

***KARYOLOGICAL ANALYSIS OF TWO SPECIES OF FAMILY SPARIDAE: SPARUS AURATUS AND LITHOGNATHUS MORMYRUS***

**BY**

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***ABSTRACT***

*Comparative karyological studies of **Sparus auratus** and **Lithognathus mormyrus** (Family Sparidae) were carried out. The diploid number of chromosomes, their morphological distribution, the arm ratio, the total and relative length of chromosomes, the total length of chromosome set and the number of arms were determined.*

*The diploid number of chromosomes was found to be 48 for both species.*

*In **sparus auratus** the chromosomes are more isobrachial than **Lithognathus mormyrus**.*

*The morphological distribution of the karyotype of the two species is different; **Sparus auratus** have two pairs of chromosomes consistently involved satellite and three pairs have chromatid bridges, but **Lithognathus mormyrus** have 6 pairs consistently involved satellite and one pair have chromatid bridge.*

***INTRODUCTION***

Karyological characteristics have been used as a valuable aid to taxonomic and evolutionary studies in many groups of plants and animals. The cytological data of fish are most lacking, [from 25000 species of teleosts about 300 species

are known cytologically so far (Michele *et al.*, 1977), because the chromosomes are small and the available techniques often yield questionable counts and minimal morphological details.

The study of chromosomes help in the areas of systematic, mutagenesis and aquaculture (Kligerman and Bloom, 1977) and in phylogenetic relationship and experimental hybridization (Roberts 1967). Besides, the study of chromosomes is necessary in artificial gynogenesis and polyploid induction in fish.

This paper concerned with data on two species of family sparidae, *Sparus auratus* and *Lithognathus mormyrus*.

The karyological studies have been undertaken in order to:

1. Obtain the standard karyotypes of the two species.
2. Compare the two species particularly from the cytotaxonomical points and to estimate possible intraspecific variation in the karyotypes.
3. Test the value of the karyotypic criterion as species specific.
4. Detect any abnormality, polymorphism, peculiarities such as super numerary chromosomes, morphologically distinguishable sex chromosomes, polyploidy which may be suitable for a more detailed study.
5. Estimate whether numerical or morphological changes and alternations in karyotype can be interpreted in terms of evolution.

### MATERIAL AND METHODS

Ten specimens of each species: *Sparus auratus* and *Lithognathus mormyrus* of weight 80-100 gm weight were used for karyological studies, 0.01 ml/g body weight colochicine solution was injected intraperitoneal (Marian and Krasznai 1978).

After 20 hours the gills and scales were prepared and processed. For hypotonising, potassium chloride 0.04% was used. Fixation was made with freshly prepared and cooled mixture of methanol and acetic acid in a ratio of 3:1. Chromosome preparations were made according to the routine air drying methods (Denton and Howell, 1969; Howell 1972; Gold 1974).

The staining was performed with 10% Giemsa solution. The chromosome set of 20 cells of each species were determined on the basis of the mode value. All long arms and short arms of the chromosome sets were measured on photoes (Denton 1973). The indices of the arm ratio, the total length of chromosome set and relative length of the chromosomes were calculated. Chromosomes were classified according to centromeric position by long arm-short arm ratio grouping of Levan *et al* (1964).

## RESULTS

The karyotype analysis include the number and morphology of chromosomes for each species: *Sparus auratus* Fig. (1,2) and *Lithognathus mormyrus* Fig. (2,3) are represented the Karyological analysis and Idiogram for each species respectively. The diploid number of chromosomes for both species was found to be 48 chromosomes and 10, 8 fragments as dots (minutes) in *Sparus auratus* and *Lithognathis mormyrus* respectively. These species show not only the constancy in the chromosome number, but also some similarities among karyotypes Fig. (5). In fact all the karyotype show rather small chromosomes which can be arranged in order of decreasing length and whose centromeric position are median, submedian, acrocentric and telocentric.

The size of chromosomes range from 3.6 to 12 $\mu$  in *Sparus auratus* and can be arranged into 5 pairs median, and submedian, 5 pairs acrocentric; 11 pairs telocentric, and three pairs have chromatid bridge.

In *Lithognathus mormyrus* the chromosomes range in size from 2.4 $\mu$  to 14.4 $\mu$  and can be arranged into 4 pairs median, submedian, 4 pairs acrocentric and 15 pairs telocentric, and one pair have chromatid bridge.

In *S. auratus* two pairs of chromosomes were consistently involved stallite, but there were 6 pairs in *L. mormyrus*.

