

Chromosome analysis in *Saccodon wagneri* (Characiformes) and insights into the karyotype evolution of Parodontidae



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Parodontidae is a relatively small group of Neotropical characiform fishes consisting of three genera (*Apareiodon*, *Parodon*, and *Saccodon*) with 32 valid species. A vast cytogenetic literature is available on *Apareiodon* and *Parodon*, but to date, there is no cytogenetic data about *Saccodon*, a genus that contains only three species with a trans-Andean distribution. In the present study the karyotype of *S. wagneri* was described, based on both conventional (Giemsa staining, Ag-NOR, C-bands) and molecular (repetitive DNA mapping by fluorescent *in situ* hybridization) methods. A diploid chromosome number of $2n = 54$ was observed in both sexes, and the presence of heteromorphic sex chromosomes of the ZZ/ZW type was detected. The W chromosome has a terminal heterochromatin band that occupies approximately half of the long arm, being this band approximately half the size of the Z chromosome. The FISH assay showed a synteny of the 18S-rDNA and 5S-rDNA genes in the chromosome pair 14, and the absence of interstitial telomeric sites. Our data reinforce the hypothesis of a conservative karyotype structure in Parodontidae and suggest an ancient origin of the sex chromosomes in the fishes of this family.

Keywords: Ag-NOR, Cytogenetics, FISH, Heterochromatin, ZW sex chromosomes.

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Parodontidae é um grupo relativamente pequeno de peixes caracíformes neotropicais que consiste em três gêneros (*Apareiodon*, *Parodon* e *Saccodon*) com 32 espécies válidas. Uma vasta literatura citogenética está disponível sobre *Apareiodon* e *Parodon*, mas até o momento não há dados citogenéticos sobre *Saccodon*, um gênero que contém apenas três espécies com distribuição transandina. No presente estudo foi descrito o cariótipo de *S. wagneri*, baseado em métodos convencionais (coloração de Giemsa, Ag-NOR, bandas C) e moleculares (mapeamento de DNA repetitivo por hibridização fluorescente *in situ*). Um número cromossômico diplóide de $2n = 54$ foi observado, e a presença de cromossomos sexuais heteromórficos do tipo ZZ/ZW foi revelada. O cromossomo W possui uma banda terminal heterocromática que ocupa aproximadamente metade do braço longo, sendo esta banda aproximadamente a metade do tamanho do cromossomo Z. O ensaio FISH mostrou uma sintonia dos genes 18S-rDNA e 5S-rDNA no par de cromossomos 14, e a ausência de sítios teloméricos intersticiais. Nossos dados reforçam a hipótese de uma estrutura cariotípica conservadora em Parodontidae e sugerem uma origem ancestral dos cromossomos sexuais nos peixes desta família.

Palavras-chave: Ag-RON, Citogenética, Cromossomos sexuais ZW, FISH, Heterocromatina.

INTRODUCTION

The Neotropical region has the largest repository of freshwater fish species that correspond to about 16% of the world's fish biodiversity (Albert, Reis, 2011; Reis *et al.*, 2016). This biodiversity has enormous ecological relevance and economic importance, as many of these species represent a fishery and aquaculture resource (Hilsdorf, Hallerman, 2017). One of the most represented fish groups present in the hydrographic basins of this geographic region is Characiformes. This order includes exclusively freshwater fishes distributed in both Africa and America and shows its greatest diversity in the Neotropical Region (Malabarba, 1998; Nelson *et al.*, 2016). Characiformes comprises 2,081 valid species grouped into 23 families, mostly in Characidae (1,214 species) (Fricke *et al.*, 2020a). Parodontidae is a relatively small family distributed throughout South America and part of Panama (Nelson *et al.*, 2016), and includes 32 species (Fricke *et al.*, 2020a) organized in three genera: *Apareiodon* Eigenmann, 1916 ($N = 15$), *Parodon* Valenciennes, 1850 ($N = 14$) and *Saccodon* Kner, 1863 ($N = 3$) that differ due to some subtle morphological characters (Pavanelli, 2003).

Cytogenetic studies in Parodontidae cover about 50% of recognized valid species (Tab. 1), representing only two genera: *Apareiodon* and *Parodon*. Although the available data show that these fishes have a conserved diploid number ($2n$) of 54 chromosomes, differences in the number of chromosome arms (FN) and extensive variation in the position of 18S and 5S rDNA sites exist. Besides this, species with proto sex chromosomes are found together with others characterized by ZZ/ZW and ZZ/ZW1W2 multiple sex chromosome systems (Tab. 1). Sex chromosomes show different sizes among the Parodontidae species (Moreira-

Filho *et al.*, 1993; Rosa *et al.*, 2006; Vicari *et al.*, 2006; Bellafronte *et al.*, 2009), but in all the ZW species, the W chromosome is a subtelocentric chromosome almost entirely heterochromatic, whereas the Z is smaller and usually shows heterochromatic regions only in the distal segmental portion of its short arms. The use of satellite DNA and transposable elements as probes showed that the differentiation of the sex chromosomes in the family is associated with the accumulation of these repeated sequences (Bellafronte *et al.*, 2011; Schemberger *et al.*, 2011, 2016; Nascimento *et al.*, 2018).

