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Inter Simple Sequence Repeat (ISSR) and Cytogenetic Analysis of Three Fish Species of Family Osphronemidae

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ABSTRACT

The ISSR-PCR and karyological analysis methods are successfully used to detect the genetic variations between the different species of fishes, especially these belonging to the same family. The aim of this study was to determine the karyotypes and molecular genetic variations of three species of family Osphronemidae, *Trichogaster trichopterus*, *Trichogaster leeri* and *Colisa lalia*by using ISSR-PCR and chromosomal analysis. Samples were collected from ornamental fish farms in Egypt. The diploid chromosome numbers and Fundamental numbers for species under study were 2n = 46 and FN = 46, 2n = 46 and FN = 46, 2n = 46 and FN = 70 respectively, the first two species have same karyotype but the third species was different. The ISSR-PCR analysis was carried out using ten primers. All primers were successfully amplified on the genomic DNA extracted from all studied fish species. These findings indicate that ISSR-PCR and cytogenetic analysis are very useful in determination of genetic molecular variations and relationship degree between the species which belong to same family.

INTRODUCTION

Perciformes is the most diverse order of ray-finned fish and include about forty percent of all species of bony fish, making it also the largest order of vertebrates. Gouramis are a group of freshwater perciformes fishes that comprise the family Osphronemidae. There are 15 genera and 46 species in the family Osphronemidae. The genus *Trichogaster* is represented by 4 species including three-spot gourami (*T. trichopterus*), peal gourami (*T. leeri*), moonlight gourami (*T. microlepis*) and snakeskin gourami (*T. pectoralis*) (Vidthayonon, 2005).

Knowledge of the genetic diversity of an organism is one of the crucial aspects to be known for both basic and applied conservational purposes. Studies on fish

genetics conducted in the region addressed mainly cytogenetic or the final product of DNA. Cytogenetic is a branch of genetics that is concerned with study of the structure and function of the cell, especially the chromosomes (Baltimore & Williams, 2006).

White (1973) reported that cytogenetic is concerned with the cytotaxonomic differences which exist between related species. The cytological evidence of value to taxonomy is of four chief types: chromosome number; chromosome morphology; chromosome behavior in crosses and aberrations during reproduction.

Chromosomes vary widely between different organisms (Paux *et al.*, 2008). Karyotyping is the process of pairing and ordering all the chromosomes of an organism, thus providing a genome-wide snapshot of an individual's chromosomes. Karyotypes are prepared using standardized staining procedures that reveal characteristic structural features for each chromosome (O`Connor, 2008).

Only 17 species of family Osphronemidae have been cytogenetically investigated reporting the diploid chromosome number (2n) that ranging from 16 to 48 including the 2n=16 of chocolate gourami (*Sphaerichthys osphromonoides*), 2n = 34 of three-lined mouth brooder (*Betta prima*), 2n = 42 of Siamese fighting fish (*B. splendens*) (Magtoon *et al.*, 2007), 2n = 44 of frail gourami (*Ctenopsnobilis*), 2n = 46 of(*Trichogaster pectoralis, T. microlepis, T. leeri, T. trichopterus T. sumatranu),* croaking gourami (*Trichopsis vittatus*), honey gourami (*Colisa chuna*), honey dwarf gourami (*C. lalia*) (Rishi *et al.*, 1997 and Rishi *et al.*, 2001).

The polymerase chain reaction PCR method has been used successfully to identify fish species and to avoid fraudulent label (Cocolin *et al.,* 2000 and Takeyama *et al.,* 2001). The ISSR-PCR strategy is especially attractive because it avoids the need to carry out costly cloning and sequencing inherent in the original microsatellite-based approach (Nagaraju *et al.,* 2002). This PCR-based method uses primers annealing to microsatellite repeats to amplify the regions between adjacent SSRs provided. They are close enough to allow exponential multiplication (Kramer *et al.,* 2007). In respect with, variable ISSR patterns have potentials as dominant markers for studying genetic diversity of many fishes (Tong *et al.,* 2005).

The aim of this study was to provide information about the chromosome numbers and karyotypes of the three species of family Osphronemidae. In addition, determine the molecular genetic variations and phylogenetic relationships among the three species under study.

MATRIALS AND METHODS

Samples of three fish species of family Osphronemidae, fresh water fishes were collected from the ornamental fish farms in Port-said; Species are *Trichogaste rtrichopterus, Trichogaster leeri* and *Colisa lalia.* They were transported to the lab and kept alive until processed. Mitotic chromosomes were prepared from head kidney, liver and gills as described by Nirchio & Cequea (1998). Each specimen was injected with 0.05% Colchicine (1ml / 100g fish weight) the fish were maintained in a well aerated aquarium and after 2hr they were sacrificed. The kidneys, liver and gills were removed and placed in a hypotonic solution of 0.56% KCl after nearly 30 min; the tissues were immersed three times in ethanol-acetic acid glacial mixture 3:1 every time was taken 20min, then they were squashed in 60% acetic acid. Three droplets of the cellular suspension were dropped on a clean microscope slide, previously chilled in a freezer, from a height of 50 cm. The slides were briefly passed over a flame and then allowed to air-dry. For conventional karyotype the preparations were stained for 40 min with 5% Giemsa in phosphate buffer ph 6.8. The slides were examined under a

research light microscope using ×10 or ×15 eyepieces, together with × 15 objectives for chromosomal analysis. Karyotypes were made from good spreads of chromosomes. Classification of chromosomes in karyotype studies relating to centromeric index was done according to (Levan *et al.*, 1964).

DNA was extracted from the three samples by DNeasy Mini Kit (Qiagen Santa Clarita, CA), this was performed following the manufacturer's instructions. Ten primers were used in ISSR- PCR analysis to study the difference between the three specimens of family Osphronemidae**,** the code and sequences of these primers are shown in table (1).

Table (1): The sequence of 10 primers used in ISSR analysis of three species of fishes (*Trichogastert richopterus, Trichogaster leeri, Colisa lalia*).

