

Phylogenetic relationships and divergence times among New World monkeys (Platyrrhini, Primates)

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Abstract

Orthologous sequences of six nuclear genes were obtained for all recognized genera of New World monkeys (Primates: Platyrrhini) and outgroups to evaluate the phylogenetic relationships and to estimate divergence times. Phylogenetic relationships were reconstructed by maximum parsimony, maximum likelihood, and Bayesian approaches. All methods resolved with 100% branch support genus-level relationships, except for the grouping of *Aotus* as a sister taxa of *Cebus* and *Saimiri*, which was supported by low bootstrap percentages and posterior probability. All approaches depict three monophyletic New World monkey families: Atelidae, Cebidae, and Pitheciidae; also within each family, all approaches depict the same branching topology. However, the approaches differ in depicting the relationships of the three families to one another. Maximum parsimony depicts the Atelidae and Cebidae as sister families next joined by the Pitheciidae. Conversely, likelihood and Bayesian phylogenetic trees group families Atelidae and Pitheciidae together to the exclusion of Cebidae. Divergence time estimations using both local molecular clock and Bayesian approaches suggest the families diverged from one another over a short period of geological time in the late Oligocene–early Miocene.

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1. Introduction

New World monkeys (NWMs) represent a monophyletic group, which inhabit South and Central America. Extant NWM species are assigned to the Infraorder Platyrrhini and Superfamily Ceboidea, which in turn divides into three families (Atelidae, Cebidae, and Pitheciidae) and 14–15 genera (Goodman et al., 1998, 1999; Wildman and Goodman, 2004). The phylogenetic relationships among NWMs have been extensively investigated using nucleotide

sequences from different genomes (nuclear and mitochondrial) and other kinds of markers like short interspersed elements (SINEs) (Ray et al., 2005). Nevertheless, despite these different studies, there still are some unresolved issues. The branching order of the three families has not been confidently resolved. Some evidence suggests that the first split separated Pitheciidae from the other two families (Fig. 1A) (Prychitko et al., 2005; Ray et al., 2005; Steiper and Ruvolo, 2003; von Dornum and Ruvolo, 1999). Conversely, families Atelidae and Pitheciidae as the sister group of Cebidae has also been proposed (Fig. 1B) (Canavez et al., 1999a; Harada et al., 1995; Porter et al., 1997a). The phylogenetic relationships among different genera of the families Atelidae ((*Lagothrix*, *Brachyteles*), *Ateles*), *Alouatta*) and

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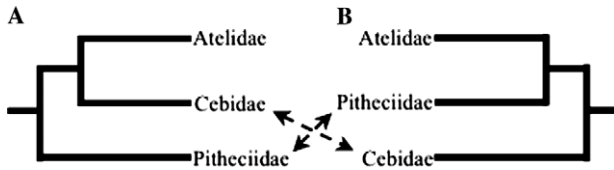


Fig. 1. Schematic representation of the two hypotheses proposed to explain the phylogenetic relationships at the familial level among New World Monkeys. (A) The first hypothesis suggests an initial split between Pitheciidae and the remaining platyrrhines, grouping Atelidae and Cebidae together. (B) The second hypothesis groups Atelidae and Pitheciidae as the sister group of the family Cebidae.

Pitheciidae (*Cacajao*, *Chiropotes*, *Pithecia*, *Callicebus*) are well characterized (Canavez et al., 1999a; Goodman et al., 1998; Harada et al., 1995; Horovitz et al., 1998; Meireles et al., 1999; Porter et al., 1997a,b, 1999; Schneider et al., 1993; von Dornum and Ruvolo, 1999); however, based on mitochondrial (COII and control region) and nuclear (aldolase A) sequences, one recent article suggests that within the family Atelidae a trichotomy among *Brachyteles*, *Lagothrix*, and *Ateles* should still be recognized (Collins, 2004). On the other hand, morphological data suggest *Ateles* and *Brachyteles* as a sister group of *Lagothrix* (Rosenberger, 2002). Within family Cebidae, most molecular phylogenetic studies agree that genera within the subfamily Callitrichinae group as follows, (*Callimico*, *Callithrix*, *Leontopithecus*, *Saguinus*) (Canavez et al., 1999a,b; Chaves et al., 1999; von Dornum and Ruvolo, 1999). However, some articles have reported alternative topologies, grouping *Leontopithecus* and *Saguinus* as a sister taxa (Goodman et al., 1998; Harada et al., 1995; Porter et al., 1997b, 1999; Schneider et al., 1993). A sister-group relationship between *Saimiri* and *Cebus* is well supported (Canavez et al., 1999a,b; Harada et al., 1995; Horovitz et al., 1998; Porter et al., 1997a,b, 1999; von Dornum and Ruvolo, 1999;

