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USING SDS-PAGE AND ISSR AS BIOCHEMICAL MARKERS FOR ASSESSMENT THE GENETIC SIMILARITY AND PROTEIN ANALYSIS OF SOME CYPRINID FISH SPECIES

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Genetics similarity and protein analysis of some cyprinid fish were studied using Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Inter-simple sequence repeated (ISSR Markers). Species *Pethia nigrofasciatus, Barbonymus schwanenfeldii, Puntius tetrazone* and *Brachydanio rerio* were collected from the fish farms in Damietta to study the genetic variability among them. Eleven ISSR primers were tested to assess the effectiveness of ISSR analysis in discriminating among the four applied fish species. We observed varied size of amplified products depending upon the sequence of ISSR primers and genotypes used. A total of 131 discrete amplified products were obtained (size 79 to 1185 bp approximately) with polymorphism 95%. Out of 131 products, 63 bands were species specific markers indicating high level polymorphism among species. The highest and lowest number of ISSR bands detected for primers ISSR 15 and ISSR16 was 18 and 17 respectively. 7 bands were most relevant as found monomorphic in all four species of family: *Cyprinidae*. Highest similarity observed between *Pethia nigrofasciatus* and *Barbonymus schwanenfeldii* 80% and lowest similarity was between *Pethia nigrofasciatus* and *Brachydanio rerio* 31 %. The protein analysis by SDS-PAGE produced 29 bands of molecular weight ranging from 11 to 132 KD with polymorphism 14%. This study concludes that ISSR-based DNA analysis bands and protein profile in muscles from *Pethia nigrofasciatus and Barbonymus schwanenfeldii* are the most closest species compared molecularly compared with other species used in this study.

Keywords: Genetics, Cyprinid fish, fingerprint, ISSR, Protein analysis.

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INTRODUCTION

Approximately fishes represent half of all the described vertebrates, and comprise of 482 families with living species. Approximately 100 new species of fresh water fishes are described each year, compared with too new bird species (MAITLAND, 1995). Cyprinids, evolutionarily young group of fish, with a significant taxonomic diversity and the distribution, are of great interest for population genetic studies. Due to the high frequency of natural hybridization (DEMARAIS *et al*., 1992).

The advantages of ISSR markers are more than other marker systems. ISSR technique is simple, quick and less costly like the RAPD technique. ISSR markers have high reproducibility than RAPD primers due to the longer primer length. (FANG and ROOSE, 1997; MORENO *et al*., 1998). Development of ISSR markers does not need prior knowledge of the genome to be analyzed; hence, it can be used universally for genome analysis. ISSR markers provide more polymorphism (WOLFE *et al*., 1998).

Different studies showed that inter simple sequence repeats markers have a high potential to identify polymorphism and determine genomic diversity across species as compared to other random primers (SOUFRAMANIEN and GOPALA, 2004).

SDS-PAGE protein marker was used extensively to identify and study the genetic characters and relationships among different species (KAKAEI and KAHRIZI, 2011; MAGED and SHAWKAT, 2012). Many authors recommended the use and applications of SDS-protein as rapid method to identify and characterize species (FREITAS *et al*., 2004 and OPPONG - KONADU *et al*., 2005).

In the present investigation, The DNA and muscle proteins of Four fish species of *Pethia nigrofasciatus, Barbonymus schwanenfeldii, Puntius tetrazone* and *Brachydanio rerio,* have been analyzed using ISSR analysis and SDS-PAGE technique and the resemblances and differences between the species was established.

MATERIALS AND METHODS

Samples collection

Four species of *Pethia nigrofasciatus, Barbonymus schwanenfeldii, Puntius tetrazone* and *Brachydanio rerio,* were collected from the fish farms in Damietta because they are available. Appropriate pieces of muscles were cut from the three different fish species and put in eppendorf tubes with a saline solution (0.85% of NaCl). The same individuals were cut and preserved with 70% ethanol into eppendorf tubes. Then, both muscle samples were frozen at -20˚C.

DNA Extraction and ISSR-Technique

DNA was extracted from the four fish species by DNeasy Mini Kit (Qiagen). The DNA amplifications were performed in an automated thermal cycle programmed for one cycle at 94˚C for 5 min followed by 40 cycles of 1 min at 94˚C, 1 min at 36˚C for ISSR, and 1 min at 72˚C. The final extension at 72˚C for 10 min using eleven ISSR primers as shown in Tables1. The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. At the first step, when each accession represents its own cluster, the distances between these accessions are defined by the

chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. This method is called Unweighted Pair Group Method using Arithmetic Average (UPGMA) (SNEATH and SOKAL, 1973).

