

An electrophoretic taxonomic study on serum proteins of *Acanthobrama marmid*, *Leuciscus cephalus*, and *Chondrostoma regium*

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Abstract

In this study, native polyacrylamide gel electrophoresis (Native-PAGE) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were applied to the serum proteins of *Leuciscus cephalus*, *Acanthobrama marmid* and *Chondrostoma regium* (Cyprinidae) fish taken from the Karakaya Dam (Malatya, Turkey). The electrophoregrams showed that there were similarities and differences in the molecular weight (MW) of the serum proteins among the three species. In the Native-PAGE, the total number of serum protein bands of *L. cephalus*, *A. marmid* and *C. regium* were 5, 8, 7, respectively. In the SDS-PAGE, 13 protein bands were obtained in *L. cephalus*, 11 in *A. marmid* and 11 in *C. regium*. In the electrophoregrams obtained from native-PAGE, the MW of the protein bands were found to be different in the fish species except the 80.3 kD protein band which was detected in the three fish species. In the SDS-PAGE, the protein band of 21.7 kD was observed in the three fish species. Protein bands of 63.4, 52.3 and 49.5 kD were present only in *L. cephalus* and *C. regium*. On the other hand, it was seen that other protein bands had similar molecular weights.

Keywords: Fish, native-PAGE, SDS-PAGE, serum proteins, taxonomy.

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INTRODUCTION

In the past, the identification of fish species was carried out mainly by examining the external morphological characteristics. In the present day, electrophoresis of sarcoplasmic proteins, serum proteins, liver proteins and a number of enzymes often have been used by some researchers as an aid in the species identification of fish (Focant et al. 1981, Miyazaki et al. 1998, Pinerio et al. 2001). Recent studies indicated that serum proteins can present taxonomic values, when the serum proteins of different fish species are examined electrophoretically (Khan and Gadru 1988). We previously showed that

serum protein bands of *Capoeta trutta* and *Capoeta capoeta umbla* were significantly different (Yilmaz et al. 2000). Unfortunately, there has been no taxonomical study with these species by serum protein electrophoresis in Turkey.

In the present investigation, the serum proteins of *Leuciscus cephalus*, *Acanthobrama marmid* and *Chondrostoma regium* have been analyzed using the native and SDS-PAGE technique and the resemblances and differences between the species was established.

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MATERIAL AND METHODS

In the study, *Leuciscus cephalus*, *Acanthobrama marmid* and *Chondrostoma regium* belonging to the Cyprinidae family were used and the fish were obtained in the summer season from Karakaya Lake (Malatya, Turkey) (Fig. 1).

The fish were transported alive to the laboratory. A 2-3 ml blood sample was taken from the dorsal aorta of the fishes and injected into tubes, which contain EDTA. The blood samples were centrifuged at 1500 Rpm for 10 min at +4°C and to separate the serum. The obtained serum was used for the analysis of proteins and electrophoresis. Protein content was determined by using the method of Lowry et al. (1952) with bovine serum albumin as a standard.

SDS-PAGE was performed according to the Laemmli (1970) and O'Farrell (1975) methods. Proteins were separated on ft-16x10 cm dimension and a 1 mm thick slab gel. Slab gel consist of stacking gel [which proteins (stocked)] and a running gel part on which proteins seperate. Running gel containing 10% acrylamide was polymerized 12 hrs before electrophoresis and stacking gel containing 4% acrylamide was poured and polymerized 2 hrs before sample application. Each sample was mixed with a sample buffer which contained 10% glycerol, 2% mercaptoethanol, 2% SDS and 0.01 brom phenol blue. Protein concentrations were adjusted to 2 µg/µl and 0.2 µg/µl, then heat-denatured and run on the SDS-PAGE. For SDS-PAGE, 20µl sample were loaded on the stacking gel. And 200 Volts applied the brom phenol blue was present on the lowest side of the gel. Following electrophoresis, the proteins were stained with 0.125% Commassie Brilliant Blue R-250 in 40% ethanol and 7% acetic acid, and then destained in acetic acid. Bovine serum albumine (66 kD) and pepsin (34.7 kD) were used as the protein standard in the SDS-PAGE electrophoresis and pepsin (34.7 kD) and β-lactoglobulin (18.4) were used as protein standart for the native PAGE. Molecular weight of the proteins were calculated according to Weber et al. (1972).