Steiper and Ruvolo, 2003). Nevertheless, the phylogenetic position of *Aotus* is still unclear, some studies have shown *Aotus* as a branch in a cebid polytomy (Schneider et al., 1993; Singer et al., 2003; von Dornum and Ruvolo, 1999), as a sister clade of the subfamily Callitrichinae (Goodman et al., 1998; Harada et al., 1995; Porter et al., 1997a,b, 1999) or as sister of all other extant Cebidae (Horovitz et al., 1998). On the other hand, morphological evidence has shown *Aotus* and *Callicebus* as either a sister group of *Saimiri* or of the subtribe Pitheciina (Rosenberger, 2002).

There is now DNA sequence data available for NWM genera from six nuclear genomic loci. In the present study, we concatenate the six loci and align the orthologous sequences among 15 NWM genera. Our objective is to estimate both phylogenetic relationships and divergence times among these NWMs. Accordingly, we have produced some sequences to fill gaps in the database.

2. Materials and methods

Sequences from ε-globin (HBE), interphotoreceptor retinol binding protein (IRBP), von Willenbrand factor (vWF), β2-microglobulin (B2M), β-globin (HBB), and glucose-6-phosphate dehydrogenase (G6PD) genes were obtained for all genera of NWMs and outgroups from GenBank (Table 1). For the vWF gene, we conducted PCRs for species of the genera *Brachyteles* (DQ129680), *Chiropotes* (DQ129681), *Pithecia* (DQ129682), and *Saimiri* (DQ129683) (Table 1). We used vWF-8 and vWF-10 primers (Chaves et al., 1999) under the following conditions: 30 cycles of denaturation, 94 °C, 1 min; annealing, 52 °C, 1 min; and extension 72 °C, 3 min; initial denaturation at 94 °C for 2 min and extension at 72 °C for 10 min were conducted. For the β-globin gene, we conducted PCRs for species in *Brachyteles* (DQ145531), *Cacajao* (DQ145529), *Chiropotes* (DQ145530), *Leontopithecus*

Table 1
Accession numbers of all sequences and taxa used in this study

Genus	ε-Globin	G6PD intron 4	G6PD intron 5	β2-Microglobulin	IRBP	vWF	β-Globin
<i>Alouatta</i>	L25370	AF028473	AF028497	AF032048	U18602	AF092837	AY279110
<i>Aotus</i>	L25371	AF028475	AF028500	AF032093	U18601	AF092812	AY279113
<i>Ateles</i>	L25369	AF028477	AF028501	AF032087	U18603	AF092813	AY279117
<i>Brachyteles</i>	L25366	AF028478	AF028502	AF032051	U18605	This study	This study
<i>Cacajao</i>	L25365	AF028484	AF028508	AF032078	U19748	AF092814	This study
<i>Callicebus</i>	L25359	AF028483	AF028507	AF069326	U18609	AF092815	AY279119
<i>Callimico</i>	L25364	AF028486	AF028510	AF032039	U19749	AF092826	AY279118
<i>Callithrix</i>	L25363	AF028488	AF028512	AF068767	U18606	AF092828	AY279111
<i>Cebus</i>	U18608	AF028479	AF028503	AF032018	U18607	AF092822	AY279115
<i>Chiropotes</i>	L25360	AF028487	AF028511	AF032075	U18612	This study	This study
<i>Lagothrix</i>	L25358	AF028489	AF028513	AF032054	U18614	AF092830	AY279114
<i>Leontopithecus</i>	L25357	AF028490	AF028514	AF032036	U19751	AF092832	This study
<i>Pithecia</i>	L25356	AF028492	AF028516	AF032072	U18615	This study	AY279112
<i>Saguinus</i>	L25355	AF028493	AF028517	AF032024	U19752	AF092836	This study
<i>Saimiri</i>	U18618	AF028496	AF028520	AF068765	U18619	This study	AY279116
Outgroups							
<i>Homo</i>	NG000007	X55448	X55448	M17987	J05253	AC006576	WGS
<i>Macaca</i>	M81364	456149763	460044619	AY091962	402318992	AY434057	X05665
<i>Tarsier</i>	M81411	NA	NA	NA	NA	NA	M33973
<i>Otolemur</i>	U60902	NA	NA	NA	NA	NA	M61740

