พันธุศาสตร์เซลล์ของปลากะรังลายตุ๊กแก โดยการย้อมสีโครโมโซมแบบธรรมดาและแถบสีแบบนอร์

Cytogenetics of Greasy Grouper, *Epinephelus tauvina* (Perciformes, Serranidae) by Conventional Staining and Ag-NOR Banding Techniques

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บทคัดย่อ

แคริโอไทป์ และอิดิโอแกรมมาตรฐานของปลากะรังลายตุ๊กแก (*Epinephelus tauvina*) ใช้ตัวอย่างปลาจากศูนย์วิจัยและพัฒนาประมงชายฝั่งภูเก็ต เตรียมโครโมโซมจากไตด้วยวิธีเตรียมโดยตรง ย้อมสีโครโมโซมแบบธรรมดาและแถบสีแบบนอร์ ผลการศึกษาพบว่าปลากะรังลายตุ๊กแกมีจำนวนโครโมโซมดิพลอยด์ (2n) เท่ากับ 48 แท่ง มีจำนวนโครโมโซมพื้นฐาน (NF) เท่ากับ 48 ทั้งในเพศผู้และเพศเมีย แคริโอไทป์ประกอบด้วยโครโมโซมชนิดเทโลเซนทริกขนาดใหญ่ 26 แท่ง เทโลเซนทริกขนาดกลาง 20 แท่ง และเทโลเซนทริกขนาดเล็ก 2 แท่ง ไม่พบความแตกต่างของโครโมโซมเพศ โครโมโซมเครื่องหมาย คือ โครโมโซมที่พบตำแหน่งของนอร์อยู่บริเวณใกล้เซนโทรเมียร์ชนิดเทโลเซนทริกขนาดเล็กที่สุดคู่ที่ 24 ปลากะรังลายเสือมีสูตรแคริโอไทป์ ดังนี้ 2n (48) = $L^t_{26} + M^t_{20} + S^t_2$

คำสำคัญ: ปลากะรังลายตุ๊กแก, แคริโอไทป์, อิดิโอแกรม

Abstract

Standardized karyotype and idiogram of the greasy grouper (*Epinephelus tauvina*) from Phuket Coastal Fisheries Research and Development Center were studied. Renal cell samples were taken from kidney. The mitotic chromosome preparation was accomplished by the direct methods, followed by conventional staining and Ag-NOR-banding techniques. The results showed that diploid chromosome number was 2n=48 and the fundamental number (NF) were 48 in both males and females. The karyotype consists of 26 large telocentrics, 20 medium telocentrics, and 2 small telocentric chromosomes. No heteromorphic sex chromosomes were found in male and female. We also detected that NOR-bearing chromosome was located on the chromosome pair 24 (subcentromeric region). The karyotype formula of *E. tauvina* is as follows: 2n (48) = $L^t_{26} + M^t_{20} + S^t_2$

Keywords: *Epinephelus tauvina,* karyotype, idiogram

Introduction

Thailand is one of the world richest places of biodiversity of animals and plants. With more than 24,000 species recorded, Thailand is one of the fresh water and marine fish species diversity centers of the world. There are more than 13,000 and 4,000 species of fishes that live in sea and coralline, respectively. Moreover, it is well known that fish is the highest species diversity (Tamrongnawasawad *et al*., 2004).

The greasy grouper (*Epinephelus tauvina*) is member of the family Serranidae. It is wildly distributed in both of marine and brackish water. The fish grows up to 75 cm in length. Its head and body are pale greenish grey or brown with round spots, varying from orange-red to dark brown. A group of black spots may be visible on the body at the base of the rear of the dorsal fin (Fig. 1) (Heemstra and Randall, 1993).

Figure 1 General characteristics of greasy grouper (*Epinephelus tauvina*), scale bar indicate 5 cm.

