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## Cytogenetic characterization of the silverside fish *Odontesthes regia* (Humboldt, 1833) (Teleostei: Atheriniformes: Atherinopsidae) from Iquique, Chile

Caracterización citogenética del pejerrey *Odontesthes regia* (Humboldt, 1833) (Teleostei: Atheriniformes: Atherinopsidae) de Iquique, Chile

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**Abstract.-** This paper describes the karyotype of *Odontesthes regia* by means of Giemsa staining, C-banding, to reveal the distribution of the constitutive heterochromatin, and by Ag-staining and fluorescent *in situ* hybridization (FISH), to locate ribosomal genes (rDNA). The chromosome diploid modal count in the species was  $2n = 48$ . The karyotype is composed of one submetacentric pair (pair 1), 16 subtelocentric pairs (pairs 2 to 17), and 7 acrocentric pairs (pairs 18 to 24). With the exception of pair 1 it was not possible to classify the homologous chromosomes accurately because differences in chromosome size were too slight between adjacent pairs. The distribution of C-banded heterochromatin allowed for a more accurate matching of the majority of chromosomes of the subtelocentric series. Silver staining of metaphase spreads allowed for the identification of Nucleolus Organizer Regions (Ag-NOR) on pair 1. FISH experiments showed that 18S rDNA sequences were located, as expected, in the same chromosome pair identified as the Ag-NOR-bearing one.

Key words: Karyotype, NOR, C-bands, FISH

**Resumen.-** Este trabajo describe el cariotipo de *Odontesthes regia*, por medio de tinción Giemsa, bandeo C, para revelar la distribución de la heterocromatina constitutiva, y por medio de tinción con nitrato de plata e hibridación fluorescente *in situ* (FISH), para localizar genes ribosomales (rADN). El recuento modal cromosómico diploide en la especie fue de  $2n = 48$ . El cariotipo está compuesto por un par submetacéntrico (par 1), 16 pares subtelocéntricos (pares 2 al 17) y 7 pares acrocéntricos (pares 18 al 24). Con excepción del par 1, no fue posible clasificar con exactitud a los cromosomas homólogos, ya que las diferencias en el tamaño fueron muy pequeñas entre pares adyacentes. La distribución de la heterocromatina por bandeo C permitió aparear a la mayoría de los cromosomas de la serie subtelocéntrica. La tinción con plata de preparaciones metafásicas permitió la identificación de las regiones organizadoras del nucléolo (Ag-NOR) en el par 1. Los experimentos FISH mostraron que las secuencias ADNr 18S estaban localizadas, como era de esperar, en el mismo par cromosómico identificado como los portadores de Ag-ROn.

Palabras clave: Cariotipo, RON, bandeo-C, FISH

## Introduction

The great importance of conservation biology and the need to develop new tools that allow understanding ecosystems, phyletic relationship among taxa and identification of new species, has encouraged scientists to perform new karyological studies in fishes.

Cytogenetic studies of fishes from the South American continent have displayed a considerable expansion in the last years. To date cytogenetic data for 47 families, 278 genera and 1,047 freshwater species

and 39 families, 73 genera and 109 marine species are registered (Nirchio & Oliveira 2006), but for Chile, few karyological studies of fish, in relation with its richness, specially of marine fishes, have been carried out. Among these studies, the contribution of the late Dr. Hugo Campos is of great importance (Arratia & Campos 1997<sup>1</sup>, Campos 1972, Cuevas *et al.* 1999,

<sup>1</sup> Arratia G & HH Campos. 1997. Karyotypes of Diplomystid and certain loricioid catfishes. International Symposium on Phylogeny and Classification of Neotropical Fishes The Neotropical Ichthyological Association, Porto Alegre, Brazil

