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Arrival and Diversification of Caviomorph Rodents and Platyrrhine Primates in South America

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Abstract.—Platyrrhine primates and caviomorph rodents are clades of mammals that colonized South America during its period of isolation from the other continents, between 100 and 3 million years ago (Mya). Until now, no molecular study investigated the timing of the South American colonization by these two lineages with the same molecular data set. Using sequences from three nuclear genes (ADRA2B, vWF, and IRBP, both separate and combined) from 60 species, and eight fossil calibration constraints, we estimated the times of origin and diversification of platyrrhines and caviomorphs via a Bayesian relaxed molecular clock approach. To account for the possible effect of an accelerated rate of evolution of the IRBP gene along the branch leading to the anthropoids, we performed the datings with and without IRBP (3768 sites and 2469 sites, respectively). The time window for the colonization of South America by primates and by rodents is demarcated by the dates of origin (upper bound) and radiation (lower bound) of platyrrhines and caviomorphs. According to this approach, platyrrhine primates colonized South America between 37.0 ± 3.0 Mya (or 38.9 ± 4.0 Mya without IRBP) and 16.8 ± 2.3 (or 20.1 ± 3.3) Mya, and caviomorph rodents between 45.4 ± 4.1 (or 43.7 ± 4.8) Mya and 36.7 ± 3.7 (or 35.8 ± 4.3) Mya. Considering both the fossil record and these molecular datings, the favored scenarios are a trans-Atlantic migration of primates from Africa at the end of the Eocene or beginning of the Oligocene, and a colonization of South America by rodents during the Middle or Late Eocene. Based on our nuclear DNA data, we cannot rule out the possibility of a concomitant arrival of primates and rodents in South America. The caviomorphs radiated soon after their arrival, before the Oligocene glaciations, and these early caviomorph lineages persisted until the present. By contrast, few platyrrhine fossils are known in the Oligocene, and the present-day taxa are the result of a quite recent, Early Miocene diversification. [Biogeography; Caviomorpha; colonization; molecular dating; nuclear genes; Platyrrhini; South America.]

South America was an isolated continent after its separation from Africa in the Cretaceous, 90 to 100 million years ago (Mya) (Smith et al., 1994), until its reconnection with North America in the Pliocene, 3 to 3.5 Mya. In the Eocene-Early Oligocene, between 50 and 30 Mya, South America was colonized by primates and rodents, of which the extant New World platyrrhines and caviomorphs are the descendants (Flynn and Wyss, 1998). No other groups of terrestrial placentals colonized South America during the same period. The times and ways of the South American colonization remain debated because of the poor fossil record available to reconstruct the evolutionary history of platyrrhines and caviomorphs from their areas of origin to South America. For platyrrhines and caviomorphs alike, the most intriguing questions are (i) When and where did they diverge from their respective sister groups? (ii) When and via which route did they reach South America? (iii) Were these colonization events synchronous or not? and (iv) When did extant platyrrhines and caviomorphs begin to diversify?

Extant platyrrhines are currently divided, according to molecular studies, into three clades: Pitheciidae (e.g., *Pithecia*, the sakis), Atelidae (e.g., *Ateles*, the spider monkeys), and Cebidae (e.g., *Cebus*, the capuchins, and *Callithrix*, the marmosets) (reviewed in Schneider, 2000). Resolving the relationships between these families is difficult due to the fast radiation of the platyrrhines. Thus, their branching order remains unclear in spite of a large number of studies based on mitochondrial (Horovitz and Meyer, 1995) and nuclear DNA (Harada et al., 1995; Schneider et al., 1996, 2001; von Dornum and Ruvolo, 1999; Schneider, 2000). Old World monkeys and apes, the catarrhines from Asia and Africa, are the sister group of New World monkeys, with which they form the anthropoid clade. The origin of stem anthropoids has not yet been elucidated because the most recent discoveries revealed early anthropoid fossils in Africa (Kay et al., 1997; Gebo et al., 2000; Gunnell and Miller, 2001; Seiffert et al., 2003) and in Asia (Beard et al., 1994; Jaeger et al., 1999; Marivaux et al., 2003). The Old World anthropoid fossils that share most similarities with South American primates come from the Jebel Qatrani Formation of Fayum in Egypt (Late Eocene–Early Oligocene) (Fleagle, 1999). This may give some support for an African rather than Asiatic origin of the New World monkeys.

Caviomorphs belong to the rodent infraorder Hystricognathi. Like for platyrrhines, the relationships within the caviomorph clade are difficult to establish, and this is again probably due to a fast radiation (Flynn and Wyss, 1998). Studies based on the mitochondrial 12S rRNA or on nuclear genes failed to give robust phylogenetic results (e.g., Nedbal et al., 1994; Catzeflis et al., 1995; Adkins et al. 2001). However, phylogenetic analyses of exon 28 of the von Willebrand factor (vWF) (Huchon and Douzery, 2001) displayed four well-defined major clades: Cavioidea (e.g., *Cavia*, the guinea pigs), Erethizontoidea (e.g., *Erethizon*, the New World porcupines), Chinchilloidea (e.g., *Chinchilla; Dinomys*, the pacaranas), and Octodontoidea (e.g., *Echimys*, the arboreal spiny rats), but their reciprocal phylogenetic

affinities remained difficult to assess. According to paleontological data, hystricognaths have an Asiatic origin (Flynn et al., 1986; Bryant and McKenna, 1995; Marivaux et al., 2002), but the origin of caviomorphs could be either Asiatic (Hussain et al., 1978; Marivaux et al., 2002) or African (Lavocat, 1969; Martin, 1994).

Platyrrhines and caviomorphs are considered to have arrived in South America around the same paleontological time, during the Late Eocene–Early Oligocene (Hoffstetter, 1972; Flynn et al., 1986). This assumption is supported by the oldest fossil findings of primates in South America at 27 Mya (Hoffstetter, 1969; Rosenberger et al., 1991), and rodents at least at 31 Mya (Wyss et al., 1993). Molecular datings may help to demarcate the periods of possible colonization by estimating the time windows between the divergence of platyrrhines and caviomorphs from their respective Old World sister groups and the subsequent radiations of extant New World monkeys and rodents. Various molecular dating studies have already attempted to assess the times of origin and diversification of platyrrhines and/or caviomorphs, as summarized in Table 1, but the results are quite inconclusive.

Not only the concomitant or independent arrival of primates and rodents in the New World remains an open question, but also the possible routes and means of colonization have to be evaluated. Various biogeographical scenarios have been proposed. The most popular hypothesis is, until now, migration by rafting via floating islands from Africa to South America (Hoffstetter, 1972; Houle, 1999). Alternative explanations for the presence of endemic rodents and primates in South America are Gondwanan vicariance or land-mediated dispersal (Arnason et al., 2000), but both would require an unrealistically early diversification of primates and rodents, even preceding their appearance during the Late Cretaceous (e.g., Springer et al., 2003).

Defining the time of colonization of "island" areas by a given taxonomic group can be subject to different interpretations when using molecular data. It has been described either as the age of divergence of the studied clade from its mainland sister group (e.g., Vences et al., 2003; Nagy et al., 2003) or as the time of initial diversification into descending lineages on the "island" (e.g., Yoder et al., 1996, 2003; Groombridge et al., 2002; Montgelard et al., 2003; Ross et al., 2003). These alternatives actually represent two extreme interpretations. Indeed, both approaches would give similar dating results only if the radiation of a taxon takes place immediately after the divergence from its closest mainland relative. However, the radiation of a taxon may take place long after the initial colonization event, or offshoots from early radiations may go extinct. Moreover, the extant mainland sister group of an insular clade is not necessarily its closest mainland relative, which may have gone extinct. When using extant taxa, as is the case in molecular dating studies, any such extinction events cannot be detected. Hence, to better capture the time of colonization from molecular studies, we propose a conservative approach by providing a time window for possible colonization demarcated by the divergence from the closest mainland sister group as an upper (i.e., oldest) bound and the ingroup diversification as a lower (i.e., most recent) bound (see Poux et al., 2005, fig. 3, for further explanation).

With this conservative approach, we estimate in the present article the dating of origin and radiation of South American primates and rodents using separate and combined sequences from three nuclear genes, coding for the alpha 2B adrenergic receptor (ADRA2B), the von Willebrand factor (vWF), and the interphotoreceptor retinoid binding protein (IRBP). The advantage of these nuclear genes is that they have already proven to be useful in solving the phylogeny of placental mammals (e.g., Madsen et al., 2001; Murphy et al., 2001; Huchon et al., 2002). This expanded data set, combined with accepted fossil calibrations (e.g., Gatesy and O'Leary, 2001; Gheerbrant et al., 2001) and a Bayesian relaxed molecular clock dating method (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002), allows for the first time to compare simultaneously the phylogeny and the evolutionary history of primates and rodents in South America using the same orthologous markers.

