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Chromosomal Formula of Four Marine Fish Species from the Mediterranean Sea in Egypt

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ABSTRACT
The karyological analysis is an important tool for the detection of biodiversity; it is also used for measuring biodiversity evolutionary aspects. Several families of Perciformes showed a remarkable degree of chromosomal conservation (2n=48, FN=48). The present study was aimed to characterize cytogenetically the chromosomal formula, chromosome numbers and karyotypes of four fish species of order Perciformes; (*Argyrosomus regius, Pomadasys stridens, Sparus aurata and Dicentrarchus labrax)* collected from the Mediterranean Sea in Port Said. The Mitotic chromosomal spreads illustrated that all chromosomes of these four species are acrocentric chromosomes, the chromosomal formula for all four fish species was 48a and the fundamental number for all four fish species is (FN=48). The relative length ranged from 1.82% to 5.61% in *Argyrosomus regius* 2.73% to 5.61% in *Pomadasys stridens*, 1.91% to 5.83% in *Sparus aurata and in Dicentrarchus labrax* it ranged from 2.48% to 5.59%. All species had centromeric index equal zero and arm ratio equal ∞.

INTRODUCTION

More than half of known vertebrates are fishes, the recognized fish species are more than 32,000 species **(Eschmeyer and Fong. 2014)**. The different fish species show a variety of behavior, morphology and habitat **(Nelson, 2006)**. Fishes in marine habitat exhibit a little diversity in chromosome numbers and the karyotype formula among different species; this is because of the absence of geographical barriers that is found in fresh water habitat hindering the gene flow among populations. So, a great variation in chromosome numbers is clearly observed in fish species of freshwater habitat and absent in that is live in marine habitat **(Bloom, *et al.*, 2013)**.

Cytogenetic data of fishes are very little; they only represent approximately 10.7% of all recognized species in the world **(Nirchio, *et al.*, 2014).** Karyological analysis is one of cytogenetic techniques; it aims to study the number, morphology and size of chromosomes for a species. Mitotic chromosomal spreads are used for this analysis **(Shalaby, *et al.*, 2020).**

Karyotyping is a process by which we can establish the chromosomes number, chromosomes length, centrosomes position, banding pattern and sex chromosomes differences. Karyotyping is a part of cytogenetic **(King, *et al.*, 2006)**. Standardized staining procedures are used in karyotype preparations **(O'Connor, 2008)**.

There is a great variation in chromosome number among marine fishes; however, there are many groups, such as Perciformes that contain more than 700 marine species including many marine teleosts that have an economic importance and little chromosome divergence. Previous studies illustrated that the karyotype of approximately 60% of fish species consists of acrocentric chromosomes such as Sciaenidae, Blenniidae, Sparidae, some pomacentridae, Serranidae, Nototheniidae, Channichthyidae **(Neto, *et al.*, 2011)**.

Order perciformes is a large order including the largest number of teleostei species. **Brum, (1995)** stated that about 420 species of 50 perciformes families were karyologically studied and 67% of the studied species had a diploid chromosome number equal 48 chromosomes, 30% had 2n less than 48 chromosomes and 3% had 2n more than 48 chromosomes. **Nirchio *et al.*, (2014)** studied the karyotype of 70 marine fish species , twenty five of them representing about 35.71% of the total species had 48 acrocentric chromosomes and approximately 60% of all studied species had a diploid chromosome number equal to 48 chromosomes.

Because of the scarcity of chromosomal evolution reports on fish species of order percifomes in Egypt, the aim of this study is to provide new chromosomal data for four fish species (*Argyrosomus regius, Pomadasys stridens, Sparus aurata and Dicentrarchus labrax)* collected from the Mediterranean Sea in Port Said in Egypt and to compare these results to previous published chromosomal data, in order to correlate chromosomal rearrangements during the evolutionary history of the order Perciformes.

MATERIALS AND METHODS

Cytogenetical investigations were performed on four fish species (*Argyrosomus regius, Pomadasys stridens, Sparus aurata and Dicentrarchus labrax)* collected from Mediterranean Sea in Port Said in Egypt.

Cytogenetical technique

Mitotic chromosomes were obtained from kidney, gills and spleen as described by **(Netto *et al.*, 2007)**. Fish samples were kept in appropriate aquarium then they were injected with 0.05% colchicine (1ml per 100g fish weight). After approximately two hours, samples were dissected and then tissues of kidney, gills and spleen were preserved in a hypotonic solution (0.56% KCL) for approximately one hour. Fixation of the tissues was performed using a mixture of ethanol and glacial acetic acid (3:1); tissues were preserved in the fixative for twenty minutes. This step was repeated three times.

The fixed tissues were squashed in 60% glacial acetic acid forming a cellular suspension. Dropping of three droplets of the cellular suspension on a microscopic slide. Passing the slides over a flame and then dried on air. Staining of slides for approximately one hour using 5% giemsa stain was performed.

