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Intraspecific karyotypic variation in the silverside fish *Chirostoma humboldtianum* (Atheriniformes: Atherinopsidae)Variación cariotípica intraespecífica en el pez blanco *Chirostoma humboldtianum* (Atheriniformes: Atherinopsidae)

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## ABSTRACT

Within its genus, *Chirostoma humboldtianum* is one of the species with the widest distribution, inhabiting lake and pond environments along the Lerma-Santiago basin in the Mexican Plateau. Although the species is of great ichthyological, economic, and cultural relevance, factors related to human activity such as habitat loss, pollution, overfishing, and introduction of non-native fish have played important roles in the decline and disappearance of populations. With the aim of describing the karyotype of the species, 22 specimens of *C. humboldtianum* were collected and their chromosomes obtained from gills based on Denton (1973). Here we reveal intraspecific chromosome variation, characterized by five cytotypes found in four studied populations: 1) Las Tazas, Tiacaque dam ( $2n = 48$ ,  $FN = 58$ ) with chromosome formula  $6m + 4sm + 38t$ ; 2) Villa Victoria dam ( $2n = 48$ ,  $FN = 54$ )  $2m + 2sm + 2st + 42t$ ; 3) Tepuxtepec dam, with two cytotypes ( $2n = 48$ ,  $FN = 50$ )  $8m + 2sm + 38t$  and ( $4n = 96$ ,  $FN = 116$ )  $8m + 2st + 38t$ ; 4) and San Pedro Lagunillas lagoon ( $2n = 48$ ,  $FN = 58$ )  $8m + 2sm + 38t$ . We observed chromosome variation in the morphology of pairs 1, 2, 4, and 5. Results allow us to suggest that pericentric inversions are the source of intraspecific chromosome variation. Comparative analyses support the hypothesis that the karyotype of the population from Villa Victoria dam represents the primitive form for the species.

**Key words:** Karyotype, Shortfin silverside, polyploidy, variation.

## RESUMEN

*Chirostoma humboldtianum* es dentro del género, una de las especies con la más amplia distribución, habita ambientes lacustres y cuerpos de agua a lo largo del sistema Lerma-Santiago en la Mesa Central de México. Aunque la especie es de gran relevancia ictiológica, económica y cultural, factores relacionados con la actividad humana como la pérdida de hábitat, contaminación, sobrepesca y la introducción de peces no-nativos, han jugado un papel importante en la disminución y desaparición de poblaciones. Con el propósito de describir el cariotipo de la especie, se colectaron 22 individuos de *C. humboldtianum* y sus cromosomas se obtuvieron a partir de branquias de acuerdo a Denton (1973). En el presente trabajo se revela variación cromosómica intraespecífica caracterizada por cinco citotipos de cuatro poblaciones estudiadas: 1) Presa Las Tazas, Tiacaque ( $2n = 48$ ,  $NF = 58$ ) con las fórmulas cromosómicas  $6m + 4sm + 38t$ ; 2) Presa Villa Victoria ( $2n = 48$ ,  $NF = 54$ )  $2m + 2sm + 2st + 42t$ ; 3) Presa Tepuxtepec, con dos citotipos ( $2n = 48$ ,  $NF = 50$ )  $8m + 2sm + 38t$  y ( $4n = 96$ ,  $NF = 116$ )  $8m + 2st + 38t$ ; 4) y Laguna San Pedro Lagunillas ( $2n = 48$ ,  $NF = 58$ )  $8m + 2sm + 38t$ . La variación cromosómica se detectó principalmente en la morfología de los pares 1, 2, 4 y 5. Los resultados encontrados permiten sugerir la presencia de inversiones pericéntricas como el principal mecanismo de variación cromosómica intraespecífica. El análisis comparativo de los datos apoya la hipótesis de que el cariotipo de la población Villa Victoria representa, la forma cromosómica primitiva de la especie.

**Palabras clave:** Citotipo, pez blanco, poliploidía, variación.

## INTRODUCTION

Among vertebrates, fish have the greatest number of species. Atheriniformes contains six, globally-recognized families, from which Atherinopsidae is exclusive to the New World (Nelson, 1994), and includes two genera restricted to inland waters of the Mexican plateau, *Chirosoma* and *Poblana*.