Material and Methods

Choice of Genes

To estimate phylogeny and divergence times, the nuclear genes for ADRA2B (intronless), vWF (exon 28), and IRBP (exon 1) were chosen for the following reasons. (i) A large number of sequences was already available, especially within rodents, and these genes have been shown to contain phylogenetic information within and between mammalian orders (e.g., Madsen et al., 2001; Murphy et al., 2001; Huchon et al., 2002). (ii) The sequenced parts of these genes are of similar lengths (around 1.2 to 1.3 kb) and have comparable numbers of variable sites, which favors their equal contribution to a combined analysis. (iii) Nuclear genes have been shown to perform better than mitochondrial markers at the phylogenetic level we are interested in (Springer et al., 2001). (iv) The three genes ADRA2B, vWF, and IRBP are not genetically linked; their location is variable, on chromosomes 2, 6, and 14 in *Mus*; chromosomes 3, 4, and 16 in *Rattus*; and chromosomes 2, 12, and 10 in *Homo*. (v) The proteins they encode do not display any biological interaction or functional relation: ADRA2B is an adrenergic receptor mainly expressed in the kidneys, the vWF protein is a blood coagulation factor, and IRBP is located in the matrix of the retina. These properties allow us to combine the three genes to obtain a more reliable estimation of the phylogenies and datings, because it provides a longer data set in which the potential influence of any contrasting evolutionary properties of each individual gene is moderated.

Taxon Sampling

For each gene, 60 mammalian species were included in our study, as presented in Table 2, selected on the

TABLE 1. Summary of molecular datings for platyrrhine and caviomorph origins and diversifications. Ages are in Mya (± standard deviation, when available). *Phiomorphs s.s. = Bathyergidae + Thryonomyidae + Petromuridae. IC: internal calibrations, i.e., within primates or rodents. EC: external calibrations, i.e., outside primates or rodents.

References	Platyrrhine/catarrhine split	Radiation of extant platyrrhines	Markers	Methods	Calibrations
Bailey et al. (1991)	27.2–34.2	12.6–15.9[a]	Coding and noncoding nuclear DNA	Local clock based on MP branch lengths	IC
Takahata and Satta (1997)	57.5	—	Nuclear DNA	Global clock, ML method	—
Goodman et al. (1998)	—	25	Coding nuclear DNA	Local clock based on NJ branch lengths	IC
Kumar and Hedges (1998)	47.6 ± 8.3	—	Nuclear proteins	Global clock and multi-protein gamma distance	EC
Arnason et al. (1998)	60	—	Complete mitochondrial proteins	Local clock based on ML branch lengths	EC
Arnason et al. (2000)	70	—	Complete mitochondrial proteins	Local clock based on ML branch lengths	EC
Schneider (2000)	—	26	Nuclear DNA	Local clock based on branches calculated with the least-squares method	IC
Nei and Glazko (2002)	32.3–35.2	—	Nuclear proteins	Global clock and multi-protein gamma distance	IC/EC
Glazko and Nei (2003)	31.9–33.0	—	Nuclear proteins	Global clock and multi-protein gamma distance	IC/EC
Adkins et al. (2003)	32.4–49.6	—	Coding nuclear DNA	Local clock and rate smoothing	IC
Schrago and Russo (2003)	32.8–41.9	—	Complete mitochondrial genes and proteins	Global clock and Bayesian relaxed clock	IC
Hasegawa et al.[c] (2003)	37.5 ± 3.1	Around 17[a,b]	Nuclear and mitochondrial DNA	Bayesian relaxed clock	EC
Yang and Yoder (2003)	53.3–61.1	—	Coding mitochondrial DNA	ML local clock and Bayesian relaxed clock	IC/EC
Yoder and Yang (2004)	43.5–55.7	-	Coding mitochondrial DNA and coding/noncoding nuclear DNA	Bayesian relaxed clock	IC/EC

References	Caviomorphs/phiomorphs s.s.*	Radiation of extant caviomorphs	Markers	Methods	Calibrations
Nebdal et al. (1994)	33–39	-	Mitochondrial rRNA	Global clock	IC
Huchon et al. (2000)	—	27.7–51	Nuclear proteins	Global clock	IC/EC
Huchon and Douzery (2001)	43–54	—	Coding nuclear proteins	ML local clock	IC
Mouchaty et al. (2001)	85		Complete mitochondrial proteins	Local clock based on ML branch lengths	EC
Springer et al. (2003)	31–46	—	Nuclear and mitochondrial DNA	Bayesian relaxed clock	IC/EC
Adkins et al. (2003)	34.7–57.1	—	Coding nuclear DNA	Local clock and rate smoothing	EC
Hasegawa et al.[c] (2003)	Around 40[b]	34.4 ± 1.6[d]	Nuclear and mitochondrial DNA	Bayesian relaxed clock	IC/EC

[a] Platyrrhine radiation age based on two species only; this date might be too young if the most basal clade is not represented.
[b] Ages deduced from the relaxed tree displayed in the article.
[c] The data set used in Hasegawa et al. (2003) is from Nikaido et al. (2001) and Murphy et al. (2001).
[d] This node was constrained to be younger than 37 Mya.

basis of the following criteria. (i) The species should represent all placental mammalian orders, as well as two divergent marsupials as outgroups. (ii) The sampling should reflect the diversity of primate and rodent taxa; sequences from primates (8 for ADRA2B and 11 for vWF) and three rodents (for ADRA2B and IRBP) were therefore newly determined to include all families and/or superfamilies, with a broad representation of platyrrhines and caviomorphs. (iii) Species should be included that enable the use of paleontological calibration constraints from various lineages, thus minimizing the dependence of the results upon a single fossil reference (Soltis et al., 2002; Douzery et al., 2003).

DNA Amplification and Sequencing

Newly determined primate sequences were obtained for the partial exon 28 of the vWF gene and for

TABLE 2. Taxonomic sampling and accession numbers for the three nuclear genes. Newly determined sequences are indicated (*).

	Species	ADRA2B	vWF	IRBP
Placentalia				
Rodentia				
Sciurognathi				
Muridae	*Mus musculus*	M94583	U27810	AF126968
	Rattus norvegicus	M32061	AJ224673	AJ429134
	Tachyoryctes sp.	AJ427264	AJ402713	AJ427231
Dipodidae	*Dipus sagitta*	AJ427263	AJ224665	AJ427232
Heteromyidae	*Dipodomys merriami*	AJ427261	AJ427226	AJ427233
Geomyidae	*Thomomys talpoides*	AJ427262	AJ427227	AJ427234
Gliridae	*Glis glis*	AJ427258	AJ224668	AJ427235
	Dryomys nitedula	AJ427257	AJ224666	AJ427236
Sciuridae	*Marmota monax*	AJ427255	AJ224671	AJ427237
Aplodontidae	*Aplodontia rufa*	AJ427256	AJ224662	AJ427238
Castoridae	*Castor canadensis*	AJ427260	AJ427228	AJ427239
Anomaluridae	*Anomalurus* sp.	AJ427259	AJ427229	AJ427230
Ctenodactylidae	*Massoutiera mzabi*	AJ427265	AJ238388	AJ427242
Hystricognathi				
Thryonomyidae	*Thryonomys swinderianus*	AJ427267	AJ224674	AJ427243
Petromuridae	*Petromus typicus*	AJ427268	AJ251144	AJ427244
Bathyergidae	*Bathyergus suillus*	AJ427252	AJ238384	AJ427251
Hystricidae	*Trichys fasciculata*	AJ427266	AJ224675	AJ427245
Chinchillidae	*Chinchilla lanigera*	AJ427271	AJ238385	AJ427246
Dinomyidae	*Dinomys branickii*	AM050859*	AJ251145	AM050862*
Echimyidae	*Echimys chrysurus*	AJ427269	AJ251141	AJ427247
Octodontidae	*Octodon lunatus*	AM050860*	AJ238386	AM050863*
Caviidae	*Cavia porcellus*	AJ271336	AJ224663	AJ427248
Agoutidae	*Agouti paca*	AM050861*	AJ251136	AM050864*
Erethizontidae	*Erethizon dorsatum*	AJ427270	AJ251135	AJ427249
Lagomorpha				
Leporidae	*Oryctolagus cuniculus*	Y15946	U31618	Z11812
	Lepus crawshayi	AJ427254	AJ224669	AJ427250
Ochotonidae	*Ochotona princeps*	AJ427253	AJ224672	AY057832
Primates				
Hominidae	*Homo sapiens*	M34041	X06828	J05253
Hylobatidae	*Hylobates lar*	AM050851*	AJ410300*	AJ313478
Cercopithecidae	*Macaca mulatta*	AM050852*	AJ410302*	AJ313476
	Cercopithecus solatus	AM050853*	AJ410301*	AJ313477
Cebidae	*Callithrix jacchus*	AM050856*	AJ410299*	AJ313472
	Cebus apella	AM050854*	AJ410297*	AJ313473
Atelidae	*Ateles* sp.[a]	AM050855*	AF061059	AJ313474
Pitheciidae	*Pithecia pithecia*	AM050857*	AJ410298*	AJ313475
Tarsiidae	*Tarsius bancanus*	AJ891081	AJ410296*	AF271423
Lemuridae	*Lemur catta*	AJ891067	AJ410292*	AJ313470
Indridae	*Propithecus verreauxi*	AJ891076	AJ410294*	AJ313471
Cheirogalidae	*Microcebus murinus*	AM050858*	AJ410295*	AJ313469
Loridae	*Nycticebus coucang*	AJ251186	AJ410291*	AJ313467
Dermoptera				
Cynocephalidae	*Cynocephalus variegatus*	AJ251182	U31606	Z11807
Scandentia				
Tupaiidae	*Tupaia* sp.[b]	AJ251187	U31624	Z11808
Pholidota				
Manidae	*Manis* sp.	AJ251185	U97535	AF025389
Carnivora				
Felidae	*Felis catus*	AJ251174	U31613	Z11811
Perissodactyla				
Equidae	*Equus* sp.[c]	Y15945	U31610	U48710
Tapiridae/Rhinocerotidae	*Tapirus* sp./*Ceratotherium*[d]	AJ315939	U31604	AF179294
Cetartiodactyla				
Bovidae	*Bos taurus*	Y15944	X63820	M20748
Physeteridae	*Physeter catodon*	AJ427417	AF108834	U50818
Hippoptamidae	*Hippopotamus amphibius*	AJ251178	AF108832	AF108837
Camelidae	*Lama* sp.[e]	AJ315941	AF108835	AF108836
Suidae	*Sus scrofa*	AJ251177	S78431	U48588
Chiroptera				
Pteropodidae	*Cynopterus sphinx*	AJ251181	U31605	U48709
Megadermatidae	*Megaderma lyra*	AF337537	U31616	AY057833
Phyllostomidae	*Tonatia bidens*	AF337541	U31622	Z11810