The total length of the chromosome set was 135.6  $\mu$ m in *Sparus auratus* (Table 1) and 175.8 $\mu$  in *Lithognathus mormyrus* (Table 2).

The number of arms (FN) were 58, 56 in *Sparus auratus* and *Lithognathus mormyrus*, respectively.

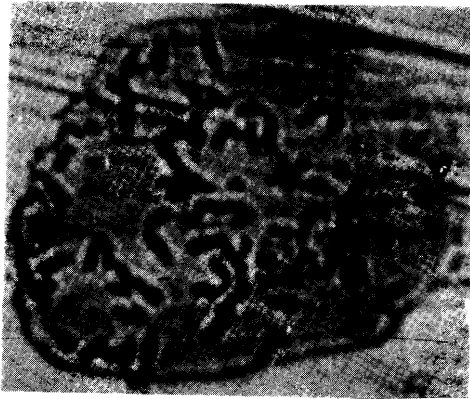


Figure (1): Karyological analysis of *Sparus auratus*

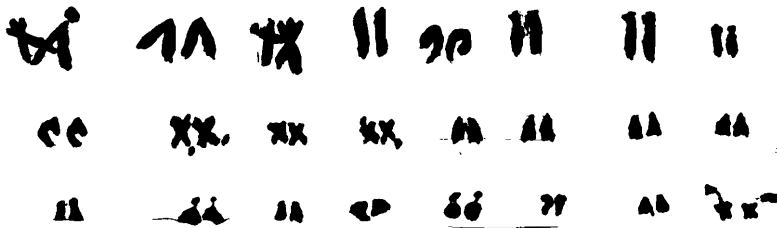
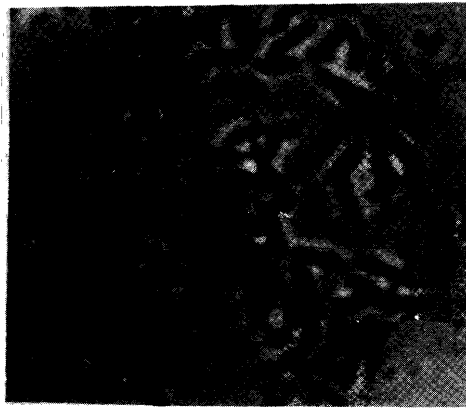


Figure (2): Karyological analysis of *Lithoganthus mormyrus*

Fig. (1)

Figure 1: Funnel-type incubators (1.5l each) employed in incubation of Nile tilapia, *O. niloticus*, eggs at water temperature 24-28°C.

**KARYOLOGICAL ANALYSIS**

**Table (1): Indices of karyological analysis of *Sparus auratus* (Sparidae):**

<i>Chromosome No.</i>	<i>Long arm</i> $\mu$	<i>Short arm</i> $\mu$	<i>Total length</i> $\mu$	<i>Relative length</i>	<i>L/S</i>	<i>Name</i>
1	12	--	12	88.4	--	A
2	10.8	--	10.8	79.6	--	A
3	9.6	2.4	9.6	70.7	--	T
4	7.2	--	9.6	70.7	--	T
5	9.6	--	9.6	70.7	--	A
6	4.2	4.2	8.4	61.9	--	Ch-B
7	7.2	1.2	8.4	61.9	6	A
8	8.4	--	8.4	61.9	--	T
9	7.2	1.2	8.4	53.3	6	A
10	4.8	2.4	7.2	53.3	2	SM
11	3.6	3.6	7.2	53.3	1	M
12	7.2	--	7.2	44.2	--	T
13	3.6	2.4	6	44.2	1.5	M
14	3.6	2.4	6	44.2	1.5	M
15	3.6	2.4	6	35.4	1.5	M
16	4.8	--	4.8	35.4	--	T
17	4.8	--	4.8	35.4	--	T
18	4.8	--	4.8	35.4	--	T
19	4.8	--	4.8	35.4	--	T
20	4.8	--	4.8	35.4	--	Ch-B
21	3.6	--	3.6	26.5	--	T
22	3.6	--	3.6	26.5	--	T
23	3.6	--	3.6	26.5	--	T
24	3.6	--	3.6	26.5	--	ChB
<b>Total</b>			<b>135.6</b>			

A = Acrocentric  
T = Telocentric  
Ch-B = Chromatid bridge  
SM = Submedian  
M = Median