No.	Name of primer	Sequence	
1	ISSR- 1	5'-AGAGAGAGAGAGAGAGYC-3'	(AG)8YC
2	ISSR- 2	5'-AGAGAGAGAGAGAGAGYG-3'	(AG)8YG
3	ISSR- 3	5'-ACACACACACACACACYT-3'	$(AC)_8$YT
4	ISSR- 4	5'-ACACACACACACACACYG-3'	$(AC)_8$YG
5	ISSR- 5	5'-GTGTGTGTGTGTGTGTYG-3'	$(GT)_8$YG
6	ISSR- 6	5'-CGCGATAGATAGATAGAT-3'	CGC$(GATA)_4$
7	ISSR- 7	5'-GACGATAGATAGATAGATA-3'	GAC$(GATA)_4$
8	ISSR- 8	5'-AGACAGACAGACAGACGC-3'	$(AGAC)_4$GC
9	ISSR- 9	5'-GATAGATAGATAGATAGC-3'	$(GATA)_4$GC
10	ISSR- 10	5'-GACAGACAGACAGACAAT-3'	$(GACA)_4$AT

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts, visualized, and documented using a gel documentation and image analysis system. The banding patterns generated by ISSR-PCR marker analyses were compared to determine the genetic relatedness of the three samples. The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient:

Dice formula: $GS_{ij} = 2a/(2a+b+c)$

Where GS_{ij} is the measure of genetic similarity between individuals *i* and *j*, a is the number of bands shared by *i* and *j*, b is the number of bands present in *i* and absent in *j*, and c is the number of bands present in *j* and absent in *i*.

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).

RESULTS

Three species of fishes, belonging to family Osphronemidae: *Trichogaster trichopterus, Trichogaster leeri* and *Colisa lalia* were cyto-and molecule-genetically studied, using air drying technique and ISSR-PCR analysis. The chromosomal numbers of all species under study were the same, with 2n = 46, but differ in the karyotype in some species. Ten single primers (ISSR-1, ISSR-2, ISSR-3, ISSR-4, ISSR-5, ISSR-6, ISSR-7, ISSR-8, ISSR-9 and ISSR-10) were used in the present

investigation to determine the genetic differences among the three species were study. Following are the karyotypes and amplification results of the three species obtained from this study.

Trichogaster trichopterus

The chromosome number of *Trichogaster trichopterus* was scored, the diploid set consists of 2n = 46 and fundamental number (FN) = 46 as shown in (Figs. 1 & 2). The karyotype consists of one group all of them are acrocentric pairs of chromosomes, with relative lengths varies from 2.35 % to 7.81%, arm ratio is ∞ and centromeric index is zero. These measurements indicated in table (2). This sample produced different ISSR band patterns number of 68 bands ranged in size from 70 bp in the primer (ISSR9) to 1200 bp in (ISSR3). The generated bands ranged in number from 2 in (ISSR6) to 11 in (ISSR9).

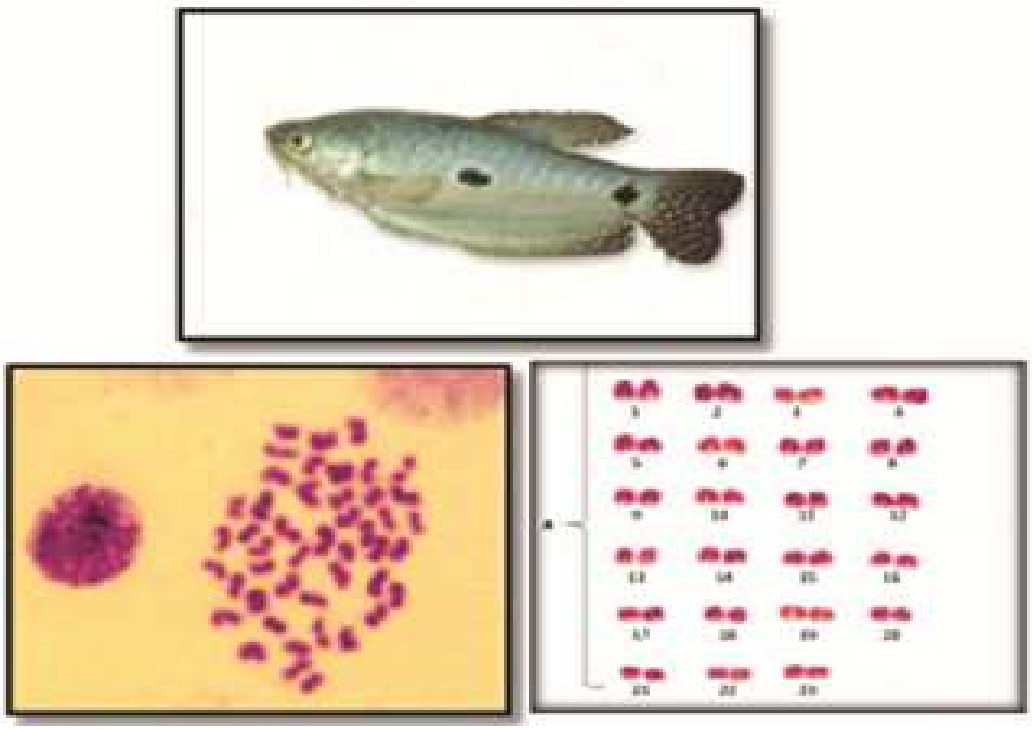

Fig. (1): A coloured photograph, chromosome spread and karyotype of *Trichogaster trichopterus.*

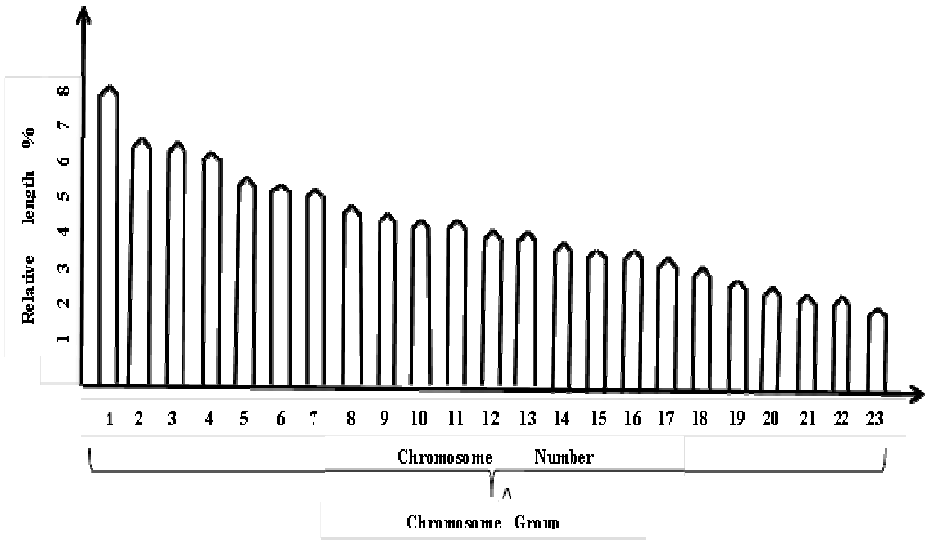

Fig. (2): Ideogram of chromosome of *Trichogaster trichopterus* which constructed in respect to relative length

