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# Chromosome evolution in fishes: a new challenging proposal from Neotropical species

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We present a database containing cytogenetic data of Neotropical actinopterygian fishes from Venezuela obtained in a single laboratory for the first time. The results of this study include 103 species belonging to 74 genera assigned to 45 families and 17 out of the 40 teleost orders. In the group of marine fishes, the modal diploid number was 2n=48 represented in 60% of the studied species, while in the freshwater fish group the modal diploid complement was 2n=54, represented in 21.21 % of the studied species. The average number of chromosomes and the mean FN were statistically higher in freshwater fish than in marine fish. The degree of diversification and karyotype variation was also higher in freshwater fish in contrast to a more conserved cytogenetic pattern in marine fish. In contrast to the assumption according to which 48 acrocentric chromosomes was basal chromosome number in fish, data here presented show that there is an obvious trend towards the reduction of the diploid number of chromosomes from values near 2n=60 with high number of biarmed chromosomes in more basal species to 2n=48 acrocentric elements in more derived Actinopterygii.

Se presenta una base de datos que contiene los datos citogenéticos de peces Actinopterigios Neotropicales de Venezuela obtenidos por primera vez en un solo laboratorio. Los resultados de este estudio incluyen 103 especies pertenecientes a 74 géneros de 45 familias contenidas en 17 de los 40 órdenes de teleósteos. En el grupo de peces marinos, el número diploide modal fue 2n=48 representado en 60% de las especies estudiadas, mientras que en el grupo de peces de agua dulce el complemento diploide modal fue 2n=54, representado en el 21,21% de las especies estudiadas. El número de cromosomas y FN promedio fueron estadísticamente superiores en peces dulceacuícolas. El grado de diversificación y variación en el cariotipo también fue mayor en peces de agua dulce en contraste con un patrón citogenético más conservado en peces marinos. En contraposición a la suposición según la cual 48 cromosomas acrocentricos era el número cromosómico basal en los peces, los datos aquí presentados muestran que existe una evidente tendencia hacia la reducción del número de cromosomas desde valores cercanos a 2n=60 con alto número de cromosomas birrámeos en las especies más basales a 2n=48 elementos acrocéntricos en los actinopterigios más derivados.

**Key words:** Actinopterygii, Ancestral karyotype, Diploid number, Fundamental number.

## Introduction

Fishes represent more than half of all extant vertebrates with more than 32,000 recognized species (Eschmeyer *et al.*, 2014), and are characterized by different morphology, behavior, and habitat (Nelson, 2006). The study of chromosomes in these animals has been expanding significantly thanks to the development of refined techniques of cell and tissue culture originally developed for mammals, but later adapted to the fish physiology (Clem *et al.*, 1961; Booke, 1968; Wolf & Quimby, 1969; Denton, 1973) and the development of less expensive *in vivo* direct methods (Osuf-Costaz & Foresti, 1992). Because of the basal position that ray-finned fishes occupy in the phylogeny of vertebrates, studies on chromosomes of this group have provided valuable information for the understanding of different aspects such as mechanisms of sex determination and evolution of sex chromosomes, distribution of the nucleolus organizers regions (NOR), existence of supernumerary chromosomes and the role of polyploidy in evolution (Pisano *et al.*, 2007).

Data on fish chromosomes are reported in a wide range of sources such as journals, proceedings and reports; however,

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these sources are often on local species not readily available for users, making it difficult for scientists or students to obtain general but detailed information on the cytogenetic characteristics of fishes, useful for comparative, evolutionary, and phylogenetic studies. Several checklists on cytogenetic data have been published (Denton, 1973; Park, 1974; Ojima *et al.*, 1976; Vasiliev, 1985; Sola *et al.*, 1981; Oliveira *et al.*, 1988; Porto *et al.*, 1992; Klinkhardt *et al.*, 1995). The most recent one (Arai, 2011) cover about 3,425 species/subspecies of agnathans, cartilaginous fish, actinopterygians (ray-finned fish) and sarcopterygians (lobe-finned fish), which represent barely 10.7% of recognized species at global level. The main problem with the available checklists is that source data were obtained by a large number of different researchers, working with different methodologies (different antimitotic concentration and time of exposure) and with different standard as reference (different criteria of chromosome classification and fundamental number, FN, calculation), generating data that are difficult to compare. Indeed, it has been reported that chromosome size may be poorly evaluated due to the selection made by individual researcher, as observed in plants (Guerra, 2012). Furthermore, data for many important fish groups are still lacking in fish lists. Thus, although the general data are very important for our knowledge of fish cytogenetics, the analyses and interpretations on chromosome evolution in the ray finned fish are actually limited by the problems referred to above.

Venezuela is ranked in the top 10 countries with the greatest biodiversity on the planet, both in aquatic and terrestrial environments (Miloslavich, 2003). The presence of a shoreline that stretches along the Caribbean Sea and Atlantic Ocean (*ca.* 3,964 Km), which includes several distinct aquatic environments (sand, rocky littorals, seagrass meadows, coral reefs, communities of soft bottom, and mangrove forests), and tracts of continental water also with a variety of environmental conditions (rivers, coastal lagoons, marshes, lakes, and reservoirs), explains the great fish diversity. Fishbase registers at least 941 freshwater and 807 marine species in Venezuela (Froese & Pauly, 2013).

In order to reach a better understanding of chromosome evolution in actinopterygian fishes, we karyotyped 103 Venezuelan species belonging to a wide range of evolutionary lineages (Table 1) with the same methodology and trying to cover a wide variety of freshwater and marine species.

