in the clade Atlantogenata, and the latter two grouped in the clade Boreoeutheria (Fig. 1; Springer et al., 2004; Asher et al., 2009; Hallstrom and Janke, 2010; Meredith et al., 2011).

Alternative hypotheses either placed Xenarthra (e.g., O'Leary et al., 2013) or Afrotheria (e.g., Hallstrom and Janke, 2010) at the base of Placentalia with the remaining clades grouped in Epitheria or Exafroplacentalia, respectively.

In this study, we seek to discuss four primary topics: 1) the interspecific differences in closure level and their relation with size; 2) the interspecific differences in the sequence of suture closure (heterochrony); 3) the intraspecific variation in postnatal ontogenetic sequence of cranial suture closure within the four major clades of placental mammals; 4) the general pattern of the suture closure of Placentalia and Marsupialia.

MATERIAL AND METHODS

We examined 1161 skulls of 38 living placental species (Fig. 1): 14 xenarthrans, 13 afrotherians, six laurasiatherians and five euarchontoglirans (Supporting Information). To compare placentals with marsupials, we examined 162 marsupial specimens distributed in 12 genera: three didelphimorphians, three dasyuromorphians, two peramelemorphians and four diprotodontians (Fig. 2; Supporting Information).

The following collections house the examined specimens: Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires (MACN); Museum für Naturkunde Berlin (ZMB), Muséum national d'Histoire naturelle, Paris (MNHN), Natural History Museum, London (BMNH), Naturhistorisches Museum Basel (NMB), Naturhistorisches Museum Bern (NMBE), Paläontologisches Institut und Museum der Universität Zürich (PIMUZ), Institut für Paläoanatomie, Domestikationsforschung und Geschichte der Tiermedizin der Ludwig-Maximilians-Universität München (SAPM), Zoologische Sammlung, Universitat Tübingen (SZ), University Museum of Zoology Cambridge (UMZC), Zoologisches Museum der Universität Zürich (ZMUZH) and the Zoologische Staatssammlung München (ZSM).

Twenty-two skull sutures and synchondroses were analysed, covering the vault, base, palatal, facial and cranio-facial areas (Fig. 3). Sutures and synchondroses are termed in this study with the names of the bones involved at the synarthrotic intersections. Sutures were coded as open (0) when more than half of the suture was visible or closed (1) when less than half of the suture was visible. This coding is simpler than the four levels of closure coding some of us used in the recent studies of rodents (Wilson and Sánchez-Villagra, 2009) and carnivorans (Goswami et al., 2013). The method we follow here is appropriate to address the questions posed and most importantly, is one that is more easily replicated with fossil forms, thus increasing the usefulness of our study as landmark reference for future palaeontological studies.

Sutures that do not exist because the adjacent bones are not in contact were coded as missing (-). Sutures that could not be clearly assessed (because of incomplete maceration, lack of visibility or some other issue) were coded as undecided (?).

Maximum Suture Closure Level and Body Mass

Suture closure level of a species was measured as the number of closed sutures [sutures coded as (1)] across all studied specimens as a percentage of the number of studied sutures. The correlation between closure level and body mass was examined using phylogenetically independent contrasts (Felsenstein, 1985) which, in contrast to other methods, takes the fact into account that phylogenetically related species cannot be treated as statistically independent. Correcting for the phylogeny prevents an overstatement of significance.
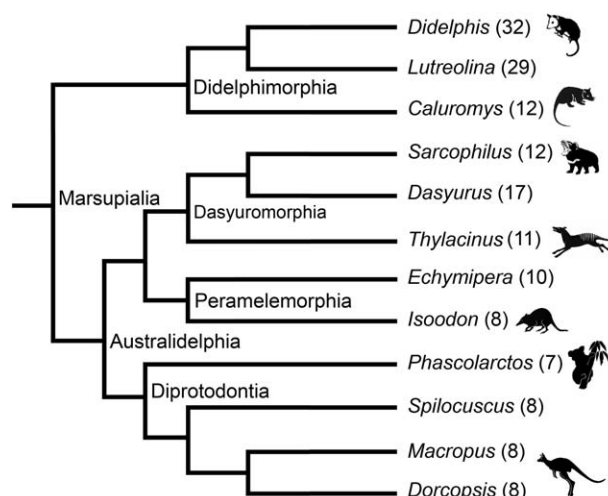

Fig. 2. Phylogenetic tree of major marsupial clades according to Meredith et al. (2011), with details on relations among families based on Wroe et al. (2000), Meredith et al. (2008), Voss and Jansa (2009). In parentheses, number of specimens studied for each taxon.

The corresponding Pearson product-Moment correlation coefficient r and its P value were calculated using the PDAP package version 1.16 (Midford et al., 2005) in Mesquite version 2.75 (Maddison and Maddison, 2011). The analysis would yield $r=1$ if the average percentage of closed sutures across all studied specimens of a taxon was perfectly correlated to the average body mass in this taxon, and it would yield $r=0$ if there was no correlation at all between the average percentage of closed sutures across all studied specimens of a taxon and the average body mass in this taxon. Body masses were obtained from the online database panTHERIA (Jones et al., 2009).

Closure Sequence and Heterochrony

The sequence of suture and synchondrosis closure was obtained by ranking them by their closure level within each species, as exemplified in Figure 4 for *Elephas maximus*. Following Wilson and Sánchez-Villagra (2009), the Kendall's coefficient of concordance W was used to assess the agreement of the sequence of cranial suture closure between two or more taxa. This analysis was performed with R version 2.14.2 R (Ihaka and Gentleman, 1996). The analysis would result in $W=1$ if the sutures closed in exactly the same order in all of the compared taxa and $W=0$ if no two sutures closed in the same order in any pair of the compared taxa.

Event-pairs were constructed to assess the order in which every possible pair among the analysed 22 suture/synchondrosis events happened. An event corresponds here to the closure of a specific cranial suture. Potential heterochronic shifts were examined using the event-pairing approach (Mabee and Trendler, 1996; Smith, 1997; Velhagen 1997). The order was coded (0) if suture A closed before suture B, (1) if the sequence of suture closure was unresolved for the pair in question and (2) if suture A closed after suture B. Event-pairing and the identification of potential heterochronic shifts was done using LEEP v. 0.1, a new software presented in this article for the first time (supplementary information).