(SDS-PAGE) Technique

One piece of muscle (0.2 g) from each species was powdered in a mortar, and extracted two times in appropriate volume of extraction saline, respectively (0.85 % of NaCl. The homogenized samples were centrifuged at 12000 rpm /15 min /4˚C. The supernatants were transferred to new eppendorf tubes, and kept in deep freezer until use (GORINSTEIN *et al.*, 1999). SDS-PAGE technique was used to compare the studied species by their protein patterns. Protein Samples were applied to a 15 % polyacrylamide gel. Gel preparation, electrophoresis conditions, staining and destaining gels were done according to (LAEMMLI, 1970 and SHARAF-ELDEEN *et al.*, 2006). Molecular weight of protein was calculated according to WEBER *et al.* (1972).

Table 1. The sequence of ISSR primers (A: Adenine,T: Thymine,G: Guanine,C: Cytosine).

Primer	Sequence
ISSR 1	5'-AGAGAGAGAGAGAGAGAC -3'
ISSR 2	5'- AGAGAGAGAGAGAGAGAG -3'
ISSR 3	5'- ACACACACACACACACAT -3'
ISSR 8	5'- AGACAGACAGACAGACGC -3
ISSR 9	5'- GATAGATAGATAGATAGC -3'
ISSR 10	5'- GACAGACAGACAGACAAT -3'
ISSR 11	5'- ACACACACACACACACAA -3'
ISSR 12	5'- ACACACACACACACACTC -3'
ISSR 14	5'- CTCCTCCTCCTCCTCTT -3'
ISSR 15	5'-CTCTCTCTCTCTCTCTAG -3'
ISSR 16	5'- TCTCTCTCTCTCTCTCAA -3'

RESULTS

Inter-simple sequence repeated (ISSR) Analysis

In this study, *Pethia nigrofasciatus, Barbonymus schwanenfeldii, Puntius tetrazone* and *Brachydanio rerio* (Family Cyprinidae) were collected from the fish farms in Damietta to study the genetic variability among them using eleven single ISSR primers (ISSR-1, ISSR-2, ISSR-3,ISSR-8,ISSR-9,ISSR-10,ISSR-11, ISSR-12, ISSR-14, ISSR-15 and ISSR-16) (Table 1).

All eleven primers generated strong amplification profiles with distinct bands that revealed extensive DNA polymorphism to four species under study. The banding patterns of these DNA fragments were analyzed by Gene profiler computer software program and summarized in (Table 2, 3 and Figures 1, 2, 3 and 4). The eleven ISSR primers detected a total of 131 DNA fragments (Table 3), with an average of 12 fragments per primer. The total number of amplified fragments varied from 8 (ISSR3 and ISSR12) to 18 (ISSR15) primers. Of the 131 amplified bands, 7 were

monomorphic bands, 61 polymorphic and 63 unique bands with polymorphism ranged from 82 to 100%.

Table 2. Survey of ISSR Markers using eleven primers, where (1) means present and (0) means absence- (1- Pethia nigrofasciatus, 2- Barbonymus schwanenfeldii 3- Puntius tetrazone and 4- Brachydanio rerio) Fr. Means Frequency