The genus *Saccodon* is cytogenetically unexplored and contains only three valid species: *S. dariensis* (Meek & Hildebrand, 1913) distributed in Colombia and Panama, *S. terminalis* (Eigenmann & Henn, 1914) that lives in the Daule River Basin in Ecuador, and *S. wagneri* Kner, 1863 that inhabits the coastal basins of Ecuador and northern Peru (Pavanelli,

TABLE 1 | Cytogenetic characteristics in Parodontidae. 1. Moreira Filho *et al.*, 1980; 2. Moreira Filho *et al.*, 1985; 3. Jesus *et al.*, 1999; 4. Jorge, Moreira-Filho, 2000; 5. Bellafronte *et al.*, 2009; 6. Bellafronte *et al.*, 2011; 7. Schemberger *et al.*, 2011; 8. Leite, Maistro, 2004; 9. Calgaro *et al.*, 2004; 10. Traldi *et al.*, 2016; 11. Traldi *et al.*, 2019; 12. Bellafronte *et al.*, 2012; 13. Moreira-Filho *et al.*, 1984; 14. Vicari *et al.*, 2006; 15. Santos *et al.*, 2019; 16. Rosa *et al.*, 2006; 17. Moreira-Filho *et al.*, 1993; 18. Centofante *et al.*, 2002; 19. Bellafronte *et al.*, 2005; 20. Paula *et al.*, 2017.

Genus/Species	Locality/River, State	2n	Karyotype formula		Sex system	Ribosomal gene-bearing chromosomes		Reference
			Male	Female		18S rDNA (pair)	5S rDNA (pair)	
<i>Apareiodon</i>								
<i>A. affinis</i> (Steindachner, 1879)	Rio Passa Cinco (SP) Brazil	54/55	50 m/sm, 4 st	51 m/sm, 4 st	ZZ/ ZW1W2	26 (st)	8 (m)	1–7
<i>A. affinis</i>	Rio Sapucaí (MG), Brazil	54/55	50 m/sm, 4 st	51 m/sm, 4 st	ZZ/ ZW1W2	26 (st)		8
<i>A. affinis</i>	Rio Paraná (MP) Argentina	54/55	40–42 m/sm, 12 st	43/47 m/sm, 8–12 st	Absence			9
<i>A. argenteus</i> Pavanelli & Britski, 2003	Rio Araguaia (GO) Brazil	54	54 m/sm	54 m/sm	Absence	2 (m)	18 (m)	10
<i>A. argenteus</i>	Rio Araguaia (GO) Brazil	54	54 m/sm	54 m/sm	Absence			10, 11
<i>A. cavalcante</i> Pavanelli & Britski, 2003	Rio Araguaia (GO) Brazil	54	52 m/sm, 2 st	52 m/sm, 2 st	Absence			10, 11
<i>A. davisii</i> Fowler, 1941	Rio Salgado (CE) Brazil	54	54 m/sm	54 m/sm	Absence	4, 9, 14, 17 (m)	4, 9, 14, 17(m)	10, 11
<i>A. hasemani</i> Eigenmann, 1916	Rio São Francisco (MG) Brazil	54	54 m/sm	54 m/sm	ZZ/ZW	7, 22, 11 (st)	14 (m)	12
<i>A. ibitiensis</i> Amaral Campos, 1944	Ribeirão Araras (MG) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	ZZ/ZW	14 (m), 26 (st)	9 (m)	5, 6
<i>A. ibitiensis</i>	Rio Passa Cinco (SP) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	Absence	26 (st)		4
<i>A. ibitiensis</i>	Rio Passa Cinco (SP) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	ZZ/ZW	6, 14, 15, 26 (st)	9 (m)	5, 6
<i>A. ibitiensis</i>	Rio Piumi (MG) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	ZZ/ZW			7
<i>A. ibitiensis</i>	Rio Verde (PR) Brazil	54	48 m/sm, 6 st	47 m/sm, 7 st	ZZ/ZW			7
<i>A. machrisi</i> Travassos, 1947	Rio Araguaia (GO) Brazil	54	52 m/sm, 2 st	52 m/sm, 2 st	Absence			10
<i>A. piracicabae</i> (Eigenmann, 1907)	Rio Passa-Cinco/Mogi-Guaçu (SP) Brazil	54	52 m/sm, 2 st	52 m/sm, 2 st	Absence	27 (st)	9 (m)	2, 3, 6, 13
<i>A. piracicabae</i>	Rio Piumhi (MG) Brazil	54	52 m/sm, 2 st	52 m/sm, 2 st	Absence	27 (st)		7
<i>Apareiodon</i> sp.	Rio Verde (PR) Brazil	54	48 m/sm, 6 st	47 m/sm, 7 st	ZZ/ZW	25 (st)	9 (m)	5, 6, 14



