ZOOLOGICAL SYSTEMATICS AND EVOLUTIONARY RESEARCH

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Chromosomal distribution of interstitial telomeric sequences in nine neotropical primates (Platyrrhini): possible implications in evolution and phylogeny

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Abstract

To localize interstitial telomeric sequences (ITSs) and to test whether their pattern of distribution could be linked to chromosomal evolution, we hybridized telomeric sequence probes (peptide nucleic acid, PNA) on metaphases of New World monkeys: *Callithrix argentata, Callithrix jacchus, Cebuella pygmaea, Saguinus oedipus, Saimiri sciureus, Aotus lemurinus griseimembra, Aotus nancymaae* (Cebidae), *Lagothrix lagotricha* (Atelidae) and *Callicebus moloch* (Pithecidae), characterized by a rapid radiation and a high rate of chromosomal rearrangements. Our analysis of the probe signal localization allowed us to show in all the species analysed, as normally, the telomeric location at the terminal ends of chromosomes and unexpected signal distributions in some species. Indeed, in three species among the nine studied, *Aotus lemurinus griseimembra, Aotus nancymaae* (Cebidae) and *Lagothrix lagotricha* (Atelidae), we showed a high variability in terms of localization and degree of amplification of interstitial telomeric sequences, especially for the ones found at centromeric or pericentromeric positions (het-ITS). A comparative analysis, between species, of homologous chromosomes to human syntenies, on which we have found positive interspersed PNA signals, allowed us to explain the observed pattern of ITS distribution as results of chromosomal rearrangements in the neotropical primates analysed. This evidence permitted us to discuss the possible implication of ITSs as phylogenetic markers for closely related species. Moreover, reviewing previous literature data of ITSs distribution in Primates and in the light of our results, we suggest an underestimation of ITSs and highlight the importance of the molecular cytogenetics approach in characterizing ITSs, which role is still not clarified.

Key words: Chromosomes – telomeric sequences – genome evolution – phylogeny – owl monkeys

Introduction

Using classic and molecular comparative cytogenetic approaches, it is possible to determine chromosomal homologies, rearrangements and cytogenetic marker variation involved in genome evolution. Recent cytogenetic and molecular studies have underlined the importance of genome variability related to repetitive sequences, such as interstitial telomeric sequences (ITSs). ITSs are a potential cause of genome plasticity and chromosomal evolution (Nergadze et al. 2004, 2007; Ruiz-Herrera et al. 2008; Nagamachi et al. 2013; Bruschi et al. 2014), but their role in these events is not fully understood. The conservation of telomeric sequences, the terminal regions of chromosomes, is well known (Meyne et al. 1989). In vertebrates such as fish, reptiles, amphibians, birds and mammals, including primates, the DNA component of telomeric sequences can also be found at intrachromosomal sites, close to the centromeres and/or between centromere and telomeres (Meyne et al. 1990; Vermeesch et al. 1996; Azzalin et al. 1997, 2001; Garagna et al. 1997; Metcalfe et al. 1997, 1998; Fagundes and Yonenaga-Yassuda 1998; Pellegrino et al. 1999; Finato et al. 2000; Go et al. 2000; Hirai 2001; Ruiz-Herrera et al. 2002, 2005, 2008; Mudry et al. 2007; Nagamachi et al. 2013). ITSs have been associated with different genome organization: (1) to chromosomal rearrangements such as fusion, fission and inversion in vertebrates, as hypothesized through the cytogenetic approach (Meyne et al. 1990; Lee et al. 1993; Vermeesch et al. 1996; Metcalfe et al. 1997; Bouffler 1998; Finato et al. 2000; Go et al. 2000; Azzalin et al. 2001; Nanda et al. 2002; Ruiz-Herrera et al. 2002, 2005, 2008; Mudry et al. 2007; Bolzán 2012); (2) to the mechanism of genome reorganization such as double DNA strand break repair, as demonstrated through a comparative molecular approach, analysing DNA sequences in Chinese hamster and in many primates (Faravelli et al. 2002; Nergadze et al. 2004, 2007; Ruiz-Herrera et al. 2008; Farré et al. 2009;). These ITSs are not related to the breaks needed to produce the rearrangements (Farré et al. 2009) but rather arise through the mechanism of DNA repair that acts by the capture of telomeric DNA fragments at the break point site or by telomerase enzymes (Nergadze et al. 2004, 2007; Ruiz-Herrera et al. 2008); these ITS behaving as transposable elements can be used as informative markers for phylogenetic studies; (3) to the process of recombination and amplification of the terminal end of chromosomes (Azzalin et al. 2001; Ruiz-Herrera et al. 2008; Bruschi et al. 2014); and 4) to a mechanism of centromere/telomere interchanges that occur during the evolution of eukaryotic chromosomes (Villasante et al. 2007). Examples with at least three centromere/telomere interchanges involving telomeric repetitive sequences have been shown in Platyrrhini (Primates) (Rocchi et al. 2012). Also the relationship between centromere and telomere sequences has recently been suggested, based on a molecular approach (Prakhongcheep et al. 2013).

To verify whether ITS distribution is correlated with rearrangements in rapid-evolving genomes, we mapped a telomeric sequence (TTAGGG)n (PNA) probe on Platyrrhini: *Callithrix argentata* (Linnaeus, 1766), *Callithrix jacchus* (Linnaeus, 1758), *Cebuella pygmaea* (Spix, 1823), *Saguinus oedipus* (Linnaeus 1758), *Saimiri sciureus* (Linnaeus, 1758), *Aotus lemurinus griseimembra* (Hershkovitz, 1983), *Aotus nancymaae* (Hershkovitz, 1983) (Cebidae), *Lagothrix lagotricha* (Humboldt, 1812) (Atelidae) *and Callicebus moloch* (Hoffmannsegg, 1812) (Pithecidae). ITSs locations have been analysed considering their possible correlation with heterochromatin regions detectable through C-banding. This work extends a previous analysis in New World monkeys (Mudry et al. 2007). The choice of these species was based on the fact they are products of a rapid radiation and are characterized by high genome plasticity and reshuffled karyotypes (Dumas et al. 2007; Stanyon et al. 2008). In this perspective, these species can be used as a promising model for the

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study of genome organization and evolution. Moreover, employing a comparative approach through the analysis of previous literature data, we report the distribution of the diverse kinds of ITSs found in Primates.

Materials and methods

Following the standard protocol (Small et al. 1985), metaphases were obtained for all specimens: *Callithrix argentata, Callithrix jacchus, Cebuella pygmaea, Saguinus oedipus, Saimiri sciureus, Aotus lemurinus griseimembra, Aotus nancymaae* (Cebidae), *Lagothrix lagotricha* (Atelidae) and *Callicebus moloch* (Pithecidae) from primary cultures of lymphoblast or fibroblast cell lines and successively fixed on slides at the National Cancer Institute, USA. The *Aotus* specimens, identified by Dumas et al. (2015) as *A. lemurinus griseimembra,* were kindly offered by professor T. Ishida from University of Tokyo. The species were karyologically analysed; the diploid number (2n) has been identified by G- and C-banding and compared with the corresponding G- and C-banding patterns previously published for the Ceboidea. Chromosomes were also identified using DAPI followed by C-banding; DAPI fluorochrome has a higher affinity for AT-rich DNA then GC as well as C-banding, and for this reason, bright DAPI regions overlap C-bands.

The distribution of the telomeric sequence (TTAGGG)n was analysed in each species above mentioned using fluorescence *in situ* hybridization (FISH) with a FITC-conjugated peptide nucleic acid (PNA) oligonucleotide probe obtained from Panagene, Cambridge Research Biochemicals. To possibly be more efficient in detecting ITSs, hybridization has been performed following the protocols purchased by Panagene, adjusting stringency conditions. Even if literature data report hybridization with telomeric probe performed in low stringency, we performed our FISH experiment both in high and low stringency. At high stringency, we have found pericentromeric ITS (het-ITS) and ITS (s-ITS) at internal position, while telomeric signals appeared to be faint or absent as shown even in previous studies on rodents (Rovatsos et al. 2011); at low stringency, we have found much more strong and larger signals at telomerics ends (subtelo-ITSs).