ISSR 1						ISSR 2					
MW (bp)	*Pethia nigrofasciatus*	*Barbonymus schwanenfeldii*	*Puntius tetrazone*	*Brachydanio rerio*	Frequency	MW (bp)	1	2	3	4	Fr.
1000	0	0	1	1	0.3	477	1	1	0	0	0.4
796	0	0	1	1	0.3	210	1	1	0	0	0.6
684	0	0	1	1	0.3	382	0	0	1	1	0.4
493	0	0	1	1	0.4	428	0	0	0	1	0.1
354	0	0	1	1	0.3	611	0	0	0	1	0.1
312	0	0	0	1	0.1	688	0	0	0	1	0.1
299	0	1	0	0	0.1	982	0	0	0	1	0.1
261	1	0	1	0	0.4	348	0	0	1	0	0.3
227	0	0	1	1	0.6	300	0	0	0	1	0.3
179	0	1	0	0	0.4	181	0	0	0	1	0.3
158	1	0	0	0	0.4	146	0	0	1	0	0.4
ISSR3						158	1	0	0	0	0.3
MW (bp)	*Pethia nigrofasciatus*	*Barbonymus schwanenfeldii*	*Puntius tetrazone*	*Brachydanio rerio*	Frequency	117	1	1	0	0	0.6
446	1	1	0	0	0.4	ISSR 8					
362	1	1	0	0	0.4	MW (bp)	1	2	3	4	Fr.
291	1	1	0	0	0.4	955	1	1	0	0	0.4
221	0	0	0	1	0.3	860	0	0	1	0	0.1
184	0	1	0	0	0.1	678	1	0	0	0	0.1
176	1	0	0	0	0.4	561	0	0	1	0	0.1
156	0	0	1	0	0.3	503	0	0	1	0	0.3
145	1	0	1	0	0.6	436	0	0	0	0	0.1
ISSR 9						396	0	0	0	1	0.1
MW (bp)	*Pethia nigrofasciatus*	*Barbonymus schwanenfeldii*	*Puntius tetrazone*	*Brachydanio rerio*	Frequency	357	1	1	1	0	0.6
584	0	1	0	0	0.4	315	0	0	0	0	0.3
413	1	1	1	0	0.6	232	1	1	1	1	0.7
344	1	1	1	1	0.9	179	0	0	0	0	0.3
538	1	1	0	0	0.4	174	0	0	0	1	0.1
309	1	1	1	1	0.9	ISSR 10					
274	1	1	0	0	0.7	MW (bp)	1	2	3	4	Fr.
237	0	0	0	1	0.3	1185	0	0	1	0	0.3
144	1	1	0	0	0.4	461	0	0	0	1	0.3
242	1	0	1	0	0.4	413	0	0	1	0	0.1
197	0	0	1	0	0.4	390	0	0	1	1	0.3
212	1	0	1	0	0.4	347	0	0	0	1	0.3

ISSR 11						314	1	1	0	0	0.4
MW (bp)	*Pethia nigrofasciatus*	*Barbonymus schwanenfeldii*	*Puntius Tetrazone*	*Brachydanio rerio*	Frequency	271	1	0	0	1	0.4
322	1	1	1	0	0.7	236	1	1	0	0	0.6
636	1	1	0	0	0.4	214	1	0	0	0	0.4
553	1	1	0	0	0.6	143	0	1	0	0	0.1
346	1	0	0	1	0.3	ISSR 12					
370	1	0	1	1	0.7	MW (bp)	1	2	3	4	Fr.
410	0	0	1	1	0.6	916	0	0	0	1	0.1
579	0	0	0	1	0.1	544	0	1	0	0	0.3
689	0	0	0	1	0.3	480	0	0	1	0	0.1
735	0	0	0	1	0.1	426	0	0	1	0	0.1
486	0	0	1	0	0.1	365	1	1	1	0	0.7
435	1	0	0	0	0.4	306	1	0	0	0	0.3
277	1	1	0	1	0.6	257	0	0	0	1	0.1
232	1	1	1	1	1.0	209	0	0	1	0	0.3
ISSR 14						ISSR 15					
MW (bp)	*Pethia nigrofasciatus*	*Barbonymus schwanenfeldii*	*Puntius tetrazone*	*Brachydanio rerio*	Frequency	MW (bp)	1	2	3	4	Fr.
510	1	0	0	0	0.3	764	1	0	1	1	0.7
381	1	1	0	0	0.4	592	0	0	1	1	0.3
352	0	0	1	0	0.1	556	1	0	1	0	0.6
319	1	1	0	0	0.4	533	0	0	0	1	0.3
285	0	0	1	1	0.3	502	1	0	0	0	0.4
267	1	1	0	0	0.4	473	0	0	1	1	0.4
253	0	0	1	1	0.3	413	1	1	0	0	0.6
231	0	0	0	1	0.1	390	1	1	0	0	0.4
211	1	1	1	0	0.9	357	0	0	1	0	0.4
182	1	1	0	0	0.6	324	1	1	1	1	0.7
166	1	1	1	0	0.7	259	0	0	0	1	0.3
148	1	1	0	0	0.6	234	1	0	0	0	0.4
130	1	1	0	0	0.6	220	1	1	1	1	1.0
ISSR 16						163	0	0	0	1	0.1
MW (bp)	*Pethia nigrofasciatus*	*Barbonymus schwanenfeldii*	*Puntius tetrazone*	*Brachydanio rerio*	Frequency	146	1	1	1	0	0.6
752	1	0	1	0	0.4	122	1	1	0	0	0.4
179	0	0	0	1	0.3	99	0	0	0	1	0.1
287	0	1	1	1	0.7	79	1	1	1	0	0.9
306	0	0	0	1	0.3						
360	0	0	0	1	0.3						
390	1	1	1	1	1.0						
491	0	0	0	1	0.3						
539	0	0	1	1	0.3						
630	0	0	0	1	0.3						
218	0	0	1	0	0.1						
244	0	0	1	1	0.6						
342	1	1	1	0	0.7						
424	0	0	1	0	0.4						
456	0	0	1	0	0.3						
601	1	0	1	0	0.6						
686	0	0	1	0	0.1						
259	1	1	0	0	0.4						

