

MOLECULES, MORPHOLOGY, AND PLATYRRHINE SYSTEMATICS

H. Schneider¹ and A. L. Rosenberger²

¹University Federal of Para
Center of Biological Sciences
Department of Genetics
Belem, Para 66075-900, Brazil

²Smithsonian Institution
Washington, DC 20008

INTRODUCTION

Phylogenetic perspective is gradually penetrating fields not always accustomed to the language of systematics, thanks, in part, to extended discussions of methodology (e.g., Harvey and Pagel, 1991; Brooks and McLennan, 1991). Two recent examples are studies of the evolution of social organization in primates: Garber's (1994) analysis of callitrichines and Di Fiore and Rendell's (1994) review of the primate order. As this welcome trend continues, the importance of classification, a reference system of ideas regarding evolution, phylogeny and adaptation, will also grow.

After decades of extensive debate, it is widely recognized that the Linnaean system of classification is imperfect in many respects. It is also no secret that taxonomic discontent is an intractable, permanent feature of evolutionary biology. The reasons for this involve analytical difficulties in choosing the best answers among several logically possible historical hypotheses, as well as issues of scholarship, and the use of a cumbersome system of linguistically and procedural rules associated with classification (that many consider arcane). In such an environment it is perhaps best for professionals to agreeably disagree. For practical purposes, however, it is also desirable to delineate classifications that are true to the better ideas, that are consistent with the broadest range of available evidence - behavioral, ecological, anatomical, paleontological, molecular, etc. This, we think, is very feasible for the platyrrhines, in spite of the fact that the co-authors of this chapter each advocate slightly different systematic schemes. The point is that classification is an organizing tool that plays a primary role in the understanding of adaptation and evolution. Given the exponential increase of information in many fields pertinent to platyrrhines, the importance of this tool will grow and its shape will be adjusted to accommodate new facts, types of analyses and interpretations.

Central to the framework of a classification is a hypothesis of phylogeny. For the New World primates, a considerable research investment has been made in recent years to reconstruct the cladistic linkages of the modern genera. This is a critical step toward a full phylogeny, which would also specify the nature of the genealogical ties between living and extinct forms, i.e., both ancestral-descendant and collateral (sister group) relationships. The cladistic approach is a suitable way to begin. It offers a rich foundation for developing a heuristically sound classification. More, it is presently the only approach that can be applied to the certain types of data that cannot yet be extracted from fossils.

Our intention is to summarize views on the higher-level systematics and classification of the platyrrhines, emphasizing a synthesis of data developed by morphologists and new studies conducted in H. Schneider's lab employing DNA sequences. We particularly seek to accomplish the following: 1) demonstrate the growing concordance of molecular and morphological evidence; and 2) dispel the notion that platyrrhine higher-level systematics is in a state of disarray, which frequently finds its way into the literature, as in the following passage:

"...the relationships of neotropical platyrrhine monkeys to other groups of primates and to each other remain perhaps the most poorly known for any major primate taxon" (Flynn et al., 1995).

The discussion emphasizes modern forms but with the conviction that the framework we establish accommodates the fossils as well.

Our preferred classifications of the extant groups are also presented (Table 1). Occasionally, these differ. We emphasize, however, that most of these differences reflect one of the most arbitrary features of the classification process, the selection of taxonomic ranks attributed to groups based on phylogenetic (non-arbitrary) criteria. This is a fact of life in the world of taxonomy. One of us may prefer to list a particular monophyletic group as a family but the other, while retaining the same etymology for that taxon, may consider it best classified as a subfamily. In such cases, when the taxonomic contents are precisely the same, we introduce taxa under both ranks (e.g., atelids/atelines; pitheciids/pitheciines; callitrichids/callitrichines) and, where necessary, employ more general common names (see Rosenberger, 1981) to simplify discussion. In the one place where our phylogenetic views are significantly incompatible, we use versions of the family-level term set off in quotation marks; "Cebidae" and "cebids". It appears likely to us that the rules of nomenclature will perpetually saddle us with a term deriving from "Cebidae", based on the genus *Cebus*. However, the composition of this taxonomic group (to include the nearest relatives of *Cebus*) has been a matter of dispute for more than a decade, and it will likely continue thusly well into the future.

TURNING POINT

Historical reviews (Hershkovitz, 1977; Rosenberger, 1981) mark the middle 1970s as the turning point when contemporary platyrrhine classification, a doctrine unchanged for generations (e.g., Pocock, 1925; Simpson, 1945; Napier and Napier, 1967), began to falter. Its heuristic value was undermined by revolutions in theory, method, and new scientific programs. The pressures of cladistic-based analyses, molecular systematics and new data from behavioral ecology, especially, proved that the familiar classification, which emphasized discontinuities among taxa (Table 2), was unworkable. Key to this re-

Table 1. Abbreviated classifications of the modern platyrrhine genera according to the authors

Schneider	Rosenberger
Family Atelidae	Family Atelidae
Subfamily Atelinae	Subfamily Atelinae
Tribe Atelini	Tribe Atelini
<i>Ateles, Brachyteles, Lagothrix</i>	<i>Ateles, Brachyteles, Lagothrix</i>
Tribe Alouattini	Tribe Alouattini
<i>Alouatta</i>	<i>Alouatta</i>
Family Pitheciidae	Subfamily Pitheciinae
Subfamily Pitheciinae	Tribe Pitheciini
Tribe Pitheciini	<i>Pithecia, Chiropotes, Cacajao</i>
<i>Pithecia, Chiropotes, Cacajao</i>	Tribe Homunculini
Tribe Callicebini	<i>Callicebus, Aotus</i>
<i>Callicebus</i>	
Family Cebidae	Family Cebidae
Subfamily Cebinae	Subfamily Cebinae
<i>Cebus, Saimiri</i>	<i>Cebus, Saimiri</i>
Subfamily Aotinae	Subfamily Callitrichinae
<i>Aotus</i>	<i>Callimico, Saguinus, Leontopithecus, Callithrix, Cebuella</i>
Subfamily Callitrichinae	
<i>Callimico, Saguinus, Leontopithecus, Callithrix, Cebuella</i>	