The location and abundance of the ITSs in the genomes have been analysed looking for the PNA signals on a large number of metaphases, 20 for each specimen, due to the fact some signals could show a different intensity between the various metaphases and even between homologues chromosomes (Hirai 2001). All digital images were obtained using a Leica DMRXA2 epifluorescence microscope equipped with appropriate filters and a CCD camera (Princeton Instruments); merging of images was performed using Adobe Photoshop software.

Results

The PNA probe in all the specimens analysed, as expected, shows telomeric signals (Fig. 1). The intensity of telomeric sequence signals, different within and between Ceboidea, is correlated with the length of the sequence repetition (Ruiz-Herrera et al. 2002, 2005, 2008).

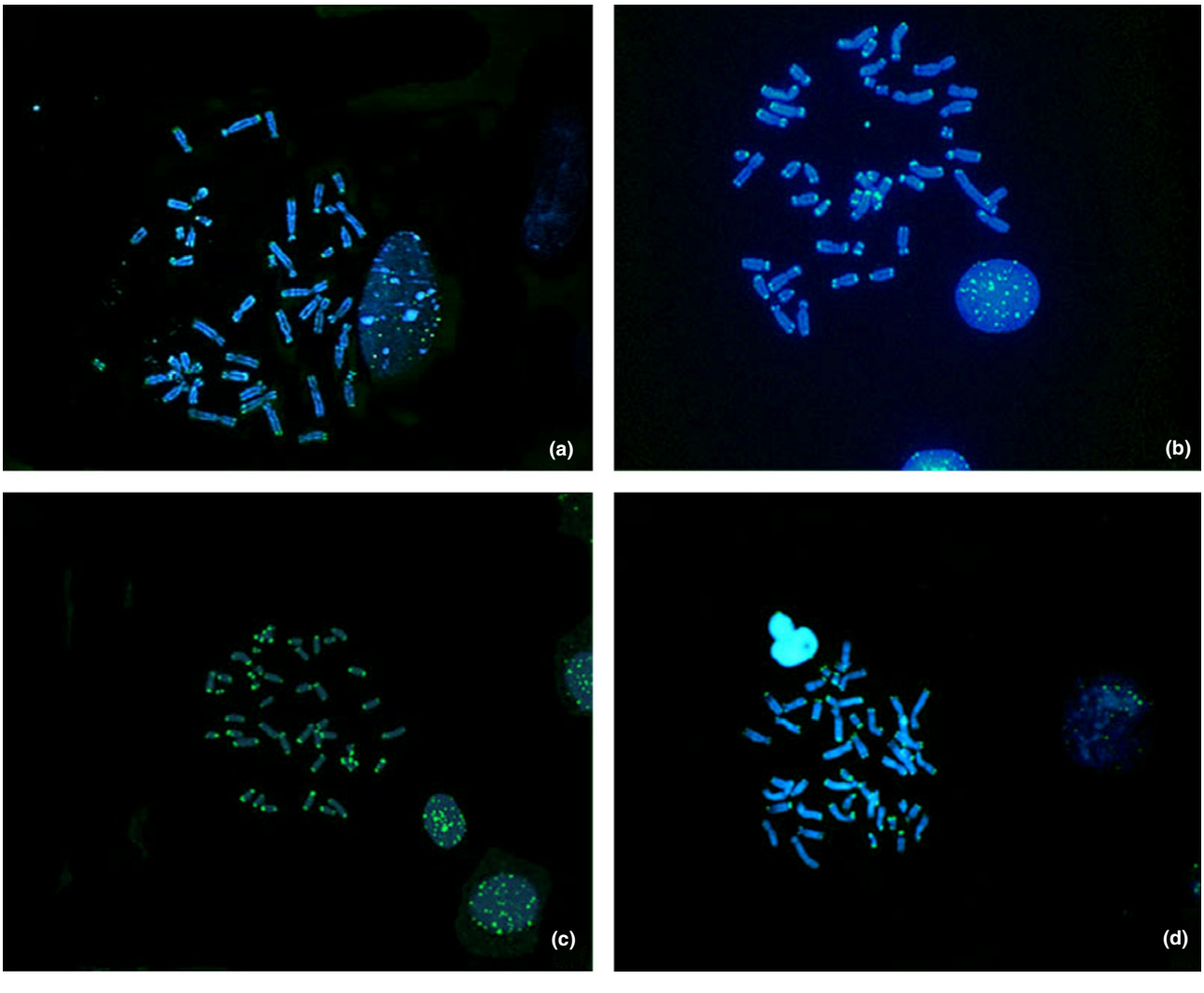

Fig. 1. Example of telomeric sequence probe (PNA) mapping on: (a) *Callithrix jacchus* (CJA), (b) *Cebuella pygmaea* (CPY), (c) *Callithrix argentata* (CAR), (d) *Saguinus oedipus* (SOE); all species showing a telomeric sequence pattern only

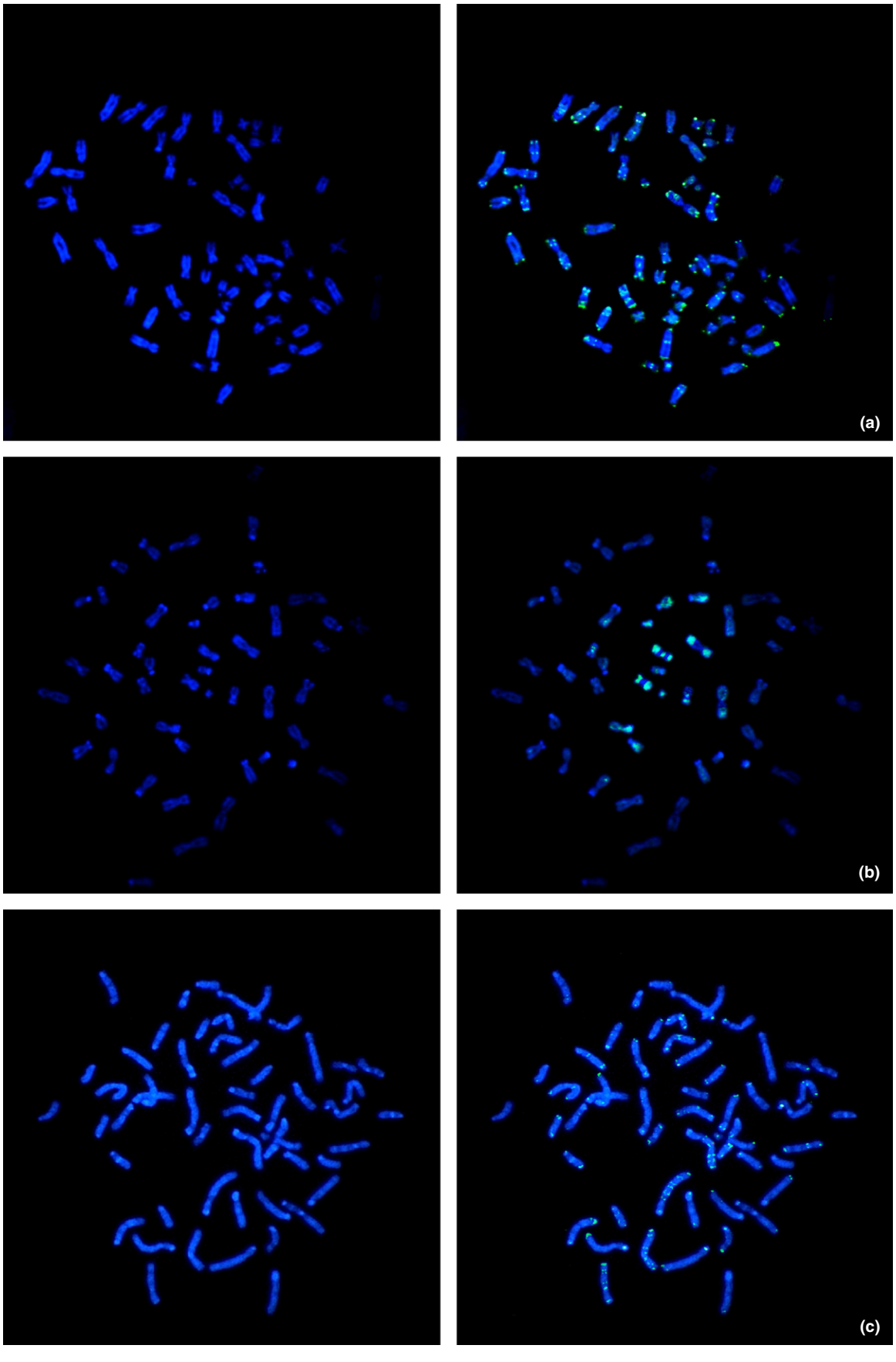

Fig. 2. The DAPI chromosomes (in blue), and DAPI/PNA combined (in blue/green) (a) *Lagothrix lagothrica* (LLA); (b) *Aotus nancymaae* (ANA), (c) *A. lemurinus griseimembra* (ALG)