Table 3. Percentage of polymorphism, molecular weight and number of total bands, monomorphic bands, polymorphic and unique bands generated by eleven ISSR primers with four fish species (1- Pethia nigrofasciatus, 2- Barbonymus schwanenfeldii 3- Puntius tetrazone and 4- Brachydanio rerio).

Primer code	No. of amplified bands				Total amplified bands	Size of amplified bands (bp)	No. of Monomorphic bands	No. of Polymorphic bands	No. of Unique bands	Polymorphic and Unique bands	Polymorphism %
	1	2	3	4							
ISSR1	2	2	7	7	11	158-1000bp	0	7	4	11	100%
ISSR2	4	3	3	7	13	117-982bp	0	4	9	13	100%
ISSR3	5	4	2	1	8	145-446bp	0	4	4	8	100%
ISSR8	4	3	5	3	9	174-955bp	1	2	6	8	89%
ISSR9	8	7	6	3	11	144-584bp	2	6	3	9	82%
ISSR10	4	3	3	4	10	143-1185bp	0	4	6	10	100%
ISSR11	8	5	5	8	13	232-755bp	1	7	5	12	92%
ISSR12	2	2	4	2	8	209-916bp	0	1	7	8	100%
ISSR14	9	8	5	3	13	130-510bp	0	10	3	13	100%
ISSR15	11	7	9	9	18	79-764bp	2	9	7	16	89%
ISSR16	5	4	11	9	17	259-752bp	1	7	9	16	94%
Total	62	48	60	56	131	79-1185bp	7	61	63	124	95%

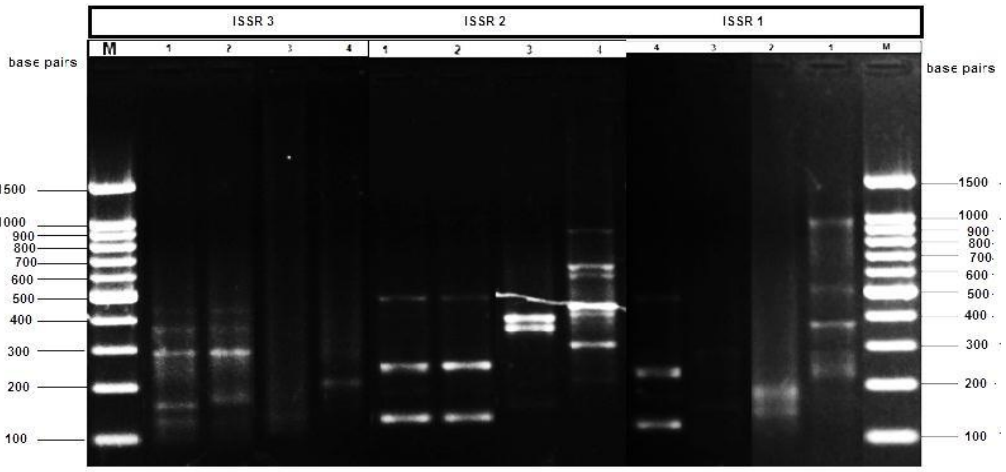

Figure 1. ISSR profile of four fish species using ISSR primers: (ISSR1, ISSR2 and ISSR3). M refers to DNA ladder marker 1 bp, (1- *Pethia nigrofasciatus*, 2- *Barbonymus schwanenfeldii* 3- *Puntius tetrazone* and 4- *Brachydanio rerio*)

