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PHYLOGENETIC RELATIONSHIPS AMONG BRAZILIAN HOWLER MONKEYS, GENUS *Alouatta* (PLATYRRHINI, ATELIDAE), BASED ON γ^1-GLOBIN PSEUDOGENE SEQUENCES

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ABSTRACT

The genus *Alouatta* (howler monkeys) is the most widely distributed of New World primates, and has been arranged in three species groups: the Central American *Alouatta palliata* group and the South American *Alouatta seniculus* and *Alouatta caraya* groups. While the latter is monotypic, the *A. seniculus* group encompasses at least three species (*A. seniculus*, *A. belzebul* and *A. fusca*). In the present study, approximately 600 base pairs of the γ^1-globin pseudogene were sequenced in the four Brazilian species (*A. seniculus*, *A. belzebul*, *A. fusca* and *A. caraya*). Maximum parsimony and maximum likelihood methods yielded phylogenetic trees with the same arrangement: {*A. caraya* [*A. seniculus* (*A. fusca*, *A. belzebul*)]}. The most parsimonious tree had bootstrap values greater than 82% for all groupings, and strength of grouping values of at least 2, supporting the sister clade of *A. fusca* and *A. belzebul*. The study also confirmed the presence of a 150-base pair *Alu* insertion element and a 1.8-kb deletion in the γ^1-globin pseudogene in *A. fusca*, features found previously in the remaining three species. The cladistic classification based on molecular data agrees with those of morphological studies, with the monospecific *A. caraya* group being clearly differentiated from the *A. seniculus* group.

INTRODUCTION

The systematics of the New World monkeys (infraorder Platyrrhini) has undergone considerable revision during the past two decades, but remains controversial (e.g. Rosenberger, 1984; Ford, 1986; Kay, 1990). Nevertheless, recent studies of DNA sequences (Schneider *et al*., 1993, 1996; Harada *et al*., 1995; Goodman, 1996; Barroso *et al*., 1997) have consistently supported an arrangement which recognizes only 15 genera, distributed in three families: Atelidae, Pitheciidae and Cebidae. Following this arrangement, the Atelidae encompasses four genera: *Alouatta* (howler monkeys), *Ateles* (spider monkeys), *Brachyteles* (muriquis) and *Lagothrix* (woolly monkeys). Relationships within the ateline clade – *Ateles*, *Brachyteles* and *Lagothrix* – have been disputed, but there is a growing consensus on the grouping of *Brachyteles* and *Lagothrix*, including conclusive evidence from gamma(γ)-globin gene sequences (Meireles *et al*., 1999). With one exception (Kay, 1990), there is also a general consensus on the distinct position of *Alouatta*, which is placed separately in either its own tribe, Alouattini (Rosenberger, 1984; Ford, 1986), or subfamily, Alouattinae (Hill, 1962; Napier and Napier, 1967; Hershkovitz, 1977).

Alouatta is the most widely distributed Neotropical primate genus (Neville *et al*., 1988), ranging from southern Mexico to northern Argentina, and is found in tropical and subtropical forest ecosystems throughout Brazil (Hirsch *et al*., 1991). Hershkovitz (1949) recognized three species groups based on the structure of the hyoid apparatus: the Central American *Alouatta palliata* group, and the South American *Alouatta seniculus* and *Alouatta caraya* groups. While the latter group is monotypic, the other two encompass a variety of different forms, whose arrangement has been subject to conflicting interpretations (e.g. Hershkovitz, 1949; Hill, 1962; Mittermeier *et al*., 1988; Groves, 1993; Bonvicino *et al*., 1995; Stanyon *et al*., 1995; Rylands and Brandon-Jones, 1998).

