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First Report of Chromosome Analysis of Two Chaetodontid Fishes (Perciformes, Chaetodontidae)

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Summary We report the first chromosome analysis in the Indian vagabond butterflyfish (*Chaetodon decussatus*) and lined butterflyfish (*C. lineolatus*) from Andaman Sea, Phuket Province, Thailand. Kidney cell samples were taken from four male and four female fishes. The mitotic chromosome preparation was directly prepared from kidney cells. The chromosomes were stained with conventional and Ag-NOR staining techniques. The results showed that the diploid chromosome number of *C. decussatus* and *C. lineolatus* was $2n=48$, and the fundamental number (NF) was 48 in both males and females. The chromosomes were present as large telocentric and medium telocentric chromosomes in numbers of 24–24 and 22–26, respectively. There was no observation of strange size chromosomes related to sex. After Ag-NOR banding technique, a single pair of nucleolar organizer regions (NORs) was observed on the long arm centromeric region of medium telocentric chromosome pair 18 in *C. decussatus* and on the long arm subcentromeric region of medium telocentric chromosome pair 17 in *C. lineolatus*. The karyotype formulas could be deduced as:

C. decussatus $2n(48)=L^t_{24}+M^t_{24}$

C. lineolatus $2n(48)=L^t_{22}+M^t_{26}$

Key words *Chaetodon decussatus*, *Chaetodon lineolatus*, Karyotype, Chromosome.

The butterflyfishes are conspicuous components of the reef community on tropical and subtropical coral reefs (Pitts 1991). Butterflyfishes include over 130 species in 13 genera and form an intricate part of the coral reef ecosystem. The genus *Chaetodon* from the family Chaetodontidae is widely distributed over major regions like the Western Pacific and the Indian Ocean (Allen *et al.* 1998).

Fish are the most primitive vertebrate group, may be found in several types of environments and show wide genetic variability both at the chromosomal and molecular levels, which makes them an interesting group for evolutionary and cytotaxonomic studies (Kosswing 1973). Recently, many studies of fish chromosomes have been reported, and a karyological approach to fish systematics has become more valuable. Cytogenetic markers have been considered as authentic tools for characterization of fish species as well as to screen putative hybrids. In addition, these markers have also been found useful for detection of intraspecific stocks and populations in some fish species and in resolving taxonomic ambiguities between some species. Further, karyotypic information can throw light on the phylogenetic relationship between different species and karyotype evolution in fish species (Nagpure *et al.* 2006).

The few cytogenetical reports on the family Chaetodontidae demonstrated a highly conserved pattern, considered basal for order Perciformes ($2n=48$, NF=48) (Arai and Inoue 1975, Arai and Yamamoto 1981, Ojima and Yamamoto 1990, Affonso *et al.* 2001, Hardie and Hebert 2004, Galetti *et al.* 2006, Nagpure *et al.* 2006). No study describing the karyotypes of the Indian vagabond butterflyfish (*Chaetodon decussatus*) and lined butterflyfish (*C. lineolatus*) have been published until the present. The present study is the first cytogenetic report of *C. decussatus* and *C. lineolatus* accomplished with the conventional staining and Ag-NOR banding techniques. The obtained results can provide increasing cy-

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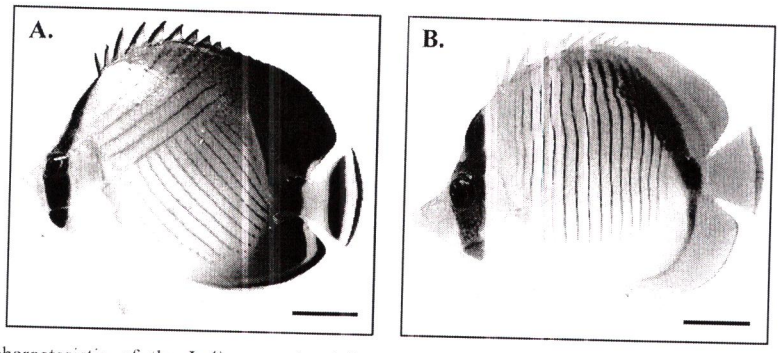

Fig. 1. General characteristic of the Indian vagabond butterflyfish, *Chaetodon decussatus* (A.), and lined butterflyfish, *C. lineolatus* (B.); scale bars indicate 5 cm.

