

# Molecular cytotaxonomy of New World monkeys (Platyrrhini) – comparative analysis of five species by multi-color chromosome painting gives evidence for a classification of *Callimico goeldii* within the family of Callitrichidae

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**Abstract.** Chromosome rearrangements are considered as “rare genomic changes” and can provide useful markers and even landmarks for reconstructing phylogenies complementary to DNA sequence data and bio-morphological comparisons. Here, we applied multi-directional chromosome painting to reconstruct the chromosome phylogeny and evolutionary relationships among the New World monkey (Platyrrhini) species *Callithrix argentata*, *Cebuella pygmaea*, *Saguinus oedipus*, *Callithrix jacchus* and *Callimico goeldii*. The results clarified several aspects of New World monkey phylogeny. In particular the phylogenetic position of *C. goeldii* was elucidated, which has been controversially discussed and variously classified in the family Callitrichidae, in the family Cebidae or in its own family Callimiconidae. Comparative genome maps were established by multi-color fluorescence *in situ* hybridization (FISH) with human, *S. oedipus* and *Lagothrix lagotricha* chromosome-

specific DNA probes. From these data we reconstructed the putative ancestral karyotype of all Callitrichidae. Various derived chromosomal synteny are shared by all five species and cytogenetically define Callitrichidae – including *Callimico goeldii* – as a distinctive group within the Platyrrhini. *C. pygmaea* and *C. argentata* share identical chromosomal synteny from which *S. oedipus* and *C. jacchus* differ by single independent translocations. A common derived chromosomal change links *Callimico* with the marmosets to the exclusion of the tamarins, however, it has further diverged from an ancestral marmoset karyotype by at least four apomorphic rearrangements. *Saimiri sciureus*, representing the Cebinae, exclusively shares a derived syntenic association with all Callitrichidae, defining the genus *Saimiri* as a sister group.

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The taxonomy and phylogeny of Neotropical primates is still subject to debate (Groves, 1989; Schneider et al., 2001). Historically, there was no agreement even on the principal taxonomic divisions. Some authors have recognized two families, Cebidae and Callitrichidae (Napier and Napier, 1967; Fleagle,

1988), while others additionally assigned *Callimico goeldii* familial status (Callimiconidae) (Hill, 1957, 1959; Hershkovitz, 1977; Mittermeier, 1988). Although the monophyly of Callitrichidae is undisputed by most authors, phylogenetic relationships within the Callitrichidae are still incongruent. Some aspects of this controversy arose from confusion about the placement of Goeldi's marmoset (*Callimico goeldii*) which was only discovered in 1904. *Callimico goeldii* shares several anatomic features either with callitrichids or cebids. It resembles tamarins and marmosets in small body size, claws and dental morphology. On the other hand it shares single births and a third molar with Cebidae. Authors recognizing two families, either placed *Callimico goeldii* into the Cebidae (Simpson, 1945; Simons, 1972; Martin, 1990) or Callitrichidae (Napier and Napier, 1967; Fleagle, 1988; Pastorini et al., 1998).

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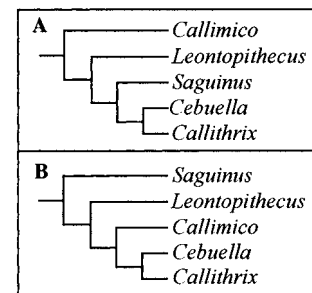
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Most studies on the comparison of morphological characteristics place *Callimico* as basal in relation to other Callitrichidae. This classification is in accordance with the “phyletic dwarfism” hypothesis, implying that marmosets and tamarins are derived and specialized primates with secondary reduced body size. After the divergence of *Callimico*, subsequent lines would in succession have given rise to the genera *Saguinus*, *Leontopithecus*, *Cebuella* and *Callithrix* (Rosenberger, 1981; Ford, 1986). Kay (1990) followed this proposal, but exchanged the position of *Leontopithecus* and *Saguinus*. Ferrari (1993) proposed that a tamarin-like ancestor gave rise to the genus *Saguinus* and *Leontopithecus*. The *Callithrix jacchus* group would have shared a common ancestor with *Leontopithecus*. From the *C. jacchus* lineage, the *Callithrix argentata* group and later *Cebuella* would have branched off. *Cebuella* would therefore represent the most recent and extreme form of phyletic dwarfism.

In contrast, molecular studies, beginning with immunological data (Baba, 1975; Cronin, 1975) have concluded that *Callimico* is not basal but instead nested within the Callitrichidae. Most publications support a phylogeny in which *Callimico* is closest to *Callithrix* and *Cebuella* (Harada et al., 1995; Horovitz and Meyer, 1995 using 16s rDNA; Porter et al., 1997, using epsilon-globin; von Dornum and Ruvolo, 1999 using G6PD). Recent morphological, cytogenetic and DNA sequence data are reviewed by Schneider et al. (2001). The contrast of alternative arrangements for the phylogenetic branching within the Callitrichidae based on morphology and molecular data are illustrated in Fig. 1.

Comparative cytogenetic studies based on chromosome banding analysis proposed further phylogenetic relationships among Callitrichidae (Dutrillaux et al., 1988; Canavez et al., 1996; Nagamachi et al., 1999). Dutrillaux et al. (1988) placed *Callimico* closest to an assumed ancestral Callitrichidae. This interpretation contrasts with Canavez et al. (1996), who classified *Callimico* nested within Callitrichidae. Nevertheless, it has been repeatedly demonstrated that banding analysis is often not informative when rearrangements are complex or translocated chromosomal fragments involved are small. Recently, comparative chromosome maps between human and a number of Platyrrhini species have been established by molecular cytogenetic methods (Consiglière et al., 1996, 1998; Richard et al., 1996; Sherlock et al., 1996; Morescalchi et al., 1997; Garcia et al., 2000; Stanyon et al., 2000, 2001). These results revealed that many New World monkeys show high rates of chromosomal evolution.

