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A phylogeny of howler monkeys (Cebidae: *Alouatta*) based on mitochondrial, chromosomal and morphological data

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Abstract: The current taxonomic status of the species and subspecies belonging to the genus *Alouatta* is addressed by combined phylogenetic analysis using morphological, kariotipyc and molecular data (mitochondrial genes cytocrome oxidase II and cytochrome B). Our result demonstrated that *Alouatta palliata* is the most basal taxon for the genus in concordance with previous studies, as well as showing the validity of the taxon *Alouatta sara* as a species. Also our analysis shows that the sex chromosome has evolved from a XY/XX system to a X1X2Y1Y2/X1X1X2X2 system within the genus, as well as an increase in the size and complexity of the hioideal bone. Rev. Biol. Trop. 52(3): 665-677. Epub 2004 Dic 15.

Key words: *Alouatta*, phylogeny, Platyrrhini, taxonomy, hyoid bone, sex chromosome system.

Palabras clave: *Alouatta*, filogenia, Platyrrhini, taxonomía, hueso hioideo, cromosomas sexuales.

The evolutionary relationships of the infraorder Platyrrhini (Primates) genera have been studied using morphological (Rosenberg 1981, 1984, Ford 1986, Zingeser 1973, Ford and Davis 1992), karyotypic and molecular data (Schneider *et al.* 1993, Horovitz *et al.* 1998, Dutrillaux *et al.* 1986). Some incongruence between morphological and molecular data has emerged in some groups, such as the subfamily Atelinae (*Lagothrix* É. Geoffroy 1812, *Brachyteles* Spix 1823, *Ateles* É. Geoffroy 1806, and *Alouatta* Lacépède 1799*)*. The clade Atelinae is considered to be a monophyletic group based on the large body size, a prehensile tail with a hairless ventral area for grasping, the frequent use of suspensory positional behaviors, and other characters (Horowitz *et al.* 1998, Rosenberg 1989).

The phylogenetic relationships among the Atelinae genera have not been explored until recently (Horovitz *et al.* 1998). According to Rosenberg (1981, 1984), Ford (1986), Zingeser (1973) and Ford and Davis (1992), reported that the genus *Alouatta* is the sister clade to a clade compose of *Lagothrix*, *Brachyteles* and *Ateles* based on morphological data (*Brachyteles* and *Ateles* are the sister clade of *Lagothrix*). In contrast, Kay (1990) found the clade *Lagothrix* + *Ateles* as the sister clade of *Alouatta* + *Brachyteles* based on craneo-dental characters.

Dutrillaux *et al.* (1986) inferring chromosome rearrangements, reported that *Brachyteles*, *Lagothrix* and *Ateles* form a separate and distinct evolutionary branch from which *Lagothrix* is more closely related to *Ateles* than *Brachyteles*. On the other hand, Schneider *et al.* (1993, 1996), using DNA sequences for the α-globin and IRBP intron-1, found that *Alouatta* is the sister taxon to the clade ((*Brachyteles*+*Lagothrix*) *Ateles*). These relationships were tested by Horovitz *et al.*

(1998) in a combined analysis of morphological and molecular data (nuclear and mitochondrial sequences), with the resulting topology identical to the one already obtained by Schneider *et al.* (1996) for the Atelinae.

The Atelinae subfamily has also been studied at the species level. Froehlich *et al.* (1991) used a multivariate approach to examine *Ateles* cranial and dental morphometric patterns and concluded that this genus may be composed of three species. A more recent study using cytogenetical data (Madeiros *et al.* 1997), however concluded that *Ateles fusciceps* maybe reproductive isolated and therefore considered as a separated species. The genus *Alouatta* is currently considered to be composed of 9 species: *Alouatta belzebul* (Linnaeus 1766), *A. seniculus* (Linnaeus 1766), *A. caraya* (Humboldt 1812), *A. fusca* (É. Geoffroy 1812), A. *palliata* (Gray 1849), *A. pigra* (Lawrence 1933), *A. sara* (Elliot 1910), *A. coibensis* (Thomas 1902), and *A. arctoidea* (Rylands 1995). Of those, *A. sara* and *A. arctoidea* were subspecies of *A. seniculus* but now considered as full species by Minezawa *et al.* (1985) and Stanyon *et al.* (1995); while *A. coibensis* was also proposed as a separate species from the formerly known *A. palliata panamensis* (Froehlich and Froehlich 1987).