We also 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 not programmed to analyse datasets with ties
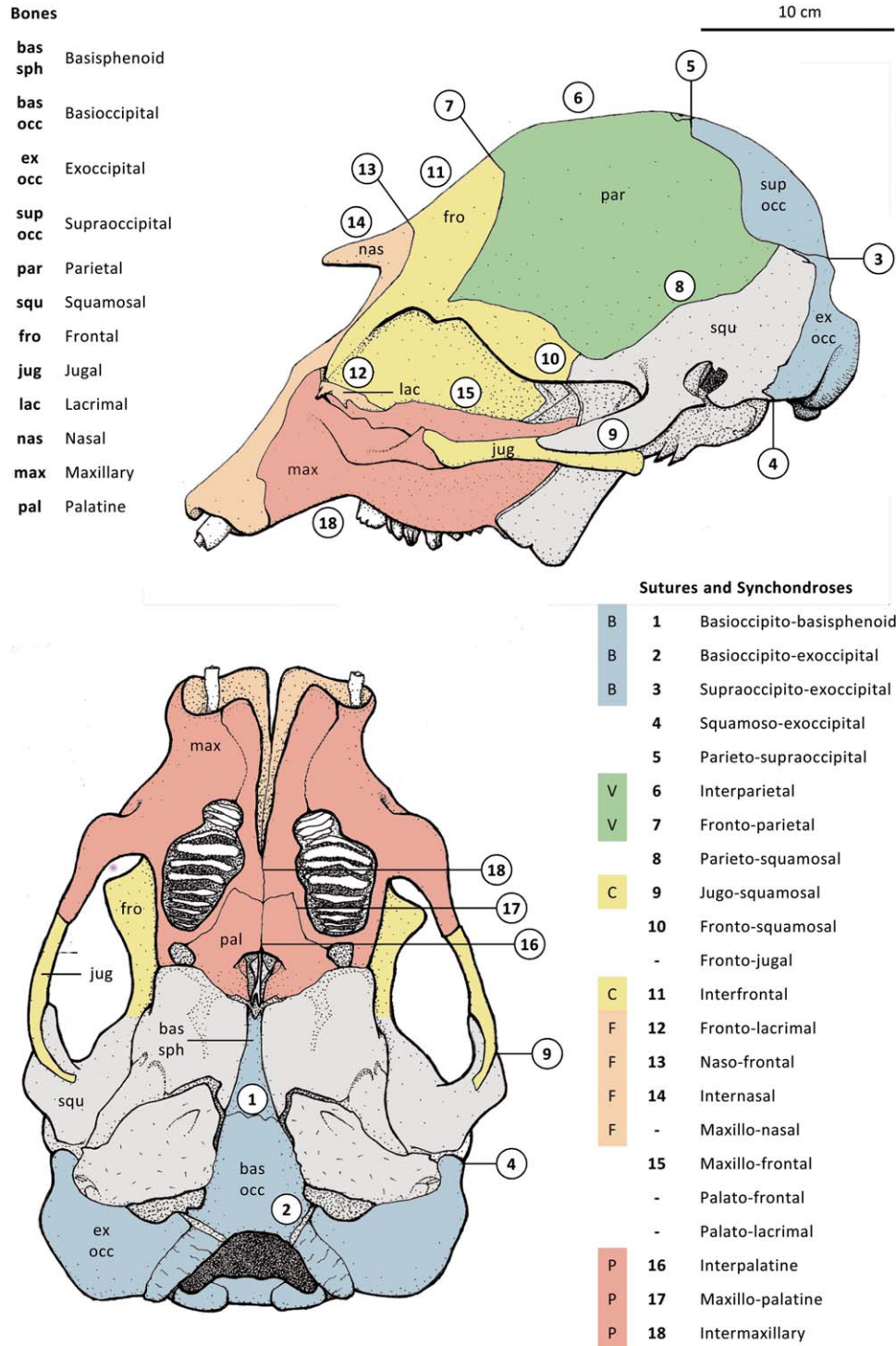
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Bones

bas sph	Basisphenoid
bas occ	Basioccipital
ex occ	Exoccipital
sup occ	Supraoccipital
par	Parietal
squ	Squamosal
fro	Frontal
jug	Jugal
lac	Lacrimal
nas	Nasal
max	Maxillary
pal	Palatine

Sutures and Synchondroses

B	1	Basioccipito-basisphenoid
B	2	Basioccipito-exoccipital
B	3	Supraoccipito-exoccipital
	4	Squamoso-exoccipital
	5	Parieto-supraoccipital
V	6	Interparietal
V	7	Fronto-parietal
	8	Parieto-squamosal
C	9	Jugo-squamosal
	10	Fronto-squamosal
	-	Fronto-jugal
C	11	Interfrontal
F	12	Fronto-lacrimal
F	13	Naso-frontal
F	14	Internasal
F	-	Maxillo-nasal
	15	Maxillo-frontal
	-	Palato-frontal
	-	Palato-lacrimal
P	16	Interpalatine
P	17	Maxillo-palatine
P	18	Intermaxillary

Fig. 3. Skull of a neoatal asian elephant, *Elephas maximus* (ZMN 17473); bones adjacent to the studied sutures and synchondroses labelled with abbreviations; studied sutures labelled with numbers, sutures not present in *Elephas maximus* but studied in other species without numbers; colours representing the regions of the skull: base (B) in blue, vault (V) in green, craniofacial (C) in yellow, facial (F) in pink, palatal (P) in red.

excluded. We ran the analyses using both ACCTRAN and DELTRAN optimisations as recommended by Jeffery et al. (2005). The ACCTRAN option assumes accelerated transformations (favoring reversals); the DELTRAN option provides delayed transformations (favouring convergences; Maddison and Maddison, 1992). Only the events that were reported using both approaches were interpreted as heterochronies. To control for the artefactual interpretation of heterochrony due to accumulation of ties across events, we ran two Parsimov analyses, one with the original data and a second with all ties converted to

suture	Fronto-parietal	Interfrontal	Interparietal	Parieto-supraoccipital	Basioccipito-exoccipital	Naso-frontal	Supraoccipito-exoccipital	Basioccipito-basisphenoid	Internasal	Parieto-squamosal	Fronto-lacrimal	Squamoso-exoccipital	Fronto-squamosal	Jugo-squamosal	Intermaxillary	Interpalatine	Maxillo-frontal	Maxillo-palatine	Fronto-jugal	Maxillo-nasal	Palato-frontal	Palato-lacrimal	closure level
(BA 5238)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	-	-	-	-	
(L 6.8.20.1)	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	-	-	-	-	
(BA -)	1	1	1	1	1	1	1	0	1	1	0	0	1	0	0	0	0	0	-	-	-	-	
(UMZC 114451)	1	1	1	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	-	-	-	-	
(BE 1023496)	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	-	-	-	-	
(BA 2513)	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	-	-	-	-	
closure level	6	6	6	6	5	5	4	4	4	3	3	2	2	1	0	0	0	0	0	0	0	0	8,83

Fig. 4. An example of how the character state for each suture and synchondrosis of each specimen was arranged in a table for each species; in this case for *Loxodonta africana*, the African elephant.

missing data (i.e., coded as "?" for unknown timing; see Sánchez-Villagra et al., 2009). Because of the uncertain phylogenetic position of the placental root, the event-pair analyses were performed in two phylogenetic contexts. 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, Hallstrom and Janke, 2010).

Intraspecific Variation in Closure Patterns

In addition to identifying suture closure sequences (e.g. Fig. 5) for comparison across species, we analysed the variation within species in the order of suture closure. To do so, we designed a method that identified specimens within each species with the same closure score and quantified their similarity in suture closure order as follows. The mean amount of gaps per specimen m_{GAsp} and the length of gaps per specimen m_{GLsp} were corrected by dividing by the maximum number of closed sutures n_{su} because they showed a significant positive correlation with the number of specimens n_{sp}. The mean amount of gaps per suture m_{GAsu} and the length of gaps per suture m_{GLsu} were corrected by dividing by the maximum number of closed sutures n_{su} and by the number of specimens n_{sp} because they showed a significant positive correlation with both values.

Gaps are character states coded as (0) and bracketed by character states coded as (1) in the row of sutures of a specimen or in the row of specimens of a suture. To make the number of gaps and the length of gaps comparable between species, the data per species were arranged in the table as follows: in specimens with identical closure level but with different sutures closed, blocks were formed for each closure pattern and the gaps were located as far to the right as possible within blocks of identical closure level; in sutures with identical closure level but with different specimens closing the suture, blocks were formed of identical specimens and the gaps were located as close as possible to the bottom. Outliers were located as far to the right and as close to the bottom as possible also, so that longer gaps were moved farther to the left and upwards. The criteria for data table arrangement were: 1) order by mean closure level/suture and mean closure level/specimen with highest closure levels first; 2) within blocks of identical closure level form blocks of identical closure pattern; 3) within blocks of identical closure level move gaps as far to the right and to the bottom as possible; 4) within blocks of identical closure level

move outliers, that is, longest gaps as far to the right and upwards as possible.

The variation in closure patterns as a measurement of intraspecific variation in closure sequence can be expressed using the variation index V (details about the calculation of the variation index and equations used can be found in Supporting Information).

