



## การวิเคราะห์ลักษณะแคริโอไทป์และตำแหน่งนอร์ (NORs) ของปลาสดหิน

### *Neoglyphidodon nigroris*

## Karyological Analysis and Nucleolar Organizer Region of Benh's damselfish,

### *Neoglyphidodon nigroris* (Perciformes, Pomacentridae)

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#### บทคัดย่อ

ศึกษาลักษณะแคริโอไทป์และตำแหน่งนอร์ของปลาสดหิน *Neoglyphidodon nigrolis* จากทะเลอันดามัน ภาคใต้ของประเทศไทย ด้วยเทคนิคการย้อมสีแบบธรรมดาและแถบสีแบบนอร์ พบว่าจำนวนโครโมโซมดิพลอยด์ ( $2n$ ) เท่ากับ 48 แห่ง มีจำนวนโครโมโซมพื้นฐาน (NF) เท่ากับ 84 แคริโอไทป์ประกอบด้วยโครโมโซมชนิดเมทาเซนทริก 8 แห่ง ซับเมทาเซนทริก 26 แห่ง อะโครเซนทริก 2 แห่ง และเทโลเซนทริก 12 แห่ง โดยส่วนใหญ่เป็นโครโมโซมชนิดสองแขน ซึ่งเกิดจากกระบวนการหักและต่อสลับโดยมีเซนโทเมียร์ร่วมด้วยหลายครั้งเกิดขึ้นภายในแคริโอไทป์ พบตำแหน่งนอร์ 1 คู่ บริเวณปลายแขนข้างสั้นของโครโมโซมชนิดอะโครเซนทริกขนาดใหญ่ คู่ที่ 18 ความรู้พื้นฐานพันธุศาสตร์เซลล์ของปลาสดหิน สามารถนำไปใช้สนับสนุนการศึกษาด้านการปรับปรุงพันธุ์ การอนุรักษ์และวิวัฒนาการของโครโมโซมในปลากลุ่มนี้ได้ สูตรแคริโอไทป์ของปลาสดหิน *N. nigrolis* คือ  $L_m^2 + L_{sm}^{10} + L_a^2 + L_t^4 + M_m^6 + M_{sm}^{16} + M_t^8$  หรือ  $8m+26sm+2a+12t$

#### ABSTRACTS

Karyotype and nucleolar organizer regions (NORs) in the pomacentrid fish *Neoglyphidodon nigrolis* were studied. The specimen collected from the Andaman Sea (Thailand). We conducted conventional cytogenetic and Ag-positive NORs analyses in *N. nigrolis*. The chromosome number was observed to be  $2n=48$ , the fundamental number was 84, and the karyotype consisted of 8 metacentric, 26 submetacentric, 2 acrocentric and 12 telocentric chromosomes with a large number of bi-armed chromosomes, where multiple pericentric inversions played a significant role in the karyotype organization. The Ag-NOR banding exhibited that a single pair of NORs was presented on telomeric regions of the short arms in a large acrocentric chromosomes pair 18. Basic knowledge on cytogenetics of damselfish would be applied for support on breeding, conservation and chromosome evolution in this fish. The karyotype formula of *N. nigrolis* is as follows:  $L_m^2 + L_{sm}^{10} + L_a^2 + L_t^4 + M_m^6 + M_{sm}^{16} + M_t^8$  or  $8m+26sm+2a+12t$

