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Scientific Report

Karyotype Analysis in *Alburnus heckeli* (Battalgil, 1943) from Lake Hazer

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Abstract: Karyotype analysis was performed on *Alburnus heckeli* (Battalgil, 1943) (Fam: *Cyprinidae*) by investigating the number and structures of its chromosomes. The fish used in this study were caught with fishing nets in Lake Hazer and were taken to our laboratory. Fish were injected intraperitoneally with doses of 0.01 ml/g body weight of 0.6% colchicine solution and left for 190 min before sacrificing. It was determined by metaphase investigation that *A. heckeli* has 2n = 50 chromosomes. Its karyotypes were determined to be composed of 7 metacentric, 9 submetacentric and 9 acrocentric chromosome pairs with NF = 82. We were unable to identify any sex-related chromosomes in this species.

Key Words: *Cyprinidae*, *Alburnus heckeli*, karyotype, Lake Hazer

Hazer Gölü'nden İnci Balığı'nda (*Alburnus heckeli* Battalgil, 1943) Karyotip Analizi

Özet: : *Alburnus heckeli* (Battalgil, 1943) (Fam: *Cyprinidae*)'nin kromozomlarının sayı ve yapıları incelenerek, karyotip analizi yapılmıştır. Bu çalışmada kullanılan balıklar Hazer Gölü'nden ağlarla yakalanarak laboratuvara getirilmiştir. Her bir gram vücut ağırlığı için 0,01 ml, % 0,6'lık kolsişin solusyonu balıkların karın boşluğuna enjekte edilmiş ve balık kesilmeden önce 190 dakika beklenilmiştir. Metafaz incelemeleri ile *A. heckeli*'nin 2n = 50 kromozoma sahip olduğu belirlenmiştir. Karyotiplerinin; 7 metasentrik, 9 submetasentrik ve 9 akrosentrik kromozom çiftinden (NF = 82) oluştuğu tespit edilmiştir. Bu türde cinsiyete bağlı herhangi bir kromozom tespit edilememiştir.

Anahtar Sözcükler: Sazangiller, İnci Balığı, karyotip, Hazer Gölü

Introduction

The family Cyprinidae is the richest and most important family of fish, and its members are distributed throughout the world (1). The vast majority of boned fish belongs to this family in Turkey, and these are distributed widely in freshwater sources. Although this family is represented by approximately 1500 species worldwide, there are only 30 geni and 70 species in Turkey (2).

Chromosomal analysis is important for fish breeding from the viewpoint of genetic control, the rapid production of inbred lines, taxonomy and evolutionary studies. Genetic divergences of populations and their local adaptation are a potential resource for breeding programs in aquaculture and for fishery management (3).

However, as happened in the Turkish Cyprinids, cytogenetic studies in fish have not been comprehensive when compared to other vertebrate groups (1-15). Standard karyotypes (chromosome and chromosome arm number) have been reported in less than 10% of the more than 20,000 species of fish. The application of chromosome banding methodologies to fish chromosomes has been minimal. The main difficulty in working with fish chromosomes is in obtaining high quality metaphase spreads. A few studies have used fish standard karyotypes to examine taxonomic or systematic problems (16).

The aim of this study was to investigate the chromosomes and karyotype of *A. heckeli* from Lake Hazer in Turkey.

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Materials and Methods

Eight *A. heckeli* females and 7 males were caught in Lake Hazer (lat 38° 31´ N, long 39° 25´ E) in eastern Turkey. The fish were transported live to the laboratory, and kept in a well-aerated aquarium at 20-25 °C before analysis.

Fish were injected intraperitoneally with doses of 0.01 ml/g body weight of 0.6% colchicine solution and left for 190 min before sacrificing. The gill filament tissues were removed and placed in hypotonic 0.046 M KCl solution for 45 min (17-25). They were then fixed in fresh, cold Carnoy (3:1) for 40 min. Staining was performed with 20% Giemsa in a Sorenson buffer solution for 7 min. The concentration of Giemsa may be reduced, but the treatment should then be longer (1).

