



Craniodental Allometry, Prenatal Growth Rates, and the Evolutionary Loss of the Third Molars in New World Monkeys

TESLA A. MONSON ^{1,2,3*} JEFFREY L. COLEMAN,¹ AND LESLEA J. HLUSKO^{1,2,3}

¹Department of Integrative Biology, University of California, Berkeley, California

²Museum of Vertebrate Zoology, University of California, Berkeley, California

³Human Evolution Research Center, University of California, Berkeley, California

ABSTRACT

A growing body of literature demonstrates that genetic patterning mechanisms underlie the relative proportions of the mammalian postcanine dentition with the third molar being key to understanding variation within the molar row. With this relatively recent insight, there has been renewed interest in mammalian taxa that have lost the third molars. Within platyrrhines, the marmosets and tamarins (*Callitrichidae* family) are characterized by small body size, claw-like nails, twinning, and reduced molar number. Small body size is hypothesized to have resulted in the third molar being crowded out of the jaws leading to its evolutionary loss in this family. To further explore this hypothesis, we measured the cranium and dentition of 142 individuals spanning all five platyrrhine families. These data reveal that callitrichids have a significantly smaller proportion of mandibular postcanine tooth row length relative to other platyrrhines, refuting the “crowding out” hypothesis. However, postcanine tooth row length is significantly correlated with mandibular length and cranial length ($P < 0.01$) across all platyrrhines providing evidence for a strong allometric association between postcanine tooth row length and body size more generally. The small body size that characterizes callitrichids results in part from slower prenatal growth rates. Given the allometric relationship between postcanine tooth row length and body size, reported here and in previous studies, we hypothesize that the evolutionary loss of the third molars in callitrichids results from the inhibition of third molar development as a consequence of the slower prenatal growth rates associated with small body size in this family. *Anat Rec*, 302:1419–1433, 2019. © 2018 Wiley Periodicals, Inc.

Key words: *Callitrichidae*; dentition; Platyrrhini; body size; twinning

INTRODUCTION

Over the last decade, it has become increasingly apparent that genetic patterning mechanisms underlie the phenotypic patterning of the mammalian dentition with the

third molar playing an important role in discerning the evolution of dental proportions in mice (Kavanagh et al., 2007) and primates (Hlusko et al., 2016). The third molars are the last of the postcanine teeth to develop in

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*Correspondence to: Tesla A. Monson, 3040 Valley Life Sciences, University of California, Berkeley, CA. E-mail: tesla.monson@berkeley.edu

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primates (e.g., Teaford et al., 2000; Swindler, 2002), and developmental work in mice has shown that the third molars are stimulated to develop through timed signals released when the more anterior teeth are nearing maturity (Kavanagh et al., 2007). These insights have led to a renewed interest in mammals that have lost the third molars over the course of their evolution and, particularly, how our understanding of these genetic patterning mechanisms may elucidate the evolutionary processes involved in reducing tooth number (Asahara, 2013; Bernal, 2013; Scott, 2015).

Marmosets and tamarins (Callitrichidae family) are characterized by the loss of the third molars in almost every species in the family, the exception being *Callimico goeldii*, Goeldi's marmoset. Callitrichids have also been noted to have relatively simpler molars (lacking development of the hypocone) as well as less-developed deciduous and permanent dentition at birth compared to other platyrrhines, and while *Callimico* has retained, or secondarily derived, third molars, these teeth are relatively simple like the molars in other callitrichids (Martin, 1992; Smith et al., 2015). Fossil and molecular evidence support the evolutionary origins of the Callitrichidae family in the early Miocene, ~20 million years ago (Ma), with seven extant genera currently recognized: *Callibella*, *Callimico*, *Callithrix*, *Cebuella*, *Leontopithecus*, *Mico*, and *Saguinus* (van Roosmalen and van Roosmalen, 2003; Rylands et al., 2012; Garbino, 2015; Schneider and Sampaio, 2015).

Other unique characteristics of the Callitrichidae family relative to other anthropoids include claw-like nails and the evolution of twinning—having two offspring in each birth (again, the exception being *C. goeldii*, Altmann et al., 1988; Rosenberger et al., 1990; Martin, 1992; Kay, 1994; Nowak, 1999; Montgomery and Mundy, 2013; Scott, 2015). Some researchers have hypothesized that the rare phenotypes seen in callitrichids (reduction of molar number, claw-like nails, and twinning) are adaptations to reductions in body size, or dwarfing (Ford 1980, 1986; Leutenegger, 1980; Rosenberger 1981, 1984; Martin, 1992). It has also been argued that the reduction of molar complexity in callitrichids is related to decreases in body size (Ford, 1980; Martin, 1992).

Third molar loss is exceptionally rare in primates (e.g., Swindler, 2002). Outside of extant callitrichids, one fossil platyrrhine, *Xenothrix*, is the only other example of evolutionary third molar loss in primates with debates about the phylogenetic placement of this extinct genus ongoing (MacPhee and Horowitz, 2004; Cooke et al., 2017; Woods et al., 2018). Debates center around orbital shape and dental morphology in these two taxa, but many studies agree that the dental formulas shared between *Xenothrix* and extant callitrichids are convergent (Rosenberger, 1977; Rosenberger et al., 1990; MacPhee and Horowitz, 2004).

Despite there being a number of fossils recovered that have been uncontroversially assigned to the callitrichid lineage (Szalay and Delson, 1979; Setoguchi and Rosenberger, 1985; Meldrum and Kay, 1997), the lack of complete dentitions for these specimens has made dating the timing of the evolutionary loss of the third molars difficult. Given the lack of fossil evidence, researchers have proposed hypotheses for the evolutionary loss of the third molars in callitrichids that focus on understanding variation in extant platyrrhine taxa. The most cited hypothesis

for the evolutionary loss of the third molars is the “crowding out” hypothesis, originally proposed by Ford (1980), which draws on the idea that phyletic nanism, or a significant decrease in body size, is correlated with a shortening of the face and mandible (Kanazawa and Rosenberger, 1988). The “crowding out” hypothesis builds off of Gould's (1975) work demonstrating a negatively allometric relationship between postcanine tooth area and body mass in dwarfed mammals, where body mass decreases more quickly than tooth size in dwarfed animals relative to their larger evolutionary ancestors, and proposes that the loss of the third molars in callitrichids is an adaptive response to rapid body size reductions leading to a postcanine occlusal surface area that maintains the same relative proportion with body size as is seen in larger animals (Ford, 1980; Martin, 1992). This hypothesis has also been applied to human evolution as an explanation for variable third molar agenesis in some populations (Kömerik et al., 2014).

Agenesis of the third molar has been reported in many humans (Garn and Lewis, 1962), although these incidents have been linked to specific genetic mutations (Haga et al., 2013; AlFawaz et al., 2015; Carter and Worthington, 2015). There is evidence for increased impaction of third molars in humans (Mucci, 1982), and some researchers hypothesize that human jaw size has decreased more quickly than tooth size (e.g., Inoue, 1980). Comparable to the “crowding out” hypothesis for platyrrhines (Ford, 1980), some researchers have hypothesized that jaw reduction and overcrowding of the dentition may be selective pressures for reduction of tooth number and tooth size in humans (e.g., Kanazawa and Rosenberger, 1988).

Earlier tests of Gould's (1975) hypothesis and the “crowding out” hypothesis (Ford, 1980) focused on associations between body size and postcanine tooth area (Martin, 1992; Plavcan and Gomez, 1993a,b), as well as comparisons of individual molar proportions to mandibular length (Kanazawa and Rosenberger, 1988), and tooth row length relative to palatal length (Scott, 2015). These studies provide important insights into allometric relationships between body size and postcanine tooth size as well as between molar proportions and mandibular length, but the majority of them skirt around the question of “crowding out” by not directly testing the available space in the cranium or mandible as associated with postcanine tooth number and length. Our study differs from previous work in taking into account cranial integration (e.g., Ackermann and Cheverud, 2004) by analyzing postcanine tooth row length within the context of the entire skull (cranial length and mandibular length) while also considering the maxilla and mandible separately, as these structures of the skull develop independently, and mandibular versus maxillary tooth row proportions may have different signals in platyrrhines (Cordero et al., 2011). Our study tests the “crowding out” hypothesis in the Callitrichidae family by comparing measurements of the postcanine tooth row to cranial and mandibular lengths in a large sample of primates spanning New World monkeys. We collected dental and craniomandibular data from 16 genera of extant platyrrhines to test two hypotheses that capture the predictions of the “crowding out” hypothesis: (1) callitrichids and other platyrrhines have statistically indistinguishable postcanine tooth row length proportions relative to cranial and mandibular lengths, and (2) tooth row length

scales with negative allometry against cranial and mandibular lengths (i.e., cranial and mandibular lengths decrease at a more rapid rate than tooth row length).