In addition, the taxonomic status of *A. seniculus macconelli* and *A. s. straminea* is in debate. According with Hill (1962), *A. s. macconelli* and *A. s. straminea* were considered as part of the *Alouatta* seniculus' 9 subspecies group. Bonvicino *et al.* (1995), however, proposed that *A. s. seniculus, A. s. stramineus* and *A. s. macconelli* should be considered as separate species basing their results in the analysis of quantitative cranial traits. In contrast, studies done by Figueiredo (1998) using mitochondrial DNA and Sampaio *et al.* (1996) using biochemical data (20 protein loci) showed that *A. s. macconelli* and *A. s. stramineus* are related closely enough to be considered a single subspecies. In addition, Lima and Seuanez (1991) found the same closeness between these two subspecies, but in this case the authors did not support the fusion of these subspecies.

The first systematic revision for the genus *Alouatta* was conducted by Hershkovitz (1949) using morphological characters. Hershkovitz (1949) proposed its division in three subgroups based on the hyoideal bone morphology, and their composition is as follows: the seniculus group (*A. seniculus*, A. *belzebul*, *A. fusca*), the *palliata* group (A. *palliata* and *A. pigra*) and finally the caraya group (*A. caraya*). More recently, Sampaio *et al.* (1996) studied the taxonomy of the Seniculus group containing the species *A. seniculus* and A. *belzebul*, based on biochemical and chromosome data. Oliveira (1996) using cytogenetical data proposed the placement of *A. belzebul nigerrima* together with *A. seniculus*. Also Gregorin (1996) proposed a phylogenetic hypothesis for the *Alouatta* species using cranial and hiodeal bone morphology which resulted in the placement as species of most of the *A. seniculus* subspecies, some of which (*A. seniculus ululata, A. s. discolor*) were closely related to A. *belzebul*. The recent study realized by Bonvicino *et al.* (2001) using Cytochrome B sequences partially support the phylogenetic relationships found previously: A. *palliata* as the basal taxon to all *Alouatta* species.

The goal of the present paper is to estimate the phylogenetic relationships among *Alouatta* species using karyotypic, morphological and DNA data on a simultaneous analysis.

MATERIAL AND METHODS

Data collection: The karyotypic data was compiled from existing literature (Table 1). Testing for chromosomal homologies was not possible because the low-resolution techniques used in most of the studies (chromosome homologies only reported by Mudry *et al.* 1994). Characteristics of the hyoideal bone were evaluated and taken from the illustrations of Hershkovitz (1949) and illustrations by Gregorin (1996); some of the Gregorin matrix characters used in this analysis as well as the hyoideal volumetric data previously reported by Crockett and Eisenberg (1986).

TABLE 1
Karyotypic information summary of the Alouatta species and the outgroup taxa

TAXON	SEX	N	2n	Me	Su	Ac	NMcr	NSY	NSX	X	Y	TR
A. belzebul belzebul 1	M	10	49	6	16	26	0		1	sm	A	P
A. belzebul belzebul	F	6	50	6	16	26	0		1			
A. belzebul	M	3	49	8	14	24	0	1	2	Sm,a	A	P
A. belzebul	F	3	50	8	14	24	0		2	Sm,a		
A. fusca clamitans 2	M	14	50-49	4-10	10-16	26-28	0	1	1-2	sm	a, sm	p
A. fusca clamitans	F	2	46	10	12	26			2	Sm		
A. seniculus seniculus 3	M	10	43to45	6	6	26	3to5	1	1	a	Sm	A
A. seniculus sara	M	13	50*	?	12	27	4	2	2	sm	a-sm	P
A. seniculus sara	F	20	50*	6	12	26	4		1	sm		
A. seniculus arctoidea	M	2	45	2	8	26	4	2	2	sm	a-sm	P
A. seniculus arctoidea	F	2	44	2	8	26	3	2	2			
A. seniculus macconelli	M	7	47-49	8	12	22-24	0-3	2	2	sm	a-sm	P
A. seniculus macconelli	F	13		8	12	22	1-3	2	2	sm		
A. seniculus stramineus	M	4	47-49	8	12	22-24	1-3	2	2	a-sm	A	P
A. seniculus stramineus	F	4	47-49	8	12	22	1-2	2	2	sm	a-sm	
A. caraya 4	M	14	52	2-6	14, 20	30	0	1	1	Sm	a-m	A
A. caraya	F	8	52	6	14, 12	32	0	1	1	Sm		A
A. palliata palliata 5	M	7	53	4	18	30	0	1	1	Sm	A	P
A. palliata palliata	F	10	54	4	18	30	0	1	1	Sm		
Ateles paniscus 6	M	1	32	12	16	1	0	1	1	M	A	A
Ateles sp.	M/F		34	12	18	2	0	1	1	M	a-sm	A
Lagothrix lagothricha	M		62	12	22	15	0	1	1	M	A	A
Brachyteles arachnoides 8	F		62	6	18	36	0	1	1	Sm	?	A