*Chirosoma* is the representative genus of the Atherinopsidae. It is endemic to Mexican ichthyofauna and includes the silversides “charales” and “peces blancos”; among the latter, *C. humboldtianum* (Valenciennes 1835) has the greatest but disjunct distribution, isolated in lakes and ponds from the Valley of Mexico to the Pacific Ocean following the Lerma-Chapala-Santiago basin system (Alvarez & Navarro, 1957; Barbour, 1973 a, b). It also has high economic and cultural value (Jiménez & Gracia, 1995; Soria-Barreto *et al.*, 1998; Barriga-Sosa, 2001; Barriga-Sosa *et al.*, 2002, 2004, 2005; Rojas & Sasso, 2005).

*Chirosoma humboldtianum* faces several problems such as habitat reduction and modification (e.g., the groundwater extraction in the Basin of Mexico, Alvarez & Navarro, 1957), pollution, introduction of exotic species (Barbour, 1973a; Berlanga-Robles *et al.*, 2002), and translocations. These in turn played important roles in the decline and extirpation of local populations (Lyons *et al.*, 1998; Soria-Barreto *et al.*, 1998; Soto-Galera *et al.*, 1998; Barriga-Sosa *et al.*, 2002, 2004, 2005; Rojas & Sasso, 2005; Mercado-Silva *et al.*, 2006). In despite of all these threats to the species, it has not been designated as threatened in the Official Mexican Standard Norms (i.e., NOM-059-Semarnat-2010) or in the International Union for the Conservation of Nature (IUCN 2015).

Morphological and genetic studies on the “*humboldtianum* group” (a group mainly made up of “peces blancos”, *C. humboldtianum* included) at the intra and interspecific level (Barriga-Sosa, 2001; Barriga-Sosa *et al.*, 2002), found the presence of significant morphological and genetic differences between populations of *C. humboldtianum*. These studies suggest that habitat diversity and geographic isolation might have played important roles on their heterogeneity. However, species distribution (populations within the same region) has been of limited interest. Only recently, García-Martínez *et al.* (2015), using mitochondrial

DNA sequences and including samples from six locations along the entire disjunct distribution of the species, have found evidence of a strong genetic structure of the species and have established the presence of at least six populations.

Where differences in the number and structure of chromosomes have been detected, cytogenetic analysis of fish have been used to characterize both populations (i.e., rainbow trout, Thorgaard, 1983; Ocalewicz & Dobosz, 2009) and species (Ariids, Uribe-Alcocer, 1988; cichlids, Uribe-Alcocer *et al.*, 1992, 1999; Hodaňová *et al.*, 2014), contributing to the knowledge and understanding of the evolutionary history and relationships of organisms (White, 1973; Huxley, 1974; Mank & Avise, 2006). For the members of *Chirosoma*, there is no information regarding intraspecific chromosome characterization, and of 18 recognized species, only five have been described as karyotypes (Uribe-Alcocer *et al.*, 2002; Uribe-Alcocer & Díaz-Jaimes, 2003). Thus, the aim of this study is to describe the karyotype of *C. humboldtianum* along its distribution in the Lerma-Chapala-Santiago basin in order to solve one important question: Does the species possess a conserved karyotype along its disjunct distribution?

## MATERIALS AND METHODS

**Fish sampling.** We obtained twenty-two *C. humboldtianum* adults from stocks maintained in culture conditions in the Planta Experimental de Producción Acuicola at the Universidad Autónoma Metropolitana-Iztapalapa campus. The original stocks were collected from four natural locations (Table 1, Fig. 1) that correspond to what hereinafter we refer to as “populations” according to García-Martínez *et al.* (2015). Our populations originated on the high, middle, and low Lerma-Chapala-Santiago basin and were identified by their morphological characters according to Barbour (1973b).

**Chromosome preparation.** We obtained chromosome spreads from gill epithelium using a 1 % sodium citrate hypotonic solution following Denton (1973). Slides with chromosomes were stained with 10 % Giemsa Sörensen solution, pH 7 for 15 min. Mitotic chromosomes were screened in an Olympus CX31 microscope equipped with a digital Infi-

Table 1. Collecting sites for the analyzed *Chirosoma humboldtianum*.