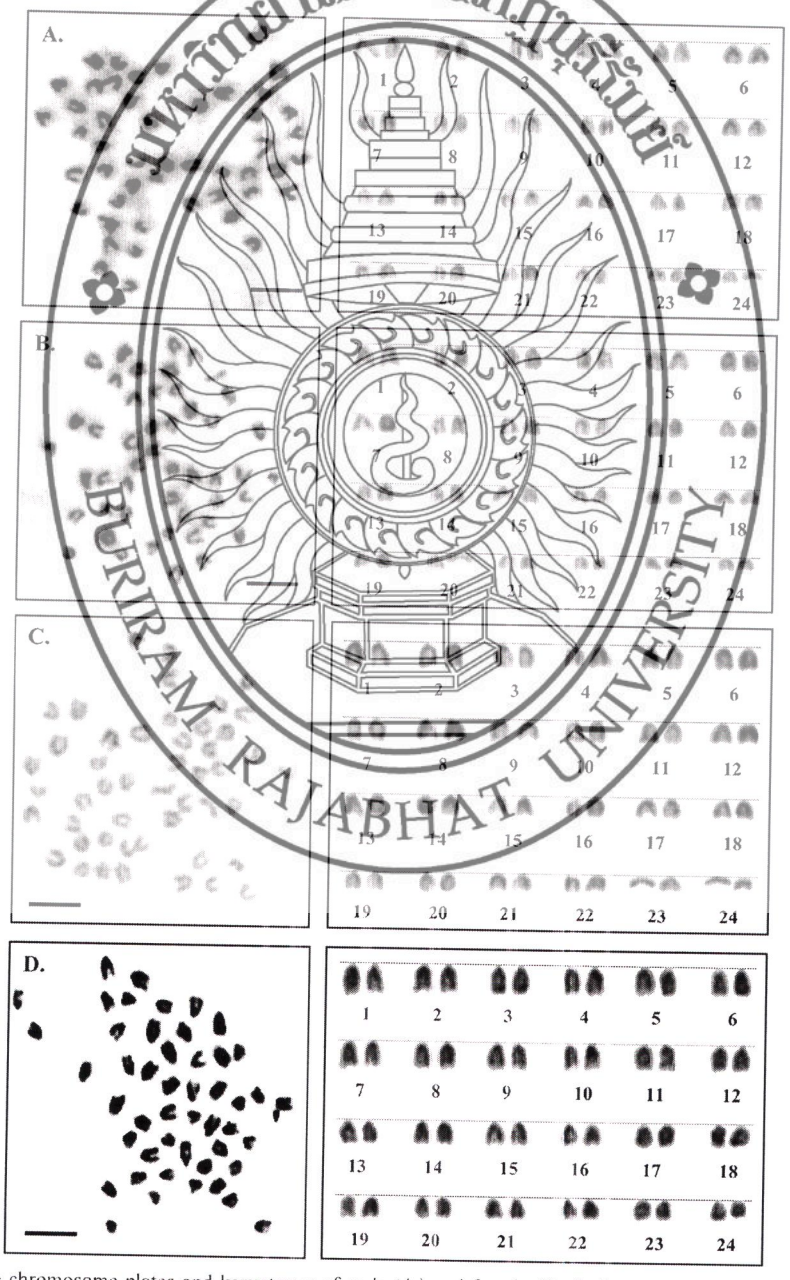

Fig. 2. Metaphase chromosome plates and karyotypes of male (A.) and female (B.) Indian vagabond butterflyfish (*Chaetodon decussatus*) and male (C.) and female (D.) lined butterflyfish (*C. lineolatus*), $2n$ (diploid)=48 by conventional staining technique (scale bars=5 μm). There is no observation of strange size chromosomes related to sex.

Table 1. Review of butterflyfish cytogenetic reports in the family Chaetodontidae.

