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## Genetic Investigation of Some Perciformes Fish Species Using Karyological Analysis

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#### ABSTRACT

In the order perciformes, there are many of fishes which have economic importance and have cytogenetic biodiversity. Cytogenetics is becoming an important biodiversity-detection tool and used to measurement biodiversity evolutionary aspects. A remarkable degree of chromosomal conservatism (2n=48, FN=48) has been identified in several families of Perciformes. However, some families exhibit greater karyotypic diversity. The present study was aimed to characterize cytogenetically the chromosomal formula, chromosome numbers and karyotypes of three fish species of order Perciformes; *Colisa chuna, Osphronemus goramy* and *Betta splendens* using karyological analysis. All Fish species were collected from ornamental fish farms in Egypt. The diploid chromosome number, chromosomal formula and fundamental numbers of the three species under study were 2n=46, 20m+26sm and FN=92, 2n=48, 2m+46a and FN=50 and 2n=42, 12sm+14st+16a and FN=68 respectively. These results may open the way for using cytogenetic analysis in the modern taxonomy.

# INTRODUCTION

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Fish are represented by 32,900 species; more than 20,000 are marine and 8,000 are living in Neotropical continental waters. The studies on the chromosomes of fishes have not been successful or widespread as in other vertebrate groups. Therefore karyological data on fish are available only for a small percentage (about 10%) of some 25,000 species (**Van Der** *et al.*, **2014**). Osphronemidae contains 15 Genera and 46 species, genus *Trichogaster* comprises of 4 species including three-spot gourami (*T. trichopterus*), peal gourami (*T. leeri*), moonlight gourami (*T. microlepis*) and snakeskin gourami (*T. pectoralis*) (**Vidthayonon**, **2005**).

Study of chromosomes using cytogeneyic analysis, were enables the development of evolutionary, taxonomic, and phylogenetic inferences (Jacobina *et al.*, 2011). Cytogenetic invesigations provide important basic knowledge which may have applications for many other studies, such as detection of ploidy in fishes (Pradeep *et al.*, 2011& 2012). Molecular cytogenetic studies on chromosomes constitute important approaches for characterizing species and reconstructing phylogenetic relationships (Ocalewicz *et al.*, 2008; Ruiz-Herrera *et al.*, 2012). Karyological features indicate the

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evolutionary distance between species of different taxonomic categories (**Dobigny** *et al.*, 2004).

The cytogenetic studies indicated that the diploid chromosome numbers of Actinopterygii ranging from 2n=16 to 2n =134 (Santini *et al.*, 2009; Sato and Nishida, 2010; Kai *et al.*, 2011; Mazzuchelli *et al.*, 2012); Guyomard *et al.*, 2012; Schartl *et al.*, 2013; Eschmeyer and Fong, 2014; Grassi *et al.*, 2017, Abu Almaaty *et al.*, 2015 & 2017a; Almeida *et al.*, 2017).

There are some species of family Osphronemidae have been cytogenetically investigated reporting the diploid chromosome number (2n) 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 (Betta splendens) (Magtoon et al., 2007).

The cytogenetic information provided us more fully explain the taxonomic and evolutionary statuses, and reveal the inherent differences of *Colisa chuna, Osphronemus goramy* and *Betta splendens*. Because of the scarcity of chromosomal evolution reports on fish species of family Osphronemidae in Egypt, so this study was aimed to provide new chromosomal data for three species of this family: *Colisa chuna, Osphronemus goramy* and *Betta splendens* by using cytogenetic analysis.

### MATERIALS AND METHODS

Samples of three species of ornamental fresh water fishes were collected from the ornamental fish farms in Port Said, Colisa chuna, Osphronemus goramy and Betta splendens of family Osphronemidae. They were caught and transported to the lab and kept alive until processed. Mitotic chromosomes were prepared from kidney as described by (Netto et al., 2007). 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 30 min. The tissues were immersed three times in a mixture of ethanolacetic acid glacial 3:1 every time was taken 20min, then the tissues 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  $\times 10$  or  $\times 15$  eyepieces, together with  $\times$  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 (Molina et al., 2012&2013).

# RESULTS

In this study the chromosomal formula of three species of family Osphronemidae: (*Colisa chuna, Osphronemus goramy* and *Betta splendens*) including chromosome number, Fundamental number and karyotypes were investigated using cytogenetic analysis.

