MARIANA CAMPANER USSO

MAPEAMENTO FÍSICO DE DIFERENTES DNAS REPETITIVOS EM PEIXES DA FAMÍLIA CICHLIDAE:

CITOGENÉTICA COMPARATIVA E EVOLUTIVA

Londrina
2019

Universidade Estadual de Londrina
Instituto Agronômico do Paraná
Empresa de Brasileira de Pesquisa Agropecuária

Mariana Campaner Usso

"Mapeamento físico de diferentes DNAs repetitivos em peixes da família Cichlidae: citogenética comparativa e evolutiva"

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Orientadora: Profa. Dra. Ana Lúcia Dias

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RESUMO

No presente trabalho foram analisadas onze espécies de peixes da família Cichlidae, pertencentes a seis gêneros e três tribos, Chaetobranchini, Cichlasomatini e Geophagini, das sete existentes, coletadas em quatro bacias hidrográficas, do Rio Paraguai-MS, Rio Paraná-PR, sistema hidrográfico Laguna dos Patos-RS e do Rio Tramandaí-RS. Os dados citogenéticos revelaram que a maioria das espécies possuem 2n=48, com exceção de *Bujurquina vittata* que apresentou 2n=44, característica cromossômica dos Ciclídeos Africanos. Apesar de um número diploide conservado na maioria das espécies analisadas, diferentes formulas cariotípicas foram observadas: *Bujurquina vittata* apresentou 30m+8sm+6st-a (NF=82), *Chaetobranchopsis australis* 48st-a (NF=48), *Cichlasoma portalegrense* 14m-sm+34st-a (NF=62), *Crenicichla jaguarensis* 4m+4sm+40st-a(NF=56), C. *lepidota* e *C. maculata* 6m+42st-a (NF=54), *Geophagus brasiliensis* 4m+44st-a (NF=52). Para as quatro espécies de *Gymnogeophagus* as constituições cromossômicas foram: 2m+46st-a (NF=50) para *G. balzani*, *G. gymnogenys* da população do saco da Alemoa com 4m+44st-a (NF=52) e 6m+42st-a (NF=54) para a população do Gasômetro, *G. labiatus* com 4m+4sm+40st-a (NF=58) e *G. rhabdotus* 4m+2sm+42st-a (NF=52). Para uma melhor compreensão da evolução cromossômica entre as diferentes espécies de Ciclideos foi realizado um mapeamento cromossômico com diferentes DNAs repetitivos, sendo os pequenos DNAs nucleares (snDNA) U1 e U2 e os DNAs ribossômicos 18S e 5S, bem como um levantamento de dados nas sete tribos da subfamília Cichlinae, relacionado aos número diploide, fórmula cariotipica, distribuição da heterocromatina e DNAs ribossômicos. Os diferentes DNAs repetitivos analisados no presente estudo evidenciaram uma plasticidade na distribuição cariotípica dessas sequênicas, podendo ser encontrados em diferentes posições e em diferentes cromossomos. Em cinco espécies da tribo Geophagini, *Crenicichla jaguarensis*, *C. lepidota*, *C. maculata*, *Gymnogeophagus balzani* e *G. labiatus*, foi observada a interação do cluster de DNAr 18S com DNAsn U2, indicando uma aparente homeologia do primeiro par, um cromossomo metacêntrico, e uma possível interação entre famílias de DNA repetitivos distintas. Rearranjos cromossômicos parecem ser responsáveis pela diversidade cariotípica entre os ciclídeos, como observado em *Gymnogeophagus gymnogenys* aqui analisada e em outras espécies, como evidenciado pelo levantamento de dados da literatura no grupo. As sequências de DNA repetitivos parecem desempenhar um importante papel na ocorrência desses eventos. O maior número de dados cariotípicos ainda se restrige às tribos Geophagini, Cichlasomatini e Heroini que, apesar de terem aumentado nos últimos anos, não refletem a real diversidade da família, principalmente quando são relacionados às sequencias de DNAs repetitivos, mas já é possível inferir que rearranjos cromossômicos estão ocorrendo de forma independente entre as espécies.

Palavras-chave: Evolução cariotípica. DNAr. DNAsn. Família multigênica. Tribos.

Usso, Mariana Campaner. **Physical mapping of different repetitive DNAs in fish of the cichlid family:** comparative and evolutionary cytogenetics. 2019. 116 p.Thesis (Doctorate in Genetics and Molecular Biology) – Universidade Estadual de Londrina, Londrina, 2019.

ABSTRACT

In the present work, eleven species of Cichlidae, belonging to six genera and to three tribes, Chaetobranchini, Cichlastomatini and Geophagini, were collected from four hydrographic basins, from the Paraguay-MS River, Paraná River , the Laguna dos Patos-RS hydrographic system and the Tramandaí-RS River. The cytogenetic data revealed that most species have 2n = 48, except for *Bujurquina vittata* that presented 2n=44, a chromosomal characteristic of the African Cichlids. *Bujurquina vittata* showed 30m+8sm+6st-a (NF=82), *Chaetobranchopisis australis* 48st-a (NF=48), *Cichlasoma portalegrense* 14m-sm+34st-a (NF = 54), *Geophagus brasiliensis* 4m+44st-a (NF=62), *Crenicichla jaguarensis* 4m+4sm+40st-a (NF=56), *C. lepidota* and *C. maculata* 6m+42st-a NF=52). For the four species of *Gymnogeophagus*, the chromosomal constitutions were: 2m+46st-a (NF=50) for *G. balzani*, *G. gymnogenys* of the population of the bag Alemoa 4m+44st-a (NF = 52) and 6m+42st-a (NF=54) for the population of Gasômetro, *G. labiatus* with 4m+4sm+40st-a (NF=58) and *G.rhabdotus* 4m+2sm+42st-a (NF=52). To better understand the chromosomal evolution between the different species of cichlids, a chromosome mapping with different repetitive DNAs was carried out, being small nuclear DNA (snDNA) U1 and U2 and the ribosomal DNAs 18S and 5S, as well as a survey of the seven tribes of the subfamily Cichlinae, related to diploid number, karyotype formula, distribution of heterochromatin and ribosomal DNAs. The different repetitive DNAs analyzed in the present study evidenced a plasticity in the karyotypic distribution of these sequences, being able to be found in different positions and in different chromosomes. In five species of the Geophagini tribe, *Crenicichla jaguarensis*, *C. lepidota*, *C. maculata*, *Gymnogeophagus balzani* and *G. labiatus*, the interaction of the 18S rDNA cluster with U2 snDNA was observed, indicating an apparent homeology of the first pair, a chromosome metacentric, and the possible interaction between distinct repetitive DNA families. Chromosomal rearrangements appear to be responsible for the karyotype diversity among cichlid species, as observed in *Gymnogeophagus gymnogenys* analyzed here and in other species, as evidenced by the literature survey of cichlids. Repetitive DNA sequences seem to play an important role in the occurrence of these events. The largest number of karyotype data still restrict the tribes Geophagini, Cichlastomatini, and Heroini, and although they have increased in recent years do not reflect the real diversity of the family, especially when repetitive DNA sequences are related, but it is already possible to infer that chromosomal rearrangements are occurring independently among species.

Keywords: Karyotype evolution. Multigenic family. rDNA. snDNA. Tribes.

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1. INTRODUÇÃO

1.1 Aspectos Gerais da Família Cichlidae

A região Neotropical abrange o território do Norte do México ao sul da América do Sul, abrigando a maior diversidade de peixes de água doce com, aproximadamente, 5160 espécies, 739 gêneros, 69 famílias e 20 ordens (REIS et al., 2016). A família Cichlidae representa o maior e mais diversificado grupo de peixes entre os Cichliformes sendo considerada um dos maiores grupos de teleósteos, com cerca de 1720 espécies válidas distribuídas em, aproximadamente, 343 gêneros, apesar de serem estimadas mais de 2275 espécies (ESCHMEYER e FONG, 2019); a grande maioria habita a África e América do Sul, com algumas espécies na América Central e do Norte e em partes da Ásia (KULLANDER, 1998, MOYLE e CECH JUNIOR, 2000).

No Brasil, os ciclídeos representam 6% da fauna de peixes de água doce de acordo com Feldberg et al. (2003). Destacam-se entre os ciclídeos neotropicais espécies dos gêneros *Cichla*, *Astronotus*, *Pterophyllum* e *Symphysodon*. Os tucunarés (*Cichla* spp) e os apaiaris ou oscar (*Astronotus* spp), são popularmente conhecidos em todo o país por serem bastante utilizados para a pesca de subsistência, esportiva e aquicultura. Já os acarás-bandeiras ou "angelfish" (*Pterophyllum* spp) e os acarás-discos (*Symphysodon* spp) são bastante valorizados na aquariofilia (GOLDSTEIN, 1988; KULLANDER, 2003), devido ao seu pequeno porte e o colorido fascinante (AXELROD, 1996).

Morfologicamente, os ciclídeos apresentam os raios anteriores da dorsal e anal, e o primeiro raio da ventral transformados em espinhos, característica esta compartilhada com os Scianidae entretanto, somente os ciclídeos apresentam a linha lateral interrompida, uma anterior, que corre mais dorsalmente e outra posterior que corre sobre o meio do pedúnculo caudal (BRITSKI et al., 1986); apresentam também uma narina em cada lado da cabeça, nadadeira dorsal geralmente com 7-25 espinhos e 5-30 raios moles, e nadadeira anal com 3-15 espinhos e 4-15 raios moles (NELSON, 2006). Outra característica da família é à saída do intestino pelo lado esquerdo do estômago, ao contrário dos demais grupos de peixes (ZIHLER, 1982).

Os ciclídeos, em sua maioria, são adaptados a ambientes lênticos, ocorrendo em uma grande diversidade de ambientes aquáticos, sendo comumente encontrado em lagoas marginais, lagos e mesmo em rios e riachos de todas as regiões do Brasil (BUCKUP, 1999; MOYLE e CECH JUNIOR, 2000). Não apresentam uma época bem definida para a

reprodução, como ocorre em outras famílias de peixes, podendo se reproduzir por um período mais longo; apresentam dimorfismo sexual, diferindo no tamanho corpóreo, onde os maiores são os machos, e durante a época de reprodução quando, então, algumas características podem se acentuar (BAUMGARTNER et al.2012).

O cuidado parental é uma das características marcante entre os indivíduos desta família, sendo que este papel é desempenhado principalmente pelas fêmeas, com exceção dos poucos casos onde o cuidado é biparental (MOYLE e CECH JUNIOR, 2000). Convencionalmente, os ciclídeos são divididos em três grupos em relação às suas estratégias reprodutivas: os de incubação bucal, os que cuidam dos ovos no substrato e um terceiro grupo que combina essas duas estratégias. (KEENLEYSIDE, 1991; MORLEY e BALSHINE, 2003). Os ciclídeos guardadores apresentam comportamentos de corte elaborados, territorialidade e algumas espécies cuidam dos ovos e das larvas (VAZZOLER, 1996).

1.2 - Aspectos filogenéticos da família Cichlidae

A filogenia da família Cichlidae teve início com base em caracteres morfológicos por Kullander (1998), que analisou 91 caracteres e propôs uma árvore filogenética para este grupo de peixes compreendendo as seguintes subfamílias: Etroplinae (*Etroplus* e *Ptychochromis*), Pseudocrenilabrinae (todos ciclídeos africanos, com exceção de *Heterochromis*, que foi considerado gênero monotípico da subfamília Heterochromidinae), Retroculinae, Cichlinae (tribos: Crenicichlini e Cichlini), Astronotinae (tribos: Astronotini e Chaetobranchini), Geophaginae (tribos: Geophagini, Acarichthyini e Crenicaratini) e Cichlasomatinae (tribos: Acaroninii, Cichlasomatini e Heroini); as cinco últimas são pertencentes ao novo mundo e as primeiras ao velho mundo (Figura 1).

Neste mesmo estudo, Kullander propôs que o gênero *Retroculus* é considerado o mais basal dos ciclídeos neotropicais, seguido por *Cichla* e *Crenicichla*, que são considerados grupos irmãos. Essa filogenia também sugeriu que as linhagens neotropicais e africanas tivessem uma origem polifilética devido à separação dos gêneros *Heterochromis* e *Cichla* das demais espécies de seus respectivos continentes (OLIVER, 1984; STIASSNY, 1987,1991; KULLANDER, 1998).

Farias et al. (2001), com base em sequências gênicas do citocromo b, encontraram o gênero *Heterochromis* entre os ciclídeos africanos, mas foi considerado como uma linhagem independente. Ciclídeos neotropicais formam um grupo monofilético tendo como grupo

basal *Retroculus* e *Cichla*. O gênero *Astronotus* está intimamente relacionado com as tribos Heroini-Cichlasomatini que são monofiléticas e grupos irmãos. Dentre os Geophagini essa ferramenta não se mostrou resolutiva para os problemas taxonômicos ainda existentes (Figura 1).

Outra análise filogenética utilizando dois genes mitocondriais (subunidade ribossomal 16S e subunidade I do complexo citocromo c oxidase) em todos os gêneros de ciclídeos da Índia e Madagascar foi realizada por Sparks e Smith (2004), que confirmaram o monofiletismo do clado e sugeriram a fragmentação da subfamília Etroplinae, proposta por Kullander (1998). Nessa família permaneceram os gêneros *Etroplus* e *Paretroplus* e uma nova família, Ptychochrominae formada pelos gêneros *Ptychochromis, Ptychochromoides* e *Oxylapia;* o gênero *Paratilapia* de Madagascar ficou sem posição definida. Nesse estudo, o gênero neotropical *Retroculus* permaneceu como grupo irmão dos demais ciclídeos neotropicais, como sugerido por Kullander (1998). A Figura 1 mostra essa e as demais hipóteses citadas acima das relações filogenéticas entre os ciclídeos.

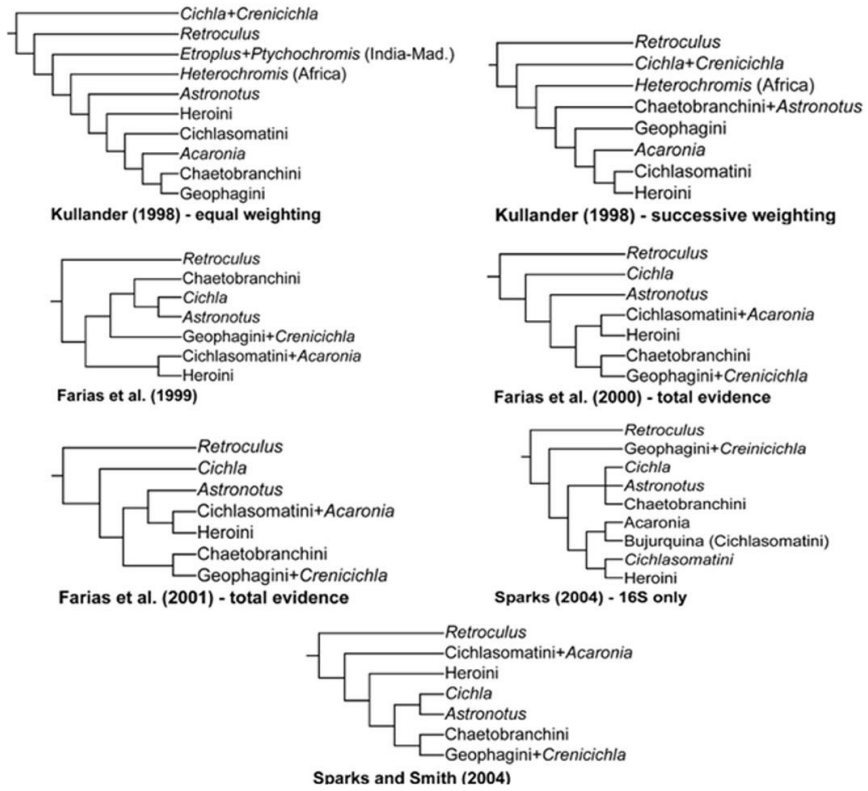

Figura 1- Hipóteses das relações filogenéticas dos ciclídeos Neotropicais baseadas em evidências morfológicas (Kullander, 1998), moleculares (Farias et al. 1999; Spark, 2004; Spark e Smith, 2004) e combinadas (Farias et al. 2000, 2001). Retirado de Smith et al., 2008).

Uma nova filogenia, bem mais abrangente e completa, baseada nos genes mitocondriais 16S, COI, Cyt-b, ND4, 4C4 e nucleares H3, M27, S7, RAG2 combinada com

análise simultânea de 6309 caracteres morfológicos foi proposta por Smith et al. (2008). Foram analisados 90 gêneros, incluindo representantes de todas as principais linhagens de ciclídeos e todos os gêneros neotropicais, resultando em uma filogenia bem estabelecida.

Com base nesse estudo, a subfamília neotropical Cichlinae foi considerada como monofilética e dividida em sete tribos: Astronotini, Chaetobranchini, Cichlasomatini, Cichlini, Geophagini, Heroini e Retroculini. Chaetobranchini + Geophagini (inclusive os creniciclineos) foram classificados como grupo irmão de Heroini + Cichlasomatini (inclusive Acaronia). Astronotini que é composto por um único gênero (*Astronotus*) foi classificado como grupo irmão destas quatro tribos anteriores. O clado composto de Ciclini + Retroculini foi proposto como grupo irmão para todos os outros Cichlinae. Nesse estudo foi incluído o fóssil de *Proterocara argentina*, posicionado junto com Geophagini, reforçando a origem a partir de Gondwana para Cichlidae (SMITH et al., 2008) (Figura 2).

Apesar de estudos mais recentes confirmarem essas sete tribos em Cichlinae, as posições filogenéticas ainda não estão totalmente esclarecidas. López-Fernández et al. (2010) realizaram estudos baseados em dados moleculares mitocondriais e nucleares, com ênfase nos Cichlinae, e propuseram o posicionamento do clado formado por Retroculini + Cichlini como grupo irmão dos demais Cichlinae no entanto, o gênero *Astronotus* foi considerado como grupo irmão do clado formado por Cichlasomatini + Heroini. Sendo assim, o clado formado por Chaetobranchini + Geophagini foi então considerado grupo irmão de Astronotini e Cichlasomatini + Heroini (Figura 2).

Recentemente, Ilves et al. (2018) realizaram uma análise filogenômica em 128 espécies de ciclídeos neotropicais, seis táxons africanos e cinco espécies da Índia e Madagascar. Em geral, esta análise molecular rendeu topologias semelhantes às relações anteriormente hipotetizadas dentro de Cichlinae, com os clados Retroculini e Cichlini como grupo irmão do restante de Cichlinae. Entretanto, algumas diferenças foram evidenciadas nas relações entre os demais grupos como mostra a Figura 2: Cichlasomatini e Heroini formam um clado monofilético e irmao do clado Astronotini, Chaetobranchini e Geophagini; Astronotini forma um clado irmao de Chaetobranchini e Geophagini. Ilves et al. (2018) sugerem que os estudos de Smith et al. (2008) e López-Fernández et al. (2010) foram baseados em um conjunto limitado de genes e muitos dados mitocondriais, não sendo suficiente para uma abordagem filogenética mais robusta em ciclídeos. Sendo assim, a classificação em sete tribos para os ciclídeos neotropicais, subfamília Cichlinae, parece ser bem estabelecida como relatado acima, contudo ainda existem conflitos a serem resolvidos dentro e entre essas tribos.

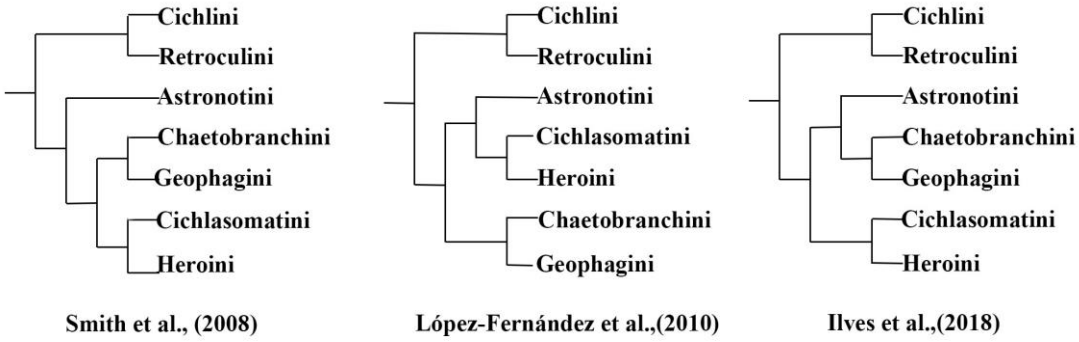

Figura 2- Hipóteses das relações filogenéticas dos ciclídeos Neotropicais baseadas em evidências morfológicas e moleculares (Smith et al. 2008), moleculares (Lópes-Fernández et al. 2010; Ilves et al.2018).