## Material and Methods

Fishes of 103 species of different orders were captured with diverse fishing methods (hook, snorkeling, cast nets, bag nets, seine nets, arrowhead fish-trap) and transported alive to laboratory where they were analyzed. Catalog numbers of the voucher specimens of species whose results are firstly presented here are given in Table 2 (LBP, Laboratório de Biologia e Genética de Peixes, UNESP, Botucatu, Brazil; ECAM, Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente, Núcleo Nueva Esparta, Venezuela).

**Table 1.** Number of analyzed freshwater and marine Actinopterygii. Classification followed Eschmeyer & Fong (2014).

| Order | Families | Genera | Species | Marine | Freshwater |
|---|---|---|---|---|---|
| Anguilliformes | 1 | 1 | 2 | 2 | 0 |
| Aulopiformes | 1 | 1 | 1 | 1 | 0 |
| Batrachoidiformes | 1 | 4 | 4 | 4 | 0 |
| Beloniformes | 2 | 3 | 4 | 4 | 0 |
| Beryciformes | 1 | 1 | 1 | 1 | 0 |
| Characiformes | 5 | 12 | 18 | 0 | 18 |
| Cyprinodontiformes | 2 | 2 | 2 | 1 | 1 |
| Elopiformes | 1 | 1 | 1 | 1 | 0 |
| Gymnotiformes | 2 | 2 | 2 | 0 | 2 |
| Lophiiformes | 1 | 1 | 1 | 1 | 0 |
| Mugiliformes | 1 | 2 | 7 | 6 | 1 |
| Perciformes | 15 | 26 | 38 | 36 | 2 |
| Pleuronectiformes | 1 | 2 | 2 | 2 | 0 |
| Scorpaeniformes | 3 | 4 | 6 | 6 | 0 |
| Siluriformes | 4 | 8 | 9 | 2 | 7 |
| Synbranchiformes | 1 | 2 | 2 | 0 | 2 |
| Tetraodontiformes | 2 | 2 | 3 | 3 | 0 |
| Total | 44 | 75 | 103 | 70 | 33 |

Mitotic chromosomes were obtained by injecting a solution of colchicine 0.0125% (1cc/100g of weight), exposure to the alkaloid for 50 min, obtaining cell suspension from kidney tissue, hypotonic treatment for 20 min and centrifugation-fixation (3 times) with Carnoy's solution (Nirchio & Oliveira, 2006). Special care was taken to standardize exposure to colchicine and hypotonic treatment times in order to achieve similar chromosomal condensation grades and to keep uniform criteria for comparisons.

Chromosomes in the karyotype were organized in a decreasing order of size and grouped by morphological types according to the ratio of lengths of their arms (r = long arm length/short arm length) as metacentric (m, r = 1.00 to 1.70), submetacentric (sm, r = 1.71 to 3.00), subtelocentric (st, r = 3.01 to 7.00) and acrocentric (a, r > 7.01) (Levan *et al.*, 1964).

Data were analyzed with non-parametric test (Sokal & Rohlf, 1995). The non-parametric variance analysis of Kruskal-Wallis was used to verify the existence of statistical differences in the diploid number (2n) of chromosomes and in the FN between freshwater and marine fish. The association between the proportion of species with 48 exclusively acrocentric chromosomes and the environment (marine or freshwater), as well as the association between the number of species with 2n=48 acrocentric chromosomes among the Perciformes and among the other orders of Actinopterygii were tested using the Chi-squared test of independence.

Absence of normality in the data examined determined an inability to perform regression analysis. Hence, scatter plots and 95% concentration ellipses were created for the diploid number of chromosomes and FN (arm number) against the Nelson's (2006) phylogenetic position of Actinopterygii families in PAST (Hammer *et al.*, 2001). Ellipses with 95% confidence are used as a correlation indicator. When two variables are not correlated, the ellipse is circular in shape and it becomes more elongated when the correlation between two variables is stronger. To establish whether there was a correlation between these parameters and the phylogenetic position of the orders, a test of correlation by Spearman rank was conducted using WinSTAT.

Additionally, data on diploid number and DNA content per haploid nucleus (C-value) of fish species from which both values are available at the Animal Genome Size Database (Gregory, 2012) were used to establish the correlations between these parameters and phylogenetic position as following: a) for those data expressed in ranges, the average value of the range was calculated; b) multiple records were averaged to provide a single value for each species. The correlation analysis was performed first considering all the species and then excluding the possible polyploids. To standardize our data, we considered the distribution of the available C-value data for non-polyploid ray-finned fish species reported by Smith & Gregory (2009) that range from 0.35 to 4.20 pg., with a mean of < 1.1 pg, and with the vast majority of species falling within a smaller range of about 0.5 to 2.0 pg. Thus we assumed that C-values = 1 represents normal values for diploids; C-values < 2 would represent partial duplications of DNA sequences and C-values < 1 represents partial DNA loss. Values > 2 would represent polyploidy events.