TABLE 1 | (Continued)

Genus/Species	Locality/River, State	2n	Karyotype formula		Sex system	Ribosomal gene-bearing chromosomes		Reference
			Male	Female		18S rDNA (pair)	5S rDNA (pair)	
<i>Apareiodon</i> sp.	Rio Aripuanã (MT) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	ZZ/ZW	2, 5, 9, 26, 27 (m)	6 (m)	15
<i>Apareiodon</i> sp. A	Rio Frio (MG) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	Absence	26 (st)		4
<i>Apareiodon</i> sp. B	Rio Barreiro (MG) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	Absence	26 (st)		45
<i>Apareiodon</i> sp. C	Rio Araguaia (GO) Brazil	54	52 m/sm, 2 st	52 m/sm, 2 st	Absence			4
<i>A. vittatus</i> Garavello, 1977	Rio Jordão (PR) Brazil	54	52 m/sm, 2 st	52 m/sm, 2 st	Absence	27 (st), additional 14 (m)	9 (m)	4, 6
<i>A. vittatus</i>	Rio Jordão (PR) Brazil	54	52 m/sm, 2 st	52 m/sm, 2 st	Absence			7
<i>A. vladii</i> Pavanelli, 2006	Rio Piquiri (PR) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	ZZ/ZW	26 (st)	9 (m), additional 3 (m)	6, 16
<i>A. vladii</i>	Rio Piquiri (PR) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	ZZ/ZW			7
<i>Parodon</i>								
<i>P. hilarii</i> Rheinhardt, 1867	Córrego do Porta (MG) Brazil	54	54 m/sm	53 m/sm, 1 st	ZZ/ZW	16 (m)	11 (m)	6, 7, 17
<i>P. moreirai</i> Ingenito & Buckup, 2005	Córrego Paiol Grande (SP) Brazil	54	54 m/sm	54 m/sm	ZZ/ZW	15 (m)	11 (m)	6, 7, 18
<i>P. nasus</i> Kner, 1859	Rio Passa Cinco (SP) Brazil	54	48 m/sm, 6 st	48 m/sm, 6 st	Absence	25 (st)	25 (st)	2, 6, 13, 19
<i>P. nasus</i>	Rio Três Bocas (PR) Brazil	54	54 m/sm	54 m/sm	Absence	2 (m)		²⁰
<i>P. nasus</i>	Rio Paraguai (MT) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	Absence			7
<i>P. pongoensis</i> (Allen, 1942)	Rio Taquaralzinho (MT) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	Absence	2 (m)	9 (m)	6, 7
<i>Parodon</i> cf. <i>pongoensis</i>	Rio Araguaia (GO) Brazil	54	50 m/sm, 4 st	50 m/sm, 4 st	Absence			10, 11
<i>Saccodon</i>								
<i>S. wagneri</i> Kner, 1863	Río Bonito (El Guabo) / El Oro Ecuador	54	32 m, 16 sm, 6 st	31 m, 16 sm, 7 st	ZZ/ZW	14 (m)	14 (m)	Present study

2003). This last one was previously known as *S. cranocephalum* Thominot, 1882 and *Parodon ecuadoriensis* Eigenmann & Henn, 1914, now considered synonym (Fricke *et al.*, 2020b). *Saccodon wagneri* is adapted to live in rivers that flow rapidly with rocky bottoms near the mountains and generally above 100 m altitude (Roberts, 1974), often forming schools when swims in rapid waters (Glodek, 1978). *Saccodon wagneri* specimens easily adapt to confinement in aquariums where they eat algae and even balanced food, so that they could also be considered as aquarium fish, similarly to other Parodontiidae. Indeed, some species of this family as *Apareiodon affinis* (Steindachner, 1879), *Parodon pongoensis* (Allen, 1942), and *P. suborbitalis* Valenciennes, 1850, are included in the pet trade (Prang, 2008) and advertised on websites dedicated to the sale of aquarium fish (https://www.aquariumglaser.de/en/fish-archives/apareiodon_affinis_en/).

