

แคโรไทป์ของปลากระบอกดำ [*Chelon subviridis* (Valenciennes, 1836)]

โดยเทคนิคการย้อมสีแบบธรรมดาและแถบสีแบบนอร์

Karyotype of Greenback Mullet [*Chelon subviridis* (Valenciennes, 1836)]

by Conventional Staining and Ag-NOR Banding Techniques

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บทคัดย่อ

การศึกษาพันธุศาสตร์เซลล์ครั้งแรกในปลากระบอกดำ [*Chelon subviridis* (Valenciennes, 1836)] โดยใช้ตัวอย่างเพศผู้และเพศเมียเพศละ 5 ตัว จากศูนย์วิจัยและพัฒนาประมงชายฝั่งภูเก็ต เตรียมโครโมโซมโดยวิธีทางตรงจากไต ย้อมสีโครโมโซมแบบธรรมดาและแถบสีแบบนอร์ พบว่าปลากระบอกดำมีจำนวนโครโมโซมดิพลอยด์ ($2n$) เท่ากับ 48 แท่ง มีจำนวนโครโมโซมพื้นฐาน (NF) เท่ากับ 48 ทั้งในเพศผู้และเพศเมีย และเป็นโครโมโซมเทโลเซนทริกทั้งหมด พบตำแหน่งนอร์ (nucleolar organizer regions, NORs) บริเวณเพริเซนโทรเมียร์ของโครโมโซมคู่ที่ 1 และไม่พบความแตกต่างของรูปร่างโครโมโซมเพศ ปลากระบอกดำมีสูตรแคโรไทป์ ดังนี้ $2n (48) = L_{24}^t + M_{18}^t + S_6^t$

คำสำคัญ: ปลากระบอกดำ แคโรไทป์ อิดิโอแกรม

Abstract

The first cytogenetics of greenback mullet [*Chelon subviridis* (Valenciennes, 1836)] from Phuket Coastal Fisheries Research and Development, Phuket province, Thailand, were studied. Renal cells of five males and five females *C. subviridis* (Valenciennes, 1836) were used to prepare mitotic chromosome by direct method. Conventional staining and Ag-NOR banding techniques were applied to stain the chromosomes. The results indicated that the diploid chromosome number of *C. subviridis* (Valenciennes, 1836) was $2n=48$ and the fundamental number (NF) was 48 in both males and females. All chromosomes were telocentrics. NOR bands were localized to pericentromeric region of chromosome pair 1. No heteromorphic sex-chromosome was observed between male and female chromosome. The karyotype formula of *C. subviridis* (Valenciennes, 1836) was $2n (48) = L_{24}^t + M_{18}^t + S_6^t$

Keywords: Greenback Mullet [*Chelon subviridis* (Valenciennes, 1836)], Karyotype, Idiogram

Introduction

The greenback mullet fish, *Chelon subviridis*, (Figure 1) is a member of the class Actinopterygii, order Mugiliformes and family Mugilidae. Mugilidae is a single family in the order Mugiliformes. Visual characteristics and habitat of *C. subviridis* are distinguished by greenish back, gelatinous membrane partially covering eye, 27-32 scales in lateral line, lacks an enlarged pointed scale at upper pectoral fin base, also tail narrowly dark-edged, found throughout the region of Indo-Chinese Pacific, and its inhabits are coastal waters including estuaries (Allen *et al.*, 1999).

