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Short Communication

Karyotype and nuclear DNA content of *Trichomycterus areolatus* (Siluriformes, Trichomycteridae)

Nelson Colihueque, Olga Corrales and Margarita Parraguez

Universidad de Los Lagos, Departamento de Ciencias Básicas, Laboratorio de Genética y Acuicultura, Osorno, Chile

Abstract

Cytogenetic analysis of *Trichomycterus areolatus*, collected from the Tijeral and Huilma Rivers in southern Chile has shown a diploid chromosome number of 2n = 54, a fundamental number of FN = 106, and a karyotypic formula of 44m + 8sm + 2st. Intra-individual polymorphism of chromosome number (2n = 54, 55 and 56) in specimens from the Huilma River has also been documented, providing further evidence of the occurrence of this phenomenon in *Trichomycterus*. The karyotype exhibited large chromosome pairs: metacentric pairs 1 (relative length 7.54%), 2 (5.75%) and 3 (5.09%), submetacentric pair 23 (5.25%), and subtelocentic pair 27 (5.28%). Nuclear DNA content analysis showed an average value of 5.04 ± 1.09 pg/nucleus. This DNA content is higher than the mean value described for other species in this genus.

Key words: karyotype, nuclear DNA content, *Trichomycterus*, Siluriformes.

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Trichomycterus areolatus is a small native catfish (< 10 cm length) that inhabits the Andean river basins in Chile (Arratia, 1981; Vila *et al*., 1999). This species belongs to the family Trichomycteridae, which is native to southern Central America and South America (Berra, 1990) and comprises nine sub-families, 37 genera and 156 species (Nelson, 1994). The subfamily Trichomycterinae includes six genera: *Bullockia*, *Eremophilus*, *Hatcheria*, *Rhizosomichthys*, *Scleronema* and *Trichomycterus* (Nelson, 1994), the latter being the most diversified, possibly with more than 75 species, widely distributed throughout South America, from the Atlantic coast to the Pacific. Chromosome data obtained for the genus *Trichomycterus* from various species distributed in Brazil and Argentina show that this group has a constant diploid chromosome number (2n = 54), but the number of chromosome arms or fundamental number (FN) varies (FN = 84, 104, 106 or 108) (Torres *et al*., 1998; Borin and Martins-Santos, 1999; Gonzo *et al*., 2000; Sato *et al*., 2004). In Chile, *T. areolatus* presents different diploid chromosome numbers: 2n = 54 (Arratia and Veloso, 1980) and 2n = 56 (Arratia and Campos, 1997). In this study, we present data on various cytogenetic parameters (diploid chromosome number, fundamental number, chromosome size, chromosome formula and nuclear DNA content) in two populations of *T. areolatus* that inhabit southern Chile. This analysis provides further information that contributes to our understanding of the evolutionary process that occurred in this species of the Trichomycteridae family.

Send correspondence to N. Colihueque. Universidad de Los Lagos, Departamento de Ciencias Básicas, P.O. Box 933, Osorno, Chile. E-mail: ncolih@ulagos.cl.

The specimens studied were collected from the Tijeral and Huilma Rivers, in the south of Chile (Bueno River hydrographic basin, Province of Osorno, 10th Region). Chromosome preparations were obtained by the squash method from bronchial epithelium of the fishes, previously treated with an intraperitoneal colchicine injection (0.5% p/v). Thirteen individuals from the Tijeral River (two males, two females and nine of unknown sex) and twelve from the Huilma River (two males, two females and eight of unknown sex) were analyzed. The chromosomes were stained with 4% Giemsa solution. Chromosome morphology was identified based on the centromeric index, following the protocol of Levan *et al*. (1964). For the fundamental number determinations, it was assumed that metacentric and submetacentric chromosomes are two-armed chromosomes, and the subtelocentric and telocentric (= acrocentric) are one-armed chromosomes. The length of the short arm (SA) and long arm (LA) of each chromosome pair of the karyotype was obtained, and the absolute values were standardized as relative lengths over the total length of the haploid chromosome complement. For each chromosome pair, the total relative length (TRL) in percentage and the total absolute length (TAL) in μm were also recorded. The nuclear DNA content of *T. areolatus* was determined by

microdensitometry, using Feulgen-stained nuclei of erythrocytes. For this analysis, quantification of the nuclear DNA content involved the use of chicken Feulgen-stained nuclei erythrocytes as a standard, which were used to transform the relative values of each nucleus measurement into absolute values in DNA picograms (pg), using the value established for this species (2.49 pg/nucleus, according to Johnston *et al*., 1999). Ten individuals were used for this analysis.

