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# *SRY* Evolution in Cebidae (Platyrrhini: Primates)

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**Abstract.** Sex determination in mammals is dependent on the presence of *SRY*, which codes for a protein with a DNA binding motif (the HMG-box domain). Here we analyze the evolution of *SRY* among seven genera of New World monkeys belonging to the family Cebidae. Estimates of the number of synonymous and nonsynonymous substitutions indicated the absence of positive selection acting on *SRY* evolution. The presence of indels at the C-terminus coding region in different genera and species maintained an open reading frame, indicating a selective pressure constraining the evolution of this coding region. Available data on the fertility of natural and captive interspecific hybrids failed to show any relationship between *SRY* evolution and speciation for the genera herein studied. Our phylogenetic arrangement for Cebidae genera was similar to previous topologies based on mitochondrial and autosomal DNA sequences. This arrangement also corroborated the division of *Cebus* into two species groups. However, for *Callithrix* the differences among *SRY* topology and those derived from autosomal and mitochondrial genes suggested a Y-chromosome ancestral polymorphism.

**Key words:** *SRY* — Sex determination — Synonymous substitutions — Nonsynonymous substitutions — Platyrrhini — Cebidae — Callitrichinae

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## Introduction

Sex determination in mammals is dependent on the presence (in males) or absence (in females) of the *SRY* gene that is located in the Y chromosome. This gene codes for a protein with a conserved DNA binding motif (Sinclair et al. 1990), which is related to members of ***h***igh-***m***obility ***g***roup **(HMG)** proteins. In man, *SRY* transcripts were detected in the genital ridge before testis formation and in many other tissues of adult individuals (Clepet et al. 1993), but in adult men, the SRY protein was detected only in the nuclei of gonadal cells (Salas-Corté et al. 1999). In *Mus musculus*, *SRY* transcripts were detected in the developing genital ridge and adult testes (Koopman et al. 1990) although, in the latter, mRNA molecules exhibited a circular conformation that probably deters translation (Capel et al. 1993).

*SRY* expression initiates a cascade of events leading to testis development. One of these events is activation of the *MIS* gene [***M***üllerian ***i***nhibiting ***s***ubstance (Haqq et al. 1994)] subsequent to binding of the SRY protein to the *MIS* promoter region. The *SRY* HMG-box protein domain (between amino acids residues 59 and 135 in humans) is responsible for binding and bending the target DNA. The majority of *SRY* mutations responsible for sex reversal in man occurs in this domain (for a review see Haqq and Danahoe 1998), although two others were also reported outside this region [L163 stop (Tajima et al. 1994); S18N (Domenice et al. 1998)]. Some of these mutations were not *de novo* and were inherited from normal males and shared by male relatives, indicating incomplete penetrance (Berta et al. 1990; Hawkins et al. 1992; Jäger et al. 1992; Domenice et al. 1998).

In all eutherian mammals analyzed so far *SRY* was found to be intronless, although in dasyurid marsupials one intron is present in the coding region of the C-terminal end (O'Neill et al. 1998). Interspecific DNA sequence comparisons showed that the *SRY* regions flanking the HMG-box coding domain have gone through a high number of nonsynonymous nucleotide substitutions (Whitfield et al. 1993; Turker and Lundrigan 1993), frequently resulting in a ratio of nonsynonymous:synonymous substitutions per site (dN/dS) > 1 between recently diverged species, suggesting positive selection related to reproductive isolation (Whitfield et al. 1993). Similarly, high-dN/dS ratios were found in other genes related to male reproduction in different animals, as was the case of *PRM-1*, *PRM-2*, and *TNP-2* in great apes (Wyckoff et al. 2000) and the gene coding for the fusagenic protein of gastropods (Swanson and Vacquier 1995). However, speciation and reproductive isolation could not be straightforwardly associated with *SRY* sequence divergence because recently diverged wallaby species (Marsupialia: Macropodidae) may share identical *SRY* sequences and naturally crossbreed producing sterile male hybrids and fertile female hybrids (O'Neill et al. 1997).

In this paper we analyze *SRY* evolution in New World monkeys (Suborder Platyrrhini) belonging to the family Cebidae (*sensu* Schneider et al. 1993). This approach included a study of related aspects of *SRY* function (existence of regions of functional relevance), evidence for positive selection (excess of nonsynonymous nucleotide substitutions), the relationship between *SRY* evolution and cebid speciation, and the construction of a phylogeny based on male lineages. The family Cebidae comprises seven genera: *Cebus* (capuchin monkeys), *Saimiri* (squirrel monkeys), *Aotus* (night monkeys), *Saguinus* (tamarins), *Leontopithecus* (lion tamarins), *Callimico* (Goeldi's marmoset), and *Callithrix* (marmosets, including *Callithrix pygmaea*, or the pygmy marmoset previously considered *Cebuella pygmaea*). All cebid genera except *Callimico* are polytypic, with several morphotypes of controversial taxonomic status (subspecies or species). Extensive molecular data supported the monophyly of this family (Schneider et al. 1993, 1996, 2001; Porter et al. 1997a, b; Canavez et al. 1999a; von Dornum and Rulovo 1999), in disagreement with other proposed topologies based on morphologic attributes (Rosenberger 1984; Ford 1986; Kay 1990).

Marmosets and tamarins comprise a tight group (the subfamily Callitrichinae *sensu* Schneider et al. 1993) which is strongly supported by molecular and morphologic analyses. Callitrichines differ from the remaining New World monkeys in their lower body weight, presence of claws instead of nails, high frequency of twin births, and absence of third molars. The latter two characteristics, however, are not shared by *Callimico*, a genus representing an early divergent callitrichine offshoot in morphologic topologies. This arrangement, however, was not supported by molecular phylogenies because these studies showed that *Callimico* was a derived lineage and a sister group of *Callithrix* (Schneider et al. 2001).

*SRY* sequence data were also used to infer intrageneric (or interspecific) relationships in *Cebus*, *Callithrix*, *Leontopithecus*, and *Aotus*. This is important because phylogenetic studies, based on morphologic or molecular data, had not been carried out previously to establish interspecific relationships in *Cebus*, a genus with a widespread geographic distribution. Moreover, phylogenetic relationships of selected species of the remaining three genera were restricted to analyses of mitochondrial DNA [in *Aotus* (Silva 1995) and *Callithrix* (Tagliaro et al. 1997)], morphologic attributes [in *Callithrix* and *Leontopithecus* (Pires, 1990)], or morphologic data [in *Callithrix* (Natori 1994)].

## Materials and Methods

### Animals and DNA Isolation

Genomic DNA was isolated from 32 animals (22 males and 10 females), listed in Table 1. DNA was isolated from liver (*Callithrix jacchus*), primary fibroblasts (*Callimico goeldii*), or blood (remaining specimens) following Sambrook et al. (1989). Samples of female DNA were obtained for representative specimens of all genera except *Callimico*; DNA was extracted from fibroblasts of the *Callithrix pygmaea* female and from blood samples of all other female specimens (Table 1).

### PCR Amplification

*SRY* was PCR-amplified with the SW2 forward primer (reported by Whitfield et al. 1993) and a reverse primer (*SRY*-: 5′-CGG-TAAAAGGAGAGTCTGCGTAG-3′), except for *Callithrix pygmaea* and *Callimico goeldii* DNAs, which were amplified with SW2 and SW3 primers (Whitfield et al. 1993). PCR reactions were carried out with 1.25 U of *Taq* DNA polymerase, a 0.25 μM concentration of each dNTP, 50 pmol of each primer, a 1.5–3.0 mM concentration of $MgCl_2$, and 1× Promega PCR reactions buffer in a final volume of 50 μl (see Table 1). Following an initial denaturation period of 5 min at 94°C, amplifications proceeded with 30 cycles at 94°C (1 min), 50–55°C (1 min), and 72°C (1 min). PCR products were visualized in 2% agarose gels, purified with the Qiaquick PCR purification Kit (Qiagen), and sequenced.

### DNA Sequencing

Reactions were carried out with the Big Dye Sequence Reaction Kit (AP-Biosystem), with the same primers used for PCR amplifications and two internal primers (5′-CAGAGTGAAGCGAC-CCATGAACGC-3′ and 5′-AGCGTTCATGGGTCGCTTCACT-CT-3′). Samples were sequenced with an ABI Prism 377 (Perkin–Elmer).

