Neotropical primate evolution and phylogenetic reconstruction using chromosomal data

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Abstract

Platyrrhini are a group of Neotropical primates living in central and south America, and have been extensively studied through morphological and molecular data in order to shed light on their phylogeny and evolution. Agreement on the main clades of Neotropical primates has been reached using different approaches, but many phylogenetic nodes remain under discussion. Contrasting hypotheses have been proposed, presumably due to different markers and the presence of polymorphisms in the features considered; furthermore, neither Neotropical primate biodiversity nor their taxonomy are entirely known. In our perspective, a cytogenetic approach can help by making an important contribution to the evaluation of the phylogenetic relationships among Platyrrhini. In this work, molecular cytogenetic data regarding the principal nodes of the Neotropical monkey tree have been reviewed; classical cytogenetic data have also been considered, especially when other data have proven elusive, permitting us to discuss highly derived karyotypes characterized by a wide range of diploid numbers of chromosomes and variable chromosomal evolution with different rearrangement and polymorphism rates.

Keywords: Neotropical monkeys, molecular cytogenetics, painting, human associations, genome

Introduction

Platyrrhini are small anthropoids of the superfamily Ceboidea, grouped into three families and many genera with about 120 living species, which inhabit the trees of tropical forests, ranging from Central to South America.

Molecular analysis has revealed a unique phylogenetic arrangement of Platyrrhini, with three monophyletic families: Cebidae, Atelidae and Pitheciidae (Schneider et al. 1993, 1996, 2001; Von Dornum & Ruvolo 1999; Ray et al. 2005; Opazo et al. 2006; Osterholz et al. 2009; Wildman et al. 2009; Perelman et al. 2011; Kiesling et al. 2015); however, the relationships among the three major clades remain under discussion. Indeed, molecular data have permitted researchers to hypothesize Cebidae as a sister clade of Atelidae and Pitheciidae (Figure 1(a); Schneider et al. 1996, 2001; Canavez et al. 1999b; Opazo et al. 2006) or, alternatively, Pitheciidae as a sister clade of the two remaining families (Figure 1(b); Ray et al. 2005; Osterholz et al. 2009; Wildman et al. 2009; Perelman et al. 2011; Kiesling et al. 2015).

In the following discussions of each family (Cebidae, Atelidae and Pitheciidae), their principal features as well as phylogenetic relationships, according to recent molecular data, are reported. Indeed, Platyrrhini have also been studied in depth at the intra-family level, analyzing intergenic and intragenus relationships. The principal works on Platyrrhini phylogeny, using different molecular markers for their analyses, at various levels are reported below (Table I). The Cebidae family includes many genera and is marked by a close relationship between Cebus (capuchin...
monkeys) and Saimiri (squirrel monkeys) (Schneider et al. 2001; Perelman et al. 2011) which share many characteristics at the morphological level that indicate a common origin, such as a high ratio of brain/body weight, pronounced sexual dimorphism, and the ability to gradually change their diet which is based mainly on fruits and insects. However, they also have some differences; for example, capuchin monkeys (Cebus and Sapajus) have a semi-prehensile tail which is unable to sustain weight, whereas Saimiri are born with prehensile tails but lose grasping ability as they age.

Among Cebidae it is also possible to distinguish Callithrix (Atlantic marmosets), Cebuella (marmosets), Saguinus (tamarins), and Leontopithecus (lion tamarins); the latter, also known as callitrichids, are characterized, with some exceptions, by small body size, claw-like nails on all digits but the hallux, two molars instead of three, and dietary exploitation of plant exudates (gums and saps). In addition, there is a peculiarity in marmosets in that they are known to give birth to twins. Another Cebidae genus is the monotypic Callimico (Goeldi’s monkeys), with the species C. goeldii characterized by small body size and claw-like nails like marmosets, as well as by other features more typical of larger bodied Platyrrhini, such as a third molar and single births. For this reason, its phylogenetic position has been highly debated, although it is now considered to be resolved (Canavez et al. 1999b; Schneider et al. 2001). Among callitrichids, indeed, molecular phylogenetic reconstructions present Saguinus as the sister taxa of Leontopithecus and Callimico as well as the Callithrix and Cebuella clades (Schneider et al. 2001; Perelman et al. 2011).

Figure 1. Phylogenetic trees reconstructed on the basis of different molecular markers: (a) with Cebidae as sister group to the Atelidae and Pitheciidae clade (Schneider et al. 1996, 2001; Canavez et al. 1999a; Opazo et al. 2006); (b) with Pitheciidae as sister clade to that of Atelidae and Cebidae (Ray et al. 2005; Osterholz et al. 2009; Wildman et al. 2009; Perelman et al. 2011; Kiesling et al. 2015).
Table I. List of platyrrhine phylogenetic studies based on molecular markers and references; letters C, A and P stand for Cebidae, Atelidae and Pitheciidae, respectively; (*) see article for species identification.

| Markers | Species analyzed | References |
|---------|------------------|------------|
| LINE-1 patterns | **C**: Callimico goeldii, Callithrix jacchus, Cebuella pygmaea, Leontopithecus rosalia, Saguinus labiatus, Sapajus apella | Seuánez et al. (1988) |
| Molecular sequences combined in tandem, ε globin and IRBP | **C**: Aotus azarae, Callimico goeldii, Callithrix jacchus, Cebuella pygmaea, Leontopithecus rosalia, Saguinus midas, Saimiri sciureus | Schneider et al. (1993) |
| | **A**: Alouatta belzebul, A. seniculus, Ateles belzebuth, A. geoffroyi, Brachyteles arachnoides, Lagothrix lagotricha | Schneider et al. (1996); Schneider et al. (2001) (*) |
| | **P**: Cacajao calvus, C. moloch, Callimico torquatus, Chiropotes satanas, Pithecia irrorata | Schneider et al. (1999) |
| Analysis of cytochrome b | **C**: Callithrix jacchus jacchus, Leontopithecus rosalia chrysomelas, L. rosalia chrysopygus, L. rosalia rosalia, Saguinus oedipus, Sapajus apella | Moreira and Seuánez (1999) |
| | **A**: - | - |
| | **P**: - | - |
| ε globin and IRBP | **C**: Callithrix goffroyi, C. jacchus, C. manesi, Cebuella pygmaea, Mico argentatus | Porter et al. (1997) |
| DNA sequences of mitochondrial genes | **C**: Callithrix argentata, C. aurita, C. goffroyi, C. humeralifer, C. jacchus, C. kuhlii, C. manesi, C. penicillata, Cebuella pygmaea, Leontopithecus chrysomelas | Tagliaro et al. (1997) |
| Mitochondrial DNA | **C**: Callimico goeldii, Callithrix jacchus, Cebuella pygmaea, Leontopithecus chrysomelas, L. rosalia, Saguinus midas, Sapajus apella | Pastorini et al. (1998) |
| | **A**: Ateles geoffroyi | - |
| | **P**: - | - |
| Genes 12S + 16S | **C**: Aotus trivirgatus, Callimico goeldii, Callithrix jacchus, Cebuella pygmaea, Leontopithecus rosalia, Saguinus goffroyi, S. oedipus, Saimiri sciureus, Sapajus apella | Horovitz et al. (1998) |
| | **A**: Ateles palliata, A. seniculus, Ateles sp., Brachyteles arachnoides, Lagothrix lagotricha | - |
| | **P**: Callimico moloch, Chiropotes satanas, Pithecia pithecia | Canavez et al. (1999b) |
| Nuclear sequences | **C**: Aotus azarae, Callimico goeldii, Callithrix humeralfera, C. kuhlii, C. penicillata, Cebuella pygmaea, Cebus olivaceus, Leontopithecus chrysopygus, Mico emiliae, Saguinus midas, Saimiri boliviensis, S. sciureus, S. ustus | - |
| | **A**: Ateles seniculus, Ateles paniscus, Brachyteles arachnoides, Lagothrix lagotricha | - |
| | **P**: Cacajao melanochephaeus, Callimico hoffmannii, C. moloch, C. personatus nigrofons, C. personatus personatus, C. torquatus, Chiropotes satanas | - |