Despite the need for further work, especially on the integration of genetic and morphological data, the most recent major revisions of the genus (Hershkovitz, 1949; Hill, 1962) all divide the *A. seniculus* group into three species: *A. seniculus*, *A. belzebul* (endemic to Brazil) and *A.* (*guariba*) *fusca*. Groves (1993) does recognize a fourth species, *Alouatta sara*, but it is endemic to Bolivia, and thus not relevant to the present study. More recent studies (Bonvicino *et al*., 1995) also allocate species status to a number of subspecific forms of both *A. seniculus* and *A. belzebul*, but in the absence of a consensus, the arrangement of Groves (1993) will be followed here.

In addition to other unresolved questions, the karyotype of *Alouatta* varies from 2n = 43 to 2n = 54 (Armada *et al*., 1987; Lima and Seuánez, 1991; Stanyon *et al*., 1995). Of the four Brazilian species, only *A. caraya* has a fixed number of chromosomes, with 2n = 52 (Pargament *et al*., 1984), whereas in *A. seniculus*, 2n varies from 43 to 49 (Yunis *et al*., 1976; Lima and Seuánez, 1991). *A. belzebul* and *A. fusca*, on the other hand, share both the same diploid number (2n = 49/50) and a translocation on the Y chromosome (Koiffmann and Saldanha, 1974; Armada *et al*., 1987).

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In humans, the γ-globin gene is located on the short arm of chromosome 11 (Lebo *et al*., 1979; Bunn and Forget, 1986). This gene is expressed embryonically in strepsirrhine primates (Tagle *et al*., 1988), but was duplicated in tandem in anthropoids (γ^1 and γ^2), becoming expressed fetally (Fitch *et al*., 1991). This globin gene is part of the beta(β)-globin gene cluster, which in primates includes the ε, γ, ψη, δ and β globin genes (Figure 1). All genes in this cluster have three exons and two introns (Lawn *et al*., 1980). In the atelids, γ^1 is a pseudogene (Meireles *et al*., 1995, 1998), whereas in the platyrrhine genera *Aotus* and *Saimiri*, the two loci are combined into a hybrid gene (Chiu *et al*., 1996).

Several recent studies (Schneider *et al*., 1993, 1996; Harada *et al*., 1995; Porter *et al*., 1995, 1997a,b; Meireles *et al*., 1999) have used DNA sequences of the globin gene to resolve phylogenetic relationships in the platyrrhines, producing results generally consistent with those of more traditional morphological studies in most (see Schneider and Rosenberger, 1996), but not all (Shoshani *et al*., 1996) cases. In the present study, sequences of the γ^1-globin pseudogene were used in a molecular analysis of the phylogenetic relationships among Brazilian howler monkeys. The aligned sequences were also used to estimate divergence times based on a local molecular clock, and to investigate the presence of the *Alu* insertion – found in other *Alouatta* species (Meireles *et al*., 1995, 1998) – in *A. fusca*.

MATERIAL AND METHODS

Study species

Howler monkeys are large-bodied, prehensile-tailed platyrrhines more specialized for folivory than frugivory, as is typical of other atelids. They are also highly sexually dimorphic in body size (adult males are 70-80% heavier than females), and two of the Brazilian species (*A. caraya* and *A. fusca*) are among only three platyrrhines (the other is *Pithecia pithecia*) to exhibit sexual dimorphism in pelage coloration (Mittermeier *et al*., 1988). *A. belzebul* also exhibits the most marked individual variation in pelage coloration of any platyrrhine (Bonvicino *et al*., 1989). Only one species (*A. belzebul*) is endemic to Brazil, but it is also the only one found in both Amazonian and Atlantic forest biomes. The remaining three species are found in Amazonia (*A. seniculus*), the Brazilian Atlantic forest and neighboring areas (*A. fusca*), and the savanna and swampy habitats of central and western Brazil (*A. caraya*).

Preparation and amplification of DNA

Genomic DNA was extracted from the peripheral blood cells of captive specimens of the four Brazilian howler monkey species (Table I), and the γ^1-globin sequences for *A. caraya*, *A. belzebul* and *A. seniculus* were determined by C.M.M. (Meireles, 1997). DNA extraction for *A. caraya* and *A. seniculus* was based on the protocol of Bell *et al*. (1981), whereas for *A. belzebul* and *A. fusca*, that of Sambrook *et al.* (1989) was used.