Both the Tijeral and the Huilma River samples had a modal diploid chromosome number of 2n = 54. The distribution of cells from specimens collected in the River Tijeral was as follows: two had 50 chromosomes, one had 51 chromosomes, seven had 52 chromosomes, 48 had 54 chromosomes, three had 55 chromosomes, and two had 56 chromosomes. The distribution of cells from specimens collected in the Huilma River was: six cells had 50 chromosomes, 12 had 51 chromosomes, nine had 52 chromosomes, two had 53 chromosomes, 62 had 54 chromosomes, 15 had 55 chromosomes, and 25 had 56 chromosomes. Thus, 76.2% (48/63) and 50.8% (62/122) of the metaphase plates studied had a modal diploid number of 54 chromosomes, in the samples from the Tijeral and the Huilma Rivers, respectively. The chromosome formula, based on centromeric index values, showed that in both populations the karyotype consisted of 44m + 8sm + 2st, with a fundamental number of FN = 106 (Table 1). The chromosome pairs with the largest TRL were metacentric pairs 1 (7.54%), 2 (5.75%), and 3 (5.09%); submetacentric pair 23 (5.25%), and subtelocentric pair 27 (5.28%) (Table 1). These pairs were easily identified in the karyotype (Figure 1a).

The diploid number of 2n = 54 that we observed in *T. areolatus* was also described by Arratia and Veloso (1980), but differs from another chromosome analysis which re-

Table 1 - Values of karyotype parameters in *T. areolatus* (2n = 54) (n = 9 chromosome metaphase plates): average relative length in percentage of short arm (SA) and long arm (LA) of each chromosome pair are shown; total relative length (TRL) and total absolute length (TAL) of each chromosome pair are also shown. The chromosome types were classified according to the centromeric index (CI). SD = standard deviation.

Chromosome pair	SA	LA				
	Av ± SD	Av ± SD	CI ± SD	TRL ± SD (%)	TAL ± SD (μm)	Chromosome type
1	3.13 ± 0.48	4.41 ± 0.88	41.23 ± 2.09	7.54 ± 1.33	2.11 ± 0.88	m
2	2.56 ± 0.49	3.19 ± 0.34	43.86 ± 2.65	5.75 ± 0.79	1.60 ± 0.62	m
3	2.31 ± 0.28	2.78 ± 0.33	44.93 ± 2.41	5.09 ± 0.50	1.39 ± 0.44	m
4	2.15 ± 0.15	2.60 ± 0.27	45.21 ± 3.04	4.75 ± 0.29	1.30 ± 0.37	m
5	2.01 ± 0.22	2.45 ± 0.11	44.40 ± 2.24	4.46 ± 0.23	1.20 ± 0.33	m
6	1.89 ± 0.24	2.30 ± 0.13	44.21 ± 2.49	4.19 ± 0.27	1.12 ± 0.30	m
7	1.75 ± 0.25	2.17 ± 0.16	44.29 ± 4.13	3.92 ± 0.29	1.16 ± 0.28	m
8	1.75 ± 0.21	2.08 ± 0.16	45.24 ± 2.47	3.83 ± 0.32	1.03 ± 0.25	m
9	1.65 ± 0.17	2.03 ± 0.12	44.33 ± 1.41	3.68 ± 0.24	0.99 ± 0.24	m
10	1.62 ± 0.15	1.96 ± 0.14	44.72 ± 2.36	3.59 ± 0.17	0.97 ± 0.24	m
11	1.64 ± 0.14	1.86 ± 0.12	46.40 ± 2.05	3.50 ± 0.19	0.94 ± 0.23	m
12	1.51 ± 0.13	1.83 ± 0.12	45.11 ± 2.44	3.34 ± 0.17	0.90 ± 0.22	m
13	1.48 ± 0.14	1.75 ± 0.12	45.63 ± 2.04	3.23 ± 0.20	0.87 ± 0.20	m
14	1.39 ± 0.22	1.73 ± 0.14	43.38 ± 1.67	3.13 ± 0.24	0.83 ± 0.20	m
15	1.43 ± 0.17	1.62 ± 0.17	46.37 ± 3.34	3.05 ± 0.21	0.81 ± 0.18	m
16	1.35 ± 0.16	1.56 ± 0.16	46.17 ± 3.22	2.90 ± 0.22	0.78 ± 0.17	m
17	1.29 ± 0.17	1.49 ± 0.10	46.16 ± 2.41	2.78 ± 0.24	0.74 ± 0.16	m
18	1.21 ± 0.20	1.46 ± 0.14	44.86 ± 3.31	2.67 ± 0.27	0.71 ± 0.15	m
19	1.19 ± 0.17	1.34 ± 0.14	46.66 ± 1.34	2.53 ± 0.28	0.67 ± 0.16	m
20	1.11 ± 0.22	1.28 ± 0.16	45.85 ± 3.87	2.39 ± 0.34	0.64 ± 0.16	m
21	1.05 ± 0.12	1.28 ± 0.13	45.11 ± 3.85	2.32 ± 0.18	0.63 ± 0.15	m
22	1.13 ± 0.37	1.50 ± 0.80	44.00 ± 4.53	2.63 ± 1.17	0.71 ± 0.35	m
23	2.00 ± 1.19	3.25 ± 0.90	35.12 ± 2.19	5.25 ± 1.34	1.35 ± 0.49	sm
24	1.34 ± 0.45	2.51 ± 0.45	32.45 ± 3.89	3.85 ± 0.72	1.05 ± 0.46	sm
25	1.15 ± 0.38	1.97 ± 0.18	34.22 ± 3.47	3.12 ± 0.31	0.83 ± 0.29	sm
26	0.95 ± 0.26	1.72 ± 0.32	33.98 ± 5.37	2.67 ± 0.32	0.71 ± 0.22	sm
27	1.55 ± 0.76	3.73 ± 1.24	26.19 ± 1.82	5.28 ± 1.63	1.43 ± 0.75	st

