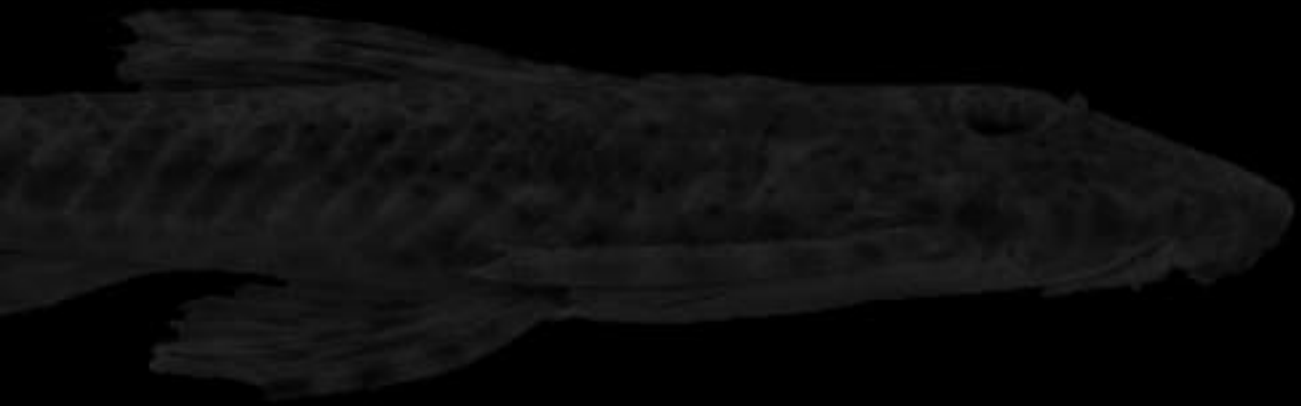
Tese de Doutorado

Relações evolutivas e história demográfica em espécies de *Harttia* Steindachner, 1876



Francisco de Menezes Cavalcante Sassi

São Carlos - 2025

UNIVERSIDADE FEDERAL DE SÃO CARLOS
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM
GENÉTICA EVOLUTIVA E BIOLOGIA MOLECULAR

Relações evolutivas e história demográfica em espécies de *Harttia* Steindachner, 1876

Francisco de Menezes Cavalcante Sassi

São Carlos – SP

2025

UNIVERSIDADE FEDERAL DE SÃO CARLOS

CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM

GENÉTICA EVOLUTIVA E BIOLOGIA MOLECULAR

Francisco de Menezes Cavalcante Sassi

Relações evolutivas e história demográfica em espécies de *Harttia* Steindachner, 1876

Tese apresentada ao Programa de Pós-Graduação em Genética Evolutiva e Biologia Molecular do Centro de Ciências Biológicas e da Saúde da Universidade Federal de São Carlos, como parte dos requisitos para obtenção do título de Doutor em Ciências (Ciências Biológicas).

Área de Concentração: Genética Evolutiva

Orientador: **Prof. Dr. Marcelo de Bello Cioffi**

São Carlos – SP

2025

Identificação Bibliográfica

Nome e sobrenome: **Francisco de Menezes Cavalcante Sassi**

Título da Dissertação: **Relações evolutivas e história demográfica em espécies de *Harttia* Steindachner, 1876**

Título da Dissertação em Inglês: **Evolutionary relationships and demographic history in species of *Harttia* Steindachner, 1876**

Programa de estudo: **Pós-Graduação em Genética Evolutiva e Biologia Molecular**

Orientador: **Prof. Dr. Marcelo de Bello Cioffi**

Ano de início: **2020**

Ano da defesa: **2025**

Palavras-chave: Evolução, Peixes, Citogenética Molecular, Sequenciamento NGS, DArT-seq, Filogeografia, Cromossomos Sexuais

Keywords: Evolution, Fishes, Molecular Cytogenetics, NGS sequencing, Phylogeography, DArT-seq, Sex Chromosomes

“Nada em biologia faz sentido exceto à luz da evolução”

Theodosius Dobzhansky

UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Genética Evolutiva e Biologia Molecular

Folha de Aprovação

Defesa de Tese de Doutorado do candidato Francisco de Menezes Cavalcante Sassi, realizada em 20/01/2025.

Comissão Julgadora:

Prof. Dr. Marcelo de Bello Cioffi (UFSCar)

Prof. Dr. José Maurício Barbanti Duarte (UNESP)

Prof. Dr. Cláudio Oliveira (UNESP)

Prof. Dr. Francisco Langeani Neto (UNESP)

Prof. Dr. Vladimir Trifonov (IAPG)

O Relatório de Defesa assinado pelos membros da Comissão Julgadora encontra-se arquivado junto ao Programa de Pós-Graduação em Genética Evolutiva e Biologia Molecular.

AGRADECIMENTOS

- À minha mãe Marina de Menezes Cavalcante e minha irmã Ana Clara Cavalcante Dias Antonio, que me apoiaram e guiaram em todas as escolhas que tive que fazer para chegar até aqui, sempre com muito amor, carinho e suporte incondicional. Esta tese é para vocês. Ao meu pai Roberto Sassi (*in memoriam*), que sempre acreditou no meu potencial e me incentivou a seguir meus sonhos; à minha vó Helena, meu avô Nick, meus tios Álvaro e Gisele, minha madrinha Arlete e Tocá, meu segundo pai Valdir Dias (*in memoriam*), por todo apoio e incentivo constante, que com toda com certeza me ajudaram a chegar aqui; aos meus irmãos Dr. Roberto Brito Sassi e Drª. Alexandra Beumer Sassi, que me inspiraram como trajetórias profissionais.

- Ao Prof. Dr. Marcelo de Bello Cioffi, pela orientação ao longo de todos esses anos. Seu constante incentivo e esforço em proporcionar oportunidades únicas na academia, aos quais serei eternamente grato, foram fundamentais para o meu crescimento científico. Obrigado por todos os conselhos, sugestões, e cobranças, além das incontáveis conversas e discussões enriquecedoras sobre o papel do cientista e do professor universitário no contexto brasileiro.

- Ao Prof. Dr. Orlando Moreira Filho, que além de um grande apaixonado pelas *Harttia*, foi força motriz desta tese, desde a coleta às publicações, além de guia para a carreira científica.

- Ao Prof. Dr. Luiz Antônio Carlos Bertollo que é inspiração para todos citogeneticistas e contribuiu para esta tese desde suas primeiras hipóteses, ao aceitar me orientar durante o início do Mestrado e me permitir integrar o grupo do Laboratório de Citogenética de Peixes, que é protagonista na história da citogenética no Brasil;

- Aos Profs. Drs. Thomas Liehr e Tariq Ezaz, e suas respectivas equipes (em destaque ao Niklas, Steffi, Luisa e Foyez Shamz), que me receberam de braços abertos durante minhas estadias na Alemanha e Austrália, respectivamente, contribuindo para os avanços técnicos e intelectuais desta tese, além de me apresentarem as realidades científicas e culturais de países tão distintos;

- Ao Dr. Alexandr Sember (Sassa) pela colaboração científica em forma de valiosas contribuições e discussões sobre evolução de cromossomos sexuais.

- À Professora Dra. Eliana Feldberg e equipe de seu laboratório, estendendo a toda equipe do Instituto Nacional de Pesquisas da Amazônia (em especial aos Drs., Jansen Zuanon, Patrik Viana, Leandro Marajó, José Francisco de Sousa e Souza, assim como os técnicos Arlindo, Arnóbio e Josedec) que me auxiliaram durante as diversas coletas e preparações de material citogenético por toda a Amazônia

- Ao Prof. Dr. Ricardo Utsunomia e sua equipe, em especial ao Dr. Caio Goes e ao Rodrigo Zeni, que me ensinaram muito sobre DNAs satélites e tornaram a estadia na Alemanha mais agradável, além da amizade e carinho que guardo por todos;

- À equipe do Laboratório de Citogenética de Peixes/Laboratório de Citogenética Evolutiva e egressos: Luiz Henrique da Silva (Piau), e Antonio Donizete da Silva (Toninho) por todas as coletas e conversas sobre a vida, à Dra. Geize Aparecida Deon pela pareceria de longa data no estudo deste grupo tão fascinante, pelas valiosas ideias que ajudaram na construção dessa tese, e pela convivência, à Dra. Renata Luiza R. de Moraes pela longa parceria no estudo dos Lebiasinidae e pela convivência em todos esses anos de laboratório, e ao Gustavo Akira Toma, Fernando H. S.de Souza, Jhon A. D. Vidal, Príncia G. Setti, Mariannah P. B. de Oliveira, Alan M. de Oliveira, Guilherme M. Souza, os Drs. Ezequiel A. de Oliveira, e Manolo F. Perez, e as Dras. Mayara Moura, Marina Chioda, e Nayore Takamiya que tornaram a rotina algo prazeroso, também me ensinando muito sobre ciência e sobre a vida;

- Ao Dr. Osvaldo T. Oyakawa, à Dra. Lucia Rapp-Py Daniel, e ao Dr. Francisco Langeani pela identificação taxonômica dos exemplares utilizados neste trabalho e pela colaboração acadêmica;

- Aos Drs. Rafael Kretschmer, Wagner Molina, Mauro Nirchio e Alongklod Tanomtong, que me permitiram colaborar em estudos com aves e peixes, marinhos e de água doce, incluindo de outros continentes;

- À Dra. Karine Kavalco e ao Dr. Rubens Pasa, que não mediram esforços durante minha iniciação científica para apresentar e ensinar os temas-base da minha carreira científica até o presente momento, com o estudo da citogenética evolutiva;

- À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e a Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), pelas bolsas de doutorado concedidas (processo nº 2020/02681-9, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP));

- À Universidade Federal de São Carlos, em especial ao Programa de Pós-graduação em Genética Evolutiva e Biologia Molecular, à University of Canberra, e à Universitätsklinikum Jena / Friedrich-Schiller-Universität Jena pelo apoio institucional fundamental para a execução deste trabalho;

- À Comissão de Pós-Graduação do Programa de Pós-graduação em Genética Evolutiva e Biologia Molecular, que garantiram uma participação equitativa das opiniões dos discentes do PPGGEv, por mim representados durante parte da execução desta tese.

- Ao Mauro Yida, Matheus Lewi, e Luiz Miotto, aos quais agradeço a amizade de longa data, pelas inúmeras conversas e conselhos, além das risadas que tornaram essa jornada mais agradável;

Agradeço a todos. Sem vocês, esse trabalho não seria possível.

RESUMO

Esta tese explorou as relações evolutivas, citogenômicas e filogeográficas do gênero *Harttia*, um gênero de peixes amplamente distribuídos no território brasileiro. Devido à sua diversidade de sistemas de cromossomos sexuais, incluindo o simples (XX/XY) e múltiplos (XX/XY_1Y_2 e $X_1X_1X_2X_2/X_1X_2Y$) encontrados em aproximadamente 28% das espécies citogeneticamente investigadas, *Harttia* é um dos poucos grupos biológicos considerados como repositório de sexuais múltiplos, fornecendo insights únicos sobre a evolução cromossômica. Investigamos a história evolutiva do gênero a partir de 16 espécies, seis destas caracterizadas citogeneticamente pela primeira vez na literatura (*H. dissidens*, *H. duriventris*, *H. guianensis*, *H. rondoni*, *H. villasboas* e *Harttia* sp. 3). A tese foi pioneira em propor uma filogenia para o gênero com dados genômicos de larga escala (DArTseq SNPs), ampliando as perspectivas das relações evolutivas ao caracterizar os três principais clados do gênero associados a diferentes regiões biogeográficas: um composto por espécies do Escudo das Guianas (Clado I), outro por espécies das drenagens do Norte do Brasil (Clado II) e o terceiro por espécies do Sul e Sudeste do Brasil (Clado III). A modelagem biogeográfica sugere que o ancestral do gênero habitava as bacias Amazônica, Paraná ou Atlântico Sul, com colonizações secundárias para as bacias do Tocantins-Araguaia, São Francisco e Atlântico Leste. As análises citogenéticas (mapeamento de DNAs repetitivos via FISH, hibridização comparativa de genomas - CGH e pintura cromossômica - WCP) revelaram que metade das espécies estudadas possuem cromossomos sexuais diferenciáveis, incluindo novas ocorrências do sistema múltiplo X_1X_2Y e a primeira ocorrência de XX/XY para uma espécie do clado II. Os dados de modelagem de número cromossômico e marcadores ligados ao sexo indicam que esses sistemas derivaram de um ancestral com cariótipo de 2n = 58 e sistema XX/XY, que compartilha marcadores ligados ao sexo entre espécies dos clados I e III, sugerindo um evento de *turnover* no clado II. Ademais, os marcadores ligados ao sexo indicam que todas as 16 espécies investigadas apresentam um sistema de cromossomos sexuais com heterogametia masculina (oito previamente conhecidas visto seu heteromorfismo), sugerindo a presença de sistemas simples homomórficos em oito delas (*H. dissidens*, *H. guianensis*, *Harttia* sp. 3, *H. kronei*, *H. longipinna*, *H. loricariformis*, *H. gracilis*, *H. fowleri*). Além disso, enquanto as fusões de cromossomos sexuais com autossomos possibilitaram o surgimento de sistemas sexuais múltiplos no clado III, dados de pintura cromossômica demonstram que as fissões cromossômicas foram cruciais para a diferenciação nos cromossomos sexuais no clado II. A diferenciação cromossômica, investigada por DNAs repetitivos (satélites, microssatélites, DNAs ribossomais), sugere que o isolamento geográfico e fatores históricos moldaram a estrutura genética e as divergências evolutivas do gênero. Conclusivamente, o trabalho contribui para o entendimento da evolução citogenômica e demográfica de *Harttia*, consolidando-o como um modelo significativo para explorar diversificação em ambientes Neotropicais.

Palavras-chave: Evolução, Peixes, Citogenética Molecular, Sequenciamento NGS, DArT-seq, Filogeografia, Cromossomos Sexuais

ABSTRACT

This thesis explores the evolutionary, cytogenomic, and filogeographic relationships of the genus *Harttia*, a genus of fish widely distributed across Brazil. Due to its diversity of sex chromosome systems, including simple (XX/XY) and multiple (XX/XY_1Y_2 and $X_1X_1X_2X_2/X_1X_2Y$) systems found in approximately 28% of cytogenetically investigated species, *Harttia* stands out as one of the few natural repositories of multiple sex chromosomes, offering unique insights into chromosomal evolution. We generated novel data for six species (*H. dissidens*, *H. duriventris*, *H. guianensis*, *H. rondoni*, *H. villasboas* and *Harttia* sp. 3), integrating cytogenetic, genomic and phylogeographic methodologies. The thesis pioneered the proposal of a phylogeny for the genus using genome-wide data (DArTseq SNPs), enhancing perspectives on evolutionary relationships by characterizing the three major clades associated with different biogeographic regions: one composed of species from the Guiana Shield (Clade I), another by species from the drainages of Northern Brazil (Clade II), and the third by species from Southern and Southeastern Brazil (Clade III). Biogeographic modeling suggests that the genus' ancestor inhabited the Amazon, Paraná, or South Atlantic coastal basins, with secondary colonization into the Tocantins-Araguaia, São Francisco, and East Atlantic basins. Cytogenetic analyses (mapping of repetitive DNAs by FISH, comparative genomic hybridization - CGH, and whole chromosome painting - WCP) revealed that half of the species studied here have distinguishable sex chromosomes, including new occurrences of the X_1X_2Y multiple system and the first occurrence of XX/XY in a clade II species. The chromosome number evolution modeling data and sex linked markers suggests that these systems derive from an ancestor with a 2n = 58 karyotype and XX/XY system, sharing sex-linked markers among species of clades I and III, and suggesting a turnover event in clade II. Additionally, sex-linked markers indicate that all 16 investigated species exhibit a male heterogametic sex chromosome system (eight previously known for their cytogenetic heteromorphism), suggesting the presence of simple homomorphic systems (XX/XY) in eight species (*H. dissidens, H. guianensis, Harttia sp. 3, H. kronei, H. longipinna, H. loricariformis*, *H. gracilis,* and *H. fowleri*). Furthermore, while the fusion of sex chromosomes with autosomes enabled the emergence of multiple sex systems in clade III, chromosome painting data show that chromosome fissions were crucial for the differentiation of sex chromosomes in clade II. Chromosomal differentiation, investigated through repetitive DNAs (satellites, microsatellites, ribosomal DNAs), suggests that geographic isolation and historical factors shaped the genetic structure and evolutionary divergence within the genus. In conclusion, this work contributes significantly to understanding the cytogenomics and demographic evolution of *Harttia*, establishing it as a valuable model for exploring diversification in Neotropical environments.

Keywords: Evolution, Fishes, Molecular Cytogenetics, NGS sequencing, Phylogeography, DArT-seq, Sex Chromosomes

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

1 Introdução

1.1 A evolução da biodiversidade e a região Neotropical

A Convenção sobre Diversidade Biológica (ONU, 1992), tratado da Organização das Nações Unidas (ONU), define biodiversidade como a diversidade de espécies, assim como a diversidade entre espécies e de ecossistemas. Em uma definição expandida, biodiversidade pode ser compreendida como a profusão da vida em suas diversas escalas, ou seja, a variação de genes, espécies e traços funcionais (Cardinale *et al*., 2012). Desta maneira, a biodiversidade é gerada pela evolução biológica, dado o acúmulo de mudanças que ocorrem ao longo de gerações em populações. A origem de novas espécies decorre de um processo evolutivo denominado especiação, que é o produto das barreiras ao fluxo gênico entre duas ou mais espécies emergentes e o acúmulo de mudanças que tornem esse fluxo completamente impedido. Este isolamento pode ocorrer de duas formas: i) pré-zigótico, onde há o impedimento do acasalamento, da fertilização, ou do encontro de parceiros e ii) pós-zigótico, onde ocorre a formação do zigoto, mas este é impedido durante o desenvolvimento ou a prole é infértil. Seguindo o conceito de espécies proposto por de Queiroz (2007), espécies são linhagens de metapopulações que evoluem independentemente. Assim, a compreensão dos mecanismos pelo qual o isolamento reprodutivo é estabelecido se torna essencial para que a delimitação das espécies seja mais bem definida.

Mecanismos genéticos podem contribuir para o isolamento reprodutivo. Em animais, rearranjos cromossômicos, principalmente envolvendo os pares sexuais, podem desempenhar um papel fundamental no isolamento pós-zigótico ao limitar a introgressão, facilitando o desenvolvimento e manutenção do isolamento reprodutivo ao suprimir a recombinação (Bateson, 1909; Dobzhansky, 1933; Muller, 1942; Coyne & Orr, 2004; Faria & Navarro, 2010; Sichová *et al*., 2015). Contudo, a forma mais comum de especiação é a alopatria (Mayr, 1942), onde o surgimento de uma barreira física é o mecanismo responsável pelo isolamento reprodutivo pré-zigótico, podendo, consequentemente, gerar um isolamento pós-zigótico. Rull (2018) sugere que as altas taxas de especiação por alopatria na região Neotropical seriam resultado dos eventos geológicos e climáticos complexos ocorridos durante o Neogeno e o Pleistoceno (23 Ma a 11,7 mil anos atrás), o que explicaria a grande biodiversidade da região.

A região Neotropical, que se estende do sul dos Estados Unidos ao sul da Argentina, se destaca em relação a outras regiões biogeográficas pela alta diversidade de espécies, sendo considerada um *hotspot* de biodiversidade (região com alto grau de endemismo de espécies *sensu* Myers, 1988). Em especial, a região Amazônica apresenta um dos maiores níveis de biodiversidade do mundo (Antonelli *et al*., 2018), gerada pela combinação de processos locais climáticos e geológicos (Albert & Reis, 2011; Rull, 2018). Por exemplo, mudanças de curso

dos rios, incursões e regressões marítimas e o fechamento do istmo do Panamá (~7-2,5 Ma), que criou um fluxo de biodiversidade entre as regiões Norte e Sul das Américas, são categorizados como eventos de extrema importância para a riqueza da biodiversidade na região Neotropical (Hubert & Renno, 2006; Bacon *et al*., 2015; Carrillo *et al*., 2020; Rull & Carnaval, 2020). Da mesma maneira, o soerguimento da Cordilheira dos Andes (~90 Ma) teve um impacto fundamental na formação das paisagens amazônicas, especialmente com as mudanças climáticas que acompanharam este evento (Hoorn *et al*., 2010).

Um dos principais atores na diversificação da fauna Neotropical são os rios. A região apresenta uma gama de bacias hidrográficas que fragmentam a paisagem e criam barreiras ao fluxo gênico, isolando populações que não são capazes de cruzar tais corpos d'água com as magnitudes dos encontrados na região (Wallace, 1852). Neste cenário, exemplos são observados em primatas da região amazônica (Boubli *et al*., 2015), assim como aves na bacia do Paraná-Paraguai (Kopuchian *et al*., 2020) e sapos na bacia do rio São Francisco (Bruschi *et al*., 2019). Além disso, a fauna aquática é diretamente afetada pelo dinamismo hidrogeológico da região Neotropical, pela recorrência de eventos de conexão e rupturas entre bacias hidrográficas (Albert e Reis, 2011) que mesclaram e isolaram as ictiofaunas.

Quatro principais bacias compõem a paisagem da região Neotropical: Amazonas, Orinoco, La Plata e São Francisco (Lundberg *et al*., 1998). A bacia Amazônica abrange uma área de mais de 7 milhões de km², com seus principais afluentes drenando do Escudo Brasileiro (e.g. Rios Tocantins-Araguaia, Xingú, Tapajós), dos Andes (e.g. Rios Purus, Japurá, Iça), e do Escudo das Guianas (e.g. Rios Negro, Branco, Uatumã, Trombetas). Esta bacia é a mais diversa em espécies de peixes de água doce do mundo, englobando juntamente à bacia do Orinoco, com a qual compartilha diversos gêneros, aproximadamente 65% das espécies da ictiofauna da região Neotropical (Reis *et al*., 2016). A bacia do Orinoco é principalmente representada pelo rio que a nomeia, que se origina das montanhas de altitudes do Escudo das Guianas e teve seu curso atual definido durante o Mioceno Tardio (11,6-5,3 Ma atrás) (Gamero, 1996). Já a segunda maior bacia do continente sul-americano, La Plata, inclui rios originários do Escudo Brasileiro (Rio Paraná), de desertos montanhosos na região andina, do Pantanal paraguaio (Rio Paraguai) e dos pampas uruguaios (Rio Uruguai) (Brea & Zucol, 2011). Por fim, a bacia do Rio São Francisco se origina na Serra da Canastra e se estende por uma área de aproximadamente 600 mil km² (Sato & Godinho, 2003). A combinação destas bacias e os limites geológicos transformam a paisagem neotropical (**Figura 1**) em uma malha de nichos, permitindo a diversificação em larga escala de espécies em ambientes terrestres e aquáticos, tornando a região de alto interesse para estudos evolutivos.

Figura 1. Mapa parcial da região Neotropical (contorno preto), com enfoque para a América do Sul, destacando as principais bacias hidrográficas da região: Amazônica (verde escuro), Orinoco (roxo), La Plata (laranja) e São Francisco (amarelo).

1.2 O uso de cromossomos para estudos da biodiversidade

Os cromossomos são estruturas compostas pela combinação do DNA, proteínas e RNA, que atuam na formação da estrutura tridimensional destas moléculas, assim como nos processos regulatórios da expressão dos genes ali contidos. Exclusivamente observados em eucariotos viventes, os cromossomos têm sua origem como material de hereditariedade na origem deste grupo, a aproximadamente 2.7 bilhões de anos (revisado em Damas *et al*., 2021).

Rearranjos cromossômicos são importantes fatores evolutivos principalmente por sua contribuição com o isolamento reprodutivo e consequentemente com o processo de especiação (Cazaux *et al*., 2011). O surgimento de sistemas de cromossomos sexuais também desempenha um papel preponderante na formação de barreiras reprodutivas entre espécies. De acordo com o modelo canônico de evolução dos cromossomos sexuais (Muller, 1914; Bull, 1983; White, 1940, revisado em Kratochvíl *et al*., 2021; Zhu *et al*., 2024), estes se diferenciam a partir de pares autossômicos, pela aquisição de um gene mestre na determinação sexual (MSD). Genes ligados ao sexo também se acumulam neste novo par sexual, até a supressão da recombinação no loci MSD, usualmente por uma inversão (Charlesworth, 2023; Olito *et al*., 2024). Em seguida, sequências repetitivas, como por exemplo elementos transponíveis, se acumulam e o processo de degeneração ocorre, levando ao heteromorfismo do par sexual, embora este processo não seja igual em todas as linhagens (Charlesworth & Charlesworth, 2000; Graves, 2006; Yazdi *et al*., 2020; Charlesworth, 2021). Em sistemas múltiplos – aqueles em que há mais de um par sexual, como por exemplo o $X_1X_1X_2X_2/X_1X_2Y$ –, alterações estruturais envolvendo um par autossômico e um sexual, levando a uma rápida redução, ou mesmo a supressão da recombinação nas regiões próximas aos pontos de quebra (revisado em Zhu *et al*., 2024). Tais rearranjos aumentam também o número de genes ligados ao sexo, acelerando assim o acúmulo de diferenças genéticas entre as populações (Vieira *et al*., 2003). Com esse processo, genes que desempenham um papel no isolamento reprodutivo podem se acumular nos cromossomos neo-sexuais, favorecendo a especiação (Kitano & Peichel, 2012). Em bichos-da-seda (*Samia cynthia*), por exemplo, é relatado o papel dos sistemas de cromossomos sexuais múltiplos associados a outros rearranjos cromossômicos para a formação de barreiras reprodutivas, sendo estas diretamente ligadas com a especiação (Yoshido *et al*. 2011).

Durante a evolução dos vertebrados, diversos rearranjos cromossômicos como duplicações e deleções, que podem ocorrer em larga escala, assim como as fusões, fissões, translocações e inversões, foram peças-chave no isolamento de espécies, atuando como importantes fontes de variação genética (Damas *et al*., 2021). Em um espectro amplo, a evolução cromossômica em vertebrados pode ser organizada em quatro principais caminhos

evolutivos: I) estase ou conservação cariotípica (e.g. Motta-Neto *et al*., 2019; Altmanová *et al*., 2023; Bredeson *et al*., 2024); II) redução de 2n (também conhecido como disploidia descendente) (e.g. Sember *et al*., 2020; Cheng *et al*., 2020; Moraes & Sassi *et al*., 2023), III) ganho de 2n (disploidia ascendente) (e.g. Molina *et al*., 2021), ou IV) especiação homoploide (e.g. Lukhtanov *et al*., 2020; Olave *et al*., 2022).

A citogenética é a subárea da genética responsável pelo estudo dos cromossomos, o principal material de herança em organismos eucariontes. Nesta área, são estudados estes elementos partindo de sua estrutura e morfologia, arranjo dentro do núcleo, organização durante a divisão celular, padrões de bandamento e posição de genes ou sequências, assim como sua variação em número e forma intra- e entre espécies. Ao combinar técnicas de genômica e análise de dados moleculares, recebe o nome de citogenômica ou cromossômica (Deakin *et al*., 2019; Liehr, 2021) (**Figura 2**).

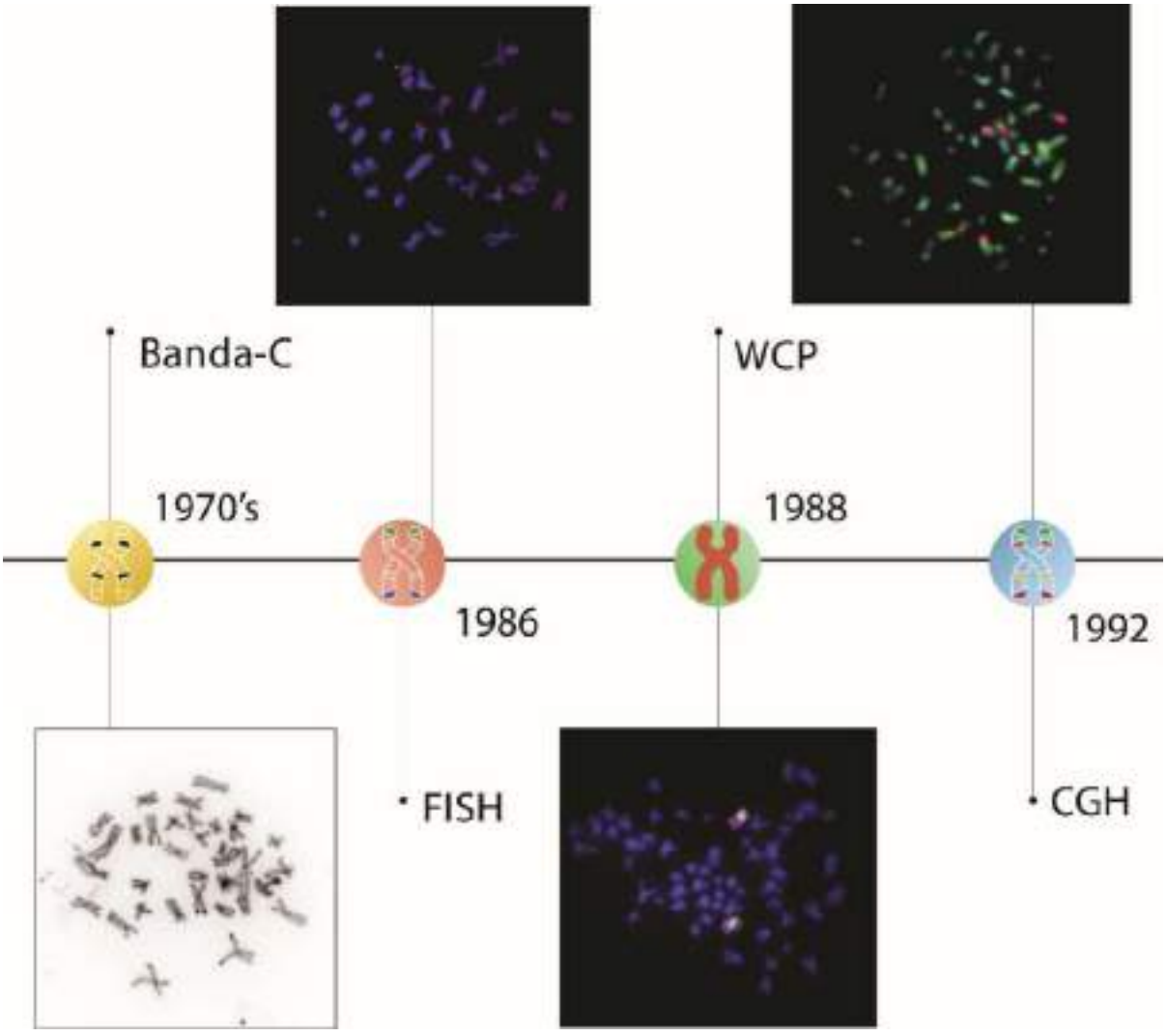


Figura 2. Linha do tempo destacando a exemplos das principais técnicas citogenéticas abordadas neste trabalho.

Inicialmente, estudos citogenéticos inferiam sobre a biodiversidade com a utilização de marcadores que permitiam o correto pareamento dos cromossomos e a comparação dos padrões entre espécies, conhecidas como técnicas de bandamento cromossômico. O método de criação destas bandas revela aspectos da composição e organização do DNA nos cromossomos. Por exemplo, ao tratar os cromossomos com um ácido, seguido do tratamento com uma base e estabilizá-lo em solução salina, conseguimos observar regiões de heterocromatina constitutiva, que são mais compactadas em relação às regiões eucromáticas (Sumner, 1972). Descrita por Sumner (1972), a técnica de bandamento C foi amplamente aplicada em estudos evolutivos, indicando para a maioria dos grupos de eucariotos as regiões pericentroméricas e terminais dos cromossomos como ricas em heterocromatina constitutiva (Liu *et al*., 2020). A aplicação desta técnica se estende por trabalhos de caracterização de cromossomos sexuais e de delimitação de espécies, como por exemplo em peixes dos gêneros *Brycon* e *Leporinus*, cuja distribuição diferencial de heterocromatina constitutiva entre espécies com cariótipos semelhantes (mesmo 2n e fórmula cariotípica) é um dos principais marcadores de distinção entre espécies (Galetti Jr. *et al*, 1991; Margarido & Galetti Jr., 2000).

Desde os primeiros trabalhos com mosca-da-fruta (Morgan *et al*., 1915), que indicaram os cromossomos como elementos carregadores de genes, a ciência dos mapeamentos *in situ* começou a se desenvolver. Com o trabalho de Watson, Crick e Franklin descrevendo a estrutura molecular de ácidos nucléicos (Watson & Crick, 1953) e avanços subsequentes em técnicas de bioquímica, como por exemplo os métodos de detecção de proteínas baseados em imunofluorescência, o mapeamento *in situ* ganhou espaço e relevância em estudos genéticos. Este tipo de mapeamento consiste na identificação física da posição de ácidos nucleicos em outros ácidos nucleicos, que podem ser cromossomos metafásicos ou interfásicos, assim como RNA. Com isso, é possível detectar a posição e quantificar ácidos nucléicos em células ou tecidos alvo. A técnica se baseia nas propriedades físicas e químicas dos ácidos nucleicos, que permitem sua desnaturação (quebra das ligações de hidrogênio entre fitas complementares) e renaturação com outras moléculas complementares sob certas condições. Os primeiros estudos foram realizados ao final da década de 1960, utilizando sondas de RNA ribossomal (rRNA) acopladas com Trítio (H^3), um isótopo radioativo de hidrogênio, para a visualização de genes de rRNA em oócitos de rã-de-unhas-africana (*Xenopus laevis*) (Pardue & Gall, 1969). Durante o mesmo período, técnicas similares com foco nos mapeamentos em células e tecidos fixados, foram publicadas (John *et al*., 1969; Buongiorno-Nardelli & Amaldi, 1970), seguindo da primeira aplicação em mamíferos (Pardue & Gall, 1970). Neste importante trabalho, os autores demonstraram pela primeira vez a ocorrência de sequências de DNA satélite, uma classe de

DNA repetitivo, na heterocromatina centromérica de cromossomos de ratos, utilizando mapeamentos DNA-DNA e RNA-DNA (Pardue & Gall, 1970).

Um importante avanço nas técnicas de mapeamento *in situ* foi o desenvolvimento de técnicas menos nocivas à saúde do pesquisador e do ambiente para a marcação fluorescente de sondas. As sequências de DNA ou RNA mapeadas neste tipo de experimentos são denominadas sondas, sendo necessária a presença de uma molécula fluorescente que permite a sua identificação visual por técnicas de microscopia. Como mencionado anteriormente, moléculas radioativas eram amplamente utilizadas, até a popularização de anticorpos como agentes de marcação fluorescente. Estes anticorpos são ligados a outras moléculas ou aplicados em *nick-translation* (técnica que permite fragmentar o DNA em porções aleatórias e ligar tais fragmentos pela incorporação de nucleotídeos que carregam moléculas fluorescentes). Com isso, a técnica de hibridização fluorescente *in situ* (FISH) foi desenvolvida e rapidamente popularizada (Pinkel *et al*., 1986), permitindo a aplicação do mapeamento de sequências conservadas ou variáveis (como DNAr e DNA satélites) em estudos de caracterização da biodiversidade (Sochorová *et al*., 2018). Além do aspecto descritivo da biodiversidade, sequências de DNAr são utilizadas em estudos evolutivos, pois apesar do alto grau de conservação a nível de sequência, os DNAr podem variar em número de cópias e na posição nos cromossomos, mesmo em populações de uma mesma espécie (Zimmer *et al*., 1981; Dover, 1982; Schubert & Wobus, 1985; Dubcovsky & Dvorak, 1995; Averbeck & Eickbush, 2005; Roy *et al*., 2005; McTaggart *et al*., 2007; Wang *et al*., 2017; Sochorová *et al*., 2018). Ademais, outras sequências repetitivas também são frequentemente mapeadas aos cromossomos para estudos evolutivos, como por exemplo microssatélites e a sequência telomérica (TTAGGG)n, que é conservada na maioria dos vertebrados (Meyne *et al*., 1989). Microssatélites são pequenas sequências de um a seis nucleotídeos que se repetem em tandem, sendo frequentemente encontradas em posições congruentes à outras sequências repetitivas e apresentando um padrão de alta variação entre espécies (Cioffi & Bertollo, 2012). Já a sequência telomérica de vertebrados também é repetida na composição do telômero, o final da fita de DNA que compõem os cromossomos, o que permite sua identificação por fluorescência. Em eventos de fusão cromossômica, resquícios dos telômeros podem ser retidos como sequências teloméricas intersticiais (ITS) (Blackburn, 1994), embora sua perda durante os rearranjos seja mais comum (Schubert *et al*., 1992). Além disso, mecanismos de reparo podem gerar ITS sem que de fato representem um telômero verdadeiro acumulado por um rearranjo prévio (revisado em Aksenova & Mirkin, 2019).

Variações da técnica de FISH foram desenvolvidas com o passar dos anos, como por exemplo a hibridização genômica comparativa (CGH) (Kallioniemi *et al*., 1992), que utiliza como sonda o DNA total extraído das espécies, hibridizando concomitantemente sequências bloqueadoras de DNAs repetitivos, como por exemplo o C_0t-1 DNA (Yano *et al*., 2017). Nesta abordagem, ficam evidenciadas regiões de maior variação entre dois genomas, destacando por exemplo regiões sexo-específicas e a extensão da homologia entre cromossomos de híbridos (Pereira *et al*., 2013; Symonová *et al*., 2013; Koubová *et al*., 2014; Sember *et al*., 2018; Sassi *et al*., 2019, 2020; Mazzoleni *et al*., 2020; Poignet *et al*., 2021). Cromossomos inteiros ou partes destes também passaram a serem utilizados como sondas nas técnicas conhecidas como pintura cromossômica total (WCP) ou parcial (PCP) (Pinkel *et al*., 1988), que quando aplicadas na comparação das homologias entre espécies distintas recebe o nome de cross-FISH ou Zoo-FISH (Yang *et al*., 2009). Tais cromossomos podem ser obtidos por microdissecção ou por citometria de fluxo, seguindo a amplificação por primers degenerados e marcação fluorescente (Yang *et al*., 2009). Com isso, é possível identificar a homologia de pares cromossômicos ao longo de espécies relacionadas e de rearranjos cromossômicos (Ried *et al*., 1998; Shetty *et al*., 1999; Yang *et al*., 2017).

1.3 Marcadores moleculares para estudos evolutivos

A união de metodologias de investigação da posição de genes (mapeamentos *in situ*) e de bandamentos cromossômicos permite a investigação comparativa da evolução do genoma sob a ótica citogenética em diversos grupos. Porém, apenas com a integração de dados filogenéticos é que se faz possível a conexão de tais eventos cromossômicos ao processo de diversificação das espécies. Filogenias são hipóteses sobre as relações evolutivas de organismos, representadas em forma de diagrama, que podem variar conforme o tamanho dos ramos (em unidades de tempo, número de gerações ou quantidade de mudanças), mas também, embora raras, em espessura (variação no tamanho populacional ao longo do tempo) (revisado em O'Meara, 2012). Com elas, podemos observar as relações de ancestralidade de espécies, correlacionando com eventos geológicos e biogeográficos, principalmente pela modelagem da história demográfica (e.g. Liu *et al*., 2006; Oliveira *et al*., 2020; Perez *et al*., 2023). Este tipo de modelagem infere os centros de origem de espécies a partir das áreas de ocorrência e uma filogenia, utilizando modelos evolutivos desenhados para diversos cenários, como por exemplo dispersão, extinção e vicariância (Matzke, 2012; Pyron *et al*., 2014; Matzke, 2016).

Filogenias podem ser construídas a partir de diversos tipos de dados, com destaque para dados de origem genômica, que permitem a comparação em larga escala tanto de organismos

quanto de marcadores (Chase *et al*., 1993; Goloboff *et al*., 2009; O'Meara, 2012). Com o desenvolvimento de metodologias de sequenciamento de nova geração (NGS) e de técnicas para enriquecimento das bibliotecas de sequenciamento para regiões específicas do genoma, o custo da produção de marcadores genômicos para filogenias (polimorfismos de nucleotídeo único - SNPs, elementos ultra conservados - UCEs, exons, genes, sequências simples repetidas - SSRs, íntrons e regiões intergênicas) em grupos de organismos não-modelo foi drasticamente reduzido (Lemmon and Lemmon, 2013). Contudo, a geração massiva de dados genômicos não implica em uma reconstrução mais acurada da história evolutiva de um grupo, dada a dificuldade do estabelecimento de um padrão de qualidade para os dados, assim como dos modelos de análise (Rodriguez-Ezpeleta *et al*. 2007, Rannala & Yang 2008, Philippe *et al*. 2011; Lemmon & Lemmon, 2013; Smith *et al*., 2020).

Neste contexto, o estabelecimento de métodos de redução da complexidade do genoma (i.e., métodos baseados na seleção e sequenciamento exclusivo de parcelas informativas do genoma como RAD-seq e DArTseq) permitem a investigação evolutiva a um custo relativamente baixo quando comparado com sequenciamento de genomas completos (Pukk *et al*., 2015). Técnicas como estas apresentam a vantagem de não exigirem um genoma de referência, pois se baseiam no uso de enzimas de restrição e sequenciamento de fragmentos de tamanhos específicos para representação do genoma (Peterson *et al*., 2012). Especificamente, o DArTseq utiliza a combinação de uma enzima de corte aleatório e da PstI, que é sensível à metilação CpG e realiza o corte preferencialmente em regiões de DNA hipometiladas. Estas por sua vez são geralmente ricas em genes e possuem alta complexidade, permitindo a identificação de marcadores sob possível influência de pressões seletivas (Kilian *et al*., 2012).

Além da utilização de marcadores moleculares na reconstrução filogenética, o advento das metodologias de NGS beneficiou a produção de marcadores cromossômicos. Por exemplo, a caracterização completa de bibliotecas de DNAs repetitivos (elementos transponíveis, minissatélites, microssatélites, satélites) permite sua utilização em estudos comparativos, dada a alta taxa de variação destas sequências mesmo entre populações de uma mesma espécie (Garrido-Ramos, 2017). Os DNAs satélites, que por muito tempo foram vistos como "DNA lixo" devido à sua aparente falta de função, agora são reconhecidos por desempenharem papéis significativos na regulação gênica, na modificação da cromatina e como componentes funcionais dos cromossomos (Kuhn, 2015; Thakur *et al*., 2021). Desta forma, diversos estudos recentes apresentam uma caracterização molecular e cromossômica mais detalhada do satelitoma (conjunto de DNAs satélites de um organismo) em trabalhos comparativos, elucidando sua contribuição para a estrutura cromossômica e seu papel na evolução do genoma

e da biodiversidade (e.g. Utsunomia *et al*., 2016; Ahmad *et al*., 2020; Goes *et al*., 2022; Gutiérrez *et al*., 2023; Lisachov *et al*., 2023; de Moraes *et al*., 2023; Rocha-Reis *et al*., 2024). Ademais, o uso do satelitoma em trabalhos de citogenômica comparativa fornece dados importantes sobre o processo de degeneração de cromossomos sexuais (e.g. Utsunomia *et al*., 2019; Serrano-Freitas *et al*., 2020; Ferretti *et al*., 2020; Cabral-de-Mello *et al*., 2021; de Oliveira *et al*., 2023).

Em consonância, a investigação de sistemas sexuais com o uso de marcadores moleculares também foi beneficiada nos avanços do NGS, permitindo a ampla investigação de marcadores ligados ao sexo (SLM), como a presença e ausência de alelos específicos ou a restrição destes a um dos sexos. Combinando métodos de redução de complexidade do genoma para o sequenciamento de regiões com baixo número de cópias (e.g. DArTseq, RAD-seq), com a análise comparativa entre sexos, estudos com SLM provém insights sobre a evolução destes sistemas especialmente em grupos cuja determinação sexual é altamente variável, como em peixes, anfíbios e répteis (e.g. Lambert *et al*., 2016; Hill *et al*., 2018; Sopniewski *et al*., 2019; Schimek *et al*., 2022; Nguyen *et al*., 2022). Em geral, a obtenção dos SLM permite a detecção de sistemas sexuais em espécies com cromossomos sexuais homomórficos, além da investigação de SLM restritos ao cromossomo sexo-específico e, na disponibilidade de um genoma montado a nível cromossômico, a identificação da região do gene MSD (e.g. Kuhl *et al*., 2024). Três principais cenários são investigados: i) marcadores restritos a um dos sexos, ii) marcadores que são homozigotos em um dos sexos e completa ou parcialmente heterozigotos no outro, e iii) marcadores com o dobro de profundidade de leitura em um dos sexos (Jeffries *et al*., 2018; Devloo-Delva *et al*., 2023).

Com a combinação de filogenias robustas e informações sobre as espécies, a aplicação de abordagens comparativas baseadas em modelos estatísticos para a predição dos processos evolutivos históricos que geraram os padrões atuais da biodiversidade se torna possível. Dentre tais metodologias, a inferência de caracteres ancestrais se destaca pela possibilidade de estimar um processo não-observável. Três principais metodologias se destacam na reconstrução dos processos evolutivos: metodologias baseadas no princípio da parcimônia evolutiva – que favorecem as hipóteses com menor número de passos evolutivos; de verossimilhança, que analisam as probabilidades de um estado de caractere ocorrer; e de processos de Markov em tempo contínuo, onde a evolução de um sistema em que o tempo é contínuo (ou seja, varia de forma contínua e não em passos discretos) faz com que o futuro estado do sistema dependa apenas do estado atual (revisados em O'Meara *et al*., 2012). Neste contexto, podemos estimar números cromossômicos ancestrais ao observar a frequência de números de cromossomos em

uma filogenia, verificando as transições (ganhos e perdas de número cromossômico por aneuploidia, fissões e fusões) em um modelo de Markov de tempo contínuo, utilizando o software ChromEvol (Glick & Mayrose, 2014). Com a utilização de tal software, estudos demonstram, por exemplo, que a variação do tamanho do genoma não está relacionada com a evolução do número cromossômico em peixes teleósteos (Kushwaha *et al*., 2023).

1.4 Biodiversidade ictiológica

Peixes são excelentes modelos para o estudo da evolução cromossômica. Além de representarem o grupo mais diverso dentre os vertebrados viventes, sua distribuição em todos os habitats aquáticos permite correlacionar a evolução biológica do grupo à evolução geológica da Terra (Lundberg, 1993). Especialmente para os peixes dulcícolas, cada continente apresenta uma fauna particular, onde os padrões distintos de distribuição são congruentes com os processos de dinamismo hidrográfico. A maioria das espécies de peixes ocorre nas regiões tropicais e subtropicais, com uma redução global da diversidade em regiões temperadas e polares (Berra, 2001). A África tropical, o sudeste asiático e a bacia do rio Amazonas se destacam pela riqueza de espécies de água doce (Lévêque *et al*., 2008, Nelson *et al*., 2016), enquanto a região da América Central é relativamente pobre em diversidade em decorrência de sua história geológica.

Estudos de sistemática indicam que "peixes" é um grupo parafilético, incluindo peixes agnatos (lampreias e peixes-bruxa), peixes cartilaginosos (tubarões e raias) e peixes ósseos (celacantos, peixes pulmonados e peixes de nadadeira raiada), mas que também deveria incluir os demais tetrápodes, já que a linhagem evoluiu a partir de ancestrais Sarcopterygii (Szarski, 1977). A maior parcela da diversidade de peixes está distribuída na infraclasse Teleostei (Actinopterygii), grupo que engloba 27 mil espécies distribuídas em mais de 450 famílias (Nelson *et al*., 2016; Fricke *et al*., 2020) que habitam águas marinhas e continentais. Este clado é definido como "o clado menos inclusivo que contém *Hiodon tergisus* Lesueur 1818 (Osteoglossomorpha), *Elops saurus* Linnaeus 1766 (Elopomorpha), *Engraulis encrasicolus* Linnaeus 1758 (Otocephala/Clupeomorpha) e *Perca fluviatilis* Linnaeus 1758 (Euteleostei)" (Near & Thacker, 2024).

Diversos eventos de duplicação completa do genoma ocorreram durante a diversificação dos teleósteos, partindo de um cariótipo ancestral composto de 26 cromossomos. Estes eventos foram cruciais para a diversificação dos vertebrados, pois permitiram a expansão de famílias gênicas essenciais para o funcionamento do genoma, como genes *Hox* e do complexo de histocompatibilidade (Castro *et al*., 2004; Dehal & Boore, 2005; Sacerdot *et al*., 2018; Simakov

et al., 2020). Além dos dois grandes eventos de duplicação completa do genoma ocorridos na evolução dos vertebrados, um terceiro grande evento ocorreu há aproximadamente 400 Ma no ancestral dos peixes teleósteos, gerando um cariótipo ancestral com 2n = 52 cromossomos (Sato & Nishida, 2010; Arai, 2011). Já no grupo monofilético Ostariophysi, diversificado a 160.6 Ma (Hughes *et al*., 2018), rearranjos cromossômicos tiveram papel preponderante na diversificação genômica do grupo, em detrimento de grandes eventos de duplicações como ocorridos no grupo ancestral (Muramoto *et al*., 1968).

1.5 A ordem Siluriformes e a família Loricariidae

Dentre os Ostariophysi, a ordem monofilética Siluriformes (Fink & Fink, 1981; Saitoh *et al*., 2003; Arratia *et al*., 2003; Sullivan *et al*., 2006) representa uma grande parcela da diversidade de peixes de água doce. Com mais de 3 mil espécies e 36 famílias (Ferraris, 2007), os animais pertencentes a tal ordem são conhecidos como bagres ou peixes-gato. Como apontado pelas filogenias de Sullivan *et al*. (2006) e Kappas (2016) (**Figura 3**), duas subordens são reconhecidas: Loricarioidei (seis famílias, mais basal e restrita à América do Sul), e Siluroidei (irmã de Diplomystidae, englobando 29 famílias distribuídas por todos os continentes, exceto a Antártida). Ao todo, 25 famílias são consideradas monofiléticas, sendo Bagridae, Schilbidae, Claroteidae e Sisoridae as exceções. A família neotropical Diplomystidae não se encontra inclusa em nenhuma subordem, apesar de formar um clado irmão à Siluroidei (Sullivan *et al*., 2006).

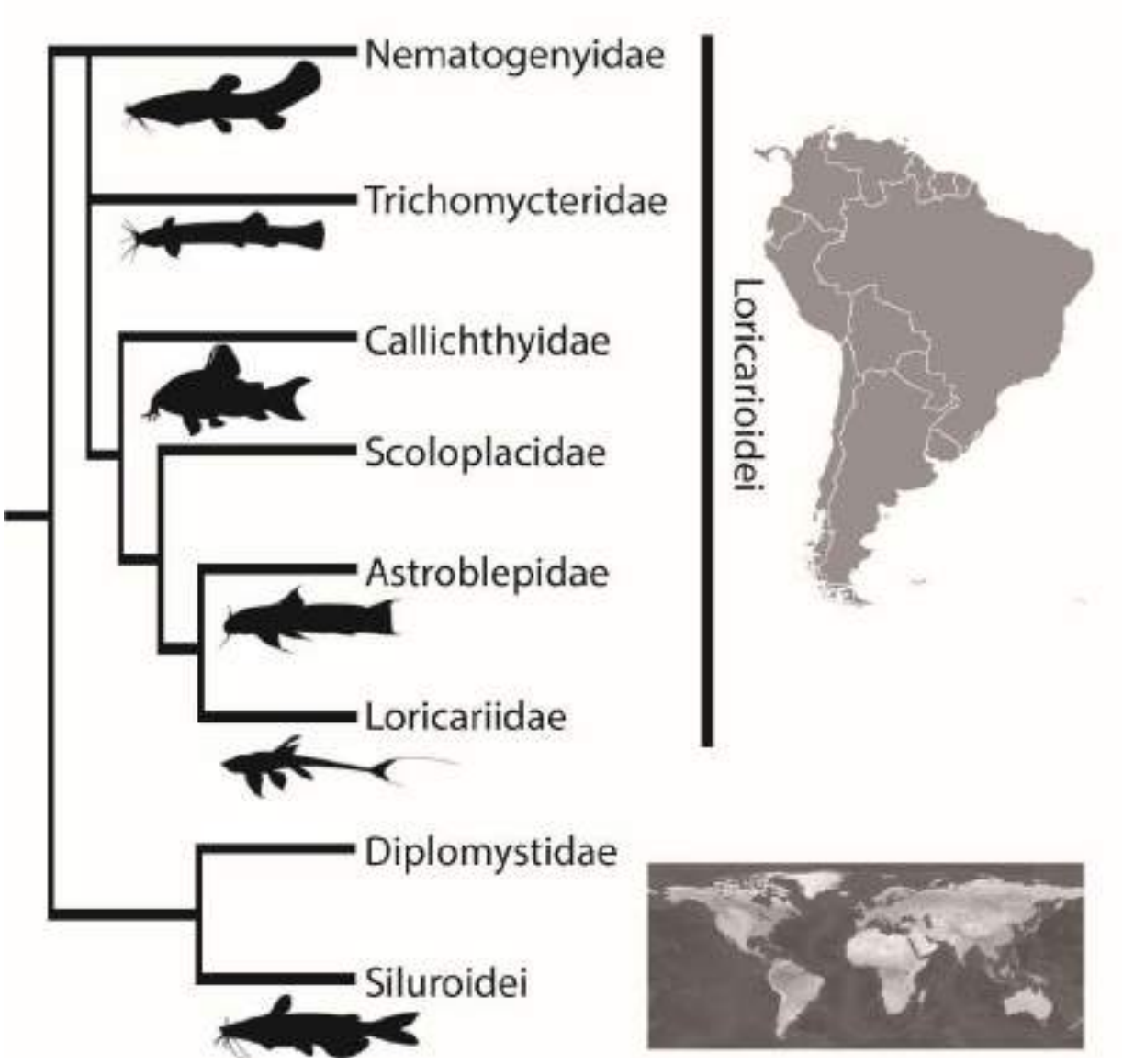


Figura 3. Relações filogenéticas da ordem Siluriformes conforme Sullivan *et al*. (2006), com silhuetas representativas de alguns clados e a respectiva distribuição de Loricarioidei + Diplomystidae (América do Sul) e Siluroidei (Mundo, exceto Antártida).

Por conta da sua distribuição global, os peixes da ordem Siluriformes são objetos frequentes de estudos biogeográficos (Briggs, 2005; Diogo, 2004; Gosline, 1944; Lundberg, 1993; Myers, 1938; Regan, 1922; Schaeffer, 1952). Estes sugerem que a América do Sul possa ter sido o centro de origem dos peixe-gato, suportada pela presença endêmica de Diplomystidae, assim como do clado Loricarioidei (Sullivan *et al*., 2006). Na América do Sul, 52 gêneros e 67% das espécies de Siluriformes são do clado Loricarioidei. A partir de espinhos peitorais de Diplomystidae encontrados na Argentina e Bolívia, datados de 83,2 a 66,0 Ma respectivamente (Cione *et al.*, 1987; Arratia & Cione, 1996; Gayet & Meunier, 1998), análises de relógio molecular sugerem a origem do grupo na América do Sul, no Cretáceo Inferior, a 121,4 Ma

(Hughes *et al*., 2018). Contudo, com a recente descrição do fóssil mais antigo para a ordem, *Afrocascudo saharensis* Brito *et al* (2024) de 112 Ma, um Loricariidae encontrado em Marrocos e o único da família observado até o momento fora da América do Sul, desafia as hipóteses de origem do grupo.

Sendo a terceira família de peixes com maior riqueza, com 1.235 espécies, Loricariidae se destaca dentre os Siluriformes (Fricke *et al*., 2024). Loricariidae é a mais representativa em número de espécies dentre os Siluriformes, representando 48% do total de novas espécies descritas ao longo de 25 anos, segundo levantamento bibliográfico realizado compreendendo o período de 1990 a 2014 (Ota *et al*., 2015). Esta família, juntamente com Characidae e Cyprinidae, são responsáveis por aproximadamente 50% de todas as espécies de Ostariophysi (Nelson *et al*., 2016). Os peixes loricariídeos, popularmente conhecidos como cascudos, bodós e acaris, são distribuídos na região Neotropical, principalmente entre o norte da Costa Rica e o sul da Argentina (Reis *et al*., 2003). Estes animais apresentam placas dérmicas revestindo o corpo e boca em formato de ventosa, localizada na região ventral. A maioria das espécies dessa família apresentam dieta iliófaga, ou bentófaga, também sendo observadas dietas exclusivas de madeira, ou algas (Buck & Sazima, 1995; Lujan *et al*., 2017). Loricariídeos apresentam uma ampla variação de tamanho corporal, com espécies menores que 5 cm (e.g. Ribeiro *et al*., 2012) a espécies que atingem aproximadamente 1 m (Lujan *et al*., 2010). Principalmente devido a atividades antrópicas, como a combinação de exploração para o comércio aquarista, destruição de habitats e poucas informações populacionais, diversos loricariídeos estão ameaçados de extinção (Barros *et al*., 2023). Por morfologia e dados moleculares, seis subfamílias são reconhecidas: Delturinae, Hypoptopomatinae, Hypostominae, Lithogeninae, Loricariinae e Rhinelepinae (Covain *et al*., 2016; Roxo *et al*., 2019).

Das seis subfamílias reconhecidas em Loricariidae, Hypostominae e Loricariinae são as que apresentam o maior número de espécies (604 e 322 espécies respectivamente) (Fricke *et al*., 2024) e a maior diversidade cariotípica (Traldi *et al*., 2019). Em espécies de Loricariinae são encontrados caracteres dimórficos entre os sexos, com a presença de odontódeos (Reis *et al*., 2003). Membros da subfamília Loricariinae podem ser facilmente identificados pelo pedúnculo caudal achatado, ausência de nadadeira adiposa e pela projeção do rostro/focinho (Ferraris, 2003; Covain, 2006). São encontrados em rios da América do Sul, além de rios do sul do México, Colômbia, Equador e Panamá (Blanco, 2012). A subfamília Loricariinae é monofilética e subdividida em duas tribos: Harttiini e Loricariini, sendo a segunda mais rica em número de espécies (Covain *et al*., 2016; Roxo *et al*., 2019). A tribo Hartiini é composta pelos

gêneros *Harttia* Steindachner (1876) (gênero tipo), *Harttiella* e *Cteniloricaria* (Montoya-Burgos *et al*., 1998; Covain *et al*., 2008; Lujan *et al*., 2015; Covain *et al*., 2016).

Estudos citogenéticos são escassos quando comparados a diversidade de espécies de Loricariidae, porém revelam uma grande diversidade de números diploide e de cariótipos, assim como de cromossomos sexuais e acessórios. Apenas 234 espécies tiveram seus cariótipos explorados, sendo a maioria representantes de Hypostominae, seguido por Loricariinae. O número diploide varia de 2n = 33 em machos de *Rineloricaria teffeana* (Marajó *et al*., 2022) a 2n = 96 em *Hemipsilichthys* sp. (Kavalco *et al*., 2004, 2005), sendo 2n = 54 o mais encontrado. Sete dos nove sistemas de cromossomos sexuais encontrados em peixes são descritos em 31 espécies de Loricariidae: os simples XX/XY, XX/X0 e ZZ/ZW, e os múltiplos $X_1X_1X_2X_2/X_1X_2Y$, XX/XY_1Y_2, ZZ/ZW_1W_2 e $Z_1Z_1Z_2Z_2/Z_1Z_2W_1W_2$.

1.6 O gênero *Harttia* Steindachner (1876)

Harttia inclui 28 espécies válidas (Fricke *et al*., 2024; **Tabela 1**). Espécies deste gênero são encontradas principalmente no Brasil, mas também na Venezuela, Guiana, Guiana Francesa e Suriname (**Figura 4**). Em geral, habitam corredeiras fortes, com fundo rochoso e arenoso, próximas a quedas d'água (Casatti & Castro, 2006; Covain & Fisch-Muller, 2007). No Brasil, habitam bacias do sudeste brasileiro, destacando-se as porções superiores das bacias dos rios São Francisco e Paraná, e as bacias costeiras dos rios Doce, Paraíba do Sul, Ribeira de Iguape, e riachos do Complexo do Espinhaço, Serra da Mantiqueira, e bacia do Leste. Ao norte do Brasil, ocorrem em diversas drenagens da bacia Amazônica (Tapajós, Trombetas, Uatumã, Xingu) e na bacia Tocantins-Araguaia, assim como em rios que drenam do escudo das Guianas e da bacia do Orinoco (rios Curuá e Maicuru, por exemplo).

469 **Tabela 1.** Espécies do gênero *Harttia* com suas respectivas distribuições geográficas em ecorregiões *sensu* Albert
470 *et al*., 2011. Adaptado de Oyakawa *et al*., 2018 e de Oliveira e Oyakawa, 2019.

Espécies	Bacia de distribuição
Harttia absaberi Oyakawa, Fichberg & Langeani 2013	Alto Paraná
H. carvalhoi Miranda-Ribeiro 1939	Paraíba do Sul
H. canastra Caldas, Cherobim, Langeani 2022	São Francisco
H. depressa Rapp Py-Daniel & Oliveira 2001	Amazonas / Escudo das Guianas
H. dissidens Rapp Py-Daniel & Oliveira 2001	Tapajós – Juruena
H. duriventris Rapp Py-Daniel & Oliveira 2001	Tocantins – Araguaia
H. fluminensis Covain & Fisch-Muller 2012	Leste da Guiana
H. fowleri Pellegrin 1908	Leste da Guiana
H. garavelloi Oyakawa 1993	São Francisco
H. gracilis Oyakawa 1993	Alto Paraná
H. guianensis Rapp Py-Daniel & Oliveira 2001	Leste da Guiana
H. intermontana de Oliveira & Oyakawa 2019	Alto Doce
H. kronei Miranda-Ribeiro 1939	Ribeira de Iguape, Alto Paraná
H. leiopleura Oyakawa 1993	São Francisco
H. longipinna Langeani, Oyakawa & Montoya-Burgos 2001	São Francisco
H. loricariformis Steindachner 1877	Paraíba do Sul
H. merevari Provenzano, Machado-Alisson, Chernoff, Willink & Petry 2005	Orinoco / Escudo das Guianas
H. novalimensis Oyakawa 1993	São Francisco
H. panara Oyakawa, Fichberg & Rapp Py-Daniel 2018	Alto Xingu
H. punctata Rapp Py-Daniel & Oliveira 2001	Tocantins – Araguaia
H. rhombocephala Miranda-Ribeiro 1939	Mata Atlântica
H. rondoni Oyakawa, Fichberg & Rapp Py-Daniel 2018	Alto Xingu
H. surinamensis Boeseman 1971	Leste da Guiana
H. torrenticola Oyakawa 1993	São Francisco
H. trombetensis Rapp Py-Daniel & Oliveira 2001	Amazonas / Escudo das Guianas
H. tuna Covain & Fish-Muller 2012	Leste da Guiana
H. uatumensis Rapp Py-Daniel & Oliveira 2001	Amazonas / Escudo das Guianas
H. villasboas Oyakawa, Fichberg & Rapp Py-Daniel 2018	Alto Xingu

Figura 4. Mapa político parcial da América do Sul, com a distribuição (círculos pretos) de todas as espécies de *Harttia* com coordenadas geográficas disponíveis na base de dados de ocorrência de peixes Neotropicais (Tonella *et al*., 2022), além de coordenadas disponíveis na base de dados do Laboratório de Citogenética Evolutiva da Universidade Federal de São Carlos. Mapa criado com QGIS 3.28.2, utilizando os shapefiles Natural Earth e rios da América do Sul do HydroSheds (Lehner *et al*., 2008).

Estudos filogenéticos baseados em caracteres morfológicos e moleculares indicam que o gênero é monofilético (Rapp Py-Daniel, 1997; Provenzano, 2012; Covain *et al*., 2016; Londoño-Burbano & Reis, 2021). Contudo, análises de DNA mitocondrial desafiam tal monofiletismo, sugerindo o reposicionamento de *H. absaberi* em um novo gênero (Cherobim, 2021). Apesar disso, as filogenias concordam na ocorrência de três principais clados em *Harttia* (**Figura 5**): o primeiro a se diversificar sendo composto por espécies do Escudo das Guianas (clado I), e dois clados irmãos compostos por espécies da região Norte/Centro-Oeste (clado II) e do Sul/Sudeste do Brasil (clado III). Oyakawa *et al*. (2018) propõe a organização das espécies em três grupos com base no padrão de cobertura de placas da região abdominal (completo, parcial ou ausente), embora este não seja demonstrado como um caractere congruente às filogenias.

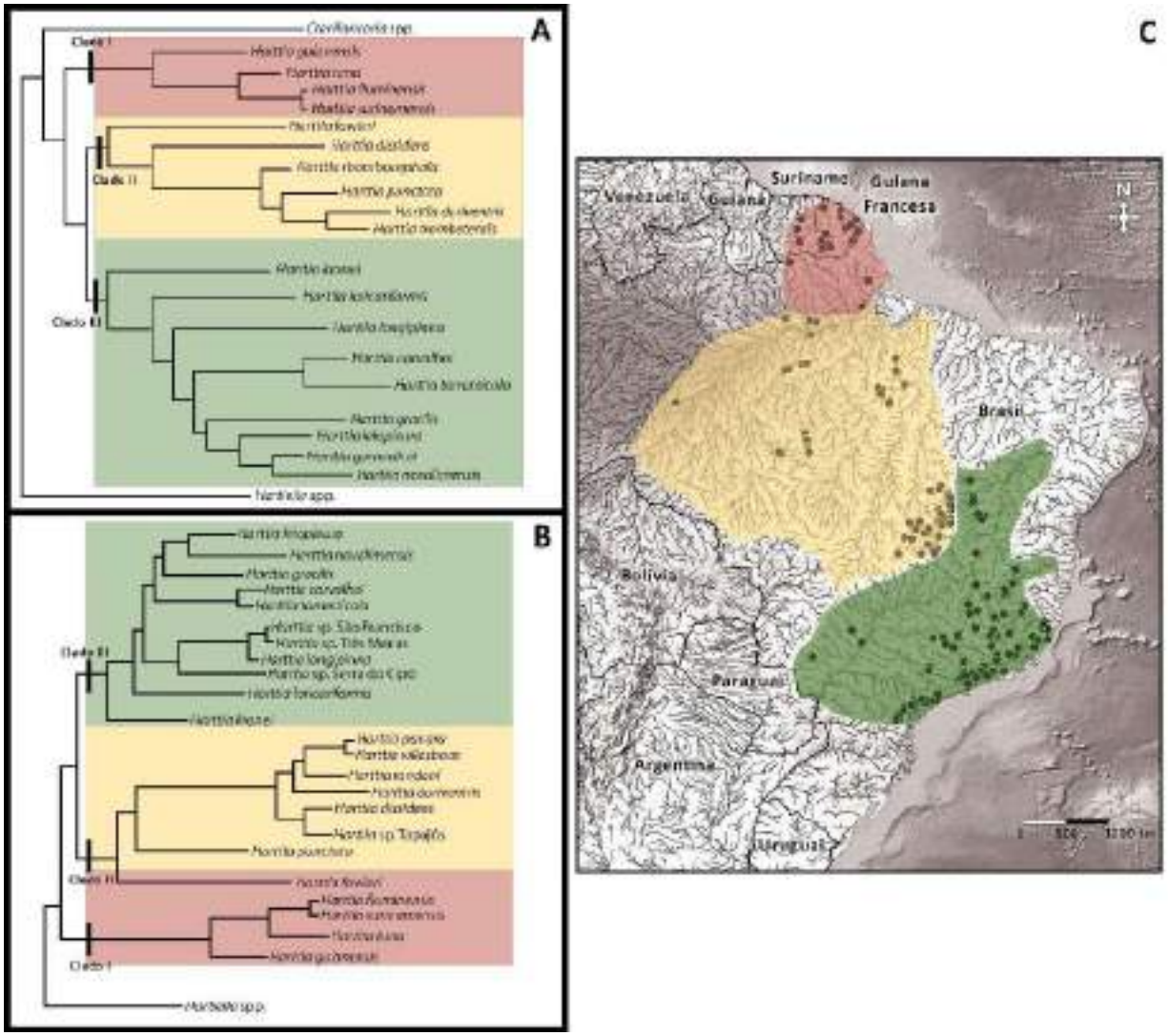


Figura 5. Relações filogenéticas de *Harttia*, baseadas em caracteres morfológicos e genéticos, segundo Covain *et al*. (2016) (A) e Londoño-Burbano e Reis (2021) (B), com a distribuição aproximada dos clados na América do Sul (C).

A ecologia de *Harttia* foi investigada sob o ponto de vista de poucas espécies, cujas características de hábito e reprodução são reportadas principalmente por taxonomistas durante a descrição das espécies. Estudos sugerem que essas espécies apresentem uma pequena capacidade de dispersão, habitando principalmente cabeceiras de pequenos riachos (Blanco, 2012). Contudo, este padrão só é verdadeiro para espécies da região Sudeste do Brasil (Oliveira & Oyakawa, 2019), já que espécies das bacias Amazônica, Orinoco e do Escudo das Guianas habitam rios de maior porte, inclusive no leito principal dos grandes afluentes destas bacias (Rapp Py-Daniel & Oliveira, 2001; Provenzano *et al*., 2005; Covain *et al*., 2012; Oyakawa *et al*., 2018). De acordo com Covain *et al*. (2012), espécies de *Harttia* são mais frequentemente encontradas em áreas com grande intensidade de luz solar, o que reflete em adaptações

morfológicas e ecológicas, como o tamanho maior dos olhos. Diversas espécies apresentam dimorfismo sexual, com a presença da hipertrofia de odontódeos em machos (Langeani *et al*., 2001; Caldas *et al*., 2022). Em *H. carvalhoi* Miranda Ribeiro, 1939, a desova é total e o período reprodutivo ocorre de setembro a fevereiro (Biagiotto *et al*., 2012). Os ovos são geralmente espessos, em especial na zona radiata, o que é uma característica comum para peixes reofílicos e indica que a desova é realizada em área aberta (Dotzer & Weidner, 2003). Já *H. torrenticola* apresenta ovos adesivos e cuidado parental (Covain & Fisch-Muller, 2007). Observações de gônadas de fêmea em estágio reprodutivo em algumas espécies, como por exemplo em *H. canastra* (comunicação pessoal), destacam a presença de ovos em distintos estágios de maturação, o que pode indicar desova parcelada em algumas espécies.

1.7 *Harttia* como modelo para estudos de evolução cariotípica e cromossomos sexuais

Até o presente momento, apenas o gênero *Harttia* foi analisado citogeneticamente dentre os membros da tribo Hartiini. Contudo, este gênero tem se mostrado extremamente interessante para análises citogenéticas, devido a presença de sistemas sexuais e de diversas outras características, como por exemplo a distribuição de DNAs ribossomais em sintenia na espécie *Harttia loricariformis* (Kavalco *et al*., 2004, 2005). Apesar da grande diversidade de espécies no gênero *Harttia*, apenas oito espécies (~28% da riqueza total do gênero) apresentavam dados citogenéticos até o início deste trabalho em 2020 (**Tabela 2**), com a ampliação para 11 espécies no trabalho de Deon *et al*. (2020), publicado após o início desta tese (~39% da riqueza total do gênero). Contudo, uma notável variação de números diploide era observada, com 2n = 52/53 em *Harttia carvalhoi* (Centofante *et al*., 2006) a 2n = 62 em *H. absaberi* (Rodrigues, 2010). Grande parte da variação de número cromossômico em *Harttia* é atribuída a eventos de fusões Robertsonianas (Centofante *et al*., 2006; Blanco *et al*., 2014, 2017). Dois sistemas sexuais múltiplos oriundos de rearranjos e quebras cromossômicas são descritos no gênero: XX/XY_1Y_2 para *H. carvalhoi* (Centofante *et al*., 2006) e $X_1X_1X_2X_2/X_1X_2Y$ para *H. punctata* (Blanco *et al*., 2014).

Ao longo do desenvolvimento desta tese, mais três espécies de *Harttia* tiveram seus cariótipos explorados por Deon *et al*. (2020), ampliando a descrição citogenética de espécies e o número de espécies com sistemas de cromossomos sexuais múltiplos. Em *Harttia* sp. 1 Ribeirão dos Macacos – SP (2n=56/57), o mesmo sistema XX/XY_1Y_2 observado em *H. carvalhoi* é encontrado. Já *H. intermontana* apresenta um sistema sexual XX/XY_1Y_2 com homologia parcial ao de *H. carvalhoi* e *Harttia* sp. 1, porém com uma translocação entre o par autossômico 9 e os cromossomos sexuais, indicada por experimentos de pintura cromossômica

(Deon *et al*., 2022a, b). Por fim, *Harttia* sp. 2 apresentou o mesmo número diploide de *H. absaberi* (2n = 62), compreendendo o maior 2n observado em espécies do gênero.

Tabela 2. Dados citogenéticos do gênero *Harttia* disponíveis na literatura científica até o ano de 2020, início da presente tese. O número diploide 2n = 52 descrito em Alves *et al*. (2003) na realidade se trata de amostras de *Harttia* carvalhoi, conforme corrigido em Centofante *et al*. (2006).

Espécie	2n	Fórmula cariotípica	Referência
Harttia kronei	♀♂58	40m/sm+18st	Alves *et al*., 2003
		14m+20sm+14st+10a	Blanco *et al*., 2013
H. loricariformis	♀♂52	32m/sm+20st/a	Alves *et al*., 2003*
	♀♂56	16m+22sm+10st+8a	Kavalco *et al*., 2004
H. carvalhoi	52♀,	18m+18sm+8st+8a ♀	Centofante *et al*., 2006
	53♂	17m+18sm+8st+10a ♂	
H. absaberi	♀♂62	13m+23sm+16st+10a	Rodrigues, 2010
H. longipinna	♀♂58	16m+12sm+16st+14a	Blanco *et al*., 2012
H. torrenticola	♀♂56	16m+10sm+16st+14a	Blanco *et al*., 2013
H. punctata	58♀,	16m+20sm+12st+10a ♀	Blanco *et al*., 2014
	57♂	16m+21sm+12st+8a ♂	
H. gracilis	♀♂58	20m+22sm+8st+8a	Blanco *et al*., 2017
H. intermontana	56♀,	14m+12sm+12st+14a	Deon *et al*., 2020
	57♂	13m+12sm+13st+15a	
Harttia sp. 1	52♀,	14m+14sm+10st+18a	Deon *et al*., 2020
	53♂	13m+14sm+10st+20a	
Harttia sp. 2	62♀♂	16m+14sm+12st+20a	Deon *et al*., 2020

2 Justificativa e objetivos

Conforme destacado, os Siluriformes oferecem um modelo particular para estudos sistemáticos, biogeográficos e evolutivos. Entretanto, dados informativos e de interesse para estas abordagens, tais como caracteres citogenéticos e genômicos, são ainda deficientes para os Siluriformes como um todo, especialmente em membros da tribo Hartiini. No gênero *Harttia*, lacunas importantes na caracterização citogenômica, principalmente em espécies da região Amazônica e do Escudo das Guianas são observadas, além da escassez de trabalhos comparativos no grupo como um todo. Em especial, as filogenias moleculares e morfológicas não englobam toda a diversidade de *Harttia* investigada pela citogenética, o que dificulta a clara identificação dos caminhos evolutivos percorridos pelo grupo. Desta maneira, a presente tese objetivou estudar a evolução citogenômica em *Harttia*, traçando paralelos com a evolução da família Loricariidae e de demais peixes Neotropicais. Para isso, utilizaremos a abordagem da citogenômica comparativa, combinando dados cromossômicos, genômicos, filogeográficos e sistemáticos. Tais procedimentos possibilitaram uma melhor compreensão dos mecanismos de evolução genômica e cromossômica destas espécies, em consonância com as seguintes propostas investigativas:

A) Citogenômica evolutiva no gênero *Harttia*

Apenas oito espécies do gênero *Harttia* apresentam dados citogenéticos descritos na literatura. Sendo assim, esta tese objetivou a expansão deste número pela caracterização de outras espécies do gênero *Harttia*, com destaque para espécies das regiões Amazônica e do Escudo das Guianas, utilizando procedimentos citogenéticos clássicos e moleculares (por exemplo, bandamento C, CGH, WCP e mapeamento de sequencias repetitivas) destacando características genômicas inter- e intraespecíficas, permitindo evidenciar o panorama da evolução cromossômica ocorrida neste grupo. Além disso, ancoramos os dados citogenômicos em uma filogenia que inclui todas as espécies analisadas pela citogenética, visando responder quais os principais rearranjos que moldaram o cariótipo das espécies de *Harttia*.

B) Investigação dos sistemas de cromossomos sexuais de *Harttia*

No gênero *Harttia*, dois sistemas sexuais múltiplos são encontrados ($X_1X_1X_2X_2/X_1X_2Y$ e XX/XY_1Y_2) dentre as oito espécies citogeneticamente analisadas. Assim, foram realizados experimentos de pintura cromossômica (WCP) com sondas obtidas por microdissecção cromossômica de cromossomos sexuais, visando elucidar os mecanismos de origem destes sistemas sexuais múltiplos presentes em diferentes espécies do gênero *Hartiia*, bem como a putativa identificação de cromossomos sexuais nas demais espécies não portadoras de

cromossomos sexuais heteromórficos. Foram também realizados experimentos de hibridização genômica comparativa (CGH) intraespecífica, visando a comparação genômica entre os sexos e buscando identificar a ocorrência de regiões sexo-especificas. Ademais, investigamos a existência de marcadores ligados ao sexo nestas espécies, almejando responder aos seguintes questionamentos: Quais são os cromossomos sexuais entre as espécies que apresentam cariótipos homomórficos entre sexos? Proto-cromossomos sexuais poderiam ser evidenciados entre as espécies destes grupos com base em metodologias mais resolutivas, fundamentadas na citogenômica? A diferenciação dos cromossomos sexuais, embora evidenciando diferentes estágios evolutivos, estaria presente nas espécies do gênero *Harttia* como um todo, não sendo, portanto, um atributo de apenas algumas de suas espécies? Qual a composição dos cromossomos sexuais e quais as diferenças entre sistemas simples e múltiplos?

C) História demográfica e filogeografia de *Harttia*

Estudar os princípios e processos que determinam a distribuição geográfica de linhagens genealógicas de *Harttia* é essencial para uma compreensão mais abrangente do seu processo evolutivo. Dada a sua distribuição pelo Brasil, pelas Guianas e Suriname, além da presença de grupos irmãos em todo o continente Sul-Americano, objetivamos estudar a colonização das bacias, investigando os centros de dispersão e os processos históricos que levaram a presente distribuição. Além disso, analisamos tais dados em conjunto aos dados de evolução cromossômica, modelando a evolução do número de cromossomos e correlacionando aos processos biogeográficos. Com isso, objetivamos responder: qual o número cromossômico ancestral do gênero? Quais eventos biogeográficos podem ser correlacionados com a idade dos rearranjos cromossômicos? Quais eventos biogeográficos determinaram a atual distribuição das espécies de *Harttia*?

3 Material e Métodos

3.1 Material

No presente estudo, amostras de espécies de *Harttia* foram coletadas em seus ambientes naturais (**Tabela 3**, **Figura 6**), com ênfase em espécies distribuídas na região Norte do Brasil. As coletas (**Figura 7**) foram realizadas em duas incursões: a primeira na Serra do Cachimbo, no distrito de Cachoeira da Serra em Altamira - PA, realizada em 2019 entre os meses de setembro e outubro. Esta região, localizada na borda norte do Escudo Brasileiro, é de grande importância ecológica dada a suas montanhas que atingem 740 m de altitude e dividem as bacias do Xingú e Tapajós. Logramos êxito na coleta de indivíduos de *H. rondoni*, *H. villasboas* e de *Harttia* sp. 3 Rio do Peixe – PA. Já a segunda expedição foi realizada no ano de 2020, entre os meses de outubro e novembro, percorrendo os municípios paraenses Alenquer, Canaã dos Carajás e Rurópolis, onde foram coletadas as espécies *H. dissidens*, *H. fowleri*, e *H. guianensis*. Esta segunda expedição focou na bacia do rio Tapajós, além de drenagens do Escudo das Guianas, na bacia Amazônica.

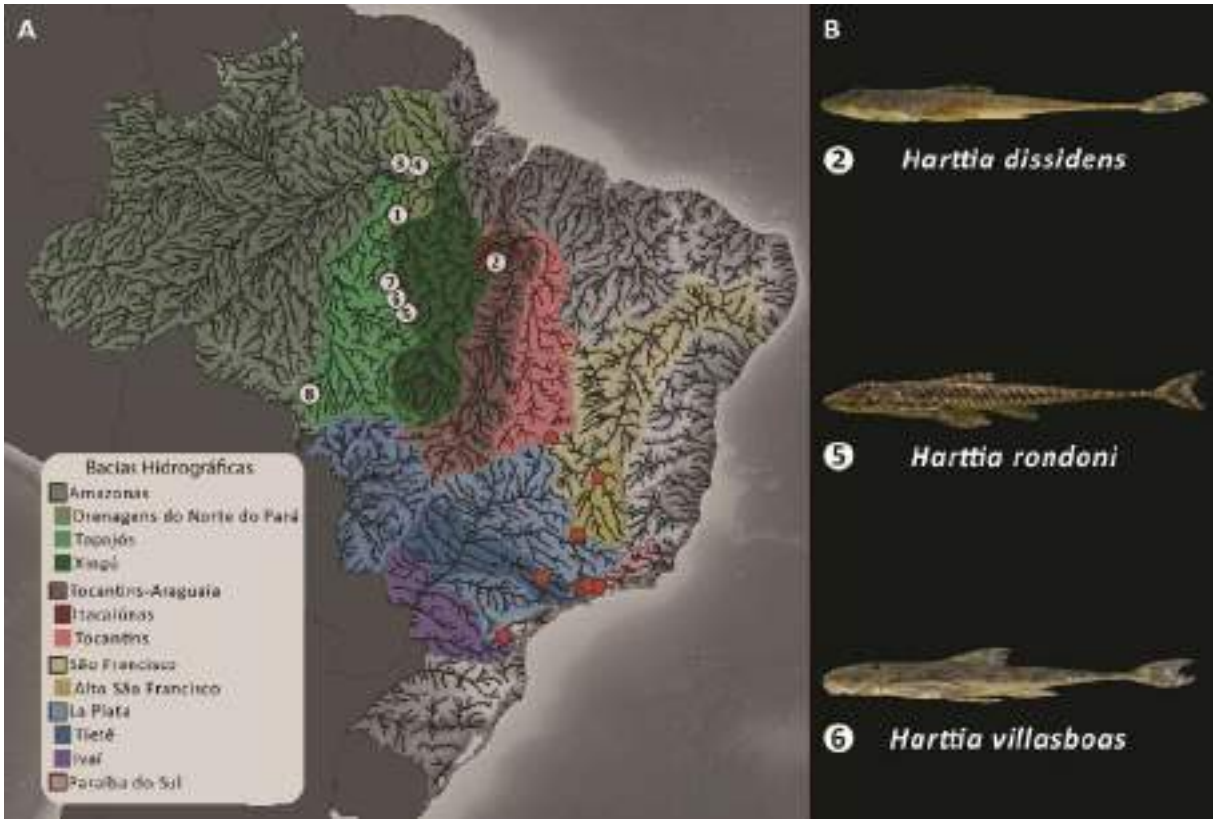


Figura 6. Mapa do Brasil (A), com a representação dos pontos de coleta amostrados durante o desenvolvimento desta tese. Localidades amostradas (círculos brancos) e suas respectivas espécies (números conforme **Tabela 3**) e demais pontos de amostras previamente coletadas utilizadas na presente tese (círculos vermelhos). São apresentadas as divisões de bacias e sub-bacias conforme HydroSheds (Lehner *et al*., 2008). Fotos representativas das espécies analisadas na presente tese (B), gentilmente cedidas pelo Dr. Osvaldo Oyakawa – MZUSP.

Tabela 3. Espécies amostradas no presente estudo, destacando as localidades amostradas, com as respectivas coordenadas geográficas e bacia hidrográfica, além do número de espécimens coletados (N) e número de voucher dos museus. Amostras previamente coletadas encontram-se indicadas com asterisco.

Espécie	Local	Bacia	Coordenadas	N	Voucher
1 - *Harttia dissidens*	Igarapé Tambor, Rurópolis - PA	Tapajós	04°5'37.8"S 55°0'30.2"W	07♀ 25♂	INPA-ICT 059577
2 - *H. duriventris*	Rio Parauapebas, Canaã dos Carajás - PA	Tocantins-Araguaia	06°30'06.5"S 50°02'35.3"W	08♀ 07♂	MZUSP 126598
3 - *H. fowleri*	Rio Maicuru, Alenquer - PA	Amazonas	01°36'32.3"S 54°22'28.5"W		-
4 - *H. guianensis*	Igarapé Paraíso, Alenquer - PA	Amazonas	01°29'02.2"S 54°50'31.2"W	06♀ 10♂	INPA-ICT 059584
5 - *H. rondoni*	Rio 13 de Maio, Altamira – PA	Xingu	08°38'53.0"S 55°01'41.0"W	15♀ 14♂	MZUSP 111385
6 - *H. villasboas*	Rio Curuá, Altamira - PA	Xingu	08°44'09.0"S 54°57'46.0"W	34♀ 38♂	MZUSP 126599
7 - *Harttia* sp. 3	Rio do Peixe, Altamira - PA	Tapajós	08°39'20.7"S 55°09'24.1"W	11♀ 15♂	MZUSP 127605
8 - *Farlowella* sp.	Igarapé Triângulo, Pontes e Lacerda - MT	Amazonas	15°10'43.7"S 59°17'01.7"W		-
**H. carvalhoi*	Ribeirão Grande, Pindamonhangaba - SP	Paraíba do Sul	22°46'2.98"S 45°26'7.05"W	17♀ 12♂	MZUSP 109782
**H. gracilis*	Córrego Machadinho, Santo Antônio do Pinhal - MG	Paraíba do Sul	22°48'31"S 45°41'21"W	18♀ 15♂	MZUSP 111384
**H. intermontana*	Rio Piranga, Carandaí - MG	Doce	20°59'34.0"S 43°43'30.0"W	20♀ 13♂	MZUSP 126520
**H. kronei*	Rio Açungui, Campo Largo – PR	Atlântico Sul	25°22'44"S 49°39'08"W	10♀ 05♂	MZUSP 109783
**H. longipinna*	Rio São Francisco, Pirapora - MG	São Francisco	17°21'22.8"S 44°51'0.2"W	13♀ 16♂	MZUSP 106767
**H. loricariformis*	Rio Paraitinga, Silveiras - SP	Paraíba do Sul	22°52'22.5"S 44°51'041"W	07♀ 03♂	MZUSP 111386
**H. punctata*	Rio Itiquira, Formosa – GO	Tocantins-Araguaia	15°19'25"S 47°25'26"W	18♀ 25♂	MZUSP 111385
**H. torrenticola*	Rio Araras, Piumhi - MG	São Francisco	20°26'16.4"S 45°55'39.8"W	08♀ 06♂	MZUSP 109704
**Harttia* sp. 1	Ribeirão dos Macacos, Silveiras - SP	Paraíba do Sul	22°40'43"S 44°51'25"W	10♀ 07♂	-
**Harttia* sp. 2	Rio Barra Grande, Prudentópolis - PR	Paraná	24°58'40.72"S 51°7'34.25"W	17♀ 11♂	MZUSP 126000

Espécies de *Harttia* previamente coletadas e disponíveis no banco de amostras do Laboratório de Citogenética Evolutiva da Universidade Federal de São Carlos foram utilizadas para análises comparativas, compreendendo um conjunto de dados final composto por 17 espécies (60,7% da riqueza do gênero). Além disso, coletamos exemplares de *Farlowella* sp. em Pontes e Lacerda – MT, para utilização como grupo-externo nas análises moleculares. Todas as coletas foram realizadas com autorização da agência de controle ambiental ICMBIO/SISBIO (Licença Nº 48628-2) e SISGEN (AA80B9E) e todos os procedimentos seguiram as condutas éticas e de anestesia aprovadas pelo Comitê de Ética em Experimentação e Uso Animal da Universidade Federal de São Carlos (Processo CEUA 1853260315).

Os animais foram coletados com o auxílio de redes do tipo tarrafa, além de peneirão. Todas as coletas foram realizadas em período diurno, já que coletas noturnas não foram bem-sucedidas na captura de *Harttia*. Durante a coleta, os peixes foram mantidos em baldes contendo água do próprio corpo d'água onde se encontravam, com oxigenação fornecida por aeradores à pilha. Sacos plásticos contendo 30% do volume total de água e completado com oxigênio puro foram utilizados para o transporte dos animais vivos até o local de obtenção dos materiais genéticos. Os animais então foram eutanasiados pela imersão em solução de Eugenol 3% e posteriormente fixados em formaldeído 10% por 48h até a sua final conservação em etanol 70%. A identificação e depósito das espécies foram realizadas pelo Dr. Osvaldo Oyakawa (Museu de Zoologia da Universidade de São Paulo - MZUSP) e pela Dra. Lúcia Helena Rapp Py-Daniel (Instituto Nacional de Pesquisas da Amazônia - INPA). As espécies *Harttia* sp. 1 Ribeirão dos Macacos - SP, *Harttia* sp. 2 Rio Barra Grande – PR, e *Harttia* sp. 3 Rio do Peixe - PA correspondem a unidades taxonômicas cuja identificação morfológica não foi suficiente para uma alocação às espécies conhecidas, cuja variação citogenética e molecular são abordadas ao longo desta tese.

3.2 Métodos

3.2.1 Revisão bibliográfica de Loricariidae

Conduzimos um levantamento bibliográfico no Google Scholar dos artigos publicados até Dezembro de 2023, utilizando como palavras-chave "cytogenetics Loricariidae" e "chromosomes Loricariidae". Durante a busca, artigos duplicados e sem informação de cariótipo foram excluídos de nossas análises (e.g. Traldi *et al*., 2019 realiza o mapeamento de sequências repetitivas em diversas espécies da família, porém não apresenta dados de 2n e estrutura cariotípica). Foram incluídos todos os artigos publicados de 1968 a 2023 em revistas científicas, de onde extraímos informações citogenéticas e biogeográficas, como o 2n, número fundamental (FN), fórmula cariotípica (KF), sistema de cromossomos sexuais (SCS) e sítios de AgNOR/18S DNAr (classificados entre simples e múltiplos).

Além disso, a localidade e as coordenadas geográficas das populações investigadas também foram compiladas, com o nome da cidade, estado e país indicados de acordo com as coordenadas fornecidas nos artigos. Os nomes das espécies e famílias foram verificados no Catálogo de Peixes de Eschmeyer (Fricke *et al*., 2023) e atualizados conforme os nomes válidos. Para os artigos em que as coordenadas geográficas estavam disponíveis, plotamos esses pontos de amostra em mapas usando o QGIS 3.28.2.

3.2.1 Obtenção cromossômica, extração de DNA e bandamento-C

As preparações de cromossomos mitóticos foram obtidas do rim anterior por meio do método convencional *air drying* (Bertollo *et al*., 1978; Bertollo *et al.*, 2015). Utilizamos a combinação de dois métodos para a obtenção a depender das condições do local de preparação, visto que alguns espécimens foram preparados em campo e outros em laboratório. Desta forma, o primeiro protocolo, mais comumente utilizado em campo, iniciou-se com a injeção intra-abdominal de 200µl de Colchicina 0,0125% e manutenção em aquário por 45 minutos, até a transferência para a solução anestésica de Eugenol 3%. Os fragmentos de rim foram removidos e transferidos para 7 ml de KCl 0,075M, prosseguindo-se para a dissociação completa pela aspiração e devolução repetida com auxílio de seringa de 5ml. Esta solução é mantida a 32-38 °C (temperatura ambiente nos meses e localidades de coleta) por 20 a 25 min, até sua prefixação pela aplicação de 1 ml de fixador Carnoy II (3:1 metanol, ácido acético) recém preparado e gelado. O material foi então mantido em temperatura ambiente por 5 min antes de sua centrifugação a 1.000 rpm por 10 min. Em seguida, o sobrenadante foi removido e substituído no mesmo volume por fixador Carnoy II gelado. Usualmente, este processo foi repetido de duas

a três vezes, até o clareamento completo do *pellet*. Por fim, o *pellet* foi ressuspendido em 1,5 ml de fixador e armazenado em freezer a -18 °C.

Já o segundo protocolo parte da anestesia dos animais, seguido da remoção do rim e transferência para solução de RPMI previamente aquecida a 37 °C. O material então foi dissociado e 40 µl de Colchicina 0,0125% foram acrescidos, prosseguindo-se a incubação em estufa a 37 °C por 25 min. O sobrenadante então foi removido após centrifugação a 1.000 rpm por 10 min e o *pellet* ressuspendido em 7 ml de KCl 0,075M. Após 20 min de incubação a 37 °C, 1 ml de fixador Carnoy II gelado foi acrescentado e ressuspendidos ao material, com incubação por 10 min em temperatura ambiente. O protocolo se encerra da mesma forma que anteriormente descrito, com lavagens sucessivas e armazenamento final em 1,5 ml de fixador.

Para as análises citogenéticas, lâminas foram preparadas utilizando vapor e aquecimento em conjunto com gotas de fixador para garantir o espalhamento do material. Em uma placa aquecedora a 55 °C, embebemos um pedaço de papel em água destilada, dobrando as extremidades para criar um suporte para a lâmina, que foi posicionada com a face voltada para baixo, permitindo o acúmulo de vapor. Com o vapor acumulado, a lâmina é desvirada e aplica-se 20 µl da preparação cromossômica. Em seguida, 10 µl de fixador Carnoy II foi aplicado sob o material, procedendo-se à incubação em uma região seca da placa aquecedora por 40s, seguindo de secagem completa em temperatura ambiente. Então, as lâminas foram coradas com 2,5 ml de Giemsa 5% por 8 minutos e verificadas em microscopia de campo claro. Para garantir a aplicação de técnicas em sequência, lâminas de referência foram criadas utilizando uma lâmina de vidro com adesivo branco. Assim que as metáfases eram encontradas, a lâmina com a preparação cromossômica é removida sem alterar a posição da platina. Em seguida, a lâmina de referência é posicionada, o potenciômetro alternado para o máximo e o diafragma fechado, criando um ponto de luz que é marcado com lápis ou caneta. Este por sua vez corresponderá completamente ao local da metáfase na lâmina com a preparação cromossômica, facilitando as análises sequenciais em microscopia de fluorescência. Para a remoção do Giemsa, as lâminas foram incubadas em fixador Carnoy II por 15 min, lavadas com água destilada e secas em temperatura ambiente.

Para a criação de bandas de heterocromatina constitutiva, seguimos o protocolo de Sumner (1972). As lâminas descoradas foram submersas em HCl 0,2 N a 42 °C por 12 min. Em seguida, transferidas para solução aquosa a 42 °C de hidróxido de bário ($Ba(OH)_2 x8H_2O$) 5% recém-preparada e filtrada, por 3 min. Para interromper a ação da solução de hidróxido de bário, as lâminas foram rapidamente lavadas em solução de HCl 0,2N, enxaguadas em água corrente e após leve secagem ao ar, transferidas para solução salina 2xSSC em estufa à 60 °C durante

45 min. Por fim, as lâminas foram lavadas em água corrente e, após secas, coradas com 20 µl de Antifanding + 0.7µl de Iodeto de Propídio (50µg/mL), seguindo Lui *et al*. (2012) para tornar as regiões heterocromáticas mais evidentes.

Durante a remoção dos fragmentos de rim para obtenção cromossômica, amostras de fígado e coração foram obtidas e preservadas em etanol absoluto a -18 °C, para posterior submissão ao protocolo de extração de DNA por fenol-clorofórmio:isoamiloálcool (Sambrook e Russel, 2001). Em resumo, os tecidos foram secos e triturados em tubo de 2 ml para a digestão a 55 °C com o mix de digestão (EDTA 0,5M, NaCl 5M, Tris HCl 2M, Proteinase K, H_2O Mili-Q, e SDS 10%). Após a digestão completa, 500 µL de solução de Fenol (2 mL Fenol + 1920 µl Cloro + 80 µl de Álcool para 8 eppendorfs, pH: 8.0) foi adicionada. O tubo foi agitado lentamente por 10 min e em seguida centrifugado a 13.000 rpm por 20 min. O sobrenadante foi transferido para um segundo tubo, onde se aplicou 1/10 do volume de NaCl 5M (geralmente, 50 µl) + 1.000 µl de Etanol Baker 100% gelado. Em seguida, o material foi centrifugado a 13.000 rpm por 10 min, o sobrenadante removido, e o *pellet* de DNA lavado com 50 µl de etanol 70% gelado. Por fim, uma nova centrifugação permitiu a remoção do etanol e secagem do *pellet* em temperatura ambiente, para a ressuspensão final em 30 a 50 µl de T.E. (Tris-HCl 10mM; EDTA 1mM). Para garantir a pureza do DNA extraído, realizamos o tratamento com RNAse A a 37 °C por 1h.

3.2.2 Hibridização Fluorescente *in situ* (FISH)

O protocolo de FISH utilizado na presente tese é o resultado da aplicação dos princípios técnicos de Pinkel *et al*. (1986) somados a modificações para obtenção das sondas, concentrações de reagentes e tempo de tratamentos utilizados em diversos trabalhos de citogenética de peixes. Todas essas modificações foram compiladas durante o desenvolvimento desta tese em um capítulo de livro (Sassi *et al*., 2023), que se encontra anexado ao material suplementar (**Anexo S1**), visto que, por questões de direitos autorais, não é possível anexá-lo ao documento principal.

Para a caracterização citogenética e análises comparativas, utilizamos sondas preparadas em mix de hibridização, que pode ser armazenado a -20 °C e utilizado posteriormente. Para a preparação do mix, dissolvemos 2g de sulfato de sódio dextrano em 10 ml de 50% formamida deionizada/2×SSC/50 mM tampão fosfato por 3h a 70 °C. O pH foi ajustado a 7 com tampão fosfato e a solução foi distribuída em tubos de 2 ml e armazenada em freezer. Sequencias de DNAs ribossomais (5S e 18S DNAr), microssatélites ($(CA)_{15}$, $(GA)_{15}$, $(A)_{30}$), e do telômero (TTAGGG)n foram utilizadas. As sondas de DNAr correspondem às

sequências previamente isoladas do genoma de *Hoplias malabaricus* (Characiformes, Erythrinidae) que estão clonadas à vetores plasmidiais e propagadas em células de *Escherichia coli* DH5α (Invitrogen, San Diego, CA, USA), disponíveis no Laboratório de Citogenômica Evolutiva da Universidade Federal de São Carlos. Enquanto a sonda de 5S DNAr inclui um fragmento de 120 pares de base (pb) do gene e outro de 200pb do espaçador não-transcrito (NTS), a sonda de 18S DNAr apresenta somente um fragmento de 1400pb do gene (Martins *et al*., 2006; Cioffi *et al*., 2009). Os produtos de PCR destas sondas foram marcados com nucleotídeos fluorescentes utilizando um kit comercial de *nick-translation* (Jena Biosciences, Jena, Alemanha) com os fluoróforos Atto550-dUTP (554-576 nm) para o 5S DNAr e Atto488-dUTP (501-523 nm) para o 18S. Já para a sequência telomérica, foi utilizado uma sonda comercial DAKO Telomere PNA FISH Kit/FITC (DAKO, Glostrup, Dinamarca), enquanto os microssatélites foram diretamente marcados com Cy3 (Sigma-Aldrich, Darmstadt, Alemanha) durante a síntese (Kubat *et al*., 2008).

Protocolo Fish-FISH (Sassi *et al*., 2023)

Dia 1

1. Aplique a preparação cromossômica em lâminas de vidro limpas;
2. Incube as lâminas por 1h a 60 °C em uma Jarra de Coplin;
3. Aplique 100 µl da solução de RNAse (1,5 µl RNAse A em 100 µl de 2×SSC) por lâmina e cubra com lamínula 24x60mm. Incube a 37 °C por 1h30min em uma câmara úmida e escura.
4. Em paralelo, coloque 1 ml de dd-H_2O + 10 µl de HCl 0,1 M para aquecer a 37 °C e, em uma Jarra de Coplin, coloque o tampão de desnaturação (70% (v/v) formamida deionizada, 20% (v/v) dd-H_2O, 10% (v/v) 20×SSC) para aquecer em banho maria a 72 °C;
5. Após o tempo de incubação da RNAse, remova a lamínula e lave a lâmina sob agitação em 1×PBS por 5 min em temperatura ambiente;
6. Adicione 3 µl de pepsina (20mg/µl) no tubo de H_2O+HCl previamente aquecido e homogeneíze. Aplique 100 µl desta solução nas lâminas ainda úmidas da etapa anterior e cubra com lamínula 24×60mm. Incube por 10 min a 37 °C em uma câmara úmida e escura;
7. Remova a lamínula e lave a lâmina em agitação em 1×PBS por 5 min em temperatura ambiente;
8. Rapidamente submerja as lâminas em água destilada e desidrate-as em série alcoólica (etanol 70%, 85%, e 100% por 2 min cada) em temperatura ambiente. Seque completamente as lâminas ao ar.
9. Desnature os cromossomos aplicados na lâmina pela submersão das lâminas no tampão de desnaturação previamente aquecido a 72 °C por 3min 15s.
10. Imediatamente após o tempo, transfira a lâmina para etanol 70% a -20 °C por 3 min, completando a série alcoólica em etanol 85% e 100% por 2 min cada em temperatura ambiente. Seque completamente as lâminas ao ar.
11. Desnature o mix de hibridização em termociclador a 85 °C por 10 min, seguido de resfriamento à 4 °C por ao menos 2 min antes da aplicação nas lâminas.
12. Aplique 20 µl do mix de hibridização (sondas + tampão de hibridização) nas lâminas e cubra com lamínula 24×50mm. Incube a 37 °C por 16 a 24h.

Dia 2

13. Aqueça uma jarra de Coplin com 1×SSC à 65 °C, remova as lamínulas e lave as lâminas por 5 min em agitação;

14. Transfira as lâminas para o tampão de lavagem (4×SSC/ 0,05% Tween) e lave por 5 min sob agitação;

15. Lave as lâminas em 1×PBS por 1 min sob agitação e após, submerja rapidamente em água destilada;

16. Desidrate-as em série alcoólica (etanol 70%, 85%, e 100% por 2 min cada) em temperatura ambiente. Seque completamente as lâminas ao ar.

17. Aplique 20 µl de DAPI+ *antifading* (Vector Laboratories, Burlingame, EUA), cubra com lamínula 24×50mm e estabilize a 4 °C por 30 min antes de observar em microscopia de fluorescência.

A depender do grau de dano aos cromossomos durante a técnica de FISH, que será influenciado pelo estado de fixação dos cromossomos, uma segunda rodada de hibridização pode ser realizada após a desmontagem da hibridização anterior. Após este processo, é possível retomar o protocolo Fish-FISH da etapa 9 (desnaturação dos cromossomos).

1. Remova a lamínula com cuidado e lave a lâmina duas vezes sob intensa agitação por 5 min em 1×SSC à 65 °C (utilize duas cubetas contendo a solução);

2. Repita o processo acima, porém em tampão de lavagem (4×SSC/Tween), a temperatura ambiente;

3. Lave as lâminas em 1×PBS por 1 min sob agitação e após, submerja rapidamente em água destilada;

4. Desidrate-as em série alcoólica (etanol 70%, 85%, e 100% por 2 min cada) em temperatura ambiente. Seque completamente as lâminas ao ar.

Com este protocolo, além da hibridização de sequências repetitivas para fins de caracterização do cariótipo, comparações interespecíficas e de prospecção de cromossomos sexuais pela diferença de marcadores entre os sexos, podemos realizar pequenas modificações para aplicação nas técnicas a seguir, baseadas na FISH.

3.2.3 Hibridização Genômica Comparativa (CGH)

Para o procedimento de CGH, utilizamos como sonda 500 ng de DNA total de cada espécie, submetido à marcação por nick-translation (Jena Biosciences, Jena, Alemanha), em uma reação de 3h30min a 15 °C, utilizando Atto550-dUTP (vermelho), Atto488-dUTP (verde) ou Atto425-dUTP (ciano). Além disso, C_0t-1 DNA não-marcado foi obtido por DOP-PCR (Zwick *et al*., 1997; Yang & Graphodatsky, 2009) e utilizado como competidor de hibridização, evitando a hibridização excessiva de regiões repetitivas ao longo do genoma. Para experimentos intraespecíficos (entre sexos), o mix de hibridização para cada lâmina (20 µl) foi composto de DNA total derivado de macho e fêmea (500 ng cada) acrescido de 25 µg de C_0t-1 DNA do sexo oposto ao da preparação cromossômica, visando identificar regiões sexo-específicas. O mix de hibridização foi precipitado a álcool utilizando 5 µl Acetato de Sódio 3M, 10 µl de tRNA (Cat. No.: R8508, Sigma, EUA), e 100 µl de etanol 100% ou isopropílico 100%. Após 14-16h a -20 °C ou 30 min a -80 °C, os tubos foram centrifugados a 13.000 rpm por 20 min e o sobrenadante descartado. O *pellet* então foi seco ao ar e ressuspendido no mix de hibridização descrito anteriormente. Para experimentos interespecíficos, utilizamos até três sondas de DNA total, mantendo a concentração de 500ng de cada espécie, sendo estas do mesmo sexo que a preparação cromossômica em que será hibridizada, e 10 µg de C_0t-1 DNA de cada espécie (totalizando 30 µg de bloqueadores), porém do sexo oposto ao da preparação cromossômica. No caso de experimentos interespecíficos com apenas duas sondas, utilizamos 15 µg de de C_0t-1 DNA de cada.

Durante a desnaturação da sonda, utilizamos 85 °C por 10 min, seguido de uma etapa de pré-anelamento do C_0t-1 DNA não marcado às sondas a 37 °C por 40 min e de resfriamento a 4 °C por 2 min. Além disso, após a aplicação do mix de hibridização nas lâminas e cobertura com lamínulas, aplicamos cola de borracha para selar as bordas (entre lamínula e lâmina) e a hibridização ocorreu em câmara úmida e escura a 37 °C por 20-40h. Após o período, as lavagens seguiram o protocolo Fish-FISH, porém com dupla lavagem na primeira etapa (PBS 1× 65°C).

3.2.4 Pintura Cromossômica Total (WCP)

Quatro sondas foram utilizadas nos experimentos de pintura cromossômica total, sendo duas derivadas de *Harttia carvalhoi* e duas de *H. punctata.* No primeiro cenário, o maior cromossomo metacêntrico (X) e o maior submetacêntrico (par 9) de *H. carvalhoi* foram selecionados, dado que ambos fazem parte das duas formas variantes do sistema múltiplo XX/XY_1Y_2 (Deon *et al*., 2022a, b). Já para *H. punctata*, os cromossomos X_1 e X_2 foram selecionados, visto que apresentam heteromorfismo (Blanco *et al*., 2014). Os cromossomos

foram microdissectados com agulha de vidro, onde 15 cópias de cada alvo foram capturadas em um microscópio invertido (Zeiss Axiovert 135). O DNA obtido foi amplificado em uma reação de DOP-PCR seguindo Yang *et al*. (2009). Uma segunda amplificação em DOP-PCR foi realizada, utilizando como molde 1 µl do produto inicialmente amplificado, para marcação fluorescente das sondas. Desta forma, cromossomos foram marcados com Spectrum Orange-dUTP (vermelho) ou Spectrum Green-dUTP (verde) (Vysis, Downers Grove, EUA).

Para as hibridizações, foram utilizados 200ng de cada sonda de pintura e 20 µg de C_0t-1 DNA derivado da espécie doadora dos cromossomos como sonda. A desnaturação seguiu o mesmo protocolo do CGH, com 45 min de pré-anelamento da sonda ao C_0t-1 DNA e a hibridização ocorreu por 48h a 37 °C em câmara úmida. As lavagens pós hibridização seguiram as mesmas para o CGH e, ao fim, 20 µl de DAPI+ Vectashield (Vector Laboratories, Burlingame, EUA) foram aplicados como contracorante de visualização em microscopia de fluorescência.

3.2.5 Satelitoma

Selecionamos quatro espécies com sistemas homólogos de cromossomos sexuais, para o isolamento da biblioteca de DNAs satélites (satDNAs = satelitoma) presentes nestas espécies. O DNA total de *H. duriventris*, *H. rondoni*, *H. villasboas*, e *H. punctata* foi extraído e posteriormente sequenciado em BGISEQ-500 (BGI Genomics, Shenzhen, China) gerando sequências de leitura curta de final emparelhado (*pair-end*) de 2 x 150 bp. Para *H. villasboas* e *H. rondoni*, machos e fêmeas foram sequenciados, enquanto para *H. duriventris* e *H. punctata*, apenas amostras de machos foram sequenciadas.

As sequências obtidas foram aparadas com Trimmomatic (Bolger *et al*., 2014) para a seleção de leituras com Q > 20 para todos os nucleotídeos, assim como para a remoção de adaptadores de sequenciamento. O satelitoma foi caracterizado pela sucessiva iteração da ferramenta TAREAN (Novák *et al*., 2017), que cria gráficos De Brujin a partir das sequências, onde apenas aquelas com alta similaridade e cobertura formam gráficos circulares, que são selecionados como sequências repetitivas. As sucessivas iterações procederam com a filtragem via DeconSeq (Schmieder & Edwards, 2011) das leituras com similaridade aos DNA satélites (satDNA) obtidos, até que nenhum satDNA seja recuperado. Estimamos a abundância e divergência de cada satDNA ao mascarar as leituras completas de machos e fêmeas (10,000,000 *reads* (2×5,000,000)) contra o satelitoma utilizando o RepeatMasker (Smit *et al*., 2013), com o script (https://github.com/fjruizruano/satminer/blob/master/repeat_masker_run_big.py). Ordenados pela abundância, nomeamos os satDNAs com a primeira letra do gênero e as duas

primeiras letras do epíteto específico, seguido do tamanho em pares de base (e.g. HviSat00-000 para *H. villasboas*, HroSat00-000, HduSat00-000 e HpuSat00-000 para *H. rondoni*, *H. duriventris* e *H. punctata*, respectivamente). Geramos *repeat landscapes* (i.e. gráficos, de divergência de satDNA considerando a distância entre sequências com base no modelo de Kimura-2-parâmetros (K2P)) (Kimura, 1980) via o script calcDivergenceFromAlign.pl no RepeatMasker. Satélites específicos ao sexo foram indicados quando a razão da abundância dos satDNA entre os sexos foi maior do que 1,2.

Buscamos por homologia entre os satelitomas de *Harttia* com o script rm_homology (Ruiz-Ruano *et al*., 2016), que realiza alinhamentos entre todas as sequências com o RepeatMasker v 4.0.5 (Smit *et al*., 2013). Para satDNAs correspondentes entre as espécies, realizamos alinhamentos par-a-par com o ClustalX (Thompson *et al*., 1997) e revisamos manualmente. A divergência de sequência entre as famílias de satDNA de cada comparação pareada foi calculada seguindo o modelo K2P no software MEGA7 (Kumar *et al*., 2016). A taxa de *turnover* consenso (CTR) foi calculada seguindo a fórmula: $CTR = K/2T$, onde T = tempo de divergência (obtido via TimeTree (Kumar *et al*., 2017)) e K = distância K2P. Além disso, para verificar a presença de satDNAs conservados, os satelitomas foram alinhados contra a coleção do NCBI via BLAST (Altschul *et al*., 1990). Utilizamos também a Repbase (Bao *et al*., 2015) para buscar por homologias com elementos transponíveis, via RepeatMasker (Smit *et al*., 2013) com as opções "no_low" e "no_is". Quadros de leitura abertos (ORF) foram prospectados nos satelitomas com "orfipy" (Singh & Wurtele, 2021) e Geneious 7.1.3, utilizando o tamanho mínimo de 100 pb. As ORF identificadas foram traduzidas para sequências de proteínas com SMS (Stothard, 2000) e buscadas na base de dados NCBI com BLASTp (Altschul *et al*., 1997), seguindo pela filtragem por *query cover* e porcentagem de identidade > 70%.

Ao longo dos experimentos de mapeamento das sequências, alguns satDNAs chamaram a atenção por sua distribuição contínua em cromossomos sexuais. Desta forma, buscamos alguns satDNAs como HviSat08-4011 entre as leituras brutas de Illumina de espécies como *H. duriventris* e *H. punctata*, das quais não conseguimos isolar sua contraparte homóloga com TAREAN, usando o script disponível publicamente mapping_blat_gs.py (https://github.com/fjruizruano/ngs-protocols/blob/master/mapping_blat_gs.py). As leituras brutas de ambas as espécies foram mapeadas e quantificadas em relação a cada duas partes descritas em HviSat08-4011 usando o script repeat_masker_run_big.py, conforme descrito acima.

Desenhamos primers para oito dos mais abundantes satDNAs de *H. villasboas*. Selecionamos essa espécie para a construção de sondas, uma vez que apresenta o sistema múltiplo X_1X_2Y e está mais intimamente relacionada à espécie portadora do sistema simples XY *H. rondoni* (quando comparada a *H. duriventris* e *H. punctata* segundo filogenias de Covain *et al*. (2016) e Londoño-Burbano & Reis (2021)), o que nos permite inferir o papel dos rearranjos que ocorreram no clado. As sequências de consenso para cada um desses satDNAs foram investigadas manualmente para o desenho de primers de PCR (**Tabela 4**). Em seguida, os satDNAs foram amplificados por PCR usando uma etapa de desnaturação inicial de 95 °C por 5 min, 30-35 ciclos a 95 °C durante 20 s, com 39,1–54,2 °C como temperatura de anelamento durante 40 s, 72 °C durante 30 s e uma etapa de extensão final de 10 min. Os produtos de PCR resultantes foram verificados em um gel de agarose a 1% para confirmar o padrão de escada típico característico dos satDNAs. As sequências foram depositadas no Genbank (Números de acesso: OR827473-OR827555).

Tabela 4. Condições ideais para amplificação por PCR dos satélites de *Harttia villasboas* (primers, temperatura, e concentração de DNA molde).

Satélite	Primer F	Primer R	Variação de temperatura de anelamento	[DNA] ng/µl
HviSat01	5’GAAACCCAGGCAAAAGTTCA	5’CCTTCATACTCCCATGTAGCT	56 °C - 48 °C	100
HviSat02	5’GGCTGAATTTTGCTGTGACAC	5’CCATAATGACGTCCCTCACC	56 °C - 48 °C	100
HviSat03	5’ACACTCATGACACTATCCCGT	5’CGGATTGTGCCCAGTAGCGTT	62.9 °C - 58 °C	100, 10, or 1
HviSat04	5’TCGGCCAAAATACAGACTGCA	5’TTCCAGACGGCTGATGGCTTG	66 °C - 58 °C	100, 10 or 1
HviSat05	5’ACGAAACTCGCATATGTCGTT	5’ACACGTACGACTTCGGCTTA	61.4 °C - 55 °C	100, or 10
HviSat08	5’ACAGAGTCCAAACTGAAGAGC	5’TCTTCTTCTTTTCCTCCACTG	62.9 °C - 58 °C	100, 10, or 1
HviSat13	5’CCACCTCTGCAAAAACAAGGG	5’TTGATCAGAGGGGTGCCGTC	64.4 °C - 58 °C	100
HviSat18	5’CGTTCCGATCGGGGAGGTCC	5’AGGCGCGTTCGGGGGACC	69°C - 62 °C	100

Para os experimentos de FISH, os produtos de PCR foram marcados com Atto550-dUTP ou Atto488-dUTP por *nick-translation* (Jena Biosciences, Jena, Alemanha). As sondas foram compostas de 100 ng de cada satDNA marcado acrescidos de 50% de formamida, 2× SSC, 10% de SDS, 10% de sulfato dextrano e tampão de Denhardt a pH 7,0 em um volume total de 20 µl. Os experimentos de FISH foram conduzidos primeiramente em lâminas de *H. villasboas* com cada sonda única, depois em FISH duplo para espécies irmãs *H. rondoni*, *H. duriventris* e *H. punctata*, além de *H. dissidens*, *H. guianensis* e *Harttia* sp. 3. Após os resultados dos satDNAs sondados em cromossomos sexuais, realizamos a pintura cromossômica completa (WCP), juntamente com um mapeamento FISH desses satélites, usando sondas derivadas da microdissecção dos cromossomos sexuais de *H. punctata* (HPU-X1 e HPU-X2), conforme descrito anteriormente. Para a investigação da evolução dos cromossomos sexuais, incluímos duas espécies (*H. kronei* e *H. intermontana*) que carregam a condição ancestral dos grupos de ligação que compõem os múltiplos X_1X_2Y (ou seja, HPU-X1 e HPU-X2 localizados em sintenia no mesmo cromossomo), seguindo os resultados dos experimentos de pintura cromossômica. Nessas duas últimas espécies, mapeamos os HviSatDNAs que foram encontrados acumulados nos cromossomos sexuais das quatro principais espécies-alvo e detectamos os grupos de ligação ancestrais por meio do WCP. As sondas foram desnaturadas a 86 °C por 8 min, então resfriadas a 4 °C por 2 min antes de serem aplicadas às lâminas. Os procedimentos de hibridização e lavagens pós-hibridização seguiram os protocolos previamente descritos nesta tese.

3.2.6 Análises de microscopia

Para todos os experimentos de microscopia de fluorescência, utilizamos dois microscópios distintos para a captura: um Olympus BX53 acoplado a uma câmera CoolSNAP (Olympus Corporation, Ishikawa, Japão) e o microscópio Axioplan II (Carl Zeiss Jena GmbH, Jena, Alemanha), ambos com filtros permitindo a aplicação de até quatro cores distintas. Verificamos os padrões de hibridização em ao menos 30 metáfases por indivíduo para confirmação dos resultados e utilizamos a classificação de cromossomos segundo de Levan *et al*. (1964), com adaptações sugeridas por Arai (2011) para peixes.

3.2.7 Reconstrução filogenética e marcadores ligados ao sexo

Para a reconstrução filogenética de *Harttia*, selecionamos todas as espécies com dados citogenéticos disponíveis (com exceção de *H. absaberi* pela ausência de amostras) e *Farlowella* sp. como grupo externo. Amostras de fígado de três machos e três fêmeas de cada espécie foram encaminhadas para genotipagem por sequenciamento na Diversity Arrays Technology (DArT)

Pty Ltd (University of Canberra, Canberra, Austrália). DArT-seq usa redução de complexidade para a identificação de regiões de baixa cópia e geralmente ativas do genoma, das quais regiões ligadas ao sexo podem ser incluídas, e NGS para a geração de milhares de polimorfismos de nucleotídeo único (SNP) de até 69 pb e loci SilicoDArT (presença-ausência). Em resumo, amostras de DNA foram digeridas com duas enzimas (PstI e SphI) antes da reação de ligação, seguidas pela amplificação dos fragmentos ligados em 30 rodadas de PCR e aplicadas ao Illumina HiSeq2000 para o sequenciamento de leitura única. Filtragem e controle de qualidade também foram realizados pela empresa, descartando quaisquer resultados com reprodutibilidade < 90% e profundidade de leitura < 3,5 para SNPs e < 5 para marcadores SilicoDArT.

Os marcadores ligados ao sexo foram obtidos pela aplicação do pipeline proposto por Lambert *et al*. (2016). Com o Microsoft Excel (Office 365, Microsoft Corporation), primeiro organizamos os SNPs por espécie e sexo de forma que machos e fêmeas fossem organizados em blocos distintos. Para cada linha, representando cada indivíduo para um alelo específico, usamos a função 'countif' para contar todos os resultados heterozigotos (2) para machos ou fêmeas, usando como contrapartida a contagem de resultados homozigotos (para a referência "0" ou o SNP "1"). Esses SNPs ligados ao sexo foram então organizados em uma segunda planilha, transformados em um arquivo ".fasta" e pesquisados via BLAST contra a coleção NCBI para verificar possível homologia com genes anotados ou regiões cromossômicas. Em seguida, usando o pacote dartR (Gruber *et al*., 2018; Mijangos *et al*., 2022), criamos um objeto "genlight" combinando os dados de SNP com informações de sexo dos indivíduos, prosseguindo com a filtragem de loci com alelo nulo com limite de 0,85 antes de executar uma Análise de Componentes Principais (PCA) para SNPs autossômicos e ligados ao sexo.

Utilizamos o método de máxima verossimilhança no software RAxML-NG (Kozlov *et al*., 2019) para a reconstrução filogenética das espécies com o modelo HKY+I+G4. O modelo foi selecionado com ModelTest-NG a partir de 203 modelos de submatrizes GTR (Darriba *et al*., 2020) e apresentou o menor AIC (238039,1556). Filtramos os dados de SNPs por *callrate* a 0,85 e removemos os marcadores sob seleção identificados com o BAYESCAN (Foll & Gaggiotti, 2008), resultando em um conjunto de dados com 1.375 sequências informativas para a reconstrução filogenética. Usando o pacote dartR (Gruber *et al*., 2018), na função "gl.2.fasta" e concatenamos as sequências, exportando em formato ".fasta" para a análise, resultando em uma sequência com 76.915 pb. No RAxML-NG construímos 50 árvores iniciais de máxima verossimilhança e 50 árvores de máxima parcimônia, gerando a melhor árvore para todas as iniciais (menor valor de log-likelihood). Então, realizamos 200 bootstraps não paramétricos e

mapeamos na melhor árvore usando o comando “raxml-ng --support". A árvore final foi então enraizada posteriormente, seguindo recomendação de Kozlov *et al*. (2019), utilizando o FigTree v1.4.4 (Rambaut *et al*., 2018).

3.2.8 Modelagens de história demográfica e evolução cromossômica

A partir da árvore final concatenada por espécie, realizamos modelagens da história demográfica utilizando o BioGeoBEARS (Matzke, 2013; Dupin *et al*., 2017). As estimativas de distribuição ancestral foram baseadas em três modelos: DEC (Ree e Smith, 2008), que modela evolução como um processo discreto mediado por dispersão, extinção local e cladogênese (Ree e Smith, 2008), DIVALIKE (Ronquist 1997), análise de dispersão e vicariância por verossimilhança, e BAYAREALIKE (Landis *et al*., 2013), que utiliza análise bayesiana para inferência biogeográfica. Considerando que a história demográfica de peixes não permite saltos de dispersão (pulo à nova área de ocorrência sem dispersão prévia e expansão de área), visto que são dependentes das conexões entre bacias, excluímos o parâmetro *j* das análises, assim como recomendado por diversos autores (Rabosky *et al*., 2014; Ree & Sanmartín, 2018; Cassemiro *et al*., 2023). Desta forma, utilizamos a delimitação de bacias disponível no HydroSHEDS (Lehner *et al*., 2008) para a delimitação das áreas biogeográficas de ocorrência, resultando em sete áreas (Guianas, Amazonas, Tocantins-Araguaia, Paraná, São Francisco, Atlântico Leste e Atlântico Sudeste). A comparação e seleção de modelos foi realizada com base no critério de informação de Akaike (AIC). O número máximo de áreas que uma espécie pode ocupar (max_range_size) foram três, seguindo o número máximo observado (2) com adição de uma área provável que pode ter ocorrido no passado, incluindo também o parâmetro nulo que permite a distribuição ser igual a zero áreas.

Para a modelagem de evolução cromossômica, utilizamos o ChromEvol (Mayrose *et al*., 2010; Glick & Mayrose, 2014). Desta forma, a análise ocorreu com a tabela de contagem de números cromossômicos haploides para cada espécie e a árvore filogenética previamente obtida. Utilizamos os seis modelos padrões, que consideram eventos de ganho e perda de cromossomos ao longo da filogenia (fissões e fusões), além de duplicações completas ou de 1,5x no genoma. A adequação de modelo foi realizada seguindo o teste nativo do software (Chromevol best model), utilizando o critério de informação de Akaike e o critério corrigido de informação de Akaike (AIC e AICc). Visto que a espécie *H. fowleri*, apesar de presente na reconstrução filogenética, não apresenta dados cromossômicos, o clado foi removido pelo software. Utilizando a ferramenta de remoção de clados disponível no ChromEvol para clados sem contagem de número cromossômico, testamos a evolução cromossômica incluindo e

excluindo o grupo-externo, além de uma terceira análise excluindo tanto o grupo-externo quanto *Harttia* sp. 2, visto que os dados cromossômicos e moleculares sugerem que tal espécie pode não representar uma espécie válida de *Harttia*. Com isso, almejamos representar os possíveis cenários de evolução cromossômica no grupo, considerando que nossas análises não incluem os gêneros irmãos de *Harttia* (i.e. *Harttiella* e *Cteniloricaria*). As probabilidades de 2n ancestral foram desenhadas aos nós das árvores filogenéticas usando RStudio com os pacotes ggplot2 (Wickham, 2016) e paletteer (Hvitfeldt, 2021), e Adobe Illustrator (Adobe Inc. 2020).