Chromosomal analysis and karyotyping:

Examination of the slides under light microscope using x10 or x15 eyepieces, with x100 objectives and a good chromosomal spread were photographed for further karyotyping. According to **(Molina *et al.*, 2012&2013),** chromosomes were classified in

the present study. Arm ratio (AR), Relative length (RL) and centromeric index (CI) were calculated.

RESULTS

The karyotype of four fish species under the present study showed that the chromosomes of those species are all acrocentric chromosomes and Ideogrames of chromosomes for all fish species were constructed in respect to relative length (Figures 2, 4, 6 and 8).

Argyrosomus regius

This species belongs to family scianidae. The photographed spreads of cells of this species (fig.1) and karyotype showed the diploid chromosome number (2n=48) and all were acrocentric chromosomes, as illustrated in (fig.1). The relative length of these chromosomes ranged from 1.82% to 5.61%, centromeric indices were equal to zero and arm ratio of ∞ as illustrated in Table (1).

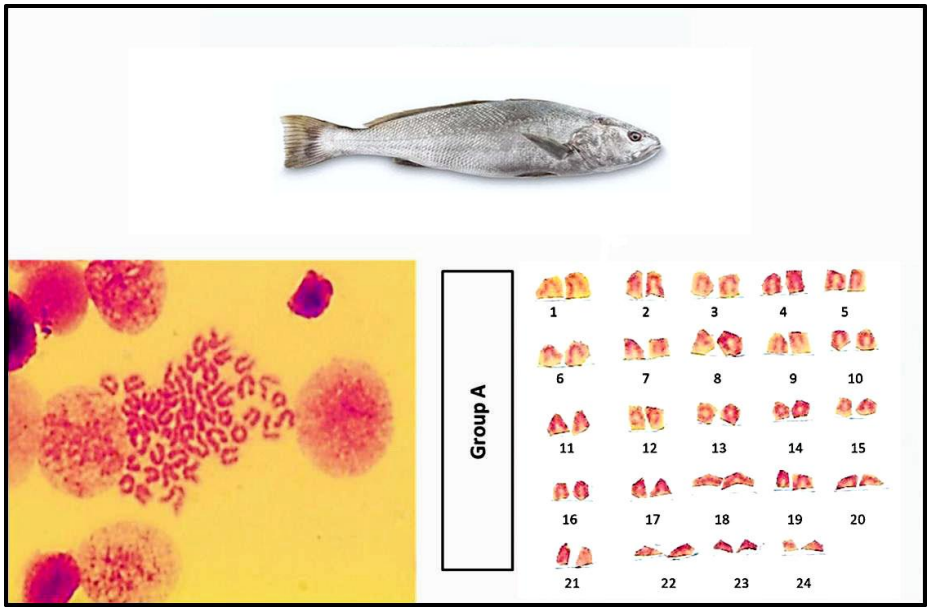

Fig. 1. A coloured photograph, chromosomes spread and karyotype of *Argyrosomus regius.*

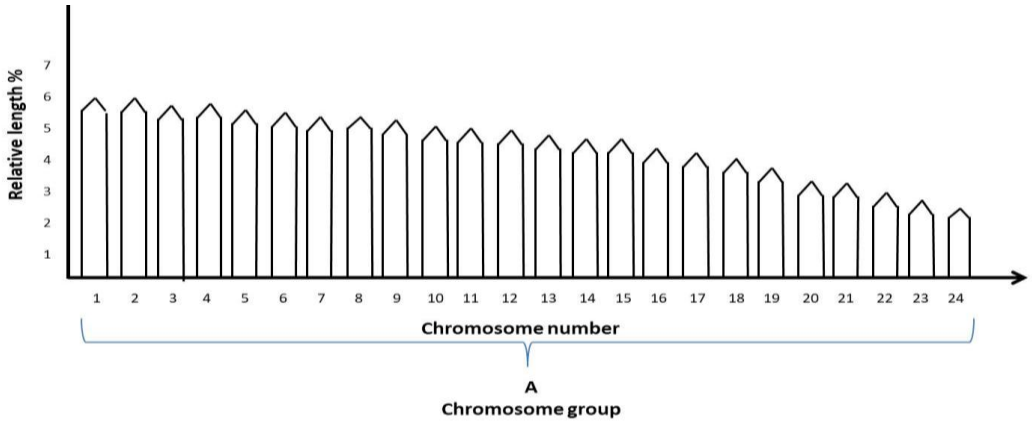

Fig. 2. Idiogram of chromosomes of *Argyrosomus regius* which constructed in respect to relative length.