The variation index is zero ($V=0$) if there is no variation (i.e., all specimens that close the same number of sutures close the same sutures and all sutures that close in the same number of specimens close in the same specimens). $V=1$ if the maximum possible variation within the sample is reached (i.e., there are no two specimens with the same sutures closed in the largest sample and there are no two sutures that close in the same specimens in the species with the highest maximum number of closed sutures).

RESULTS
Closure Level

Maximum closure levels range between 9% in the reindeer *Rangifer tarandus* and 91% in the two-toed sloth *Choloepus didactylus* and the golden mole *Chrysospalax trevelyani*. They are generally higher in the studied Afrotheria and Xenarthra. All maximum closure levels above 50% are recorded in these two clades. Laurasiatherians have maximum closure levels of 13–16%, whereas the leopard *Panthera pardus* has a value of 27%. Among Euarchontoglires, the maximum closure level only ranges between 14% in the capybara *Hydrochoerus hydrochaeris* and 22% in the crab-eating macaque *Macaca fascicularis*.

Among Afrotheria, the recorded maximum closure levels range between 14% in the cape hyrax *Procavia capensis* and 91% in the golden mole *Chrysospalax trevelyani*. Rather low values (around 20%) were recorded among the remaining Afroinsectiphilia, in the rock hyrax *Heterohyrax*
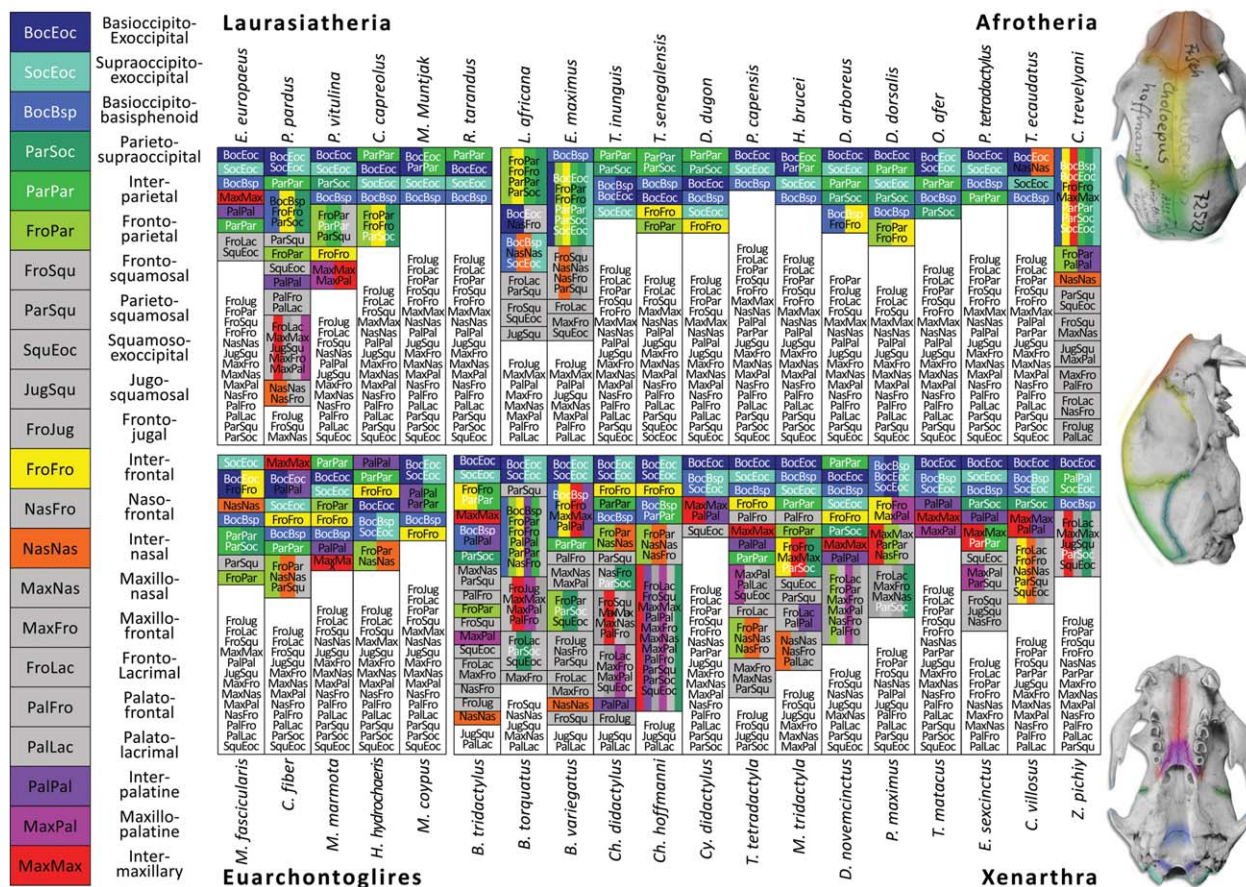
Fig. 5. The sequence of cranial suture and synchondrosis closure: one column for each of the studied placental species from top (first to close) to bottom (last to fuse), on white background sutures and synchondroses that do not close in any of the studied specimens, vertical colour-coding for unresolved closure of two or more sutures and synchondroses; key to abbreviations for each suture and synchondrosis and colour-coding in the table on the left; location of colour-coded sutures and synchondroses on a skull of Hoffmann's two-toed sloth *Choloepus hoffmanni* (MFN 72522) on the right; colours representing the regions of the skull: base in blue, vault in green, craniofacial in yellow, facial in orange, palatal in purple.

brucei and in one of the sea cows (*Trichechus inunguis*), higher values in the hyrax *Dendrohyrax* spp. and sea cow *Trichechus* spp. and *Dugon dugon*, and exceptionally high values (above 50%) in the elephants *Loxodonta africana* and *Elephas maximus*.

Xenarthrans showed generally high closure levels above 50% with the exception of 23% maximum closure level in the pygmy anteater *Cyclopes didactylus* and medium values in the smaller armadillos. The highest closure levels were recorded among sloths; it ranges between 77% in the three toed sloth *Bradypus torquatus* and 91% in the two-toed sloth *Choloepus didactylus*. All the other sloth species (*B. tridactylus*, *B. variegatus* and *Choloepus hoffmanni*) display a maximum closure level of 86%.

Maximum closure level and mean body mass of each species are presented in Table 1. The correlation between the two is presented in Table 2: The Pearson product-Moment correlation coefficient *r*, corrected for phylogeny using Felsenstein's phylogenetically independent contrast shows a weak significant positive correlation in Eutheria as a whole. A significant correlation was also detected among Xenarthra, but only among the Cingulata and Paenungulata a strong correlation was to be seen.

Closure Sequence and Heterochronies

Despite the variation among species, some patterns are still detectable among clades (Fig. 5). The basioccipio-exoccipital synchondrosis is the first or second one to close in all species except for the Paenungulata (*Loxodonta africana, Elephas maximus, Trichechus inguinus, T. senegalensis* and *Dugong dugon*) and the capybara *Hydrochoeris hydrochaeris*. In most species, the supraoccipito-exoccipital synchondrosis is the second one to close. The African elephant *Loxodonta africana* is the only species in which this joint is not among the first five to close. The parieto-supraoccipital suture closes fourth or later in all studied species but the common seal *Phoca vitulina* and some afrotherians.

