 Cytologia **80**(1): 111–116

First Karyological Analysis of the Vermiculate Spinefoot, *Siganus vermiculatus* (Perciformes, Siganidae) from Thailand

Sarun Jumrusthanasan[1], Weerayuth Supiwong[2], Aphinun Suvarnaraksha[3], Wirat Jiwyam[2], Krit Pinthong[4] and Alongklod Tanomtong[1]*

[1] Applied Taxonomic Research Center (ATRC), Department of Biology, Faculty of Science, Khon Kaen University, Muang, Khon Kaen, 40002, Thailand
[2] Faculty of Applied Science and Engineering, Khon Kaen University, Nong Khai Campus, Muang, Nong Khai 43000, Thailand
[3] Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Sansai, Chiangmai 50290, Thailand
[4] Biology Program, Faculty of Science and Technology, Surindra Rajabhat University, Muang, Surin 32000, Thailand

Received May 24, 2014; accepted November 7, 2014

Summary Standardized karyotype and idiogram of the vermiculate spinefoot, *Siganus vermiculatus* (Valenciennes, 1835) from Andaman Sea, Southern Thailand were established. The mitotic chromosome preparations were prepared directly from kidney cells of two male and two female fish. The chromosomes were stained by using conventional and Ag-NOR banding techniques. Its diploid chromosome number was $2n$=48 and the fundamental number (NF) was 50 in both males and females. The types of chromosomes were 2 large acrocentric, 30 large telocentric, 12 medium telocentric, and 4 small telocentric chromosomes. No strange size chromosomes related to sex were observed. The region adjacent to the telomere of the short arm of chromosome pair 1 showed clearly observable nucleolar organizer regions (NORs). The karyotype formula could be inferred as:

$2n$ (diploid) $48=L^{a}_{2}+L^{t}_{30}+M^{t}_{12}+S^{t}_{4}$

Key words Vermiculate spinefoot, *Siganus vermiculatus*, Chromosome, Karyotype.

Marine fishes are especially important as they provide a high quality source of protein as well as food source for people who live near the coast. To date, about 8% of the species in the order Perciformes have been karyotyped, revealing a model diploid chromosome number of $2n$=48 (Le Grande and Fitzsimons 1988, Affonso *et al.* 2001). However, karyotypes difference from the typical order Perciformes pattern have frequently been detected, including Robertsonian rearrangements as the preferential process in some groups (Ueno and Takai 2000, Molina and Galetti 2002).

The family Siganidae (rabbitfishes) is classified into the order Perciformes. The genus *Siganus* is the only one member of the family. It comprises 21 species, namely, *S. argenteus*, *S. corallines*, *S. doliatus*, *S. canaliculatus*, *S. fuscescens*, *S. guttatus*, *S. javus*, *S. labyrinthodes*, *S. lineatus*, *S. niger*, *S. puellus*, *S. punctatus*, *S. punctatissimus*, *S. randalli*, *S. spimus*, *S. stellatus*, *S. unimaculatus*, *S. uspi*, *S. vergatus*, *S. vermiculatus*, and *S. vulpinus*. Rabbitfishes are plant feeders, sometimes forming large schools as they roam over the reef. They are also sometimes called "spinefeet" in reference to the unusual arrangement of two pelvic-fin spines separated by three soft rays. Another peculiarity is the high (seven) number of anal fin spines. All dorsal, anal and pelvic

* Corresponding author, e-mail: tanomtong@hotmail.com
DOI: 10.1508/cytologia.80.111

Table 1. Cytogenetic reviews of fishes in the family Siganidae (Perciformes).

Species	2*n*	NF	Karyotype formula	NOR	Locality	Reference
Siganus vermiculatus	48	50	2a+46t	pair 1 (SA)	Thailand	Present study
S. fuscescens	48	50	2a+46t	—	Japan	Kitada *et al.* (1979)
S. javus	48	48	48t	—	India	Choudhury *et al.* (1979)
S. spinus	42	48	6m+36t	—	Japan	Ojima and Yamamoto (1990)

Remarks: 2*n*=diploid chromosome number, NF=fundamental number, NOR=nucleolar organizer region, m=metacentric, a=acrocentric, t=telocentric chromosome, SA=short arm chromosome, and — =not available.