In the present study, we performed a cytogenetic survey of *S. wagneri* based on both conventional (Giemsa staining, silver staining, C-banding) and molecular (repetitive DNA mapping methods). The study aims to verify whether morphologically

differentiated sex chromosomes, that are present in some *Apareiodon* and *Parodon* species, can be identified also in the genus *Saccodon* and whether chromosome number and main karyotype structure are conserved in this genus. A comparative analysis of cytogenetic data on this species and the remaining Parodontidae is presented here.

MATERIAL AND METHODS

Eleven individuals (2 males and 9 females) of *S. wagneri*, from the Río Bonito, El Guabo, El Oro Province, 03°07'55"S 79°45'00"W, were sampled (Fig. 1). The fishes were collected with cast nets and placed in plastic bags filled up to a third of their capacity with water and oxygen the remaining two thirds, transported in cardboard boxes to the laboratory where they were confined in aquariums provided with constant aeration until they were processed.

Mitotic chromosomes were obtained from kidney cells suspension following the conventional air-drying method (Nirchio, Oliveira, 2006). The animals were stimulated to increase the number of metaphases with an injection of yeast-glucose suspension (Lozano *et al.*, 1988) in the caudal peduncle 48 h before being processed. Each fish was injected with 0.0125% colchicine (1.0 ml/100 g of body weight) 50 min before being sacrificed with an overdose of benzocaine (Leary *et al.*, 2013).

Voucher specimens are preserved and deposited in the Ichthyology Collection of the Laboratório de Biologia e Genética de Peixes (LBP) of Universidade Estadual Paulista, Botucatu, São Paulo, Brazil (UNESP) (collection numbers LBP 26871–26874) and Universidad Técnica de Machala, El Oro, Ecuador (collection numbers UTMACH–0398–0399).

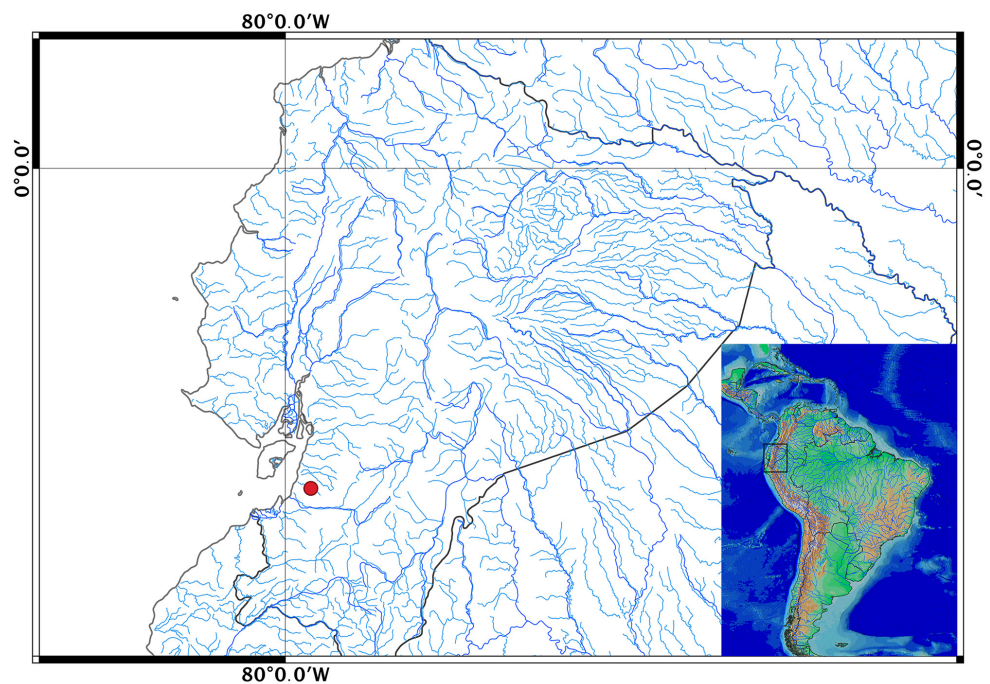


FIGURE 1 | Map of Ecuador, highlighting the sampling site of *Saccodon wagneri*.

The metaphases were stained with 5% Giemsa solution to define the $2n$ and the karyotype formula. C-positive heterochromatic regions were identified by the C-banding procedure, following Sumner (1972), while the nucleolus organizer regions (NORs) were identified using silver nitrate impregnation (Howell, Black, 1980) after Giemsa staining.