RESULTS

In the native-PAGE, the protein bands of *Leuciscus cephalus*, *Acanthobrama marmid* and *Chondrostoma regium* were 5, 8, 7, respectively (Fig. 2). Molecular weights of the protein bands were 80.3, 61.2, 53.8, 41.5 and 30.4 kD in *Leuciscus cephalus*, 80.3, 76.4, 70.6, 68.8, 52.4, 45.5, 43.1, and 34.1 kD in *Acanthobrama marmid*, 80.3, 66.1, 56.6, 51.8, 44.2, 34.7, and 27 kD in *Chondrostoma regium*. In the SDS-PAGE, protein bands of *Leuciscus cephalus*, *Acanthobrama marmid* and *Chondrostoma regium* were 13, 11, 11, respectively (Fig. 3). Molecular weights of the protein bands were found to be 98.3, 89.2, 68.8, 65.1, 63.4, 52.3, 49.5, 43.1, 31.9, 31, 27, 21.7, and 9 kD in *Leuciscus cephalus*, 101, 76.8, 66.7, 61.7, 51, 38.2, 33.7, 31.1, 27.4, 21.7, and 9.2 kD in *Acanthobrama marmid*, 95.6, 63.4, 56.8, 52.3, 49.5, 36.6, 31.4, 28.8, 21.7, and 9.5 kD in *Chondrostoma regium*.

DISCUSSION

In general, taxonomic studies are based on morphometric measurements and anatomical characteristics. Electrophoresis of serum proteins have been widely used in the classification of fish. These kinds of studies brought about a new look to taxonomical evaluation. Discrimination of related taxa can be easily made according to their electrophoretic results of serum proteins (Theophilus and Rao 1998). In a previous study by using SDS-PAGE, the serum protein bands of *Capoeta trutta* and *Capoeta capoeta umbla* were separated and analyzed by using SDS-PAGE. The serum protein band numbers of these fishes were 16 and 11, respectively (Yilmaz et al. 2000). In the other research, the sarcoplasmic proteins of *Orthrias insignis euphraticus* and *Cyprinion macrostomus* were separated by using SDS-PAGE and, the electrophoregram showed that there were differences between the two species in both the number of bands and the molecular weight of the sarcoplasmic proteins (Yilmaz et al. 2005).