## Results

The results of this study include 103 species (70 marine and 33 freshwater) belonging to 74 genera ascribed to 45 families and 17 out of the 40 teleost orders (Table 1). Diploid number (2n), karyotype formula, and arm number (FN) are shown in Table 2. The diploid number ranged from 2n = 24 in *Mugil curema* Valenciennes, 1836 (Mugilidae: Mugiliformes) to 2n = 100 in *Potamorhina* sp. (Curimatidae: Characiformes) and *Prionotus punctatus* (Bloch, 1793) (Triglidae: Scorpaeniformes), with a mode of 48 chromosomes represented in 45 of the 103 studied species (43.7%). FN ranged from 110 in *Pygocentrus cariba* (Humboldt, 1821), *Serrasalmus irritans* Peters, 1877 and *S. rhombeus* (Linnaeus, 1766) (Characiformes) to 33-34 in *Stephanolepis setifer* (Bennet, 1831) (Tetraodontiformes). In 26 out of 103 species (25.24%) a karyotype composed of 48 exclusively acrocentric chromosomes (2n=48, FN = 48) was observed. In the group of marine fishes, the modal diploid number was 2n = 48 represented in 60% of the studied species, while in the freshwater fish group the modal diploid complement was 2n = 54, represented in 21.21% of the studied species. Both the number of chromosomes and FN were statistically higher in freshwater fishes than in marine fishes (H= 32.730; $P = 1.06x10^{-8}$ and H = 39.007; $P = 4.22 \text{ x } 10^{-10}$ respectively).

Statistical significant results were obtained also when numbers of chromosomes were compared with the species habitat (Table 3). Twenty-five out of 70 marine fish species (corresponding to 35.71%) show 48 acrocentric chromosome, compared to a very low frequency (3.03%) observed in freshwater fishes ($\chi^2 = 12.696$; $P \leq 0.0004$). The relative proportions of species with 48 exclusively acrocentric chromosomes became even higher (57.89%) and dependency stronger ($\chi^2 = 34.02$; $P \leq 0.0001$) when the group of Perciformes was compared to the other orders of Actinopterygii.

The scatter plot of the diploid chromosome numbers (2n) and the arm number (FN) against Nelson's (2006) phylogenetic position for the Actinopterygii (Fig. 1) provides an overview of the relationship between those two variables. Spearman rank test revealed a significant inversely proportional relationship between phylogenetic position and diploid number ($\rho$= -0,5126; $P = 1.55x10^{-8}$) and also between phylogenetic position and chromosome arm number ($\rho$= -0.7106; $P = 2.0953x10^{-17}$).

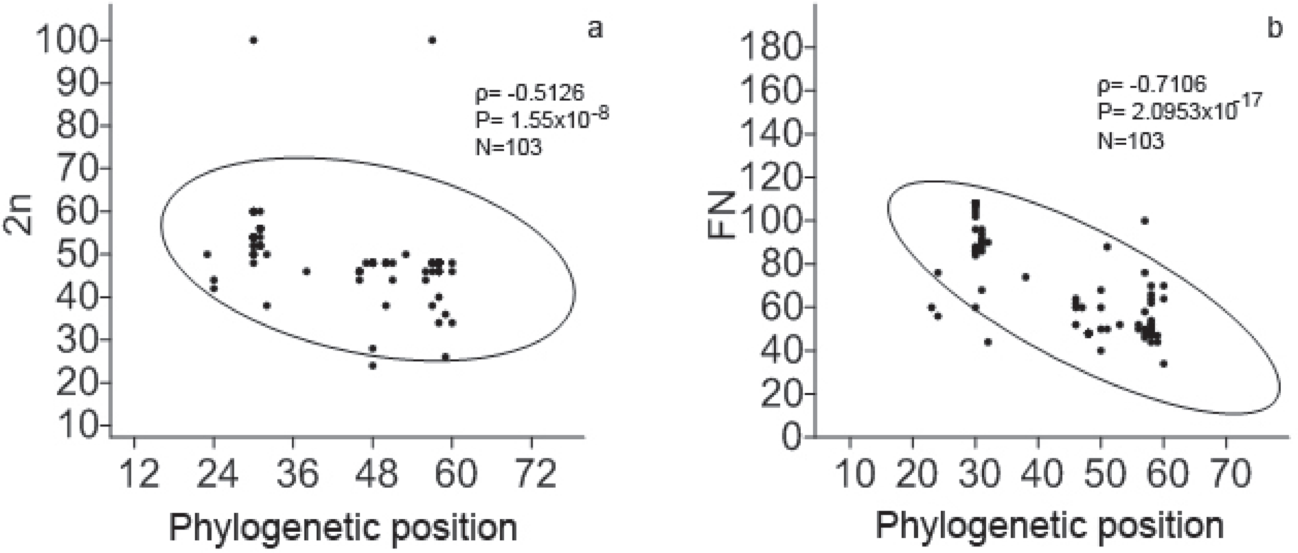

**Fig. 1.** Scatter-plot of (a) diploid number (2n) and (b) fundamental number (FN), against the phylogenetic position of Actinopterygii families presented by Nelson (2006) for 103 fish species. Ellipses with 95% confidence are used as a correlation indicator.

