Assiut Journal of Agricultural Science, 37 (4) (37-49)

CHROMOSOMAL COMPLEMENT, C-BANDING AND AG-NOR IN NILE PERCH, *Lates niloticus* (Centropomidae, Perciformes)

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Abstract: The present study was carried out on Nile perch, *Lates niloticus* to determine its standard karyotype as well as the number and localization of nucleolus organizer regions (NORs) in its chromosomes. The distribution of heterochromatin in *Lates niloticus* chromosomes was also investigated. Specimens used in this study were treated with the suitable methods for normal chromosomal analysis and banding studies in fish. The obtained results showed that the normal diploid chromosome number in mitotic cells of this species is 2n = 48. This diploid chromosome number was confirmed by studying meiotic chromosomes prepared from gonad cells of both males and females. The formula proposed for *Lates niloticus* is n = 1M + 1SM + 1 ST + 21 A with FN = 52. Comparison of karyotypes from male and female somatic cells did not reveal any specific heteromorphic sex chromosomes.

C-banding studies showed slightly stained centromeric heterochromatin in seven pairs of acrocentric chromosomes. One of these chromosomes also showed heterochromatin in telomeric region.

NOR-banding analysis revealed one Ag-NOR band located in telomeric region of the long arm of one large-sized telocentric chromosome pair. No differences were detected between males and females in the distribution of the C-heterochromatin and NORs banding pattern.

Key words: *Lates niloticus*, Standard karyotype, C-banding, NORs banding

Introduction

Karyological studies in fish provide basic information on the number, size and morphology of chromosomes which characterize a specific species, and is important to undertake chromosome manipulation in fish (Uribe-Alcocer *et al.*, 1999; Khan *et al.*, 2000). Moreover, karyotype analysis helps to predict, with considerable certainty, the fertility or sterility of hybrids by comprising the number and morphology of the chromosomes of parental species (Serebryakova, 1972). The Nile perch (*Lates niloticus*) is a species of freshwater fish of order Perciformes, family Centropomidae. It is widespread through much of the Afrotropic ecozone, being native to Nile and other river basins. It also

occurs in the brackish water of Lake Maryut in Egypt and represents one of the well marketable fish species in Egypt (Bishai *et al*., 2000).

In spite of the importance of *Lates niloticus* as one of the well marketable fish species, no karyotypic information regarding it has been reported in the literature. Therefore, the present work was conducted to investigate: the standard chromosome complement of *Lates niloticus*, in addition to the number and localization of NORs and distribution of heterochromatin in chromosomes of this species.

Materials and Methods

The experimental materials used in the present study were ten adult specimens, of *Lates niloticus* including males and females obtained from Lake Nasser, Aswan, Egypt during summer, 2004.

Specimens were intraperitonealy injected with colchicine solution at a dose of 0.02 mg/g body weight for 2-3 hours. Fish were sacrificed by decapitation and their sex was determined by histological analysis of gonads. Then, kidney, intestine, gills and parts of gonads were removed and sliced into 0.5 cm segments for hypotonic treatment (45 to 60 min. in 56 % KCl at 25°C). Tissues were removed from hypotonic solution and fixed in cold freshly prepared Carnoy's fixative, then additional fixative was replaced twice with fresh Carnoy's for 30 min.. Slides were prepared according to the method of solid tissue technique (Kligerman and Bloom, 1977). For conventional karyotype, slides were stained with Giemsa for 20-30 min.

For C-banding, old (3-4 weeks) air dried chromosome preparations were stained according to Sumner (1972) with few modifications. Briefly, the slides were treated with 0.2 N H Cl for 15-20 min at 37°C, rinsed with distilled water and placed in a freshly prepared 5 % aqueous solution of barium hydroxid octahydrate ($Ba(OH)_2$) at 37°C for 5-10 min. After thorough rinsing in several changes of distilled water, slides were incubated in 2X SSC (0.3 M sodium chloride containing 0.03 M tri-sodium citrate) for 60-75 min. at 60 °C. Slides were then stained for 30-60 min. in 20 % Giemsa.

