

First Report of Chromosome Analysis of Two Siganid Fishes (Perciformes, Siganidae)

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ABSTRACT

*This is the first report of chromosome analysis in the white-spotted rabbitfish (*Siganus canalicutus*) and the orange-spotted spinefoot (*S. gattatus*) from the Andaman Sea, Thailand. Kidney cell samples were taken from three male and three female fishes. The mitotic chromosome preparation was created through direct engineering on kidney cells. Conventional and Ag-NOR staining techniques were applied so as to stain the chromosomes. The results showed that the diploid chromosome number of both *S. canalicutus* and *S. gattatus* was $2n=48$, that the fundamental number (NF) was 50 in *S. canalicutus* and 54 in *S. gattatus*. Among the chromosomes present, both acrocentric and telocentric ones were identified as 2- 46 and 6- 42 respectively. No heteromorphic chromosomes were observed which could be considered as sex-chromosomes. After Ag- NORs banding technique, a single pair of nucleolar organiser regions/ NORs was observed on the short arm telomeric region of large acrocentric chromosome Pair 1 in both species. The karyotype formulas could be deduced as:*

$$S. canalicutus \ 2n \ (48) = L^a_2 + L^t_{22} + M^t_{16} + S^t_8$$

$$S. gattatus \ 2n \ (48) = L^a_4 + L^t_{18} + M^a_2 + M^t_{22} + S^t_2$$

Keywords: *Siganus canalicutus*, *Siganus gattatus*, Karyotype, Chromosome

INTRODUCTION

The family Siganidae (Rabbitfishes, Spinefoot) is classified into the order Perciformes. The genus *Siganus* is the only one member of the family. It comprises 21 species, namely *Siganus argenteus*, *S. corallinus*, *S. doliatus*, *S. canaliculatus*, *S. fuscescens*, *S. gattatus*, *S. javus*, *S. labyrinthodes*, *S. lineatus*, *S. niger*, *S. puellus*, *S. punctatus*, *S. punctatissimus*, *S. randalli*, *S. spinus*, *S. stellatus*, *S. unimaculatus*, *S. uspi*, *S. vergatus*, *S. vermiculatus*, and *S. vulpinus* (Allen et al., 1999). Fishes in the family Siganidae are generally characterised by a laterally compressed body which is shaped like a torpedo, a small jaw and a dorsal fin with thirteen strong spines and ten soft rays. This fin is also preceded by a short, sharp, forwardly projecting spine, usually protruding slightly from its pocket but sometimes completely hidden. They have an anal fin with seven strong spines and nine rays. The pelvic fins comprise seven spines, a character unique to this family. All dorsal, anal and pelvic fin spines are grooved and contain venom glands. If handled carelessly, they are capable of inflicting very painful wounds. They have very small cycloid scales, sometimes absent from isthmus and midthoracic regions, but if present on the head, they don't extend beyond the suborbital area of the cheek. As for their colour, most species are usually brightly colored and ornately patterned whereas other ones are often drab and tend to become mottled with brown at death (Carpenter and Niem, 2001).

There were approximately eight percent of the Perciformes order that were recorded for their karyological characteristics, revealing a model with a diploid chromosome number of $2n=48$ (Affonso et al., 2001). However, different karyotypes from the typical order Perciformes pattern had frequently been detected, indicating Robertsonian rearrangements as the preferential process in some groups, such as the families Labridae and Pomacentridae (Ueno and Takai, 2000; Molina and Galetti, 2002). The cytogenetical reports on the family Siganidae demonstrated a highly conserved pattern, considered basal for the order Perciformes ($2n=48$), except for *S. spinus* ($2n=42$) (Kitada et al., 1979; Choudhury et al., 1979; Ojima and Yamamoto, 1990; Jumrusthanasan et al., 2015). No study describing the karyotypes of the white-spotted rabbitfish (*Siganus canalicutus*) and orange-spotted spinefoot (*S. gattatus*) had been published previously. Almost, these fishes are importance species as food and sometime, they are kept in aquarium as ornamental fish but the knowledge of these species has no data especially biology and cytogenetics. These present study is the first report of cytogenetic studies of *S. canalicutus* and *S. gattatus* performed using conventional staining and Ag-NOR banding techniques. The obtained results attempt to provide further cytogenetic information for future studies on taxonomy and evolutionary relationships between these fishes.

MATERIAL AND METHODS

Materials

Three males and three females *S. canalicutus* and *S. gattatus* were obtained from the Andaman Sea in Southern Thailand (Figure 1). These fishes were transferred to some laboratory aquaria and were kept under standard conditions for three days prior to the experiment. Isolation procedures involving fish chromosomes were carried out directly on kidney cells (Supiwong et al., 2012; 2013; 2015). The chromosome preparations were stained with 10% Giemsa for 30 minutes and NORs were identified by Ag-NOR staining (Howell and Black, 1980). The metaphase figures were analysed according to the chromosome classification after Turpin and Lejeune (1965). The centromeric index (CI) for chromosomes situated in the ranges 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 respectively. The Fundamental number (NF) was obtained by assigning a value of two to metacentric, submetacentric and acrocentric chromosomes and a value of one to telocentric chromosomes. Thirty metaphase plates per specimen were used for classify chromosome types.