**Table 2.** Karyotype characteristics for 103 species of fishes from Venezuela. M = marine, F = freshwater, m= meta-, sm = submeta-, st = subtelo- and a = acrocentric chromosomes. In the last column the catalog numbers of the voucher specimens of species whose results are firstly presented here. Classification follows Eschmeyer & Fong (2014).

| Order | Family | Species | Habitat | 2n | Karyotype formulae | FN | Reference or catalog number of voucher |
|---|---|---|---|---|---|---|---|
| Anguilliformes | Muraenidae | *Gymnotorax moringa* | M | 44 | 12m+32a | 56 | ECAM 661 |
| | | *G. ocellatus* | M | 42 | 34m/sm+8a | 76 | ECAM 682 |
| Aulopiformes | Synodontidae | *Synodus foetens* | M | 46 | 28m/sm+18a | 74 | LBP 6095 |
| Batrachoidiformes | Batrachoididae | *Amphichthys cryptocentrus* | M | 46 | 4m+2sm+40a | 52 | Nirchio *et al*. 2001 |
| | | *Batrachoides manglae* | M | 46 | 8m+6sm+32a | 60 | Nirchio *et al*. 2001 |
| | | *Porichthys plectrodon* | M | 44 | 8m+10sm+6st+20a | 68 | Nirchio *et al*. 2004 b |
| | | *Thalassophryne maculosa* | M | 46 | 12m+6sm+20st+8a | 84 | Nirchio *et al*. 2004 a |
| Beloniformes | Belonidae | *Strongylura marina* | M | 48 | 2m+46a | 50 | LBP 6077 |
| | | *S. timucu* | M | 48 | 12m+36a | 60 | LBP 6078 |
| | | *Tylosurus crocodrilus* | M | 48 | 8m+12sm+28a | 68 | LBP 6100 |
| | Hemiramphidae | *Hyporhamphus unifasciatus* | M | 38 | 2m+36a | 40 | LBP 6099 |
| Beryciformes | Holocentridae | *Holocentrus adscensionis* | M | 50 | 2m+6st+42a | 58 | LBP 6072 |
| | Bryconidae | *Brycon amazonicus* | F | 50 | 22m+14sm+14st | 100 | Mariguela *et al*. 2010 |
| | | *B. falcatus* | F | 50 | 18m+16sm+16st | 100 | LBP 7686 |
| Characiformes | Characidae | *Astyanax bimaculatus* | F | 50 | 4m+6sm+28st+12a | 88 | LBP 13292 |
| | Curimatidae | *Potamorhina altamazonica* | F | 100 | 4sm+96a | 104 | LBP 3056 |
| | Erythrinidae | *Hoplerythrinus unitaeniatus* | F | 48 | 32m+16sm | 96 | ECAM 317 |
| | Prochilodontidae | *Prochilodus mariae* | F | 54 | 40m+14sm | 108 | Oliveira *et al*. 2003 |
| | | *Prochilodus reticulatus* | F | 54 | 34m+20 sm | 108 | LBP 6127 |
| | | *Semaprochilodus kneri* | F | 54 | 40m+14sm | 108 | Oliveira *et al*. 2003 |
| | | *S. laticeps* | F | 54 | 40m+14sm | 108 | Oliveira *et al*. 2003 |
| | | *Colossoma macropomum* | F | 54 | 54m | 108 | Nirchio *et al*. 2003 b |
| | | *Mylossoma duriventris* | F | 54 | 54m/53m+1a | 108/107 | LBP 7206 |
| | | *Piaractus brachypomus* | F | 54 | 54m | 108 | Nirchio *et al*. 2003 b |
| | Serrasalmidae | *Pygocentrus cariba* | F | 60 | 18m+30sm+2st+ 10a | 110 | Gaviria *et al*. 2005 |
| | | *Serrasalmus altuvei* | F | 60 | 26m+22st+12a | 108 | ECAM 1015 |
| | | *S. irritans* | F | 60 | 22m+20sm+8st+10a | 110 | ECAM 997 |
| | | *S. rhombeus* | F | 60 | 30m+16sm+4st+10a | 110 | Nirchio *et al*. 2002 |
| | Triportheidae | *Triportheus orinocensis* | F | 52 | 20m+14sm+12st+6a | 98 | LBP 2663 |
| | | *T. venezuelensis* | F | 52 | 20m+16sm+ 16st | 104 | Nirchio *et al*. 2007a |
| Cyprinodontiformes | Cyprinodontidae | *Cyprinodon dearborni* | M | 48 | 2m+38sm+8a | 88 | Nirchio *et al*. 2003 a |
| | Rivulidae | *Rivulus hartii* | F | 44 | 2m+4sm+8st+30a | 58 | Nirchio *et al*. 2005 b |
| Elopiformes | Elopidae | *Elops saurus* | M | 50 | 6m+4t+40a | 60 | LBP 6042 |
| Gymnotiformes | Rhamphichthyidae | *Rhamphichthys sp* | F | 50 | 22m+18sm+6st+4a | 96 | LBP 9949 |
| | Sternopygidae | *Eigenmannia* sp | F | 38 | 4m+2sm+6st+26a | 50 | LBP 2225 |
| Lophiiformes | Antennariidae | *Antennarius multiocelatus* | M | 48 | 12m+16st-20a | 76 | LBP 6087 |
| Mugiliformes | Mugilidae | *Agonostomus monticola* | F | 48 | 2st+46a | 50 | Nirchio *et al*. 2008 b |
| | | *Mugil curema* | M | 24 | 22m+2sm | 48 | Rossi *et al*. 2005 |
| | | *M. incilis* | M | 48 | 48a | 48 | Het *et al*. 2011 |
| | | *M. liza* | M | 48 | 48a | 48 | Rossi *et al*. 2005 |
| | | *M. rubrioculus* | M | 48 | 48a | 48 | Nirchio *et al*. 2007 b |
| | | *Mugil sp.* | M | 28 | 20m+4st+4a | 52 | LBP 6128 |
| | | *M. trichodon* | M | 48 | 48a | 48 | Nirchio *et al*. 2005 a |
| | Carangidae | *Selene vomer* | M | 46 | 2st+46a | 50 | LBP 6068 |
| | | *Trachinotus falcatus* | M | 48 | 2m+2st+44a | 52 | LBP 6082 |
| | Centropomidae | *Centropomus undecimalis* | M | 48 | 48a | 48 | LBP 6046 |
| | Cichlidae | *Cichla orinocensis* | F | 48 | 48a | 48 | ECAM 387 |
| | | *Geophagus surinamensis* | F | 48 | 2m+2sm+12st+32a | 64 | ECAM 379 |
| | Ephippidae | *Chaetodipterus faber* | M | 48 | 48a | 48 | Narváez 2000 |
| | Gerreidae | *Eucinostomus argenteus* | M | 48 | 48a | 48 | LBP 6090 |
| | Haemulidae | *Haemulon aurolineatum* | M | 48 | 48a | 48 | Nirchio *et al*. 2006a |
| | | *H. bonariensis* | M | 48 | 48a | 48 | Nirchio *et al*. 2006a |
| | | *H. flavolineatum* | M | 48 | 48a | 48 | Ron & Nirchio 2005 |
| | | *H. plumierii* | M | 48 | 48a | 48 | Nirchio *et al*. 2006 a |
| | | *H. sciurus* | M | 48 | 48a | 48 | ECAM 1021 |
| | | *H. steindachneri* | M | 46 | 46a | 46 | ECAM 1022 |
| | | *H. striatum* | M | 48 | 48a | 48 | ECAM 1019 |
| | | *Orthopristis ruber* | M | 48 | 48a | 48 | ECAM 1017 |
| | Labridae | *Halichoeres bivittatus* | M | 48 | 48a | 48 | LBP 6056 |
| | | *Lachnolaimus maximus* | M | 48 | 6m+16st+26a | 70 | ECAM 1018 |
| | | *Xyrichthys novacula* | M | 46 | 2m+4sm+40a | 52 | ECAM 1031 |

