

Chromosomes of *Cichlasoma istlanum* (Perciformes: Cichlidae) and karyotype comparison of two presumed subspecies

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Abstract: *Cichlasoma istlanum* (Jordan & Snyder, 1900) is a freshwater cichlid from the Balsas river province in the Pacific Basin. Two subspecies: *C. istlana istlana*, from the Ixtla river in the state of Morelos, Mexico, and *C. istlana fusca*, from the Huámito river near the town of La Huacana, in the state of Michoacán, were named half a century ago on the basis of meristic characters. In this work, the karyotype of the species was established by conventional and G-banding cytogenetic procedures and a comparative analysis of karyotypes from the two populations, previously proposed as subspecies, was performed. Ten females were collected in the Amacuzac river, and nine specimens (two females), were collected in the Huámito river. Based on the count of 264 mitotic fields of the former and 203 of the latter, the modal number of $2n=48$ was established in every sample and considered as the diploid number of both populations. The karyotype analysis was based on ten karyotypes prepared from Morelos and eight from Michoacán, which included three from females and five from males. The chromosome formula thus established was of $8sm+40st$. The G-banding pattern was similar in both populations and the comparison of the mean lengths of the chromosome pairs did not reveal statistically significant differences between both populations. The presence of a practically identical karyotype does not support the subspecific division. The morphometric analyses made by other authors, which detected overlapping in the characters that were proposed as distinctive of the two subspecies, agree with the results of this study: not enough divergence has been found to substantiate the subdivision of *C. istlanum*. The lack of population divergence might have been brought about by an abated geographic isolation caused by gene flow among contiguous populations along their continuous distribution in the Balsas Basin regions, by the relatively small divergence time since their distribution in these regions, and/or, less probably, by a recent historical replacement of one population by the other. The absence of karyotype differences might also be attributed to characteristics inherent to the genome organization in the genus *Cichlasoma* still to be identified and understood.

Key words: *Cichlasoma istlanum*, cytogenetics, chromosomes, G-bands, Cichlidae, Balsas River.

The family Cichlidae is represented in Mexico by the two autochthonous genera: *Petenia* Günther 1862 and *Cichlasoma* Swainson 1839, and by the introduced *Tilapia* Smith 1840 and *Oreochromis* Günther 1889.

The genus *Cichlasoma* is found only in the New World where it comprises approximately 49 species, whereas the genus *Petenia* includes only one (Nelson 1994). These fishes have been the subject of several regional studies

dealing with their taxonomy (Vega-Bravo 1973, Arredondo-Figueroa & Guzmán-Arroyo 1986, Mejía-Mójica 1997), genetics (Uribe-Alcocer *et al.* 1992), feeding habits (Trujillo-Jiménez 1995), ecology (Sánchez-Salazar 1984), reproduction and growth (Martínez-Palacios & Ross 1992, Contreras-McBeath 1997, Cruz-Gómez & Rodríguez-Varela 1997, Luna-Figueroa & Figueroa-Torres 1997), parasitism (Lamothe-Argumedo & Aguirre-Macedo 1991, García 1993), and potential for aquaculture (Martínez-Palacios *et al.* 1993).

Cichlasoma istlanum (Jordan & Snyder, 1900) is a freshwater cichlid from the Balsas River province in the Pacific Basin. It has been collected from the high river sources to the mouth river. Its distribution area includes also the small basins of the Armería river in the states of Colima and Jalisco, and some other small basins of the state of Guerrero, such as those of the Nexapa, Papagayo, and Coyuca rivers (De Buen 1946, Miller 1966, Alvarez del Villar 1970, 1972a, b, 1977). Recent reports have shown its drastic displacement by the exotic cichlid *C. nigrofasciatum* (Contreras-McBeath 1997).

De Buen (1946) based on differences of the fin-rays formulas and on the number of spines divided the species *C. istlanum* into two subspecies: *C. istlana istlana*, from the Ixtla river in the state of Morelos, and *C. istlana fusca*, from the Huámito river near the town of La Huacana, in the state of Michoacán.

The karyotype might characterize a species through the determination of the number, size and structure of its chromosomes. Although in vertebrates it constitutes a particularly stable feature because it does not seem to be subject to direct selective pressures, chromosome variability has been shown in some species through the detection of polymorphisms in the chromosome diploid number, like in *Noturus albatro* Taylor 1969 (LeGrande 1981), *Gymnotus carapo* L. 1758 (Foresti *et al.* 1984), *Gobius niger* L. 1758 (Vitturi & Catalano 1989), *Gobius paganellus* L. 1758 (Amores *et al.* 1990) and in the cichlid *C. friedrichsthalii* (Heckel, 1840) (Salas &

Boza 1991) or by the presence of supernumerary chromosomes, like in *Prochilodus scrofa* Steindachner 1881 (Pauls & Bertollo 1983) and in the cichlid *Gymnogeophagus balzanii* (Perugia 1891) (Feldberg & Bertollo 1984). These antecedents justify the search of chromosome differences that might be coincident with the spatial separation of the subspecies proposed by De Buen (1946).