# Colisa chuna

The chromosomal formula of *Colisa chuna* was reported, the diploid chromosomes number found 2n=46, fundamental number (FN) =92 and ideogram of chromosomes as shown in (Figs.1, 2). The karyotype consists of two different groups formed: group A composed of ten metacentric pairs of chromosomes with relative lengths varies from 2.94 % to 5.6%, arm ratios ranging from 1.18 to 1.7 centromeric indices from 36.95 to 45.83 and group B is composed of 13 subtelocentric pairs of chromosomes with relative lengths varies from 3.43 % to 5.89%, arm ratios ranging from 3 to 6 centromeric indices from 14.28 to 25 in Table (1).



Fig. 1. A coloured photograph, Chromosomes spread and karyotype of Colisa chuna.



Fig. 2. Ideogram of chromosomes of *Colisa chuna* which constructed in respect to relative length.

	Chromosome Length			Relative Length %					<u> </u>
Chromosome Number	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.	Arm Ratio Mean ± S.D.	Centromeric Index Mean ± S.D.	)lassificati on
1	0.29±0.04	0.17±0.05	0.46±0.03	3.56±0.13	2.08±0.15	5.6±0.25	1.7±0.11	36.95±1.16	Μ
2	0.27±0.03	0.16±0.04	0.43±0.05	3.31±0.14	1.96±0.17	5.27±0.23	1.68±0.12	37.20±1.13	Μ
3	0.25±0.03	0.15±0.05	0.40±0.04	3.07±0.12	1.84±0.14	4.91±0.21	1.66±0.13	37.5±1.09	М
4	0.19±0.02	0.14±0.04	0.33±0.03	2.33±0.15	1.71±0.18	4.04±0.26	1.35±0.12	42.42±1.07	М
5	0.18±0.03	0.13±0.03	0.31±0.05	2.21±0.14	1.59±0.16	3.8±0.23	1.38±0.11	41.93±1.08	М
6	0.17±0.05	0.13±0.04	0.30±0.04	2.08±0.13	1.59±0.14	3.67±0.24	1.30±0.15	43.33±1.06	М
7	0.16±0.04	0.12±0.05	0.28±0.03	1.96±0.12	1.47±0.20	3.43±0.25	1.33±0.14	42.85±1.05	Μ
8	0.15±0.03	0.12±0.07	0.27±0.04	1.84±0.15	1.47±0.21	3.31±0.21	1.25±0.13	44.44±1.03	Μ
9	0.14±0.03	0.11±0.08	0.25±0.05	1.71±0.14	1.35±0.21	3.06±0.20	1.27±0.11	44.00±1.04	Μ
10	0.13±0.02	0.11±0.07	0.24±0.04	1.59±0.17	1.35±0.16	2.94±0.19	1.18±0.12	45.83±1.05	М
11	0.36±0.04	0.12±0.03	0.48±0.04	4.42±0.16	1,47±0.17	5.89±0.18	3.00±0.13	25.00±1.07	ST
12	0.35±0.03	0.11±0.04	0.46±0.03	4.29±0.18	1.35±0.18	5.64±0.17	3.18±0.11	23.91±1.09	ST
13	0.34±0.05	0.10±0.04	0.44±0.04	4.17±0.13	1.22±0.17	5.39±0.12	3.40±0.14	22.72±1.03	ST
14	0.33±0.04	0.09±0.05	0.42±0.05	4.05±0.12	1.10±0.15	5.15±0.19	3.66±0.12	21.42±1.02	ST
15	0.32±0.03	0.09±0.04	0.41±0.03	3.93±0.11	1.10±0.16	5.03±0.21	3.55±0.15	21.95±1.04	ST
16	0.31±0.03	0.08±0.03	0.39±0.02	3.80±0.14	0.98±0.17	4.78±0.12	3.87±0.13	20.51±1.03	ST
17	0.30±0.02	0.07±0.03	0.37±0.04	3.68±0.12	0.85±0.13	4.53±0.19	4.28±0.14	18.91±1.03	ST
18	0.29±0.04	0.07±0.02	0.36±0.05	3.56±0.14	0.85±0.14	4.41±0.18	4.14±0.13	19.44±1.05	ST
19	0.28±0.03	0.06±0.03	0.34±0.03	3.43±0.12	0.73±0.13	4.16±0.17	4.66±0.12	17.64±1.06	ST
20	0.27±0.02	0.05±0.02	0.32±0.04	3.31±0.11	0.61±0.14	3.92±0.16	5.40±0.11	15.62±1,03	ST
21	0.26±0.05	0.05±0.02	0.31±0.03	3.19±0.12	0.61±0.15	3.8±0.18	5.20±0.13	16.12±1.06	ST
22	0.25±0.05	0.05±0.02	0.30±0.04	3.07±0.14	0.61±0.16	3.68±0.19	5.00±0.14	16.66±1.04	ST
23	0.24±0.03	0.04±0.02	0.28±0.05	2.94±0.15	0.49±0.14	3.43±0.21	6.00±0.13	14.28±1.07	ST
Sum			8.14±0.27						

