Retrotransposon mapping in spider monkey genomes of the family Atelidae (Platyrrhini, Primates) shows a high level of LINE-1 amplification

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Abstract

To investigate the distribution of LINE-1 repeat sequences, a LINE-1 probe was Fluorescence *In Situ* Hybridized (FISH) on the chromosomes of *Ateles geoffroyi* and *Ateles fusciceps* (Atelidae); a LINE-1 probe was also mapped on *Cebuella pygmaea* (Cebidae) and used as an outgroup for phylogenetic comparison. *Ateles* spider monkeys have a highly rearranged genome and are an ideal model for testing whether LINE-1 is involved in genome evolution. The LINE-1 probe has been mapped in the two Atelidae species for the first time, revealing a high accumulation of LINE-1 sequences along chromosomal arms, including telomeres, and a scarcity of LINE-1 signals at centromere positions. LINE-1 mapping in *C. pygmaea* (Cebidae) revealed signals at centromere positions and along chromosome arms, which was consistent with previous published data from other Cebidae species. In a broader sense, the results were analyzed in light of published data on whole-chromosomal human probes mapped in these genomes. This analysis allows us to speculate about the presence of LINE-1 sequences at the junction of human chromosomal syntenies, as well as a possible link between these sequences and chromosomal rearrangements.

Introduction

About 45% of the human genome is made up of repetitive sequences, such as rDNA, interstitial telomeric sequences, and Transposable Elements (TEs).[1] These elements were initially thought to be merely junk DNA and self-centered DNA parasites; while they are now known to be associated with genome function, chromosome evolution, speciation, and diversity.[2–5] Among repetitive sequences in particular TEs play a role in genome structure, including DNA packaging, centromere stability, and plasticity; furthermore, it has been hypothesized that TEs are involved in evolution, promoting the occurrence of chromosomal rearrangements and disease.[6–12]

Among TEs, those of the family LINE-1 are the most abundant in primate and mammalian genomes.[13-17] The principal approaches previously used to study LINE-1 distribution were the restriction enzyme method or whole genome screening.[18,19] First investigations of LINE-1 sequences of Primates were performed among Old World Monkeys (OWMs) in Hominidae, including humans.[14,16] The LINE-1 sequence comparisons permitted researchers to group them into subfamilies with a high rate of amplification.[14,15] Other studies suggest that rates of LINE-1 amplification differ substantially between taxa, indicating that LINE-1 amplification changed rap-

idly during primate evolution.[19-21] Numerous additional species-specific LINE-1 retrotransposons have been discovered in chimpanzees, gorillas (Hominidae), and representative species of the Cercopithecidae family in recent studies using advanced sequencing methods. These new methods allow a better detection of repetitive sequences that traditionally have not been easily detected.[22-23] Through molecular sequence data analysis, LINE-1 activity has also been shown in New World Monkeys (NWMs) of the genera *Saimiri* and *Saguinus*, but with contrasting results in the *Ateles* lineage.[19,20]

Another approach used to study LINE sequence distribution is the molecular cytogenetic approach by Fluorescence *In Situ* Hybridization (FISH). This approach through chromosomal mapping of LINE-1 has shown different distribution patterns in mammals;[24-32] some LINE-1 are insertions in Adenine and Thymine (AT) rich regions, in euchromatic G-positive bands, on some autosomes and on the X chromosome;[25,26] many other LINE-1 insertions occur in heterochromatic regions, especially in the centromeric regions presumably having a role at the centromeric position.[27-31] Furthermore, it has been hypothesized through the FISH approach that LINE-1 play a role during evolution and are linked with chromosomal rearrangements.[8,9,32] Platyrrhini are small anthropoids grouped into three families: Cebidae, Atelidae and Pitheciidae.[33] FISH has been performed on many platyrrhine species, mapping specific sequences onto chromosomes such as, Bacterial Artificial Chromosomes (BAC) with specific human sequences used as probes,[34,35] repetitive sequences such as rDNA loci, telomeric sequences,[2-4] LINE-1,[36-39] and painting chromosome probes[40] showing that highly rearranged genomes with many inter and intrachromosomal rearrangements occurred during their radiation.