The purpose of this study was to establish the karyotype and the G-banding pattern of *C. istlanum*, obtained from organisms of the same regions where De Buen (1946) collected the samples that led him to establish the subspecific division of the species, and to compare the chromosome diploid number, the morphology, relative size and banding patterns of the chromosomes from both samples, in order to provide arguments to verify or deny the subdivision of the species.

MATERIALS AND METHODS

Ten females were collected in the Amacuzac river, near the town of La Fundición, in the state of Morelos, Mexico, (18.52° N and 99.22° W) and nine organisms: two females and seven males in the Huámito river near the town of La Huacana, in the state of Michoacán (18.95° N and 101.87° W). These sample sizes fall within the range used in several cytogenetic studies, due to the karyotype stability generally found.

The cytogenetic methods followed to obtain the mitotic fields have been described by Uribe-Alcocer *et al.* (1983) and Maldonado-Monroy *et al.* (1985). Briefly, they consist of a pretreatment with calcium chloride and colchicine, gentle scraping of the gill epithelium followed by a hypotonic shock, fixation in a methanol-acetic acid solution, air slide drying and Giemsa staining. The method of Levan *et al.* (1964), based on the centromere position, assessed in this work through the determination of the centromeric index (short arm length (p)/total chromosome length (p+q)), was followed to classify chromosomes. The G-banding

patterns were obtained by incubation of the slides in 2xSSC at 60° C for 2 hr, followed with digestion with a mixture of trypsin 0.03% and Giemsa stain 3.3% in buffer phosphate pH 7.2 (Ruiz-Carús, 1995).

RESULTS

Chromosomes of 467 mitotic fields, derived from both populations, were counted to identify the modal number, 48, which was considered as the diploid number of the species. Numbers under the mode were considered as chromosome losses during the cytogenetic technique rather than aneuploidies, due to the decreasing frequencies of chromosome numbers below the modal diploid num-

ber in all specimens studied and to the lack of high frequencies indicative of a different chromosome number in any particular specimen.

Eighteen mitotic fields were studied to determine the karyotype of the species. In both samples it was composed by four submetacentrics pairs (sm), and 20 subtelo-acrocentric (stt) chromosome pairs. The chromosome formula derived from both samples was $8sm+40stt$. The fundamental number found (number of chromosome arms: two for submetacentrics and one for subtelo-centric and acrocentric chromosomes) was 56. Figs. 1 and 2 show the chromosome groups as well as their G-banding pattern. A digitally composed karyotype, which shows an element of each population for every chromosome pair, is depicted in Fig. 3.

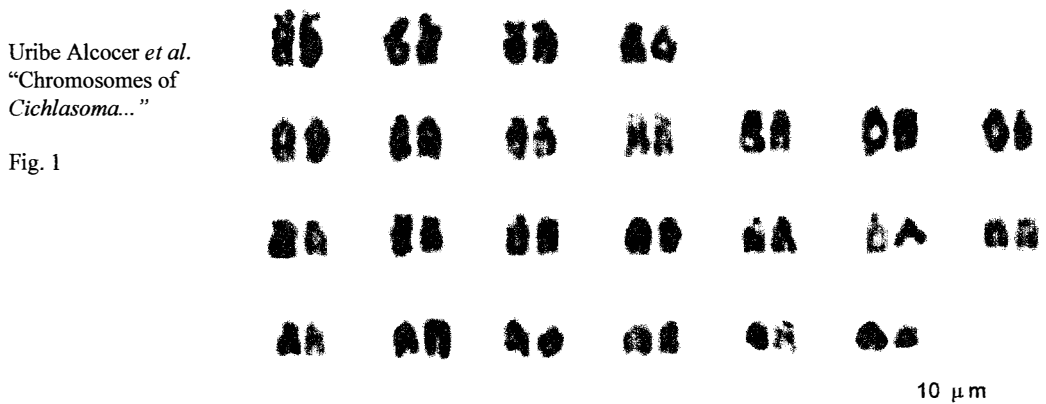


Fig. 1. G-banded karyotype of *C. istlanum* from Morelos sample. The upper row shows the submetacentric chromosomes, other rows, subtelo-centric and acrocentric chromosomes.

