Karyological Analysis of Hongkong Buterflyfish, *Chaetodon wiebeli* (Perciformes, Chaetodontidae) from Thailand

Khunapat Sonsrin[1] & Krit Pinthong[2]*

บทคัดย่อ

การศึกษาพันธุศาสตร์เซลล์ของปลาผีเสื้อชุมพร (*Chaetodon wiebeli* Kaup, 1863) เก็บตัวอย่างปลาเพศผู้และเพศเมียอย่างละ 2 ตัว จากอ่าวไทย ย้อมสีโครโมโซมด้วยเทคนิคการย้อมสีแบบธรรมดา และแถบสีแบบนอร์ เตรียมโครโมโซมจากการแบ่งเซลล์ไมโทซีสด้วยวิธีตรงจากเซลล์ไต ผลการศึกษาพบว่าปลาผีเสื้อชุมพรมีจำนวนโครโมโซมดิพลอยด์เท่ากับ 48 แท่ง โครโมโซมเป็นชนิดเทโลเซนทริกทั้งหมด มีจำนวนโครโมโซมพื้นฐานเท่ากับ 48 ทั้งในเพศผู้และเพศเมีย ตรวจไม่พบความแตกต่างของโครโมโซมเพศระหว่างปลาเพศผู้และเพศเมีย จากการย้อมแถบสีแบบนอร์พบตำแหน่งโครโมโซมเครื่องหมายอยู่บนแขนข้างยาวบริเวณใกล้เซนโทรเมียร์ของโครโมโซมชนิดเทโลเซนทริกขนาดเล็กคู่ที่ 24 เป็นรายงานของการศึกษาพันธุศาสตร์เซลล์ปลาผีเสื้อชุมพรด้วยวิธีการย้อมแถบสีแบบธรรมดา และแถบสีแบบนอร์

คำสำคัญ : *Chaetodon wiebeli*, แคริโอไทป์, โครโมโซม

[1] Dr. Department of Basic Science, Faculty of Science and Technology, Surindra Rajabhat University, Muang, Surin 32000, Thailand

[2] Assistant Professor, Dr. Department of Basic Science, Faculty of Science and Technology, Surindra Rajabhat University, Muang, Surin 32000, Thailand

* Corresponding Author, Email: k_pinthong@yahoo.com

Abstract

The cytogenetic study in Hongkong butterflyfish (*Chaetodon wiebeli* Kaup, 1863), occurring in the Gulf of Thailand, by using conventional staining and Ag-NORs banding techniques as carried out analysed in the present study. Two male and two female fish samples were directly used in the chromosome preparation. The mitotic chromosome preparations were conducted directly from kidney cells. The results showed that the diploid chromosomes number (2*n*) in *C. wiebeli* was found to be 48. Base on the karyomorphology, the fundamental number (NF) were 48 in both sexes and the karyotype formula was derived as 48t (48 telocentric chromosomes). No different sized of chromosomes related to sex are observed. Silver staining revealed that presence of one pair of NORs is located on the long arm subcentomeric region of small telocentric chromosome pair 24. This cytogenetics data was the first description of karyotype in this species using conventional staining and Ag-NORs banding techniques.

Keywords: *Chaetodon wiebeli*, Karyotype, Chromosome.

Introduction

Thailand is one of the world richest places of biodiversity, especially for marine fish species. Interesting that of about 13,000 marine fish species that have been recorded (Nelson, 1994) less than 8% of these have been studied cytogenetically (Brum, 1996). Cytogenetic markers have been considered as authentic tools for characterization of fish species as well as to screen putative hybrids (Manna and Khuda-Bukhsh, l974; Amemiya and Gold 1988). In addition, these markers have also been found useful for detection of intraspecific stocks and populations in some fish species (Phillips *et al.*, 1988) and in resolving taxonomic ambiguities between some species (Kushwaha *et al.*, 2001). Further, karyotypic information can throw light on phylogenetic relationship between different species and karyotype evolution in fish species. In view of the above, cytogenetic characterization has been carried out in *Chaetodon wiebeli*. he brief description of this fish is as follow.

The butterflyfishes are a group of conspicuous tropical marine fish of the family Chaetodontidae, the bannerfishe and coralfishesare also included in this group. The Chaetodontidae family contains 12 genera with 129 species, the majority in the genus *Chaetodon* (Allen 1985). In the genus *Chaetodon*, cytogenetic studies have been performed in 15 species as follow in Table 1

Table 1.

Review of cytogenetic publications of genus *Chaetodon* (Perciformes, Chaetodontidae).