Table (2): Averages of chromosomes measurements and classification, obtained from observations on ten cell spreads of *Trichogaster trichopterus.*

Chromosome Number	Chromosome Length			Relative Length %			Arm Ratio Mean ±S.D.	Centromeric Index Mean ± S.D.	Classification
	Long Arm Mean ± S.D.	Short Arm Mean± S.D.	Total Mean ± S.D.	Long Arm Mean ± S.D.	Short Arm Mean ± S.D.	Total Mean ± S.D.			
1	0.53±0.07	Zero	0.53±0.06	7.81±0.04	Zero	7.81±0.03	∞	Zero	Acro.
2	0.44±0.09	Zero	0.44±0.09	6.48±0.05	Zero	6.48±0.05	∞	Zero	Acro.
3	0.43±0.06	Zero	0.43±0.05	6.34±0.09	Zero	6.34±0.08	∞	Zero	Acro.
4	0.42±0.04	Zero	0.42±0.04	6.19±0.06	Zero	6.19±0.06	∞	Zero	Acro.
5	0.37±0.06	Zero	0.37±0.09	5.45±0.08	Zero	5.45±0.07	∞	Zero	Acro.
6	0.36±0.08	Zero	0.36±0.08	5.30±0.03	Zero	5.30±0.03	∞	Zero	Acro.
7	0.35±0.04	Zero	0.35±0.03	5.16±0.05	Zero	5.16±0.06	∞	Zero	Acro.
8	0.33±0.06	Zero	0.33±0.06	4.86±0.04	Zero	4.86±0.04	∞	Zero	Acro.
9	0.31±0.02	Zero	0.31±0.04	4.57±0.06	Zero	4.57±0.06	∞	Zero	Acro.
10	0.30±0.09	Zero	0.30±0.09	4.42±0.08	Zero	4.42±0.06	∞	Zero	Acro.
11	0.29±0.04	Zero	0.29±0.07	4.27±0.07	Zero	4.27±0.07	∞	Zero	Acro.
12	0.28±0.08	Zero	0.28±0.08	4.12±0.08	Zero	4.12±0.04	∞	Zero	Acro.
13	0.27±0.06	Zero	0.27±0.04	3.98±0.05	Zero	3.98±0.05	∞	Zero	Acro.
14	0.26±0.07	Zero	0.26±0.07	3.83±0.06	Zero	3.83±0.08	∞	Zero	Acro.
15	0.25±0.05	Zero	0.25±0.05	3.68±0.04	Zero	3.68±0.04	∞	Zero	Acro.
16	0.24±0.04	Zero	0.24±0.04	3.53±0.03	Zero	3.53±0.06	∞	Zero	Acro.
17	0.23±0.06	Zero	0.23±0.06	3.39±0.08	Zero	3.39±0.08	∞	Zero	Acro.
18	0.22±0.03	Zero	0.22±0.04	3.24±0.04	Zero	3.24±0.03	∞	Zero	Acro.
19	0.20±0.03	Zero	0.20±0.03	2.94±0.03	Zero	2.94±0.03	∞	Zero	Acro.
20	0.19±0.05	Zero	0.19±0.05	2.80±0.08	Zero	2.80±0.06	∞	Zero	Acro.
21	0.18±0.04	Zero	0.18±0.08	2.65±0.06	Zero	2.65±0.06	∞	Zero	Acro.
22	0.17±0.03	Zero	0.17±0.03	2.50±0.04	Zero	2.50±0.05	∞	Zero	Acro.
23	0.16±0.07	Zero	0.16±0.06	2.35±0.05	Zero	2.35±0.03	∞	Zero	Acro.
Sum			6.78±0.27						

Trichogaster leeri

The chromosomal analysis of the studied samples demonstrated that the diploid chromosomal number was 2n = 46 and fundamental number (FN) = 46 (Figs. 3 & 4). Chromosomes were arranged in one group, all of them were acrocentric pairs, with relative lengths varies from 2.68 % to 6.44%, arm ratio was ∞ and centromeric index was zero. These measurements are shown in table (3). Regarding the ISSR band patterns the number of bands was 61. They were ranged in size from 70 bp in the primer (ISSR9) to 910 bp in (ISSR6). The generated bands ranged in number from three in (ISSR6) to eight in (ISSR2and ISSR7).

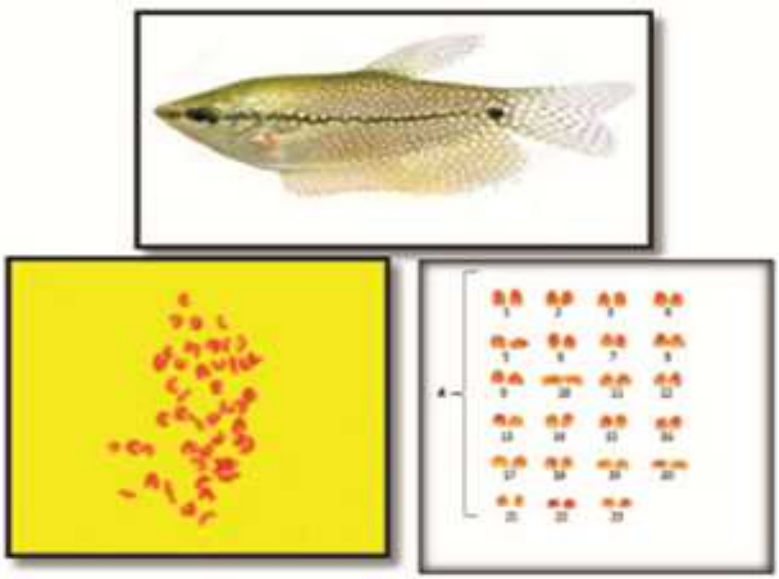

Fig. (3): A coloured photograph, chromosome spread and karyotype of *Trichogaster leeri.*