The γ^1 fragment was obtained using the PCR protocol described in Meireles *et al*. (1995), and consisted of an initial denaturation of 3 min at 94°C followed by 30 cycles of denaturation at 94°C (30 s), annealing at 55°C (45 s), extension at 72°C (45 s) and a final extension of 10 min at 72°C. The amplification products were separated by agarose gel electrophoresis. The DNA primers (synthesized at the Center for Molecular Medicine and Genetics, Wayne State University, Detroit, USA) were:
R1 5' -AAT GTG GAA GAT GCT GGG- 3' and
R2 5' -GTC ATG TCT GAG CAA CAA AC- 3'

Despite the presence of a 1.8-kb γ^1 deletion in all four species (Figure 1), these primers amplified a ~600-bp fragment in all cases.

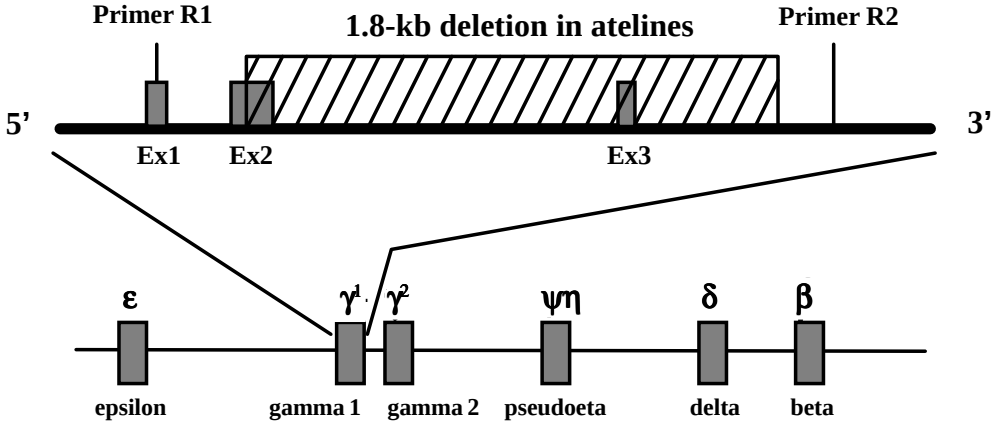

Figure 1 - Model of the β-cluster showing the five globin genes. The γ^1-globin pseudogene is amplified exhibiting the primers R1 and R2 used in this study and also the 1.8-kb deletion shared by all four *Alouatta* species as well as by the ateline group (Meireles *et al*., 1995, 1998).

Table I - Code, origin and GenBank accession number for specimens analyzed in the present study.

Species	Code	Origin[a]	GenBank accession number	Source[b]
Alouatta caraya	Aca	Los Angeles Zoo	AF030095	1
Alouatta belzebul	Abe	CENP	AF030096	1
Alouatta seniculus	Ase	Los Angeles Zoo	AF030097	1
Alouatta fusca	Afu	CPRJ	AF068573	1
Outgroups				
Cebus albifrons	Cal	Unknown	M81409	2
Macaca mulatta	Mmu	Unknown	J03938	3
Homo sapiens	Hsa	Unknown	J00179	4

[a]CENP = Brazilian National Primate Center, Ananindeua, Pará; CPRJ = Rio de Janeiro Primate Center, Magé, Rio de Janeiro. [b]1 = Present study; 2 = Hayasaka *et al.* (1993); 3 = Slightom *et al.* (1988); Fitch *et al.* (1991); 4 = Slightom *et al*. (1980); Shen *et al*. (1981); Rogan *et al.* (1987).