**Table 2 (cont.).** Karyotype characteristics for 103 species of fishes from Venezuela. M = marine, F = freshwater, m= meta-, sm = submeta-, st = subtelo- and a = acrocentric chromosomes. In the last column the catalog numbers of the voucher specimens of species whose results are firstly presented here. Classification follows Eschmeyer & Fong (2014).

| Order | Family | Species | Habitat | 2n | Karyotype formulae | FN | Reference or catalog number of voucher |
|---|---|---|---|---|---|---|---|
| Perciformes | Lutjanidae | *Lutjanus analis* | M | 48 | 48a | 48 | Nirchio *et al*. 2008 |
| | | *L. apodus* | M | 48 | 48a | 48 | LBP 6052 |
| | | *L. griseus* | M | 48 | 48a | 48 | Nirchio *et al*. 2008 |
| | | *L. synagris* | M | 47 / 48 | 1m+46a/48a | 48 | Nirchio *et al*. 2008 |
| | | *Ocyurus chrysurus* | M | 48 | 48a | 48 | Nirchio *et al*. 2009 |
| | | *Rhomboplites aurorubens* | M | 48 | 2st+46a | 50 | Nirchio *et al*. 2009 |
| | Opistognathidae | *Opistognathus macrognathus* | M | 40 | 1m+6sm+2st+28a | 46 | Nirchio & Oliveira 2012 |
| | Pomacanthidae | *Pomacanthus arcuatus* | M | 48 | 48a | 48 | LBP 6071 |
| | | *P. paru* | M | 48 | 2st+46a | 50 | LBP 6070 |
| | Scaridae | *Nicholsina usta* | M | 48 | 8m+10sm+6st+24a | 72 | LBP 6054 |
| | | *Sparisoma aurofrenatum* | M | 46 | 12m+12sm+12st+10a | 82 | LBP 6055 |
| | | *S. chrysopterum* | M | 46 | 6m+12sm+10st+18a | 74 | LBP 6096 |
| | Scianidae | *Bairdiella ronchus* | M | 48 | 48a | 48 | LBP 6436 |
| | | *B. sanctaluciae* | M | 48 | 48a | 48 | LBP 6435 |
| | | *Ophioscion punctatissimus* | M | 48 | 48a | 48 | LBP 6085 |
| | | *Stellifer sp.* | M | 48 | 48a | 48 | LBP 6437 |
| | Serranidae | *Diplectrum formosum* | M | 48 | 2m+46a | 50 | LBP 6093 |
| | | *Paralabrax dewegeri* | M | 48 | 48a | 48 | LBP 6097 |
| | Sparidae | *Archosargus rhomboidalis* | M | 48 | 14m-sm+34a | 62 | LBP 6439 |
| Pleuronectiformes | Paralichthyidae | *Citharichthys spilopterus* | M | 26 | 14m+4sm+4st+4a | 48 | ECAM 1016 |
| | | *Etropus crossotus* | M | 36 | 11m+14st+11a | 61 | LBP 6049 |
| Scorpaeniformes | Dactylopteridae | *Dactylopterus volitans* | M | 48 | 14m+14sm+6st+14a | 82 | ECAM 901 |
| | Scorpaenidae | *Pterois volitans* | M | 48 | 2m+8sm+8st+30a | 66 | LBP 13291 |
| | | *Scorpaena brasiliensis* | M | 46 | 2sm+44st-a | 48 | LBP 6044 |
| | | *S. isthmensis* | M | 38 | 8m+10st+20a | 56 | LBP 6045 |
| | | *S. plumieri* | M | 48 | 2m+32st+14a | 82 | ECAM 1072 |
| | Triglidae | *Prionotus punctatus* | M | 100 | 100a | 100 | ECAM 900 |
| Siluriformes | Ariidae | *Cathorops spixii* | M | 52 | 14m+20sm+18st | 104 | Nirchio *et al*. 2010 |
| | | *Sciades herzbergii* | M | 54 | 14m+20sm+18st+2a | 106 | Nirchio *et al*. 2010 |
| | Callichthyidae | *Hoplosternum littorale* | F | 60 | 6m+2sm+2st+50a | 70 | Nirchio *et al*. 2006 b |
| | Loricariidae | *Glyptoperichthys gibbiceps* | F | 52 | 20m+24sm+8st | 104 | Alves *et al*. 2006 |
| | | *Liposarcus multiradiatus* | F | 52 | 22m+18sm+12st | 104 | Alves *et al*. 2006 |
| | Pimelodidae | *Pimelodus blockii* | F | 56 | 18m+16sm+10st+12a | 100 | LBP 3038 |
| | | *Pseudoplatystoma metaense* | F | 56 | 24m+18 sm + 14a | 98 | Nirchio *et al*. 2013 |
| | | *P. orinocoense* | F | 56 | 24m+18 sm + 14a | 98 | Nirchio *et al*., 2013 |
| | Pseudopimelodidae | *Cephalosilurus apurensis* | F | 54 | 6 m + 28sm+14st+6a | 102 | Martinez *et al*. 2008 |
| Synbranchiformes | Synbranchidae | *Ophisternon aenigmaticum* | F | 45/46 | 6m+1st+38a/6m+2st+38a | 52<br>54 | Nirchio *et al*. 2011 |
| | | *Synbranchus marmoratus* | F | 44 | 6m+38a | 50 | LBP 2227 |
| Tetraodontiformes | Monachantidae | *Stephanolepis setifer* | M | 33/34 | 1m+32a/34a | 34 | Nirchio & Oliveira, 2007 |
| | Tetraodontidae | *Sphoeroides testudineus* | M | 46 | 24m+22a | 70 | LBP 6059 |
| | | *S. greeleyi* | M | 48 | 10m+8sm+12st+16a | 76 | LBP 6058 |