Interestingly, apart from those signals classically localized at the terminal ends of all chromosomes, several interspersed telomeric sequences (ITSs), especially centromeric and pericentric (het-ITSS), have been found in *Lagothrix lagotricha* (LLA), *Aotus nancymaae* (ANA) and *Aotus l. griseimembra* (ALG), (Fig. 2). Karyotypes have been reconstructed (Fig. 3) through the analysis of C- and G-banding for the species *Lagothrix lagotricha* (*2n=62*), *Aotus l. griseimembra* (2n=53) and *Aotus nancymaae* (2n=54) in accordance with previous cytogenetics data (Ma et al. 1976; Dutrillaux et al. 1980; Pieczarka et al. 1998) and following previous chromosomes number identification (Stanyon et al. 2001, 2004, 2011; Ruiz-Herrera et al. 2005; Dumas et al. 2015). In *Lagothrix lagotricha,* ITS signals have been found on at least seven couple of bi-armed chromosomes, on chromosome X and at least three couple of acrocentric chromosomes (Fig. 2a).

In *Aotus nancymaae,* ITS signals have been found on at least five couple of bi-armed chromosomes and on at least two couple of acrocentric chromosomes (Fig. 2 b). ITS signals in this species have been found vary intensive and particularly diffused.

In *Aotus l. griseimembra,* ITS signals have been found on at least five couple of bi-armed chromosomes, plus one, the polymorphic chromosome 1 previously described (Stanyon et al. 2011; Dumas et al. 2015) and on the two unpaired acrocentric chromosomes (Fig. 2c).

ITS sequences on chromosomes could be underestimated because we considered just PNA bright signals found on the majority of the metaphases analysed.

Discussion

Het-ITS and Platyrrhini

Among Platyrrhini species, telomeric sequences location has been previously analysed in *Alouatta caraya*, *Alouatta palliate, A. guariba clamitans, Ateles chamek* (Atelidae), *Cebus apella* (Ruiz-Herrera et al. 2005), *Cebus nigritus, Cebus paraguayanus, Aotus azarae* and *Saimiri boliviensis boliviensis* (Cebidae) (Mudry et al. 2007). We extended these analyses, identifying ITSs localizations in other New World monkeys by hybridizing the PNA probe on *Callithrix argentata, Callithrix jacchus, Cebuella pygmaea, Saguinus oedipus, Saimiri sciureus, Aotus lemurinus griseimembra, Aotus nancymaae, Lagothrix lagotricha* and *Callicebus moloch.* Apart from the normal signal at terminal ends of chromosomes in all species, we showed many interspersed telomeric sequences, mainly heterochromatic, centromeric and pericentromeric, with different frequency, intensity and localization into three species: *Lagothrix lagotricha, Aotus nancymaae* and *Aotus l. griseimembra* (Fig. 2). Also in *Callithrix argentata,* we found very bright PNA signals at terminal ends of many bi-armed and acrocentric chromosomes (Fig. 1c), but, in the present study, we took into consideration mainly ITSs at inner positions. ITS regions in those ceboidea specimens analysed, *L. lagotricha* and *Aotus*, mostly correspond with heterochromatin, even if, as expected, it does not precisely correspond with the whole C-pattern. These results, occurring especially in species in which karyotypes are among the most rearranged in Platyrrhini, lead us to propose that ITSs could be related to genome evolution and provide phylogenetic information. Our preliminary comparative analysis, among species, of dapi banding homologous chromosomes on which PNA signals have been found, allows us to propose, as it is possible to appreciate observing ideograms reconstructed for each species karyotype of *Lagothrix lagotricha, Aotus nancymaae* and *Aotus l. griseimembra* (Fig. 3), which the telomeric sequence repeats, fall on chromosomes formed by human ancestral associations of primates (3/21, 14/15) as well as platyrrhine ones (2/16, 5/7, 8/18 10/16) present in the hypothetical ancestral karyotypes previously proposed (Stanyon et al. 2008); at least on chromosomes where these associations are conserved as occur especially in *L. lagotricha* (Fig. 3a). These results lead us to hypothesize ITS as linked with rearrangements responsible of the formation of such associations. Our data are in agreement with similar reports that found ITS on *C. apella* chromosomes 1, 4, 5, 7 and 11, respectively, formed by associated fragments of human syntenies 5/7, 10/16, 2/16, 8/18 and 3/21 (Ruiz-Herrera et al. 2005). A slightly different ITS pattern has been shown in *A. nancymaae* and *A. l. greisemembra* where these associations have been reorganized through other rearrangements (Fig. 3); in particular, we showed a very peculiar ITS pattern with intensive and diffused ITS signals on many chromosomes of *A. nancymaae* (Fig. 3b), probably due to the many fusion events characterizing its derivative karyotype (Stanyon et al. 2004). This pattern of distribution of ITSs observed explained as the result of chromosomal rearrangements, especially fusions and fissions, leads us to support the hypothesis that interstitial telomeric sequences should be considered as remnants of ancestral and more recent chromosomal rearrangements (Slijepcevic 1998; Ruiz-Herrera et al. 2005). Indeed, a part from these signals found on ancestral associations, ITS presumably could be also results of rearrangements forming new associations. For example, a well-defined PNA signal at the centromere has been found on metacentric chromosomes 5, 6 of *A. l. greisemembra,* probably originated by a fusion of human syntenies forming the more recent associations 16/1 and 16/22 (Fig. 3c); these results are in agreement with data previously found in *Macaca fascicularis* where new associations 7/21 and 20/22, respectively, on ch. 2 and ch. 13, both originated by fusion, showed ITS at centromeric position (Ruiz-Herrera et al. 2002, 2005, 2008). Moreover, we have found a PNA signal at pericentromeric positions of the polymorphic chromosome 1 of *A. l. greisemembra,* presumably originated by fusion of two acro/submetacentric chromosomes (Fig. 3c). The putative mechanism of the origin of these ITS, by centric fusions of two chromosomes, occurs in our opinion or (Fig. 3d) conserving the normal telomeric sequences (resulting with a centromeric ITS), as seen in the previous case for chromosomes 5 and 6 of *A. l. greisemembra*; or (Fig. 3e) with the loss of part of the ITS sequences (resulting with a pericentromeric ITS) due to a breakage within satellite DNA and elimination of normal telomeric sequences as seen, for example in *A. l. greisemembra* chromosome 1, in accordance with what previously proposed (Garagna et al. 2001; Rovatsos et al. 2011).

Other diverse ITSs in *Lagothrix* and *Aotus* species could be the result of different rearrangements such as fission, inversions, formation of new evolutionary centromeres or of other mechanism involving transposons (Nergadze et al. 2004) or due to 'amplification', as demonstrated also for other animals (Adegoke et al. 1993; Garrido-Ramos et al. 1998; Wijayanto et al. 2005; Rovatsos et al. 2011). For example, in *Lagothrix lagotricha* we have found the PNA signals, at the centromere of the metacentric chromosome 14 and on the upper part of the acrocentric chromosome 19, both homologous to human chromosome 4 (Fig. 3a), originated by fission of a bi-armed chromosome resulting into three fragments (Stanyon et al. 2001); our PNA signals are presumably in correspondence with the putative new centromere demonstrated through BACs mapping on LLA ch. 19 (Rocchi et al. 2012) as well with the old one presumably present on LLA ch. 14. The presence of the ITS sequences both at old and new centromeric positions leads us to support the hypothesis about a correlation of ITSs sequences with the evolutionary new centromere activation process.