MATERIALS AND METHODS

Materials

Platyrrhines are a diverse clade of anthropoid primates spanning a range of body sizes and diets and found exclusively in Central and South America (e.g., Nowak, 1999). For this study, we examined the skeletal remains of 142 individuals representing 35 species and 16 genera of New World monkey (NWM) held in the collections of the Museum of Vertebrate Zoology (MVZ), the California Academy of Sciences (CAS), and the National Museum of Natural History (NMNH) in Washington DC (Table 1). To compare proportions of postcanine tooth row length, we

took four linear craniodental measurements for each individual: cranial length, mandibular length, maxillary post-canine tooth row length, and mandibular postcanine tooth row length.

A subset of these specimens (n = 34) were measured from photographs. All photographs were taken by TAM, in standard occlusal view, according to published protocols (Grieco et al., 2013), and the measurements were taken by JLC in ImageJ v1.48 (Rasband, 2016). Before including these photographed specimens in the sample, we conducted an intermethodology error test to confirm that measurements taken from specimens were not significantly different from measurements taken from photographs using ImageJ. JLC photographed n = 10 specimens at the MVZ using the standardized photograph protocols and measured them in ImageJ using the same cranial measurement definitions as for handled specimens. These

TABLE 1. Primate species sampled for this study^a

Family	Genus	Species	N	Repository
Aotidae	<i>Aotus</i>	<i>nigriceps</i>	1	MVZ
		<i>trivirgatus</i>	1	MVZ
		<i>vociferans</i>	12	MVZ
		Total	14	
Atelidae	<i>Alouatta</i>	<i>caraya</i>	1	CAS
		<i>palliata</i>	4	MVZ
		<i>pigra</i>	1	CAS
		<i>seniculus</i>	4	MVZ
	<i>Ateles</i>	<i>belzebuth</i>	1	CAS
		<i>geoffroyi</i>	14	MVZ
		spp.	1	MVZ
	<i>Lagothrix</i>	<i>lagotricha</i>	2	CAS, MVZ
		<i>poepigii</i> ^b	1	MVZ
		Total	29	
Callitrichidae	<i>Callithrix</i>	<i>jacchus</i>	8	MVZ, NMNH
		<i>penicillata</i>	7	CAS, MVZ, NMNH
		spp.	1	NMNH
		Total	16	
	<i>Cebuella</i>	<i>pygmaea</i> ^b	5	MVZ
		<i>rosalia</i>	10	NMNH
		<i>argentatus</i> ^b	6	NMNH
	<i>Leontopithecus</i>	<i>humeralifer</i> ^b	1	NMNH
		<i>intermedius</i>	1	NMNH
		<i>melanurus</i>	4	NMNH
		<i>fuscicollis</i>	2	MVZ
		<i>geoffroyi</i>	1	CAS
		<i>imperator</i>	3	MVZ
		<i>oedipus</i>	6	CAS, MVZ
	spp.	1	CAS	
Total	56			
Cebidae	<i>Cebus</i>	<i>albifrons</i>	6	MVZ
		<i>capucinus</i>	2	MVZ
		<i>olivaceus</i>	2	MVZ
	<i>Saimiri</i>	<i>boliviensis</i>	1	MVZ
		<i>sciureus</i>	7	CAS, MVZ
	<i>Sapajus</i>	<i>apella</i> ^b	4	MVZ
		Total	22	
Pitheciidae	<i>Cacajao</i>	<i>calvus</i>	2	CAS, MVZ
		<i>cupreus</i>	12	MVZ
	<i>Callicebus</i>	<i>moloch</i>	2	MVZ
		<i>satanas</i>	1	MVZ
	<i>Chiropotes</i>	<i>monachus</i>	2	MVZ
		<i>pithecia</i>	2	MVZ
	Total	21		
	TOTAL		142	

^aAbbreviations as follows: N is sample size, MVZ is Museum of Vertebrate Zoology, CAS is California Academy of Sciences, NMNH is National Museum of Natural History.

^bSuperscript indicates taxa with an alternate genus-level designation in the 10kTrees phylogenetic tree database (Arnold et al., 2010).

TABLE 2. Descriptions of measurements taken for this study^a

Measurement	Definition
Maxillary postcanine tooth row length	Maximum length of the maxillary postcanine tooth row, from the anterior of the second premolar to the posterior of the distal molar (M ³ in most taxa, M ² in some Callitrichidae).
Mandibular postcanine tooth row length	Maximum length of the mandibular postcanine tooth row, from the anterior of the second premolar to the posterior of the distal molar (M ₃ in most taxa, M ₂ in some Callitrichidae).
Mandibular length	The length of the mandible, from the superior infradentale to the midpoint of the superior left condyloid.
Calvarial length	The length of the calvarium, from the midpoint of the frontonasal suture to the inion.
Cranial length	Maximum cranial length, from prosthion to inion.

^aAll measurements were taken by JLC. Abbreviations as follows: M is molar, number indicates tooth position, superscript indicates maxillary, subscript indicates mandibular. E.g., M³ is maxillary third molar.

measurements from the photographs were statistically compared to measurements taken with calipers on the same specimens at the MVZ using a *t* test in the R statistical environment (R Core Team, 2016). The four craniodental measurements were taken by JLC three times, and the average of the measurements was used in the analyses. All measurements were taken with Mitutoyo digital calipers, and definitions for all measurements are available in Table 2.

Analytical Methods

We ran a series of analyses to assess variation in platyrrhine craniodental morphology. All statistical analyses were run in R v3.1.2 (R Core Team, 2016), and all measurements were size corrected using geometric mean (GM) adjustment prior to analysis. GM adjustment was calculated by dividing the value of each trait (for each individual) by the GM of that trait value across all individuals sampled (calculated using the *geom* function in Microsoft Excel). The GM adjustment gives values that are relative to the sample average for each phenotype, centered around a value of 1 (greater than 1 is larger than the sample average across all genera, smaller than 1 is smaller than the sample average across all genera).

First, we ran descriptive statistics on all cranial and dental measurements using the *describeBy* function in *psych* (Revelle, 2017). Next, we calculated the relative proportions of postcanine tooth row using two ratios: maxillary postcanine tooth row length to cranial length, and mandibular postcanine tooth row length to mandibular length. In order to more directly compare cranial and mandibular lengths with the postcanine tooth row, we limited our measurements to lengths and did not collect data on tooth surface area. The ratios of the postcanine

tooth row describe the proportion of the cranium or mandible that is occupied by the postcanine tooth row length, or how much of the cranial and mandibular lengths are dedicated to chewing (as a product of tooth length).

Family-level differences were analyzed with boxplots using the *qplot* function in *ggplot2* (Wickham, 2009), ANOVA using the *aov* function in *psych* (Revelle, 2017), and phylogenetic ANOVA using the *aov.phylo* function in *geiger* (Harmon et al., 2016). Phylogenetic ANOVA tests statistically compare the averages of the operational taxonomic units of interest while taking into account the structure of the phylogenetic tree (Harmon et al., 2016). The phylogenetic tree for sampled platyrrhines was built using molecular data (14 mitochondrial and 6 nuclear genes) from the 10kTrees database (Arnold et al., 2010) and was visualized in R using *phytools* (Revell, 2012). We compared family-level variation in relative proportions of postcanine tooth row length (maxillary and mandibular) across our five sampled platyrrhine families using species averages for the phenotypic data and representative species for the molecular data (one per family), a requirement for the method.

Next, we looked at the relative proportions of postcanine tooth row length, comparing mandibular postcanine tooth row length to mandibular length, and maxillary postcanine tooth row length to cranial length, in a bivariate regression. To test for correlation within a phylogenetic framework, we ran phylogenetic regressions using Phylogenetic Generalized Least Squares (PGLS) in *caper* (Orme et al., 2015). The PGLS method takes into account nonindependence of trait values from phylogenetic structure through the joining of species averages of the phenotypes of interest and a phylogeny with branch lengths (Orme et al., 2015). The results of the PGLS analysis give the fit of the linear model, here the fit of postcanine tooth row length to cranial or mandibular length, as the coefficient of determination (R^2) as well as the slope and intercept of the best-fit line (Orme et al., 2015). We also ran correlations between traits (r) calculated using *rcorr* in *Hmisc* (Harrell and Dupont, 2012). Both correlation (r) and PGLS (R^2) analyses detail the relationship between the traits of interest ($r = \sqrt{R^2}$), and therefore both were included here.