TABLE 1. *Body mass (kg) (from the web data base panTHERIA Jones et al., 2009) and maximum closure level (%) (maximum number of closed sutures and synchondroses among all studied specimens of a species as a percentage of the number of studied sutures) of each of the 38 studied species; number of studied specimens in each species in parentheses*

Taxon (no. of specimens)	Maximum closure level (%)	Mean body mass (kg)
Laurasiatheria		
Erinaceus europaeus (75)	12.68	0.78
Panthera pardus (35)	27.01	52.40
Phoca vitulina (39)	15.79	87.32
Capreolus capreolus (68)	14.30	22.50
Muntiacus muntjak (19)	16.27	17.61
Rangifer tarandus (11)	9.09	109.09
Euarchontoglires		
Macaca fascicularis (25)	21.82	4.57
Castor fiber (37)	16.46	19.00
Marmota marmota (54)	18.77	4.06
Hydrochoeris hydrochaeris (53)	14.32	48.14
Myocastor coypus (44)	16.53	6.36
Afrotheria		
Loxodonta africana (6)	54.55	3824.54
Elephas maximus (9)	63.64	3269.79
Trichechus inunguis (9)	22.73	418.00
Trichechus senegalensis (15)	27.27	454.00
Dugong dugon (38)	27.27	295.00
Procavia capensis (73)	13.64	2.95
Heterohyrax brucei (29)	18.18	2.45
Dendrohyrax arboreus (22)	31.82	2.98
Dendrohyrax dorsalis (24)	31.82	3.17
Orycteropus afer (26)	22.73	56.18
Petrodromus tetradactylus (18)	18.18	0.20
Tenrec ecaudatus (12)	18.18	0.89
Chrysospalax trevelyani (9)	90.91	0.43
Xenarthra		
Bradypus tridactylus (58)	86.36	4.38
Bradypus torquatus (12)	77.27	4.47
Bradypus variegatus (38)	86.36	4.14
Choloepus didactylus (33)	90.91	6.65
Choloepus hoffmanni (8)	86.36	5.89
Cyclopes didactylus (14)	22.73	0.26
Tamandua tetradactyla (68)	63.64	4.80
Myrmecophaga tridactyla (53)	59.09	29.53
Dasypus novemcinctus (85)	50.00	3.95
Priodontes maximus (6)	54.55	40.64
Tolypeutes matacus (12)	27.27	1.30
Euphractus sexcinctus (25)	59.09	4.73
Chaetophractus villosus (9)	36.36	4.37
Zaedyus pichiy (8)	27.27	1.44

Kendall's coefficient of concordance. The Kendall's coefficient (W) shows that there is a highly significant concordance ($P < 0,001$) among sequences of the different species of Placentalia and among all higher clades studied (Table 3). There is no phylogenetic signal in the level of agreement of the sequence between species among different taxa. The sequence is highly conserved among all taxa.

Heterochronic shifts as detected by event-pairing. No heterochronic shift was detected in the sequence of cranial suture closure of the hypothetical last common ancestors of any of the major clades of Placentalia using Leep v. 0.1. Heterochronic shifts were detected for several

TABLE 2. *Phylogenetically independent Product-Moment Correlation Coefficient r indicating the correlation between body mass and suture and synchondrosis closure level among the studied specimens of placental clades and the associated P-values; number of studied species and specimens in each clade in parentheses*

Clade (no. of species, no. of specimens)	PDAP r	P-value
Eutheria (38, 1126)	0.352	0.030
Boreoeutheria (11, 443)	−0.438	0.178
Atlantogenata (27, 719)	0.364	0.062
Laurasiatheria (6, 229)	−0.388	0.447
Euarchontoglires (5, 213)	−0.736	0.156
Afrotheria (13, 290)	0.419	0.154
Xenarthra (14, 719)	0.535	0.049
Paenungulata (9, 225)	0.856	0.003
Afroinsectiphilia (4, 65)	−0.105	0.895
Pilosa (8, 284)	0.162	0.702
Cingulata (6, 145)	0.981	0.001

clades (Laurasiatheria excluding Lipotyphla or Scrotifera, Carnivora, Cervidae and the subgroup Cervinae, Rodentia, *Marmota marmota* + Hystricognathi, Hystricognathi, *Dendrohyrax*, Bradypodidae, Megalonychidae, *Bradypus tridactylus* + *Bradypus variegatus*, Myrmecophagidae, Tolypeutinae + Euphractinae, Tolypeutinae and Euphractinae) and single species. The xenarthrans are characterised by more heterochronic shifts than afrotherians, and all the species that are not distinguished from their sister taxa by any heterochronic shift belong to afrotherian or boreoeutherian clades. The specific sequence of cranial suture closure is most variable in sloths.

Notable exceptions in the early sequence of suture closure concerned exclusively the internasal suture and sutures of the palatal region. The internasal suture closes first in the common tenrec *Tenrec ecaudatus* and was also observed to close very early in other tenrec species. The internasal also closes earlier in the only studied

TABLE 3. *Kendall's coefficient of concordance W indicating agreement between suture and synchondrosis closure sequences (W = 1 means complete agreement, W = 0 means no agreement) between the studied species in higher placental clades with the associated P-values; number of studied species and specimens in each clade in parentheses*

Clade (no. of species, no. of specimens)	W	P-value
Eutheria(38, 1162)	0.609	1.30E-089
Boreoeutheria (11, 443)	0.707	4.84E-024
Atlantogenata (27, 719)	0.588	4.52E-058
Laurasiatheria (6, 229)	0.738	5.02E-011
Euarchontoglires (5, 213)	0.799	1.77E-009
Afrotheria (13, 290)	0.701	1.71E-029
Xenarthra (14, 429)	0.621	9.50E-028
Paenungulata (9, 225)	0.753	5.20E-020
Afronisectiphilia (4, 65)	0.707	1.58E-005
Pilosa (8, 284)	0.696	2.53E-015
Cingulata (6, 145)	0.673	1.28E-009
Folivora (5, 149)	0.781	6.73E-009
Vermilingua (3, 135)	0.773	5.49E-004

TABLE 4. Heterochronies in the onset of suture and synchondrosis closure in afrotherians, xenarthrans, and major clades of mammals using ACCTRAN and DELTRAN consensus obtained from Parsimov analyses; (*) indicates shifts involving bones that ossify at the same rank in most of the species of the considered group

Hypothesis	Clade	Event	Shift	Related to
Atlantogenata, including ties	Atlantogenata		No shift	
	Afrotheria	Intermaxillary	Late	Fronto-jugal*, Fronto-parietal, Fronto-squamosal*, Internasal*, Maxillo-nasal*, Naso-frontal*, Palato-frontal*, Palato-lacrimal*, Parieto-squamosal*, Squamoso-exoccipital*
		Interpalatine	Late	Fronto-jugal*, Fronto-parietal, Fronto-squamosal*, Internasal*, Maxillo-palatine*, Parieto-squamosal*
	Xenarthra	Fronto-lacrimal	Early	Fronto-jugal, Palato-lacrimal
		Intermaxillary	Early	Maxillo-palatine
		Interpalatine	Early	Maxillo-nasal, Naso-frontal, Palato-frontal, Palato-lacrimal, Squamoso-exoccipital
		Naso-frontal	Early	Fronto-jugal, Maxillo-nasal, Squamoso-exoccipital
	Boreoeutheria		No shift	
	Laurasiatheria		No shift	
	Euarchontoglires	Fronto-parietal	Early	Fronto-squamosal, Jugo-squamosal, Maxillo-frontal, Maxillonasal, Maxillo-palatine, Naso-frontal, Palato-frontal, Palato-lacrimal
Atlantogenata, ties as missing data	Atlantogenata	Palato-lacrimal	Late	Jugo-squamosal, Maxillo-nasal
	Afrotheria	Fronto-squamosal	Early	Maxillo-frontal, Maxillo-palatine, Palato-frontal
		Internasal	Early	Maxillo-palatine, Palato-frontal, Parieto-squamosal, Squamoso-exoccipital
		Jugo-squamosal	Early	Palato-frontal
	Xenarthra		No shift	
	Laurasiatheria	Internasal	Late	Fronto-lacrimal
	Euarchontoglires	Interfrontal	Early	Basioccipito-basisphenoid, Parieto-supraoccipital
		Internasal	Early	Maxillo-palatine, Palato-frontal, Parieto-squamosal, Parieto-supraoccipital, Squamoso-exoccipital
Exafroplacentalia, including ties	Afrotheria		No shift	
	Xenarthra	Fronto-lacrimal	Early	Palatolacrimal
		Naso-frontal	Early	Fronto-jugal, Maxillo-nasal, Squamoso-exoccipital
	Boreoeutheria		No shift	
	Laurasiatheria		No shift	
	Euarchontoglires	Fronto-parietal	Early	Maxillo-frontal, Maxillo-palatine, Naso-frontal, Palato-frontal
Exafroplacentalia, ties as missing data	Afrotheria	Fronto-squamosal	Early	Jugo-squamosal, Maxillo-frontal
		Internasal	Early	Parieto-squamosal, Squamoso-exoccipital
		Jugo-squamosal	Early	Palato-lacrimal
	Xenarthra		No shift	
	Boreoeutheria		No shift	
	Laurasiatheria		No shift	
	Euarchontoglires	Internasal	Early	Moved E relative to Maxillo-palatine, Palato-frontal, Parieto-squamosal, Parieto-supraoccipital, Squamoso-exoccipital

