

## เซลล์พันธุศาสตร์ของปลาผีเสื้อลายทแยง

(*Chaetodon vagabundus* Linnaeus, 1758) ด้วยเทคนิคการย้อมสี

แบบธรรมดาและแถบสีแบบนอร์

Cytogenetics of the Vagabond Butterflyfish, *Chaetodon vagabundus*

(Perciformes, Chaetodontidae) by Conventional Staining

and Ag-NOR Banding Techniques

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### บทคัดย่อ

การศึกษาแคโรไทป์และอิดิโอแกรมมาตรฐานของปลาผีเสื้อลายทแยง (*Chaetodon vagabundus* Linnaeus, 1758) เพศผู้และเพศเมียจำนวนอย่างละ 5 ตัว จากศูนย์วิจัยและพัฒนาประมงชายฝั่งภูเก็ตโดยเตรียมโครโมโซมจากไตด้วยวิธีการบดขยี้เซลล์ ย้อมสีโครโมโซมแบบธรรมดาและแถบสีแบบนอร์ พบว่าปลาผีเสื้อลายทแยงมีจำนวนโครโมโซมดิพลอยด์ ( $2n$ ) เท่ากับ 48 แห่ง มีจำนวนโครโมโซมพื้นฐาน (NF) เท่ากับ 48 ทั้งในเพศผู้และเพศเมีย ประกอบด้วยโครโมโซมชนิดเทโลเซนทริกขนาดใหญ่ 26 แห่ง และเทโลเซนทริกขนาดกลาง 22 แห่ง ไม่พบความแตกต่างของโครโมโซมเพศระหว่างปลาเพศผู้และเพศเมีย พบตำแหน่งของนอร์บริเวณใกล้เซนโทรเมียร์ของโครโมโซมคู่ที่ 20 และปลาผีเสื้อลายทแยงมีสูตรแคโรไทป์ คือ  $2n (48) = L_{26}^t + M_{22}^t$

**คำสำคัญ:** ปลาผีเสื้อลายทแยง โครโมโซม แคโรไทป์ อิดิโอแกรม

### Abstract

The present study on standardized karyotype and idiogram of the vagabond butterflyfish (*Chaetodon vagabundus* Linnaeus, 1758) from Phuket Coastal Fisheries Research and Development Center (Phuket, Thailand) was accomplished by conventional staining and Ag-NOR banding techniques. The mitotic chromosome preparation was performed by air-drying technique of renal cells which were obtained from each five male and five female specimens, followed by the staining techniques mentioned above. The findings indicated that the diploid chromosome number ( $2n$ ) and the fundamental number (NF) were 48 in both males and females. The chromosome comprises 26 large telocentric and 22 medium telocentric chromosomes. However, no of strange sized chromosomes related to sex were observed. NOR-bearing chromosome was found at

pericentromeric region of the chromosome 20. The karyotype formula of *C. vagabundus* was  $2n$  (48)  
 $= L_{26}^t + M_{22}^t$

**Keywords:** *Chaetodon vagabundus*, chromosome, karyotype, idiogram

### Introduction

The vagabond butterflyfish (*Chaetodon vagabundus* Linnaeus, 1758) is member of the family Chaetodontidae, and genus *Chaetodon*. Its distribution is found in tropical zone of Thailand, Myanmar, and Malaysia. This fish are most common in shallow coral reefs. Some species are found at depths of 60 meters or more. Its color displayed by mostly white and bright yellow with black lines on body diagonal in 2 sets perpendicular to each other. There are black ocular bar as well as caudal peduncle and end of caudal fin bars while dorsal fin and anal fin rays pose on yellow and black (Figure1) (Allen *et al.* 1999; Carpenter and Niem, 2001).