**Table 1.** Animal samples, PCR conditions for *SRY* amplification, and GenBank accession numbers of sequenced PCR products

| Animal samples[a] | PCR conditions[b] | GenBank accession no. |
|---|---|---|
| *Aotus azarae* M[4,9] and F[4,9] | $MgCl_2$ = 1.5 m*M*; Ta = 54°C; SW2/SRY- | AF338375 |
| *Aotus infulatus* M[2,8] and F[2,8] | $MgCl_2$ = 3.0 m*M*; Ta = 58°C; SW2/SRY- | AF338376 |
| *Aotus lemurinus griseimembra* M[3] | $MgCl_2$ = 1.5 m*M*; Ta = 54°C; SW2/SRY- | AF338374 |
| *Callimico goeldii* M[2] | $MgCl_2$ = 3.0 m*M*; Ta = 55°C; SW2/SW3B | AF338383 |
| *Callithrix aurita* M[1] and F[1] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338377 |
| | | AF338392[c] |
| *Callithrix geoffroyi* M[1] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338378 |
| *Callithrix jacchus* M[6] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338379 |
| *Callithrix kuhlii M*[1] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338380 |
| *Callithrix penicillata* M[6] and F[4] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338381 |
| *Callithrix pygmaea* M[3] and F[2] | $MgCl_2$ = 3.0 m*M*; Ta = 50°C; SW2/SW3B | AF338382 |
| *Cebus albifrons* M1[5] and M2[3] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338386 |
| | | AF338387 |
| *Cebus apella apella* M1[3,7], M2[5,8], and F[5,8] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338384 |
| | | AF338385 |
| *Cebus apella xanthosternos* M[4] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338390 |
| *Cebus capucinus* M[3] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338388 |
| *Cebus nigrivitattus* M[4] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338389 |
| *Leontopithecus chrysomelas* M[1] and F[1] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338370 |
| *Leontopithecus chrysopygus* M[1] and F[1] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338371 |
| *Leontopithecus rosalia* M1[1], M2[1], and F[1] | $MgCl_2$ = 1.5 m*M*; Ta = 55°C; SW2/SRY- | AF338372 |
| | | AF338373 |
| *Saguinus midas midas* M[1] and F[1] | $MgCl_2$ = 3.0 m*M*; Ta = 50°C; SW2/SW3B | AF338391 |

[a] M, male; F, female. Animal origin: [1]Centro de Primatologia do Rio de Janeiro, Mage, RJ, Brazil; [2]primary fibroblast cell culture, National Cancer Institute/National Institutes of Health (USA); [3]DNA sample provided by Universidade Federal do Pará (Genetics Department), Belém, PA, Brazil; [4]São Paulo Zoo, São Paulo, SP, Brazil; [5]Centro Nacional de Primatas, Belem, PA, Brazil; [6]Rio de Janeiro Zoo, Rio de Janeiro, RJ, Brazil; [7]animal collected at Jamari river, RO, Brazil; [8]animal collected in Tucuruí, PA, Brazil; [9]animal collected in Samuel, RO, Brazil.

[b] Ta, annealing temperature, Primer pairs used, SW2/SW3B or SW2/SRY-.

[c] GenBank accession number from the *SRY* sequence of the female *Callithrix aurita*.

## SRY *Sequence Analysis and Phylogenetic Inferences*

Sequences were aligned by eye with data from *Hylobates lar* (GenBank accession No. X86384), *Pongo pygmaeus* (X86383), *Pan paniscus* (X86381), *Homo sapiens* (X5372), and *Saimiri sciureus* (AF151695; partial sequence) and with tandemly aligned sequences of *Papio* sp./*Papio hamadryas anubis* (X86386/AJ008918), *Pan troglodytes* (X86380/AF008917), and *Gorilla gorilla* (X86382/AJ003068).

Phylogenetic analyses were carried out with PAUP 3.1.1 (Swofford 1993) using catarrhine genera as outgroups. Parsimony trees were obtained by branch and bound, with bootstrap estimates based on 1000 replicates, analyzed by heuristic search, and with 10 replications of sequence random addition. Decay indexes were obtained as described by Bremer (1988). *Saimiri sciureus SRY* sequence data included only part of the HMG-box domain and the C-*terminus* coding region but not the N-terminus coding region and the 5′ region. Therefore, two parsimony analyses were carried out, one excluding and another including *Saimiri sciureus SRY* sequence data. The latter analysis included an aligned sequence region between nucleotide (nt) 584 and nt 838 (Fig. 1).

A topology, based on previous molecular analyses (Schneider et al. 2001; Goodman et al. 1998) and on *SRY* data, was used to assess evidence of positive selection. *Callithrix aurita* was not included in this topology due to the presence of indels at the 3′ coding region affecting sequence alignment. Reconstructions of ancestral *SRY* states at internal nodes were carried out by maximum likelihood with the program CODEML from PAML 3.1 [phylogenetic analyses by maximum-likelihood (Yang 1997)] or by parsimony with PAUP 3.1.1 (Swofford 1993). The number of synonymous and nonsynonymous substitutions and the ratio of nonsynonymous and synonymous substitutions per site (dN/dS) for each branch were estimated with CODEML for maximum-likelihood reconstruction and with MEGA 2.0 (Kumar et al. 2001) using the modified Nei–Gojobori method (Zang et al. 1998) for parsimony. The analyzed region comprised a homologous coding sequence spanning between the start codon and the last amino acid coding codon in man.

The significance of maximum-likelihood estimates was assessed through the log-likelihood values obtained under a free-ratio model (with the dN/dS ratio varying among branches) and a one-ratio model (with the same dN/dS ratio for all branches). Twice the difference of the log-likelihood values [$2\Delta l = 2(l_1 - l_0)$] was compared with a $\chi^2$ distribution (Yang 1998) with 36 degrees of freedom. For parsimony reconstruction of internal nodes, evidence for positive selection (dN/dS > 1) for branches was assessed by Fisher's exact test (Zhang et al. 1997). The same approach was carried out considering only HMG-box flanking regions.

## Results

PCR reactions with SW2/SW3 or SW2/SRY(-) primers amplified a single product of the expected size (*ca.* 850 bp) with male DNA samples of all species. Conversely, amplifications were not observed with female DNA extracted from blood samples of *Aotus* and *Cebus* and from primary fibroblasts of *Callithrix pygmaea*. However, amplified products were obtained

with DNA extracted from blood samples of three female callitrichines (*Leontopithecus rosalia*, *Callithrix aurita*, and *Saguinus midas midas*). All male PCR products and the *Callithrix aurita* female product were sequenced.

Aligned sequences comprised a data set of 838 bp with 257 variable and 180 informative sites (excluding indels) in parsimony analysis (Fig. 1). This data set consisted of four segments: (1) from nucleotide (nt) 1 to nt 178, corresponding to the untranslated region upstream of the start codon; (2) from nt 179 to nt 352, corresponding to the coding region upstream of the HMG box; (3) from nt 353 to nt 584, comprising the HMG-box domain; and (4) from nt 585 to nt 838, corresponding to the downstream coding region. Sequence alignment indicated presence of indels in the first and fourth segments. The two male specimens of *Leontopithecus rosalia* and the single male *L. chrysopygus* shared an identical sequence, as was the case for two *Aotus* (*A. azarae* and *A. infulatus*), four callitrichines (*Callithrix jacchus*, *C. penicillata*, *C. geoffroyi*, and *C. kuhlii*), and two *Cebus apella apella*. Moreover, male and female *Callithrix aurita SRY* sequences were also identical. Deduced amino acid sequences (Fig. 2) did not provide information on the C-terminal end in any species sequenced herein due to the absence of a stop codon.

Parsimony analysis excluding *Saimiri sciureus* data (GenBank accession No. AF151695) resulted in six most parsimonious trees with 332 steps (CI = 0.907, RI = 0.965, RC = 0.875); the strict consensus tree is shown in Fig. 3. Differences between trees were due to variable positions of *Hylobates* and *Pongo* among catarrhines and of *Saguinus* among platyrrhines. The formers either grouped with the ((*Homo*, *Pan*) *Gorilla*) clade or, alternatively, in a sister clade with hominids ((*Hylobates*, *Pongo*) ((*Homo*, *Pan*) *Gorilla*)). With respect to *Saguinus*, it grouped either in a trichotomy with *Leontopithecus* and (*Callimico*, *Callithrix*) or with *Leontopithecus* (albeit sustained by ambiguous synapomorphies). All nodes were supported by bootstrap values above 70% except for *Homo*/*Pan*.