Table 1

## Chromosome data of Atheriniformes

Datos cromosómicos de Atheriniformes

| Species                        | 2n | NF  | Karyotype formula      | Reference  |
|--------------------------------|----|-----|------------------------|--|
| <i>Atherion elymus</i>         | 48 | 50  | 2SM + 10ST + 36A       | Arai & Fujiki (1978)                             |
| <i>Atherina monchon</i>        | 48 | 54  | 6SM + 42 ST-A          | Vasiliev (1980, 1985)                            |
| <i>Basilichthys australis</i>  | 48 | 57  | 4M + 5SM + 39ST-A      | Gajardo (1992)                                   |
| <i>B. banariensis</i>          | 48 | 52  | 4M + 44 ST-A           | Arai & Koike (1980)                              |
| <i>B. microlepidotus</i>       | 46 | 62  | 2M + 14SM + 30ST-A     | Gajardo (1992)                                   |
| <i>Bedotia geayi</i>           | 48 | 72  |                        | Scheel (1972) in Klinkhardt <i>et al.</i> (1995) |
| <i>Chirostoma attenuatum</i>   | 48 | 76a | 4M + 24SM + 2ST + 18A  | Alvarez-Espíndola (1994)                         |
| <i>C. estor</i>                | 48 | 68  | 12M + 8SM + 12ST + 16A | Uribe-Alcocer <i>et al.</i> (2002)               |
| <i>C. jordani</i>              | 48 | 68  | 8M + 12SM + 10ST + 18A | Uribe-Alcocer <i>et al.</i> (2002)               |
| <i>C. patzcuaro</i>            | 44 | 44  | 12ST + 32A             | Uribe-Alcocer <i>et al.</i> (2002)               |
| <i>C. grandocule</i>           | 48 |     |                        | Durán-González <i>et al.</i> (1997)              |
| <i>Labidesthes sicculus</i>    | 48 | 82  | 12M + 22SM + 14ST-A    | Korth & Fitzsimons (1987)                        |
| <i>Membras martinico</i>       | 48 | 84  | 18M + 18SM + 12ST-A    | Korth & Fitzsimons (1987)                        |
| <i>Menidia berilina</i>        | 48 | 74  | 8M + 18SM + 22ST-A     | Korth & Fitzsimons (1987)                        |
| <i>M. berilina</i>             | 48 | 82  | 10M + 24SM + 14ST-A    | Korth & Fitzsimons (1987)                        |
| <i>M. mendia</i>               | 48 | 66  | 4M + 14SM + 12ST + 18A | Warkentine <i>et al.</i> (1987)                  |
| <i>Odontesthes bonariensis</i> | 48 | 52  | 4SM + 44 + 46ST        | Sola <i>et al.</i> (1988)                        |
| <i>O. bonariensis</i>          | 48 | 52  | 4SM + 44ST-A           | Arai & Kioke (1980)                              |
| <i>O. regia</i>                | 48 | 50  | 2SM+32ST+14A           | Present study                                    |
| <i>Telmatherina ladiges</i>    | 48 | 86  |                        | Scheel (1972) in Klinkhardt <i>et al.</i> (1995) |

Valenzuela *et al.* 1992<sup>2</sup>, Campos *et al.* 1995<sup>3</sup>). A recent paper by Winkler *et al.* (2004) described the karyotype of three species of flounders from Chile.

*Odontesthes regia* (Humboldt, 1833) (Teleostei: Atheriniformes) belongs to the Atherinopsidae family and is locally known as “pejerrey” or “pejerrey marino,” and internationally as “sea silverside.” This is an epipelagic species distributed from the north Pacific coast of Peru to the Aysen region, in Chile (Dyer 2000). The species has great economic importance as a fresh staple food (Froese & Pauly 2005). Although the

family Atherinopsidae includes 104 species (13 genera), only 17 species have been cytogenetically studied to date (Table 1), demonstrating that cytogenetic studies in this group are still scarce.

As part of a project to study the cytogenetic biodiversity of the Chilean ichthyofauna, this paper is the first report of the karyotype of *O. regia* from Iquique, Chile by means of Giemsa staining, C-banding, silver staining to locate active Nucleolus Organizing Regions (NORs), and FISH to locate the NOR cistrons on the chromosomes.

## Materials and methods

A sample comprising five specimens (2 females and 3 males) of *O. regia* was analyzed. Specimens were seined off the coast of Iquique Bay (20°10.15'S 70°10.18'W). Voucher specimens were kept at the fish collection of Departamento de Ciencias del Mar (MUAP-PO: 0893, 0894, 0895, 0896, 0897), Universidad Arturo Prat, Iquique, Chile.

