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Short Communication

Nuclear DNA content in *Galaxias maculatus* (Teleostei: Osmeriformes: Galaxiidae)

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ABSTRACT. The nuclear DNA content (2C value) was determined in the commercial fish *Galaxias maculatus* (Galaxiidae) was determined by microdensitometry of erythrocyte nuclei after Feulgen staining; rainbow trout erythrocytes with a known 2C value were used as a standard. The 2C value of *G. maculatus* was 2.21 ± 0.12 pg and its C value was equivalent to 1.105 pg (1,082.9 Mbp). This C value is within the range recorded for other osmeriform species (0.62-3.2 pg). The average sperm head diameter of *G. maculatus* is lower than the average sperm head diameter of rainbow trout (used as a standard), which coincides with the differences observed in the nuclear DNA content of both species. This information increases the genome data available for *G. maculatus* and might be useful in future programs dealing with its genetic manipulation.

Keywords: *Galaxias maculatus*, nuclear DNA content, nucleotypic effect, Galaxiidae, Osmeriformes, Chile.

Contenido de ADN nuclear en *Galaxias maculatus* (Teleostei: Osmeriformes: Galaxiidae)

RESUMEN. El contenido de ADN nuclear (valor 2C) fue determinado en el pez comercial *Galaxias maculatus* (Galaxiidae) usando microdensitometría de núcleos de eritrocitos sometidos a tinción de Feulgen, utilizando como estándar eritrocitos de trucha arco iris con un valor 2C conocido. El valor 2C de *G. maculatus* fue 2,21 ± 0,12 pg y su valor C es equivalente a 1,105 pg (1.082,9 pMb). Este valor C está dentro del rango registrado para otras especies de osmeriformes (0,62-3,2 pg). El diámetro promedio de la cabeza del espermatozoide de *G. maculatus* es menor al promedio descrito para la trucha arco iris utilizado como estándar, lo que coincide con las diferencias observadas en el contenido de ADN nuclear entre ambas especies. Estos datos contribuyen a ampliar los antecedentes genómicos disponibles para *G. maculatus* y podrían ser útiles en futuros programas tendientes a su manipulación genética.

Palabras clave: *Galaxias maculatus*, contenido de ADN nuclear, efecto nucleotípico, Galaxiidae, Osmeriformes, Chile.

Galaxias maculatus (Galaxiidae), is a small short-life fish, that inhabits in freshwater, estuarine and marine environments, having circum-antarctic distribution, and occurring in South America, Oceania and South Africa (Campos, 1984). In Chile, *G. maculatus* is distributed from central to the southern region (32°-53°S) (Campos, 1970), and in recent years estuarine and inland water populations have been cultured with commercial purpose. At present, many data from this species are widely known such as distribution, systematic, biogeography and reproduction (Campos, 1970, 1973, 1979, 1984, 1985; Peredo & Sobarzo, 1993, 1994; Barriga *et al*., 2002; Cussac *et al*., 2004), and have been applied in programs focused to its intensive production (Barile *et al*., 2003). However, other attractive antecedents for aquaculture such as the genome structure has not been studied for *G. maculatus*. The only genome data known for this species include chromosome number (2n = 22), karyotype morphology (Campos, 1972; Merri-

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lees 1975; Johnson *et al*., 1981), and mitochondrial DNA sequences, but all have been focused to its biosystematics circumscription within the eusteleostean (Waters *et al*., 2000; Ishiguro *et al*., 2003).

Respect to the genome structure, the DNA content information is used in a wide number of biological fields, since it is positively correlated with a wide variety of cellular and organismal parameters, which is known as nucleotypic effect (Bennett, 1972). Regarding to fishes, the DNA content of about 1,354 species has been estimated and represents the largest data set for any vertebrate group (Gregory *et al*., 2007). Within the Osteichthyes, the Salmoniformes are one of the best-studied fish groups with regard to DNA content, with about 37 species assessed. Many of these species belong to the genera *Oncorhynchus*, *Salmo* and *Salvelinus*, all of them of interest for aquaculture field. Different is the case of the close-related order Osmeriformes with 240 recognized species, where the DNA content has been assessed just in 10 species (Gregory, 2007), but the genus *Galaxias* has not been examined.

The aim of the present study is to estimate the DNA content of the diploid genome in freshwater individuals of *G. maculatus* from a natural population in Hornopiren locality, Región de Los Lagos, Chile. The specimens have been maintained in hatcheries at the Aquaculture School of the Universidad Católica de Temuco.