primate species *Macaca fascicularis*. Sutures of the palatal region were recorded to close exceptionally early in the european hedgehog *Erinaceus europaeus* and two rodent species. The intermaxillary suture closes first in the beaver *Castor fiber*, and the interpalatine suture closes first in the capybara *Hydrochoeris hydrochaeris*.

Heterochronic shifts as detected by Parsimov. With ties included (i.e., not treated as missing data) or not, Parsimov analysis does not identify any heterochronic shift for the nodes representing the Laurasiatheria and Boreoeutheria (Table 4) regardless of the position of the placental root (i.e., Atlantogenata versus Exafroplacentalia). With ties included only one heterochronic shift

characterised the Euarchontoglires, namely an early closure of the fronto-parietal suture. However, different heterochronic shifts are detected for this clade when ties are treated as missing data: an early ossification of the interfrontal and internasal in relation to the bones of the craniofacial, palatal, vault and basicranial regions.

By retaining ties in the dataset, a late shift of the intermaxillary and interpalatine with respect to several bones (craniofacial, facial, and palatal regions) is detected for Afrotheria. However, most of these shifts occur at a single developmental stage (Table 4) and should be considered as a methodological artefact. This artefact becomes evident by running the analyses with ties coded as

missing data, the Afrotheria being then characterised by different heterochronic shifts involving the fronto-squamosal, internasal and jugo-squamosal sutures whatever the phylogenetic context. Only few heterochronic shifts were detected for Xenarthra which appear slightly different depending on the position of the placental root. None of these heterochronic shifts were retrieved when ties were treated as missing data (Table 4).

Intraspecific Variation in Closure Patterns

Intraspecific variation in the pattern of closed cranial sutures occurs in 32 of the 38 species studied (84%). Only two laurasiatherians (the barking deer *Muntiacus muntjak* and the reindeer *Rangifer tarandus*) and four afrotherians (the African manatee *Trichechus senegalensis*, the cape hyrax *Procavia capensis*, the four-toed sengi *Petrodromus tetradactylus* and the common tenrec *Tenrec ecaudatus*) lack variation in the sequence of suture closure. The most variable afrothere is *Loxodonta* ($V = 0.30$). Variation parameters are given in Tables 5 and 6.

The variation index in laurasiatherians ranged from 0 (for some cervids) to 0.43 for *Panthera pardus*. Among Euarchontoglires, variation indices range from relatively low ($V=0.18$) in the alpine marmot *Marmota marmota* to moderate ($V=0.33$) in the beaver *Castor fiber* and the crab-eating macaque *Macaca fascicularis*. Variation indices are rather similar in the two hystricognath rodent species: the coypu *Myocastor coypus* ($V=0.26$) and the capybara *Hydrochoeris hydrochaeris* ($V=0.32$).

Among the four major clades of placentals, the largest range of variation in the index V occurs in Xenarthra (0.64). It is much lower in the Afrotheria (0.30) and Boreoeutheria (0.15 in Euarchontoglires and 0.43 in Laurasiatheria).

Both atlantogenatan clades show exceptional variation indices in comparison to Boreoeutheria. A visual parameter for intraspecific variation in the sequence of cranial suture closure is the frequency and distribution of gaps in the ordered data tables. This was measured by the amount of gaps per specimen and per suture and the length of gaps per specimen and per suture in each species. The patterns just described above for the variation indices of Xenarthra and Afrotheria are also reflected in the amount and length of gaps in each species.

Marsupial Patterns

In marsupials, most skull sutures remain open, even in the largest specimens (Fig. 6). The mean closure level per specimen is 2.63 sutures closed which represents 12% of the total sutures analysed (Supporting Information). The highest value of suture closure was found in the carnivorous Tasmanian devil (*Sarcophilus harrisi*) with an

TABLE 5. *Variation index* V *and the components of which it is the mean value: the variation index in specimens* V$_{sp}$ *and the variation index in sutures* V$_{su}$*; number of studied specimens in each species in parentheses*

Taxon (no. of specimens)	V	Vsp	Vsu
Laurasiatheria			
Erinaceus europaeus (57)	0.17	0.33	0.00
Panthera pardus (35)	0.43	0.29	0.57
Phoca vitulina (39)	0.27	0.17	0.37
Capreolus capreolus (68)	0.10	0.21	0.00
Muntiacus muntjak (19)	0.00	0.00	0.00
Rangifer tarandus (22)	0.00	0.00	0.00
Euarchontoglires			
Macaca fascicularis (25)	0.33	0.21	0.45
Castor fiber (37)	0.33	0.28	0.37
Marmota marmota (54)	0.18	0.36	0.00
Hydrochoeris hydrochaeris (53)	0.32	0.23	0.40
Myocastor coypus (44)	0.26	0.35	0.18
Afrotheria			
Loxodonta africana (6)	0.30	0.10	0.49
Elephas maximus (9)	0.12	0.07	0.18
Trichechus inunguis (9)	0.15	0.05	0.25
Trichechus senegalensis (15)	0.00	0.00	0.00
Dugong dugon (38)	0.16	0.33	0.00
Procavia capensis (73)	0.00	0.00	0.00
Heterohyrax brucei (29)	0.06	0.12	0.00
Dendrohyrax arboreus (22	0.22	0.08	0.35
Dendrohyrax dorsalis (24)	0.09	0.19	0.00
Orycteropus afer (26)	0.06	0.13	0.00
Petrodromus tetradactylus (18)	0.00	0.00	0.00
Tenrec ecaudatus (12)	0.00	0.00	0.00
Chrysospalax trevelyani (9)	0.28	0.00	0.56
Xenarthra			
Bradypus tridactylus (58)	0.57	0.58	0.55
Bradypus torquatus (12)	0.23	0.12	0.35
Bradypus variegatus (38)	0.61	0.35	0.86
Choloepus didactylus (33)	0.41	0.34	0.48
Choloepus hoffmanni (8)	0.24	0.00	0.48
Cyclopes didactylus (14)	0.21	0.11	0.30
Tamandua tetradactyla (68)	0.81	0.82	0.79
Myrmecophaga tridactyla (53)	0.82	0.83	0.80
Dasypus novemcinctus (85)	0.59	1.00	0.18
Priodontes maximus (6)	0.22	0.06	0.39
Tolypeutes matacus (12)	0.18	0.06	0.30
Zaedyus pichiy (8)	0.27	0.14	0.40
Euphractus sexcinctus (25)	0.34	0.29	0.38
Chaetophractus villosus (9)	0.30	0.15	0.45

average of 4.25 sutures closed (19.32%) and the herbivore cuscus (*Spilocuscus* spp*.)* with an average of 4.75 sutures closed (21.6%). *Didelphis* is by far the best represented marsupial taxon with 32 specimens at different growth stages. In *Didelphis*, skull size varies across the size range (e.g., *Didelphis albiventris*) and the number of sutures that fuse is low (average of 2.15 sutures closed to a maximum of five sutures closed in one specimen).