Up to the present, there are only 11 of 49 butterflyfish species that have been cytogenetically investigated. This information is scarce number for Chaetodontidae in this field. Arai and Enoue (1975) reported the study on cytogenetics of seven butterflyfishes, namely threadfin butterflyfish (*Chaetodon auriga*), oriental butterflyfish (*C. auripes*), raccoon butterflyfish (*C. lunula*), blueblotch butterflyfish (*C. plebeius*), chevron butterflyfish (*C. strigangulus*), melon butterflyfish (*C. trifasciatus*), and vagabond butterflyfish (*C. vagabundus*) from Japan, and revealed that all of them have diploid chromosome number of 48. In addition, Ojima and Yamamoto (1988) showed that *C. auripes* from Japan has diploid chromosome number of 48. This is the same with previous of Affonso *et al.* (2001) for the banded butterflyfish (*C. striatus*), Galetti *et al.* (2006) for the reef butterflyfish (*C. sedentarius*) from Brazil as well as Nagpure *et al.* (2006) for the redtail butterflyfish (*C. collare*). However, there are only two species (*C. collare* and *C. striatus*) which were examined their karyotype using Ag-NOR banding. All previous studies showed that the karyotype of butterflyfishes had all telocentric chromosomes except for *C. plebeius*, which had 2 metacentric and 46 telocentric chromosomes and *C. strigangulus* that comprised of 2 submetacentric and 46 telocentric chromosomes.

### Materials and methods

#### 1. Sample collection

The samples, *C. vagabundus*, were collected from Phuket Coastal Fisheries Research and Development Center in Phuket province, Thailand. They were fed in the morning and evening in

aquarium under the salinity of 26 – 32 ppt, temperature of 25°C, 16 light hours: 8 dark hours for seven days. Then they were identified following Carpenter and Niem (2001).

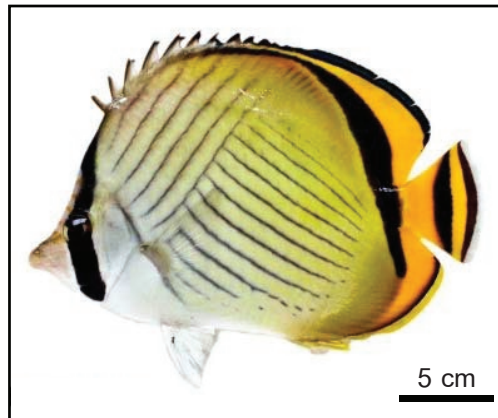


Figure 1 Vagabond butterflyfish (*Chaetodon vagabundus*).

## 2. Chromosome preparation

Chromosomes were directly prepared according to Chen and Ebeling (1968); Nanda *et al.*, (1995). Colchicine was injected to fish's abdominal cavity then it was left for one hour. Kidney was cut into small pieces then mixed with 0.075 M KCl. All large pieces cells were discarded. Fifteen ml of cell sediment were transferred to a centrifuge tube and incubated for 25 – 35 minutes. Then, the fresh and so cool fixative (methanol: glacial acetic acid; 3: 1) were added to stop KCl activities. The cell sediments were centrifuged at 1,200 – 1,500 rpm for 10 minutes. The supernatant were discarded, and seven ml of fixative were added again and centrifuged again to wash the cell sediments. The obtained cell sediments were used for further chromosome studies.

## 3. Chromosome staining

Conventional staining technique (Rooney, 2001)

The mixture was dropped onto a clean and cold slide by micropipette following by air-drying technique. The slide were conventionally stained with 20% Giemsa's solution for 30 minutes.

Ag-NOR banding technique (Howell and Black, 1980)

The two drops of each 50% silver nitrate and 2% gelatin were added on slides, respectively. The slides were sealed with cover glasses and incubated at 60°C for 5 – 10 minutes, then soaked in distilled water until cover glasses are separated.

## 4. Karyotyping

Chromosome counting was performed on mitotic metaphase cells under light microscope. Twenty clearly observable and spread chromosomes of each male and female were selected and

photographed. The length of short arm chromosome (Ls), long arm chromosome (Ll), total length of chromosome arm (LT = Ls + Ll), relative length (RL), the centromeric index (CI), standard deviation (SD) of RL and CI were calculated. CI was computed according to the method of Turpin and Lejeune (1965).

### Results and discussion

Chromosome counting of the 100 metaphase cells of *C. vagabundus* revealed the  $2n$  of 48. This number was found in 91 cells of this fish. Its NF was 48. It comprised 48 telocentric chromosomes regards to centromeric index (CI) value. The chromosomes were divided into two groups of size including large (LT of 2.25 – 2.90) and medium (LT of 1.58 – 2.24).