(DQ145532), and *Saguinus* (DQ145533) (Table 1). We used U61661, U62476, L62612, U63482, L63588, and L63981 primers (Prychitko et al., 2005), under the following conditions: 94 °C, 5 min; touchdown PCR (4 cycles for each temperature) 94 °C, 45 s; 60–53 °C, 45 s; 68 °C, 2 min; and final extension: 68 °C, 10 min. PCR products were extracted from gel using Qiaquick (Qiagen, Valencia, CA) gel extraction kit, DNA sequencing reactions were done using Big Dye (Applied Biosystems, Foster City, CA) cycle sequencing kit according to the manufacturer's specifications.

2.1. Phylogenetic analysis

Sequences obtained in this work were aligned with those previously published using ClustalX (Thompson et al., 1997). *Homo* and *Macaca* were used as outgroups. Maximum parsimony analysis was performed using PAUP* 4.0b10 (Swofford, 2002), and support for internal nodes was assessed by bootstrap analysis after 1000 heuristic replicates, using the TBR branch-swapping algorithm. Maximum likelihood analysis was also done in PAUP* 4.0b10 (Swofford, 2002), the TVM + Γ model was chosen in a non-nested framework by Akaike Information Criterion (AIC) using Modeltest 3.06 (Posada and Crandall, 1998). Support of internal nodes was assessed by 1000 heuristic replicates, using the TBR branch-swapping algorithm. A Bayesian approach as implemented in Mr. Bayes 3.1.0 (Ronquist and Huelsenbeck, 2003) was also used to infer phylogenetic relationships. Markov Chain Monte Carlo (MCMC) simulations were run for 2,000,000 generations, with four simultaneous chains, using a sample frequency of 100 and a burn-in of 200,000. Default settings for the prior probabilities on the model parameters (GTR + Γ_4) were used.

2.2. Divergence times

Parsimony-based local molecular clocks as described by Goodman (1986), examples of which are shown in Bailey et al. (1991, 1992), were used to estimate divergence times. Additionally, MULTIDIVTIME (Kishino et al., 2001; Thorne et al., 1998) was used to estimate divergence dates. We chose the earliest known fossil platyrrhine (*Branisella boliviana*) found in the Deseadan fauna of La Salla, Bolivia, approximately 26 Mya (MacFadden, 1990) as a calibration point. We chose a single calibration point for two reasons. First, we wanted our results to be comparable to those of other studies. Second, *B. boliviana* is a stem platyrrhine, while the phylogenetic position of many of the early crown platyrrhines (e.g., *Chilecebus*, *Tremacebus*) is contentious (Rosenberger, 2002). Estimations were made using maximum parsimony, and maximum likelihood and Bayesian tree topologies obtained from phylogenetic analyses. In the maximum parsimony approach we estimated branch lengths using both the ACCTRAN and DELTRAN algorithms, and each node value was estimated as the average branch length of all branches

that descend from the node of interest. Branch lengths were estimated by maximum likelihood using the TVM model of evolution suggested by Modeltest. As with the parsimony tree, each node value was estimated as the average branch length of all branches that descend from the node of interest.