The study of fish and other aquatic animal chromosomes has become an active area of research in recent decades. Karyological studies have provided basic information on the diploid chromosome number (2n), fundamental number (NF, number of chromosome arms), sex determination and morphology of chromosomes. It may be useful for addressing a variety of evolutionary and genetic questions about animals and may permit a detection of changes that modified an ancestral karyotype as it evolved into new lines. These studies are also an important step towards the establishment of genetic improvement techniques involved in chromosome manipulation techniques, including inter or intra-species hybridizations, sex determination, gynogenesis, androgenesis and induction of polyploidy. These genetic techniques have been widely applied to improve farmed stocks in many aquaculture species in the world (Na-Nakhon, 2000).

Cytogenetic study of *E. tauvina* is the only one previous report (Rodríguez-daga *et al*., 1993), that the diploid chromosome number (2*n*) was 48 chromosomes. The fundamental number (NF) was 50. Moreover, the standardizations of chromosome including karyotyping and idiograming

have not been studied in any previous reports. It is interestingly that there were few cytogenetic studies of *E. tauvina*. Moreover, there is no record of cytogenetics of this species in Thailand. Accordingly, the present study aimed at cytogenetical analyzing of *E. tauvina* by conventional staining and Ag-NOR banding techniques. In this study, the nucleolar organizer region (NOR) in *E. tauvina* by Ag-NOR banding technique was presented for the first time.

Research methodology

Sample collection

The five males and five females of *Epinephelus tauvina* were collected from Phuket Coastal Fisheries Research and Development Center, Phuket, Thailand.

Chromosome preparation

The fish were transferred to laboratory aquaria and were kept under standard conditions for seven days prior to the experiments. The experiments followed ethical protocols, and anesthesia with clove oil was administered prior to sacrificing the animals to minimize suffering. Mitotic chromosomes were obtained from cell suspensions of the anterior kidney, using the conventional air-drying method (Chen and Ehbeling, 1968; Nanda *et al*., 1995). The specimens were deposited in the fish collection of the Cytogenetic Laboratory, Department of Biology, Faculty of Science, Khon Kaen University.

Chromosome staining

The chromosomes were stained accomplished with the two following techniques.

1. Conventional staining technique

The mixture was dropped onto a clean and cold slide by micropipette following by the direct methods. The slide was conventionally stained with 20% Giemsa's solution for 30 minutes (Rooney, 2001).

2. Ag-NOR banding technique

The two drops of each 50% silver nitrate and 2% gelatin were added on slide, respectively. Then it was sealed with cover glass and incubated at 60 °C for 5 minutes following Howell and Black (1980). After that it was soaked in distilled water until the cover glass was separated.

Karyotype analysis

Chromosome counting was performed on mitotic metaphase cells under light microscope. Twenty clearly observable and well spread chromosomes of each male and female were selected and photographed. The length of short arm chromosome (Ls) and long arm chromosome (Ll) were

measured and calculated to the length of total arm chromosome (LT, LT = Ls+Ll). The relative length (RL), the centromeric index (CI) and standard deviation (SD) of RL and CI were estimated. CI was also computed to classify the types of chromosomes according to Chaiyasut (1989) and Chen and Ebeling (1968). All parameters were used in karyotyping.

Results and discussion

Karyological analysis of the *E. tauvina* using direct methods revealed that the chromosome number was 2n (diploid) = 48 in both males and females (Fig. 2). This number was the same with the previous report of Rodríguez-daga *et al.* (1993). The types of chromosome were 26 large, 20 medium, and 2 small telocentrics (Table 1). It is inconsistent to the report of Rodríguez-daga *et al.* (1993) that revealed the chromosomes of *E. tauvina* were as follow: 2 submetacentric and 46 telocentrics. The NF of *E. tauvina* was 48 in both males and females, which differ from the report of Rodríguez-daga *et al.* (1993) that demonstrated the NF of 50.