Table 2. Comparison of selected classifications of the modern genera

Martin, 1990	Fleagle, 1988	Hershkovitz, 1977
Family Cebidea	Family Cebidea	Family Cebidea
Subfamily Cebinae	Subfamily Cebinae	Subfamily Cebinae
<i>Cebus, Saimiri</i>	<i>Cebus, Saimiri</i>	<i>Cebus</i>
Subfamily Aotinae	Subfamily Aotinae	Subfamily Aotinae
<i>Aotus, Callicebus</i>	<i>Aotus, Callicebus</i>	<i>Aotus</i>
Subfamily Pitheciinae		Subfamily Pitheciinae
<i>Pithecia, Chiropotes, Cacajao</i>		<i>Pithecia, Chiropotes, Cacajao</i>
Subfamily Atelinae	Family Atelidae	Subfamily Atelinae
<i>Ateles, Brachyteles, Lagothrix</i>	Subfamily Atelinae	<i>Ateles, Brachyteles, Lagothrix</i>
	<i>Ateles, Brachyteles, Lagothrix, Alouatta</i>	
Subfamily Alouattinae	Subfamily Pitheciinae	Subfamily Saimiriinae
<i>Alouatta</i>	<i>Pithecia, Chiropotes, Cacajao</i>	<i>Saimiri</i>
Subfamily Callimiconinae		Subfamily Callicebinae
<i>Callimico</i>		<i>Callicebus</i>
		Subfamily Alouattinae
		<i>Alouatta</i>
Family Callitrichidae	Family Callitrichidae	Family Callitrichidae
<i>Callithrix, Cebuella, Leontopithecus, Saguinus</i>	<i>Callithrix, Cebuella, Leontopithecus, Saguinus, Callimico</i>	<i>Callithrix, Cebuella, Leontopithecus, Saguinus</i>
		Family Callimiconidae
		<i>Callimico</i>

vision was the recognition that marmosets and tamarins, often called Callitrichidae (formerly Hapalidae), were not an isolated, primitive line. Rather, it seemed likely to many investigators that callitrichines were a group sharing a complex of derived anatomical and behavioral features. Furthermore, it appeared they could be linked genealogically with other platyrrhine groups, based on a reinterpretation of the evolution of their unique blend of traits. The implications forced a paradigm shift, a reorganization of platyrrhine classification based on a phylogenetic perspective. Single or pairs of genera could no longer be treated routinely as subfamilies because of their anatomical differences. Rather, the ranks of family, subfamily, tribe and subtribe would be invoked to reflect genealogical coherence and adaptive continuity.

Not surprisingly, as the old structure toppled the higher-level systematics of the New World monkeys became the subject of rich debate. A variety of classifications were proposed, leading some workers to abstain altogether from selecting any one in particular. Today, there are camps supporting the division of platyrrhines into two or three families, with differing content (Table 2). However, in contrast to the prevailing views of prior generations, which split families into many subfamilies along the taxonomic lines of genera (see Hershkovitz, Table 2), there is a growing consensus that clusters of genera should be assigned to a smaller number of coherent subfamilies.

This new paradigm is a distinctly phylogenetic approach. There is a broad consensus which recognizes three monophyletic groups within Platyrrhini, with a relatively stable albeit "new" nomenclature. 1) The smallest-bodied, clawed monkeys, callitrichines (*Cebuella*, *Callithrix*, *Leontopithecus*, *Saguinus*, *Callimico*). 2) The specialized seed-predators, pitheciines (*Pithecia*, *Chiropotes*, *Cacajao*). 3) The largest-bodied, prehensile-tailed monkeys, atelines (*Alouatta*, *Lagothrix*, *Ateles*, *Brachyteles*). Within these groups there remains only a few stubborn cladistic problems, but none really affect the bigger picture of platyrrhine relationships and classification.

That there is universal consensus about the essential phylogenetics of these three groups is a significant shift and advance. For example, Hershkovitz (1977) divided callitrichines into two families, one alone for *Callimico*. He argued they were two lineages that may have arisen independently from non-platyrrhine stock. Similarly, *Alouatta* was long maintained, by almost unanimous consent, as a subfamily separate from *Lagothrix*, *Ateles*, and *Brachyteles*. Only the morphologically bizarre pitheciines have been consistently united as a group. St. George Mivart set them aside as a subfamily in 1865 and there they remained, until new ideas began to broaden the concept.

Concerning the overarching cladistics, the picture is indeed muddled by significant disagreements about the inter-relationships of the three clades (Fig. 1). Among morphologists focusing on the 16 modern genera, Rosenberger (1981 *et seq.*) and Ford (1986; Ford and Davis, 1992) place pitheciines close to the atelines, while Kay (1990) argues that atelines and callitrichines are a monophyletic group trichotomized by the unresolved position of *Aotus*. The specific causes of this disagreement are unclear. Rosenberger's data principally comes from the dentition and skull. Ford's data are derived from several long bones and tarsal elements, but also involve a reanalysis of Rosenberger's original dental characters. These dental features form the basis of Kay's study, too.

An important source of the discrepancies may be the methodological differences involved in these studies. Rosenberger employed a synthetic approach to character analysis, using distributional information such as in-group and out-group commonality, time, ontogeny in the few instances where the data exists, functional morphology and behavioral ecology to assess the evolution of each character or complex individually, and thus build



Figure 1. Hypotheses of the cladistic relationships of modern platyrrhines based on morphology, after Rosenberger (a), Ford (b) and Kay (c).

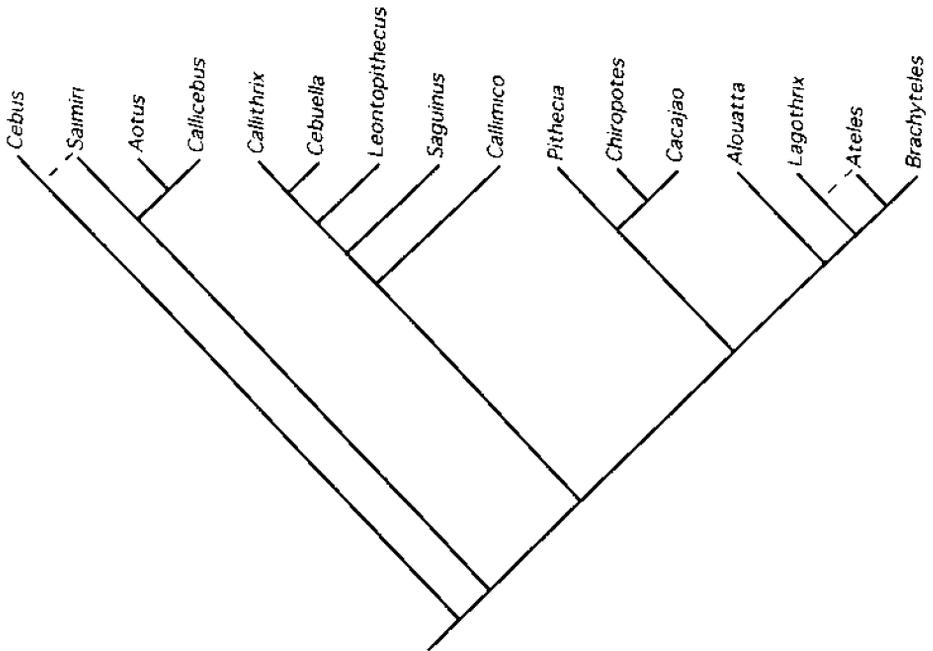


Figure 1b.

