UNIVERSIDADE ESTADUAL DO OESTE DO PARANÁ
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO STRICTO SENSU EM CONSERVAÇÃO E MANEJO DE RECURSOS NATURAIS – NÍVEL MESTRADO

CHRYSTIAN APARECIDO GRILLO HAERTER

# INVESTIGAÇÃO DA DISTRIBUIÇÃO DE ELEMENTOS REPETITIVOS NO GENOMA DE ESPÉCIES DE *TRACHELYOPTERUS* (VALENCIENNES, 1840)

CASCAVEL-PR
Agosto/2021

UNIVERSIDADE ESTADUAL DO OESTE DO PARANÁ
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO STRICTO SENSU EM CONSERVAÇÃO E MANEJO DE RECURSOS NATURAIS – NÍVEL MESTRADO

# INVESTIGAÇÃO DA DISTRIBUIÇÃO DE ELEMENTOS REPETITIVOS NO GENOMA DE ESPÉCIES DE *TRACHELYOPTERUS* (VALENCIENNES, 1840)

CHRYSTIAN APARECIDO GRILLO HAERTER

Dissertação apresentado ao Programa de Pós-graduação Stricto Sensu em Conservação e Manejo de Recursos Naturais – Nível Mestrado, do Centro de Ciências Biológicas e da Saúde, da Universidade estadual do Oeste do Paraná, como requisito para a obtenção do título de Mestre em Conservação e Manejo de Recursos Naturais

Área de Concentração: Ciências Ambientais

Orientador: Roberto Laridondo Lui

CASCAVEL-PR
Agosto/2021

**CHRYSTIAN APARECIDO GRILLO HAERTER**

**Investigação da distribuição de elementos repetitivos no genoma de espécies de *Trachelyopterus* (Valenciennes, 1840)**

Dissertação apresentada ao Programa de Pós-Graduação em Conservação e Manejo de Recursos Naturais em cumprimento parcial aos requisitos para obtenção do título de Mestre em Conservação e Manejo de Recursos Naturais, área de concentração Ciências Ambientais, linha de pesquisa Biologia comparada e indicadores de qualidade no ambiente aquático, APROVADO pela seguinte banca examinadora:

Orientador - Roberto Laridondo Lui

Universidade Estadual do Oeste do Paraná - Campus de Cascavel (UNIOESTE)

Eliana Feldberg

Instituto Nacional de Pesquisas da Amazônia (INPA)

Cesar Martins

Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP)

Cascavel, 27 de agosto de 2021

Ficha de identificação da obra elaborada através do Formulário de Geração Automática do Sistema de Bibliotecas da Unioeste.

Haerter, Chrystian Aparecido Grillo
Investigação da distribuição de elementos repetitivos no genoma de espécies de *Trachelyopterus* (Valenciennes, 1840) / Chrystian Aparecido Grillo Haerter; orientador Roberto Laridondo Lui. -- Cascavel, 2021.
129 p.

Dissertação (Mestrado Acadêmico Campus de Cascavel) -- Universidade Estadual do Oeste do Paraná, Centro de Ciências Biológicas e da Saúde, Programa de Pós-Graduação em Conservação e Manejo de Recursos Naturais, 2021.

1. DNA ribossomal 18S e 5S. 2. Histonas H3 e H4. 3. Small Nuclear RNA U2. 4. Microssatélite (GATA)n. I. Lui, Roberto Laridondo, orient. II. Título.

# Sumário

*“A Ciência não resolve um problema sem criar outros dez”*

*George Bernard Shaw*

## AGRADECIMENTOS

Agradeço à minha querida mãe, dona Marlene, por todo incentivo, apoio e ensinamento durante toda a minha jornada. Também por todo amor, carinho e cuidado durante toda a vida acadêmica e vida fora dela.

Agradeço também ao meu pai, que mesmo distante nunca deixou de acreditar em mim, em minha capacidade profissional e pessoal.

Agradeço também, e muito, a minha querida Dhyovana, por me ouvir nos momentos mais difíceis, me incentivar naqueles em que eu pensava em desistir, por me animar nos momentos tristes e dividir comigo um pouco da sua imensa alegria, carinho, dedicação e amor.

Agradeço imensamente ao meu professor e orientador Roberto Laridondo Lui por todo incentivo profissional e pessoal durante os últimos três anos, que nunca duvidou da minha capacidade e me auxiliou nessa jornada de muito aprendizado e bons resultados.

Meus sinceros agradecimentos também ao professor Vladimir Pavan Margarido, pelas conversas, orientações e incentivos. Assim, como aos professores Daniel Rodrigues Blanco, Josiane Baccarin Traldi e Fabio Hiroshi Takagui pelos ensinamentos e conversas e Alberto Sergio Fenocchio pela hospitalidade e compartilhamento de experiência durante a coleta e preparo de exemplares.

Agradeço aos meus primeiros colegas de laboratório, Sandro, Amanda e Evandro pelo suporte, assim como a todos os outros que vieram e também participaram da minha formação: Gabrielle, Cleisson, Letícia, Gabriel, Natália, Simone, Diana, Treicy, Luiz e Samantha.

Agradeço à Universidade Estadual do Oeste do Paraná e o Programa de Mestrado em Conservação e Manejo de Recursos Naturais – PPRN/UNIEOSTE por possibilitar a graduação e também a pós-graduação do autor, assim como a Coordenação de Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES pelo suporte financeiro que possibilitou o desenvolvimento na integra deste projeto.

## RESUMO

*Trachelyopterus* é um gênero de bagre Neotropical com história taxonômica controversa e mais de 200 anos de revisões devido à morfologia semelhante entre as espécies. Citogeneticamente, *Trachelyopterus* ainda é pouco explorado e não há mapeamento além dos DNAs ribossomais 18S e 5S. Apenas o microssatélite $(GATA)_n$ foi analisado em algumas espécies, tendo papel crucial na origem e evolução de cromossomos B, mas sem eficiência em distinguir as espécies através dos cromossomos do complemento A. Neste estudo, o mapeamento dos elementos repetitivos 18S e 5S DNAr, genes de histonas H3 e H4, snRNA U2 e o microssatélite $(GATA)_n$ foi realizado em seis espécies de *Trachelyopterus*: *T. striatulus*, *T. galeatus* e *T. porosus* da Bacia hidrográfica do Rio Amazonas; *T. coriaceus* e *Trachelyopterus* aff. *galeatus* (possível nova espécie) da bacia do Rio Araguaia; e *Trachelyopterus* aff. *coriaceus* (possível nova espécie) da bacia do Rio Paraguai. O DNAr 18S foi encontrado em apenas um par de cromossomos. O 5S DNAr foi evidenciado em 1-3 pares cromossômicos. Os genes de histonas H3 e H4 foram encontrados em 1-2 pares cromossômicos e, na maioria das espécies, sintênicos com o DNAr 18S. O snRNA U2 foi encontrado em apenas um par de cromossomos, todavia, *Trachelyopterus* aff. *galeatus* teve um polimorfismo de inversão cromossômica, que está em equilíbrio de Hardy-Weinberg e cromossomos homólogos com tamanho distinto. Citotaxonomicamente, o DNAr 5S revelou autapomorfias de quatro espécies, os genes de histonas H3 e H4 e o DNAr 18S de duas espécies e o snRNA U2 de apenas uma espécie. Como resultado, todas as espécies puderam ser distinguidas, incluindo *Trachelyopterus* aff. *coriaceus* e *Trachelyopterus* aff. *galeatus*, ambos possíveis novas espécies. Uma vez conhecido o padrão de distribuição dos genes 18S e 5S DNAr, snRNA U2 e histonas H3 e H4, este foi usado como guia para comparar o padrão de distribuição do microssatélite $(GATA)_n$ nos cromossomos homeólogos entre as seis espécies. Como resultado, a maioria das espécies teve um padrão de distribuição semelhante da sequência $(GATA)_n$ nos portadores do DNAr 18S. Porém, *T. galeatus* apresentou polimorfismo cromossômico, que está em equilíbrio de Hardy-Weinberg e foi possivelmente originado através de eventos de amplificação. Por outro lado, o mapeamento da sequência $(GATA)_n$ nos portadores dos genes 5S DNAr e H3 / H4 não revelou novas informações sobre a estrutura desses pares cromossômicos, todavia, permitiu indicar com maior precisão as possíveis homeologias cromossômicas entre as espécies e reafirmou a existência do polimorfismo cromossômico em *T. galeatus*. Semelhante aos portadores de DNAr 18S, a maioria das espécies apresentou um padrão de distribuição conservado do microssatélite $(GATA)_n$ nos cromossomos do snRNA U2. No entanto, *Trachelyopterus* aff. *galeatus* apresentou um novo polimorfismo cromossômico em desequilíbrio de Hardy-Weinberg e seis possíveis citótipos. Assim, através do mapeamento combinado de marcadores, pudemos evidenciar autapomorfias citotaxonômicas que distinguiem todas as seis espécies, incluindo as possíveis novas espécies, como também evidenciamos novos aspectos evolutivos e de diversidade no controverso gênero neotropical *Trachelyopterus*, o que pode levar a um melhor entendimento de sua taxonomia, evolução e biodiversidade.



**PALAVRAS-CHAVE:** Genes de Histona H3 e H4; 18S DNAr; 5S DNAr; snRNA U2; SSR$(GATA)_n$.

HAERTER, C.A.G. Investigação da distribuição de elementos repetitivos no genoma de espécies de *Trachelyopterus* (Valenciennes, 1840). Dissertação (Mestrado em Conservação e Manejo de Recursos Naturais) - Centro de Ciências Biológicas e Saúde, Universidade Estadual do Oeste do paraná. Cascavel, p. 123. 2021.

INVESTIGATION OF THE DISTRIBUTION OF REPETITIVE ELEMENTS IN THE GENOME OF *TRACHELYOPTERUS* SPECIES (VALENCIENNES, 1840)

## ABSTRACT

*Trachelyopterus* is a Neotropical catfish genus with a controversial taxonomic history and more than 200 years of revisions due to similar morphology among species. Cytogenetically, it is still poorly explored and there is no physical mapping of repetitive elements beyond the rDNAs 18S and 5S. Only the microsatellite $(GATA)_n$ distribution pattern was analyzed in some species, having a crucial role in the origin and evolution of B chromosomes, but without efficiency in distinguishing species through complement A chromosomes. In this study, the mapping of the repetitive elements 18S and 5S rDNA, H3 and H4 histone genes, snRNA U2 and the microsatellite $(GATA)_n$ were performed in six *Trachelyopterus* species: *T. striatulus*, *T. galeatus* and *T. porosus* of the Amazon River Basin; *T. coriaceus* and *Trachelyopterus* aff. *galeatus* (possible new species) from the Araguaia River basin; and *Trachelyopterus* aff. *coriaceus* (possible new species) of the Paraguay River basin. The 5S DNAr was evidenced in 1-3 chromosome pairs. The H3 and H4 histone genes were found in 1-2 chromosome pairs and, in most species, synthenic with DNAr 18S. The U2 snRNA was found in only one chromosome pair, however, *Trachelyopterus* aff. *galeatus* had a chromosomal inversion polymorphism, which is in Hardy-Weinberg equilibrium and had homologous chromosomes with distinct size. Cytotaxonomically, the 5S rDNA revealed autapomorphies of four species, while the H3 and H4 histone genes and 18S rDNA distinguished two species and U2 snRNA only one species. As a result, all species could be distinguished, including *Trachelyopterus* aff. *coriaceus* and *Trachelyopterus* aff. *galeatus*, both possible new species. Once the distribution pattern of the 18S and 5S rDNA, U2 snRNA and H3/H4 histone genes was known, it was used as a guide to compare the microsatellite $(GATA)_n$ distribution pattern in the homologous chromosomes of the six species. As a result, most species had a similar $(GATA)_n$ distribution pattern in the 18S rDNA chromosome carriers. However, *T. galeatus* presented a chromosomal polymorphism, which is in Hardy-Weinberg equilibrium and possibly originated through amplification events. On the other hand, the mapping of the $(GATA)_n$ sequence in the carriers of the 5S rDNA and H3 / H4 histone genes did not reveal new information about the structure of these chromosomal pairs, however, it indicated a more precisely chromosomal homeologies between the species and reaffirmed the existence of the chromosomal polymorphism in *T. galeatus*. Similar to the carriers of the 18S rDNA, most species presented a conserved distribution pattern of microsatellite $(GATA)_n$ on the chromosomes of the U2 snRNA. However, *Trachelyopterus* aff. *galeatus* presented a new chromosomal polymorphism in Hardy-Weinberg disequilibrium and six possible cytotypes. Thus, through combined mapping of these markers, we could evidence cytotaxonomic autapomorphies that can distinguish all species, including both possible new species, as well as new evolutive e diversity aspects in the controvertial neotropical genus *Trachelyopterus*, which can lead to a better understanding of its taxonomy, evolution and biodiversity.

**KEY WORDS:** H3 and H4 Histone genes, 18S rDNA, 5S RDNA, U2 snRNA, SSR $(GATA)_n$.

HAERTER, C.A.G. Investigação da distribuição de elementos repetitivos no genoma de espécies de *Trachelyopterus* (Valenciennes, 1840). Dissertação (Mestrado em Conservação e Manejo de Recursos Naturais) - Centro de Ciências Biológicas e Saúde, Universidade Estadual do Oeste do paraná. Cascavel, p. 123. 2021

## LISTA DE TABELAS



### CAPÍTULO I



### CAPÍTULO II

## LISTA DE FIGURAS



## CAPITULO I



## CAPITULO II

## LISTA DE ABREVIAÇÕES

| | |
|---|---|
| **2n** | Número diploide |
| **AgNO3** | Nitrato de prata |
| **DAPI** | 4’,6-diamino-2-fenilindol |
| **DNA** | Ácido desoxirribonucleico |
| **DNAr** | DNA ribossomal |
| **DNAsat** | DNA satélite |
| **ET** | Elemento Transponível |
| **ETs** | Elementos Transponíveis |
| **FISH** | Hibridização *in situ* Fluorescente (Fluorescence *in situ* Hybridization) |
| **$(GATA)_n$** | Microsatélite GATA |
| **ITS** | Sítios Teloméricos Intersticiais |
| **LINEs** | Long Interspersed Nuclear Elements |
| **LTR** | Long Terminal Repeat |
| **NCBI** | National Center for Biotechnology Information |
| **FN** | Número fundamental |
| **non-LTR** | Non-Long Terminal Repeat |
| **NTS** | Non-Transcribed Spacer |
| **PCR** | Polymerase Chain Reaction |
| **rDNA** | Ribosomal DNA |
| **RNA** | Ácido ribonucleico |
| **RON** | Região Organizadora de Nucléolo |
| **RONs** | Regiões Organizadoras de Nucléolos |
| **TEs** | Transposable Elements |
| **(TTAGGG)n** | Sequência telomérica |
| **UNIOESTE** | Universidade Estadual do Oeste do Paraná |

## 1. INTRODUÇÃO

Peixes compreendem cerca de metade das espécies de vertebrados do mundo com mais de 32.000 espécies catalogadas. Sua origem e irradiação é datada de cerca de 500 milhões de anos atrás e tanto agora quanto no passado exibem uma incomparável diversidade (NELSON, 2006; HELFMAN et al., 2009). Evolutivamente este grupo prosperou por meio da aparição dos mais impressionantes e variados aspectos morfológicos, fisiológicos, comportamentais e ecológicos ao longo dos milhões de anos (NELSON, 2006; HELFMAN et al., 2009). Atualmente, cerca de 43% dos espécimes de peixes catalogados são pertencentes a ambientes de água doce, os quais apesar de representarem uma porção extremamente pequena quando comparados a ambientes marinhos, contém um número desproporcional das espécies de peixe do mundo (WOOTTON, 1990; NELSON, 2006; HELFMAN et al., 2009).

Nas últimas décadas o número de espécies de peixes tem sido constantemente atualizado. Mais de 1,7 milhões de novas espécies foram descritas e catalogadas, entretanto, este ainda é um número extremamente baixo considerando toda a diversidade biológica existente no planeta (BLAXTER, 2003; WILSON, 2003). Além disso, não obstante o pouco conhecimento acerca da diversidade do planeta, um número significativo já catalogado pode apresentar problemas em sua descrição e taxonomia, sejam por erros na interpretação de dados ou por variabilidade críptica e, como resultado, a diversidade pode ser ainda maior. Portanto, estudos visando melhor compreensão da diversidade ictiofaunística são essenciais, uma vez que, contribuem significativamente na sistemática dos grupos, na tomada de decisões e no manejo e na conservação de espécies, principalmente as que possuem alto valor econômico e biológico (HAY et al., 2009; HELFMAN et al., 2009; BICKFORD et al., 2006). Ademais, compreender a organização tanto morfológica quanto genética permite desenvolver hipóteses filogenéticas mais claras e contundentes, minimizando os efeitos das constantes mudanças e reorganizações de táxons, historicamente comuns na literatura e que causam grande confusão durante a interpretação.

Recentemente, um novo meio além da morfologia tem permitido a caracterização, segregação ou agrupamento de espécies, a citogenética. Utilizando de marcadores moleculares, como os elementos repetitivos, a citogenética permite adentrar o genoma e evidenciar diferenças e semelhanças não possíveis de serem

identificadas apenas com base em morfologia, gerando novos dados capazes inclusive de diferir espécies morfologicamente idênticas (BICKFORD et al., 2006). Exemplos a serem ressaltados são os complexos de espécies em *Astyanax scabripinnis* (MOREIRA-FILHO; BERTOLLO, 1991), *Hoplias malabaricus* (BERTOLLO et al., 2000), as espécies crípticas do gênero *Pimelodella* (Siluriformes: Heptapteridae) encontradas no Rio Miranda, Bacia do Rio Paraguai (SOUZA-SHIBATTA et al., 2013), as espécies crípticas descritas dentro de *Sorubim* Cuvier (Siluriformes: Pimelodidae) da porção superior e meio da bacia Amazônica (LITTMAN et al., 2001) e as espécies crípticas de *Imparfinis* (Siluriformes, Heptapteridae) (FERREIRA et al., 2014).

Dessa forma, a análise de elementos repetitivos por meio da citogenética é uma importante ferramenta durante a descrição de grupos, podendo contribuir para com problemáticas taxonômicas (NUNES et al., 2007; VENTURA, 2009; BLANCO et al., 2011; LUI, 2011; CHIRINO et al., 2013; RINCÓN, 2016), caracterização evolutiva de cromossomos sexuais (MARTINS, 2007; VICARI et al., 2008), descrição de cromossomos B (LUI et al., 2009; LUI, 2013; FELICETTI, 2018) e a caracterização da influência de sequências repetitivas na organização e arquitetura genômica e expressão gênica (SHAPIRO; STERNBERG, 2005; LYNCH, 2007; SECCO et al., 2011). Além disso, a caracterização de elementos repetitivos também é uma ferramenta de grande importância na medida em que contribui com a descrição de novas espécies (HIRMAN et al., 2018) e com novos dados e conhecimento para os grupos de análise (COLUCCIA et al., 2011), visto que, a citogenética ainda é um campo relativamente novo, principalmente de peixes, e ainda carente de informações.

## 1.1 Região Neotropical

A região Neotropical compreende as áreas desde o Deserto de Sonora, no sul dos Estados Unidos, até à Terra do Fogo, no extremo sul da América do Sul (MORRONE et al., 2014). Em decorrência do isolamento da América do Sul da América Central e África durante o período terciário, a biota neotropical pode evoluir de forma totalmente isolada e independente (JOLY, 2008). Em razão disto, é tida como uma das mais ricas áreas em termos de diversidade e riqueza de espécies para diversos grupos (JOLY, 2008).

Em grande parte do planeta, os peixes são considerados um dos grupos com maior sucesso adaptativo nos sistemas aquáticos, e o mesmo também pode ser observado na região Neotropical, com cerca de 5.160 espécies descritas e estimativas de mais de 9000 espécies (NELSON et al*.,* 2016). Todavia, apesar do crescente número de descrições formais, o conhecimento da ictiofauna neotropical ainda se mostra incompleto, principalmente pela falta de consenso sobre a validade taxonômica de várias espécies, deficiência no número de pesquisas e revisões sistemáticas (BUCKUP et al., 2007; LANGEANI et al., 2009). Atualmente, a região Neotropical tem Ostariophysi como a classe de peixes com o maior número de representantes, em que se destacam as ordens Characiformes, Siluriformes e Gymnotiformes, sendo Siluriformes o segundo maior grupo em número de espécies (FRICKE, 2021).

## 1.2 Ordem Siluriformes

Pertencente à classe Actinopterygii, os Siluriformes, também chamados peixes de couro, bagres, ou *catfishes*, apresentam cerca de 3.730 espécies validadas distribuídas em 40 famílias e 490 gêneros, sendo Hypsidoridae, Andinichthyidae e Bachmanniidae consideradas extintas, caracterizando-se como o segundo maior grupo de peixes da região Neotropical (NELSON et al., 2016; FRICKE, 2021). No Brasil, a ordem representa em torno de 40% das espécies, com mais de 1.000 identificadas e distribuídas em 11 famílias e cerca de 200 gêneros (BUCKUP et al., 2007).

Os Siluriformes possuem hábitos sedentários e são geralmente encontrados no fundo de rios (FROESE, 2021). São animais de hábitos primariamente noturnos caracterizados pela ausência de escamas pelo corpo, recoberto por pele espessa e placas ósseas. Normalmente, dispõe de quatro pares de barbilhões, um no maxilar e outro na mandíbula, maxilares pequenos e dentes ausentes (FROESE, 2021). São geralmente orientados por sentido químico e possuem o primeiro raio das nadadeiras dorsal e peitoral transformados em acúleo, além de nadadeira adiposa normalmente estar presente (MOYLE; CHECH, 1988; FROESE, 2021). A ordem é composta por indivíduos que podem não ultrapassar 20 milímetros de comprimento quando adultos (como alguns Scoloplacidae) e outros que podem ultrapassar 2-3 metros de

comprimento (como em Siluridae, Pimelodidae e Pangasiidae). Apresentam ampla distribuição pela América do Sul, África e poucos representantes na Ásia e Europa. Com exceção de Ariidae e Plotosidae que são exclusivamente marinhos e Auchenipteridae, Aspredinidae e Pangasiidae que apresentam espécies de estuário com movimentações periódicas ao mar, todos os outros representantes conhecidos são dulcícolas (PINNA, 1998; NIRCHIO, 2010). Tanta variedade morfológica, comportamental e ecológica reflete diretamente em sua complexidade taxonômica, historicamente confusa (NELSON et al., 2016).

## 1.3 Família Auchenipteridae

Na ordem dos Siluriformes, Auchenipteridae é uma família restrita a região Neotropical e representada por cerca de 26 gêneros e 125 espécies validadas (FRICKE et al., 2021). Junto de Doradidae e Aspridinidae, Auchenipteridae faz parte da superfamília Doradoidea (NELSON et al., 2016). Este grupo pode ser dividido na subfamília Centromochlinae, composta pelos gêneros *Centromochlus*, *Gelanoglanis*, *Glanidium* e *Tatia,* e na subfamilia Auchenipterinae, que agrupa todos os outros gêneros (FERRARIS JR, 2003). Esta última abrigaria o gênero *Parauchenipterus*, um gênero historicamente confuso e que apresenta problemas taxonômicos e de validação desde o momento de sua criação (AKAMA, 2004, LUI, 2010). Do total de espécies de válidas, cerca de 70 já foram registradas no território brasileiro (AKAMA, 2004; NELSON et al., 2016).

As espécies de Auchenipteridae*,* conhecidas popularmente como carataí, fidalgo, mandubé, palmito, judeu, ximbé, cangati e cachorro-de-padre, são caracterizadas pela inseminação interna e dimorfismo sexual, que geralmente se apresenta na nadadeira anal nos machos sexualmente maduros, mas podendo ocorrer também na nadadeira dorsal ou barbilhões durante períodos reprodutivos (AKAMA, 2004; BIRINDELLI, 2010). Apresentam o olho coberto por tecido adiposo, sem borda nítida e a nadadeira adiposa é reduzida ou ausente (AKAMA, 2004). O barbilhão maxilar é mais longo, desenvolvendo fileiras de espinhos. Possuem acúleo na nadadeira dorsal ossificado (FERRARIS JR, 1988). Possuem hábitos alimentares diversos, estando incluído em suas dietas principalmente pequenas frutas e insetos na superfície da água. Todavia, alguns poucos representantes do grupo são

primariamente planctívoros e/ou piscívoros (FERRARIS JR, 2003). Espécies desta família podem também emitir sinais sonoros em contextos agonísticos, reprodutivos e de perturbações, além de exibirem habilidade de distinção de seus próprios sons, podendo então ser considerado um caráter taxonômico (KAATZ; STEWART, 2012).

## 1.4 Estudos citogenéticos em Auchenipteridae

Apesar do avanço da ciência e da citogenética de peixes, Auchenipteridae ainda é uma família pouco explorada. Os dados citogenéticos disponíveis em literatura fazem menção à apenas uma pequena fração de espécies dos gêneros *Glanidium* (Lütken, 1874), *Parauchenipterus* (*Trachelyopterus*), *Tatia* (Miranda-Ribeiro, 1911), *Tympanopleura* (Eigenmann, 1912), *Auchenipteru*s (Miranda Ribeiro, 1918) e *Entomocorus* (*Eigenmann,* 1917) (FENOCCHIO; BERTOLLO, 1992; RAVEDUTTI; JÚLIO JR, 2001; FENOCCHIO et al., 2008; LUI et al., 2009, 2010, 2013a, 2013a, 2015; MACHADO et al., 2018; FELICETTI et al. 2021; LUI et al. 2021; SANTOS et al., 2021; MACHADO et al., 2021). Para Auchenipteridae, o número diploide (2n) de 58 cromossomos são assíduos, entretanto, diploidia de 56 cromossomos já foram identificadas em algumas populações de *Ageneiosus inermis* (Linnaeus, 1766) (citado como *A*. *brevifilis*), *Tympanopleura atronasus* (citado como *Ageneiosus atronases*) oriundos da bacia Amazônica (FENOCCHIO; BERTOLLO, 1992) e de 46 cromossomos em *Centromochlus heckelii* (KOWALSKI et al., 2020).

Com relação às exceções ao número diploide padrão para Auchenipteridae, *Ageneiosus inermis* e *Tympanopleura atronasus*, além da variação no número de cromossomos, curiosamente as AgRONs apresentam-se localizadas em cromossomos submetacêntricos e na posição terminal do braço curto para *Ageneiosus inermis* e intersticial no braço longo para *Tympanopleura atronasus* (FENOCCHIO; BERTOLLO, 1992). Baseado nessas informações, estudos posteriores encontraram o mesmo número diploide (2n=56) para uma população de *Ageneiosus inermis* coletada no rio Araguaia, e coincidentemente obtiveram o mesmo padrão de distribuição de AgRONs (LUI et al., 2013b). A partir disso, utilizando-se de sondas teloméricas, foi comprovado a ocorrência de fusão cromossômica como fonte da variação do número diploide de *Ageneiosus inermis* e *Tympanopleura atronasus*, evidenciada pela presença destas sequências em posição centromérica dos

cromossomos do par 1 do cariótipo (LUI et al., 2013b). Da mesma maneira, o número menor número diploide já registrado para Auchenipteridae, em *Centromochlus heckelii,* também parece estar relacionado a eventos de fusão cromossômica, o qual também pode estar relacionado com o surgimento de um sistema sexual do tipo ZZ/ZW (KOWALSKI et al., 2020).

Para o gênero *Tatia,* estudos citogenéticos foram desenvolvidos tendo como base as espécies de *Tatia jaracatia* Pavanelli and Bifi, 2009 do rio Iguaçu e *Tatia neivai* Ihering, 1930 do Rio Machado, afluente do Rio Bugres, bacia do Rio Paraguai (LUI et al., 2013a). Para ambas as espécies estudadas o número diploide de 2n=58 foi descrito, não diferindo dos outros gêneros de *Auchenipteridae*, exceto de *Ageneiosus inermis* e *Tympanopleura atronasus* com 2n=56 (LUI et al., 2013a). Para estes estudos, a distribuição da heterocromatina foi encontrada predominantemente em regiões terminais dos cromossomos, entretanto, blocos menores também foram encontrados na região centromérica de *Tatia jaracatiá* e dois blocos conspícuos foram identificados em região intersticial do braço longo do par submetacêntrico 15 e na posição terminal do braço curto do par subtelocêntrico 28 de *Tatia Neiva,* constituindo um padrão não comum aos outros representantes da família *Auchenipteridae* (LUI et al., 2013a). Condizente a dados de hibridização de sondas sítio específicas, para *Tatia jaracatiá* sondas fluorescentes 18S rDNA mostraram marcação única no par cromossômico 28, correspondendo a marcação por nitrato de prata (AgRONs). Por outro lado, marcações com sondas de rDNA 5S se mostraram presentes nos pares cromossômicos 4,18,19, e curiosamente e diferente dos demais, a marcação também foi localizada no par subtelocêntrico 29, sendo até o momento uma característica particular de *Tatia jaracatiá* (FELICETTI, 2018). Para *Tatia Neiva* a sonda de rDNA 18S foi localizada apenas no par subtelocêntrico 28, enquanto marcações por sondas rDNA 5S foram detectadas nos pares 4, 21 e 22 (LUI et al., 2013a). Contudo, não houve polimorfismo intraespecífico em relação a número diploide, fórmula cariotípica, bandamento C, rDNA 5S e 18s em ambas as espécies (LUI et al., 2013a).

Analises citogenéticas para o gênero *Glanidium* são relatadas para populações de *Glanidium ribeiroi* provenientes de do Rio Iguaçu e coletadas no reservatório Salto de Caxias - PR (RAVEDUTTI; JULIO JR, 2001); reservatório Segredo – PR e reservatório Salto Osório - PR (FENOCCHIO et al., 2008); e Capanema – PR (LUI et al. 2013a). Em todos os estudos o número diploide de 2n=58 cromossomos é relatado. Pequenas alterações no número fundamental e na fórmula cariotípica foram

observadas para espécimes do reservatório Salto Caxias, sendo explicada por possíveis evento evolutivos, tais como inversões pericêntricas, duplicações e deleções (RAVEDUTTI; JÚLIO JR, 2001). Os padrões de heterocromatina mostraram marcações majoritariamente teloméricas e pequenas frações distribuídas em regiões centroméricas, não se diferenciando muito em relação a outros representantes de *Auchenipteridae,* exceto por um bloco conspícuo de heterocromatina correspondente a Ag-NORs e outro bloco heterocromático adjacente a esse mesmo local na população oriunda de Capanema (LUI et al., 2015). A hibridização por sondas específicas foi realizada pela primeira vez no grupo para as populações do reservatório de Segredo e Salto Osório (FENOCCHIO et al., 2008) com sondas DNAr 18S. As sondas evidenciaram sítios no braço curto e em cromossomos submetacêntricos para ambas as populações (FENOCCHIO et al., 2008).

Para a região de Capanema, um estudo mais recente, as análises foram realizadas utilizando DNAr 18S, 5S, sondas teloméricas $(TTAGGG)_n$ e sequências $(GATA)_n$. Para esta população a hibridização das sondas de DNAR 18S se mostrou presente apenas no braço curto e em posição intersticial do par submetacêntrico 14, de forma muito semelhante as populações do reservatório de Segredo e Salto Osório (LUI et al., 2015). Por outro lado, com a hibridização da sonda de DNAr 5S foi observada marcação somente na porção intersticial do braço longo do par metacêntrico 16, sem diferenças entre as populações (LUI et al., 2015). Do mesmo modo, sondas teloméricas não demonstraram variações quanto ao esperado para essa fração repetitiva, e sequências $(GATA)_n$ se mostram dispersas por todo o genoma e com elevada afinidade por porções cromossômicas terminais (LUI et al., 2015), sendo semelhante a outras análises para a família, diferindo significativamente apenas da marcação pericentromérica identificada em *Ageneiosus inermis* (LUI et al., 2013a). Todavia, ainda há grande dificuldade em realizar comparações dentro da família em relação a satélites, microssatélites e minissatélites, uma vez que, estudos contendo tais marcadores ainda são escassos para *Auchenipteridae*, sugerindo a necessidade de estudos utilizando essas frações de DNA repetitivo.

Para o gênero *Trachelyopterus,* as principais análises tiveram como espécie alvo *Trachelyopterus galeatus* (comumente citado como *Parauchenipterus galeatus*). Os estudos citogenéticos foram conduzidos no Rio Paraná – Porto Rico/PR (RAVEDUTTI; JULIO JR, 2001); Reservatório Jupiá do Rio Paraná - MS; Lagoa dos Tropeiros da bacia do rio Piumi - MG; Lagoa Marginal ao Rio São Francisco - MG (LUI

et al., 2010); e Rio Piumhi, bacia do Atlântico NE Oriental – RN (ARAÚJO; MOLINA, 2013). Nestas análises, o número cromossômico diploide relatado para todas as populações foi 2n=58 cromossomos, não diferindo do padrão para a família. O mesmo pode ser observado quanto a seu número fundamental e fórmula cariotípica, divergindo apenas para a população coletada no Rio Paraná – Porto Rico/PR, sendo tal discordância provinda de possíveis rearranjos cromossômicos ocorridos durante o percurso evolutivo (RAVEDUTTI; JULIO JR, 2001). AgRONs simples foram identificadas em posição terminal do braço curto do par subtelocêntrico, diferindo apenas o cromossomo portador da marcação, sendo 23 ou 24 ou 25 (RAVEDUTTI; JULIO Jr, 2001; LUI et al., 2009, LUI et al., 2010). A heterocromatina foi localizada predominantemente em posição terminal dos cromossomos do complexo A, com algumas exceções, como a marcação bitelomérica em alguns cromossomos metacêntricos, submetacêntricos e subtelocêntricos e marcação pericentromérica em alguns cromossomos acrocêntricos (LUI et al., 2009, LUI et al., 2010).

Para populações do Reservatório Jupiá do rio Paraná, Lagoa dos Tropeiros da bacia do rio Piumhi e Lagoa Marginal ao rio São Francisco, marcações das sondas de DNAr 5S identificaram sítios localizados em dois pares cromossômicos submetacêntricos nas três populações, diferindo apenas o cromossomo portador da sequência (LUI et al., 2009; LUI, et al., 2010). Tal variação também foi associada a eventos evolutivos e rearranjos cromossômicos (LUI et al., 2009; LUI et al., 2010), assim como em *Glanidium riberoi* (RAVEDUTTI; JÚLIO Jr, 2001). Por outro lado, marcações com DNAr 18S revelaram apenas um único par cromossômico marcado, correspondendo com os dados de impregnação por nitrato de prata (LUI et al., 2009; LUI et al., 2010). É válido ressaltar também que, neste grupo ocorreu a primeira descrição de cromossomos B para a família Auchenipteridae (LUI et al., 2009).

Recentemente, exemplares *Trachelyopteryus galeatu*s (citado como *Parauchenipterys galeatus*) coletados na bacia do rio Piumhi no Estado do Rio Grande do Norte também foram analisados (ARAÚJO; MOLINA, 2013), resultando em um número diploide de 2n=58, no entanto, com fórmula cariotípica diferente de outras populações, como do Amazonas e São Franciso (ARAÚJO; MOLINA, 2013). A heterocromatina foi localizada predominantemente em regiões centroméricas e pericentroméricas, diferindo da maioria dos estudos para a família *Auchenipteridae* que relatam predominância por frações cromossômicas terminais (LUI et al., 2009, LUI et al., 2010), porém, assemelhando-se a espécies de *Auchenipterus* e

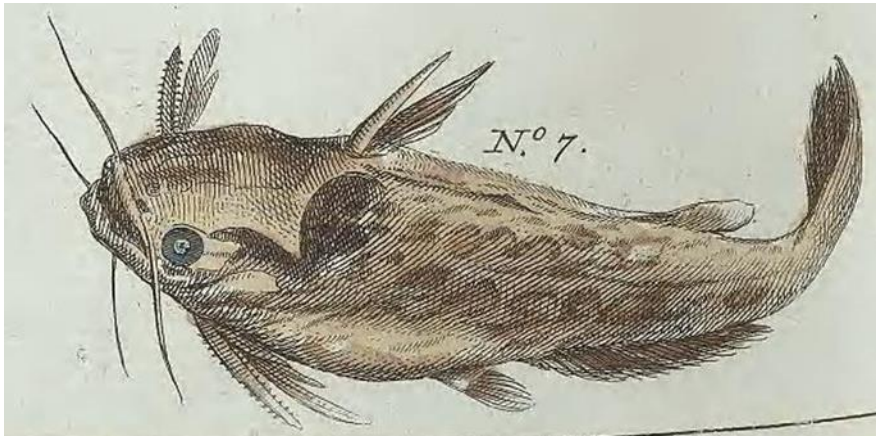
*Entomocorus* oriundas do baixo rio Paraná e rio Araguaia, em que foram localizados blocos heterocromáticos proeminentes nas regiões intersticial e pericentromérica (MACHADO et al., 2018). Além das variações na localização, são relatadas diferenças também na composição da heterocromatina, evidenciando variabilidade possivelmente ainda maior para esta população em razão das outras (ARAÚJO; MOLINA, 2013). Por final, AgRONs foram localizadas em posição terminal de um par de cromossomos submetacêntricos, padrão este confirmado pela hibridização de sondas DNAr 18S (ARAÚJO; MOLINA, 2013).

Da mesma forma, foram também analisados exemplares de *T. striatulus* da bacia do Rio Doce, Minas Gerais, e *T. coriaceus* e *Trachelyopterus* aff. *galeatus*, da bacia do rio Araguaia (SANTOS et al. 2021). Todas as espécies apresentaram heterocromática em região terminal de quase todos os cromossomos e em região pericentromérica quando acrocêntricos. AgRONs simples foram encontradas em cromossomos subtelocêntricos e confirmadas por sondas de DNA ribossomal 18S. Todavia, *T. striatulus* apresentou 3 pares cromossômicos portadores do DNA ribossomal 5S, inédito para o grupo, que normalmente apresenta apenas dois pares. Da mesma forma, *Trachelyopterus* aff. *Galeatus* apresentou apenas um par cromossômico portador do DNA ribossomal 5S, também inédito para o grupo, o que o levou a ser sugerido como um possível novo táxon.

## 1.5 Incongruências taxonômicas em "*Parauchenipterus" Trachelyopterus*

*Parauchenipterus* Bleeker, 1862 é um gênero conhecido por sua confusa e volátil história taxonômica. A primeira ilustração conhecida (Fig. 1) de um exemplar do gênero foi realizada por Seba (1759). Contudo, em decorrência da alta similaridade entre as espécies e a não existência de dados genéticos, ainda se desconhecia a real diversificação das espécies do gênero. Portanto, a representação possui caracteres morfológicos que correspondem a qualquer uma das espécies atualmente descritas. Além disso, o exemplar utilizado por Seba como base para a ilustração foi leiloado e seu destino é incerto, impossibilitando a identificação da espécie ilustrada (AKAMA, 2004).