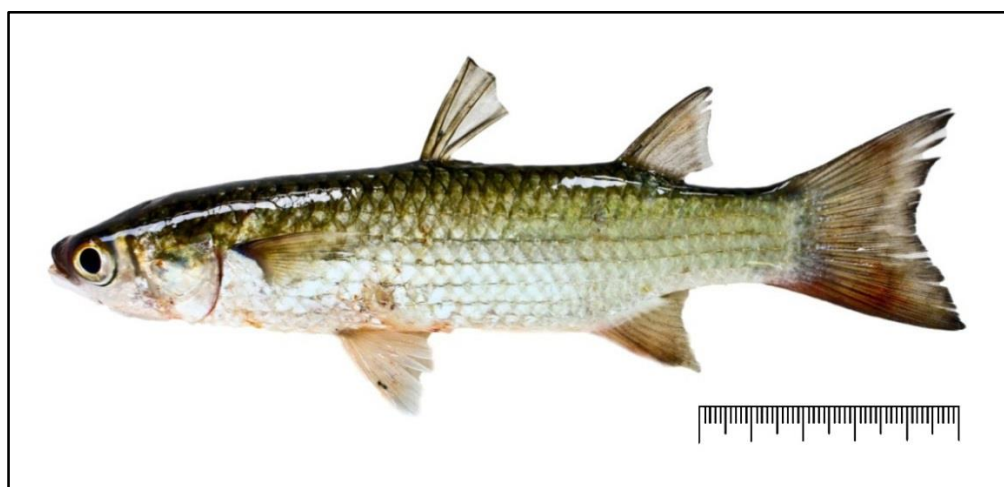


Figure 1 General characteristic of the greenback mullet, *Chelon subviridis* (Mugilidae, Mugiliformes) from Phuket marine, Thailand (Scale bar = 5 cm).

To date chromosome number of 18 mullets fish species have been cytogenetically reported from 24 to 48 chromosomes including the *Agonostomus monticola*, $2n=48$ (Nirchio *et al.*, 2009); *Chelon labrosus*, $2n=48$ (Cataudella and Capanna, 1973); *L. aurata*, $2n=48$ (Cataudella *et al.*, 1974); *L. haematocheila*, $2n=48$ (Yu *et al.*, 1995); *L. parsia*, $2n=48$ (Khuda-Bukhsh and Manna, 1976); *L. ramada*, $2n=48$ (Cataudella and Capanna, 1973); *L. saliens*, $2n=48$ (Cataudella *et al.*, 1974; Gornung *et al.*, 2001); *M. cephalus*, $2n=48$ (Lakra and Krishna, 1995; Rossi *et al.*, 1996); *M. curema*, $2n=28$ (LeGrande and Fitzsimons, 1976; Nirchio *et al.*, 2005a) and $2n=24$ (Nirchio *et al.*, 2003; Nirchio *et al.*, 2005a); *M. gaimardianus*, $2n=48$ (Nirchio *et al.*, 2003); *M. liza*, $2n=48$ (Rossi *et al.*, 2005); *M. platanus*, $2n=48$ (Jordao *et al.*, 1992); *M. rubrioculus*, $2n=48$ (Nirchio *et al.*, 2007); *M. trichodon*, $2n=48$ (Nirchio *et al.*, 2005b); *Oedalechilus labeo*, $2n=48$ (Cataudella *et al.*, 1974); *Paramugil parmatus*, $2n=48$ (Choudhury *et al.*, 1979); *Rhinomugil corsula*, $2n=48$ (Nayak and Khuda-Bukhsh, 1991) and *Valamugil speigleri*, $2n=48$ (Rishi and Singh, 1982).

In case of dyeing with the conventional staining technique, the Giemsa's stain always stains on histone protein area, which scatters around the chromosome; so, the whole piece of chromosome can be visible with the Giemsa's stain; on the other hand, dyeing with the NOR banding technique with silver nitrate, the dye always stains on non-histone area, which is the position of NOR or the secondary constriction (Campiranon, 2003).