(Continued)
Table I. (Continued).

| Markers | Species analyzed | References |
|---------|------------------|------------|
| **Genetic loci** | | |
| C: | Ateles paniscus chamek | Seuánez et al. (2001) |
| P: | - |  |
| **SRY (The region of the Y chromosome in sex determination)** | | |
| C: | Aotus azarae, Callimico goeldii, Callithrix jacchus, C. geoffroyi, C. capucinus, C. nigrivittatus, Leontopithecus chrysomelas, L. chrysopygus, L. rosalia, Saguinus midas midas, Sapajus apella apella, S. a. xanthosternos | Moreira (2002) |
| A: | - |  |
| **Alu elements** | | |
| G: | Aotus azarai, Callimico goeldii, Cebidae pygmaea, Leontopithecus chrysomelas, L. chrysopygus, L. rosalia, Saguinus bicolor, S. fuscicollis, S. f. lagotricha, S. labiatus, S. midas, S. oedipus, Saimiri sciureus, Sapajus apella | Singer et al. (2003) |
| A: | Alouatta belzebul, Ateles fusciceps, Lagothrix lagotricha |  |
| P: | Cacajao calus, Calliebus cupreus, Chiroptes satanas albinaurus, Pithecia pithecia |  |
| **Sequences from two non-coding regions of nuclear genes** | | |
| C: | Saimiri sciureus/Cebus | Lima et al. (2007) |
| A: | Alouatta belzebul, Ateles geoffroyi, Ateles sp., Brachytes arachnoides, Lagothrix lagotricha |  |
| **SINE** | | |
| C: | Aotus azarae, Callimico goeldii, Cebidae pygmaea, Leontopithecus chrysomelas, Saguinus imperator, Saimiri sciureus, Sapajus apella | Osterholz et al. (2009) |
| A: | Alouatta belzebul, Ateles fusciceps, Lagothrix lagotricha |  |
| **Nuclear DNA markers derived from a random genomic shotgun library** | | |
| C: | Aotus lemurinus, A. azarae, Callimico goeldii, Callithrix penicillata, Leontopithecus chrysomelas, Saguinus midas, Saimiri sciureus, Sapajus apella | Wildman et al. (2009) |
| A: | Alouatta belzebul, Ateles belzebul, Brachytes arachnoides, Lagothrix lagotricha |  |
| P: | Cacajao calus, Calliebus personatus, C. torquatus, Chiroptes satanas, Pithecia irrorata |  |
| **Large-scale concatenated data set of multiple nuclear/mitochondrial regions** | | |
| C: | Aotus azarae boliviensis, A. azarae infundatus, A. azarae, A. nancymae, A. lemurinus griseimembra, A. trivirgatus, Callimico goeldii, Callithrix jacchus, C. penicillata, C. geoffroyi, C. kuhlii, C. aurita, Cebidae pygmaea, Cebus robustus, C. xanthosternos, C. capucinus, C. albifrons, C. olivaceus, Leontopithecus chrysomelas, L. rosalia, Mico argentata, M. humeralifer, Saguinus bicolor, S. martini, S. midas, S. geoffroyi, S. oedipus, S. labiatus, S. imperator, S. fuscicollis, Saimiri boliviensis boliviensis, S. oestarii oestarii, S. sciureus, S. ustus, Sapajus apella | Perelman et al. (2011) |
| A: | Alouatta belzebul, A. caraya, A. sara, A. palliata, Ateles belzebul, A. geoffroyi, A. hybridus, A. fusciceps, A. chamek, A. paniscus, Brachyteles arachnoides, B. hypocestus, Lagothrix cana, L. lagotricha |  |
| **P: Cacajao melanochephalus, C. calus, Calliebus brunnneus, C. moloch, C. cupreus, C. caligatus, C. donacophilus, C. coinbrati, C. personatus, C. nigrifrons, Chiroptes israelita, C. satanas chiroptes, Pithecia irrorata, P. pithecia** |  |

(Continued)
Table I. (Continued).

| Markers | Species analyzed | References |
|---------|------------------|------------|
| C: Callimico goeldii, Callithrix aurita, C. geoffroyi, C. jacchus, C. kuhlii, C. penicillata, Cebuella pygmaea, Leontopithecus chrysomelas, Mico argentatus, M. humeralifer, M. mauesi, M. humilis, M. saterei | Schneider et al. (2012) | |
| P: - | | |
| C: Callimico goeldii, Callithrix aurita, C. geoffroyi, C. jacchus, C. penicillata, Cebuella pygmaea, Leonopithecus catusara, L. chrysomelas, L. chryopygus, L. rosalia, Mico argentatus, M. emiliae, M. humeralifer, M. mauesi, M. saterei, Saginus nigricollis nigricollis, S. nigricollis graellisi, S.fuscicolli illigeri, S. tripartitus, S.fuscicolli lagomontis, S. weddeli weddeli, S. weddeli melanoleucus, S.fuscicolli nigrifrons, S.fuscicolli leucogenys, S.fuscicolli fuscicolli, S. imperator, S. labiatus, S. inustus, S. mystax, S. leucopus, S. oedipus, S. geoffroyi, S. niger, S. midas, S. bicolor, S. martinsi | Buckner et al. (2014) | |
| A: - | | |
| P: - | | |
| C: Aotus azarae, A. lemurinus, A. nancymaeae, Callithrix flaviceps, C. geoffroyi, C. jacchus, C. kuhlii, C. penicillata, Callimico goeldii, Cebus albifrons, C. nigritus, C. olivaceus, C. santrosterno, Leonopithecus chrysomelas, Mico argentatus, Saginus fuscicolli, S. imperator, S. martinsi, S. niger, Saimiri sciureus, S. ustus, Sapajus apella | Kiesling et al. (2015) | |
| A: Ateles belzebul, A. palliata, Ateles belzebul, A. geoffroyi, A. paniscus, Brachyteles arachnoideus, Lagotheres lagotricha | | |
| P: Cacajao calvus, Callithriches donacophilus, C. nigricollis, Chiropotes uahuicki, Pithecia irrorata | | |