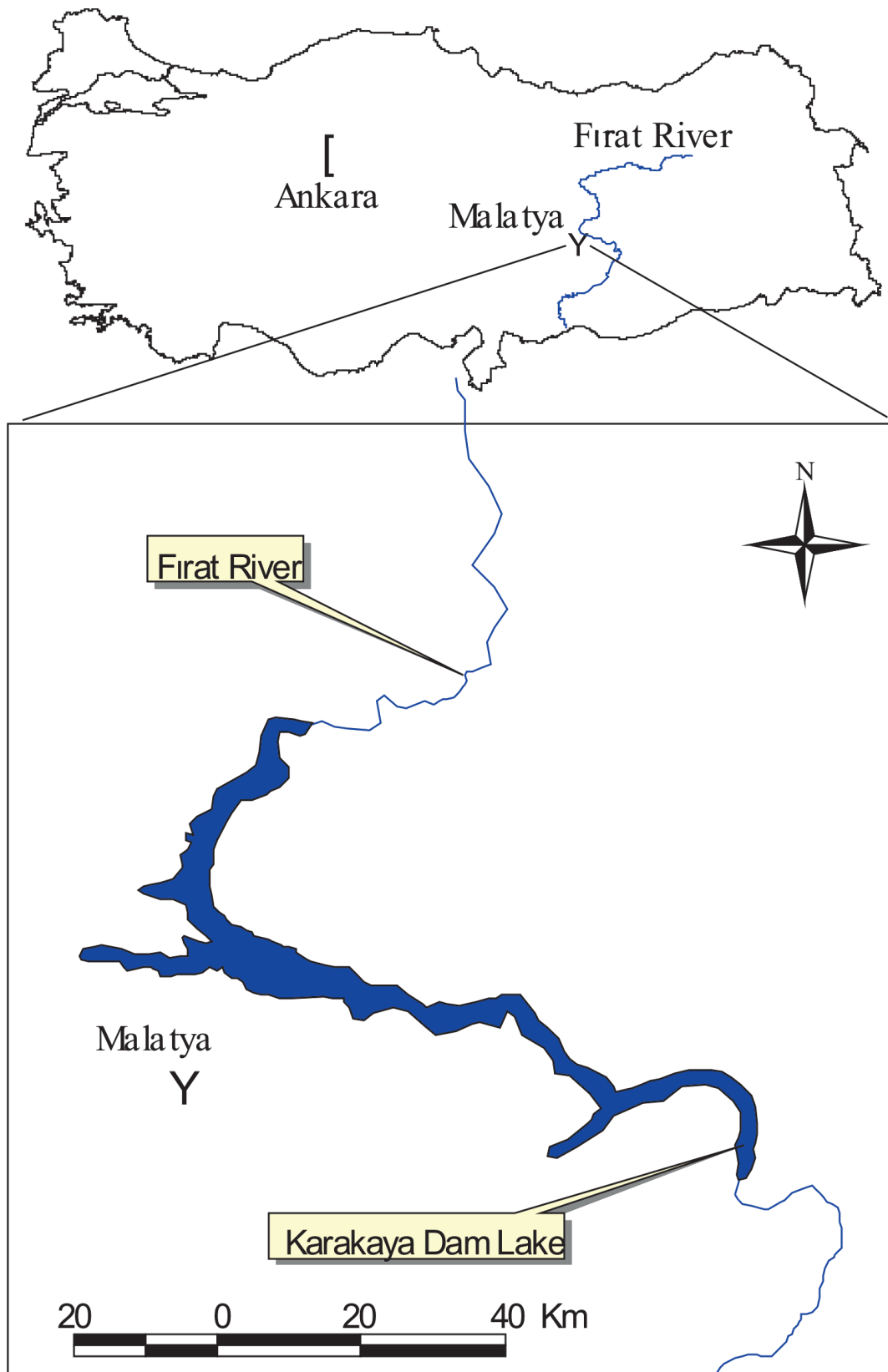


Fig 1. Map of study area.

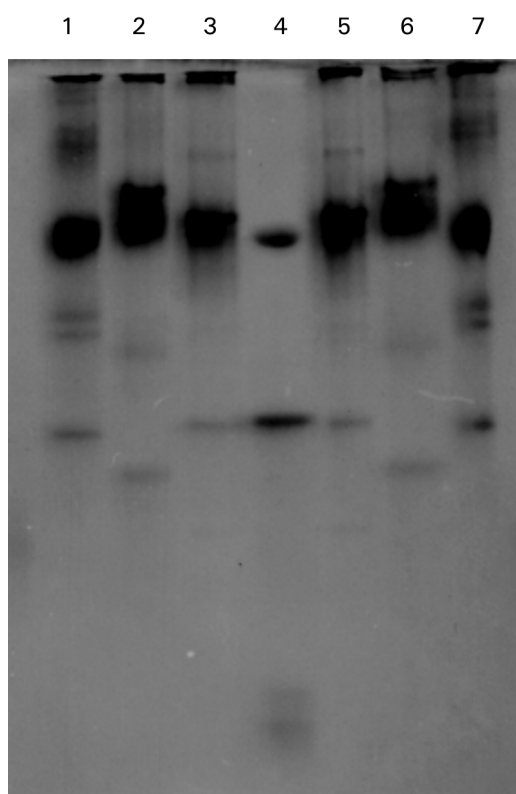


Fig 2. Native-PAGE of the serum proteins of *Leuciscus cephalus*, *Acanthobrama marmid* and *Chondrostoma regium* (1st lane belongs to *Acanthobrama marmid*, 2nd lane *Leuciscus cephalus* 3rd lane *Chondrostoma regium*, 4th lane the standard proteins, 5th lane *Chondrostoma regium*, 6th lane *Leuciscus cephalus* and 7th lane *Acanthobrama marmid*).

Similarly, the liver proteins of six species belonging to the Cyprinidae family, Acheilognathinae, Leuciscinae and Gobioninae subfamilies were separated using SDS-PAGE and *Cyprinus carpio* and *Pseudogobius esocinus esocinus* gave the smallest genetic distance. Nevertheless, *Tribolodon hakonensis* had almost equal genetic distances to the three other species (Miyazaki et al. 1998). In an other investigation, the serum proteins of the female *Cyprinus carpio*. And male *Ctenopharyngodon idella* were analysed using SDS-PAGE and it was stated that there were differences in the electrophoregrams of each species (Li 1991). Sexual dimorphism in electrophoretic patterns of blood serum proteins of a smelt [*Hypomesus nipponensis*] was investigated (Komagata et al. 1991). They found that in the electrophoretic

