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CYTOGENETICS OF CHILEAN FISHES: A COMMENTED DATABASE

CITOGENETICA DE PECES CHILENOS: UNA BASE DE DATOS COMENTADA

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ABSTRACT

A database containing data from cytogenetic studies of Chilean fish species is documented for the first time. The cytogenetic data compiled for Chilean fishes include 28 species belonging to 11 families, 9 orders and 16 genera, taking as reference 18 publications since 1972. The application of a variety of cytogenetic methods has provided information on chromosome number, karyotype morphology, genome size, and /or location of different DNA sequences. These data represent only ca. 2.7% of Chile's fish diversity.

Key words: Fishes, chromosome number, karyotype morphology, chromosome banding, genome size

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RESUMEN

Se documenta por primera vez una base de datos sobre estudios citogenéticos de peces chilenos. Los datos citogenéticos recopilados para peces chilenos incluyen 28 especies pertenecientes a 11 familias, 9 órdenes y 16 géneros, tomando como referencia 18 publicaciones desde 1972. La aplicación de variados métodos citogenéticos ha entregado información sobre número cromosómico, morfología del cariotipo, tamaño genómico y/o localización cromosómica de diferentes secuencias de ADN. Estos datos sólo representan ca. 2,7% de la diversidad íctica chilena.
Palabras clave: Peces, número cromosómico, morfología del cariotipo, bandeo cromosómico, tamaño genómico.

INTRODUCTION

Fishes are the most diverse group of vertebrates, with ca. 25,000 species characterized by the great diversity of their morphology, physiology, ecology, life history and behavior. Furthermore, great variation in genome size has recently been recognized among fishes (Smith and Gregory, 2009). However, despite progress in genome size estimation in fishes, basic data such as chromosome number and/or karyotypes are not available for a large number of species. This gap in chromosome studies has encouraged the collection and analysis of new datasets obtained from different sources, in which important progress can be observed. The first compilation on chromosome numbers in fishes was documented by Lagler et al. (1977), who estimated the number of species studied around the world to that decade at 400 (ca. 1.6%). Numerous works have been done since then, and these were compiled in a review documented by Klinkhardt et al. (1995). Sola et al., (1981) analyzed the role of karyotype diversity in the speciation patterns of bony-fish, contrasting hypotheses of chromosome change mechanisms vs. polyploidization. Polyploidization as a cyto-evolutionary process in fishes was initially discussed by Schultz (1980), and later by Comber and Smith (2004), and from these works it may be concluded that nine of the 45 extant orders belonging to the Teleostomi include polyploid species (ca. 20% of the total orders). It may be noted that some South American taxa have been included in these reviews, and current estimations show that around 1,047 species have been examined cytogenetically (Nirchio and Oliveira, 2006). Nevertheless, it is a cause for concern that Chilean taxa are little represented in these datasets, despite the efforts of a number of Chilean researchers to supply data for some species, e.g. in the pioneering work documented by Campos (1972), and Arratia and Veloso (1980), followed by additional contributions in later decades.

Current estimations on fish diversity in Chile show that the number of species is relatively low (ca. 1,027 species) compared with tropical zones, however endemism is high, especially in continental and insular environments (Pequeño, 1989; Habit et al., 2006; Vila et al., 2006; Manzur, 2008). These characteristics make Chilean fishes an interesting model for the study of cyto-evolutionary patterns at different spatial scales. However this task depends on knowledge of advances in the cytogenetics of Chilean fishes, which requires the available information to be collected and stored in databases with easy access for researchers.

The object of this work is to show what cytogenetic data are available for Chilean fishes, focusing on the number of publications on the subject, the taxonomic representation and the chromosome markers analyzed. A detailed database of species which have been examined cytogenetically is also given.

NUMBER OF PUBLICATIONS

The data were compiled from varied sources, including personal literature, access to animal databases (Gregory, 2011), and searches of specialized bibliography in on-line directories (Science Direct, JStore, Web of Science). The compiled data are summarized in Table 1.

Eighteen articles on the cytogenetics of Chilean fishes were compiled, dated from 1972 to 2011. A strong increase in the number of publications was recorded in the period 2001-2011, with 11 reports that included 18 additional species. Cytogenetic data have been published in four Chilean journals; however the majority of the reports were published in nine foreign journals. On the other hand, the majority of the publications were authored by Chilean cytogeneticists (at least eight Chilean groups), while few studies have been reported by foreign specialists.