Moreover, Cebidae includes the genus *Aotus* (owl/night monkeys), the only representative platyrhine species having nocturnal habits, characterized by monogamous social organization in small groups. *Aotus* is primarily frugivorous, but these primates also consume leaves and insects. The phylogenetic position of *Aotus* has been highly debated and still remains unclear; it is supposed to be a sister clade to either *Cebus*/Saimiri (Opazo et al. 2006; Wildman et al. 2009) or callitrichine (Perelman et al. 2011; Kiesling et al. 2015).

Moreover, other research has led to conflicting phylogenetic reconstructions on the basis of the markers analyzed (Perez et al. 2012).

Three additional Cebidae genera have also been proposed and supported by molecular-level factors: *Mico* (from Callithrix) (Lynch Alfaro et al. 2012a,b), *Leonopithecus* (from Saginus), (Buckner et al. 2014; Sampaio et al. 2015) and *Sapajus* (from *Cebus*) (Schneider et al. 2012; Schneider & Sampaio 2015).

Atelidae includes four genera: *Alouatta* (howler monkeys), its sister clade *Ateles* (spider monkeys), and the *Brachyteles* (muriquis)/Lagotheres (woolly monkeys) clade (Schneider et al. 1996; Lima et al. 2007). These monkeys are characterized by large body size, frugivorous–folivorous diet, and a muscular prehensile tail used to support their weight.

Pithecidae includes four genera: the highly specialized seed predators *Pithecia* (saki monkeys), *Chiropotes* (bearded sakis), and Cacajao (ukarisis) which have the *Callicebus* genus (titi monkeys) as their sister taxon (Canavez et al. 1999b; Schneider et al. 2001; Opazo et al. 2006; Wildman et al. 2009; Perelman et al. 2011).

**Comparative cytogenetics and phylogenetic reconstructions**

Classic cytogenetic studies using banding analysis allowed researchers to demonstrate that primate chromosomes have been conserved during evolution (Dutrillaux 1979, 1988; Dutrillaux & Couturier 1981; Dutrillaux et al. 1986). Since the 1990s (Wienberg et al. 1990), the karyotypes of different primate species have also been compared at the molecular level, applying fluorescent in situ hybridization (FISH) with human chromosome probes. This molecular cytogenetic approach is known as “chromosome painting” and consists of the hybridization of the human
DNA probes of a whole chromosome (labeled with a fluorescent substance) with the DNA of a target species, taking advantage of their complementary nature. Chromosome painting allows researchers to determine chromosomal homologies at the level of whole or partial chromosomes, as well as interchromosomal rearrangements (translocations, fissions and fusions) that have occurred during evolution; chromosomal painting permits the determination of which chromosomes, or chromosomal syntenies (the localization of two or more genes on the same chromosome), have been conserved or reshaped, identifying syntenic associations in the genomes of the species being compared.

Subsequently, it has been possible to hybridize not only human probes but even other animal probes (zoo-FISH) made through flow sorting. Human and other primate probes have been reciprocally hybridized through chromosome painting, permitting the detection of real homologies in two reciprocal experiments and rearrangement breakpoints (Stanyon et al. 2001; Dumas et al. 2007). In recent years, sub-regional or locus-specific probes, produced by microdissection or by cloning DNA within vectors, have also been used for FISH. These probes have demonstrated a high resolving power, identifying intrachromosomal rearrangements and breakpoints that are not detectable through painting (Stanyon et al. 2008).

Chromosomal data obtained through comparative cytogenetics have been used for phylogenetic reconstructions using the cladistic approach and the principle of parsimony. The first step in this field is making the distinction between homology due to shared ancestry and homoplasy due to parallel or convergent evolution; thus, among homologies, it is necessary to distinguish ancestral chromosomal syntenies (synapomorphies) from new shared syntenic associations (symplesiomorphies) formed as a consequence of chromosomal rearrangements (Wienberg & Stanyon 1995; Rokas & Holland 2000). Since rearrangements are rare events in mammals (two for every 10 million years, Froenicke 2005; Murphy et al. 2005), the common derivative syntenic associations between two species are useful for phylogenetic reconstructions. Through this analysis, in a comparative perspective, it has been possible to reconstruct the hypothetical ancestral karyotype of all primates and of the main nodes of the primate evolutionary tree (Stanyon et al. 2008). To distinguish conserved from derived characteristics, a comparison with an outgroup – a closely related species that is considered external to the group under examination – is used. According to the principle of parsimony, the interpretation that involves the least likely number of steps is preferred among the various possible interpretations of a phenomenon (chromosomal organization). Another aspect to take into account in phylogenetic reconstructions, and which complicates the analysis, is the distinction of hemiplasy due to the phylogenetic sorting of a genetic polymorphism (Avise & Robinson 2008; Robinson et al. 2008).

**Ancestral platyrrhini karyotype**

The pioneering studies on the chromosomes of Neotropical primates through classical cytogenetic analysis started in the 1970s (Dutrillaux 1979, 1988; Dutrillaux & Couturier 1981; Dutrillaux et al. 1986), while the molecular cytogenetic approach was first applied in the 1990s to Callithrix jacchus (Sherlock et al. 1996), Cebus capucinus (Richard et al. 1996), Alouatta seniculus arctoides, A. sara (Consiglieri et al. 1996) and Ateles paniscus chamek (Canavez et al. 1998, 1999a). These works allowed scientists to verify the chromosomal homologies proposed in the literature, based on comparative banding analysis (Stanyon et al. 1995). Furthermore, chromosomal painting studies applied to many primates permitted researchers to reconstruct the putative ancestral karyotype of New World monkeys characterized by diploid number 2n = 54, with the following syntenies or human syntenic associations: 1a, 1b, 1c, 2a, 2b/16b, 3a, 3b, 3a/21, 4, 5/7a, 6, 7b, 8b, 8a/18, 9, 10p 10a/16a, 11, 12, 13, 14/15a, 15b, 17, 19, 20, 22, X and Y (Neussler et al. 2001; Stanyon et al. 2008). This karyotype derives from the hypothetical one of all primates (2n = 50, with chromosomes 1, 2a, 2b, 3/21, 4, 5, 6, 7a, 7b/16b, 8, 9, 10a, 10b, 11, 12a/22a, 12b/22b, 13, 14/15, 16a, 17, 18, 19a, 19b, 20, X and Y) via six fissions in four chromosomes (1, 3/21, 8 and 14/15) and by four fusions which form syntenic associations (2b/16b, 5/7a, 8a/18 and 10a/16a). Some ancestral primate associations are not present in New World monkeys due to previous rearrangements which occurred in the anthropoid branch – in particular, a fission of 7b/16b, a reciprocal translocation producing chromosome 12, and a fusion giving the whole chromosome 19.
Discussion