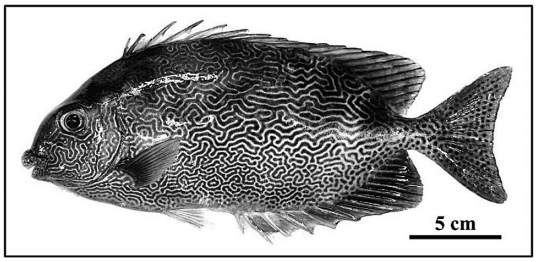

Fig. 1. General characteristic of the vermiculate spinefoot, *Siganus vermiculatus* (Valenciennes, 1835) from Andaman Sea, Thailand.

fin spines are grooved and contain venom glands. If handled carelessly, they are capable of inflicting very painful wounds (Allen *et al.* 1999).

Up to the present, it is interesting that there were few cytogenetic studies of fishes in the family Siganidae. Moreover, there are scarce records of cytogenetics of this family (Table 1). In this article, we report the karyotype and other chromosomal markers such as Ag-stained nucleolar organizer region (Ag-NOR) in *S. vermiculatus* population from Andaman Sea, Southern Thailand. In the future, the obtained knowledge on basic cytogenetics could be applied to numerous breeding studies and also provide advantage in the species conservation and chromosome evolution studies.

Materials and methods

Two males and two females of *S. vermiculatus* were obtained from Andaman Sea, Southern Thailand (Fig. 1). The fish were transferred to laboratory aquaria and were kept under standard conditions for 7 d prior to the experiments. Procedures for fish chromosome were prepared directly from kidney cells (Chen and Ehbeling 1968, Nanda *et al.* 1995). The chromosome preparations were stained with 10% Giemsa's for 30 min and NORs were identified by Ag-NOR staining (Howell and Black 1980). 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.

Results and discussion

The present study revealed that the somatic chromosome number of *S. vermiculatus* is 2*n*=48, and the NF was 50 in both males and females. The types of chromosomes were 2 large acrocentric,

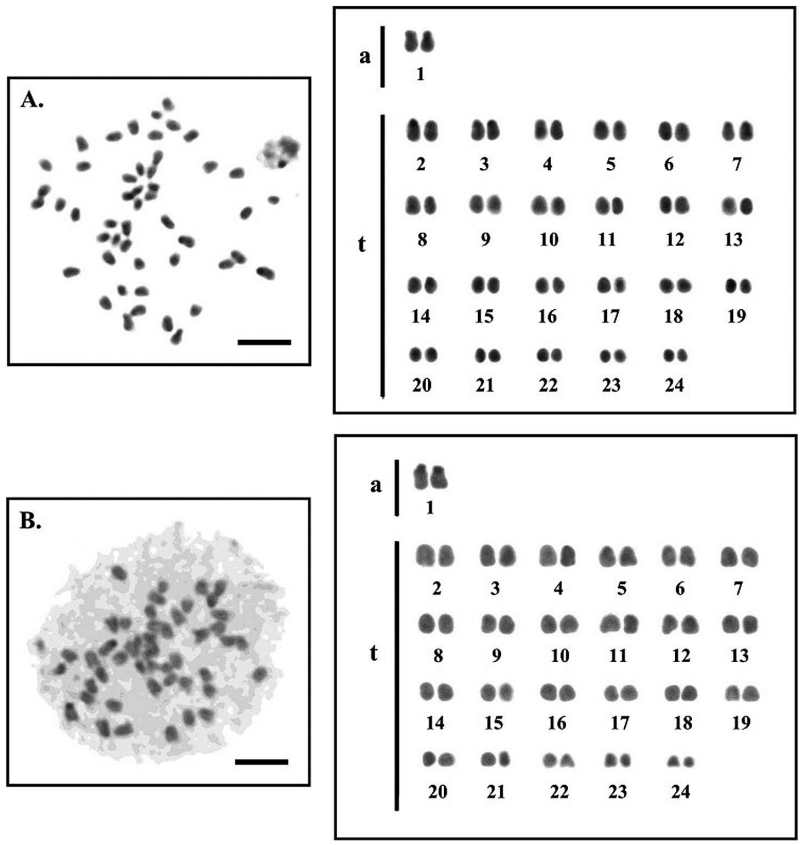

Fig. 2. Metaphase chromosome plates and karyotypes of the male (A) and female (B) vermiculate spinefoot (*Siganus vermiculatus*), 2*n* (diploid)=48 by conventional staining technique. Scale bars indicate 10 μm.

