การศึกษาลักษณะแคริโอไทป์ของปลาการ์ตูนมะเขือเทศและปลาการ์ตูนแก้มหนาม

Karyological characteristics of *Amphiprion frenatus* and *Premnas biaculeatus* (Perciformes, Amphiprioninae)

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บทคัดย่อ

การศึกษาลักษณะแคริโอไทป์ของปลาการ์ตูนมะเขือเทศและปลาการ์ตูนแก้มหนามจากทะเลอันดามัน ภาคใต้ของประเทศไทย เตรียมโครโมโซมด้วยวิธีตรงจากเนื้อเยื่อไต หลังจากฉีดสารละลายโครชิซิน หยดเซลล์ลงบนสไลด์และตากให้แห้ง ย้อมสีโครโมโซมแบบ ธรรมดาด้วยสารละลายสีจิมซ่า ผลการศึกษาพบว่าปลาการ์ตูนทั้ง 2 ชนิด มีจำนวนโครโมโซมดิพลอยด์ (2*n*) เท่ากับ 48 แท่ง มีจำนวนโครโมโซมพื้นฐาน (NF) เท่ากับ 94 ทั้งในเพศผู้และเพศเมีย ชนิดโครโมโซมประกอบด้วยโครโมโซมชนิดเมทาเซนทริก ซับเมทาเซนทริก อะโครเซนทริก และเทโลเซนทริก เท่ากับ 16-20-10-2 และ 14-26-6-2 ตามลำดับ ไม่พบความแตกต่าง ของโครโมโซมในปลาทั้ง 2 เพศ ปลาการ์ตูนมีสูตรคาริโอไทป์ คือ

ปลาการ์ตูนมะเขือเทศ; $2n$ (48) = $L^{m}_{10}+L^{sm}_{14}+ L^{a}_{10}+L^{t}_{2}+M^{m}_{6}+M^{sm}_{6}$

$2n$ (48) = 16m+20sm+10a+2t

ปลาการ์ตูนแก้มหนาม; $2n$ (48) = $L^{m}_{6}+L^{sm}_{10}+ L^{a}_{4}+M^{m}_{8}+M^{sm}_{16}+M^{a}_{2}\ M^{t}_{2}$

$2n$ (48) = 14m+26sm+6a+2t

ABSTRACT

The karyotypic characteristics of tomato anemonefish, *Amphiprion frenatus* and spine cheek anemonefish, *Premnas biaculeatus* from Andaman sea, Southern of Thailand were obtained from the present study. The mitotic chromosomes were prepared by direct method from kidney tissues after *in vivo* colchicine treatment. Conventional staining technique was used to stain the metaphase chromosome with Giemsa's solution. The results showed that the diploid chromosome number (2n) of *A. frenatus* and *P. biaculeatus* were 48, with the fundamental numbers (NF) being 94 in both males and females. The chromosomes of *A. frenatus* and *P. biaculeatus* present metacentric, submetacentric, acrocentric, and telocentric chromosomes as 16-20-10-2

and 14-26-6-2, respectively. No morphologically differentiated sex chromosomes were detected in both sexes. The karyotype formulae were as follows:

A. frenatus; 2*n* (48) = $L^{m}_{10}+L^{sm}_{14}+ L^{a}_{10}+L^{t}_{2}+M^{m}_{6}+M^{sm}_{6}$

2*n* (48) = 16m+20sm+10a+2t

P. biaculeatus; 2*n* (48) = $L^{m}_{6}+L^{sm}_{10}+ L^{a}_{4}+M^{m}_{8}+M^{sm}_{16}+M^{a}_{2}\ M^{t}_{2}$

2*n* (48) = 14m+26sm+6a+2t

คำสำคัญ: ปลาการ์ตูนมะเขือเทศ ปลาการ์ตูนแก้มหนาม แคริโอไทป์ โครโมโซม อิดิโอแกรม

Keywords: *Amphiprion frenatus*, *Premnas biaculeatus*, Karyotype, Chromosome, ideogram

INTRODUCTION

Pomacentridae (Perciformes) consists of 28 genera and around 320 species known as damselfishes and anemonefishes. This family is one of the most diverse among seawater fishes, with species broadly dispersed in several tropical seas of the world (Nelson, 2006). Species of the Subfamily several species raise a great ornamental importance because of their various color forms, and this has led to their commercial exploitation (Molina and Galetti, 2004). In Thailand, 2 genera and 29 Amphiprioninae can be found.

