Using Telomeric Length Measurements and Methylation to Understand The Karyotype Diversification of *Ctenomys Minutus* (A Small Fossorial Mammals)

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Abstract

The genus *Ctenomys* has been widely used in karyotype evolution studies due to the variation in their diploid numbers (2n), which range from 2n = 10 to 2n = 70. *Ctenomys minutus* is characterized by intraspecific variation in diploid number (2n = 42, 46, 48, and 50), which makes it an interesting model to investigate the genomic instability mechanisms that have led to different cytotypes in this species. We aimed to contribute to the knowledge about telomeres' role in chromosomal instability and global DNA methylation in the genome evolution of *C. minutus*. This study found that telomere length differs between cytotypes, but only for females (50a<46a,48a,42), although methylation was also higher, no significant difference was shown. It was also shown that young individuals, regardless of cytotype, had the longest telomere and the most methylated DNA, although only the last was statistically significant. Despite this, there is still much to be answered, although new cytotypes seem to have emerged within the distribution of parental cytotypes by the accumulation of different chromosomal rearrangements.

1. Introduction

Several mammalian species present repetitions of short blocks of telomeric-like DNA in intrachromosomal sites (ITSs) [1], including those repeats located close to the centromeres and those found between the centromeres and the telomeres [2]. These sequences can be generated by mechanisms of mutation and repair mistake, inadequate crossing over, transposition, or they may be indicative of a chromosomal rearrangement such as inversion, centric or tandem fusion, which can arise during karyotype evolution [3–5]. Chromosomal sites composed of ITSs have been detected in widely different vertebrate species [5], fishes [6], anuran [7,8], squamate reptiles [9], birds [10], marsupials [11–13], rodents [14,15], and plants [16,17]. The function of the telomerase enzyme, together with a variety of telomere-binding proteins, is required to maintain adequately long telomeres, certifying stability to the linear eukaryotic chromosomes. Nergadze et al. [18] proposed that telomerase was used, in some cases, to repair double-stranded DNA breaks that arose in the rodent and primate genomes during chromosomal evolution, that the ITSs were inserted into the repair, and that the ITSs could arise from the capture of telomeric fragments or telomerase action and that they were fixed during genome evolution.

Gonzalo et al. [19] showed that mouse telomeric and subtelomeric chromatin contains histone modifications commonly found in heterochromatin and that subtelomeric DNA can be methylated. DNA methylation, the most known epigenetic mark, is the addition of a methyl group (CH_3) to cytosine at the CpG dinucleotide, forming 5-methyl-2'-deoxycytidine, or 5-mdC by a DNA (cytosine-5)-methyltransferase 1 (DNMT1) enzyme [20]. Histone modifications in telomeric chromatin or DNA methylation in subtelomeric regions were correlated with telomere length deregulation in the study by Blasco [21]. Increasing evidence indicates the existence of functional links between these epigenetic marks and the homeostasis of telomere length [22].

Rodents have strongly contrasting genomic organizations and are models to study the role of chromosomal rearrangements in speciation. Studies by classical cytogenetics show that rodents display large karyotypic diversity, with diploid numbers ranging from 2n = 10 in *Ctenomys steinbachi* (Ctenomyidae) [23] to 2n = 102 in *Tympanoctomys barrerae* (Octodontidae) [24,25]. The genus *Ctenomys* has about 65 species described [26] with high rates of chromosomal variation that vary from 2n = 10 to 2n = 70 [27]. Additionally, there are species with significant chromosomal variation such as *C. pearsoni* (2n = 56, 64, and 70), *C. boliviensis* (2n = 42, 44, 45, and 46), *C. rionegrensis* (2n = 48, 50, 56, and 58), *C. minutus* (2n = 42, 46, 48, and 50), *C. lami* (2n = 54, 55, 56, 57, and 58), *C. talarum* (2n = 44, 45, 46, 47, and 48) and *C. perrensis* (2n = 50, 54, 56, and 58) [28–33]. Populations of *C. minutus* have autosomal chromosomes characterized by the morphological variation between biarmed (from 14 to 17 pairs) and acrocentric (from 3 to 10 pairs) chromosomes, thus forming different karyotypes [34]. This species has chromosomal variation, comprising seven parapatrically dispersed parental karyotypes (2n = 50a, 46a, 48a, 42, 46b, 48b and 50b) and six intraspecific hybrid zones with transitional karyotypes: (I) 50a x 48a = 49a; (II) 46a x 48a = 47a; (III) 48a x 42 = 43, 44, 45, and 46; (IV) 42 x 46b = 43, 44, and 45; (V) 46b x 48b = 47b; and (VI) 48b x 50b = 49b [31,35–40]. The karyotypes show an instigating geographic distribution, where 2n = 50 are found at the extremes of the distribution, and this number is reduced to approximately the central region of the distribution, presenting 2n = 42. Studies show Robertsonian rearrangements, fissions and fusions or tandem fusion, and a pericentromeric inversion, which occurred in the chromosomes, originating even the 'a' and 'b' systems [31,37,41].

Considering the high frequency of chromosomal rearrangements among *C. minutus* populations, this species is an excellent model for studying the mechanism of genomic instability that originated and continues to originate different karyotypes in this species. In addition, there is a lack of studies with molecular cytogenetic techniques to explain these different cytotypes that occur in this species. Thus, this study aims to contribute to the knowledge about the role of telomeres in chromosomal instability and global DNA methylation in the karyotype diversity of *C. minutus* throughout its geographic distribution.

2. Results

2.1 Karyotype description and telomere sequence mapping

A tuco-tuco specimen 2n = 45b was collected in Tavares, the Rio Grande do Sul State, where 2n = 48b is commonly found; however, there is a contact zone between 2n = 48b and 42 karyotypic forms in this local [31,37]. The signals of the (TTAGGG)n sequences were observed on both telomeric ends of all chromosomes of seven different karyotypic forms of *Ctenomys minutus* (2n = 50a, 50b; 48a,48b; 46a, 45b, and 42) tested here (shown in Fig. 2a-g). Interstitial signals (ITS) were found only in the pericentromeric region of chromosome 1 of cytotype 2n = 50b (shown in Fig. 2c). There were no ITSs in the sex chromosomes.