The aim of this work is to review the molecular cytogenetic data available in the literature for any major lineages of the platyrrhine tree (Table II) while also considering useful classical cytogenetic data. We report human associations and evolutionary rearrangements characterizing the principal nodes of the Neotropical primate tree. The tree adopted is the one proposed by Perelman et al. (2011), in terms of the families recognized, but with some modifications made in order to take into account chromosomal data at the inter- and intrageneric levels. Furthermore, we also point out principal cytogenetic data that are in conflict with molecular data. In particular, the New World monkey data gathered are discussed below, for each family (Cebidae, Atelidae and Pithecidae) and for each genus within it, following the tree reported in Figure 2(a–c). This phylogenetic tree was drawn using Mesquite, a software program for evolutionary biology designed to help biologists organize and analyze comparative data. A previous review of the same topic (De Oliveira et al. 2012) has been published, explaining conflicting features through traditional interpretative hypotheses, taking into account the distinction between homologies and homoplasy. In addition to this, we explain the possible evolutionary scenarios, considering hemiplasy in addition to homology and homoplasy. Some discordance in evolutionary interpretation can occur when a tree constructed through chromosomal data is not in accordance with a species tree due to the phylogenetic sorting of a genetic polymorphism; this kind of evolutionary event is termed hemiplasy.

Cebidae family

Cytogenetic analysis of the data found in the literature shows low variability within and between species, in both the number and structure of chromosomes in the Cebidae family (Dutrillaux & Couturier 1981; Seuánez et al. 1988; Nagamachi et al. 1997a,b, 1999), showing highly conservative genomes with diploid numbers of chromosomes ranging between 44 and 54. This has led researchers to assume that the adaptive radiation of Cebidae was characterized by a limited number of chromosomal rearrangements.

Chromosomal painting data enabled us to show that all of the syntenies in the putative ancestral platyrrhine karyotype (i.e., 3a/21, 5/7a, 2b/16b, 8a/18, 14/15a, and 10a/16a) were conserved in Cebus, and Mico. C. capucinus, C. albifrons and even Sapajus apella, previously known as C. apella (Richard et al. 1996; Garcia et al. 2002; Amaral et al. 2008), share a pericentric inversion of a submetacentric chromosome formed by 14/15a association, resulting in the form 14/15a/14. In particular, C. capucinus presents the most conserved karyotype among all Platyrrhini (Richard et al. 1996; Garcia et al. 2002; Amaral et al. 2008), while the other gracile/un-tufted Cebus and the robust-tufted Sapajus species are more derived (Figure 2(a)). The karyotype of C. albifrons differs from that of C. capucinus by another pericentric inversion in the 14/15a human association which results in a metacentric chromosome with 15a/14/15a/14 in tandem, and by a fusion followed by a pericentric inversion involving the homologous-to-human chromosomes 15b and 8b (8/15/8) (Amaral et al. 2008). The C. olivaceus (also known as C. nigrivittatus) and S. apella subspecies group are linked by a chromosomal inversion homologous to human synteny 20; they differ through another diverse pericentric inversion in the association 14/15a/14, resulting in a metacentric chromosome, and an apomorphic robertsonian rearrangement in the chromosomes homologous to human 12 and 15b (12/15) in C. olivaceus; the interchromosomal rearrangements mentioned above (not shown in Figure 2) in C. albifrons and C. olivaceus (2n = 52) explain their different diploid number when compared with that of the other Cebus capuchin species (2n = 54) (Richard et al. 1996; Garcia et al. 2002; Amaral et al. 2008).

Chromosome painting on Saimiri sciureus shows the 2a/15b human association that could represent a link (synapomorphy) between marmosets and tamarins (Figure 2(a)), (Neusser et al. 2001; Dumas et al. 2005, 2007). Moreover, all species of the Saimiri genus possess the same diploid number of chromosomes, 2n = 44, although pericentric inversions characterize three geographically distinct karyotypes (Ma et al. 1974; Jones & Ma 1975).

Chromosomal painting data on both marmosets and tamarins (callitrichids), specifically Callithrich jacchus (Sherlock et al. 1996; Neusser et al. 2001), Cebuella pygmaea, Mico argentatus (Neusser et al. 2001), Saguinus oedipus (Neusser et al. 2001) and Leontopithecus chrysomelas (Gerbault-Serreau et al. 2004), allow researchers to show the chromosomal associations phylogenetically linking these species (13/17/20, 13/9/22, 2a/15b), later confirmed by reciprocal chromosome painting (Dumas et al. 2007) (Figure 2(a)). In particular, comparative analysis permits us to identify S. oedipus as the sister group of the remaining callitrichids, having human synteny 1a and 10b not fused (Neusser et al. 2001); the other callitrichid species are
Table II. List of platyrrhine species analyzed using a molecular cytogenetic approach and considering the principal published classical cytogenetic data. Chromosome painting studies with human probes, reciprocal chromosome painting, and multidirectional probes (New World monkey probes used are reported in parentheses) are highlighted in bold. Notes: *Homo sapiens* (HSA), *Aotus nancymaae* (ANA), *Lagothrix lagotricha* (LLA), *Saguinus oedipus* (SOE), *Callimico goeldii* (CGO), *Cebuella pygmaea* (CPY), *Mico argentata* (MAR), *Saimiri sciureus* (SSC), *Gallicebus donacophilus pallecens* (CPA).