<sup>2</sup> Valenzuela AC, HF Alay, EJ Gavilan & CH Campos 1992. Contribución al estudio citogenético de peces dulceacuícolas en Chile: Los cromosomas somáticos de *Cheirodon galusdae*, *C. australae* y *Percichthys trucha*. XXV Reunión anual Sociedad de Genética de Chile, La Serena, Chile, 26-29 de agosto, 1992 (<http://www.conicyt.cl/bases/bibfon/0/0/920200.html>)

<sup>3</sup> Campos CH, C Cuevas & EJ Gavilán. 1995. Cariotipos de *Aplocheilichthys zebra* y *Aplocheilichthys taeniatus* (Pisces: Salmoniformes). Proyecto FONDECYT: 92/0200. (<http://www.conicyt.cl/bases/bibfon/0/0/920200.html>)

Chromosome preparations were carried out following the technique described by Bertollo *et al.* (1978). For the conventional karyotype, the preparations were stained for 20 minutes with 5% Giemsa in a phosphate buffer at pH 6.88. C-bands were obtained according to Sumner (1972). Detection of the Nucleolus Organizer Regions (NORs) was carried out following the silver staining method of Howell & Black (1980), and by FISH according Pinkel *et al.* (1986). A tilapia (*Oreochromis niloticus*) 18S rDNA sequence (about 1,800 base pairs) cloned in pGEM-T was labeled by nick translation with biotin-14-dATP according to the manufacturer instructions (Bionick™ Labelling System-Gibco. BRL). The 18S rDNA sequences were located in the chromosomes by Avidin-N-fluorescein Isothiocyanate (FITC) conjugate, and the signal was enhanced by using biotinylated Anti-avidin goat antibodies following a second round of the Avidin-FITC detection. Chromosomes were counter-stained with Propidium Iodide (50µg/ml) diluted in Antifade.

Mitotic chromosomes were photographed using a digital camera and images were digitally processed with Adobe Photoshop v. 7.0. The karyogram was constructed with chromosomes organized in size-decreasing order. Chromosomes were classified following Levan *et al.* (1964). Metaphases analyzed by FISH were examined in a Zeiss Axiophot photomicroscope and pictures were taken with a Kodak Gold Ultra 400 ASA film.

## Results and discussion

Chromosome diploid modal count was  $2n=48$ , obtained in 82% of all the (107) cells examined. The representative karyogram for the species is shown in Fig. 1A. The karyotype of this species consists of one submetacentric pair (pair 1), 16 subtelocentric pairs (pairs 2 to 17), and 7 acrocentric pairs (pairs 18 to 24). With the exception of pair 1, it was not possible to carry out an accurate classification of chromosomes as homologous pairs due to their similar morphology and slight differences in chromosome size.

According to the most recent review of fish chromosome data, 11.5% of the extant fish species possess a karyotype with  $2n = 48$  and a variable Fundamental Number (FN) (Klinkhardt *et al.* 1995). A recent study (Miya *et al.* 2003) suggests that orders Atheriniformes, Cyprinodontiformes, and Beloniformes constitute a monophyletic group closely related to

Mugiliformes and some Perciformes. Considering that the most common diploid number found in Mugiliformes and Perciformes is  $2n = 48$  (Klinkhardt *et al.* 1995), it is possible to suggest that the presence of  $2n = 48$  chromosomes, as observed in *O. regia*, represents a primitive characteristic of the order Atheriniformes. Despite the conserved diploid number in the genus *Odonthestes*, a clear difference in chromosome formulae is observed between *O. bonariensis* and *O. regia*. Sola *et al.* (1988) and Arai & Kioke (1980) reported the karyotype of *O. bonariensis* as possessing 48 chromosomes with 4 submetacentric and 44 subtelocentric-acrocentric and  $FN = 52$ , so determined by considering subtelocentric elements as unarmed-chromosomes. If for comparative purposes we consider the subtelocentric chromosomes of *O. regia* as unarmed, the species would have a  $FN=50$ , so the differences in chromosome formulae between these two species suggest that their karyotypes could have diverged by rearrangements involving a pericentric inversion to convert a subtelocentric chromosome into a submetacentric or into an acrocentric one, respectively (or vice versa) while at the same time keeping the numeric hypothetical chromosome condition  $2n = 48$ .

C-banded heterochromatin is distributed in a pericentromeric position in all chromosomes except a large positive interstitial band near the centromere in pair 6, and a large telomeric segments in chromosome pairs 3 and 7. Such distribution of C-banded heterochromatin allows for a more accurate, tentatively homologous chromosome pairing in the majority of chromosomes of the subtelocentric series (Fig. 1B).