To test for allometry, the traits were log-transformed and plotted in log-log space with the regression line generated by the PGLS analyses plotted over the bivariate graph. In log-log space, a slope greater than 1 indicates positive allometry, a slope of 1 indicates isometry, and a slope of less than 1 indicates negative allometry (Nunn and Barton, 2001). We ran PGLS for the entire sample to generate a phylogenetic regression for platyrrhines as well as for callitrichids and non-callitrichids separately. We then ran an ANCOVA to compare slopes of callitrichids and non-callitrichids using the *aov* and *anova* functions (Chambers and Hastie, 1992; Revelle, 2017). Additionally, we visualized craniofacial variation in platyrrhines using principle components analysis (PCA) with the *prcomp* function in *psych* (Revelle, 2017).

As a way to further investigate the evolution of third molar loss in callitrichids in light of the limited platyrrhine fossil record, we ran an ancestral state reconstruction (ASR) in Mesquite using the Mk1 likelihood model (Maddison and Maddison, 2015) and the phylogenetic tree from 10kTrees (Arnold et al., 2010). All species in our sample are available in the 10kTrees database except

TABLE 3. Descriptive statistics by platyrrhine family^a

Family	Stat.	RXPC	LXPC	RDPC	LDPC	Man. length	Cranial length
Aotidae	N	11	12	11	12	12	14
	mean	14.2	14.2	15.8	15.8	38.7	60.3
	SD	0.5	0.5	0.4	0.5	1.2	1.5
	range	1.9	1.8	1.4	1.7	4.1	4.7
	SE	0.2	0.1	0.1	0.1	0.4	0.4
Atelidae	N	20	20	25	25	29	29
	mean	26.6	27.6	30.4	30.9	75.7	108.5
	SD	4.3	5.0	5.7	5.9	9.6	6.6
	range	12.1	15.4	18.9	18.1	38.0	26.7
	SE	1.0	1.1	1.1	1.2	1.8	1.2
Callitrichidae	N	53	52	52	54	53	56
	mean	9.4	9.4	10.7	10.7	31.0	45.3
	SD	1.6	1.6	1.8	1.7	4.2	5.9
	range	7.1	7.0	7.1	6.8	18.7	25.9
	SE	0.2	0.2	0.2	0.2	0.6	0.8
Cebidae	N	12	15	17	16	22	22
	mean	16.6	17.7	20.5	20.2	49.7	80.4
	SD	4.3	4.4	4.8	4.8	12.0	14.1
	range	10.1	11.1	13.0	11.8	36.3	43.8
	SE	1.2	1.1	1.2	1.2	2.6	3.0
Pitheciidae	N	17	15	18	16	19	21
	mean	16.9	16.9	18.9	19.0	47.4	69.0
	SD	2.3	2.5	2.8	3.0	9.8	12.3
	range	7.2	7.3	9.6	9.9	33.0	41.2
	SE	0.6	0.6	0.7	0.7	2.2	2.7

^aAbbreviations as follows: Stat. is statistic, N is sample size, SD is standard deviation, SE is standard error, RXPC is right maxillary postcanine tooth row length, LXPC is left maxillary postcanine tooth row length, RDPC is right mandibular postcanine tooth row length, LDPC is left mandibular postcanine tooth row length, Man. length is mandibular length. All measurements are in millimeters.

Callicebus cupreus and *Pithecia monachus*. Additionally, as *C. goeldii* is an integral species in the investigation of third molar loss in callitrichids, we included this species in the ASR although it is not included in our craniodental metric dataset. The Mk1 likelihood model estimates the likelihood of a binary phenotypic state at each ancestral node in the phylogeny but is limited to a single transition rate. In order to account for the possibility of multiple transition rates, we also ran the ASR using the AsymmMk1 model (Maddison and Maddison, 2015). We then compared fit of the Mk1 model and the AsymmMk1 model using a likelihood ratio test in Mesquite (Maddison and Maddison, 2015). Species were coded as either a 0 or a 1, where 0 is the presence of three molars, and 1 is the presence of only two molars.

RESULTS

Callitrichids are the smallest New World monkeys in all aspects of their craniodental morphology (Table 3). Using standard ANOVA, the relative proportions of postcanine tooth row length are significantly lower in both the mandible and maxilla of callitrichids compared to other New World monkeys, moreso in the mandible (Fig. 1), consistent with what has been reported in previous studies (e.g., Natori, 2002; Scott, 2015). Using phylogenetic ANOVA, differences between proportions of maxillary postcanine tooth row length are insignificant across families, but callitrichids have a significantly lower proportion of mandibular postcanine tooth row length relative to mandibular length compared to other platyrrhines even when accounting for phylogeny. The proportion of mandibular

tooth row length to mandibular length is arguably a better test of the “crowding out” hypothesis than the proportion of maxillary tooth row length to the maxilla, because platyrrhine cranial length varies significantly, a result of functional and morphological adaptations in the calvarium and rostrum (Fleagle, 2013; Marroig and Cheverud, 2005, 2009), and we find that this mandibular phenotype is significantly smaller in callitrichids. This significant result reflects the fact that the postcanine tooth row is smaller in callitrichids relative to the mandible and cranium, likely related to the loss of the third molars, and refutes the predictions of the “crowding out” hypothesis. Atelidae has the highest proportions of postcanine tooth row length relative to cranial and mandibular lengths, likely driven by *Alouatta*, a genus with large teeth relative to cranial and mandibular lengths (Fig. 2).

Mandibular postcanine tooth row length is significantly correlated with mandibular length across platyrrhines ($r = 0.99, P < 0.001$) as is maxillary postcanine tooth row length with cranial length ($r = 0.97, P < 0.001$). There is also a strong fit between postcanine tooth row length and cranial/mandibular length along the regression line when assessed in a phylogenetic context (maxillary coefficient of determination, $R^2 = 0.89, P < 0.001$, mandibular coefficient of determination, $R^2 = 0.94, P < 0.001$), indicating that correlation between these traits is still very strong when phylogenetic relatedness is included in the model. The positive slopes reported for fit between maxillary postcanine tooth row length and cranial length ($y = 1.16 \times -0.93$), and mandibular postcanine tooth row length and mandibular length ($y = 1.12 \times -0.60$), indicate an allometric relationship