Measurements of nuclear DNA content (2C-value) were done microdensitometrically in erythrocytes obtained from adult specimens, using the software Image Pro-Plus 4.0. The blood was dispersed on slides, air dried, fixed in methanol-acetic acid (3:1 v/v) at 4ºC for 24 h and stained with the Feulgen reaction (hydrolysis with 5N HCl for 60 min at room temperature, staining with Schiff´s reagent for 60 min, followed for three washes of 5 min each in sulphurous water). The software captures black and white image from the microscope Nikon Eclipse 400 and analyses the different structures visible on the images. Nuclear optic density (OD) is calculated by the software according to the formula $OD = \log_{10}(1/T) = -\log_{10}T$; where T = intensity of transmitted light/intensity of incident light. From this estimation, the computer integrates the values of OD obtained for each one of the pixels and it calculates the integrated optical density (IOD = ΣOD). For *G. maculatus*, values of IOD of 199 individuals nuclei were determined. The IOD values were converted to absolute mass of DNA by comparison with erythrocyte smears of rainbow trout (*Oncorhynchus mykiss,* 2C = 5.5 pg, 2n = 58-60) (Hartley & Horne, 1985) a common standard to determine DNA content in fishes using flow cytometry (Tiersch *et al*., 1989) or Feulgen microdensitometry (Carvalho *et al*., 2002). The standard erythrocyte smears were included at the same staining runs and IOD estimations that the cells of *G. maculatus*. The 2C-value was determined using the equation CVu = CVs x (IODu/IODs). In the equation, CVu = 2C value of *G. maculatus*; CVs = 2C value of standard; IODu = average IOD of *G. maculatus*; IODs = average IOD of standard. DNA content of the haploid genome (or C-value) was indirectly estimated as 2C/2, where 2C corresponds to the estimated somatic DNA content and 2 is the number of chromosome sets of the genome (2n = 22, n = 11). The C-value in picograms (mass of ADN in pg) was expressed in megabase pairs (Mbp) using the relations 1 pg = 980 Mbp proposed by Cavallier-Smith (1985). To evaluate the nucleotypic effect over cell dimensions, the average sperm heads diameter [(higher length in μm + lower length μm)/ 2] was determined in *G. maculatus* and compared with the average sperm heads diameter of the standard rainbow trout (*t*-Student at confidence level of 95%). The sperm smears of both species were done by semen dilution in freshwater, and then the sperm heads were observed in phase contrast with a Nikon Eclipse 400 microscope. The sperm heads were measured using the software QCapture Pro 5.1 and photographed with a digital camera QImaging Micropublisher 3.3.

The 2C-value of *G. maculatus* measured in erythrocytes was estimated to be 2.21 ± 0.12 pg (average IOD = 4.99 arbitrary units), with a coefficient of variation of 5.4%. Due to *G. maculatus* is diploid (2n = 22, n = 11) (Campos, 1972; Merrilees, 1975; Johnson *et al*., 1981) its C-value is 1.105 pg and is equivalent to 1,082.9 megabase pairs (Mbp). The IOD value for erythrocytes of rainbow trout was 12.44 ± 1.12 arbitrary units with a coefficient of variation of 9% (Figs. 1a and 1b). The average sperm head diameter of *G. maculatus* (2.18 ± 0.07 μm) was lower than the average sperm head diameter determined for the standard rainbow trout (2.93 ± 0.08 μm) ($p < 0.05$) (Figs. 2a and 2b), which is coincident with the differences observed between their C-values.

In fishes, the C-value varies in different degrees among the different groups examined. In the case of the teleostean order Osmeriformes, the lower C-value has been described for the anadromous *Osmerus eperlanus* (Osmeridae) with 0.62 pg (607.6 Mbp) whereas the higher C-value was described for the marine *Bathylagus pacificus* (Bathylagidae) with 3.2 pg (3,136 Mbp) (Gregory, 2007). The C-value of *G. maculatus* documented here for the first time, is within the range previously described for osmeriform species and near to the average C-value of 1.2 pg described for teleost. It is remarkable that the smallest fish C-value has been described for the puffer-fish *Fugu rubripes* with C = 0.4 pg (392 Mbp) (Tetraodontidae), whereas the largest C-value has been reported for the lung-fish *Protopterus aethiopicus* with C = 130 pg (127,400 Mbp) (Protopteridae) (Gregory, 2007). In an updated eukaryotic genome size database was reported that the average C-value for all fish groups pooled is 20.6 pg (Gregory *et al*., 2006)

It is widely known that the C-value is a genomic character positively correlated with phenotypic parameters such as cell size, duration of mitotic cycle, meiosis and gametogenesis, embryo development complexity, metabolic rate, oxygen consumption and the life cycle span (Gregory, 2002a, 2002b; Olmo, 2003; Gallardo *et al*., 2003, 2004). At present, the relationship between genome size and cell size is well established in fishes (Gregory, 2007). The present study, shows the significant differences in average sperm head diameter between *G. maculatus* and *O. mykiss* to be coincident with the differences observed in their C-values. These differences suggest the occurrence of so-called nucleotypic effect in gametes of those Protacanthopterygii fishes, such as has been previously described by comparing erythrocyte dimensions between both species (Jaramillo, 2005). Additionally, lower haematological parameters (*e.g*. low erythrocyte number per plasma volume, low hemoglobine content per cell) were