1.3 - Aspectos Citogenéticos da família Cichlidae

Dentro da ordem Cichliformes os ciclídeos são os peixes mais estudados citogeneticamente, segundo o levantamento de Pires (2013) que mostrou dados cariotípicos para 149 espécies da família Cichlidae. As análises nesta família tiveram início com Thompson (1979) que cariotipou 41 espécies Neotropicais e, dentre estas, 31 apresentaram 2n=48 e, entre as demais, 7 espécies com 2n abaixo (2n = 38, 44 e 46) e 3 espécies com 2n acima desse valor (2n = 50, 52 e 60). De acordo com Thompson (1979), o número diplóide igual 48 cromossomos subtelo-acrocêntricos, é o mais basal em Cichlidae, este é encontrado em 98 das 149 espécies de ciclídeos analisadas até o momento (65,77%), segundo o levantamento recente realizado por Pires (2013).

Outros autores seguiram acrescentando dados citogenéticos para o grupo (GROSS et al.,2010; SCHNEIDER et al., 2013; PERAZZO et al.,2013; ALVES-SILVA et al., 2015; OLIVEIRA et al., 2016, entre outros) e, segundo Feldberg e Bertollo (1985a), a evolução cromossômica em Cichlidae ocorre de modo conservativo mantendo o 2n=48 para muitas espécies, entretanto variações de 32 a 60 cromossomos foram evidenciadas em algumas espécies, provavelmente devido a ocorrência de rearranjos, como fusões e fissões. Variações no número fundamental de 44 a 118 também são observadas e, neste caso, as inversões pericêntricas seriam as responsáveis (PIRES et al., 2013).

Feldberg et al. (2003) sugeriram três tendências evolutivas para Cichlidae: a primeira é marcada pela manutenção de 2n=48 acrocêntricos, com poucos cromossomos meta-submetacêntricos, possivelmente devido a inversões pericêntricas. Este cariótipo está

presente em espécies das tribos Cichlini, Astronotinae, Geophagini e Cichlasomatini (segundo classificação de SMITH et al., 2008). A segunda tendência inclui um decréscimo no 2n e um maior número de cromossomos de dois braços (m/sm), sugerindo fusões cromossômicas e inversões pericêntricas, como relatado no gênero *Crenicichla* (REZENDE, 1996) *e Apistogramma* (THOMPSON 1979; RONCATI et al., 2007) pertencente a tribo Geophagini e em *Bujurquina vittata* (MARESCALCHI, 2005; RONCATI et al., 2007) e em *Laetacara* (MARTINS-SANTOS et al., 2005; HODANOVA et al., 2014) da tribo Cichlasomatini. A terceira tendência resulta em um aumento no 2n (igual 50 e 52), com manutenção de cromossomos acrocêntricos, possivelmente devido a ocorrência de inversões pericêntricas no ancestral desse grupo com cromossomos m/sm, seguidas de fissões cêntricas que levaram ao aumento do número diplóide. Este cariótipo pode ser encontrado em espécies da tribo Cichlasomatini.

Segundo levantamento feito por Pires (2013) um menor número de estudos citogenéticos pode ser observado nas tribos Cichlini, Retroculini e Chaetobranchini e, até o momento, uma manutenção do cariótipo plesiomórfico parece ser característico dessas tribos. As demais tribos, Geophagini, Cichlastomatini e Heroni concentram o maior número de análises, deixando mais evidente a ocorrência dos rearranjos cromossômicos.

Esses rearranjos cromossômicos foram relatados por Martins-Santos et al. (2005), que encontraram para a espécie *Laetacara* cf. *dorsigera* (tribo Cichlasomatini) 4 citótipos diferentes, com 2n=46, 45, 44 e 43. As diferenças nas fórmulas cariotípicas estavam relacionadas com o número de cromossomos metacêntricos, sendo estes inversamente proporcionais ao seu número diplóide, levando assim a um NF constante igual 48. Segundo os autores, eventos de fusões cêntricas, como translocação Robertsoniana e formação de isocromossomo, estariam envolvidos neste processo de polimorfismo nesta espécie. Mais recentemente, Valente et al. (2012) também relataram para *Geophagus proximus* (Geophagini) dois citótipos diferentes, mas com o mesmo número diplóide (2n=48) e os autores consideraram que este polimorfismo foi consequência de translocações Robertsonianas entre dois cromossomos subtelo-acrocêntricos, resultando em um cromossomo metacêntrico grande e um elemento puntiforme.

Geophagus brasiliensis é considerado um complexo de espécies (FARIAS et al., 2000) pois, apesar de todas as populações analisadas até o presente momento apresentarem 2n=48, diferentes fórmulas cariotípicas foram relatadas entre e dentro destas populações (PERAZZO et al., 2013; ALVES-SILVA et al.,2015; OLIVEIRA et al., 2016), evidenciando

a ocorrência de rearranjos cromossômicos como as inversões pericêntricas que, segundo Perazzo et al. (2013) podem contribuir para o isolamento reprodutivo das espécies.

Na tribo Heroini encontra-se o gênero *Symphysodon*, o qual apresenta um cariótipo bastante incomum, com 2n=60 cromossomos com a presença, principalmente, de cromossomos do tipo m/sm, além de alguns st/a e microcromossomos (MESQUITA et al., 2008; GROSS et al., 2009, 2010). Em estudos meióticos de *S. aequifasciatus* e *S. haraldi* foi relatada a ocorrência da formação de uma grande cadeia cromossômica durante a meiose, com a presença de até 20 elementos, sendo a maior encontrada em vertebrados. A origem desta cadeia seria baseada numa série de translocações envolvendo regiões heterocromáticas de vários cromossomos (GROSS et al., 2009). Além disso, uma grande variação foi observada no número de sítios de RONs nestas duas espécies, em que pelo menos sete pares de cromossomos homólogos estavam envolvidos com esta região (MESQUITA et al., 2008).

A impregnação pelo nitrato de prata evidenciou a RON em um par de cromossomos na maioria das espécies analisadas (MARTINS et al., 1995; LOUREIRO et al., 2000; LORSCHEIDER, 2004; MIZOGUCHI et al., 2007, PIRES et al., 2008, 2010, 2019) entretanto, padrão múltiplo desta região e variação entre populações já foram observados. Em *Crenicichla niederleinii,* por exemplo, Martins et al. (1995) evidenciaram RONs múltiplas, com dois pares portadores e uma outra população analisada por Pires et al. (2019) evidenciaram apenas um par. Diferentes populações de *Geophagus brasiliensis* também apresentaram padões distintos de distribuição da AgRON. As seis populações analisadas por Perazzo et al. (2013) apresentaram apenas 1 par cromossômico com marcação no braço curto, exceto a população do Parque Nacional que foi na região terminal do braço longo; diferentes populações desse complexo de espécies apresentaram um mosaico de AgRONs, de 2 a 5 marcações na região terminal do braço curto de cromossomos subtelo-acrocentricos (ALVES-SILVA et al., 2015; OLIVEIRA et al., 2016) .

Outros exemplos de RONs múltiplas também podem ser citados como em *Cichlasoma paranaense* (Loureiro, 1999; Pires, 2013), *Symphysodon aequifasciatus* e *S. haraldi* (GROSS et al., 2010), *Gymnogeophagus rhabdotus* (PIRES, 2013), *Uaru amphiacanthoides* (SCHNEIDER et al., 2013) e *Crenicichla britskii* (PIRES et al., 2019).

Estudos com o fluorocromo cromomicina (CMA) evidenciaram regiões mais brilhantes coincidentes com as regiões organizadoras de nucléolos em muitas espécies de Cichlidae como em: *Geophagus brasiliensis*, *Cichlasoma paranaense*, *Crenicichla* sp e C. *niederleinii* (LOUREIRO, 1999; LOUREIRO et al., 2000); *Geophagus brasiliensis*, *Gymnogeophagus gymnogenys*, *G. labiatus*, *Crenicichla lepidota*, *C. punctata* e *C. maculata*

por Pires et al. (2008, 2010, 2019); *Crenicichla lepidota* e *Australoheros facetus* (PERAZZO et al., 2011); todas as 10 espécies (*Cichla piquiti*, *Retroculus lapidifer*, *Biotodoma cupido*, *Crenicichla strigata*, *Geophagus proximus*, *Satanoperca jurupari*, *Aequidens tetramerus*, *Laetacara araguaiae*, *Heros efasciatus* e *Mesonauta festivus*) analisadas por Valente et al. (2012), entre outros.

As análises acima confirmam a relação entre blocos CMA_3^+ e AgRON nos ciclideos, e a não associação desses blocos parecem ser uma exceção no grupo sugerindo que essas regiões podem estar relacionadas ao dinamismo cariotípico observado no cariótipo de Cichlidae (VALENTE et al., 2012).

Análises de bandamento C evidenciaram a heterocromatina distribuída em região pericentromérica na maioria das espécies de Cichlidae (MARTINS et al., 1995; PIRES et al., 2008, 2010, 2019; VALENTE et al., 2012, entre outros) contudo, existem variações quanto aos cromossomos portadores e a quantidade de heterocromatina distribuída. Diferentes populações de *Geophagus brasiliesis* analisadas apresentaram a heterocromatina preferencialmente na região pericentromérica e centromérica (ALVES-SILVA et al.,2015; OLIVEIRA et al., 2016) porém, os exemplares do Rio Doce apresentaram um par de cromossomos subtelocêntrico com um forte bloco de heterocromatina na região do braço curto. Outro padrão de distribuição da heterocromatina foi observado em *G. brasiliensis* do Rio Jaguariaíva, que, além da heterocromatina na região pericentromérica observada em maior quantidade quando comparada com as outras populações, apresentou também bandas intersticiais (VICARI et al., 2006) *Crenicichla* sp 2, além das marcações pericentroméricas, apresentou marcações em ambas regiões terminais de alguns cromossomos, padrão este que não tinha sido observado ainda no gênero *Crenicichla* (MIZOGUCHI et al., 2007). Para muitas espécies, alguns blocos heterocromáticos estão relacionados à AgRON, como em *Geophagus brasiliensis* (LOUREIRO, 1999; PIRES et al.,2010) e *Gymnogeophagus labiatus* (PIRES et al.,2010) e para todas as epécies analisadas por Valente et al. (2012), entre outras.

1.4 DNAs Repetitivos

O DNA repetitivo compreende segmentos de diferentes tamanhos, que se repetem de dezenas a milhões de vezes no genoma e compõem uma grande parcela do genoma dos eucariotos (TAFT et al., 2007), podendo ser dividido em moderadamente repetitivo com 10 a 10^5 cópias por genoma e sequências altamente repetitivas com mais de 10^5 por genoma.

Por muitos anos, o DNA repetitivo foi considerado como um “DNA lixo” (DOOLITTLE e SAPIENZA, 1980), entretanto, este tipo de sequências pode estar envolvido em rearranjos cromossômicos, tais como deleções, duplicações, inversões e translocações recíprocas, sendo responsável por proporções significativas das variações cariotípicas observadas em muitos grupos (KIDWELL, 2002). O mapeamento cromossômico de DNAs repetitivos tem se mostrado um marcador citogenético muito importante no grupo dos peixes, podendo auxiliar na compreensão de problemas taxonômicos (BLANCO et al., 2011), filogenéticos (TEIXEIRA et al., 2009), diferenciações populacionais (MARTINEZ et al., 2011), evolução de cromossomos sexuais (VICARI et al., 2008) e de cromossomos B (SILVA et al., 2014).

Sequências de DNA repetitivo podem ser divididas em dois grupos principais: repetições em *tandem*, representadas pelos DNA satélites, microssatélites e minisatélites, e repetições interespaçadas dispersas, representadas por elementos transponíveis (ET) (TIMBERLAKE, 1978; CHARLESWORTH et al., 1994; JURKA et al., 2005).

Existem ainda as famílias multigênicas, que são um tipo de DNA moderadamente repetitivo, que compreende um grupo de genes todos descendentes de um ancestral comum, com sequências similares e funcionalmente relacionadas (NEI e ROONEY, 2005). São seqüências repetidas de DNA, que codificam importantes moléculas como os RNAs ribossômicos (RNAr) (MARTINS, 2007), histonas (CHILDS et al., 1981) e pequenos DNAs nucleares (UDNAsn) (VALADKHAN, 2005). Eventos de duplicação podem ser responsáveis pela formação das famílias multigênicas e as diferenças observadas entre esses genes podem ser causadas por acúmulo de mutações ao longo do tempo. Pseudogenes são comuns nas famílias multigênicas, possuindo grande semelhança com os genes funcionais da mesma família, mas perderam sua capacidade de expressão devido à mutações adquiridas (FARAH, 2007).

Os RNAs ribossômicos são os mais abundantes RNAs na célula eucariótica (MARTINS e WASKO, 2004) e podem ser divididos em duas famílias multigênicas, repetidas em tandem: a primeira representada pelo DNAr 45S, que compreende as regiões que transcrevem os genes RNAs 18S , 28S e 5,8S, além de espaçadores intergênicos (Figura 3); e a segunda, DNAr 5S, que consiste de uma sequência altamente conservada de 120 pares de bases e espaçadores não transcritos (NTS) de tamanho variável (MARTINS, 2007). Os quatro genes de RNAr em eucariotos são transcritos por duas RNAs polimerase distintas: os genes do DNAr 45S pela polimerase I e o gene de DNAr 5S pela polimerase III (MARTINS e WASKO, 2004).

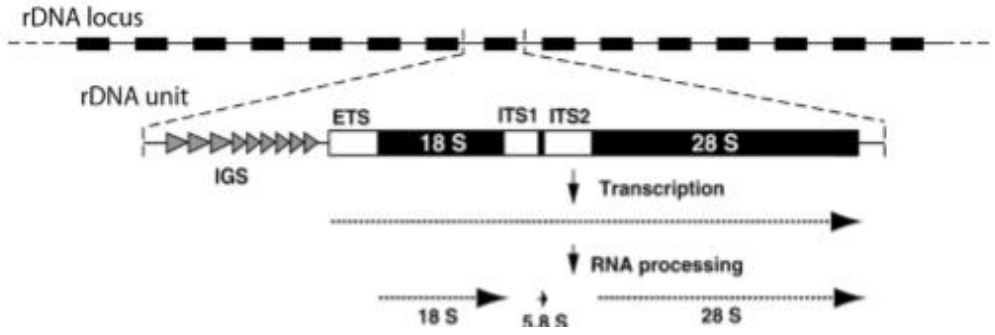

Figura 3-Organização do DNAr 45S em Eucariotos, retirado de Eickbush & Eickbush (2007).

A unidade de repetição do DNAr 45S situa-se em locais específicos do cromossomo, formando muitas vezes uma constrição secundária, denominada região organizadora de nucléolo (RON) (LONG e DAVID, 1980). Essa região pode ser detectada pela impregnação com nitrato de prata contudo, esta técnica só permite observar RONs que foram ativas na interfase anterior (MILLER et al., 1976). A hibridação fluorescente in situ (FISH) com sonda de DNAr da família 45S, principalmente o DNAr 18S, permite a localização precisa da RON independente de sua atividade de transcrição (OLIVEIRA, 1999). Por sua vez, a família do DNAr 5S não está associada à formação do nucléolo contudo, em alguns casos, pode se encontrar intercalada com outras famílias multigênicas (DROUIN e MONIZ DE SÁ, 1995).

O mapeamento cromossômico dos DNAr 5S e 18S, pela técnica de FISH, vem sendo realizado com grande freqüência em estudos citogenéticos de peixes, mostrando ser um importante marcador cromossômico, uma vez que sua localização pode diferir entre espécies, assim como entre populações da mesma espécie, podendo estar em um único par cromossômico, vários pares, em sintenia entre eles e com outros DNAs repetitivos (TRALDI et al., 2013; PANSONATO-ALVES et al., 2013; SILVA et al., 2014).

Os pequenos DNAs nucleares (DNAsn) pertencem a uma família multigênica de RNAs não codificantes que compõem o spliceossomo, um grande complexo de ribonucleoproteinas que realiza o *splicing* (remoção dos íntrons do pré-RNAm), sendo a maior e mais complicada maquinaria celular conhecida (VALADKHAN, 2005). A remoção de íntrons é uma etapa crucial e onipresente na expressão genética dos eucariotos e quase todos os transcritos primários sofrem múltiplos eventos de *splicing* e *splicing* alternativo. Os DNAsn podem ser subdivididos em 5 tipos: U1, U2, U4, U5 e U6 (VALADKHAN, 2005). A maioria dos RNAsn são transcritos pela polimerase II, exceto o U6 RNAsn que é transcrito pela polimerase III (McNAMARA-SCHROEDER et al., 2001).

A utilização destes DNAs como marcadores citogenéticos é recente em peixes, sendo que os trabalhos realizados até então, somente analisaram o DNAsn U1 e U2 e estão restritos a poucas famílias: Batrachoididae, Moronidae, Bagridae, Cichlidae, Haemulidae, Gymnotidae, Characidae, Crenuchidae, Cyprinodontidae, Triportheidae e Heptapteridae (MERLO et al., 2010; ÚBEDA-MANZANARO et al., 2010;; CABRAL-DE-MELO et al., 2012; SUPIWONG et al., 2013; UTSUNOMIA et al., 2014b; SILVA et al., 2015; SCACCHETT et al., 2015; ARAYA-JAIME et al., 2017; SERRANO et al., 2017; dos SANTOS et al., 2017; YANO et al., 2017; USSO et al., 2019).

Diferentes padrões de distribuição de DNAsn U2 já foram observados: ocorrência em um par de cromossomos em espécies das famílias Moronidae (MERLO et al., 2010), Synbranchidae (UTSUNOMIA et al., 2014), Characidae (SILVA et al., 2015; dos SANTOS et al., 2017), Crenuchidae (SCACCHETT et al., 2015); sequencia dispersa pelos cromossomos na família Batrochoididae (ÚBEDA-MANZANARO et al., 2010); em vários cromossomos como em *Gymnotus pantanal* com sinais de DNAsn U2 em 12 cromossomos acrocêntricos nas fêmeas e em 11 nos machos, diferença esta devida a ocorrência de cromossomos sexuais nesta espécie (UTSUNOMIA et al., 2014). Em *Triportheus albus*, YANO et al. (2017) evidenciaram essa sequencia repetitiva no cromossomos sexual W, entre os 4 cromossomos portadores. Usso et al (2019) analisando diferentes populações de *Rhamdia quelen*, observaram variações interpopulacionais em relação ao número de cromossomos portadores de DNAsn U2.

Análises com DNAsn U1 são ainda mais escassas em peixes e os dados evidenciados por Cabral de Melo et al. (2012) em 12 espécies de ciclideos mostraram sítios dessa sequência em um par de cromossomos homólogos. Silva et al. (2015) analisando 5 espécies de *Astyanax* detectaram sítios de DNAsn U1 em 3 pares de cromossomos homólogos. Ambos os estudos mostraram variação dessa sequência em relação ao tipo de cromossomo portador e a posição no cromossomo, podendo apresentar sítios terminal, intersticial e proximal.

A sintenia dos pequenos DNAs nucleares com outra sequência de DNA repetitivo parece não ser comum em peixes (ÚBEDA-MANZANARO et al., 2010; MERLO et al., 2010; SUPIWONG et al., 2013; UTSUNOMIA et al., 2014) contudo, sintenia de DNAsn U2 com DNAr 5S já foi observada em 10 espécies de *Characidium* (SCACCHETT et al., 2015), quatro espécies de *Triportheus* (YANO et al., 2017) e em *Eigenmannia* aff. *trilineata* (ARAYA-JAIME et al., 2017). Em *Thalassophryne maculosa* foi observada a co-localização de DNAr 18S e DNAsn U2 (ÚBEDA-MANZANARO et al., 2010), e a sintenia

entre essas sequencias foi evidenciada em *Bryconamericus ecai* cytotype VI (dos SANTOS et al., 2017); em algumas espécies de *Triportheus*: *T. auritus*, *T. nematurus*, *T. signatus* e *T. trifurcatus* (YANO et al., 2017) e em *Rhamdia quelen* (USSO et al., 2019). Essas sequências de DNAsn, comparadas ao DNAr, possuem um menor número de dados em peixes, mas é evidente que, a cada análise, novas contribuições sobre a distribuição destas sequências vão se somando e uma real proposta evolutiva para sua localização cromossômica em peixes poderá ser estabelecida.

1.5 DNAs Repetitivos na Família Cichlidae

Dentre os poucos estudos com DNAs repetitivos em ciclídeos, apesar da grande diversidade de espécies, DNAr 18s é o mais estudado e, até o momento, os sítios dessa sequência se encontram mais frequentemente distribuídos em um par de cromossomos homólogos, geralmente em região terminal como em: *Cichla kelberi* (TEIXEIRA et al., 2009), *Cichla monoculus* (SCHENIDER et al., 2013a), *Geophagus brasiliensi*s (VICARI et al., 2006; NETO et al., 2010; PERAZZO et al., 2013), várias espécies de *Crenicichla* (PIRES et al 2015, 2019), entre outros.