4 Resultados e Discussão

Os resultados e discussões serão apresentados no formato de capítulos, que resumem os trabalhos já publicados derivados desta tese, assim como resultados submetidos e/ou em fase final de preparação para submissão (**Tabela 4**). Destaca-se que todas as publicações foram ou serão realizadas em periódicos *open access*.

No primeiro capítulo "Caracterização citogenética de espécies de *Harttia* no contexto da evolução cromossômica de Loricariidae" abordaremos os trabalhos de descrição citogenética (Sassi *et al*., 2020, 2021), unindo ambos os trabalhos em uma discussão ampla em conjunto ao trabalho de revisão citogenética de Loricariidae (Sassi *et al*., 2024 *in press*). Similarmente, o segundo capítulo "Evolução de cromossomos sexuais em *Harttia*" compila os trabalhos baseados em WCP (Sassi *et al*., 2023a, b) e na caracterização do satelitoma (artigo em revisão no periódico Communications Biology). Por fim, o quarto capítulo "Evolução citogenômica e história demográfica em *Harttia*" abarca dados referentes a um único trabalho, que está em fase final de preparação. Ao final, no tópico **V - Considerações Finais**, será apresentada uma discussão geral englobando todos os três capítulos.

Tabela 5. Artigos científicos derivados da presente tese, detalhados em título e revista de publicação, destacando a editora entre parênteses, e organização quanto aos capítulos apresentados e respectivo número de anexo para as versões completas.

Capítulo	***Título***	***Periódico***	***Referência***	***Anexo***
1	Multiple Sex Chromosomes and Evolutionary Relationships in Amazonian Catfishes: The Outstanding Model of the Genus *Harttia* (Siluriformes: Loricariidae)	*Genes* (MDPI)	Sassi *et al*., 2020	I
	Adding New Pieces to the Puzzle of Karyotype Evolution in Harttia (Siluriformes, Loricariidae): Investigation of Amazonian Species	*Biology* (MDPI)	Sassi *et al*., 2021	II
	A state-of-art review of Loricariidae (Ostariophysi: Siluriformes) cytogenetics	*Neotropical Ichthyology* (SBI)	Sassi *et al.* (2024)	III
2	Turnover of multiple sex chromosomes in *Harttia* catfish (Siluriformes, Loricariidae): a glimpse from whole chromosome painting	*Frontiers in Genetics* (Frontiers)	Sassi *et al*., 2023a	IV
	Homeology of sex chromosomes in Amazonian *Harttia* armored catfishes supports the X-fission hypothesis for the X_1X_2Y sex chromosome system origin	*Scientific Reports* (Springer-Nature)	Sassi *et al*., 2023b	V
	Independent evolution of satellite DNA sequences in homologous sex chromosomes	*Communications Biology* (Springer-Nature)	Sassi *et al*., (em revisão)	VI
3	Evolutionary cytogenomics and demographic history in *Harttia* (Siluriformes, Loricariidae)	*In prep*	Sassi *et al.*	

Capítulo 1

Caracterização citogenética de espécies de *Harttia* no contexto da evolução cromossômica em Loricariidae

Este primeiro capítulo compila os resultados e discussões dos seguintes trabalhos derivados desta tese que já se encontram publicados: "*Multiple Sex Chromosomes and Evolutionary Relationships in Amazonian Catfishes: The Outstanding Model of the Genus Harttia (Siluriformes: Loricariidae)"*, publicado no periódico Genes (MDPI, IF 2023 = 2,8) (**Anexo I**) e "*Adding New Pieces to the Puzzle of Karyotype Evolution in Harttia (Siluriformes, Loricariidae): Investigation of Amazonian Species"*, publicado na revista Biology (MDPI, IF 2023 = 3,6) (**Anexo II**). Além disso, compilamos os resultados destes trabalhos com todos os artigos de citogenética da família Loricariidae publicados até o ano de 2023 no artigo "*A state-of-art review of Loricariidae (Ostaryophysi: Siluriformes) cytogenetics"*, publicado na revista Neotropical Ichthyology (SBI, IF 2023 = 2,0) (**Anexo III**).

No primeiro trabalho, caracterizamos citogeneticamente e pela primeira vez na literatura científica, as espécies *H. duriventris*, *H. rondoni* e *H. villasboas*, com enfoque na descrição dos cromossomos sexuais presentes nas três espécies (Sassi *et al*., 2020). Já o segundo trabalho teve como foco as espécies *H. dissidens*, *H. guianensis*, e *Harttia* sp. 3 (Sassi *et al*., 2021), que não apresentaram cromossomos sexuais detectáveis pelas metodologias utilizadas. A caracterização destas espécies se trata da primeira ampla amostragem de *Harttia* realizada na região Norte do Brasil para fins de investigação citogenética, preenchendo importantes lacunas no conhecimento da evolução cromossômica deste gênero. Para isso, combinamos a análise de cariótipo via Giemsa e a caracterização da heterocromatina por bandamento C, com o mapeamento de sequências repetitivas por FISH (DNAr 5S e 18S, microssatélites $(CA)_{15}$, $(GA)_{15}$ e $(A)_{30}$, e sequências teloméricas (TTAGGG)n). Além disso, realizamos experimentos de CGH intraespecíficos (para comparações entre os sexos) e interespecíficos (entre as distintas espécies) para a caracterização da história evolutiva do grupo.

Descrevemos o 2n = 54, inédito para o gênero, em três espécies: *H. rondoni* (XX/XY) (**Figura 7d-f)**, *H. dissidens* e *Harttia* sp. 3 (**Figura 8A e C**). Enquanto ambas *H. duriventris* (16m + 16sm + 16st + 8a) e *H. villasboas* (18m + 24sm + 6st + 8a) apresentaram 2n = 56 para fêmeas, encontramos 2n = 55 em machos (17m + 16sm + 16st + 6a em *H. duriventris* e 19m +

24sm + 6st + 6a em *H. villasboas*), devido a um sistema de cromossomos sexuais múltiplos $X_1X_1X_2X_2/X_1X_2Y$ (**Figura 7a-c e g-i**). Por fim, *H. guianensis* apresentou 2n = 58 (20m + 26sm + 2st + 10a) para ambos os sexos (**Figura 8B)**. Em concordância com as demais espécies do gênero, bandas de heterocromatina C-positiva foram observadas na região pericentromérica da maioria dos cromossomos do complemento, com poucos cromossomos apresentando padrões distintos, como as marcações terminais observadas no primeiro par de acrocêntricos de *H. guianensis*. A distribuição simples de DNAr foi observada em todas as espécies, com exceção de *H. guianensis*, que apresenta um sítio extra de DNAr 5S em sintenia ao par do DNAr 18S (par 25).

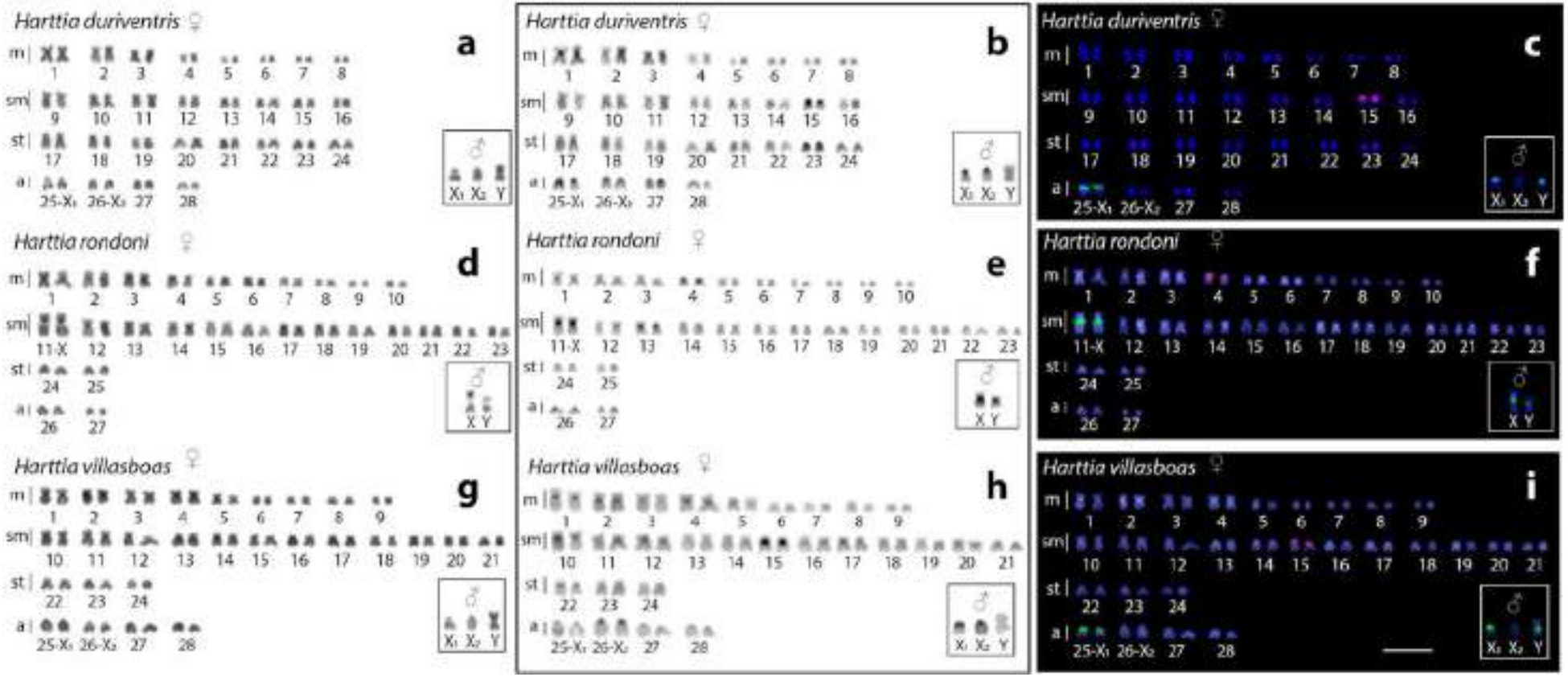


Figura 7. Reprodução da Figura 2 do artigo Sassi *et al*. (2020) (**Anexo I**). Cariótipos de *H. duriventris* (a-c), *H. villasboas* (d-f) e *H. rondoni* (g-i), organizados sequencialmente em Giemsa (a, d, e g), bandamento-C (b, e, e h) e FISH com DNAr 5S (vermelho) e DNAr 18S(verde) (c, f, e i). Cromossomos sexuais se encontram destacados. Barra de escala = 5 µm.

Figura 8. Reprodução das Figuras 2 e 3 do artigo Sassi *et al*. (2021) (**Anexo II**). Cariótipos de *H. dissidens* (A), *H. guianensis* (B) e *Harttia* sp. 3 (C), organizados sequencialmente em Giemsa e bandamento-C (correspondentes a Figura 2 do artigo) e FISH com DNAr 5S (vermelho) e DNAr 18S (verde) (Figura 3 do artigo). Barra de escala = 10 µm.

A distribuição de microssatélites variou conforme a presença ou ausência de cromossomos sexuais. O $(A)_{30}$ demonstrou sinais positivos em nove cromossomos em machos e dez cromossomos em fêmeas das espécies *H. duriventris*, *H. rondoni* e *H. villasboas* (**Figura 9**) devido à ausência de sinais no cromossomo Y destas espécies. Além disso, *H. rondoni* demonstrou sinais dispersos em todos os outros cromossomos para este mesmo microssatélite. Já nas espécies sem cromossomos sexuais detectados (*H. dissidens*, *H. guianensis* e *Harttia* sp. 3 – **Figura 10**), o $(A)_{30}$ também apresentou um padrão disperso na maioria dos cromossomos, com sinais mais proeminentes na região pericentromérica de alguns pares em *Harttia* sp. 3. Em relação ao $(CA)_{15}$ e $(GA)_{15}$, estes se apresentaram como dispersos nos cromossomos, com preferência de acumulação nas regiões terminais dos cromossomos, em especial nos cromossomos sexuais (na região telomérica dos cromossomos X_2 e Y de *H. duriventris*, nas regiões teloméricas de X_1, X_2 e Y de *H. villasboas*, e fortemente acumulado na região telomérica dos cromossomos X e Y de *H. rondoni*).

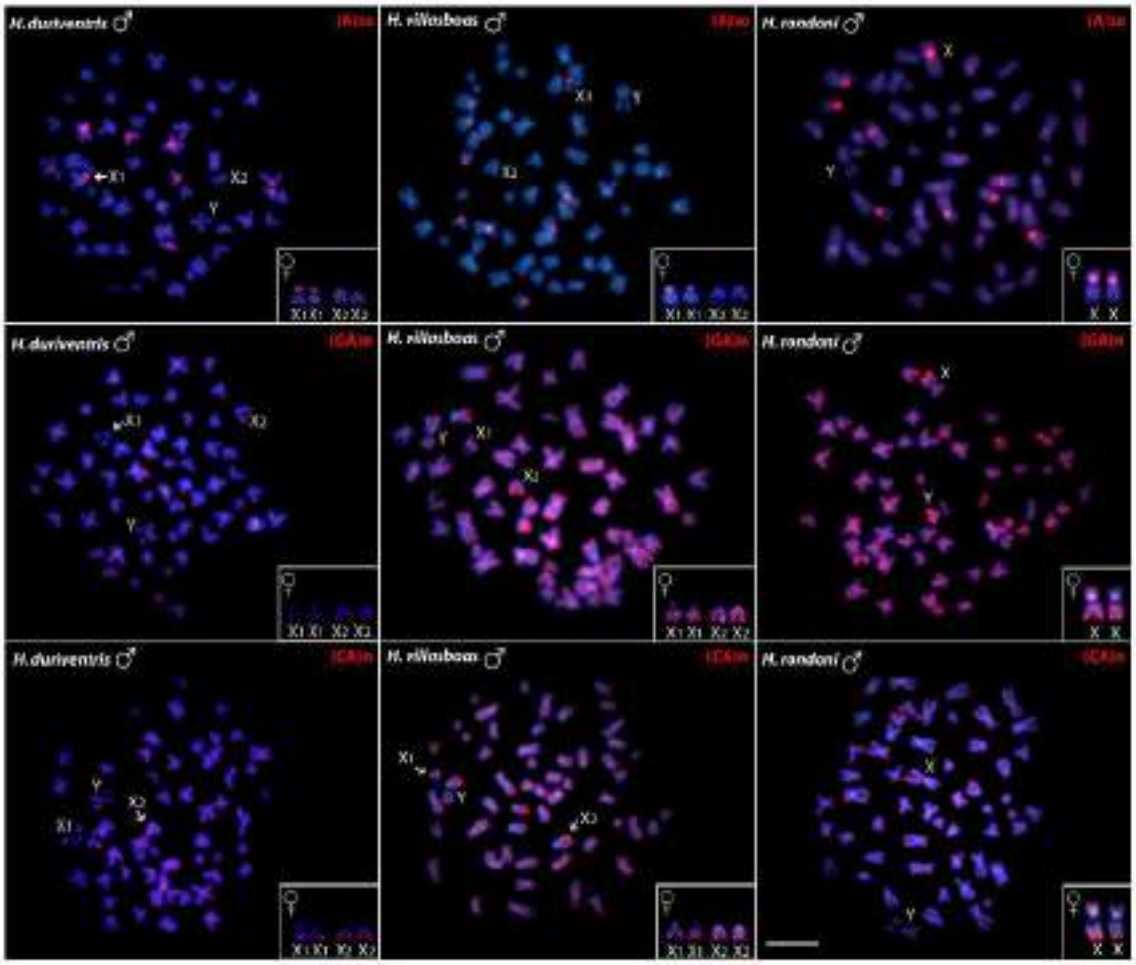


Figura 9. Reprodução da Figura 3 do artigo Sassi *et al*. (2020) (**Anexo I**). Cromossomos em metáfase de machos de *H. duriventris*, *H. villasboas* e *H. rondoni* hibridizados com sondas de microssatélites. Os cromossomos foram contracorados com DAPI (azul) e as sondas de microssatélites foram diretamente marcadas com Cy3 durante a síntese (sinais vermelhos). Os cromossomos sexuais femininos são mostrados em caixas. Barra = 5 µm.

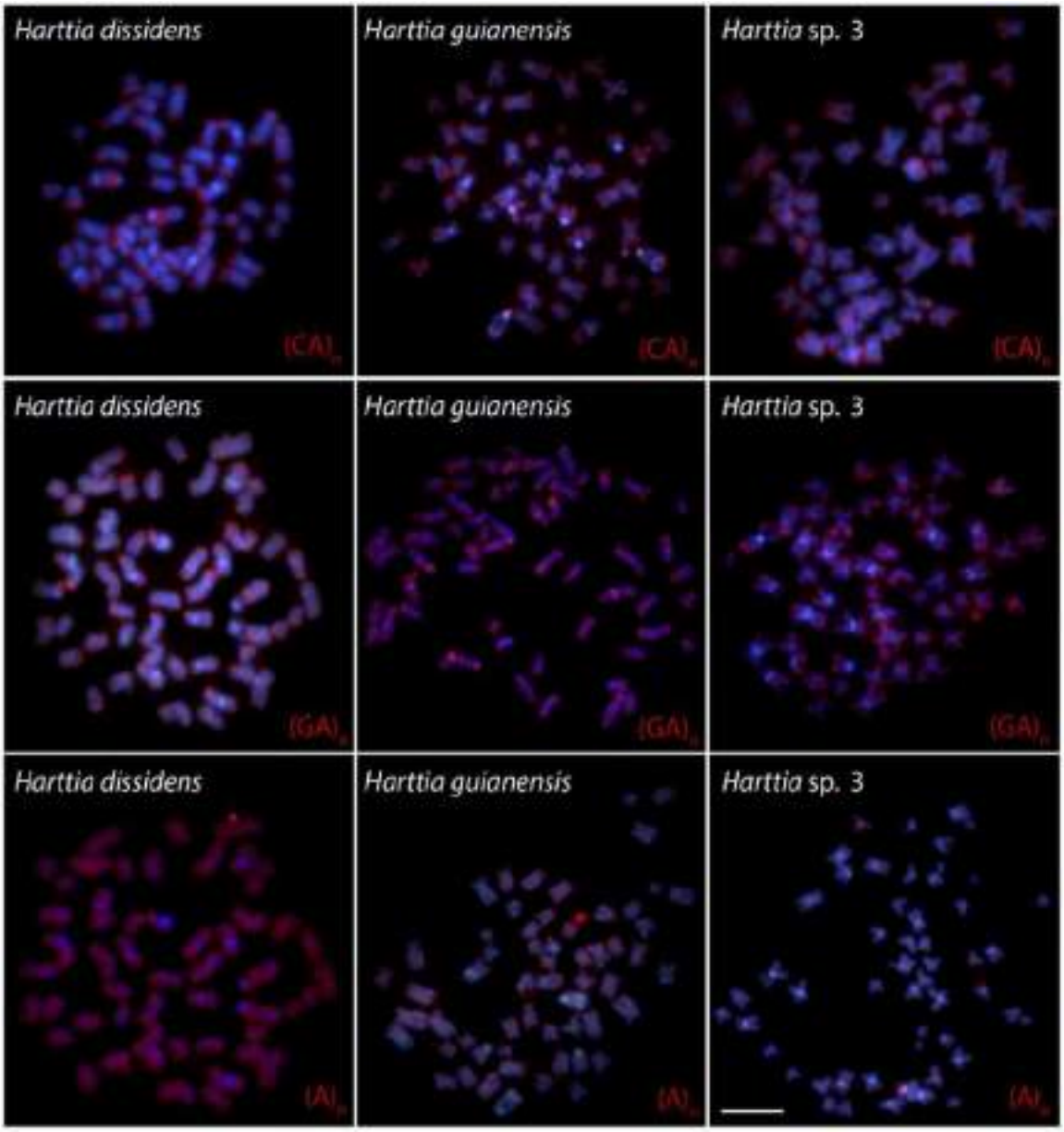


Figura 10. Reprodução da Figura 4 do artigo Sassi *et al*. (2021) (**Anexo II**). Mapeamento cromossômico de três sequências de microssatélites em cromossomos mitóticos de *Harttia*. Sondas (sinais vermelhos): $(A)_{30}$, $(CA)_{15}$ e $(GA)_{15}$. Os cromossomos foram contracorados com DAPI (azul). Barra de escala = 10 µm.

Os experimentos de CGH entre os sexos não demonstraram regiões sexo específicas em nenhuma das espécies (**Figuras 11 e 12**). Contudo, um pequeno acúmulo de sequencias é observada nos cromossomos Y de *H. villasboas*, *H. rondoni* e *H. duriventris* (**Figura 11**). Curiosamente em *Harttia* sp. 3, a sonda genômica derivada do genoma dos machos hibridizou predominantemente (mas não exclusivamente) na região pericentromérica do par 2.

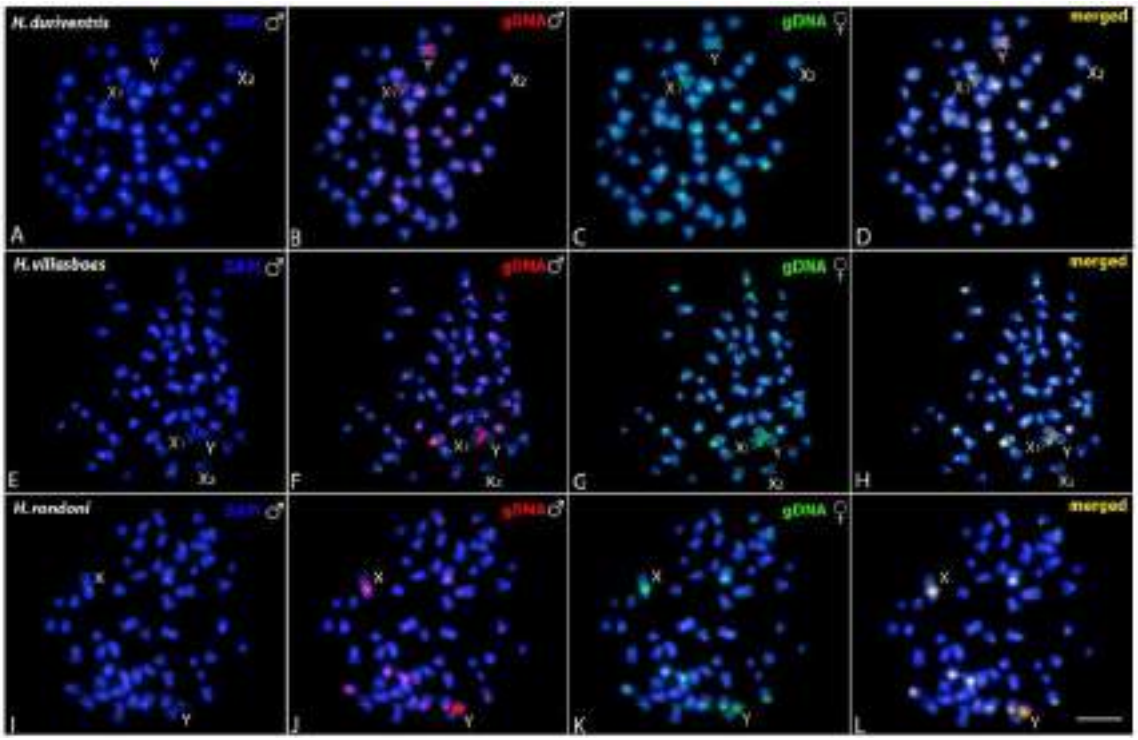


Figura 11. Reprodução da Figura 5 do artigo Sassi *et al*., 2020 (**Anexo I**). Cromossomos mitóticos de machos de *H. duriventris* (A–D), *H. villasboas* (E–H) e *H. rondoni* (I–L) submetidos a CGH. Sondas genômicas derivadas de machos e fêmeas foram hibridizadas concomitantemente para cada espécie. Primeira coluna (A, E e I): DAPI (azul); segunda coluna (B, F e J): padrão de hibridização da sonda derivada de machos (vermelho); terceira coluna (C, G e K): padrão de hibridização da sonda derivada de fêmeas (verde). Quarta coluna (D, H e L): imagens mescladas de ambas as sondas genômicas e coloração DAPI. As regiões genômicas comuns para machos e fêmeas são representadas em amarelo. Os cromossomos sexuais são indicados. Barra = 10 μm.

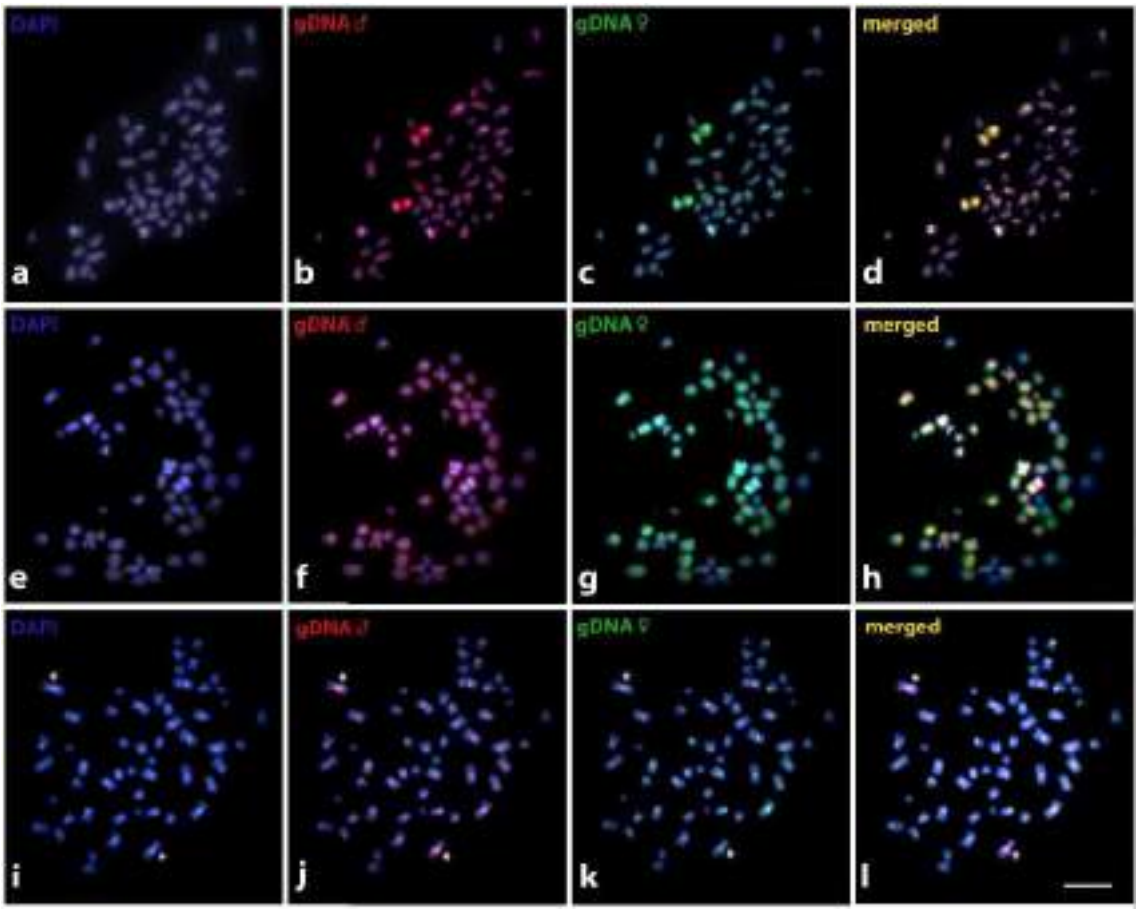


Figura 12. Reprodução da Figura 5 do artigo Sassi *et al*., 2021 (**Anexo II**). Metáfases mitóticas de machos de *Harttia* após procedimento CGH intraespecífico. *H. dissidens* (a–d), *H. guianensis* (e–h) e *Harttia* sp. 3. (i–l). Primeira coluna (a, e, i): imagens DAPI (azul); segunda coluna (b, f, j): padrões de hibridização de sondas de gDNA derivadas de machos; terceira coluna (c, g, k): padrões de hibridização de sondas de gDNA derivadas de genoma de fêmeas; quarta coluna (d, h, l): imagens mescladas de ambas as sondas genômicas e contracoloração DAPI. Regiões com hibridização concomitante de ambas as sondas genômicas são amarelas (ou seja, combinação de verde e vermelho). Asteriscos destacam as regiões com acúmulo preferencial de sonda de gDNA derivadas de machos quando comparadas a padrões de hibridização de sonda de gDNA derivada do genoma de fêmea. Barra de escala = 10 µm.

Para os CGH interespecíficos, focamos na comparação entre os genomas das espécies investigadas e o sistema de cromossomos sexuais múltiplo X_1X_2Y. No primeiro experimento (**Figura 13**), hibridizamos o DNA total de todas as espécies portadoras do sistema (*H. duriventris*, *H. villasboas* e *H. punctata*) contra os cromossomos de *H. villasboas*, que indicou alto grau de compartilhamento entre os três sistemas, sugerindo uma origem comum. Já no segundo experimento (**Figura 14**), encontramos padrões uniformes de hibridização de ambas as sondas de *H. dissidens* e *H. guianensis* em todo o complemento cromossômico de machos *H. villasboas*. A sonda derivada do genoma de machos de *H. villasboas*, no entanto, destacou predominantemente as regiões de alto conteúdo repetitivo, apontando para o fato de que os outros dois genomas comparados não compartilham (qualitativa ou quantitativamente) essa porção específica das classes de DNA repetitivo de *H. villasboas*.

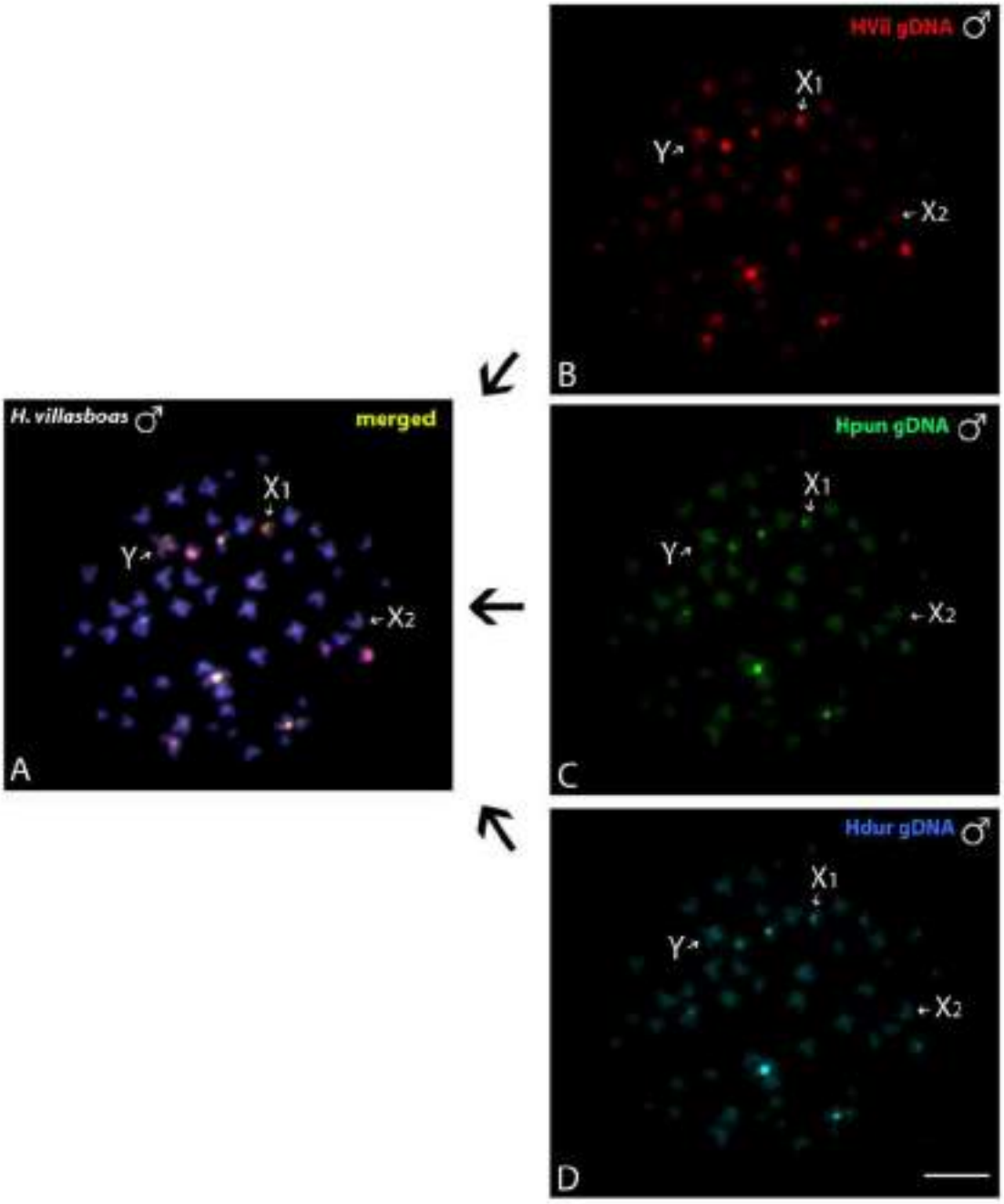


Figura 13. Reprodução da Figura 6 do artigo Sassi *et al*., 2020 (**Anexo I**). Hibridização Genômica Comparativa (CGH) de espécies de *Harttia* com sistema de cromossomo sexual X_1X_2Y. Cromossomos masculinos mitóticos de *H. villasboas* (A) hibridizados contra sondas genômicas derivadas de machos de *H. villasboas* (B) *H. punctata* (C) e H. duriventris (D). Os cromossomos sexuais são indicados e as regiões genômicas comuns para as três espécies são representadas em amarelo em (A). Barra = 10 μm.

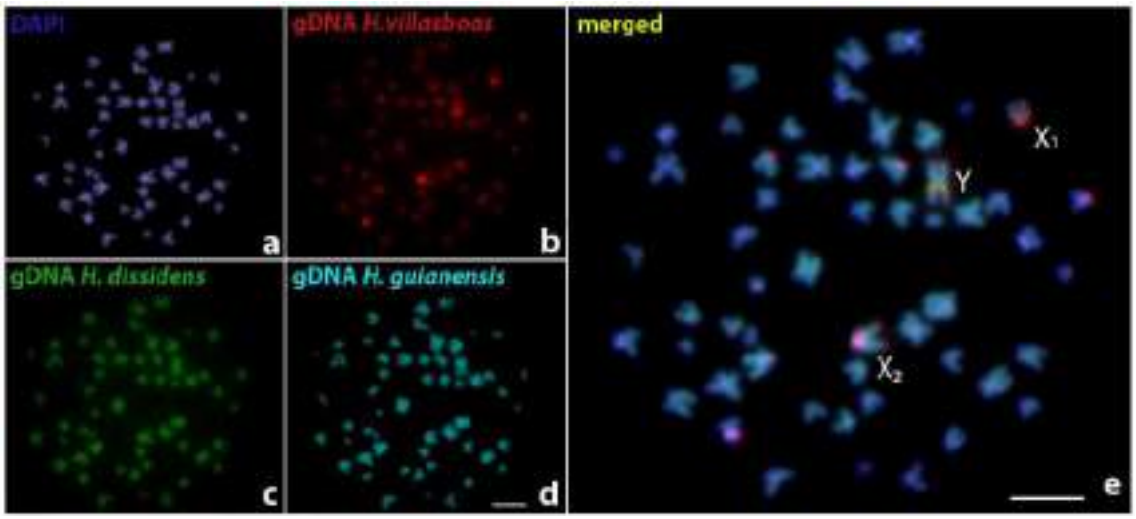


Figura 14. Reprodução da Figura 6 do artigo Sassi *et al*., 2021 (**Anexo II**). Hibridização Genômica Comparativa (CGH) de espécies de *Harttia* com sistema de cromossomo sexual X_1X_2Y. Cromossomos masculinos mitóticos de *H. villasboas* (a) hibridizados contra genoma de machos de *H. villasboas* (vermelho) (b), *H. dissidens* (verde) (c) e *H. guianensis* (azul claro) (d). A imagem composta (e) contém todas as três sondas genômicas mescladas e contracoloração DAPI (a). As regiões genômicas com hibridização compartilhada de sondas são amareladas. Barra de escala = 10 µm.

Com esta caracterização citogenética, ampliamos o número de espécies com dados cromossômicos, preenchendo uma importante lacuna para as espécies do Norte do Brasil. O gênero *Harttia* é definido por três principais clados: um composto por espécies do Sul e Sudeste do Brasil, outro por espécies das drenagens do Norte do Brasil e o terceiro por espécies do Escudo das Guianas (Covain *et al*., 2016). Anteriormente, apenas a espécie *H. punctata* tinha seu cariótipo conhecido (Blanco *et al*., 2014, 2017) e a descrição de *H. guianensis* reforça a proposta de 2n = 58 como número diploide ancestral do gênero (Blanco *et al*., 2017). O clado que inclui *H. guianensis* é apontado por reconstruções filogenéticas como clado mais basal (Covain *et al*., 2016; Londoño-Burbano e Reis, 2021), e dado que essa espécie compartilha o mesmo 2n com outras espécies de *Harttia* de outros clados, como *H. gracilis*, *H. kronei*, *H. longipinna* e fêmeas de *H. punctata*, é provável que este corresponda ao 2n ancestral para o gênero como um todo. Apesar do 2n ancestral para Loricariidae ser proposto como 2n = 54 (Artoni & Bertollo, 2001), a descrição citogenética de clados basais na família – como Harttiini e outros clados basais de Farlowellinii – tem desafiado tal proposta, sugerindo que outros 2n possam representar o caractere plesiomórfico do grupo (Takagui *et al*., 2020). De fato, o levantamento bibliográfico conduzido durante a presente tese indica que este 2n é o mais amplamente distribuído em Loricariidae (**Figura 15**). Porém, ao considerarmos que o número de

espécies cariotipadas ainda é extremamente baixo comparado à diversidade da família (234 spp. com dados citogenéticos vs. 1051 espécies válidas), os caminhos evolutivos que levaram à diversidade de 2n e estruturas cariotípicas ainda são obscuros.

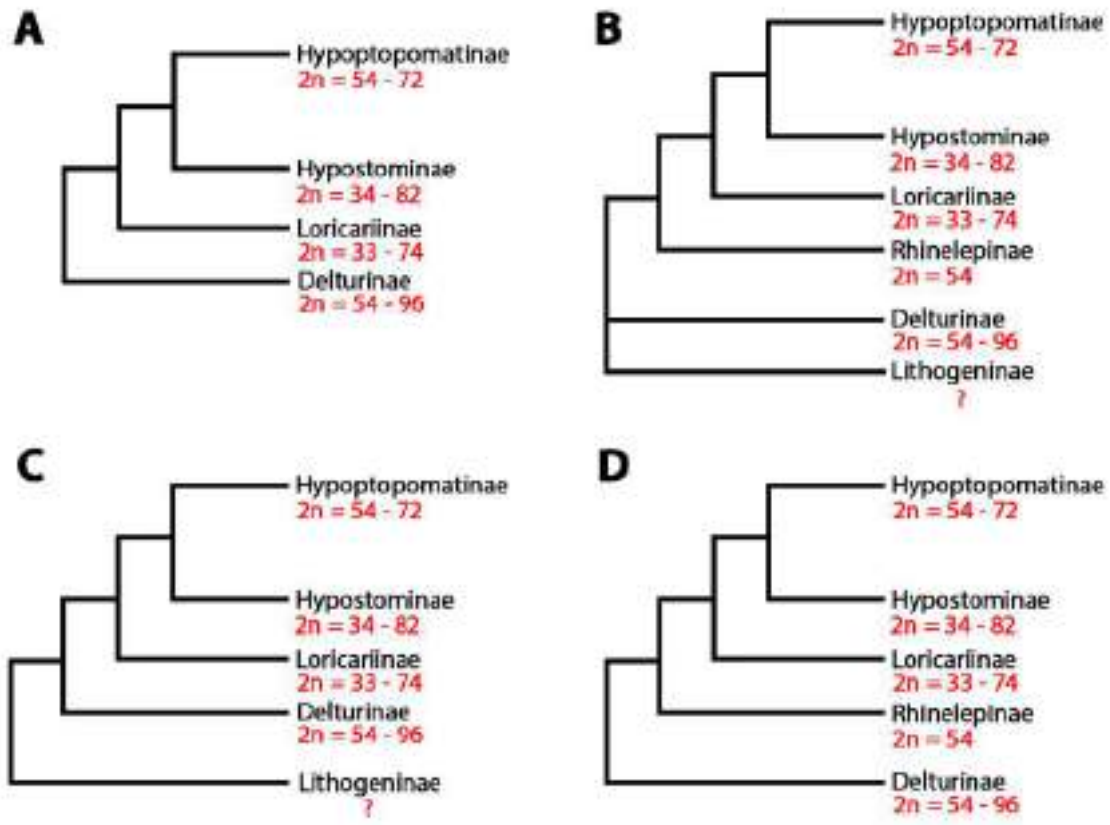


Figura 15. Reprodução da Figura 3 do artigo aceito para publicação no periódico Neotropical Ichthyology (Sassi *et al*., *in press*) (**Anexo III**). Relações filogenéticas de Loricariidae com dados citogenéticos aqui compilados em vermelho. Filogenias moleculares modificadas de: A. Roxo *et al*. 2014; B. Lujan *et al*., 2015; C. Pereira e Reis 2017; D. Roxo *et al*. 2019.

Enquanto a estabilidade é restrita ao 2n em alguns gêneros, com alta divergência na estrutura do cariótipo, como 2n = 58 em *Farlowella*, 2n = 54 em *Corumbataia* e 2n = 52 em *Pterygoplichthys*, a conservação de 2n e estrutura cariotípica também são observados, como 2n = 52 (26m + 20sm + 6st/a) em duas espécies de *Panaque*. Além disso, variações populacionais podem ser observadas na família, como em *Rhinelepis aspera* do mesmo rio que diferem na fórmula cariotípica (Artoni & Bertollo, 2001; Endo *et al*., 2012), sugerindo que inversões pericêntricas desempenharam um papel importante na evolução cromossômica da espécie. Tal mecanismo não é restrito a Rhinelepinae, mas também observado em outros loricariídeos, como *Ancistrus* (Mariotto *et al*., 2009),

Loricariichthys (Takagui *et al*., 2014) e *Rineloricaria* (Rosa *et al*., 2012; Primo *et al*., 2018). Além disso, os múltiplos Ag-NOR observados em *Pogonopoma wertheimeri* Steindachner 1867 (Artoni & Bertollo, 2001) quando comparados à distribuição simples em outras espécies de Rhinelepinae sugerem que outros mecanismos também são importantes para a evolução cromossômica no grupo. Notavelmente, polimorfismos numéricos e estruturais são frequentemente observados em loricariídeos, por exemplo, nos múltiplos cariomorfos de *Rineloricaria pentamaculata* (Glugoski *et al*., 2023), e a presença de cromossomos B em *Harttia longipinna* (Blanco *et al*., 2012). Essa diversidade cromossômica foi provavelmente gerada por uma combinação de rearranjos que incluem fusões e fissões Robertsonianas, inversões paracêntricas e pericêntricas e translocações (Artoni & Bertollo, 2001; Kavalco *et al*., 2004; Ziemniczak *et al*., 2012).

Três principais caminhos evolutivos são descritos para *Harttia*: (i) a manutenção do ancestral 2n = 58; (ii) a elevação de 2n por fissões cêntricas, e (iii) a redução de 2n por fusões (Deon *et al*., 2020). Para Loricariidae, é proposto que pontos de quebra evolutivas ocorram adjacentes a sítios de DNAr, promovendo quebras em dupla fita e a reorganização dos sítios de ribossomais, devido a instabilidade gerada pela alta atividade transcricional da região ribossomal (Berthelot *et al*., 2015; Barros *et al*., 2017; Glugoski *et al*., 2018; Schöfer & Weipoltshammer, 2018; Warmerdam & Wolthuis *et al*., 2019; Deon *et al*., 2020). Em nossas análises, observamos que embora 2n = 54 seja compartilhado em três espécies (*H. dissidens*, *H. rondoni* e *Harttia* sp. 3), é altamente provável que o par cromossômico carregador do locus de DNAr 18S esteja envolvido em eventos de fusão em *H. dissidens* e *Harttia* sp. 3, dada sua distribuição em cromossomos meta- ou submetacêntricos em detrimento da sua presença em acrocêntricos nas demais espécies de *Harttia*. Embora não seja uma característica comum para o genoma dos peixes, a presença de sítios sintênicos de DNAr 5S e 18S é considerada uma característica cariotípica basal para Loricariidae (Ziemniczak *et al*., 2012). Essa característica também é observa em *H. guianensis*, no par 25, assim como em *H. carvalhoi* (Centofante *et al*., 2006), *Harttia* sp. 1 (Deon *et al*., 2020), e *H. loricariformis* (Kavalco *et al*., 2004). Dada a posição de *H. guianensis* na base da filogenia de *Harttia*, parece provável que este segundo sítio de DNAr 5S tenha sido eliminado durante a evolução em muitas espécies, enquanto foi retido em um subconjunto destas. Curiosamente, o sítio simples de DNAr 5S é hipoteticamente localizado em cromossomos homólogos (par 19 em *H. dissidens*, 4 em *H. guianensis* e *H. rondoni*, e 15 em *H. villasboas*, *H. duriventris* e *Harttia* sp. 3). Os pares de cromossomos mencionados apresentam pequenas diferenças em forma e

tamanho entre as espécies, diferindo também quanto à posição do 5S DNAr (terminal vs. proximal), o que poderia ser explicado por inversões paracêntricas.

Além de autossomos, sequências de DNAr aparentam ter um importante papel na evolução de cromossomos sexuais em *Harttia* e outros Loricariidae. Considerando a diversidade de sistemas sexuais múltiplos observados no gênero, *Harttia* pode ser considerado um repositório de sistemas múltiplos, empatando em número de casos por espécie com o gênero *Notobranchius* (Cyprinodontiformes, Nothobranchiidae) (Sember *et al*., 2021). Embora o rearranjo mais comumente observado na origem de sistemas múltiplos seja a fusão entre o cromossomo Y e um par autossômico (Pennell *et al*., 2015; Sember *et al*., 2021), a presença do sistema XY em *H. rondoni* em estágios iniciais de diferenciação cria duas principais hipóteses. A hipótese do proto-XY sugere que o sistema observado em *H. rondoni* represente um estágio da diferenciação de XY pela amplificação e contração de repetições em tandem. Dessa forma, este seria o caractere ancestral e o sistema X_1X_2Y originado por fissões cêntricas. Já a hipótese do neo-XY sugere a fusão de um par ancestral XY com autossomos, originando o sistema múltiplo das demais espécies do Norte (i.e. *H. duriventris*, *H. villasboas* e *H. punctata*) e uma fusão secundária em *H. rondoni* originando o neo-XY, explicando a redução cariotípica ocorrida nesta espécie. Almejando esclarecer tais cenários evolutivos, realizamos análises com foco na origem e evolução dos cromossomos sexuais no gênero, que serão apresentadas a seguir.

Capítulo 2

Evolução de cromossomos sexuais em *Harttia*

Neste capítulo são apresentados os resultados e discussões de três trabalhos científicos cujo foco é a caracterização da história evolutiva dos cromossomos sexuais no gênero. Estes compreendem dois trabalhos já publicados "*Turnover of multiple sex chromosomes in Harttia catfish (Siluriformes, Loricariidae): a glimpse from whole chromosome painting*", publicado no periódico Frontiers in Genetics (Frontiers, IF 2023 = 2,8) (**Anexo IV**) e "*Homeology of sex chromosomes in Amazonian Harttia armored catfishes supports the X-fission hypothesis for the X_1X_2Y sex chromosome system origin*", publicado na revista Scientific Reports (Springer-Nature, IF 2023 = 3,8) (**Anexo V**), além do trabalho "*Independent evolution of satellite DNA sequences in homologous sex chromosomes*", que se encontra em revisão no periódico Communications Biology (Springer-Nature, IF 2023 = 5,2).

Nos dois primeiros trabalhos, realizamos o mapeamento de dois conjuntos de sondas de pintura cromossômica, descrevendo os padrões de hibridização ao longo de espécies com e sem sistemas de cromossomos sexuais detectáveis por citogenética. O primeiro grupo de sondas se refere aos pares de cromossomos X (HCA-X) e 9 (HCA-9), constituintes das duas formas variantes do sistema XY_1Y_2, encontrados em algumas espécies do Sul e Sudeste Brasileiro. Enquanto *H. carvalhoi* e *Harttia* sp. 1 compartilham inteiramente a identidade dos cromossomos XY_1Y_2, o mesmo sistema em *H. intermontana* apresenta uma translocação com o grupo de ligação correspondente ao cromossomo 9 em *H. carvalhoi*, o que implica duas fusões X-autossomo independentes na origem desse sistema de cromossomos sexuais (Deon *et al*., 2022a). Dessa forma, aplicamos esse conjunto de sondas de WCP nas seis espécies citogeneticamente descritas por essa tese (i.e. *H. villasboas*, *H. duriventris*, *H. rondoni*, *Harttia* sp. 3, *H. dissidens* e *H. guianensis*) para estudar a dinâmica desses grupos de ligação em outros clados que não apresentam esse tipo de sistema múltiplo XY_1Y_2 (**Figura 16**). Cada sonda hibridizou completamente dois pares distintos de cromossomos (um metacêntrico e um submetacêntrico) em *H. villasboas*, *H. duriventris*, *H. rondoni* e *Harttia* sp. 3. Além desses pares, *H. guianensis* também demonstrou um pequeno par metacêntrico com

hibridização adicional na sonda HCA-9. Em *H. rondoni*, sinais de HCA-X adicionais foram encontrados na região pericentromérica dos cromossomos sexuais XY, provavelmente devido ao conteúdo compartilhado de DNAr que foi amplificado neste par. Finalmente, em *H. dissidens*, a sonda HCA-9 marcou inteiramente um par submetacêntrico, mas apenas braços longos de um par metacêntrico, enquanto a sonda HCA-X revelou os mesmos padrões da maioria das espécies estudadas, com um par metacêntrico e um submetacêntrico.

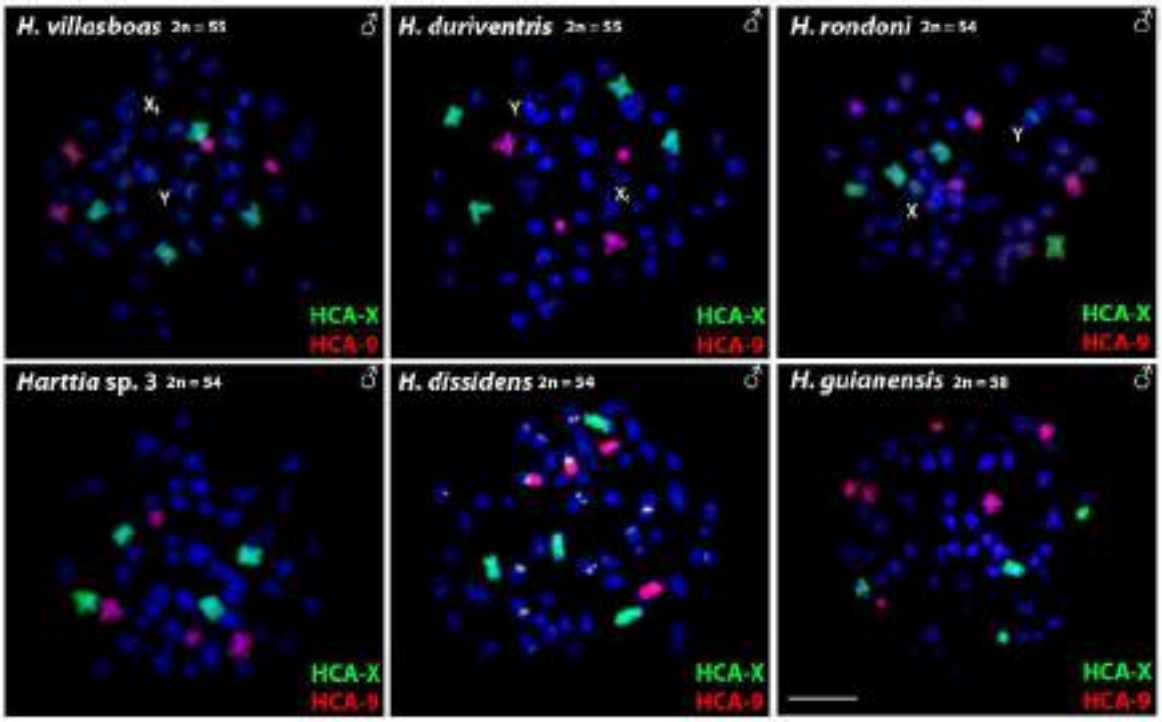


Figura 16. Reprodução da Figura 2 do artigo Sassi *et al*., 2023a (**Anexo IV**). Pintura cromossômica total (WCP) com HCA-X (verde) e HCA-9 (vermelho) em cromossomos de machos de seis espécies de *Harttia*, indicadas no canto superior esquerdo. Cromossomos sexuais estão indicados se presentes e detectáveis (primeira linha). Barra de escala = 10 µm.

Estes resultados indicam que os grupos de ligação envolvidos nas duas formas independentes do sistema XY_1Y_2 não fazem parte dos cromossomos sexuais encontrados nas espécies aqui trabalhadas. Além disso, demonstramos que o par 9 de *H. carvalhoi* está presente em um rearranjo encontrado em *H. dissidens*, indicando a propensão desse par a rearranjar no cariótipo. Baseando-se nos padrões encontrados, é possível inferir que que a redução no 2n a partir de um ancestral 2n = 58 pela fusão de HCA-9 apenas ocorreu em *H. dissidens*, já que nas demais espécies com cariótipo reduzido (i.e., 2n = 56 em *H. loricariformis*, *H. villasboas* e *H. duriventris*, e 2n = 54 em *H. rondoni* e *Harttia* sp. 3)

este cromossomo está presente como grupos de ligação independentes (Deon *et al*., 2022). Considerando que o cariótipo de *H. guianensis* possa representar o estado ancestral dos grupos de ligação, a presença de três pares com homologia à HCA-9, incluindo um par acrocêntrico, sugere que fusões cêntricas de cromossomos acrocêntricos foram responsáveis pela redução do 2n nas demais espécies. Concordantemente, *H. guianensis* é a espécie com maior número de cromossomos acrocêntricos (cinco pares), quando comparada com espécies com menor 2n (Blanco *et al*., 2017; Deon *et al*., 2020; Sassi *et al*., 2020, 2021).

O segundo trabalho focou na comparação entre as espécies que apresentam o sistema X_1X_2Y, usando sondas de WCP derivadas do sistema sexual de *H. punctata* (HPU-X1 e HPU-X2) nas seis espécies alvo desta tese. Encontramos homologia completa entre espécies com o mesmo sistema múltiplo (i.e. *H. duriventris*, *H. villasboas* e *H. punctata*), além do sistema XY presente em *H. rondoni* (**Figura 17**). Para as espécies sem cromossomos sexuais citogeneticamente distinguíveis, — *H. dissidens*, *H. guianensis* e *Harttia* sp. 3 – ambas as sondas de pintura hibridizaram em um único par de cromossomos metacêntricos, em geral com a sonda HPU-X2 correspondendo aos braços curtos e HPU-X1 nos braços longos (**Figura 17**).

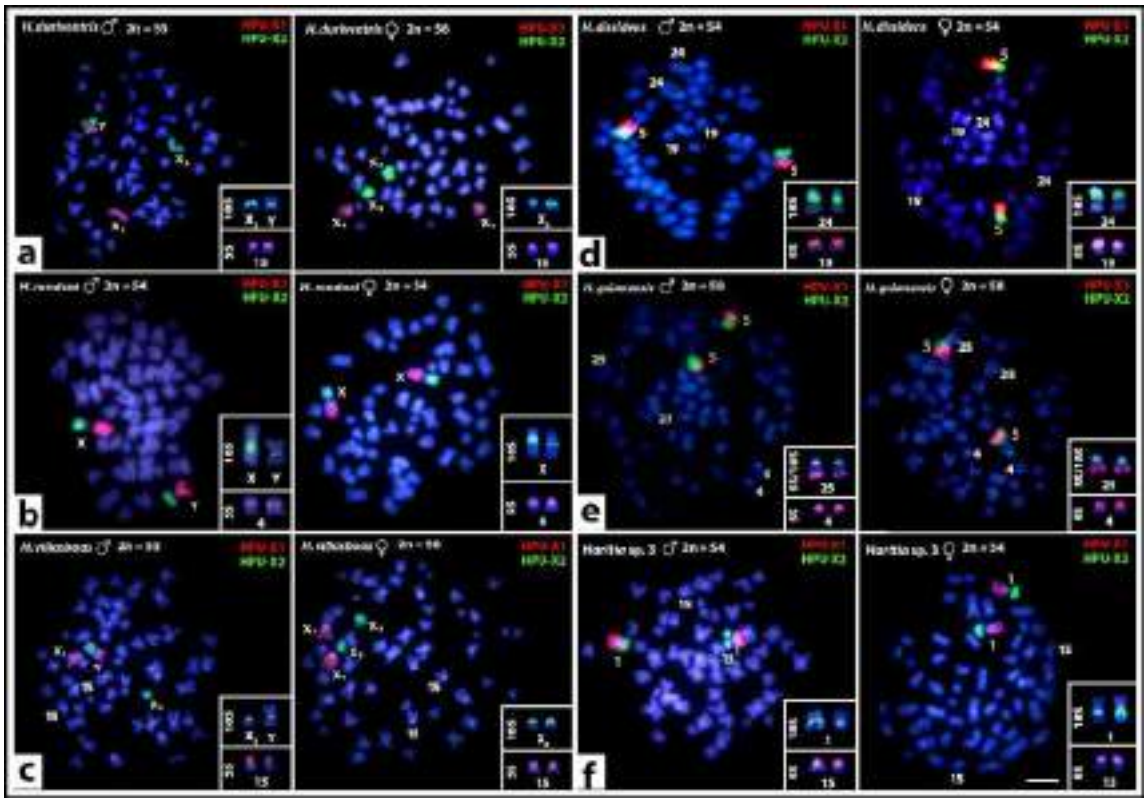


Figura 17. Reprodução da Figura 1 do artigo Sassi *et al*., 2023b (**Anexo V**). Zoo-FISH com sondas de pintura HPU-X1 e HPU-X2 em metáfases mitóticas de machos (primeira e terceira colunas) e fêmeas (segunda e quarta colunas) de *Harttia duriventris* (a), *H. dissidens* (d), *H. rondoni* (b), *H. guianensis* (e), *H. villasboas* (c) e *Harttia* sp. 3 (f). Os cromossomos que carregam clusters de DNAr 5S (vermelho) e 18S (verde) são destacados em caixas. Os cromossomos foram contracorados com DAPI (azul). Barra de escala = 10 µm.

No trabalho de descrição do cariótipo de *H. punctata*, os autores discutem a hipótese de translocação Robertsoniana entre dois cromossomos acrocêntricos (um ancestral Y e um autossomo) como mecanismo de origem do sistema múltiplo X_1X_2Y. Este rearranjo seria acompanhado da perda de sequências do 18S DNAr no cromossomo neo-Y recém originado, visto que essa sequência não está presente no Y de *H. punctata*, porém está presente no X_2 (Blanco *et al*., 2014). Contudo, os dados aqui apresentados indicam que os grupos de ligação que representam os cromossomos X_1 e X_2 formam braços do mesmo cromossomo no cariótipo ancestral, visto que essa condição está presente em *H. guianensis* e nas espécies do clado Norte. Dessa forma, é altamente provável que o mecanismo de origem deste sistema múltiplo seja devido à ocorrência de uma fissão cêntrica em detrimento da translocação anteriormente proposta (**Figura 18**).

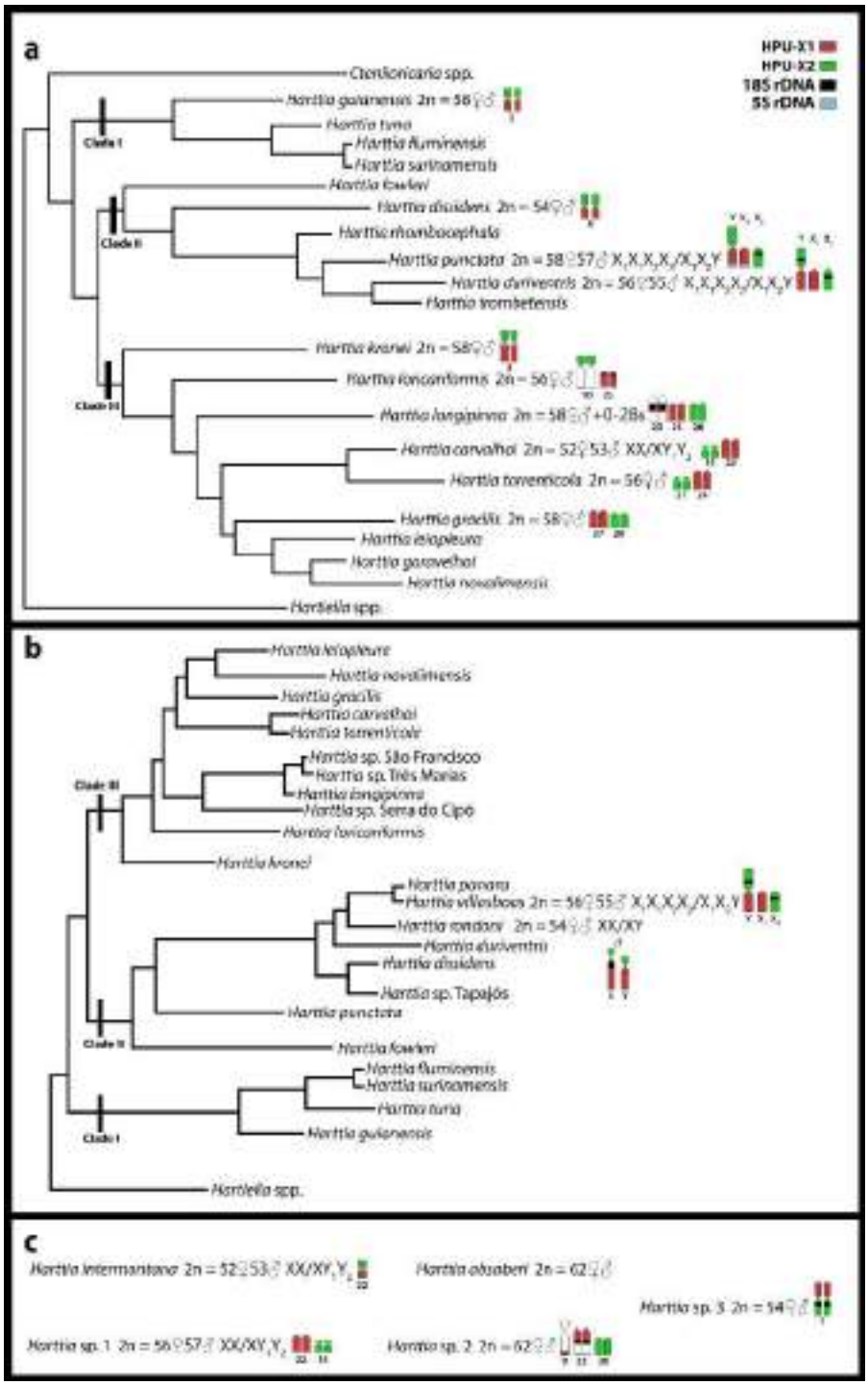


Figura 18. Reprodução da Figura 3 do artigo Sassi *et al*., 2023b (**Anexo V**). Relações filogenéticas entre peixes Hartiini com base em dados morfológicos e moleculares, juntamente com características citogenéticas ancoradas. A filogenia segue a) Londoño-Burbano e Reis (2021) e b) Covain *et al*. (2016), enquanto (c) apresenta os dados cromossômicos de espécies que não foram incluídas nas respectivas reconstruções filogenéticas. Características citogenéticas indicadas: 2n; sistemas de cromossomos sexuais; ideogramas parciais representam a organização de blocos de sintenia mapeados, conforme revelado pelas sondas de pintura derivadas de cromossomos sexuais HPU-X1 (vermelho) e HPU-X2 (verde); sítios de DNAr 5S (azul) e 18S (preto). A atribuição de sinais a pares de cromossomos específicos no clado III seguiu os padrões encontrados em Deon *et al*. (2022a, b).

Estudos teóricos indicam que em cenários de eventos de fissão cêntrica originando cromossomos sexuais múltiplos, a espécie mais próxima que carrega o sistema simples relacionado deve ter um 2n menor do que as que carregam o múltiplo (Kitano & Peichel, 2012). Este é o cenário observado em *Harttia*, visto que *H. rondoni* (2n = 54, XX/XY) apresenta um 2n menor do que as demais espécies com o sistema múltiplo $X_1X_1X_2X_2/X_1X_2Y$ (*H. punctata*, *H. duriventris* e *H. villasboas*). Em conjunto com a análise de CGH previamente realizada (**Capítulo 1**, **Figura 13**), é possível observar que a provável região diferenciada do cromossomo Y neste sistema múltiplo é localizada próxima ao centrômero, região do cromossomo que é propensa ao estabelecimento de regiões sexo-determinantes em sistemas recém originados (Charlesworth, 2019).

Para uma caracterização mais completa da composição deste sistema múltiplo, caracterizamos o satelitoma das quatro espécies (i.e. *H. villasboas*, *H. rondoni*, *H. duriventris* e *H. punctata*). Todas demonstraram a predominância de satélites longos (> 100bp) em seus genomas, porém variando em outras características como número total de satélites, proporção de bases A+T nas sequências e tamanho da unidade de repetição (**Tabela 6, 7**).

Tabela 6. Principais características dos satelitomas de *Harttia*: N = número de sequências de DNA satélite; RUL = tamanho da unidade de repetição (bp); A + T = proporção de nucleotídeos A + T nos satélites; Max = máximo; Min = mínimo; Med = mediana.

Espécie	N	Max RUL	Min RUL	Med RUL	Max A + T	Min A + T	Med A + T
Harttia duriventris	18	2707	21	262	81%	23.6%	56%
Harttia punctata	19	2750	21	177	72%	39%	59%
Harttia villasboas	21	4011	32	730	68.1%	24.2%	52%
Harttia rondoni	25	4165	19	372	81%	25.1%	57.8%

Tabela 7. Catálogos de satelitomas de *Harttia*. Para *H. villasboas* e *H. rondoni*, as características dos satelitomas de machos e fêmeas são indicados, enquanto *H. duriventris* e *H. punctata* foram caracterizadas apenas a partir de amostras de machos. satDNA = DNA satélite; A+T(%) = proporção de adeninas e timinas nos satélites; AM = abundância em machos; AF = abundância em fêmeas; M/F = diferenças entre os catálogos de machos e fêmeas, coloridos em laranja caso apresentem distribuição maior em fêmeas e em verde para machos; Σ = somatório AM+AF; DivM = divergência em machos; DivF = divergência em fêmeas.

Espécie	satDNA	A+T (%)	AM	AF	M/F	Σ	DivM	DivF
Harttia villasboas	HviSat01-2531	57.1	0.00651051	0.0068585	0.9492965017	0.013368758	11.69	11.79
	HviSat02-2707	52.1	0.005255686	0.0055203 8	0.9520513206	0.010776066	3.77	3.65
	HviSat03-997	39.7	0.003744728	0.0037817 2	0.9902181535	0.007526449	10.75	10.81
	HviSat04-2959	54.4	0.001384657	0.0016507 2	0.8388176423	0.003035382	7.48	7.18
	HviSat05-177	65.5	0.001118204	0.0012361 2	0.9046074034	0.002354324	5.54	5.06
	HviSat06-200	68	0.00084482	0.0007746 2	1.0906256510	0.001619439	5.19	5.12
	HviSat07-93	38.7	0.00079764	0.0008376 2	0.9522667171	0.001635263	7.32	7.29
	HviSat08-4011	56.2	0.000566046	0.0006423 3	0.8812338169	0.001208379	5.12	5.79
	HviSat09-815	59.5	0.000424192	0.0004643 3	0.9135553754	0.000888522	5.06	4.97
	HviSat10-1666	56.5	0.000417871	0.0003944 5	1.0593771297	0.00081232	9.41	11.01
	HviSat11-1338	58.1	0.000393588	0.0003573 9	1.1012749266	0.000750982	4.27	4.38
	HviSat12-144	54.2	0.000366828	0.0003902 4	0.9400009094	0.00075707	10.94	10.76
	HviSat13-730	36	0.000363972	0.0004182 5	0.8702192128	0.000782225	4.61	4.55
	HviSat14-32	60.5	0.000351162	0.0003428 3	1.0243007323	0.000693993	9.76	9.88

	HviSat15-347	53.1	0.000347879	0.00034702	1.0024863247	0.000694896	20.81	20.6
	HviSat16-2480	60.3	0.000306566	0.00032959	0.9301348259	0.00063616	5.15	7.31
	HviSat17-49	55.1	0.000135999	0.00013343	1.0192599992	0.000269427	5.2	5.14
	HviSat18-1068	24.2	0.000133953	0.00015103	0.8869528437	0.000284979	1.45	1.37
	HviSat19-589	61.5	0.000109466	0.00012288	0.8908375343	0.000232347	12.14	11.86
	HviSat20-47	68.1	0.000106951	6.0566E-05	1.7658675724	0.000167517	3.35	3.21
	HviSat21-575	45.7	9.21198E-05	0.00011101	0.8298533977	0.000203127	3.15	3.15
Harttia rondoni	HroSat01-93	37.63	0.003789909	0.00437880 5	0.865512183	0.008168715	7.74	7.4
	HroSat02-1662	56.38	0.003764020	0.00354314 7	1.062338085	0.007307167	11.43	11.35
	HroSat03-372	59.14	0.002637919	0.00276887 1	0.952705857	0.005406790	9.13	8.88
	HroSat04-920	35.98	0.002201956	0.00226880 2	0.970536874	0.004470758	3.49	3.38
	HroSat05-515	58.83	0.002185305	0.00265562 6	0.822896497	0.004840931	18.43	17.51
	HroSat06-1634	55.75	0.002106668	0.00212171 3	0.992909032	0.004228381	19.62	19.41
	HroSat07-19	68.42	0.001634906	0.00392817 4	0.416200027	0.005563080	5.74	5.18
	HroSat08-347	60.23	0.001563824	0.00323719 8	0.483079458	0.004801022	7.86	7.38
	HroSat09-2961	54.47	0.001291713	0.00142239 9	0.908123061	0.002714112	8.61	9.59
	HroSat10-21	80.95	0.001091777	0.00575085 9	0.189845948	0.006842637	5.78	4.13
	HroSat11-534	25.09	0.001041824	0.00138155 3	0.754096078	0.002423377	2.15	1.55

	HroSat12-114	30.70	0.001006509	0.001241631	0.810634573	0.002248139	3.42	3.32
	HroSat13-1440	58.82	0.000727660	0.000592262	1.228612492	0.001319922	5.19	5.27
	HroSat14-4165	56.42	0.000666859	0.000920301	0.724610028	0.001587160	5.75	6.46
	HroSat15-93	34.41	0.000605190	0.000661809	0.914448541	0.001266999	8.04	8.5
	HroSat16-177	64.97	0.000497806	0.000567514	0.87716955	0.001065320	4.3	3.91
	HroSat17-2481	60.34	0.000398167	0.000471484	0.844496667	0.000869651	7.35	10.73
	HroSat18-1194	61.39	0.000281495	0.000275020	1.02354253	0.000556515	7.03	7.18
	HroSat19-815	60.25	0.000270435	0.000503189	0.537441531	0.000773624	5.33	5.09
	HroSat20-45	57.78	0.000257782	0.000215465	1.196396821	0.000473247	4.19	4.19
	HroSat21-50	60	0.000205573	0.000252480	0.814217275	0.000458053	6.14	5.51
	HroSat22-211	62.09	0.000151882	0.000174905	0.868366557	0.000326787	7.12	7.15
	HroSat23-576	46.53	0.000140003	0.000091847	1.524309737	0.000231849	3.2	3.39
	HroSat24-48	56.25	0.000109270	0.000123891	0.881982123	0.000233161	9.23	7.1
	HroSat25-35	57.14	0.000081886	0.000132465	0.618170838	0.000214351	8.12	8.04

	SatDNA	**A+T (%)**	**Abundância**	**Div**
Harttia duriventris	HduSat01-2707	52	0.005296699	3.49
	HduSat02-347	62.20	0.003393394	5.13
	HduSat03-2143	57.10	0.00187602	11.58
	HduSat04-31	67.70	0.001730222	7.26
	HduSat05-93	37.60	0.001116687	9.71
	HduSat06-177	65.00	0.000884022	5.4

	HduSat07-21	81.00	0.000851979	5.76
	HduSat08-1754	55.80	0.000790658	6.04
	HduSat09-771	34.80	0.000719605	3.39
	HduSat10-1449	59.10	0.000559583	4.83
	HduSat11-815	60.50	0.000538898	5.5
	HduSat12-1670	56.10	0.000507084	10.65
	HduSat13-144	54.20	0.000359392	10.76
	HduSat14-32	53.10	0.000357065	20.36
	HduSat15-534	23.60	0.000346816	1.13
	HduSat16-114	28.90	0.00030558	2.98
	HduSat17-150	52.00	0.000136754	11.15
	HduSat18-45	57.80	0.000132868	4.63
Harttia punctata	HpuSat01-21	71.43	0.015663791	6.99
	HpuSat02-177	65.54	0.014303394	3.62
	HpuSat03-144	54.17	0.012200361	5.29
	HpuSat04-2707	51.64	0.008121138	3.18
	HpuSat05-1068	40.26	0.004088409	11.39
	HpuSat06-105	44.76	0.002966126	10.25
	HpuSat07-2750	54.22	0.002216664	7.56
	HpuSat08-1546	60.03	0.001745528	7.34
	HpuSat09-33	39.39	0.001719941	7.97
	HpuSat10-781	59.67	0.000967001	2.01
	HpuSat11-32	53.13	0.000878637	19.3
	HpuSat12-1973	54.84	0.000869407	3.03
	HpuSat13-60	71.67	0.000788852	4.77
	HpuSat14-503	58.85	0.000642217	5.64
	HpuSat15-93	62.37	0.000615839	6.53
	HpuSat16-33	60.61	0.000476905	6.49
	HpuSat17-52	53.85	0.000397269	13.95
	HpuSat18-680	60.74	0.000171358	7.05
	HpuSat19-794	63.73	0.000129686	5.55

Com base no catálogo de *H. villasboas*, obtivemos sondas que foram mapeadas *in situ* nos cromossomos das seis espécies caracterizadas nesta tese (i.e. *H. duriventris*, *H. dissidens*, *H. guianensis*, *Harttia* sp. 3, *H. rondoni*). Na espécie *H. villasboas* (**Figura 19**), apenas HviSat13-730 e HviSat18-1068 foram mapeados na constrição secundária do X_2 e do cromossomo Y. Entre os autossomos, HviSat01-2531 foi encontrado nas regiões pericentroméricas de cinco pares, enquanto HviSat02-2707 demonstrou sinais dispersos em todos os pares. Tanto HviSat04-2959 quanto HviSat05-177 foram hibridizados nos braços curtos de um pequeno par metacêntrico, e HviSat08-4011 foi encontrado na região terminal dos braços longos de um par submetacêntrico. Nenhum sinal visível foi identificado com a sonda HviSat03-997 (**Figura 19**).

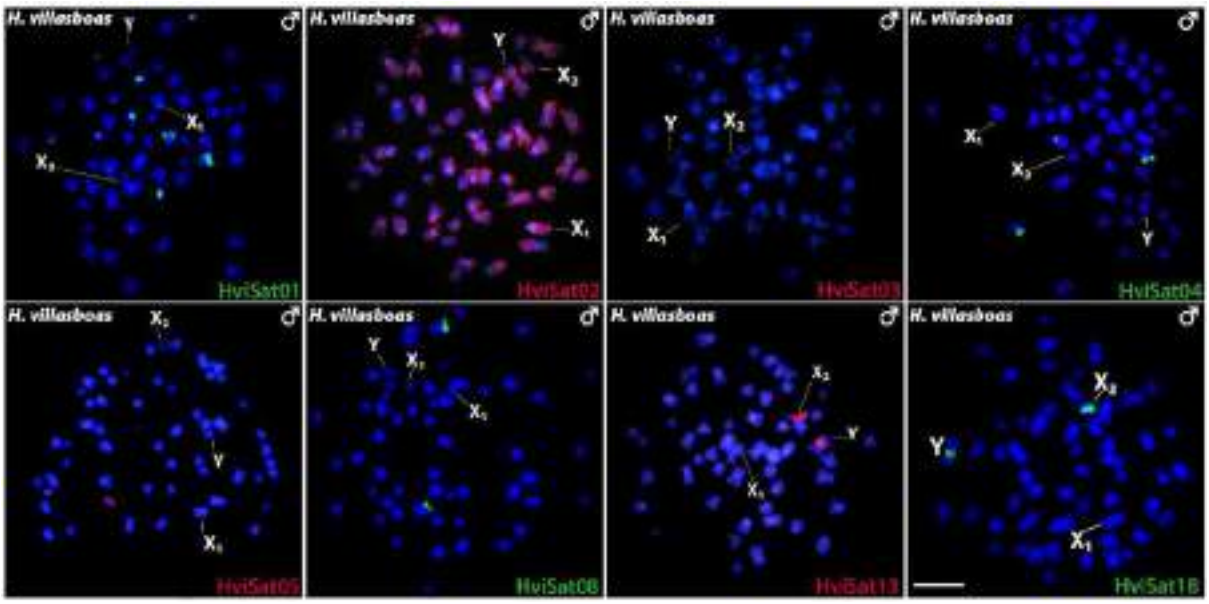


Figura 19. Reprodução da Figura 2 do artigo Sassi *et al*. (em revisão, Communications Biology (Springer-Nature)) (**Anexo VI**). Cromossomos mitóticos de *Harttia villasboas* após hibridização com sequências distintas de DNAs satélites, indicadas no canto inferior direito. Cromossomos sexuais são indicados nas metáfases. Barra de escala = 5 µm.

Tanto *H. rondoni* quanto *H. duriventris* apresentaram padrões de distribuição semelhantes de HviSatDNAs. Apenas HviSat13-730 e HviSat18-1068 foram mapeados nos cromossomos sexuais X e Y de *H. rondoni* e X_2 e Y de *H. duriventris* (**Figura 20**). HviSat08-4011 foi hibridizado na porção terminal dos braços longos de um par submetacêntrico, além de um sinal na região pericentromérica no cromossomo X_1 de *H. duriventris*. HviSat04-2959 e HviSat05-177 foram mapeados nos braços curtos de um pequeno par metacêntrico. HviSat02-2707 foi encontrado disperso em todos os cromossomos e HviSat03-997 não produziu sinais

visíveis, enquanto HviSat01-2531 foi hibridizado em regiões pericentroméricas de seis e três pares em *H. rondoni* e *H. duriventris*, respectivamente.

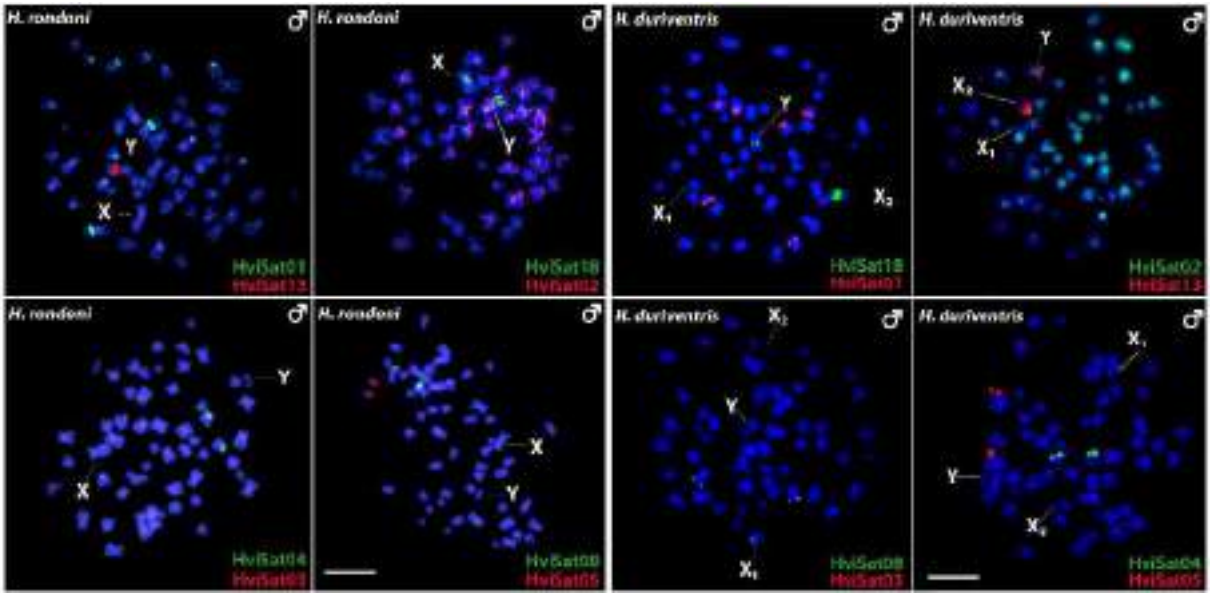


Figura 20. Reprodução da Figura 3 do artigo Sassi *et al*. (em revisão, Communications Biology (Springer-Nature)) (**Anexo VI**). Cromossomos mitóticos de *H. rondoni* e *H. duriventris* após hibridização com sequências distintas de DNA satélite de *H. villasboas*, conforme indicado no canto inferior direito. Os cromossomos sexuais são indicados. Barra de escala = 5 µm.

Quando hibridizados aos cromossomos de *H. punctata* (**Figura 21**), os HviSatDNAs produziram resultados inesperados. Os cromossomos sexuais desta espécie acumulam várias sequências, incluindo HviSat08-4011 em um grande bloco pericentromérico no X_1, HviSat13-730 na constrição secundária do X_2, HviSat04-2959 também em um grande bloco pericentromérico no X_1, um pequeno sinal pericentromérico em X_2 e Y, e HviSat05-177 centromérico no X_1 e X_2, além de sinais terminais no X_2 e Y, o último restrito aos braços longos. HviSat04-2959 também foi encontrado em um pequeno par de autossomos metacêntricos e HviSat05-177 nos braços curtos de seis autossomos. HviSat01-2531 se demonstrou restrito a um grande par de cromossomos submetacêntricos, localizado em posição intersticial nos braços longos, enquanto HviSat02-2707 produziu pequenos sinais na região pericentromérica de alguns pares de cromossomos. A espécie *Harttia* sp. 3 (**Figura 21**) produziu padrões semelhantes a *H. villasboas* e *H. rondoni*. O HviSat01-2531 foi encontrado em três pares de cromossomos na região pericentromérica, enquanto HviSat03-997 demonstrou-se disperso em quase todos os cromossomos. HviSat18-1068 e HviSat13-730 foram encontrados na constrição

secundária do primeiro par de cromossomos na região pericentromérica. Por outro lado, HviSat02-2707 foi hibridizado a alguns pares de cromossomos, mas apenas em um dos homólogos do primeiro par de cromossomos, todos na posição pericentromérica. Tanto HviSat04-2959 quanto HviSat05-177 seguiram o padrão de um único par de cromossomos hibridizados com cada sonda, enquanto HviSat08-4011 foi encontrado nos braços longos de um grande par submetacêntrico.

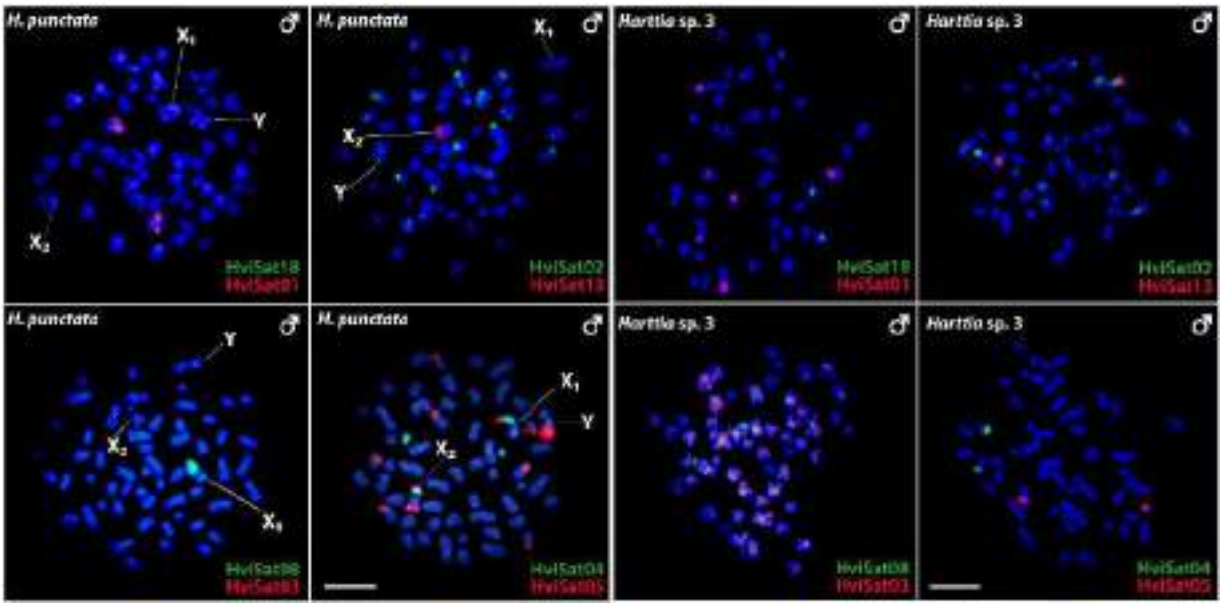


Figura 21. Reprodução da Figura 4 do artigo Sassi *et al*. (em revisão, Communications Biology (Springer-Nature)) (**Anexo VI**). Metáfases mitóticas de *H. punctata* e *Harttia* sp. 3 após hibridização com distintos HviSatDNAs, conforme indicado no canto inferior direito. Os cromossomos sexuais são indicados. Barra de escala = 5 µm.

Por fim, para *H. dissidens* e *H. guianensis* (**Figura 22**), HviSat01-2531 produziu fortes sinais de hibridização em cinco pares de cromossomos na primeira espécie e foi restrito a um único par submetacêntrico em posição intersticial na segunda espécie. O HviSat13-730 foi restrito à constrição secundária da NOR em um único par em ambas as espécies. O HviSat08-4011 seguiu o padrão para a maioria das outras espécies, sendo acumulado na região telomérica dos braços longos de um par submetacêntrico em ambas as espécies. Enquanto HviSat05-177 foi encontrado em um único par de *H. dissidens*, quatro pares foram observados com sinais em *H. guianensis*. Exclusivamente em *H. dissidens*, um padrão disperso foi observado para HviSat03-997, e um único par foi marcado com HviSat04-2959, uma vez que nenhum sinal visível foi encontrado em *H. guianensis* para ambos os satDNAs. Tanto HviSat18-1068 quanto HviSat02-2707 não produziram sinais visíveis.

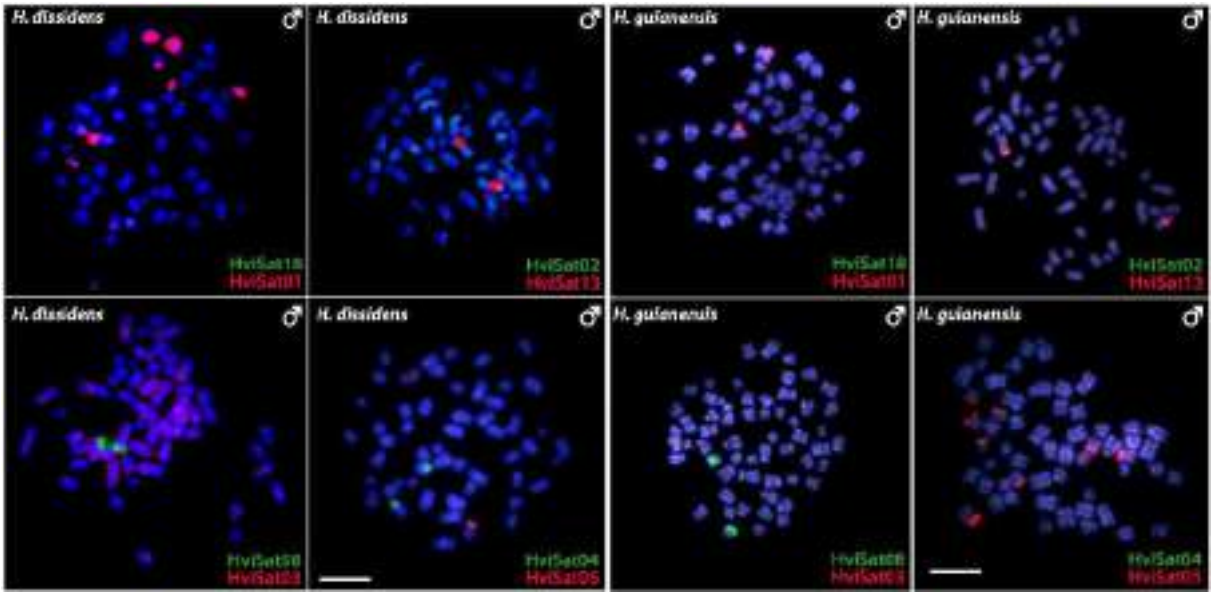


Figura 22. Reprodução da Figura 5 do artigo Sassi *et al*. (em revisão, Communications Biology (Springer-Nature)) (**Anexo VI**). DNA satélites de *Harttia villasboas* hibridizados em *H. dissidens* e *H. guianensis*. Barra de escala = 5 µm.

As análises comparativas indicaram que 19 dos 21 satDNAs de *H. villasboas* apresentam uma contraparte homóloga em uma, duas ou três outras espécies de *Harttia* (**Tabelas 8-10**). Além de satélites compartilhados entre todas as espécies, encontramos satDNAs espécie-específicos: duas sequências – HviSat17-49 e HviSat20-47 – somente são encontrados em *H. villasboas*, enquanto *H. rondoni* apresenta seis sequências específicas, quatro em *H. punctata* e uma em *H. duriventris*. Neste contexto, encontramos alguns satélites que são específicos da linhagem *H. villasboas*-*H. rondoni*: HviSat08-4011, HviSat16-2480, HviSat19-589 e HviSat21-575 têm homólogos apenas em *H. rondoni*, espécie filogeneticamente mais próxima de *H. villasboas*. Esta tabela também reflete o processo saltacional da evolução de alguns satélites que formam os satellitomes dessas espécies, uma vez que há satDNAs compartilhados por duas ou mais espécies para as quais o padrão de compartilhamento não está em conformidade com as filogenias: entre vários outros, um exemplo é o HviSat02-2702 para o qual encontramos homólogos em *H. duriventris* e *H. punctata*, mas não em *H. rondoni*. Esses dados refletem amplificações diferenciais desses satélites em diferentes espécies em diferentes tempos evolutivos (**Figura 23**). A Tabela 9 apresenta as distâncias genéticas entre os satDNAs de *H. villasboas* e os satélites homólogos correspondentes, evidenciando que, em geral, quanto maior a distância filogenética, maior a distância genética entre os satélites homólogos. Isso é especialmente verdadeiro em comparações com *H. punctata*. Contudo, os dados também indicam taxas de mudança altamente

aceleradas ou desaceleradas para algumas linhagens de satDNAs, com distâncias genéticas que não são consistentes com a proximidade filogenética em algumas comparações. Por exemplo, o homólogo HviSat01-2531 em *H. rondoni* ou o homólogo HviSat04-2959 em *H. duriventris* mostram maior divergência de *H. villasboas* do que homólogos de espécies filogeneticamente mais distantes (*H. punctata*). Tomando como referência o único nó filogenético no qual temos uma datação clara para comparar duas espécies (linhagem *H. punctata* e demais espécies), a Tabela 10 mostra a taxa de turnover consenso (CTR) para cada satDNA assumindo um tempo de divergência de 17,5 my entre *H. villasboas* e *H. punctata* (Kumar *et al*., 2022), demonstrando taxas de mudança de sequências muito distintas em diferentes satDNAs.

Tabela 8. Reprodução da tabela 4 do artigo Sassi *et al*. (em revisão, Communications Biology (Springer-Nature)). Famílias homólogas de DNA satélites em *Harttia*.

Species			
H. villasboas	***H. rondoni***	***H. duriventris***	***H. punctata***
HviSat01-2531	HroSat02-1662	HduSat03-2143	HpuSat12-1973
	HroSat03-372		
	HroSat05-515		
HviSat02-2707		HduSat01-2707	HpuSat04-2707
HviSat03-997			HpuSat05-1068
HviSat04-2959	HroSat09-2961	HduSat08-1754	HpuSat07-2750
HviSat05-177	HroSat16-177	HduSat06-177	HpuSat02-177
HviSat06-200		HduSat04-31	
HviSat07-93	HroSat01-93	HduSat05-93	HpuSat06-105
HviSat08-4011	HroSat14-4165		
HviSat09-815	HroSat19-815	HduSat11-815	HpuSat10-781
HviSat10-1666	HroSat06-1634	HduSat12-1670	HpuSat14-503
HviSat11-1338	HroSat13-1440	HduSat10-1449	HpuSat08-1546
HviSat12-144		HduSat13-144	HpuSat03-144
HviSat13-730	HroSat04-920	HduSat09-771	
	HroSat12-144	HduSat16-144	
HviSat18-1068	HroSat11-534	HduSat15-534	
HviSat14-347	HroSat08-347	HduSat02-347	
HviSat15-32		HduSat14-32	HpuSat11-32
HviSat16-2480	HroSat17-2481		
HviSat19-589	HroSat18-1194		
HviSat21-575	HroSat23-576		
	HroSat10-21	HduSat07-21	HpuSat01-21
	HroSat20-45	HduSat18-45	

Tabela 9. Reprodução da tabela 5 do artigo Sassi *et al*. (em revisão, *Communications Biology* (Springer-Nature)). Comparação das distâncias genéticas entre DNA satélites homólogos entre as espécies de *Harttia*. As distâncias genéticas são apresentadas entre pares de espécies, seguindo: (1) Hvi-Hro, (2) Hvi-Hdu, (3) Hvi-Hpu, (4) Hro-Hdu, (5) Hdu-Hpu, (6) Hro-Hpu.

		satDNA homólogos	
Harttia villasboas	***Harttia rondoni***	***Harttia duriventris***	***Harttia punctata***
HviSat01-2531	0.081(1)	0.023(2)	0.189(3)
	0.068(4)	0.198(5)	
		0.128(6)	
HviSat02-2707		0.004(2)	0.025(3)
		0.023(5)	
HviSat03-997			0.296(3)
HviSat04-2959	0.018(1)	0.390(2)	0.180(3)
	0.393(4)	0.498(5)	
		0.156(6)	
HviSat05-177	0.006(1)	0.006(2)	0.163(3)
	0.000(4)	0.156(5)	
		0.156	
HviSat06-200		0.067(2)	
HviSat07-93	0.044(1)	0.116(2)	0.316(3)
	0.142(4)	0.350(5)	
		0.367(6)	
HviSat08-4011	0.016(1)		
HviSat09-815	0.030(1)	0.030(2)	0.271(3)
	0.043(4)	0.280(5)	
		0.275(6)	
HviSat10-1666	0.016(1)	0.023(2)	0.498(3)
	0.020(4)	0.480(5)	
		0.471(6)	
HviSat11-1338	0.070(1)	0.065(2)	0.771(3)
	0.025(4)	0.775(5)	
		0.768(6)	
HviSat12-144		0.007(2)	0.007(3)
		0.000(5)	
HviSat13-730	0.092(1)	0.109(2)	
	0.048(4)		
HviSat14-347	0.012(1)	0.032(2)	
	0.038(4)		
HviSat15-32		0.000(2)	0.000(3)
		0.000(5)	
HviSat16-2480	0.015(1)		
HviSat18-1068	0.038(1)	0.067(2)	
	0.061(4)		
HviSat19-589	0.107(1)		
HviSat21-575	0.007(1)		

Tabela 10. Reprodução da tabela 6 do artigo Sassi *et al*. (em revisão, Communications Biology (Springer-Nature)). Taxa de turnover consenso (CTR) entre DNA satélites de *H. villasboas* (HviSatDNA) e *H. punctata* (HpuSatDNA).

HviSatDNA	HpuSatDNA	CTR
HviSat01-2531	0.189	0.54×10^{-8}
HviSat02-2707	0.025	0.07×10^{-8}
HviSat03-997	0.296	0.85×10^{-8}
HviSat04-2959	0.180	0.51×10^{-8}
HviSat05-177	0.163	0.47×10^{-8}
HviSat07-93	0.316	0.90×10^{-8}
HviSat09-815	0.271	0.78×10^{-8}
HviSat10-1666	0.498	1.42×10^{-8}
HviSat11-1338	0.771	2.20×10^{-8}
HviSat12-144	0.007	0.2×10^{-8}
HviSat15-32	0.000	0
	Média	0.71×10^{-8}

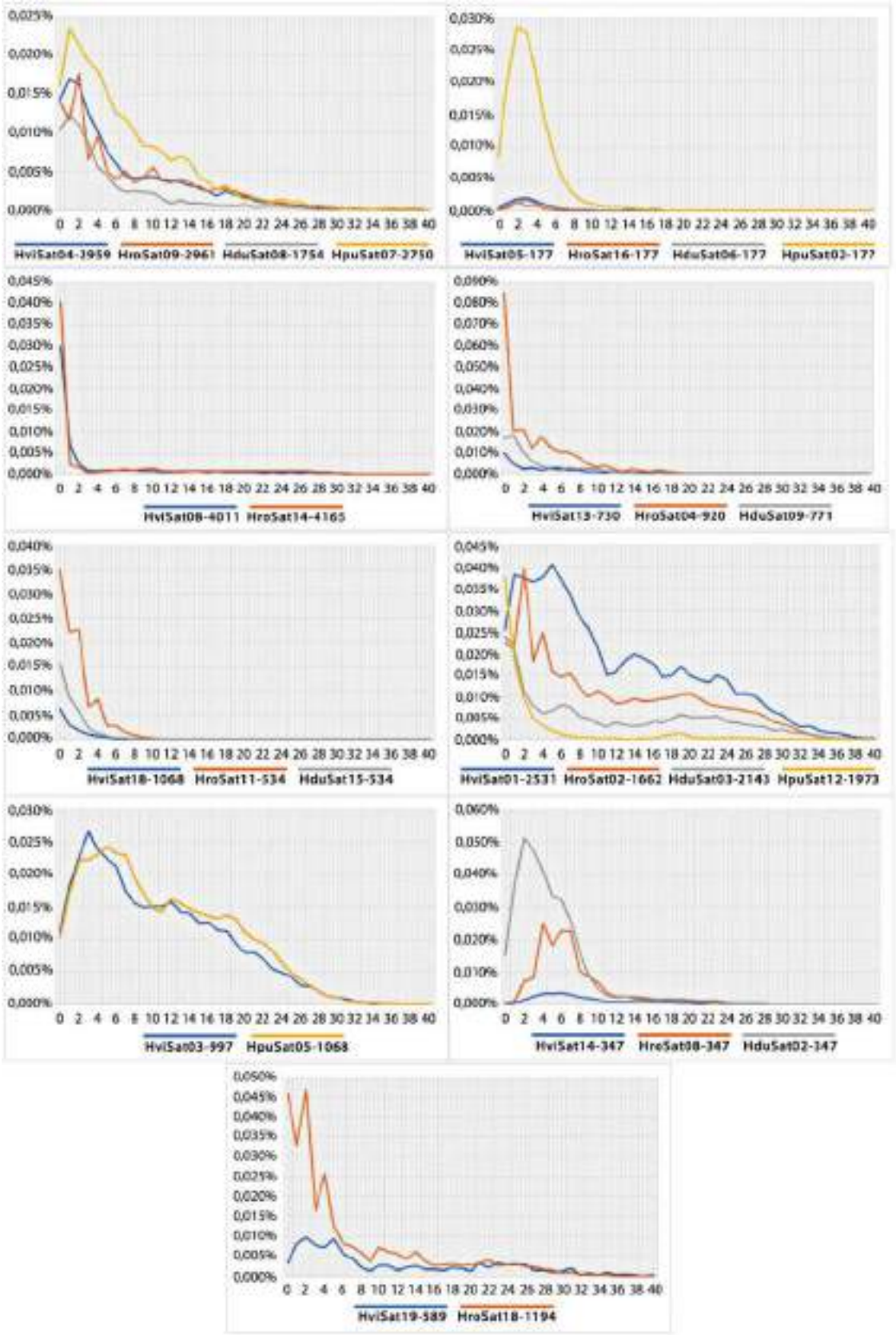


Figura 23. Reprodução da Figura 7 do artigo Sassi *et al*. (em revisão, Communications Biology (Springer-Nature)) (**Anexo VI**). Gráficos de paisagem de repetitivos (RL) para DNAs satélites (satDNA) homólogos de *Harttia*. Abundância (eixo y) e divergência (eixo x) em relação a sequência consenso construída para cada satDNA.

Considerando que os satDNAs degeneram por mutações pontuais (divergência crescente) e homogeneíza por amplificação (divergência decrescente) (Garrido-Ramos, 2017), presume-se que picos em valores de divergência mais baixos nos perfis de paisagens de repetitivos são o produto de amplificações recentes, enquanto aqueles em valores de divergência mais altos são provavelmente variantes mais antigas degeneradas pelo acúmulo de mutações. Seguindo esse argumento, analisamos os gráficos de satDNAs encontrados nos cromossomos sexuais de espécies de *Harttia* (**Figura 24**). Assim, o satélite HviSat08-4011 e sua contraparte HroSat14-4165 mostram um pico de amplificação único em 0% de divergência. Então, embora o gráfico mostre variantes de sequência desses satélites com divergências de até 30%, a amplificação que resultou no status atual deste satélite na linhagem *H. villasboas-H. rondoni* deve ter sido muito recente após a divisão da linhagem que levou a *H. duriventris*. No caso dos satélites HviSat13-730 e HviSat18-1068 e suas contrapartes homólogas nas demais espécies, os diversos picos observados podem refletir diferentes ondas de amplificação recentes (os picos mais altos estão em 0% de divergência) desses satélites em diferentes momentos em cada espécie. Os RLs de HviSat04-2959 e HviSat05-177, e suas contrapartes, mostram vários picos, mas os maiores picos dos satélites HpuSat02-177 e HpuSat07-2750 se destacam, o que pode estar relacionado à sua incorporação e amplificação nos cromossomos sexuais de *H. punctata* (esses satélites estão em autossomos nas outras espécies) possivelmente após a separação das linhagens (17,5 mya), uma vez que a divergência de 2% desses picos corresponderia a um tempo de divergência de cerca de 8 mya (**Tabela 9**). Por fim, os demais gráficos RL na Figura 23 representam exemplos de quatro satélites autossômicos (HviSat01-2531, HviSat03-997, HviSat14-347 e HviSat19-589, e suas contrapartes). Todos os satélites apresentam vários picos que podem refletir diferentes ondas de expansão, a maioria das quais são muito antigas (há picos de divergência de mais de 20%).

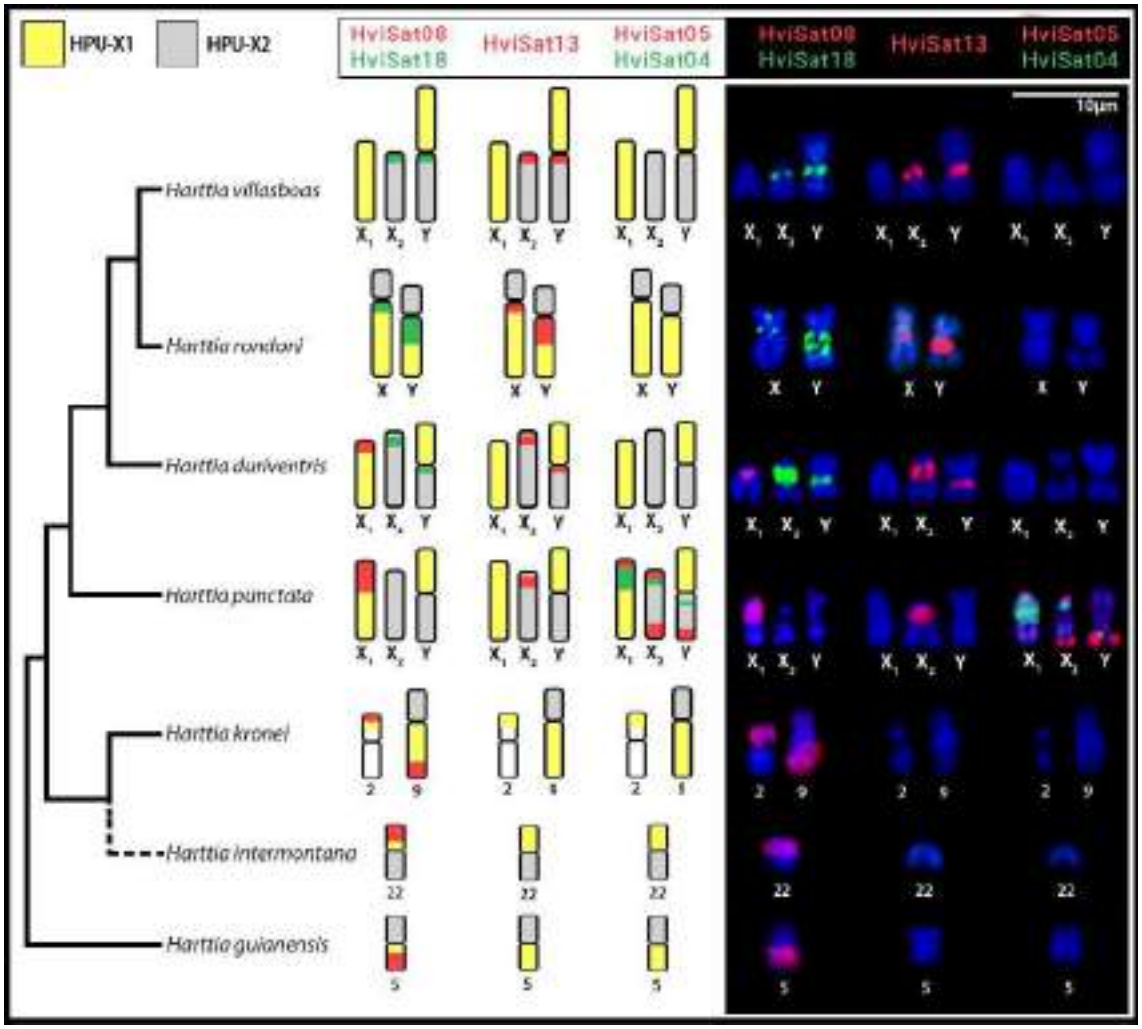


Figura 24. Reprodução da Figura 6 do artigo Sassi *et al*. (em revisão, Communications Biology (Springer-Nature)) (**Anexo VI**). Sequências satélite de *H. villasboas* hibridizadas aos cromossomos sexuais em distintas espécies de *Harttia*, esquematizadas de acordo com suas relações filogenéticas (Covain *et al*., 2016). *H. intermontana* foi adicionada manualmente à árvore com base na distribuição geográfica e características cromossômicas, mas sua posição deve ser confirmada, visto que não está presente na análise de Covain *et al*. (2016). A combinação de sondas é indicada acima de cada ideograma/imagem de FISH. As cores dos idiogramas seguiram a homologia dos cromossomos sexuais obtidos da pintura de cromossomos inteiros, conforme descrito na metodologia da presente tese. Barra de escala = 10 µm.

Como podemos observar na imagem acima, o HviSat08-4011 é amplamente distribuído ao longo da filogenia, no mesmo grupo de ligação que dá origem ao sistema múltiplo X_1X_2Y presente em *H. duriventris*, *H. villasboas* e *H. punctata*. Conforme indicado, satélites homólogos ao satélite HviSat08-4011 e sua contraparte HroSat14-4165 em *H. rondoni* não foram isolados dos genomas de *H. duriventris* e *H. punctata*. No entanto, a FISH com HviSat08-4011 detectou grandes blocos de hibridização deste satélite no cromossomo X_1 de ambas as espécies, além de um locus autossômico (apenas em *H. duriventris*), que contrasta com a

localização autossômica única deste satélite em *H. villasboas* e *H. rondoni* (bem como em outras espécies sem cromossomos sexuais heteromórficos) (**Figura 24**). A busca via BLASTn e BLASTx de HviSat08-4011 revela que este é um satDNA complexo, composto de pelo menos duas partes diferenciadas (**Figura 25A**). A maior parte (cerca de 2300 pb) é uma sequência sem homologia com nenhuma outra sequência disponível na base de dados. No entanto, cerca de 1700 pb mostra homologia e alta identidade (~80%) com o gene da "proteína de ligação ao esperma da zona pelúcida 4-like" (ZP4) de diversas espécies das ordens Siluriformes (como *Ictalurus*, *Silurus*, *Neoarius* e *Tachysurus*) e Characiformes (como *Colossoma*, *Pygocentrus* e *Astyanax*). Como mostra a **Figura 25A**, as partes homólogas são descontínuas, separadas por sequências desconhecidas. No entanto, as partes homólogas se alinham continuamente com ~90% da sequência traduzida do gene nessas outras espécies, com pequenas discrepâncias nos limites.

A região análoga do gene ZP4 é onde os primers utilizados em nossa estratégia de FISH inicial foram construídos (**Figura 25A**, F1R1). Dois pares de primers adicionais foram construídos, um localizado na região não homóloga à ZP4 (**Figura 25A**, F2R2), enquanto o outro é composto por um primer reverso dentro da região homóloga à ZP4 e um primer direto fora dela (**Figura 25A**, F3R3). No primeiro caso, os resultados diferem dos encontrados inicialmente (**Figura 24**): a) as duas partes do satélite HviSat08-4011 (ZP4-homóloga e não-ZP4-homóloga) hibridizam apenas em um par de autossomos em *H. rondoni* e *H. villasboas*; b) a parte satélite homóloga de ZP4 hibridiza em X_1 e um par autossômico de *H. duriventris*, mas a parte do satélite não-homóloga à ZP4 hibridiza apenas no par de autossomos; c) a parte do satélite homóloga à ZP4 hibridiza apenas ao X_1 de *H. punctata* e a parte do satélite não-homóloga à ZP4 não hibridiza em nenhum cromossomo. No segundo caso, com os primers F3R3-HviSat08-4011, apenas *H. villasboas* e *H. rondoni* produziram fragmentos visíveis no gel de agarose (**Figura 25B**). Essas últimas amplificações do produto de PCR foram então sequenciadas para verificar a composição dos fragmentos amplificados, e eles coincidiram totalmente com a região esperada.

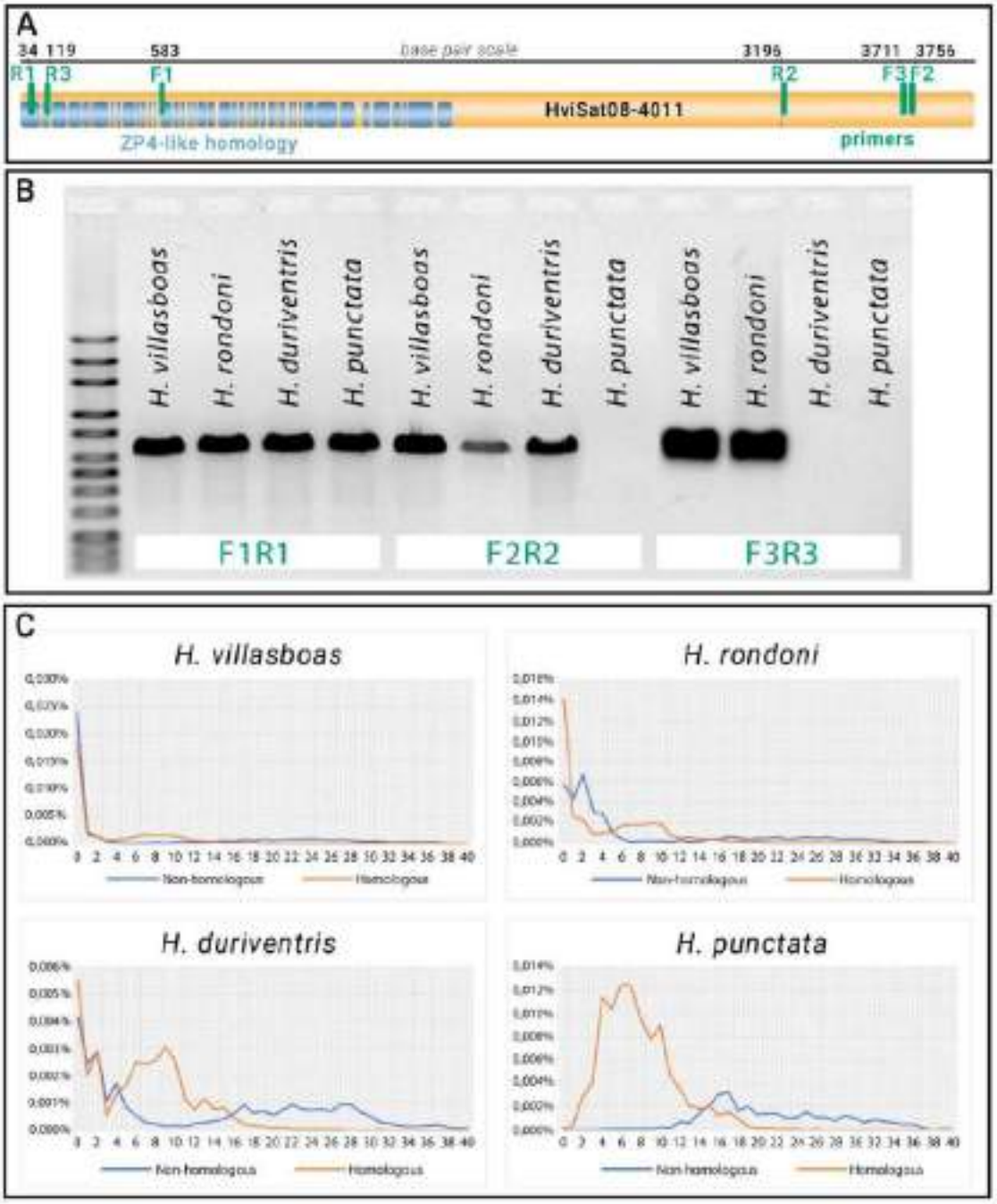


Figura 25. Reprodução da Figura 8 do artigo Sassi *et al*. (em revisão, Communications Biology (Springer-Nature)). Estrutura do HviSat08-4011 (A), indicando as regiões homólogas com o gene ZP4 (azul) e os três conjuntos de primers (F1R1, F2R2 e F3R3, em verde) usados para PCR, conforme demonstrado pela imagem em gel de agarose (B). Gráficos de paisagens de repetitivos (C) representando, para *H. villasboas*, *H. rondoni*, *H. duriventris* e *H. punctata*, abundância (eixo y) e divergência (eixo x) em relação a sequência de consenso construída para cada parte da unidade de repetição do HviSat08-4011, sendo as retas azuis para a porção não-homóloga à ZP4 e laranja para a porção homóloga.

Além disso, investigamos a relação entre os satDNAs e elementos transponíveis (TEs) (**Tabelas 11-14**). Dos diversos satDNAs encontrados nos cromossomos sexuais de *Harttia*, apenas HviSat04-2959 e seus homólogos nas demais espécies, é relacionado com TEs. A maioria dos satDNAs relacionados a TEs são complexos, compostos por diferentes partes derivadas de elementos SINE e/ou LINE, além de genes de tRNA. Também observamos que HviSat02-2707 e seus homólogos são relacionados a elementos Penelope, enquanto o satélite exclusivo de *H. duriventris* HduSat17-150 é relacionado a elementos Helitron. TEs são conhecidos por contribuir para mecanismos de expansão do genoma, principalmente por meio de retrotransposições. Além disso, também contribuem pela criação de novas repetições em tandem (Ahmed e Liang, 2012) por duplicação parcial de TE seguida por crossing-over desigual (Wong & Choo, 2004), ou por quebras induzidas por transposases e mecanismos de reparo (Kapitonov & Jurka, 1999).

Tabelas 11-14. Resultados do BLASTn de Hvi (11), Hro (12), Hdu (13) e HpuSatDNAs (14), respectivamente, com correspondência positiva em sequências repetitivas. Colunas indicam os seguintes dados: pontuação Smith-Waterman da correspondência, com complexidade ajustada (SW); porcentagem de substituições na região correspondente em comparação ao consenso (Div); porcentagem de bases opostas a uma lacuna na sequência de consulta (pb deletados); porcentagem de bases opostas a uma lacuna no consenso de repetição (pb inseridos); posição na consulta (início-fim); fita (fita de transcrição (+) ou fita complementar (C)); repetição correspondente (Rep); classe/família de repetição (Classe); porcentagem de sequência de satDNA correspondente (%) e porcentagem total por satDNA (Total).