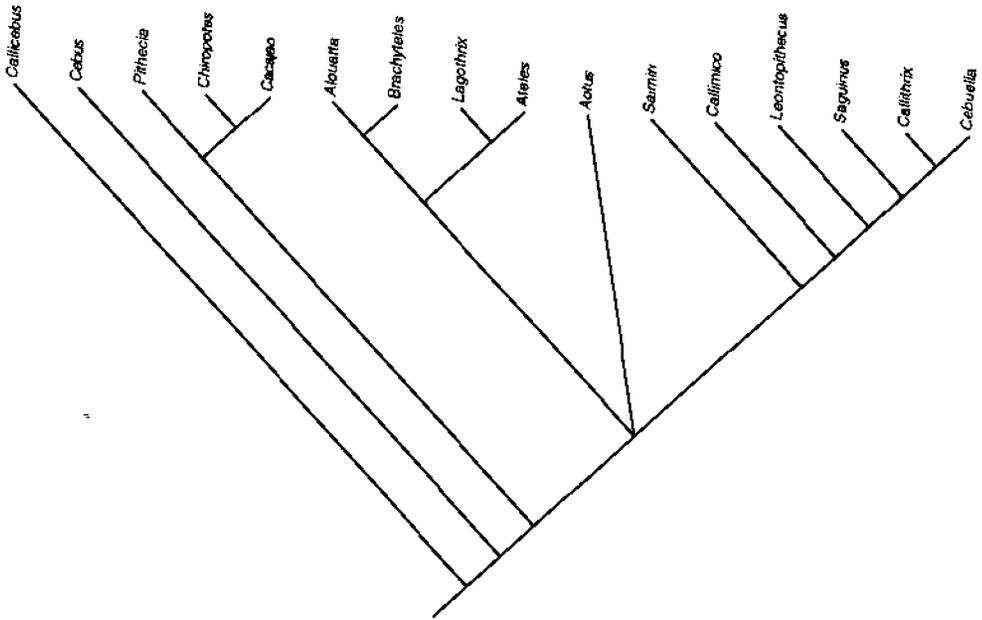


Figure 1c.

the tree. Both Ford and Kay, in contrast, employed numerical cladistic, parsimony algorithms to reconstruct relationships based on a matrix of traits.

The underlying systematic problem concerns the interpretation of four genera, *Cebus*, *Saimiri*, *Aotus*, and *Callicebus* (Fig. 1). Rosenberger (1981, 1984) proposed that *Cebus* and *Saimiri* are most closely related to the callitrichines, and *Aotus* and *Callicebus* to the saki-uakaris. Kay (1990) considered *Callicebus* and *Cebus* as two independent lineages that emerged one after the other at the beginning of the modern radiation, outside the more conventional groupings of callitrichines, atelines and pitheciines. Like Rosenberger, Kay also linked *Saimiri* with callitrichines but the affinities of *Aotus* were to remain indeterminate: Owl monkeys were rooted in a single trichotomous node shared also with atelines and callitrichines. Ford (1986; Ford and Davis, 1992) proposed two solutions for *Cebus* and *Saimiri*. One had them as independent clades, with *Cebus* a stem group of the radiation and *Saimiri* a relative of the *Aotus*-*Callicebus* clade. The alternative had *Cebus* and *Saimiri* united as the first-branching clade.

MOLECULAR EVIDENCE

Recently, Schneider's team analyzed the cladistic relationships of the 16 living New World monkey genera using 3.7 kilobases of DNA encompassing the whole Epsilon-globin gene (1.9 kilobases [kb]; Schneider *et al.*, 1993), and the intron 1 of the Interphotoreceptor Retinoid Binding Protein (IRBP) gene (1.8 kb; Schneider *et al.*, in preparation). Procedures for extracting, amplifying and aligning DNA to prepare a data matrix are explained elsewhere (Sambrook *et al.*, 1989; Schneider *et al.*, 1993; Cabot and Beckenbach, 1989). Cladograms were constructed using a variety of the maximum parsimony

algorithms (e.g., DNAPARS of Felsenstein, 1989; PTRALL, SURF and CONSEN of J. Czelusniak; see Schneider *et al.*, 1993 for descriptions). Distance matrix methods were also used (Kimura, 1980) to examine the data. Bootstrap analyses were performed with 2000 replications for both neighbor-joining (Saitou and Nei, 1987) and parsimony trees using the programs MEGA (Kumar *et al.*, 1993) and PHYLIP 3.5 (Felsenstein, 1989). This is a re-sampling procedure that tests for consistency by iteratively deleting and replacing characters randomly and comparing the results. As shown in Figures 2 and 3, consensus trees (maximum parsimony and neighbor-joining trees, respectively) generated for the Epsilon and IRBP genes provide strong evidence for three major clades.

THREE MAJOR CLADES

Clade I - Atelids/Atelines

Cladistic arrangements obtained by both maximum parsimony and neighbor-joining distances unite *Alouatta* with *Ateles*, *Brachyteles*, and *Lagothrix*. Seventeen sites were identified at which *Alouatta* shares derived characters with the others, grouping this genus with the three other atelines in all bootstrap iterations. The monophyly of this group is also strongly supported by morphology (Rosenberger, 1981, 1984; Ford, 1986; Kay *et al.* 1987), as well as behavior and ecology (Rosenberger and Strier, 1989). The connection between *Alouatta* and the others has a long history (Rosenberger, 1981), although it has been uncommon until recently to assemble the four genera into one family or subfamily.

The first-branch position of *Alouatta* is corroborated cladistically by molecular and morphological data, with few exceptions. Dunlap *et al.* (1985) placed *Alouatta* as the sister group of *Lagothrix* on the basis of forelimb musculature, while Kay (1990; but see Kay *et al.*, 1987) grouped *Alouatta* with *Brachyteles* on the basis of dental characteristics. Another discrepancy concerns the relationships of *Brachyteles*. Our molecular evidence suggests that *Brachyteles* and *Lagothrix* share a unique common ancestor, to the exclusion of *Ateles*. This *Brachyteles/Lagothrix* clade is supported by eleven base-pair synapomorphies, as opposed to three possibly derived sites that would link *Lagothrix* with *Ateles*, and four favoring a *Brachyteles/Ateles* clade. There are also cytogenetic similarities, recently found by determining and verifying the correct karyotype for *Brachyteles* (Koiffman and Saldhana, 1978) which was incorrectly reported in the literature since the early 1960s. *Brachyteles* and *Lagothrix* share a karyotype with $2n=62$ chromosomes, while the diploid number in *Ateles* varies from $2n=32$ to $2n=34$ (Dutrillaux, 1988; Pieczarka *et al.*, 1989).

Thus within atelids/atelines, these molecular and chromosomal data disagree with the morphology. Zingesser's (1973) view of an *Ateles-Lagothrix* sister-group has never been independently corroborated. The recent review by Rosenberger and Strier (1989) supported an *Ateles/Brachyteles* link based on an extensive series of derived postcranial traits and complexes, balanced by the demonstration of convergence in the dental morphology of *Alouatta* and *Brachyteles*. Ford's (1986) early analysis of the craniodental data produced an undefined *Ateles/Brachyteles/Lagothrix* trichotomy, which was later (Ford and Davis, 1992) revised to the *Ateles/Brachyteles* concept. Kay (Kay *et al.*, 1987; Kay, 1990) also discussed two alternative solutions to the internal relationships of this group. The *Ateles/Brachyteles* linkage proved most compelling, based on non-dental data.