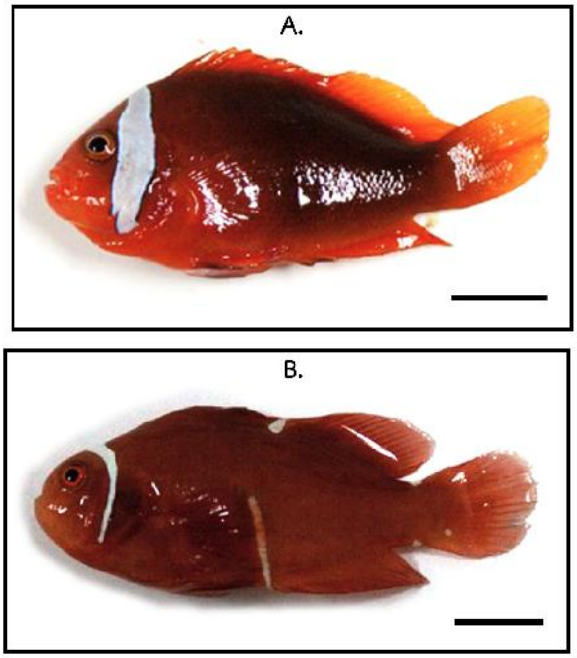


Figure 1 General characteristics of the tomato anemonefish (*Amphiprion frenatus*) (A.) and spine cheek anemonefish (*Premnas biaculeatus*) (B.), scale bars indicate 2 centimeters.

Up to now, cytogenetic analyses has being conducted in 30 Pomacentridae species , and among them, only 5 species belong to the subfamily Amphiprioninae (Takai and Kosuga 2007). All species present a diploid chromosome number (2n) of 48 chromosomes with the fundamental numbers (NF) ranging from 78 to 96 ((Molina and Galetti, 2004, Takai and Kosuga , 2007)). Pericentric inversions are the main responsible for changes in NF observed in five *Amphiprion* (*A. perideraion*, *A. polymnus*, *A. clarkii*, *A. ocellaris* and *A. frenatus*) (Table 1). Here we performed conventional cytogenetic analyzed two

anemonefishes, named: the tomato anemonefish (*Amphiprion frenatus*) and spine cheek anemonefish (*Premnas biaculeatus*)

RESEARCH METHODOLOGY

Fourteen specimens (3 females and 4 males) of *A. frenatus* and (3 females and 4 males) of *P. biaculeatus* were obtained from Phuket Marine Biological Center and Phang Nga Coastal Research and Development Center (Figure 1).

Chromosome were prepared according to the direct method with colchicine injected into the specimens (Chen and Ehbeling, 1968, Nanda *et al*. 1995). Briefly, the fishes were injected on their intramuscular or abdominal cavity with 0.01 ml/g (body weight) of colchicine. After 1 hour, kidney tissues were taken out, placed into a petridish and cut into small pieces. The cells were inclubated with hypotonic solution (0.075M KCl) for 30 min and fixed with Carnoy's fixative (a mixture of acetic acid and methyl alcohol (1:3) freshly prepared). Then, cell suspensions were dropped on clear slides, and after air-dried, stained slide with 20% Giemsa solution diluted with phosphate buffered saline. Chromosome checking was performed on mitotic metaphase cells under a light microscope, conventional staining chromosomes on the same metaphase plates were examined and agree with the chromosome nomenclature well-known as Turpin and Lejeune (1965). For the estimate of fundamental number (NF), the NF was obtained by assigning a value of two to metacentric, submetacentric and acrocentrics and one to telocentrics chromosomes. The idiogram was constructed using a model drawing of karyotype and accomplished by a computer program.

Table 1. Review of anemonefishes cytogenetic reports in the subfamily Amphiprioninae.