2.2 Telomere Length (TL)

A total of 134 individuals of *C. minutus* DNA were used to analyze telomere length in different cytotypes. As the data distribution was asymmetric, we used log transformation in the first comparisons among cytotypes, sexes, and age groups. No difference between the cytotypes was statistically significant (ANOVA P = 0.473) (shown in Fig. 3a). Age groups and sexes did not either differ (ANOVA p values 0.410 and 0.076, respectively) (shown in Fig. 3b-c). However, using GZLM analysis, we observed statistically significant interactions between cytotype and sex (P = 0.015), indicating sex-dependent differences among cytotypes in TL length. In the multiple pairwise comparisons, no differences between cytotypes were observed in males (P > 0.20 in all comparisons). However, in females, the telomere length of 2n = 50a was shorter than that of 2n = 48a (P = 0.008), 2n = 46a (P = 0.048) and 2n = 42 (P = 0.001) (shown in Fig. 4). Concerning rearrangement characters, no statistically significant difference was found between them and the telomere length: character 01 (P = 0.09), character 04 (P = 0.937), character 07 (P = 0.289), character 10 (P = 0.85) and character 15 (P = 0.757) (shown in Fig. 5a-e).

2.3 Global DNA Methylation

We measured global DNA methylation in a total of 124 individuals of *C. minutus* DNA by quantifying 5-mdC in different cytotypes. In the first analysis, we did not observe significant differences among cytotypes (ANOVA KW P = 0.088) (shown in Fig. 6a) or between sexes (ANOVA KW P = 0.097) (shown in Fig. 6c). The difference among age groups was statistically significant (ANOVA Kruskal-Wallis P = 0.048) (shown in Fig. 6b). Pairwise WMW indicated a significant difference between juveniles and subadults (P = 0.011) and between juveniles and adults (P = 0.048). When analyzing all factors together in the GZLM model, we found no suggestion of interaction effects. The P values of the main effects were cytotype (P = 0.187), sex (P = 0.199), and age group (P = 0.087). Regarding the rearrangements, there were no statistically significant differences between the characters and global DNA methylation: character 01 (P = 0.05), character 04 (P = 0.269), character 07 (P = 0.847), character 10 (P = 0.367) and character 15 (P = 0.283) .MW and ANOVA (Kruskal-Wallis) (shown in Fig. 7a-e). We did not observe a correlation between TL and 5-mdC values (Spearman´s r = 0.065; P = 0.501).

3. Discussion

In this study, we analyzed cytotypes of *C. minutus* using FISH, telomeric length, and global DNA methylation methods to better understand the karyotype evolution in this species. We observed that all the cytotypes of *C. minutus* included in our study showed telomeric FISH signals at the end of all chromosomes. In addition, contrary to the finding by Freygang et al. [41], ITS signal was found only in the pericentromeric region on chromosome 1 of cytotype 2n= 50b (shown in Fig. 2g). This ITS region may indicate a fusion between chromosomes 7 and 5 of *C. flamarioni*, found recently in the cytotype 2n= 46a of *C. minutus* by Kubiak et al. [42]. De Freitas [43] comparing the karyotypes of *C. lami* 2n=54 and 2n=46a of *C. minutus* found the fusion of chromosomes 13 and 23 of *C. lami* to form pair 1 of *C. minutus*. They may be a remnant of the chromosomal rearrangement produced during karyotype evolution, as ITS regions are considered fragile sites and susceptible to spontaneous and induced chromosomal breaks [44]. The absence of ITS in the other cytotypes suggests that the telomeric repetitions were lost due to a

progressive reduction or degeneration through small chromosomal organizations and point mutations [45]. This absence of ITS after chromosomal rearrangements has also been reported in *Ctenomys magellanicus* [46] and in species of Sigmodontinae rodents, *Oligoryzomys* [47], *Nectomys* [48], and *Rhipiidomys* [49]. In addition, some studies demonstrated that the telomeric sequence is not always retained during fusion events [11,50].

Telomeres protect the ends of chromosomes to stabilize the nuclear genome with high fidelity during youth but usually decreasing during aging and with the influence of environmental agents and diseases [51,52]. The relationship between TL shortening and aging was not observed in this study, although juvenile organisms presented larger telomeres than subadults and adults (shown in Fig. 3b). Studies have been observed in several species, with more significant attrition in the telomeres in males than in females [53], including this study (shown in Fig. 3c). We found that the differences between the cytotypes were sex-dependent (shown in Fig. 4). Although chromosomal rearrangements that differentiate cytotypes also occur in males, differences in TL were observed only in some cytotypes in females. Even observing a great variability of telomeric size within each cytotype, the most interesting was to observe that cytotype 50a had the smallest size, being even significantly smaller than 46a, 48a, and 42 among females. There are some hypotheses to this, the inactivation on the X chromosome [54], as well as the sex size dimorphism, where males are larger than females, and that end up presenting more duplication of cells [55]. Despite 46a, 48a, and 42 present characters 01 and 07 (fusion of chromosomes 20/17 and 23/19) different from 50a (fission of chromosomes 20/17 and 23/19), no statistically significant difference was found for telomere length and different character.

DNA global methylation is an epigenetic alteration that plays a role in regulating cellular processes, including genomic instability and gene expression [56]. This study found a significant decrease in global DNA methylation in subadults, and adults compared to juveniles (shown in Fig. 6b). This decrease is explained because, during aging, mammalian cells undergo a DNA methylation deviation, which alters the 5-methyl-cytosine distribution, resulting in this decrease. This decline occurs mainly in domains with repetitive sequences and constitutive heterochromatin, facilitating heterochromatin decondensation. It has been proposed that this occurs because of the loss of effectiveness of DNMT1 [57].

In this study, signals of ITSs were observed in chromosome one of cytotype 2n= 50b, indicating remnants of a chromosomal fusion. However, future studies are needed to address the chromosomal mechanism that maintained ITSs in the cytotype 2n= 50b and not in the 2n= 50a. There are reasons to think that there are some advantages to *C. minutus* that present high rates of chromosomal variation. One obvious advantage seems to be the possibility of rapidly accruing a phenotypic effect from combinatorial acquisition of smaller-effect genetic changes. In *C. minutus* species, we showed significant differences between the karyotypes and telomere length sex-dependent, although we have not found any relationship between global methylation in genomic DNA and cytotype variation in this species.

4. Material And Methods

Animals

The rodents were caught and handled using Oneida-Victor number 0 Snap traps according to the recommendations of the capture of the Animal Care Committee [58].

Ethics

The authors confirm that the study was reported in accordance with ARRIVE guidelines. Permission for fieldwork was granted by the Brazilian Ministry of Environment - IBAMA/ICMBio permanent collection permits 14690-1; in accordance with the precepts of National and International Guidelines and Norms, especially Law 11,794 of November 8, 2008, Decree 6899 of July 15, 2009, and the norms published by the National Council for the Control of Animal Experimentation (CONCEA). Field procedures with rodents were approved by institutional ethical committee (Ethics Committee on the Use of Animals of the Universidade Federal do Rio Grande do Sul - CEUA -31925).