A second parsimony analysis was carried out with *SRY* sequence data of *Saimiri sciureus* (GenBank accession No. AF151695), which included only positions 584 to 838. This analysis resulted in 100 most parsimonious trees (165 steps, CI = 0.903, RI = 0.960, RC = 0.867) and a strict consensus tree very similar to the previous one (Fig. 3). In this analysis, *Saimiri* and *Cebus* were grouped with a bootstrap value of 82%.

Reconstruction of ancestral states with maximum likelihood did not indicate positive selection acting on *SRY* evolution. The log-likelihood values for the free-ratio model (ln = −2156.44) and one-ratio model (ln = −2141.06; with a dN/dS ratio = 0.605) did not show significant differences between ancestral reconstructions under these different assumptions ($2\Delta l = 30.8$, $p > 0.05$, with 36 df). However, the free-ratio model showed some branches with dS/dN ratio >1 (Fig. 4), most of them leading to terminal nodes lacking synonymous substitutions and to the branch leading to callitrichines.

Parsimony reconstruction of ancestral sequence states resulted in similar numbers of synonymous and nonsynonymous substitutions and dN/dS ratios for the same branches (Fig. 4). A Fisher's exact test did not indicate significant differences between the number of synonymous and that of nonsynonymous substitutions for branches with dN/dS > 1.

Similar analyses were performed considering only the HMG-box flanking regions. The maximum-likelihood approach did not show significant differences between the free-ratio model (ln = −1579.04) and the one-ratio model (ln = −1594.33; with dN/dS = 0.784). For parsimony reconstruction, Fisher's exact test did not show significance between the number of synonymous and that of nonsynonymous substitutions for branches with dN/dS > 1 (data not shown).

## Discussion

### *DNA and Deduced Protein Sequences*

The high frequency of twin births among marmosets and tamarins (except for Goeldi's marmoset; genus *Callimico*) and the occurrence of placental anastomoses resulting in bone marrow chimerism (Benirschke et al. 1962; Benirschke and Brownhill 1962) explained why *SRY* amplifications occurred in some blood DNA samples extracted from female animals. This occurred in *Callithrix* and *Leontopithecus* samples and also in the single female specimen of *Saguinus*.

Comparisons of the 5′ region upstream of the start codon allowed the identification of two conserved motifs similar to the ones described previously by Veitia et al. (1997), corresponding to two putative Sp1 transcription factor binding motifs ($Sp1^2$, from nt 21 to nt 26 and $Sp1^1$, from nt 43 to nt 51). In all platyrrhines analyzed herein $Sp1^2$ was identical (GGGCGG). Conversely, the putative $Sp1^1$ region showed two motifs among platyrrhines: one (GGGGGGCGG) was restricted to all species of the genus *Callithrix*, while the other (GGGGGCGG), in all other platyrrhine species, was identical to the homologous *Papio* motif. A third motif highlighted by Veitia et al. (1997), including the human transcription start site AACAAAG (nt 85–91), showed C or T replacements at position 90. The latter substitution was shared by all *Cebus* specimens, while the former was present in all other platyrrhines.

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                                                                                                             TRANSCRIPTION
                                                  SP1[2]                   SP1[1]                              START SITE     11111111111111111111111111111111111111111111111111
                                    111111111122222222223333333333444444444455555555556666666666777777777788888888889999999999000000000011111111112222222222333333333344444444444
                           12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123456789
Homo sapiens               TACTAGGGGGTAGGCTGGTTGGGCGGGGTTGAGGGGG-TGTTGAGGG-CGGAGAAATG-CAAGTTTCATTACAAAGTTAACGTAACAAAGAATCTGGTAGAAGTGAGTTTTGGATAGTAAAATAAGTTTCGAACTCTGGCACCTTTCA
Pan troglodytes            .......................A...................................A............G.....................C.....................................................A
Pan paniscus               ?????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????
Gorilla gorilla            C...............................C.........................A..........................................................................................
Pongo pygmaeus             ?????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????
Hylobates lar              ?????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????
Pappio sp                  C............C............A......A..G.....G................A.....................G.......................A................T.G......A..A....A.........
Leontopithecuschrysomelas  C.G.T..A.........................A..G..A..G.............C.TT.....G..C............G.......C....T..........A...-G.............G.A...CC...G...T...T..GG.
L.chrysopygus              C.G.T..A.........................A..G..A..G.............C.TT.....G..C............G.......C....T..........A...-G.............G.A...CC...G...T...T..GG.
L.rosalia  1               C.G.T..A.........................A..G..A..G.............C.TT.....G..C............G.......C....T..........A...-G.............G.A...CC...G...T...T..GG.
L.rosalia  2               C.G.T..A.........................A..G..A..G.............C.TT.....G..C............G.......C....T..........A...-G.............G.A...CC...G...T...T..GG.
Callithrix aurita          C.G.T..AT........................A.....C..G...G.........G..T.....G...............G.......C....T.....A....A.....G............G.....CC...G...T...T..GG.
C.geoffroyi                C.G.T..AT........................A.....C..G...G.........G..T.....G...............G.......C....T.....A....A.....G............G.....CC...G...T...T..GG.
C.jacchus                  C.G.T..AT........................A.....C..G...G.........G..T.....G...............G.......C....T.....A....A.....G............G.....CC...G...T...T..GG.
C.kulhii                   C.G.T..AT........................A.....C..G...G.........G..T.....G...............G.......C....T.....A....A.....G............G.....CC...G...T...T..GG.
C.penicillata              C.G.T..AT........................A.....C..G...G.........G..T.....G...............G.......C....T.....A....A.....G............G.....CC...G...T...T..GG.
C.pygmaea                  C.G.T..AT....................T.AAC.....CC.G...G.........G..T.....G...............G.......C....T.....A....A.....G............G.....C....G...T...T..GG.
Callimico goeldii          C.G.T..AT........................A..G..C..G.............C..T.....G...............G.......C....T.....A....A.A...G..................CC...G...T...T..GG.
Saguinus m. midas          C.G.T..A.........................A..G..C..G.............C..T.....G..A............G.......C....T.......T..A.....G............G.....CC...G...T...T..GG.
Aotus l.griseimembra       C.G.T..A.........................A..G.....G.............CATT.....G...............G.......C....T..........A.....G.........GTG.G-...ACT..G...T...T...G.
A.azarae                   C.G.T..A.........................A..G.....G.............CATT.....G...............G.......C....T..........A.....G.........GTG.G-...ACT..G...T...T...G.
A.infulatus                C.G.T..A.........................A..G.....G.............CATT.....G...............G.......C....T..........A.....G.........GTG.G-...ACT..G...T...T...G.
Cebus albifrons 1          C.G.T..A......A..................A..G..C..G.............A..T.....G...............G.......T....T..........A.A...G............G.....CC.......T...T...G.
Cebus albifrons 2          C.G.T..A......A..................A..G..C..G.............A..T.....G...............G.......T....T..........A.A...G............G.....CC.......T...T...G.
Cebus apella apella 1      C.G.T..A...G..A..................A..G..C..G.............A..T.....G...............G.......T....T..........A.A...G............G.....CC...G...T...T...G.
Cebus apella apella 2      C.G.T..A...G..A..................A..G..C..G.............A..T.....G...............G.......T....T..........A.A...G............G.....CC...G...T...T...G.
Cebus capucinus            C.G.T..A......A..................A..G..C..G.............A..T.....G...............G.......T....T..........A.A...G............G.....CC.......T...T...G.
Cebus nigrivitattus        C.G.T..A....C.A..................A..G..C..G.............A..T.....G...............G.......T....T..........A.A...G............G.....CC.......T...T...G.
Cebus a. xanthosternos     C.G.T..A......A..................A..G..C..G.............A..T.....G...............G.......T....T..........A.A...G............G.....CC...G...T...T...G.
Saimiri sciureus           ?????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????

