Physical mapping of repetitive DNA suggests 2n reduction in Amazon turtles *Podocnemis* (Testudines: Podocnemidae)

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Abstract

Cytogenetic studies show that there is great karyotypic diversity in order Testudines (2n = 26–68), and that this may be mainly attributed to the presence/absence of microchromosomes. Members of the Podocnemididae family have the smallest diploid numbers of this order (2n = 26–28), which may be a derived condition of the group. Diverse studies suggest that repetitive-DNA-rich sites generally act as hotspots for double-strand breaks and chromosomal reorganization. In this context, we used fluorescent *in situ* hybridization (FISH) to map telomeric sequences (TTAGGG)n, 45S rDNA, and the genes encoding histones H1 and H3 in two species of genus *Podocnemis*. We also observed conservation of the 45S rDNA and H1 histone sequences (probable case of conserved synteny), but multiple conserved and non-conserved clusters of H3 genes, which colocalized with the interstitial telomeric sequences in the *Podocnemis* genome. Our results suggest that fusions have occurred between macro and microchromosomes or between microchromosomes, leading to the observed reduction in diploid number in the family Podocnemididae.

Introduction

The members of order Testudines may be subdivided in two suborders (Cryptodira and Pleurodira) and comprise one of the oldest lineages of existing vertebrates [1]. Studies have revealed a high degree of karyotypic variation in this order; the diploid numbers (2n) range from 26 in *Peltcephalus dumerilianus* (Pleurodira, Podocnemidae) [2,3] to 68 in *Carettochelys insculpta* (Cryptodira, Carettochelyidae) [4,5], with 2n = 52 reported as the most frequent diploid number [5]. The karyotypic diversity of the Testudines is attributed mainly to the presence/absence of microchromosomes. In suborder Cryptodira, the 2n ranges from 48 to 68 and numerous microchromosomes are seen [4–6]. In suborder Pleurodira, representatives of Chelidae have high diploid numbers and observable microchromosomes, with the 2n ranging from 50 to 58.
Species of superfamily Pelomedusoidea have the smallest diploid numbers: the 2n ranges from 34 to 36 in Pelomedusidae, which have a few microchromosomes [7], and from 26 to 28 in Podocnemididae, which lack microchromosomes [3,8–10]. Cytogenetic studies of the Podocnemididae (Erymnochelys, Peltocephalus and Podocnemis) have suggested that their smaller diploid numbers represent a derived condition (chromosomal reduction) that was likely caused by multiple chromosomal rearrangements [5,7,9,11].

Chromosome mapping of telomeric sequences has been widely used to identify chromosomal rearrangements between the karyotypes of different vertebrate lineages, including various mammals [12–15] amphibians [16,17] and fishes [18,19]. In diverse organisms, the presence of interstitial telomeric sequences, often in association with heterochromatic regions, appear to represent remnants of chromosomal rearrangements that have contributed to reorganizing the genomic architecture and providing new chromosomal forms during evolution [17,20–24]. In chelonians, interstitial telomeric sequences have been identified and examined in Podocnemis unifilis; the authors of these studies proposed that the interstitial telomeric sequences were due to the amplification of telomere-like sequences [10] or represented remnants of chromosomal fusions that reduced the diploid number [5,10].

The grouped organization of rDNA and histone genes makes these sequences useful as chromosomal markers for the study of chromosomal variation and genomic organization in many groups of eukaryotes [25]. High mutation rates in intergenic regions of multigenic families represent an important source of genetic variability and can generate sites that are prone to undergoing double-strand breaks (DSB), which also promotes chromosomal reorganization during karyotypic evolution [19,22,26,27]. In family Podocnemididae, studies suggest that the 45S rDNA located on the first chromosome pair is conserved [3,5,10]. Histone genes have been mapped in diverse organisms [25,28–31], but physical chromosome mapping of histone genes had not previously been reported in any member of order Testudines.

It has been suggested that sites rich in repetitive DNA act as hotspots for DSB and chromosomal reorganization [19,32–34]. This proposal has been supported by data from the in situ mapping of multigenic families, microsatellite expansions and transposable elements in the regions of syntenic breaks, as well as by studies of the chromosomal organizations of many groups [19,32–36]. Because repetitive-DNA-rich regions contain many paralogous genes copies, they facilitate DSB, non-homologous recombination and Robertsonian fusion-based rearrangements [19,33,37]. These regions also undergo sequence exchanges and duplications of subtelomeric regions, such as expansions of multigenic families located near telomeres [38].