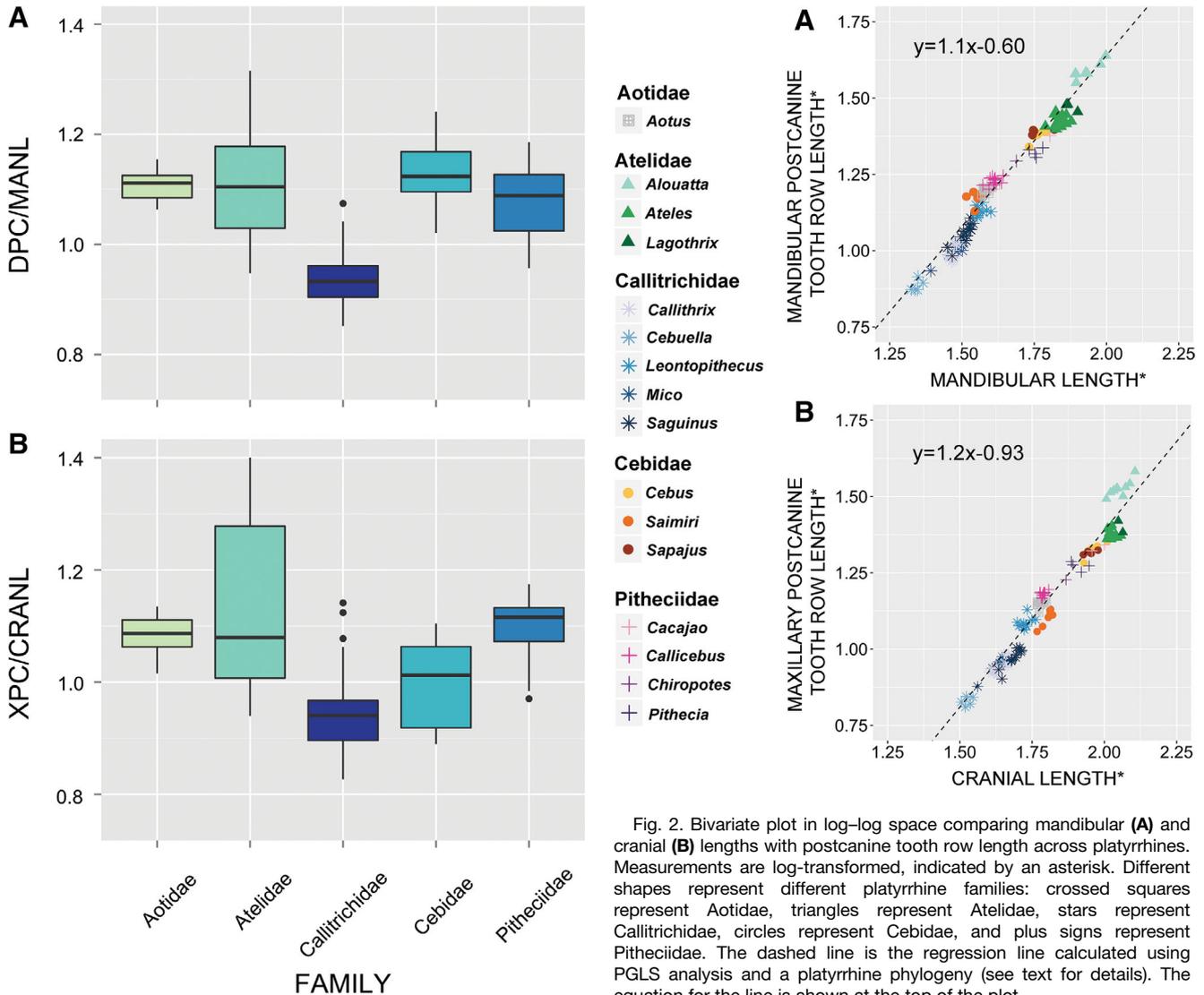


Fig. 1. Boxplot comparing relative proportions of mandibular (A) and maxillary (B) postcanine tooth row lengths across platyrrhines. Relative proportion of postcanine tooth row length is calculated as either the mandibular postcanine tooth row length (DPC) divided by mandibular length (MANL) or the maxillary postcanine tooth row length (XPC) divided by cranial length (CRANL). All measurements are geometric mean size corrected. Postcanine tooth row length was averaged across left and right sides. See Table 1 for sample sizes. Family Callitrichidae has a significantly lower relative proportion of mandibular postcanine tooth row length than all other platyrrhine families (ANOVA and phylogenetic ANOVA, $P < 0.05$), and a significantly lower relative proportion of maxillary postcanine tooth row length than all platyrrhine families except Cebidae (ANOVA, $P < 0.05$), driven by the relatively longer cranium in *Saimiri*. Callitrichids do not have statistically significantly lower proportions of maxillary postcanine tooth row length when compared using phylogenetic ANOVA ($P = 0.09$).

between these traits where postcanine tooth row length decreases more quickly than cranial or mandibular length, contrary to the predictions of the “crowding out” hypothesis (Fig. 2). We find that the slope of the regression between these traits is significantly different ($P = 0.04$) between callitrichids (maxilla: $y = 1.12 \times -0.90$, mandible: $y = 1.02 \times -0.49$) and

Fig. 2. Bivariate plot in log-log space comparing mandibular (A) and cranial (B) lengths with postcanine tooth row length across platyrrhines. Measurements are log-transformed, indicated by an asterisk. Different shapes represent different platyrrhine families: crossed squares represent Aotidae, triangles represent Atelidae, stars represent Callitrichidae, circles represent Cebidae, and plus signs represent Pitheciidae. The dashed line is the regression line calculated using PGLS analysis and a platyrrhine phylogeny (see text for details). The equation for the line is shown at the top of the plot.

non-callitrichids (maxilla: $y = 1.06 \times -0.74$, mandible: $y = 1.00 \times -0.24$), and is greater than 1, indicating that postcanine tooth row length is relatively shorter in platyrrhines with smaller crania and mandibles. While callitrichids have higher slopes than other platyrrhines, confidence intervals indicate that none of the slopes can be statistically distinguished from isometry except in the mandibular analysis of the platyrrhine sample as a whole (Table 4).

The GM size corrected measurements taken for this study are all higher in *Alouatta* than in other New World monkey taxa with the exception of cranial length, which is comparable to other genera of family Atelidae, indicating that the cranium is shorter relative to the mandible and postcanine tooth row length in *Alouatta* than in other platyrrhines (Fig. 3A–D). All calculated ratios of morphological measurements are also higher in *Alouatta* with the exception of cranial length to mandibular length which is much lower in *Alouatta* due to the positioning of the mandibular condyle and shortened cranium in this genus (Bruner et al., 2004, Fig. 3E). At the other end of the spectrum, the ratio of cranial length to mandibular

TABLE 4. Results of the PGLS analyses^a

Sample	Traits	Intercept (95% CI)	Slope (95% CI)
Platyrrhines	XPC vs. CRANL	-0.930 ± 0.307	1.158 ± 0.166
	DPC vs. MANL	-0.603 ± 0.193	1.118 ± 0.115
Aotidae, Atelidae, Cebidae, Pitheciidae	XPC vs. CRANL	-0.735 ± 0.557	1.058 ± 0.293
	DPC vs. MANL	-0.389 ± 0.283	1.001 ± 0.163
Callitrichidae	XPC vs. CRANL	-0.896 ± 0.358	1.124 ± 0.214
	DPC vs. MANL	-0.487 ± 0.230	1.020 ± 0.154

^aAbbreviations as follows: PGLS is phylogenetic generalized least squares, XPC is maxillary postcanine tooth row length, CRANL is cranial length, DPC is mandibular postcanine tooth row length, MANL is mandibular length, CI is confidence interval.

length is much higher in *Saimiri* indicating a relatively longer cranium compared to mandible in this genus (Fig. 3E).

Visualizing craniodental variation in PCA space demonstrates clear discrimination between genera with particularly tight clusters of *Cebuella*, *Callicebus*, and *Callithrix* (Fig. 4). At the family-level, there is still distinction between NWM families, but there is more overlap, particularly between Cebidae, Pitheciidae, and Aotidae. *Alouatta* is visualized separately from all other taxa in PC space giving Atelidae the widest distribution across PC space (Fig. 4). This distinction is driven by differences between *Alouatta* and other genera in cranial and mandibular lengths and maxillary and mandibular postcanine tooth row lengths (Fig. 2–3, Table 5). More than 98% of the variation in the PCA is explained by PC1, likely capturing differences in body size with Callitrichidae, the smallest family, sitting in negative PC1 space to the left of the graph and Atelidae sitting in positive PC1 space to the right. The loadings for PC1 are 0.50 for maxillary and mandibular postcanine tooth row lengths, mandibular length, and cranial length, indicating that all phenotypes have similar contributions to PCA variation. The loadings for PC2 are more variable: -0.45 and -0.48 for maxillary and mandibular postcanine tooth row lengths, respectively, 0.20 for mandibular length, and 0.73 for cranial length, with variation in Atelidae accounting for almost all variation in PC2.

Ancestral State Reconstruction

Our ASR supports that the ancestor of platyrrhines had three molars with greater than 99.0% likelihood and that the ancestor of Callitrichidae + *Aotus* had three molars with greater than 97.0% likelihood (Fig. 5, Node 1). In contrast, the ancestor of callitrichids is supported to have a reduced molar number with greater than 91% likelihood, a number that increases to greater than 99% likelihood in the ancestor of *Cebuella*, *Mico*, *Callithrix*, and *Callibella* (not sampled here) approximately 12 Ma (Opazo et al., 2006; Perelman et al., 2011). The ancestor of *Callimico* and other marmosets is also supported to have a reduced molar number with greater than 91% likelihood providing evidence to support the secondary evolution of the third molars in *Callimico* (see also: Scott, 2015). ASR using the AsymmMk1 model generates statistically similar results ($P = 0.342$), although the likelihood of the dominant phenotype at each numbered node within the Callitrichidae family (having reduced molar number) is higher using this model. A full list of likelihoods supported by the ASR is available in Table 6.

DISCUSSION

The proportion of postcanine tooth row length relative to mandibular length is significantly smaller in callitrichids refuting the predictions of the “crowding out” hypothesis. Additionally, while we find an allometric relationship between postcanine tooth row length and cranial and mandibular lengths that characterizes platyrrhines broadly, this relationship is isometric rather than negatively allometric further refuting the predictions of the “crowding out” hypothesis.