Materials and Methods

C. subviridis (five males and five females) were obtained from the Phuket Coastal Fisheries Research and Development, Phuket province, Thailand. The fish were transferred to laboratory aquaria and were kept under standard condition for seven days prior to the experimentations. Chromosome preparation was conducted by the colchicine-hypotonic-fixation-air drying technique, from kidney. The chromosomes were stained with 20% Giemsa's for 30 min and NORs were identified through Ag-NOR staining (Howell and Black, 1980). Many pictures of the chromosomes in metaphase cells were taken for counting the number of chromosomes. The length of short arm (Ls) and long arm (Ll) chromosomes were measured and calculated for the length of total arm chromosomes (LT, $LT=Ls+Ll$). Relative length (RL) and centromeric index (CI) were also calculated. CI was also computed to classify the types of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiogramming. In karyotype formula, classify the size of chromosome, which the symbol 'L' represents a large-size chromosome, the symbol 'M' represents a medium-size chromosome and the symbol 'S' represents a small-size chromosome. Classify the type of the chromosome, which the symbol 'm' represents metacentric chromosome, the symbol 'sm' represents submetacentric chromosome, the symbol 'a' represents acrocentric chromosome and the symbol 't' represents telocentric chromosome.

Results

The maximum frequencies of chromosome number per cell (Table 1) and the karyotype showed that the number of diploid chromosome of *C. subviridis* was $2n=48$ consisting of all telocentric chromosome, the fundamental number (NF, number of chromosome arms) was 48 in both males and females (Figure 2). The chromosome types were present as 24 large telocentric, 18 medium telocentric, and six small telocentric chromosomes. No heteromorphic sex-chromosome was observed between male and female chromosome. NOR bands were localized to pericentromeric region of chromosome pair 1 (Figure 3). The 10 metaphase cells of each male and female were measured for individual length of both short arm and long arm. The length of short arm chromosome (Ls) and

long arm chromosome (LI) were calculated for the length of total arm chromosome (LT=Ls+LI). The size and type of chromosomes were showed in the table 2. The karyotype formula can be deduced as: $2n (48) = L_{24}^t + M_{18}^t + S_6^t$

Table 1 Frequencies of chromosome number per cell's counting of greenback mullet (*C. subviridis*)

| Number of chromosome | Number of cell | |
|----------------------|----------------|------------|
| | Male (%) | Female (%) |
| < 46 | 10 (8.13) | 9 (7.76) |
| 46 | 7 (5.69) | 5 (4.31) |
| 47 | 14 (11.38) | 13 (11.21) |
| 48 | 82 (66.67) | 78 (67.24) |
| > 48 | 10 (8.13) | 11 (9.48) |
| Total cell | 123 | 116 |