Simbology: 1. Armada *et al.* 1987, Lima and Seuanez 1989; 2. Koiffman and Saldanha 1974, Koiffman 1977, Oliveira et 1996; 3. Yunis *et al.* 1976, Minezawa et al. 1985, Stanyon et al 1995, Vassar *et al.* 1996, Lima et al 1990, Lima and Seuanez 1991, Consigliere et tal 1996; 4. Egozcue and Egoszcue et tal 1966, Egozcue 1969, Mundry *et al.* 1994, 5. Ma *et al.* 1975; 6. Pieczarka *et al.* 1989, Egozcue 1969, Dutrillaux *et al.* 1986, 7. Viegas-Pequignot *et al.* 1985, Dutrillaux *et al.* 1980, 8. Viegas-Pequignot *et al.* 1985. a: acrocentric; A: absent; Ac: Number of acrocentrics; m: metacentric; Mcr: Presence/Absence of Microchromosomes; Me: Number of metacentrics; N: number of individuals analyzed; NA: not apply; NMcr: Microchromosome Number; NOR: Nuclear Organized Region number; NSX: Number of X Chromosomes; NSY: Number of Y Chromosomes; P: present ; sm: submetacentric; Su: Number of submetacentrics; TR: Presence/Absence of translocations; X: X chromosome morphology; Y: Y-chromosome morphology; 2n: diploid number; ?: unreported/unknown.

DNA sequences from mitochondrial cytochrome B gene (CYTB) and cytochrome oxidase II (COII) were obtained from genebank and other sources (Table 2). DNA sequence alignments were performed using CLUSTAL W, ver. 1.75. (Thompson *et al.* 1994) and each gene was independently aligned and then concatenated. Because the genes are from independent studies, the sequences were not from the same localities, therefore the criterion used to concatenate was geographical proximity. The karyotypic and morphological data were added *a posteriori* to each taxon therefore all the sequences for a species had the same information. For *Alouatta* pigra and *A. coibensis*, no available data have been reported from the revised literature.

Phylogenetic analysis and sequence alignment: The phylogenetic analyses for the simultaneous combined matrix were performed using PIWEE, ver. 2.83. (Goloboff 1993). The search strategy consisted in 10000 TBR replicates (mult*), keeping 2 trees by replicate. All the uninformative characters were removed before the analysis. The Bremer decay index was calculated using a sample of 15000 suboptimal trees up to 15% less fit. The preformed homogeneity tests (I.L.D.L test by Farris *et al.* 1995) were also performed using Winclada ver. 1.00.08 with 1000 replicas.

TABLE 2

Species, GenBank Accession numbers, and geographic origins of the specimens sampled