Chromosomal rearrangements can be attributed special clastic weight, because they can be considered as “rare genomic changes” (see Rokas and Holland, 2000, for review) with very low levels of convergence. Karyological differences can be helpful complementary markers to DNA sequences, especially when the latter analyses are contradictory or not yet conclusive. Therefore we compared the karyotypes of five species of Callitrichidae by multi-color, “multi-directional chromosome painting” between these species and humans. “Reciprocal” or “multi-directional chromosome painting” is now accepted as the method of choice to establish comparative chromosome maps (Arnold et al., 1996; Goureau et al., 1996; Müller et al., 1998,



**Fig. 1.** Two alternative phylogenetic arrangements of Callitrichidae, based on (A) morphological studies (Kay, 1990) and (B) multiple DNA data sets (Schneider et al., 2001). The two phylogenies differ by the position of *Saguinus* and *Callimico*.

1999; Stanyon et al., 2001). We employed human, *Saguinus oedipus* and *Lagothrix lagothricha* chromosome painting probes to establish chromosomal homologies. These experiments allowed the verification of chromosome homologies and precise sub-chromosomal definition for the species investigated.

## Materials and methods

### Cell samples, tissue culture and chromosome preparation

Metaphase preparations were obtained from established fibroblast cell lines of one male individual of the species *Callimico goeldii*, *Callithrix jacchus*, *Cebuella pygmaea* and a female individual of *Callithrix argentata*. The cell lines were kindly provided by S. O'Brien, Laboratory of Genomic Diversity, National Cancer Institute, Frederick MD, USA. *Saguinus oedipus* peripheral blood metaphases were obtained from the same individual as described in Müller et al. (2001). Male *Cebus apella paraguayanus* (Capuchin monkey) metaphases from an individual kept at the Curitiba Zoological Garden, Brazil, were directly prepared from blood cultures (kindly provided by E. de Oliveira). All metaphase preparations and G-banding followed standard protocols. A listing of the species including their common name, Latin name, diploid numbers and literature on the karyotype nomenclature is given in Table 1.

### Composition and labeling of multi-color chromosome painting probe sets

Human and New World monkey chromosome-specific painting probes were the same as described before (Stanyon et al., 2001; Müller et al., 2001). Probe labeling was performed by DOP-PCR in the presence of hapten or fluorochrome conjugated dUTPs as described earlier (Telenius et al., 1992). For multiplex FISH with *S. oedipus* chromosome-specific probes, probe sets S1–S4 were composed of either four to seven differentially labeled probes (Table 2). *S. oedipus* painting probes were labeled in Boolean combinations with Biotin-dUTP, Digoxigenin-dUTP (Roche) and Rhodamine 110-dUTP (Perkin Elmer) to visualize up to seven chromosomes simultaneously (Ried et al., 1992). Six color probe sets H1–H4 were established from human chromosome-specific paints according to Müller et al. (2001). Human multi-color probe sets were labeled using Cy3-dUTP (Amersham) instead of Rhodamine 110-dUTP.

### In situ hybridization and probe detection

Hybridization *in situ* and probe detection were carried out using a modification of the procedure described previously (Cremer et al., 1988). In double and triple hybridization experiments with human and *L. lagothricha* probes, 100–300 ng of each labeled DOP-PCR product was diluted in 15 µl hybridization buffer (50% deionized formamide, 10% dextran sulphate, 2× SSC). For FISH experiments with combinatorially labeled human and *S. oedipus* probe sets, 200 ng of each painting probe were used together with 10 µg unlabeled human competitor DNA (Cot-1 DNA, Gibco BRL) or 10 µg

**Table 1.** Summary of taxonomy, diploid chromosome number and references on chromosome nomenclature and published cross-species chromosome painting of New World primates included in this study

Common species name	Scientific name	Taxonomy	2 N	Chromosome nomenclature	Cross-species FISH
Cotton-top tamarin	<i>Saguinus oedipus</i>	Callitrichidae	46	Nagamachi et al., 1997	Müller et al., 2001
Common marmoset	<i>Callithrix jacchus</i>	Callitrichidae	46	Sherlock et al., 1996	Sherlock et al., 1996
Bare-ear marmoset	<i>Callithrix argentata</i>	Callitrichidae	44	Canavez et al., 1996	—
Pygmy marmoset	<i>Cebuella pygmaea</i>	Callitrichidae	44	Canavez et al., 1996	—
Goeldi's monkey	<i>Callimico goeldii</i>	Callitrichidae/Cebidae	47	Seuánez et al., 1989	—

unlabeled *S. oedipus* genomic DNA, respectively. DNA probes were denatured at 68 °C for 7 min, preannealed at 37 °C for 30 min and hybridized for 48 h at 37 °C. Post-hybridization washes included 2 × 5 min in 50% formamide, 1 × SSC, 45 °C, 2 × 5 min in 2 × SSC, 45 °C and 1 × 5 min in 0.1 × SSC, 60 °C. Depending on the fluorochrome/hapten combination of multiplex probes, biotinylated DNA probes were detected by Avidin-Cy3 or Avidin-Cy5 (Vector Laboratories). Digoxigenin-labeled probes were visualized by mouse anti-Dig-Cy5 (Jackson Immuno Research) or sheep anti-Dig-FITC (Roche) antibodies. For chromosome identification slides were counter stained with DAPI (4',6-diamidino-2-phenyl-indol, Sigma).

#### Microscopy and image analysis

Metaphases were captured with a cooled CCD camera (Photometrics C250/A equipped with a KAF1400 chip, Kodak) coupled to a Zeiss Axiophot microscope. Camera control, digital image acquisition, merging of gray-scale images and false color assignment was performed using SmartCapture VP 1.4 software (DigitalScientific, Cambridge, UK).

