Standardized karyotype and idiogram of Andaman damselfish (*Pomacentrus alleni*) by conventional cytogenetic analyses

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Abstract

This research aimed to study concerning karyotyping and idiograming of Andaman damselfish (*Pomacentrus alleni*) from the Andaman Sea (Indian Ocean). Chromosomes were prepared from kidney tissues of fishes. The mitotic chromosomes were harvested by colchicines-hypotonic-fixation-air-drying method, employing conventional cytogenetic analyses with Giemsa stain. Standardized karyotype and idiogram were constructed. The results presented that *P. alleni* has a diploid chromosome number of 2n=48 (48t), the fundamental number (NF) has 48. The karyotypes of sex chromosome in *P. alleni* is not different in both males and females.

The karyotype formula of *P. alleni* is as follows: 2n (48) = $L^{t}_{28} + M^{t}_{20}$

Keyword: *Pomacentrus alleni*, Andaman damselfish, Chromosome, Karyotype

Introduction

The family Pomacentridae (Perciformes) contains 28 genera and approximately 320 species known as damselfishes. This family is one of the most diverse among marine teleost which is widely distributed in tropical seas of the world (Nelson, 1994). Species of the family Pomacentridae are found in coastal waters associated with rocky substrates, usually occurring at low depths and often assembling in large fish schools. The taxonomy of damselfishes is complicated by the large number of complex species and the color patterns that vary among individuals and populations of the same species. Several species are growing economic interest because of their diverse color patterns, and this has led to their exploitation. (Molina and Galetti, 2004)

Karyological studies of fish can contribute significantly to understand of many problems in areas of research ranging from taxonomy, phylogenetics and environmental toxicology (Al-Sabti, 1985). However, the small size and large number of chromosomes in fish and the lack of a standard technique for fish chromosome preparation make their evaluations difficult (Denton, 1973; Thorgaard and Disney, 1990). Chromosomal analysis is interest in fish breeding from the viewpoint

of genetic control, the rapid production of inbred lines, taxonomy and evolutionary studies (Al-Sabti, 1987).

About 13,000 marine fish species that have been recorded, fewer than 2% of these have been studied cytogenetically. Most marine fish studied have a diploid complement of 48 acrocentric chromosomes (Brum, 1996). In the subfamily pomacentrinae, There are only 10 species in the genus *Pomacentrus* that have already been considered through cytogenetic studies such as whitetail damselfish (*P. chrysurus*), neon damselfish (*P. coelestis*), lamon damselfish (*P. moluccensis*), tree line damselfish (*P. trilineatus*), dusky damselfish (*P. fuscus*), philippinen damselfish (*P. philippinus*), wedgespot damselfish (*P. cuneatus*), yellow bellied damselfish (*P. caeruleus*), similar damsel (*P. similis*) and goldbelly damsel (*P. auriventris*) (Table 1.).

This study aiming to clarify the actual chromosomal rearrangements showed by Andaman damselfish (*P. alleni*) (Fig. 1) employing conventional cytogenetic analyses.

Table 1. Review of damselfish cytogenetic reports in the genus *Pomacentrus*

Species	2*n*	NF	m	sm	a	t	References
P. auriventris	48	48	-	-	-	48	Getlekha (2017)
P. caeruleus	48	68	6	12	8	16	Getlekha and Tanomtong (2013)
P. chrysurus	48	48	-	-	4	44	Takai and Ojima (1991)
P. coelestis	48	48	-	-	-	48	Takai and Ojima (1991)
P. cuneatus	48	86	8	16	14	10	Getlekha and Tanomtong (2013)
P. fuscus	48	48	-	-	-	48	Takai and Ojima (1995)
P. philippinus	48	76	6	22	-	20	Takai and Ojima (1991)
P. moluccensis	48	84	8	28	-	12	Takai and Ojima (1991)
	48	94	10	26	10	2	Getlekha (2017)
P. similis	48	48	-	-	-	48	Getlekha (2017)
P. trilineatus	50	58	8	-	-	42	Rishi (1973)
P. alleni	48	48	-	-	-	48	Present study

Remark: 2*n* = diploid chromosome number, NF = fundamental number, m = metacentric, sm = submetacentric, a = acrocentric and t = telocentric

Fig. 1 General characteristics of the Andaman damselfish (*P. alleni*), scale bar indicates 1 cm.