**คำสำคัญ:** ปลาสลิดหิน แครีโอไทป์ โครโมโซม อิติโอแกรม

**Keywords:** *Neoglyphidodon nigroris*, Benh's damselfish, Karyotype, Chromosome, Idiogram

## INTRODUCTION

The family Pomacentridae represents the dominant group both in number and diversity in the bulk of the coral environments. This group presents many taxonomic problems due to the species complexities and their body color patterns, which are both geographical and ontogenetically quite variable (Menezes and Figueiredo, 1985). Benh's damselfish (*Neoglyphidodon nigroris*) is one of the species that belong to the genus *Neoglyphidodon* that is a genus of fish in the family Pomacentridae. This species occasionally makes its way into the aquarium trade in Thailand as an ornamental fish. The *N. nigroris* is found throughout the Indo-Pacific region and live in coral reefs and lagoons that are rich in corals. They are found at depths of 2–21 metres. Adults' individuals of Benh's damselfish can grow up to 13 centimetres. There are two varieties of adult coloration. The adult individuals from the western Pacific Ocean have a tan front and a yellow back. In the area ranging from the Andaman Sea to Japan, adults are black. The two varieties overlap around the island of Bali. Juveniles have a yellow coloration with horizontal black stripes. Cytogenetic investigations of Pomacentridae have revealed the karyotypes of 49 species. The chromosomal locations of nucleolar organizer regions (NORs) have been reported in 35 species. These investigations showed a diverse karyotype with a wide range of chromosome numbers (28–50) and fundamental numbers (arm numbers) (48–96). Most of the species investigated have diploid complements with  $2n=48$ , eight of them having karyotypes composed exclusively of uni-armed chromosomes (Alvarez et al., 1980; Arai and Inoue, 1976; Arai et al., 1976; Molina and Galletti Jr, 2002, 2004;

Ojima and Kashiwagi, 1981; Takai and Ojima, 1987, 1991a, 1991b, 1995; Takai and Kosuga, 2007; Takai, 2011). Cytogenetic reviews of the genus *Neoglyphidodon* (Pomacentridae), there are five report in three species in genus *Neoglyphidodon*. All of them have diploid chromosome with  $2n=48$  and the fundamental numbers ranged from 70 to 88. Only one species have been reported the chromosomal locations of nucleolar organizer regions (Table 1) (Takai and Ojima, 1991a; Takai, 2011; Getlekha, 2018). These provided interesting findings that help in understanding karyotypic evolution and phylogenetic relationships. The present study deals with the karyotype and NORs in a species of Pomacentridae, *N. nigroris*.

## RESEARCH METHODOLOGY

The cytogenetic analyses were carried out on the *N. nigroris* (six male and four female) from Pacific Ocean, Southern Thailand (Fig. 1). The chromosomes were obtained from anterior kidney tissue dissociated in 7 ml of hypotonic solution (0.075M KCl) after *in vivo* colchicine treatment for 45 min. The hypotonization was 30 min at room temperature. The material was fixed with methanol/acetic acid (3:1). The slides were stained by 20% Giemsa's solutions, used for banding chromosomes. The NORs regions were visualized according to the techniques described by Howell and Black (1980). The metaphases were photographed using a light microscope with 1,000X magnification with Canon EOS 550D DSLR camera. The chromosome pairs were arranged size order and classified centromeric index (CI) between 0.50–0.59 as metacentric (m), 0.60–0.69 as submetacentric (sm), 0.70–0.89 as acrocentric (a) and 0.90–1.00 as telocentric (t) (Chaiyasut, 1989). The fundamental number (number of chromosome arm, NF)

obtained by assigning a value of two to metacentric, submetacentric and acrocentric chromosomes and one to telocentric chromosome. All parameters were used

in karyotyping (Chooseangjaew et al., 2017). The idiogram was constructed using a model drawing of karyotype and accomplished by a computer program.

**Table 1** Cytogenetic reviews of the Genus *Neoglyphidodon* (Pomacentridae).

No.	Species	2n	NF	NORs	Formula	Locality	Reference
1	<i>N. melas</i>	48	82	–	8m+26sm+14st/a	Japan	Takai and Ojima (1991)
		48	80	2	6m+26sm+16st/a	Japan	Takai (2011)
		48	88	–	8m+28sm+4a+8t	Thailand	Getlekha (2018)
2	<i>N. nigroris</i>	48	82	–	8m+26sm+14st/a	Japan	Takai and Ojima (1991)
		48	84	2	8m+26sm+2a+12t	Thailand	Present study
3	<i>N. oxyodon</i>	48	70	–	2m+20sm+16st/a	Japan	Takai and Ojima (1991)

Remarks: 2n = diploid chromosome number, NF = fundamental number (number of chromosome arm), m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric, t = telocentric chromosome, NORs = nucleolar organizer regions and – = not available.