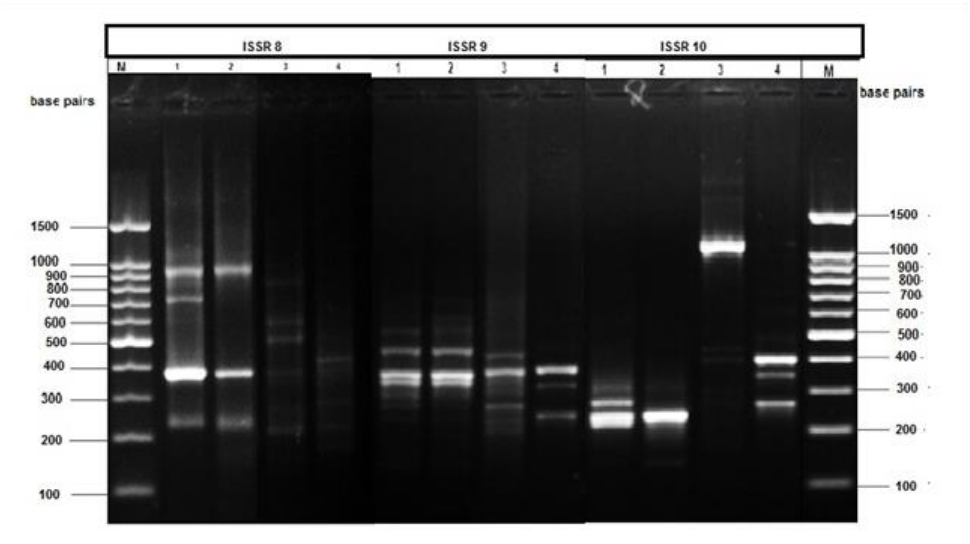

Figure 2. ISSR profile of four fish species using ISSR primers: (ISSR8, ISSR9 and ISSR10). M refers to DNA ladder marker 1 bp, (1- *Pethia nigrofasciatus*, 2- *Barbonymus schwanenfeldii* 3- *Puntius tetrazone* and 4- *Brachydanio rerio*).

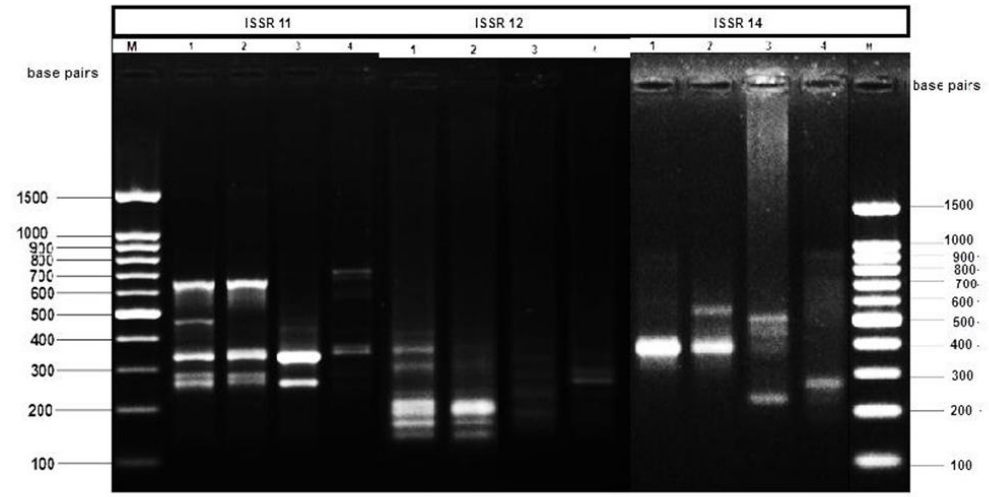

Figure 3. ISSR profile of four fish species using ISSR primers: (ISSR11, ISSR12 and ISSR14). M refers to DNA ladder marker 1 bp, (1- *Pethia nigrofasciatus*, 2- *Barbonymus schwanenfeldii* 3- *Puntius tetrazone* and 4- *Brachydanio rerio*)