4.1 Specimens studied

In this study, we used fresh biological material to obtain chromosome preparations, and biological material from the animal captured using Oneida-Victor number 0 Snap traps under the guidelines of the American Society of Mammologists' Animal Care Committee [58]. The animals were collected in the municipalities of Jaguaruna, Praia do Barco, Bacupari, Mostardas, Tavares, Bojuru, and São José do Norte.

Ctenomys minutus age groups were established according to the Wilks [59] method, which is centered on body weight. Three age groups were defined: juveniles, females up to 125 g and males up to 135 g; subadults, females from 125 to 185 g and males from 135 to 225 g; and adults, above the limits of the subadults [60].

4.2 Cell culture and chromosome preparations

Lung biopsies were collected from one adult *C. minutus* per cytotype in the geographic regions related to the different cytotypes, except in Tavares (two adults) (see Fig. 1). To execute cell culture, according to Verma and Babu [61], the fibroblast cultures were grown at 37°C in medium-high glucose (Dulbecco's Modified Eagle's, Gibco) enriched with 15% fetal bovine serum (GIBCO), penicillin (100 units/ml) and streptomycin (100 mg/ml). Chromosome preparations were made following standard methods, which consist of 1 hour in colchicine, 15 minutes in hypotonic solution (0.075 M KCl), and fixation. The diploid number and chromosomal morphology were established from at least 50 metaphase chromosomes per individual, stained with Giemsa 10% followed by air drying.

To determine telomere length and global DNA methylation, we used tissue samples available in the animal collection of the Laboratório de Citogenética e Evolução of the Universidade Federal do Rio Grande do Sul for DNA extraction.

4.3 Chromosome Rearrangements

Freygang et al. [41] observed specific chromosomal rearrangements that differentiate the *C. minutus* karyotypes, and we analyzed them for TL and 5-mdC. The analyzed rearrangements are characterized according to the study by Freygang et al. [41]: Character 01 (pairs 20 and 17 separated in the karyotype 2n = 50a and fused in the other karyotypes); character 04 (metacentric chromosome 2 in karyotypes 2n = 50a, 46a, and 42; acrocentric showing inversion in arm 2p in karyotypes 2n = 46b, 48b, and 50b; chromosome 2 acrocentric in karyotype 2n = 48a); character 07 (pairs 23 and 19 separated in karyotypes 2n = 50a and 50b and fused in the other karyotypes); character 10 (pairs 22, 24, and 16 fused in karyotype 2n = 42; pairs 24 and 16 fused in karyotype 2n = 46b; separate pairs in karyotypes 2n = 50a, 48a, 46a, 48b, and 50b); character 15 (positive signal of constitutive heterochromatin (CH) of chromosome pair 08 in karyotypes 2n = 50a, 48a, 46a, and 42).

4.4 DNA Extraction

DNA extraction was performed on the tissues from 150 individuals (90 females and 60 males). Total DNA was extracted from muscle tissue samples using the standard phenol: chloroform protocol [62].

4.5 Fluorescence *in situ* hybridization (FISH)

Biotin-labeled (TTAGGG)n probes were obtained by PCR using the DNA of *C. minutus* as DNA template and detected with Cy3-streptavidin [63] to detect the location of telomeric sequences on the chromosomes of seven different cytotypes of *C. minutus* (2n=50a and b; 48a and b; 46a and b, and 42). At least 10 metaphase spreads per individual were analyzed to confirm the FISH results. The slides were analyzed using a Zeiss Imager 2 microscope, 63x objective, and Axiovision 4.8 software (Zeiss, Germany).

4.6 Telomere Length Analysis by qPCR

Telomere length (TL) was quantified from the total *C. minutus* genomic DNA by using a real-time quantitative Polymerase Chain Reaction (qPCR) method following the protocol according to Callicott and Womack [64], with minor modifications by Matzenbacher et al. [52]. The forward and reverse primer sequences for the telomeric region gene were 5’ CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG TTT GGG TT 3’ and 5’ GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT 3’, respectively. The mouse 36B4 gene primers are related to the acidic ribosomal phosphoprotein PO (36B4) gene, which is well conserved. The forward and reverse primers used in the 36B4 fraction of the assay were 5’ ACT GGT CTA GGA CCC GAG AAG 3’ and 5’ TCA ATG GTG CCT CTG GAG ATT 3’, respectively. Each reaction analyzing the telomere and 36B4 fragments comprised 12.5 µl SYBR Green PCR Master Mix (Quatro G), 300 nM telomere primers (forward and reverse), 300 nM 36B4 forward primer and 500 nM 36B4 reverse primer, 20 ng genomic DNA, and an adequate amount of water was added to yield a 25 µl reaction. Three 20 ng samples of each DNA mixture were put in adjacent wells of a 96-well plate for the telomere and 36B4 assays and analyzed using the Step One Plus TM Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). For the telomere amplicons, qPCR was done using the following reaction conditions: set at 95°C for 10 min; followed by 30 cycles of denaturation at 95°C for 15 s, and annealing

and extension at 56°C for 1 min. For the 36B4 amplicons, the reaction conditions were an initial step at 95°C for 10 min followed by 35 cycles of data collection at 95°C for 15 s, with 52°C annealing for 20 s, followed by extension at 72°C for 30 s.

Serially diluted DNA standards ranging from 0.384 to 37.5 ng/µl (3-fold dilution; six data points) were used to produce the standard curves for the telomere and 36B4 fragments on each 96-well plate. The Tel STD curve was used to quantify the telomeric content per sample in kilobases (kb), while the 36B4 STD curve was used to quantify the number of diploid genome copies per sample. The qPCR method used to assess TL was adapted in our laboratory, and our results presented reproducible and consistent standard curves for both the telomere and 36B4 (single-copy gene) standards (Supplementary Fig. 1). The telomere qPCR's cycle threshold (Ct) ranged from 7 to 13, and all target samples were within the standard linear range. All samples were evaluated in triplicate, with negative and reference controls as standard curves. The Ct point of each sample was used to calculate the TL in kb per *C. minutus* diploid genome. Individual samples with a standard deviation of Ct < 1 for the triplicate samples were included in the complete evaluation.