For NOR-banding, slides were stained according to Howell and Black (1980) procedure with minor modifications. Two drops of colloidal developer plus four drops of 50 % silver nitrate solution were pipetted onto the surface of the slide. The slides were placed on a slide warmer 56°C. Within 30 sec., the silver-staining mixture will turn yellow, and within 3 min. it will become golden-brown. The cover slides were removed by swirling the slide in distilled water and air dried.

Metaphases were examined using a bright field Olympus microscope

and photographs of suitable mitotic metaphases were taken on Kodak color film (ASA 200, Kodak Limited, England) at a total magnification of 1500X. Length features of chromosomes (the total length of chromosome, the length of long and short arms and the relative length in relation to the total haploid length) were recorded. The Excel application paired up all the chromosomes using criteria of maximum resemblance based on the total length and the centromere position. These chromosome measurements were made on best ten chosen metaphase spreads using the computer application Micro-Measure version 3.3.

Chromosome pairs were classified following the recommendations of Levan *et al*. (1964). Chromosome arm number (FN) was determined considering M/Sm chromosomes to have two arms and St/A chromosomes to have one arm.

Results

a. Karyotype studies:

65.58 % of all examined mitotic cells from different tissues of both sexes were found to possess 48 chromosomes. Only 0.97 % of the cells showed hyperdiploidy (i.e. 48 chromosomes) while 33.45 % of the cells had a hypodiploidy chromosome numbers (i.e. < 48) (Table 1). These results suggested that the diploid chromosome number in *Lates niloticus* is 48. In order to confirm this conclusion, meiotic chromosomes prepared from gonad cells of both males and females were examined. Table 2 presents the distribution of bivalents in metaphase-1 cells of *Lates niloticus*. From the data presented in the table, it is evident that 67.33 % of metaphase figures posses 24 bivalents. These results, taken together with that obtained for the mitotic cells, confirm that the diploid chromosome number of *Lates niloticus* is 48.