Species	2*n*	NF	Karyotype formula	NOR banded	Locality	Reference
C. andamannesis	48	52	2m+2a+44t	2	Thailand	Boonsuk (2013)
C. auriga	48	48	48t	-	Japan	Arai and Inoue (1975)
	48	48	48t	2	Thailand	Na Nongkhai (2014)
C. auripes	48	48	48t	-	Japan	Arai and Inoue (1975)
	48	48	48t	-	Japan	Ojima and Yamamoto (1990)
C. collare	48	48	48t	-	India	Nagpure *et al.* (2006)
	48	48	48t	2	Thailand	Boonsuk (2013)
C. decussatus	48	48	48t	2	Thailand	Boonsuk (2013)
C. lineolatus	48	48	48t	2	Thailand	Boonsuk (2013)
C. lunula	48	48	48t	-	Japan	Arai and Inoue (1975)
	48	48	48t	2	Thailand	Na Nongkhai (2014)
C. ocellatus	48	48	48t	2	Brazil	Molina *et al.* (2013)
C. plebeius	48	50	2m+46t	-	Japan	Arai and Inoue (1975)
C. sedentarius	48	48	48t	-	Brazil	Galetti *et al.* (2006)
C. striatus	48	48	48t	-	Brazil	Affonso *et al.* (2001)
	48	48	48t	2	Brazil	Molina *et al.* (2013)
C. strigangulus	48	50	2sm+46t	-	Japan	Arai and Inoue (1975)
C. triangulum	48	48	48t	2	Thailand	Boonsuk (2013)
C. trifasciatus	48	48	48t	-	Japan	Arai and Inoue (1975)
	48	48	48t	2	Thailand	Na Nongkhai (2014)
C. vagabundus	48	48	48t	-	Japan	Arai and Inoue (1975)
	48	48	48t	2	Thailand	Boonsuk (2013)
C. wiebeli	48	48	48t	2	Thailand	**Present study**

Remarks: 2*n* = diploid chromosome number, NF = fundamental number (number of chromosome arms), m = metacentric chromosome, sm = submetacentric chromosome, a = acrocentric chromosome, t = telocentric chromosome, NOR = nucleolar organizer region and - = not available.

Chaetodontids form one of the most colorful fishes of the coral reefs with a fantastic range in color and pattern. These are very popular as marine aquarium fishes. Commonly known as butterfly fish, *C. wiebeli* is distributed in Western Pacific (Japan to Thailand) including the Ryukyu Islands, Taiwan, the South China Sea and the Gulf of Thailand. Inhabits coral reefs at depths of 4 to 25 meters. The maximum total length about 18 cm. The species is recognized by its white and back coloration on the tail (Fig. 1).

Fig. 1. General characteristics of the Honhkong buterflyfish (*Chaetodon wiebeli*).

They are seen in pairs or several aggregations in coral reefs and juveniles are exported in large numbers to Western countries for aquarium trade, even though acclimatizing these fishes in aquarium conditions is often difficult (Allen 1985).

This present study aim to analysis the karyotype and chromosomal characteristics

of nucleolar organizer regions/ NORs in *C. wiebeli* by conventional staining and Ag-NORs banding techniques. We provide the first report on chromosome standardization, including chromosome measurements of shape and size, karyotype formulation and idiograming. The results obtained can provide more cytogenetic information for future studies on taxonomy and evolutionary relationships in this genus. Moreover, it provides useful basic information for conservation and breeding practices, as well as for studies on the chromosome evolution of this fish.

Materials and methods

Live specimens of *C. wiebeli* (n=4, two males and two females) were collected from Chumphon Province, Gulf of Thailand. Fishes were injected intramuscularly with 0.01% colchicine (1.0 mL/l00g body weight) to stop the nuclear division and maintained alive for one hour in a plastic tub. The specimens were then sacrificed and the kidney and gill tissues were processed for chromosome preparations using hypotonic treatment, acetic acid and methanol fixation, air-drying technique (Phimphan *et al.*, 2013; Pinthong *et al.*, 2017; Supiwong *et al.*, 2017c). The chromosome slides were stained with 10% Giemsa's. The chromosome slides were also stained by silver staining technique for detection of nucleolar organizer region (NORs). Ag-NORs banding was carried out according to the method of Howell and Black (1980) with minor modifications for getting crisp and clear banding effects. The chromosome pattern was determined by studying a minimum of 20 metaphase spreads per fish specimens. For karyotyping, the chromosomes were grouped as per the classification proposed by Turpin and Lejeune (1965). The length of short arm (Ls) and long arm (Ll) chromosome were calculated for the length of total arm chromosome (LT, LT = Ls+ Ll). Relative length (RL) and centromeric index (CI) were also calculated. All parameters were used in karyotyping and idiograming.

