Turk J Biol
35 (2011) 79-87

doi:10.3906/biy-0903-4

Chromosomal differentiation between populations of *Clarias gariepinus* (Burchell, 1822) from the Göksu Delta and Orontes River (Turkey)

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Received: 10.03.2009

Abstract: The karyotype of *Clarias gariepinus* was described by means of G-banding, C-banding, Q-banding, and Ag-NORs (NORs) staining in this study. Samples were collected from 2 regions of Turkey: the Göksu Delta and Orontes River. Although samples from both regions had the same diploid chromosome number and fundamental arm number, (2n = 56, FN = 100), they had remarkable differences in karyotype formula and distribution of rDNA sites, according to silver nitrate staining and G-banding. While the karyotype formula for Orontes River samples was 24m + 10sm + 10st + 12a, the Göksu Delta samples' was 28m + 6sm + 10st + 12a. Comparative analysis showed that there was intraspecific variability in NORs and different NOR numbers in the 2 populations. The study's findings show that the Göksu Delta and Orontes River samples differed from each other.

Key words: *Clarias gariepinus*, Cytogenetic analyses, Göksu Delta, Orontes River, Turkey

Göksu Deltası ve Asi Nehri'ndeki *Clarias gariepinus* (Burchell, 1822) populasyonları arasındaki kromozomal farklılık (Türkiye)

Özet: Bu çalışmada Giemsa bant, C-bant, Ag-NOR bant ve Quinacrine bant teknikleri ile *Clarias gariepinus*'un karyolojik analizi yapılmıştır. Örnekler Türkiye'nin iki farklı bölgesinden yakalandı: Göksu Delta'sı ve Asi Nehri. Her ne kadar her iki bölge örneklerinde diploid kromozom sayısı ve kromozom kol sayıları aynı bulunmuş olsa da (2n = 56, NF = 100) karyotip formüllerinin, gümüş nitrat boyama ile belirlenen rDNA bölgelerinin ve G-bant bölgelerinin oldukça farklı olduğu belirlenmiştir. Asi Nehri örneklerinin karyotipi 24m + 10sm + 10st + 12a kromozom olarak tanımlanmış iken, Göksu Deltası örneklerinde karyotip 28m + 6sm + 10st + 12a kromozom olarak tanımlanmıştır. Karşılaştırmalı analizler NOR bölgelerinin tür içi çeşitliliğini ortaya çıkarmıştır ve iki populasyon için farklı sayılarda NOR bölgeleri belirlenmiştir. Bu çalışmadaki bulgular Göksu Deltası ile Asi Nehri örneklerinin birbirlerinden farklı olduklarını göstermiştir.

Anahtar sözcükler: *Clarias gariepinus,* sitogenetik analiz, Göksu Deltası, Asi Nehri, Türkiye

Introduction

Fish are the most primitive vertebrate group and exhibit wide genetic variability, both at the chromosomal and molecular levels, which make them an interesting group for evolutionary and cytotaxonomic studies (1). The study of fish

chromosomes has received considerable attention in recent years because of its usefulness in classification, and evolutionary and heredity analysis (2,3).

Clarias gariepinus is of significant economic importance and is widely consumed in the southern part of Turkey. Several researchers (4-8) have reported its chromosome complements (Table 1). All these karyotype studies were based on the conventional standard karyotype. The qualification of specific characteristics of an individual or group of individuals can demonstrate the degree of speciation induced by biotic and abiotic conditions, and contributes to the definition of different stocks of species (9). Nucleolar organizing regions (NORs) may be used as important chromosomal markers in groups of fishes in which the number and position of NORs change according to genus, species, and population. The number and position of heterochromatin regions are very important in speciation (10).

The aim of the present study was to determine the level of differentiation between 2 populations of *C. gariepinus* using Ag-NORs staining, and G-, C-, and Q-banding techniques, and to evaluate the obtained cytotaxonomic data to further our understanding of the evolution of this species.