The classify for chromosome sizes is as:

$$L > \frac{\text{mean of LT of the largest pair} + \text{mean of LT of the smallest pair}}{2}$$

$$M \leq \frac{\text{mean of LT of the largest pair} + \text{mean of LT of the smallest pair}}{2}$$

$$S < \frac{\text{mean of LT of the largest pair}}{2}$$

RESULTS

Diploid number, fundamental number and karyotype of *S. canalicutus* and *S. gattatus*

The present cytogenetic description on type and size of chromosome was done by using the conventional staining and Ag-NOR banding techniques. The findings show that *S. canalicutus* and *S. gattatus* have the modal $2n$ of 48 and the respective NF of 50 and 54 in both sexes (Figure 2). There was no observation of unusual size chromosome related to sex for all analysed species. This report constitutes the first mention of the modal of $2n=48$ for the pertinent members of the family Siganidae.

The karyotype formulas for *S. canalicutus* and *S. gattatus* are as follows:

$$S. canalicutus \ 2n \ (48) = L^a_2 + L^t_{22} + M^t_{16} + S^t_8$$

$$S. gattatus \ 2n \ (48) = L^a_4 + L^t_{18} + M^a_2 + M^t_{22} + S^t_2$$

However, there are no significant differences in chromosome size between ‘large’ and ‘medium’ and between ‘medium’ and ‘small’, the presentation in Table 2 and 3 is not recommended.

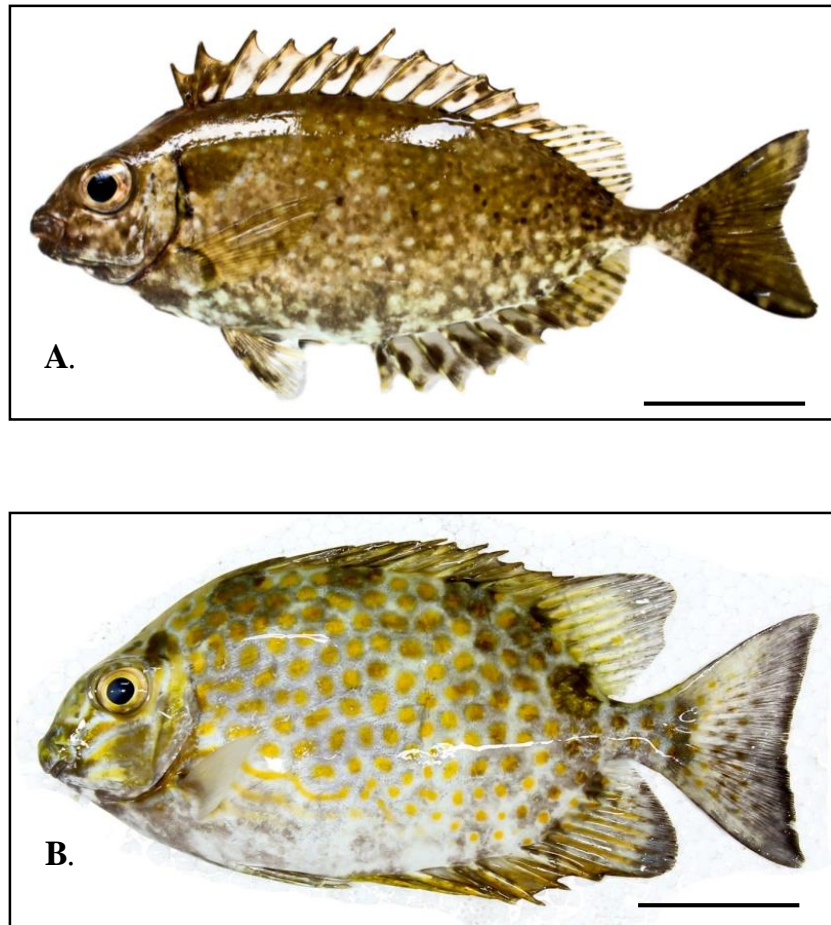


Figure 1. General characteristics of *Siganus canalicutus* (A.) and *S. gattatus* (B.). Scale bars indicate five centimeters.