Results and discussion

The typical diploid metaphase chromosome complement in both male and female *C. wiebeli* containing 48 chromosomes. The fundamental number (NF, number of chromosome arm) was 48.

The chromosome type in this species was all telocentric chromosome in morphology (Fig. 2A and 2B), with all telocentric chromosomes seems to be characteristic of majority of marine species (Singh *et al*., 1997). Among the non-native fishes, approximately 60% of the perciform species so far studied show a karyotype of characterized by 48 uni-armed (telocentric) chromosomes (Galetti Jr. *et al*., 2000).

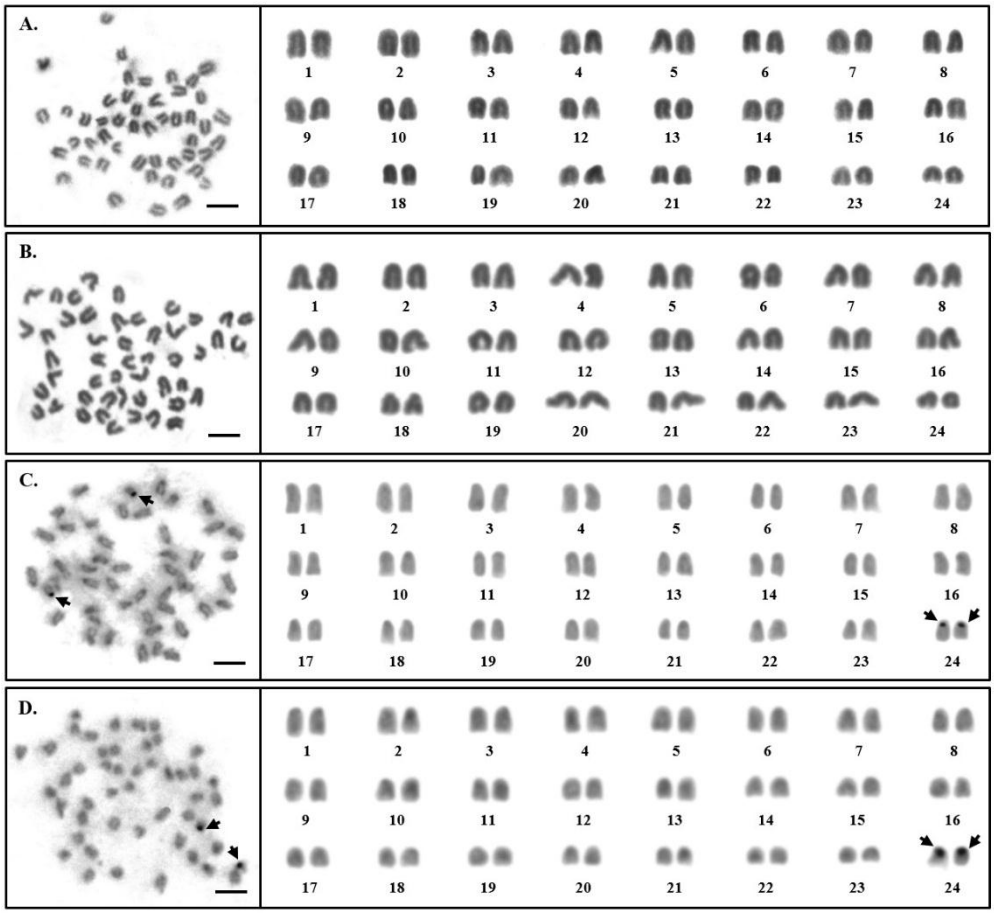

Fig. 2. Metaphase chromosome plates and karyotypes of male (A. and C.) and female (B. and D.) Hongkong buterflyfish (*Chaetodon wiebeli*), 2*n*=48 by conventional straining and Ag-NORs banding techniques. The arrows indicate nucleolar organizer regions/NORs (scale bars = 5 micrometers).

Presence of all uni- armed chromosomes has been considered as primitive character (Gold and Amemiya, 1986; Ozouf-costaz *et a1.*, 1997), hence *C. wiebeli* can be considered as primitive in the evolution order owing to the presence of 48 telocentric chromosomes.

The chromosome lengths in centimeters of 20 cells (males and females) in mitotic metaphase were measured. The Ls, Ll, LT, RL, CI, standard deviation of RL and CI, size and type of chromosome are presented in Table 2.

Table 2.

Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosomes (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphases of male and female Hongkong butterflyfish (*Chaetodon wiebeli*), 2*n*=48.