Materials and methods

Clarias gariepinus specimens were collected from the Göksu Delta and Orontes River using a fyke (Figure 1). Cytogenetic analysis was conducted on 20 specimens (10 from the Orontes River and 10 from the Göksu Delta). Chromosome preparation was carried out according to the technique described by Ergene et al. (4). Detection of NORs was performed following the silver ($AgNO_3$) staining method of Howell and Black (11). C-bands were obtained according to the method described by Sumner (12). Q-banding was performed according to Schmid (13). Metaphase chromosomes were banded using the conventional trypsin-Giemsa banding technique (14). G-banded, Ag-NORs-stained, Q-banded, and C-banded mitotic chromosomes were photographed using a digital camera, and the images were digitally processed with Adobe Photoshop v.7.0 software. The karyogram and the chromosomes were classified according to Levan et al. (15). Arm ratios were determined using the Micro-Measure program. The fundamental number (FN) was determined by considering the metacentric, submetacentric, and subtelocentric chromosomes with 2 arms and acrocentric chromosomes with only 1 arm.

Results

The present study's finding show that the 2 populations of *C. gariepinus* have 2n = 56 chromosomes (FN = 100), without differentiation between the sexes (Table 2). Although the chromosome numbers were uniform, they exhibited

Table 1. Cytogenetical data of *Clarias gariepinus*.

No	Species	Locality	2n	FN	Chromosome formula	Sex chromosomes	References
1	*Clarias gariepinus*	Africa	56	89	8m+24sm+24st/a ♂		8
				88	8m+25 sm+23a ♀	ZW-ZZ	
2	*Clarias gariepinus*	Unspecified	56	89	8m+24sm+28 st/a		5
3	*Clarias lazera*	Turkey	56	100	18m+26sm+12a		4
4	*Clarias gariepinus*	Ivory Coast	56	89	8m+25sm+23st/a ♀	ZW-ZZ	6
5	*Clarias gariepinus*	Ivory Coast	56	88	8m+24sm+24st/a ♂	ZW-ZZ	7
6	*Clarias gariepinus*	Göksu River, Turkey	56	100	28m+6sm+10st+12a		Present study
7	*Clarias gariepinus*	Orontes River, Turkey	56	100	24m+10sm+10st+12a		Present study