Silver staining revealed that the Ag-NOR clusters are localized on pair 1 with the Ag-NOR signal located on the tip of their short arms (Fig. 2). Sola *et al.* (1988) reported the single pair of NORs in *O. bonariensis* as terminal on the short arm of a small-sized submetacentric chromosome.

NOR-silver staining is one of the methods used to locate 18S and 28S rRNA cistrons in chromosomes (Howell & Black 1980). Silver stainability of NORs requires transcriptional activity of the ribosomal genes during the preceding interphase (see Hubbel 1985, Sánchez-Pina *et al.* 1984, Jimenez *et al.* 1988), whereas FISH allows for the exact detection of the location and number of ribosomal genes in the chromosome complement.



Figure 1

Cytogenetic characteristics of *Odontesthes regia*. (A) Giemsa stained karyotype. Above pair 1 the same chromosome pair stained with silver nitrate to show the Ag-NORs (black dots). (B) C-banded karyotypes

Características citogenéticas de *Odontesthes regia*. (A) Cariotipo teñido con Giemsa. Sobre el par 1, el mismo par de cromosomas teñido con nitrato de plata para mostrar las Ag-NOR (puntos negros). (B) Cariotipos de bandeo C

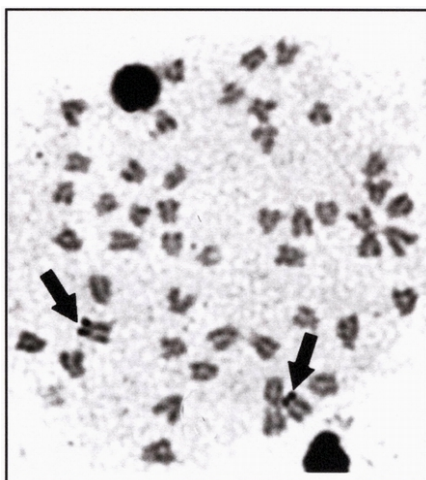


Figure 2

Silver-stained metaphase of *Odontesthes regia* showing Ag-NOR-bearing chromosomes (arrows)

Metafasas de *Odontesthes regia* teñidas con plata mostrando los cromosomas que tienen Ag-NOR (flechas)

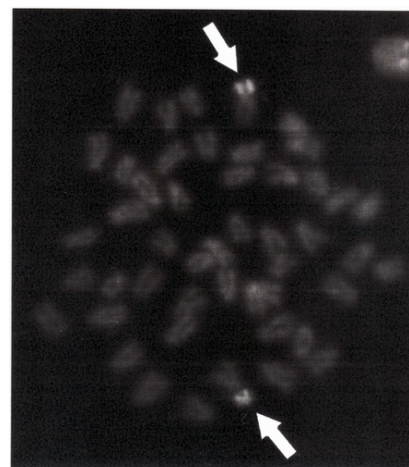


Figure 3

Metaphase of *Odontesthes regia* showing chromosomes after fluorescent *in situ* hybridization with the 18S rDNA gene. Arrows point out the 18S-bearing chromosomes

Metafase de *Odontesthes regia* mostrando los cromosomas después de hibridación fluorescente *in situ* con el gen 18S rADN. Las flechas indican los cromosomas que llevan el 18S

As far as ribosomal cistrons are concerned, the most frequent localization of NORs in fish chromosomes is terminal on a single chromosome pair (Galetti *et al.* 1984, Birstein & Vasilev 1987, Klinkhardt *et al.* 1995), although some taxa exhibit multiple NORs (Foresti *et al.* 1981, Nirchio *et al.* 2002, Gaviria *et al.* 2005, Phillips *et al.* 1988, Castro *et al.* 1996). The use of FISH with 18S rDNA probing is a technique extremely well suited to characterize a species; it allows researchers to know whether differences in Ag-NOR number are related only with the differential activities of these regions or with different numbers of NORs in the genome of different specimens (Gornung *et al.* 1997, Nirchio *et al.* 2003). The FISH experiments performed here show that 18S rDNA signals coincide with signals seen in the Ag-NOR-bearing chromosomes (Fig. 3), indicating that *O. regia* does not possess additional NOR sites and that all NOR cistrons are active.

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