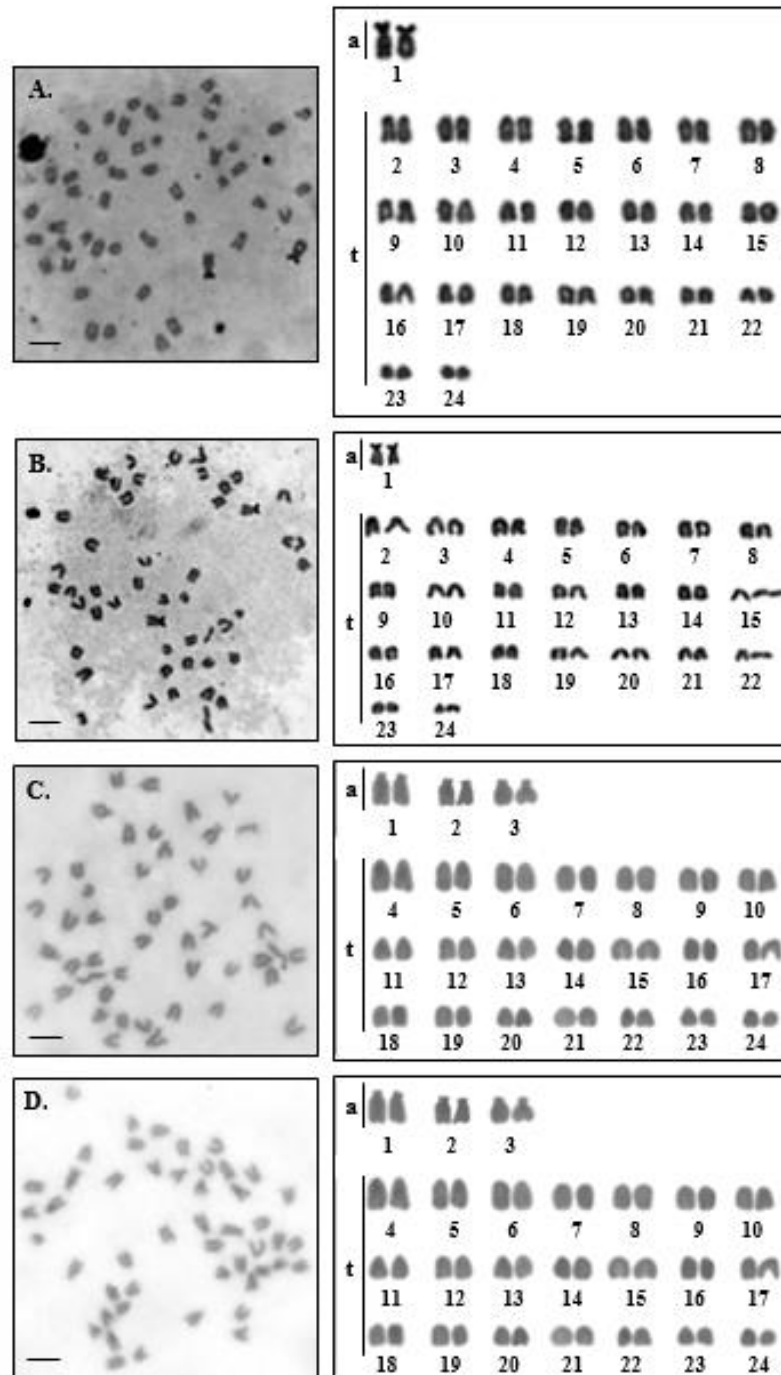


Figure 2. Metaphase chromosome plates and karyotypes of male (A.) and female (B.) *Siganus canalicutus*, $2n=48$ and male (C.) and female (D.) *S. gattatus*, $2n=48$ by conventional straining technique. Scale bars indicate five micrometers.

Chromosome markers of *S. canalicutus* and *S. gattatus*

The objective of Ag-NOR banding technique is to reach out the nucleolar organizer region (NOR) which is the representative location of genes (loci) that function in ribosome synthesis (18S and 28S ribosomal RNA) (Sharma et al., 2002; Khakhong et al., 2014). If these are regions are active during the interphase prior to mitosis, they could be detected by silver nitrate staining (Howell and Black, 1980) since it specifically stains a set of acidic protein related to ribosomal synthesis process; this technique actually reveals active NORs (Ag-NORs) and not the rDNA associated to NORs (Jordan, 1987).

Through Ag-NORs banding technique, a single pair of NOR was observed on the short arm telomeric region of the large acrocentric chromosome pair 1 in both species (Figures 3). The chromosome markers determination of these two species showed the positive Ag-NOR marks on pairs of homologous chromosomes. They are distinguished into the group depending on NOR locations which have the short arm of telomeric NOR.

The asymmetrical karyotypes of *S. canalicutus* and *S. gattatus*, and the only two types of chromosomes (acrocentric and telocentric) that were found, are important chromosome markers. The idiogram shows continuous length gradation of chromosomes. The largest and smallest chromosomes present size differences (approximately threefolds). Data of chromosomal checks on mitotic metaphase cells of *S. canalicutus* and *S. gattatus* are shown in Tables 1, 2 and 3, respectively. Figures 4 and 5 show the idiograms obtained by conventional staining and Ag-NOR banding techniques, respectively.

According to previous reports, there are four species in the family Siganidae, namely *S. fuscescens*, *S. javus*, *S. spinus* and *S. vermiculatus* which were analysed for their karyotype information. The present study, the hypothesis model for the chromosome evolution of the family Siganidae on the basis of cytogenetic descriptions of six fishes including *S. canalicutus*, *S. fuscescens*, *S. gattatus*, *S. javus*, *S. spinus* and *S. vermiculatus* is proposed (Figures 6).