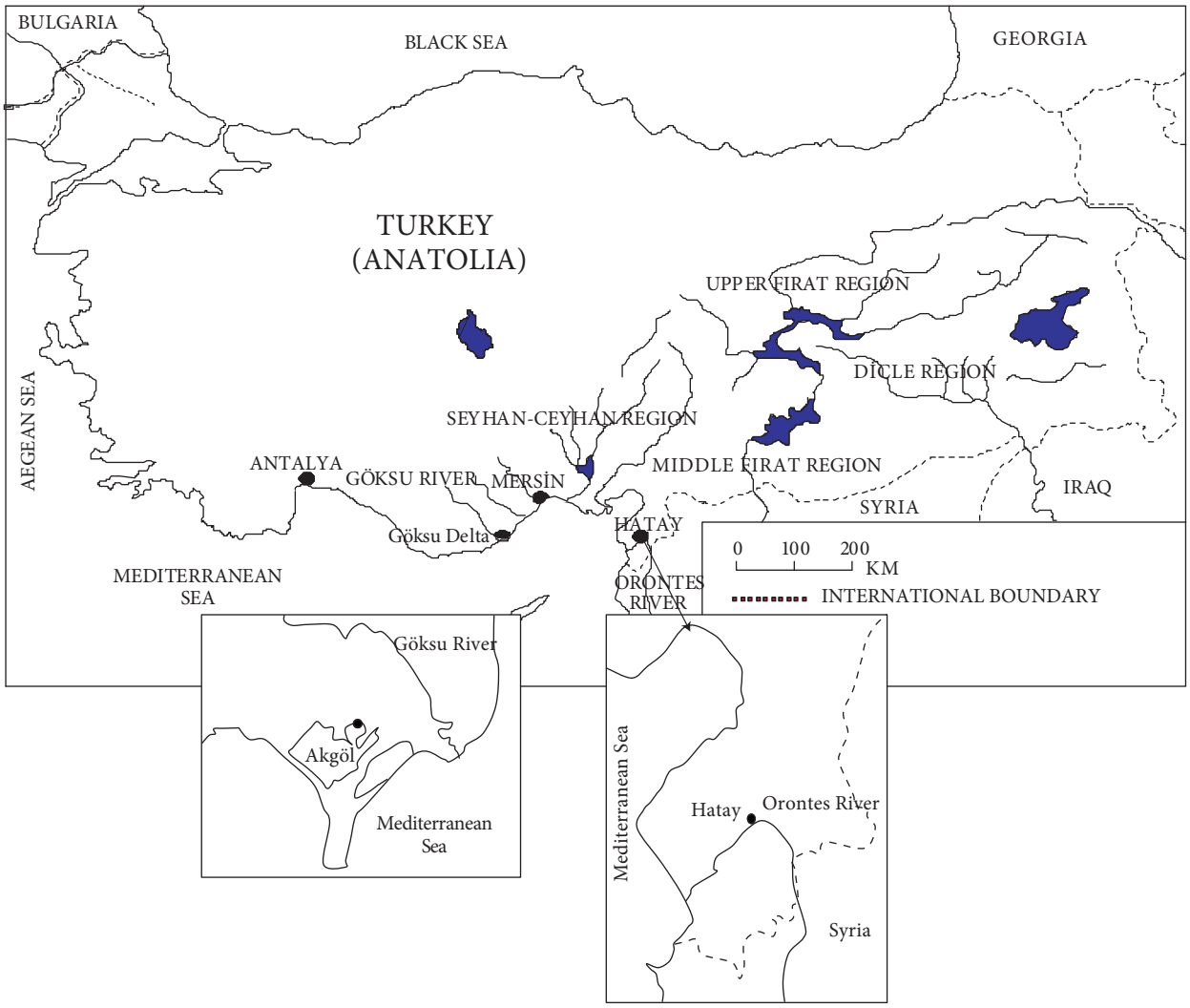

Figure 1. Collecting sites of *C. gariepinus* samples.

Table 2. The frequency analyses of chromosome number.

Göksu River					Orontes River				
NA	DM	CN (2N)	%	SE	NA	DM	CN (2N)	%	SE
	4	50	0.9	4.2		3	51	0.7	3.5
	5	52	1.1	2.8		4	52	1.0	2.8
	8	54	1.8	1.4		7	54	1.7	1.4
10	414	56	94.3	0.1	10	398	56	94.5	0.0
	5	58	1.1	1.5		3	57	0.7	0.7
	3	60	0.7	2.9		6	60	1.4	2.9
	439		100			421		100	

NA: Number of analyzed individuals, DM: Determining metaphase. CN: Chromosome number. SE: Standard error.

different karyotypes. The chromosome characteristics of *C. gariepinus* analyzed in the present study and in previous studies are summarized in Table 1.

The karyotype formula for the Orontes River samples was 24m + 10sm + 10st + 12a. Multiple NORs were observed in the terminal region on the short arm of submetacentric chromosome pair 13, on the long arm of subtelocentric chromosome pair 22, and on the long arm of acrocentric chromosome pairs 27 and 28 (Figure 2a). The C-band-positive heterochromatin regions were distributed in centromeric positions (Figure 2b). The heterochromatin blocks of