TABLE 2. Taxonomic sampling and accession numbers for the three nuclear genes. Newly determined sequences are indicated (*). *(Continued)*

	Species	ADRA2B	vWF	IRBP
Eulipotyphla				
Erinaceidae	*Erinaceus europaeus*	Y12521	U97536	AF025390
Proboscidea				
Elephantidae	*Elephas/Loxodonta*[f]	Y12525	U31611	U48711
Sirenia				
Dugongidae	*Dugong dugon*	Y15947	U31608	U48583
Hyracoidea				
Procaviidae	*Procavia capensis*	Y12523	U31619	U48586
Tubulidentata				
Orycteropodidae	*Orycteropus afer*	Y12522	U31617	U48712
Xenarthra				
Bradypodidae	*Bradypus tridactylus*	AJ251179	U31603	U48708
Marsupialia				
Didelphimorphia	*Didelphis* sp.[g]	Y15943	AF226848	Z11814
Diprotodontia	*Macropus* sp.[h]	AJ251183	AJ224670	AJ429135

[a] *Ateles paniscus* (IRBP, ADRA2B) or *A. belzebuth* (vWF).
[b] *Tupaia tana* (ADRA2B) or *T. glis* (vWF, IRBP).
[c] *Equus asinus* (vWF) or *E. caballus* (IRBP, ADRA2B).
[d] *Tapirus pinchaque* (IRBP) or *T. terrestris* (ADRA2B), and *Ceratotherium simum* (vWF).
[e] *Lama glama* (vWF, IRBP) or *L. pacos* (ADRA2B).
[f] *Elephas maximus* (vWF, ADRA2B) or *Loxodonta africana* (IRBP).
[g] *Didelphis virginiana* (vWF, IRBP) or *D. marsupialis* (ADRA2B).
[h] *Macropus giganteus* (vWF, IRBP) or *M. rufus* (ADRA2B).

the ADRA2B gene. PCR reactions on the vWF gene provided two overlapping products using the primer pairs V1 (5′-TGTCAACCTCACCTGTGAAGCCTG-3′)/W4 (5′-TTGTTTTCAGGGGCCTGCTT-3′) and V2 (5′-CCCTCAGAGCTGCGGCGCAT-3′)/W1 (5′-TGCAGGACCAGGTCAGGAGCCTCTC-3′), and a program of 29 cycles of 20 s at 94°C, 30 s at 47°C, and 2 min at 68°C, and one final cycle of 10 min at 68°C. The PCR products were purified on Ultrafree-DA Amicon columns (Millipore) and concentrated on Microcon filterable columns (Millipore). Manual sequencing was conducted with the dideoxy chain termination method with α^{33}P-ddNTP and the Thermo Sequenase Cycle Sequencing Kit (Amersham) on both strands, using the external primers just mentioned plus the internal primers V30 (5′-AAMTCCRTGGTTCTGGAYGTGG-3′) and V40 (5′-GAGAAGCAGGCCCCNGAGAACAAGG-3′). The almost complete coding region of the ADRA2B gene was amplified with the primers ADRA2BFOR (5′-ASCCCTACTCNGTGCAGGCNACNG-3′) and ADRA2BREV (5′-CTGTTGCAGTAGCCDATCCARAA RAARAAYTG-3′). The program was 2 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 54°C, and 1 min 30 s at 72°C, and finally 10 min at 72°C. The PCR products were reamplified when necessary, and subsequently sequenced with the Big Dye Terminator Sequencing Kit (Applied Biosystems) and the PCR primers above, in combination with the two internal primers ADRA2B5-2 (5′-GCARGTAVACNAGRATCATG-3′) and ADRA2B3-2 (5′-ATCATGATYCTNGTBTACYTGC-3′). For the newly determined rodent ADRA2B sequences, the same protocol as given for primates was used, whereas the amplification and sequencing procedure for rodent IRBP followed Huchon et al. (2002). Species names and accession numbers of the newly determined sequences are given in Table 2.

Phylogenetic Analyses

The sequences of the three nuclear exons were highly conserved in length and easily aligned by hand with the ED editor of the MUST package, version 2000 (Philippe, 1993). Nonsequenced positions and gaps were coded as missing data. Amino acids repeats and sites not sequenced or gapped in more than 25% of the taxa were excluded from analysis. The final alignment included 1188 sites for ADRA2B, 1281 sites for vWF, and 1299 sites for IRBP. Phylogenetic reconstructions were performed on the complete DNA data set by maximum likelihood (ML) with PAUP* (version 4 beta 10) (Swofford, 1999) and by Bayesian analyses with MrBayes (version 3.0 beta 4) (Ronquist and Huelsenbeck, 2003).

The ML assumptions were chosen after running ModelTest 3.5 (Posada and Crandall, 1998). The Akaike information criterion (AIC) applied to the complete data set showed that the best fitting model of DNA sequence evolution was general time reversible (GTR) with a gamma distribution (Γ) coupled to a fraction of invariable (INV) sites to describe the substitution rate heterogeneities among sites (Yang, 1996). Maximum likelihood parameters and best topology were estimated by PAUP* using a loop approach on the concatenated ADRA2B + vWF + IRBP genes. First, the ML parameters were optimized on an NJ topology derived from ML distances obtained using the selected model from ModelTest. Second, an ML heuristic search was conducted with tree bisection-reconnection (TBR) branch swapping to identify the optimal tree. Then, the likelihood parameters were reestimated on the new topology, and a new heuristic tree search was run under these new parameters. This loop procedure was performed until stabilization of both topology and parameters. The stability of the nodes was estimated by bootstrap (Felsenstein, 1985), with 100 replicates of heuristic searches. For each replicate, we

used NJ starting trees, with ML parameters identically set to their optimal value previously estimated during the loop procedure, and TBR branch swapping limited to 1000 rearrangements.

For the Bayesian inference, the nuclear DNA data set was divided into 9 partitions (3 codon positions × 3 genes). According to the best fitting models proposed by ModelTest 3.5 for each codon position separately, two different models were combined: one using GTR+Γ+INV parameters independently estimated for the two first codon position of the three exons, and one using GTR+Γ parameters independently estimated for the third codon positions of each gene. Posterior distributions were approximated by a Metropolis-coupled Markov chain Monte Carlo (MCMCMC) technique. Five incrementally heated chains were sampled every 20 generations during 1,000,000 generations ("short-run," burn-in on the first 1500 trees) for the first analysis, and every 100 generations during 10,000,000 generations ("long-run," burn in on the first 50,000 trees) for the second. This second analysis was conducted to verify that a 10-fold increase of the number of MCMCMC generations did not affect the phylogenetic conclusions. We used Dirichlet priors for base frequencies (1,1,1,1) and for GTR parameters (1,1,1,1,1) scaled to the G-T rate, a uniform (0.05,50.00) prior for the Γ shape, and an exponential (10.0) prior for branch lengths. All topologies were a priori equally probable.

The DNA character matrix and tree are available from TreeBASE under study accession number S1389 and matrix accession number M2481.

Molecular Dating Analyses

The molecular dating analyses were performed according to the Bayesian relaxed molecular clock approach (Thorne et al., 1998; Kishino et al., 2001), using the MULTIDIVTIME package (Thorne and Kishino, 2002). First, the best fitting parameters for each of the three codon positions in the ADRA2B, vWF, and IRBP genes (9 partitions in total) were calculated via PAML (Yang, 1997), using the F84 model (because more complex models are not incorporated in the ESTBRANCHES program) and five discrete gamma categories. These parameters were then entered in the ESTBRANCHES program to calculate the branch lengths of the rooted ML tree, shown in Figure 1, and their variance-covariance matrix under each of the 9 partitions. Second, a priori knowledge was incorporated about the gamma distributions of (i) the root age, (ii) the substitution rate at the root, and (iii) the substitution rate autocorrelation along branches. These priors were specified as means and standard deviations (SD) of the three distributions according to the MULTIDIVTIME guidelines. The posterior distributions of node times were approximated through MCMC runs using the MULTIDIVTIME program. To check that the data provide significant dating information, we computed both prior and posterior divergence time distributions. Posterior distributions were also computed twice for the combined data set using all fossil calibrations, starting the MCMC runs from different initial values. The calculations have been done on each gene independently as well as on the combined data set, using the topology obtained from the concatenated alignment (Fig. 1). Furthermore, the priors were the same for all 9 partitions, except for the substitution rate at the root, for which priors were recalculated for each gene separately.