Observations and microphotographs were taken with a Nikon light microscope. Chromosomes were classified on the basis of the arm-length ratio (21).

Results

Relatively small and high numbers of chromosomes were observed in *A. heckeli*. In 68 metaphases from the gill epithelial cells of 15 *A. heckeli* specimens, the diploid number was 2n = 50 (Figures 1 and 2). In 3 metaphase cells from the *A. heckeli* specimens the chromosome number was 49 (Figure 3). Different chromosome numbers in a total of 12 metaphase cells were recorded, ranging from 48 to 52 (Table 1).

Cells lacking a normal number of chromosomes values (2n = 48-52) were probably caused by losses during preparation or additions from nearby cells. The karyotype consists of 7 pairs of metacentric, 9 pairs of submetacentric and 9 pairs of acrocentric chromosomes (Figure 4). The number of chromosome arms was therefore determined to be NF = 82.

Discussion

Karyotypes are prepared from metaphases with well spread chromosomes. The major difficulty encountered is the morphological variation existing even between homologous chromosomes in the same nucleus (1,20). Sometimes it could happen that some chromosomes are more contracted than others, so chromosome measurements are very difficult, especially in fish, which have very small chromosomes compared to those of man and mammals. Another problem is that fish karyotypes are not identical, as in human beings or in other animal species, so we cannot have a standard karyotype for fish because not only are there differences between species, but polymorphism often occurs within the same fish species (1).

Several incomplete metaphases were encountered in the preparation, and these probably resulted from hypotonic overtreatment (19).

The majority of authors classify uni-armed and bi-armed chromosomes according to the guidelines of Levan

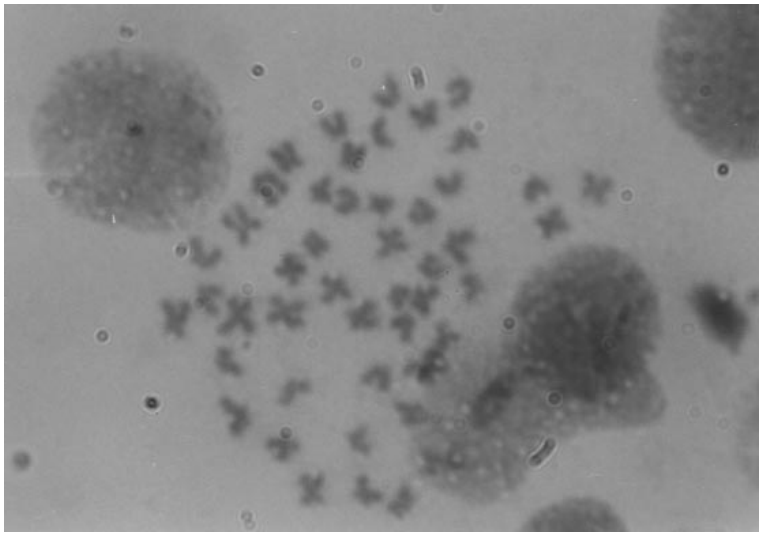

Figure 1. Metaphase spread from gill epithelial tissue of *A. heckeli* from Lake Hazer (Turkey). x 1,600.

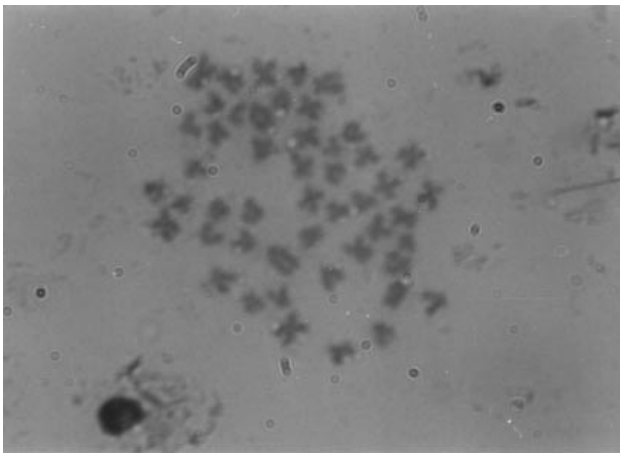

Figure 2. Metaphase spread from gill epithelial tissue of *A. heckeli* from Lake Hazer (Turkey). x 1,600.