Species	N	COII*	COII localities	CYTB **	CYTB localities
Alouatta belzebul belzebul	1	Figueiredo (1999)	Amapá, Brazil	AF289515[b]	Pacatuba Farm, Sapé (07°05__S, 35°13__W), Paraíba State, Brazil
	2	Figueiredo (1999)	Amapá, Brazil	AF289515	
	3	Figueiredo (1999)	Amapá, Brazil	AF289515	
	4	Figueiredo (1999)	Marajó Island, Pará, Brazil	AF289513[b]	Tucuruí Dam reservoir (03°45__S, 49°40__W), Pará State, Brazil
	5	Figueiredo (1999)	Marajó Island, Pará, Brazil	AF289512[b]	Tucuruí Dam reservoir, Pará State, Brazil
	6	Figueiredo (1999)	Rio Grande do Norte, Brazil	AF289515	
	7	Figueiredo (1999)	Tocantins River, right bank, Para, Brazil	AF289514[b]	Tucuruí Dam reservoir, Pará State, Brazil
	8	Figueiredo (1999)	Tocantins River, Left bank, Para, Brazil	AF289511[b]	Tucuruí Dam reservoir, Pará State, Brazil
Alouatta caraya	1	Figueiredo (1999)	Corrientes, Argentina	AF289519[b]	Rio Casca, Manso Dam reservoir, Chapada dos Guimarães, Mato Grosso State, Brazil
	2	Figueiredo (1999)	Serra da Mesa, Goiás, Brazil	AF289518[b]	Serra da Mesa Dam reservoir, Goiás State, Brazil
Alouatta fusca clamitans	1	Figueiredo (1999)	Espírito Santo, Brazil	AF289986[b]	Unknown
	2	Figueiredo (1999)	Santa Catarina, Brazil	AF289986	
	3	Figueiredo (1999)	São Paulo, Brazil	AF289987[b]	Unknown
Alouatta seniculus macconelli	1	AF054291[a]	Jari Rive, Left bank, Amazonas State, Brazil	AF289984[b]	Rio Jari, Amazonas State, Brazil
	2	AF054292[a]	Jari Rive, Left bank, Amazonas State, Brazil	AF289984	
	3	AF054293[a]	Jari River, Right bank, Amazonas State, Brazil	AF289984	
	4	AF054294[a]	Trombetas River, Left bank, Para, Brazil	AF289984	
	5	AF054295[a]	Trombetas River, Left bank, Para, Brazil	AF289984	
	6	AF 054296[a]	Trombetas River, Right bank, Para, Brazil	AF289984	
	7	AF054297[a]	Uatumã River, Left bank, Amazonas State, Brazil	AF289984	
	8	AF054298[a]	Uatumã River, Left bank, Amazonas State, Brazil	AF289984	
	9	AF 054299[a]	Uatumã River, Right bank, Amazonas State, Brazil	AF289984	
	10	AF 054300[a]	Uatumã River, Right bank, Amazonas State, Brazil	AF289984	
Alouatta seniculus sara	1	Figueiredo (1999)	Bolívia (Los Angeles Zoo)	AF245095[e]	San Diego Zoological Society
Alouatta palliata	1	AF 216252[c]	Costa Rica		
	2	L22774[d]	Costa Rica		
Ateles paniscus	1	AF054301[a]	Trombetas River, Para, Brazil		
	2	AF216250[c]	Trombetas River, Para, Brazil		
Brachyteles arachnoides	1	AF216253[c]	Fazenda Esmeralda, Minas Gerais State, Brazil	AF289989[b]	Unknown
Lagothrix lagothrica	1	AF216251[c]	Unknown		
	2	L22779[d]	Unknown		

Simbology: [a] Figueiredo *et al.* (1998); [b] Bonvicino et al (2001); [c] Collis and Dubach (2000); [d] Adkins and Honeycutt (1994); [e] Mundy *et al.* (2000). * COII: cytochrome oxidase II, **CYTB: cytochrome B

According to White (1978) the karyotypic information for some taxa shows some discernible directional tendencies but all the characters were stated as unordered because in most cases the available information lacked the resolution needed to infer direction for the chromosomal changes. The genera *Lagothrix*, *Brachyteles*, and *Ateles* were used as the outgroup, and their relationships to *Alouatta* followed the phylogenetic hypotheses proposed by Horowitz *et al.* (1998) and Schneider *et al.* (1993). Some morphological characters were treated as additive (Table 3 and Appendix for karyiotipic and morphological part of the matrix).

We choose the use of simultaneous analysis of different data sets because it could improve the phylogenetic signal because of the possible common shared history of different data sets. The homoplasy distribution is likely to be different in each data set because they are subject to different constrains, therefore when different data sets are analyzed simultaneously the signal common to all of them is more likely to overwhelm the homoplasy signal on the data (Kluge 1989).

RESULTS

The molecular partition was found to be incompatible with the morphological partition according to the I.L.D. test (Farris *et al.* 1975) (P value= 0.0102) as well as the cytochrome oxidase II gene with respect to the cytochrome B gene (P value= 0.0102). According to Cunningham (p.739, 1997), however, when the P value of the IDL test is greater than 0.01 the combination of the "incompatible" data sets improved, or in the worst case did not affected the accuracy of the phylogenetic reconstruction.

From the 1675 characters used, only 264 were informative. The phylogenetic analysis using the whole data set resulted in 3 equally parsimonious trees from which a strict consensus was calculated (Fig. 1, Length= 463, CI= 0.69; RI= 0.90, RC= 0.621, Fit value= 2315.0). All the obtained trees only differ on the relationships among DNA sequences within the taxon *A. seniculus macconelli*.

A. palliata is placed as the sister taxon to all other *Alouatta* species. In addition, *A. s. sara* is placed as the sister taxon of *A. seniculus macconelli*, more than 15 fit units would needed to collapse all *A. s. macconelli* to conform a single clade with *A. s. sara* (Fig.1). Also, the phylogenetic relationship among *A. fusca*, A. *belzebul* and the clade conformed by *A. s. sara* and *A. s. macconelli* on the consensus appears to be resolved. 4.5 fit units would be needed to collapse the clade conformed by A. *belzebul*, *A. s. sara* and *A. s. macconelli* (Fig. 1).