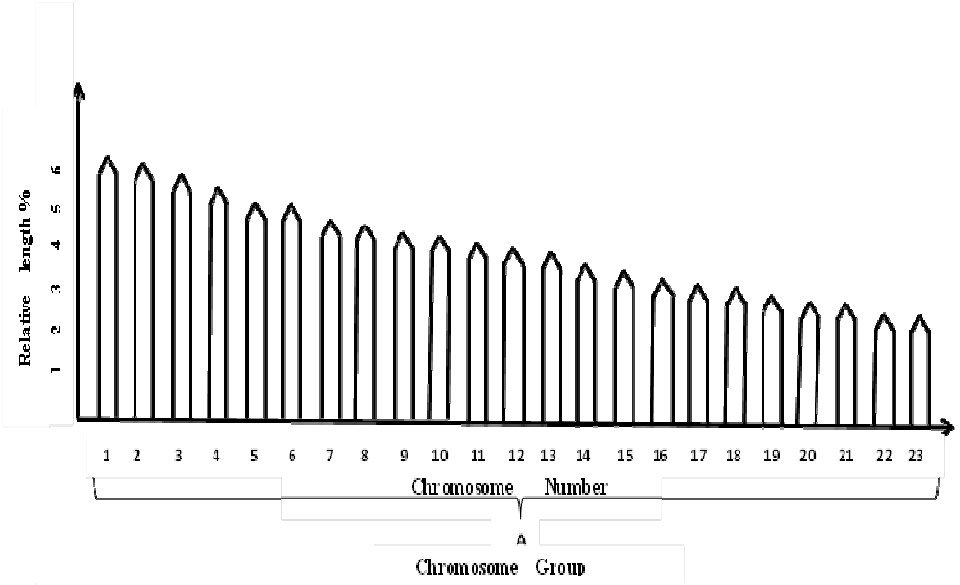

Fig. (4): Ideogram of chromosome of *Trichogaster leeri* which constructed in respect to relative length

Table (3): Averages of chromosomes measurements and classification, obtained from observations on ten cell spreads of *Trichogaster leeri.*

Chromosome Number	Chromosome Length			Relative Length %			Arm Ratio Mean ± S.D.	Centromeric Index Mean ± S.D.	Classification
	Long Arm Mean ± S.D.	Short Arm Mean ± S.D.	Total Mean ± S.D.	Long Arm Mean ± S.D.	Short Arm Mean ± S.D.	Total Mean ± S.D.			
1	0.48±0.03	Zero	0.48±0.08	6.44±0.08	Zero	6.44±0.07	∞	Zero	*Acro.*
2	0.47±0.05	Zero	0.47±0.04	6.30±0.06	Zero	6.30±0.06	∞	Zero	Acro.
3	0.45±0.08	Zero	0.45±0.02	6.04±0.07	Zero	6.04±0.05	∞	Zero	Acro.
4	0.42±0.09	Zero	0.42±0.08	5.63±0.03	Zero	5.63±0.03	∞	Zero	Acro.
5	0.41±0.04	Zero	0.41±0.06	5.50±0.05	Zero	5.50±0.04	∞	Zero	Acro.
6	0.39±0.06	Zero	0.39±0.05	5.23±0.06	Zero	5.23±0.06	∞	Zero	Acro.
7	0.37±0.07	Zero	0.37±0.07	4.96±0.04	Zero	4.96±0.03	∞	Zero	Acro.
8	0.36±0.08	Zero	0.36±0.08	4.83±0.04	Zero	4.83±0.04	∞	Zero	Acro.
9	0.35±0.03	Zero	0.35±0.03	4.69±0.09	Zero	4.69±0.08	∞	Zero	Acro.
10	0.34±0.09	Zero	0.34±0.06	4.56±0.03	Zero	4.56±0.03	∞	Zero	Acro.
11	0.33±0.07	Zero	0.33±0.04	4.42±0.06	Zero	4.42±0.05	∞	Zero	Acro.
12	0.32±0.05	Zero	0.32±0.07	4.29±0.07	Zero	4.29±0.07	∞	Zero	Acro.
13	0.31±0.07	Zero	0.31±0.09	4.16±0.03	Zero	4.16±0.05	∞	Zero	Acro.
14	0.29±0.06	Zero	0.29±0.06	3.89±0.05	Zero	3.89±0.05	∞	Zero	Acro.
15	0.28±0.05	Zero	0.28±0.05	3.75±0.05	Zero	3.75±0.08	∞	Zero	Acro.
16	0.27±0.04	Zero	0.27±0.04	3.62±0.03	Zero	3.62±0.09	∞	Zero	Acro.
17	0.26±0.08	Zero	0.26±0.08	3.48±0.07	Zero	3.48±0.05	∞	Zero	Acro.
18	0.25±0.03	Zero	0.25±0.05	3.35±0.04	Zero	3.35±0.04	∞	Zero	Acro.
19	0.24±0.04	Zero	0.24±0.09	3.22±0.03	Zero	3.22±0.03	∞	Zero	Acro.
20	0.23±0.07	Zero	0.23±0.07	3.08±0.07	Zero	3.08±0.09	∞	Zero	Acro.
21	0.22±0.09	Zero	0.22±0.05	2.95±0.03	Zero	2.95±0.03	∞	Zero	Acro.
22	0.21±0.06	Zero	0.21±0.06	2.81±0.08	Zero	2.81±0.07	∞	Zero	Acro.
23	0.20±0.08	Zero	0.20±0.08	2.68±0.05	Zero	2.68±0.04	∞	Zero	Acro.
Sum			7.45±0.09						

Colisa lalia

The photographs of cell spread and karyotype of this species was found to have a diploid chromosome number of 2n = 46 and fundamental number (FN) = 70 as shown in (Figs. 5 & 6).The karyotype consists of two different groups: group A composed of 12 metacentric pairs of chromosomes with relative lengths varies from 3,71 % to 6,84% , arm ratios ranging from 1,45 to 1,66 centromeric indices from 37,5 to 40,67. Group B is composed of 11 acrocentric pairs of chromosomes with relative lengths varies from 2, 2 % to 4, 41%, arm ratio of ∞ and centromeric index zero as shown in table (4). This sample produced ISSR band patterns of 77 bands ranged in

size from 70 bp in the primer (ISSR9) to 1300 bp in (ISSR7). The generated bands ranged in number from four in (ISSR6) to 13 in (ISSR4).