Common features with placentals are still detectable. Similar to placentals the first of the 22 sutures/synchondroses analysed to close postnatally is the basioccipital-exoccipital synchondrosis. The alisphenoid-basisphenoid synchondrosis closes during the embryonic development in *Monodelphis* (Clark and Smith, 1993). This suture was not

TABLE 6. *Corrected values for: the amount of gaps per specimen* m_{GAsp}, *the length of gaps per specimen* m_{GLsp}, *the amount of gaps per suture* m_{GAsu} *and the length of gaps per suture* m_{GLsu}; *number of studied specimens in each species in parentheses*

Taxon (no. of specimens)	$mGAsp$	$mGLsp$	$mGAsu$	$mGLsu$
Laurasiatheria				
Erinaceus europaeus (57)	0.048	0.061	5.9809E-04	3.3892E-03
Panthera pardus (35)	0.020	0.042	1.2303E-03	3.6910E-03
Phoca vitulina (39)	0.010	0.013	6.8182E-04	2.1591E-03
Capreolus capreolus (68)	0.002	0.002	9.5493E-05	1.9099E-04
Muntiacus muntjak (19)	0.000	0.000	0.0000E+00	0.0000E+00
Rangifer tarandus (22)	0.000	0.000	0.0000E+00	0.0000E+00
Euarchontoglires				
Macaca fascicularis (25)	0.022	0.036	1.0101E-03	3.4343E-03
Castor fiber (37)	0.024	0.027	8.5995E-04	2.9484E-03
Marmota marmota (54)	0.021	0.032	4.2088E-04	7.4705E-03
Hydrochoeris hydrochaeris (53)	0.009	0.009	2.1441E-04	4.2882E-04
Myocastor coypus (44)	0.076	0.095	1.0331E-03	5.5096E-03
Afrotheria				
Loxodonta africana (6)	0.071	0.155	3.2468E-03	4.8701E-03
Elephas maximus(9)	0.008	0.016	3.6075E-04	1.4430E-03
Trichechus inunguis (9)	0.022	0.022	1.0101E-03	1.0101E-03
Trichechus senegalensis (15)	0.000	0.000	0.0000E+00	0.0000E+00
Dugong dugon (38)	0.013	0.013	5.9809E-04	5.9809E-04
Procavia capensis (73)	0.000	0.000	0.0000E+00	0.0000E+00
Heterohyrax brucei (29)	0.017	0.017	3.9185E-04	3.9185E-04
Dendrohyrax arboreus (22)	0.006	0.006	2.9516E-04	3.2468E-03
Dendrohyrax dorsalis (24)	0.012	0.012	5.4113E-04	8.1169E-04
Orycteropus afer (26)	0.015	0.015	3.4965E-04	2.4476E-03
Petrodromus tetradactylus (18)	0.000	0.000	0.0000E+00	0.0000E+00
Tenrec ecaudatus (12)	0.000	0.000	0.0000E+00	0.0000E+00
Chrysospalax trevelyani (9)	0.033	0.044	1.2626E-03	1.2626E-03
Xenarthra				
Bradypus tridactylus (58)	0.031	0.068	1.4107E-03	3.6050E-03
Bradypus torquatus (12)	0.009	0.023	1.0522E-03	1.2626E-03
Bradypus variegatus (38)	0.024	0.039	1.0167E-03	1.9139E-03
Choloepus didactylus (33)	0.023	0.058	7.5758E-04	3.9945E-03
Choloepus hoffmanni (8)	0.026	0.039	8.9713E-04	8.9713E-04
Cyclopes didactylus (14)	0.048	0.048	1.6234E-03	2.1645E-03
Tamandua tetradactyla (68)	0.021	0.047	1.1141E-03	5.6818E-03
Myrmecophaga tridactyla (53)	0.029	0.066	1.2864E-03	5.1458E-03
Dasypus novemcinctus (85)	0.024	0.043	8.6384E-04	8.7207E-03
Priodontes maximus (6)	0.042	0.111	1.2626E-03	1.8939E-03
Tolypeutes matacus (12)	0.014	0.014	6.3131E-04	6.3131E-04
Zaedyus pichiy (8)	0.042	0.097	2.5253E-03	3.7879E-03
Euphractus sexcinctus (25)	0.031	0.058	1.2587E-03	5.8741E-03
Chaetophractus villosus (9)	0.040	0.182	2.7548E-03	5.9688E-03

observed in any specimen sampled, suggesting an embryonic closure in all marsupials.

The sutures/synchondroses that generally close postnatally are included in the cranial base (basioccipital-exoccipital, basioccipital-basisphenoid and exoccipital-supraoccipital), cranial vault (interparietals, fronto-parietals) and craniofacial part of the skull (e.g., inter-frontals). Sutures of the facial region always remain open. Some sutures of the palatal region close in a few taxa. Among the sutures of the cranial base, the basioccipital-exoccipital synchondrosis closes in all the taxa analysed, while the basioccipital-basisphenoid and exoccipital-suproccipital synchondroses appear more variable. The basioccipital-exoccipital synchondrosis always closes and is first to close in the basicranium, usually followed by the exoccipital-suproccipital and then the basioccipital-basisphenoid synchondroses, except in some didel-

phids and *Thylacinus* that present a reverse sequence.

In the vault and cranio-facial areas, the inter-frontal suture closes before the inter-parietal in the sampled didelphids and is reversed in order with the Australasian marsupials. Examination of *Dromiciops gliroides* would be important to establish if a synapomorphy of australidelphians exists in this context given the basal position of this species in the clade (e.g., Nilsson et al., 2010; Beck, 2012). The inter-frontal suture closes before the fronto-parietal in all marsupials but *Dasyurus* and *Spilocuscus*.

DISCUSSION

The cranial suture closure pattern in Placentalia exhibits the following characteristics: 1) a higher maximum closure levels among Xenarthra than

Didelphis		Lutreolina		Caluromys		Sarcophilus		Dasyurus		Thylacinus	
BocEoc	28	BocEoc	28	SocEoc	12	ParPar	12	ParPar	15	ParPar	10
FroFro	23	ParPar	21	BocEoc	12	BocEoc	12	SocEoc	13	BocEoc	10
ParPar	18	FroFro	20	BocBsp	5	SocEoc	8	BocEoc	13	FroFro	8
NasNas	0	BocBsp	3	FroFro	1	FroFro	4	FroPar	3	BocBsp	6
NasFro	0	SocEoc	1	FroPar	1	BocBsp	4	FroFro	2	SocEoc	5
FroPar	0	PalPal	1	ParSoc	1	FroPar	3	NasNas	0	FroPar	4
SocEoc	0	NasNas	0	NasNas	0	MaxPal	3	NasFro	0	ParSoc	1
ParSqu	0	NasFro	0	NasFro	0	PalLac	3	ParSqu	0	PalLac	1
JugSqu	0	FroPar	0	ParPar	0	MaxFro	1	JugSqu	0	NasNas	0
MaxFro	0	ParSqu	0	ParSqu	0	PalFro	1	MaxFro	0	NasFro	0
FroLac	0	JugSqu	0	JugSqu	0	NasNas	0	FroLac	0	ParSqu	0
MaxPal	0	MaxFro	0	MaxFro	0	NasFro	0	MaxPal	0	FroSqu	0
MaxPal	0	FroLac	0	FroLac	0	ParSoc	0	MaxPal	0	JugSqu	0
PalPal	0	MaxPal	0	MaxPal	0	ParSqu	0	PalPal	0	MaxFro	0
BocBsp	0	MaxPal	0	MaxPal	0	JugSqu	0	BocBsp	0	FroLac	0
PalFro	0	PalFro	0	PalPal	0	FroLac	0	PalFro	0	MaxPal	0
PalLac	0	PalLac	0	PalFro	0	MaxPal	0	PalLac	0	MaxPal	0
MaxNas	0	MaxNas	0	PalLac	0	PalPal	0	MaxNas	0	PalPal	0
ParSoc	–	ParSoc	–	MaxNas	0	MaxNas	0	SquEoc	–	PalFro	0
SquEoc	–	SquEoc	–	SquEoc	–	SquEoc	–	FroSqu	–	MaxNas	0
FroSqu	–	FroSqu	–	FroSqu	–	FroSqu	–	FroJug	–	SquEoc	–
FroJug	–	FroJug	–	FroJug	–	FroJug	–	ParSoc	–	FroJug	–