TAXONOMIC REPRESENTATION

In our literature search we found cytogenetic data for 28 species, belonging to 11 families, 9 orders and 16 genera, representing ca. 2.7% of Chilean fishes. The orders are Atheriniformes, Clupeiformes, Cyprinodontiformes, Mugiliformes, Ophidiiformes, Osmeriformes, Perciformes, Pleuronectiformes and Siluriformes. Of these, eight are marine species inhabiting the Chilean coast, while 20 live in continental environments. It must be clarified that the available data for *Mugil cephalus* Linnaeus, 1758 belong to populations inhabiting the Mediterranean Sea, however this cosmopolitan species is also present along the Chilean coast. On the other hand, although the geographical range for those Chilean fishes cytogenetically examined is wide, covering from 18ºS to 40ºS, samplings are discontinuous since only local populations of each species have been examined. At present there are no cytogenetic data for insular species.

It was found that the families whose cytogenetic characteristics have been most studied in Chile are the Cyprinodontidae, Galaxiidae and Trichomycteridae. The percentage of species with cytogenetic data per family was 100% for Cyprinodontidae, 71.4% for Trichomycteridae and 44.4% for Galaxiidae. In other families, the percentage of species cytogenetically studied was below 33% (based on Habit et al., 2006; G. Pequeño, personal communication).

Only four species of Chilean fishes have been re-studied. For *M. cephalus*, there are two reports mentioned in this work and seven other references cited by Rossi et al. (1996). In the case of *Galaxias maculatus* Jenyns, 1842, *Galaxias platei* Steindachner, 1898 and *Brachigalaxias bullockii* Regan, 1908, there are two studies of each species with different cytogenetic data documented by various authors, including karyotype morphology, C and Ag-NOR banding, and/or genome size (Campos, 1972; Cuevas et al., 1999; Jara-Seguel et al., 2008a, 2008b).

CHROMOSOME NUMBER

The most frequent chromosome number found for Chilean taxa was 2n = 48, present in nine of the examined species (33%), of which five are marine and four are continental. The range of chromosome numbers for marine species varies from 46 to 48, whereas for continental species the range was broader, varying between 22-30 chromosomes in *Galaxias* species (Campos, 1972) to 94 in *Nematogenys inermes* Guichenot, 1848 (Arratia and Veloso, 1980). The high chromosome number described for *N. inermes* may due to polyploidy, as has been described for other Siluriformes (Comber and Smith, 2004). However, this supposition is not discussed for *N. inermes* in the original source.

Among the taxa most studied for chromosome number, studies in three families are of particular interest. In Galaxiidae, with two genera studied, the two *Brachigalaxias* species both present chromosome numbers 2n = 38, whereas the numbers for the two *Galaxias* species are 22 and 30. These chromosome

numbers recorded for Chilean Galaxiidae are in addition to the 2n = 32 and 44 found for Tasmanian species (Johnson et al., 1981). The Galaxiidae thus present high polymorphism in their chromosome numbers, which may be related to the wide geographical distribution of these species around South America and Oceania.

In the case of Trichomycteridae, a complex series of chromosome numbers has been described for the genera *Trichomycterus* and *Bullockia* (52, 54, 55, 56, 58, 60 and 62). In *Trichomycterus areolatus* Valenciennes, 1846, interpopulation and intrapopulation polymorphism in 2n number were found (2n = 54, 55 and 56) (Colihueque et al., 2006), however 54 is the most frequently observed number in this genus, being present in Chilean, Brazilian and Argentinean taxa (total 13 species) (Arratia and Veloso, 1980; Arratia and Campos, 1997; Ramos et al., 2004; Colihueque et al., 2006). This framework of cytogenetic data on Trichomycteridae has supported interesting cytogeographical interpretations; the geographical distribution of the groups recognized within the genus *Trichomycterus* on the basis of karyotype characteristics coincides with cis and trans-Andean location. These interpretations can be reviewed in Ramos et al., (2004).

Within the Cyprinodontidae, interspecific polymorphism in chromosome number has also been described in the genus *Orestias* with the series 2n = 48, 52 and 55. Interestingly, a special case of polymorphism in 2n number has been described between males (2n = 51) and females (2n = 50) of *Orestias laucaensis* Arratia, 1982, which was not observed in sister species (Vila, 2006). It is possible that the high polymorphism found in chromosome numbers among *Orestias* species may be a consequence of its geographical isolation in Andean Plateau lakes (Vila and Pardo, 2008). However, phylogeographical studies based on DNA sequences may provide new insights into evolutionary processes in *Orestias*.