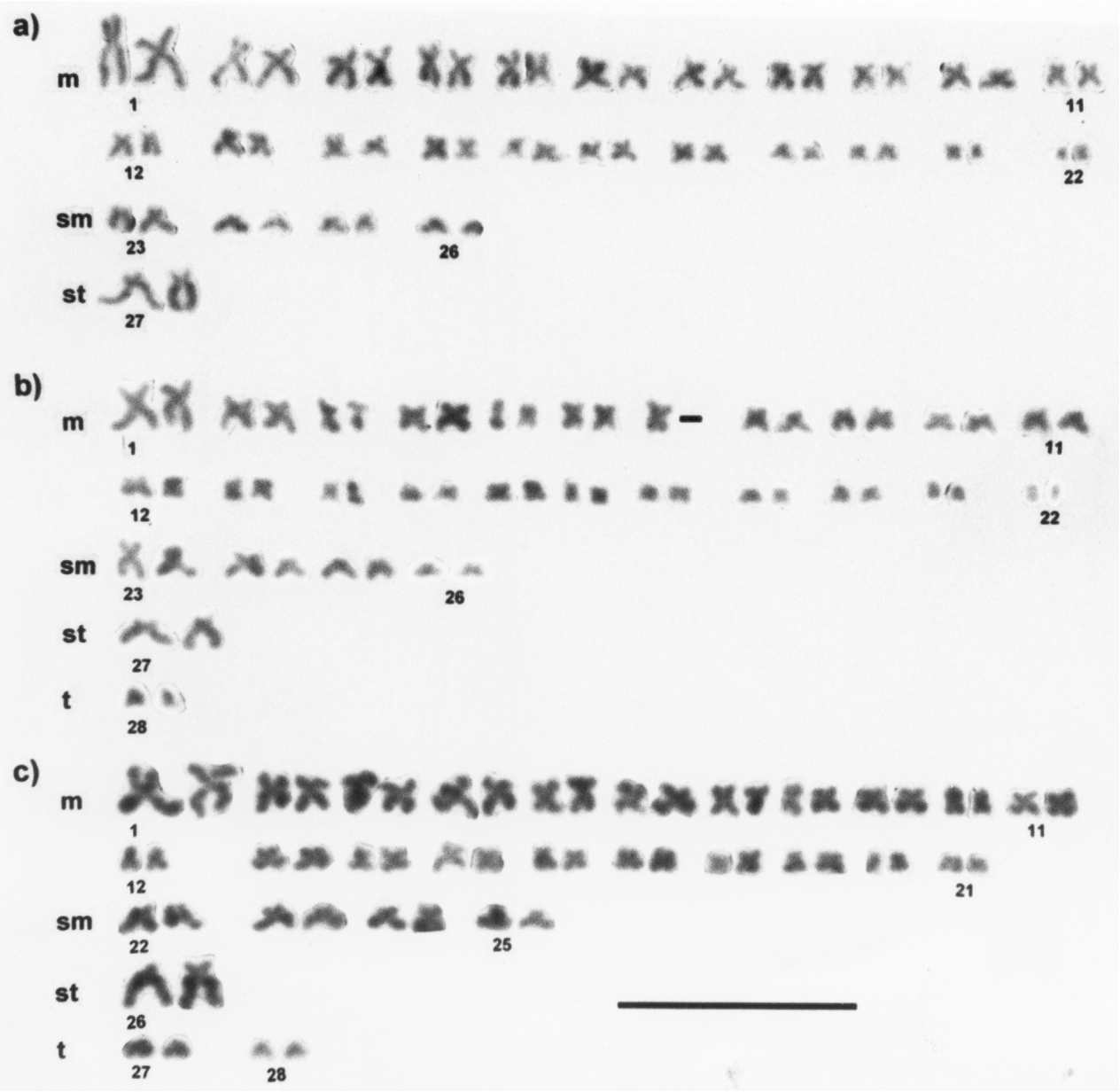

Figure 1 - Karyotypes of *T. areolatus* from Chile. a) Karyotype with 2n = 54 chromosomes of the Tijeral and Huilma River populations. In b) and c) karyotypes with 2n = 55 and 2n = 56 chromosomes, respectively, representing the intra-individual numerical chromosome variation observed in some specimens of the Huilma River. Chromosome morphologies: m = metacentric, sm = submetcentic, st = subtelocentric, and t = telocentric (= acrocentric). Bar represents 5 μm.

ported 2n = 56 (Arratia and Campos, 1997). These data indicate the occurrence of intraspecific polymorphism for diploid chromosome number in Chilean *T. areolatus*. Further studies are necessary to determine the extent of this type of chromosome variation.

The 2n = 54 diploid number here described in *T. areolatus* has been reported in most of the *Trichomycterus* species (Torres *et al*., 1998; Borin and Martins-Santos, 1999; Gonzo *et al*., 2000; Sato *et al*., 2004). Therefore, this diploid number appears to be conserved within the genus. However, not all the *Trichomycterus* species with 2n = 54 have the same chromosome formula. This becomes apparent by comparing *T. areolatus* [44m + 8sm + 2st] (this study) with *T. paolence* [46m + 6sm + 2st] (Torres *et al*., 1998), *T. Stawiarski* [42m + 8sm + 4st] (Borin and Martins-Santos, 1999) and *T. davisi* [42m + 10sm + 2st] (Sato *et al*., 2004). These interspecific chromosome differences suggest that structural rearragements must have occurred during the chromosomal evolution of these species.

Sato *et al*. (2004) grouped the cis-Andean *Trichomycterys* species into two groups, according to the size of the first metacentric chromosome pairs and the loca-