30 large telocentric, 12 medium telocentric, and 4 small telocentric chromosomes (Fig. 2). To our knowledge, this is the first karyological study of *S. vermiculatus*. Compared with other studies in the family Siganidae (genus *Siganus*), *S. vermiculatus* has a different chromosome number and fundamental number; for instance, in *S. spinus*, 2*n*=42 (NF=48) (Ojima and Yamamoto 1990); *S. fuscescens*, 2*n*=48 (NF=50) (Kitada *et al.* 1979), and *S. javus*, 2*n*=48 (NF=48) (Choudhury *et al.* 1979). A karyotype with 2*n*=48 is considered as ancestral condition for the Teleosts (Ohno 1974), and occurs in 211 of the 660 Perciformes species analyzed so far (Klinkhardt *et al.* 1995).

Similar to other species in the family Siganidae, no cytologically distinguishable sex chromosome was observed (Choudhury *et al.* 1979, Kitada *et al.* 1979, Ojima and Yamamoto 1990). It is possible that the fish's sex-chromosomes are in the initiation phase of differentiation and hence these chromosomes which contain the sex-determination gene cannot be detected by cytogenetic analyses (Na-Nakron 2000). The origin and development of sex-chromosomes have been reported for neotropical fish in Brazil (Bertollo *et al.* 2004).

Cytogenetic study of *S. vermiculatus* was accomplished here by using the Ag-NOR staining technique. The region adjacent to the telomere of short arm of chromosome pair 1 showed clearly observable nucleolar organizer regions/NORs (Figs. 3 and 5). The objective of this technique was to define the nucleolar organizer regions representing the location of genes (loci) functioning in ribosome synthesis (18S and 28S ribosomal RNA). Normally, most fishes have only one pair of small NORs on chromosomes. If some fishes have more than two NORs, it may be caused by the

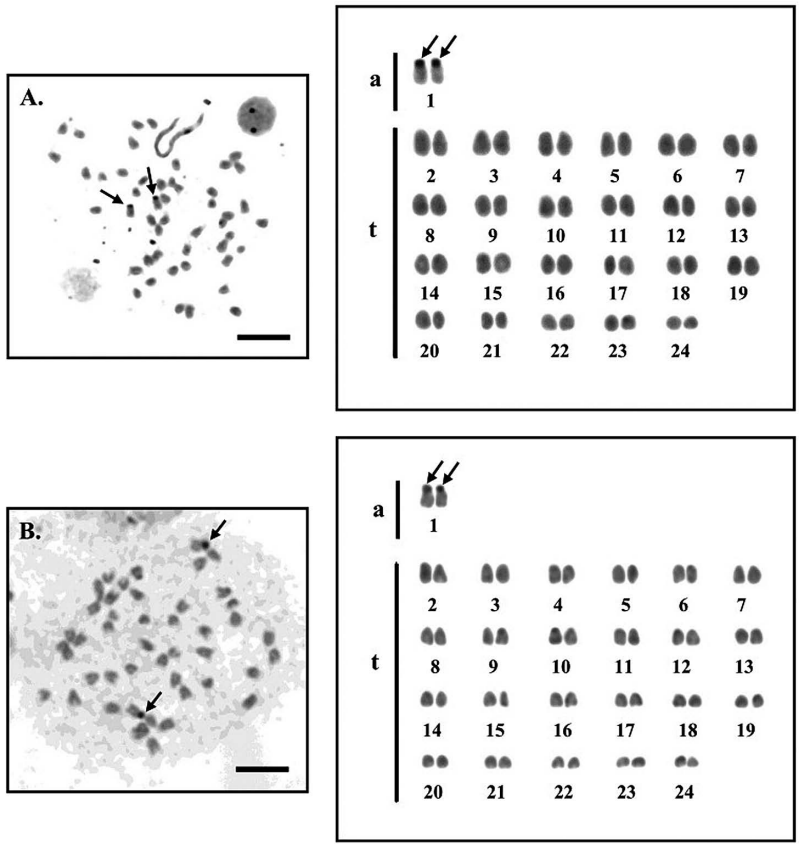

Fig. 3. Metaphase chromosome plates and karyotypes of the male (A) and female (B) vermiculate spinefoot (*Siganus vermiculatus*), 2*n* (diploid)=48 by Ag-NOR banding technique. Arrows indicate nucleolar organizer regions/NORs (scale bars = 10 μm).