4.7 Global DNA Methylation Analysis by HPLC

Global DNA methylation (5-mdC) levels were quantified in the isolated DNA based on the proportional quantification of 5-mdC using high-performance liquid chromatography (HPLC) as described in Berdasco M, Fraga MF [65], and Cappetta et al. [66]. Quickly, DNA was hydrolyzed with nuclease P1 and alkaline phosphatase to yield 2-deoxymononucleosides, which were isolated by HPLC and detected by ultraviolet (UV) light. A mixture of deoxyadenosine, deoxythymidine, deoxyguanosine, deoxycytidine, 5-methyl-2-deoxycytidine, and deoxyuridine (Sigma-Aldrich) was used as a standard. Global genomic DNA methylation percentages were calculated by integrating the 5-mdC peak area (obtained from the HPLC analysis) relating to global cytidine (methylated or not). The median for each sample was calculated, and duplicated samples, indicating a difference in 5-mdC greater than 3% or with low HPLC resolution were eliminated.

4.8 Statistical Analysis

The telomere length and 5-mdC data presented asymmetric distribution and/or heterogeneity of variances. Then we used a one-way ANOVA with Brown-Forsythe (B-F) robust test for equality of means and Tamhane test for multiple comparisons to evaluate differences among karyotypes, sex, and age groups. We checked the results using Kruskal-Wallis (KW) nonparametric ANOVA, followed by pairwise Wilcoxon-Mann-Whitney (WMW) tests. Next, we employed Generalized Linear Models (GZLM) to evaluate the effects of the three factors karyotype, sex, and age group in the same model, to investigate possible interaction effects. We chose a gamma distribution with a log link for global DNA methylation, and telomere length values were transformed into natural Log (Ln) based on the deviant results. All interactions between the factors were initially considered in the models but dropped in sequence if statistically non-significant. Using the GZLM approach, adjustments for multiple comparisons between groups were performed using the sequential Bonferroni procedure. We evaluated whether there was any

correlation between TL and the 5-mdC data using the nonparametric Spearman rank correlation coefficient. To analyze the rearrangements, we used Kruskal- Wallis, ANOVA (Tukey), Mann- Whitney, and t-test. P values ≤ 0.05 were considered statistically significant. The analyses were performed with SPSS version 18 and GraphPad PRISM software, version 5.01 (GraphPad Inc., San Diego, CA).

Declarations

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AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. T.R.O.F. and J.S. conceived of the original idea. Material preparation, data collection and analysis were performed by A.L.H.G., C.A.M., R.K., and M.C. The first draft of the manuscript was written by C.A.M., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

COMPETING INTEREST

The authors declare no competing interests.

ADDITIONAL INFORMATION

Data Availability

The datasets generated during, and current study are available from the corresponding author on reasonable request.

All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).

Readers are welcome to comment on the online version of the paper

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Figures

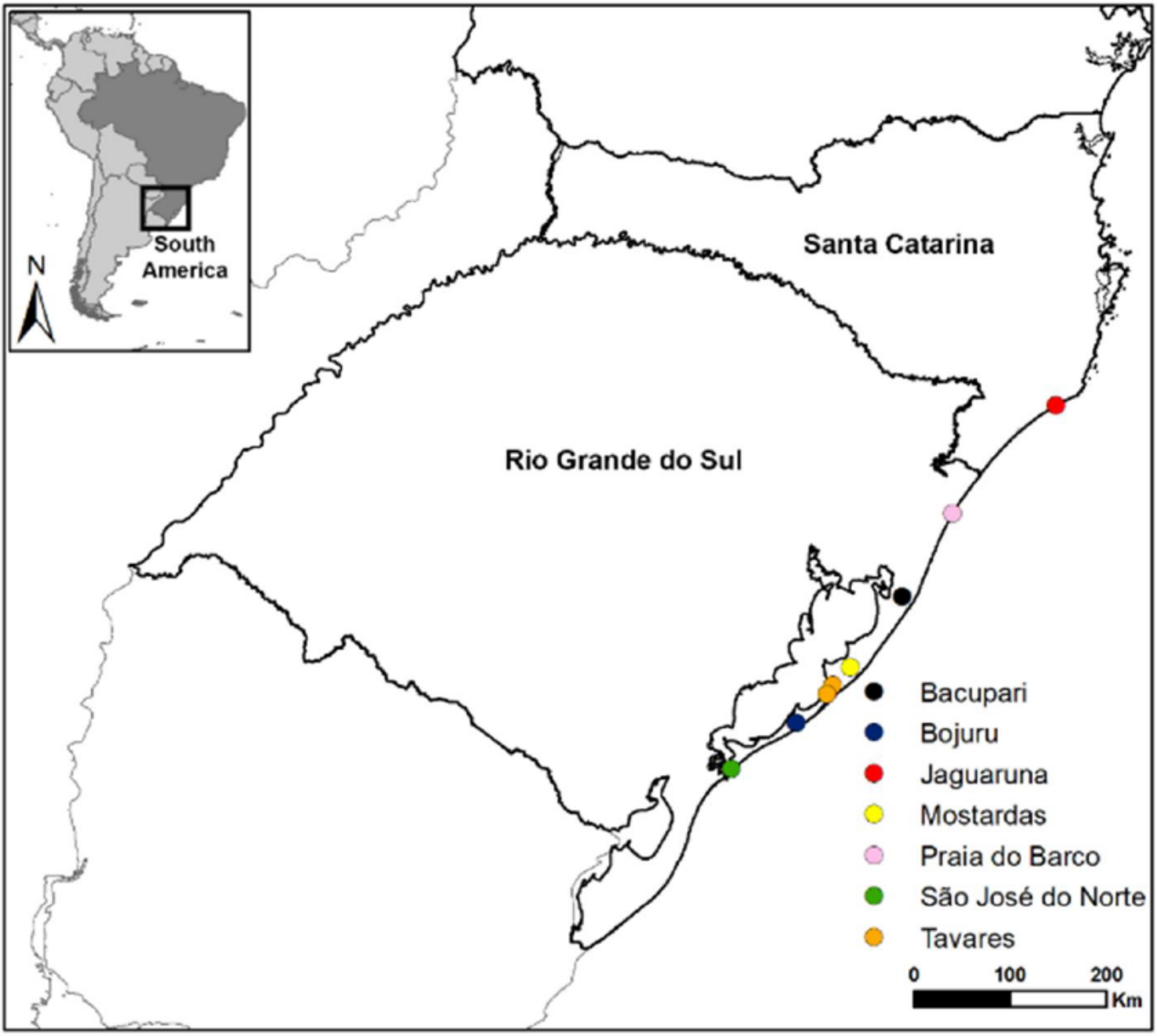

Figure 1

Locations where collections were made for the FISH experiment. Bacupari (2n = 48a), Bojuru (2n = 48b), Jaguaruna (2n = 50a), Mostardas (2n = 42), Praia do Barco (2n = 46a), São José do Norte (2n = 50b) and Tavares (2n = 46b).