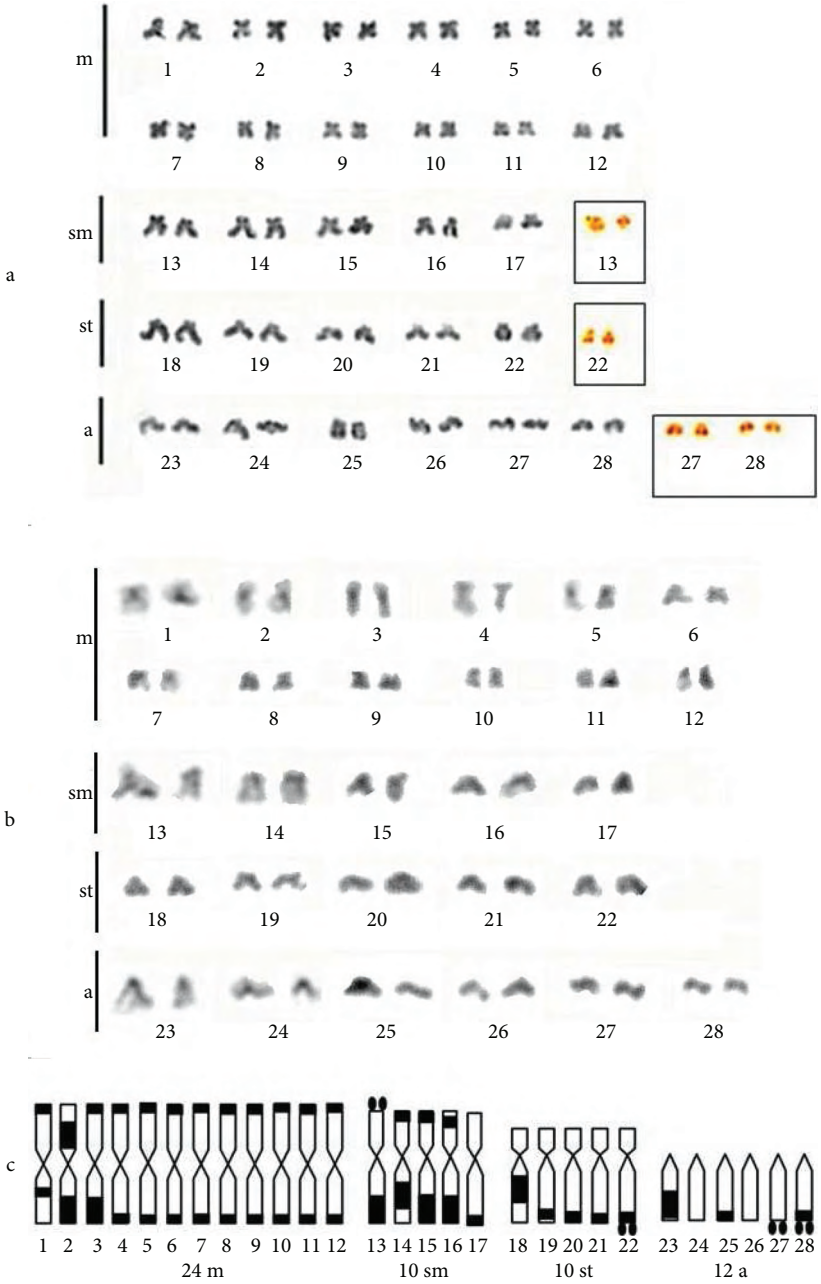

Figure 2. Karyotypes of *C. gariepinus* from Orontes River. (a) G-banded and (b) C-banded. In the inset, chromosomes bearing Ag-NORs (c) Ideogram: G-banded region.

chromosome pairs 2, 3, 13, 14, 15, 16, 18, and 23 were much larger than those of the other chromosomes. Other smaller blocks were observed in the pericentromeric and terminal regions of many chromosomes using G-banding (Figure 2a and c). The karyotype was created according to arm measurement.

The karyotype formula for the Göksu Delta samples was 28m + 6sm + 10st + 12a. Multiple NORs were observed in the terminal region of the short arm of metacentric chromosome pair 13 and on the long arm of subtelocentric chromosome pairs 21 and 22 (Figure 3a). The karyotype was made according to C-banding measurement. The detailed C-banded karyotype is presented in Figure 3b and c. C-band-positive heterochromatic regions were distributed in centromeric and pericentromeric positions. Clear C-bands were observed at the centromeric regions of many chromosomes and were interstitial on the short arm of metacentric chromosome pair 1 (Figure 3b and c). The heterochromatic blocks on chromosome pairs 2, 3, 8, 20, and 23 were much larger than those on the other chromosomes. In the present study smaller blocks were observed in the pericentromeric and terminal regions of many chromosomes using G-banding (Figure 3a and c)

Although the Göksu Delta and Orontes River samples have the same diploid number, they have very distinct constitutive heterochromatin distribution patterns. The largest chromosome pair of the complements was characterized as subtelocentric for both regions' samples (Figure 4a and b). According to Q-banding, a special band region was not observed on the chromosomes in the 2 regions' samples.

Discussion

In previous cytogenetics studies of *C. gariepinus* the chromosome number was reported as 2n = 56, with differences in the karyotypic formula: (FN = 89) 8m + 24sm + 28st/a (5), (NF = 89) 8m + 25sm + 23st/a (6), (FN = 88) 8m + 24sm + 24 st/a (7), and (FN = 88) 8m + 24sm + 24st/a (male) (8) (Table 1). In the present study the diploid chromosome number for the 2 regions' samples was 56, but the karyotypes differed. While the karyotype of Göksu Delta samples was 28m + 6sm + 10st +12a, the karyotype of the Orontes River samples was 24m + 10sm + 10st + 12a.