Echymipera		Isoodon		Phascolarctos		Spilocuscus		Macropus		Dorcopsis	
BocEoc	8	BocEoc	8	BocEoc	6	BocEoc	7	BocEoc	7	SocEoc	6
ParPar	6	ParPar	6	SocEoc	4	SocEoc	6	SocEoc	5	BocEoc	6
FroFro	1	SocEoc	1	PalPal	1	ParPar	5	BocBsp	3	NasNas	0
NasNas	0	NasNas	0	NasNas	0	ParSoc	5	ParSoc	1	NasFro	0
NasFro	0	NasFro	0	NasFro	0	BocBsp	4	NasNas	0	FroFro	0
FroPar	0	FroFro	0	FroFro	0	FroPar	3	NasFro	0	FroPar	0
ParSoc	0	FroPar	0	FroPar	0	SquEoc	3	FroFro	0	ParPar	0
SocEoc	0	ParSoc	0	ParPar	0	FroFro	2	FroPar	0	ParSqu	0
ParSqu	0	ParSqu	0	ParSqu	0	ParSqu	2	ParPar	0	JugSqu	0
FroSqu	0	FroSqu	0	JugSqu	0	MaxPal	1	ParSqu	0	MaxFro	0
JugSqu	0	JugSqu	0	MaxFro	0	NasNas	0	JugSqu	0	FroLac	0
MaxFro	0	MaxFro	0	FroLac	0	NasFro	0	MaxFro	0	MaxPal	0
FroLac	0	FroLac	0	MaxPal	0	JugSqu	0	FroLac	0	MaxPal	0
MaxPal	0	MaxPal	0	MaxPal	0	MaxFro	0	MaxPal	0	PalPal	0
MaxPal	0	MaxPal	0	BocBsp	0	FroLac	0	MaxPal	0	BocBsp	0
PalPal	0	PalPal	0	PalFro	0	MaxPal	0	PalPal	0	PalFro	0
BocBsp	0	BocBsp	0	MaxNas	0	PalPal	0	PalFro	0	MaxNas	0
PalFro	0	PalFro	0	ParSoc	–	PalFro	0	MaxNas	0	ParSoc	–
PalLac	0	PalLac	0	SquEoc	–	MaxNas	0	SquEoc	–	SquEoc	–
MaxNas	0	MaxNas	0	FroSqu	–	FroSqu	–	FroSqu	–	FroSqu	–
SquEoc	–	SquEoc	–	FroJug	–	FroJug	–	FroJug	–	FroJug	–
FroJug	–	FroJug	–	PalLac	–	PalLac	–	PalLac	–	PalLac	–

Fig. 6. The sequence of cranial suture and synchondrosis closure: one column for each of the studied marsupial species from top (first suture to close) to bottom (last suture to fuse), on white background sutures and synchondroses that do not close in any of the studied specimens. Abbreviations and color-coding in Figure 3. For each suture, we indicate the numbers of specimens with the suture closed for that particular species.

among sampled Boreoeutheria, with Afrotheria also showing high levels; 2) the maximum closure levels are non-significantly correlated to body mass; 3) the basioccipito-basisphenoid and basioccipito-exoccipital synchondroses are the only ones that close in all species; the supraoccipito-exoccipital synchondrosis and the inter-parietal suture close in most species; 4) the first five sutures to obliterate are almost in all cases the same; 5) the amount of intraspecific variation differs among species being exceptionally high among xenarthrans and excep-tionally low among afrotherians. The comparison of placentals with marsupials reveals a lower level of closure of the latter. The exceptions to the trends within each clade are revealing of potential biome-chanical factors influencing suture closure level, as described below.

Suture Closure Level and Size

Bärmann and Sánchez-Villagra (2012) hypothes-ised that thinner skull bones in smaller species may be functionally coupled with lower closure

levels. Likewise, they reported a significant positive correlation between closure level and body mass in artiodactyls (Bärmann and Sánchez-Villagra, 2012). In contrast, a weak and non-significant negative correlation between suture closure and skull length (a common proxy of body mass) was reported across Carnivora (Goswami et al., 2013). In the present study, we only found a clear correlation between size and closure level in armadillos, a clade with several species of different sizes but similar ecology.

The giant golden mole *Chrysospalax trevelyani* has the highest closure level of all the species studied. *C. trevelyani* represents the most obvious outlier concerning closure level and is also the only fully fossorial species in our sample. Most likely associated with their burrowing behaviour (Wake, 1993), the peccaries *Dicotyles tajacu* and *Tayassu pecari* (Herring, 1974) exhibit high levels of fusion, consistent with the golden mole condition. Apart from *Chrysospalax trevelyani*, only xenarthrans close more than 30% of the 22 sutures studied. Large numbers of closed sutures occur in two sloths (*Bradypus tridactylus* and *Choloepus didactylus*). High closure level also occurs in the two largest species studied: the elephants *Elephas maximus* and *Loxodonta africana*. The massive size and strength of the elephant and the mechanical use of some elements such as tusks and the trunk produce great stress on the skull (Laursen and Bekoff, 1978; Shoshani and Eisenberg, 1982).

Compared to other paenungulates, sirenians (*Dugong dugon*, *Trichechus senegalensis* and *Trichechus inunguis*) show very low closure levels; similarly, the closure level of the common seal (*Phoca vitulina*) is lower than that of the studied terrestrial carnivore species (*Panthera pardus*). Bärmann and Sánchez-Villagra (2012) reached analogous conclusions by comparing the pattern of suture closure of four species of cetaceans with their artiodactyl relatives. They showed that only three synchondroses and one suture studied here close in the common dolphin *Delphinus delphis*, the pilot whale *Globicephala melas* and the rough-toothed dolphin *Steno bredanensis*: the basioccipito-basisphenoid, the basioccipito-exoccipital, the supraoccipito-exoccipital and the parieto-supraoccipital. Goswami et al. (2013) also found that most pinnipeds showed lower levels of suture closure than terrestrial carnivorans, with the most extreme example being the elephant seal, *Mirounga leonina*, in which only three sutures close fully. However, a large amount of variation across pinnipeds was evident, with the walrus, *Odobenus rosmarus*, displaying one of the highest levels of suture closure among carnivorans, possibly coupled with their skull high bone density.

The overall conclusion of this study supports higher levels of suture closure in atlantogenatan than in boreoeutherian placentals. This has the caveat that the sampling excludes some boreoeutherians of high closure levels, including some rodents (Wilson and Sánchez-Villagra, 2012) and some carnivorans (Goswami et al., 2013). However, the high levels of closure found in xenarthrans are clear and uncontested.

Sequence and Heterochrony

We detected more heterochronic shifts for species, genera and families than for high-level clades, a result that mirrors the patterns observed in previous studies focusing on sequence heterochrony in tetrapods (Werneburg et al., 2009; Weisbecker and Mitgutsch, 2010; Hautier et al., 2011; Bärmann and Sánchez-Villagra, 2012; Goswami et al., 2013). The Parsimov analysis identified only a few heterochronies among the major groups of mammals examined (Table 4), except for the Boreoeutheria and the Laurasiatheria. This method has been shown to be overly conservative and to suffer from low sequence resolution and accumulation of ties (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, 2013). Given the variable resolution across species in our dataset and given the differences in the sample sizes, we acknowledge that the Parsimov results obtained should be treated as one of many measures of heterochrony. With these qualifications in mind, it is noteworthy here that Parsimov retrieved more consistent heterochronic shifts for southern placental mammals (Afrotheria and Xenarthra) than for their northern relatives (Laurasiatheria and Euarchontoglires).