                           11111111111111111111111111111 || 5555555555555556666666666666666666666666666666666666666666666666666666666666666666666666666666666666666666666667
                           55555555556666666666777777777 || 8888899999999990000000000111111111122222222223333333333444444444455555555556666666666777777777788888888889999999999990
                           01234567890123456789012345678 || 5678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Homo sapiens               ATTTTGTCGCACTCTCCTTGTTTTGACA  || AGATGCTGCCGAAGAATTGCAGTTTGCTTCCCGCAGATCCCGCTTCGGTA-CTCTGCAGCGAAGTGC---AACTGGACAACAGGTTGTACA-----------GGGATGACTGTAC
Pan troglodytes            ............C................ || .C..................................................................................................................
Pan paniscus               ????????????????????????????? || .C..................................................................................................................
Gorilla gorilla            ............C................ || .................................................................................C..................................
Pongo pygmaeus             ????????????????????????????? || .........A.....G........C.........................................TGC...............................................
Hylobates lar              ????????????????????????????? || .........A.....G...............T.....T.G....................A.......................................................
Pappio sp                  G..........GC................ || .........AA..C.G...............G........T...........C......A......TA.----CA.........................................
Leontopithecuschrysomelas  ........T..C..A.............. || .C.......A...C...GA.........AAT..T......T...A.A.......TG.T.....A.........G.A..GG...........CCTTCTCATACA.T..A........
L.chrysopygus              ........T..C..A.............. || .C.......A...C...GA.........AAT..T......T...A.A.......TG.T.....A.........G.A..GG...........CCTTCTCATACA.T..A........
L.rosalia  1               ........T..C..A.............. || .C.......A...C...GA.........AAT..T......T...A.A.......TG.T.....A.........G.A..GG...........CCTTCTCATACA.T..A........
L.rosalia  2               ........T..C..A.............. || .C.......A...C...GA.........AAT..T......T...A.A.......TG.T.....A.........G.A..GG...........CCTTCTCATACA.T..A........
Callithrix aurita          ........T..C..A............A. || .T.......A...C...GA..........A....C.....AT..A.A.A......-----------.......GCA..GG...........CCTTCTCATACA.T..A...A...A
C.geoffroyi                ........T..C..A............A. || .T.......A...C...GA..........A....C.....AT..A.A.......TG.T.....A.........GCA..GG.........T.CCTTCTCATACA.T..A...A...A
C.jacchus                  ........T..C..A............A. || .T.......A...C...GA..........A....C.....AT..A.A.......TG.T.....A.........GCA..GG...........CCTTCTCATACA.T..A...A...A
C.kulhii                   ........T..C..A............A. || .T.......A...C...GA..........A....C.....AT..A.A.......TG.T.....A.........GCA..GG.........T.CCTTCTCATACA.T..A...A...A
C.penicillata              ........T..C..A............A. || .T.......A...C...GA..........A....C.....AT..A.A.......TG.T.....A.........GCA..GG.........T.CCTTCTCATACA.T..A...A...A
C.pygmaea                  ........T..C..A............A. || .T.......A...C...GA..........A....C.....AT..A.A.......TG.T.....A.........GCA..GG...........CCTTCTCATACA.T..A...A...A
Callimico goeldii          ........T.....A.............. || .C.......A...C...GA..........A....CA....AT..A.A.......TG.T.....A.........G.A..GG.....T.....CCTTCTCATACC.T..A...A....
Saguinus m. midas          ........T..C..A.............. || .C.......A...C.----..........A....C.....T...A.A......ATG.T.....A.........G.A..GG...........CCTTCTCATACA.T..A........
Aotus l.griseimembra       ........T..C..A.............. || .CG......A...C...GAG.....T........C.....T...A.A.......T........A.........G.A..GG.....C.....CC...TCATACA....A........
A.azarae                   ........T..C..A.............. || .CG......AA..C...GAG.....T........C.....T...A.A.......T........A.........G.A..GG.....C.....CC...TCATACA....A........
A.infulatus                ........T..C..A.............. || .CG......AA..C...GAG.....T........C.....T...A.A.......T........A.........G.A..GG.....C.....CC...TCATACA....A........
Cebus albifrons 1          .....TCA.T..C..G............. || .CT.A....A...C...GA........G......TC....T...A.AG.......T.A.....A.........G.A..AG...........CCTTCTCATACA....A........
Cebus albifrons 2          .....TCA.T..C..G............. || .CT.A....A...C...GA........G......TC....T...A.AG.......T.A.....A.........G.A..AG...........CCTTCTCATACA....A........
Cebus apella apella 1      .....TAA.T..C..G............. || .CT.A....A...TG..GA........G......C.....T...A.AG.......T.A.....A...........A..AG...........CCTTCTCATACA....A........
Cebus apella apella 2      .....TAA.T..C..G............. || .CT.A....A...TG..GA........G......C.....T...A.AG.......T.A.....A...........A..AG...........CCTTCTCATACA....A........
Cebus capucinus            .....TCA.T..C..G............. || .CT.A....A...C...GA........G......TC....T...A.AG.......T.A.....A.........G.A..AG...........CCTTCTCATACA....A........
Cebus nigrivitattus        .....TCA.T..C..G............. || .CT.A....A...C...GA........G......C.....T...A.AG.......T.A.....A.........G.A..AG...........CCTTCTCATACA....A........
Cebus a. xanthosternos     .....TCA.T..C..G............. || .CT.A....A...CG..GA........G......C.....T...A.AG.......T.A.....A.........G.A..AG...........CCTTCTCATACA....A........
Saimiri sciureus           ????????????????????????????? || .C.AA....A...C...GA...............C.....T...A.A........C.T.....A.........G.A..GG..G......T.CCTTCTCATACA.A..A........
```

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                           777777777777777777777777777777777777777777777777777777777777777777777777777777777777777777777777777888888888888888888888888888888888888888
                           000000000111111111122222222223333333333444444444455555555556666666666777777777788888888889999999999000000000011111111112222222222333333333
                           123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678
Homo sapiens               GAAAGCCACACACTCAAGAATGGAGCACCAGCTAGGCCACTTACCGCCCATCAACGCAGCCAGCTCACCGCAGCAACGGGACCGCTACAGCCACTGGACAAAGCTGTAGGACAATCGGGTAACATTGGCTACAAAGAC
Pan troglodytes            .............................................................................................................?????????????????????????????
Pan paniscus               .................C....................................................................G......................?????????????????????????????
Gorilla gorilla            .............................................................................................................?????????????????????????????
Pongo pygmaeus             ...................C...................................A.......................................C....G.......C.............................
Hylobates lar              A......................................................A.....................A.....A...........C.............?????????????????????????????