Species	2*n*	NF	m	sm	t	NORs	Locality	Reference
Chaetodon decussatus	48	48	0	0	48	2 (CR)	Thailand	Present study
C. lineolatus	48	48	0	0	48	2 (SCR)	Thailand	Present study
C. auriga	48	48	0	0	48	—	Japan	Arai and Inoue (1975)
	48	48	0	0	48	—	Japan	Hardie and Hebert (2004)
C. auripes	48	48	0	0	48	—	Japan	Arai and Inoue (1975)
	48	48	0	0	48	—	Japan	Ojima and Yamamoto (1990)
C. collare	48	48	0	0	48	2	India	Nagpure *et al.* (2006)
C. lunula	48	48	0	0	48	—	Japan	Arai and Inoue (1975)
C. plebeius	48	50	2	0	46	—	Japan	Arai and Inoue (1975)
C. sedentarlus	48	48	0	0	48	—	Brazil	Galetti *et al.* (2006)
C. striatus	48	48	0	0	48	2	Brazil	Affonso *et al.* (2001)
C. strigangulus	48	50	0	2	46	—	Japan	Arai and Inoue (1975)
C. trifasciatus	48	48	0	0	48	—	Japan	Arai and Inoue (1975)
C. vagabundus	48	48	0	0	48	—	Japan	Arai and Inoue (1975)
	48	48	0	0	48	—	Japan	Hardie and Hebert (2004)
Heniochus acuminatus	48	48	0	0	48	—	Japan	Arai and Yamamoto (1981)

Remarks: 2*n*=diploid chromosome number, NF=fundamental number, m=metacentric chromosome, sm=submetacentric chromosome, t=telocentric chromosome, NORs=nucleolar organizer regions, CR=centromeric region, SCR=subcentromeric region and —=not available.

togenetic information for future studies on the taxonomy and evolutionary relationships of these fishes.

Materials and methods

Four males and four females of *C. decussatus* and *C. lineolatus* were obtained from Phuket province, Andaman Sea, Southern Thailand (Fig. 1). The fish were transferred to laboratory aquaria and were kept under standard conditions for seven days prior to the experiment. Procedures for fish chromosome were prepared directly from kidney cells (Chen and Ebeling 1968, Nanda *et al.* 1995, Kasiroek *et al.* 2017). The chromosome preparations were stained with 10% Giemsa's for 30 min (Chooseangjaew *et al.* 2017) and NORs were identified by Ag-NOR staining (Howell and Black 1980, Sangpakdee *et al.* 2017). The metaphase figures were analyzed according to the chromosome classification after Chaiyasut (1989). The centromeric index (CI) between 0.50–0.59, 0.60–0.69, 0.70–0.89, and 0.90–0.99 were described as metacentric, submetacentric, acrocentric, and telocentric chromosomes, respectively. Fundamental number, NF (number of chromosome arm), is obtained by assigning a value of two to metacentric, submetacentric and acrocentric chromosomes and one to telocentric chromosomes (Chooseangjaew *et al.* 2017).

Results and discussion

Diploid number, fundamental number and karyotype of C. decussatus *and* C. lineolatus

The diploid chromosome number (2*n*) found in *C. decussatus* and *C. lineolatus* was 48 chromosomes in both males and females (Fig. 2). In comparison with the family Chaetodontidae, it is the same diploid chromosome number as *C. auriga* (Arai and Inoue 1975, Hardie and Hebert 2004); *C. auripes* (Arai and Inoue 1975, Ojima and Yamamoto 1990); *C. lunula*; *C. plebeius*; *C. strigangulus*; *C. trifasciatus*; *C. vagabundus* (Arai and Inoue 1975); *Heniochus acuminatus* (Arai and Yamamoto 1981); *C. sedentarlus* (Galetti *et al.* 2006); *C. striatus* (Affonso *et al.* 2001) and *C. collare* (Nagpure *et al.* 2006) (Table 1).

We found that the fundamental number (NF, number of chromosome arms) of *C. decussatus* and *C. lineolatus* was 48 in both males and females. The comparative studies with others in the family Chaetodontidae showed the similar NF as those found in *C. auriga*, *C. auripes*, *C. collare*, *C. lunula*, *C. sedentarlus*, *C. striatus*, *C. trifasciatus*, *C. vagabundus*, and *H. acuminatus* with NF=48 (Arai and Inoue 1975, Arai and Yamamoto 1981, Ojima and Yamamoto 1990, Affonso *et al.* 2001, Hardie and Hebert 2004, Galetti *et al.* 2006, Nagpure *et al.* 2006), but the NF was different from *C. plebeius* and *C. strigangulus* with NF=50 (Arai and Inoue 1975).