Collecting site	Geographic Coordinates (DMS)	MASL (m)	<i>n</i> Sex	TL (cm)	TW (g)
1. Las Tazas Tiacaque dam Jocotitlán, State of Mexico (T) (OBS at PExPA)	19° 38' 29" N 99° 42' 27" W	2540	6 ♀ 6 ♂	10.5-19.2	5-20
2. Villa Victoria dam, State of Mexico (VV) (OBS at PExPA)	19° 26' N 100° 00' W	2570	4 ♂	9-11.5	5-10
3. Tepuxtepec dam, Contepec, Michoacan (Tx) (OBS at PExPA)	20° 00' 09" N 100° 12' 45" W	2361	2 ♀ 2 ♂	10-13	10-12
4. San Pedro Lagunillas lagoon, Nayarit (SP) (OBS at PExPA)	21° 11' 39" N 104° 43' 44" W	1248	2 ♂	8.3-9.5	4-9

OBS = Original brood stock collected in the referred site; PExPA = Planta Experimental de Producción Acuicola; MASL = Meters above sea level; *n* = Number of organisms analyzed

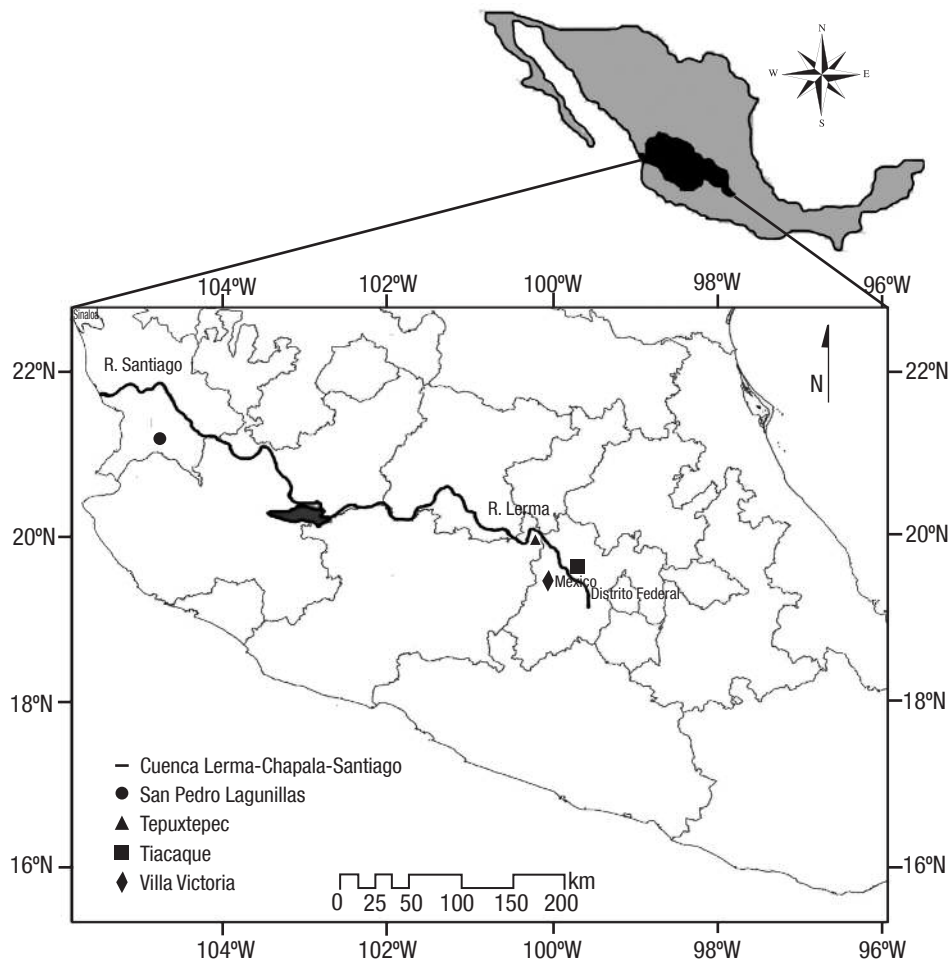


Figure 1. Map showing the collected localities (populations) from *Chiostoma humboldtianum* specimens.

nity 1 camera and 7.5X zoom. All images were processed using Adobe Photoshop CS5 Version 8.0.1. Ten mitoses of Las Tazas, Tiacaque dam population, four of Villa Victoria dam, six of Tepuxtepec dam and five of San Pedro Lagunillas, were measured to determine chromosome relative length. Centromeric index and arm ratios of chromosomes were used to classify them following Levan *et al.* (1964): a ratio from 1.0 to 1.7 for short to long arms (p and q) corresponded to metacentric (m); 1.71 to 3.0 to submetacentric (sm); between 3.1 and 7.0 to subtelocentric (st) and higher than 7.0 to telocentric (t) chromosomes. The fundamental number (FN) was determined as the total number of chromosome arms (Matthey, 1973). Ideograms were prepared according to the relative lengths of the short and long arms, as well as the centromere position (Denton, 1973) of each chromosome pair.