Pappio sp                  C........G............C........T...............T.......A.......................................C.............?????????????????????????????
Leontopithecuschrysomelas  ....TG...C..A....C.....T.....C...G....T..C......AG.....C...AG..............G..............T....C......C...CG...................AG.....C..T
L.chrysopygus              ....TG...C..A....C.....T.....C...G....T..C......AG.....C...AG..............G..............T....C......C...CG...................AG.....C..T
L.rosalia  1               ....TG...C..A....C.....T.....C...G....T..C......AG.....C...AG..............G..............T....C......C...CG...................AG.....C..T
L.rosalia  2               ....TG...C..A....C.....T.....C...G....T..C......AG.....C...AG..............G..............T....C......C...CG...................AG.....C..T
Callithrix aurita          ....T....C..A....C......A....C........T..C..T...AG.....C...A...............G..........G........C......C...C....................A.........T
C.geoffroyi                ....T....C..A....C......A....C........T..C......AG.....C...A...............G..........G........C......T...C....................A.........T
C.jacchus                  ....T....C..A....C......A....C........T..C......AG.....C...A...............G..........G........C......T...C....................A.........T
C.kulhii                   ....T....C..A....C......A....C........T..C......AG.....C...A...............G..........G........C......T...C....................A.........T
C.penicillata              ....T....C..A....C......A....C........T..C......AG.....C...A...............G..........G........C......T...C....................A.........T
C.pygmaea                  ....T....C..A....C......A....C........T..C......AG.....C..AA........G......G...A......G........C......C...C........???????????????????????
Callimico goeldii          ....T....C..A....C......A....C........T..C......AG.....C...A..A............G...................C......C...C........???????????????????????
Saguinus m. midas          ....T....CG.A....C...........C........T..C......AG.....CT..A..............GG...................C......C...C........???????????????????????
Aotus l.griseimembra       ....T....C..A....C.......................C......AC.....CG..........A.......G..............T.C..C......C...C....................A.........T
A.azarae                   ....T....C..A....C.......................C......AC.....CG..................G..............T.C..C......C...C................T...A.........T
A.infulatus                ....T....C..A....C.......................C......AC.....CG..................G..............T.C..C......C...C................T...A.........T
Cebus albifrons 1          ....T....C..A....T.................T.....C...T..AG.....C...................G...............A...C......C...C...G................A.........G
Cebus albifrons 2          ....T....C..A....T.................T.....C...T..AG.....C...................G...............A...C......C...C...G................A.........G
Cebus apella apella 1      ....T....C..A....T.................T.....C...T..AG.....C...................G...............A...C......C...C...G................A.........G
Cebus apella apella 2      ....T....C..A....T.................T.....C...T..AG.....C...................G...............A...C......C...C...G................A.........G
Cebus capucinus            ....T....C..A....T.................T.....C...T..AG.....C...................G...............A...C......C...C...G................A.........G
Cebus nigrivitattus        ....T....C..A....T.................T.....C...T..AG.....C...................G...............A...C......C...C....................A.........G
Cebus a. xanthosternos     ....T....C..A....T.................T.....C...T..AG.....C...................G...............A...C......C...C...G................A.........G
Saimiri sciureus           A...T....C..A....T..................T....C..T...AG...G.C...................G......T....T..............C...C...G???????????????????????????
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**Fig. 1.** Alignment of *SRY* sequences comprising 3′ untranslated and untranscribed regions (positions, nt 1 to nt 178) and C-terminal regions downstream of the HMG-box domain (positions, nt 585 to nt 838). *Underlined* regions correspond to Sp1 binding motifs (Sp1[1] and Sp1[2]); regions in *boldface* indicate homologies to the human transcription start site. *Dashes* indicate alignment gaps. *Dots* indicate nucleotides identical to the human sequence (GenBank accession number X5372). *Question marks* indicate unknown nucleotides. Positions between nt 179 and nt 584 did not present alignment gaps and are not shown.

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                                                                                                                                1 1111111111
                                    1 1111111112 2222222223 3333333334 4444444445 5555555556 6666666667 7777777778 8888888889 9999999990 0000000001
                           1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
Homo sapiens               MQSYASAMLS VFNSDDYSPA VQENIPALRR SSSFLCTESC NSKYQCETGE NSKGNVQDRV KRPMNAFIVW SRDQRRKMAL ENPRMRNSEI SKQLGYQWKM LTEAEKWPFF
Pan troglodytes            .......... .......... ..Q....... .........Y .....R.... ...DS..... .......F.. .......... .......... .......... ..........
Pan paniscus               .......... .........G ..Q....... .........Y .....R.... ...DS..... .......F.. .......... .......... .......... ..A.......
Gorilla gorilla            .......... .......... ..QT...H.. .......... .......... ....S..... .......... .......... .......... .......... ..........
Pongo pygmaeus             .......... .......... ..Q....... ....I...Y. .......... ....S..... .......... .......... ...K...... .......... ..........
Hylobates lar              .......... .....Y.... ..Q.T..... ....I..... ....P..... ...VS..... .......... .......... ...K...... ......R... ..........
Pappio sp                  ......T... ...T.G.... A.Q....P.. ....I..... S......A.. ....S..... .......... .......... ...K...... .......... ....D.....
Leontopithecus chrysomelas .......V.R .....E.N.. A.Q...DSGK ...VIW.D.S S..DK.Q... ....S..N.. .......... .........V ...Q...... ..R......L ..........
L. chrysopygus             .........R .....E.N.. A.Q...DSGK ...VIW.D.S S..DK.Q... ....S..N.. .......... .........V ...Q...... ..R......L ..........
L. rosalia 1               .........R .....E.N.. A.Q...DSGK ...VIW.D.S S..DK.Q... ....S..N.. .......... .........V ...Q...... ..R......L ..........
L. rosalia 2               .........R .....E.N.. A.Q...DSGK ...VIW.D.S S..DK.Q... ....S..N.. .......... .........V ...Q...... ..R......L ..........
Callithrix aurita          .........R .....E.N.. ALQ...DSGK ...VIW.DN. S..DPWQ... ....S..N.. .......... .........V ...Q...... ..R......L ..........
C. geoffroyi               .........R .....E.N.. ALQ...DSGK ...VIW.DNS S..D.WQ... ....S..N.. .......... .........V ...Q...... ..R......L ..........
C. jacchus                 .........R .....E.N.. ALQ...DSGK ...VIW.DNS S..D.WQ... ....S..N.. .......... .........V ...Q...... ..R......L ..........
C. penicillata             .........R .....E.N.. ALQ...DSGK ...VIW.DNS S..D.WQ... ....S..N.. .......... .........V ...Q...... ..R......L ..........
C. kuhlii                  .........R .....E.N.. ALQ...DSGK ...VIW.DNS S..D.WQ... ....S..N.. .......... .........V ...Q...... ..R......L ..........