Chro. pair	Ls	Ll	LT	RL±SD	CI±SD	Chro. size	Chro. type
1	0.000	5.633	5.633	0.054±0.003	1.000±0.000	Large	Telocentric
2	0.000	5.490	5.490	0.053±0.001	1.000±0.000	Large	Telocentric
3	0.000	5.104	5.104	0.049±0.002	1.000±0.000	Large	Telocentric
4	0.000	5.092	5.092	0.049±0.001	1.000±0.000	Large	Telocentric
5	0.000	4.993	4.993	0.048±0.002	1.000±0.000	Large	Telocentric
6	0.000	4.803	4.803	0.046±0.003	1.000±0.000	Large	Telocentric
7	0.000	4.745	4.745	0.046±0.001	1.000±0.000	Large	Telocentric
8	0.000	4.689	4.689	0.045±0.002	1.000±0.000	Large	Telocentric
9	0.000	4.595	4.595	0.044±0.001	1.000±0.000	Large	Telocentric
10	0.000	4.527	4.527	0.044±0.001	1.000±0.000	Large	Telocentric
11	0.000	4.381	4.381	0.042±0.001	1.000±0.000	Medium	Telocentric
12	0.000	4.306	4.306	0.042±0.001	1.000±0.000	Medium	Telocentric
13	0.000	4.243	4.243	0.041±0.001	1.000±0.000	Medium	Telocentric
14	0.000	4.110	4.110	0.040±0.000	1.000±0.000	Medium	Telocentric
15	0.000	4.071	4.071	0.039±0.002	1.000±0.000	Medium	Telocentric
16	0.000	3.966	3.966	0.038±0.001	1.000±0.000	Medium	Telocentric
17	0.000	3.990	3.990	0.038±0.002	1.000±0.000	Medium	Telocentric
18	0.000	4.016	4.016	0.039±0.003	1.000±0.000	Medium	Telocentric

Chro. pair	Ls	Ll	LT	RL±SD	CI±SD	Chro. size	Chro. type
19	0.000	3.890	3.890	0.038±0.002	1.000±0.000	Medium	Telocentric
20	0.000	3.700	3.700	0.036±0.002	1.000±0.000	Medium	Telocentric
21	0.000	3.517	3.517	0.034±0.001	1.000±0.000	Medium	Telocentric
22	0.000	3.313	3.313	0.032±0.002	1.000±0.000	Medium	Telocentric
23	0.000	3.280	3.280	0.032±0.002	1.000±0.000	Medium	Telocentric
24*	0.000	3.205	3.205	0.031±0.003	1.000±0.000	Medium	Telocentric

Remarks: Chro. = chromosome and * = NORs bearing chromosomes (satellite chromosome).

The idiogram of *C. wiebeli* shows the gradually decreased length of the chromosomes (Fig. 3).

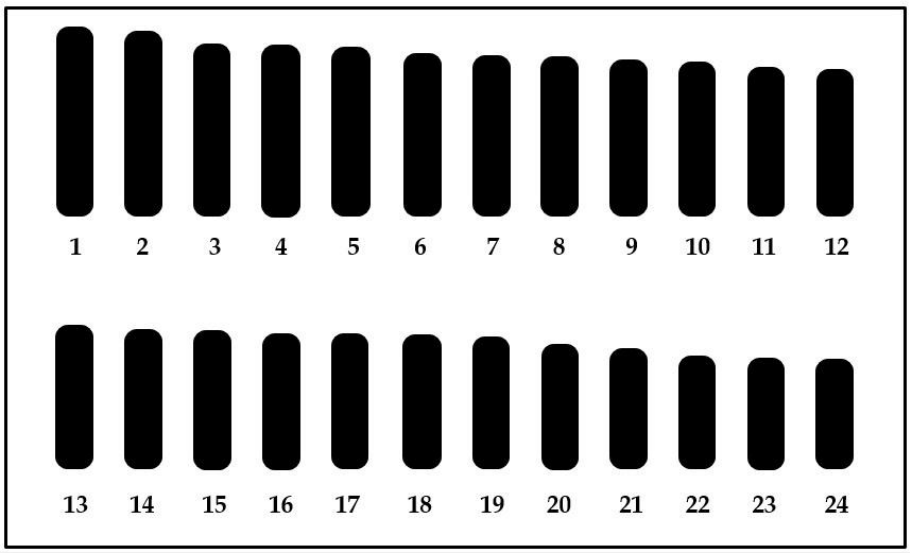

Fig. 3. Idiogram showing lengths and shapes of chromosomes of Hongkong buterflyfish (*Chaetodon wiebeli*), 2*n*=48 by conventional staining technique.