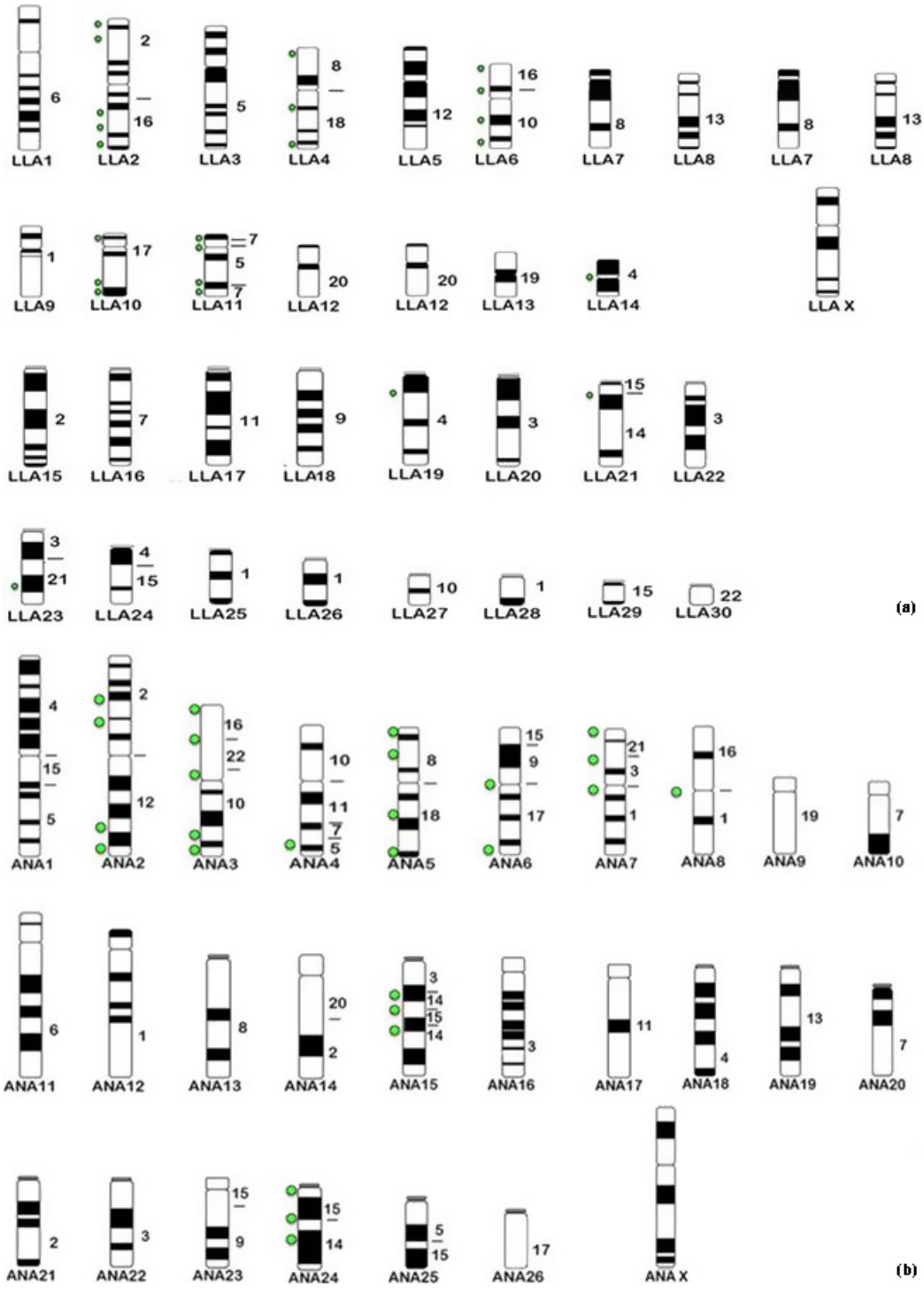

Fig. 3. Chromosome ideograms for the three species karyotypes (reconstructed in agreement with Stanyon et al. 2001, 2004, 2011 and Dumas et al. 2015) showing the location of ITS on the left (green dots) and the homology with human syntenic associations on the right; PNA probe signals, identified analysing many metaphases for each species, have been localized on: (a) *Lagothrix lagotrica* (LLA) homologous chromosomes formed by human platyrrhini associations 2/16, 5/7, 8/18, 10/16, and human primates associations 3/21, 15/14, respectively, on ch. 2, 11, 4, 6 and 23, 21. Also note PNA signals on LLA ch. 14 and 19 probably involved in the new centromere activation process as previously shown (Rocchi et al. 2012). (b) *A. nancymaae* (ANA), homologous chromosomes formed by human association 5/7, 8/18, 10/16, respectively, found on ch 4, 5 and 3 (last one interrupted by a fragment of human synteny 22); on ancestral human associations 3/21 and 15/14, respectively, on ch. 7 and 15, 24 (note that the putative ancestral platyrrhini association 2/16, is not present in ANA); on new associations 16/1 and 9/17, respectively, on ch. 8 and 6 (larger dots for this species stand for stronger and diffuse signals). (c) *A. l. greisememb*ra homologous chromosomes formed by human association 5/7 found on ch. 3 as well on the ancestral associations 3/21 and 15/14 found, respectively, on ch. 4 and 8 (note that the putative ancestral platyrrhini associations 2/16, 8/18, 10/16 are not present in ALG); on new human associations 16/1 and 16/22 localized on ch. 5, 6. (d, e) Putative mechanism of origin of het-ITS by centric fusions of acrocentric and/or submetacentric chromosomes containing het-ITSs; this fusion might occur: (d) following breakage within satellite DNA and elimination of normal telomeric sequences or alternatively conserving the normal telomeric sequences, as previously shown (Garagna et al. 2001; Rovatsos et al. 2011); (f) Graphic representation of the different kinds of ITSs: Het, s and subtelo