C. pygmaea                 .........R .....E.N.. ALQ...DSGK ...VIW.DNS S..D.WQ... ....S..N.. .......... .........V ...Q...... ..W......L ..........
Callimico goeldii          .........R .....E.N.. ALQ...DSGK N..VIW.DNS S..D..Q... ....S..N.. .......... .........V ...Q...... ..R......L ..........
Saguinus_m. midas          .........R ....NE.N.. A.Q...DSGK ...VIW.DNS S..D..Q.R. ....S..N.. .......... .........V ...Q...... ..R......L ..........
Aotus l. griseimembra      .........R .....E.... A.Q....S.K ...VIW.DNS S.N....... ...DS..N.. .......... .........V ...Q...... ..R......L ..........
A. azarae                  .........R .....E.... A.Q....S.K ...VIW.DNS S.NH...... ...DS..N.. .......... .........V ...Q...... ..R......L ..........
A. infulatus               .........R .....E.... A.Q....S.K ...VIW.DNS S.NH...... ...DS..N.. .......... .........V ...Q...... ..R......L ..........
Cebus albifrons 1          ......T..R ...C.E.... A.Q....SGK ...VVW..NS S......... .......N.. .......... .........V Q..Q...... ..R......L ..........
Cebus albifrons 2          ......T..R ...C.E.... A.Q....SGK ...VVW..NS S......... .......K.. .......... .........V ...Q...... ..R......L ..........
Cebus apella apella 1      ......T..R .....E.... A.Q....SGK ...VIW..NS S......... .......N.. .......... .........V ...Q...... ..R......L .S........
Cebus apella apella 2      ......T..R .....E.... A.Q....SGK ...VIW..NS S......... .......N.. .......... .........V ...Q...... ..R......L .S........
Cebus capucinus            ......T..R ...C.E.... A.Q....SGK ...VVW..NS S......... .......N.. .......... .........V ...Q...... ..R......L ..........
Cebus nigrivitattus        ......T..R ...C.E.... A.Q....SGK ...VVW..NS S......... .......N.. .......... .........V ...Q...... ..R......L ..........
Cebus a. xanthosternos     .P....T..R .....E.... A.Q....SGK ...VIW..NS S......... .......N.. .......... .........V ...Q...... ..R......L .S........
Saimiri sciureus           ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ????.....L ......R...

                           1111111111 1111111111 1111111111 1111111111 1111111111 1111111111 1111111111 1111111111 1111111112 2222222222 2222222222
                           1111111112 2222222223 3333333334 4444444445 5555555556 6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
                           1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
Homo sapiens               QEAQKLQAMH REKYPNYKYR PRRKAKMLPK NCSLLPADPA SV-LCSEVQ- LDNRLY---- RDDCTKATHS RMEHQLGHLP PINAASSPQQ RDRYSHWTKL *?????????
Pan troglodytes            .......... .......... .....N.... .......... .......... .......... .......... .......... .......... .......... *.........
Pan paniscus               .......... .......... .....N.... .......... .......... .......... .......... T......... .......... ...C...... *.........
Gorilla gorilla            .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... *.........
Pongo pygmaeus             .......... .......... ........Q. S..S...... ........LQ .......... .......... .L........ ...T...... ......S.E. YDNRVTLATK
Hylobates lar              .......... .......... ........Q. S.......S. ......K... .......... .......... .......... ...T...... Q.H...S... *.........
Pappio sp                  .......... .......... ........QN S........S ...P.R..Y. -N........ .......... ..Q....... L..T...... ......S... *.........
Leontopithecus chrysomelas .......... .......... .....N..QN .D...N...S .E...G.M.. VED...TFSY S.N...C.Q. T.V.P..LS. .V.PE..... ......S.N. RDNRVTLSTT
L. chrysopygus             .......... .......... .....N..QN .D...N...S .E...G.M.. VED...TFSY S.N...C.Q. T.V.P..LS. .V.PE..... ......S.N. RDNRVTLSTT
L. rosali 1                .......... .......... .....N..QN .D...N...S .E...G.M.. VED...TFSY S.N...C.Q. T.V.P..LS. .V.PE..... ......S.N. RDNRVTLSTT
L. rosalia 2               .......... .......... .....N..QN .D...N...S .E...G.M.. VED...TFSY S.N...C.Q. T.V.P..LS. .V.PE..... ......S.N. RDNRVTLSTT
Callithrix aurita          .......... .......... .....N..QN .D...T...S .ET.Q----. AED...TFSY S.NSK.S.Q. T...P..LSL .V.PD..... ...C..S.N. QDNRVTLTTK
C. geoffroyi               .......... .......... .....N..QN .D...T...S .E...G.M.. AED..FTFSY S.NSK.S.Q. T...P..LS. .V.PD..... ...C..S.N. QDNRVTLTTK
C. jacchus                 .......... .......... .....N..QN .D...T...S .E...G.M.. AED..FTFSY S.NSK.S.Q. T...P..LS. .V.PD..... ...C..S.N. QDNRVTLTTK
C. penicillata             .......... .......... .....N..QN .D...T...S .E...G.M.. AED..FTFSY S.NSK.S.Q. T...P..LS. .V.PD..... ...C..S.N. QDNRVTLTTK
C. kuhlii                  .......... .......... .....N..QN .D...T...S .E...G.M.. AED..FTFSY S.NSK.S.Q. T...P..LS. .V.PD..... ...C..S.N. QDNRVTLTTK
C. pygmaea                 .......... .......... .....N..QN .D...T...S .E...G.M.. AED...TFSY S.NSK.S.Q. T...P..LS. .V.PN..R.. .N.C..S.N. QDN.......
Callimico goeldii          .......... .......... .....N..QN .D...T.N.S .E...G.M.. VED.F.TFSY ..NS..S.Q. T...P..LS. .V.PDN.... ......S.N. QDN.......
Saguinus_m. midas          .......... .......... .....N..QN .-...T...S .E..YG.M.. VED...TFSY S.N...S.E. T...P..LS. .V.LD....R ......S.N. QDN.......
Aotus l. griseimembra      .......... .......... .....NV.QN .E.F.....S .E.....M.. VED...T.SY ..N...S.Q. T.......S. .L.R...T.. .....PS.N. QDNRVTLTTK
A. azarae                  .......... .......... .....NV.QN .E.F.....S .E.....M.. VED...T.SY ..N...S.Q. T.......S. .L.R...... .....PS.N. QDNRVTLTTK
A. infulatus               .......... .......... .....NV.QN .E.F.....S .E.....M.. VED...T.SY ..N...S.Q. T.......S. .L.R...... .....PS.N. QDNRVTLTTK
Cebus albifrons 1          .......... .......... .....NL.QN .D..R.V..S .E...N.M.. VED...TFSY ..N...S.Q. I.....V.S. .V.P...... .....NS.N. QGNRVTLTTK
Cebus albifrons 2          .......... .......... .....NL.QN .D..R.V..S .E...N.M.. VED...TFSY ..N...S.Q. I.....V.S. .V.P...... .....NS.N. QGNRVTLTTK
Cebus apella apella 1      .......... .......... .....NL.QN DD..R....S .E...N.M.. .ED...TFSY ..N...S.Q. I.....V.S. .V.P...... .....NS.N. QGNRVTLTTK
Cebus apella apella 2      .......... .......... .....NL.QN DD..R....S .E...N.M.. .ED...TFSY ..N...S.Q. I.....V.S. .V.P...... .....NS.N. QGNRVTLTTK
Cebus capucinus            .......... .......... .....NL.QN .D..R.V..S .E...N.M.. VED...TFSY ..N...S.Q. I.....V.S. .V.P...... .....NS.N. QGNRVTLTTK
Cebus nigrivitattus        .......... .......... .....NL.QN .D..R....S .E...N.M.. VED...TFSY ..N...S.Q. I.....V.S. .V.P...... .....NS.N. QDNRVTLTTK
Cebus a. xanthosternos     .......... .......... .....NL.QN DD..R....S .E...N.M.. VED...TFSY ..N...S.Q. I.....V.S. .V.P...... .....NS.N. QGNRVTLTTK
Saimiri sciureus           .......... .......... .....NK.QN .D.......S .E..R.M... VEDG..TFSY ..N...S.Q. I.......SL .VSP...... ..C.....N. Q.........
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**Fig. 2.** Alignment of *SRY*-deduced amino acid sequences. The *underlined* region corresponds to the HMG-box domain; the *boldface* region is homologous to the SIP-1 interacting motif.