Table 1. Average of ten spreads of chromosomes measurements and classification of *Argyrosomus regius.*

Chromosome number	Chromosome length			Relative length			Arm ratio	Centromeric Index%	Classification
	Long arm	Short arm	Total	Long arm	Short arm	Total			
	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	
1	0.68±0.05	0.0	0.68±0.05	5.61±0.04	0.0	5.61±0.04	∞	0.0	Acro.
2	0.67±0.05	0.0	0.67±0.05	5.53±0.04	0.0	5.53±0.04	∞	0.0	Acro.
3	0.66±0.04	0.0	0.66±0.04	5.45±0.04	0.0	5.45±0.04	∞	0.0	Acro.
4	0.65±0.04	0.0	0.65±0.04	5.36±0.03	0.0	5.36±0.03	∞	0.0	Acro.
5	0.64±0.04	0.0	0.64±0.04	5.28±0.05	0.0	5.28±0.05	∞	0.0	Acro.
6	0.62±0.03	0.0	0.62±0.03	5.12±0.03	0.0	5.12±0.03	∞	0.0	Acro.
7	0.61±0.05	0.0	0.61±0.05	5.03±0.03	0.0	5.03±0.03	∞	0.0	Acro.
8	0.60±0.03	0.0	0.60±0.03	٤.95±0.04	0.0	5.95±0.04	∞	0.0	Acro.
9	0.58±0.04	0.0	0.58±0.04	4.78±0.03	0.0	4.78±0.03	∞	0.0	Acro.
10	0.57±0.04	0.0	0.57±0.04	4.70±0.04	0.0	4.70±0.04	∞	0.0	Acro.
11	0.56±0.05	0.0	0.56±0.05	4.62±0.04	0.0	4.62±0.04	∞	0.0	Acro.
12	0.55±0.05	0.0	0.55±0.05	4.54±0.05	0.0	4.54±0.05	∞	0.0	Acro.
13	0.53±0.03	0.0	0.53±0.03	4.37±0.05	0.0	4.37±0.05	∞	0.0	Acro.
14	0.51±0.02	0.0	0.51±0.02	4.21±0.03	0.0	4.21±0.03	∞	0.0	Acro.
15	0.50±0.05	0.0	0.50±0.05	4.13±0.04	0.0	4.13±0.04	∞	0.0	Acro.
16	0.47±0.05	0.0	0.47±0.05	3.88±0.04	0.0	3.88±0.04	∞	0.0	Acro.
17	0.45±0.04	0.0	0.45±0.04	3.71±0.05	0.0	3.71±0.05	∞	0.0	Acro.
18	0.42±0.04	0.0	0.42±0.04	3.47±0.05	0.0	3.47±0.05	∞	0.0	Acro.
19	0.38±0.03	0.0	0.38±0.03	3.14±0.03	0.0	3.14±0.03	∞	0.0	Acro.
20	0.36±0.03	0.0	0.36±0.03	2.97±0.03	0.0	2.97±0.03	∞	0.0	Acro.
21	0.35±0.03	0.0	0.35±0.03	2.89±0.05	0.0	2.89±0.05	∞	0.0	Acro.
22	0.30±0.04	0.0	0.30±0.04	2.48±0.04	0.0	2.48±0.04	∞	0.0	Acro.
23	0.25±0.05	0.0	0.25±0.05	2.06±0.04	0.0	2.06±0.04	∞	0.0	Acro.
24	0.22±0.05	0.0	0.22±0.05	1.82±0.03	0.0	1.82±0.03	∞	0.0	Acro.
Sum.			12.12±0.03						

Dicentrarchus labrax

This species belongs to family moronidae. The photographed spreads of cells of this species (Fig.3) and karyotype showed the diploid chromosome number (2n=48) and all were acrocentric chromosomes, as illustrated in (Fig.3). The relative length of these chromosomes ranged from 2.48% to 5.95%, centromeric indices were equal to zero and arm ratio of ∞ as shown in Table (2).

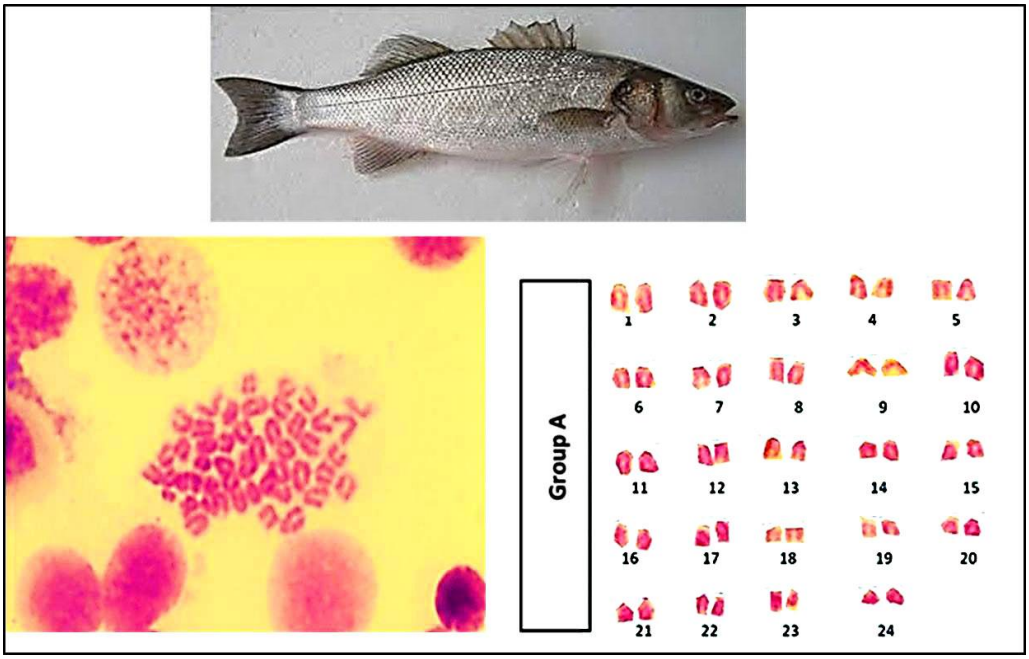

Fig. 3. A coloured photograph, chromosomes spread and karyotype of *Dicentrarchus labrax*.