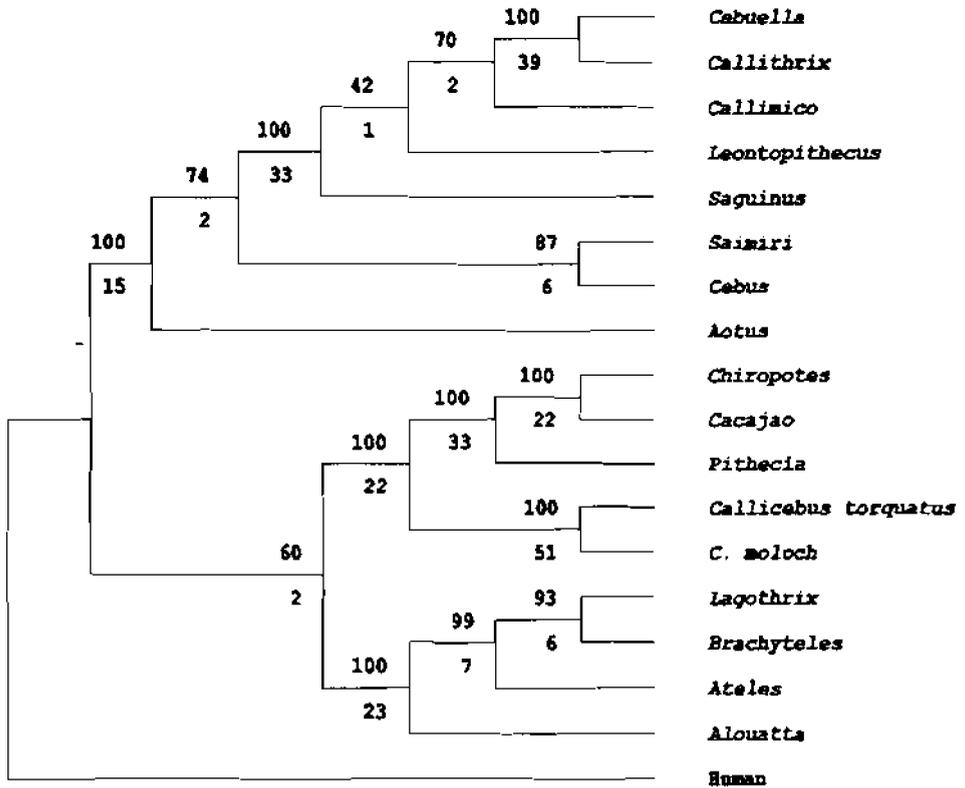


Figure 2. Phylogenetic tree produced by maximum parsimony method using DNAPARS from PHYLIP 3.5 (Felsenstein, 1989) and J. Czelusniak's programs (PTRALL, SURF, CONSEN). Numbers above nodes represent bootstrap values of 2,000 replications (SEQBOOT program). Numbers below the nodes are strength of grouping values (number of extramutations required to break the respective clade) estimated by J. Czelusniak's programs.

A potential source of the conflicting molecular and chromosomal information is the apparently very short span of time separating the emergence of the three lineages. This explanation is indicated by the very small differences in nucleotide diversity that separates the three genera. By comparison with the strongest generic dyads in the tree (Fig. 2), *Cacajao/Chiropotes* and *Cebuella/Callithrix*, the *Brachyteles/Lagothrix* linkage is less secure. Although the pair emerged as sister taxa in 93% of bootstrap iterations, it requires only 6 alternative substitutions to break the implied monophyly, compared with values of 100% & 39 and 100% & 22, respectively, for the other pairs. The *Saimiri/Cebus* link appeared in fewer iterations, 87%, and is supported at the same strength, requiring 6 "hits" to be broken.

Clade II - Pitheciids/Pitheciines

The cladistic arrangement obtained with molecular data clusters *Chiropotes* with *Cacajao* and *Pithecia* as the collateral relatives of the atelid/ateline clade, but with a twist.

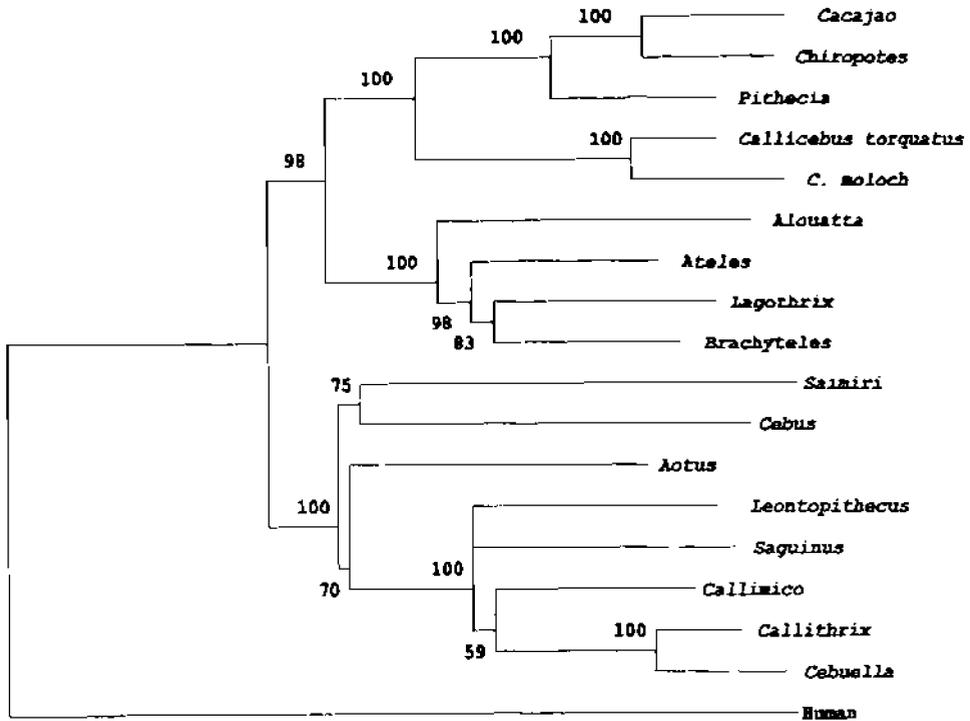


Figure 3. Phylogenetic tree obtained by the neighbor-joining method. Numbers at nodes represent bootstrap values pertaining to 2,000 replications.

The monophyly of the saki-uakaris seems unequivocal, based on morphological (Rosenberger, 1981; Ford, 1986; Kay, 1990; Kinzey, 1992; Hershkovitz, 1987) and biochemical markers (Schneider et al., 1995). Studies of immunological distances and karyotypes have reached similar conclusions using only two of the three genera; *Pithecia* and *Cacajao* (Cronin and Sarich, 1975, Dutrillaux, 1988) or *Cacajao* and *Chiropotes* (Baba et al., 1979). Most previous arrangements have identified *Chiropotes* and *Cacajao* as the most closely related internal lineages, but de Boer (1974) proposed an alternative arrangement based on chromosomal data, grouping *Pithecia* ($2n=46$) with *Cacajao* ($2n=44$ and 46), and identifying *Chiropotes* ($2n=54$) as the oldest member of the clade. It seems more likely that the unusual diploid number in *Chiropotes* is autapomorphic, having no immediate cladistic relevance.