The karyotypes of *C. decussatus* and *C. lineolatus* consisted of 48 telocentric chromosomes. According to several authors, the karyotype composing of 48 telocentric chromosomes (NF=48) should be regarded as basal for the order Perciformes. Such karyotypical constitution seems to be common in marine species, which are generally more cytogenetically conserved than continental ones. At the moment, cytogenetical studies carried out on the family Chaetodontidae and related species revealed that this scenario is maintained even in morphologically and/or ecologically derived species (Arai and Yamamoto 1981, Affonso *et al.* 2001, 2002).

Both species investigated have no cytologically distinguishable sex chromosome. This characteristic is similar to others in the family Chaetodontidae (Arai and Inoue 1975, Arai and Yamamoto 1981, Ojima and Yamamoto 1990, Affonso *et al.* 2001, Hardie and Hebert

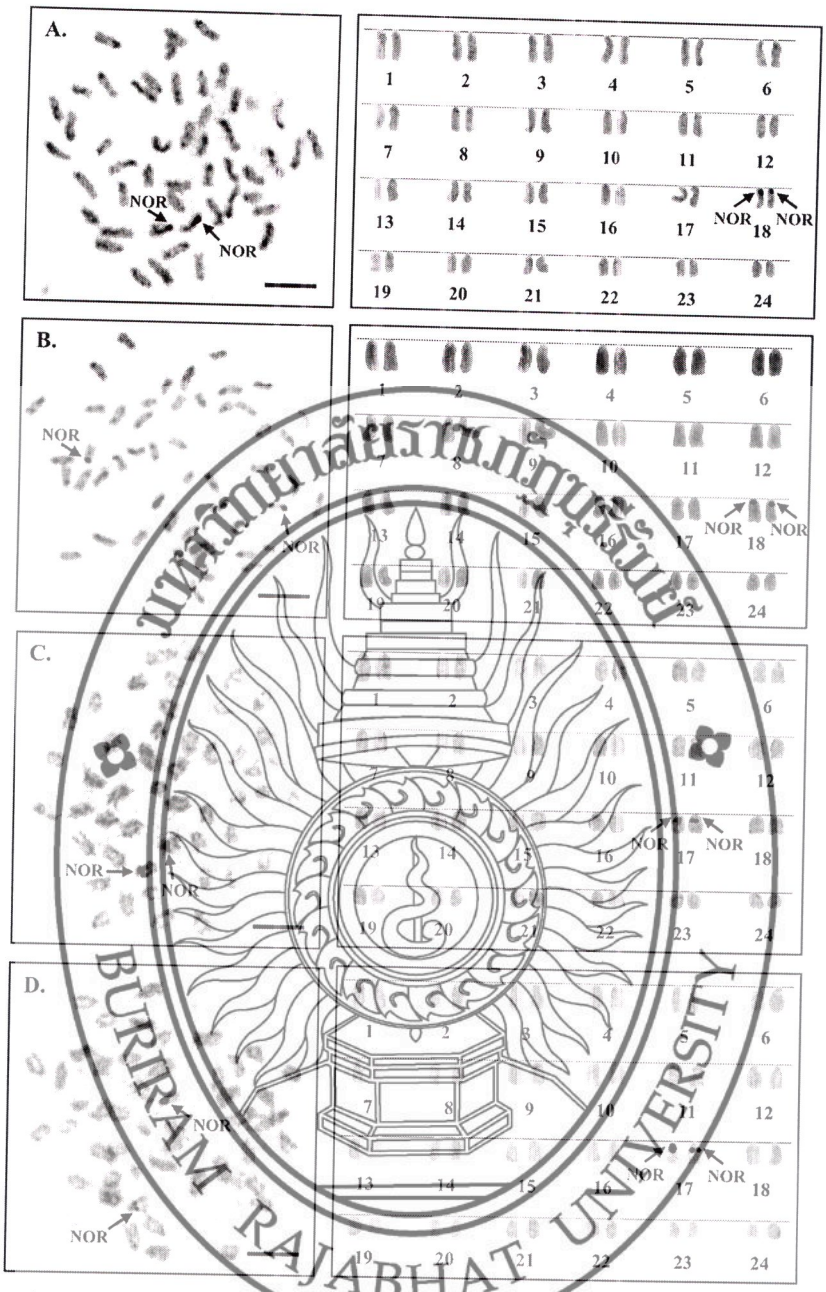

Fig. 3. Metaphase chromosome plates and karyotypes of male (A.) and female (B.) Indian vagabond butterflyfish (*C. decussatus*) and male (C.) and female (D.) lined butterflyfish (*C. lineolatus*), $2n$ (diploid)=48 by Ag-NOR banding technique; scale bars indicate 5 μm. The region adjacent to the long arm centromeric region of medium telocentric chromosome pair 18 in *C. decussatus* and on the long arm subcentromeric region of medium telocentric chromosome pair 17 in *C. lineolatus* showed clearly observable nucleolar organizer regions (NORs).