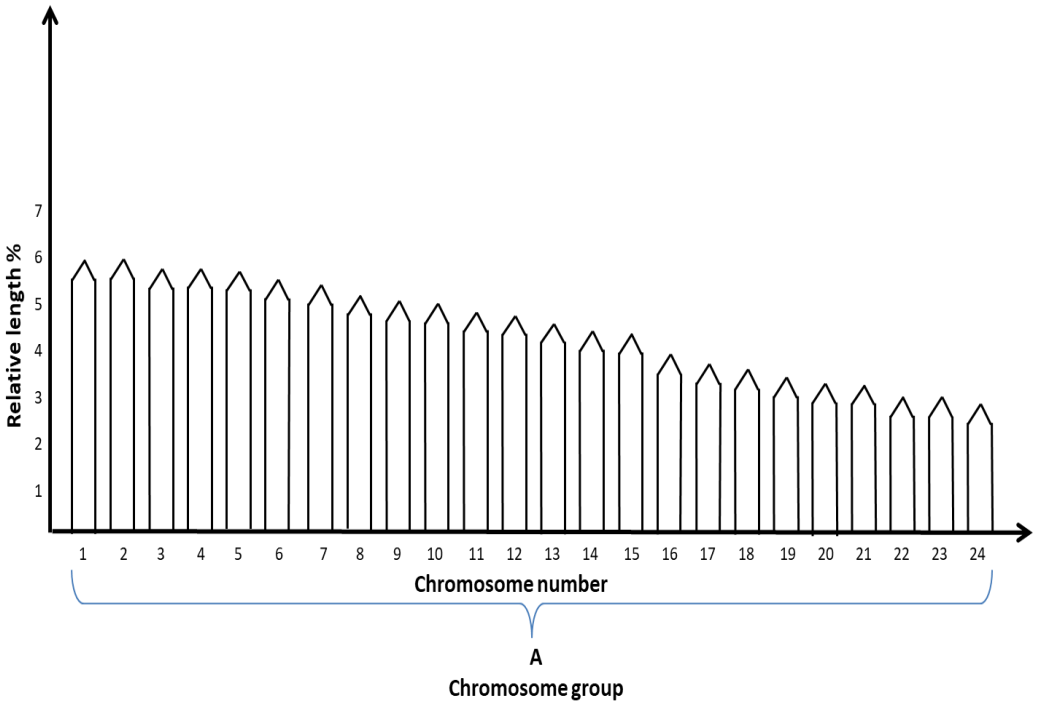

Fig. 4. Idiogram of chromosomes of *Dicentrarchus labrax* which constructed in respect to relative length.

Table 2. Average of ten spreads of chromosomes measurements and classification of *Dicentrarchus labrax.*

Chromosome number	Chromosome length			Relative length			Arm ratio	Centromeric Index%	Classification
	Long arm	Short arm	Total	Long arm	Short arm	Total			
	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	
1	0.70±0.04	0.0	0.70±0.04	5.59±0.03	0.0	5.59±0.03	∞	0.0	Acro.
2	0.69±0.04	0.0	0.69±0.04	5.51±0.03	0.0	5.51±0.03	∞	0.0	Acro.
3	0.68±0.04	0.0	0.68±0.04	5.43±0.04	0.0	5.43±0.04	∞	0.0	Acro.
4	0.67±0.05	0.0	0.67±0.05	5.35±0.03	0.0	5.35±0.03	∞	0.0	Acro.
5	0.66±0.05	0.0	0.66±0.05	5.27±0.04	0.0	5.27±0.04	∞	0.0	Acro.
6	0.64±0.03	0.0	0.64±0.03	5.11±0.05	0.0	5.11±0.05	∞	0.0	Acro.
7	0.62±0.04	0.0	0.62±0.04	4.95±0.05	0.0	4.95±0.05	∞	0.0	Acro.
8	0.61±0.04	0.0	0.61±0.04	4.87±0.05	0.0	4.87±0.05	∞	0.0	Acro.
9	0.60±0.03	0.0	0.60±0.03	4.79±0.05	0.0	4.79±0.05	∞	0.0	Acro.
10	0.58±0.03	0.0	0.58±0.03	4.63±0.04	0.0	4.63±0.04	∞	0.0	Acro.
11	0.57±0.03	0.0	0.57±0.03	4.55±0.04	0.0	4.55±0.04	∞	0.0	Acro.
12	0.55±0.05	0.0	0.55±0.05	4.39±0.03	0.0	4.39±0.03	∞	0.0	Acro.
13	0.53±0.04	0.0	0.53±0.04	4.23±0.05	0.0	4.23±0.05	∞	0.0	Acro.
14	0.51±0.05	0.0	0.51±0.05	4.07±0.04	0.0	4.07±0.04	∞	0.0	Acro.
15	0.49±0.05	0.0	0.49±0.05	3.91±0.03	0.0	3.91±0.03	∞	0.0	Acro.
16	0.45±0.05	0.0	0.45±0.05	3.59±.0.03	0.0	3.59±.0.03	∞	0.0	Acro.
17	0.42±0.05	0.0	0.42±0.05	3.35±0.03	0.0	3.35±0.03	∞	0.0	Acro.
18	0.41±0.04	0.0	0.41±0.04	3.27±0.05	0.0	3.27±0.05	∞	0.0	Acro.
19	0.39±0.03	0.0	0.39±0.03	3.12±0.04	0.0	3.12±0.04	∞	0.0	Acro.
20	0.38±0.03	0.0	0.38±0.03	3.04±0.03	0.0	3.04±0.03	∞	0.0	Acro.
21	0.37±0.03	0.0	0.37±0.03	2.95±0.04	0.0	2.95±0.04	∞	0.0	Acro.
22	0.35±0.04	0.0	0.35±0.04	2.79±0.04	0.0	2.79±0.04	∞	0.0	Acro.
23	0.34±0.04	0.0	0.34±0.04	2.71±0.05	0.0	2.71±0.05	∞	0.0	Acro.
24	0.31±0.04	0.0	0.31±0.04	2.48±0.05	0.0	2.48±0.05	∞	0.0	Acro.
Sum.			12.52±0.04						