According to the previous reports, there were total 26 species of the genus *Epinephelus* which have been studied. All of them had the number of diploid chromosome of 48, however, they had different fundamental number (NF) which can be divided into three groups those with:1) NF=48 with all telocentrics 2) NF=50–56 with metacentrics, submetacentrics, and telocentrics and 3) NF=62 with metacentrics, submetacentrics, acrocentrics, and telocentrics (Table 2). Because of chromosome aberrations such as fission, fusion, and pericentric inversion, fishes in the genus *Epinephelus* have a variety of chromosome types (Pinthong *et al.*, 2013). Similar to other species in family Epinephelidae, no cytologically distinguishable sex chromosome was observed (Pinthong *et al.*, 2013). This suggested that the fish's sex-chromosomes were at the initiation of differentiation and hence these chromosomes which contained the sex-determination gene could not be detected by conventional cytogenetic analyses (Na-Nakron, 2000).

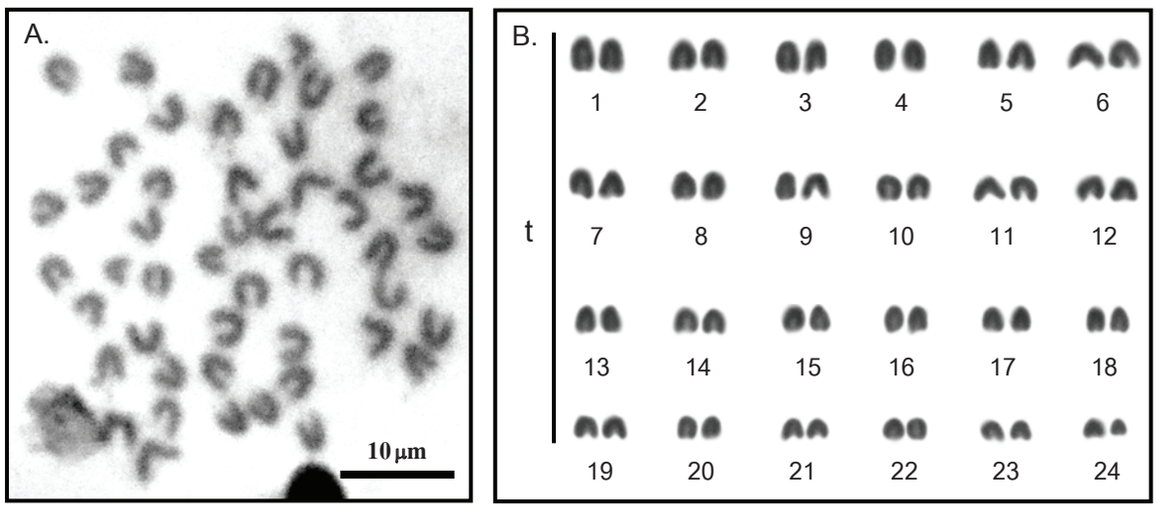

Figure 2 Metaphase chromosome plate (A.) and karyotype (B.) of greasy grouper (*Epinephelus tauvina*), 2n=48 by conventional staining technique.

The results which were obtained from Ag-NOR banding showed that *E. tauvina* had the clearly observable NOR-bearing chromosome (Fig. 3); on the smallest telocentric chromosome pair 24 in the subcentromeric region (SCR). This was the first study to reveal Ag-NOR position of *E. tauvina*. This feature was consumed in fish of *Epinephelus* in *E. tauvina*, *E. alexandrinus*, *E. guaza*, *E. caninus*, *E. fasciatomaculatus*, *E. fasciatus*, *E. marginatus*, *E. akaara* and *E. adscensionis*. However, NORs were located on the secondary constriction in the subcentromeric region (SC) in short arms in *E. awoara*; telomeric region (TR) in *E. malabaricus* and shot arm in *E. guttatus*, *E. bruneus*, and *E. coioides* (Table 2).