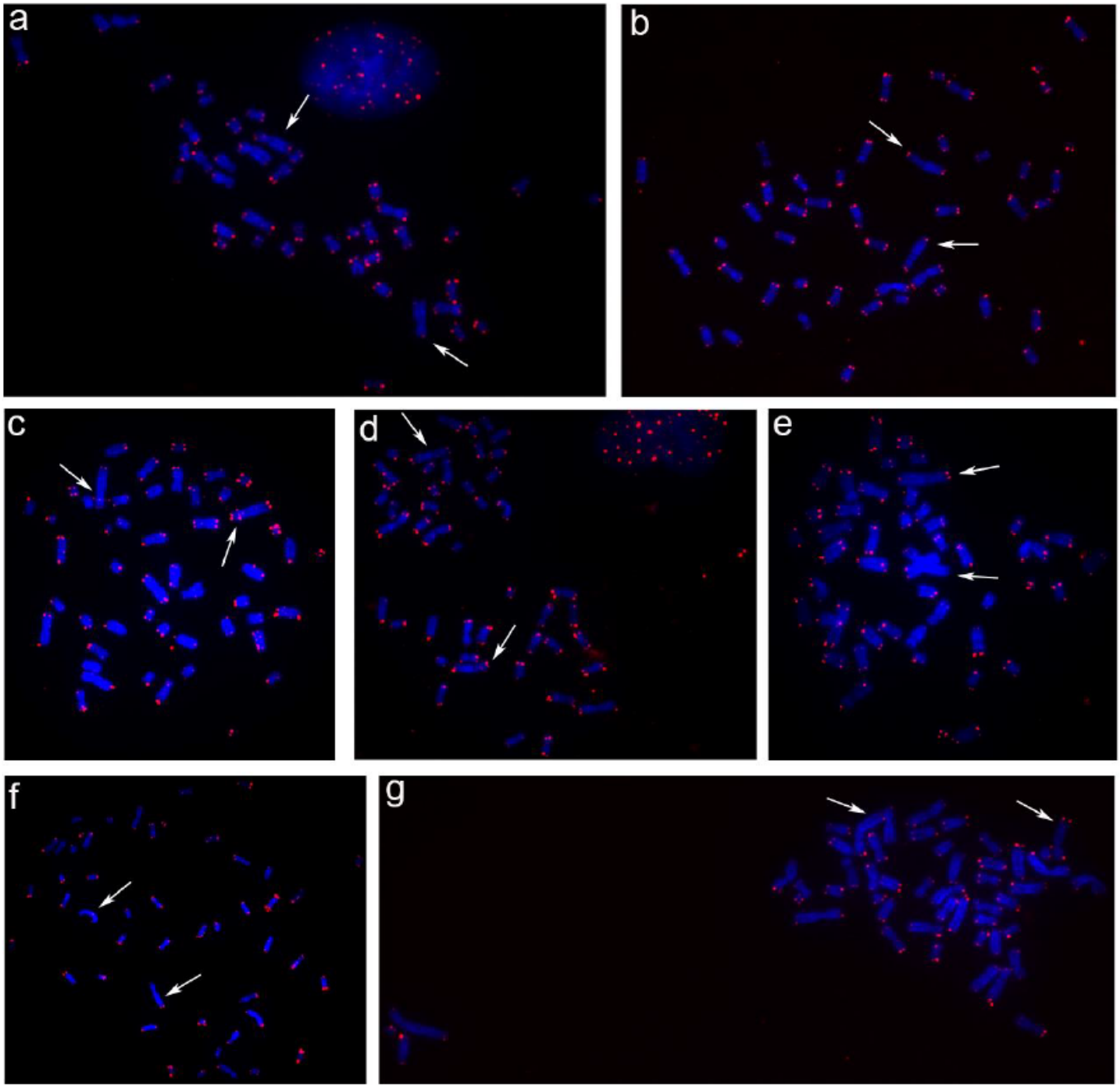

Figure 2

Fluorescence in situ localization of the telomeric sequence (TTAGGG)n in C. minutus with different diploid numbers: a) 2n = 42; b) 2n = 46a; c) 2n = 50b; d) 2n = 45b; e) 2n = 48b; f) 2n =48a; g) 2n = 50a. Arrows indicate chromosome 1 in all karyotypes.