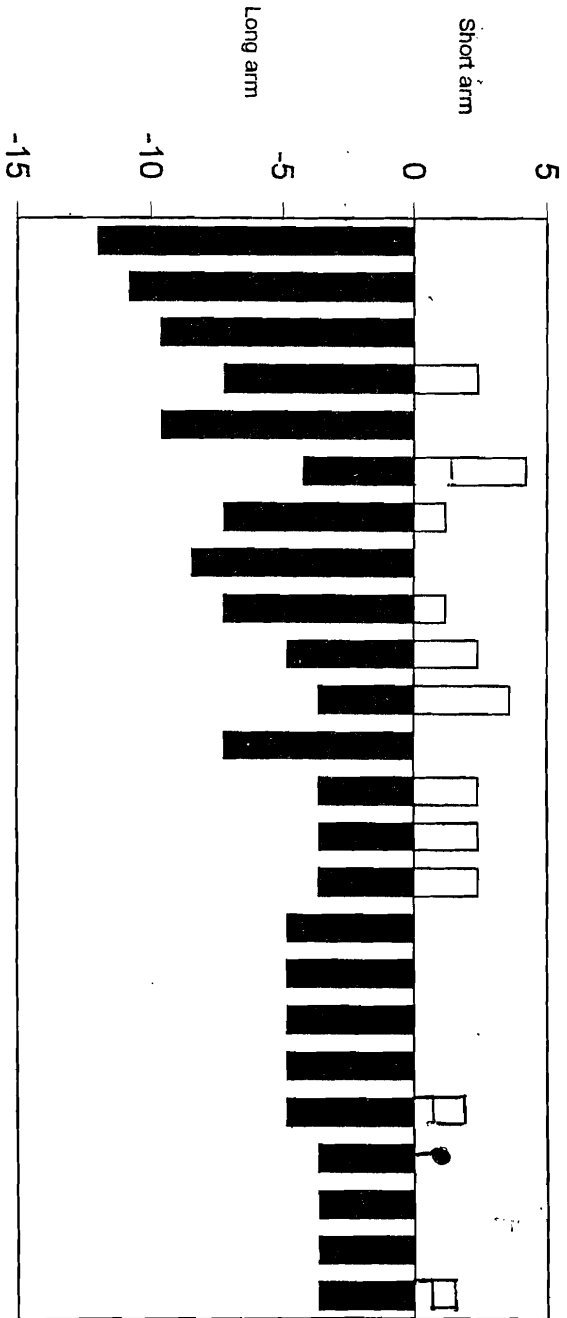


Figure (3): Idiogram of *Sparus auratus*.

KARYOLOGICAL ANALYSIS

Table (2): Indices of karyological analysis of *Lithoganthus mormyrus* (Sparidae):

Chromosome No.	Long arm $\mu$	Short arm $\mu$	Total length $\mu$	Relative length	L/S	Name
1	12	2.4	14.4	81.9	5	A
2	12	--	12	68.2	--	A
3	8.4	3.6	12	68.2	2.3	SM
4	12	--	12	68.2	--	T
5	10.2	--	10.2	58	--	A
6	10.2	--	10.2	58	--	T
7	10.2	--	10.2	58	--	T
8	8.4	--	8.4	47.7	--	T
9	8.4	--	8.4	47.7	--	A
10	4.2	4.2	8.4	47.7	1	M
11	3.6	3.6	7.2	40.9	1	M
12	3.6	3.6	7.2	40.9	1	M
13	6	--	6	34.1	--	T
14	6	--	6	34.1	--	T
15	6	--	6	34.1	--	T
16	4.8	--	4.8	27.3	--	T
17	4.8	--	4.8	27.3	--	T
18	4.8	--	4.8	27.3	--	T
19	4.8	--	4.8	27.3	--	T
20	4.8	--	4.8	27.3	--	T
21	3.6	--	3.6	20.4	--	T
22	3.6	--	3.6	20.4	--	T
23	3.6	--	3.6	20.4	--	T
24	2.4	--	2.4	13.6		Ch-B
Total			175.8			