**Table 1.** Averages of chromosomes measurements and classification, obtained from observations on ten cell spreads of *Colisa chuna*.

### Osphronemus goramy

The metaphase chromosomes of *Osphronemus goramy* was scored, the diploid set consists of 2n=48, fundamental number (FN) =50 and ideogram of chromosomes as shown in (Figs. 3, 4). The karyotype consists of two different groups formed: group A composed of one metacentric pair of chromosomes with relative length 5.16 %, arm ratio 1.42 and centromeric index 41.26 and group B is composed of 23 acrocentric pairs of chromosomes with relative lengths varies from 2.45 % to 6.22%, arm ratio of  $\infty$  and centromeric index zero in Table (2).



Fig. 3. A coloured photograph, Chromosomes spread and karyotype of Osphronemus goramy.



Fig. 4. Ideogram of chromosomes of Osphronemus goramy which constructed in respect to relative length.

Table 2. Averages of a	chromosomes measured	urements and	classification,	obtained from	observations
on ten cell sp	oreads of Osphroner	mus goramy.			

	Ch	romosome Len	ıgth	R	elative Length	%			
Chromosome Number	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.	Arm Ratio Mean± S.D.	Centromeric Index Mean ± S.D.	Classification
1	0.37±0.03	0.26±0.05	0.63±0.03	3.03±0.05	2.13±0.03	5.16±0.05	1.42±0.07	41.26±1.03	М
2	0.76±0.06	Zero	0.76±0.06	6.22±0.06	Zero	6.22±0.06	x	Zero	Acro.
3	0.73±0.07	Zero	0.73±0.07	5.98±0.07	Zero	5.98±0.07	x	Zero	Acro.
4	0.69±0.09	Zero	0.69±0.09	5.65±0.09	Zero	5.65±0.09	x	Zero	Acro.
5	0.66±0.03	Zero	0.66±0.03	5.40±0.05	Zero	5.40±0.05	x	Zero	Acro.
6	0.63±0.08	Zero	0.63±0.08	5.16±0.07	Zero	5.16±0.07	x	Zero	Acro.
7	0.61±0.06	Zero	0.61±0.06	5.00±0.08	Zero	5.00±0.08	x	Zero	Acro.
8	0.59±0.08	Zero	0.59±0.08	4.83±0.04	Zero	4.83±0.04	x	Zero	Acro.
9	0.57±0.04	Zero	0.57±0.04	4.67±0.06	Zero	4.67±0.06	x	Zero	Acro.
10	0.55±0.06	Zero	0.55±0.06	4.50±0.06	Zero	4.50±0.06	x	Zero	Acro.
11	0.53±0.07	Zero	0.53±0.07	4.34±0.08	Zero	4.34±0.08	x	Zero	Acro.
12	0.51±0.08	Zero	0.51±0.08	4.18±0.04	Zero	4.18±0.04	x	Zero	Acro.
13	0.49±0.03	Zero	0.49±0.03	4.01±0.05	Zero	4.01±0.05	x	Zero	Acro.
14	0.47±0.04	Zero	0.47±0.04	3.85±0.06	Zero	3.85±0.06	x	Zero	Acro.
15	0.45±0.06	Zero	0.45±0.06	3.68±0.07	Zero	3.68±0.07	x	Zero	Acro.
16	0.44±0.08	Zero	0.44±0.08	3.60±0.03	Zero	3.60±0.03	x	Zero	Acro.
17	0.42±0.06	Zero	0.42±0.06	3.44±0.06	Zero	3.44±0.06	x	Zero	Acro.
18	0.41±0.05	Zero	0.41±0.05	3.36±0.09	Zero	3.36±0.09	x	Zero	Acro.
19	0.39±0.08	Zero	0.39±0.08	3.19±0.04	Zero	3.19±0.04	x	Zero	Acro.
20	0.37±0.07	Zero	0.37±0.07	3.03±0.03	Zero	3.03±0.03	x	Zero	Acro.
21	0.35±0.06	Zero	0.35±0.06	2.86±0.09	Zero	2.86±0.09	x	Zero	Acro.
22	0.33±0.05	Zero	0.33±0.05	2.70±0.07	Zero	2.70±0.07	x	Zero	Acro.
23	0.32±0.04	Zero	0.32±0.04	2.62±0.08	Zero	2.62±0.08	x	Zero	Acro.
24	0.30±0.03	Zero	0.30±0.03	2.45±0.06	Zero	2.45±0.06	x	Zero	Acro.
Sum			12.2±0.13						

