Journal of Genetic and Environmental Resources Conservation, 2013,1(1):1-5.
www.jgerc.com

Classification of Bunnei *Barbus sharpeyi* (Gunther, 1874) and Gattan *Barbus xanthopterus* (Heckel, 1843) Iraqi fishes by using proteins electrophoretic pattern

Faris A. Al-Obaidi

Iraq Natural History Research Center & Museum, University of Baghdad, Baghdad, Iraq.

Abstract

The objective of this study was to classification of Bunnei (*Barbus sharpeyi*) and Gattan (*Barbus xanthopterus*) Iraqi fishes by using muscle proteins electrophoretic pattern. Ten individuals of Bunni and twelve of Gattan were collected from the region of Al-Siweira in Wasit province at a weight (2000 ± 36) g and an average total length (28 ± 2.5) cm, samples of 1.0 gm of fish muscle were taken from individuals to determined electrophoretic pattern of muscle proteins. Results revealed that electrophoretic pattern of muscle proteins were similar between the two fish species. But, Gattan predominant Bunnei in the number of separated proteins. Gattan muscle proteins were separated into 21 protein bands, whereas Bunni muscle proteins were separated into 16 protein bands. Also, the two fish species were differed in the percentage values of the separated protein bands. No significant variations in the electrophoretic pattern of muscle proteins due to sex within species were noticed. In conclusion, the ability of using electrophoretic pattern of muscle proteins as a good diagnostic tool for Bunnei and Gattan fishes classification.

Key words: Classification, Bunnei, Gattan, Iraqi fishes, Proteins electrophoresis.

Introduction

Bunnei (*Barbus sharpeyi*) and Gattan (*Barbus xanthopterus*) are actually members of the fishes of Iraq, these species are found in the Tigris-Euphrates basin and they have well adapted in freshwater of Iraq (Beckmann, 1962; Al-Hazzaa, 2005).

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; Pineiro *et.al.*, 2001).

Electrophoresis is being used with increasing frequency by vertebrate taxonomists (Roman *et.al.*, 2009). This technique takes advantage of the different migration rates of protein molecules in an electric field, electrophoresis is one of the most effective methods for the separation of ionic components of a mixture, the resolving power of different electrophoretic methods is quite variable. To separate two component ions, it is necessary to permit migration to continue until one of the kinds of ions has traveled further than the other (Ordonneau *et.al.*, 2005). Muscle protein electrophoresis is an invaluable diagnostic tool in fish classification, Its a powerful technique that has been applied widely in population biology (Chen *et.al.*, 2003; Smith, 2009).

The objective of this study was to classification of Bunnei (*Barbus sharpeyi*) and Gattan (*Barbus xanthopterus*) Iraqi fishes by using muscle proteins electrophoretic pattern.

Materials and Methods

Ten individuals of Bunnei (*Barbus sharpeyi*) and twelve of Gattan (*Barbus xanthopterus*) Iraqi fishes were collected from the region of Al-Siweira in Wasit province at a weight (2000 ± 36) g and an average total length (28 ± 2.5) cm, samples of 1.0 ml of fish muscle were taken from the individuals.

Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS - PAGE) was performed

according to the Laemmli (1970) methods, proteins were separated by using a ten column electrophoresis apparatus utilizing stainless steel wires electrodes and two cubic reservoirs (17 cm deep and 11 X 16 cm in dimensions) constructed from 12 cm glass tubing. Acrylamide gels (0.5 cm diameter x 10 cm length). Gel consist of stacking gel which proteins (stocked) and a running gel part on which proteins separate. Running gel containing 10% acrylamide was polymerized 2 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 bromphenol 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 mA per sample appilied for 150 min, by EISCO power supply. The bromphenol 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. Protein bands and its percentages were determined according to the Schematic diagrams and parameters were obtained by Photo Capt Molecular Weight Software (Photo Capt Molecular Weight Software, 2001).

Results and Discussion

Eelectrophoretic patterns of Bunnei muscle proteins were similar to that of Gattan fish (Fig.1). But, Gattan predominant Bunnei in the number of separated proteins. Gattan muscle proteins were separated into 21 protein bands, whereas Bunni muscle proteins were separated into 16 protein bands (Fig.2). No significant variations in the electrophoretic pattern of muscle proteins due to sex within species were noticed.