Materials and Methods

Chromosome preparation and Conventional staining

The *P. alleni* was analyzed: 4 male and 4 female from the Andaman Sea (Indian Ocean) (Fig. 1). The methods followed ethical protocols and anesthesia with clove oil was used prior to sacrificing the animals to minimize suffering. The 0.02% colchicine (1 ml/100 g body weight) was injected to its intramuscular or abdominal cavity and left for 1 hour. Mitotic chromosomes were obtained from the cell suspensions of the anterior kidney, using the conventional air-drying method (Bertollo et al., 1978). Conventional staining was done using 20% Giemsa solution in phosphate buffer pH 6.8 for 10 min.

Chromosome checking, Karyotyping and Idiograming

Standardized karyotypes and idiogram of the *P. alleni* were constructed. Next, chromosome checking was performed on mitotic metaphase cells under light microscope. After that, the frequencies of chromosome number per cell were counting. The maximum frequency of chromosome number per cell is the chromosome diploid number of *P. alleni*. Then, ten cells of each male and female with clearly observable and well-spread chromosome were selected and photographed.

The length of short arm chromosome (Ls) and the length of long arm chromosome (Ll) were measured and calculated to the length of total arm chromosome (LT, LT=Ls+Ll). Next, the relative length (RL), the centromeric index (CI) and standard deviation (S.D.) of RL and CI were estimated. The CI (q/p+q) 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 (Chaiyasut, 1989). The fundamental number (number of chromosome arm, NF) was obtained by assigning a value of 2 to metacentric, submetacentric and acrocentric chromosomes and 1 to telocentric

chromosome. All parameters were used in karyotyping. Furthermore, the idiogram was constructed using a model drawing of karyotype and accomplished by a computer program.

Results

The results showed that the number of diploid chromosome of *P. alleni* was 2n=48, the fundamental number was 48 in both males and females. The chromosome types were presented as 28 large telocentric (LT>1.79) and 10 medium telocentric chromosomes (1.79>LT>1.17) (Fig. 2 and 3). No strange sized chromosomes related to sex were observed. The karyotype formula of *P. alleni* is as follows: 2n (48) = L^{t}_{28} + M^{t}_{20}