Scatter-plot of diploid number (Figs. 2a-2c) and C-Value data (Figs. 2b-2d) were taken from the Animal Genome Size Database (Gregory, 2012), *versus* the phylogenetic position of Actinopterygii families. Again an inversely proportional relationship with highly significant correlation coefficients was obtained both when all the 646 species were considered and when the probable 93 polyploid species were excluded.

## Discussion

In freshwater fishes both the average number of chromosomes and the FN were higher than in marine fishes, and a general higher degree of cytogenetic diversification and karyotype variation is observed, compared to a more conserved cytogenetic pattern in marine fishes. Few decades ago the difference between karyotypes of freshwater and marine fishes was already observed and considered related to a more stable environment at sea as compared to inland waters, with some exceptions (Nikolsky, 1976). More recently, it has been reported that the differences in chromosome number and arm number between marine and freshwater fishes could be explained taking into account topographic barriers common to freshwater environments that would hamper the gene flow between populations, leading to fixation of macro-structural alterations in chromosomes. On the contrary, in the marine environment, the occurrence of large populations, the absence of well-defined geographical barriers, and the large capacity of dispersal would contribute to homogenize populations, reducing karyotype diversification (Molina, 2007). This is congruent with the idea that speciation rates are higher in freshwater than in marine

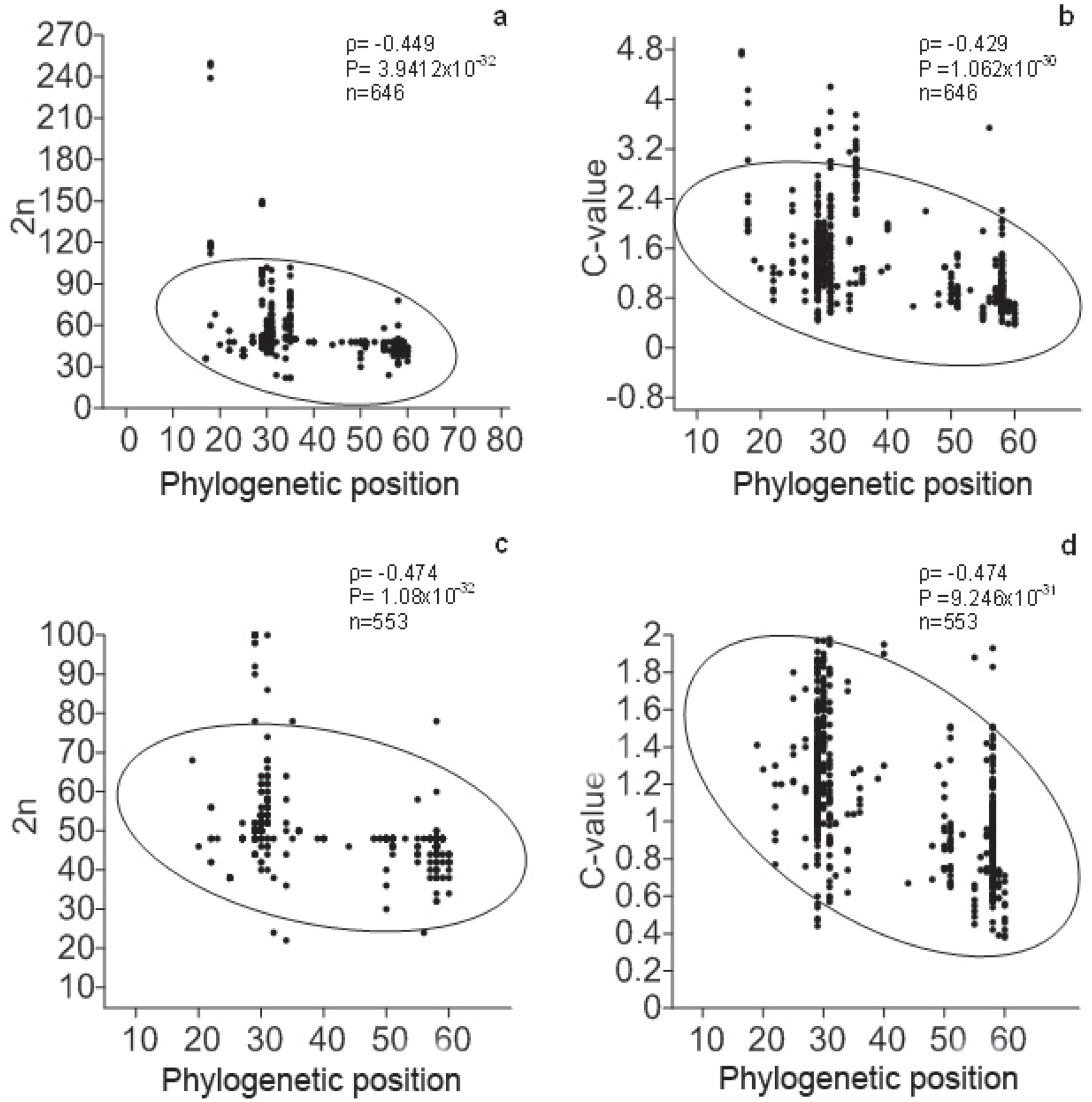

**Fig. 2.** Scatter-plot of (a, c) diploid number (2n), and (b, d) pg of DNA per haploid nucleus (C-Value), against the phylogenetic position of Actinopterygii families presented by Nelson (2006). Data include all available species (a, b) or exclude possible polyploidy species (c, d). Ellipses with 95% confidence are used as a correlation indicator.

fish lineages as the result of a greater number of barriers in the first habitat related to the latter (Bloom *et al.*, 2013). The effects of mobility and population dimension on chromosome diversification seem to be confirmed when the analysis is limited to marine species that show different biological traits. Indeed marine taxa that have high mobility (eggs, larvae, or adults), like Haemulidae, Sciaenidae, Lutjanidae, and Serranidae (Perciformes) show 48 acrocentric chromosomes and reduced frequency of chromosomal macro-structural reorganizations. On the contrary groups such as Muraenidae, Batrachoididae, and Scorpaenidae that do not form schools, are characterized by benthic habits, smaller populations, and more limited spatial locomotion, display a more extensive chromosomal diversity. However the apparent overall karyotype stability of Perciformes should be considered with caution, and could hide a more dynamic situation, *i.e.*, the presence of microstructural rearrangements undetectable by conventional cytogenetic methods but visible after molecular banding, as reported over