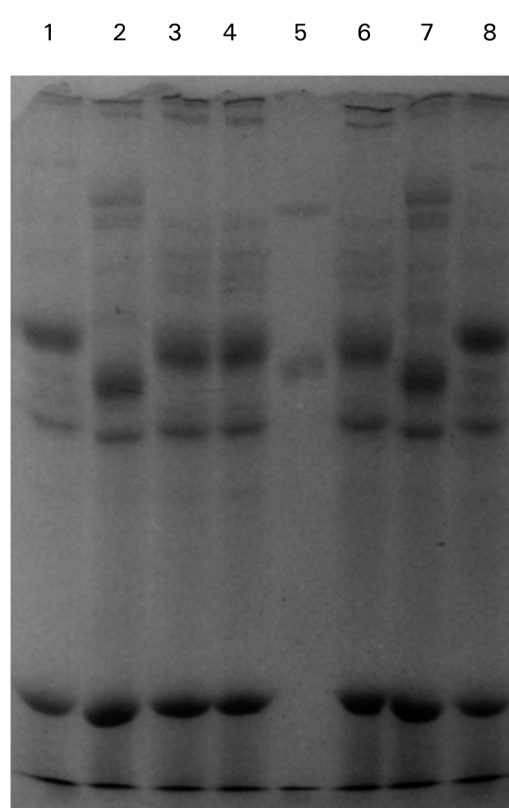


Fig 3. SDS-PAGE of the serum proteins of *Leuciscus cephalus*, *Acanthobrama marmid* and *Chondrostoma regium* (1st lane belongs to *Acanthobrama marmid*, 2nd lane *Leuciscus cephalus* 3rd and 4th lane *Chondrostoma regium*, 5th lane the standard proteins, 6th lane *Chondrostoma regium*, 7th lane *Leuciscus cephalus* and 8th lane *Acanthobrama marmid*).

analyses of blood serum proteins were different in the female and male *Hypomesus nipponensis*. In other research, the serum protein profiles of parr and smolt in masu salmon (*Oncorhynchus masou*) have been analysed by two-dimensional SDS-PAGE and they have identified two proteins (43.80 kD) as possible smolt-specific serum proteins, these proteins were not detected in parr. The presence of differences in serum protein profiles between parr and smolt were confirmed using two-dimensional SDS-PAGE (Ura et al. 1994).

The serum proteins of five species of freshwater fish (*Sarotherodon galilaeus*, *Tilapia zillii*, *Oreochromis niloticus*, *Clarias lazera*, and *Barbus bynni*) were studied, electrophoretically. Eight fractions of serum

proteins in *S. galilaeus*, *T. zillii* and *O. niloticus* were reported. In the same research, seven and five fractions of serum proteins were found in *C. lazera* and *B. bynni*, respectively (Zowail and Baker 1998). In other investigation, genetic variations about the relationship among the characid fish *Alestes dentex* and cyprinid fishes *Barbus bynni*, and *Labeo niloticus* common in the River Nile at El Minia, Egypt, and the Chinese grass carp, *Ctenopharyngodon idella* were studied. Variations are found in fourteen enzymatic and two non-enzymatic proteins encoded by twenty-one structural loci. The characid fish *A. dentex* demonstrated the minimum levels

of polymorphism. According to genetic similarity and distance between characid and cyprinid fishes, they closely related to each other (Shahin 1999).

In the study of mentioned, serum protein band numbers of *Acanthobrama marmid* and *Chondrostoma regium* have shown a similarity to serum protein band numbers of *Capoeta capoeta umbla* mentioned above. However, serum protein band patterns of the *Leuciscus cephalus* studied were found to be different. In conclusion, these fishes are easily distinguished by native and SDS-PAGE, taxonomically.

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***Acanthobrama marmid*, *Leuciscus cephalus* ve *Chondrostoma regium*'un Serum Proteinleri Uzerine Taksonomik Bir Calisma**

Ozet

Bu calismada, Karakaya Baraj Golu (Malatya)'nden yakalanan *Leuciscus cephalus*, *Acanthobrama marmid* and *Chondrostoma regium* (Cyprinidae)'nin serum proteinleri dogal poliakrilamid gel elektroforez (Native-PAGE) ve Sodyum dodeosil sulfat-poliakrilamid jel elektroforezine (SDS-PAGE) uygulandi. Elektroforegramlar uc turun serum proteinlerinin molekuler agirliklari arasinda benzerlik ve farkliliklerin varligini gostermistir. *L. cephalus*, *A. marmid* and *C. regium*'un total serum protein bandlari Native-PAGE'de sirasiyla 5, 8, 7 ' dir. SDS-PAGE'de *L. cephalus* icin 13, *A. marmid* icin 11 ve *C. regium* icin 11 protein bandi elde edildi. Native-PAGE'den elde edilen elektroforegramlarda protein bandlarinin molekuler agirliklari 80.3 kD protein bandi haric, uc balik turunde farkli bulundu. Uc balik turunde protein bandlarinin molekuler agirliklari SDS-PAGE'de 21.7 kD olarak belirlendi. 63.4, 52.3 and 49.5 kD protein bandlari sadece *L. cephalus* ve *C. regium*'da mevcuttu. Diger taraftan, diger protein bandlarinin molekuler agirliklarına yakin oldugu goruldu.

Anahtar Kelimeler: Balik, native-PAGE, SDS-PAGE, serum proteinleri, taxonomi.