2004, Galetti *et al.* 2006, Nagpure *et al.* 2006). It is possible that the fish's sex chromosomes are dependent on an initiation of differentiation. Therefore, chromosomes containing the sex-determination gene cannot be found by cytogenetic analyses (Bertollo *et al.* 2004). The karyotype formulas for *C. decussatus* and *C. lineolatus* are as follows:

C. decussatus $2n\,(48)=L^t_{24}+M^t_{24}$

C. lineolatus $2n\,(48)=L^t_{22}+M^t_{26}$

Chromosome markers of C. decussatus *and* C. lineolatus

Our present information was obtained by using the Ag-NOR banding technique. The objective of this technique was to present nucleolar organizer regions (NORs) representing the location of genes (loci) that function in ribosome synthesis (18S and 28S ribosomal RNA) (Sharma *et al.* 2002). The regions adjacent to the long arm centromeric region of medium telocentric chromosome pair 18 in *C. decussatus* and on the long arm subcentromeric region of medium telocentric chromosome

Table 2. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphases of the Indian vagabond butterflyfish (*Chaetodon decussatus*), $2n=48$.

Chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	Chromosome size	Chromosome type
1	0.00	2.52	2.52	0.027±0.001	1.000±0.000	Large	Telocentric
2	0.00	2.41	2.41	0.026±0.001	1.000±0.000	Large	Telocentric
3	0.00	2.33	2.33	0.025±0.001	1.000±0.000	Large	Telocentric
4	0.00	2.28	2.28	0.024±0.001	1.000±0.000	Large	Telocentric
5	0.00	2.22	2.22	0.024±0.001	1.000±0.000	Large	Telocentric
6	0.00	2.18	2.18	0.023±0.001	1.000±0.000	Large	Telocentric
7	0.00	2.15	2.15	0.023±0.001	1.000±0.000	Large	Telocentric
8	0.00	2.11	2.11	0.023±0.000	1.000±0.000	Large	Telocentric
9	0.00	2.07	2.07	0.022±0.000	1.000±0.000	Large	Telocentric
10	0.00	2.03	2.03	0.022±0.000	1.000±0.000	Large	Telocentric
11	0.00	2.00	2.00	0.022±0.000	1.000±0.000	Large	Telocentric
12	0.00	1.96	1.96	0.021±0.000	1.000±0.000	Large	Telocentric
13	0.00	1.92	1.92	0.021±0.000	1.000±0.000	Medium	Telocentric
14	0.00	1.89	1.89	0.020±0.000	1.000±0.000	Medium	Telocentric
15	0.00	1.84	1.84	0.020±0.000	1.000±0.000	Medium	Telocentric
16	0.00	1.81	1.81	0.019±0.001	1.000±0.000	Medium	Telocentric
17	0.00	1.77	1.77	0.019±0.001	1.000±0.000	Medium	Telocentric
18*	0.00	1.73	1.73	0.019±0.001	1.000±0.000	Medium	Telocentric
19	0.00	1.70	1.70	0.018±0.001	1.000±0.000	Medium	Telocentric
20	0.00	1.65	1.65	0.018±0.000	1.000±0.000	Medium	Telocentric
21	0.00	1.61	1.61	0.017±0.001	1.000±0.000	Medium	Telocentric
22	0.00	1.55	1.55	0.017±0.001	1.000±0.000	Medium	Telocentric
23	0.00	1.45	1.45	0.016±0.001	1.000±0.000	Medium	Telocentric
24	0.00	1.32	1.32	0.014±0.001	1.000±0.000	Medium	Telocentric

Remark: *=NOR-bearing chromosome (satellite chromosome).