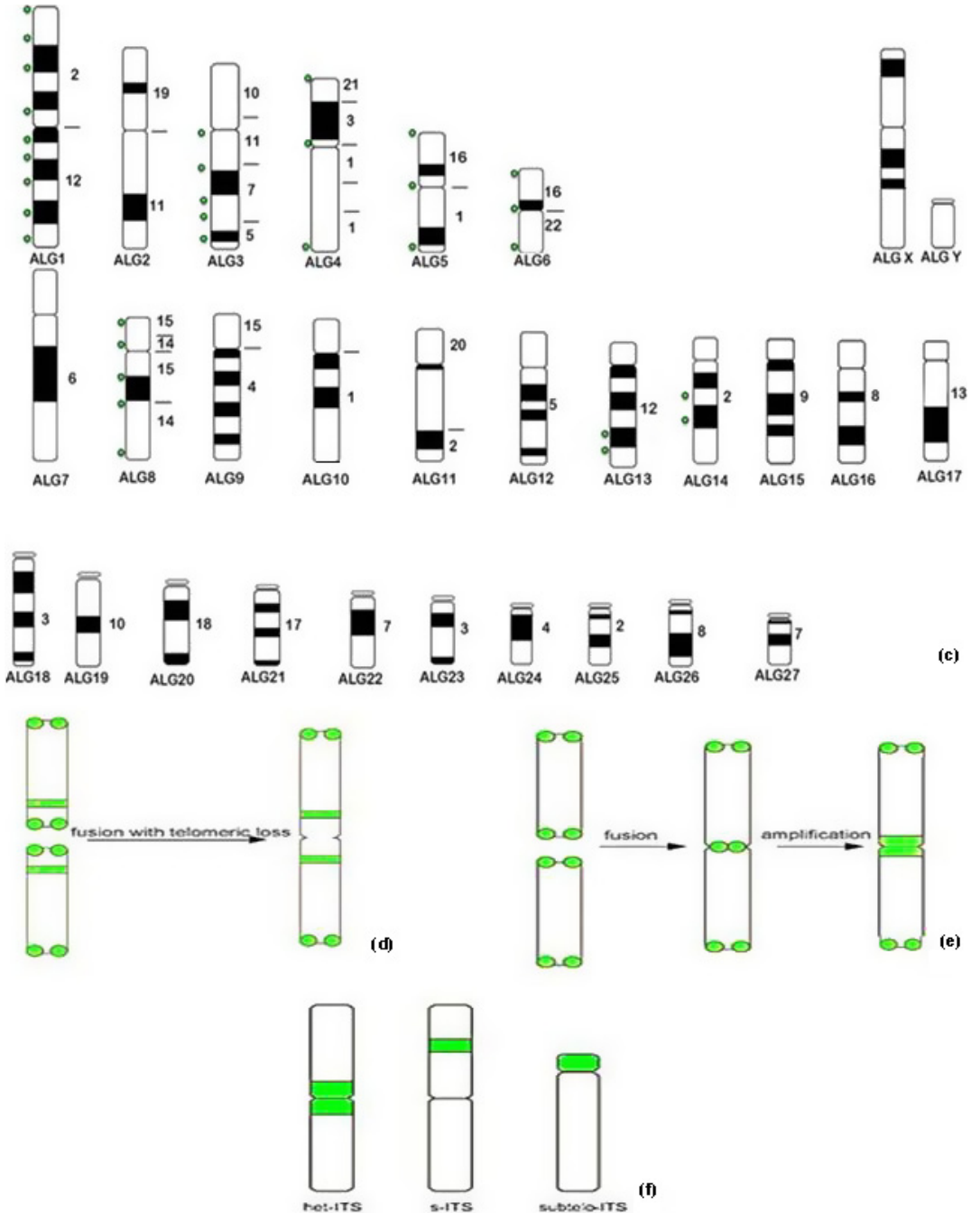

Fig. 3. (continued)

The data presented here on the possible identification of the exact chromosomes on which PNA signals fall are only preliminary results, and further analyses should be performed through BAC (bacterial artificial chromosomes) and painting probes to define without any doubt human homologues chromosomes, especially the little acrocentric chromosomes not always easily identifiable. Moreover, it is also to take into account that some ITS could have not been detected even in this work, probably, as proposed by others researchers, due to problems with the resolution limit of the methods and/or the insufficient repetition of the sequences. As occurred, for example, in two other *Cebus* species previously analysed, *Cebus nigritus* and *Cebus paraguayanus* (Mudry et al. 2007).

Het-ITS and *Aotus*

Comparing the ITS patterns among species of the same genus *Aotus,* we showed a different distribution of ITS not only in *A. nancymaae* and *A. l. greisememembra* but also in a previously studied species, *Aotus azarae,* in which it was reported ITS sequences present at the pericentromeric region of a pair of submetacentric chromosomes (het-ITS), probably as researchers suggested, representing a remnant of an ancestral fusion event that occurred during karyotype evolution (Mudry et al. 2007). We correlated such different ITSs patterns of distributions with the evolutionary history of *Aotus*. The monogamous *Aotus*, the only nocturnal among Platyrrhini, is characterized by a deep phyloge-

Table 1. Presence/absence and different kinds of ITSs in all the species analysed, in the present work and literature

	2n	ITSs	References
Strepsirrhini			
Cheirogaleidae			
Microcebus murinus (MIM)	34	–	Meyne et al. (1990)
Lorisidae			
Otolemur crassicaudatus (OCR)	62	–	Go et al. (2000)[2]
Perodicticus potto (PPO)	62	–	Meyne et al. (1990)
Lemuridae			
Daubentonia madagascariensis (DMA)	30	–	Rakotoarisoa et al. (2000)[2]
Eulemur coronatus (ECO)	46	het-ITS subtelo-ITS	Garagna et al. (1997) Go et al. (2000)[2]
Eulemur fulvus fulvus (EFF)	60	het-ITS subtelo-ITS	Garagna et al. (1997) Go et al. (2000)[2]
Eulemur macaco (EMA)	44	het-ITS subtelo-ITS	Meyne et al. (1990) Garagna et al. (1997)
Eulemur rubriventer (ERU)	50	het-ITS	Garagna et al. (1997)
Hapalemur griseus alaotrensis (HGA)	54	het-ITS s-ITS[1] subtelo-ITS	Go et al. (2000)[2]
Hapalemur simus (HSI)	60	subtelo-ITS	Go et al. (2000)[2]
Lemur catta (LCA)	56	het-ITS[1] subtelo-ITS	Go et al. (2000)[2]
Varecia variegata variegata (VVV)	64	het-ITS[1]	Go et al. (2000)[2]
Indridae			
Propithecus verreauxi verreauxi (PVV)	72	het-ITS s-ITS[1] subtelo-ITS[1]	Go et al. (2000)[2]
Haplorrhini			
Platyrrhini			
Cebidae			
Aotus azarae (AAZ)	49/50	het-ITS[1]	Mudry et al. (2007)
Aotus lemurinus griseimembra (ALG)	52/53/54	het-ITS s-ITS subtelo-ITS	Present work
Aotus nancymaae (ANA)	54	het-ITS s-ITS	Present work
Callithrix argentata (CAR)	44	subtelo-ITS	Present work
Callithrix jacchus (CJA)	46	–	Present work
Cebuella pygmaea (CPY)	44	–	Present work
Cebus apella (CAP)	28	het-ITS s-ITS subtelo-ITS	Ruiz-Herrera et al. (2005)
Cebus nigritus (CNI)	54	–	Mudry et al. (2007)
Cebus paraguayanus (CPA)	54	–	Mudry et al. (2007)
Saguinus oedipus (SOE)	46	–	Present work
Saimiri boliviensis (SBO)	44	–	Mudry et al. (2007)
Saimiri sciureus (SSC)	44	–	Present work
Pitechiidae			
Callicebus moloch (CMO)	50	–	Present work
Atelidae			
Alouatta caraya (ACA)	52	–	Mudry et al. (2007)
Alouatta guariba clamitans (AGU)	45/46	–	Mudry et al. (2007)
Alouatta palliata (APA)	53/54	–	Mudry et al. (2007)
Ateles chamek (ACH)	34	–	Mudry et al. (2007)
Lagothrix lagotricha (LLA)	62	het-ITS s-ITS subtelo-ITS	Present work
Catarrhini			
Cercopithecidae			
Macaca fascicularis (MFA)	44	het-ITS s-ITS subtelo-ITS	Ruiz-Herrera et al. (2002)
Hylobatidae			
Hylobates agilis (HAG)	44	–	Wijayanto et al. (2005)[2]
Symphalangus syndactylus (SSY)	50	s-ITS[1] subtelo-ITS	Hirai (2001)[2] Wijayanto et al. (2005)[2]
Hominidae			
Gorilla gorilla (GGO)	48	het-ITS s-ITS[1] subtelo-ITS	Hirai (2001)[2]

Table 1. (continued)

	2n	ITSs	References
Pan paniscus (PPA)	48	het-ITS s-ITS subtelo-ITS	Hirai (2001)[2]
Pan troglodytes (PTR)	48	het-ITS s-ITS subtelo-ITS	Hirai et al. (2005)[2]
Pongo pygmaeus (PPY)	48	–	Meyne et al. (1990)
Homo sapiens (HSA)	46	het-ITS s-ITS subtelo-ITS	Azzalin et al. (2001)

[1]Signal present in one couple of chromosomes
[2]Studies performed with PRINS (Primed *in situ* labelling) technique

netic split between northern 'grey-necked' (*A. nancymaae* and *A.l. griseimembra,* 2n=54, 53) and southern Amazonian 'red-necked' (*A. azarae,* 2n=50), and by the separation of *A. nancymaae,* that became genetically isolated, as a result of a western migration (Plautz et al. 2009; Menezes et al. 2010;). The greater antiquity, and geographic isolation with evolutionary pressures, of northern *Aotus* taxa, would explain the many morphological, immunological and karyotype differences seen in owl monkey species (Ford 1994; Defler and Bueno 2007; Fernandez-Duque 2011). On the contrary, the majority of southern species are identical from a karyological point of view; almost all possess the same diploid number, suggesting that the southern expansion and radiation of *Aotus* was gradual and with a role of gene flow. One simple explanation for the current distribution of telomere sequences in *Aotus* species would be linked with their evolutionary history; in the common ancestor of closely related species, ITSs were probably present, whose origins and history are presumably linked with rearrangements and amplification; subsequently, the different evolutionary history and distance of species were reflected in the frequency and distribution of ITSs. Indeed, in the species analysed belonging to the two groups described above, we have *A. nancymaae,* the most ancient showing a high reshuffled karyotype showing both intact platyrrhini ancestral human associations as well a lot of new ones, colocalized with abundant and diffused ITSs; *A. l. griseimembra* having an intermediate position in terms of conserved human associations and abundance of ITS, and *A. azarae* with just one ITS. This perspective would be in agreement with molecular phylogenetic reconstruction (Babb et al. 2011); unlikely in *A. azarae* chromosomes, human homologues have not yet been establish and it is not possible to identify in which chromosome exactly the ITSs fall. For this reason, further analysis is needed to better elucidate this model.