The fusion of microchromosomes between themselves and/or with macrochromosomes is considered to be the main mechanism of diploid number reduction in amniotes and tetrapods [39]. In scaled reptiles, it is believed that the large numbers of microchromosomes predicted as the ancestral state were reduced by such fusions [40–42]. In Testudines, some cytogenetic data strengthen the chromosome evolution hypothesis of the group, as ribosomal DNA and nucleolus organizer region, localized in microchromosomes in testudinatas with high diploid number (2n = 50–58) [5,43,44], while for the family Podocnemididae the same markers are reported located on the first chromosome pair [3,5,10].

Here, in an effort to improve our understanding of the chromosomal evolution and genomic dynamics of Podocnemis (Pleurodira, Podocnemidae), we used fluorescent in situ hybridization (FISH) to probe the telomeric, 45S rDNA and histone H1 and H3 sequences in two species of the genus (Podocnemis expansa and Podocnemis unifilis).
Materials and methods

Specimens and approval

We studied two species of genus *Podocnemis*, *Podocnemis expansa* and *Podocnemis unifilis*, utilizing specimens kept in the Zoobotanical Park Adhemar Monteiro, Capitão Poço, Pará, Brazil. This study was conducted in strict accordance with the ethical recommendations for the use and management of chelonians in research, under a protocol approved by Ethics Committee on Experimental Animal Research (license number 68–2015) and Biodiversity Information and Authorization System (SISBIO; license number 42642–5).

Chromosomal preparation, DNA extraction and probe production

Lymphocyte culture and chromosomal preparation were performed as described by Viana et al. [45]. Genomic DNA was purified from the muscle tissues and blood specimens using the conventional proteinase K and phenol/chloroform extraction method [46]. The obtained DNA was diluted in elution buffer and kept at 20°C until use. The genes encoding histones H1 and H3 were polymerase chain reaction (PCR) amplified using the following primers: 5'-AGA RGA GCG GCG TGT-3' and 5'-CYT CTT CRC CTT CYT KG-3' for histone H1; and 5'-ATG GCT CGT ACC AAG CAG AC(ACG) GC-3' and 5'-ATA TCC TT(AG) GGC AT(AG) AT(AG) GTG AC-3' for histone H3, both designed by Cabral-de-Mello et al. [47]. The amplification reaction set up: genomic DNA = 80 ng, forward primer = 0.2 μM, reverse primer = 0.2 μM, dNTPs = 0.16 mM, Taq DNA Polymerase (Invitrogen) = 1 U, MgCl2 = 1.5 mM, reaction buffer 1× (200 mM Tris, pH 8.4, 500 mM KCl). The amplification program set up: 4min–95°C/(1min—95°C / 1min—60°C / 2min—74°C) 30 cycles / 5min—74°C. The general telomeric sequence of vertebrates (TTAGGG)n was obtained as described by Ijdo et al. [48]. To construct the 45S rDNA probe, we used the pTa71 plasmid, which contains the 5.8S, 18S and 28S genes and their respective intergenic spacers from *Triticum aestivum* [49]. The probes were nick-translation-labeled with biotin 14-dATP or digoxigenin 16-dUPT using the BioNick Labeling System (Invitrogen) and a DIG-Nick kit (Roche Applied Science), respectively.

Fluorescence in situ hybridization (FISH)

FISH was performed as described by Pinkel et al. [50], with some modification. The signals were detected with avidin-CY3 (Sigma) and antidigoxigenin-FITC (Roche). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; 0.2 μg mL−1) in Vectashield H-100 mounting medium (Vector) and analyzed under an epifluorescence microscope (Nikon H550S). The chromosomes were organized by size and categorized as metacentric (m), sub-metacentric (sm), subtelocentric (st) or acrocentric (a) as previously described [51]. Approximately 30 metaphase spreads of each species were analyzed to determine the diploid number, karyotypic formula and the presence/absence of interstitial telomeric sequences, rDNA and histones H1 or H3.

