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Karyotype of River Loach *Turcinoemacheilus kosswigi* Bănărescu and Nalbant, 1964 (Cypriniformes, Balitoridae) from the Euphrates River, Turkey

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ABSTRACT

Karyotype of river loach *Turcinoemacheilus kosswigi* from the Euphrates River, Turkey was investigated using conventional Giemsa-staining and C-banding. Two females and two males were analyzed. Diploid chromosome number was $2n=50$ in all specimens. The karyotype consisted of four pairs of metacentric (m), seven pairs of submeta (sm)-subtelocentric (st) and 14 pairs of acrocentric (a) chromosomes, the number of chromosome was $NF=72$. No heteromorphic sex chromosomes were found. C-banded positive constitutive heterochromatin was found in the entire short arms of a pair of large m-sm chromosomes and in the centromeres of several chromosome pairs though to a lesser extent. Karyotype and C-banding patterns of *T. kosswigi* were compared with those of other loaches.

Keywords: Balitorid loaches, Chromosome banding, Cytotaxonomy, Fish cytogenetics.

INTRODUCTION

River loach *Turcinoemacheilus kosswigi* Bănărescu and Nalbant, 1964 was first described from the Tigris River in Hakkari Province, Anatolia (Bănărescu and Nalbant, 1964). *T. kosswigi* (Kuru, 1996) was claimed to be limited to the Tigris River but was also recorded in the Euphrates River by Breil and Bohlen (2001). Genus *Turcinoemacheilus* contains a single species *T. kosswigi* distributed in the Tigris and Euphrates R. basin. *Turcinoemacheilus* was formerly considered to be a member of the family Cobitidae: Cobitoidea (Bănărescu and Nalbant, 1964; Nalbant and Bianco, 1998). However, it has been recently shown that this species belongs to Balitoridae: Nemacheilinae (Breil and Bohlen, 2001).

A high number of loach species, which also include *Turcinoemacheilus* species,

occur in rivers and lakes of Turkey, and still new species are being identified (e.g. Erk'akan *et al.*, 2007; Erk'akan *et al.*, 2008). Although karyotype and C-banding studies have not been conducted on *T. kosswigi* yet, there are some studies concerning other balitorid and cobitid loaches. Some of these are *Misgurnus anguillicaudatus*, *Barbatula toni*, *Lefua nikkosis*, *Cobitis delicata* and *C. biwae* (Hitotsumachi *et al.*, 1969), *Barbatula barbatula* (*Noemacheilus barbatulus*) (Boroň, 1995a), *C. taenia* (Boroň, 1995b; Boroň, 1999; Boroň, 2003; Janko *et al.*, 2005; Boroň *et al.*, 2008) and *Orthrias angorae* (Kaya *et al.*, 2005).

The aim of the present study was to describe the karyotype and distribution of C-banded positive constitutive heterochromatin in *T. kosswigi* from the Euphrates R.

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MATERIALS AND METHODS

Two females and two males of *Turcinoemacheilus kosswigi* from Karakaya Dam Lake, the Euphrates River, Malatya, Turkey, (38°29' N, 38°15' E) were analyzed. The fish were transported live to the laboratory, and kept in well-aerated aquaria until processing. Slides with mitotic chromosome were prepared according to Collares-Pereira (1992) from kidney cells. The fish were injected intraperitoneally with 0.1% colchicine solution per 100 g of body weight per 1 ml and sacrificed after 2.5 hours. The kidneys were removed and minced with mesh in 0.075 M KCl for 30 minutes at room temperature, and then fixed with cold fresh 1:3 acetic acid: methanol fixative. The solutions were centrifuged for 10 minutes. After the fixative was changed twice, the cell suspension was dropped onto slides. The air dried chromosomes were stained with 10% Giemsa for 10-15 minutes. C-banding protocols followed are as described by Sumner (1972). Briefly, slides were hydrolyzed for 15-20 minutes in 0.2N

HCl at room temperature and washed in distilled water, and denatured in a saturated Ba(OH)₂ solution at 40°C for 10 minutes. Then slides were incubated in 2XSSC at 65°C for 70 minutes and stained with 10% Giemsa for 10-15 minutes. At least 25 metaphases were studied per specimen. Chromosomes were classified using the nomenclature proposed by Levan *et al.* (1964). The preparations were observed and photographed digitally using a Leica DMLB research microscope. The specimens analyzed are deposited in the Cytogenetics Laboratory of the Department of Biology, Faculty of Science and Arts, Ahi Evran University, 40100, Kirsehir, Turkey, M. Gaffaroğlu (M. G. 30).