| Cebidae                  | Chromosome painting                                      | Notes                                      |
|--------------------------|----------------------------------------------------------|--------------------------------------------|
| *Aotus nancymaae*        | 54 C, G banding and NOR staining                          | Pieczarka et al. (1992)                    |
|                          | Multidirectional chromosome painting (HSA-ANA, LLA)       | Ruiz - Herrera et al. (2004)               |
| *A. karyomorphs*         | 50 Chromosome painting                                    |                                            |
| *Aotus l. griseimembra*  | 54 Chromosome painting                                    | Stanyon et al. (2011)                      |
| *Callimico goeldii*      | 47/48 G, C banding                                       | Dutrillaux et al. (1988)                   |
|                          | Multidirectional chromosome painting (HSA, SOE, LLA)      | Neusser et al. (2001)                      |
|                          | Reciprocal chromosome painting (CGO-HSA)                 | Dumas et al. (2007)                        |
| *Callithrix jacchus*     | 46 C, T and Q banding                                     | Dutrillaux and Couturier (1991)            |
|                          | G banding and NOR staining                               | Ardito et al. (1987)                       |
|                          | G banding                                                | Seuanez et al. (1988)                      |
|                          | Chromosome painting                                      | Sherlock et al. (1996)                     |
|                          | Multidirectional chromosome painting (HSA, SOE, LLA)      | Neusser et al. (2001)                      |
| *Cebuella pygmaea*       | 44 G banding                                             | Dumas et al. (2007)                        |
|                          | Multidirectional chromosome painting (HSA, SOE)          |                                            |
|                          | Reciprocal chromosome painting (CPY-HSA)                 |                                            |
| *Cebus albifrons*        | 52 Multidirectional chromosome painting (HSA, SOE)       | Amaral et al. (2008)                       |
| *Cebus capucinus*        | 54 Q, R, C banding and NOR staining                      | Dutrillaux (1979)                         |
|                          | Q, R, C banding and NOR staining                         | Dutrillaux & Rumpler (1980)                |
|                          | G, R banding and NOR staining                            | Garcia et al. (1983)                       |
|                          | G banding and NOR staining                               | Richard et al. (1996)                      |
|                          | Chromosome painting                                      | Neusser et al. (2001)                      |
| *Cebus olivaceus*        | 52 C banding                                             | Dumas et al. (2004)                        |
| (nigrivittatus)           | G banding and chromosome painting                        | Amaral et al. (2008)                       |
|                          | G and C banding                                          |                                            |
|                          | Chromosome painting                                      |                                            |
| *Leontopithecus rosalia* | 46 G banding                                             | Nagamachi et al. (1997)                    |
| *Leontopithecus chrysomelas* | Chromosome painting                                   | Gerbault - Serreau et al. (2004)           |
| *Mico argentatus*        | 44 G, C and NOR banding                                  | Pieczarka et al. (1996)                    |
|                          | G, C banding                                             | Nagamachi et al. (1996)                    |
|                          | Multidirectional chromosome painting (HSA, SOE)          | Canavez et al. (1996)                      |
|                          | Reciprocal chromosome painting (MAR- HSA)                | Neusser et al. (2001)                      |
| *Saguinus oedipus*       | 46 G banding                                             | Dumas et al. (2007)                        |
|                          | Reciprocal Chromosome painting (HSA-SOE)                 |                                            |
|                          | Chromosome painting                                      |                                            |
| *Saimiri sciureus*       | 44 Giemsa                                                |                                             |
|                          | C, T, and Q banding                                      |                                             |
|                          | Chromosomal features                                    |                                             |
|                          | C banding and NOR staining                               |                                             |
|                          | Chromosome painting                                      |                                             |
|                          | Reciprocal chromosome painting (HSA-SSC)                 |                                             |
| *Sapajus apera*          | 54 G, C banding and NOR staining                         | Freitas & Seuanez (1982)                   |
|                          | C banding                                                |                                              |
|                          | G, R banding and NOR staining                            |                                              |
|                          | G and C banding                                          |                                              |
|                          | Q, C and G banding                                       |                                              |
|                          | Chromosome painting                                      |                                              |
| *Sapajus a. robustus*    | 54 Multidirectional chromosome painting (HSA, SOE)       | Amaral et al. (2008)                       |
| *Sapajus a. paraguayanus* | 54 Multidirectional chromosome painting (HSA, SOE)       | Amaral et al. (2008)                       |
linked by the 1a/10b association. Particularly noteworthy is *Mico argentatus*, a species of the newly recognized genus, which shows the same chromosomal syntenies with respect to *Cebuella pygmaea* (Neusser et al. 2001), but has a large amount of heterochromatin at the terminal ends of two chromosomes; the *C. jacchus* karyotype differs from that of other species by a single fission. In particular, a comparison of classical cytogenetic data on tamarins, *Saguinus* and *Leontopithecus*, shows

| Chromosomal evolution in New World monkeys |
|------------------------------------------|

Table II. (Continued).

| Atelidae |  |
|----------|---|
| **Alouatta belzebul** | 49/50 Chromosome painting |
| **Alouatta caraya** | 52 G banding C and G banding Multidirectional chromosome painting (HSA, SOE, LLA) Chromosome painting |
| **Alouatta fusca clamitans** | 50 Multidirectional chromosome painting (HSA, SOE, LLA) |
| **Alouatta fusca (guariba guariba)** | 49/50 Chromosome painting |
| **Alouatta sara** | 50 G banding Chromosome painting |
| **Alouatta seniculus arctoidea** | 50 G banding 44/45 Chromosome painting |
| **Alouatta seniculus macconnelli** | 52 Multidirectional chromosome painting (HSA, SOE, LLA) |
| **Ateles belzebuth marginatus** | 34 C, G banding and NOR staining Multidirectional chromosome painting (HSA, SOE, LLA) |
| **Ateles belzebuth hybridus** | 34 Chromosome painting |
| **Ateles geoffroyi** | 32 C, G banding and NOR staining C, G banding and NOR staining Multidirectional chromosome painting (HSA, SOE, LLA) |
| **Brachyteles arachnoides** | 62 C banding and NOR staining Multidirectional chromosome painting (HSA, SOE, LLA) |
| **Lagothrix lagotricha** | 62 C banding Banding and NOR staining G and C banding Reciprocal chromosome painting (HSA-LLA) |

| Pithecidae |  |
|-----------|---|
| **Cacajao calvus rubicundus** | 45/46 C, G banding and NOR staining and Multidirectional chromosome painting (HSA, SOE) |
| **Callirhinchus cupreus** | 46 Multidirectional chromosome painting (HSA, CPA) |
| **Callirhinchus donacophilus pallescens** | 50 Multidirectional chromosome painting (HSA, SOE) |
| **Callirhinchus lugens** | 16 G banding Chromosome painting |
| **Callirhinchus moloch** | 50 Chromosome painting |
| **Callirhinchus pallescens** | 50 Chromosome painting Reciprocal chromosomal painting (HSA-CPA) |
| **Callirhinchus personatus** | 44 C, G banding, NOR staining Chromosome painting |
| **Chiropotes israelita** | 54 G banding Chromosome painting |
| **Chiropotes satanas utahicki** | 54 G banding G banding Chromosome painting |
| **Pithecia irrorata** | 48 C, G banding and NOR staining and chromosome painting |