For the remaining Chilean fishes with percentage representation within their families lower than 33%, interesting cyto-evolutionary interpretations can be reviewed in the original sources (see Table 1).

In general, the polymorphism in 2n numbers mentioned above for three families of Chilean fishes may be explained by the occurrence of chromosome re-arrangements i.e. Robertsonian translocation or pericentric inversions, such as have been described in other taxa (e.g. Ophidiidae, Paralichthyidae, Atherinidae) using conventional and/or modern methods (Vitturi et al., 1988; Winkler et al., 2004; Muñoz et al., 2006). The available data would suggest that polyploidy is infrequent in Chilean fishes. It is thus clear that the study of karyotype morphology is crucial to understanding the processes underlying the numerical variations described in the chromosome complements within each family.

KARYOTYPE MORPHOLOGY

The first karyotypes reported for Chilean fishes (including the first chromosome numbers) were obtained using the drip method with gill cells (Campos, 1972; Arratia and Veloso, 1980). Later, drip and squash methods have been performed using gill epithelium, kidney, spleen and liver tissues. As a general protocol, the collected individuals are treated with an anti-mitotic reagent (colchicine) by intraperitoneal injection, or by submersion in an antimitotic solution prepared in the medium (water) with constant aeration. Later, small pieces of organs are excised by dissection, hypothonized and fixed using standard methods (Gold, 1974; Fan and Fox, 1990). The stain methods vary, using Giemsa or Feulgen reaction. The nomenclature to describe the chromosome morphology basically follows Levan et al., (1964).

Karyotype morphology has been described in detail for 18 Chilean species belonging to seven families. In all of them the karyotype composition was well defined. However, for *N. inermes* the details of karyotype composition are incomplete, perhaps due to the presence of a high number of micro chromosomes that may hinder the quantitative study of chromosome morphology.

In marine species the presence of subtelocentric (st) or telocentric (t) chromosomes in the karyotype was predominant (with 95-100% of t-st chromosomes), whereas in continental species submetacentric (sm) and metacentric (m) chromosomes predominated (range between 34 to 96% of sm-m chromosomes), except for *Basilichthys australis* Eigenmann, 1928 with only 18% of sm-m. These karyotype characteristics observed in Chilean fishes agree with the hypothesis proposed by Ohno (1974), who suggests that an acrocentric complement 2n = 48 may be an ancestral characteristic of teleost fishes, given that this number is present in ca. 11.5% of extant fishes around the world (Klinkhardt et al.,1995). As additional evidence in support of this hypothesis, we found that 2n = 48 is present in species with different evolutionary origins, such as the continental Cyprinodontidae, derived from neotropical ancestors, and the Atherinidae, which are derived from Pacific Ocean ancestors (Vila and Pardo, 2008). This would imply that the separation of different fish lineages was subsequent to the existence of a primitive complement 2n = 48. This is envisaged by Muñoz et al. (2006) to explain the status of *Odontesthes regia* Humboldt, 1821 in regard to ancestral forms with 48 chromosomes (e.g. Mugiliformes, Perciformes).

Another karyotype characteristic little studied in fishes is the absolute chromosome size (in µm). An example of the usefulness of these data has been documented for the Ophidiidae species *Ophidion barbatum* Linnaeus, 1758 and *Parophidium vassali* (Risso), both of which present polymorphism in their 2n numbers, with individuals reported having 43 and 44 chromosomes. In individuals where 2n = 43, the largest chromosome pairs are bi-armed, with a size of 2.45 µm in *O. barbatum* and 3.85 µm in *P. vassali*, while the remaining chromosomes decrease progressively in size. Thus, the occurrence of Robertsonian translocation among uni-armed chromosomes can be detected using relative (in percentage) or absolute (in µm) size relation among the chromosomes (Vitturi and Catalano, 1988). Moreover, the absolute total haploid set length (the sum of the sizes in µm of all the chromosomes of the haploid set) may be positively or negatively correlated with genome size (C-value); this is an interesting parameter for comparing karyotypes, in addition to chromosome morphology. This relation has been widely discussed for gastropod mollusks (Pascoe et al., 2004; Libertini et al., 2009). In this respect, accurate chromosome measurements can be facilitated using specialized computer programs which have been broadly applied to the study of karyomorphometric features in mollusks for several decades (Zhang et al., 1999).

On the above basis, robust karyotype affinities based on quantitative and/or qualitative analyses have been established within Chilean fish families, in many cases allowing accurate cyto-evolutionary and cytotaxonomic descriptions.