#### Betta splendens

The chromosome number of *Betta splendens* was 2n=42, fundamental number (FN) = 68 and ideogram of chromosomes as shown in (Figs.5, 6). The karyotype consists of three different groups formed: group A composed of sex submetacentric pairs of chromosomes with relative lengths varies from 4.49 % to 5.68%, arm ratios ranging from 1.86 to 2.09 and centromeric indices from 32.35 to 35 and group B: is composed of seven subtelocentric pairs of chromosomes with relative lengths varies from 3.97 % to 6.34%, arm ratios ranging from 3 to 5 and centromeric indices from 16.66 to 25 and group C: composed of eight acrocentric pairs of chromosomes with relative lengths varies from 3.44 % to 5.69%, arm ratios  $\infty$  and centromeric index zero in Table (3).



Fig. 5. A coloured photograph, Chromosomes spread and karyotype of Betta splendens.



Fig. 6. Ideogram of chromosomes of *Betta splendens* which constructed in respect to relative length.

e	Chromosome Length			Relative Length %					0
Chromosom Number	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.	Arm Ratio Mean ± S.D.	Centromeric Index Mean ± S.D.	Jassification
1	0.28±0.03	0.15±0.03	0.43±0.06	3.70±0.10	1.98±0.09	5.68±0.09	1.86±0.04	35.00±1.06	SM
2	0.27±0.05	0.14±0.02	0.41±0.05	3.57±0.09	1.85±0.05	5.42±0.06	1.92±0.05	34.14±1.04	SM
3	0.26±0.06	0.14±0.04	0.40±0.03	3.44±0.07	1.85±0.08	5.30±0.05	1.86±0.03	35.00±1.05	SM
4	0.25±0.03	0.13±0.05	0.38±0.07	3.31±0.08	1.72±0.04	5.03±0.08	1.92±0.06	34.21±1.03	SM
5	0.24±0.08	0.12±0.03	0.36±0.05	3.17±0.06	1.58±0.07	4.75±0.07	2.00±0.04	33.33±1.09	SM
6	0.23±0.06	0.11±0.06	0.34±0.04	3.04±0.09	1.45±0.06	4.49±0.08	2.09±0.02	32.35±1.06	SM
7	0.36±0.04	0.12±0.04	0.48±0.06	4.76±0.05	1.58±0.08	6.34±0.06	3.00±0.05	25.00±1.08	ST
8	0.30±0.03	0.09±0.02	0.39±0.03	3.97±0.08	1.19±0.03	5.16±0.07	3.33±0.03	23.07±1.04	ST
9	0.29±0.06	0.08±0.03	0.37±0.08	3.84±0.06	1.05±0.07	4.89±0.06	3.62±0.07	21.62±1.05	ST
10	0.28±0.07	0.08±0.02	0.36±0.06	3.70±0.07	1.05±0.05	4.75±0.08	3.50±0.05	22.22±1.09	ST
11	0.27±0.04	0.07±0.03	0.34±0.05	3.57±0.06	0.92±0.05	4.49±0.05	3.85±0.04	20.58±1.07	ST
12	0.26±0.05	0.06±0.02	0.32±0.03	3.44±0.09	0.79±0.06	4.23±0.09	4.33±0.06	18.75±1.05	ST
13	0.25±0.03	0.05±0.02	0.30±0.05	3.31±0.05	0.66±0.07	3.97±0.06	5.00±0.09	16.66±1.07	ST
14	0.43±0.02	Zero	0.43±0.02	5.69±0.07	Zero	5.69±0.07	œ	Zero	Acro.
15	0.38±0.06	Zero	0.38±0.06	5.03±0.07	Zero	5.03±0.05	œ	Zero	Acro.
16	0.36±0.05	Zero	0.36±0.03	4.76±0.04	Zero	4.76±0.04	œ	Zero	Acro.
17	0.34±0.04	Zero	0.34±0.07	4.50±0.09	Zero	4.50±0.07	œ	Zero	Acro.
18	0.32±0.07	Zero	0.32±0.04	4.23±0.08	Zero	4.23±0.05	œ	Zero	Acro.
19	0.30±0.06	Zero	0.30±0.05	3.97±0.05	Zero	3.97±0.06	œ	Zero	Acro.
20	0.28±0.03	Zero	0.28±0.03	3.70±0.04	Zero	3.70±0.04	œ	Zero	Acro.
21	0.26±0.04	Zero	0.26±0.04	3.44±0.06	Zero	3.44±0.08	œ	Zero	Acro.
Sum			7.55±0.26						