In this study, the LINE-1 probe was mapped by FISH onto chromosomes of *Ateles geoffroyi*, *Ateles fusciceps* (Atelidae), and *Cebuella pygmaea* (Cebidae) in order to analyze LINE-1 chromosomal distribution and discuss its possible role. Among Platyrrhini, Atelidae species have the most rearranged genomes and represent an optimal model for testing whether LINE-1 elements are linked to genome evolution and to chromosomal rearrangements.

Materials and Methods

Following standard protocols,[38] metaphases were obtained from fibroblast cell lines by a cell bank conserved in Liquid Nitrogen at the Palermo University: *Ateles geoffroy*-AGO, *Ateles fusciceps*-AFC (Atelidae), and *Cebuella pygmaea*-CPY (Cebidae); these cell lines come from animals or samples as detailed in Table 1.

Metaphases of the three analyzed species, obtained through cell culture and chromosome harvesting, were treated to obtain G bands. Furthermore, other metaphases were stained pre-FISH using CMA3 and DAPI. DAPI and CMA3 staining was useful for detecting AT and GC-rich regions. DAPI images were inverted with a photo editing program (Adobe Photoshop CC 2022 V23.3.2); inverted gray bands generally correspond to dark G bands; chromosomes here were arranged according to published karyotypes for *Ateles*[41-43] and *Cebuella*[40,44] species that are suggested to have almost the same set of chromosomes as the *A. geoffroyi*, *A. fusciceps*, and *C. pygmaea* karyotypes.

C banding was done sequentially post-FISH through a protocol which included denaturation with formamide.[45] The post-FISH C banding pattern obtained was compared with previously published C banding obtained by standard methods for *Ateles* species[42,46-48] and *C. pygmaea*.[40,44]

DNA from the cell culture pellet derived from the fibroblast cell line was extracted according to the basic protocol using the Pure Link DNA kit from Invitrogen. The LINE-1-like repeat sequence (LINE-1) was amplified from each species's own DNA through Polymerase Chain Reaction (PCR) using the universal set of primers: L1R, 5′-ATTCTRTTCCATTGGTCT A-3′ and L1F, 5′-CCATGCTCATSGATTGG -3′ developed for mammals including primates. The genomic DNA was amplified in 50 µL reactions in PCR using an Applied Biosystems SimpliAmp Thermo-Cycler (Thermo Fisher Scientific): five units of Taq DNA Polymerase were incubated together with the template DNA, 500 nM of each primer, 200 µM each of dATP, dCTP, dTTP and dGTP in 10 mM TRIS-HCl, pH 8.3, 1.5 mM MgCl2, 50 mM KCl. Cycling parameters were 30 cycles of 94°C for 30 s; 52.5°C for 30 s; 72°C for 30 s, following a 2 min denaturation at 94°C.

Products were visualized on 1% agarose gel. The PCR amplification products were labeled through nick translation using 11-dUTP-Cy-5.

FISH was performed following previously described protocols;[49-51] at least 10 metaphase spreads were analyzed from each sample with DAPI and CMA3 staining according to a recent protocol, with some adjustments.[52]

The chromosomes with the LINE-1 probe signals were identified using inverted DAPI. Homologies with human chromosomes were taken into account by extrapolating data from painting results for *Ateles* species[42,43] and *C. pygmaea*.[40,44]

After FISH, the metaphases were analyzed under a Zeiss Axio2 epifluorescence microscope. Images were captured using a coupled Zeiss digital camera.

Results

Reconstructed inverted DAPI karyotypes of all studied species were in agreement with the previously published data;[42,43] *A. geoffroyi* and *A. fusciceps*, have 2n = 34, and all chromosomes were bi-armed except for chromosome pair 16 and the Y chromosome (Figure 1a); *C. pygmaea*, have a karyotype with the diploid number of 2n = 44 as previously described,[40,44] with the majority of chromosome pairs being bi-armed but for 5 acrocentric pairs and the Y chromosome (Figure 1b).

The reconstructed karyotypes with C-bands after FISH (Figure 1a), demonstrated that the results in *Ateles* were in agreement with previous literature data:[46,47] heterochromatin C positive bands were distributed at the centromeres of some chromosomes (Chr 8,

Table 1. Analysed samples: species and fibroblast cell lines used in this study.