For fossil calibrations we selected eight time constraints that have been used already widely in molecular dating studies (e.g., Douady and Douzery, 2003; Douzery et al., 2003; Springer et al., 2003). As calibration constraints outside the primate and rodent clades we took the diversification age of Paenungulata (54 to 65 Mya; Gheerbrant et al., 2001), Perissodactyla (54 to 58 Mya; Garland et al., 1993), Cetartiodactyla (55 to 65 Mya; Gatesy and O'Leary, 2001) and Lagomorpha (minimum age, i.e., lower boundary of 37 Mya; identification of ochotonids since Late Eocene; McKenna and Bell, 1997). Within the rodents we used the split *Glis/Dryomys* (minimum age of 28.5 Mya; identification of first Glirinae in Late Oligocene; Hartenberger, 1994) and the split *Aplodontia/Marmota* (minimum age of 37 Mya; identification of first Sciuridae in Late Eocene; McKenna and Bell, 1997). Within primates we used the basal primate radiation (63 to 90 Mya; Martin, 1993; Gingerich and Uhen, 1994; Tavaré et al., 2002) and the Cercopithecoidea/Hominoidea divergence (25 to 35 Mya; Shoshani et al., 1996; Fleagle, 1999).

Additional analyses were conducted to assess the impact on dating estimates of (i) a reduced taxon sampling among platyrrhines, and (ii) the use of taxonomic chimeras. Present-day platyrrhines and caviomorphs are divided into three and four well-defined clades, respectively. Our study comprises only four species as representatives of platyrrhines (one Atelidae, one Pitheciidae, and two Cebidae), versus seven for caviomorphs (one Erethizontoidea, two Cavioidea, two Chinchilloidea, and two Octodontoidea). To assess the potential impact on dating estimates of having a smaller taxon sampling within primates, we reestimated divergence times after removing three of the caviomorphs (*Cavia, Dinomys, Octodon*), in order to reach a minimal taxon sampling (each of the four superfamilies of caviomorphs is then represented by only one species).

Moreover, in order to evaluate the impact on dating estimates of the use of taxonomic chimeras among placentals, we constructed a 67-taxon supermatrix of characters without chimera. In this matrix, all chimera of the previous analysis were disassembled and replaced by two or three sequences depending on the number of sequences used to construct the chimera. Missing data were incorporated when a given species was not represented for a given gene. In other words, we included *Ateles paniscus* and *A. belzebuth, Tupaia tana* and *T. glis, Equus asinus* and *E. caballus, Tapirus pinchaque, T. terrestris*, and *Ceratotherium simum, Lama glama* and *L. pacos, Loxodonta africana* and *Elephas maximus*, respectively, instead of *Ateles* sp., *Tupaia* sp., *Equus* sp., Ceratomorpha, *Lama* sp., and Elephantidae. When a species was not scored for a given character partition during the Bayesian relaxed

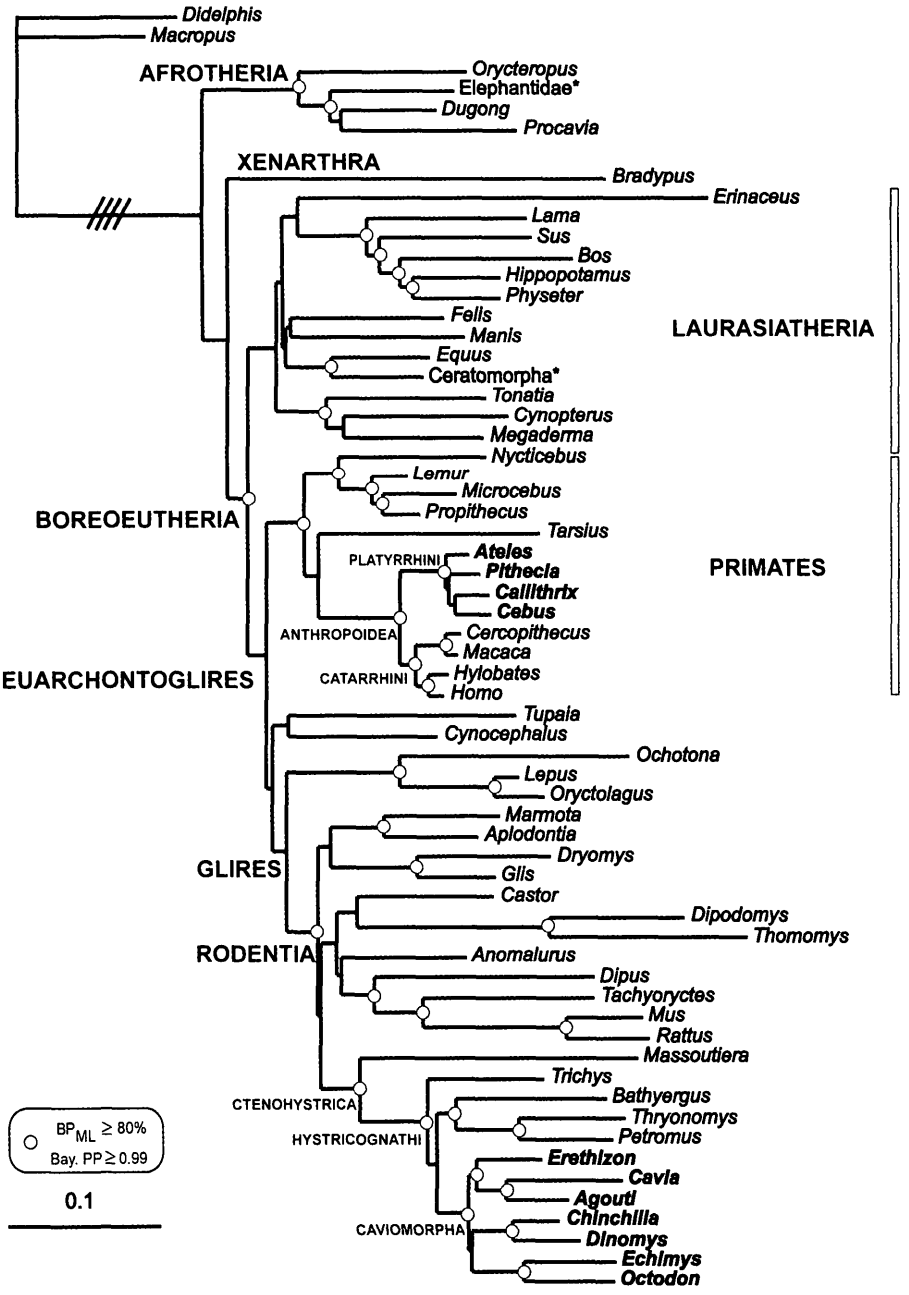

FIGURE 1. Phylogenetic tree reconstructed by ML analysis of the three concatenated markers ADRA2B, vWF, and IRBP ($-\ln L$ 71,720.63). The Bayesian analyses gave the same topology. Nodes getting a bootstrap support (BP) ≥80% and a posterior probability (PP) ≥0.99 are marked with an open circle. The length of the branch connecting the eutherians to the marsupial outgroup has been reduced four times. The names of South American primates and rodents are in bold. *Elephantidae and Ceratomorpha are represented in the data set by concatenated sequences from different genera (see Table 2). The names of supraordinal clades are documented in Springer et al. (2004b) and references therein. Ctenohystrica is according to Huchon et al. (2000) and Hystricognathi according to Tullberg (1899). Within primates the higher taxon names are given according to Fleagle (1999), and for Caviomorpha, see McKenna and Bell (1997).

molecular clock analysis, it was automatically removed by the ESTBRANCHES program for the branch lengths computation under that partition.

Statistical Tests of Compatibility of Calibration Constraints

The reciprocal compatibility of the eight calibration constraints was analyzed by repeating the dating calculations after removal of each one of the calibration constraints in turn. To assess the stability of the dating results when removing a specific calibration point, we performed an analysis of variance (ANOVA) and an H.S.D. Tukey's test (Tukey's Honestly Significantly Difference test) with the program SPSS 10.0.7 for Windows (SPSS Inc., Chicago, IL). The tests were performed on the difference between the dates calculated with all the calibration constraints and the dates found after removal of a specific calibration point.

RESULTS

Phylogenetic Relationships

The ML and long-run Bayesian analysis of the three concatenated genes ADRA2B, vWF, and IRBP yield identical topologies. The four major mammalian clades (Murphy et al., 2001) are recovered: Afrotheria (ML bootstrap percentage [BP] = 100; posterior probability [PP] = 1.00), Xenarthra (only represented here by *Bradypus*), Laurasiatheria (BP = 74; PP = 1.00), and Euarchontoglires (BP = 66; PP = 1.00). Boreoeutheria, comprising Euarchontoglires and Laurasiatheria, is also strongly supported. Within primates and rodents the monophyly of platyrrhines and caviomorphs, respectively, get maximal support (BP = 100; PP = 1.00). The anthropoid clade that joins platyrrhines to catarrhines also receives maximal support. Amongst the rodent nodes of direct interest, Hystricognathi and Phiomorpha s.s. (Bathyergidae plus Thryonomyoidea) get maximal support as well; only the phylogenetic relation between Caviomorpha and Phiomorpha s.s. is somewhat less supported (BP = 69 and PP = 1.00).

Very minor differences were found between the maximum posterior probability topologies of the short-run and long-run Bayesian analyses—i.e., 1,000,000 versus 10,000,000 MCMCMC generations. For example, *Echimys* and *Octodon* are either in basal position within caviomorphs (PP = 0.51 for the shortest run) or sister group of *Chinchilla* + *Dinomys* (PP = 0.53 for the longest run). Moreover, the interpretation of posterior probabilities in terms of node support was identical for the two Bayesian analyses. The support for the strongest nodes (PP $\geq$ 0.99) remained unaffected by longer runs. Posterior probabilities of weaker nodes (PP $\leq$ 0.98) appeared more variable, as illustrated by the *Dugong* + *Procavia* association (short run: PP = 0.79; long run: PP = 0.90). However, all nodes in our phylogenetic tree (Fig. 1) that are relevant to the understanding of the South American migration and diversification of primates and rodents are well supported, providing a reliable phylogenetic framework for the assessment of divergence times.