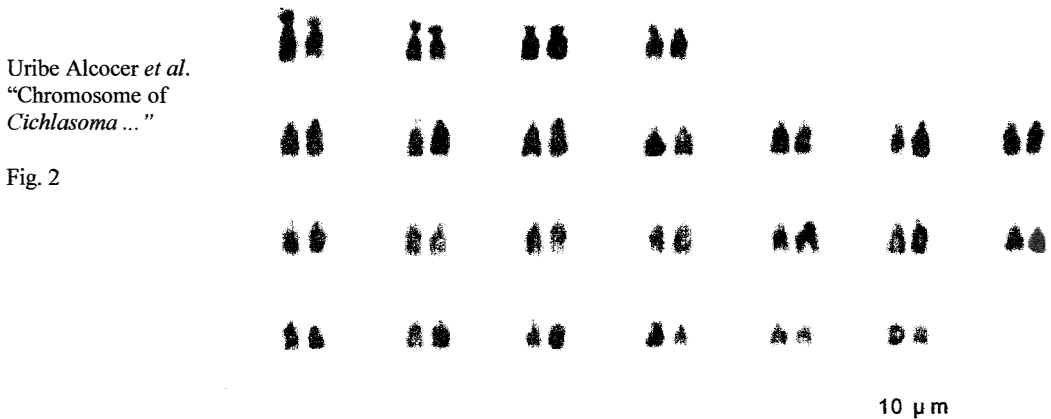


Fig. 2. G-banded karyotype of *C. istlanum* from the sample of Michoacán.

Uribe Alcocer *et al*
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Fig. 3

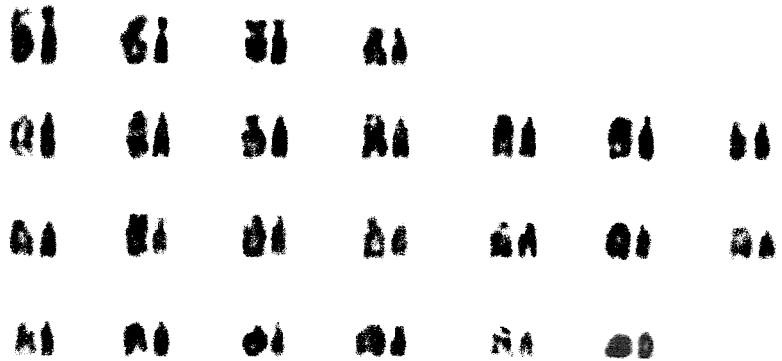


Fig. 3. Digitally composed karyotype of *C. istlanum*, which shows at left and at right of every chromosome pair an element of the Morelos and of the Michoacán populations respectively. The chromosomes of the latter have been artificially enlarged to the approximate same size of its counterpart to emphasize the correspondence of the G-bands.

Mean relative length of the chromosome pairs corresponding to both samples and their standard deviations are shown in Table 1. They were derived from the measurements of ten karyotypes from the sample of the state of Morelos and eight from the state of Michoacán. No dependence among the chromosome diploid number counts and the specimens analyzed nor the collecting sites was found (Contingency table $p > 0.05$). Data corresponding to chromosome and arm lengths were assayed for goodness of fit to a normal distribution and no statistical differences were found (Shapiro Wilk W

$p > 0.05$). Similarly no differences were found between the natural logarithms of the standardized whole length chromosome ($p+q$) of every pair, nor between the short (p) and long arms (q) of the four banded chromosome pairs of both populations (Two-way ANOVA $p > 0.05$).

Consistently with the lack of reports on sex chromosomes in all other cichlid species studied, heteromorphic chromosome elements, indicative of differentiated sex-chromosomes, were not detected in the sample of the Huámto river, the only site in which both sexes were represented.

TABLE 1

Mean relative lengths of the chromosome pairs of the karyotypes of *C. istlanum* from the Amacuzac river (Morelos) and the Huámto river (Michoacán)

Chromosome pairs	Morelos population		Michoacan population		Chromosome classification (2)
	Relative±standard length deviation	Centromeric index ₍₁₎	Relative±standard length deviation	Centromeric index ₍₁₎	
1	58.12 ± 4.62	26.22	54.60 ± 5.30	28.48	sm
2	50.70 ± 2.16	27.21	50.21 ± 4.58	28.28	sm
3	46.49 ± 2.16	28.17	46.81 ± 5.54	27.44	sm

Continúa...