Results

The two species presented a diploid number of 28 chromosomes. *P. expansa* had a fundamental number (FN) of 54 and a karyotypic formula of 24m/sm + 2st + 2a, while *P. unifilis* had FN = 52 and a karyotypic formula of 22m/sm + 2st + 4a (Figs 1–3). The main karyotypic difference between the two species was in chromosome pair 9, which was submetacentric in *P. expansa* and acrocentric in *P. unifilis*. Both species also showed a size heteromorphism for pair 10. No heteromorphic sex chromosome was found in either species.
Telomeric (TTAGGG)n signals were observed in the distal regions of all chromosome pairs in *P. expansa* and *P. unifilis*. In addition, interstitial telomeric sequences signals were detected on pairs 1–5, 7 and 13 in both species; besides those signals, it was detected interstitial telomeric sequences in a chromosome of pair 6, in a single homologue, in the two species (Figs 1–3).

The 45S rDNA sites were found in the proximal region of the short arm of submetacentric pair 1 in both species. Double-FISH showed that the 45S rDNA and the interstitial telomeric sequences signals in the first chromosome pair are adjacent in *P. expansa* and *P. unifilis* (Fig 1).

Similar to the results obtained from FISH with rDNA, the H1 histone sequence was localized in the proximal region of the short arm of the first chromosome pair in both species.
Double-FISH revealed that the signals of the histone H1-encoding genes are adjacent with the interstitial telomeric sequences in both species (Fig 2).

*In situ* localization of the histone H3-encoding sequences revealed that clustered in the peri-centromeric regions of six chromosome pairs (pairs 1–5 and 7) in *P. unifilis* (Fig 3B). Similar signals were observed in *P. expansa* specimens, and also revealing clusters of histone H3-encoding sequences in an additional chromosome pair (pairs 1–5, 7 and 13) (Fig 3A). Double-FISH showed that the histone H3 signals consistently colocalized with the interstitial telomeric sequences of both species, except for the interstitial telomeric sequences present in the single chromosome from pair 6 in both species and in pair 13 of *P. unifilis* (Fig 3).
Discussion

Podocnemididae have the smallest diploid numbers of the order Testudines, with 2n ranging from 26 to 28 chromosomes [3,10]. Our results corroborate those previously obtained for *P. expansa* and *P. unifilis*, in that we observed 2n = 28 chromosomes, with no microchromosomes [7–11,52]. We also observed evidence of possible chromosome fusions in these species. Our data support the hypothesis that the diploid number has undergone reduction in Podocnemididae and suggest a few chromosomal sites that may have been involved in these genomic reorganization events.
Our cytogenetic data revealed the presence of size heteromorphism for pair 10 in the karyotypes of *P. expansa* and *P. unifilis*, which is consistent with the data obtained by Noronha et al. [10] for *P. unifilis*. The authors of the prior paper suggested that this might reflect a size variation in the constitutive heterochromatin of one of the homologous chromosomes, which would have originated through uneven crossover(s), transposition(s), and/or duplication(s) in *cis*. However, whereas Noronha et al. [10] did not observe heteromorphism of chromosome pair 10 in *P. expansa*, we observed such heteromorphism in the present study. This apparent discrepancy can be explained by the shortening of the chromosomes that occurs during the chromosomal preparation method used in the previous paper, complicating the identification of heteromorphism. Our analysis further showed that this karyotypic variation did not involve the 45S rDNA, (TTAGGG)n or histone H1 and H3 sequences.

Our identification of interstitial telomeric sequences sites in the pericentromeric regions of both species corroborates the findings of Montiel et al. [5] and Noronha et al. [10] for specimens of *P. unifilis*, but contrasts with the lack of such sites reported by Noronha et al. [10] for *P. expansa*. We speculate that the interstitial telomeric sequences of the previously studied examples of *P. expansa* could have undergone successive losses and/or degenerations, leading to a gradual shortening of non-functional telomeric matrices [53]. In this context, such interstitial telomeric sequences would be very short and might not be detected by the techniques previously used for their visualization [54,55]. The shortening of the non-functional telomeric matrix could be a possible cause for the visualization of the interstitial telomeric sequences in a single homologue of the chromosome pair 6 in the present study. In addition, not all chromosomal fusions retain telomeric DNA repeats at the fusion points. The lack of telomeric hybridization signals at putative fusion sites may therefore suggest that the chromosome breakage that preceded the fusion event occurred within the chromatin proximal to the telomeric region [54].