Our data indicate that cranial length and maxillary postcanine tooth row length, as well as mandibular length and mandibular postcanine tooth row length, are highly correlated across all platyrrhine families, including the callitrichids, adding evidence to the growing body of literature demonstrating allometry between traits of the skeleton and dentition (e.g., Gould, 1975; Martin, 1992; Hlusko et al., 2006; Copes and Schwartz, 2010). However, contrary to the assumptions of the “crowding out” hypothesis, the proportions of postcanine tooth row length relative to cranial and mandibular lengths are much smaller in callitrichids, driven by the reduced molar number in this family. Our data definitively show that the total length of the postcanine tooth row has not increased as a way to maintain a proportional chewing surface as proposed by the “crowding out” hypothesis. This is consistent with previous studies that have failed to find a relationship between available space in the jaw and timing of tooth development in primates (Boughner and Dean, 2004). However, it is possible that the postcanine teeth were already crowded in the past and there was selection for reduced tooth number in callitrichids. Given the paucity of callitrichid fossils with dental remains (Szalay and Delson, 1979; Setoguchi and Rosenberger, 1985; Meldrum and Kay, 1997), this hypothesis is currently untestable, but continuing paleontological excavations in Central and South America will hopefully contribute additional fossil materials that allow for more in-depth testing of this evolutionary hypothesis.

The tight correlation between postcanine tooth row length and cranial/mandibular length ($r > 0.90$) indicates that a reduction in cranial and mandibular lengths is highly correlated with a reduction in postcanine tooth row length. This mirrors previous studies that have shown a strong correlation between body size, postcanine tooth row area, and tooth size, with callitrichids falling near the best fit line along with other primates (Martin, 1992; Plavcan and Gomez, 1993a,b). Based on the scaling between body mass and postcanine tooth row area, Martin (1992) hypothesized that, comparable to the

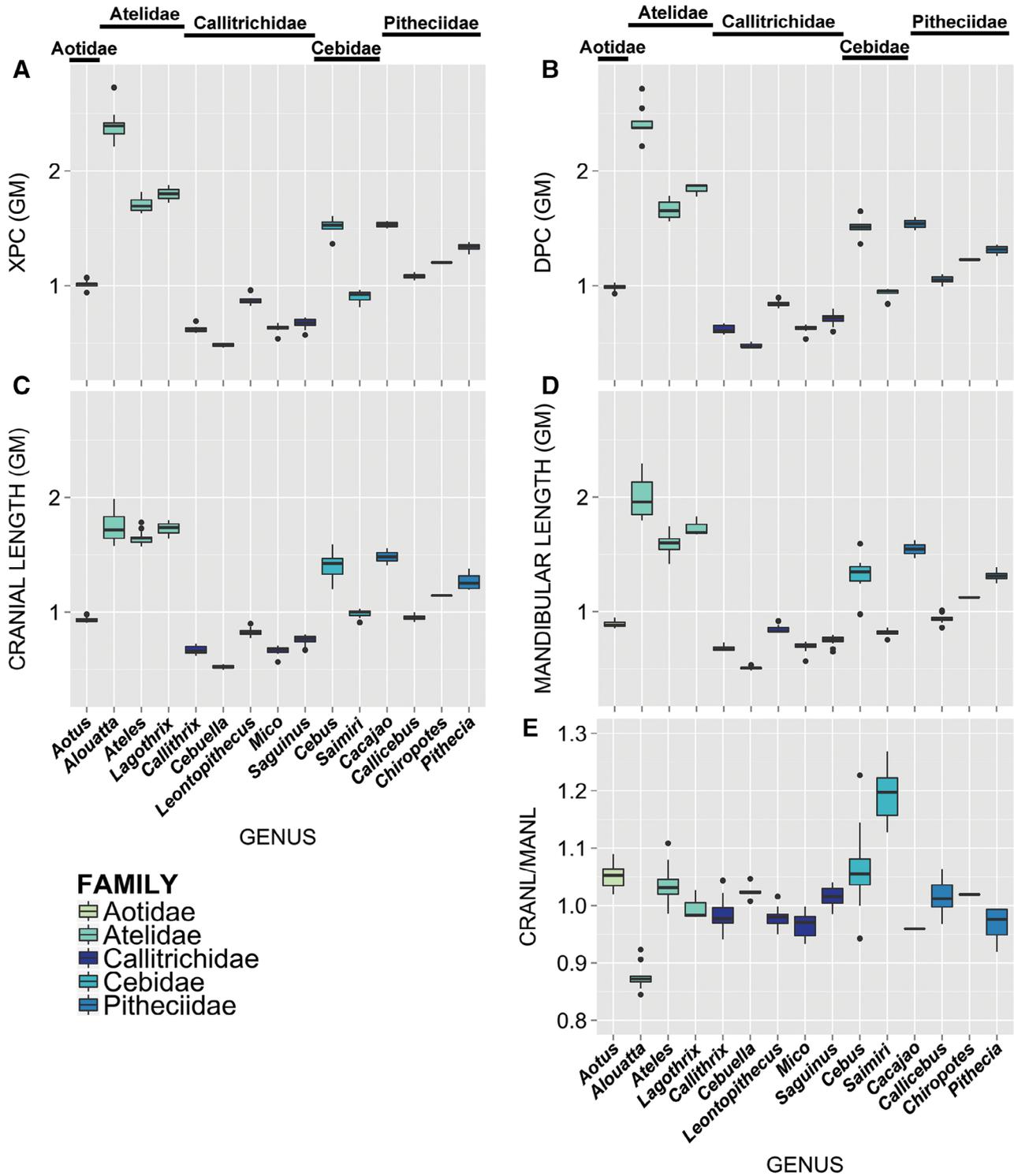


Fig. 3. Series of boxplots comparing measurements across genera. (A) Maxillary postcanine tooth row length (XPC, averaged left and right sides), (B) Mandibular postcanine tooth row length (DPC, averaged left and right sides), (C) Cranial length, (D) Mandibular length, (E) Ratio of cranial length (CRANL) to mandibular length (MANL). Genera are colored by family. See legend in figure. All measurements are geometric mean size corrected. Callitrichids are much smaller in measurements except the ratio of cranial to mandibular length. *Alouatta* has the highest XPC, DPC, and mandibular length, and the smallest ratio of cranial to mandibular length. Cebidae has the highest ratio of cranial to mandibular length, driven by *Saimiri* (E).

“crowding out” hypothesis, reduction of the molar row in callitrichids is related to reduction of body size and developmental schedules of tooth formation and eruption.

However, tests of Gould’s (1975) hypothesis that dwarfed species have proportionally larger molars relative to body size than other species have found that callitrichids do not

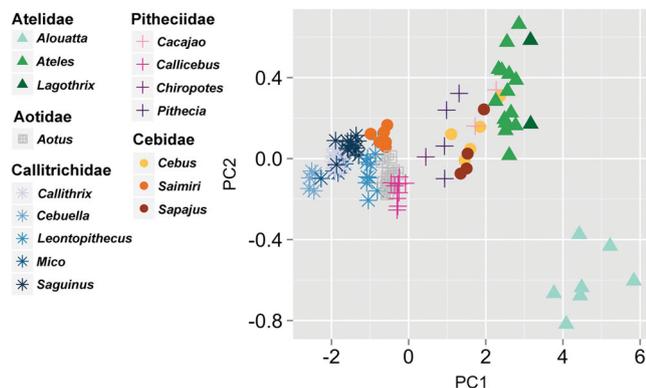


Fig. 4. Principle components analysis comparing craniodental variation in platyrrhines. Traits included are cranial length, mandibular length, maxillary postcanine tooth row length, and mandibular postcanine tooth row length. All measurements are geometric mean size corrected. See legend in figure. PC1 captures 98.27% of the variation. PC2 captures 1.3% of the variation. Loadings for PC1 indicate that all traits have equal contribution to the variation. See text for details.

have proportionally larger molars, and in some cases have smaller molars relative to body size than other platyrrhines, leading to rejection of the hypothesis that third molar loss in callitrichids results from overcrowding (Martin, 1992; Plavcan and Gomez, 1993a,b; Scott, 2015).