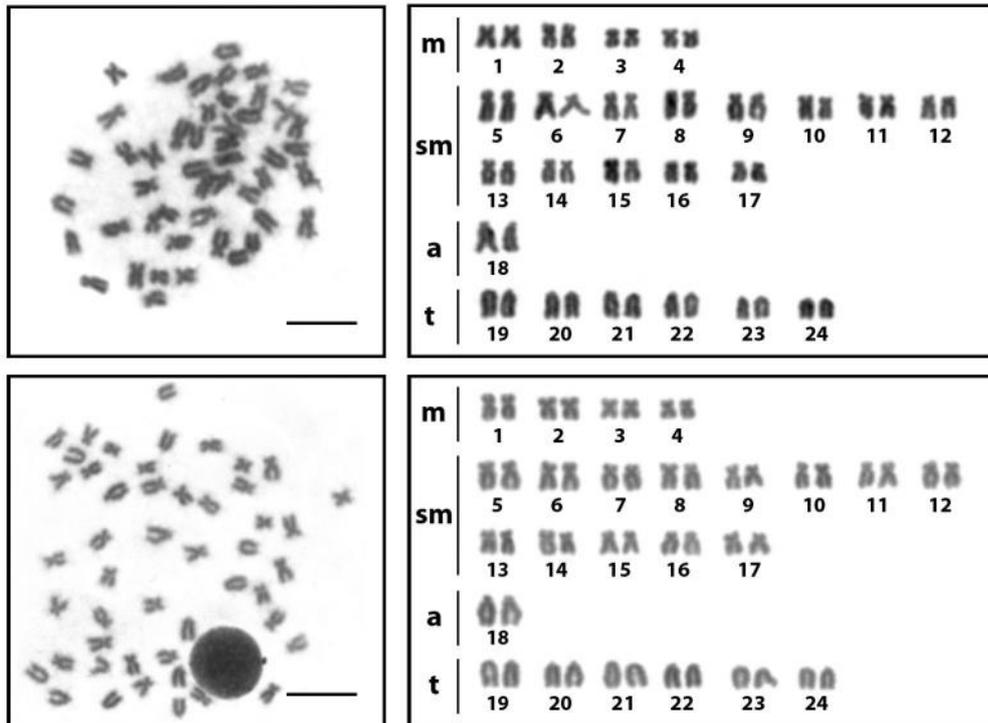
**Figure 1** General characteristic of the Benh's damselfish (*Neoglyphidodon nigroris*).

## RESULTS

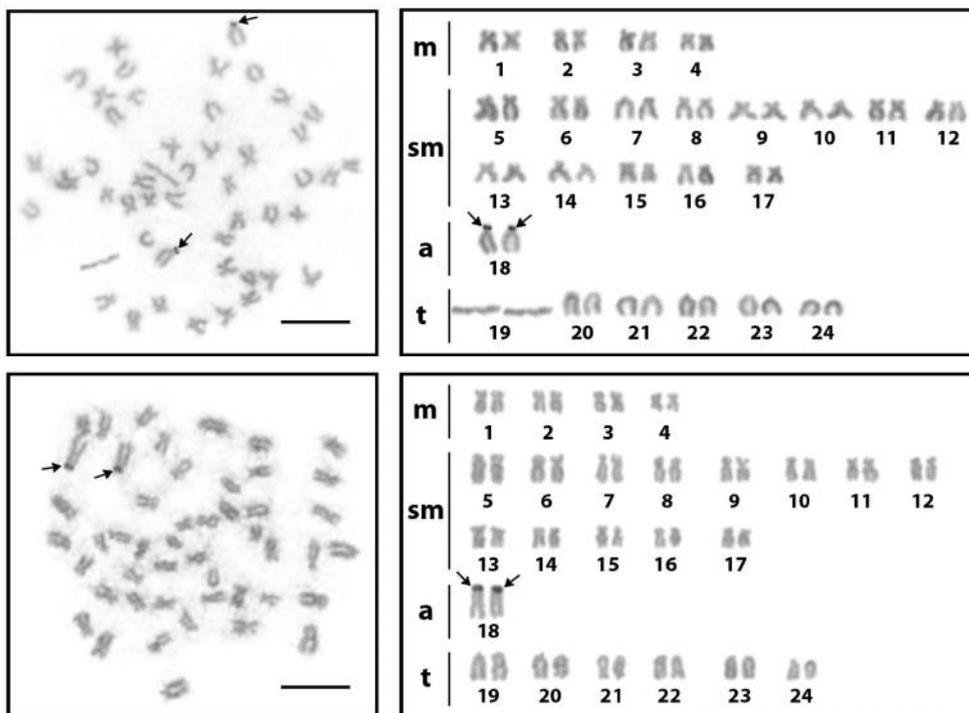
The *N. nigrolis* exhibits 2n=48, with asymmetrical karyotypes. A karyotype consisting of two large metacentric, ten large submetacentric, two large acrocentric, four large telocentric, six medium metacentric, 16 medium submetacentric and eight medium telocentric chromosomes and a fundamental number is 88 (Fig. 2). The chromosomes showed a gradual change in size. The Ag-NOR banding exhibited that a single pair of NORs was presented on telomeric regions of the short arms (telomeric NOR) large acrocentric chromosomes pair 18 (Fig. 3). No heteromorphic chromosome pair was observed. The karyotype formula of *N. nigrolis* as follow:  $L_m^2 + L_{sm}^{10} +$