For *E. tauvina* the chromosome markers are chromosome pair 1 and pair 24 which are the largest telocentric chromosome and the smallest telocentric chromosome, respectively. The important karyotype features the asymmetrical karyotype, which was found in only one type of the telocentric chromosomes. The largest chromosome is two times larger than the smallest chromosomes. The idiogram showed gradually decreasing length of the chromosomes (Fig. 4). The karyotype formula of *E. tauvina* could be deduced as:

$$2n\ (48) = L^{t}_{26} + M^{t}_{20} + S^{t}_{2}$$

Remarks: L = large chromosome, M = medium chromosome, S = small chromosome, 2*n* = diploid chromosome number, and t = telocentric chromosome.

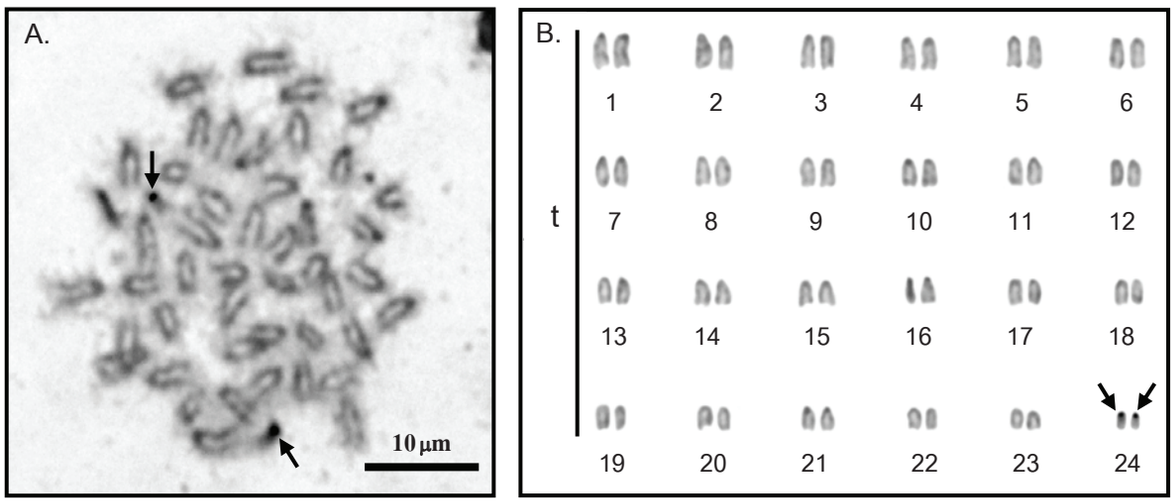

Figure 3 Metaphase chromosome plate (A.) and karyotype (B.) of greasy grouper (*Epinephelus tauvina*), 2*n*=48 by Ag-NOR banding technique, arrows indicate nucleolar organizer regions/NORs.

Conclusions

Standardized karyotype and idiogram of the *E. tauvina* accomplished by the direct methods, followed by conventional staining and NOR-banding techniques showed that the number of diploid chromosome was 48 (NF=48) in both males and females. The karyotype consisted of 26 large, 20 medium, and 2 small telocentric chromosomes. No heteromorphic sex chromosomes were observed in male and female. Moreover, we also suggest that the detection of the NOR-bearing chromosome on the smallest telocentric chromosome pair 24 of greasy grouper was the first report. Accordingly, the cytogenetic study of greasy grouper in which we have conducted was the first to utilize the Ag-NOR banding technique.