The deduced amino acid sequence (Fig. 2) showed seven substitutions at the HMG-box region in comparisons of man and platyrrhines. These amino acid replacements were unrelated to previously described substitutions associated with sex reversal in man. Interestingly, among *Cebus* species, two amino acid replacements were observed in the HMG-box domain, at positions 81 and 102 (Fig. 2). The former, in one specimen of *Cebus albifrons* (*C. albifrons* 1), was a substitution of glutamine for glutamic acid (a hydrophilic, uncharged amino acid for a hydrophilic, acid amino acid). The latter, shared by the tufted *Cebus* species (*C. apella apella* and *C. a. xanthosternos*), was a substitution of serine for threonine (both hydrophilic, uncharged amino acids). An autapomorphic replacement was also observed in *Callithrix pygmaea* at position 93: one arginine, present in all other platyrrhines, was replaced with tryptophan, representing a substitution of a hydrophilic, basic amino acid for a hydrophobic amino acid. Replacements were not observed for amino acid residues of the human-homologous SRY-HMG box which interact with DNA target sites such as DNA bases (N65, F67, I68, S88, I90, S91, Y129), phosphates (R59, R62, R72, R75, K92, R121, K128), or sugars [M64, F67, A79, W98 (see Werner et al. 1995)].

The only case of an amino acid substitution associated with sex reversion in man (S18N) was found in callitrichines (Fig. 2), while in all other platyrrhines, serine was present at position 18. This substitution was described previously in a human patient with gonadal dysgenesis and was shared by his normal father and one normal brother, indicating that this mutation had not occurred *de novo* (Domenice et al. 1998). The finding of an identical missense substitution in callitrichines, albeit without any relation to gonadal dysgenesis, suggested that the effect of this mutation in man might be determined by other

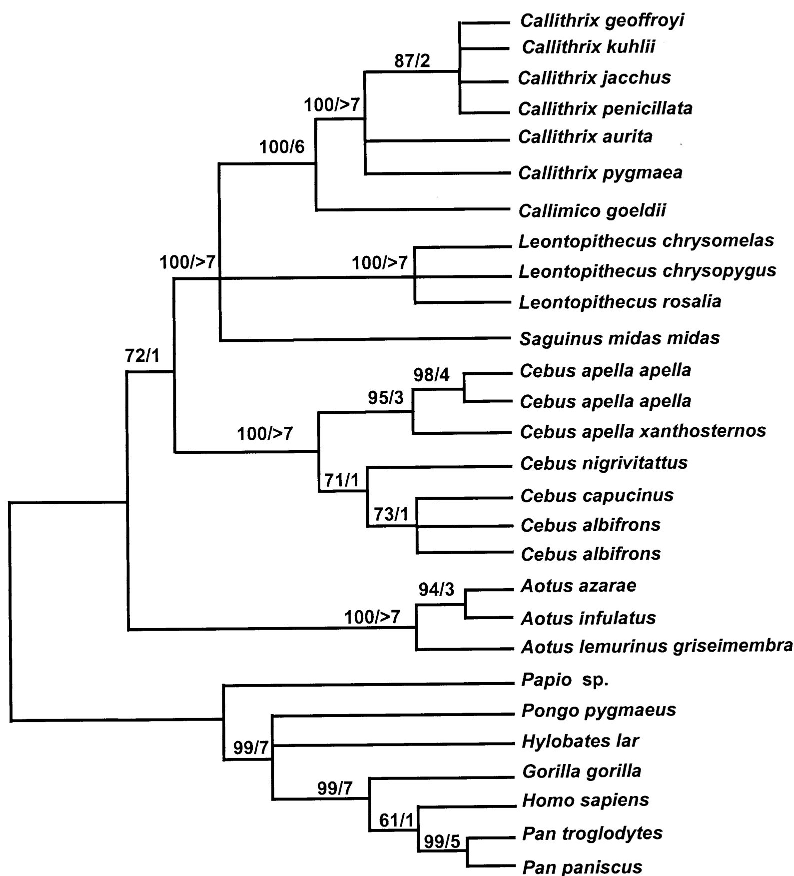


**Fig. 3.** Consensus tree of the six most parsimonious trees (332 steps; CI = 0.907; RI = 0.965; RC = 0.875) obtained from analyses of *SRY* aligned sequences excluding *Saimiri sciureus* data. Numbers at nodes are bootstrap values (%) and decay indexes, respectively.

genetic factors: allelic variants of proteins interacting with SRY or DNA differences at SRY-binding motifs resulting in either normal male development or gonadal disgenesis. Similar events were reported in a comparative study of the pyrin gene showing that the presence of some amino acids residues at positions known to cause familial Mediterranean fever in man lead to asymptomatic phenotypes in other primates (Schaner et al. 2001).

In the C-terminal region, downstream of the HMG-box, nucleotide sequence alignment showed five indels among platyrrhines. One of these events, extending from nt 676 to nt 687, was either a deletion shared by all catarrhines or, alternatively, an insertion shared by all platyrrhines. Comparisons with other available *SRY* sequences of mammals outside the primate order did not allow us to determine the ancestral state due to the high variability of the C-terminal region. The remaining indels were one deletion from nt 601 to nt 603 in *Saguinus*, one punctual insertion at position 635 and one deletion from nt 642 to nt 651 in *C. aurita*, and one deletion from nt 678 to nt 680 in all *Aotus* species.

Most indels at the coding region encompassed segments of either three or multiples of three nucleotides except in *C. aurita*, where we found one punctual insertion and a 10-bp deletion, resulting in net loss of 9 bp with respect to the remaining *Callithrix* species. However, none of these events or any other nucleotide substitution among species generated a stop codon upstream of human positions 807–809 (TAG; Fig. 1), indicating that the C-terminal region of the SRY protein must be functionally relevant. This was evident in *Mus musculus musculus*, in which sex reversion did not occur in transgenic females expressing an HMG-box domain lacking either the C-terminal end of the SRY protein or the glutamine repeat region of this end (Bowles et al. 1999). Furthermore, sex reversion resulting in an XY human was also associated with a mutation at the C-terminal end generating a stop codon and a truncated SRY protein lacking the last 41 amino acids (Tajima et al. 1994). The human SRY C terminus (particularly the terminal seven-amino acid-long oligopepitid YSHWTKL) was shown to be involved in protein-protein interactions with SIP-1 [SRY interacting protein 1 or $Na^{+}/H^{+}$ exchanger regulatory factor-NHERF-2 (see Poulat et al. 1997)]. Our studies indicated, however, that the deduced amino acid sequences of this homologous region were highly variable among species because only the second (serine), fifth (threonine), and seventh (leucine) residues were evolutionary conserved. These variations, however, did not prevent the interaction of the homologous seven-residue oligopeptidie of *Callithrix*, *Pongo*, *Hylobates*/*Papio*, *Pan paniscus*, and *P. troglodytes* with human SIP-1 (Poulat et al. 1997).

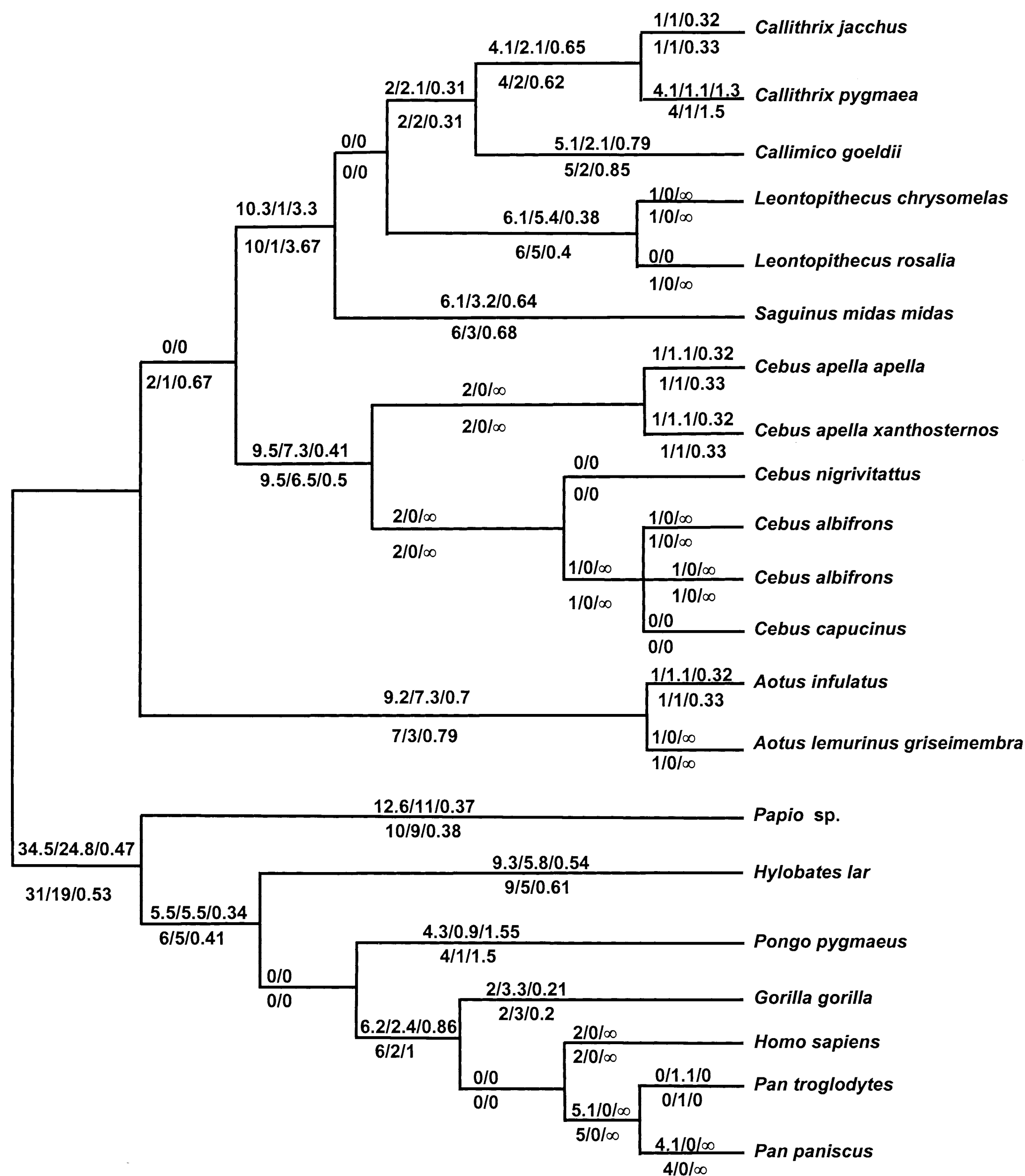


**Fig. 4.** Topology used to estimate the number of synonymous and nonsynonymous substitutions for branches. Values above branches indicate the numbers of nonsynonymous and synonymous substitutions and the dN/dS ratio, respectively, for maximum-likelihood reconstruction using the free-ratio model. Values below branches indicate the same estimates for parsimony reconstruction.

In the topology used to infer ancestral states (Fig. 4) most nodes were strongly supported (Schneider et al. 2001; Goodman et al. 1998) except for the one representing the common ancestor of *Cebus* and Callitrichines. Most molecular topologies, however, showed the association of *Cebus*, *Aotus*, and the callitrichines as a trichotomy. With respect to *Cebus* species, we used our topology resulting from parsimony analysis in view of the lack of previous phylogenetic arrangements.

Estimates of the number of synonymous and nonsynonymous substitutions and dN/dS ratios for branches did not show evidence for positive selection acting on *SRY* in analyses including all coding regions or only HMG-box flanking regions. Both methods used for reconstructing ancestral states (maximum likelihood and parsimony) led to this conclusion. The maximum-likelihood approach also showed that the differences observed among dN/dS values for branches using the free-ratio model were not significant. On the other hand, the presence of six indels in the C-terminal region without disruption of the open reading frames indicated that this region might be under selective constrains, despite the fact that most nonsynonymous substitutions were found in HMG-box flanking regions. Moreover, our results contradict the conclusions of Whitfield et al. (1993) for positive selection or neutral evolution acting on HMG-box flanking regions in primates. Altogether, the absence of *SRY* sequence variation among four *Callithrix* species (*C. jacchus*, *C. penicillata*, *C. kuhlii*, and *C. geoffroyi*), between two *Leontopithecus* species (*L. ro-*

*salia* and *L. chrysoppygus*), and between two *Aotus* species (*A. azarae* and *A. infulatus*) did not confirm an association between *SRY* variation and speciation events as postulated by Whitfield et al. (1993) in apes.