Family	Species name	Code	Sample	Sample/cell line acknowledgement
Atelidae	*Ateles geoffroyi*	AGO	Male	Melody Roelker-Parker (Leidos, NCI, USA);
	Ateles fusciceps	AFC	Female	June Bellizzi and Richard Hahn (Catoctin Zoo, MD, USA)
Cebidae	*Cebuella pygmaea*	CPY	Male	Stephen O'Brien (Laboratory of Genomic Diversity, National Cancer Institute, Frederick MD, USA)

14-16), more evident on chromosome 8 in *A. fusciceps*; furthermore, other C bands were interstitial (Chr 1) or at the terminal ends of p arms (Chr 3, 6, 9-13; Figure 1a).

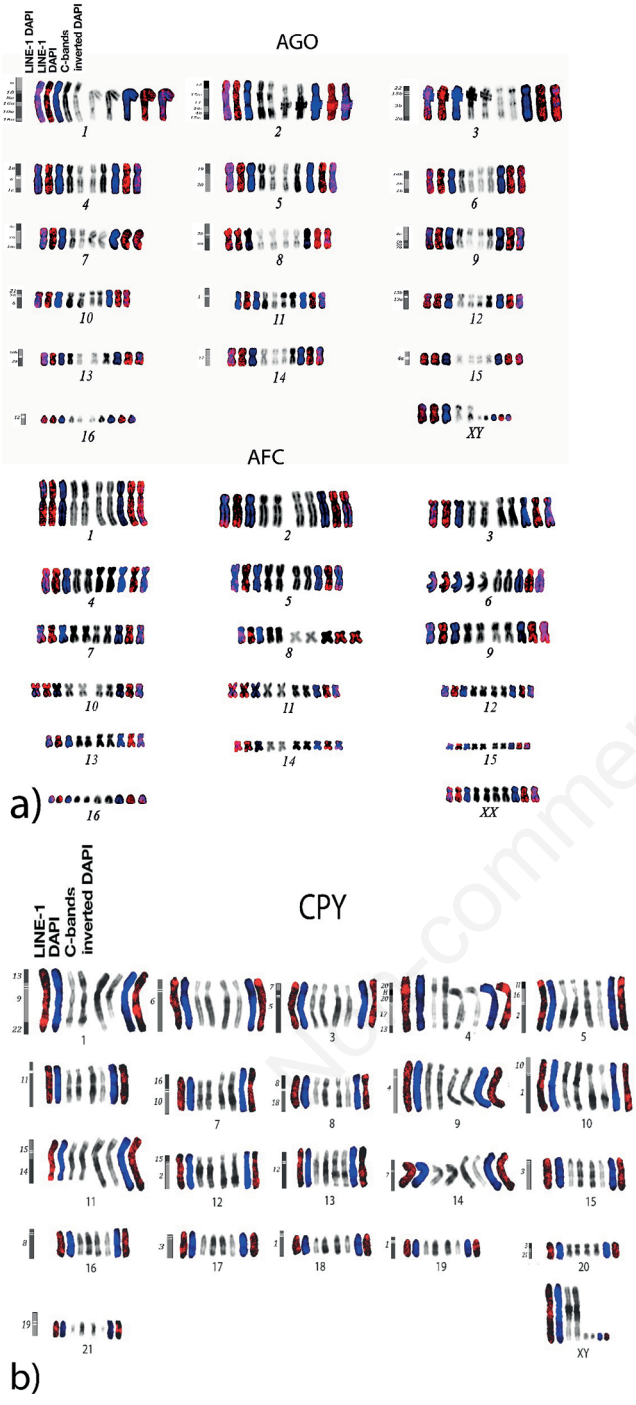

Figure 1. LINE-1 pattern on diploid inverted DAPI karyotypes of a) *A. geoffroyi (AGO), A. fusciceps* (AFC), and b) *Cebuella pygmaea*(CPY); see chromosomes legends with different staining and probe localization over chromosomes 1. Human syntenies and evolutionary breakpoint regions are indicated by numbers and bars at the left of chromosomes in AGO (in AFC they are the same) and CPY; the painting data on human syntenies are extrapolated from previous publications[42,43] for *Ateles* and *Cebuella*.[40,44]

In *C. pygmaea*, the reconstructed karyotype with C bands (Figure 1b) were in agreement with previous results;[40,44] C positive bands were on centromeric and acrocentric chromosomes (Chr 15, 17, 18, 19) and of a metacentric one (Chr 21), while other blocks were interstitial (Chr 4p) or at the terminal edge of p arms (Chr 2, 3, 5).