The 5S rDNA and 18S rDNA (ribosomal genes), and telomeric repeats were mapped onto chromosomes by fluorescence *in situ* hybridization (FISH) (Pinkel *et al.*, 1986). Probes were obtained and labeled by PCR from the genome of *Hypsolebias flagellatus* (Costa, 2003) using the primers described by Pendas *et al.* (1995) for 5S rDNA, Utsunomia *et al.* (2016) for 18S rDNA and Ijdo *et al.* (1991) for telomeric repeats. The 5S rDNA and telomeric probes were labeled with biotin-16-dUTP (2'-deoxyuridine 5'-triphosphate), and the 18S rDNA probes were labeled with digoxigenin-11-dUTP. Signals were detected with fluorescein-conjugated avidin (Sigma-Aldrich, www.sigma-aldrich.com) and antidigoxigenin-rhodamine conjugate (Roche Diagnostics, www.roche.com), respectively. Chromosomes were counterstained with 4,6-diamidino-2-phenylindole included in the Vectashield mounting medium (Vector Laboratories, Ltd., Peterborough, UK).

Images capture of chromosome spread after Giemsa, silver staining (Ag-NORs), and C-bands (constitutive heterochromatin), was performed under a CX31 Olympus microscope equipped with a Moticam 10+ digital camera coupled to a Motic Images Plus 2.0 software. FISH metaphases were analyzed under an Olympus BX53 epifluorescence microscope (Olympus Corporation, Ishikawa, Japan) with the appropriate filters; images were captured with an Olympus DP73 digital camera coupled to cellSens Dimension Software (Olympus) for image acquisition. Images were merged and edited to optimize the brightness and contrast using the Photoshop CS5 program (Adobe Systems, www.adobe.com). At least 30 metaphase spreads per individual were analyzed to confirm the diploid number, karyotype structure and FISH results. Chromosomes were classified as metacentric (m), submetacentric (sm), or subtelocentric (st) according to their arm ratios (Levan *et al.*, 1964).

RESULTS

The diploid number of *S. wagneri* is $2n = 54$ chromosomes for males and females, although differences in the FN are present between sexes. Indeed, the karyotype is composed of $31m + 16sm + 7st$ chromosomes in females, with $FN = 101$ (Fig. 2A), and of $32m + 16sm + 6st$, $FN = 102$ (Fig. 2B) in males. This is due to the presence of morphologically differentiated sex chromosomes, *i.e.*, to a heteromorphic ZZ/ZW sex chromosome system. The Z chromosome is submetacentric while the W is metacentric and almost twice as large as the Z (Fig. 2).

Sequential Giemsa and silver nitrate staining revealed a single pair of Ag-NOR positive marks located at the tip of the short arms of a small metacentric chromosome pair, probably pair 14 (Fig. 2, boxes).

C-banding revealed regions of centromeric heterochromatin in most chromosomes, as well as the presence of interstitial and terminal C-positive bands (Fig. 3). A large heterochromatic block is present on the half-distal part of the long arms of W chromosome in the female metaphases (Fig. 3B); a similar band is absent in the Z chromosome.