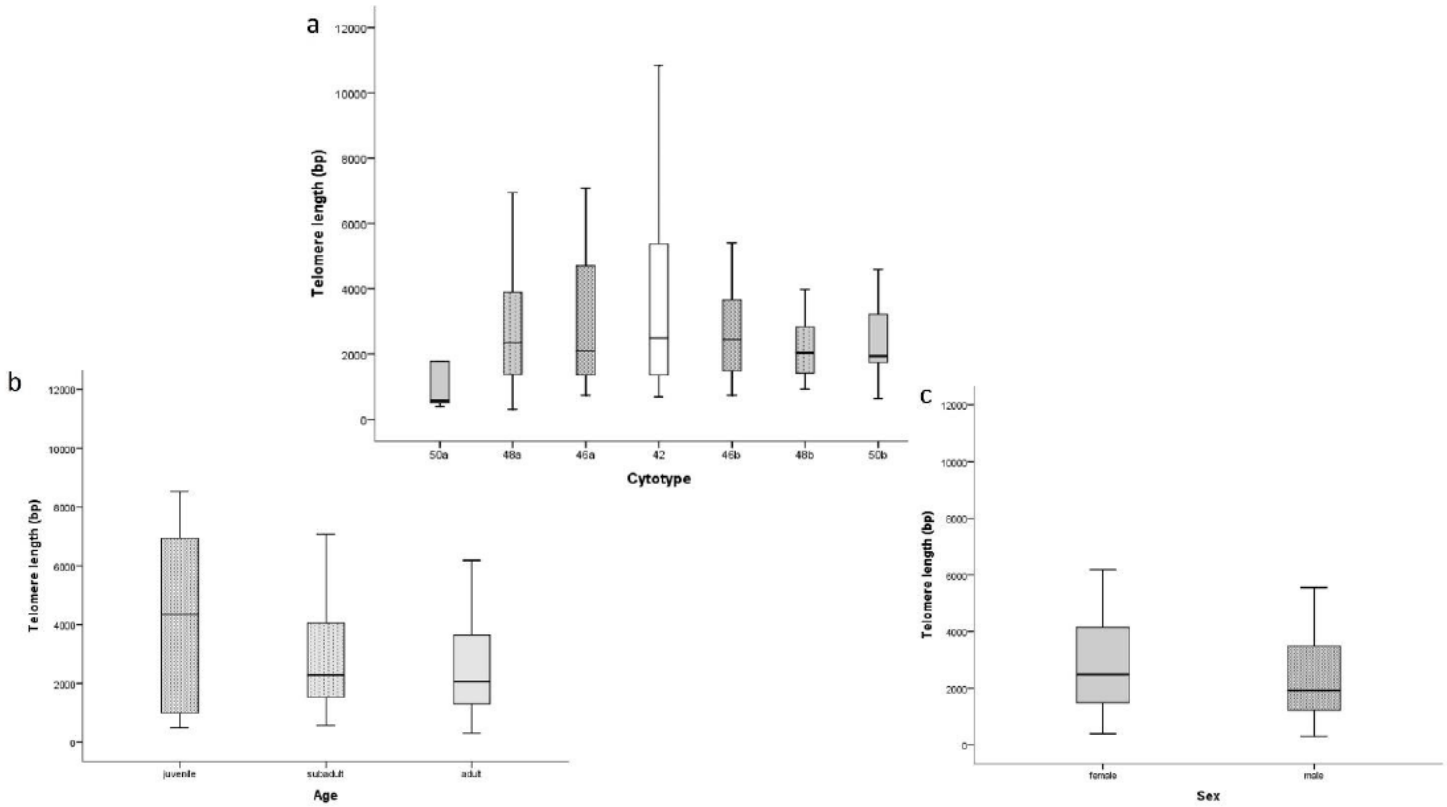

Figure 3

Mean values, standard deviation, minimum and maximum telomere length among the C. minutus cytotypes: a) 2n = 50a (04), 2n = 46a (22), 2n = 48a (34), 2n = 42 (11), 2n = 46b (16), 2n = 48b (17) and 2n = 50b (18) N of each karyotype; b) age classes: juvenile (09), subadult (51) and adult (62) N of juvenile, subadult and adult, respectively; and c) sex: female (71) and male (51) N of female and male, respectively. Telomere length values expressed in base pairs (bp).

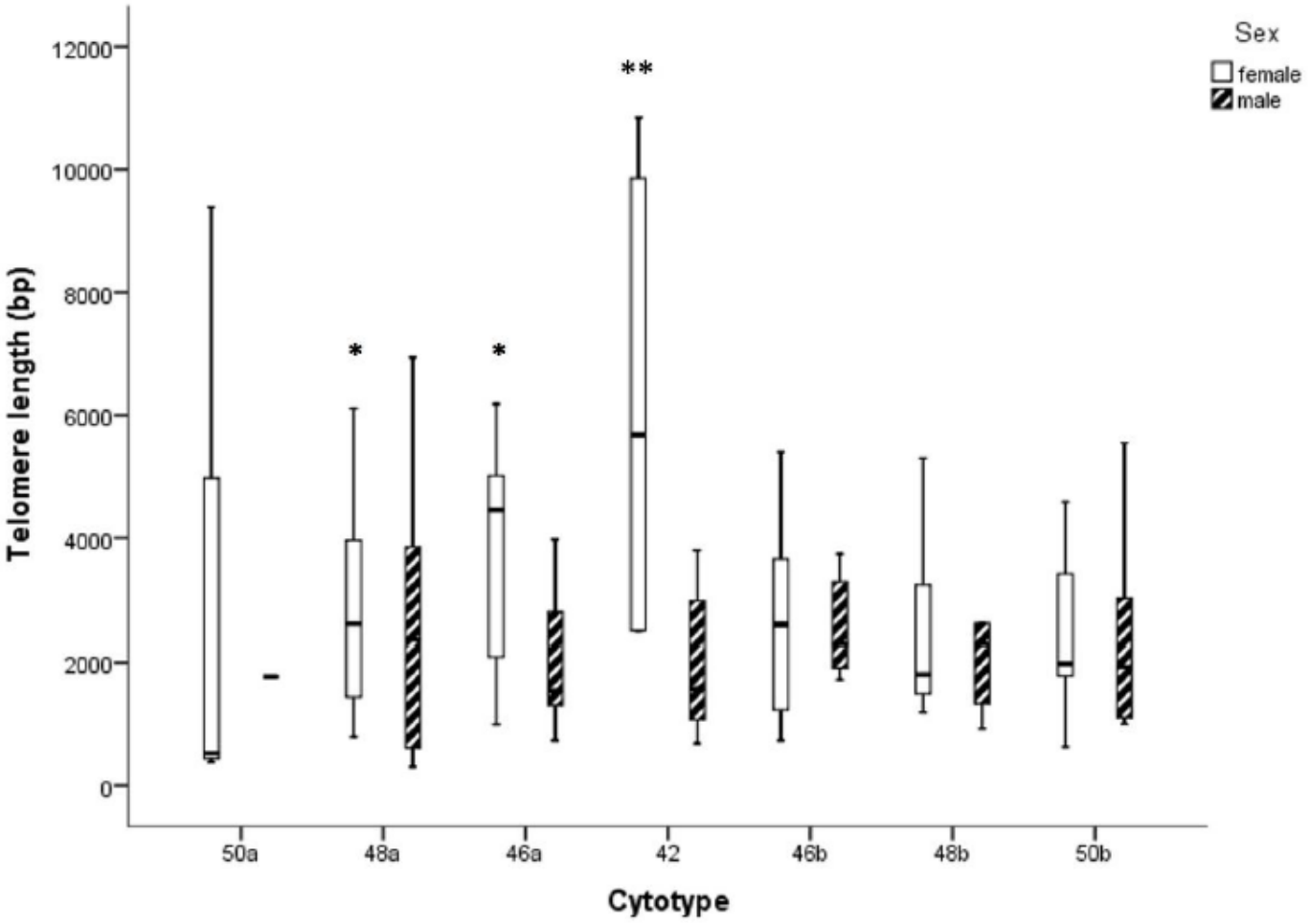

Figure 4

Telomere length among the C. minutus cytotype. Mean values, standard deviation, minimum and maximum by sex: 2n = 50a (04, 01), 2n = 46a (10, 14), 2n = 48a (22, 17), 2n = 42 (04, 07), 46b (13, 05), 2n = 48b (11, 06) and 2n = 50b (14, 05). N of females and males, respectively. * P < 0.05 and ** P < 0.001 in relation to 2n = 50a. Telomere length values expressed in base pairs (bp).