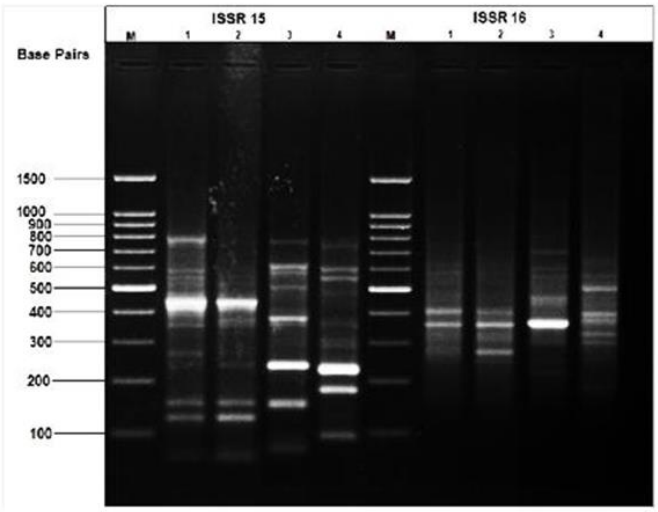

Figure 4. ISSR profile of four fish species using ISSR primers: (ISSR15 and ISSR16). M refers to DNA ladder marker 1 bp, (1- *Pethia nigrofasciatus*, 2- *Barbonymus schwanenfeldii* 3- *Puntius tetrazone* and 4- *Brachydanio rerio*).

Following are the amplification results of four fish species obtained by the examined primers:
Pethia nigrofasciatus

ISSR with this species produced different band patterns of 62 bands ranged in size from 79 bp in the primer (ISSR15) to 955 bp in (ISSR8). The generated bands ranged in number from 2 in (ISSR1 and ISSR12) to 11 in (ISSR15).

Barbonymus schwanenfeldii

The number of different ISSR band patterns in this species was 48 bands ranged in size from 79 bp in the primer (ISSR15) to 955bp in (ISSR8). The generated bands ranged in number from 2 in (ISSR1 and ISSR12) to 7 in (ISSR9 and ISSR15).

Puntius tetrazone

This sample produced different ISSR band patterns number of 60 bands ranged in size from 79 bp in the primer (ISSR15) to 1185 bp in (ISSR10). The generated bands ranged in number from 2 in (ISSR3) to 11 in (ISSR16).

Brachydanio rerio

This species with ISSR produced different band patterns number of 56 bands ranged in size from 99 bp in the primer (ISSR15) to 1000 bp in (ISSR1). The generated bands ranged in number from 1 in (ISSR3) to 9 in (ISSR15 and ISSR16).

Genetic distance was lowest between *Pethia nigrofaciatus* and *Brachydanio rerio*, while highest between *Pethia nigrofasciatus* and *Barbonymus schwanenfeldii*. Genetic similarity index was from 31% to 80% (Table 4).The phylogenetic tree constructed from genetic distance showed that the dendrogram is divided in to two clusters group in (Fgure 5).

Table 4. Averages of genetic similarities (%) estimated by molecular ISSR primers, adopting the arithmetic complement of Jaccard coefficient for four fish species of Cyprinidae.

	Pethia nigrofasciatus	*Barbonymus schwanenfeldii*	*Puntius tetrazone*	*Brachydanio rerio*
Pethia nigrofasciatus	100			
Barbonymus schwanenfeldii	80	100		
Puntius tetrazone	50	38	100	
Brachydanio rerio	31	32	43	100

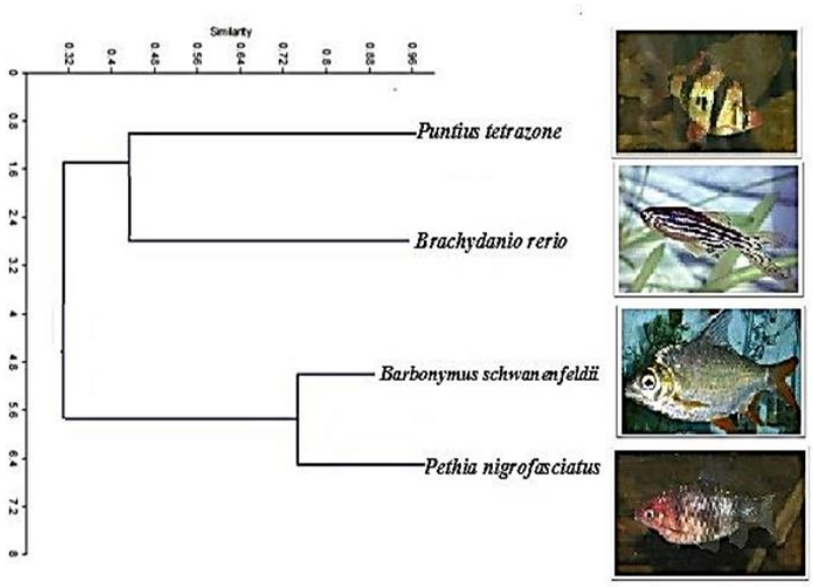

Figure 5. Dendrogram showing Cluster analysis for four fish species (1- *Pethia nigrofasciatus*, 2- *Barbonymus schwanenfeldii* 3- *Puntius tetrazone* and 4- *Brachydanio rerio*) based on ISSR molecular markers.