**Figura 1.** Primeira ilustração de *Parauchenipterus*




**Fonte**: Seba (1759).

Em 1766, tendo como base a ilustração realizada por Seba, a primeira descrição formal foi realizada por Linnaeus, que não possuía informações morfológicas ou local de coleta do animal, o que tornaria nos próximos anos a taxonomia do gênero ainda mais duvidosa. Não obstante, a taxonomia de *Parauchenipterus* também é agravada quando Bloch (1794) passa a utilizar *Silurus galeatus* Linnaeus, 1766 como espécie-tipo para caracterizar *Parauchenipterus*, a mesma espécie descrita por Linnaeus (1766). Dessa forma, as primeiras descrições do gênero *Parauchenipterus* foram baseadas em uma cascata de potenciais erros e poderiam ser equivalentes a qualquer espécie atual do gênero, culminando em uma fragilidade taxonômica que perdura até a atualidade (AKAMA, 2004).

Devido à taxonomia incontundente e carência de informações, o gênero *Parauchenipterus* foi revisado diversas vezes (Vide tabela 1 para informação resumida). A primeira tentativa de adequação foi realizada por Gunther (1864) que cunhou *Parauchenipterus* como sinônimo de *Auchenipterus* Valenciennes, 1840. Mais de 20 anos depois, Eigenmann e Eigennman (1888) propuseram o gênero como equivalente a *Trachycorystes* Bleeker, 1862, removendo a validação anterior cunhada por Gunther (1864). Todavia, a problemática ainda não havia sido resolvida e após quase um século, Mess (1974) revalidou o gênero *Parauchenipterus* e retirou a sinonímia para com *Trachycorystes* Bleeker. Entretanto, quase duas décadas depois, Ferraris (1988) propôs nova sinonímia para o gênero *Parauchenipterus*, desta vez com *Trachelyopterus* (AKAMA, 2004).

**Tabela 1**. Resumo histórico das considerações taxonômicas em *Trachelyopterus* e *Parauchenipterus*.

| ANO | AUTOR | CONSIDERAÇÕES |
|---|---|---|
| 1759 | **Seba** | Pela primeira vez ilustrou *Parauchenipterus* |
| 1766 | **Linnaeus** | Descreveu a espécie |
| 1794 | **Bloch** | Utilizou como espécie-tipo *Silurus galeatus* para caracterizar *Parauchenipterus* |
| 1864 | **Gunther** | Sinonimizou *Parauchenipterus* com *Auchenipterus* |
| 1888 | **Eignmann e Eignmann** | Retirou *Parauchenipterus* de *Auchenipterus,* considerando-o sinônimo de *Trachycorystes* |
| 1972 | **Britski** | Corrobora a hipótese de Eignmann e Eignmann |
| 1974 | **Mees** | Revalidou o gênero *Parauchenipterus* |
| 1988 | **Ferraris Jr** | Propôs pela primeira vez que *Parauchenipterus* seria sinônimo de *Trachelyopterus* |
| 1989 | **Curran** | *Parauchenipterus* foi desconsiderado como sinônimo de *Trachelyopterus,* validando os dois gêneros |
| 1999 | **Royero** | Considerou *Parauchenipterus* e *Trachelyopterus* gêneros distintos e válidos |
| 2003 | **Ferraris Jr** | Considerou os gêneros *Parauchenipterus* e *Trachelyopterus* sinônimos, colocando todas as espécies dentro de *Trachelyopterus* |
| 2004 | **Akama** | Novamente separou *Parauchenipterus* e *Trachelyopterus,* como gêneros distintos e válidos |
| 2007 | **Ferraris Jr** | Reafirmou *Parauchenipterus* e *Trachelyopterus* como sinônimos e todas as espécies destes dois gêneros em *Trachelyopterus* |
| 2007 | **Buckup et al.** | Consideram os dois gêneros válidos |
| 2007 | **Graça e Pavanelli** | Consideram os dois gêneros válidos |
| 2010 | **Birindelli et al.** | Consideram os dois gêneros válidos |
| 2019 | **Calegari et al.** | Consideram os dois gêneros Sinônimos |

**Fonte:** Adaptado de Felicetti (2018).

Desde então, o principal desafio envolvendo o gênero encontra-se na dificuldade em esclarecer as relações taxonômicas entre *Parauchenipterus* e *Trachelyopterus.* Após a proposição de sinonímia entre os gêneros por Ferraris Jr (1988), Curran (1989) e Royero (1999) revalidaram em seus trabalhos os dois gêneros como unidades distintas, porém, Ferraris Jr (2003) reforçou novamente sua decisão no livro "*Check Listo of Freshwater Fishes of South and Central America*", mantendo a sinonímia. Contudo, Akama (2004) através de um estudo filogenético baseado em morfologia, reconsiderou os gêneros como unidades distintas, em que *Parauchenipterus* seria validado abrangendo cinco espécies (*P. galeatus*,

*Parauchenipterus* sp. n., *P. ceratophysus*, *P. porosus* e *P. striatulus*), enquanto *Trachelyopterus* abrangeria duas espécies (*T. coriaceus* e *Trachelyopterus* sp. n.). Ainda assim, Ferraris Jr (2007) em novo trabalho publicado manteve sua proposição inicial, considerando novamente *Parauchenipterus* equivalente a *Trachelyopterus.* As últimas atualizações envolvendo os gêneros foram realizadas por Buckup et al. (2007), Graça e Pavanelli (2007) e Birindelli (2010), que reapresentam ambos os gêneros como válidos, considerando a classificação proposta por Akama (2004). Todavia, principalmente ao estudo desenvolvido por Calegari et al. (2019), as bases de dados *Eschmeyer's Catalog of Fishes* (FRICKE, 2021) e *Fishbase* (FROESE, 2021) continuam por adotar em sua base de dados *Parauchenipterus* como sinônimo de *Trachelyopterus* demonstrando as incertezas e lacunas taxonômicas ainda presentes.

Como resultado atual das incertezas envolvendo a filogenia de *Trachelyopterus* e *Parauchenipterus*, além da identificação de novas espécies com o uso de ambas as nomenclaturas, gerando discordância na literatura, durante a proposição de sua revisão taxonômica Akama (2004) considerou nestes gêneros um total de 7 espécies, um número extremamente reduzido comparado a outros autores, como Ferraris Jr (2003, 2007), sugerindo um agrupamento significativo, baseado em morfologia, das espécies atualmente válidas para o gênero *Trachelyopterus.* Se comparado a dados do presente momento (Tabela 2), 18 espécies são validadas em *Trachelyopterus* pelo *Eschmeyer's Catalog of Fishes* (FRICKE, 2021), enquanto a *Fishbase* considera válido para o gênero um total de 16 espécies (FROESE, 2021), um número quase três vezes maior ao descrito por Akama (2004). Nesse contexto, a principal problemática envolvendo tal discrepância em números se revela na medida que não há plena concordância na comparação entre as espécies descritas por Akama (2004) e as espécies validadas atualmente para o gênero *Trachelyopterus*. Uma menor fração pode ser identificada e equiparada por meio da similaridade de nomenclatura, visto que, mantiveram epiteto específico, alterando somente a referência ao gênero. Todavia, uma porção substancial não possui nenhuma similaridade de nomenclatura ou indícios de onde estariam inseridas, dificultando identificar quais espécies atualmente válidas foram agrupadas para compor as novas espécies propostas por Akama (2004).

Além disso, Akama (2004) sugere a descrição de um novo gênero, ao qual cunhou de “*Amplexiglanis*”, grupo irmão de *Ageneiosus* e *Tetranematichthys*. Este novo gênero, apesar de não validado, também possui importante papel na discussão

envolvendo *Trachelyopterus*, pois, parte das espécies que compunham o gênero *Parauchenipterus* (atual *Trachelyopterus*) foram remanejadas de acordo com Akama (2004) e Royero (1999) para este novo grupo, impactando ainda mais na organização e filogenia por ele proposta. Dessa forma, *Amplexiglanis* constitui um importante elo para localizar e identificar parte do rearranjo realizado por Akama (2004), pois, assim como para parte das novas espécies designadas pelo autor para os gêneros *Parauchenipterus* e *Trachelyopterus,* o epiteto específico foi mantido, permitindo localizá-las na literatura atual (Tab. 2).

**Tabela 2**. Espécies consideradas válidas atualmente para o gênero *Trachelyopterus* em comparação a revisão filogenética de Akama (2004).

| **Akama (2004) – Última revisão** | ***Eschmeyer's Catalog of Fishes* (2021)** | ***FishBase* (2021)** |
|---|---|---|
| *Parauchenipterus galeatus* | *Trachelyopterus galeatus* | *Trachelyopterus galeatus* |
| | *Trachelyopterus lacustris* | *Trachelyopterus lacustris* |
| | *Trachelyopterus isacanthus* | *Trachelyopterus isacanthus* |
| | -- | *Trachelyopterus analis* |
| | *Trachelyopterus albicrux* | *Trachelyopterus albicrux* |
| | *Trachelyopterus leopardinus* | *Trachelyopterus leopardinus* |
| | *Trachelyopterus cratensis* | *Trachelyopterus cratensis* |
| *Parauchenipterus* sp. | -- | -- |
| *Parauchenipterus striatulus* | *Trachelyopterus striatulus* | *Trachelyopterus striatulus* |
| *Parauchenipterus porosus* | *Trachelyopterus porosus* | -- |
| | *Trachelyopterus lucenai* | *Trachelyopterus lucenai* |
| *Parauchenipterus ceratophysus* | *Trachelyopterus ceratophysus* | *Trachelyopterus ceratophysus* |
| | -- | *Trachelyopterus brevibarbis* |
| *Trachelyopterus coriaceus* *Trachelyopterus* sp. | *Trachelyopterus coriaceus* | *Trachelyopterus coriaceus* |
| "*Amplexiglanis*" *fisheri* | *Trachelyopterus fisheri* | *Trachelyopterus fisheri* |
| "*Amplexiglanis*" *amblops* | *Trachelyopterus amblops* | *Trachelyopterus amblops* |
| "*Amplexiglanis*" *teaguei* | *Trachelyopterus teaguei* | *Trachelyopterus teaguei* |
| "*Amplexiglanis*" *insignis* | *Trachelyopterus insignis* | *Trachelyopterus insignis* |
| | *Trachelyopterus peloichthys* | *Trachelyopterus peloichthys* |
| "*Amplexiglanis*" sp. | -- | -- |

**Fonte**: Dados oriundos de *Eschmeyer's Catalog of Fishes* (FRICKE, 2021), *Fishbase* (FROESE, 2021) e Akama (2004). Edição do autor. Não identificada relação até o presente momento (para comparações entre Akama e atuais), não se faz presente ou não é validado pelo catálogo (para comparação entre catálogos) (--).

Entretanto, um número expressivo de espécies atualmente válidas se encontra avulsa em comparação a sua revisão taxonômica. Portanto, a diversidade por traz da

descrição realizada por Akama (2004) é possivelmente muito maior. Em vista disso, é de grande importância analisar e compreender essa diversidade, tanto para contribuir com as incongruências envolvendo os gêneros, quanto para melhorar a qualidade das informações taxonômicas e evolutivas de *Trachelyopterus.*

## 1.6 DNAs repetitivos e evolução do genoma

O genoma das espécies tem se demonstrado altamente variável e com grandes diferenças na quantidade de DNA. Em animais, esta variação pode ir de 0,02 pg em *Pratylenchus coffeae* (nemátode) à 132,83 pg em *Protopterus aethiopicus* (peixe) (www.genomesize.com). Contudo, ao contrário do que se imaginava, tal variação não tem demonstrado qualquer relação com a complexidade do organismo e nem ao menos com a quantidade de genes (GREGORY, 2005; CABRAL-DE-MELLO, 2011). Em vez disso, atualmente a diversidade no tamanho genômico das espécies tem sido amplamente associada a uma quantidade substancial de cópias múltiplas e repetidas de DNA alinhadas em tandem ou dispersas, denominadas elementos repetitivos ou DNA repetitivo, podendo ocupar 80% do genoma (MARTINS et al., 2011; PLOHL et al., 2008).

Por muito tempo os DNAs repetitivos foram considerados erroneamente como *junk* DNAs (DNA lixo) (DOOLITTLE; SAPIENZA, 1980; MARTINS et al., 2011). Contudo, tem-se conhecimento atualmente de que estas frações genômicas repetitivas diferem de genes de cópia única por serem influenciadas de forma menos agressiva por pressões seletivas, apresentando então taxas de mutações significativamente maiores. Em razão disto, podem facilmente e rapidamente divergir domínios cromossômicos equivalentes, tanto em indivíduos da mesma espécie quanto de espécies diferentes (ARCHIDIACONO et al., 1995; GOSÁLVES et al., 2010; COLUCCIA et al., 2011). Dessa forma, cresce constantemente o número de estudos que realizam a conexão entre essas sequências e eventos evolutivos que resultam em variabilidade genética, como rearranjos cromossômicos, incluindo deleções, duplicações, inversões e translocações, dando possíveis sentidos a sua existência (KIDWELL, 2002).

Além disso, sendo uma sequência ubíqua em eucariotos, estas frações de DNA podem estar envolvidas em diversos outros aspectos genômicos ainda não

conhecidos (CABRAL-DE-MELLO, 2011). Recentemente, estudos têm evidenciado possível conexão de elementos repetitivos na organização do genoma, no processo de replicação, expressão gênica, recombinação (BIET et al., 1999, LIU et al., 2001; LI et al., 2002; CABRAL-DE-MELLO, 2011), na formação da heterocromatina, e também na regulação da expressão gênica (SHAPIRO; STERNBERG, 2005; BIÉMONT; VIEIRA, 2006; FESCHOTTE; PRITHAM, 2007; SLOTKIN; MARTIENSSEN, 2007; CABRAL-DE-MELLO, 2011; SECCO et al., 2011). Portanto, a caracterização desses elementos não se restringe somente a apresentar a variabilidade genética existente, mas também na medida que pode contribuir no entendimento das funções, organização e arquitetura genômica das espécies (LYNCH, 2007).

## 1.7 Classificação e organização do DNA repetitivo no genoma eucarioto

Os DNAs repetitivos podem ser classificados em sequências codificantes e não codificantes, em tandem ou dispersas. Os DNAs repetitivos codificantes são representados principalmente pelas famílias multigênicas (DNA ribossômico, histonas e pequenos DNAs nucleares - snDNAs), enquanto as não codificantes são representadas principalmente por repetições em tandem, tais quais os satélites, minissatélites, microssatélites e também as chamadas repetições “egoístas”, conhecidas coletivamente como elementos transponíveis - ETs (CHARLESWORTH et al., 1994; SUMNER et al. 2003; LÓPEZ-FLORES; GARRIDO-RAMOS, 2012).

As famílias multigênicas correspondem a um grupo de genes originados a partir de um gene ancestral comum, a como consequência, possuem funções similares (MARTINS; WASKO, 2004; NEI; ROONEY, 2005). Dentro das famílias multigênicas, o DNA ribossômico eucariótico (DNAr) é representado por duas classes de unidades repetidas em tandem. A porção principal (DNAr 45S) compreende os genes DNAr 18S, 5.8S e 28S e a classe secundária é representada pelo DNAr 5S (LONG E DAWID, 1980). Os clusters de DNA ribossomal 45S são separados por espaçadores transcritos externos (ETS-External Transcribed Spacer) e por espaçadores intergênicos (IGS – Intergenic Spacer), enquanto as porções menores 18S, 5.8S e 28S são separadas por espaçadores intergênicos transcritos internos (ITS-Internal Transcribed Spacer) (EICKBUSH; EICKBUSH, 2007). Por outro lado, o arranjo do DNA ribossomal 5S é caracterizado por copias múltiplas de uma unidade

transcricional altamente conservada de aproximadamente 120 pares de base, separados por um espaçador não transcrito (NTS-Non Transcribed Spacer) que varia em tamanho e composição da sequência de espécie para espécie (REBORDINOS et al., 2013).

Os genes de histonas (H1, H2A, H2B, H3 e H4) estão entre os primeiros genes eucarióticos a serem caracterizados (WOLFFE, 2001). Em grande parte dos organismos elas são encontradas organizadas em clusters e em repetições de 5 a 10 vezes. Seu produto é responsável por atuar diretamente na formação do nucleossoma, que contém cerca de 150 pb de DNA envolto 1,7 ao redor de um cerne de proteínas histonas. O cerne por sua vez contem oito histonas, duas subunidades de cada uma das quatro histonas, organizadas como dois dímeros de H2A e H2B e um tetrâmero de H3 e H4. Por final, encontra-se uma histona com propriedades ligantes, H1, que termina o processo de compactação da fibra de cromatina em estruturas de ordem superior responsáveis por condensar ainda mais o DNA (GRIFFITHS et al., 2016).

Também sendo constituinte das famílias multigênicas, as pequenas sequências de RNA nuclear (snRNA) são consideradas uma das menores sequências de RNA eucarioto, com tamanho médio de cerca de 150 bases (HARI; PARTHASARATHY, 2018). Os snRNAs estão localizados no núcleo celular e possuem funções essenciais durante o processo de splicing e processamento do RNA mensageiro, sendo um dos principais componentes da maquinaria do spliceossomo (MANCHADO et al., 2006), composto por um grande complexo RNA-proteína e cinco tipos de RNAs não codificantes (U1, U2, U4, U5 e U6) (VALADKHAN, 2005; ÚBEDA-MANZANARO et al., 2010). Todavia, além do papel no processamento do mRNA, essencial durante o processo de transcrição, evidencias indicam atividade na maturação de transcritos primários em mRNA e também na regulação da expressão gênica, cumprindo papel essencial na maquinaria celular (revisado em HARI; PARTHASARATHY, 2018).

Por outro lado, o DNA repetitivo organizado em tandem, pode ser dividido em três classes principais de acordo com seu tamanho. Os satélites possuem cerca de 100 a 300 pb, minissatélites 15 a 150 pb e os microssatélites cerca de 6 pb (LEVINSON, GUTMAN, 1987). Em especial, os microssatélites, também chamadas de repetições curtas em tandem (STRs) ou repetições de sequência simples (SSRs) (ELLEGREN, 2004) são compostos de frações de DNA repetido em mono-, di-, tri-, tetra- ou penta-nucleotideos distribuídos através de todo o genoma da maioria dos

organismos eucariotos (ELLEGREN 2004; KALIA et al., 2011). Eles são um dos mais abundantes e variaveis tipos de sequência de DNA no genoma e, embora a atividade de transposons também tenha sido relatada como fonte de novos microssatélites, (MESSIER et al., 1996; LI et al., 2002; KALIA et al., 2011; GRANDI, 2013), eles são resultado principalmente do emparelhamento incorreto da dupla fita de DNA e subsequentes erros durante o reparo do DNA (LEVINSON; GUTMAN, 1987; KALIA et al., 2011).

Dentro das sequências classificadas como não codificantes e dispersas, os elementos transponíveis são os únicos capazes de realizar a transposição para regiões distintas do genoma (KAZAZIAN, 2004; MARTINS et al., 2011). Exceto o elemento transponível *Pokey* encontrado no microcrustáceo *Daphnia* que possui afinidade por uma região específica dentro da sequência de DNA ribossomal 18S, todas as outras sequências conhecidas apresentam movimentação randômica pelo genoma (LYNCH, 2007). Dessa forma, são frações de DNA com enorme potencial evolutivo, pois, não há consideração com a relevância do novo sítio em que estarão se inserindo ou com as consequências geradas, sendo, portanto, uma grande fonte de mutações, matéria-prima para eventos evolutivos nas espécies (LYNCH, 2007).

Elementos transponíveis podem ser classificados de acordo com seu mecanismo de movimentação pelo genoma, sendo replicativos (*copy and paste* – classe I) ou conservadores (*cut and paste* – classe II) (FINNEGAN, 1989; LYNCH, 2007). Para os que utilizam de mecanismos conservativos, denominados transposons, a forma de movimentação se dá através de remoção e inserção, em que a sequência repetitiva é deslocada de um ponto do genoma para outro na forma de DNA (LYNCH, 2007). Por outro lado, aqueles que utilizam mecanismos replicativos, denominados retrotransposons, utilizam de transcrição reversa para movimentar-se pelo genoma. Para isso, a partir da sequência de DNA original é produzido um RNA intermediário, mantendo a cópia original, então o RNA intermediário é transcrito reversamente em uma nova cópia de DNA, que por sua vez se insere em outra parte do genoma, resultando assim em duas cópias do elemento transponível (LYNCH, 2007; MARTINS et al., 2011).

Os transposons são conhecidos classicamente como a segunda classe (classe II) dos elementos repetitivos dispersos do DNA. Este grupo pode ser dividido atualmente em duas subclasses: **a)** TIR e Crypton e **b)** Helitron e Maverick/Polinton. Dentro dessas subclasses estão inseridas 12 superfamílias (Tc1/Mariner, hAT,

Mutator, Merlin, Transib, P, PiggyBac, PIF/Harbinger, CACTA, Crypton, Helitron e Maverick) (FESHOTTE; PRITHMAN, 2007; CALIXTO, 2013; CLARO, 2013). Evolutivamente e filogeneticamente a análise desses elementos não apresenta grande relevância no que condiz a tamanhos genômicos, pois, devido à movimentação não replicativa esta classe de elementos transponíveis não favorece a diferenciação no tamanho e quantidade de material genético (MARTINS et al., 2011). Todavia, durante seu movimento pode causar mutações e afetar diretamente a expressão e atividade gênica, haja vista que, há a possibilidade de alterar a estrutura de genes e inclusive levá-los a inativação (JURKA, 2007; CALIXTO, 2013; CLARO, 2013).

Por outro lado, os retrotransposons são conhecidos classicamente como a classe II de elementos genéticos móveis e podem ser classificados atualmente em cinco ordens (LTR, DIRS, PLE, LINE e SINE) englobando um total de 16 superfamílias (gypsy, bel-pao, retrovirus, ERV, DIRS, Ngaro, VIPER, Penelope, R2, RTE, Jockey, L1, I, tRNA, 7SL e 5S). Evolutivamente, as consequências da movimentação dos retrotransposons são muito semelhantes à movimentação de transposons, porém, adicionalmente, durante sua movimentação são produzidas novas cópias da sequência original, aumentando assim a quantidade de DNA no genoma da espécie e impactando diretamente na sua organização. Portanto, os retrotransposons são grandes influenciadores no tamanho, estruturação e organização do genoma dos táxons ao longo do percurso evolutivo (LYNCH, 2007).

De maneira geral, elementos transponíveis são sequências com enorme potencial evolutivo. Podem agir adicionando rotas regulatórias alternativas, éxons e sítios de *splicing* a genes (HANCKS; KAZAZIAN, 2010), ou proporcionando rearranjos cromossômicos, recombinações não homologas, deleções, duplicações, inversões, translocações, quebras cromossômicas, variação no tamanho do genoma e inativação de genes (BIET et al., 1999, LIU et al., 2001; LI et al., 2002; ZHANG; PETERSON, 2004; JURKA, 2007; LYNCH, 2007; CABRAL-DE-MELLO, 2011; CLARO, 2013). Em casos mais complexos, por apresentarem regiões promotoras e elementos regulatórios próprios, a inserção desses elementos a genes pode resultar nas mais variadas modificações no processo de transcrição e expressão gênica, até mesmo se inseridos adjacentemente (JORDAN et al., 2003; HAN et al., 2004; LESAGE; TODESCHINI, 2005; MARINO-RAMIREZ et al., 2005; LYNCH, 2007). Além disso, devido às interações únicas em cada grupo, elementos transponíveis também estão

associados com o surgimento de complexas redes de regulação gênica transcricional e pós transcricional (FESCHOTTE; PRITHAM, 2007; VENÂNCIO NETO, 2019) e com a estruturação e estabilidade de cromossomos em um número considerável de eucariotos (LYNCH, 2007).

# 2. OBJETIVOS

## 2.1 Objetivo Geral

a) Investigar a distribuição de elementos repetitivos no genoma de espécies de *Trachelyopterus*

## 2.2 Objetivos Específicos

a) Analisar de forma integrada os padrões de distribuição dos elementos repetitivos DNAr 18S, DNAr 5S, snRNA U2, genes de histona H3 e H4 em espécies de *Trachelyopterus* e seus possíveis aspectos citotaxonômicos e evolutivos;
b) Comparar os padrões de distribuição do microssatélite (GATA)n nos cromossomos portadores dos elementos repetitivos DNAr 18S, DNAr 5S, snRNA U2, genes de histona H3 e H4 em espécies de *Trachelyopterus* assim como seus possíveis aspectos evolutivos.

# 3. MATERIAIS E MÉTODOS

## 3.1 Amostragem

Foram analisados indivíduos que contemplam 5 dos 7 táxons (71,43%) pertencentes aos gêneros *Trachelyopterus* e *Parauchenipterus* descritos em revisão taxonômica realizada por Akama (2004). Os espécimes são pertencentes à 7 populações provenientes de pontos de coleta distribuídos em 7 bacias hidrográficas da América do Sul, incluindo a bacia do Rio da Prata, bacia do Rio Piumhi, bacia dos Rios Tocantins-Araguaia, bacia do Rio Cuiabá, bacia do Rio Doce, bacia do Rio Paraguai e bacia do Rio Amazonas (Tab. 4).

**Tabela 4**. Populações analisadas e disponibilizadas pelo Laboratório de Citogenética do grupo de Biologia e Conservação de Anamniotas da Universidade Estadual do Oeste do Paraná (UNIOESTE).

| Espécie | Local | Município | Estado | GPS | BH | Sexo | | Voucher |
|---|---|---|---|---|---|---|---|---|
| | | | | | | ♂ | ♀ | |
| *Trachelyopterus galeatus* Linnaeus, 1766 | Lago Catalão, Bacia do Rio Amazonas | Manaus | AM | 03° 09' 47" S 59° 54' 29" W | AM | 6 | 8 | INPA 57939 |
| *Trachelyopterus porosus* Eigenmann e Eigenmann, 1888 | Lago Catalão, Bacia do Rio Amazonas | Manaus | AM | 03° 09' 47" S 59° 54' 29" W | AM | 4 | 4 | INPA 57940 |
| *Trachelyopterus* aff. *galeatus* Linnaeus, 1766 | Córrego do Medo (Fazenda Ana Maria), Rio Araguaia | São Miguel do Araguaia | GO | 13º 08’ 52,7” S 50º 25’ 02,8” O | A | 9 | 10 | MZUSP 110803 |
| *Trachelyopterus striatulus* Steindachner, 1877 | Lagoa Verde (PERD*), Bacia do Rio Doce | Marliéria | MG | 19º 49’ 44,5” S 42º 37’ 52.5” O | C | 3 | 3 | MZUSP 109798 |
| *Trachelyopterus coriaceus* Valenciennes, 1840 | Córrego do Medo (Fazenda Ana Maria), Rio Araguaia | São Miguel do Araguaia | GO | 13º 08’ 52,7” S 50º 25’ 02,8” O | A | 4 | 3 | MZUSP 106766 |
| *Trachelyopterus* aff. *coriaceus* | Lagoa Arrombado, Bacia do Rio Bento Gomes | Poconé | MT | 16º 25’ 40,9” S 56º 25’ 07,4” O | PG | 2 | 1 | MZUSP 110806 |

* PERD – Parque Estadual do rio Doce; AM: Amazonas GO – Goiás; MT – Mato Grosso; MG – Minas Gerais; GPS: Global Positioning System; BH – Bacia Hidrográfica; A Araguaia; PA – Paraná; PG – Paraguai; SF – São Francisco; C – Costeira; INPA: Instituto Nacional de Pesquisas da Amazônia; MZUSP – Museu de Zoologia da Universidade de São Paulo; NUP - Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura da Universidade Estadual de Maringá.

## 3.2 Suspenção celular de cromossomos mitóticos

### 3.2.1 Indução do número de mitoses – Lee e Elder (1980)

Para obtenção de uma preparação cromossômica com maior quantidade e qualidade de células metafásicas, induziu-se a função hematopoiética (mitótica) do rim anterior através da aplicação intraperitonial na proporção de 1 mL/100 gramas de suspensão de levedura (0,5 g de fermento de padaria + 1,5 g de dextrose + 6 ml de $H_2O$). Os animais injetados com a solução foram mantidos em aquário arejado ininterruptamente durante 24-72 horas e então submetidos ao tratamento com solução aquosa de colchicina.

### 3.2.2 Tratamento *in vivo* – Bertollo et al. (1978)

Os exemplares foram tratados com solução aquosa intra-abdominal de colchicina 0.02% (1mL/100g de peso corporal) e mantidos em aquário arejado ininterruptamente por 30-40 minutos. Após o período de absorção sistêmica da colchicina os espécimes foram sacrificados por overdose de óleo de cravo (GRIFFITHS, 2000). Para obtenção dos cromossomos mitóticos foram retiradas porções do rim (tecido hematopoiético), o qual foi lavado em solução hipotônica (KCl 0.075M) e transferido para cubas de vidro contendo aproximadamente 10mL da mesma solução. O material foi então fragmentado com auxílio de pinças e seringa hipodérmica desprovida de agulha até a obtenção de uma solução homogênea. As soluções obtidas foram transferidas para *Falcons* de vidro e incubadas em estufa BOD à 37º C por 30 minutos. Passado o período de incubação, 10 gotas de fixador recém preparado (3 metanol: 1 ácido acético) foi adicionado e o material ressuspendido e centrifugado por 10 minutos à 500-800 rpm. Com cuidado, o sobrenadante foi então removido e vagarosamente e 5-7 mL de fixador foram adicionadas pelas paredes do tubo. O material foi então novamente homogeneizado com pipeta *Pasteur* e centrifugado por 10 minutos a 500-800 rpm. O procedimento foi repetido 1-2 vezes. Finalizadas as centrifugações, o sobrenadante foi descartado e aproximadamente 1 mL de fixador foi adicionado.

As suspensões celulares foram transferidas para tubos do tipo *Ependorff* e armazenadas em freezer.

### 3.2.3 Tratamento *in vitro* – Moreira-Filho e Bertollo (1990)

Para obtenção dos cromossomos mitóticos in vitro, foram retiradas porções do rim (tecido hematopoiético) e mantidas em placas de petri contendo solução salina de Hanks. O material foi então fragmentado com auxílio de pinças e seringas hipodérmicas desprovidas de agulha e transferido para tubos de centrífuga juntamente com 1-2 gotas de colchicina 0.0125%. A solução foi incubada em estufa BOD a 37ºC por 30 minutos. Após a incubação o material foi centrifugado durante 10 minutos a 900 rpm. O sobrenadante foi descartado e adicionou-se 10 ml de solução hipotônica (KCl 0,075M) seguido de homogeneização com pipeta Pasteur até obter uma solução homogênea. A solução foi incubada em estufa BOD a 37ºC por 30 minutos. Após o período de incubação foram adicionadas 9-10 gotas de fixador (3 metanol: 1 ácido acético) recém-preparado e o material foi novamente centrifugado durante 10 minutos a 900 rpm. O sobrenadante foi descartado, foram adicionados 10 ml de fixador, seguido de homogeneização e nova centrifugação por 10 minutos a 900 rpm. O procedimento foi repetido 1-2 vezes. Finalizadas as centrifugações, o sobrenadante foi descartado e cerca de 1 ml de fixador foi adicionado. As suspensões celulares foram transferidas para tubos do tipo *Ependorff* e armazenadas em freezer.

## 3.3 Obtenção e marcação das sondas

### 3.3.1 DNAr 18S e DNAr 5S

Sondas de DNAr 18S de *Prochilodus argenteus*, obtidas por Hatanaka e Galetti Jr (2004) e preservadas em plasmídeo foram utilizadas para marcação por *nick-translation* com Biotina-16-dUTP e/ou Digoxigenina-11-dUTP (*Bio-Nick-Translation* e/ou *Dig-Nick-Translation mix* – Roche), de acordo com as instruções

do fabricante (1 hora e 30 minutos para DNAr 18S e 1 hora e 15 minutos para sondas de DNAr 5S). Sondas de DNAr 18s ou 5s marcadas com Biotina-16-dUTP foram detectadas com antibiotina-avidina-FITC/ antiavidina-Biotina (Roche). Sondas de DNAr 5S foram produzidas a partir de fragmentos de DNAr 5S amplificados por PCR. Para isso foram utilizados os primers 5SA (5'- TAC GCC CGA TCT CGT CCG ATC - 3') e 5SB (5' - CAG GCT GGT ATG GCC GTA AGC - 3') (MARTINS; GALETTI, 1999) para amplificar os fragmentos a partir da solução contendo plasmídeos com a sequência de interesse. Essa região foi amplifica por uma reação de PCR (25 µL) contendo 5 µL de tampão de reação 5X, 1,5 µL $MgCl_2$ (25mM), 0,4 µL dNTP Mix (10 mM – 2,5 mM de cada nucleotídeo), 0,4 µL de cada primer (10 µm), 2 µL DNA plasmidial (300 ng/µL), 0,2 µL Taq Polimerase (5 U/µL), 0,025 mM de Tetrametilrodamina-5-dUTP e 14,3 µL de $H_2O$ Mili-Q. As condições de PCR foram: 95 °C (5 minutos), 30 ciclos de 95 °C (30 segundos), 56 ºC (45 segundos), 72 °C (2 minutos), seguido de extensão final por 72 °C (7 minutos).

### 3.3.2 snRNA U2

Os fragmentos de snDNA U2 foram amplificados a partir do genoma total de *Trachelyopterus Galeatus* com auxílio dos primers U2F (5'- ATC GCT TCT CGG CCT TAT G -3') e U2R (3'- TCC CGG CGG TAC TGC AAT A -5') (BUENO et al., 2013). Essa região foi amplifica por uma reação de PCR contendo 5 µL de tampão de reação 5X, 1,5 µL $MgCl_2$ (25mM), 0,4 µL dNTP Mix (10 mM – 2,5 mM de cada nucleotídeo), 0,5 µL de cada primer (10 µm), 0.5 µL DNA genômico total (800 ng/µL), 0,2 µL Taq Polimerase (5 U/µL) e 15,8 µL de $H_2O$ Mili-Q. As condições de PCR foram: 95 °C (5 minutos), 30 ciclos de 95 °C (45 segundos), 51,7 ºC (45 segundos), 72 °C (1 minuto e 20 segundos), seguido de extensão final por 72 °C (7 minutos). As sondas foram marcadas a partir de nova reação de PCR seguindo os mesmos parâmetros (25 µL de reação total para hibridação em até 12 lâminas) tendo como *template* os fragmentos isolados a partir do genoma total. A marcação foi realizada com o uso de Fluoresceína-12-dUTP (Roche) na quantidade de 0,6 µL (1mM) a ser descontada do volume de água da reação de amplificação.

### 3.3.3 Histonas H3 e H4

Os fragmentos de Histonas H3 e H4 foram amplificados a partir do genoma total de *Trachelyopterus Galeatus* com auxílio dos primers H3F (5'-ATG GCT CGT ACC AAG CAG ACV GC-3') e H3R (5' - ATG GCT CGT ACC AAG CAG ACV GC - 3') (COLGAN et al., 1998); H4F2s (5′ - TSC GIG AYA ACA TYC AGG GIA TCA C - 3′) e H4F2er (5′-CKY TTI AGI GCR TAI ACC ACR TCC AT-3′) (PINEAU et al., 2004). Essa região foi amplifica por uma reação de PCR contendo 5 µL de tampão de reação 5X, 2,0 µL $MgCl_2$ (25mM), 0,4 µL dNTP Mix (10 mM – 2,5 mM de cada nucleotídeo), 0,5 µL de cada primer (10 µm), 0.5 µL DNA genômico total (800 ng/µL), 0,2 µL Taq Polimerase (5 U/µL) e 15,8 µL de $H_2O$ Mili-Q. As condições de PCR foram: 95 °C (5 minutos), 30 ciclos de 95 °C (30 segundos), 52 ºC (45 segundos), 72 °C (1 minuto e 20 segundos), seguido de extensão final por 72 °C (7 minutos). As sondas foram marcadas a partir de nova reação de PCR seguindo os mesmos parâmetros (25 µL de reação total para hibridação em até 12 lâminas) tendo como *template* os fragmento isolados a partir do genoma total (200ng). A marcação de ambas as sondas foi realizada com o uso de Tetrametil-Rodanima-5-dUTP (Roche) na quantidade de 0,6 µL (1mM) a ser descontada do volume de água da reação de amplificação.

### 3.3.4 Microssatélite $(GATA)_n$

Os fragmentos do microssatélite (GATA)n foram amplificados e marcados através de reação de PCR utilizando os primers como *template* de acordo com Ijdo et al. (1991). As sondas foram marcadas com Tetrametil-Rodamina-5-dUTP ou Fluroesceína-11-dUTP utilizando os primers $(GATA)_7$ e $(TATC)_7$ (Ijdo et al., 1991). A reação de marcação (12,5 µL de reação total para até 12 lâminas) foi composta por: 2,5 µL de tampão de reação 5X, 0,75 µL $MgCl_2$ (25mM), 0,25 µL dNTP Mix (10 mM – 2,5 mM de cada nucleotídeo), 1,25 µL de cada primer como template (10 µm), 0,1 µL Taq Polimerase (5 U/µL), 6,1 µL de $H_2O$ Mili-Q e 0,3 µL (10µM) de Tetrametil-Rodamina-5-dUTP ou Fluroesceína-11-dUTP. As condições de PCR foram: 95 °C (5 minutos), 10 ciclos de 95 °C (1 minuto), 55 ºC (30 segundos), 72 °C

(1 minuto); 30 ciclos de 95 °C (1 minuto), 60 ºC (30 segundos), 72 °C (1 minuto e 30 segundos) seguido de extensão final por 72 °C (7 minutos).

## 3.4 Hibridação *in situ* Fluorescente (FISH)

### 3.4.1 Preparo das lâminas

A FISH foi conduzida de acordo com Pinkel et al. (1986) e Margarido e Moreira-Filho (2008) sob altas condições de estringência. Para isso, as lâminas contendo DNA cromossômico foram incubadas a 37ºC em solução contendo 0.4% RNAse/2xSSC em câmara úmida e escura por 60 minutos. Em seguida, foram lavadas em 2xSSC e incubadas por 45 minutos a 60ºC em 2xSSC. Terminado o período, foram submetidas a série alcoólica de 70% e 100% por 5 minutos. O DNA cromossômico foi então desnaturado em 0.05M NaOH/2xSSC por 3 minutos exatos e novamente desidratado em série alcoólica 70% e 100% por 5 minutos cada. A solução de hibridação (Anexo I para concentrações) contendo as sondas foi desnaturada à 100ºC por 10 minutos e submetida a choque térmico em gelo. Posteriormente, as lâminas contendo o DNA cromossômico foram incubadas por 12-14 horas em câmara úmida e escura a 37ºC juntamente com 58 µL da solução de hibridação sob altas condições de estringência (200ng de cada sonda, formamida deionizada 50%, sulfato dextrano 10%, 2x SSC, pH 7.0–7.2, 37º overnight).

### 3.4.2 Detecção do sinal de sondas marcadas por PCR

Terminado o período de incubação (12-14 horas) com a solução de hibridação, as lâminas foram submetidas a lavagem em solução de 1xSSC a 37ºC por 5 minutos. Em seguida, as lâminas foram (i) lavadas novamente em solução de 1xSSC em temperatura ambiente; (ii) lavadas duas vezes em solução de Tween 0.05%/4xSSC por 5 minutos cada; (iii) lavadas uma vez em solução 4xSSC em temperatura ambiente e (iv) mantidas em solução 1xSSC por 5 minutos. Após

secarem, as lâminas foram coradas em 25 uL de 4' 6-diamidino-2-phenylindole dihydrochloride – DAPI (200 µl de *Antifading* + 1 µl de DAPI 0.2 mg/mL).