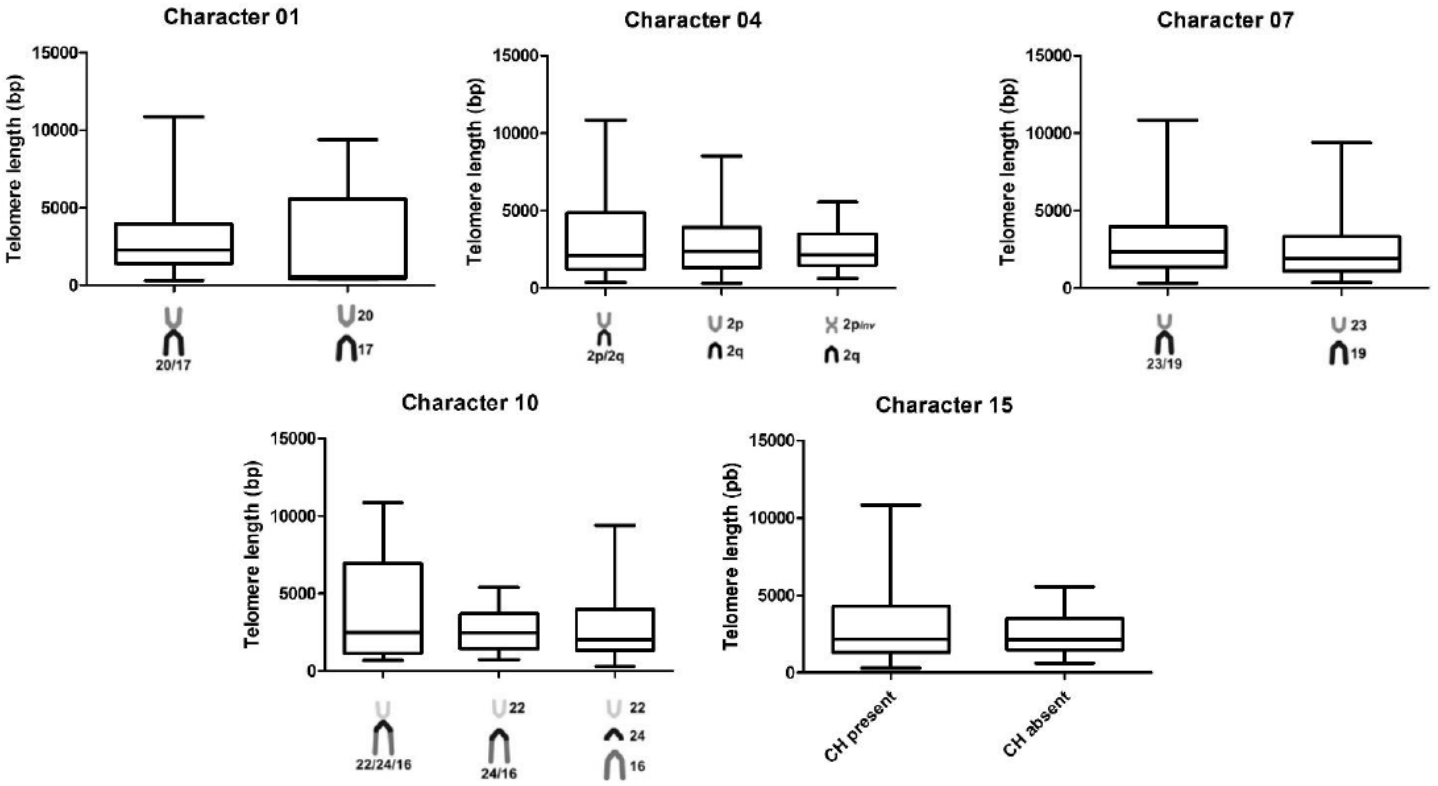

Figure 5

Comparison between telomere length and different rearrangements found in C. minutus cytotypes. a) chromosomes 20 and 17 fused (N = 129), separated (N =5); b) chromosome 2 metacentric (N = 41), acrocentric (N = 39) and 2p with inversion (N = 54); c) chromosomes 23 and 19 fused (N = 110), separated (N = 24); d) chromosomes 22, 24 and 16 fused (N = 11), 24 and 16 fused (N = 18), separated (N = 105); e) heterochromatin present (N = 80), absent (N =54). Telomere length values expressed in base pairs (bp).