Bright LINE-1 probe signals were obtained for all species (Figure 2): in the two Atelidae species (Figure 1a, 2a) and in *C. pygmaea* (Figure 1b, 2d); in the two Atelidae, the LINE-1 patterns were very similar: many interspersed signals were found in almost all chromosome pairs, especially along chromosomal arms, but very few at centro-pericentromeric positions. However it has to be noted that the limit of resolution of FISH experiments might fail to identify smaller clusters of repetitive elements, which might still be present at the centromeric position. A bright signal was also present on the Y chromosome in *A. geoffroyi*.

In *C. pygmaea*, LINE-1 signals, instead, were found both at the centromere position and along the chromosome arms (Figure 1b, 2d).

The X chromosome in all three species was rich in LINE-1 along both arms.

Furthermore, the reconstruction of the additional G banded and

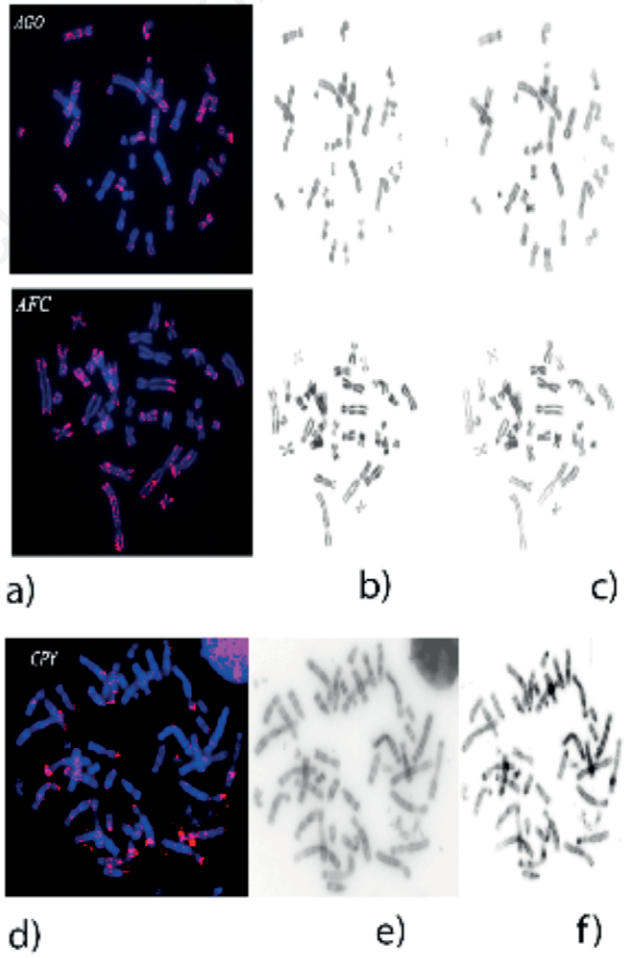

Figure 2. LINE-1 probe mappingonto mitotic metaphases of: a) *A. geoffroyi* (AGO), *A. fusciceps* (AFC); from the same methaphases there are also showed: b) DAPI inverted chromosomes before FISH and c) sequential C bands after FISH. LINE-1 probe mapping is also showed in d) *C. pygmaea* (CPY); from the same methaphases there are also showed: e) the DAPI inverted chromosomes before FISH and f) sequential C bands after FISH.

CMA3/DAPI karyotype for the species analyzed (Figure 3), was useful for identifying chromosomes and preferential insertion sites of LINE-1 sequences. It is possible to analyse that C bands (Figure 2c) were not in correspondence with CMA3 staining for the Atelidae species (Figure 3b). Also in *C. pygmaea* C positive bands (Figure 2f) seems to be not always a correspondence of the CMA3 positive region especially at not centromeric position (Figure 3d).

Discussion

Platyrrhine genomes are characterized by a high evolutionary rate of inter and intra chromosomal rearrangements[53] and are a good model for understanding how LINE-1 repetitive sequences may contribute to the evolution and architecture of the genome. To test whether LINE-1 sequences were important modifiers of genomes, FISH was used to characterize the distribution of LINE-1 sequences in genomes of three representative species of NWMs within a phylogenetic framework, also considering previous painting data. Among NWMs, Atelidae is a peculiar group with its species having the highest rate of chromosomal rearrangements, as previously shown through FISH with whole chromosome painting probes.[41-43]

The analysis of the results obtained for the three species allows us to show a different pattern of LINE-1 distribution between Atelidae and Cebidae species, with a very rich signal pattern along chromosomes and almost no centromeric signals in the former (Figure 1a, 2a), while the latter having both centromeric and interstitial signals with a slighter accumulation (Figure 1b, 2d).