Consigliere et al. (1998) Mudry et al. (1990) Mudry et al. (1998) De Oliveira et al. (2002) Stanyon et al. (2011) De Oliveira et al. (2002) Stanyon et al. (2011) Stanyon et al. (1995) Consigliere et al. (1996) De Oliveira et al. (2002) Medeiros et al. (1997) Cecchi et al. (1994) Pieczarka, et al. (1989) Medeiros et al. (1997) De Oliveira et al. (2005) Viegas Pequignot et al. (1985) De Oliveira et al. (2005) Garcia et al. (1983) Clemente et al. (1987) Stanyon et al. (2001)
that they have similar karyotypes \((2n = 46)\) and differ only by para- and pericentric inversions detected on at least four acrocentric chromosomes (data not shown in Figure 2) (Nagamachi et al. 1997b; Neusser et al. 2001).

The *Callimico* genus – with one species, *Callimico goeldii*, whose phylogenetic position has been highly discussed – is characterized by a translocation involving the Y chromosome and an autosome. Consequently, males may have a diploid number of 47 or 48 chromosomes (Dutrillaux et al. 1988; Margulis et al. 1995). Chromosome painting permitted researchers to definitely demonstrate that *Callimico* is phylogenetically linked with *Callithrix* and *Cebuella* (marmosets) by sharing the same human chromosomal association 1a/10b (Neusser et al. 2001), which characterizes all callitrichids (Figure 2(a)), eliminating any previous doubts.

The taxonomy of the *Aotus* genus of owl monkeys has been debated since it was first described by the Spanish naturalist Félix de Azara in 1802, especially in terms of the number of species and subspecies recognized. Initially, just one species was recognized, *Aotus trivirgatus*; subsequently, on the basis of chromosomal characteristics and geographical distribution, nine species and four subspecies have been recognized due to the presence of sibling species (species seemingly identical from a morphological point of view but which possess divergent karyotypes). The karyotypes of these species are characterized by many polymorphisms and a variable diploid number, ranging from 46 to 59 chromosomes (Galbreath 1983; Torres et al. 1998), with differences as well between males and females in the diploid number due to a translocation between chromosome Y and an autosome (Ma et al. 1976; Pieczarka & Nagamachi 1988). It has been suggested that the karyotypes of these species originated from the ancestral platyrrhine karyotype \((2n = 54)\), passing through fissions, translocations and inversions (De Boer 1974; Ma et al. 1976; Mudry et al. 1984;
Chromosomal painting was first applied to metaphases of *Aotus nancymaeae*, showing that the karyotype of owl monkeys is highly derived. Successively, two other *Aotus* karyotypes have been reconstructed: one of a karyomorph (Ruiz-Herrera et al. 2005) as well as that of *A. lemurinus griseimembra* (Stanyon et al. 2011). These three *Aotus* samples share the following derived associations: 1/3, 1/16, 2/20, 4/15, 7/11, 10/11, 14/15 twice and 16/22, and the loss of the ancestral New World monkey associations 2b/16b, 10a/16a (Figure 2(a)). Presumably the loss of the 10a/16a association occurred by fusion with synteny 22 and a successive inversion to give 10a/22/16a. *Aotus l. griseimembra* has the least derived karyotype, while the karyomorph and *A. nancymaeae* share four derived associations (2/12, 5/15, 9/15, 10/22) indicating a sister-clade relationship between them (Stanyon et al. 2011). A peculiarity arises in the syntenic association 10/11 shown in the karyotypes of *Aotus* and *Callicebus* (Pitheciidae) (Dumas et al. 2005); this association could be either a real homology phylogenetically linking the two genera or a homoplasic result of convergent evolution, or even a hemiplasy; thus, further analyses are needed through Bacterial Artificial Chromosome mapping in order to test these possible explanations.

**Atelidae family**

Chromosomal painting data on *Lagotrichia lagotricha* (Stanyon et al. 2001), *Ateles geoffroyi* (Morescalchi et al. 1997), *A. belzebuth hybridus* (Garcia et al. 2002), *A. paniscus paniscus, Brachyteles arachnoides* (De Oliveira et al. 2005) and six *Alouatta* species (Consigliere et al. 1996, 1998; De Oliveira et al. 2002; Stanyon et al. 2011) compared with the ancestral platyrrhine associations (3a/21, 5/7a, 10a/16a, 8a/18, 2b/16b, 14/15a) show that these are conserved in Atelidae. In addition, Atelidae exclusively share derived fissions of human chromosome homologs 1, 4, 5 and 15a that resulted in chromosome forms 1a1, 1a2, 4a, the association of 4b/15a2, 4c, the inversion 7a/5a/7a and 5b, 15a1/14, indicating the monophyletic origin of this group (Figure 2(b)). Thus, painting data allowed researchers to propose the hypothetical ancestral Atelidae karyotype (2n = 62), almost identical to those of *Lagotrichia lagotricha* and *Brachyteles arachnoides* (Stanyon et al. 2001; De Oliveira et al. 2005); the two species, having the same karyotypes, are considered sister clades, differing by just five intrachromosomal rearrangements detected by G banding (data not shown in Figure 2) (Stanyon et al. 2001; De Oliveira et al. 2005).

While painting data support the monophyly of Atelidae, they do not help in resolving the branching genera sequence. In support of the previous approach, classical cytogenetic data analysis on Atelidae permitted researchers to identify a derived inversion involving the 10a/16a association linking *Alouatta, Brachyteles* and *Lagotrichia*, resulting in 16a/10a/16a/10a, which was presumably lost in *Ateles* which instead presents the association 16a/10a/16a; moreover, researchers have formulated an evolutionary tree with four branches in the following order, *Alouatta, Brachyteles, Lagotrichia*, and *Ateles*, due to two inversions of human synteny 8b linking *Brachyteles, Lagotrichia* and *Ateles*, and of human synteny 13 linking *Lagotrichia* and *Ateles* (De Oliveira et al. 2005). These inversions need to be further tested with BAC probes to check whether they share the same breakpoints and can be considered real homologies.