## Results

We hybridized the complete set of human and *S. oedipus* chromosome-specific paints to *Callimico goeldii* and the Callitrichidae species *Cebuella pygmaea*, *Callithrix argentata* and *Callithrix jacchus*. *C. jacchus* has previously been investigated with human painting probes (Sherlock et al., 1996). The results were further compared to those obtained by reciprocal painting between *S. oedipus* and human (Müller et al., 2001). Human chromosome-specific paints were hybridized to New World monkey chromosomes in four experiments in sets of six combinatorially labeled probes. Highly reproducible results were obtained for all chromosomes with the exception of the Y-chromosome. Even hybridization with paints derived from human chromosomes involved in small translocations in Platyrrhini homologs (human chromosomes 3/21 and 5/7) gave signals bright enough to be distinguished from unspecific background. Centromeric as well as other heterochromatic chromosome regions were not hybridized by any human probe. Chromosome homologies were readily established from ten hybridized metaphases.

Compared to human probes, *S. oedipus* chromosome-specific probes provided even higher signal intensity and signal to noise ratios. As with the human paints centromeric regions were either not painted or showed reduced fluorescence intensity. The *S. oedipus* Y chromosome-specific probe yielded no hybridization signal in any other species analyzed (Fig. 2).

Characterization of the *S. oedipus* karyotype with human probes, together with reverse *S. oedipus* probe assignment to human metaphases were recently described (Müller et al., 2001). These data were used to verify present hybridization

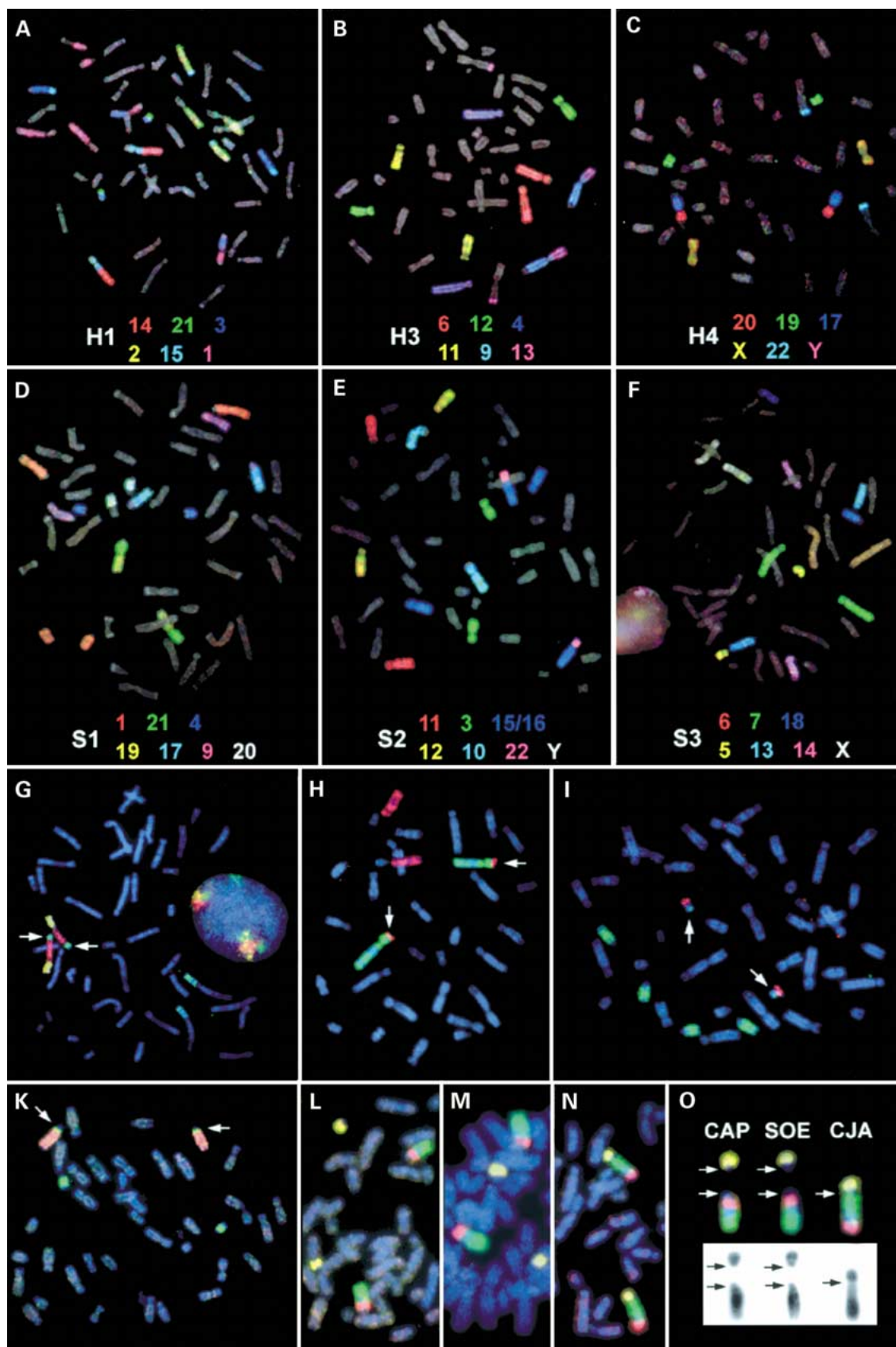
**Table 2.** Multiplex probe composition and false color assignment of *S. oedipus* (S1–S4) chromosome-specific probe sets. In total, probe sets S1–S4 represent all *S. oedipus* chromosomes 1–22, X and Y. Probes marked <sup>a</sup> and <sup>b</sup> contained two chromosomes or derivative chromosomes (Müller et al., 2001).