SDS-PAGE Technique

The SDS-protein profiles of four fish species of cyprinidae used in this study demonstrate in Table 5 and Figure 6. A maximum of 29 bands were detected with molecular weights ranging from 11 to 132 KD. The monomorphic bands were 25. Only four polymorphic bands were recorded with a percentage of 14%. *Pethia nigrofasciatus* produced 28 bands ranged in size from 11 KD to 132 KD, *Barbonymus schwanenfeldii* produced 28 bands ranged in size from 11 KD to 132 KD, *Puntius tetrazone* produced 27 bands ranged in size from 11 KD to132KD and *Brachydanio rerio* produced 26 bands ranged in size from 11 KD to132KD (Table 5).

Table 5. SDS-PAGE protein bands of four fish species of Cyprinidae

zero
one

MW (KD)	*Pethia nigrofasciatus*	*Barbonymus schwanenfeldii*	*Puntius tetrazone*	*Brachydanio rerio*	Frequency
132	1	1	1	1	1.0
123	1	1	1	1	1.0
116	1	1	1	1	1.0
107	1	1	1	1	1.0
94	1	1	1	1	1.0
85	1	1	1	1	0.9
79	1	1	1	1	1.0
69	1	1	1	1	1.0
62	1	1	1	1	1.0
52	1	1	1	1	1.0
50	1	1	1	1	1.0
45	1	1	1	1	1.0
44	1	1	1	1	1.0
39	1	1	1	1	1.0
35	1	1	1	1	1.0
33	1	1	1	1	0.9
30	1	1	1	1	1.0
28	1	1	1	1	1.0
23	1	1	1	1	1.0
21	1	1	0	1	0.6
21	0	0	1	0	0.4
19	1	1	1	1	0.9
18	1	1	0	0	0.6
17	1	1	1	1	1.0
13	1	1	1	1	1.0
13	1	1	1	1	1.0
12	1	1	1	0	0.7
11	1	1	1	1	0.9
11	1	1	1	1	1.0

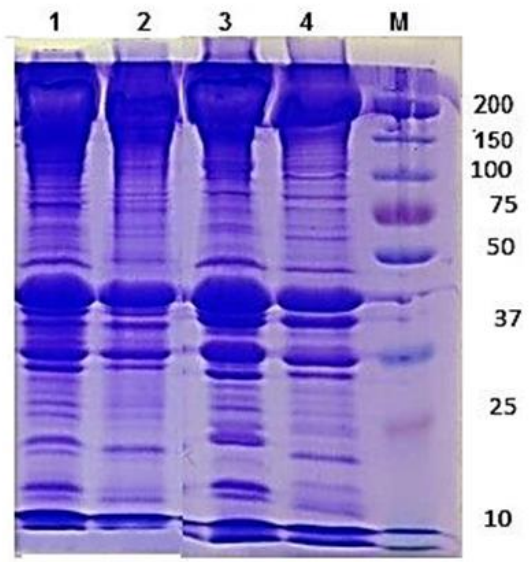

Figure 6. SDS-PAGE protein banding patterns (1- *Pethia nigrofasciatus*, 2- *Barbonymus schwanenfeldii* 3- *Puntius tetrazone* and 4- *Brachydanio rerio*).

DISSCUSION

This study offered some detailed analysis of the genetic variation and structure for four fish species of family cyprinidae, *Pethia nigrofasciatus, Barbonymus schwanenfeldii, Puntius tetrazone* and *Brachydanio rerio* based on biochemical and molecular markers that have been proven to be valuable for the determination of genetic variability.

ISSR is one of the simplest and widely used techniques, which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. Though ISSR markers are dominant like RAPD, they are more stable and reproducible. Because of these properties ISSR markers have recently been found using extensively for finger printing, phylogenetic analysis, population structure analysis, varietal/line identification and genetic mapping (VIJAYAN, 2005).