Contudo, outros perfis de distribuição dessa sequência podem ser observados. Gross et al. (2010) analisando três espécies do gênero *Symphysodon: S. aequifasciatus*, *S. discus* e *S. haraldi* observaram uma variação inter e intraespecífica quanto à localização de DNAr 18S. Em *S. aequifasciatus* dois a três sítios foram encontrados, exibindo três perfis cariotípicos de distribuição na região terminal do braço curto dos pares 3, 8 e 19. Em *S. discus* foram encontrados de dois a cinco sítios, com quatro perfis diferentes de distribuição, envolvendo a região terminal do braço curto dos cromossomos 6, 17 e 23. Em *S. haraldi* foram evidenciados dois a três sítios, com quatro perfis cariotípicos diferentes, ocorrendo na região terminal dos pares 4 e 6 e na região intersticial dos pares 1 e 28.

Múltiplos sítios de DNAr 18S ocorrem também em outras espécies como *Crenicichla britskii* (PIRES et al., 2019), *C. paranaense*, *Gymnogeophagus rhabdotus* (Pires 2013), *Uaru amphiacanthoides* (SCHNEIDER et al., 2013) que apresentaram dois pares portadores dessa sequência. No complexo de espécies *Geophagus brasiliensis*, o número de sítios variou entre as populações analisadas, de 2 a 6 cromossomos portadores de DNAr 18S (ALVES-SILVA et al., 2015; OLIVEIRA et al., 2016). O caso mais diferente foi em *Pterophyllum leopoldi* onde 21 sítios foram observados (SCHNEIDER et al., 2013).

Em relação ao DNAr 5S, foram observadas marcações intersticiais no braço longo em um par de cromossomos para diferentes populações de *Gymnogeophagus brasiliensis* (VICARI et al., 2006, NETO et al., 2010, PERAZZO et al., 2013; ALVES-SILVA et al., 2015; OLIVEIRA et al., 2016) e *Cichlasoma facetum* (VICARI et al., 2006)*;* em região terminal também em um par de cromossomos em *Symphysodon aequifasciatus*, *S. discus* e *S. haraldi* (GROSS et al., 2009). Somente três espécies, até o momento, apresentaram mais de um par de cromossomos portadores de DNAr 5S: *Aequidens tetramerus* (POLETTO et al., 2010a), *C. lepidota* (PERAZZO et al, 2011), *Caquetaia spectabilis* (SCHNEIDER et al., 2013).

Nakajima et al. (2012), realizaram o mapeamento citogenético de genes de DNAr 5S em 18 ciclídeos Sul Americanos, 22 Africanos e uma espécie Asiática e do DNAR 18S de três espécies africanas. Nos ciclídeos Neotropicais, o número de "clusters" de DNAr 5S variou de 2 a 15, sendo mais comum a presença de 2 cromossomos portadores do sítio e em 76% das espécies essa sequência está em região intersticial (NAKAJIMA et al., 2012). Quanto ao DNAr 18S, houve variação de 2 a 6 sítios, com 2 sendo mais comum e os ciclídeos neotropicais apresentaram um perfil mais variável que os africanos mas, preferencialmente, o DNAr 18S se encontra na região terminal do braço curto de 1 par de cromossomos (NAKAJIMA et al., 2012).

Schneider et al. (2013) ao analisarem essas duas sequências ribossomais em 13 ciclídeos neotropicais, observaram duplicações do DNAr 18S, bem como sítios múltiplos na maioria dos táxons analisados. O DNAr 5S mostrou-se localizado na região intersticial de um par de cromossomos homólogos, embora variações tenham sido observadas. Os sítios teloméricos intersticiais também foram observados e parecem estar envolvidos em eventos de rearranjo cromossômico e no acúmulo de sequências de DNA satélites ricas em repetição. Os autores citados acima sugerem que a maior parte dessa diversidade é devida às seqüências repetitivas presentes em regiões heterocromáticas, as quais influenciaram muito a evolução cariotípica destes peixes.

Em relação aos pequenos DNAs nucleares, somente uma análise foi realizada em ciclídeos por Cabral de Melo et al. (2012). Nas 12 espécies analisadas os autores utilizaram sonda de DNAsn U1 e todas elas apresentaram pequenos sítios em um par de cromossomos homólogos, nas posições terminal, intersticial ou proximal. As espécies africanas apresentaram esta sequência na região terminal do braço longo de um par subtelo/acrocêntrico. Em *Etroplus maculatus*, uma espécie asiática, esta sequência ocupa a região intersticial do braço longo de um pequeno subtelo/acrocêntrico. Nas espécies da

América do sul um perfil mais variado foi observado: em *Retroculus lapidifer, Cichla kelberi, Biotodoma cupido, Aequidens tetramerus, Heros efasciatus, Geophagus brasiliensis, G. proximus* e *Satanoperca jurupari* o cístron está no braço longo de um par subtelo/acrocêntrico, em região terminal nas cinco primeiras espécies, intersticial em *Geophagus brasiliensis* e proximal nas últimas duas espécies. Em *Astronotus ocellatus* e *Mesonauta festivus* o gene de RNAsn U1 está localizado na região terminal de um par meta/submetacêntrico, em *A. ocellatus* no braço longo e em *M. festivus* no braço curto (CABRAL DE MELO et al., 2012).

Embora a família Cichlidae seja a mais estudada citogeneticamente dentro da ordem Cichliformes, os números ainda são pouco representativos em vista da ampla diversidade do grupo. Ainda existem dúvidas em relação aos mecanismos cromossômicos que tiveram envolvimento na diversificação cariotípica dos ciclídeos. Dessa forma, a expansão das análises citogenéticas convencional e molecular, com realização de mapeamento físico cromossômico de genes e sequências de DNA que podem ser integrados a dados de sequenciamento nucleotídico completo de genomas, podem permitir interpretações acerca da biologia e da complexa história evolutiva deste grupo.

2. OBJETIVOS

2.1 GERAL:

O presente estudo teve por objetivo geral realizar um mapeamento físico do genoma de diferentes espécies da família Cichlidae, utilizando sondas de diferentes sequências de DNA repetitivo, visando contribuir para o entendimento dos processos de diferenciação e evolução cariotípica desta família.

2.2 ESPECÍFICOS:

O presente trabalho teve por objetivos específicos:

- Caracterizar os perfis cromossômicos de peixes da família Cichlidae de diferentes bacias hidrográficas, a partir da análise citogenética;
- Analisar a distribuição e localização cromossômica dos cístrons ribossômicos 18S e 5S;
- Analisar a distribuição e localização cromossômica dos cístrons de DNAsn U1 e U2.
- Estabelecer possíveis homeologias cromossômicas entre diferentes espécies da família Cichlidae e identificar possíveis marcadores cromossômicos para essas espécies.
- Identificar tendências da evolução cromossômica no grupo e o papel exercido pelos DNAs repetitivos, por meio de uma análise comparativa de diferentes espécies.

3. ESPÉCIES ESTUDADAS E LOCAIS DE COLETA

3.1 Material e Locais de Coleta

Para o presente estudo foram analisadas onze espécies de peixes da família Cichlidae, pertencendo a três tribos: Chaetobranchini, Cichlasomatini, Geophagini (Figura 1). Estas espécies foram coletadas em diferentes pontos de quatro bacias hidrográficas distintas (Tabela 1).

Tabela 1 - Locais de coleta, bacias hidrográficas e espécies analisadas de Cichlidae.

Espécies	Tribos	Local de coleta	Bacia Hidrográfica	Número de indivíduos
Bujurquina vittata	Cichlasomatini	Miranda river-MS 19°31'24.96"S/57°02'25.51"W	Alto rio Paraguai Rio Paraguai	2?,3♀
Chaetobranchopsis australis	Chaetobranchini	Miranda river-MS 19°31'24.96"S/57°02'25.51"W	Alto rio Paraguai Rio Paraguai	4?
Cichlasoma portalegrense	Cichlasomatini	Barra do Joao Pedro 29º46'21.2 'S 50º05'08.0"W	Laguna dos Patos/RS	3♀,4♂
Crenicichla jaguarensis	Geophagini	Laranjinha river -PR 23º24'53.47 'S/ 50º27'08.28"W	Rio Paranapanema Rio Paraná	1♂,3♀
Crenicichla lepidota	Geophagini	Barra do Joao Pedro 29º46'21.2 'S/50º05'08.0"W	Laguna dos Patos/RS	4♀,4♂,3?
Crenicichla maculata	Geophagini	Rio Maquiné 29º39'10.4''S/50º12'31.8''W	Tramandaí/RS	4♀,2♂
Geophagus brasiliensis	Geophagini	Ribeirão Cambezinho 23º17'8.28'' S/51º16'67.7''W	Paraná River/PR	6♀,4♂
Geophagus brasiliensis	Geophagini	Saco da Alemoa 29º22'08.0''S/51º14'24.1''W	Laguna dos Patos/RS	3♀,5♂
Geophagus brasiliensis	Geophagini	Charco 29º46'15.98''S/ 50º03'55.34''W	Laguna dos Patos/RS	1♀,1♂,1?
Gymnogeophagus balzani	Geophagini	Miranda river-MS 19°31'24.96"S/57°02'25.51"W	Alto rio Paraguai Rio Paraguai	2?,2♀
Gymnogeophagus gymnogenys	Geophagini	Saco da Alemoa 29º22'08.0''S/51º14'24.1''W	Laguna dos Patos/RS	2♀,2♂,2?
Gymnogeophagus gymnogenys	Geophagini	Gasomêtro 30°02'06.3''S/51°14'29.12''W	Laguna dos Patos/RS	1♀,2♂
Gymnogeophagus labiatus	Geophagini	Rio Forqueta 29°22'08.0''S/52°03'30.0''W	Laguna dos Patos/RS	1♀,3♂
Gymnogeophagus rhabdotus	Geophagini	Estação UFRS 30º5'38.38''S/ 51º40'22.4''W	Laguna dos Patos/RS	1♀,3♂
		Total of individuals: 79		

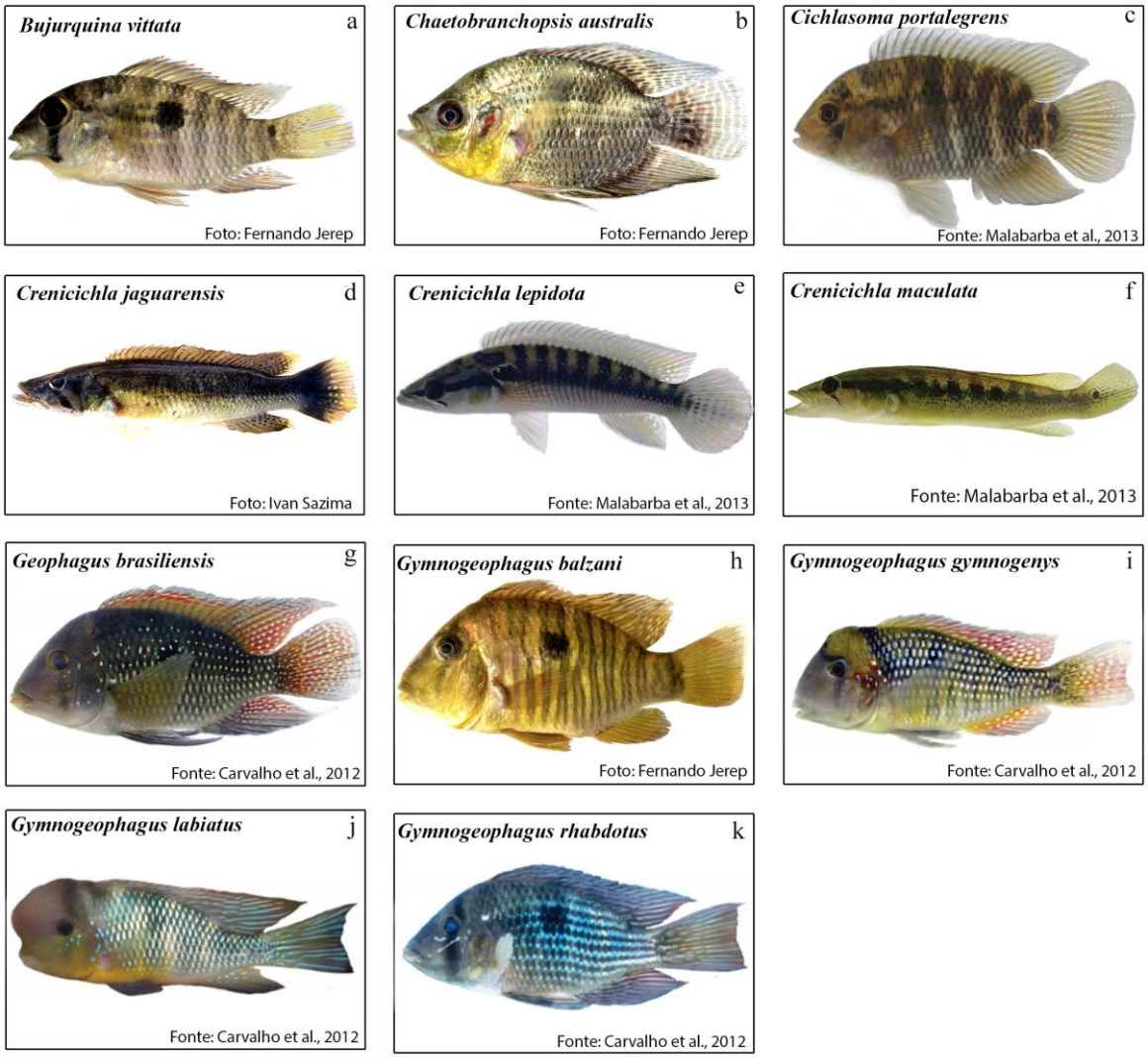

Figura 1 - Espécies de peixes analisadas da família Cichlidae.

CAPÍTULO 1

Physical mapping of rDNA, U1 and U2 snDNAs in four species of Cichlidae: evidence of syntenia and independent localization among different repetitive DNA sequences.

***This article will be submitted to the Journal of Heredity**

Physical mapping of rDNA, U1 and U2 snDNAs in four species of Cichlidae: evidence of synteny and independent localization among different repetitive DNA sequences.

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Abstract

Cichlids represent one of the most species-rich families of fishes which have been considered a model for evolutionary studies because their morphological and behavioral adaptations to diverse ecological niches. To further understanding of chromosome evolution among cichlid species, we provide comparative mapping of U2 and U1 small nuclear RNA (snRNA) and 18S and 5S ribosomal DNA (rDNA) for four Neotropical cichlids species. These are the first fluorescence in situ hybridization (FISH) analyzes for *Bujurquina vittata*, *Chaetobranchopsis australis*, *Crenicichla jaguarensis* and *Gymnogeophagus balzani*, which presented 2n=48, with exception of *B. vittata* that showed a karyotype similar to African cichlids (2n = 44). The distribution of small nuclear RNA genes and 5S rDNA was more conserved, with only one carrier pair in all species, independent of the location. The 18S rDNA sites besides showing greater variability among species were syntenic with U2 snRNA gene in *C. jaguarensis* e *G. balzani*, indicating a apparent homeology in first metacentric pair, and a possible interaction between distinct repetitive DNA families. The data presented here provide new information on the structure and organization of repetitive DNA sequences of cichlids and contribute to a better understanding of the karyotype evolution of the group.

Subject area: Genomics and gene mapping

Key words: Cichlasomatini, Chaetobranchini, Geophagini, genome evolution, multigene family.

Introduction

Cichlidae is among the largest lineages of freshwater fishes, including approximately 1.700 validated species so far (Eschmeyer and Fong 2018). Their species are distributed on Central and South America and across Africa to Madagascar and southern India (Kullander 1998; Kocher 2004) and thus this is the third most diverse family of fishes of Neotropical region (Reis 2016). Phylogenetic analyses support the subfamily Cichlinae (Neotropical) as monophyletic, and as a sister to the monophyletic African Pseudocrenilabrinae. Both sisters form a paraphyletic arrangement of Indian and Malagasy lineages in the subfamilies Etroplinae and Ptychochrominae (Stiassny 1991; Sparks 2008; Smith et al. 2008; López-Fernández et al. 2010; McMahan et al. 2013; Friedman et al. 2013; Matschiner et al. 2017; Ilves et al. 2018). The subfamily Cichlinae *sensu* Sparks and Smith 2004, Neotropical clade, includes about 60 genera with many unknown species (Reis et al. 2003; López-Fernández et al. 2010). Neotropical species are located within the three largest tribes, Geophagini, Cichlasomatini, and Heroini, and the other species are distributed in the tribes Cichlini, Retroculini, Chaetobranchini, and Astronotini (Kullander 1998; Smith et al. 2008; López-Fernández et al. 2010).

There has been increasing scientific interest on Cichlids due to their rapid adaptive radiation, species richness and the diversity of their ecological niches (López-Fernández et al., 2005). Despite the karyotype evolution for Neotropical cichlids three profile were proposed: the first is characterized by the maintenance of 2n = 48 acrocentrics, or with few metacentric-submetacentric (m-sm) chromosomes. The second is a decrease in diploid number in parallel with a larger number of bi-armed chromosomes (m-sm) and the third pattern results from an increase in diploid number (2n = 50 and 52), although most of the chromosomes remain acrocentric, suggesting a derived character (Feldberg et al. 2003). The first profile is observed in most species of Cichlinae, however, the fundamental number (FN) and, consequently, the karyotype formula, presents great variation, with the majority of

the species presenting an FN equal to or greater than 48 (Feldberg et al. 2003). Although there is a predominance of the diploid number, it can vary from 38 chromosomes, like in *Apistogramma borellii*, to 60 chromosomes, like in the genus *Symphysodon*, evidencing a dynamic karyotype in cichlids (Feldberg et al. 2003).

Recent studies with repetitive DNA corroborated this dynamics, showing variations in the number and location sites of ribosomal DNA (Schneider et al. 2013a, Gross et al. 2010) and some differences in the profile of distribution of TEs among species, that can be dispersed and compartmentalized (Mazzuchelli and Martins 2009, Teixeira et al. 2009, Gross et al. 2009, Schneider et al. 2013b). Cichlids have the largest volume of cytogenetic data of the order Cichliformes, however, information on the repetitive DNA sequences mapping is still scarce, especially in Neotropical members. The results obtained in the present work, through a classical approach and the physical mapping of different repetitive DNA sequences (18S and 5S rDNA, U1 and U2snRNA) in four cichlid species from South America, allowed an evaluation of the processes involved in karyotype rearrangement and the role of these sequences in the evolution of cichlid genomes.

Material and Methods

Origin of the samples

The species studied were collected in two hydrographic basins (Table 1) with the permission of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), protocol number 11399-1. We also obtained permission from the research ethics committee of the University Estadual of Londrina (animal ethic use number: CEUA **5579.2018.72**). The specimens were deposited in the Museum of Zoology at the State University of Londrina, Parana, Brazil.

Table 1 – Collection sites and hydrographic basins of Cichlidae specimens analyzed. MS = Mato Grosso do Sul state; PR = Paraná state

Species	Tribes	Collection sites	Hydrographic basins	Number of individuals
Bujurquina vittata	Cichlasomatini	Miranda river-MS 19°31'24.96"S/57°02'25.51"W	Paraguai river	2?,3♀
Chaetobranchopsis australis	Chaetobranchini	Miranda river-MS 19°31'24.96"S/57°02'25.51"W	Paraguai river	4?
Crenicichla jaguarensis	Geophagini	Laranjinha river -PR 23º24'53.47 'S/ 50º27'08.28"W	Paraná river	1♂,3♀
Gymnogeophagus balzani	Geophagini	Miranda river-MS 19°31'24.96"S/57°02'25.51"W	Paraguai river	2?,2♀
		Total of individuals: 17		

Conventional cytogenetic analysis and chromosome banding

Before undergoing euthanasia (48 h), the specimens received an intraperitoneal injection of 2mL of Broncho-vaxom (bacterial lysate) to trigger an inflammatory process and hence increase the number of renal cells in mitotic division (Molina et al. 2010). Mitotic chromosomes were obtained by direct preparation removing the anterior kidney, as proposed by Bertollo et al. (1978), and then stained with 5 % Giemsa in phosphate buffer (pH 6.8). Metaphase spreads from different individuals were analyzed to confirm the diploid number and karyotype structure. Chromosomes were classified according to the protocol of Levan et al. (1964). For determination of the fundamental number (FN), the metacentric (m) and submetacentric chromosomes (sm) were considered biarmed and subtelocentric (st) and acrocentric chromosomes (a) uniarmed. The nucleolus organizer regions (AgNORs) were detected by the silver nitrate impregnation technique according to the protocol of Howell and Black (1980).The images were captured with Moticam Pro 282B. Karyotype mounting and image brightness and contrast adjustments were performed in Adobe Photoshop CS6.

Repetitive sequences probes and FISH experiments

Genomic DNA from *Cichlasoma* was extracted from the muscles using phenol/chloroform procedure described by Sambrook and Russel (2001). 18S and 5S rDNA,

and U2 and U1 snRNA probes were obtained by PCR using the genomic DNA, as template primers listed in the Table 2.