### 3.4.3 Detecção do sinal de sondas Marcadas por *nick-translation*

Terminado o período de incubação (12-14 horas) com a solução de hibridação, as lâminas foram submetidas a lavagem em solução de 1xSSC a 37ºC por 5 minutos. Em seguida, as lâminas foram (i) lavadas novamente em solução de 1xSSC em temperatura ambiente; (ii) lavadas duas vezes em solução de Tween 0.05%/4xSSC por 5 minutos cada; (iii) incubadas em tampão NFDM por 15 minutos; (iv) lavadas duas vezes em solução de Tween 0.05%/4xSSC por 5 minutos cada. Para a detecção das sondas foram utilizadas Antidigoxi Rodamina (Roche) para sondas marcadas com *Dig-Nick-Translation Mix* (Roche) e Antibiotina Avidina – FITC (Roche) para sondas marcadas com *Biotin-Nick-Translation Mix* (Roche). Quando necessário realizou-se à amplificação do sinal de sondas marcadas por *Biotin-Nick-Translation Mix* (Roche) com auxílio de *Anti-avidin-Biotin Mix* (Roche). As lâminas foram incubadas durante 60/30 minutos em câmara úmida e escura a temperatura ambiente com o respectivo mix de detecção. Terminado o período foram (v) lavadas duas vezes em solução de Tween 0.05%/4xSSC por 5 minutos cada; (vi) lavadas uma vez em solução 4xSSC em temperatura ambiente com agitação por 5 minutos e (vii) incubadas por 5 minutos em solução 1xSSC em temperatura ambiente. Após secarem, as lâminas foram coradas com uma mistura de 25 uL contendo 4' 6-diamidino-2-phenylindole dihydrochloride – DAPI (200 µl de Antifading + 1 µl de DAPI 0.2 mg/mL).

### 3.4.4 Análise das lâminas

As imagens digitais foram capturadas pelo *software* DP Controller 3.2.1.276 usando câmera digital Olympus DP71 conectada ao microscópio de epifluorescência BX61 (Olympus America Inc., Center Valley, PA, Estados Unidos da América).

## 4. REFERENCIAS


ARAÚJO, W.C.; MOLINA, W.F. Citótipo exclusivo para *Parauchenipterus galeatus* (Siluriformes, Auchenipteridae) na Bacia do Atlântico NE Oriental do Brasil: Indicações de um complexo de espécies. **Biota Amazônia**, v. 3, n. 2, p. 33-39, 2013.

AKAMA, A; BRITSKI, H. A. **Revisão sistemática dos gêneros *Parauchenipterus* Bleeker, 1862 e *Trachelyopterus* Valenciennes, 1840 (Siluriformes, Auchenipteridae)**. Tese de doutorado. 2004.Universidade de São Paulo, São Paulo, 2004.

ARCHIDIACONO, N.; ANTONACCI, R.; MARZELLA, R.; FINELLI, P.; LONOCE, A.; ROCCHI, M. Comparative mapping of human alphoid sequences in great apes using fluorescence in situ hybridization. **Genomics**, Louisiana, v. 25, n. 2., p. 477-484, 1995.

BERTOLLO, L. A. C., BORN G. G., DERGAM J. A., FENOCCHIO A. S.; MOREIRA-FILHO, O. A biodiversity approach in the neotropical Erythrinidae fish, *Hoplias malabaricus*. Karyotypic survey, geographic distribution of cytotypes and cytotaxonomic considerations. **Chromosome Research**, v. 8, n, 7, p. 603-613, 2000.

BICKFORD, D.; LOHMAN, D.J.; SODHI, N.S.; NG, P.K.L.; MEIER, R.; WINKER, K.; INGRAM, K.K. Cryptic species as a window on diversity and conservation. **Trends in Ecology & Evolution**, v. 22, n. 3, p. 148-155, 2007.

BIÉMONT, C. Genome size evolution: Within-species variation in genome size. **Heredity**, v. 101, n. 4, p.297-298, 2008.

BIET, E.; SUN, J.S.; DUTREIX, M. Conserved sequence preference in DNA binding among recombination proteins: an effect of ssDNA secondary structure. **Nucleic Acids Research**, v. 27, n. 2, p.596-600, 1999.

BIRINDELLI, J.L.O. **Relações filogenéticas da superfamília Doradoidea (Ostariophysi, Siluriformes).** 2010. Tese (Doutorado em Ciências) - Instituto de Biociências, Universidade de São Paulo, São Paulo.

BORATYN, G.M.; THIERRY-MIEG, J., THIERRY-MIEG, D.; BUSBY, B.; MADDEN, T.L. Magic-BLAST, an accurate RNA-seq aligner for long and short reads. **BMC Bioinformatics**, v. 20, n.1, 2019.

BLANCO, D. R.; LUI, R. L. ; VICARI, M. R. ; BERTOLLO, L. A. C. ; MOREIRA-FILHO, O. Comparative Cytogenetics of Giant Trahiras *Hoplias aimara* and *H. intermedius* (Characiformes, Erythrinidae): Chromosomal Characteristics of Minor and Major Ribosomal DNA and Cross-Species Repetitive Centromeric Sequences Mapping Differ among Morphologically Identical Karyotypes. **Cytogenetic and Genome Research**, v. 132, p. 71-78, 2011.

BLAXTER, M. Counting angels with DNA. **Nature,** Londres, v. 421, n. 6919, p. 122-123, 2003.

BLOCH, M.E. **Naturgeschichte der ausländischen Fische**. Berlin: 1794.

BUCKUP, P.A.; MENEZES, N.A..; GHAZZI, M.S. **Catálogo das Espécies de Peixes de Água Doce do Brasil**. Rio de Janeiro: Museu Nacional – Universidade Federal do Rio de Janeiro, Série Livros 23, 2007.

BUENO, D.; PALACIOS-GIMENEZ, O.M.; CABRAL-DE-MELLO, D.C. Chromosomal Mapping of Repetitive DNAs in the Grasshopper *Abracris flavolineata* Reveal Possible Ancestry of the B Chromosome and H3 Histone Spreading. **Plos One**, v. 8, n. 6, 2013.

CABRAL-DE-MELLO, D. **Organização cromossômica de elementos repetitivos de DNA em representantes da subfamília scarabaeinae (coleoptera: scarabaeidae).** 2011. 233 f. Tese (Doutorado) - Programa de Pós-graduação em Ciências Biológicas, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, 2011.

CALEGARI, B.B.; VARI, R.P.; REIS, R.E. Phylogenetic systematics of the driftwood catfishes (Siluriformes: Auchenipteridae): a combined morphological and molecular analysis. **Zool J Linnean Soc**., v. 187, p. 661-773, 2019.

CALIXTO, M. S. **Mapeamento cromossômico de DNA repetitivos em espécies de morcegos da família Phyllostomidae.** 2013. 138 f. Tese (Doutorado) - Programa de Pós-Graduação em Genética, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Recife, 2013.

COLGAN D.; MCLAUCHLAN, A.; WILSON, G.;LIVINGSTON, S. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. **Aust J Zool,** v.46, p.419-437, 1998.

CHARLESWORTH, B.; SNIEGOWSKI P.; STEPHAN, W. The evolutionary dynamics of repetitive DNA in eukaryotes. **Nature,** v. 371, pp. 215–220, 1994.

CHIRINO, M.; PAPESCHI, A.; BRESSA, M. The significance of cytogenetics for the study of karyotype evolution and taxonomy of water bugs (Heteroptera, Belostomatidae) native to Argentina. **Comparative Cytogenetics**, v. 7, n. 2, p.111-129, 2013.

CLARO, F. L. **Estudos do DNA repetitivo no gênero Eigenmannia.** 2013. 150 f. Tese (Doutorado) - Programa de Pós-Graduação em Ciências, Departamento de Genética e Biologia Evolutiva, Universidade de São Paulo, São Paulo, 2013.

COLUCCIA, E.; PICHIRI, G.; NIEDDU, M.; CONI, P.; MANCONI, S., DEIANA, A. M., MEZZANOTTE, R. Identification of two new repetitive elements and chromosomal mapping of repetitive DNA sequences in the fish *Gymnothorax unicolor* (Anguilliformes: Muraenidae). **European Journal of Histochemistry**, Pávia, v. 55, n. 2, 2011.

DOOLITTLE, W. F.; SAPIENZA, C. Selfish genes, the phenotype paradigm and genome evolution. **Nature**, v. 284, n. 5757, p.601-603, 1980.

CURRAN, D.J. **Phylogenetic Relationships Among the Catfish Genera of the family Auchenipteridae (Teleostei: Siluroidea)**. Kansas: Copeia, 1989.

EICKBUSH, T.H.; EICKBUSH, D.G. Finely orchestrated movements: Evolution of the ribossomal RNA genes. **Genetics**, v. 175, n.2, p. 477-485, 2007.

EIGENMANN, C.H.; EIGENMANN, R.S. Preliminary notes on South American Nematognathi. **Proceedings of the California Academy of Sciences***,* v. 1, p. 119-172, 1888.

ELLEGREN, H. Microsatellites: simple sequences with complex evolution. Nature **Reviews Genetics**, 5:435–445, 2004.

FELICETTI, D. **análise genética de populações simpátricas de Trachelyopterus valenciennes, 1840 (auchenipteridae) das bacias Amazônica e Paraguai:** contribuições à evolução de cromossomos B e taxonomia do grupo. 2018. Dissertação (Mestrado Conservação e Manejo de Recursos Naturais) – Centro de Ciências Biológicas e Saúde, Universidade Estadual do Oeste do Paraná, Paraná.

FELICETTI, D.; HAERTER, C.A.G.; BAUMGÄRTNER, L.; PAIZ, L. et al. A New Variant B Chromosome in Auchenipteridae: The Role of (GATA)n and (TTAGGG)n Sequences in Understanding the Evolution of Supernumeraries in *Trachelyopterus*. **Cytogenet Genome Res**., v. 161, p. 70-81, 2021.

FERRARIS JR, C.J. **The auchenipteridae: Putative monophyly and sistematics, with a classification of the neotropical Doradoid catfishes (Ostariophysi: Siluriformes).** Tese de doutorado não publicada. City University of New York. New York, p. 229, 1988.

FERRARIS JR, C.J. **Family Auchenipteridae**. *In*: Check List of Freshwater Fishes of South and Central America (Eds. Reis RE, Kullander SO, Ferraris Jr CJ). EDIPUCRS: Porto Alegre, p. 470-482, 2003.

FERRARIS JR, C.J. Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. **Zootaxa**, Auckland, v. 1418, p. 1-628, 2007.

FERREIRA, M. KAVALCO, K.F. ALMEIRA-TOLEDO, L.F.; GARCIA, C. Cryptic Diversity Between Two Imparfinis Species (Siluriformes, Heptapteridae) by Cytogenetic Analysis and DNA Barcoding. **Zebrafish**, Nova York, v. 11, n. 4, p. 306-317, 2014.

FENOCCHIO, A.S.; BERTOLLO, L.A.C. Karyotype, C-bands and NORs of the neotropical siluriform fish *Ageneiosus brevifilis* and *Ageneiosus atronases* (Ageneiosidae). **Cytobios**, v.72, n. 288, p. 19-22, 1992.

FENOCCHIO, A. S.; DIAS, A. L.; MARGARIDO, V. P.; SWARÇA, A. C. Molecular cytogenetic characterization of *Glanidium ribeiroi* (Siluriformes) endemic to the Iguaçu river, Brazil. **Chromosome Science**, v. 11, p. 61-66, 2008.

FESCHOTTE, C.; PRITHAM, E. J. DNA Transposons and the Evolution of Eukaryotic Genomes. **Annual Review of Genetics**, v. 41, n. 1, p.331-368, 2007.

FINNEGAN, D. J. Eukaryotic transposable elements and genome evolution. **Trends in Genetics**, v. 5, p.103-107, 1989.

FORESTI, F.; OLIVEIRA, C.; ALMEIDA-TOLEDO, L. F. A method for chromosome preparations from large specimens of fishes using *'in vitro'* short treatment with colchicine. **Experientia**. v. 49, p. 810– 813, 1993.

FRICKE, R.; ESCHMEYER, W. N.; VAN DER LAAN, R. (eds) 2019. ESCHMEYER'S CATALOG OF FISHES: GENERA, SPECIES, REFERENCES. Disponível em: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp**.** Acesso em: 31 jan. 2021.

FROESE, R.; PAULY, D. FishBase. World Wide Web electronic publication. Disponível em: www.fishbase.org. Acesso em 15 jun. 2020.

GARRIDO-RAMOS, M.A. Satellite DNA: an evolving topic. **Genes,** v.8, p. 230, 2017.

GIBBS R. H. JR.; COLLETTE B. B. Comparative anatomy and systematies of the tunas, genus Thunnus. **U.S. Fish Wildl. Serv., Fish. Bull**, v. 66, p. 65-130, 1967.

GOSÁLVES, J.; CRESPO, F.; VEGA-PLA, J.L.; LOPEZ-FERNÁNDEZ, C.; CORTÉS-GUTIÉRREZ, E.L.; DEVILA-RODRIGUEZ, M.I.; MEZZANOTE, R. Shared Y chromosome repetitive DNA sequences in stallion and donkey as visualized using whole-genomic comparative hybridization**. European Journal of Histochemistry**, Pávia v. 54, n. 2, p. 10-11, 2010.

GRANDI, F.C.; An, W. Non-LTR retrotransposons and microsatellites. **Mob Genet Elements,** 3:e25674, 2013.

GREGORY, T.R. Genome size evolution in animals. *In*: Gregory TR (Ed.). The Evolution of the Genome. **Elsevier**. San Diego, pp. 3-87, 2005.

GRIFFITHS, A.J.F.; WESSLER, S.R.; CARROLL, S.B.; DOEBLEY, J. **Introdução a genética.** Editora Guanabara Koogan LTDA, Rio de Janeiro, 2016.

GÜNTHER, A. **Catalogue of the fishes in the British Museum. Catalogue of the Physostomi, containing the families Siluridae, Characinidae, Haplochitonidae, Sternoptychidae, Scopelidae, Stomiatidae in the collection of the British Museum**. v. 5. Londres : British Museum Trustees, 1864.

HARI, R.; PARTHASARATHY, S. Prediction of Coding and Non-Coding RNA. **Reference Module in Life Sciences**, v. 3, p. 230-240, 2018.

HAN, J.S.; BOEKE, J.D. A highly active synthetic mammalian retrotransposon. **Nature**, v. 429, n. 6989, p.314-318, maio 2004.

HATANAKA, T.; GALETTI JR, P.M. Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). **Genetica,** v. 122, p. 239-244, 2004.

HAY, J.M.; SARRE, S.D.; LAMBER, D.M.; ALLENDORF, F.W.; DAUGHERTY, C.H. Genetic diversity and taxonomy: a reassessment of species designation in tuatara (Sphenodon: Reptilia). **Conservation Genetics**, Netherlands, v. 11, n. 3, p. 1063-1081, 2009.

HIRMAN, M.; KOTYK, M.; VARADÍNOVÁ, Z. K.; ŠŤÁHLAVSKÝ, F. First Cytogenetic Study of a Member of the Harvestman Family Neogoveidae (Opiliones: Cyphophthalmi. **Annales Zoologici**, Museum and Institute of Zoology at the Polish Academy of Sciences, v. 68, n. 4, p. 867-877, 2018.

HELFMAN, G.; COLLETTE, B. B.; FACEY, D. E; BOWEN, B. W. **The Diversity of Fishes : Biology, Evolution, and Ecology**. 2. ed. Hong Kong: Wiley-Blackwell, p. 1-736, 2004.

IJDO, J.W.; WELLS, R.A.; BALDINI, A.; REEDERS, S.T. Improved telomere detection using a telomere repeat probe (TTAGGG)n generated by PCR. **Nucleic Acids Res**, v. 19, p. 4780, 1991.

JOLY, C.A. Biodiversity and climate change in the Neotropical region. **Biota Neotrop**, v.8, n.1, 2008.

JORDAN, I.K.; ROGOZIN. I.B.; GLAZKO. G.V.; KOONIN, E.V. Origin of a substantial fraction of human regulatory sequences from transposable elements. **Trends Genet**. v. 19, p. 68–72, 2003.

JURKA, J.; KAPITONOV, V.V.; PAVLICEK, A.; KLONOWSKI, P.; KOHANY, O.; WALICHIEWICZ, J. Repbase Update, uma base de dados de elementos eucarióticos repetitivos. **Cytogenetic and Genome Research**, Basélia*,* v. 100, n.1, p. 462–467, 2005.

KAATZ, M.I.; STEWART, D.J.Bioacoustic variation of swimbladder disturbance sounds in Neotropical doradoid catfishes (Siluriformes: Doradidae, Auchenipteridae): Potential morphological correlates. **Current Zoology**, v. 58, n. 1, p. 171–188, 2012.

KALIA, R.K.; RAI, M. .K.; KALIA, S.; SINGH, R.; DHAWAN, A.K. Microsatellite markers: an overview of the recent progress in plants. **Euphytica**, v. 177, pp. 309-334, 2010.

KAZAZIAN, H. H. Mobile Elements: Drivers of Genome Evolution. **Science**, v. 303, n. 5664, p.1626-1632, 2004.

KIDWELL, M. G. Transposable elements and the evolution of genome size in eukaryotes. **Nature Reviews Genetics,** v. 115, n. 1, p. 49-63, 2002.

KOWALSKI, S.; PAIZ. L;M.; SILVA, M; MACHADO, A.S.; FELDBERG, E.; TRALDI, J.B.; MARGARIDO, V.P.; LUI, R.L. Chromosomal analysis of *Centromochlus heckelii* (Siluriformes: Auchenipteridae), with a contribution to Centromochlus definition. **Neotrop Ichthyol**. v. 18:e200009, 2020.

LANGEANI, F.; BUCKUP, P. A.; MALABARBA, L. R.; PYDANIEL, L. H. R.; LUCENA, C. A. S.; ROSA, R. S.; ZUANON, J. A. S.; LUCENA, Z. M. S.; BRITTO,M. R.; OYAKAWA, O. T.; GOMES-FILHO, G. Peixes de Água Doce. In: ROCHA, R. M.; BOEGER, W. A. P. (Eds.). **Estado da Arte e perspectivas para a Zoologia no Brasil**. Curitiba, 2009. p. 209-230.

LEE, M.R.; ELDER, F.F.B. Yeast stimulation of bone marrow mitosis for cytogenetic investigation. **Cytogenetic Cell Genetic**, v. 26, p. 36-40, 1980.

LESAGE, P.; TODESCHINI, A.l. Happy together: the life and times of Ty retrotransposons and their hosts. **Cytogenetic and Genome Research**, v. 110, n. 1-4, p.70-90, 2005

LEVINSON, G.; GUTMAN, G.A. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. **Mol Biol Evol**, v. 4, p. 203-21, 1987.

LINNAEUS, C. **Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis**. 12º ed. Estocolmo, 1766.

LITTMANN, M. W.; BUR, B. M.; BUITRAGO-SUAREZ, U. A. A new cryptic species of Sorubim Cuvier (Siluriformes: Pimelodidae) from the upper and middle Amazon Basin. **Proceedings of The Academy of Natural Sciences of Philadelphia**, Philadelphia, v. 151, n. 1, p. 87-93, 2001.

LI, Y.; KOROL, A.B.; FAHIMA, T.; BEILES, A.; NEVO, E. Microsatellites: genomic distribution, putative functions and mutational mechanisms. **Molecular Ecology**, v. 11, n. 12, p.2453-2465, 2002.

LIU, Z.; LI, P.; KOCABAS, A.; KARSI, A.; JU, Z. Microsatellite-Containing Genes from the Channel Catfish Brain: Evidence of Trinucleotide Repeat Expansion in the Coding Region of Nucleotide Excision Repair Gene RAD23B. **Biochemical And Biophysical Research Communications**, v. 289, n. 2, p.317-324, 2001.

LÓPEZ-FLORES, I.; GARRIDO-RAMOS, M.A. The repetitive DNA content of eukaryotic genomes. **Genome Dyn** v. 7, p. 1-28, 2012.

LUI, R. L.; BLANCO, D. R.; MARGARIDO, V. P.; MOREIRA-FILHO, O. First description of B chromosomes in the family Auchenipteridae, *Parauchenipterus galeatus* (Siluriformes) of the São Francisco River basin (MG, Brazil). **Micron**, v. 40, p. 552-559, 2009.

LUI, R. L.; BLANCO, D. R.; MARGARIDO, V. P.; MOREIRA-FILHO, O. Chromosome characterization and biogeographic relations among three populations of the driftwood catfish *Parauchenipterus galaetus* (Linnaeus, 1766)

(Siluriformes: Auchenipteridae) in Brazil. **Biological Journal of the Linnean Society,** v. 99, p. 648-656, 2010.

LUI, R. L.; BLANCO, D. R.; MOREIRA-FILHO, O; MARGARIDO, V. P. Propidium iodide for making heterochromatin more evident in the C-banding technique. **Biotechnic *&* Histochemistry**, v. 87, p. 433–438, 2012.

LUI, R. L.; BLANCO, D. R.; MARGARIDO, V. P.; TROY, W. P.; MOREIRA-FILHO, O. Comparative chromosomal analysis concerning two species of genus *Tatia* (Siluriformes, Auchenipteridae). **Comparative Cytogenetics**, v. 7, p. 63-71, 2013a.

LUI, R. L.; BLANCO, D. R.; MARTINEZ, J. F.; MARGARIDO, V. P.; VENERE, P. C.; MOREIRA-FILHO, O. The role of chromosomal fusion in the karyotypic evolution of the genus *Ageneiosus* (Siluriformes, Auchenipteridae). **Neotropical Ichthyology**, v. 11, p. 327-334, 2013b.

LUI, R. L.; BLANCO, D. R.; TRALDI, J. B.; MARGARIDO, V. P.; MOREIRA-FILHO, O. Karyotypic variation of *Glanidium ribeiroi* Haseman, 1911 (Siluriformes, Auchenipteridae) along the Iguazu river basin. **Brazilian Journal of Biology**, v. 75, p. 215-221, 2015.

LUI, R.L. **Estudos evolutivos em Auchenipteridae (Siluriformes): citogenética, DNA mitocondrial e DNA satélite**. Tese (Doutorado em Genética Evolutiva e Biologia Molecular) — Programa de Pós-Graduação em Genética Evolutiva e Biologia Molecular, Universidade Federal de São Carlos, São Carlos.

LYNCH, M. **The origins of genome architecture**. Sunderland: Sinauer Associates, 2007. p. 1-494.

LUI, R.L.; TRALDI, J.B.; BLANCO, D.R.; MARGARIDO, V.P., et al. Possible Common Origin of B Chromosomes in Neotropical Fish (Siluriformes, Auchenipteridae) Reinforced by Repetitive DNA Mapping. **Braz Arch Biol Technol** v. 64:e21190494, 2021.

MACHADO, A.S.; KOWALSKI, S.; PAIZ, L.M.; MARGARIDO, V.P., et al. Comparative cytogenetic analysis between species of Auchenipterus and Entomocorus (Siluriformes, Auchenipteridae). **Caryologia**, v. 74, n. 2, p. 89-101, 2021.

MARIÑO-RAMÍREZ, L.; LEWIS, K.C.; LANDSMAN, D.; JORDAN, I.K. Transposable elements donate lineage-specific regulatory sequences to host genomes. **Cytogenetic and Genome Research**. v. 110, n. 1-4, p.333-341, 2005.

MARTINS C; GALETTI PM JR. Chromosomal localization of 5S rDNA genes in Leporinus fish (Anostomidade, Characiformes). **Chromosome Research**, v. 7, p. 363-367, 1999.

MARTINS, C.; MELLO, D.C.C.; VALENTE, G.T.; MAZZUCHELLI, J.M.; OLIVEIRA, S.G. Cytogenetic mapping and its contribution to the knowledge of animal

genomes. In: **Advances in Genetics Research.** Hauppauge: Nova Science Publishers, 2011. p.1-81.

MARTINS, C.; WASKO, A. P. Organization and evolution of 5S ribosomal DNA in the fish genome, in Focus on Genome Research, ed. C. Williams, (New York, NY: Nova Science Publishers), p. 335–363, 2004.

MARTINS, C., Chromosomes and repetitive DNAs: a contribution to the knowledge of fish genome. *In*: **Fish Cytogenetics**. Eds: Pisano E, OzoufCostaz C, Foresti F and Kapoor BG. USA: Science Publisher, Inc., 2007. p.1-80.

MEES, G.F. The Auchenipteridae and Pimelodidae of Suriname (Pisces, Nemat,ognathi). **Zoologische Verhandelingen**, v. 132, p. 1-246, 1974.

MEES, G.F. The Auchenipteridae and Pimelodidae of Suriname (Pisces, Nematognathi). **Zoologische Verhandelingen**, v. 132, p.1-246, 1974.

MESSIER, W.;LI, S.H.; STEWAR, C.B. The birth of microsatellites. **Nature,** v. 6, pp. 381, 1996.

MILHOMEM, S.S.R.; SOUZA, A.C.P., NASCIMENTO, A.L.; CARVALHO, JR.; FELDBERG, E.; PIECZARKA. J.C.; NAGAMACHI, C.Y. Cytogenetic studies in fishes of the genera *Hassar, Platydoras* and *Opsodoras* (Doradidae, Siluriformes) from Jarí and Xingú rivers, Brazil. **Genetics and Molecular Biology,** v. 31: pp. 256-260, 2008.

MOREIRA-FILHO, O.; BERTOLLO, L.A.C. *Astyanax scabripinnis* (Pisces, Characidae): a species complex. **Revista Brasileira de Genética**, v. 14, n. 2, p. 331-357, 1991.

MORRONE, J. J. Biogeographical regionalization of the neotropical region. **Zootaxa**, v. 3782, n. 1, 2014.

MOYLE, P.B.; CHECH, J.J. **Fishes - an introduction to ichthyology**. Upper Saddle River: Prentice Hall. 2. ed., 1988.

NELSON J.S. **Fishes of the World**. New Jersey: John Wiley & Sons, 2006.

NEI, M.; ROONEY, A. P. Concerted and birth-and-death evolution of multigene families. **Annu. Rev. Genet**., v.39, p. 121–152, 2005.

NOVÁK, P.; NEUMANN, P.; MACAS, JIRI. Graph-based clustering and characterization of repetitive sequences in next-generation sequencing data. **BMC Bioinformatics**, v. 11, v. 378, 2010.

NUNES, J.D.; TORRES, G.A.; DAVIDE, L.C.; SALGADO, C.C. Citogenética de *Piper hispidinervum* e *Piper aduncum*. **Pesquisa Agropecuária Brasileira**, v. 42, n. 7, p.1049-1052, 2007.

NIRCHIO, M. MARTINEZ, E.R.M.; FORESTI, F. OLIVEIRA, C. Cytogenetic analysis of three sea catfish species (Teleostei, Siluriformes, Ariidae) with the first

report of Ag-NOR in this fish family. **Genetics and Molecular Biology**, São Paulo, v. 33, n. 2, p. 262-265, 2010.

PAVANELLI, C.S.; BIFI, A.G. A new *Tatia* (Ostariophysi: Siluriformes: Auchenipteridae) from the rio Iguaçu basin, Paraná state, Brazil. **Neotropical Ichthyology** v. 7, p. 199-204, 2009.

PINEAU, P.; HENRY, M.; SUSPENE, R.; MARCHIO, A.; DETTAI, A.; DEBRUYNE, R.; PETIT, T.; LECU, A.; MOISSON, P.; DEJEAN, A.; WAIN-HOBSON, S.; VARTANIAN, J.P. A universal primer set for PCR amplification of nuclear histone H4 genes from all animal species. **Mol Biol Evol**, v.22, p. 582-588, 2005.

PINNA, M.C.C. Phylogenetics relationships of Neotropical Siluriformes: historical overview and synthesis of hypothesis. *In*: **Phylogeny and classification of Neotropical Fishes** (Eds. Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS). Edipucrs: Porto Alegre, 1998, p. 279-330.

PLOHL, M.; LUCHETTI A.; MESTROVIC, N.; MANTOVANI, B. Satellite DNAs between selfishness and functionality: Structure, genomics and evolution of tandem repeats in centromeric (hetero)chromatin. **Gene**, v. 409, n. 1-2, p.72-82, fev. 2008.

RAVEDUTTI, C. G.; JÚLIO JR., H. F. Cytogenetic Analysis of Three Species of the Neotropical Family Auchenipteridae (Pisces, Siluriformes) from the Paraná River Basin, Brazil. **Cytologia**, v. 66, p. 65-70, 2001.

REBORDINOS, L., CROSS, I., AND MERLO, A. High evolutionary dynamism in 5S rDNA of ish: state of the art. **Cytogenet. Genome Res***.* v. 141, p. 103–113, 2013.

REED, K. M. Tc1-Like Transposable Elements in the Genome of Lake Trout (Salvelinus namaycush). **Marine Biotechnology**, v. 1, n. 1, p.60–67, 1999.

RINCÓN, A.C. **Caracterização morfológica, citogenética e molecular de Mazama Americana (Artiodactyla:Cervidae) a partir de um topótipo atual**. 118 f. Dissertação (mestrado) - Programa de Pós-graduação em Genética e Melhoramento animal, Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, São Paulo, 2016.

ROYERO, R.L. **Studies on the systematics and phylogeny of the catfish family Auchenipteridae (Teleostei: Siluriformes).** Tese (Doutorado em Zoologia) - Departamento de zoologia, University of Bristol, Bristol.

SANTOS, D.P.; FELICETTI, D.; BAUMGÄRTNER, L.; MARGARIDO, V.P. Contributions to the taxonomy of Trachelyopterus (Siluriformes): comparative cytogenetic analysis in three species of Auchenipteridae. **Neotropical Ichthyology**, v. 19:e200115, 2021.

SANTOS EO, DEON GA, ALMEIDA RB, OLIVEIRA EA, NOGAROTO V, SILVA HP, PAVANELLI CA, CESTARI MM, BERTOLLO LAC, MOREIRA-FILHO O,

VICARI MR. Cytogenetics and DNA barcode reveal an undescribed *Apareiodon* species (Characiformes: Parodontidae). **Genet Mol Biol,** v. 42, p. 365–373, 2019.

SCOLES, D. R.; GRAVES, J. E. Genetic analysis of the population structure of yellowfin tuna, *Thunnus albacares*, from the Pacific Ocean. **Fisheries Bulletin**, v. 91, p. 690-698, 1993.

SCHEMBERGER, M. O.; NOGAROTO, V.; ALMEIDA, M. C.; ARTONI, R. F.; VALENTE, G. T.; MARTINS, C. MOREIRA-FILHO, O.; CESTARI, M. M.; VICARI, M. R. Sequence analyses and chromosomal distribution of the Tc1/Mariner element in Parodontidae fish (Teleostei: Characiformes). **Gene**, v. 593, p. 308-314, 2016.

SCHWARZACHER, T.; HESLOP-HARRISON, J.S. **Practical in situ Hybridization**. (Oxford, UK: Bios), 2000.

SEBA, A. **Locupletissimi rerum naturalium thesauri accurata descriptio et iconibus artificiosissimis expressio per universam physices historian**. v. 3, Amstelaedami.

SECCO, D.; WANG, C.; SHOU, H.; SCHULTZ, M.D.; CHIARENZA, S.; NUSSAUME, L.; ECKER, J.R.; WHELAN, J.; LISTER, R. Stress induced gene expression drives transient DNA methylation changes at adjacet repetitive elements, **eLife**, v. 4, p. 4, 2015.

SILVA, D.M.Z.A.; USTUNOMIA, R.; PANSONATO-ALVES, J.C.; OLIVEIRA, C.; FOREST, F. Chromosomal Mapping of Repetitive DNA Sequences in Five Species of *Astyanax* (Characiformes, Characidae) Reveals Independent Location of U1 and U2 snRNA Sites and Association of U1 snRNA and 5S rDNA. **Cytogenetic and Genome Research**, v. 146, n. 2, p.144-152, 2015.

SHAPIRO, J. A.; VON STERNBERG, R. Why repetitive DNA is essential to genome function. **Biological Reviews**, v. 80, n. 2, p.227-250, 2005

SLOTKIN, R. K.; MARTIENSSEN, R. Transposable elements and the epigenetic regulation of the genome. **Nature Reviews Genetics**, v. 8, n. 4, p.272-285, 2007.

SMIT, A.F.A.; HUBLEY, R.; GREEN, P. RepeatMasker Open-4.0. 2013-2015. Disponível em: http://www.repeatmasker.org. Acesso em 15 Jan 2020.

SOUZA-SHIBATTA, L.; PEZENTI, L. F.; FERREIRA, D. G.; ALMEIDA F. S.; SOFIA, S. H.; SHIBATTA, O. A. Cryptic species of the genus Pimelodella (Siluriformes: Heptapteridae) from the Miranda River, Paraguay River basin, Pantanal of Mato Grosso do Sul, Central Brazil. **Neotropical Ichthyology**, São Paulo, v. 11, n. 1, p. 101-109, mar./2013.

STACE, C.A. Cytology and cytogenetics as a fundamental taxonomic resource for the 20 th and 21 st centuries. **Taxon**, v. 49, n. 3, p.451-477, 2000.

SUMNER, A.T. **Chromosomes organization and function**. Blackwell, Malden, 2003.

ÚBEDA-MANZANARO, M.; MERLO, M.A.; PALAZÓN, J.L.; CROSS, I.; SARASQUETE, C.; REBORDINOS, L. Chromosomal mapping of the major and minor ribosomal genes, (GATA)n and U2 snRNA gene by double-colour FISH in species of the Batrachoididae family. **Genetica,** v. 138, p. 787-794, 2010.

VALADKHAN, S. snRNAs as the catalysts of pre-mRNA splicing. **Curr Op Chem Biol,** v. 9, p. 603-608, 2005.

VENÂNCIO NETO, S. **Estudo cromossômico em espécies do gênero *boana* (hylidae, anura): diversidade cariotípico e distribuição de DNAS repetitivos**. 2019. 70 f. Tese (Doutorado) - Programa de Pos-graduação em Biologia Evolutiva, Chromosome Biology: Structure & Function Lab, Universidade Estadual de Ponta Grossa, Ponta Grossa, 2019.

VENTURA, K**. Estudos de citogenética e de filogenia molecular em roedores da tribo Akodontini**. 2009. 156 f. Tese (Doutorado) - Programa de Pós-graduação em Ciências, Instituto de Biociências da Universidade de São Paulo. Departamento de Genética e Biologia Evolutiva, São Paulo, 2009.

VICARI, M. R.; ARTONI, R.F.; MOREIRA-FILHO, O.; BERTOLLO, L.A.C. Diversification of a ZZ/ZW sex chromosome system in Characidium fish (Crenuchidae, Characiformes). **Genética**, v. 134, n. 3, p.311-317, 2008.

VOLFF, J.N.; KORTING, K.; SCHARTL, M. Multiple lineages of the non-LTR retrotransposon Rex1 with varying success in invading fish genomes. **Mol Biol Evol**, v.17, p.1673-1684, 2000.

VOLFF, J.-N.; KÖRTING, C.; FROSCHAUER, A.; SWEENEY, K.; SCHARTL, M. Non-LTR Retrotransposons Encoding a Restriction Enzyme-Like Endonuclease in Vertebrates. **Journal of Molecular Evolution**, v. 52, n. 4, p.351-360, 2001.

VOLFF J.N.; KÖRTING, C.; SWEENEY K.; SCHARTL, M. The non-LTR retrotransposon Rex3 from the fish Xiphophorusis widespread among teleosts. **Mol. Biol. Evol**. v. 16, p.1427–1438 , 1999.

WILSON, E. The encyclopedia of life. **Trends in Ecology & Evolution**, Amsterdã, v. 18, n. 2, p. 77-80, 2003.

WOOTTON, R. J. **Ecology of Teleost Fishes**. 2. ed. Nova York: Chapman and Hall Ltd, 1990. p. 1-403.

WOLFFE, A. P. Histone Genes. **Encyclopedia of Genetics**, p. 948–952, 2001.

YUAN Z.; LIU, S.; ZHOU, T.; TIAN, C.; BAO, L.; DUNHAM, R. Comparative genome analysis of 52 fish species suggests differential associations of repetitive elements with their living aquatic environments. **BMC Genomics**, Londres, v. 19, n. 1, 2018.

ZHANG J.; PETERSON T. Transposition of reversed Ac element ends generates chromosome rearrangements in maize. **Genetics**. v.167, p.1929–1937, 2004.

## 5. RESULTADOS

Os resultados do desenvolvimento dessa pesquisa e dissertação resultaram em dois trabalhos independentes, que são apresentados a seguir em dois capítulos: **(Capitulo I)** "*Contributions to Trachelyopterus species diagnosis by cytotaxonomic autapomorphies: from snRNA U2 chromosome polymorphism to rDNA and Histone genes synteny*" e **(Capitulo II)** "*Gene cluster as guide to physical mapping of a widely spread microsatellite: new insights about Trachelyopterus (Siluriformes, Auchenipteridae) diversity*". Cada capítulo foi desenvolvido em língua inglesa e conta com estrutura e normatização independentes, estruturados de acordo com a revista que serão submetidos. Ao longo dos dois capítulos são discutidos aspectos relacionados a evolução cariotípica, distribuição de elementos repetitivos, evolução cromossômica, citotaxonomia e diferenciação de espécies de *Trachelyopterus*.

# CAPITULO I

Chrystian AG Haerter
Roberto Laridondo Lui

## Contributions to *Trachelyopterus* species diagnosis by cytotaxonomic autapomorphies: from snRNA U2 chromosome polymorphism to rDNA and Histone genes synteny

*Artigo em normas de submissão à revista Chromosome Research
Fonte e espaçamento do corpo do texto ajustados para a dissertação.
Imagens e tabelas incluídas ao final do corpo do texto.

**Cascavel**
**2021**

Title: Contributions to *Trachelyopterus* species diagnosis by cytotaxonomic autapomorphies: from snRNA U2 chromosome polymorphism to rDNA and Histone genes synteny

Authors: Chrystian Aparecido Grillo Haerter[1], Roberto Laridondo Lui[1]

Affiliations:
[1]Universidade Estadual do Oeste do Paraná, Centro de Ciências Biológicas e da Saúde, Cascavel, Brazil

Short Title: *Trachelyopterus* diversity through integrative mapping with repetitive elements

*Corresponding Author
Full name: Roberto Laridondo Lui
Department: Centro de Ciências Biológicas e Saúde
Institute/University/Hospital: Universidade Estadual do Oeste do Paraná
Street Name and Number: Rua Universitária, 1619
City, State, Postal code, Country: Cascavel, Paraná, 85819-110, Brasil
Tel:+5545999222055
Fax: (45) 3324-4590
E-mail: roberto.lui@unioeste.br
Chrystian Aparecido Grillo Haerter ORCID ID: 0000-0002-8772-978X
Roberto Laridondo Lui ORCID ID: 0000-0003-4310-4865

**Abstract:** *Trachelyopterus* is a Neotropical catfish genus with a controversial taxonomic history due to the highly similar morphology among the species. Cytogenetically, it is still poorly explored and there is no physical mapping of repetitive elements beyond the rDNAs. Therefore, through an integrative approach with the 18S and 5S rDNA, here we describe for the first time the distribution pattern of U2 snRNA, H3 and H4 histone genes and discuss cytotaxonomic aspects of six *Trachelyopterus* species: *T. striatulus*, *T. galeatus* and *T. porosus* from the Amazonas River basin; *T. coriaceus* and *Trachelyopterus* aff. *galeatus* from the Araguaia River basin; and *Trachelyopterus* aff. *coriaceus* from the Paraguay River basin. The 18S rDNA was found in only one chromosome pair in all species. The 5S rDNA was evidenced in 1-3 chromosome pairs. The H3 and H4 histone genes were found in 1-2 chromosome pairs, syntenic with the 18S rDNA in most species. The U2 snRNA was found in only one chromosome pair in all species, however, *Trachelyopterus* aff. *galeatus* had an inversion polymorphism, which is in Hardy-Weinberg equilibrium and also exhibited two different homologous sizes. Cytotaxonomically, the 5S rDNA was the most efficient marker and revealed autapomorphies of four species. The synteny of H3 and H4 histone genes with the 18S rDNA was used as a single marker and revealed autapomorphies of two species, whereas the U2 snRNA distinguished only one species. As a result, all species could be distinguished, including *Trachelyopterus* aff. *coriaceus* and *Trachelyopterus* aff. *galeatus*, both suggested as new species.