Most chromosome banding studies on fish used C-bands or silver- and chromomycin A3-bands for identifying NORs, while descriptions of the distinct structural Q-, G-, and replication-banding patterns are limited (16). Weak compartmentalization of the genomes due to the base composition (AT- or GC-rich DNA) of cold-blooded vertebrates has been reported (17,18). Weak compartmentalization of fish chromosomes is thought to be a primary cause of the limited number of reports on the distinct structural Q- and G-banding patterns.

G-banding can be used to identify chromosomal abnormalities, such as translocation, because there is a unique pattern of light and dark bands for each chromosome (16). In one study the karyotypic macrostructure of *Leporinus* fish was relatively constant, but it was reported that differences related to heterochromatin among the species were observed (19). Heterochromatin regions have been shown to be useful in the identification of polymorphisms and characterization of intrapopulation variability in some species (20). Ueda et al. (21) reported that the composition of heterochromatin had to be investigated more effectively in order to clarify the karyotypic evolution in this fish group. According to Giemsa-banding in the present study, very large heterochromatin regions were observed on metacentric chromosome pairs 2 and 8, subtelocentric chromosome pair 20, and acrocentric chromosome pair 23 in the Göksu Delta samples, and on metacentric chromosome pairs 2 and 3, submetacentric chromosome pairs 13, 14, 15, and 16, subtelocentric chromosome pair 18, and acrocentric chromosome pair 23 in the Orontes River samples. Localization of large heterochromatin regions varied widely between the 2 regions' samples. These chromosome pairs can be used as markers for the 2 regions' samples. In the present study the Q-band technique was performed on the metaphase plate of the 2 regions' samples, but a special band region was not observed.

NORs are an indicator of certain rewiring chromosomal polymorphism in and between species among many fish groups, and it was reported that this variety can affect the position on the chromosome,