We analyzed differences between relative lengths of the most variable chromosome pairs in morphology of different cytotypes by a Wilcoxon-Mann-Whitney test (STATISTICA Version 10).

A UPGMA dendrogram was constructed in order to form groups based on cytotype similarities. A similarity matrix was built containing

the following characters: 1) fundamental number; 2) numbers of metacentric, submetacentric, subtelocentric, and telocentric chromosomes; 3) morphology of pairs 1, 2, 4, and 5; and 4) the relative lengths of each chromosome pairs (1 to 24) and using the Bray-Curtis coefficient (MVSP Version 3.0, Kovach, 1998). We used the Similarity Percentage Analysis (SIMPER) (Clark, 1993) to determine the relative contribution of populations to dissimilarity between the resolved groups and average dissimilarity; the analysis was conducted in Past 3.03 (Hammer *et al.*, 2001).

## RESULTS

From 429 mitotic spreads analyzed from the four populations of *C. humboldtianum*, 70.39 % revealed a diploid number of  $2n = 48$  chromosomes. One specimen from Tepuxtepec dam exhibited a tetraploid karyotype ( $4n = 96$ ) 57.14 % of the analyzed mitosis (12 out of 21 mitosis) for that specimen (Table 2).

Five cytotypes were resolved in the four populations analyzed: one cytotype for each one of the populations, Las Tazas, Tiacaque, and Villa

Table 2. Number of individuals, preparations and mitosis analyzed per population of *C. humboldtianum*.

Population	n	Chromosome slides	Mitoses spreads	Measured mitoses
Las Tazas Tiacaque dam (T)	12	12	295	10
Villa Victoria dam (VV)	4	4	42	4
Tepuxtepec dam (Tx)	4	4	51	6
San Pedro Lagunillas lagoon (SP)	2	2	41	5