Table 3. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphases of the lined butterflyfish (*Chaetodon lineolatus*), $2n=48$.

Chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	Chromosome size	Chromosome type
1	0.00	2.62	2.62	0.027±0.001	1.000±0.000	Large	Telocentric
2	0.00	2.47	2.47	0.025±0.000	1.000±0.000	Large	Telocentric
3	0.00	2.38	2.38	0.024±0.001	1.000±0.000	Large	Telocentric
4	0.00	2.32	2.32	0.024±0.000	1.000±0.000	Large	Telocentric
5	0.00	2.29	2.29	0.023±0.000	1.000±0.000	Large	Telocentric
6	0.00	2.24	2.24	0.023±0.000	1.000±0.000	Large	Telocentric
7	0.00	2.20	2.20	0.023±0.000	1.000±0.000	Large	Telocentric
8	0.00	2.18	2.18	0.022±0.000	1.000±0.000	Large	Telocentric
9	0.00	2.15	2.15	0.022±0.000	1.000±0.000	Large	Telocentric
10	0.00	2.11	2.11	0.022±0.000	1.000±0.000	Large	Telocentric
11	0.00	2.08	2.08	0.021±0.000	1.000±0.000	Large	Telocentric
12	0.00	2.05	2.05	0.021±0.000	1.000±0.000	Medium	Telocentric
13	0.00	2.02	2.02	0.021±0.000	1.000±0.000	Medium	Telocentric
14	0.00	1.99	1.99	0.020±0.000	1.000±0.000	Medium	Telocentric
15	0.00	1.96	1.96	0.020±0.000	1.000±0.000	Medium	Telocentric
16	0.00	1.93	1.93	0.020±0.000	1.000±0.000	Medium	Telocentric
17*	0.00	1.90	1.90	0.019±0.000	1.000±0.000	Medium	Telocentric
18	0.00	1.87	1.87	0.019±0.000	1.000±0.000	Medium	Telocentric
19	0.00	1.83	1.83	0.019±0.000	1.000±0.000	Medium	Telocentric
20	0.00	1.78	1.78	0.018±0.000	1.000±0.000	Medium	Telocentric
21	0.00	1.73	1.73	0.018±0.000	1.000±0.000	Medium	Telocentric
22	0.00	1.67	1.67	0.017±0.001	1.000±0.000	Medium	Telocentric
23	0.00	1.59	1.59	0.016±0.001	1.000±0.000	Medium	Telocentric
24	0.00	1.48	1.48	0.015±0.001	1.000±0.000	Medium	Telocentric

Remark: *=NOR-bearing chromosome (satellite chromosome).

pair 17 in *C. lineolatus* showed clearly observable NOR (Fig. 3). In all species (four species) of the family Chaetodontidae investigated to date, the single NOR-bearing telocentric chromosome pair (centromeric and subcentromeric regions) is conserved (Affonso *et al.* 2001, Nagpure *et al.* 2006). Normally, most fishes have only one pair of small NOR (single NOR) on chromosomes. However, some fishes have more than two NORs, which