Table 2 – Primers used to PCR amplification for gene fragments 18S rDNA, 5S rDNA, U2 snRNA and U1 snRNA.

Gene	Primers sequences	References
5S rDNA	5SA 5'TCAACCAACCACAAAGACATTGGCAC-3' 5SB 5'TAGACTTCTGGGTGGCCAAAGGAATCA-3'	Pendás et al. (1994)
18S rDNA	18SF 5'-GTAGTCATATGCTTGTCTC-3' 18SR 5'-TCCGCAGGTTCACCTACGGA-3'	White et al. (1990)
U2snRNA	U2 F 5'-ATCGCTTCTCGGCCTTATG-3' U2 R 5'-TCCCGGCGGTACTGCAATA-3'	Bueno et al. (2013)
U1snRNA	U1 F 5'- GCAGTCGAGATTCCCACATT-3' U1 R 5'-NCTTACCTGGCAGGGGAGATA-3'	Cabral-de-Melo et al. (2012)

The 18S rDNA and U1 snRNA probes were labeled by PCR with biotin-16-dUTP, and the 5S rDNA and U2 snRNA probes were labeled by PCR with digoxigenin-11-dUTP. FISH was performed under high-stringency conditions using the method described by Pinkel et al. (1986) with modifications. Slides were incubated with RNAse (50µg/ml) for 1 h at 37°C. Next, the chromosomal DNA was denatured in 70% formamide/2×SSC for 5 min at 70°C, and the slides were taken through an ice-cold ethanol series (70%-80%-100%). For each slide, 30µl of hybridization solution containing 200 ng of each labeled probe, 50% formamide, 2×SSC and 10% dextran sulfate were denatured for 10 min at 95°C, dropped onto the slides and hybridized overnight at 37°C in a 2×SSC moist chamber. After hybridization, slides were washed in 0.2×SSC/15% formamide for 20 min at 42°C, followed by a second wash in 0.1×SSC for 15 min at 60°C and a final wash at room temperature in 4×SSC/0.5% Tween for 10 min. Probe detection was carried out with avidin-FITC (Life Technologies, Carlsbad, CA) or anti-digoxigenin-rhodamine (Roche Applied Science, Indianapolis, IN). Chromosomes were counterstained with DAPI (4', 6-diamidino-2-phenylindole, Vector Laboratories). The images were captured with an Olympus camera

(DP70) coupled to an Olympus BX61 photomicroscope. The construction of the idiogram was performed using the Easy Ideo.

Results

Bujurquina vittata presented a diploid number (2n) equal to 44 chromosomes with karyotype formula 30m+8sm+6st-a and fundamental number 82 (Figure 1a); the other three species revealed a 2n=48 with different karyotype formula: 48st-a and FN=48 in *Chaetobranchopsis australis* (Figure 1b); 4m+4sm+40st-a and FN=56 for *Crenicichla jaguarensis* (Figure 1c) and 2m+46st-a and FN =50 for *Gymnogeophagus balzani* (Figure 1d).

Nucleolar organizer region (AgNOR) was observed on long arm of the first subtelo-acrocentric pair of chromosomes in *Chaetobranchopsis australis,* and in *Crenicichla jaguarensis* on short arm of first metacentric chromosome, both in terminal region (Figure 1b,c- box). *Gymnogeophagus balzani* AgRONs were observed on the short arm of metacentric pair 1, and on the short arm on only one homologous of pair 7, all in interstitial region. *Bujurquina vittata* presented two pairs with AgNORs in the terminal region of the short arm of metacentric pair 8, and subtelo-acrocentric pair 21 (Figure 1a - box). FISH mapping with the 18S rDNA probe was consistent with Ag-NOR patterns in all species.

The 5S rDNA was located in the interstitial region of subtelo-acrocentric pair 22 of *B. vittata* and *C. australis* (Figure 1a, b – boxes, respectively). In *C. jaguarensis* and *G. balzani* 5S rDNA sites were observed in interstitial region of subtelo-acrocentric pair 6 and 5, respectively (Figure 1c, d – boxes, respectively).

FISH using U2 snDNA probe located sequences in the terminal region of the short arm of metacentric pair 2 in *B. vittata* and subtelo-acrocentric pair 3 in *Chaetobranchopsis australis* (Figure 1a,b – boxes, respectively). In *Crenicichla jaguarensis* and *G. balzani* the

U2 snRNA clusters were associated with 18S rDNA in the short arm of metacentric pair 1 (Figure 1c, d – boxes, respectively).

The U1 snDNA clusters were located in the terminal region of metacentric pair 4 of *B. vittata* (Figure 1a-box), in the interstitial region in long arm of subtelo-acrocentric pair 17 of *Chaetobranchopsis australis* (Figure 1b–box), and in interstitial region of subtelo-acrocentric pair 6 and 10 of *Crenicichla jaguarensis* and *G. balzani*, respectively (Figure 1c, d – boxes, respectively); in *C. jaguarensis* U1snRNA clusters were co-localized with 5S rDNA (Figure 1c-box).

Discussion

Karyotypic analysis

As observed to the most of South American cichlids the diploid number of 48 chromosomes was constant in Cichlidae species , corroborating with the general karyotypic pattern for family (Feldberg et al. 2003), except to *Bujurquina vittata* with 2n=44, which is the most commonly number found in African cichlids (Feldberg et al. 2003). Few variations in the karyotype formulas were found in relation to other populations of the same species (Feldberg and Bertollo, 1985; Marescalchi 2005; Roncati et al. 2007; Lorscheider 2008). According to Schneider et al. (2013a), these variations are due to differences in the quality of the chromosomal preparations, chromatin condensation, and errors in chromosome arm measurements, which may generate differences in karyotype interpretations, resulting in intraspecific differences.

The karyotype formula observed in *C. australis*, tribe Chaetobranchini, with 48 subtelo-acrocentric chromosomes, is considered a plesiomorphic characteristic in Neotropical cichlids, but it has been observed in all species of two other Neotropical tribes: Cichlini and Retroculini (Poletto et al. 2010; Valente et al. 2012). This characteristic is consistent with the phylogenetic analysis where Chaetobranchini occupies a basal position

of Neotropical cichlids (Ilves et al. 2018). *Crenicichla jaguarensis* and *G. balzani*, both Geophagini, also maintain 2n = 48 with a few metacentric-submetacentric chromosomes, due mainly to pericentric inversions.

The lower chromosome number of *Bujurquina vittata* (Cichlasomatini) associated with the few acrocentrics chromosomes, suggest that complex rearrangements like pericentric inversion and fusions may be contributing the evolution of this species. Eight metacentric chromosome pairs presented a much smaller size than the other metacentric chromosomes (Figure 1a). Something similar was observed by Roncati et al. (2007), which besides the reduced diploid number classified seven chromosome pairs as microchromosomes in *B. vittata* from the Paraná river, reinforcing the occurrence of these chromosomal rearrangements.

This karyotype of *B. vittata* is uncommon among Neotropical cichlids, however, a reduction of 2n also was observed in *Nannacara* (Thompson 1979; Hodanová et al. 2014) and *Laetacara* (Poletto et al. 2010, Santos et al. 2005; Valente et al. 2012; Marescalchi 2005). Molecular analyses with the mitochondrial 16S gene also showed that *Bujurquina* and *Laetacara* are sister groups (Marescalchi 2005), which is evidence of the ancestry of this reduction in the 2n for the group. However, more recent cytogenetic data have shown 2n=50 for two Cichlasomatini species: *Bujurquina peregrinabunda* and *Acaronia nassa*, with an increase in diploid number (Schneider et al. 2013a), although most of the chromosomes remained acrocentric, suggesting a derived character. The chromosomal rearrangements and the formation of the karyotype similar to that of *B. vittata*, analyzed in this study, occurred several times independently in the evolution of cichlids (Hodanová et al. 2014). The appearance of this karyotype probably represents a secondary change back to the "common teleost karyotype", since the karyotype with most subtelocentric-acrocentric chromosomes, considered ancestral for cichlids, it is not usually an ancestral trait for other fish groups (Thompson, 1979, Arai, 2011).

Repetitive DNA families distribution

A simple NOR in the short arm is common in Neotropical cichlids (Poletto et al. 2010), as observed in *Crenicichla jaguarensis. Chaetobranchopsis australis* also presents a single 18S rDNA site, however, it is found in the region terminal of the long arm of pair 1, a characteristic observed only in *Cichla monoculus*, *Acaronia nassa* (Schneider et al. 2013a), *Gymnogeophagus setequedas* (Paiz et al. 2017), *Cichlasoma paranaense* and *Gymnogeophagus rhabdotus* (Pires, 2013), and *Crenicichla britski* (Pires et al. *in press*). All of these species have a large number of st-a chromosomes, which seems to be a derived condition for the group (Poletto et al. 2010). *Bujurquina vittata* and *G. balzani* showed multiple 18S rDNA sites and in *G. balzani* only one homologous of pair 7 presented this site, due probably to the terminal location of the ribosomal sites. This could facilitate the transfer of genetic material in interphasic core due to the nuclear proximity and orientation of the chromosomes, as proposed by the Rabl model (Cowan et al. 2001). This situation has already been observed in other species of fish as *Bryconamericus* sp (Santos et al. 2017) and *Rhamdia quelen* (Usso et al. 2018).

Intraspecific and interspecific variability in the distribution of 18S rDNA, along with the presence of one of the homologous chromosomes carrying this sequence, was observed by Gross et al. (2010) in species of *Symphysodon*. The authors also suggested that terminal location of 18S rDNA could facilitate its transposition to other chromosome pairs. Additionally, the species analyzed maybe susceptible to environmental variations. During evolution of this group, an association of rDNA with heterochromatin and transposable elements may have favored structural polymorphisms of the rDNA sites, suggesting that the genome of these species may have been continuously modified by repetitive DNA variation (Schneider et al. 2013a, 2013b). The variability described for the 18S rDNA here and observed in other Neotropical cichlid (Poletto et al. 2010, Gross et al. 2010) confirms that

this represents one of the most dynamic components in the evolution of eukaryote genomes (Gornung 2013).

In contrast to the 18S rDNA, the distribution of the 5S rRNA genes was more conservative, with only one chromosomal pair bearing the site in the 4 species analyzed, with variations only in the position and the pair carrier of the sites. These information reinforce the conservative evolution of 5S rDNA in Neotropical cichlids in general, where the most common finding is the presence of this site in subtelo-acrocentric chromosomes in the interstitial position of the long arm (Nakajima et al. 2012). The presence of 5S rDNA sites in two chromosomes pairs or more is not common in Neotropical cichlids and it has been observed only in *Crenicichla lepidota* (Perazzo et al. 2011) and *Caquetaia spectabilis* (Schneider et al. 2013a).

Although scarce, data on physical mapping of U snRNA genes in fish have shown a strong conservation in the sites number per genome, and suggested that they tend to accumulate exclusively in one chromosomal pair (Merlo et al. 2010, 2012a, b; Úbeda-Mazanaro et al. 2010; Supiwong et al. 2013; Utsunomia et al. 2014; Scacchetti et al. 2015; Santos et al. 2017; Araya-Jaime et al. 2017). These are the first data concerning U2 snDNA in Neotropical cichlids and their distribution was highly conserved, always in a single chromosome pair as in the species analyzed so far. However, their differential dispersion and organization has already been described as in Úbeda-Manzanaro et al. (2010) that analyzed species of Batrachoididae and observed: (i) compartmentalization of this sequence in a single pair, (ii) a very widely scattered distribution throughout all chromosomes, or (iii) simultaneous scattered and compartmentalized organization. *Gymnotus pantanal* analysed by Utsunomia et al. (2014) was the species that presented more sites of U2 snDNA, in 12 pairs and a sexual chromosome. Usso et al. (2018) showed differences in U2 snDNA accumulation between populations of *Rhamdia quelen.*

The conservation of U1 chromosomal cluster numbers among distantly related cichlid has already been reported by Cabral-de-Mello et al. (2012). According to these authors, the African species showed clusters of the U1 snRNA gene exclusively in the terminal position, among Asiatic species in the interstitial position, and among Neotropical cichlids in the terminal, interstitial and proximal position, all in the long arm of one st/a chromosomal pair. Also, in two Neotropical cichlids, clusters of U1 were located terminally on the m/sm pair and on the long and short chromosomal arms (Cabral-de-Mello et al. 2012). The greatest variation in the position of the U1snDNA clusters in Neotropical cichlids in relation to the Asiatics and Africans could be attributed to the specific chromosomal rearrangements during the evolutionary diversification of the group, a variation also observed in the species analyzed here (Figure 2).

Most cichlids species had their U1 and U2 snDNA sites located on a single chromosomal pair, but there are a few reports linking these sequences to other multigenic families, such as rDNA, or different snDNAs. Species studied here presented their snDNA sites U1 and U2 located in different chromosomes, a result also observed in *Astyanax* (Silva et al. 2015) and grasshoppers (Bueno et al. 2013; Palacios-Gimenez et al. 2013). However, a possible interaction between the distinct repetitive DNA families, U2 snRNA and 18S rDNA, was observed in the first metacentric pair in *C. jaguarensis* and *G. balzani* in the short arm; both belong to the tribe Geophagini, showing a possible homology (Figure 2). This is not a very common situation in fish but it has recently been observed in some species of *Triportheus* (Yano et al. 2017) and *Rhamdia quelen* (Usso et al. 2018).

Although double FISH for 5S and U1 snDNA was not performed, this cluster seems to be in the same chromosome in *C. jaguarensis* but in different regions (Figure 2). The molecular association between these two sequences has already been reported in the fishes as *Solea senegalensis* (Pelliccia et al. 2001; Manchado et al. 2006), *Astyanax jordani*, *A. paranae*, *A. bockmanni*, and *A. fasciatus* (Silva et al. 2015). Some species of *Triportheus*

(Yano et al. 2017) show a very rare situation for fishes, in which U2 snDNA clusters are closely located near both rDNAs. Whether this association provides any selective advantage remains unclear (Silva et al. 2015).

The karyotypic macrostructure in cichlids reflects a conservative group from a cytogenetic point of view (Feldberg and Bertollo 1985), with a few exceptions, like *B. vittata*. However, when analyses with different classes of repetitive DNAs were performed, this group showed high evolutionary rates at a microstructural level, confirming that these sequences have greatly influenced the karyotypic evolution of these fishes as evidenced in our study and by other authors (Gross et al. 2009, 2010; Teixeira et al. 2009; Mazzuchelli and Martins 2009; Valente et al. 2012).

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Conflict of Interest

The authors have no conflicts of interest to declare.

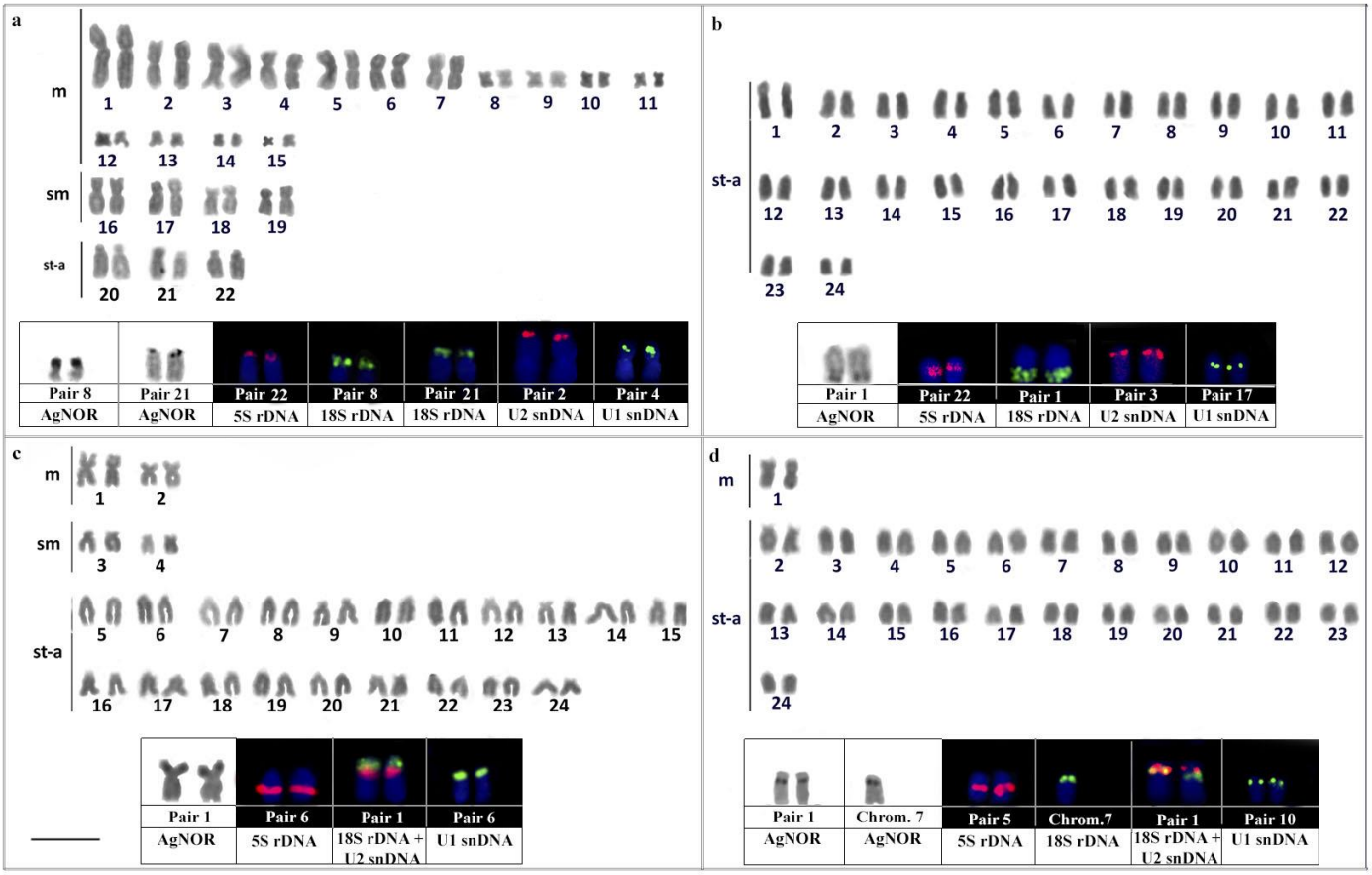

Figure 1 - Karyoytpes arranged from Giemsa-stained metaphases of (a) *Bujurquina vittata*; (b) *Chaetobranchopsis australis;* (c) *Crenicichla jaguarensis* and (d) *Gymnogeophagus balzani*. Inset box shows location of nucleolar organizer regions with impregnation of silver nitrate (Ag-NOR); in situ hybridization (FISH) with probes of 5S rDNA, 18S rDNA, U2 snDNA and U1 snDNA. In *C. jaguarensis* (c) and *G. balzani* (d) was observed synteny between 18S rDNA+U2 snDNA. Bar 10 µm.