Cloning of PCR products

The PCR products were separated in 1.0% agarose gels in TBE buffer (89 mM Tris-base, 89 mM boric acid and 2 mM EDTA, pH 8.0) containing 0.5 µg ethidium bromide/ml. The amplified fragments were excised and purified using a Qiaex II gel extraction kit (Qiagen). The purified DNA fragments were cloned into the pGEM-T vector system I (Promega) and transformed into *Escherichia coli* host JM109 (Promega) according to the protocol supplied by the vendors. Single-stranded DNA was prepared from selected clones using helper phage M13K07 (Promega) and purified by PEG-NaCl precipitation followed by phenol-chloroform extraction and ethanol precipitation.

Sequencing

Nucleotide sequences were determined for at least three clones of each species by the dideoxy chain-termination method (Sanger *et al*., 1977) using a Sequenase version 1.0 kit (United States Biochemical). The sequences were aligned by eye using version 3.0 of the ESEE sequence editor (Cabot and Beckenbach, 1989). Gaps were inserted to minimize the number of nucleotide substitutions and indels (insertions/deletions) needed to account for the descent of the aligned sequences. As PCR reactions commonly result in slightly different sequences for the same cloned fragments, a consensus sequence of three or more clones was defined for each species. These consensus sequences were then aligned against the known orthologous sequences which represented the outgroups for the phylogenetic analysis: *Cebus albifrons* (Cal), *Macaca mulatta* (Mmu) and *Homo sapiens* (Hsa).

Phylogenetic analysis

Phylogenetic analyses were performed using the maximum parsimony and maximum likelihood methods. The most parsimonious and the maximum likelihood trees were determined using the phylogenetic analysis using parsimony (PAUP) parsimony program, version 4.0 for DOS (written by David Swofford, Smithsonian Institute, Washington, DC, USA). For both trees, the strength of grouping values or Bremer decay indices were estimated using the PAUP program, and bootstrap analysis was applied to test the support for each grouping. Two thousand bootstrap replications with one shuffle per replication were carried out on the seven data sets. The number of parsimony-informative characters (synapomorphies) was established by eye and confirmed by the PAUP program. Divergence times were estimated using the branch lengths of the maximum likelihood tree constructed from a distance matrix calculated using Kimura's (1980) model. The molecular clock was calibrated using the local clock procedure, based on Goodman's (1996) estimate of 21 MYA for the divergence between *Cebus* and *Alouatta*.

RESULTS AND DISCUSSION

Analysis of γ^1-pseudogene sequences

PCR amplification using the 5' R1 and 3' R2 primers produced two fragments for all four genera. The fragment corresponding to γ^1 contained approximately 600 bp, and that of γ^2 about 2100 bp (Figure 2). The sequences indicated that this difference in size was due to a ~1.8-kb deletion at the γ^1 locus, which is shared by all four atelid genera (Meireles *et al*. 1995, 1998). These authors also encountered a ~150-bp insertion, described as a monomeric *Alu* element by Drs. Roy Britten (CIT, Pasadena, USA) and Jerzy Jurka (UC, Berkeley, USA), in *A. caraya*, *A. belzebul* and *A. seniculus*. In the present study, this same 150-bp *Alu* insertion was also found in *A. fusca*, indicating that it is an important synapomorphic marker for the analysis of *Alouatta* phylogeny and evolution. Figure 2 also shows a clear difference in the size of the γ^1-globin fragment in two representative atelid genera, *Ateles* (~450 bp) and *Alouatta* (~600 bp).

