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# Cytogenetic Analysis of *Garra variabilis* (Heckel, 1843) (Pisces, Cyprinidae) from Savur Stream (Mardin), Turkey

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**Abstract**

In this study cytogenetic analysis were carried out on *Garra variabilis* (Heckel, 1843) individuals from Savur stream (Mardin). The karyotype of *G. variabilis* was analyzed using G-banding, C-banding, Ag-NOR staining and Q-banding techniques. Diploid chromosomes number of specimens was determined as 2n=102. However, two karyotype formulae were identified in the *G. variabilis* specimens: female 42m+18sm+24st+18a (FN = 186) and males 41m+18sm+24t+19a (FN = 185). Nucleolar organizer regions (NORs) were identified by silver nitrate staining. NOR regions were observed on many chromosome pairs. The largest chromosome pair was defined as submetacentric. The detail karyotype of *G. variabilis* is reported for the first time.

*Keywords*: *Garra variabilis,* karyotype, G-, C- , Q- banding, Ag-NOR staining, Turkey.

**Savur Irmağı'nda (Mardin) yaşayan Garra variabilis (Helkel, 1843) (Pisces, Cyprinidae)'in Sitogenetik Analizi, Türkiye**

**Özet**

Bu çalışmada Savur Irmağı'nda (Mardin) yaşayan *Garra variabilis* (Helkel, 1843) bireylerinin sitogenetik analizi yapılmıştır. *G. variabilis*'in karyolojik analizi G-bantlama, C-bantlama, Ag-NOR boyama ve Q-bantlama teknikleri kullanılarak belirlenmiştir. Örneklerin diploit kromozom sayısı 2n=102 olarak tanımlanmıştır. Bununla birlikte *G. variabilis* bireylerinde iki farklı karyotip görülmüştür: dişi 42m+18sm+24st+18a (FN=186), erkekler 41m+18sm+24st+19a (FN=185). Çekirdekçik yapıcı bölgeler (NORs) gümüş boyama tekniği ile belirlenmiştir. Birçok kromozom üzerinde NOR bölgeleri görülmüştür. En büyük kromozom submetasentrik olarak tanımlanmıştır. *G. variabilis*'in ayrıntılı karyotipi bu çalışma ile ilk kez belirlenmiştir.

*Anahtar Kelimeler*: *Garra variabilis*, karyotip, G-, C-, Q- bantlama, Ag-NOR boyama, Türkiye.

## Introduction

Cyprinid fishes comprise a major element of the ichthyofauna of Africa, Asia, Europe, and North America. The family Cyprinidae has about 220 genera and about 2,420 species, and is the largest family of freshwater fishes (Nelson, 2006). More than 95 cyprinid species have been reported from Turkey (Kuru, 2004). Despite the diversity of Turkey's fishes, little is known about the genetic structure of Turkey freshwater fishes. Recently, cytogenetic markers have been used for genetic structure analyses in different fish species (Karahan and Ergene, 2009; Ergene *et al*., 2010), but the studies are still insufficient.

Generally, a large number and small chromosomes characterizes fish karyoptypes. This discourages many researchers from pursuing fish karyotypic analysis and therefore, karyological data on fish are available for only a small percentage (about 10%) of some 25,000 taxonomically known species (Nelson, 2006).

Genus *Garra* has 103 species (Arkhipchuk, 1999). However, 11 species of *Garra* were cytogenetically studied up to now (Table 1). Detailed chromosome analyses of the genus *Garra* are rare. The diploid chromosome number of *G. rufa* which is relative with *G. variabilis,* was notified as 2n=46-50 by Karahan and Ergene (2009), 2n=44 (NF=85, 22m+20sm+2a) by Ergene Gözükara and Cavas (2004); 2n=44 (NF=87, 16m+26sm+1st+1a) by Kılıç-Demirok (2000); 2n=44–52 by Arkhipchuk (1999) and 2n = 44-52 by Vasil'ev (1980).

The study on fish chromosome has received considerable attention in recent years because of their importance in classification, evolution and heredity (Gold *et al*., 1990; Ozouf–Costaz and Foresti, 1992; Barat, 2002). Moreover cytogenetic studies of fish have been used as biological indicator for determination of water pollution (Klinkhardt, 1993).

An important characteristic of nucleolar organizer regions (NORs) in fish is related to that it has inter- and intra-species polymorphism. NOR characterization can be a cytogenetic marker of for cytotaxonomic studies and can even aid in constructing phylogenetic hypotheses (cytosystematics) for several fish groups (Amemyia and Gold, 1988; Galetti Jr, 1998; Almeida-Toledo, 2000). Some fish groups have a simple NOR system characterized by ribosomal cistrons on only one chromosome pair, whereas others have a multiple NOR system composed of cistrons dispersed over several chromosomes (Galetti Jr, 1998).