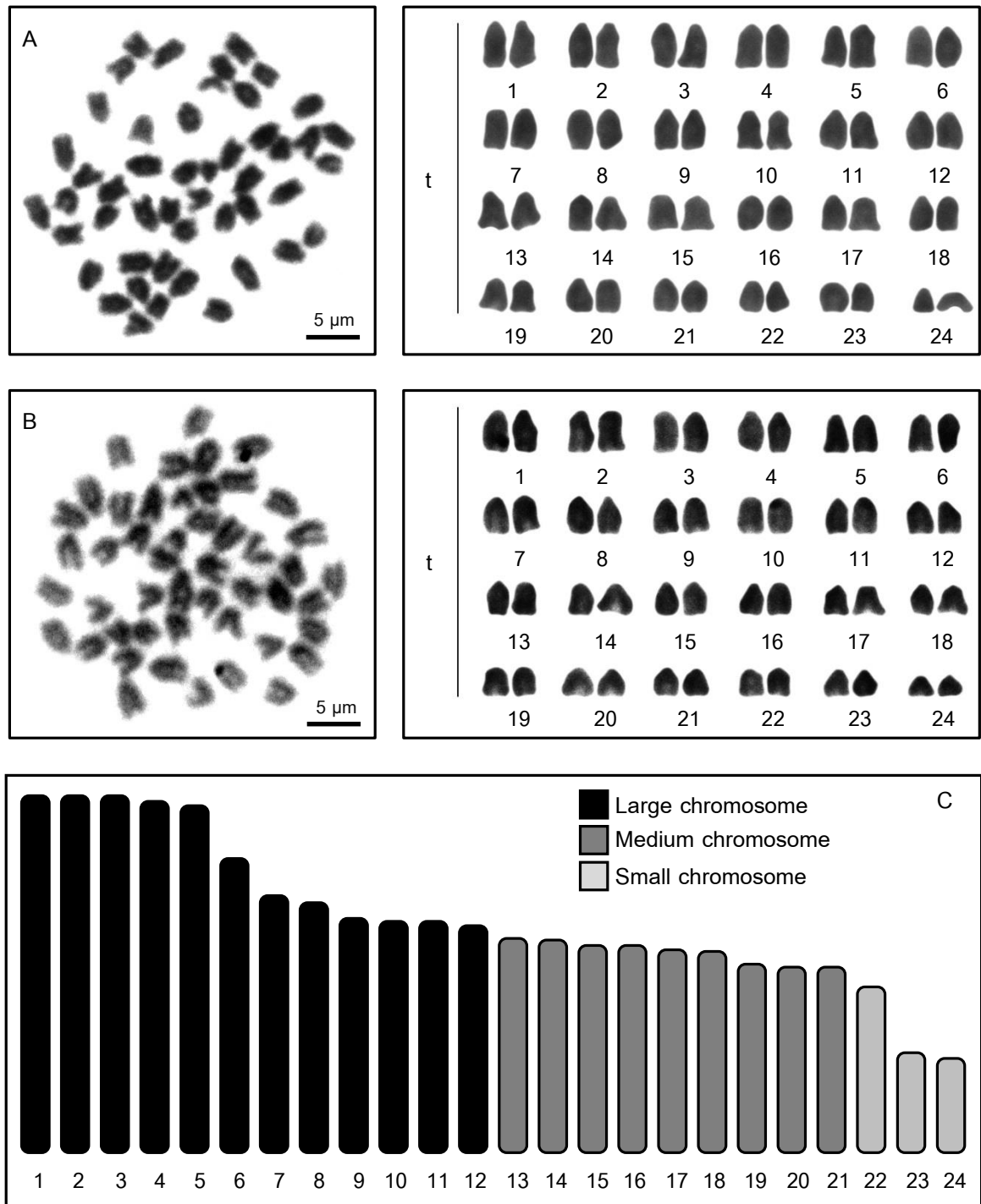


Figure 2 Metaphase chromosome plates and karyotype of male (A) and female (B), and idiogram (C) of greenback mullet (*Chelon subviridis*) $2n=48$ by conventional staining technique