Molecular Datings Based on Three Combined Genes and Using All Calibration Constraints

The log-likelihood of the best tree without clock constraint was $\ln L_{NO\ CLOCK} = -71{,}720.63$, against $\ln L_{CLOCK} = -72{,}690.56$ under the global clock constraint. A likelihood-ratio test significantly rejected the hypothesis of a clock-like behavior of our data: $\delta = 2\times(\ln L_{NO\ CLOCK} - \ln L_{CLOCK}) = 1{,}939.86$; d. f. = 60; $P < 0.001$. We therefore proceeded to a relaxation of the molecular clock hypothesis through a Bayesian approach of substitution rate autocorrelation.

First of all, the combined ADRA2B, vWF, and IRBP genes contain dating signal because the prior and posterior distributions of the divergence times are markedly different, and divergence times are converging toward the same estimates when the MCMC are run from different starting states (data not shown). The following informations from the dating results (Table 3 and Fig. 2) are most relevant for our purposes: (i) the age of the stem groups, i.e., the time at which platyrrhines and caviomorphs diverged from their closest extant sister group—catarrhines and phiomorphs, respectively—before they arrived in South America: this will be the upper (= deeper) bound of the estimated time of arrival in South America; (ii) the age of the crown groups, i.e., the time of the earliest diversification of platyrrhines and caviomorphs (here represented by extant species only), which must have occurred after their arrival in South America: this will be the lower bound of the estimated time of arrival in South America; and (iii) the time interval between these two events, which demarcates the period during which primates and rodents may have reached South America. This interval should capture with sufficient statistical significance the actual time window during which the colonization took place.

According to our datings and their standard deviations (Table 3, all calibration constraints), the platyrrhines arrived within a time window of maximally 25.5 My, between 37.0 $\pm$ 3.0 (i.e., 34.0 to 40.0) Mya—the age of the Catarrhini/Platyrrhini divergence—and 16.8 $\pm$ 2.3 (i.e., 14.5 to 19.1) Mya—the earliest diversification of the extant platyrrhines. One should note that 25.5 My is most probably an overestimation of the time frame for the possible arrival of platyrrhines, because extant platyrrhines may not represent the first event of diversification of the platyrrhines in South America (see Discussion). By contrast, the arrival of caviomorphs in South America must have taken place in a time window of maximally 16.5 My during the Middle Eocene, between 45.4 $\pm$ 4.1 Mya—the Phiomorpha/Caviomorpha split—and 36.7 $\pm$ 3.7 Mya—the earliest radiation of extant caviomorphs. Taking $\pm$1 SD into account, there is an overlap of 7.0 My (indicated by the dark gray zone in Fig. 2) between the latest possible arrival time of caviomorphs (33.0 Mya) and the earliest possible arrival of platyrrhines (40.0 Mya). Based on our data, we therefore cannot rule out the possibility of a concomitant arrival of primates and rodents in South America.

Influence of Individual Calibration Constraints

To test the possibility that individual calibration constraints may have disproportional effects on the obtained platyrrhine and caviomorph divergence time estimates, we repeated the dating analysis after removing each of the eight calibration constraints in turn (Table 3, upper part). In all instances, the posterior divergence ages are highly concordant with those obtained with the complete set of calibration constraints, with the exception of the calibration corresponding to the divergence between Cercopithecoidea and Hominoidea. When this calibration information is removed (Table 3, upper part, in bold), the date estimates for platyrrhines become different (more recent) relative to the other posterior divergence time estimations and in strong disagreement with the anthropoid fossil record (Fleagle, 1999) (see Discussion). The times estimated for the caviomorphs become

TABLE 3. Posterior divergence times in Mya (±1 standard deviation) as inferred by the Bayesian relaxed molecular clock approach from the concatenated ADRA2B, IRBP, and vWF sequences. Upper part: ages pertaining to the platyrrhine and caviomorph nodes under study. Lower part: ages pertaining to the eight fossil calibration constraints.

			Removal of the following calibration constraint during the analysis[c]:							
Radiation or branching (/)	Calibration time frame[a] (Mya)	All calibration constraints[b]	Paenungulata	Cetartiodactyla	Perissodactyla	Primates	Cercopithecoidea/ Hominoidea split	*Ochotona*/ Leporidae split	*Marmota*/ *Aplodontia* split	*Dryomys*/ *Glis* split
Platyrrhini radiation		16.8 ± 2.3	16.7 ± 2.2	17.0 ± 2.3	16.6 ± 2.2	16.8 ± 2.3	**14.9 ± 2.3**	16.8 ± 2.3	16.7 ± 2.3	16.8 ± 2.2
Platyrrhini/Catarrhini		37.0 ± 3.0	36.9 ± 2.9	37.4 ± 3.0	36.8 ± 2.9	37.0 ± 2.9	**32.2 ± 3.7**	37.0 ± 3.0	37.0 ± 3.0	37.0 ± 2.9
Caviomorpha radiation		36.7 ± 3.7	36.5 ± 3.6	37.2 ± 3.9	36.4 ± 3.6	36.7 ± 3.6	**35.1 ± 3.5**	36.6 ± 3.6	36.6 ± 3.7	36.6 ± 3.6
Phiomorpha s.s./Caviomorpha		45.4 ± 4.1	45.1 ± 4.1	45.9 ± 4.1	45.0 ± 4.1	45.3 ± 4.1	**43.9 ± 4.0**	45.2 ± 4.1	45.2 ± 4.1	45.2 ± 4.1
Paenungulata	54–65	59.1 ± 3.0	58.4 ± 6.0							
Cetartiodactyla	55–65	59.4 ± 2.6		60.0 ± 3.0						
Perissodactyla	54–58	55.9 ± 1.1			54.6 ± 4.7					
Primates	63–90	77.5 ± 4.4				77.5 ± 4.5				
Cercopithecoidea/Hominoidea	25–35	26.8 ± 1.5					**21.4 ± 3.0**			
Ochotona/Leporidae	>37	51.1 ± 4.8						51.0 ± 4.8		
Marmota/Aplodontia	>37	49.9 ± 4.5							49.7 ± 4.5	
Dryomys/Glis	>28.5	38.9 ± 4.3								38.6 ± 4.3

[a] Paleontological calibration time frames in the order in which they appear in Figure 2 (from top to bottom).

[b] Underlined are the time estimates of platyrrhine and caviomorph nodes obtained when using all calibration points.

[c] The influence of each calibration point was tested by computing divergence ages after removing that calibration point. The upper part of the table shows that only the removal of the Cercopithecoidea/Hominoidea calibration point has a significant effect, by lowering the calculated times of radiation and divergence of platyrrhines and catarrhines (in bold; ANOVA $P < 0.001$ and H.S.D. Tuckey's test $P < 0.01$). The diagonal of the table shows that seven calibration points are correctly recovered (falling within the calibration time frame as set for that point, and close to the times obtained with all calibration points) when the point itself is excluded from the constraints. However, the Cercopithecoidea/Hominoidea split (in bold) is dated more recent (21.4 Mya) than allowed by its calibration time frame (25 to 35 Mya), and also more recent than the recovered time when all calibrations are used (26.8 Mya).