TABLE 3

Data Matrix. This data matrix includes only the cytogenetic and morphological data used in the analysis discussed in the text. Polymorphic data is enclosed in parenthesis

Taxa	Characters*
Ateles paniscus	5(45)0000010000000001010000??????0?0000???0
Brachyteles arachnoides	256800020???000????10000??????????????0
Lagothrix lagotrichia	571800010???000??????????????????????????
Alouatta belzebul	(23)(34)(234)(45)021(02)13423222303011112111110112110 10
Alouatta caraya	(012)(2346)560002021111?0???01111200010?11100011
Alouatta fusca	(14)(1234)(234)(23456)?(01)(01)21222321120201111201011011110011
Alouatta palliata	155700021130211011101111100000?00000000
Alouatta sara	223511121??????????????????????????????????
Alouatta seniculus	(023)(0245)(23)(1234)111(02)(01)32343222030111121201111111100

*See appendix 1 for character and character states description.

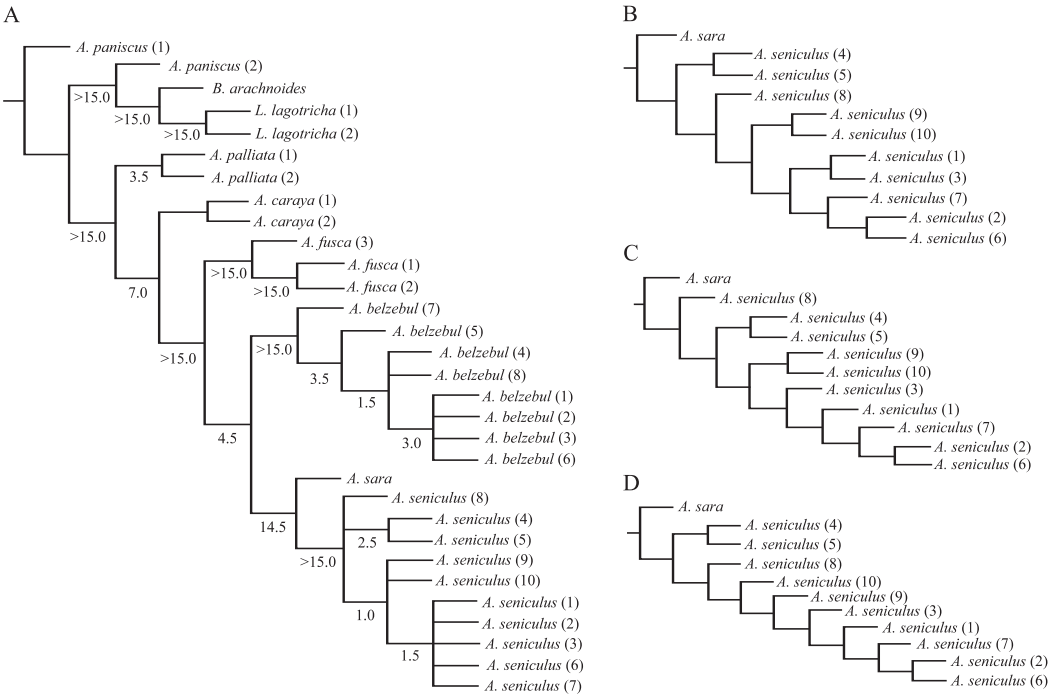

Fig. 1. Phylogenies obtained for the genus *Alouatta*. A. Bremer Decay Indexes for the strict consensus tree; B-D, Topogical differences among the obtained 3 equally most parsimonious trees.

DISCUSSION

Taxonomic implications: According to Hershkovitz (1949), the *Alouatta* genus could be divided in three different groups based on the hyoid bone morphology. These are: the seniculus group (*A. seniculus*, A. *belzebul* and *A. fusca*); the *palliata* group (A. *palliata*) (Mittermeier *et al.* 1988, included *A. pigra* and *A. coibensis*); and finally the caraya group (*A. caraya*). Later, Sampaio *et al.* (1996), using biochemical and chromosome data, found that *A. seniculus* and A. *belzebul* are not the closest species at the seniculus group. Meanwhile, the chromosome data compiled by them supports the notion of a closest relationship between *A. belzebul* and *A. fusca*.

Recent independent molecular analysis using the pseudogene α-1-globin (Meireles *et al*. 1999) and cytochrome B sequences (Bonvicino *et al.* 2001) produced partially congruent topologies to the one found with the combined data approach. These studies did not included all the species of the genus, therefore *A. palliata* was never included, not was *A. sara*. Figueiredo (1998) using the COII gene included this species in her analysis, the trees found could not resolve the relationships among *A. belzebul*, *A. fusca* and a polytomy conformed by *A. seniculus, A. sara, A. nigerrima* and *A. caraya*. Figueredo's (1998) analysis placed *A. palliata* as the sister taxa to all other species. Morphological analysis based on the hyoid bone was also incongruent with the molecular studies: Gregorin (1999) found *A. belzebul* and *A. seniculus* conforming a clade with *A. fusca* as the sister taxon. Our results support this relationship (Fig. 1).