To use *B. boliviana* as a calibration point for Bayesian analysis, we added a tarsier and strepsirrhine species to estimate the branch length for the stem platyrrhine lineage (Table 1). We used the ϵ -globin and β -globin genes to make these estimations because of the six genes only these two loci were represented in non-anthropoid primate species as well as the anthropoids. Parameter estimation for each gene was obtained using the BASEML module of PAML 3.14 (Yang, 1997). This information was used to estimate branch lengths and the variance–covariance matrix for each gene separately using the ESTBRANCHES program. The MULTIDIVTIME program was used to estimate divergence times using both variance–covariance matrices. After a burnin of 2,000,000 cycles, the Markov chain was sampled 200,000 times every 5 cycles. Time units between the tip and the root were 57.2 Mya; however, according to author's recommendation we converted these units such that rtime was 2. The rtrate (= rtratesd) value was set as the average of the median values of the amount of evolution from the root to the tip for both genes (Jeffrey Thorne, personal communication). To check the consistency of the results, we ran the program three times. Brownmean (= brownmeansd) was 0.5. We chose 125 Mya as the largest possible amount of time between the tip and the root.

3. Results

Maximum parsimony analysis supports (with 100% bootstrap values) the three proposed platyrrhine families (Atelidae, Cebidae, and Pitheciidae). In the maximum parsimony tree, the two most closely related families are Atelidae and Cebidae. However, this grouping has only moderate bootstrap (72%) support (Fig. 2). Genus-level relationships were mostly well resolved (Fig. 2), except for the low bootstrap support (63%) of *Aotus* as a sister of *Cebus* and *Saimiri* (Fig. 2). The maximum likelihood and Bayesian topologies were identical to each other, but they differ from the maximum parsimony topology in the grouping of the three families. Likelihood and Bayesian approaches group families Atelidae and Pitheciidae together. However, this grouping has low bootstrap (58%) and posterior probability (87%) support (Fig. 3). In agreement with maximum parsimony results, the likelihood and Bayesian results recognized the three proposed platyrrhine families with 100% bootstrap and posterior probability support (Fig. 3). As in the parsimony results, genus-level relationships are mostly well resolved except for the moderate bootstrap and posterior probability support of *Aotus* as a sister of *Cebus* and *Saimiri* (Fig. 3).

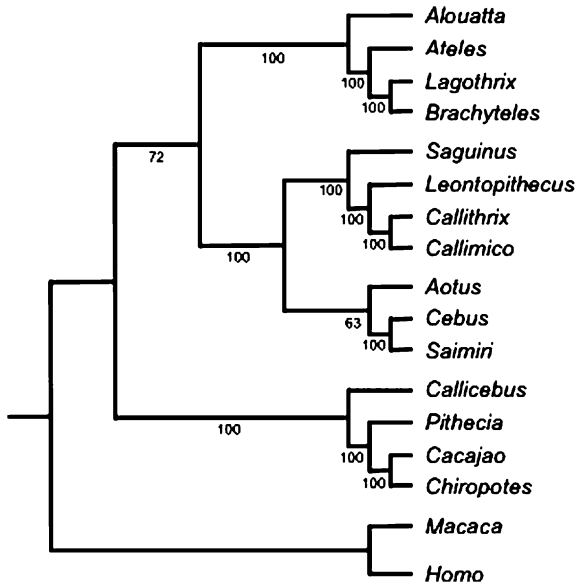


Fig. 2. Tree topology obtained by maximum parsimony for all New World monkey genera. Numbers below branches represent bootstrap support percentage values.