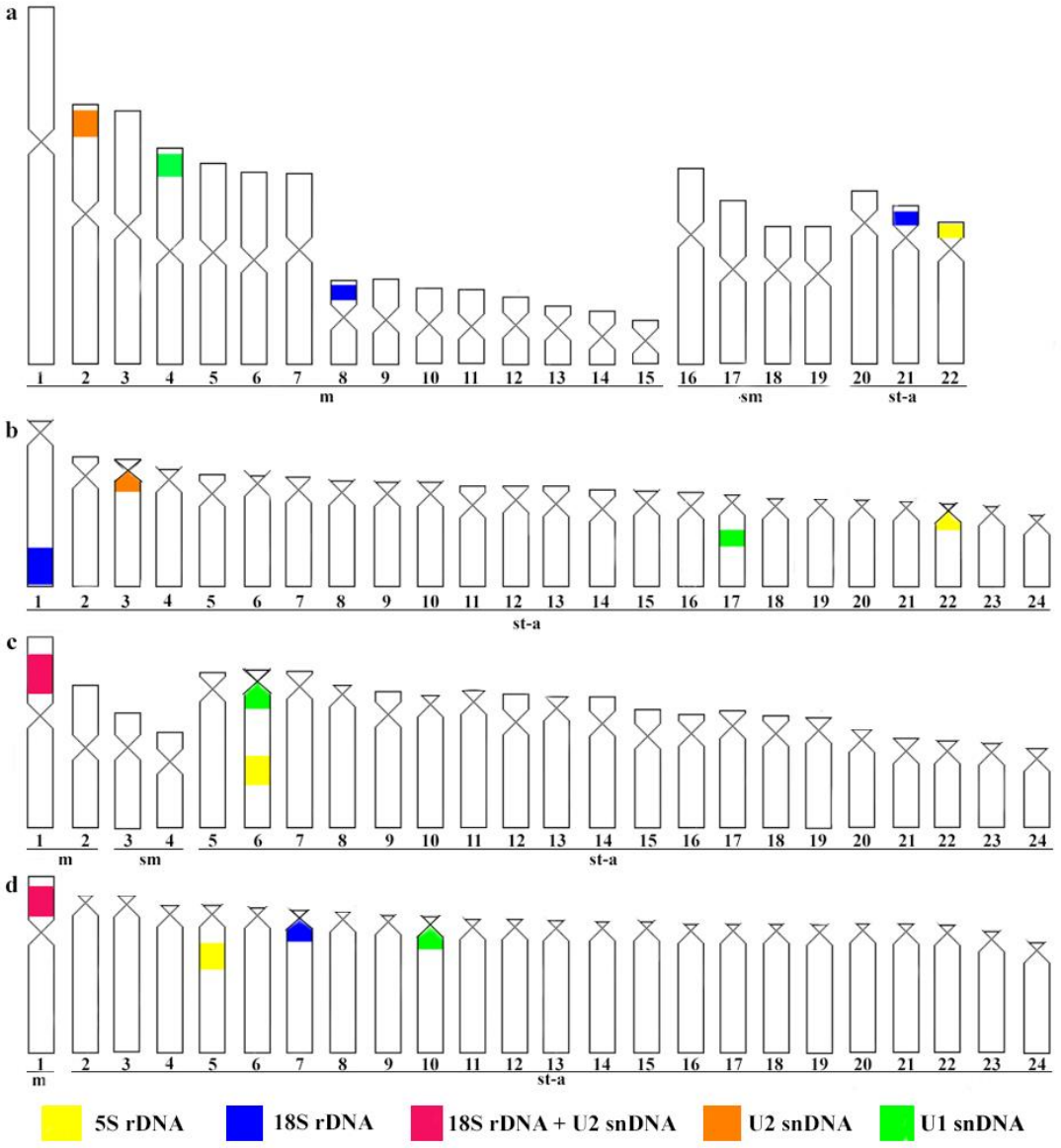

Figure 2 - Representative ideogram of karyotype: (a) *Bujurquina vittata* ;(b) *Chaetobranchopsis australis;(*c) *Crenicichla jaguarensis* and (d) *Gymnogeophagus balzani* showing accumulation of distribution of different repetitive DNAs: 5S rDNA, 18S rDNA, synteny between 18S rDNA+U2 snDNA, U2 snDNA and U1 snDNA.

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CAPÍTULO 2

Repetitive DNA families mapping reveals patterns for genome organization and chromosomal evolution in Cichlidae (Cichliformes)

***This article will be submitted to the Chromosome Research.**

Repetitive DNA families mapping reveals patterns for genome organization and chromosomal evolution in Cichlidae (Cichliformes)

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Abstract

The fishes of the Cichlidae family were considered to have low karyotypic variability due to their conserved diploid number of 48. In this study seven species of Neotropical Cichlids have the karyotype investigated. All species present 2n=48, however, different karyotype formulae were observed. In situ hybridization (FISH) analyzes with probes of small nuclear RNA (snRNA) and 18S and 5S ribosomal DNA (rDNA) showed an evolutionary dynamism regarding the localization of sequences on karyotypes. In most species, the rDNA and snDNA were observed in one homologous pair, however, *Geophagus brasiliensis*, *Gymnogeophagus gymnogenys* and *Gymnogeophagus rhabdotus* presentfrom 4 to 6 sites of 18S rDNA and *Crenicichla lepidota* present 4 sites of 5S rDNA, numbers not observed in most of Cichlidae. Besides that *Crenicichla lepidota, C. maculata* and *Gymnogeophagus labiatus* present 18S rDNA and snDNA in a sintenic condition, not commonly observed in fishes. The occurrence of these distinct karyotypes in the species analyzed, indicate the action of chromosomal rearrangements, such as the pericentric inversion involving 18S rDNA in *Gymnogeophagus gymnogenys.* The distribution of repetitive DNA, which may be located in different types and positions in the chromosomes, is corroborating with other studies, that suggest the karyotypic plasticity in Cichlidae. These sequences play an important role in the karyotype evolution of the group.

Subject area: Genomics and gene mapping

Key words: genome evolution, karyotype evolution, multigene family, repetitive DNA

Introduction

From an evolutionary point of view, the Cichlidae family is an interesting group to study because their adaptive radiation, species richness and diversity of ecological niches (López-Fernández et al. 2005). This is one of the most species-rich families of fishes (Eschmeyer and Fong 2019), and presents a wide distribution that can be found in Central and South America and across Africa to Madagascar and southern India (Kullander 1998; Kocher 2004). Most species are found in Africa and many endemic to lakes in the central region, forming extremely specialized groups (Fryer and Iles 1972; Ribbink 1990), where a predominantly intralacustrian speciation is triggered by sexual selection and trophic specialization (Kullander 1983).

Unlike African cichlids, most South American species are widely distributed and, moreover, this group does not have a specific period for reproduction, being able to have several generations in a year, allowing the fixation of new genetic recombination (Martins-Santos et al. 2005). Speciation in Neotropical cichlids probably occurred due to the accumulation of genetic mutations in allopatric populations living in different rivers, but not associated with large divergences in feeding mode (Kullander 1983). This group of fishes appears to have undergone lower rates of extinction and speciation than African cichlids, preserving primitive traits and accumulating higher levels of genetic divergence in some strains (Farias et al. 1999).

Repetitive DNA comprises segments of different sizes, which repeat tens of millions of times in the genome and make up a large portion of the eukaryotic genome (Taft et al. 2007). Among these DNA sequences are the multigenic families, moderately repetitive, which comprise a group of genes descended from a common ancestor, and with similar sequences and functionally (Nei and Rooney 2005), such as ribosomal DNAs (Martins 2007) and small nuclear DNAs (U DNAsn) (Valadkhan 2005).

In recent years, there were an increase in the number of studies in fish with rDNA probes including Cichlidae. This repetitive sequence , has shown to be a good chromosomal marker and seems to play an important role in the karyotype rearrangements of this group, as well as other repetitive DNAs (Teixeira et al. 2009; Gross et al. 2010; Schneider et al. 2013a). A large rate of mutations is observed in the intergenic regions of these sequences, and during karyotype evolution these changes have been shown to be important in chromosome reorganization (Carvalho et al. 2011; Georgiev and Karagyozov 2012). The accumulation of mutation is the source of the genetic variability, which can generate sites that are prone to double-stranded ruptures (DSB), which promote chromosomal plasticity during karyotype evolution and speciation (Barros et. 2017; Bruschi et al. 2014; Carvalho et al. 2011).

Although the Cichlids are one of the most species rich families of fish, the cytogenetic information regarding the mapping of the repetitive DNAs and their functions are still punctual. These elements are known to be dynamically present in the genome of neotropical cichlids, but most studies in this group are restricted to the distribution of ribosomal DNA, showing 18S rDNA in the terminal region of the short arm of subtelo-acrocentric chromosomes and 5S rDNA in a single pair in interstitial region of the long arm (Gross et al. 2009, Mazzuchelli and Martins 2009, Teixeira et al. 2009, Nakajima et al. 2012; Schneider et al. 2013a). So far, a single study has been carried to verify patterns of distribution of snDNA in the Cichlidae family. Cabral de Melo et al. (2012), hybridized U1snDNA in 19 species from South America, Asia and Africa and observed a high conservation of this sequence in a pair of chromosomes with few variations in position and chromosome type.

Considering the lack of analyses of repetitive DNAs in Cichlidae, compared to the great diversity of the family, this study brings the physical mapping of repetitive DNA sequences (18S and 5S rDNA and U2snRNA) of seven South American species, in an effort

to improve our understanding of the chromosomal evolution and genomic dynamics in the cichlids.

Material and Methods

Origin of the samples

The species studied belong two tribes of Cichlidae and were collected in different hydrographic basins (Table 1) with the permission of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), protocol number 11399-1. We also obtained permission from the research ethics committee of the State University of Londrina (animal ethic use number: CEUA **5579.2018.72**). The specimens were deposited in the Museum of Zoology at the State University of Londrina, Parana, Brazil.

Table 1 – Tribes, collection sites, hydrographic basins and number of individuals of Cichlidae species analyzed. PR = Paraná; RS= Rio Grande do Sul

Species	Tribes	Collection sites	Hydrographic basins	Number of individuals
Cichlasoma portalegrense	Cichlasomatini	Barra do Joao Pedro 29º46'21.2 'S 50º05'08.0"W	Laguna dos Patos/RS	3♀,4♂
Crenicichla lepidota	Geophagini	Barra do Joao Pedro 29º46'21.2 'S 50º05'08.0"W	Laguna dos Patos/RS	4♀,4♂,3?
Crenicichla maculata	Geophagini	Rio Maquiné 29º39’10.4’’S 50º12’31.8’’W	Laguna dos Patos/RS	4♀,2♂
Geophagus brasiliensis	Geophagini	Cambezinho Stream 23º17’8.28’’ S/51º16’67.7’’W	Paraná River/PR	6♀,4♂
Geophagus brasiliensis	Geophagini	Saco da Alemoa 29º22’08.0’’S / 51º14’24.1’’W	Laguna dos Patos/RS	3♀,5♂
Geophagus brasiliensis	Geophagini	Charco 29º46’15.98’’S/ 50º03’55.34’’W	Laguna dos Patos/RS	1♀,1♂,1?
Gymnogeophagus gymnogenys	Geophagini	Saco da Alemoa 29º22’08.0’’S /51º14’24.1’’W	Laguna dos Patos/RS	2♀,2♂,2?
Gymnogeophagus gymnogenys	Geophagini	Gasomêtro 30°02’06.3’’S/51°14’29.12’’W	Laguna dos Patos/RS	1♀,2♂
Gymnogeophagus labiatus	Geophagini	Forqueta river 29°22’08.0’’S/52°03’30.0’’W	Laguna dos Patos/RS	1♀,3♂
Gymnogeophagus rhabdotus	Geophagini	Estação UFRGS 30º5’38.38’’S/ 51º40’22.4’’W	Laguna dos Patos/RS	1♀,3♂
		Total of individuals: 62		

Conventional cytogenetic analysis and chromosome banding

Before undergoing euthanasia (48h), the specimens received an intraperitoneal injection of 2mL of Broncho-vaxom (bacterial lysate) to trigger an inflammatory process and hence increase the number of renal cells in mitotic division (Molina et al. 2010). Mitotic chromosomes were obtained by direct preparation removing the anterior kidney, as proposed by Bertollo et al. (1978), and then stained with 5 % Giemsa in phosphate buffer (pH 6.8). Metaphase spreads from different individuals were analyzed to confirm the diploid number and karyotype structure. Chromosomes were classified according to the protocol of Levan et al. (1964). For determination of the fundamental number (FN), the metacentric (m) and submetacentric chromosomes (sm) were considered biarmed and subtelocentric (st) and acrocentric chromosomes (a) uniarmed. The images were captured with a Moticam Pro 282B. Karyotype mountingand image brightness and contrast adjustments were performed in Adobe Photoshop CS6.

Repetitive sequences probes and FISH experiments

Genomic DNA from *Cichlasoma* was extracted from the muscles using phenol/chloroform procedure described by Sambrook and Russel (2001). 18S and 5S rDNA, and U2 snRNA probes were obtained by PCR using the genomic DNA, as template and the primers are listed in the Table 2.

Table 2 - Primers used to PCR amplification for gene fragments 18S rDNA, 5S rDNA and U2 snRNA.

Gene	Primers sequences	References
5S rDNA	5SA 5'-TCAACCAACCACAAAGACATTGGCAC-3' 5SB 5'-TAGACTTCTGGGTGGCCAAAGGAATCA-3'	Pendás et al. (1994)
18S rDNA	18SF 5'-GTAGTCATATGCTTGTCTC-3' 18SR 5'-TCCGCAGGTTCACCTACGGA-3'	White et al. (1990)
U2snRNA	U2 F 5'-ATCGCTTCTCGGCCTTATG-3' **U2 R 5'-TCCCGGCGGTACTGCAATA-3'**	Bueno et al. (2013)

The 18S rDNA probe were labeled by PCR with biotin-16-dUTP, and the 5S rDNA and U2 snRNA probes were labeled by PCR with digoxigenin-11-dUTP. FISH was performed under high-stringency conditions using the method described by Pinkel et al. (1986) with modifications. Slides were incubated with RNAse (50µg/ml) for 1h at 37°C. Next, the chromosomal DNA was denatured in 70% formamide/2×SSC for 5min at 70°C, and the slides were taken through an ice-cold ethanol series (70%-80%-100%). For each slide, 30µl of hybridization solution containing 200ng of each labeled probe, 50% formamide, 2×SSC and 10% dextran sulfate were denatured for 10min at 95°C, dropped onto the slides and hybridized overnight at 37°C in a 2×SSC moist chamber. After hybridization, slides were washed in 0.2×SSC/15% formamide for 20min at 42°C, followed by a second wash in 0.1×SSC for 15min at 60°C and a final wash at room temperature in 4×SSC/0.5% Tween for 10 min. Probe detection was carried out with avidin-FITC (Sigma) or anti-digoxigenin-rhodamine (Roche). Chromosomes were counterstained with DAPI (4',6-diamidino-2-phenylindole, Vector Laboratories). The images were captured with an Olympus camera (DP70) coupled to an Olympus BX61 photomicroscope. The construction of the idiogram was performed using the Easy-Ideo.

Results

All species shared a diploid number of 2n=48, independently collection sites, and no differentiated sex chromosomes were observed, although interspecific and intraspecific variations in karyotype formula were present: *Cichlasoma portalegrense* 14m+34st-a and FN=62 (Fig. 1a), *Crenicichla lepidota* and *Crenicichla maculata* with 6m+42st-a and FN=54 (Fig. 1b,c, respectively), *Geophagus brasiliensis* 4sm+44st-a and FN=52 (Fig. 1d, e, f), *Gymnogeophagus gymnogenys* from Gasômetro presented 6m+42st-a and FN=54 and from Saco da Alemoa 4m+44st-a and FN=52 (Fig. 1g, h, respectively), *G. labiatus*

4m+4sm+40st-a (Fig. 1i) and FN=56 and *G. rhabdotus* 4m+2sm+42st-a and FN=54 (Fig. 1j).

The simultaneous hybridization of 18S and 5S rDNA probes showed that these sequences are non-syntenic in all analyzed species (Fig. 1 – boxes). The 18S rDNA probe was located in the terminal region of the short arm of metacentric pair 1 in *Crenicichla lepidota*, *C. maculata* and *Gymnogeophagus labiatus* (Fig. 1b, c, i - boxes) and pair 5 of *Cichlasoma portalegrense* (Fig. 1a - box).

The three populations of *Geophagus brasiliensis* showed differences in the location of 18S rDNA: population of the Cambezinho stream presented this ribosomal site in the terminal region of the short arm of pair 2, submetacentric (Fig. 1d – box); in the Charco population was found in the long arm of the interstitial region of pair 6, subtelo-acrocentric (Fig. 1f – box) and in Saco da Alemoa in pair 6 and in one of the homologous of pair 8 (Fig.1e–box). *Gymnogeophagus gymnogenys* from Gasômetro presented this probe in pair 20 subtelocentric, in terminal region of the short arm (Fig. 1g box) and from Saco da Alemoa in pairs 3, 9 and 13 subtelo-acrocentric chromosomes in the long arm, interstitial region (Fig. 1h box). *G. rhabdotus* also presented three pairs with rDNA18S, the pairs subtelo-acrocentric 5 and 12 in the long arm in interstitial region and pair 15 in both terminal regions. (Fig. 1j-box).

The 5S rDNA was located in the interstitial region of all species: in *Cichlasoma portalegrense* in the long arm of pair 6; in *Crenicichla lepidota* in the pairs 11 and 12; in *Crenicichla maculata* in the pair 16*;* in *Geophagus brasiliensis* from Cambezinho in the pair 9 and pair 3, from Charco and Saco da Alemoa population; in *Gymnogeophagus gymnogenys* in the pair 5; pair 9 in *G. labiatus* and pair 11 in *G. rhabdotus* (Fig.1- boxes) in all species in subtelo-acrocentric pair exept *Cichlasoma portalegrense* which was located on a metacentric chromosome.

The simultaneous hybridization using 18S rDNA and U2snRNA probes showed that this genes are syntenic in *Crenicichla lepidota, Crenicichla maculata* and *Gymnogeophagus labiatus* located in the terminal region of the short arm of metacentric pair 1 (Fig. 1b,c, I - boxes, respectively). For the other species, U2snRNA probe was located in interstitial region of subtelo-acrocentric pair 15 in *Cichlasoma portalegrense* (Fig. 1a - box), pair 13 in *G. rhabdotus* (Fig. 1j - box) and pair 12 for all populations of *Geophagus brasiliensis* and *Gymnogeophagus gymnogenys* (Fig. 1 d-h – boxes, respectively).

Discussion

All species reported in this study, presented the diploid number in agreement with the conserved 2n=48, commonly found in South American cichlids and in contrast with the presence of 2n=44 in African cichlids (Feldberg et al. 2003). Despite the conservation of the diploid number in the neotropical species, variations were observed by others authors, such as in *Laetacara* cf. *dorsigera* with a reduction of the diploid number to 44 chromosomes with an intraspecific variation from 2n=43 to 2n=46 (Martins-Santos et al. 2005), *Bujurquina vittata* that also presented a reduction for 2n=44 (Usso et al. in preparation). On the other hand, species like *Symphysodon* present high diploid number of 60 chromosomes (Gross et al. 2009) and, other genera of Neotropical cichlids species as *Acaronia nassa*, *Bujurquina peregrinabunda* and *Caquetaia spectabilis* also showed a increase in diploid number to 2n=50 (Schneider et al. 2013a). Although most Neotropical cichlids conserve 2n=48, these variations evidenced the occurrence of chromosomal rearrangements in the family.

More than 60% of karyotypes in Cichlidae followed the plesiomorphic condition proposed for the order Cichliformes, with 48 chromosomes, mostly acrocentric (Thompson 1979; Feldberg et al. 2003; Poletto et al. 2010, 2012) however, in spite of that, other karyotype formulas have already been observed, as in the present study, where none of the

species presented this pattern, showing a greater number of metacentric chromosomes in *Cichlasoma portalegrense*, although, intra and interspecific variations have been observed. The formation of different karyotype formulas can occur due to specific chromosomal rearrangements, such as pericentric inversions, translocations and fission, such or fusion as already reported by several authors (Feldberg et al. 2003; Mesquita et al. 2008; Poletto et al. 2010). The plesiomorphic pattern with 48 chromosomes its more commonly observed among tribes Cichlini (Teixeira et al. 2009;Poletto et al. 2010; Valente et al. 2012; Schneider et al. 2013a) and Retroculini (Poletto et al. 2010; Valente et al. 2012). These tribes, together with Astronotini, were considered basal lineages of Neotropical Cichlidae (Farias et al. 2001).

Among the repetitive DNAs analyzed, the 18S rDNA was the most dynamic (Fig. 2), being observed in both groups of chromosomes m-sm or st-a, in the short or long arm, also varying in terminal and interstitial position, and in more than one pair in 3 species analyzed, ranging from 2 to 6 carrier chromosomes, including in both terminal regions of the pair 15 of *Gymnogeophagus rhabdotus.* In *Geophagus brasiliensis* from Saco da Alemoa only one of the homologous of pair 8 presented 18S rDNA, in terminal region, this position may favor the transference of genetic material from one homologous chromosome to another due to proximity of the interphase nucleus, according to the Rabl model (Cowan et al. 2001).

Nakajima et al. (2012), analyzing this same probe had already proposed this characteristic more variable of Neotropical Cichlids in comparison to the Africans, where the 18S rDNA tends to be in the terminal region of the short arm of the st-a chromosomes. According to the same author, the distribution of 5S rDNA tends to be in a single interstitial pair in the long arm, a pattern observed in most of the species analyzed in this study except *C. lepidota*, which presented more than one 5S carrier pair, also observed in other population in the same pairs 11 and 21 (Perazzo et al. 2011), a pattern observed only in plus

two other species, *Aequidens tetramerus* (Poletto et al. 2010a), and *Coquetaia spectabilis* (Schneider et al. 2013a).

Pires et al. (2010) proposed the possible occurrence of a pericentric inversion on pair 20 in *G. gymnogenys* from Gasômetro, with the AgNOR in terminal position, originating the pair 3 in the population Saco da Alemoa, with the AgNOR in interstitial position. The FISH with probe 18S rDNA showed the same result, evidencing alteration of the karyotype formula of the species involving this sequence. In *Gymnogeophagus gymnogenys* and *Geophagus brasiliensis* from Saco da Alemoa and *Gymnogeophagus rhabdotus* was evidenced the presence of more sites bearing rDNA than that evidenced by the impregnation of silver nitrate for the same populations (Pires et al. 2010; Pires 2013). The most common explanation is that only NORs that were transcriptionally active in the previous cell cycle appears positive after silver staining (Mais et al. 2005). However, this difference may be associated with heterochromatic regions, presence of transposable elements and the position terminal of 18S rDNA favoring the transposition to other chromosome pairs through translocation events, like reported for *Symphysodon* (Gross et al. 2009a, b). Another hypothesis would be origin of pseudogenes, as proposed for the 5S rDNA in *Ancistrus* sp. (Barros et al. 2017).