**Keywords:** Auchenipteridae, Chromosomal inversion polymorphism, Cytotaxonomy, Neotropical region, *Parauchenipterus*

## Introduction

There is an extraordinary degree of variation in genome size among eukaryotes (Charlesworth et al. 1994; Gregory 2001). However, repetitive DNA is the major fraction for most organisms (Bostock 1980; Charlesworth et al. 1994; Flynn et al. 2018), which can be repeated hundreds or thousands of times and may represent more than half of the nucleus's total DNA content (Biscotti et al. 2015). It includes tandem repeats (satellites, minisatellites, and microsatellites), multigene families (ribosomal DNA, histone genes and small nuclear RNAs) and interspersed “selfish” repeats, collectively known as transposable elements (TEs) (Charlesworth et al. 1994; Kidwell 2002; Sumner 2003; López-Flores and Garrido-Ramos 2012).

The repetitive nature and high variability rates of repeated sequences compared to single-copy elements generally increase the differences between species and make them a valuable tool to understand evolutionary aspects (Garrido-Ramos 2017; Pereira et al. 2020). In fish, repetitive cytogenetic markers have allowed better characterization of the biodiversity and karyoevolution of the ichthyofauna (Vicari et al. 2010). They can be used to identify karyotype rearrangements and diversity (e.g. Blanco et al. 2017; Santos et al. 2019), the origin and evolution of B chromosomes (e.g. Malimpensa et al. 2018; Coan et al. 2018; Felicetti et al. 2021), heteromorphic sex chromosome systems (e.g. Bertollo et al. 2004; Blanco et al. 2014; Schemberger et al. 2019), non-sexual chromosome polymorphisms (Traldi et al. 2016) and to contribute to taxonomic and phylogenetic conflicts (Blanco et al. 2011; Da Silva et al. 2020). However, most mapping studies in fish have focused on ribosomal genes (Gornung, 2013) and there is still a lack of information about other types of sequence (Utsunomia et al. 2014).

Histone genes are ubiquitous sequences in eukaryote genomes. Their products (H1, H2A, H2B, H3, H4 and the linker histone H1) are highly conserved and organize the DNA into its fundamental unit, the nucleosome (Hentschel and Birnstiel 1981; Eirín-López et al. 2009). Cytogenetically, histone genes sites are usually found clustered and/or colocalized in one or two chromosome pairs (Piscor and Parise-Maltempi 2016), although scattered arrays have also been reported (e.g. Utsunomia et al. 2014; Costa et al. 2014; Traldi et al. 2019). Furthermore, they may also be co-located with ribosomal DNA, as can be seen with the 5S rDNA (e.g Silva et al. 2013; Costa et al. 2014; Piscor and Parise-Maltempi 2016) or the 18S rDNA (e.g. Hashimoto et al. 2013; Costa et al. 2014; Nascimento et al. 2020). In fish, studies with histone genes are still scarce (e.g.

Pendás et al. 1994; Malimpensa et al. 2018; Traldi et al. 2019; Malimpensa et al. 2020) and there is a great lack of knowledge about their organization and distribution patterns (Hashimoto et al. 2013).

Small nuclear RNA sequences (snRNAs) are the main components of the spliceosome machinery. It consists of five types of non-coding RNAs (U1, U2, U4, U5 and U6), directly related to the splicing process of mRNA precursors (Nilsen 2003; Valadkhan 2005; Wahl et al. 2009; Will and Lührmann 2011). In fish, the snRNAs have been poorly explored and are restricted mainly to U1 and U2 (e.g. Merlo et al. 2012; Utsunomia et al. 2014; Usso et al. 2018). Some species have shown a highly conserved distribution pattern of these snRNAs (e.g Ponzio et al. 2018); however, this multigene family can be remarkably variable in both location and number of pairs (e.g. Utsunomia et al. 2014; Pucci et al. 2018) and can be found clustered (e.g. Cabral-de-Mello et al. 2012; Yano et al. 2017; Pucci et al. 2018) or scattered throughout the genome (e.g. Úbeda-Manzanaro et al. 2010; Palacios-Gimenez et al. 2013; Anjos et al. 2015). The colocalization between snRNAs and rDNAs has been reported in different organisms (e.g. Pelliccia et al. 2001; Cross and Rebordinos 2005; Manchado et al. 2006; Vierna et al. 2011), but only a few fish species have exhibited the synteny between U2 snRNA and rDNA (e.g. Manchado et al. 2006; Úbeda-Manzanaro et al. 2010; Merlo et al. 2012). Furthermore, they can also be found in sex chromosomes (e.g. Palacios-Gimenez et al. 2013) and B chromosomes (e.g. Bueno et al. 2013).

Auchenipteridae, known as the driftwood catfish family, is one of the most representative groups of Siluriformes in the neotropics. It is restricted but widely distributed in South America and consists of 125 species divided into 26 genera (Fricke et al. 2021). Among them, with 17 valid species (Fricke et al. 2021), *Trachelyopterus* is known to have highly similar morphology, a great obstacle in identifying characters that can be used to distinguish them. As a result, the genus has a long and controversial taxonomic history and several revisions have been carried out (e.g. Akama, 2004; Calegari et al. 2019). Cytogenetically, *Trachelyopterus* is one of the most studied genera of Auchenipteridae. However, only a few species have been studied and the cytogenetic aspects reflect the morphological similarity, since not many differences among the species have been found so far. Currently, the genus has only been studied using classic cytogenetic techniques (Giemsa, C-Band and Ag-NORs), 18S and 5S rDNA, telomeric probes and the Simple Sequence Repeats SSR - $(GATA)_n$ (e.g. Fenocchio and Bertollo 1992; Ravedutti and Júlio Jr 2001; Fenocchio et al. 2008;

Lui et al. 2010; Araújo and Molina 2013; Lui et al. 2013; Lui et al. 2015, Lui et al. 2021, Santos et al. 2021; Felicetti et al. 2021). Furthermore, as most fish species, there is no information about snRNA or histone genes distribution pattern. Consequently, there are taxonomic problems still unresolved and *Trachelyopterus* species remain weakly differentiated even through cytogenetic analysis.

Considering that the chromosomal markers investigated until then revealed greater similarity than differences, as well as the gap of information for most species, we decided to apply to *Trachelyopterus* species markers not yet described (histone genes H3 and H4 and snRNA U2) integrated with previously performed markers (18S rDNA, 5S rDNA). We intended to test whether the integrative mapping of multiple markers could expose unknown chromosomal arrangements and improve the understanding of the *Trachelyopterus* species diversity through a cytotaxonomic approach.

**Materials and Methods**

Six species of *Trachelyopterus* from different Brazilian basins were cytogenetically analyzed (Tab 01). Four species are formally described and two are suggested as possible new species: *Trachelyopterus* aff. *galeatus* (cited as *Trachelyopterus galeatus* - suggested in Santos et al. 2021) and *Trachelyopterus* aff. *coriaceus* (cited as *Trachelyopterus* sp. n. - suggested in Akama 2004) (SISBio license nº 49379-1). The genomic DNA was extracted from the liver of *Trachelyopterus galeatus* according to the method of Sambrook et al. (2001) and used to generate the U2 snRNA, H3 and H4 histone genes probes.

The 18S rDNA fragments were amplified with the primers NS1 and NS8 (White et al. 1990) directly from a plasmid solution (Hatanaka and Galetti Jr. 2004), labeled with Biotin-16-dUTP (Bio-Nick-Translation, Roche) according to manufacturer's instructions and detected with Antibiotin-Avidin-FITC / Antiavidin-Biotin (Roche). The 5S rDNA fragments were amplified with the primers 5SA and 5SB directly from a plasmid solution (Martins and Galetti Jr. 1999), labeled through PCR in a final volume of 25 µL, containing 200 ng of genomic DNA, 0.2 µM of each primer, 0.16 mM of dNTPs mix (Roche), 1U of Taq DNA polymerase (GoTaq® DNA Polymerase - Promega), 1.5 mM of magnesium chloride, 5x Buffer (Colorless GoTaq® Reaction Buffer - Promega), 0.025 mM of Tetramethyl-Rhodamine-5-dUTP and distilled water. The PCR cycle

conditions for the 5S rDNA were: (i) 95°C (5 minutes); (ii) 30 cycles at 95°C (30 sec), 56°C (45 min) 72°C (2 min); and (iv) final extension cycle at 72°C (7 min).

The histone genes H3 and H4 fragments were amplified from *T. galeatus* genome with the primers H3F and H3R (Colgan et al. 1998); and H4F2s and H4F2er (Pineau et al. 2004). The H3 and H4 histone probes were amplified and labeled through PCR in a final volume of 25 µL, containing 200 ng of DNA, 0.2 µM of each primer, 0.16 mM of dNTPs mix (Roche), 1 U of Taq DNA polymerase (GoTaq® DNA Polymerase - Promega), 2.0 mM of magnesium chloride, 5x Buffer (Colorless GoTaq® Reaction Buffer - Promega), 0.025 µM of Tetramethyl-Rhodamine-5-dUTP (Roche) and distilled water. PCR cycle conditions were: (i) 95°C (5 minutes); (ii) 30 cycles at 95°C (30 sec), 52°C (45 sec), 72°C (1 min 20 sec); and (iii) final extension cycle at 72°C (7 min).

The U2 snRNA fragments were amplified from *T. galeatus* genome with the primers U2F and U2R (Bueno et al. 2013). The U2 snRNA probes were amplified and labeled through PCR in a final volume of 25 µL, containing 200 ng of DNA, 0.2 µM of each primer, 0.16 mM of dNTPs mix (Roche), 1 U of Taq DNA polymerase (GoTaq® DNA Polymerase - Promega), 1.5 mM of magnesium chloride, 5x Buffer (Colorless GoTaq® Reaction Buffer - Promega), 0.025 µM of Fluorescein-12-dUTP (Roche) and distilled water. PCR cycle conditions were: (i) 95°C (5 minutes); (ii) 30 cycles at 95°C (45 sec), 51,7°C (45 sec), 72°C (1 min 20 sec); and (iii) final extension cycle at 72°C (7 min).

The PCR products of H3 and H4 histone genes and U2 snRNA were sequenced in both ways, forward and reverse, using the ABI 3730 DNA Analyzer with the BigDye Terminator v3.1 and Cycle Sequencing Kit (code 4337456). The sequences were analyzed by the Sequencing Analysis software 5.3.1 using the Base Caller KB. The nucleotide composition and identities were confirmed using BLASTn 2.11.0 (National Center for Biotechnology Information) (Altschul et al. 1997) using the consensus sequence generated by the Bioedit Sequence Alignment Editor (Hall 1999).

For the cytogenetic analyses, the specimens were treated with a 0.02% colchicine solution (1 mL/100g of body weight) 30-40 min before the euthanizing process by clove oil overdose (Griffiths 2000a) (according to the ethics committee on animal experimentation and practical classes at Unioeste: 09/13 - CEEAAP / Unioeste). The mitotic chromosomes were obtained from anterior kidney cells (Bertollo et al. 1978). The chromosome morphology was classified according to Levan et al. (1964). Fluorescent *in situ* hybridization (FISH) was carried out according to Pinkel et

al. (1986) with modifications suggested by Margarido and Moreira-Filho (2008). Stringency of 77% was used for all hybridization processes (200 ng of each probe, 50% formamide, 10% dextran sulfate, 2xSSC, pH 7.0 - 7.2, at 37°C overnight). The images were captured by the DP Controller 3.2.1.276 software using an Olympus DP71 digital camera connected to the BX61 epifluorescence microscope (Olympus America Inc., Center Valley, PA, United States of America). The Hardy–Weinberg equilibrium (HWE) and Chi-squared test were performed using the Hardy-Weinberg (HW) testing program (Santos et al. 2020).

## Results

### DNA sequence analysis

The DNA sequencing of the H3 histone gene resulted in a 415-bp consensus sequence, which had approximately 91.35% similarity with the *Pimelodus microstoma* (Siluriformes: Pimelodidae) H3 histone gene (Sequence ID: MT094432.1). The H4 histone gene resulted in a 254-bp consensus sequence, which had 90.09% similarity with the *Pimelodus microstoma* H4 histone gene (MT094433.1). The U2 snRNA resulted in a 197-bp consensus sequence and 97.84 % similarity with the *Parabotia fasciatus* (Cypriniformes: Botiidae) U2 snRNA gene (Sequence ID: MG874999.1).

### Cytogenetic Analysis

*Trachelyopterus porosus* from the Amazonas River basin had 2n=58 chromosomes for both sexes (Fig. 1a). The 18S rDNA sites were found clustered in the terminal position of the short arm of the subtelocentric chromosome pair 23. FISH with 5S rDNA probes revealed sites in the interstitial position of the short arm of the metacentric chromosome pair 3 and terminal position of the short arm of the metacentric chromosome pair 4. The U2 snRNA sites were found in the terminal position of the short arm of the acrocentric chromosome pair 26. FISH with H3 and H4 histone genes probes revealed sites in the terminal position of the short arm of the subtelocentric chromosome pair 23, colocalized with the 18S rDNA, and in the short arm of the subtelocentric chromosome pair 24. There were no differences between

males and females. The B chromosomes of this population, recently described (Felicetti et al. 2021), did not present any of the mapped sequences.

*Trachelyopterus coriaceus* from the Araguaia River basin had 2n=58 chromosomes for both sexes (Fig. 1b). The 18S rDNA sites were found clustered in the terminal position of the short arm of the subtelocentric chromosome pair 23. FISH with 5S rDNA probes revealed sites in the terminal position of the short arm of the metacentric chromosome pair 3 and long arm of the submetacentric chromosome pair 16. FISH with U2 snRNA probes revealed sites in the terminal position of the short arm of the acrocentric chromosome pair 28. Both H3 and H4 histone genes were only found in the terminal position of the short arm of the subtelocentric chromosome pair 23, co-localized with 18S rDNA. No differences between males and females were revealed.

*Trachelyopterus* aff. *coriaceus* (*Trachelyopterus* sp. n. according to Akama 2004) from the Bento Gomes River basin had 2n=58 chromosomes for both sexes (Fig. 1c). The 18S rDNA sites were found clustered in the terminal position of the short arm of the subtelocentric chromosome pair 22. FISH with 5S rDNA probes revealed sites in the interstitial position of the long arm of the submetacentric chromosome pair 16 and terminal position of the short arm of the submetacentric chromosome pair 18. FISH with U2 snRNA probes revealed sites in the terminal position on the short arm of the acrocentric chromosome pair 27. Both H3 and H4 histone genes were found only in the terminal position of the short arm of the subtelocentric chromosome pair 23. No differences between males and females were revealed. The B chromosomes of this population, recently described (Felicetti et al. 2021), did not present any of the mapped sequences.

*Trachelyopterus striatulus* from the Doce River basin had 2n=58 chromosomes for both sexes (Fig. 2a). The 18S rDNA sites were found clustered in the terminal position of the short arm of the subtelocentric chromosome pair 23. FISH with 5S rDNA probes revealed sites in the interstitial position of the long arm of the submetacentric chromosome pairs 10 and 15, and in the interstitial position of the short arm of the submetacentric chromosome pair 13. The U2 snRNA sites were found in the terminal position of the short arm of the acrocentric chromosome pair 28. FISH with H3 and H4 histone genes probes revealed sites in the terminal position of the short arm of the subtelocentric chromosome pair 23, co-localized with 18S rDNA, and in the short arm of the submetacentric chromosome pair 18. No differences between males and females were revealed.

*Trachelyopterus galeatus* from the Amazonas River basin had 2n=58 chromosomes for both sexes (Fig. 2b). The 18S rDNA sites were found clustered in terminal position of the short arm of the subtelocentric chromosome pair 20. FISH with 5S rDNA probes revealed sites in terminal position of the short arm of the submetacentric chromosome pair 14 and long arm of the submetacentric chromosome pair 16. FISH with U2 snRNA probes revealed sites in terminal position of the short arm of the acrocentric chromosome pair 28. Both H3 and H4 histone genes were found in the terminal position of the short arm of the subtelocentric chromosome pair 20, co-localized with 18S rDNA, and in the terminal position of the short arm of the subtelocentric chromosome pair 21. No differences between males and females were revealed. The B chromosomes of this population, recently described (Lui et al. 2021), did not present any of the mapped sequences.

*Trachelyopterus* aff. *galeatus* from the Araguaia River basin had 2n=58 chromosomes for both sexes (Fig. 2c). FISH with 18S rDNA probes revealed clustered sites in the terminal position of the short arm of the subtelocentric chromosome pair 24. The 5S rDNA probes revealed sites in the interstitial position of the long arm of the metacentric chromosome pair 3. Both H3 and H4 histone genes were found in terminal position of the short arm of the subtelocentric chromosome pairs 24 and 25. For this species, the U2 snRNA sites were found in chromosome pair 26. However, it has shown a polymorphism with two different organizations of U2 snRNA in the chromosome: clustered U2 snRNA sites in interstitial position of the short arm of the chromosome (A form) and clustered U2 snRNA sites in interstitial position (proximal) of the long arm of the chromosome (B form). Three combinations of these polymorphic chromosomes were found (Fig. 2b): (AA) both chromosomes with clustered U2 snRNA sites in interstitial position of the short arm; (AB) one chromosome with clustered U2 snRNA sites in interstitial position of the short arm and one chromosome with clustered U2 snRNA sites in interstitial position (proximal) of the long arm; and (BB) both chromosomes with clustered U2 snRNA sites in interstitial position (proximal) of the long arm. The frequency of AA form was 10 individuals (03 males and 07 females), AB form had 05 individuals (02 males and 03 females) and BB form had 01 individual (female). The Hardy–Weinberg equilibrium (HWE) and Chi-squared test value was 0.116 (Df = 2; p=<0.05). Furthermore, the chromosome with U2 snRNA in the short arm presented an acrocentric morphology and a smaller size compared to the chromosome with the U2 snRNA in the long arm, which had a subtelocentric

morphology and a larger size. No differences between males and females were revealed.

## Discussion

The diploid number of 58 chromosomes and small karyotype formula differences were found for all analyzed species and confirmed previously results (Fig. 1, 2). Although divergent results can be seen in *Ageneiosus inermis* with 56 chromosomes (Fenocchio and Bertollo 1992; Lui et al. 2013) and in *Centromochlus heckelii* with 46 chromosomes (Kowalski et al. 2020), the diploid number of 58 chromosomes might be a plesiomorphic trait of Auchenipteridae catfishes (e.g., Lui et al. 2013; Lui et al. 2015; Felicetti 2018; Santos et al. 2021; Lui et al. 2021), as even most species of Doradidae, sister-group of Auchenipteridae (Sullivan et al. 2006; Nelson et al. 2016), have this same diploid number (e.g., Eler et al. 2007; Milhomem et al. 2008; Baumgärtner et al. 2016; Takagui et al. 2017, 2019). Although the presence of previously described B chromosomes (Felicetti et al. 2021; Lui et al. 2021) was notable, none of the markers were found on them.

As well as the diploid number, the 18S rDNA and 5S rDNA distribution pattern confirmed previously described results (Fig. 1, 2; Tab. 2). The 18S rDNA sites were found in terminal position of the short arm of only one chromosome pair, whereas the 5S rDNA sites were found in 1-3 chromosome pairs (Lui et al. 2013; Lui et al. 2015; Felicetti 2018; Santos et al. 2021; Lui et al. 2021). In contrast to some species of the sister-group Doradidae (Baumgärtner et al. 2018; Takagui et al. 2019), there was no synteny between the rDNAs in these species of Auchenipteridae. Although multiple Ag-NORs have already been reported in Auchenipteridae (Kowalski et al. 2021), these *Trachelyopterus* species have a very conservative 18S rDNA distribution pattern, usually with only one chromosomal pair, and small differences in the chromosome morphology and karyotype position, typically associated to non-Robertsonian rearrangements (Lui et al. 2010; Santos et al. 2021) or amplification events. Unlike the 18S rDNA, the 5S rDNA has been shown to be more diverse. Commonly, the 5S rDNA sites are found in two chromosome pairs, as can be seen in *T. galeatus*, *T. coriaceus* (Santos et al. 2021), *Trachelyopterus* aff. *coriaceus* (cited as *Trachelyopterus* sp.) (Lui et al. 2021) and *T. porosus* (Felicetti 2018). Nevertheless, two species with different numbers of 5S rDNA chromosome carriers have been recently reported: *T. striatulus*

with three chromosome pairs and *Trachelyopterus* aff. *galeatus* from Araguaia basin (suggested as possible new species) with only one chromosome pair (Santos et al. 2021).

The 5S rDNA and 18S rDNA patterns have contributed to several studies about diversity, taxonomy and evolution of Auchenipteridae (e.g. Lui et al. 2010; Araújo and Molina 2013; Lui et al. 2013; Lui et al. 2015; Kowalski et al. 2020; Lui et al. 2021; Santos et al. 2021). However, new markers besides the rDNA are essential to improve the capacity to distinguish species, since they were very alike even in the genome composition and classic cytogenetic and ribosomal markers were unable to distinguish all of them. So, the results we discuss henceforth reflect the efficiency of integrative mapping to expose unknown chromosomal arrangements, which are impossible to visualize when classic cytogenetic and usual markers are seen in an isolated perspective.

**Interaction patterns between Histone genes H3, H4 and the 18S rDNA**

Cytogenetically, the histone genes distribution pattern can be widely variable. Most species have been found with simple (e.g. Cabral-de-Mello et al. 2011; Neto et al. 2013) or multiple sites (e.g. Carrilho et al. 2011; Rincão et al. 2020), but dispersal arrays throughout the chromosomes have also been reported (Utsunomia et al. 2014; Traldi et al. 2019). In *Trachelyopterus*, both histones H3 and H4 were found colocalized and clustered in one (*Trachelyopterus* aff. *coriaceus* and *T. coriaceus*) or two chromosome pairs (*T. galeatus* from Amazon basin*, Trachelyopterus* aff. *galeatus* from Araguaia basin*, T. striatulus* and *T. porosus*) with preference for terminal position in the short arm of the chromosomes (Fig. 1, 2). In addition, the combined mapping revealed the existence of synteny between the H3 and H4 histone genes and the 18S rDNA (Fig. 1, 2), except in *Trachelyopterus* aff. *coriaceus*. All species had subtelocentric carriers of the histone genes, except *T. striatulus* (Fig. 2a) that exhibited one submetacentric pair (13) and one subtelocentric pair (23), possibly related to non-Robertsonian rearrangements or amplification events. In Neotropical fish, little is known about the histone genes distribution pattern, but simple/multiple sites are the most recurrent so far (e.g. Pendás et al. 1994; Hashimoto et al. 2011; 2013; Piscor and Parise-Maltempi 2016), which now also includes these *Trachelyopterus* species.

In Neotropical fish, the colocalization of H3 and H4 as well as the occurrence of single locus or a few loci are a recurrent pattern, as can be seen in Pimelodidae (Malimpensa et al. 2018), Parodontidae (Pucci et al. 2018), *Characidium* (Pansonato-Alves et al. 2013a), Loricariidae (Pansonato-Alves et al. 2013b) and *Astyanax* (Silva et al. 2013). On the other hand, the synteny between histone genes and the 18S rDNA are not usual in Neotropical fish. It is commonly found with the 5S rDNA (e.g. Pansonato-Alves et al. 2013b; Silva et al. 2013; Piscor and Parise-Maltempi 2016; Malimpensa et al. 2018) whereas with the 18S rDNA the synteny appear less frequently (e.g. Hashimoto et al. 2013; Nascimento et al. 2020). Furthermore, the colocalization of histone genes and the 18S rDNA in the same chromosome arm found in these *Trachelyopterus* species agree with the hypothesis of clustering of housekeeping genes. When a gene is expressed in most tissues and with high expression rates, it might show strong clustering, which apparently is responsible for increases in the expression rate (Lercher et al. 2002). It was proposed based on humans but was also used to explain colocalization in a closer fish species, *Bergiaria westermanni (*Pimelodidae, Siluriformes) (Malimpensa et al. 2018).

**The role of pericentric inversion into snRNA U2 chromosome polymorphism and size difference**

The *Trachelyopterus* species analyzed presented the U2 snRNA clustered in only one chromosome pair with preference for terminal position of the short arm, which seems to be homeologous chromosomes among all species (Fig. 1, 2). The highly conserved distribution pattern of the U2 snRNA found in *Trachelyopterus* seems not to be an exclusive characteristic of the genus but a commonly feature of fish genomes so far, since most species tend to have a strong conservation in the number of sites and to accumulate exclusively within only one chromosomal pair (e.g. Piscor et al. 2016; Yano et al. 2017; Pucci et al. 2018; Usso et al. 2018). Despite the conserved U2 snRNA pattern of all species, *Trachelyopterus* aff. *galeatus* from the Araguaia River basin presented a non-related to sex chromosome polymorphism (Fig. 2c).

The most parsimonious hypothesis to explain the *Trachelyopterus* aff. *galeatus* U2 snRNA polymorphism origin is related to a pericentric chromosome inversion; followed by chromosome size differentiation owing to accumulation of sequences/amplification and/or duplications (See Hoffman et al. 2004; Hoffmann and

Rieseberg 2008). This type of chromosome polymorphism does not affect the diploid number but can have an impact on the chromosome morphology and gene position (Andolfatto et al. 2001; Hoffman et al. 2004). These two characteristics can be seen in *Trachelyopterus* aff. *galeatus* from the Araguaia River basin: (1) the same diploid number (2n=58) of all other genus species and (2) different chromosome morphology, size and position of the gene.

Chromosomal inversions were first described in *Drosophila melanogaster* (Sturtevant 1917). Since then, they are the topic of many analysis (Corbett-Detig and Hartl 2012) and have long been implicated as drivers of evolutionary change (Hoffmann et al. 2004; Hoffmann and Rieseberg 2008; Kirkpatrick 2010; Kirkpatrick 2017; Wellenreuther and Bernatchez 2018; Kapun and Flatt 2019). Although the natural selection mechanisms that affect the inversion process remain unknown (Hoffmann end Rieseberg 2008; Corbett-Detig and Hartl 2012), many studies reported that it may play different roles in the evolution process (Corbett-Detig and Hartl 2012; Wellenreuther and Bernatchez 2018); including the reallocation of genes along a chromosome (Andolfatto et al. 2001). Small inversions are the most recurrent events, but recent genomic-based studies have shown that large inversions are also frequent and can correspond to a substantial proportion of the entire genome (Wellenreuther and Bernatchez 2018). As example, all inversions found in *D. melanogaster* can represent 43% of the genome (Wellenreuther and Bernatchez 2018). Usually the inversion polymorphisms are related to paracentric inversions, but they can also be a product of pericentric inversions (Krimbas et al. 1992; Hoffmann et al. 2004). They occur when a large inverted region of a chromosome segregates within populations with its non-inverted counterpart (Hoffmann et al. 2004). Therefore, the polymorphism found for *Trachelyopterus* aff. *galeatus* might be a new case of such chromosome rearrangement: a pericentric chromosome inversion polymorphism.

Since the A form can be found in all other *Trachelyopterus* species, the size difference between the homologous appears to be related to the increase of the chromosome form B, implying that it is a derived trait. The sequence accumulation/duplication that possibly determined the size difference between the homologous chromosomes may be explained by two consequences of the chromosome rearrangement: (1) reduced frequency of recombination and crossing-over between homologous and (2) inverted chromosomal loops during the cell reproduction cycle (see Charlesworth and Charlesworth 1973; Griffiths et al. 2000b;

Hoffmann et al. 2004; Kirkpatrick 2010; Torgasheva and Borodin 2010; Kapun and Flatt 2019). These consequences are related mainly to the heterozygote. It can inhibit or cause a (1) dramatic reduction in the frequency of recombination and crossing-over between 'standard' and inverted arrangements (Charlesworth and Charlesworth 1973; Griffiths et al. 2000b; Hoffmann et al. 2004; Kirkpatrick 2010; Kapun and Flatt 2019). As a result, the difference between the chromosomes also tends to increase, including the accumulation of repetitive sequences/duplications that can lead to size differences, as can be seen in *Trachelyopterus* aff. *galeatus.* Furthermore, to minimize the effects of differences between homologous chromosomes, heterozygotes tend to form (2) inverted chromosomal loops during mitosis and meiosis, which may allow the polymorphism to exist in the genome but can also increase the differences between the homologous chromosomes (Griffiths et al. 2000b; Torgasheva and Borodin 2010). Pairing events in pericentric inversions can produce four products: two chromosomes with the standard and inverted gene order and two duplication and/or deletion products, leading to the generation of inviable gametes. The outcome could be the selective recovery of non-cross-over chromosomes leading to viable progeny and consequently maintenance of the polymorphism (reviewed by Wellenreuther and Bernatchez 2018).

When a chromosome inversion polymorphism emerges in a population, it can follow several ways. One of them is the maintenance of the new trait within the population. The maintenance might be a consequence of six main hypothesis: (1) reduced recombination and local selection, (2) reduced recombination and epistatic selection, (3) inversion itself is under selection, (4) inversion is neutral (5) overdominance and (6) underdominance (see Hoffmann and Rieseberg 2008). The U2 snRNA inversion polymorphism of *Trachelyopterus* aff. *galeatus* was found in Hardy-Weinberg (HWE) equilibrium ($\chi 2 = 0.116$; Df = 2; $p=<0.05$) and may be better explained by the (4) neutrality of the rearrangement, as it suggests that there is no change in adaptability between the genotypes in this population and it does not seem to affect the host fitness positively or negatively. The same traits could also be seen in water beetles (Aradottir and Angus 2004) and in blackflies (Kuvangkadilok et al. 2003), in which intraspecific variation in chromosomal rearrangements largely follows Hardy-Weinberg (HW) equilibrium, suggesting that there is no strong selection on the rearrangements. In this state, the inversion polymorphism behaves like a neutral

marker and depends essentially on genetic drift and migration between populations (Hoffmann and Rieseberg 2008).

**Integrative mapping contributions to *Trachelyopterus* taxonomy and diversity**

*Trachelyopterus* is an enigmatic genus due to high morphology similarity and controversial taxonomic history (Akama 2004; Calegari et al. 2019). Most differences identified in previous research (classic cytogenetic, 18S rDNA and 5S rDNA) are mainly related to the chromosome pair number in the karyotype; therefore, in this approach we will not focus on it, since we understand that the karyotypes are assembled by different authors and the high similarity between the species can lead to inconsistencies that make it difficult to use as strong cytotaxonomic features. The aspects we discuss henceforth will consider mainly autapomorphies and synapomorphies with potential to be used as cytotaxonomic traits not only in *Trachelyopterus* but also in other species/genera of Auchenipteridae. The discussion will be focused only on the species of this study because there is no data about these new markers for any other population/species in Auchenipteridae and even for the superfamily Doradoidea.

Cytotaxonomically, the 18S rDNA was the less efficient repetitive element in *Trachelyopterus* due to the presence of only one chromosomal pair carrier with mostly differences in the karyotype position; and the histone genes were able only to distinguish *T. striatulus*, with one submetacentric pair. However, the syntenic interaction with the histone genes (H3 and H4) revealed new autapomorphies that turned both markers into a valuable tool to distinguish these *Trachelyopterus* species. The combination of 18S rDNA and H3/H4 histone genes divided the species into four classes: (1) only one subtelocentric chromosome pair with the 18S rDNA in synteny with the H3 and H4 histone genes (*T. coriaceus*); (2) a subtelocentric chromosome pair with the 18S rDNA and another chromosome pair with the H3 and H4 histones genes (*Trachelyopterus* aff. *coriaceus*); (3) one subtelocentric chromosome pair carrying 18S rDNA in synteny with the H3 and H4 histones genes and a second subtelocentric chromosome pair carrying the H3 and H4 genes histones (*T. porosus*, *Trachelyopterus galeatus* from Amazon River basin and *Trachelyopterus* aff. *galeatus* from Araguaia River basin) and (4) one subtelocentric chromosome pair with the 18S rDNA in synteny with the H3 and H4 histone genes and a second pair of chromosomes with the H3 and

H4 histone genes, but submetacentric (*T. striatulus*) (Tab. 2). *Trachelyopterus coriaceus*, *Trachelyopterus* aff. *coriaceus* and *Trachelyopterus striatulus* could be distinguished with this combination of markers. Curiously, *T. coriaceus* from Araguaia River diverged from *Trachelyopterus* aff. *coriaceus* from Bento River (suggested as a new species), which did not have the same syntenic pattern with the 18S rDNA. Nevertheless, *Trachelyopterus* aff. *galeatus* from the Araguaia River basin, *T. galeatus* from the Amazon River basin and *T. porosus* were still gathered in the same group, demonstrating that only these three markers were still not enough to differentiate all species.

On the other hand, the U2 snRNA has been less efficient and divided the species into only two classes: (1) presence of the chromosome inversion polymorphism (*Trachelyopterus* aff. *galeatus* from Araguaia River basin) or (2) absence of the chromosome inversion polymorphism (*T. coriaceus, T. porosus, T. striatulus*, *Trachelyopterus* aff. *coriaceus* and *T. galeatus* from Amazon River basin) (Tab. 2). However, unlike the 18S rDNA and histone genes, the U2 snRNA was able to separate the *Trachelyopterus* aff. *galeatus* (Araguaia River basin) from *T. galeatus* (Amazonas River basin) and *T. porosus*. Particularly, the presence of U2 snRNA chromosome polymorphism were found only in the *Trachelyopterus* aff. *galeatus* from Araguaia River (suggested as new species) whereas *T. galeatus* from Amazonas River basin had the same pattern as the other species. In this scenario, using the chromosomal physical mapping with 18S rDNA, H3 and H4 histone genes and U2 snRNA, five species would be distinguished. However, they would not be sufficient to distinguish the six species in the sample, since *T. galeatus* (Amazonas River basin) and *Trachelyopterus porosus* would still be gathered in the same group even after the combination of five chromosomal markers through repetitive elements.

In this way, the 5S rDNA was one of the most efficient cytogenetic markers in *Trachelyopterus* species. It was able to distinguish *T. porosus* from *T. galeatus* (Amazon River basin) and divided the species into five classes: (1) one chromosome pair with the sites in the short arm and a second chromosome pair with the sites in the long arm, both submetacentric (*T. galeatus* from Amazonas River basin and *Trachelyopterus* aff. *coriaceus*; (2) one metacentric chromosome pair with the sites in the short arm and a second submetacentric chromosome pair with the sites in the long arm (*T. coriaceus*); (3) two metacentric chromosome pairs with sites in the long arm (*T. porosus*); (4) only one submetacentric chromosome pair with sites in the short arm

(*Trachelyopterus* aff. *galeatus* from Araguaia River basin); and (5) three submetacentric chromosome pairs, two with sites in the short arm and one with sites in the long arm (*T. striatulus*) (Tab. 2). Nevertheless, the 5S rDNA was not able to differentiate *T. galeatus* from Amazonas River basin and *Trachelyopterus* aff. *coriaceus*), but the U2 snRNA and 18S rDNA integrated with histone genes H3 and H4 could do it, reinforcing the importance of all markers in cytotaxonomic diagnosis of these *Trachelyopterus* species. As a final result, all species including *Trachelyopterus* aff. *coriaceus* and *Trachelyopterus* aff. *galeatus* were differentiated by the combination of 18S rDNA, histone genes, U2 snRNA and 5S rDNA.

Thus, since all species were distinguished, the aspects evidenced by the integrative mapping allowed us to infer new insights about *Trachelyopterus* diversity. Among them: (1) reiterate the existence of a possible new species, *Trachelyopterus* aff. *galeatus* from Araguaia River basin, suggested by Santos et al. (2021) based previously on ribosomal marker; (2) corroborate with the hypothesis of synonymy between *Parauchenipterus* and *Trachelyopterus*, since the species of the genus *Trachelyopterus* (see Akama 2004) proved to be more similar to *Parauchenipterus* species than between itself; and that (3) the sample of our study identified by morphology as *Trachelyopterus* aff. *coriaceus* may in fact constitute a new species, which were proposed to be found in the Paraná and Paraguay rivers basins (the population of this study is from Paraguay River basin) (see Akama 2004).

## Conclusions

The mapping of U2 snRNA and H3/H4 histone genes is unprecedented for the superfamily Doradoidea. The integrative mapping data allowed us to distinguish all species in this study, including the suggested new ones, which was not possible until then with ribosomal markers and classic cytogenetic. Furthermore, our data revealed the existence of a unique inversion chromosome polymorphism in Auchenipteridae and reiterated the synonymy of *Parauchenipterus* with *Trachelyopterus*. The same approach also reiterated the existence of a possible new species proposed by Santos et al. (2021), as well as another unpublished species initially proposed by Akama (2004). So, integrative mapping seems to be promising in *Trachelyopterus* and has potential for other Auchenipteridae species. It can contribute significantly to future

taxonomic reviews, studies about evolutionary aspects and genome architecture/organization.

**References**

Akama A (2004) Sistemática dos gêneros *Parauchenipterus* Bleeker, 1862 e *Trachelyopterus* Valenciennes, 1840 (Siluriformes, Auchenipteridae). Dissertation, Universidade de São Paulo

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research 25:3389-3402. https://doi.org/10.1093/nar/25.17.3389

Andolfatto P, Depaulis F, Navarro A (2001) Inversion polymorphisms and nucleotide variability in Drosophila. Genetical Research 77:1-8. https://doi.org/10.1017/S0016672301004955

Anjos A, Rocha GC, Paladini A, Mariguela TC, Cabral-de-Mello DC (2016) Karyotypes and Repetitive DNA Evolution in Six Species of the Genus *Mahanarva* (Auchenorrhyncha: Cercopidae). Cytogenet Genome Res 149:321-327. https://doi.org/10.1159/000450730

Aradottir GI, Angus RB (2004) A chromosomal analysis of some water beetle species recently transferred from Agabus Leach to Ilybius Erichson, with particular reference to the variation in chromosome number shown by *I. montanus Stephens* (Coleoptera: Dytiscidae). Hereditas 140:185-92. https://doi.org/10.1111/j.1601-5223.2004.01837.x

Araújo WC, Molina WF (2013) Citótipo exclusivo para *Parauchenipterus galeatus* (Siluriformes, Auchenipteridae) na Bacia do Atlântico NE Oriental do Brasil: Indicações de um complexo de espécies. Biota Amazônia 3:33-39. https://doi.org/10.18561/2179-5746

Baumgärtner L, Paiz LM, Margarido VP, Portela-Castro ALB (2016) Cytogenetics of the Thorny Catfish *Trachydoras paraguayensis* (Eigenmann & Ward, 1907), Siluriformes, Doradidae): Evidence of pericentric inversions and chromosomal fusion. Cytogenet Genome Res 149:201-206. https://doi.org/10.1159/000448126

Bertollo LAC, Takahashi CS, Moreira-Filho O (1978) Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Rev Bras Genet 1:103-120.