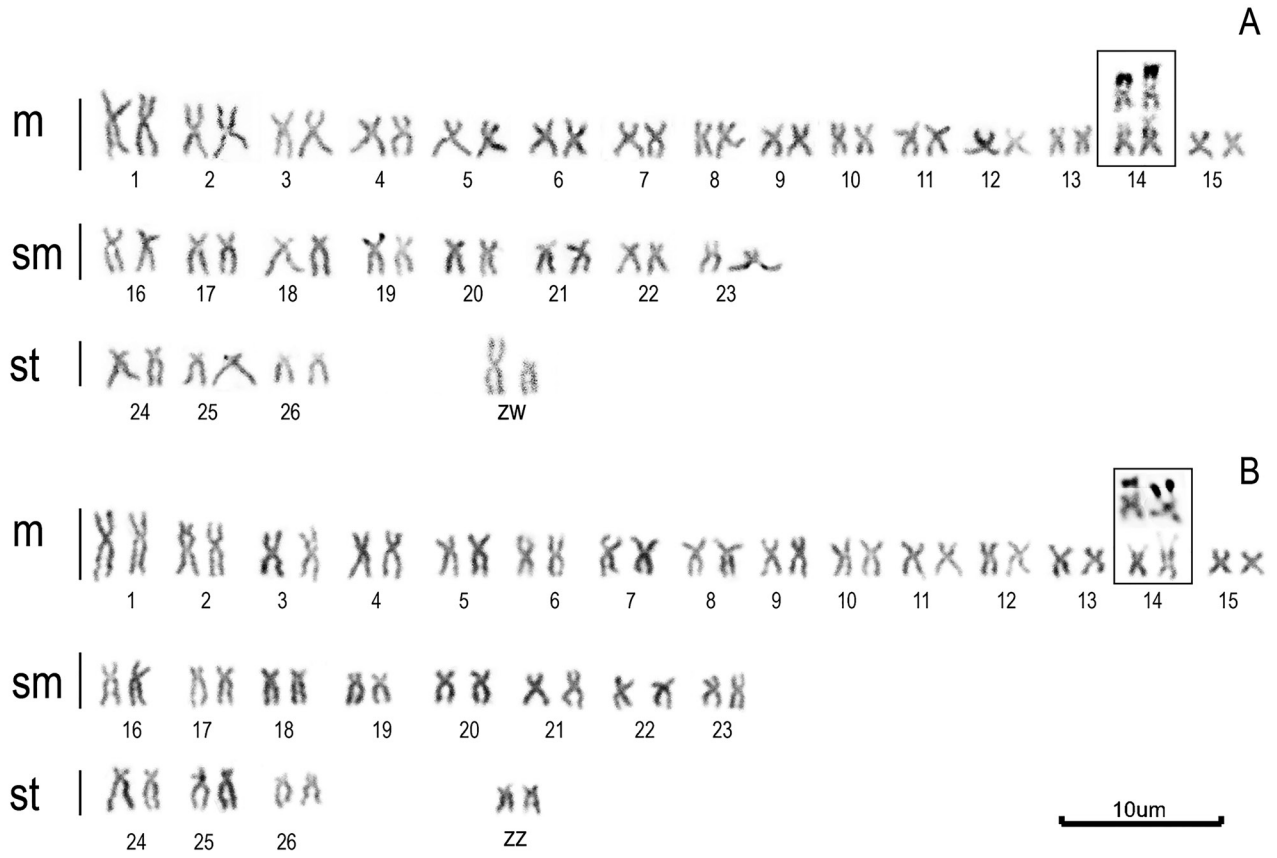


FIGURE 2 | *Saccodon wagneri* Giemsa karyotypes. A. Female; B. Male. Sex chromosomes are indicated. The NOR-carrying chromosomes, after silver staining, are boxed.

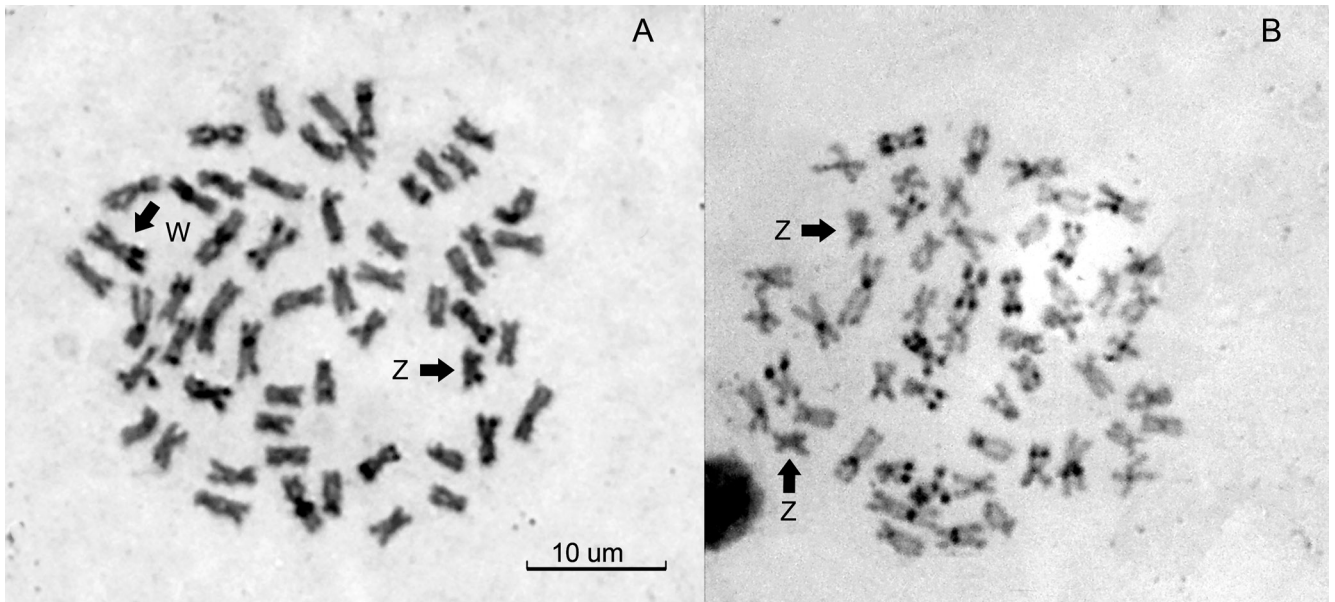


FIGURE 3 | *Saccodon wagneri* C-banded metaphases. A. Female; B. Male. The arrows indicate the sex chromosomes.