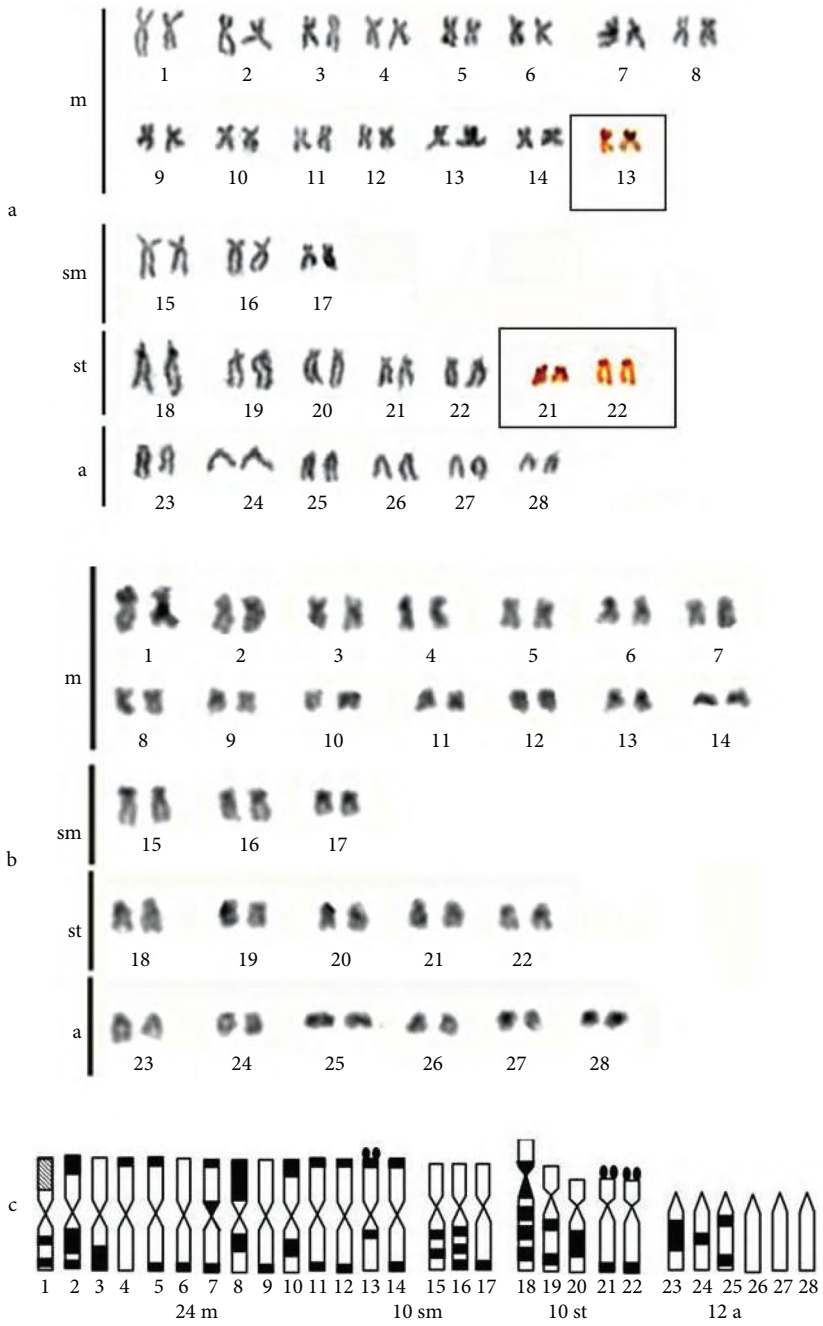

Figure 3. Karyotypes of *C. gariepinus* from Göksu Delta. (a) G-banded and (b) C-banded. In the inset, chromosomes bearing Ag-NORs (c) Ideogram: G-banded region, heterochromatin region outside the centromer.

and size and active number of NORs in the entire genome (22). Although NORs are commonly observed on the short arm of chromosomes, sometimes they can be observed on the long arm of m and a chromosomes (23). In the present study NORs in the Orontes River samples were observed on the short arm of submetacentric chromosome pair 13, and on the long arm of acrocentric chromosome pairs 22, 27, and 28 (Figure 2a). Göksu Delta specimens had 3 NORs, which were on the short arm of metacentric

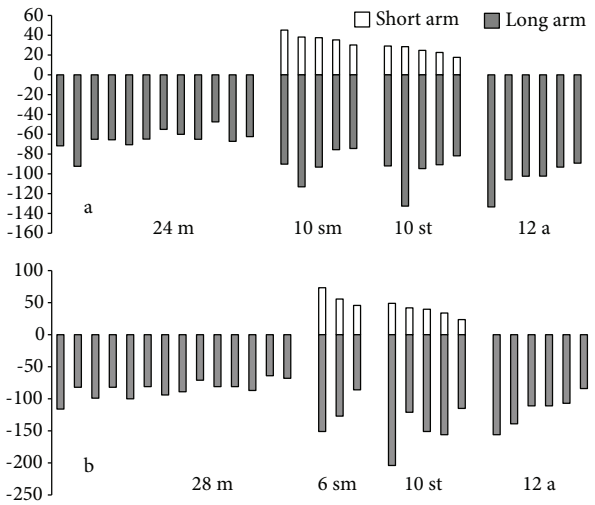

Figure 4. Arm ratio of *C. gariepinus* from (a) Orontes River, (b) Göksu Delta.

chromosome pair 13, and submetacentric chromosome pairs 20 and 21 (Figure 3a).

In general, C-band distribution patterns in many fishes are simple. Most species seem to have the centromeric C-band patterns characterized by similar size distributed in almost all chromosomes (24); however, in the present study different patterns were observed in the Göksu Delta samples. Most fishes with such characteristic C-band patterns belong to Cyprinidae and other lower teleostean groups. As such, C-band distribution in Cyprinidae is fairly interesting. Generally, karyotypic features in the lower teleostean group are more complicated than those of the intermediate and higher teleostean groups (24). According to our findings, special C-band patterns in the Göksu Delta samples can be used as a marker for this region (Figure 3b and c).

Thode et al. (25) reported that 2 *Scorpaena* species were very different, not only in chromosome number, but also in C-banding patterns. Ueda-Ojima (26) and Ojima-Takai (27) reported differences in the C-banding patterns among the *Carassius auratus* subspecies. Ueda and Ojima (28) reported geographical variation in the C-banding patterns in *Salvelinus leucomaenis*. Thus, changes in C-band patterns are useful for species differentiation. In the present study the C-band distribution patterns in the 2 regions' samples were very different.