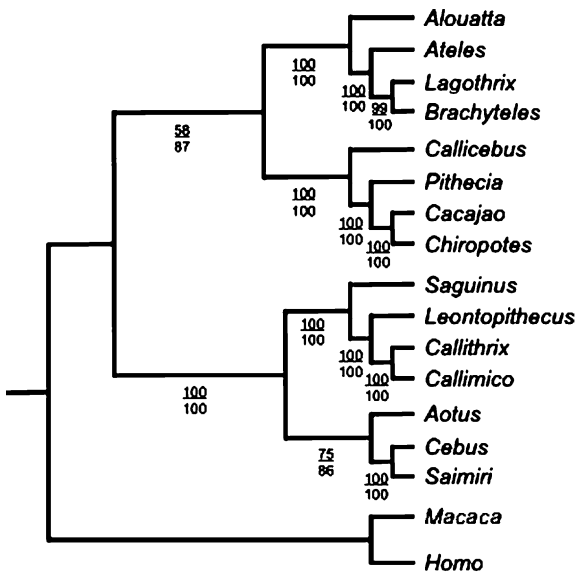


Fig. 3. Tree topology obtained by maximum likelihood and Bayesian approaches for all New World monkey genera. Numbers below branches represent bootstrap (upper) and posterior probability (lower) support.

Divergence time estimates using both topologies suggest the families diverged in the late Oligocene–early Miocene (Figs. 4 and 5). According to both topologies, the lineages to most genera separated from one another in the middle–late Miocene between 16.4 and 5.3 Mya, the exceptions being the lineages to *Aotus* and *Callicebus* which separated from one another and from the lineages to all other genera in the late Oligocene–early Miocene (Figs. 4 and 5). The last common ancestor (LCA) of *Cebus* and *Saimiri* existed at near the early–middle Miocene boundary according to the maximum parsimony tree (Fig. 4); however, according to the maximum likelihood and Bayesian tree this LCA existed in the early Miocene (Fig. 5).

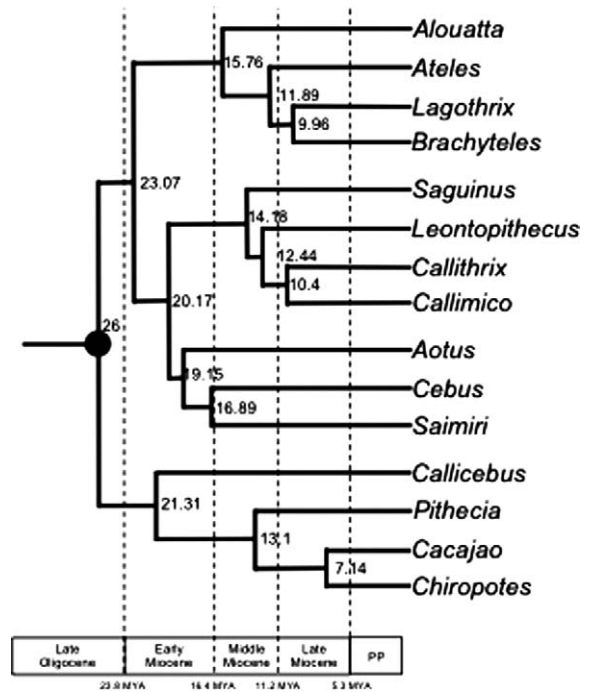


Fig. 4. Divergence time estimations based on the maximum parsimony tree topology. Each value represents the average between the estimation based on local molecular clocks and Bayesian approaches. Individual estimation results are given in Supplementary Figures 1 and 2. Geological times are according to the 1999 geologic time scale of the Geological Society of America (www.geosociety.org/science/timescale/timescl.pdf) (PP, Plio-Pleistocene).