**Table 3.** Results of Chi-square ($\chi^2$) test between freshwater and marine environment and between Perciformes and other Orders. N = Number of species.

| Environment | Diploid number | | N | $\chi^2$ | D.F. | *P*-value |
|---|---|---|---|---|---|---|
| | $2n \neq 48$ | $2n = 48A$ | | | | |
| Freshwater | 32 | 1 | 33 | 12.6957 | 1 | 0.0004 |
| Marine | 45 | 25 | 70 | | | |
| Total | 77 | 26 | 103 | | | |
| Order | | | | | | |
| Perciformes | 16 | 22 | 38 | 34.0203 | 1 | <0.0001 |
| Other orders | 61 | 4 | 65 | | | |
| Total | 75 | 26 | 103 | | | |

the last decade in marine fishes with conserved karyotypes like Serranidae (see references in Galetti *et al.*, 2000), Haemulidae (Nirchio *et al.*, 2006) and Lutjanidae (Nirchio *et al.*, 2008) among others. This has also been observed in Mugiliformes, an order considered to be karyotypically conserved but in which recent studies carried out by the application of differential staining techniques and fluorescence *in situ* hybridization (FISH) with several DNA probes allowed the detection of a considerable number of microstructural changes among species (Sola *et al.*, 2007, 2008).

The highly significant probability (more than 99% confidence) of the Spearman correlation analysis, and the scatter-plot derived from our data clearly indicate that the most ancestral fishes, tend to have higher chromosome number compared to the more derived fishes (Figs. 1-2), and that a karyotype with $2n = 48$ characterize the more derived groups, mainly Perciformes (Table 3). Moreover our estimates on the number of species belonging to this order showing a karyotype composed of 48 acrocentric chromosomes (57.89%) is comparable to that (60%) provided on a global level (Galetti *et al.*, 2000).

The evidence presented herein challenge both the long-lasting hypothesis that a karyotype composed of 48 acrocentric chromosomes could be ancestral for all teleost (Ohno, 1970), and the estimates of the chromosome number of the Actinopterygii proto-karyotype, dated 450 million, based on an analysis of the sequence of the genome of *Tetraodon nigroviridis* (Tetraodontiformes) (Jaillon *et al.*, 2004). Furthermore present data do not agree with the indication of an ancestral karyotype probably composed of less than 48 chromosomal elements (Mank & Avise, 2006), obtained mapping the number of chromosomes on the topology generated by molecular phylogenetic study for the Actinopterygii (Mank *et al.*, 2005).