This paper reports the result of a cytogenetic study on Savur stream population of *G. variabilis*. Chromosomes were analyzed by $G^-$, $C^-$, $Q^-$ banding and $Ag^-$ staining techniques. Detailed karyotype of *G. variabilis* was determined for the first time with this study.

## Materials and Methods

Basic cytogenetic analysis methods were performed on 34 specimens of *G. variabilis* (1 female and 33 males), which were collected from Savur Stream in Mardin (Figure 1). The preparation of the mitotic chromosome suspension was performed using cells from the fin epithelium according to Ergene *et al*. (1999). Chromosome morphology was determined based on the arm relationship proposed by Levan *et al*. (1964). The fundamental number (FN) was calculated considering metacentric (m), submetacentric (sm) and subtelocentric (st) chromosomes with two arms and acrocentric (a) chromosomes with only one arm. Nucleolar organizer regions (NORs) were identified following the silver ($AgNO_3$) staining method of Howell and Black (1980). C-bands were obtained according to Sumner (1972). Q-banding with quinacrine was performed according to Schimid (1980). Metaphase chromosomes were banded using the conventional Trypsin-Giemsa banding technique (Seabright, 1971). Giemsa-stained, G-banded, Ag-NOR-stained, Q-banded, and C-banded mitotic chromosomes were photographed using a digital camera and the images were digitally processed using Adobe Photoshop v.7.0 software. The arm ratio was determined using the Micro-Measure program. A haploid ideogram was prepared based on the measurements of C-banded, Ag-NOR stained, and G banded chromosomes.

## Results

The diploid chromosome number of *G. variabilis* is 2n=102 (within 229 metaphase with 75.98%), comprising 42 metacentric, 18 submetacentric, 24 subtelocentric, 18 acrocentric, FN = 186 at female; 41 metacentric, 18 submetacentric, 24 subtelocentric, 19 acrocentric, FN = 185 at males. Among the 34 specimens analyzed, one specimen was female and 33 were males.

According to G-banding, four band regions were determined on st chromosome arms 32., tree band regions m chromosome arms 16., 18., two band regions m chromosome arms 1., 2., 5., 6., 17., sm chromosome arms 21., 23., st chromosome arms 30., 31., 33., 34. and one band region m chromosome arms 3., 7., 8., 9., 10., 11., 12., 19., 20., sm chromosome arms 22., 24., 27., st chromosome arms 36., 37., 38., 39., a chromosome arms 42., 44., 46. In addition, two large band regions were seen on the metacentric chromosome arms 51 (Figure 2a, Figure 3).

Karyotype was determined according to the arm measurements which were the consequence of C-band (Figure 4). C-banding heterochromatin was observed at the terminal region of many chromosomes, as well as on the interstitial region of some pairs (No. 2, 4, 6,