S1	S2	S3	S4	Color
1	11	6	2	red
21	3	7		green
4	15+16 <sup>a</sup>	18	15 <sup>a</sup>	blue
19	12	5	8 <sup>b</sup>	yellow
17	10	13		cyan
9	22	14	16 <sup>a</sup>	magenta
20	Y	X		white

<sup>a</sup> Discrimination of chromosome 15 and 16 was possible through probe set S4 by co-hybridization with two derivative chromosome 8 probes t(8;16) and del(8).

<sup>b</sup> Chromosome 15+16 were digoxigenin labeled, (blue false color), the derivative chromosome 8 probe t(8;16), was biotin-dUTP labeled (red false color). A second derivative chromosome 8 probe del(8) was rhodamine 110-dUTP labeled (green false color). Consequently, chromosome 15 appears blue, chromosome 16 magenta and chromosome 8 yellow, except for the region deleted in the derivative probe del(8), which appears red.

**Fig. 2.** Representative FISH experiments with human and *S. oedipus* multi-color probe sets to *C. goeldii* (A–D), *C. pygmaea* (B+E) and *C. argentata* (C+F) metaphases are illustrated in (A–F). Below each metaphase the respective probe composition and false color assignment is given (H = human, S = *S. oedipus*). DAPI banded chromosomes are shown in gray. (G–I) Control experiments on *C. jacchus* reveal associations not proposed by Sherlock et al. (1996): (G) Three color hybridization with human chromosome 13 (green), 17 (red) and 20 (yellow) probes delineates association of human chromosome 13/17/20 homologs (arrows). (H) Dual color hybridization with human chromosome 5 (green) and 7 (red) specific probes visualize association 5/7 (arrows). (I) Assignment of human chromosomes 3 (green) and 21 (red), associated on *C. jacchus* chromosome 21 (arrows). (K–O) Supplementary experiments with *L. lagotheria* painting probes for sub-chromosomal definition of certain chromosomes. (K) Cohybridization of *L. lagotheria* chromosome 3 (red) and 11 (green) specific probes delineates different fission breakpoints (arrows) in *L. lagotheria* and *C. goeldii* indicating a different evolutionary origin of chromosome 5/7 homologs. (L–N) Three color hybridization with *L. lagotheria* chromosome 9 (green), 27 (yellow) and 28 (red) to metaphases of (L) *C. apella*, (M) *S. oedipus* and (N) *C. jacchus*. Chromosomal counterstain is shown blue. (O) Summary of these results together with corresponding DAPI stained chromosomes (inverted), visualizing identical sub-chromosomal organization in *C. apella* (CAP) and *S. oedipus* (SOE), in contrast to that observed in *C. jacchus* (CJA). Arrows point to centromeres, highlighting different probe assignment in relation to the centromere and a fusion in *C. jacchus*.



**Table 3.** Inferred ancestral platyrrhine chromosome forms and their human homologous chromosomes and chromosome segments

Platyrrhini <sup>a</sup>	Human <sup>b</sup>	Platyrrhini <sup>a</sup>	Human <sup>b</sup>
1a	1p	9	9
1b	1q32 qter	10a/16a	10q/16p
1c	1q21 q31	10b	10p
2a	2q13 qter	11	11
2b/16b	2pter q12/16q	12	12
3a/21	3p12/21	13	13
3b	3pter p24; p21 p12; q12 q13; q27 qter	14/15a	14/15q14 q24
3c	3p24 q21; q13 q26	15b	15q11 q13; q25 qter
4	4	17	17
5/7a	5/7p22; q11; q21	19	19
6	6	20	20
7b	7p21 p11; q11 q21; q22 qter	22	22
8a /18	8p/18	X	X
8b	8q	Y	Y

<sup>a</sup> Abbreviated nomenclature for ancestral Platyrrhini chromosome forms.

<sup>b</sup> Human chromosome regions homologous to ancestral Platyrrhini chromosomes.

experiments with other species. For example, *S. oedipus* chromosome 15 was hybridized by human chromosome 10 and 16 specific probes. Reverse painting of *S. oedipus* chromosome 15 probe painted human chromosome 16p and 10q. Thus, painting these probes to any other New World monkey not only defines the respective homologous chromosomes to *S. oedipus*, but also the homologous sub-regions in humans. Accordingly, human chromosome 16p and 10q/*S. oedipus* chromosome 15 defined the 10a/16a-chromosome form found in other monkey species (Table 3). Hybridizations with probes from the woolly monkey (*Lagothrix lagothricha*, Atelinae) provided further information on sub-regional homologies (Stanyon et al., 2001).

Figure 2A–C illustrates representative FISH experiments with human probe sets, Fig. 2D–F hybridizations with *S. oedipus* probes. Figure 3 summarizes the assignment of all human and *S. oedipus* chromosome-specific probes to G-banded chromosomes of the species investigated. Homologous chromosome regions of all five species were aligned in order to facilitate a comparative banding analysis. Reinvestigation of previously published chromosome painting of *C. jacchus* (Sherlock et al., 1996) with human painting probes are documented in Fig. 2G–I. Supplementary fine analysis of various species with probes derived from *L. lagothricha* is shown in Fig. 2K–O. Chromosomes were identified by inverted DAPI banding in all FISH experiments.