We suggest here that no cytologically distinguishable sex- chromosome was observed which is consistent to *C. andamanensis*, *C. auriga*, *C. auripes*, *C. collare*, *C. decussatus*, *C. lineolatus*, *C. lunula*, *C. ocellatus*, *C. sedentarius*, *C. striatus*, *C. triangulum*, *C. trifasciatus* and *C. vagabundus* (Arai and Inoue, 1975; Nagpure *et al.*, 2006; Boonsuk, 2013; Na Nongkhai, 2014; Supiwong *et al.*, 2017a, 2017b) and other fishes in the order Perciformes. It may be possible that the fish's sex- chromosomes are at the initiation of differentiation and hence these chromosomes which contain the sex determination gene cannot be detected by cytogenetic analyses. The origin and development of sex-chromosomes had been reported for Neotropical fish in Brazil (Bertollo *et al.*, 2004; Kaewmad *et al.*, 2014; Kasiroek *et al.*, 2017).

The development of silver staining technique (Howell and Black 1980) to detect metaphase chromosome sites of NORs has greatly facilitated comparative studies of NORs variation. Silver staining of NORs is considered as one of the standard banding methods and has assumed considerable importance in the characterization of a species karyotype. The results of NORs in *C. wiebeli* could be observed in one pair of chromosomes in both male and female at the near top (subcentomeric region) of the long arm of telocentric chromosome pair 24 (Figs. 2C and 2D). Their respective ideogram of the *C. wiebeli* from Ag-NOR banding technique is shown in Figure 4.

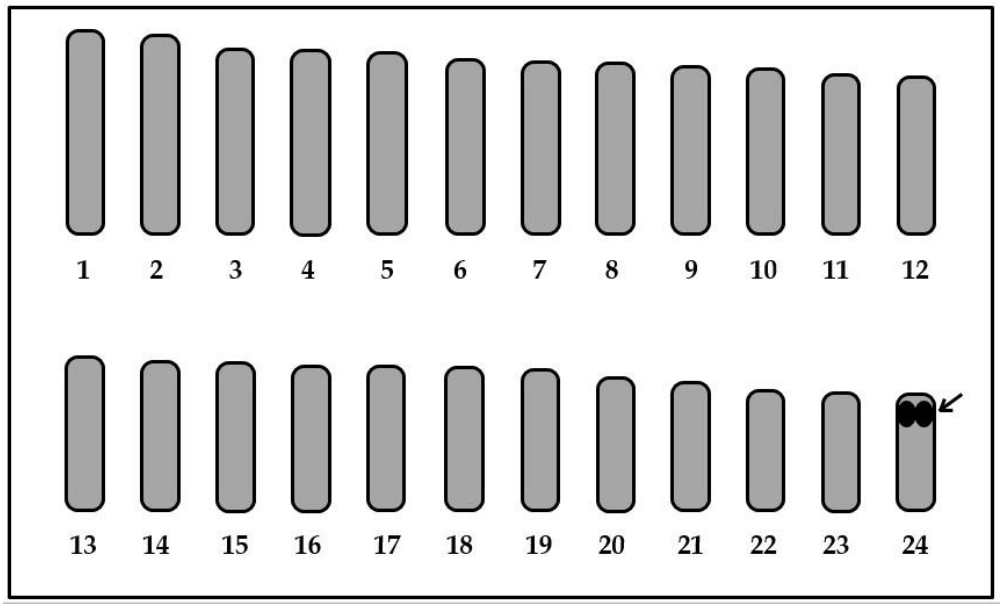

Fig. 4. Idiogram showing length and shape of chromosomes of Hongkong buterflyfish (*Chaetodon wiebeli*), 2*n*= 48 by Ag-NOR banding technique. The arrows indicate nucleolar organizer region/NOR.

No report has been published on the NORs of this species. Such studies are useful in resolving taxonomic ambiguities among closely related fish species and can also throw light on karyoevolution and speciation of the marine species. Cytogenetic markers have found useful for identification of intra-specific stocks and populations in some fish species (Phillips *et al.*, 1988) and can also aid in identification of putative hybrids between closely related species. Information on the cytogenetic markers can also be used for molecular cytogenetic assignment of genes on chromosomes (Donate *et al.*, 2003). To date, it seems to be no information on karyomorphology and chromosome banding studies. This study describes, for the first time, the karyotype and the localization of NORs in the *C. wiebeli* species from the Gulf of Thailand.

Acknowledgment

This work has supported by Department of Fundamental Science, Faculty of Science and Technology, Surindra Rajabhat University, Surin, Muang 32000, Thailand

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