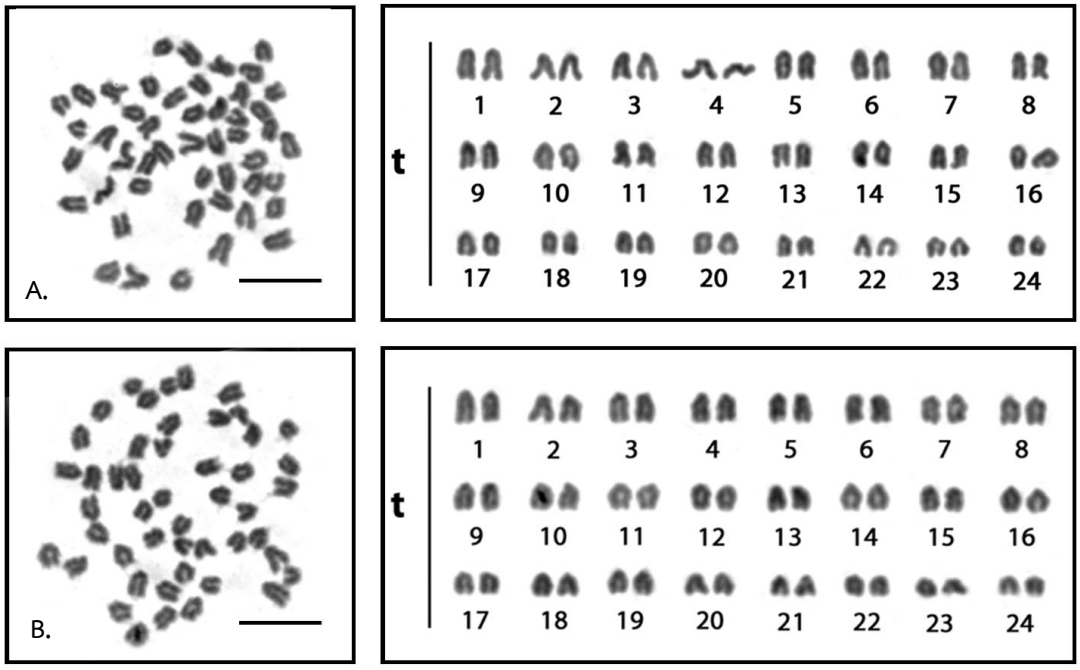

Fig. 2 Metaphase chromosome plates and karyotypes of male (A.) and female (B.) Andaman damselfish (*P. alleni*), 2n=48 by conventional straining technique (scale bars 5 micrometers). There is no observation of strange size chromosomes related to sex.

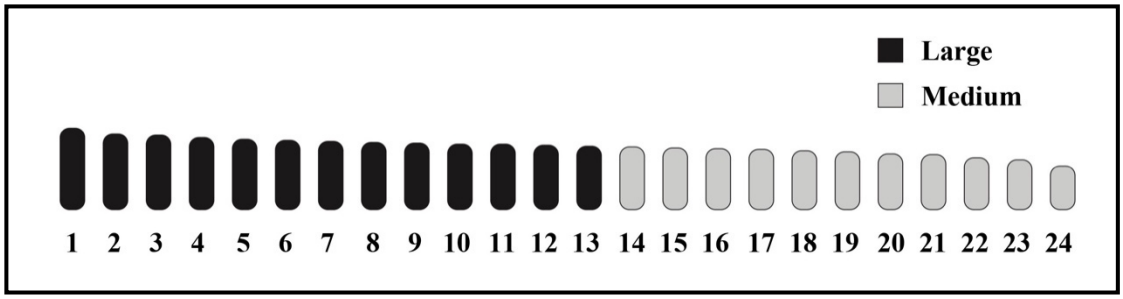

Fig. 3. Idiogram showing lengths and shapes of chromosomes of the Andaman damselfish (*P. alleni*), 2n=48 by conventional staining technique.

The 10 metaphase cells of each male and female were measured for individual length of both short arm and long arm. The length of short arm chromosome (Ls) and long arm chromosome (Ll) were calculated to the length of total arm chromosome (LT, LT = Ls+Ll). The size and type of chromosomes were showed in the Tables 2.

Discussions

The *P. alleni* has a chromosome number of 2n = 48 similar to the reports on table 1 that presented the studies karyotype of 9 damselfish in genus *pomacentrus* have a chromosome number of 2n=48, in 4 damselfish (*P. auriventris*, *P. coelestis*, *P. fuscus* and *P. similis*) have 48 telocentric chromosomes (Getlekha, 2017; Takai and Ojima, 1991, 1995). In contrast, there are 5 damselfish have a different of chromosomes type in *P. caeruleus* (6m+12sm+8a+16t) (Getlekha and Tanomtong, 2013), *P. chrysurus* (4a+44t) (Takai and Ojima, 1991), *P. cuneatus* (8m+16sm+14a+10t) (Getlekha and Tanomtong, 2013), *P. philippinus* (6m+22sm+20t) (Takai and Ojima, 1991) and *P. moluccensis* (8m+28sm+12t and 10m+26sm+10a+2t) (Takai and Ojima, 1991; Getlekha, 2017). The reasons that make the different types of chromosomes due to the use of different criteria for classification of chromosome type or due to pericentric inversion of chromosomes that still have 48 of diploid chromosome but increase of the fundamental number.

In turn, the present study is different from Rishi (1973) that studied karyotype of damselfish in the same genus that reported the chromosome of *P. trilineatus* have 2n=50 consist of 8 metacentric and 42 telocentric chromosomes, the increase of the diploid chromosome number not only because of pericentric inversion but also chromosome fission, it make diverse characteristic of chromosome compared to the ancestral 48A karyotype (NF=48).