A = Acrocentric  
T = Telocentric  
Ch-B = Chromatid bridge  
SM = Submedian

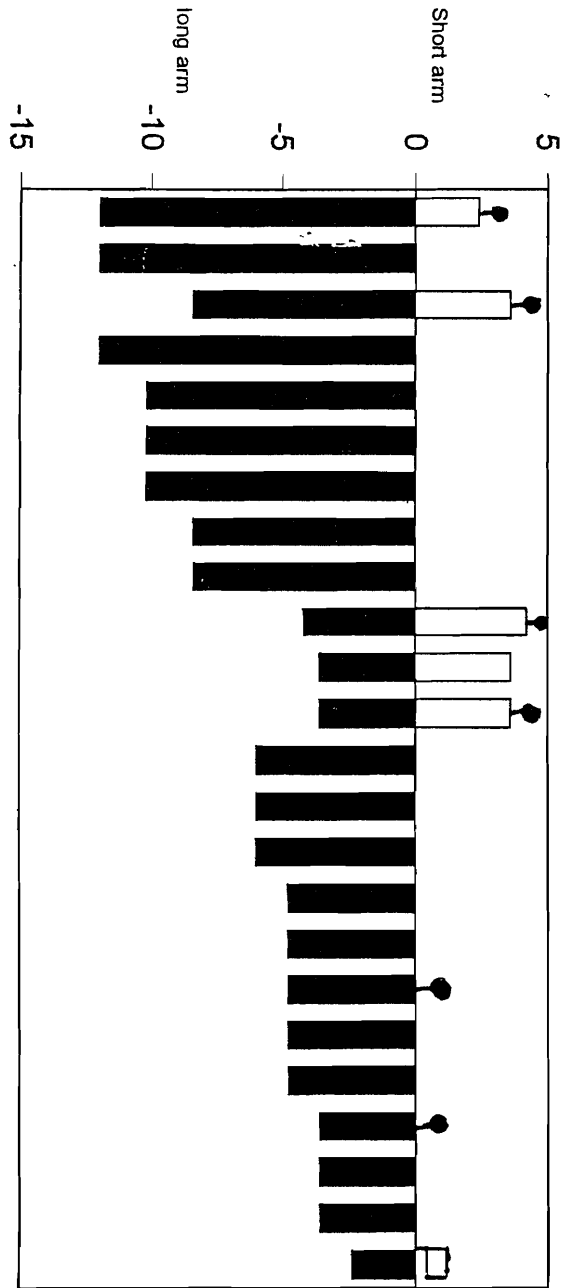


Figure (4): Idiogram of *Lithoganthus normyrus*.



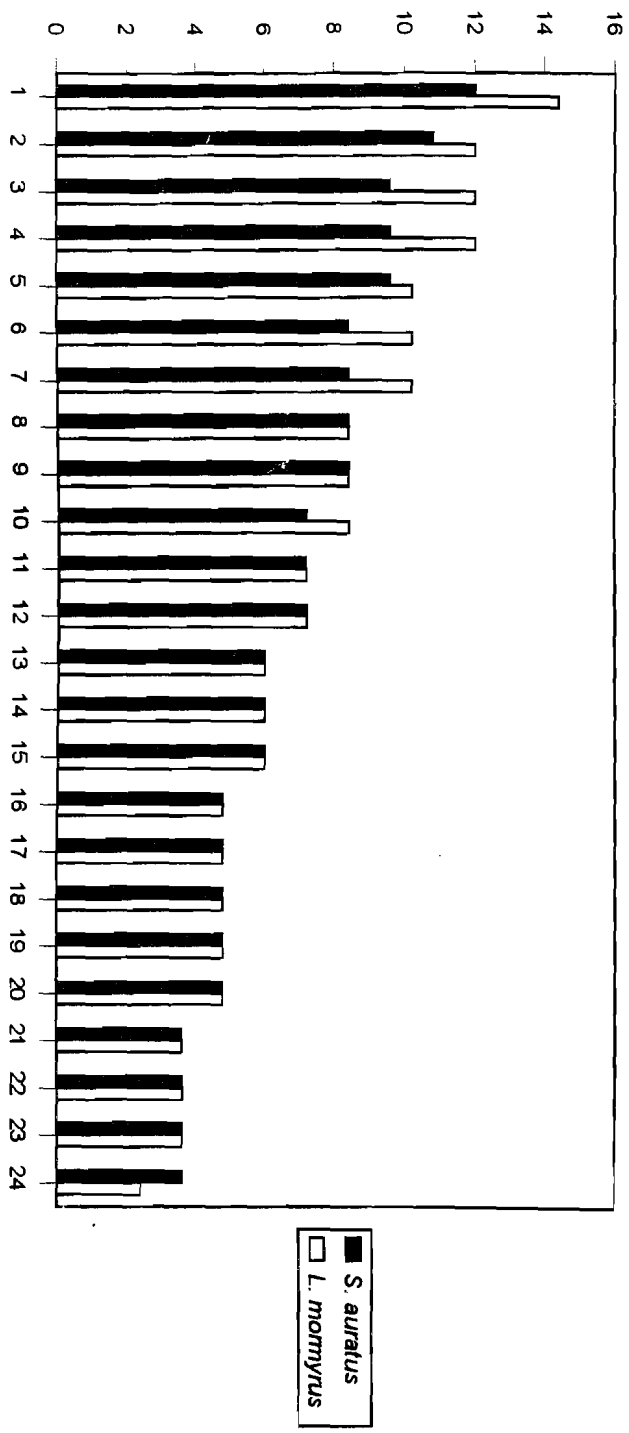


Figure (5): Comparative Idiogram of *Sparus auratus* and *Lithogamithis mormyrus*.