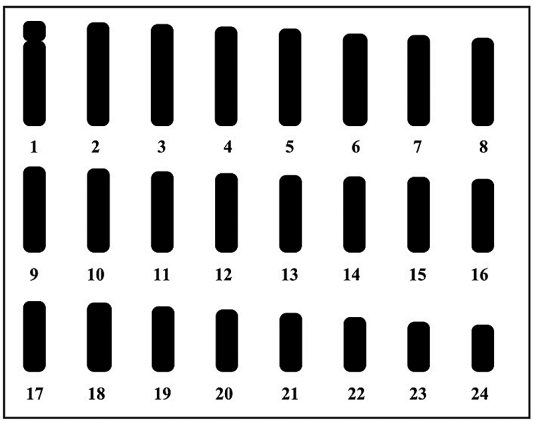

Fig. 4. Idiogram showing lengths and shapes of chromosomes of the vermiculate spinefoot (*Siganus vermiculatus*), 2*n* (diploid)=48, by conventional staining technique.

translocation between some parts of chromosomes which have NOR and another chromosome. Furthermore, NOR is usually located close to the telomere of the chromosome arm. If NOR appears between the centromere and telomere (interstitial NOR), it may be the result of the tandem fusion

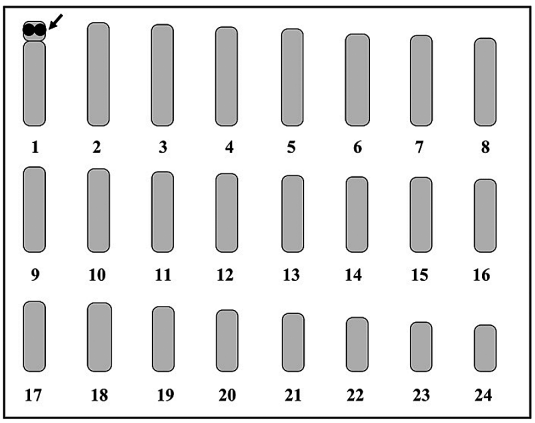

Fig. 5. Idiogram of the vermiculate spinefoot (*Siganus vermiculatus*), 2*n* (diploid)=48, by Ag-NOR banding technique. Arrow indicates nucleolar organizer region (NOR).

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 vermiculate spinefoot (*Siganus vermiculatus*), 2*n*=48.

Chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	Chromosome size	Chromosome type
1*	0.95	2.14	3.09	0.055±0.004	0.693±0.007	Large	Acrocentric
2	0.00	3.07	3.07	0.055±0.002	1.000±0.000	Large	Telocentric
3	0.00	3.01	3.01	0.054±0.002	1.000±0.000	Large	Telocentric
4	0.00	2.94	2.94	0.052±0.001	1.000±0.000	Large	Telocentric
5	0.00	2.89	2.89	0.051±0.001	1.000±0.000	Large	Telocentric
6	0.00	2.73	2.73	0.049±0.002	1.000±0.000	Large	Telocentric
7	0.00	2.68	2.68	0.048±0.001	1.000±0.000	Large	Telocentric
8	0.00	2.59	2.59	0.046±0.001	1.000±0.000	Large	Telocentric
9	0.00	2.53	2.53	0.045±0.002	1.000±0.000	Large	Telocentric
10	0.00	2.47	2.47	0.044±0.002	1.000±0.000	Large	Telocentric
11	0.00	2.39	2.39	0.042±0.002	1.000±0.000	Large	Telocentric
12	0.00	2.34	2.34	0.041±0.002	1.000±0.000	Large	Telocentric
13	0.00	2.28	2.28	0.040±0.002	1.000±0.000	Large	Telocentric
14	0.00	2.24	2.24	0.040±0.002	1.000±0.000	Large	Telocentric
15	0.00	2.23	2.23	0.039±0.005	1.000±0.000	Large	Telocentric
16	0.00	2.17	2.17	0.038±0.002	1.000±0.000	Large	Telocentric
17	0.00	2.09	2.09	0.037±0.003	1.000±0.000	Medium	Telocentric
18	0.00	2.04	2.04	0.036±0.002	1.000±0.000	Medium	Telocentric
19	0.00	1.93	1.93	0.034±0.002	1.000±0.000	Medium	Telocentric
20	0.00	1.83	1.83	0.032±0.002	1.000±0.000	Medium	Telocentric
21	0.00	1.73	1.73	0.031±0.001	1.000±0.000	Medium	Telocentric
22	0.00	1.60	1.60	0.028±0.002	1.000±0.000	Medium	Telocentric
23	0.00	1.46	1.46	0.026±0.002	1.000±0.000	Small	Telocentric
24	0.00	1.38	1.38	0.024±0.001	1.000±0.000	Small	Telocentric