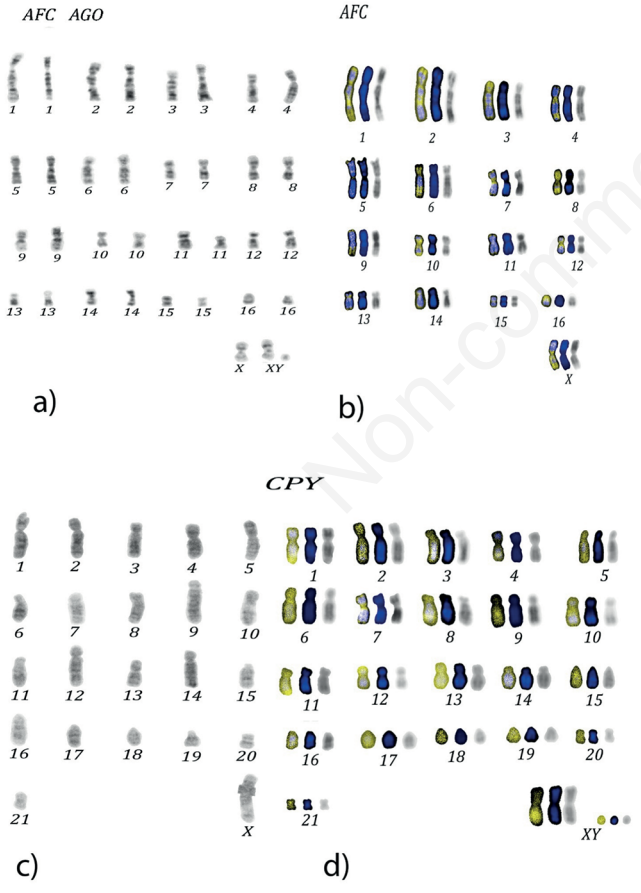

Figure 3. a) Haploid G-banded karyotypes of *A. fusciceps* (AFC) and *A. geoffroyi* (AGO); b) Haploid CMA3/DAPI stained karyotypes of *A. fusciceps* (AFC); c) Haploid G banded karyotype of *C. pygmaea* (CPY); d) Haploid CMA3/DAPI stained karyotypes of *C. pygmaea*.

In both *A. geoffroyi* and *A. fusciceps* (Figure 1a, 2a), we found almost all non-centromeric LINE-1 signals along chromosomal arms on the X chromosome and in autosomes with a few exception; these signals were, in euchromatic regions either in DAPI positive bands or in CMA3-positive regions and also with a partial correspondence with C positive bands (Figure 2a, b, c; Figure3b), in agreement with what was observed in many mammals groups,[25-27,33] including NMWs.[37-38]

These interstitial LINE-1 signals found along chromosomes at non-centromeric regions, through a comparison with the human chromosomal homology extrapolated from previously obtained painting data for *A. geoffroyi* and *A. fusciceps,*[42,43] led us to show they overlap with breakpoint regions at the junction of human syntenic blocks (Figure 1a). For example, they were found on *Ateles* chromosomes 1 and 2, which are composed of many human associations as previously demonstrated by painting (respectively by 9/18/8a/16a/10a/16a and 12/15a1/14/1a1/4b/15a2), or on chromosome 5 composed of human association 5b/8b, where signals are between the fusion point of human syntenies (Figure 1a).[42,43]

Further many other LINE-1 signals were surprising in *A. geoffroyi* and *A. fusciceps*; indeed they were found interstitially along chromosomes but not at the junction of the human sytenies. This evidence could be explained due to intrachromosomal rearrangements characterizing these species. However the painting data can not show these intrachromosomal differences, thus the classic banding pattern can give information on these events; for example, were found other LINE-1 signals on chromosomes 6, 8, and 13 which have variable banding patterns due to intrachromosomal rearrangements (Figure 1a), in agreement with previous G/C banding analysis that showed the occurrence of inversions.[46-48] This difference is reflected in the LINE patterns of distribution: in *A. geoffroyi* on those chromosomes LINE-1 signals as well as C bands are interstitially along chromosome arms, while the LINE-1 are at centromeric positions in *A. fusciceps* presumably as result of the inversions (Figure 1a, 2a, b, c). All these data are in agreement with what has already been hypothesized for other NWMs[37,38] and support the hypothesis about the link between LINE-1 location and evolutionary rearrangements[8,9,37,38] being these both intra or interchromosomal changes.