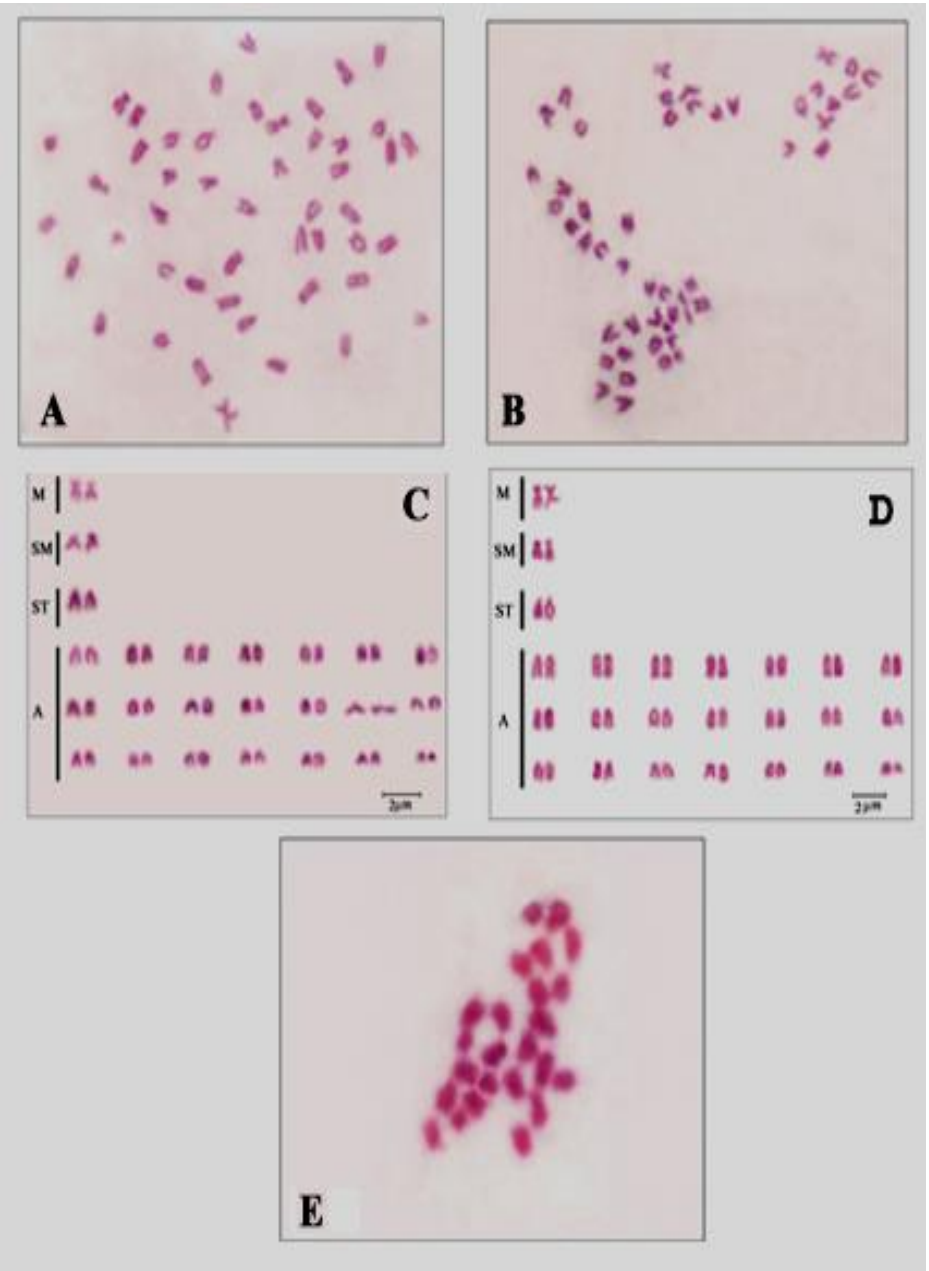
The karyotypes of male and female *lates niloticus* (figures 1 c and 1d) revealed 24 pairs of chromosomes all of which are homomorphic, i.e. no heteromorphic pairs were observed in both sexes. The first three chromosome pairs could be distinguished from the rest of the chromosomes by being metacentric, submetacentric and subtelocentric respectively. The size of chromosomes varied from 0.387 to 1.025µ m (Table 3).

Table(1): Distribution of diploid chromosome number in *Lates niloticus*

Fish No.	Sex	Number of cells with chromosomes number						Total
		45	46	47	48	49	50	
1	Male	5	5	2	20	-	-	32
2	Male	3	2	4	19	-	1	29
3	Male	4	6	1	22	-	-	33
4	Male	5	5	1	19	-	-	30
5	Male	5	4	1	22	1	-	33
6	Female	4	2	2	21	1	-	30
7	Female	4	5	2	20	-	-	31
8	Female	5	1	3	21	-	-	30
9	Female	3	3	3	22	-	-	31
10	Female	8	5	-	16	-	-	29
Total		46	38	19	202	2	1	308
%		14.94	12.34	6.17	65.58	0.65	0.32	100

Table (2): Distribution of bivalents in metaphase-I cells of *Lates niloticus*:

Fish No.	Sex	Number of metaphase-I cells showing bivalent numbers						Total
		21	22	23	24	25	26	
1	Male	4	2	1	22	-	-	29
2	Male	8	3	-	19	-	-	30
3	Male	7	1	1	18	1	-	28
4	Male	4	6	1	18	-	1	30
5	Male	4	5	-	22	-	-	31
6	Female	5	1	4	21	-	-	31
7	Female	4	4	2	21	-	-	31
8	Female	6	2	3	18	-	1	30
9	Female	4	4	1	21	-	-	30
10	Female	2	2	4	22	-	-	30
Total		48	30	17	202	1	2	300
%		16	10	5.67	67.33	0.33	0.67	100



Figure(1): A mitotic metaphase spreads, (2n = 48) from (A) male, (B) female of *L. niloticus*, karyograme from a male (C) and a female (D) and meiotic chromosomes, (n = 24) from a male (E).