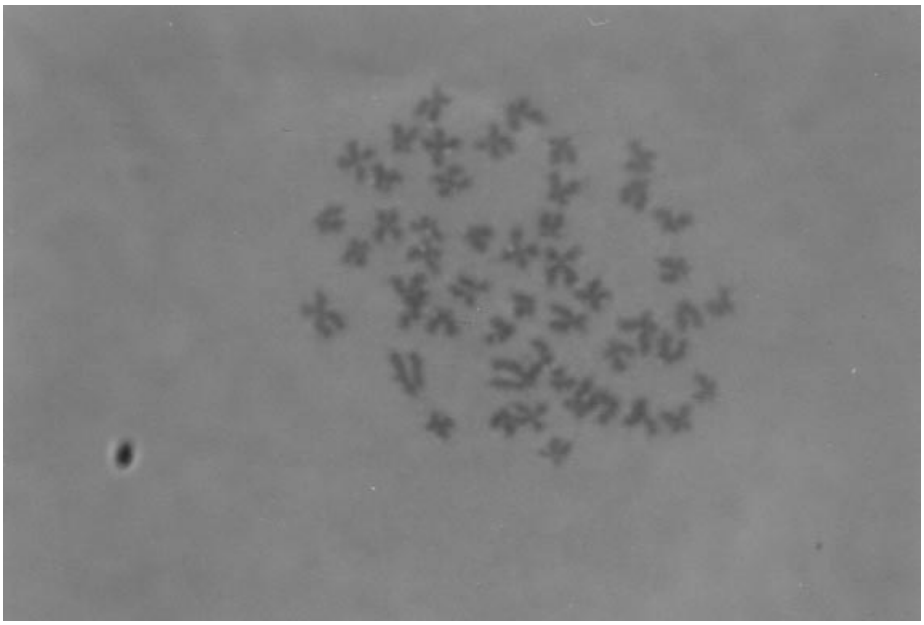

Figure 3. The number of *A. heckeli* chromosomes was found to be 49 in 3 metaphase cells x 1,600.

et al. (21). Where differences in the number of chromosome arms have been reported for the same species, this is usually the result of a difference in the scoring of subtelocentric chromosomes by different authors (3).

The majority of cyprinid species have 2n = 50 chromosomes (1), while *Cyprinus carpio* has 2n = 98-100 (22) and the polyploid Barbus species from southern Africa has 2n = 148 or 150 chromosomes (23). Fourty-eight chromosomes were found in a few species, such as *Chalcalburnus mossulensis* (24) and *Ctenopharyngodon idella* (2).

Karyotypes have been described for all major species of Alburnus (Table 2) (25). The chromosome numbers for all of the Alburnus species vary between 48 and 52, with NF numbers around 80.

Heteromorphic sex chromosomes have been identified in *Coregonus sardinella*, *Oncorhynchus mykiss*, *O. nerka*

Table 1. Chromosome complement of *A. Heckeli.*

	Chromosome number						karyotype (2n = 50)			
Number of fish	48	49	50	51	52	total metaphases	m	sm	a	NF
1	1		4	1		6	14	18	18	82
2			8		1	9				
3		1	5			6				
4			2	1		3				
5	1		7			8				
6		1	6			7				
7			4	1		5				
8			3			3				
9			4			4				
10	1		2			3				
11		1	3		1	5				
12			6			6				
13			4			4				
14	1		5			6				
15			5			5				
Totals	4	3	68	3	2	80				