Tabela 11

Porcentagem Busca	Posição SW	Div	Del	Ins	Início	Fim	Fita	Rep	Classe	%	Total
HviSat01- 2531	354	20.0	0.0	0.0	78	137	C	tRNA-ValGTG	tRNA	2,33	
HviSat01- 2531	573	11.0	0.0	0.0	264	336	C	tRNA-ValGTG	tRNA	2,84	
HviSat01- 2531	405	18.3	0.0	0.0	1868	1938	C	tRNA-AspGAY	tRNA	2,77	
HviSat01- 2531	420	19.4	0.0	0.0	2438	2509	C	tRNA-AspGAY	tRNA	2,81	10,75
HviSat02- 2707	5446	29.9	0.0	2.6	1	2403	+	Penelope- 5_XT	LINE/Penelope	88,73	
HviSat02- 2707	355	24.7	0.0	2.0	2551	2699	+	Penelope- 5_XT	LINE/Penelope	5,47	94,20
HviSat04- 2959	4989	28.6	2.4	1.9	54	2055	+	Rex1- 3_EL	LINE/Rex-Babar	67,62	
HviSat04- 2959	338	27.1	9.3	0.0	2233	2302	+	tRNA-IleATC	tRNA	2,33	
HviSat04- 2959	591	9.6	5.1	0.0	2423	2495	C	tRNA-AlaGCY	tRNA	2,43	
HviSat04- 2959	302	26.8	0.0	1.1	2869	2955	+	MarinerN4_SSa	DNA/TcMarISRm11	2,91	
HviSat04- 2959	568	7.2	0.0	0.0	2891	2959	+	tRNA-IleATT	tRNA	2,30	77,59
HviSat06- 200	370	4.5	3.4	10. 7	1	197	+	MOSAT_D R	SatDNA	98,00	98,00
HviSat09- 815	597	4.3	0.0	0.0	1	70	+	tRNA-GlyGGY	tRNA	8,47	
HviSat09- 815	385	23.8	30.7	0.0	71	84	+	DNA- 1_Gav	DNA	1,60	10,06
HviSat10- 1666	646	4.9	0.0	0.0	46	127	+	Nimb- 4_DR	LINE/I	4,86	
HviSat10- 1666	595	10.8	0.0	0.0	633	715	C	tRNA-LeuCTG	tRNA	4,92	
HviSat10- 1666	349	17.1	13.6	0.0	1516	1585	C	Nimb- 4_DR	LINE/I	4,14	13,93
HviSat11- 1338	629	0.0	0.0	0.0	250	322	+	tRNA- 3_DRe	tRNA	5,38	5,38
HviSat16- 2480	628	4.2	0.0	0.0	12	83	+	tRNA-GlyGGA	tRNA	2,86	
HviSat16- 2480	275	26.1	0.0	0.0	519	587	+	tRNA-LysAAA	tRNA	2,74	
HviSat16- 2480	600	5.6	0.0	0.0	748	819	C	tRNA-TrpTGG	tRNA	2,86	
HviSat16- 2480	397	19.0	0.0	3.7	1704	1785	+	tRNA- 1_DRe	tRNA	3,27	
HviSat16- 2480	630	6.7	0.0	0.0	1723	1797	+	tRNA-LysAAA	tRNA	2,98	
HviSat16- 2480	249	30.3	5.7	1.0	1798	1822	+	Ves2_ML	SINE/tRNA	0,97	15,69
HviSat19- 589	359	21.9	6.8	0.0	55	75	C	tRNA- 3_DRe	tRNA	3,40	
HviSat19- 589	363	20.8	0.0	0.0	76	147	C	tRNA-ArgCGY	tRNA	12,09	

HviSat19- 589	397	15.2	8.0	5.2	361	457	C	tRNA- 3_DRe	tRNA	16,30	31,75
HviSat21- 575	596	8.8	0.0	0.0	5	84	+	tRNA- 1_DRe	tRNA	13,74	
HviSat21- 575	665	0.0	0.0	0.0	320	393	C	tRNA-AsnAAC	tRNA	12,70	26,43

Tabela 12

Porcentagem Busca	Posição SW	Busca	SW	Busca	SW	Busca	SW	Busca	SW	Busca	SW
HroSat02-1662	592	5.6	0.0	0.0	28	99	+	tRNA-AspGAY	tRNA	4,27	
HroSat02-1662	523	15.1	0.0	0.0	1344	1416	+	tRNA-ValGTG	tRNA	4,33	
HroSat02-1662	244	26.4	7.1	1.1	1350	1441	+	SINE_FR2	SINE/tRNA -V-RTE	5,47	
HroSat02-1662	531	12.3	0.0	0.0	1523	1595	+	tRNA-ValGTY	tRNA	4,33	18,41
HroSat03-372	359	20.6	0.0	0.0	89	151	C	tRNA-ValGTG	tRNA	16,66	
HroSat03-372	458	16.7	0.0	0.0	290	361	C	tRNA-AspGAY	tRNA	19,08	35,75
HroSat05-515	411	16.1	0.0	0.0	32	93	+	tRNA-ValGTY	tRNA	11,84	
HroSat05-515	225	15.4	4.9	0.0	66	104	C	Gypsy- 11_XL-LTR	LTR/Gypsy	7,37	
HroSat05-515	375	21.4	0.0	0.0	178	247	+	tRNA-ValGTG	tRNA	13,39	
HroSat05-515	371	18.8	0.0	0.0	335	398	+	tRNA-AspGAY	tRNA	12,23	44,85
HroSat06-1634	673	3.7	0.0	0.0	149	230	+	Nimb-4_DR	LINE/I	4,95	
HroSat06-1634	615	9.6	0.0	0.0	723	805	C	tRNA-LeuCTG	tRNA	5,01	9,97
HroSat09-2961	1870	27.5	0.0	0.8	2	500	+	REX1-5_DR	LINE/RexBabar	16,81	
HroSat09-2961	337	27.1	0.0	0.0	678	747	+	tRNA-IleATC	tRNA	2,33	
HroSat09-2961	591	9.6	0.0	0.0	868	940	C	tRNA-AlaGCY	tRNA	2,43	
HroSat09-2961	603	8.1	0.0	0.0	1336	1409	+	tRNA-IleATT	tRNA	2,46	
HroSat09-2961	563	18.1	0.0	1.1	1340	1434	+	TguSINE1	SINE/tRNA -CR1	3,17	
HroSat09-2961	3250	28.3	6.7	2.4	1449	2961	+	Rex1-3_EL	LINE/RexBabar	51,06	78,28
HroSat13-1440	629	0.0	0.0	0.0	7	79	C	tRNA- 3_DRe	tRNA	5	
HroSat13-1440	323	26.6	1.3	11. 5	614	787	+	SAT_LM	Satellite	12,01	17,01
HroSat17-2481	256	32.0	4.8	0.0	103	130	C	Ves2_ML	SINE/tRNA	1,08	
HroSat17-2481	623	4.2	0.0	0.0	131	202	C	tRNA-LysAAA	tRNA	2,86	
HroSat17-2481	398	16.7	1.3	4.9	140	221	C	tRNA- 1_DRe	tRNA	3,26	
HroSat17-2481	611	5.6	0.0	0.0	1104	1175	+	tRNA-TrpTGG	tRNA	2,86	
HroSat17-2481	275	26.1	0.0	0.0	1336	1404	C	tRNA-LysAAA	tRNA	2,74	
HroSat17-2481	333	25.7	0.0	1.3	1622	1696	C	tRNA-GlyGGA	tRNA	2,98	
HroSat17-2481	628	4.2	0.0	0.0	1840	1911	C	tRNA-GlyGGA	tRNA	2,86	18,66

HroSat18-1194	281	23.1	0.0	0.0	212	276	C	SINE2- 1_SSc	SINE/tRNA	5,36	
HroSat18-1194	471	20.2	1.0	3.9	791	893	C	tRNA- 3_DRe	tRNA	8,54	
HroSat18-1194	620	4.2	0.0	0.0	1109	1180	C	tRNA- 3_DRe	tRNA	5,94	19,84
HroSat19-815	379	24.7	1.2	0.0	12	25	C	DNA-1_Gav	DNA	1,59	
HroSat19-815	564	8.4	0.0	0.0	26	96	C	tRNA-GlyGGY	tRNA	8,58	10,18
HroSat23-576	596	8.8	0.0	0.0	60	139	+	tRNA- 1_DRe	tRNA	13,71	
HroSat23-576	665	0.0	0.0	0.0	375	448	C	tRNA-AsnAAC	tRNA	12,67	26,38

Tabela 13

Porcentagem Busca	Posição SW	Busca	SW	Busca	SW	Busca	SW	Busca	SW	Busca	SW
HduSat01-2707	355	24.7	9.3	2.0	9	157	C	Penelope- 5_XT	LINE/Penel ope	5,47	
HduSat01-2707	5420	30.2	2.3	2.6	305	2707	C	Penelope- 5_XT	LINE/Penel ope	88,73	94,20
HduSat03-2143	531	15.1	0.0	0.0	1532	1604	+	tRNA-ValGTG	tRNA	3,36	
HduSat03-2143	268	25.3	7.1	1.1	1538	1629	+	SINE_FR2	SINE/tRNA -V-RTE	4,25	
HduSat03-2143	323	23.6	1.4	0.0	1711	1782	+	tRNA-ValGTY	tRNA	3,31	
HduSat03-2143	404	18.3	0.0	0.0	2073	2143	+	tRNA-AspGAY	tRNA	3,27	14,19
HduSat08-1754	569	10.8	0.0	0.0	139	212	C	tRNA-Ile-ATT	tRNA	4,16	
HduSat08-1754	285	28.4	3.6	1.2	148	229	C	MarinerN4_SSa	DNA/TcMar -ISRm11	4,62	
HduSat08-1754	264	21.9	4.7	2.4	596	606	C	tRNA-2_DRe	tRNA	0,57	
HduSat08-1754	591	9.6	0.0	0.0	607	679	+	tRNA-AlaGCY	tRNA	4,10	
HduSat08-1754	364	25.7	0.0	0.0	800	869	C	tRNA-Ile-ATC	tRNA	3,93	
HduSat08-1754	1370	30.6	3.9	3.2	990	1732	C	Rex1-3_EL	LINE/RexBabar	42,30	59,69
HduSat10-1449	318	25.6	1.3	1.3	483	561	C	SAT_LM	Satellite	5,38	
HduSat10-1449	605	2.7	0.0	0.0	1197	1269	+	tRNA-3_DRe	tRNA	4,97	
HduSat10-1449	237	27.5	0.0	2.4	1198	1279	C	LTR10A_BT	LTR/ERV1	5,59	15,94
HduSat11-815	458	16.4	0.0	0.0	742	814	+	tRNA-GlyGGY	tRNA	8,83	8,83
HduSat12-1670	661	4.9	0.0	0.0	355	436	C	Nimb-4_DR	LINE/I	4,85	
HduSat12-1670	340	19.2	13.1	0.0	563	635	+	tRNA-SerAGY	tRNA	4,31	
HduSat12-1670	615	9.6	0.0	0.0	1435	1517	+	tRNA-LeuCTG	tRNA	4,91	14,07
HduSat17-150	279	21.2	3.9	2.0	43	143	C	Helitron- 2_DR	RC/Helitron	66,67	66,67

Tabela 14

Porcentagem Busca	Posição SW	Busca	SW	Busca	SW	Busca	SW	Busca	SW	Busca	SW
HpuSat04-2707	355	21.8	7.3	2.3	4	183	C	Penelope-5_XT	LINE/Penel ope	6,61	
HpuSat04-2707	5241	30.8	2.2	2.5	331	2707	C	Penelope-5_XT	LINE/Penel ope	87,77	94,38
HpuSat07-2750	482	5.2	0.0	0.0	1	58	C	tRNA-Ile-ATT	tRNA	2,07	
HpuSat07-2750	541	13.7	0.0	0.0	462	534	+	tRNA-AlaGCY	tRNA	2,62	
HpuSat07-2750	275	20.6	1.6	0.0	910	972	C	REX1-5_DR	LINE/RexBabar	2,25	
HpuSat07-2750	4491	28.4	3.8	1.8	984	2595	C	Rex1-3_EL	LINE/RexBabar	58,58	65,53
HpuSat08-1546	286	27.7	0.0	0.0	555	619	+	SAT_LM	Satellite	4,14	
HpuSat08-1546	656	3.5	0.0	2.3	781	867	C	tRNA-3_DRe	tRNA	5,56	9,70
HpuSat10-781	604	4.2	0.0	0.0	557	627	C	tRNA-GlyGGY	tRNA	8,96	
HpuSat10-781	287	17.4	4.2	2.8	565	635	C	Bov-tA2	SINE/tRNA -Core-RTE	8,96	17,93
HpuSat12-1973	646	4.1	0.0	0.0	1475	1547	+	tRNA-ValGTG	tRNA	3,65	
HpuSat12-1973	228	27.8	5.3	2.2	1481	1572	+	SINE_TE	SINE/tRNA -V	4,61	
HpuSat12-1973	569	9.6	0.0	0.0	1655	1727	+	tRNA-ValGTG	tRNA	3,65	
HpuSat12-1973	592	5.6	0.0	0.0	1821	1892	+	tRNA-AspGAY	tRNA	3,60	15,51
HpuSat19-794	635	2.8	0.0	0.0	159	230	+	tRNA-ArgAGG	tRNA	8,94	
HpuSat19-794	233	21.5	5.8	4.4	239	306	+	tRNA-Met-i	tRNA	8,44	17,38

Em *Harttia*, esses elementos têm uma relação intrincada com a formação de satDNAs, conforme demonstrado por sequências complexas compostas de partes distintas nas quais motivos derivados de elementos intercalados curtos (SINE) e/ou elementos intercalados longos (LINE) e genes de RNAt. Por exemplo, HviSat02-2707 e seus homólogos em outras espécies estão relacionados a elementos Penelope, uma classe de elementos repetitivos amplamente distribuídos em genomas de peixes, vistos em medaka, baiacus e ciclídeos (Volff *et al*., 2001; Eickbush & Malik, 2007). Elementos semelhantes a Penelope (PLE) são encontrados predominantemente em genomas animais, com perdas ocasionais em algumas linhagens (Arkhipova, 2006). Por outro lado, HduSat17-150 está relacionado a Helitron, um grupo de TEs que conserva sequências nas extremidades, mas com motivos centrais repetidos em tandem semelhantes a satDNA (Thomas & Pritham, 2015), incluindo satDNAs reais embutidos em tal posição central em Bivalves (Satović *et al*., 2016; Vojvoda *et al*., 2020). Isso demonstra que a história evolutiva das sequências de satDNA também está relacionada a TEs, refletindo a história complexa de rearranjos cromossômicos que ocorreram no gênero. No entanto, tais TEs não parecem participar da diferenciação dos cromossomos sexuais, conforme discutido na próxima seção. A maioria dos satDNAs neste gênero apresentou taxas de divergência entre sequências homólogas que variam de acordo com as relações filogenéticas, mas RLs demonstraram eventos de amplificação e homogeneização específicos de cada espécie, especialmente nas repetições encontradas nos cromossomos sexuais. Tais padrões indicam um cenário de evolução contingente para os satDNAs de *Harttia*, como também visto no conteúdo repetitivo de gafanhotos (Camacho *et al*., 2022).

Por outro lado, dos vários satélites encontrados nos cromossomos sexuais de *H. punctata* (ver **Figura 24**), apenas HviSat04-2959 e seus homólogos (HroSat09-2961, HduSat08-1754 e HpuSat07-2750) estão relacionados a TEs. Este satélite tem homologia parcial com os elementos LINE-Rex-Babar e Tc/Mariner, que geralmente são inativados ou degenerados em genomas de peixes, mas também podem acumular mutações em taxas neutras até perder sua identidade molecular (Isvák *et al*., 1995; Muñoz-Lopez & Garcia-Perez, 2010; Schemberger *et al*., 2019). Ambos os elementos foram identificados em espécies relacionadas de Loricariidae, mas como sequências dispersas sem qualquer acumulação em um par de cromossomos específico (Ayres-Alves *et al*., 2017). Em bagres, elementos Tc1-Mariner podem ser encontrados associados à variação de loci de DNAr, sugerindo atividade no sistema de transposição (Gouveia *et al*., 2017). Ambos os satélites HviSat04-2959 e HviSat05-177 e suas contrapartes homólogas, localizadas em cromossomos sexuais em *H. punctata*, mas em

autossomos no resto das espécies, têm se expandido por mais de ~20 milhões de anos nas quatro espécies. No entanto, houve um aumento significativo no número desses satélites em *H. punctata*, o que se alinha com a observação de loci extras no cromossomo X_2 desta espécie. TEs são menos propensos a se acumular em cromossomos X jovens em peixes em comparação com autossomos (Chalopin *et al*., 2015), e esta pode ser a razão para a escassez de sequências de satélite derivadas de TE nos cromossomos sexuais de *Harttia*.

Embora as espécies de *Harttia* compartilhem cromossomos sexuais homólogos, conforme indicado por nossos dados de pintura cromossômica, cada uma demonstrou uma composição distinta de satDNAs, indicando evolução independente dessas sequências em cada linhagem. A diferenciação dos cromossomos sexuais por acumulação independente de satDNAs em um tempo evolutivo recente pode refletir a origem *de novo* de tais sistemas, como também visto em outros peixes com cromossomos sexuais com origem recente, como *Megaleporinus elongatus* e *Nothobranchius* spp. (Crepaldi & Parise-Maltempi, 2020; Voleníková *et al*., 2023). Em *Harttia*, a discrepância nos perfis de satDNA pode ser observada especialmente em sequências que não seguem um arranjo filogenético, por exemplo, HviSat08-4011, que provavelmente foi amplificado em *H. duriventris* e *H. punctata* após sua divisão, além de HviSat13-730 e HviSat18-1068. Os cromossomos sexuais de *H. villasboas* carregam apenas dois satDNAs aqui mapeados: HviSat13-730 e HviSat18-1068. Esses satélites compartilham uma pequena parte de sua sequência (cerca de 6%) com 84% de similaridade, sugerindo uma origem comum. No entanto, tanto os satDNAs quanto suas contrapartes homólogas em cada espécie têm sinais de amplificações recentes em *H. villasboas*, *H. rondoni* e *H. duriventris*, além de eventos independentes de amplificações anteriores. Para HviSat18-1068 em relação aos seus homólogos HroSat11-534 e Hdu15-534, que são menores em tamanho, um processo de duplicação em *H. villasboas* foi responsável por sua diferença de tamanho. Este satélite é apresentado na constrição secundária formada pelo 18S DNAr, onde eventos de crossing-over desigual foram propostos como os principais mecanismos para diferenças no acúmulo desta sequência ribossômica entre os homólogos. Como esses satélites não estão ligados a TEs, a recombinação desigual pode ter causado a duplicação, conforme descrito para outros satDNAs em arroz e macacos (Cardone *et al*., 2004; Ma & Jackson, 2006). No entanto, a replicação em círculo rolante também pode ser responsável pela duplicação de tais sequências repetitivas (Plohl *et al*., 2014). Em relação a HviSat13-730 e seus homólogos HroSat04-920 e HduSat09-771, que apresentaram sinais FISH positivos em todas as quatro espécies, não conseguimos identificar sequências homólogas no genoma de *H. punctata*. Nessa

espécie, o sinal FISH é restrito ao cromossomo X_2 e ausente no Y, correspondendo ao padrão de acumulação de DNAr 18S (Blanco *et al*., 2014), sugerindo que o evento de fissão que criou os múltiplos cromossomos sexuais também pode explicar essa variação, conforme proposto anteriormente.

Por fim, a relação mais intrigante entre uma sequência de satDNA e cromossomos sexuais no gênero foi observada com o HviSat08-4011, que tem uma homologia parcial com o gene ZP4, responsável pela formação da zona pelúcida. Glicoproteínas da zona pelúcida são geradas no ovário ou fígado pelos genes ZP (Bausek *et al*., 2000). RLs mostram um padrão gráfico simétrico para as porções homólogas e não-homólogas à ZP4 em *H. villasboas* e *H. rondoni* (**Figura 25**), o que indicaria um único satélite autossômico recentemente originado em ambas as espécies. No entanto, o gráfico em *H. duriventris* seria compatível com uma amplificação mais antiga de um satélite formado a partir de sequências parciais do gene ZP4 no X_1, possivelmente em adição a uma amplificação mais recente do satélite composto (composto de duas partes, as partes homólogas e não homólogas ao gene ZP4) em autossomos, conforme revelado por FISH. Isso é mais evidente em *H. punctata*, onde há vários picos principais representando o conjunto mais abundante de sequências homólogas ao gene ZP4, em uma divergência entre 4 e 10%, compatível com várias ondas de amplificação de um satDNA derivado de ZP4 no cromossomo X_1. A parte não homóloga ao gene ZP4, não detectada por FISH em *H. punctata*, mostra picos de amplificação relativamente menores em divergências maiores que 16%. Os genes ZP em vertebrados sofrem frequentes eventos de duplicação e perda de genes, sugerindo que são genes reprodutivos que evoluem rapidamente (Feng *et al*., 2018). Essa rápida evolução pode permitir que eles se adaptem a vários ambientes ecológicos (Cao *et al*., 2016) e sirvam como barreiras à fertilização (Bianchi & Wright, 2015; Claw & Swanson, 2012), tornando-os alvos potenciais para a seleção natural. Um gene relacionado à fertilização no mesmo grupo de ligação que o ancestral dos três clados de *Harttia* pode indicar um sistema ancestral de cromossomos sexuais. De fato, o grupo de ligação mencionado é recrutado exclusivamente como cromossomos sexuais nas espécies do clado II (**Figura 4**). Isso sugere um evento de *turnover* no surgimento de múltiplos cromossomos em relação aos demais sistemas encontrados no gênero (revisado em Deon *et al*., 2020).

Capítulo 3

Evolução citogenômica e história demográfica em *Harttia*

Neste último capítulo, apresentamos os resultados e discussões referentes a um último trabalho, que está em fase final de construção, que será intitulado "*Cytogenomic evolution and demographic history in Harttia*". Utilizamos dados de sequenciamento de genoma completo com redução de complexidade via DArTseq (Diversity Arrays Technology, Canberra, Australia) para reconstruir as relações evolutivas das espécies investigadas pela citogenética, buscando posicionar as variantes identificadas, porém não reconhecidas ao nível de espécie (*Harttia* sp. 1, *Harttia* sp. 2 e *Harttia* sp. 3). Também utilizamos metodologias que buscam por marcadores ligados ao sexo, visto que este método de sequenciamento tem como alvo principal as regiões de baixo número de cópias ou hipometiladas do genoma. Por fim, utilizamos modelagens para reconstruir a evolução cromossômica do grupo e as áreas de ocorrência ancestrais, visando a reconstrução da história demográfica do gênero.

Recuperamos 95.305 SNPs e 282.275 SilicoDArT loci (presença-ausência) para 107 indivíduos de 18 espécies (17 espécies de *Harttia* e *Farlowella* sp. como grupo-externo). A probabilidade de que um único locus exibisse um marcador ligado ao sexo por acaso foi calculada em $5{,}87\times10^{-8}$, o que indica que de todos os 95.305 SNPs, espera-se que apenas 0,005 indicarão por acaso ligação ao sexo, o que indica que nosso tamanho amostral foi suficiente para recuperar marcadores verdadeiramente ligados ao sexo. O gráfico PCA (**Figura 26**) baseado em SNPs autossômicos com os PCs mais representativos (PC1 – 28,5%, PC2 – 18,9%) demonstrou cinco grupos distintos de espécies, concordando com a distribuição geográfica das amostras, com três grandes grupos incluindo as espécies de *Harttia*, outro formado exclusivamente por *Harttia* sp. 2, e outro formado pelo grupo externo *Farlowella* sp. Encontramos 419 marcadores ligados ao sexo (SLM) para o gênero *Harttia*, 95 indicando um sistema com heterogametia masculina (XY) e 16 indicando um sistema com heterogametia feminina (ZW) para os dados de SNP, e 292 XY e 16 ZW para os dados de PA. Cada espécie apresentou um número único de marcadores, com apenas *Harttia* sp. 2 não demonstrando SLMs em nenhuma das análises. O gráfico PCA dos SNPs ligados ao sexo (**Figura 26**) indicou três grupos principais: um formado somente por *H. intermontana* (XY_1Y_2), outro formado por *H.*

carvalhoi (XY_1Y_2), *Harttia* sp. 1 (XY_1Y_2) e *H. torrenticola* (XY), e o último agrupando todas as espécies restantes, com um pequeno subagrupamento de amostras de *H. gracilis*.

A reconstrução filogenética agrupou todas as espécies de *Harttia*, exceto *Harttia* sp. 2, em três clados principais com alta congruência geográfica. O clado I é composto por espécies com distribuição nas drenagens das Guianas (*H. fowleri* e *H. guianensis*), o clado II por espécies encontradas em drenagens do norte do Brasil, ou seja, Amazonas e Tocantins-Araguaia (*H. duriventris*, *H. dissidens*, *H. punctata*, *H. rondoni*, *H. villasboas* e *Harttia* sp. 3), e o clado III por representantes do sudeste e sul do Brasil (*H. carvalhoi*, *H. torrenticola*, *H. intermontana*, *H. longipinna*, *H. gracilis*, *H. loricariformis*, *H. kronei* e *Harttia* sp. 1) (**Figura 27**). Em geral, a configuração da filogenia nos três principais clados seguiu o padrão observado em reconstruções prévias baseadas em dados morfológicos e genéticos (Covain *et al*., 2016; Londoño-Burbano e Reis, 2021). Contudo, as relações interespecíficas diferem das reconstruções prévias. Em ambos os trabalhos de Covain *et al*. (2016) e Londoño-Burbano e Reis (2021), *H. fowleri* foi considerada pertencente ao clado II (espécies do norte do Brasil), enquanto em nossa reconstrução a espécie é mais próxima de *H. guianensis*, ambas compondo o clado I (espécies das Guianas). Já *H. dissidens* forma um clado irmão às espécies *H. duriventris*, *H. rondoni* e *H. villasboas* em Covain *et al*. (2016), enquanto em nossa reconstrução *H. dissidens* é irmã do clado *H. rondoni* + *H. villasboas* e todas estas, por sua vez, formam um grupo irmão do clado de *H. duriventris*. Em Londoño-Burbano & Reis (2021), *H. dissidens* forma um clado irmão às espécies *H. rhombocephala*, *H. punctata*, *H. duriventris* e *H. trombetensis*. Diferenças na configuração dos clados também foram observadas para o clado III (espécies do Sul e Sudeste do Brasil), apesar das relações estarem mais congruentes em uma ampla observação. A análise conduzida na presente tese inclui pela primeira vez a espécie *H. intermontana* em uma reconstrução filogenética do gênero, posicionando a em um clado irmão às espécies *H. torrenticola*, *H. carvalhoi* e *Harttia* sp. 1.

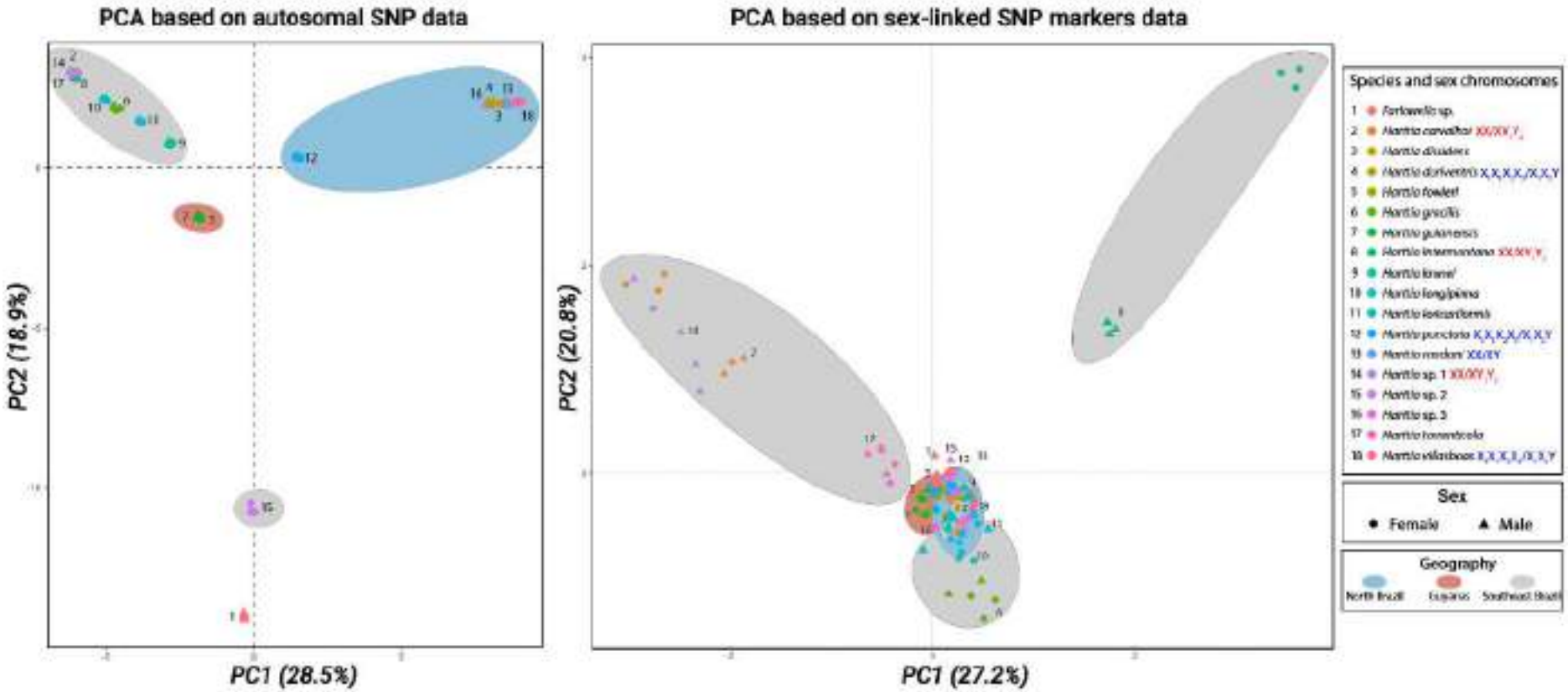


Figura 26. Gráfico PCA de dados de marcadores SNP autossômicos e ligados ao sexo. Os clusters apresentam a distribuição geográfica das espécies, seguindo o azul para o Norte do Brasil, o vermelho para as Guianas e o cinza para o Sul e Sudeste do Brasil. Cada espécie é representada por um círculo colorido e um número, seguindo a legenda à direita. O sexo dos indivíduos segue círculos para fêmeas e triângulos para machos.

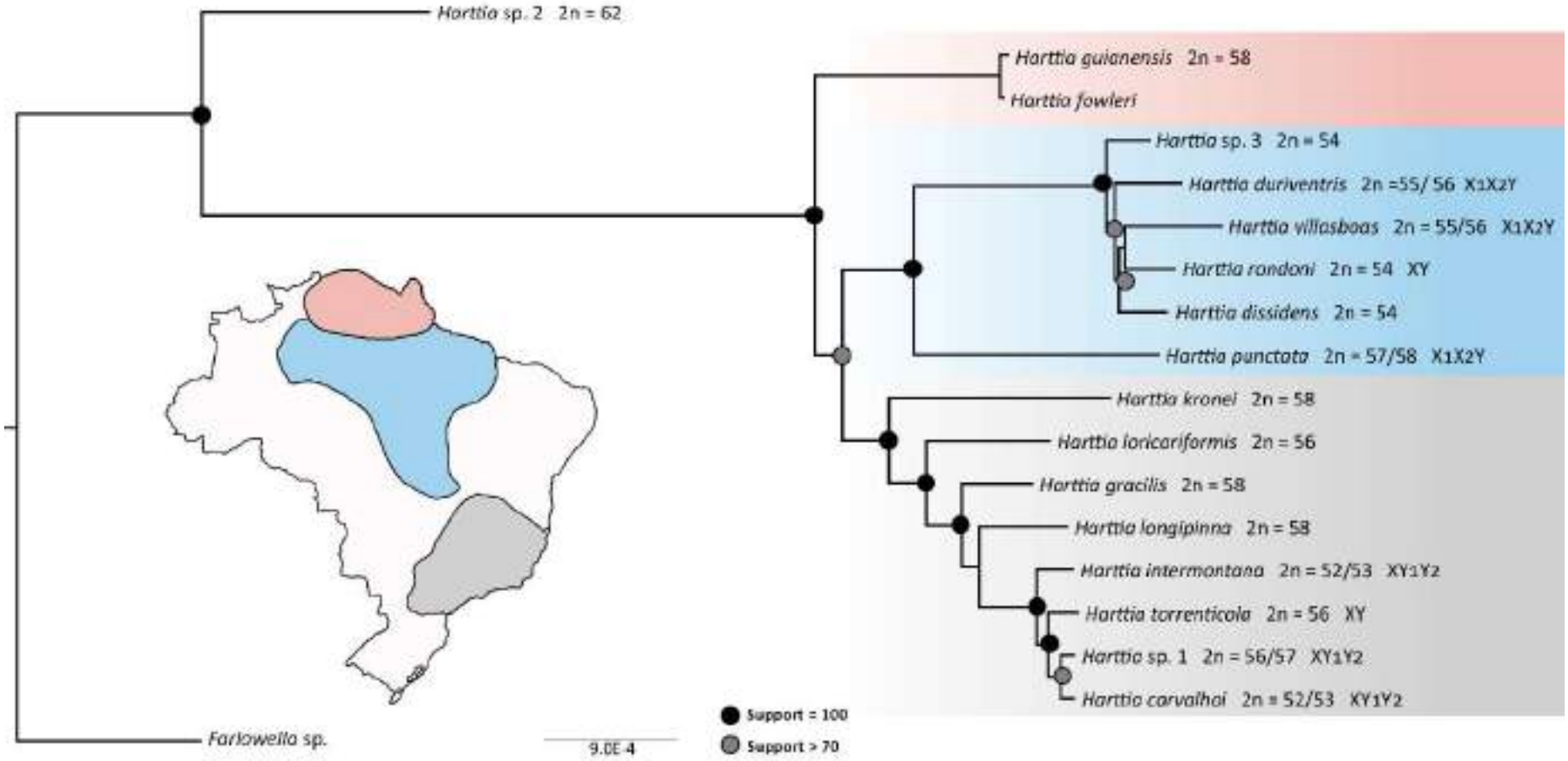


Figura 27. Relações filogenéticas de máxima verossimilhança de espécies de *Harttia*, usando *Farlowella* sp. como um grupo externo, recuperadas de 1.375 SNPs gerados por DArT-Seq de 107 indivíduos. Número diploide e sistemas de cromossomos sexuais são exibidos juntamente ao nome da espécie. O suporte dos clados é exibido pelos círculos nos nós, seguindo círculos pretos para 100 de bootstrap e cinza para > 70. A distribuição aproximada dos clados no território brasileiro é apresentada. Barra de escala em tamanho dos ramos.

A discordância das reconstruções filogenéticas era esperada, devido aos distintos marcadores utilizados para recuperar tais relações. Aqui, apresentamos a primeira hipótese filogenética para o gênero baseada em dados moleculares derivados de sequenciamento amplo de genoma, enquanto as reconstruções prévias basearam-se em dados mitocondriais ou poucos genes nucleares, além de dados morfológicos. A amostragem incompleta das linhagens e outros problemas filogenéticos podem ser resolvidos com o uso de múltiplos marcadores genéticos nucleares (Mirarab *et al*., 2016; Simmons & Gatesy, 2021), principalmente ao considerarmos que análises baseadas em um único locus representam uma única amostragem de um processo ancestral (ou genealógico) altamente estocástico (Degnan & Rosenberg, 2009; Leaché & Oaks, 2017). Desta forma, mesmo que a filogenia aqui apresentada seja baseada um número elevado de marcadores, não é possível afirmar que esta representa a definição dos clados de *Harttia*, sendo necessário ampliar a amostragem e o número de marcadores para uma compreensão mais clara da história evolutiva do grupo.

Utilizando a filogenia aqui produzida como base, modelamos a evolução do número de cromossomos no gênero (**Figuras 28-30**), utilizando três configurações da árvore filogenética: i) completa (**Figura 28**), ii) sem o grupo-externo (**Figura 29**) e iii) sem o grupo-externo e *Harttia* sp. 2 (**Figura 30**), todos com o modelo "Dys" indicado pelo software como o mais apropriado para os dados. Em todos os cenários, 2n = 56 foi encontrado como 2n ancestral mais provável para o clado mais inclusivo que engloba *Harttia* sp. 3, *H. duriventris*, *H. dissidens*, *H. villasboas* e *H. rondoni*, assim como 2n = 58 para o clado que inclui *H. loricariformis*, *H. gracilis* e *H. longipinna*, e 2n = 56 para o clado *H. intermontana*, *H. torrenticola*, *H. carvalhoi* e *Harttia* sp. 1. O 2n ancestral para toda a filogenia variou conforme os cenários, sendo 2n = 62 quando incluídas todas as espécies, 2n = 64 quando analisado sem *Farlowella* sp., e o terceiro, 2n = 60. Desta forma, eventos de disploidia descendente (fusões cromossômicas) foram predominantes em todas as análises. Contudo, os processos de fusões não foram iguais em todos os clados. Enquanto no clado das espécies do sul e sudeste brasileiro, as fusões ocorridas envolveram a formação do sistema sexual XY_1Y_2 (Deon *et al*., 2022a, b), fusões no clado norte envolveram outros pares (veja resultados dos experimentos de pintura cromossômica no capítulo 2).

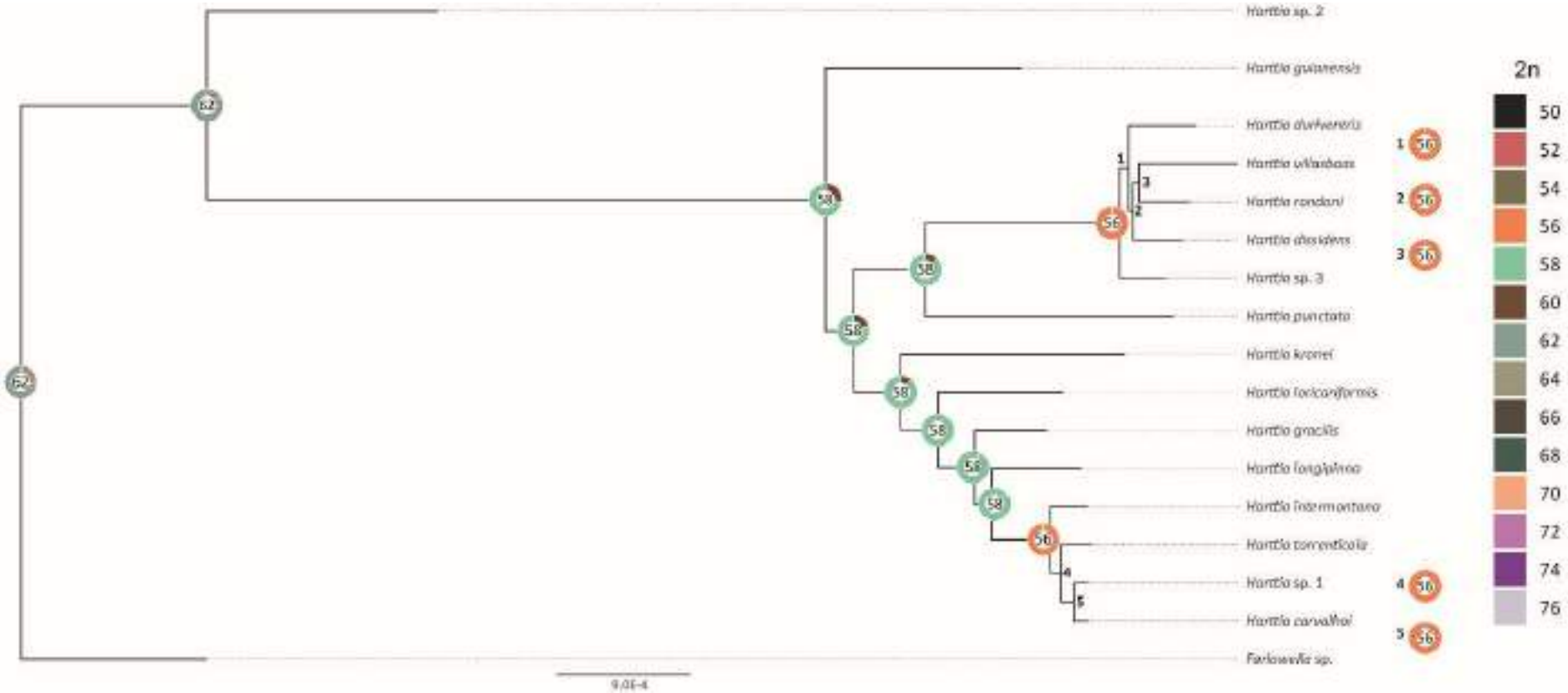


Figura 28. Relações filogenéticas de *Harttia* com o número diploide (2n) ancestral inferido pelas análises do ChromEvol. Os gráficos de pizza indicam a probabilidade de 2n ancestral. Barra de escala em comprimento dos ramos.

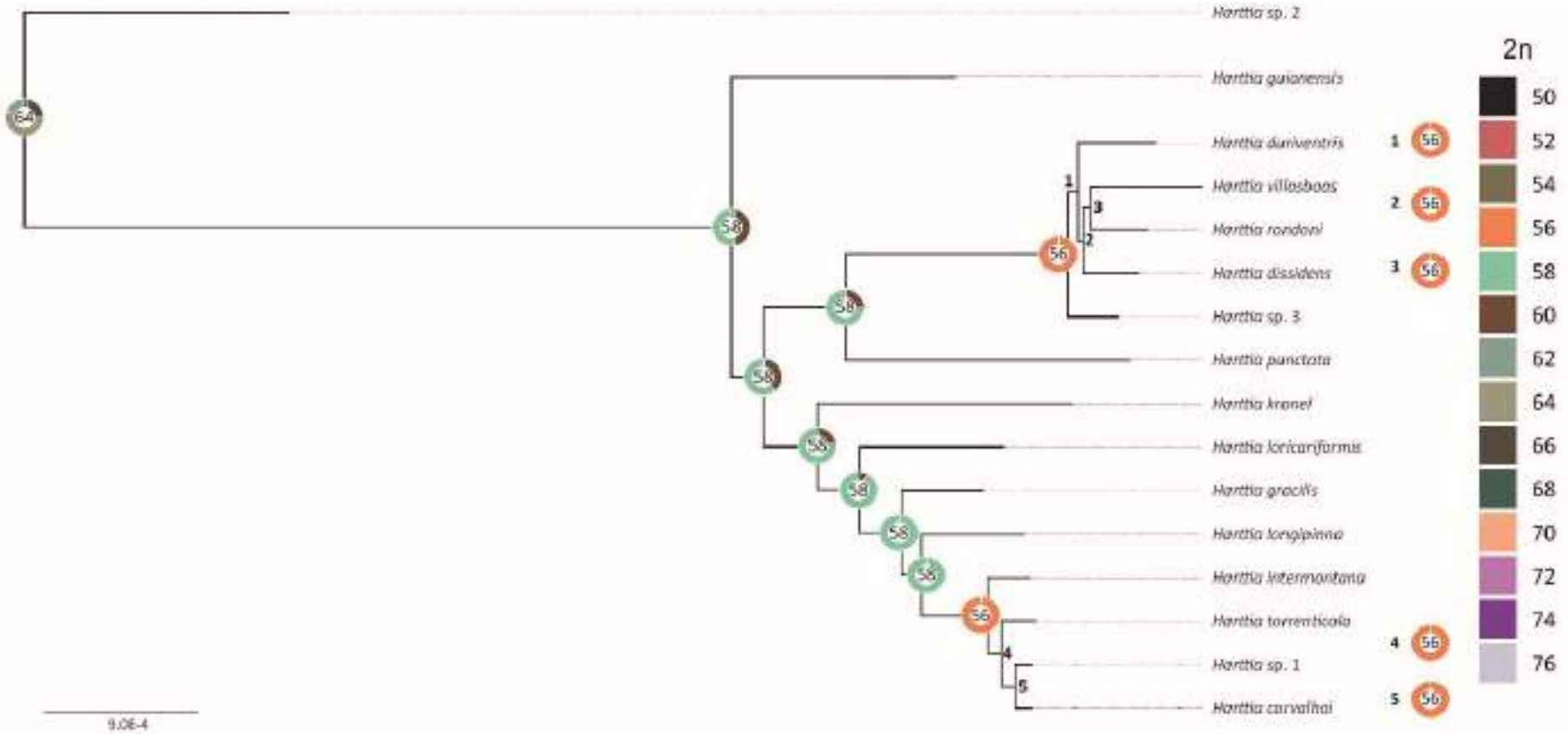


Figura 29. Relações filogenéticas de *Harttia* sem o grupo-externo (*Farlowella* sp.), com o número diploide (2n) ancestral inferido pelas análises do ChromEvol. Os gráficos de pizza indicam a probabilidade de 2n ancestral. Barra de escala em comprimento dos ramos.

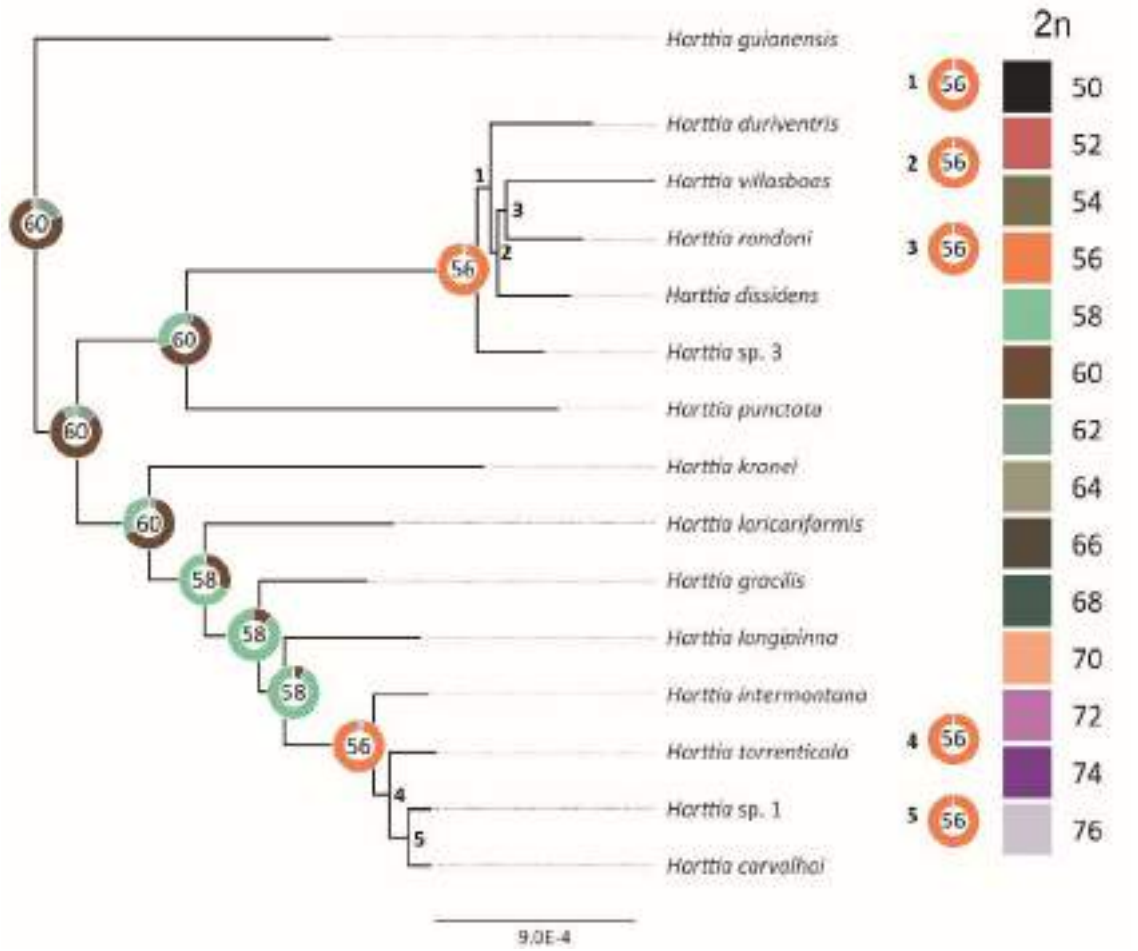


Figura 30. Relações filogenéticas de *Harttia* sem o grupo-externo (*Farlowella* sp.) e sem *Harttia* sp. 2, com o número diploide (2n) ancestral inferido pelas análises do ChromEvol. Os gráficos de pizza indicam a probabilidade de 2n ancestral. Barra de escala em comprimento dos ramos.

A reconstrução utilizando a filogenia completa (**Figura 28**) sugere que o 2n ancestral do grupo seja 62 cromossomos. Este é observado em duas espécies de *Harttia*: *H. absaberi* (Rodrigues, 2010) e *Harttia* sp. 2 Rio Barra Grande – PR (Deon *et al*., 2020). A espécie *H. absaberi* foi inclusa apenas na filogenia de Cherobim (2021), que sugere a sua alocação em um novo gênero, dadas as distâncias filogenéticas observadas. Curiosamente, o padrão aqui observado para *Harttia* sp. 2 indica que esta espécie é mais próxima do grupo externo do que das demais espécies do gênero *Harttia*. Dessa forma, é possível sugerir que *Harttia* sp. 2 possa representar mais uma espécie ainda não descrita do novo gênero que *H. absaberi* pertence, caracterizado pelo 2n = 62.

Considerando que *Harttia* sp. 2 também pode ser um grupo externo para a análise em *Harttia*, ambas as modelagens com dois ou apenas um grupo externo (**Figuras 28 e**

29), são consistentes em apontar 2n = 58 como número diploide ancestral para os três clados do gênero. Este já havia sido considerado como número ancestral visto que é o 2n mais frequente no gênero (Blanco *et al*., 2017; Deon *et al*., 2020). Além disso, a hipótese do proto-XY apresentada no capítulo 1 desta tese considera o cariótipo ancestral composto por 2n = 58 e cromossomos sexuais XX/XY, em estágios iniciais de diferenciação, sendo corroborada pelo resultado de modelagem assim como pelos marcadores ligados ao sexo.

Ao observarmos o número de SLM em ambos os dados de SNP e PA (**Figura 31B**) e sua distribuição em todas as espécies de *Harttia* exceto *Harttia* sp. 2, é possível corroborar a hipótese proto-XY. Assim, sistemas de cromossomos sexuais eram reconhecidos pelo seu heteromorfismo no cariótipo de oito espécies (*H. carvalhoi*, *H. intermontana*, *Harttia* sp. 1, *H. torrenticola*, *H. duriventris*, *H. rondoni*, *H. villasboas* e *H. punctata*) e, aqui, incluímos mais oito espécies com sistema homomórfico XX/XY reconhecido por SLM (*H. dissidens*, *H. guianensis*, *Harttia* sp. 3, *H. kronei*, *H. longipinna*, *H. loricariformis*, *H. gracilis*, *H. fowleri*). Curiosamente, três SLM se apresentaram compartilhados entre as espécies *H. gracilis* e *H. guianensis* (**Tabela 15**), o que reforça a hipótese de um sistema proto-XY compartilhado em *Harttia*, indicando também que um evento de *turnover* ocorreu no clado das espécies amazônicas. Contudo, não foi possível estabelecer algum possível gene sexo determinante, visto que a busca pelos SLM na base de dados NCBI via BLAST indicou homologia com regiões do genoma de peixes sem anotação e regiões anotadas a nível de gene que não apresentam relação com determinação do sexo (**Tabelas 16-17**).

Tabela 15. Relação de SNPs ligados ao sexo compartilhados entre espécies de *Harttia*. Cada coluna apresenta o conjunto de espécies e as sequências que compartilham.

Espécies	*H. gracilis* *H. guianensis*	*H. rondoni* *H. villasboas* *Harttia* sp. 3	*H. carvalhoi* *H. intermontana*
SNP	TGCAGCCTAAAACAAGT ACACTGAGGTTTTATGT AGCATTTTATTATAAATT GTGCTCAATCTGCATGA	TGCAGGTAAATACAA CAGAGAACATACACG CATG	TGCAGGTTCTTCTCTGA TAGCCGCATG
	TGCAGCCAGGAACTACA ACTCCCACCATGCAACG TGCATG		TGCAGACAAACAGCAA TGTTCTTTTTGAAGAGC GATGGCTTTCTCCATGC AACCCTGCTATGCATGA GC
	TGCAGTATTTTATCAGT AGTAGTGGAAAAGAGA GTGCATG		

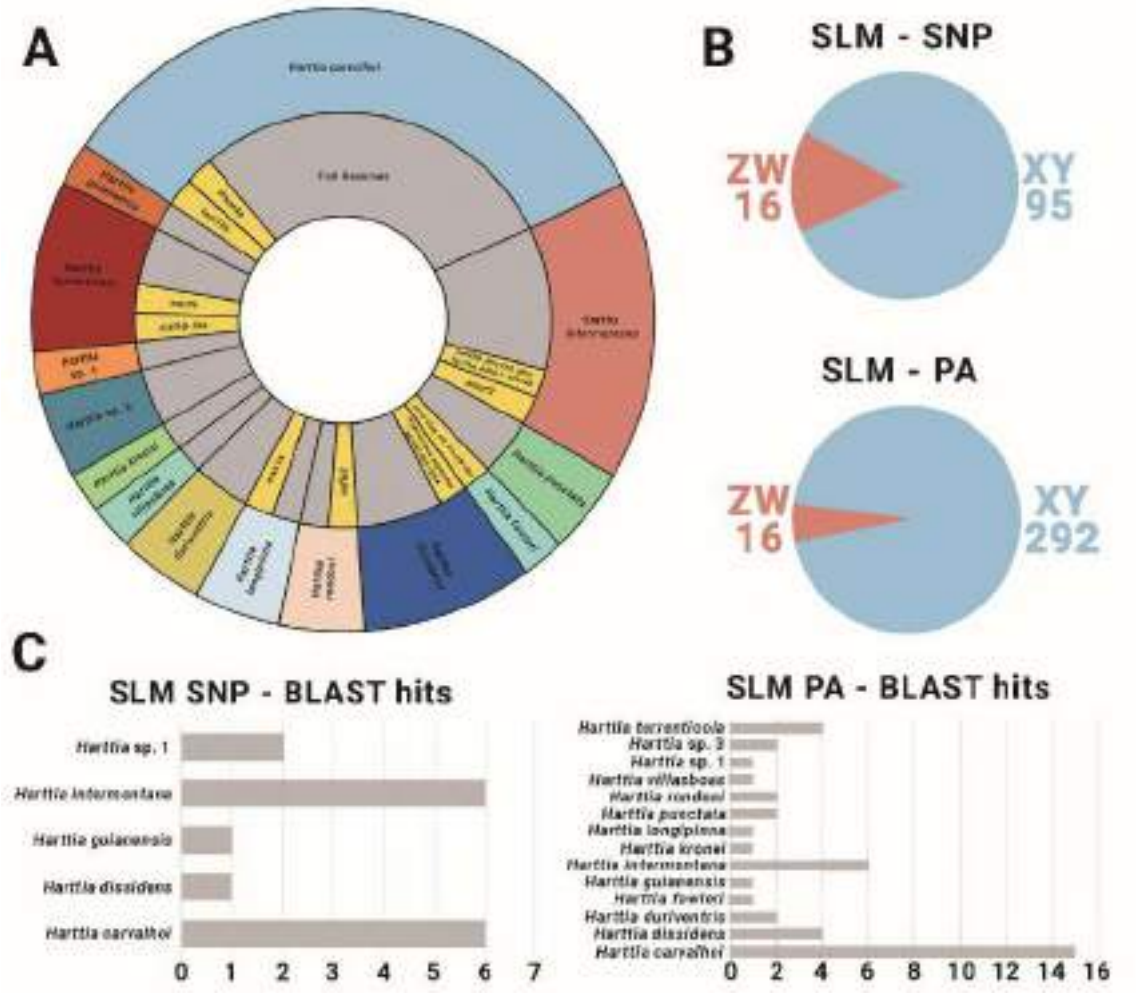


Figura 31. Resumo dos resultados de marcadores ligados ao sexo (SLM). A) Busca na base de dados NCBI com BLAST dos SNPs ligados ao sexo, com o tamanho refletindo o número de marcadores com resultados positivos por espécie, detalhado em C). Em B) são apresentados os números de SLMs que indicam sistemas de heterogametia masculina (XY) ou feminina (ZW).

Tabela 16. Resultados do BLAST dos SNPs ligados ao sexo (SLM) identificados em *Harttia*.

Espécie	SLM	BLAST
H. carvalhoi	TGCAGCATTAATCAATTGGAAATGTGCTGCAT TAGCGTGCTGCATTTCCGATGGCTGCCACCCGT CTGT	Fish genomes
H. carvalhoi	TGCAGTCATCTGTCCTGAACCCCATCTAGCATT TATGGGGGCACTTGAAGACAGAGAAAGCCAA ACATT	*Conger conger* genome
H. carvalhoi	TGCAGTGACACTGATGTGGTACTGGTATGTTG GTCTGTGTTGTGGTGGTACAAATGGATCAGAC AGCAG	Fish genomes, mRNA
H. carvalhoi	TGCAGATAGAGGGTGCCAAATGTCCTTTCAGT GTAATTTAAATTGTGGTTCTGGCAAAACAATG GGTAG	gad2
H. carvalhoi	TGCAGCCAATGCATTGCCAAAGGAGGCTCCTG TGACTGACACCCTCTGTCTCATCTTGGGTGGCT CCTG	Fish genomes, mainly Siluriformes, mRNA
H. carvalhoi	TGCAGAGAACACATCTCCACTGATGTAGTGTC CAGTGGCAGCATGAGCGGTTCAGCAGGAATGC CGAGA	Fish genomes
H. dissidens	TGCAGCGGTATTTGCCTCAGCAGCTGCTGACC CCGCTCTGTCATTTTACGTGGCCTATGAGTTGC AGTC	Fish genomes, mRNA
H. guianensis	TGCAGACCCTGCTCCAGCATCTCAAGTCTCAC AGCCTAACCATCGTGAGCAACGCCTGCGGAAC CCTCT	apc2, mRNA
H. intermontana	TGCAGAGGGATCTGAAACGTGTGGTTGATGCC AGACTCAGACTCAGCGAGGAGCTCAGTGGAGG ACGGA	actr3b, mRNA
H. intermontana	TGCAGGTTCTTCTCTGATAGCCGCATG	Fish genomes
H. intermontana	TGCAGAGTTCCAAACCTCTTCTGGCATTAACAT TAGCACAAAAACTGAGCAGTTGCATG	*Ocypus olens* genome assembly
H. intermontana	TGCAGCACCGCTTTAAATCAAACACTGGACCT CGTGTATACCTGCATG	Fish genomes
H. intermontana	TGCAGGAAATTATCAACTTTTTGTAAATAACTT GCAGCATG	Fish genomes, Siluriformes mainly, mRNA
H. intermontana	TGCAGTTGGGTCCTCCTTTCCATCTCACCCCCA ACTCATAACACCGACTTGCTCAATTAGGGCTTT GAG	Fish genomes
Harttia sp. 1	TGCAGTTTACTAAAGATCACGTGGACAAGCCA GAAGGCTTGTTGGAGAATGTTTTGTGGACGGA TGAGA	Fish genomes, mainly Siluriformes, mRNA
Harttia sp. 1	TGCAGCTGCACCTCATGCTGGCGCGTGAGCAC TTCGTGTTGCTTCTGGAACTCGGCGAAGAGTA GCTGT	hdac5, fish genomes, mRNA

Tabela 17. Resultados do BLAST dos marcadores ligados ao sexo (SLM) identificados com presença e ausência (PA) em *Harttia*.

Espécie	SLM	BLAST
H. carvalhoi	TGCAGTTAACAGTGAGTGCTTACAGGCTTAATGAGCTGTTGGACTTGGCTAATTGACAGAAAACACGGC	Fish genomes
H. carvalhoi	TGCAGTCTTGCCTGCGTGGTCAACCTTAATTAACATCCAAAACTAGACTCAACATGAAACTAAAAGAAA	*Trachurus trachurus* genome assembly
H. carvalhoi	TGCAGTAGGAATGATGGTATTTACATGTATCTGGTAGGAGCACCAACGCTGCCCAGCCGGCCACTAGAG	tnni1b, mRNA
H. carvalhoi	TGCAGATACTGCATGGATGACTTCAGGAGATGTAGGGCTAGCTAAGATGGCTTTCTTCCTCCTCCACCT	Fish genomes
H. carvalhoi	TGCAGTTCTGAAGGTCAAACGAAGTCCAACATGCTACTAGATCTCTAATAAAAGTAGCCATTCAGTGCA	*Thalassophryne amazonica* genome assembly
H. carvalhoi	TGCAGGTACATATATACACCCCAGACAAATAAATAGTCATCTTGGGTGTTTAATGTGGTACTAACTTCA	Fish genomes
H. carvalhoi	TGCAGTGCGAGTCCACATTCGTGTTAGACTTGGTCTACCTATGGCCAGGGTAGGACTAAGTTTCACTCG	plppr4a
H. carvalhoi	TGCAGCACATTCCAATAAAAGTTAGGCCAGGGACAAGGGCTAATGATGTGATTTCAGACGGGTGATGTG	Fish genomes
H. carvalhoi	TGCAGGGAGAGGGAGAGAGAAAGGCCAAGACATCCAAGTCGAGCTCCATCAGAGCACCATAACGCTATC	*Valencia hispanica* genome assembly
H. carvalhoi	TGCAGATGTTGTGTGCAGTAATGTAAAATGTGACTGTAATGTGATGTAATGTTTTTAAATCTTAATTAT	Fish genomes, mRNA
H. carvalhoi	TGCAGAAGGCGTTGCATGAGGATGATCAGAATGTAAATACCTGCGTGGTATGCGACAGCATTCATAGAT	Fish genomes
H. carvalhoi	TGCAGGACATCGTACTTCACTTTTGTGAGCTTGGAGCATAGACCACTTACATATATCAAACTGGTATCA	*Conger conger* genome assembly
H. carvalhoi	TGCAGGCGCGTGCCGATAATCAACCTCACACACACACTCGCATG	Fish genomes
H. carvalhoi	TGCAGAAACGTGGACACTACTGTGAACAGGTCATGCAATCCAGCATACGTACGAGCACCATGCACGTGT	Fish and parasites genomes
H. carvalhoi	TGCAGACAAGTGAAAGTATGGGAAGGGAGTGAAGGGAAGAGGGAGTGCGCACACGTAGGTTAGAGGAAG	*Silurus aristotelis* genome assembly
H. dissidens	TGCAGCCATGCCTTTTTAATGTGTGCATCATGTGGTTTTGCATTGTTTTGTTAAAAAATGCATG	*Thalassophryne amazonica* genome assembly
H. dissidens	TGCAGGGCTGCTCTCTGCTCAGCACTCAGTGTCAGTCCGTTTCCACATACCAACTCCAGTGAATAATGC	Fish genomes
H. dissidens	TGCAGTGGCACGTCCGTATAGGACGATTATATGGCATGACTGACCGGGCCACTAAAAATGTATAAGAAC	*Hypostomus asperatus* partial 28S rRNA gene, fish genomes

H. dissidens	TGCAGTGTGCACTCCTGTTTGAGCAGCAGGTTC ACTTCCTCAAGGTCCAGCTTGGAGGGGTCGGG AAGC	Fish genomes
H. duriventris	TGCAGAGATCCGCAGCTTAGGTAGGAGTTCTG TCCACAGTACAACTATTAGTCGTGCATG	*Raja brachyura* genome assembly
H. duriventris	TGCAGCAGTGGAAAAATGTTTCCTGGAGTGAT TAATCATTTTTCACTATCTGGTAGTCTGATGGA GAAG	Fish genomes
H. fowleri	TGCAGCTTTAAGCTCAAAACATTCACAGCTAA AAGAATTGGACCTCAGCTACAATCACCCAGGA GAGTC	NLRC3-like, NLR, TRIM39-like, fish genomes
H. guianensis	TGCAGGACGGGCGTATGCATGAGCGGTTCAGC AGGAATGCCGAGACCGATCTCGTATGCCGTCT TCTGC	Fish genomes
H. intermontana	TGCAGTAAAAAATGCACCTGCCATATTGCATT AATCTCAGAATATACACTATATTGCCAAAAGT ATTCG	Fish genomes
H. intermontana	TGCAGTTCTAGGTCAGGTTATGAGCCCAAAGA ATGAGGTCAGCTGATACCTGAATATACTGAAC CACCA	Fish genomes
H. intermontana	TGCAGCTTTTTTGTCAGCACAAAGCAGTGAGTT TTCCTTTATTGCACCATTCTGAGTAAACTCCAG AGA	Fish genomes
H. intermontana	TGCAGTAATGCTTGAGTAGATGGTACTGGTCA AAGTAACATCCACATGAATGCCAGGACTCTAG TTCTC	Fish genomes
H. intermontana	TGCAGTCTCTAACTGTAGAGTTAAGAGTACAA AGTGCACCTATATGGTAAGTGGAGCTGATAAA ATGAT	mterf2, Fish genomes
H. intermontana	TGCAGTCTGTAATTGTAGAACTACAAAGTGTG TCTAATGGTAAGTGGAGCTGATAAAATGGACA GTGAG	znf420, bloc1s4, fip1l1a, ghra, cdc14b, pdlim1, fish genomes
H. kronei	TGCAGTATTAGTACTAAAGCGTTGAATTCCGCT GGGTTTAAGTAATGCATCATGTAAATGCAAAA AAAT	*Silurus aristotelis* genome assembly
H. longipinna	TGCAGGGAGGGCTGGAACCGCTACGTCTGCGA CTGCGCAGCCACCGGCTTCCTGGGACGCTCGT GTGAA	nrxn1a, fish genomes
H. punctata	TGCAGAAGTTTTTTCTGCTGGCCCTGCAATGGA CTGGCATCCTATCCAGGGCATG	Fish genomes
H. punctata	TGCAGCAACCTGATAGTAGAAATGTTCAATAG GTGCCTTGCAGCCAGATGCAGCATG	Fish genomes
H. rondoni	TGCAGGCAAGCAGATAGAGTGTCAGATGGAGT GGTGTAAAGCATG	Fish genomes
H. rondoni	TGCAGCATATATGCAAATATGTGGGAGGAATG TATGAGAAAACTGGAATACCTGGAAAAAAACT CAAGC	nufip2, *Scleropages formosus* genome
Harttia sp. 1	TGCAGAGGTTGTAGAAGTGGGAGGTCAACCTG TCAGTACTCAGACCATACGCCACACTGCATCA ATTGG	Fish genomes
Harttia sp. 3	TGCAGTTGTTCAGGTTTAGGTTCAGCAACATTC TGTCTCCAATGAATGAGGTCAGCTGACTAACT GAAT	Fish genomes
Harttia sp. 3	TGCAGTTGGTCAGGTTTAGGTTCAGAAACATT ATGTGCATAAACAATGAGGTCAGCTGAAACCT GAATA	Fish genomes

Harttia torrenticola	TGCAGCGTTTCCCATACATTGATTTATTTATTA TCAACACTGATTGACATGTTTATTTCATTGATT CAC	Fish genomes
Harttia torrenticola	TGCAGGAGGAAAAGCGCACCTGAAGCAGTGC GAGCAGATGCAGCTGACGCGGCGTCAGAAGC GCGCGTG	wnt9b, Fish genomes
Harttia torrenticola	TGCAGTAATTCATGCAAAAAAAGGCCCAAGCA AATATTGAGTGCATAGAAATGAACATACTTTT CAGAA	Fish genomes
Harttia torrenticola	TGCAGCAATGTCAGCGTCTTTCAGGACACTAG CGAATGCCATCTTTGCTACATGAGGCGGGGGG GGAAA	PVALB-β-like
H. villasboas	TGCAGATGTGCTGAAGTCATTCAAAGCAAAGG CATG	Fish genomes

Por fim, a modelagem de áreas ancestrais indicou o modelo DEC (cladogênese por dispersão e extinção) como o que melhor representa os dados, visto seu menor AIC em relação aos demais modelos (**Tabela 17**). A análise demonstrou que o ancestral de *Harttia* distribuía-se pela bacia Amazônica, Paraná ou Atlântico Sudeste, excluindo as drenagens das Guianas como possível centro de origem do grupo (**Figura 32**).

Tabela 17. Análise de modelos biogeográficos implementados no BioGeoBEARS, indicando a escolha do modelo DEC como mais representativo dos dados, visto o menor valor de AIC. LnL = log-verossimilhança dos dados em relação ao modelo; AIC = critério de informação de Akaike.

Modelo	LnL	AIC
DEC	-56,68819	117,3764
DIVALIKE	-70,877	145,754
BAYAREALIKE	-102,9561	209,9122

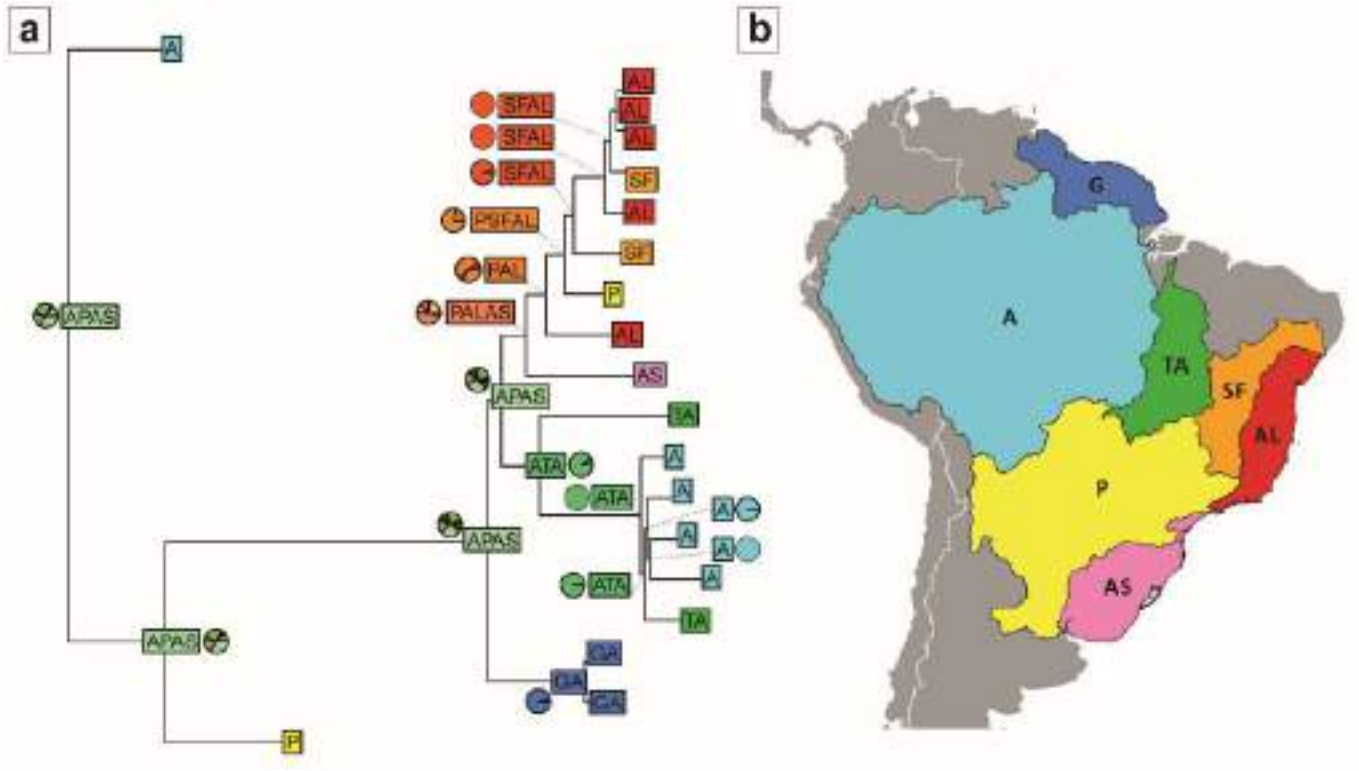


Figura 32. Estimativas de distribuição ancestral indicadas pelo modelo DEC implementado no BioGeoBEARS. A) filogenia de *Harttia* (conforme **Figura 28**) indicando as áreas de distribuição inferidas pela análise, com as probabilidades indicadas pelos gráficos de pizza. As áreas utilizadas estão apresentadas em b).

Observando os padrões da modelagem na filogenia (**Figura 32a**), as bacias dos rios Tocantins-Araguaia e São Francisco, além das drenagens do Atlântico Leste e das Guianas, foram colonizadas a partir do ancestral inferido. Concordantemente, eventos biogeográficos de colonização de espécies distribuídas na bacia La Plata às drenagens do Atlântico Leste e Sul, interrompidos após o soerguimento da Serra do Mar e Serra da Mantiqueira (30-10 Ma), assim como entre espécies do leste amazônico às drenagens das Guianas e do escudo brasileiro (~10 Ma), são descritos como cruciais para a diversificação de peixes da América do Sul (Cassemiro *et al*., 2023), em especial membros da família Loricariidae. Assim, o ancestral de *Harttia* pode ter se diversificado entre 30 e 23 milhões de anos atrás, quando o a bacia La Plata se desconectou da Amazônia e se ligou aos rios da costa atlântica, em concordância com a data de divergência média sugerida entre *Harttia* e *Farlowella* de 30 Ma (Kumar *et al*., 2017 com dados de Rabosky *et al*., 2013; Betancur-R *et al*., 2015; Cassemiro *et al*., 2023).

5 Considerações finais

A presente tese investigou as relações evolutivas, citogenômicas e biogeográficas de espécies do gênero *Harttia*, que fica evidenciado como um modelo exemplar para estudos evolutivos. Por conta da diversidade de sistemas de cromossomos sexuais, que incluem tanto o sistema simples XX/XY quanto múltiplos (XX/XY_1Y_2 e $X_1X_1X_2X_2/X_1X_2Y$) em ~28% das espécies citogeneticamente investigadas, o gênero é considerado um repositório de cromossomos sexuais múltiplos, sendo um modelo único dentre os peixes Neotropicais.

A pesquisa revelou que *Harttia* possui uma estrutura filogenética complexa, onde nossa reconstrução baseada em múltiplos marcadores nucleares oferece novas perspectivas sobre as relações evolutivas no grupo. Este estudo foi o primeiro a propor uma hipótese filogenética para o gênero usando dados genômicos de larga escala, diferenciando-se dos estudos moleculares anteriores que empregavam dados mitocondriais ou de genes nucleares isolados. Dessa forma, demonstramos a ocorrência de três principais clados que também apresentam congruência com a distribuição geográfica das espécies. O primeiro é composto por espécies distribuídas nas drenagens das Guianas, cujo cariótipo foi aqui investigado pela primeira vez para a literatura científica com a descrição citogenética de *H. guianensis*. Já o segundo engloba as espécies distribuídas nas bacias do escudo Brasileiro que drenam para o rio Amazonas ou o Oceano Atlântico (i.e., bacias Tapajós, Tocantins-Araguaia e Xingú), que teve cinco espécies aqui descritas citogeneticamente pela primeira vez (*H. dissidens*, *H. duriventris*, *H. villasboas*, *H. rondoni* e *Harttia* sp. 3). Por fim, o terceiro clado inclui as espécies encontradas nas bacias do Paraná e São Francisco, além das drenagens do Atlântico Leste e Sul. Nossa modelagem biogeográfica indica que o ancestral se distribuía ou pela bacia Amazônica, ou Paraná, ou Atlântico Sul, enquanto as bacias do Tocantins-Araguaia, São Francisco e Atlântico Leste foram colonizadas secundariamente.

Em metades das espécies caracterizadas na presente tese, observamos cromossomos sexuais diferenciáveis por técnicas citogenéticas, incluindo duas ocorrências do sistema X_1X_2Y, que anteriormente era conhecido apenas para *H. punctata*, e uma do sistema simples XX/XY, inédito para espécies do clado II. As análises de modelagem revelam que, embora esses sistemas variem entre espécies, há indícios de que todos derivam de um ancestral comum com cariótipo de 2n = 58 e com um sistema

simples XX/XY. A presença desse sistema de cromossomos sexuais ancestral é evidenciada pelo compartilhamento de marcadores ligados ao sexo entre espécies do clado I e III, o que também indica que um evento de *turnover* XY-XY ocorreu no clado II. Enquanto para as espécies do clado III, as fusões de cromossomos sexuais com pares autossômicos foram fundamentais para o surgimento de sistemas sexuais múltiplos, apresentamos evidências que fissões cromossômicas contribuíram para a evolução dos cromossomos sexuais nas espécies do clado II. A diferenciação dos cromossomos sexuais ocorreu independentemente em cada espécie, conforme apontado pelas análises de DNA repetitivo conduzidas ao longo da tese, indicando que fatores ambientais e históricos, como o isolamento geográfico, influenciaram as divergências evolutivas e a estruturação genética entre as espécies de *Harttia*.

Em conclusão, o trabalho contribui significativamente para o entendimento da evolução citogenômica e demográfica de *Harttia*, sendo um modelo relevante para estudos de diversificação em ambientes Neotropicais. Esta tese abre espaço para investigações adicionais, como a exploração dos fatores moleculares que regulam a diferenciação dos cromossomos sexuais e a análise das interações entre mudanças cromossômicas, adaptações ecológicas e a origem de novas espécies. Além disso, estudos comparativos com gêneros relacionados, como *Harttiella* e *Cteniloricaria*, além dos demais Loricariidae, e análises em áreas pouco estudadas da bacia Amazônica e Escudo das Guianas, podem enriquecer o conhecimento sobre a história evolutiva dos peixes e permitir uma compreensão mais ampla dos processos biogeográficos que moldam a biodiversidade na América do Sul.

6 Referências

AHMAD, S. F. *et al.* Dark Matter of Primate Genomes: Satellite DNA Repeats and Their Evolutionary Dynamics. **Cells**, v. 9, n. 12, p. 2714, 2020.

AKSENOVA, A. Y.; MIRKIN, S. M. At the Beginning of the End and in the Middle of the Beginning: Structure and Maintenance of Telomeric DNA Repeats and Interstitial Telomeric Sequences. **Genes**, v. 10, n. 2, p. 118, 2019.

ALBERT, J. S.; REIS, R. **Historical biogeography of Neotropical freshwater fishes**.: Univ of California Press, 2011.

ALTMANOVÁ, M. *et al.* Karyotype stasis but species-specific repetitive DNA patterns in *Anguis* lizards (Squamata: Anguidae), in the evolutionary framework of Anguiformes. **Zoological Journal of the Linnean Society**, p. zlad153, 2023.

ALTSCHUL, S. F. *et al.* Basic local alignment search tool. **Journal of Molecular Biology**, v. 215, n. 3, p. 403–410, 1990.

ALTSCHUL, S. F. *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. **Nucleic Acids Research**, v. 25, n. 17, p. 3389–3402, 1997.

ALVES, A. L.; OLIVEIRA, C.; FORESTI, F. Karyotype variability in eight species of the subfamilies Loricariinae and Ancistrinae (Teleostei, Siluriformes, Loricariidae). **Caryologia**, v. 56, n. 1, p. 57–63, 2003.

ANTONELLI, A. *et al.* Amazonia is the primary source of Neotropical biodiversity. **Proceedings of the National Academy of Sciences**, v. 115, n. 23, p. 6034–6039, 2018.

ARAI, R. **Fish karyotypes: a check list**.: Springer Science & Business Media, 2011.

ARKHIPOVA, I. R. Distribution and Phylogeny of Penelope-Like Elements in Eukaryotes. **Systematic Biology**, v. 55, n. 6, p. 875–885, 2006.

ARRATIA, G.; CIONE, A. The record of fossil fishes of southern South America. **Münchner Geowissenschaftliche Abhandlungen**, v. 30, n. A, p. 9-72, 1996.

ARRATIA, G *et al.* **Catfishes**.: Science Publishers, Inc., 2003. v. 1

ARTONI, R. F.; BERTOLLO, L. A. C. Trends in the karyotype evolution of Loricariidae fish (Siluriformes). **Hereditas**, v. 134, n. 3, p. 201–210, 2001.

AVERBECK, K. T.; EICKBUSH, T. H. Monitoring the Mode and Tempo of Concerted Evolution in the *Drosophila melanogaster* rDNA Locus. **Genetics**, v. 171, n. 4, p. 1837–1846, 2005.

AYRES-ALVES, T. *et al.* Karyotypic Evolution and Chromosomal Organization of Repetitive DNA Sequences in Species of *Panaque*, *Panaqolus*, and *Scobinancistrus*

(Siluriformes and Loricariidae) from the Amazon Basin. **Zebrafish**, v. 14, n. 3, p. 251–260, 2017.

BACON, C. D. *et al.* Biological evidence supports an early and complex emergence of the Isthmus of Panama. **Proceedings of the National Academy of Sciences**, v. 112, n. 19, p. 6110–6115, 2015.

BARROS, A. V. *et al.* Fragile sites, dysfunctional telomere and chromosome fusions: What is 5S rDNA role? **Gene**, v. 608, p. 20–27, 2017.

BARROS, F. J. T. *et al.* Weight-Length and Length-Length Relationships of the Endangered Zebra Pleco *Hypancistrus zebra* (*Siluriformes*, *Loricariidae*) from the Xingu River, Amazon, Brazil. **Journal of Applied Ichthyology**, v. 2023, p. e5158180, 2023.

BATESON. The Influence of Heredity on Disease, with Special Reference to Tuberculosis, Cancer and Diseases of the Nervous System. **Proceedings of the Royal Society of Medicine**, v. 2, p. 22–30, 1909.

BAUSEK, N. *et al.* The Major Chicken Egg Envelope Protein ZP1 Is Different from ZPB and Is Synthesized in the Liver. **Journal of Biological Chemistry**, v. 275, n. 37, p. 28866–28872, 2000.

BERRA, T. M. **Freshwater Fish Distribution**.: Academic Press, 2001.

BERTHELOT, C. *et al.* The 3D organization of chromatin explains evolutionary fragile genomic regions. **Cell reports**, v. 10, n. 11, p. 1913–1924, 2015.

BERTOLLO, L. A. C.; CIOFFI, M. B.; MOREIRA-FILHO, O. Direct chromosome preparation from Freshwater Teleost Fishes. *In*: OZOUF-COSTAZ, C. *et al.* (org.). **Fish cytogenetic techniques (Chondrichthyans and Teleosts)**. Enfield USA: CRC Press, 2015. p. 21–26. Disponível em: https://doi.org/10.1201/b18534-4.

BERTOLLO, L. A. C.; TAKAHASHI, C.; MOREIRA-FILHO, O. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). **Brazil J Genet**, v. 1, 1978.

BETANCUR-R., R. *et al.* Phylogenomic incongruence, hypothesis testing, and taxonomic sampling: The monophyly of characiform fishes. **Evolution**, v. 73, n. 2, p. 329–345, 2019.

BIAGIOTTO, R. H. **Aspectos reprodutivos de *Harttia carvalhoi* Miranda-Ribeiro, 1939, um pequeno loricariídeo de riachos do leste da Serra da Mantiqueira, SP, Brasil**. Tese de Doutorado - Universidade Estadual Paulista Júlio de Mesquita Filho, 2012.

BIANCHI, E.; WRIGHT, G. J. Cross-species fertilization: the hamster egg receptor, Juno, binds the human sperm ligand, Izumo1. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 370, n. 1661, p. 20140101, 2015.

BLACKBURN, E. H. Telomeres: No end in sight. **Cell**, v. 77, n. 5, p. 621–623, 1994.

BLANCO, D. R. *et al.* Chromosomal Characterization of Armored Catfish *Harttia longipinna* (Siluriformes, Loricariidae): First Report of B Chromosomes in the Genus. **Zoological Science**, v. 29, n. 9, p. 604–609, 2012.

BLANCO, D. R. **Estudos citogenéticos clássicos e moleculares em espécies do gênero *Harttia* (Siluriformes, Loricariidae), com enfoque no papel dos DNAs repetitivos na evolução cariotípica do grupo**. 2012. 7162 f. Tese de Doutorado - Universidade Federal de São Carlos, 2012.

BLANCO, D. R. *et al.* Karyotype Diversity and Evolutionary Trends in Armored Catfish Species of the Genus *Harttia* (Siluriformes: Loricariidae). **Zebrafish**, v. 14, n. 2, p. 169–176, 2017.

BLANCO, D. R. *et al.* Origin of the $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system of *Harttia punctata* (Siluriformes, Loricariidae) inferred from chromosome painting and FISH with ribosomal DNA markers. **Genetica**, v. 142, n. 2, 2014.

BLANCO, D. R. *et al.* The role of the Robertsonian rearrangements in the origin of the XX/XY_1Y_2 sex chromosome system and in the chromosomal differentiation in *Harttia* species (Siluriformes, Loricariidae). **Reviews in Fish Biology and Fisheries**, v. 23, n. 1, p. 127–134, 2013.

BOLGER, A. M.; LOHSE, M.; USADEL, B. Trimmomatic: a flexible trimmer for Illumina sequence data. **Bioinformatics**, v. 30, n. 15, p. 2114–2120, 2014.

BOUBLI, J. P. *et al.* Spatial and temporal patterns of diversification on the Amazon: A test of the riverine hypothesis for all diurnal primates of Rio Negro and Rio Branco in Brazil. **Molecular Phylogenetics and Evolution**, v. 82, Phylogeny and Biogeography of Neotropical Primates, p. 400–412, 2015.

BREA, M.; ZUCOL, A. F. Four. The Paraná-Paraguay Basin: Geology and Paleoenvironments. *In*: ALBERT, J. S.; REIS, R. (org.). **Historical Biogeography of Neotropical Freshwater Fishes**.: University of California Press, 2011. p. 69–88. Disponível em: https://www.degruyter.com/document/doi/10.1525/9780520948501-006/pdf?licenseType=restricted. Acesso em: 28 out. 2024.

BREDESON, J. V. *et al.* Conserved chromatin and repetitive patterns reveal slow genome evolution in frogs. **Nature Communications**, v. 15, n. 1, p. 579, 2024.

BRIGGS, J. C. The biogeography of otophysan fishes (Ostariophysi: Otophysi): a new appraisal. **Journal of Biogeography**, v. 32, n. 2, p. 287–294, 2005.

BRITO, P. M. *et al.* A Saharan fossil and the dawn of Neotropical armoured catfishes in Gondwana. **Gondwana Research**, v. 132, p. 103–112, 2024.

BRUSCHI, D. P. *et al.* Signature of the Paleo-Course Changes in the São Francisco River as Source of Genetic Structure in Neotropical *Pithecopus nordestinus* (Phyllomedusinae, Anura) Treefrog. **Frontiers in Genetics**, v. 10, 2019. Disponível em:

https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2019.00728/full. Acesso em: 28 out. 2024.

BUCK, S.; SAZIMA, I. An assemblage of mailed catfishes (Loricariidae) in southeastern Brazil: distribution, activity, and feeding. **Ichthyological Exploration of Freshwaters**, v. 6, p. 325–332, 1995.

BULL, J. J. **Evolution of sex determining mechanisms**. Menlo Park, California, USA: The Benjamin/Cummings Publishing Company, Inc., 1983. v. 1 Disponível em: https://www.cabidigitallibrary.org/doi/full/10.5555/19840180646.

BUONGIORNO-NARDELLI, M.; AMALDI, F. Autoradiographic Detection of Molecular Hybrids between rRNA and DNA in Tissue Sections. **Nature**, v. 225, n. 5236, p. 946–948, 1970.

CABRAL-DE-MELLO, D. C. *et al.* The Role of Satellite DNAs in Genome Architecture and Sex Chromosome Evolution in Crambidae Moths. **Frontiers in Genetics**, v. 12, 2021. Disponível em: https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2021.661417/full. Acesso em: 13 ago. 2024.

CALDAS, L.; CHEROBIM, A. M.; LANGEANI, F. A new species of *Harttia* from the rio São Francisco basin (Siluriformes: Loricariidae). **Neotropical Ichthyology**, v. 20, 2022.

CAMACHO, J. P. M. *et al.* Satellitome comparison of two oedipodine grasshoppers highlights the contingent nature of satellite DNA evolution. **BMC Biology**, v. 20, n. 1, p. 36, 2022.

CAO, L. *et al.* Neofunctionalization of zona pellucida proteins enhances freeze-prevention in the eggs of Antarctic notothenioids. **Nature Communications**, v. 7, n. 1, p. 12987, 2016.

CARDINALE, B. J. *et al.* Biodiversity loss and its impact on humanity. **Nature**, v. 486, n. 7401, p. 59–67, 2012.

CARDONE, M. F. *et al.* Evolution of Beta Satellite DNA Sequences: Evidence for Duplication-Mediated Repeat Amplification and Spreading. **Molecular Biology and Evolution**, v. 21, n. 9, p. 1792–1799, 2004.

CARRILLO, J. D. *et al.* Disproportionate extinction of South American mammals drove the asymmetry of the Great American Biotic Interchange. **Proceedings of the National Academy of Sciences**, v. 117, n. 42, p. 26281–26287, 2020.

CASATTI, L.; CASTRO, R. M. C. Testing the ecomorphological hypothesis in a headwater riffles fish assemblage of the rio São Francisco, southeastern Brazil. **Neotropical Ichthyology**, v. 4, p. 203–214, 2006.

CASSEMIRO, F. A. S. *et al.* Landscape dynamics and diversification of the megadiverse South American freshwater fish fauna. **Proceedings of the National Academy of Sciences**, v. 120, n. 2, p. e2211974120, 2023.

CASTRO, L. F. C.; FURLONG, R. F.; HOLLAND, P. W. H. An antecedent of the MHC-linked genomic region in amphioxus. **Immunogenetics**, v. 55, n. 11, p. 782–784, 2004.

CAZAUX, B. *et al.* Are ribosomal DNA clusters rearrangement hotspots? A case study in the genus *Mus* (Rodentia, Muridae). **BMC Evolutionary Biology**, v. 11, n. 1, p. 1–14, 2011.

CENTOFANTE, L.; BERTOLLO, L. A. C.; MOREIRA-FILHO, O. Cytogenetic characterization and description of an XX/XY_1Y_2 sex chromosome system in catfish *Harttia carvalhoi* (Siluriformes, Loricariidae). **Cytogenetic and Genome Research**, n. 112, p. 320–324, 2006.

CHALOPIN, D. *et al.* Transposable elements and early evolution of sex chromosomes in fish. **Chromosome research**, v. 23, n. 3, p. 545–560, 2015.

CHARLESWORTH, B.; CHARLESWORTH, D. The degeneration of Y chromosomes. **Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences**, v. 355, n. 1403, p. 1563–1572, 2000.

CHARLESWORTH, D. The timing of genetic degeneration of sex chromosomes. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 376, n. 1832, p. 20200093, 2021.

CHARLESWORTH, D. Why and how do Y chromosome stop recombining?. **Journal of Evolutionary Biology**, v. 36, n. 3, p. 632–636, 2023.

CHARLESWORTH, D. Young sex chromosomes in plants and animals. **New Phytologist**, v. 224, n. 3, p. 1095–1107, 2019.

CHASE, M. W. *et al.* Phylogenetics of Seed Plants: An Analysis of Nucleotide Sequences from the Plastid Gene rbcL. **Annals of the Missouri Botanical Garden**, v. 80, n. 3, p. 528–580, 1993.

CHENG, Y. *et al.* Whole genome-wide chromosome fusion and new gene birth in the *Monopterus albus* genome. **Cell & Bioscience**, v. 10, n. 1, p. 67, 2020.

CHEROBIM, A. M. Phylogenetic analysis of *Harttia* Steindachner, 1877 (Siluriformes: Loricariidae: Loricariinae). 2021. Tese de Doutorado – Universidade Estadual Paulista Júlio de Mesquita Filho. Disponível em: http://hdl.handle.net/11449/216922. Acesso em: 29 out. 2024.

CIONE, A. L. *et al.* Marine oberjurassische Plattenkalke in Argentinien (ein erster Forschungsbericht). **Archaeopteryx**, v. 5, p. 13-22, 1987.

CIOFFI, M. de B. *et al.* Chromosomal variability among allopatric populations of Erythrinidae fish *Hoplias malabaricus*: Mapping of three classes of repetitive DNAs. **Cytogenetic and Genome Research**, p. 132–141, 2009.

CIOFFI, M. de B.; BERTOLLO, L. A. C. Chromosomal distribution and evolution of repetitive DNAs in fish. *In*: GARRIDO-RAMOS, M. A. (org.). **Repetitive DNA**. Basel: Karger, 2012. p. 197–221.

CLAW, K. G.; SWANSON, W. J. Evolution of the Egg: New Findings and Challenges. **Annual Review of Genomics and Human Genetics**, v. 13, n. Volume 13, 2012, p. 109–125, 2012.

COVAIN, R. *et al*. Les espèces du genre *Harttia* (Siluriformes: Loricariidae) en Guyane française: morphologie, taxinomie et distribution. **Cybium**, v. 30, n. 1, p. 3-18, 2006.

COVAIN, R. *et al.* Assessing phylogenetic dependence of morphological traits using co-inertia prior to investigate character evolution in Loricariinae catfishes. **Molecular Phylogenetics and Evolution**, v. 46, n. 3, p. 986–1002, 2008.

COVAIN, R. *et al.* Molecular phylogeny of the highly diversified catfish subfamily Loricariinae (Siluriformes, Loricariidae) reveals incongruences with morphological classification. **Molecular Phylogenetics and Evolution**, v. 94, p. 492–517, 2016.

COVAIN, R. *et al.* The Harttiini (Siluriformes, Loricariidae) from the Guianas: A multi-table approach to assess their diversity, evolution, and distribution. **Cybium**, v. 36, n. 1, p. 115–161, 2012.

COVAIN, R.; FISCH-MULLER, S. The genera of the Neotropical armored catfish subfamily Loricariinae (Siluriformes: Loricariidae): A practical key and synopsis. **Zootaxa**, v. 40, n. 1462, p. 1–40, 2007.

COYNE, J. A.; ORR, H. A. Patterns of Speciation in *Drosophila*. **Paleobiology**, v. 43, n. 2, p. 362–381, 1989.

CREPALDI, C.; PARISE-MALTEMPI, P. P. Heteromorphic Sex Chromosomes and Their DNA Content in Fish: An Insight through Satellite DNA Accumulation in *Megaleporinus elongatus*. **Cytogenetic and Genome Research**, v. 160, n. 1, p. 38–46, 2020.

DAMAS, J.; CORBO, M.; LEWIN, H. A. Vertebrate Chromosome Evolution. **Annual Review of Animal Biosciences**, v. 9, n. Volume 9, 2021, p. 1–27, 2021.

DARLINGTON, P. J. Area, climate, and evolution. **Evolution**, p. 488-510, 1959.

DARLINGTON, P. J. Interconnected Patterns of Biogeography and Evolution. **Proceedings of the National Academy of Sciences**, v. 68, n. 6, p. 1254–1258, 1971.

DARRIBA, D. *et al.* ModelTest-NG: A New and Scalable Tool for the Selection of DNA and Protein Evolutionary Models. **Molecular Biology and Evolution**, v. 37, n. 1, p. 291–294, 2020.

DE MORAES, R. L. R. *et al.* Chromosomal Rearrangements and Satellite DNAs: Extensive Chromosome Reshuffling and the Evolution of Neo-Sex Chromosomes in the Genus *Pyrrhulina* (Teleostei; Characiformes). **International Journal of Molecular Sciences**, v. 24, n. 17, p. 13654, 2023a.

DE MORAES, R. L. R. *et al.* Small Body, Large Chromosomes: Centric Fusions Shaped the Karyotype of the Amazonian Miniature Fish *Nannostomus anduzei* (Characiformes, Lebiasinidae). **Genes**, v. 14, n. 1, p. 192, 2023b.

DE OLIVEIRA, M. P. B. *et al.* Following the Pathway of W Chromosome Differentiation in *Triportheus* (Teleostei: Characiformes). **Biology**, v. 12, n. 8, p. 1114, 2023.

DE OLIVEIRA, J. C.; OYAKAWA, O. T. New loricariid fishes from headwaters on Serra da Mantiqueira and Complexo do Espinhaço, Minas Gerais state, Brazil (Teleostei: Siluriformes: Loricariidae). **Zootaxa**, v. 4586, n. 3, p. 401–424, 2019.

DE QUEIROZ, K. Species Concepts and Species Delimitation. **Systematic Biology**, v. 56, n. 6, p. 879–886, 2007.

DEAKIN, J. E. *et al.* Chromosomics: Bridging the Gap between Genomes and Chromosomes. **Genes**, v. 10, n. 8, p. 627, 2019.

DEGNAN, J. H.; ROSENBERG, N. A. Gene tree discordance, phylogenetic inference and the multispecies coalescent. **Trends in Ecology and Evolution,** v. 24, n. 6, p. 332-340, 2009.

DEHAL, P.; BOORE, J. L. Two Rounds of Whole Genome Duplication in the Ancestral Vertebrate. **PLOS Biology**, v. 3, n. 10, p. e314, 2005.

DEON, G. A. *et al.* Chromosomal Rearrangements and Origin of the Multiple XX/XY_1Y_2 Sex Chromosome System in *Harttia* Species (Siluriformes: Loricariidae). **Frontiers in Genetics**, v. 13, 2022a. Disponível em: https://www.frontiersin.org/articles/10.3389/fgene.2022.877522.

DEON, G. A. *et al.* Evolutionary breakpoint regions and chromosomal remodeling in *Harttia* (Siluriformes: Loricariidae) species diversification. **Genetics and Molecular Biology**, v. 45, 2022b.

DEON, G. A. *et al.* Highly Rearranged Karyotypes and Multiple Sex Chromosome Systems in Armored Catfishes from the Genus *Harttia* (Teleostei, Siluriformes). **Genes**, v. 11, n. 11, p. 1366, 2020.

DEVLOO-DELVA, F. *et al.* An R-based tool for identifying sex-linked markers from restriction site-associated DNA sequencing with applications to elasmobranch conservation. **Conservation Genetics Resources**, v. 16, n. 1, p. 11–16, 2024.

DÍAZ DE GAMERO, M. L. The changing course of the Orinoco River during the Neogene: a review. **Palaeogeography, Palaeoclimatology, Palaeoecology**, v. 123, n. 1, p. 385–402, 1996.

DIOGO, R.; CHARDON, M.; VANDEWALLE, P. Osteology and myology of the cephalic region and pectoral girdle of *Batrochoglanis raninus*, with a discussion on the synapomorphies and phylogenetic relationships of the Pseudopimelodinae and Pimelodidae (Teleostei: Siluriformes). **Animal Biology**, v. 54, n. 3, p. 261-280, 2004. Disponível em: https://brill.com/view/journals/ab/54/3/article-p261_4.xml. Acesso em: 28 out. 2024.

DOBZHANSKY, Th. On the Sterility of the Interracial Hybrids in *Drosophila pseudoobscura*. **Proceedings of the National Academy of Sciences**, v. 19, n. 4, p. 397–403, 1933.

DOTZER, P.; WEINER, T. *Harttia guianensis*, Die Pflege und die Zucht sind swar schwierig, aber doch möglich. **Das Aquarium**, p. 20–26, 2003.

DOVER, G. A. Molecular drive: A cohesive model of species evolution. **Nature**, v. 199, p. 111–117, 1982.

DUBCOVSKY, J.; DVORÁK, J. Ribosomal RNA multigene loci: nomads of the Triticeae genomes. **Genetics**, v. 140, n. 4, p. 1367–1377, 1995.

DUPIN, J. *et al.* Bayesian estimation of the global biogeographical history of the Solanaceae. **Journal of Biogeography**, v. 44, n. 4, p. 887–899, 2017.

EICKBUSH, T. H.; MALIK, H. S. Origins and Evolution of Retrotransposons. *In*: MOBILE DNA II.: John Wiley & Sons, Ltd, 2007. p. 1111–1144. Disponível em: https://onlinelibrary.wiley.com/doi/abs/10.1128/9781555817954.ch49. Acesso em: 13 ago. 2024.

ENDO, K. S. *et al.* Karyotype description of possible new species of the *Hypostomus ancistroides* complex (Teleostei: Loricariidae) and other Hypostominae. **Acta Scientiarum. Biological Sciences**, v. 34, n. 2, p. 181–189, 2012.

FARIA, R.; NAVARRO, A. Chromosomal speciation revisited: rearranging theory with pieces of evidence. **Trends in Ecology & Evolution**, v. 25, n. 11, p. 660–669, 2010.

FENG, J.-M. *et al.* Evolution and multiple origins of zona pellucida genes in vertebrates. **Biology Open**, v. 7, n. 11, p. bio036137, 2018.

FERRARIS JR, C. J. Subfamily Loricariinae (armored catfishes). **Check List of the Freshwater Fishes of South and Central America, Edipucrs, Porto Alegre, Brazil**, p. 330-350, 2003.

FERRARIS, C. J. **Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types**. 2007-. ISSN 11755326.

FERRETTI, A. B. S. M. *et al.* High dynamism for neo-sex chromosomes: satellite DNAs reveal complex evolution in a grasshopper. **Heredity**, v. 125, n. 3, p. 124–137, 2020.

FINK, S. V.; FINK, W. L. Interrelationships of ostariophysan fishes (Teleostei). *In*: INTERRELATIONSHIPS OF FISHES. 1. ed.: Stiassny MLJ, Parenti LR, Johnson GD, 1996.

FINK, S. V.; FINK, W. L. Interrelationships of the ostariophysan fishes (Teleostei). **Zoological Journal of the Linnean Society**, v. 72, n. 4, p. 297–353, 1981.

FOLL, M.; GAGGIOTTI, O. A Genome-Scan Method to Identify Selected Loci Appropriate for Both Dominant and Codominant Markers: A Bayesian Perspective. **Genetics**, v. 180, n. 2, p. 977–993, 2008.

FRICKE, R.; ESCHMEYER, W. N.; VAN DER LAAN, R. **Catalog of Fishes: Genera, Species, References.** 2023. Disponível em: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. Acesso em: 19 dez. 2023.

FRICKE, R.; ESCHMEYER, W. N.; VAN DER LAAN, R. **Eschmeyer's catalog of fishes: Genera, Species, References**. 2024. Disponível em: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. Acesso em: 24 maio 2024.

GALL, J. G.; PARDUE, M. L. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. **Proceedings of the National Academy of Sciences**, v. 63, n. 2, p. 378–383, 1969.

GALETTI Jr., P. M. *et al.* Heterochromatin and karyotype reorganization in fish of the family Anostomidae (Characiformes). **Cytogenetics and Cell Genetics**, v. 56, n. 2, p. 116-121, 1991.

GARRIDO-RAMOS, M. A. Satellite DNA: An evolving topic. **Genes**, v. 8, n. 9, 2017.

GAYET, M.; MEUNIER, F. J. Maastrichtian to early late Paleocene freshwater Osteichthyes of Bolivia: additions and comments. **Phylogeny and classification of Neotropical fishes**, p. 85-110, 1998.

GLICK, L.; MAYROSE, I. ChromEvol: Assessing the Pattern of Chromosome Number Evolution and the Inference of Polyploidy along a Phylogeny. **Molecular Biology and Evolution**, v. 31, n. 7, p. 1914–1922, 2014.

GLUGOSKI, L. *et al.* Co-located hAT transposable element and 5S rDNA in an interstitial telomeric sequence suggest the formation of Robertsonian fusion in armored catfish. **Gene**, v. 650, p. 49–54, 2018.

GLUGOSKI, L. *et al.* Robertsonian Fusion Site in Rineloricaria pentamaculata (Siluriformes: Loricariidae): Involvement of 5S Ribosomal DNA and Satellite Sequences. **Cytogenetic and Genome Research**, v. 162, n. 11–12, p. 657–664, 2023.

GODINHO, H.; DOS SANTOS, J.; SATO, Y. Ontogênese larval de cinco espécies de peixes do São Francisco. **Águas, peixes e pescadores do São Francisco das Minas Gerais**, , p. 133–148,

GOES, C. A. G. *et al.* Revealing the Satellite DNA History in *Psalidodon* and *Astyanax* Characid Fish by Comparative Satellitomics. **Frontiers in Genetics**, v. 13, 2022. Disponível em: https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2022.884072/full. Acesso em: 13 ago. 2024.

GOSLINE, W. A. The problem of the derivation of the South American and African fresh-water fish faunas. **Anais da Academia Brasileira de Ciências**, v. 16, p. 211-223, 1944.

GOUVEIA, J. G. *et al.* Repetitive DNA in the Catfish Genome: rDNA, Microsatellites, and Tc1-Mariner Transposon Sequences in *Imparfinis* Species (Siluriformes, Heptapteridae). **Journal of Heredity**, v. 108, n. 6, p. 650–657, 2017.

GRAVES, J. Sex Chromosome Specialization and Degeneration in Mammals. **Cell**, v. 124, p. 901–914, 2006.

GRUBER, B. *et al.* dartR: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. **Molecular Ecology Resources**, v. 18, n. 3, p. 691–699, 2018.

GOLOBOFF, P. A. *et al.* Phylogenetic analysis of 73 060 taxa corroborates major eukaryotic groups. **Cladistics**, v. 25, p. 211-230, 2009. Disponível em: https://onlinelibrary.wiley.com/doi/10.1111/j.1096-0031.2009.00255.x.

GUTIÉRREZ, J. *et al.* Satellitome Analysis on *Talpa aquitania* Genome and Inferences about the satDNAs Evolution on Some Talpidae. **Genes**, v. 14, n. 1, p. 117, 2023.

HILL, P. L. *et al.* Conservation of Sex-Linked Markers among Conspecific Populations of a Viviparous Skink, *Niveoscincus ocellatus*, Exhibiting Genetic and Temperature-Dependent Sex Determination. **Genome Biology and Evolution**, v. 10, n. 4, p. 1079–1087, 2018.

HOORN, C. *et al.* Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. **Science**, v. 330, n. 6006, p. 927–931, 2010.

HUBERT, N.; RENNO, J.-F. Historical biogeography of South American freshwater fishes. **Journal of Biogeography**, v. 33, n. 8, p. 1414–1436, 2006.

HUGHES, L. C. *et al*. Comprehensive phylogeny of ray-finned fishes (Actinopterygii) based on transcriptomic and genomic data. **Proceedings of the National Academy of Sciences**, v. 115, n. 24, p. 6249-6254, 2018.

JEFFRIES, D. L. *et al.* A rapid rate of sex-chromosome turnover and non-random transitions in true frogs. **Nature Communications**, v. 9, n. 1, p. 4088, 2018.

JOHN, H. A.; BIRNSTIEL, M. L.; JONES, K. W. RNA-DNA hybrids at the cytological level. **Nature**, v. 223, p. 582-587, 1969.

KALLIONIEMI, A. *et al.* Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. **Science**, v. 258, n. 5083, p. 818–821, 1992.

KAVALCO, K. F. *et al.* Heterochromatin characterization of four fish species of the family Loricariidae (Siluriformes). **Hereditas**, v. 141, p. 237–242, 2004.

KAVALCO, K. F. *et al.* Karyotypic diversity and evolution of Loricariidae (Pisces, Siluriformes). **Heredity**, v. 94, n. 2, p. 180–186, 2005.

KILIAN, A. *et al.* Diversity Arrays Technology: A Generic Genome Profiling Technology on Open Platforms. *In*: POMPANON, F.; BONIN, A. (org.). **Data Production and Analysis in Population Genomics: Methods and Protocols**. Totowa, NJ: Humana Press, 2012. p. 67–89. Disponível em: https://doi.org/10.1007/978-1-61779-870-2_5. Acesso em: 28 out. 2024.

KIMURA, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. **Journal of Molecular Evolution**, v. 16, n. 2, p. 111–120, 1980.

KITANO, J.; PEICHEL, C. L. Turnover of sex chromosomes and speciation in fishes. **Environmental biology of fishes**, v. 94, n. 3, p. 549–558, 2012.

KOPUCHIAN, C. *et al.* A test of the riverine barrier hypothesis in the largest subtropical river basin in the Neotropics. **Molecular Ecology**, v. 29, n. 12, p. 2137–2149, 2020.

KOUBOVÁ, M. *et al.* Sex determination in Madagascar geckos of the genus *Paroedura* (Squamata: Gekkonidae): are differentiated sex chromosomes indeed so evolutionary stable? **Chromosome Research**, v. 22, n. 4, p. 441–452, 2014.

KOZLOV, A. M. *et al.* RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. **Bioinformatics**, v. 35, n. 21, p. 4453–4455, 2019.

KRATOCHVÍL, L. *et al.* Expanding the classical paradigm: what we have learnt from vertebrates about sex chromosome evolution. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 376, n. 1833, p. 20200097, 2021.

KUBAT, Z. *et al.* Microsatellite accumulation in the Y chromosome of *Silene latifolia*. **Genome**, v. 51, p. 350–356, 2008.

KUHL, H. *et al.* A candidate sex determination locus in amphibians which evolved by structural variation between X- and Y-chromosomes. **Nature Communications**, v. 15, n. 1, p. 4781, 2024.

KUHN, G. C. S. Satellite DNA transcripts have diverse biological roles in *Drosophila*. **Heredity**, v. 115, n. 1, p. 1–2, 2015.

KUMAR, S.; STECHER, G.; TAMURA, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. **Molecular biology and evolution**, v. 33, n. 7, 2016.

KUMAR, S. *et al.* TimeTree: A Resource for Timelines, Timetrees, and Divergence Times. **Molecular Biology and Evolution**, v. 34, n. 7, p. 1812–1819, 2017.

KUMAR, S. *et al.* TimeTree 5: An Expanded Resource for Species Divergence Times. **Molecular Biology and Evolution**, v. 39, n. 8, p. msac174, 2022.

KUSHWAHA, B. *et al.* Genome size estimation and its associations with body length, chromosome number and evolution in teleost fishes. **Gene**, v. 864, p. 147294, 2023.

LAMBERT, M. R.; SKELLY, D. K.; EZAZ, T. Sex-linked markers in the North American green frog (*Rana clamitans*) developed using DArTseq provide early insight into sex chromosome evolution. **BMC Genomics**, v. 17, n. 1, p. 844, 2016.

LANDIS, M. J. *et al.* Bayesian Analysis of Biogeography when the Number of Areas is Large. **Systematic Biology**, v. 62, n. 6, p. 789–804, 2013.

LANGEANI, F.; OYAKAWA, O. T.; MONTOYA-BURGOS, J. I. New Species of *Harttia* (Loricariidae, Loricariinae) from the Rio São Francisco Basin. **Copeia**, v. 2001, n. 1, p. 136–142, 2001.

LEACHÉ, A. D.; OAKS, J. R. The Utility of Single Nucleotide Polymorphism (SNP) Data in Phylogenetics. **Annual Review of Ecology, Evolution, and Systematics**, v. 48, n. Volume 48, 2017, p. 69–84, 2017.

LEHNER, B.; VERDIN, K.; JARVIS, A. New Global Hydrography Derived From Spaceborne Elevation Data. **Eos, Transactions American Geophysical Union**, v. 89, n. 10, p. 93–94, 2008.

LEMMON, E. M.; LEMMON, A. R. High-Throughput Genomic Data in Systematics and Phylogenetics. **Annual Review of Ecology, Evolution, and Systematics**, v. 44, n. Volume 44, 2013, p. 99–121, 2013.

LUNDBERG, J. G. African-South American freshwater fish clades and continental drift: problems with a paradigm. **Biological relationships between Africa and South America**, p. 156-199, 1993.

PUKK, L. *et al.* Less is more: extreme genome complexity reduction with ddrad using ion torrent semiconductor technology. **Molecular Ecology Resources**, v. 15, p. 1145-1152, 2015.

LÉVÊQUE, C. *et al.* Global diversity of fish (Pisces) in freshwater. **Hydrobiologia**, v. 595, n. 1, p. 545–567, 2008.

LIEHR, T. Chapter 1 - A definition for cytogenomics - Which also may be called chromosomics. *In*: LIEHR, T. (org.). **Cytogenomics**.: Academic Press, 2021. p. 1–7. Disponível em: https://www.sciencedirect.com/science/article/pii/B9780128235799000011. Acesso em: 28 out. 2024.

LISACHOV, A. *et al.* Conservation of Major Satellite DNAs in Snake Heterochromatin. **Animals**, v. 13, n. 3, p. 334, 2023.

LIU, J.; ALI, M.; ZHOU, Q. Establishment and evolution of heterochromatin. **Annals of the New York Academy of Sciences**, v. 1476, n. 1, p. 59–77, 2020.

LIU, F.-G. R.; MOLER, P. E.; MIYAMOTO, M. M. Phylogeography of the salamander genus *Pseudobranchus* in the southeastern United States. **Molecular Phylogenetics and Evolution**, v. 39, n. 1, p. 149–159, 2006.

LONDOÑO-BURBANO, A.; REIS, R. E. A combined molecular and morphological phylogeny of the Loricariinae (Siluriformes: Loricariidae), with emphasis on the Harttiini and Farlowellini. **PloS one**, v. 16, n. 3, p. e0247747, 2021.

LUI, R. L. *et al.* Propidium iodide for making heterochromatin more evident in the C-banding technique. **Biotechnic & Histochemistry**, v. 87, n. 7, p. 433–438, 2012.

LUJAN, N. K. *et al.* Multilocus molecular phylogeny of the ornamental wood-eating catfishes (Siluriformes, Loricariidae, *Panaqolus* and *Panaque*) reveals undescribed diversity and parapatric clades. **Molecular Phylogenetics and Evolution**, v. 109, p. 321–336, 2017.

LUJAN, N. K. *et al.* Multilocus molecular phylogeny of the suckermouth armored catfishes (Siluriformes: Loricariidae) with a focus on subfamily Hypostominae. **Molecular Phylogenetics and Evolution**, v. 82, p. 269–288, 2015.

LUJAN, N. K.; HIDALGO, M.; STEWART, D. J. Revision of *Panaque*, with Descriptions of Three New Species from the Amazon Basin (Siluriformes, Loricariidae). **Copeia**, v. 2010, n. 4, p. 676–704, 2010.

LUKHTANOV, V. A. *et al.* Incomplete Sterility of Chromosomal Hybrids: Implications for Karyotype Evolution and Homoploid Hybrid Speciation. **Frontiers in Genetics**, v. 11, 2020. Disponível em: https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2020.583827/full. Acesso em: 28 out. 2024.

LUNDBERG, J. G. *et al.* The stage for Neotropical fish diversification: a history of tropical South American rivers. *In*: MALABARBA, L. R. *et al.* (org.). **Phylogeny and classification of Neotropical fishes**. Porto Alegre: Edipucrs Porto Alegre, 1998. v. 27, p. 13–48.

MA, J.; JACKSON, S. A. Retrotransposon accumulation and satellite amplification mediated by segmental duplication facilitate centromere expansion in rice. **Genome Research**, v. 16, n. 2, p. 251–259, 2006.

MARAJÓ, L. *et al.* Chromosomal rearrangements and the first indication of an ♀$X_1X_1X_2X_2$/♂X_1X_2Y sex chromosome system in *Rineloricaria* fishes (Teleostei: Siluriformes). **Journal of Fish Biology**, v. 102, n. 2, p. 443–454, 2023.

MARGARIDO, V. P.; GALETTI JUNIOR, P. M. Amplification of a GC-rich heterochromatin in the freshwater fish *Leporinus desmotes* (Characiformes, Anostomidae). **Genetics and Molecular Biology**, v. 23, p. 569–573, 2000.

MARIOTTO, S. *et al.* Chromosome polymorphism in *Ancistrus cuiabae* Knaack, 1999 (Siluriformes: Loricariidae: Ancistrini). **Neotropical Ichthyology**, v. 7, n. 4, p. 595–600, 2009.

MATZKE, N. J. Founder-event speciation in BioGeoBEARS package dramatically improves likelihoods and alters parameter inference in Dispersal-Extinction-Cladogenesis (DEC) analyses. **Frontiers of Biogeography**, v. 4, n. suppl 1, p. 210, 2012.

MATZKE, N. J. Stochastic mapping under biogeographical models. **PhyloWiki BioGeoBEARS website**, 2016.

MATZKE, N. J.; WRIGHT, A. Inferring node dates from tip dates in fossil Canidae: the importance of tree priors. **Biology Letters**, v. 12, n. 8, p. 20160328, 2016.

MAYROSE, I.; BARKER, M. S.; OTTO, S. P. Probabilistic Models of Chromosome Number Evolution and the Inference of Polyploidy. **Systematic Biology**, v. 59, n. 2, p. 132–144, 2010.

MAZZOLENI, S. *et al.* Sex is determined by XX/XY sex chromosomes in Australasian side-necked turtles (Testudines: Chelidae). **Scientific Reports**, v. 10, n. 1, p. 4276, 2020.

MCTAGGART, S. J. *et al.* Rates of Recombination in the Ribosomal DNA of Apomictically Propagated *Daphnia obtusa* Lines. **Genetics**, v. 175, n. 1, p. 311–320, 2007.

MEYNE, J.; RATLIFF, R. L.; MOYZIS, R. K. Conservation of the human telomere sequence (TTAGGG)n among vertebrates. **Proceedings of the National Academy of Sciences of the United States of America**, v. 86, 1989. Disponível em: https://doi.org/10.1073/pnas.86.18.7049.

MIJANGOS, J. L. *et al.* dartR v2: an accessible genetic analysis platform for conservation, ecology and agriculture - Methods in Ecology and Evolution - Wiley online library., v. 13, p. 2150-2158, 2022. Disponível em: https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/2041-210X.13918. Acesso em: 29 out. 2024.

MIRARAB, S.; BAYZID, M. S.; WARNOW, T. Evaluating Summary Methods for Multilocus Species Tree Estimation in the Presence of Incomplete Lineage Sorting. **Systematic Biology**, v. 65, n. 3, p. 366–380, 2016.

MONTOYA-BURGOS, J. *et al.* **Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences**. p. 363-374. In Malabarba, L. R. L. *et al.* Phylogeny and classification of neotropical fishes. Porto Alegre: EDIPUCRS, 1998.

MORGAN, T. H. Localization of the Hereditary Material in the Germ Cells. **Proceedings of the National Academy of Sciences**, v. 1, n. 7, p. 420–429, 1915.

MOTTA-NETO, C. C. da *et al.* Overview on Karyotype Stasis in Atlantic Grunts (Eupercaria, Haemulidae) and the Evolutionary Extensions for Other Marine Fish Groups. **Frontiers in Marine Science**, v. 6, 2019. Disponível em: https://www.frontiersin.org/journals/marine-science/articles/10.3389/fmars.2019.00628/full. Acesso em: 28 out. 2024.

MÜLLER, H. J. A Factor for the Fourth Chromosome of *Drosophila*. **Science**, v. 39, n. 1016, p. 906–906, 1914.

MÜLLER, H. J. Isolating mechanisms, evolution, and temperature. **Biology Symposium**, v. 6, p. 71-125, 1942. Disponível em: https://cir.nii.ac.jp/crid/1370290617850391810. Acesso em: 28 out. 2024.

MUNOZ-LOPEZ, M.; GARCIA-PEREZ, J. L. DNA Transposons: Nature and Applications in Genomics. **Current Genomics**, v. 11, n. 2, p. 115–128, 2010.

MURAMOTO, J.; OHNO, S.; ATKIN, N. B. On the diploid state of the fish order Ostariophysi. **Chromosoma**, v. 24, n. 1, p. 59–66, 1968.

MYERS, G. S. **Fresh Water Fishes and West Indian Zoogeography**.: U.S. Government Printing Office, 1938.

MYERS, N. Threatened biotas: “Hot spots” in tropical forests. **Environmentalist**, v. 8, n. 3, p. 187–208, 1988.

NEAR, T. J.; THACKER, C. E. Phylogenetic Classification of Living and Fossil Ray-Finned Fishes (Actinopterygii). **Bulletin of the Peabody Museum of Natural History**, v. 65, n. 1, p. 3–302, 2024.

NELSON, J. S.; GRANDE, T. C.; WILSONI, M. V. H. **Fishes of the World**.: John Wiley & Sons, 2016.

NGUYEN, D. H. M. *et al.* Genome-Wide SNP Analysis of Hybrid Clariid Fish Reflects the Existence of Polygenic Sex-Determination in the Lineage. **Frontiers in Genetics**, v. 13, 2022. Disponível em: https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2022.789573/full. Acesso em: 28 out. 2024.

NOVÁK, P. *et al.* TAREAN: a computational tool for identification and characterization of satellite DNA from unassembled short reads. **Nucleic Acids Research**, v. 45, n. 12, p. e111, 2017.

OLAVE, M. *et al.* Early stages of sympatric homoploid hybrid speciation in crater lake cichlid fishes. **Nature Communications**, v. 13, n. 1, p. 5893, 2022.

OLITO, C. *et al.* Consequences of partially recessive deleterious genetic variation for the evolution of inversions suppressing recombination between sex chromosomes. **Evolution**, v. 78, n. 8, p. 1499–1510, 2024.

OLIVEIRA, E. A. *et al.* Historical demography and climate driven distributional changes in a widespread Neotropical freshwater species with high economic importance. **Ecography**, v. 43, n. 9, p. 1291–1304, 2020.

O'MEARA, B. C. Evolutionary Inferences from Phylogenies: A Review of Methods. **Annual Review of Ecology, Evolution, and Systematics**, v. 43, n. Volume 43, 2012, p. 267–285, 2012.

ONU. **Report of the United Nations Conference on Environment and Development**, Rio de Janeiro, 3-14 June 1992. Volume 2, Proceedings of the Conference. *In*: UN CONFERENCE ON ENVIRONMENT AND DEVELOPMENT (1992 : RIO DE JANEIRO, BRAZIL), 1992. **Anais**: UN, 1992. Disponível em: https://digitallibrary.un.org/record/168679. Acesso em: 28 out. 2024.

OTA, R. R. *et al.* Neotropical Siluriformes as a Model for Insights on Determining Biodiversity of Animal Groups. **PLoS ONE**, v. 10, n. 7, p. e0132913, 2015.

OYAKAWA, O. T.; FICHBERG, I.; PY-DANIEL, L. R. Three new species of *Harttia* (Loricariidae: Loricariinae) from Serra do Cachimbo, Rio Xingu basin, Pará, Northern Brazil. **Zootaxa**, v. 4387, n. 1, p. 75–90, 2018.

PARDUE, M. L.; GALL, J. G. Chromosomal Localization of Mouse Satellite DNA. **Science**, v. 168, n. 3937, p. 1356–1358, 1970.

PENNELL, M. W. *et al.* Y Fuse? Sex Chromosome Fusions in Fishes and Reptiles. **PLoS Genetics**, v. 11, n. 5, p. 1–17, 2015.

PEREIRA, C. S. A.; RÁB, P.; COLLARES-PEREIRA, M. J. Chromosomes of Iberian Leuciscinae (Cyprinidae) Revisited: Evidence of Genome Restructuring in Homoploid Hybrids Using Dual-Color FISH and CGH. **Cytogenetic and Genome Research**, v. 141, n. 2–3, p. 143–152, 2013.

PEREZ, M. F. *et al.* Integrating Genomic and Cytogenetic Data to Study the Evolutionary History of Arapaimas and Arowanas in the Neotropics. *In*: GALETTI JR., P. M. (org.). **Conservation Genetics in the Neotropics**. Cham: Springer International Publishing, 2023. p. 523–537. Disponível em: https://doi.org/10.1007/978-3-031-34854-9_21. Acesso em: 28 out. 2024.

PETERSON, B. K. *et al*. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. **PloS one**, v. 7, n. 5, p. e37135, 2012.

PHILIPPE, H. *et al.* Resolving Difficult Phylogenetic Questions: Why More Sequences Are Not Enough. **PLOS Biology**, v. 9, n. 3, p. e1000602, 2011.

PINKEL, D. *et al.* Fluorescence in situ hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. **Proceedings of the National Academy of Sciences**, v. 85, n. 23, p. 9138–9142, 1988.

PINKEL, D.; STRAUME, T.; GRAY, J. W. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. **Proceedings of the National Academy of Sciences**, v. 83, n. 9, p. 2934–2938, 1986.

PLOHL, M.; MEŠTROVIĆ, N.; MRAVINAC, B. Centromere identity from the DNA point of view. **Chromosoma**, v. 123, n. 4, 2014.

POIGNET, M. *et al.* Comparison of Karyotypes in Two Hybridizing Passerine Species: Conserved Chromosomal Structure but Divergence in Centromeric Repeats. **Frontiers in Genetics**, v. 12, 2021. Disponível em: https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2021.768987/full. Acesso em: 28 out. 2024.

PRIMO, C. C. *et al.* Mechanisms of Chromosomal Diversification in Species of *Rineloricaria* (Actinopterygii: Siluriformes: Loricariidae). **Zebrafish**, v. 14, n. 2, p. 161–168, 2017.

PROVENZANO R., F. **Estudio sobre las relaciones filogenéticas de las especies incluidas en la subfamilia Loricariinae (Siluriformes, Loricariidae)**. 2012. Doctoral Thesis, 2012. Disponível em: http://saber.ucv.ve/handle/10872/1552. Acesso em: 29 out. 2024.

PROVENZANO R., F. *et al. Harttia merevari*, a new species of catfish (Siluriformes: Loricariidae) from Venezuela. **Neotropical Ichthyology**, v. 3, n. 4, p. 519–524, 2005.

PUKK, L. *et al.* Less is more: extreme genome complexity reduction with dd RAD using Ion Torrent semiconductor technology. **Molecular Ecology Resources**, v. 15, n. 5, p. 1145-1152, 2015.

PY-DANIEL, L. R. **Phylogeny of the Neotropical armored catfishes of the subfamily Loricariinae (Siluriformes: Loricariidae).** PhD. Thesis - The University of Arizona, 1997.

PYRON, R. A. *et al.* Effectiveness of phylogenomic data and coalescent species-tree methods for resolving difficult nodes in the phylogeny of advanced snakes (Serpentes: Caenophidia). **Molecular Phylogenetics and Evolution**, v. 81, p. 221–231, 2014.

RABOSKY, D. L. *et al.* Analysis and Visualization of Complex Macroevolutionary Dynamics: An Example from Australian Scincid Lizards. **Systematic Biology**, v. 63, n. 4, p. 610–627, 2014.

RABOSKY, D. L. *et al.* Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. **Nature Communications**, v. 4, n. 1, p. 1958, 2013.

RAMBAUT, A. *et al.* Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. **Systematic Biology**, v. 67, n. 5, p. 901–904, 2018.

RANNALA, B.; YANG, Z. Phylogenetic Inference Using Whole Genomes. **Annual Review of Genomics and Human Genetics**, v. 9, n. Volume 9, 2008, p. 217–231, 2008.

RAPP PY-DANIEL, L. H.; OLIVEIRA, E. C. Seven new species of *Harttia* from the Amazonian-Guyana region (Siluriformes: Loricariidae). **Ichthyological Exploration of Freshwaters**, v. 12, n. 1, p. 79–96, 2001.

REE, R. H.; SANMARTÍN, I. Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. **Journal of Biogeography**, v. 45, n. 4, p. 741–749, 2018.

REE, R. H.; SMITH, S. A. Maximum Likelihood Inference of Geographic Range Evolution by Dispersal, Local Extinction, and Cladogenesis. **Systematic Biology**, v. 57, n. 1, p. 4–14, 2008.

REIS, R. E. *et al.* Fish biodiversity and conservation in South America. **Journal of fish biology**, v. 89, n. 1, p. 12–47, 2016.

REIS, R. E.; KULLANDER, S.; FERRARIS, C. J. **Check list of the freshwater fishes of South and Central America**.: Edipucrs, 2003.

REGAN, C. The distribution of the Fishes of the order Ostariophysi. **Bijdragen tot de dierkunde**, v. 22, n. 1, p. 203–208, 1922.

RIBEIRO, A. C.; LIMA, F. C. T.; PEREIRA, E. H. L. A New Genus and Species of a Minute Suckermouth Armored Catfish (Siluriformes: Loricariidae) from the Rio Tocantins Drainage, Central Brazil: The Smallest Known Loricariid Catfish. **Copeia**, v. 2012, n. 4, p. 637–647, 2012.

RIED, T. *et al.* Chromosome Painting: A Useful Art. **Human Molecular Genetics**, v. 7, n. 10, p. 1619–1626, 1998.

ROCHA-REIS, D. A. *et al.* In Silico characterization of satellitomes and cross-amplification of putative satDNAs in two species of the *Hypostomus ancistroides* complex (Siluriformes, Loricariidae). **Cytogenetic and Genome Research**, p. 1, 2024.

RODRÍGUEZ-EZPELETA, N. *et al*. Detecting and overcoming systematic errors in genome-scale phylogenies. **Systematic Biology**, v. 56, n. 3, p. 389-399, 2007.

RODRIGUES, R. M. **Estudos cromossômicos e moleculares em Loricariinae com ênfase em espécies de Rineloricaria (Siluriformes, Loricariidae): uma perspectiva evolutiva**. Tese de Doutorado - Universidade de São Paulo, 2010.

RONQUIST, F. Dispersal-Vicariance Analysis: A New Approach to the Quantification of Historical Biogeography. **Systematic Biology**, v. 46, n. 1, p. 195–203, 1997.

ROSA, K. O. *et al.* Numeric and structural chromosome polymorphism in *Rineloricaria lima* (Siluriformes: Loricariidae): fusion points carrying 5S rDNA or telomere sequence vestiges. **Reviews in Fish Biology and Fisheries**, v. 22, n. 3, p. 739–749, 2012.

ROXO, F. F. *et al.* Phylogenomic reappraisal of the Neotropical catfish family Loricariidae (Teleostei: Siluriformes) using ultraconserved elements. **Molecular Phylogenetics and Evolution**, v. 135, p. 148–165, 2019.

ROY, V. *et al.* Evolution of the chromosomal location of rDNA genes in two *Drosophila* species subgroups: *ananassae* and *melanogaster*. **Heredity**, v. 94, n. 4, p. 388–395, 2005.

RULL, V. Neotropical diversification: historical overview and conceptual insights. 2018. Disponível em: https://doi.org/10.7287/peerj.preprints.27294v1.

RULL, V.; CARNAVAL, A. C. (org.). **Neotropical Diversification: Patterns and Processes**. Cham: Springer International Publishing, 2020. (Fascinating Life Sciences). Disponível em: https://link.springer.com/10.1007/978-3-030-31167-4. Acesso em: 28 out. 2024.