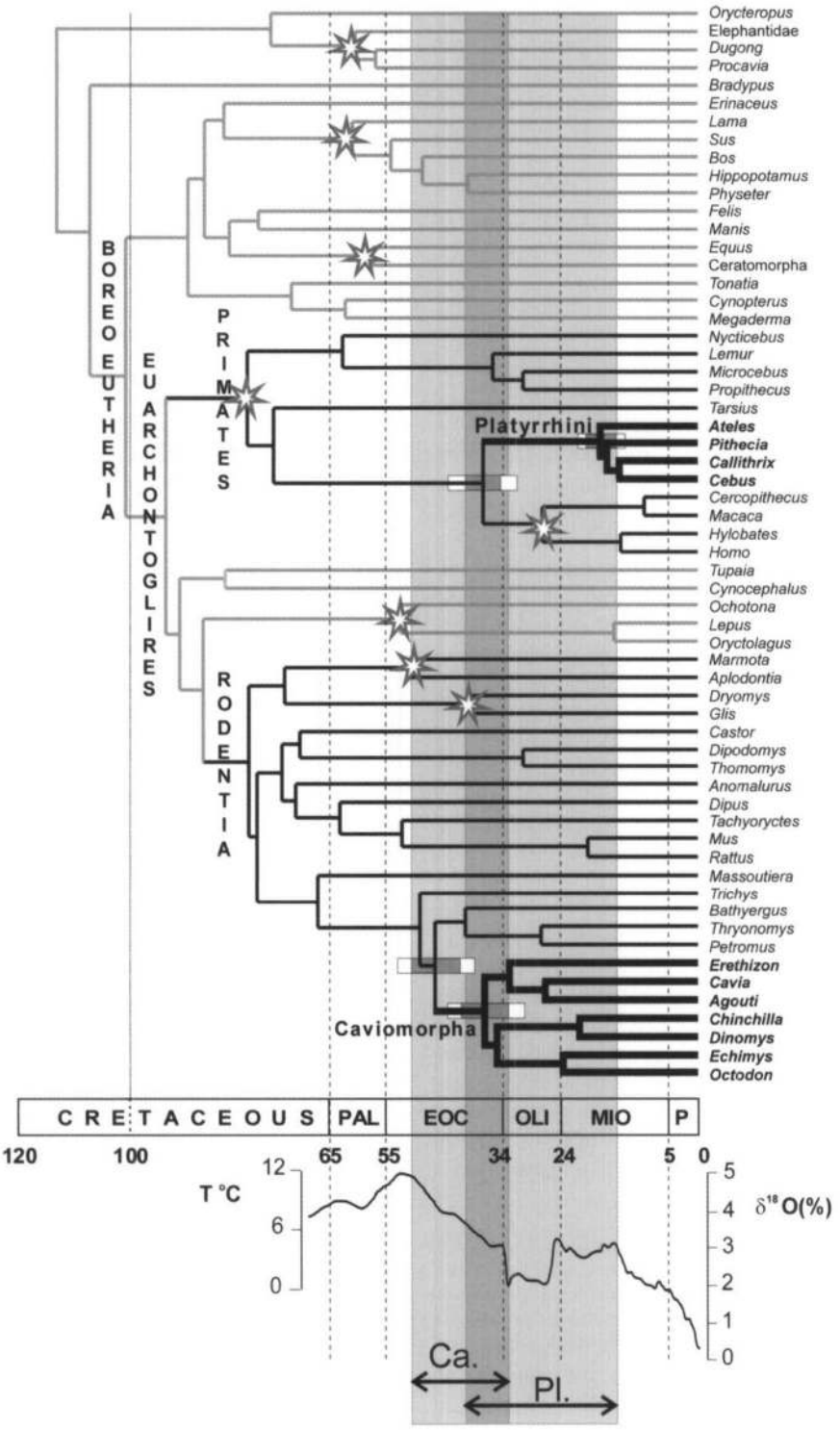

FIGURE 2. Chronogram showing the posterior divergence ages of placental taxa. The topology corresponds with the ML tree in Figure 1. Divergence times have been estimated from the concatenated ADRA2B, IRBP, and vWF sequences by a Bayesian relaxed molecular clock method with eight fossil calibration time constraints (nodes indicated by a star). For the nodes demarcating the period during which platyrrhines and caviomorphs may have reached South America, ±1 standard deviation and 95% credibility intervals are indicated by dark and light rectangles, respectively. The vertical gray zone spans the periods between the origin and radiation of Caviomorpha (Ca) and Platyrrhini (Pl), whereas the dark gray zone indicates the overlapping period during which Caviomorpha and Platyrrhini could have reached South America synchronously. The chronostratigraphic scale is given with absolute geological ages, and vertical dashed lines separate the epochs. PAL, EOC, OLI, MIO, and P stand for Paleocene, Eocene, Oligocene, Miocene, and PlioPleistocene, respectively. The black curve represents the variation of deep-sea $\delta^{18}O$ (from Zachos et al., 2001). From these values the absolute deep-sea temperature can directly be read until the Early Oligocene (34 Mya); from that period to the present the variations of deep-sea $\delta^{18}O$ are the result of both changes in temperature and in ice volume in Antarctica and the Northern Hemisphere. The curve shows that caviomorphs diversified before the Oligocene cooling down, whereas the platyrrhines diversified after the Oligocene, during the relatively warm first half of the Miocene.

more recent as well, compared to the other datings, but the discrepancies are smaller than for the primates, and the results are not in disagreement with the fossil record.

Also the posterior divergence time estimate for each calibration node remains correctly recovered by all other calibrations, again with the unique exception of the Cercopithecoidea/Hominoidea divergence (Table 3, lower part: see the diagonal). This divergence now drops to 21.4 ± 3.0 Mya, whereas the time range used as calibration constraint is 25 to 35 Mya.

Molecular Datings Based on Separate Genes

To explore the contributions of the three genes in our calculations, we estimated the divergence dates from each gene separately, with and without the Cercopithecoidea/Hominoidea calibration point (Table 4). Using all calibration constraints, ADRA2B indicates that rodents and primates may have reached South America concomitantly between 44.6 to 39.2 Mya. Similarly, the vWF data set shows an approximately concomitant migration between 38.3 to 33.1 Mya. However, absolute ages estimated by ADRA2B for rodents and the New World/Old World primates split are around 6 My deeper as compared with the vWF. Finally, IRBP gene suggests that platyrrhines originated after the radiation of caviomorphs, which would consequently exclude a concomitant migration of primates and rodents to South America.

Removing the Cercopithecoidea/Hominoidea calibration point had no effect on vWF estimates, resulted in slightly younger estimates for ADRA2B, but had the greatest impact for IRBP estimates (Table 4). In the latter case, the datings within the anthropoids become exceedingly young as compared to the fossil record. It estimates, for example, the split between Cercopithecoidea and Hominoidea at 16.5 ± 3.6 Mya, whereas the oldest Proconsulidae (hominoids) is already present in the fossil record around 25 Mya (Fleagle, 1999). Relative rate tests and nonsynonymous-to-synonymous ratios determined from the IRBP sequences (results not shown) indicate that the younger dates found within anthropoids could be explained by an increase of the molecular evolutionary rate of IRBP within this clade. This assumption is also supported by Poux and Douzery (2004), who described a higher IRBP nucleotide substitution rate along the anthropoid and platyrrhine ancestral branches. Using the Cercopithecoidea/Hominoidea calibration point allowed us to reduce the impact of the IRBP high molecular evolutionary rate on the datings.

Molecular Datings with Combined ADRA2B and vWF

Considering that the faster evolution of IRBP in anthropoids might have affected the combined results as given in Table 3, we also estimated divergence times using the combined ADRA2B and vWF genes alone (Table 4). With all calibration constraints included, the results for caviomorphs become slightly younger (order of magnitude ca. 1 My) than with the three combined genes. The impact of the IRBP removal is stronger on the platyrrhine datings, and actually makes them deeper, now placing the platyrrhine/catarrhine split at 38.9 ± 4.0 Mya and the platyrrhine radiation at 20.1 ± 3.3 Mya. These results are 1.9 My and 3.3 My older, respectively, than found with the three genes combined. The overlap between the time frames for arrival of rodents and primates now becomes larger: 11.4 My (between 42.9 My and 31.5 My, taking ±1 SD into account) versus 7.0 My.

However, the maximum time intervals during which rodents and primates may have reached South America remain concordant with the first calculations, namely 17.0 and 26.1 My, versus 16.5 and 25.5 My, respectively. The longer lag time between origin and diversification of the extant platyrrhines compared to caviomorphs is an aspect in which the three genes consistently agree. The dating estimates of the caviomorph and platyrrhine radiations never overlap, and it does not make any difference whether or not we omit IRBP, incorporate the Cercopithecoidea/Hominoidea split in the calibration, or use genes separately or in combination (Tables 3 and 4).

We also tested with this ADRA2B + vWF data set whether we could recover the individual calibration

TABLE 4. Posterior divergence ages in Mya (± standard deviation) for the primate and rodent nodes of interest, as inferred by the Bayesian relaxed molecular clock approach from the ADRA2B, vWF, and IRBP sequences separately, and for the combined ADRA2B + vWF sequences, with or without the Cercopithecoidea/Hominoidea calibration point. In bold: impact of the removal of the Cercopithecoidea/Hominoidea calibration on IRBP dating estimates. The results with all calibration constraints but without IRBP are underlined; they are compared, in the text, with the underlined results from Table 3 corresponding to the calculations computed with all calibration points and all genes.

	ADRA2B		vWF		IRBP		ADRA2B + vWF	
	All calibrations	Without Cercopithecoidea/ Hominoidea split	All calibrations	Without Cercopithecoidea/ Hominoidea split	All calibrations	Without Cercopithecoidea/ Hominoidea split	All calibrations	Without Cercopithecoidea/ Hominoidea split
Platyrrhini radiation	20.4 ± 5.0	19.1 ± 5.0	22.8 ± 4.7	22.8 ± 5.1	15.7 ± 3.2	**12.3 ± 2.8**	20.1 ± 3.3	19.4 ± 3.5
Platyrrhini/Catarrhini	44.6 ± 6.3	40.8 ± 7.4	38.3 ± 4.5	38.3 ± 6.3	37.2 ± 4.2	**27.5 ± 4.8**	38.9 ± 4.0	37.2 ± 5.0
Caviomorpha radiation	39.2 ± 6.6	38.5 ± 6.5	33.1 ± 5.0	33.1 ± 5.0	42.8 ± 6.1	41.5 ± 5.9	35.8 ± 4.3	35.4 ± 4.4
Phiomorpha s.s./Caviomorpha	46.6 ± 7.0	45.9 ± 6.9	40.6 ± 5.6	40.6 ± 5.5	52.4 ± 6.7	50.7 ± 6.4	43.7 ± 4.8	43.2 ± 4.9
Cercopithecoidea/ Hominoidea	28.7 ± 2.6	23.4 ± 6.0	29.9 ± 2.8	30.0 ± 5.9	26.9 ± 1.7	**16.5 ± 3.6**	28.7 ± 2.8	26.7 ± 4.4
Primates	77.5 ± 6.1	76.0 ± 6.3	79.4 ± 5.3	79.3 ± 5.6	76.4 ± 6.2	70.7 ± 5.7	78.5 ± 4.9	77.5 ± 5.2

constraints after removing each of them in turn. A reciprocal compatibility of all the calibrations points was observed, including that for the Cercopithecoidea/Hominoidea divergence (Table 4: 26.7 ± 4.4, to be compared to Table 3). Even though the datings after IRBP removal seem to give more appropriate results within anthropoids, the differences between the dates, with or without IRBP, will not alter our overall conclusions.