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 Andaman damselfish (*P. alleni*), 2n=48

Chromosome pairs	Ls	Ll	LT	CI±SD	RL±SD	Chromosome size	Chromosome type
1	0.00	2.34	2.34	0.054±0.004	1.000±0.000	large	telocentric
2	0.00	2.17	2.17	0.050±0.003	1.000±0.000	large	telocentric
3	0.00	2.14	2.14	0.049±0.002	1.000±0.000	large	telocentric
4	0.00	2.07	2.07	0.047±0.001	1.000±0.000	large	telocentric
5	0.00	2.02	2.02	0.046±0.001	1.000±0.000	large	telocentric
6	0.00	1.99	1.99	0.046±0.001	1.000±0.000	large	telocentric
7	0.00	1.96	1.96	0.045±0.001	1.000±0.000	large	telocentric
8	0.00	1.93	1.93	0.044±0.001	1.000±0.000	large	telocentric
9	0.00	1.91	1.91	0.044±0.001	1.000±0.000	large	telocentric
10	0.00	1.88	1.88	0.043±0.001	1.000±0.000	large	telocentric
11	0.00	1.88	1.88	0.043±0.001	1.000±0.000	large	telocentric
12	0.00	1.85	1.85	0.042±0.001	1.000±0.000	large	telocentric
13	0.00	1.82	1.82	0.042±0.001	1.000±0.000	large	telocentric
14	0.00	1.8	1.8	0.041±0.001	1.000±0.000	large	telocentric
15	0.00	1.77	1.77	0.040±0.001	1.000±0.000	medium	telocentric
16	0.00	1.75	1.75	0.040±0.001	1.000±0.000	medium	telocentric
17	0.00	1.73	1.73	0.039±0.001	1.000±0.000	medium	telocentric
18	0.00	1.69	1.69	0.039±0.001	1.000±0.000	medium	telocentric
19	0.00	1.66	1.66	0.038±0.001	1.000±0.000	medium	telocentric
20	0.00	1.6	1.6	0.037±0.001	1.000±0.000	medium	telocentric
21	0.00	1.58	1.58	0.036±0.001	1.000±0.000	medium	telocentric
22	0.00	1.49	1.49	0.034±0.001	1.000±0.000	medium	telocentric
23	0.00	1.43	1.43	0.033±0.002	1.000±0.000	medium	telocentric
24	0.00	1.25	1.25	0.028±0.002	1.000±0.000	medium	telocentric

Sex chromosome can not be detected in this study. It may be possible that the sex chromosomes of the fish are the initiation of differentiation and hence these chromosomes which contain the sex-determination gene cannot be detected by cytogenetic analyses (Na-Nakron, 2000).

In pomacentrid fish, including damselfish, the karyotypes of 30 species has been reported and these are characterized by chromosome numbers varying from 27 to 50 and NF varying from 48 to 94 (Rishi, 1973; Arai and Inoue, 1976; Arai et al., 1976; Ojima and Kashiwagi, 1981; Takai and Ojima, 1987, 1991a, 1991b, 1995; Molina and Galetti, 2004; Takai and Kosuga, 2007; Getlekha and Tanomtong, 2013; Getlekha, 2017). Takai and Ojima (1987) suggested that karyotypes of pomacentrid fish have diversified from the ancestral karyotype, consisting of 48 acrocentric chromosomes. In *P. alleni* exhibit marked chromosome conservatism from ancestral karyotype. On the other hand, some pomacentrid fish increase of the fundamental number (NF) involving pericentric inversions. Therefore, damselfish with higher NF may be the most advanced group in the karyotypic evolution in family Pomacentridae.

Moreover, adults of *P. alleni* inhabit rubble and dead reef of outer slopes and inshore areas. Usually in small groups along the edges of reef to sand on the tropical sea of the world show that no geographic barrier that case by high gene flow, make the diploid chromosome number still have 48t compared to the ancestral karyotype (NF=48).

Suggestion

In the future, karyological studies may provide interesting findings related to phylogenetic relationships. Cytogenetics about damselfish will be applied for basic knowledge to study of breeding, conservation and chromosome evolution in this fish.

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