Neither the taxonomy nor the phylogenetic relationships within the genus *Alouatta* are clear, and there is no agreement among researchers; indeed, from nine to 19 species have been recognized. The classical cytogenetic studies allowed researchers to highlight in this genus a large variation in the diploid number, from 2n = 44 to 2n = 58, and two unusual features: a system of multiple sex chromosomes involving a translocation between Y chromosomes and an autosome (Ma et al. 1975; Armada et al. 1987; Mudry et al. 1998, 2001; Steinberg et al. 2008), and the presence of various microchromosomes (Lima & Seuánez 1991), probably composed of repetitive DNA. In the *Alouatta* genus, *Alouatta sara* and *A. seniculus arctoidea* were the first New World monkey species to be analyzed through chromosome painting (Consigliere et al. 1996). They are characterized by high chromosomal variability; indeed, the chromosomal rearrangements responsible for the differences between the karyotypes of these two species are two robertsonian translocations, five tandem translocations and five intrachromosomal rearrangements. Later, human chromosomal probes hybridized on metaphases of *Alouatta belzebul* (Consigliere et al. 1996) permitted researchers to show a less rearranged karyotype than the species mentioned above. Chromosome painting has been performed on more species: *A. fusca* (guariba) (De Oliveira et al. 2002; Stanyon et al. 2011), *A. caraya, A. seniculus macconnelli* (De Oliveira et al. 2002) and *A. g. clamtians* (Stanyon et al. 2011). Through these works, it has been shown that the *Alouatta* monophyletic group is linked by a Y-autosomal translocation (Y/15b) as well as by the loss of the ancestral association 2b/16b and the presence of the association 3c/15b (except in *A.
belzebuth). Through these studies, it has also been possible to distinguish two species subgroups, one formed by A. caraya and A. belzebuth linked by human associations 2a/20, 5b/7a/5a/7a and 4c/16, while A. seniculus arctoida, A. s. macconnelli, A. sara and A. g. guariba are linked by the 2a/4b/15a2 association and the fission resulting in the double 10a/16a associations (Consigliere et al. 1996, 1998; De Oliveira et al. 2002; Stanyon et al. 2011). Moreover, A. seniculus arctoida, A. s. macconnelli and A. sara are linked by the associations 20/1c, 8b/7a/5a/7a, while A. guariba guariba and A. g. clamitans are linked by the syntenic associations 1/14, 6/15, 7/15, 10/22 and 17/18 (De Oliveira et al. 2002) (Figure 2(b)).

The diploid number of Ateles species varies from 32 to 34 chromosomes (Piecarka et al. 1989; Morescalchi et al. 1997). The karyotypes of Ateles geoffroyi (Morescalchi et al. 1997) and Ateles belzebuth hybrida studied by chromosome painting (Garcia et al. 2002) differ only by inversions. Chromosome painting was also applied to A. paniscus paniscus and A. belzebuth marginatus, allowing researchers to reconstruct the hypothetical karyotype of all atelids, characterized by a highly reshuffled genome with human associations 9/18/8a/16a/10a/16a, 12/15a2/14/1a1/4b/15a2, 22/15b/3b/2a, 3c/7b/1a2, 5b/8b, 16b/2b/1b, 10b/2a, 1a/6/1c, 4c/7a/5a/7a, 19/20 and 21/3a/6 (Figure 2(b)). Moreover, on the basis of classical cytogenetic analysis, a tree with the following branches has been proposed: A. belzebuth marginatus, A. paniscus paniscus, A. belzebuth hybrida and A. geoffroyi, with the last three species linked by the inversion of the 1a1/6/1c/6/1c association present in the ancestral atelid karyotype; A. belzebuth hybrida and A. geoffroyi are further linked by an inversion of the ancestral 16b/2b/16b/2b/1b association (De Oliveira et al. 2005).

**Pitheciidae family**

The first comparative cytogenetic studies on representatives of the pitheciid families, including Pithecia irrorata, Chiropotes satanas chiropterus, Chiropotes satanas utahicki and Cacajao calvus rubicundus, showed their monophony (Moura-Pensin et al. 2001).

Human chromosome probes hybridized to Chiropotes utahicki and C. israelita gave the same pattern of hybridization, and comparison with the ancestral hypothetical platyrhine karyotype indicates the Chiropotes karyotype is very conserved (Stanyon et al. 2004). Classical and molecular cytogenetics have also been applied to Pithecia irrorata (2n = 48) and Cacajao calvus rubicundus (2n = 45 in males, 2n = 46 in females) using human and Saguinus oedipus whole chromosome probes (Finotelo et al. 2010). These analyses indicate that the chromosomal differences found among these three taxa are consequences of centric fusions and fissions, pericentric and paracentric inversions, tandem fusions and a Y-autosome translocation; furthermore, these three species are linked by the 2a/10b human association, and Chiropotes and Cacajao are linked by a fission of the ancestral New World association 5/7a (giving association 5a/7a and synteny 5b) and by a fusion leading to the association of human synteny 20/15a/14 (Figure 2(c)). It should be noted that the 5/7a fission in pithecide species and in Atelidae is a homoplasys since it has already been shown that they have different breakpoints (De Oliveira et al. 2005; Finotelo et al. 2010). In addition, an inversion of the human association 10a/16a has been found in Chiropotes, Pithecia and Cacajao through G banding data analysis; this result apparently is a synapomorphic feature linking all pithecids (including Calliebus) (Finotelo et al. 2010), so this association is worthy of further investigation in order to test whether the same breakpoints are shared by the different species.

For *Calliebus*, from 28 to 32 species have been recognized (Van Roosmalen et al. 2002; Van Roosmalen & Van Roosmalen 2013), with chromosomal diploid numbers ranging from 16 chromosomes in *C. lugens* (Bonvicino et al. 2003) to 50 chromosomes in *C. donacophilus pallescens*, *C. pallescens* and *C. hoffmannsi* (De Boer 1974; Minezawa & Borda 1984; Stanyon et al. 2000; Rodrigues et al. 2001). *Calliebus lugens* is the species with the most derived karyotype and lowest diploid number of chromosomes found among all primates. Chromosome painting has been applied to six *Calliebus* species – *Calliebus moloch*, *C. lugens*, *C. cupreus*, *C. pallescens*, *C. d. pallescens* and *C. personatus* (Stanyon et al. 2000, 2003; Barros et al. 2003; Dumas et al. 2005; Rodrigues et al. 2011) – showing that fusions are the predominant rearrangements responsible for the karyotype evolution of these species; moreover, it has been shown that three new human associations, 7/15, 10/11 and 22/2, and two inversions involving the 2/22 (22/2/22) and 16/2 human associations (16/2/16/2), characterize the hypothetical ancestral *Calliebus* karyotype (Figure 2(c)). Apart from these ancestral associations shared by all of the species analyzed, further comparison permits the identification of other specific associations and arrangements, such as: 12/19 linking all species but *C. lugens*; 13/17 present in *C. cupreus*, *C. pallescens*, and *C. d. pallescens*; 17/20 association present in all species except *C. d. pallescens*, *C. personatus* and *C. moloch*; 9/7/5a shared by *C. cupreus* and *C. d. pallescens* (not reported in Figure 2). These
analyses also permitted the demonstration that *C. pallescens* is a different taxon if compared with *C. d. pallescens*, and therefore it is possible to assume that they are two different species (Dumas et al. 2005). The data discussed highlight very highly rearranged karyotypes among *Callicebus* species; however, from the data gathered so far, it has not been possible to find any human syntenic associations, apart from the above-mentioned inversion of human association 10a/16a, linking *Callicebus* to other pithecids. On the other hand, the 13/17 association found in *Callicebus* needs further investigation since a similar association has been found in callitrichids (Cebidae), although, as unpublished data suggest, this could be the result of convergent evolution (De Oliveira et al. 2012). Moreover, as mentioned previously, the 10/11 associations found in *Callicebus* could be a cytogenetic link with *Aotus* (Cebidae), so they must be better analyzed in order to test breakpoints and real homology.