Bunnei and Gattan fishes muscle proteins were separated into several bands, these bands were differed in quantitative parameters (Tables: 1-4) and they belong to muscle tissue. There are a number of proteins that make up muscle tissue. Following is a list of some muscle proteins that may be present as bands in the fish protein fingerprints, along with their approximate molecular weights in kilodaltons: actin (42 kDa), myosin heavy chain (210 kDa), myosin light chains (15, 17, and 24 kDa), titin (3000 kDa), dystrophin (400 kDa), filamin (270 kDa), spectrin (265 kDa), nebulin (107 kDa), α-actinin (100 kDa), gelsolin (90 kDa), fimbrin (68 kDa), tropomyosin (35 kDa), troponin T (30 kDa), thymosin (5 kDa). Since all of the fish protein samples are from muscle tissue, there will be some expected similarities. Nevertheless, difference in the protein banding patterns will also be apparent and these differences can be used to assess evolutionary relatedness (CBSC, 2005).

To compare protein profiles between fishes, scientists separate the mixture of protein molecules in a particular tissue (such as muscle tissue) by gel electrophoresis. This creates a unique pattern of bands for each fish, called a protein fingerprint. The individual bands correspond to different proteins and may vary in intensity between species. In addition, some bands (i.e.,proteins) may be visible in one species fingerprint but not in another. In general, protein fingerprint patterns obtained from different species are more similar when the species are more closely related and less similar when they are more distantly relate. The variations between the two Iraqi fishes in muscle proteins were encoded by gene structural loci. The similarity between sex within species demonstrated the minimum levels of polymorphism. According to genetic similarity and distance between characid and cyprinid fishes, they are not closely related to each other (Shahin, 1999). In conclusion, these fishes are easily distinguished by SDS-PAGE, taxonomically (Yilmaz *et.al.*, 2007).

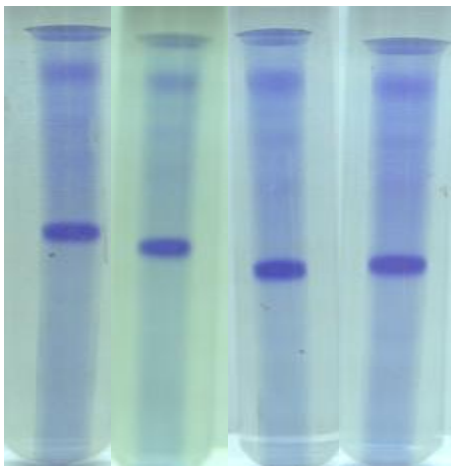
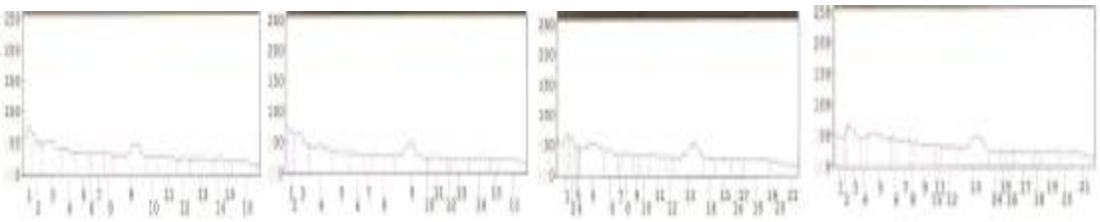


Fig. (1): electrophoretic pattern of Bunnei (1: male, 2 : female) and Gattan (3: male, 4 : female) muscle proteins.



1 2 3 4

Fig. (2): Schematic diagrams of electrophoretic pattern of Bunnei (1: male, 2 : female) and Gattan (3: male, 4 : female) muscle proteins.

Table (1): Quantitative parameters of electrophoretic pattern of Bunnei (male) muscle proteins.

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	84554	101	1260		169.333	9	178621	103	5040		59.286
2	78714	111	1470		163.333	10	49541	105	1785		50.589
3	138312	113	2940		147.317	11	78844	75	3045		41.308
4	82475	67	2205		131.225	12	46065	63	1890		28.063
5	74283	63	2205		119.179	13	89866	81	3780		20.918
6	3586	81	105		105.168	14	39966	61	1680		16.188
7	70580	81	2100		94.537	15	24066	69	1050		12.966
8	42613	81	1365		88.578	16	77767	73	4335		8.621

Table (2): Quantitative parameters of electrophoretic pattern of Bunnei (female) muscle proteins.

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	43382	83	630		172.000	9	164278	97	5040		57.130
2	13428	77	210		167.333	10	2271	41	105		40.035
3	121364	97	2100		159.333	11	69950	59	2940		36.880
4	149998	99	3570		137.927	12	11431	57	525		28.063
5	99181	69	2730		126.537	13	61686	65	2730		23.876
6	63672	63	2100		109.832	14	76914	75	3360		18.252
7	37522	67	1260		91.886	15	25733	67	1155		12.345
8	97737	75	3465		84.614	16	83481	69	4409		10.403

Table (3): Quantitative parameters of electrophoretic pattern of Gattan (male) muscle proteins.