Table 1. Review of siganid fishes cytogenetic reports (Perciformes, Siganidae).

Species	2n	NF	Karyotype	NORs	Locality	Reference
<i>Siganus canalicutus</i>	48	50	2a+46t	1(TR)	Thailand	Present study
<i>S. fuscescens</i>	48	50	2a+46t	N/A	Japan	Kitada et al. (1979)
<i>S. gattatus</i>	48	54	6a+42t	1(TR)	Thailand	Present study
<i>S. javus</i>	48	48	48t	N/A	Japan	Choudhury et al. (1979)
<i>S. spinus</i>	42	48	6m+36t	N/A	Japan	Ojima and Yamamoto (1990)
<i>S. vermiculatus</i>	48	50	2a+46t	1(TR)	Thailand	Jumrusthanasan et al. (2015)

Notes: 2n = diploid chromosome number, NF = fundamental number (number of chromosome arms), m = metacentric chromosome, a = acrocentric chromosome, t = telocentric chromosome, NORs = nucleolar organizer regions, TR = telomeric region, and N/A = not analysed yet.

Table 2. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), and centromeric index (CI) from 20 metaphases of the white-spotted rabbitfish (*Siganus canaliculatus*), $2n=48$.

Chro. pair	Ls	Ll	LT	RL	CI	Chro. Size	Chro. type
1*	0.23	0.61	0.84	0.065	0.724	Large	Acrocentric
2	0.00	0.72	0.72	0.056	1.000	Large	Telocentric
3	0.00	0.69	0.69	0.053	1.000	Large	Telocentric
4	0.00	0.67	0.67	0.052	1.000	Large	Telocentric
5	0.00	0.65	0.65	0.050	1.000	Large	Telocentric
6	0.00	0.64	0.64	0.049	1.000	Large	Telocentric
7	0.00	0.62	0.62	0.048	1.000	Large	Telocentric
8	0.00	0.60	0.60	0.04	1.000	Large	Telocentric
9	0.00	0.58	0.58	0.045	1.000	Large	Telocentric
10	0.00	0.56	0.56	0.043	1.000	Large	Telocentric
11	0.00	0.55	0.55	0.042	1.000	Large	Telocentric
12	0.00	0.55	0.55	0.042	1.000	Large	Telocentric
13	0.00	0.54	0.54	0.042	1.000	Medium	Telocentric
14	0.00	0.53	0.53	0.041	1.000	Medium	Telocentric
15	0.00	0.52	0.52	0.040	1.000	Medium	Telocentric
16	0.00	0.51	0.51	0.039	1.000	Medium	Telocentric
17	0.00	0.49	0.49	0.038	1.000	Medium	Telocentric
18	0.00	0.48	0.48	0.037	1.000	Medium	Telocentric
19	0.00	0.47	0.47	0.036	1.000	Medium	Telocentric
20	0.00	0.42	0.42	0.032	1.000	Medium	Telocentric
21	0.00	0.40	0.40	0.031	1.000	Small	Telocentric
22	0.00	0.36	0.36	0.028	1.000	Small	Telocentric
23	0.00	0.33	0.33	0.025	1.000	Small	Telocentric
24	0.00	0.26	0.26	0.020	1.000	Small	Telocentric

Notes: * = NOR-bearing chromosome (satellite chromosome) and chro. = chromosome.

Table 3. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), and centromeric index (CI) from 20 metaphases of the orange-spotted spinefoot (*Siganus guttatus*), $2n=48$.

Chro. pair	Ls	Ll	LT	RL	CI	Chro. Size	Chro. type
1*	0.270	0.708	0.978	0.055	0.724	Large	Acrocentric
2	0.216	0.624	0.840	0.048	0.743	Large	Acrocentric
3	0.195	0.518	0.713	0.04	0.727	Medium	Acrocentric
4	0.000	0.875	0.875	0.05	1.000	Large	Telocentric
5	0.000	0.850	0.850	0.048	1.000	Large	Telocentric
6	0.000	0.838	0.838	0.047	1.000	Large	Telocentric
7	0.000	0.818	0.818	0.046	1.000	Large	Telocentric
8	0.000	0.798	0.798	0.045	1.000	Large	Telocentric
9	0.000	0.775	0.775	0.044	1.000	Large	Telocentric
10	0.000	0.765	0.765	0.043	1.000	Large	Telocentric
11	0.000	0.763	0.763	0.043	1.000	Large	Telocentric
12	0.000	0.733	0.733	0.041	1.000	Large	Telocentric
13	0.000	0.725	0.725	0.041	1.000	Medium	Telocentric
14	0.000	0.715	0.715	0.04	1.000	Medium	Telocentric
15	0.000	0.713	0.713	0.04	1.000	Medium	Telocentric
16	0.000	0.705	0.705	0.04	1.000	Medium	Telocentric
17	0.000	0.690	0.690	0.039	1.000	Medium	Telocentric
18	0.000	0.673	0.673	0.038	1.000	Medium	Telocentric
19	0.000	0.650	0.650	0.037	1.000	Medium	Telocentric
20	0.000	0.643	0.643	0.036	1.000	Medium	Telocentric
21	0.000	0.603	0.603	0.034	1.000	Medium	Telocentric
22	0.000	0.588	0.588	0.033	1.000	Medium	Telocentric
23	0.000	0.558	0.558	0.031	1.000	Medium	Telocentric
24	0.000	0.475	0.475	0.027	1.000	Small	Telocentric

Notes: * = NOR-bearing chromosome (satellite chromosome) and chro. = chromosome.