SACERDOT, C. *et al.* Chromosome evolution at the origin of the ancestral vertebrate genome. **Genome Biology**, v. 19, n. 1, p. 166, 2018.

SAITOH, K. *et al.* Mitochondrial Genomics of Ostariophysan Fishes: Perspectives on Phylogeny and Biogeography. **Journal of Molecular Evolution**, v. 56, n. 4, p. 464–472, 2003.

SAMBROOK, J.; RUSSELL, D. W. **Molecular Cloning, A Laboratory Manual**. New York: Cold Spring Harbor Laboratory Press, 2001.

SASSI, F. M. C. *et al.* Adding New Pieces to the Puzzle of Karyotype Evolution in *Harttia* (Siluriformes, Loricariidae): Investigation of Amazonian Species. **Biology**, v. 10, n. 9, p. 922, 2021.

SASSI, F. M. C. *et al.* An insight into the chromosomal evolution of Lebiasinidae (Teleostei, Characiformes). **Genes**, v. 11, n. 4, p. 365, 2020.

SASSI, F. M. C. *et al.* Chromosomal Evolution and Evolutionary Relationships of *Lebiasina* Species (Characiformes, Lebiasinidae). **International Journal of Molecular Sciences**, v. 20, n. 2944, p. 1–17, 2019.

SASSI, F. M. C. *et al.* Evolutionary cytogenomics and demographic history in *Harttia* (Siluriformes, Loricariidae). *In prep*.

SASSI, F. M. C. *et al.* Homeology of sex chromosomes in Amazonian *Harttia* armored catfishes supports the X-fission hypothesis for the X_1X_2Y sex chromosome system origin. **Scientific Reports**, v. 13, p. 15756, 2023.

SASSI, F. M. C. *et al.* Independent Evolution of satellite DNA sequences in homologous sex chromosomes. **Communications Biology** (Em Revisão).

SASSI, F. M. C. *et al.* Multiple sex chromosomes and evolutionary relationships in amazonian catfishes: The outstanding model of the genus *Harttia* (siluriformes: Loricariidae). **Genes**, v. 11, n. 10, 2020.

SASSI, F. M. C. *et al.* Turnover of multiple sex chromosomes in *Harttia* catfish (Siluriformes, Loricariidae): a glimpse from whole chromosome painting. **Frontiers 3in Genetics**, v. 14, p. 1226222, 2023.

SASSI, F. M. C.; TOMA, G. A.; CIOFFI, M. B. FISH—in Fish Chromosomes. *In*: **Cytogenetics and Molecular Cytogenetics**. 1. ed.: Thomas Liehr, 2023. p. 281–293. Disponível em: https://www.taylorfrancis.com/chapters/edit/10.1201/9781003223658-24/fish%E2%80%94in-fish-chromosomes-francisco-de-sassi-gustavo-toma-marcelo-de-bello-cioffi.

SASSI, F. M. C.; CIOFFI, M. B.; MOREIRA-FILHO, O. A state-of-art review of Loricariidae (Ostariophysi: Siluriformes) cytogenetics. **Neotropical Ichthyology**, 2024.

SATO, Y.; NISHIDA, M. Teleost fish with specific genome duplication as unique models of vertebrate evolution. **Environmental Biology of Fishes**, v. 88, n. 2, p. 169–188, 2010.

SATO, Y.; GODINHO, H. P. **Migratory fishes of the São Francisco River**. In: Carolsfiled J, Harvey B, Ross C and Baer A (eds) Migratory fishes of South America. World Fisheries Trust, IDRC, World Bank, New York, USA, pp 199–232, 2003.

SATOVIĆ, E. *et al.* Adjacent sequences disclose potential for intra-genomic dispersal of satellite DNA repeats and suggest a complex network with transposable elements. **BMC Genomics**, v. 17, n. 1, p. 997, 2016.

SCHAEFFER, B. Rates of Evolution in the Coelacanth and Dipnoan Fishes. **Evolution**, v. 6, n. 1, p. 101–111, 1952.

SCHEMBERGER, M. O. *et al.* DNA transposon invasion and microsatellite accumulation guide W chromosome differentiation in a Neotropical fish genome. **Chromosoma**, v. 128, n. 4, p. 547–560, 2019.

SCHIMEK, C. *et al.* Sex-linked markers in an Australian frog *Platyplectrum ornatum* (Limnodynastidae) with a small genome and homomorphic sex chromosomes. **Scientific Reports**, v. 12, n. 1, p. 20934, 2022.

SCHMIEDER, R.; EDWARDS, R. Fast Identification and Removal of Sequence Contamination from Genomic and Metagenomic Datasets. **PLOS ONE**, v. 6, n. 3, p. e17288, 2011.

SCHÖFER, C.; WEIPOLTSHAMMER, K. Nucleolus and chromatin. **Histochemistry and cell biology**, v. 150, p. 209-225, 2018.

SCHUBERT, I.; WOBUS, U. In situ hybridization confirms jumping nucleolus organizing regions in *Allium*. **Chromosoma**, v. 92, n. 2, p. 143–148, 1985.

SCHUBERT, I. *et al.* Telomeric signals in Robertsonian fusion and fission chromosomes: implications for the origin of pseudoaneuploidy. **Cytogenetic and Genome Research**, v. 59, n. 1, p. 6-9, 1992.

SEMBER, A. *et al.* Centric Fusions behind the Karyotype Evolution of Neotropical *Nannostomus* Pencilfishes (Characiforme, Lebiasinidae): First Insights from a Molecular Cytogenetic Perspective. **Genes**, v. 11, n. 1, 2020.

SEMBER, A. *et al.* Multiple sex chromosomes in teleost fishes from a cytogenetic perspective: state of the art and future challenges. **Philosophical Transactions of the Royal Society B**, v. 376, n. 1833, p. 20200098, 2021.

SEMBER, A. *et al.* Sex Chromosome Evolution and Genomic Divergence in the Fish *Hoplias malabaricus* (Characiformes, Erythrinidae). **Frontiers in Genetics**, v. 9, n. March, p. 1–12, 2018.

SERRANO-FREITAS, É. A. *et al.* Satellite DNA content of B chromosomes in the characid fish *Characidium gomesi* supports their origin from sex chromosomes. **Molecular Genetics and Genomics**, v. 295, n. 1, p. 195–207, 2020.

SHETTY, S.; GRIFFIN, D. K.; GRAVES, J. A. M. Comparative Painting Reveals Strong Chromosome Homology Over 80 Million Years of Bird Evolution. **Chromosome Research**, v. 7, n. 4, p. 289–295, 1999.

SIMAKOV, O. *et al.* Deeply conserved synteny resolves early events in vertebrate evolution. **Nature Ecology & Evolution**, v. 4, n. 6, p. 820–830, 2020.

SIMMONS, M. P.; GATESY, J. Collapsing dubiously resolved gene-tree branches in phylogenomic coalescent analyses. **Molecular Phylogenetics and Evolution**, v. 158, p. 107092, 2021.

SINGH, U.; WURTELE, E. S. orfipy: a fast and flexible tool for extracting ORFs. **Bioinformatics**, v. 37, n. 18, p. 3019–3020, 2021.

SMIT, A.; HUBLEY, R.; GREEN, P. **RepeatMasker Open**. Versão 4.0., 2013. Disponível em: http://www.repeatmasker.org.

SOCHOROVÁ, J. *et al.* Evolutionary trends in animal ribosomal DNA loci: introduction to a new online database. **Chromosoma**, v. 127, p. 141–150, 2018.

SOPNIEWSKI, J. *et al.* Identifying sex-linked markers in *Litoria aurea*: a novel approach to understanding sex chromosome evolution in an amphibian. **Scientific Reports**, v. 9, n. 1, p. 16591, 2019.

STOTHARD, P. The Sequence Manipulation Suite: JavaScript Programs for Analyzing and Formatting Protein and DNA Sequences. **BioTechniques**, v. 28, n. 6, p. 1102–1104, 2000.

SULLIVAN, J. P.; LUNDBERG, J. G.; HARDMAN, M. A phylogenetic analysis of the major groups of catfishes (Teleostei: Siluriformes) using rag1 and rag2 nuclear gene sequences. **Molecular Phylogenetics and Evolution**, v. 41, n. 3, p. 636–662, 2006.

SUMNER, A. T. A simple technique for demonstrating centromeric heterochromatin. **Experimental Cell Research**, v. 75, n. 1, p. 304–306, 1972.

SYMONOVÁ, R. *et al.* Molecular cytogenetics in artificial hybrid and highly polyploid sturgeons: an evolutionary story narrated by repetitive sequences. **Cytogenetic and genome research**, v. 141, n. 2–3, p. 153–162, 2013.

SZARSKI, H. K. Sarcopterygii and the origin of tetrapods. In: **Major patterns in vertebrate evolution**. Boston, MA: Springer US, p. 517-540, 1977.

TAKAGUI, F. H. *et al.* The Importance of Pericentric Inversions in the Karyotypic Diversification of the Species *Loricariichthys anus* and *Loricariichthys platymetopon*. **Zebrafish**, v. 11, n. 4, p. 300–305, 2014.

TAKAGUI, F. H. *et al.* Unrevealing the Karyotypic Evolution and Cytotaxonomy of Armored Catfishes (Loricariinae) with Emphasis in *Sturisoma*, *Loricariichthys*, *Loricaria*, *Proloricaria*, *Pyxiloricaria*, and *Rineloricaria*. **Zebrafish**, v. 17, n. 5, p. 319–332, 2020.

THAKUR, J.; PACKIARAJ, J.; HENIKOFF, S. Sequence, Chromatin and Evolution of Satellite DNA. **International Journal of Molecular Sciences**, v. 22, n. 9, p. 4309, 2021.

THOMAS, J.; PRITHAM, E. J. Helitrons, the Eukaryotic Rolling-circle Transposable Elements. *In*: **MOBILE DNA III**.: John Wiley & Sons, Ltd, 2015. p. 891–924. Disponível em: https://onlinelibrary.wiley.com/doi/abs/10.1128/9781555819217.ch40. Acesso em: 13 ago. 2024.

THOMPSON, J. D. *et al.* The CLUSTAL_X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools. **Nucleic Acids Research**, v. 25, n. 24, p. 4876–4882, 1997.

TRALDI, J. B. *et al.* Chromosomal distribution of the retroelements Rex1, Rex3 and Rex6 in species of the genus *Harttia* and *Hypostomus* (Siluriformes: Loricariidae). **Neotropical Ichthyology**, v. 17, n. 2, p. 1–10, 2019.

UTSUNOMIA, R. *et al.* A glimpse into the satellite DNA library in characidae fish (Teleostei, Characiformes). **Frontiers in Genetics**, v. 8, n. AUG, 2017.

UTSUNOMIA, R. *et al.* Satellitome landscape analysis of *Megaleporinus macrocephalus* (Teleostei, Anostomidae) reveals intense accumulation of satellite sequences on the heteromorphic sex chromosome. **Scientific Reports**, v. 9, n. 1, p. 5856, 2019.

VIEIRA, C. P. *et al.* Inferences on the evolutionary history of the *Drosophila americana* polymorphic X/4 fusion from patterns of polymorphism at the X-linked paralytic and elav genes. **Genetics**, v. 164, n. 4, p. 1459-1469, 2003.

VOJVODA ZELJKO, T. *et al.* Satellite DNA-like repeats are dispersed throughout the genome of the Pacific oyster *Crassostrea gigas* carried by Helentron non-autonomous mobile elements. **Scientific Reports**, v. 10, n. 1, p. 15107, 2020.

VOLENÍKOVÁ, A. *et al.* Fast satellite DNA evolution in *Nothobranchius* annual killifishes. **Chromosome Research**, v. 31, n. 4, p. 33, 2023.

VOLFF, J.-N.; HORNUNG, U.; SCHARTL, M. Fish retroposons related to the Penelope element of *Drosophila virilis* define a new group of retrotransposable elements. **Molecular Genetics and Genomics**, v. 265, n. 4, p. 711–720, 2001.

WALLACE, A. R. On the Monkeys of the Amazon. **Annals and Magazine of Natural History**, 1854. Disponível em: https://www.tandfonline.com/doi/abs/10.1080/037454809494374. Acesso em: 28 out. 2024.

WANG, L.-B. *et al.* A sustainable mouse karyotype created by programmed chromosome fusion. **Science**, v. 377, n. 6609, p. 967–975, 2022.

WANG, C. *et al.* Disentangling sources of variation in SSU rDNA sequences from single cell analyses of ciliates: impact of copy number variation and experimental error. **Proceedings of the Royal Society B: Biological Sciences**, v. 284, n. 1859, p. 20170425, 2017.

WARMERDAM, D. O.; WOLTHUIS, R. M. F. Keeping ribosomal DNA intact: a repeating challenge. **Chromosome Research**, v. 27, n. 1, p. 57–72, 2019.

WATSON, J. D.; CRICK, F. H. C. Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. **Nature**, v. 171, n. 4356, p. 737–738, 1953.

WHITE, M. The origin and evolution of multiple sex chromosome mechanisms. **Journal of Genetics**, 1940. p. 303–306.

YANG, F. *et al.* Generation of paint probes by flow-sorted and microdissected chromosomes - **Fluorescence In Situ Hybridization (FISH) -- Application Guide**. *In*: LIEHR, T. (org.). 2009. Disponível em: https://doi.org/10.1007/978-3-540-70581-9_3.

YANG, F.; GRAPHODATSKY, A. S. Animal Probes and ZOO-FISH. *In*: LIEHR, T. (org.). **Fluorescence In Situ Hybridization (FISH) — Application Guide**. Berlin, Heidelberg: Springer Berlin Heidelberg, 2009. p. 323–346. Disponível em: https://doi.org/10.1007/978-3-540-70581-9_29.

YANO, C. F.; BERTOLLO, L. A. C.; CIOFFI, M. B. Fish-FISH: molecular cytogenetics in fish species. *In*: LIEHR, T. (org.). **Fluorescence In Situ Hybridization (FISH)- Application Guide**. Berlin: Springer, 2017. p. 429–444.

YAZDI, H. P.; SILVA, W. T. A. F.; SUH, A. Why Do Some Sex Chromosomes Degenerate More Slowly Than Others? The Odd Case of Ratite Sex Chromosomes. **Genes**, v. 11, n. 10, p. 1153, 2020.

YOSHIDO, A. *et al.* Step-by-step evolution of neo-sex chromosomes in geographical populations of wild silkmoths, *Samia cynthia* ssp. **Heredity**, v. 106, n. 4, p. 614–624, 2011.

ZHU, Z.; YOUNAS, L.; ZHOU, Q. Evolution and regulation of animal sex chromosomes. **Nature Reviews Genetics**, p. 1–16, 2024.

ZIEMNICZAK, K. *et al.* Comparative cytogenetics of Loricariidae (Actinopterygii: Siluriformes): emphasis in Neoplecostominae and Hypoptopomatinae. **Italian Journal of Zoology**, v. 79, n. 4, p. 492–501, 2012.

ZIMMER, E. A. *et al.* Rapid duplication and loss of genes coding for the alpha chains of hemoglobin. **Proceedings of the National Academy of Sciences**, v. 77, p. 2158–2162, 1980.

ZWICK, M. S. *et al.* A rapid procedure for the isolation of C_0t-1 DNA from plants. **Genome**, v. 40, p. 138–142, 1997.

7 Anexos

I – Sassi *et al.* (2020) Multiple Sex Chromosomes and Evolutionary Relationships in Amazonian Catfishes: The Outstanding Model of the Genus *Harttia* (Siluriformes: Loricariidae). Genes 11(10), 1179.

genes

MDPI

Article

Multiple Sex Chromosomes and Evolutionary Relationships in Amazonian Catfishes: The Outstanding Model of the Genus *Harttia* (Siluriformes: Loricariidae)

Francisco de M. C. Sassi [1], Geize A. Deon [1,2], Orlando Moreira-Filho [1], Marcelo R. Vicari [2], Luiz A. C. Bertollo [1], Thomas Liehr [3,*], Ezequiel Aguiar de Oliveira [4] and Marcelo B. Cioffi [1]

[1] Laboratório de Citogenética de Peixes, Departamento de Genética e Evolução, Universidade Federal de São, Carlos, São Carlos - SP 13565-905, Brazil; francisco.sassi@hotmail.com (F.d.M.C.S.); geizedeon@hotmail.com (G.A.D.); omfilho@ufscar.br (O.M.-F.); bertollo@ufscar.br (L.A.C.B.); mbcioffi@ufscar.br (M.B.C.)

[2] Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Ponta Grossa - PR 84010-330, Brazil; vicarimr@yahoo.com.br

[3] Institute of Human Genetics, University Hospital Jena, 07747 Jena, Germany

[4] Secretaria de Estado de Educação do Mato Grosso–SEDUC-MT, Cuiabá, MT 78049-909, Brazil; ezekbio@gmail.com

* Correspondence: thomas.liehr@med.uni-jena.de; Tel.: +49-3641-9396850; Fax: +49-3641-9396852

Received: 14 September 2020; Accepted: 8 October 2020; Published: 10 October 2020

Abstract: The armored *Harttia* catfishes present great species diversity and remarkable cytogenetic variation, including different sex chromosome systems. Here we analyzed three new species, *H. duriventris*, *H. villasboas* and *H. rondoni*, using both conventional and molecular cytogenetic techniques (Giemsa-staining and C-banding), including the mapping of repetitive DNAs using fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization (CGH) experiments. Both *H. duriventris* and *H. villasboas* have 2n = ♀56/♂55 chromosomes, and an $X_1X_1X_2X_2$ /X_1X_2Y sex chromosome system, while a proto or neo-XY system is proposed for *H. rondoni* (2n = 54♀♂). Single motifs of 5S and 18S rDNA occur in all three species, with the latter being also mapped in the sex chromosomes. The results confirm the general evolutionary trend that has been noticed for the genus: an extensive variation on their chromosome number, single sites of rDNA sequences and the occurrence of multiple sex chromosomes. Comparative genomic analyses with another congeneric species, *H. punctata*, reveal that the X_1X_2Y sex chromosomes of these species share the genomic contents, indicating a probable common origin. The remarkable karyotypic variation, including sex chromosomes systems, makes *Harttia* a suitable model for evolutionary studies focusing on karyotype differentiation and sex chromosome evolution among lower vertebrates.

Keywords: Cytogenetics; ribosomal DNA; comparative genomic hybridization; Neotropical fishes

1. Introduction

Siluriformes (Actinopterygii; Teleostei) is a monophyletic order that covers a large deal of the freshwater fish diversity, with more than 3000 species and 36 families [1,2]. Of the six Loricariidae subfamilies currently recognized, Hypostominae and Loricariinae are the most representative ones, with the highest number of species (579 and 302, respectively) and a remarkable karyotypic diversity [2,3]. Loricariinae fishes are distributed throughout South and Central American rivers, including

two tribes: Harttiini and Loricariini. The genus *Harttia*, popularly known as armored catfishes, is represented by 27 valid species [2,4].

Despite such great species diversity, cytogenetic studies are available for only eight of them, all from Brazilian Southern regions, with exception for *Harttia punctata* [3,5–13]. However, although still limited, the chromosome data are already enough to notice a conspicuous variation of the diploid number (2n) in the genus, ranging from 2n = 52♀/53♂ in *H. carvalhoi* [8] to 2n = 62♀♂ in *H. absaberi* [9]. Chromosomal breaks and rearrangements as Robertsonian fusions, fissions, as well inversions were proposed to occur in the karyotype diversification of *Harttia* species [13]. In addition, two multiple XY-derived sex chromosome systems were also identified: ♀XX/♂XY_1Y_2 in *H. carvalhoi* [8] and ♀$X_1X_1X_2X_2$/♂X_1X_2Y in *H. punctata* [12].

In recent years, a range of molecular cytogenetic investigations (e.g., repetitive DNA mapping based on fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH) and whole chromosome painting (WCP) has been applied in different fish groups, providing new insights into the evolutionary relationships among them [14–18]. However, the ribosomal genes are the only repetitive DNA class that has been analyzed in *Harttia* till now [3,8,12,13]. The rDNA mapping shows that the 18S sites presents a conserved pattern in number, with a single chromosome pair carrying these sequences in all analyzed species, but with variation in location and chromosome carrying them [13]. Similarly, the 5S rDNA is also found in a single chromosome pair in most species, with exception for *H. carvalhoi*, in which signals occur in both chromosome pairs 03 and 23 [8,13]. Besides, WCP analyzes are confined to only one species, *H. punctata*, highlighting the main chromosomal rearrangements involved in the origin of the X_1X_2Y sex system in this species [12].

In the most updated phylogenetical reconstruction based on mtDNA, three distinct clades can be recognized for the genus *Harttia* involving: (i) species that inhabit rivers of Guyana shield; (ii) species occurring in north Brazil, especially in Amazonas river basin and its tributaries and (iii) species distributed throughout southeast/south Brazilian rivers, such as Grande, São Francisco and Paraná river basins [19]. However, despite largely widespread in South America, only *Harttia* species from southern rivers have been cytogenetically studied to date. Here we analyzed three new species sampled from Northern Brazilian river basins by performing an extensive cytogenetic investigation using conventional cytogenetic protocols (Giemsa-staining and C-banding) combined with molecular cytogenetic ones, including CGH experiments and mapping of several repetitive DNA classes using FISH. The results allowed us to open a new chapter on the evolutionary history of *Harttia* fishes, besides describing new sex chromosome systems.

2. Materials and Methods

2.1. Sampling

The collection sites, number and gender of the specimens investigated are presented in Figure 1 and Table 1. Samples were collected with the authorization of the environmental agency ICMBIO/SISBIO (License nº 48628-2) and SISGEN (A96FF09). The specimens were proper identified by evaluation of their meristic characters by Dr. Osvaldo Takeshi Oyakawa, curator of the Museu de Zoologia da Universidade de São Paulo (MZUSP) and specialist in this fish group. Samples from *H. punctata* were used for gDNA extraction and CGH experiments.

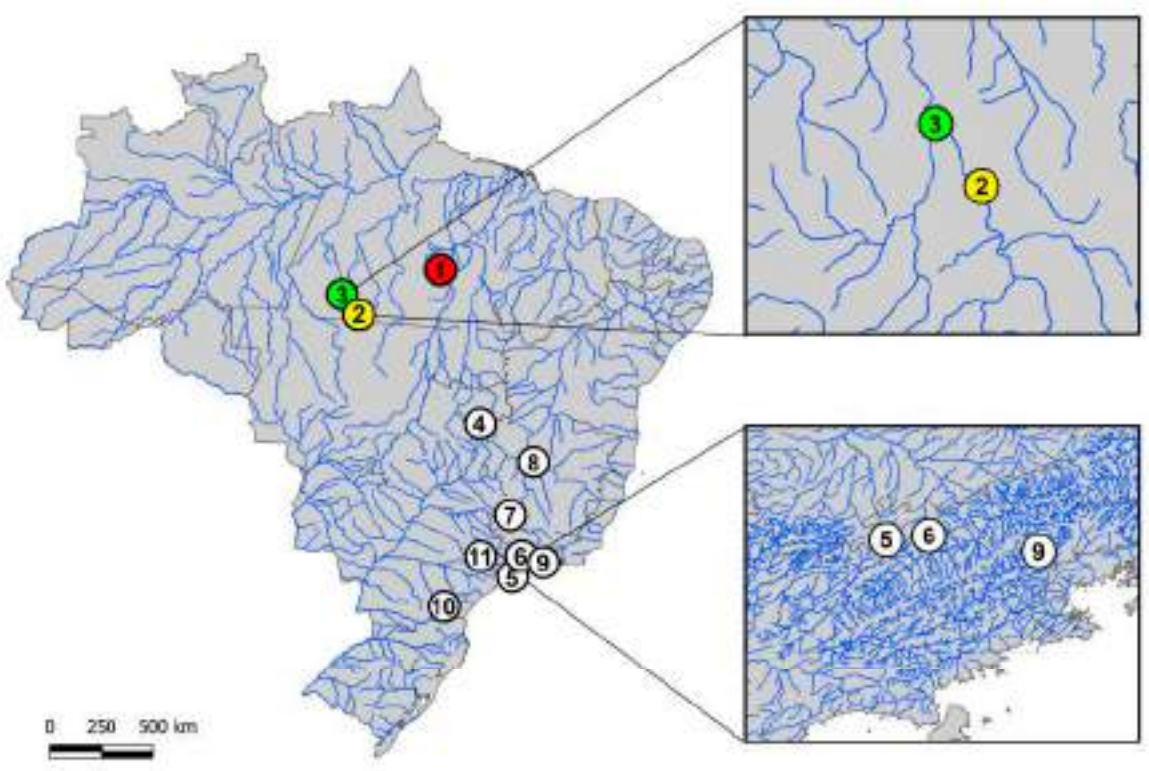


Figure 1. Brazilian collection sites of the three *Harttia* species cytogenetically investigated in the present study (colored circles) and the ones previously cytogenetically analyzed (white circles: data from [9,13]). **1**. *H. duriventris* (red circle); **2**. *H. villasboas* (yellow circle); **3**. *H. rondoni* (3-green circle); **4**. *H. punctata*; **5**. *H. gracilis*; **6**. *H. carvalhoi*; **7**. *H. torrenticola*; **8**. *H. longipinna*; **9**. *H. loricariformis*; **10**. *H. kronei*; **11**. *H. absaberi*. The boxes highlight the non-sympatric distribution of some species.

Table 1. Collection sites and samples size (N) of the species analyzed.

Species	Locality	N
1. *H. duriventris*	Parauapebas River, Canaã dos Carajás-PA (Brazil) (6°30′06.5′′ S 50°02′35.3′′ W)	08♀, 07♂
2. *H. villasboas*	Curuá River, Cachoeira da Serra-PA (Brazil) (8°44′09.0′′ S 54°57′46.0′′ W)	34♀, 38♂
3. *H. rondoni*	13 de Maio River, Cachoeira da Serra-PA (Brazil) (8°38′53.0′′ S 55°01′41.0′′ W)	15♀, 14♂
4. *H. punctata*	Itiquira river, Formosa—GO (Brazil) (15°19′25′′ S 47°25′26′′ W)	10♀, 12♂

2.2. Chromosome Preparation and C-Banding

Mitotic chromosomes were obtained by the protocol described in [20]. Briefly, the animals were first injected in the abdominal region with a 0.025% aqueous solution of colchicine at a dose of 1 ml/100 g of weight. After 50–60 min, the specimens were euthanized, and the chromosomal preparations were obtained from cells of the anterior kidney. The experiments followed ethical and anesthesia conducts and were approved by the Ethics Committee on Animal Experimentation of the Universidade Federal de São Carlos (Process number CEUA 1853260315). C-positive heterochromatin (C-banding) was identified according to [21]. Briefly, slides were treated with 0,2 N HCl for 10 min, followed by a short wash in water; incubated in 5% $Ba(OH)_2$ for 3 min at 42 °C ; rinsed in water for 1 min and then incubated in 2× SSC (pH 7.0) for 1h at 60 °C. The slides were then stained in 5% Giemsa solution (phosphate buffer, pH 6.8).

2.3. Fluorescence In Situ Hybridization (FISH) for Repetitive DNA Mapping

The 5S rDNA probe included 120 base pairs (bp) of the 5S rRNA codificant gene and 200bp of a non-transcribed spacer (NTS) isolated according to [22]. In its turn, the 18S rDNA probe contained a 1400 bp segment of the 18S rRNA gene and was isolated following [23]. These probes were directly labeled with the Nick-Translation mix kit (Jena Bioscience, Jena, Germany). The 5S rDNA was labeled with ATTO550-dUTP and the 18S rDNA with AF488-dUTP, according to the manufacturer's manual. The microsatellite sequences $(A)_{30}$, $(CA)_{15}$ and $(GA)_{15}$ were directly labeled with Cy-3 during their synthesis, as described by [24]. These sequences were selected once they are among the most abundant ones in fish genomes [14] and generated well visible hybridization patterns in our experiments. Telomeric (TTAGGG)n sequences were mapped using the DAKO Telomere PNA FISH Kit/FITC (DAKO, Glostrup, Denmark). The FISH experiments followed the methodology described in [25]. Briefly, metaphase chromosome slides were incubated with RNAse (40 μg/mL) for 1.5 h at 37 °C. After the denaturation of the chromosomal DNA in 70% formamide/2× SSC at 70 °C for 3 min, the hybridization mixture (2.5 ng/μL probes, 2 μg/μL C0t-1 DNA, 50% deionized formamide, 10% dextran sulphate) was dropped on the slides, and the hybridization was performed overnight at 37 °C in a moist chamber containing 2× SSC. The first post-hybridization wash was performed with 2× SSC for 5 min at 65 °C, and a final wash was performed at room temperature in 1× SSC for 5 min. Finally, the slides were counterstained with DAPI and mounted in an antifade solution (Vectashield from Vector Laboratories, Burlingame, CA)

2.4. Comparative Genomic Hybridization (CGH)

The total genomic DNAs (gDNAs) from male and female specimens of *H. duriventris*, *H. villaboas*, *H. rondoni* and *H. punctata* were extracted from liver tissue by the standard phenol-chloroform-isoamyl alcohol method [26]. It was focused on inter-and intraspecific comparisons, with special emphasis on molecular composition of the putative and multiple sex chromosomes. In the first set of experiments (intraspecific genomic comparisons), the male-derived gDNAs of all species were labelled with Atto550-dUTP and the female gDNAs with Atto488-dUTP, by means of nick translation (Jena Bioscience, Jena, Germany). For blocking repetitive sequences, it was used unlabeled C0t-1 DNA in all experiments (i.e. fraction of genomic DNA enriched with highly and moderately repetitive sequences), prepared according to [27]. The final hybridization mixture for each slide (20 μL) was composed of male- and female-derived gDNAs (500 ng each), plus 25 μg of female-derived C0t-1 DNA from the respective species. The probe was ethanol-precipitated, and the dry pellets were resuspended in hybridization buffer containing 50% formamide, 2× SSC, 10% SDS, 10% dextran sulfate and Denhardt´s buffer, pH 7.0. In the second set of experiments (interspecific genomic comparisons), the gDNA of all male specimens now analyzed, plus the gDNA of *H. punctata* (a species harboring multiple X_1X_2Y sex system), were hybridized against metaphase chromosomes of *H. villasboas*. For this purpose, male-derived gDNA of *H. villasboas* was labeled with Atto550-dUTP, while the gDNAs of the other three species were labeled with Atto488-dUTP (*H. duriventris* and *H. rondoni*) or Atto425-dUTP (*H. punctata*) both by means of nick translation (Jena Bioscience, Jena, Germany). In a first slide, the final probe mixture was composed of 500 ng of male-derived gDNA of each *H. villasboas*, *H. duriventris* and *H. punctata* and 10 μg of female-derived C0t-1 DNA of each species. In a second slide, the final probe mixture was composed of 500 ng of male-derived gDNA of *H. villasboas* and *H. rondoni* and 15 μg of female-derived C0t-1 DNA of each species. The chosen ratio of probe vs. C0t-1 DNA amount was based on previous experiments performed in our fish studies [18,28,29]. The CGH experiments followed the methodology described in [30].

2.5. Microscopic Analysis and Image Processing

At least 30 metaphase spreads per individual were analyzed to confirm the 2n, karyotype structure and CGH results. Images were captured using Olympus BX50 microscope (Olympus Corporation, Ishikawa, Japan), with CoolSNAP and the images were processed using Image Pro

Plus 4.1 software (Media Cybernetics, Silver Spring, MD, USA). Chromosomes were classified as metacentric (m); submetacentric (sm); subtelocentric (st) or acrocentric (a) according to [31]. The maps were created using the following software's: QGis 3.4.3 and Adobe Photoshop CC 2020.

3. Results

3.1. Karyotypes, C-banding and Sex Chromosomes

Harttia rondoni has 2n = 54 chromosomes (20m + 26sm + 4st + 4a) in both sexes. In turn, *H. villasboas* and *H. duriventris* have 2n = 56 chromosomes in the female specimens (18m+24sm+6st+8a in *H. villasboas* and 16m + 16sm + 16st + 8a in *H. duriventris*), but 2n = 55 chromosomes in the male specimens (19m + 24sm + 6st + 6a in *H. villasboas* and 17m + 16sm + 16st + 6a in *H. duriventris*). These specific male karyotypes are due to characteristics $X_1X_1X_2X_2/X_1X_2Y$ multiple sex chromosome systems, where the Y corresponds to a medium-sized m chromosome (Figure 2).

In all species, C-positive heterochromatic bands are found in the pericentromeric region of all chromosomes and in the telomeric region of the acrocentric pairs (Figure 2). In general, C-banding does not identify any chromosome heteromorphism in *H. rondoni* females. However, in males, a slight heteromorphic pattern occurs at the proximal C-positive bands on the long arms of the pair 11 (Figure 2). This points to a likely XX/XY sex chromosome system in this species. On the other hand, no conspicuous heterochromatin accumulation is observed in the Y chromosome of *H. duriventris* and *H. villasboas*.

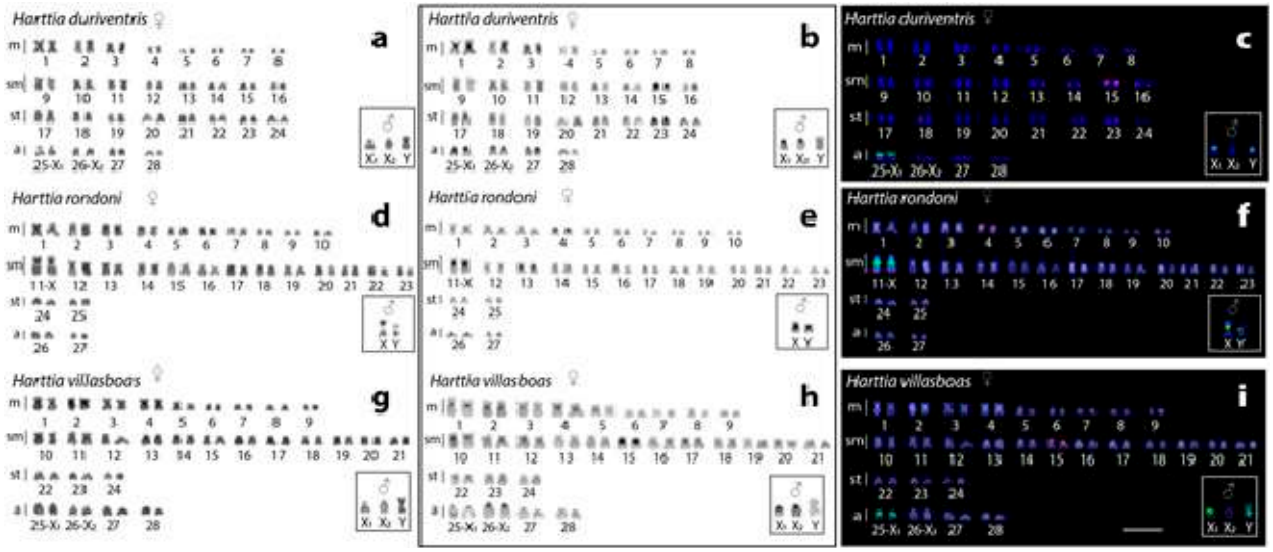


Figure 2. Karyotypes of *H. duriventris* (**a–c**) *H. villasboas* (**d–f**) and *H. rondoni* (**g–i**) arranged by sequentially Giemsa-stained (**a**, **d** and **g**) and C-banded chromosomes (**b**, **e** and **h**) and hybridized with 5S rDNA (red) and 18S rDNA (green) probes after a double-FISH analysis (**c**, **f**, and **i**). Boxes depict the male sex chromosomes. Bar = 5 μm.

3.2. Chromosomal Distribution of rDNAs, Microsatellite motifs and Telomeric Repeats

Cytogenetic mapping of the 18S and 5S rDNA sequences showed single sites in corresponding chromosomes of all three analyzed species. Specifically, the 18S rDNA sequences are mapped in the pericentromeric region of the XY chromosomes of *H. rondoni*, where a clear polymorphic state occurs in males (Figure 2). In both *H. duriventris* and *H. villasboas*, these sequences are located in both X_1 (in females) and in the X_1 and Y (in males). The statement that these rDNA loci are located on the X_1 chromosome instead of in the X_2 is based on available data showing that these sequences are also found in the corresponding X chromosome of the sister species, *H. rondoni*.

The $A_{(30)}$ motif showed hybridization in nine male chromosomes and in ten female chromosomes in all three species, this difference being due to the absence of signals in the Y

chromosomes. Besides, *H. rondoni* also presents some small scattered marks in all other chromosomes for this same microsatellite. For the $GA_{(15)}$ probe, small scattered signals occur in the chromosomes of all species, with accumulation on telomeric regions. However, the sex chromosomes also present different accumulation patterns for this microsatellite: on the telomeric region of the X_2 and the Y chromosomes of *H. duriventris*, on the telomeric regions of the X_1, X_2 and Y of *H. villasboas*, and strongly accumulated in the telomeric region of both X and Y chromosomes of *H. rondoni*. Additionally, $CA_{(15)}$ were identified in the telomeric regions of almost all chromosomes, of *H. villasboas*, including the sex ones (Figure 3).

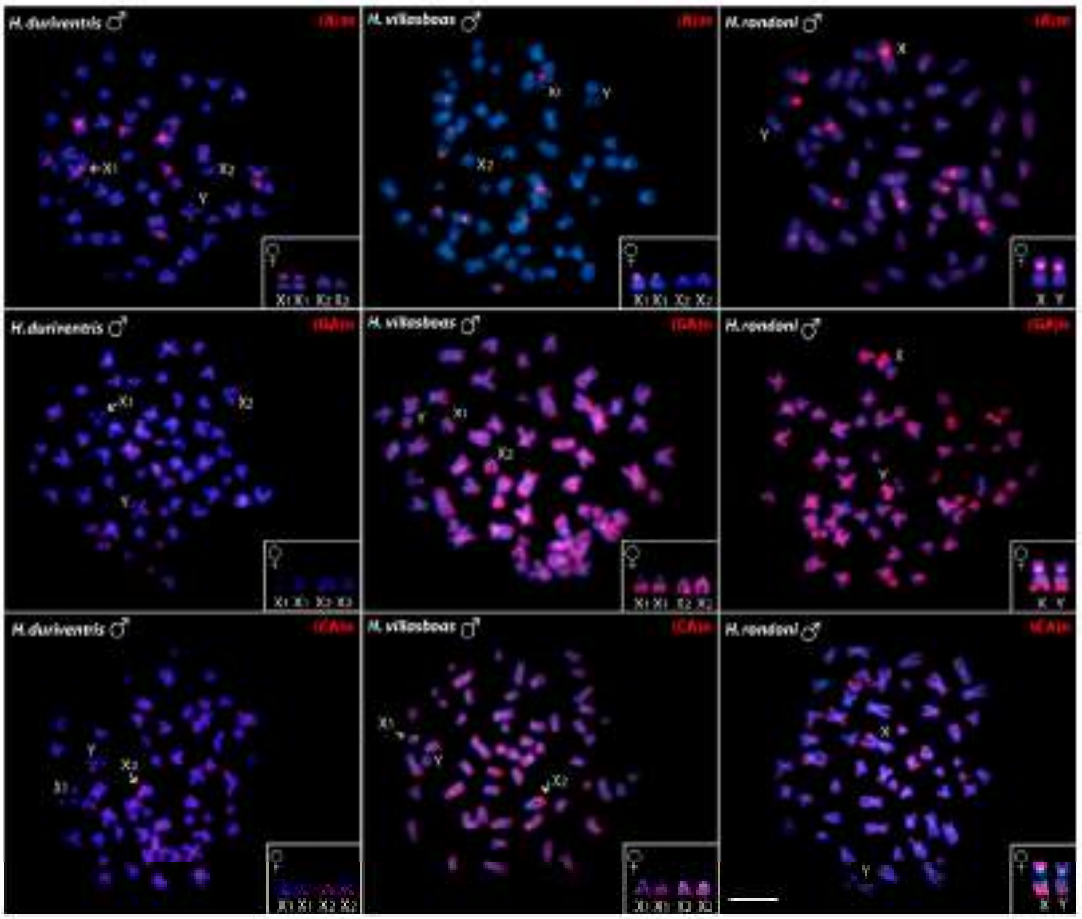


Figure 3. Metaphase chromosomes of males *H. duriventris*, *H. villasboas* and *H. rondoni* hybridized with microsatellite-containing oligonucleotides. Chromosomes were counterstained with DAPI (blue) and microsatellite probes were directly labeled with Cy3 during synthesis (red signals). The female sex chromosomes are shown in boxes. Bar = 5 μm.

FISH with telomeric (TTAGGG)n probe applied to male metaphases of *H. duriventris*, *H. villasboas* and *H. rondoni* revealed hybridization signals on the telomeres of all chromosomes in both species, with no additional interstitial telomeric sites (ITS) (Figure 4).

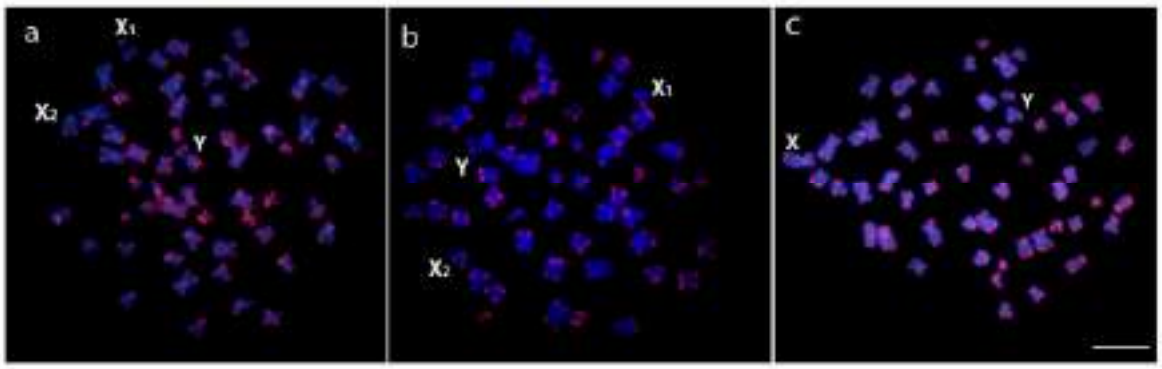


Figure 4. Male metaphase plates of *H. duriventris* (**a**), *H. villasboas* (**b**) and *H. rondoni* (**c**) showing telomeric hybridization signals on both telomeres of all chromosomes. Bar = 5 μm.

3.3. Comparative Genomic Hybridization (CGH)

3.3.1. Intraspecific Genomic Relationships: Detecting Male-Specific Regions

In all species, CGH procedure failed to detect any conspicuous sex-specific region on male chromosomes (Figure 5). However, a slight binding preference for the male-derived probe to the pericentromeric region of the Y chromosome of *H. villasboas* and *H. duriventris*, and to the Y chromosome of *H. rondoni* was evidenced (Figure 5). Female-derived probe produced only a faint hybridization signal in such regions, while both probes matched equally the large heterochromatic pericentromeric segment in the X_1 chromosome of *H. villasboas* and *H. duriventris*, and in the X chromosome of *H. rondoni* (Figure 5).

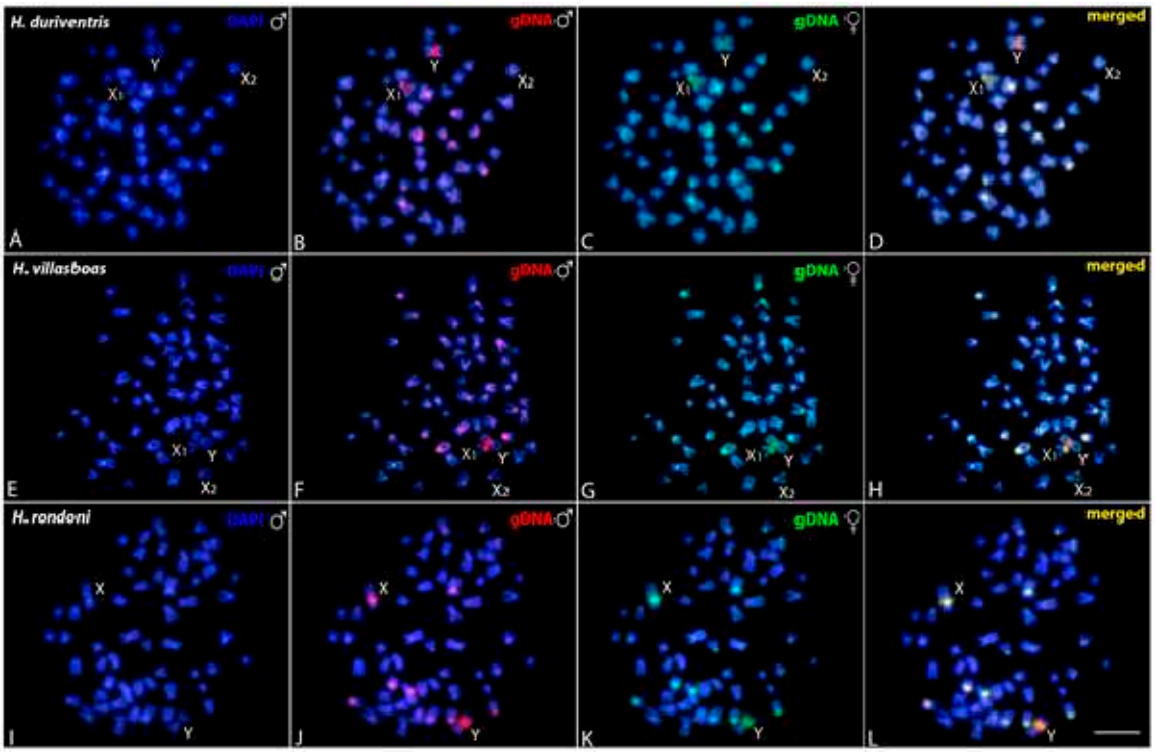


Figure 5. Mitotic chromosome spreads of males *H. duriventris* (**A–D**), *H. villasboas* (**E–H**) and *H. rondoni* (**I–L**) after intraspecific CGH procedures. Male- and female-derived genomic probes were hybridized together for each species. First column (**A**, **E** and **I**): DAPI images (blue); Second column (**B**, **F**, and **J**): hybridization pattern of the male-derived probe (red); Third column (**C**, **G**, and **K**): hybridization pattern of the female-derived probe (green). The fourth column (**D**, **H**, and **L**): merged images of both genomic probes and DAPI staining. The common genomic regions for males and females are depicted in yellow. Sex chromosomes are indicated. Bar = 10 μm.

3.3.2. Interspecific Genomic Relationships, Focusing on the Multiple X1X2Y Sex System

When the gDNA of *H. duriventris*, *H. villasboas* and *H. punctata* (all X_1X_2Y-species) was compared, no species-specific region in the sex-chromosomes were observed, thus pointing to their common genomic content (Figure 6).

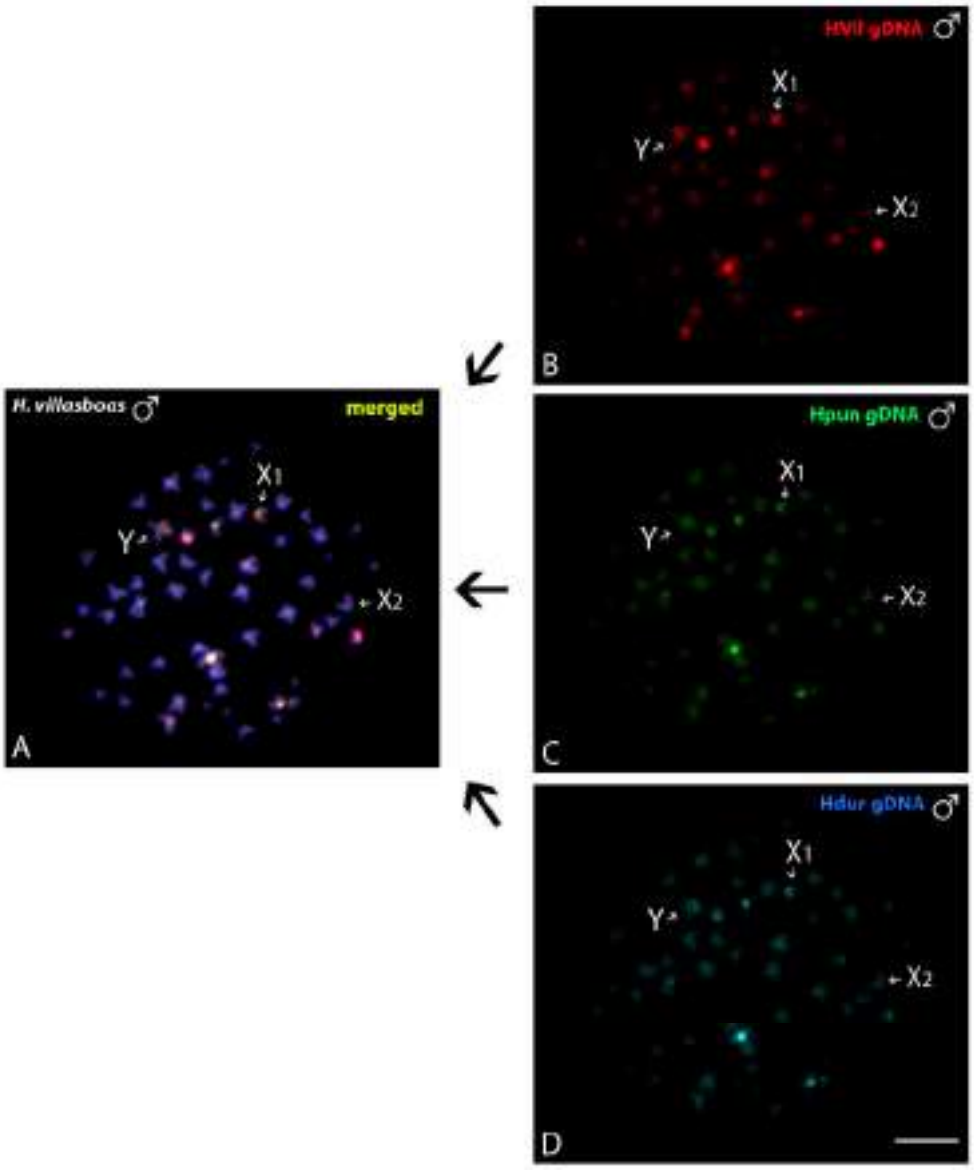


Figure 6. Comparative Genomic Hybridization (CGH) of *Harttia* species bearing X_1X_2Y sex chromosome system. Mitotic male chromosome spreads of *H. villasboas* (**A**) hybridized against male-derived genome probes of *H. villasboas* (**B**), *H. punctata* (**C**) and *H. duriventris* (**D**). Sex chromosomes are indicated and the common genomic regions for the three species are depicted in yellow on (**A**). Bar = 10 μm.

4. Discussion

4.1. Evolutionary Relationships among Harttia Species

Chromosome data have shown that *Harttia* species, as a whole, presents an extensive variation in the diploid number, karyotype composition, in addition to multiple sex chromosomes in some species [5–8,10–12], the latter corresponding to an uncommon condition among fishes. Here we brought new data for three yet unexplored Amazonian species. If we consider previous available data, it is noticeable that the chromosome numbers of all species now investigated fit into the range

of variation previously described for the genus, i.e., 2n = 52♀/53♂ in *H. carvalhoi* [8], and 2n = 62♀♂ in *H. absaberi* [9]. In addition, the general trend of single rDNA locus occurring in rearranged chromosomes is maintained within the genus [13]. However, a striking feature is that they all present new cases of sex chromosomes: an $X_1X_1X_2X_2/X_1X_2Y$ system in *H. duriventris* and *H. villasboas* and a proto or a neo-XY system in *H. rondoni* (discussed below).

The karyotype diversification and morphological patterns are often indicators of the lifestyle of a species [32,33]. Although *Harttia* have a wide geographic distribution in many South American rivers and small streams, their low vagility fosters the fixation of chromosomal rearrangements into small populations, thus promoting chromosomal diversity. Indeed, several other fish species also presenting the same above characteristics, have been evidenced as carriers of a wide variety of karyotypes: *A. fasciatus*, for example, presents karyotypes with 2n = 45, 46, 47, 48 and 50 chromosomes [34–36] and *Hoplias malabaricus*, with seven main karyotypes including multiple and simple sex chromosome systems [23,37–39]. In Late Cretaceous and Cenozoic, large-scale tectonic events led to changes in river courses and watershed limits, resulting in complex river dynamisms [40], and affecting the distribution of fish populations. For example, the Serra do Cachimbo region (Pará-Brazil), presents highlands that reach 740 m of altitude, divided between Xingu and Tapajós river basins [41]. Located at the northern border of the Brazilian shield, this region has a high number of endemic species such as *Leporinus guttatus* [42], three species of *Lebiasina* genus [43], and *Harttia villasboas* and *H. panara* [41]. This high number of endemic species can be attributed by the high number of headwaters of the Xingu and Tapajós rivers [41]. There, *H. rondoni* and *H. villasboas* have a probable geographic barrier due to a series of waterfalls 40–60 m height, over a 50 m stretch, where *H. rondoni* and *H. villasboas* occur below and above this set of waterfalls, respectively [41,44]. Despite some karyotype similarities that they share, such as the higher number of m and sm chromosomes, a vicariance event may have facilitated the fixation of another series of chromosomal rearrangements in both groups, including the origin of two distinct, but related sex chromosome systems (a proto or- neo-XY system in *H. rondoni* and a multiple $X_1X_1X_2X_2/X_1X_2Y$ system in *H. villasboas*). The generation of multiple sex chromosome systems usually involves centric fusions or fissions events and may retain vestiges of interstitial telomeric sequences (ITS) [45]. Our chromosomal mapping of telomeric sequences only reveal the expected terminal signals, without any ITS in the three species. However, it is also known that Robertsonian rearrangements can also lead to the loss or reduction of sequences close to the chromosomal breakpoints [45], and it is likely that this particular condition is responsible for the absence of ITS, as well as of microsatellite sequences and C-positive heterochromatin that, although identified on the X_1 and X_2 chromosomes of *H. duriventris* and *H. villasboas*, they are missing in the Y chromosome.

Blanco [13] discussed the split of *H. punctata* from a southeast clade with the origin of its multiple sex chromosome system being a characteristic determinant, probably originated from an ancestor with 2n = 58 chromosomes without a differentiated sex chromosome system. Now, one specific chromosomal pair present in *H. rondoni* shares similar patterns to the X_1X_2Y sex chromosomes present in the other closely related species, opening two main scenarios: (i) a proto-XY hypothesis: The X and Y chromosomes differ only slightly due to the amplification of repetitive sequences (major rDNAs) on the X chromosome, thus representing an early stage of differentiation. As all its closely related species until now studied have sex chromosomes (Figure 7, dotted line), we could hypothesize that this 2n = 58 ancestor have also carried a proto-XY sex system, as is the case of *H. rondoni*. Afterwards, chromosomal fissions on the X chromosomes may have created the X_1 and X_2 chromosomes present in *H. punctata*, *H. duriventris* and *H. villasboas*. (ii) A Neo-XY hypothesis: chromosomal fusions involving the ancestral X and Y chromosomes and a pair of autosomes could create such a neo-XY sex system, leading to the reduction of the 2n from 56 (present in the sister species) to 54 in *H. rondoni*. Besides, the bigger size of the neo-X chromosome in comparison with the Y and the loss of a A(n) rich region closer to the ribosomal loci in the later support this hypothesis. Among fishes, some cases of neo-XY sex chromosomes emerging from X and/or Y-autosomal translocations have been also observed [46–48]. Neo-sex chromosomes may not necessarily lead to the emergence of multiple sex chromosomes, as equal addition of autosomal segments to both sex

chromosomes generates neo-XY or neo-ZW systems. Complementary studies, focusing on whole chromosome painting and in the genomic organization of these sex chromosomes, are necessary to clarify these two abovementioned hypotheses.

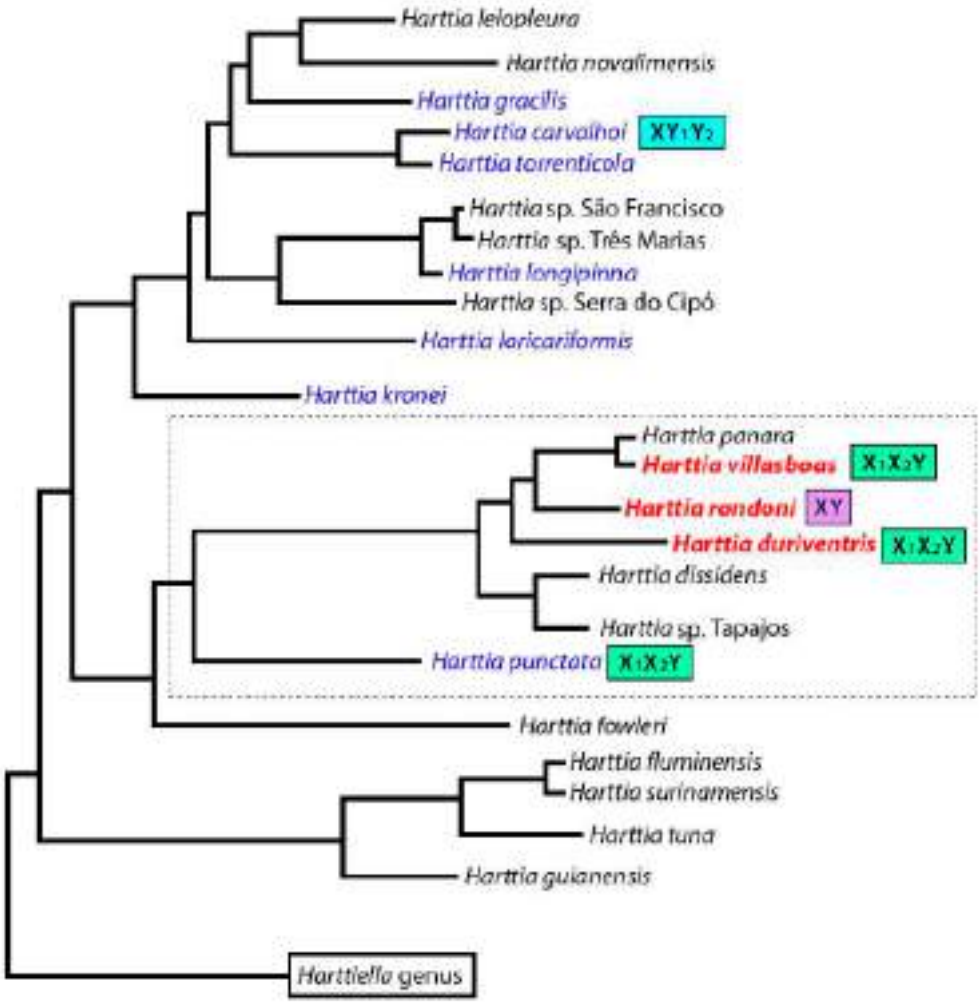


Figure 7. Adapted phylogenetic tree for the genus *Harttia*, based on the molecular-phylogenetic data generated by Covain [19]. Previous and now cytogenetic analyzed species are indicated in blue and red, respectively. The multiple X_1X_2Y (green boxes) and XY_1Y_2 (blue box) sex chromosome found in *Harttia* is also indicated, together with the putative proto or neo XY system (purple box). Species from northern Brazilian region are highlighted with the dotted line.

4.2. The Genus Harttia as A Repository of Multiple Sex Chromosome Systems

Teleost fishes represent one of the most diverse groups in terms of sex determination and differentiation [49,50]. Heteromorphic sex chromosomes are identified in about 5% of so far analyzed species [51], mostly corresponding to simple systems, the XX/XY being the most frequent one [52]. Multiple sex chromosomes occurs in a much lower number and, according to Pennel [53], 47 of such occurrences had been so far recorded, encompassing five different kinds of systems, named $♀X_1X_1X_2X_2/♂X_1X_2Y$, $♀XX/♂XY_1Y_2$, $♀X_1X_1X_2X_2/♂X_1Y_1X_2Y_2$, $♂ZZ/♀ZW_1W_2$ and $♂Z_1Z_1Z_2Z_2/♀Z_1W_1Z_2W_2$, the first of them being the most prevalent one [54]. Here, a marked feature of both *H. villasboas* and *H. duriventris* karyotypes is an X1X2Y sex chromosome system. Such occurrences, together with the two previous multiple systems described for *H. punctata* (X_1X_2Y) and *H. carvalhoi* (XY_1Y_2), makes *Harttia* the genus with the most abundant number of multiple sex chromosome systems up to now identified among fishes [53].

It is known that Y-A fusions are the most common rearrangements related to the origin of X_1X_2Y systems [53] and it is likely that this is also true for both *H. duriventris* and *H. villasboas* cases. But the

find of the concomitant proto/neo XY-system in the sister species, *H. rondoni*, adds a relevant question in this evolutionary puzzle. In such system the sex pair differ only slightly by the accumulation of repetitive sequences in only one of the chromosomes, i.e., the Y one. This heteromorphism was not always clearly detected on the pattern of the C-positive heterochromatin, but it was evident after the 18S rDNA mapping and the intraspecific CGH experiments. This scenario alone could raise doubts about the existence of such a proto-XY system, reinforcing the probable neo-origin to this chromosomal sex system. However, when the chromosomal pattern of *H. rondoni* is compared with those of *H. villasboas* and *H. duriventris*, it is possible to establish the karyological relationships among these species supporting the real occurrence of the putative sex chromosomes in *H. rondoni*, and the origin of the multiple X_1X_2Y sex chromosome systems in the two latter species. In fact, comparative genomic hybridization (CGH), plus chromosomal mapping of microsatellites and rDNAs repeats highlight that these three *Harttia* species share the same distribution pattern in the sex chromosomes. Although *H. punctata* also presents an X_1X_2Y system, its rDNA distribution includes both 5S and 18S sequences on the sex chromosomes [11,13], which is different from *H. duriventris* and *H. villasboas* where only 18S motifs occur on them, probably by a translocation of the 5S rDNA motif, present in the X_1 of *H. punctata*, to autosomal chromosomes during lineage diversification in *H. duriventris*, *H. villasboas* and *H. rondoni*.

Meaningly, a close association between microsatellites, rDNAs and multiple sex chromosomes has been reported for many fish taxa [55–59], which highlights the probable role of these sequences in the genesis of such systems. Despite our CGH data do not reveal any conspicuous Y-specific region, neither in both Y chromosomes of *H. duriventris* and *H. villasboas*, nor in the proto/neo-Y of *H. rondoni*, a slight binding preference for the male-derived probe occur at the pericentromeric region of all these chromosomes (Figure 5). Potential effects of repetitive DNA accumulation on recombination rate have been considered [60], what could explain their initial accumulation on the sex chromosomes. When sex chromosomes stop recombination, repetitive sequences are predicted to have a rapid accumulation on them [61], and microsatellite repeats seem to play a key role as "early colonizers" in their differentiation [24,62]. Numerous examples in animals and plants document a massive and differential accumulation of such small motifs in sex-specific chromosomes, particularly in simple (XY or ZW) systems [24,56,63–67]. However, most X_1X_2Y systems lack substantial differentiation in the neo-Y, since the accumulation of repetitive DNAs (= large blocks of heterochromatin) would impair the proper pairing of the neo-sex chromosomes into a stable trivalent form, thus disturbing the meiotic process [68]. Here, no significant differences in microsatellites distribution could be identified concerning autosomes and sex chromosomes of the *Harttia* species. In general, while $(A)_{30}$ sequences presented a strong accumulation pattern in the pericentromeric regions of some chromosomal pairs, both $(CA)_{15}$ and $(GA)_{15}$ displayed a widespread distribution pattern, with preferential accumulation in some telomeric regions (Figure 3). Besides, as evidenced by C-banding and CGH experiments, all the X_1, X_2 and Y chromosomes do not accumulate repetitive sequences, and the Y chromosome of *H. rondoni* is even missing $(A)_{30}$ sequences probably lost after the chromosomal rearrangements related to its genesis. Thus, the recombination suppression in multiple sex chromosome system is a question that deserves to be better clarified.

According to the most updated phylogeny proposed for the genus *Harttia* [19]. *H. duriventris*, *H. villasboas*, and *H. punctata*, all harboring multiple X_1X_2Y sex system, are evolutionary related and belong to the same major clade. In accordance with, our CGH experiments showed that they sex chromosomes share genomic content, thus pointing to their relatedness and to a probable common origin. This is a characteristic that, in general, is not found among teleosts species. Although some *Megaleporinus* [69], *Parodon* [70] and *Characidium* species [71], as well as the whole genus *Triportheus* [66,72], have their sex chromosomes with a common origin and differentiation, this is an exceptional scenario [73]. In fact, an independent origin is more commonly found, even among congeneric species [49,52,74–78]. According to Schartl [79], such a situation could be explained by the emergence of sex-determining genes on different chromosomes which, by mutation, would promote male or female development and giving rise to new sex chromosomes. Next steps, implying

finer-scale approaches such as Zoo-FISH experiments with whole chromosome painting (WCP), coupled with recent genome sequencing procedures, will shed more light on this issue, especially in the recent or ancient origin of the XY system of *H. rondoni* and its significance to the group.

5. Conclusions

Chromosomal data of the Brazilian northern *Harttia* species (*H. duriventris*, *H. villaboas*, *H. rondoni* and *H. punctata*) support the common origin for their sex chromosome systems. Additionally, the results also allowed us: (i) to track their evolutionary relationships with other *Harttia* species, adding new light on their relatedness inside the genus; (ii) to describe two new multiple sex chromosomes systems of the X_1X_2Y type; and (iii) to highlight a proto or neo-XY system in *H. rondoni* and its close association with the aforementioned multiple sex systems. The remarkable variation on karyotypic organization, in addition to the frequency and different types of sex chromosomes systems inside the genus, makes *Harttia* an useful model for evolutionary studies among fish focusing on karyotype differentiation and sex chromosomes evolution.

Author Contributions: Conceptualization, F.d.M.C.S.; G.A.D.; O.M.-F.; M.R.V.; M.d.B.C..; Formal analysis, F.d.M.C.S., G.A.D.; O.M.-F., M.R.V., T.L., E.A.d.O., M.d.B.C.; Funding acquisition, O.M.-F., L.A.C.B., M.d.B.C.; Investigation, F.d.M.C.S., O.M.-F., M.d.B.C.; Methodology, F.d.M.C.S., G.A.D., E.A.d.O; Project administration, L.A.C.B., T.L., M.d.B.C.; Resources, T.L.; Supervision, O.M.-F., M.d.B.C; Validation, T.L., E.A.O.; Visualization, F.d.M.C.S., G.A.D., O.M.-F., M.R.V., L.A.C.B., T.L., E.A.d.O., M.d.B.C.; Writing—original draft, F.d.M.C.S.; G.A.D., M.R.V., M.d.B.C.; Writing—review and editing, F.d.M.C.S., G.A.D., O.M.-F., M.R.V., L.A.C.B., T.L., E.A.d.O., M.d.B.C. All authors have read and agreed to the published version of the manuscript.

Funding: MBC was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (302449/2018-3) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (2018/22033-1). FMCS was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (2020/02681-9). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil (CAPES), Finance Code 001.

Acknowledgments: The authors are grateful to Osvaldo Oyakawa (curator of the fish collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP) for the proper specimens´ identification.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Ferraris, C.J. *Checklist of Catfishes, Recent and Fossil (Osteichthyes: Siluriformes), and Catalogue of Siluriform Primary Types*; Magnolia Press, Auckland, New Zealand, 2007; ISBN 9781869770587.
2. Fricke, R.; Eschmeyer, W.N.; van der Laan, R. Eschmeyer's Catalog of Fishes: Genera, Species. Available online: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (accessed on 12 February 2020).
3. Traldi, J.B.; Lui, R.L.; Martinez, J.d.F.; Vicari, M.R.; Nogaroto, V.; Filho, O.M.; Blanco, D.R. Chromosomal distribution of the retroelements Rex1, Rex3 and Rex6 in species of the genus *Harttia* and *Hypostomus* (Siluriformes: Loricariidae). *Neotrop. Ichthyol.* **2019**, *17*, 1–10.
4. De Oliveira, J.C.; Oyakawa, O.T. New loricariid fishes from headwaters on Serra da Mantiqueira and Complexo do Espinhaço, Minas Gerais state, Brazil (Teleostei: Siluriformes: Loricariidae). *Zootaxa* **2019**, *4586*, 401–424.
5. Alves, A.L.; Oliveira, C.; Foresti, F. Karyotype variability in eight species of the subfamilies Loricariinae and Ancistrinae (Teleostei, Siluriformes, Loricariidae). *Caryologia* **2003**, *56*, 57–63.
6. Kavalco, K.F.; Pazza, R.; Bertollo, L.A.C.; Moreira-Filho, O. Heterochromatin characterization of four fish species of the family Loricariidae (Siluriformes). *Hereditas* **2004**, *141*, 237–242.
7. Kavalco, K.F.; Pazza, R.; Bertollo, L.A.C.; Moreira-Filho, O. Karyotypic diversity and evolution of Loricariidae (Pisces, Siluriformes). *Heredity* **2005**, *94*, 180–186.

8. Centofante, L.; Bertollo, L.A.C.; Moreira-Filho, O. Cytogenetic characterization and description of an XX/XY1Y2 sex chromosome system in catfish *Harttia carvalhoi* (Siluriformes, Loricariidae). *Cytogenet. Genome Res.* **2006**, *112*, 320–324.
9. Rodrigues, R.M. *Estudos Cromossômicos e Moleculares em Loricariinae Com Ênfase em Espécies de Rineloricaria (Siluriformes, Loricariidae): Uma Perspectiva Evolutiva*; Universidade de São Paulo: São Paulo, Brazil, 2010.
10. Blanco, D.R.; Vicari, M.R.; Artoni, R.F.; Traldi, J.B.; Moreira-Filho, O. Chromosomal Characterization of Armored Catfish *Harttia longipinna* (Siluriformes, Loricariidae): First Report of B Chromosomes in the Genus. *Zool. Sci.* **2012**, *29*, 604–609.
11. Blanco, D.R.; Vicari, M.R.; Lui, R.L.; Bertollo, L.A.C.; Traldi, J.B.; Moreira-Filho, O. The role of the Robertsonian rearrangements in the origin of the XX/XY1Y2 sex chromosome system and in the chromosomal differentiation in *Harttia* species (Siluriformes, Loricariidae). *Rev. Fish Biol. Fish.* **2013**, *23*, 127–134.
12. Blanco, D.R.; Vicari, M.R.; Lui, R.L.; Artoni, R.F.; de Almeida, M.C.; Traldi, J.B.; Margarido, V.P.; Moreira-Filho, O. Origin of the X1X1X2X2/X1X2Y sex chromosome system of *Harttia punctata* (Siluriformes, Loricariidae) inferred from chromosome painting and FISH with ribosomal DNA markers. *Genetica* **2014**, *142*, 119–126.
13. Blanco, D.R.; Vicari, M.R.; Lui, R.L.; Traldi, J.B.; Bueno, V.; Martinez, J.d.F.; Brandão, H.; Oyakawa, O.T.; Moreira Filho, O. Karyotype Diversity and Evolutionary Trends in Armored Catfish Species of the Genus *Harttia* (Siluriformes: Loricariidae). *Zebrafish* **2017**, *14*, 169–176.
14. Cioffi, M.B.; Bertollo, L.A.C. *Chromosomal Distribution and Evolution of Repetitive DNAs in Fish*; Garrido-Ramos, M.A., Ed.; Genome Dynamics, v. 7: Karger, Basel, Switzerland, 2012, pp. 197–221.
15. Barby, F.F.; Bertollo, L.A.C.; de Oliveira, E.A.; Yano, C.F.; Hatanaka, T.; Ráb, P.; Sember, A.; Ezaz, T.; Artoni, R.F.; Liehr, T.; et al. Emerging patterns of genome organization in Notopteridae species (Teleostei, Osteoglossiformes) as revealed by Zoo-FISH and Comparative Genomic Hybridization (CGH). *Sci. Rep.* **2019**, *9*, 1112.
16. de Menezes Cavalcante Sassi, F.; Aguiar de Oliveira, E.; Bertollo, L.A.C.; Nirchio, M.; Hatanaka, T.; Marinho, M.M.F.; Moreira-Filho, O.; Aroutiounian, R.; Liehr, T.; Al-Rikabi, A.B.H.; et al. Chromosomal evolution and evolutionary relationships of *Lebiasina* species (Characiformes, Lebiasinidae). *Int. J. Mol. Sci.* **2019**, *20*, 2944.
17. Moraes, R.L.R. de; Sember, A.; Bertollo, L.A.; De Oliveira, E.A.; Rab, P.; Hatanaka, T.; Marinho, M.M.; Liehr, T.; Al-Rikabi, A.B.; Feldberg, E.; et al. Comparative cytogenetics and neo-Y formation in small-sized fish species of the genus *Pyrrhulina* (Characiformes, Lebiasinidae). *Front. Genet.* **2019**, *10*, 1–13.
18. Toma, G.A.; De Moraes, R.L.R.; Sassi, F.D.M.C.; Bertollo, L.A.C.; De Oliveira, E.A.; Rab, P.; Sember, A.; Liehr, T.; Hatanaka, T.; Viana, P.F.; et al. Cytogenetics of the small-sized fish, *Copeina guttata* (Characiformes, Lebiasinidae): Novel insights into the karyotype differentiation of the family. *PLoS ONE* **2019**, *14*, 1–13.
19. Covain, R.; Fisch-Muller, S.; Oliveira, C.; Mol, J.H.; Montoya-Burgos, J.I.; Dray, S. Molecular phylogeny of the highly diversified catfish subfamily Loricariinae (Siluriformes, Loricariidae) reveals incongruences with morphological classification. *Mol. Phylogenetics Evol.* **2016**, *94*, 492–517.
20. Bertollo, L.A.C.; Cioffi, M. de B.; Galetti, P.M.; Filho, O.M. Contributions to the cytogenetics of the Neotropical fish fauna. *Comp. Cytogenet.* **2017**, *11*, 665–690.
21. Sumner, A.T. A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* **1972**, *75*, 304–306.
22. Pendás, A.M.; Móran, P.; Freije, J.P.; Garcia-Vásquez, E. Chromosomal location and nucleotide sequence of two tandem repeats of the Atlantic salmon 5S rDNA. *Cytogenet. Genome Res.* **1994**, *67*, 31–36.
23. Cioffi, M.d.B.; Martins, C.; Centofante, L.; Jacobina, U.P.; Bertollo, L.A.C. Chromosomal variability among allopatric populations of Erythrinidae fish *Hoplias malabaricus*: Mapping of three classes of repetitive DNAs. *Cytogenet. Genome Res.* **2009**, *125*, 132–141.
24. Kubat, Z.; Hobza, R.; Vyskot, B.; Kejnovský, E. Microsatellite accumulation in the Y chromosome of *Silene latifolia*. *Genome* **2008**, *51*, 350–356.
25. Yano, C.F.; Bertollo, L.A.C.; Cioffi, M.B. Fish-FISH: Molecular cytogenetics in fish species. In *Fluorescence in Situ Hybridization (FISH)- Application Guide*; Liehr, T., Ed.; Springer: Berlin, Germany, 2017; pp. 429–444.
26. Sambrook, J.; Russell, D.W. *Molecular Cloning, A Laboratory Manual*; Cold Spring Harbor Laboratory Press: New York, NY, USA, 2001.

27. Zwick, M.S.; Hanson, R.E.; Mcknight, T.D.; Islam-Faridi, M.H.; Stelly, D.M.; Wing, R.A.; Price, H.J. A rapid procedure for the isolation of C 0 t-1 DNA from plants. *Genome* **1997**, *40*, 138–142.
28. Moraes, R.L.R. de; Bertollo, L.A.C.; Marinho, M.M.F.; Yano, C.F.; Hatanaka, T.; Barby, F.F.; Troy, W.P.; Cioffi, M.D.B. Evolutionary relationships and cytotaxonomy considerations in the genus *Pyrrhulina* (Characiformes, Lebiasinidae). *Zebrafish* **2017**, *14*, 536–546.
29. Sassi FMC, Hatanaka T, Moraes RLR, Toma GA, Oliveira EA, Liehr T, Rab P, Bertollo LAC, Viana PF, Feldberg E, Nirchio MT, Marinho MMF, Souza JFS, Cioffi MB. An insight into the chromosomal evolution of Lebiasinidae (Teleostei, Characiformes). *Genes* **2020**, *11*, 365
30. Symonová, R.; Sember, A.; Majtánová, Z.; Ráb, P. Characterization of fish genome by GISH and CGH. In *Fish Cytogenetic Techniques. Ray-Fin Fishes and Chondrichthyans*; Ozouf-Costaz, C., Pisano, E., Foresti, F., de Almeida-Toledo, L.F., Eds.; CCR Press: Boca Raton, FL, USA, 2015; pp. 118–131.
31. Levan, A.; Fredga, K.; Sandberg, A.A. Nomenclature for centromeric position on chromosomes. *Hereditas* **1964**, *52*, 201–220.
32. Karr, Jr. and James, F.C. Eco-morphological configurations and convergent evolution in species and communities. In: *Ecology and evolution of communities*; Cody, M.L., Diamond, J.M., Eds.; Harvard University Press, Cambridge, UK, 1975; pp. 258–291.
33. Wainwright, P.C.; Reilly, S.M. *Ecological Morphology: Integrative Organismal Biology*; University of Chicago Press: Chicago, IL, USA, 1994.
34. Pazza, R.; Kavalco, K.F.; Bertollo, L.A.C. Chromosome polymorphism in *Astyanax fasciatus* (Teleostei, Characidae). 1. Karyotype analysis, Ag-NORs and mapping of the 18S and 5S ribosomal genes in sympatric karyotypes and their possible hybrid forms. *Cytogenet. Genome Res.* **2006**, *112*, 313–319.
35. Ferreira-Neto, M.; Artoni, R.; Vicari, M.; Moreira Filho, O.; Camacho, J.; Bakkali, M.; Oliveira, C.; Foresti, F. Three sympatric karyomorphs in the fish *Astyanax fasciatus* (Teleostei, Characidae) do not seem to hybridize in natural populations. *Comp. Cytogenet.* **2012**, *6*, 29–40.
36. Pazza, R.; Dergam, J.A.; Kavalco, K.F. Trends in karyotype evolution in *Astyanax* (Teleostei, Characiformes, Characidae): Insights from molecular data. *Front. Genet.* **2018**, *9*, 131.
37. 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 Res.* **2000**, *8*, 603–613.
38. Vicari, M.R.; Artoni, R.F.; Bertollo, L.A.C. Comparative cytogenetics of *Hoplias malabaricus* (Pisces , Erythrinidae): A population analysis in adjacent hydrographic basins. *Genet. Mol. Biol.* **2005**, *110*, 103–110.
39. Ibagón, N.; Maldonado-Ocampo, J.A.; Cioffi, M.d.B.; Dergam, J.A. Chromosomal Diversity of *Hoplias malabaricus* (Characiformes, Erythrinidae) Along the Magdalena River (Colombia—Northern South America) and Its Significance for the Neotropical Region . *Zebrafish* **2020**, *17*, 211–219.
40. Lundberg, J.G.; Marshall, L.G.; Guerrero, J.; Horton, B.; Malabarba, M.; Wesselingh, F. The stage for Neotropical fish diversification: A history of tropical South American rivers. In *Phylogeny and Classification of Neotropical Fishes*; Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, C.A.S., Eds.; Edipucrs Porto Alegre: Porto Alegre, Brazil, 1998; Volume 27, pp. 13–48.
41. Oyakawa, O.T.; Fichberg, I.; Py-Daniel, L.R. Three new species of *Harttia* (Loricariidae: Loricariinae) from Serra do Cachimbo, Rio Xingu basin, Pará, Northern Brazil. *Zootaxa* **2018**, *4387*, 75–90.
42. Birindelli, J.L.O.; Britski, H.A. New species of the genus *Leporinus* Agassiz (Characiformes: Anostomidae) from the rio Curuá, rio Xingu basin, Serra do Cachimbo, Brazil, with comments on *Leporinus reticulatus*. *Neotrop. Ichthyol.* **2009**, *7*, 1–10.
43. Netto-Ferreira, A.L. Three new species of *Lebiasina* (Characiformes: Lebiasinidae) from the Brazilian shield border at Serra do Cachimbo, Pará, Brazil. *Neotrop. Ichthyol.* **2012**, *10*, 487–498.
44. Varella, H.R.; Sabaj Pérez, M.H. A titan among dwarfs: *Apistogramma kullanderi*, new species (Teleostei: Cichlidae). *Ichthyol. Explor. Freshw.* **2014**, *25*, 243–258.
45. Meyne, J.; Ratliff, R.L.; Moyzis, R.K. Conservation of the human telomere sequence (TTAGGG)n among vertebrates. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 7049–7053.
46. de Freitas, N.L.; Al-Rikabi, A.B.H.; Bertollo, L.A.C.; Ezaz, T.; Yano, C.F.; de Oliveira, E.A.; Hatanaka, T.; de Bello Cioffi, M. Early Stages of XY Sex Chromosomes Differentiation in the Fish *Hoplias malabaricus* (Characiformes, Erythrinidae) Revealed by DNA Repeats Accumulation. *Curr. Genom.* **2017**, *19*, 216–226.
47. Gammerdinger, W.J.; Kocher, T.D. Unusual Diversity of Sex Chromosomes in African Cichlid Fishes. *Genes* **2018**, *9*, 480.

48. Kottler, V.; Schartl, M. The Colorful Sex Chromosomes of Teleost Fish. *Genes* **2018**, *9*, 233.
49. Schartl, M.; Schmid, M.; Nanda, I. Dynamics of vertebrate sex chromosome evolution: From equal size to giants and dwarfs. *Chromosoma* **2016**, *125*, 553–571.
50. Guiguen, Y.; Pasquier, J.; Fostier, A.; Bobe, J. Evolution of Sex Hormone Binding Globulins Reveals Early Gene Duplication at the Root of Vertebrates. *bioRxiv* **2020**, doi:10.1101/2020.07.02.184432.
51. Arai, R. *Fish Karyotypes: A Check List*; Springer Science & Business Media: Heidelberg, Germany, 2011.
52. Devlin, R.H.; Nagahama, Y. Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* **2002**, *208*, 191–364.
53. Pennell, M.W.; Kirkpatrick, M.; Otto, S.P.; Vamosi, J.C.; Peichel, C.L.; Valenzuela, N.; Kitano, J. Y Fuse? Sex Chromosome Fusions in Fishes and Reptiles. *PLoS Genet.* **2015**, *11*, 1–17.
54. Kitano, J.; Peichel, C.L. Turnover of sex chromosomes and speciation in fishes. *Environ. Biol. Fishes* **2012**, *94*, 549–558.
55. Cioffi, M.B.; Bertollo, L.A.C. Initial steps in XY chromosome differentiation in *Hoplias malabaricus* and the origin of an X1X2Y sex chromosome system in this fish group. *Heredity* **2010**, *105*, 554–561.
56. Cioffi, M.B.; Camacho, J.P.M.; Bertollo, L.A.C. Repetitive DNAs and differentiation of sex chromosomes in neotropical fishes. *Cytogenet. Genome Res.* **2011**, *132*, 188–194.
57. Bitencourt, J.A.; Sampaio, I.; Ramos, R.T.C.; Vicari, M.R.; Affonso, P.R.A.D.M. First Report of Sex Chromosomes in Achiridae (Teleostei: Pleuronectiformes) with Inferences about the Origin of the Multiple X1X1X2X2/X1X2Y System and Dispersal of Ribosomal Genes in Achirus achirus. *Zebrafish* **2017**, *14*, 90–95.
58. Ferreira, M.; Garcia, C.; Matoso, D.A.; de Jesus, I.S.; Feldberg, E. A new multiple sex chromosome system X1X1X2X2/X1Y1X2Y2 in Siluriformes: Cytogenetic characterization of *Bunocephalus coracoideus* (Aspredinidae). *Genetica* **2016**, *144*, 591–599.
59. Xu, D.; Sember, A.; Zhu, Q.; De Oliveira, E.A.; Liehr, T.; Al-Rikabi, A.B.H.; Xiao, Z.; Song, H.; De Bello Cioffi, M. Deciphering the Origin and Evolution of the X1X2Y System in Two Closely-Related *Oplegnathus* Species (Oplegnathidae and Centrarchiformes). *Int. J. Mol. Sci.* **2019**, *20*, 1–16.
60. Reed, K.M.; Phillips, R.B. Polymorphism of the nucleolus organizer region (NOR) on the putative sex chromosomes of Arctic char (*Salvelinus alpinus*) is not sex related. *Chromosome Res.* **1997**, *5*, 221–227.
61. Charlesworth, D.; Charlesworth, B.; Marais, G. Steps in the evolution of heteromorphic sex chromosomes. *Heredity* **2005**, *95*, 118–128.
62. Cioffi, M.B.; Moreira-Filho, O.; Almeida-Toledo, L.F.; Bertollo, L.A.C. The contrasting role of heterochromatin in the differentiation of sex chromosomes: An overview from Neotropical fishes. *J. Fish Biol.* **2012**, *80*, 2125–2139.
63. Phillips, B.C.; Edmands, S. Does the speciation clock tick more slowly in the absence of heteromorphic sex chromosomes? *BioEssays* **2012**, *34*, 166–169.
64. Poltronieri, J.; Marquioni, V.; Bertollo, L.A.C.; Kejnovsky, E.; Molina, W.F.; Liehr, T.; Cioffi, M.B. Comparative chromosomal mapping of microsatellites in *Leporinus* species (Characiformes, Anostomidae): Unequal accumulation on the W chromosomes. *Cytogenet. Genome Res.* **2013**, *142*, 40–45.
65. Sabath, N.; Itescu, Y.; Feldman, A.; Meiri, S.; Mayrose, I.; Valenzuela, N. Sex determination, longevity, and the birth and death of reptilian species. *Ecol. Evol.* **2016**, *6*, 5207–5220.
66. Yano, C.F.; Bertollo, L.A.C.; Liehr, T.; Troy, W.P.; Cioffi, M.D.B. W Chromosome Dynamics in *Triportheus* Species (Characiformes, Triportheidae): An Ongoing Process Narrated by Repetitive Sequences. *J. Hered.* **2016**, *107*, 342–348.
67. Viana, P.F.; Ezaz, T.; de Bello Cioffi, M.; Liehr, T.; Al-Rikabi, A.; Goll, L.G.; Rocha, A.M.; Feldberg, E. Landscape of snake' sex chromosomes evolution spanning 85 MYR reveals ancestry of sequences despite distinct evolutionary trajectories. *Sci. Rep.* **2020**, *10*, 1–14.
68. Moreira-Filho, O.; Bertollo, L.A.C.; Galetti, P.M. Distribution of sex chromosome mechanisms in neotropical fish and description of a ZZ/ZW system in *Parodon hilarii* (Parodontidae). *Caryologia* **1993**, *46*, 115–125.
69. Ramirez, J.L.; Birindelli, J.L.; Carvalho, D.C.; Affonso, P.R.; Venere, P.C.; Ortega, H.; Carrillo-Avila, M.; Rodríguez-Pulido, J.A.; Galetti, P.M., Jr. Revealing hidden diversity of the underestimated neotropical ichthyofauna: DNA barcoding in the recently described genus Megaleporinus (Characiformes: Anostomidae). *Front. Genet.* **2017**, *8*, 149.

70. Schemberger, M.O.; Bellafronte, E.; Nogaroto, V.; Almeida, M.C.; Schühli, G.S.; Artoni, R.F.; Moreira-Filho, O.; Vicari, M.R. Differentiation of repetitive DNA sites and sex chromosome systems reveal closely related group in Parodontidae (Actinopterygii: Characiformes). *Genetica* **2011**, *139*, 1499–1508.
71. Pucci, M.B.; Nogaroto, V.; Bertollo, L.A.C.; Moreira-Filho, O.; Vicari, M.R. The karyotypes and evolution of ZZ/ZW sex chromosomes in the genus *Characidium* (Characiformes, Crenuchidae). *Comp. Cytogenet.* **2018**, *12*, 421.
72. Yano, C.F.; Bertollo, L.A.C.; Ezaz, T.; Trifonov, V.; Sember, A.; Liehr, T.; Cioffi, M.B. Highly conserved Z and molecularly diverged W chromosomes in the fish genus *Triportheus* (Characiformes, Triportheidae). *Heredity* **2017**, *118*, 276–283.
73. Cioffi, M.D.B.; Yano, C.F.; Sember, A.; Bertollo, L.A.C. Chromosomal evolution in lower vertebrates: Sex chromosomes in neotropical fishes. *Genes* **2017**, *8*, 258.
74. Takehana, Y.; Naruse, K.; Hamaguchi, S.; Sakaizumi, M. Evolution of ZZ/ZW and XX/XY sex-determination systems in the closely related medaka species, *Oryzias hubbsi* and *O. dancena*. *Chromosoma* **2007**, *116*, 463–470.
75. Tanaka, K.; Takehana, Y.; Naruse, K.; Hamaguchi, S.; Sakaizumi, M. Evidence for different origins of sex chromosomes in closely related oryzias fishes: Substitution of the master sex-determining gene. *Genetics* **2007**, *177*, 2075–2081.
76. Mank, J.E.; Avise, J.C. Evolutionary diversity and turn-over of sex determination in teleost fishes. *Sex. Dev.* **2009**, *3*, 60–67.
77. Schultheis, C.; Böhne, A.; Schartl, M.; Volff, J.N.; Galiana-Arnoux, D. Sex determination diversity and sex chromosome evolution in poeciliid fish. *Sex. Dev.* **2009**, *3*, 68–77.
78. Cioffi, M.B.; Liehr, T.; Trifonov, V.; Molina, W.F.; Bertollo, L.A.C. Independent sex chromosome evolution in lower vertebrates: A molecular cytogenetic overview in the erythrinidae fish family. *Cytogenet. Genome Res.* **2013**, *141*, 186–194.
79. Schartl, M. Sex chromosome evolution in non-mammalian vertebrates. *Curr. Opin. Genet. Dev.* **2004**, *14*, 634–641.

II – Sassi *et al.* (2021) Adding New Pieces to the Puzzle of Karyotype Evolution in Harttia (Siluriformes, Loricariidae): Investigation of Amazonian Species. Biology 10(9), 922.

biology

MDPI

Article

Adding New Pieces to the Puzzle of Karyotype Evolution in *Harttia* (Siluriformes, Loricariidae): Investigation of Amazonian Species

Francisco de M. C. Sassi [1], Orlando Moreira-Filho [1], Geize A. Deon [1,2], Alexandr Sember [3], Luiz A. C. Bertollo [1], Thomas Liehr [4,*], Vanessa C. S. Oliveira [1], Patrik F. Viana [5], Eliana Feldberg [5], Marcelo R. Vicari [2] and Marcelo de B. Cioffi [1]

1 Laboratório de Citogenética de Peixes, Departamento de Genética e Evolução, Universidade Federal de São Carlos, São Carlos 13565-905, SP, Brazil; fmcsassi@estudante.ufscar.br (F.d.M.C.S.); omfilho@ufscar.br (O.M.-F.); gdeon@estudante.ufscar.br (G.A.D.); bertollo@ufscar.br (L.A.C.B.); vcsoliveira@estudante.ufscar.br (V.C.S.O.); mbcioffi@ufscar.br (M.d.B.C.)
2 Laboratório de Biologia Cromossômica, Estrutural e Função, Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Ponta Grossa 84030-900, PR, Brazil; vicarimr@pq.cnpq.br
3 Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Rumburská 89, 277-21 Liběchov, Czech Republic; sember@iapg.cas.cz
4 Institute of Human Genetics, University Hospital Jena, Friedrich Schiller University, 07747 Jena, Germany
5 Laboratório de Genética Animal, Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Manaus 69067-375, AM, Brazil; patrik.biologia@gmail.com (P.F.V.); feldberg@inpa.gov.br (E.F.)
* Correspondence: Thomas.Liehr@med.uni-jena.de; Tel.: +49-3641-9396850; Fax: +49-3641-9396852

Simple Summary: Fishes represent a useful model for evolutionary studies, given their diversity of species and habitats. In this study, we investigate the chromosomes of three unexplored *Harttia* fish species from the Amazonian region and compare the obtained data with previous analyses. Our data reveal that both *Harttia dissidens* and *Harttia* sp. 3 exhibit the same number of chromosomes in their cells (54), but that they differ in the karyotype organization. *Harttia guianensis* possesses 58 chromosomes, being thus the first representative from north Brazil to present this feature for both sexes. Although otherwise rather common in *Harttia* species, we observed no chromosomal differences between sexes in all but one species. Namely in *Harttia* sp. 3, we revealed signs of initial differentiation between homologues of one chromosome pair in males but not in females. Altogether, our data bring new evidence strengthening the view that *Harttia* spp. represent an informative model for studying patterns of karyotype and sex chromosome dynamics in teleost fishes.

Abstract: A remarkable morphological diversity and karyotype variability can be observed in the Neotropical armored catfish genus *Harttia*. These fishes offer a useful model to explore both the evolution of karyotypes and sex chromosomes, since many species possess male-heterogametic sex chromosome systems and a high rate of karyotype repatterning. Based on the karyotype organization, the chromosomal distribution of several repetitive DNA classes, and the rough estimates of genomic divergences at the intraspecific and interspecific levels via Comparative Genomic Hybridization, we identified shared diploid chromosome numbers (2n = 54) but different karyotype compositions in *H. dissidens* (20m + 26sm + 8a) and *Harttia* sp. 3 (16m + 18sm + 14st + 6a), and different 2n in *H. guianensis* (2n = 58; 20m + 26sm + 2st + 10a). All species further displayed similar patterns of chromosomal distribution concerning constitutive heterochromatin, 18S ribosomal DNA (rDNA) sites, and most of the surveyed microsatellite motifs. Furthermore, differences in the distribution of 5S rDNA sites and a subset of microsatellite sequences were identified. Heteromorphic sex chromosomes were lacking in *H. dissidens* and *H. guianensis* at the scale of our analysis. However, one single chromosome pair in *Harttia* sp. 3 males presented a remarkable accumulation of male genome-derived probe after CGH, pointing to a tentative region of early sex chromosome differentiation. Thus, our data support already previously outlined evidence that *Harttia* is a vital model for the investigation of teleost karyotype and sex chromosome dynamics.

Citation: Sassi, F.d.M.C.; Moreira-Filho, O.; Deon, G.A.; Sember, A.; Bertollo, L.A.C.; Liehr, T.; Oliveira, V.C.S.; Viana, P.F.; Feldberg, E.; Vicari, M.R.; et al. Adding New Pieces to the Puzzle of Karyotype Evolution in *Harttia* (Siluriformes, Loricariidae): Investigation of Amazonian Species. *Biology* **2021**, *10*, 922. https://doi.org/10.3390/biology10090922

Academic Editor: Jordi Garcia-Fernàndez

Received: 25 August 2021
Accepted: 13 September 2021
Published: 16 September 2021

Keywords: chromosomes; comparative genomic hybridization (CGH); repetitive DNA; sex chromosomes

1. Introduction

With more than 35,000 species [1], fishes have the richest biodiversity among vertebrates [2,3]. The evolutionary success of ray-finned and especially teleost fishes seem to be, at least partially, related to a higher plasticity of their genomes when compared to other vertebrates [4–7], going hand in hand with their propensity towards polyploidization and hybridization events [8,9]. Another factor at play is the wealth of aquatic niches fishes adapted to [2,10–12], and the geomorphological parameters affecting the dispersion and populational dynamics in particular species (e.g., [13,14]). In effect, at the level of genome organization, fishes present a wide range of chromosome counts and genome sizes [15,16] and the karyotype dynamics may differ considerably among lineages [17]. Finally, fishes encompass astounding variability in mechanisms of sex determination and differentiation [18–22].

Siluriformes (Teleostei, Ostariophysi) represent a lineage with high diversification, comprising more than 3000 species distributed among 39 families [1]. Inside Siluriformes, the family Loricariidae whose representatives are commonly known as "armored catfishes" (due to ossified plates covering their bodies; [23]) encompasses 1015 recognized species included in more than 100 genera [1]. It is hence the most species-rich siluriform family. These fishes are distributed throughout the Neotropical region, but with the greatest diversity being found from north Costa Rica to south Argentina [24]. Notably, the diversification is associated with huge karyotype variability in Loricariidae, with diploid chromosome numbers (2n) ranging from 36 in *Rineloricaria latirostris* to 74 in *Sturisoma* cf. *nigrirostrum* [25–27]. Additionally, several types of sex chromosome systems including standard, derived, and multiple ones have been described for Loricariidae, as exemplified by *Hypostomus* genus with different ZW and XY cases [28–31], XX/XY, XX/X0, ZZ/ZW, and $Z_1Z_1Z_2Z_2/Z_1Z_2W_1W_2$ in several *Ancistrus* species (reviewed in ref. [32]), and three male-heterogametic systems in the genus *Harttia* (see below).

In this context, the genus *Harttia* can be recognized as a remarkable model for cytogenetic studies, especially to investigate the evolutionary processes related to sex chromosomes, diversification and karyotype dynamics [33–36]. *Harttia* harbors 27 recognized species [1,37], of which only 14 have been cytogenetically analyzed to date, along with several other *Harttia* spp. waiting for proper taxonomic description (summarized in [35]). This genus also encompasses the second-largest variation in the 2n among Loricariidae, ranging from 2n = 52 (females)/53 (males) in *Harttia carvalhoi* [33] to 2n = 62 in *H. absaberi* and *Harttia* sp. 2 [35,38]. Furthermore, three different male-heterogametic sex chromosome systems are known or have been suggested to exist among *Harttia* species: XX/XY_1Y_2 in *H. carvalhoi*, *H. intermontana*, and *Harttia* sp. 1 [33,35], $X_1X_1X_2X_2/X_1X_2Y$ in *H. punctata*, *H. villasboas,* and *H. duriventris* [34,36], in addition to a putative XX/XY in *H. rondoni* [36].

Harttia kronei was the first species analyzed cytogenetically in the genus [39] and, for a long time, a single representative of *Harttia* was studied from this perspective in the northern Brazilian region: the species *H. punctata* [34]. However, this gap has been progressively closed with our previous studies on *Harttia duriventris*, *H. rondoni*, and *H. villasboas* [36]. Here, we aim to cytogenetically investigate another three representatives from this region. For this, we applied C-banding, repetitive DNA mapping, and comparative genomic hybridization (CGH) experiments in *H. dissidens*, *H. guianensis*, and *Harttia* sp. 3. Our results highlight the high chromosomal dynamics within the investigated species and a putative early stage of sex chromosome differentiation, particularly in *Harttia* sp. 3.

2. Materials and Methods

2.1. Sampling

Three collection sites in the Brazilian Pará state (Figure 1, Table 1) were accessed, allowing the sampling of three species: *Harttia dissidens*, *H. guianensis*, and another yet undescribed taxon herein referred to as *Harttia* sp. 3.

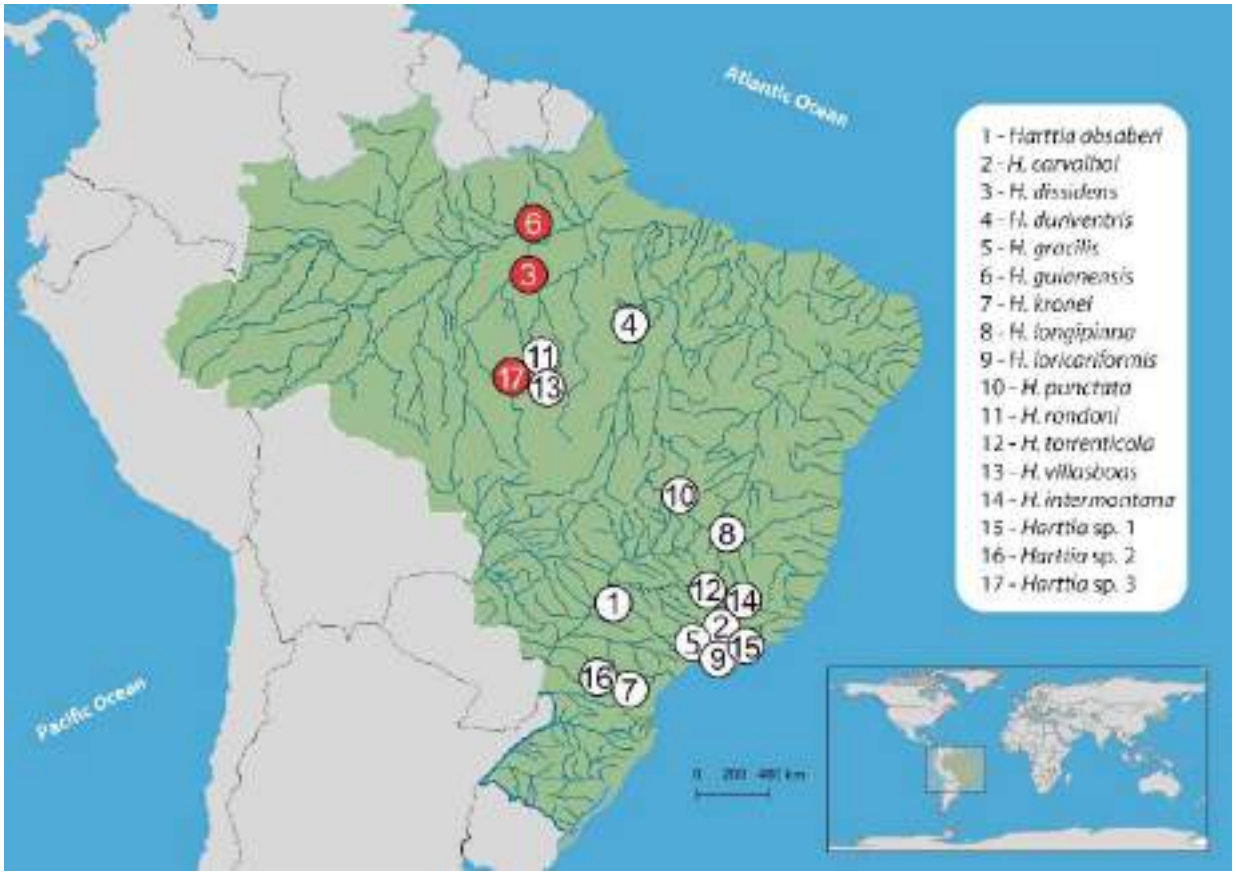


Figure 1. Brazilian territory (green), and the *Harttia* species cytogenetically analyzed in former reports [34–36,38] (white circles), including the present study (red circles). Each *Harttia* species is presented by a certain number; the table with the coding system is presented in the white frame on the right.

Table 1. Geographic coordinates of the collection sites and the number of specimens per species analyzed in this study. The number codes placed before the species names correspond to the numbering system in Figure 1.

Species	Locality	n
3-*Harttia dissidens*	Upper section of Grim waterfall, Tambor stream, Rurópolis–PA, Brazil (4°5′37.8″ S 55°0′30.2″ W)	25♂, 07♀
6-*Harttia guianensis*	Paraíso stream (formerly known as Inferno stream), Alenquer–PA, Brazil (1°29′02.2″ S 54°50′31.2″ W)	10♂, 06♀
17-*Harttia* sp. 3	Rio do Peixe, Cachoeira da Serra, Altamira–PA (08°39′20.7″ S 55°09′24.1″ W)	15♂,11♀

Sampling was done with the authorization of the environmental agency ICMBIO/SISBIO (License 48628-14) and SISGEN (A96FF09). The species *Harttia dissidens* and *H. guianensis* were identified by Dr. Lúcia Helena Rapp-Py Daniel, curator of the fish collection of the Instituto Nacional de Pesquisa da Amazônia (INPA) and deposited there under the voucher numbers INPA-ICT 059577 and INPA-ICT 059584, respectively. The specimens

of *Harttia* sp. 3 were scientifically described by Dr. Oswaldo Takeshi Oyakawa, from the Museu de Zoologia da Universidade de São Paulo (MZUSP).

2.2. Chromosome Obtainment and C-Banding

The living animals were anesthetized with Eugenol before the anterior kidney extraction for the obtainment of mitotic chromosomes following the classical air-drying protocol [40]. Experiments followed the ethical conducts approved by the Ethics Committee on Animal Experimentation of the Universidade Federal de São Carlos (Process number CEUA 1853260315). The detection of constitutive heterochromatin regions followed the C-banding protocol [41]. The C-banding procedure was performed on the slides previously used for the mapping of ribosomal DNAs, to allow a sequential analysis of the same metaphase plates.

2.3. Fluorescence In Situ Hybridization (FISH)-Based Experiments

Ribosomal DNA (rDNA), microsatellite, and the telomeric $(TTAGGG)_n$ sequences were used as probes for chromosomal mapping by FISH. The 5S rDNA probe was prepared according to Pendás et al. [42] from *Hoplias malabaricus* genome, containing a 120-base pair (bp) segment of the 5S rDNA transcribed region and 200 bp of a non-transcribed spacer (NTS). This probe was red-labeled with ATTO550-dUTP using the Nick-Translation mix kit (Jena Bioscience, Jena, Germany). The 18S rDNA probe, containing a 1400 bp-long segment of the transcribed region was also isolated from *H. malabaricus* following Cioffi et al. [43] and green-labeled with AF488-dUTP using the Nick-Translation mix kit (Jena Bioscience). The microsatellites $(A)_{30}$, $(CA)_{15}$, and $(GA)_{15}$ were selected based on their abundance and patterns of chromosome distribution encountered in previous studies in different fish species [36,44], and the corresponding probes were directly labeled with Cy3 (Sigma-Aldrich, Darmstadt, Germany) during their synthesis, as described in [45]. Telomeric sequence $(TTAGGG)_n$ was in situ located using the DAKO Telomere PNA FISH Kit/FITC (DAKO, Glostrup, Denmark). High stringency conditions described in [46] were used in all FISH experiments. As a final step for all fluorescence assays, chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI), and the slides were mounted in an antifade solution (VECTASHIELD; Vector Laboratories, Burlingame, CA, USA). Two sets of comparative genomic hybridization (CGH) experiments were designed to seek for the occurrence of sex chromosomes, both following the protocol of Sember et al. [47], with adaptations on probe/$C_0t{-}1$ DNA ratio based on previous *Harttia* studies [35,36]. In the first set of experiments, male and female genomic probes were co-hybridized to the male chromosomes (i.e., the expected heterogametic sex), in each species separately, to detect regions with specific or biased hybridization of male probe which might point to regions with sex-specific repetitive DNA accumulation on sex chromosomes. For this experimental scheme, male and female-derived whole-genome DNAs (gDNAs) of all analyzed species were extracted from the liver tissue by the standard phenol-chloroform-isoamyl-alcohol method [48] and labeled with ATTO550-dUTP (male gDNA in red) and AF488-dUTP (female gDNA in green) using the Nick Translation mix kit (Jena Bioscience). To block the excess of shared repetitive sequences, an unlabeled C_0t-1 DNA, obtained from each species according to Zwick et al. [49] was included in the final probe mixture. For each slide, male and female genomic probe (500 ng each) and 25 µg of female-derived $C_0t{-}1$ DNA from the respective species were co-precipitated in 96% ethanol, and the dry pellets were resuspended in 20 µL of the hybridization buffer containing 50% formamide, 2×SSC, 10% dextran sulfate, and Denhardt's buffer (pH 7.0). In the second CGH assay, the genomes of *H. dissidens* and *H. guianensis* were compared to one of *H. villasboas*, which possesses an $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system and whose chromosome preparations were previously obtained by our research group [36]. For this purpose, male-derived gDNAs from *H. dissidens*, *H. guianensis*, and *H. villasboas* were extracted as described above and labeled with fluorochromes emitting green (AF488-dUTP), cyan (ATTO425-dUTP), and red (ATT0550-dUTP) fluorescence, with the Nick Translation mix kit (Jena Bioscience). The

final probe mixture was composed of 500 ng of male-derived gDNA of each species and 10 µg of female-derived C_0t-1 DNA of each species. This probe was then hybridized to the male metaphase plates of *H. villasboas*.

2.4. Image Analysis and Processing

The 2n, karyotype structure, distribution of repetitive DNAs, and CGH results were confirmed by the evaluation of results in at least 30 metaphase spreads per individual. Images were captured using an Olympus BX50 microscope (Olympus Corporation, Ishikawa, Japan), coupled with a CoolSNAP camera, and processed using Image-Pro Plus 4.1 software (Media Cybernetics, Silver Spring, MD, USA). Chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st), or acrocentric (a) according to their arm-ratios [50]. Figures were organized with Adobe Photoshop CC 2020.

3. Results

Harttia dissidens and *Harttia* sp. 3 exhibited the same 2n = 54 chromosomes but differed in the karyotype composition: 20m + 26sm + 8a and 16m + 18sm + 14st + 6a, respectively. On other hand, *H. guianensis* differed from the other two species both in 2n and karyotype constitution: 2n = 58, and 20m + 26sm + 2st + 10a chromosomes (Figure 2). In *H. dissidens* and *H. guianensis*, constitutive heterochromatin was located preferentially in the pericentromeric and terminal regions of several chromosomes, but with distinct patterns of distribution between the species. In turn, *Harttia* sp. 3 differed by having larger C-positive bands, with signals distributed in the pericentromeric regions of almost all chromosomes (Figure 2). The mapping of the 18S rDNA sequence revealed a single chromosome pair bearing these sites in all three species (pair 24 in *H. dissidens*, pair 25 in *H. guianensis*, and pair 1 in *Harttia* sp. 3). The 5S rDNA cluster was found in a single chromosome pair in *H. dissidens* (pair 19) and *Harttia* sp. 3 (pair 15), and in two chromosome pairs (4 and 25) in *H. guianensis*. It is also notable that both 5S and 18S rDNA sites present a syntenic location on chromosome pair 25 in *H. guianensis* (Figure 3b).

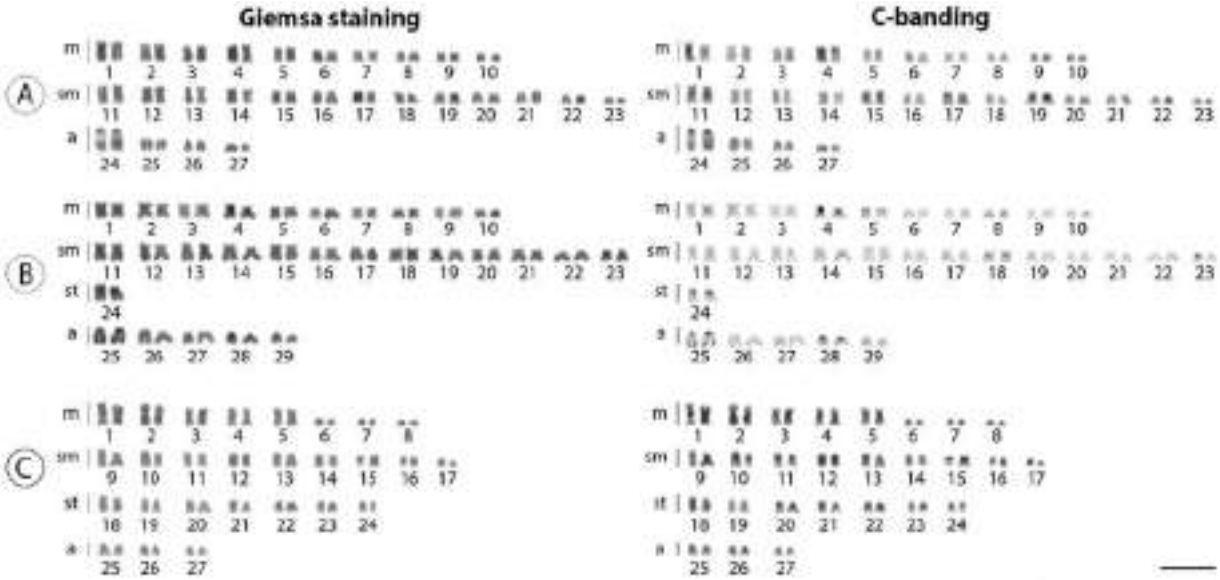


Figure 2. Karyotypes of three *Harttia* species arranged from mitotic metaphases after Giemsa staining, and C-banding. *Harttia dissidens* (**A**), *H. guianensis* (**B**), and *Harttia* sp. 3 (**C**). Scale bar = 10 µm.

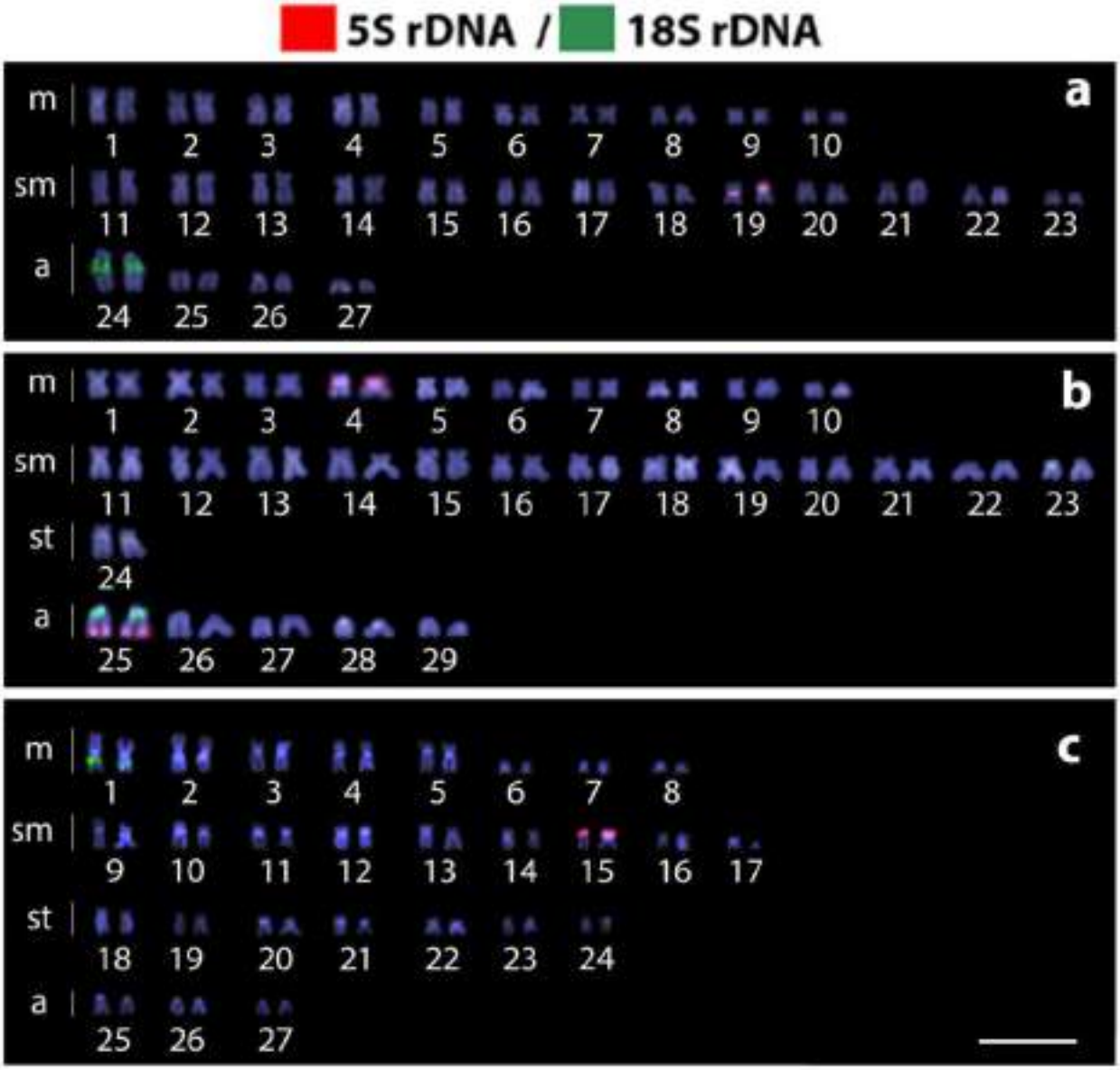


Figure 3. Karyotypes of *Harttia dissidens* (**a**), *H. guianensis* (**b**), and *Harttia* sp. 3 (**c**) after dual-color FISH: 5S (red signals) and 18S (green signals) rDNA. Note the adjacent position of 5S and 18S rDNA signals on chromosome pair No. 25 in *H. guianensis* (**b**). Chromosomes were counterstained with DAPI (blue). Scale bar = 10 μm.

The mapping of three microsatellite sequences revealed a similar pattern for $(CA)_{15}$ and $(GA)_{15}$, with accumulation at the terminal regions of all chromosomes. The last studied motif, $(A)_{30}$, presented a dispersed distribution throughout the chromosome complement of *H. dissidens* and *H. guianensis*, while it was accumulated in the pericentromeric regions of few chromosome pairs in *Harttia* sp. 3 (Figure 4). The mapping of the telomeric $(TTAGGG)_n$ sequence revealed only the standard pattern in all the three species, i.e., positive signals at the physical ends of all chromosomes (Figure A1).

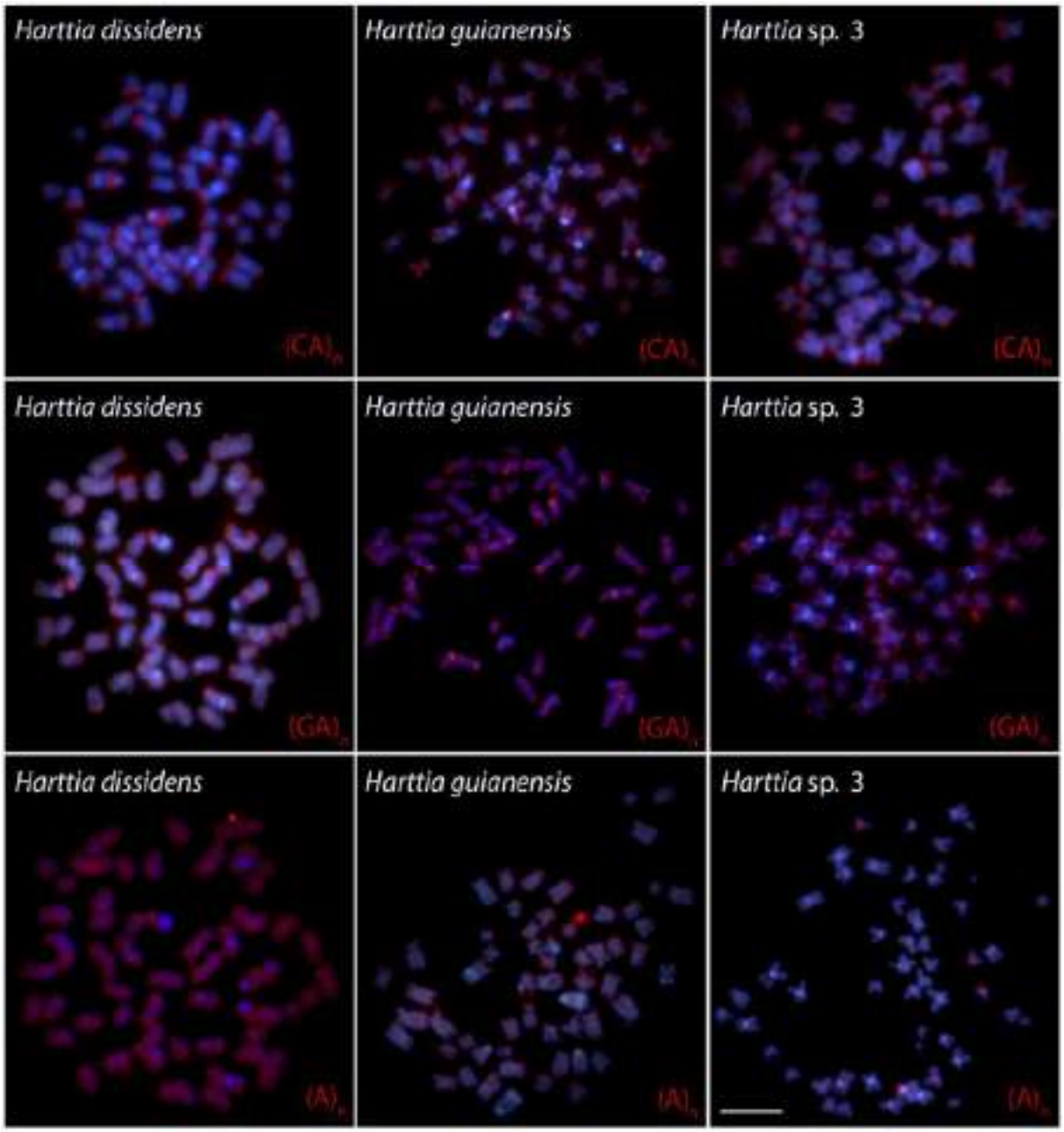


Figure 4. Chromosomal mapping of three microsatellite sequences on *Harttia* mitotic chromosomes. Probes (red signals): $(A)_{30}$, $(CA)_{15}$, and $(GA)_{15}$. Chromosomes were counterstained with DAPI (blue). Scale bar = 10 μm.

The CGH-based experiments between sexes in each of the three analyzed species showed approximately equal binding of both male- and female-specific probes to the vast majority of chromosomes in the male complement, with preferential location to regions with high content of repetitive DNA (yellow signals, i.e., combination of green and red fluorescence). In *Harttia dissidens* and *H. guianensis*, CGH failed to reveal any region with predominant or exclusive hybridization of the male-specific probe, thus we did not observe any signs of potential sex chromosome molecular differentiation at the level detectable by this methodological approach. In *Harttia* sp. 3, however, the male genomic probe predominantly (but not exclusively) marked the pericentromeric region of one large-sized metacentric chromosome pair (Figure 5).

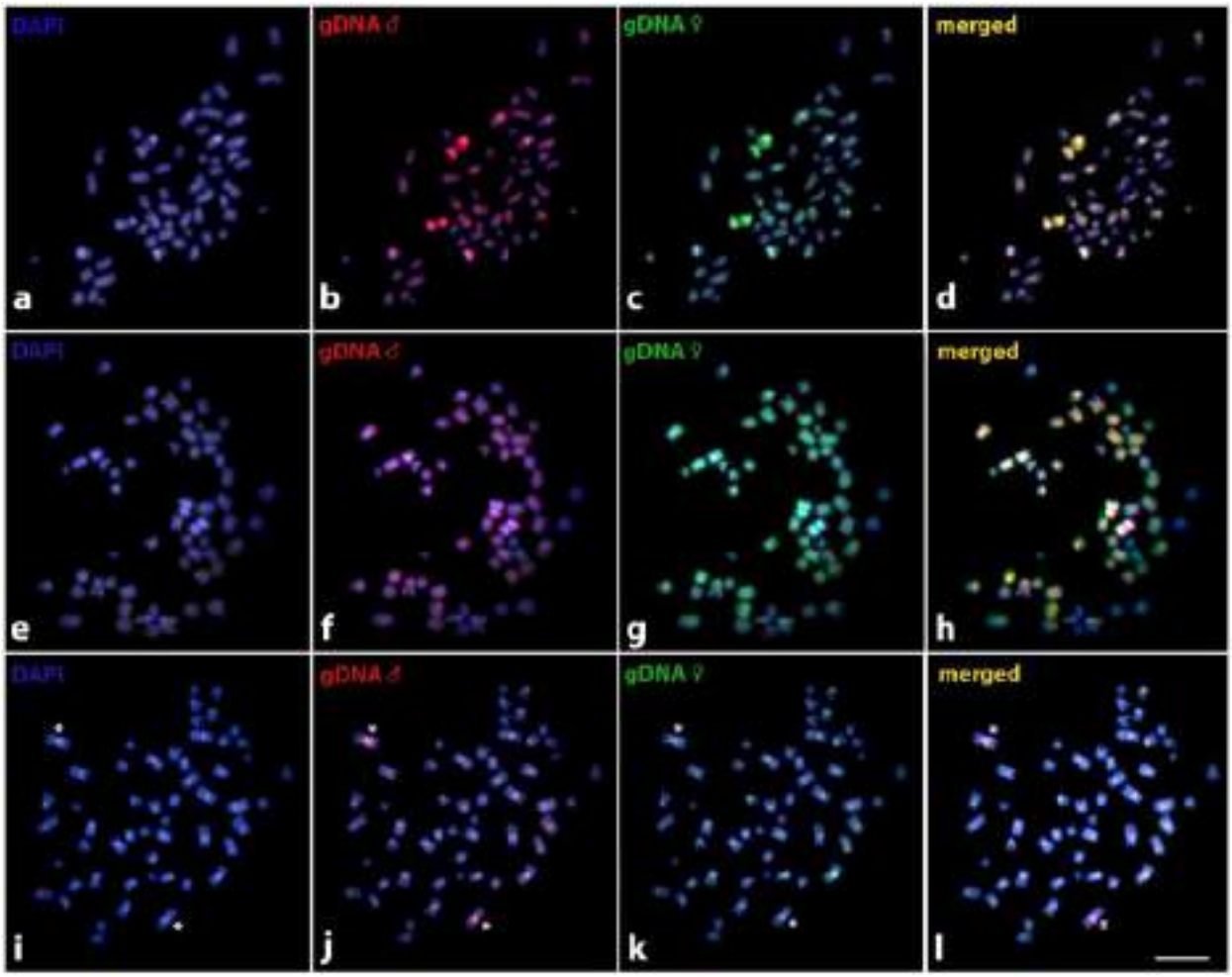


Figure 5. Mitotic chromosome spreads of *Harttia* males after intraspecific (male-to-female) CGH procedure. *Harttia dissidens* (**a**–**d**), *H. guianensis* (**e**–**h**), and *Harttia* sp. 3. (**i**–**l**). First column (**a**,**e**,**i**): DAPI images (blue); second column (**b**,**f**,**j**): hybridization patterns of male gDNA probes; third column (**c**,**g**,**k**): hybridization patterns of female gDNA probes; fourth column (**d**,**h**,**l**): merged images of both genomic probes and DAPI counterstaining. Regions with excessive hybridization of both genomic probes are yellow (i.e., combination of green and red). Asterisks highlight the regions with preferential accumulation of male gDNA probe when compared to patterns of a female gDNA probe hybridization. Scale bar = 10 μm.