Our preliminary analysis indicated that the interstitial telomeric sequences in the pericentromeric chromosomal regions of *P. expansa* and *P. unifilis* can be categorized as heterochromatic interstitial telomeric sequences. This suggests that these regions may have been involved in the diploid number reduction of Podocnemididae, since they are considered to be unstable regions where chromosomal rearrangements may occur [20,56,57]. The fusion of microchromosomes between themselves and/or with macrochromosomes is considered to be the main mechanism of diploid number reduction in amniotes and tetrapods [39]. In scaled reptiles, it is believed that the large numbers of microchromosomes predicted as the ancestral state were reduced by such fusions [40–42]. In lizards, few microchromosomes are found, and some chromosomal pairs are composed of tandem-fused chromosome segments that have homologies with microchromosomes; this suggests that the karyotypes of lizards probably arose via the in-tandem fusion of microchromosomes [58]. In this context, our detection of interstitial telomeric sequences in the pericentromeric region of seven chromosome pairs of *P. expansa* and *P. unifilis* reinforce the hypothesis that these interstitial telomeric sequences represent telomeric DNA remnants at points where micro- and macrochromosomes, or in tandem between microchromosomes, underwent fusion during evolution. However, it is important to emphasize that the interstitial repetitions of TTAGGG observed in this manuscript may also represent effect of telomeric sequence amplification, or like-telomeres regions, because generally these repetitions are lost, as previously reported in specimens of *P. expansa* [10].

Studies have demonstrated that the 45S rDNA is localized in chromosome pair 1 of Podocnemididae [3,5,10]. In *P. expansa* and *P. unifilis* the nucleolus organizer region (NOR) is flanked by regions that display CMA3 signals, indicating that the 45S rDNA region of the first chromosomal pair in these species is rich in GC base pairs [10]. In this context, we propose
that the first chromosome pair can be considered as a marker, with synapomorphic characteristic to the Podocnemididae family. Or yet, it is possible that genes preserved between representatives of the Podocnemididae family to signal a case of conserved synteny, because some genes tend to stay together throughout evolution and remain as conserved synteny blocks in a wide range of species [59–62]. So we constructed the ideogram that represent the physical chromosome mapping indicating a probable conserved synteny segment for the family Podocnemididae (Fig 4).

Previous studies found that 45S rDNA sites were localized in microchromosomes of the following: *Hydromedusa tectifera* (Pleurodira, Chelidae), which has 2n = 58 [43]; members of genus *Trachemys* (Cryptodira, Emydidae), which has 2n = 50 [44]; *Sternotherus odoratus* (Cryptodira, Kinosternidae), which has 2n = 56; *Emydra macquarii* (Pleurodira, Chelidae), which has 2n = 50; and *Chelodina oblonga* (Pleurodira, Chelidae), which has 2n = 54 [5]. The microchromosome localizations of 45S rDNA sequences in species with higher diploid numbers strongly support the idea that chromosomal fusions took place between rDNA-carrying microchromosomes and macrochromosomes during the evolution of chromosome pair 1 of Podocnemididae.

Although the physical mapping of histone genes have been done in some organisms, as invertebrates [25,28,31] and fishes [29,30], the present work is the first to report the in situ location of histone gene sequences in members of order Testudines. We found genes encoding histones H1 and H3 in the proximal region of the short arm of the first chromosome pair, indicating that this site is likely to be the main histone cluster for *P. expansa* and *P. unifilis*. This reinforces the notion that this region houses several repetitive sequences and represents a synapomorphic characteristic of family Podocnemididae, or yet a case of conserved synteny. However, the most striking case was the location of histone H3. Although the histone genes are very conserved within species, the organization of their clusters within the genome may be heterogeneous [28,30,63]. The difference in the distribution pattern of many H3 sites not correlated with H1 sites suggests an evolutionary dichotomy between those sequences in genome of...
P. expansa and P. unifilis. Some studies have suggested that the H3 sequences may be dispersed throughout genomes by ectopic recombination, invasion of transposable elements (TE), and/or circular DNA [30,31]. In fishes, Pucci et al. [64] demonstrated that parts of TE may be found in the intergenic regions of histone sequences, and suggested that such elements could help disperse copies of histone genes throughout a genome. Thus, it is likely that the dispersion of histone H3 in the studied species may be associated with TE insertions and/or genetic hitchhiking.