## DISCUSSION

Karyological characteristics have been used as a valuable aid to taxonomic and evolutionary studies of fishes. Very little published evidence is available about Sparidae karyotypes. A comparison of karyotypes among related fishes may emphasize chromosome number, arm number or DNA volume. Studies on a single species can cause misleading conclusions. Centromeric fusion can reduce chromosome number without an equivalent fundamental change in chromatin content. Similarly unequal reciprocal translocations can alter the arm number but not alter chromatin significantly (Booke, 1968). Polyploidy can cause marked changes that imply greater phylogenetic effects than have occurred (Ohno *et al.*, 1967).

From comparison studies between the two species it was found that both have 48 chromosomes occur in homologous pairs, but the chromosomes in *Sparus auratus* were more isobrachial than *Lithognathus mormyrus*. The number of arms FN were (58) and 10 dots or minutes; and 56 and 8 dots respectively.

Most teleost fishes have  $n=24$  chromosomes and the majority of those that do not, have a fewer number (Post, 1965 and Roberts, 1967). Therefore, reduction in chromosome number occurs more often than an increase. Vitture *et al* (1992) found that *Sparus auratus* contains FN=56, the diploid number of chromosome=48 and one pair is consistently involved in the nucleolus organization.

The morphological distribution of the karyotype of the two species is different. *S. auratus* has two pairs of chromosomes consistently involved satellite and three pairs have chromatide bridge, but *L. mormyrus* has 6 pairs consistently involved satellite and one pair has chromatide bridge.

(Whitehouse, 1973) found that the crossing over within paracentric inversion led to the production of a chromatid with two centromers (dicentric) and another with out a centromere (acentric). The acentric chromatid appeared as a chromosomal fragment. The dicentric chromatid formed a bridge joining the two chromosomes.

The differences in karyotype may be due to two mechanisms:

Changes in karyotypes with or without increasing in DNA content, by duplication of parts of chromosome segments and Polyploidization (Muramoto *et al.*, 1968, Muromata, *et al.*, 1968).

The differences in FN. and the average size of chromosomes pairs in the two species can be explained by duplication of chromosome segments or translocation or pericentric inversion.

Inter individual Robertsonian translocation occurred in *Diplodus annularis* (Sparidae) along with two other chromosome polymorphism, one being attributed to pericentric inversion and the other involving both the number and location of nucleolus organizer regions (Vitturi *et al.* 1993).

In *Sparus auratus* and *Lithognathus mormyrus* the dots or minutes may be due to duplication of chromosome segments. Also Amores *et al.* (1993) found scarce heterochromatic areas irregularly distributed and up to 4 nucleolus organizer regions in *D. bellottii* (Sparidae) by using conventional staining and Ag-NoR banding. The karyotype evolution of *D. bellottii* involved, centric fusion giving rise to a large metacentric pair and several pericentric inversions.

Garrido-Ramos *et al.* (1994) found a highly repetitive DNA sequence in a family from the genus of *Sparus auratus*. The family is composed of repeated units of 186 bp in length, and it accounts for 2% of the fish genome. The repetitive units are randomly arranged at the centromeres of all the chromosomes in this species. The repetitive sequence is AT rich 67% and is characterized by short stretches of constitutive AT base pairs and by short direct and inverted repeats. Sequence analysis of six cloned monomers of the family reveals some variation among clones at random positions and also distinguishes two subfamilies of repeats that differ in highly divergent block of 31 bp. These two subfamilies do not seem to be located in separate domains but occur together in the centromere of each chromosome pair. The presence of this repeat family in the genome of other sparidae species, some of which are relatively distant from *S. auratus*, indicated that this repetitive sequence could be an important component of the centromere in this fish family.

It is worthnoticing that sex chromosomes can not be distinguished in the two species.

It is necessary to know which genus, of those chromosomically characterized, is the more primitive, so that the direction of the chromosomal transformation can be ascertained. In fact if the mechanisms which are able to transform a metacentric into acrocentric or telocentric chromosome is definitely that of pericentric inversion in the event of Sparus-lithogonthus transformation which would the direction be.

Finally the karyological study of the two species can however offer some interesting details about the cytological aspects of micro evolution processes. Also it can be used as a species and specific differentiate between the two species.

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