Bertollo LAC, Oliveira C, Molina W, Margarido VP, Fontes MS, Pastori MC, Falcão JN, Fenocchio AS (2004) Chromosome evolution in the erythrinid fish, *Erythrinus erythrinus* (Teleostei: Characiformes). Heredity 93:228-233. https://doi.org/10.1038/sj.hdy.6800511

Biscotti MA, Olmo E, Heslop-Harrison JS ( 2015) Repetitive DNA in eukaryotic genomes. Chromosome Res 23:415–420. https://doi.org/10.1007/s10577-015-9499-z

Blanco DR, Lui RL, Vicari MR, Bertollo LAC, Moreira-Filho O (2011) Comparative Cytogenetics of Giant Trahiras *Hoplias aimara* and *H. intermedius* (Characiformes, Erythrinidae): Chromosomal Characteristics of Minor and Major Ribosomal DNA and Cross-Species Repetitive Centromeric Sequences Mapping Differ among Morphologically Identical Karyotypes. Cytogenet Genome Res 132:71-78. https://doi.org/10.1159/000320923

Blanco DR, Vicari MR, Lui RL, Artoni RF, Almeida MC, Traldi JB, Margarido VP, Moreira- Filho O (2014) Origin of the X1X1X2X2/X1X2Y sex chromosome system of *Harttia punctata* (Siluriformes, Loricariidae) inferred from chromosome painting and FISH with ribosomal DNA markers. Genetica 142:119-26. https://doi.org/10.1007/s10709-014-9759-4

Blanco DR, Vicari MR, Lui RL, Traldi JB, Bueno V, Martinez JF, Moreira Filho O (2017) Karyotype Diversity and Evolutionary Trends in Armored Catfish Species of the Genus *Harttia* (Siluriformes: Loricariidae). Zebrafish 14:169-176. https://doi.org/10.1089/zeb.2016.1377

Bostock C (1980) A function for satellite DNA? Trends Biochem Sci 5:117-119. https://doi.org/10.1016/0968-0004(80)90050-X

Bueno D, Palacios-Gimenez OM, Cabral-de-Mello DC (2013) Chromosomal mapping of repetitive DNAs in the grasshopper *Abracris flavolineata* reveal possible ancestry of the B chromosome and H3 histone spreading. PLoS One 8:e66532. https://doi.org/10.1371/journal.pone.0066532

Cabral-de-Mello DC, Moura RC, Martins C (2011) Cytogenetic Mapping of rRNAs and Histone H3 Genes in 14 Species of *Dichotomius* (Coleoptera, Scarabaeidae, Scarabaeinae) Beetles. Cytogenet Genome Res 134:127-135. https://doi.org/10.1159/000326803

Cabral-de-Mello DC, Valente GT, Nakajima RT, Martins C (2012) Genomic organization and comparative chromosome mapping of the U1 snRNA gene in cichlid fish, with an emphasis in *Oreochromis niloticus*. Chromosome Res 20:279-292. https://doi.org/10.1007/s10577-011-9271-y

Calegari BB, Vari RP, Reis RE (2019) Phylogenetic systematics of the driftwood catfishes (Siluriformes: Auchenipteridae): a combined morphological and molecular analysis. Zool J Linnean Soc 187:661-773. https://doi.org/10.1093/zoolinnean/zlz036

Carrilho J, Pérez-García C, Leitão A, Malheiro I, Pasantes JJ (2011) Cytogenetic characterization and mapping of rDNAs, core histone genes and telomeric sequences in Venerupis aurea and Tapes rhomboides (Bivalvia: Veneridae). Genetica 139:823. https://doi.org/10.1007/s10709-011-9587-8

Charlesworth B, Charlesworth D (1973) Selection of new inversions in multi-locus genetic systems. Genetical Research 21:167-183. https://doi.org/10.1017/S0016672300013343

Charlesworth B, Sniegowski P, Stephan W (1994) The evolutionary dynamics of repetitive DNA in eukaryotes. Nature 371:215-220. https://doi.org/10.1038/371215a0

Coan R, Martins C (2018) Structural and functional analysis of transposable elements focusing on B chromosomes of the cichlid fish *Astatotilapia latifasciata*. Genes 9:269. https://doi.org/10.3390/genes9060269

Colgan D, McLauchlan A, Wilson G, Livingston S, Edgecombe G, Macaranas J, Cassis G, Gray M (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46:419-437. https://doi.org/10.1071/ZO98048

Corbett-Detig RB, Hartl DL (2012) Population Genomics of Inversion Polymorphisms in *Drosophila melanogaster*. PLoS Genetics 8:e1003056. https://doi.org/10.1371/journal.pgen.1003056

Costa GWWF, Cioffi MB, Bertollo LAC, Molina WF (2014) Unusual dispersion of histone repeats on the whole chromosomal complement and their colocalization with ribosomal genes in *Rachycentron canadum* (Rachycentridae, Perciformes). Cytogenet Genome Res 144: 62-67. https://doi.org/10.1159/000366301

Cross I, Rebordinos L (2005) 5S rDNA and U2 snRNA are linked in the genome of *Crassostrea angulata* and *Crassostrea gigas* oysters: does the (CT)n(GA)n microsatellite stabilize this novel linkage of large tandem arrays. Genome 48:1116-1119. https://doi.org/10.1139/g05-075

da Silva MJ, Fogarin Destro R, Gazoni T, Narimatsu H, Pereira dos Santos PS, Haddad CFB, Parise-Maltempi PP (2020) Great Abundance of Satellite DNA in *Proceratophrys* (Anura, Odontophrynidae) Revealed by Genome Sequencing. Cytogenet Genome Res 160:141-147. https://doi.org/10.1159/000506531

Eirín-López JM, González-Romero R, Dryhurst D, Méndez J, Ausió J (2009) 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, 1rd edn. Springer, Berlin, pp 139-162

Eler ES, Dergam JA, Venere PC, Paiva LC, Miranda GA, Oliveira AA (2007) The karyotypes of the thorny catfishes *Wertheimeria maculata* Steindachner, 1877 and *Hassar wilderi* Kindle, 1895 (Siluriformes, Doradidae) and their relevance in doradids chromosomal evolution. Genetica 130: 99-103. https://doi.org/10.1007/s10709-006-0023-4

Felicetti D (2018) Análise genética de populações simpátricas de *Trachelyopterus* Valenciennes, 1840 (auchenipteridae) das bacias Amazônica e Paraguai: contribuições à evolução de cromossomos b e taxonomia do grupo. Dissertation, Universidade Estadual do Oeste do Paraná

Felicetti D, Haerter CAG, Baumgärtner L, Paiz L, M Takagui FH, Margarido VP, Blanco DR, Feldberg E, da Silva M, Lui RL (2021) A New Variant B Chromosome in Auchenipteridae: The Role of (GATA)n and (TTAGGG)n Sequences in Understanding

the Evolution of Supernumeraries in *Trachelyopterus*. Cytogenet Genome Res 161:70-81. https://doi.org/10.1159/000513107

Fenocchio AS, Bertollo LAC (1992) Karyotype, C-bands and NORs of the neotropical siluriform fish *Ageneiosus brevifilis* and *Ageneiosus atronases* (Ageneiosidae). Cytobios 72:19-22.

Fenocchio AS, Dias AL, Margarido VP, Swarça AC (2008) Molecular cytogenetic characterization of *Glanidium ribeiroi* (Siluriformes) endemic to the Iguaçu river, Brazil. Chromosome Science 11:61-66.

Flynn JM, Lower SE, Barbash DA, Clark AG (2018) Rates and Patterns of Mutation in Tandem Repetitive DNA in Six Independent Lineages of *Chlamydomonas reinhardtii*. Genome Biol Evol 10:1673-1686. https://doi.org/10.1093/gbe/evy123

Fricke R, Eschmeyer WN, R van der Laan (eds) 2021 ESCHMEYER'S CATALOG OF FISHES: GENERA, SPECIES. Available in: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. Electronic version accessed in 10 may 2021.

Garrido-Ramos MA (2017) Satellite DNA: an evolving topic. Genes 8:230. https://doi.org/10.3390/genes8090230

Gornung E (2013) Twenty years of physical mapping of major ribosomal RNA genes across the teleosts: A review of research. Cytogenet Genome Res 41:90-102. https://10.1159/000354832

Gregory TR (2001) Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. Biol Rev Camb Philos Soc 76:65-101. https://10.1017/s1464793100005595

Griffiths SP (2000a) The use of clove oil as an anaesthetic and method or sampling intertidal rockpool fishes. J Fish Biol 57:1453-1464. https://doi.org/10.1111/j.1095-8649.2000.tb02224.x

Griffiths AJF, Miller JH, Suzuki DT, Lewontin RC, Gelbart WM (2000b) An Introduction to Genetic Analysis. W. H. Freeman, New York

Hall TA (1999) BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98. https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29

Hashimoto DT, Ferguson-Smith MA, Rens W, Foresti F, Porto-Foresti F (2011) Chromosome mapping of H1 histone and 5S rRNA gene clusters in three species of *Astyanax* (Teleostei, Characiformes). Cytogenet Genome Res 134:64-71. https://doi.org/10.1159/000323512

Hashimoto DT, Ferguson-Smith MA, Rens W, Prado FD, Foresti F, Porto-Foresti F (2013) Cytogenetic mapping of H1 histone and ribosomal RNA genes in hybrids

between catfish species *Pseudoplatystoma corruscans* and *Pseudoplatystoma reticulatum*. Cytogenet Genome Res 139:102-106. https://doi.org/10.1159/000345299

Hatanaka T, Galetti Jr PM (2004) Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). Genetica 122:239-244. https://doi.org/10.1007/s10709-004-2039-y

Hentschel CC, Birnstiel ML (1981) The organization and expression of histone gene families. Cell 25:301-313. https://doi.org/10.1016/0092-8674(81)90048-9

Hoffmann AA, Sgrò CM, Weeks AR (2004) Chromosomal inversion polymorphisms and adaptation. Trends Ecol Evol 19:482-488. https://doi.org/10.1016/j.tree.2004.06.013

Hoffmann AA, Rieseberg LH (2008) Revisiting the impact of inversions in evolution: from population genetic markers to drivers of adaptive shifts and speciation? Annu Rev Ecol Evol Syst 39:21-42. https://doi.org/10.1146/annurev.ecolsys.39.110707.173532

Kapun M, Flatt T (2018) The adaptive significance of chromosomal inversion polymorphisms in *Drosophila melanogaster* . Molecular Ecology 28:1263-1282. https://doi.org/10.1111/mec.14871

Kidwell MG (2002) Transposable elements and the evolution of genome size in eukaryotes. Genetica 115:49-63. https://doi.org/10.1023/A:1016072014259

Kirkpatrick M (2010) How and why chromosome inversions evolve. PLoS Biology 8:e1000501. https://doi.org/10.1371/journal.pbio.1000501

Kirkpatrick M (2017) The Evolution of Genome Structure by Natural and Sexual Selection. Journal of Heredity 108:3-11. https://doi.org/10.1093/jhered/esw041

Kowalski S, Paiz LM, Silva M, Machado AS, Feldberg E, Traldi JB, Margarido VP, Lui RL (2020) Chromosomal analysis of *Centromochlus heckelii* (Siluriformes: Auchenipteridae), with a contribution to *Centromochlus* definition. Neotrop Ichthyol 18:e200009. https://doi.org/10.1590/1982-0224-2020-0009

Krimbas CB, Powell JR (1992) Drosophila inversion polymorphism. CRC Press, Boca Raton

Kuvangkadilok C, Boonkemtong C, Phayuhasena S, Baimai V (2003) Larval polytene chromosomes of black flies (Simulium) from Thailand. I. Comparison among five species in the subgenus *Gomphostilbia Enderlein*. Genetica 118:69-81. https://doi.org/10.1023/A:1022909018987

Lercher MJ, Urrutia AO, Hurst LD (2002) Clustering of housekeeping genes provides a unified model of gene order in the human genome. Nat Genet 31:180-183. https://doi.org/10.1038/ng88

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:201-22. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

López-Flores I, Garrido-Ramos MA (2012) The repetitive DNA content of eukaryotic genomes. Genome Dyn 7:1-28. https://doi.org/10.1159/000337118

Lui RL, Blanco DR, Margarido VP, Moreira-Filho O (2010) Chromosome characterization and biogeographic relations among three populations of the driftwood catfish *Parauchenipterus galeatus* (Linnaeus, 1766) (Siluriformes: Auchenipteridae) in Brazil. Biol J Linn Soc 99: 648-56. https://doi.org/10.1111/j.1095-8312.2009.01389.x

Lui RL, Blanco DR, Martinez JF, Margarido VP, Venere PC, Moreira-Filho O (2013) The role of chromosomal fusion in the karyotypic evolution of the genus *Ageneiosus* (Siluriformes: Auchenipteridae). Neotrop. Ichthyol 11:327-334. https://doi.org/10.1590/S1679-62252013005000004

Lui RL, Blanco DR, Traldi JB, Margarido VP, Moreira-Filho O (2015) Karyotypic variation of *Glanidium ribeiroi* Haseman, 1911 (Siluriformes, Auchenipteridae) along the Iguazu river basin. Braz J Bio 75:215-221. https://doi.org/10.1590/1519-6984.10714

Lui RL, Traldi JB, Blanco DR, Margarido VP, Mariotto S, Centofante L, Artoni RF, Moreira-Filho O (2021) Possible Common Origin of B Chromosomes in 3 Neotropical Fish (Siluriformes, Auchenipteridae) 4 Reinforced by Repetitive DNA Mapping. Braz Arch Biol Technol 64:e21190494. https://doi.org/10.1590/1678-4324-2021190494

Malimpensa GC, Traldi JB, Toyama D, Henrique-Silva F, Vicari MR, Moreira-Filho O (2018) Chromosomal mapping of repeat DNA in *Bergiaria westermanni* (Pimelodidae, Siluriformes): localization of 45S rDNA in B chromosomes. Cytogenet Genome Res 154:99-106. https://doi.org/10.1159/000487652

Malimpensa GC, Traldi JB, Martinez JF, Deon G, Azambuja M, Nogaroto V, Moreira-Filho O (2020) Chromosomal Diversification in Two Species of *Pimelodus* (Siluriformes: Pimelodidae): Comparative Cytogenetic Mapping of Multigene Families. Zebrafish 17:278-286. https://doi.org/10.1089/zeb.2020.1892

Manchado M, Zuasti E, Cross I, Merlo A, Infante C, Rebordinos L (2006) Molecular characterization and chromosomal mapping of the 5S rRNA gene in *Solea senegalensis*: a new linkage to the U1, U2, and U5 small nuclear RNA genes. Genome 49:79-86. https://doi.org/10.1139/g05-068

Margarido VP, Moreira-Filho O (2008) Karyotypic differentiation through chromosome fusion and number reduction in I*mparfinis hollandi* (Ostariophysi, Heptapteridae). Genet Mol Biol 31:235-238. https://doi.org/10.1590/S1415-47572008000200012

Martins C and Galetti Jr PM (1999) Chromosomal localization of 5S rDNA genes in *Leporinus* fish (Anostomidae, Characiformes). Chromosome Res 7:363-367. https://10.1023/a:1009216030316

Merlo MA, Cross I, Palazón JL, Úbeda-Manzanaro M, Sarasquete C, Rebordinos L (2012) Evidence for 5S rDNA horizontal transfer in the toadfish *Halobatrachus didactylus* (Schneider, 1801) based on the analysis of three multigene families. BMC Evol Biol 12:201. https://doi.org/10.1186/1471-2148-12-201

Milhomem SSR, Souza ACP, Nascimento AL, Carvalho Jr. JR, Feldberg E, Pieczarka JC, Nagamachi CY (2008) Cytogenetic studies in fishes of the genera *Hassar*, *Platydoras* and *Opsodoras* (Doradidae, Siluriformes) from Jarí and Xingú rivers, Brazil. Genet Mol Biol 31: 256-60. https://doi.org/10.1590/S1415-47572008000200017

Nascimento CN, Troy WP, Alves JCP, Carvalho ML, Oliveira C, Foresti F (2020) Molecular cytogenetic analyses reveal extensive chromosomal rearrangements and novel B chromosomes in *Moenkhausia* (Teleostei, Characidae). Genet Mol Bio 43:e20200027. https://doi.org/10.1590/1678-4685-gmb-2020-0027

Nelson JS, Grande TC, Wilson MVH. Fishes of the world. Wiley, Washington

Neto MR, de Souza MJ, Loreto V (2013) Chromosomal evolution of rDNA and H3 histone genes in representative Romaleidae grasshoppers from northeast Brazil. Mol Cytogenet 6:41. https://doi.org/10.1186/1755-8166-6-41

Nilsen TW (2003) Thespliceosome:the mostcomplex macromolecular machine in the cell? BioEssays 25:1147-1149. https://doi.org/10.1002/bies.10394

Palacios-Gimenez OM, Castillo ER, Martí DA, Cabral-de-Mello DC (2013) Tracking the evolution of sex chromosome systems in *Melanoplinae* grasshoppers through chromosomal mapping of repetitive DNA sequences. BMC Evol Biol 13:167. https://doi.org/10.1186/1471-2148-13-167

Pansonato-Alves JC, Hilsdorf AWS, Utsunomia R, Silva DMZA, Oliveira C, Foresti F (2013a) Chromosomal mapping of repetitive DNA and cytochrome C oxidase I sequence analysis reveal differentiation among sympatric samples of *Astyanax fasciatus* (Characiformes, Characidae). Cytogenet Genome Res 141:133-142. https://doi.org/10.1159/000354885

Pansonato-Alves JC, Serrano EA, Utsunomia R, Scacchetti PC, Oliveira C, Foresti, F (2013b) Mapping five repetitive DNA classes in sympatric species of *Hypostomus* (Teleostei, Si- luriformes, Loricariidae): analysis of chromosomal vari- ability. Rev Fish Biol Fisher 23:477-489. https://doi.org/10.1007/s11160-013-9303-0

Pelliccia F, Barzotti R, Bucciarelli E, Rocchi A (2001) 5S ribosomal and U1 small nuclear RNA genes: a new linkage type in the genome of a crustacean that has three different tandemly repeated units containing 5S ribosomal DNA sequences. Genome 44:331-335. https://doi.org/10.1139/g01-012

Pendás AM, Morán P, García-Vázquez E (1994) Organization and chromosomal location of the major histone cluster in brown trout, Atlantic salmon and rainbow trout. Chromosoma 103:147152. https://doi.org/10.1007/BF00352324

Pereira JA, Salomão TMF, Lopes DM (2020) Different repetitive DNA sequences make up heterochromatin in Meliponini. Apidologie 51:855-860. https://doi.org/10.1007/s13592-020-00766-1

Pineau P. Henry M. Suspène R, Marchio A. Dettai A. Debruyne R. Petit T. Lécu A. Moisson P. Dejean A. Wain-Hobson S. Vartanian JP (2004) A universal primer set for PCR amplification of nuclear histone H4 genes from all animal species. Mol Biol Evol 22:582-588. https://doi.org/10.1093/molbev/msi053

Pinkel D, Straume T, Gray J (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83:2934-2938. https://doi.org/10.1073/pnas.83.9.2934

Piscor D, Parise-Maltempi PP (2016) Chromosomal mapping of H3 histone and 5S rRNA genes in eight species of *Astyanax* (Pisces, Characiformes) with different diploid numbers: syntenic conservation of repetitive genes. Genome 59:167-72. https://doi.org/10.1139/gen-2015-0112

Ponzio JC, Piscor D, Parise-Maltempi PP (2018) Chromosomal locations of U2 snDNA clusters in *Megaleporinus*, *Leporinus* and *Schizodon* (Characiformes: Anostomidae). Biologia 73:295-298. https://doi.org/10.2478/s11756-018-0031-8

Pucci MB, Nogaroto V, Moreira-Filho O, Vicari MR (2018) Dispersion of transposable elements and multigene families: Microstructural variation in *Characidium* (Characiformes: Crenuchidae) genomes. Genet Mol Biol 41:585-592. https://doi.org/10.1590/1678-4685-gmb-2017-0121

Ravedutti CG, Júlio Jr HF (2001) Cytogenetic analysis of three species of the neotropical family Auchenipteridae (Pisces, Siluriformes) from the Paraná river basin, Brazil. Cytologia 66: 65-70. https://doi.org/10.1508/cytologia.66.65

Rincão MP, Brescovit AD, Dias AL (2020) Insights on repetitive DNA behavior in two species of Ctenus Walckenaer, 1805 and Guasuctenus Polotow and Brescovit, 2019 (Araneae, Ctenidae): Evolutionary profile of H3 histone, 18S rRNA genes and heterochromatin distribution. PLOS ONE 15:e0231324. https://doi.org/10.1371/journal.pone.0231324

Sambrook JF, Russell DW. Molecular Cloning: A Laboratory Manual. Laboratory Press, New York

Santos FAB, Lemes RB, Otto PA (2021) HW_TEST, a program for comprehensive HARDY-WEINBERG equilibrium testing. Genet. Mol Biol 43:02. https://doi.org/10.1590/1678-4685-GMB-2019-0380

Santos EO, Deon GA, Almeida RB, Oliveira EA, Nogaroto V, Silva HP, Pavanelli CA, Cestari MM, Bertollo LAC, Moreira-Filho O, Vicari MR (2019) Cytogenetics and DNA barcode reveal an undescribed *Apareiodon* species (Characiformes: Parodontidae). Genet Mol Biol 42:365–373. https://doi.org/10.1590/1678-4685-gmb-2018-0066

Santos DP, Felicetti D, Baumgärtner L, Margarido VP, Blanco DR, Moreira-Filho O, Lui RL (2021) Contributions to the taxonomy of *Trachelyopterus* (Siluriformes): comparative cytogenetic analysis in three species of Auchenipteridae. Neotropical Ichthyology 19:e200115. https://doi.org/10.1590/1982-0224-2020-0115

Schemberger MO, Nascimento VD, Coan R, Ramos E, Nogaroto V, Ziemniczak K, Valente GT, Moreira-Filho O, Martins C, Vicari MR (2019) DNA transposon invasion and microsatellite accumulation guide W chromosome differentiation in a Neotropical fish genome. Chromosoma 128:547-560. https://doi.org/10.1007/s00412-019-00721-9

Silva DMZA, Pansonato-Alves JC, Utsunomia R, Daniel SN, Hashimoto DT, Oliveira C, Porto-Foresti F, Foresti F (2013) Chromosomal organization of repetitive DNA sequences in *Astyanax bockmanni* (Teleostei, Characiformes): dispersive location, association and co-localization in the genome. Genetica 141:329-36. https://doi.org/10.1007/s10709-013-9732-7

Sturtevant AH (1917) Genetic factors affecting the strength of linkage in Drosophila. Proc Natl Acad Sci USA 3:555-558. https://doi.org/10.1073/pnas.3.9.555

Sullivan JP, Lundberg JG, Hardman M (2006) A phylogenetic analysis of the major groups of catfishes (Teleostei: Siluriformes) using rag1 and rag2 nuclear gene sequences. Mol Phylogenet Evol 41:636-662. https://doi.org/10.1016/j.ympev.2006.05.044

Sumner AT (2003) Chromosomes organization and function. Blackwell, Malden

Takagui FH, Dias AL, Birindelli JLO, Swarca AC, Rosa R, Lui RL, Fenocchio AS, Giuliano-Caetano L (2017) First report of B chromosomes in three neotropical thorny catfishes (Siluriformes, Doradidae). Comp Cytogenet 11:55-64. https://doi.org/10.3897/CompCytogen.v11i1.10496

Takagui FH, Baumgärtner L, Baldissera JN, Laridondo Lui R, Margarido VP, Fonteles SBA, Garcia C, Birindelli JO, Moreira-Filho O, Almeida FS, Giuliano-Caetano L (2019) Chromosomal Diversity of Thorny Catfishes (Siluriformes-Doradidae): A Case of Allopatric Speciation Among Wertheimerinae Species of São Francisco and Brazilian Eastern Coastal Drainages. Zebrafish 16:477-485. https://doi.org/10.1089/zeb.2019.1769

Torgasheva AA, Borodin PM (2010) Synapsis and recombination in inversion heterozygotes. Biochem Soc Trans 38:1676-1680. https://doi.org/10.1042/BST0381676

Traldi JB, Vicari MR, Martinez JDF, Blanco DR, Lui RL, Moreira-Filho O (2016) Chromosome analyses of *Apareiodon argenteus* and *Apareiodon davisi* (Characiformes, Parodontidae): an extensive chromosomal polymorphism of 45S and 5S ribosomal DNAs. Zebrafish 13:19-25. https://doi.org/10.1089/zeb.2015.1124

Traldi JB, Ziemniczak K, de Fátima Martinez J, Blanco DR, Lui RL, Schemberger MO, Nogaroto V, Moreira-Filho O, Vicari MR (2019) Chromosome Mapping of H1 and H4 Histones in *Parodontidae* (Actinopterygii: Characiformes): Dispersed and/or Co-Opted

Transposable Elements? Cytogenet Genome Res 158:106-113. https://doi.org/10.1159/000500987

Úbeda-Manzanaro M, Merlo MA, Palazón JL, Cross I, Sarasquete C, Rebordinos L (2010) Chromosomal mapping of the major and minor ribosomal genes, (GATA)n and U2 snRNA gene by double-colour FISH in species of the Batrachoididae family. Genetica 138:787-794. https://doi.org/10.1007/s10709-010-9460-1

Usso MC, Santos ARD, Gouveia JG, Frantine-Silva W, Araya-Jaime C, Oliveira MLM, Foresti F, Giuliano-Caetano L, Dias AL (2018) Genetic and Chromosomal Differentiation of *Rhamdia quelen* (Siluriformes, Heptapteridae) Revealed by Repetitive Molecular Markers and DNA Barcoding. Zebrafish 16:87-97. https://doi.org/10.1089/zeb.2018.1576

Utsunomia R, Pansonato-Alves JC, Scacchetti PC, Oliveira C, Foresti F (2014) Scattered organization of the histone multigene family and transposable elements in *Synbranchus*. Genet Mol Biol 37(1):30-36. http://dx.doi.org/10.1590/S1415-47572014000100007

Valadkhan S (2005) snRNAs as the catalysts of pre-mRNA splicing. Curr Op Chem Biol 9:603-608. https://doi.org/10.1016/j.cbpa.2005.10.008

Vicari MR, Nogaroto V, Noleto RB, Cestari MM, Cioffi MB, Almeida MC, Moreira-Filho O, Artoni RF (2010) Satellite DNA and chromosomes in Neotropical fishes: Methods, applications and perspectives. J Fish Biol 76:1094-1116. https://doi.org/10.1111/j.1095-8649.2010.02564.x

Vierna J, Jensen KT, Martínez-Lage A, GonzálezTizón AM (2011) The linked units of 5S rDNA and U1 snDNA of razor shells (Mollusca: Bivalvia: Pharidae). Heredity 107:127-142. https://doi.org/10.1038/hdy.2010.174

Yano CF, Bertollo LAC, Rebordinos L, Merlo MA, Liehr T, Portela-Bens S, Cioffi MB (2017) Evolutionary dynamics of rDNAs and U2 small nuclear DNAs in *Triportheus* (Characiformes, Triportheidae): high variability and particular syntenic organization. Zebrafish 14:146-154. https://doi.org/10.1089/zeb.2016.1351

Wahl MC, Will CL, Lührmann R (2009) The spliceosome: design principles of a dynamic RNP machine. Cell 136:701-718. https://doi.org/10.1016/j.cell.2009.02.009

Wellenreuther M, Bernatchez L. Eco-Evolutionary (2018) Genomics of Chromosomal Inversions. Trends Ecol Evol 33:427-440. https://10.1016/j.tree.2018.04.002

Will CL, Lührmann R (2011) Spliceosome structure and function. Cold Spring Harb Perspect Biol 3:a003707. https://doi.org10.1101/cshperspect.a003707

White T, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J. (Eds) PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

## Figures

**Fig. 1** Karyotypes of *Trachelyopterus porosus* (a), *T. galeatus coriaceus* (b) and *Trachelyopterus* aff. *coriaceus* (c). m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric. Probes: 5S rDNA and snDNA U2 (green signal); 18S rDNA and Histone genes H3/H4 (red signal). Bar=5 µm

**Fig. 2** Karyotypes of *T. striatulus* (a), *T. galeatus* (b) and *Trachelyopterus* aff. *galeatus* (c). m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric. Probes: 5S rDNA and snRNA U2 (green signal); and 18S rDNA and Histone genes H3/H4 (red signal) µm. Bar=5 µm

## Tables

**Tab. 1** *Trachelyopterus* species and number of individuals hybridized with 18S rDNA, 5S rDNA, snRNA U2, Histones H3 and H4. PERD – Parque Estadual do rio Doce; AM: Amazonas GO – Goiás; MT – Mato Grosso; MG – Minas Gerais; GPS: Global Positioning System; HB – Hydrographic Basin; A – Araguaia; PA – Paraná; PG – Paraguai; SF – São Francisco; C – Costeira; INPA: Instituto Nacional de Pesquisas da Amazônia; MZUSP – Museu de Zoologia da Universidade de São Paulo; NUP - Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura da Universidade Estadual de Maringá

**Tab. 2** Cytogenetic data available for *Trachelyopterus* species. FN: fundamental number; 2n: diploid number; Ref.: References; m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric; p: short arm; q: long arm; GO: Góias state; AM: Amazonas state; PR: Paraná state; MS: Mato Grosso do Sul state; RN: Rio Grande do Norte state; PY: Paraguay. References: 1. Santos et al. (2021); 2. Felicetti et al. (2018); 3. Ravedutti and Júlio Jr (2001); 4. Lui et al. (2010); 5. Araújo and Molina (2013); 6. Lui et al. (2021); 7. Present study

## Declarations

## Funding

The research leading to these results received funding from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná (FA) and Postgraduate Program in Conservação e Manejo de Recursos Naturais (Unioeste-PPRN)

## Conflicts of interest/Competing interests

The authors have no conflicts of interest/competing interest to declare

## Availability of data and material

The data that support the findings of this study are available from the corresponding author, RLL, upon reasonable request. It can also be found partially within the article and specific public repository

**Code availability**

Not applicable

**Authors' contributions**

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by CAGH and RLL. The first draft of the manuscript was written by CAGH and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

**Ethics approval**

Fish collections were authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, Permit number 49379-1), and the experimental procedures were approved by the Ethics Committee on Animal Experimentation and Practical Classes at Unioeste (09/13-CEEAAP/Unioeste)

**Consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Acknowledgments**

We thank to Dr. Heraldo Antonio Britski and Dr. José Luís Olivan Birindelli for the identification of the specimens; Pedro Luis Gallo and Luis Henrique da Silva for their assistance in sampling; to Chico Mendes Institute for Biodiversity Conservation (Permit number 10538-1) for authorizing the collection of the specimens; to Minas Gerais State Forestry Institute for assisting the specimens collections (Rio Doce State Park); Sr. Osmar Pereira de Barros, owner of the Ana Maria farm in São Miguel do Araguaia, who helped us collect the fish in this region. We also thank to Mestrado em Conservação e Manejo de Recursos Naturais (PPRN-Unioeste), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná (FA) for supporting this study.

**Tab 1.** *Trachelyopterus* species and number of individuals hybridized with 18S rDNA, 5S rDNA, snRNA U2, Histones H3 and H4. *PERD – Parque Estadual do rio Doce; AM: Amazonas GO – Goiás; MT – Mato Grosso; MG – Minas Gerais; GPS: Global Positioning System; INPA: Instituto Nacional de Pesquisas da Amazônia; MZUSP – Museu de Zoologia da Universidade de São Paulo; NUP - Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura da Universidade Estadual de Maringá.

| Species | Locality | Municipality/ State | GPS | Sex | | Voucher |
|---|---|---|---|---|---|---|
| | | | | ♂ | ♀ | |
| *Trachelyopterus porosus* (Eigenmann and Eigenmann, 1888) | Catalão lake (Amazonas River basin, Brazil) | Manaus - AM | 03° 09' 47" S 59° 54' 29" W | 4 | 4 | INPA 57940 |
| *Trachelyopterus coriaceus* (Valenciennes, 1840) | Lagoon near Córrego do Medo, Araguaia River tributary (Araguaia River basin, Brazil) | São Miguel do Araguaia - GO | 13º 08’ 52,7” S 50º 25’ 02,8” W | 4 | 3 | MZUSP 106766 |
| *Trachelyopterus* aff. *coriaceus* (Eigenmann and Eigenmann, 1888) | Arrombado lagoon, Bento Gomes River basin, Cuiabá River tributary (Paraguay River basin, Brazil) | Poconé - MT | 16º 25’ 40,9” S 56º 25’ 07,4” W | 2 | 1 | MZUSP 110806 |
| *Trachelyopterus striatulus* (Steindachner, 1877) | Verde lagoon (PERD*) (Doce River basin, Brazil) | Marliéria - MG | 19º 49’ 44,5” S 42º 37’ 52.5” W | 3 | 3 | MZUSP 109798 |
| *Trachelyopterus galeatus* (Linnaeus, 1766) | Catalão lake (Amazonas River basin, Brazil) | Manaus - AM | 03° 09' 47" S 59° 54' 29" W | 6 | 8 | INPA 57939 |
| *Trachelyopterus* aff. *galeatus* (Linnaeus, 1766) | Lagoon near Córrego do Medo, Araguaia River tributary (Araguaia River basin, Brazil) | São Miguel do Araguaia - GO | 13º 08’ 52,7” S 50º 25’ 02,8” W | 9 | 10 | MZUSP 110803 |

**Tab2.** Cytogenetic data available for *Trachelyopterus* species. FN: fundamental number; 2n: diploid number; Ref.: References; m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric; p: short arm; q: long arm; GO: Góias state from Brazil; AM: Amazonas state from Brazil; PR: Paraná state from Brazil; MS: Mato Grosso do Sul state from Brazil; RN: Rio Grande do Norte state from Brazil; PY: Paraguay. References: 1. Santos et al. (2021); 2. Felicetti et al. (2018); 3. Ravedutti and Júlio Jr (2001); 4. Lui et al. (2010); 5. Araújo and Molina (2013); 6. Lui et al. (2021); 7. Present study.

| Species/populations | Locality | FN | 2n | Karyotype Formula | AgNORs/18S rDNA | 5S rDNA | Histone H3 | Histone H4 | snRNA U2 | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Trachelyopterus coriaceus*** | Araguaia River, Araguaia-Tocantins river basin - GO | 108 | 58 | 20m+18sm+12st+8a | pair 23, p, st | pair 3, p, m / pair 16, q, sm | pair 23, p, st | pair 23, p, st | pair 28, p ,a | 1, 7 |
| ***Trachelyopterus* aff. *galeatus*** | Araguaia River, Araguaia-Tocantins river basin - GO | 108 | 58 | 20m+18sm+12st+8a | pair 24, p, st | pair 3, p, m | pair 24, p, st/ pair 25, p, st | pair 24, p, st/ pair 25, p, st | pair 26, q/p, a | 2, 7 |
| ***Trachelyopterus galeatus*** | Catalão Lake, Amazonas River basin - AM | 106 | 58 | 20m+12sm+18st+8a | pair 20, p, st | pair 14, p, sm/ pair 17, q, sm | pair 20, p, st/ pair 21, p, st | pair 20, p, st/ pair 21, p, st | pair 28, p, a | 2, 7 |
| | Miranda River, Paraguay River basin - PY | 108 | 58 | 24m+12sm+14st+8a | pair 24, p, st | pair 14, p, sm/ pair 17, q, sm | - | - | - | 2 |
| | Paraná River, Paraná River basin - PR | 98 | 58 | 22m+12sm+6st+18a | pair 23, p, a | - | - | - | - | 3 |
| | Paraná River, Paraná River basin - MS | 108 | 58 | 24m+18sm+8st+8a | pair 25, p, st | pair 16, p, sm / pair 17, q, sm | - | - | - | 4 |
| | Piumhi River, Paraná River basin - MG | 108 | 58 | 20m+16sm+14st+8a | pair 24, p, st | pair 15, p, sm / pair 16, q, sm | - | - | - | 4 |
| | Lagoa da Prata – São Francisco River basin - MG | 108 | 58 | 22m+16sm+12st+8a | pair 23, p, st | pair 16, p, sm / pair 17, q, sm | - | - | - | 4 |
| | Pium River, Parnamirim - RN | 108 | 58 | 24m+16sm+10st+8a | p, sm | - | - | - | - | 5 |
| ***Trachelyopterus porosus*** | Catalão Lake, Amazonas River basin - AM | 106 | 58 | 22m+16sm+10st+10a | pair 23, p, st | pair 3, p, m/ pair 4, p, m | pair 23, p, st/ pair 24, p, st | pair 23, p, st/ pair 24, p, st | pair 26, p, a | 2,7 |
| | Miranda River, Paraguay River basin - PY | 106 | 58 | 22m+16sm+10st+10a | pair 23, p, st | pair 3, p, m/ pair 4, p, m | - | - | - | 2 |
| ***Trachelyopterus* aff. *coriaceus*** | Arrombado lagoon, Bento Gomes River basin- MT | 108 | 58 | 22m+20sm+8st+8a | pair 22, p, st | pair 16, p, sm/ pair 18, q, sm | pair 23, p, st | pair 23, p, st | pair 27, p, a | 6, 7 |
| ***Trachelyopterus striatulus*** | Verde lagoon, Doce River basin - MG | 106 | 58 | 18m+20sm+10st+10a | par 23, p, st | pair 10, p, sm/ pair 13, p, sm/ pair 15, q, sm | pair 18, p, sm/ pair 23, p, st | pair 18, p, sm/ pair 23, p, st | pair 28, p, a | 6, 7 |

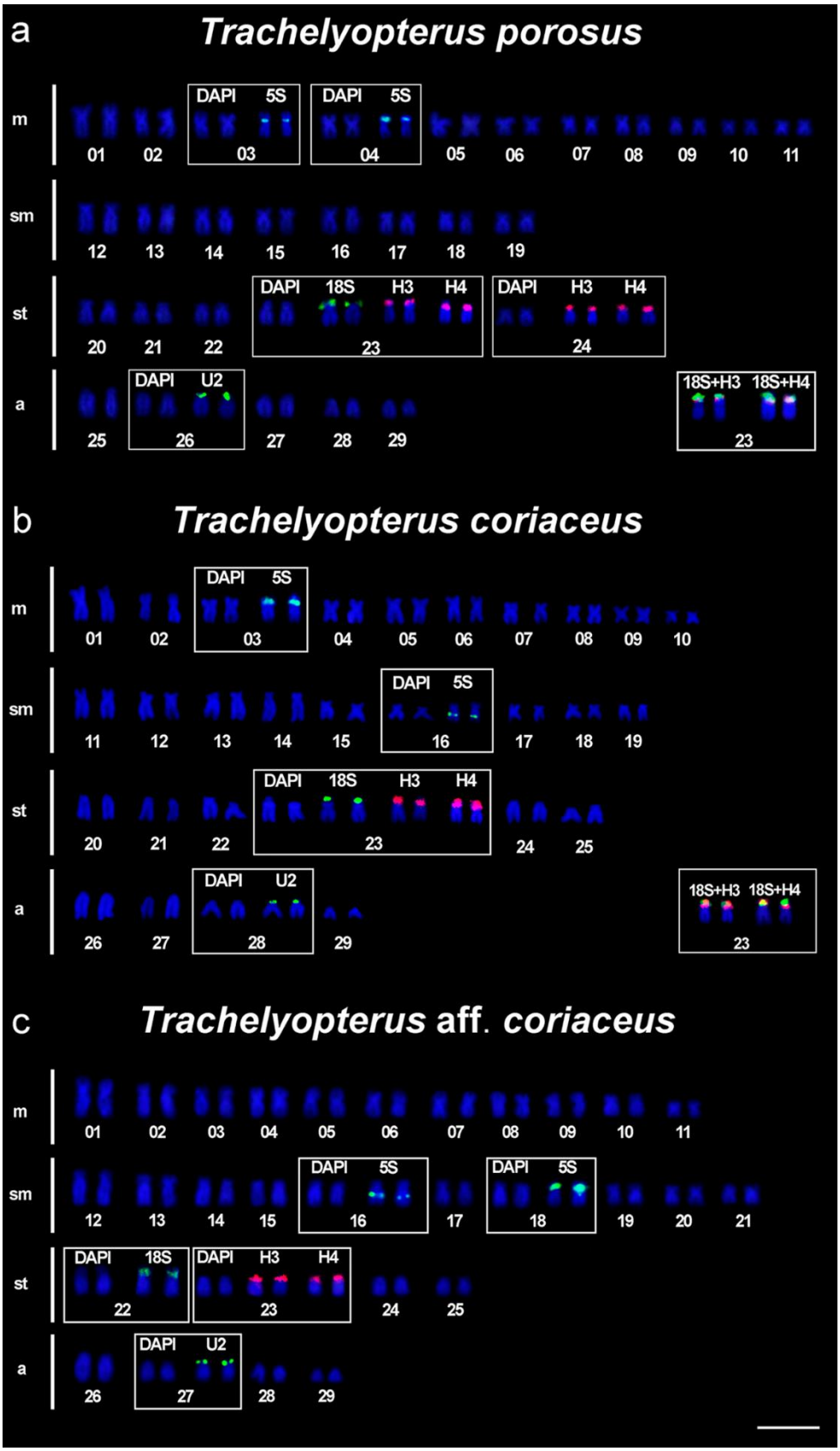



**Fig 1.** Karyotypes of *T. porosus* (a), *T. coriaceus* (b) and *Trachelyopterus* aff. *coriaceus* (c). m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric. Probes: 5S rDNA, U2 snRNA and 18S rDNA with green signal and histone genes H3/H4 with red signal. Bar = 5 µm.