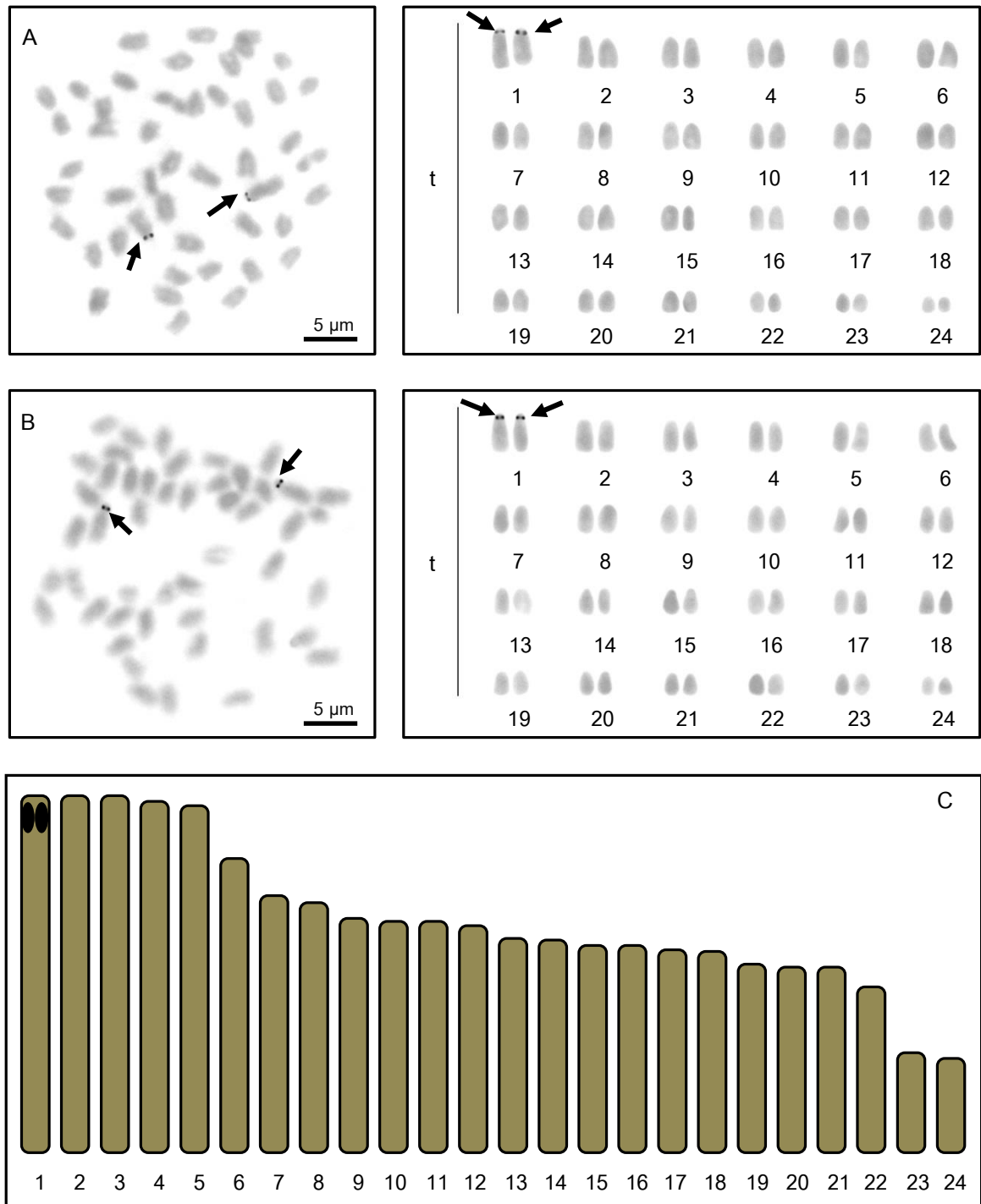


Figure 3 Metaphase chromosome plates and karyotype of male (A) and female (B), and idiogram (C) of greenback mullet (*Chelon subviridis*) $2n=48$ by NOR-banding technique (arrows indicated NOR)

Table 2 Mean length of short arm chromosome (Ls), long arm chromosome (LI), total arm chromosome (LT), relative length (RL) and centromeric index (CI) from 20 metaphases of male and female greenback mullet (*Chelon subviridis*), $2n=48$