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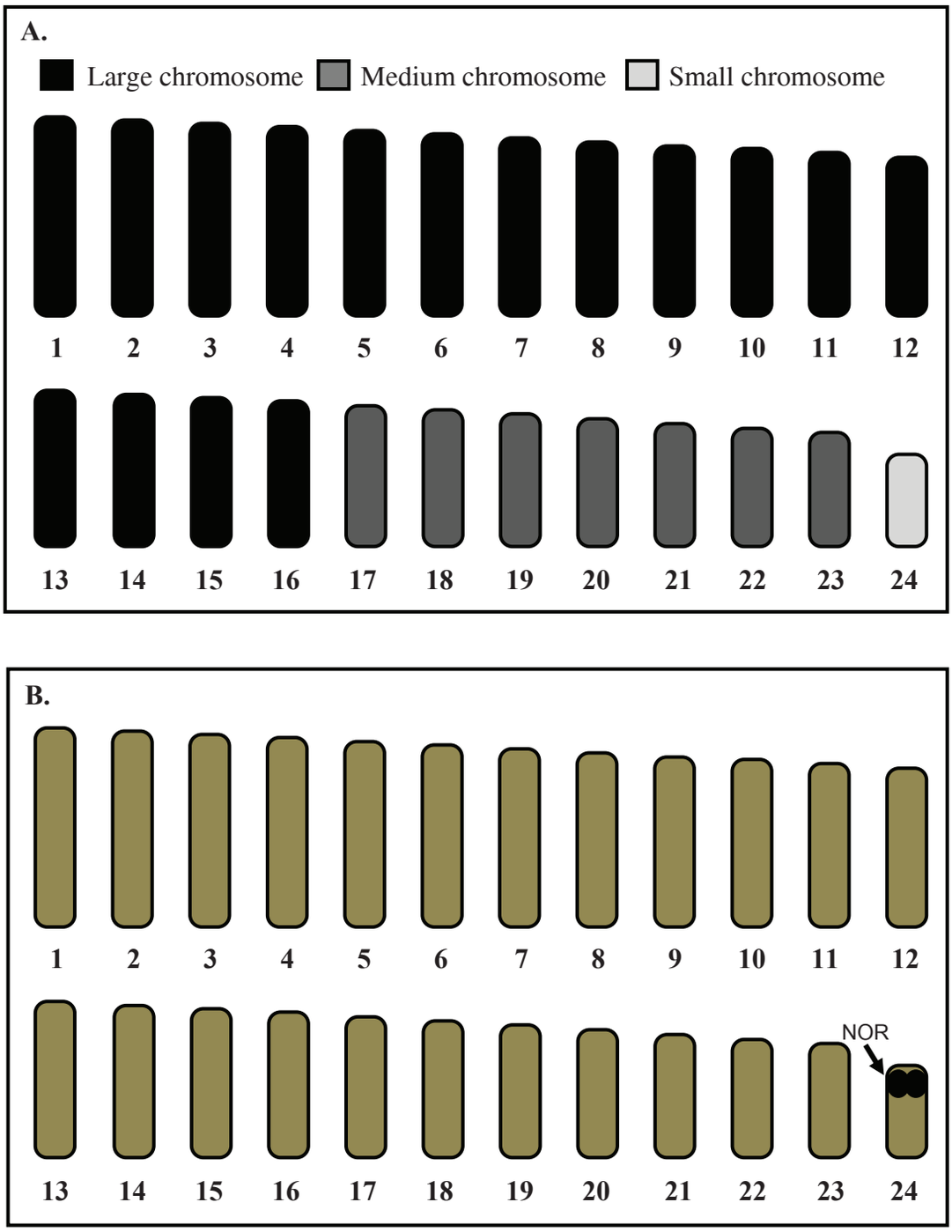

Figure 4 Idiograms of the greasy grouper (*Epinephelus tauvina*), 2n=48; by conventional staining (A.) and Ag-NOR banding techniques (B.), arrow indicates nucleolar organizer region.

Table 1 Mean of length 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 and CI from metaphase chromosome of 20 cells in greasy grouper (*Epinephelus tauvina*), 2n=48.

Chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	Size	Type
1	0.0000	1.3375	1.3375	0.0557±0.0018	1.0000±0.0000	Large	Telocentric
2	0.0000	1.2850	1.2850	0.0535±0.0013	1.0000±0.0000	Large	Telocentric
3	0.0000	1.2560	1.2560	0.0523±0.0010	1.0000±0.0000	Large	Telocentric
4	0.0000	1.2295	1.2295	0.0512±0.0010	1.0000±0.0000	Large	Telocentric
5	0.0000	1.2040	1.2040	0.0501±0.0010	1.0000±0.0000	Large	Telocentric
6	0.0000	1.1820	1.1820	0.0492±0.0009	1.0000±0.0000	Large	Telocentric
7	0.0000	1.1600	1.1600	0.0483±0.0040	1.0000±0.0000	Large	Telocentric
8	0.0000	1.1350	1.1350	0.0472±0.0040	1.0000±0.0000	Large	Telocentric
9	0.0000	1.1135	1.1135	0.0463±00006	1.0000±0.0000	Large	Telocentric
10	0.0000	1.0855	1.0855	0.0452±0.0006	1.0000±0.0000	Large	Telocentric
11	0.0000	1.0590	1.0590	0.0441±0.0005	1.0000±0.0000	Large	Telocentric
12	0.0000	1.0340	1.0340	0.0430±0.0008	1.0000±0.0000	Large	Telocentric
13	0.0000	1.0135	1.0135	0.0422±0.0008	1.0000±0.0000	Large	Telocentric
14	0.0000	0.9900	0.9900	0.0412±0.0007	1.0000±0.0000	Large	Telocentric
15	0.0000	0.9710	0.9710	0.0404±0.0006	1.0000±0.0000	Large	Telocentric
16	0.0000	0.9470	0.9470	0.0394±0.0007	1.0000±0.0000	Large	Telocentric
17	0.0000	0.9225	0.9225	0.0384±0.0008	1.0000±0.0000	Medium	Telocentric
18	0.0000	0.9005	0.9005	0.0375±0.0007	1.0000±0.0000	Medium	Telocentric
19	0.0000	0.8747	0.8747	0.0364±0.0010	1.0000±0.0000	Medium	Telocentric
20	0.0000	0.8460	0.8460	0.0352±0.0010	1.0000±0.0000	Medium	Telocentric
21	0.0000	0.8125	0.8125	0.0338±0.0013	1.0000±0.0000	Medium	Telocentric
22	0.0000	0.7875	0.7875	0.0328±0.0017	1.0000±0.0000	Medium	Telocentric
23	0.0000	0.7640	0.7640	0.0318±0.0017	1.0000±0.0000	Medium	Telocentric
24*	0.0000	0.6490	0.6490	0.0270±0.0020	1.0000±0.0000	Small	Telocentric

Remark: * = NOR-bearing chromosome.

Table 2 Review of cytogenetic reports of the genus *Epinephelus* (Perciformes, Serranidae).

Genus/Species	2n	NF	Karyotype	Ag-NORs	Reference
Epinephelus tauvina	48	50	2sm+46t	–	Rodríguez-daga *et al*. (1993)
	48	48	48t	24(SCR)	Present study
E. fuscoguttalus	48	50	2sm+46t	–	Liao *et al*. (2006)
E. adscensionis	48	48	48t	24(SCR), 2(TR)	Molina *et al*. (2002)
E. diacanthus	48	50	2sm+46t	–	Natarajan and Subrahmanyan (1974)
E. awoara	48	48	48t	24(SC)	Hong and Yang (1988)
E. guttatus	48	48	48t	24(SA)	Medrano *et al*. (1988)
E. guaza	48	48	48t	24(SCR)	Martinez *et al*. (1989)
E. alexandrinus	48	48	48t	24(SCR)	Martinez *et al*. (1989)
E. sexfasciatus	48	50	2sm+46t	–	Chen *et al*. (1990)
E. caninus	48	48	48t	24(SCR)	Rodríguez-daga *et al*. (1993)
E. fasciatomaculatus	48	48	48t	24(SCR)	Li and Peng (1994)
E. fasciatus	48	48	48t	24(SCR)	Li and Peng (1994)
E. marginatus	48	48	48t	24(SCR)	Aguilar and Galetti Jr. (1997)
	48	48	48t	24(SCR)	Martinez *et al*. (1989)
	48	48	48t	24(SCR), 2(TR)	Sola *et al*. (2000)
	48	50	2sm+46t	–	Wei *et al*. (2009)
E. malabaricus	48	48	48t	24(TR)	Ueno and Jarayabhand (1991)
E. akaara	48	48	48t	24(SCR), 5(?)	Zou *et al*. (2005)
	48	48	5a+43t	–	Wang *et al*. (2004)
E. fario	48	62	4m+6sm+4a+34t	–	Zheng *et al*. (2005)
E. merra	48	62	4m+6sm+ 4a+34t	–	Zheng *et al*. (2005)
E. moara	48	48	48t	–	Guo *et al*. (2006)
E. bruneus	48	54	2m+4sm,42t	2, 24, 9(SA)	Guo *et al*. (2008)
E. coioides	48	50	2sm+46t	24(SA)	Shifeng *et al*. (2010)
E. ongus	48	48	48t	–	Rishi and Haobam (1984)
E. lanceolatus	48	56	8sm+40t	–	Jiun and Mei (2009)