Non-reciprocal sequence exchanges and duplications of subtelo melan regions are frequent, especially when there is expansion of multigenic families close to telomeres [38]. Histone sequences have features that are common to chromosome breakage regions, in that they are arranged in tandem repeats, localized at pericentromeric or subtelo melan chromosome regions, display transposition ability when invaded by TE, and exhibit high intra- and inter-chromosomal recombination rates. In a similar pathway, interstitial telomeric sequences are associated with hotspots for chromosomal breakage and are involved in DSB repair; they appear to represent a favorable substrate for chromosome breakage and may thus promote genomic instability (for details, see [55]). In the present study, the colocalization of H3 histone with interstitial telomeric sequences in pericentromeric regions of the two species also suggests that non-homologous recombination may have acted in the dispersion of these sequences. Such sequences would logically trigger chromosomal rearrangements [20], since interstitial telomeric sequences create chromosomal instability and are prone to DSB [65,66]. This would support mainly end-to-end fusions, which could cause the observed reduction to 2n = 28.

Conclusions

In conclusion, we herein report that the karyotypes of two representative members of Podocnemis lack microchromosomes but harbor interstitial telomeric sequences. We provide evidence that the fusions of macro- and microchromosomes or in tandem between microchromosomes have occurred during the chromosomal evolution of this group, reducing the diploid number (2n = 28). Furthermore, the genomic locations of rDNA and genes encoding histone H1 are conserved on the first chromosome pair of Podocnemis, may represent conserved synteny blocks, whereas the genes encoding histone H3 are distributed in multiple conserved and non-conserved clusters that colocalized with interstitial telomeric sequences, can indicate non-homologous recombination or associated with TEs insertions and genetic hitchhiking.

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References
1. Ferri V. Turtles & Tortoises: A Firefly Guide. Firefly Books. 2002. 256p.
2. Killebrew FC. Mitotic chromosomes of turtles. Part 1: The Pelomedusidae. J Herpetol. 1975; 9:281–285.
3. Ventura K, Moreira CN, Moretti R, Yonenag-Yassuda Y, Rodrigues MT. The lowest diploid number in Testudines: Banding patterns, telomeric and 45S rDNA FISH in Peltocephalus dumerilianus, 2n = 26 and FN = 52 (Pleurodira, Podocnemididae). Genet. Mol. Biol. 2014; vol. 37 no.1 Ribeirão Preto. ISSN 1415–4757.
4. Bickham JW, Carr JL. Taxonomy and Phylogeny of the Higher Categories of Cryptodiran Turtles Based on a Cladistic Analysis of Chromosomal Data. Copeia, n. 4. 1983. p. 918–932.
5. Montiel EE, Badenhorst D, Lee LS, Litterman R, Trifonov V, Valenzuela N. Cytogenetic Insights into the Evolution of Chromosomes and Sex Determination Reveal Striking Homology of Turtle Sex Chromosomes to Amphibian Autosomes. Cytogenet Genome Res. 2016. https://doi.org/10.1159/000447478 PMID: 27423490
6. Bickham JW. Two-Hundred-Million-Year-Old Chromosomes: Deceleration of the Rate of Karyotypic Evolution in Turtles. Science. 1981; 212:1291–1293. https://doi.org/10.1126/science.212.4500.1291 PMID: 17738838
7. Bull JJ, Legler JM. Karyotypes of side necked turtles (Testudines, Pleurodira). Can J Zool. 1980; 58:828–884.
8. Ayres M, Sampaio MM, Barros RMS, Dias LB, Cunha OR. A karyological study of turtles from the Brazilian Amazon River. Cytogenetics. 1969; 8: 401–409. PMID: 5365245
9. Gunski RJ, Cunha IS, Degranni TM, Ledesma M, Garnero ADV. Cytogenetic comparison of Podocnemis expansa and Podocnemis unifilis: A case of inversion and duplication involving constitutive heterochromatin. Genet Mol Biol. 2013; Sep; 36(3): 353–356. https://doi.org/10.1590/S1415-47572013005000029 PMID: 24130442
10. Noronha RCR, Barros LMR, Araujo REF, Marques DF, Nagamachi CY, Martins C, Pieczarka JC. New insights of karyoevolution in the Amazonian turtles Podocnemis expansa and Podocnemis unifilis (Testudines, Podocnemididae). Molecular Cytogenetics. 2016; 9:73 https://doi.org/10.1186/s13039-016-0281-5 PMID: 27708713
11. Fantin C, Monjelô LAS. Cytogenetic studies in Podocnemis expansa and Podocnemis sextuberculata (Testudines, Podocnemididae), turtles of the Brazilian Amazon. Caryologia. 2011; 64(2):154–7.
12. Tsipouri V, Schueller MG, Hu S, NISC Comparative Sequencing Program, Dutra A, Pak E, Riethman H, Green ED. Comparative sequence analyses reveal sites of ancestral chromosomal fusions in the Indian muntjac genome. Genome Biol. 2008; 9:R155. https://doi.org/10.1186/gb-2008-9-10-r155 PMID: 18957082
13. Nagamachi CY, Pieczarka JC, O’Brien PC, Pinto JA, Malcher SM, Pereira AL, et al. FISH with whole chromosome and telomeric probes demonstrates huge karyotypic reorganization with ITS between two species of Oryzomyini (Sigmodontinae, Rodentia): Hylaemys megacephalus probes on Cerradomyrmex langguthi karyotype. Chromosoma Res. 2013; Apr; 21(2):107–19. https://doi.org/10.1007/s10577-013-9341-9 PMID: 23494775