RESULTS

The diploid chromosome number of all specimens of examined *T. kosswigi* was invariably $2n = 50$. The karyotype consisted of four pairs of m, seven pairs of sm-st and 14 pairs of a chromosomes; fundamental arm number (NF) was 72 (Figure 1). The

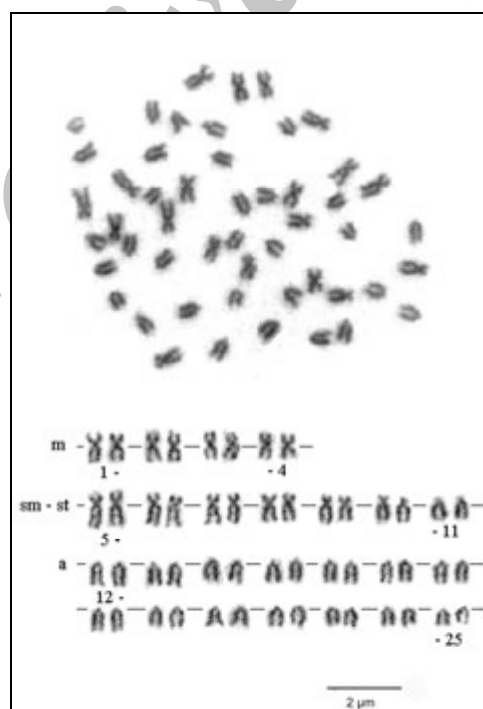


Figure 1. Metaphase and corresponding karyotype of female *Turcinoemacheilus kosswigi* arranged from Giemsa-stained chromosomes (m: Metacentric; sm-st: Submeta-subtelocentric, a: Acrocentric).

heteromorphic sex chromosomes were not detected in species examined.

C-banded positive constitutive heterochromatins were observed throughout the short arms and centromere of one pair of m-sm chromosomes in some metaphases (Figure 2). Additionally, C-banded positive heterochromatin was observed, though relatively less intensely, in centromeres of several chromosomes. Positive C-band distribution was observed to be the same even in male and female-chromosomes.

DISCUSSION

The karyotype of *T. kosswigi* showed that chromosome set is dominated by acrocentric chromosomes. The number of acrocentric elements appeared to be a characteristic of its karyotype. In this respect, *T. kosswigi* was similar to other loach species (Boroñ, 1995a; Boroñ, 1995b; Boroñ, 1999; Ráb *et al.*, 2000). In contrast to a gradual size differentiation, chromosomes did not show a considerable difference in size, as present in karyotypes of some other fish groups. In other words, only a slight difference was observed in the expansion of chromosomes. Although differences in the number of chromosome arms have been reported for

some species, this is usually the result of a difference in the scoring of subtelocentric chromosomes by different authors (Ráb *et al.*, 2000; Gaffaroglu *et al.*, 2006; Gaffaroglu *et al.*, 2009). The majority of authors classify uniarmed and biarmed chromosomes according to Levan *et al.* (1964). In the present study, subtelocentric chromosomes were considered as biarmed (Figure 1).

Chromosome number of many loaches is $2n=50$, as is the case of *Turcinoemacheilus* (Cobitidae (Acanthopsis, Cobitis, Iksookimia, Koreocobitis, Lepidocephalichthys), Nemacheilidae (Acanthocobitis, Barbatula, Schistura, Triplophysa) and Botiidae (Leptobotia, Parabotia)) (Bohlen and Ráb, 2001; Bohlen *et al.*, 2008). Besides, there are also cyprinids such as Cyprinion, Acanthobrama and many others which have $2n=50$ chromosomes (Ráb *et al.*, 1991; Ráb *et al.*, 2000; Gaffaroglu *et al.*, 2006).