The ideogram produced from the data presented in Table (3) is shown in Figure (2) which reflects the representative karyotype for *Lates niloticus*. The karyotype formula proposed for *Lates niloticus* is n = 1 M + 1 SM + 1 ST + 21 A, FN =52.

Figure (2): Idiograme of *L. niloticus* karyotype

b. C-banding:

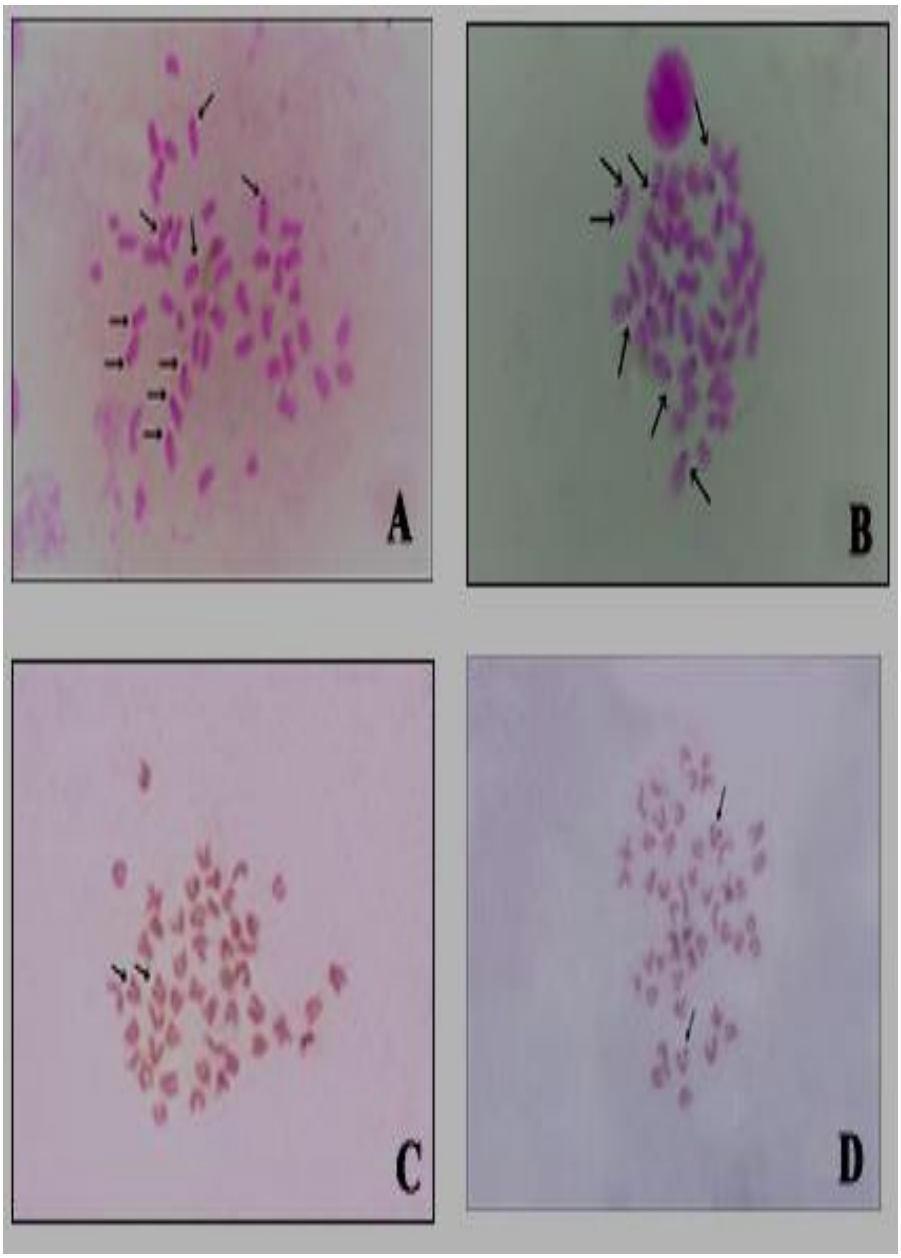
The constitutive heterochromatin, revealed by C-banding (Fig 3 a and b), was localized in and around centromeric regions of seven pairs of acrocentric chromosomes (i.e. chromosomes 4, 5, 6, 12, 13, 15 and 16). One pair of the acrocentric chromosomes (chromosome no. 15) showed an identifiable C-band in telomeric region of the long arm.