There has also been some suggestion that dietary adaptations to gouguing and insectivory may be associated with third molar loss (Rosenberger, 1992; Scott, 2012). While diet and tooth number may be associated, as reductions in tooth number and tooth length reduce grinding area and change the biomechanics of chewing and maximum bite force, there is no clear relationship between these traits in primates (see Scott, 2015). Given that cranial and mandibular lengths can serve as proxies for body size (Fitch, 2000; Sears et al., 2008), our results provide additional evidence for a relationship between body size and reduction in tooth number in mammals (e.g., Hanken, 1984; Natori and Shigehara, 1992), but we suggest that this tooth number reduction is not a product of dietary adaptation or selection on a dental phenotype *per se* but is rather more likely the result of shared genetic effects underlying body size, cranial length, and postcanine tooth length.

As the “crowding out” hypothesis cannot explain differences in tooth number, we explore prenatal growth rates as a hypothesis for the evolutionary loss of third molars in callitrichids.

Prenatal Growth Rates, Body Size, and Twinning

Marmosets and tamarins (Callitrichidae family) are unique among anthropoids in having only two molars and in having sets of twins instead of single offspring (Martin, 1992, 2013; Rutherford and Tardif, 2009). Many researchers have hypothesized that ecological pressures on life history traits have resulted in an adaptive increase in reproductive potential through twinning in the callitrichids (Martin, 1992; Caine, 1993; Rutherford and Tardif, 2009). This distinctive twinning process leads to dizygotic twins formed in a hematopoietic chimeric system where cells are exchanged between the twins during development

(Benirschke and Brownhill, 1963; Gengozian et al., 1964; Martin, 1992; Rutherford and Tardif, 2009).

Twinning may have evolved first in the callitrichid ancestor, followed by parallel episodes of decreasing body size, with some researchers hypothesizing that phyletic nanism is an adaptation to twinning (Leutenegger, 1973, 1980) and that ecological pressures independently led to the evolution of body size reduction within platyrrhines generally (particularly Aotidae, Callitrichidae, and Cebidae) with the most extreme body size changes seen in the phyletic nanism that characterizes callitrichids (Marroig and Cheverud, 2005; Montgomery and Mundy, 2013). Anthropoids are derived compared to strepsirrhines in that they have a fused single uterine chamber, an adaptation to single births (Martin 1992). Callitrichids also have a single uterine chamber, in contrast to the bicornuate uterus seen in strepsirrhines and other mammals, providing evidence that twinning is derived in this clade (Martin, 1992).

Leutenegger (1973) demonstrated a negatively allometric relationship between neonatal birth weight and maternal body weight in platyrrhines, where smaller primates (Callitrichidae in particular) have infants with a neonatal body mass that is proportionally larger relative to maternal body mass. Leutenegger (1973) concluded from this that birthing is more difficult in smaller primates (the “obstetrical hypothesis”) and hypothesized that twinning is an adaptation to smaller pelvic outlets, where reducing the body size of neonates reduces risk during delivery. However, more recent studies have found no significant decrease in relative neonatal birth weight in marmosets and tamarins, despite the phenomenon of twinning in these genera (Martin, 1992), although increased litter sizes are seen in captive animals and are associated with lower birth weights (Chambers and Hearn, 1985; Rutherford and Tardif, 2009). Additionally, *Callimico* has offspring with neonatal birth weights that are not greater than other callitrichids, despite only having singleton births (Martin, 1992).

Unlike other platyrrhines, the relationship between gestation length and body size is not correlated in callitrichids, likely associated with the reproductive strategy of twinning in this clade. Additionally, callitrichids have relatively longer gestational periods than would be predicted from their body masses based on the relationship between body size and gestation length seen in other platyrrhines (Martin, 1992). *Leontopithecus*, the largest callitrichid, has the shortest gestation length resulting in a poor association between body size and gestation length in callitrichids compared to other New World monkeys (Martin, 1992).

Investigations of heterochrony in platyrrhines have identified paedomorphic cranial features in callitrichids and cheirogaleids (mouse and dwarf lemurs), although cheirogaleids retain all three molars (Shea, 1988; Marroig and Cheverud, 2009, Montgomery and Mundy, 2013). Marroig and Cheverud (2009) demonstrated that the small body size of callitrichids (including *Callimico*) is likely related to slow intrauterine growth rates as evidenced by the low neonatal birth weight relative to gestation length in this clade. Montgomery and Mundy (2013) further investigated body mass reductions in callitrichids by comparing prenatal and postnatal growth rates in callitrichids and cheirogaleids, finding that these clades have different growth strategies that contributed evolutionarily to their convergent body mass reduction. Reduced body size in callitrichids is a result of reduction

TABLE 5. Descriptive statistics by platyrrhine genus^a

Family	Genus	Measurement	N	Mean	SD	Median	Min.	Max.	SE	
Aotidae	<i>Aotus</i>	XPC	12	14.2	0.5	14.1	13.2	15.0	0.1	
		DPC	12	15.8	0.5	15.8	14.9	16.5	0.1	
		ML	12	38.7	1.2	38.3	37.1	41.2	0.4	
	<i>Ateles</i>	CL	14	60.3	1.5	60.2	58.4	63.1	0.4	
		XPC	11	24.0	0.8	23.8	22.9	25.5	0.2	
		DPC	14	26.6	1.1	26.6	25.3	28.5	0.3	
		ML	16	69.1	3.4	69.4	61.5	75.6	0.9	
Atelidae	<i>Alouatta</i>	CL	16	105.9	3.5	105.8	101.0	114.5	0.9	
		XPC	7	34.0	2.3	33.7	30.8	38.2	0.9	
		DPC	8	38.9	2.3	38.3	35.5	43.4	0.8	
	<i>Lagothrix</i>	ML	10	86.4	7.6	84.9	77.9	99.4	2.4	
		CL	10	112.1	8.9	110.4	101.3	127.7	2.8	
		XPC	2	25.3	1.5	25.3	24.2	26.3	1.0	
		DPC	3	29.6	1.3	30.3	28.1	30.4	0.8	
		ML	3	75.2	3.7	73.4	72.6	79.5	2.2	
		CL	3	111.0	5.2	111.7	105.5	115.8	3.0	
		XPC	16	8.7	0.5	8.6	8.2	9.7	0.1	
Callitrichidae	<i>Callithrix</i>	DPC	15	9.9	0.5	9.7	9.3	10.8	0.1	
		ML	14	29.6	0.9	29.5	28.7	31.7	0.2	
		CL	16	43.1	2.1	42.4	39.8	46.6	0.5	
	<i>Cebuella</i>	XPC	5	6.7	0.3	6.9	6.3	7.0	0.1	
		DPC	5	7.7	0.3	7.5	7.5	8.1	0.1	
		ML	5	22.1	0.8	22.2	21.1	23.2	0.3	
	<i>Leontopithecus</i>	CL	5	33.6	1.2	33.3	32.0	35.1	0.5	
		XPC	10	12.2	0.5	12.0	11.5	13.3	0.2	
		DPC	10	13.4	0.4	13.4	12.8	14.2	0.1	
	<i>Mico</i>	ML	10	36.7	1.5	35.9	35.5	39.8	0.5	
		CL	10	53.3	2.4	53.1	49.9	57.8	0.8	
		XPC	11	8.8	0.5	8.8	7.5	9.4	0.1	
		DPC	12	10.0	0.5	10.1	8.6	10.6	0.2	
		ML	11	30.0	2.0	30.3	24.7	32.0	0.6	
		CL	12	42.7	2.4	43.5	36.5	45.7	0.7	
	<i>Saguinus</i>	XPC	10	9.5	0.7	9.7	7.9	10.2	0.2	
		DPC	12	11.5	0.9	11.6	9.5	12.8	0.3	
		ML	13	32.4	1.9	32.8	28.2	34.6	0.5	
		CL	13	48.7	2.6	49.4	43.1	51.8	0.7	
		XPC	5	21.2	1.4	21.5	19.0	22.5	0.6	
Cebidae	<i>Cebus</i>	DPC	5	23.9	1.2	23.9	22.0	25.3	0.5	
		ML	10	57.5	6.8	58.5	42.4	69.1	2.2	
		CL	10	90.3	7.4	91.6	77.1	102.3	2.3	
	<i>Saimiri</i>	XPC	6	12.7	0.9	12.9	11.4	13.5	0.3	
		DPC	7	15.0	0.7	15.1	13.5	15.6	0.3	
		ML	8	35.5	1.4	35.7	32.8	37.3	0.5	
	<i>Sapajus</i>	CL	8	63.5	2.5	64.4	58.5	66.2	0.9	
		XPC	4	20.8	0.3	20.9	20.4	21.1	0.2	
		DPC	4	24.6	0.5	24.7	23.9	25.0	0.2	
	Pitheciidae	<i>Cacajao</i>	ML	4	58.3	5.1	55.9	55.4	66.0	2.6
			CL	4	89.3	4.4	88.8	84.6	95.1	2.2
			XPC	2	21.5	0.7	21.5	21.0	22.0	0.5
		<i>Callicebus</i>	DPC	2	24.7	1.2	24.7	23.9	25.6	0.8
			ML	2	67.0	4.7	67.0	63.7	70.4	3.3
			CL	2	95.3	6.7	95.3	90.6	100.0	4.7
		<i>Chiropotes</i>	XPC	9	15.1	0.3	15.1	14.7	15.6	0.1
			DPC	9	16.7	0.5	16.6	15.7	17.6	0.2
			ML	12	40.8	1.7	40.9	37.4	43.9	0.5
CL			14	61.3	1.4	61.4	58.9	64.2	0.4	
XPC	0		NA	NA	NA	NA	NA	NA		
DPC	1		19.6	NA	19.6	19.6	19.6	NA		
ML	1		48.8	NA	48.8	48.8	48.8	NA		
<i>Pithecia</i>	CL	1	73.6	NA	73.6	73.6	73.6	NA		
	XPC	4	18.7	0.6	18.8	17.9	19.3	0.3		
	DPC	4	20.9	0.7	21.0	20.1	21.5	0.3		
	ML	4	57.0	2.5	56.8	54.1	60.2	1.2		
		CL	4	81.6	5.4	80.5	76.8	88.6	2.7	