tion of NORs. In group 1, the first metacentric pair was considerably larger than the second, and the Ag-NOR was located in a pericentromeric position on the short arm of a large submetacentric pair. In group 2, the first and second metacentric pairs were similar in size and larger than the other metacentrics; the NOR was pericentromeric, but located on the long arm of a large metacentric pair. Our data on relative chromosome sizes (Table 1) would place *T. areolatus* in the first group, since its first metacentric pair is larger than the second metacentric pair (TRL 7.54% *vs.* 5.75%). However, our preliminary unpublished data show that Ag-NOR location in this species is similar to that observed in group 2. This suggests that in Chile *T. areolatus* has particular cytogenetic characteristics, in line with the great chromosomal variability observed in other Chilean species of *Trichomycterus*, such as *T. chiltoni* [2n = 52] and *T. laucaensis* [2n = 62] (Arratia and Campos, 1997), and with a distinctive phylogenetic branch within Trichomycteridae, according to Wosiacki (2002).

Oliveira and Gosztonyi (2000) proposed that the ancestral karyotype of Siluriformes had 2n = 56. Based on the cytogenetic information available on Ictaluridae at the time, LeGrande (1981) proposed a basal 2n = 58 karyotype and a FN > 80 for this group. The 2n = 54 and FN = 106 found in *T. areolatus* suggest that the karyotype of this species has primitive characteristics. This is further supported by the karyotypic characteristics of the most primitive trichomycterid species karyotyped to date, *Trichogenes longipinis*, with 2n = 54 and a high fundamental number (FN = 102) (Lima and Galetti Jr., 1990).