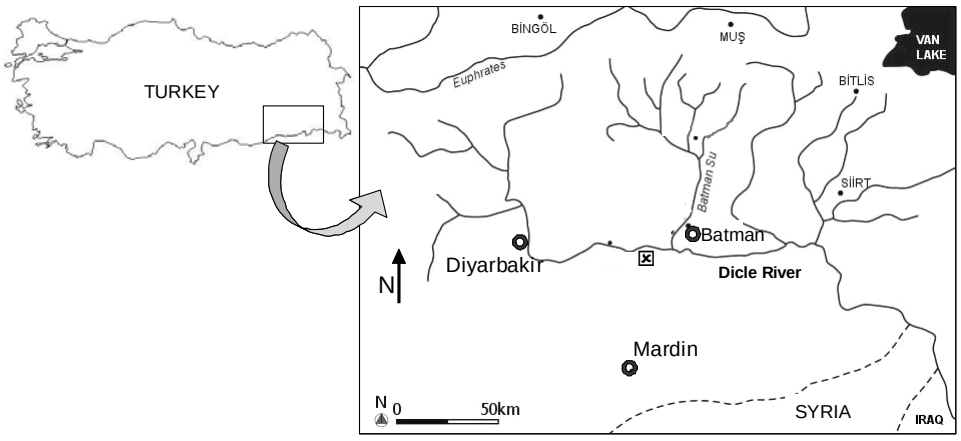


**Figure 1.** Collection site of *G. variabilis* samples. (The sampling site ⊠),

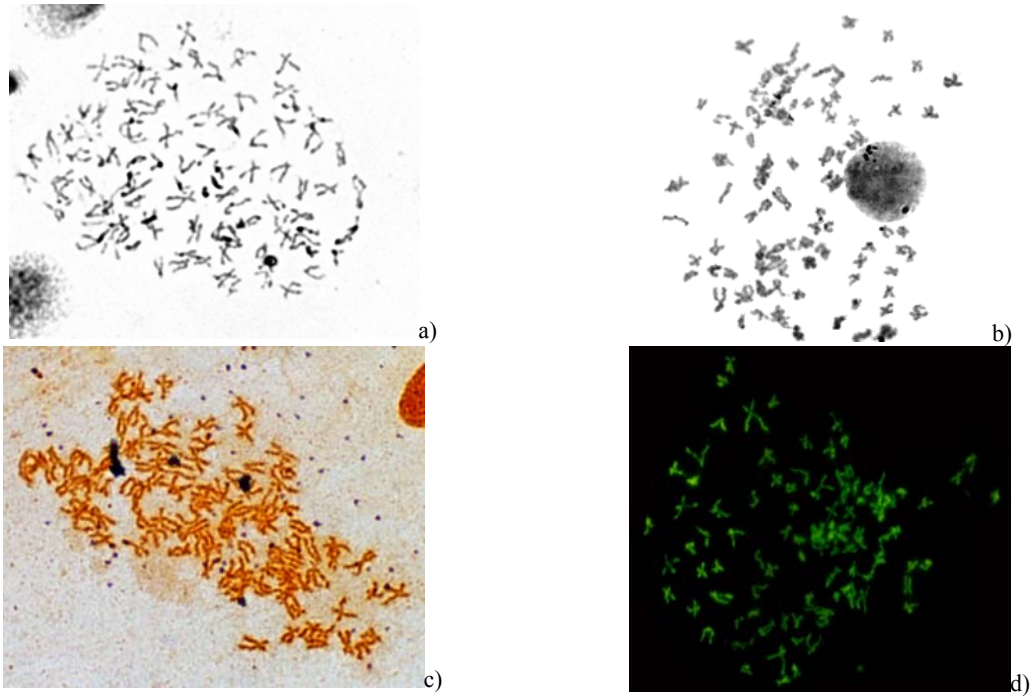


**Figure 2.** Metaphase images of *G. variabilis* a) G-banded (male); b) C-banded (male); c) Ag-NOR staining (female); d) Q-banded (male).

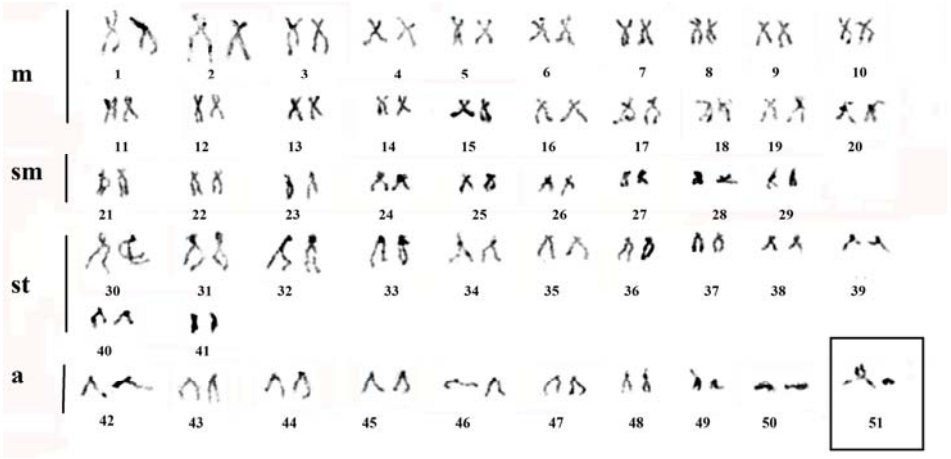


**Figure 3.** G-banded karyotype image of *G. variabilis* (male) (sex chromosomes are in the square).

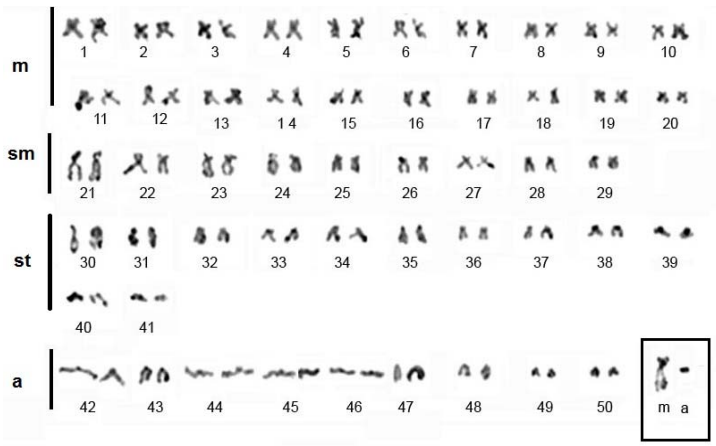


**Figure 4.** C-banded karyotype image of *G. variabilis* (male) (sex chromosomes are in the square).

**Table 1.**Chromosome characteristics in the genus *Garra* (2n=diploid number; KF=karyotypic formula; FN=fundamental number)

