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An Electrophoretic Taxonomic Study on Sarcoplasmic Proteins of Some *Balithorid* and *Cobitid* Fishes Habitated in Kars Stream, Turkey.

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Özet

Bu çalışma da, *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* ve *Cobitis taenia*'nın sarkoplazmik proteinleri Sodyum dodesil sülfat- poliakrilamid jel elektroforezine (SDS-PAGE) uygulandı. Elde edilen elektroforegramda, *Orthrias angorae bureschi* ve *Orthrias panthera*'da 13 sarkoplazmik protein bandı elde edildi. Bunun yanısıra, bu balıkların 5 sarkoplazmik protein bandının aynı moleküler ağırlıkta olduğu bulundu ve benzerlik katsayısı (SC): 0.384 olarak bulundu. *Orthrias tigris*'te 8 ve *Cobitis taenia*'da ise 12 sarkoplazmik protein bandı bulundu. *Orthrias tigris* ve *Cobitis taenia* arasında 2 protein bandı benzerlik gösterdi ve benzerlik katsayısı 0.166 olarak bulundu. Bununla birlikte, *Cobitis taenia* ve *Orthrias angorae bureschi*'nin 2 sarkoplazmik protein bandının benzer olduğu bulundu ve benzerlik katsayısı 0.153 olarak bulundu. *Cobitis taenia* ve *Orthrias panthera*'nın tüm sarkoplazmik protein bandları farklı olarak bulundu. *Orthrias angorae bureschi* ve *Orthrias tigris* arasında 1 sarkoplazmik protein bandı benzerdi ve benzerlik katsayısı 0.076 bulundu. *Orthrias tigris* ve *Orthrias panthera* arasında 3 protein bandı benzerdi ve benzerlik katsayısı 0.230 olarak bulundu.

Morfolojik olarak birbirine çok benzeyen *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* ve *Cobitis taenia*'ların sarkoplazmik protein bandları yönünden elektroforetik olarak benzerlikleri ve farklılıkları ortaya çıkarıldı. Elde edilen sonuçlara göre, *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* ve *Cobitis taenia*'ların morfolojik özelliklerinden yaralanılarak yapılan taksonomik çalışmaların doğru olduğu ortaya çıkarıldı.

Anahtar Kelimeler: Kars Çayı, *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera, Cobitis taenia,* sarkoplazmik protein, elektroforez, taksonomi.

Absract

In this study, sarcoplasmic proteins of *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* and *Cobitis taenia* were analyzed by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In the electrophoregram, 13 sarcoplasmic protein bands were obtained from *Orthrias angorae bureschi* and *Orthrias panthera.* Nevertheless, 5 protein bands of these fishes were mutually calculated and similarity coefficient (SC) was found as 0.384. Sarcoplasmic protein bands of *Orthrias tigris* and *Cobitis taenia* were 8 and 12, respectively. 2 sarcoplasmic protein bands of *Orthrias tigris* and *Cobitis taenia* were found to be similar and similarity coefficient (SC) was 0.166. However, 2 sarcoplasmic protein bands of *Cobitis taenia* and *Orthrias angorae bureschi* were similar and similarity coefficient (SC) was found as 0.153. All the sarcoplasmic protein bands of *Cobitis taenia* and *Orthrias panthera* were observed to be different. On the other hand, 1 sarcoplasmic protein band of *Orthrias angorae bureschi* and *Orthrias tigris* was found to be similar and similarity coefficient (SC) was found as 0.076. 3 sarcoplasmic protein bands of *Orthrias tigris* and *Orthrias panthera* were found to be similar and similarity coefficient (SC) was found as 0.230.

Similarities and differences of *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* and *Cobitis taenia* which highly resemble each other morphologically in term of sarcoplasmic protein bands were electrophoretically found out. According to the results of scientific researches, it was found out that taxonomic studies, which were obtained by benefitting from morphological characteristic of *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* and *Cobitis taenia*, were right.

Key Words: Stream of Kars province, *Orthrias tigris, Orthrias angorae bureschi*, *Orthrias panthera*, *Cobitis taenia,* Sarcoplasmic proteins, SDS-PAGE, Taxonomy.