In most of the species previously examined (Brunner et al., 2004; Wilson and Sánchez-Villagra, 2009; Sánchez-Villagra, 2010; Bärmann and Sánchez-Villagra, 2012; Goswami et al., 2013; Segura, 2013) a subgroup of sutures/synchondroses, that is, the interoccipital, the occipito-sphenoid, the occipito-parietal and the interparietal, were among the first five to close (with the exception of the occipito-sphenoid in carnivorans) and greater variability was observed in the later sequence, which is consistent with this study.

The bones of the base of the skull are usually the earliest to close their synchondrosis (basioccipital-exoccipital, supraoccipital-exoccipital and basioccipital-basisphenoid). The two elephant species show remarkable exceptions in the sequence of cranial suture closure. The African elephant *Loxodonta africana* is the only studied species in which the supraoccipito-exoccipital synchondrosis is not among the first five bone intersections to close. The basioccipito-exoccipital synchondrosis closes fifth or sixth in *L. africana* and fourth to seventh in the asian elephant *Elephas maximus*.

This synchondrosis closes very early in the other studied species.

Intraspecific Variation

The low intraspecific variation of most afrotherians may be due to the fact that they fuse only few sutures. However, species that close more sutures can show low variation indices (e.g., *Erinaceus europaeus*, *Marmota marmota*), while others with similar amounts of suture closure have very different variation indices (e.g., sloths, elephants).

In contrast, xenarthrans show high intraspecific variation. De Moraes-Barros et al. (2011) reported inconsistencies regarding *Bradypus* taxonomic attribution that could explain part of the sloth intraspecific variation reported here. However, they showed that almost all misidentified specimens were *B. variegatus* erroneously attributed to *B. tridactylus*, while we observed a high intraspecific variation accross all sloth species. Several studies have recently shown increased variation in morphology of extant sloth species in comparison to other living mammalian species (Buchholtz and Stepien, 2009; Billet et al., 2012). Billet et al. (2012) hypothesised that the extreme low speed and lack of movement in sloths could induce reduced functional demand on the semi-circular canals, which detect rotational acceleration of the head, and decrease evolutionary constraints allowing for phenotypic variation. Here, we describe an increased intraspecific variation throughout the sequence of cranial suture closures comparable to that seen in the inner ear. By extending the variation to parts of skulls other than the inner ear, these results seem to contradict Billet et al. (2012) functional interpretations that reduced demands relieves selection pressure, allowing an increase in variation. However, contrary to the inner ear variation, the presence of intraspecific variation in the sequence of cranial suture closure was not restricted to sloths, the highest intraspecific variation being recorded among two anteater genera (the tamandua *Tamandua tetradactyla* and the giant anteater *Myrmecophaga tetradactyla*). Anteaters are toothless and feed mainly by using their tongue musculature (Naples, 1999) and this could partly explain the lack of conserved pattern in their closure sequence. However, both the pygmy anteater *Cyclopes didactylus* and the myrmecophagous afrotherian aardvark *Orycteropus afer* show lower levels of intraspecific variation. The smaller *C. didactylus* has thinner skull bones, with fewer sutures to close with consequent less intraspecific variation. *O. afer* has a more generalised diet than the xenarthran anteaters and shows less intraspecific variation than other afrotherians.

No single factor fully explains the differences in intraspecific variation. The amount of intraspecific variation in the sequence of cranial suture closure is distributed on phylogenetic lines: exceptionally high in many xenarthran species, exceptionally low in many afrotherian species and medium in most northern placental mammal species. There is a similarity between the distribution of variation in the sequence of cranial suture closure and variations in the vertebral counts among mammalian clades: the variation in thoracolumbar vertebral count is higher in Xenarthra than in Afrotheria (Asher et al., 2011).

Comparison between Placentals and Marsupials

On average, placental species showed more sutures closed (20.6%) than marsupials (11.97%). Our observations agree with Lowrance (1949) who noticed that in the opossum *Didelphis virginiana* linear skull dimensions vary more than in the placentals he studied. The sutures in marsupials could remain for a longer period of time or even throughout life as sites of interstitial growth (Lowrance, 1949). Both marsupials and placentals exhibit interspecific variation in the sequence of cranial suture closure and heterochronic shifts were clearly detected in lower level clades and within species. The sequence classically accepted for placentals (vault, base, circum-meatal, palatal, facial, cranio-facial; Krogman, 1930) is variable for the sampled placentals and marsupials. Alternatively, we found that the interoccipital synchondroses at the base of the skull obliterate first in all mammalian groups starting by the basioccipito-exoccipital synchondrosis and reveals a common pattern (Segura, 2013).

The Tasmanian devil *Sarcophilus* is the largest extant carnivorous marsupial (8.35 kg mean adult body mass). It is a specialised osteophage that can consume a whole carcass including teeth, bones and fur (Owen and Pemberton, 2005). *Sarcophilus* can produce the highest bite pressure among living mammals studied to date (Attard et al., 2011) which induces high stress levels in the skull. *Sarcophilus* has a high number of closed sutures (19.32%) and this number is slightly larger than the values found in *Thylacinus cynocephalus* (18.59%), the recently extinct Tasmanian wolf with about three times larger body-mass (30 kg). Our data for placentals demonstrate that there is no significant correlation between the number of closed sutures and body mass, which is also the case for marsupials.

Sutures and Functional Aspects

A functional influence on the timing and level of suture closures is expected to be related to mastication (Herring, 1972, 1974; Jaslow, 1990; Jaslow and Biewener, 1995; Flores et al., 2006; Cray et al., 2008; Rice, 2008; Moazen et al., 2009;

Goswami et al., 2013). Perhaps sutural patterns are not directly selected for but rather reflect over-all osteogenicity related to biomechanical factors. Using computer models of lizards Moazen et al. (2009) showed that stresses increased in skulls with increased number of closed sutures, suggesting that open sutures mitigate the stress on the skull. Bright (2012; see also Wang et al., 2010) found that in pigs the fit of finite element models to experimental data does not improve with the inclusion of suture data. It seems that a productive approach would be a comparative/phylogenetic one (Attard et al., 2011; Cray et al., 2012) in which finite element modeling is used in closely related species with differing sutural patterns.

The material properties of bone (Blob and Snelgrove 2006) including thickness at different areas of the skull (Sun et al., 2004) and the geometry of the suture (Miura et al., 2009; Jasinoski et al., 2010) may be more important predictors of function than simple overall degree of suture closure is. We did not gain insights about the function of sutures but the large amount of data presented in this systematic fashion study provides patterns in phylogeny that functional biologists can use to formulate hypotheses and tests on them.

CONCLUSIONS

The number of closed sutures is not proportional to species body mass, perhaps because of the multiple variables involved in such a relation. Furthermore, size may play a role only within some ranges of body size when a mechanical threshold is crossed (Meier et al. 2013). The timing patterns of some suture closures can serve to characterise some clades, but the sequence pattern of closure of areas of the skull is not shared among clades. Intraspecific variation in the specific sequence of cranial suture closure was found to be high among xenarthrans, and exceptionally low among afrotherians. This result is inconsistent with the Atlantogenata hypothesis and mirrors the findings of Hautier et al. (2013) on sequence of skeletal ossification. Compared to their placental relatives the marsupial species show a low number of closed sutures. Aquatic forms such as sirenians and seals exhibit low levels of suture closure. Species with very specialised dietary behaviour within their clades are outliers in degree of suture closure, demonstrating that suture closure has a functional component.

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