Remarks : 2*n* = diploid chromosome number, NF = fundamental number (number of chromosome arm), m = metacentric, sm = submetacentric, a = acrocentric, t = telocentric chromosome, NORs = nucleolar organizer regions, SC = the secondary constriction in the subcentromeric region, SA = short arm, TR = telomeric region, SCR = subcentromeric region, and - = not available.

References

Aguilar, C. T. and Galetti Jr., P. M. 1997. Chromosomal studies in South Atlantic serranids (Pisces, Perciformes). Cytobios 89: 105-114.

Bertollo, L. A. C., Oliveira, C., Molina, W. F., Margarido, V. P., Fontes, M. S. M., Pastori, C., Falcão, J. N. and Fenocchio, A. S. 2004. Chromosome evolution in the erythrinid fish, *Erythrinus erythrinus* (Teleostei: Characiformes). Heredity 93: 228-233.

Chaiyasut, K. 1989. Cytogenetics and Cytotaxonomy of the Family Zephyranthes. Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok.

Chen, T. R. and Ebeling, A. W. 1968. Karyological evidence of female heterogamety in the mosquito fish, *Gambusia affinis*. Copeia 1: 70-75.

Guo, F., Wang, J., Su, Y. Q., Wang, D. X. and Xu, L. N. 2006. Study on the karpyotype of *Epinephelus moara*. Marine Sciences 8: 1-3.

Guo, M., Wang, S., Su, Y. and Ding, S. 2008. The cytogenetic studies of the longtooth (*Epinephelus bruneus*). Journal of Biotechnology 136S: S527-S540.

Heemstra, P. C. and Randall, J. E. 1993. FAO Species Catalogue. 6th ed. Rome, Italy.

Hong, M. X. and Yang, J. H. 1988. Studies on the karyotypes of *Epinephelus awoara*. Journal of Xiamen University (Natural Science) 27: 714-715.

Howell, W. M. and Black, D. A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36: 1014-1015.

Jiun, C. S. and Mei, C. T. 2009. Karyotype Identification of *Epinephelus lanceolatus*. 4th Academic Seminar, Department of Aquaculture, National Pintung University of Science and Technology, pp. F33-37.

Liao, J. Q., Yin, S. W., Chen, G. H., Huang, H., Lei, C. G. and Lou, T. T. 2006. The karyotype of grouper *Epinephelus fuscoguttatus*. Fisheries science 25: 567-569.

Li, X. Q. and Peng, Y. D. 1994. Studies on karyotype of *Epinephelus Fasciatomaculatus* and *Epinephelus fasciatus*. Journal of Zhanjiang Fisheries College 14: 22-26.