**Conclusion**

The taxonomic and phylogenetic relationships of Platyrhini have been difficult to reconstruct on the basis of morphological characteristics because of problems in distinguishing homology from convergence. On the other hand, at the molecular level, difficulties in finding accurate relationships within and among taxa have been probably due to a rapid separation of lineages during radiation and the low number of nucleotide differences between species. Indeed, even if phylogenetic reconstructions agree regarding the identification of three main branches – Pithecidae, Atelidae, Cebidae – their relationships are still debated, as are some unresolved nodes at inter- and intrageneric levels as well. In this perspective, classical and molecular cytogenetics are useful tools to help in the phylogenetic reconstruction of New World monkeys.

In this work, we review the molecular cytogenetic data available in the literature for principal nodes of the platyrrhine tree, also considering informative chromosomal banding patterns; this analysis has permitted us to report the main objectives reached so far through this approach and to discuss divergent data in respect to recent molecular claims. The principal issues are listed below:

1. Classical and molecular cytogenetics have shown that Neotropical primates are karyologically significantly variable and derivative with respect to the average of primates (one rearrangement for every 10 million years), but with clear differences between families.

2. Comparative cytogenetics indicates that the biodiversity of this group of species is not entirely known, for example as has been demonstrated with the description of diverse species of owl monkeys (*Aotus*), howler monkeys (*Alouatta*) and titi monkeys (*Callicebus*). This situation may occur because New World monkeys often present a condition of “sibling species”.

3. Chromosome painting has permitted researchers to show the monophyly of New World primates since all share the syntenic associations 8a/18, 10a/16a, 2b/16b, 5/7a (Neusser et al. 2001) characterizing the hypothetical platyrrhine ancestral karyotype (2n = 54); these results are also supported through reciprocal chromosome painting applied to *Lagotricha lagostricha* (Stanyon et al. 2001), *Saguinus oedipus* (Müller et al. 2001), *Aotus nancymae* (Stanyon et al. 2004), *Callicebus pallescens* (Dumas et al. 2005), *Mico argentatus* (*Callithrix argentata*), *Cebuella pygmaea*, *Callimico goeldii* and *Saimiri sciureus* (Dumas et al. 2007).

4. The *Cebus* (Cebidae) karyotype is the most similar to that of the hypothetical ancestral platyrrhine, but *Chiropotes* (Pithecidae) also shows a conserved karyotype. On the other hand, among Atelidae highly derived karyotypes have been found, especially in *Ateles* and *Alouatta*. Moreover, *Callicebus* species (Pithecidae) also show highly derived karyotypes.

5. Tamarins and marmosets (callitrichids, Cebidae) constitute a monophyletic group sharing the following derived chromosomal associations: 13/17/20, 13/19/22 and 2a/15b. The 2/15b human syntenic association of marmosets and tamarins has also been found in *Saimiri sciureus*, indicating a possible link between them; since molecular data instead link *Saimiri* to *Cebus*, BAC mapping is required to test whether this 2a/15b association may represent a real synapomorphy, or homoplasy (the result of a convergence event).

6. Chromosome painting has resolved the debate on the phylogenetic placement of *Callimico goeldii* (Cebidae). The presence of the human association 1a/10b phylogenetically links *Callimico* to marmosets (Neusser et al. 2001), in agreement with molecular data.

7. Even if the position of *Callicebus* among Pithecidae is supported at the molecular level, only an inversion of the 16a/10a ancestral platyrrhine association permits the inference of a cytogenetic link with other
Pithecids, while on the other hand the syntenic associations 13/17 and 17/20 present in some Callicebus species could link this genus with callitrichids (Cebidae). As seen in the case of Saimiri, all of these associations (inv 10a/16a, 13/17, 17/20) are worthy of further investigation to test for real homologies, or the presence of homoplasy, or if they could be explained as consequences of hemiplasy.

8. The phylogenetic relationships of Aotus among Cebidae has always been controversial when reconstructed through molecular and morphological data. Even from a cytogenetic point of view, no synapomorphies have been detected to link it to the Cebus/Samiri clade or to the callitrichids. On the contrary, the 10/11 associations instead link owl monkeys (Cebidae) and Callicebus (Pithecidae), even if molecular analyses do not provide similar evidence. In addition, this association needs to be checked in order to verify whether it shares identical breakpoints and could, then, represent a real synapomorphy, or whether it could be homoplasy or hemiplasy.

9. The 10a/16a inversion present in Atelidae and Pithecidae also needs further investigation in order to test whether it shares the same breakpoints and there could thus be a synapomorphy between the two families, or if it could instead be a homoplasy.

10. No human associations have been provided to corroborate the molecular phylogenetic recognition of two new genera, Mico and Sapajus, apart from some intrachromosomal rearrangements. The same is true for Leontocebus (S. fuscicollis), as no molecular cytogenetic mapping has so far been performed on it.

In conclusion, we would like to stress the importance of recognizing hemiplasy because “phylogenetic discordance” due to chromosomal traits could be explained through evolutionary interpretations that take into account homology or homoplasy not only due to convergence or parallelism but also due to polymorphisms in random lineage sorting. For example, the human associations reported above which link Cebidae and Pithecidae (10/11) or Pithecidae and Atelidae (inv 10a/16a), apparently in discordance, could be considered a consequence of polymorphic lineage sorting rather than a contrast, as previously hypothesized (De Oliveira et al. 2012). Moreover due to the demonstrated complexity of the evolutionary radiation in Neotropical monkeys, we emphasize the necessity of employing multidisciplinary and comparative approaches in order to clarify phylogenetic assessments.

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