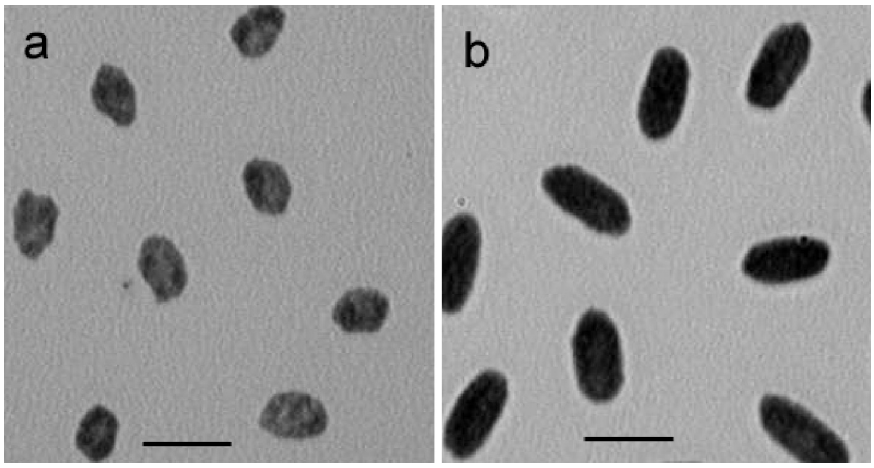

Figure 1. Erytrhocyte nuclei of a) *Galaxias maculatus,* and b) *Oncorhynchus mykiss* stained with the Feulgen reaction. Bar = 5 *µ*m.

Figura 1. Núcleos de eritrocitos de a) *Galaxias maculatus,* y b) *Oncorhynchus mykiss* sometidos a tinción con la reacción de Feulgen. Barra = 5 *µ*m.

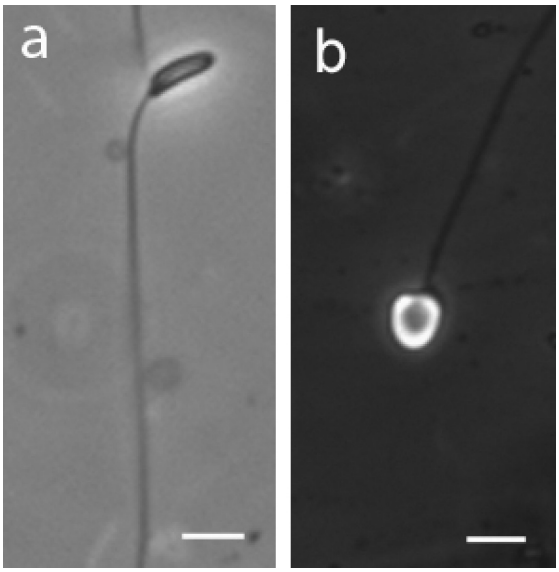

Figure 2. Sperm heads of a) *Galaxias maculatus,* and b) *Oncorhynchus mykiss* observed with phase contrast microscope. Bar = 2 *µ*m.

Figura 2. Cabezas de espermatozoides de a) *Galaxias maculatus,* y b) *Oncorhynchus mykiss* observadas con microscopio de contraste de fase. Barra = 2 *µ*m.

observed in *G. maculatus* when compared with *O. mykiss* and other salmoniform species (Jaramillo, 2005), which can also be evidence of nucleotypic effect regarding a physiological level. Although obvious physiological implications (*e.g.* swimming daily activity, metabolic rate, oxygen consumption) might be related with those lower haematological parameters of *G. maculatus* (Jaramillo, 2005), little has been investigated in fishes (Lay & Baldwin, 1999), but abundant information on nucleotypic effect regarding physiologic patterns is available for amphibians, reptiles, birds and mammals (Gregory, 2001, 2002b). Therefore, all the information available for vertebrate classes might be used as theoretical basis to understand the fish tendency relationship among DNA content and physiological parameters, data that should be complemented with experimental assays in species maintained in controlled systems such as the hatcheries.

In the future, other species of the family Galaxiidae could be included in studies on DNA content (*e.g. Galaxias platei*, *G. globiceps*, *Brachigalaxias bullocki*, *B. gothei*, *Aplochiton zebra, A. marinus*, *A. taeniatus*), where the nucleotypic effect in co-family members analyzed together may be assessed by mean of cell dimensions or by the efficiency in physiologic (metabolic rate, oxygen consumption, salinity equilibrium, haematological parameters) and reproductive processess (meiosis and gametogenesis, embryonic development), all phenotypic characters of importance in aquaculture production. On the other hand, the study of nucleotypic effect might be an important parameter to evaluate future results of possible polyploidy induction in *Galaxias* cultured species (*e.g. G. maculatus*, *G. platei*), on the basis that polyploidy induction is a chromosome manipulation technique whose purpose is increasing the chromosome sets (and nuclear DNA content) and consequently the body-biomass of fishes. Body-biomass increase obtained by polyploidization which has been of valuable interest for salmonids and cyprinids aquaculture (Chorrout *et al*., 1986; Valdebenito *et al*., 1996; Gomelsky, 2003; Comber & Smith, 2004; Pineda *et al*., 2004), would be also applicable to other fishes species.

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