Martinez, G., Thode, G., Alvarez, M. C. and Lopez, J. R. 1989. C-banding and Ag-NOR reveal a certain heterogeneity among karyotypes of serranids (Perciformes). Cytobios 58: 53-60.

Medrano, L., Bernardi, G., Couturier, J., Dutrillaux, B. and Bernardi, G. 1988. Chromosome banding and genome compartimentalization in fishes. Chromosoma 96: 178-183.

Molina, W. F., Maia-Lima, F. A. and Affonso, P. R. A. M. 2002. Divergence between karyotypical pattern and speciation events in Serranidae fish (Perciformes). Caryologia 55: 299-305.

Na-Nakhon, U. 2000. Cytogenetics of aquatic animals. 2nd ed. Kasetsart University, Bangkok.

Natarajan, R. and Subrahmanyan, K. 1974. A karyotype study of some teleosts from Portonovo waters. Proseedings of the Indian Acadamy of sciences 79: 173-196.

Pinthong, K., Gomontean, B., Kongim, B., Khakhong, S., Sriveerachai, T. and Supiwong, W. 2013. Cytogenetic comparison and chromosome localization of the nucleolar organizer region of four grouper genera (Pisces, Epinephelinae) from Thailand. Cytologia 78(3): 223-234.

Rishi, K. K. and Haobam, M. S. 1984. Karyological analysis of two marine fishes. Perspectives in Cytology and Genetics 4: 425-428.

Rodríguez-Daga, R., Amores, A. and Thode, G. 1993. Karyotype and nucleolus organizer regions in *Epinephelus caninus* (Pisces, Serranidae). Caryologia 46: 71-76.

Rooney, D. E. 2001. Human Cytogenetics: Constutitional Analysis: a Practical Approach. Oxford University Press, London.

Shifeng, S. W., Yongquan, S., Shaoxiong, D., Yan, C. and Jun, W. 2010. Cytogenetic analysis of orange–spotted grouper, *Epinephelus coioides*, using chromosome banding and fluorescence in situ hybridization. Hydrobiologia 638: 1-10.

Sola, L., Innocentiis, S. D., Gornung, E., Papalia, S., Rossi, A. R., Marino, G., Marco, P. D. and Cataudella, S. 2000. Cytogenetic analysis of *Epinephelus marginatus* (Pisces: Serranidae), with the chromosome localization of the 18S and 5S rRNA genes and of the $(TTAGGG)_n$ telomeric sequence. Marine Biology 137: 47-51.

Tamrongnawasawad, T., saisaeng, A., Temboonkiet, B. and Sumanatemeya, N. 2004. Marine fish of Thailand. 2nd ed. Banpra-Artith press, Bangkok.

Ueno, K. and Jarayabhand, P. 1991. Karyotype of a seranid, *Epinephelus malabaricus*, and a centropomid, *Psammoperca waigiensis* (Pisces, Perciformes). Vol. 51. Chromosome information service, Society of chromosome research, Higashi–Hiroshima, Japan, pp. 23-25.

Wang, Y. X., Wang, H. D., Zhang, H. F. and Liu–Fu, Y. Z. 2004. Karyotypes of *Epinephelus coioides* and *Epinephelus akaara*. Journal of Zhanjiang Ocean University 24: 4-8.

Wei, Y., Fan, T., Jiang, G., Sun, A., Xu, X. and Wang, J. 2009. Establishment of a novel fin cell line from brown-marbled grouper, *Epinephelus fuscoguttatus* (Forsskal), and evaluation of its viral susceptibility. Aquaculture Research 40: 1523-1531.

Zheng, L., Liu, C., W. and Li, C. L. 2005. Studies on the karyotype of 4 groupers. Marine Science 29: 51-55.

Zou, J. X., Yu, Q. X. and Zhou, F. 2005. The karyotypes C-bandspatterns and Ag-NORs of *Epinephelus malabaricus*. Journal of Fisheries of China 29: 33-37.