14. Rodrigues da Costa MJ, Siqueira do Amaral PJ, Pieczarka JC, Sampaio MI, Rossi RV, Mendes-Oliveira AC, et al. Cryptic Species in Proechimys goeldii (Rodentia, Echimyidae)? A Case of Molecular and Chromosomal Differntiation in Allopatric Populations. Cyto genetic Genome Res. 2016; 148(2–3):199–210. https://doi.org/10.1159/000446562 PMID: 27255109

15. de Araújo RE, Nagamachi CY, da Costa MJ, Noronha RC, Rodrigues LR, Pieczarka JC. First description of multivalent ring structures in euthenic mammalian meiosis: new chromosomal characterization of Cormura brevirostris (Emballonuridae, Chiroptera). Genetica. 2016; Aug; 144(4):407–15. https://doi.org/10.1007/s10709-016-9909-y PMID: 27300547

16. Schimid M, Steinlein C, Bogart JP, Feichtinger W, León P, Marca EL, et al. The chromosomes of terraran frogs. Insights into vertebrate cytogenetics. Cyto genetic Genome Res. 2010; 130–131:1–568. https://doi.org/10.1159/000301339 PMID: 21063086

17. Suárez P, Cardozo D, Baldó D, Pereyra MO, Faivovich J, Oriño VGD, et al. Chromosome evolution in Dendropsophiini (Amphibia, Anura, Hylinae), Cyto genetic Genome Res. 2013; 141:295–308. https://doi.org/10.1159/000354997 PMID: 21063086

18. Scacchetti PC, Pansonato-Alves JC, Utsunomia R, Oliveira C, Foresti F. Karyotypic diversity in four species of the genus Gymnotus Linnaeus, 1758 (Teleostei, Gymnotiformes, Gymnotidae): physical mapping of ribosomal genes and telomic sequences. Comp Cytogen. 2011; 5(3):223–235.

19. Barros AV, Wolski MAV, Nogaroto V, Almeida MC, Moreira-Filho O, Vicari MR. Fragile sites, dysfunctional telomere and chromosome fusions: What is SS rDNA role? Gene. 2017; 608: 20–27. https://doi.org/10.1016/j.gene.2017.01.013 PMID: 28111257

20. Ruiz-Herrera A, Nergadze SG, Santagostino M, Giulotto E. Telomeric repeats far from the ends: mechanisms of origin and role in evolution. Cyto genetic Genome Res. 2008; 122: 219–225. https://doi.org/10.1159/000167807 PMID: 19188690

21. Rosa KO, Ziemiczak K, Barros AV, Nogaroto V, Almeida MC, Cestari MM, et al. Numerical and structural chromosome polymorphism in Rineloricaria lima (Siluriformes: Loricariidae): fusion points carrying SS rDNA or telomere sequence vestiges. Rev Fish Biol Fisheries. 2012; 22:739–749 https://doi.org/10.1007/s11160-011-9250-6

22. Bruschi DP, Rivera M, Lima AP, Züñiga AB, Recco-Pimentel SM. Intersitial Telomeric Sequences (ITS) and major rDNA mapping reveal insights into the karyotypic evolution of Neotropical leaf frogs species (Phyllomedusa, Hylidae, Anura), Molecular Cytogenetics. 2014; 7:22. https://doi.org/10.1186/1755-8166-7-22 PMID: 24602295