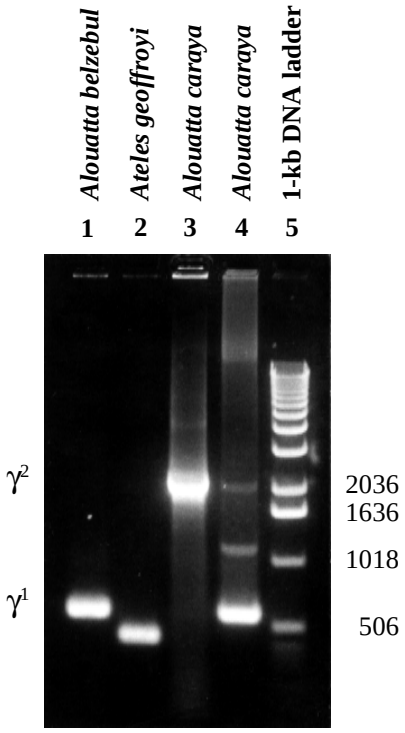

Figure 2 - PCR-amplified γ gene DNA in atelids: 1 - γ^1 fragment of the purified PCR product of *Alouatta belzebul*; 2 - γ^1 fragment of the purified PCR product of *Ateles geoffroyi*; 3 - γ^2 fragment of the purified PCR product of *Alouatta caraya*; 4 - PCR products of the genomic DNA of *Alouatta caraya*; 5 - 1-kb DNA ladder.

The nucleotide sequences bordered by primers R1 and R2 in the seven primate species studied here spanned a total of 2,482 aligned base positions (Figure 3) consisting of the following γ^1 gene regions: the 5' coding region of exon 1 (1-35), intron 1 (36-160), exon 2 (161-211), a ~1.8-kb deletion (212-2092), a region between the deletion and insertion (2093-2149), a ~150-bp insertion (2150-2298), and the 3' flanking region (2299-2482). In the phylogenetic analysis, all sequences except that of *A. fusca* were also represented by a 1.8-kb region extending upstream from the R1 primer. The sequences in this region are described in Meireles *et al.* (1999). The DNA sequences were deposited in GenBank (Table I), and are also available from the authors on request, or can be accessed via internet at http://ns.med.wayne.edu/.

Phylogeny of Brazilian howler monkeys

The maximum parsimony consensus tree (Figure 4), constructed using the PAUP program, had a nucleotide substitution score of 813 (length). The numbers above the lines show the minimum number of additional substitutions required to break up the clade (strength of grouping – SOG – value or Bremer decay index), while the numbers below the lines show the bootstrap values (number of times the grouping was encountered in 2,000 replicates) as percentages. The arrangement of howler species was: {*A. caraya* [*A. seniculus* (*A. fusca*, *A. belzebul*)]}, with SOG values of 66, 2 and 2, respectively.

All bootstrap values were greater than the critical value of 75% proposed by Hillis and Bull (1993), with a minimum value of 82% for the *A. seniculus*-*A. belzebul*/*A. fusca* clade. The *A. belzebul*-*A. fusca* clade was supported by a value of 88%, while 100% of the replications identified *A. caraya* as the basal species. The number of shared-derived sequence changes (synapomorphies) found by the PAUP analysis also supported this arrangement. The positions of the synapomorphies for the *Alouatta* species clades (Figure 3) were:
A. fusca x *A. belzebul* - 48 (G→A), 77 (C→G), 79 (G→A) with homoplasy and 2318 (A→C) with homoplasy;
Alouatta species - 212 (C→1.8-kb deletion), 2095 (del→A), 2096 (del→G), 2117 (T→G), 2145 (G→T), 2150 (del→150-bp *Alu* insertion), 2324 (C→T) with homoplasy, 2335 (C→T), 2349 (C→G), 2401 (G→A), 2403 (G→A), 2405 (G→A), 2411 (G→A), 2416 (A→del) and 2451 (C→T).

The natural logarithm (ln) of the likelihood for the best maximum likelihood tree using the aligned γ-globin sequences was -9329.13944. Bootstrap values (as a percentage of 2,000 replicates) are shown below the lines (Figure 5). Although the topologies of the tree and bootstrap values for the first (88) and last (100) clades were the same, the *A. seniculus*-*A. belzebul*/*A. fusca* clade had a borderline bootstrap value of 72. This analysis confirmed the cladistic groupings shown by the maximum parsimony tree (Figure 4).