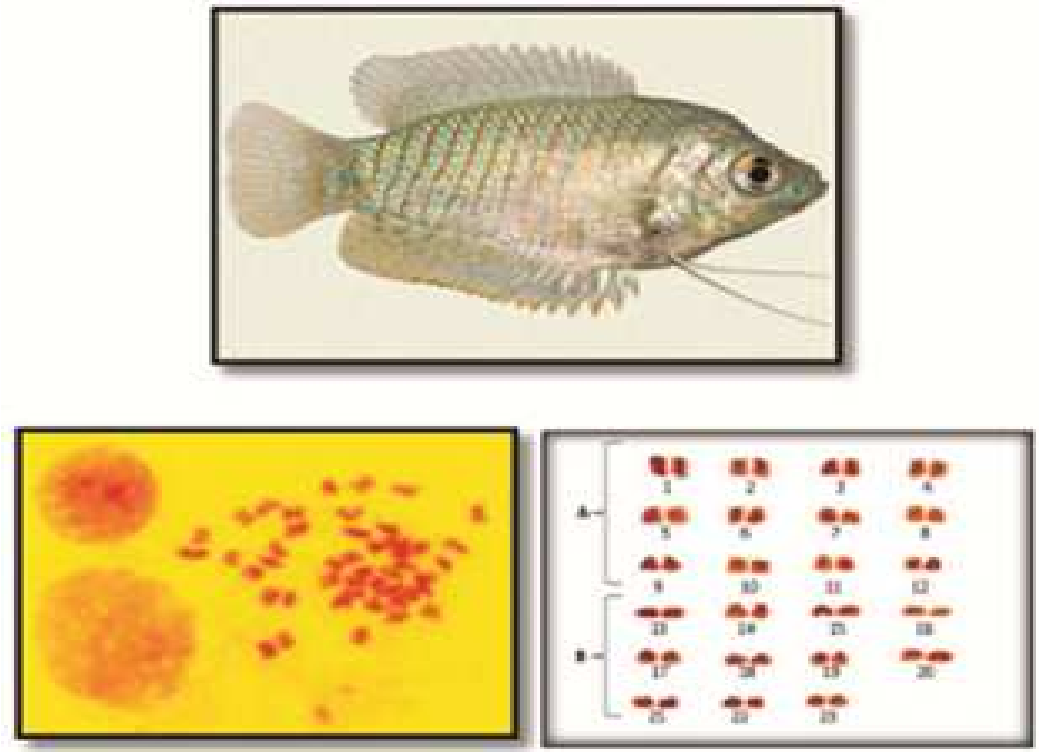

Fig. (5): A coloured photograph, chromosome spread and karyotype of *Colisa lalia*.

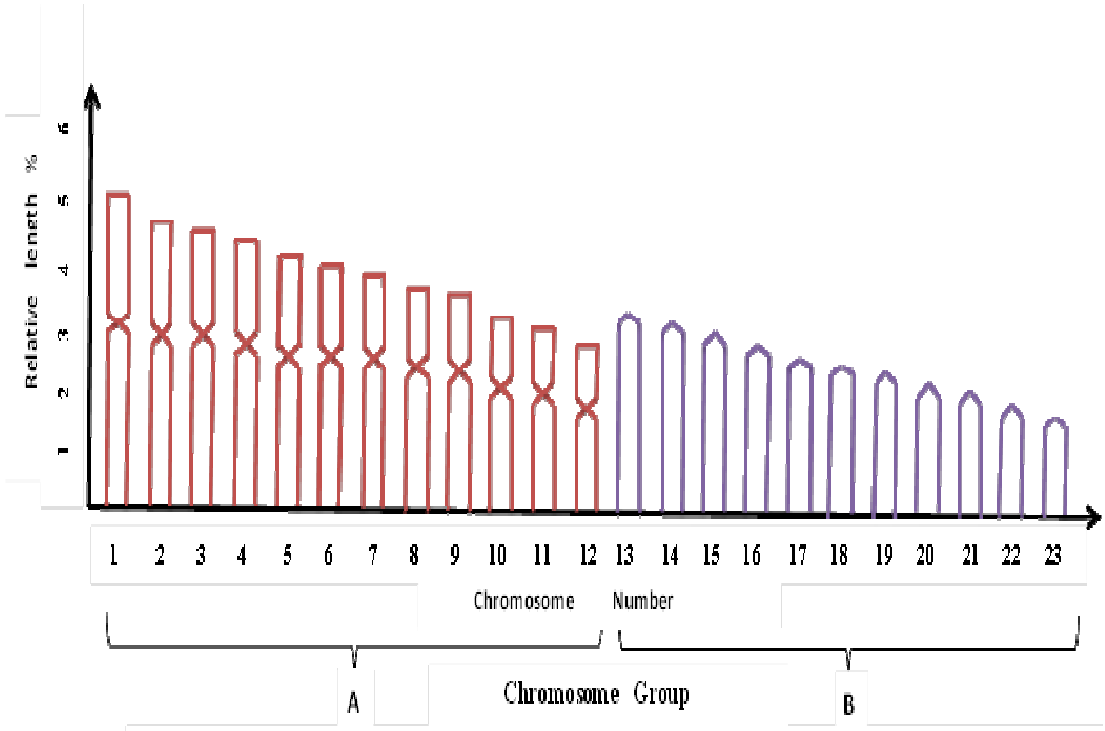

Fig. (6): Ideogram of chromosome of *Colisa lalia* which constructed in respect to relative length

Table (4): Averages of chromosomes measurements and classification, obtained from observations on ten cell spreads of *Colisa lalia.*

Chromosome Number	Chromosome Length			Relative Length %			Arm Ratio Mean± S.D.	Centromeric Index Mean± S.D.	Classification
	Long Arm Mean± S.D.	Short Arm Mean± S.D.	Total Mean ± S.D.	Long Arm Mean ± S.D.	Short Arm Mean ± S.D.	Total Mean ± S.D.			
1	0.35±0.05	0.24±0.03	0.59±0.03	4.06±0.04	2.78±0.04	6.84±0.05	1.45±0.05	40.67±1.06	M
2	0.33±0.04	0.22±0.02	0.55±0.04	3.83±0.03	2.55±0.03	6.38±0.04	1.5±0.06	4.000±1.08	M
3	0.32±0.03	0.21±0.04	0.53±0.02	3.71±0.02	2.43±0.09	6.14±0.03	1.52±0.09	39.62±1.07	M
4	0.31±0.04	0.20±0.05	0.51±0.03	3.60±0.03	2.32±0.08	5.92±0.09	1.55±0.07	39.21±1.03	M
5	0.30±0.05	0.19±0.03	0.49±0.04	3.48±0.04	2.20±0.04	5.68±0.08	1.57±0.08	38.77±1.09	M
6	0.29±0.04	0.18±0.04	0.47±0.05	3.36±0.02	2.09±0.02	5.45±0.07	1.61±0.07	38.29±1.02	M
7	0.28±0.03	0.17±0.03	0.45±0.02	3.25±0.05	1.97±0.09	5.22±0.09	1.64±0.04	37.77±1.05	M
8	0.27±0.02	0.16±0.02	0.43±0.09	3.13±0.03	1.85±0.04	4.98±0.04	1.68±0.08	37.20±1.06	M
9	0.26±0.04	0.16±0.03	0.41±0.04	3.01±0.06	1.85±0.03	4.86±0.06	1.62±0.06	39.02±1.04	M
10	0.23±0.03	0.14±0.05	0.37±0.03	2.67±0.08	1.62±0.05	4.29±0.08	1.64±0.04	37.83±1.09	M
11	0.22±0.04	0.13±0.03	0.35±0.08	2.55±0.04	1.50±0.05	4.05±0.06	1.65±0.03	37.45±1.05	M
12	0.20±0.03	0.12±0.03	0.32±0.07	2.32±0.03	1.39±0.03	3.71±0.08	1.66±0.04	37.5±1.04	M
13	0.38±0.05	zero	0.38±0.05	4.41±0.04	zero	4.41±0.04	∞	zero	Acro.
14	0.36±0.06	zero	0.36±0.06	4.18±0.03	zero	4.18±0.03	∞	zero	Acro.
15	0.34±0.04	zero	0.34±0.04	3.94±0.07	zero	3.94±0.07	∞	zero	Acro.
16	0.32±0.03	zero	0.32±0.03	3.71±0.04	zero	3.71±0.04	∞	zero	Acro.
17	0.30±0.02	zero	0.30±0.02	3.48±0.03	zero	3.48±0.03	∞	zero	Acro.
18	0.29±0.05	zero	0.29±0.05	3.36±0.07	zero	3.36±0.07	∞	zero	Acro.
19	0.27±0.04	zero	0.27±0.04	3.13±0.02	zero	3.13±0.02	∞	zero	Acro.
20	0.25±0.03	zero	0.25±0.03	2.90±0.05	zero	2.90±0.05	∞	zero	Acro.
21	0.23±0.06	zero	0.23±0.06	2.67±0.06	zero	2.67±0.06	∞	zero	Acro.
22	0.21±0.08	zero	0.21±0.08	2.43±0.04	zero	2.43±0.04	∞	zero	Acro.
23	0.19±0.05	zero	0.19±0.05	2.20±0.03	zero	2.20±0.03	∞	zero	Acro.
Sum			8.61±0.12						