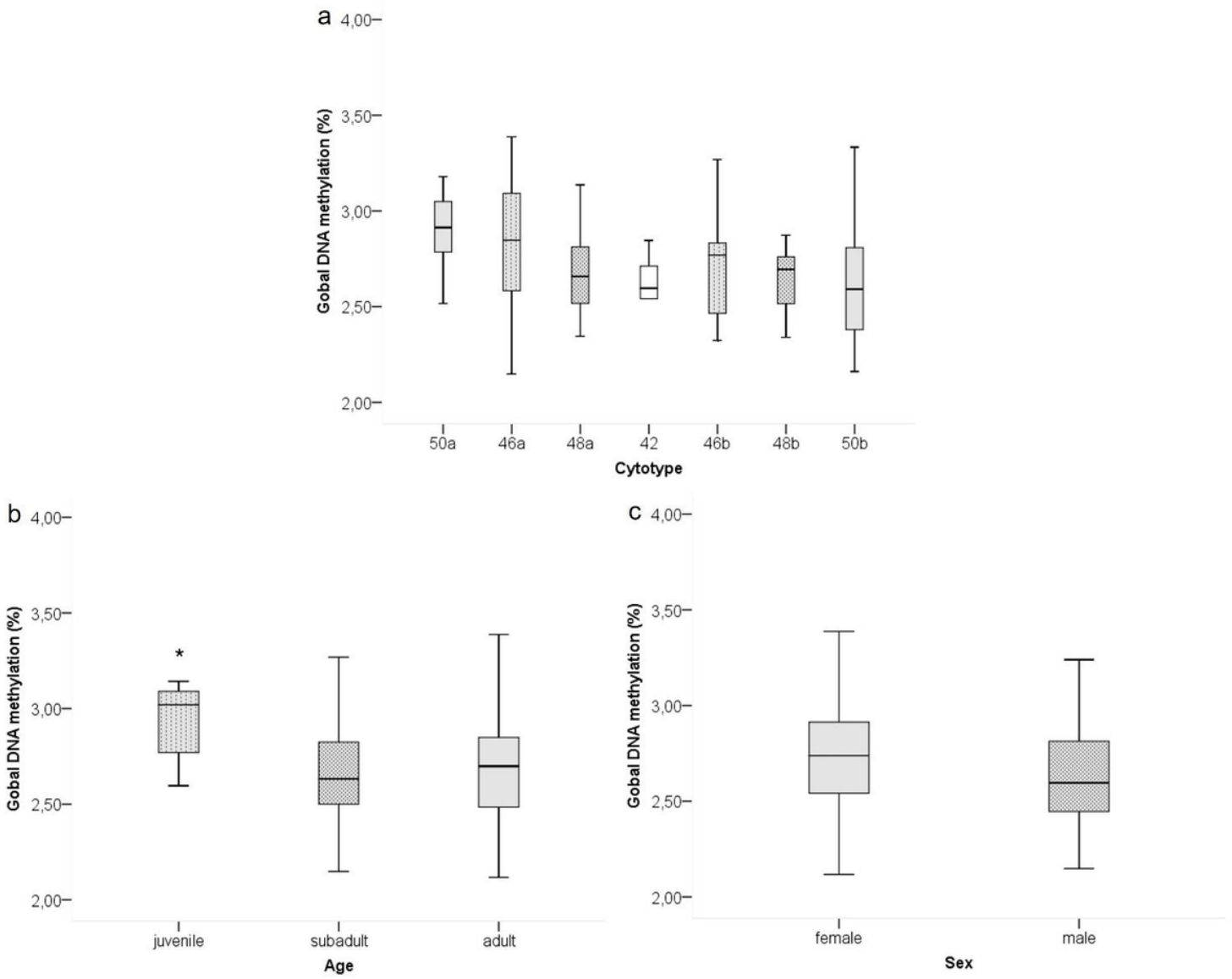

Figure 6

Mean values, standard deviation, minimum and maximum of 5-mdC. a) among the C. minutus cytotypes: 2n = 50a (06), 2n = 46a (19), 2n = 48a (35), 2n = 42 (13), 2n = 46b (14), 2n = 48b (14) and 2n = 50b (16) N of each karyotype; b) among age groups: juvenile (08), subadult (48) and adult (61); c) between sexes: female (73) and male (44).* P < 0.005 in relation to subadults (P WMW = 0.011) and adults (P WMW = 0.048)

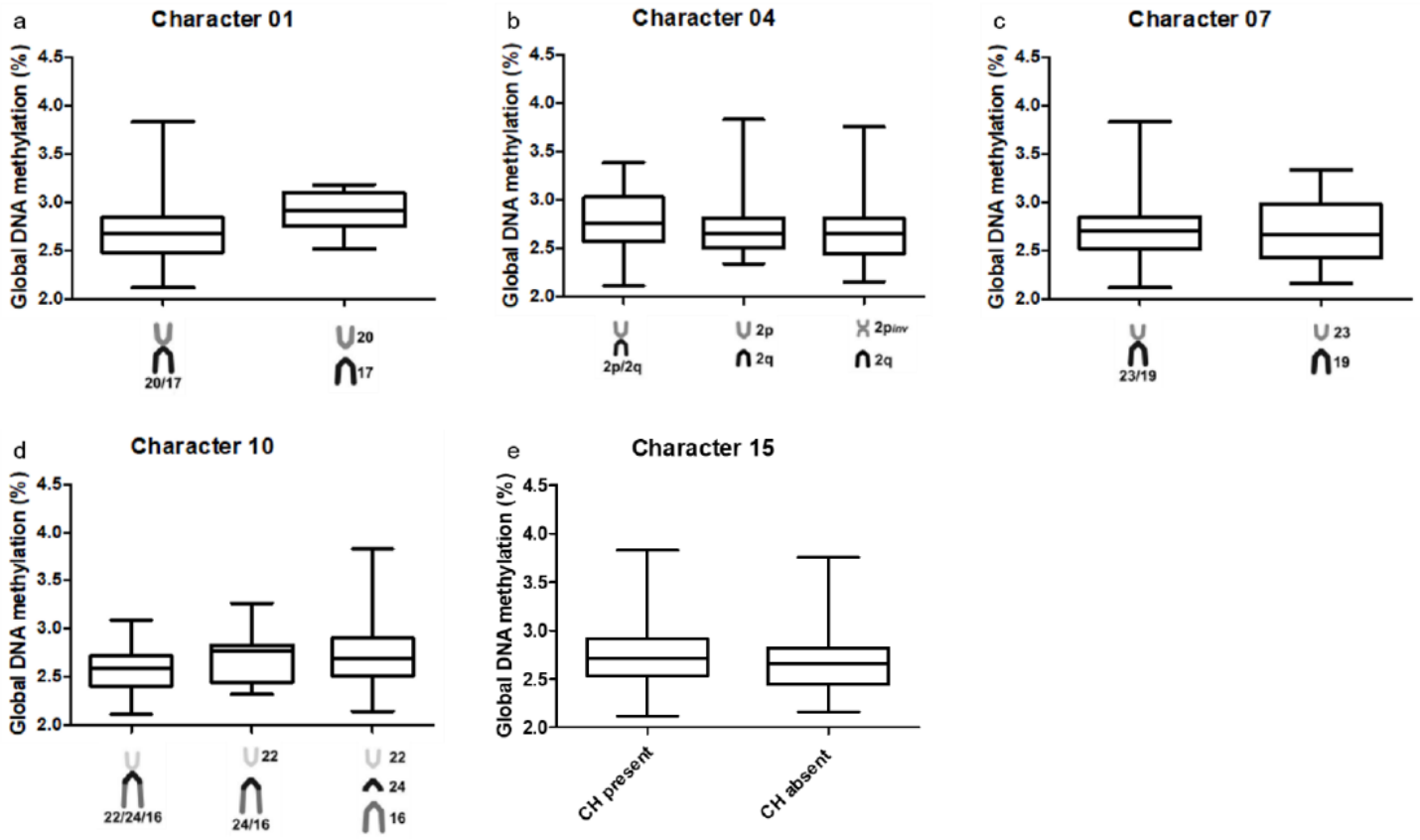

Figure 7

Comparison between global DNA methylation and different rearrangements found in C. minutus cytotypes. a) chromosomes 20 and 17 fused (N = 117), separated (N =7); b) chromosome 2 metacentric (N = 40), acrocentric (N = 38) and 2p with inversion (N = 46); c) chromosomes 23 and 19 fused (N = 100), separated (N = 24); d) chromosomes 22, 24 and 16 fused (N = 13), 24 and 16 fused (N = 15), separated (N = 96); e) heterochromatin present (N = 78), absent (N =46).

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