...Continue table 1

Chromosome pairs	Morelos population		Michoacan population		Chromosome classification (2)
	Relative±standard length deviation	Centromeric index ₍₁₎	Relative±standard length deviation	Centromeric index ₍₁₎	
4	42.77 ± 1.51	29.28	40.10 ± 5.11	30.49	sm
5	51.91 ± 4.88		54.92 ± 3.95		stt
6	47.80 ± 2.19		47.76 ± 2.78		stt
7	47.82 ± 2.83		47.59 ± 1.98		stt
8	45.83 ± 1.70		45.66 ± 2.57		stt
9	44.92 ± 2.93		46.94 ± 2.12		stt
10	44.25 ± 1.45		45.19 ± 2.82		stt
11	43.40 ± 2.01		44.25 ± 2.66		stt
12	43.14 ± 1.65		43.26 ± 1.77		stt
13	41.58 ± 1.42		42.02 ± 2.07		stt
14	41.13 ± 1.53		40.74 ± 2.11		stt
15	40.38 ± 1.12		40.14 ± 2.16		stt
16	39.43 ± 1.29		39.14 ± 2.51		stt
17	38.64 ± 1.03		37.98 ± 2.47		stt
18	37.78 ± 1.31		37.05 ± 2.39		stt
19	37.00 ± 1.74		36.62 ± 2.39		stt
20	36.42 ± 1.71		35.27 ± 1.94		stt
21	33.76 ± 0.67		34.06 ± 2.28		stt
22	32.04 ± 1.82		32.48 ± 3.10		stt
23	28.91 ± 1.81		29.74 ± 2.38		stt
24	25.76 ± 3.09		27.48 ± 1.74		stt

(1) CI = Centromeric index = length of the short arm/total length

(2) according to Levan *et al.* (1964)

DISCUSSION

The karyotypes of the *C. istlanum* populations of the rivers Amacuzac (Morelos) and Huámico (Michoacán) have a common complement of $2n = 48$. The assumption that no chromosomal change has occurred is supported by the coincidence in the number of submetacentric and subtelo-acrocentric chromosomes and in the centromere position of the submetacentrics, by the lack of significant differences in chromosome lengths, as well as by an overall similarity of their G-banding patterns. Alternatively, it could also be considered that actual modifications could be too small to be detected by the methodology followed in this work. The assumption of populational chromosome divergence between the populations would be based on frail evidence.

G-band studies in fishes have been limited (Hartley & Thorne 1985, Gold *et al.* 1990) because, for reasons probably related to intrinsic chromosome structure or to their size, do not show the abundance nor the resolution found in other animal groups, like mammals, particularly when the chromosome material has been obtained through direct techniques, but for a few exceptional cases (e.g. Sola *et al.* 1984). In this study, the agreement in the G-banding patterns of both populations supports the lack of chromosome diversification and, hence, of population divergence.

The morphometric analyses performed by Bonilla-Ruz (1982) and by Sánchez-Salazar (1984) detected overlapping in the characters that, according to De Buen (1946), were distinctive of the two proposed subspecies. These analyses agree with the results of this study in

that not enough differences were found to substantiate the subdivision of *C. istlanum*. The absence of population diversification, as estimated through chromosome and morphometric analyses, might have been brought about by an abated geographic isolation due to gene flow among contiguous populations along their continuous distribution in the Balsas Basin regions, by the relatively small divergence time since their distribution in these regions and/or, less probably, by a recent historical replacement of one population by the other, event of which there is no evidence.

The number of banded chromosomes of the *Cichlasoma* species has been related to their presumed geographic radiation center: the species considered to be of Central American origin, such as *C. istlanum*, *C. beani* (Jordan, 1888), *C. labridens* (Pellegrin, 1903) and *C. managuense* (Günther, 1862) (Regan, 1906-1908) (Thompson 1979, Salas & Boza 1991) have a smaller number of metacentric chromosomes than those of South American affinity, which show more metacentrics, except for some species, such as *C. bimaculatum* (L. 1758) and *C. severum* (Heckel, 1840), which show a smaller number, mainly through robertsonian rearrangements and pericentric inversions (Thompson 1979).