ITS and Primates

Our results, combined with data from the literature, permitted us to review, in Primates, the chromosomal distribution of the different types of ITSs (Fig. 2f) as previously defined (Azzalin et al. 2001; Ruiz-Herrera et al. 2008): (1) ITSs in subtelomeric position (subtelo-ITSs) as a possible result of recombination or amplification processes, (2) het-ITSs, large telomeric repeats that are mainly pericentromeric, possibly remnants of chromosomal rearrangements such as fusion or inversions (Ruiz-Herrera et al. 2002, 2005, 2008), with just a few deriving from end-to-end fusion like that occurred for HSA2 (Ijdo et al. 1991; Ventura et al. 2012), (3) short ITSs (s-ITSs), small repeated sequences at an internal chromosomal position considered to be relics or scars of ancient double-strand breakage identified through the molecular approach (Fig. 2f). Reviewing data in literature, we acknowledge that chromosomal localizations of telomeric sequences have been determined with HSA telomeric probes in other primates: among Strephirrhini in *Microcebus murinus (Cherogaleida*e) (Meyne et al. 1990), *Daubentonia madagascariensis (*Rakotoarisoa et al. 2000), Lorisiforms, *Perodicticus potto* (Meyne et al. 1989) and Oto*lemur crassicaudatus* (Go et al. 2000), Lemuriformes, *Lemur macaco rufus* (Meyne et al. 1989, 1990; Garagna et al. 1997), *Eulemur fulvus fulvus, Eulemur coronatus* (Garagna et al. 1997; Go et al. 2000), *Eulemur rubriventer, Lemur catta, Hapalemur griseus alaotrensis, H. sinus, Varecia variegata variegata* (Lemuridae), *Propithecus verreauxi verreauxi* (Indridae) (Go et al. 2000); among Haplorrhni in Catarrhini on: *Macaca fascicularis* (Cercopithecidae), (Ruiz-Herrera et al. 2002b) *Hylobates syndactylus* (Hylobatidae), (Hirai 2001; Wijayanto et al. 2005) and *Gorilla gorilla, Pongo pygmaeus, Pan troglodytes, Pan paniscus,* (Hominidea) (Meyne et al. 1989; Hirai 2001; Hirai et al. 2005). The data reported in the present study represent a useful refinement of genome variability and plasticity in Primates, showing the amount of ITS occurrence and variability, and helpful to discern the various kinds of ITSs, are particularly the ones that can provide phylogenetic information (het-ITS and s-ITS). Summarizing the literature and our study, the presence/absence and the different kinds of ITSs in Primates are shown (Table 1): among Strephirrini, ITSs have not been found in *Otolemur crassicaudatus, Perodicticus potto, Daubentonia madagascariensis* and *Varecia variegata variegata* (except for a pericentromeric ITSs in the *V. variegata* X chromosome). In the other Strephirrini, two principal kinds of ITS are present: ITS sequences associated with heterochromatin region (het-ITS) and acro or p-subtelomeric ITS (p-subtelomeric-ITSs). Indeed in the lemur species, *Eulemur macaco* (Meyne et al. 1990; Garagna et al. 1997), *E. fulvus fulvus, E. coronatus* (Garagna et al. 1997; Go et al. 2000), *Hapalemur griseus alaotrensis, H. sinus, Lemur catta* (Go et al. 2000) signals have been found at pericentromeric regions involving constitutive heterochromatin of some bi-armed chromosomes, and on a large region of the short arm of acrocentric chromosomes; *Propithecus verreauxi verreauxi* shows some peculiarities: pericentromeric signals on two submetacentric chromosome pairs (het-ITS), a large and intense signal in the pericentromeric region of a metacentric chromosome pair but far from the centromeric heterochromatin region (s-ITS), and one signal in an acrocentric chromosome pair (acro, psubtelo-ITS) (Go et al. 2000). In *E. rubriventer,* only a very weak signal was detected at the pericentromeric region of a few bi-armed chromosomes (het-ITS) (Garagna et al. 1997). Among

Haplorrini, in Catarrhini, on M*acaca fascicularis* (Cercopithecidae), many ITS are present in almost all chromosomes with various hybridization frequencies: het-ITS, s-ITS and distal, and few ITS are conserved in *Homo sapiens* (Ruiz-Herrera et al. 2002, 2005, 2008). In Hominidae, many internal locations of the telomeric sequences corresponding with regions of constitutive heterochromatin have been shown in *Pan paniscus* and *Pan troglodytes:* s-ITS, acrocentric/subtelocentric ITSs, het-ITS (Hirai 2001; Hirai et al. 2005); in *Gorilla gorilla* het-ITS, acrocentric or subtelocentric (distal) but no interstitial s-ITS (except on the Y chromosome) are found (Hirai 2001). In Hylobatidae (*Symphalangus syndactylus* and *Hylobates agilis*), subtelo-ITSs have been found in almost all acrocentric chromosomes, s-ITS were found in a few metacentric chromosomes, and an het-ITS (Hirai 2001; Wijayanto et al. 2005). In these studies, researchers hypothesized an evolutionary correlation between rearrangements and ITS distribution and also between ITSs, C distribution and phylogenetic distance. However, this phylogenetic linkage remains a debated issue, since some studies propose a phylogenetic value among genera, for example in Hylobatidae (Wijayanto et al. 2005), while others propose, instead, a linkage valid for closely related species of the same genus as shown, for example, in Rodents (Rovatsos et al. 2011). Arguing the evolutionary implication of ITSs in Primates from our observations is not feasible because of incomplete current knowledge on the exact ITSs position in different homologues and on the exact mechanism generating the diverse ITSs; however, we can hypothesize that signals detected in the bi-armed chromosomes at centromeric position (het-ITS) fall in the same human ancestral primates association as we detect in our study, but comparisons with human homologues have not been performed in the majority of the species and further analysis are needed; from the analysis of s-ITSs distribution, we emphasize that they are not observed in Strepsirrhini, while they are present in some Haplorrhini. This evidence is not surprising and can be explained with the limitation of PNA probe mapping which detects large stretches of telomeric repeats corresponding to large blocks (spanning several hundred kb) of telomeric-like DNA mainly localized in centromeric or pericentromeric chromosome regions (het-ITS). In contrast, short ITSs (s-ITSs) are stretches of limited numbers of telomeric hexamers distributed at internal positions that could be normally detected using either low-stringency FISH or molecular approaches and sequence database analysis.

In the light of our results, we support the hypothesis, in agreement with previous cytogenetics and molecular data (Ruiz-Herrera et al. 2008; Rovatsos et al. 2011), about ITSs related to rearrangements, ancestral and new ones, at least in the cases here detected. ITS. Furthermore on the base of our data, we support the hypothesis about usefulness of ITS in phylogenetic studies; indeed ITS patterns of distribution, their presence or absence could be used as phylogenetic markers especially in closely related species because as vary recent underlined 'sharing even a polymorphic mutation in closely related species may represent parsimonially as a strong signature of persistence since last common ancestor' (Dobigny et al. 2015).

In our opinion, ITSs in Primates have been underestimated (both because of low sequence repetition and the approach resolution limit) and we suggest, in a multidisciplinary prospective, to increase through cytogenetics and molecular approaches the knowledge on ITSs distribution in Primates to better clarify their implication in evolutionary and phylogenetic studies. Moreover, this kind of approach advance understanding of ITSs role and function; some ITSs may represent junk DNA while others can be inserted in regions that modify gene expression, others could promote illegitimate recombination, genome rearrangements, whereas others may be linked with the occurrence of new centromeres and telomeres, all aspects worthy to be addressed.