Boroñ (1995b) found that the diploid chromosome morphology of $2n=48$ chromosome diploid spined loach *Cobitis taenia* consisted of six pairs of metacentric, nine pairs of submetacentric and nine pairs of subtelo-acrocentric chromosomes, while $2n=50$ chromosome *Barbatula barbatula* was found to have four pairs of metacentric,

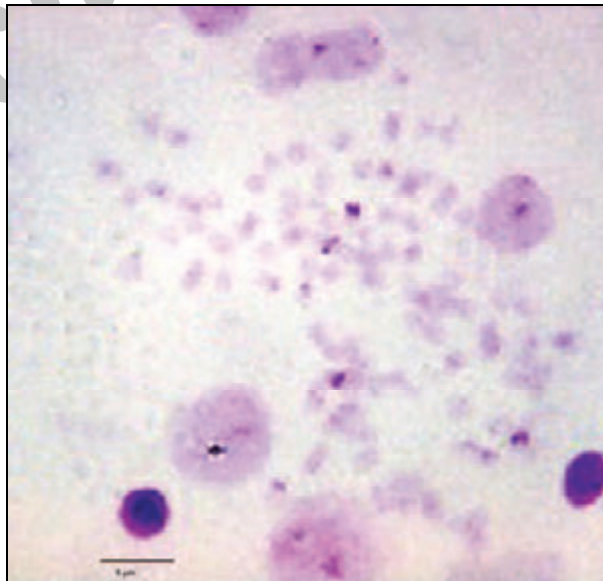


Figure 2. C-band metaphase cell of male *Turcinoemacheilus kosswigi*.



10 pairs of submetacentric and 11 pairs of subtelocentric chromosomes. Both total and metacentric chromosome numbers of *B. barbatula* are consistent with this study. However, differences were observed in other aspects of morphological chromosome groups. *C. taenia* and *T. kosswigi* display more similarities as to chromosome morphology. In *C. elongatoides* ($2n=50$, 15 pairs of metacentric, eight pairs of submetacentric, one pair of subtelocentric, one pair of acrocentric), metacentric chromosomes account for more than half of the chromosomes (Ráb et al., 2000). Although results of Kaya et al. (2005), who found seven pairs of metacentric, seven pairs of submetacentric and 11 pairs of acrocentric chromosomes in *Orthrias angorae*, seem different from ours, this difference is not substantial.

It was reported that C-band was observed in the centromeres of the largest single pair of the metacentric chromosome in *Sabanejewia aurata balcanica* (Ráb et al., 1991), metacentric chromosome of *C. taenia* (Saitoh and Aizawa, 1987) and banded chromosomes of *C. taenia* (Boroñ, 1995b). These identified heterochromatin blocks are similar to *B. barbatula* (Boroñ, 1995a), but are different from those of *C. taenia* (Boroñ, 1995b). The presence of heterochromatin blocks on some chromosomes (Figure 2) suggests the existence of some type of intrachromosomal rearrangement, such as pericentric inversions or centric fusions (Boroñ, 1995a).

Diploid ($2n=48, 50$), triploid ($3n=74$) and tetraploid ($4n=96$) forms can be observed in *Cobitis taenia* species complex (Boroñ, 1999; Ráb et al., 2000). In order to establish whether this condition, which is present in some cobitid loaches, applies to *Turcinoemacheilus*, it is necessary to collect samples from several populations and compare their karyotypes. Furthermore, further and more detailed studies carried out using different banding methods on this species will be useful for cytogenetics.

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Bănărescu and Nalbant, 1964 (Cypriniformes, کاریوتایپ ماهی لوچ رودخانه فرات ترکیه)

***Turcinoemacheilus kosswigi* Balitoridae)**

م. غفاراقلو، م. کاراسو و س. اونال

چکیده

در این تحقیق کاریوتایپ یک گونه ماهی لوچ رودخانه ای با نام *Turcinoemacheilus kosswigi* واقع در رودخانه فرات ترکیه با استفاده از روشهای رنگ آمیزی گیمسا و بانددهی نوع C مورد بررسی



قرار گرفت. برای این منظور دو نمونه ماده و دو نمونه نر انتخاب شدند. در تمامی نمونه های مورد بررسی تعداد کروموزوم برابر $2n=50$ بود. کاریوتیپ شامل ۴ جفت کروموزوم متاسانتریک، ۷ جفت کروموزوم ساب متا-تلوسانتریک و ۱۴ جفت کروموزوم اگروسانتریک با تعداد کروموزوم های $2n=72$ بود. هیچگونه کروموزوم جنسی هترومورفیک مشاهده نگردید. هتروکروماتین فعال با باندهای نوع C، در تمامی بازوهای کوتاه کروموزوم ها متا و ساب متاسانتریک و همچنین در حد کمتر در ناحیه سانترومری تعدادی زیادی از جفت کروموزوم ها مشاهده گردید. در این تحقیق الگوهای کاریوتیپی و باندهای نوع C کروموزومها در گونه *T. kosswigi* با گونه های دیگر ماهی لوچ مورد مقایسه قرار گرفته است.

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