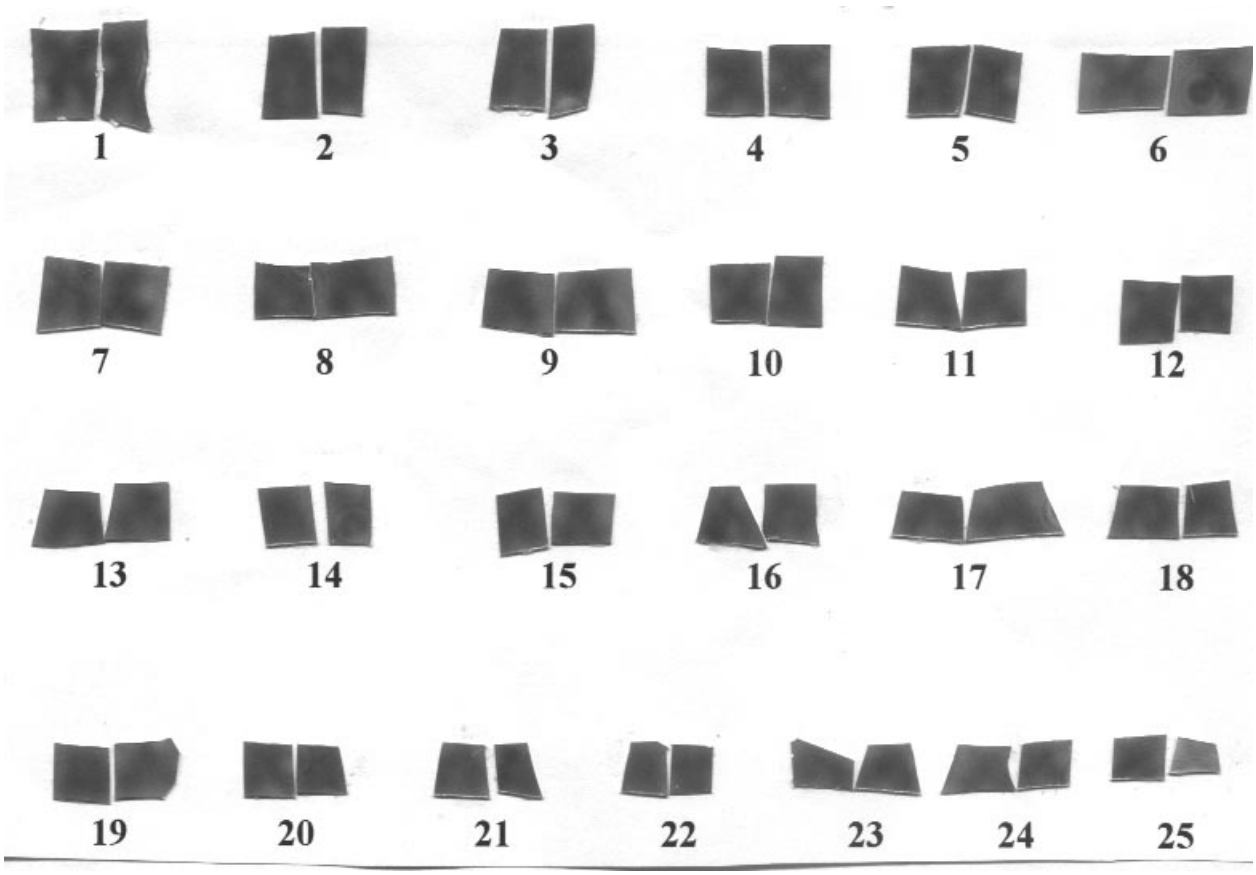

Figure 4. Karyotypes of a metaphase from *A. heckeli.* x 1,600.

Table 2. Karyotypes of *Alburnus* species.

Species	2n	No.M	No.SM	No.St	No.A	NF
Alburnus alburnus	50	16	10	16	8	76
A. bipunctatus	50	38 M,SM			12	90
A. heckeli	50	14	18		18	82

and *Salvelinus namaycush*. There is an XY/XX system in *S. namaycush* and *O. mykiss*, and an XYY system in *C. sardinella*. The formation of heteromorphic sex chromosomes often involves heterochromatin addition, as in other animals, and this appears to be the case in *S. namaycush* and *O. mykiss* (3). There was no evidence of sexual dimorphism of the chromosomes in *A. heckeli*. Similar results were also observed in many fish species (1,2,6-13,20,22,24-26).

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