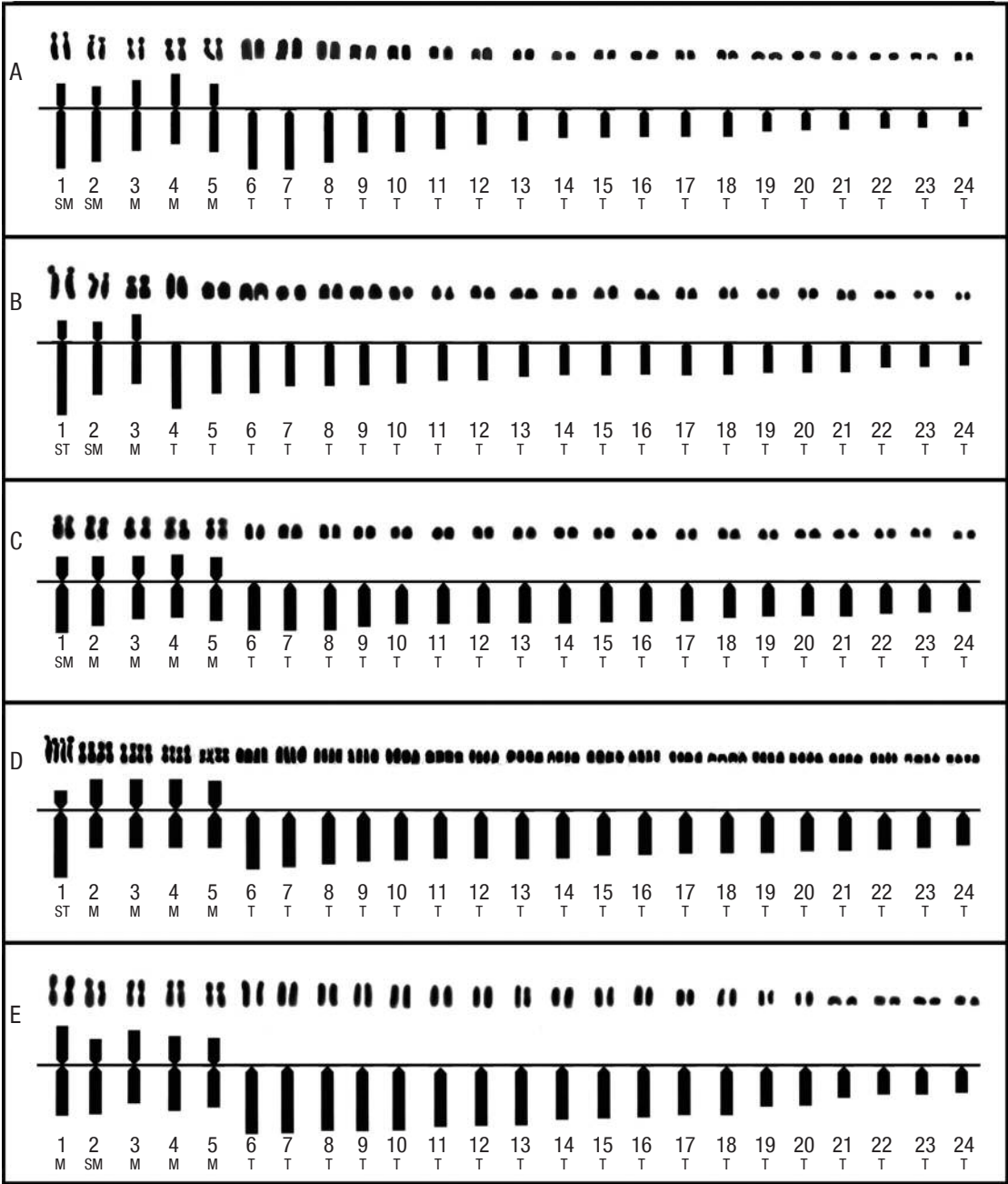


Figure 2. Karyotypes and idiograms of the five cytotypes resolved for *Chirostoma humboldtianum*. A. Female karyotype and idiogram from Las Tazas Tiacaque dam (2n = 48, FN = 58); B. Male karyotype and idiogram from Villa Victoria dam (2n = 48, FN = 54); C. Female karyotype and idiogram from Tepuxtepec dam (CI, 2n = 48, FN = 58); D. Male karyotype and idiogram from Tepuxtepec dam (CII, 4n = 96, FN = 116); E. Male karyotype and idiogram from San Pedro Lagunillas lagoon (2n = 48, FN = 58).

Table 3. Chromosomal characters of the five cytotypes described for *C. humboldtianum*.

Cytotypes	Sex	DN	FN	CF	Pair morphology					
					1	2	3	4	5	6-24
T	♀	2n=48	58	6M+4Sm+38T	Sm	Sm	M	M	M	T
VV	♂	2n=48	54	2M+2Sm+2St+42T	St	Sm	M	T	T	T
Tx CI	♀	2n=48	58	8M+2Sm+38T	Sm	M	M	M	M	T
Tx CII	♂	4n=96	116	8M+2St+38T	St	M	M	M	M	T
SP	♂	2n=48	58	8M+2Sm+38T	M	Sm	M	M	M	T

T = Las Tazas Tiacaque dam; VV = Villa Victoria dam; Tx CI = Tepuxtepec dam, Cytotype I; Tx CII = Tepuxtepec dam, Cytotype II; SP = San Pedro Lagunillas lagoon; DN = Diploid number; FN = Fundamental number; CF = Chromosome formulae; M = Metacentric; Sm = submetacentric; St = subtelocentric; T = telocentric; ♀ = Female; ♂ = male.

Victoria dams and San Pedro Lagunillas lagoon, and two cytotypes in Tepuxtepec dam (Tables 2, 3, and Fig. 2).

No pairs of heteromorphic chromosomes that could correspond to sex chromosomes were identified in any of the studied populations.

All chromosome pairs were ordered by size, from the largest to the smallest (Table 4 and Fig. 2).

Chromosome pairs 1, 2, 4, and 5 were the most variable in morphology. Pair 1 was submetacentric and subtelocentric for the cytotype-

Table 4. Relative chromosome length averages of the cytotypes of *Chirostoma humboldtianum*.