Our molecular studies also group *Callicebus*, in all bootstrap iterations, as the sister-group to the saki-uakaris. This connection is as strong as that binding *Chiropotes* and *Cacajao*, involving 22 sites. Rosenberger (1981) came to the same conclusion based on dental morphology. He later modified the hypothesis by recognizing *Aotus* as a stem of the *Callicebus* lineage (Rosenberger, 1984; Rosenberger et al. 1990), a linkage endorsed by Ford and Davis (1992) and also acknowledged in many early classifications. As discussed above, the morphological evidence generated mixed views of the affinities of both

Callicebus and *Aotus* whereas the molecular evidence, while strongly supporting a *Callicebus/saki-uakari* clade, also suggests *Aotus* is related to the another group. We return to this matter below.

Clade III - "Cebids"

Callitrichines are the third group long recognized as a coherent unit, even before cladistic analysis deepened this understanding. Although our molecular data do not resolve well the internal cladistics of callitrichines, it confirms the morphological consensus that *Callimico* is genealogically integral to this group, a point no longer questioned seriously (but see Hershkovitz, 1977). The DNA data robustly supports the monophyly of the five callitrichine genera, at the same level of assurance found among the three monophyletic saki-uakaris (Fig. 2).

How callitrichines are related to other platyrrhines, on the other hand, is a matter of deep debate. Rosenberger argued that callitrichines are the sister-group of cebines; Ford regarded them as relatives of a pitheciine/ateline clade; Kay linked them to atelines. At one level, the DNA sequence evidence supports Rosenberger's interpretation (1981, 1990) that callitrichines and a *Cebus/Saimiri* clade form a monophyletic group. However, this node, which occurs in 74% of bootstrap trees, would collapse into an unresolved link if only two base-pair substitutions were altered.

A second matter relates to our finding that *Aotus* is universally linked with the callitrichine/cebine clade by the DNA sequences. This grouping has not been suggested previously by any of the morphological work. While it is not highly supported in comparison to the other higher taxa discussed previously (15 sites versus 33 in callitrichines, 33 in saki-uakaris, 22 in *Callicebus/sakis-uakaris*, 23 in atelines), it merits further consideration and testing. Alternative interpretations, such as aligning *Aotus* as the sister group of *Callicebus* using either Epsilon or IRBP data sets, would require a considerable increase in the number of nucleotide substitutions above those required by the most parsimonious trees (Harada et al., 1995). There are thus no potential synapomorphies in either gene that would specifically group *Aotus* with *Callicebus*, as some of the morphological data indicate (Rosenberger, 1984; Ford, 1986; Ford and Davis, 1992).

The internal bootstrap values within the "cebid" clade are also variable and, in three cases, they are relatively low. They average 79% over six nodes. In contrast, values among the six atelid internal nodes average 99%. So, although the basal nodes uniting these groups seem very different - 100% bootstraps & 15 substitutions at the *Aotus* linkage, and 60% bootstraps & 2 substitutions at the atelid dichotomy - our confidence is tempered by the weaker internal structure of the "cebid" branching sequence.

RELATIONSHIPS WITHIN THE MAJOR CLADES

Cebus and *Saimiri*

In our previous analysis of Epsilon-globin gene sequences (Schneider et al., 1993), the most parsimonious tree grouped *Cebus*, *Saimiri*, *Aotus*, and the callitrichine clades as an unresolved tetrachotomy. However, after enlarging the data set with the IRBP sequences, we discovered that *Cebus* and *Saimiri* link strongly with one another (Harada et al., 1995). This corroborates one of the traditional taxonomic schemes that was confirmed

cladistically by Rosenberger (1981) using craniodental and other evidence. Often overlooked is a body of neurological evidence which strongly corroborates the linkage of *Cebus* and *Saimiri* (Armstrong and Shea, in press) as well. Postcranial information (Ford, 1986) also supports a *Cebus/Saimiri* clade as an option (Ford and Davis, 1992), although Kay's (1990) numerical cladistic analysis of dental characters posits these genera as distant relatives: *Saimiri* as the sister-group to callitrichines deep within Platyrrhini, and *Cebus* as a mono-generic branch rooted at the second internal node of the platyrrhine radiation, outside 14 of the 16 modern genera. Ford's (1986) postcranial data, as well as her re-analysis of Rosenberger's morphological data set using numerical cladistics, allowed for a similar interpretation of *Cebus* as an early branch, as did the forearm muscle-based study of Dunlap et al., (1985).

In our view, the "outlier" hypothesis is untenable, for several reasons: 1) It is thoroughly inconsistent with the positive evidence of fine-grained analyses that seem to require a *Cebus/Saimiri* clade, based on a diversity of independent data sets (i.e., DNA, morphology, ecology, and behavior (see Janson and Boinski, 1989; Rosenberger, 1992). 2) A *Cebus/Saimiri* clade accords well with the broader picture of platyrrhine relationships and adaptations, which points to a close genealogical connection between (at least) *Saimiri* and the callitrichines. 3) Other evolutionary models can explain the anatomical and life-history differences that now distinguish these genera from their shared ancestral pattern (e.g., Hartwig, in press), differences which heretofore have led to doubt about their potentially close linkage. 4) The proposal that *Cebus* is an isolated outlier seems explicable by a likely methodological bias appearing in the three contravening studies where this was indicated. All used numerical cladistic procedures in which a key part of the analysis polarized traits by out-group comparison to catarrhines. In the studies of Ford and Kay this led to an over-emphasis on molar and long bone similarities of *Cebus* and parapi- thecids. In the case of Dunlap et al. (1985), the resemblances were between *Cebus* and living terrestrial cercopithecines. This comparative framework probably resulted in a failure to identify nonhomologies between in- and out-group, making some specialized features of *Cebus* appear more primitive than they are.

Callitrichines

The monophyly of the callitrichine clade (*Callithrix*, *Cebuella*, *Leontopithecus*, *Saguinus* and *Callimico*) is hardly disputed (Ford, 1986; Seuánez et al., 1988, 1989), although the phylogenetic relationships between genera are still controversial. Martin (1990; Table 2) is the rare exception, classifying *Callimico* among the non-clawed monkeys while hinting it "...might in due course prove to be justifiable" (pg. 715) to reassign the species to the callitrichine group.

Ford (1986) and Rosenberger et al. (1990) considered *Leontopithecus* as the lineage most closely related to the *Callithrix-Cebuella* clade, followed by *Saguinus* and *Callimico*. Snowden (1994) corroborated this branching sequences in his analysis of long call vocalizations. On the other hand, Kay (1990) suggested *Saguinus* as the sister group of *Callithrix-Cebuella*, followed by *Leontopithecus* and *Callimico*. The latter view is also consistent with Garber's (1994) character analysis concerning the evolution of callitrichin mating and social systems.