| Species | Locality | 2n | FN | KF | Sex | References |
|---|---|---|---|---|---|---|
| *G. cambodgiensis* | Unspecified | 52 | | | | Vasil'ev, 1980 |
| *G. dembeensis* | Unspecified | 50 | 82 | | | Golubtsov and Krysanov, 1993 |
| *G. gotyla gotyla* | India Ooty, TN. | 50 | 90 | 14m+26sm+10t,a | | Barat, 1985 |
| *G. gotyla gotyla* | India Jammu, J&K | 50 | 74 | 14sm+10sm+10st+16t, a | No heteromorphic sex chromosomes in male. | Bukhsh, 1984 |
| *G. gotyla gotyla* | India, Itanagar, A.P | 50 | 70 | 12m+8sm+8st+22T | | Kumari Sahooet al., 2007 |
| *G. gotyla gotyla* | India, India | 50 | 90 | 14m+26sm+10t,a | | Barat, 1985 |
| *G. kempi* | India, Itanagar, A.P | 50 | 78 | 14m+14sm+10st+12t | | Kumari Sahoo *et al*., 2007 |
| *G. lissorhynchus* | India, Itanagar, A.P | 50 | 82 | 16m+16sm+6st+12t | | Kumari Sahoo *et al*., 2007 |
| *G. mullya* | India Chalakkudy river, Kerala | 50 | 82 | 18m+14sm+10st+8t | | Nagpure *et al*., 2006 |
| *G. imberba* | Unspecified | 50 | | | | Arkhipchuk, 1999 |
| *G. lamta* | India Bihar | 50 | 86 | 6m+18sm+12st+14 t,a | | Arkhipchuk, 1999 |
| *G. lamta* | India Simlipal Hills Orissa | 50 | 86 | 12m+24sm+2st+12t,a | Female,ZW Male WW | Barat, 1985 |
| *G. lamta* | Unspecified | 50 | 74 | 6m+18sm+12st+14t,a | | Khuda-Bukhsh, 1980 |
| *G. makiensis* | Unspecified | 50 | 84 | 34m,sm+16 t,a | | Barat, 1985 |
| *G. quadrimaculata* | Ethiopia | 50 | 88 | 38m,sm+12 t,a | | Golubtsov and Krysanov, 1993 |
| *G. rufa* | Unspecified | 44-50 | | | | Post, 1965 |
| *G. rufa* | Unspecified | 44-52 | | | | Vasil'ev, 1980 |
| *G. rufa* | Turkey Mersin | 44 | 85 | 22m+20sm+2a | | Ergene Gözükara and Çavaş, 2004 |
| *G. rufa* | Turkey Dicle River | 44 | 87 | 16m+26 sm+1st+1a | | Kılıç and Demirok, 2000 |
| *G. rufa* | Turkey, Mersin | 50 | 94 | 26m+10sm+8st+6a | Female | Karahan and Ergene, 2009 |
| *G. rufa* | Turkey Hatay | 46 | 88<br>87 | 22m+12sm+8st+4a<br>22m+12sm+7st+5a | Female XX<br>Male XY | Karahan and Ergene, 2009 |
| *G. rufa* | Turkey Kahramanmaras | 46 | 90<br>89 | 32m+6sm+6st+2a<br>31m+6sm+6st+3a | Female XX<br>Male XY | Karahan and Ergene, 2009 |
| *G. rufa* | Turkey Sivas | 50 | 96 | 28m+14sm+4st+4a | Female | Karahan and Ergene, 2009 |
| *G. variabilis* | Turkey Savur Stream | 102 | 186<br>185 | 42m+18sm+24st+18a<br>41m+18sm+24t+19a | Female<br>Male | Present study |

32) from the regular complement. On the other hand, the single acrocentric chromosome, which was determined at male, was completely heterochromatic (Figure 2b, 4).