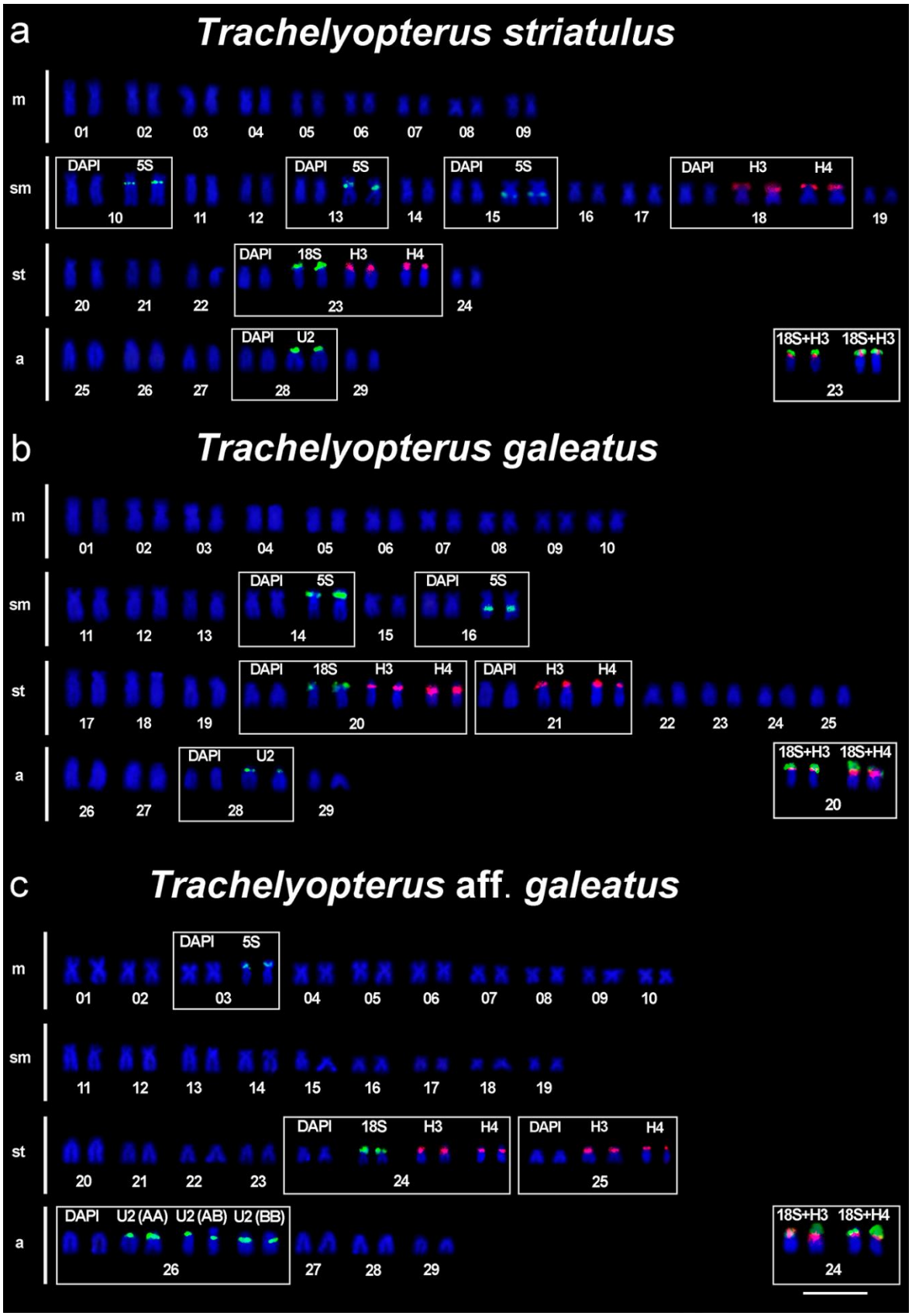



**Fig 2.** Karyotypes of *T. striatulus* (a), *T. galeatus* (b) and *Trachelyopterus* aff. *galeatus* (c). m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric. Probes: 5S rDNA, U2 snDNA and 18S rDNA with green signal and histone genes H3/H4 with red signal. Bar = 5 µm.

# CAPITULO II

Chrystian AG Haerter
Roberto Laridondo Lui

## Gene cluster as guide to physical mapping of a widely spread microsatellite: new insights about *Trachelyopterus* (Siluriformes, Auchenipteridae) diversity

*Artigo em normas de submissão à revista Comparative Cytogenetic
Fonte e espaçamento do corpo do texto ajustados para a dissertação.
Imagens e tabelas incluídas ao final do corpo do texto.

**Cascavel**
**2021**

Title: Gene cluster as guide to physical mapping of a widely spread microsatellite: new insights about *Trachelyopterus* (Siluriformes, Auchenipteridae) diversity

**Authors**:

[1]Chrystian Aparecido Grillo Haerter ORCID ID: 0000-0002-8772-978X

[1]Roberto Laridondo Lui* ORCID ID: 0000-0003-4310-4865

**Affiliations**:

[1]Universidade Estadual do Oeste do Paraná, Centro de Ciências Biológicas e da Saúde, Cascavel, Brazil

*Corresponding Author

Full name: Roberto Laridondo Lui

Department: Centro de Ciências Biológicas e Saúde

Institute/University/Hospital: Universidade Estadual do Oeste do Paraná

Street Name and Number: Rua Universitária, 1619

City, State, Postal code, Country: Cascavel, Paraná, 85819-110, Brasil

Tel:+5545999222055

Fax: (45) 3324-4590

E-mail: roberto.lui@unioeste.br

**Abstract**

The scattered distribution pattern of microsatellites is a challenging problem in fish cytogenetics. It can hardly be used as a complement A chromosomal marker due to the apparently random distribution. However, several studies in the past few decades have shown that the distribution pattern of microsatellites is non-random. Thus, here we tested whether a scattered microsatellite could have distinct distribution patterns on homeologous chromosomes of different species. The clustered sites of 18S and 5S rDNA, U2 snRNA and H3/H4 histone genes were used as a guide to compare the $(GATA)_n$ microsatellite distribution pattern on the homeologous chromosomes of six *Trachelyopterus* species: *T. coriaceus* and *Trachelyopterus* aff. *galeatus* from the Araguaia River basin*; T. striatulus*, *T. galeatus* and *T. porosus* from the Amazonas River basin; and *Trachelyopterus* aff. *coriaceus* from the Paraguay River basin. Most species had a similar distribution pattern of the $(GATA)_n$ sequence in the 18S rDNA carriers, with two conspicuous blocks only in the long arm and none in the short arm. However, *Trachelyopterus galeatus* presented a chromosomal polymorphism, which is in Hard-Weinberg equilibrium and possibly originated through amplification events. The $(GATA)_n$ chromosomal mapping on the 5S rDNA and H3/H4 histone gene carriers did not reveal new information about the structure of these chromosomal pairs, but using the $(GATA)_n$ distribution pattern, the possible chromosome homeologies between the species could be inferred, and through the combined mapping with the histone genes the chromosomal polymorphism of *T. galeatus* from the Amazon River basin could be confirmed. Similar to in the 18S rDNA carriers, most species had a conserved $(GATA)_n$ distribution pattern in the U2 snRNA chromosomes, with only two conspicuous blocks in the long arm. However, *Trachelyopterus* aff. *galeatus* presented a chromosome polymorphism, which combined with an inversion polymorphism of the U2 snRNA in the same chromosome pair resulted in six possible cytotypes, which are in Hardy-Weinberg disequilibrium. Thus, the guided mapping revealed new chromosomal arrangements even in populations that were already mapped with the $(GATA)_n$ sequence, showing potential to be used with other widely spread microsatellites, clustered gene sites and species.

**Keywords:** Histone genes, 18S rDNA, 5S rDNA, U2, SSR $(GATA)_n$.

## Introduction

Microsatellites, also known as short tandem repeats (STRs, Edwards et al. 1991) or simple sequence repeats (SSRs, Jacob et al. 1991), are stretches of DNA that consist of tandemly repeating mono-, di-, tri-, tetra- or penta-nucleotide motifs arranged through eukaryotic genomes (Powell et al. 1996; Ellegren 2004; Kalia et al. 2011) in both coding and noncoding regions (Toth et al., 2000; Kalia et al. 2011). The majority of the microsatellites are located in the nucleus (nuSSR), although they can also be found in mitochondria (mtSSR) and chloroplasts (cpSSR) (Kalia et al. 2011). They are one of the most abundant and variable types of DNA sequences in the genome (Weber 1990; Tóth et al. 2000; Ellegren 2004) and occur primarily due to slipped-strand mispairing and subsequent error(s) during DNA replication, repair or recombination (Levinson and Gutman 1987; Kalia et al. 2011). However, the activity of transposable elements, mainly non-LTR retrotransposons, has also been reported as a major source of new microsatellites and their movement throughout the genome (Messier et al. 1996; Nadir et al. 1996; Li et al. 2002; Kalia et al. 2011; Grandi and An 2013).

SSRs are recognized as powerful informative markers of genetic diversity and variability in both animal and plants (Peakall et al. 1998; Cuadrado and Jouve 2007, 2011; Ernetti et al. 2019). In chromosomal mapping, although studies are still scarce for most species, they have provided a useful tool for understand the genome and chromosomal evolution of different taxa (e.g., Piscor et al. 2016; Travenzoli et al. 2019; Bueno et al. 2021). They can be found clustered in a few chromosomal pairs (e.g., Viana et al. 2019; Bueno et al. 2021), but are usually reported throughout the chromosomes (e.g., Felicetti et al. 2021; Lui et al. 2021; Bueno et al. 2021). Usually, microsatellites are described with crucial roles in the origin and evolution of specific chromosomes, mainly B chromosomes (e.g., Milani and Cabral-de-Mello et al. 2014; Felicetti et al. 2021; Lui et al. 2021) and sex chromosomes (e.g., Barbosa et al. 2017; Barcellos et al. 2019; Viana et al. 2019). However, in complement A chromosomes and populations without sex or B chromosomes, the studies are still concentrated only in the type of array and presence or absence of the marker, considering the whole karyotype (e.g., Lui et al. 2013; Traldi et al. 2013; Sassi et al. 2021).

One of the most challenging problems in expanding the use of microsatellites is the scattered distribution pattern. It is reported for several species worldwide (e.g.,

Silva et al. 2020; Yeesin et al. 2021; Saenjundaeng et al. 2020) and is predominant in most analyzed Neotropical fish species (Tab. 1). This type of array hampers the comparison between karyotypes and can hardly be used as complement A chromosomal markers, since it has an apparently random distribution in these chromosomes. However, different lines of evidence indicated that the distribution of microsatellites is nonrandom (Li et al. 2002; Li et al. 2004). They can influence in several aspects of the genome, including the nucleosome packing (Iyer and Struhl 1995), methylation (Fukuda et al. 2013), high order chromatin structure (Pathak et al. 2013; McNeil et al. 2006; Subramanian et al. 2003a) and splicing (Cuppens et al. 1998, Hefferon et al. 2004, Hui et al. 2005). Microsatellites can also have enhancer functions (Kumar et al. 2013; Gebhardt et al. 1999; Gymrek et al. 2016), modulate gene expression (Chen et al. 2007; Kumar and Bhatia 2016; Joshi-Saha and Reddy 2015) and participate of gene activity, recombination, DNA replication, cell cycle and mismatch repair (MMR) system (reviewed in Li et al. 2002; Li et al. 2004; Bagshaw 2017). Thus, it seems that microsatellites, once thought of as generally neutral, retain considerable diversity and functional significance among the genome of eukaryotic organisms (Bagshaw et al. 2017), hence, it is unlikely to believe that their scattered distribution pattern in cytogenetic mappings is random. In this scenario, considering that the scattered pattern consists in signals throughout the chromosomes, the association or colocalization with clustered sites in cytogenetic mapping, as already reported with several other types of DNA sequences (e.g., Cuadrado and Jouve 2007; Grandi and An 2013; Ruiz-Ruano et al. 2015), may provide an additional method to compare species and karyotypes. The clusters of already mapped repetitive elements can be used as guide to homeologous chromosomes among species or populations and comparative analyses besides sex and B chromosomes could be carried out.

Auchenipteridae, known as the driftwood catfishes, is one of the 43 Siluriformes families. They are restricted but widely distributed in South America and have 26 genera and 125 valid species (Fricke et al. 2021). Among them, *Trachelyopterus* (with 17 valid species) is one of the most studied genera owing to high morphological similarity and, hence, a controversial taxonomic history (Akama 2004). The physical localization of 18S rDNA, 5S rDNA, H3/H4 histone genes and snRNA U2 is well documented in six species: *T. coriaceus*, *T. striatulus, Trachelyopterus* aff. *galeatus* (cited as *Parauchenipterus galeatus*) (Lui et al. 2010; Santos et al. 2021; Lui et al. 2021; Haerter et al. 2021), *T. porosus* (Felicetti et al. 2018; Haerter et al. 2021), *T.*

*galeatus* (cited as *P. galeatus*), *Trachelyopterus* aff. *coriaceus* (cited as *Trachelyopterus* sp.) (Lui et al. 2021; Haerter et al. 2021); whereas the SSR $(GATA)_n$ sites were described in only three species: *T. galeatus* (Felicetti et al. 2021; Lui et al. 2021), *T. porosus* (Felicetti et al. 2021) and *Trachelyopterus* aff. *coriaceus* (cited as *Trachelyopterus* sp.) (Lui et al. 2021). In B chromosomes, the $(GATA)_n$ sequence has a particularly pattern, with clustered blocks, and provided valuable data about the origin and evolution of these extra chromosomes in Auchenipteridae. On the other hand, in complement A chromosomes it presented a scattered distribution pattern and was not able to reveal diversity or specific traits (Felicetti et al. 2021; Lui et al. 2021). Therefore, these *Trachelyopterus* species constitutes an excellent model to be investigated through the integrated mapping, since it has (1) scattered signal of a microsatellite in all complement A chromosomes; (2) well known clusters of 18S rDNA, 5S rDNA, H3/H4 histone genes and U2 snRNA and; (3) highly similar karyotypes, which could test the integrated mapping efficiency.

In this paper the microsatellite $(GATA)_n$ distribution pattern was compared among the carriers of the 5S and 18S rDNA, H3/H4 histone genes and U2 snRNA sites, in six *Trachelyopterus* species. The clustered repetitive elements sites were used as a guide to specific chromosomal pairs. We intended to test whether a widely distributed microsatellite could have distinct distribution patterns on homeologous chromosomes of different species, revealing unknown chromosome arrangements as well as architecture interaction between them.

## Methods

### *Specimens and DNA extraction*

Four *Trachelyopterus* species, including two possible new ones, were analyzed: *Trachelyopterus galeatus* Linnaeus, 1766 (04 males and 07 females) from Catalão lake, Manaus, Amazon River basin, 03°09'47''S; 59°54'2''W (Instituto Nacional de Pesquisas na Amazônia - INPA 57939); *Trachelyopterus coriaceus* Valenciennes, 1840 (04 males and 03 females) from Araguaia River, São Miguel do Araguaia, 13º08'52,7''S; 50º25'02,8''W (Museu da Universidade de São Paulo - MZUSP 106766); *Trachelyopterus porosus* Eigenmann and Eigenmann, 1888 (04 males and 04 females) from Catalão lake, Manaus, Amazon River basin, 03°09'47''S; 59°54'29''W

(Instituto Nacional de Pesquisas da Amazônia - INPA 57940); *Trachelyopterus* aff. *galeatus* (06 males and 10 females) from Araguaia River (suggested as new species), São Miguel do Araguaia, 13º08'52,7''S; 50º25'02,8'W (MZUSP 110803); *Trachelyopterus striatulus* Steindachner, 1877 (03 males and 03 females) from Verde lagoon, Doce River basin, Marliéria, 19º49'44,5''S; 42º37'52.5''W (MZUSP 109798); and *Trachelyopterus* aff. *coriaceus* (02 males and 01 female) from Arrombado lagoon (suggested as new species), Bento Gomes River basin, Poconé, 16º25'40,9''S; 56º25'07,4''W (MZUSP 110806). Genomic DNA was extracted from the liver of all species according to Sambrook et al. (2001).

*Probes labeling and sequencing*

The 18S rDNA and 5S rDNA probes were obtained through polymerase chain reaction - PCR according to Haerter et al. (2021) using the primers NS1 and NS8 (White 1990), 5SA and 5SB (Martins and Galetti 1999). The 18S rDNA probes were labeled with Biotin-16-dUTP (Bio-Nick-Translation, Roche) according to manufacturer's instructions and detected with Antibiotin-Avidin-FITC / Antiavidin-Biotin (Roche). The 5S rDNA were labeled through PCR with Tetramethyl-Rhodamine-5-dUTP (Roche). The H3 and H4 histone genes fragments The U2 snRNA fragments were amplified from *Trachelyopterus galeatus* genome using the primers H3F and H3R (Colgan et al. 1998); and H4F2s and H4F2er (Pineau et al. 2004). Both probes were labeled through PCR with Tetramethyl-Rhodamine-5-dUTP (Roche) according to Haerter et al. (2021). The U2 snRNA fragments were amplified from *Trachelyopterus galeatus* genome with the primers U2F and U2R (Bueno et al. 2013). The U2 snRNA probes were labeled through PCR with Fluorescein-12-dUTP (Roche) according to Haerter et al. (2021). The $(GATA)_n$ probes were generated through PCR in the absence of a template, using only the primers according to Ijdo et al. (1991) and labeled with Tetramethyl-Rhodamine-5-dUTP (Roche).

All PCR products were sequenced in both ways, forward and reverse, using the ABI 3730 DNA Analyzer with the BigDye Terminator v3.1 Cycle Sequencing Kit (code 4337456) and the Sequencing Analysis software 5.3.1. The consensus sequence was generated by the Bioedit Sequence Alignment Editor (Hall 1999). The fragments identity was confirmed through BLASTn 2.11.0 (National Center for Biotechnology Information) (Altschul et al. 1997).

*Cytogenetic analyses and Fluorescent in situ Hybridization*

The samples were treated with a 0.02% colchicine solution (1 ml/100g of body weight) for 30-40 min and sequentially euthanized by clove oil overdose (Griffiths 2000a) (according to the ethics committee on animal experimentation and practical classes at Unioeste: 09/13 - CEEAAP / Unioeste). The mitotic chromosomes were obtained from anterior kidney cells (Bertollo et al. 1978). The chromosome morphology was classified according to Levan et al. (1964).

Fluorescent in situ hybridization (FISH) was carried out with 77% of stringency according to Pinkel et al. (1986) with modifications suggested by Margarido and Moreira-Filho (2008). Clusters of 18S rDNA, 5S rDNA, snRNA U2, H3 and H4 histone genes were used as guide to integrative (guided) mapping with the SSR $(GATA)_n$. The digital images were captured by the DP Controller 3.2.1.276 software using an Olympus DP71 digital camera connected to the BX61 epifluorescence microscope (Olympus America Inc., Center Valley, PA, United States of America). The Hardy–Weinberg equilibrium (HWE) and Chi-squared test were performed using the Hardy-Weinberg (HW) testing program (Santos, Lemes and Otto 2020).

**Results**

*DNA sequencing analysis*

The U2 snRNA and both H3 and H4 histone genes were sequenced with forward and reverse primers. The U2 snRNA had a 199-bp consensus sequence with 96,4% similarity with *Parabotia fasciatus* U2 snRNA gene (Sequence ID: MG874999.1). The histone gene H3 had a 448-bp consensus sequence with 91.57% similarity with the *Pimelodus microstoma* H3 histone gene (Sequence ID: MT094432.1). The histone gene H4 had a 269-bp consensus sequence with 90.09% similarity with the *Pimelodus microstoma* H4 histone gene (MT094433.1).

*Cytogenetic analysis*

*Trachelyopterus* aff. *galeatus* – Araguaia River basin

This species had 2n=58 chromosomes for both sexes (7 males and 9 females). The microsatellite $(GATA)_n$ were found spread throughout the chromosomes. The chromosomal pair 24 (18S rDNA and H3/H4 histone genes) had two blocks of the SSR $(GATA)_n$ in the terminal long arm and none in the short arm (Fig. 1, 4). The chromosomal pair 25 (H3 and H4 histone genes) also had two blocks of the SSR $(GATA)_n$ in the long arm and none in the short arm (Fig. 4). The chromosomal pair 26 (U2 snRNA) had a chromosome polymorphism associated to the SSR $(GATA)_n$ distribution pattern (Fig 2), which combined with the U2 snRNA distribution pattern evidenced three chromosomal forms, including a new one, here referred as C chromosomal form: (A) U2 snRNA sites in the short arm with SSR $(GATA)_n$ blocks only in the long arm of the chromosome; (B) U2 snRNA sites in the long arm with SSR $(GATA)_n$ blocks only in the long arm of the chromosome; and (C) U2 snRNA sites in the short arm with SSR $(GATA)_n$ blocks in both the short and long arm of the chromosome. From all chromosomal forms, six combinations could be hypothesized and four of them were found in the sample: AA (1 female), AB (2 males and 3 females), AC, BB (1 male and 5 females), BC, CC (2 males and 2 females) (Fig. 2; Tab. 2; Fig. 5). The $\chi 2$ value for Hardy-Weinberg equilibrium (HWE) was 15.99 (Df=03; p=<0.05). The chromosomal pair 3 (5S rDNA) had three blocks of the SSR $(GATA)_n$, two in the short arm (one in the terminal position and one in interstitial position) and one in the terminal position of the long arm (Fig. 3).

*Trachelyopterus galeatus* – Amazon River basin

This species had 2n=58 chromosomes for both sexes (7 males and 9 females). The chromosomal pair 20 (18S rDNA and H3/H4 histone genes) has shown a chromosome polymorphism (Fig. 1). It consists in two different organizations of the SSR $(GATA)_n$ in the chromosome: two clustered SSR $(GATA)_n$ sites in the terminal and interstitial position of long arm of the chromosome (A form); or two clustered SSR $(GATA)_n$ in the terminal position and interstitial position of the long arm and one in the interstitial position of the short arm of the chromosome (B form). Three combinations of these polymorphic forms were found in this population: (AA) both chromosomes with two clustered SSR $(GATA)_n$ sites in the terminal and proximal position of long arm of the chromosome (2 males and 1 female); (AB) one chromosome with two clustered

SSR $(GATA)_n$ sites in the terminal and proximal position of long arm of the chromosome and another chromosome with two clustered SSR $(GATA)_n$ in the terminal and proximal position of the long arm of the chromosome and one in the proximal position of the short arm of the chromosome (2 males and 5 females); (BB) both chromosomes with two clustered SSR $(GATA)_n$ in the terminal and proximal position of the long arm of the chromosome and one in the proximal position of the short arm of the chromosome (1 male). The chromosomal pair 28 (U2 snRNA) had two blocks of the SSR $(GATA)_n$ in the long arm and one in terminal position of the short arm. The chromosome polymorphism was found in Hardy-Weinberg equilibrium (HWE) ($\chi 2$=1.099; Df=01; p=<0.05). The chromosomal pair 24 (H3 and H4 histone genes) had two blocks of the SSR $(GATA)_n$ in the long arm and none in the short arm (Fig. 2). The chromosomal pair 14 (5S rDNA) had three blocks of the SSR $(GATA)_n$, two in the short arm (one in the terminal position and one in interstitial position) and one in the terminal position of the long arm (Fig. 4). The chromosomal pair 16 (5S rDNA) had two blocks of the SSR $(GATA)_n$, one in the terminal position of the short and another in the long arm (Fig. 3).

*Trachelyopterus porosus* – Amazon River basin

This species had 2n=58 chromosomes for both sexes (4 males and 4 females). The microsatellite $(GATA)_n$ were found spread throughout the chromosomes. The chromosomal pair 23 (18S rDNA and H3/H4 histone genes) had two blocks of the SSR $(GATA)_n$ in the long arm and none in the short arm (Fig. 1, 4). The chromosomal pair 24 (H3 and H4 histone genes) had two blocks of the SSR $(GATA)_n$ in the long arm and none in the short arm (Fig. 4). The chromosomal pair 26 (U2 snRNA) had two blocks of the SSR $(GATA)_n$ in the long arm and none in the short arm (Fig. 2). The chromosomal pair 3 (5S rDNA) had two blocks of the SSR $(GATA)_n$, one in the terminal position of the short and another in the long arm. The chromosomal pair 4 (5S rDNA) had three blocks of the SSR $(GATA)_n$, two in the short arm (one in the terminal position and one in interstitial position) and one in the terminal position of the long arm (Fig. 3).

*Trachelyopterus coriaceus* – Araguaia River basin

This species had 2n=58 chromosomes for both sexes (4 males and 3 females). The microsatellite $(GATA)_n$ were found spread throughout the chromosomes. The chromosomal pair 23 (H3 and H4 histone genes and 18S rDNA) had two blocks of the SSR $(GATA)_n$ in the long arm and none in the short arm (Fig. 1, 4). The chromosomal pair 28 (U2 snRNA) had two blocks of the SSR $(GATA)_n$ in the long arm and none in the short arm (Fig. 2). The chromosomal pair 3 (5S rDNA) had three blocks of the SSR $(GATA)_n$, two in the short arm (one in the terminal position and one in interstitial position) and one in the terminal position of the long arm. The chromosomal pair 16 (5S rDNA) had two blocks of the SSR $(GATA)_n$, one in the terminal position of the short and another in the long arm (Fig. 3).

*Trachelyopterus* aff. *coriaceus* – Paraguay river basin

This species had 2n=58 chromosomes for both sexes (2 males and 1 female). The microsatellite $(GATA)_n$ were found spread throughout the chromosomes. The chromosomal pair 22 (18S rDNA) had two blocks of the SSR $(GATA)_n$ in the long arm and none in the short arm (Fig. 1). The chromosomal pair 23 (H3 and H4 histone genes) had two blocks of the SSR $(GATA)_n$ in the long arm and none in the short arm (Fig. 4). The chromosomal pair 27 (U2 snRNA) had two blocks of the SSR $(GATA)_n$ in the long arm and none in the short arm (Fig. 2). The chromosomal pair 16 (5S rDNA) had two blocks of the SSR $(GATA)_n$, one in the terminal position of the short and another in the long arm. The chromosomal pair 18 (5S rDNA) had three blocks of the SSR $(GATA)_n$, two in the short arm (one in the terminal position and one in interstitial position) and one in the terminal position of the long arm (Fig. 3).

*Trachelyopterus striatulus* – Doce River basin

This species had 2n=58 chromosomes for both sexes (3 males and 3 females). The microsatellite $(GATA)_n$ were found spread throughout the chromosomes. The chromosomal pair 23 (18S rDNA and H3/H4 histone genes) had two blocks of the SSR $(GATA)_n$ in the long arm and none in the short arm (Fig. 1, 4). The chromosomal pair 18 (H3 and H4 histone genes) also had had two blocks of the SSR $(GATA)_n$; however, one in terminal position of the long arm and another in the interstitial position of the short arm (Fig. 4). The chromosomal pair 28 (U2 snRNA) had two blocks of the SSR

$(GATA)_n$ in the long arm and none in the short arm (Fig. 2). The chromosomal pair 10 (5S rDNA) had three blocks of the SSR $(GATA)_n$, two in the long arm (one in the terminal position and one in interstitial position) and one in the terminal position of the short arm. The chromosomal pair 13 and 15 (5S rDNA) had two blocks of the SSR $(GATA)_n$, one in the terminal position of the short and another in the long arm (Fig. 3).

## Discussion

The $(GATA)_n$ repeats, molecular components of the Bkm satellite DNA (Banded krait minor-satellite DNA), are widely distributed in the genome of higher organisms (Morescalchi et al. 2011). As expected, in all *Trachelyopterus* species of this study it was found scattered throughout the chromosomes. This type of array and has been described to different SSRs and taxa, such as plants (Chang et al. 2008; Kubat et al. 2008), amphibians (Bueno et al. 2021), fungus (Micolino et al. 2019) and fish (Traldi et al. 2013; Felicetti et al. 2021; Lui et al. 2021; Bueno et al. 2021). In Auchenipteridae, it was already reported in *T. porosus* (Felicetti et al. 2021), *T. galeatus* (Felicetti et al. 2021) and *Trachelyopterus* aff. *coriaceus* (cited as *Trachelyopterus* sp.) (Lui et al. 2021), and now it can also be seen in *T. coriaceus*, *T. striatulus* and *Trachelyopterus* aff. *galeatus*. This type of scattered array could be explained by the activity of transposable elements, mainly non-LTR retrotransposons, which are a major source of new microsatellites and can also drive it throughout the genome (see Messier et al. 1996; Nadir et al. 1996; Li et al. 2002; Grandi and An 2013), and/or to chromosomal rearrangements, such as: fusion, fission, duplication, deletion, amplification, translocation, paracentric and pericentric inversions, as already proposed for a close genus, *Hypostomus* (Traldi et al. 2013). Furthermore, the $(GATA)_n$ repeats have been associated to sex chromosome in different organisms (e.g. Singh et al. 1976; Viana et al. 2019; Mazzoleni et al. 2020); however, no differences between males and females have been found in these populations.

In the integrated mapping with the 18S rDNA, the chromosomal pair of all species had one clustered block in the interstitial position, another in the terminal position of the long arm, and none in the short arm (Fig. 1). The absence of the microsatellite in the short arm was also reported in a close species, *Glanidium ribeiroi* (Lui et al. 2015), but it was unexplored yet. Usually, SSRs can constitute a larger fraction of noncoding DNA, but are rare in protein-coding regions (Li et al. 2002) mainly

due to negative selection against frameshift mutations in coding regions, as evidenced in plants, primates, and microorganisms (see Metzgar et al. 2000). Comparative studies showed that only repeats in multiples of three may develop evenly in both regions (Tóth et al., 2000; Metzgar et al., 2000), since RNA bases are read as triplets and other types could result in frameshift mutations (Metzgar et al. 2000; Oliveira et al. 2005). In this way, the absence of the $(GATA)_n$ sequence in the short arm of the 18S rDNA chromosomes, might suggest a negative impact of the microsatellite near the coding areas.

However, this hypothesis is contradicted by some evidences: (a) *T. galeatus* from the Amazon River basin presented a chromosome polymorphism in the chromosomal pair 20 (18S rDNA), in which the B chromosomal form is represented by an additional clustered $(GATA)_n$ block in the short arm; (b) there is overlaid signal between the $(GATA)_n$ sequence and the 5S rDNA and U2 snRNA sites; and (c) the $(GATA)_n$ sequence is distributed throughout the chromosomes, indicating that the $(GATA)_n$ sequence could also be near other unmapped gene sequences. Therefore, in these *Trachelyopterus* species, the $(GATA)_n$ may not have a negative impact near coding areas. The non-existence of $(GATA)_n$ signal in the short arm might only be related to a spatial issue, since besides the 18S rDNA, the short arm of most species also carries the H3 and H4 histone genes and, consequently, there could not be enough space for large amounts of $(GATA)_n$ sequence detectable through Fluorescent *in situ* Hybridization, which needs targets of at least 1kb to express significative results (see Schwarzacher and Heslop-Harrison 2000).

The integrated mapping with 18S rDNA revealed a chromosome polymorphism associated with the $(GATA)_n$ distribution pattern in the chromosomal pair 20 (18S rDNA) of *T. galeatus* from the Amazon River basin, which is apparently neutral, since the Hardy-Weinberg equilibrium suggests that the heterozygous has no different adaptative value compared to other forms ($\chi 2$ = 1.099; Df = 01; p=<0.05). It is represented by two chromosomal forms, A and B, in which the A chromosomal form is also present in all other species of this study, whereas the B chromosomal form is exclusive of *T. galeatus* and may be considered an apomorphic trait. Two main aspects could be evidenced in the $(GATA)_n$ polymorphism. First, it depends on the presence of the $(GATA)_n$ sequence at a point that has not been reported in any species so far. Second, the size of the additional block suggests that it may have also expanded after its origin.

While minor chromosomal structural changes, primarily amplification/duplications, may account for the increase in size, the origin of the extra block is still uncertain. Since it involves the creation of a whole new block of sequences, rather than the relocation of an existing one, as in the case of paracentric or pericentric inversion polymorphisms (see Hoffman et al. 2004), it might be a result of many different events. Evolutive processes that results in movements of sequences, such as paracentric/pericentric inversions (see Hoffman et al. 2004; Hoffmann and Rieseberg 2008) or activity of transposable elements (see Messier et al. 1996; Li et al. 2002; Grandi and An 2013) seems to be the most parsimonious hypotheses. However, the amplification of little amounts of $(GATA)_n$ sequences already present in the short arm, and non-detectable through Fluorescent *in situ* Hybridization, should not be discarded.

The $(GATA)_n$ distribution pattern in the U2 snRNA chromosomes was conserved among most species, with clustered blocks only in the long arm of the chromosomes. However, *Trachelyopterus* aff. *galeatus* from Araguaia River basin presented a chromosomal polymorphism, which considering only the $(GATA)_n$ sequence distribution, consists in the presence of an additional clustered block in the short arm. The same chromosomal pair (26) were also reported to have a U2 snRNA chromosomal polymorphism (Haerter et al. 2021) and the combined polymorphic markers resulted in three chromosomal forms. The B chromosomal form is exclusive of this population; whereas the C chromosomal form was also found in *T. galeatus* from Amazon River basin and the and A form is present in *T. striatulus, T. coriaceus*, *T*. *porosus*, and *Trachelyopterus* aff. *coriaceus*. Although both polymorphic states interact to compose the chromosome arrangement, they seem to be originated in different evolutive events. In the U2 snRNA polymorphism, the new arrangement is characterized by the presence of U2 snRNA sites in the long arm of the chromosomes, while all other species had sites in the short arm. Until now, it is an exclusive trait of *Trachelyopterus* aff. *galeatus* and seems to be a product of a pericentric inversion (Haerter et al. 2021). On the other hand, the variation of the $(GATA)_n$ sequence, with sites in the short arm and in polymorphic state in *Trachelyopterus* aff. *galeatus,* can also be seen in *T. galeatus* from Amazon River, therefore, it is not an exclusive character. It could be a product of independent events in both species, *T. galeatus* and in *Trachelyopterus* aff. *galeatus*, however, the most parsimonious hypothesis is through hybridization in secondary contact zones (see Abbot et al. 2013; Sedghifar et al. 2015), since it could be facilitated by the historical and geomorphological aspects

of the Araguaia River floodplains, known by constant ichthyofaunistic exchange across surrounding hydrographic systems during the neotectonics reactivations in the Transbrasiliano Lineament during the formation of the Araguaia depression (see Saadi 1993; Saadi et al. 2005; Lima and Ribeiro 2011).

Interestingly, the U2 snRNA inversion polymorphism in *Trachelyopterus* aff. *galeatus* was reported in Hardy-Weinberg equilibrium (Haerter et al. 2021), in which, the spread of the polymorphism could be associated to the neutrality of the rearrangement, since it suggests that there is no change in adaptative value among the genotypes or in the host fitness (see Hoffmann and Rieseberg 2008), as reported for water beetles (Aradottir and Angus 2004) and blackflies (Kuvangkadilok et al. 2003). In this state, the polymorphism is essentially influenced by genetic drift and migration (Hoffmann and Rieseberg 2008). However, the polymorphic state of U2 snRNA with the $(GATA)_n$ sequence, resulted in Hardy-Weinberg disequilibrium ($\chi 2=15.99$; Df=03; p=<0.05), suggesting that the combined arrangement may be under the effect of different forces beyond just genetic drift and gene flow.

Furthermore, all genotypes were found in a similar proportion compared to the expected by the Hardy-Weinberg equilibrium test (Tab. 2), except the ones with the C chromosomal form, in which, none of the heterozygous were found in the sample (AC and BC) and the homozygous (CC) presented a three times higher frequency than expected. In some cases, heterozygous originated from chromosome rearrangements can suffer severe reductions in fitness (White 1978), zygotic lethality (Searle 1993) or hybrid sterility (Stebbins 1950; Levin 2002), especially when it involves change in gene order within a chromosome (inversions) (White 1978) or when the hybrids carry multiple rearrangements (White 1978; Searle 1993). In this scenario, the presence of multiple chromosomal polymorphism in the same chromosomal pair, which origin of both could be related to major chromosome rearrangements (inversions), associated to the absence of the C heterozygous and higher frequency of the C homozygous, may suggest the existence of distinct evolutive pressures over it compared to other genotypes. However, more specific analyses are still required to establish whether there is in fact a host fitness effect in the presence of the C form in heterozygosity or homozygosity.