CHROMOSOME BANDING AND FLUORESCENT METHODS

Among the specific methods of chromosome analysis, fluorescent banding techniques such as DAPI, CMA_3 and/or FISH have been important for studying genome characteristics in *M. cephalus* and *O. regia*. The available data are restricted to physical chromosome mapping of ribosomal genes or specific DNA sequences (AT and CG sequences, 18S rDNA and telomeric sequences). The results of these techniques have opened the way to comprehensive studies on genome structure and function. For example, a single 18S rDNA signal is co-located with an Ag-NOR+ band, showing that ribosomal cistrons are active in *M. cephalus* and *O. regia*. This has allowed interesting progress to be made in learning about the cyto-evolutionary processes of these two species at family level (Rossi et al., 1996; Gornung et al., 2004; Muñoz et al., 2006). In addition, C-banding patterns have revealed valuable information on constitutive heterochromatin location in the chromosomes of these species, and others to which the method has been applied (e.g. *B. bullockii* and *Brachygalaxias gothei* Busse, 1982, Cuevas et al., 1999). In such cases, homologous chromosomes have been paired easily during karyotype construction. All these advances using specific cytogenetic methods should be applied to the study of more Chilean species. In the future, these techniques may be of use in identifying sex chromosomes in Chilean fishes, as has already been done for species of at least seven orders of Teleostomi (see Ueno and Takai, 2008).

Table 1. Cytogenetic data of Chilean fishes. 2n, diploid number; FN, basic number of arms; HKF, haploid karyotype formula; CV, haploid DNA C-value in picograms (pg); FISH, fluorescent in situ hybridization; RF, representation of species with cytogenetic studies within the family in Chile (%).
m, metacentric; sm, submetacentric; st, subtelocentric; t, telocentric; acr, acrocentric; mic, micro chromosomes

Tabla 1. Datos citogenéticos de peces chilenos. 2n, número diploide; FN, número fundamental de brazos; HKF, fórmula haploide del cariotipo; CV, valor C haploide de ADN en picogramos (pg); FISH, hibridación in situ fluorescente; RF, representación de especies con estudios citogenéticos dentro de la familia en Chile (%).
m, metacéntrico; sm, submetacéntrico; st, subtelocéntrico; t, telocéntrico; acr, acrocéntrico; mic, microcromosomas

Family/species	2n (FN)	HKF	Banding	CV	FISH	RF	Reference
Atherinidae						18.2	
(= Atherinopsidae)							
Basilichthys microlepidotus (Jenyns, 1841)	46	1m, 7sm, 15st					Gajardo (1992)
B. australis	48	2n = 4m, 5sm, 39st					Gajardo (1992)
Odontesthes regia	48 (50)	1m, 16st, 7t	C, Ag-NOR		18S rDNA		Muñoz et al. (2006)
Clupeidae						14.3	
Sardinops sagax (Jenyns, 1842)	48			1.01			Hardie and Hebert (2004)
Cyprinodontidae						100	
Orestias agassii Valenciennes, 1846	48						Vila (2006)
O. ascotaensis Parenti, 1984	48						Vila (2006)
O. chungaraensis	55						Vila (2006)
O. laucaensis	♂:51 ♀:50						Vila (2006)
O. parinacotensis Arratia, 1982	48						Vila (2006)
O. piacotensis Vila, 2006	52						Vila (2006)
Galaxiidae						44.4	
Brachigalaxias bullockii	38	5m, 8sm, 6t					Campos (1972)
B. bullockii	38		C, Ag-NOR				Cuevas et al. (1999)
B. gothei	38	5m, 8sm, 6t	C, Ag-NOR				Cuevas et al. (1999)
Galaxias maculatus	22	4m, 6sm, 1t					Campos (1972)
G. maculatus				1.105			Jara-Seguel et al. (2008a)
G. platei	30	2n = 1m, 18sm, 11t					Campos (1972)
G. platei				0.94			Jara-Seguel et al. (2008b)
Kyphosidae						14.3	
Girella laevifrons (Tschudi, 1846)	48 (48)	24 acr					Northland-Leppe et al. (2010)