The nucleolar organizing regions were detected in the telomeric portion of the short arm 3., 6., 7., 8., 9., 10., 28., 29. chromosome pairs (Figure 2c). Clear Q-band regions were seen on long arm sm chromosomes 22., 23., 27. and short arm sm chromosomes 24., 25., 26. (Figure 2d, Figure 5). Positive Q-band, G-band and NOR regions schema was given at Figure 6. Chromosomes arm of *G. variabilis* were obtained by Micro Measure program which was given in Figure 7. The largest chromosome was determined as non homologue m chromosome at males (Figure 7).

## Discussion

In fish, most of the chromosome banding studies are related to C-bands or silver- and chromomycin$A_3$ bands to identify nucleolar organizing regions, while descriptions of the distinct structural Q-, G- and replication banding patterns are limited (Ueda and Naoi, 1999). Weak compartmentalization of the genomes due to the base composition (AT- or GC-rich DNA) of cold-blooded vertebrates has been reported (Medrano *et al*., 1988). Weak compartmentalization in the fish chromosomes is thought to be a main cause of limited reports about the distinct structural Q- and G-banding patterns. In this study, specific Q-band patterns were identified (Figure 2d, 5). These band regions can be determining as marker for this species or population. Likewise, G-band pattern was described and these band regions can be used to distinguish populations from each other.

The majority of chromosomes numbers of Cyprinid species is between 2n=50 and 2n=100 (Oelerman and Skelton, 1990). The diploid chromosomes number of *G. variabilis* was found as 102. This result is consistent with Cyprinid chromosomes. Diploid chromosomes number which cytogenetically studied 11 species of *Garra*, was consistent 50 (Table 1), excluding the species *G. rufa* and *G. combodgiensis*. These data indicate that *G.*

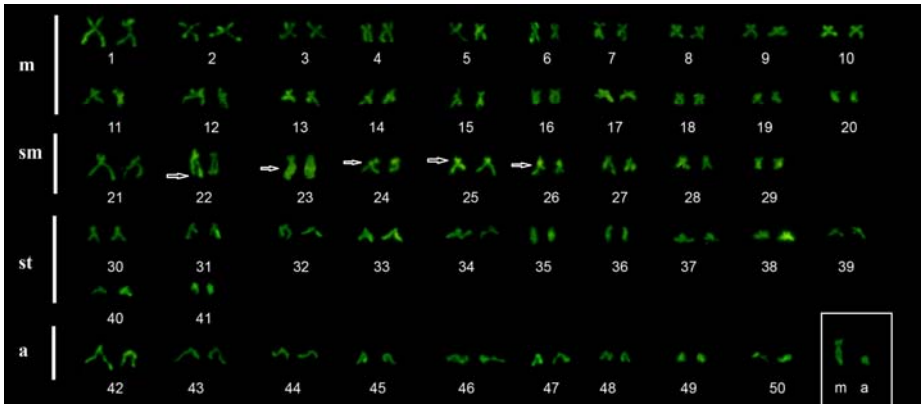


**Figure 5.** Q-banded karyotype image of *G. variabilis* (male) (sex chromosomes are in the square).

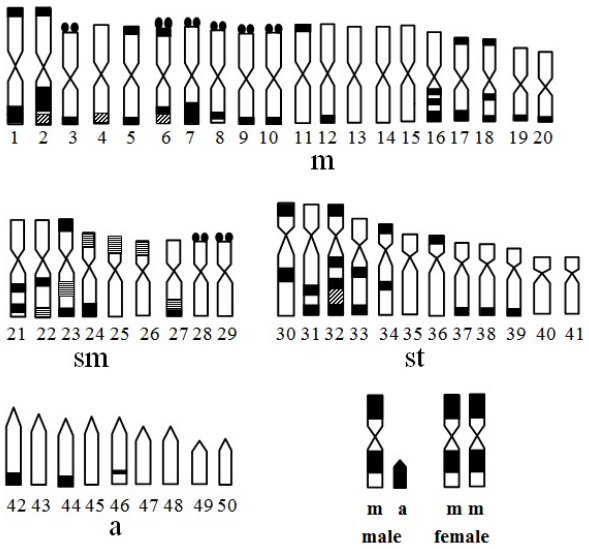


**Figure 6.** Ideogram of *G. variabilis* according to G-, C, Q banding and Ag-NOR staining (■ Giemsa band regions; Q-band regions;interstitial C-band regions).