While there is high amplification of LINE-1 and C bands along chromosome arms in Atelidae, on the other hand there is weaker presence of LINE-1 signals and C bands at centromeric levels (Figure 1a, 2a, c). This evidence is in agreement with the high rate of chromosome rearrangements in Atelidae that could have changed a hypothetical ancestral condition of the centromeric LINE-1 accumulation charatherizing all other Platyrrhini[37,38] including the *Cebuella* here analysed (Figure 1b, 2d, f).

The rich peculiar LINE-1 and C band distributions described for the two *Ateles* species could be linked to the fact that the *Ateles* karyotypes are quite different from any other Platyrrhini. These species are indeed characterized by many rearrangements that gave rise to a reduced number of chromosome pairs covered by many human syntenic associations with many that are unique (presumably in association also with apomorfism LINE-1 signals) and that could also be related to the high degree of ecological specialization of these species.[41-43,46-48] Also chromosome Y in *A. geoffroyi* shows LINE-1 amplified signals in the big C positive band (Figure 1), presumably related to the history of this chromosome in *Ateles*, characterized by a translocation with an autosomal chromosome

and changes in size linked to the presence of variable amounts of heterochromatic sequences.[47]

In *C. pygmaea*, non-centromeric signals as well as centromeric or pericentromeric blocks were found (Figure 1b, 2d); the LINE-1 accumulation occurred in almost all the centromeres on both bi-armed and acrocentric chromosomes (Figure 1b, 2d), co-localized with heterochromatin (Figure 1b, 2f) but with a partial corrispondence between them and CMA3 positive bands (Figure 3d), and with few exceptions in agreement with what has previously been shown in many other mammals[27-29,31] and primates.[31,37-39] The obtained results, together with previous literature data, support the hypothesis about LINE-1 elements at centromeres or pericentromeric regions in CMA3 and C positive bands in Cebidae. Thus, these signals at centromeres possibly indicate that LINE-1, and not just the classic alpha satellite DNA,[54] are presumably responsible for the architecture of almost all bi-armed or acrocentric chromosomes in platyrrhines.[37-39] The presence of LINE-1 at centromeres is not surprising since LINE-1-like elements have also been shown in other primates;[30] moreover, an innovative sequencing method applied to the entire human chromosome 8 and on some acrocentrics has recently demonstrated that LINE-1 sequences are part of the centromeric region.[55,56]

The interstitial LINE-1 signals found along chromosomes at non-centromeric regions, through a comparison with the human chromosomal homology extrapolated from previously obtained painting data for *C. pygmaea,*[44] support the link between LINE-1 and rearrangements.[8,9,32,37,38] Indeed, for example on chromosomes 1 and 4 in CPY covered respectively, by human syntenic associations 13/9/22 and 20/17/13 (Figure 1b), LINE-1 signals presumably fall at the junction of human syntenies as it occurs for example on their homologues, respectively chromosome 1 and 2 in *Saguinus mystax, Leontocebus fuscicollis,* and *Leontopithecus rosalia.*[37] This distribution at the junction of human syntenies in CPY also are in agreement with the above observations in Atelidae.

Conclusions

The data analysis for the two *Ateles* species in a phylogenetic framework suggests that the rich LINE-1 amplification presumably at the junction point of human syntenic associations is linked to inter-chromosomal rearrangements; other signals, such as some at telomeric positions, could be linked to intra-chromosomal rearrangements such as inversions; thus, the rich pattern observed in these species could be linked to genome reshuffling in these highly derived genomes. The absence of centromere signals could also be a consequence of this reshuffling.

Furthermore, our data analysis on *C. pygmaea* show both non-centromeric LINE-1 and centromeric signals, in agreement with data obtained in other Platyrrhini species, supporting previous hypotheses regarding the link between LINE-1 with chromosomal rearrangements and also with centromeric regions.

Despite all this evidence, however, further studies in more species are needed in order to clarify the role of LINE-1.

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