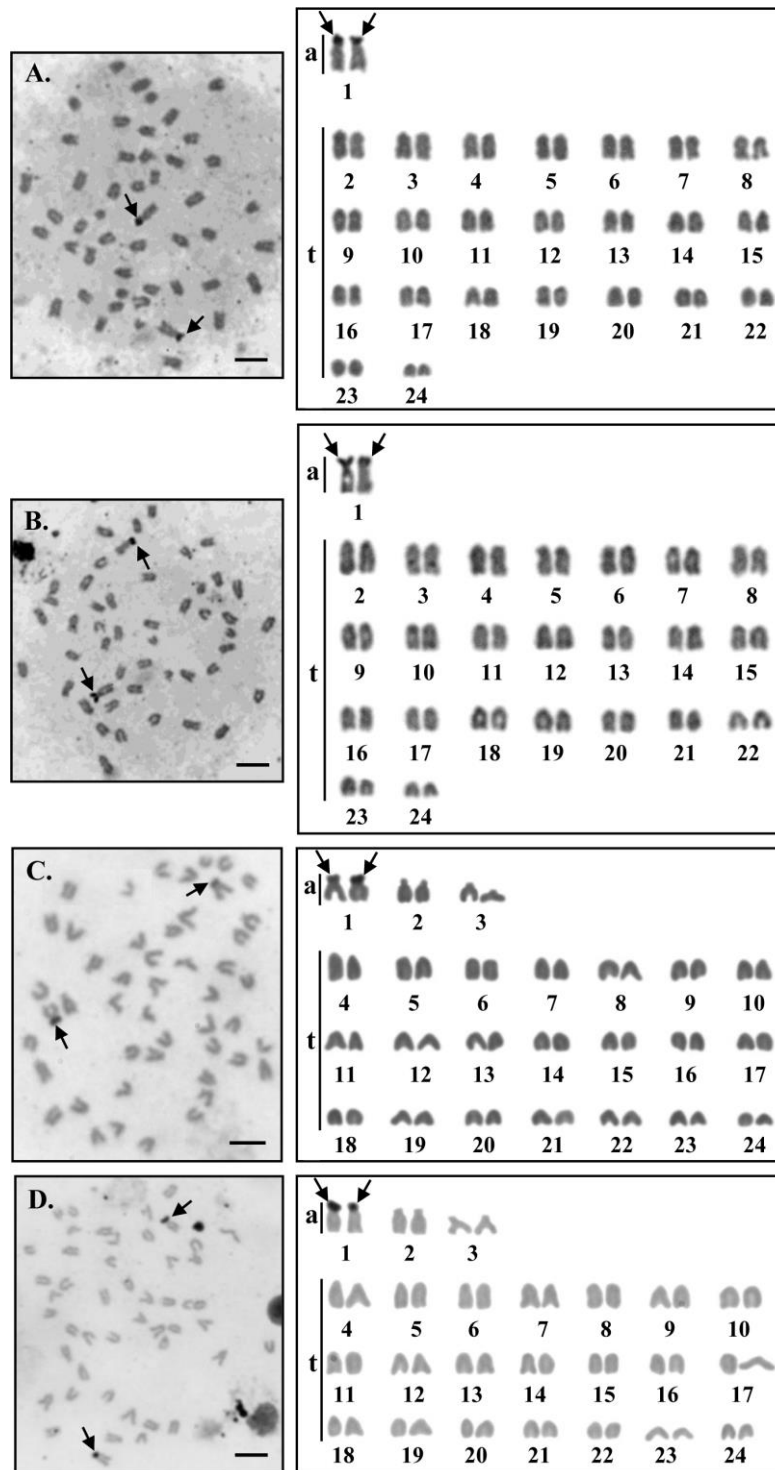


Figure 3. Metaphase chromosome plates and karyotypes of male (A.) and female (B.) *Siganus canalicutus*, $2n=48$ and male (C.) and female (D.) *S. gattatus*, $2n=48$ by Ag-NOR banding technique. Arrows indicate NORs bearing (scale bars = 5 micrometers).