In the sample of 12 individuals from the Huilma River, four specimens studied had chromosome metaphase plates with a high frequency of 54 (44.4%), 55 (19.5%) and 56 (23.85%) chromosomes, and NF values of about 106. The increased chromosome number, from 54 to 56 within the same individual, involved the gain of two small acrocentric pairs and the loss of a pair of metacentrics (Figure 1 b, c). Intra-individual chromosome polymorphism has also been described in other species of the genus *Trichomycterus*, such as *T. paolence* (Torres *et al*., 2002) and *T. davisi* (Borin and Martins-Santos, 2000). In *T. davisi*, this type of chromosome polymorphism was attributed to the occurrence of postzygotic non disjunction. In our case, the intra-individual chromosome polymorphism could have resulted from Robertsonian rearrangements (centric fusions or fissions), given that this type of rearrangement does not alter the FN value. This chromosome variation is not unusual in fishes, and it has been reported in various salmonid species (Hartley and Horne, 1984).

The average value of nuclear DNA content in *T. areolatus* was 5.04 ± 1.09 pg/nucleus (Table 2), with a coefficient of variation (CV) = 21.16%, this variability being higher than that reported in other fishes at the intraspecific level (CV = 4%-6%, Carvalho *et al*., 1998). However, if two individuals from the Tijeral River sample (n. 448 and 449, Table 2) that presented a higher value of nuclear DNA content than the others individuals (around 60%) were not included, the average value for this parameter would be 4.54 ± 0.44 pg/nucleus and the coefficient of variation would be lower (CV = 9.69%). In this scenario, there is no statistically significant difference between the Tijeral and Huilma River samples (4.63 ± 0.45 pg/nucleus *vs.* 4.39 ± 0.40 pg/nucleus, $p < 0.05$). This nuclear DNA content value that we found for *T. areolatus* is higher than that described in two other congeneric species that inhabit Brazil, *Trichomycterus sp.* (average = 2.62 ± 0.19 pg/nucleus) and *T. cf. iheringi* (average = 2.3 ± 0.23 pg/nucleus) (Fenerich *et al.*, 2004). These findings show that this cellular parameter varies widely in *Trichomycterus*. Of note are the considerable variations in nuclear DNA contents of three species of the same genus, even though they possess the same diploid number (2n = 54). However, this is not atypical in fishes, given that this type of interspecific variation is often observed at the genus level (Carvalho *et al*., 1998; Fenerich *et al*., 2004), and can result from different processes, such as duplication and/or loss of chromosome segments, or differential accumulation of repeated DNA elements in the genome, as already well documented in higher vertebrates (Hartl, 2000). The nuclear DNA content

Table 2 - DNA content analysis of two populations of *T. areolatus* from Chile. DNA content was determined by microdensitometry of Feulgen-stained nuclei of erythrocytes.

Population/ specimen identification	N. of nuclei analyzed	Average relative value	Average diploid DNA content (pg)
Chicken	200	31.27	2.49*
Tijeral River			
435	100	48.99	4.70
436	61	61.44	4.89
437	57	54.42	4.33
438	69	62.02	4.94
440	68	43.19	4.24
Average (A)			4.63 ± 0.45[a]**
Huilma River			
444	71	51.86	4.13
448	73	87.64	6.98
449	67	89.00	7.09
451	67	58.50	4.66
452	64	55.20	4.40
Average (B)			5.47 ± 1.37[b]
Average (C) without specimens 448 and 449			4.39 ± 0.40[a]
Both populations			
A + B (n = 10)			5.04 ± 1.09
A + C (n = 8)			4.54 ± 0.44

*Johnston *et al*. (1999).
**Different letters in column indicate significant difference ($p < 0.01$).

values described for *Trichomycterys* so far (2.3-5.04 pg/nucleus) are higher than the average described for fishes (2 pg/nucleus; Hinegardner and Rosen, 1972), although they fall within the upper range described for various species of Siluriformes (1.04-8.75 pg/nucleus; Carvalho *et al*., 1998). This high nuclear DNA content may be related to the fact that Siluriformes, as a group belonging to the superorder Ostariophysi, is a primitive group of fishes (Saitoh *et al*., 2003).

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