We further performed a second CGH assay with hybridization of the male genomic probes from *Harttia dissidens*, *H. guianensis*, and *H. villasboas* against the chromosomal set of *H. villasboas* (i.e., the species previously reported to have $X_1X_1X_2X_2/X_1X_2Y$ multiple sex chromosome system; see ref. [36]). We found uniform hybridization patterns of both *H. dissidens* and *H. guianensis* probes across the chromosome complement of *H. villasboas* males. The *H. villasboas* male probe seldom predominantly highlighted the regions of high repetitive content, pointing to the fact that the other two compared genomes do not (qualitatively or quantitatively) share this specific portion of *H. villasboas* repetitive DNA classes (Figure 6).

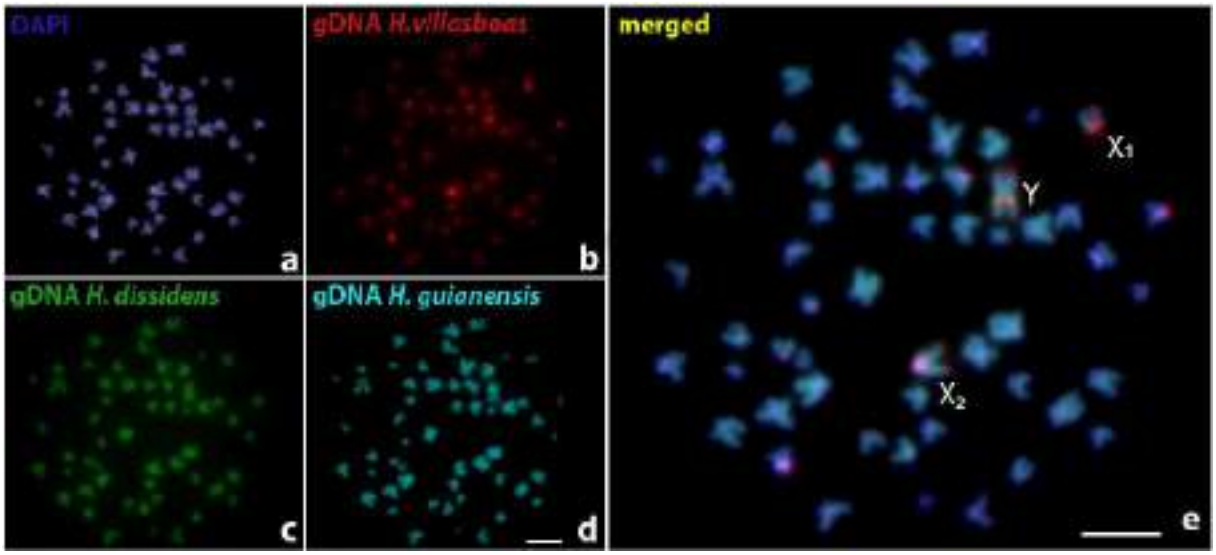


Figure 6. Mitotic metaphase from *Harttia villasboas* male after interspecific comparative genomic hybridization (CGH). Chromosome spreads were probed with male-derived genomic probes from *Harttia villasboas* (red) (**b**), *H. dissidens* (green) (**c**), and *H. guianensis* (light blue) (**d**). The composed image (**e**) contains all three merged genomic probes and DAPI counterstaining (**a**). The genomic regions with shared excessive hybridization of applied male genomic probes are yellowish. Scale bar = 10 µm.

4. Discussion

4.1. New Pieces into the Chromosomal Evolution of Harttia

In a previous chromosomal study in *Harttia* species, 2n = 58 was proposed as the likely ancestral 2n for this genus [34]. A few years later, the karyotype diversity among *Harttia* species was described as the result of three different evolutionary pathways: (i) the maintenance of the ancestral 2n = 58 up to today; (ii) the elevation of 2n by centric fissions, and (iii) the 2n reduction by fusions [35]. Here, we add new pieces to this puzzle by analyzing the karyotypes of another three *Harttia* species.

In the most recent phylogenetic analysis of the subfamily Loricariinae [51], the existence of three clades within the *Harttia* genus was proposed. The earliest-diverging clade is composed of *H. guianensis*, *H. tuna*, *H. fluminensis*, and *H. surinamensis* which inhabit waters from the Guiana shield. The two more recently diverging clades display a well-defined and non-overlapping geographical distribution. Their species are found in southeast and south Brazilian regions, which are separated from the northern rivers. Until the present study, cytogenetical investigations only comprised fishes from the latter two clades [34–36].

The description of the *H. guianensis* karyotype in the present study reinforces the proposal of 2n = 58 as the ancestral 2n for the genus [34]. If we keep in mind that this species is basalmost in the clade, as pointed by phylogenetic reconstructions [51,52], and that it shares the same 2n with other *Harttia* species from other clades, such as *H. gracilis*, *H. longipinna*, and *H. punctata*, such hypothesis is still valid but lacks profound reconstruction analysis. Furthermore, although not a common occurrence, the syntenic organization of at least some sites of both rDNA classes is also considered as a basal karyotype feature for Loricariidae fishes [53]; this is a pattern that we also found in the karyotype of *H. guianensis*, in which 5S and 18S rDNA site share the location on the chromosome pair 25. Interestingly, all three *Harttia* species under study share one 5S rDNA site, which might be hypothetically located on homeologous chromosomes (pairs 19, 4, and 15 in *H. dissidens*, *H. guianensis*, and *Harttia* sp. 3, respectively). The mentioned chromosome pairs only show mild changes in shape and size between species and they differ also regarding the position of an 5S rDNA site (terminal vs. more proximal), which could be explained by paracentric inversions. The second extra 5S rDNA site found in *H. guianensis* only, i.e., the one found in synteny with 18S rDNA cluster on chromosome pair 25 is a karyotype trait that has been so far reported

only for two other *Harttia* species: *H. carvalhoi* [34] and *Harttia* sp. 1 [35]. Given the position of *H. guianensis* at the base of *Harttia* phylogeny [51], it seems probable that this second 5S rDNA site has been eliminated during evolution in many *Harttia* species while it was retained in a subset of them.

The 5S rDNA site has been either entirely eliminated or reduced in copy number of repeats below the resolution of the standard FISH protocol. Based on tracking the location of the 18S rDNA site, we may infer that particularly this linkage group underwent a dynamic evolution (see below). Based on the comparison of 18S rDNA patterns, it is highly probable that the chromosome pair bearing the 18S rDNA site was involved in a fusion event in *H. dissidens* and *Harttia* sp. 3. This may be inferred from the terminal location of 18S rDNA site on a rather middle-sized chromosome pair in *H. guianensis* vs. an interstitial position of 18S rDNA cluster on large-sized chromosomes in the remaining two species. While the presence of a large metacentric pair in *Harttia* sp. 3 supports centric fusion as an underlying mechanism, the large 18S rDNA-bearing chromosome in *H. dissidens* is acrocentric and hence either tandem fusion took place here or the situation was more complex (i.e., involving more consecutive rearrangements).

For loricariid genomes, it was proposed that the evolutionary breakpoint regions occur adjacent to rDNA sites, promoting double-strand breaks and the reorganization of these sequences in *Ancistrus* [54], *Harttia* [35,36], and *Rineloricaria* [55]. The number of studies showing rDNA sites inside or nearby a predicted fusion points is steadily growing for fishes [56–59] and a high potential of rDNAs to facilitate chromosome rearrangements has also been shown in mice and humans (e.g., [60,61]). A possible explanation for rDNA instability might be a high transcriptional activity of tandemly arrayed rDNA genes due to the necessity to synthetize high amounts of ribosomes in the cell [62]. Long stretches of transcriptionally active euchromatin might be prone to double-strand breaks [63,64]. Interestingly, mapping of telomeric repeats did not reveal any interstitial telomeric sites (ITSs), which could point to regions of former rearrangements, in our case specifically fusions or inversions; therefore, it seems that the mechanisms of these rearrangements in *Harttia* usually do not preserve these sequences in the fusion points [65,66].

The two species analyzed herein, *H. dissidens* and *Harttia* sp. 3, share the same chromosome count (2n = 54) as was previously only described in *H. rondoni* [36], a species that also inhabits the northern Brazilian region. Indeed, *H. dissidens* occurs in the Tapajós river basin [67], and *H. rondoni* inhabits the Xingu River basin [68]. The range of distribution of *Harttia* sp. 3 cannot be inferred from the current data. This suspected new *Harttia* species was collected in a small rocky-bottom river located on the edge of Serra do Cachimbo, 700 m highlands dividing the Tapajós and Xingu basins. Therefore, it is not yet known whether it flows into the Tapajós River basin or the Xingu River basin.

Although the karyotypes of *H. dissidens*, *H. rondoni*, and *Harttia* sp. 3 share 2n equal to 54 chromosomes, notable differences can be found between the species at the level of karyotype composition, and distribution of repetitive DNA classes–either specific ones or the uncharacterized repetitive DNA fraction revealed by CGH. Taking an important role in the initial steps of sex chromosome differentiation, in post-zygotic reproductive isolation, or by contributing to local adaptation in certain populations, chromosomal inversions can suppress the recombination, especially around the rearrangement breakpoints [69–77]. In addition, repetitive DNAs may also play an important role in promoting biodiversity, in the differentiation of sex-specific chromosomal regions, and speciation of diverse eukaryotic organisms including fishes [78–81]. As evidenced in our present study, the differences in karyotype organization and number and distribution of rDNA sites are instrumental as chromosomal markers for the determination of *Harttia* species with 2n = 54 chromosomes. The fixation of such karyotype differences might be facilitated by vicariant events after the Serra do Cachimbo uplifting [36]. This event seems to have played a significant role in the diversification of *Harttia* species, as pointed out by the position of the clades in the phylogenetic reconstruction of this genus. Species from Xingu and Tocantins-Araguaia basins, which have at least two different sex chromosome systems [36], form a sister clade

to the branch which encompasses species from the Tapajós basin [57]. One member of the latter group investigated herein, *H. dissidens*, does not present a cytologically detectable sex chromosome system. Additionally, *H. punctata*, a species from the Tocantins-Araguaia basin which possesses an $X_1X_1X_2X_2/X_1X_2Y$ multiple sex chromosome system, corresponds to the sister group of all those above-mentioned species in their phylogenetic reconstruction ([52], Figure 7).

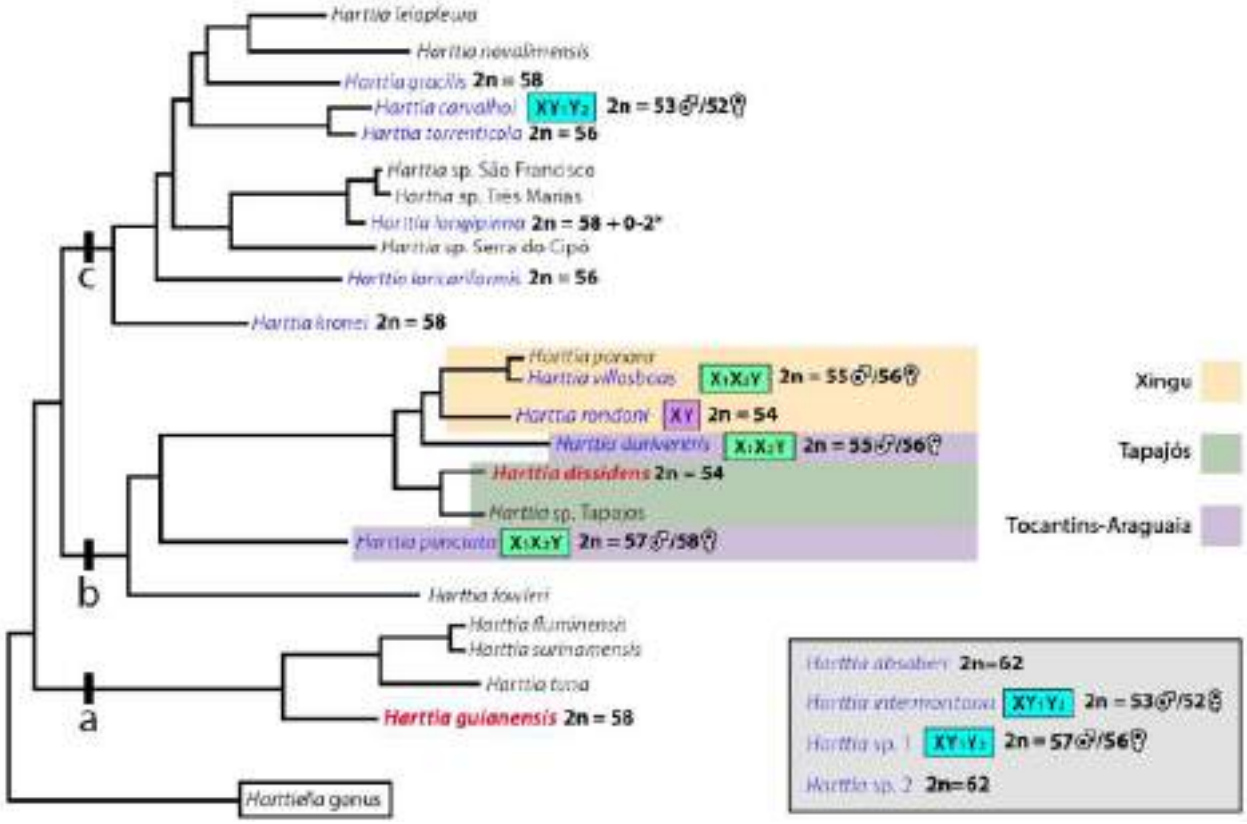


Figure 7. Updated schematic representation of the phylogenetic relationships between *Harttia* species (modified from our formerly published scheme—see ref. [36], based on the molecular data (see ref. [52]), and with plotted cytogenetic characteristics based on previous cytogenetic studies (blue), and the present study (red). Differentiated sex chromosomes are indicated in boxes as follows: X_1X_2Y (green box), XY_1Y_2 (light blue box), and XY (pink box). Species listed in the gray box (placed at the bottom-right corner) were cytogenetically investigated but their position in the phylogenetic reconstruction needs yet to be determined. River basins where norther *Harttia* species occur are highlighted as follows: Xingu (yellow), Tapajós (green), and Tocantins-Araguaia (purple). Asterisks correspond to extra B chromosomes that can be found in *H. longipinna* karyotype [82].

4.2. Sex Chromosomes in Harttia species

At least nine distinct sex chromosome systems are described among fishes, so far known to be distributed in approx. 1% of teleost species (440 cases according to [83]). Many of these systems present only subtle genetic differentiation, which is not detectable by standard cytogenetic methods [19,83].

Until now, *Harttia* has presented three distinct male-heterogametic sex chromosome systems: (i) $X_1X_1X_2X_2/X_1X_2Y$ found in *H. punctata* [34], *H. duriventris* and *H. villasboas* [36], (ii) XX/XY_1Y_2 found in *H. carvalhoi* [33,34], *H. intermontana* and *Harttia* sp. 1 [35], and (iii) the putative XX/XY sex chromosome system at an early stage of differentiation proposed for *H. rondoni* [36]. Consequently, *Harttia* can be recognized as a taxon with high karyotype dynamics, which is prone to formation of multiple sex chromosome systems. Up to now, 75 cases of multiple sex chromosomes with predicted 60 independent origins have been reviewed by Sember et al. [83] and another two systems have been concomitantly

described in *Harttia* [35]. Together with *Nothobranchius*, *Harttia* is the genus with highest number (N = 6) of multiple sex chromosomes (reviewed in ref. [83]).

Interestingly, although largely different from many viewpoints, both mentioned teleost taxa (*Harttia* and *Nothobranchius*) share the evolution in small allopatric populations with restricted gene flow and propensity to fusions and fissions. It is becoming increasingly apparent that Robertsonian rearrangements might have a significant effect on the nuclear organization of chromosomes along with gene evolution and expression (e.g., [84]). Although present in seven out of the 14 karyotyped species, differentiated sex chromosomes are still intensely discussed concerning the ancestral karyotype of *Harttia*. For this, two main scenarios have been already proposed [36]. The first one—"the proto-XY hypothesis"—considers both X and Y chromosomes to differ only by amplification or contraction of tandem repeats, thus representing an early stage of sex chromosome differentiation. Following this presumption, a proto-XY sex chromosome system, like the one found in *H. rondoni*, could be present in the ancestral karyotype and, consequently, would have originated the X_1X_2Y sex chromosome system found in *H. duriventris*, *H. punctata*, and *H. villasboas* via centric fissions [36]. A possible second hypothesis, named "the neo-XY system", considers the fusions of ancestral X and Y chromosomes with an autosome pair, thus leading to the formation of neo-XY sex chromosomes accompanied by the reduction of 2n [36]. A third hypothesis partially overlaps with the second one and it can be considered for the southeast Brazilian clade of *Harttia*, which does not present any proto-sex, or heteromorphic XY sex chromosomes identified so far. In this case, similar CGH patterns and the phylogenetic relationships between *H. torrenticola* (without differentiated sex chromosomes) and *H. carvalhoi* (with an XY_1Y_2 sex system), point to a Robertsonian fusion originating the X-chromosome of *H. carvalhoi*, *H. intermontana*, and *Harttia* sp. 1, as well as the first chromosome pair of *H. torrenticola* [35]. Such hypotheses, especially the first two of them, were proposed assuming the scenario in which all species from the northern Brazilian region present differentiated sex chromosomes in their karyotypes. However, this is not the case, as the present CGH experiments confirmed that *H. dissidens* and *H. guianensis* do not possess differentiated sex chromosomes, at least not at the stage which could be detected by CGH [47,85]. These data suggest rather early steps of differentiation in the *Harttia* species with XX/XY_1X_2 sex chromosome system when compared to the karyotypes with homomorphic sex chromosomes in the clade from northern Brazilian region.

Recent genomic studies continue to reveal homomorphic sex chromosome systems among fishes, thus pointing to a potentially gross underestimation regarding the counts of fish sex chromosomes cases by previous cytogenetic reports [86,87]. This might especially apply to the *Harttia* genus, where several cryptic and undescribed species occur, in addition to the scarcity of cytogenetic (and a complete absence of genomic) data, especially for species from both northern Brazil and Guyana shield. Our CGH experiments utilizing male and female genomic probes to be compared on the male chromosome background in *Harttia* sp. 3, revealed a specific pericentromeric region in one metacentric pair (pair 2) highlighted more (but not exclusively) by the male genomic probe. This observation might point to a nascent region of suppressed recombination on homomorphic sex chromosomes [47], bearing in mind that specific repetitive DNA accumulations might happen on corresponding regions of both sex chromosomes [88]; however, we cannot entirely discard the possibility of intraspecific variability in the copy number and distribution of certain repetitive DNAs.

Author Contributions: Formal analysis, F.d.M.C.S., O.M.-F., G.A.D., A.S., T.L., V.C.S.O., P.F.V., M.R.V. and M.d.B.C.; Funding acquisition, M.d.B.C.; Investigation, F.d.M.C.S., O.M.-F., G.A.D., A.S., L.A.C.B., T.L., P.F.V., E.F. and M.d.B.C.; Methodology, F.d.M.C.S., G.A.D., T.L., V.C.S.O., P.F.V. and M.d.B.C.; Project administration, O.M.-F., T.L. and M.d.B.C.; Validation, F.d.M.C.S., O.M.-F., G.A.D., A.S., L.A.C.B., T.L., V.C.S.O., P.F.V., E.F., M.R.V. and M.d.B.C.; Visualization, F.d.M.C.S., A.S., L.A.C.B., T.L., V.C.S.O., E.F., M.R.V. and M.d.B.C.; Writing—original draft, F.d.M.C.S. and M.d.B.C.; Writing—review & editing, O.M.-F., G.A.D., A.S., L.A.C.B., V.C.S.O., P.F.V., E.F. and M.R.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by FAPESP, grant numbers 2020/02681-9 and 2020/11772-8. A.S. was supported by RVO: 67985904 of IAPG CAS, Liběchov.

Institutional Review Board Statement: Sampling was done with the authorization of the environmental agency ICMBIO/SISBIO (License 48628-14) and SISGEN (A96FF09). Experiments followed the ethical conduct approved by the Ethics Committee on Animal Experimentation of the Universidade Federal de São Carlos (Process number CEUA 1853260315).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: Special thanks to Alany Pedrosa Gonçalves (Mamirauá Sustainable Development Reserve) and Cárlison Silva de Oliveira for the indication of sampling sites, to Marcella Costa Radael (Federal University of West Pará-UFOPA) for the support getting chemical reagents during our fieldwork, and to Lucia Rapp-Py Daniel (National Institute of Amazonian Research–INPA) and Oswaldo Oyakawa (USP Zoology Museum) for species identification.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

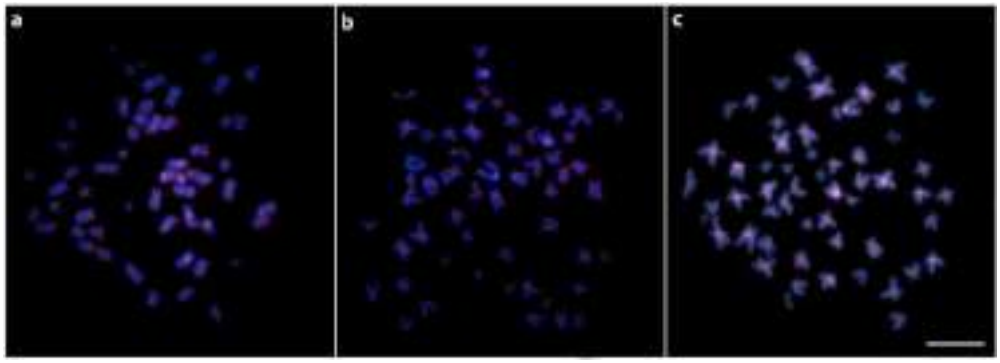


Figure A1. Distribution of the telomeric probe $(TTAGGG)_n$ directly labeled with Cy3-dUTP (red) and hybridized against the DAPI-counterstained (blue) chromosomes of *Harttia dissidens* (**a**), *H. guianensis* (**b**) and *Harttia* sp. 3 (**c**). Bar scale = 10 μm.

References

1. Fricke, R.; Eschmeyer, W.N.; van der Laan, R. Eschmeyer's Catalog of Fishes: Genera, Species, References. Available online: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (accessed on 24 August 2020).
2. Dudgeon, D.; Arthington, A.H.; Gessner, M.O.; Kawabata, Z.-I.; Knowler, D.J.; Lévêque, C.; Naiman, R.J.; Prieur-Richard, A.-H.; Soto, D.; Stiassny, M.L.J. Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biol. Rev.* **2006**, *81*, 163–182. [CrossRef]
3. Nelson, J.S.; Grande, T.C.; Wilson, M.V.H. *Fishes of the World*, 5th ed.; John Wiley & Sons: Hoboken, NJ, USA, 2016; ISBN 111834233X.
4. Volff, J.N. Genome evolution and biodiversity in teleost fish. *Heredity* **2005**, *94*, 280–294. [CrossRef] [PubMed]
5. Ravi, V.; Venkatesh, B. Rapidly evolving fish genomes and teleost diversity. *Curr. Opin. Genet. Dev.* **2008**, *18*, 544–550. [CrossRef] [PubMed]
6. Lee, A.P.; Kerk, S.Y.; Tan, Y.Y.; Brenner, S.; Venkatesh, B. Ancient vertebrate conserved noncoding elements have been evolving rapidly in teleost fishes. *Mol. Biol. Evol.* **2011**, *28*, 1205–1215. [CrossRef]
7. Sallan, L.C. Major issues in the origins of ray-finned fish (Actinopterygii) biodiversity. *Biol. Rev.* **2014**, *89*, 950–971. [CrossRef] [PubMed]
8. Mable, B.K.; Alexandrou, M.A.; Taylor, M.I. Genome duplication in amphibians and fish: An extended synthesis. *J. Zool.* **2011**, *284*, 151–182. [CrossRef]
9. Braasch, I.; Postlethwait, J.H. Polyploidy in fish and the teleost genome duplication. In *Polyploidy and Genome Evolution*; Springer: Berlin/Heidelberg, Germany, 2012; pp. 341–383.
10. Bernardi, G. Speciation in fishes. *Mol. Ecol.* **2013**, *22*, 5487–5502. [CrossRef]

11. Pörtner, H.O.; Schulte, P.M.; Wood, C.M.; Schiemer, F. Niche dimensions in fishes: An integrative view. *Physiol. Biochem. Zool.* **2010**, *83*, 808–826. [CrossRef]
12. Reis, R.E.; Albert, J.S.; Di Dario, F.; Mincarone, M.M.; Petry, P.; Rocha, L.A. Fish biodiversity and conservation in South America. *J. Fish Biol.* **2016**, *89*, 12–47. [CrossRef]
13. Albert, J.S.; Reis, R. *Historical Biogeography of Neotropical Freshwater Fishes*; Univ of California Press: Berkeley, CA, USA, 2011; ISBN 0520948505.
14. Albert, J.S.; Tagliacollo, V.A.; Dagosta, F. Diversification of Neotropical freshwater fishes. *Annu. Rev. Ecol. Evol. Syst.* **2020**, *51*, 27–53. [CrossRef]
15. Arai, R. *Fish Karyotypes: A Check List*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2011.
16. Gregory, T.R. Animal Genome Size Database. Available online: http://www.genomesize.com (accessed on 19 August 2021).
17. Yoshida, K.; Kitano, J. Tempo and mode in karyotype evolution revealed by a probabilistic model incorporating both chromosome number and morphology. *PLoS Genet.* **2021**, *17*, e1009502. [CrossRef]
18. Devlin, R.H.; Nagahama, Y. Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* **2002**, *208*, 191–364. [CrossRef]
19. Schartl, M.; Schmid, M.; Nanda, I. Dynamics of vertebrate sex chromosome evolution: From equal size to giants and dwarfs. *Chromosoma* **2016**, *125*, 553–571. [CrossRef] [PubMed]
20. Guiguen, Y.; Fostier, A.; Herpin, A. Sex determination and differentiation in fish: Genetic, genomic, and endocrine aspects. In *Sex Control in Aquaculture*, 1st ed.; Wang, H.-P., Piferrer, F., Chen, S.-L., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2018; pp. 35–63.
21. Shen, Z.; Wang, H. Environmental sex determination and sex differentiation in teleost—How sex is established. In *Sex Control in Aquaculture*, 1st ed.; Wang, H.-P., Piferrer, F., Chen, S.-L., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2018; pp. 85–115.
22. Kuwamura, T.; Sunobe, T.; Sakai, Y.; Kadota, T.; Sawada, K. Hermaphroditism in fishes: An annotated list of species, phylogeny, and mating system. *Ichthyol. Res.* **2020**, *67*, 341–360. [CrossRef]
23. Schaefer, S.A.; Lauder, G.V. Historical transformation of functional design: Evolutionary morphology of feeding mechanisms in loricarioid catfishes. *Syst. Zool.* **1986**, *35*, 489–508. [CrossRef]
24. Reis, R.E.; Kullander, S.; Ferraris, C.J. *Check List of the Freshwater Fishes of South and Central America*; Edipucrs: Porto Alegre, Brazil, 2003; ISBN 8574303615.
25. Kavalco, K.F.; Pazza, R.; Bertollo, L.A.C.; Moreira-Filho, O. Karyotypic diversity and evolution of Loricariidae (Pisces, Siluriformes). *Heredity* **2005**, *94*, 180–186. [CrossRef]
26. Takagui, F.H.; Baumgärtner, L.; Venturelli, N.B.; Paiz, L.M.; Viana, P.; Dionísio, J.F.; Pompeo, L.R.S.; Margarido, V.P.; Fenocchio, A.S.; da Rosa, R. Unrevealing the karyotypic evolution and cytotaxonomy of armored catfishes (Loricariinae) with emphasis in *Sturisoma*, *Loricariichthys*, *Loricaria*, *Proloricaria*, *Pyxiloricaria*, and *Rineloricaria*. *Zebrafish* **2020**, *17*, 319–332. [CrossRef]
27. Giuliano-Caetano, L. *Polimorfismo Cromossômico Robertsoniano Em Populações de Rineloricaria latirostris (Pisces, Loricariinae)*; Universidade Federal de São Carlos: São Carlo, Brazil, 1998.
28. de Lara Kamei, M.C.S.; Baumgärtner, L.; Paiva, S.; Zawadzki, C.H.; Martins-Santos, I.C.; de Portela-Castro, A.L.B. Chromosomal diversity of three species of *Hypostomus* Lacépède, 1803 (Siluriformes, Loricariidae), from the Paraná River Basin, Brazil: A species complex in *Hypostomus Ancistroides* reinforced by a ZZ/ZW sex chromosome system. *Zebrafish* **2017**, *14*, 357–363. [CrossRef] [PubMed]
29. Takagui, F.H.; de Moura, L.F.; Ferreira, D.C.; Centofante, L.; de A. Vitorino, C.; Bueno, V.; Margarido, V.P.; Venere, P.C. Karyotype diversity in Doradidae (Siluriformes, Doradoidea) and presence of the heteromorphic ZZ/ZW sex chromosome system in the family. *Zebrafish* **2017**, *14*, 236–243. [CrossRef] [PubMed]
30. Rocha-Reis, D.A.; de Oliveira Brandão, K.; de Almeida-Toledo, L.F.; Pazza, R.; Kavalco, K.F. The persevering cytotaxonomy: Discovery of a unique XX/XY sex chromosome system in catfishes suggests the existence of a new, endemic and rare species. *Cytogenet. Genome Res.* **2018**, *156*, 45–55. [CrossRef] [PubMed]
31. de Oliveira, L.C.; Ribeiro, M.O.; de Medeiros Costa, G.; Zawadzki, C.H.; Prizon-Nakajima, A.C.; Borin-Carvalho, L.A.; Martins-Santos, I.C.; de Brito Portela-Castro, A.L. Cytogenetic characterization of *Hypostomus soniae* Hollanda-Carvalho & Weber, 2004 from the Teles Pires River, southern Amazon basin: Evidence of an early stage of an XX/XY sex chromosome system. *Comp. Cytogenet.* **2019**, *13*, 411.
32. Glugoski, L.; Deon, G.; Schott, S.; Vicari, M.R.; Nogaroto, V.; Moreira-Filho, O. Comparative cytogenetic analyses in *Ancistrus* species (Siluriformes: Loricariidae). *Neotrop. Ichthyol.* **2020**, *18*, e200013. [CrossRef]
33. Centofante, L.; Bertollo, L.A.C.; Moreira-Filho, O. Cytogenetic characterization and description of an XX/XY_1Y_2 sex chromosome system in catfish *Harttia carvalhoi* (Siluriformes, Loricariidae). *Cytogenet. Genome Res.* **2006**, 320–324. [CrossRef]
34. Blanco, D.R.; Vicari, M.R.; Lui, R.L.; Traldi, J.B.; Bueno, V.; de Martinez, J.F.; Brandão, H.; Oyakawa, O.T.; Moreira Filho, O. Karyotype diversity and evolutionary trends in armored catfish species of the genus *Harttia* (Siluriformes: Loricariidae). *Zebrafish* **2017**, *14*, 169–176. [CrossRef] [PubMed]
35. Deon, G.A.; Glugoski, L.; Vicari, M.R.; Nogaroto, V.; Sassi, F.M.C.; Cioffi, M.B.; Liehr, T.; Bertollo, L.A.C.; Moreira-Filho, O. Highly rearranged karyotypes and multiple sex chromosome systems in armored catfishes from the genus *Harttia* (Teleostei, Siluriformes). *Genes* **2020**, *11*, 1366. [CrossRef] [PubMed]

36. Sassi, F.M.C.; Deon, G.A.; Moreira-Filho, O.; Vicari, M.R.; Bertollo, L.A.C.; Liehr, T.; de Oliveira, E.A.; Cioffi, M.B. Multiple sex chromosomes and evolutionary relationships in Amazonian catfishes: The outstanding model of the genus *Harttia* (Siluriformes: Loricariidae). *Genes* **2020**, *11*, 1179. [CrossRef]
37. De Oliveira, J.C.; Oyakawa, O.T. New loricariid fishes from headwaters on Serra da Mantiqueira and Complexo do Espinhaço, Minas Gerais State, Brazil (Teleostei: Siluriformes: Loricariidae). *Zootaxa* **2019**, *4586*, 401–424. [CrossRef] [PubMed]
38. Rodrigues, R.M. Estudos Cromossômicos e Moleculares em Loricariinae Com Ênfase em Espécies de *Rineloricaria* (Siluriformes, Loricariidae): Uma Perspectiva Evolutiva. Ph.D. Thesis, Universidade de São Paulo, São Paulo, Brazil, 2010.
39. Alves, A.L.; Oliveira, C.; Foresti, F. Karyotype variability in eight species of the subfamilies Loricariinae and Ancistrinae (Teleostei, Siluriformes, Loricariidae). *Caryologia* **2003**, *56*, 57–63. [CrossRef]
40. Bertollo, L.A.C.; Cioffi, M.B.; Moreira-Filho, O. Direct chromosome preparation from freshwater teleost fishes. In *Fish Cytogenetic Techniques (Chondrichthyans and Teleosts)*; Ozouf-Costaz, C., Pisano, E., Foresti, F., Almeida Toledo, L.F., Eds.; CRC Press: Boca Raton, FL, USA, 2015; pp. 21–26.
41. Sumner, A.T. A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* **1972**, *75*, 304–306. [CrossRef]
42. Pendás, A.M.; Móran, P.; Freije, J.P.; Garcia-Vásquez, E. Chromosomal location and nucleotide sequence of two tandem repeats of the Atlantic salmon 5S rDNA. *Cytogenet. Cell Genet.* **1994**, *67*, 31–36. [CrossRef]
43. Cioffi, M.B.; Martins, C.; Centofante, L.; Jacobina, U.P.; Bertollo, L.A.C. Chromosomal variability among allopatric populations of Erythrinidae fish *Hoplias malabaricus*: Mapping of three classes of repetitive DNAs. *Cytogenet. Genome Res.* **2009**, *125*, 132–141. [CrossRef] [PubMed]
44. Phimphan, S.; Chaiyasan, P.; Suwannapoom, C.; Reungsing, M.; Juntaree, S.; Tanomtong, A.; Supiwong, W. Comparative karyotype study of three Cyprinids (Cyprinidae, Cyprininae) in Thailand by classical cytogenetic and FISH techniques. *Comp. Cytogenet.* **2020**, *14*, 597. [CrossRef] [PubMed]
45. Kubat, Z.; Hobza, R.; Vyskot, B.; Kejnovský, E. Microsatellite accumulation in the Y chromosome of *Silene latifolia*. *Genome* **2008**, *51*, 350–356. [CrossRef]
46. Yano, C.F.; Bertollo, L.A.C.; Cioffi, M.B. Fish-FISH: Molecular cytogenetics in fish species. In *Fluorescence In Situ Hybridization (FISH)—Application Guide*; Liehr, T., Ed.; Springer: Berlin, Germany, 2017; pp. 429–444.
47. Sember, A.; Bertollo, L.A.C.; Ráb, P.; Yano, C.F.; Hatanaka, T.; de Oliveira, E.A.; Cioffi, M.B. Sex chromosome evolution and genomic divergence in the fish *Hoplias malabaricus* (Characiformes, Erythrinidae). *Front. Genet.* **2018**, *9*, 71. [CrossRef]
48. Sambrook, J.; Russell, D.W. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: New York, NY, USA, 2001; Volume 855.
49. Zwick, M.S.; Hanson, R.E.; Mcknight, T.D.; Islam-Faridi, M.H.; Stelly, D.M.; Wing, R.A.; Price, H.J. A rprocedure for the isolation of C_0t-1 DNA from plants. *Genome* **1997**, *40*, 138–142. [CrossRef]
50. Levan, A.; Fredga, K.; Sandberg, A.A. Nomenclature for centromeric position on chromosomes. *Hereditas* **1964**, *52*, 201–220. [CrossRef]
51. Londoño-Burbano, A.; Reis, R.E. A combined molecular and morphological phylogeny of the Loricariinae (Siluriformes: Loricariidae), with emphasis on the Harttiini and Farlowellini. *PLoS ONE* **2021**, *16*, e0247747. [CrossRef] [PubMed]
52. Covain, R.; Fisch-Muller, S.; Oliveira, C.; Mol, J.H.; Montoya-Burgos, J.I.; Dray, S. Molecular phylogeny of the highly diversified catfish subfamily Loricariinae (Siluriformes, Loricariidae) reveals incongruences with morphological classification. *Mol. Phylogenet. Evol.* **2016**, *94*, 492–517. [CrossRef]
53. Ziemniczak, K.; Barros, A.V.; Rosa, K.O.; Nogaroto, V.; Almeida, M.C.; Cestari, M.M.; Moreira-Filho, O.; Artoni, R.F.; Vicari, M.R. Comparative cytogenetics of Loricariidae (Actinopterygii: Siluriformes): Emphasis in Neoplecostominae and Hypoptopomatinae. *Ital. J. Zool.* **2012**, *79*, 492–501. [CrossRef]
54. Barros, A.V.; Wolski, M.A.V.; Nogaroto, V.; Almeida, M.C.; Moreira-Filho, O.; Vicari, M.R. Fragile sites, dysfunctional telomere and chromosome fusions: What is 5S rDNA role? *Gene* **2017**, *608*, 20–27. [CrossRef]
55. Glugoski, L.; Giuliano-Caetano, L.; Moreira-Filho, O.; Vicari, M.R.; Nogaroto, V. Co-located hAT transposable element and 5S rDNA in an interstitial telomeric sequence suggest the formation of Robertsonian fusion in armored catfish. *Gene* **2018**, *650*, 49–54. [CrossRef]
56. Molina, W.F.; Galetti-Jr, P.M. Robertsonian rearrangements in the reef fish *Chromis* (Perciformes, Pomacentridae) involving chromosomes bearing 5S rRNA genes. *Genet. Mol. Biol.* **2002**, *25*, 373–377. [CrossRef]
57. Rosa, K.O.; Ziemniczak, K.; de Barros, A.V.; Nogaroto, V.; Almeida, M.C.; Cestari, M.M.; Artoni, R.F.; Vicari, M.R. Numeric and structural chromosome polymorphism in *Rineloricaria lima* (Siluriformes: Loricariidae): Fusion points carrying 5S rDNA or telomere sequence vestiges. *Rev. Fish. Biol. Fish.* **2012**, *22*, 739–749. [CrossRef]
58. Kushwaha, B.; Baisvar, V.S.; Kumar, R. 18S rDNA mapping revealed conservation and rearrangements of chromosome segments in two *Channa* species. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci.* **2021**, 1–5. [CrossRef]
59. Getlekha, N.; Molina, W.F.; Cioffi, M.B.; Yano, C.F.; Maneechot, N.; Bertollo, L.A.C.; Supiwong, W.; Tanomtong, A. Repetitive DNAs highlight the role of chromosomal fusions in the karyotype evolution of *Dascyllus* species (Pomacentridae, Perciformes). *Genetica* **2016**, *144*, 203–211. [CrossRef]
60. Cazaux, B.; Catalan, J.; Veyrunes, F.; Douzery, E.J.P.; Britton-Davidian, J. Are ribosomal DNA clusters rearrangement hotspots? A case study in the genus *Mus* (Rodentia, Muridae). *BMC Evol. Biol.* **2011**, *11*, 124. [CrossRef] [PubMed]

61. Tchurikov, N.A.; Uroshlev, L.A.; Klushevskaya, E.S.; Alembekov, I.R.; Lagarkova, M.A.; Kravatskaya, G.I.; Makeev, V.Y.; Kravatsky, Y. V Chromosomal translocations in NK-cell lymphomas originate from inter-chromosomal contacts of active rDNA clusters possessing hot spots of DSBs. *Cancers* **2021**, *13*, 3889. [CrossRef]
62. Schöfer, C.; Weipoltshammer, K. Nucleolus and chromatin. *Histochem. Cell Biol.* **2018**, *150*, 209–225. [CrossRef] [PubMed]
63. Berthelot, C.; Muffato, M.; Abecassis, J.; Crollius, H.R. The 3D organization of chromatin explains evolutionary fragile genomic regions. *Cell Rep.* **2015**, *10*, 1913–1924. [CrossRef]
64. Warmerdam, D.O.; Wolthuis, R.M.F. Keeping ribosomal DNA intact: A repeating challenge. *Chromosome Res.* **2019**, *27*, 57–72. [CrossRef]
65. Slijepcevic, P. Telomeres and mechanisms of Robertsonian fusion. *Chromosoma* **1998**, *107*, 136–140. [CrossRef] [PubMed]
66. Ocalewicz, K. Telomeres in fishes. *Cytogenet. Genome Res.* **2013**, *141*, 114–125. [CrossRef] [PubMed]
67. Rapp Py-Daniel, L.H.; Oliveira, E.C. Seven new species of *Harttia* from the Amazonian-Guyana region (Siluriformes: Loricariidae). *Ichthyol. Explor. Freshw.* **2001**, *12*, 79–96.
68. Oyakawa, O.T.; Fichberg, I.; Py-Daniel, L.R. Three new species of *Harttia* (Loricariidae: Loricariinae) from Serra do Cachimbo, Rio Xingu basin, Pará, Northern Brazil. *Zootaxa* **2018**, *4387*, 75–90. [CrossRef]
69. Lahn, B.T.; Page, D.C. Retroposition of autosomal mRNA yielded testis-specific gene family on human Y chromosome. *Nat. Genet.* **1999**, *21*, 429–433. [CrossRef]
70. Charlesworth, D.; Charlesworth, B.; Marais, G. Steps in the evolution of heteromorphic sex chromosomes. *Heredity* **2005**, *95*, 118–128. [CrossRef] [PubMed]
71. Bergero, R.; Charlesworth, D. The evolution of restricted recombination in sex chromosomes. *Trends Ecol. Evol.* **2009**, *24*, 94–102. [CrossRef]
72. Ortiz-Barrientos, D.; Engelstädter, J.; Rieseberg, L.H. Recombination rate evolution and the origin of species. *Trends Ecol. Evol.* **2016**, *31*, 226–236. [CrossRef]
73. Charlesworth, D. The guppy sex chromosome system and the sexually antagonistic polymorphism hypothesis for Y chromosome recombination suppression. *Genes* **2018**, *9*, 264. [CrossRef]
74. Wellenreuther, M.; Bernatchez, L. Eco-evolutionary genomics of chromosomal inversions. *Trends Ecol. Evol.* **2018**, *33*, 427–440. [CrossRef]
75. Connallon, T.; Olito, C.; Dutoit, L.; Papoli, H.; Ruzicka, F.; Yong, L. Local adaptation and the evolution of inversions on sex chromosomes and autosomes. *Philos. Trans. R. Soc. B Biol. Sci.* **2018**, *373*, 20170423. [CrossRef] [PubMed]
76. Natri, H.M.; Merilä, J.; Shikano, T. The evolution of sex determination associated with a chromosomal inversion. *Nat. Commun.* **2019**, *10*, 145. [CrossRef]
77. Peichel, C.L.; McCann, S.R.; Ross, J.A.; Naftaly, A.F.S.; Urton, J.R.; Cech, J.N.; Grimwood, J.; Schmutz, J.; Myers, R.M.; Kingsley, D.M. Assembly of the threespine stickleback Y chromosome reveals convergent signatures of sex chromosome evolution. *Genome Biol.* **2020**, *21*, 177. [CrossRef] [PubMed]
78. Cioffi, M.B.; Bertollo, L.A.C. Chromosomal distribution and evolution of repetitive DNAs in fish. In *Repetitive DNA*; Garrido-Ramos, M.A., Ed.; Karger: Basel, Switzerland, 2012; pp. 197–221. ISBN 9783318021509.
79. Blommaert, J.; Riss, S.; Hecox-Lea, B.; Welch, D.B.M.; Stelzer, C.-P. Small, but surprisingly repetitive genomes: Transposon expansion and not polyploidy has driven a doubling in genome size in a metazoan species complex. *BMC Genom.* **2019**, *20*, 466. [CrossRef]
80. Bracewell, R.; Chatla, K.; Nalley, M.J.; Bachtrog, D. Dynamic turnover of centromeres drives karyotype evolution in *Drosophila*. *eLife* **2019**, *8*, e49002. [CrossRef] [PubMed]
81. Ávila Robledillo, L.; Neumann, P.; Koblížková, A.; Novák, P.; Vrbová, I.; Macas, J. Extraordinary sequence diversity and promiscuity of Ccentromeric Ssatellites in the Llegume tribe Fabeae. *Mol. Biol. Evol.* **2020**, *37*, 2341–2356. [CrossRef] [PubMed]
82. Blanco, D.R.; Vicari, M.R.; Artoni, R.F.; Traldi, J.B.; Moreira-Filho, O. Chromosomal characterization of armored catfish *Harttia longipinna* (Siluriformes, Loricariidae): First report of B chromosomes in the genus. *Zoolog. Sci.* **2012**, *29*, 604–609. [CrossRef]
83. Sember, A.; Nguyen, P.; Perez, M.F.; Altmanová, M.; Ráb, P.; Cioffi, M.B. Multiple sex chromosomes in teleost fishes from a cytogenetic perspective: State of the art and future challenges. *Philos. Trans. R. Soc. B* **2021**, *376*, 20200098. [CrossRef]
84. Vara, C.; Paytuví-Gallart, A.; Cuartero, Y.; Álvarez-González, L.; Marín-Gual, L.; Garcia, F.; Florit-Sabater, B.; Capilla, L.; Sanchéz-Guillén, R.A.; Sarrate, Z. The impact of chromosomal fusions on 3D genome folding and recombination in the germ line. *Nat. Commun.* **2021**, *12*, 2981. [CrossRef]
85. Traut, W.; Winking, H. Meiotic chromosomes and stages of sex chromosome evolution in fish: Zebrafish, platyfish and guppy. *Chromosome Res.* **2001**, *9*, 659–672. [CrossRef] [PubMed]
86. Gamble, T. Using RAD-seq to recognize sex-specific markers and sex chromosome systems. *Mol. Ecol.* **2016**, *25*, 2114–2116. [CrossRef] [PubMed]
87. Kuhl, H.; Guiguen, Y.; Höhne, C.; Kreuz, E.; Du, K.; Klopp, C.; Lopez-Roques, C.; Yebra-Pimentel, E.S.; Ciorpac, M.; Gessner, J. A 180 Myr-old female-specific genome region in sturgeon reveals the oldest known vertebrate sex determining system with undifferentiated sex chromosomes. *Philos. Trans. R. Soc. B* **2021**, *376*, 20200089. [CrossRef] [PubMed]
88. Chalopin, D.; Volff, J.-N.; Galiana, D.; Anderson, J.L.; Schartl, M. Transposable elements and early evolution of sex chromosomes in fish. *Chromosome Res.* **2015**, *23*, 545–560. [CrossRef] [PubMed]

III – Sassi *et al.* (2024) A state-of-art review of Loricariidae (Ostariophysi: Siluriformes) cytogenetics. Neotropical Ichthyology (*in press*).

Neotropical Ichthyology

Review article

https://doi.org/10.1590/1982-0224-2024-0050

A state-of-art review of Loricariidae (Ostariophysi: Siluriformes) cytogenetics

Correspondence:
Marcelo de Bello Cioffi
mbcioffi@ufscar.br

Francisco de Menezes Cavalcante Sassi, Marcelo de Bello Cioffi and Orlando Moreira-Filho

Loricariidae is a Neotropical fish family divided into six subfamilies and ranking in third among the most biodiverse fish groups. This study conducts an updated review of the cytogenetic investigations within the family, discussing the trends in chromosomal evolution and the main gaps and future directions for studies. Covering 125 publications that analyzed 234 species from all subfamilies except Lithogeninae, corresponding to about 21% of the valid species diversity, our study revealed samples from six different river basins in Argentina, Brazil, Ecuador, Paraguay, and Venezuela. There was a dearth of data for northeast Brazil, the Western Amazon, the Guianas Shield, and other Neotropical countries. In loricariids, there are seven different sex chromosome systems and a variety of diploid numbers (2n) ranging from 33 to 96 as a result of different chromosomal rearrangements such as fusions, fissions, translocations, and inversions. We recorded more simple nucleolar organizer regions (Ag-NOR) compared to multiple ones, and the fundamental number (FN) varied between 34 and 142. Populational studies have been conducted only in a few taxa, but a remarkable karyotype variation that includes B chromosomes is shown. Despite continuous efforts, cytogenetics still does not adequately capture the diversity of Loricariidae.

Keywords: Ag-NOR, B chromosomes, Karyotype, Plecos, Sex chromosomes.

Submitted June 6, 2024
Accepted October 28, 2024
by Claudio Oliveira
Epub ???, 2024

Online version ISSN 1982-0224
Print version ISSN 1679-6225

Neotrop. Ichthyol.
vol. 22, no. 4, Maringá 2024

Laboratório de Citogenética de Peixes, Departamento de Genética e Evolução, Universidade Federal de São Carlos, Rodovia Washington Luis, km 235, 13565-905 São Carlos, SP, Brazil. (FMCS) francisco.sassi@hotmail.com, (MBC) mbcioffi@ufscar.br (corresponding author), (OMF) omfilho@ufscar.br.

Loricariidae é uma família de peixes Neotropicais, dividida em seis subfamílias e ranqueada na terceira posição dentre os grupos mais biodiversos de peixes. Este estudo conduz uma revisão atualizada das investigações citogenéticas na família, discutindo as tendências evolutivas cromossômicas e as principais lacunas e direções futuras para estudos. Cobrindo 125 publicações que analisam 234 espécies de todas as subfamílias exceto Lithogeninae, correspondendo a aproximadamente 21% da diversidade de espécies válidas, nosso estudo revelou amostragens em seis bacias hidrográficas distintas na Argentina, Brasil, Equador, Paraguai e Venezuela. Há uma escassez de dados para o nordeste do Brasil, o oeste da Amazônia, o escudo das Guianas e outros países Neotropicais. Em loricariídeos, há sete sistemas de cromossomos sexuais distintos e uma variedade de números diploides (2n), variando de 33 a 96 como resultado de rearranjos cromossômicos diferentes como fusões, fissões, translocações e inversões. Nós registramos mais regiões organizadoras de nucléolo (Ag-RON) simples quando comparado com múltiplas, e o número fundamental (NF) variou entre 34 e 142. Estudos populacionais foram realizados em poucos organismos, mas uma extraordinária variação cariotípica que inclui cromossomos B é demonstrada. Apesar de esforços contínuos, a citogenética ainda não captura adequadamente a diversidade de Loricariidae.

Palavras-chave: Ag-RON, Cariótipo, Cascudos, Cromossomos B, Cromossomos sexuais.

INTRODUCTION

Among vertebrates, the paraphyletic group of fish outstands because of its high richness and diversity of morphology and ecology (Nelson *et al*., 2016). They can be found all over the Earth in freshwater and marine environments, and humans exploit them widely as primary food sources or as ornamental species for the aquarium trade (Evers *et al*., 2019; Novák *et al*., 2020, 2022). Such diversity is observed in about 36,000 valid species, with almost half of them belonging to the 450 families that make up the infraclass Teleostei (Fricke *et al*., 2023). More than 18,000 species are found in freshwater environments, the majority of which are found in tropical and subtropical regions, including the Amazon River Basin, tropical Africa, and southeast Asia (Lévêque *et al*., 2008; Nelson *et al*., 2016). The monophyletic order Siluriformes (popularly known as catfishes), is home to a large portion of this variety (Fink, Fink, 1981, 1996; Arratia *et al*., 2003; Saitoh *et al*., 2003; Sullivan *et al*., 2006). This order is composed by the suborder Loricarioidei, which consists of six families that are endemic to South America, and Siluroidei, which includes 29 families that are spread over all continents except Antarctica (Sullivan *et al*., 2006). Due to their widespread distribution (Fig. 1), research focusing on the biogeography of siluriform fishes often indicates that South America may have served as the group's center of origin (Briggs, 2005; Sullivan *et al*., 2006).

Loricariidae species, which rank third among the most biodiverse fish families, are known by several names, including armored catfishes, plecos, "cascudos", "bodós", and

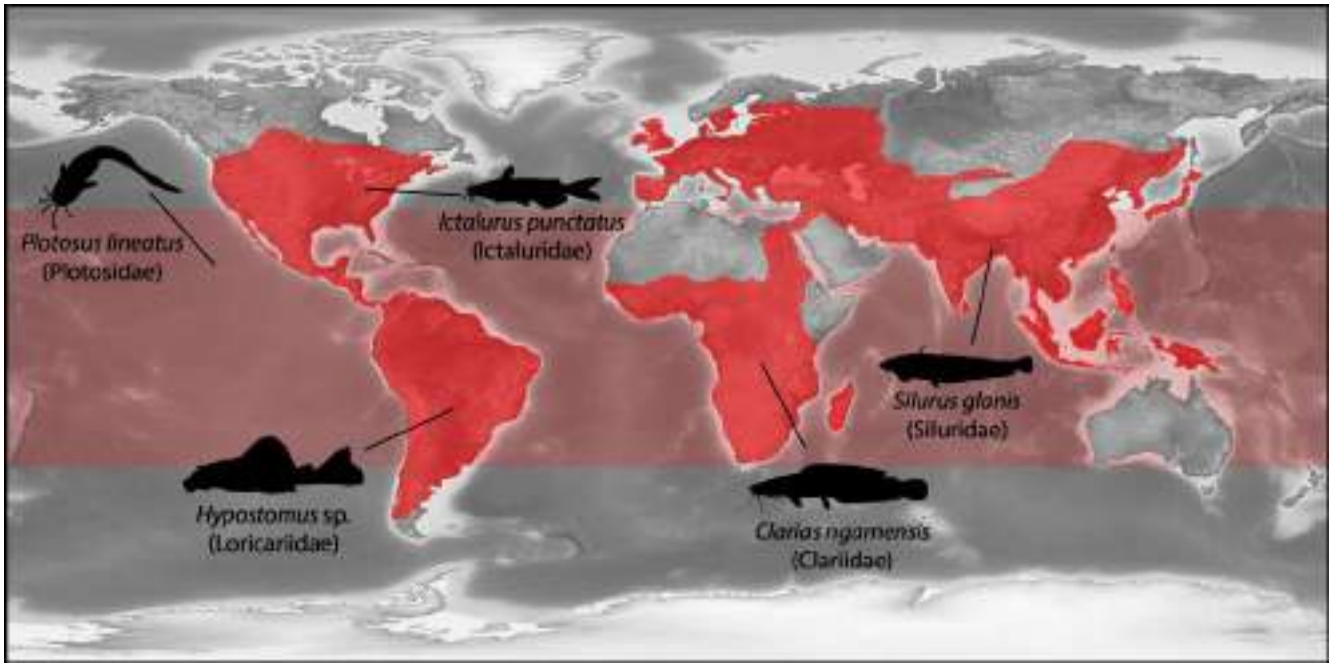


FIGURE 1 | World map showing the distribution of Siluriformes (red areas) according to Nelson *et al.* (2016) and GBIF (2023), with illustrations of important families in distinct regions.

"acaris". Together with Characidae and Cyprinidae, it corresponds for about 50% of all Ostariophysi species (Nelson *et al*., 2016). According to Fricke *et al*. (2023), 1,051 valid species are divided into six subfamilies: Delturinae, Hypoptopomatinae, Hypostominae, Lithogeninae, Loricariinae, and Rhinelepinae. Their distinct anatomy, which includes a suckermouth positioned ventral and dermal plates covering the body, allows them to be recognized despite their widespread distribution in the Neotropical region. Loricariids are iliophagous, with a vast diversity of feeding habitats ranging from wood to detritus and algae (Buck, Sazima, 1995; Lujan *et al*., 2017). They demonstrate a broad range of sizes, from less than 5 cm (*e.g*., Ribeiro *et al*., 2012) to approximately 1 m (Lujan *et al*., 2010). Given a combination of excessive reaping for the aquarium trade and the destruction of their habitats, some loricariids are threatened (Barros *et al*., 2023). Indeed, the high ornamental trade of plecos led to the development of the L-code (or L-number). This code was developed by aquarists, aiming to create a temporary "voucher number" for morphological variants identified by aquarium trade that lack a proper taxonomic identification or are difficult to classify only by morphological features (Stawikowski *et al*., 2004). Recently, 223 Loricariidae species have been assigned from their L-numbers to valid species name (Novák *et al*., 2022).

The description of fish species is often complex. While some species present a conservation of morphological features, body-color variation and polymorphisms are frequently observed in fish (Price *et al*., 2008). In this context, the use of complementary methods is fundamental to assessing all biodiversity, in especial cytogenetics (Stace, 2000), the field of genetics that studies chromosomes from their structure and molecular composition to their organization and variation among species. Cytogenetics is an important tool for discovering the Neotropical fish biodiversity, as exemplified by the

outstanding number of new and/or cryptic species in addition to karyomorphs with absence of hybrid forms reported (*e.g.*, Pazian *et al*., 2012; Ferreira *et al*., 2014; Oliveira *et al*., 2016; Ramirez *et al*., 2017; Rocha-Reis *et al*., 2018). Among loricariids, Artoni, Bertollo (2001) suggest that 2n = 54 might represent the basal diploid number for this family, with distinct trends in evolution among its subfamilies. Considering the continuous growing number of studies involving Loricariidae species, here we review for the first time the scientific literature that includes cytogenetic data for Loricariidae species. We discussed the trends in chromosomal evolution in this representative fish group and the main gaps and future directions for studies.

MATERIAL AND METHODS

A detailed search was conducted on Google Scholar until December of 2023, using the keywords "Cytogenetics Loricariidae" and "Chromosomes Loricariidae". During the screening of research papers, all the duplicate papers and cytogenetic papers without karyotype information were excluded from our compilation (for example Traldi *et al*., 2019, where only mapping of repetitive sequences was conducted, without addressing the diploid number — 2n and karyotype structure). We included all papers found published between 1968 and 2023 in journals in any language available. For more assurance on the quality of results, the data analyzed was double-checked by the authors: the resulted table comprising the cytogenetic features was checked by each author, comparing with the original papers to determine whether such articles would comply with the inclusion and exclusion criteria. We followed the classification of chromosomes by the centromeric position and arm ratios, proposed by Levan *et al*. (1964) and standardized for fish in Arai (2011) as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a).

From each paper, we extracted cytogenetic and biogeographic data such as diploid number (2n), fundamental number (NF), karyotype formula (KF), sex chromosome system (SCS) and AgNOR/18S rDNA sites (simple or multiple). Additionally, locality and geographical coordinates of the populations investigated were also compiled, with the city name, state, and country indicated according to the coordinates provided in the papers. Species and family names were checked against Eschmeyer's Catalog of Fishes (Fricke *et al*., 2023) and updated according to the valid names. Taxonomic abbreviations were maintained as present in the papers, following: "sp." for specimens not fully identified, "prope" for specimens with resemblance to a described taxon but not identical, "aff." for closely related to an identified taxon or with uncertainty in taxonomic identification, "cf." for specimens similar to or comparable to a taxon but with doubts, "n.sp." for proposed new species, and "L." for locally variants of specimens. For the papers in which the geographical coordinates were available, we plotted those sample points into maps using QGIS 3.28.2.

RESULTS

We compiled 125 papers that included cytogenetic data on Loricariidae species. These studies comprised a diversity of 234 species, here included those identified as "sp.", *prope*, "aff.", "cf.", "n.sp.", and "L" as distinct species, from 48 genus. Hypostominae was the subfamily most represented with 142 species karyotyped, followed by Loricariinae (54 spp.), Hypoptopomatinae (34 spp.), and Delturinae and Rhinelepinae with 2 spp. each, with absence of data for Lithogeninae. The *Hypostomus* genus was the most represented with 26 valid species karyotyped, in a total of 159 records when including all populational data and those identified as "sp.", *prope*, "aff.", "cf.", "n. sp.", and "L". The geographical coordinates plotted into maps show a distribution of species cytogenetically investigated in Brazil, Argentina, and Paraguay, comprising six distinct river basins (Fig. 2). Although species from Ecuador and Venezuela were also compiled, those papers do not present geographical coordinates of the sample sites.

Diploid number varied from 2n = 33 in males of *Rineloricaria teffeana* (Steindachner, 1879) (Marajó *et al*., 2022) to 2n = 96 in *Hemipsilichthys* sp. (Kavalco *et al*., 2004, 2005). The fundamental number ranged from 34 in *R. teffeana* (Marajó *et al*., 2022) to 142 in *Hypostomus topavae* (Godoy, 1969) (Kamei *et al*., 2017), and the simple distribution of Ag-NOR/18S rDNA was the most common with 269 records, against 110 records of multiple sites. Seven sex chromosomes systems were described for 31 Loricariidae species, the simple XX/XY, XX/X0, and ZZ/ZW, and the multiples $X_1X_1X_2X_2/X_1X_2Y$, XX/XY_1Y_2, ZZ/ZW_1W_2, and $Z_1Z_1Z_2Z_2/Z_1Z_2W_1W_2$. B chromosomes were found in five species, varying from 1B (*e.g*., de Souza *et al*., 2009) to 3B chromosomes (*e.g*., Porto *et al*., 2010). Complete results were compiled in Tab. 1.

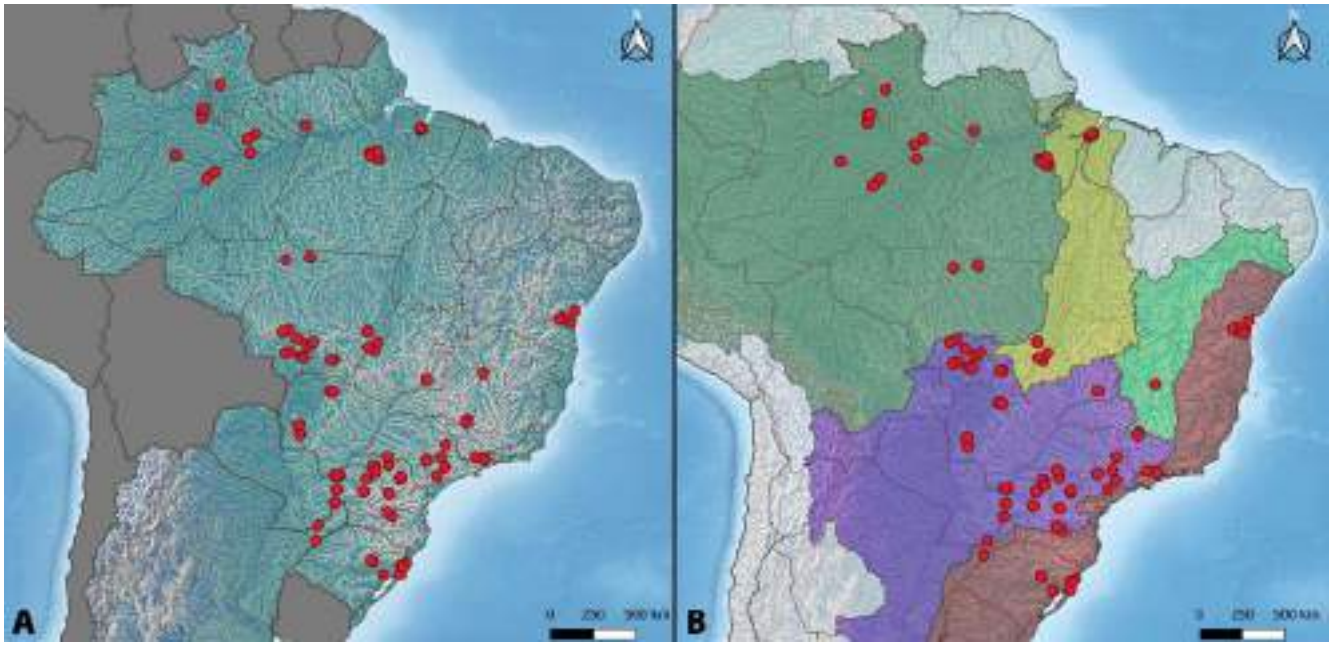


FIGURE 2 | Partial maps of South America showing the distribution of Loricariidae species (red dots) investigated by cytogenetics that have the geographical coordinates published together. **A.** Map highlighting the geopolitical regions of South America, with emphasis on Brazil, Paraguay, and part of Argentina territory; **B.** Map highlighting the hydrographic basins of South America, with emphasis on Amazonas (green), Tocantins-Araguaia (yellow), Paraná/Paraguay (purple), São Francisco (cyan), Atlantic East and Atlantic South (red) River Basins.

TABLE 1 | Review of cytogenetic data by subfamily of Loricariidae published as articles. Geographical coordinates ending with asterisk indicates mismatched coordinates. Diploid number (2n), fundamental number (NF), karyotype formula (KF), sex chromosome system (SCS) are displayed.

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
Loricariinae											
Brochiloricaria	*macrodon* (Kner, 1853)					58♀♂	78		18m + 2sm + 38st/a		Michele *et al.* (1977). Reported as Loricaria macrodon
Farlowella	*amazonum* (Günther, 1864)	Agua Boa stream, Mundo Novo, MS, Brazil				58♀♂	110	Simple	6m + 38sm + 8st + 6a		Fernandes *et al.* (2012)
	amazonum	Dourado stream, Japorã, MS, Brazil	23°51'04.9"S	54°09'51.1"W	Iguatemi - Paraná	58♀♂	110	Simple	12m + 30sm + 10st + 6a		Fernandes *et al.* (2015)
	cf. *amazonum*	Paraná do Piloto, Barcelos, AM, Brazil	0°56'04.8"S	62°58'01.6"W	Negro - Amazonas	58♀♂	116	Simple	14m + 30sm + 14st		Marajó *et al.* (2018)
	schreitmuelleri Arnold, 1936	Jundiá stream, Manaus, AM, Brazil	2°19'43.8"S	60°04'40.4"W	Cuieiras - Amazonas	58♀♂	112	Simple	10m + 30sm + 14st + 4a		Marajó *et al.* (2018)
	hahni Meinken, 1937	Dourado stream, Mundo Novo, MS, Brazil	23°51'04.9"S	54°26'31.4"W	Paraná	58♀♂	110	Simple	12m + 30sm + 10st + 6a		Fernandes *et al.* (2021)
	hahni	Iguaçu River, Capanema, PR, Brazil	25°38'18.7"S	54°28'01.7"W	Paraná	58♀♂	112	Simple	12m + 20sm + 22st + 4a		Fernandes *et al.* (2021)
Harttia	*carvalhoi* Miranda Ribeiro, 1939	Ribeirão grande, Pindamonhangaba, SP, Brazil	22°46'03.0"S	45°26'07.1"W	Paraíba do Sul	52♀, 53♂		Simple	18m + 18sm + 8st + 8a or 17m + 18sm + 8st + 10a	XX/XY_1Y_2	Centofante *et al.* (2006)
	carvalhoi	Pindamonhangaba, SP, Brazil			Paraíba do Sul	52♀, 53♂		Simple	16m + 16sm + 12st + 8a ♀, 15m + 16sm + 12st + 10a ♂	XX/XY_1Y_2	Blanco *et al.* (2013, 2017); Deon *et al.* (2022a,b)
	carvalhoi					52♀♂		Simple	30m/sm + 20st/a		Alves *et al.* (2003). Reported as Harttia loricariformis, corrected in Centofante et al. (2006)
	dissidens Rapp Py-Daniel & Oliveira, 2001	Tambor stream, Rurópolis, PA, Brazil	4°05'37.8"S	55°00'30.2"W		54♀♂	92	Simple	20m + 26sm + 8a		Sassi *et al.* (2021, 2023a,b)
	duriventris Rapp Py-Daniel & Oliveira, 2001	Parauapebas River, Canaã dos Carajás, PA, Brazil	6°30'06.5"S	50°02'35.3"W	Tocantins-Araguaia	55♂, 56♀	96, 98	Simple	16m + 16sm + 16st + 8a ♀, 17m + 16sm + 16st + 6a ♂	$X_1X_1X_2X_2/X_1X_2Y$	Sassi *et al.* (2020, 2023a,b)
	gracilis Oyakawa, 1993	Machadinho stream, Santo Antonio do Pinhal, MG, Brazil	22°48'31.0"S	45°41'21.0"W	Sapucaí-Mirim	58♀♂		Simple	20m + 22sm + 8st + 8a		Blanco *et al.* (2017); Deon *et al.* (2022a,b)
	guianensis Rapp Py-Daniel & Oliveira, 2001	Paraíso stream, Alenquer, PA, Brazil	1°29'02.2"S	54°50'31.2"W		58♀♂	96	Simple	20m + 26sm + 2st + 10a		Sassi *et al.*, 2021, 2023a,b
	intermontana Oliveira & Oyakawa, 2019	Piranga River, Carandaí, MG, Brazil	20°59'34.0"S	43°43'30.0"W		52♀, 53♂	90, 91		14m + 12sm + 12st + 14a ♀, 13m + 12sm + 13st + 15a ♂	XX/XY_1Y_2	Deon *et al.* (2020, 2022)
	kronei Miranda Ribeiro, 1908					58♀♂		Simple	40m/sm + 18st/a		Alves *et al.* (2003)
	kronei	Açungui River, Campo Largo, PR, Brazil			Ribeira	58♀♂	106	Simple	14m + 20sm + 14st + 10a		Blanco *et al.* (2013, 2017); Deon *et al.* (2022a,b)
	longipinna Langeani, Oyakawa & Montoya-Burgos, 2001	São Francisco River, Pirapora, MG, Brazil	17°21'22.3"S	44°56'59.5"W	São Francisco	58♀♂	102	Simple	16m + 12sm + 16st + 14a + 0-2Bs		Blanco *et al.* (2013, 2017); Deon *et al.* (2022a,b)
	loricariformis Steindachner, 1877	Paraitinga River, Silveiras, SP, Brazil	22°52'22.5"S	44°51'00.4"W	Paraíba do Sul	56♀♂	106		16m + 22sm + 10st + 8a		Kavalco *et al.* (2004, 2005); Blanco *et al.* (2017); Deon *et al.* (2022a,b)
	punctata Rapp Py-Daniel & Oliveira, 2001	Itiquira River, Formosa, GO, Brazil	15°19'25.0"S	47°25'26.0"W	Tocantins-Araguaia	57♂, 58♀	116	Simple	16m + 20sm + 12st + 10a ♀, 16m + 21sm + 12st + 8a ♂	$X_1X_1X_2X_2/X_1X_2Y$	Blanco *et al.* (2014, 2017); Deon *et al.* (2022); Sassi *et al.* (2021, 2023a)
	rondoni Oyakawa, Fichberg & Rapp Py-Daniel, 2018	13 de Maio River, Cachoeira da Serra, PA, Brazil	8°38'53.0"S	55°01'41.0"W	Xingu - Amazon	54♀♂	100	Simple	20m + 26sm + 4st + 4a	XX/XY	Sassi *et al.* (2020, 2023a,b)
	sp. 1	Macacos stream, Silveiras, SP, Brazil	22°40'43.0"S	44°51'25.0"W		56♀, 57♂	94		14m + 14sm + 10st + 18a ♀, 13m + 14sm + 10st + 20a ♂	XX/XY_1Y_2	Deon *et al.* (2020, 2022a,b)
	sp. 2	Barra Grande River, Prudentópolis, PR, Brazil	24°58'40.7"S	51°07'34.3"W		62♀♂	104		16m + 14sm + 12st + 20a		Deon *et al.* (2020, 2022a,b)
	sp. 3	Rio do Peixe, Cachoeira da Serra, PA, Brazil	8°39'20.7"S	55°09'24.1"W		54♀♂	96	Simple	16m + 18sm + 14st + 6a		Sassi *et al.* (2021, 2023a,b)
	torrenticola Oyakawa, 1993	Araras stream, Brazil			São Francisco	56♀♂	98	Simple	16m + 10sm + 16st + 14a		Blanco *et al.* (2012a, 2017); Deon *et al.* (2022a,b)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	villasboas Oyakawa, Fichberg & Rapp Py-Daniel, 2018	Curuá River, Cachoeira da Serra, PA, Brazil	8°44'09.0"S	54°57'46.0"W	Xingu - Amazon	55♂, 56♀	96, 98	Simple	18m + 24sm + 6st + 8a ♀, 19m + 24sm + 6st + 6a ♂	$X_1X_1X_2X_2/X_1X_2Y$	Sassi *et al.* (2020, 2023a,b)
Hemiodontichthys	sp. 1	São Francisco stream, Brazil			Purus	46♀♂			42m/sm + 4st/a		Carvalho *et al.* (2018)
	sp. 2	Iquiri stream, Brazil			Purus	58♀♂			28m/sm + 30st/a		Carvalho *et al.* (2018)
Loricaria	*cataphracta* Linnaeus, 1758	Córrego da Onça, Coxim, MS, Brazil			Paraguai	64♀♂	86	Simple and Multiple	12m + 8sm + 2st + 42a		Porto *et al.* (2014b)
	cataphracta					64	76		12m/sm + 52st/a		Fenocchio *et al.* (2003). Reported as Loricaria carinata
	cf. *cataphracta*	Iguaçu River, Brazil			Paraná	64♀♂		Simple	12m + 8sm + 2st + 42a		Takagui *et al.* (2020)
	simillima Regan, 1904	Paraná River, Brazil	27°06'22.5"S	55°31'20.0"W	Paraná	64♀♂	88	Simple	12m + 12sm + 40st/a		Benitez *et al.* (2017)
	simillima	Miranda River, Brazil			Paraguai	64♀♂		Simple	12m + 8sm + 2st + 42a		Takagui *et al.* (2020)
Loricariichthys	*anus* (Valenciennes, 1835)	Capivara stream, Laguna dos Patos system, RS, Brazil	30°17'34.0"S	51°19'21.2"W		54♀♂	82	Simple	10m + 18sm + 26a		Takagui *et al.* (2014)
	anus	Itapeva Lagoon, Tramandaí system, Brazil	29°32'11.9"S	49°55'47.9"W		54♀♂	80	Simple	10m + 16sm + 28a		Takagui *et al.* (2014)
	anus	Chimiray stream, Argentina	28°05'49.4"S	55°41'37.3"W	Uruguay	54♀♂	82	Simple	10m + 18sm + 26a		Takagui *et al.* (2014)
	anus	Iguaçu River, Brazil			Paraná	54♀♂		Simple	14m + 14sm + 26a		Takagui *et al.* (2020)
	maculatus (Bloch, 1794)				La Plata	56	78		22m/sm + 34st/a		Fenocchio *et al.* (2003)
	platymetopon Isbrücker & Nijssen, 1979	Jacutinga River, Brazil	23°13'25.3"S	50°58'47.4"W	Paranapanema	54♀♂	80	Simple	10m + 16sm + 28a		Takagui *et al.* (2014)
	platymetopon	Paranapanema River, Brazil	22°42'30.3"S	51°04'08.4"W		54♀♂	80	Simple	10m + 16sm + 28a		Takagui *et al.* (2014)
	platymetopon	Miranda River, Brazil				54♀♂		Simple	10m + 16sm + 28a		Takagui *et al.* (2020)
Pyxiloricaria	*menezesi* Isbrücker & Nijssen, 1984	Miranda River, Brazil			Paraná	68♀♂		Simple	8m + 6sm + 2st + 52a		Takagui *et al.* (2020)
Proloricaria	*prolixa* (Isbrücker & Nijssen, 1978)	Três Bocas stream, Brazil			Paraná	62♀♂		Simple	20m + 6sm + 36a		Takagui *et al.* (2020)
Rineloricaria	*aequalicuspis* Reis & Cardoso, 2001	Maquine River, RS, Brazil	29°39'10.4"S	50°12'31.8"W	Southeast Atlantic	68♀♂		Simple	68st/a		Venturelli *et al.* (2021)
	cadeae (Hensel, 1868)					66♀♂			2m + 64st/a		Alves *et al.* (2003)
	cadeae	Guaíba Lake, RS, Brazil				64♀♂		Simple	2m/sm + 62st/a		Maia *et al.* (2010)
	cadeae	Forquetinha River, RS, Brazil	29°24'22.4"S	52°03'19.2"W		64♀♂		Simple	64st/a		Venturelli *et al.* (2021)
	capitonia Ghazzi, 2008	Uruguai River, São Carlos, SC, Brazil			Uruguai	64♀♂	70	Simple	4m + 2sm + 58st/a		Primo *et al.* (2017)
	kronei (Miranda Ribeiro, 1911)	Itapocu River, Santa Catarina, Brazil				64♀♂			6m/sm + 58st/a		Alves *et al.* (2003)
	lanceolata (Günther, 1868)	Onça Stream, Coxim, MS, Brazil	18°32'20.0"S	54°33'43.0"W		48♀♂	54	Multiple	4m + 2st + 42a		Porto *et al.* (2014a)
	lanceolata	Onça Stream, Coxim, MS, Brazil	18°32'20.0"S	54°33'43.0"W		48♀♂	53	Multiple	3m + 2st + 43a		Porto *et al.* (2014a)
	lanceolata	Onça Stream, Coxim, MS, Brazil	18°32'20.0"S	54°33'43.0"W		48♀♂	55	Multiple	3m + 2sm + 2st + 41a		Porto *et al.* (2014a)
	lanceolata	Onça Stream, Coxim, MS, Brazil	18°32'20.0"S	54°33'43.0"W		47♀♂	54	Multiple	4m + 1sm + 2st + 40a		Porto *et al.* (2014a)
	lanceolata	Onça Stream, Coxim, MS, Brazil	18°32'20.0"S	54°33'43.0"W		47♀♂	53	Multiple	3m + 1sm + 2st + 41a		Porto *et al.* (2014a)
	lanceolata	Onça Stream, Coxim, MS, Brazil	18°32'20.0"S	54°33'43.0"W		47♀♂	52	Multiple	2m + 1sm + 2st + 42a		Porto *et al.* (2014a)
	lanceolata	Onça Stream, Coxim, MS, Brazil	18°32'20.0"S	54°33'43.0"W		46♀♂	54	Multiple	4m + 2sm + 2st + 38a		Porto *et al.* (2014a)
	lanceolata	Onça Stream, Coxim, MS, Brazil	18°32'20.0"S	54°33'43.0"W		46♀♂	53	Multiple	3m + 2sm + 2st + 39a		Porto *et al.* (2014a)
	lanceolata	Onça Stream, Coxim, MS, Brazil	18°32'20.0"S	54°33'43.0"W		46♀♂	52	Multiple	2m + 2sm + 2st + 40a		Porto *et al.* (2014a)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	lanceolata	Onça Stream, Coxim, MS, Brazil	18°32'20.0"S	54°33'43.0"W		45	53		4m + 2sm + 37a		Porto *et al.* (2014a)
	latirostris (Boulenger, 1900)	Laranjinha River, Ventania, PR, Brazil			Cinzas	46♀♂	60	Simple	10m + 4sm + 32st/a		Primo *et al.* (2017)
	latirostris	Barra Grande River, Prudentópolis, PR, Brazil			Ivaí	46♀♂	60	Simple	10m + 4sm + 32st/a		Primo *et al.* (2017)
	latirostris	Das Pedras River, Ventania, PR, Brazil	24°07'39.29"S	50°14'16.21"W		46♀♂	60	Simple	10m + 4sm + 32st/a		Glugoski *et al.* (2018)
	latirostris	Piumhi River, Piumhi, MG, Brazil	20°31'55"S	46°02'42"W		48♀♂	60	Simple	6m + 6sm + 36st/a		Glugoski *et al.* (2018)
	latirostris	Laranjinha River, Ventania, PR, Brazil	23°58'13"S	50°14'01"W		46♀♂	60	Simple	10m + 4sm + 32st/a		Glugoski *et al.* (2022)
	latirostris	Piumhi River, Piumhi, MG, Brazil	20°31'55"S	46°02'42"W		48♀♂	60	Simple	6m + 6sm + 36st/a		Glugoski *et al.* (2022)
	lima (Kner, 1853)	Ribeira River, Areia stream, or Açungui River Ponta Grossa, PR, Brazil			Paraná	70♀♂	72	Simple	2st + 68a		Rosa *et al.* (2012)
	lima	Ribeira River, Areia stream, or Açungui River Ponta Grossa, PR, Brazil			Paraná	70♀♂	74	Simple	2m + 2st + 66a		Rosa *et al.* (2012)
	lima	Ribeira River, Areia stream, or Açungui River Ponta Grossa, PR, Brazil			Paraná	69♀♂	72	Simple	1m + 2st + 66a		Rosa *et al.* (2012)
	lima	Ribeira River, Areia stream, or Açungui River Ponta Grossa, PR, Brazil			Paraná	69♀♂	73	Simple	1m + 1sm + 2st + 65a		Rosa *et al.* (2012)
	lima	Ribeira River, Areia stream, or Açungui River Ponta Grossa, PR, Brazil			Paraná	68♀♂	72	Simple	2m + 2st + 64a		Rosa *et al.* (2012)
	lima	Ribeira River, Areia stream, or Açungui River Ponta Grossa, PR, Brazil			Paraná	68♀♂	74	Simple	1m + 1m + 2sm + 2st + 62a		Rosa *et al.* (2012)
	lima	Ribeira River, Areia stream, or Açungui River Ponta Grossa, PR, Brazil			Paraná	66♀♂	72	Simple	2m + 1m + 1m + 2st + 60a		Rosa *et al.* (2012)
	lima	Ribeira River, Areia stream, or Açungui River Ponta Grossa, PR, Brazil			Paraná	66♀♂	72	Simple	1m + 1m + 1m + 1st + st + 60a		Rosa *et al.* (2012)
	longicauda Reis, 1983	Cerquinha Lagoon, RS, Brazil	30°13'56.5"S	50°15'40.9"W	Southeast Atlantic	70♀♂		Simple	8m/sm + 62st/a		Venturelli *et al.* (2021)
	malabarbai Rodriguez & Reis, 2008	Forquetinha River, RS, Brazil	29°24'22.4"S	52°03'19.2"W	Southeast Atlantic	64♀♂		Simple	4m/sm + 60st/a		Venturelli *et al.* (2021)
	microlepidogaster (Regan, 1904)	Forquetinha River, RS, Brazil	29°24'22.4"S	52°03'19.2"W	Southeast Atlantic	68♀♂		Simple	68st/a		Venturelli *et al.* (2021)
	n. sp.	Betari River, São Paulo, Brazil				70♀♂			2sm + 68a		Alves *et al.* (2003)
	parva (Boulenger, 1895)					48♀♂					Hinegardner, Rosen (1972). Reported as Loricaria parva
	parva	Miranda River, Brazil			Paraguai	60♀♂		Simple	6m/sm + 54st/a		Takagui *et al.* (2020)
	pentamaculata Langeani & de Araujo, 1994	Tauá stream, Brazil			Paraná	56-59♀♂	64-65		8m/sm + 48st/a or 9m/sm + 47st/a + 0-3Bs		Porto *et al.*, 2010
	pentamaculata	Jacuaca stream, tributary of Tibagi River, PR, Brazil				56♀♂			8m/sm + 48st/a		Maia *et al.* (2010)
	pentamaculata	Água do Oito stream, tributary of Tibagi River, PR, Brazil				56♀♂			8m/sm + 48st/a		Maia *et al.* (2010)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	pentamaculata	Tatupeba stream, Brazil			Paraná	56♀♂	64	Multiple	8m/sm + 48st/a		Porto *et al.* (2011)
	pentamaculata	Tauá stream, Brazil			Paraná	56♀♂	64-65	Simple	8m/sm + 48st/a or 9m/sm + 47st/a		Porto *et al.* (2011)
	pentamaculata	Keller River, Brazil			Paraná	56♀♂	64	Simple	8m/sm + 48st/a		Porto *et al.* (2011)
	pentamaculata	Barra Grande River, Prudentópolis, PR, Brazil			Ivaí	56♀♂	70	Simple	4m + 10sm + 42st/a		Primo *et al.* (2017)
	pentamaculata	Barra Grande River, Prudentópolis, PR, Brazil			Ivaí	54♀♂	64	Simple	6m + 4sm + 44st/a		Primo *et al.* (2018)
	pentamaculata	Juruba River, Apucarana, PR, Brazil			Tibagi	56♀♂	70	Simple	4m + 10sm + 42st/a		Primo *et al.* (2017)
	pentamaculata	Itiz stream, Brazil			Ivaí	56♀♂	64	Simple	8m/sm + 48st/a		Cius *et al.* (2022)
	pentamaculata karyomorph A	Barra Grande river, Prudentópolis, PR, Brazil	25°05'21.0"S	50°57'22.0"W	Ivaí	56♀♂	64	Simple			Glugoski *et al.* (2023)
	pentamaculata karyomorph B	Barra Grande river, Prudentópolis, PR, Brazil	25°05'21.0"S	50°57'22.0"W	Ivaí	55♀♂	64	Simple			Glugoski *et al.* (2023)
	pentamaculata karyomorph C	Barra Grande river, Prudentópolis, PR, Brazil	25°05'21.0"S	50°57'22.0"W	Ivaí	54♀♂	64	Simple			Glugoski *et al.* (2023)
	quadrensis Reis, 1983	Quadros lagoon, RS, Brazil	29°44'42.8"S	50°06'54.3"W	Southeast Atlantic	70♀♂		Simple	6m/sm + 64st/a		Venturelli *et al.* (2021)
	reisi Ghazzi, 2008	Chimiray stream, Brazil			Uruguai	60♀♂		Simple	60st/a		Takagui *et al.* (2020)
	stellata Ghazzi, 2008	Uruguai River, São Carlos, SC, Brazil			Uruguai	54♀♂	74	Simple	6m + 14sm + 34st/a		Primo *et al.* (2017)
	strigilata (Hensel, 1868)	Forquetinha River, RS, Brazil			Southeast Atlantic	68♀♂		Simple	6m/sm + 62st/a		Maia *et al.* (2010)
	teffeana (Steindachner, 1879)	Tefé River, Tefé, AM, Brazil	3°21'14.9"S	64°40'27.9"W	Amazon	33♂, 34♀	34	Simple	1sm + 32a	$X_1X_1X_2X_2$/ X_1X_2Y	Marajó *et al.* (2022)
Spatuloricaria	sp.	Xingu River, Altamira, PA, Brazil	3°19'30.0"S	52°11'10.0"W	Xingu - Amazon	66♀♂	92	Simple	4m + 7sm + 6st + 16a		Ferreira *et al.* (2014)
	sp.	Caripetuba River, Abaetuba, PA, Brazil	1°37'23.5"S	48°55'33.0"W		66♀♂	82		6m + 10sm + 50a		Almeida *et al.* (2023)
Sturisoma	*barbatum* (Kner, 1853)	Miranda River, Brazil			Paraguai	66♀♂		Simple	20m + 18sm + 16st + 12a		Takagui *et al.* (2020)
	cf. *nigrirostrum* Fowler, 1940	Araguaia River, Barra do Garças, MT, Brazil			Tocantins-Araguaia	74♀♂			20m + 18sm + 36st/a		Artoni, Bertollo (2001)
Hypoptopomatinae											
Corumbataia	*cuestae* Britski, 1997	Alambari stream, Botucatu, SP, Brazil				54♀♂	108	Simple	34m + 20sm		Cristina *et al.* (2005)
	cuestae	Lapa stream, SP, Brazil			Paraná	54♀♂		Simple	14m + 10sm + 3st/a		Camilo, Moreira Filho (2005)
	tocantinensis Britski, 1997	Vermelho River, Goiás, GO, Brazil				54♀♂	108	Simple	28m + 26sm		Cristina *et al.* (2005)
Hisonotus	*depressicauda* (Miranda Ribeiro, 1918)	Santo Inácio River, Bofete, SP, Brazil				54♀♂		Simple	14m + 18sm + 2st + 10a		Andreata *et al.* (1994). Reported as *Microlepidogaster depressicauda*
	leucofrenatus (Miranda Ribeiro, 1908)	Poço Grande stream, Juquiá, SP, Brazil				54-56♀♂		Simple	24m + 26sm + 4st + 2a	ZZ/ZW	Andreata *et al.* (1993). Reported as *Microlepidogaster leucofrenatus*
	leucofrenatus	Marumbi River, Morretes, PR, Brazil				54-56♀♂		Simple	22m + 24sm + 4st + 2a	ZZ/ZW (discarded by Andreata *et al.*, 2010)	Andreata *et al.* (1993). Reported as *Microlepidogaster leucofrenatus*
	leucofrenatus	Cavalo stream, Jaraguá do Sul, SC, Brazil				54♀♂		Simple	22m + 24sm + 6st + 2a		Andreata *et al.* (2006)
	leucofrenatus	Marumbi River, Morretes, PR, Brazil				54-55♀♂		Simple	22m + 24sm + 4st + 2a		Andreata *et al.* (2010)
	leucofrenatus	Cavalo stream, Jaraguá do Sul, SC, Brazil				54♀♂		Simple	22m + 24sm + 6st + 2a		Andreata *et al.* (2010)
	nigricauda (Boulenger, 1891)	Guaíba River, Eldorado do Sul, RS, Brazil				54♀♂		Simple	26m + 20sm + 8st		Andreata *et al.* (2006)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	sp. A	Paraitinga River, Salesópolis, SP, Brazil				54♀♂		Simple	26m + 26sm + 2st		Andreata *et al.* (2006)
	sp. D	Grande stream, Pindamonhangaba, SP, Brazil				54♀♂		Simple	26m + 26sm + 2st		Andreata *et al.* (2006)
Hypoptopoma	*inexspectatum* (Holmberg, 1893)	Piraí River, Poconé, MT, Brazil				54♀♂	82	Simple	10m + 18sm + 8st + 18a		Cristina *et al.* (2005). Reported as *Hypoptopoma guentheri*
Isbrueckerichthys	*duseni* (Miranda Ribeiro, 1907)	Açungui River, Brazil			Ribeira	54♀♂	108	Simple	20m + 20sm + 14st		Ziemniczak *et al.* (2012)
	duseni	Betari River, Iporanga, SP, Brazil				54♀♂			20m + 20sm + 14st		Alves *et al.* (2005)
Kronichthys	*lacerta* (Nichols, 1919)	Marumbi River, Morretes, PR, Brazil				54♀♂			20m + 20sm + 14st		Alves *et al.* (2005)
	lacerta	Marumbi River, Morretes, PR, Brazil				54♀♂		Simple	20m + 20sm + 14st		Alves *et al.* (2012a)
	lacerta	Açungui River, Brazil			Ribeira	54♀♂	108	Simple	22m + 22sm + 10st		Ziemniczak *et al.* (2012)
	subteres Miranda Ribeiro, 1908	Betari River, Iporanga, SP, Brazil				54♀♂			20m + 20sm + 14st		Alves *et al.* (2005)
Microlepidogaster	sp. A	Alambari River, Botucatu, SP, Brazil				54♀♂		Multiple	30m + 20sm + 4st		Andreata *et al.* (1994)
	sp. B	Móia stream, Penápolis, SP, Brazil				54♀♂		Simple	22m + 28sm + 4st		Andreata *et al.* (1994)
Neoplecostomus	*microps* (Steindachner, 1877)	Paraitinga River, Brazil	22°52'22.5"S	44°51'41.0"W	Paraíba do Sul	54♀♂	108		24m + 20sm + 10st		Kavalco *et al.* (2004)
	microps	Paraitinga River, Brazil	22°52'22.5"S	44°51'41.0"W	Paraíba do Sul	54♀♂	108	Simple	24m + 20sm + 10st		Kavalco *et al.* (2005)
	microps	Grande stream, Campos do Jordão, SP, Brazil				54♀♂			20m + 20sm + 14st		Alves *et al.* (2005)
	microps	Paraíba do Sul River, Brazil				54♀♂		Simple	24m + 20sm + 10st		Alves *et al.* (2012a)
	paranensis Langeani, 1990	Sapateiro stream, Barbacena, MG, Brazil				54♀♂			20m + 20sm + 14st		Alves *et al.* (2005)
	paranensis	Hortelã stream, Botucatu, SP, Brazil				54♀♂			20m + 20sm + 14st		Alves *et al.* (2005)
	yapo Zawadzki, Pavanelli & Langeani, 2008	Verde River, PR, Brazil			Tibagi	54♀♂	108	Simple	18m + 20sm + 16st		Ziemniczak *et al.* (2012)
Otocinclus	aff. *vestitus*	Livramento River, Belém, PA, Brazil				72♀♂		Simple	22m + 12sm + 4st + 34a		Andreata *et al.* (1994)
	affinis Steindachner, 1877	Biguá River, Miracatu, SP, Brazil				54♀♂		Simple	46m + 8sm		Andreata *et al.* (1994)
	affinis	Bonito River, Niterói, RJ, Brazil				54♀♂		Simple	40m + 12sm + 12st		Andreata *et al.* (1994)
	flexilis Cope, 1894	Santo Antônio da Patrulha, RS, Brazil				54♀♂	108	Simple	36m + 18sm		Cristina *et al.* (2005)
	vittatus Regan, 1904	Taquari River, Coxim, MS, Brazil				54♀♂	108	Multiple	36m + 18sm		Cristina *et al.* (2005)
	vittatus	Cuiabá River, Santo Antônio do Leverger, MT, Brazil				54♀♂	76	Simple	12m + 10sm + 14st + 18a		Cristina *et al.* (2005)
Otothyris	*juquiae* Garavello, Britski & Schaefer, 1998	Rio Preto stream, Itanhaém, SP, Brazil				54♀♂	96	Simple	32m + 10sm + 12st		Cristina *et al.* (2005)
	travassosi Garavello, Britski & Schaefer, 1998	Ribeira da Terra Firme River, Canavieiras, BA, Brazil				54♀♂	96	Simple	26m + 16sm + 12st		Cristina *et al.* (2005)
Otothyropsis	cf. *polyodon*	Córrego Dourado, MT, Brazil	23°51'04.9"S	54°25'13.9"W		54♀♂	108	Simple	18m + 28sm + 8st		Fernandes *et al.* (2016)
Pareiorhaphis	*splendens* (Bizerril, 1995)	Marumbi River, Morretes, PR, Brazil				54♀♂			20m + 30sm + 4st		Alves *et al.* (2005). Reported as *Hemipsilichthys splendens*
	splendens	Marumbi River, Morretes, PR, Brazil				54♀♂		Simple	20m + 20sm + 14st		Alves *et al.* (2012a)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	splendens	São João River, Garuva, SC, Brazil				54♀♂			20m + 30sm + 4st		Alves *et al.* (2005). Reported as *Hemipsilichthys splendens*
	steindachneri (Miranda Ribeiro, 1918)	Cavalo stream, Jaraguá do Sul, SC, Brazil				54♀♂			20m + 20sm + 14st		Alves *et al.* (2005). Reported as *Hemipsilichthys steindachneri*
	vestigipinnis (Pereira & Reis, 1992)	Caveiras River, Painel, SC, Brazil				54♀♂			20m + 20sm + 14st		Alves *et al.* (2005). Reported as *Hemipsilichthys vestigipinnis*
Pareiorhina	*brachyrhyncha* Chamon, Aranda & Buckup, 2005	Ribeirão Grande creek, Pinda-monhangaba, SP, Brazil			Paraíba do Sul	54♀♂		Simple	18m + 30sm + 6st		Centofante *et al.* (2011)
	rudolphi (Miranda Ribeiro, 1911)	Convento stream, Pinda-monhangaba, SP, Brazil				54♀♂			26m + 16sm + 12st		Alves *et al.* (2005)
	rudolphi	Marimbondo creek, Visconde de Mauá, RJ, Brazil			Paraíba do Sul	54♀♂		Simple	18m + 32sm + 4st		Centofante *et al.* (2011)
Parotocinclus	*maculicauda* (Steindachner, 1877)	Açungui River, Brazil			Ribeira	54♀♂	108	Simple	20m + 20sm + 14st		Ziemniczak *et al.* (2012)
	maculicauda	Poço Grande stream, Juquiá, SP, Brazil				54♀♂		Simple	20m + 32sm + 2st		Andreata *et al.* (1994)
Pseudotocinclus	*tietensis* (Ihering, 1907)	Tietê River, Paranapiacaba, SP, Brazil				54♀♂		Simple	26m + 20sm + 6st	XX/XY	Andreata *et al.* (1992)
	n. sp.	Juquiá River, Juquitiba, SP, Brazil				54♀♂		Simple	22m + 24sm + 8st		Cristina *et al.* (2005)
Pseudotothyris	*obtusa* (Miranda Ribeiro, 1911)	Tributary of Itanhaém River, Itanhaém, SP, Brazil				54♀♂		Simple	26m + 18sm + 4st + 6a		Andreata *et al.* (1994)
Schizolecis	*guentheri* (Miranda Ribeiro, 1918)	Parati-Mirim stream, Parati, RJ, Brazil				54♀♂	102	Simple	30m + 18sm + 6a		Cristina *et al.* (2005)
	guentheri	Sítio do Meio stream, Mongaguá, SP, Brazil				54♀♂	102	Simple	30m + 18sm + 6a		Cristina *et al.* (2005)
	guentheri	Descoberto stream, Guaratuba, PR, Brazil				54♀♂	102	Simple	30m + 18sm + 6a		Cristina *et al.* (2005)
	guentheri	Garuva River, Garuva, SC, Brazil				54♀♂	102	Simple	30m + 18sm + 6a		Cristina *et al.* (2005)
Hypostominae											
Ancistomus	*spilomma* (Cardoso & Lucinda, 2003)	Araguaia River, Pontal do Araguaia, MT, Brazil	15°50'15.0"S	51°58'43.0"W		52♀♂		Multiple	25m + 21sm + 6st ♀, 24m + 22sm + 6st ♂	ZZ/ZW	Oliveira *et al.* (2006). Reported as *Hemiancistrus spilomma*
	spinosissimus (Cardoso & Lucinda, 2003)	Araguaia River, Pontal do Araguaia, MT, Brazil	15°50'15.0"S	51°58'43.0"W		52♀♂		Simple	26m + 22sm + 4st		Oliveira *et al.* (2006). Reported as *Hemiancistrus spinosissimus*
	feldbergae	PA, Brazil				52♀♂		Simple	38m/sm + 14st		Pety *et al.* (2018). Reported as *Peckoltia feldbergae*
Ancistrus	*abilhoai* Bifi, Pavanelli & Zawadzki, 2009	Iguaçu River, União da Vitória, PR, Brazil	26°15'64"S*	51°06'28"W*		48♀♂	90	Simple	22m + 14sm + 6st + 6a		Ribeiro *et al.* (2015)
	abilhoai	Timbó River, Santa Cruz do Timbó, SC, Brazil	26°30'37.0"S	50°46'55.0"W		48♀♂	90	Simple	22m + 14sm + 6st + 6a		Ribeiro *et al.* (2015)
	aff. *dolichopterus* Kner, 1854	Aiapuá Lake, Piagaçu-Purus Sustainable Development Reserve, AM, Brazil	4°27'26.0"S	62°11'56.0"W		52♀♂	78, 79	Simple	16m + 8sm + 2st + 26a ♂, 16m + 9sm + 2st + 25a ♀	ZZ/ZW	Oliveira *et al.* (2007)
	aff. *dolichopterus*	Aiapuá Lake, Piagaçu-Purus Sustainable Development Reserve, AM, Brazil	4°27'26.0"S	62°11'56.0"W		52♀♂	78, 79	Simple	16m + 8sm + 2st + 26a ♂, 16m + 9sm + 2st + 25a ♀	ZZ/ZW	Favarato *et al.* (2016). Reported as *Ancistrus* sp. Balbina
	aguaboensis Fisch-Muller, Mazzoni & Weber, 2001	Ribeirão Bandeirinha, GO, Brazil			Tocantins-Araguaia	50♀♂	86	Simple	16m + 10sm + 4st + 20a		Glugoski *et al.* (2020)
	aguaboensis	Ribeirão Bandeirinha, GO, Brazil			Tocantins-Araguaia	50♀♂	86	Simple	16m + 10sm + 4st + 20a		Schott *et al.* (2022)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	cf. *dubius Eigenmann & Eigenmann, 1889*	Serra das Araras, Barra do Bugres, MT, Brazil				44♀♂		Simple	18m + 10sm + 16st/a	ZZ/ZW	Mariotto *et al.* (2004)
	cf. *dubius*	Coxipó River, Chapada dos Guimarães, MT, Brazil				42♀♂	84		24m + 10sm + 8st		Mariotto *et al.* (2006)
	cf. *dubius*	Pari creek, Cuiabá, MT, Brazil				42♀♂	84		24m + 10sm + 8st	XX/XY	Mariotto *et al.* (2006)
	cf. *dubius*	Flechas creek, Cáceres, MT, Brazil				42♀♂	84		24m + 10sm + 8st	XX/XY	Mariotto *et al.* (2006)
	cf. *dubius*	Fundo creek, Poconé, MT, Brazil				42♀♂	84		24m + 10sm + 8st	XX/XY	Mariotto *et al.* (2006)
	cf. *dubius*	Flechas stream, Brazil	15°58'07.0"S	57°19'07.0"W	Paraguai	42♀♂	84	Simple	24m + 10sm + 8st	XX/XY	Mariotto *et al.* (2011)
	cf. *dubius*	Fundo stream, Brazil	16°14'17.0"S	56°37'31.0"W	Paraguai	42♀♂	84	Simple	24m + 10sm + 8st	XX/XY	Mariotto *et al.* (2011)
	cf. *dubius*	Pari stream, Brazil	15°36'06.0"S	56°12'19.0"W	Paraguai	42♀♂	84	Simple	24m + 10sm + 8st	XX/XY	Mariotto *et al.* (2011)
	cf. *multispinis* (Regan, 1912)	Ribeirão Grande, SP, Brazil			Paraíba do Sul	52♀♂	84	Simple	16m + 10sm + 6st + 20a		Glugoski *et al.* (2020)
	cf. *multispinis*	Ribeirão Grande, SP, Brazil			Paraíba do Sul	52♀♂	84	Simple	16m + 10sm + 6st + 20a		Schott *et al.* (2022)
	cirrhosus (Valenciennes, 1836)	Maracapucú River, Abaetuba, PA, Brazil	1°45'29.2"S	48°56'57.0"W	Tocantins-Araguaia	34♀♂	68	Simple	20m + 14sm		Santos da Silva *et al.* (2023)
	cirrhosus	Ilha do Capim, Abaetuba, PA, Brazil	1°34'02.8"S	48°51'49.1"W	Tocantins-Araguaia	34♀♂	68	Simple	20m + 14sm		Santos da Silva *et al.* (2023). Reported as *Ancistrus* sp. 2
	cirrhosus	Maracapucú River, Abaetuba, PA, Brazil	1°45'29.2"S	48°56'57.0"W	Tocantins-Araguaia	34♀♂	68	Simple	20m + 14sm		Santos da Silva *et al.* (2022a). Reported as *Ancistrus* sp. 2
	cirrhosus	Ilha do Capim, Abaetuba, PA, Brazil	1°34'02.8"S	48°51'49.1"W	Tocantins-Araguaia	34♀♂	68	Simple	20m + 14sm		Santos da Silva *et al.* (2022a)
	claro Knaack, 1999	Coxipó River, Brazil	15°21'59.0"S	55°57'11.0"W	Paraguai	54♀♂	84	Simple	14m + 8sm + 8st + 24a		Mariotto *et al.* (2011)
	claro	Coxipó River, Chapada dos Guimarães, MT, Brazil	15°21'00.0"S	55°57'00.0"W	Paraguai	54♀♂	84	Simple	14m + 8sm + 8st + 24a		Mariotto *et al.* (2013)
	clementinae Rendahl, 1937	Río Palenque (Cantón Pasaje), Ecuador				52♂, 53♀	100, 101	Simple	48m/sm + 5a ♀, 48m/sm + 4st/a ♂	ZZ/ ZW_1W_2	Nirchio *et al.* (2023)
	clementinae	Río La Moquillada (Cantón Las Lajas), Ecuador				52♂, 53♀	100, 101	Simple	48m/sm + 5a ♀, 48m/sm + 4st/a ♂	ZZ/ ZW_1W_2	Nirchio *et al.* (2023)
	cuiabae Knaack, 1999	Arrombado, Poconé, MT, Brazil	16°21'21.0"S	56°27'55.0"W		34♀♂	68	Simple	20m + 8sm + 6st		Mariotto *et al.* (2009)
	cuiabae	Arrombado, Poconé, MT, Brazil	16°21'21.0"S	56°27'55.0"W		34♀♂	67	Multiple	19m + 8sm + 6st + 1a		Mariotto *et al.* (2009)
	cuiabae	Arrombado, Poconé, MT, Brazil	16°21'21.0"S	56°27'55.0"W		34♀♂	66	Simple	18m + 8sm + 6st + 2a		Mariotto *et al.* (2009)
	cuiabae	Arrombado bay, Brazil	16°21'21.0"S	56°27'55.0"W	Paraguai	34♀♂	68	Simple	20m + 8sm + 6st		Mariotto *et al.* (2011)
	dolichopterus Kner, 1854	Demeni River, Barcelos, AM, Brazil	0°25'19.0"S	62°54'42.0"W	Negro	52♀♂	79, 8	Simple	12m + 12sm + 4st + 24a ♂, 11m + 12sm + 4st + 25a ♀	$Z_1Z_1Z_2Z_2$/ $Z_1Z_2W_1W_2$	Oliveira *et al.* (2008)
	dolichopterus	Demeni River, Barcelos, AM, Brazil	0°25'19.0"S	62°54'42.0"W	Negro	52♀♂	79, 8	Simple	12m + 12sm + 4st + 24a ♂, 11m + 12sm + 4st + 25a ♀	$Z_1Z_1Z_2Z_2$/ $Z_1Z_2W_1W_2$	Favarato *et al.* (2016)
	dubius Eigenmann & Eigenmann, 1889	Barretinho stream, Presidente Figueiredo, AM, Brazil	1°58'20.0"S	59°29'48.0"W		38♀, 39♂	76, 78	Simple	27m + 10sm + 2st ♂, 26m + 10sm + 2st ♀	XX/ XY_1Y_2	Oliveira *et al.* (2008)
	dubius	Barretinho stream, Presidente Figueiredo, AM, Brazil	1°58'20.0"S	59°29'48.0"W		38♀, 39♂	76, 78	Simple	27m + 10sm + 2st ♂, 26m + 10sm + 2st ♀	XX/ XY_1Y_2	Favarato *et al.* (2016). Reported as *Ancistrus* sp. Barcelos
	maximus de Oliveira, Zuanon, Zawadzki & Rapp Py-Daniel, 2015	Igarapé Macoari, RR, Brazil	1°10'00.0"N	61°51'00.0"W		46♀♂	81, 82	Simple	18m + 11sm + 6st + 11a ♂, 18m + 12sm + 6st + 10a ♀	XX/XY	Oliveira *et al.* (2010)
	maximus	Igarapé Macoari, RR, Brazil	1°10'00.0"N	61°51'00.0"W		46♀♂	81, 82	Simple	18m + 11sm + 6st + 11a ♂, 18m + 12sm + 6st + 10a ♀	XX/XY	Favarato *et al.* (2016)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	multispinis (Regan, 1912)	Itapocu River, SC, Brazil				52♀♂		Simple	28m/sm + 24st/a		Alves *et al.* (2003)
	n. sp. 1	São Francisco River, AC, Brazil				38♀♂		Simple	30m/sm + 8st		Alves *et al.* (2003)
	n. sp. 1	Vermelho River, GO , Brazil				39♂, 40♀		Simple	33m + 6sm or 34m + 6sm	XX/X0	Alves *et al.* (2006)
	n. sp. 2	Betari River, SP, Brazil				52♀♂		Simple	30m/sm + 20st/a		Alves *et al.* (2003)
	n. sp. 2	Garuva River, SC, Brazil				52♀♂		Simple	10m + 16sm + 12st + 14a		Alves *et al.* (2006)
	ranunculus Muller, Rapp Py-Daniel & Zuanon, 1994	Xingu River, Altamira, PA, Brazil	3°15'21.0"S	52°12'45.0"W	Xingu	48♀♂	82	Simple	20m + 8sm + 6st + 14a ♂, 19m + 9sm + 6st + 14a ♀	ZZ/ZW	Oliveira *et al.* (2007)
	ranunculus	Xingu River, Altamira, PA, Brazil	3°15'21.0"S	52°12'45.0"W	Xingu	48♀♂	82	Simple	20m + 8sm + 6st + 14a ♂, 19m + 9sm + 6st + 14a ♀	ZZ/ZW	Favarato *et al.* (2016). Reported as *Ancistrus* sp. Piagaçu
	sp.	Criminoso stream, MS, Brazil	18°29'20.0"S	54°45'14.0"W	Paraguai	42♀♂	84	Simple	18m + 16sm + 8st		Prizon *et al.* (2016)
	sp.	Ivaí River, Brazil			Ivaí	50♀♂	88		20m + 12sm + 6st + 12a		Barros *et al.* (2017)
	sp.	Ivaí River, Brazil			Ivaí	50♀♂	88		20m + 12sm + 6st + 12a		Schott *et al.* (2022)
	sp. 01	Pipa stream, Serra de São Vicente, MT, Brazil	14°33'00.0"S	57°24'00.0"W	Paraguai	54♀♂	84	Simple	14m + 8sm + 8st + 24a		Mariotto *et al.* (2013)
	sp. 03	Pari stream, Cuiabá, MT, Brazil	15°36'00.0"S	56°12'00.0"W	Paraguai	54♀♂	84	Simple	14m + 8sm + 8st + 24a		Mariotto *et al.* (2013)
	sp. 04	Sepotuba River, Brazil	14°41'35.0"S	57°48'14.0"W	Paraguai	52♀♂	82	Simple	16m + 8sm + 6st + 22a		Mariotto *et al.* (2011)
	sp. 04	São José stream, Sepotuba River, Tangará da Serra, MT, Brazil	14°41'00.0"S	57°48'00.0"W	Paraguai	52♀♂	82	Simple	16m + 8sm + 6st + 22a		Mariotto *et al.* (2013)
	sp. 06	Matrixã River, Brazil	10°03'07.0"S	57°36'27.0"W	Amazon	50♀♂	86	Simple	18m + 10sm + 8st + 14a		Mariotto *et al.* (2011)
	sp. 06	Matrixã River, Nova Monte Verde, MT, Brazil	10°03'00.0"S	57°36'00.0"W	Amazon	50♀♂	86	Simple	18m + 10sm + 8st + 14a		Mariotto *et al.* (2013)
	sp. 08	Currupira River, Brazil	15°07'59.0"S	56°49'47.0"W	Paraguai	44♀♂	80	Simple	18m + 10sm + 8st + 8a	ZZ/ZW	Mariotto *et al.* (2011)
	sp. 1	Quianduba River, Abaetuba, PA, Brazil	1°45'18.2"S	49°00'38.8"W	Tocantins-Araguaia	38♀♂	72	Simple	20m + 14sm + 4st	XX/XY	Santos da Silva *et al.* (2022a)
	sp. 1	Quianduba River, Abaetuba, PA, Brazil	1°45'18.2"S	49°00'38.8"W	Tocantins-Araguaia	38♀♂	72	Simple	20m + 14sm + 4st	XX/XY	Santos da Silva *et al.* (2023)
	sp. 1	Maracapucú River, Abaetuba, PA, Brazil	1°45'29.2"S	48°56'57.0"W	Tocantins-Araguaia	38♀♂	72	Simple	20m + 14sm + 4st	XX/XY	Santos da Silva *et al.* (2023)
	sp. 1	Ilha do Capim, Abaetuba, PA, Brazil	1°34'02.8"S	48°51'49.1"W	Tocantins-Araguaia	38♀♂	72	Simple	20m + 14sm + 4st	XX/XY	Santos da Silva *et al.* (2023)
	sp. 13	Salgadinho stream, Brazil	14°40'14.0"S	52°21'50.0"W	Tocantins-Araguaia	40♀♂	80	Simple	26 m + 10sm + 4st		Mariotto *et al.* (2011)
	sp. 13	Salgadinho stream, Nova Xavantina, MT, Brazil	14°40'00.0"S	52°21'00.0"W	Tocantins-Araguaia	40♀♂	80	Simple	30m + 6sm + 4st		Mariotto *et al.* (2013)
	sp. Catalão	Catalão Lake, AM, Brazil	3°10'45.0"S	59°54'25.0"W	Amazonas	34♀♂		Simple	22m + 8sm + 4st	XX/XY	Favarato *et al.* (2016)
	sp. Dimona	Fazenda Dimona, Brazil	2°19'00.0"S	60°04'00.0"W		52♀♂	78	Simple	16m + 8sm + 2st + 26a		Oliveira *et al.* (2010)
	sp. L1	Mourão River, Brazil				50♀♂		Simple	12m + 18sm + 12st + 8a		Prizon *et al.* (2017)
	sp. L10	São Francisco Falso River, Brazil				50♀♂		Simple	10m + 18sm + 16st + 6a		Prizon *et al.* (2017)
	sp. L2	Stream 19, Brazil				50♀♂		Simple	12m + 18sm + 12st + 8a ♀, 11m + 18sm + 13st + 8a ♂	XX/XY	Prizon *et al.* (2017)
	sp. L3	Keller River, Brazil				50♀♂		Simple	12m + 18sm + 12st + 8a ♀, 11m + 18sm + 13st + 8a ♂	XX/XY	Prizon *et al.* (2017)
	sp. L6	São Francisco Verdadeiro River, Brazil				50♀♂		Simple	14m + 16sm + 14st + 6a		Prizon *et al.* (2017)
	sp. L8	Arroyo San Juan, Misiones, Posada, Argentina				50♀♂		Simple	10m + 14sm + 12st + 14a		Prizon *et al.* (2017)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	sp. L9	Ocoí River, Brazil			Paraná	50♀♂		Simple	10m + 18sm + 16st + 6a		Prizon *et al.* (2017)
	sp. Purus	Purus River, AM, Brazil	4°51'00.0"S	62°38'00.0"W		34♀♂	68	Simple	21m + 11sm + 2st ♂, 20m + 12sm + 2st ♀	XX/XY	Oliveira *et al.* (2010)
	sp. Purus	Purus River, AM, Brazil	4°51'00.0"S	62°38'00.0"W		34♀♂	68	Simple	21m + 11sm + 2st ♂, 20m + 12sm + 2st ♀	XX/XY	Favarato *et al.* (2016). Reported as *Ancistrus* sp. Macoari
	sp. Trombetas	Trombetas River, PA, Brazil	1°27'00.0"S	56°21'00.0"W	Trombetas	38♀♂	73	Simple	22m + 8sm + 5st + 3a		Oliveira *et al.* (2010)
	sp. Vermelho	Demeni River, middle Negro River, AM, Brazil	0°23'00.0"S	62°50'00.0"W	Negro	42♀♂	78	Simple	26m + 6sm + 4st + 6a		Oliveira *et al.* (2010)
	taunayi Miranda Ribeiro, 1918	Cascalho stream, Mondaí, SC, Brazil			Uruguai	50♀♂		Simple	22m + 10sm + 10st + 8a	ZZ/ZW	Konerat *et al.* (2015)
	tombador Fisch-Muller, Cardoso, da Silva & Bertaco, 2005	Preto River, Diamantino, MT, Brazil	14°40'00.0"S	52°21'00.0"W	Amazon	50♀♂	84	Simple	14m + 12sm + 8st + 16a		Mariotto *et al.* (2013)
Aphanotorulus	*emarginatus* (Valenciennes, 1840)	Araguaia River, Barra do Garças, MT, Brazil				52♀♂		Simple	16m + 30sm + 6st		Artoni, Bertollo (2001). Reported as *Hypostomus emarginatus*
Araichthys	*loro* Zawadzki, Bifi & Mariotto, 2016	Papagaio River, Brazil	12°79'67"S*	58°39'02"W*	Amazon	54♀♂	108	Simple	36m + 12sm + 6st		Mariotto *et al.* (2019)
Baryancistrus	aff. *niveatus*	Xingu River, Altamira, PA, Brazil	3°12'48.0"S	52°12'41.7"W	Xingu - Amazon	52♀♂	104	Simple	16m + 32sm + 4st		Souza *et al.* (2004)
	xanthellus Rapp Py-Daniel, Zuanon & de Oliveira, 2011	Volta Grande do Xingu, Altamira, PA, Brazil	3°36'31.5"S	51°34'57.4"W	Xingu-Amazon	52♀♂	104	Simple	16m + 28sm + 8st		Medeiros *et al.* (2016)
	xanthellus	Volta Grande do Xingu, Altamira, PA, Brazil	3°23'28.2"S	51°44'29.3"W	Xingu-Amazon	52♀♂	104	Simple	16m + 28sm + 8st		Medeiros *et al.* (2016)
	xanthellus	Volta Grande do Xingu, Altamira, PA, Brazil	3°22'29.7"S	51°42'25.0"W	Xingu-Amazon	52♀♂	104	Simple	16m + 28sm + 8st		Medeiros *et al.* (2016)
	xanthellus	Volta Grande do Xingu, Altamira, PA, Brazil	3°35'38.6"S	51°49'36.0"W	Xingu-Amazon	52♀♂	104	Simple	16m + 28sm + 8st		Medeiros *et al.* (2016)
Corymbophanes	n. sp.	Chopotó River, Desterro de Melo, MG, Brazil				54♀♂			20m + 20sm + 14st		Alves *et al.* (2005)
Hemiancistrus	sp.	Araguaia River, Barra do Garças, MT, Brazil				52♀♂		Simple	20m + 20sm + 12st/a		Artoni, Bertollo (2001)
Hypancistrus	cf. *debilittera* Armbruster, Lujan & Taphorn, 2007	Uatumã River, AM, Brazil			Uatumã-Amazon	52♀♂	104	Multiple	34m/sm + 18st		Santos *et al.* (2023)
	cf. *debilittera*	Uatumã River, AM, Brazil			Uatumã-Amazon	52♀♂	105	Multiple	34m/sm + 18st		Silva *et al.* (2014)
	L066	Xingu River, PA, Brazil			Xingu-Amazon	52♀♂	104	Multiple	40m/sm + 12st/a		Cardoso *et al.* (2016)
	L333	Xingu River, PA, Brazil			Xingu-Amazon	52♀♂	104	Multiple	40m/sm + 12st/a		Cardoso *et al.* (2016)
	sp. "Pão"	Xingu River, Belo Monte hydroeletric plant	3°06'12.8"S	51°43'53.9"W		52♀♂	94		40m/sm + 12st/a		Almeida *et al.* (2023)
	sp. "Pão"	Altamira, PA				52♀♂	104		22m + 18sm + 12st		Santos *et al.* (2023)
	zebra Isbrücker & Nijssen, 1991	Xingu River, PA, Brazil			Xingu-Amazon	52♀♂	104	Multiple	34m/sm + 18st		Silva *et al.* (2014)
	zebra	Xingu River, PA, Brazil			Xingu-Amazon	52♀♂	104	Multiple	40m/sm + 12st/a		Cardoso *et al.* (2016)
	zebra	Xingu River, Belo Monte hydroeletric plant	3°06'12.8"S	51°43'53.9"W		52♀♂	94		40m/sm + 12st/a		Almeida *et al.* (2023)
	zebra	Altamira, PA				52♀♂	104		16m + 20sm + 16st		Silva *et al.* (2014)
Hypostomus	aff. *agna* (Miranda Ribeiro, 1907)	Cavalo stream, Jaraguá do Sul, SC, Brazil			Southern Brazil Coast	74♀♂		Multiple	8m + 10sm + 32st + 24a		Martinez *et al.* (2011)
	aff. *ancistroides* (Ihering, 1911)	São Miguel Arcanjo, SP, Brazil	23°54'14.3"S	47°55'46.8"W	Parapanema	66♀♂		Multiple	16m + 12sm + 12st + 26a ♀, 17m + 12sm + 12st + 25a ♂	XX/XY	Rocha-Reis *et al.* (2018)
	aff. *ancistroides*	Keller River, Brazil	23°38'25.9"S	51°51'32.8"W		68♀♂	119, 118	Multiple	16m + 13sm + 22st + 17a ♀, 16m + 12sm + 22st + 18a ♂	ZZ/ZW	Kamei *et al.* (2017)
	aff. *ancistroides*	Ponta Grossa, PR, Brazil			Tibagi	66♀♂	104	Multiple	12m + 16sm + 10st + 28a		Maurutto *et al.* (2012)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	aff. *cochliodon* Kner, 1854	Esparramo stream, Rondonópolis, MT, Brazil	16°30'19.6"S	54°40'31.6"W	Paraguai	64♀♂		Multiple	18m + 20sm + 26st/a		Becker *et al.* (2014)
	aff. *cochliodon*	Pitaluga stream, Rondonópolis, MT, Brazil	16°28'38.1"S	54°32'08.3"W	Paraguai	64♀♂		Multiple	18m + 20sm + 26st/a		Becker *et al.* (2014)
	aff. *hermanni* (Ihering, 1905)	Keller River, Brazil	23°38'25.9"S	51°51'32.8"W		72♀♂	124	Multiple	12m + 22sm + 18st + 20a		Kamei *et al.* (2017)
	aff. *paulinus* (Ihering, 1905)	Piquiri River, PR, Brazil				74♀♂		Simple	10m + 12sm + 20st + 32a		Bueno *et al.* (2013)
	aff. *paulinus*	Piracicaba River, Piracicaba, SP, Brazil	22°43'07.0"S	47°39'19.0"W		76♀♂	102	Simple	8m + 18sm + 50st/a		Rubert *et al.* (2016)
	aff. *tietensis* (Ihering, 1905)	Pirapó River, Brazil	23°18'15.0"S	51°53'40.0"W	Paranapanema	76♀♂	114	Simple	8m + 6sm + 24st + 38a		Paula *et al.* (2022)
	aff. *tietensis*	Do Campo River, Brazil	24°04'41.0"S	52°26'12.0"W	Ivaí	76♀♂	114	Multiple	8m + 6sm + 24st + 38a		Paula *et al.* (2022)
	aff. *topavae* (Gody, 1969)	Mogi Guaçu River, Pirassununga, SP, Brazil				70♀♂	102	Multiple	18m + 14sm + 38st/a		Artoni, Bertollo (1996). Reported as *Hypostomus* sp. A
	aff. *topavae*	Mogi Guaçu River, Pirassununga, SP, Brazil				70♀♂	102	Multiple	18m + 14sm + 38st/a		Lorscheider *et al.* (2015)
	aff. *unae* (Steindachner, 1878)	Contas River, Brazil	13°51'51.0"S	40°04'54.0"W	Contas	76♀♂	104	Simple	12m + 16sm + 48st/a		Bitencourt *et al.* (2012)
	aff. *unae*	Preto do Costa River, Brazil	13°45'84"S*	39°56'47"W*	Contas	76♀♂	108	Simple	12m + 20sm + 44st/a		Bitencourt *et al.* (2012)
	aff. *unae*	Oricó River, Brazil	14°08'03.0"S	39°21'30.0"W	Contas	76♀♂	100	Simple	10m + 14sm + 52st/a		Bitencourt *et al.* (2012)
	aff. *unae*	Preto do Criciúma River, Brazil	13°55'45.0"S	39°57'57.0"W	Contas	76♀♂	106	Simple	10m + 20sm + 46st/a		Bitencourt *et al.* (2012)
	affinis (Steindachner, 1877)	Jacuí stream, Brazil	22°52'00.0"S	44°51'00.0"W	Paraíba do Sul	66♀♂	106		14m + 14sm + 12st + 12a		Kavalco *et al.* (2004)
	affinis	Jacuí stream, Brazil	22°52'00.0"S	44°51'00.0"W	Paraíba do Sul	66♀♂	106	Multiple	14m + 14sm + 12st + 12a		Kavalco *et al.* (2005)
	affinis	Jacuí creek, Cunha, SP, Brazil	23°02'25.9"S	44°56'02.7"W	Paraíba do Sul	66♀♂	104	Multiple	12m + 12sm + 14st + 28a		Brandão *et al.* (2018)
	affinis	Paraíba do Sul River, Itaocara, RJ, Brazil	21°39'41.1"S	42°04'28.3"W	Paraíba do Sul	66♀♂	104	Multiple	12m + 12sm + 14st + 28a		Brandão *et al.* (2018)
	albopunctatus (Regan, 1908)	Mogi Guaçu River, Pirassununga, SP, Brazil				74♀♂	104	Multiple	10m + 20sm + 44st/a		Artoni, Bertollo (1996)
	albopunctatus	Piquiri River, Brazil				74♀♂		Multiple	8m + 14sm + 16st + 36a		Bueno *et al.* (2013)
	albopunctatus	Piquiri River, Nova Laranjeiras, Brazil	24°56'54.0"S	52°35'49.0"W		74♀♂		Simple	8m + 14sm + 16st + 36a		Bueno *et al.* (2014)
	albopunctatus	Piracicaba River, Piracicaba, SP, Brazil	22°43'07.0"S	47°39'19.0"W		74♀♂	104	Multiple	10m + 20sm + 44st/a		Rubert *et al.* (2016)
	albopunctatus	Mogi Guaçu River, Pirassununga, SP, Brazil				74♀♂	104	Multiple	10m + 20sm + 44st/a		Lorscheider *et al.* (2015)
	albopunctatus	Piracicaba River, Piracicaba, SP, Brazil	22°43'07.0"S	47°39'19.0"W		74♀♂			10 + 18sm + 46st/a		Rubert *et al.* (2022)
	ancistroides (Ihering, 1911)					68♀♂	105M, 106F		10m + 27sm + 31st/a ♂, 10m + 28sm + 30st/a ♀		Michele *et al.* (1977)
	ancistroides	Rio Monjolinho, São Carlos, SP, Brazil				68♀♂	102	Multiple	16m + 18sm + 34st/a		Artoni, Bertollo (1996). Reported as *Plecostomus strigaticeps*
	ancistroides	Araquá River, Botucatu, SP, Brazil				68♀♂		Multiple	18m + 10sm + 12st + 28a		Alves *et al.* (2006)
	ancistroides				Paranapanema	68♀♂	104	Multiple	10m + 26sm + 32st/a		Rubert *et al.* (2011)
	ancistroides	Corumbataí River, SP, Brazil				68♀♂		Multiple	16m + 4sm + 16st + 32a		Alves *et al.* (2012b)
	ancistroides	Piquiri River, Formosa do Oeste, PR, Brazil				68♀♂			14m + 14sm + 8st + 32a		Bueno *et al.* (2012)
	ancistroides	Dourados stream, Mandaguari, PR, Brazil			Pirapó	68♀♂		Multiple	14m + 12sm + 18st + 24a		Endo *et al.* (2012)
	ancistroides	Maringá stream, Maringá, PR, Brazil			Pirapó	68♀♂		Multiple	16m + 12sm + 18st + 22a		Endo *et al.* (2012)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	ancistroides	Ximbaúva stream, Ourizona, PR, Brazil			Ivaí	68♀♂		Multiple	8m + 10sm + 18sm + 32a		Endo *et al.* (2012)
	ancistroides	Piquiri River, Brazil				68♀♂		Multiple	14m + 14sm + 8st + 32a		Bueno *et al.* (2013)
	ancistroides	Água Boa stream, Mundo Novo, MS, Brazil				68♀♂	116	Multiple	14m + 24sm + 10st + 20a		Fernandes *et al.* (2012)
	ancistroides	Dourado stream, Mundo Novo, MS, Brazil				68♀♂	116	Multiple	10m + 22sm + 16st + 20a		Fernandes *et al.* (2012)
	ancistroides	Dourado stream, Mundo Novo, MS, Brazil				68♀♂	120	Multiple	14m + 16sm + 22st + 16a		Traldi *et al.* (2013)
	ancistroides	Hortelã stream, Botucatu, SP, Brazil	22°56'28.9"S	48°35'03.2"W		68♀♂		Multiple	10m + 20sm + 10st + 28a		Pansonato-Alves *et al.* (2013)
	ancistroides	Piquiri River, Nova Laranjeiras, Brazil	24°56'54.0"S	52°35'49.0"W		68♀♂		Multiple	14m + 14sm + 8st + 32a		Bueno *et al.* (2014)
	ancistroides	Monjolinho River, São Carlos, SP, Brazil				68♀♂	102	Multiple	16m + 18sm + 34st/a		Lorscheider *et al.* (2015)
	cf. *heraldoi* Zawadzki, Weber & Pavanelli, 2008	Mogi Guaçu River, Pirassununga, SP, Brazil			Mogi Guaçu	72♀♂		Simple	6m + 6sm + 26st + 34a		Martinez *et al.* (2011)
	cf. *plecostomus* (Linnaeus, 1758)	Severo stream, Brazil	9°54'30.8"S	56°03'33.9"W	Amazon	68♀♂	120/ 121	Multiple	14m + 24sm + 14st + 16a ♂, 15m + 24sm + 14st + 15a ♀	ZZ/ZW	Oliveira *et al.* (2015)
	cf. *topavae*	Carrapato stream, Penápolis, SP, Brazil			Paraná	80♀♂		Multiple	6m + 8sm + 42st + 24a		Martinez *et al.* (2011)
	cf. *wuchereri* (Günther, 1864)	Mutum River, Jequié, BA, Brazil	13°43'18.0"S	39°51'20.0"W	Contas	76♀♂	104	Simple	10m + 18sm + 48st/a		Bitencourt *et al.* (2011b)
	cf. *wuchereri*	Una River, Valença, BA, Brazil	13°21'55.0"S	39°04'35.0"W	Recôncavo Sul	76♀♂	104	Simple	10m + 18sm + 48st/a		Bitencourt *et al.* (2011b)
	cochliodon Kner, 1854	Iguaçu River, Brazil				64♀♂		Simple	12m + 16sm + 16st + 20a		Bueno *et al.* (2014)
	cochliodon	Iguaçu River, Foz do Iguaçu, PR, Brazil	25°38'53.0"S	54°27'28.0"W		64♀♂		Simple	12m + 16sm + 16st + 20a		Rubert *et al.* (2016)
	cochliodon	Piraputanga River, Cáceres, MT, Brazil	16°03'33.0"S	57°40'33.0"W	Paraguai	64♀♂	100	Multiple	16m + 20sm + 28st/a		Rubert *et al.* (2016)
	commersoni Valenciennes, 1836	Iguaçu River, Brazil				68♀♂		Multiple	12m + 14sm + 14st + 28a		Bueno *et al.* (2013)
	commersoni	Lake of Ney Braga hydroeletric plant, Mangueririnha, PR, Brazil				68♀♂	100	Multiple	12m + 12sm + 8st + 36a		Maurutto *et al.* (2012)
	commersoni	Piquiri River, Nova Laranjeiras, Brazil	24°56'54.0"S	52°35'49.0"W		68♀♂		Multiple	12m + 14sm + 14st + 28a		Bueno *et al.* (2014)
	commersoni	Iguaçu River, Foz do Iguaçu, PR, Brazil	25°38'53.0"S	54°27'28.0"W		68♀♂		Multiple	12m + 14sm + 14st + 28a		Bueno *et al.* (2014)
	commersoni	Iguaçu River, PR, Brazil	26°15'01.1"S	51°06'10.7"W	Paraná	68♀♂	106	Multiple	12m + 12sm + 14st + 30a		Lorscheider *et al.* (2018)
	derbyi (Haseman, 1911)	Iguaçu River, Curitiba, PR, Brazil				66♀♂	82	Multiple	6m + 10sm + 20st + 30a		Maurutto *et al.* (2012)
	derbyi	Iguaçu River, PR, Brazil	26°15'01.1"S	51°06'10.7"W	Paraná	68♀♂	102	Simple	12m + 12sm + 10st + 34a		Lorscheider *et al.* (2018)
	faveolus Zawadzki, Birindelli & Lima, 2008	Taquaralzinho River, MT, Brazil				64♀♂		Simple	18m + 8sm + 22st + 16a		Bueno *et al.* (2013)
	faveolus	Taquaralzinho River, Barra do Garças, MT, Brazil	15°40'42.0"S	52°17'52.0"W		64♀♂		Simple	18m + 8sm + 22st + 16a		Bueno *et al.* (2014)
	goyazensis (Regan, 1908)	Vermelho River, GO, Brazil				72♀♂		Simple	10m + 16sm + 10st + 36a		Alves *et al.* (2006)
	hermanni (Ihering, 1905)	Piquiri River, PR, Brazil				72♀♂		Multiple	10m + 8sm + 32st + 22a		Bueno *et al.* (2013)
	hermanni	Piquiri River, Nova Laranjeiras, Brazil	24°56'54.0"S	52°35'49.0"W		72♀♂		Multiple	10m + 8sm + 32st + 22a		Bueno *et al.* (2014)
	hermanni	Piracicaba River, Piracicaba, SP, Brazil	22°43'07.0"S	47°39'19.0"W		72♀♂	98	Simple	8m + 18sm + 46st/a		Rubert *et al.* (2016)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	hermanni	Piracicaba River, Piracicaba, SP, Brazil	22°43'07.0"S	47°39'19.0"W		72♀♂			8m + 18sm + 46st/a		Rubert *et al.* (2022)
	iheringii (Regan, 1908)	da Lapa stream, Ipeúna, SP, Brazil				80♀♂	132	Multiple	8m + 16sm + 28st + 28a		Traldi *et al.* (2012)
	iheringii	Piracicaba River, Piracicaba, SP, Brazil	22°43'07.0"S	47°39'19.0"W		80♀♂	102	Simple	8m + 14sm + 58st/a		Rubert *et al.* (2016)
	iheringii	Mogi Guaçu River, Pirassununga, SP, Brazil				76♀♂	114	Simple	8m + 30sm + 38st/a		Artoni, Bertollo (1996). Reported as *Hypostomus* aff. *auroguttatus*
	iheringii	Mogi Guaçu River, Pirassununga, SP, Brazil				76♀♂	114	Simple	8m + 30sm + 38st/a		Lorscheider *et al.* (2015)
	iheringii	Piracicaba River, Piracicaba, SP, Brazil	22°43'07.0"S	47°39'19.0"W		80♀♂			8m + 14sm + 58st/a		Rubert *et al.* (2022)
	jaguar Zanata, Sardeiro & Zawadzki, 2013					76♀♂		Multiple	10m + 20sm + 46st/a		Anjos *et al.* (2020)
	macrops (Eigenmann & Eigenmann, 1888)					68♀♂	92		10m + 14sm + 44st/a		Michele *et al.* (1977)
	mutucae Knaack, 1999	Claro/Mutuca River, Chapada dos Guimarães, MT, Brazil	15°20'13.0"S	55°53'45.0"W	Paraguai	82♀♂	104	Simple	4m + 18sm + 60st/a		Rubert *et al.* (2016)
	mutucae	Claro River, Brazil	15°20'13.0"S	55°53'45.0"W		82♀♂		Simple	4m + 18sm + 60st/a		Rubert *et al.* (2022)
	myersi (Gosline, 1947)	Iguaçu River, PR, Brazil	26°15'01.1"S	51°06'10.7"W	Paraná	74♀♂	114	Simple	12m + 16sm + 12st + 34a		Lorscheider *et al.* (2018)
	nigromaculatus (Schubart, 1964)	Mogi Guaçu River, Cachoeira de Emas, Pirassununga, SP, Brazil				76♀♂	104	Multiple	8m + 20sm + 48st/a		Rubert *et al.* (2008)
	nigromaculatus	Três Bocas stream, Londrina, PR, Brazil				76♀♂	102	Multiple	6m + 20sm + 50st/a		Rubert *et al.* (2008)
	nigromaculatus	dos Apertados stream, Londrina, PR, Brazil				76♀♂	102	Multiple	6m + 20sm + 50st/a		Rubert *et al.* (2008)
	nigromaculatus	Lapa stream, Ipeúna, SP, Brazil				76♀♂	140	Simple	12m + 22sm + 30st + 12a		Traldi *et al.* (2013)
	nigromaculatus	Hortelã stream, Botucatu, SP, Brazil	22°56'28.9"S	48°35'03.2"W		76♀♂		Simple	8m + 18sm + 20st + 30a		Pansonato-Alves *et al.* (2013)
	nigromaculatus	Pirapitinga River, Caldas Novas, GO, Brazil	17°43'37.0"S	48°32'54.0"W		74♀♂	104	Simple	8m + 22sm + 44st/a		Rubert *et al.* (2016)
	paulinus (Ihering, 1905)					74♀♂	104		10m + 20sm + 44st/a		Michele *et al.* (1977). Reported as *Plecostomus ancistroides*
	paulinus	Três Bocas stream, PR, Brazil			Paranapanema	76♀♂	98	Simple	6m + 16sm + 54st/a		Rubert *et al.* (2011)
	paulinus	Apertados stream, PR, Brazil			Paranapanema	76♀♂	98	Simple	6m + 16sm + 54st/a		Rubert *et al.* (2011)
	paulinus					74♀♂		Simple	10m + 12sm + 20st + 32a		Bueno *et al.* (2014)
	paulinus	Piracicaba River, Piracicaba, SP, Brazil	22°43'07.0"S	47°39'19.0"W		76♀♂	98	Simple	6m + 16sm + 54st/a		Rubert *et al.* (2016)
	paulinus	Mogi Guaçu River, Pirassununga, SP, Brazil				72♀♂	100	Multiple	10m + 18sm + 44st/a		Artoni, Bertollo (1996). Reported as *Hypostomus* sp. C
	paulinus	Mogi Guaçu River, Pirassununga, SP, Brazil				72♀♂	100	Multiple	10m + 18sm + 44st/a		Lorscheider *et al.*, 2015
	paulinus	Mogi Guaçu River, Pirassununga, SP, Brazil				72♀♂	106	Multiple	14m + 20sm + 38st/a		Artoni, Bertollo (1996). Reported as *Hypostomus* sp. D2
	paulinus	Mogi Guaçu River, Pirassununga, SP, Brazil				72♀♂	106	Multiple	14m + 20sm + 38st/a		Lorscheider *et al.* (2015)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	paulinus	Piracicaba River, Piracicaba, SP, Brazil	22°43'07.0"S	47°39'19.0"W		76♀♂			6m + 16sm + 54st/a		Rubert *et al.* (2022)
	plecostomus (Linnaeus, 1758)					54♀♂					Hinegardner, Rosen (1972)
	plecostomus					54♀♂			24m/sm + 12st + 18a		Muramoto *et al.* (1968). Reported as *Plecostomus paulinus*
	prope *iheringii*	Corumbataí River, SP, Brazil				74♀♂		Multiple	10m + 14sm + 20st + 30a		Alves *et al.* (2012b)
	prope *paulinus*	Corumbataí River, SP, Brazil				76♀♂		Simple	6m + 18sm + 12st + 40a		Alves *et al.* (2012b)
	prope *paulinus*	Corumbataí River, SP, Brazil				76♀♂		Simple	6m + 18sm + 12st + 40a		Alves *et al.* (2012b)
	prope *plecostomus*	Orinoco River, Bolivar, Venezuela				68♀♂		Simple	12m + 16sm + 12st + 28a		Alves *et al.* (2012b)
	prope *unae*	Contas River, Brazil	13°51'51.0"S	40°04'54.0"W	Contas	76♀♂	104	Multiple	12m + 16sm + 48st/a		Bitencourt *et al.* (2011a)
	prope *unae*	Contas River, Brazil	13°51'51.0"S	40°04'54.0"W	Contas	76♀♂	108	Multiple	12m + 20sm + 44st/a		Bitencourt *et al.* (2011a)
	prope *unae*	Oricó River, Brazil	14°08'03.0"S	39°21'30.0"W	Contas	76♀♂	100	Multiple	10m + 14sm + 52st/a		Bitencourt *et al.* (2011a)
	prope *unae*	Preto do Criciúma River, Brazil			Contas	76♀♂	106	Multiple	10m + 20sm + 46st/a		Bitencourt *et al.* (2011a)
	regani (Ihering, 1905)					72♀♂			10m + 20sm + 42st/a		Artoni, Bertollo (1996)
	regani	Araquá River, Botucatu, SP, Brazil				72♀♂		Multiple	12m + 18sm + 26st + 16a		Alves *et al.* (2006)
	regani	Jacutinga River, Brazil			Paranapanema	72♀♂	100	Multiple	10m + 18sm + 44st/a		Rubert *et al.* (2011)
	regani	Piumhi River, Piumhi, MG, Brazil	20°20'31.0"S	45°59'03.4"W	São Francisco	72♀♂	116	Simple	8m + 16sm + 20st + 28a		Mendes-Neto *et al.* (2011)
	regani	Mogi Guaçu River, Pirassununga, SP, Brazil				72♀♂		Simple	6m + 6sm + 32st + 28a		Martinez *et al.* (2011)
	regani	Ivaí River, Floresta, PR, Brazil			Ivaí	72♀♂		Multiple	12m + 14sm + 26st + 20a		Endo *et al.* (2012)
	regani	Piquiri River, Brazil				72♀♂		Multiple	12m + 8sm + 10st + 42a		Bueno *et al.* (2013)
	regani	Piquiri River, Nova Laranjeiras, Brazil	24°56'54.0"S	52°35'49.0"W		72♀♂		Multiple	12m + 8sm + 10st + 42a		Bueno *et al.* (2014)
	regani	Mogi Guaçu River, Pirassununga, SP, Brazil	21°55'37.7"S	47°22'02.6"W		72♀♂	100	Simple	10m + 20sm + 42st/a		Rubert *et al.* (2016)
	regani	Pirapitinga River, Caldas Novas, GO, Brazil	17°43'37.0"S	48°32'54.0"W		72♀♂	106	SImple	12m + 22sm + 38st/a		Rubert *et al.* (2016)
	regani	Onça stream, Coxim, MS, Brazil	18°32'18.0"S	54°33'43.0"W	Paraguai	72♀♂		Simple	12m + 14sm + 18st + 28a		Ferreira *et al.* (2019)
	regani	Onça stream, Coxim, MS, Brazil	18°32'18.0"S	54°33'43.0"W	Paraguai	72♀♂		Simple	13m + 14sm + 17st + 18a		Ferreira *et al.* (2019)
	soniae Hollanda Carvalho & Weber, 2005	Alta Floresta, MT, Brazil	9°54'30.8"S	56°03'33.9"W	Teles Pires	64♀♂	112	Multiple	12m + 22sm + 14st + 16a	XX/XY	Oliveira *et al.* (2019)
	soniae	Alta Floresta, MT, Brazil	9°53'50.5"S	56°03'39.5"W	Teles Pires	64♀♂	112	Multiple	12m + 22sm + 14st + 16a	XX/XY	Oliveira *et al.* (2019)
	soniae	Alta Floresta, MT, Brazil	9°53'30.5"S	56°04'18.8"W	Teles Pires	64♀♂	112	Multiple	12m + 22sm + 14st + 16a	XX/XY	Oliveira *et al.* (2019)
	sp.	Mogi Guaçu River, Pirassununga, SP, Brazil				72♀♂	108	Multiple	10m + 26sm + 36st/a		Artoni, Bertollo (1996). Reported as *Hypostomus* sp. D1
	sp.	Mogi Guaçu River, Pirassununga, SP, Brazil				72♀♂	108	Multiple	10m + 26sm + 36st/a		Lorscheider *et al.* (2015)
	sp.	Fundo stream, Barra do Garças, MT, Brazil			Tocantins-Araguaia	64♀♂		Simple	14m + 24sm + 26st/a ♂, 15m + 24sm + 25st/a ♀	ZZ/ZW	Artoni *et al.* (1998)
	sp.	Mogi Guaçu River, Pirassununga, SP, Brazil			Mogi Guaçu	68♀♂		Multiple	6m + 6sm + 32st + 24a		Martinez *et al.* (2011)