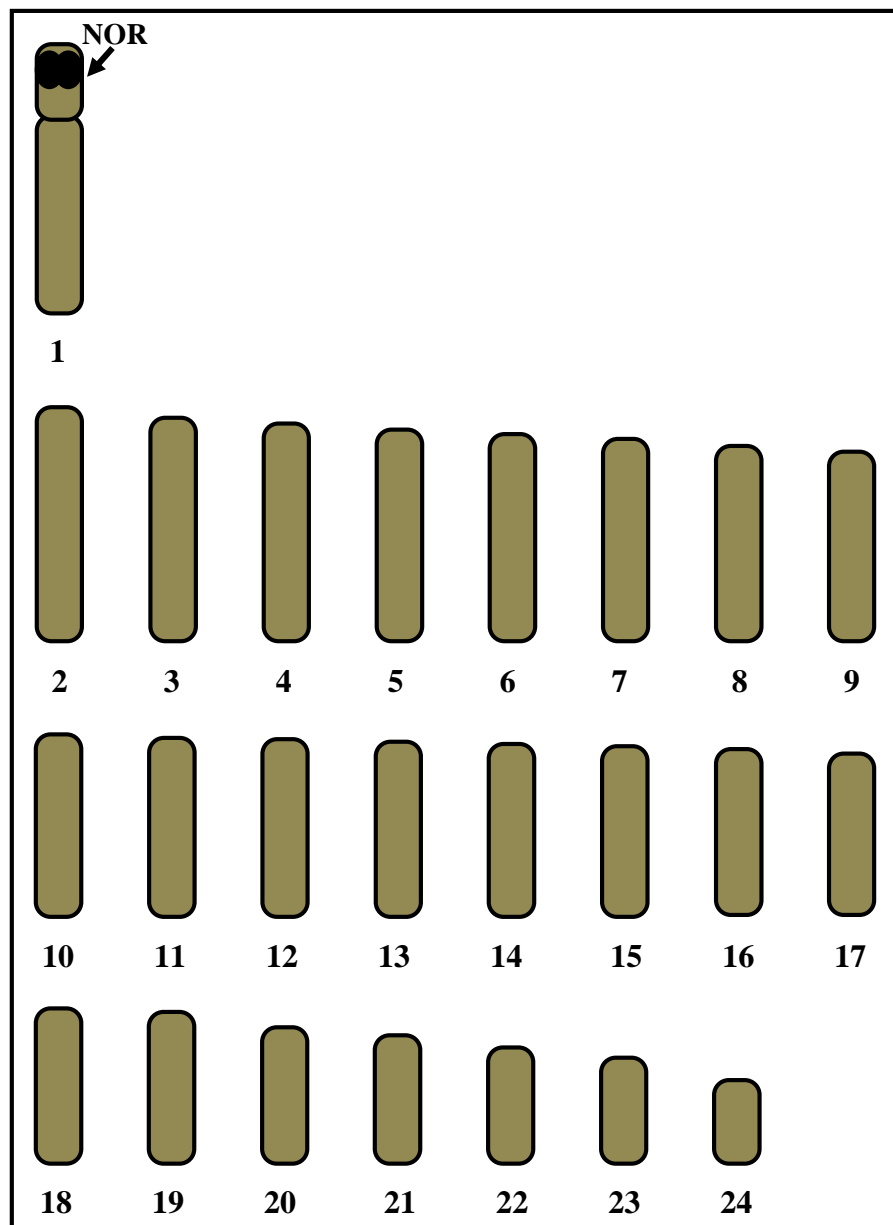


Figure 4. Idiogram showing lengths and shapes of chromosomes of *Siganus canaliculus*, $2n=48$. Arrow indicates NORs bearing chromosome pair 1.