The DNA fragments generated by the ten ISSR primers from the genomic DNA of the three species were separated using agarose gel electrophoresis and illustrated in figures (7, 8 and 9). The banding patterns of these DNA fragments were analyzed by Gene profiler computer software program and summarized with each primer in table (5).

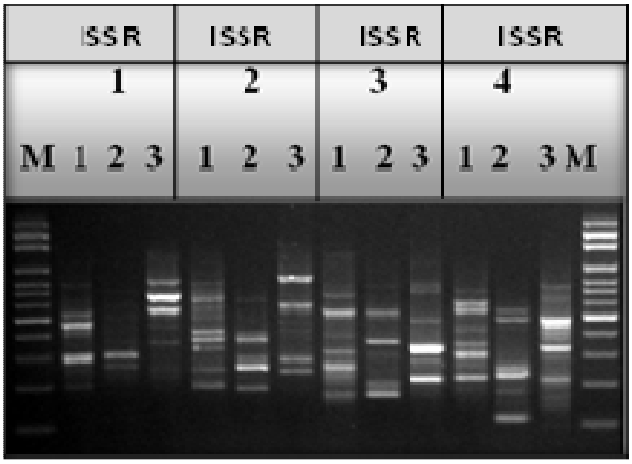

Fig. (7): Agrose-gel electrophoresis of ISSR product generated with the primers (ISSR-1, ISSR-2, ISSR-3 and ISSR-4) in species 1- *Trichogaster trichopterus* 2- *Trichogaster leeri* 3- *Colisa lalia.*

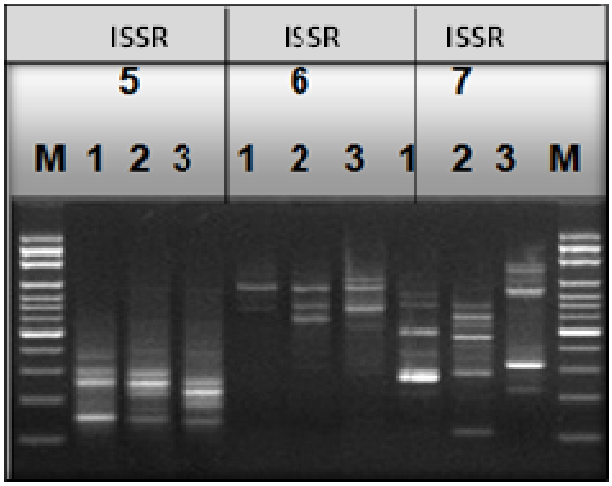

Fig. (8): Agrose-gel electrophoresis of ISSR product generated with the primers (ISSR-5, ISSR-6 and ISSR-7) in species *Trichogaster trichopterus*, *Trichogaster leeri* and *Colisa lalia*.

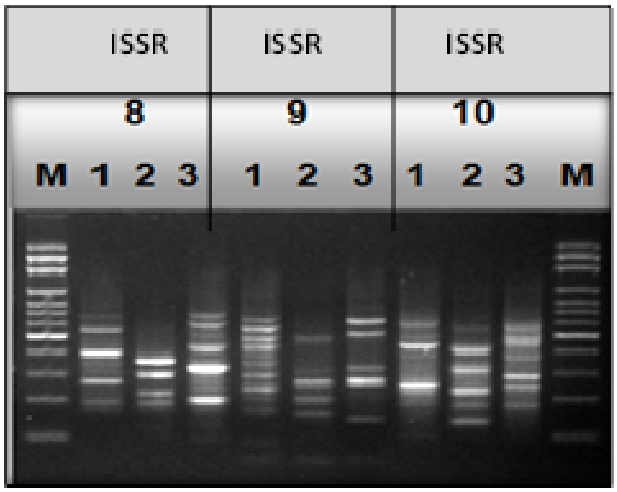

Fig. (9): Agrose-gel electrophoresis of ISSR product generated with the primers (ISSR-8, ISSR-9 and ISSR-10) in species *Trichogaster trichopterus*, *Trichogaster leeri* and *Colisa lalia*.

Table (5): Survey of ISSR Markers using ten primers. In (1-*Trichogaster trichopterus*, 2-*Trichogaster leeri* and 3- *Colisa lalia* of Family*:* Osphronemidae) where (1) means present and (0) means absence.