Our results placed *A. palliata* as the sister taxon to the remainder taxa agreeing with previously reported topologies (Meireles *et al.* 1999, Bonvicino *et al.* 2000). Moreover, *A. sara* was the sister taxon to *A. seniculus macconelli*, with a strong support (decay index

value of 14.5), confirming its separate species status as previous works (i.e. Minezawa *et al.*1985, Stanyon *et al.* 1996). Stanyon *et al.* (1996) sugested the specific status based on more than 10 chromosomal rearrangements between *A. s. sara* and *A. s. arctoidea*.

Hyoid evolution: The hyoid bone is a large thin walled subglobular capsule that acts as a resonator and amplifier of *Alouatta*'s characteristic loud calls during territorial delimitation and appears to be a very specialized structure (Hershkovitz 1949, Crockett and Eisemberg 1986, Rosenberg 1989). Also, it has been considered a key character for *Alouatta's* species identification (Hershkovitz 1949). There have been few studies so far discussing the evolution of this structure.

In general, our results suggest a tendency towards an increasing complexity for the hyoid bone. There has been a trend towards a more globular hyoid bone, which is observed as an increase in the volume of the bone (with its highest volume exhibited by *A. seniculus*). Also, the tentorium has undergone a size increase in *A. seniculus* (Fig 3). The hyiod opening, the cornum and corniculum has been reduced most probably as a consequence of the increasing bulla development (i.e. the hioyd lateral shape changed from a "J" shape in *A. palliata* to a globular structure in *A. seniculus*).

The mentioned morphological modifications could account for the present acoustic differences among the species calls. In *A. seniculus*, the entering of air acoustically activated air by the glottis into the hyoid bulla and lateral air sacs amplify some frequencies, and increase the amplitude of the calls (Schon-Ybarra 1986). The *A. seniculus* barks range from 350 to 3500 Hz, with a spectral energy concentration on 350-1100 Hz (Schon-Ybarra 1986) while in *A. caraya*, which lacks of a complex development in the hyoid bone, produce lower call frequencies. The strongest frequencies in barks and long calls of *A. caraya* are in the range of 310-328 Hz (Thorington *et al.* 1984). Also, *A. palliata*, which is the species with the least complex hyoid bone, produces barks and roars at lower frequencies than *A. caraya* and *A. seniculus* (Eisenberg 1976, Thorington *et al.* 1984, Schön-Ybarra 1986).

Karyotypic evolution: Several tendencies were found for the *Alouatta* species like the reduction in the diploid number, which is seen as a further gain for *A. caraya* and *A. palliata* (Fig. 2). These tendencies have also been observed in *Ateles* species (2n= 34-33) in comparison with the genera *Lagothrix* and

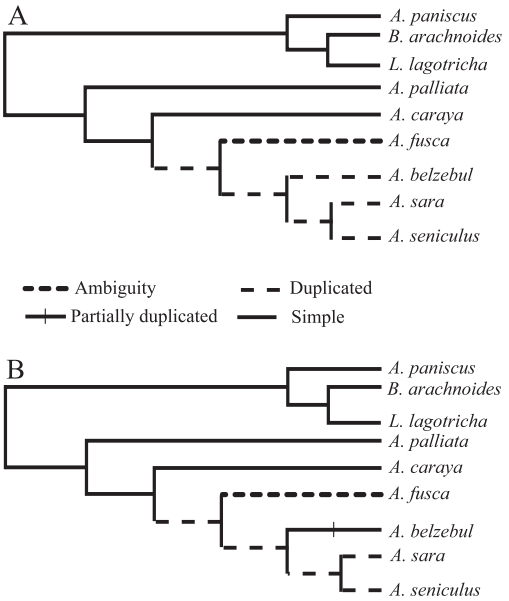

Fig. 2. Sex chromosome system evolution for the females (A) and males (B) of the genus *Alouatta*.