### *Species Hybridization and* SRY

The lack of sequence variation among *C. jacchus*, *C. penicillata*, *C. geoffroyi*, and *C. kuhlii* indicated that these species have diverged recently. Together with *C. aurita,* these species are included in the *jacchus* species group, whose monophyly is strongly supported by karyologic and molecular data (Nagamachi et al. 1997; Tagliaro et al. 1997; Canavez et al. 1999a, b). These species presently exhibit a parapatric distribution, with *Callithrix penicillata* occupying a geographic area bordering the distribution of the four other species studied herein. Evidence of natural cross hybrids between *C. penicillata* and *C. jacchus* has been well documented (Alonso et al. 1987), while captive-bred male and female hybrids of any cross among *C. jacchus*, *C. penicillata*, *C. geoffroyi*, and *C. kuhlii* were found to be fertile (Coimbra-Filho et al. 1993). The same authors also reported cross hybridizations between a male *C. aurita* and a female *C. kuhlii* producing two female litters but without data on hybrid fertility. For this reason, *SRY* differences between *C. aurita* and other species in the *jacchus* group could not be assessed with respect to sex differentiation and fertility in the male sex. With respect to *C. penicillata*/*C. jacchus* natural hybrids, their area of occurrence is very restricted and strongly modified by anthropic action, suggesting that reproductive isolation between these species in other boundaries of their distribution must be maintained by behavioral mechanisms (Alonso et al. 1987).

Sequence analyses also indicated that lion tamarin species have diverged recently. *Leontopithecus rosalia* and *L. chrysopygus* shared the same *SRY* sequence but diverged from *L. chrysomelas* in one nonsynonymous substitution (M8V; Fig. 2). This amino acid substitution coexisted with normal male and female development and with normal fertility in captive-bred hybrids resulting from a cross of a male *L. chrysomelas* with a female *L. rosalia* (Coimbra-Filho et al. 1991).

Similar findings of natural or captive hybrids are not available for *Aotus* and *Cebus*. Therefore, for these genera, differences between congeneric species did not allow us to establish a relationship among nucleotide substitutions, *SRY* function in different genetic backgrounds, and speciation.

### *The Phylogeny Based on Male Lineages*

The consensus parsimony tree (Fig. 3) grouping the New World monkeys studied herein was similar to previously reported DNA sequence topologies (Schneider et al. 1993, 1996, 2001; Horovitz and Meyer 1995; Porter et al. 1997a, b; Pastorini et al. 1998; Canavez et al. 1999a, b; Moreira and Seuánez 1999). Within callitrichines, the relationships among *Saguinus*, *Leontopithecus*, and *Callithrix*/*Callimico* could not be solved using *SRY* data, despite the fact that molecular phylogenies showed *Saguinus* as an earlier callitrichine offshoot. The *SRY* topology showed a close association of *Callimico* and *Callithrix*, in agreement with immunological and molecular studies (Cronin and Sarich 1975; Seuánez et al. 1989; Schneider et al. 2001) except for the IRBP (interphotoreceptor retinoid-binding protein) topology, in which *Callimico* and *Leontopithecus* were closely associated (Schneider et al. 1996). Most molecular topologies also associated *Cebus* with *Saimiri* as in our tree including sequence data of *Saimiri sciureus*. However, a well-supported arrangement establishing the relationships among *Cebus*/*Saimiri*, *Aotus,* and callitrichines has not been reported.

The *SRY* topology also showed a trichotomy formed by *Callithrix pygmaea*, *C. aurita*, and the remaining species in the *jacchus* group (*C. jacchus*, *C. penicillata*, *C. kuhlii*, and *C. geoffroyi*). However, the monophyletic arrangement of *C. aurita* with the remaining species in the *jacchus* group has been well supported by karyologic comparisons (Nagamachi et al. 1997) and molecular analyses based on mitochondrial (Tagliaro et al. 1997) and nuclear sequences [$\beta_2$-microglobulin (Canavez et al. 1999a, b)].

Contrary to that found in *Callithrix* species, the internal arrangement of the capuchin monkeys clade was well resolved and in agreement with the subdivision of the genus *Cebus* into two groups based on the presence or absence of head tufts (Hershkovitz 1949). The untufted group, distributed in the Amazonian forest and Central America, was represented by three species: *Cebus albifrons*, *C. nigrivittatus*, and *C. capucinus* (Mittermeier et al. 1988). The association of these species was supported by moderate bootstrap (71%) and decay index (1) values. The tufted group comprises several morphotypes distributed from Amazonian forest to the north of Argentina. The two mophotypes herein studied (*C. apella apella* and *C. a. xanthosternos*, distributed, respectively, in the Amazonian forest and Bahia state, Brazil) were associated with a higher bootstrap value (95%) strongly supporting the monophyly of this group.

Similarities between phylogenies derived from mitochondrial and those from male-specific genes (Fig. 3) did not indicate different evolutionary histories of male and female lineages. However, *Callithrix* may be an exception because the male-derived topology presents an arrangement different from that of the mitochondrial and autosome gene-derived trees. In the latter topologies, the association of *C. aurita* with the remaining *jacchus*-species group was strongly sup-

ported, contrary to what was observed in the *SRY* phylogeny. This fact suggests two alternative hypotheses: (i) an ancestral Y-chromosome polymorphism in male ancestral populations of *Callithrix* with random evolutionary sorting of different Y-chromosome lineages in *C. aurita* from the remaining *jacchus*-species group; or (ii) insufficient sequence data to clarify the trichotomy *C. pygmae*, *C. aurita*, and the remaining *jacchus* species.

## Conclusions

1. Analyses of synonymous and nonsynonymous substitutions did not show evidence of positive selection acting on *SRY* evolution in the ancestral lineages studied herein. Additionally, the presence of six indels at the C-terminal coding region without disruption of the open reading frame indicated that this region must be functionally important and under selective constrains.

2. The lack of *SRY* variation among closely related *Callithrix*, *Aotus* and *Leontopithecus* species ruled out that *SRY* change could be associated with speciation in these genera, contrary to the postulates of Whitfield et al. (1993) for apes. On the other hand, the existence of captive cross hybrids of species differing at the SRY protein sequence level (*L. chrysomelas* and *L. rosalia*) and the fact that male hybrids show normal sex differentiation and fertility also raise doubt that *SRY* variation and speciation could be related.

3. Phylogenetic analysis of *SRY* resulted in arrangements similar to previous topologies based on nuclear or mitochondrial DNA sequences concerning inter- and intrageneric relationships. In *Cebus*, the division of species into two monophyletic groups (tufted and untufted species) supported the monophyly of the taxonomic arrangement of Hershkovitz (1949) based on morphologic data. However, for *Callithrix* the differences among the *SRY* topology and those derived from autosomal and mitochondrial genes indicated an ancestral Y-chromosome polymorphism.

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