Although the *A. belzebul*-*A. fusca* clade was relatively well defined, with a high bootstrap value (88%) and four shared synapomorphies, the analysis did not permit identification of the species closest to *A. seniculus*, despite the relative geographic proximity of *A. belzebul* in southwestern Amazonia (Hirsch *et al.*, 1991). The data, nevertheless, supported the presence of a well-defined *A. seniculus* group [*A. seniculus* (*A. belzebul*, *A. fusca*)] as proposed by Hershkovitz (1949) and still widely accepted (e.g. Mittermeier *et al.*, 1988). There is little doubt from these data that *A. caraya* should be placed in a distinct, monotypic group, and that *Alouatta* is monophyletic, with 15 synapomorphies.

The divergence times between the different *Alouatta* lineages (Table II) were calculated by the local molecular clock method, based on Goodman's (1996) estimate of 21 MYA for the split between *Alouatta* and *Cebus*, using the maximum likelihood branch lengths for the sequences of the γ^1-globin pseudogene. This analysis indicated that the different *Alouatta* lineages diverged relatively recently in relation to the putative ± 11 MYA split of the alouattine and ateline groups (Meireles *et al.*, 1999).

Overall, then, the results of the present study corroborate the phylogenetic relationships among Brazilian howler monkey species suggested by morphological characteristics (Mittermeier *et al.*, 1988), principally the existence of distinct *A. caraya* and *A. seniculus* groups, which diverged approximately 2.5 MYA (Table II).

```
        AATGTGGAAGATGCTGGG R1              <----INTRON 1
Abe     AATGTGGAAGATGCTGGGGGAGAAACCCTGGGAAGGTAGGCTCTGGTAACCAGGACAAGGGAGGGAAGGAAGGAACGTATGC--CTGGCAAAAGTCCAGGCTGCCTCTCAGGATTTGTGG
Afu     ..................................................................................--....................................
Ase     ...............................................G.........................G..C.C.CAGG...-................................
Aca     ...............................................G............................C.....--....................................
Cal     ...............................................G............................C.G...--......G............................A
Mmu     .................A...................-.........G............A.............C.C.G...--.................CA.T...T...........
Hsa     .................A.............................G..........................C.C.G...--................TC..T...............
        ----+---10----+---20----+---30----+---40----+---50----+---60----+---70----+---80----+---90----+--100----+--110----+--120

                                                <----EXON 2                                        <----ATELINE 1.8-KB DELETION
Abe     CACCTTCTGACTTTCAAACTGCTATTGTTCAATTTCACAGGCTCCTGGTTGTGTACCCATGGACCCAGAGGTTCTTTGACAGCTTTGGCAG-----------------------------
Afu     T................................C.........................................................-----------------------------
Ase     .................................C.........................................................-----------------------------
Aca     .................................C.....................T...................................-----------------------------
Cal     T.....TC.........................C..........................................C..............CCTGTCCTCTCCTTCTGCCATCATGGGCA-//-
Mmu     ...T........G..........C.........C..................C.....................................ACCTGTCCTCTGCCTCTGCCATCATGGGCA-//-
Hsa     ............G........T.C....-....C..................C.....................................ACCTGTCCTCTGCCTCTGCCATCATGGGCA-//-
        ----+--130----+--140----+--150----+--160----+--170----+--180----+--190----+--200----+--210----+--220----+--230----+--240