$$L_a^2 + L_t^4 + M_m^6 + M_{sm}^{16} + M_t^8 \text{ or } 8m+26sm+2a+12t$$

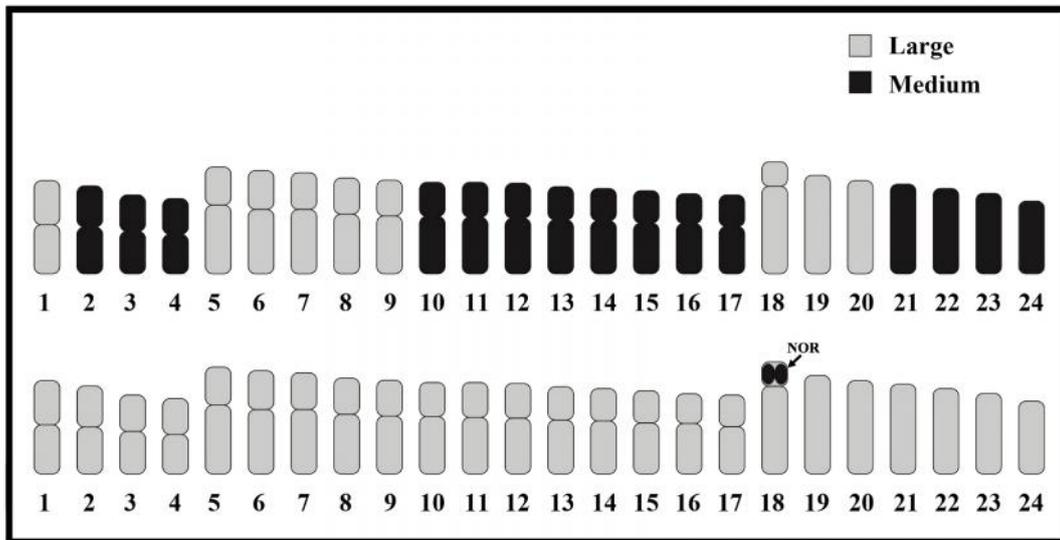
The ten metaphase cells of each male and female were measured for individual length of both short arm and long arm. The length of short arm chromosome (Ls) and long arm chromosome (Ll) were calculated to the length of total arm chromosome (LT,  $LT=Ls+Ll$ ). The size and type of chromosomes were in the Table 2. The idiogram of *N. nigrolis* shows a gradually decreasing length of the chromosomes with classify type and size of chromosome, respectively. Size of chromosomes are the large size ( $LT > 2.11 \mu\text{m}$ ), the medium size ( $2.11 > LT > 1.28 \mu\text{m}$ ) and the small size ( $LT < 1.28 \mu\text{m}$ ) (Fig. 4)



**Figure 2** Metaphase chromosome plates and karyotypes of male (A) and female (B) of the Benh's damselfish (*Neoglyphidodon nigroris*,  $2n=48$ ) by conventional staining technique (scale bars indicate  $10\ \mu\text{m}$ ).



**Figure 3** Metaphase chromosome plates of male (A) and female (B) of the Benh's damselfish (*Neoglyphidodon nigroris*,  $2n=48$ ) by Ag-NOR banding technique; scale bars indicate  $10\ \mu\text{m}$ . The telomeric regions of the short arms in a pair of large acrocentric chromosomes showed clearly observable nucleolar organizer regions/NORs (arrows).