Table 1. Continuation

Tabla 1. Continuación

Family/species	2n(FN)	HKF	Banding	CV	FISH	RF	Reference
Mugilidae						50.0	
Mugil cephalus	48	4t	Ag-NOR, CMA_3, DAPI		18S rDNA		Rossi et al. (1996)
M. cephalus					$(TTAGGG)_n$		Gornung et al. (2004)
Ophidiidae						9.1	
Genypterus blacodes				0.6			Hardie and Hebert (2004)
(Forster in Bloch and Schneider, 1801)							
G. chilensis (Guichenot, 1881)				0.5			Jara-Seguel et al. (2011)
Paralichthyidae						37.5	
Hyppoglosinna macrops Steindachner, 1876	48 (48)	24t					Winkler et al. (2004)
Paralichthys adspersus (Steindachner,	46 (48)	1m, 22t					Winkler et al. (2004)
1867)							
P. microps (Günther, 1881).	46 (48)	1m, 22t					Winkler et al. (2004)
Dyplomistidae						33.3	
Dyplomistes camposensis Arratia, 1987	56	8m, 12sm, 4st, 4t					Campos and Arratia (1997)
Nematogenyidae						100	
Nematogenys inermes	94	t; mic					Arratia and Veloso (1980)
Trichomycteridae						71.4	
Bullockia maldonadoi (Eigenmann, 1928)	60	23m-sm, 7st-t					Arratia and Campos (1997)
B. maldonadoi	60	m, sm					Arratia and Veloso (1980)
Hatcheria macraei (Girard, 1855)	52	15m-sm, 11st-t					Arratia and Veloso (1980)
Trichomycterus areolatus	54, 55 (106)	22m, 4sm, 1st		2.52			Colihueque et al. (2006)
T. areolatus	56						Arratia and Veloso (1980)
T. chiltoni (Eigenmann, 1928)	52	22m-sm, 4st-t					Arratia and Campos (1997)
T. laucaensis Arratia, 1983	58	21m-sm, 8st-t					Arratia and Campos (1997)
T. laucaensis	62	m, sm, t					Arratia and Veloso (1980)

GENOME SIZE

At present, the genome size of ca. 1,300 fish species has been studied worldwide, showing both the smallest and the largest C-values among vertebrates. For example the puffer fish *Takifugu rubripes* Temminck and Schlegel, 1850 has the smallest 1C-value with 0.4 pg, while the lungfish, *Protopterus aethiopicus* Heckel, 1851, has the largest 1C-value with 130 pg. In the case of teleost fishes, the range varies from 0.4 to 4.4 pg (Gregory, 2011).

In Chilean fishes, genome size (DNA content or C-value) has been estimated in only six species. The method used for Chilean species has been Feulgen image analysis densitometry of red blood cells, following the protocol described by Hardie et al., (2002) with little modification. This requires reference to standard species with a known C-value, the most frequently used being erythrocytes of rainbow trout and

chicken. Specialized computer programs are used to measure the optical density of the nuclei of many cells in a short time, increasing the efficiency of DNA estimation protocols.

The 1C-values estimated in Chilean species are close to the mean of 1.2 pg described for teleost fishes (Gregory, 2011); only *T. areolatus* presents double this value with 2.5 pg. Fishes have traditionally been the least investigated vertebrates in terms of the patterns and consequences of C-value diversity. Thus the study of Chilean species, especially endemic taxa, may to contribute interesting insights into processes related with genome size evolution among Osteichthyes (Smith and Gregory, 2009). Possible cases of polyploidy, such as the high chromosome number in *N. inermes*, can also be studied using nuclear DNA estimations.

CONCLUDING COMMENTS

The focus of this work is on compiling and commenting advances in cytogenetic studies of Chilean fish species. Nevertheless, it is evident that despite all the progress achieved, cytogenetic information is lacking for a high percentage of species (ca. 97%), especially insular taxa. Moreover, since the total sampled population in each species is small (only one or two), cytogeographical studies cannot be representative, especially in those genera with greater diversity and a wider distribution.

From an applied point of view, many fish species are edible and an unreasonably high number is extracted from the habitat (Habit et al., 2002), undermining conservation. In addition, a large number of species in continental environments are threatened, principally by human activity (Vila et al., 2006). In this scenario of irrational exploitation of resources, where the warnings of the global warming and its effects are recognized by the scientific community, we need to increase our knowledge of fishes, especially in relation to genetic diversity at different levels (i.e. molecular, cytogenetic and phenotype). The aim is therefore to compile information to protect and/or manage fish species. In this sense, it is broadly accepted that cytogenetics has made important contributions to knowledge about patterns of genetic variation, phylogeny, taxonomy and evolution of fishes around the world, and is a tool with great potential for improving aquaculture and the genome characterization of fishery resources (Martínez, 2005). We therefore hope that knowledge of the cytogenetics of Chilean fishes can be increased in the future, by current research groups and young specialists who follow up the study of native species, including taxa of all classes.

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