It is evident the involvement of repetitive DNA sequences in the chromosomal rearrangements. Pericentric inversions, transfer between non-homologous chromosomes, TE mediated or due to the positioning of the sequence in a terminal region of the chromosome, could favors these events are accumulate high mutation rates, observed in repetitive DNA sequences such as 5S and 18S rDNA, which can generate sites that are prone to double-stranded ruptures (DSB) (Bruschi et al. 2014/Barros et. 2017); these breaks, in these regions, that can be for process of repair by homologous recombination or gene conversion (Stults et al. 2008; Cazaux et al. 2011)

Studies with DNA in fishes are scarce if compared to the great diversity of the Cichlidae, but it is already possible to observe by the analyzes carried out here and in the literature that the pattern of localization of U1 and U2 snDNA in Cichlidae is in a single chromosomal pair (Cabral de Melo et al.2012; Usso et al. in preparation). This is apparently a pattern for fishes, since most of the species analyzed so far shown a strong conservation in the sites number per genome with accumulate exclusively in one chromosomal pair (Merlo et al. 2010, 2012a, b; Úbeda-Mazanaro et al. 2010; Supiwong et al. 2013; Utsunomia et al. 2014; Scacchetti et al. 2015; Santos et al. 2017; Araya-Jaime et al. 2017). In this study, as observed by Cabral de Melo et al. (2012) for U1 snDNA, a variation in the position and type of chromosome bearing the site of U2 snDNA was characterized. This sequence seems to be acting on chromosomal rearrangements as in *Gymnogeophagus gymnogenis* where a translocation or a paracentric inversion seems to have occurred between the two populations.

In Neotropical Cichlids, this variation in relation to snDNA is higher to African Cichlids (Cabral de Melo et al. 2012) and may be related to different types of speciation in these groups and the accumulating higher levels of genetic divergence in the Neotropical group (Farias et al. 1999).

The association of snDNA with other repetitive DNAs seems not to be very common so far in fishes, but some cases have already been observed (Yano et al. 2017; Usso et al. 2019). In *Crenicichla* the association between U2 snDNA and 18S rDNA in the first metacentric pair seems to be a marker for the genus, observed in the two species analyzed here and in *C. jaguarensis* (Usso et al. in preparation); as in two species of genus *Gymnogeophagus*, *G. labiatus* (Fig. 2) and *G. balzani* also analysed by Usso et al. (in preparation), that suggest a possible homology of chromosome 1 in these genera belonging to the Geophagini tribe, however, other species of the same tribe did not present this pattern.

In conclusion, Neotropical Cichlids species analyzed in this study showed evolutionary dynamism regarding the structural patterns of the karyotype and repetitive DNA (18SrDNA, 5S rDNA and U2 snDNA). The results may contribute to elucidate the organization of repetitive elements in cichlid genomes and also the repetitive DNA accumulation in the terminal region, how it occurs with 18S rDNA may facilitate chromosomal rearrangement and play an important role in divergent karyotype evolution of this species.

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Conflict of Interest The authors have no conflicts of interest to declare.

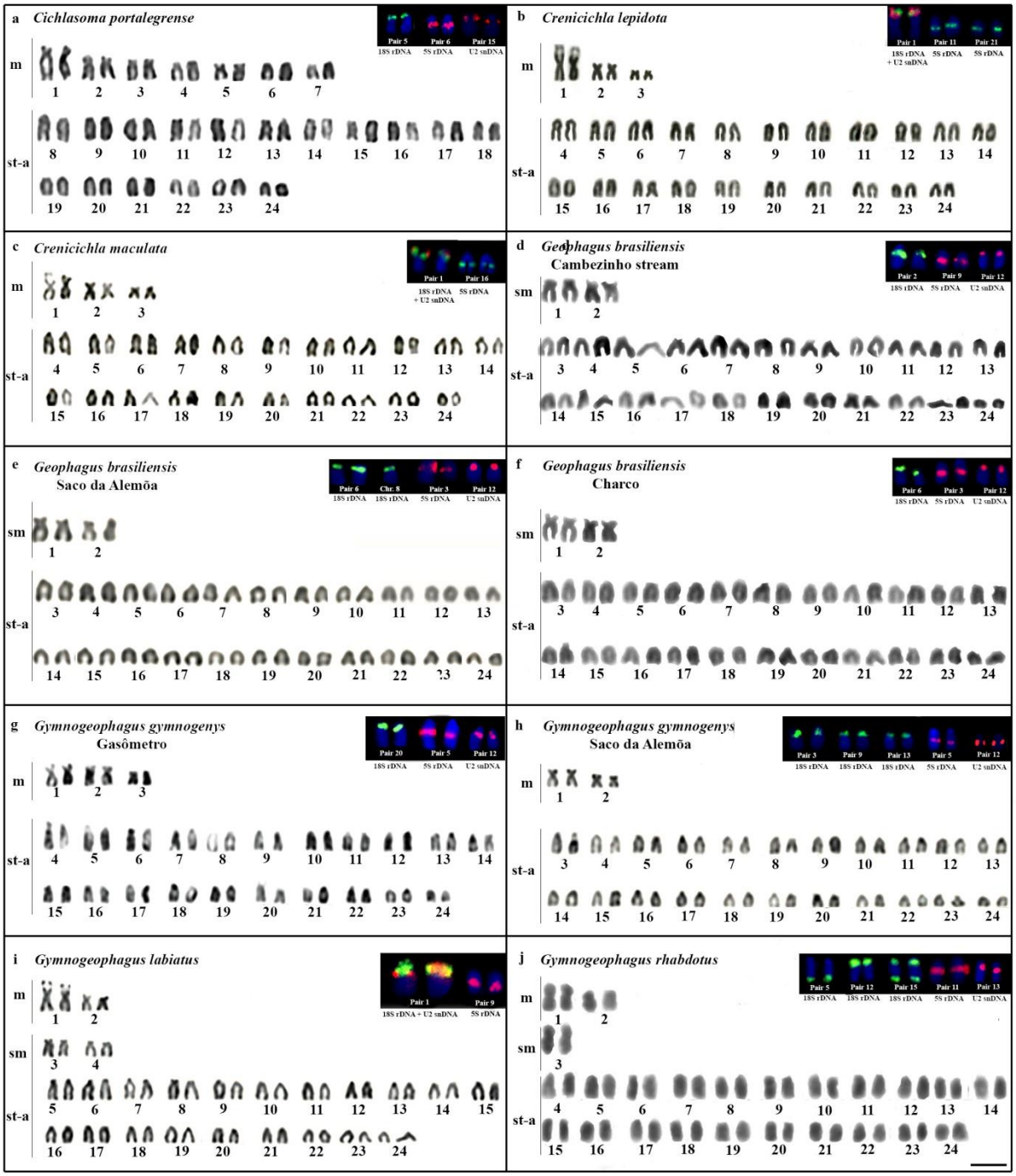

Figure 1 - Karyoytpes arranged from Giemsa-stained metaphases of (a) *Cichlasoma portalegrense*;(b) *Crenicichla lepidota;(*c) *Crenicichla maculata; Geophagus brasiliensis* from (d) Cambezinho, (e) Charco and (f) Saco da Alemoa; *Gymnogeophagus gymnogenis* from (g) Gasômetro and (h) Saco da Alemoa; (i) *Gymnogeophagus labiatus* and (j) *Gymnogeophagus rhabdotus* Inset box shows location of in situ hybridization with probes of 5S rDNA, 18S 5S rDNA and U2 snDNA.

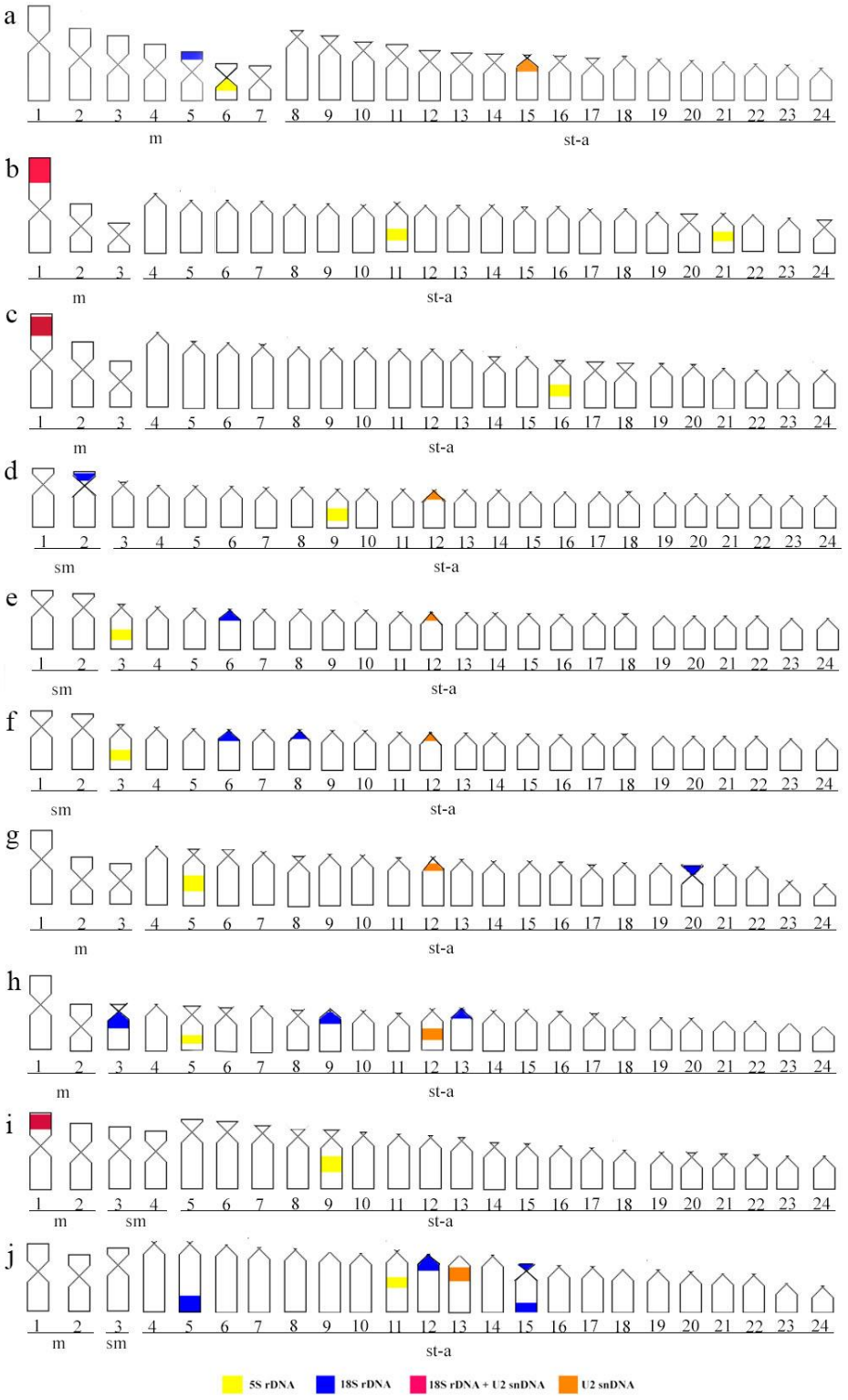

Figure 2 - Representative ideogram of karyotype: (a) *Cichlasoma portalegrense*;(b) *Crenicichla lepidota;(*c) *Crenicichla maculata; Geophagus brasiliensis* from (d) Cambezinho, (e) Charco and (f) Saco da Alemoa; *Gymnogeophagus gymnogenis* from (g) Gasômetro and (h) Saco da Alemoa; (i) *Gymnogeophagus labiatus* and (j) *Gymnogeophagus rhabdotus.* The ideogram shows accumulation of distribution of different repetitive DNAs (5S rDNA, 18S 5S rDNA and U2 snDNA).

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CAPÍTULO 3

An Update Review Cytogenetic data in Cichlinae: insights into chromosome evolution and phylogenetic relationships in the group

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An Update Review Cytogenetic data in Cichlinae: insights into chromosome evolution and phylogenetic relationships in the group

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Abstract

Cichlinae is a neotropical subfamily of Cichlidae, divided into seven tribes: Cichlini, Retroculini, Chaetobranchini, Astronotini, Geophagini, Cichlasomatini and Heroini. This paper shows a review of the last 50 years of cytogenetic analyzes in the subfamily and so far 137 species were analyzed belonging to 41 genera. The diploid number, 2n=48 was predominat, in more than 80% of the species, wich can be considered plesiomorphic for the group. However, intra and interspecific differences were observed regarding the karyotype formula. Heterochromatin was distributed in the pericentromeric or centromeric regions of the chromosomes in most of the analyzed species. More than 80 percent of the species with data of in situ hybridization showed the sequence of 18S rDNA in on chromosome pair, in the terminal region. The 5S rDNA was found also in a pair of homologous in 90% of the species, and in 80% of them in the interstitial position. Although there is a more common karyotype pattern, variations are observed among the Cichlinae species and, particularly, these repetitive DNA sequences appear to act directly in the group diversification through chromosomal rearrangements. The three largest tribes (Geophagini, Cichlasomatini, Heroin) present a greater chromosome diversity that seems to be occurring independently between species.

Key words: Cichlids, Chromosome differentiation, genome evolution, repetitive DNA, ribosomal DNA, tribes.

Introduction

Cichlidae is the largest lineage of freshwater fishes (Eschmeyer & Fong, 2019) with more than 1700 species. A phylogeny of this family began with studies based on 91 morphological characters by Kullander (1998), that proposed phylogenetic tree comprising the following subfamilies: Etroplinae (*Etroplus* and *Ptychochromis*), Pseudocrenilabrinae, Cichlinae (tribes: Crenicichlini and Cichlini), Astronotinae (tribes: Astronotini and Chaetobranchini), Geophaginae (tribes: Geophagini, Acarichthyini and Crenicaratini) and Cichlasomatinae (tribes: Acaroninii, Cichlasomatini and Heroini).

These initial analyzes proposed that the neotropical and African lineages had a polyphyletic origin due to the separation of the genera *Heterochromis* and *Cichla* from the other species of their respective continents (Oliver, 1984; Stiassny, 1987, 1991; Kullander, 1998). However, more recent studies at the family using molecular data have suggested a monophyletic origin, with the exception of Madagascar and India (Farias *et al*., 1999; Sparks, 2004; Sparks & Smith, 2004). Currently, Cichlidae is separated into four subfamilies: Etroplinae (Indians), Ptychochrominae (Malagasy), Cichlinae (Neotropical) and Pseudocrenilabrinae (African) (Sparks & Smith, 2004; Smith *et al*., 2008). The neotropical subfamily Cichlinae includes approximately 60 genera with many species not yet described (Reis *et al*., 2003; López-Fernández *et al*., 2010). Most of the neotropical species are located within the three largest tribes, Geophagini, Cichlasomatini and Heroini, with the other species distributed in the Cichlini, Retroculini, Chaetobranchini and Astronotini tribes (Kullander, 1998; Smith *et al*., 2008; López-Fernández *et al*., 2010).

At the beginning of cytogenetic studies, the family Cichlidae was considered a group with conserved karyotype, where 60% of the analyzed species presented a diploid number equal 48, withal the ancestral karyotype consisted of 48 acrocentric chromosomes (Thompson, 1979, Kornfield, 1981). After more than 50 years of studies, different authors suggested significant changes in the karyotype structure in several cichlids groups, involving

different chromosomal rearrangements, including variability diploid number and chromosome formula (Gross *et al.,* 2009; Poletto *et al.,* 2010; Schneider *et al.,* 2013). More recently, analyzes with repetitive DNA, 18s rDNA and 5S rDNA, have evidenced the role of these sequences in the karyotype diversification of Neotropical Cichlids (Schneider *et al.*, 2013; Perazzo *et al*., 2013; Alves Silva *et al*., 2015; Paiz *et al.,* 2017).

The aim of the present paper was to carry out a survey of the cytogenetic data of Cichlinae using published articles, theses, dissertations, monographs and abstracts of unpublished congresses, and to promote a broad analysis on diploid number, karyotype formula, distribution of constitutive heterochromatin, 18S rDNA and 5S rDNA, in the different groups of this subfamily. Based on these data, it was intended to observe some trends in the chromosomal evolution and genomic dynamics of Cichlinae, and the role of the repetitive DNA in these process. Besides these analyzes and discussions can be used as support for future studies on karyotype structure and evolution in this group of fishes.

Discussion

The subfamily Cichlinae has a wide distribution in the rivers of South and Central America, with species being distributed from Patagonia to Texas (Reis *et al*., 2003). This group a monophyletic clade and sister group of the monophyletic clade of African Cichlids (Sparks & Smith, 2004). Karyotype studies in the subfamily began more than fifty years ago with Ohno & Atkin (1966), and up to the present time there are cytogenetic reports to 41 genera and 137 species (Table 1). According to Reis et al. (2003), the neotropical clade includes approximately 60 genera, thus over 65% of the genera have cytogenetic studies (Table 1). The greatest diversity of species is found within three tribes: Geophagini, restricted to South America and southern Panama with approximately 18 genera and 250 species, Cichlasomatini includes 11 described genera and more than 70 species and Heroini with approximately 30 genera and 150 spp (Lopes-Fernández *et al*., 2010). Consequently,

the largest number of karyotypeanalyzes is concentrated in these tribes, with 50% of the genera of Geophagini, 82% of the genera of Cichlasomatini and 53% of Heroini. Less than 10% of the total species with described karyotypes are distributed in the four smaller tribes, Cichlini, Retroculini, Chaetobranchini and Astronotini.

Three trends of karyotype differentiation in Neotropical cichlids were related from different studies (Thompson 1979, Feldberg *et al*., 2003 Hodaňová *et al*., 2014): the first is the pleisiomorphic condition of 2n=48 with mostly subtelo-acrocentric chromosomes, presence of pericentric inversions, which may lead to changes in chromosome morphology, such as the appearance of meta and submetacentric chromosomes. Approximately 80% of the species analyzed exibited the diploid number with 48 chromosomes; the second pattern is the reduction of 2n accompanied by increase in the number of meta and submetacentric chromosomes probably caused by rearrangements centric fusions (Thompson 1979, Poletto et al., 2010a); this pattern is most observed in African cichlids where the presence of 2n=44 is more common (Feldberg et al., 2003). Among the Ciclinae 13% presented a 2n lower than 48 chromosomes, ranging from 38 in *Laetacara curviceps* and *Apistograma borellii* to 46 chromosomes in *A. agassizii, A. borellii, A. ortmanni, A. steindachneri, A. trifasciata, Laetacara dorsigera* and *Uaru amphiacanthoides (*Table 1)

The last pattern is an increasing of diploid number, occurring only in 7% of Cichlinae, ranging from 50 chromosomes in *Acaronia nassa*, *Bujurquina peregrinabunda*, *Cleithracara maronii*, *Caquetaia kraussii* and *Caquetaia spectabilis* to 60 chromosomes in all species of *Symphysodon* analyzed, which chromosomal breakage/fission events has an important role in this karyotype type,dominated by uniarmed chromosomes. However, in *Symphysodon* that has high diploid number, predominates meta-submetacentric chromosomes, suggesting occurrence of polyploidization in this genus, as well as chromosomal rearrangements, such as pericentric inversions, translocations and fissions/fusions (Thompson, 1976; Mesquita *et al.*, 2008).

In fact it is important to note that chromosome rearrangements and formation of new karyotypes seems to be occurred independently in cichlid evolution, from 137 examined Cichlinae. Tribes Cichlini, Retroculini and Chaetobranchini present 2n=48 with mostly subtelocentric-acrocentric chromosomes, the pleisiomorphic condition of Neotropical Cichlids. These tribes are the least studied cytogenetically, and it is not clear whether this chromosomal stability is characteristic of the group or lack of sampling. Currently, Cichlini, Retroculini and Astronotini were considered basal lineages of Neotropical Cichlidae (Farias *et al.*, 2001) and Chaetobranchini as monophyletic and sister to Geophagini (Smith *et al*., 2008). Due to recent phylogenetic changes in Cichlinae proposed by Smith et al. (2008), *Astronotus*, which formerly belonged to Chaetobranchini, now belongs to Astronotini and before these analyzes chaetobranchins and geophagins formed a single group (Sparks and Smith, 2004).

Geophagini is divided in three subtribes: Acarichthyina, Crenicaratina and Geophagina, and only in the last two were found species with a diploid number smaller than the plesiomorphic characteristic (Table 1). *Crenicichla* sp and *Dicrossus filamentosus*, Crenicaratina subtribe show 46 chromosomes and in subtribe Geophagina, *Apistograma agassizi*, *A.ortmanni*, *A.steindachneri* and *A.trifasciata* also present 2n=46 (Table 1). *A. borellii* of Comercial Fonte and Lagoa Comprida-MS present 2n=38 and 46, respectively (Thompson, 1979).