The evidence obtained from Epsilon and IRBP sequences, while not very strong (70% of trees, 2 sites), places *Callimico* as the sister group of the *Cebuella-Callithrix* clade (Schneider, et al., 1993), followed by *Leontopithecus* (42% of trees, 1 site) and

Saguinus (100%, 33 sites). As noted above, it is important to emphasize that this internal cladistic structure is not a robust outcome. There are no bootstrap values above 70% and two nodes are held together by only 1 or 2 sites (Figure 2), meaning only one or two mutations would be enough to change the position of *Leontopithecus* and *Saguinus*. In fact, since the base of the callitrichine tree is bounded by three taxa with relatively low rooting robusticity (33 for the *Saguinus* root, 1 for *Leontopithecus*, 2 for *Callimico*), and this particular topology has not appeared before in any of the morphological trees, it is likely that alternative solutions will be found with additional DNA data.

Callithrix and *Cebuella*

The sister-group relationship of *Callithrix* and *Cebuella* is unanimously agreed upon by nearly all the aforementioned researchers (Ford, Garber, Kay, Rosenberger, Snowdon), but not by Hershkovitz (1977). This linkage is the most robust of any based on our DNA sequences. Actually, the divergence values we obtained from IRBP nucleotide sequences for *Cebuella pygmaea* and *Callithrix jacchus* are within the range of values expected for species of the same genus, corroborating our previous findings with the Epsilon gene (Schneider et al., 1993). This re-opens discussion of the monophyletic status of the genus *Callithrix*, for it is possible that *Callithrix jacchus* (and close allies) are more closely related to *Cebuella pygmaea* than to other species now classified in the genus *Callithrix*. Analyzing morphological data, both Moynihan (1976) and Rosenberger (1981) suggested that *pygmaea* should appear as a congener, a practice advocated by some systematists for years.

Recently, Nagamachi et al. (1992), observed that the karyotypes of *Cebuella pygmaea* ($2n=44$) and *Callithrix emiliae* ($2n=44$) differed from that of *Callithrix jacchus* ($2n=46$) by a pericentric inversion of chromosome 19 and a Robertsonian translocation (20/16 in *Cebuella* and 22/16 in *Callithrix emiliae*). *Cebuella* and the Amazonian *Callithrix emiliae* differ from each other by only a reciprocal translocation between an acrocentric autosome and the short arm of the submeracentric chromosome, which distinguishes their karyotype from that of *Callithrix jacchus*. Seuánez et al., (1989) also observed a close karyotypic resemblance between *Callithrix argentata* and *Cebuella*. In our opinion, the mounting evidence (morphological, cytogenetic and molecular) requires a detailed analysis of the interrelationships of these species, with the possibility that some will have to be shifted taxonomically from one genus to the other.

FOSSILS AND PHYLOGENY

Although our emphasis has been the modern forms, absent input from the fossil record, our picture of platyrrhine relationships and the material basis for a classification remains incomplete. Fossils hold their own clues to phylogenetic relationships. Their analysis serves to validate morphotype reconstructions by identifying intermediate morphological linkages between anatomically disparate modern forms, and by expanding our knowledge of morphological combinations. Below we discuss briefly how platyrrhine fossils contribute specifically to our knowledge of phylogeny. In this discussion, the temporal dimension provided by geological context is also highly pertinent. It may, for example, contribute explanations to straighten likely cladistic linkages, or clarify the ambiguities of others.

The earliest fossil platyrrhines (Rosenberger et al., 1991) shed little light on the relationships of later forms. The most that can be said of them now is they reinforce the likelihood that callitrichines are not the ancestral stock. Although negative evidence, this lends confidence to the prevailing interpretations of the major outlines of character evolution among New World monkeys, and their contingent cladistic hypotheses.

A striking picture that emerges from the fossil record beginning in the early Miocene is the number of the lineages and modern genera which appear to be long lived (Delson and Rosenberger, 1984). Cebines have been traced back to the early Miocene, when they are represented by *Dolichocebus* and possibly *Chilecebus* (see Flynn et al., 1995), and by *Leventiana* (Rosenberger et al., 1991b) from the middle Miocene. *Saimiri* is related to a middle Miocene species that we place in the same genus, *S. fieldsi* (Rosenberger et al., 1991a); others (e.g., Takai et al., 1994) agree with the phylogeny but maintain the original genus name for the species (*Neosaimiri*). We interpret *Callimico* as phylogenetically linked with *Mohanamico herskovitzi* (Rosenberger et al. 1990) from the middle Miocene, although this point is disputed by Kay (1990). If correct, *Mohanamico* is the best preserved evidence demonstrating the existence of callitrichines in the fossil record. Saki-uakaris are easily recognized in the middle Miocene in *Cebupithecia sarmientoi*, whose precise affinities are still unclear. Earlier, less saki-like pitheciines are also known from Argentina (see Rosenberger et al., 1990). The genus *Aotus* is known by the species *A. dindensis*, although this interpretation is also debated (cf. Rosenberger et al., 1990; Kay, 1990). *Alouatta* relatives are well recognized in the middle Miocene by two species of *Stirtonia* (see Kay et al., 1987). It is also possible that one or both of these species could just as well be classified in the same genus as the living howler monkeys.

Is there a pattern in the fossil record that we can relate to DNA sequence data, or to other questions regarding platyrrhine cladogeny? Realistically, we understand too little to draw firm inferences. Nor would we expect simple explanations to fit complex historical puzzles. However, it would appear that the most confounding higher taxa, the close relatives of the *Callicebus*, *Aotus*, *Cebus* and *Saimiri* lines, are drawn from ancient lineages. They may be difficult to place not only because their phylogenetic signals have been obscured by the passage of time, but also because of the probability that a variety of intervening, intermediate clades have since become extinct and have not yet been resurrected in the form of a fossil. As noted below, a succession of early and rapid branching events may be difficult to tease apart with molecular data at this time.

PLATYRRHINE CLASSIFICATION: TWO OR THREE FAMILIES?

How to classify three distinct clades when their interrelationship are still murky? Our work with Epsilon sequences supports the view that the New World monkeys should be divided into two families; the Atelidae, with two subfamilies Atelinae (*Ateles*, *Brachyteles*, *Lagothrix* and *Alouatta*) and Pitheciinae (*Pithecia*, *Chiropotes*, *Cacajao* and *Callicebus*); and the "Cebidae", with *Aotus*, *Cebus*, *Saimiri*, and the callitrichines. Analyzed independently, the IRBP data places pitheciines closer to the "cebids", but the IRBP and Epsilon, when analyzed together, groups pitheciines with atelines, as suggested by the Epsilon gene. However, in this case the bootstrap values are low.

These results cannot dichotomously resolve the relationships among the three groups. Perhaps there is a message here regarding evolutionary tempo. For example, Nei (1986) proposed a method for inferring the number of cladistically informative nucleotides necessary to resolve three taxa into a dichotomous branching model. According to

this method, the probability of finding the correct topology depends on the number of nucleotides examined and the trees' summed branch length. In our previous paper (Schneider *et al.*, 1993), we estimated the split between Cebidae and Atelidae occurred at 20 MYA and the Atelinae-Pitheciinae split at 17 MYA. If the temporal difference separating the origins of these two divisions were smaller than 3 MYA, we would need far more than 6,000 nucleotides to find the correct topology with a probability of 90%.