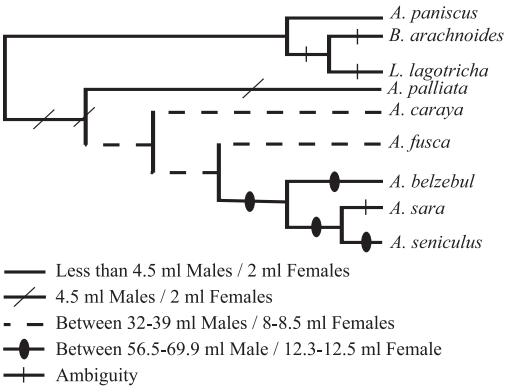

Fig. 3. Evolution of hioideal bone's volume for males and females of the genus *Alouatta*.

Brachyteles who have a diploid number of 62. Pieczarka *et al.* (1989) reported for *Ateles paniscus* paniscus a similar situation as a result of the fusion between the chromosomes 4 and 12. Fusion of chromosomes was also found in *A. belzebul belzebul*, *A. b. hybridus*, and *A. geoffroyi* (Garcia *et al.* 1975, Benirshke 1975, Kunkel *et al.* 1980). Nevertheless, it is important to mention that the diploid number for the species is the result of a population sampling process, making this character extremely variable (Ma *et al.* 1975).

The sex chromosome system in the genus *Alouatta* is highly diverse in comparison with other Neotropical primates. A. *palliata* has been described to have an XY system for males and XX for females (Ma *et al.* 1975) from a population studied in Barra Colorado Island (Panama). *A. caraya* has also been reported to have this same sex chromosome system (Oliveira 1996). *A. fusca*, however, shows at least 4 different karyomorphic groups: 2n=52 XY/XX; 2n= 48 or 50 XY/XX; 2n=49, X1X2Y/50,X1X1X2X2 and 2n=45 X1X2Y/ 46 X1X1X2X2 (Oliveira *et al.* 1995, 1998). It seems like the origin of these groups in *A. fusca* appear independently where the *de novo* sex chromosomes came from different autosomes (Bonvicino *et al.* 2001) *A. belzebul* also present a similar sex chromosome system of 50 X1X1X2X2/49X1X2Y (Armada *et al.* 1987), while *A. macconelli*, *A. sara* and *S. arctoidea* showed a X1X2Y1Y2/X1X1X2X2 system (Consigliere *et al.* 1996, Stanyon *et al.* 1995, Lima *et al.* 1990).

According with our results, the sex chromosome has evolved from a XY/XX system to a X1X2Y1Y2/X1X1X2X2 system. *A. fusca* is a polymorphic species with regard to the sex chromosome system, and due to its phylogenetic position in our results it was expected to present a XY/XX system. Due to the polymorphic status of this species it is not possible to asset unambiguously a character state at the node shared with the clade composed by *A. belzebul*, *A. sara* and *A. macconelli*.

The existence of microchromosomes in some species of the genus *Alouatta* has been another distinctive feature. These chromosomes have been found in *A. sara*, *A. s. artoidea* (Consigliere *et al.* 1996, Minezawa *et al.* 1985), *A. s. seniculus* (Yunis *et al.* 1976) and *A. s. macconelli* (Lima *et al.* 1990, Vassart *et al.*, 1996). For A. *palliata* (Ma *et al.* 1975), *A. caraya* (Egozcue and Egozcue 1966, Mudry *et al.* 1994, Egozcue 1969) and A. *belzebul belzebul* (Armada *et al.* 1987), however the presence of microchromosomes has not been reported. In *A. fusca*, the absence of microchromosomes has been reported (Koiffman 1982, Koiffman and Saldanha 1974), but the authors mention a numerical variation of karyotype in the number of the smallest pair. Minezawa *et al.* (1985) pointed out that this variation could be due to the presence of "accessory chromosomes" (microchromosomes). According with the present results, if the absence of microchromosomes in *A. fusca* is considered, the microchromosomes would have evolve once in the genus for the taxa *A. sara* and *A. seniculus*. Conversely, if the numerical variation on the karyotype of *A. fusca* is considered as proof of the microchromosome presence then the microchromosomes would have acquired independently by *A. fusca* and the clade *A. sara* and *A. seniculus*.

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RESUMEN

El estado taxonómico actual de las especies y subespecies del genero *Alouatta* (Lacépède, 1799) fue estudiado empleando un análisis filogenético combinado de datos morfológicos, cariotipicos y moleculares (genes mitocontridales del Citocromo Oxidasa II y el Citocromo B). Nuestros resultados demuestran que *Alouatta palliata*

(Gray 1949) es la especie mas basal del genero en concordancia con propuestas previas para el grupo, también muestran la valides de *Alouatta sara* (Elliot 1910) como una especie. Nuestros análisis también muestra que los cromosomas sexuales evolucionaron de un sistema XY/XX a un sistema X1X2Y1Y2/X1X1X2X2 dentro del genero así como también un incremento en el tamaño y complejidad del hueso hioideo.