**Figure 4** Standardized idiogram showing lengths and shapes of chromosomes of the Benh's damselfish (*Neoglyphidodon nigroris*,  $2n=48$ ) by conventional staining and Ag-NOR banding techniques. The arrow indicates nucleolar organizer regions.

## DISCUSSION

### Chromosome number, fundamental number and karyotype of *N. nigroris*

Cytogenetic analyses were conducted on the *N. nigroris* from Thailand (Indo-Pacific). The number of chromosomes ( $2n$ ) of *N. nigroris* is 48 chromosomes in all specimens which show a high number of bi-brachial chromosomes. The karyotypes consist of four pairs of metacentric, 13 pairs of submetacentric, one pair of acrocentric and six pairs of telocentric chromosomes (NF=84). The chromosomes showed a gradual change in size (Fig. 2 and Table 2). This result similar to Takai and Ojima (1991a) that analysis karyotype in *N. nigroris* from Japan (Osaka), the result shown, *N. nigroris* had  $2n=48$ , and the karyotype consisted of four pairs of metacentric, 13 pairs of submetacentric, and seven pairs of subtelo-or acrocentric chromosomes (NF=82). The differences of chromosome type and the fundamental

number of the chromosome due to the chromosomal classification system of each person that make a reported by a different types of chromosomes. These results in agreement with the data of the genus *Neoglyphidodon* in the previous studies indicated that the  $2n=48$  in *N. melas*, *N. nigroris* and *N. oxyodon* (Takai and Ojima, 1991a; Getlekha, 2018) demonstrating a highly conserved karyotype occur among these fishes, a karyotype pattern with  $2n=48$  is an ancestor of the group. In the genus *Neoglyphidodon*, the NF between 70-88 for example *N. melas* had NF=82 (Takai and Ojima, 1991a) and NF=88 (Getlekha, 2018), *N. nigroris* had NF=82 and *N. oxyodon* had NF=70 (Takai and Ojima, 1991a) display a high number of bi-arms chromosomes. They markedly differ from an ancestor group with  $2n=48$  (NF=48) However, a high NF suggests that pericentric inversion event in the chromosome rearrangement of *Neoglyphidodon*.

**Table 2** Mean length of short arm chromosome (Ls), length long arm chromosome (LL), length total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphase cells of the male and female the Benh's damselfish (*Neoglyphidodon nigroris*), 2n=48.

Chro. pair	Ls	LL	LT	CI	RL	Chro. size	Chro. type
1	1.0092	1.1219	2.1311	0.043±0.001	0.527±0.006	large	metacentric
2	0.9390	1.0717	2.0107	0.041±0.002	0.534±0.008	medium	metacentric
3	0.8268	0.9687	1.7955	0.037±0.003	0.540±0.004	medium	metacentric
4	0.8172	0.8958	1.7130	0.035±0.002	0.523±0.012	medium	metacentric
5	0.8700	1.5688	2.4388	0.050±0.002	0.644±0.022	large	submetacentric
6	0.8933	1.4708	2.3641	0.048±0.001	0.623±0.022	large	submetacentric
7	0.8366	1.4675	2.3041	0.047±0.002	0.639±0.017	large	submetacentric
8	0.8347	1.3584	2.1931	0.044±0.001	0.619±0.022	large	submetacentric
9	0.8176	1.3158	2.1334	0.043±0.001	0.616±0.027	large	submetacentric
10	0.8030	1.2731	2.0760	0.042±0.002	0.626±0.011	medium	submetacentric
11	0.7986	1.2902	2.0888	0.042±0.001	0.617±0.008	medium	submetacentric
12	0.7808	1.3147	2.0956	0.042±0.001	0.614±0.012	medium	submetacentric
13	0.7611	1.2328	1.9939	0.040±0.001	0.618±0.025	medium	submetacentric
14	0.7389	1.2132	1.9521	0.040±0.003	0.622±0.016	medium	submetacentric
15	0.7410	1.1646	1.9056	0.039±0.002	0.610±0.015	medium	submetacentric
16	0.6754	1.1489	1.8243	0.037±0.002	0.629±0.017	medium	submetacentric
17	0.7167	1.0848	1.8016	0.037±0.001	0.602±0.008	medium	submetacentric
18	0.5627	2.0046	2.5673	0.052±0.003	0.782±0.017	large	acrocentric
19	0.0000	2.2540	2.2540	0.046±0.003	1.000±0.000	large	telocentric
20	0.0000	2.1305	2.1305	0.043±0.001	1.000±0.000	large	telocentric
21	0.0000	2.0539	2.0539	0.042±0.001	1.000±0.000	medium	telocentric
22	0.0000	1.9514	1.9514	0.040±0.001	1.000±0.000	medium	telocentric
23	0.0000	1.8380	1.8380	0.037±0.002	1.000±0.000	medium	telocentric
24	0.0000	1.6575	1.6575	0.034±0.001	1.000±0.000	medium	telocentric

Remark: Chro. = chromosome, \* = NOR-bearing chromosome.