^aAbbreviations as follows: N is sample size, SD is standard deviation, Min. is minimum, Max. is maximum, SE is standard error, XPC is maxillary postcanine tooth row length (averaged left and right sides), DPC is mandibular postcanine tooth row length (averaged left and right sides), ML is mandibular length, CL is cranial length. All measurements are in millimeters.

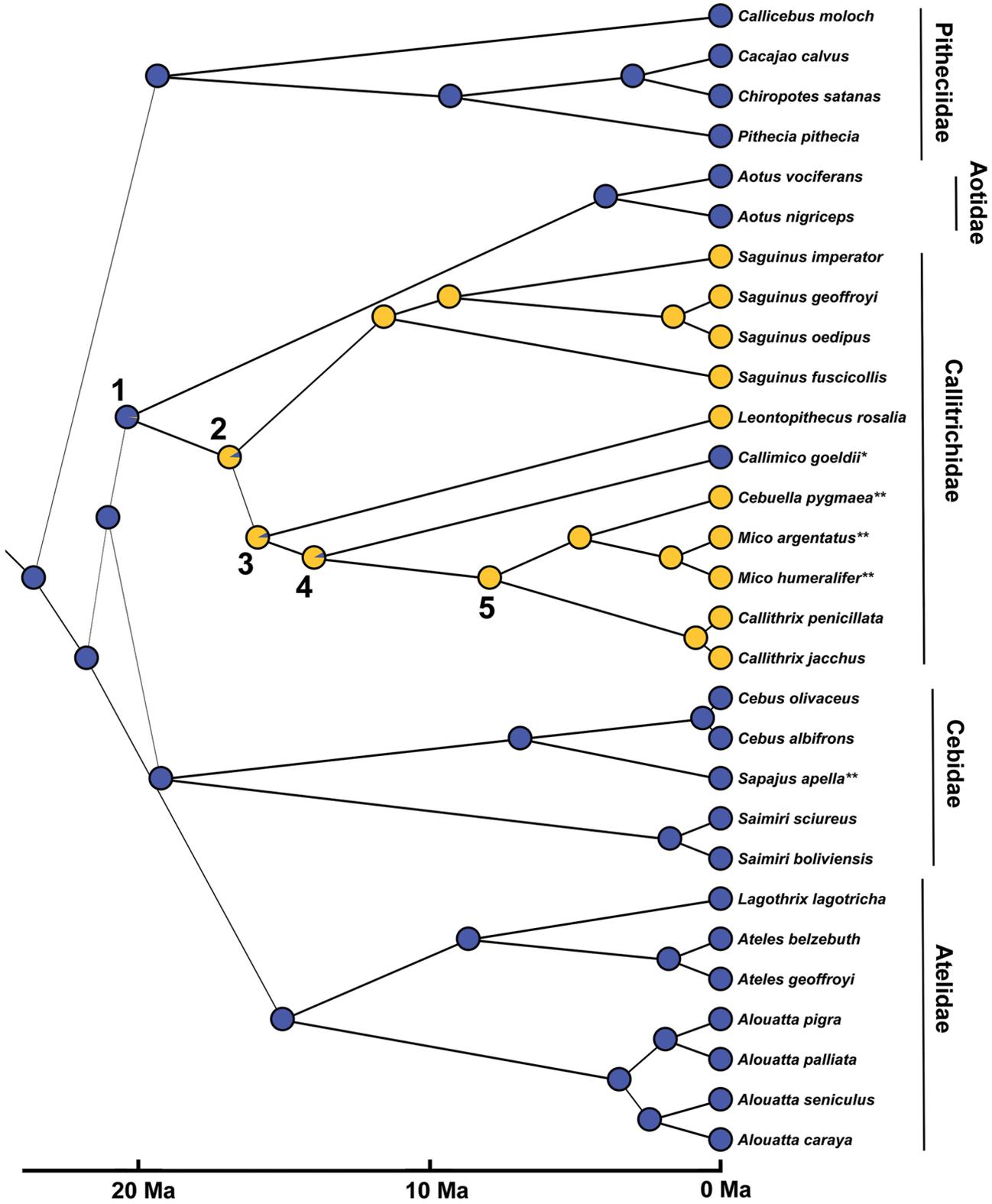


Fig. 5. Ancestral state reconstruction for the evolutionary reduction of molar number in callitrichids using the Mk1 method. AsymmMk1 method gives similar results (see text for details). Node numbers refer to Table 6. Blue nodes denote taxa with three molars. Yellow nodes denote taxa with two molars. Phylogeny was built from molecular data for representative species of each genus, from 10kTrees (Arnold et al., 2010). A single asterisk indicates a species that was not measured in this study but was included in the ancestral state reconstruction. Double asterisks indicate species with alternate genus-level designations in the 10kTrees database. Branch lengths are equal to divergence time. Ma is millions of years ago.

TABLE 6. Results of the ancestral state reconstruction^a

ASR node ^a	Support for three molars (Mk1)	Support for two molars (Mk1)	Support for three molars (AsymmMk1)	Support for two molars (AsymmMk1)	Diverging clade	Geologic date ^a
1	97.9%	2.2%	95.9%	4.1%	Aotidae	~19–22 Ma
2	9.1%	90.9%	2.7%	97.3%	<i>Saguinus</i>	~15–16 Ma
3	8.0%	92.0%	2.0%	98.0%	<i>Leontopithecus</i>	~13–16 Ma
4	8.4%	91.6%	2.3%	97.7%	<i>Callimico</i>	~10–14 Ma
5	0.1%	99.9%	0.1%	99.9%	<i>Callithrix</i>	~6–12 Ma

^aASR node numbers refer to Figure 5. Divergence dates from Opazo et al. (2006) and Perelman et al. (2011). Abbreviations as follows: Mk1 is Maximum Likelihood model, AsymmMk1 is Asymmetrical Maximum Likelihood model, Ma is millions of years ago. A likelihood ratio test of the Mk1 and AsymmMk1 models indicates that they are not significantly different ($P = 0.342$).

in the prenatal, not the postnatal, growth rate, while cheirogaleids have a shortened period of postnatal growth (Montgomery and Mundy, 2013; Masters et al., 2014).

We hypothesize that the loss of third molars is associated with slower prenatal growth rates in callitrichids. Teeth form *in utero* (e.g., Swindler, 2002), and slowed prenatal growth in callitrichids may inhibit organogenesis of later-developing phenotypes like the third molars which are initiated via signals from the developing second molars (Kavanagh et al., 2007), serving as the proximate mechanism for the loss of these teeth. This mechanism explains why cheirogaleids, a clade that achieves small body size through reduction of the postnatal (rather than prenatal) growth period, retain all three molars (Montgomery and Mundy, 2013) and why callitrichids do not have the proportionally larger postcanine teeth relative to body size predicted for dwarfed species with slow or shortened postnatal growth (Gould, 1975; Plavcan and Gomez, 1993a,b).