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APPENDIX

Karyotipic and morphological character description

Character 0. Number of metacentric chromosomes: with two metacentric pairs (0)-2, four and five metacentrics (1), six metacentrics (2), eight metacentrics (3), 10 metacentrics (4), 12 metacentrics (5).

Character 1. Number of submetacentric chromosomes: with six and eight submetacentrics (0), 10 submetacentrics (1), 12 submetacentrics (2), 14 submetacentrics (3), 16 submetacentrics (4), 18 submetacentrics (5), 20 submetacentrics (6), 22 submetacentrics (7).

Character 2. Number of acrocentric chromosomes: one or two acrocentrics (0), 15 acrocentrics (1), 22 or 24 acrocentrics (2), 26 or 27 acrocentrics (3), 28 acrocentrics (4), 30 or 32 acrocentrics (5), 36 acrocentrics (6).

Character 3. Diploid number: 0-32 y 34, 1- 43 y 44, 2-45 y 46, 3-47 y 48, 4-49, 5-50, 6-52, 7-53 y 54, 8-62.

Character 4. Presence of microchromosomes: absence (0), presence (1).

Character 5. Ordered. Type of duplication in male sex chromosome: simple (xy) (0), duplicated (xxyy) (1), partially duplicated (xxy) (2).

Chracater 6 Ordered. Type of female sex chromosomes: simple (XX) (0), duplicated (XXXX) (2).

Character 7. X chromosome morphololgy: acrocentric (0), metacentric (1), submetacentric (2).

Character 8. Ordered. Presence of Y chromosome translocations: absent (0), present (1).

Character 9. Ordered. Hioid volumen (ml) for male and female: 4.5/2 or less (0), 4.5/2 (1), 23-39/8-8.5 (2), 56.5-69.9/12.3-12.5 (3).

Character 10. Male hioid bulla ventral shape: no developed (0), flat (1), round but not acute (2), round but acute (3), "w" shape (4).

Character 11. Male tentorium development: absent or no developed (0), rudimentario (1), evident without a chamber and slidely inflated (2), conspicous with an inflated chamber (3) .

Character 12. Male hioid shape aberture: without aperture (0), subrectangular (1), suboval (2), subpentameral with convex dorsal edge (3).

Character 13. Male hioid lateral shape: "l" shape (0), "j" shape (1), subtriangular (2), subrectangular (3).

Character 14. Ordered. Female hioid lateral shape: absent (0), round with curved edges in the last 1/3 (1), round with strait edges on the last 1/3 (2).

Character 15. Female tentorium dorsal part shape: absent (0), concavous (2), strait or flat (3).

Character 16. Female hioid aperture shape: without aperture (0), subrectangular (1), suboval (2), subpentameral (3).

Character 17. Ordered. Cornu development: reduced (0), prominent (1).

Character 18. Female hioid lateral shape: "l" shape (0), "j" shape (1), subtriangular (2), subrectangular (3).

Character 19. Ordered. Paraconule development: reduced (0), expanded (1).

Character 20. Ordered. Metaconule development: reduced (0), evident (1).

Character 21. Ordered. Presence of a folivorous diet: absent or infrequent (0), frecuent (1).

Character 22. Ordered. Occipital condile position: ventral (0), posterior (1).

Character 23. Ordered. Hioid shape: compresed plate like (0), chamber like (1).

Character 24. Ordered. Male hioid bulla development: absent (0), reduced (1), developed (2).

Character 25. Presence of male medial constriction on the hioid bone: absent (0), present (1).

Character 26. Male bulla aperture's shape: developed and cuadrangular (0), oval (1), semicircular (2).

Character 27. Presence of a lateral furrow on the hioid bone: absent (0), present (1).

Character 28. Bulla dorsal wall inclination: expanded (0), strait (1).

Character 29. Ordered. Presence of the tentorium in males: absent (0), present (1).

Character 30. Male tentorium shape: concavous (0), convex and oval (1).

Character 31. Ordered. Male cornu development: developed (0), reduced (1).

Character 32. Ordered. Male hioid posterior shape: bowed (0), convex (1).

Character 33. Ordered. Male curniculum development: developed (0), reduced (1), absent (2).

Character 34. Ordered. Presence of female tentorium: absent (0), present (1).

Character 35. Female hiodeal bulla shape: rounded (0), elongated (1).

Character 36. Presence of a dorso-ventral constriction: absent (0), present (1).

Character 37. Female corniculum development: developed (0), reduced (1).

Character 38. Ordered. Presence of sexual dicromatism: absent (0), present (1).