Sparus aurata

This species belongs to family sparidae. The photographed spreads of cells of this species (Fig.5) and karyotype showed the diploid chromosome number (2n=48) and all were acrocentric chromosomes, as illustrated in (Fig.5). The relative length of these chromosomes ranged from 1.91% to 5.83%, centromeric indices were equal to zero and arm ratio of ∞ as illustrated in Table (3).

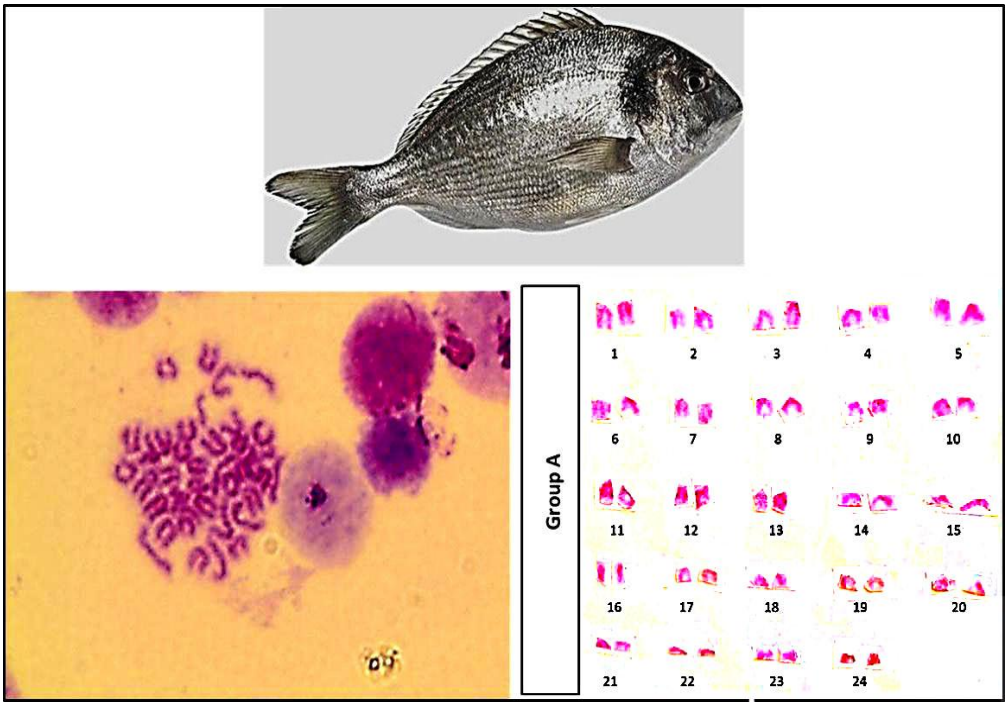

Fig. 5. A coloured photograph, chromosomes spread and karyotype of *Sparus aurata.*