The present result was the first report on NOR analysis for *C. vagabundus*. This species has NOR at pericentromeric region of the chromosome 20. This characteristic is the chromosome marker of this fish. However, no of strange sized chromosomes related to sex was observed. Its karyotype formula could be deduced as:  $2n (48) = L_{26}^t + M_{22}^t$

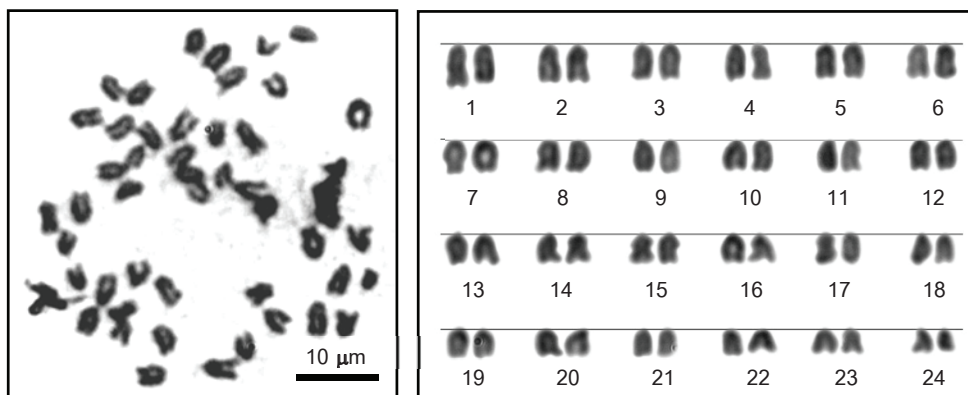


Figure 2 Metaphase cell plate (left) and karyotype of the male vagabond butterflyfish (*Chaetodon vagabundus*),  $2n = 48$  by Giemsa (right).

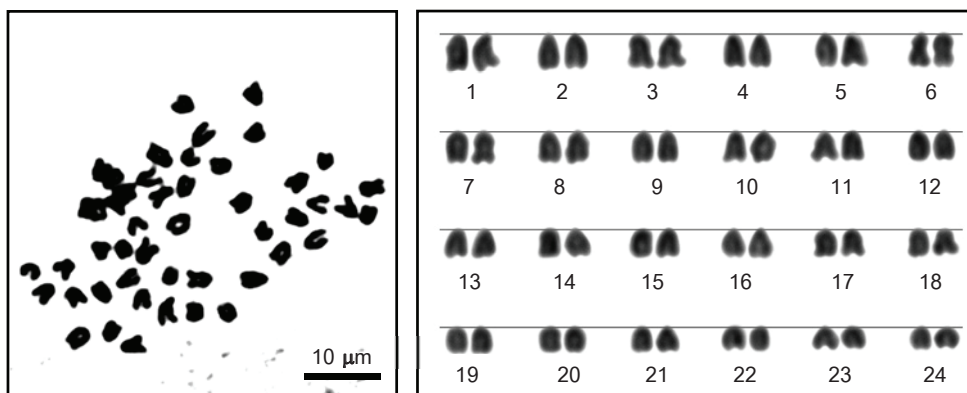


Figure 3 Metaphase cell plate (left) and karyotype of the female vagabond butterflyfish (*Chaetodon vagabundus*),  $2n = 48$  by Giemsa (right).

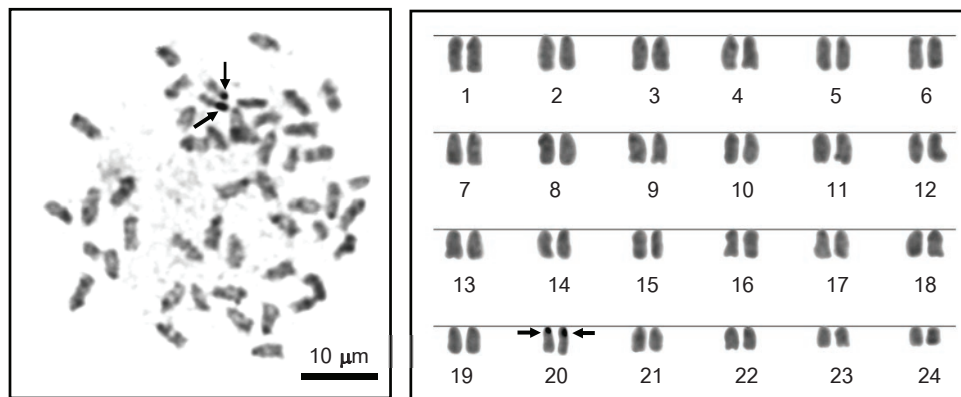


Figure 4 Metaphase cell plate (left) and karyotype of the vagabond butterflyfish (*Chaetodon vagabundus*),  $2n = 48$  by Ag-NOR banding technique (right). Arrows indicate nucleolar organizer regions/NORs.