23. Porto FE, Vieira MM, Barbosa LM, Borin-Carvalho LA, Vicari MR, Portela-Castro AL, Martins-Santos IC. Chromosomal Polymorphism in Rineloricaria Lanceolata Günther, 1868 (Loricariidae: Loricariinae) of the Paraguay Basin (Mato Grosso do Sul, Brazil): Evidence of Fusions and Their Consequences in the Population. Zebrafish. 2014; 11:318–324. https://doi.org/10.1089/zeb.2014.0996 PMID: 25069031

24. Primo CC, Glugoski L, Almeida MC, Zawadzki CH, Moreira-Filho O, Vicari MR, Nogaroto V. Mechanisms of Chromosomal Diversification in Species of Rineloricaria (Actinopterygy: Siluriformes: Loricariidae). ZEBRAFISH. 2017; Volume 14, Number 2, Mary Ann Liebert, Inc. https://doi.org/10.1089/zeb.2016.1386 PMID: 28027029

25. Cabral-de-Mello DC, Moura RC, Martins C. Cytogenetic Mapping of rRNAs and Histone H3 Genes in 14 Species of Dichotomius (Coleoptera, Scarabaeidae, Scarabaeinae) Beetles. Cyto genetic Genome Res. 2011; 127:135–179 https://doi.org/10.1159/000326803 PMID: 21555878

26. Carvalho A, Guedes-Pinto H, Lima-Brito J. Physical localization of NORs and ITS length variants in old Portuguese durum wheat cultivars. J Genet. 2011; 90(1):95–101. PMID: 21677393

27. Georgiev O, Karagyozev L. Structure of the intergenic spacer of barley ribosomal DNA repeat units: evidence for concerted evolution. Genetics and Plant Physiology. 2012; 2:145–150.

28. Cabrero J, López-León MD, Teruel M, Camacho JP. Chromosome mapping of H3 and SS rRNA gene clusters in 35 species of acridid grasshoppers. Chromosoma Res. 2009; 17:397–404. https://doi.org/10.1007/s10577-009-9030-5 PMID: 19337846

29. Hashimoto DT, Ferguson-Smith MA, Rens W, Foresti F, Porto-Foresti F. Chromosome mapping of H1 histone and SS rRNA gene clusters in three species of Astyanax (Teleostei, Characiformes). Cyto genetic Genome Res. 2011; 134:64–71. https://doi.org/10.1159/000323512 PMID: 21252491

30. Costa GW, Cioffi MB, Bertollo LA, Molina WF. Unusual dispersion of histone repeats on the whole chromosomal complement and their colocalization with ribosomal genes in Rachycentron canadum
31. Almeida BRR, Milhomem-Paixão SSR, Noronha RCR, Nagamachi CY, Costa MJR, Pardal PPO, et al. Karyotype diversity and chromosomal organization of repetitive DNA in *Tityus obscurs* (Scorpiones, Buthidae). BMC Genetics. 2017; 18:35. https://doi.org/10.1186/s12863-017-0494-6 PMID: 28412934

32. Huang J, Ma L, Yang F, Fei SZ, Li L. 45S rDNA regions are chromosome fragile sites expressed as gaps in vitro on metaphase chromosomes of root-tip meristematic cells in *Lolium* spp. Plos One. 2008; 3:e2167. https://doi.org/10.1371/journal.pone.0002167 PMID: 18478113

33. Cazaux B, Catalán J, Veyrunes F, Douzery EJP, Britton-Davidian J. Are ribosomal DNA clusters rearrangement hotspots? A case in the genus *Mus* (Rodentia, Muridae). BMC Evol Biol. 2011; 11:124. https://doi.org/10.1186/1471-2148-11-124 PMID: 21569527

34. Srikulnath K, Nishida C, Matsuda Y. Karyotype evolution in monitor lizards: cross-species chromosome mapping . PLoS ONE. 2012; 7(12): e53027. https://doi.org/10.1371/journal.pone.0053027 PMID: 23030852

35. Fantin C, Giuliano-Caetano L. Cytogenetic characterization of two turtle species: *Trachemys scripta elegans* and *Trachemys dorbigni* (Testudines: Podocnemididae). Cytogenet Genom. 2008; 13: 975–986. https://doi.org/10.1007/s10577-008-9398-0 PMID: 25341625

36. Ijdo JW, Wells RA, Baldini A, Reeders ST. Improved telomere detection using a telomere repeat probe (TTAGGG)ₙ generated by PCR. Nucleic Acids Res. 1991; Set 11; 19(17): 4780. PMID: 18913730
49. Gerlach WL, Bedbrook JR. Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res. 1979; 7:1885–69.

50. Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci. 1986; 83:2934–2938. PMID: 3458254

51. Levan A, Fredga K, Sandberg AA. Nomenclature for centromeric position on chromosomes. Hereditas. 1964; Volume 52, Issue 2. Pages 201–220.

52. Huang C, Clark CHF. Chromosome studies of the cultured cells of two species of side-necked turtles (Podocnemis unifilis and Podocnemis expansa). Chromosoma. 1969; 26: 245–253. PMID: 5800666

53. Slijepcevic P, Hande MP, Bouffler SD, Lansdorp P, Bryant PE. Telomere length, chromatin structure and chromosome fusigenic potential. Chromosoma. 1998; 106:413–421.

54. Ocalewicz K. Telomeres in Fishes. Cytogenet Genome Res. 2013; 141:114–125 https://doi.org/10.1159/000354278 PMID: 23988378

55. Bolzán AD. Interstitial telomeric sequences in vertebrate chromosomes: origin, function, instability and evolution. Mutat Res. 2017; 775:51–65. https://doi.org/10.1016/j.mrrev.2017.04.002 PMID: 28927537

56. Schmidt M, Steinlein C. Chromosome banding in amphibia. XXXIV. Intrachromosomal telomeric DNA sequences in Anura. Cytogenet Genome Res. 2016; 148:211–226. https://doi.org/10.1159/000446298 PMID: 27230250

57. Shampay J, Schmitt M, Bassham S. A novel minisatellite at a cloned hamster telomere. Chromosoma. 1995; 104:29–38. PMID: 7587592

58. Srikulnath K, Uno Y, Nishida C, Ota H, Matsuda Y. Karyotype Reorganization in the Hokou Gecko (Gekko hokouensis, Gekkonidae): The Process of Microchromosome Disappearance in Gekkota. PLoS ONE. 2015; 10(8): e0134829. https://doi.org/10.1371/journal.pone.0134829 PMID: 26241471

59. Kirk IK, Weinhold N, Brunak S, Belling K. The impact of the protein interactome on the syntenic structure of mammalian genomes. PLoS One. 2017; Sep 14; 12(9):e0179112. https://doi.org/10.1371/journal.pone.0179112 eCollection 2017. PMID: 28910296

60. López MD, Guerra JJM, Samuelsson T. Analysis of gene order conservation in eukaryotes identifies transcriptionally and functionally linked genes. PLoS ONE. 2010; 5: e10654 https://doi.org/10.1371/ journal.pone.0010654 PMID: 20498846

61. Kikuta H, Laplante M, Navratilova P, Komisarczuk AZ, Engström PG, Fredman D, et al. Genomic regulatory blocks encompass multiple neighboring genes and maintain conserved synteny in vertebrates. Genome Res. Cold Spring Harbor Lab; 2007; 17: 545–555. https://doi.org/10.1101/gr.6086307 PMID: 17387144

62. Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, et al. A gene atlas of the mouse and human protein-encoding transcripts. Proc Natl Acad Sci USA. National Acad Sciences; 2004; 101: 6062–6067. https://doi.org/10.1073/pnas.0400782101 PMID: 15075390

63. Eiríñ-López JM, González-Romero R, Dryhurst D, Méndez J, Ausió J. Long-term evolution of histone families: old notions and new insights into their mechanisms of diversification across eukaryotes, in Pontarotti P (ed): Evolutionary Biology: Concept, Modeling and Application. 2009. pp 139–162.

64. Pucci MB, Nogaroto V, Moreira-Filho O, Vicari MR. Dispersion of transposable elements and multigene families: Microstructural variation in Characidium (Characiformes: Crenuchidae) genomes. Genetics and Molecular Biology, (in press).

65. Bolzán AD. Chromosomal aberrations involving telomeres and interstitial telomeric sequences. Mutagenesis. 2012; 27 (1), 1–15. https://doi.org/10.1093/mutage/ger052 PMID: 21857006

66. Aksenova AY, Greenwell PW, Dominska M, Shishkin AA, Kim JC, Petes TD, Mirkin SM. Genome rearrangements caused by interstitial telomeric sequences in yeast. Proc. Natl. Acad. Sci. U. S. A. 2013; 110: 19866–19871. https://doi.org/10.1073/pnas.1319313110 PMID: 24191060