		ISSR1				ISSR5	
MW	1	2	3	MW	1	2	3
850	0	0	1	350	1	1	1
790	1	0	1	280	1	1	0
700	0	0	1	240	1	1	1
650	1	0	1	200	0	1	1
610	0	0	1	170	0	0	1
600	0	1	1	140	1	1	1
540	1	1	1			ISSR6	
460	1	1	1	MW	1	2	3
420	1	0	0	1050	0	0	1
380	0	1	1	910	1	1	1
300	1	1	0	710	1	1	1
270	0	1	1	590	0	1	0
230	0	0	1	520	0	0	1
200	1	0	0			ISSR7	
		ISSR2		MW	1	2	3
MW	1	2	3	1300	0	0	1
910	0	0	1	1200	0	0	1
840	1	0	1	960	0	0	1
760	1	0	0	860	1	0	1
670	1	1	0	740	1	1	0
630	0	0	1	630	0	1	0
560	1	0	1	540	0	1	0
510	1	1	0	520	1	0	0
490	0	0	1	460	0	1	0
420	1	1	1	400	0	1	0
380	1	1	0	390	0	0	1
340	1	0	0	370	1	0	0
300	0	1	1	340	0	1	0
270	0	1	0	310	0	0	1
250	1	1	1	280	1	1	0
200	0	0	0	250	1	0	0
190	1	1	0	200	0	0	1
		ISSR3		120	0	1	0
MW	1	2	3			ISSR8	
1200	1	0	0	MW	1	2	3
820	1	1	1	660	1	0	1
730	0	0	1	530	1	0	0
660	1	0	0	570	0	0	1
580	1	1	0	410	0	0	1
520	1	1	0	380	1	0	1
490	0	0	1	270	0	1	0
420	0	0	1	330	0	1	0
390	1	1	0	290	0	0	1
360	0	0	1	240	1	0	0
320	1	1	1	210	0	0	1
300	0	0	1	200	0	1	0
260	1	0	0	170	1	1	1
220	0	0	1			ISSR9	
200	0	1	0	MW	1	2	3
180	1	1	0	630	1	0	1
		ISSR4		560	1	0	0
MW	1	2	3	470	1	1	1
800	0	0	1	410	1	0	0
740	1	0	1	340	1	0	0
670	1	0	1	310	1	0	0
560	1	1	0	280	1	1	1
500	0	1	1	240	1	1	1
450	1	0	1	210	0	1	1
410	0	0	1	200	1	0	0
380	0	0	1	180	0	1	0
350	1	0	1	150	1	1	0
330	1	0	0	130	0	0	1
300	1	0	1	70	1	1	1
260	0	1	0			ISSR10	
240	1	1	1	MW	1	2	3
200	0	0	1	600	1	0	0
190	0	1	0	510	0	1	1
180	0	0	1	470	1	0	0
160	0	0	1	420	1	0	1
130	0	1	0	370	0	1	0
				340	0	0	1
				290	0	1	0
				270	0	0	1
				230	1	0	1
				200	1	1	1
				190	0	1	0
				160	1	1	1
				120	0	1	0

A total of 131 DNA bands were generated by the 10 primers for all studied fishes; From ISSR data 15% were monomorphic bands, 28% polymorphic ones and 57% unique bands were observed among samples. The number of total fragments amplified per primer varied between five (ISSR-6) and 18 (ISSR-4, ISSR-7) (Table 6). Genetic distance was lowest between *Trichogaster leeri***,** and *Colisa lalia*, while highest between *Trichogaster trichopterus*, and *Trichogaster leeri*. Genetic similarity index was from 39% to 51% (Table7), and Dendrogram (Fig. 10).

Table (6): Number of amplified and polymorphic DNA-fragments in the three species. (1-*Trichogaster trichopterus*, 2-*Trichogaster leeri* and 3- *Colisa lalia*).

No. of primer	Primer code	No. of amplified bands			Total amplified bands	No. of monomorphic bands	No. of polymorphic bands	No. of unique bands	Polymorphism %
		1	2	3					
1	ISSR1	7	6	11	14	2	6	6	86%
2	ISSR2	10	8	8	15	2	7	6	87%
3	ISSR3	9	7	8	16	2	4	10	88%
4	ISSR4	8	6	13	18	1	7	10	95%
5	ISSR5	4	5	5	6	3	2	1	50%
6	ISSR6	2	3	4	5	2	0	3	60%
7	ISSR7	6	8	7	18	0	3	15	100%
8	ISSR8	5	4	7	12	1	2	9	92%
9	ISSR9	11	7	7	14	4	3	7	72%
10	ISSR10	6	7	7	13	2	3	8	85%
total		68	61	77	131	19	37	75	86%

Table (7): Similarity matrix UPGWA Dice coefficient. (1-*Trichogaster trichopterus*, 2-*Trichogaster leeri* and 3-*Colisa lalia*).

Similarity Matrix			
	1	2	3
1	100		
2	51	100	
3	47	39	100

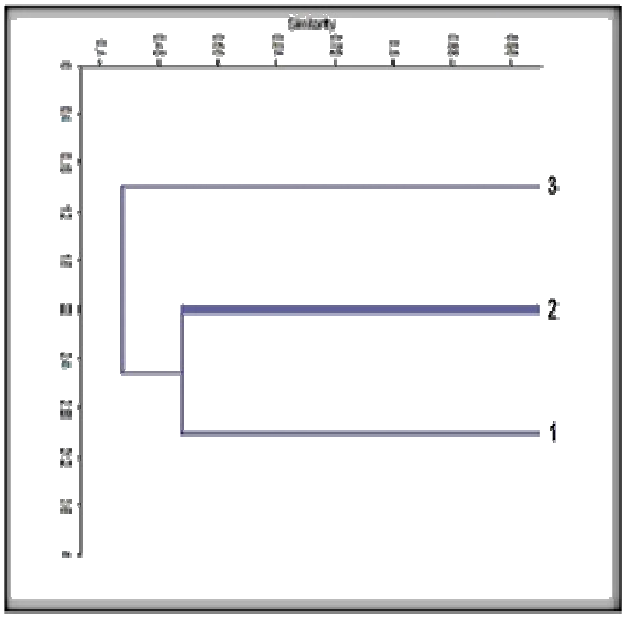

Fig. (10): Dendrogram for three species of fishes constructed from the ISSR data using similarity matrices computed according to dice coefficients. (1-*Trichogaster trichopterus*, 2-*Trichogaster leeri* and 3- *Colisa lalia*).

The results of cytogenetic analysis (karyotyping) and of ISSR-PCR analysis were compared with those obtained from the classical methods in taxonomy using morphological and anatomical characters. This research is an initial study reporting the chromosome numbers, karyotypic characters and ISSR analysis of three species, *Trichogaster trichopterus, Trichogaster leeri* and *Colisa lalia* in Egypt.

DISCUSSION

Cytogenetic studies of chromosome number and karyotypes of fish are of special interest to taxonomists and evolutionists because of the number of species and varieties of this phylum have extreme diversity in their morphology and anatomy.

Cytogenetic studies of fresh water fishes have resulted in the analysis of approximately 1040 species, of which more than 70% correspond to the orders Characiformes and Siluriformes (Oliveira *et al.,* 1988 and Oliveira *et al.,* 2009). Cyprinodontiform fishes comprise approximately 850 species, of which only 67 species have cytogenetic information (Costa, 1998 and Oliveira *et al.,* 2009). Most of the Poeciliidae species are with diploid chromosomes number 48, and the diploid chromosome numbers ranging from 2n = 46 to 2n = 48 (Abu-Almaaty *et al.,* 2015).