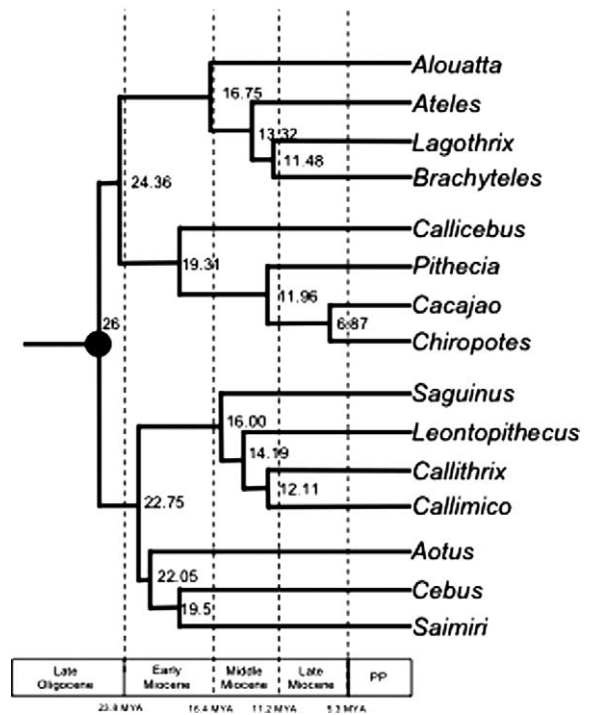


Fig. 5. Divergence time estimations based on the maximum likelihood and Bayesian approach tree topology. Each value represents the average between the estimation based on local molecular clocks and Bayesian approaches. Individual estimation results are given in Supplementary Figures 1 and 2. Geological times are according to the 1999 geologic time scale of the Geological Society of America (www.geosociety.org/science/timescale/timescl.pdf). (PP, Plio-Pleistocene).

4. Discussion

Two compelling hypotheses have been proposed regarding phylogenetic relationships at the familial level in New World monkeys (Figs. 1A and B). Some evidence suggests a first split between the Pitheciidae and the remaining platyrrhines, grouping families Atelidae and Cebidae together (Fig. 1A) (Chaves et al., 1999; Prychitko et al., 2005; Ray et al., 2005; Steiper and Ruvolo, 2003; von Dornum and Ruvolo, 1999). Conversely, a clade comprising families Atelidae and Pitheciidae as sister group of the family Cebidae have also been proposed (Fig. 1B) (Canavez et al., 1999a; Harada et al., 1995; Porter et al., 1997a; Schneider et al., 1993). Individually, among the six loci used in our study, two of them (vWF and HBB) support the first hypothesis, and one (B2M) the second one. The IRBP locus depicts a third hypothesis by first separating Atelidae from the rest of the platyrrhines and then grouping Pitheciidae and Cebidae together (Supplementary Table 1). G6PD and HBE loci were unable to resolve familial relationships. Apparently due to accumulated homoplasy in the NWM DNA sequences, our results fail to resolve the familial branching order; the maximum parsimony result agrees with the first hypothesis (Fig. 2), while the maximum likelihood and Bayesian results agree with the second one (Fig. 3). However, phylogenetic evidence from another kind of marker (presence or absence of specific Alu sequences) supports the sister grouping of Atelidae and Cebidae (Ray et al., 2005). What our results do indicate is that an initial radiation in the early Miocene separated the three familial clades from one another. These separations occurred over a relatively short period of geological time as evident in either the maximum parsimony topology or the alternative topology (Figs. 4 and 5; Supplementary Figures 1 and 2).

In our study, phylogenetic relationships among genera within Atelidae (*(Lagothrix, Brachyteles, Ateles)*, *Alouatta*) and similarly within Pitheciidae (*(Cacajao, Chiropotes, Pithecia)*, *Callicebus*), agree with the previous published molecular evidence (Canavez et al., 1999a; Goodman et al., 1998; Harada et al., 1995; Horovitz et al., 1998; Meireles et al., 1999; Porter et al., 1997a,b; Porter et al., 1999; Schneider et al., 1993; von Dornum and Ruvolo, 1999). An exception was in the study by Collins (2004) in which some of the results supported a trichotomy (*Brachyteles, Lagothrix*, and *Ateles*) of atelins within family Atelidae. It shall be noted that the molecular studies, which support the sister grouping of *Brachyteles* and *Lagothrix*, employed a broader (i.e., not just atelids) taxonomic sampling of platyrrhines. Also altogether at least several species of *Ateles* were represented in the molecular studies that depicted *Ateles* as sister to a *Brachyteles–Lagothrix* clade. The molecular evidence including our present results does not support the morphological evidence, which has a clade of *Ateles* and *Brachyteles* as the sister group of *Lagothrix* (Rosenberger, 2002).