Chromosome number and morphology, which are obtained from chromosomal analysis, are used for identification of species, and define the relationship and differences between species. The chromosome number and morphology can vary between fish species. This variation can be used to investigate the evolutionary relationship between interpopulation and intrapopulation (29). Comparison of the Orontes River and Göksu Delta samples showed that their chromosome formula patterns differed. Orontes River and Göksu Delta *C. gariepinus* samples were previously compared by Ergene et al. (30), in terms of morphometric characters, which were reported to be very different; the present study's results are in agreement.

The Mediterranean Sea was at least 100 m lower than present on several occasions during the Pleistocene (31). Ueda et al. (32) reported that these conditions might have allowed Nilotic specimens to expand northward via freshwater connections that are presently submerged or via massive freshwater runoff from the Nile during wet paleoclimatic periods. Alternatively, the slip tectonic fault between the Gulf of Aqaba, via the Jordan, Litani, and Orontes valleys might have provided a vehicle for northward migration. Several typical freshwater African elements are known from the Levant up to the Orontes (*Trionyx*) (32). According to Arndt et al. (33), DNA analysis was not useful to address the question of whether the *C. gariepinus* presently found south of Sagalassos in the Aksu River (Antalya) is an ancient population or represents a rather recent human introduction. From the earliest period onwards, marine fish have been exported to the site from the Mediterranean coast approximately 40 km to the south (33). In that coastal area *C. gariepinus* is currently found in the Akgöl (34).

Chromosomal distribution of heterochromatin regions and rDNA sites were analyzed in *C. gariepinus* samples collected from 2 regions in Turkey using C-, G-, and Q-banding, and Ag-NOR staining. These species exhibited various patterns according to the distribution of these bands. According to the findings, the 2 regions' species have differentiated. Diversification can be the result of chromosomal rearrangement, such as inversion, Robersonian translocation, or incorrect pairing during cell division.

The degree of differentiation indicates that isolation between the 2 populations may have occurred recently or that gene flow is occurring.

Acknowledgements

We thank Zafer Oranlı for aiding in the collection of *C. gariepinus*. This study was supported by Mersin University, Turkey.