                                              DELETION ENDS>                                                         <----Alouatta
Abe     ----------------------------------------------------TTAGTTTCATTAACTATAGTGAAGGGATCCTTACTTTACTAAGGAACTTTTCTTTTTCTTTTTTTTTT
Afu     ----------------------------------------------------....................................................................
Ase     ----------------------------------------------------....................................................................
Aca     ----------------------------------------------------....................................................................
Cal     TTTGGGGTACTTCAGGTGTTAGAGATCAGAGCAGGAAACAGATTTTTTTTTT..CT..........C..G......TC.............A...........TG....-----------
Mmu     TATGGGGAGGTTGAGGTGTTAGAGATCAGAGCAGGAAACAGATTTTTCTTTCCA-----..G.....GC.A.....T..................A........G....-----------
Hsa     TACGGGGAGGTTGAGGTGTTAGAGATCAGAGCAGGAAACAGGTTTTTCTTTCCA-----.GG......C.A.....T...........................A....-----------
        ----+-2050----+-2060----+-2070----+-2080----+-2090----+-2100----+-2110----+-2120----+-2130----+-2140----+-2150----+-2160

        Alu INSERTION ELEMENT
Abe     TATATTAAGGCGGCGTTTCACCATGTTGGTCAGGCTGGTCTTGAACTCCTGACCTCAGGCGATCCGCCCGCCTTGGCCTTCAAAGTGCTTGGATTACAGGCGTGAGCACTGCACC-GGCC
Afu     ...........................................................T.................................................C.....C....
Ase     ...........................................................T.................................................C.....-....
Aca     ...........................................................T.................................................C.....-....
Cal     ------------------------------------------------------------------------------------------------------------------------
Mmu     ------------------------------------------------------------------------------------------------------------------------
Hsa     ------------------------------------------------------------------------------------------------------------------------
        ----+-2170----+-2180----+-2190----+-2200----+-2210----+-2220----+-2230----+-2240----+-2250----+-2260----+-2270----+-2280

           INSERTION ENDS>
Abe     CCTACTAAGGAACTTTTCAAGGGTTGATGCATACTTACAGAAGTGAAATTAATCTCATGCCCTCAAGTGTGCAGACTGGTCACAGCATTTCAAGGAAGAGAACTCATTGTAAGCTTCTGG
Afu     ......................................G.................................................................................
Ase     .....................................TG.................................................................................
Aca     ......................................G............-....................................................................
Cal     ------------------.......C---........TG....C....C.....C.............T......................G...........G................
Mmu     ------------------...T..........G.C..A...G.C..........C........T..-------------......................C................A.
Hsa     ------------------...T.....C....G.C..A...G............C...A....T....C.A.........................G....C................A.
        ----+-2290----+-2300----+-2310----+-2320----+-2330----+-2340----+-2350----+-2360----+-2370----+-2380----+-2390----+-2400

                                                                      GTCATGTCTGAGCAACAAAC R2
Abe     AGAAAATGGGAACTT-------------GAGCCAGCAGAGGCTCACAAGTTAGCATCAGTGTGTCATGTCTGAGCAACAAAC
Afu     ...............-------------...........A..........................................
Ase     ...............-------------......................................................
Aca     ...............-------------......................................................