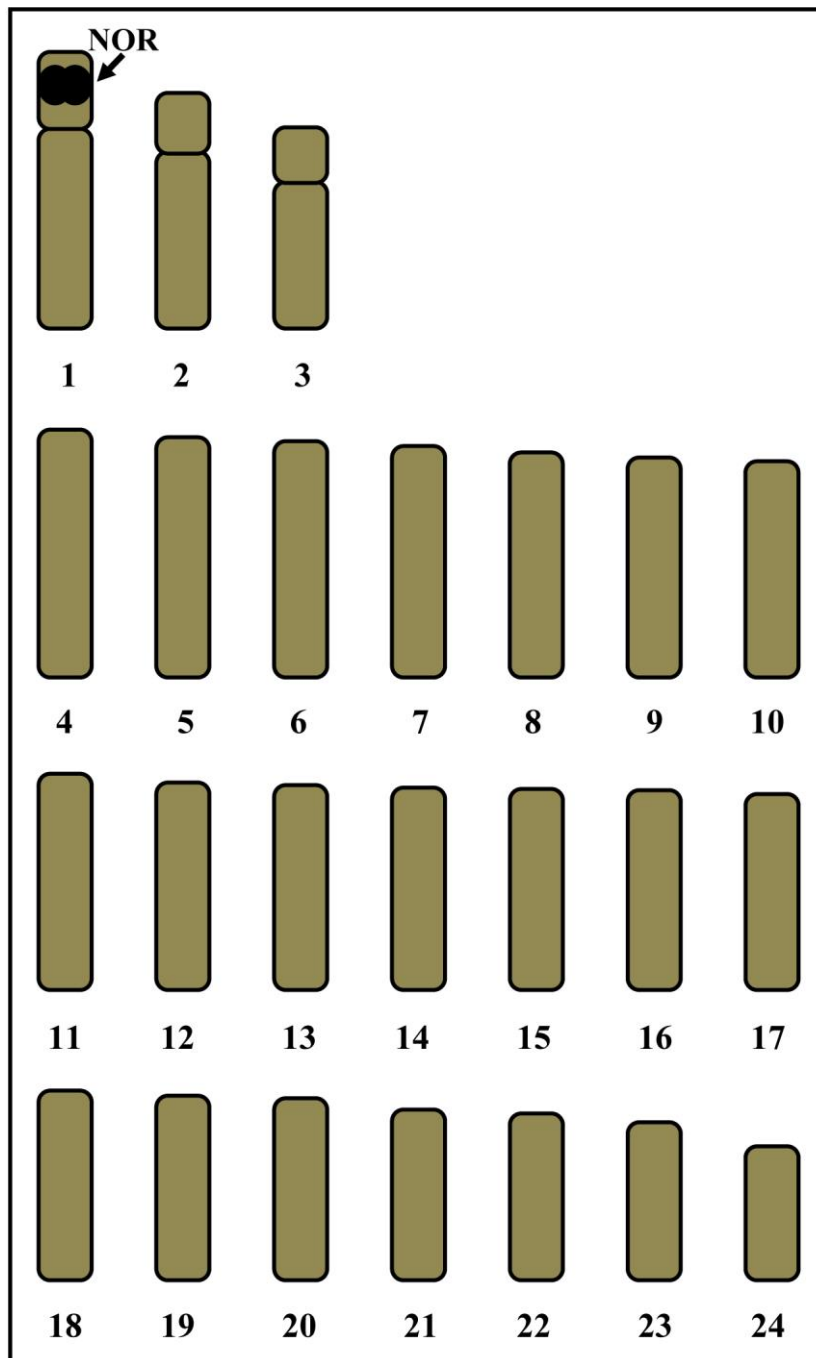


Figure 5. Idiogram showing lengths and shapes of chromosomes of *Siganus gattatus*, $2n=48$. Arrow indicates NORs bearing chromosome pair 1.