In fact, in the more basal Actinopterygii as the Polypteriformes and Acipenseriformes, the number of chromosomes is usually larger, ranging from 120-260 for Acipenseridae (Fontana *et al.*, 1998) to 120 for Polyodontidae and 56-68 for Lepisosteidae (see references in Sola *et al.*, 1981). Although extant Acipenseriformes families may have originated from a tetraploid ancestor with a probable karyotype of 120-macro and micro-chromosomes, it is presumed that the tetraploidization occurred at an early stage during the evolution of the group (Birstein *et al.*, 1997) from an ancestor with 60 chromosomes (Dingerkus & Howell, 1976; Carlson *et al.*, 1982). On the other hand the presence of a karyotype close to $2n = 60$ in the most ancient orders, is congruent with other studies. Indeed, the original idea that the ancestral karyotype of the Teleostei had approximately 60 elements and that the reduction in the number of chromosomes to 48 occurred independently in more than one phylogenetic line through fusions and deletions (Dingerkus, 1979) was proposed by Brum & Galetti (1997). These authors suggested that the 48 chromosomes would be a synapomorphy extended among the more apical branches of the extant Teleostei (Clupeomorpha and Euteleostei) that was preserved mainly in marine species of Atherinomorpha and Percomorpha.

A general evolutionary tendency towards a reduction of chromosome number in fishes was also estimated by Nakatani *et al.* (2007) on the base of genome analysis suggesting that after two rounds of whole genome duplication (WGD), one occurred in the vertebrate proto-karyotype and one occurred in the Gnathostomata proto-karyotype, the number of chromosomes in the osteichthyan ancestor (after the divergence of Chondrichthyes, about 450 Mya) was $n \approx 31$. After the divergence of ray-finned and lobe-finned fishes, along the Actinopterygii lineage, chromosome fusions reduced this number to $n \approx 13$, in teleost ancestor about 350 Mya. A third round of whole genome duplication (3R-WGD) in the teleost ancestor doubled the number of chromosomes to $n \approx 26$. Since then, the number of chromosomes in the teleost lineage has remained nearly unchanged. These hypotheses are largely congruent with the present results.

Our data indicated a trend towards simplification of the karyotype along the phylogeny of the Actinopterygii, both in terms of the chromosome number and in the genome size (DNA content/nucleus). Although remarkable modification in genome size were reported to be unconnected with chromosome number in ray-finned fish (Mank & Avise, 2006), the assays of DNA content of over 200 teleost fishes, suggested a correlation between DNA content and evolution in this group (Hinegardner, 1968). That is, the more derived species of fishes have less DNA content per nucleus than ancestral ones, suggesting implicitly that evolution and specialization in teleosts had been accompanied by loss of DNA (Hinergardner, 1968), as also recently confirmed by measures of DNA bp content on a dataset from 1,043 species of Actinopterygii representing 190 families (Yi & Streelman, 2005). As an example the haploid genome of *Acipenser brevirostrum* Lesuer, 1818 belonging to one of the most basal orders (Acipenseriformes), is 20 times greater than that found in *Arothron meleagris* (Anonymous, 1798) which belongs to Tetraodontiformes, the most derived teleost order. Molecular analysis suggests that this reduction is the consequence of loss of repetitive sequences and/or other non-coding DNA sequences (Neafsey & Palumbi, 2003), including satellite DNA, ribosomal genes and transposable elements (TEs) (Biémont, 2008; Fedoroff, 2012).

Recognizing that several evolutionary events at the chromosomal level (polyploidization, fusions, fissions) have occurred in different lineages of ray-finned fishes, data here provided reveals a trend towards simplification of the karyotype along the phylogeny of the Actinopterygii from the more basal to the more derived lineages with a general conservation of chromosome numbers around 2n=48 acrocentric elements in the last ones. This different point of view challenges the currently accepted hypothesis, overall, based on oversimplification of chromosomal data, that a karyotype composed of 48 acrocentric chromosomes could be ancestral for all teleosts.

Although certainly limited by the number of species examined, data here reported represent also an initial reference database for Neotropical fishes from Venezuela that can be used for future comparative analyses aimed at better understanding the evolution of fishes, on the light of the huge expanding researches on genomes. Indeed, recent literature based on genetic maps and sequencing provided new evidence on debated aspects of genome evolution. For instances many authors argued that the 3R-WGD could be responsible for "species richness" in teleosts, but doubt about this direct association were raised (Santini *et al.*, 2009; Sato & Nishida, 2010). Moreover, although fishes were reported as having high rates of chromosomal rearrangements and low rates of synteny within vertebrates (Robinson-Rechavi *et al.*, 2001a, 2001b; Venkatesh, 2003; Ravi & Venkatesh, 2008), more recent investigation indicated both the presence of large syntenic blocks within Teleosts and between Teleosts and other vertebrates (Mazzuchelli *et al.*, 2012). This indicates the stability of the genome of these fishes over long evolutionary time scales (Guyomard *et al.*, 2012; Schartl *et al.*, 2013). In addition a higher conserved gene order was observed in fishes than in mammals by genome comparison of three teleost species (fugu, *Tetraodon*, and medaka) with those of three mammalian lineages (human, mouse, and opossum), and authors reported these results as "largely due to a lower rate of interchromosomal rearrangements in the teleosts" (Kai *et al.*, 2011).

Thus an effort is necessary to fill the gap between chromosome and genomic data on fishes, and to integrate cytogenetics and genomics in comparative and evolutionary studies.

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