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	59673	111	945		166.000	12	24867	75	840		61.047
2	49938	113	840		159.333	13	172795	103	4620		43.918
3	14985	91	315		154.667	14	49073	107	1785		34.652
4	14985	81	315		152.667	15	62047	69	2415		28.063
5	176574	99	3675		141.200	16	34557	73	1365		22.854
6	84261	83	2205		127.207	17	10071	67	420		19.775
7	14164	77	420		110.499	18	95952	67	3885		16.593
8	62454	81	1785		107.166	19	14334	61	630		10.652
9	26444	75	840		97.854	20	38638	69	1785		8.414
10	45460	73	1575		85.274	21	32055	57	1995		6.346
11	83157	75	2730		78.698						

Table (4): Quantitative parameters of electrophoretic pattern of Gattan (female) muscle proteins.

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	48894	89	1050		170.000	12	74701	79	2415		65.907
2	16503	119	315		163.333	13	160043	93	4410		45.273
3	113661	103	1995		159.333	14	36336	57	1575		38.043
4	38547	79	840		143.963	15	16591	57	735		30.543
5	169369	99	3465		131.225	16	20950	55	945		28.063
6	42823	105	945		118.510	17	66715	65	2940		21.868
7	69820	69	1680		108.498	18	18454	67	840		18.252
8	11999	75	315		102.506	19	56944	63	2625		13.172
9	103804	95	2835		96.527	20	35071	65	1680		9.862
10	12612	75	315		81.317	21	57890	65	3234		6.966
11	21255	73	630		76.746						

References

Al-Hazzaa, R., 2005. Some Biological Aspects of the Himri Barbel, Barbus luteus, in the Intermediate Reaches of the Euphrates River. Turk. J. Zool., 29 : 311-315.

Beckmann, W.C., 1962. The freshwater fishes of Syria and their general biology and management. FAO fisheries biology, technical paper, 8: 297.

Carolina Biological Supply Company (CBSC), 2005. Fish Protein Fingerprinting on Agarose and Polyacrylamide Gels, Teacher's manual with student guide, www.carolina.com

Chen, T.Y., Shiau C.Y., Noguchi T., Wei C. and Hwang D.F., 2003. Identification of puffer fish species by native isoelectric focusing technique. Food Chem., 83(3):475-479.

Focant, B., Jacob M.F. and Huriaux F., 1981. Electrophoretic comparison of the proteins of some perch (*Perca fluviatilis L.*) head muscles. J. Muscle Res. Cell Motil., 2(3):295-305.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assemble, of the head of bacteriophage T4. Nature, 227: 680-288.

Miyazaki, J.I., Hirabayashi T., Hosoya K. and Iwami T.A., 1998. Study of the systematics of cyprinid fishes by two dimensional gel electrophoresis. Environ. Biol. Fish., 52:173-179.

Ordonneau, D., Roman Y., Chaste-Duvernoy D. and Bomsel-Demontoy M.C., 2005. Plasma electrophoresis reference ranges in various bird species. In: Proceedings of the 8th EAAV conference, Arles: 283-289. (cited from Roman *et.al.*, 2009).

Pineiro, C., Vazquez J., Marina A.I., Barros-Velazquez J. and Gallardo J.M., 2001. Characterization and partial sequencing of species-specific sarco- plasmic polypeptides from commercial hake species by mass spectrometry following two-dimensional electrophoresis. Electrophoresis, 22(8): 1545-1552.

Photo Capt Molecular Weight Software, 2001. Version 10.01 Copyright 1999 – 2001.

Roman, Y., Levrier J., Ordonneau D., Chaste-Duvernoy D., Bomsel-Demontoy M.C. and Jalme M.S., 2009. Location of the fibrinogen and albumin fractions in plasma protein electrophoresis agarose gels of five taxonomically distinct bird species. Revue Méd. Vét., 160(3):160-165.

Shahin, A.A., 1999. Phylogenetic relationship between the characid nile fish *Alestes dentex*, cyprinid *Barbus bynni*, *Labeo niloticus*, and the introduced grass carp, *Ctenopharyngodon idella* elucidated by protein electrophoresis. J. Egypt Ger. Soc. Zool., 29:21-42.

Smith, P.J., 2009. Protein Electophoresis for identification of Australasian fish stocks. Austr. J. Marine Freshwater Res., 41(6): 823 – 833.

Yilmaz, M., Yilmaz H.R. and Alas A., 2007. An electrophoretic taxonomic study on serum proteins of *Acanthobrama marmid*,*Leuciscus cephalus*, and *Chondrostoma regium*. EurAsia J. Biol. Sci., 3:22-27.