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Riassunto

La distribuzione delle sequenze telomeriche intersperse sui cromosomi di nove Platyrrhinae: possibili implicazioni evolutive e filogenetiche

Al fine di localizzare le sequence telomeriche intersperse (ITS) e verificare se il loro pattern di distribuzione è ricollegabile all'evoluzione cromosomica sono state ibridate sonde telomeriche (TTAGGG)n sulle metafasi di specie Platyrrhinae caratterizzate da una rapida radiazione ed da un alto tasso di riarrangiamenti cromosomomici: *Callithrix argentata, Callithrix jacchus, Cebuella pygmaea, Saguinus oedipus, Saimiri sciureus, Aotus lemurinus griseimembra, Aotus nancymaae* (Cebidae), *Lagothrix lagotricha* (Atelidae), e *Callicebus moloch* (Pithecidae). L'analisi del segnale della sonda PNA mappato sui cromosomi ha permesso di dimostrare, in tutte le specie, come atteso, la normale localizzazione delle sequenze telomeriche sulle estremità terminali dei cromosomi e solo su alcune di esse, invece, una distribuzione peculiare delle sequenze telomeriche intersperse. Infatti in tre specie tra le nove analizzate *Aotus lemurinus griseimembra, Aotus nancymaae* (Cebidae), e *Lagothrix lagotricha* (Atelidae) è stata dimostrata un'alta variabilità nella distribuzione e nel grado di amplificazione delle ITS, in special modo per quelle riscontrate in regioni centro o pericentromeriche (het-ITS). L'analisi comparativa dei cromosomi delle specie in esame, omologhi alle sintenie cromosomiche umane, sui quali si sono riscontrati segnali della sonda PNA interspersi, ha permesso di spiegare il *pattern* di distribuzione delle ITS osservato come risultato di riarrangiamenti cromosomici verificatesi nel corso dell'evoluzione in Primates, in particolar modo in Platyrrhinae. Queste evidenze hanno inoltre permesso di discutere le possibili implicazioni delle ITS come *marker* filogenetici. In ultimo, da un'analisi dei dati presenti in letteratura sulla distribuzione delle ITS in Primates ed alla luce dei risultati ottenuti si ipotizza che gli ITS possano essere stati sottostimati e si evidenzia l'importanza dell'approccio citogenetico nello studio degli ITS, sequenze il cui ruolo è ancora poco conosciuto e che meriterebbe maggiori approfondimenti.

References

Adegoke JA, Arnason U, Widegren B (1993) Sequence organization and evolution, in all extant whalebone whales, of a satellite DNA with terminal chromosome localization. Chromosoma **102**:382–388.

Azzalin CM, Mucciolo E, Bertoni L, Giulotto E (1997) Fluorescence in situ hybridization with a synthetic (T2AG3)n polynucleotide detects several intrachromosomal telomere-like repeats on human chromosomes. Cytogenet Cell Genet **78**:112–115.

Azzalin CM, Nergadze SG, Giulotto E (2001) Human intrachromosomal telomeric-like repeats: sequence organization and mechanisms of origin. Chromosoma **110**:75–82.

Babb PL, Fernandez-Duque E, Baiduc CA, Gagneux P, Evans S, Schurr TG (2011) mtDNA diversity in Azara's owl monkeys (*Aotus azarai azarai*) of the Argentinean Chaco. Am J Phys Anthropol **146**:209–224.

Bolzán AD (2012) Chromosomal aberrations involving telomeres and interstitial telomeric sequences. Mutagenesis **27**:1–15.

Bouffler SD (1998) Involvement of telomeric sequences in chromosomal aberrations. Mutat Res/Fundament Mol Mech Mutag **404**:199–204.

Bruschi DP, Rivera M, Lima AP, Zuniga AB, Recco-Pimentel SM (2014) Interstitial Telomeric Sequences (ITS) and major rDNA mapping reveal insights into the karyotypical evolution of Neotropical

leaf frogs species (Phyllomedusa, Hylidae, Anura). Mol Cytogenet **7**:22.

Defler TR, Bueno ML (2007) *Aotus* diversity and the species problem. Primate Conserv **22**:55–70.

Dobigny G, Britton-Davidian J, Robinson TJ (2015) Chromosomal polymorphism in mammals: an evolutionary perspective. Biol Rev 3 august 2015. [Epub ahead of print]

Dumas F, Stanyon R, Sineo L, Stone G, Bigoni F (2007) Phylogenomics of species from four Genera of New World monkeys by flow sorting and reciprocal chromosome painting. BMC Evol Biol **7**(Suppl 2):S11.

Dumas F, Sineo L, Ishida T (2015) Identificazione tassonomica di Aotus (Platyrrhinae) mediante la citogenetica. J Biol Res **88**:5161.

Dutrillaux B, Couturier J, Fosse AM (1980) The use of high resolution banding in comparative cytogenetics: comparison between man and *Lagothrix lagotricha* (Cebidae). Cytogenet Cell Genet **27**:45–51.

Fagundes V, Yonenaga-Yassuda Y (1998) Evolutionary conservation of whole homologous chromosome arms in the Akodont rodents *Bolomys* and *Akodon* (Muridae, Sigmodontinae): maintenance of interstitial telomeric segments (ITBs) in recent event of centric fusion. Chromosome Res **6**:643–648.

Faravelli M, Azzalin CM, Bertoni L, Chernova O, Attilini C, Mondello C, Giulotto E (2002) Molecular organization of internal telomeric sequences in Chinese hamster chromosomes. Gene **283**:11–16.

Farré M, Ponsà M, Bosch M (2009) Interstitial telomeric sequences (ITSs) are not located at the exact evolutionary breakpoints in primates. Cytogenet Genome Res **124**:128–131.

Fernandez-Duque E (2011) The Aotinae: social monogamy in the only nocturnal haplorhines. In: Campbell CJ, Fuentes A, MacKinnon KC, Bearder SK, Stumpf RM (eds), Primates in Perspective. Oxford University Press, Oxford, pp 140–154.1.

Finato AO, Varella-Garcia M, Tajara EH, Taddei VA, Morielle-Versute E (2000) Intrachromosomal distribution of telomere-like repeats in *Eumops glaucinus* and *Euntops perotis* (Molossidae, Chiroptera). Chromosome Res **8**:563–569.

Ford SM (1994) Taxonomy and distribution of the owl monkey. In: Baer JF, Weller RE, Kakoma I (eds), Aotus: The owl Monkey. Academic Press, San Diego, pp 1–57.

Garagna S, Ronchetti E, Mascheretti S, Crovell S, Formenti D, Rumpler Y, Manfredi Romanini MG (1997) Non-telomeric chromosome localization of (TTAGGG)n repeats in the genus *Eulemu*r. Chromosome Res **5**:487–491.

Garagna S, Marziliano N, Zuccotti M, Searle JB, Capanna E, Redi CA (2001) Pericentromeric organization at the fusion point of mouse Robertsonian translocation chromosomes. Proc Natl Acad Sci USA **98**:171–175.

Garrido-Ramos MA, de la Herran R, Ruiz Rejon C, Ruiz Rejon M (1998) A satellite DNA of the Sparidae family (Pisces, Perciformes) associated with telomeric sequences. Cytogenet Cell Genet **83**:3–9.

Go Y, Rakotoarisoa G, Kawamoto Y, Randrianjafy A, Koyama N, Hirai H (2000) PRINS analysis of the telomeric sequence in seven lemurs. Chromosome Res **8**:57–65.

Hirai H (2001) Relationship of telomere sequence and constitutive heterochromatin in the human and apes as detected by PRINS. Methods Cell Sci **23**:29–35.

Hirai H, Matsubayashi K, Kumazaki K, Kato A, Maeda N, Kim HS (2005) Chimpanzee chromosomes: retrotransposable compound repeat DNA organization (RCRO) and its influence on meiotic prophase and crossing-over. Cytogenet Genome Res **108**:248–254.

Ijdo JW, Baldini A, Ward DC, Reeders ST, Wells RA (1991) Origin of human chromosome 2: an ancestral telomere-telomere fusion. Proc Natl Acad Sci USA **88**:9051–9055.

Lee C, Sasi R, Lin CC (1993) Interstitial localization of telomeric DNA sequences in the Indian muntjak chromosomes; further evidence for tandem chromosome fusions in the karyotype evolution of the Asian muntjaks. Cytogenet Cell Genet **63**:156–159.