Knowledge of the proximate composition of fishes can be used to estimate the food value of fishes and to plan the most appropriate industrial and commercial processing. Fish proteins contain all the essential amino acids (not synthesized and need to be provided in the diet) in the required proportion and hence have a high nutritional value, which contribute to their high biological value (JAN *et al*., 2012).

ISSR analysis was used to detect genetic polymorphism, and to determine the variability among three species of fish family Osphronemidae, *Trichogaster trichopterus*, *Trichogaster leeri* and *Colisa lalia* (ABU ALMAATY *et al.*, 2017). 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).

SDS-PAGE patterns of serum proteins of five species of *Schizothorax, S. niger, S. curvifrons, S. esocinus, S. labiatus and S. plagiostomus* (family cyprinidae) were 7, 5, 5, 6, and 6 respectively and their molecular weights ranged from 41.5 to 121 KD (GANAI *et al.*, 2014).

The muscle protein bands of *Puntius filamentosus*, *Puntius tambraparniei* and *Puntius bimaculatus* were separated and analyzed by using SDS-PAGE. The protein band numbers of these fishes were 8, 7 and 6 respectively, their size ranged from 5KD to 90 KD (JESSLIN *et al.*, 2013). SDS-PAGE was separated the sarcoplasmic proteins of *Orthrias insignis euphyraticus* and *Cyprinion macrostomus* 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). Different PCR markers for studying genetic similarity among the investigated species are useful tools for estimating the genetic variability and degree of similarity among species (ABU-ALMAATY *et al.*, 2019).

The results indicated that molecular analysis by using ISSR and SDS-PAGE techniques may be good tools for DNA fingerprinting, detecting the genetic variations and identifying of cyprinid fish species collected from different locations. Variation in DNA sequences lead to polymorphism. Greater polymorphism reflects higher genetic variation. Polymorphism percentages of different used markers (ISSR and SDS-PAGE) were recorded as follow 95% and 13% respectively.

CONCLUSION

This study summaries that *Pethia nigrofasciatus* and *Barbonymus schwanenfeldii are very close* to each other, however, *Pethia nigrofasciatus*, *Puntius tetrazone and Brachydanio rerio* are very different based on DNA bands and muscles protein numbers. In conclusion, these fishes are easily distinguished by ISSR and SDS-PAGE, taxonomically.

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KORIŠĆENJE SDS-A I ISSR-A KAO BIOHEMIJSKIH MARKERA ZA PROCENU GENETIČKE SLIČNOSTI I PROTEINSKA ANALIZA NEKIH VRSTA ŠARANA

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Izvod

Genetička sličnost i analiza proteina šarana proučavane su primenom elektroforeze *SDS-PAGE* i *ISSR* markera. Vrste *Pethia nigrofasciatus, Barbonymus schwanenfeldii, Puntius tetrazone* i *Brachydanio rerio* sakupljene sun a farmi riba u Damijeti u cilju proučavanja njihove genetičke varijabilnosti. Jedanaest ISSR prajmera testirano je za procenu efikasnosti ISSR analize u diskriminaciji između četiri proučavane vrste riba. Primetili smo različitu veličinu amplifikovanih proizvoda u zavisnosti od sekvence ISSR prajmera i genotipova. Dobijen je 131 amplifikovan proizvod (veličine od 79 do 1185 bp) sa polimorfizmom od 95%. Od toga su 63 trake bile specifične za vrstu ukazujući na visok nivo polimorfizma između vrsta. Najveći i najmanji broj ISSR traka bio je utvrđen za prajmere ISSR 15 i ISSR16, I iznosio je 18, odnosno 17. Najrelevantnijih 7 traka je bilo monomorfno u sve četiri vrste familije *Cyprinidae*. Najveća sličnost utvrđena je između vrsta *Pethia nigrofasciatus* i *Barbonymus schwanenfeldii* i iznosila je 80%, dok je najniža sličnost utvrđena između vrsta *Pethia nigrofasciatus* i *Brachydanio rerio* i iznosila je 31 %. Proteinska SDS-PAGE analiza dala je 29 traka molekulske težine od 11-132 kD sa polimorfizmom od 14%. DNK analize zasnovane na ISSR-u i proteinski profili u mišićima iz vrsta *Pethia nigrofasciatus* i *Barbonimus schvanenfeldii* ukazali su da su to najsrodnije vrste u poređenju sa drugim vrstama koje su se proučavale.

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