| Chromosome pair | Ls | LI | LT | CI±SD | RL±SD | Chromosome size | Chromosome type |
|-----------------|-------|--------|--------|-------------|-------------|-----------------|-----------------|
| 1* | 0.000 | 17.009 | 17.009 | 1.000±0.000 | 0.063±0.020 | Large | Telocentric |
| 2 | 0.000 | 17.004 | 17.004 | 1.000±0.000 | 0.063±0.008 | Large | Telocentric |
| 3 | 0.000 | 16.987 | 16.987 | 1.000±0.000 | 0.063±0.008 | Large | Telocentric |
| 4 | 0.000 | 16.741 | 16.741 | 1.000±0.000 | 0.062±0.007 | Large | Telocentric |
| 5 | 0.000 | 16.528 | 16.528 | 1.000±0.000 | 0.061±0.010 | Large | Telocentric |
| 6 | 0.000 | 14.006 | 14.006 | 1.000±0.000 | 0.052±0.010 | Large | Telocentric |
| 7 | 0.000 | 12.197 | 12.197 | 1.000±0.000 | 0.045±0.009 | Large | Telocentric |
| 8 | 0.000 | 11.900 | 11.900 | 1.000±0.000 | 0.044±0.008 | Large | Telocentric |
| 9 | 0.000 | 11.173 | 11.173 | 1.000±0.000 | 0.042±0.007 | Large | Telocentric |
| 10 | 0.000 | 11.017 | 11.017 | 1.000±0.000 | 0.041±0.006 | Large | Telocentric |
| 11 | 0.000 | 11.011 | 11.011 | 1.000±0.000 | 0.041±0.007 | Large | Telocentric |
| 12 | 0.000 | 10.833 | 10.833 | 1.000±0.000 | 0.04±0.008 | Large | Telocentric |
| 13 | 0.000 | 10.196 | 10.196 | 1.000±0.000 | 0.038±0.007 | Medium | Telocentric |
| 14 | 0.000 | 10.100 | 10.100 | 1.000±0.000 | 0.037±0.007 | Medium | Telocentric |
| 15 | 0.000 | 9.859 | 9.859 | 1.000±0.000 | 0.037±0.008 | Medium | Telocentric |
| 16 | 0.000 | 9.850 | 9.850 | 1.000±0.000 | 0.037±0.006 | Medium | Telocentric |
| 17 | 0.000 | 9.614 | 9.614 | 1.000±0.000 | 0.036±0.007 | Medium | Telocentric |
| 18 | 0.000 | 9.600 | 9.600 | 1.000±0.000 | 0.035±0.008 | Medium | Telocentric |
| 19 | 0.000 | 8.978 | 8.978 | 1.000±0.000 | 0.033±0.009 | Medium | Telocentric |
| 20 | 0.000 | 8.836 | 8.836 | 1.000±0.000 | 0.033±0.009 | Medium | Telocentric |
| 21 | 0.000 | 8.800 | 8.800 | 1.000±0.000 | 0.032±0.010 | Medium | Telocentric |
| 22 | 0.000 | 7.899 | 7.899 | 1.000±0.000 | 0.028±0.009 | Small | Telocentric |
| 23 | 0.000 | 4.749 | 4.749 | 1.000±0.000 | 0.021±0.007 | Small | Telocentric |
| 24 | 0.000 | 4.450 | 4.450 | 1.000±0.000 | 0.016±0.007 | Small | Telocentric |

Remark: * = NOR-bearing chromosomes.

Discussion

The present result obtained here is in agreement with the previous reports (Cataudella and Capanna, 1973) in the number of diploid chromosome of genus *Chelon* was $2n=48$. However, our result shows a contrary in the type of chromosome and fundamental number with the reports of *C. labrosus* (Cataudella and Capanna, 1973).

Table 3 Cytogenetic publications of mullets fish (Mugiliformes, Mugilidae).