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References

1. Kosswig G. The role of fish in research on genetics and evolution. In: Schroder JH (ed) Genetics and Mutagenesis of Fish. Springer-Verlag; Berlin, 1973; pp 3-16.
2. Barat A, Sahoo PK, Ponniah AG. Karyotype and Nucleolar Organizer Regions (NORs) in a few hill stream fishes. In: Ayyappan, S., Jena, J. K., Joseph, M. M. (eds) The Fifth Indian Fisheries Forum Proceedings, AFSIB, Mangalore and AoA, Bhubaneswar, 2002; pp. 111-114.
3. Gold JR, Li YC, Shipley NS et al. Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. J Fish Biol 37: 563-575, 1990.
4. Ergene S, Portakal E, Karahan A. Karyological analysis and body proportion of catfish (Clariidae, *Clarias lazera*, Valenciennes, 1840) in the Göksu Delta, Turkey. Tr J of Zoology 23: 423-426, 1999.
5. Hinegardner R, Rosen DE. Cellular DNA content and the evolution of Teleostean fishes. Am Nat 106: 621-644, 1972.
6. Teugels GG, Ozouf-Costaz C, Legendre M et al. A karyological analysis of the artificial hybridization between *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* Valenciennes, 1840 (Pisces; Clariidae). J Fish Biol 40: 81-86, 1992.
7. Klinkhardt M, Tesche M, Greven H. Database of fish chromosomes. Westarp Wissenschaften, 1995.
8. Ozouf-Costaz C, Teugels GG, Legendre M. Karyological analysis of three strains of the African catfish, *Clarias gariepinus* (Clariidae), used in aquaculture. Aquaculture 87: 271-277, 1990.
9. Palma J, Andrade JP. Morphological study of *Diplodus sargus, Diplodus puntazzo* and *Lithognathus mormrus* (Sparidae) in the Eastern Atlantic and Mediterranean Sea. Fisheries Res 57: 1-8, 2002.
10. John B, Milkos GLG. Functional aspects of satellite DNA and heterochromatin. Int Rev Cytol 58: 1-114, 1979.
11. Howell WM, Black DA. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer. 1 Step Method Experiential 36: 1014-1015, 1980.
12. Sumner AT. A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75: 304-306, 1972.
13. Schmid M. Chromosomal banding an Amphibia IV. Differentiation of GC and AT rich regions in Anura. Chromosoma 77: 83-103, 1980.
14. Seabright MR. A rapid banding technique for human chromosomes. Lancet 2: 971-972, 1971.
15. Levan A, Fredga K, Sandberg AA. Nomenclature for centromer position on chromosomes. Hereditas 52: 201-220, 1964.
16. Ueda T, Naoi H. BrdU-4Na-EDTA-Giemsa band karyotypes of 3 small freshwater fish, *Danio rerio, Oryzias latipes,* and *Rhodeus ocellatus.* Genome 42: 531-535, 1999.
17. Hudson AP, Cuny G, Cortadas J et al. An analysis of fish genomes by density gradient centrifugation. Eur J Biochem 112: 203-210, 1980.
18. Medrano L, Bernardi G, Couturier J et al. Chromosome banding and genome compartmentalization in fishes. Chromosoma 96: 178-183, 1988.
19. Galettı PM, Cesar ACG, Venere PC. Heterochromatin and NORs variability in *Leporinus* fish (Anostomidae, Characiformes). Caryologia 44: 287-292, 1991.
20. Mantovani M, Abel LDS, Mestriner CA et al. Accentuated polymorphism of heterochromatin and nuclear organizer regions in *Astyanax scabripinnis* (Pisces, Characidae): Tools for understanding karyotypic evolution. Genetica 109: 161-168, 2000.
21. Ueda T, Naoi H, Arai R. Flexibility on the karyotype evolution in bitterlings (Pisces, Cyprinidae). Genetica 111: 423-432, 2001.
22. Ozouf-Costaz C, Foresti F. Fish cytogenetic research advances applications and perspectives. Netherlands Journal of Zoology 42: 277-290, 1992.
23. Sola LS, Bressanello A, Rossi R et al. A karyotype analysis of the genus *Dicentrarchus* by different staining techniques. J Fish Biol 43: 329-337, 1993.
24. Takai A, Ojima Y. Chromosomal Distribution o f C-banded Heterochromatin in Cyprinid Fishes Proc. Japan Acad 64, Ser. B. 49, 1988.
25. Thode G, Alvarez MC, Giles V et al. Chromosome complement C-banding and Ag-NOR location in *Ophysurus serpens* (Ophichthidae, Anguilliformes). Cytobios 43: 73-77, 1985.

26. Ueda T, Ojima Y. Differential chromosomal characteristics in the funa subspecies (Carassius) Proc Japan Acad 54B, 283-288, 1978.

27. Ojima Y, Takai A. Further cytogenetical studies on the origin of the gold-fish. Proc Jpn Acad 55B, 346-350, 1979.

28. Ueda T, Ojima Y. Karyotypes with C-banding patterns of two species in the genus Salvelinus of the family Salmonidae. Proc Japan Acad 59, 343-346, 1983.

29. Thorgard GH, Dısney JE. Chromosome preparation and analysis methods for fish biology. American Fisheries Society, Bethesda, Maryland, USA, 1990; 171-187.

30. Ergene S, Yalcın S, Karahan A. A Preliminary Study on Metric and Meristic Variation of *Clarias lazera* (Valeciennes, 1840) which are Living Two Distinct Localities, III. National Aquaculture Symposium, 10-12. June, Erzurum 1998; 569-575.

31. Belknap DF, Mart Y. Sea-level low stand in the eastern Mediterranean: Late Pleistocene coastal terraces offshore northern Israel. J Coast Res 15: 399-412, 1999.

32. Por FD. The Legacy of Tethys: An Aquatic Biogeography of the Levant, Kluwer Academic, Dordrecht/Boston; 1989.

33. Arndt A, Neer WV, Hellemans B et al. Roman trade relationships at Sagalassos (Turkey) elucidated by ancient DNA of fish remains. J Arch Sci 30: 1095-1105, 2003.

34. Geldiay R, Balık S. Freshwater Fishes of Turkey. Ege University Press, Bornova, İzmir, Turkey, 1996. pp: 410-411.