Species	2*n*	NF	m	sm	a	t	NORs	Reference
Pink (*A. perideraion*)	48	96	12	26	10	-	2 (a)	Takai and Kosuga (2013)
Saddleback (*A. polymnus*)	48	96	20	20	8	-	2 (a)	Tanomtong *et al*. (2012)
Clak's (*A. clarkii*)	48	78	14	16	18	-	-	Arai and Inoue (1976)
	48	86	12	26	10	-	2 (a)	Takai and Kosuga (2007)
False clown (*A. ocellaris*)	48	84	14	22	12	-	-	Arai *et al*. (1976)
Tomato (*A. frenatus*)	48	92	14	22	8	4	2 (a)	Molina and Galetti (2004)
	48	86	12	26	10	-	2 (a)	Takai and Kosuga (2007)
	48	94	16	22	8	2	-	Present study
Spine cheek (*P. biaculeatus*)	48	94	14	26	6	2	-	Present study

Remark: 2*n* = diploid chromosome number, NF = fundamental number, m = metacentric chromosome, sm = submetacentric chromosome, a = acrocentric chromosome, t = telocentric chromosome, NORs = nucleolar organizer regions, and - = not available.

RESULTS

Both species presented a 2*n*=48 with the NF of 94 in both male and females. No cytological visible heteromorphic sex chromosomes were identified. The chromosomes of *A. frenatus* and *P. biaculeatus* present metacentric, sub-metacentric, acrocentric, and telocentric chromosomes as 16-20-10-2 and 14-26-6-2, respectively. The karyotype formulae were as follows: *A. frenatus*; $2n = L^{m}_{10}+L^{sm}_{14}+ L^{a}_{10}+L^{t}_{2}+ M^{m}_{6}+M^{sm}_{6}$ or 16m+20sm+10a+2t and *P. biaculeatus*; $2n = L^{m}_{6}+ L^{sm}_{10}+L^{a}_{4}+M^{m}_{8}+M^{sm}_{16}+M^{a}_{2}\ M^{t}_{2}$ or 14m+26sm+6a+2t (Table 2) (Figure 2-4).