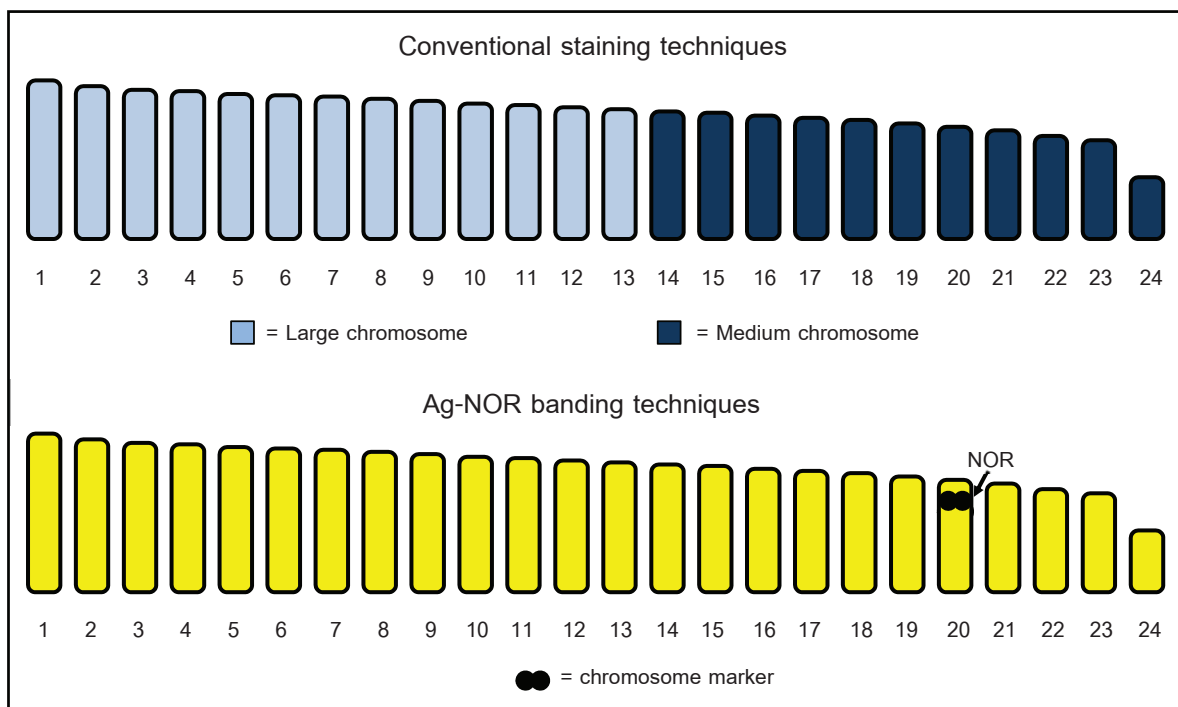


Figure 5 Standardized idiogram of the vagabond butterflyfish (*Chaetodon vagabundus*),  $2n = 48$  by Giemsa and Ag-NOR banding techniques. The arrow indicates nucleolar organizer region (NOR) on pericentromeric region chromosome 20.

Table 1 Karyotype of butterflyfish in the family Chaetodontidae.

Species	2n	NF	Type				NOR	Reference
			m	sm	a	t		
Threadfin butterflyfish ( <i>Chaetodon auriga</i> )	48	48	-	-	-	48	-	Arai and Inoue (1975)
Oriental butterflyfish ( <i>C. auripes</i> )	48	48	-	-	-	48	-	Arai and Inoue (1975)
	48	48	-	-	-	48	-	Ojima and Yamamoto (1988)
Raccoon butterflyfish ( <i>C. lunula</i> )	48	48	-	-	-	48	-	Arai and Inoue (1975)
Blueblotch butterflyfish ( <i>C. plebeius</i> )	48	50	2	-	-	46	-	Arai and Inoue (1975)
Chevron butterflyfish ( <i>C. strigangulus</i> )	48	50	-	2	-	46	-	Arai and Inoue (1975)
Melon butterflyfish ( <i>C. strifasciatus</i> )	48	48	-	-	-	48	-	Arai and Inoue (1975)
Vagabond butterflyfish ( <i>C. vagabundus</i> )	48	48	-	-	-	48	-	Arai and Inoue (1975)
	48	48	-	-	-	48	2 (PCR)	Present study
Banded butterflyfish ( <i>C. striatus</i> )	48	48	-	-	-	48	2	Affonsoet al. (2001)
Reef butterflyfish ( <i>C. sedentarius</i> )	48	48	-	-	-	48	-	Galettiet al. (2006)
Red tail butterflyfish ( <i>C. collare</i> )	48	48	-	-	-	48	2	Nagpureet al. (2006)