#### *Callimico goeldii*

**Hybridization with human paints:** In total 38 homologous chromosome segments were distinguishable per haploid *C. goeldii* chromosome set. Probes derived from chromosomes 4, 6, 11, 12, 19, 20 and X hybridized to entire chromosomes indicating that these chromosomes have been conserved entirely. Human chromosome 14, 17, 18, 21 and 22 probes identified single homologous regions in only one chromosome pair, but associated with other chromosomes or chromosome segments. Painting probes 1, 2, 3, 5, 7, 8, 9, 10, 13, 15 and 16 gave signals

on 2–4 chromosomes. Association of homologs to human chromosomes 1/3, 1/10, 2/15, 2/16, 3/15, 3/21, 5/7, 8/18, 9/13, 9/22, 13/17, 10/16 and 14/15 were defined. The human chromosome 1-specific probe identified the Y/autosomal translocation and hybridized to the long arm of X<sub>2</sub> and the Y chromosome described by Hsu and Hampton (1970).

**Hybridizations with *S. oedipus* probes:** *S. oedipus* chromosomes 4, 5, 7, 8, 10–15, 18 and X were found entirely conserved in *C. goeldii*. Chromosome 1, 6, 9, 2, 16, 17, 19, 21 and 22 specific probes delineated inter-chromosomal rearrangements. Chromosome 20 probe mapped to the entire X<sub>2</sub> and the Yq chromosome. *S. oedipus* chromosome 3-specific probe gave a signal on the terminal Yp region. Most of Yp was not hybridized by any *S. oedipus* painting probe.

#### *Cebuella pygmaea*

**Hybridization with human paints:** The painting results showed that the human chromosome 4, 6, 11, 12, 19 and X homologs were fully conserved. Human chromosome-specific probes 14, 17, 18, 20, 21 and 22 hybridized to sub-regions of a single chromosome pair. Disrupted synteny was revealed for human chromosome 1, 2, 3, 5, 7, 8, 9, 10, 13, 15 and 16 homologs. Associations of human homologous chromosomes or chromosome segments 13/17/20, 13/9/22, 5/7, 8/18, 2/15, 2/16, 10/16, 1/10, 14/15 and 3/21 were observed. *C. pygmaea* chromosome pairs 4, 5, 7 and 12 were not hybridized entirely, presumably due to heterochromatin comprised of repetitive sequences not present in the human genome. Chromosome pairs 4 and 7 further showed size polymorphisms between the two homologs.

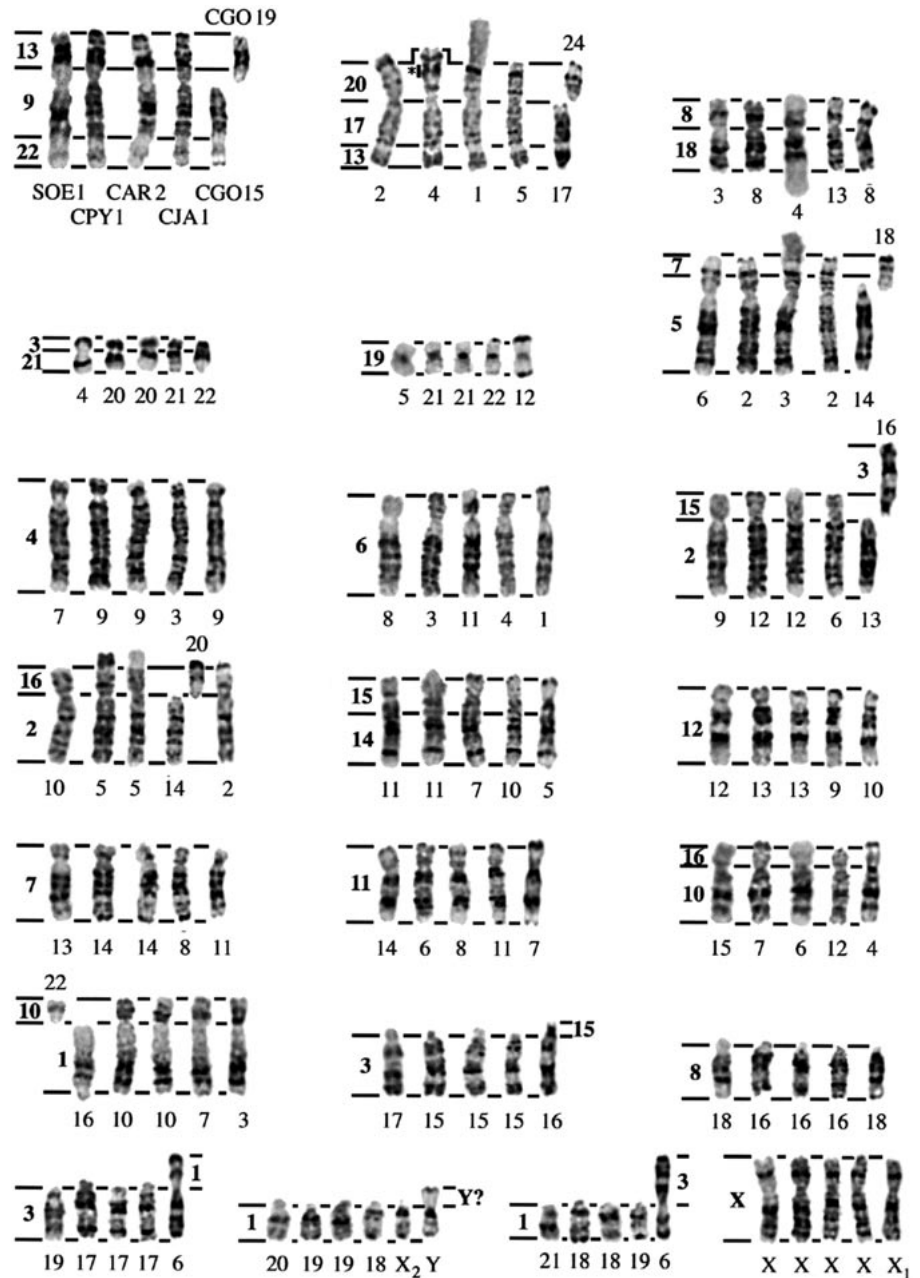
**Hybridizations with *S. oedipus* probes:** With the exception of *S. oedipus* chromosome 16 and 22 specific probes, each paint completely hybridized one homologue, indicating that these chromosomes have been conserved entirely. *S. oedipus* chromosome 16 and 22 homologs were found associated in *C. pygmaea*. Assumed heterochromatic regions not hybridized by human probes (see above) were also not hybridized by any *S. oedipus* probe.