Ma NSF, Jones TC, Miller AC, Morgan LM, Adams EA (1976) Chromosome polymorphism and banding patterns in the Owl monkey (Aotus). Lab Anim Sci **26**:1022–1036.

Menezes AN, Bonvicino CR, Seuanez HN (2010) Identification, classification and evolution of owl monkeys (Aotus. Illiger 1811). BMC Evol Biol **10**:248–263.

Metcalfe CJ, Eldridge MD, McQuade LR, Johnston PG (1997) Mapping the distribution of the telomeric sequence (T2AG3)n in rock-wallabies, *Petrogale* (Marsupialia: Macropodidae), by fluorescence in situ hybridization. I. The penicillata complex. Cytogenet Cell Genet **78**:74–80.

Metcalfe CJ, Eldridge MD, Toder R, Johnston PG (1998) Mapping the distribution of the telomeric sequence (T2AG3)n in the Macropodoidea (Marsupalia), by fluorescence in situ hybridization. I. The swamp wallaby, *Wallabia bicolor*. Chromosome Res **6**:603–610.

Meyne J, Ratliff RL, Moyzis RK (1989) Conservation of the human telomere sequence (TTAGGG)n among vertebrates. Proc Natl Acad Sci USA **86**:7049–7053.

Meyne J, Baker RJ, Hobart HH, Hsu TC, Ryder OA, Ward OG, Wiley JE, Wurster-Hill DH, Yates TL, Moyzis RK (1990) Distribution of non-telomeric sites of the (TTAGGG)n telomeric sequence in vertebrate chromosomes. Chromosoma **99**:3–10.

Mudry MD, Nieves M, Bolzán AD (2007) Chromosomal localization of the telomeric (TTAGGG) n sequence in eight species of New World Primates (Neotropical Primates, Platyrrhini). Cytogenet Genome Res **119**:221–224.

Nagamachi CY, Pieczarka JC, O'Brien PC, Pinto JA, Malcher SM, Pereira AL, Rissino Jd, Mendes-Oliveira AC, Rossi RV, Ferguson-Smith MA (2013) FISH with whole chromosome and telomeric probes demonstrates huge karyotypic reorganization with ITS between two species of Oryzomyini (Sigmodontinae, Rodentia): *Hylaeamys megacephalus* probes on *Cerradomys langguthi* karyotype. Chromosome Res **21**:107–119.

Nanda I, Schrama D, Feichtinger W, Haaf T, Schartl M, Schmid M (2002) Distribution of telomeric (TTAGGG)(n) sequences in avian chromosomes. Chromosoma **111**:215–227.

Nergadze SG, Rocchi M, Azzalin CM, Mondello C, Giulotto E (2004) Insertion of telomeric repeats at intrachromosomal break sites during primate evolution. Genome Res **14**:1704–1710.

Nergadze SG, Santagostino M, Salzano A, Mondello C, Giulotto E (2007) Contribution of telomerase RNA retrotranscription to DNA double-strand break repair during mammalian genome evolution. Genome Biol **8**:R260.

Pellegrino KC, Rodrigues MT, Yonenaga-Yassuda Y (1999) Chromosomal evolution in the Brazilian lizards of genus *Leposoma* (Squamata, Gymnophthalmidae) from Amazon and Atlantic rain forests: banding patterns and FISH of telomeric sequences. Hereditas **131**:15–21.

Pieczarka JC, Nagamachi CY, Muniz JA, Barros RM, Mattevi MS (1998) Analysis of constitutive heterochromatin of Aotus (Cebidae, Primates) by restriction enzyme and fluorochrome bands. Chromosome Res **6**:77–83.

Plautz HL, Goncalves EC, Ferrari SF, Schneider MPC, Silva AL (2009) Evolutionary inferences of the genus Aotus (Platyrrhini. Cebidae) from cytochrome c oxidase subunit II gene sequences. Mol Phylogenet Evol **51**:382–387.

Prakhongcheep O, Chaiprasertsri N, Terada S, Hirai Y, Srikulnath K, Hirai H, Koga A (2013) Heterochromatin blocks constituting the entire short arms of acrocentric chromosomes of Azara's owl monkey: formation processes inferred from chromosomal locations. DNA Res **20**:461–470.

Rakotoarisoa G, Hirai Y, Go Y, Kawamoto Y, Shima T, Koyama N, Randrianjafy A, Mora R, Hirai H (2000) Chromosomal localization of 18S rDNA and telomere sequence in the aye-aye, *Daubentonia madagascariensis*. Genes Genet Syst **75**:299–303.

Rocchi M, Archidiacono N, Schempp W, Capozzi O, Stanyon R (2012) Centromere repositioning in mammals. Heredity **108**:59–67.

Rovatsos MT, Marchal JA, Romero-Fernández I, Fernández FJ, Giagia-Athanosopoulou EB, Sánchez A (2011) Rapid, independent, and extensive amplification of telomeric repeats in pericentromeric regions in karyotypes of arvicoline rodents. Chromosome Res **19**:869–882.

Ruiz-Herrera A, Garcia F, Azzalin CM, Giulotto E, Egozcue J, Ponsà M, Garcia M (2002) Distribution of intrachromosomal telomeric sequences (ITS) on *Macaca fascicularis* (Primates) chromosomes and their implication for chromosome evolution. Hum Genet **110**:578–586.

Ruiz-Herrera A, Garcia F, Giulotto E, Attolini C, Egozcue J, Ponsa M, Garcia M (2005) Evolutionary breakpoints are co-localized with fragile

sites and intrachromosomal telomeric sequences in primates. Cytogenet Genome Res **108**:234–247.

Ruiz-Herrera A, Nergadze SG, Santagostino M, Giulotto E (2008) Telomeric repeats far from the ends: mechanisms of origin and role in evolution. Cytogenet Genome Res **122**:219–228.

Slijepcevic P (1998) Telomeres and mechanisms of Robertsonian fusion. Chromosoma **107**:136–140.

Small MF, Stanyon R, Smith DG, Sineo L (1985) High resolution chromosomes of rhesus macaques (*Macaca mulatta*). Am Journal Primatol **9**:63–67.

Stanyon R, Consigliere S, Bigoni F, Ferguson-Smith M, O'Brien PCM, Wienberg J (2001) Reciprocal chromosome painting between a New World primate, the woolly monkey, and humans. Chromosome Res **9**:97–106.

Stanyon R, Bigoni F, Slaby T, Muller S, Stone G, Bonvicino CR, Neusser M, Seuánez HN (2004) Multi-directional chromosome painting maps homologies between species belonging to three genera of New World monkeys and humans. Chromosoma **113**:305–315.

Stanyon R, Rocchi M, Capozzi O, Roberto R, Misceo D, Ventura M, Cardone MF, Bigoni F, Archidiacono N (2008) Primate chromosome evolution: ancestral karyotypes, marker order and neocentromeres. Chromosome Res **16**:17–39.

Stanyon R, Garofalo F, Steinberg ER, Capozzi O, Di Marco S, Nieves M, Archidiacono N, Mudry MD (2011) Chromosome painting in two genera of South American monkeys: species identification, conservation, and management. Cytogenet Genome Res **134**:40–50.

Ventura M, Catacchi CR, Sajjadia S, Vives L, Sudmant PH, Marques-Bonet T, Graves TA, Wilson RK, Eichler EE (2012) The evolution of African great ape subtelomeric heterochromatin and the fusion of human chromosome 2. Genome Res **22**:1036–1049.

Vermeesch JR, De Meurichy W, Van Den Berghe H, Marynen P, Petit P (1996) Differences in the distribution and nature of the interstitial telomeric (TTAGGG)n sequences in the chromosomes of the Giraffidae, okapai (Okapia johnstoni), and giraffe (Giraffa camelopardalis): evidence for ancestral telomeres at the okapi polymorphic rob (5;26) fusion site. Cytogenet Cell Genet **72**:310–315.

Villasante A, Abad JP, Mendez-Lago M (2007) Centromeres were derived from telomeres during the evolution of the eukaryotic chromosome. Proc Natl Acad Sci USA **104**:10542–10547.

Wijayanto H, Hirai Y, Kamanaka Y, Katho A, Sajuthi D, Hirai H (2005) Patterns of C-heterochromatin and telomeric DNA in two representative groups of small apes, the genera Hylobates and Symphalangus. Chromosome Res **13**:715–722.