Callitrichids also deviate from other anthropoids in having less developed deciduous and permanent dentition at birth (Smith et al., 2015), and they develop and erupt their teeth earlier than other anthropoids (Macho, 2001; Smith, 1989) consistent with our hypothesis posed here. However, it is important to note that generalizing the hypothetical relationship between dental development and body size distills callitrichid development down to overall growth rate, and it has been shown that growth rates of the skeleton and dentition vary among callitrichids (Glaser, 1970; Smith et al., 2015), although they do tend to have a slower growth rate than other anthropoids (e.g., Montgomery and Mundy, 2013). Overall, the timing of dental development likely has a phylogenetic signal in primates comparable to what is seen for other ontogenetic

dental traits, such as permanent tooth eruption sequence (Monson and Hlusko, 2018), with callitrichids deviating from other anthropoids in having slower growth rates and earlier tooth eruption (Smith, 1989; Macho, 2001; Montgomery and Mundy, 2013).

Our results also provide additional evidence for Martin's (1992) observation that tooth area and body size are allometrically associated in callitrichids, as has been noted in other primates, although the relationship between these traits appears to be statistically indistinguishable from isometry in this family. This isometric relationship suggests underlying genetic and/or developmental coordination between body size (represented here by cranial and mandibular lengths) and total tooth row length, lending further evidence to support that the developmental mechanisms promoting third molar formation may not be initiated in smaller anthropoids (represented in this study by callitrichids). While *Callimico* is the only genus of callitrichid that does not twin, and they likewise have third molars, their gestation length is not significantly different from that of other callitrichids (Montgomery and Mundy, 2013), and we hypothesize that the slower prenatal growth rate associated with twinning and small body size underlies the loss of the third molars more so than does twinning itself.

Given the current understanding of phylogenetic relationships in Callitrichidae, *Callimico* likely secondarily evolved third molars and lost twinning sometime since the divergence of this genus from other marmosets in the Miocene, approximately 12 Ma. Reports comparing relative birth weights of *Callimico* to neonatal weight in other callitrichids can be conflicting (e.g., Marroig and Cheverud, 2009; Montgomery and Mundy, 2013) with some suggesting that neonatal weight is higher in *Callimico*

TABLE 7. Variation in body mass and growth rates in Callitrichidae^a

Species	Adult body mass (g)	Neonate body mass (g)	Age at sexual maturity (days)	Gestation length (days)	Prenatal growth rate	Postnatal growth rate
<i>Callimico goeldii</i>	558.00	50.50	413.84	153.99	0.33	1.23
<i>Saguinus fuscicollis</i>	393.99	39.18	406.61	148.00	0.26	0.87
<i>Saguinus oedipus</i>	462.04	41.00	680.38	166.49	0.25	0.62
<i>Leontopithecus rosalia</i>	592.52	51.89	890.34	134.00	0.39	0.61
<i>Saguinus midas</i>	540.56	39.78	841.82	138.24	0.29	0.59
<i>Callithrix jacchus</i>	290.21	27.74	455.99	144.00	0.19	0.58
<i>Callithrix pygmaea</i>	123.94	14.50	708.50	134.44	0.11	0.15

^aRates calculated from data from Montgomery and Mundy (2013) Supporting Information. A gram is abbreviated as “g”.

(Montgomery and Mundy, 2013). Given the values cited by Montgomery and Mundy (2013), we calculated prenatal growth rate (neonatal weight/gestation length) and postnatal growth rate (adult body mass/age at sexual maturity) and found that *Callimico* has one of the highest prenatal growth rates (second only to *Leontopithecus*) and the highest postnatal growth rate of the Callitrichidae family (Table 7). A faster prenatal growth rate relative to most other callitrichids, in combination with a fast postnatal growth rate, may facilitate stimulation of the dental inhibitory cascade (Kavanagh et al., 2007) in *Callimico* and may be why this genus forms reduced third molars where other callitrichids do not. Both prenatal and postnatal growth rates may contribute to the reduction in size and loss of the third molars in primates. Secondary evolution of the ultimate molar (M_2) has also been proposed for other mammals (Werdelin, 1987; Scott, 2015), and further investigation of tooth number variation in mammals will likely contribute to our understanding of the evolutionary processes influencing dental variation more broadly.

Montgomery and Mundy (2013) pointed out that dwarfed domestic dog breeds achieve their small body mass through similar mechanisms as callitrichids, with a slower prenatal growth rate (Wayne, 1986a,b; Marroig and Cheverud, 2009), a phenotype that has been traced back to variation at a single gene locus (Sutter et al., 2007). Animals in the Canidae family are characterized by the loss of the maxillary third molars and reduction of the mandibular third molars (e.g., Line, 2003; Hillson, 2005). Domestic dogs, particularly smaller dogs, are frequently noted to have agenesis of one or more postcanine teeth (e.g., Arnall, 1961), although this hypodontia cannot be directly linked to body size, particularly as dramatic artificial selection on these animals may also be contributing to variation in tooth number. *Speothos* (bush dog) is a dwarfed canid that has been shown to have the same developmental strategy as callitrichids, where a slower prenatal growth rate is implicated in the small body size of this taxon (Wayne, 1986a; Montgomery and Mundy, 2013). Like callitrichids, *Speothos* also has reduced molar number compared to other canids, with only one maxillary molar and a highly reduced mandibular second molar (e.g., Beisiegel and Zuercher, 2005; Asahara, 2013). In contrast, *Nyctereutes* does not have reduced molar number or second molar size compared to other canids (Asahara, 2013) despite also being suggested to have a slower prenatal growth rate (Wayne, 1986a). *Cuon alpinus*, the dhole, also has a reduced molar tooth row but is of average canid body size (Chacon, 2000). Third molars are likely lost for different reasons in different mammalian lineages, but by considering tooth patterning in the larger biological context, and in light of what we are now learning about the underlying genetic patterning mechanisms, we are increasingly able to figure out the various pressure points. Further investigation of body size, tooth number, and life history in Callitrichidae as well as Canidae may provide further insight into the relationship between body size, prenatal growth rate, and reduction of the dentition in mammals.

It is unclear whether ecological pressures first selected for twinning as an adaptation for increased reproductive fitness or for reduced body size as an adaptation to limited resources in an insular forest environment (Foster, 1964; Niven, 2007; Weston and Lister, 2009). Likewise, it is unclear whether the slower prenatal growth rate seen in callitrichids is an adaptation to twinning or neonatal

body mass, or some combination of these phenotypes (e.g., Leutenegger, 1973, 1980; Martin, 1992; Montgomery and Mundy, 2013). These traits may have evolved in tandem in callitrichids. Some analyses suggest that phyletic nanism occurred in parallel in callitrichids over the last 25 million years (Montgomery and Mundy, 2013). However, parallel evolution of twinning in callitrichids is much more unlikely (Martin, 1992). Given that the distribution of twinning across the extant callitrichid phylogeny is the same as the distribution of taxa with only two molars, ASR supports the hypothesis that the ancestor of callitrichids was characterized by both twinning and two molars with greater than 90% likelihood. Twinning, associated with slower prenatal growth rates in callitrichids, likely further influenced body size in marmosets and tamarins. Given the association between twinning and the evolutionary loss of the third molars in callitrichids, we hypothesize that these traits coevolved in the Miocene. Because the limited number of cases of evolutionary loss of the third molars in primates makes it unfeasible to test the relationship between life history and this phenotype in a phylogenetic context, we present here a mechanistic hypothesis that will require further elaboration and testing in future studies, potentially in model organisms with extrapolation to primates.

Studies of molar development in mice have shown that development of the third molar is initiated by signals from the second molar (Kavanagh et al., 2007), and more recent work has identified genetic patterning mechanisms that underlie molar proportions in primates and likely other mammals (Hlusko et al., 2016). Our results accord with previous observations about the allometric relationships between postcanine tooth row area and body size and suggest that third molar development may be inhibited at a certain prenatal rate and period of growth. We hypothesize that the unique life history strategy of the Callitrichidae family, where small body size is associated with slower prenatal growth rates rather than slowed or shorter postnatal growth (Marroig and Cheverud, 2009; Montgomery and Mundy, 2013), is the cause of evolutionary third molar loss in this clade. Consequently, the loss of the third molars in callitrichids is not the phenotypic target of selection, nor lost due to deleterious effects of crowding predicted from a decrease in body size, but is rather a “spandrel” arising from a pleiotropic cascade of effects resulting from slower prenatal growth rates associated with nanism and twinning in this family. From our data, we highlight the tangled relationship between body size, prenatal growth rates, twinning, and the evolutionary loss of the third molars in platyrrhines.

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