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INTRODUCTION

Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera (Balitoridae) and *Cobitis taenia* (Cobitidae) are belong to Cypriniformes order. They are morphologically very close species each other. Moreover, Balitoridae family have a number of similarities with the sibling family of loaches (Cobitidae). In solving this problem, the use of electrophoretic techniques becomes inevitable. For this reason, In the past, as an aid in the species identification of fish are used mainly electrophoresis of sarcoplasmic proteins, serum proteins, and a number of enzymes [1,2]. From these techniques, sarcoplasmic protein electrophoresis is often used in the species identification of fish.

In the present study, sarcoplasmic proteins of *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* and *Cobitis taenia* have been analyzed by SDS-PAGE technique and thus, resemblances and differences between these species have been tried to establish.

MATERIALS AND METHODS

In this study, specimens of *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* and *Cobitis taenia* were collected from Kars stream (TURKEY). Fish samples which has no dermal infection and parasite from each groups were alive when they were transported to the laboratory. Then, a piece of white muscle tissue were taken from the fish by means of a bistoury. Tissue sample of the all fish were taken from left dorsal. After that, these samples cleaned of the skin and fish bone and homogenized in Tris-HCl buffer (0.5 M, pH: 6.8) for 1 minute. The homogenates were centrifuged for 15 minutes at +4°C and 20.000 g. The obtained supernatants were used for the analysis of proteins and SDS-PAGE. Protein content determined by using the method of Lowry et al. [3] with bovine serum albumin as a standard.

SDS-PAGE was performed according to the Laemmli and O'Farrell's methods[4,5]. Proteins were separated on 12x8 cm dimension and 1 mm thick slab gel. Slab gel consist of stacking gel which proteins stocked and running gel part on which proteins separate. Running gel (contains 10% acrylamide) was polymerized 12 hr before from electrophoresis and stacking gel (contains 4% acrylamide) was poured and polymerized 2 hr before sample application. Each sample mixed with sample buffer which contains 10% glycerol, 2% mercaptoethanol, 2% SDS, 0.01 brom phenol blue and protein concentration adjusted to 2 µg/µL with Tris-HCl buffer, then heat-denatured and run on SDS-PAGE. For SDS-PAGE, 20µl sample were loaded on the stacking gel. 200 V given until brom phenol blue come to lowest side of gel. Following electrophoresis, the proteins were stained with 0.125% commassie brilliant blue R-250 in 40% ethanol and 7% acetic acid for 12 hr, and then, destained in acetic acid. Excess stain in the gel was removed from the gel by waiting in the solution (5% methanol + 7.5% acetic acid) for 24 hours. That is, it was decolored. Bovine albumin (66 kD), egg albumin (44 kD), trypsinogen (24 kD) and lisosyme (14 kD) were used as protein standards in electrophoresis. Molecular weights of proteins were calculated according to Weber et al.[6].

RESULTS

Sarcoplasmic protein bands of *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* and *Cobitis taenia* were analysed by SDS-PAGE. Molecular weights of sarcoplasmic proteins of these fishes were showed in table 1. 13 sarcoplasmic protein bands were found in *Orthrias angorae*

bureschi and *Orthrias panthera.* Nevertheless, 5 protein bands of these fishes were mutually calculated and, similarity coefficient (SC) was found as 0.384. Sarcoplasmic protein bands of *Orthrias tigris* and *Cobitis taenia* were 8 and 12, respectively. 2 sarcoplasmic protein bands of *Orthrias tigris* and *Cobitis taenia* were found to be similar and similarity coefficient (SC) was 0.166. However, 2 sarcoplasmic protein bands of *Cobitis taenia* and *Orthrias angorae bureschi* were similar and similarity coefficient (SC) was found as 0.153. All the sarcoplasmic protein bands of *Cobitis taenia* and *Orthrias panthera* were observed to be different. On the other hand, 1 sarcoplasmic protein band of *Orthrias angorae bureschi* and *Orthrias tigris* was found to be similar and similarity coefficient (SC) was found as 0.076. 3 sarcoplasmic protein bands of *Orthrias tigris* and *Orthrias panthera* were found to be similar, and similarity coefficient (SC) was found as 0.230 (figure 1).

Table 1. Molecular weights of sarcoplasmic protein bands of *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* and *Cobitis taenia*.