### Chromosome markers of *N. nigroris*

This is the first report that revealed that NORs of *N. nigroris*. The present study was accomplished by using the Ag-NOR staining technique which represent the location of genes (loci) that function in ribosome synthesis (18S and 28S ribosomal RNA), and a positive NOR is detected when these genes are functioning (Sharma et al., 2002). From the result, NORs were located on a single pair of chromosomes, at the terminal region of the short arms of acrocentric chromosome pair 18 without size heteromorphism. It is

the same as previous studies in *N. melas* with two NOR bearing chromosomes, Ag-positive NORs were located at terminal regions of the short arms of a pair of large subtelocentric chromosomes (Takai, 2011). Kashiwagi et al. (2005) suggested that NOR-bearing chromosomes showed size variation among the species, but no intra-specific variation, and these chromosomes have been differentiated involving heterochromatin amplifications or translocation of NORs in some genus. However, genus *Neoglyphidodon*, this is a usual state in Pomacentridae. Occurrence of active chromosomal changes has been

demonstrated that it is an interesting phenomenon that the number of NORs have been conserved throughout these changes (Takai, 2011).

#### Evolution of the genus *Neoglyphidodon*

According to Ohno (1974) the karyotype consisting of only 48 acrocentric chromosomes may have been ancestral in fish evolution, and this presumed ancestral karyotype was also ancestral in many fish groups at family and order levels. Pomacentridae has several species with the 48A karyotype. Therefore, the 48A karyotype was also ancestral in Pomacentridae (Takai, 2011). Most Pomacentrid fishes display a conserved  $2n=48$ , but vary in their fundamental number ( $NF=48-90$ ) possibly due to the occurrence of pericentric inversions (Takai and Ojima, 1987). In previous studies on cytogenetics of the genus *Neoglyphidodon*, karyotypes of three species for example *N. melas* ( $NF=80, 82$  and  $88$ ) (Takai and Ojima, 1991a; Takai, 2011; Getlekha, 2018), *N. oxyodon* ( $NF=70$ ) (Takai and Ojima, 1991a) and *N. nigroris* ( $NF=82$  and  $84$ ) (Takai and Ojima, 1991a; present study) have been reported to have high NFs (Table 1). Thus far, it is evident that *Neoglyphidodon* is a karyologically highly differentiated genus, demonstrated that there is one pattern of its chromosome evolution. Therefore, the occurrence of pericentric inversion at telocentric chromosomes provide the bi-arms chromosomes (metacentric, submetacentric and acrocentric chromosome), which does not affect the chromosome number but provides an increment of fundamental numbers.

Moreover, in other genera, *Amphiprion* and *Chrysiptera* species have high NF. Conversely, *Dascyllus* and *Abudefduf* species have low NF, ranging between 48 and 52. NF of *Pomacentrus* species range from low to high (48–84) (Takai, 2011). In Pomacentrinae, a large number of pericentric inversions and possibly also

paracentric ones corresponds to primary mechanisms of karyotype differentiation, as shown in some *Pomacentrus* species. Such rearrangements, besides structural modifications (e.g., changes in the centromere position), may promote additional effects (e.g., changes in gene expression, meiotic pairing anomalies, and reduced recombination). The reduction of recombination increases adaptive alleles in local populations, resulting in genomic differentiation and evolutionary divergence of lineages (Getlekha et al., 2017). Up to the present, there are three species of the genus *Neoglyphidodon* cytogenetically analyzed. *Neoglyphidodon* species provides remarkable karyotype features for chromosome evolutionary discussion. Further studies of other species as well as additional information and molecular techniques for chromosome analyses are expected to clarify and explain the reasons to support the karyotype polymorphism and chromosomal evolution in these fishes.

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