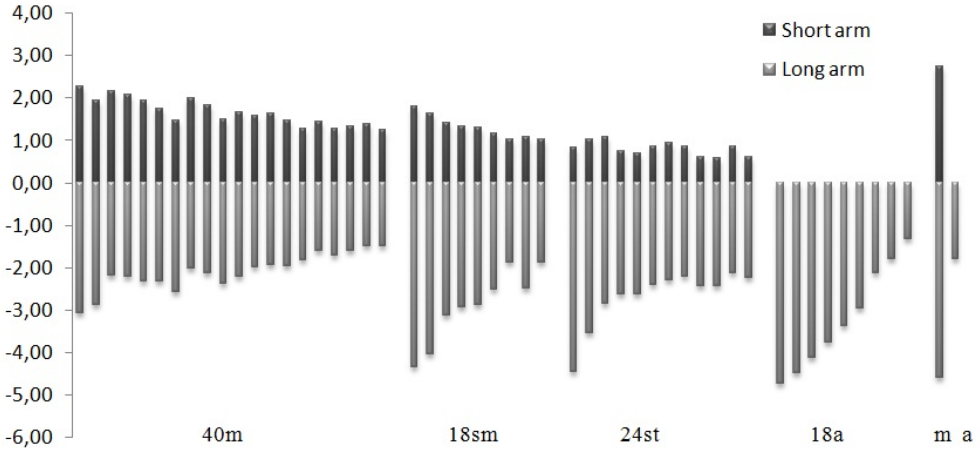


**Figure 7.** Arm ration of *G. variabilis* (male).

*variabilis* can be polyploidy.

G-banding can be used to identify chromosomal anomalies such as translocations, due to unique pattern of light and dark bands for each chromosome (Ueda and Naoi, 1999). C-banding and Ag-NOR staining to be useful for the investigation of the karyotype evolution of bitterlings. Heterochromatin has played an important role in the karyotypic diversification of fish (Ueda *et al*., 2001). In this study, quite large heterochromatin region was seen on

metacentric chromosomes arms 2, 7 and 51 consequence of G-banding (Figure 2a, 3, 6). These chromosomes (2 and 7) can be defined as a marker for *G. variabilis.*

John and Miklos (1979) suggested that the quantitative and positional changes in constitutive heterochromatin are one of the important factors for speciation. Meyne *et al*. (1990) have reported that the interstitial sites of the (TTAGGG)n telomeric sequence enable greater flexibility for the karyotype change. However, C-banding was important to enable identification of the centric fusion. At this study, clear C-bands regions were seen at centromeric position on many chromosomes and interstitial position at the long arm of chromosome pairs 2, 4, 6, 32 (Figure 2b, 4, 6). These heterochromatic regions could have been formed by tandem duplication or pericentric inversion of heterochromatic DNA.

Sex chromosomes of *G. rufa* populations which are living in two different regions (Hatay and Kahramanmaras) were identified by Karahan and Ergene (2009). According to this study, *G. rufa* has XX/XY heteromorphic sex chromosomes system. In the present study, two chromosomes, which are two metacentric in female; one metacentric and one small acrocentric in male, are thought to be sex chromosomes. All acrocentric and most of the metacentric chromosomes consisted heterochromatin.

According to Khuda-Bukhsh (1984); Khuda-Bukhsh and Barat (1987); Ergene-Gözükara and Cavas (2004); Nagpure *et al*. (2006); Kumari Sahoo *et al*. (2007), the species of *G. rufa* has the largest submetacentric chromosome pair in the karyotype. Therefore, they have pointed out that submetacentric chromosome pair can be defined as a marker for genus *Garra*. However, the largest chromosome pairs were identified as metacentric of this study. Four *G. rufa* populations from four distinct localities in Turkey were compared with each other by Karahan and Ergene (2009). According to this study, the largest chromosome pair of the complements was characteristically submetacentric for Mersin and Kahramanmaras *G. rufa* populations, metacentric for Sivas and Hatay populations. As a result of this, it can be concluded that *G. variabilis* is compatible with Sivas and Hatay *G. rufa* populations.

NOR regions are used as important chromosomal markers in fishes, the number and position of NORs change according to genus, species and population. The NOR is a certain indicator for rewiring chromosomal polymorphism in and between species in many fish groups and it is noted that this variety can affect the position on the chromosome, size and active number of NOR's in the whole genome (Ozouf–Costaz and Foresti, 1992). Although the NOR is especially seen at the short arm of chromosome, sometimes it can be seen on the long arm of m and a chromosomes (Rab *et al*., 1996). Furthermore, these can be seen between telomere and centromere (Jankun *et al*., 1998). Amemiya and Gold (1988) have hypothesized that single NOR located terminally on acrocentric chromosome represents the plesiomorphic state for Cyprinidae. Two or multiple pairs of chromosome bearing rDNA sites seem to represent a derived condition among Cyprinidae as well as among Leuciscinae (Boron, 2001). In the present study, eight NOR regions were observed at the terminal region of the short arm in the metacentric chromosome pairs 3, 6, 7, 8, 9, 10 and submetacentric 28, 29 (Figure 2c, 6).