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	sp.	Esparramo stream, Rondonópolis, MT, Brazil	16°30'00.0"S	54°40'00.0"W	Paraguay	74♀♂		Multiple	12m + 20sm + 42st/a		Becker *et al.* (2014)
	sp. 2 Rio Perdido	Perdido River, Planalto da Bodoquena, MS, Brazil	21°17'09.0"S	56°41'46.0"W		84♀♂	106	Simple	6m + 16sm + 62st/a		Cereali *et al.* (2008)
	sp. 3 Córrego Salobrinha	Salobra River, Planalto da Bodoquena, MS, Brazil	20°41'34.0"S	56°44'25.0"W		82♀♂	100-102		6m + 12sm + 64st/a + 1-2aBs		Cereali *et al.* (2008)
	sp. 3 Córrego Salobrinha	Salobrinha stream, Planalto da Bodoquena, MS, Brazil	20°41'07.0"S	56°46'44.0"W		82♀♂	100-102		6m + 12sm + 64st/a + 1-2aBs		Cereali *et al.* (2008)
	sp. B	Mogi Guaçu River, Pirassununga, SP, Brazil				72♀♂		Simple	12m + 18sm + 42st/a		Artoni *et al.* (1999a)
	sp. E	Mogi Guaçu River, Pirassununga, SP, Brazil				80♀♂		Multiple	8m + 16sm + 56st/a		Artoni *et al.* (1999a)
	sp. F	São Francisco River, Brazil				75, 76♀♂			10m + 16sm + 50st/a or 10m + 17sm + 48st/a		Artoni *et al.* (1999a)
	sp. Xingu-1	Xingu River, Altamira, PA, Brazil	3°12'48.0"S	52°12'41.7"W	Xingu - Amazon	64♀♂		Simple	32m/sm + 32st/a		Milhomem *et al.* (2010)
	sp. Xingu-2	Xingu River, Altamira, PA, Brazil	3°12'48.0"S	52°12'41.7"W	Xingu - Amazon	66♀♂		Simple	32m/sm + 34st/a		Milhomem *et al.* (2010)
	sp. Xingu-3	Xingu River, Altamira, PA, Brazil	3°12'48.0"S	52°12'41.7"W	Xingu - Amazon	65♀♂		Simple	38m/sm + 26st/a + 1B		Milhomem *et al.* (2010)
	spiniger (Hensel, 1870)	Forquetinha River, Forquetinha, RS, Brazil	29°21'43.5"S	52° 07'39.6"W		66♀♂		Multiple	10m + 16sm + 14st + 26a		Takagui *et al.* (2023)
	spiniger	Quadros Lagoon, RS, Brazil	29°21'43.5"S	52° 07'39.6"W		66♀♂		Multiple	10m + 16sm + 14st + 26a		Takagui *et al.* (2023)
	spiniger	Forquetinha River, Forquetinha, RS, Brazil	29°21'43.5"S	52°07'39.6"W		66♀♂	92	Multiple	10m + 16sm + 40st/a		Rubert *et al.* (2016). Reported as *Hypostomus commersoni*, corrected in Takagui et al. (2023)
	strigaticeps (Regan, 1908)					74♀♂	86		8m + 4sm + 62st/a		Michele *et al.* (1977). Reported as *Plecostomus macrops*
	strigaticeps				Paranapanema	72♀♂	98	Multiple	10m + 16sm + 46st/a		Rubert *et al.* (2011)
	strigaticeps	Corumbataí River, SP, Brazil				74♀♂		Multiple	10m + 14sm + 14st + 36a		Alves *et al.* (2012b)
	strigaticeps	Ivaí River, Floresta, PR, Brazil				72♀♂		Multiple	10m + 14sm + 18st + 30a		Endo *et al.* (2012)
	strigaticeps	Piquiri River, Brazil				72♀♂		Multiple	12m + 12sm + 18st + 30a		Bueno *et al.* (2013)
	strigaticeps	Água Boa stream, Mundo Novo, MS, Brazil				72♀♂	122	Multiple	12m + 18sm + 20st + 22a		Fernandes *et al.* (2012)
	strigaticeps	Hortelã stream, Botucatu, SP, Brazil	22°56'28.9"S	48°35'03.2"W		72♀♂		Multiple	10m + 18sm + 18st + 26a		Pansonato-Alves *et al.* (2013)
	strigaticeps	Piquiri River, Nova Laranjeiras, Brazil	24°56'54.0"S	52°35'49.0"W		72♀♂		Multiple	12m + 12sm + 18st + 30a		Bueno *et al.* (2014)
	strigaticeps	Atlântico stream, PR, Brazil	23°18'13.0"S	52°01'51.0"W	Paraná	72♀♂		Multiple	12m + 12sm + 18st + 30a		Baumgärtner *et al.* (2014)
	strigaticeps	Piquiri River, PR, Brazil	24°56'54.0"S	52°35'49.0"W	Paraná	72♀♂		Multiple	12m + 12sm + 18st + 30a		Baumgärtner *et al.* (2014)
	strigaticeps	Paraná River, PR, Brazil	24°48'43.0"S	54°19'16.0"W	Paraná	72♀♂		Multiple	12m + 12sm + 18st + 30a		Baumgärtner *et al.* (2014)
	strigaticeps	Iguassu River, PR, Brazil	25°39'02.0"S	54°27'25.0"W	Paraná	72♀♂		Multiple	12m + 12sm + 18st + 30a		Baumgärtner *et al.* (2014)
	strigaticeps	Mogi Guaçu River, Pirassununga, SP, Brazil	21°55'37.7"S	47°22'02.6"W		72♀♂	98	Simple	10m + 16sm + 46st/a		Rubert *et al.* (2016)
	strigaticeps	Mogi Guaçu River, Pirassununga, SP, Brazil				72♀♂	102	Simple	12m + 18sm + 42st/a		Lorscheider *et al.* (2015)
	strigaticeps	Mogi Guaçu River, Pirassununga, SP, Brazil				72♀♂	102	Simple	12m + 18sm + 42st/a		Artoni, Bertollo (1996). Reported as *Hypostomus* sp. B

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	tapijara Oyakawa, Akama & Zanata, 2005	Ribeira de Iguape River, Registro, SP, Brazil				66♀♂	118	Multiple	14m + 24sm + 14st + 14a		Traldi *et al.* (2013)
	tietensis (Ihering, 1905)	Piraí River, Brazil	23°22'22.0"S	47°22'13.0"W	Paraná	72♀♂	108	Simple	8m + 8sm + 20st + 36a		Anjos *et al.* (2020)
	tietensis	Piraí River, Brazil	23°22'22.0"S	47°22'13.0"W	Paraná	72♀♂	109	Simple	8m + 8sm + 20st + 36a		Paula *et al.* (2022)
	topavae (Godoy, 1969)	Mogi Guaçu River, Pirassununga, SP, Brazil				80♀♂	104	Multiple	8m + 16sm + 56st/a		Artoni, Bertollo (1996). Reported as *Hypostomus* sp. E
	topavae	Mogi Guaçu River, Pirassununga, SP, Brazil				80♀♂	104	Multiple	8m + 16sm + 56st/a		Lorscheider *et al.* (2015)
	topavae	Piquiri River, Nova Laranjeiras, PR, Brazil				80♀♂			14m + 10sm + 26st + 30a		Bueno *et al.* (2012)
	topavae	Piquiri River, PR, Brazil				80♀♂		Simple	14m + 10sm + 26st + 30a		Bueno *et al.* (2013)
	topavae	Piquiri River, Nova Laranjeiras, PR, Brazil	24°56'54.0"S	52°35'49.0"W	Paraná	80♀♂		Simple	14m + 10sm + 26st + 30a		Bueno *et al.* (2014)
	topavae	Keller River, Brazil	23°38'25.9"S	51°51'32.8"W		80♀♂	142	Multiple	14m + 30sm + 18st + 18a		Kamei *et al.* (2017)
	unae (Steindachner, 1878)					76♀♂			10m + 20sm + 46st/a		Anjos *et al.* (2020)
Lasiancistrus	*schomburgkii* (Günther, 1864)	Massangana River, Brazil	9°80′48″S*	63°08′90″W*	Amazon	54♀♂	108	Simple	28m + 16sm + 10st		Mariotto *et al.* (2019)
	sp.	Cachoeira River, Brazil	14°64′66″S*	52°35′50″W*	Tocantins-Araguaia	54♀♂	108	Simple	28m + 16sm + 10st		Mariotto *et al.* (2019)
Megalancistrus	sp.	Cuiabá River, Brazil	15°62′90″S*	56°08′70″W*	Paraguai	52♀♂	104	Simple	28m + 16sm + 8st		Mariotto *et al.* (2019)
	parananus (Peters, 1881)	Piquiri River, Nova Laranjeiras, PR, Brazil				52♀♂		Simple	18m + 24sm + 10st		Bueno *et al.* (2018)
Panaqolus	sp.	Camarapi River, Portel, PA, Brazil				52♀♂		Simple	24m + 18sm + 10st/a		Ayres-Alves *et al.* (2017)
	tankei Cramer & Sousa, 2016	Xingu River, Brazil				52♀♂	104	Simple	6m + 38sm + 8st		Ferreira *et al.* (2021)
Panaque	*armbrusteri* Lujan, Hidalgo & Stewart, 2010	Xingu River, Altamira, PA, Brazil				52♀♂		Simple	26m + 20sm + 6st/a		Ayres-Alves *et al.* (2017)
	armbrusteri	Gorgulho da Rita, Altamira, PA, Brazil				52♀♂		Simple	26m + 20sm + 6st/a		Ayres-Alves *et al.* (2017)
	cf. *nigrolineatus*	Araguaia River, Barra do Garças, MT, Brazil				52♀♂		Simple	26m + 20sm + 6st/a		Artoni, Bertollo (2001)
Peckoltia	*cavatica* Armbruster & Werneke, 2005	PA, Brazil				52♀♂		Simple	38m/sm + 14st		Pety *et al.* (2018)
	multispinis (Holly, 1929)	PA, Brazil				52♀♂		Simple	28m/sm + 24st		Pety *et al.* (2018)
	oligospila (Günther, 1864)	PA, Brazil				52♀♂		Multiple	38m/sm + 14st		Pety *et al.* (2018)
	sabaji Armbruster, 2003	PA, Brazil				52♀♂		Multiple	38m/sm + 14st		Pety *et al.* (2018)
	sp. 1 Jari river	Jari River, Monte Dourado, PA, Brazil	03°18'14.9"N	52°03'29.3"W		52♀♂	102	Multiple	44m/sm + 6st + 2a + 1B		Souza *et al.* (2009)
	sp. 2 Jari river	Jari River, Monte Dourado, PA, Brazil	03°18'14.9"N	52°03'29.3"W		52♀♂	102		32m/sm + 18st + 2a		Souza *et al.* (2009)
	sp. 3 Jarumã	Abaetuba, PA, Brazil	1°42'41.9"S	48°51'45.9"W		52♀♂		Simple	46m/sm + 6st		Silva *et al.* (2021)
	sp. 4 Caripetuba	Abaetuba, PA, Brazil	1°37'23.5"S	48°55'33.0"W		52♀♂		Multiple	40m/sm + 12st		Silva *et al.* (2021)
	vittata (Steindachner, 1881)	Xingu River, Altamira, PA, Brazil	03°12'4"N	52°12'41.7"W		52♀♂	102	Simple	16m + 20sm + 14st + 2a		Souza *et al.* (2009)
	vittata	PA, Brazil				52♀♂		Simple	32m/sm + 18st + 2a		Pety *et al.* (2018)
Pseudacanthicus	*leopardus* (Fowler, 1914)	Xingu River, Brazil			Xingu - Amazon	52♀♂	104	Simple	18m + 34sm		Santos da Silva *et al.* (2022b)
	sp.	Xingu River, Brazil			Xingu - Amazon	52♀♂	104	Simple	18m + 34sm		Santos da Silva *et al.* (2022b)
	spinosus (Castelnau, 1855)	Tocantins River, Brazil			Tocantins-Araguaia	52♀♂	104	Simple	18m + 34sm		Santos da Silva *et al.* (2022b)
Pterygoplichthys	*ambrosettii* (Holmberg, 1893)	Preto River, Mirassolândia, SP, Brazil				52♀♂		Simple	16m + 24sm + 8st + 4a		Artoni *et al.* (1999b). Reported as *Liposarcus anisitsi*
	ambrosettii	Tietê River, Botucatu, SP, Brazil				52♀♂		Simple	28m + 12sm + 8st + 4a		Alves *et al.* (2006). Reported as *Liposarcus anisitsi*

TABLE 1 | (Continued)

Subfamily/ Genus	Species	Locality	Latitude	Longitude	Basin	2n	NF	Ag-NOR - 18S	KF	SCS	Reference/ Observation
	ambrosettii	Miranda River, MS, Brazil				52♀♂		Simple	8m + 14sm + 14st + 16a		Alves *et al.* (2006). Reported as *Liposarcus anisitsi*
	ambrosettii	Agua Boa stream, Brazil	23°50'16.7"S	54°20'55.5"W		52♀♂	100	Simple	14m + 26sm + 8st + 4a		Fernandes *et al.* (2015). Reported as *Pterygoplichthys anisitsi*
	ambrosettii	Paraná River, Guaíra, PR, Brazil				52♀♂		Simple	16m + 24sm + 8st + 4a		Bueno *et al.*, 2018
	chrysostiktos (Birindelli, Zanata & Lima, 2007)					52♀♂		Simple	22m + 20sm + 10st		Anjos *et al.* (2020). Reported as *Hypostomus chrysostiktos*
	gibbiceps (Kner, 1854)	Orinoco River, Venezuela				52♀♂		Simple	20m + 24sm + 8st		Alves *et al.* (2006). Reported as *Glyptoperichthys gibbiceps*
	joselimaianus (Weber, 1991)	Quatro Bocas Lake, Araguaiana, MT, Brazil	15°23'20.1"S	51°42'45.9"W		52♀♂		Simple	28m + 16sm + 8st/a		Oliveira *et al.* (2006)
	multiradiatus (Hancock, 1828)	Orinoco River, Venezuela				52♀♂		Simple	22m + 18sm + 12st		Alves *et al.* (2006). Reported as *Liposarcus multiradiatus*
	multiradiatus	Orinoco River, Venezuela				52♀♂		Simple	22m + 18sm + 12st		Alves *et al.* (2012a). Reported as *Liposarcus multiradiatus*
	pardalis (Castelnau, 1855)	Porto dos Milagres, Amazonas River, Santarém, PA, Brazil				52♀♂		Simple	18m + 18sm + 8st + 8a		Guimarães *et al.* (2023)
	pardalis	Arapixuna, Amazonas River, Santarém, PA, Brazil				52♀♂		Simple	18m + 18sm + 8st + 8a		Guimarães *et al.* (2023)
	pardalis	Grande Lake, Amazonas River, Santarém, PA, Brazil				52♀♂		Simple	18m + 18sm + 8st + 8a		Guimarães *et al.* (2023)
	pardalis	Pacoval Lake, Amazonas River, Santarém, PA, Brazil				52♀♂		Simple	18m + 18sm + 8st + 8a		Guimarães *et al.* (2023)
Scobinancistrus	*aureatus* Burgess, 1994	Xingu River, Brazil			Xingu-Amazon	52♀♂		Simple	22m + 20sm + 10st		Cardoso *et al.* (2013)
	aureatus	Xingu River, Brazil			Xingu-Amazon	52♀♂		Simple	22m + 20sm + 10st		Ayres-Alves *et al.* (2017)
	aureatus	Xingu River, Belo Monte hydroeletric plant, Brazil	3°06'12.8"S	51°43'53.9"W		52♀♂			24m + 18sm + 10st		Almeida *et al.* (2023)
	aureatus	Xingu River, Gorgulho da Rita, Brazil	3°20'06.2"S	52°10'32.9"W		52♀♂			24m + 18sm + 10st		Almeida *et al.* (2023)
	pariolispos Isbrücker & Nijssen, 1989	Xingu River, Brazil			Xingu-Amazon	52♀♂		Simple	24m + 18sm + 10st		Cardoso *et al.* (2013)
	pariolispos	Xingu River, Brazil			Xingu-Amazon	52♀♂		Simple	24m + 18sm + 10st		Ayres-Alves *et al.* (2017)
	pariolispos	Xingu River, Belo Monte hydroeletric plant, Brazil	3°06'12.8"S	51°43'53.9"W		52♀♂			22m + 20sm + 10st		Almeida *et al.* (2023)
Transancistrus	*santarosensis* (Tan & Armbruster, 2012)	Río Dos Bocas, Río Palenque, Ecuador				54♀♂	106	Multiple	25m + 27sm + 2a ♀, 26m + 26sm + 2a ♂	ZZ/ZW	Tursellino *et al.* (2023)
Delturinae											
Hemipsilichthys	sp.	Paraitinga River, Brazil	22°52'22.5"S	44°51'00.4"W	Paraíba do Sul	96♀♂	120		16m + 8sm + 72a		Kavalco *et al.* (2004). Reported as *Upsilodus* sp.
	sp.	Paraitinga River, Brazil	22°52'22.5"S	44°51'00.4"W	Paraíba do Sul	96♀♂	120	Simple	16m + 8sm + 72a		Kavalco *et al.* (2005)
	n. sp.	Patos River, Lapa, PR, Brazil				54♀♂			20m + 30sm + 4st		Alves *et al.* (2005)
Rhinelepinae											
Pogonopoma	*wertheimeri* (Steindachner, 1867)	Mucurí River, Taquarinha, BA, Brazil				54♀♂		Multiple	20m + 30sm + 4st		Artoni, Bertollo (2001)
Rhinelepis	*aspera* Spix & Agassiz, 1829	Paraná River, Guaíra, PR, Brazil				54♀♂		Simple	20m + 26sm + 8st		Artoni, Bertollo (2001). Reported as *Rhinelepis aspera*
	aspera	Paraná River, Porto Rico, PR, Brazil				54♀♂		Simple	24m + 18sm + 12st		Endo *et al.* (2012)
	aspera	Paraná River, Santa Helena, PR, Brazil				54♀♂		Simple	20m + 26sm + 8st		Bueno *et al.* (2018)

DISCUSSION

The origin of the chromosomal diversity of Loricariidae is attributed to both ecological and molecular factors. Small and isolated populations, in addition to the low vagility, are the main ecological characteristics that have allowed the fixation of chromosomal rearrangements in Loricariidae, as suggested for *Farlowella* (Marajó *et al*., 2018), *Harttia* (Sassi *et al*., 2023), and *Rineloricaria* (Rosa *et al*., 2012). The diploid number 2n = 54 is often considered the ancestral diploid number for the family, since it is observed in most Loricariidae subfamilies and present in other Siluriformes (Artoni, Bertollo, 2001). However, there is no consensus on the matter particularly considering the new karyotypic description of basal taxa within subfamilies. Takagui *et al*. (2020) in a review of Loricariinae karyotypes argue that although predominant, 2n = 54 should not be considered the basal diploid number for the family because multiple divergences in the microstructure of karyotypes within the same 2n are recurrently seen throughout the family. Notably, the 2n range in Loricarioidei suggest that other numbers rather than 2n = 54 can be considered the plesiomorphic ones, as Astroblepidae presents 2n = 52–54, Scoloplacidae 2n = 50, Callichthyidae 2n = 40–134, and Trichomycteridae 2n = 32–62 (reviewed by Conde-Saldaña *et al*., 2018). Beyond that, the Diplomystidae (Siluroidei) presents 2n = 56 chromosomes (Campos *et al*., 1997; Oliveira, Gosztonyi, 2000), which might also suggest that as the ancestral 2n.

We plotted the 2n range per subfamily into the main molecular phylogenetic reconstructions of Loricariidae (Fig. 3) and 2n = 54 is the most widespread, being conserved in Rhinelepinae and present in all other subfamilies. Regardless matter whether 2n = 54 or 2n = 56 is the ancestral diploid number, it is noteworthy that chromosomal evolution in Loricariidae is complex. Considering the lower number of karyotyped species when compared to the valid richness, 234 spp. with cytogenetic data against 1,051 valid species (Fricke *et al*., 2023), it is difficult to establish the evolutionary pathways that led to the observed variability. Notably, while the stability is restricted to 2n in some genera, with high divergence in karyotype structure, as the 2n = 58 in *Farlowella*, 2n = 54 in *Corumbataia*, and 2n = 52 in *Pterygoplichthys*, stable 2n and karyotype is also observed, as the 2n = 52 (26m+20sm+6st/a) in two species of *Panaque*. Our compilation reveals that processes of ascending and descending dysploidy (*i.e*., the increase or decrease of 2n while preserving the genomic content) were frequent in most subfamilies but Rhinelepinae, which may exhibit the constant 2n = 54 as a symplesiomorphic trait.

Despite the 2n conservation in Rhinelepinae, variations in the karyotype structure are observed within and between species. Populations of *Rhinelepis aspera* Spix & Agassiz, 1829 from the same river differ in the karyotype formula (Artoni, Bertollo, 2001; Endo *et al*., 2012), suggesting that pericentric inversions played an important role in the chromosomal evolution of the species. Such mechanism is not restricted to Rhinelepinae but also observed in other loricariids, such as *Ancistrus* (Mariotto *et al*., 2009), *Loricariichthys* (Takagui *et al*., 2014), and *Rineloricaria* (Rosa *et al*., 2012; Primo *et al*., 2018). In addition, the multiple Ag-NOR observed in *Pogonopoma wertheimeri* (Steindachner, 1867) (Artoni, Bertollo, 2001) when compared to the simple distribution in other Rhinelepinae species suggest that other mechanisms are also important for the chromosomal evolution in the group. Notably, numeric and structural polymorphisms

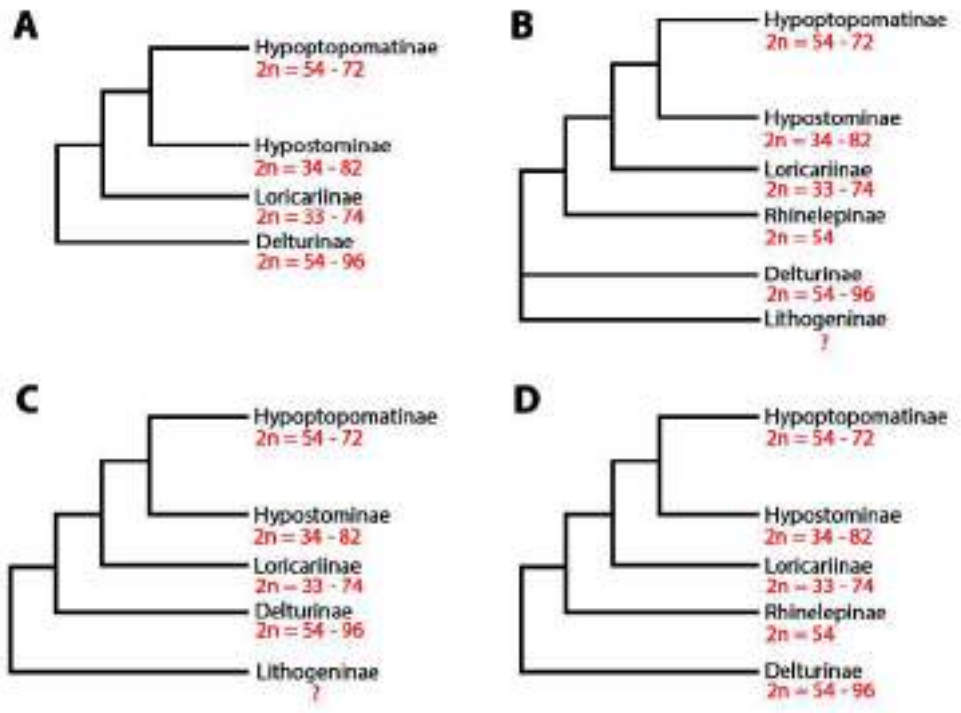


FIGURE 3 | Phylogenetic relationships of Loricariidae with cytogenetic data herein compiled in red. Molecular phylogenies modified from: **A.** Roxo *et al.* (2014); **B.** Lujan *et al.* (2015); **C.** Pereira, Reis (2017); **D.** Roxo *et al.* (2019).

are frequently observed in loricariids, for example in the multiple karyomorphs of *Rineloricaria pentamaculata* Langeani & de Araujo, 1994 (Glugoski *et al.*, 2023), and the presence of B chromosomes in *Harttia longipinna* Langeani, Oyakawa & Montoya-Burgos, 2001 (Blanco *et al.*, 2012). This chromosomal diversity was probably generated by a combination of rearrangements that include Robertsonian fusions and fissions, paracentric and pericentric inversions, and translocations (Artoni, Bertollo, 2001; Kavalco *et al.*, 2004; Ziemniczak *et al.*, 2012).

Although rare in most fish species, being observed in only about 5% of Teleostei species (Arai, 2011; Sember *et al.*, 2021), our compilation shows that Loricariidae species carry seven out of the nine known sex chromosome systems observed among fishes. *Ancistrus* was demonstrated to harbor the largest diversity of sex chromosomes with six of the seven recognized systems for the family distributed in 18 species, corresponding to about 23% of the valid species (Neuhaus *et al.*, 2023). The simple XX/XY was the most predominant in the genus, recorded in *A. maximus* de Oliveira, Zuanon, Zawadzki & Rapp Py-Daniel, 2015 (Oliveira *et al.*, 2010; Favarato *et al.*, 2016), *Ancistrus* cf. *dubius* (Mariotto *et al.*, 2011), *Ancistrus* sp. 1 Quianduba River (Silva *et al.*, 2022, 2023), *Ancistrus* sp. 1 Maracapucú River (Santos da Silva *et al.*, 2023), *Ancistrus* sp. 1 Ilha do Capim (Santos da Silva *et al.*, 2023), *Ancistrus* sp. Catalão (Favarato *et al.*, 2016), *Ancistrus* sp. L2 (Prizon *et al.*, 2017), *Ancistrus* sp. L3 (Prizon *et al.*, 2017), and *Ancistrus* sp. Purus (Oliveira *et al.*, 2010; Favarato *et al.*, 2016). Additionally, two multiple sex chromosome systems that were not observed in any other Loricariidae are recorded in *Ancistrus*: ZZ/ZW_1W_2 in *A. clementinae* Rendahl, 1937 (Nirchio *et al.*, 2023), and $Z_1Z_1Z_2Z_2/Z_1Z_2W_1W_2$ (Oliveira

et al., 2008; Favarato *et al*., 2016). On other hand, *Harttia* harbor the highest number of multiple sex chromosome systems when compared to the number of valid species, with six occurrences representing about 25% of valid species (compiled in Sassi *et al*., 2021): XX/XY_1Y_2 in *H. carvalhoi* Miranda Ribeiro, 1939, *H. intermontana* Oliveira & Oyakawa, 2019, and *Harttia* sp. 1 (Centofante *et al*., 2006; Deon *et al*., 2020); $X_1X_1X_2X_2/X_1X_2Y$ in *H. duriventris* Rapp Py-Daniel & Oliveira, 2001, *H. punctata* Rapp Py-Daniel & Oliveira, 2001, and *H. villasboas* Oyakawa, Fichberg & Rapp Py-Daniel, 2018 (Blanco *et al*., 2014; Sassi *et al*., 2020), in addition to putative simple XX/XY in *H. rondoni* Oyakawa, Fichberg & Rapp Py-Daniel, 2018 and *H. torrenticola* Oyakawa, 1993 (Deon *et al*., 2020; Sassi *et al*., 2020). The Loricariidae diversity of sex chromosomes was originated by rearrangements that include translocations (Blanco *et al*., 2014), centric fissions (Sassi *et al*., 2023); centric fusions (Centofante *et al*., 2006), and pericentric inversions (Artoni *et al*., 1998), including the combination of distinct rearrangements especially in the origin of multiple sex chromosome systems (Oliveira *et al*., 2008; Deon *et al*., 2022). Sex chromosomes in Loricariidae seems to have independent origins, but further research is required to explore the genomic content of those sex chromosomes and its origin. Indeed, there is a recognized lack of information regarding the effects of environmental cues and molecular/gene mechanisms in sex determination of Neotropical fishes (Fernandino, Hattori, 2019).

Most Loricariidae species present a single 18S rDNA/Ag-NOR site, which is also considered a plesiomorphic character in the group (Artoni, Bertollo, 2001; Kavalco *et al*., 2004), and the standard distribution in most vertebrates (Sochorová *et al*., 2018). Notably, such region in loricariids is involved in several chromosomal rearrangements, including the origin and differentiation of sex chromosomes, being considered evolutionary breakpoint regions in *Ancistrus*, *Harttia*, and *Rineloricaria* (Glugoski *et al*., 2018; Deon *et al*., 2022). Size heteromorphism in the Ag-NOR site is also common, probably because of unequal crossing-over between homologs (Takagui *et al*., 2020). According to our review, some genus conserved the simple Ag-NOR locus in all analyzed species to date (here included only those genera with more than one species karyotyped), namely as *Baryancistrus*, *Corumbataia*, *Farlowella*, *Harttia*, *Hisonotus*, *Lasiancistrus*, *Loricaria*, *Loricariichthys*, *Neoplecostomus*, *Panaqolus*, *Panaque*, *Pareiorhina*, *Pseudacanthicus*, *Pterygoplichthys*, and *Scobinancistrus*. On other hand, the multiple distribution of Ag-NOR seems to be conserved only in *Hypancistrus*, while other genus as *Ancistrus*, *Hypostomus*, and *Rineloricaria* present both simple and multiple distributions on chromosomes.

Although distributed throughout the Neotropical region, there is a predominance of cytogenetic studies in Loricariidae species from Brazil (Fig. 2). Few studies were conducted in other countries that includes Argentina, Ecuador, Paraguay, and Venezuela. Notably, those in Argentina and Paraguay were mostly restricted to the frontier region with Brazil. When accounting the Brazilian territory, is also notable that some regions are poorly represented in cytogenetic studies, especially the northeast in which at least nine states have not been included in the cytogenetic samplings. In addition, the Guianas Shield and Western Amazon regions are largely recognized as neglected regions in biogeographical and evolutionary studies (Cassemiro *et al*., 2023), also with little or absent cytogenetic information for Loricariidae. Despite the regional sampling gap problem, Loricariidae diversity is still insufficiently represented by cytogenetic

studies. Our compilation recorded 234 species assessed by cytogenetic studies, that in comparison to the 1,051 valid species (Fricke *et al*., 2023), represents 22.26% of the family species richness. The diversity of genus assessed by cytogenetic studies was the highest in Hypoptopomatinae (42.1%), followed by Rhinelepinae (28.5%), Hypostominae (27.2%), Loricariinae (24.4%), Delturinae (20%), and Lithogeninae (0%). Besides Lithogeninae that do not have any species karyotyped to date, the subfamily Delturinae has only one genus and two unidentified species karyotyped: *Hemipsilichthys* sp. Paraitinga River (Kavalco *et al*., 2004, 2005), and *Hemipsilichthys* n. sp. Patos River (Alves *et al*., 2005). We suggest that further cytogenetic studies focus on expand the sampling in the northeast Brazil, the Western Amazon, the Guianas Shield, and other Neotropical countries, in addition to evaluate a more representative portion of the diversity.

ACKNOWLEDGMENTS

The authors are grateful to all cytogeneticists who contributed to the increase of the knowledge about Loricariidae cytogenetics. We also thank to the Fundação de Amparo à Pesquisa do Estado de São Paulo (FMCS 2020/02681–9, 2022/04261–2, and 2023/08116–0; MBC AR-23/00955–2), to Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPQ (MBC 302928/2021–9), and Instituto Nacional de Ciência e Tecnologia da Biodiversidade e uso Sustentável de Peixes Neotropicais – INCT Peixes (405706/2022–7).

REFERENCES

- **Almeida BRR, Souza LF, Alves TA, Cardoso AL, Oliveira JA, Ribas TFA *et al*.** Chromosomal organization of multigene families and meiotic analysis in species of Loricariidae (Siluriformes) from Brazilian Amazon, with description of a new cytotype for genus *Spatuloricaria*. Biol Open. 2023; 12(11):bio060029. https://doi.org/10.1242/bio.060029
- **Alves AL, Borba RS, Pozzobon APB, Oliveira C, Nirchio M, Granado A *et al*.** Localization of 18S ribosomal genes in suckermouth armoured catfishes Loricariidae (Teleostei, Siluriformes) with discussion on the Ag-NOR evolution. Comp Cytogen. 2012a; 6(3):315–21. https://doi.org/10.3897/CompCytogen.v6i3.2667
- **Alves AL, Borba RS, Oliveira C, Nirchio M, Granado A, Foresti F.** Karyotypic diversity and evolutionary trends in the Neotropical catfish genus *Hypostomus* Lacépède, 1803 (Teleostei, Siluriformes, Loricariidae). Comp Cytogen. 2012b; 6(4):443–52. https://doi.org/10.3897/CompCytogen.v6i4.4028
- **Alves AL, Oliveira C, Foresti F.** Karyotype variability in eight species of the subfamilies Loricariinae and Ancistrinae (Teleostei, Siluriformes, Loricariidae). Caryologia. 2003; 56(1):57–63. https://doi.org/10.1080/00087114.2003.10589308
- **Alves AL, Oliveira C, Foresti F.** Comparative cytogenetic analysis of eleven species of subfamilies Neoplecostominae and Hypostominae (Siluriformes: Loricariidae). Genetica. 2005; 124:127–36. https://doi.org/10.1007/s10709-004-7561-4
- **Alves AL, Oliveira C, Nirchio M, Granado A, Foresti F.** Karyotypic relationships among the tribes of Hypostominae (Siluriformes:Loricariidae) with description of XO sex chromosome system in a Neotropical fish species. Genetica. 2006; 128:1–09. https://doi.org/10.1007/s10709-005-0715-1

- **Andreata AA, Almeida-Toledo LF, Oliveira C, Toledo Filho SA.** Chromosome Studies in Hypoptopomatinae (Pisces, Siluriformes, Loricariidae): I. XX/XY Sex Chromosome Heteromorphism in *Pseudotocinclus tietensis*. Cytologia. 1992; 57(3):369–72. https://doi.org/10.1508/cytologia.57.369
- **Andreata AA, Almeida-Toledo LF, Oliveira C, Toledo Filho SA.** Chromosome studies in Hypoptopomatinae (Pisces, Siluriformes, Loricariidae) II. ZZ/ZW sex-chromosome system, B chromosomes, and constitutive heterochromatin differentiation in *Microlepidogaster leucofrenatus*. Cytogenet Genome Res. 1993; 63(4):215–20. https://doi.org/10.1159/000133538
- **Andreata AA, Almeida-Toledo LF, Oliveira C, Toledo Filho AS.** Cytogenetic studies on the subfamily Hypoptopomatinae (Pisces, Siluriformes, Loricariidae). III. Analysis of seven species. Caryologia. 1994; 47(1):27–37. https://doi.org/10.1080/00087114.1994.10797279
- **Andreata AA, Ferreira DC, Foresti F, Oliveira C.** Molecular cytogenetic study of heterochromatin in *Hisonotus leucofrenatus* (Teleostei, Loricariidae, Hypoptopomatinae). Hereditas. 2010; 147(1):10–17. https://doi.org/10.1111/j.1601-5223.2009.2149.x
- **Andreata AA, Oliveira C, Foresti F.** Karyological characterization of four Neotropical fish species of the genus *Hisonotus* (Teleostei, Loricariidae, Hypoptopomatinae) from distinct Brazilian river basins. Genet Mol Biol. 2006; 29(1):62–66. https://doi.org/10.1590/S1415-47572006000100013
- **Anjos MS, Bitencourt JA, Nunes LA, Sarmento-Soares LM, Carvalho DC, Armbruster JW *et al*.** Species delimitation based on integrative approach suggests reallocation of genus in Hypostomini catfish (Siluriformes, Loricariidae). Hydrobiologia. 2020; 847:563–78. https://doi.org/10.1007/s10750-019-04121-z
- **Arai R.** Fish karyotypes a check list. Springer: Tokyo, Japan; 2011.
- **Arratia G, Kapoor BG, Chardon M, Diogo R.** Catfishes Vol. 1 & 2. Enfield, NH: Science Publishers; 2003.
- **Artoni RF, Bertollo LAC.** Cytogenetic studies on Hypostominae (Pisces, Siluriformes, Loricariidae). Considerations on karyotype evolution in the genus *Hypostomus*. Caryologia. 1996; 49(1):81–90. https://doi.org/10.1080/00087114.1996.10797353
- **Artoni RF, Bertollo LAC.** Nature and distribution of constitutive heterochromatin in fishes, genus *Hypostomus* (Loricariidae). Genetica. 1999; 106:209–14. https://doi.org/10.1023/A:1003957719178
- **Artoni RF, Bertollo LAC.** Trends in the karyotype evolution of Loricariidae fish (Siluriformes). Hereditas. 2001; 134(3):201–10. https://doi.org/10.1111/j.1601-5223.2001.00201.x
- **Artoni RF, Venere PC, Bertollo LAC.** A heteromorphic ZZ/ZW sex chromosome system in fish, genus *Hypostomus* (Loricariidae). Cytologia. 1998; 63(4):421–25. https://doi.org/10.1508/cytologia.63.421
- **Ayres-Alves T, Cardoso AL, Nagamachi CY, Sousa LM, Pieczarka JC, Noronha RCR.** Karyotypic evolution and chromosomal organization of repetitive DNA sequences in species of *Panaque*, *Panaqolus*, and *Scobinancistrus* (Siluriformes and Loricariidae) from the Amazon basin. Zebrafish. 2017; 14(3):251–60. https://doi.org/10.1089/zeb.2016.1373
- **Barros AV, Wolski MAV, Nogaroto V, Almeida MC, Moreira-Filho O, Vicari MR.** Fragile sites, dysfunctional telomere and chromosome fusions: what is 5S rDNA role? Gene. 2017; 608:20–27. https://doi.org/10.1016/j.gene.2017.01.013
- **Barros FJT, Rodrigues ELC, Moura MCS, Torres RA, Paula EA, Sousa LM.** Weight-length and length-length relationships of the endangered zebra pleco *Hypancistrus zebra* (Siluriformes, Loricariidae) from the Xingu River, Amazon, Brazil. J Appl Ichthyol. 2023; 5158180. https://doi.org/10.1155/2023/5158180
- **Baumgärtner L, Paiz LM, Zawadzki CH, Margarido VP, Castro ALBP.** Heterochromatin polymorphism and physical mapping of 5S and 18S ribosomal DNA in four populations of *Hypostomus strigaticeps* (Regan, 1907) from the Paraná River basin, Brazil: evolutionary and environmental correlation. Zebrafish. 2014; 11(5):479–87. https://doi.org/10.1089/zeb.2014.1028

- **Becker QMC, Castro RJ, Silva AM, Vizzotto PC.** Cytogenetic characterization of two species of *Hypostomus* (Siluriformes, Loricariidae) from tributaries of the Vermelho river, upper Paraguay river basin. Biodiversidade. 2014; 13(1):1–13.
- **Benitez MF, Pastori MC, Garrido GG, Takagui F, Giuliano L, Fenocchio AS.** First cytogenetic characterization of *Loricaria simillima* (Loricariidae, Siluriformes) from Paraná River (Argentina) with emphasis in cytotaxonomy of *Loricaria*. Caryologi. 2017; 70(1):29–33. https://doi.org/10.1080/00087114.2016.1258158
- **Bitencourt JA, Affonso PRAM, Giuliano-Caetano L, Dias AL.** Heterochromatin heterogeneity in *Hypostomus* prope *unae* (Steindachner, 1878) (Siluriformes, Loricariidae) from Northeastern Brazil. Comp Cytogen. 2011a; 5(4):329–44. https://doi.org/10.3897/CompCytogen.v5i4.1149
- **Bitencourt JA, Affonso PRAM, Giuliano-Caetano L, Dias AL.** Identification of distinct evolutionary units in allopatric populations of *Hypostomus* cf. *wuchereri* Günther, 1864 (Siluriformes: Loricariidae): karyotypic evidence. Neotrop Ichthyol. 2011b; 9(2):317–24. https://doi.org/10.1590/S1679-62252011000200008
- **Bitencourt JA, Affonso PRAM, Giuliano-Caetano L, Carneiro PLS, Dias AL.** Population divergence and peculiar karyoevolutionary trends in the loricariid fish *Hypostomus* aff. *unae* from northeastern Brazil. Genet Mol Res. 2012; 11(2):933–43. http://doi.org/10.4238/2012.April.13.1
- **Blanco DR, Vicari MR, Artoni RF, Traldi JB, Moreira-Filho O.** Chromosomal characterization of armored catfish *Harttia longipinna* (Siluriformes, Loricariidae): first report of B chromosomes in the genus. Zool Sci. 2012; 29(9):604–09. http://doi.org/10.2108/zsj.29.604
- **Blanco DR, Vicari MR, Lui RL, Bertollo LAC, Traldi JB, Moreira-Filho O.** The role of the Robertsonian rearrangements in the origin of the XX/XY_1Y_2 sex chromosome system and in the chromosomal differentiation in *Harttia* species (Siluriformes, Loricariidae). Rev Fish Biol Fish. 2013; 23(1):127–34. http://doi.org/10.1007/s11160-012-9283-5
- **Blanco DR, Vicari MR, Lui RL, Artoni RF, Almeida MC, Traldi JB *et al*.** Origin of the $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system of *Harttia punctata* (Siluriformes, Loricariidae) inferred from chromosome painting and FISH with ribosomal DNA markers. Genetica. 2014; 142(2):119–26. http://doi.org/10.1007/s10709-014-9759-4
- **Blanco DR, Vicari MR, Lui RL, Traldi JB, Bueno V, Martinez JF *et al*.** Karyotype diversity and evolutionary trends in armored catfish species of the genus *Harttia* (Siluriformes: Loricariidae). Zebrafish. 2017; 14(2):169–76. http://doi.org/10.1089/zeb.2016.1377
- **Brandão KO, Rocha-Reis DA, Garcia C, Pazza R, Almeida-Toledo LF, Kavalco KF.** Studies in two allopatric populations of *Hypostomus affinis* (Steindachner, 1877): the role of mapping the ribosomal genes to understand the chromosome evolution of the group. Comp Cytogen. 2018; 12(1):1–12. http://doi.org/10.3897/CompCytogen.v12i1.22052
- **Briggs JC.** The biogeography of Otophysan fishes (Ostariophysi: Otophysi): a new appraisal. J Biogeography. 2005; 32(2):287–94. https://doi.org/10.1111/j.1365-2699.2004.01170.x
- **Buck S, Sazima I.** An assemblage of mailed catfishes (Loricariidae) in southeastern Brazil: distribution, activity and feeding. Ichthyol Explor Freshw. 1995; 6(4):325–32.
- **Bueno V, Zawadzki CH, Margarido VP.** Trends in chromosome evolution in the genus *Hypostomus* Lacépède, 1803 (Osteichthyes, Loricariidae): a new perspective about the correlation between diploid number and chromosomes types. Rev Fish Biol Fish. 2012; 22:241–50. http://doi.org/10.1007/s11160-011-9215-9
- **Bueno V, Venere PC, Zawadzki CH, Margarido VP.** Karyotypic diversification in *Hypostomus* Lacépède, 1803 (Siluriformes, Loricariidae): biogeographical and phylogenetic perspectives. Rev Fish Biol Fish. 2013; 23(1):103–12. http://doi.org/10.1007/s11160-012-9280-8
- **Bueno V, Venere PC, Konerat JT, Zawadzki CH, Vicari MR, Margarido VP.** Physical mapping of the 5S and 18S rDNA in ten species of *Hypostomus* Lacépède 1803 (Siluriformes: Loricariidae): evolutionary tendencies in the genus. Sci World J. 2014; 943825. http://doi.org/10.1155/2014/943825

- **Bueno V, Konerat JT, Zawadzki CH, Venere PC, Blanco DR, Margarido VP.** Divergent chromosome evolution in Hypostominae tribes (Siluriformes: Loricariidae): correlation of chromosomal data with morphological and molecular phylogenies. Zebrafish. 2018; 15(5):492–503. http://doi.org/10.1089/zeb.2018.1612
- **Camilo FM, Moreira-Filho O.** Karyotypic description of *Corumbataia cuestae* (Pisces, Loricariidae, Hypoptopomatinae). Cytologia. 2005; 70(1):47–51. https://doi.org/10.1508/cytologia.70.47
- **Campos H, Arratia G, Cuevas C.** Karyotypes of the most primitive catfishes (Teleostei: Siluriformas: Diplomystidae). J Zool System Evol Res. 1997; 35:113–19. https://doi.org/10.1111/j.1439-0469.1997.tb00412.x
- **Cardoso AL, Carvalho HLS, Benathar TCM, Serrão SMG, Nagamachi CY, Pieczarka JC *et al*.** Integrated cytogenetic and mitochondrial DNA analyses indicate that two different phenotypes of *Hypancistrus* (L066 and L333) belong to the same species. Zebrafish. 2016; 13(3):209–16. https://doi.org/10.1089/zeb.2015.1213
- **Cardoso AL, Sales KAH, Nagamachi CY, Pieczarka JC, Noronha RCR.** Comparative cytogenetics of two species of genus *Scobinancistrus* (Siluriformes, Loricariidae, Ancistrini) from the Xingu River, Brazil. Comp Cytogen. 2013; 7(1):43–51. https://doi.org/10.3897/CompCytogen.v7i1.4128
- **Carvalho ML, Silva GJC, Melo S, *et al*.** The non-monotypic status of the Neotropical fish genus *Hemiodontichthys* (Siluriformes, Loricariidae) evidenced by genetic approaches. Mitochondrial DNA Part A. 2018; 29(8):1224–30. https://doi.org/10.1080/24701394.2018.1431230
- **Cassemiro FAS, Albert JS, Antonelli A, Graham CH.** Landscape dynamics and diversification of the megadiverse South American freshwater fish fauna. PNAS. 2023, 120(2):e2211974120. https://doi.org/10.1073/pnas.2211974120
- **Centofante L, Bertollo LAC, Moreira-Filho O.** Cytogenetic characterization and description of an XX/XY_1Y_2 sex chromosome system in catfish *Harttia carvalhoi* (Siluriformes, Loricariidae). Cytogen Genome Res. 2006; 112(3–4):320–24. https://doi.org/10.1159/000089887
- **Centofante L, Vicari MR, Artoni RF, Buckup PA, Bertollo LAC, Moreira-Filho O.** Chromosome analyses in species of *Pareiorhina* (Siluriformes, Loricariidae) and cytotaxonomic considerations on the group. Nucleus. 2011; 54(2):65–70. https://doi.org/10.1007/s13237-011-0035-z
- **Cereali SS, Pomini E, Rosa R, Zawadzki CH, Froehlich O, Giuliano-Caetano L.** Karyotype description of two species of *Hypostomus* (Siluriformes, Loricariidae) of the Planalto da Bodoquena, Brazil. Genet Mol Res. 2008; 7(3):583–91. https://doi.org/10.4238/vol7-3gmr404
- **Cius A, Lorscheider CA, Barbosa LM, Zawadzki CH, Borin-Carvalho LA, Porto FE *et al*.** Contributions of species *Rineloricaria pentamaculata* (Loricariidae:Loricariinae) in a karyoevolutionary context. Caryologia. 2022; 75(3):39–46. https://doi.org/10.36253/caryologia-1611
- **Cristina FD, Chiachio MC, Takako AK, Andreata AA, Foresti F, Oliveira C.** Comparative cytogenetics of nine species of Hypoptopomatinae (Teleostei: Siluriformes: Loricariidae): the importance of structural rearrangements in chromosome evolution. Caryologia. 2005; 58(4):387–95. https://doi.org/10.1080/00087114.2005.10589478
- **Deon GA, Glugoski L, Vicari MR, Nogaroto V, Sassi FMC, Cioffi MB *et al*.** Highly rearranged karyotypes and multiple sex chromosome systems in armored catfishes from the genus *Harttia* (Teleostei, Siluriformes). Genes. 2020; 11(11):1366. https://doi.org/10.3390/genes11111366
- **Deon GA, Glugoski L, Sassi FMC, Hatanaka T, Nogaroto V, Bertollo LAC *et al*.** Chromosomal rearrangements and origin of the multiple XX/XY_1Y_2 sex chromosome system in *Harttia* Species (Siluriformes: Loricariidae). Front Genet. 2022a; 13:877522. https://doi.org/10.3389/fgene.2022.877522
- **Deon GA, Glugoski L, Hatanaka T, Sassi FMC, Nogaroto V, Bertollo LAC *et al*.** Evolutionary breakpoint regions and chromosomal remodeling in *Harttia* (Siluriformes: Loricariidae) species diversification. Genet Mol Biol. 2022b; 45(2):e20210170. https://doi.org/10.1590/1678-4685-GMB-2021-0170

- **Endo KS, Martinez ERM, Zawadzki CH, Paiva LRS, Júlio Junior HF.** Karyotype description of possible new species of the *Hypostomus ancistroides* complex (Teleostei: Loricariidae) and other Hypostominae. Acta Scient Biol Sci. 2012; 34(2):181–89. https://doi.org/10.4025/actascibiolsci.v34i2.9318
- **Evers H, Pinnegar JK, Taylor MI.** Where are they all from? – sources and sustainability in the ornamental freshwater fish trade. J Fish Biol. 2019; 94(6):909–16. https://doi.org/10.1111/jfb.13930
- **Favarato RM, Silva M, Oliveira RR, Artoni RF, Feldberg E, Matoso DA.** Cytogenetic diversity and the evolutionary dynamics of rDNA genes and telomeric sequences in the *Ancistrus* genus (Loricariidae: Ancistrini). Zebrafish. 2016; 13(2):103–11. https://doi.org/10.1089/zeb.2015.1140
- **Fenocchio AS, Pastori MC, Roncati HÁ, Moreira-Filho O, Bertollo LAC.** A cytogenetic survey of the fish fauna from Argentina. Caryologia. 2003; 56(2):197–204. https://doi.org/10.1080/00087114.2003.10589325
- **Fernandes CA, Alves DS, Guterres ZR, Martins-Santos IC.** Cytogenetic analysis of two locariid species (Teleostei, Siluriformes) from Iguatemi River (Paraná River drainage) in Brazil. Comp Cytogenet. 2015; 9(1):67–78. https://doi.org/10.3897/CompCytogen.v9i1.8804
- **Fernandes CA, Bailly D, Sant'Ana DRA, Alves DS.** First cytogenetic record for a species of *Otothyropsis* Ribeiro, Carvalho & Melo, 2005 (Loricariidae, Hypoptopomatinae). Neotrop Ichthyol. 2016, 14(1):e150123. https://doi.org/10.1590/1982-0224-20150123
- **Fernandes CA, Damásio JF, Martins-Santos IC.** Cytogenetic studies in species of family Loricariidae (Osteichthyes, Siluriformes) from Iguatemi river basin, Brazil. First cytogenetic report in *Farlowella amazonum* (Günther, 1864). Caryologia. 2012; 65(4):276–80. https://doi.org/10.1080/00087114.2012.752913
- **Fernandes CA, Paiz LM, Piscor D, Gavazzoni M, Carvalho LAB, Portela-Castro ALB *et al*.** Chromosomal diversity in two allopatric populations of *Farlowella hahni* Meinken 1937 (Teleostei: Siluriformes): cytogenetics and cytochrome *b* analyses. Zebrafish. 2021; 18(1):66–72. https://doi.org/10.1089/zeb.2020.1966
- **Fernandino JI, Hattori RS.** Sex determination in Neotropical fish: Implications ranging from aquaculture technology to ecological assessment. Gen Comp Endocrinol. 2019; 273:172–83. https://doi.org/10.1016/j.ygcen.2018.07.002
- **Ferreira AMV, Viana PF, Zuanon J, Ezaz T, Cioffi MB, Takagui FH *et al*.** Cytogenetic analysis of *Panaqolus tankei* Cramer & Sousa, 2016 (Siluriformes, Loricariidae), an ornamental fish endemic to Xingu River, Brazil. Cytogenet Genome Res. 2021; 161(3–4):187–94. https://doi.org/10.1159/000514061
- **Ferreira GEB, Barbosa LM, Prizon-Nakajima AC, Paiva S, Vieira MMR, Gallo RB *et al*.** Constitutive heterochromatin heteromorphism in the Neotropical armored catfish *Hypostomus regani* (Ihering, 1905) (Loricariidae, Hypostominae) from the Paraguay River basin (Mato Grosso do Sul, Brazil). Comp Cytogenet. 2019; 13(1):27–39. https://doi.org/10.3897/CompCytogen.v13i1.30134
- **Ferreira RO, Pereira AL, Nagamachi CY, Pieczarka JC, Sousa LM, Noronha RCR.** Caracterização citogenética de uma espécie de *Spatuloricaria* (Siluriformes, Loricariidae) do rio Xingu, (Pará, Amazônia, Brasil). Biota Amazôn. 2014; 4(1):30–36. http://dx.doi.org/10.18561/2179-5746/biotaamazonia.v4n1p30-36
- **Fink S, Fink W.** Interrelationships of the ostariophysan fishes (Teleostei). ZJLS. 1981; 72:297–353.
- **Fink S, Fink W.** Interrelationships of ostariophysan fishes (Teleostei). In: Stiassny MLJ, Parenti LR, Johnson GD, editors. Interrelationships of fishes. New York: Academic Press; 1996. p.209–45.
- **Fricke R, Eschmeyer WN, Van der Laan R.** Eschmeyer's catalog of fishes: genera, species, references [Internet]. San Francisco: California Academy of Science; 2023. Available from: https://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp
- **Glugoski L, Giuliano-Caetano L, Moreira-Filho O, Vicari MR, Nogaroto V.** Co-located *hAT* transposable element and 5S rDNA in an interstitial telomeric sequence suggest the formation of Robertsonian fusion in armored catfish. Gene. 2018; 650:49–54. https://doi.org/10.1016/j.gene.2018.01.099

- **Glugoski L, Deon G, Schott S, Vicari MR, Nogaroto V, Moreira-Filho O.** Comparative cytogenetic analyses in *Ancistrus* species (Siluriformes: Loricariidae). Neotrop Ichthyol. 2020; 18(2):e200013. https://doi.org/10.1590/1982-0224-2020-0013
- **Glugoski L, Nogaroto V, Deon GA, Azambuja M, Moreira-Filho O, Vicari MR.** Enriched tandem repeats in chromosomal fusion points of *Rineloricaria latirostris* (Boulenger, 1900) (Siluriformes: Loricariidae). Genome. 2022; 65(9):479–89. https://dx.doi.org/10.1139/gen-2022-0043
- **Glugoski L, Deon GA, Nogaroto V, Moreira-Filho O, Vicari MR.** Robertsonian fusion site in *Rineloricaria pentamaculata* (Siluriformes: Loricariidae): involvement of 5S rDNA and satellite sequences. Cytogenet Genome Res. 2023; 162(11–12):657–64. https://doi.org/10.1159/000530636
- **Guimarães AS, Maciel LAM, Souza MFB, Rodrigues LRR.** Karyotypic and molecular analysis of *Pterygoplichthys pardalis* (Castelnau 1855) from the lower Amazon River. Animals. 2023; 13(9):1533. https://doi.org/10.3390/ani13091533
- **Hinegardner R, Rosen DE.** Cellular DNA content and the evolution of teleostean fishes. The American Naturalist. 1972; 106(951):621–44. https://doi.org/10.1086/282801
- **Kamei MCSL, Baumgärtner L, Paiva S, Zawadzki CH, Martins-Santos IC, Portela-Castro ALB.** Chromosomal diversity of three species of *Hypostomus* Lacépède, 1803 (Siluriformes, Loricariidae), from the Paraná River basin, Brazil: a species complex in *Hypostomus ancistroides* Reinforced by a ZZ/ZW sex chromosome system. Zebrafish. 2017; 14(4):357–63. https://doi.org/10.1089/zeb.2017.1429
- **Kavalco KF, Pazza R, Bertollo LAC, Moreira-Filho O.** Heterochromatin characterization of four fish species of the family Loricariidae (Siluriformes). Hereditas. 2004; 141(3):237–42. https://doi.org/10.1111/j.1601-5223.2004.01850.x
- **Kavalco KF, Pazza R, Bertollo LAC, Moreira-Filho O.** Karyotypic diversity and evolution of Loricariidae (Pisces, Siluriformes). Heredity. 2005; 94(2):180–86. https://doi.org/10.1038/sj.hdy.6800595
- **Konerat JT, Bueno V, Margarido VP, Portela-Castro ALB, Martins-Santos IC.** Diversity of sex chromosome systems in Ancistrini (Loricariidae, Hypostominae): ZZ/ZW in *Ancistrus taunayi* Miranda Ribeiro, 1918. Cytogenet Genome Res. 2015; 146(4):306–10. https://doi.org/10.1159/000441431
- **Levan A, Fredga K, Sandberg AA.** Nomenclature for centromeric position on chromosomes. Hereditas. 1964; 52:201–20.
- **Lévêque C, Oberdorff T, Paugy D, Stassny MLJ, Tedesco PA.** Global diversity of fish (Pisces) in freshwater. Hydrobiologia. 2008; 595:545–67. https://doi.org/10.1007/s10750-007-9034-0
- **Lorscheider CA, Oliveira JIN, Dulz TA, Nogaroto V, Martins-Santos IC, Vicari MR.** Comparative cytogenetics among three sympatric *Hypostomus* species (Siluriformes: Loricariidae): an evolutionary analysis in a high endemic region. Braz Arch Biol Technol. 2018; 61:e18180417. http://dx.doi.org/10.1590/1678-4324-2018180417
- **Lorscheider CA, Zawadzki CH, Vicari MR, Martins-Santos IC, Artoni RF.** Karyotypic diversity of the armoured catfish genus *Hypostomus* (Siluriformes: Loricariidae) in the context of its occurrence and distribution. J Fish Biol. 2015; 87(4):1099–110. https://doi.org/10.1111/jfb.12762
- **Lujan NK, Armbruster JW, Lovejoy NR, López-Fernández H.** Multilocus molecular phylogeny of the suckermouth armored catfishes (Siluriformes: Loricariidae) with a focus on subfamily Hypostominae. Mol Phylogenet Evol. 2015; 82:269–88. https://doi.org/10.1016/j.ympev.2014.08.020
- **Lujan NK, Cramer CA, Covain R, Fisch-Muller S, López-Fernández H.** Multilocus molecular phylogeny of the ornamental wood-eating catfishes (Siluriformes, Loricariidae, *Panaqolus* and *Panaque*) reveals undescribed diversity and parapatric clades. Mol Phylogenet Evol. 2017; 109:321–36. https://doi.org/10.1016/j.ympev.2016.12.040
- **Lujan NK, Hidalgo M, Stewart DJ.** Revision of *Panaque* (*Panaque*), with descriptions of three new species from the Amazon Basin (Siluriformes, Loricariidae). Copeia. 2010; 4(2010):676–704. https://doi.org/10.1643/CI-09-185

- **Maia TPA, Giuliano-Caetano L, Rodriguez MS, Rubert M, Takagui FH, Dias AL.** Chromosomal banding in three species of the genus *Rineloricaria* (Siluriformes, Loricariidae, Loricariinae). Ichthyol Res. 2010; 57(2):209–13. https://doi.org/10.1007/s10228-009-0145-7
- **Marajó L, Viana PF, Ferreira M, Rapp Py-Daniel LH, Feldberg E.** Cytogenetics of two *Farlowella* species (Loricariidae: Loricariinae): implications on the taxonomic status of the species. Netrop Ichthyol. 2018; 16(4):e180029. https://doi.org/10.1590/1982-0224-20180029
- **Marajó L, Viana PF, Ferreira AMV, Rapp Py-Daniel LH, Cioffi MB, Sember A *et al*.** Chromosomal rearrangements and the first indication of an $♀X_1X_1X_2X_2/♂X_1X_2Y$ sex chromosome system in *Rineloricaria* fishes (Teleostei: Siluriformes). J Fish Biol. 2022; 102(2):443–54. https://doi.org/10.1111/jfb.15275
- **Mariotto S, Artoni RF, Miyazawa CS.** Occurrence of sexual chromosome, of the type ZZ/ZW, in *Ancistrus* cf. *dubius* (Loricariidae, Ancistrinae) of the Paraguay River Basin, Mato Grosso, Brazil. Caryologia. 2004; 57(4):327–31. https://doi.org/10.1080/00087114.2004.10589413
- **Mariotto S, Centofante L, Miyazawa CS, Bertollo LAC, Moreira-Filho O.** Chromosome polymorphism in *Ancistrus cuiabae* Knaack, 1999 (Siluriformes: Loricariidae: Ancistrini). Netrop Ichthyol. 2009; 7(4):595–600. https://doi.org/10.1590/S1679-62252009000400006
- **Mariotto S, Centofante L, Moreira-Filho O.** Diversity and chromosomal evolution in the genus *Ancistrus* Kner, 1854 (Loricariidae: Ancistrini) from three hydrographic basins of Mato Grosso State, Brazil. Netrop Ichthyol. 2013; 11(1):125–31. https://doi.org/10.1590/S1679-62252013000100015
- **Mariotto S, Centofante L, Venere PC, Ferreira DC, Artoni RF.** New comparative cytogenetic data on three genera of armored catfishes of Ancistrini (Loricariidae: Hypostominae). Cytogenet Genome Res. 2019; 159(4):208–14. https://doi.org/10.1159/000504723
- **Mariotto S, Centofante L, Vicari MR, Artoni RF, Moreira-Filho O.** Chromosomal diversification in ribosomal DNA sites in *Ancistrus* Kner, 1854 (Loricariidae, Ancistrini) from three hydrographic basins of Mato Grosso, Brazil. Comp Cytogenet. 2011; 5(4):289–300. https://doi.org/10.3897/CompCytogen.v5i4.1757
- **Mariotto S, Miyazawa CS.** *Ancistrus* cf. *dubius* (Siluriformes, Ancistrinae), a complex of species. 1. Chromosomic characterization of four populations and occurence of sexual chromosomes of type XX/XY, in the Pantanal basin of Mato Grosso, Brazil. Caryologia. 2006, 59(4):299–304. https://doi.org/10.1080/00087114.2006.10797929
- **Martinez ERM, Zawadzki CH, Foresti F, Oliveira C.** Cytogenetic analysis of five *Hypostomus* species (Siluriformes, Loricariidae). Genet Mol Biol. 2011; 34(4):562–68. https://doi.org/10.1590/S1415-47572011005000038
- **Maurutto FAM, Manvailer LFS, Sczepanski TS, Cestari MM, Artoni RF.** Cytogenetic characterization of three allopatric species of *Hypostomus* Lacépède (1803) (Teleostei, Loricariidae). Caryologia. 2012; 65(4):340–46. https://doi.org/10.1080/00087114.2012.760882
- **Medeiros LA, Ginani EG, Sousa LM, Rapp Py-Daniel LH, Feldberg E.** Cytogenetic analysis of *Baryancistrus xanthellus* (Siluriformes: Loricariidae: Ancistrini), an ornamental fish endemic to the Xingu River, Brazil. Neotrop Ichthyol. 2016; 14(2):e150108. https://doi.org/10.1590/1982-0224-20150108
- **Mendes-Neto EO, Vicari MR, Artoni RF, Moreira-Filho O.** Description of karyotype in *Hypostomus* regani (Ihering, 1905) (Teleostei, Loricariidae) from the Piumhi river in Brazil with comments on karyotype variation found in *Hypostomus*. Comp Cytogenet. 2011; 5(2):133–42. https://doi.org/10.3897/CompCytogen.v5i2.964
- **Michele JL, Takahashi CS, Ferrari I.** Karyotypic study of some species of the family Loricariidae. Cytologia. 1977; 42:539–46. https://doi.org/10.1508/cytologia.42.539

- **Milhomem SSR, Castro RR, Nagamachi CY, Souza A, Feldberg E, Pieczarka J.** Different cytotypes in fishes of the genus *Hypostomus* Lcépède, 1803, (Siluriformes: Loricariidae) from Xingu river (Amazon region, Brazil). Comp Cytogenet. 2010; 4(1):45–54. https://doi.org/10.3897/compcytogen.v4i1.31
- **Muramoto J, Ohno S, Atkin NB.** On the Diploid State of the Fish Order Ostariophysi. Chromosoma. 1968; 24:59–66. https://doi.org/10.1007/BF00329607
- **Nelson JS, Grande TC, Wilson MV.** Fishes of the World. Hoboken, New Jersey: John Wiley & Sons; 2016.
- **Nirchio M, Oliveira C, Cioffi MB, Sassi FMC, Valdiviezo J, Paim FG *et al*.** Occurrence of sex chromosomes in fish of the genus *Ancistrus* with a new description of multiple sex chromosomes in the Ecuadorian endemic *Ancistrus clementinae* (Loricariidae). Genes. 2023; 14:306. https://doi.org/10.3390/genes14020306
- **Novák J, Hofmann J, Hohl D, Magalhães ALB, Patoka J.** Enigmatic armoured catfishes (Siluriformes: Callichthyidae and Loricariidae) in ornamental aquaculture: a new insight into Neotropical fish diversity. Aquaculture. 2022; 547:737460. https://doi.org/10.1016/j.aquaculture.2021.737460
- **Novák J, Kalous L, Patoka J.** Modern ornamental aquaculture in Europe: early history of freshwater fish imports. Rev Aquaculture. 2020; 12(4):2042–60. https://doi.org/10.1111/raq.12421
- **Oliveira C, Gosztonyi AE.** A cytogenetic study of *Diplotnystes mesembrinus* (Teleostei, Siluriformes, Diplomystidae) with a discussion of chromosome evolution in siluriforms. Caryologia. 2000; 53(1):31–37. https://doi.org/10.1080/00087114.2000.10589178
- **Oliveira LC, Ribeiro MO, Costa GM, Zawadzki CH, Prizon-Nakajima AC, Borin-Carvalho LA *et al*.** Cytogenetic characterization of *Hypostomus soniae* Hollanda-Carvalho & Weber, 2004 from the Teles Pires River, southern Amazon basin: evidence of an early stage of an XX/XY sex chromosome system. Comp Cytogenet. 2019; 13(4):411–22. https://doi.org/10.3897/CompCytogen.v13i4.36205
- **Oliveira LC, Ribeiro MO, Dutra ES, Zawadzki CH, Portela-Castro ALB, Martins-Santos IC.** Karyotype structure of *Hypostomus* cf. *plecostomus* (Linnaeus, 1758) from Tapajós River basin, Southern Amazon: occurrence of sex chromosomes (ZZ/ZW) and their evolutionary implications. Genet Mol Res. 2015; 14(2):6625–34. http://dx.doi.org/10.4238/2015.June.18.5
- **Oliveira RR, Feldberg E, Anjos MB, Zuanon J.** Mechanisms of chromosomal evolution and its possible relation to natural history characteristics in *Ancistrus* catfishes (Siluriformes: Loricariidae). J Fish Biol. 2009; 75(9):2209–25. https://doi.org/10.1111/j.1095-8649.2009.02450.x
- **Oliveira RR, Feldberg E, Anjos MB, Zuanon J.** Karyotype characterization and ZZ/ZW sex chromosome heteromorphism in two species of the catfish genus *Ancistrus* Kner, 1854 (Siluriformes: Loricariidae) from the Amazon basin. Neotrop Ichthyol. 2007; 5(3):301–06. https://doi.org/10.1590/S1679-62252007000300010
- **Oliveira RR, Feldberg E, Anjos MB, Zuanon J.** Occurrence of multiple sexual chromosomes (XX/XY_1Y_2 and $Z_1Z_1Z_2Z_2/Z_1Z_2W_1W_2$) in catfishes of the genus *Ancistrus* (Siluriformes: Loricariidae) from the Amazon basin. Genetica. 2008; 134(2):243–49. https://doi.org/10.1007/s10709-007-9231-9
- **Oliveira RR, Souza IL, Venere PC.** Karyotype description of three species of Loricariidae (Siluriformes) and occurrence of the ZZ/ZW sexual system in *Hemiancistrus spilomma* Cardoso & Lucinda, 2003. Neotrop Ichthyol. 2006; 4(1):93–97. https://doi.org/10.1590/S1679-62252006000100010
- **Pansonato-Alves JC, Serrano EA, Utsunomia R, Scacchetti P, Oliveira C, Foresti F.** Mapping five repetitive DNA classes in sympatric species of *Hypostomus* (Teleostei: Siluriformes: Loricariidae): analysis of chromosomal variability. Rev Fish Biol Fish. 2013; 23:477–89. https://doi.org/10.1007/s11160-013-9303-0
- **Paula GBN, Gavazzoni M, Zawadzki CH, Fernandes CA, Portela_castro ALB, Lui RL *et al*.** Identification of cryptic species in allopatric populations of *Hypostomus tietensis* (Siluriformes: Loricariidae) through cytogenetics analyses. Neotrop Ichthyol. 2022; 20(2):e210158. https://doi.org/10.1590/1982-0224-2021-0158

- **Pazian MF, Pereira LHG, Shimabukuru-Dias CK, Oliveira C, Foresti F.** Cytogenetic and molecular markers reveal the complexity of the genus *Piabina* Reinhardt, 1867 (Characiformes: Characidae). Neotrop Ichthyol. 2012; 10(2):329–40. https://doi.org/10.1590/S1679-62252012005000015
- **Pereira EH, Reis RE.** Morphology-based phylogeny of the suckermouth armored catfishes, with emphasis on the Neoplecostominae (Teleostei: Siluriformes: Loricariidae). Zootaxa. 2017; 4264(1):1–104. https://doi.org/10.11646/zootaxa.4264.1.1
- **Pety AM, Cardoso AL, Nagamachi CY, Pieczarka JC, Sousa LM, Rodrigues RC.** *In situ* localization of ribosomal sites in *Peckoltia* and *Ancistomus* (Loricariidae: Hypostominae) from the Amazon basin. Zebrafish. 2018; 15(3):263–69. https://doi.org/10.1089/zeb.2017.1523
- **Porto FE, Portela-Castro ALB, Martins-Santos IC.** Possible origins of B chromosomes in *Rineloricaria pentamaculata* (Loricariidae, Siluriformes) from the Paraná River basin. Genet Mol Res. 2010; 9(3):1654–59. https://doi.org/10.4238/vol9-3gmr859
- **Porto FE, Portela-Castro ALB, Martins-Santos IC.** Chromosome polymorphism in *Rineloricaria pentamaculata* (Loricariidae, Siluriformes) of the Paraná River basin. Ichthyol Res. 2011; 58:225–31. https://doi.org/10.1007/s10228-011-0215-5
- **Porto FE, Vieira MMR, Barbosa LM, Borin-Carvalho LA, Vicari MR, Portela-Castro ALB *et al*.** Chromosomal polymorphism in *Rineloricaria lanceolata* Günther, 1868 (Loricariidae: Loricariinae) of the Paraguay basin (Mato Grosso do Sul, Brazil): evidence of fusions and their consequences in the population. Zebrafish. 2014a; 11(4):318–24. https://doi.org/10.1089/zeb.2014.0996
- **Porto FE, Gindri BS, Vieira MMR, Borin LA, Portela-Castro ALB, Martins-Santos IC.** Polymorphisms of the nucleolus organizing regions in *Loricaria cataphracta* (Siluriformes, Loricariidae) of the upper Paraguay River basin indicate an association with transposable elements. Genet Mol Res. 2014b; 13(1):1627–34. http://dx.doi.org/10.4238/2014.March.12.15
- **Price AC, Weadick CJ, Shim J, Rodd FH.** Pigments, patterns, and fish behavior. Zebrafish. 2008; 5(4):297–307. https://doi.org/10.1089/zeb.2008.0551
- **Primo CC, Glugoski L, Almeida MC, Zawadzki CH, Moreira-Filho O, Vicari MR *et al*.** Mechanisms of chromosomal diversification in species of *Rineloricaria* (Actinopterygii: Siluriformes: Loricariidae). Zebrafish. 2017; 14(2):161–68. https://doi.org/10.1089/zeb.2016.1386
- **Primo CC, Glugoski L, Vicari MR, Nogaroto V.** Chromosome mapping and molecular characterization of the *Tc1/Mariner* element in *Rineloricaria* (Siluriformes: Loricariidae). Braz Arch Biol Technol. 2018; 61:e18170623. http://dx.doi.org/10.1590/1678-4324-2018170623
- **Prizon AC, Borin-Carvalho LA, Bruschi DP, Ribeiro MO, Barbosa LM, Ferreira GEB *et al*.** Cytogenetic data on *Ancistrus* sp. (Siluriformes, Loricariidae) of the Paraguay River basin (MS) sheds light on intrageneric karyotype diversification. Comp Cytogenet. 2016; 10(4):625–36. https://doi.org/10.3897/CompCytogen.v10i4.8532
- **Prizon AC, Bruschi DP, Borin-Carvalho LA, Cius A, Barbosa LM, Ruiz HB *et al*.** Hidden diversity in the populations of the armored catfish *Ancistrus* Kner, 1854 (Loricariidae, Hypostominae) from the Paraná River basin revealed by molecular and cytogenetic data. Front Genet. 2017; 8:185. https://doi.org/10.3389/fgene.2017.00185
- **Ramirez JL, Birindelli JL, Carvalho DC, Affonso PRAM, Venere PC, Ortega H *et al*.** Revealing hidden diversity of the underestimated Neotropical ichthyofauna: DNA barcoding in the recently described genus *Megaleporinus* (Characiformes:Anostomidae). Front Genet. 2017; 8:149. https://doi.org/10.3389/fgene.2017.00149
- **Ribeiro AC, Lima FCT, Pereira EHL.** A new genus and species of a minute suckermouth armored catfish (Siluriformes: Loricariidae) from the rio Tocantins drainage, Central Brazil: the smallest known loricariid catfish. Copeia. 2012; 2012(4):637–47. https://doi.org/10.1643/CI-11-137
- **Ribeiro MO, Noleto RB, Lorscheider CA, Porto FE, Prizon AC, Zawadzki CH *et al*.** Cytogenetic description of *Ancistrus abilhoai* (Siluriformes: Loricariidae) from Iguaçu River basin, southern Brazil. Genet Mol Res. 2015; 14(2):4051–57. http://dx.doi.org/10.4238/2015.April.27.20

- **Rocha-Reis D, Brandão KO, Almeida-Toledo LF, Pazza R, Kavalco KF.** The persevering cytotaxonomy: discovery of a unique XX/XY sex chromosome system in catfishes suggests the existence of a new, endemic and rare species. Cytogenet Gen Res. 2018; 156(1):45–55. https://doi.org/10.1159/000492959
- **Rosa KO, Ziemniczak K, Barros AV, Nogaroto V, Almeida M, Cestari MM *et al*.** Numeric and structural chromosome polymorphism in *Rineloricaria lima* (Siluriformes: Loricariidae): fusion points carrying 5S rDNA or telomere sequence vestiges. Rev Fish Biol Fish. 2012; 22(3):739–49. https://doi.org/10.1007/s11160-011-9250-6
- **Roxo FF, Albert JS, Silva GS, Zawadzki CH, Foresti F, Oliveira C.** Molecular phylogeny and biogeographic history of the armored Neotropical catfish subfamilies Hypoptopomatinae, Neoplecostominae and Otothyrinae (Siluriformes: Loricariidae). PLoS ONE. 2014; 9(8):e105564. https://doi.org/10.1371/journal.pone.0105564
- **Roxo FF, Ochoa LE, Sabaj MH, Lujan NK, Covain R, Silva GSC *et al*.** Phylogenomic reappraisal of the Neotropical catfish family Loricariidae (Teleostei: Siluriformes) using ultraconserved elements. Mol Phylogenet Evol. 2019; 135:148–65. https://doi.org/10.1016/j.ympev.2019.02.017
- **Rubert M, Rosa R, Jerep FC, Bertollo LAC, Giuliano-Caetano L.** Cytogenetic characterization of four species of the genus *Hypostomus* Lacépède, 1803 (Siluriformes, Loricariidae) with comments on its chromosomal diversity. Comp Cytogenet. 2011; 5(5):397–410. https://doi.org/10.3897/CompCytogen.v5i5.1589
- **Rubert M, Rosa R, Zawadzki CH, Mariotto S, Moreira-Filho O, Giuliano-Caetano L.** Chromosome mapping of 18S ribosomal RNA genes in eleven *Hypostomus* species (Siluriformes, Loricariidae): diversity analysis of the sites. Zebrafish. 2016; 13(4):360–68. https://doi.org/10.1089/zeb.2016.1279
- **Rubert M, Takagui FH, Santos KF, Pompeo LRS, Rosa R, Zawadzki CH *et al*.** Topotype-based chromosomal diversity among five species of freshwater armored catfishes in the *Hypostomus auroguttatus* supergroup (Actinopterygii: Siluriformes). Zool Sci. 2022; 39(5):446–52. https://doi.org/10.2108/zs210103
- **Rubert M, Zawadszki CH, Giuliano-Caetano L.** Cytogenetic characterization of *Hypostomus nigromaculatus* (Siluriformes, Loricariidae). Neotrop Ichthyol. 2008, 6(1):93–100. https://doi.org/10.1590/S1679-62252008000100011
- **Saitoh K, Miya M, Inoue JG, Ishiguro NB, Nishida M.** Mitochondrial genomics of ostariophysan fishes: perspectives on phylogeny and biogeography. J Mol Evol. 2003; 56(4):464–72. https://doi.org/10.1007/s00239-002-2417-y
- **Santos CEV, Almeida BRR, Tavares FS, Frade LSF, Cardoso AL, Sá ALA *et al*.** Chromosomal mapping of the histone multigene family and U2 snRNA in *Hypancistrus* species (Siluriformes, Loricariidae) from the Brazilian Amazon. Zebrafish. 2023; 20(1):28–36. https://doi.org/10.1089/zeb.2022.0030
- **Santos da Silva K, Souza ACP, Pety AM, Noronha RCR, Vicari MR, Pieczarka JC *et al*.** Comparative cytogenetics analysis among *Peckoltia* species (Siluriformes, Loricariidae): insights on karyotype evolution and biogeography in the Amazon region. Front Genet. 2021; 12:779464. https://doi.org/10.3389/fgene.2021.779464
- **Santos da Silva K, Glugoski L, Vicari MR, Souza ACP, Noronha RCR, Pieczarka JC *et al*.** Chromosomal diversification in *Ancistrus* species (Siluriformes: Loricariidae) inferred from repetitive sequence analysis. Front Genet. 2022a; 13:838462. https://doi.org/10.3389/fgene.2022.838462
- **Santos da Silva K, Souza ACP, Rodrigues LRR, Pieczarka JC, Nagamachi CY.** Chromosomal diversification in *Pseudacanthicus* species (Loricariidae, Hypostominae) revealed by comparative mapping of repetitive sequences. Animals. 2022b; 12:2612. https://doi.org/10.3390/ani12192612
- **Santos da Silva K, Glugoski L, Vicari MR, Souza ACP, Akama A, Pieczarka JC *et al*.** Mechanisms of karyotypic diversification in *Ancistrus* (Siluriformes, Loricariidae): inferences from repetitive sequence analysis. Int J Mol Sci. 2023; 24(18):14159. https://doi.org/10.3390/ijms241814159
- **Sassi FMC, Deon GA, Moreira-Filho O, Vicari MR, Bertollo LAC, Liehr T *et al*.** Multiple sex chromosomes and evolutionary relationships in Amazonian catfishes: the outstanding model of the genus *Harttia* (Siluriformes: Loricariidae). Genes. 2020; 11(10):1179. https://doi.org/10.3390/genes11101179

- **Sassi FMC, Moreira-Filho O, Deon GA, Sember A, Bertollo LAC, Liehr T *et al*.** Adding new pieces to the puzzle of karyotype evolution in *Harttia* (Siluriformes, Loricariidae): investigation of Amazonian species. Biology. 2021; 10(9):922. https://doi.org/10.3390/biology10090922
- **Sassi FMC, Sember A, Deon GA, Liehr T, Padutsch N, Oyakawa OT *et al*.** Homeology of sex chromosomes in Amazonian *Harttia* armored catfishes supports the X-fission hypothesis for the X_1X_2Y sex chromosome system origin. Sci Rep. 2023; 13(1):15756. https://doi.org/10.1038/s41598-023-42617-w
- **Schott SCQ, Glugoski L, Azambuja M, Moreira-Filho O, Vicari MR, Nogaroto V.** Comparative cytogenetic and sequence analysis of u small nuclear RNA genes in three *Ancistrus* species (Siluriformes: Loricariidae). Zebrafish. 2022; 19(5):200–09. https://doi.org/10.1089/zeb.2022.0040
- **Sember A, Nguyen P, Perez MF, Altmanová M, Petr R, Cioffi MB.** Multiple sex chromosomes in teleost fishes from a cytogenetic perspective: state of the art and future challenges. Philos Trans Royal Soc London B Biol Sci. 2021; 376(1833):20200098. https://doi.org/10.1098/rstb.2020.0098
- **Silva KS, Souza ACP, Pety AM, Noronha RCR, Vicari MR, Pieczarka JC *et al*.** Comparative cytogenetics analysis among *Peckoltia* species (Siluriformes, Loricariidae): insights on karyotype evolution and biogeography in the Amazon region. Front Genet. 2021; 12:779464. http://dx.doi.org/10.3389/fgene.2021.779464
- **Silva M, Ribeiro ED, Matoso DA, Sousa LM, Hrbek T, Rapp Py-Daniel L *et al*.** Chromosomal polymorphism in two species of *Hypancistrus* (Siluriformes: Loricariidae): an integrative approach for understanding their biodiversity. Genetica. 2014; 142(2):127–39. https://doi.org/10.1007/s10709-014-9760-y
- **Sochorová J, Garcia S, Gálvez F, Symnonová R, Kovarík A.** Evolutionary trends in animal ribosomal DNA loci: introduction to a new online database. Chromosoma. 2018; 127:141–50. https://doi.org/10.1007/s00412-017-0651-8
- **Souza ACP, Nagamachi CY, Milhomem SSR, Feldberg E, Pieczarka JC.** Cytogenetic analysis in catfish species of the genus *Peckoltia* Miranda Ribeiro, 1912 (Teleostei: Siluriformes: Loricariidae). Comp Cytogenet. 2009; 3(2):103–09. https://doi.org/10.3897/compcytogen.v3i2.17
- **Souza ACP, Nascimento AL, Carvalho Jr. JR, Barros RMS, Feldberg E, Nagamachi CY *et al*.** Karyotypic analysis of *Baryancistrus* aff. *niveatus* (Ancistrinae, Loricariidae) by C-banding, AgNOR, CMA3, DAPI and FISH. Caryologia. 2004; 57(3):219–23. https://doi.org/10.1080/00087114.2004.10589396
- **Stace CA.** Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21st centuries. Taxon. 2000; 49:451–77. https://doi.org/10.2307/1224344
- **Stawikowski R, Werner A, Seidel I.** Fünfzehn jahre l-nummern/fifteen years of l-numbers. Die Aquarien- und Terrarienzeitschrift Special L-Nummern/L-Numbers, 4–5. Verlag Eugen Ulmer, Stuttgart, Germany; 2004.
- **Sullivan JP, Lundber JG, Hardman M.** A phylogenetic analysis of the major groups of catfishes (Teleostei: Siluriformes) using rag1 and rag2 nuclear gene sequences. Mol Phylogenet Evol. 2006; 41(3):636–62. https://doi.org/10.1016/j.ympev.2006.05.044
- **Takagui FH, Baumgärtner L, Venturelli NB, paiz LM, Viana P, Dionísio JF *et al*.** Unrevealing the karyotypic evolution and cytotaxonomy of armored catfishes (Loricariinae) with emphasis in *Sturisoma*, *Loricariichthys*, *Loricaria*, *Proloricaria*, *Pyxiloricaria*, and *Rineloricaria*. Zebrafish. 2020; 17(5):319–32. https://doi.org/10.1089/zeb.2020.1893
- **Takagui FH, Rubert M, Dionisio JF, Baumgärtner L, Cardoso YP, Jerep FC *et al*.** Cytogenetic markers reinforce the redescription of the armored pleco *Hypostomus spiniger* (Loricariidae - Hypostominae), an endemic species in the Uruguay River basin and Patos Lagoon system. Braz Arch Biol Technol. 2023; 66:e23220154. https://doi.org/10.1590/1678-4324-2023220154

- **Takagui FH, Venturelli NB, Dias AL, Swarça AC, Vicari MR, Fenocchio AS *et al*.** The Importance of pericentric inversions in the karyotypic diversification of the species *Loricariichthys anus* and *Loricariichthys platymetopon*. Zebrafish. 2014; 11(4):300–05. https://doi.org/10.1089/zeb.2014.0985
- **Traldi JB, Blanco DR, Vicari MR, Martinez JF, Lui RL, Barros AV *et al*.** Chromosomal diversity in *Hypostomus* (Siluriformes, Loricariidae) with emphasis on physical mapping of 18S and 5S rDNA sites. Genet Mol Res. 2013; 12(1):463–71. http://dx.doi.org/10.4238/2013.February.8.11
- **Traldi JB, Lui RL, Martinez JF, Vicari MR, Nogaroto V, Moreira-Filho O *et al*.** Chromosomal distribution of the retroelements *Rex*1, *Rex*3 and *Rex*6 in species of the genus *Harttia* and *Hypostomus* (Siluriformes: Loricariidae). Neotrop Ichthyol. 2019; 17(2):e190010. https://doi.org/10.1590/1982-0224-20190010
- **Traldi JB, Vicari MR, Blanco DR, Martinez JF, Artoni RF, Moreira-Filho O.** First karyotype description of *Hypostomus iheringii* (Regan, 1908): a case of heterochromatic polymorphism. Comp Cytogenet. 2012; 6(2):115–25. https://doi.org/10.3897/CompCytogen.v6i2.2595
- **Tursellino MN, Cioffi MB, Sassi FMC, Deon GA, Oliveira C, Kuranaka M *et al*.** Integrating genomic and chromosomal data: a cytogenetic study of *Transancistrus santarosensis* (Loricariidae: Hypostominae) with characterization of a ZZ/ZW sex chromosome system. Genes. 2023; 14:1662. https://doi.org/10.3390/genes14091662
- **Venturelli NB, Takagui FH, Pompeo LRS, Rodriguez MS, Rosa R, Giuliano-Caetano L.** Cytogenetic markers to understand chromosome diversification and conflicting taxonomic issues in *Rineloricaria* (Loricariidae: Loricariinae) from Rio Grande do Sul coastal drainages. Biologia. 2021; 76:2561–72. https://doi.org/10.1007/s11756-021-00748-3
- **Ziemniczak K, Barros AV, Rosa KO, Nogaroto V, Almeida MC, Cestari MM *et al*.** Comparative cytogenetics of Loricariidae (Actinopterygii: Siluriformes): emphasis in Neoplecostominae and Hypoptopomatinae. Italian J Zool. 2012; 79(4):492–501. https://doi.org/10.1080/11250003.2012.676677

AUTHORS' CONTRIBUTION

Francisco de Menezes Cavalcante Sassi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing-original draft, Writing-review and editing.

Marcelo de Bello Cioffi: Conceptualization, Funding acquisition, Project administration, Supervision, Writing-original draft, Writing-review and editing.

Orlando Moreira-Filho: Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Writing-review and editing.

ETHICAL STATEMENT

Not applicable.

COMPETING INTERESTS

The author declares no competing interests.

HOW TO CITE THIS ARTICLE

- **Sassi FMC, Cioffi MB, Moreira-Filho O.** A state-of-art review of Loricariidae (Ostariophysi: Siluriformes) cytogenetics. Neotrop Ichthyol. 2024; 22(4):e240050. https://doi.org/10.1590/1982-0224-2024-0050

Neotropical Ichthyology

OPEN ACCESS

SBI | Official Journal of the Sociedade Brasileira de Ictiologia

IV – Sassi *et al.* (2023a) Turnover of multiple sex chromosomes in *Harttia* catfish (Siluriformes, Loricariidae): a glimpse from whole chromosome painting. Frontiers in Genetics 14:1226222.

frontiers | Frontiers in Genetics

TYPE Original Research
PUBLISHED 28 July 2023
DOI 10.3389/fgene.2023.1226222

OPEN ACCESS

EDITED BY
Vindhya Mohindra,
National Bureau of Fish Genetic Resources (ICAR), India

REVIEWED BY
Kris Christensen,
Fisheries and Oceans Canada (DFO), Canada
Julio Cesar Pieczarka,
Federal University of Pará, Brazil
Duílio M. Z. A. Silva,
São Paulo State University, Brazil

*CORRESPONDENCE
Thomas Liehr,
thomas.Liehr@med.uni-jena.de

RECEIVED 20 May 2023
ACCEPTED 17 July 2023
PUBLISHED 28 July 2023

CITATION
Sassi FdMC, Deon GA, Sember A, Liehr T, Oyakawa OT, Moreira Filho O, Bertollo LAC, Vicari MR and Cioffi MdB (2023), Turnover of multiple sex chromosomes in *Harttia* catfish (Siluriformes, Loricariidae): a glimpse from whole chromosome painting. *Front. Genet.* 14:1226222. doi: 10.3389/fgene.2023.1226222

Turnover of multiple sex chromosomes in *Harttia* catfish (Siluriformes, Loricariidae): a glimpse from whole chromosome painting

Francisco de Menezes Cavalcante Sassi[1], Geize Aparecida Deon[1], Alexandr Sember[2], Thomas Liehr[3]*, Osvaldo Takeshi Oyakawa[4], Orlando Moreira Filho[1], Luiz Antonio Carlos Bertollo[1], Marcelo Ricardo Vicari[5] and Marcelo de Bello Cioffi[1]

[1]Laboratório de Citogenética de Peixes, Departamento de Genética e Evolução, Universidade Federal de São Carlos, São Carlos, Brazil, [2]Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Liběchov, Czechia, [3]Institut für Humangenetik, Universitätsklinikum Jena, Jena, Germany, [4]Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil, [5]Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Ponta Grossa, Brazil

The remarkable fish biodiversity encompasses also great sex chromosome variability. *Harttia* catfish belong to Neotropical models for karyotype and sex chromosome research. Some species possess one of the three male-heterogametic sex chromosome systems, XY, X_1X_2Y or XY_1Y_2, while other members of the genus have yet uncharacterized modes of sex determination. Particularly the XY_1Y_2 multiple sex chromosome system shows a relatively low incidence among vertebrates, and it has not been yet thoroughly investigated. Previous research suggested two independent X-autosome fusions in *Harttia* which led to the emergence of XY_1Y_2 sex chromosome system in three of its species. In this study, we investigated evolutionary trajectories of synteny blocks involved in this XY_1Y_2 system by probing six *Harttia* species with whole chromosome painting (WCP) probes derived from the X (HCA-X) and the chromosome 9 (HCA-9) of *H. carvalhoi*. We found that both painting probes hybridize to two distinct chromosome pairs in Amazonian species, whereas the HCA-9 probe paints three chromosome pairs in *H. guianensis,* endemic to Guyanese drainages. These findings demonstrate distinct evolutionary fates of mapped synteny blocks and thereby elevated karyotype dynamics in *Harttia* among the three evolutionary clades.

KEYWORDS

microdissection, WCP, chromosomal rearrangements, karyotype, evolution

1 Introduction

The Loricariidae family, which includes the Neotropical armored catfishes, is a promising group for evolutionary studies. Slightly above 1,000 species are distributed among 115 genera and most of this diversity occurs in the subfamilies Hypostominae, Hypoptopomatinae, and Loricariinae (Fricke et al., 2023). Loricariids have spread to almost all freshwater environments during their evolutionary history, being thus especially valuable

in the artisanal fishery and the ornamental fish trade (Novák et al., 2022). Despite their great diversity in morphology, ecology, and habitats, Loricariidae is recovered as a monophyletic group in both morphological and molecular phylogenetic reconstructions, though the relationships between the tribes and genera have not been fully resolved so far (Covain et al., 2016; Londoño-Burbano and Reis, 2021). In this context, cytogenetic research provides important landmarks for solving issues related to taxonomy, phylogeny, and biodiversity in loricariids (e.g., Rocha-Reis et al., 2018; Glugoski et al., 2020; Takagui et al., 2020; Takagui et al., 2023; de Paula et al., 2022) as well as in other teleosts (Bellafronte et al., 2005; Cioffi et al., 2018; Gavazzoni et al., 2023). Moreover, particularly in the Neotropical region, with the richest freshwater biodiversity on the planet (Albert et al., 2020), cytogenetic investigation has abundant opportunities to deeply scrutinize the relationship between karyotype and sex chromosome evolution and reproductive isolation/diversification (Cioffi et al., 2017; Cioffi et al., 2018; de Souza et al., 2022).

Armored catfishes exhibit a wide variation in diploid chromosome numbers, ranging from 2n = 34 in *Rineloricaria latirostris* (Giuliano-Caetano, 1998) to 2n = 96 in *Hemipsilichthys gobio* (published as *Upsilodus* sp. in Kavalco et al., 2005). Some reports also documented population polymorphisms in the karyotype organization and repetitive DNA distribution, and the presence of B and sex chromosomes, the latter in different stages of differentiation (Scavone and Julio, 1994; Centofante et al., 2006; Rosa et al., 2012; Porto et al., 2014; Glugoski et al., 2018; Marajó et al., 2023). While the plesiomorphic 2n for loricariids has been supposed to be 2n = 54 (Artoni and Bertollo, 2001), this issue is still under intense debate, particularly considering the karyotypic features of species from the early-diverging clades (Takagui et al., 2020; Sassi et al., 2021).

Teleost fishes are well known for remarkable diversity of sex determination mechanisms (Devlin and Nagahama, 2002; Godwin and Roberts, 2018; Shen and Wang, 2018; Guiguen et al., 2019; Schartl et al., 2023). To date, more than 500 species have been found to carry one of nine so far known sex chromosome systems (El Taher et al., 2021; Sember et al., 2021), five of which are so-called multiple sex chromosome systems as they involve more than two chromosomes. Among 81 currently known cases of teleost multiple sex chromosomes (Deon et al., 2020; Sember et al., 2021; Ferchaud et al., 2022; de Araújo et al., 2023; Marajó et al., 2023; Nirchio et al., 2023), only nine are of the XY_1Y_2 type and evolved either via Y-fission or X-autosome fusion (Deon et al., 2020; Sember et al., 2021), Intriguingly, three of these systems have been found in *Harttia* spp., all from the Southeastern Brazilian drainages (Centofante et al., 2006; Blanco et al., 2013; Deon et al., 2020).

The genus *Harttia* (Loricariidae, Loricariinae) currently harbors 28 valid species (Oyakawa et al., 2018; Caldas et al., 2022; Fricke et al., 2023) as well as three other predicted species based on the cytogenetic data (Sassi et al., 2021). *Harttia* catfish are organized into three phylogenetic clades grouping representatives from Guyanese, Amazonian, and Southeastern Brazilian drainages (Covain et al., 2016). *Harttia* spp. are excellent models for studying interplay between chromosomal and species diversity in Neotropical fishes as they underwent extensive karyotype repatterning. Their 2n varies from 2n = 52/53 in *H. carvalhoi* (Centofante et al., 2006) to 2n = 62 in *H. absaberi* and *Harttia* sp. 2 (Rodrigues, 2010; Deon et al., 2020) and at least three distinct sex chromosome systems have been described in some species: an XX/XY sex chromosome system in *H. rondoni* (Sassi et al., 2020), and the two following multiple sex chromosome systems: $X_1X_1X_2X_2/X_1X_2Y$ in *H. duriventris*, *H. punctata*, and *H. villasboas* (Blanco et al., 2013; Sassi et al., 2020), and XX/XY_1Y_2 in *H. carvalhoi*, *H. intermontana* and *Harttia* sp. 1 (Centofante et al., 2006; Deon et al., 2020). Studies using whole chromosome painting (WCP) probes have recently demonstrated that both the XY and X_1X_2Y sex chromosome systems are homologous, while the XY_1Y_2 system is formed by different linkage groups and thus has evolved independently (Deon et al., 2022a; b). Intriguingly, the three XY_1Y_2-bearing species share the ancestral sex chromosomes but differ by autosomal additions. While *H. carvalhoi* and *Harttia* sp. 1 entirely share the identity of XY_1Y_2 chromosomes, the same system in *H. intermontana* involves linkage group corresponding to chromosome 9 in *H. carvalhoi*, which implies two independent X-autosome fusions behind the origin of known *Harttia* XY_1Y_2 sex chromosomes (Deon et al., 2022a).

Here, we investigate six *Harttia* species from the Guyanese (*H. guianensis*) and Amazonian (*H. villasboas, H. duriventris, H. rondoni, Harttia* sp., and *H. dissidens*) clades using cross-species (i.e., Zoo-FISH) WCP experiments to examine the trajectory of karyotype and sex chromosome evolution. For this, we used the probes derived from the largest chromosomes (pairs X and 9) of *H. carvalhoi* (2n = 52/53 XY_1Y_2), bearing in mind that chromosome 9 is involved in the XY_1Y_2 sex chromosome system of *H. intermontana*. The results allowed us to demonstrate distinct evolutionary fates of mapped synteny blocks and thereby elevated karyotype dynamics in *Harttia*.

2 Materials and methods

2.1 Sampling and chromosome preparation

Individuals belonging to seven species (including *H. carvalhoi* used solely for the WCP probes preparation) were collected in distinct locations in Amazonas and Tocantins-Araguaia River basins (Figure 1; Table 1), with the approval of the Brazilian environmental agencies ICMBIO/SISBIO (License 48628-14) and SISGEN (A96FF09). We used cells from the posterior kidney and spleen tissue to obtain metaphase chromosomes by the classical air-drying technique (Bertollo et al., 2015). The specimens were deposited in the fish collection of the Instituto Nacional de Pesquisa da Amazônia (INPA) and the Museu de Zoologia da Universidade de São Paulo (MZUSP) as indicated in Table 1. The anesthesia and ethical practices used during the procedures were previously approved by the Ethics Committee on Animal Experimentation of the Universidade Federal de São Carlos (Process number CEUA 1853260315).

2.2 Microdissection and preparation of hybridization probes

We selected the largest metacentric (X) and the largest submetacentric (No. 9) chromosomes of *H. carvalhoi* since both chromosomes are involved in the two variant forms of the XY_1Y_2 system in *Harttia* species (Deon et al., 2022b). Glass-needle-based

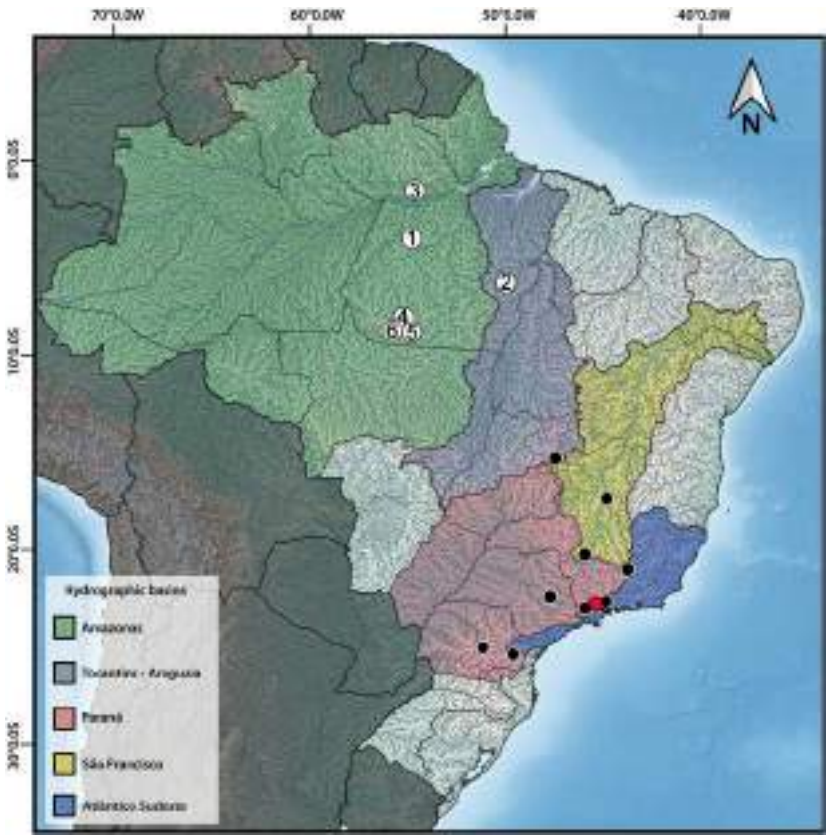


FIGURE 1
Distribution map of *Harttia* species with available cytogenetic data, highlighting the six species studied herein: 1 – *H. dissidens*, 2 – *H. duriventris*, 3 – *H. guianensis*, 4 – *H. rondoni*, 5 – *H. villasboas*, 6 – *Harttia* sp. 3. The red circle indicates the collection site of *H. carvalhoi*, a species used to construct WCP probes, while the black circles correspond to the distribution of other *Harttia* species which were not included in the present investigation. Major Brazilian hydrographic basins are highlighted: Amazonas in green, Tocantins-Araguaia in purple, Paraná in red, São Francisco in yellow, and Atlântico-Sudeste in blue. The map was created in QGIS 3.20 using Natural Earth package.

TABLE 1 ***Harttia*** **species, collection sites, diploid chromosome numbers and sex chromosomes, sampling, and voucher numbers.**

Species	Locality	2n	N	Voucher
1 - *Harttia dissidens*	Rurópolis – PA 4°5'37.8"S 55°0'30.2"W	54♀♂	07♀, 25♂	INPA-ICT 059577
2 - *H. duriventris*	Canaã dos Carajás – PA 6°30'06.5"S 50°02'35.5"W	56♀, 55♂ (X_1X_2Y)	08♀, 07♂	MZUSP 126598
3 - *H. guianensis*	Alenquer – PA 1°29'02.2"S 54°50'31.2"W	58♀♂	06♀, 10♂	INPA-ICT 059584
4 - *H. rondoni*	Altamira – PA 8°38'53"S 55°01'41"W	54♀♂ (XY)	15♀, 14♂	MZUSP 127606
5 - *H. villasboas*	Altamira – PA 8°44'09"S 54°57'46"W	56♀, 55♂ (X_1X_2Y)	34♀, 38♂	MZUSP 126599
6 - *Harttia* sp. 3	Altamira – PA 08°39'20.7"S 55°09'24.1"W	54♀♂	11♀, 15♂	MZUSP 127605

microdissection was used to isolate 15 copies of each target chromosome under an inverted microscope (Zeiss Axiovert 135). The obtained DNA was amplified using a degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) following Yang et al. (2009). The probes were labeled with Spectrum Green-dUTP and Spectrum Orange-dUTP (Vysis, Downers Grove, United States), in a secondary DOP-PCR reaction using 1 µL of the first amplification as template (Yang and Graphodatsky, 2009).