In situ hybridization using the 18S rDNA probe confirmed the presence of a single cluster of major ribosomal genes, localized on a small metacentric chromosome pair, likely coinciding with the Ag-NOR signals. Minor ribosomal genes were located on this same chromosome pair, just below the major rDNA cluster, in a syntenic condition (Fig. 4A).

FISH with the telomeric repeat probe (TTAGGG)_n (Fig. 4B) revealed hybridization signals only in the telomeric regions of all chromosomes, without the presence of interstitial telomeric sites (ITSs).

DISCUSSION

Recent characiform phylogenomic studies showed that Parodontidae originated about 70 million years ago (mya) and the first recognized cladogenesis occurred about 40 mya, separating *Saccodon* (an exclusive trans-Andean group) from *Parodon* and *Apareiodon* (wide-spread groups in the Neotropical region) (Bruno F. Melo, 2020, pers. comm.). The cytogenetics data on Parodontidae reveal a conservative $2n = 54$ karyotype, that is composed predominantly of metacentric and submetacentric chromosomes (except for *Apareiodon affinis*, where females present $2n = 55$ due to the unique ZW_1W_2 sex system) (Moreira Filho *et al.*, 1980) (Tab. 1). Results here obtained on *Saccodon wagneri* reinforce this picture, despite the ancient divergence of this genus within the family. Moreover, other Neotropical fishes closely related to Parodontidae, *e.g.*, families Anostomidae, Prochilodontidae, Chilodontidae, and Curimatidae (Betancur *et al.*, 2019; Bruno F. Melo, 2020, pers. comm.), also share this feature, *i.e.*, almost all species with 54

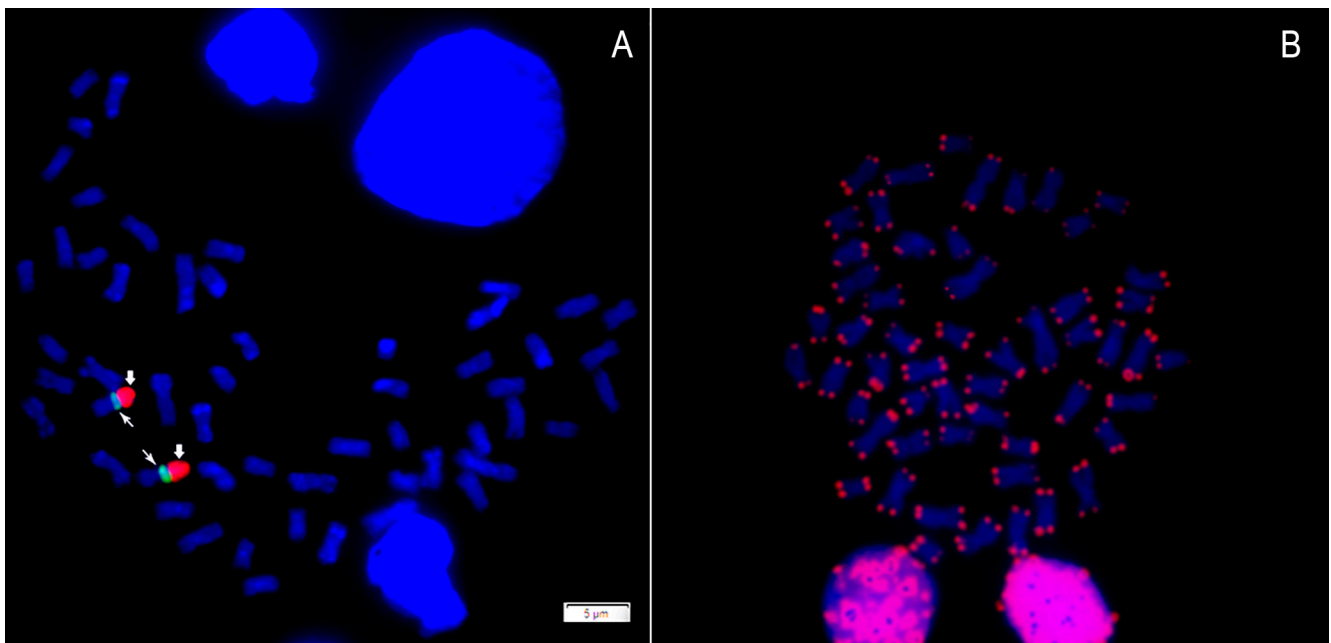


FIGURE 4 | *Saccodon wagneri* metaphase plates after **A.** Double FISH with 5S rDNA (green-thin arrows) and 18S rDNA (red-thick arrows) probes; **B.** FISH using telomeric probes showing positive signals in the terminal positions of all chromosomes.

chromosomes, mainly metacentrics and submetacentrics, with a few exceptions (Arai, 2011). These data indicate an ancient origin of such a karyotype, whose conservatism has been related to the population structures of these fishes, as they include many long migratory species able to form large schools (Oliveira *et al.*, 1988).