Chromosome Pair	T	VV	Tx CI	Tx CII	SP
1	8.24 ±2.673	9.16 ±3.490	7.10 ±1.145	7.35 ±1.383	6.82 ±1.938
2	7.20 ±1.628	7.01 ±2.952	6.62 ±1.083	5.76 ±1.449	5.53 ±2.249
3	6.79 ±4.071	6.73 ±0.552	6.15 ±0.849	5.75 ±0.873	5.50 ±1.007
4	6.62 ±2.071	6.56 ±4.324	6.15 ±1.501	5.02 ±0.702	5.42 ±0.469
5	6.27 ±1.071	5.06 ±0.143	6.13 ±0.532	4.66 ±0.623	5.21 ±1.671
6	6.02 ±2.584	5.05 ±0.566	4.51 ±0.695	4.65 ±0.893	5.10 ±0.679
7	5.99 ±1.206	4.42 ±0.525	4.49 ±0.569	4.37 ±0.701	5.10 ±0.211
8	5.24 ±1.490	4.41 ±0.659	4.45 ±0.657	4.50 ±0.881	4.83 ±0.720
9	4.26 ±±1.73	4.29 ±1.759	4.06 ±1.115	4.22 ±0.508	4.82 ±0.886
10	4.22 ±0.898	3.81 ±2.569	3.88 ±0.984	4.13 ±0.443	4.74 ±0.301
11	3.76 ±1.095	3.77 ±1.385	3.75 ±0.465	4.01 ±0.719	4.40 ±1.198
12	3.45 ±0.615	3.65 ±1.446	3.74 ±0.626	4.01 ±0.409	4.39 ±0.952
13	3.18 ±1.584	3.37 ±2.174	3.73 ±0.920	3.98 ±0.858	4.38 ±1.183
14	2.82 ±0.571	3.34 ±0.627	3.72 ±0.398	3.84 ±0.600	4.10 ±1.233
15	2.82 ±0.880	3.33 ±1.072	3.59 ±0.436	3.73 ±0.789	3.90 ±0.884
16	2.79 ±0.692	3.29 ±1.421	3.53 ±0.272	3.72 ±0.826	3.85 ±0.008
17	2.79 ±0.243	3.29 ±0.329	3.53 ±0.277	3.53 ±0.312	3.61 ±0.008
18	2.74 ±0.645	3.28 ±0.795	3.23 ±0.393	3.52 ±0.847	3.60 ±0.160
19	2.59 ±1.125	3.04 ±1.065	3.15 ±0.637	3.43 ±0.911	3.10 ±2.131
20	2.56 ±0.626	2.96 ±0.627	3.10 ±0.612	3.39 ±1.021	2.91 ±1.056
21	2.53 ±0.800	2.94 ±0.444	2.97 ±0.437	3.38 ±0.686	2.31 ±0.776
22	2.43 ±0.509	2.54 ±1.620	2.93 ±0.544	3.16 ±0.868	2.10 ±0.845
23	2.37 ±0.455	2.43 ±0.803	2.89 ±0.469	3.01 ±0.541	2.09 ±0.752
24	2.33 ±0.848	2.26 ±0.131	2.60 ±0.664	2.90 ±0.540	1.98 ±0.735

Las Tazas Tiacaque dam (T), Villa Victoria dam (VV), Tepuxtepec dam, Cytotype I (TxCI), Tepuxtepec dam, Cytotype II (TxCII), San Pedro Lagunillas lagoon (SP).



pes from Tepuxtepec. Pair 2 was metacentric in both cytotypes from Tepuxtepec dam and submetacentric in the cytotypes from Las Tazas, Tiacaque, and Villa Victoria dams and San Pedro Lagunillas lagoon. Pairs 4 and 5 were metacentric in Las Tazas, Tiacaque, and Tepuxtepec dams and San Pedro Lagunillas lagoon, and acrocentric in Villa Victoria dam. However, their relative lengths did not show differences ( $P = 0.591, 0.9079, 0.591, \text{ and } 0.6099$ , for pairs 1, 2, 4, & 5, respectively).

The constructed dendrogram using 33 chromosome characters allowed the identification of three groups. Based on their similarity, Las Tazas, Tiacaque, and Villa Victoria dams made up group I. The cytotypes from Tepuxtepec dam and San Pedro Lagunillas lagoon were placed in group II (Fig. 3). Geographically, group I corresponds to individuals from the high Lerma-Chapala-Santiago basin, and group II to individuals from the middle and lower basin. The SIMPER analysis identified the population that contributed most strongly to the configuration of groups. Average dissimilarity for both groups was 35%. The contribution of dissimilarity of the populations of *C. humboldtianum* from Villa Victoria dam (35.42%) and San Pedro Lagunillas (35.46%) are close to the overall average dissimilarity, thus, these populations appear to contribute the most to the conformation of Groups I and II.