c. Ag-NORs:

As indicated in Table (4) and Fig (4 c and d) there was only one telocentric pair of chromosomes which had intensive silver staining region at telomeres (arrow) i.e. the location of NOR is terminal.

Table (4): chromosomal location of NORs in *Lates niloticus*:

Fish No.	Sex	No. of Cells examined	No. of NORs Chromosome pairs
1	Male	23	1
2	Male	19	1
3	Male	13	1
4	Female	29	1
5	Female	27	1
Total		111	



Figure(3): C-band stained metaphases of a male (A) and a female (B) and a Silver-staining metaphases of a male (C) and a female (D) of *L. niloticus*

Discussion

A karyotype consisting of 2n = 48 rod-like chromosomes has been suggested by many authors to constitute the primitive condition in fish (Ohno *et al*., 1968). Manna and Khuda-Bukhsh (1977) found that about 262 species belonging to 53 families out of 108 families studied had 2n = 48 chromosomes. So, the modal number in fishes could reasonably be described as 2n = 48. The cytogenetic data currently available for marine Perciformes indicate a high degree of chromosomal conservation in which a large number of species show only minor deviations in the chromosomal organization and fundamental number. Almost all the species of Perciformes that have been analyzed cytogenetically have 2n = 48, with fundamental numbers varying between 48 and 92 (Khuda-Bukhsh 1979, Carey and Mather 1999, Duran-Gonzalez *et al*. 1990, Caputo *et al*. 2001 and Molina and Galetti 2004 a and b). A karyotype with 2n = 48 chromosomes and FN = 48 is considered ancestral in this group (Ohno, 1974) and has been observed in 211 of the 660 Perciformes species analyzed so far (Klinkhardt *et al*., 1995).

The present study revealed that *Lates niloticus* has 2n = 48 chromosomes and FN = 52. This represents the first report of *Lates niloticus* karyotype which coincide with the ancestral karyotype in the number of chromosomes. *Lates niloticus* has 1 metacentric (number 1), 1 submetacentric (number 2), 1 subtelocentric (number 3) and 21 acrocentric (number 4 to 24) chromosome pairs. No heteromorphic sex chromosomes were detected. According to the classification proposed by Thompson (1979) based on the number of biarmed chromosomes, the karyotype of *Lates niloticus* is type "A" since it has less than five meta-submetacentric chromosomes, differing from karyotype "B" that has five or more meta-submetacentric chromosome pairs.

Most of Perciformes studied have shown a small heterochromatic content (Molina, 2000). In this group, the heterochromatin is restricted to the centromeric and pericentromeric regions and apparently has a reduced influence in the process of karyotypic differentiation (Molina and Galetti, 2004 a).

In the present investigation, C-banding pattern obtained for *Lates niloticus* show constitutive heterochromatin in centromeric regions of seven pairs of chromosomes, in addition to heterochromatin in the telomeric region of one of them. These results are in agreement with that obtained by Salvadori *et al*., (2003) who found that heterochromatin was localized in all the centromeres and in the short arm of pair 3 of *Lepomis*

gibbosus (Perciformes, Centrachidae). On the other hand, the constitutive heterochromatin pattern obtained by Brinn *et al*., (2004) for *Cichla monoculus* and *C. temensis* (Perciformes, Cichlidae) and their hybrid, showed heterochromatin blocks located preferentially in the pericentromeric region of all chromosomes in addition to an interstitial C-band on the largest chromosome pair. However, a faint distal C-band was also found in the NOR sites of *C. monoculus*, *C. temensis* and Cichla hybrids.