**Table 3.** Averages of chromosomes measurements and classification, obtained from observations on ten cell spreads of *Betta splendens*.

The chromosomal analysis of this study was compared with those obtained from the classical methods in taxonomy using morphological and anatomical characters. This study indicated that each species from three fish species under study has special chromosomal formula. This work could be considered a pilot that reporting the chromosomal characterization of three species under study in Egypt.

# DISCUSSION

Fresh water fish offer extremely informative models to investigate geologic background and connectivity among basins during the identification of biogeographic processes. The Neotropical region offers excellent opportunities to the study on diversification mechanisms among freshwater fish. The region holds the biggest biodiversity in the world due to complex ecological and historical processes that deal with isolation and specialization (**Reis** *et al.*, **2003**). More than 1,000, out of the 8,000 estimated species, already have information about their karyotype and demonstrate great

diversity in diploid values. It covers from 2n=20 chromosomes in Pterolebias longipinnis (Rivulidae) up to 2n=134 in catfish Corydoras aeneus (**Oliveira** *et al.*, **2007**).

Most of cytogenetic researches on Characiformes order (40 species), Siluriformes (24 species) and Percifomes (10 species). Diploid numbers ranged from 2n=36 (*Astyanax schubarti*) to 2n=102 (*Potamorhina squamoralaevis*) and three modal numbers were determined 2n=54 (27 species), 2n=48 (11 species) and 2n=56 (10 species). The modal diploid number 2n=54 is a frequent feature among the Characiformes (Anostomidae, Curimatidae, Parodontidae, Prochilodontidae and Serrasalmidae) (Voltolin *et al.*, 2013).

Several authors including (Santini et al., 2009; Sato and Nishida, 2010; Kai et al., 2011; Mazzuchelli et al., 2012; Guyomard et al., 2012; Schartl et al., 2013; Eschmeyer and Fong, 2014, Grassi, et al., 2017, Abu Almaaty et al., 2015 & 2017a; Almeida, et al., 2017; reported that chromosome numbers confirm a large diversity in species of fishes in Class Actinopterygii which exhibit wide range, ranging from 2n=16 in (*Sphaerichthys osphromenoides*) to 2n=134 (*Corydoras aeneus*).

**Calado, (2014),** reported that chromosome numbers confirm a large diversity in species of fishes in Order perciformes which exhibit wide range, ranging from 2n = 20 chromosomes in *Pterolebias longipinnis* (Rivulidae), up to 2n = 134 in catfish Corydoras aeneus.

**Magtoon** *et al.*, (2007), concluded that the number of metaphase chromosomes of *Betta splendens* was 2n=42. The results in this study concluded that the diploid chromosome number of *Colisa chuna* is 2n=46, this agrees with the previous studies made by (**Rishi** *et al.*, 2001). The diploid chromosome numbers and Fundamental numbers of *Trichogaster trichopterus*, *Trichogaster leeri* and *Colisa lalia*, were 2n = 46 and FN = 46, 2n = 46 and FN = 70 respectively (**Abu Almaaty** *et al.*, 2017b)

### CONCLUSION

The present data reveal the high rate of chromosomal evolution in Osphronemidae when compared to their families of Perciformes and related groups. The results of this study indicated that the three species *Colisa chuna*, *Osphronemus goramy* and *Betta splendens*; have different diploid chromosome number and karyotype. The diploid chromosome number and fundamental numbers of the three species under study were 2n=46 and FN=92, 2n=48 and FN=50 and 2n=42 and FN=68 respectively. It can be concluded also that, studying of karyotypes structure could prove to be a useful tool for estimating the variability and various taxonomical degree among fish species.

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