The diploid chromosome number of *C. istlanum* ($2n = 48$) agrees with that of practically all the *Cichlasoma* species studied. According to the classification proposed by Thompson (1979), based on the number of banded chromosomes, the karyotype of *C. istlanum* is type "A" since it has less than five meta-submetacentric chromosomes, differing from the karyotypes "B" that have five or more meta-submetacentric chromosome pairs. The common occurrence of karyotype "A" in some species is no evidence of a close phylogenetic relationship among them because, although they share this karyotype configuration, they belong to different species groups of the genus *Cichlasoma* (Regan 1905, Miller & Nelson 1961, Miller, 1966). If the classification method of Levan *et al.* (1964) is followed, the karyotype of *C. istlanum* is coincident with

those of *C. dovii* (Günther, 1864) (Thompson, 1979, Salas & Boza, 1991), *C. citrinellum* (Günther, 1864), *C. festivum* (Heckel, 1840) (Thompson 1979), *C. trimaculatum* (Günther, 1866) (Uribe-Alcocer *et al.* 1992), *C. managuense* (Salas & Boza 1991) and *C. facetum* (Jenyns, 1842) (Feldberg & Bertollo 1985). Furthermore, these species not only belong to different species groups, but some of them have also been considered to belong to the South American groups while others have been considered to have Central American affinities.

Kornfield *et al.* (1979), Thompson (1979), Vervoort (1980) and Uribe-Alcocer & Arre-guín-Espinosa (1989) have pointed out that some cichlid species, close or distant phylogenetically, do not show large karyotype differences. Existent variability might be due to the acquisition or loss of small chromosome fragments, to non-reciprocal translocations, to pericentric inversions, and to tandem duplications or differential crossing-overs (Uribe-Alcocer *et al.* 1992). It is not likely that these rearrangements have been relevant to their evolution, since changes in the structure or size of chromosomes have not modified the diploid nor the fundamental chromosome numbers of their populations and, for this reason, their reproductive isolation has been probably brought about by other means. Notwithstanding some reports of chromosome variability in cichlid species (Feldberg & Bertollo 1984, Salas & Boza 1991), which justified the search undertaken in this study, the karyotype in this genus seems to be a conservative feature of low modification rate that apparently accompanies the evolution of the species, but does not bring about their reproductive isolation, nor the evolution of other biological characters. The widespread absence of significant karyotype differences in the genus *Cichlasoma* might also be attributed to characteristics inherent to the genome organization still to be identified and understood.

Genetic studies of the neotropical cichlids by methodologies that allow the detection of existing variations, for instance in their allozymes, their DNA (Sültmann *et al.* 1995),

or in their microsatellite markers (Lee & Kocher 1996), are considered necessary to establish, confirm or modify our knowledge of the phylletic relationships of this important group of neotropical cichlids.

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RESUMEN

Cichlasoma istlanum (Jordan & Snyder, 1900) es un cíclido dulceacuícola que se encuentra en la provincia del Río Balsas en la Cuenca del Pacífico mexicano. De Buen (1946), basado en caracteres merísticos de la especie, propuso la división en dos subespecies: *C. istlana istlana*, procedente del Río Ixtla, en el estado de Morelos, y *C. istlana fusca*, del Río Huámito, Michoacán. En este trabajo se estableció el cariotipo de la especie por medio de procedimientos citogenéticos convencionales y de bandejo G, y se realizó un análisis comparativo de los cariotipos provenientes de las muestras de las dos poblaciones previamente propuestas como subespecies. Se recolectaron diez hembras en el Río Amacuzac, en el estado de Morelos, y nueve especímenes en el Río Huámito: dos hembras y siete machos. Mediante la cuenta de 264 campos mitóticos de la primera muestra y 203 de la segunda, se pudo establecer un número modal de $2n=48$ en cada una, moda que se consideró correspondía al número diploide de la especie. El análisis cariotípico se basó en diez cariogramas preparados de la población de Morelos y de ocho de la de Michoacán, que incluyó tres provenientes de hembras y cinco de machos. La fórmula cromosómica encontrada fue de $8sm+40stt$. El patrón de bandas G de ambas poblaciones fue similar y las comparaciones estadísticas de las longitudes promedio de los pares cromosómicos no mostraron diferencias significativas concluyentes entre ambas poblaciones. La existencia de un cariotipo prácticamente idéntico concuerda con la falta de diversificación subespecífica entre las poblaciones estudiadas. Los análisis morfométricos realizados por otros autores que encontraron sobreposición en los caracteres que fueron propuestos como distintivos de las dos subespecies, concuerdan con los resultados del presente estudio en que no se detectó divergencia suficiente para fundamentar la subdivisión de

C. istlanum. La falta de divergencia poblacional encontrada puede deberse a un aislamiento geográfico disminuido por la existencia de flujo genético entre poblaciones contiguas a lo largo de su distribución en las diferentes regiones del Río Balsas, por un lapso de divergencia evolutiva relativamente corto a partir de su distribución en estas regiones y/o, con menor probabilidad, por un reemplazo de una población por la otra. La estabilidad carotípica encontrada puede ser debida también a características, aun por identificarse y definirse, inherentes a la organización del genoma en este género.

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