A morphologically well-differentiated ZZ/ZW sex chromosome system is present in approximately half of all *Parodon* and *Apareiodon* species analyzed so far (Moreira-Filho *et al.*, 1993; Rosa *et al.*, 2006; Vicari *et al.*, 2006; Bellafronte *et al.*, 2009; Kitano, Peichel, 2012). The occurrence of such sex system, characterized by an enlarged metacentric W chromosome, in *S. wagneri* points to its old origin inside Parodontidae. Besides this, and as frequently observed in higher vertebrates (Schartl *et al.*, 2016), rather than showing a size reduction, the sex-specific W chromosome in Parodontidae is larger than the Z, because of a huge heterochromatin amplification. Despite these common features, the W chromosomes have evolved to different shapes and sequence contents among Parodontidae species. Two main questions remain unanswered, *i.e.*, whether (i) the Z and W chromosomes have a common origin, representing the same linkage group in all species and (ii) the absence of sex chromosomes in some species may represent a derived character, probably related to sex chromosomes turnovers, as already documented in other fishes (Kitano, Peichel, 2012). Our data reinforce the hypothesis that this common ZW system has an ancient origin and it seems possible that the putative absence in some Parodontidae species would be related to subsequent specific chromosome differentiation. Further studies will make it possible to confirm the validity of this hypothesis.

In all the Parodontidae species studied so far, the presence of a single pair of NOR bearing chromosomes is the common condition, with few exceptions (Bellafronte *et al.*, 2011). However, different locations of these genes have been observed among the species, probably as the result of chromosomal rearrangements (pericentric inversion), occurred along with the diversification of their karyotypes. The presence of multiple sites reported in *Apareiodon davisi* (Traldi *et al.*, 2016), and *A. ibitiensis* (Bellafronte *et al.*, 2009; Bellafronte *et al.*, 2011) represents an exception, that has been attributed to the presence of transposable elements (Bellafronte *et al.*, 2011). The syntenic arrangement of the 18S and the 5S rDNA genes detected in *S. wagneri*, has only been reported in two other Parodontidae species, named *A. davisi* (Traldi *et al.*, 2016) and *P. nasus* (Bellafronte *et al.*, 2005), and does not represent a common condition in fishes (Sochorová *et al.*, 2018). Indeed, the presence of these genes on different chromosomes/sites in fishes and in the majority of vertebrates has been interpreted in the light of their functional dynamics (Martins, Galetti, 1999) and efficiency in evolution processes associated with multiple tandem arrays (Martins, Wasko, 2004).

FISH with the telomeric probe (TTAGGG)_n in *S. wagneri* revealed hybridization signals only in the telomeric regions of all chromosomes in females and males, without Interstitials Telomeric Sequences (ITSs) that might result from the occurrence of recent Robertsonian fusions or other chromosomal rearrangements (Ocalewicz, 2013). This evidence, the common localization of constitutive heterochromatin (Moreira-Filho *et al.*, 1984; Jesus, 2000; Jesus, Moreira-Filho, 2000; Vicente *et al.*, 2001, 2003; Centofante *et al.*, 2002; Bellafronte *et al.*, 2005; Rosa *et al.*, 2006; Vicari *et al.*, 2006), and the constancy of 2n suggest that diversification in Parodontidae karyotypes has not involved macrostructural reorganizations but rather microstructural ones.

In conclusion, our study, the first one to report cytogenetic data on a *Saccodon* species both by conventional and molecular protocols, reinforces the hypothesis of karyotype homeostasis in fishes of the family Parodontidae, by conserving the basic diploid number and chromosome formulae. The synteny of both 18S and 5S rDNA found in *S. wagneri* represents an uncommon trait, and its presence in species of the other two genera (*A. davisi* and *P. nasus*), suggests its ancient origin, *i.e.*, that this is a symplesiomorphic character within the family. As an alternative hypothesis, this similarity could be due to a homoplastic condition, obtained by parallelism. Further studies with chromosomal painting, sequence analysis of microdissected sex chromosomes, and comparative mapping of transposable elements will be useful to obtain a more complete picture of the evolution of karyotype and sex chromosomes within Parodontidae.

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COMPETING INTERESTS

The authors declare no competing interests.

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