#### *Callithrix argentata*

*C. argentata* displayed a karyotype very similar to *C. pygmaea*. With the exception of presumably heterochromatic regions, the results revealed that the hybridization pattern of *C. argentata* is identical to that of *C. pygmaea* with both human and *S. oedipus* probes. Chromosome pairs 1, 3 and 4 showed large heterochromatic segments not painted by any human or *S. oedipus* probe. Respective regions on chromosomes 1 and 3 showed size polymorphism between the two homologs.

#### *Callithrix jacchus*

**Hybridization with human paints:** Except for the proposed triple association 8/18/13 and separate human 3, 21, 5 and 7 homologs, our hybridization results confirmed those of Sherlock et al. (1996). We performed dual and three color hybridization experiments with respective human chromosome-specific probes (Fig. 2G–I). These experiments delineated association of human 5/7, 3/21 and 20/17/13, whereas clearly no chromo-



**Fig. 3.** Alignment of homologous *S. oedipus* (SOE), *C. pygmaea* (CPY), *C. argentata* (CAR), *C. jacchus* (CJA) and *C. goeldii* (CGO) (left to right) G-banded chromosomes, together with the assignment of human chromosome-specific painting probes. Chromosome numbering (below or above chromosomes) follows citations given in Table 1. Numbers to the left or right of chromosomes indicate human homologous chromosome regions, horizontal bars the borders of homologous regions, respectively. Chromosomal regions outside these borders were not painted by any probe and represent terminal heterochromatin. Asterisked bar to the left of chromosome 4 of *C. pygmaea* marks an interstitial heterochromatin region, not hybridized either. No signal was observed with human or *S. oedipus* Y specific probes. *C. goeldii* Y homologous material presumably involved in a Y/autosomal translocation is marked as "Y?".

some 13 homologous material was found associated with the human 8/18 homologue.

**Hybridization with *S. oedipus* probes:** With the exception of probe 10, 16 and 22, all probes identified single homologous chromosomes which were entirely conserved between the two species. *S. oedipus* probe 10 entirely hybridized two acrocentric chromosome pairs, whereas probes 16 and 22 were found associated on one chromosome pair of *C. jacchus*.

#### Supplementary FISH experiments

Previous painting of *L. lagotricha* and present results on *C. goeldii* with human probes showed an association of human 5 and 7 homologs on a small submetacentric chromosome in

both species. To improve the sub-chromosomal definition of this association, double hybridization of *L. lagotricha* chromosome 3 (human 5pter→q31.2) and 11 (human 5q31.3→qter/7pter→p22/7q11/q21) specific probes was performed on *C. goeldii*. The results revealed that the 5/7 association originated from different breakpoints in both species and thus was derived by evolutionarily independent chromosome rearrangements (Fig. 2K).

In order to confirm the orientation of the association of human chromosome 1 and 10 homologous segments relative to the centromere, *L. lagotricha* chromosome 9 (human 1p34.3→p12), 27 (human 10p) and 28 (human 1pter→p35) painting probes were applied in triple hybridization experi-



ments. These probes were hybridized to *Cebus apella paraguayanus*, *S. oedipus* and *C. jacchus* metaphases (illustrated in Fig. 2L–N and summarized in Fig. 2O). *L. lagothericha* chromosome 9 and 28 revealed an identical sub-chromosomal orientation in *C. a. paraguayanus* and *S. oedipus*. In contrast, the *C. jacchus* homolog showed an inverse hybridization pattern compared to these species.

## Discussion

### *Molecular cytogenetics in the study of New World monkey phylogeny*

The experiments demonstrate that hybridization in a multi-color format is also applicable for more distantly related primate species. Human multiplex probe sets gave reproducible results despite an evolutionary distance of about 30–40 million years between human and platyrrhines. The application of both human and *S. oedipus* probes and in some experiments those of *L. lagothericha* provided a sub-chromosomal definition of homologous chromosome segments. This additional sub-chromosomal mapping information allowed us to determine more confidently whether chromosomal associations were the result of rearrangements in the karyotype of a common ancestor and therefore truly homologous (Müller et al., 1999). Consequently, it allowed a more reliable interpretation of phylogenetic linkage. In some cases, the orientation of chromosomes and chromosome segments in fusions and fissions could also be identified. *L. lagothericha* probes 9, 27 and 28 revealed that the fusion of human 1 and 10 homologs was not a simple Robertsonian fusion, but was of more complex nature. Presumably a head to tail tandem fusion occurred, accompanied by the loss of centromeric function in the human chromosome 1 homolog (Fig. 4A). An alternate explanation is a Robertsonian type fusion, followed by a large paracentric inversion.

### *Revision of the inferred ancestral karyotype of all New World primates*

Associations of homologs to human chromosomes are particularly informative in comparative karyotype analysis since conserved chromosomal synteny found in species of particular phylogenetic lines may be an indication for their common evolutionary origin (Wienberg and Stanyon, 1998; Wienberg et al., 2000; for reviews). Thus, the first step in using chromosome data in phylogenetic reconstructions is to propose the ancestral condition from the appropriate outgroup comparisons. Common derived traits can then be used to link species phylogenetically.