Still, there are no simple solutions to the question of classification. H. Schneider favors a three-family system while A.L. Rosenberger favors a two-family system, as summarized in Table 1. In either case, we agree on the composition of most taxonomic units below the family-level. These, we propose, have significant utility for a wide range of applications. Of great theoretical interest to us both is the alignment of *Aotus* with callitrichines and cebines based on the molecules. We are less worried about the inconsistencies of morphology- and molecule-based branching patterns within callitrichines.

CONCLUDING REMARKS

Relative to the morphological cladistic studies that have a longer history, we conclude that, despite several unanswered questions, cladistic analysis of Epsilon-globin and IRBP gene sequences provides important complementary information on the major genealogical outlines in the phylogeny of New World monkeys. These are quite consistent with evidence from the fossil record, which means that the modern forms provide a good basis for developing a platyrrhine classification, and also that understanding the relationships of fossils may best proceed by including the living genera in the analysis of fossils. The molecular and morphological studies strengthen the idea of three major modern groups, possibly diverging closely spaced in time.

Concerning what has been the muddle in the middle of platyrrhine systematics for decades - *Cebus*, *Saimiri*, *Aotus*, *Callicebus* - the combined evidence definitely places *Callicebus* as a relative of pitheciines. They reinforce the connection between *Saimiri* and callitrichines, the linkage of *Cebus* with *Saimiri*, and their association with callitrichines as a monophyletic "cebid" group. However, the data diverge in that the DNA adds *Aotus* as the stem group of this cluster, an interpretation that is inconsistent with morphology.

The DNA also points to the need for a reconsideration of the taxonomy of genus *Callithrix*, which may not be monophyletic. It partially confirms the branching patterns of the atelid clade, placing *Alouatta* as the oldest lineage. Problems remaining within callitrichines and atelines include: 1) precise affinities among the atelins, *Lagothrix*, *Ateles* and *Brachyteles*; 2) the branching sequence within callitrichines, i.e., *Callithrix/Cebuella*, *Leontopithecus*, *Saguinus*, and *Callimico*.

ACKNOWLEDGMENTS

We would like to thank Dr. Eric Cabot for the sequence editor (ESEE200c), Dr. José Carneiro Muniz (Centro Nacional de Primatas - Belém, Pará, Brazil), Dr. Ademar Coimbra-Filho and Dr. Alcides Pissinatti (Centro de Primatologia do Rio de Janeiro, Rio de Janeiro, RJ, Brazil), and Dr. Filomeno Encarnación, Proyecto Peruano de Primatologia, Iquitos, Peru) for the samples used in this work. We also thank Dr. John Czelusniak for the parsimony programs PTRALL, CONSEN, SURF, and Steve Ferrari for his reading of

the manuscript. This research was made possible thanks to grants from CNPq-Brazil (910043/91.4; 201142/91.0; 201596/92.0), NSF-USA (DEB9116098), and FINEP-Brazil (6.6.94.0034.00). A.L. Rosenberger's work on platyrrhine systematics has been supported by NSF, University of Illinois, the L.S.B. Leakey Foundation, the National Zoological Park and the Smithsonian Institution. He thanks the many curators and museums that made his research possible.

REFERENCES

- Armstrong, E. and Shea, M.A. (in press) Brains of New and Old World Monkeys. In *New World Primates: Ecology, Evolution; Behavior*. (W.G. Kinsey, Ed.), Aldine, NY.
- Baba, M.L., Darga, L., and Goodman, M. (1979). Biochemical evidence on the phylogeny of Anthropeidea. In "Evolutionary Biology of the New World Monkeys and Continental Drift" (R. Ciochon and A.B. Chiarelli, Eds.), pp. 423-443, Plenum Press, New York.
- Brooks, D.R. and McLennan, D.H. (1991) *Phylogeny, Ecology, and Behavior: A Research Program in Comparative Biology*. University of Chicago Press, Chicago.
- Beckenbach, A.T. (1989). Simultaneous editing of multiple nucleic acid and protein sequences with FSEE. *Comp. Applic. Biosc.* 5: 233-234.
- Cabrera, A. (1958). Catalogo de los mamiferos de America del Sur. *Rev. Mus. Argentino Cienc. Nat. Bernardino Rivadavia* 4 1 307.
- Cronin, J. E., Sarich, V. S. (1975). Molecular systematics of the New World monkeys. *J. Hum. Evol.*: 4:357-375.
- De Boer, L. E. M. (1974). Cytotaxonomy of the Platyrrhini (Primates). *Genen Phaenen*, 17:1-115.
- Delson, E. & A.L. Rosenberger. (1984) Are there any anthropoid primate "living fossils"? In: *Casebook on Living Fossils*. N. Eldredge & S. Stanley, Eds. Fischer Publishers, New York, pp. 50-61.
- Di Fiore, A. and Rendell, D. (1994) Evolution of social organization: A reappraisal for primates by using phylogenetic methods. *Proc. Nat. Acad. Sci.*, 91: 9941-9945.
- Dunlap, S. S., Thorington, R. W. Jr., and Aziz, M. A. (1985). Forelimb anatomy of New World monkeys: myology, and the interpretation of primitive anthropoid models. *Am. J. Phys. Anthropol.* 6: 499-517.
- Dutrillaux, B. (1988). Chromosome evolution in primates. *Folia Primatol.* 50: 134-135.
- Felsenstein, J. (1989). PHYLIP - Phylogeny inference package (version 3.2). *Cladistics* 5:164-166.
- Fleagle, J.G. (1988) *Primate Adaptation and Evolution*. Academic Press, New York., pp., xix + 486.
- Flynn, J.J., Wyss, A.R., Charrier, R. and Swisher, C.C. (1995) An early Miocene anthropoid skull from the Chilean Andes. *Nature* 373: 603-607.
- Ford, S.M. (1986). Systematics of the New World monkeys. In "Comparative Primate Biology, Volume I: Systematics, Evolution and Anatomy" (D.R. Swindler and J. Erwin, Eds.), pp. 73-135, Alan R. Liss, New York.
- Ford, S.M. and Davis, L.C. (1992) Systematics and body size: implication for feeding adaptations in New World monkeys. *Am. J. Phys. Anthropol.* 88: 415-568.
- Garber, P.A. (1994) Phylogenetic approach to the study of tamarin and marmoset social behavior. *Am. J. Primatol.* 34: 199-219.
- Harada, M. L., Schneider, H., Schneider, M.P.C., Sampaio, I., Czelusniak, J., and Goodman, M. (1995). DNA Evidence on the Phylogenetic Systematics of the New World Monkeys: Support for the Sister-Grouping of *Cebus* and *Saimiri* from two Unlinked Nuclear Genes. *Molecular Phylogenetics and Evolution*. 4:331-349.
- Hartwig, W.C. (in press) The effect of life history on the squirrel (Platyrrhine, *Saimiri*) monkey cranium. *Am. J. Phys. Anthropol.*
- Harvey, P. and Pagel, M. (1991) *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.
- Hershkovitz, P. *Living New World Monkeys*. University of Chicago Press, Chicago, 1977.
- Hershkovitz, P. (1987) The taxonomy of South American sakis, genus *Pithecia* (Cebidae, Platyrrhini): a preliminary report and critical review with the description of a new species and a new subspecies. *Am. J. Primatol.* 12: 387-468.
- Hill, W. C. O. (1962). *Primates, comparative anatomy and taxonomy. Vol.V. Cebidae, Part B*. Univ. Press, Edinburgh.
- Janson, C.H. and Boinski, S. (1992) Morphological and behavioral adaptations for foraging in generalist primates. The case of the ebinés. *Am. J. Phys. Anthropol.* 88: 483-498.