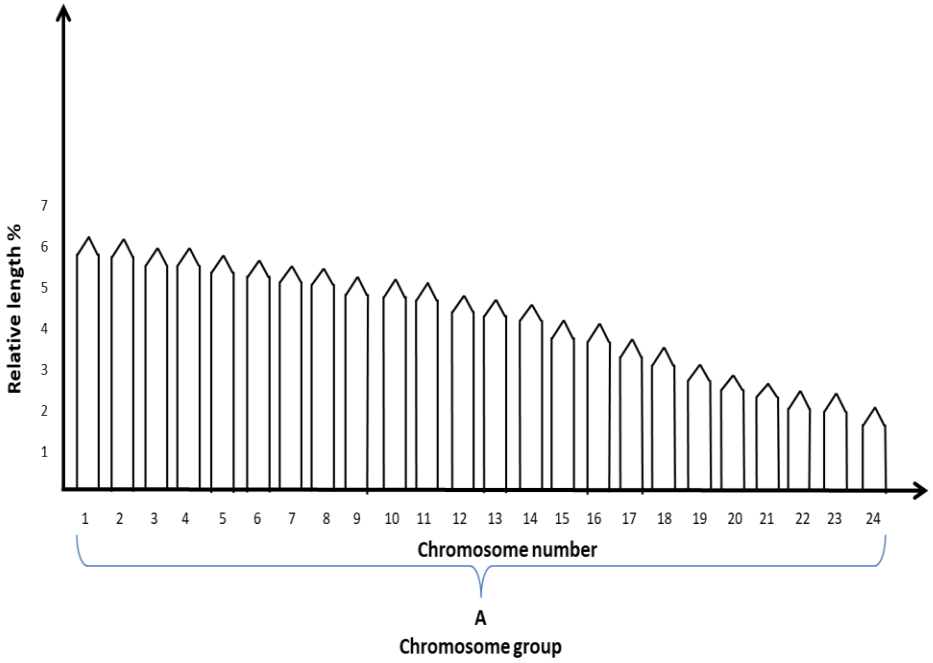

Fig. 6. Idiogram of chromosomes of *Sparus aurata* which constructed in respect to relative length.

Table 3. Average of ten spreads of chromosomes measurements and classification of *Sparus aurata*.

Chromosome number	Chromosome length			Relative length			Arm ratio	Centromeric Index%	Classification
	Long arm	Short arm	Total	Long arm	Short arm	Total			
	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	
1	0.61±0.03	0.0	0.61±0.03	5.83±0.05	0.0	5.83±0.05	∞	0.0	Acro.
2	0.60±0.04	0.0	0.60±0.04	5.73±0.03	0.0	5.73±0.03	∞	0.0	Acro.
3	0.59±0.04	0.0	0.59±0.04	5.64±0.04	0.0	5.64±0.04	∞	0.0	Acro.
4	0.58±0.05	0.0	0.58±0.05	5.54±0.05	0.0	5.54±0.05	∞	0.0	Acro.
5	0.57±0.03	0.0	0.57±0.03	5.44±0.03	0.0	5.44±0.03	∞	0.0	Acro.
6	0.56±0.03	0.0	0.56±0.03	5.35±0.04	0.0	5.35±0.04	∞	0.0	Acro.
7	0.54±0.03	0.0	0.54±0.03	5.16±0.04	0.0	5.16±0.04	∞	0.0	Acro.
8	0.53±0.05	0.0	0.53±0.05	5.06±0.04	0.0	5.06±0.04	∞	0.0	Acro.
9	0.52±0.05	0.0	0.52±0.05	4.96±0.04	0.0	4.96±0.04	∞	0.0	Acro.
10	0.51±0.05	0.0	0.51±0.05	4.87±0.05	0.0	4.87±0.05	∞	0.0	Acro.
11	0.50±0.04	0.0	0.50±0.04	4.78±0.05	0.0	4.78±0.05	∞	0.0	Acro.
12	0.47±0.05	0.0	0.47±0.05	4.48±0.03	0.0	4.48±0.03	∞	0.0	Acro.
13	0.46±0.04	0.0	0.46±0.04	4.39±0.03	0.0	4.39±0.03	∞	0.0	Acro.
14	0.45±0.03	0.0	0.45±0.03	4.29±0.03	0.0	4.29±0.03	∞	0.0	Acro.
15	0.42±0.05	0.0	0.42±0.05	4.01±0.04	0.0	4.01±0.04	∞	0.0	Acro.
16	0.40±0.04	0.0	0.40±0.04	3.82±0.03	0.0	3.82±0.03	∞	0.0	Acro.
17	0.36±0.05	0.0	0.36±0.05	3.44±0.03	0.0	3.44±0.03	∞	0.0	Acro.
18	0.33±0.05	0.0	0.33±0.05	3.15±0.05	0.0	3.15±0.05	∞	0.0	Acro.
19	0.30±0.03	0.0	0.30±0.03	2.86±0.04	0.0	2.86±0.04	∞	0.0	Acro.
20	0.28±0.03	0.0	0.28±0.03	2.67±0.03	0.0	2.67±0.03	∞	0.0	Acro.
21	0.25±0.03	0.0	0.25±0.03	2.39±0.04	0.0	2.39±0.04	∞	0.0	Acro.
22	0.23±0.04	0.0	0.23±0.04	2.19±0.03	0.0	2.19±0.03	∞	0.0	Acro.
23	0.21±0.05	0.0	0.21±0.05	2.00±0.03	0.0	2.00±0.03	∞	0.0	Acro.
24	0.20±0.03	0.0	0.20±0.03	1.91±0.05	0.0	1.91±0.05	∞	0.0	Acro.
Sum.			10.47±0.05						

Pomadasys stridens

This species belongs to family haemulidae. The photographed spreads of cells of this species (Fig.7) and karyotype showed the diploid chromosome number (2n=48) and all were acrocentric chromosomes, as illustrated in (Fig.7). The relative length of these

chromosomes ranged from 2.73% to 5.61%, centromeric indices were equal to zero and arm ratio of ∞ as shown in Table (4).