Nucleolus organizer regions (NOR´s) can be identified on metaphase chromosomes with the selective silver staining procedure first described by Goodpasture and Bloom (1975). NOR´s of fish chromosomes have been investigated in several species (Foresti *et al*., 1981; Uwa and Ojima, 1981; Amemiya and Gold 1988 and 1990) and they usually appear to be located near the telomeric regions of satellite chromosomes except those of *Fandulus diaphanus* (Howell and Black, 1979), which are located on the secondary constrictions of the sex chromosomes, and those of *Carassius auratus langsdorfii* (Ojima and Yamano, 1980) which are characterized by a pair of NOR´s at midpoint of the submetacentric chromosomes.

In the present study, silver staining showed that *Lates niloticus* posses a single NOR-bearing long sized telocentric pair and that NORs are terminal on the long arm.

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الهيئة الكروموسومية وحزم الهتروكروماتين ومناطق تنظيم النوية في أسماك قشر البياض

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أجريت هذه الدراسة علي سمك قشر البياض الذي يعد واحد من أهم الانواع الاقتصادية في مصر وقد استهدفت هذه الدراسة بيان الطراز الكروموسومي القياسي لهذه الاسماك بالاضافة الي دراسة توزيع الهتروكروماتين في كروموسومات هذه الاسماك فضلا عن تحديد عدد ومواقع مناطق تنظيم النوية في هذه الكروموسومات. وقد تمت معاملة الافراد المستخدمة في الدراسة والتي تم تجميعها من بحيرة ناصر بالطرق و الاساليب المناسبة للوصول الي الاهداف المشار اليها سابقا وقد اوضحت النتائج المتحصل عليها ان العدد الكروموسومي الثنائي (2 n) في هذه الاسماك هو 48 كروموسوم سواء في الذكور او في الاناث تنتظم في 24 زوج من ازواج الكروموسومات النظيرة وقد تم تاكيد هذا العدد من دراسة خلايا الانقسام الميوزي حيث احتوت غالبية الخلايا التي تم فحصها علي 24 وحدة ثنائية الكروموسوم. وقد توزعت هذه الـ 24 زوج من الكروموسومات والتي تشكل الهيئة الكروموسومية لهذه الاسماك الي زوج واحد من الكروموسومات وسطي السنترومير و زوج واحد من الكروموسومات تحت وسطي السنترومير و زوج واحد من الكروموسومات تحت طرفي السنترومير بالاضافة الي 21 زوج من ازواج الكروموسومات طرفية السنترومير وكان العدد الكلي لاذرع الكروموسومات 52 ذراع. وقد اظهرت الدراسة ايضا عدم وجود فروق شكلية او مظهرية في الهيئة الكروموسومية السابق الاشارة اليها بين الذكور والاناث مما يشير الي عدم وجود كروموسومات جنس مميزة شكليا بين الذكور و الاناث.

و قد اظهرت حزم الـ C اجزاء هتروكروماتينية في المناطق القريبة من السنترومير في سبعة ازواج من الكروموسومات طرفية السنترومير وقد احتوي احد هذه الكروموسومات طرفية السنترومير علي اجزاء هتروكروماتينية في نهاية الذراع الطويل و قد اظهر صبغ مناطق تنظيم النوية باستخدام نترات الفضة وجود زوج واحد من الكروموسومات الكبيرة طرفي السنترومير يحتوي علي منطقة تنظيم النوية في المنطقة الطرفية من الذراع الطويل ولم يلاحظ وجود اي اختلافات بين الذكور و الاناث في مناطق توزيع الهتروكروماتين او عدد اومواقع مناطق تنظيم النوية.