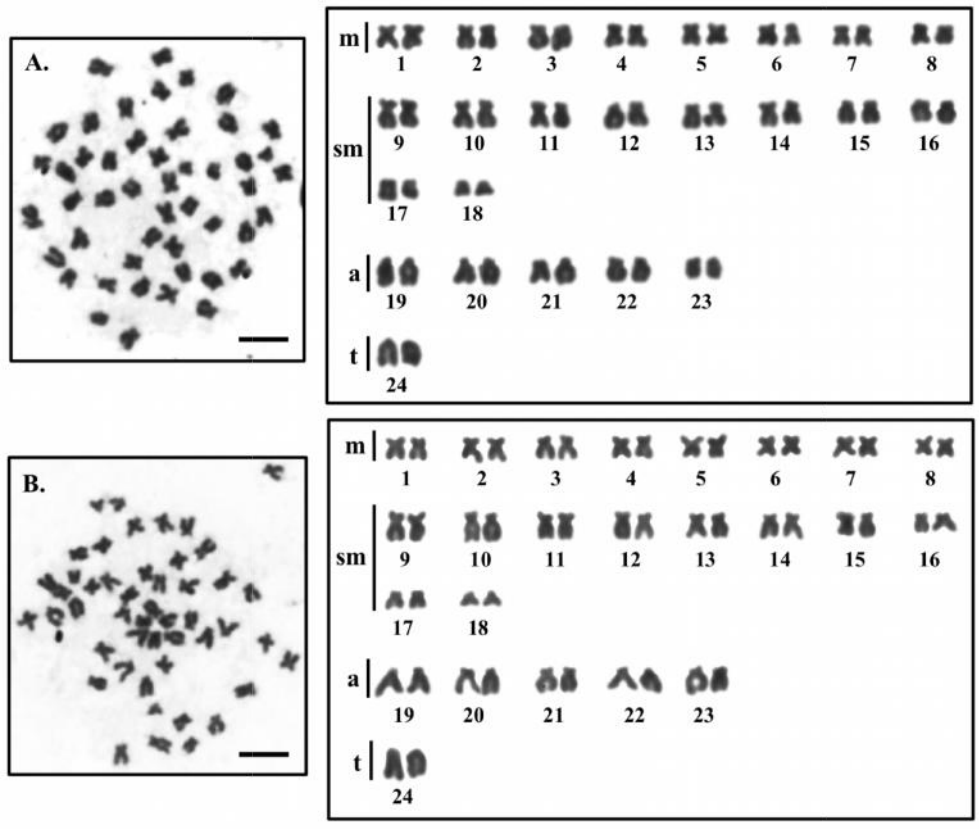


Figure 2 Metaphase chromosome plates and karyotypes of male (A.) and female (B.) of tomato anemonefish (*Amphiprion frenatus*), 2*n* = 48 by conventional staining technique (scale bars 5 micrometers).

DISCUSSION

The present study represents the first cytogenetic report on conducted on the cheek anemonefish, (*P. biaculeatus*) and on the spine tomato anemonefish, (*A. frenatus*), both from the Andaman Sea, Southern Thailand. Their 2n of 48 chromosomes are in agreement with the previous reports from Japan (Molina and Galetti, 2004, Takai and Kosuga , 2007). In fact, this represent the most usual condition found for Pomacentridae species and for most marine fishes (reviewed in Arai, 2011) The fundamental number (NF) of *A. frenatus* was 94 and the karyotype comprises 46 bi-armed and 2 mono-armed chromosomes (Fig. 2). These results different from the studies of Molina and Galetti (2004) and Takai and Kosuga (2007) that reported the fundamental number of *A. frenatus* with 44 bi-armed and 4 mono-armed chromosomes and 38 bi-armed and 10 mono-armed chromosomes, respectively (Table 1).

According to Ojima (1983), the karyotype containing of only 48 telocentric chromosomes (NF=48) represent the ancestral condition for fishes Pomacentridae has several species with conserved karyotype of 48 chromosomes (Ojima and Kashiwagi, 1981). Therefore, we believe the 48 telocentric chromosomes were also ancestor in Pomacentridae (Takai and Ojima, 1987). Thus, karyotypes of several pomacentrid fishes have been characterized as having a stable chromosome number of 2*n* = 48 and broadly ranging NFs between 48 and 88. These results indicate that karyotype differentiation in pomacentrid fishes has occurred preferentially due to of the occurrence of pericentric inversions (Takai and Ojima, 1991a, b). Here, pericentric inversions occurred in both *A. frenatus* and *P. biaculeatus* rearrangement resulted

in the increasing number of chromosome arms (NF=94) compared to the ancestral karyotype (NF=48).

Further, the application of other cytogenetic techniques such as C-banding, NOR-banding techniques may offer interesting discoveries linked to phylogenetic relationships.