| Species | 2n | NF | Karyotype formula | NORs number | Reference |
|------------------------------|-----------|-----------|----------------------|----------------|--|
| <i>Agonostomus monticola</i> | 48 | 50 | 2a+46t | 2 | Nirchio <i>et al.</i> (2009) |
| <i>Chelon labrosus</i> | 48 | 50 | 2a+46t | - | Cataudella and Capanna (1973) |
| <i>C. subviridis</i> | 48 | 48 | 48t | 2 | Present study |
| <i>Liza aurata</i> | 48 | 50 | 2a+46t | - | Cataudella <i>et al.</i> (1974) |
| <i>L. haematocheila</i> | 48 | 48 | 48t | - | Yu <i>et al.</i> (1995) |
| <i>L. parsia</i> | 48 | 48 | 48t | - | Khuda-Bukhsh and Manna (1976) |
| <i>L. ramada</i> | 48 | 50 | 2a+46t | - | Cataudella and Capanna (1973) |
| <i>L. saliens</i> | 48 | 50 | 2a+46t | 2 | Cataudella <i>et al.</i> (1974); Gornung <i>et al.</i> (2001) |
| <i>Mugil cephalus</i> | 48 | 48 | 48t | - | Lakra and Krishna (1995) |
| | 48 | 48 | 48t | 1-2 | Rossi <i>et al.</i> (1996) |
| <i>M. curema</i> | 28 | 52 | 20m+4a+4t | - | LeGrande and Fitzsimons (1976) |
| | 28 | 52 | 20m+4a+4t | 2 | Nirchio <i>et al.</i> (2005a) |
| | 24 | 48 | 22m+2sm | 2 | Nirchio <i>et al.</i> (2003); Nirchio <i>et al.</i> (2005a) |
| <i>M. gaimardianus</i> | 48 | 48 | 48t | - | Nirchio <i>et al.</i> (2003) |
| <i>M. liza</i> | 48 | 48 | 48t | 2 | Rossi <i>et al.</i> (2005) |
| <i>M. platanus</i> | 48 | 48 | 48t | 2 | Jordao <i>et al.</i> (1992) |
| <i>M. rubrioculus</i> | 48 | 48 | 48t | 2 | Nirchio <i>et al.</i> (2007) |
| <i>M. trichodon</i> | 48 | 48 | 48t | 2 | Nirchio <i>et al.</i> (2005b) |
| <i>Oedalechilus labeo</i> | 48 | 50 | 2a+46t | 2 | Cataudella <i>et al.</i> (1974) |
| <i>Paramugil parmatus</i> | 48 | 48 | 48t | - | Choudhury <i>et al.</i> (1979) |
| <i>Rhinomugil corsula</i> | 48 | 48 | 48t | - | Nayak and Khuda-Bukhsh (1991) |
| <i>Valamugil speigleri</i> | 48 | 48 | 48t | - | Rishi and Singh (1982) |

Remark: 2n = diploid chromosome, NF = fundamental number, m = metacentric chromosome, sm = submetacentric chromosome, a = acrocentric chromosome, t = telocentric chromosome, and - = not available.

Previous studies of mullets's karyotypes revealed that most of them have the number of diploid chromosome of 48, while their fundamental numbers are seem to be similar. The *M. curema* has 2n=24 (Nirchio *et al.*, 2003; Nirchio *et al.*, 2005), this is the minimal chromosome number of the family Mugilidae while majority of the members of the family Mugilidae have 2n=48 (Cataudella and Capanna, 1973; Cataudella *et al.*, 1974; Khuda-Bukhsh and Manna, 1976; Choudhury *et al.*, 1979; Rishi and Singh, 1982; Nayak and Khuda-Bukhsh, 1991; Jordao *et al.*, 1992; Lakra and Krishna, 1995; Yu *et al.*, 1995; Rossi *et al.*, 1996; Gornung *et al.*, 2001; Nirchio *et al.*, 2003; Rossi *et al.*, 2005; Nirchio *et al.*, 2005b; Nirchio *et al.*, 2007; Nirchio *et al.*, 2009). This karyotype information described above

indicates that members of the family Mugilidae have a close chromosome evolutionary. To the present study, the karyotype of *C. subviridis* shows much consistent characteristics to the others members in the same family (table 3).

According to the type of karyotype of the fish that has been brought to this study, it was in conformity with the most of marine fishes, which the diploid number of chromosome contains 48 chromosomes, and $NF = 48$, which expresses the high conservative character of karyotype in Marine fishes, as a result of the nature of the habitat, due to the sea water has connected as the continents; therefore, it causes of gene flow at all times; while evolution of the character of karyotype has rather a little change.

This cytogenetic knowledge, a report on karyotype analysis and chromosomal characteristic of the nucleolar organizer regions (NORs) of *C. subviridis*, is the first record. Our knowledge is advantage cytogenetic information for further study on taxonomy and evolutionary relationship. Moreover, this provides useful basic information for genetic studying in the future.

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