Influence of Taxon Sampling and Use of Chimeras

Divergence times estimated with only one species sampled from each of the four well-defined caviomorph superfamilies—*Erethizon* (Erethizontoidea), *Agouti* (Cavioidea), *Chinchilla* (Chinchilloidea), and *Echimys* (Octodontoidea)—lead to very similar results as compared to the datings based on the complete taxonomic set of seven species. Sampling four instead of seven caviomorphs yields the following divergence time estimates: 44.2 ± 4.0 Mya instead of 45.4 ± 4.1 Mya for the caviomorph/phiomorph split, and 36.1 ± 3.6 Mya instead of 36.7 ± 3.7 Mya for the caviomorph radiation. Extrapolating this observation to the platyrrhines, we thus can reasonably assume that the inclusion of only one species of Pitheciidae and Atelidae has only had a minor influence on the results. Moreover, given that all three platyrrhine clades were sampled, and apparently radiated in a very short time span, a reduced sampling within each family is not expected to strongly influence the results.

Apart from the problem of a reduced taxon sampling among platyrrhines, the construction of taxonomic chimeras in order to improve the nuclear gene coverage may have biased our dating estimates. Divergence times were therefore estimated from a 67-taxon supermatrix of characters; i.e., without chimeras of placental species, and using a reference topology in which the respective monophyly of *Ateles paniscus* + *A. belzebuth, Tupaia tana* + *T. glis, Equus asinus* + *E. caballus, Tapirus pinchaque* + *T. terrestris* + *Ceratotherium simum, Lama glama* + *L. pacos*, and *Loxodonta africana* + *Elephas maximus* was assumed. We observed that the dating results are essentially identical to those estimated from the original 60-taxon matrix, containing eight chimeras: 45.1 ± 4.1 Mya and 36.5 ± 3.6 Mya for the origin and radiation of caviomorphs, and 37.0 ± 2.9 Mya and 16.8 ± 2.3 Mya for the origin and radiation of platyrrhines. The finding that the use of composite taxa did not influence our dating results is in agreement with previous analyses showing the positive contribution of composite taxa in phylogeny reconstruction, as long as the species used to build chimeras are known to be monophyletic relative to the other species in the dataset (e.g., Springer et al., 2004a).

DISCUSSION

Contribution of Each Gene and Calibration Constraint

Two major difficulties of molecular dating are (i) the variation of evolutionary rate, which can be both gene-specific and lineage-specific, and (ii) the paleontological uncertainties associated with the calibration constraints (for reviews see Bromham and Penny, 2003; Graur and Martin, 2004). With regard to rate variation, a global molecular clock certainly does not fit our data (see Results), in agreement with the fact that extensive rate variations have been shown in primates and rodents, both with nuclear and mitochondrial data (e.g., Liu et al., 2001; Adkins et al., 2003; Douzery et al., 2003; Poux and Douzery, 2004). We therefore used the Bayesian method of Thorne et al. (1998) and Kishino et al. (2001), which is based on a probabilistic model of evolutionary rate autocorrelation, and has already been used in various animal groups (e.g., Bossuyt and Milinkovitch, 2001; Hasegawa et al., 2003; Hassanin and Douzery, 2003; Springer et al., 2003; Yoder et al., 2003). In this model, rates are allowed to vary over time and lineages, and rate changes along descending branches are autocorrelated according to a log-normal model. Moreover, a distinct model of nucleotide substitution can be defined for each selected gene partition (Thorne and Kishino, 2002).

The three nuclear markers used in this study do not lead to concordant datings and colonization scenarios, arguing for the combination of multiple genes to obtain an averaged representation of the underlying evolutionary processes, and thus divergence times. Our results show that, even with the use of more realistic evolutionary models, strong rate variation cannot be completely taken into account (see Table 2, Cercopithecoidea/Hominoidea split). To compensate to some extent for this effect, we will use in the following biogeographic sections both the datings obtained with the three-gene and with the two-gene (ADRA2B + vWF) combinations. An unexplained observation is that the datings obtained with the three- or two-gene combinations are always more recent than the average dates based on the separate genes (Tables 3 and 4). This intriguing feature can in fact also be observed in other dating studies where the results from combined genes might be markedly different from the average dates calculated with the separated genes (e.g., Yoder and Yang, 2004).

The problem of the paleontological uncertainties is reduced by Kishino et al.'s (2001) method because it handles calibration constraints as time ranges instead of time points. It has indeed been shown that the precision of the divergence time estimates is substantially enhanced when constraints are included (Kishino et al., 2001). In our analysis it only is the Cercopithecoidea/Hominoidea calibration point that has a major influence, but only on some of the anthropoid datings. Because this calibration point is located inside the anthropoid crown group, it is the only one that can counterbalance the influence of the rate acceleration in the IRBP sequences of anthropoids. This illustrates the importance of taking calibration constraints close to the nodes to be dated, in order to reduce the influence of local deviations in the evolutionary properties of the genes involved. The Cercopithecoidea/Hominoidea calibration point has already been used in recent molecular studies, but with quite different assigned times: e.g., Yang and Yoder (2003) used

a range of 32 to 38 Mya (partially based on molecular datings), whereas Schrago and Russo (2003) and Adkins et al. (2003) used 25 Mya based on paleontological data. As this calibration point is crucial for our datings, we used a conservative range of 25 to 35 Mya, especially in the light of the fossil record and a recent molecular study (Steiper et al. 2004). It is interesting to note that in our calculations the age of the Cercopithecoidea/Hominoidea split is never older than 30.0 Mya (Tables 3 and 4). Using a range of 32 to 38 Mya could therefore lead to biased conclusions because this time frame seems too deep, or at least too narrow. The difference in the assigned age of the Cercopithecoidea/Hominoidea calibration point could explain why Schrago and Russo (2003) and Yang and Yoder (2003) obtained such strikingly different results for the platyrrhine/catarrhine split (see Table 1), even though the same methods and mitochondrial DNA data were used.

Finally, the importance of a broad taxon sampling in combination with various calibration constraints must be emphasized. This allows the breaking of long branches, and consequently a better coping with the rate variations along such branches. Unfortunately, in our case it was not possible to break the long branch leading to the anthropoids because there are no additional extant species available.

Could the Colonizations of South America by Primates and Rodents Have Been Synchronous or Not?

The phylogenetic relationships among primates as shown in Figure 1 agree with the current consensus from nuclear DNA phylogenies (i.e., Goodman et al., 1998; Poux and Douzery, 2004), and rodent relationships are congruent with those in Adkins et al. (2001), Huchon and Douzery (2001), Huchon et al. (2002), and DeBry (2003). Given that platyrrhines and caviomorphs are restricted to South America, the monophyly of both groups suggests a single colonization event for each of them. A double invasion event has been claimed for primates on the basis of antigenic determinants (Bauer and Schreiber, 1997) and for rodents on the basis of paleontological evidence (e.g., Bryant and Mc Kenna, 1995), but this is not supported by our or any other molecular data. If other clades of primates and rodents reached South America during its period of isolation, they left no extant representatives.

A general agreement was found among divergence time estimates from different studies on primates and rodents (Table 1). Our estimated dates for the catarrhine/platyrrhine split (37.0 ± 3.0 Mya for the ADRA2B + vWF + IRBP analysis/38.9 ± 4.0 Mya for the ADRA2B + vWF analysis) are in perfect agreement with the results of Hasegawa et al. (2003) (37.5 ± 3.1 Mya), and around the average dates of the other studies based on either nuclear or mitochondrial genes, or a combination thereof. Similarly, our datings of the caviomorph/phiomorph split (45.4 ± 4.1 Mya/43.7 ± 4.8 Mya) fit with those of Huchon and Douzery (2001), and are slightly older than the dates obtained by Hasegawa et al. (2003) and Springer et al. (2003). However, there are three exceptions with deviating time estimates based on mitochondrial sequences. Arnason et al. (1998, 2000) estimated the catarrhine/platyrrhine (i.e., anthropoid) split at 60 to 70 Mya, whereas Yang and Yoder (2003: table 7) estimated it between 53.3 Mya and 61.1 Mya. Mouchaty et al. (2001) estimated the caviomorph/phiomorph split at 85 Mya. These discrepancies are likely to be the result of differences in the (i) methodology used for the calculations (global, local, or relaxed clocks); (ii) choice of calibration references (far from the clades under focus); and (iii) degree of accuracy and precision of these calibrations. It has indeed been suggested that the results obtained with nuclear and mitochondrial markers become consistent if appropriate methods and calibrations are used (e.g., Hasegawa et al., 2003).

Our dating estimates do not clearly advocate either a synchronous or an asynchronous colonization of South America by primates and rodents. As already mentioned, the periods of time during which primates and rodents could have reached South America overlap for 7.0 to 11.4 My, depending on the nuclear genes used. If we take into account that during this period, from 40.0/42.9 Mya (Middle Eocene) to 33.0/31.5 Mya (Early Oligocene), the geographic and environmental conditions allowing colonization may only temporarily have existed, a more or less synchronous arrival can be conceived. In that case, the caviomorphs diverged from their sister group (45.4/43.7 Mya) some time before they reached South America and radiated (36.7/35.8 Mya) soon after their arrival. In contrast, the primates should have colonized South America just after the divergence from their sister clade (37.0/38.9 Mya), whereas the radiation of extant platyrrhines (16.8/20.1 Mya) began much later, any lineages resulting from earlier diversification now being extinct. In conclusion, if suitable conditions and opportunities for primates and rodents to reach South America have been extremely rare, perhaps only occurring from the Middle Eocene until the Early Oligocene (the dark gray zone in Fig. 2), our datings may be in favor of a synchronous colonization; if suitable conditions existed repeatedly throughout the Eocene and Oligocene, our data would rather favor asynchronous colonizations.