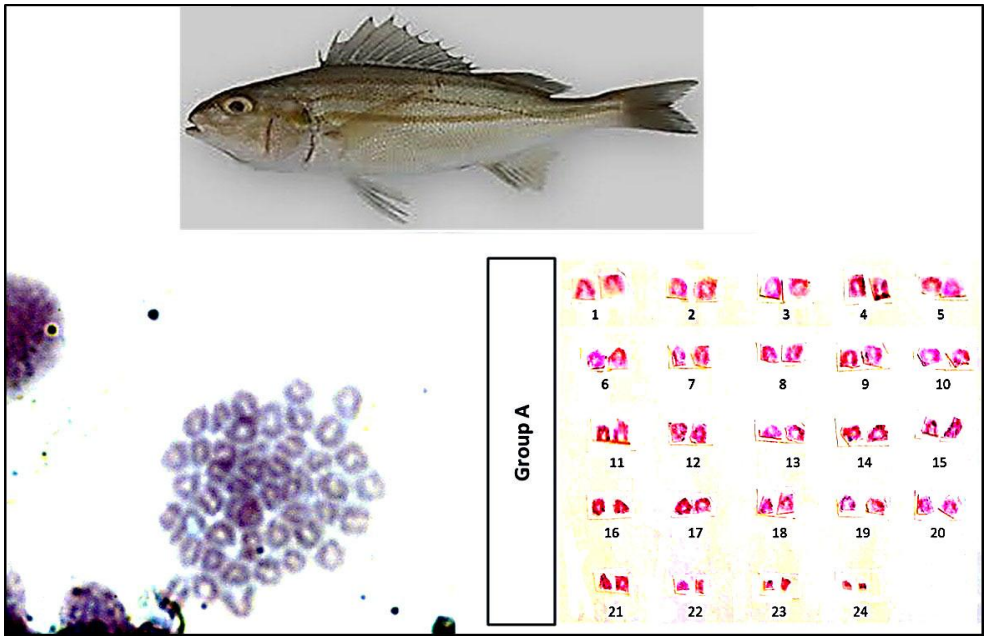

Fig. 7. A coloured photograph, chromosomes spread and karyotype of *Pomadasys stridens.*

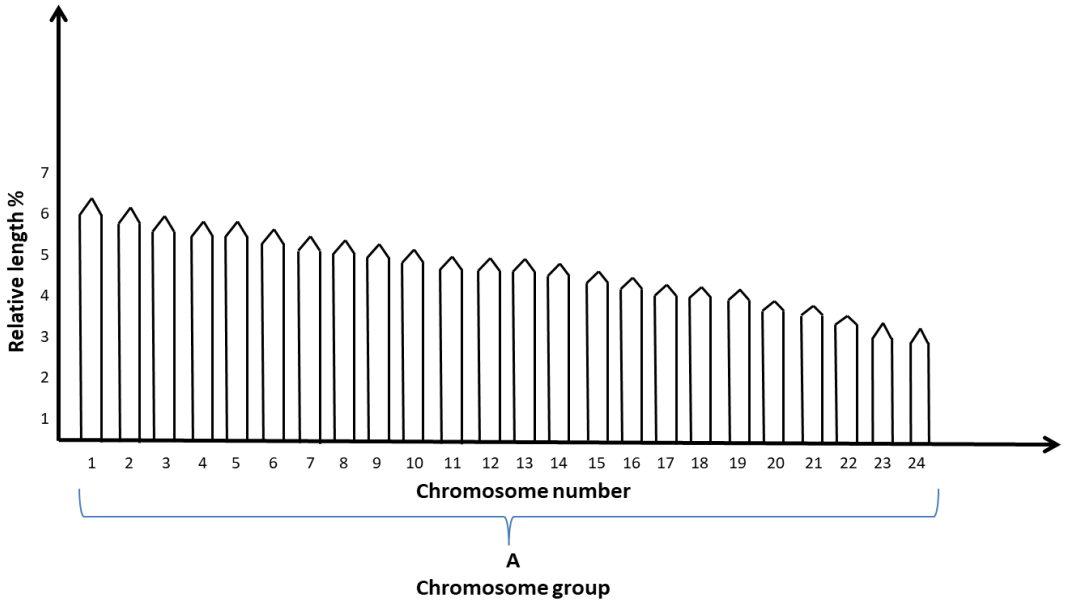

Fig. 8. Idiogram of chromosomes of *Pomadasys stridens* which constructed in respect to relative length.