2.3 Whole chromosome painting (WCP)

Chromosome preparations were aged at 60 °C for 1h, then treated with RNAse solution [1.5 µL RNase A (10 mg/mL) in 1.5 mL 2×SSC] for 1 h 30min. Next, chromosomes were treated with 0.005% (v/v) pepsin solution [99 µL H_2O, 10 µL 1M HCl, and 2.5 µL pepsin (20 mg/mL)]. Slides were denatured in 70% formamide in 2× SSC at 72 °C for 3 min. The probe mix per each slide contained 200 ng of each painting probe and 20 µg of

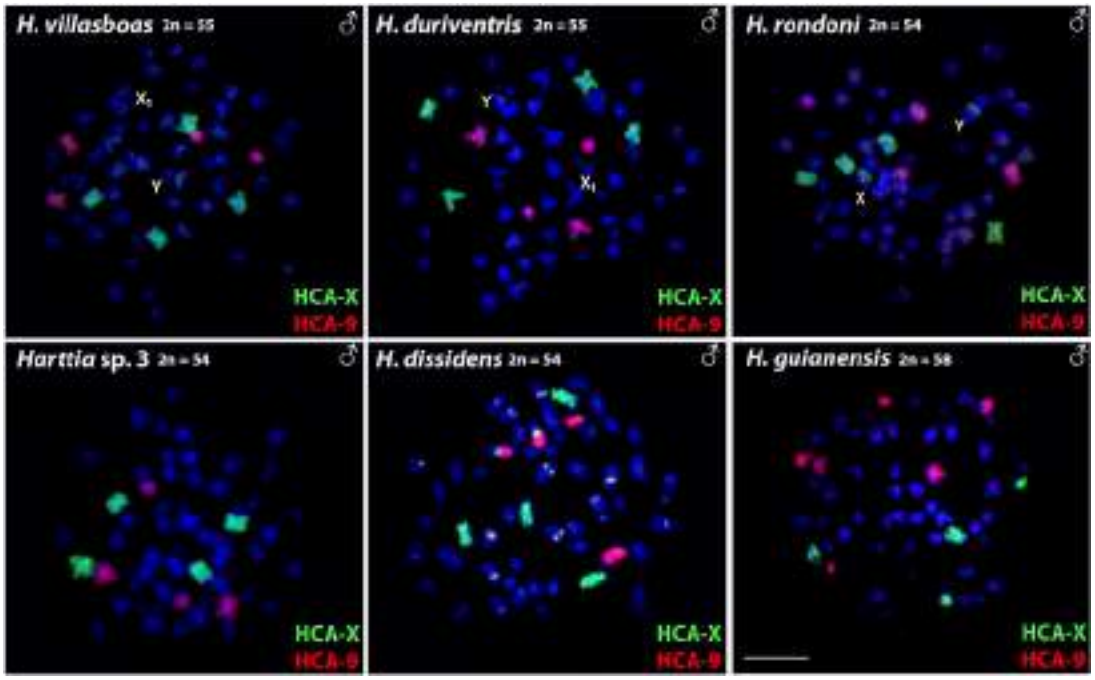


FIGURE 2
Whole chromosome painting with HCA-X (green) and HCA-9 (red) probes on the male chromosomes of six *Harttia* species. Sex chromosomes are indicated if present and detectable in the given species (first line). Scale Bar = 10 μm.

blocking DNA which was obtained by DOP-PCR from *H. carvalhoi* genome and used as a blocker to highly and moderated repeated sequences (Yang et al., 2009). The final probe mixture was dissolved in the hybridization buffer containing 50% formamide, 2× SSC and 10% dextran sulfate, then denatured for 10 min at 86°C, cooled at 4°C for 2 min, and pre-annealed for 40 min at 37°C before being applied onto denatured chromosome slides. Hybridization took place in a dark moist chamber at 37°C for 72 h, followed by a series of post-hybridization washes in 1× SSC, 4× SSC/Tween, and 1× PBS solutions (for details, see Sassi et al., 2023). Chromosomes were finally counterstained with 4', 6-diamidino-2-phenylindole (DAPI) 0.2 μg/mL in Vectashield mounting medium (Vector, California, United States).

2.4 Microscopic analyses and image processing

At least 30 metaphase spreads per individual were evaluated to confirm the WCP results. Metaphases were captured in an Axioplan II microscope (Carl Zeiss Jena GmbH, Germany) fluorescence microscope using the ISIS software (MetaSystems, Silver spring, MD, USA).

3 Results

Both HCA-X and HCA-9 probes hybridized to the chromosomes of all analyzed species (Figure 2). Each probe completely painted from end to end two distinct chromosome pairs (one metacentric and one submetacentric in both cases) in *H. villasboas*, *H. duriventris*, *H. rondoni*, and *Harttia* sp. 3. In addition to those pairs, *H. guianensis* also demonstrated a small metacentric pair probed with HCA-9 (Figure 2). In *H. rondoni*, additional HCA-X signals were found in pericentromeric regions of XY sex chromosomes, probably due to shared ribosomal DNA (rDNA) content which has been amplified on sex chromosomes in this species. Finally, in *H. dissidens*, the HCA-9 probe stained entirely one submetacentric pair but further only long arms of one metacentric pair, while the HCA-X probe revealed the same patterns as for the majority of studied species, with a metacentric and a submetacentric pair being stained. In this species, repetitive DNA sequences contained in both painting probes form distinct clusters, revealed as additional hybridization signals on several chromosomes. An ideogram compiling the present WCP results is provided in Figure 3.

4 Discussion

In the present study, we mapped two WCP probes prepared from *H. carvalhoi* chromosomes, where the chromosomes X and 9 were involved in rearrangements leading to emergence of the XY_1Y_2 sex chromosome system which is present in three *Harttia* species (Deon et al., 2022a). Both probes painted mostly entire chromosomes, thus pointing to preserved synteny blocks. The probes hybridized only to chromosomes without detectable sex-specific differences in size and shape (and therefore do not represent differentiated sex chromosomes) in species from the Amazonian and Guyanese clades (Figures 2, 3). These findings

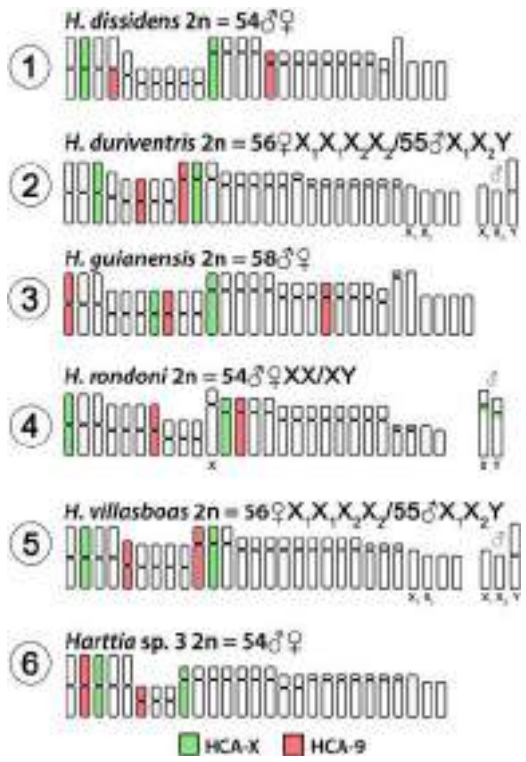


FIGURE 3
Idiogram summarizing obtained WCP/Zoo-FISH patterns by HCA-X and HCA-9 probes. HCA = *Harttia carvalhoi*.

corroborate the view of independent events behind X_1X_2Y and XY_1Y_2 origin in *Harttia* (Deon et al., 2022a; b). We also show that chromosome 9 of *H. carvalhoi*, which has been fused to X chromosome in *H. intermontana*, is not involved in any other known sex chromosome system in *Harttia*, however, its propensity to fuse with other chromosomes has been recorded in *H. dissidens*.

The ancestral karyotype of *Harttia* is thought to be 2n = 58, with poorly differentiated or absent sex chromosomes (Blanco et al., 2017; Sassi et al., 2021). Until now, four species – *H. gracilis*, *H. guianensis*, *H. kronei*, *H. longipinna*–from the three phylogenetic clades are known to share these karyotype characteristics. In addition, *H. punctata* exhibits 58 chromosomes in females, but 57 in males due to the presence of X_1X_2Y sex chromosome system. Except for *H. guianensis*, which possesses three chromosome pairs labeled with the HCA-9 probe, all remaining five examined species, including *H. punctata* (Deon et al., 2022b), share the pattern of two distinct chromosome pairs being painted either with HCA-X or HCA-9 probe. Based on this evidence, it is possible to infer that the chromosome number reduction from the probable ancestral 2n = 58 to 2n = 56 in *H. loricariformis*, *H. villasboas*, and *H. duriventris*, and to 2n = 54 in *H. rondoni* and *Harttia* sp. 3 was achieved by chromosome fusions which did not involve the herein mapped chromosomes. On the other hand, in *H. dissidens* (2n = 54♀♂, Figure 2) the HCA-9 probe mapped to a submetacentric pair and the short arms of a metacentric pair, thus indicating that a fusion event involving the chromosome 9 of *H. carvalhoi* was responsible for its 2n reduction in relation to the ancestral karyotype. In support of this hypothesis, *H. guianensis* (2n = 58) also possesses more acrocentric chromosomes – five pairs – than the other species with lower 2n (Blanco et al., 2017; Deon et al., 2020; Sassi et al., 2020; Sassi et al., 2021). While the trajectory via centric fusions of acrocentric chromosomes seems highly probable, based on the available data we cannot entirely rule out also the possible involvement of biarmed (metacentric and submetacentric) chromosomes in the (specifically end-to-end/tandem) fusion events (cf. Schubert and Lysak, 2011) in the evolutionary history of *Harttia*.

It is notable that the patterns of hybridization in the Zoo-FISH experiments follows a biogeographical pattern, with *H. guianensis* (distributed in Guyanese drainages) showing three chromosomal pairs labeled with HCA-9, while the species from Amazonian and Tocantins-Araguaia River basins share the pattern of two chromosomal pairs being stained with the same particular WCP probe. Indeed, species from the Amazonian and Tocantins-Araguaia River basins exhibit more similar cytogenetic features when compared to *H. guianensis*, the only species from the Guyanese drainages karyotyped to date (discussed also in Sassi et al., 2021). Based on the cytogenetic and phylogenetic data from nuclear and mitochondrial DNA (Covain et al., 2016), it is possible to suggest that species from Guyanese drainages carry the ancestral-like karyotype arrangement (Sassi et al., 2021). As *H. guianensis* with the most diversified karyotype is the only species from the left bank of the Amazon River, it is tempting to speculate about the possible role of Amazon River as a barrier for gene flow. However, it is difficult to address this issue in the light of the still growing list of *Harttia* species which have been described within the last few years (Oyakawa et al., 2018; Caldas et al., 2022), and the existence of at least three karyotype forms proposed to represent new *Harttia* species (see Deon et al., 2020; Sassi et al., 2021). Moreover, at least seven *Harttia* species are known to occur in Guyanese drainages (Py-Daniel et al., 2001), but only *H. guianensis* has been investigated cytogenetically thus far. It is also noteworthy that the phylogenetic patterns of *Harttia* spp. are not well resolved, with the previously recognized monophyletic status of the genus (Covain et al., 2016) being questioned in a recent study (Cherobim, 2022).

Both the X and Y chromosomes of *H. rondoni* exhibit a positive HCA-X signal in the pericentromeric region of their long arms. As our previous study showed that pericentromeric regions of these chromosomes are enriched with tandem repeats of the major rDNA gene cluster (Sassi et al., 2020), the observed signal pattern might be attributed rather to shared repeat content between the HCA-X probe and the X and Y chromosomes and therefore does not represent homology of synteny blocks. However, the chromosome X of *H. carvalhoi* does not carry major rDNA site, neither do the chromosomes composing both forms of the XY_1Y_2 sex system of *Harttia* sp. 1 and *H. intermontana* (Blanco et al., 2017; Deon et al., 2020; 2022a). Bearing also in mind that rDNA sites outside the homeologous synteny blocks were not revealed in other herein studied species by the painting probes, it is highly probable that other repeat class interspersed with rDNA on the XY sex chromosomes of *H. rondoni* is responsible for the observed signal pattern. Analogous situation has been observed after application of HCA-X or HCA-9 probe in other *Harttia* spp. (Deon et al., 2022b). Similarly, an unknown

repeat sequence contained in both painting probes demonstrated homology with certain regions on multiple chromosomes of *H. dissidens*.

Sex chromosomes in Loricariidae are promising targets for evolutionary research, since this family is the most diverse among Neotropical fishes (Fricke et al., 2023) and exhibits a wide range of sex chromosomes at distinct stages of differentiation (reviewed in Sember et al., 2021). Taking *Harttia* as an example, although inhabiting large Brazilian rivers, such as São Francisco, Paraná and Araguaia (Caldas et al., 2022), most species are found in riffle areas where they form isolated subpopulations with restricted/absent gene flow. This condition is being further pronounced by low vagility of these fishes, favoring a faster fixation of chromosomal rearrangements by genetic drift (Lande, 1977; King, 1993; Araya-Jaime et al., 2017), and thus also faster species divergence. Structuring into small, geographically isolated populations may also facilitate sex chromosome differentiation and transition between sex chromosome systems (e.g., Primo et al., 2017; Cioffi et al., 2018; Krysanov & Demidova, 2018; Glugoski et al., 2020; Štundlová et al., 2022; Marajó et al., 2023) To answer the question whether indeed genetic drift is a major force in sex chromosomes fixation in *Harttia* and other fish lineages with similar eco-geographical and demographic features and whether certain selective forces and ecological factors (cf. Pennell et al., 2015; Veller et al., 2017; Saunders et al., 2018; Meisel, 2022) might have played a role in this process, we have to acquire much deeper knowledge especially concerning genetic content and degree of sex chromosome differentiation, presence/absence of sex chromosomes in wide range of conspecific populations, and their phylogenetic distribution based on well-resolved phylogeny (cf. Sember et al., 2021). Particularly in *Harttia*, most of the phylogenetic reconstructions do not include all valid species, neither those recognized by cytogenetic studies (Covain et al., 2016; Roxo et al., 2019; Londono-Burbano and Reis, 2021; Sassi et al., 2021; Cherobim, 2022). In this context, given the collection efforts performed within the frame of the recent cytogenetic studies, distinct evolutionary units were identified in *Harttia* (Deon et al., 2020; Sassi et al., 2020) and their recognition as valid species remains to be investigated.

In the present study, we provide another piece of evidence strengthening the proposed view about 2n reduction in *Harttia* by fusions and about independent evolution of *Harttia* multiple sex chromosomes, with repeated origins involving different synteny blocks among species. A thorough phylogenetic reconstruction including all currently recognized representatives of *Harttia* is urgently needed for achieving a complete picture of sex chromosome evolution and karyotype reshaping in this noteworthy group of the Neotropical ichthyofauna.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Ethics Committee on Animal Experimentation of the Universidade Federal de São Carlos (Process number CEUA 1853260315).

Author contributions

FS, GD, and MC conceived and designed research. FS and GD conducted experiments. FS, GD, AS, OM, LB, MV, and MC analysed the data. AS, TL, OO contributed with new methods. FS, AS, GD, LB, MV, and MC wrote the paper. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by São Paulo Research Foundation (FAPESP) grants 2020/02681-9 and 2022/04261-2 (FS), 2020/11772-8 (MC) and Brazilian National Council for Scientific and Technological Development (CNPq), grant number 302928/2021-9 (MC). AS was supported by Czech Academy of Sciences (RVO: 67985904 of IAPG CAS, Liběchov). We also acknowledge support for TL by the German Research Foundation Projekt-Nr. 512648189 and the Open Access Publication Fund of the Thueringer Universitaets-und Landesbibliothek Jena. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil (CAPES), Finance Code 001. This study was supported by INCT - Peixes, funded by MCTIC/CNPq (proc. 405706/2022-7).

Acknowledgments

The authors are grateful to Eliana Feldberg and Lúcia Helena Rapp Py-Daniel from National Institute of Amazonian Research who provided assistance in the collection and identification of animal samples. This paper is dedicated in memory of Daniel Rodrigues Blanco, who contributed significantly to the research of *Harttia* chromosomes.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Albert, J. S., Tagliacollo, V. A., and Dagosta, F. (2020). Diversification of Neotropical freshwater fishes. *Annu. Rev. Ecol. Evol. Syst.* 51, 27–53. doi:10.1146/annurev-ecolsys-011620-031032

Araya-Jaime, C., Mateussi, N. T. B., Utsunomia, R., Costa-Silva, G. J., Oliveira, C., and Foresti, F. (2017). ZZ/Z0: The new system of sex chromosomes in *Eigenmannia* aff. *Trilineata* (Teleostei: Gymnotiformes: Sternopygidae) characterized by molecular cytogenetics and DNA barcoding. *Zebrafish* 14 (5), 464–470. doi:10.1089/zeb.2017.1422

Artoni, R. F., and Bertollo, L. A. C. (2001). Trends in the karyotype evolution of Loricariidae fish (Siluriformes). *Hereditas* 134 (3), 201–210. doi:10.1111/j.1601-5223.2001.00201.x

Bellafronte, E., Margarido, V. P., and Moreira-Filho, O. (2005). Cytotaxonomy of *Parodon nasus* and *Parodon tortuosus* (Pisces, Characiformes). A case of synonymy confirmed by cytogenetic analyses. *Genet. Mol. Biol.* 28, 710–716. doi:10.1590/S1415-47572005000500010

Bertollo, L. A. C., Cioffi, M. B., and Moreira-Filho, O. (2015). "Direct chromosome preparation from freshwater teleost fishes," in *Fish cytogenetic techniques*. Editors C. Ozouf-Costaz, E. Pisano, F. Foresti, and L. F. Almeida-Toledo (Boca Raton, FL: CRC Press), 31–36.

Blanco, D. R., Vicari, M. R., Lui, R. L., Bertollo, L. A. C., Traldi, J. B., and Moreira-Filho, O. (2013). The role of the Robertsonian rearrangements in the origin of the XX/XY_1Y_2 sex chromosome system and in the chromosomal differentiation in *Harttia* species (Siluriformes, Loricariidae). *Rev. Fish. Biol. Fish.* 23, 127–134. doi:10.1007/s11160-012-9283-5

Blanco, D. R., Vicari, M. R., Lui, R. L., Traldi, J. B., Bueno, V., Martinez, J. D. F., et al. (2017). Karyotype diversity and evolutionary trends in armored catfish species of the genus *Harttia* (Siluriformes: Loricariidae). *Zebrafish* 14 (2), 169–176. doi:10.1089/zeb.2016.1377

Caldas, L., Cherobim, A. M., and Langeani, F. (2022). A new species of *Harttia* from the rio São Francisco basin (Siluriformes: Loricariidae). *Neotrop. Ichthyol.* 20 (4), e220051. doi:10.1590/1982-0224-2022-0051

Centofante, L., Bertollo, L. A. C., and Moreira-Filho, O. (2006). Cytogenetic characterization and description of an XX/XY_1Y_2 sex chromosome system in catfish *Harttia carvalhoi* (Siluriformes, Loricariidae). *Cytogenet. Genome Res.* 112 (3-4), 320–324. doi:10.1159/000089887

Cherobim, A. M. (2022). *Phylogenetic analysis of* Harttia *Steindachner, 1877 (Siluriformes: Loricariidae: Loricariinae)*. [PhD Thesis]. [São José do Rio Preto (SP)]: Universidade Estadual Paulista.

Cioffi, M. B., Moreira-Filho, O., Ráb, P., Sember, A., Molina, W. F., and Bertollo, L. A. C. (2018). Conventional cytogenetic approaches—useful and indispensable tools in discovering fish biodiversity. *Curr. Genet. Med. Rep.* 6, 176–186. doi:10.1007/s40142-018-0148-7

Cioffi, M. B., Yano, C. F., Sember, A., and Bertollo, L. A. C. (2017). Chromosomal evolution in lower vertebrates: Sex chromosomes in neotropical fishes. *Genes* 8 (10), 258. doi:10.3390/genes8100258

Covain, R., Fisch-Muller, S., Oliveira, C., Mol, J. H., Montoya-Burgos, J. I., and Dray, S. (2016). Molecular phylogeny of the highly diversified catfish subfamily Loricariinae (Siluriformes, Loricariidae) reveals incongruences with morphological classification. *Mol. Phylogenet. Evol.* 94, 492–517. doi:10.1016/j.ympev.2015.10.018

de Araújo, L., Ramos, L. I., Vieira, M. M. D. R., Oliveira, A. V. D., Portela-Castro, A. L. D. B., Borin-Carvalho, L. A., et al. (2023). Cytogenetic and molecular characterization of *Eigenmannia* aff. *desantanai* (gymnotiformes: Sternopygidae): A first report of system of sex chromosomes ZW_1W_2/ZZ in Gymnotiformes. *Zebrafish* 20 (2), 77–85. doi:10.1089/zeb.2022.0059

de Paula, G. B., Gavazzoni, M., Zawadzki, C. H., Fernandes, C. A., Portela-Castro, A. L., Lui, R. L., et al. (2022). Identification of cryptic species in allopatric populations of *Hypostomus tietensis* (Siluriformes: Loricariidae) through cytogenetics analyses. *Neotrop. Ichthyol.* 20 (2), e210158. doi:10.1590/1982-0224-2021-0158

de Souza, F. H. S., Sassi, F. M. C., Ferreira, P. H. N., Bertollo, L. A. C., Ezaz, T., Liehr, T., et al. (2022). Integrating cytogenetics and population genomics: Allopatry and neo-sex chromosomes may have shaped the genetic divergence in the *Erythrinus erythrinus* species complex (Teleostei, Characiformes). *Biology* 11, 315. doi:10.3390/biology11020315

Deon, G. A., Glugoski, L., Hatanaka, T., Sassi, F. M. C., Nogaroto, V., Bertollo, L. A. C., et al. (2022a). Evolutionary breakpoint regions and chromosomal remodeling in *Harttia* (Siluriformes: Loricariidae) species diversification. *Genet. Mol. Biol.* 45 (2), e20210170. doi:10.1590/1678-4685-GMB-2021-0170

Deon, G. A., Glugoski, L., Sassi, F. M. C., Hatanaka, T., Nogaroto, V., Bertollo, L. A. C., et al. (2022b). Chromosomal rearrangements and origin of the multiple XX/XY_1Y_2 sex chromosome system in *Harttia* species (Siluriformes: Loricariidae). *Front. Genet.* 13, 877522. doi:10.3389/fgene.2022.877522

Deon, G. A., Glugoski, L., Vicari, M. R., Nogaroto, V., Sassi, F. M. C., Cioffi, M. B., et al. (2020). Highly rearranged karyotypes and multiple sex chromosome systems in armored catfishes from the genus *Harttia* (Teleostei, Siluriformes). *Genes* 11 (11), 1366. doi:10.3390/genes11111366

Devlin, R. H., and Nagahama, Y. (2002). Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* 208 (3–4), 191–364. doi:10.1016/S0044-8486(02)00057-1

El Taher, A., Ronco, F., Matschiner, M., Salzburger, W., and Böhne, A. (2021). Dynamics of sex chromosome evolution in a rapid radiation of cichlid fishes. *Sci. Adv.* 7 (36), eabe8215. doi:10.1126/sciadv.abe8215

Ferchaud, A. L., Mérot, C., Normandeau, E., Ragoussis, J., Babin, C., Djambazian, H., et al. (2022). Chromosome-level assembly reveals a putative Y-autosomal fusion in the sex determination system of the Greenland halibut (*Reinhardtius hippoglossoides*). *G3 (Bethesda)* 12 (1), jkab376. doi:10.1093/g3journal/jkab376

Fricke, R., Eschmeyer, W. N., and Van der Laan, R. (2023). ESCHMEYER'S catalog of fishes: GENERA, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (Accessed April 15, 2023).

Gavazzoni, M., Pavanelli, C. S., Graça, W. J., De Oliveira, E. A., Moreira-Filho, O., and Margarido, V. P. (2023). Species delimitation in *Psalidodon fasciatus* (Cuvier, 1819) complex (Teleostei: Characidae) from three hydrographic basins. *Biol. J. Linn. Soc.* 138 (1), 51–67. doi:10.1093/biolinnean/blac139

Giuliano-Caetano, L. (1998). *Polimorfismo cromossômico Robertsoniano em populações de* Rineloricaria latirostris *(Pisces, Loricariinae)*. [Ph.D Thesis]. [São Carlos (SP)]: Universidade Federal de São Carlos.

Glugoski, L., Deon, G., Schott, S., Vicari, M. R., Nogaroto, V., and Moreira-Filho, O. (2020). Comparative cytogenetic analyses in *ancistrus* species (Siluriformes: Loricariidae). *Neotrop. Ichthyol.* 18 (2), e200013. doi:10.1590/1982-0224-2020-0013

Glugoski, L., Giuliano-Caetano, L., Moreira-Filho, O., Vicari, M. R., and Nogaroto, V. (2018). Co-located *hAT* transposable element and 5S rDNA in an interstitial telomeric sequence suggest the formation of Robertsonian fusion in armored catfish. *Gene* 650, 49–54. doi:10.1016/j.gene.2018.01.099

Godwin, J., and Roberts, R. (2018). "Environmental and genetic sex determining mechanisms in fishes," in *Transitions between sexual systems*. Editor J. Leonard (Cham: Springer), 311–344. doi:10.1007/978-3-319-94139-4_11

Guiguen, Y., Fostier, A., and Herpin, A. (2019). "Sex determination and differentiation in fish: Genetic, genomic, and endocrine aspects," in *Sex control in aquaculture*. Editors H. P. Wang, F. Piferrer, and S. L. Chen (Hoboken: John Wiley & Sons), 35–63. doi:10.1002/9781119127291.ch2

Kavalco, K. F., Pazza, R., Bertollo, L. A. C., and Moreira-Filho, O. (2005). Karyotypic diversity and evolution of Loricariidae (Pisces, Siluriformes). *Heredity* 94 (2), 180–186. doi:10.1038/sj.hdy.6800595

King, M. (1993). *Species evolution: The role of chromosome change*. Cambridge: Cambridge University Press.

Krysanov, E., and Demidova, T. (2018). Extensive karyotype variability of African fish genus *Nothobranchius* (Cyprinodontiformes). *Comp. Cytogenet.* 12 (3), 387–402. doi:10.3897/CompCytogen.v12i3.25092

Lande, R. (1977). The influence of the mating system on the maintenance of genetic variability in polygenic characters. *Genetics* 86 (2), 485–498. doi:10.1093/genetics/86.2.485

Londoño-Burbano, A., and Reis, R. E. (2021). A combined molecular and morphological phylogeny of the Loricariinae (Siluriformes: Loricariidae), with emphasis on the Harttiini and Farlowellini. *PloS One* 16 (3), 0247747. doi:10.1371/journal.pone.0247747

Marajó, L., Viana, P. F., Ferreira, A. M. V., Py-Daniel, L. H. R., Cioffi, M. B., Sember, A., et al. (2023). Chromosomal rearrangements and the first indication of an $♀X_1X_1X_2X_2/♂X_1X_2Y$ sex chromosome system in *Rineloricaria* fishes (Teleostei: Siluriformes). *J. Fish. Biol.* 102 (2), 443–454. doi:10.1111/jfb.15275

Meisel, R. P. (2022). Ecology and the evolution of sex chromosomes. *J. Evol. Biol.* 35 (12), 1601–1618. doi:10.1111/jeb.14074

Nirchio, M., Oliveira, C., Cioffi, M. B., Sassi, F. M. C., Valdiviezo, J., Paim, F. G., et al. (2023). Occurrence of sex chromosomes in fish of the genus *Ancistrus* with a new description of multiple sex chromosomes in the Ecuadorian endemic *Ancistrus clementinae* (Loricariidae). *Genes* 14 (2), 306. doi:10.3390/genes14020306

Novák, J., Hofmann, J., Hohl, D., Magalhães, A. L. B., and Patoka, J. (2022). Enigmatic armoured catfishes (Siluriformes: Callichthyidae and Loricariidae) in ornamental aquaculture: A new insight into neotropical fish diversity. *Aquaculture* 547, 737460. doi:10.1016/j.aquaculture.2021.737460

Oyakawa, O. T., Fichberg, I., and Py-Daniel, L. R. (2018). Three new species of *Harttia* (Loricariidae: Loricariinae) from serra do Cachimbo, rio Xingu basin, Pará, Northern Brazil. *Zootaxa* 4387 (1), 75–90. doi:10.11646/zootaxa.4387.1.3

Pennell, M. W., Kirkpatrick, M., Otto, S. P., Vamosi, J. C., Peichel, C. L., Valenzuela, N., et al. (2015). Y fuse? Sex chromosome fusions in fishes and reptiles. *PLoS Genet.* 11 (5), e1005237. doi:10.1371/journal.pgen.1005237

Porto, F. E., Gindri, B. S., Vieira, M. M. R., Borin, L. A., Portela-Castro, A. L. B., and Martins-Santos, I. C. (2014). Polymorphisms of the nucleolus organizing regions in *Loricaria cataphracta* (Siluriformes, Loricariidae) of the upper Paraguay River basin indicate an association with transposable elements. *Genet. Mol. Res.* 13, 1627–1634. doi:10.4238/2014.March.12.15

Primo, C. C., Glugoski, L., Almeida, M. C., Zawadski, C. H., Moreira-Filho, O., Vicari, M. R., et al. (2017). Mechanisms of chromosomal diversification in species of

Rineloricaria (Actinopterygii: Siluriformes: Loricariidae). *Zebrafish* 14 (2), 161–168. doi:10.1089/zeb.2016.1386

Rocha-Reis, D. A., Brandão, K. O., Almeida-Toledo, L. F., Pasa, R., and Kavalco, K. F. (2018). The persevering cytotaxonomy: Discovery of a unique XX/XY sex chromosome system in catfishes suggests the existence of a new, endemic and rare species. *Cytogenet. Genome Res.* 156, 45–55. doi:10.1159/000492959

Rodrigues, R. M. (2010). *Estudos cromossômicos e moleculares em Loricariinae com ênfase em espécies de* Rineloricaria *(Siluriformes, Loricariidae): uma perspectiva evolutiva*. São Paulo: Universidade de São Paulo. [PhD Thesis]. [São Paulo (SP)].

Rosa, K. O., Ziemniczak, K., de Barros, A. V., Nogaroto, V., Almeida, M. C., Cestari, M. M., et al. (2012). Numeric and structural chromosome polymorphism in *Rineloricaria lima* (Siluriformes: Loricariidae): Fusion points carrying 5S rDNA or telomere sequence vestiges. *Rev. Fish. Biol. Fish.* 22, 739–749. doi:10.1007/s11160-011-9250-6

Roxo, F. F., Ochoa, L. E., Sabaj, M. H., Lujan, N. K., Covain, R., Silva, G. S., et al. (2019). Phylogenomic reappraisal of the Neotropical catfish family Loricariidae (Teleostei: Siluriformes) using ultraconserved elements. *Mol. Phylogenet. Evol.* 135, 148–165. doi:10.1016/j.ympev.2019.02.017

Sassi, F. M. C., Deon, G. A., Moreira-Filho, O., Vicari, M. R., Bertollo, L. A. C., Liehr, T., et al. (2020). Multiple sex chromosomes and evolutionary relationships in Amazonian catfishes: The outstanding model of the genus *Harttia* (Siluriformes: Loricariidae). *Genes* 11 (10), 1179. doi:10.3390/genes11101179

Sassi, F. M. C., Moreira-Filho, O., Deon, G. A., Sember, A., Bertollo, L. A. C., Liehr, T., et al. (2021). Adding new pieces to the puzzle of karyotype evolution in *Harttia* (Siluriformes, Loricariidae): Investigation of Amazonian species. *Biology* 10 (9), 922. doi:10.3390/biology10090922

Sassi, F. M. C., Toma, G. A., and Cioffi, M. B. (2023). "FISH—In fish chromosomes," in *Cytogenetics and molecular cytogenetics*. Editor T. Liehr (Boca Raton, FL: CRC Press), 281–296.

Saunders, P. A., Neuenschwander, S., and Perrin, N. (2018). Sex chromosome turnovers and genetic drift: A simulation study. *J. Evol. Biol.* 31 (9), 1413–1419. doi:10.1111/jeb.13336

Scavone, M. D. P., and Júlio, H. F., Jr (1994). Cytogenetic analysis and probable supernumerary chromosomes of *Loricaria prolixa* and *Loricaria* sp. females (Loricariidae-Siluriformes) from the Paraná River basin. *Rev. Ictiol.* 2 (3), 41–47.

Schartl, M., Georges, A., and Graves, J. A. M. (2023). Polygenic sex determination in vertebrates–is there any such thing? *Trends Genet.* 39, 242–250. doi:10.1016/j.tig.2022.12.002

Schubert, I., and Lysak, M. A. (2011). Interpretation of karyotype evolution should consider chromosome structural constraints. *Trends Genet.* 27 (6), 207–216. doi:10.1016/j.tig.2011.03.004

Sember, A., Nguyen, P., Perez, M. F., Altmanová, M., Ráb, P., and Cioffi, M. B. (2021). Multiple sex chromosomes in teleost fishes from a cytogenetic perspective: State of the art and future challenges. *Phil. Trans. R. Soc. B Biol. Sci.* 376 (1833), 20200098. doi:10.1098/rstb.2020.0098

Shen, Z. G., and Wang, H. P. (2018). "Environmental sex determination and sex differentiation in teleosts–how sex is established," in *Sex control in aquaculture*. Editors H-P. Wang, F. Piferrer, S-L. Chen, and Z-G. Shen (Hoboken, NJ: John Wiley & Sons), 85–115.

Štundlová, J., Hospodářská, M., Lukšíková, K., Voleníková, A., Pavlica, T., Altmanová, M., et al. (2022). Sex chromosome differentiation via changes in the Y chromosome repeat landscape in African annual killifishes *Nothobranchius furzeri* and *N. kadleci*. *Chromosome Res.* 30 (4), 309–333. doi:10.1007/s10577-022-09707-3

Takagui, F. H., Baumgärtner, L., Venturelli, N. B., Paiz, L. M., Viana, P., Pompeo, L. R. S., et al. (2020). Unrevealing the karyotypic evolution and cytotaxonomy of armored catfishes (Loricariinae) with emphasis in *Sturisoma, Loricariichthys, Loricaria, Proloricaria, Pyxiloricaria*, and *Rineloricaria*. *Zebrafish* 17, 319–332. doi:10.1089/zeb.2020.1893

Takagui, F. H., Rubert, M., Dionisio, J. F., Baumgärtner, L., Cardoso, Y. P., Jerep, F. C., et al. (2023). Cytogenetic markers reinforce the redescription of the armored pleco *Hypostomus spiniger* (Loricariidae-Hypostominae), an endemic species in the Uruguay River basin and Patos Lagoon system. *Braz. Arch. Biol. Tech.* 66, e23220154. doi:10.1590/1678-4324-2023220154

Veller, C., Muralidhar, P., Constable, G. W., and Nowak, M. A. (2017). Drift-induced selection between male and female heterogamety. *Genetics* 207 (2), 711–727. doi:10.1534/genetics.117.300151

Yang, F., and Graphodatsky, A. S. (2009). "Animal probes and ZOO-FISH," in *Fluorescence in situ hybridization (FISH)—application guide*. Editor T. Liehr (Berlin: Springer), 323–346.

Yang, F., Trifonov, V., Ng, B. L., Kosyakova, N., and Carter, N. P. (2009). "Generation of paint probes by flow-sorted and microdissected chromosomes," in *Fluorescence in situ hybridization (FISH)—application guide*. Editor T. Liehr (Berlin: Springer).

V – Sassi *et al.* (2023b) Homeology of sex chromosomes in Amazonian *Harttia* armored catfishes supports the X-fission hypothesis for the X_1X_2Y sex chromosome system origin. Scientific Reports 13:15756.

scientific reports

Check for updates

OPEN

Homeology of sex chromosomes in Amazonian *Harttia* armored catfishes supports the X-fission hypothesis for the X_1X_2Y sex chromosome system origin

Francisco de Menezes Cavalcante Sassi[1], Alexandr Sember[2], Geize Aparecida Deon[1], Thomas Liehr[3]✉, Niklas Padutsch[3], Osvaldo Takeshi Oyakawa[4], Marcelo Ricardo Vicari[5], Luiz Antonio Carlos Bertollo[1], Orlando Moreira-Filho[1] & Marcelo de Bello Cioffi[1]

The Neotropical monophyletic catfish genus *Harttia* represents an excellent model to study karyotype and sex chromosome evolution in teleosts. Its species split into three phylogenetic clades distributed along the Brazilian territory and they differ widely in karyotype traits, including the presence of standard or multiple sex chromosome systems in some members. Here, we investigate the chromosomal rearrangements and associated synteny blocks involved in the origin of a multiple X_1X_2Y sex chromosome system present in three out of six sampled Amazonian-clade species. Using 5S and 18S ribosomal DNA fluorescence in situ hybridization and whole chromosome painting with probes corresponding to X_1 and X_2 chromosomes of X_1X_2Y system from *H. punctata*, we confirm previous assumptions that X_1X_2Y sex chromosome systems of *H. punctata*, *H. duriventris* and *H. villasboas* represent the same linkage groups which also form the putative XY sex chromosomes of *H. rondoni*. The shared homeology between X_1X_2Y sex chromosomes suggests they might have originated once in the common ancestor of these closely related species. A joint arrangement of mapped *H. punctata* X_1 and X_2 sex chromosomes in early diverging species of different *Harttia* clades suggests that the X_1X_2Y sex chromosome system may have formed through an X chromosome fission rather than previously proposed Y-autosome fusion.

Teleost fishes display an astounding variety of sex determination systems, involving genetic and environmental mechanisms or a combination thereof[1–3]. These mechanisms may largely differ among fish lineages and even among populations of the same species[4–7]. Particularly genetic sex determination is in fishes mainly governed by one of the nine presently known sex chromosome systems which evolved independently multiple times in different lineages[6–9] and carry different sex-determining genes[2,10]. The majority of these systems show little genetic differentiation[8,11,12] and are therefore prone to frequent sex chromosome turnovers[7,13–15]. These properties make teleost sex chromosomes a well-suited model for studying early phases of sex chromosome differentiation[16,17] and causes and consequences of sex chromosome turnovers (whereby the newly evolved system replaces the former one)[15,18,19], and the bearing of sex chromosome evolution to the establishment of reproductive barriers between incipient species[20–22].

The study of sex chromosomes has undergone a remarkable transformation in recent years as genome sequencing, assembly and scaffolding techniques rapidly improved. Despite these advances, several unique biological features of sex chromosomes are still hardly tractable by computational tools, or their analysis requires multiple integrated methodologies and/or large number of individuals to be analyzed[18,23]. Cytogenetics and

[1]Laboratório de Citogenética de Peixes, Departamento de Genética e Evolução, Universidade Federal de São Carlos, São Carlos, SP 13565-905, Brazil. [2]Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Rumburská, 89, Liběchov, Czech Republic. [3]Institut für Humangenetik, Universitätsklinikum Jena, 07747 Jena, Germany. [4]Museu de Zoologia, Universidade de São Paulo, São Paulo, SP 04263-000, Brazil. [5]Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil. ✉email: thomas.liehr@med-uni.jena.de

particularly the use of whole chromosome painting (WCP) probes enables comparative study among multiple (closely) related species, and it can be narrowed down specifically to linkage group(s) representing sex chromosomes[8,24]. This approach enables to determine whether sex chromosomes originated independently from different linkage groups or are formed by the same synteny blocks. The latter situation points either on a single shared origin of sex chromosomes or repeated and independent co-option of the same synteny blocks for the sex-determining role[8–10]. The use of cytogenetics may also avoid misinterpretations related to accidental involvement of sex-reversed individuals or the intra-specific variability in the sex-determining systems[8,24].

The Neotropical armored catfish genus *Harttia* (Siluriformes, Loricariidae, Loricariinae) presently harbors 28 valid species[25–27] together with three *Harttia* spp. determined based on cytogenetic features but waiting for a proper taxonomic description[28]. After *Rineloricaria* (reviewed in[29]), *Harttia* displays the second-largest variation in diploid chromosome number (2n) among Loricariidae fishes, ranging from 2n = 52♀/53♂ in *H. carvalhoi*[30], to 2n = 62♀♂ in *H. absaberi*[31] and *Harttia* sp. 2[32]. Furthermore, the following three male-heterogametic sex chromosome systems have been identified in a subset of surveyed species: XX/XY_1Y_2 in *H. carvalhoi*, *H. intermontana*, and *Harttia* sp. 1[30,32,33]; $X_1X_1X_2X_2/X_1X_2Y$ in *H. duriventris*, *H. punctata*, and *H. villasboas*[34,35], and a putative XX/XY in *H. rondoni*[35]. Together with African *Nothobranchius* killifishes[36], *Harttia* represents a genus with the highest incidence of multiple sex chromosomes among teleosts to date[8,32]. It is therefore highly informative lineage for investigating underlying evolutionary forces that drive transitions from standard sex chromosomes (or other forms of sex determination) to multiple sex chromosome systems.

The most updated phylogeny of Loricariinae[37], though not including all valid species, recognized the monophyly of *Harttia*, with the occurrence of three distinct evolutionary lineages: Clade I is composed of the species from Guyanese shield, Clade II includes the species from the Amazonian and Tocantins-Araguaia river basins, and Clade III harbors the species from the southern/southeastern Brazil. When complemented with a species set from a former phylogenetic study[38] it is clear that *Harttia* species with known sex chromosomes, though nested within species lacking them based solely on a cytogenetic evidence, are grouped according to the type of their sex chromosome systems: those with an $X_1X_1X_2X_2/X_1X_2Y$ system or tentative XX/XY sex chromosomes are placed in the clade II, and those with an XX/XY_1Y_2 system in the clade III[28,32].

In our former studies, we demonstrated by WCP probes used in cross-species experiments (Zoo-FISH) that X_1X_2Y and XY_1Y_2 sex chromosome systems represent different linkage groups and therefore evolved independently[39–41]. While we also revealed by the same method the full or partial homeology between XY_1Y_2 systems among the three *Harttia* spp.[39,41], similar information is lacking for XY and X_1X_2Y systems as yet. Indirect evidence based on ribosomal DNA (rDNA) physical mapping and comparative genomic hybridization (CGH) suggested that these systems might be potentially homeologous[35]. In this study, we aim to investigate the mechanism(s) of origin and the relationships between the sex chromosome systems in *Harttia* species belonging to the Amazonian clades I and II where three members are known to carry an X_1X_2Y sex chromosome system and a single species possesses putative XY sex chromosomes. We therefore probed altogether six related *Harttia* species with WCP probes derived from the X_1 and X_2 sex chromosomes of the X_1X_2Y system in *H. punctata* thus complementing our former study[40]. The analysis in the present study was complemented by mapping of rDNA clusters as these usually locate on *Harttia* sex chromosomes[34,35,42]. Our data show homeology between X_1X_2Y sex chromosome systems and also the putative XY sex chromosome system of *H. rondoni*. Among the two formerly proposed hypotheses on X_1X_2Y sex chromosome origin i.e. the Y-autosome fusion[34,40] and X fission[35], our results support the latter scenario.

Results

Cross-hybridization with HPU-X_1 and HPU-X_2 painting probes revealed full homeology between X_1X_2Y sex chromosome systems of *H. punctata* (analyzed by us formerly[40]), *H. duriventris* and *H. villasboas* (Fig. 1a,c). In *H. duriventris* and *H. villasboas* the HPU-X_1 probe entirely hybridized to X_2 chromosome and conversely, HPU-X_2 probe painted X_1 chromosome when following the nomenclature by Sassi et al.[35]. As the location of sex-determining region has not been identified yet, the assignation of X_1 (ancestral) and X_2 (neo) sex chromosomes in different species was done arbitrarily in former studies[34,35]. Hence, to avoid confusion in designation of demonstrably the same synteny blocks, we unified the nomenclature of sex chromosomes according to X_1X_2Y system of *H. punctata*[34,40].

The painting probes also labelled different portions of putative XY sex chromosome in *H. rondoni* (Fig. 1b) whose identity was confirmed by 18S rDNA mapping (see below). While the HPU-X_1 probe painted the long (q) arms of these supposed X and Y chromosomes, the HPU-X_2 probe painted short (p) arms of them both. Remarkably, a (peri)centric region of both chromosomes was left unstained by these probes. For the species without cytologically distinguishable sex chromosomes—*H. dissidens H. guianensis*, and *Harttia* sp. 3 (Fig. 1d–f) both painting probes, again, hybridized to a single metacentric chromosome pair. In *H. dissidens* and *H. guianensis* the hybridization pattern was the same as in *H. rondoni* (i.e. HPU-X_1 covered q-arms while HPU-X_2 stained p-arms of the fifth and fourth chromosome pair in *H. dissidens* and *H. guianensis*, respectively; compare Fig. 1d,e with Fig. 1b) but this time without the unstained region in (peri)centromeres. In *Harttia* sp. 3 (Fig. 1f) the painted chromosome corresponded to the 18S rDNA-bearing chromosome pair 1, with the HPU-X_1 probe hybridizing on its p-arms and the HPU-X_2 probe on its q-arms (i.e. the opposite scenario to the one found in *H. rondoni*, *H. dissidens* and *H. guianensis*). The (peri)centromeric region of this chromosome pair was left unstained by the painting probes.

18S rDNA signals were placed in the pericentromeric regions of X_2 and Y chromosomes of *H. villasboas* and *H. duriventris* (Supplementary Fig. 1). These clusters also occupied (peri)centromeric positions in putative XY sex chromosomes in *H. rondoni*. Corroborating the previous study[35], 18S rDNA cluster showed size heteromorphism with the X-linked site being notably extended compared to the Y-linked one. 18S rDNA probe further

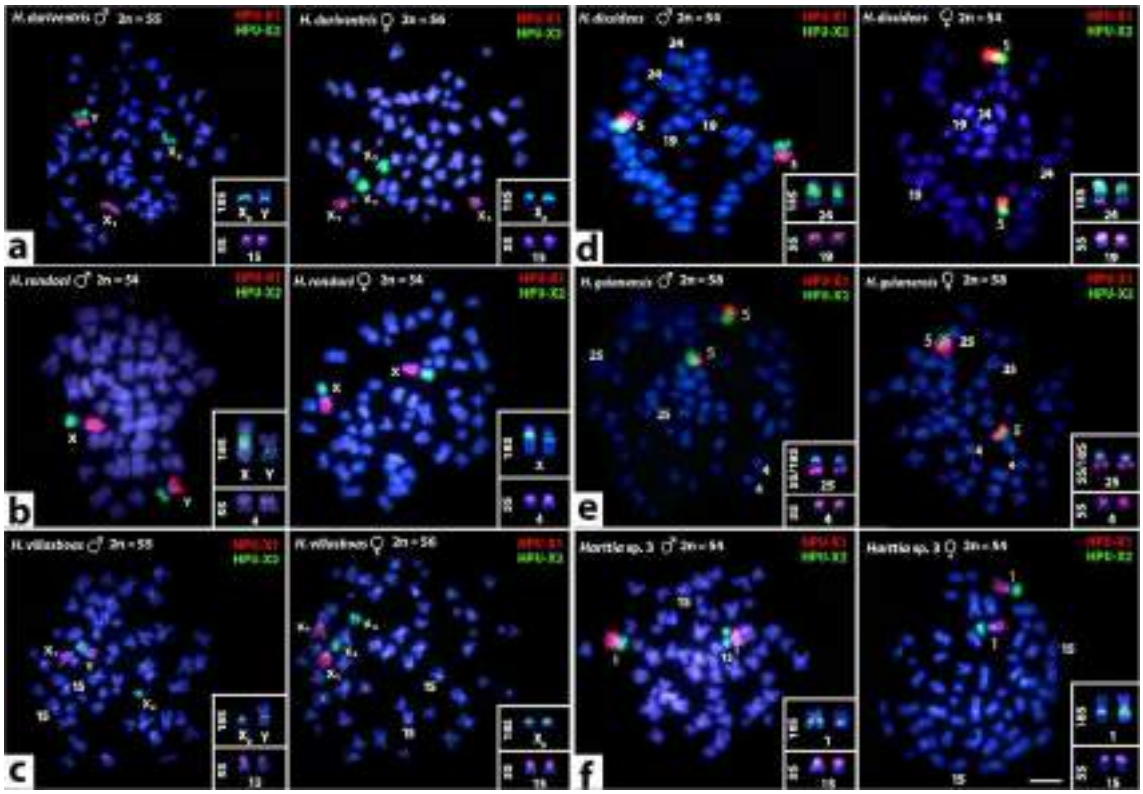


Figure 1. Zoo-FISH with HPU-X_1 and HPU-X_2 painting probes in male (first and third column) and female (second and fourth column) mitotic metaphases of *Harttia duriventris* (**a**), *H. dissidens* (**d**), *H. rondoni* (**b**), *H. guianensis* (**e**) *H. villasboas* (**c**), and *Harttia* sp. 3 (**f**). Chromosomes bearing 5S (red) and 18S (green) rDNA clusters as revealed after reprobing in the second FISH round are highlighted in boxes. The assignment of signals to specific chromosome pairs was performed based on data in our previous studies[28,35]. Full metaphase images are provided in Supplementary Fig. 1. Chromosomes were counterstained with DAPI (blue). Bar 10 µm.

co-localized with the painting probes only in *Harttia* sp. 3 where it revealed a site on both homologs positioned on q-arms closely downstream of the centromere. Finally, in *H. dissidens* and *H. guianensis*, we observed a single pair of 18S rDNA-bearing chromosomes (pair 24 and 25, respectively, according to Sassi et al.[28]), with the centromere-proximal signal on the q-arms. In *H. guianensis*, the 18S rDNA-bearing chromosome pair also bore 5S rDNA cluster at the terminal portion of q-arms. Second pair of 5S rDNA signals in this species was located interstitially on the p-arms of metacentric chromosome pair 4. The same site was present also on chromosome pair 4 with the same morphology in *H. rondoni* where no additional 5S rDNA signals were detected. *H. villasboas*, *H. duriventris* and *Harttia* sp. 3 exhibited a single chromosome pair (no. 15) bearing 5S rDNA arrays on its p-arms. The same pattern but on chromosome pair 19 was found in *H. dissidens*.

All hybridization patterns are summarized in an ideogram (Fig. 2). Full metaphase images with rDNA hybridization patterns are provided in the Supplementary Fig. 1.

Discussion

We have shown herein by Zoo-FISH that *Harttia* species with X_1X_2Y sex chromosome system entirely share the synteny blocks by which these sex chromosomes are formed. Our findings also corroborate the existence of previously proposed[35] XY sex chromosome system in *H. rondoni*, as also these chromosomes were stained by the same sex chromosome-derived painting probes.

Since the identification of the multiple XX/XY_1Y_2 sex chromosome system in *H. carvalhoi*[30], *Harttia* catfish genus became an excellent model for studying sex chromosome evolution in Neotropical fishes, with three species having the X_1X_2Y sex chromosome system, other three the XY_1Y_2 one, and yet one another representative featuring tentative XY sex chromosomes. A series of cytogenetic studies relying mostly on repetitive DNA mapping, CGH and Zoo-FISH experiments provided already evidence that XY_1Y_2 systems found in *H. carvalhoi*, *H. intermontana* and *Harttia* sp. 1 are fully or partially homeologous among each other but are non-homologous to the X_1X_2Y system of *H. punctata*[28,32,34,35,39–42]. Homeology between X_1X_2Y sex chromosomes in *H. punctata*, *H. duriventris* and *H. villasboas*, and their close relationship to putative XY sex chromosomes in *H. rondoni* were previously proposed based on the shared presence of 18S rDNA clusters and CGH patterns[35].

Blanco et al.[34] initially hypothesized that the X_1X_2Y sex chromosomes in *H. punctata* originated from a Robertsonian translocation between the two acrocentic chromosomes—an ancestral Y and an autosome. This rearrangement would be accompanied by the loss of 18S rDNA arrays from the emerging neo-Y chromosome. Nonetheless, the chromosome painting data from the work by Deon et al.[39] and our present study, once anchored

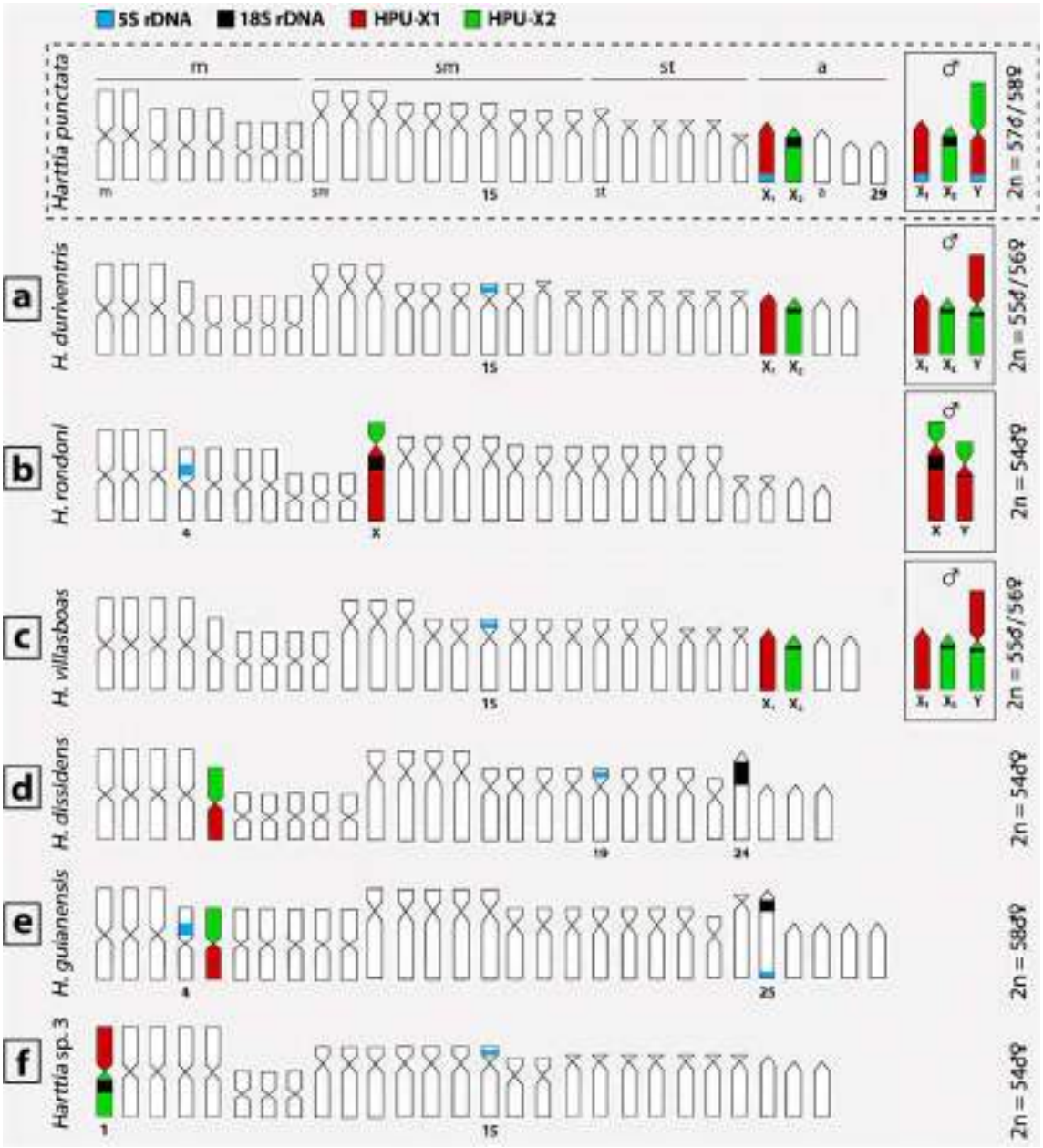


Figure 2. Schematic representation of hybridization results on chromosomes of studied *Harttia* species. For *Harttia punctata* the hybridization pattern was adopted from our previous study utilizing the same probes[40]. Letters correspond to those on Fig. 1. The assignment of signals to specific chromosome pairs, as well as the arrangement of chromosomes into a karyotype, was performed based on data in our previous studies[28,35].

to the current phylogenetic analysis ([37]Fig. 3A, [38]Fig. 3B), clearly show that the linkage groups representing X_1 and X_2 chromosomes were ancestrally forming arms of the same chromosome. Besides the early diverging *H. guianensis*, this pattern has been found also in other *Harttia* lineages ([39]Fig. 3). This means that more probably the X_1X_2Y system emerged after a centric fission in the ancestral X chromosome.

When a fission event creates an X_1X_2Y multiple sex chromosome system, a closely related species carrying the ancestral XY sex chromosomes is expected to have a lower 2n in the karyotype[43]. This is what can be inferred from the comparison of *H. rondoni* with 2n = 54 (XY/XX) and the species with multiple X_1X_2Y/♀$X_1X_1X_2X_2$ sex chromosomes: *H. punctata* with (2n = 57♂/58♀), *H. villasboas* and *H. duriventris* (2n = 55♂/56♀).

CGH analysis has formerly shown that a probable region of differentiation on the Y chromosome might be located proximally to the centromere[28]. Indeed, the centromeric regions have been widely shown to suppress recombination[44] and therefore are thought to be suitable regions for establishment of a new sex-determining region[17]. It is further intriguing that *H. villasboas* and *H. duriventris* share the presence of centromere-proximal 18S rDNA site on their Y chromosomes and that both XY chromosomes in *H. rondoni* share a pericentromeric 18S rDNA cluster being consistently larger on the X chromosome ([35]this study). It is tempting to hypothesize that the ancestral situation would be close to the scenario found in *H. rondoni* and the X-linked amplified 18S

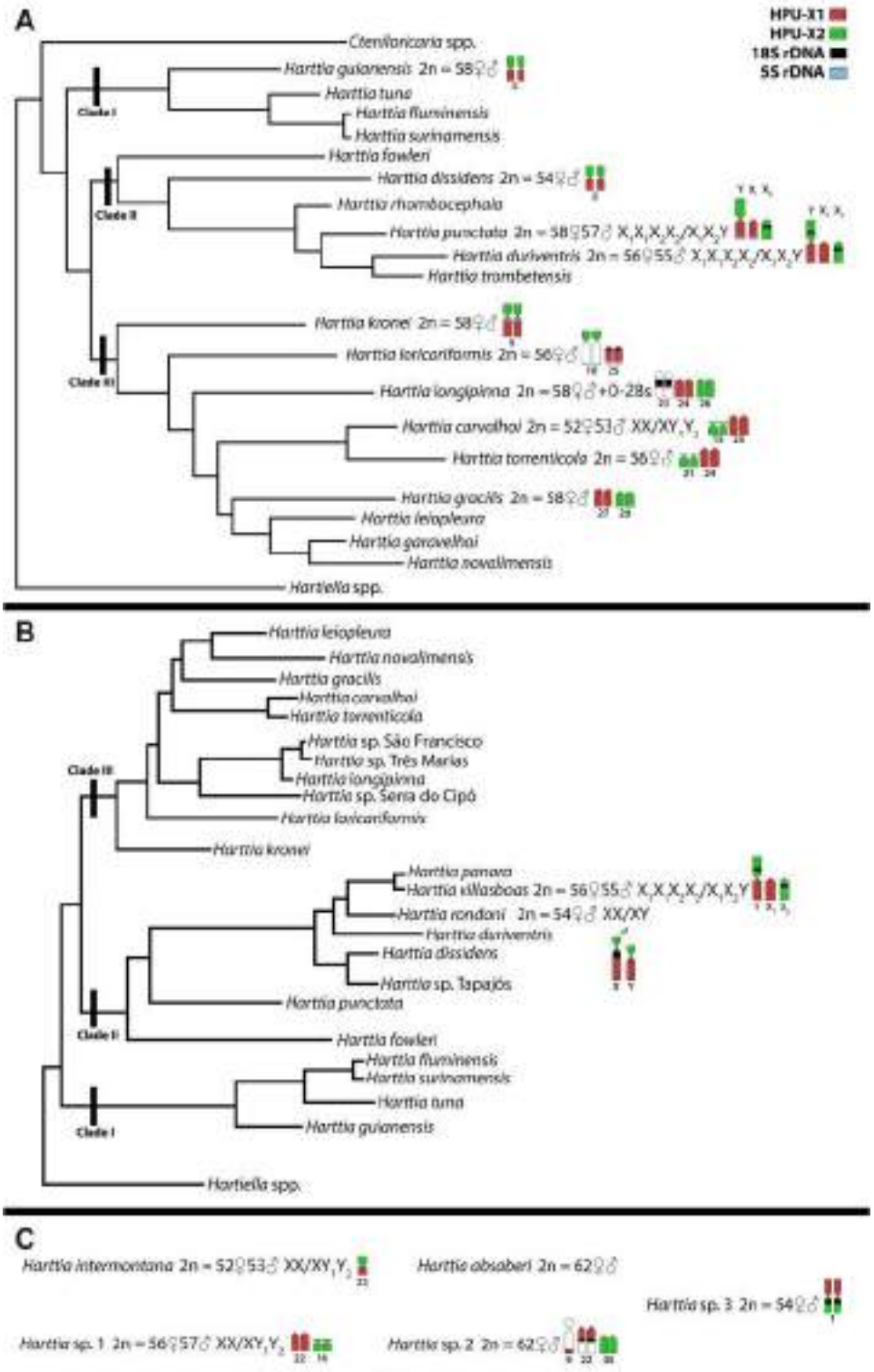


Figure 3. Phylogenetic relationships among Hartiini fishes based on morphological and molecular data along with anchored cytogenetic characteristics. Phylogeny follows[37] (**a**), and[38] (**b**), while (**c**) presents the chromosomal data from species that were not included in the respective phylogenetic reconstructions. Indicated cytogenetic characteristics: 2n; sex chromosome systems; partial ideograms represent the organization of mapped synteny blocks as revealed by the sex chromosome-derived painting probes HPU-X_1 (red) and HPU-X_2 (green); 5S rDNA (blue), and 18S rDNA (black) sites. The assignment of signals to specific chromosome pairs was performed based on data in our previous studies[28,32,35,40]. More specifically, the cytogenetic data synthesis has been undertaken as follows: *H. guianensis*, *H. dissidens* and *Harttia* sp. 3 ([28]this study); *H. punctata*, *H. kronei*, *H. loricariformis*, *H. longipinna*, *H. carvalhoi*, *H. torrenticola* and *H. gracilis*[40]; *H. duriventris*, *H. villasboas* and *H. rondoni* ([35]this study); *H. intermontana*, *Harttia* sp. 1 and *Harttia* sp. 2[32,40]; *H. absaberi*[31].

rDNA region might cause instability around the centromere, leading eventually to the fission, while selection would counteract similar fission to happen on the Y chromosome, to preserve the linkage disequilibrium in/around the sex-determining region.

rDNA clusters have been abundantly shown to cause chromosomal instability due to heavy transcription and organization into long tandem arrays[45]. More specifically, the following conditions collectively provide ample opportunities for DNA damage to happen: (1) highly decondensed DNA, (2) exposure of non-templated DNA strand and its tendency to form various secondary structures and (3) increased probability of collision between transcription and replication machineries. Consequent DNA repair may accidentally lead to rearrangements[46–48]. In the frame of the X-fission scenario, loss of rDNA sequences is among the possible consequences of double-stranded breaks in these tandem repeats[49]. Moreover, fission itself may lead to a partial degradation of exposed chromosomal ends until new telomeres are being established[50]. rDNA dynamics in *Harttia* is further evidenced by a complete loss of rDNA sites on certain linkage groups and their emergence on another chromosome pairs ([40]this study—see Fig. 2). Notably, besides *Harttia* spp.[32,40], rDNA sites operated as breakpoint regions independently in many fish groups[51–55].

While *H. rondoni* has not been involved in the current phylogenetic analysis[37], former work[38] proposed the phylogenetic position of this species being nested within the species carrying X_1X_2Y sex chromosomes, which might either mean that (1) ancestral XY system has been preserved in *H. rondoni* while X_1X_2Y system evolved repeatedly in separate evolutionary events in the closely related species or (2) there was a single origin of X_1X_2Y system and X_1 and X_2 fused secondarily back again in *H. rondoni* creating a neo-XY system, or (3) the phylogenetic relationships of *H. rondoni* with closely related species are not interpreted correctly by Covain et al.[38]. Regarding the last point, although geographical distribution and morphological characters reinforce the Covain's proposition[25], further phylogenetic studies involving the species in question are necessary to untangle this issue. Noteworthily, if X_1X_2Y sex chromosomes evolved multiple times independently in this *Harttia* lineage, then the repeated fusion of ancestral Y with always the same autosome is rather improbable[9,56–58], which, again, reinforces the X-fission hypothesis.

The origin of multiple X_1X_2Y sex chromosome system in the three *Harttia* species seems not to follow the common evolutionary pathway. A centric or tandem fusion of the original Y chromosome with an autosome has been proposed (and in several cases empirically confirmed) as an underlying mechanism leading to emergence of X_1X_2Y sex chromosomes in the remaining 62 teleosts cases reported to date[8,55,59]. It is also the commonest mechanism of multiple sex chromosome creation in other cold-blooded vertebrates[60,61]. Sex chromosome fissions are much less common and in teleosts they have been proposed thus far only in five cases (four times as Y-fission and once as W-fission; reviewed in[8]). Another tentative W-fission might have taken place in *Ancistrus clementinae*[62]. Finally, a Y-fission has been proposed also for the XY_1Y_2 system in *H. carvalhoi*[33], however, a more recent study[32] suggested X-autosome fusion to be responsible instead, which has been further reinforced by Zoo-FISH showing that a probable ancestral X chromosome fused with two different autosomes within the set of three XY_1Y_2-bearing *Harttia* species[39].

Fissions are generally hard to track in the species' karyotypes as they generate less noticeable products compared to large chromosomes derived from fusions[50,63]. Hence, unfortunately, our understanding of the genomic properties of fission sites and the etiology of this rearrangement type are still rather limited, despite the steadily growing number of sequenced genomes in non-model organisms. Studies combining chromosomal painting with specific sex-chromosome probes and other cytogenetic markers, such as the distribution of repetitive DNAs, are useful to indicate major fission events, as already proven in fishes[64,65], lizards[56,66], and birds[67]. Such an approach is desirable as fissions have been associated with chromosomal evolution and speciation events in several metazoans[68–70] and they have been also, for instance, associated with the evolution of the olfactory system in carnivores[71].

While sex chromosome-autosome fusions have been much more explored regarding their possible effects on reproductive isolation, adaptation and radiation[8,20,57,60] fissions might have a similar effect[72]. In the case of *Harttia* species, given that these fishes form rather small, fragmented populations with restricted/absent gene flow, multiple sex chromosomes might have emerged and get fixed rather under the major effect of genetic drift[73] which highly likely applies also to several other Neotropical fishes with multiple sex chromosomes[55,65,74]. The possible contribution of natural selection in this process will require further studies particularly oriented towards a detailed characterization of genetic content of sex-determining regions and neighboring chromosomal areas.

Conclusion

The chromosomal evolution in *Harttia* species has been shaped by numerous inter-chromosomal rearrangements giving rise to a complex karyotype variability, including the emergence of different male-heterogametic sex chromosome systems. In this study, we demonstrated that the X_1X_2Y and XY sex chromosomes, as well as some autosomes, share several homologies among *Harttia* species. This strengthens our previously proposed theory that the X_1X_2Y system emerged after a centric fission in the ancestral X chromosome and thus represents a derivation of the ancestral XY sex chromosome system. Although the amount of chromosomal data has significantly increased for *Harttia* species during the recent years, the genus still lacks a robust phylogenetic reconstruction that would include all recognized species along with the other emerging but yet undescribed species whose existence has been proposed by their distinct cytogenetic features[28].

Methods

Sampling and chromosome preparation

Species were gathered from seven distinct localities (Fig. 4, Table 1), with the authorization of the Brazilian environmental agency ICMBIO/SISBIO (License 48628-14) and SISGEN (A96FF09). Mitotic chromosomes were

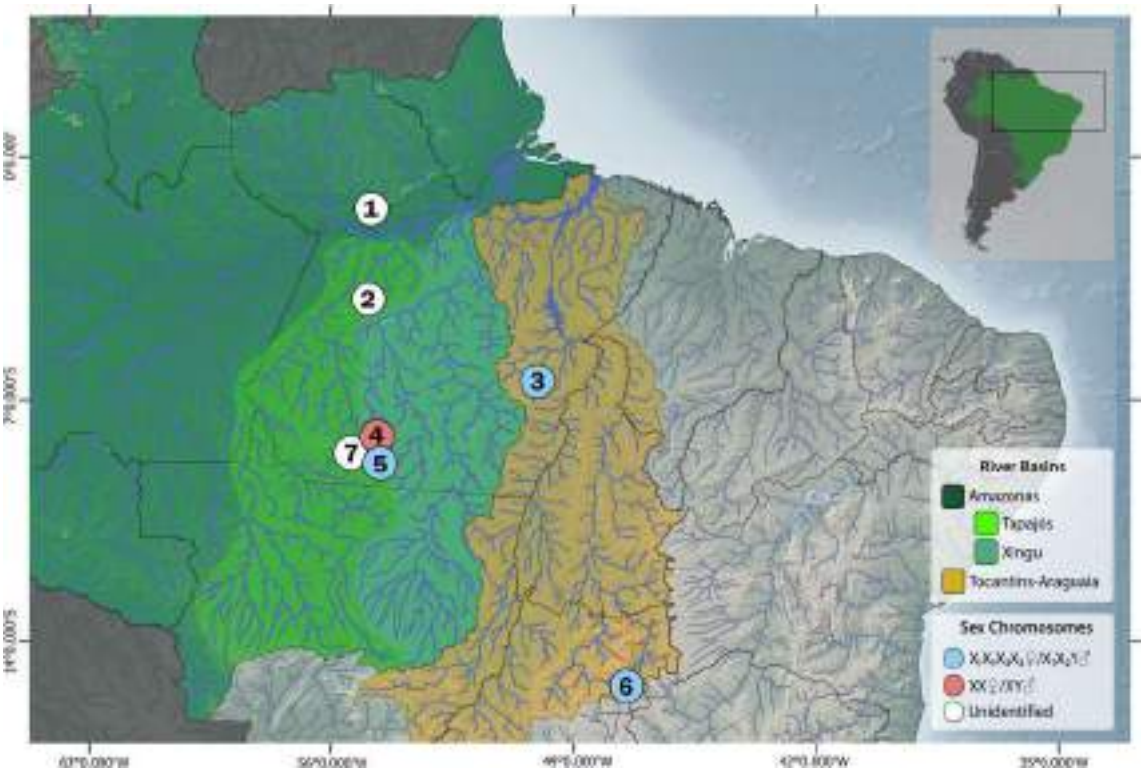


Figure 4. Partial map of Brazil highlighting the Amazonian (green) and Tocantins-Araguaia (orange) river basins. Circles correspond to sampling sites of *Harttia* species whose sex chromosome systems are indicated by specific colors. The color-coding system for river basins and sex chromosomes is presented in frames (bottom right). 1 = *H. guianensis*; 2 = *H. dissidens*; 3 = *H. duriventris*; 4 = *H. rondoni*; 5 = *H. villasboas*; 6 = *H. punctata* and 7 = *Harttia* sp. 3. The map was created with QGIS 3.22 with the package Natural Earth.

Species	Geographic Coordinates	2n	Karyotype composition	N	Voucher
2—*Harttia dissidens* **Clade II**	4° 5′ 37.8″ S 55° 0′ 30.2″ W	54♀♂	20 m + 26sm + 8a	07♀25♂	INPA-ICT 059577
3—*H. duriventris* **Clade II**	6° 30′ 06.5″ S 50° 02′ 35.5″ W	56♀55♂	16 m + 16sm + 16st + 8a♀ 17 m + 16sm + 16st + 6a♂	08♀07♂	MZUSP 126598
1—*H. guianensis* **Clade I**	1° 29′ 02.2″ S 54° 50′ 31.2″ W	58♀♂	20 m + 26sm + 2st + 10a	06♀10♂	INPA-ICT 059584
6—*H. punctata* **Clade II**	15° 19′ 25″ S 47° 25′ 26″ W	58♀57♂	16 m + 16sm + 16st + 8a♀ 17 m + 16sm + 16st + 6a♂	10♀12♂	MZUSP 111385
5—*H. villasboas* **Clade II**	8° 44′ 09″ S 54° 57′ 46″ W	56♀55♂	18 m + 24sm + 6st + 8a♀ 19 m + 24sm + 6st + 6a♂	34♀38♂	MZUSP 126599
4—*H. rondoni* **Clade II**	8° 38′ 53″ S 55° 01′ 41″ W	54♀♂	20 m + 26sm + 4st + 4a	15♀14♂	MZUSP 127606
7—*Harttia* sp. 3	08° 39′ 20.7″ S 55° 09′ 24.1″ W	54♀♂	16 m + 18sm + 14st + 6a	11♀15♂	MZUSP 127605

Table 1. Geographic coordinates, diploid chromosome numbers (2n), karyotype composition, sample sizes (**N**), and voucher numbers of the sampled species. *Harttia* sp. 3 is still waiting for a proper taxonomic identification. Numbers that precede the species names correspond to those in Fig. 1. The species were assigned to their phylogenetic clades following[37].

obtained by the classic air-drying method[75], using the anterior kidney cells as the main source material, being occasionally supplemented with the cells of the spleen tissue. All procedures followed the ethical and anesthesia conducts approved by the Ethics Committee on Animal Experimentation of the Universidade Federal de São Carlos (Process number CEUA 1853260315). The authors complied with ARRIVE guidelines. Specimens were fixed in 10% formalin and deposited in the fish collections of the Instituto Nacional de Pesquisa da Amazônia (INPA-ICT) and Museu de Zoologia da Universidade de São Paulo (MZUSP). Their voucher numbers are provided in Table 1.

Probe preparation for Zoo-FISH and rDNA FISH

Sex chromosomes of *H. punctata* were selected to be used as probes since this species exhibits the closest 2n relative to the proposed ancestral state for the genus and, at the same time, it possesses a multiple sex chromosome system of the $X_1X_1X_2X_2/X_1X_2Y$ type[28,32,34,35,42]. Fifteen copies of the X_1 and X_2 chromosomes were isolated by glass-needle-based microdissection under an inverted microscope (Zeiss Axiovert 135). The collected DNA material was then amplified in a primary degenerated oligonucleotide-primed polymerase chain reaction (DOP-PCR)[76]. The probes were then labeled in the secondary DOP-PCR reaction using 1 µL of the initial amplified product as a template DNA[77]. The probe derived from the X_1 chromosome (HPU-X_1) was labeled with Spectrum Orange-dUTP (red), and the one derived from the X_2 chromosome (HPU-X_2) with Spectrum Green-dUTP (green) (Vysis, Downers Grove, United States).

The 5S and 18S rDNA fragments were obtained by PCR from the wolf fish *Hoplias malabaricus* genome using primers and thermal profiles described in previous studies[78–80]. The labelling was done by nick translation using Atto550-dUTP (red) for the 5S rDNA and Alexa Fluor 488-dUTP (green) for the 18S rDNA (both Jena Biosciences, Jena, Germany), according to manufacturer's protocol.

FISH experiments

Zoo-FISH followed the protocol described by Sassi et al.[81]. C_0t-1 DNA prepared from *H. punctata* male genome was used as a blocker to high-copy repeat sequences[82]. Slides with metaphase chromosomes of *H. dissidens*, *H. duriventris*, *H. guianensis*, *H. villasboas*, *H. rondoni* and *Harttia* sp. 3 were denatured in 70% formamide/2 × SSC at 72 °C for 3 min. For each assay, the hybridization solution (200 ng of HPU-X_1, 200 ng of HPU-X_2 and 20 µg of C_0t-1 DNA in 50% formamide + 2 × SSC + 10% dextran sulfate; final volume 20 µL) was denatured for 10 min at 85 °C, cooled at 4 °C for 2 min, and allowed to pre-anneal for 45 min at 37 °C in a thermocycler. Next, the probes were spotted onto the denatured slides, and the hybridization process took place in a dark moist chamber for 48 h at 37 °C. To remove unspecific hybridization signals, slides were washed twice with 1 × SSC at 65 °C (5 min each), and then in 4 × SSC/Tween (5 min) and 1 × PBS (1 min), at room temperature. After capturing the resulting images, the slides were washed for the second round of hybridization[80], in which 100 ng of each 5S and 18S rDNA probe was applied after being dissolved in the hybridization solution (50% formamide and 10% dextran sulfate in 2 × SSC), in the final volume 20 µL. The rDNA FISH experiments followed the same protocol as described above, except for the hybridization time which was 24 h. In all experiments, chromosomes were finally counterstained with VECTASHIELD® Antifade Mounting Medium with DAPI (4′,6-diamidino-2-phenylindole) (Vector Laboratories, California, United States).

Microscopy and image analysis

Hybridization patterns were verified in at least 30 metaphase spreads per experiment. Images were captured using an Olympus BX50 microscope (Olympus Corporation, Ishikawa, Japan), coupled with a CoolSNAP camera. Images were processed with Ikaros/ISIS (MetaSystems, Germany). The assignment of signals to specific chromosome pairs, as well as the arrangement of chromosomes into a karyotype, was performed based on data in our previous studies[28,35].

Ethical approval

Sample was approved by the Brazilian Environmental Agency ICMBIO/SISBIO (License 48628-14) and SISGEN (A96FF09). All experiments followed the guidelines and were approved by the Ethics Committee on Animal Experimentation of the Universidade Federal de São Carlos (Process number CEUA 1853260315 and 7994170423).

Data availability

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

Received: 14 April 2023; Accepted: 12 September 2023
Published online: 21 September 2023

References

1. Devlin, R. H. & Nagahama, Y. Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* **208**, 191–364 (2002).
2. Guiguen, Y., Fostier, A. & Herpin, A. Sex determination and differentiation in fish: Genetic, genomic, and endocrine aspects. In *Sex Control in Aquaculture* (eds Wang, H. P. *et al.*) 35–63 (Wiley, 2019). https://doi.org/10.1002/9781119127291.ch2.
3. Shen, Z. G. & Wang, H. P. Environmental sex determination and sex differentiation in teleosts—how sex is established. In *Sex Control in Aquaculture* (eds Wang, H. P. *et al.*) 85–115 (Wiley, 2019). https://doi.org/10.1002/9781119127291.ch4.
4. Wilson, C. A. *et al.* Wild sex in zebrafish: Loss of the natural sex determinant in domesticated strains. *Genetics* **198**, 1291–1308 (2014).
5. Yamamoto, Y., Zhang, Y., Sarida, M., Hattori, R. S. & Strüssmann, C. A. Coexistence of genotypic and temperature-dependent sex determination in pejerrey *Odontesthes bonariensis*. *PLoS One* **9**, e102574 (2014).
6. Myosho, T., Takehana, Y., Hamaguchi, S. & Sakaizumi, M. Turnover of sex chromosomes in *celebensis* group medaka fishes. *G3 (Bethesda)* **5**, 2685–2691 (2015).
7. El Taher, A., Ronco, F., Matschiner, M., Salzburger, W. & Böhne, A. Dynamics of sex chromosome evolution in a rapid radiation of cichlid fishes. *Sci. Adv.* **7**, eabe8215 (2021).
8. Sember, A. *et al.* Multiple sex chromosomes in teleost fishes from a cytogenetic perspective: State of the art and future challenges. *Philos. Trans. R. Soc. B* **376**, 20200098 (2021).

9. Jeffries, D. L., Mee, J. A. & Peichel, C. L. Identification of a candidate sex determination gene in *Culaea inconstans* suggests convergent recruitment of an *Amh* duplicate in two lineages of stickleback. *J. Evol. Biol.* **35**, 1683–1695 (2022).
10. Pan, Q. *et al.* Evolution of master sex determiners: TGF-β signalling pathways at regulatory crossroads. *Philos. Trans. R. Soc. B Biol. Sci.* **376**, 20200091 (2021).
11. Gamble, T. Using RAD-seq to recognize sex-specific markers and sex chromosome systems. *Mol. Ecol.* **25**, 2114–2116 (2016).
12. Schartl, M., Schmid, M. & Nanda, I. Dynamics of vertebrate sex chromosome evolution: From equal size to giants and dwarfs. *Chromosoma* **125**, 553–571 (2016).
13. Mank, J. E. & Avise, J. C. Evolutionary diversity and turn-over of sex determination in teleost fishes. *Sex. Dev.* **3**, 60–67 (2009).
14. Kabir, A. *et al.* Repeated translocation of a supergene underlying rapid sex chromosome turnover in *Takifugu* pufferfish. *Proc. Natl. Acad. Sci. USA* **A119**, e2121469119 (2022).
15. Vicoso, B. Molecular and evolutionary dynamics of animal sex-chromosome turnover. *Nat. Ecol. Evol.* **3**, 1632–1641 (2019).
16. Kamiya, T. *et al.* A Trans-species missense SNP in *Amhr2* is associated with sex determination in the tiger pufferfish, *Takifugu rubripes* (Fugu). *PLoS Genet.* **8**, e1002798 (2012).
17. Charlesworth, D. Young sex chromosomes in plants and animals. *New Phytol.* **224**, 1095–1107 (2019).
18. Palmer, D. H., Rogers, T. F., Dean, R. & Wright, A. E. How to identify sex chromosomes and their turnover. *Mol. Ecol.* **28**, 4709–4724 (2019).
19. Saunders, P. A. Sex chromosome turnovers in evolution. *eLS* **20**, 1–8. https://doi.org/10.1002/9780470015902.a0028747 (2019).
20. Kitano, J. *et al.* A role for a neo-sex chromosome in stickleback speciation. *Nature* **461**, 1079–1083 (2009).
21. O'Neill, M. J. & O'Neill, R. J. Sex chromosome repeats tip the balance towards speciation. *Mol. Ecol.* **27**, 3783–3798 (2018).
22. Payseur, B. A., Presgraves, D. C. & Filatov, D. A. Introduction: Sex chromosomes and speciation. *Mol. Ecol.* **27**, 3745–3748 (2018).
23. Carey, S. B. *et al.* Representing sex chromosomes in genome assemblies. *Cell Genom.* **2**, 100132 (2022).
24. Deakin, J. E. *et al.* Chromosomics: Bridging the gap between genomes and chromosomes. *Genes* **10**, 627 (2019).
25. Oyakawa, O. T., Fichberg, I. & Py-Daniel, L. R. Three new species of *Harttia* (Loricariidae: Loricariinae) from Serra do Cachimbo, Rio Xingu basin, Pará, Northern Brazil. *Zootaxa* **4387**, 75–90 (2018).
26. Caldas, L., Cherobim, A. M. & Langeani, F. A New species of *Harttia* from the rio São Francisco basin (Siluriformes: Loricariidae). *Neotrop. Ichthyol.* **20**, 25 (2022).
27. Fricke, R., Eschmeyer, W. N. & van der Laan, R. Eschmeyer's catalog of fishes: Genera, Species, References. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (2023).
28. Sassi, F. M. C. *et al.* Adding new pieces to the puzzle of karyotype evolution in *Harttia* (Siluriformes, Loricariidae): Investigation of Amazonian species. *Biology* **10**, 922 (2021).
29. Takagui, F. H. *et al.* Unrevealing the karyotypic evolution and cytotaxonomy of armored catfishes (Loricariinae) with emphasis in *Sturisoma*, *Loricariichthys*, *Loricaria*, *Proloricaria*, *Pyxiloricaria*, and *Rineloricaria*. *Zebrafish* **17**, 319–332 (2020).
30. Centofante, L., Bertollo, L. A. C. & Moreira-Filho, O. Cytogenetic characterization and description of an XX/XY_1Y_2 sex chromosome system in catfish *Harttia carvalhoi* (Siluriformes, Loricariidae). *Cytogenet. Genome Res.* **112**, 320–324 (2006).
31. Rodrigues, R. M. Estudos cromossômicos e moleculares em Loricariinae com ênfase em espécies de *Rineloricaria* (Siluriformes, Loricariidae): Uma perspectiva evolutiva (Ph.D. Thesis, Universidade de São Paulo, 2010).
32. Deon, G. A. *et al.* Highly rearranged karyotypes and multiple sex chromosome systems in armored catfishes from the genus *Harttia* (Teleostei, Siluriformes). *Genes* **11**, 1366 (2020).
33. Blanco, D. R. *et al.* The role of the Robertsonian rearrangements in the origin of the XX/XY_1Y_2 sex chromosome system and in the chromosomal differentiation in *Harttia* species (Siluriformes, Loricariidae). *Rev. Fish Biol. Fish.* **23**, 127–134 (2013).
34. Blanco, D. R. *et al.* Origin of the $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system of *Harttia punctata* (Siluriformes, Loricariidae) inferred from chromosome painting and FISH with ribosomal DNA markers. *Genetica* **142**, 119–126 (2014).
35. Sassi, F. M. C. *et al.* Multiple sex chromosomes and evolutionary relationships in amazonian catfishes: The outstanding model of the genus *Harttia* (Siluriformes: Loricariidae). *Genes* **11**, 1179 (2020).
36. Krysanov, E. & Demidova, T. Extensive karyotype variability of African fish genus *Nothobranchius* (Cyprinodontiformes). *Comp. Cytogenet.* **12**, 387 (2018).
37. Londoño-Burbano, A. & Reis, R. E. A combined molecular and morphological phylogeny of the Loricariinae (Siluriformes: Loricariidae), with emphasis on the Harttiini and Farlowellini. *PLoS One* **16**, e0247747 (2021).
38. Covain, R. *et al.* Molecular phylogeny of the highly diversified catfish subfamily Loricariinae (Siluriformes, Loricariidae) reveals incongruences with morphological classification. *Mol. Phylogenet. Evol.* **94**, 492–517 (2016).
39. Deon, G. A. *et al.* Chromosomal rearrangements and origin of the multiple XX/XY_1Y_2 sex chromosome system in *Harttia* species (Siluriformes: Loricariidae). *Front. Genet.* **13**, 877522 (2022).
40. Deon, G. A. *et al.* Evolutionary breakpoint regions and chromosomal remodeling in *Harttia* (Siluriformes: Loricariidae) species diversification. *Genet. Mol. Biol.* **45**, e20210170 (2022).
41. Sassi, F. M. C. *et al.* Turnover of multiple sex chromosomes in *Harttia* catfish (Siluriformes, Loricariidae): A glimpse from whole chromosome painting. *Front. Genet.* **14**, 1226222 (2023).
42. Blanco, D. R. *et al.* Karyotype diversity and evolutionary trends in armored catfish species of the genus *Harttia* (Siluriformes: Loricariidae). *Zebrafish* **14**, 169–176 (2017).
43. Kitano, J. & Peichel, C. L. Turnover of sex chromosomes and speciation in fishes. *Environ. Biol. Fish.* **94**, 549–558 (2012).
44. Nambiar, M. & Smith, G. R. Repression of harmful meiotic recombination in centromeric regions. *Mech. Cancer Cachexia* **54**, 188–197 (2016).
45. Schöfer, C. & Weipoltshammer, K. Nucleolus and chromatin. *Histochem. Cell Biol.* **150**, 209–225 (2018).
46. Potapova, T. A. & Gerton, J. L. Ribosomal DNA and the nucleolus in the context of genome organization. *Chromosome Res.* **27**, 109–127 (2019).
47. Warmerdam, D. O. & Wolthuis, R. M. F. Keeping ribosomal DNA intact: A repeating challenge. *Chromosome Res.* **27**, 57–72 (2019).
48. Goffová, I. & Fajkus, J. The rDNA loci—intersections of replication, transcription, and repair pathways. *Int. J. Mol. Sci.* **22**, 1302 (2021).
49. Warmerdam, D. O., van den Berg, J. & Medema, R. H. Breaks in the 45S rDNA lead to recombination-mediated loss of repeats. *Cell Rep.* **14**, 2519–2527 (2016).
50. Perry, J., Slater, H. R. & Choo, K. H. A. Centric fission—simple and complex mechanisms. *Chromosome Res.* **12**, 627–640 (2004).
51. Cioffi, M. B. & Bertollo, L. A. C. Initial steps in XY chromosome differentiation in *Hoplias malabaricus* and the origin of an X_1X_2Y sex chromosome system in this fish group. *Heredity* **105**, 554–561 (2010).
52. Barros, A. V. *et al.* Fragile sites, dysfunctional telomere and chromosome fusions: What is 5S rDNA role?. *Gene* **608**, 20–27 (2017).
53. Supiwong, W. *et al.* Karyotype diversity and evolutionary trends in the Asian swamp eel *Monopterus albus* (Synbranchiformes, Synbranchidae): A case of chromosomal speciation?. *BMC Evol. Biol.* **19**, 73 (2019).
54. Sember, A. *et al.* Centric fusions behind the karyotype evolution of Neotropical *Nannostomus* pencilfishes (Characiforme, Lebiasinidae): First insights from a molecular cytogenetic perspective. *Genes* **11**, 91 (2020).
55. Marajó, L. *et al.* Chromosomal rearrangements and the first indication of an $♀X_1X_1X_2X_2/♂X_1X_2Y$ sex chromosome system in *Rineloricaria* fishes (Teleostei: Siluriformes). *J. Fish Biol.* **102**, 443–454 (2023).
56. Giovannotti, M. *et al.* New insights into sex chromosome evolution in anole lizards (Reptilia, Dactyloidae). *Chromosoma* **126**, 245–260 (2017).

57. Carabajal Paladino, L. Z. *et al.* Sex chromosome turnover in moths of the diverse superfamily Gelechioidea. *Genome Biol. Evol.* **11**, 1307–1319 (2019).
58. Oliveira da Silva, W. *et al.* Identification of two independent X-autosome translocations in closely related mammalian (*Proechimys*) species. *Sci. Rep.* **9**, 4047 (2019).
59. Ferchaud, A.-L. *et al.* Chromosome-level assembly reveals a putative Y-autosomal fusion in the sex determination system of the Greenland Halibut (*Reinhardtius hippoglossoides*). *G3 (Bethesda)* **12**, jkab376 (2022).
60. Pennell, M. W. *et al.* Y fuse? Sex chromosome fusions in fishes and reptiles. *PLoS Genet.* **11**, e1005237 (2015).
61. Ma, W. J. & Veltsos, P. The diversity and evolution of sex chromosomes in frogs. *Genes* **12**, 483 (2021).
62. Nirchio, M. *et al.* Occurrence of sex chromosomes in fish of the genus *Ancistrus* with a new description of multiple sex chromosomes in the Ecuadorian endemic *Ancistrus clementinae* (Loricariidae). *Genes* **14**, 306 (2023).
63. Imai, H. T., Satta, Y. & Takahata, N. Integrative study on chromosome evolution of mammals, ants and wasps based on the minimum interaction theory. *J. Theor. Biol.* **210**, 475–497 (2001).
64. Schemberger, M. O. *et al.* Differentiation of repetitive DNA sites and sex chromosome systems reveal closely related group in Parodontidae (Actinopterygii: Characiformes). *Genetica* **139**, 1499–1508 (2011).
65. de Oliveira, E. A. *et al.* Tracking the evolutionary pathway of sex chromosomes among fishes: Characterizing the unique XX/XY_1Y_2 system in *Hoplias malabaricus* (Teleostei, Characiformes). *Chromosoma* **127**, 115–128 (2018).
66. Gladkikh, O. L. *et al.* Rapid karyotype evolution in *Lasiopodomys* involved at least two autosome – sex chromosome translocations.*PLoS ONE.* **11**, e0167653 (2016)
67. Kretschmer, R. *et al.* Extensive chromosomal fissions and repetitive DNA accumulation shaped the atypical karyotypes of two Ramphastidae (Aves: Piciformes) species. *Biol. J. Linn. Soc. Lond.* **130**, 839–849 (2020).
68. Voss, S. R. *et al.* Origin of amphibian and avian chromosomes by fission, fusion, and retention of ancestral chromosomes. *Genome Res.* **21**, 1306–1312 (2011).
69. de Vos, J. M., Augustijnen, H., Bätscher, L. & Lucek, K. Speciation through chromosomal fusion and fission in Lepidoptera. *Philos. Trans. R. Soc. B Biol. Sci.* **375**, 20190539 (2020).
70. Huang, Z. *et al.* Recurrent chromosome reshuffling and the evolution of neo-sex chromosomes in parrots. *Nat. Commun.* **13**, 944 (2022).
71. Fan, H. *et al.* Chromosome-level genome assembly for giant panda provides novel insights into Carnivora chromosome evolution. *Genome Biol.* **20**, 267 (2019).
72. Yoshido, A. *et al.* Evolution of multiple sex-chromosomes associated with dynamic genome reshuffling in *Leptidea* wood-white butterflies. *Heredity* **125**, 138–154 (2020).
73. Saunders, P. A., Neuenschwander, S. & Perrin, N. Sex chromosome turnovers and genetic drift: A simulation study. *J. Evol. Biol.* **31**, 1413–1419 (2018).
74. de Souza, F. H. S. *et al.* Integrating cytogenetics and population genomics: Allopatry and neo-sex chromosomes may have shaped the genetic divergence in the *Erythrinus erythrinus* species complex (Teleostei, Characiformes). *Biology* **11**, 315 (2022).
75. Bertollo, L. A. C., Cioffi, M. B. & Moreira-Filho, O. Direct chromosome preparation from freshwater teleost fishes. In *Fish Cytogenetic Techniques* (eds Ozouf-Costaz, C. *et al.*) 21–26 (CRC Press, 2015). https://doi.org/10.1201/b18534-4.
76. Yang, F., Trifonov, V., Ng, B. L., Kosyakova, N. & Carter, N. P. Generation of paint probes by flow-sorted and microdissected chromosomes. In *Fluorescence In Situ Hybridization (FISH)—Application Guide* (ed. Liehr, T.) 35–52 (Springer, 2009). https://doi.org/10.1007/978-3-540-70581-9_3.
77. Yang, F. & Graphodatsky, A. S. Animal probes and ZOO-FISH. In *Fluorescence In Situ Hybridization (FISH)—Application Guide* (ed. Liehr, T.) 323–346 (Springer, 2009). https://doi.org/10.1007/978-3-540-70581-9_29.
78. Pendás, A. M., Móran, P., Freije, J. P. & Garcia-Vásquez, E. Chromosomal location and nucleotide sequence of two tandem repeats of the Atlantic salmon 5S rDNA. *Cytogenet. Cell Genet.* **67**, 31–36 (1994).
79. Cioffi, M. B., Martins, C., Centofante, L., Jacobina, U. & Bertollo, L. A. C. Chromosomal variability among allopatric populations of Erythrinidae fish *Hoplias malabaricus*: Mapping of three classes of repetitive DNAs. *Cytogenet. Genome Res.* **125**, 132–141 (2009).
80. Yano, C. F., Bertollo, L. A. C. & Cioffi, M. B. Fish-FISH: Molecular cytogenetics in fish species. In *Fluorescence In Situ Hybridization (FISH)—Application Guide* (ed. Liehr, T.) 429–444 (Springer, 2017).
81. Sassi, F. M. C., Toma, G. A. & Cioffi, M. B. FISH—in fish chromosomes. In *Cytogenetics and Molecular Cytogenetics* (ed. Liehr, T.) 281–293 (CRC Press, 2023).
82. Zwick, M. S. *et al.* A rapid procedure for the isolation of C0t–1 DNA from plants. *Genome* **40**, 138–142 (1997).

Acknowledgements

The authors are grateful to Eliana Feldberg and Lúcia Helena Rapp Py-Daniel from National Institute of Amazonian Research who provided assist in the collection and identification of animal samples. We also thanks to FAPESP for the funding.

Author contributions

F.M.C.S., G.A.D., O.M.F. and M.B.C. conceived and designed research. F.M.C.S. and G.A.D. conducted experiments. F.M.C.S., G.A.D., T.L., A.S., O.M.F., L.A.C.B., M.R.V., and M.B.C. analyzed the data. A.S., T.L., O.T.O. contributed with new methods. F.M.C.S., A.S., G.A.D., L.A.C.B., M.R.V., T.L. and M.B.C. wrote the paper.

Funding

Open Access funding enabled and organized by Projekt DEAL. This work was supported by São Paulo Research Foundation (FAPESP) grants 2020/02681-9, 2022/04261-2, and 2023/08116-0 (FMCS), 2020/11772-8 (MBC), Brazilian National Council for Scientific and Technological Development (CNPq), Grant number 302928/2021-9 (MBC) and Czech Academy of Sciences (RVO: 67985904 of IAPG CAS, Liběchov) (AS).

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to T.L.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

VI – Sassi *et al.* (em revisão). Independent evolution of satellite DNA sequences in homologous sex chromosomes. Communications Biology (*under review*).

Independent evolution of satellite DNA sequences in homologous sex chromosomes

Francisco de M. C. Sassi[1], Manuel A. Garrido-Ramos[2], Ricardo Utsunomia[3], Rodrigo Zeni dos Santos[3], Tariq Ezaz[4], Geize A. Deon[1], Fábio Porto-Foresti[3], Thomas Liehr[5*], Marcelo de B. Cioffi[1]

[1] Laboratory of Evolutionary Cytogenetics, Department of Genetics and Evolution, Universidade Federal de São Carlos, 13565-905 São Carlos - SP, Brazil.
[2] Departamento de Genética, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain.
[3] Faculdade de Ciências, UNESP, 17033-360 Bauru - SP, Brazil.
[4] Institute for Applied Ecology, University of Canberra, 2617 Canberra - ACT, Australia.
[5] Universitätsklinikum Jena, Friedrich-Schiller Universität, Institut für Humangenetik, 07747 Jena - Thüringen, Germany.

[*]Corresponding author: thomas.liehr@med-uni.jena.de

Abstract

The Neotropical armored catfish *Harttia* is a valuable model for studying sex chromosome evolution, featuring two independently evolved male-heterogametic systems. This study examined satellitomes—sets of satellite DNAs—from four Amazonian species: *H. duriventris* (X_1X_2Y), *H. rondoni* (XY), *H. punctata* (X_1X_2Y), and *H. villasboas* (X_1X_2Y). These species share homologous sex chromosomes, with their satellitomes showing a high number of homologous satellite DNAs (satDNAs), primarily located on centromeres or telomeres, and varying by species. Whole chromosome painting and bioinformatics revealed that in *Harttia* species without heteromorphic sex chromosomes, a specific satDNA (HviSat08-4011) is amplified in the same linkage group associated with sex chromosomes, suggesting an ancestral system. The study indicates that these homologous sex chromosomes have diverged rapidly, recently, and independently in their satDNA content, with transposable elements playing a minor role compared to autosomal chromosome evolution.

Keywords: repetitive DNA; transposable elements; FISH; short-reads; ZP4.

Introduction

Multiple sex chromosome systems arise from rearrangements between XY or ZW chromosomes and autosomes, this being the most common way, observed in several animal and plant groups [1–5], or even from fissions within the ancestral sex pairs without the involvement of additional autosomes [6–12]. In contrast, the differentiation of sex chromosomes in simple sex chromosome systems (i.e., XX female/XY male; ZZ male/ZW female), which is based on autosomal ancestry, is more extensively clarified [13–17]. An autosomal pair generally acquires a sex-determining locus, which may be a dosage-dependent or sex-determining allele. Subsequent modifications include the suppression of recombination either via the accumulation of various repetitive DNA classes or via chromosome rearrangements [18–23]. Undoubtedly, compared to the extensive research that has already been done on simple XY and ZW systems, multiple systems still have significant shortcomings in evolutionary research.

While some taxa, like birds and mammals, have retained the same mode of sex chromosome system (i.e., ZW and XY, respectively) in most of their extant species throughout their evolutionary history, fish and amphibians show regular turnovers, with a large number of

species presenting homomorphic, simple and multiple sex chromosomes, including alternative modes of sex chromosome system even within closely related species [5,24–27]. Among vertebrates, multiple sex chromosome systems derived from the ZZ/ZW system are extremely rare, only known in a few birds, lizards, and fish species (revised in [5]). On the other hand, the $X_1X_1X_2X_2/X_1X_2Y$ system is the most widespread XY-derived system, which has been found in several mammals and reptiles, frogs, and fishes [3,5]. Fishes possess the most abundant and diverse range of multiple sex chromosome systems among vertebrates. Approximately 5% of the teleost species examined had heteromorphic sex chromosomes, with a total of 81 instances of multiple systems [2,4,5,28–31].

Harttia (Siluriformes, Loricariidae) is a Neotropical fish genus with significant karyotype diversity among its 28 recognized and several cryptic species, which are widespread in the waters of the Orinoco and Guyana shields, as well as most Brazilian rivers [32,33]. The genus *Harttia* records the highest number of species with multiple sex chromosomes among fishes, with six male-heterogametic occurrences, comprising two independently evolved sex chromosome systems: XX/XY_1Y_2 and $X_1X_1X_2X_2/X_1X_2Y$ (Table 1, Figure 1). Whole chromosome painting (WCP) studies combined with the fluorescence in situ (FISH) mapping of repetitive sequences such as ribosomal DNA (rDNA) and microsatellites highlighted the putative origins and evolutionary relationships of the sex chromosomes, indicating that independent fission events occurred in the origin of the multiple systems [10,11,34,35].

Table 1. Distribution of sex chromosome systems in *Harttia* species. Cell colors reflect the homology of the sex chromosome systems. Data from [10,34] and [11,35].

Sex Chromosome System	Species	2n
$X_1X_1X_2X_2/X_1X_2Y$	*H. duriventris*	56♀/55♂
$X_1X_1X_2X_2/X_1X_2Y$	*H. villasboas*	56♀/55♂
$X_1X_1X_2X_2/X_1X_2Y$	*H. punctata*	58♀/57♂
XX/XY	*H. rondoni*	54♀♂
XX/XY_1Y_2	*H. carvalhoi*	52♀/53♂
XX/XY_1Y_2	*H. intermontana*	52♀/53♂
XX/XY_1Y_2	*Harttia* sp. 1	56♀/57♂
XX/XY	*H. torrenticola*	56♀♂

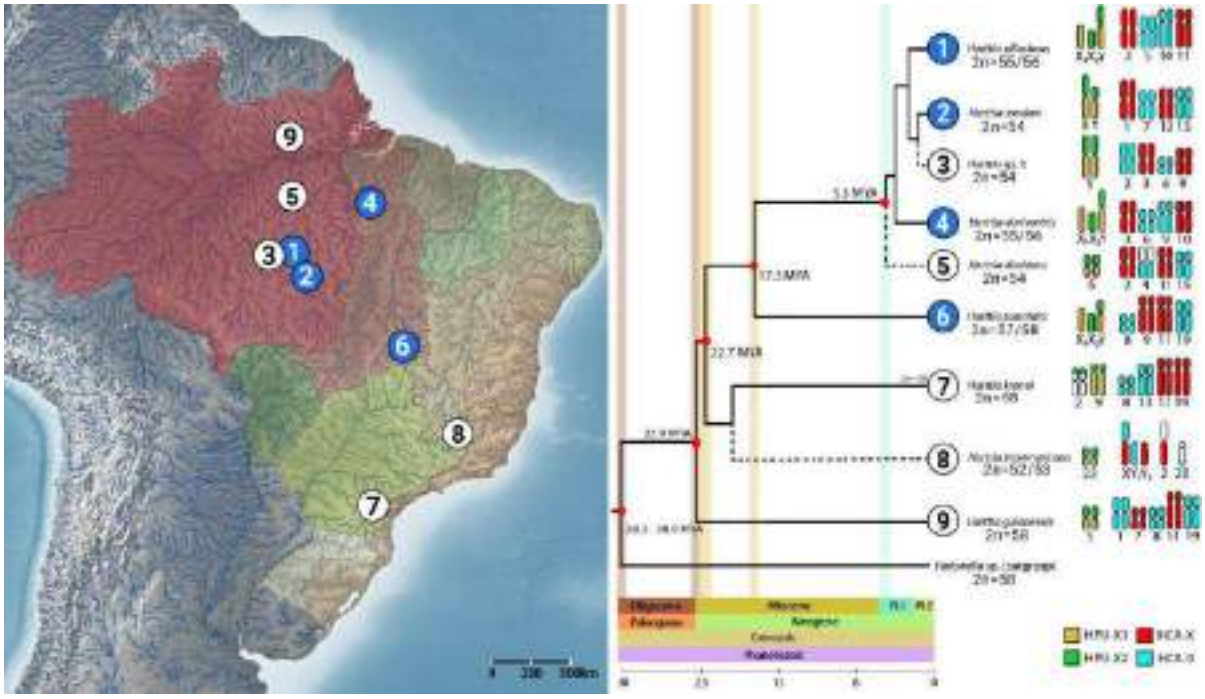


Figure 1. **Distribution map of *Harttia* species herein investigated, with their respective phylogenetic relationships modified from Covain et al. (2016).** The map highlights the hydrographic basins of Brazil and the rivers of South America. Dashed lines indicate the inferred relationships of species based on chromosomal data [10,11,34,36–38] that are not included in the previous [39] phylogeny. Numbers on the map indicate the collection sites (detailed in the material and methods section Table 8) of the same species presented in phylogeny. Idiograms illustrate the homologous sex chromosome systems according to their linkage groups, following [10,34] and [11,35]. Nodes marked in red indicate the divergence between clades recovered from TimeTree 5 [40]. Blue dots indicate the species targeted for satellitome characterization, while the white dots indicate the ones used for comparative FISH experiments.

SatDNAs are fast-evolving repetitive sequences that show species-specific profiles [41,42]. Notwithstanding, these sequences might have a particular role in gene regulation, chromatin modification, or as chromosomal functional components [41,43]. The extensive mapping of repetitive DNA sequences in fish chromosomes has provided an unparalleled opportunity to understand the role of such sequences in the evolutionary process [44]. Although fish genomics has been studied since the late 1980s, the broad incorporation of next-generation sequencing methods in the study of non-model organisms (e.g., [45–48]) allowed the expansion of chromosomics [49]. More recently, this combination of techniques (cytogenetics and genomics) is being used to characterize and map full catalogs of satellite DNA sequences (satDNAs) in several non-model fish (e.g. [46,50–53]), revealing the essential role of those repetitive sequences in sex chromosomal differentiation [54–58].

In standard XX/XY and ZZ/ZW systems, the association of satDNA sequences with the differentiation of the sex-specific (Y or W) chromosome has been clearly demonstrated (e.g., [54,55,57,59–64], among several others). The few studies on the satDNA content of multiple sex chromosome systems also demonstrate an accumulation of these repetitive sequences in distinct regions of the sex chromosomes, as demonstrated for the cricket *Eneoptera surinamensis* (X_1X_2Y) that carries satDNAs in the Y [65] and the anostomid *Megaleporinus elongatus* ($Z_1W_1Z_2W_2$) whose accumulation occurs in the W_1 [46]. The sorrel plant (*Rumex acetosa*, XY_1Y_2) presents satDNAs accumulated at both Y_1 and Y_2 sex chromosomes with a

different satDNA profile from those at the X chromosome, suggesting an older origin when compared to other dioecious plants with simple sex chromosomes [66–72]. The *Oxycarenus hyalinipennis* (X_1X_2Y) bug has a satDNA family distributed through the entire length of the Y chromosome and at least 26 satDNA families with a bias towards the male genome [73]. Although lacking Y-specific motifs, several satDNAs of *Pyrrhulina semifasciata* (X_1X_2Y) are accumulated in the proto-sex pairs in relative species [74]. Except for the few studies described above, no research on fish has been reported comparing species satellitomes with homologous multiple sex chromosomes. By integrating genomic and chromosomal data, we uncovered and compared the complete catalogs of satDNA families of four *Harttia* species named *H. duriventris, H. punctata,* and *H. villasboas* (X_1X_2Y) and *H. rondoni* (XY). This allowed us to investigate the evolutionary trajectory of satDNA families and expose their recent and accelerated patterns of evolution in these rearranged multiple sex chromosomes.

Results

Characteristics of Harttia satellitomes

The main features of the satellitomes are compiled in Table 2 and Supplementary Table 2, including the number of satellite sequences, the maximum and minimal values for the A + T proportion, and the range of repeat unit lengths. The maximum number of satellites was recovered in *H. rondoni*, with 25 satDNA families. This species also has the largest variation in the repeat unit length, with satDNAs varying from 4165 bp to 19 bp. Long satDNAs predominate among the four satellitomes, corresponding to 76.2% of the sequences in *H. villasboas*, 64% in *H. rondoni*, 61.2% in *H. duriventris*, and 52.6% in *H. punctata*. Putative ORFs were identified in four HviSatDNAs, five in HroSatDNAs, three in HduSatDNAs, and four in HpuSatDNAs (Supplementary Table 3). We have further investigated the satellitome of *H. villasboas* and studied it comparatively with the rest of the species.

Table 2. Main features of the *Harttia* satellitomes herein obtained: N = number of satellite sequences; RUL = repeat unit length (bp); A + T = proportion of A + T nucleotides in the satellite; Max = maximum; Min = minimal; Med = median.

Species	N	Max RUL	Min RUL	Med RUL	Max A + T	Min A + T	Med A + T
Harttia duriventris	18	2707	21	262	81%	23.6%	56%
Harttia punctata	19	2750	21	177	72%	39%	59%
Harttia villasboas	21	4011	32	730	68.1%	24.2%	52%
Harttia rondoni	25	4165	19	372	81%	25.1%	57.8%

Comparative fluorescence in situ hybridization

The FISH mapping of satellite sequences revealed distinct patterns for each *Harttia* species. Here we present the results in males, while hybridizations on females are presented in Supplementary Figures 2 and 3. In the donor species *H. villasboas* (Figure 2), concerning the sex chromosomes, only HviSat13-730 and HviSat18-1068 were present both at the secondary constriction of X_2 and Y. Among autosomes, HviSat01-2531 was found in the

pericentromeric regions of five chromosome pairs, while HviSat02-2707 was scattered in all pairs in a non-clustered pattern. Both HviSat04-2959 and HviSat05-177 were hybridized in the short arms of a small metacentric pair, and HviSat08-4011 was found in the long arms at the terminal region of a submetacentric pair. No visible signals were identified with the HviSat03-997 probe.

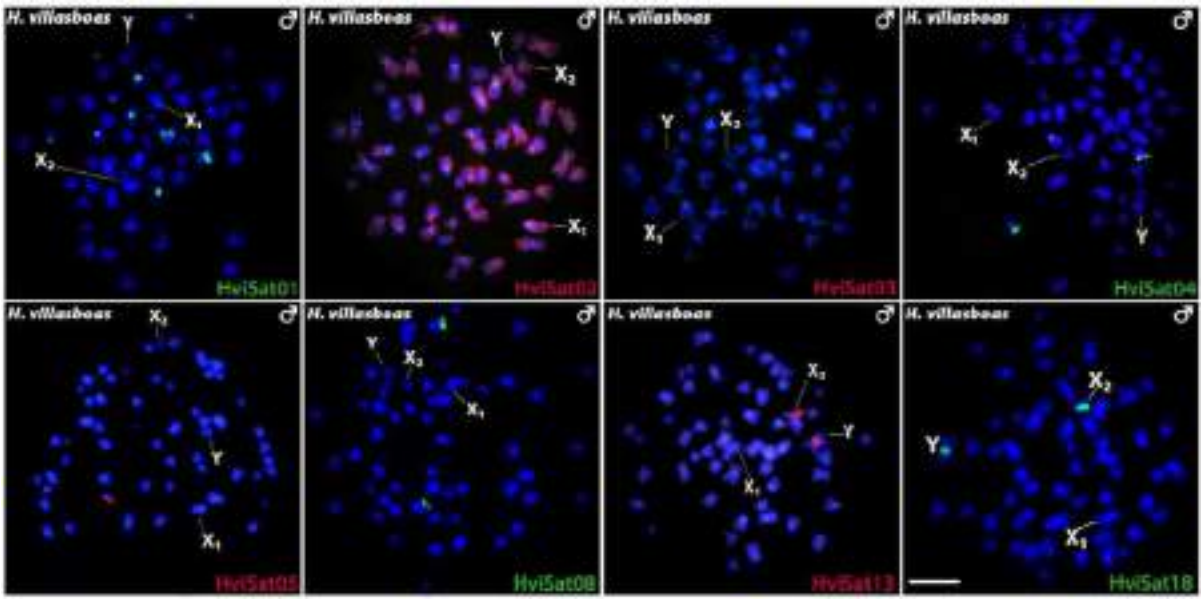


Figure 2. **Distribution of satDNAs in *H. villasboas*.** Male mitotic chromosomes of *H. villasboas* after hybridization with distinct satellite DNA sequences, indicated in the lower right corner. Sex chromosomes are indicated in the metaphase. Scale bar = 5 µm.

Both *H. rondoni* and *H. duriventris* share similar distribution patterns of HviSatDNAs. Only HviSat13-730 and HviSat18-1068 were mapped in the X and Y sex chromosomes of *H. rondoni* and X_2 and Y of *H. duriventris* (Figure 3). HviSat08-4011 was hybridized to the terminal portion of the long arms of a submetacentric pair, in addition to a pericentromeric signal on the X_1 chromosome of *H. duriventris*. Following this, HviSat04-2959 and HviSat05-177 were found on the short arms of a small metacentric pair. HviSat02-2707 was found scattered in all chromosomes and HviSat03-997 does not produce visible signals, while HviSat01-2531 was hybridized to pericentromeric regions of six and three pairs in *H. rondoni* and *H. duriventris,* respectively.

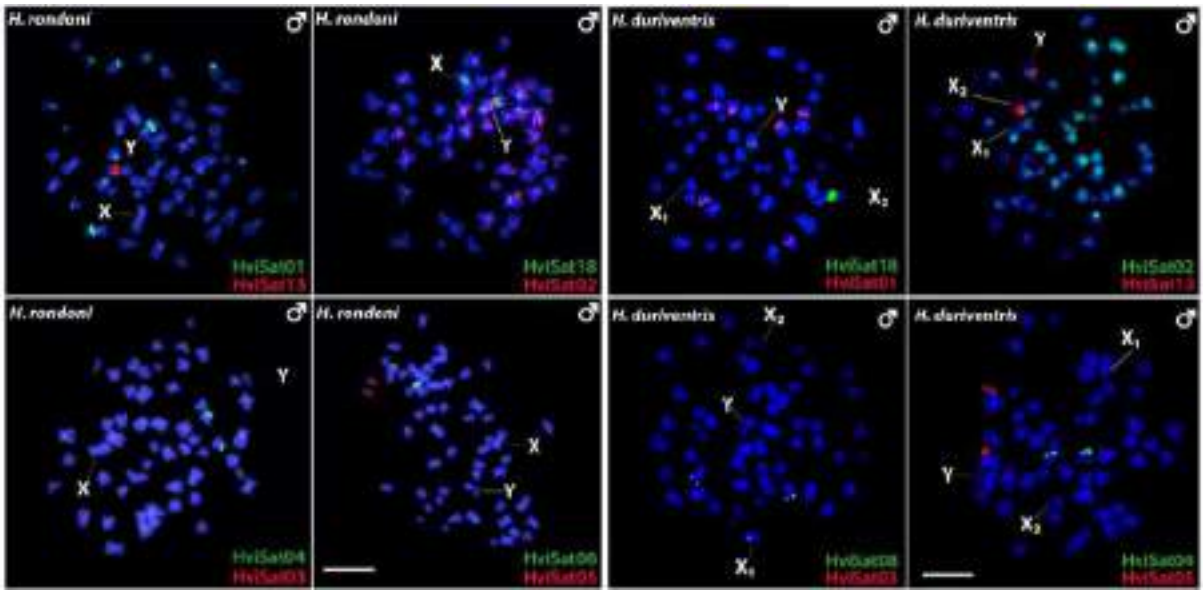


Figure 3. **Distribution of satDNAs in *H. rondoni* and *H. duriventris*.** Male mitotic chromosomes of *H. rondoni* and *H. duriventris* after hybridization with distinct satellite DNA sequences from *H. villasboas*, as depicted in the lower right corner. Sex chromosomes are indicated. Scale bar = 5 µm.

When hybridized with *H. punctata* chromosomes (Figure 4), the HviSatDNAs produced unexpected results. The sex chromosomes of this species accumulate several sequences, including HviSat08-4011 in a large pericentromeric block on X_1, HviSat13-730 at the secondary constriction of X_2, HviSat04-2959 also in a large pericentromeric block on X_1, a small pericentromeric signal on X_2 and Y, and HviSat05-177 at the centromere of X_1 and X_2, in addition to telomeric signals on X_2 and in the Y, the last restricted to long-arms. HviSat04-2959 was also found in a small metacentric autosome pair and HviSat05-177 in the short arms of six autosomes. HviSat01-2531 was restricted to a large sm chromosome pair in an interstitial position on long arms, while HviSat02-2707 produced small signals in the pericentromeric region of a few chromosome pairs. The species *Harttia* sp. 3 (Figure 4) produced similar patterns to *H. villasboas* and *H. rondoni*. The at01-2531 was found in three chromosome pairs in the pericentromeric region, while HviSat03-997 was scattered in almost all chromosomes. HviSat18-1068 and HviSat13-730 were found in the secondary constriction of the first chromosome pair in the pericentromeric region. On the other hand, HviSat02-2707 was found slightly hybridized to some chromosome pairs, but only in one of the homologs of the first chromosome pair, all in the pericentromeric position. Both HviSat04-2959 and HviSat05-177 followed the pattern of a single chromosome pair hybridized with each probe, while HviSat08-4011 was found in the long arms of a submetacentric large pair.

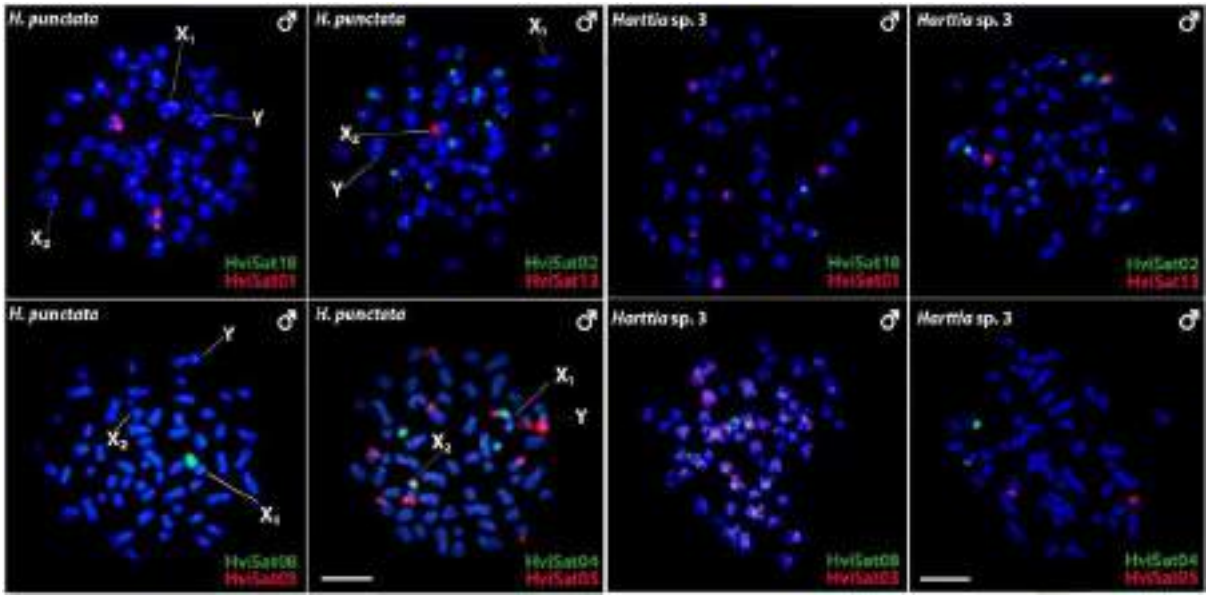


Figure 4. **Distribution of satDNAs in *H. punctata* and *Harttia* sp. 3.** FISH mapping of satellite sequences from *H. villasboas* against male *H. punctata* and *Harttia* sp. 3 chromosomes. Each probe combination is identified in the respective box, and sex chromosomes are indicated in each metaphase spread. Scale bar = 5 µm.

For *H. dissidens* and *H. guianensis* (Figure 5), HviSat01-2531 produced strong hybridization signals in five chromosome pairs in the first and was restricted to a single sm pair at an interstitial position in the last. The HviSat13-730 was restricted to the secondary NOR constriction in a single pair in both species. The HviSat08-4011 followed the pattern for most other species, being accumulated in the telomeric region of the long arms of a submetacentric pair in both species. While HviSat05-177 was found in a single pair of *H. dissidens*, four pairs were probed in *H. guianensis*. Exclusively in *H. dissidens*, a scattered pattern was observed for HviSat03-997, and a single pair was labeled with HviSat04-2959, since no visible signals were found in *H. guianensis* for both satDNAs. Both HviSat18-1068 and HviSat02-2707 do not produce visible FISH signals. Figure 6 highlights *H. villasboas* satellite sequences that were hybridized to sex chromosomes in distinct selected *Harttia* species were schematized according to their phylogenetic relationships by Covain et al. (2016).

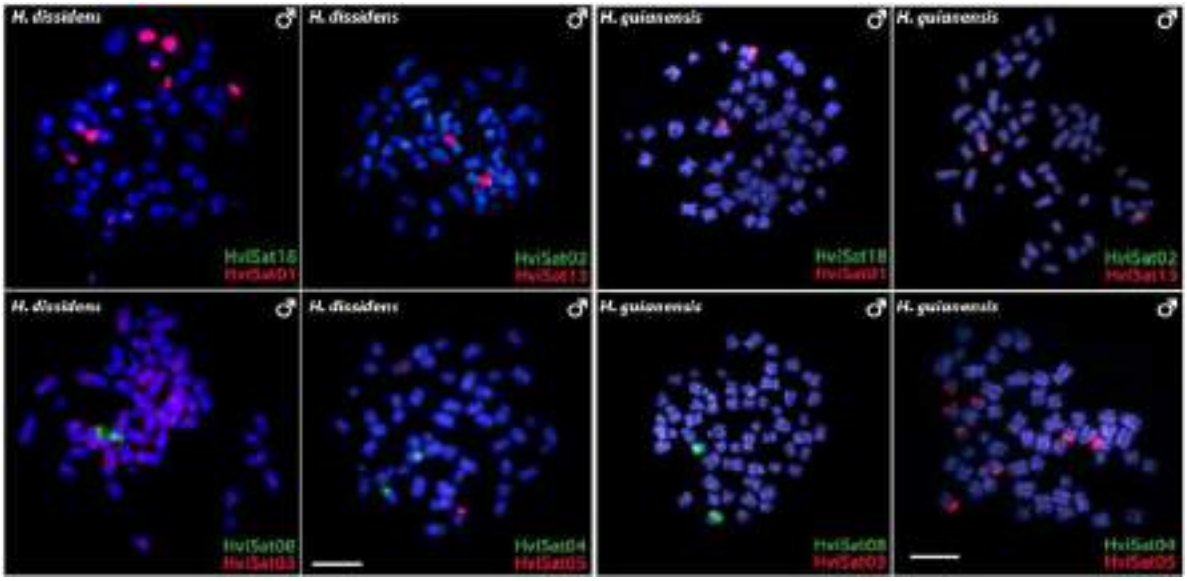


Figure 5. **Distribution of satDNAs in *H. dissidens* and *H. guianensis*.** Satellite sequences of *H. villasboas* hybridized against male *H. dissidens* and *H. guianensis* chromosomes. Each probe combination is identified in the respective box. Scale bar = 5 µm.