- Kay, R.F., Madden, R.H., Plaven, J.M., Cifelli, R.L. and Guerro-D, J. (1987) *Stirtonia victorae*, a new species of Miocene Colombian primate. *J. Hum. Evolu.* 16: 173–196.
- Kay, R.F. (1990). The phyletic relationships of extant and fossil Pitheciinae (Platyrrhini, Anthropoidea). *J. Human Evol.* 19: 175–208.
- Koiffman and Saldhana (1978) Cytogenetics of Brazilian monkeys. *J. Hum. Evol.* 3: 275–282.
- Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.*, 16:111–120.
- Kinzey, W.G. (1992) Dietary and dental adaptations in the Pitheciinae. *Am. J. Phys. Anthrop.* 88: 499–514.
- Kumar, S., Tamura, K., and Nei, M. (1993). MEGA: Molecular Evolutionary Analysis, version 1.2. The Pennsylvania State University, University Park, PA.
- Martin, R.D. (1990) Primate Origins and Evolution. A Phylogenetic Reconstruction. Princeton University Press, Princeton, pp. xiv + 804.
- Moynihan, M. (1976). The New World primates: adaptive radiation and the evolution of social behavior, languages, and intelligence. Princeton University Press, New Jersey.
- Nagamachi, C.Y.; Pieczarka, J.C., and Barros, R.M.S. (1992). Karyotypic comparison among *Cebuella pygmaea*, *Callithrix jacchus* and *emiliae* (Callitrichidae, Primates) and its taxonomic implications. *Genetica*, 85: 249–257.
- Napier, J.R., Napier, P.H. (1967). *A handbook of living primates*. Academic Press, New York.
- Nei, M. (1986). *Stochastic errors in DNA evolution and molecular phylogeny*. Evolutionary perspectives and the New genetics. Alan R. Liss, pp. 133–147.
- Pieczarka, J., Nagamachi, C.Y., and Barros, R.M.S. (1989). The karyotype of *Ateles paniscus paniscus* (Cebidae, Primates): 2n=32. *Rev. Brasil. Genet.* 12: 543–551.
- Pocock, R.I. (1925) Additional notes on the external characters of some platyrrhine monkeys. *Proc. Zoo. Soc. London*, 1925: 27–47.
- Rosenberger, A.L. (1981). *Systematics: the higher taxa*. In "Ecology and behavior of Neotropical Primates, Vol. 1, (A.F. Coimbra-Filho and R. Mittermeier, Eds.), pp. 9–27, Academia Brasileira de Ciencias, Rio de Janeiro.
- Rosenberger, A.L. (1983) Aspects of the systematics and evolution of marmosets. In: *A Primatologia no Brasil*. M.T. de Mello, Ed. Universidad Federal Distrito Federal, Brasilia, pp. 159–180.
- Rosenberger, A.L. (1984) Fossil New World monkeys dispute the molecular clock. *Journal of Human Evolution*, 13:737–742.
- Rosenberger, A.L. (1992) The evolution of feeding niches in New World monkeys. *American Journal of Physical Anthropology*. 88:525–562.
- Rosenberger, A.L., Hartwig, W.C., Takai, M., Setoguchi, T. and Shigehara, N. (1991a) Dental variability in *Saimiri* and the taxonomic status of *Neosaimiri filedsi*, as early squirrel monkey from La Venta, Colombia. *Int. J. Primatol.* 12: 291–301.
- Rosenberger, A.L., Setoguchi, T. and Hartwig, W.C. (1991b) *Laventiana annectens*, new genus and species: fossil evidence for the origins of callitrichine monkeys. *Proc. Nat. Acad. Sci.* 28: 315–356.
- Rosenberger, A.L., W.C. Hartwig & R. Wolff. (1991) *Scalotavus attricuspis*, an early platyrrhine primate from Salla, Bolivia. *Folia Primatologica*, 56:221–233.
- Rosenberger, A. L., Strier, K. B. (1989). Adaptive radiation of the ateline primates. *J. Hum. Evol.*, 18:717–750.
- Rosenberger, A.L., Setoguchi, T., and Shigehara, N. (1990). The fossil record of callitrichine primates. *J. Human Evol.* 19: 209–236.
- Saitou, N., and Nei, M. (1987). The Neighbor-joining method; a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406–425.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Simons, E.L. *Primate evolution: an introduction to man's place in nature*. Macmillan, New York, 1972.
- Simpson, G. G. (1945). The principles of classification and classification of mammals. *Amer. Mus. Nat. Hist.* 85: 1–350.
- Schneider, H., Schneider, M.P.C., Sampaio, M.I.C.; Harada, M.L., Stanhope, M. and M. Goodman. (1993). Molecular phylogeny of the New World monkeys (Platyrrhini, Primates). *Molecular Phylogenetic and Evolution*, 2: 225–242.
- Schneider, M.P.C., Schneider, H., Sampaio, M.I.C., Carvalho-Filho, N.M., Encarnación, F., Montoya, E. and Salzano, F.M. 1995 Biochemical Diversity and Genetic Distances in the Pitheciinae Subfamily (Primates, Platyrrhini). *Primates* 36:129–134.
- Seuanez, H.N., Forman, L., and Alves, G. (1988). Comparative chromosome morphology in three callitrichid genera: *Cebuella*, *Callithrix*, and *Leontopithecus*. *J. Hered.* 79: 418–424.
- Seuanez, H. N., Forman, L., Matayoshi, T., Fanning, T. G. (1989). The *Callimico goeldii* (Primates, Platyrrhini) genome: karyology and middle repetitive (LINE-1) DNA sequences. *Chromosoma*, 98:389–395.

- Snowden, C.T. (1993) A vocal taxonomy of callitrichids. In *Marmosets and Tamarins. Systematics, Behaviour, and Ecology*. (A.B. Rylands, ed.), pp. 78–94, Oxford University Press, Oxford.
- Takai, M. (1994) New specimens of *Neosaimia fieldsi* from La Venta, Colombia: a middle Miocene ancestor of living squirrel monkeys. *J. Hum. Evol.* 27: 329–360.
- Zingales, M.R. (1973). Dentition of *Brachyteles arachnoides* with reference to alouattine and ateline affinities. *Folia Primatol.* 20: 351–390.