## DISCUSSION

The four populations of *C. humboldtianum* analyzed in this study showed a diploid number ( $2n = 48$ ), which concurs with the most frequent diploid number found in Atheriniformes (Atherinopsidae, in *Labidesthes*, *Membras* and *Menidia*, Jeffrey & Fitzsimons, 1987; Warkentine *et al.*, 1987; *Odontesthes*, Sola *et al.*, 1988; *Basilichthys*, Gajardo, 1992; and *Atherinella*, Da Silva Cortinhas *et al.*, 2003; Sczepanski *et al.*, 2007). It

is also considered to be the ancestral number for several freshwater teleost fish (i.e., salmonids, Amaro *et al.*, 1996; cichlids, Thompson, 1979; Arias-Rodríguez *et al.*, 2006) and even marine fishes (Galetti *et al.*, 2000). Overall, within the family, the diploid number  $2n = 48$  is a conserved characteristic, except for *C. patzcuaro* ( $2n = 44$ ) (Uribe-Alcocer *et al.*, 2002; Uribe-Alcocer & Díaz-Jaimes, 2003).

On the other hand, the fundamental number (FN) in *Chirostoma humboldtianum* populations was variable (FN = 54, 58 and 116). Variation in FN can be explained either by the occurrence of chromosomal rearrangements consistent with pericentric inversions and/or heterochromatin additions (Jackson, 1971; Thitiot-Quévieux, 1994; Appels *et al.*, 1998; Sobti *et al.*, 2002). These rearrangements can affect only the fundamental number, but not the diploid number (Appels *et al.*, 1998; Sobti *et al.*, 2002). These patterns are common in fish and play a significant role in fish chromosomal evolution (i.e., Cyprinidae, Uyeno & Miller, 1973; Cichlidae, Thompson, 1979; Atherinopsidae, Jeffrey & Fitzsimons, 1987; Uribe-Alcocer *et al.*, 2002; Muñoz *et al.*, 2006; Carangidae, Lobotidae and Sciaenidae, Tripathy & Das, 1988; and Batrachoididae, Merlo *et al.*, 2005). Particularly within Atheriniformes the FN is very variable, with chromosome arms recorded from 44 to 86, and such variation has been related to pericentric inversion rearrangements (Jeffrey & Fitzsimons, 1987; Warkentine *et al.*, 1987; Sola *et al.*, 1988; Gajardo, 1992; Uribe-Alcocer *et al.*, 2002; Da Silva Cortinhas *et al.*, 2003; Sczepanski *et al.*, 2007).

Although morphological variation was detected in chromosomes pairs 1, 2, 4, and 5 of *Chirostoma humboldtianum*, non-significant differences were observed when their relative lengths were compared; hence we explain the changes in the morphology of these pairs by