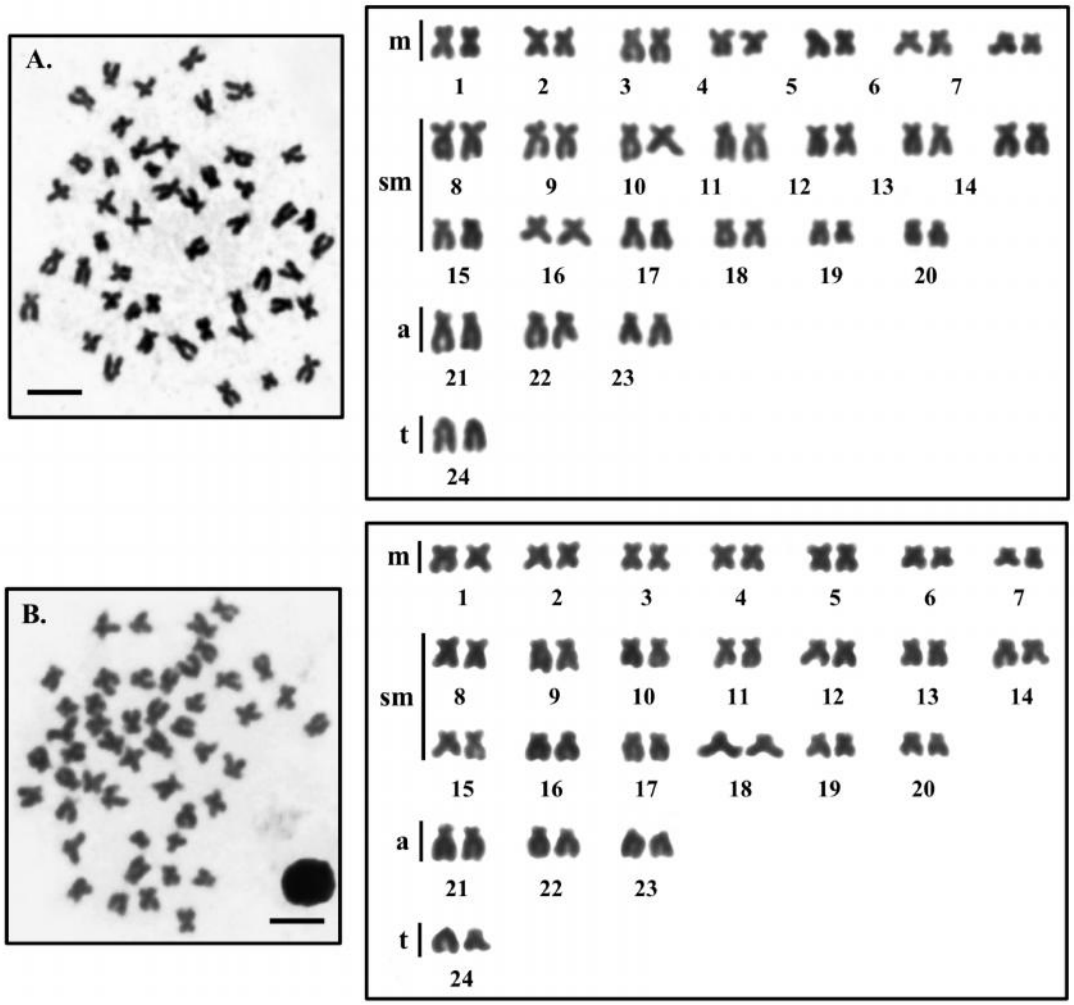
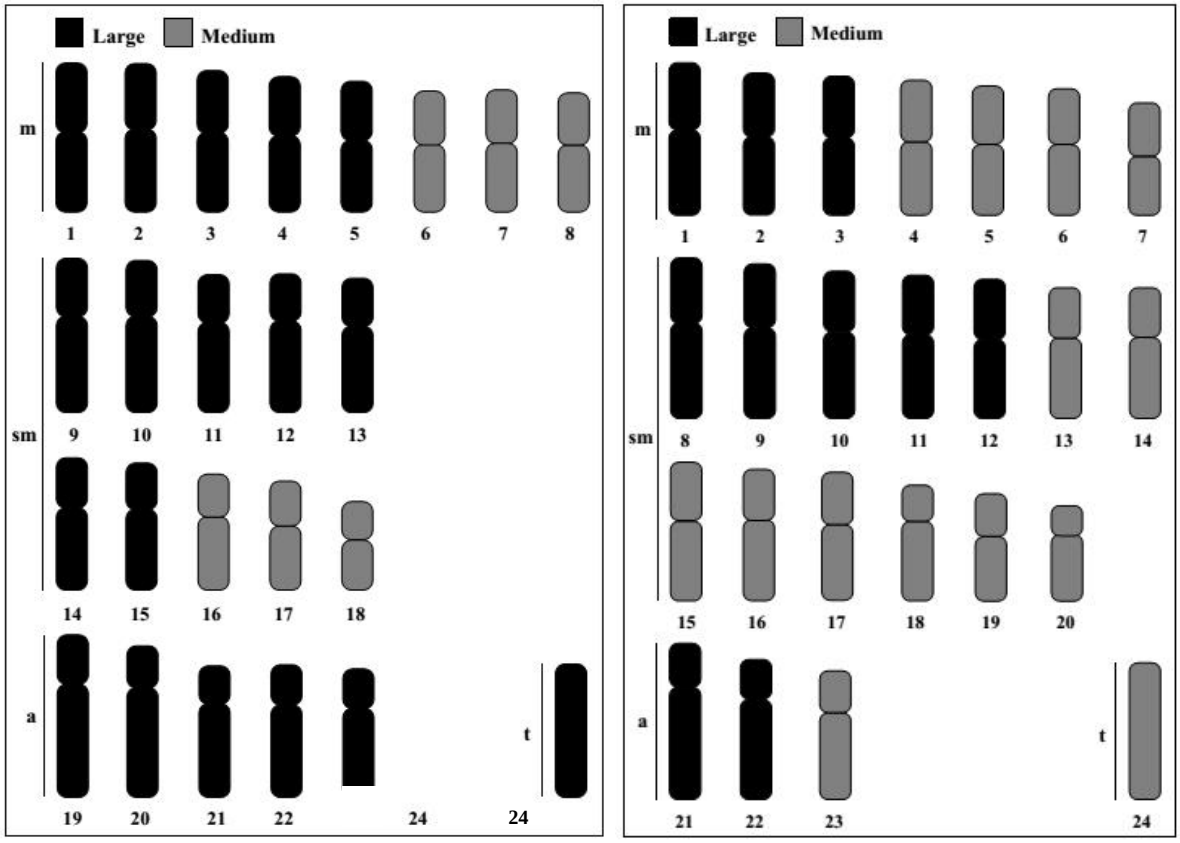