In contrast to the previously discussed markers, the $(GATA)_n$ chromosomal mapping on the 5S rDNA and H3/H4 histone gene carriers did not reveal new information about the structure of these chromosomal pairs. However, using the

$(GATA)_n$ distribution pattern on the 5S rDNA carriers, the possible chromosome homeologies between the species could be inferred (Fig. 3). Since this is a marker normally found on multiple chromosomal pairs in *Trachelyopterus*, the chromosomal correspondence is difficult to suggest without additional information about the organization of each chromosome, a gap that could be partially filled with the distribution of the $(GATA)_n$ sequence on these chromosomes. Through the $(GATA)_n$ mapping on the 5S rDNA chromosome pairs, three main chromosomal arrangements could be evidenced: (1) 5S rDNA site in the interstitial position of the long arm of the chromosome; with two blocks of the SSR $(GATA)_n$, one in the terminal position of the short arm another in the terminal position of the long arm; (2) 5S rDNA site in the proximal position of the short arm; with three blocks of the SSR $(GATA)_n$, one in terminal position of the short arm and two in the long arm (one in the terminal position and another in the proximal position); (3) 5S rDNA site in the proximal position of the short arm; with two blocks of the SSR $(GATA)_n$, one in the terminal position of the short arm and another in terminal position of the long arm (Fig. 3). The chromosomal arrangement (1) is present in all species, whereas the chromosomal arrangement (2) is present in *T. striatulus*, *T. galeatus*, *Trachelyopterus* aff*. coriaceus* and *T. coriaceus*, and the chromosomal arrangement (3) is present only in *T. striatulus* and *T. porosus*.

The microsatellite mapping combined with the histone genes confirmed the distribution of SSR $(GATA)_n$ already pointed out through the integration with 18S rDNA for the species that have this synteny (*T. striatulus*, *T. galeatus*, *Trachelyopterus* aff*. galeatus*, *T. porosus* and *T. coriaceus*) as well as the polymorphism in the chromosomal pair 20 of *T. galeatus* from the Amazon River basin. No new arrangement could be observed, and even for species that have multiple sites of H3 and H4 histone genes (*T. striatulus*, *T. galeatus*, *Trachelyopterus* aff*. galeatus* and *T. porosus*), both chromosomal pairs showed the same microsatellite distribution pattern, proximal and distal in the long arm.

*Integrated mapping perspectives to scattered microsatellites in Neotropical fishes*

To date, there is no similar approach in cytogenetic mapping of microsatellites comparing Neotropical fish species. Most studies have focused in the origin and evolution of specific chromosomes, mainly B chromosomes (12 out of 88 studied species - 13,64%) and sex chromosomes (41 out of 88 studied species – 46,59%);

whereas others focused only on the type of array and presence or absence of the marker (35 out of 88 studied species – 39,77%) (Tab. 1). Nonetheless, of all cytogenetically analyzed species through physical mapping of microsatellites, 76.14% had at least one scattered microsatellite (67 out of 88 analyzed species), and in most studies, they could not be used to differ the species or populations.

Although the integrated mapping did not reveal large chromosomal rearrangements and most species had a similar distribution pattern of the $(GATA)_n$ sequence in the analyzed chromosomes, it proved that the scattered microsatellite $(GATA)_n$ has a non-random distribution, reiterating the existence of organization even in scattered microsatellites, which can be better described through a smaller scale analysis, comparing specific chromosomes between species. Through the integrated mapping a more accurate $(GATA)_n$ pattern were described to the chromosome carriers of the 18S and 5S rDNA, H3 and H4 histone genes and U2 snRNA, and even though three species/populations used in this study were already mapped with $(GATA)_n$, none of them were able to detect the $(GATA)_n$ polymorphism in *T. galeatus* from Amazonas River basin and the presence of three chromosomal arrangements of the $(GATA)_n$ sequence in the 5S rDNA carrier. Likewise, the $(GATA)_n$ polymorphism in the U2 snRNA chromosomes of *Trachelyopterus* aff. *galeatus* from the Araguaia River basin would possibly go unnoticed without using the U2 snRNA site as a guide. Therefore, the integrated mapping (guided) proved to be an efficient methodology to reveal cryptic chromosomal arrangements of scattered microsatellites.

Cytotaxonomically, the integrated mapping showed the divergence in one more marker between *T. galeatus* from the Amazon River basin and *Trachelyopterus* aff. *galeatus* from the Araguaia River, which reiterate the existence of a possible new species, as already proposed through ribosomal markers (Santos et al. 2021) and integrated mapping with H3/H4 histone genes and U2 snRNA (Haerter et al. 2021). Methodologically, the integrated mapping turned the widely distributed SSR $(GATA)_n$, with contributions in *Trachelyopterus* only to B chromosomes origin and evolution, into promising marker to distinguish other Auchenipteridae species. Thus, with the advancement of repetitive elements cytogenetic, we expect that the integrated mapping can further add to *Trachelyopterus* and also to other species with scattered distributed microsatellites.

**References**

Schnitzler CK, Turchetto C, Teixeira MC, Freitas LB (2020) What could be the fate of secondary contact zones between closely related plant species?. Genetics and Molecular Biology 43:e20190271. Available from: https://doi.org/10.1590/1678-4685-GMB-2019-0271

Abbott R, Albach D, Ansell S, Arntzen JW, Baird SJ, Bierne N, Boughman J,.…Zinner D (2013) Hybridization and speciation. J Evol Biol 26:229-246. https://10.1111/j.1420-9101.2012.02599.x

Akama A (2004) Sistemática dos gêneros *Parauchenipterus* Bleeker, 1862 e *Trachelyopterus* Valenciennes, 1840 (Siluriformes, Auchenipteridae). Ph.D. Dissertation, Universidade de São Paulo: Museu de Zoologia, 375pp. [In Portuguese].

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research 25:3389-3402. https://doi.org/10.1093/nar/25.17.3389

Aradottir GI, Angus RB (2004) A chromosomal analysis of some water beetle species recently transferred from Agabus Leach to Ilybius Erichson, with particular reference to the variation in chromosome number shown by *I. montanus Stephens* (Coleoptera: Dytiscidae). Hereditas 140:185-92. https://doi.org/10.1111/j.1601-5223.2004.01837.x

Bagshaw ATM (2017) Functional mechanisms of microsatellite DNA in eukaryotic genomes. Genome Biol Evol 9:2428–2443. https://doi.org/10.1093/gbe/evx164

Barbosa P, Leal EV, da Silva M, de Almeida MC, Moreira-Filho O, Artoni RF (2017) Variability and evolutionary implications of repetitive DNA dynamics in genome of Astyanax scabripinnis (Teleostei, Characidae). Comp Cytogenet 11:143-162. https://10.3897/CompCytogen

Barcellos SA, Kretschmer R, de Souza MS, Costa AL, Degrandi TM, dos Santos MS, de Oliveira EHC, Cioffi MB, Gunski RJ and Garnero ADV (2019) Karyotype evolution and distinct evolutionary history of the W chromosomes in swallows (Aves, Passeriformes). Cytogenet Genome Res 158:98-105. https://10.1159/000500621

Bertollo LAC, Takahashi CS, Moreira-Filho O (1978) Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Rev Bras Genet 1:103-120.

Blanco DR, Vicari MR, Lui RL, Artoni RF, de Almeida MC, Traldi JB, Margarido VP, Moreira-Filho O (2014) Origin of the X1X1X2X2/X1X2Y sex chromosome system of *Harttia punctata* (Siluriformes, Loricariidae) inferred from chromosome painting and FISH with ribosomal DNA markers. Genetica 142:119-26. https://10.1007/s10709-014-9759-4

Bueno D, Palacios-Gimenez OM, Cabral-de-Mello DC (2013) Chromosomal mapping of repetitive DNAs in the grasshopper *Abracris flavolineata* reveal possible ancestry of

the B chromosome and H3 histone spreading. PLoS One 8:e66532. https://doi.org/10.1371/journal.pone.0066532

Bueno GDP, Gatto KP, Gazolla CB, Leivas PT, Struett MM, Moura M, et al. (2021) Cytogenetic characterization and mapping of the repetitive DNAs in *Cycloramphus bolitoglossus* (Werner, 1897): More clues for the chromosome evolution in the genus Cycloramphus (Anura, Cycloramphidae). PLoS ONE 16:e0245128. https://doi.org/10.1371/journal.pone.0245128

Carvalho PC, Oliveira EA, Bertollo LAC, Yano CF, Oliveira C, Decru E, … Cioffi MB (2017) First Chromosomal Analysis in Hepsetidae (Actinopterygii, Characiformes): Insights into Relationship between African and Neotropical Fish Groups. Frontiers in Genetics 8:203. https://10.3389/fgene.2017.00203

Cerqueira FJ, Fernandes CA, Lui RL, Parise-Maltemp PP, Margarido VP (2020) Chromosomal mapping of repetitive sequences in *Hyphessobrycon eques* (Characiformes, Characidae): a special case of the spreading of 5S rDNA clusters in a genome. Genetica 148, 25-32. https://doi.org/10.1007/s10709-020-00086-3

Chang SB, Yang TJ, Datema E, van Vugt J, Vosman B, Kuipers A, Meznikova M, Szinay D, Lankhorst RK, Jacobsen E, de Jong H (2008) FISH mapping and molecular organization of the major repetitive sequences of tomato. Chromosome Res 16:919-33. https://10.1007/s10577-008-1249-z

Chen T-M, Kuo P-L, Hsu C-H, Tsai S-J, Chen M-J, Lin C-W, Sun HS (2007) Microsatellite in the 3′ untranslated region of human fibroblast growth factor 9 (FGF9) gene exhibits pleiotropic effect on modulating FGF9 protein expression. Hum Mutat 28:98. https://doi.org/10.1002/humu.9471

Cioffi MB, Kejnovsky E, Bertollo LAC (2011) The Chromosomal Distribution of Microsatellite Repeats in the Genome of the Wolf Fish *Hoplias malabaricus*, Focusing on the Sex Chromosomes. Cytogenetic and Genome Research 132:289-296. https://10.1159/000322058

Cioffi M, Fernanda Yano C, Bertollo L, Molina W, Liehr T (2014) Genomic organization of repetitive DNAs and its implications for male karyotype and the neo-Y chromosome differentiation in *Erythrinus erythrinus* (Characiformes, Erythrinidae). Comparative Cytogenetics 8:139-151. https://doi.org/10.3897/compcytogen.v8i2.7597

Colgan D, McLauchlan A, Wilson G, Livingston S, Edgecombe G, Macaranas J, Cassis G, Gray M (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46:419-437. https://doi.org/10.1071/ZO98048

Conde-Saldaña CC, Cunha MS, Albornoz-Garzón JG, Barreto CAV, Ibagón N, Villa-Navarro FA, Dergam JA (2018) Karyotypic Divergence of Two Co-Occurring Species of Andean Climbing Catfishes (Siluriformes: Loricarioidei: Astroblepidae). Zebrafish 16. https://10.1089/zeb.2018.1659

Conde-Saldaña CC, Barreto CAV, Villa-Navarro FA, Dergam JA (2018) An Unusual Accumulation of Ribosomal Multigene Families and Microsatellite DNAs in the XX/XY

Sex Chromosome System in the Trans-Andean Catfish *Pimelodella cf. chagresi* (Siluriformes:Heptapteridae). Zebrafish 15:55–62. https://10.1089/zeb.2017.1469

Cuadrado A and Jouve N (2007) Similarities in the chromosomal distribution of AG and AC repeats within and between Drosophila, human and barley chromosomes. Cytogenetic and Genome Research 119:91–99. https://doi:10.1159/000109624

Cuadrado A and Jouve N (2011) Novel simple sequence repeats (SSRs) detected by ND-FISH in heterochromatin of *Drosophila melanogaster*. BMC Genomics 12:205. https://doi:10.1186/1471-2164- 12-205

Cuppens H, Lin W, Jaspers M, Costes B, Teng H, Vankeerberghen A, Jorissen M, Droogmans G, Reynaert I, Goossens M, Nilius B, Cassiman JJ (1998) Polyvariant mutant cystic fibrosis transmembrane conductance regulator genes. The polymorphic (Tg)m locus explains the partial penetrance of the T5 polymorphism as a disease mutation. J Clin Invest. 101:487-496. https://10.1172/JCI639

Edwards A, Civitello A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49:746–756.

Ellegren H (2004) Microsatellites: simple sequences with complex evolution. Nature Reviews Genetics 5:435–445. https://10.1038/nrg1348

Ernetti JR, Gazolla CB, Recco-Pimentel SM, Luca EM, Bruschi DP (2019) Non-random distribution of microsatellite motifs and (TTAGGG)n repeats in the monkey frog Pithecopus rusticus (Anura, Phyllomedusidae) karyotype. Genetics and Molecular Biology 42: e20190151. https://doi.org/10.1590/1678-4685-GMB-2019-0151

Felicetti D, Haerter CAG, Baumgärtner L, Paiz L, M Takagui FH, Margarido VP, Blanco DR, Feldberg E, da Silva M, Lui RL (2021) A New Variant B Chromosome in Auchenipteridae: The Role of (GATA)n and (TTAGGG)n Sequences in Understanding the Evolution of Supernumeraries in *Trachelyopterus*. Cytogenet Genome Res. https://doi.org/10.1159/000513107

Ferreira AMV, Viana PF, Zuanon J, Ezaz T, Cioffi MB, Takagui FH, Feldberg E (2021) Cytogenetic Analysis of *Panaqolus tankei* Cramer & Sousa, 2016 (Siluriformes, Loricariidae), an Ornamental Fish Endemic to Xingu River, Brazil. Cytogenetic and Genome Research 161:187-194. https://doi.org/10.1159/000514061

Fricke, R., Eschmeyer, W. N. & R. van der Laan (eds) 2021. ESCHMEYER'S CATALOG OF FISHES: GENERA, SPECIES. Available in: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. Electronic version accessed in 10 may 2021.

Fukuda K, Ichiyanagi K, Yamada Y, Go Y, Udono T, Wada S, Maeda T, Soejima H, Saitou N, Ito T, Sasalo H (2013) Regional DNA methylation differences between humans and chimpanzees are associated with genetic changes, transcriptional divergence and disease genes. J Hum Genet 58:446-454. https://10.1038/jhg.2013.55

Gebhardt F, Zanker KS, Brandt B (1999) Modulation of epidermal growth factor receptor gene transcription by a polymorphic dinucleotide repeat in intron 1. J Biol Chem 274:13176-13180. https://doi.org/10.1074/jbc.274.19.13176

Gouveia JG, Wolf IR, Vilas-Boas LA, Heslop-Harrison JS, Schwarzacher T, Dias AL (2017) Repetitive DNA in the Catfish Genome: rDNA, Microsatellites, and Tc1-Mariner Transposon Sequences in *Imparfinis* Species (Siluriformes, Heptapteridae). Journal of Heredity 108:650-657. https://10.1093/jhered/esx065

Grandi FC and An W (2013) Non-LTR retrotransposons and microsatellites. Mob Genet Elements 3:e25674. https://10.4161/mge.25674

Griffiths SP (2000) The use of clove oil as an anaesthetic and method or sampling intertidal rockpool fishes. J Fish Biol 57:1453-1464. https://doi.org/10.1111/j.1095-8649.2000.tb02224.x

Gymrek M, Willems T, Guilmatre A, Zeng H, Markus B, Georgiev S, Daly MJ, Price AL, Pritchard JK, Sharp AJ, Erlich Y (2016) Abundant contribution of short tandem repeats to gene expression variation in humans. Nat Genet. 48:22-29. https://doi.org/10.1038/ng.3461

Hall TA (1999) BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98. https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29

Hefferon TW, Groman JD, Yurk CE, Cutting GR (2004) A variable dinucleotide repeat in the CFTR gene contributes to phenotype diversity by forming RNA secondary structures that alter splicing. Proc Natl Acad Sci U S A 101:3504-3509. https://doi.org/10.1073/pnas.0400182101

Hoffmann AA, Sgrò CM, Weeks AR (2004) Chromosomal inversion polymorphisms and adaptation. Trends Ecol Evol 19:482-488. https://doi.org/10.1016/j.tree.2004.06.013

Hoffmann AA, Rieseberg LH (2008) Revisiting the impact of inversions in evolution: from population genetic markers to drivers of adaptive shifts and speciation? Annu Rev Ecol Evol Syst 39:21-42. https://doi.org/10.1146/annurev.ecolsys.39.110707.173532

Hui J, Hung LH, Heiner M, Schreiner S, Neumuller N, Reither G, Haas SA, Bindereif A (2005) Intronic CA-repeat and CA-rich elements: a new class of regulators of mammalian alternative splicing. EMBO J 24:1988–1998. https://doi.org/10.1038/sj.emboj.7600677

Ijdo JW, Wells RA, Baldini A and Reeders ST (1991) Improved telomere detection using a telomere repeat probe (TTAGGG)n generated by PCR. Nucleic Acid Res 19:4780.

Iyer V, Struhl K (1995) Poly(dA: dT), a ubiquitous promoter element that stimulates transcription via its intrinsic DNA structure. EMBO J 14:2570-2579.

Jacob HJ, Lindpaintnesr K, Kusumir EL, Bunkery K, Mao IP, Gantenv D, Dzau J, Lander ES (1991) Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. Cell 67:213-224. https://10.1016/0092-8674(91)90584-l

Joshi-Saha A, Reddy KS (2015) Repeat length variation in the 5′UTR of myo-inositol monophosphatase gene is related to phytic acid content and contributes to drought tolerance in chickpea (*Cicer arietinum L*.). J Exp Bot 66:5683–5690. https://doi.org/10.1093/jxb/erv156

Kalia RK, Rai M K, Kalia S, Singh R, Dhawan AK (2010) Microsatellite markers: an overview of the recent progress in plants. Euphytica 177:309-334. https://10.1007/s10681-010-0286-9

Kubat Z, Hobza R, Vyskot B, Kejnovsky E (2008) Microsatellite accumulation on the Y chromosome in Silene latifolia. Genome 51: 350–356. https://doi.org/10.1139/G08-024

Kumar RP, Krishnan J, Pratap Singh N, Singh L, Mishra RK (2013) GATA simple sequence repeats function as enhancer blocker boundaries. Nat Commun 4:1844. https://doi.org/10.1038/ncomms2872

Kumar S, Bhatia S (2016) A polymorphic (GA/CT)n- SSR influences promoter activity of Tryptophan decarboxylase gene in *Catharanthus roseus L*. Don. Sci Rep. 6:33280 https://doi.org/10.1038/srep33280

Kuvangkadilok C, Boonkemtong C, Phayuhasena S, Baimai V (2003) Larval polytene chromosomes of black flies (Simulium) from Thailand. I. Comparison among five species in the subgenus *Gomphostilbia Enderlein*. Genetica 118:69-81. https://doi.org/10.1023/A:1022909018987

Laing WA Bulley S Wright M Cooney J Jensen D Barraclough D MacRae E (2004) A highly specific L-galctose-1-phosphate phosphatase on the path to ascorbate biosynthesis. Proceedings of the National Academy of Sciences USA 101:16976-16981. https://doi.org/10.1073/pnas.0407453101

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:201-22. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

Levin DA (2012) The long wait for hybrid sterility in flowering plants. New Phytologist 196:666–670. https://doi:10.1111/j.1469-8137.2012.04309.x

Levinson G, Gutman GA (1987) Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol Biol Evol 4:203-21. https://10.1093/oxfordjournals.molbev.a040442

Lima FCT, Ribeiro AC (2011) Continental-scale tectonic controls of biogeography and ecology. In: Albert JS, RE Reis (Eds) Historical Biogeography of Neotropical Freshwater Fishes. California: University of California Press.

Lima-Filho PA, Amorim KDJ, Cioffi MB, Bertollo LAC, Molina WF (2015) Chromosomal Mapping of Repetitive DNAs in *Gobionellus oceanicus* and *G. stomatus* (Gobiidae; Perciformes): A Shared XX/XY System and an Unusual Distribution of 5S rDNA Sites on the Y Chromosome. Cytogenetic and Genome Research 144:333–340. https://10.1159/000373909

Li YC, Korol AB, Fahima T, Beiles A, Nevo E (2002)Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Mol Eco. 11:2453–2465. https://10.1046/j.1365-294x.2002.01643.x

Lui RL, Blanco DR, Margarido VP, Moreira-Filho O (2010) Chromosome characterization and biogeographic relations among three populations of the driftwood catfish *Parauchenipterus galeatus* (Linnaeus, 1766) (Siluriformes: Auchenipteridae) in Brazil. Biol J Linn Soc 99: 648-56. https://doi.org/10.1111/j.1095-8312.2009.01389.x

Lui RL, Blanco DR, Martinez JF, Margarido VP, Venere PC, Moreira-Filho O (2013) The role of chromosomal fusion in the karyotypic evolution of the genus *Ageneiosus* (Siluriformes: Auchenipteridae). Neotrop. Ichthyol 11:327-334. https://doi.org/10.1590/S1679-62252013005000004

Lui RL, Blanco DR, Traldi JB, Margarido VP, Moreira-Filho O (2015) Karyotypic variation of *Glanidium ribeiroi* Haseman, 1911 (Siluriformes, Auchenipteridae) along the Iguazu river basin. Brazilian Journal of Biology 75:215–221. https://doi:10.1590/1519-6984.10714

Lui RL, Traldi JB, Blanco DR, Margarido VP, Mariotto S, Centofante L, Artoni RF, Moreira-Filho O (2021) Possible Common Origin of B Chromosomes in Neotropical Fish (Siluriformes, Auchenipteridae) Reinforced by Repetitive DNA Mapping. Braz Arch Biol Technol 64. https://doi.org/10.1590/1678-4324-2021190494

Malimpensa GC, Traldi JB, Toyama D, Henrique-Silva F, Vicari MR, Moreira-Filho O (2018) Chromosomal Mapping of Repeat DNA in *Bergiaria westermanni* (Pimelodidae, Siluriformes): Localization of 45S rDNA in B Chromosomes. Cytogenetic and Genome Research 154:99-106. https://10.1159/000487652

Margarido VP, Moreira-Filho O (2008) Karyotypic differentiation through chromosome fusion and number reduction in I*mparfinis hollandi* (Ostariophysi, Heptapteridae). Genet Mol Biol 31:235-238. https://doi.org/10.1590/S1415-47572008000200012

Martins C, Galetti Jr PM (2001) Organization of 5S rDNA in species of the fish Leporinus: Two different genomic locations are characterized by distinct nontranscribed spacers. Genome 44:903-910. https://doi.org/10.1139/g01-069

Mazzoleni S, Augstenová B, Clemente L, Auer M, Fritz U, Praschag P, Protva T, Valensky P, Kratochvíl L, Rovatsos M (2020) Sex is determined by XX/XY sex chromosomes in Australasian side-necked turtles (Testudines: Chelidae). Sci Rep 10, 4276 (2020). https://doi.org/10.1038/s41598-020-61116-w

McNeil JA, Smith KP, Hall LL, Lawrence JB (2006) Word frequency analysis reveals enrichment of dinucleotide repeats on the human X chromosome and [GATA]n in the X escape region. Genome Res 16:477-484. https://doi/10.1101/gr.4627606

Messier W, Li SH, Stewar CB (1996) The birth of microsatellites. Nature 6:381. https://10.1038/381483a0

Milani D, Cabral-de-Mello DC (2014) Microsatellite Organization in the Grasshopper *Abracris flavolineata* (Orthoptera: Acrididae) Revealed by FISH Mapping: Remarkable Spreading in the A and B Chromosomes. PLoS ONE 9:e97956. https://10.1371/journal.pone.0097956

Metzgar D, Bytof J, Wills C (2000) Selection against frameshift mutations limits microsatellite expansion in coding DNA. Genome Res. 10, 72–80.

Moraes RLR, Bertollo LAC, Marinho MMF, Yano CF, Hatanaka T, Barby FF, … Cioffi MB (2017) Evolutionary Relationships and Cytotaxonomy Considerations in the Genus *Pyrrhulina* (Characiformes, Lebiasinidae). Zebrafish 14:536–546. https://10.1089/zeb.2017.1465

Moraes RLR, Sember A, Bertollo LAC, Oliveira EA, Ráb P, Hatanaka T, … Cioffi MB (2019) Comparative Cytogenetics and Neo-Y Formation in Small-Sized Fish Species of the Genus Pyrrhulina (Characiformes, Lebiasinidae). Frontiers in Genetics 10:678. https://10.3389/fgene.2019.00678

Morescalchi MA, Stingo V, Capriglione T (2011) Cytogenetic analysis in *Polypterus ornatipinnis* (Actinopterygii, Cladistia, Polypteridae) and 5S rDNA. Marine Genomics 4:25-31. https://10.1016/j.margen.2010.12.002

Nadir E, Margalit H, Gallily T, Ben-Sasson SA (1996) Microsatellite spreading in the human genome: evolutionary mechanisms and structural implications. Proc Natl Acad Sci USA 93:6470-6475. https://10.1073/pnas.93.13.6470

Oliveira EA, Bertollo LAC, Yano CF, Liehr T, Cioffi MB (2015) Comparative cytogenetics in the genus Hoplias (Characiformes, Erythrinidae) highlights contrasting karyotype evolution among congeneric species. Mol Cytogenet 8:56. https://doi.org/10.1186/s13039-015-0161-4

Oliveira EA, Sember A, Bertollo LAC, Yano CF, Ezaz T, Moreira-Filho O, Hatanaka T, Trifonov V, Liehr T, Al-Rikabi ABH, Ráb P, Pains H, Cioffi MB (2018) Tracking the evolutionary pathway of sex chromosomes among fishes: characterizing the unique XX/XY1Y2 system in *Hoplias malabaricus* (Teleostei, Characiformes). Chromosoma 127:115-128. https://10.1007/s00412-017-0648-3

Pathak RU, Mamillapalli A, Rangaraj N, Kumar R, Vasanthi D, Mishra K, Mishra RK (2013) AAGAG repeat RNA is an essential component of nuclear matrix in Drosophila. RNA Biol 10:564-571. https://doi.org/10.4161/rna.24326

Peakall R, Gilmore S, Keys W, Morgante M, Rafalski A (1998) Cross-species amplification of soybean (Glycine max) simple sequence repeats (SSRs) within the

genus and other legume genera: implications for the transferability of SSRs in plants. Molecular Biology and Evolution, 15:1275-1287. https://10.1093/oxfordjournals.molbev.a025856

Pineau P. Henry M. Suspène R, Marchio A. Dettai A. Debruyne R. Petit T. Lécu A. Moisson P. Dejean A. Wain-Hobson S. Vartanian JP (2005) A universal primer set for PCR amplification of nuclear histone H4 genes from all animal species. Mol Biol Evol 22:582-588. https://doi.org/10.1093/molbev/msi053

Pinkel D, Straume T, Gray J (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83:2934-2938. https://doi.org/10.1073/pnas.83.9.2934

Piscor D, Parise-Maltempi PP (2016) Microsatellite Organization in the B Chromosome and A Chromosome Complement in *Astyanax* (Characiformes, Characidae) Species. Cytogenetic and Genome Research 148:44-51. https://10.1159/000444728

Poltronieri J, Marquioni V, Bertollo LAC, Kejnovsky E, Molina WF, Liehr T, Cioffi MB (2014) Comparative Chromosomal Mapping of Microsatellites in *Leporinus* Species (Characiformes, Anostomidae): Unequal Accumulation on the W Chromosomes. Cytogenetic and Genome Research 142:40-45 https://10.1159/000355908

Prizon AC, Bruschi DP, Gazolla CB, Borin-Carvalho LA, Portela-Castro ALB (2018) Chromosome Spreading of the Retrotransposable Rex-3 Element and Microsatellite Repeats in Karyotypes of the Ancistrus Populations. Zebrafish 15:504-514. https://10.1089/zeb.2018.1620

Pucci MB, Barbosa P, Nogaroto V, Almeida MC, Artoni RF, Scacchetti PC, … Vicari, MR (2016) Chromosomal Spreading of Microsatellites and (TTAGGG)n Sequences in the *Characidium zebra* and *C. gomesi* Genomes (Characiformes: Crenuchidae). Cytogenetic and Genome Research 149:182-190. https://10.1159/000447959

Ruiz-Ruano FJ, Cuadrado Á, Montiel EE, Camacho JPM and López-León MD (2015) Next generation sequencing and FISH reveal uneven and nonrandom microsatellite distribution in two grasshopper genomes. Chromosoma 124:221-234. https://10.1007/s00412-014-0492-7

Saadi A (1993) Neotectônica da plataforma Brasileira: Esboço e Interpretação preliminares. Geonomos 1. https://doi.org/10.18285/geonomos.v1i1e2.233

Saadi A, Bezerra FHR, COSTA RD, IGREJA HLS, FRANZINELLI E (2005) Neotectõnica da Plataforma Brasileira. In: Souza CLG, Suguio K, Oliveira AMS, Oliveira PE. Quaternário do Brasil. Ribeirão Preto: Editora Holos, 211-234 pp.

Scacchetti PC, Utsunomia R, Pansonato-Alves JC, Vicari MR, Artoni RF, Oliveira C, Foresti F (2015) Chromosomal Mapping of Repetitive DNAs in *Characidium* (Teleostei, Characiformes): Genomic Organization and Diversification of ZW Sex Chromosomes. Cytogenetic and Genome Research, 146:136–143. https://10.1159/000437165

Salgado FS, Cunha MS, Melo S, Dergam JA (2021) Cytogenetic analysis of *Hypomasticus copelandii* and *H. steindachneri*: relevance of cytotaxonomic markers in the Anostomidae family (Characiformes). Comparative Cytogenetics 15(1): 65-76. https://doi.org/10.3897/compcytogen.v15.i1.61957

Santos DP, Felicetti D, Baumgärtner L, Margarido Margarido VP, Blanco DR, Moreira-Filho O, Lui RL (2021) Contributions to the taxonomy of *Trachelyopterus* (Siluriformes): comparative cytogenetic analysis in three species of Auchenipteridae. Neotropical Ichthyology 19:e200115. https://doi.org/10.1590/1982-0224-2020-0115

Saenjundaeng P, Supiwong W, Sassi FMC, Bertollo LAC, Rab P, Kretschmer R, Tanomtong A, Suwannapoom C, Reungsing M, Cioffi MB (2020) Chromosomes of Asian cyprinid fishes: Variable karyotype patterns and evolutionary trends in the genus Osteochilus (Cyprinidae, Labeoninae, “Osteochilini”). Genetics and Molecular Biology 43:e20200195. https://doi.org/10.1590/1678-4685-GMB-2020-0195

Santos FAB, Lemes RB, Otto PA (2021) HW_TEST, a program for comprehensive HARDY-WEINBERG equilibrium testing. Genet. Mol Biol 43:02. https://doi.org/10.1590/1678-4685-GMB-2019-0380

Sassi O, Bertollo N, Hatanaka M, et al (2019) Chromosomal Evolution and Evolutionary Relationships of Lebiasina Species (Characiformes, Lebiasinidae). Int J Mol Sci 20:2944. https://doi.org/10.3390/ijms20122944

Sassi FMC, Deon G A, Moreira-Filho O, Vicari MR, Bertollo LAC, Liehr T., … Cioffi MB (2020) Multiple Sex Chromosomes and Evolutionary Relationships in Amazonian Catfishes: The Outstanding Model of the Genus *Harttia* (Siluriformes: Loricariidae). Genes 11:1179. https://10.3390/genes11101179

Sassi FMC, Perez MF, Oliveira VCS, Deon GA, Souza FHS, Ferreira PHN, Oliveira EA, Hatanaka T, Liehr T, Bertollo LAC, Cioffi MB (2021) High Genetic Diversity despite Conserved Karyotype Organization in the Giant Trahiras from Genus Hoplias (Characiformes, Erythrinidae). Genes 12:252. https://doi.org/10.3390/genes12020252

Schwarzacher T, Heslop-Harrison P (2000) BIOS Scientific Publishers. Oxford, 2000 pp. 203.

Searle JB (1993) Chromosomal hybrid zones in eutherian mammals. In: RG Harrison (Eds) Hybrid zones and the evolutionary process. Oxford University Press. New York, 309-353.

Silva MJD, Araújo Vieira AP, Galvão Cipriano FM, Santos Cândido MR, Oliveira EHC, Pinheiro TG, Silva EL (2020) The Karyotype of Salvator merianae (Squamata, Teiidae): Analyses by Classical and Molecular Cytogenetic Techniques. Cytogenetic and Genome Research 160:94-99. https://doi:10.1159/000506140

Singh L, Purdom IF, Jones KW (1976) Satellite DNA and evolution of sex chromosomes. Chromosoma. 1 59:43-62. https://10.1007/BF00327708

Stebbins GL Jr (1950) Variation and evolution in plants. Columbia University Press, 644 pp. https://doi.org/10.7312/steb94536

Subramanian S, Mishra RK, Singh L (2003) Genome-wide analysis of Bkm sequences (GATA repeats): predominant association with sex chromosomes and potential role in higher order chromatin organization and function. Bioinformatics 19:681-685. https://doi.org/10.1093/bioinformatics/btg067

Supiwong W, Liehr T, Cioffi MB, Chaveerach A, Kosyakova N, Pinthong K, … Tanomtong A (2013) Karyotype and cytogenetic mapping of 9 classes of repetitive DNAs in the genome of the naked catfish *Mystus bocourti* (Siluriformes, Bagridae). Molecular Cytogenetics 6:51. https://10.1186/1755-8166-6-51

Toth G, Gaspari Z, Jurka J (2000) Microsatellites in different eukaryotic genomes: survey and analysis. Genome Res. 10:967–981. https://10.1101/gr.10.7.967

Traldi JB, Blanco DR, Vicari MR, Martinez JF, Lui RL, Artoni RF, Moreira-Filho O (2013) Physical mapping of (GATA) n and (TTAGGG) n sequences in species of Hypostomus (Siluriformes, Loricariidae). J Genet 92:127-130. https://doi.org/10.1007/s12041-013-0224-4

Traldi JB, Vicari MR, Fátima JM, Blanco DR, Lui RL, Azambuja M, … Moreira-Filho O (2020) Recent Apareiodon species evolutionary divergence (Characiformes: Parodontidae) evidenced by chromosomal and molecular inference. Zoologischer Anzeiger 289:166-176. https://10.1016/j.jcz.2020.10.010

Travenzoli NM, Lima BA, Cardoso DC, Dergam JA, Fernandes-Salomão TM, Lopes DM (2019) Cytogenetic Analysis and Chromosomal Mapping of Repetitive DNA in Melipona Species (Hymenoptera, Meliponini). Cytogenet Genome Res 158:213-224. https://doi.org/10.1159/000501754

Utsunomia R, Melo S, Scacchetti PC, Oliveira C, Machado MA, Pieczarka JC, … Foresti F (2018) Particular Chromosomal Distribution of Microsatellites in Five Species of the Genus *Gymnotus* (Teleostei, Gymnotiformes). Zebrafish 15:398-403. https://10.1089/zeb.2018.1570

Vanzela ALL, Swarça AC, Dias AL, Stolf R, Ruas PM, Ruas CF, Sbalgueiro IJ, Giuliano-Caetano L (2002) Differential distribution of (GA)9 + C microsatellite on chromosomes of some animal and plant species. Cytologia 67:9-13. https://10.1508/cytologia.67.9

Viana P, Ezaz T, de Bello Cioffi M, Jackson Almeida B, Feldberg E (2019) Evolutionary Insights of the ZW Sex Chromosomes in Snakes: A New Chapter Added by the Amazonian Puffing Snakes of the Genus Spilotes. Genes 10:288. https://doi.org/10.3390/genes10040288

Yano CF, Poltronieri J, Bertollo LAC, Artoni RF, Liehr T, Cioffi MB (2014) Chromosomal Mapping of Repetitive DNAs in *Triportheus trifurcatus* (Characidae, Characiformes): Insights into the Differentiation of the Z and W Chromosomes. PLoS ONE 9:e90946. https://doi.org/10.1371/journal.pone.0090946

Yeesin P, Buasriyot P, Ditcharoen S, Chaiyasan P, Suwannapoom C, Juntaree S, Jantarat S, Talumphai S, Cioffi MB, Liehr T, Tanomtong A, Supiwong W. Comparative study of four *Mystus* species (Bagridae, Siluriformes) from Thailand: insights into their karyotypic diversity (2021) Comp Cytogenet 15:119-136. https://10.3897/CompCytogen.v15i2.60649

Weber JL (1990) Informativeness of human (dC-dA)n (dGdT)n polymorphisms. Genomics 7:524–530. https://10.1016/0888-7543(90)90195-z

White S (1978) Modes of Speciation. Michael J. D. White W. H. Freeman and Co., 456 pp. https://doi:10.1017/S0094837300006072

White T, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J. (Eds) PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Ziemniczak K, Traldi JB, Nogaroto V, Almeida MC, Artoni RF, Moreira-Filho O, Vicari MR (2015) In situ Localization of (GATA)n and (TTAGGG)n Repeated DNAs and W Sex Chromosome Differentiation in Parodontidae (Actinopterygii: Characiformes). Cytogenetic and Genome Research 144:325–332. https://10.1159/000370297

**Figures:**

**Figure 1**. SSR $(GATA)_n$ distribution pattern in the 18S rDNA carrier of *Trachelyopterus* species. 18s rDNA probes in green signal and $(GATA)_n$ probes in red signal.

**Figure 2**. SSR (GATA)n distribution pattern in the U2 snRNA carrier of *Trachelyopterus* species. U2 snRNA probes in green signal and $(GATA)_n$ probes in red signal. AA: Both chromosomes with U2 snRNA sites in the short arm and SSR $(GATA)_n$ only in the long arm; AB: One chromosome with U2 snRNA site in the long arm and another chromosome with the U2 snRNA site in the short arm, both with the SSR $(GATA)_n$ only in the long arm of the chromosome; BB: both chromosomes with U2 snRNA sites in the long arm and the SSR $(GATA)_n$ only in the long arm; CC: both chromosomes with U2 snRNA sites in the short arm of the chromosome and the SSR $(GATA)_n$ in the long and short arm of the chromosome. Bar = 5 uM.

**Figure 3**. SSR (GATA)n distribution pattern in the 5S rDNA carrier of *Trachelyopterus* species. 5S rDNA probes in green signal and (GATA)n probes in red signal.

**Figure 4**. SSR $(GATA)_n$ distribution pattern in the H3 and H4 histone genes carrier of *Trachelyopterus* species. H3/H4 histone genes in red signal, 18S rDNA in green signal and $(GATA)_n$ probes in green signal.

**Figure 5.** Ideogram of the $(GATA)_n$ distribution pattern in the chromosomes carrying the 5S rDNA, 18S rDNA, H3 and H4 histone genes and U2 snRNA

**Tables:**

**Table 1.** An overview of published cytogenetic mappings with microsatellites in neotropical fish species and their distribution patterns. 2n: Diploid number; Ref: references.

**Table 2.** Hardy-Weinberg Equilibrium test for *Trachelyopterus galeatus* (GATA)n polymorphism from Amazonas River basin and *Trachelyopterus* aff. *galeatus* U2 snRNA and $(GATA)_n$ polymorphism from Araguaia River basin.

**Funding:**

The research leading to these results received funding from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Universidade Estadual do Oeste do Paraná (Unioeste) and Postgraduate Program in Conservação e Manejo de Recursos Naturais (Unioeste-PPRN).

**Competing interests:**

The authors have no conflicts of interest/competing interest to declare.