There are 12 genera and 46 species in the family Osphronemidae. The genus *Trichogaster* comprises of four species including three-spot gourami (*T. trichopterus*), peal gourami (*T. leeri*), moonlight gourami (*T. microlepis*) and snakeskin gourami (*T. pectoralis*) (Vidthayonon, 2005).

Only 17 species of this family (Osphronemidae) have been cytogenetically investigated, the diploid chromosome number (2n) of these species ranging from 16 to 48 according to (Calton & Denton, 1974). Number of chromosomes have been reported in fishes such as Siamese fighting fish (*B. splendens*) 2n = 42 accepted with (Magtoon *et al.,* 2007). Four species of fishes *Trichogaster trichopterus*, *Trichogaster leeri*, Colisa *chuna*, and *C. leeri*, have same chromosome number 2n=46, and the number of chromosomes have been reported in fish *Osphronemus goramy* 2n=48. This is agreement with the previous studies made by (Donsakul *et al.,* 1978, Rishi *et al.,* 1997 and Rishi *et al.,* 2001).

The ISSR-PCR strategy is especially attractive because it avoids the need to carry out costly cloning and sequencing inherent in the original microsatellite-based approach (Nagaraju *et al.,* 2002). This PCR-based method uses primers annealing to microsatellite repeats to amplify the regions between adjacent, inversely orientated SSRs, provided they are close enough to allow exponential multiplication (Kramer *et al.,* 2007). The polymorphism between the studied species by using ISSR-1, ISSR-2, ISSR-3, ISSR-4, ISSR-5, ISSR-6, ISSR-7, ISSR-8, ISSR-9 and ISSR-10 was 86%, 81%, 88%, 95%, 50%, 60%, 100%, 92%, 72%, and 77%, respectively. It can be concluded also that ISSR-PCR is a useful tool for estimating the genetic variability and degree of similarity among fish species.

CONCLUSION

The results of this study indicated that *Trichogaster trichopterus* and *Trichogaster leeri* were identical in their karyotypes, but the karyotype of *Colisa lalia* was different. In addition, to the results indicated that each species has different molecular genetic characteristics. The cluster analysis clearly differentiated *Trichogaster trichopterus* and *Trichogaster leeri* from *Colisa lalia*. The molecular

genetic taxonomic relationship among three species of Osphronemidae fishes were investigated using cytogenetic analysis and ISSR markers for first time in Egypt. ISSR markers are one of the cheapest and easiest marker systems with high efficiency in generating polymorphism among closely related varieties. A further the molecular genetic studies would play a major role in fish genome analysis in the future.

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ARABIC SUMMARY

التحليل التتابعي التكراري البسيط والوراثي الخلوي لثلاثة أنواع من الأسماك (عائلة أسفرونيميدي)

علي حسين أبو المعاطي – محمد كامل حسن – إيمان محمد بهجت – مريم السعيد المحمدي سليمان
قسم علم الحيوان – كلية العلوم ببورسعيد – جامعة بورسعيد

في هذا البحث تم استخدام التحليل الوراثي الخلوى وتقنيه التحليل التتابعى التكراري البسيط ISSR لدراسة الانماط الوراثيه والعلاقة الجزيئية الوراثية ودرجة القربي لثلاثة أنواع من ألاسماك التي تنتمي الى عائلة أسفرونيميدي، وقد اظهرت الدراسة النتائج التالية :

١- ترايكوجاستر ترايكوبتيريس : *Trichogaster trichopterus*

أوضحت دراسة التحليل الكروموسومى أن عدد الكروموسومات هو ٢ ن =٤٦، وقد تم ترتيب الصبغيات فى مجموعه واحده جميعها طرفية السنترومير. وبإستخدام عشر بادئات بطول عشرة نيكليوتيدات تم الحصول على طرز حزمية مميزه لكل بادئ، تراوحت اعداد هذه الحزم من حزمتين مع ISSR-6 إلى ١١حزمة مع ISSR-9 ، بينما تراوحت احجام هذه الحزم من٧٠ زوج من القواعد مع ISSR-9 الى ١٢٠٠ زوج من القواعد مع ISSR-3.

٢- ترايكوجاستر ليري : *Trichogaster leeri*

أوضحت دراسة التحليل الكروموسومى أن عدد الكروموسومات هو ٢ ن = ٤٦. وقد تم ترتيب الصبغيات فى مجموعه واحده جميعها طرفية السنترومير. وبإستخدام عشرة بادئات بطول عشر نيكليوتيدات تم الحصول على طرز حزمية مميزه لكل بادئ، تراوحت اعداد هذه الحزم من ثلاث مع ISSR-6 الى ثماني حزم مع ISSR-7 وISSR-2 ، بينما تراوحت احجام هذه الحزم من ٧٠ زوج من القواعد مع ISSR-9 الي٩١٠ زوج من القواعد مع ISSR-6.

٣- كوليسا لاليا : *Colisa lalia*

أوضحت دراسة التحليل الكروموسومى أن عدد الكروموسومات هو ٢ ن = ٤٦. وقد تم ترتيب الصبغيات فى مجموعتين الاولي ١٢ زوج وسطي السنترومير والثانيه ١١ زوج طرفي السنترومير. استخدام

عشرة بادئات بطول عشر نيكليوتيدات تم الحصول على طرز حزمية مميزه لكل بادئ ، تراوحت اعداد هذه الحزم من أربع مع ISSR-6 إلى ١٣حزمة مع ISSR-4 ، بينما تراوحت احجام هذه الحزم من٧٠ زوج من القواعد مع ISSR-9 إلى ١٣٠٠ زوج من القواعد مع ISSR-7.

أوضح التحليل التتابعي التكراري البسيط لهذه الأنواع وجود درجات متفاوتة من التقارب و الاختلاف بين هذه الانواع، فمثلا درجة التقارب بين ترايكوجاستر ترايكوبتيريس و ترايكوجاستر ليري كانت ٥١% وبين ترايكوجاستر ترايكوبتيريس و كوليسا لاليا ٤٧% وبين ترايكوجاستر ليري و كوليسا لاليا ٣٩% وتمثل هذه الدراسة أهميه كبيره في علم التصنيف الحديث الذي اصبح يستخدم الانماط الوراثيه والاختلافات الجزيئيه بين الانواع بالاضافه الى الصفات المورفولوجيه والتشريحه للانواع في تصنيفها وتعد هذه اول دراسه وراثيه خلويه وجزيئيه على هذه الانواع في مصر.