Orthrias angorae bureschi **(kD)**	*Orthrias tigris* **(kD)**	*Cobitis taenia* **(kD)**	*Orthrias panthera* **(kD)**
85.0	79.5	86.1	79.5
82.8	**69.6**	79.5	**77.4**
77.4	51.9	71.5	**69.6**
69.6	**50.5**	**60.9**	66.0
60.9	48.6	**50.5**	**47.9**
49.2	**29.6**	**49.2**	46.0
47.9	**28.1**	45.4	**41.9**
43.9	20.9	34.0	36.7
41.9		30.4	35.7
33.0		29.6	**28.1**
32.1		22.1	27.4
20.5		14.8	19.9
11.6			**11.6**

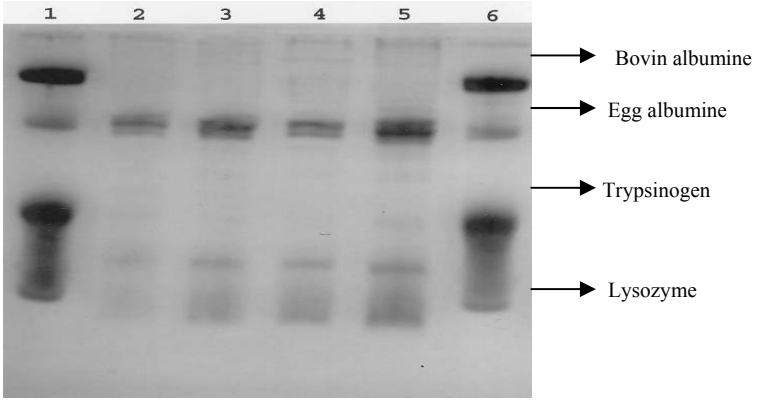

Figure. 1. Comparison of sarcoplasmic proteins of *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* and *Cobitis taenia*. 1. Standard proteins, 2. *Cobitis taenia,* 3. *Orthrias tigris,* 4. *Orthrias panthera,* 5. *Orhtrias angorae bureschi*, 6. Standard proteins.

DISCUSSION

Electrophoretic analyses of sarcoplasmic proteins have been prevalently used for fish taxonomy. Thus, definition of relative taxons can be made[7].

Although the identification of fish species belong to Balitoridae family have made various studies for different species, unfortunately, there is no taxonomical study on sarcoplasmic protein electrophoresis of *Orthrias angorae bureschi*, *Orthrias panthera, Orthrias tigris* and *Cobitis taenia*. In a research, phylogenetic relationships and intraspecific variations of loaches of the genus *Lefua* (Balitoridae) were investigated by using two dimensional electrophoresis and DNA analyses. Protein analyses showed that genetic distances between *Lefua* sp. and *L. echigonia s. str.* and between *Lefua sp.* and *L. nikkonis* were as large as that between *L. echigonia s. str.* and *L. nikkonis*. DNA analyses showed that *Lefua sp.* was more closely related to *L. echigonia s. str.* than to the *L. nikkonis-L. costata* complex[8]. In an another study, DNA analyses *of Lefua nikkonis*, *Lefua echigonia* and *Lefua costata* were investigated. This study showed that each species of *Lefua* formed a monophyletic group, indicating clearly that *Lefua* species can be genetically distinguished from one another[9]. In an other research, sarcoplasmic proteins of *Orthrias insignis euphyraticus* and *Cyprinion macrostomus* were applied by SDS-PAGE, and total number of the sarcoplasmic protein bands of *Orthrias insignis euphyraticus* were 20 while *Cyprinion macrostomus* had 18 protein bands. In conclusion, since the number of the sarcoplasmic protein bands differs between *Orthrias insignis euphyraticus* and *Cyprinion macrostomus,* two fish species are easily distinguished from each other taxonomically[10]. The present study, The sarcoplasmic proteins of *Orthrias angorae bureschi, Orthrias panthera, Orthrias tigris* and *Cobitis taenia* were found to be different from *Orthrias insignis euphyraticus*. In an another study, carried out on Serum Proteins of *Acanthobrama marmid, Leuciscus cephalus,* and *Chondrostoma regium,* serum protein band numbers of *Acanthobrama marmid* and *Chondrostoma regium* have shown a similarity to serum protein band numbers of *Capoeta capoeta umbla*. However, serum protein band patterns of *Leuciscus cephalus* studied were found to be different. In conclusion, these fishes are easily distinguished by native and SDS-PAGE, taxonomically[11].

Similarities and differences of *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* and *Cobitis taenia* which highly resemble each other morphologically in term of sarcoplasmic protein bands were electrophoretically found out. According to the results of scientific researches, it was found out that taxonomic studies, which were obtained by benefitting from morphological characteristic of *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* and *Cobitis taenia*, were right.

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