Among members of the family Cebidae, phylogenetic results in the subfamily Callitrichinae (*(Callimico, Calli-*

thrix), *Leontopithecus)*, *Saguinus*) agree with results of Canavez et al. (1999a,b) and von Dornum and Ruvolo (1999). However, other studies have supported, grouping *Leontopithecus* and *Saguinus* as sister genera, however, with low bootstrap support (Goodman et al., 1998; Harada et al., 1995; Porter et al., 1997b, 1999; Schneider et al., 1993) in contrast to the 100% support for each branch point within the (*(Callimico, Callithrix)*, *Leontopithecus)*, *Saguinus*) clade provided in our study by the concatenated data. In the subfamily Cebinae, the sister-group relationship between *Cebus* and *Saimiri* is well supported, in agreement with previously results (Canavez et al., 1999a,b; Harada et al., 1995; Horovitz et al., 1998; Porter et al., 1997a,b, 1999; Steiper and Ruvolo, 2003; von Dornum and Ruvolo, 1999). However, the position of *Aotus* is still not fully resolved. Some studies have shown *Aotus* as a branch in a cebid polytomy (Canavez et al., 1999a,b; Harada et al., 1995; Schneider et al., 1993; Singer et al., 2003; Steiper and Ruvolo, 2003; von Dornum and Ruvolo, 1999), as sister of the subfamily Callitrichinae (Goodman et al., 1998; Porter et al., 1997a,b, 1999) or as sister of all other extant Cebidae (Harada et al., 1995; Horovitz et al., 1998). Among the six loci used in this study, G6PD and B2M support the cebid polytomy, IRBP supports *Aotus* as sister of all other cebids, and HBE as a sister of the subfamily Callitrichinae (Supplementary Table 2). In turn, like our results, vWF and HBB loci support *Aotus* as sister to the clade of *Cebus* and *Saimiri*, however, with low branch support (Figs. 2 and 3) (Supplementary Table 2). According to Steiper and Ruvolo (2003), who employed G6PD sequences, this sister-group relationship between *Aotus* and the *Cebus–Saimiri* clade is favored by maximum parsimony analysis, but not by other analyses. Prychitko et al. (2005), who employed HBE sequences, also support the sister grouping of *Aotus* with the *Cebus–Saimiri* clade.

We conducted two types of local molecular clock divergence time estimations (parsimony and Bayesian). In general, the results from each of these methods agree with one another (Supplementary Figures 1 and 2). However, the local clock results disagree with results obtained using global molecular clock estimations (data not shown). Global molecular clocks assume a uniform rate of nucleotide substitution on all branches of the tree. In this study, the global molecular clock using 26 Mya for the LCA of extant platyrrhines mostly overestimated the times of divergence, but it should be noted that statistical tests show that the global molecular clock model fits the data much more poorly than a no clock model ($p < 10^{-3}$).

As would be expected our divergence time estimations are comparable to previous estimations made by the local molecular clock procedure. For example, previously the *Cebus/Saimiri* split was placed at approximately 20 Mya (Goodman et al., 1998, 1999; Wildman and Goodman, 2004). According to our present results, this split would have occurred between 16.15 and 19.95 Mya (Supplementary Figures 1 and 2). Previous studies placed the *Callimico/Callithrix* divergence between 10.9 and 11.1 Mya (Chaves et al., 1999). Our results suggest this divergence occurred

between 10.04 Mya to 12.21 Mya (Supplementary Figures 1 and 2). Furthermore, our estimations of divergence times agree with the idea of time-based phylogenetic classification, in which divergence times older than ~6 Mya indicate that the taxa belong to different genera (Goodman et al., 1998). Whether *Cacajao* and *Chiropotes* should be treated as subgenera of the same genus (Goodman et al., 1998; Wildman and Goodman, 2004) or as separated genera, in our study, depends on the method that we used to do the estimation. Local clock estimates place the LCA of *Cacajao* and *Chiropotes* between 6.39 and 6.68 Mya (Supplementary Figures 1a and 2a), which according to the time-based classification criteria is on the boundary of generic or subgeneric rank (Goodman et al., 1998). However, the Bayesian method places the LCA of *Cacajao* and *Chiropotes* between 7.06 and 7.89 Mya (Supplementary Figures 1b and 2b), with a 95% confidence interval between 4.67 and 10.98 Mya, which does not allow us to differentiate the rank. Otherwise, the NWM grouping into separated genera would be the same as in Goodman et al. (1998, 1999) and Wildman and Goodman (2004).