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

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

Chromosome pair	Ls	LI	LT	RL±SD	CI±SD	Size	Type
1	0.00	2.90	2.90	0.0541±0.0018	1.0000±0.000	Large	Telocentric
2	0.00	2.73	2.73	0.0502±0.0018	1.0000±0.000	Large	Telocentric
3	0.00	2.66	2.66	0.0488±0.0015	1.0000±0.000	Large	Telocentric
4	0.00	2.59	2.59	0.0476±0.0016	1.0000±0.000	Large	Telocentric
5	0.00	2.55	2.55	0.0469±0.0014	1.0000±0.000	Large	Telocentric
6	0.00	2.51	2.51	0.0460±0.0012	1.0000±0.000	Large	Telocentric
7	0.00	2.48	2.48	0.0455±0.0012	1.0000±0.000	Large	Telocentric
8	0.00	2.45	2.45	0.0450±0.0009	1.0000±0.000	Large	Telocentric
9	0.00	2.40	2.40	0.0458±0.0006	1.0000±0.000	Large	Telocentric
10	0.00	2.36	2.36	0.0433±0.0006	1.0000±0.000	Large	Telocentric
11	0.00	2.33	2.33	0.0428±0.0007	1.0000±0.000	Large	Telocentric
12	0.00	2.30	2.30	0.0438±0.0005	1.0000±0.000	Large	Telocentric
13	0.00	2.26	2.26	0.0414±0.0007	1.0000±0.000	Large	Telocentric
14	0.00	2.22	2.22	0.0408±0.0007	1.0000±0.000	Medium	Telocentric
15	0.00	2.18	2.18	0.0400±0.0009	1.0000±0.000	Medium	Telocentric
16	0.00	2.14	2.14	0.0393±0.0010	1.0000±0.000	Medium	Telocentric
17	0.00	2.10	2.10	0.0393±0.0012	1.0000±0.000	Medium	Telocentric
18	0.00	2.06	2.06	0.0377±0.0010	1.0000±0.000	Medium	Telocentric
19	0.00	2.01	2.01	0.0370±0.0011	1.0000±0.000	Medium	Telocentric
20*	0.00	1.95	1.95	0.0358±0.0010	1.0000±0.000	Medium	Telocentric
21	0.00	1.89	1.89	0.0346±0.0012	1.0000±0.000	Medium	Telocentric
22	0.00	1.80	1.80	0.0336±0.0015	1.0000±0.000	Medium	Telocentric
23	0.00	1.71	1.71	0.0315±0.0019	1.0000±0.000	Medium	Telocentric
24	0.00	1.58	1.58	0.0290±0.0025	1.0000±0.000	Medium	Telocentric

Remark: \* = NOR-bearing chromosome.

The present result obtained here was in agreement with the report of Arai and Inoue (1975) which indicated the diploid chromosome number and the fundamental number of 48 for *C. vagabundus* from Japan. Nevertheless, this information differs from those of fish in genus *Chaetodon*, *C. plebeius* and *C. strigangulus* (Arai and Inoue, 1975). This different NF was due to the difference in type of chromosome. Because they had 46 mono-armed and 2 bi-armed chromosomes, their NF was of 50.

Up to the present, only two species which examined for NOR analysis (Affonso *et al.*, 2001; Nagpure *et al.*, 2006). They show a pair of NOR but no of strange sized chromosomes related to sex

was observed. This information was consistent to our present study. There is an assumption that sex chromosome of fish is being in initiation stage of chromosome change (Na-Nakhon, 2000).

So, the present study was the first cytogenetically analysis of *C. vagabundus* from Thailand by conventional staining and Ag-NOR banding techniques. We suggest that there is no cytogenetic information of the genus *Chaetodon* in Thailand. The results obtained here can be used to support the further investigation on taxonomy and evolutionary relationship among the family Chaetodontidae and others. In addition, this information could be applied to numerous breeding studies and this could also provide insights into species conservation and chromosome evolution studies of the family Chaetodontidae.

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