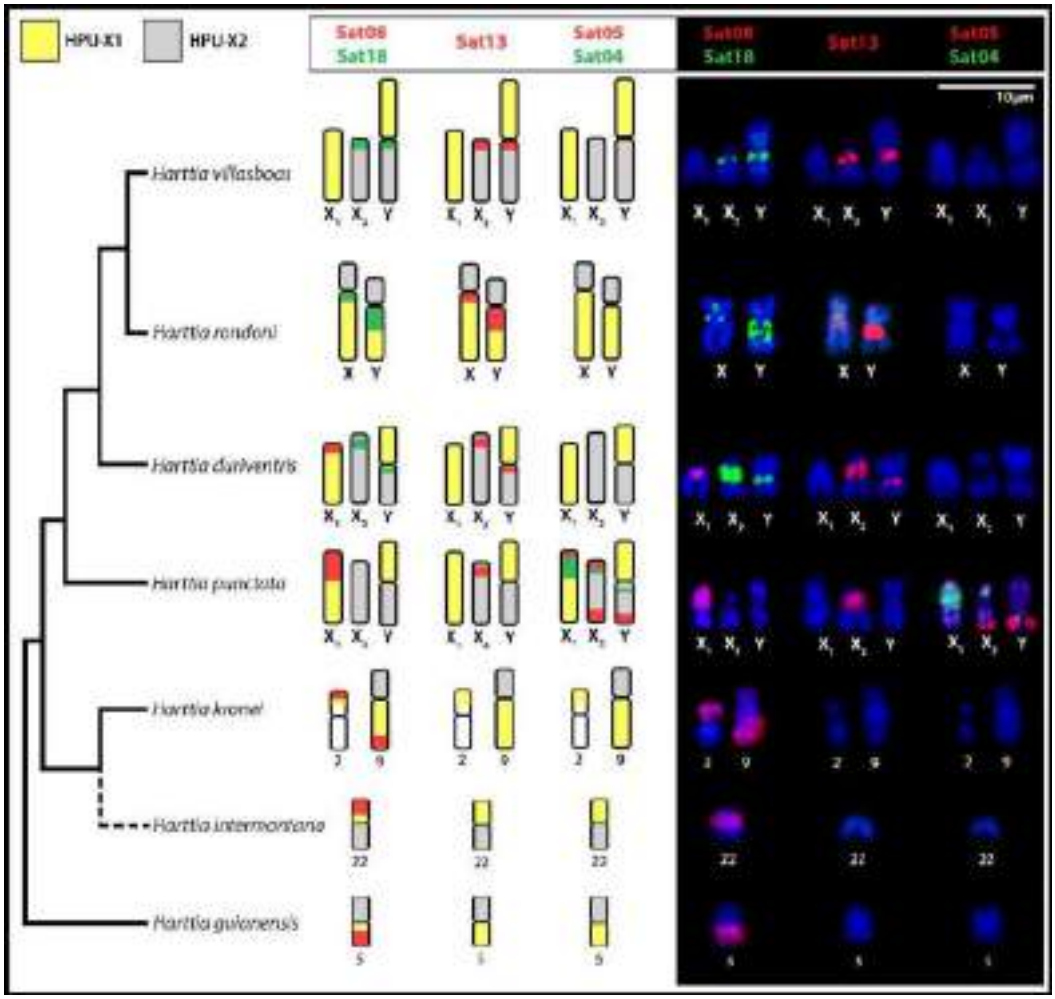


Figure 6. **HviSatDNAs hybridized to sex chromosomes and homologous autosomes of seven *Harttia* species.** A highlight of *H. villasboas* satellite sequences that were hybridized to sex chromosomes in distinct selected *Harttia* species schematized according to their

phylogenetic relationships [39]. *H. intermontana* was manually added to the tree based on geographical distribution and chromosomal characteristics, but its position must be confirmed since it was not included in Covain's analysis. The combination of probes is indicated above each ideogram/FISH image. The colors of idiograms followed the homology of sex chromosomes obtained from whole chromosome painting with HPU-X_1 (yellow) and HPU-X_2 (gray) described by [10,34] and [11]. See Supplementary Figure 4 for WCP + HviSat08-4011 on species without heteromorphic sex chromosomes. Scale bar = 10 µm.

Comparative satellitomics

19 of the 21 *H. villasboas* satDNAs have a homologous counterpart in one, two, or three other *Harttia* species (Table 4). Table 4 reflects that there are conserved satellites in all four species analyzed (e.g., the homologs of HviSat01-2531, HviSat04-2959 or HviSat05-177, among others). However, there are lineage-specific satellites: two satDNAs, HviSat17-49 and HviSat20-47, appear to be specific to *H. villasboas*, six satDNAs appear to be specific to *H. rondoni*, 1 to *H. duriventris* and 4 to *H. punctata*. In this context, we have found some satellites that are specific to the *H. villasboas-H. rondoni* lineage: HviSat08-4011, HviSat16-2480, HviSat19-589, and HviSat21-575 have homologues only in *H. rondoni*, the closest relative to *H villasboas*. But this table also reflects the saltational process of evolution of some satellites that form the satellitomes of these species since there are satDNAs shared by two or more species for which the pattern of sharing does not conform to the phylogeny of Figure 1: among several others, one example is HviSat02-2702 for which we have found homologs in *H. duriventris* and *H. punctata* but not in *H. rondoni*. These data reflect differential amplifications of these satellites in different species at different evolutionary times.

Table 4. Homologous satDNA families of *Harttia*.

Species			
H. villasboas	***H. rondoni***	***H. duriventris***	***H. punctata***
HviSat01-2531	HroSat02-1662	HduSat03-2143	HpuSat12-1973
	HroSat03-372		
	HroSat05-515		
HviSat02-2707		HduSat01-2707	HpuSat04-2707
HviSat03-997			HpuSat05-1068
HviSat04-2959	HroSat09-2961	HduSat08-1754	HpuSat07-2750
HviSat05-177	HroSat16-177	HduSat06-177	HpuSat02-177
HviSat06-200		HduSat04-31	
HviSat07-93	HroSat01-93	HduSat05-93	HpuSat06-105
HviSat08-4011	HroSat14-4165		
HviSat09-815	HroSat19-815	HduSat11-815	HpuSat10-781
HviSat10-1666	HroSat06-1634	HduSat12-1670	HpuSat14-503
HviSat11-1338	HroSat13-1440	HduSat10-1449	HpuSat08-1546
HviSat12-144		HduSat13-144	HpuSat03-144
HviSat13-730	HroSat04-920	HduSat09-771	
	HroSat12-144	HduSat16-144	
HviSat18-1068	HroSat11-534	HduSat15-534	
HviSat14-347	HroSat08-347	HduSat02-347	
HviSat15-32		HduSat14-32	HpuSat11-32
HviSat16-2480	HroSat17-2481		
HviSat19-589	HroSat18-1194		
HviSat21-575	HroSat23-576		
	HroSat10-21	HduSat07-21	HpuSat01-21
	HroSat20-45	HduSat18-45	

Table 5 shows the genetic distances between the satDNAs of *H. villasboas* and the corresponding homologous satellites. In general, the greater the phylogenetic distance, the greater the genetic distance between homologous satellites. This is especially true in comparisons with *H. punctata*. But Table 5 also shows that there are highly accelerated or slowed rates of change for some satDNAs lineages, with genetic distances that are not consistent with phylogenetic proximity in some comparisons. For example, the homologous HviSat01-2531 in *H. rondoni* or the homologous HviSat04-2959 in *H. duriventris* show greater divergence from *H. villasboas* than homologs of phylogenetically more distant species. The table, in general, shows differential divergence patterns between different satellites and between different lineages. Taking as a reference the only node in which we have a clear dating to compare two species, Table 6 shows CTR for each satDNA assuming a divergence time of 17.5 my between *H. villasboas* and *H. punctata* (Figure 1), demonstrating very different rates of sequence change in different satDNAs.

Table 5. A comparison of the genetic distances between the homologous satDNAs of *Harttia villasboas* and the other three species (*H. rondoni*, *H. duriventris*, and *H. punctata*). Genetic distances between (1) Hvi-Hro, (2) Hvi-Hdu, (3) Hvi-Hpu, (4) Hro-Hdu, (5) Hdu-Hpu, (6) Hro-Hpu.

		Homologous satDNA	
Harttia villasboas	***Harttia rondoni***	***Harttia duriventris***	***Harttia punctata***
HviSat01-2531	0.081(1)	0.023(2)	0.189(3)
	0.068(4)	0.198(5)	
		0.128(6)	
HviSat02-2707		0.004(2)	0.025(3)
		0.023(5)	
HviSat03-997			0.296(3)
HviSat04-2959	0.018(1)	0.390(2)	0.180(3)
	0.393(4)	0.498(5)	
		0.156(6)	
HviSat05-177	0.006(1)	0.006(2)	0.163(3)
	0.000(4)	0.156(5)	
		0.156	
HviSat06-200		0.067(2)	
HviSat07-93	0.044(1)	0.116(2)	0.316(3)
	0.142(4)	0.350(5)	
		0.367(6)	
HviSat08-4011	0.016(1)		
HviSat09-815	0.030(1)	0.030(2)	0.271(3)
	0.043(4)	0.280(5)	
		0.275(6)	
HviSat10-1666	0.016(1)	0.023(2)	0.498(3)
	0.020(4)	0.480(5)	
		0.471(6)	
HviSat11-1338	0.070(1)	0.065(2)	0.771(3)
	0.025(4)	0.775(5)	
		0.768(6)	
HviSat12-144		0.007(2)	0.007(3)
		0.000(5)	
HviSat13-730	0.092(1)	0.109(2)	
	0.048(4)		
HviSat14-347	0.012(1)	0.032(2)	
	0.038(4)		
HviSat15-32		0.000(2)	0.000(3)
		0.000(5)	
HviSat16-2480	0.015(1)		
HviSat18-1068	0.038(1)	0.067(2)	
	0.061(4)		
HviSat19-589	0.107(1)		
HviSat21-575	0.007(1)		

Table 6. Consensus turnover rate (CTR) of satDNA sequences between *Harttia villasboas* (HviSatDNA) and *H. punctata* (HpuSatDNA).

HviSatDNA	HpuSatDNA	CTR
HviSat01-2531	0.189	0.54×10^{-8}
HviSat02-2707	0.025	0.07×10^{-8}
HviSat03-997	0.296	0.85×10^{-8}
HviSat04-2959	0.180	0.51×10^{-8}
HviSat05-177	0.163	0.47×10^{-8}
HviSat07-93	0.316	0.90×10^{-8}
HviSat09-815	0.271	0.78×10^{-8}
HviSat10-1666	0.498	1.42×10^{-8}
HviSat11-1338	0.771	2.20×10^{-8}
HviSat12-144	0.007	0.2×10^{-8}
HviSat15-32	0.000	0
	Average	0.71×10^{-8}

Repeat landscapes of Harttia satDNAs

Figure 7 displays repeat landscape (RL) plots representing, for several *Harttia* satDNAs, abundance (y-axis) and divergence (x-axis) to the consensus sequence built for each satDNA repeat unit. satDNA degenerates through point mutation (increasing divergence) and homogenizes through amplification (decreasing divergence). Then, it is assumed that peaks at lower divergence values in the repeat landscape profiles are the product of recent amplifications, whereas those at higher divergence values are probably older variants degenerated by the accumulation of mutations. Following this argument, we have analyzed the RL plots of satDNAs found in the sex chromosomes of *Harttia* species. Thus, the satellite HviSat08-4011 and its counterpart HroSat14-4165 show a unique amplification peak at 0% divergence. Then, although the graph shows sequence variants of these satellites with divergences of up to 30%, the amplification that resulted in the current status of this satellite in the *H. villasboas-H. rondoni* lineage must have been very recent after the lineage split that led to *H. duriventris*. In the case of the HviSat13-730 and HviSat18-1068 satellites and their counterparts, several peaks are observed that could reflect different, recent (the highest peaks are at 0% divergence) amplification waves of these satellites at different times in the distinct species. The RLs of HviSat04-2959 and HviSat05-177, and their counterparts, show several peaks. But the greatest peaks of the satellites HpuSat02-177 and HpuSat07-2750 stand out, which could be related to their incorporation and amplification in the sex chromosomes of *H. punctata* (these satellites are in autosomes in the other species) possibly after the separation of the lineages (17.5 mya; Figure 1) since the divergence of 2% of these peaks would correspond to a divergence time of about 8 my (Table 5). Finally, the rest of the RL plots in Figure 7 represent examples of four autosomal satellites (HviSat01-2531, HviSat03-997, HviSat14-347, and HviSat19-589, and their counterparts). As shown, all these satellites show several peaks that could reflect different expansion waves, most of which are very old (there are divergence peaks of more than 20%), differentiated in time between the compared species.

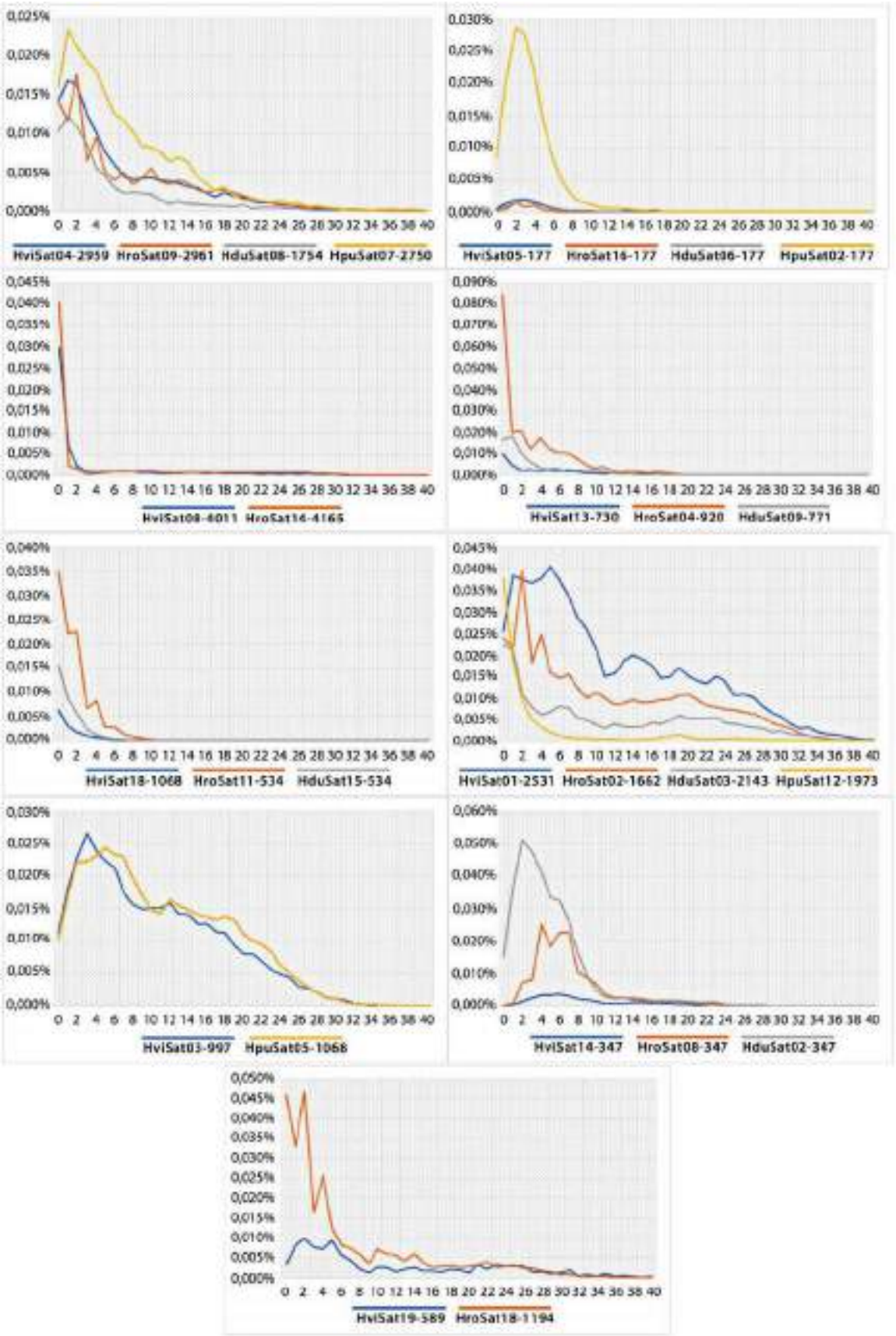


Figure 7. Repeat landscape plots for *Harttia* satDNAs. Repeat landscape plots representing, for several *Harttia* satDNAs, abundance (y-axis) and divergence (x-axis) to a consensus sequence built for each satDNA repeat unit.

The complex nature of HviSat08-4011

As indicated, homologous satellites to the satellite HviSat08-4011 and its counterpart HroSat14-4165 in *H. rondoni* were not isolated from the genomes of *H. duriventris* and *H. punctata*. However, FISH with probes derived from HviSat08-4011 detected large blocks of hybridization of this satellite on chromosome X_1 of both species, in addition to an autosomal locus (only in *H. duriventris*), which contrasts with the unique autosomal location of this satellite in *H. villasboas* and *H. rondoni* (as well as in other species lacking sex-chromosomes) (Figure 6). BLASTn and BLASTx search of HviSat08-4011 reveal a complex satDNA composed of at least two differentiated parts (Figure 8A). The largest part (about 2300 bp) is a sequence with no homology to any other known sequence. However, about 1700 bp of HviSat08-4011 shows homology and high identity (~80%) with the zona pellucida sperm-binding protein 4-like (*ZP4*) gene of diverse species of the orders Siluriformes (such as *Ictalurus, Silurus*, *Neoarius* and *Tachysurus*) and Characiformes (such as *Colossoma*, *Pygocentrus* and *Astyanax*). As Figure 8A shows, the homologous parts are discontinuous, separated by intervening unknown sequences. However, the homologous parts align continuously with ~90% of the translated sequence of the gene in those other species, with small discrepancies at the boundaries.

The analogous region of the ZP4 gene is where the primers utilized in our initial FISH strategy were constructed (Figure 8A, F1R1). Two additional primer pairings have been put to the test. One is located in the ZP4 non-homologous region (Figure 8A, F2R2), while the other is made up of a reverse primer inside the ZP4 homologous region and a forward primer outside of it (Figure 9A, F3R3). In the first case, the results differ from those initially found (Table 7 and Figure 8B): a) the two satellite parts (*ZP4*-homologous and non-*ZP4*-homologous) hybridize only on one autosome in *H. rondoni* and *H. villasboas*; b) the *ZP4*-homologous satellite part hybridizes on X_1 and one autosome of *H. duriventris* but the satellite part not homologous to *ZP4* hybridizes only on the autosome; c) the satellite part homologous to *ZP4* hybridizes only on X_1 of *H. punctata* and the satellite part not homologous to *ZP4* does not hybridize on any chromosome. In the second case, with the F3R3-HviSat08-4011 primers, only *H. villasboas* and *H. rondoni* produced visible fragments in the agarose gel (Table 6 and Figure 9B). Those last PCR product amplifications were then sequenced to check the composition of the amplified fragments, and they fully coincided with the expected region.

Table 7. Summary of PCR and FISH results according to the distribution on autosomes (A) or sex chromosome (X_1). Probes using F1R1 primers were called HviZP4, while those using F2R2 primers Hvi-non-ZP4, and F3R3 HviSat08-4011. Colored cells indicate positive results.

		FISH HviZP4		FISH Hvi-nonZP4		PCR		
Species	**SatDNA**	**A**	**X_1**	**A**	**X_1**	**HviZP4**	**Hvi-non-ZP4**	**HviSat08-4011**
H. villasboas	HviSat08-4011							
H. rondoni	HroSat14-4165							
H. duriventris								
H. punctata								

We have searched for HviSat08-4011 among the Illumina reads of *H. duriventris* and *H. punctata* using the script mapping_blat_gs.py (see Materials and Methods) but we obtained no results other than partial sequences with homology to the portion homologous to ZP-4 (mostly) and some with homology to the portion not homologous to ZP-4. We then mapped and quantified the raw reads of both species against both regions of HviSat08-4011 (repeat_masker_run_big.py) and obtained FISH-compatible results. Specifically, while this

satDNA represents 0.05% and 0.01% of the genome of *H. villasboas* and *H. rondoni*, respectively (Supplementary Table S1), the part homologous to ZP-4 accounts for 0.1% of the genome of *H. punctata* while the non-homologous part accounts for 0.03% of its genome, these percentages being 0.03% in both cases in *H. duriventris*. Furthermore, we have found different evolutionary trajectories for each part (homologous to ZP-4 and non-homologous to ZP-4) in *H. duriventris* and *H. punctata*. Thus, Figure 8C shows repeat landscape plots representing, for *H. villasboas*, *H. rondoni*, *H. duriventris,* and *H. punctata*, abundance (y-axis) and divergence (x-axis) with respect to a consensus sequence built for each part of the HviSat08-4011 repeat unit.

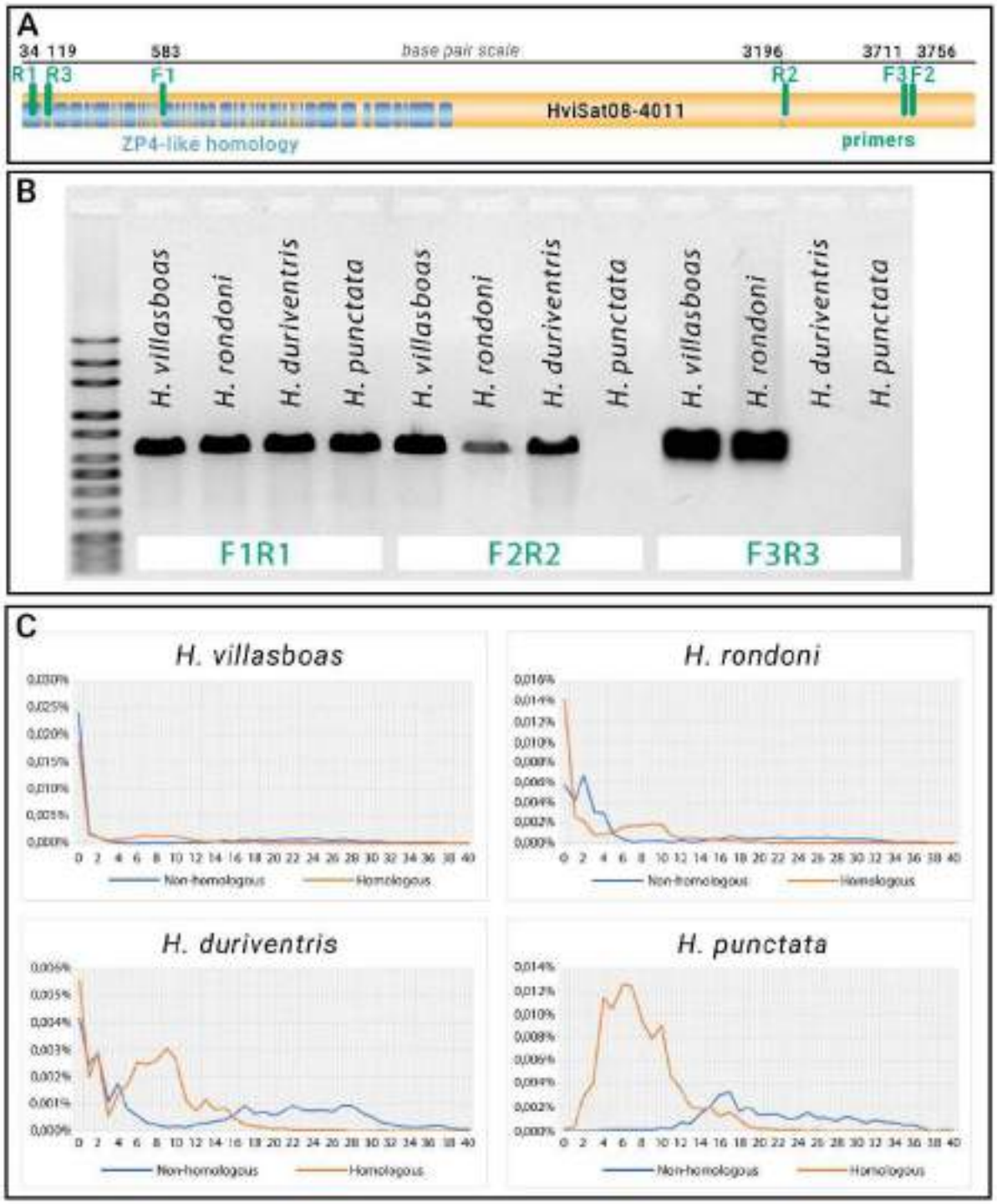


Figure 8. **Composition, distribution and evolution of HviSat08-4011**. Structure of HviSat08-4011 (**a**), indicating the homologous regions with the *ZP4*-like gene (blue) and the three sets of primers (F1R1, F2R2, and F3R3, in green) used for PCR, as demonstrated by the agarose-gel image (**b**). Repeat landscape plots (**c**) representing, for *H. villasboas*, *H. rondoni*, *H. duriventris,* and *H. punctata*, abundance (y-axis) and divergence (x-axis) to a consensus sequence built for each part of the HviSat08-4011 repeat unit, being blue lines for the non-homologous to *ZP4* and orange for the homologous portion.

Relationship of Harttia satDNAs with transposable elements (TEs)

Supplementary Tables 4-7 show the relationships between transposable elements (TEs) and the various groups of *Harttia* homologous satellites. The most remarkable results are: a) out of the several satellites found in the sex chromosomes in this study, only the satellite HviSat04-2959, and its homologs in the rest of the species, is related to this type of elements; b) most of the satellites are complex ones composed of different parts in which sequences derived from SINE and/or LINE elements and tRNA genes (and satDNAs derived from tRNAs) are intermingled with intervening sequences; c) HviSat02-2707 and its homolog are connected to Penelope elements, whereas HduSat17-150, which has no homologs in other species, is related to Helitron elements; d) HviSat06-200, but not its shorter homologous HduSat04-31, is analogous to a mosaic zebrafish satDNA.

Discussion

Our results show that the complex history of chromosomal evolution in *Harttia* is also reflected in its satDNA content. We demonstrated the occurrence of distinct profiles of satDNAs on homologous sex chromosomes, suggesting that these repeats diverged fast, recently, and independently in each species. SatDNA sequences are abundant on sex chromosomes and may play an important role in gene regulation and dosage compensation, resulting in hybrid incompatibilities [41,75]. Moreover, our data demonstrated that in other *Harttia* without heteromorphic sex chromosomes, a satDNA (HviSat08-4011) is amplified in the same linkage group that creates the sex chromosomes seen in the four target species of this study, suggesting an ancestral sex chromosome system, which will be discussed below.

Evolution of satellite DNAs in Harttia genomes

A large number of satDNA families are shared among the four *Harttia* species investigated in this study. This finding supports the library hypothesis [76], which has already been reported for other groups, including fishes [74,77,78], mammals [79,80], reptiles [81], insects [82,83,] and plants [84–86]. The library hypothesis states that species possess a repertoire of satellite DNA (satDNA) families, which exhibit variations in both the number of copies and their arrangement within the genome [76]. As seen in Table 4, which is the result of differential amplifications of each satDNA in different species at different evolutionary times, thus supports this hypothesis. Roughly, genetic distances between homologous satellites agree with the phylogenetic relationships shown in Figure 1. Therefore, the distances between *H. villasboas* and *H. rondoni* are consistently the shortest, indicating that these two species are the closest in phylogenetic proximity. The primary differences consistently occurred between *H. punctata* and any other species, indicating that this lineage split from the lineage that originated the clade consisting of the other three species a long time ago (Figure 1). However, in agreement with the library hypothesis, differential amplifications lead to accelerated rates of change in different lineages as disparate genetic distances that do not fit with phylogenetic relationships are observed (Table 5). In this context, the repeat landscapes indicated peaks in both higher and lower divergence values, probably due to the presence of older variants degenerated by the accumulation of mutations in addition to recent amplification and homogenization events (Figure 7).

Satellite DNAs (satDNAs) exhibit variation in their location on *Harttia* chromosomes, ranging from being scattered in a few places throughout the chromosome to being mostly confined to either the pericentromeric or telomeric regions (Figures 2-5), the preferential places of clusterization of satDNAs [41,87]. It has been proposed that satDNAs may play a

significant role in the structure and operation of both centromeric and telomeric regions since such distribution lines up within all eukaryotes [88,89]. In terms of size, there is a prevalence of long satDNAs (>100bp), which is consistent with what has been seen in the related species *H. carvalhoi* [78].

Some satDNAs of *Harttia* also share the position with the 18S rDNA secondary constriction, following HviSat13-730 and HviSat18-1068 associated with the secondary NOR constriction of most species, with the last being especially highlighted in *H. villasboas*, *H. rondoni*, *H. duriventris*, and *Harttia* sp. 3 (Figures 2-5). All four species that harbor the multiple X_1X_2Y sex chromosome system demonstrated some ribosomal DNA loci in both X and Y chromosomes. While *H. punctata* [90] carries both 5S (X_1 and Y) and 18S rDNA (X_2), only the 18S rDNA is found in the sex chromosomes of *H. duriventris* and *H. villasboas* (X_2 and Y) [36]. The sister species with the homologous simple XY system (*H. rondoni* [36]) has the 18S rDNA locus in both sex chromosomes. Ribosomal sequences were suggested to increase the instability of genomes due to their high transcriptional activity, which can facilitate double-strand breaks [91,92], as suggested for other *Harttia* species [38], and also other loricariids such as *Ancistrus* [93] and *Rineloricaria* [94,95]. In plants, numerous satDNAs arise from the intergenic spacer of the ribosomal locus [96,97], which was not observed in *Harttia* genomes. Since the rDNAs located in the sex chromosomes of *Harttia* were suggested to surround or be inserted in an evolutionary breakpoint region that is reused in the karyotypic diversification of the genus [34], both HviSat13-730 and HviSat18-1068 may likely have contributed to the genomic instability that led to the fission process the originates the multiple sex chromosomes herein investigated. SatDNAs are also associated with the formation of chromocenters and micronuclei (reviewed in [98]), which can trigger DNA breakage and incompatibilities between populations, leading to reproductively isolated populations [99].

Transposable elements (TEs) are known to contribute to genome expansion mechanisms primarily through retrotranspositions, but also by creating new tandem repeats [100] by partial TE duplication followed by uneven crossing-over [101], or by transposase-induced breaks and repair mechanisms [102]. In *Harttia*, these elements have an intricate relation with the formation of satDNAs, as demonstrated by complex sequences composed of distinct parts in which motifs derived from short-interspersed elements (SINE) and/or long interspersed elements (LINE) and tRNA genes. For example, HviSat02-2707 and its homologs in other species are related to Penelope elements, a class of repetitive elements widely distributed in fish genomes, seen in medaka, pufferfishes, and cichlids [103]. Penelope-like elements (PLE) are predominantly found in animal genomes, with occasional losses in some lineages [104,105]. On the other hand, the *H. duriventris*-specific satellite HduSat17-150 is related to Helitron, a group of TEs that conserves sequences at ends but with satDNA-like central tandem-repeated motifs [106], including real satDNAs embedded in such central position in Bivalves [107,108]. This demonstrates that the evolutionary history of satDNA sequences is also related to TEs, reflecting the complex history of chromosomal rearrangements that occurred in the genus. However, such TEs do not seem to participate in sex chromosome differentiation, as discussed in the next section. Most satDNAs in this genus presented rates of divergence between homologous sequences that vary according to the phylogenetic relationships, but repeat landscapes demonstrated species-specific amplification and homogenization events, especially in the repeats found in sex chromosomes. Such patterns indicate a contingent evolution scenario for the satDNAs of *Harttia*, as also seen in the repetitive content of grasshoppers [109].

The role of satDNAs in the evolution of Harttia´s sex chromosomes

Although all *Harttia* species from Clade III share homologous sex chromosomes, as indicated by chromosome painting studies [11], each demonstrated a distinct composition of satDNAs, indicating independent evolution of these sequences in each lineage. Sex chromosome differentiation by independent accumulation of satDNAs in a recent evolutionary time may reflect the neo origin of such systems, as also seen in other fish with recently originated sex chromosomes, such as *Megaleporinus elongatus* and *Nothobranchius* spp. [46,48,52]. In *Harttia*, the discrepancy in satDNA profiles can be observed, especially in sequences that do not follow a phylogenetic arrangement, for example, HviSat08-4011, which was probably amplified in *H. duriventris* and *H. punctata* after their split, besides HviSat13-730 and HviSat18-1068, which we will discuss below.

The sex chromosomes of *H. villasboas* harbor only two satDNAs: HviSat13-730 and HviSat18-1068. These satellites share a minor part of their repeat sequence (about 6%) with 84% similarity, suggesting an ancient common origin. However, both satDNAs and their homologous counterparts in each species, have signals of recent amplifications in *H. villasboas*, *H. rondoni*, and *H. duriventris*, in addition to independent events of previous amplifications (Figure 7). For HviSat18-1068 and its homologs HroSat11-534 and Hdu15-534, which are smaller in size, a duplication process in *H. villasboas* was responsible for their difference in size. This satellite is presented in the secondary constriction formed by the 18S rDNA, where events of unequal crossing-over were proposed as the main driver for differences in the accumulation of this ribosomal sequence between the homologs [36]. Since these satellites are not linked to TEs, unequal recombination may have caused the duplication, as described for other satDNAs in rice and monkeys [110,111]. However, rolling circle replication may also be responsible for the duplication of such repetitive sequences [112]. Regarding HviSat13-730 and its homologs HroSat04-920 and HduSat09-771, which showed positive FISH signals in all four species, we could not identify homolog sequences in *H. punctata* genome. In this species, the FISH signal is restricted to the X_2 chromosome and lacking in the Y, matching the 18S rDNA accumulation pattern [90], suggesting that the fission event that created the multiple sex chromosomes may also explain this variation [11].

On the other hand, out of the several satellites found in *H. punctata* sex chromosomes (see Figure 6) only HviSat04-2959 and its homologs (HroSat09-2961, HduSat08-1754, and HpuSat07-2750), are related to TEs. This satellite has partial homology with LINE-Rex-Babar and Tc/Mariner elements (see Supplementary Tables 4-7), which are usually inactivated or degenerated in fish genomes but can accumulate mutations at neutral rates until losing their molecular identity [113–116]. Both elements have been identified in related Loricariidae species, but as dispersed sequences without any accumulation on a specific chromosome pair [117]. In catfishes, Tc1-Mariner elements can be found associated with rDNA loci variation, suggesting activity in the transposition system [118]. Both satellites HviSat04-2959 and HviSat05-177 and their homologous counterparts, located on sex chromosomes in *H. punctata* but on autosomes in the rest of the species, have been expanding over more than ~20 million years in the four species. However, there has been a significant increase in the number of these satellites in *H. punctata*, which aligns with the observation of extra loci on the X_2 chromosome of this species. TEs are less likely to build up on young X chromosomes in fishes compared to autosomes [119], and this might be the reason for the scarcity of TE-derived satellite sequences in the sex chromosomes of *Harttia*.

The most intriguing relation between a satDNA sequence and sex chromosomes in the genus was observed with HviSat08-4011, which has a partial homology with the ZP4 gene responsible for the formation of the egg envelope. Zona pellucida glycoproteins are generated

in the ovary or liver by ZP genes [120]. The HviSat08-4011 is consistently mapped to autosomes and the linkage group is mapped by the whole chromosome painting probe HPU-X1 across the *Harttia* phylogeny (Figure 7), except in *H. villasboas* and *H. rondoni,* where only autosomal FISH signals were detected. Repeat landscapes show a symmetrical graphical pattern for both homologous and non-homologous ZP4 satDNAs in *H. villasboas* and *H. rondoni*, which would indicate a single autosomal satellite recently originated in both species. However, the graph in *H. duriventris* would be compatible with an older amplification of a satellite formed from partial sequences of the *ZP4* gene in X_1, possibly in addition to a more recent amplification of the composite satellite (composed of the two parts, the homologous and the non-homologous parts to *ZP4* gene) in autosomes as revealed by FISH. This is more evident in *H. punctata* where there are several major peaks representing the most abundant set of sequences homologous to the ZP4 gene, at a divergence between 4 and 10%, compatible with several waves of amplification of a ZP4-derived satDNA on chromosome X_1. The non-homologous to ZP4 gene part, not detected by FISH in *H. punctata*, shows relatively smaller amplification peaks at divergences higher than 16%. The ZP genes in vertebrates undergo frequent gene duplication and loss events, suggesting that they are reproductive genes that evolve rapidly [121]. This rapid evolution may enable them to adapt to various ecological environments [122] and serve as barriers to fertilization [123,124], making them potential targets for natural selection. A fertilization-related gene in the same linkage group as the ancestral of the three *Harttia* clades may imply an ancestral sex chromosome system. Indeed, the linkage group mentioned is exclusively recruited as sex chromosomes in Clade III, which includes the species under investigation. This suggests a turnover event in the emergence of multiple XY_1Y_2 chromosomes in Clade II [11,34,35,78,95].

The species with the most satDNAs accumulated in sex chromosomes (*H. punctata*) is also the oldest divergent in the clade (17.5 My), when compared to a much more recent diversification of *H. villasboas*, *H. rondoni*, and *H. duriventris* clade at 5.5 My [125]. However, each species revealed a distinct satDNA profile, with independent amplification and homogenization events occurring, suggesting an important role of these repetitive sequences in sex chromosome differentiation in a short evolutionary time, especially in recently originated sex chromosomes. Besides, satDNAs may have contributed to the speciation process in *Harttia*, since these sequences play important roles in chromosome pairing and segregation, indicating that the distinct profiles on homologous sex chromosomes may have contributed to reproductive isolation.

Material and methods

Specimens, chromosomal obtainment, and DNA sequencing

We collected samples of nine *Harttia* species, namely *H. dissidens*, *H. duriventris*, *H. guianensis*, *H. kronei*, *H. intermontana*, *H. punctata*, *H. rondoni*, *H. villasboas*, and *Harttia* sp. 3 (a distinct karyomorph from Rio do Peixe, PA, Brazil, described in [37] that is not assembled to any known species), in distinct localities inside the Brazilian territory according to Figure 1 and Table 8. After anesthesia with clove oil (Eugenol diluted in 95% ethanol in a final concentration of 60mg/L in water), as approved by the Ethics Committee on Animal Experimentation of the Universidade Federal de São Carlos (Process number CEUA 7994170423), chromosomes were obtained from the anterior kidney following the classical air-drying technique [126]. The liver and muscle tissues were stored at 4 °C in 100% ethanol for molecular analysis. Total DNA was extracted from these tissues using a silica spin-column-based kit (Cellco Biotech, São Carlos – SP, Brazil). The purified DNA of species with described sex chromosomes (i.e. *H. duriventris*, *H. rondoni*, *H. villasboas*, and *H. punctata*) was

sequenced on BGISEQ-500 platform (BGI Genomics, Shenzhen, China), generating 2 x 150 bp short read sequences. For *H. villasboas* and *H. rondoni*, males and females were sequenced, while for *H. duriventris* and *H. punctata*, only male samples were targeted.

Table 8. Geographical coordinates, diploid number (2n), sex chromosome system, and the number of individuals (n) analyzed in this study. The number before the species name corresponds to the same presented in Figure 1. Specimens were deposited in the Museum of Zoology of the University of São Paulo (MZUSP) and the Ichthyological Collection of the National Institute for Amazonian Research (INPA-ICT). Species highlighted with asterisks correspond to those whose satellitomes were generated, while others were used only for FISH experiments.

Species	2n	Locality	Coordinates	n	Voucher
*1 - *Harttia villasboas*	56♀55♂ X_1X_2Y	Rio Curuá, Altamira (PA)	8°44'09''S 54°57'46''W	34♀38♂	MZUSP 126599
*4 - *Harttia duriventris*	56♀55♂ X_1X_2Y	Canaã dos Carajás (PA)	6°30'06.5''S 50°02'35.5''W	08♀07♂	MZUSP 126598
*2 - *Harttia rondoni*	54♀♂ XY	Rio Curuá, Altamira (PA)	8°38'53''S 55°01'41''W	15♀14♂	MZUSP 127606
*6 - *Harttia punctata*	58♀57♂ X_1X_2Y	Formosa (PA)	15°19'25''S 47°25'26''W	10♀12♂	MZUSP 111385
5 - *Harttia dissidens*	54♀♂	Igarapé Tambor, Rurópolis (PA)	4°5'37.8''S 55°0'30.2''W	07♀25♂	INPA-ICT 059577
9 - *Harttia guianensis*	58♀♂	Rio Curuá, Alenquer (PA)	1°29'02.2''S 54°50'31.2''W	06♀10♂	INPA-ICT 059584
3 - *Harttia* sp. 3	54♀♂	Rio do Peixe, Altamira (PA)	08°39'20.7''S 55°09'24.1''W	11♀15♂	MZUSP 127605
7 - *Harttia kronei*	58♀♂	Rio Açungui, Campo Largo (PR)	25º22'44''S 49º39'0.8''W	10♀, 5♂	MZUSP 109783
8 - *Harttia intermontana*	52♀/53♂ XY1Y2	Piranga river, Carandaí (MG)	20°59'34.0''S 43°43'30.0''W	20♀, 13♂	MZUSP 126520

Satellite DNA library, sequence analysis and primer design

The raw reads obtained after the shotgun sequencing were trimmed with Trimmomatic [127] to select pair-end reads with Q > 20 for all nucleotides. Then, the catalogs of satellite DNAs (satellitome) of each analyzed species (i.e. *H. duriventris*, *H. rondoni*, *H. punctata*, and *H. villasboas*) were characterized by running several iterations of the TAREAN tool [128], each followed by filtering out the reads with similarity to the identified satDNAs using DeconSeq [129], until no satDNA was found. Subsequently, we performed a homology search with RepeatMasker [130] to group the sequences into variants, families, and superfamilies, as suggested by [131]. Abundance and divergence values of each satDNA were estimated by masking male and female genomic libraries against the catalogs of satDNAs with RepeatMasker [130] with the publicly available script (https://github.com/fjruizruano/satminer/blob/master/repeat_masker_run_big.py), using 10,000,000 reads (2×5,000,000). Additionally, repeat landscapes were generated to estimate the average divergence of satDNAs considering the distances between sequences based on the Kimura-2-parameter model using the script calcDivergenceFromAlign.pl of the

RepeatMasker suite. Based on the abundance, satellites were named using the first letter of the genus and the two first letters of the specific epithet (HviSat for *H. villasboas*, HroSat for *H. rondoni*, Hdu for *H. duriventris*, and Hpu for *H. punctata*). To detect sex-specific accumulated satDNAs, we selected those with an F/M ratio (quotient of satDNA female and male abundances) higher than 1.2.

We searched for homology between *Harttia* satellitomes with the rm_homology script [131] that makes all-to-all alignments with RepeatMasker v4.0.5 [130]. For correspondent satellites between species, we did pairwise alignments using ClustalX [132], which were manually revised. Sequence divergence between satDNA families of each pairwise comparison was calculated following Kimura's two-parameter method (K2P) [133] using MEGA11 [134]. A consensus turnover rate (CTR) was calculated using the CTR = K/2T equation, where T = divergence time (see nodes in the phylogenetic tree of Figure 1) between species and K = K2P distance [109].

To check the presence of conserved satDNAs, the whole satellitome was BLAST-searched [135] against NCBI nucleotide collection. Putative open reading frames were prospected in the satellitomes using "orfipy" [136] and Geneious 7.1.3, with a minimum size of 100 bp. Identified ORFs were translated to protein sequences using SMS [137] and searched against the NCBI database using BLASTp [138], following filtering for query cover and percentage identity >70%.

In addition, we searched Repbase [139] for homologies with transposable elements with RepeatMasker [130] with "no_low" and "no_is" options.

We searched for some satDNAs like HviSat08-4011 among the Illumina reads of species like *H. duriventris* and *H. punctata*, from which we could not isolate its homologous counterpart with TAREAN, using the publicly available script mapping_blat_gs.py (https://github.com/fjruizruano/ngs-protocols/blob/master/mapping_blat_gs.py). Raw reads of both species were mapped and quantified against every two parts described in HviSat08-4011 using the script repeat_masker_run_big.py, as described above.

Primers were designed for the eight most abundant of the total 21 satDNAs identified in *H. villasboas* using the abovementioned process. We selected *H. villasboas* for the construction of probes since this species has multiple X_1X_2Y and is more closely related to the XY-carrier species *H. rondoni* (when compared to *H. duriventris* and *H. punctata*), which allows us to infer the role of the rearrangements that occurred in the clade. The consensus sequences for each of those satDNAs were manually investigated to design PCR primers (Supplementary Table 1). Then, satDNAs were amplified by PCR using a starting denaturation step of 95 °C for 5 min, 30-35 cycles with 95 °C during 20 s, with 39.1–54.2°C as annealing temperature during 40 s, 72 °C during 30 s, and a final extension step of 10 min (Supplementary Table 1). The resulting PCR products were checked on a 1% agarose gel to confirm the typical ladder pattern characteristic of satDNAs (Supplementary Figure 1). Sequences were deposited on Genbank (Access numbers: OR827473-OR827555).

Fluorescence in situ Hybridization

According to the manufacturer's instructions, the selected PCR-amplified satellites of *H. villasboas* were directly labeled with Atto550-dUTP or Atto488-dUTP by Nick-Translation (Jena Biosciences, Jena, Germany). Probes were composed of 100 ng of each labeled satellite DNA plus 50% formamide, 2× SSC, 10% SDS, 10% dextran sulphate, and Denhardt's buffer at pH 7.0 in a total volume of 20 µl. FISH experiments were conducted firstly in *H. villasboas* slides with every single probe, then in double-FISH for sister species *H. rondoni*, *H. duriventris,* and *H. punctata,* in addition to *H. dissidens*, *H. guianensis*, and *Harttia* sp. 3. Following the results of satDNAs probed on sex chromosomes, we performed whole chromosome painting (WCP), together with a FISH mapping of these satellites, using probes

derived from microdissection of the sex chromosomes of *H. punctata* (HPU-X1 and HPU-X2), as previously described in [34] and [11]. For the investigation of sex chromosome evolution, we included two species (*H. kronei* and *H. intermontana*) that carry the ancestral condition of the linkage groups that compose the multiple X_1X_2Y (i.e., both HPU-X1 and HPU-X2 located in synteny on the same chromosome) [34]. In these last two species, we mapped the HviSatDNAs that were found accumulated in the sex chromosomes of the four main targeted species and detected the ancestral linkage groups through WCP. Chromosomes were denatured in 70% Formamide/2×SSC at 72 °C for 3 min, probes at 86 °C for 8 min, then cooled at 4 °C for 2 min before being applied to slides. The hybridization was carried out for 16h in a dark, moist chamber at 37 °C. Following a 5-min post-hybridization wash with 1×SSC at 65 °C, next with a 4×SSC/Tween solution at room temperature. Finally, chromosomes were counterstained with DAPI mounted in Vectashield (Vector Laboratories, Burlingame, USA). For the WCP experiment, chromosomes were denatured as abovementioned, but the probes were denatured at 85 °C for 8 min, cooled at 4 °C for 2 min, and pre-hybridized with the blocking sequence (unlabeled C0t-1 DNA, following [140]) at 37 °C for 1 h. Hybridization occurred for 48h overnight at 37 °C in a dark, moist chamber. The final wash was performed using the same procedure as described above.

Images and analysis

To confirm the 2n number, karyotype structure, and FISH results, at least 30 metaphase spreads per individual were examined. Images were captured with CoolSNAP on an Axioplan II microscope (Carl Zeiss Jena GmbH, Germany) and processed with ISIS (MetaSystems Hard & Software GmbH, Altlussheim, Germany). The map was generated using QGIS 3.32 (Lima) with a Natural Earth package and riverine information from HydroRIVERS [141].

Data availability

Sequencing data that support the findings of this study have been deposited in Genbank, with their accession codes OR827473-OR827555 (http://www.ncbi.nlm.nih.gov/nuccore/OR827473).

References

1. White, M. The origin and evolution of multiple sex chromosome mechanisms. *Journal of Genetics* 303–306 (1940).
2. Kitano, J. & Peichel, C. L. Turnover of sex chromosomes and speciation in fishes. *Environmental biology of fishes* **94**, 549–558 (2012).
3. Pokorná, M., Altmanová, M. & Kratochvíl, L. Multiple sex chromosomes in the light of female meiotic drive in amniote vertebrates. *Chromosome Res* **22**, 35–44 (2014).
4. Pennell, M. W. *et al.* Y Fuse? Sex Chromosome Fusions in Fishes and Reptiles. *PLoS Genetics* **11**, 1–17 (2015).
5. Sember, A. *et al.* Multiple sex chromosomes in teleost fishes from a cytogenetic perspective: state of the art and future challenges. *Philosophical Transactions of the Royal Society B* **376**, 20200098 (2021).
6. Ming, R., Bendahmane, A. & Renner, S. S. Sex Chromosomes in Land Plants. *Annual Review of Plant Biology* **62**, 485–514 (2011).

7. Blanco, D. R. *et al.* The role of the Robertsonian rearrangements in the origin of the XX/XY1Y2 sex chromosome system and in the chromosomal differentiation in *Harttia* species (Siluriformes, Loricariidae). *Reviews in Fish Biology and Fisheries* **23**, 127–134 (2013).
8. Šíchová, J. *et al.* Fissions, fusions, and translocations shaped the karyotype and multiple sex chromosome constitution of the northeast-Asian wood white butterfly, *Leptidea amurensis*. *Biological Journal of the Linnean Society* **118**, 457–471 (2016).
9. Giovannotti, M. *et al.* New insights into sex chromosome evolution in anole lizards (Reptilia, Dactyloidae). *Chromosoma* **126**, 245–260 (2017).
10. Deon, G. A. *et al.* Chromosomal Rearrangements and Origin of the Multiple XX/XY1Y2 Sex Chromosome System in *Harttia* Species (Siluriformes: Loricariidae). *Frontiers in Genetics* **13**, (2022).
11. Sassi, F. M. C. *et al.* Homeology of sex chromosomes in Amazonian *Harttia* armored catfishes supports the X-fission hypothesis for the X1X2Y sex chromosome system origin. *Sci Rep* **13**, 15756 (2023).
12. Dutrillaux, B., Dutrillaux, A.-M., Salazar, K. & Boucher, S. Multiple Chromosome Fissions, Including That of the X Chromosome, in *Aulacocyclus tricuspis* Kaup (Coleoptera, Passalidae) from New Caledonia: Characterization of a Rare but Recurrent Pathway of Chromosome Evolution in Animals. *Genes* **14**, 1487 (2023).
13. Muller, H. J. A Factor for the Fourth Chromosome of *Drosophila*. *Science* **39**, 906–906 (1914).
14. Ohno, S. Conservation of the Original Z-Chromosome by Diverse Avian Species and Homology of the Z-linked Genes. In *Sex Chromosomes and Sex-linked Genes* (ed. Ohno, S.) 73–81 (Springer, Berlin, Heidelberg, 1966). doi:10.1007/978-3-662-35113-0_6.
15. Bull, J. J. *Evolution of Sex Determining Mechanisms*. vol. 1 (The Benjamin/Cummings Publishing Company, Inc., Menlo Park, California, USA, 1983).
16. Charlesworth, B. The Evolution of Sex Chromosomes. *Science* **251**, 1030–1033 (1991).
17. Graves, J. A. M. Evolution of vertebrate sex chromosomes and dosage compensation. *Nat Rev Genet* **17**, 33–46 (2016).
18. Fisher, R. A. The evolution of dominance. *Biological Reviews of the Cambridge Philosophical Society* **6**, 345–368 (1931).
19. Nei, M. Linkage modification and sex difference in recombination. *Genetics* **63**, 681–699 (1969).
20. Rice, W. R. Sex Chromosomes and the Evolution of Sexual Dimorphism. *Evolution* **38**, 735–742 (1984).
21. Charlesworth, B., Jordan, C. Y. & Charlesworth, D. The evolutionary dynamics of sexually antagonistic mutations in pseudoautosomal regions of sex chromosomes. *Evolution* **68**, 1339–1350 (2014).
22. Schartl, M., Schmid, M. & Nanda, I. Dynamics of vertebrate sex chromosome evolution: from equal size to giants and dwarfs. *Chromosoma* **125**, 553–571 (2016).
23. Kratochvíl, L. *et al.* Expanding the classical paradigm: what we have learnt from vertebrates about sex chromosome evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* **376**, 20200097 (2021).
24. Nanda, I., Schartl, M., Feichtinger, W., Epplen, J. T. & Schmid, M. Early stages of sex chromosome differentiation in fish as analysed by simple repetitive DNA sequences. *Chromosoma* **101**, 301–310 (1992).
25. Rovatsos, M., Vukić, J., Lymberakis, P. & Kratochvíl, L. Evolutionary stability of sex chromosomes in snakes. *Proceedings of the Royal Society B: Biological Sciences* **282**, 20151992 (2015).
26. Stöck, M. *et al.* Sex chromosomes in meiotic, hemiclonal, clonal and polyploid hybrid vertebrates: along the 'extended speciation continuum'. *Philosophical Transactions of the Royal Society B: Biological Sciences* **376**, 20200103 (2021).
27. Pla, S., Benvenuto, C., Capellini, I. & Piferrer, F. Switches, stability and reversals in the evolutionary history of sexual systems in fish. *Nat Commun* **13**, 3029 (2022).

28. Arai, R. *Fish Karyotypes: A Check List*. (Springer Science & Business Media, 2011).
29. Marajó, L. *et al.* Chromosomal rearrangements and the first indication of an ♀X1X1X2X2/♂X1X2Y sex chromosome system in *Rineloricaria* fishes (Teleostei: Siluriformes). *Journal of Fish Biology* **102**, 443–454 (2023).
30. Nirchio, M. *et al.* Occurrence of Sex Chromosomes in Fish of the Genus *Ancistrus* with a New Description of Multiple Sex Chromosomes in the Ecuadorian Endemic *Ancistrus clementinae* (Loricariidae). *Genes* **14**, (2023).
31. de Araújo, L. *et al.* Cytogenetic and Molecular Characterization of *Eigenmannia* aff. *desantanai* (Gymnotiformes: Sternopygidae): A First Report of System of Sex Chromosomes ZW1W2/ZZ in Gymnotiformes. *Zebrafish* **20**, 77–85 (2023).
32. Oyakawa, O. T., Fichberg, I. & Py-Daniel, L. R. Three new species of *Harttia* (Loricariidae: Loricariinae) from Serra do Cachimbo, Rio Xingu basin, Pará, Northern Brazil. *Zootaxa* **4387**, 75–90 (2018).
33. Fricke, R., Eschmeyer, W. N. & van der Laan, R. Eschmeyer's catalog of fishes: Genera, Species, References. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (2024).
34. Deon, G. A. *et al.* Evolutionary breakpoint regions and chromosomal remodeling in *Harttia* (Siluriformes: Loricariidae) species diversification. *Genetics and Molecular Biology* **45**, (2022).
35. Sassi, F. M. C. *et al.* Turnover of multiple sex chromosomes in *Harttia* catfish (Siluriformes, Loricariidae): a glimpse from whole chromosome painting. *Front. Genet.* **14**, 1226222 (2023).
36. Sassi, F. M. C. *et al.* Multiple sex chromosomes and evolutionary relationships in amazonian catfishes: The outstanding model of the genus *Harttia* (siluriformes: Loricariidae). *Genes* **11**, (2020).
37. Sassi, F. M. C. *et al.* Adding New Pieces to the Puzzle of Karyotype Evolution in *Harttia* (Siluriformes, Loricariidae): Investigation of Amazonian Species. *Biology* **10**, (2021).
38. Deon, G. A. *et al.* Highly Rearranged Karyotypes and Multiple Sex Chromosome Systems in Armored Catfishes from the Genus *Harttia* (Teleostei, Siluriformes). *Genes* **11**, 1366 (2020).
39. Covain, R. *et al.* Molecular phylogeny of the highly diversified catfish subfamily Loricariinae (Siluriformes, Loricariidae) reveals incongruences with morphological classification. *Molecular Phylogenetics and Evolution* **94**, 492–517 (2016).
40. Kumar, S., Stecher, G., Suleski, M. & Hedges, S. B. TimeTree: A Resource for Timelines, Timetrees, and Divergence Times. *Molecular Biology and Evolution* **34**, 1812–1819 (2017).
41. Garrido-Ramos, M. A. Satellite DNA: An evolving topic. *Genes* **8**, (2017).
42. Thakur, J., Packiaraj, J. & Henikoff, S. Sequence, Chromatin and Evolution of Satellite DNA. *International Journal of Molecular Sciences* **22**, 4309 (2021).
43. Kuhn, G. C. S. Satellite DNA transcripts have diverse biological roles in *Drosophila*. *Heredity* **115**, 1–2 (2015).
44. Cioffi, M. de B. & Bertollo, L. A. C. Chromosomal distribution and evolution of repetitive DNAs in fish. in *Repetitive DNA* (ed. Garrido-Ramos, M. A.) 197–221 (Karger, Basel, 2012). doi:10.1159/000337950.
45. Gaffaroglu, M. *et al.* Present and Future Salmonid Cytogenetics. *Genes* **11**, 1462 (2020).
46. Crepaldi, C. & Parise-Maltempi, P. P. Heteromorphic Sex Chromosomes and Their DNA Content in Fish: An Insight through Satellite DNA Accumulation in *Megaleporinus elongatus*. *Cytogenetic and Genome Research* **160**, 38–46 (2020).
47. Favarato, R. M. *et al.* Comparative cytogenetics of Serrasalmidae (Teleostei: Characiformes): The relationship between chromosomal evolution and molecular phylogenies. *PLOS ONE* **16**, e0258003 (2021).
48. Voleníková, A. *et al.* Fast satellite DNA evolution in *Nothobranchius* annual killifishes. *Chromosome Res* **31**, 33 (2023).

49. Deakin, J. E. *et al.* Chromosomics: Bridging the Gap between Genomes and Chromosomes. *Genes* **10**, 627 (2019).
50. Serrano-Freitas, É. A. *et al.* Satellite DNA content of B chromosomes in the characid fish *Characidium gomesi* supports their origin from sex chromosomes. *Mol Genet Genomics* **295**, 195–207 (2020).
51. Goes, C. A. G. *et al.* Revealing the Satellite DNA History in *Psalidodon* and *Astyanax* Characid Fish by Comparative Satellitomics. *Front. Genet.* **13**, (2022).
52. Štundlová, J. *et al.* Sex chromosome differentiation via changes in the Y chromosome repeat landscape in African annual killifishes *Nothobranchius furzeri* and *N. kadleci*. *Chromosome Res* **30**, 309–333 (2022).
53. Rocha-Reis, D. A. *et al.* In Silico characterization of satellitomes and cross-amplification of putative satDNAs in two species of the *Hypostomus ancistroides* complex (Siluriformes, Loricariidae). *Cytogenetic and Genome Research* 1 (2024) doi:10.1159/000539429.
54. Utsunomia, R. *et al.* Satellitome landscape analysis of *Megaleporinus macrocephalus* (Teleostei, Anostomidae) reveals intense accumulation of satellite sequences on the heteromorphic sex chromosome. *Sci Rep* **9**, 5856 (2019).
55. Ferretti, A. B. S. M., Milani, D., Palacios-Gimenez, O. M., Ruiz-Ruano, F. J. & Cabral-de-Mello, D. C. High dynamism for neo-sex chromosomes: satellite DNAs reveal complex evolution in a grasshopper. *Heredity* **125**, 124–137 (2020).
56. Shatskikh, A. S., Kotov, A. A., Adashev, V. E., Bazylev, S. S. & Olenina, L. V. Functional Significance of Satellite DNAs: Insights From *Drosophila*. *Front. Cell Dev. Biol.* **8**, (2020).
57. Cabral-de-Mello, D. C., Zrzavá, M., Kubíčková, S., Rendón, P. & Marec, F. The Role of Satellite DNAs in Genome Architecture and Sex Chromosome Evolution in Crambidae Moths. *Front. Genet.* **12**, (2021).
58. de Oliveira, M. P. B. *et al.* Following the Pathway of W Chromosome Differentiation in *Triportheus* (Teleostei: Characiformes). *Biology* **12**, 1114 (2023).
59. Yunis, J. J. & Yasmineh, W. G. Heterochromatin, Satellite DNA, and Cell Function. *Science* **174**, 1200–1209 (1971).
60. Bonaccorsi, S. & Lohe, A. Fine mapping of satellite DNA sequences along the Y chromosome of *Drosophila melanogaster*: relationships between satellite sequences and fertility factors. *Genetics* **129**, 177–189 (1991).
61. Harvey, S. C. *et al.* Analysis of repetitive DNA sequences in the sex chromosomes of *Oreochromis niloticus*. *Cytogenetic and Genome Research* **101**, 314–319 (2003).
62. Krzywinski, J., Sangaré, D. & Besansky, N. J. Satellite DNA From the Y Chromosome of the Malaria Vector *Anopheles gambiae*. *Genetics* **169**, 185–196 (2005).
63. Schemberger, M. O. *et al.* Construction and Characterization of a Repetitive DNA Library in Parodontidae (Actinopterygii: Characiformes): A Genomic and Evolutionary Approach to the Degeneration of the W Sex Chromosome. *Zebrafish* **11**, 518–527 (2014).
64. Kretschmer, R. *et al.* Satellitome analysis illuminates the evolution of ZW sex chromosomes of Triportheidae fishes (Teleostei: Characiformes). *Chromosoma* **131**, 29–45 (2022).
65. Palacios-Gimenez, O. M. *et al.* High-throughput analysis of the satellitome revealed enormous diversity of satellite DNAs in the neo-Y chromosome of the cricket *Eneoptera surinamensis*. *Sci Rep* **7**, 6422 (2017).
66. Navajas-Pérez, R. *et al.* The Evolution of Reproductive Systems and Sex-Determining Mechanisms Within *Rumex* (Polygonaceae) Inferred from Nuclear and Chloroplastidial Sequence Data. *Molecular Biology and Evolution* **22**, 1929–1939 (2005).
67. Navajas-Pérez, R. *et al.* The origin and evolution of the variability in a Y-specific satellite-DNA of *Rumex acetosa* and its relatives. *Gene* **368**, 61–71 (2006).
68. Navajas-Pérez, R., Quesada del Bosque, M. E. & Garrido-Ramos, M. A. Effect of location, organization, and repeat-copy number in satellite-DNA evolution. *Mol Genet Genomics* **282**, 395–406 (2009).

69. Navajas-Pérez, R., Schwarzacher, T., Rejón, M. R. & Garrido-Ramos, M. A. Molecular cytogenetic characterization of *Rumex papillaris*, a dioecious plant with an XX/XY1Y2 sex chromosome system. *Genetica* **135**, 87–93 (2009).
70. Hobza, R. *et al.* An accumulation of tandem DNA repeats on the Y chromosome in *Silene latifolia* during early stages of sex chromosome evolution. *Chromosoma* **115**, 376–382 (2006).
71. Steflova, P. *et al.* Contrasting Patterns of Transposable Element and Satellite Distribution on Sex Chromosomes (XY1Y2) in the Dioecious Plant *Rumex acetosa*. *Genome Biology and Evolution* **5**, 769–782 (2013).
72. Jesionek, W. *et al.* Fundamentally different repetitive element composition of sex chromosomes in *Rumex acetosa*. *Annals of Botany* **127**, 33–47 (2021).
73. Cabral-de-Mello, D. C. *et al.* The spread of satellite DNAs in euchromatin and insights into the multiple sex chromosome evolution in Hemiptera revealed by repeatome analysis of the bug *Oxycarenus hyalinipennis*. *Insect Molecular Biology* **32**, 725–737 (2023).
74. De Moraes, R. L. R. *et al.* Chromosomal Rearrangements and Satellite DNAs: Extensive Chromosome Reshuffling and the Evolution of Neo-Sex Chromosomes in the Genus *Pyrrhulina* (Teleostei; Characiformes). *IJMS* **24**, 13654 (2023).
75. Ramos, L. & Antunes, A. Decoding sex: Elucidating sex determination and how high-quality genome assemblies are untangling the evolutionary dynamics of sex chromosomes. *Genomics* **114**, 110277 (2022).
76. Fry, K. & Salser, W. Nucleotide sequences of HS-α satellite DNA from kangaroo rat *Dipodomys ordii* and characterization of similar sequences in other rodents. *Cell* **12**, 1069–1084 (1977).
77. Utsunomia, R. *et al.* A glimpse into the satellite DNA library in Characidae fish (Teleostei, Characiformes). *Frontiers in Genetics* **8**, (2017).
78. Deon, G. A. *et al.* The role of satellite DNAs in the chromosomal rearrangements and the evolution of the rare XY1Y2 sex system in *Harttia* (Siluriformes: Loricariidae). *Journal of Heredity* esae028 (2024) doi:10.1093/jhered/esae028.
79. Ahmad, S. F. *et al.* Dark Matter of Primate Genomes: Satellite DNA Repeats and Their Evolutionary Dynamics. *Cells* **9**, 2714 (2020).
80. Gutiérrez, J. *et al.* Satellitome Analysis on *Talpa aquitania* Genome and Inferences about the satDNAs Evolution on Some Talpidae. *Genes* **14**, 117 (2023).
81. Lisachov, A., Rumyantsev, A., Prokopov, D., Ferguson-Smith, M. & Trifonov, V. Conservation of Major Satellite DNAs in Snake Heterochromatin. *Animals* **13**, 334 (2023).
82. Mestrović, N., Plohl, M., Mravinac, B. & Ugarković, D. Evolution of satellite DNAs from the genus *Palorus*--experimental evidence for the 'library' hypothesis. *Molecular Biology and Evolution* **15**, 1062–1068 (1998).
83. Palacios-Gimenez, O. M. *et al.* Comparative analysis of morabine grasshopper genomes reveals highly abundant transposable elements and rapidly proliferating satellite DNA repeats. *BMC Biol* **18**, 199 (2020).
84. Quesada del Bosque, M. E., Navajas-Pérez, R., Panero, J. L., Fernández-González, A. & Garrido-Ramos, M. A. A satellite DNA evolutionary analysis in the North American endemic dioecious plant *Rumex hastatulus* (Polygonaceae). *Genome* **54**, 253–260 (2011).
85. Quesada del Bosque, M. E., López-Flores, I., Suárez-Santiago, V. N. & Garrido-Ramos, M. A. Differential spreading of HinfI satellite DNA variants during radiation in Centaureinae. *Annals of Botany* **112**, 1793–1802 (2013).
86. del Bosque, M. E. Q., López-Flores, I., Suárez-Santiago, V. N. & Garrido-Ramos, M. A. Satellite-DNA diversification and the evolution of major lineages in Cardueae (Carduoideae Asteraceae). *J Plant Res* **127**, 575–583 (2014).
87. Šatović-Vukšić, E. & Plohl, M. Satellite DNAs—From Localized to Highly Dispersed Genome Components. *Genes* **14**, 742 (2023).

88. Casacuberta, E. Drosophila: Retrotransposons Making up Telomeres. *Viruses* **9**, 192 (2017).
89. Talbert, P. B. & Henikoff, S. What makes a centromere? *Experimental Cell Research* **389**, 111895 (2020).
90. Blanco, D. R. *et al.* 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**, (2014).
91. Berthelot, C., Muffato, M., Abecassis, J. & Crollius, H. R. The 3D organization of chromatin explains evolutionary fragile genomic regions. *Cell reports* **10**, 1913–1924 (2015).
92. Warmerdam, D. O. & Wolthuis, R. M. F. Keeping ribosomal DNA intact: a repeating challenge. *Chromosome Research* **27**, 57–72 (2019).
93. Barros, A. V. *et al.* Fragile sites, dysfunctional telomere and chromosome fusions: What is 5S rDNA role? *Gene* **608**, 20–27 (2017).
94. Glugoski, L., Giuliano-Caetano, L., Moreira-Filho, O., Vicari, M. R. & Nogaroto, V. Co-located hAT transposable element and 5S rDNA in an interstitial telomeric sequence suggest the formation of Robertsonian fusion in armored catfish. *Gene* **650**, 49–54 (2018).
95. Glugoski, L., Deon, G. A., Nogaroto, V., Moreira-Filho, O. & Vicari, M. R. Robertsonian Fusion Site in *Rineloricaria pentamaculata* (Siluriformes: Loricariidae): Involvement of 5S Ribosomal DNA and Satellite Sequences. *Cytogenet Genome Res* **162**, 657–664 (2022).
96. Lim, K. Y. *et al.* Dynamic Changes in the Distribution of a Satellite Homologous to Intergenic 26-18S rDNA Spacer in the Evolution of *Nicotiana*. *Genetics* **166**, 1935–1946 (2004).
97. Jo, S.-H. *et al.* Evolution of ribosomal DNA-derived satellite repeat in tomato genome. *BMC Plant Biol* **9**, 42 (2009).
98. Gerbi, S. A. Bundling up DNA. *eLife* **7**, e37234 (2018).
99. Jagannathan, M., Cummings, R. & Yamashita, Y. M. A conserved function for pericentromeric satellite DNA. *eLife* **7**, e34122 (2018).
100. Ahmed, M. & Liang, P. Transposable Elements Are a Significant Contributor to Tandem Repeats in the Human Genome. *International Journal of Genomics* **2012**, 947089 (2012).
101. Wong, L. H. & Choo, K. H. A. Evolutionary dynamics of transposable elements at the centromere. *Trends in Genetics* **20**, 611–616 (2004).
102. Kapitonov, V. V. & Jurka, J. The Long Terminal Repeat of an Endogenous Retrovirus Induces Alternative Splicing and Encodes an Additional Carboxy-Terminal Sequence in the Human Leptin Receptor. *J Mol Evol* **48**, 248–251 (1999).
103. Volff, J.-N., Hornung, U. & Schartl, M. Fish retroposons related to the Penelope element of *Drosophila virilis* define a new group of retrotransposable elements. *Mol Gen Genomics* **265**, 711–720 (2001).
104. Eickbush, T. H. & Malik, H. S. Origins and Evolution of Retrotransposons. in *Mobile DNA II* 1111–1144 (John Wiley & Sons, Ltd, 2007). doi:10.1128/9781555817954.ch49.
105. Arkhipova, I. R. Distribution and Phylogeny of Penelope-Like Elements in Eukaryotes. *Systematic Biology* **55**, 875–885 (2006).
106. Thomas, J. & Pritham, E. J. Helitrons, the Eukaryotic Rolling-circle Transposable Elements. in *Mobile DNA III* 891–924 (John Wiley & Sons, Ltd, 2015). doi:10.1128/9781555819217.ch40.
107. Satović, E., Vojvoda Zeljko, T., Luchetti, A., Mantovani, B. & Plohl, M. Adjacent sequences disclose potential for intra-genomic dispersal of satellite DNA repeats and suggest a complex network with transposable elements. *BMC Genomics* **17**, 997 (2016).
108. Vojvoda Zeljko, T., Pavlek, M., Meštrović, N. & Plohl, M. Satellite DNA-like repeats are dispersed throughout the genome of the Pacific oyster *Crassostrea gigas* carried by Helentron non-autonomous mobile elements. *Sci Rep* **10**, 15107 (2020).
109. Camacho, J. P. M. *et al.* Satellitome comparison of two oedipodine grasshoppers highlights the contingent nature of satellite DNA evolution. *BMC Biol* **20**, 36 (2022).

110. Cardone, M. F. *et al.* Evolution of Beta Satellite DNA Sequences: Evidence for Duplication-Mediated Repeat Amplification and Spreading. *Molecular Biology and Evolution* **21**, 1792–1799 (2004).
111. Ma, J. & Jackson, S. A. Retrotransposon accumulation and satellite amplification mediated by segmental duplication facilitate centromere expansion in rice. *Genome Res.* **16**, 251–259 (2006).
112. Plohl, M., Meštrović, N. & Mravinac, B. Centromere identity from the DNA point of view. *Chromosoma* (2014) doi:10.1007/s00412-014-0462-0.
113. Izsvák, Z., Ivics, Z. & Hackett, P. B. Characterization of a Tc1-like transposable element in zebrafish (*Danio rerio*). *Molec. Gen. Genet.* **247**, 312–322 (1995).
114. Munoz-Lopez, M. & Garcia-Perez, J. L. DNA Transposons: Nature and Applications in Genomics. *Current Genomics* **11**, 115–128 (2010).
115. Primo, C. C., Glugoski, L., Vicari, M. R. & Nogaroto, V. Chromosome Mapping and Molecular Characterization of the Tc1/Mariner Element in *Rineloricaria* (Siluriformes: Loricariidae). *Braz. arch. biol. technol.* **61**, (2018).
116. Schemberger, M. O. *et al.* DNA transposon invasion and microsatellite accumulation guide W chromosome differentiation in a Neotropical fish genome. *Chromosoma* **128**, 547–560 (2019).
117. Ayres-Alves, T. *et al.* Karyotypic Evolution and Chromosomal Organization of Repetitive DNA Sequences in Species of *Panaque* , *Panaqolus* , and *Scobinancistrus* (Siluriformes and Loricariidae) from the Amazon Basin. *Zebrafish* **14**, 251–260 (2017).
118. Gouveia, J. G. *et al.* Repetitive DNA in the Catfish Genome: rDNA, Microsatellites, and Tc1-Mariner Transposon Sequences in *Imparfinis* Species (Siluriformes, Heptapteridae). *Journal of Heredity* **108**, 650–657 (2017).
119. Chalopin, D., Volff, J.-N., Galiana, D., Anderson, J. L. & Schartl, M. Transposable elements and early evolution of sex chromosomes in fish. *Chromosome research* **23**, 545–560 (2015).
120. Bausek, N., Waclawek, M., Schneider, W. J. & Wohlrab, F. The Major Chicken Egg Envelope Protein ZP1 Is Different from ZPB and Is Synthesized in the Liver. *Journal of Biological Chemistry* **275**, 28866–28872 (2000).
121. Feng, J.-M., Tian, H.-F., Hu, Q.-M., Meng, Y. & Xiao, H.-B. Evolution and multiple origins of zona pellucida genes in vertebrates. *Biology Open* **7**, bio036137 (2018).
122. Cao, L. *et al.* Neofunctionalization of zona pellucida proteins enhances freeze-prevention in the eggs of Antarctic notothenioids. *Nat Commun* **7**, 12987 (2016).
123. Bianchi, E. & Wright, G. J. Cross-species fertilization: the hamster egg receptor, Juno, binds the human sperm ligand, Izumo1. *Philosophical Transactions of the Royal Society B: Biological Sciences* **370**, 20140101 (2015).
124. Claw, K. G. & Swanson, W. J. Evolution of the Egg: New Findings and Challenges. *Annual Review of Genomics and Human Genetics* **13**, 109–125 (2012).
125. Cassemiro, F. A. S. *et al.* Landscape dynamics and diversification of the megadiverse South American freshwater fish fauna. *Proceedings of the National Academy of Sciences* **120**, e2211974120 (2023).
126. Bertollo, L. A. C., Cioffi, M. de B. & Moreira-Filho, O. Direct chromosome preparation from Freshwater Teleost Fishes. in *Fish cytogenetic techniques (Chondrichthyans and Teleosts)* (eds. Ozouf-Costaz, C., Pisano, E., Foresti, F. & Almeida Toledo, L. F.) 21–26 (CRC Press, Enfield USA, 2015). doi:10.1201/b18534-4.
127. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
128. Novák, P. *et al.* TAREAN: a computational tool for identification and characterization of satellite DNA from unassembled short reads. *Nucleic Acids Research* **45**, e111 (2017).
129. Schmieder, R. & Edwards, R. Fast Identification and Removal of Sequence Contamination from Genomic and Metagenomic Datasets. *PLOS ONE* **6**, e17288 (2011).
130. Smit, A., Hubley, R. & Green, P. RepeatMasker Open. (2013).

131. Ruiz-Ruano, F. J., López-León, M. D., Cabrero, J. & Camacho, J. P. M. High-throughput analysis of the satellitome illuminates satellite DNA evolution. *Scientific Reports* **6**, (2016).
132. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. The CLUSTAL_X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools. *Nucleic Acids Research* **25**, 4876–4882 (1997).
133. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120 (1980).
134. Kumar, S., Stecher, G. & Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular biology and evolution* **33**, (2016).
135. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *Journal of Molecular Biology* **215**, 403–410 (1990).
136. Singh, U. & Wurtele, E. S. orfipy: a fast and flexible tool for extracting ORFs. *Bioinformatics* **37**, 3019–3020 (2021).
137. Stothard, P. The Sequence Manipulation Suite: JavaScript programs for analyzing and formatting protein and DNA sequences. (2000).
138. Altschul, S. F. *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**, 3389–3402 (1997).
139. Bao, W., Kojima, K. K. & Kohany, O. Repbase Update, a database of repetitive elements in eukaryotic genomes. *Mobile DNA* **6**, 11 (2015).
140. Zwick, M. S. *et al.* A rapid procedure for the isolation of C 0 t-1 DNA from plants. *Genome* **40**, 138–142 (1997).
141. Lehner, B. & Grill, G. Global river hydrography and network routing: baseline data and new approaches to study the world's large river systems. *Hydrological Processes* **27**, 2171–2186 (2013).

Acknowledgements

The authors are grateful to Flávio Henrique Silva and Eduardo Pereira de Souza for the help with sequencing, to Foyez Shamz for the valuable insights, and to Jhon Alex Dziechciarz Vidal for the assistance in the ORFs identification. We are also grateful to Eliana Feldberg, Osvaldo Takeshi Oyakawa and Lúcia Helena Rapp Py-Daniel for the identification of the samples, and to Luiz Henrique da Silva, Antônio Aparecido Donizetti da Silva, Ezequiel Aguiar de Oliveira and Patrik Viana for assistance in field work. Francisco de M. C. Sassi was supported by FAPESP (Proc. numbers 2020/02681-9; 2022/04261-2 and 2023/08116-0). MBC was founded by FAPESP (2023/00955-2) and CNPq (302928/2021-9). TL was founded by the German Research Foundation Projekt-Nr. 512648189 and the Open Access Publication Fund of the Thueringer Universitaets- und Landesbibliothek Jena. This study was partially funded by CAPES Finance code 001 and INCT – Peixes (proc. 405706/2022-7).

Author contributions

FMCS, MAGR, GAD, and MBC conceived and designed research. FMCS, MAGR, RZS, RU and GAD conducted experiments. FMCS, GAD, RZS, RU, TE, FPF, TL, and MBC analyzed the data. MAGR, RZS, and RU contributed with new methods. FMCS, MAGR, RZS, RU, TE, GAD, FPF, TL, and MBC wrote the paper.

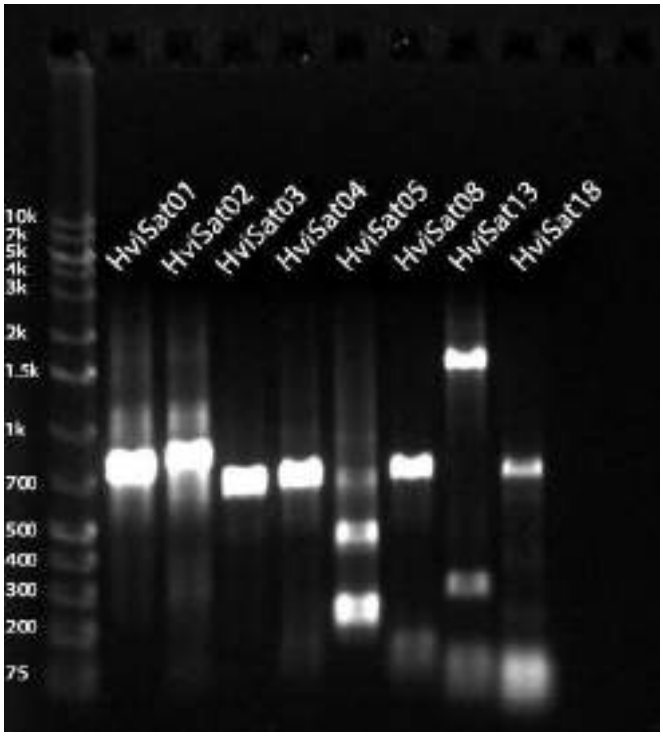
Competing interests

The authors declare no competing interests. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

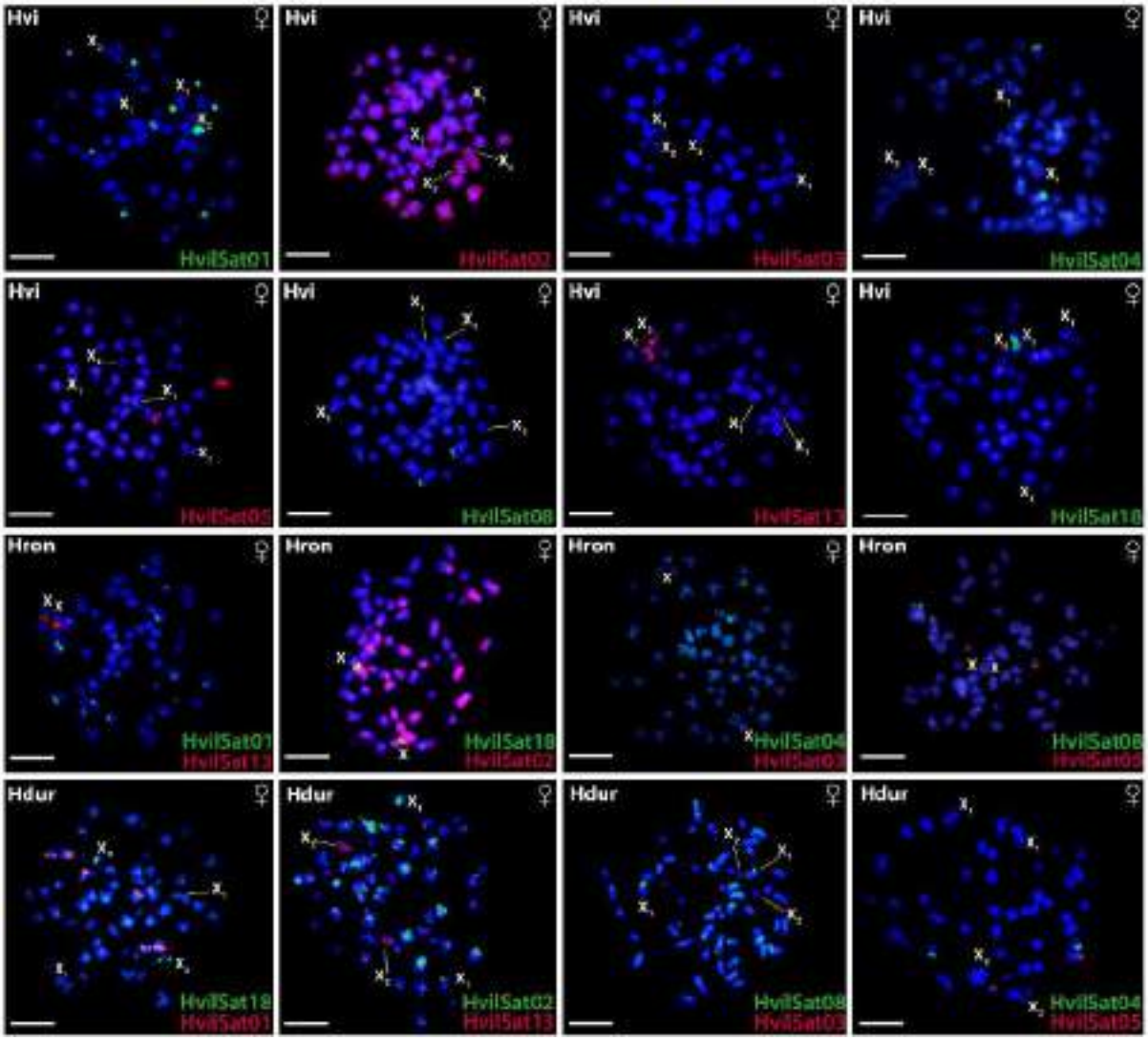
Supplementary Information

Independent evolution of satellite DNA sequences in homologous sex chromosomes

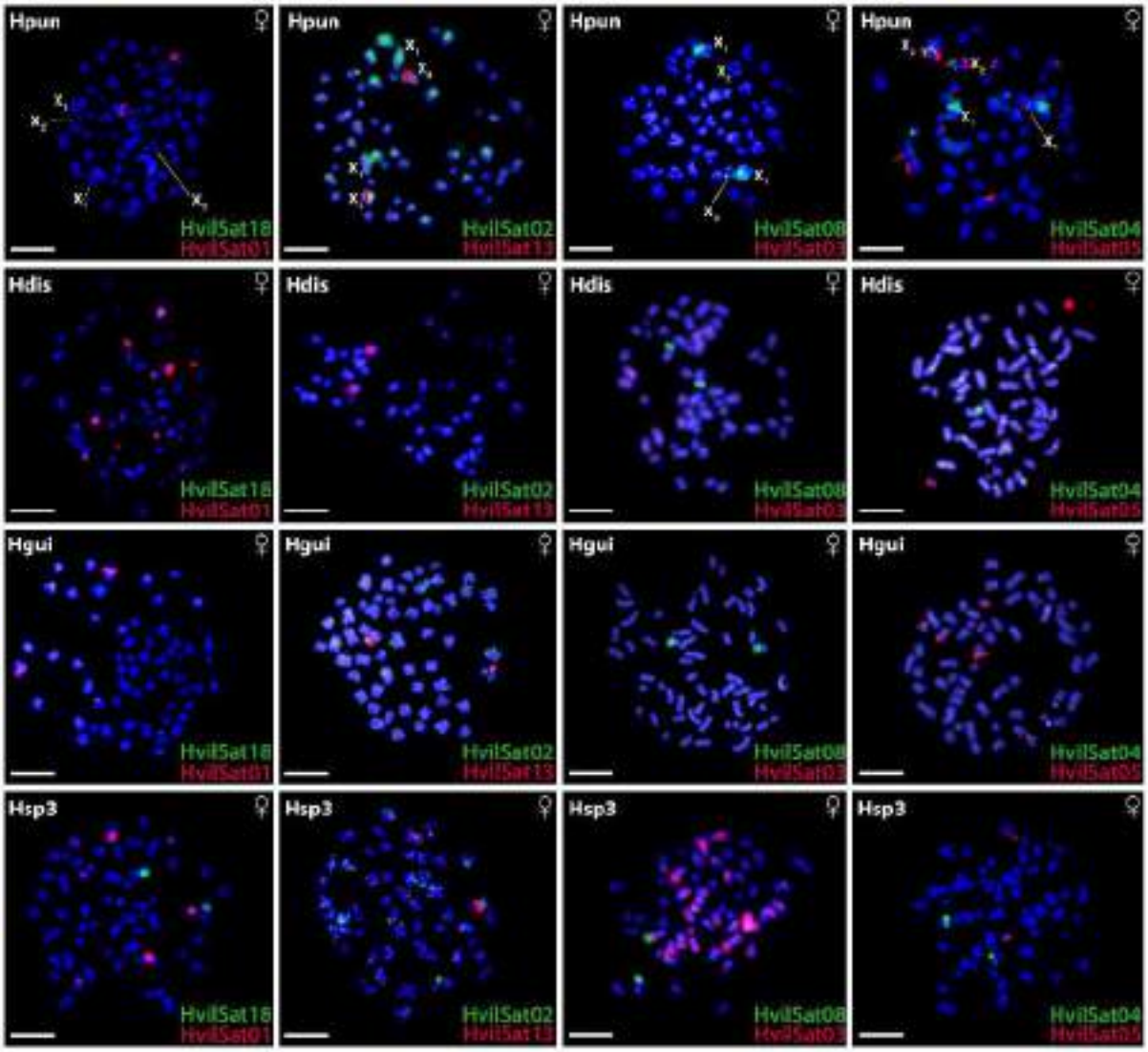
Francisco de M. C. Sassi[1], Manuel A. Garrido-Ramos[2], Rodrigo Zeni dos Santos[3], Ricardo Utsunomia[3], Tariq Ezaz[4], Geize A. Deon[1], Fábio Porto-Foresti[3], Thomas Liehr[5*], Marcelo de B. Cioffi[1]



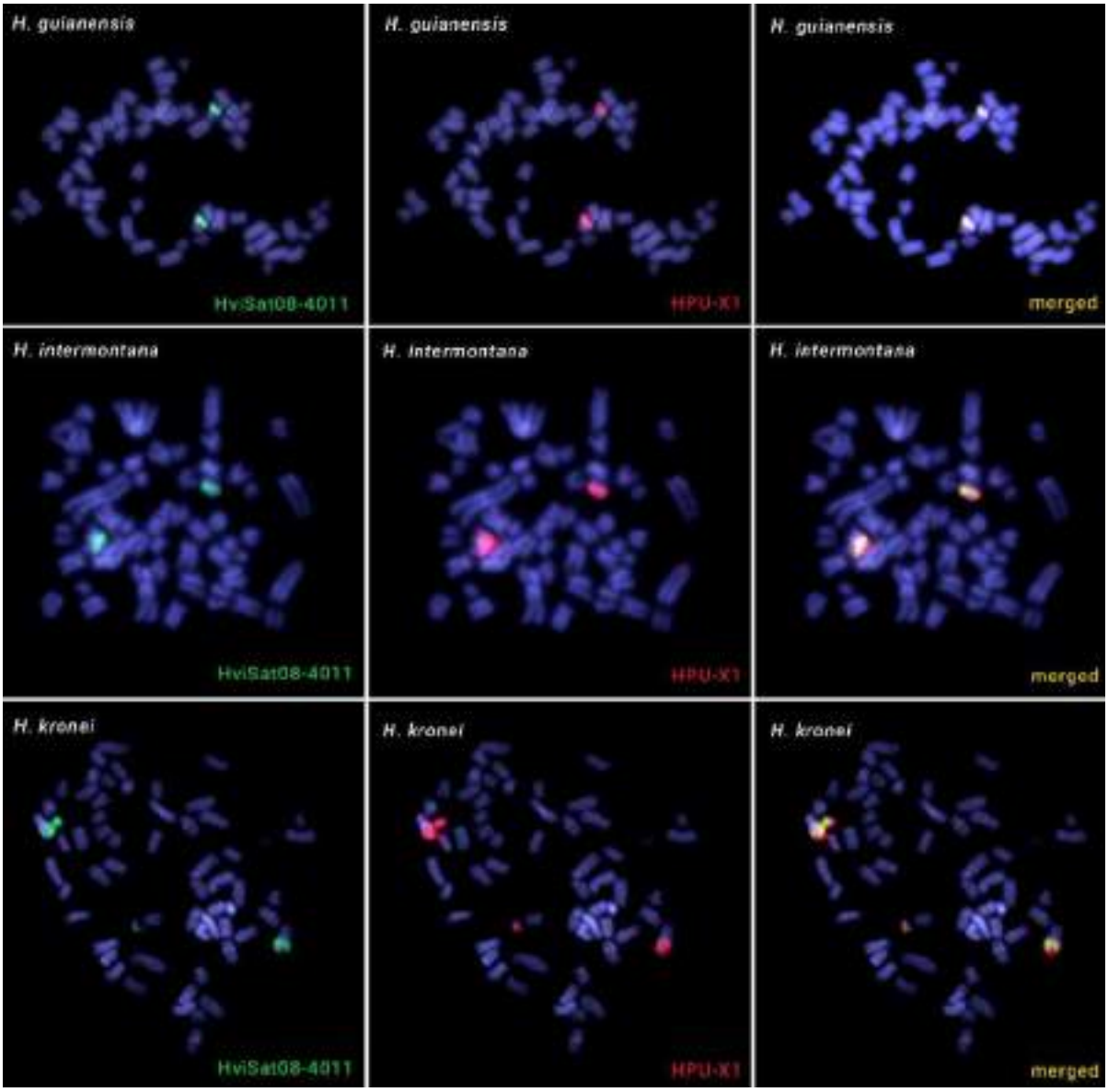
Supplementary Figure 1. Band patterns of satellites obtained by PCR with *Harttia villasboas* DNA as template in Agarose 1% gel with a 1kb ladder.



Supplementary Figure 2. FISH with *H. villasboas* satellitome probes on female metaphase chromosomes of *Harttia villasboas* (Hvi), *H. rondoni* (Hron), and *H. duriventris* (Hdur). Probes and their respective colors are indicated in the lower right corner and species in the upper left. Scale bar = 5 µm.



Supplementary Figure 3. FISH with *H. villasboas* satellitome probes on female metaphase chromosomes of *H. punctata* (Hpun), *H. dissidens* (Hdis), *H. guianensis* (Hgui), and *Harttia* sp. 3 (Hsp3). Probes and their respective colors are indicated in the lower right corner and species in the upper left. Scale bar = 5 µm.



Supplementary Figure 4. FISH mapping of HviSat08-4011 (first column) and sequential WCP with HPU–X1 (second column) on species of *Harttia* without heteromorphic sex chromosomes derived from the linkage group that forms the multiple sex chromosomes in the four main target species of this study (lines). The third column correspond to both hybridizations merged in a single image to demonstrate the congruence in the position of both mapped probes.

Supplementary Table 1. PCR conditions (primers, temperature, and concentration of template DNA) for the optimal amplification of satellite DNAs from *Harttia villasboas* genome.

Satellite	Primer F	Primer R	Annealing temperature range	[DNA] ng/µl
HviSat01-2531	5'GAAACCCAGGCAAAAGTTCA	5'CCTTCATACTCCCATGTAGCT	56 °C - 48 °C	100
HviSat02-2707	5'GGCTGAATTTTGCTGTGACAC	5'CCATAATGACGTCCCTCACC	56 °C - 48 °C	100
HviSat03-997	5'ACACTCATGACACTATCCCGT	5'CGGATTGTGCCCAGTAGCGTT	62.9 °C - 58 °C	100, 10, or 1
HviSat04-2959	5'TCGGCCAAAATACAGACTGCA	5'TTCCAGACGGCTGATGGCTTG	66 °C - 58 °C	100, 10 or 1
HviSat05-177	5'ACGAAACTCGCATATGTCGTT	5'ACACGTACGACTTCGGCTTA	61.4 °C - 55 °C	100 or 10
HviSat08-4011	5'ACAGAGTCCAAACTGAAGAGC	5'TCTTCTTCTTTTCCTCCACTG	62.9 °C - 58 °C	100, 10 or 1
HviSat13-730	5'CCACCTCTGCAAAAACAAGGG	5'TTGATCAGAGGGGTGCCGTC	64.4 °C - 58 °C	100
HviSat18-1068	5'CGTTCCGATCGGGGAGGTCC	5'AGGCGCGTTCGGGGGACC	69°C - 62 °C	100

Supplementary Table 2. Satellitome catalogues of four *Harttia* species. For *H. villasboas* and *H. rondoni*, the characteristics of both female and male satellitomes are displayed, while *H. duriventris* and *H. punctata* were based only in male samples. satDNA = satellite DNA; A+T(%) = percentage of adenine and thymine nucleotides; AM = abundance in males; AF = abundance in females; M/F = difference between male and female catalogues; Σ = sum AM+AF; DivM = divergence in males; DivF = divergence in females.

Species	SatDNA	A+T (%)	AM	AF	M/F	Σ	Div M	Div F
Harttia villasboas	HviSat01-2531	57.1	0.00651051	0.00685825	0.9492965017	0.013368758	11.69	11.79
	HviSat02-2707	52.1	0.005255686	0.00552038	0.9520513206	0.010776066	3.77	3.65
	HviSat03-997	39.7	0.003744728	0.00378172	0.9902181535	0.007526449	10.75	10.81
	HviSat04-2959	54.4	0.001384657	0.00165072	0.8388176423	0.003035382	7.48	7.18
	HviSat05-177	65.5	0.001118204	0.00123612	0.9046074034	0.002354324	5.54	5.06
	HviSat06-200	68	0.00084482	0.00077462	1.0906256510	0.001619439	5.19	5.12
	HviSat07-93	38.7	0.00079764	0.00083762	0.9522667171	0.001635263	7.32	7.29
	HviSat08-4011	56.2	0.000566046	0.00064233	0.8812338169	0.001208379	5.12	5.79
	HviSat09-815	59.5	0.000424192	0.00046433	0.9135553754	0.000888522	5.06	4.97
	HviSat10-1666	56.5	0.000417871	0.00039445	1.0593771297	0.00081232	9.41	11.01
	HviSat11-1338	58.1	0.000393588	0.00035739	1.1012749266	0.000750982	4.27	4.38
	HviSat12-144	54.2	0.000366828	0.00039024	0.9400009094	0.00075707	10.94	10.76
	HviSat13-730	36	0.000363972	0.00041825	0.8702192128	0.000782225	4.61	4.55
	HviSat14-32	60.5	0.000351162	0.00034283	1.0243007323	0.000693993	9.76	9.88
	HviSat15-347	53.1	0.000347879	0.00034702	1.0024863247	0.000694896	20.81	20.6
	HviSat16-2480	60.3	0.000306566	0.00032959	0.9301348259	0.00063616	5.15	7.31
	HviSat17-49	55.1	0.000135999	0.00013343	1.0192599992	0.000269427	5.2	5.14

	HviSat18-1068	24.2	0.000133953	0.00015103	0.886952843	0.000284979	1.45	1.37
	HviSat19-589	61.5	0.000109466	0.00012288	0.890837534	0.000232347	12.14	11.86
	HviSat20-47	68.1	0.000106951	6.0566E-05	1.765867572	0.000167517	3.35	3.21
	HviSat21-575	45.7	9.21198E-05	0.00011101	0.829853397	0.000203127	3.15	3.15
Harttia rondoni	HroSat01-93	37.63	0.003789909	0.004378805	0.865512183	0.008168715	7.74	7.4
	HroSat02-1662	56.38	0.003764020	0.003543147	1.062338085	0.007307167	11.43	11.35
	HroSat03-372	59.14	0.002637919	0.002768871	0.952705857	0.005406790	9.13	8.88
	HroSat04-920	35.98	0.002201956	0.002268802	0.970536874	0.004470758	3.49	3.38
	HroSat05-515	58.83	0.002185305	0.002655626	0.822896497	0.004840931	18.43	17.51
	HroSat06-1634	55.75	0.002106668	0.002121713	0.992909032	0.004228381	19.62	19.41
	HroSat07-19	68.42	0.001634906	0.003928174	0.416200027	0.005563080	5.74	5.18
	HroSat08-347	60.23	0.001563824	0.003237198	0.483079458	0.004801022	7.86	7.38
	HroSat09-2961	54.47	0.001291713	0.001422399	0.908123061	0.002714112	8.61	9.59
	HroSat10-21	80.95	0.001091777	0.005750859	0.189845948	0.006842637	5.78	4.13
	HroSat11-534	25.09	0.001041824	0.001381553	0.754096078	0.002423377	2.15	1.55
	HroSat12-114	30.70	0.001006509	0.001241631	0.810634573	0.002248139	3.42	3.32
	HroSat13-1440	58.82	0.000727660	0.000592262	1.228612492	0.001319922	5.19	5.27
	HroSat14-4165	56.42	0.000666859	0.000920301	0.724610028	0.001587160	5.75	6.46
	HroSat15-93	34.41	0.000605190	0.000661809	0.914448541	0.001266999	8.04	8.5
	HroSat16-177	64.97	0.000497806	0.000567514	0.877169555	0.001065320	4.3	3.91

	HroSat17-2481	60.34	0.000398167	0.000471484	0.844496667	0.000869651	7.35	10.73
	HroSat18-1194	61.39	0.000281495	0.000275020	1.02354253	0.000556515	7.03	7.18
	HroSat19-815	60.25	0.000270435	0.000503189	0.537441531	0.000773624	5.33	5.09
	HroSat20-45	57.78	0.000257782	0.000215465	1.196396821	0.000473247	4.19	4.19
	HroSat21-50	60	0.000205573	0.000252480	0.814217275	0.000458053	6.14	5.51
	HroSat22-211	62.09	0.000151882	0.000174905	0.868366557	0.000326787	7.12	7.15
	HroSat23-576	46.53	0.000140003	0.000091847	1.524309737	0.000231849	3.2	3.39
	HroSat24-48	56.25	0.000109270	0.000123891	0.881982123	0.000233161	9.23	7.1
	HroSat25-35	57.14	0.000081886	0.000132465	0.618170838	0.000214351	8.12	8.04

Species	SatDNA	A+T (%)	Abundance	Div
Harttia duriventris	HduSat01-2707	52	0.005296699	3.49
	HduSat02-347	62.20	0.003393394	5.13
	HduSat03-2143	57.10	0.00187602	11.58
	HduSat04-31	67.70	0.001730222	7.26
	HduSat05-93	37.60	0.001116687	9.71
	HduSat06-177	65.00	0.000884022	5.4
	HduSat07-21	81.00	0.000851979	5.76
	HduSat08-1754	55.80	0.000790658	6.04
	HduSat09-771	34.80	0.000719605	3.39
	HduSat10-1449	59.10	0.000559583	4.83

	HduSat11-815	60.50	0.000538898	5.5
	HduSat12-1670	56.10	0.000507084	10.65
	HduSat13-144	54.20	0.000359392	10.76
	HduSat14-32	53.10	0.000357065	20.36
	HduSat15-534	23.60	0.000346816	1.13
	HduSat16-114	28.90	0.00030558	2.98
	HduSat17-150	52.00	0.000136754	11.15
	HduSat18-45	57.80	0.000132868	4.63
Harttia punctata	HpuSat01-21	71.43	0.015663791	6.99
	HpuSat02-177	65.54	0.014303394	3.62
	HpuSat03-144	54.17	0.012200361	5.29
	HpuSat04-2707	51.64	0.008121138	3.18
	HpuSat05-1068	40.26	0.004088409	11.39
	HpuSat06-105	44.76	0.002966126	10.25
	HpuSat07-2750	54.22	0.002216664	7.56
	HpuSat08-1546	60.03	0.001745528	7.34
	HpuSat09-33	39.39	0.001719941	7.97
	HpuSat10-781	59.67	0.000967001	2.01
	HpuSat11-32	53.13	0.000878637	19.3
	HpuSat12-1973	54.84	0.000869407	3.03

HpuSat13-60	71.67	0.000788852	4.77
HpuSat14-503	58.85	0.000642217	5.64
HpuSat15-93	62.37	0.000615839	6.53
HpuSat16-33	60.61	0.000476905	6.49
HpuSat17-52	53.85	0.000397269	13.95
HpuSat18-680	60.74	0.000171358	7.05
HpuSat19-794	63.73	0.000129686	5.5

Supplementary Table 3. Putative Open Reading Frames (ORFs) identified in the four *Harttia* satellitomes, their BLASTp match (>70% Query Cover and >70% Percentage of Identity), and the start and stop bp in the satellite sequence.

Satellite	ORF	BLASTp	Start	Stop
HviSat02-2707	5	Uncharacterized protein from fish species	56	2456
HviSat04-2959	5	Hypothetical protein from *Prochilodus magdalenae*	1671	2001
HviSat04-2959	12	Putative nuclease HABI1 from *Trichomycterus rosablanca*, hypothetical protein from fish species	521	713
HviSat04-2959	14	Hypothetical protein from *Prochilodus magdalenae*	1901	2111
HviSat08-4011	23	ZP4-like from fish	168	384
HviSat08-4011	29	ZP4-like from fish	665	788
HviSat08-4011	34	ZP4-like from fish	808	1063
HviSat10-1666	1	Hypothetical protein from eukaryotes	21	141
HviSat10-1666	11	Mucin-1-like from *Cervus elaphus*, basic proline-rich protein-like from several mammals, dual specificity protein phosphatase 18 from *Kryptolebias marmoratus*	615	747
HroSat06-1634	2	Hypothetical protein from eukaryotes	124	244
HroSat09-2961	8	Hypothetical protein, putative RNA-directed DNA polymerase from transposon BS, F-actin-uncapping protein, RAC serine/threonine-protein kinase, all from fish	28	181
HroSat09-2961	13	Hypothetical protein from *Prochilodus magdalenae*	113	446
HroSat09-2961	17	Putative nuclease HABI1 from *Trichomycterus rosablanca*, hypothetical protein from *Prochilodus magdalenae*, uncharacterized protein from *Anguilla anguilla*, and putative RNA-directed DNA polymerase from transposon BS from *Labeo rohita*	1925	2117
HroSat14-4165	5	ZP4-like from fish	2652	2907
HroSat14-4165	13	ZP4-like from fish	3331	3547
HroSat14-4165	19	ZP4-like from fish	2927	3050
HroSat17-2481	3	Uncharacterized protein from fish, hypothetical protein from spider	1062	1209
HroSat23-576	3	Uncharacterized protein from vertebrates, hypothetical protein from vertebrates, NADH dehydrogenase iron-sulfur protein 7 from rabbit	327	444
HduSat01-2707	17	Uncharacterized Fish Protein	251	2651
HduSat08-1754	7	Putative nuclease HABI1 from *Trichomycterus rosablanca*, hypothetical protein from fish species	1184	1376
HduSat12-1670	10	Hypothetical protein from eukaryotes	340	460
HpuSat04-2707	12	Uncharacterized protein from fish species	277	2677
HpuSat07-2750	14	Hypothetical and uncharacterized fish proteins, zinc finger BED domain-containing protein 4 from *Anabarilius grahami*, putative RNA-directed DNA polymerase from transposon BS from *Labeo rohita*, transposon Tf2-8 polyprotein from *Labeo rohita*, hydrocephalus-inducing protein homolog from *Tachysurus fulvidraco*, gastrula zinc finger protein XlCGF28.1-like from *Silurus meridionalis*, LINE-1 retrotransposable element ORF2 protein from *Labeo rohita*	2432	2567
HpuSat07-2750	22	Hypothetical and uncharacterized fish proteins, putative RNA-directed DNA polymerase from transposon BS from *Labeo rohita*,	1537	1648

Satellite	ORF	BLASTp	Start	Stop
HviSat02-2707	5	Uncharacterized protein from fish species	56	2456
HviSat04-2959	5	Hypothetical protein from *Prochilodus magdalenae*	1671	2001
HviSat04-2959	12	Putative nuclease HABI1 from *Trichomycterus rosablanca*, hypothetical protein from fish species	521	713
HviSat04-2959	14	Hypothetical protein from *Prochilodus magdalenae*	1901	2111
		adhesion G protein-coupled receptor E1-like from *Megalobrama amblycephala*, galactokinase isoform X1 from *Phyllopteryx taeniolatus*, teneurin-3-like from *Syngnathus typhle*, transposon Tf2-6 polyprotein from *Labeo rohita*, gastrula zinc finger protein XlCGF28.1-like from *Silurus meridionalis*, chloride channel protein 2b isoform X2 from *Entelurus aequoreus*		
HpuSat07-2750	27	putative RNA-directed DNA polymerase from transposon BS from *Labeo rohita*, hypothetical protein from *Hemibagrus guttatus*	1059	1230
HpuSat10-781	3	cilia- and flagella-associated protein 46 from *Pseudobagrus ichikawai*, hypothetical protein from *Channa argus* and *Tachysurus vachellii*	541	673
HpuSat12-1973	8	Uncharacterized protein from *Corvus cornix cornix*	1789	1936

Supplementary Tables 4-7. BLASTn results of Hvi (4), Hro (5), Hdu (6), and HpuSatDNAs (7), respectively, with positive match in repetitive sequences. Different columns indicate the following data: Smith-Waterman score of the match, complexity adjusted (SW); percentage of substitutions in matching region compared to the consensus (Div); percentage of bases opposite a gap in the query sequence (deleted bp); percentage of bases opposite a gap in the repeat consensus (inserted bp); position in query (begin-end); strand (transcript strand (+); complementary strand (C); matching repeat (Rep); repeat class/family (Class); percentage of matching satDNA sequence (%), and total percentage per satDNA (Total).

Supplementary Table 4

		Percentage			Position						
Query	**SW**	**Div**	**Del**	**Ins**	**Begin**	**End**	**Strand**	**Rep**	**Class**	**%**	**Total**
HviSat01-2531	354	20.0	0.0	0.0	78	137	C	tRNA-Val-GTG	tRNA	2,33	
HviSat01-2531	573	11.0	0.0	0.0	264	336	C	tRNA-Val-GTG	tRNA	2,84	
HviSat01-2531	405	18.3	0.0	0.0	1868	1938	C	tRNA-Asp-GAY	tRNA	2,77	
HviSat01-2531	420	19.4	0.0	0.0	2438	2509	C	tRNA-Asp-GAY	tRNA	2,81	10,75
HviSat02-2707	5446	29.9	0.0	2.6	1	2403	+	Penelope-5_XT	LINE/Penelope	88,73	
HviSat02-2707	355	24.7	0.0	2.0	2551	2699	+	Penelope-5_XT	LINE/Penelope	5,47	94,20
HviSat04-2959	4989	28.6	2.4	1.9	54	2055	+	Rex1-3_EL	LINE/Rex-Babar	67,62	
HviSat04-2959	338	27.1	9.3	0.0	2233	2302	+	tRNA-Ile-ATC	tRNA	2,33	
HviSat04-2959	591	9.6	5.1	0.0	2423	2495	C	tRNA-Ala-GCY	tRNA	2,43	
HviSat04-2959	302	26.8	0.0	1.1	2869	2955	+	Mariner-N4_SSa	DNA/TcMar-ISRm11	2,91	
HviSat04-2959	568	7.2	0.0	0.0	2891	2959	+	tRNA-Ile-ATT	tRNA	2,30	77,59
HviSat06-200	370	4.5	3.4	10.7	1	197	+	MOSAT_DR	SatDNA	98,00	98,00
HviSat09-815	597	4.3	0.0	0.0	1	70	+	tRNA-Gly-GGY	tRNA	8,47	
HviSat09-815	385	23.8	30.7	0.0	71	84	+	DNA-1_Gav	DNA	1,60	10,06
HviSat10-1666	646	4.9	0.0	0.0	46	127	+	Nimb-4_DR	LINE/I	4,86	
HviSat10-1666	595	10.8	0.0	0.0	633	715	C	tRNA-Leu-CTG	tRNA	4,92	
HviSat10-1666	349	17.1	13.6	0.0	1516	1585	C	Nimb-4_DR	LINE/I	4,14	13,93
HviSat11-1338	629	0.0	0.0	0.0	250	322	+	tRNA-3_DRe	tRNA	5,38	5,38
HviSat16-2480	628	4.2	0.0	0.0	12	83	+	tRNA-Gly-GGA	tRNA	2,86	
HviSat16-2480	275	26.1	0.0	0.0	519	587	+	tRNA-Lys-AAA	tRNA	2,74	
HviSat16-2480	600	5.6	0.0	0.0	748	819	C	tRNA-Trp-TGG	tRNA	2,86	
HviSat16-2480	397	19.0	0.0	3.7	1704	1785	+	tRNA-1_DRe	tRNA	3,27	
HviSat16-2480	630	6.7	0.0	0.0	1723	1797	+	tRNA-Lys-AAA	tRNA	2,98	
HviSat16-2480	249	30.3	5.7	1.0	1798	1822	+	Ves2_ML	SINE/tRNA	0,97	15,69
HviSat19-589	359	21.9	6.8	0.0	55	75	C	tRNA-3_DRe	tRNA	3,40	
HviSat19-589	363	20.8	0.0	0.0	76	147	C	tRNA-Arg-CGY	tRNA	12,09	
HviSat19-589	397	15.2	8.0	5.2	361	457	C	tRNA-3_DRe	tRNA	16,30	31,75
HviSat21-575	596	8.8	0.0	0.0	5	84	+	tRNA-1_DRe	tRNA	13,74	
HviSat21-575	665	0.0	0.0	0.0	320	393	C	tRNA-Asn-AAC	tRNA	12,70	26,43

Supplementary Table 5

Query	SW	Percentage Div	Del	Ins	Position Begin	End	Strand	Rep	Class	%	Total
HroSat02-1662	592	5.6	0.0	0.0	28	99	+	tRNA-Asp-GAY	tRNA	4,27	
HroSat02-1662	523	15.1	0.0	0.0	1344	1416	+	tRNA-Val-GTG	tRNA	4,33	
HroSat02-1662	244	26.4	7.1	1.1	1350	1441	+	SINE_FR2	SINE/tRNA-V-RTE	5,47	
HroSat02-1662	531	12.3	0.0	0.0	1523	1595	+	tRNA-Val-GTY	tRNA	4,33	18,41
HroSat03-372	359	20.6	0.0	0.0	89	151	C	tRNA-Val-GTG	tRNA	16,66	
HroSat03-372	458	16.7	0.0	0.0	290	361	C	tRNA-Asp-GAY	tRNA	19,08	35,75
HroSat05-515	411	16.1	0.0	0.0	32	93	+	tRNA-Val-GTY	tRNA	11,84	
HroSat05-515	225	15.4	4.9	0.0	66	104	C	Gypsy-11_XL-LTR	LTR/Gypsy	7,37	
HroSat05-515	375	21.4	0.0	0.0	178	247	+	tRNA-Val-GTG	tRNA	13,39	
HroSat05-515	371	18.8	0.0	0.0	335	398	+	tRNA-Asp-GAY	tRNA	12,23	44,85
HroSat06-1634	673	3.7	0.0	0.0	149	230	+	Nimb-4_DR	LINE/I	4,95	
HroSat06-1634	615	9.6	0.0	0.0	723	805	C	tRNA-Leu-CTG	tRNA	5,01	9,97
HroSat09-2961	1870	27.5	0.0	0.8	2	500	+	REX1-5_DR	LINE/Rex-Babar	16,81	
HroSat09-2961	337	27.1	0.0	0.0	678	747	+	tRNA-Ile-ATC	tRNA	2,33	
HroSat09-2961	591	9.6	0.0	0.0	868	940	C	tRNA-Ala-GCY	tRNA	2,43	
HroSat09-2961	603	8.1	0.0	0.0	1336	1409	+	tRNA-Ile-ATT	tRNA	2,46	
HroSat09-2961	563	18.1	0.0	1.1	1340	1434	+	TguSINE1	SINE/tRNA-CR1	3,17	
HroSat09-2961	3250	28.3	6.7	2.4	1449	2961	+	Rex1-3_EL	LINE/Rex-Babar	51,06	78,28
HroSat13-1440	629	0.0	0.0	0.0	7	79	C	tRNA-3_DRe	tRNA	5	
HroSat13-1440	323	26.6	1.3	11.5	614	787	+	SAT_LM	Satellite	12,01	17,01
HroSat17-2481	256	32.0	4.8	0.0	103	130	C	Ves2_ML	SINE/tRNA	1,08	
HroSat17-2481	623	4.2	0.0	0.0	131	202	C	tRNA-Lys-AAA	tRNA	2,86	
HroSat17-2481	398	16.7	1.3	4.9	140	221	C	tRNA-1_DRe	tRNA	3,26	
HroSat17-2481	611	5.6	0.0	0.0	1104	1175	+	tRNA-Trp-TGG	tRNA	2,86	
HroSat17-2481	275	26.1	0.0	0.0	1336	1404	C	tRNA-Lys-AAA	tRNA	2,74	
HroSat17-2481	333	25.7	0.0	1.3	1622	1696	C	tRNA-Gly-GGA	tRNA	2,98	
HroSat17-2481	628	4.2	0.0	0.0	1840	1911	C	tRNA-Gly-GGA	tRNA	2,86	18,66
HroSat18-1194	281	23.1	0.0	0.0	212	276	C	SINE2-1_SSc	SINE/tRNA	5,36	
HroSat18-1194	471	20.2	1.0	3.9	791	893	C	tRNA-3_DRe	tRNA	8,54	
HroSat18-1194	620	4.2	0.0	0.0	1109	1180	C	tRNA-3_DRe	tRNA	5,94	19,84
HroSat19-815	379	24.7	1.2	0.0	12	25	C	DNA-1_Gav	DNA	1,59	
HroSat19-815	564	8.4	0.0	0.0	26	96	C	tRNA-Gly-GGY	tRNA	8,58	10,18
HroSat23-576	596	8.8	0.0	0.0	60	139	+	tRNA-1_DRe	tRNA	13,71	
HroSat23-576	665	0.0	0.0	0.0	375	448	C	tRNA-Asn-AAC	tRNA	12,67	26,38

Supplementary Table 6

		Percentage			Position						
Query	**SW**	**Div**	**Del**	**Ins**	**Begin**	**End**	**Strand**	**Rep**	**Class**	**%**	**Total**
HduSat01-2707	355	24.7	9.3	2.0	9	157	C	Penelope-5_XT	LINE/Penelope	5,47	
HduSat01-2707	5420	30.2	2.3	2.6	305	2707	C	Penelope-5_XT	LINE/Penelope	88,73	94,20
HduSat03-2143	531	15.1	0.0	0.0	1532	1604	+	tRNA-Val-GTG	tRNA	3,36	
HduSat03-2143	268	25.3	7.1	1.1	1538	1629	+	SINE_FR2	SINE/tRNA-V-RTE	4,25	
HduSat03-2143	323	23.6	1.4	0.0	1711	1782	+	tRNA-Val-GTY	tRNA	3,31	
HduSat03-2143	404	18.3	0.0	0.0	2073	2143	+	tRNA-Asp-GAY	tRNA	3,27	14,19
HduSat08-1754	569	10.8	0.0	0.0	139	212	C	tRNA-Ile-ATT	tRNA	4,16	
HduSat08-1754	285	28.4	3.6	1.2	148	229	C	Mariner-N4_SSa	DNA/TcMar-ISRm11	4,62	
HduSat08-1754	264	21.9	4.7	2.4	596	606	C	tRNA-2_DRe	tRNA	0,57	
HduSat08-1754	591	9.6	0.0	0.0	607	679	+	tRNA-Ala-GCY	tRNA	4,10	
HduSat08-1754	364	25.7	0.0	0.0	800	869	C	tRNA-Ile-ATC	tRNA	3,93	
HduSat08-1754	1370	30.6	3.9	3.2	990	1732	C	Rex1-3_EL	LINE/Rex-Babar	42,30	59,69
HduSat10-1449	318	25.6	1.3	1.3	483	561	C	SAT_LM	Satellite	5,38	
HduSat10-1449	605	2.7	0.0	0.0	1197	1269	+	tRNA-3_DRe	tRNA	4,97	
HduSat10-1449	237	27.5	0.0	2.4	1198	1279	C	LTR10A_BT	LTR/ERV1	5,59	15,94
HduSat11-815	458	16.4	0.0	0.0	742	814	+	tRNA-Gly-GGY	tRNA	8,83	8,83
HduSat12-1670	661	4.9	0.0	0.0	355	436	C	Nimb-4_DR	LINE/I	4,85	
HduSat12-1670	340	19.2	13.1	0.0	563	635	+	tRNA-Ser-AGY	tRNA	4,31	
HduSat12-1670	615	9.6	0.0	0.0	1435	1517	+	tRNA-Leu-CTG	tRNA	4,91	14,07
HduSat17-150	279	21.2	3.9	2.0	43	143	C	Helitron-2_DR	RC/Helitron	66,67	66,67

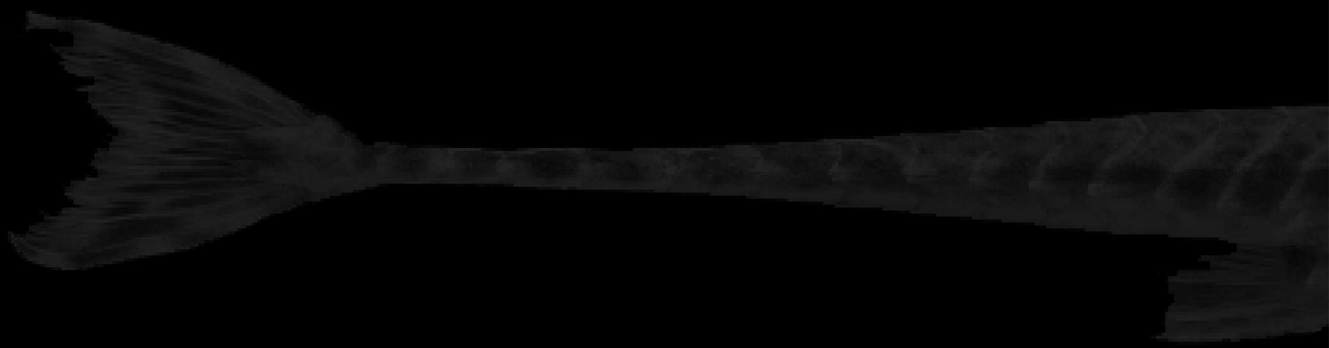
Supplementary Table 7

Query	SW	Percentage Div	Del	Ins	Position Begin	End	Strand	Rep	Class	%	Total
HpuSat04-2707	355	21.8	7.3	2.3	4	183	C	Penelope-5_XT	LINE/Penelope	6,61	
HpuSat04-2707	5241	30.8	2.2	2.5	331	2707	C	Penelope-5_XT	LINE/Penelope	87,77	94,38
HpuSat07-2750	482	5.2	0.0	0.0	1	58	C	tRNA-Ile-ATT	tRNA	2,07	
HpuSat07-2750	541	13.7	0.0	0.0	462	534	+	tRNA-Ala-GCY	tRNA	2,62	
HpuSat07-2750	275	20.6	1.6	0.0	910	972	C	REX1-5_DR	LINE/Rex-Babar	2,25	
HpuSat07-2750	4491	28.4	3.8	1.8	984	2595	C	Rex1-3_EL	LINE/Rex-Babar	58,58	65,53
HpuSat08-1546	286	27.7	0.0	0.0	555	619	+	SAT_LM	Satellite	4,14	
HpuSat08-1546	656	3.5	0.0	2.3	781	867	C	tRNA-3_DRe	tRNA	5,56	9,70
HpuSat10-781	604	4.2	0.0	0.0	557	627	C	tRNA-Gly-GGY	tRNA	8,96	
HpuSat10-781	287	17.4	4.2	2.8	565	635	C	Bov-tA2	SINE/tRNA-Core-RTE	8,96	17,93
HpuSat12-1973	646	4.1	0.0	0.0	1475	1547	+	tRNA-Val-GTG	tRNA	3,65	
HpuSat12-1973	228	27.8	5.3	2.2	1481	1572	+	SINE_TE	SINE/tRNA-V	4,61	
HpuSat12-1973	569	9.6	0.0	0.0	1655	1727	+	tRNA-Val-GTG	tRNA	3,65	
HpuSat12-1973	592	5.6	0.0	0.0	1821	1892	+	tRNA-Asp-GAY	tRNA	3,60	15,51
HpuSat19-794	635	2.8	0.0	0.0	159	230	+	tRNA-Arg-AGG	tRNA	8,94	
HpuSat19-794	233	21.5	5.8	4.4	239	306	+	tRNA-Met-i	tRNA	8,44	17,38

BIOGRAFIA

Nasceu no litoral paulista (Guarujá – SP) e, no ano de 2014, ingressou no curso de bacharelado em Ciências Biológicas com ênfase em conservação da biodiversidade na Universidade Federal de Viçosa, *Campus* Rio Paranaíba (UFV-CRP). Neste mesmo ano, iniciou seus estudos e seu primeiro estágio, no Laboratório de Genética Ecológica e Evolutiva (LaGEEvo), onde foi apresentado pela Dr. Karine Kavalco e pelo Dr. Rubens Pasa à citogenética evolutiva, inicialmente auxiliando nos trabalhos de citogenética vegetal (*Brassica napus*). Ao longo dos quatro anos de graduação, desenvolveu projetos com diversos organismos (*Astyanax*, *Hoplias*, *Hypostomus*) na citogenética animal. Também atuou em projetos de divulgação científica já existentes no laboratório (Biologia na Web, Rock com Ciência, Folha Biológica). Também na UFV-CRP, participou da fundação e como Diretor Administrativo Financeiro no Centro Acadêmico de Ciências Biológicas – CABio Colmeia (2017). Por fim, desenvolveu sua monografia com morfometria geométrica em espécies do gênero *Hypostomus*, avaliando a diversidade e configuração corporal desses animais na bacia do rio Paranaíba. Em 2018, graduou-se e retornou ao estado de São Paulo, em São Carlos, onde ingressou no mestrado em Genética Evolutiva e Biologia Molecular pela Universidade Federa de São Carlos, sob orientação do professor Dr. Luiz Antonio Carlos Bertollo. Ao longo do mestrado também desenvolveu atividades de divulgação científica, nos projetos Poeira Estelar, Próxima Parada: Cerrado e Rock com Ciência. Já no ano de 2019, passou a ser orientado pelo professor Dr. Marcelo de Bello Cioffi, concluindo o mestrado no início do ano seguinte. Sob a mesma orientação, ingressa em março de 2020 no Doutorado pelo mesmo programa, para estudar a evolução cromossômica em *Harttia*. Atuou como representante discente (2023-2025) e foi suplente (2021-2023) do PPGGEv e realizou períodos sanduíche (dez/22-jun/23 e set/23-jan/24, BEPE FAPESP) na Universitätsklinikum da Friedrich-Schiller-Universität Jena (Alemanha) e University of Canberra (Austrália), respectivamente. Com parte das análises conduzidas durante o Doutorado e as estadias no exterior, venceu o Prêmio Horácio Schneider de melhor trabalho em Genética e Melhoramento Animal pela Sociedade Brasileira de Genética (2024). Durante o Doutorado, participou também de pesquisas em peixes, répteis e aves de diversos continentes, sempre atuando na investigação dos mecanismos genéticos de evolução da

biodiversidade, em especial no papel dos cromossomos e do sexo neste processo. Fruto destas análises produziu um total de 28 artigos publicados em coautoria.



Tese de Doutorado - 2025