New World monkeys like caviomorph rodents (South American hystricomorph rodents) are a monophyletic group and share a comparable biogeographic history; it has been suggested that both groups dispersed to South America in a single colonization event from Africa (Flynn and Wyss, 1998). As a consequence of this colonization episode, both groups of mammals would have replaced the endemic fauna then present in the Neotropical region (Flynn and Wyss, 1998). Accordingly, it would be interesting to compare the times when both platyrrhine and caviomorph radiations occurred. Members of the superfamily Caviioidea (guinea pigs and allies) represent a good opportunity because a recent estimation of divergence times included all recognized genera (like this study), all generic rankings agree with the time-based phylogenetic classification, and the superfamily has an origin as a crown group similar to the superfamily Ceboidea (Opazo, 2005). Moreover, coincident with our estimations for New World monkeys, a first radiation that originated the three major groups of cavioid rodents took place in the late Oligocene (Opazo, 2005). Coincident with our estimations of the time of origin of New World monkey genera, the origin of all genera within the superfamily Caviioidea would have occurred in the middle-late Miocene (Opazo, 2005).

In general, our divergence time estimations are in good agreement with the known New World monkey fossil record. Palaeontological evidence describes *Dolichocebus* (Szalay and Delson, 1979) as a cebine fossil, in the clade that includes living members of the genus *Saimiri*, and its age has been estimated between 18 and 20 Mya (MacFadden, 1990). Based on our estimations of the origin of the subfamily Cebinae (18.05 Mya) this fossil would be in the stem of the subfamily. Other sources have dated this fossil older than 20.5 Mya (Kay et al., 1999), in which case the position would be ambiguous. *Stirtonia* (13.15 Mya) (Szalay and Delson, 1979) has been considered an alouattin fossil,

according to our results, the split between *Alouatta* and the remaining members of the family (*Ateles*, *Lagothrix*, and *Brachyteles*) occurred 16.26 Mya (midpoint), a result that agrees with the fossil description. The fossil pitheciid *Cebupithecia* (13.5 Mya) (Szalay and Delson, 1979) represents one of the most complete skeletons of a fossil platyrrhine, and like *Nuciraptor* (13.5 Mya) (Meldrum and Kay, 1997) and *Soriacebus* (17.25 Mya) (Fleagle et al., 1987; Fleagle, 1990) has been dated near the time of the most recent common ancestor of the total group which includes as its extant members *Pithecia*, *Cacajao*, and *Chiropotes*. According to our estimates, the split between the lineage that eventually led to *Callicebus* and the lineage leading to the other pitheciid genera (*Pithecia*, *Cacajao*, and *Chiropotes*) occurred approximately 20.35 Mya, which places these extinct taxa after the divergence of *Callicebus* but before the diversification of *Pithecia*, *Cacajao*, and *Chiropotes*. This close correspondence between the NWM fossil evidence and divergence dates calculated by either the local molecular clock or Bayesian procedures is not obtained by the global molecular clock procedure.

The concatenated data set provides greater bootstrap support (Figs. 2 and 3) than do orthologous alignments of individual gene loci (Supplementary Tables 1 and 2 and Supplementary Figure 3).

Finally, we want to sound a note of caution about the use of New World monkey common names, as if they reflect natural groups. In the case of tamarins, the group contains members of the genera *Saguinus* (tamarins) and *Leontopithecus* (lion tamarins), but not the other two genera of the subfamily Callithrichinae (*Callithrix* and *Callimico*). As can be seen from this and previous studies, tamarins are a paraphyletic group. Similarly, within family Pitheciidae, saki monkeys commonly refer to members of the genera *Pithecia* (sakis) and *Chiropotes* (bearded sakis), but not to *Callicebus* (titi monkeys) nor *Cacajao* (uakari monkeys). Based on our phylogenetic results, it is clear that saki monkeys, in the broad sense, are a paraphyletic group.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ympev.2005.11.015](https://doi.org/10.1016/j.ympev.2005.11.015).

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