Figure 3 Metaphase chromosome plates and karyotypes of male (A.) and female (B.) of spine cheek anemonefish (*Premnas biaculeatus*), 2*n* = 48 by conventional staining technique (scale bars 5 micrometers).



A B

Figure 4. Standardized idiogram showing lengths and shapes of chromosomes of the tomato anemonefish (*Amphiprion frenatus*), 2*n* = 48 (A.) and the spine cheek anemonefish (*Premnas biaculeatus*), 2*n* = 48 (B.) by conventional staining technique.

Table 2 Mean length of short arm chromosomes (Ls), length long arm chromosomes (Ll), length total arm chromosomes (LT), centromeric index (CI), and relative length (RL) from 20 metaphases of the tomato anemonefish (*Amphiprion frenatus*), 2*n*=48.

Chro. pair	Ls	Ll	LT	CI	RL	Chro. size	Chro. type
1	1.9794	2.2944	4.2738	0.5375	0.0468	Large	Metacentric
2	1.8923	2.3620	4.2543	0.5559	0.0466	Large	Metacentric
3	1.8063	2.2641	4.0704	0.5576	0.0445	Large	Metacentric
4	1.6906	2.1830	3.8736	0.5622	0.0424	Large	Metacentric
5	1.6915	2.0504	3.7419	0.5486	0.0409	Large	Metacentric
6	1.5633	1.9195	3.4827	0.5509	0.0381	Medium	Metacentric
7	1.5280	1.9600	3.4880	0.5661	0.0382	Medium	Metacentric
8	1.4886	1.9084	3.3970	0.5616	0.0371	Medium	Metacentric
9	1.6456	2.7549	4.4005	0.6241	0.0481	Large	Submetacentric
10	1.6110	2.7440	4.3550	0.6275	0.0475	Large	Submetacentric
11	1.3553	2.5857	3.9410	0.6517	0.0429	Large	Submetacentric
12	1.3620	2.6152	3.9772	0.6553	0.0434	Large	Submetacentric
13	1.3796	2.4648	3.8444	0.6383	0.0420	Large	Submetacentric

Remark: Chro. = chromosome

Table 2 Mean length of short arm chromosomes (Ls), length long arm chromosomes (Ll), length total arm chromosomes (LT), centromeric index (CI), and relative length (RL) from 20 metaphases of the tomato anemonefish (*Amphiprion frenatus*), 2*n*=48. (continue)