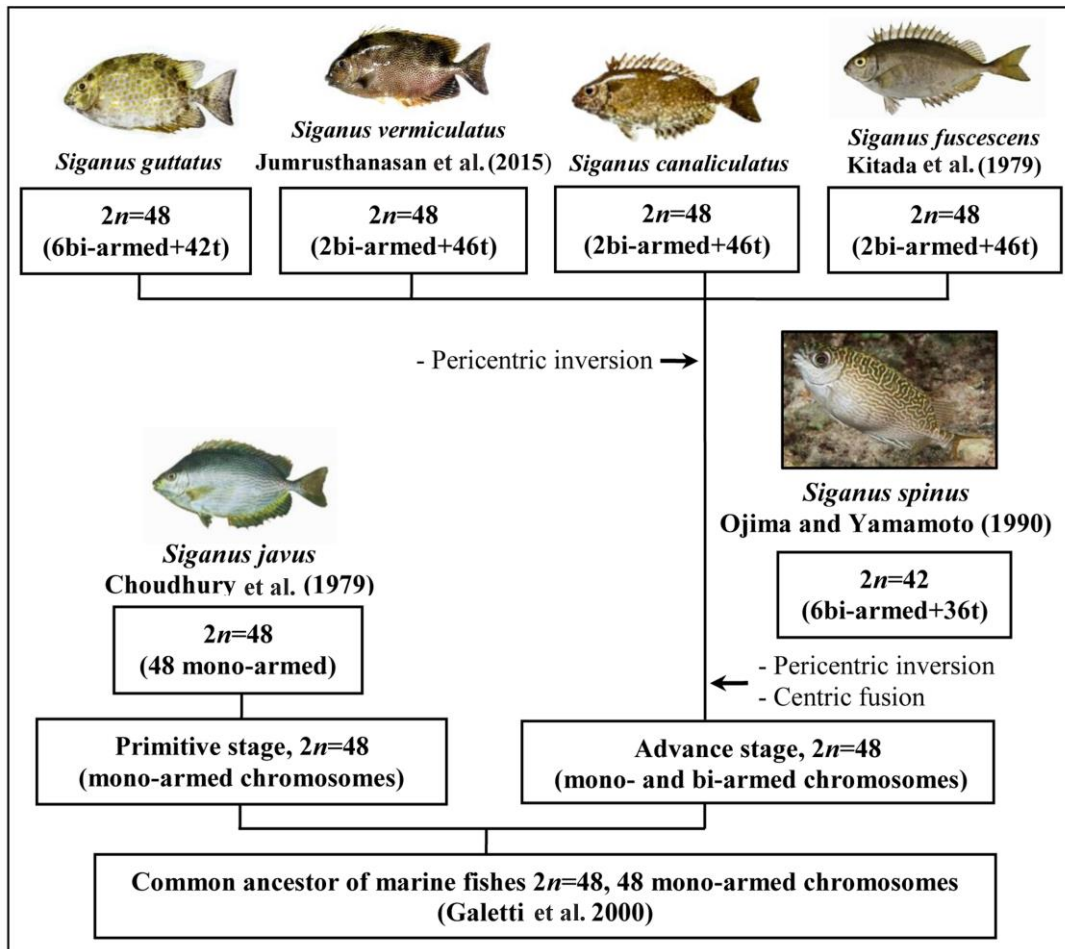


Figure 6. The hypothesis model for the chromosome evolution of the family Siganidae.

DISCUSSION

In the present study, the findings pertaining to *S. canaliculatus* and *S. guttatus* are consistent with those published in the chromosomal characteristic reports on the Siganidae fishes including *S. fuscescens* (Kitada et al., 1979), *S. javus* (Choudhury et al., 1979) and *S. vermiculatus* (Jumrusthanasan et al., 2015). However, different information on the diploid chromosome number was found in a chromosomal examination of *S. spinus* which revealed the $2n$ of 42 (Ojima and Yamamoto, 1990). The NF was 50 in *S. canaliculatus* and 54 in *S. gattatus*. The differences of karyological characteristics between both species show variations in the number of chromosome arms which are similar to those stated in the previous reports of NF for the other siganid fishes, namely *S. fuscescens*, *S. javus*, *S. spinus*, and *S. vermiculatus* that have their NF ranging from 48 to 50 (Choudhury et al., 1979; Kitada et al., 1979; Ojima and Yamamoto, 1990; Jumrusthanasan et al., 2015).

Both investigated species have no cytologically distinguishable sex chromosome. This characteristic is similar to other ones in the family Siganidae (Choudhury et al., 1979; Kitada et al., 1979; Ojima and Yamamoto, 1990; Jumrusthanasan et al., 2015). It is possible that the fish's sex-chromosomes are dependent upon an initiation of differentiation. Therefore, chromosomes containing sex-determination genes cannot be found by cytogenetic analyses (Bertollo et al., 2004; Kaewmad et al., 2014; Kasiroek et al., 2017).

Chromosome markers of *S. canalicutus* and *S. gattatus*, the location of NOR in a terminal position and close to the centromeres are also founded upon a primitive feature (Vitturi et al., 1988). NOR is frequently used to compare variations, as well as to identify and explain specifications. Species, which have limited gene exchange due to geographical isolation, have elevated karyotype numbers and NOR variations. The use of NORs in explaining kinships depends on a large extent on the uniformity of this characteristic and on the degree of variety within a taxon (Yüksel and Gaffaroğlu, 2008).

The cytogenetic information which was obtained in this research, i.e. $2n$, NF, type and sizes of chromosomes of two analysed fishes of the family Siganidae, namely *S. canalicutus* and *S. gattatus* exhibited all of them have the same diploid chromosomes number of 48. Nevertheless, differences in NF and type as well as size of chromosome were as shown. These variations on each member's chromosomal characteristics revealed the occurrence of chromosomal evolution among the species. Although most Siganid species have an NF of 48 or 50, with most telocentric chromosomes in the complement, the number of chromosome arms in the *Siganus* genus varies from 48 to 54. Under the assumption that species with a larger NF are more advanced in evolutionary terms, the most recent species within the *Siganus* genus would be *S. fuscescens*, NF=50; *S. javus*, NF=48 and *S. vermiculatus*, NF=50. However, despite differences in NF, all *Siganus* species share the same diploid chromosome number ($2n=48$). Such changes in chromosome arm number appear to be related to the occurrence of pericentric inversions, which are among the most common modifications contributing to karyotypic rearrangement in fishes and other vertebrates (Galetti et al., 2000).

That is the good recommendation. However, we discuss the chromosomal evolution in this group, only species that have been cytogenetic reported. We only hypothesize the trend of chromosomal evolution which is possible to occur in this family if there are more data from more species, they will be good hypothesis for chromosomal evolution.

CONCLUSION

The diploid chromosome number of *S. canalicutus* and *S. gattatus* as $2n=48$. The chromosomes were identified as belonging to the acrocentric and telocentric types defined as 2-46 and 6-42, respectively. No heteromorphic chromosomes were observed that could be considered as sex-chromosomes. Following an Ag-NORs banding technique, a single pair of nucleolar organiser regions/ NORs was observed on the short arm telomeric region of large acrocentric chromosome pair 1 in both species. The karyotype formulas could be deduced as:

$$S. canalicutus\ 2n\ (48) = L^a_2 + L^t_{22} + M^t_{16} + S^t_8$$

$$S. gattatus\ 2n\ (48) = L^a_4 + L^t_{18} + M^a_2 + M^t_{22} + S^t_2$$

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