**Ethics approval**:

Fish collections were authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, Permit number 49379-1), and the experimental procedures

were approved by the Ethics Committee on Animal Experimentation and Practical Classes at Unioeste (09/13-CEEAAP/Unioeste)

**Acknowledgements**

We thank to Dr. Heraldo Antonio Britski and Dr. José Luís Olivan Birindelli for the identification of the specimens; Pedro Luis Gallo and Luis Henrique da Silva for their assistance in sampling; to Chico Mendes Institute for Biodiversity Conservation (Permit number 10538-1) for authorizing the collection of the specimens; to Minas Gerais State Forestry Institute for assisting the specimens collections (Rio Doce State Park); Sr. Osmar Pereira de Barros, owner of the Ana Maria farm in São Miguel do Araguaia, who helped us collect the fish in this region. We also thank to Mestrado em Conservação e Manejo de Recursos Naturais (PPRN-Unioeste), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná (FA) for supporting this study.

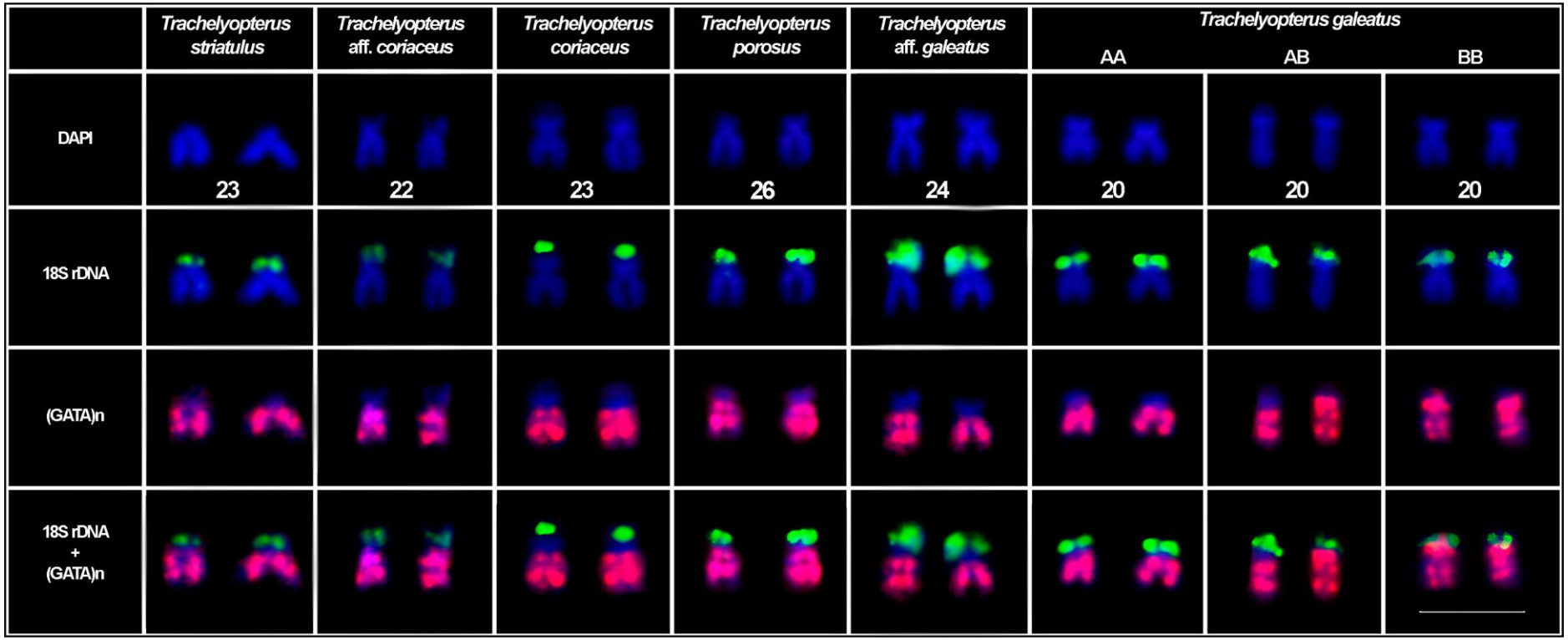



**Figure 1**. SSR (GATA)n distribution pattern in the 18S rDNA carrier of *Trachelyopterus* species. 18S rDNA probes in green signal and (GATA)n probes in red signal. Bar = 5 uM.

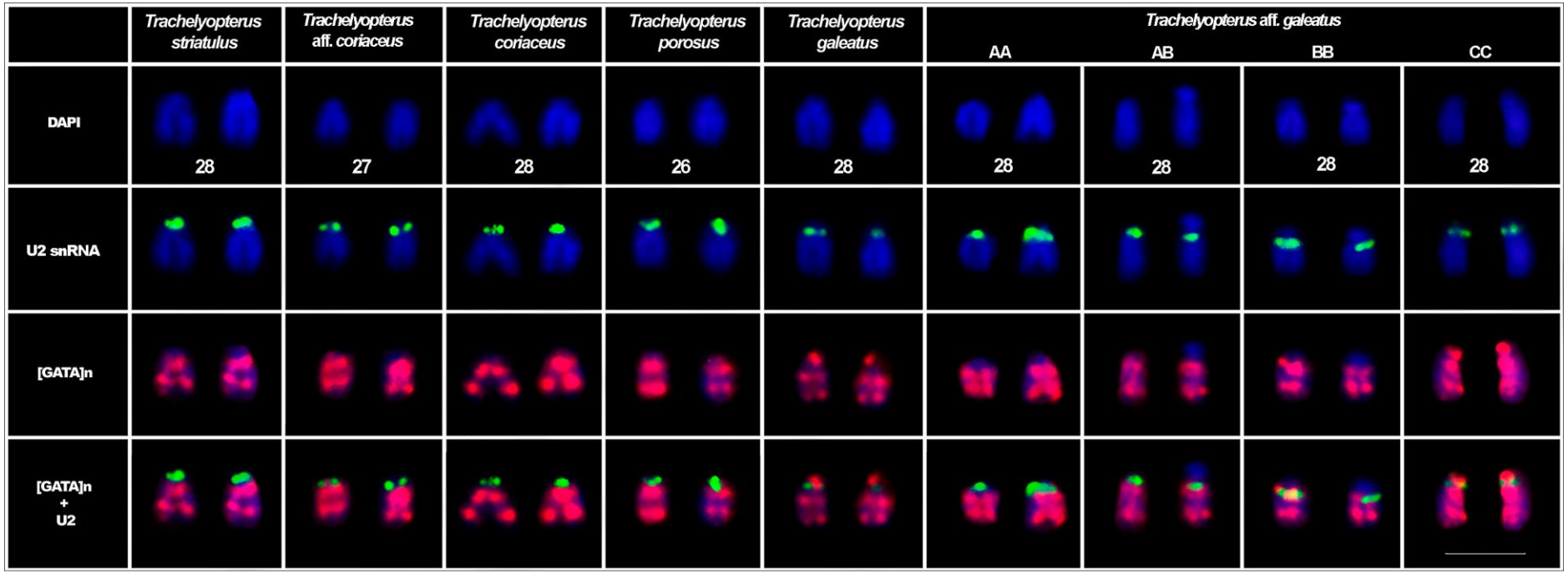



**Figure 2**. SSR (GATA)n distribution pattern in the U2 snRNA carrier of *Trachelyopterus* species. U2 snRNA probes in green signal and (GATA)n probes in red signal. AA: Both chromosomes with U2 snRNA sites in the short arm and SSR (GATA)n only in the long arm; AB: One chromosome with U2 snRNA site in the long arm and another chromosome with the U2 snRNA site in the short arm, both with the SSR (GATA)n only in the long arm of the chromosome; BB: both chromosomes with U2 snRNA sites in the long arm and the SSR (GATA)n only in the long arm; CC: both chromosomes with U2 snRNA sites in the short arm of the chromosome and the SSR (GATA)n in the long and short arm of the chromosome. Bar = 5 uM.

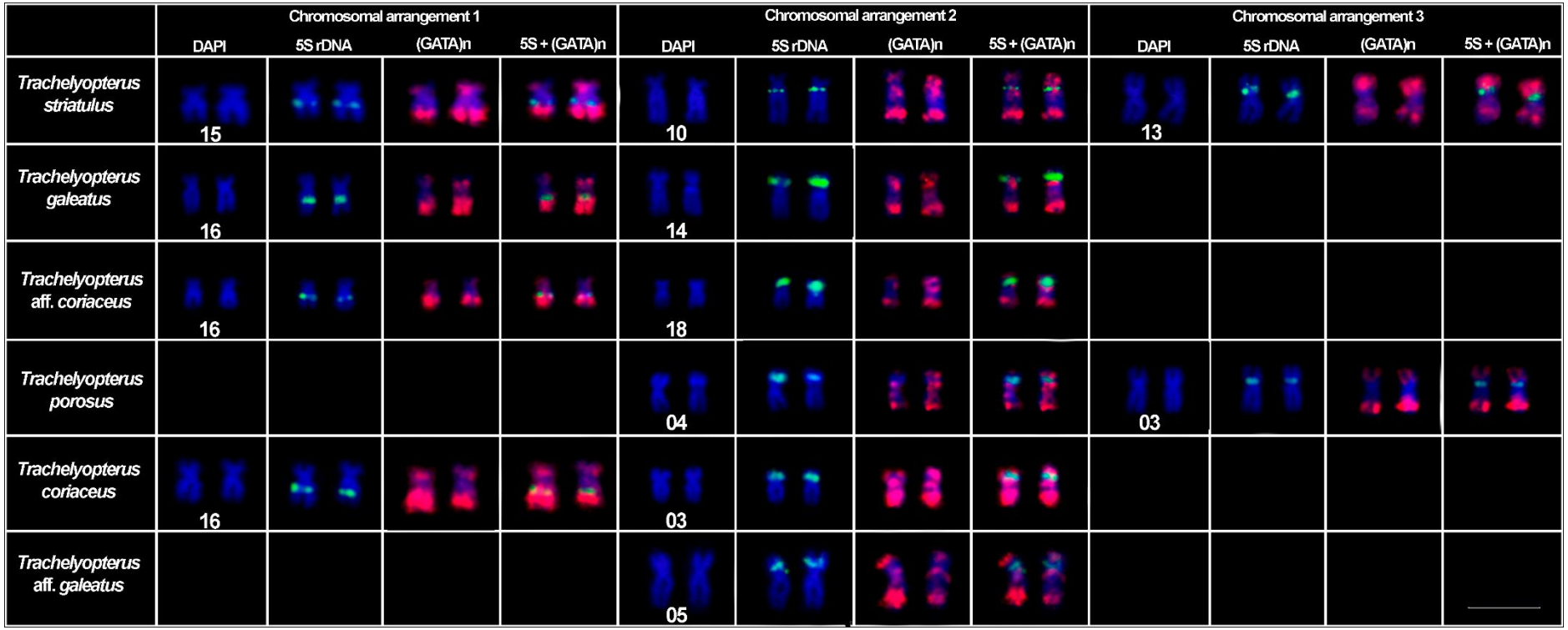



**Figure 3**. SSR (GATA)n distribution pattern in the 5S rDNA carrier of *Trachelyopterus* species. 5S rDNA probes in green signal and (GATA)n probes in red signal. Bar = 5 uM.

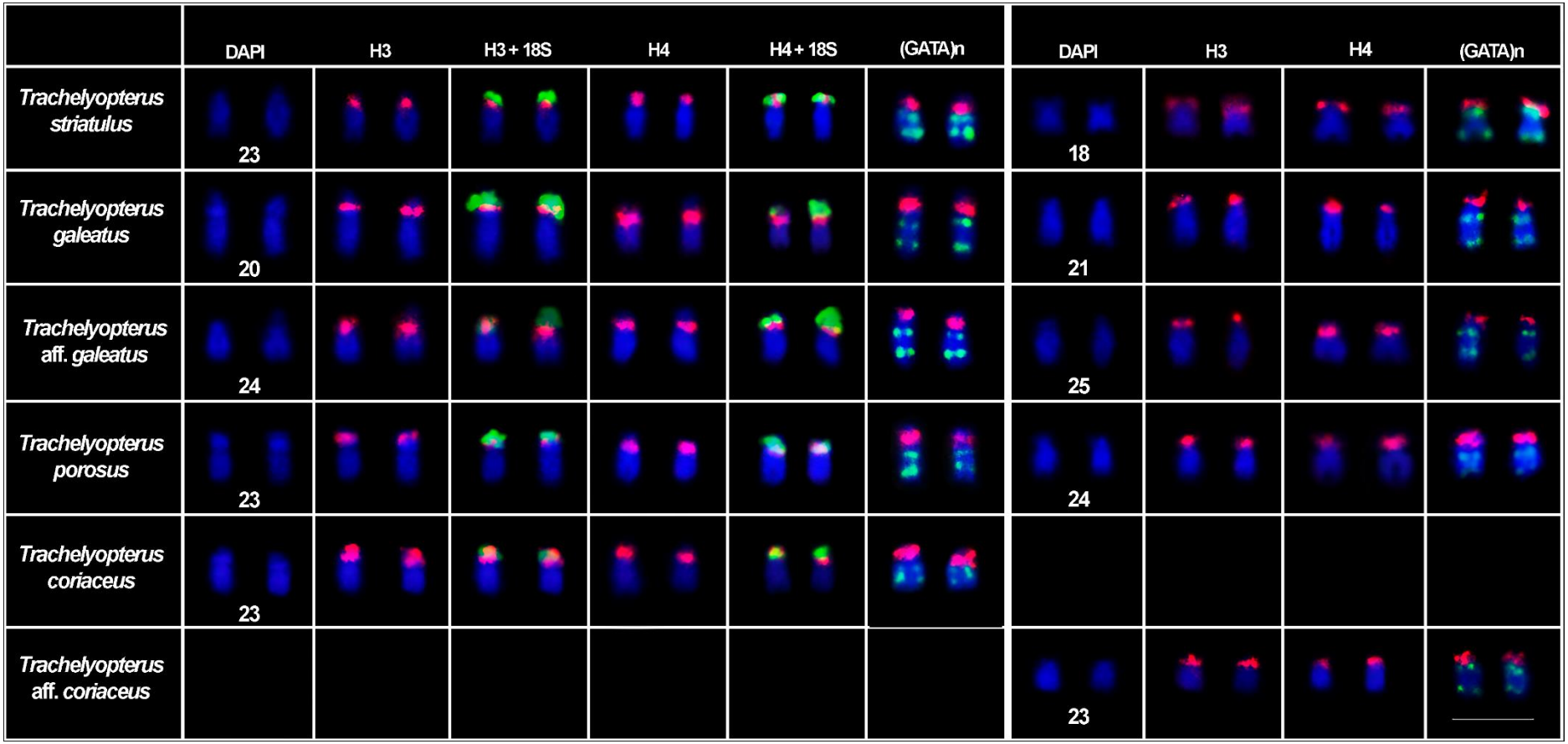



**Figure 4**. SSR (GATA)n distribution pattern in the H3 and H4 histone genes carrier of *Trachelyopterus* species. H3/H4 histone genes in red signal, 18S rDNA in green signal and (GATA)n probes in green signal. Bar = 5 uM.

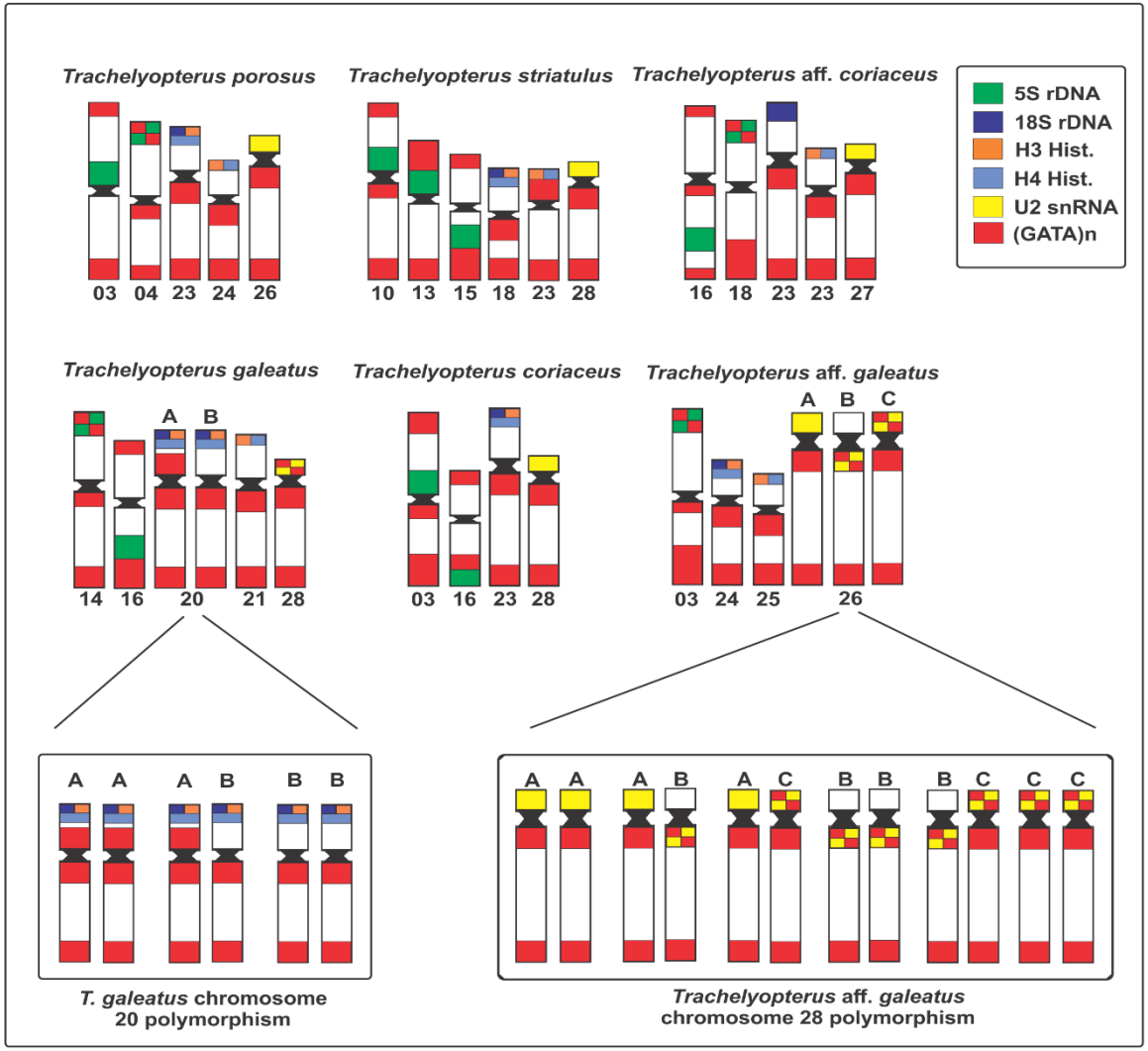



**Figure 5.** Ideogram of the $(GATA)_n$ distribution pattern in the chromosomes carrying the 5S rDNA, 18S rDNA, H3 and H4 histone genes and U2 snRNA.

**Table 1.** An overview of published cytogenetic mappings with microsatellites in neotropical fish species and their distribution patterns. 2n: Diploid number; Ref: references. Karyoevolution: evolution of the karyotype as a whole, without highlighting a particular chromosome or event.

| Specie | 2n | Microsatellite/taxon | Distribution pattern | Main Contributions | Ref |
|---|---|---|---|---|---|
| | | **SILURIFORMES** | | | |
| | | Auchenipteridae | | | |
| *Ageneiosus inermis* | 56 | $(GATA)_n$ | Scattered | Karyoevolution | 6 |
| *Glanidium riberoi* | 58 | $(GATA)_n$ | Scattered | Karyoevolution | 3 |
| *Trachelyopterus galeatus* | 58 | $(GATA)_n$ | Scattered | B chromosomes | 1,2 |
| *Trachelyopterus* aff. *galeatus* | 58 | $(GATA)_n$ | Scattered | - | 1 |
| *Trachelyopterus coriaceus* | 58 | $(GATA)_n$ | Scattered | - | 1 |
| *Trachelyopterus* aff. *coriaceus* | 58 | $(GATA)_n$ | Scattered | B chromosomes | 1,4 |
| *Trachelyopterus striatulus* | 58 | $(GATA)_n$ | Scattered | B chromosomes | 1,4 |
| *Trachelyopterus porosus* | 58 | $(GATA)_n$ | Scattered | B chromosomes | 1,2 |
| | | Loricariidae | | | |
| *Ancistrus* | - | $(GA)_{15}$ and $(CA)_{15}$ | Scattered | Sex chromosomes | 16 |
| *Astroblepus grixalvii* | 52 | $(GA)_{15}$ and $(CA)_{15}$ | Subtelomeric | Karyoevolution | 11 |
| *Astroblepus homodon* | 54 | $(GA)_{15}$ and $(CA)_{15}$ | Subtelomeric | Karyoevolution | 11 |
| *Harttia punctata* | 58 | $(GATA)_n$ | No signal | - | 7 |
| *Harttia duriventris* | 55/56 | $(A)_{30}$, $(CA)_{15}$ and $(GA)_{15}$ | Scattered/clustered | Sex chromosomes | 12 |
| *Harttia villasboas* | 55/56 | $(A)_{30}$, $(CA)_{15}$ and $(GA)_{15}$ | Scattered/clustered | Sex chromosomes | 12 |
| *Harttia rondoni* | 54 | $(A)_{30}$, $(CA)_{15}$ and $(GA)_{15}$ | Scattered/clustered | Sex chromosomes | 12 |
| *Hypostomus ancistroides* | 68 | $(GATA)_n$ | Scattered/dispersal | Karyoevolution | 5 |
| *Hypostomus iheringii* | 80 | $(GATA)_n$ | Scattered/dispersal | Karyoevolution | 5 |
| *Hypostomus nigromaculatus* | 76 | $(GATA)_n$ | Scattered/dispersal | Karyoevolution | 5 |
| *Hypostomus tapijara* | 66 | $(GATA)_n$ | Scattered/dispersal | Karyoevolution | 5 |
| *Panaqolus tankei* | 52 | $(AC)_{15}$, $(GA)_{15}$ and $(GT)_{15}$ | Scattered/subtelomeric | Karyoevolution | 14 |
| *Rinelocaria latirostris* | 44 | $(GA)_{15}$ | Dispersal/clustered | Karyoevolution | 9 |
| | | Heptapteridae | | | |
| *Imparfins schubarti* | 58 | $(GA)_{15}$, $(GACA)_4$, $(GAA)_7$, $(CAC)_5$, and $(CA)_8$ | Dispersal/clustered | Karyoevolution | 9,10 |
| *Imparfinis borodini* | 50 | $(GA)_{15}$, $(GACA)_4$, $(GAA)_7$, $(CAC)_5$, and $(CA)_8$ | Scattered/clustered | Karyoevolution | 10 |
| *Pimelodella cf. chagresi* | | $(CA)_{15}$ and $(GA)_{15}$ | Subtelomeric | Sex chromosomes | 13 |
| | | Pimelodidae | | | |
| *Bergiaria westermanni* | 56 | $(GATA)_n$ | Scattered | Sex chromosomes | 15 |
| *Steindachneridion scripta* | 56 | $(GA)_{15}$ | Dispersal/clustered | Karyoevolution | 9 |

| Specie | 2n | Microsatellite/taxon | Distribution pattern | Main Contributions | Ref |
|---|---|---|---|---|---|
| | | **GYMNOTIFORMES** | | | |
| | | Gymnotidae | | | |
| *Gymnotus sylvius* | 40 | $(A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(GA)_{15}$, $(GC)_{15}$, $(TTA)_{10}$, $(CAA)_{10}$, and $(GAG)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 17 |
| *Gymnotus cuia* | 54 | $(A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(GA)_{15}$, $(GC)_{15}$, $(TTA)_{10}$, $(CAA)_{10}$, and $(GAG)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 17 |
| *Gymnotus pantanal* | 39/40 | $(A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(GA)_{15}$, $(GC)_{15}$, $(TTA)_{10}$, $(CAA)_{10}$, and $(GAG)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 17 |
| *Gymnotus capanema* | 34 | $(A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(GA)_{15}$, $(GC)_{15}$, $(TTA)_{10}$, $(CAA)_{10}$, and $(GAG)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 17 |
| *Gymnotus carapo* | 42 | $(A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(GA)_{15}$, $(GC)_{15}$, $(TTA)_{10}$, $(CAA)_{10}$, and $(GAG)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 17 |
| | | **GOBIIFORMES** | | | |
| | | Gobiidae | | | |
| *Gobionellus oceanicus* | 56 | $(CA)_{15}$ | Subtelomeric/dispersal | Sex chromosomes | 18 |
| *Gobionellus stomatus* | 56 | $(CA)_{15}$ | Subtelomeric/dispersal | Sex chromosomes | 18 |
| | | **CHARACIFORMES** | | | |
| | | Anostomidae | | | |
| *Leporinus elongatus* | 54 | $(A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(GC)_{15}$, $(GA)_{15,}$ $(GAA)_{10}$, $(CAG)_{10}$, and $(CAT)_{10}$ | Scattered, clustered, and mixed | Sex chromosomes | 19 |
| *Leporinus conirostris* | 54 | $(A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(GC)_{15}$, $(GA)_{15,}$ $(GAA)_{10}$, $(CAG)_{10}$, and $(CAT)_{10}$ | Scattered, clustered, and mixed | Sex chromosomes | 19 |
| *Leporinus obtusidens* | 54 | $(A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(GC)_{15}$, $(GA)_{15,}$ $(GAA)_{10}$, $(CAG)_{10}$, and $(CAT)_{10}$ | Scattered, clustered, and mixed | Sex chromosomes | 19 |
| *Leporinus reinhardti* | 54 | $(A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(GC)_{15}$, $(GA)_{15,}$ $(GAA)_{10}$, $(CAG)_{10}$, and $(CAT)_{10}$ | Scattered, clustered, and mixed | Sex chromosomes | 19 |
| *Astyanax altiparanae* | 50 | $(CA)_{15}$, $(GA)_{15}$, $(CG)_{15}$, $(GACA)_4$ and $(GATA)_8$ | Scattered, clustered, and mixed | B chromosome | 20 |
| *Astyanax fasciatus* | 46 | $(CA)_{15}$, $(GA)_{15}$, $(CG)_{15}$, $(GACA)_4$ and $(GATA)_8$ | Scattered, clustered, and mixed | B chromosome | 20 |
| *Astyanax marionae* | 48 | $(CA)_{15}$, $(GA)_{15}$, $(CG)_{15}$, $(GACA)_4$ and $(GATA)_8$ | Scattered, clustered, and mixed | B chromosome | 20 |
| *Astyanax. schubarti* | 36 | $(CA)_{15}$, $(GA)_{15}$, $(CG)_{15}$, $(GACA)_4$ and $(GATA)_8$ | Scattered, clustered, and mixed | B chromosome | 20 |

| Specie | 2n | Microsatellite/taxon | Distribution pattern | Main Contributions | Ref |
|---|---|---|---|---|---|
| *Astyanax mexicanus* | 50 | $(CA)_{15}$, $(GA)_{15}$, $(CG)_{15}$, $(GACA)_4$ and $(GATA)_8$ | Scattered, clustered, and mixed | B chromosome | 20 |
| Lebiasinidae | | | | | |
| *Pyrrhulina australis* | 40 | $(CA)_{15}$, $(GA)_{15}$ | Scattered, clustered, and mixed | Sex chromosomes | 21, 26 |
| *Pyrrhulina aff. australis* | 40 | $(CA)_{15}$, $(GA)_{15}$ | Scattered, clustered, and mixed | Sex chromosomes | 21, 26 |
| *Pyrrhulina brevis* | 42 | $(CA)_{15}$, $(GA)_{15}$ | Scattered, clustered, and mixed | Sex chromosomes | 21 |
| *Pyrrhulina semifasciata* | 41 | $(CA)_{15}$, $(GA)_{15}$ | Scattered, clustered, and mixed | Sex chromosomes | 21 |
| *Lebiasina bimaculata* | 36 | $(CA)_{15}$, $(GA)_{15}$, $(CAT)_{10}$, and $(CGG)_{10}$ | Scattered, clustered, and mixed | Sex chromosomes | 27 |
| *Lebiasina melanoguttata* | 36 | $(CA)_{15}$, $(GA)_{15}$, $(CAT)_{10}$, and $(CGG)_{10}$ | Scattered, clustered, and mixed | Sex chromosomes | 27 |
| Erythrinidae | | | | | |
| *Erythrinus erythrinus* | 51 | $(CA)_{15}$, $(CAA)_{10}$, $(CAC)_{10}$, $(CAG)_{10}$, $(CAT)_{10}$, $(CGG)_{10}$, $(GA)_{15}$, $(GAA)_{10}$, $(GAG)_{10}$ and $(TA)_{15}$ | Scattered, clustered, and mixed | Sex chromosomes | 22, 38 |
| *Hoplias intermedius* | 50 | $(CAA)_{10}$, $(GA)_{15}$ and $(CA)_{15}$ | Scattered, clustered, and mixed | Karyoevolution | 23 |
| *Hoplias brasiliensis* | 50 | $(A)_{30,}$ $(CA)_{15}$, $(CAA)_{10}$, $(GA)_{15,}$ $(CA)_{15,}$ and $(CAC)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 23, 41 |
| *Hoplias aimara* | 50 | $(A)_{30,}$ $(CA)_{15}$, $(CAA)_{10}$, $(GA)_{15,}$ $(CA)_{15,}$ and $(CAC)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 23, 41 |
| *Hoplias lacerdae* | 50 | $(A)_{30,}$ $(CA)_{15}$, $(CAA)_{10}$, $(GA)_{15,}$ $(CA)_{15,}$ and $(CAC)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 23, 41 |
| *Hoplias malabaricus* | 50 | $(A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(GA)_{15}$, $(TA)_{15}$, $(CAC)_{10}$, $(CAT)_{10}$, $(GAC)_{10}$,$(GAG)_{10}$, and $(CGG)_{10,}$ $(GC)_{15}$, $(CAA)_{10}$, $(CAC)_{10}$, $(CAT)_{10}$, $(TAA)_{10}$ | Scattered, clustered, and mixed | Sex chromosomes | 29, 40, 42 |
| *Hoplias australis* | 50 | $(A)_{30}$, $(CA)_{15}$, $(GA)_{15}$, and $(CAC)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 41 |
| *Hoplias curupira* | 50 | $A)_{30}$, $(CA)_{15}$, $(GA)_{15}$, and $(CAC)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 41 |
| *Hoplias intermedius* | 50 | $A)_{30}$, $(CA)_{15}$, $(GA)_{15}$, and $(CAC)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 41 |
| Crenuchidae | | | | | |

| Specie | 2n | Microsatellite/taxon | Distribution pattern | Main Contributions | Ref |
|---|---|---|---|---|---|
| *Characidium zebra* | 50 | $(A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(CAA)_{10}$, $(CAC)_{10}$, $(CAG)_{10}$, $(CAT)_{10}$, $(CG)_{15}$, $(CGG)_{10}$, $(GA)_{15}$, $(GAA)_{10}$, $(GAC)_{10}$, $(GACA)_4$, $(GAG)_{10}$ ,$(TA)_{15}$, $(TAA)_{10}$ and $(GATA)_7$ | Scattered, clustered, and mixed | Karyoevolution | 24 |
| *Characidium gomesi* | 50 | $A)_{30}$, $(C)_{30}$, $(CA)_{15}$, $(CAA)_{10}$, $(CAC)_{10}$, $(CAG)_{10}$, $(CAT)_{10}$, $(CG)_{15}$, $(CGG)_{10}$, $(GA)_{15}$, $(GAA)_{10}$, $(GAC)_{10}$, $(GACA)_4$, $(GAG)_{10}$ ,$(TA)_{15}$, $(TAA)_{10}$ and $(GATA)_7$ | Scattered, clustered, and mixed | Sex chromosomes | 24, 32 |
| *Hepsetus odoe* | 58 | $(A)_{30}$, $(CA)_{15}$, $(GA)_{15}$, $(CAC)_{10}$, $(CGG)_{10}$, $(GAA)_{10}$ and $(GAG)_{10}$ | Scattered, clustered, and mixed | Karyoevolution | 28 |
| *Characidium cf. zebra* | 50 | $(CA)_{15}$, $(GA)_{15}$, $(CG)_{15}$, and $(TTA)_{10}$ | Subtelomeric/clustered | Sex chromosomes | 32 |
| *Characidium vidali* | 50 | $CA)_{15}$, $(GA)_{15}$, $(CG)_{15}$, and $(TTA)_{10}$ | Subtelomeric/clustered | Sex chromosomes | 32 |
| *Characidium pterostictum* | 50 | $CA)_{15}$, $(GA)_{15}$, $(CG)_{15}$, and $(TTA)_{10}$ | Subtelomeric/clustered | Sex chromosomes | 32 |
| *Characidium timbuiense* | 50 | $CA)_{15}$, $(GA)_{15}$, $(CG)_{15}$, and $(TTA)_{10}$ | Subtelomeric/clustered | Sex chromosomes | 32 |
| *Characidium lanei* | 50 | $CA)_{15}$, $(GA)_{15}$, $(CG)_{15}$, and $(TTA)_{10}$ | Subtelomeric/clustered | Sex chromosomes | 32 |
| *Characidium alipioi* | 50 | $(CA)_{15}$, $(GA)_{15}$ and $(GAG)_{10}$ | Scattered/clustered | B chromosomes | 37 |
| Anostomidae | | | | | |
| *Hypomasticus copelandii* | 54 | $(CA)_{15}$, $(GA)_{15}$ | Subtelomeric | Karyoevolution | 25 |
| *Hypomasticus steindachneri* | 54 | $(CA)_{15}$, $(GA)_{15}$ | Subtelomeric | Karyoevolution | 25 |
| Characidae | | | | | |
| *Astyanax scabripinnis* | 50 | $(CA)_{15}$, $(CAC)_{10}$, $(CAG)_{10}$, $(CAT)_{10}$, $(GA)_{15}$, $(GAA)_{10}$, $(GAG)_{10}$, $(GC)_{15}$ and $(GATA)_n$ | Scattered, clustered, and mixed | Karyoevolution | 34 |
| *Astyanax bockmanni* | - | $(AG)_{15}$ | Abundant in B chromosomes | B chromosomes | 36 |
| *Astyanax fasciatus* | 45-47 | $(AG)_{15}$ | Abundant in B chromosomes | B chromosomes | 36 |
| *Astyanax paranae* | - | $(AG)_{15}$ | Abundant in B chromosomes | B chromosomes | 36 |
| *Triportheus trifurcatus* | 52 | $(CA)_{15}$, $(CAA)_{10}$, $(CAC)_{10}$, $(CAG)_{10}$, $(CAT)_{10}$, $(CGG)_{10}$, $(GA)_{15}$, $(GAA)_{10}$ and $(TA)_{15}$ | Scattered, clustered, and mixed | Sex chromosomes | 30 |
| *Hyphessobrycon eques* | 52 | $(A)_{30}$, $(CA)_{15}$, $(CAG)_{10}$ and $(GATA)_8$ | Scattered, clustered, and mixed | Karyoevolution | 31 |
| Parodontidae | | | | | |
| *Apareiodon* sp. | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 35, 43 |
| *Apareiodon piracicabae* | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 44 |
| *Apareiodon hasemani* | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 44 |
| *Apareiodon ibitiensis* | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 44 |

| Specie | 2n | Microsatellite/taxon | Distribution pattern | Main Contributions | Ref |
|---|---|---|---|---|---|
| *Apareiodon affinis* | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 44 |
| *Parodon* cf. *pongoensis* | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 44 |
| *Parodon nasus* | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 44 |
| *Parodon hilarii* | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 44 |
| *Apareiodon machrisi* | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 44 |
| *Apareiodon cavalcante* | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 44 |
| *Apareiodon* sp. 1 | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 44 |
| *Apareiodon* sp. 2 | 54 | $(GATA)_n$ | Scattered, clustered, and mixed | Sex chromosomes | 44 |
| Prochilodontidae | | | | | |
| *Semaprochilodus insignis* | 54 | $(CA)_{22}$, $(GA)_{20}$, $(GT)_{17}$, $(CT)_{26}$, $(CT)_{14}GT(CT)_5(CG)_2(CT)_9$, $(GT)_9CA(GT)_7CG(GT)_{19}$ | Dispersal/clustered | Sex chromosomes | 39 |
| *Semaprochilodus taeniurus* | 54 | $CA)_{22}$, $(GA)_{20}$, $(GT)_{17}$, $(CT)_{26}$, $(CT)_{14}GT(CT)_5(CG)_2(CT)_9$, $(GT)_9CA(GT)_7CG(GT)_{19}$ | Dispersal/clustered | Sex chromosomes | 39 |

**Table 2.** Hardy-Weinberg Equilibrium test for *Trachelyopterus galeatus* (GATA)n polymorphism from Amazonas River basin and *Trachelyopterus* aff. *galeatus* U2 snRNA and $(GATA)_n$ polymorphism from Araguaia River basin.

| Genotype | Genotype Absolut frequencies | | Genotype relative frequencies | | χ2 | p value* |
|---|---|---|---|---|---|---|
| *Trachelyopterus galeatus* $(GATA)_n$ polymorphism - Amazonas | | | | | | |
| | **observed** | **expected** | **observed** | **expected** | | |
| **AA** | 3 (2♂ 1♀) | 3.841 | 0.273 | 0.349 | 0.184 | |
| **AB** | 7(2♂ 5♀) | 5.318 | 0.636 | 0.483 | 0.531 | |
| **BB** | 1(1♂) | 1.841 | 0.091 | 0.167 | 0.384 | |
| | | | | **Total** | **1.099** | **0.2942** |
| *Trachelyopterus* aff. *galeatus* U2 snRNA and $(GATA)_n$ polymorphism - Araguaia | | | | | | |
| | **observed** | **expected** | **observed** | **expected** | | |
| **AA** | 1 (1♀) | 0.766 | 0.063 | 0.048 | 0.071 | |
| **AB** | 5 (2♂ 3♀) | 3.719 | 0.313 | 0.232 | 0.441 | |
| **AC** | 0 | 1.75 | 0 | 0.109 | 1.75 | |
| **BB** | 6 (1♂ 5♀) | 4.516 | 0.375 | 0.282 | 0.487 | |
| **BC** | 0 | 4.25 | 0 | 0.266 | 4.25 | |
| **CC** | 4 (2♂ 2♀) | 1 | 0.25 | 0.063 | 9 | |
| | | | | **Total** | **15.999** | **0.0011** |

## 6. CONCLUSÕES

Os aspectos evidenciados por este estudo permitiram inferir novas informações sobre a diversidade de *Trachelyopterus*, entre elas: (1) reiterar a existência de uma possível nova espécie, *Trachelyopterus* aff. *galeatus* da bacia do rio Araguaia, sugerido por Santos et al. (2021) com base em marcadores ribossomais; (2) corroborar com a hipótese de sinonímia entre "*Parauchenipterus*" e *Trachelyopterus*, uma vez que as espécies do gênero *Trachelyopterus* (ver Akama, 2004) se mostraram mais semelhantes às espécies de "*Parauchenipterus*" do que entre si; que (3) a amostra de nosso estudo identificada pela morfologia como *Trachelyopterus* aff. *coriaceus* pode de fato constituir uma nova espécie, que foi proposta para ser encontrada nas bacias dos rios Paraná e Paraguai (a população deste estudo é da bacia do rio Paraguai) (ver Akama, 2004); e que (4) o mapeamento integrado (guiado) mostrou-se uma metodologia eficiente para estudar microssatélites dispersos, uma vez que, por meio dele, novos arranjos cromossômicos, incluindo dois polimorfismos, foram evidenciados nessas espécies de *Trachelyopterus*.