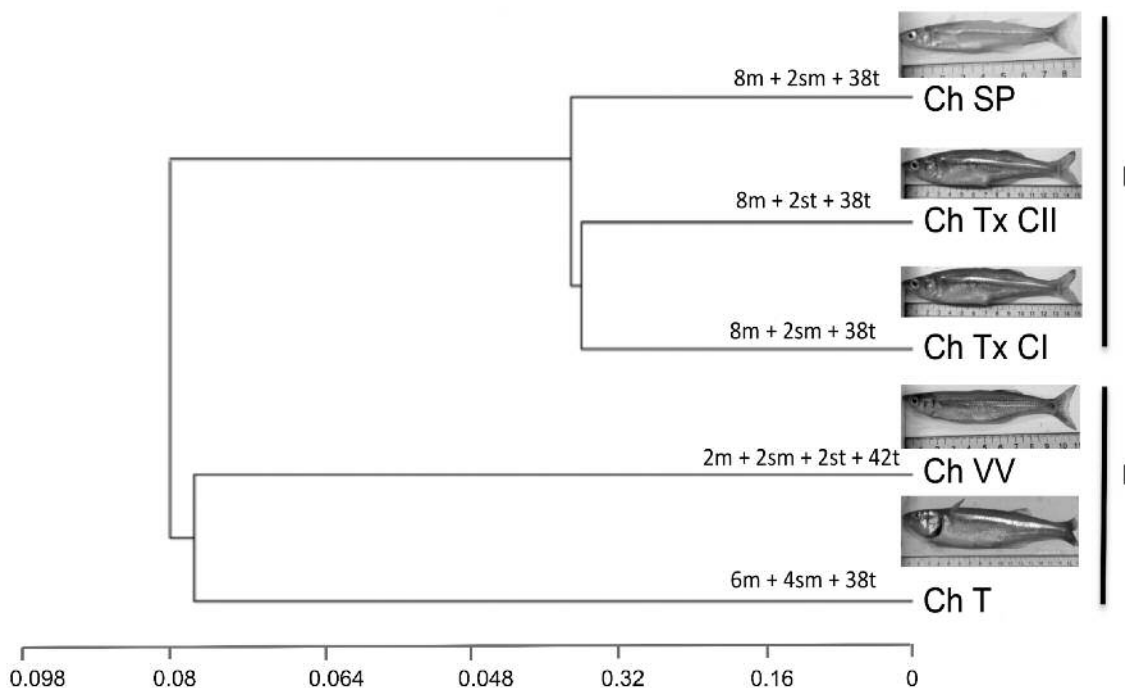


Figure 3. Dendrogram based on chromosomal characters of the five cytotypes for *C. humboldtianum* (FN, morphology of the pairs 1, 2, 4 and 5; and relative length of all chromosome pairs) and using Bray-Curtis distances. The chromosome formulae resolved for each cytotype are shown.

rearrangements of the pericentric-inversion type rather than by heterochromatin addition.

Considering that pericentric inversions are the most frequent rearrangements detected on the resolved karyotype variation of *C. humboldtianum*, then the hypothesis of a process of orthoselection, in which only one type of chromosome rearrangement occurs repeatedly within a species (White, 1973, 1978, & 1978a), could be proposed as the predicted mutation model for the species. Although karyotype analysis with a larger sample size is suggested to estimate chromosome mutation rate (King, 1993), the presence of at least two different cytotypes in the population in the Tepuxtepec dam population allows us to support the proposed hypothesis.

The arrangement observed in the inferred dendrogram, conformed by two resolved groups, supported by the SIMPER average dissimilarity concurs with the preliminary layout of disjunct distribution of the species, where each population possesses a different cytotype, indicating geographic isolation and a process where intraspecific karyotype diversification might promote allopatric speciation (Key 1968, *vide in* King 1993). This proposal is supported by intraspecific variation previously reported for the species at the morphological, meristic, and allozyme level, which were also explained by the geographic isolation of the studied populations (Barriga-Sosa 2001; Barriga-Sosa *et al.*, 2002), and more recently with sequences of the mitochondrial genome by García-Martínez *et al.* (2015), where a high degree of genetic divergence between the studied populations was confirmed.

The results presented here allow us to suggest that the cytotype from the Villa Victoria dam could represent the most primitive karyotype described so far for *Chirostoma humboldtianum* and for the genus; this is so basically because it contains the largest number of single-armed chromosomes, it coincides with the diploid number of 48, also considered the primitive teleost karyotype in about 200 fish species (Nayyar, 1966; Gyldenholm & Scheel, 1971; Thompson, 1979; Rab *et al.*, 1983; Feldberg & Bertollo, 1985; Salas & Boza, 1991; Martins *et al.*, 1995; Uribe-Alcocer *et al.*, 1992, 1999; Arias-Rodríguez *et al.*, 2006), and is supported by the relative contribution of this population to the SIMPER average dissimilarity.

With respect to the polyploid organism that we found at the Tepuxtepec dam, we explain its presence by a possible autopolyploid mechanism, according to Leggett & Iwama (2003) and Vasil'ev (2009), where the entire chromosomal complement could be derived from only one parental species. In this species, the fertilization of the haploid egg occurs by haploid sperm with subsequent errors in meiotic reduction, due to abrupt changes in experimental conditions, such as temperature or exposure to high hydrostatic pressure (Arai, 2001), which in turn, leads to spontaneous autotetraploids, that are not morphologically different from their diploid counterparts. The tetraploid specimen described in the present study was a male and showed no apparent morphological differences to their diploid counterparts from the same population (Table 1). Spontaneous autopolyploids have been reported in a closely related silversides, *Menidia sp.*, *Odontesthes x Patagonina* (Schultz, 1980; Strüssmann *et al.*, 1993, 1997), and in other teleost fish, (i.e., cyprinids, Machordom & Doadrio, 2001; Leggett & Iwama, 2003; Steven & Smith, 2004; and acipenserids, Schreier *et al.*, 2013); however, in order to test this hypothesis, further studies are required (i.e., crosses between populations and/or polyploid induction).

In summary, the most common diploid number in the genus *Chirostoma* is  $2n = 48$ . The intraspecific karyotype variation encountered in *C. humboldtianum* was caused by rearrangements of the pericentric-type inversions and could be associated to its disjunct distribution. The chromosome changes observed within the species could respond to a process of karyotype orthoselection. The tetraploid organism encountered in Tepuxtepec is likely to have resulted from an autopolyploid mechanism.

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