Table 4. Average of ten spreads of chromosomes measurements and classification of *Pomadasys stridens.*

Chromosome number	Chromosome length			Relative length			Arm ratio	Centromeric Index%	Classification
	Long arm	Short arm	Total	Long arm	Short arm	Total			
	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	Mean± S.D	
1	0.78±0.05	0.0	0.78±0.05	5.61±0.04	0.0	5.61±0.04	∞	0.0	Acro.
2	0.76±0.04	0.0	0.76±0.04	5.47±0.03	0.0	5.47±0.03	∞	0.0	Acro.
3	0.73±0.04	0.0	0.73±0.04	5.25±0.04	0.0	5.25±0.04	∞	0.0	Acro.
4	0.72±0.03	0.0	0.72±0.03	5.19±0.05	0.0	5.19±0.05	∞	0.0	Acro.
5	0.70±0.04	0.0	0.70±0.04	5.04±0.05	0.0	5.04±0.05	∞	0.0	Acro.
6	0.69±0.05	0.0	0.69±0.05	4.97±0.05	0.0	4.97±0.05	∞	0.0	Acro.
7	0.66±0.05	0.0	0.66±0.05	4.75±0.03	0.0	4.75±0.03	∞	0.0	Acro.
8	0.64±0.03	0.0	0.64±0.03	4.61±0.04	0.0	4.61±0.04	∞	0.0	Acro.
9	0.63±0.03	0.0	0.63±0.03	4.54±0.03	0.0	4.54±0.03	∞	0.0	Acro.
10	0.62±0.04	0.0	0.62±0.04	4.47±0.05	0.0	4.47±0.05	∞	0.0	Acro.
11	0.60±0.05	0.0	0.60±0.05	4.32±0.02	0.0	4.32±0.02	∞	0.0	Acro.
12	0.59±0.04	0.0	0.59±0.04	4.25±0.05	0.0	4.25±0.05	∞	0.0	Acro.
13	0.58±0.03	0.0	0.58±0.03	4.18±0.04	0.0	4.18±0.04	∞	0.0	Acro.
14	0.55±0.05	0.0	0.55±0.05	3.96±0.04	0.0	3.96±0.04	∞	0.0	Acro.
15	0.54±0.05	0.0	0.54±0.05	3.89±0.04	0.0	3.89±0.04	∞	0.0	Acro.
16	0.53±0.04	0.0	0.53±0.04	3.82±0.03	0.0	3.82±0.03	∞	0.0	Acro.
17	0.51±0.05	0.0	0.51±0.05	3.67±0.04	0.0	3.67±0.04	∞	0.0	Acro.
18	0.50±0.03	0.0	0.50±0.03	3.60±0.04	0.0	3.60±0.04	∞	0.0	Acro.
19	0.48±0.03	0.0	0.48±0.03	3.46±0.05	0.0	3.46±0.05	∞	0.0	Acro.
20	0.45±0.02	0.0	0.45±0.02	3.24±0.03	0.0	3.24±0.03	∞	0.0	Acro.
21	0.44±0.04	0.0	0.44±0.04	3.17±0.04	0.0	3.17±0.04	∞	0.0	Acro.
22	0.40±0.05	0.0	0.40±0.05	2.88±0.03	0.0	2.88±0.03	∞	0.0	Acro.
23	0.39±0.03	0.0	0.39±0.03	2.81±0.03	0.0	2.81±0.03	∞	0.0	Acro.
24	0.38±0.04	0.0	0.38±0.04	2.73±0.05	0.0	2.73±0.05	∞	0.0	Acro.
Sum.			13.88±0.04						

DISCUSSION

This study presented cytogenetic information for four fish species (*Argyrosomus regius* of family Scianidae, *Pomadasys stridens* of family Haemulidae, *Sparus aurata* of family Scianidae and *Dicentrarchus labrax* of family Sparidae). There is a little information about the karyotype of these species. This study presented the differences in karyotype and idiograms of these four fish species.

Most studies concluded that the diploid chromosome numbers of fishes ranging from 2n=16 to 2n =134. (**Grassi *et al.*, 2017, Abu Almaaty *et al.*, 2015, 2017a &2017b; Almeida *et al.*, 2017).**

The diploid chromosome numbers have 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 *Corydoras aeneus* **(Calado, 2014)**. The diploid chromosome number of perciformes fish species was 48 chromosomes **(Brum, 1995**; **Nirchio *et al*., 2014)**

Our results are agree with results of (**Nirchio *et al*., 2007; Accioly and Molina, 2008; Merlo *et al*., 2010; Neto *et al*., 2012; Nirchio *et al*., 2014; and Motta-Neto *et al*., 2019)** which they reported that the diploid chromosomes number for four species under study was 48 chromosomes.

Karyological studies for determination of chromosome number and chromosomal formula of fish are of special interest to taxonomists because of the number of species and varieties of fish species have extreme diversity in their morphology.

CONCLUSION

Our results of this study indicated that the four species (*Argyrosomus regius, Pomadasys stridens, Sparus aurata and Dicentrarchus labrax)*; have the same diploid chromosome number, chromosomal formula and fundamental number 48. Further studies are needed to different molecular techniques to investigate if some genes have specific sequence in each species.

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