The chromosome number and morphology from chromosomal analyses are used in the identification of species easily and in defining the relationship and differences between varied species. The chromosome number and morphology can change among fish species. This variation can be used in the search of evolutional relationship between inter- and intra-populations (Thorgard and Disney, 1990). The formation of the heterochromatin could be useful for determination of chromosome reconstruction. The composition of heterochromatin has to be better investigated to further clarify the karyotype evolution in fish groups. This paper appears to be first report of banding pattern within the *G. variabilis*. This information can provide useful data for the fish cytogenetic database. The detection of the cytogenetic pattern of *G. variabilis* from Savur stream might constitute important information to better understand the population biology of this fish.

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## References

Almeida-Toledo, L.F. 2000. Karyotypic evolution in Neotropical freshwater fish. Chrom. Today, 13: 169-181.

Amemiya, C.T. and Gold, J.R. 1988. Chromosomal NORs as taxonomic and systematic characters in North American Cyprinid fishes. Genetica, 76: 81–90.

Arkhipchuk, V.V. 1999. Chromosome database. Database of Dr. Victor Arkhipchuk. In: R. Froese and D. Pauly (Eds.), Fishbase World Wide Web electronic publication. Version (02/2007).

Barat, A., Sahoo, P.K. and Ponniah, A.G. 2002. Karyotype and Nucleolar Organizer Regions (NORs) in a few hill stream fishes. In: S. Ayyappan, J.K. Jena, M.M. Joseph (Eds.), The Fifth Indian Fisheries Forum Proceedings, AFSIB, Mangalore and AoA, Bhubaneswar: 111–114.

Barat, A. 1985. A study of chromosomes in some Indian Teleost (Pisces). PhD thesis dissertation. West Bengal: Kalyani University.

Boroń, A. 2001. Comparative chromosomal studies on two minnow fish, *Phoxinus phoxinus* (Linnaeus, 1758) and *Eupallasella perenurus* (Pallas, 1814); an associated cytogenetic-taxonomic consideration. Genetica, 111: 387–395.

Ergene, S. and Karahan, A. 1999. Karyological analyses of *Tilapia rendalli* (Boulenger, 1897) (Pisces: Cichlidae). Gazi University Journal of Education Faculty, 19(2): 161-165.

Ergene Gözükara, S. and Çavaş, T. 2004. A Karyological Analysis of *Garra rufa* (Heckel, 1843) (Pisces, Cyprinidae) from the Eastern Mediterranean River Basin in Turkey. Turk. J. Vet. Anim. Sci., 28: 497-500.

Ergene, S., Karahan, A. and Kuru, M. 2010. Cytogenetic analysis of *Pseudophoxinus antalyae*, Bogustkaya, 1992 (Pisces: Cyprinidae) from the Eastern Mediterranean River Basin, Turkey. Turk. J. Zool., 34: 111-117.

Galetti Jr, P.M. 1998. Chromosome diversity in neotropical fish. NOR studies. Ital. J. Zool., 65: 53-56.

Gold, J.R., Li, Y.C., Shipley, N.S. and Powers, P.K. 1990. Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. J. Fish. Biol., 37(4): 563–575.

Golubtsov, A.S. and Krysanov, E.Y. 1993. Karyological study of some cyprinid species from Ethiopia. The ploidy differences between large and small *Barbus* of Africa. J. Fish Biol., 42(3): 445-455.

Howell, W.M. and Black, D.A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1 step method. Experientia (Basel.), 36: 1014-1015.

Jankun, M., Woznicki, P., Dajnowicz, G., Demska-Zakes, K. and Luczynski, M.J. 1998. Heterochromatin and NOR location in northern Pike (*Esox lucius*). Acquit. Sci., 60: 17-21.

John, B. and Miklos, G.L.G. 1979. Functional aspects of satellite DNA and heterochromatin. Int. Rev. Cytol. 58: 1–114.

Karahan, A. and Ergene, S. 2009. Cytogenetic Variation of Geographically Isolated Four Populations of *Garra rufa* [(Heckel, 1843) (Pisces, Cyprinidae)] in Turkey, Caryologia, 62(4): 276-287.

Khuda-Bukhsh. A.R., Gupta, S.K. and Goswami S. 1980. Karyotypic studies in *Garra lamta* and *Mystus cavassius* (Pisces). Proc Ind. Acad. Sci., 89(6): 557-562.