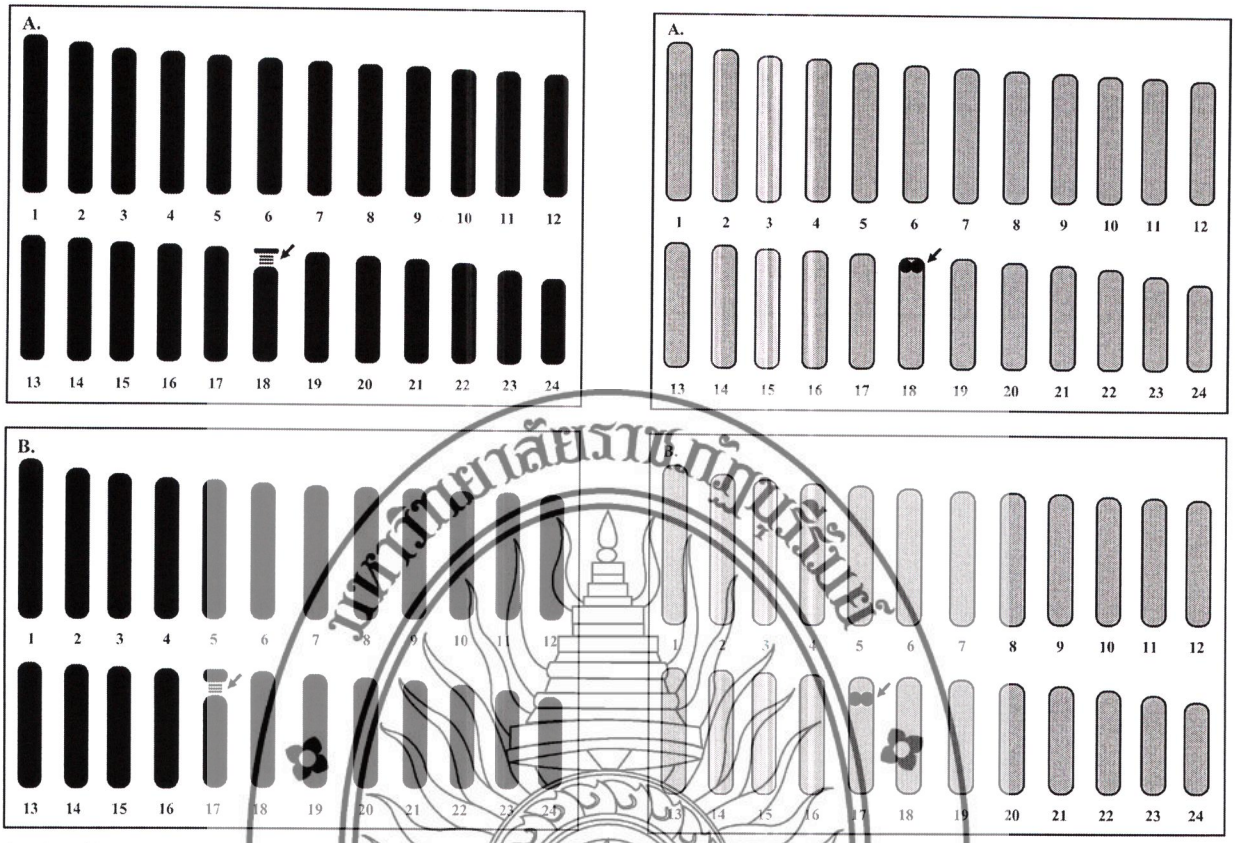

Fig. 4. Idiograms showing lengths and shapes of chromosomes of the Indian vagabond butterflyfish, *Chaetodon decussatus* (A.) and lined butterflyfish, *C. lineolatus* (B.), 2*n* (diploid) = 48 by conventional staining technique. Arrows indicate nucleolar organizer region (NOR).

Fig. 5. Idiograms of the Indian vagabond butterflyfish, *Chaetodon decussatus* (A.) and lined butterflyfish, *C. lineolatus* (B.), 2*n* (diploid) = 48 by Ag-NOR banding technique. Arrows indicate nucleolar organizer region (NOR).

may be caused by the translocation between some part of the chromosome having a NOR and another chromosome. Furthermore, NOR is usually located close to the telomere of the chromosome arm (Sharma *et al.* 2002).

The localization of NOR sites is an important tool in certain studies, such as those on evolution and cytotaxonomy, and those on gene expression (Galetti 1998). The detection of NORs in *C. decussatus* and *C. lineolatus* has proved to be useful to evaluate the mechanisms of chromosomal differentiation within the family Chaetodontidae. Several Perciformes species and correlated groups that present a karyotype exclusively composed by telocentric chromosomes are also characterized by a single NOR site at interstitial position, close to centromere (Feldberg and Bertollo 1985, Delgado *et al.* 1994, Brum 1996, Affonso *et al.* 2002), including examples in the family Chaetodontidae. It is suggested that such a NOR location could represent a basal condition for these fishes (Affonso *et al.* 2001, Nagpure *et al.* 2006).

The asymmetrical karyotypes of *C. decussatus* and *C. lineolatus*, and the one and only type of chromosomes (telocentric chromosomes) that we found are important chromosome markers. The idiogram shows a continuous length gradation of chromosomes. The largest and smallest chromosomes show size differences (approximately two-fold). Data of chromosomal checks on mitotic metaphase cells of *C. decussatus* and *C. lineolatus* are shown in Tables 2 and 3, respectively. Figures 4 and 5 show the idiograms obtained by conventional staining and Ag-NOR banding techniques, respectively.

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