Chro. pair	Ls	Ll	LT	CI	RL	Chro. size	Chro. type
14	1.4309	2.3475	3.7784	0.6203	0.0413	Large	Submetacentric
15	1.3503	2.3022	3.6525	0.6280	0.0399	Large	Submetacentric
16	1.2235	2.0938	3.3173	0.6291	0.0364	Medium	Submetacentric
17	1.2772	1.8327	3.1099	0.6095	0.0340	Medium	Submetacentric
18	1.1044	1.4236	2.5280	0.6034	0.0277	Medium	Submetacentric
19	1.4110	3.2292	4.6403	0.7174	0.0507	Large	Acrocentric
20	1.2178	3.1226	4.3404	0.7194	0.0475	Large	Acrocentric
21	1.0851	2.6725	3.7576	0.7097	0.0411	Large	Acrocentric
22	1.1532	2.6266	3.7799	0.7038	0.0413	Large	Acrocentric
23	1.1337	2.5301	3.6638	0.7006	0.0400	Large	Acrocentric
24	0.0000	3.7944	3.7944	1.0000	0.0416	Large	Telocentric

Remark: Chro. = chromosome

Table 3 Mean length of short arm chromosomes (Ls), length long arm chromosomes (Ll), length total arm chromosomes (LT), centromeric index (CI), and relative length (RL) from 20 metaphases of the spine cheek anemonefish (*Premnas biaculeatus*), 2*n*=48.

Chro. Pair	Ls	Ll	LT	CI	RL	Chro. size	Chro. type
1	1.8590	2.3385	4.1976	0.5578	0.0465	Large	Metacentric
2	1.7529	2.1350	3.8288	0.5577	0.0426	Large	Metacentric
3	1.6964	2.1629	3.9159	0.5519	0.0434	Large	Metacentric
4	1.6938	2.0268	3.7232	0.5453	0.0414	Medium	Metacentric
5	1.5886	1.9553	3.5439	0.5509	0.0395	Medium	Metacentric
6	1.5181	1.9550	3.4731	0.5619	0.0386	Medium	Metacentric
7	1.4689	1.6193	3.0882	0.5253	0.0345	Medium	Metacentric
8	1.8132	2.4990	4.4465	0.6001	0.0493	Large	Submetacentric
9	1.7523	2.3861	4.2513	0.6066	0.0472	Large	Submetacentric
10	1.6573	2.3023	4.0434	0.6092	0.0447	Large	Submetacentric
11	1.6496	2.1802	3.9519	0.6272	0.0438	Large	Submetacentric
12	1.6521	2.2140	3.7529	0.6189	0.0424	Large	Submetacentric
13	1.5389	2.2261	3.7121	0.6381	0.0414	Medium	Submetacentric
14	1.4859	2.1974	3.7945	0.6292	0.0409	Medium	Submetacentric
15	1.5971	2.1873	3.6816	0.6469	0.0419	Medium	Submetacentric
16	1.5944	2.0872	3.5209	0.6253	0.0409	Medium	Submetacentric
17	1.3351	2.1858	3.7490	0.6216	0.0391	Medium	Submetacentric
18	1.5084	2.2406	3.1919	0.6168	0.0417	Medium	Submetacentric
19	1.4196	1.7723	3.0952	0.6053	0.0354	Medium	Submetacentric
20	1.2625	1.8327	4.2876	0.6004	0.0344	Medium	Submetacentric

Remark: Chro. = chromosome

Table 3 Mean length of short arm chromosomes (Ls), length long arm chromosomes (Ll), length total arm chromosomes (LT), centromeric index (CI), and relative length (RL) from 20 metaphases of the spine cheek anemonefish (*Premnas biaculeatus*), 2*n*=48. (continue)

Chro. Pair	Ls	Ll	LT	CI	RL	Chro. size	Chro. type
21	1.2003	3.0873	3.8409	0.7220	0.0474	Large	Acrocentric
22	1.0941	2.7468	3.5262	0.7164	0.0424	Large	Acrocentric
23	1.1351	2.3911	3.4380	0.7088	0.0391	Medium	Acrocentric
24	0.0000	3.7512	3.7512	1.0000	0.0415	Medium	Telocentric

Remark: Chro. = chromosome

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