Khuda-Bukhsh, A.R. 1984. Karyotype studies in two species of hill stream fishes, *Labeo diplostomus* and *Garra gotyla gotyla* (Pisces,Cyprinidae). Proc. 4*th* All India Cong. Of Cytol. And Genet. In Perspectives in Cytology and Genetics (G.K. Manna and U. Sinha Eds.,). Hindasia Publishers, Delhi, 4: 421-424.

Khuda-Bukhsh, A.R. and Barat, A. 1987. Chromosomes in fifteen species of Indian Teleosts (Pisces). Caryologia., 40(1-2): 131–144.

Kılıç-Demirok, N. 2000. Dicle Nehrinde yaşayan bazı Cyprinidae tür ve alt türleri üzerine kromozomal çalışma (Chromosomal works on certain Cyprinids species and subspecies from Dicle River basin, PhD, thesis Diyarbakır: Dicle University Institute of Science and Technology.

Klinkhardt, M. 1993. Fish chromosomes as sensitive toxicity indicators - possibilities and limits. In: T.H. Braunbeck, W. Hanke and S. Segner (Eds.,) Fish in ecotoxicology and ecophysiology; proceedings of an international symposium, Heidelberg: 45-54

Kumari Sahoo, P., Nanda, P. and Barat, A. 2007. Karyotypic diversity among three species of *Garra* (Family: Cyprinidae) from river Dikrong, Arunachal Pradesh, India. Cytologia, 72(3): 259–263.

Kuru, M. 2004. Recent systematic status of inland Water fishes of Turkey, Gazi University Journal of Gazi Educational Faculty, 3: 1-21.

Levan, A., Fredga, K. and Sandberg, A.A. 1964. Nomenclature for centromer position on chromosomes. Hereditas, 52: 201-220.

Medrano, L., Bernardi, G., Couturier, J., Dutrillaux, B. and Bernardi, G. 1988. Chromosome banding and genome compartmentalization in fishes. Chromosoma, 96: 178–183.

Meyne, J., Baker, R.J., Hobart, H.H., Hsu, T.C., Ryder, O.A., Ward, O.G., Wiley, J.E., Wurster-Hill, D.H., Yates, T.L. and Moyzis, R.K. 1990. Distribution of non-telomeric sites of the (TTAGGG)n telomeric sequences in vertebrate chromosomes. Chromosoma. 99(1): 3–10.

Nagpure, N.S., Kumar, R. and Srivastava, S.K. 2006. Cytogenetic studies of two cyprinids *Garra mullya* and *Barilius bakeri* from the Western Ghats of India. J. Cytol. Genet., 7: 69–78.

Nelson, J.S. 2006. Fishes of the world, John Wiley and Sons Inc., 4th edition. New York. 601 pp.

Oelerman, L.K. and Skelton, PH. 1990. Hexaploidy in yelowfish species (Barbus, Pisces, Cyprinidae) from Southern Africa. J. of Fish Biology, 37: 105-115.

Ozouf–Costaz, C., Foresti, F. 1992. Fish cytogenetic research, advances, applications and perspectives. Netherlands Journal of Zoology, 42, (2-3): 277-290.

Post, A. 1965. Vergleichende Untersuchungen der Chromosomenzahlen bei Süßwasser-Teleosteern. Z. Zool. Syst. Evol. Forsch., 3: 47-93.

Rab, P., Karakousis, Y.P. and Rabova, M. 1996. Karyotype NOR phenotype and C banding study of *Barbus cyclolepis* from Greece. Folia Zool., 45: 77-83.

Schimid, M. 1980. Chromosoma banding an Amphibia IV. Differentiation of GC and AT rich regions in Anura. Chromosoma, 77: 83–103.

Seabright, M.R. 1971. A rapid banding technique for human chromosomes. Lancet, 2: 971–972.

Sumner, A.T. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res., 75: 304-306.

Thorgard, G.H., and Disney, J.E. 1990. Chromosome preparation and analysis. In: C.B. Schreck and P.B. Moyle (Eds.), Method for fish biology. American Fisheries Society, Bethesda: 171-190.

Ueda, T. and Naoi, H. 1999. BrdU-4Na-EDTA-Giemsa band karyotypes of 3 small freshwater fish, *Danio rerio, Oryzias latipes,* and *Rhodeus ocellatus.* Genome, 42: 531–535.

Ueda, T., Naoi, H. and Arai, R. 2001. Flexibility on the karyotype evolution in bitterlings (Pisces, Cyprinidae). Genetica, 111: 423–432.

Vasil'ev, V.P. 1980. Chromosome numbers in fish-like vertebrates and fish. J. Ichthyol., 20(3): 1-38.