Remark: *=satellite chromosome (nucleolar organizer region).

between this chromosome with NOR and another one. However, it may be caused by the centric fusion or pericentric inversion between two telocentric chromosomes that one chromosome has NOR at the telomere (Sharma *et al.* 2002).

The asymmetrical karyotype of *S. vermiculatus*, two types of chromosomes (two acrocentric and 46 telocentric chromosomes), found in this study is the important chromosome markers. The

idiogram shows continuous length gradation chromosomes (Fig. 4). A size difference of the largest and the smallest chromosomes is approximately two-fold. The chromosome marker of *S. vermiculatus*, chromosome pair 1, is the largest acrocentric chromosome, while chromosome pair 24 is the smallest telocentric chromosome. Data of the chromosomal checks on mitotic metaphase cells are shown in Table 2. The karyotype formula for *S. vermiculatus* is as follows: 2*n* (diploid) $48=L_2^a+L_{30}^t+M_{12}^t+S_4^t$.

Acknowledgment

This work was supported by grants from the Applied Taxonomic Research Center (ATRC), Khon Kaen University grant ATRC_R5801, faculty of Science, Khon Kaen University.

References

Affonso, P. R. A. M., Guedes, W., Pauls, E. and Galetti, P. M., Jr. 2001. Cytogenetic analysis of coral reefs fishes from Brazil (families Pomacanthidae and Chaetodontidae). Cytologia **66**: 379–384.

Allen, G., Swainston, R. and Ruse, J. 1999. Marine Fishes of South-East Asia. Berkeley Books, Singapore.

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 Ehbeling, A. W. 1968. Karyological evidence of female heterogamety in the mosquito fish, *Gambusia affinis*. Copeia **1**: 70–75.

Choudhury, R. C., Prasad, R. and Das, C. C. 1979. Chromosomes of six species of marine fishes. Caryologia **32**: 15–21.

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.

Kitada, J., Tatewaki, R. and Tagawa, M. 1979. The karyotype of the common aigo, *Siganus fuscescens*. Chromosome Information Serv. **27**: 12–13.

Klinkhardt, M., Tesche, M. and Greven, H. 1995. Database of Fish Chromosome. Westarp Wissenschaften, Magdeburg.

Le Grande, W. H. and Fitzsimons, J. M. 1988. Chromosome numbers of some gulf coast sciaenid fishes. Copeia **2**: 491–493.

Molina, W. F. and Galetti, P. M., Jr. 2002. Robertsonian rearrangements in the reef fish *Chromis* (Perciformes, Pomacentridae) involving chromosomes bearing 5s rRNA genes. Genet. Mol. Biol. **25**: 373–377.

Na-Nakron, U. 2000. Fishes Genetics. Kasetsart University Press, Bangkok.

Nanda, I., Schsrtl, M., Fiechtinger, W., Schlupp, I., Parzefall, J. and Schmid, M. 1995. Chromosomal evidence for laboratory synthesis of triploid hybrid between the gynogenetic teleost Poecilia Formosa and its host species. J. Fish Biol. **47**: 619–623.

Ojima, Y. and Yamamoto, K. 1990. Cellular DNA contents of fishes determined by flow cytometry. La Kromosomo II **57**: 1871–1888.

Ohno, S. 1974. Animal Cytogenetics. Chordata 1—Protochordata, Cyclostomata and Pisces, V. 4. Gebrüder Burntraeger, Berlin.

Sharma, O. P., Tripathi, N. K. and Sharma, K. K. 2002. A review of chromosome banding in fishes. In: Sobti, R. C., Obe, G. and Athwal, R. S. (eds.). Some Aspects of Chromosome Structure and Functions. Narosa Publishing House, New Delhi. pp. 109–122.

Ueno, K. and Takai, A. 2000. Chromosome evolution involving Robertsonian rearrangements in *Xirichthys* fish (Labridae, Perciformes). Cytobios **103**: 7–15.