On the basis of our results and published chromosome painting data from other primates we can refine two aspects of the hypothetical ancestral karyotype of New World monkeys we have proposed previously (Stanyon et al., 2000). It was assumed to have a diploid number of  $2N = 56$  chromosomes. i) The association 2/16 is found in all platyrrhine genera except the genus *Alouatta*. Moreover, it is found in four Callitrichidae species, but not in *C. jacchus*. The direction of this chromosomal rearrangement is more likely to be an independent loss of the 2/16 association in *C. jacchus* and the genus *Alouatta*, than

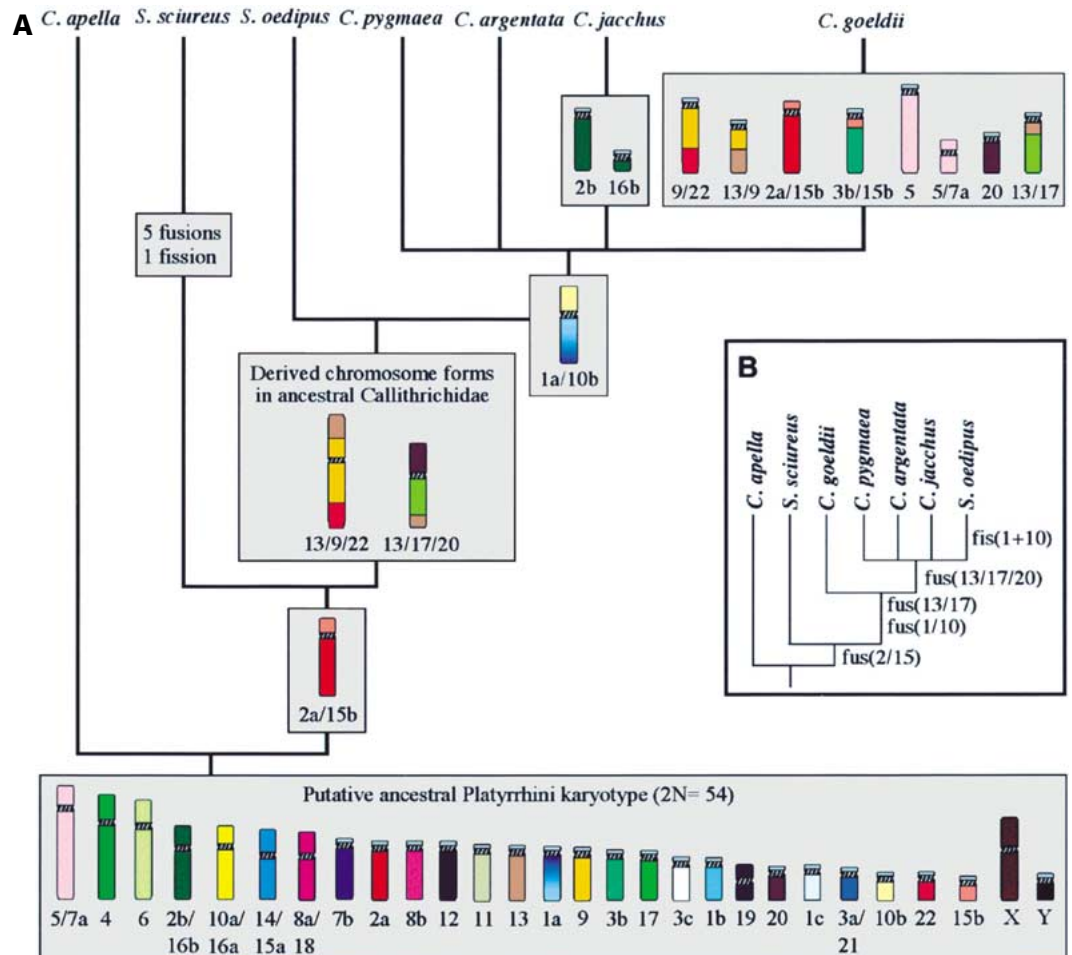
a convergent fusion of 2 and 16 in all other species. We thus consider this association to be ancestral for all New World monkeys. ii) The association 5/7 should also be included, although it was not detected in all species. As demonstrated for *C. jacchus* in our study, the translocated chromosome 7 homologous fragment is minute and may therefore have escaped detection in previous chromosome painting experiments (Sherlock et al., 1996). Consequently, the inferred ancestral New World monkey karyotype would have had a diploid number of  $2N = 54$  chromosomes (Table 3 and Fig. 4A). The assumed ancestral karyotype is found conserved in *Cebus capucinus* and *C. apella* except for a derived pericentric inversion of the human chromosome 14/15a homolog (Richard et al., 1996; Garcia et al., 2000).

### *The phylogeny of the Callitrichidae based on chromosome painting results*

Interspecies chromosome homology maps were analyzed in order to reconstruct the direction of chromosome changes. Shared ancestral traits were discriminated from derived characters by comparison with the proposed ancestral Platyrrhini karyotype and using *Cebus* as outgroup (Richard et al., 1996; Garcia et al., 2000). The relevant ancestral Platyrrhini chromosome forms informative for Callitrichidae would include homologs to human chromosomes 13, 9, 22, 20, 17, 1a, 10b, 2a and 15b (Table 3, Fig. 4A). The association of human 2a/15b homologs, observed in all species investigated here and in *S. sciureus* (Stanyon et al., 2000), is most probably a common derived trait of these species and defines them as a distinctive group of New World monkeys. No other species characterized by comparative chromosome painting to date, showed this association. This trait gives evidence for a closer relationship of *S. sciureus* to Callitrichidae than to any other group of Platyrrhini (Fig. 4A), a conclusion also supported by some morphological comparisons (Rosenberger, 1981; Kay, 1990).