Crenicichla, subtribe Crenicaratina, is the genus with more cytogenetic analyzes, with 28 species, and all have 48 chromosomes, except *Crenicichla* sp (Table 1). Although the diploid number remains conserved in this genus, several karyotype formulas were observed with the number of m-sm chromosomes varying from 4 in *Crenicichla saxantilis* to 14 in *C. niederleinii* (Table 1), but most of the analyzed species present 6 or 8 m-sm. According Mizoguchi et al. (2007) may be a measurement error or chromosomes

condensation differences, but rearrangements can not be discarded and, in fact, are the most likely events to explain the chromosome differences.

In subtribe Geophagina, *Stanoperca, Geophagus* and *Gymnogeophagus* genera also maintains diploid number of 48 with karyotypic variations, mainly in *Geophagus brasiliensis*, which is considered a species complex (Farias *et al.*, 2000; Feldberg *et al.*, 2003). Sympatric populations presented different karyotypic patterns, probably due to pericentric inversions, which could be contributing to the reproductive isolation in the species as proposed by Perazzo et al. (2013).

The greatest variation of diploid number occurs in Cichlasomatini (Table 1), in which is evident that karyotypic evolution occurs independently, especially when analyzing some genera such as *Bujurquina*, in which *B. peregrinabunda* presents an increase in the diploid number (2n=50) and *B. vittata* a decrease (2n=44); this group also shows *Laetacara* and *Nannacara* with a diploid number ranging from 38 to 46 and *Acaronia nassa*, *Cichlasoma salvini* and *Cleithacara maronii* showing increase of 2n=50 and 52 (Table 1).

Besides karyotypic diversity, Cichlasomatini presents some taxonomic problems as in the genus *Aequidens* that could to synonymize with the genus *Cichlasoma*, based on morphological and molecular analyzes (Musilova *et al.*, 2009). Another example is *Laetacara*, sister to the *Cleithracara–Nannacara* clade for Smith et al. (2008) and in recent phylogenomics analyses performed by Ilves et al. (2018) defined a well-supported sister relationship between *Andinoacara* and *Bujurquina*, just as they defined *Acaronia* and *Laetacara* as sisters to the "andinoacarines"; however, the placement of the genera in relation to the others was not supported by their analysis. The karyotypic divergence and low bootstrap values observed in molecular analysis may indicate possible species identification errors and a need for further studies within these genera to resolve these conflicts.

In Heroini, as well as in Cichlasomatini, a large diploid number variation can be observed, from 2n=42 in *Hypselecara coryphaenoides* to 2n=60 in *Symphysodon* (Table 1). The increased diploid number (from 48 to 60 chromosomes) in *Symphysodon* due to polyploidy (Thompson, 1976) or chromosomal rearrangements (Mesquita *et al.*, 2008), probable occur in ancestor of the genus, leading to the maintenance of this 2n in all genus, according Gross et al. (2009) *S. discus* is probably the oldest species, that could have hybridized with an ancestor *Discus* species that may now be extinct. For the karyotype differentiation in *Symphysodon* it is possible that a convergence occurred for the same number of chromosomes and different from the plesiomorphic stage, leading to the maintenance of this 2n in all genus. However in other cases of Cichlinae, like *Bujurquina*, *Cichlasoma*, *Hypselecara* and *Laetacara* where three trends in karyotype differentiation are observed, confirm that the events of karyotypic evolution occurs independently in each species.

Valente et al. (2012) made a survey in the literature of the distribution of heterochromatin in the seven Cichlinae tribes in a total of 41 species and observed a preference to pericentromeric and centromeric distribution. Currently, there are data for 49 species (Table 1). This heterochromatic distribution seems to be common to all cichlids, since it is also observed in African group (Kornfield *et al.*, 1979; Majumdar and McAndrew, 1986). The authors also observed that only 14 species present no heterochromatin related with nucleolar organizer region (NOR). Several studies in fish have shown that the association of heterochromatin and NORs is an important element in chromosome differentiation, favored breaks and consequent rearrangements during the chromosomal evolution (Galetti *et al.*, 1991). Thus, this association in neotropical cichlids or several other fish groups may be related to the variability in location and number of the active NORs. Despite having a conserved position in most of the analyzed Cichlidae, heterochromatin plays an important role in the karyotype evolution of the group.

Despite this conservation in the distribution of heterochromatin in Cichlinae, variations were evidenced in different populations of species complex *Geophagus brasiliensis,* which showed differences in quantity and location of heterochromatin with additional telomeric and interstitial bands, indicating duplication events and pericentric inversions may be occurring associated with the sympatric speciation of this species (Perazzo *et al*., 2013).

In recent years, there has been an increase in physical mapping of repetitive DNAs sequences, and as useful markers provided a substantial increase to the knowledge of biodiversity and evolution of genome structure and organization, and detection karyotypic rearrangements (Ferreira *et al.,* 2010). For Cichlinae most studies have focused in the description of karyotypes where, only 31% of the species analyzed predented has a 18S rDNA mapping and 26% de 5S rDNA. In this detailed analysis, an intense dynamism of these sequences was observed mainly regarding the position and the type of carrier chromosome (Table 1).

The most common condition for 18S rDNA in Cichlinae, approximately 80% of the species, was the presence in two clusters in homologous chromosomes, in terminal position, with variation in the chromosome type. Despite the large majority of species to present the same distribution pattern of the 18 rDNA, intraspecific and interspecific variability in the distribution of this sequence was observed in the complex *Geophagus brasiliensis*, presenting from 2 to 6 chromosomes carrying this site in different analyzed populations (Table 1). Other species also showed more than two cistrons of this sequence, like *Gymnogeophagus balzanii*, *G gymnogenis*, *G. rhabdotus* and *G. stequedas* (Geophagini), *Bujurquina vittata*, *Cichlasoma sanctifranciscense* (Cichlasomatini), *Mesonaua festivus*, *Symphysodon aequifasciatus*, *S.haraldi*, *S. discus* and *Uaru amphiaconthoides* (Heroini), which vary in their position (terminal or interstitial) and the chromosome type (Table 1).

The variability in the distribution of 18S rDNA in Neotropical cichlid, can be favored by presence of this sequence in terminal position facilitating its transposition to other pairs of chromosomes (Gross *et al.,* 2010).The association of that region with transposable elements, as reported by Schneider et al. (2013a, 2013b), may have favored structural polymorphisms of the rDNA sites. Translocation events, as already reported for Neotropical Cichlidae by Gross et al. (2009) and accumulation of mutations, a common event in multigenic families, may be lead to the formation of pseudogenes and sites prone to double-strand rupture (Barros *et al.,* 2017), wich may be continuously modifying the genome of these species.

Another fact that reinforces the accumulation of mutations and events of translocations involving the 18S rDNA sequence is the presence of twenty-one 18S rDNA sites in *Pterophyllum leopoldi,* analysed by Schneider et al. (2013a). According to the authors, this segment has probably undergone a duplication and dispersion in genome of the species and besides, the 18S rRNA site on chromosome 9 is the only one transcriptionally active, corresponding to the AgNOR; based on Rooney and Ward (2005). The authors infered that the additional 18S rDNA sites in *P. leopoldi* may be related to natural processes of the birth and death of repetitive sequences in the genome.

Among Cichlinae species with cytogenetic analyzes, only 25% have 5S rDNA data, whose location occurs in a single pair of chromosomes in about 90% of the analyzed species and 80% in the interstitial position (Table 1), as also observed by Nakajima et al. (2012). The majority ofspecies (19), representing 41% of the species analyzed, exhibited 5S rDNA sites on a st-a chromosome in the interstitial region of the long arm. However, nine different patterns regarding the position and number of chromosome carrying this ribosomal site were observed, leading to the believe that this sequence, as well as 18S rDNA and heterochromatin, are involved in chromosomal rearrangements in Cichlinae. Only three species show four chromosomes bearing this site ribosomal: *Aequidens tetramerus,*

Caquetaia spectabilis and *Crenicichla lepidota* (Table 1). The presence of 5S sequence at the interstitial region may be responsible for protection from events such as unequal exchange that could act in the dispersion of sequences (Martins and Wasko, 2004).

In contrast to the large diversity of species in the subfamily, there are still few cytogenetic data involving rDNA, as well as other repetitive DNAs, which have been studied in the group even less frequently. For example, Teixeira et al. (2009) and Schneider et al. (2013b) analysed the genomic organization of transposable elements (Rex 1, Rex3, Rex 6) in Cichlidae species. These authors observed different distribution patterns in the chromosomes, as dispersed or blocks in the centromeric and terminal portions, associated with heterochromatic and euchromatic regions. The mapping with other repetitive DNAs, such as U1 snDNA, was performed for 12 species and sites were observed in a pair of homologous, in the terminal, interstitial or proximal position (Cabral de Melo *et al*., 2012). These results showed that the increase of analyzes with different repetitive DNA sequences may make even more apparent the occurrence of a karyotype variability in the group of cichlids.

Cichlidae was considered, for a long time, a group conservative karyotypes, mainly due to the diploid number (Thompson, 1979; Kornfield, 1984). Currently, with the increase of chromosome data and with more detailed analyzes, itwas observed that, although 80% of the analyzed species of Cichlinae present 2n=48, several karyotype formulas are observed.Repetitive sequences of DNA, such as heterochromatic regions, 18S and 5S rDNA, are distributed in the genome of the group with variability in relation to the number, type, position in the chromosomes. Recent studies have shown that these sequences act on karyotypic differentiation due to intra-chromosomal rearrangements (Gross *et al*., 2009, Valente *et al*., 2012; Schneider *et al*., 2013).

In some tribes, such as Geophagini, Cichlasomatini and Heroin, a greater karyotype variability is observed, and appears to be occurring independently in the species.

These tribes are also the most studied cytogenetically and despite the increase of data, the quantity is not representative of the high species diversity in the group. The increase in the number of species analyzed, especially in relation to the chromosomal mapping of different types of repetitive DNAs, may help to understand the karyotype evolution of the group, confirming probably a more divergent evolution, as well as the resolution of some taxonomic and phylogenetic conflicts.

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Conflict of Interest

The authors have no conflicts of interest to declare.

Table 1- Compilation of cytogenetic data for neotropical subfamily Cichlinae species including analyses for diploid number (2n); karyotype formulae, fundamental number (FN), heterochromatin distribution (C Band) 18S and 5S rDNA location; m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric; p = short arm; q = long arm; t = terminal position; i = interstitial position; c = centromeric position; pc= pericentromeric; chr = chromosomes. Classification follows Smith et al. (2008).

Tribes/ Species	2n	Karyotipic formulae	FN	C Band	18S rDNA	5S rDNA	Ref.
Cichlini							
Cichla sp.	48	48 *st-a*	48	---	----	---	14;/26
Cichla kelberi	48	48 *st-a*	48	c	2chr;t-p;*st-a*	---	58
Cichla monoculus	48	48 *st-a*	48	pc	2chr;t-q;*st-a*	2chr;i-q;*st-a*	26; 34; 62
Cichla piquiti	48	48 *st-a*	48	pc	---	---	53;60
Cichla temensis	48	48 *st-a*	48	pc	---	---	7;26; 53;34
Cichla orinocensis	48	48 *st-a*	48	---	---	---	53
Retroculini							
Retroculus lapidifer	48	48 *st-a*	48	pc	2chr;t-p;*st-a*	2chr;i-q;*st-a*	53;60
Astronotini							
Astronotus sp.	48	---		---	---	---	23
Astronotus crassipinnis	48	18*m-sm*+30*st-a*	66	---	---	---	22
Astronotus ocellatus	48	16 *m-sm* +32*st-a*	64	PC	2chr;i-p;*m-sm*	2chr;i-q;*st-a*	50;56;62
Astronotus ocellatus	48	12 *m-sm* +36*st-a*	60	---	---	---	11;12
Astronotus ocellatus	48	-	96	---	---	---	6
Astronotus ocellatus	48	6*m-sm* + 42 *st-a*	54	--	---	---	7
Astronotus ocellatus	48	48*st-a*	48	---	---	---	11;12
Astronotus ocellatus	48	6*m-sm* + 42 *st-a*	54	---	2chr;t-p;*m-sm*	---	53
Chaetobranchini							
Chaetobranchopsis australis	48	48*st-a*	48	---	2chr;t-q;*st-a*	2chr;i-q;*st-a*	11;12;71
Chaetobranchus flavescens	48	6*m-sm*+42*st-a*	54	---	2chr;i-p;*m-sm*	---	53;56;57
Geophagini							
Acarichthys heckelli	48	6*m-sm*+42*st-a*	54	---	---	---	7
Apistogramma agassizii	46	*24m-sm*+ 22*st-a*	70	---	---	---	7
Apistogramma borellii	38	*22m-sm*+ 16*st-a*	60	---	---	---	7
Apistogramma borellii	46	*16m-sm*+ 30*st-a*	62	---	---	----	53
Apistogramma ortmanni	46	*24m-sm*+ 22*st-a*	70	---	---	---	7
Apistogramma steindachneri	46	-	-	---	---	---	6
Apistogramma trifasciata	46	*16m-sm-30st-a*	62	pc	---	---	42
Biotodoma cupido	48	4*m-sm* +44 *st-a*	52	---	2chr;t-p;*m-sm*	---	52;59
Biotodoma cupido	48	4*m-sm* +44 *st-a*	52	pc	2chr;t-p;*m-sm*	---	60
Crenicichla sp.	46	-	-	---	---	---	23
Crenicichla sp.	48	6*m-sm*+42*st-a*	54	---	---	---	24
Crenicichla sp.	48	8*m-sm* +40 *st-a*	56	pc	---	---	36
Crenicichla sp.1	48	8*m-sm* +40 *st-a*	56	pc	---	---	41
Crenicichla sp.2	48	8*m-sm* +40 *st-a*	56	pc	---	---	41
Crenicichla sp.A	48	6*m-sm*+42*st-a*	54	---	---	---	19
Crenicichla sp.B	48	8*m-sm* +40 *st-a*	56	---	---	---	19
Crenicichla britskii	48	8*m-sm* +40 *st-a*	56	pc	---	---	40
Crenicichla britskii	48	6*m-sm*+42*st-a*	54	---	---	---	53
Crenicichla aff. *britskii*	48	6*m-sm*+42*st-a*	54	---	---	---	52;59
Crenicichla cincta	48	8*m-sm* +40 *st-a*	56	pc	---	----	40
Crenicichla aff. haroldoi	48	6*m-sm*+42*st-a*	54	---	----	----	53
Crenicichla iguassuensis	48	6*m-sm*+42*st-a*	54	---	----	----	33
Crenicichla iguassuensis	48	8*m-sm* +40 *st-a*	56	pc	----	---	41
Crenicichla inpa	48	6*m-sm*+42*st-a*	54	pc	----	---	40
Crenicichla cf. johanna	48	8*m-sm* +40 *st-a*	56	pc	---	---	40
Crenicichla juaguarensis	48	4*m* +4*sm* +40*st-a*	56	---	2chr;t-p;*m*	2chr;i-q;*st-a*	71
Crenicichla jupiaensis	48	4*m*+4*sm* +10*st*+*32a*	64	---	---	----	46
Crenicichla lacustris	48	6*m-sm*+42*st-a*	54	---	---	---	11;12
Crenicichla lacustris	48	10*m-sm*+38*st-a*	58	---	---	---	41
Crenicichla lepidota	48	6*m* + 42 *st-a*	54	---	---	---	7
Crenicichla lepidota	48	6*m* + 42 *st-a*	54	---	---	---	11;12
Crenicichla lepidota	48	6*m* + 42 *st-a*	54	pc	---	---	42
Crenicichla lepidota	48	6*m* + 42 *st-a*	54	pc/i			20
Crenicichla lepidota	48	6*m* + 42 *st-a*	54	---	---	---	32
Crenicichla lepidota	48	8*m-sm* +40 *st-a*	56	pc	2chr;i-p;*m-sm*	4chr;i-q;*st-a*	]57
Crenicichla lepidota	48	6*m* + 42 *st-a*	54	---	2chr;t-p;*m*	4chr;i-q;*st-a*	72

Crenicichla lepidota	48	6*m* + 42 *st-a*	54	---	2chr;t-p;*m-sm*	---	53;59
Crenicichla lepidota	48	6*m* + 42 *st-a*	54	pc	2chr;t-p;*m-sm*	---	66
Crenicichla lucius	48	6*m* + 42 *st-a*	54		---	---	7
Crenicichla lugubris	48	8*m-sm* +40 *st-a*	56	pc	---	---	40
Crenicichla maculate	48	6*m* + 42 *st-a*	54		2chr;t-p;*m*	2chr;i-q;*st-a*	72
Crenicichla menezesi	48	4m+44st-a	52	pc	---	---	64
Crenicichla niederleinii	48	6*m-sm* + 42 *st-a*	54	---	---	----	32
Crenicichla niederleinii	48	6*m-sm* + 42 *st-a*	54	pc	---	---	42
Crenicichla niederleinii	48	6*m-sm* + 42 *st-a*	54	c	---	---	33
Crenicichla niederleinii	48	10*m-sm* + 38 *st-a*	58	pc	---	---	36
Crenicichla niederleinii	48	14*m-sm* + 34 *st-a*	62	---	---	---	20
Crenicichla notophthalmus	48	6m-s*m* + 42 *st-a*	54	---	---	---	7
Crenicichla reticulata	48	6*m-sm* + 42 *st-a*	54	pc	---	---	40
Crenicichla reticulata	48	6*m-sm* + 42 *st-a*	54	---	---	---	35
Crenicichla "saxatilis"	48	4m-*sm* +44*st-a*	52	---	---	---	4
Crenicichla cf. saxatilis	48	-	-	---	---	---	31
Crenicichla semifasciata	48	6-sm*m* + 42 *st-a*	54	---	---	---	11;12
Crenicichla semifasciata	48	6-sm*m* + 42 *st-a*	54	---	---	---	32
Crenicichla strigata	48	6*m-sm* + 42 *st-a*	54	---	---	---	7
Crenicichla strigata	48	6*m-sm* + 42 *st-a*	54	---	---	---	53
Crenicichla strigata	48	6*m-sm* + 42 *st-a*	54	pc			60
Crenicichla vittata	48	6*m-sm* + 42 *st-a*	54	---	---	---	11;12
Dicrossus filamentosus	46	12*m-sm* + 34 *st-a*	58	---	---	---	7
Geophagus sp.	48	-	50	---	---	---	24
Geophagus altifrons	48	4*m-sm* +44 *st-a*	52	---	---	---	21
Geophagus brasiliensis	48	-	90	---	---	---	28
Geophagus brasiliensis	48	-	92	---	---	---	6
Geophagus brasiliensis	48	6*sm* +42*st*	54	pc/c/i	2chr;t-p;*st-a*	2chr;i-q;*st-a*	39
Geophagus brasiliensis	48	3*m-sm* +45*st-a*	51	---	---	----	5
Geophagus brasiliensis	48	2*m-sm* +46*st-a*	50	---	---	----	11;12
Geophagus brasiliensis	48	2*m-sm* +46*st-a*	50		---	----	52;58;59
Geophagus brasiliensis	48	4*m-sm* +44 *st-a*	52	---	---	---	7;33
Geophagus brasiliensis	48	8*m-sm* +40 *st-a*	56	pc	---	---	20
Geophagus brasiliensis	48	8*m-sm* +40 *st-a*	56	---	---	---	27
Geophagus brasiliensis	48	6*m-sm* + 42 *st-a*	54	---	---	---	27
Geophagus brasiliensis	48	6*m-sm* + 42 *st-a*	54	pc/c	2chr;t-p;*st-a*	2chr;i-q;*st-a*	55
Geophagus brasiliensis	48	8*m-sm* +40 *st-a*	56				18
		2*m-sm* +46*st-a*	50				
Geophagus brasiliensis	48	4*m-sm* +44 *st-a*	54	pc/t	2chr;t-p;*st-a*	2chr;i-q;*st-a*	63
		6-sm*m* + 42 *st-a*	52				
		2*m-sm* +46*st-a*	50				
Geophagus brasiliensis	48	4*m-sm* +44 *st-a*	54	pc/t	2chr;t-p;*st-a*	2chr;i-q;*st-a*	63
		6-sm*m* + 42 *st-a*	52				
Geophagus brasiliensis	48	2*m-sm* +46*st-a*	50	pc/t	2chr;t-p;*st-a*		63
Geophagus brasiliensis	48	2*m-sm* +46*st-a*	50	pc/t	2chr;t-q;*st-a*	2chr;t-q;*st-a*	63
Geophagus brasiliensis	48	2*m-sm* +46*st-a*	50	pc/t	2chr;t-p;*st-a*	2chr;t-q;*st-a*	63
		6-sm*m* + 42 *st-a*	52				
		2*m-sm* +46*st-a*	50				
Geophagus brasiliensis	48	4*m-sm* +44 *st-a*	54	pc/t	2chr;t-p;*st-a*	2chr;i-q;*st-a*	63
		6-sm*m* + 42 *st-a*	52				
		3*sm*+18*st*+27*a*	50				
Geophagus brasiliensis	48	*2sm*+20*st*+26*a*	51	pc/c	5chr;t-p;*st*	2chr;i-q;*st-a*	67
		4*sm*+18*st*+26*a*	52				
		3*sm*+18*st*+27*a*	50				
Geophagus brasiliensis	48	*2sm*+20*st*+26*a*	51	pc/c	4chr;t-p;*st*	2chr;i-q;*st-a*	67
		4*sm*+18*st*+26*a*	52				
		3*sm*+18*st*+27*a*	50				
Geophagus brasiliensis	48	*2sm*+20*st*+26*a*	51	pc/c	6chr;t-p;*st*	2chr;i-q;*st-a*	67
		4*sm*+18*st*+26*a*	52				
		3*sm*+18*st*+27*a*	50				
Geophagus brasiliensis	48	*2sm*+20*st*+26*a*	51	pc/c	2chr;t-p;*st*	2chr;i-q;*st-a*	67
		4*sm*+18*st*+26*a*	52				
		3*sm*+18*st*+27*a*	50				
Geophagus brasiliensis	48	*2sm*+20*st*+26*a*	51	pc/c	4chr;t-p;*st*	2chr;i-q;*st-a*	67
		4*sm*+18*st*+26*a*	52				
		3*sm*+18*st*+27*a*	50				
Geophagus brasiliensis	48	*2sm*+20*st*+26*a*	51	pc/c	4chr;t-p;*st*	2chr;i-q;*st-a*	67
		4*sm*+18*st*+26*a*	52				