Possible Migration Histories

Given that representatives of both primate and rodent orders did not reach South America before the Eocene, a land-bridge dispersal during the Late Cretaceous–Early Paleocene via the Rio Grande Rise and the Walvis Ridge, as proposed for primates by Arnason et al. (2000), can be dismissed. Various other biogeographical hypotheses remain open to explain the colonization of South America by primates and rodents. These hypotheses are based, first, on the locations of the oldest anthropoid, platyrrhine, and hystricognath fossils in the Old World (parsimoniously assuming that these locations might be the centers of origin of these clades) and, secondly, on the climatic and geographic conditions during the migration period to South America.

One hypothesis assumes an African origin for caviomorphs and platyrrhines, from phiomorph (Lavocat, 1969; Martin, 1994) and anthropoid stocks (Fleagle, 1999), respectively, followed by a transatlantic migration. Despite the distance between the two continents during the Middle Eocene–Early Oligocene, colonization could have occurred, aided by marine currents, palaeowinds, or "stepping stone" islands along with rafts (Wyss et al., 1993; Flynn and Wyss, 1998; Houle, 1999). Transoceanic dispersals have also been suggested for a variety of other taxonomic groups (de Queiroz, 2005), including Africa/South America exchanges for squamates and angiosperms, and Africa/Madagascar exchanges for squamates and amphibians (e.g., Vences et al., 2003) and mammals (e.g., Yoder et al., 1996, 2003, Poux et al., 2005). The transatlantic route is the preferred hypothesis concerning platyrrhine migration for two reasons. First, fossils considered as early platyrrhines (parapithecids or proteopithecids from the Late Eocene Fayum formation in Egypt; Simons, 1997; Fleagle, 1999; Ross, 2000) and early catarrhines (*Aegyptopithecus* from the Early Oligocene Fayum formation in Egypt; Fleagle, 1999) have so far only been found in Africa. Secondly, migration through Antarctica is unlikely for this group because at the time of platyrrhines/catarrhines divergence—at most, 37 Mya—Australia, Antarctica, and South America were no longer strongly connected, while Antarctica was, moreover, covered by ice sheets (Zachos et al., 2001).

With respect to rodents, an Asiatic origin of hystricognaths is broadly supported (Flynn et al., 1986; Bryant and McKenna, 1995; Marivaux et al., 2002), implying that South American caviomorphs and their sister group, the African phiomorphs, share an Asian hystricognath ancestor (Marivaux et al., 2002). However, it is not clear whether caviomorphs and phiomorphs diverged already in Asia or after migration of their hystricognath ancestor into Africa, which leaves different dispersion routes to South America open to speculation. Indeed, the dispersal of the caviomorph ancestor to South America might have occurred from Africa (Lavocat, 1969), but also from Asia, with a subsequent migration via Antarctica (Huchon and Douzery, 2001) or North America (e.g., Hussain et al., 1978). A weak point of the latter two proposals is that no protocaviomorph remains have been reported from Antarctica or Australia, and the ones discovered in North America appear to have been misinterpreted (e.g., Martin, 1994). However, in contrast to North America, the fossil record of Antarctica is relatively unexplored, and it has recently indeed been shown that transantarctic dispersal has been quite frequent in the southern hemisphere (Sanmartín and Ronquist, 2004). Moreover, colonization via Antarctica was physically possible for rodents because South America was connected to Antarctica until 37 to 30 Mya (e.g., Barker et al., 1991; Lawver et al., 1992; Lawver and Gahagan, 2003), and even though Australia became separated from Antarctica around 90 Mya, the two continents remained fairly close together until the opening of the Tasman Sea, 35 Mya (Lawver et al., 1992). During this period, the climate was still temperate, and angiosperms flourished on Antarctica. However, the sea barrier between Asia and Australia was at that time wider than the Atlantic Ocean and, because of this major problem, Houle (1999) refuted this possibility. From our study it is not possible to decide between a rodent colonization of South America via Africa or via Antarctica; the discovery of protocaviomorph fossils might shed more light on this issue.

When Did Platyrrhines and Caviomorphs Diversify in South America?

The oldest caviomorph fossil found in South America is estimated at 31 Mya and is considered to belong to the extant family Dasyproctidae (Wyss et al., 1993) or Dinomyidae (Vucetich et al. 1999). This means that at 31 Mya the caviomorphs had probably already started to diversify, and implies that the arrival of caviomorphs in South America predated the Early Oligocene. Such a view is supported by our Late Eocene dating of the caviomorph radiation ($36.7 \pm 3.7/35.8 \pm 4.3$ Mya) and is in agreement with the paleontological analysis of Vucetich et al. (1999). This result also implies that extant caviomorph lineages derive from early diversification events.

The oldest primate fossils from South America, *Branisella* and *Szalatavus* (27 Mya, Bolivia) (Rosenberger et al., 1991), considered as a single genus by Takai and Anaya (1996), are plesiomorph platyrrhines and have no direct relation with living platyrrhines (Fleagle, 1999). The Patagonian primate fossils from the Early and Middle Miocene (21 to 14 Mya) are considered either as sister group of extant platyrrhines or as nested within this clade (Fleagle, 1999). Thus, there are still paleontological uncertainties about the time of radiation of living South American primates. However, the primate fossils from the later Middle Miocene of La Venta (12 to 13 Mya, Colombia) are highly similar to modern platyrrhines (Fleagle, 1999). Our dating of the platyrrhine diversification, during the Early Miocene ($16.8 \pm 2.3/20.1 \pm 3.3$ Mya), is concordant with the view that the La Venta fossils belong to the modern lineages, and that only the oldest of the Patagonian fossils might not belong to any of the extant families, but originated earlier. The platyrrhine diversity before the Early Miocene is quite poor either because of gaps in the fossil record or because platyrrhines did not undergo an explosive radiation as the caviomorphs did. Extinction events likely occurred in both platyrrhine and caviomorph lineages but, because of the poor diversity of platyrrhines, only one lineage resulting from the early platyrrhine radiation survived. Present-day platyrrhines would actually derive from a late diversification event during the Early Miocene.

Platyrrhine and Caviomorph Radiations and Global Climatic Changes

The dating of the platyrrhine and caviomorph arrivals and their subsequent diversification can be correlated with global climatic changes (see Fig. 2) (Zachos et al., 2001). Such an influence has already been proposed for another South American mammalian clade,

the xenarthrans (Delsuc et al., 2004). The diversification of extant caviomorphs started in the Late Eocene, before the beginning of the Oligocene glaciation periods. It appears that several caviomorph lineages were able to adapt to the Oligocene climate changes, and an example of such adaptation might be the evolution of hypsodont teeth among caviomorphs (Vucetich et al., 1999). In fact, around 31 to 35 Mya, hypsodont herbivores were dominating the mammalian fauna in South America (Tinguiririan fauna); these herbivores are usually considered as grazers, implying that open habitats (woodlands to savanna) and grasslands were present (Flynn and Wyss, 1998). Rodents probably radiated by exploiting these and other new niches opened by climatic changes.

According to our dating, all present-day platyrrhines result from an Early Miocene diversification. This suggests that Oligocene platyrrhines underwent extinction events. Primates extinctions have frequently been explained by climatic changes, such as the general cooling down in the beginning of the Oligocene, leading to a strong decline of primate diversity in the northern continents (Gingerich, 1986; Fleagle, 1999). The African ancestors of neotropical monkeys were arboreal quadrupeds (Fleagle, 1999), and all extant platyrrhines are still arboreal; only *Branisella*, the first recorded primate in South America (27 Mya), has been suggested to be semiterrestrial and its dentition adapted to abrasive food (Takai et al., 2000). It thus seems likely that Oligocene glaciations (26.5 to 34.0 Mya), resulting in the transformation of forests into open areas, may explain why early platyrrhine lineages are semi-terrestrial lineages that are not representative of the extant diversity. McKenna and Bell (1997) recognized only one genus of primates in the Oligocene and five in the Early Miocene, as compared to 16 caviomorph genera in the Oligocene and 25 in the Early Miocene. These differences in number of genera may illustrate the fact that the climatic conditions were probably not appropriate to allow primates to realize a similar explosive radiation as caviomorphs did.

CONCLUSIONS

This study is the first one comparing the colonization histories of platyrrhine primates and caviomorph rodents using the same set of (nuclear) genes. Considering both the fossil record and our molecular dating estimates, the most plausible scenario for primates suggests a transatlantic migration at the end of the Eocene, followed by the extinction of all but one of the few earlier diverging lineages, and the radiation of extant platyrrhines during the Early Miocene. Our results also show that the arrival of rodents and primates in South America might have been contemporaneous. However, in contrast to platyrrhines, representatives of the early diversification of caviomorphs, which occurred before the Oligocene glaciations, survive until the present. The absence of fossil information about caviomorphs outside South America, which could have been combined with our molecular data, allows only speculations about their migration history. A better understanding of this open biogeographical question awaits the discovery of new fossils, phylogenetically close to the caviomorphs.

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