Derived associations of human chromosome 9/13, 9/22 and 13/17 homologs were exclusively identified in *C. jacchus*, *C. argentata*, *C. pygmaea*, *S. oedipus* and *C. goeldii*. We consider these shared derived associations to be phylogenetic landmarks that represent a clear division between Callitrichidae and Cebidae and support a classification of *Callimico* within Callitrichidae.

Some additional chromosome associations at first appearance seem to link *C. goeldii* with one Cebidae or another. For example, *C. goeldii*, *Alouatta seniculus sara* and *Ateles geoffroyi* show an association between segments homologous to human 3 and 15 (Consiglière et al., 1996; Morescalchi et al., 1997). The respective *S. oedipus* painting probe clearly demonstrated that in *A. seniculus* the human chromosome 3 segment was not homologous to that found associated in *C. goeldii*. Furthermore, in *C. goeldii* the 3/15b homolog is a secondary product, derived from the chromosome 2a/15b homolog, whereas in *Ateles* and *Alouatta* it is a direct fusion product of chromosome 3 and 15 segments. Additionally, in contrast to the association 5/7a found in other Callitrichidae, *Callimico* appears to share fragmented chromosome forms 5a/7a and 5b with Atelinae, for example *L. lagothericha* (Stanyon et al., 2001). Hybridization of *C. goeldii* with respective *L. lagothericha* probes (Fig. 2K) ascer-



**Fig. 4.** The reconstruction of chromosomal changes in Callitrichidae leads to two most parsimonious interpretations (phylogenetic tree 1, **(A)** and phylogenetic tree 2, **(B)**, see text for details). **(A)** Numbering indicates human homologous chromosome forms according to Table 3, color code refers to the putative ancestral Platyrrhini karyotype. According to phylogenetic tree 1, *S. oedipus* conserved the ancestral karyotype, since it shares separate human chromosome 1a and 10b homologs and the sub-chromosomal organization with the *C. apella* (phylogenetic outgroup) chromosome 1a homolog. From this, all other Callitrichidae would be derived by a synapomorphic “head to tail” fusion involving human 1a and 10b homologs. Consequently, *C. goeldii* would constitute the sister clade to genus *Callithrix* and *Cebuella*, but accumulated autapomorphisms.

tained different breakpoints in *C. goeldii* and *L. lagothrica*. These experiments showed that association of 3b/15b and fission products 5a/7a and 5b are the result of independent rearrangements in different phylogenies and do not reflect a common evolutionary origin of *Callimico* and *Atelinae*. Instead they appear to constitute autapomorphisms (derived species-specific chromosome forms) in *C. goeldii*. In conclusion, *C. goeldii* shares derived chromosome forms exclusively with Callitrichidae (Fig. 4A). This observation provides further evidence for the taxonomic and phylogenetic integration of *Callimico* within Callitrichidae.

Several different phylogenies can be proposed for genus *Callithrix*, *Cebuella*, *Saguinus* and *Callimico*, taking *Cebus* as an outgroup. The two most parsimonious trees are illustrated in Fig. 4. Both trees require the same number of common derived rearrangements, which however differ in their branching position. In tree 1 (Fig. 4A) five rearrangements would link all Calli-

trichidae, which include fusions of 2a/15b, 9/22, 13/17/20 and an additional translocation of 13 to form chromosome 9/22/13. In contrast to all other Callitrichidae *S. oedipus* would have conserved separate human 1a and 10b homologous segments (*S. oedipus* chromosome 16 and 22, Fig. 3) from the ancestral platyrrhine karyotype. The derived fusion of 1a/10b would then link *Callithrix*, *Cebuella* and *Callimico* (Fig. 4A). In contrast, in tree 2 (Fig. 4B), association of human 1a/10b homologs is assumed to be ancestral for all Callitrichidae and the fusion of the chromosome 20 homolog to 13/17 would constitute a synapomorphism of *Callithrix*, *Cebuella* and *Saguinus*. *Callimico* would have conserved the ancestral 13/17 and 20 chromosome form.

Both trees apparently require homoplasy. Tree 1 requires one derived reverse fission of the chromosome 20 homolog from the 13/17 association in *C. goeldii*, while tree 2 requires a reverse fission of 1a/10b in *S. oedipus*. Homoplasy has been



reported for Robertsonian transformations (Nash et al., 1998; Yang et al., 2000). However, for other types of chromosome rearrangements like tandem translocations, reciprocal translocations and inversions homoplasy appears to be extremely rare (Wienberg et al., 2000).

Phylogeny 1 can be favored because the 1a/10b association would result from a "head to tail" tandem fusion as shown by the sub-chromosomal probes from *L. lagotherichia* (Fig. 2O, Fig. 4A). Moreover, the 1a homolog in *S. oedipus* shows the same orientation as the putative ancestral form in New World monkeys found in *Cebus*. It seems implausible that two "rare genomic events", a fission and a generation of a new centromere in the same position as in the outgroup *Cebus apella* would have reversed independently, as would be required for tree 2 (Fig. 4B). It is much more likely, that separate 1a and 10b homologous chromosomes conserved in *Saguinus* and outgroup *Cebus apella* represent the ancestral condition, as proposed in tree 1 (Fig. 4A).

In conclusion, this study provided detailed insight into several aspects of Callitrichidae chromosomal phylogeny, which may contribute to resolution of some open questions concerning the evolutionary branching sequence. Firstly, a common derived rearrangement shared by *S. sciureus* and Callitrichidae indicates a closer relationship of *Saimiri* to Callitrichidae than to any other group of Platyrrhini. Secondly, our data suggest the taxonomic integration of *C. goeldii* in the family Callitrichidae. *S. oedipus* would constitute the most basal clade, *C. goeldii* the sister clade to genus *Callithrix* and *Cebuella*, being more closely related to marmosets than tamarins. Finally, no chromosomal evidence was found either supporting the "phyletic dwarfism" or the "primitivity" hypotheses.

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