Cal     G.G.G.....G....AGGTGAAGGAAAT......................C...............................
Mmu     G.G.GG....G....AGGTGAAGGAAAT......................C.......TA......................
Hsa     -.G.GG....G....AGGTGAAGGAAAT...........A..........C........C......................
        ----+-2410----+-2420----+-2430----+-2440----+-2450----+-2460----+-2470----+-2480--
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Figure 3 - Aligned DNA sequences of the γ^1-globin pseudogene corresponding to the region between the R1 and R2 primers in four simian genera, including four *Alouatta* species (see abbreviations in Table I). Dots indicate the same nucleotide as that found in the first (Abe) sequence, and dashes designate gaps resulting from insertions/deletions in the alignment. Sequences corresponding to positions 2041-3720 in *Cebus albifrons* (Cal), *Macaca mulatta* (Mmu) and *Homo sapiens* (Hsa) were published by Bailey *et al*. (1992) and are thus omitted here.

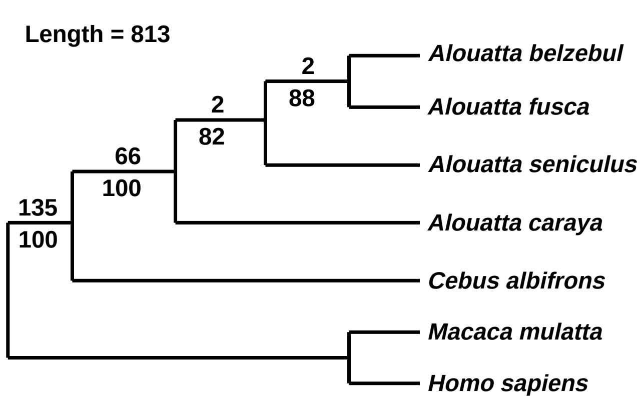

Figure 4 - Maximum parsimony tree derived from the aligned γ^1-globin nucleotide sequences in seven simian species. The length, or nucleotide substitution score, is 813. Values above the lines represent the minimum number of additional substitutions required to break up the clade designated by that node and those below the lines, the percentage of bootstrap values obtained in 2,000 replicates.

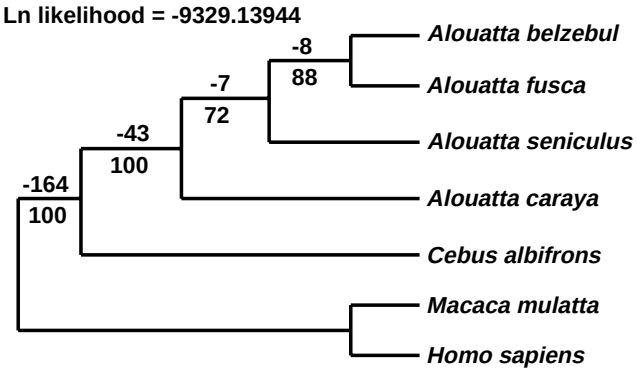

Figure 5 - Maximum likelihood tree derived from the aligned γ^{1}-globin nucleotide sequences in seven simian species. Log likelihood is -9329.13944. Values above the lines represent the minimum decrease in the log likelihood required to break up the clade designated by that node and those below the lines, the percentage of bootstrap values obtained in 2,000 replicates.

Table II - Local molecular clock estimates of the divergence times of *Alouatta* species lineages based on the maximum likelihood branch lengths and Goodman's (1996) estimate of the split between *Alouatta* and *Cebus*.

Branching point	Mean branch length	Divergence (MYA)
(*A. fusca*, *A. belzebul*)	0.0029	1.0
[*A. seniculus* (*A. fusca*, *belzebul*)]	0.0040	1.5
{*A. caraya* [*A. seniculus* (*A. fusca*, *A. belzebul*)]}	0.0047	2.4
(*Cebus*, *Alouatta*)	0.0383	21.0

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RESUMO

Os guaribas, do gênero *Alouatta*, que são os primatas do Novo Mundo com maior distribuição geográfica, têm sido colocados em três grupos de espécies: o grupo *Alouatta palliata* da América central, e os grupos sulamericanos *Alouatta seniculus* e *Alouatta caraya*. Este último é monotípico, mas o grupo *A. seniculus* inclui pelo menos três espécies (*A. seniculus*, *A. belzebul* e *A. fusca*). Neste estudo, foram seqüenciados aproximadamente 600 pares de base do pseudogene globina γ^{1} nas quatro espécies brasileiras (*A. seniculus*, *A. belzebul*, *A. fusca* e *A. caraya*). Os métodos de máxima parcimônia e máxima verossimilhança produziram árvores filogenéticas com o mesmo arranjo: {*A. caraya* [*A. seniculus* (*A. fusca*, *A. belzebul*)]}. A árvore mais parcimoniosa apresentou valores de bootstrap maiores de 82% para todos os agrupamentos, e valores de força de ligação de pelo menos 2, apoiando o agrupamento irmão de *A. fusca* e *A. belzebul*. O estudo também confirmou a presença em *A. fusca* do elemento de inserção *Alu*, com 150 pares de base, e uma deleção de 1,8 kb no pseudogene globina γ^{1} já conhecidos nas demais espécies de guaribas. A classificação cladística baseada em dados moleculares é congruente com as de estudos morfológicos, com um isolamento claro do grupo monoespecífico *A. caraya* em relação ao grupo *A. seniculus*.

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