Geophagus brasiliensis	48	4*sm* +44*st-a*	52	pc	2chr;t-p;*sm*		43
Geophagus brasiliensis	48	4*sm* +44*st-a*	52	pc	2chr;t-p;*sm*	2chr;i-q;*st-a*	43;72
Geophagus brasiliensis	48	4*sm* +44*st-a*	52		2chr;i-q;*st-a*	2chr;i-q;*st-a*	72
Geophagus brasiliensis	48	4*sm* +44*st-a*	52	pc	4chr;i-q;*st-a*	2chr;i-q;*st-a*	51;72
Geophagus brasiliensis	48	2*sm*+46*st-a*	50	pc/c	2chr;i-q;*st-a*	2chr;i-q;*st-a*	69
Geophagus brasiliensis	48	2*sm*+46*st*	50	pc/c	5Chr;i-q;*st-a*	2chr;i-q;*st-a*	69
Geophagus brasiliensis	48	2*sm*+46*st*	50	pc/c	2chr;i-q;*st-a*	2chr;i-q;*st-a*	69
Geophagus brasiliensis	48	2*sm*+46*st*	50	pc/c	6chr;i-q;*st-a*	2chr;i-q;*st-a*	69
Geophagus brasiliensis	48	2*sm*+46*st*	50	pc/c	5chr;i-q;*st-a*	2chr;i-q;*st-a*	69
Geophagus brasiliensis	48	2*sm*+46*st*	50	pc/c	3chr;i-q;*st-a*	2chr;i-q;*st-a*	69
Geophagus brasiliensis	48	2*sm*+46*st*	50	pc/c	2chr;i-q;*st-a*	2chr;i-q;*st-a*	69
Geophagus itapicuruensis	48	2*sm*+46*st*	50	pc/c	3chr;i-q;*st-a*	2chr;i-q;*st-a*	69
Geophagus proximus	48	12*m-sm* +36*st-a*	60	c	2chr;i-p;*m-sm*	2chr;i-q;*st-a*	62
Geophagus proximus	48	4*m-sm* +44 *st-a*	52	pc	---	---	60
Geophagus proximus	48	5*msm*+42*sta*+1*micr*		pc	---	---	60
Geophagus aff. proximus	48	4*m-sm* +44 *st-a*	52	---	---	---	52
Geophagus cf. proximus	48	12*m-sm*+36*st-a*	60	---	---	---	52;59;61
Geophagus surinamensis	48	4*m-sm* +44 *st-a*	52	---	---	---	7;11;12;52;53
Guianacara sp.	48	4*m-sm* +44 *st-a*	52	---	---	---	15
Gymnogeophagus sp.	48	-	-	---	---	---	38
Gymnogeophagus balzanii	48	2*m* + 46*st-a*	50	pc	3chrs; i-p;*m*/i-p;*st-a*	2chr;i-q;*st-a*	10;12;42;72
Gymnogeophagus gymnogenis	48	6*m* +42*st-a*	54	pc	2chr;t-p;*st-a*	2chr;i-q;*st-a*	51;72
Gymnogeophagus gymnogenis	48	4*m* +44*st-a*	52	pc	6chr;i-q;*st-a*	2chr;i-q;*st-a*	51;72
Gymnogeophagus labiatus	48	4*m* +4*sm*+ 40*st-a*	58	pc	2chr;t-p;*m*	2chr;i-q;*st-a*	51;72
Gymnogeophagus labiatus	48	4*m* +44*st-a*	52	---	---	---	13
Gymnogeophagus lacustris	48	4*m* +44*st-a*	52	---	---	---	13
Gymnogeophagus rhabdotus	48	4*m* +2*sm*+ 42*st-a*	54		2chr t-p/q;*st-a*/4chr;i-q;*st-a*	2chr;i-q;*st-a*	72
Gymnogeophagus rhabdotus	48	4*m* +44*st-a*	52	---	---	---	13
Gymnogeophagus setequedas	48	4*sm* +24*st* + 20*a*	76		5chr; t-q;*st-a*	2chr;i-q;*st-a*	70
Stanoperca acuticeps	48	-	-	---	---	---	35
Stanoperca jurupari	48	-	-	---	---	---	8
Stanoperca jurupari	48	4*m-sm* +44 *st-a*	52	pc	2chr;t-p;*st-a*		53;60
Stanoperca jurupari	48	5*m-sm* +43 *st-a*	53	---	---	---	32
Stanoperca jurupari	48	6*m-sm* +42 *st-a*	54	---	---	---	19;32
Stanoperca pappaterra	48	6*m-sm* +42 *st-a*	54	pc	-	-	20
Cichlasomatini							
Acaronia nassa	50	4*m-sm* +46 *st-a*	54	p	2chr; i-q;*st-a*	2chr;i-q;*st-a*	62
Acaronia nassa	50	50 *st-a*	50	p	---	---	19;40
Aequidens sp.	48	48 *st-a*	48	---	---	---	21
Aequidens metae	48	6*m-sm* +42 *st-a*	54	---	---	---	7;36
Aequidens plagiozonatus	48	-	-	---	---	---	16
Aequidens plagiozonatus	48	12*m-sm*+36*st-a*	60	---	---	---	53
Aequidens pulcher	48	4*m-sm* +44 *st-a*	52	---	---	---	36
Aequidens rivalatus	48	8*m-sm* +40 *st-a*	56	---	---	---	36
Aequidens tetramerus	48	12*m-sm* +36 *st-a*	60	---	2chr;t-p;*st-a*	4chr;i-q;*st-a*	52;60
Bujurquina sp	48	8*m-sm* +40 *st-a*	56	---	---	---	38
Bujurquina peregrinabunda	50	10*m-sm* +40 *st-a*	60	pc	---	---	40
Bujurquina peregrinabunda	50	20*m-sm* +30 *st-a*	70	pc	2chr;t-p;*m-sm*	2chr;t-q;*st-a*	62
Bujurquina vittata	44	22*m-sm*+8*st-a*+1-4 Bs	66	pc	---	---	42
Bujurquina vittata	44	26*m-sm* +18 *st-a*	70	---	---	---	7;36
Bujurquina vittata	44	30*m* + 8*sm* +6*st-a*	82	---	4chr; t-p;*m*/t-p;*st-a*	2chr;i-p; *st-a*	71
Cichlasoma sp. C	46	-		---	---	---	23
Cichlasoma amazonarum	48	2*m-sm* +46 *st-a*	50	---	---	---	21
Cichlasoma beani	48	6*m-sm* +42 *st-a*	54	---	---	---	7
Cichlasoma bimaculatum	44	44 *st-a*	44	---	---	---	30
Cichlasoma bimaculatum	48	6*m-sm* +42 *st-a*	54	---	---	---	7
Cichlasoma dimerus	48	8*m-sm* +40 *st-a*	56	pc	---	---	42
Cichlasoma facetum	48	8*m-sm* +40 *st-a*	56	---	---	---	4
Cichlasoma facetum	48	*10m-sm* +38 *st-a*	58	---	2chr;t-p;*st-a*	---	11;12;29;39
Cichlasoma facetum	48	6*m-sm* +42 *st-a*	54	---	---	---	53
Cichlasoma istlanum	48	8*m-sm* +40 *st-a*	56	---	---	---	34
Cichlasoma nigrofasciatum	48	8*m-sm* +40 *st-a*	56	---	---	---	53
Cichlasoma octofasciatus	48	-	96	---	---	---	6
Cichlasoma octofasciatus	48	6*m-sm* +42 *st-a*	54	---	---	---	7
Cichlasoma orientale	48	6m+10st+32a	54	p/c	---	---	64

Cichlasoma paranaense	48	14*m-sm* +34 *st-a*	62	---	---	---	29
Cichlasoma paranaense	48	20*m-sm* +28 *st-a*	68	---	---	---	20
Cichlasoma paranaense	48	6*m-sm* +42 *st-a*	54	---	---	---	53
Cichlasoma portalegrense	48	14*m* +34*st-a*	62	---	2chr;t-p;*m*	2chr;i-q;*m*	72
Cichlasoma salvini	52	-	104	---	---	---	6
Cichlasoma salvini	52	28*m-sm* +24 *st-a*	80	---	---	---	7
Cichlasoma sanctifranciscense	48	10*sm*+28*st*+10*a*	86	---	4chrs;t-p;*sm-st*	2chrs;i-q;*a*	68
Cichlasoma trimaculatus	48	6*m-sm* +42 *st-a*	54	---			6
C. (Parachromis) dovii	48	8m/sm+40st/a	56	---	---	---	17
Parachromis (Cichlasoma) friedrichsthalii	48	6m/sm+42st/a	54	---	---	---	17
Cichlasoma istlanum	48	8m/sm+40st/a	56	---	---	---	34
Nandopsis (*Cichlasoma*) *tetracanthus*	48	6*m-sm*+28*st*+14*a*	82	---	---	---	9
Cleithracara maronii	50	-	100	---	---	---	6
Cleithracara maronii	50	12*m-sm*+38*st-a*	62	---	---	---	36
Cleithracara maronii	50	14*m-sm*+36*st-a*	64	---	----	---	65
Ivanacara adoketa	48	16m-sm+32st-a	64	---	---	---	65
Laetacara araguaiae	44	4*m-sm*+40*st-a*	48	pc	---	---	60
Laetacara curviceps	38			---	---	--	3
Laetacara dorsigera	44	4*m-sm*+40*st-a*	48	---	2chr,t-p;*st-a*	---	52
Laetacara cf. *dorsigera*	46	2*m* +44*a*	48	c	---	---	37
Laetacara cf. *dorsigera*	45	3*m* +42*a*	48	c	---	---	37
Laetacara cf. *dorsigera*	44	4*m* +40-*a*	48	c	---	---	37
Laetacara cf. *dorsigera*	43	5*m* +38*a*	48	c	---	---	37
Nannacara anomala	44	18m-sm+26st-a	62	---	---	---	7;65
Nannacara aureocephalus	44	18m-sm+26st-a	62	---	---	---	65
Nannacara taenia	44	16m-sm+28st-a	60	---	---	---	65
Heroini							
Amphilophus citrinellus	48	-	96	---	---	---	6
Amphilophus citrinellus	48	8*m-sm* +40 *st-a*	56	---	---	---	7
Amphilophus citrinellus	48	36*m-sm* +12 *st-a*	84	---	---	---	2
Amphilophus macracanthus	48	-	96	---	---	---	6
Amphilophus macracanthus	48	6*m-sm*+42*st-a*	54	---	---	---	7
Archocentrus centrarchus	48	6*m-sm*+42*st-a*	54	---	---	---	7
Archocentrus nigrofasciatus	48	-	96	---	---	---	6
Archocentrus nigrofasciatus	48	4*m-sm* +44 *st-a*	52	---	---	---	7
Archocentrus septemfasciatus	48	6*m-sm*+42*st-a*	54	---	---	---	7
Australoheros angiru	48	18*sm* +30 *st-a*	66	---	2chr;t-p;*st-a*	2chr;i-q;*st-a*	39
Australoheros facetus	48	22*sm* +26 *st-a*	70	pc-c	2chr; t-p;*m*	---	57
Caquetaia kraussii	50	6*m-sm* +44 *st-a*	56	---	---	---	7
Caquetaia spectabilis	50	12*m-sm* +38 *st-a*	62	c-i	2chr;t-p;*st-a*	4chr;t-p;i-q;st-a	62
Caquetaia spectabilis	50	50 *st-a*	50	---	---	---	21
Herichthys cyanoguttatus	48	-	94	---	---	---	6
Herichthys cyanoguttatus	48	6*m-sm*+42*st-a*	54	---	---	---	7
Herichthys labridens	48	6*m-sm*+42*st-a*	54	---	---	---	7
Herichthys minckleyi	48	6*m-sm*+42*st-a*	54	---	---	---	7
Heros sp.	48	6*m-sm*+42*st-a*	54	---	---	---	19
Heros efasciatus	48	8*m-sm* +40 *st-a*	56	---	2chr;t-p;*st-a*	2chr;i-q;*st-a*	52
Herotilapia multispinosa	48	-	96	---	---	---	6
Herotilapia multispinosa	48	6*m-sm*+42*st-a*	54	---	---	---	7
Hoplarchus psittacus	48	16*m-sm* +32 *st-a*	64	c	2chr; t-p;*m-sm*	2chr;t-p;*st-a*	62
Hypselecara coryphaenoides	42	16*m-sm* +26 *st-a*	58	c	2chr; i-p;*m-sm*	2chr; i-p;*m-sm*	62
Hypselecara coryphaenoides	42	6*m-sm* +42 *st-a*	54	---	---	---	7
Hypselecara temporalis	48	16*m-sm* +32st-a	64	c	2chr;i-p;*m-sm*	2chr;i-p;*m-sm*	62
Mesonauta festivus	48	14*m-sm* +34 *st-a*	62	---	5chr;t-p;st-a	2chr;i-q;*st-a*	53;60
Mesonauta festivus	48	-	96	---	---	---	6
Mesonauta festivus	48	8m,sm+40st,a	56	---	---	---	7
Mesonauta festivus	48	12m,sm+36st,a	60	---	---	---	39
Mesonauta insignis	48	12m,sm+36st,a	60	---	---	---	39
Nandopsis tetracanthus	48	6*m-sm*+42*st-a*	54	---	---	---	9
Neetroplus nematopus	48	8*m-sm*+40*st-a*	56	---	---	---	7
Parachromis dovii	48	8*m-sm*+40*st-a*	56	---	---	---	7
Parachromis managuensis	48	-	96	---	---	---	6
Parachromis managuensis	48	6*m-sm*+42*st-a*	54	---	---	---	7;53
Petenia splendid	48	6*m-sm*+42*st-a*	54	---	---	---	44

Pterophyllum leopoldi	48	16*m-sm* +32*st-a*	64	c	21 chr	2chr;i-p;*m-sm*	62
Pterophyllum scalare	48	16*m-sm* +32 *st-a*	64	c	2chr; t-p;*m-sm*	2chr;i-q;*st-a*	62
Pterophyllum scalare	48	4*m-sm*+44*st-a*	52	---	---	---	7;22
Pterophyllum scalare	48	6*m-sm*+42*st-a*	54	---	---	---	53
Symphysodon aequifasciatus	60	42*m-sm*+18*micr*	102	---	---	---	37
Symphysodon aequifasciatus	60	42m-sm+18micr	102	---	---	---	25
Symphysodon aequifasciatus	60	44*m-sm*+16*st-a*	104	---	---	---	1
Symphysodon aequifasciatus	60	44*m-sm*+16*micr*	104	---	---	---	21
Symphysodon aequifasciatus	60	58*m-sm*+2*st-a*	118	---	---	---	7
Symphysodon aequifasciatus	60	46*m-sm+4sta*+4*micr*	106	---	---	---	53, 58
Symphysodon aequifasciatus	60	48*m-sm+8sta*+4*micr*	108	---	---	---	42
Symphysodon aequifasciatus	60	*50msm+6sta+4micr*	110	pc	2-3chr; t-p;*m-sm*	2chr; t-p;*m-sm*	45;47;54
Symphysodon haraldi	60	52*m-sm*+4*sta*+4*micr*	112	pc	2-3chr; t-p;*m-sm*/i-p;*m-sm-st-a*	2chr; t-p;*m-sm*	45;47;54
Symphysodon discus	60	50*m-sm* +10 *st-a*	110	pc	2-5chr; t-p;*m-sm*	2chr; t-p;*m-sm*	47;54
Symphysodon discus	60	42*m-sm*+18*micr*	102	---	---	---	19
Symphysodon discus	60	C1: 46*m-sm*+14*micr*	106	---	---	---	25
Symphysodon discus	60	C2: *48*m-sm+12*micr*	108	---	---	---	25
Symphysodon discus	60	C1: 50*m-sm*+10*st-a*	110	---	---	---	45
Symphysodon discus	60	C2: 54*m-sm*+6*st-a*	114		---	---	45
Uaru amphiaconthoides	46	16*m-sm* +30 *st-a*	62	pc/c	4chr; t-p;*m-sm*	2chr;i-q;*m-sm*	62
Uaru amphiacanthoides	46	8*m-sm*+38*st-a*	54		---	---	7

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5. CONSIDERAÇÕES FINAIS

1. Todas as espécies analisadas apresentaram um número diplóide de 48 cromossomos, característico da família Cichlidae, com exceção de *Bujurquina vittata* que apresentou 2n=44. Entretanto, foram encontradas diferenças interespecíficas nas fórmulas cariotípicas devido, provavelmente, à ocorrência de rearranjos cromossômicos, como translocações ou inversões;

2. 80% das espécies da subfamília ichlinae apresentam 2n=48, contudo redução e aumento no numero diploide forão observados e parecem estar ocorrendo independentemente entre as espécies. As tribos Cichlini, Retroculini and Chaetobranchini, em sua maioria, apresentaram 2n=48, com cromossomos subtelo-acrocentricos, característica que parece ser plesiomórfica para os Ciclideos Neotropicais.

3. A impregnação por nitrato de prata (AgRON) evidenciou um sistema simples em *Chaetobranchopsis australis e Crenicichla jaguarensis*, e um sistema múltiplo com 4 e 3 cromossomos portadores de AgRON evidenciados em *Bujurquina vittata* e *Gymnogeophagus balzani* respectivamente, e confirmados com a FISH de DNAr 18S.

4. Aproximadamente 80% das espécies da subfamília Cichlinae apresentam um único par portador de sítios de DNAr 18S e 87% destas espécies na região terminal do cromossomo. Entre as espécies aqui analisadas, *Bujurquina vittata, Geophagus brasiliensis, Gymnogeophagus balzani, G. gymnogenys* e *G. rhabdotus* apresentaram mais de um par portador dessa sequência. A posição terminal de DNAr 18S nos cromossomos, pode estar favorecendo a ocorrência de eventos de translocação, bem como a associação com elementos transponíveis pode levar ao aparecimento de mais de um sítio de DNAr 18S.

5. Aproximadamente 90% das espécies da subfamília Cichlinae apresentam um único par portador de sítios de DNAr 5S e entre as 11 espécies aqui analisadas apenas *Crenicichla lepidota* não apresentou essa característica. Contudo, variações entre os tipos e a posição da sequência nos cromossomos foram evidenciadas. A posição intersticial dessa sequencia na maioria das espécies analisadas, 82%, pode favorecer a não dispersão dessa sequencia pelo genoma.

6. As onze espécies apresentaram o DNAsn U2 em um único par cromossômico e não foi observada sintenia entre U1 e U2, nas quatro espécies analisadas com estas duas sequencias. Contudo, a interação entre famílias de DNA repetitivos distintas, como DNAr 18S com DNAsn U2, foi observada em cinco espécies da tribo Geophagini, sendo elas *Crenicichla jaguarensis, C. lepidota, C. maculata, Gymnogeophagus balzani* e *G. labiatus.*

7. A hibridação com sonda de DNAsn U1 evidenciou a presença de um único par cromossômico portador do sítio em *Bujurquina vittata, Chaetobranchopisis australis, Crenicichla jaguarensis, Gymnogeophagus balzani*, padrão observado para outras espécies de ciclideos e em *C. jaguarensis* o cluster de DNAsn U1 estava co-localizado com DNAr 5S.

8. O maior número de dados cariotípicos ainda se restrigem às tribos Geophagini, Cichlastomatini e Heroini e nessas tribos foi observada uma maior variabilidade cariotípica. Um maior número de análises, principalmente em tribos menos estudadas, poderiam reforçar a tendência cariotípica não conservativa da subfamília Cichlinae.

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