NOR Polymorphism and Chromosome Analysis of Banggai Cardinalfish, *Pterapogon kauderni* (Perciformes, Apogonidae)

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Summary This is the first nucleolar organizer region (NOR) polymorphism and chromosome analysis of Banggai cardinalfish (*Pterapogon kauderni* Koumans, 1933). Kidney cell samples were taken from 10 male and 10 female fish. Mitotic chromosome preparations were prepared directly from kidney cells. Conventional and Ag-NOR banding techniques were applied to stain the chromosomes. The results showed that the diploid chromosome number of *P. kauderni* was $2n=46$, and the fundamental number (NF) was 92 in both males and females. The types of chromosomes were 6 large acrocentric, 4 medium metacentric, 14 medium submetacentric, and 22 medium acrocentric chromosomes. The results indicated that the short arm subtelomeric of the acrocentric chromosome pair 13 showed clearly observable NORs. This finding exhibited that three NOR polymorphism patterns were found: 1) homomorphic which shows an equal size of both chromosome pair 13 (13a13a), 2) heteromorphic that displays different sizes of NORs of chromosome pair 13 (13a13c) and 3) heteromorphic which is found in only one homologous chromosome pair 13 (13a13b). There was no observation of strange size chromosomes related to sex. The karyotype formula for *P. kauderni* was:

$$2n \text{ (diploid) } 46 = L_6^a + M_4^m + M_{14}^{sm} + M_{22}^a$$

Key words *Pterapogon kauderni*, Chromosome, NOR polymorphism, Karyotype.

The Banggai cardinalfish (*Pterapogon kauderni* Koumans, 1933) (Fig. 1) belongs to the class Actinopterygii (ray-finned fishes), superorder Percomorpha, order Perciformes, suborder Percoidei, and family Apogonidae (Nelson 2006). Cardinalfishes are a family Apogonidae found in the Atlantic, Indian, and Pacific Oceans; they are chiefly marine, but some species are found in brackish water and a few are found in fresh water. A handful of species are kept in the aquarium and are popular as small, peaceful, and colorful fish. Most species live in tropical or subtropical waters, where they inhabit coral reefs and lagoons (Johnson and Gill 1998, Kasiroek *et al.* 2017).

Nucleolar organizer regions (NORs) are parts of chromosomes in which there are ribosomal ribonucleic acid (rRNA) encoding genes (5.8S, 18S, and 28S). In all

eukaryotic organisms, rRNA genes occur in many copies, thus reflecting high cell demand for rRNA. NORs, as ribosomal gene clusters that were active in previous interphase, form prominent cytogenetic features, namely secondary constrictions (Andraszek *et al.* 2009). An important characteristic of NORs in fish is its inter- and intra-species polymorphism. NOR characterization can be a cytogenetic marker for cytotaxonomic studies and can even aid in constructing phylogenetic hypotheses for several fish groups. Some fish groups present a simple NOR system characterized by ribosomal cistrons on only one chromosome pair, whereas others have multiple NOR systems composed of cistrons dispersed over several chromosomes (Galetti 1998).

Although basic cytogenetic information is available for Apogonidae (Table 1), little is known about the karyotypic features of apogonids in the Pacific. The present study is the first report on chromosomal char-

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Fig. 1. General characteristic of the Banggai cardinalfish, *Pterapogon kauderni* Koumans, 1933 (Perciformes: Apogonidae). Scale bar indicates 2 cm.

Table 1. Review of fish cytogenetic reports in the family Apogonidae (genera; *Apogon*, *Nectamia*, *Phaeoptyx*, *Pterapogon*, and *Sphaeramia*).

Species	2n	NF	Karyotype	Ag-NORs	Locality	Reference
<i>Apogon americanus</i>	36	70	12m+6sm+16a+2t	2 (TR)	Brazil	Araújo <i>et al.</i> (2010)
<i>A. binotatus</i>	36	50	14m/sm+22a/t	—	USA	Rivlin <i>et al.</i> (1987)
	36	62	26m/sm+10a/t	—	USA	Rivlin <i>et al.</i> (1987)
	35	49	14m/sm+21a/t	—	USA	Rivlin <i>et al.</i> (1987)
<i>A. doederleini</i>	46	54	2m+6sm+38a/t	—	Japan	Ojima and Kojima (1985)
<i>A. endekataenia</i>	46	46	46a/t	—	India	Rishi (1973)
	46	52	2m+4sm+16a+24t	—	Japan	Murofushi (1986)
<i>A. imberbis</i>	36	56	—	—	Spain	Alvarez <i>et al.</i> (1991)
<i>A. lineatus</i>	46	52	2m+4sm+2a+38t	—	Japan	Murofushi (1986)
<i>A. maculatus</i>	34	61	27m/sm+7a/t	—	Puerto Rico	Rivlin <i>et al.</i> (1988)
<i>A. moluccensis</i>	46	46	46a/t	—	India	Rishi (1973)
<i>A. notatus</i>	46	52	2m+4sm+40a/t	—	Japan	Ojima and Kojima (1985)
	46	53	2m+5sm+39a/t	—	Japan	Ojima and Kojima (1985)
	46	52	2m+4sm+40a/t	—	Japan	Ojima and Kojima (1985)
	46	52	2m+4sm+40a/t	—	Japan	Murofushi (1986)
<i>A. nubilis</i>	46	92	2m+36sm+8a	—	USA	Rivlin <i>et al.</i> (1986)
<i>A. pseudomaculatus</i>	36	66	30m/sm+2a+4t	—	Puerto Rico	Rivlin <i>et al.</i> (1986)
<i>A. semilineatus</i>	46	52	2m+4sm+20a+20t	—	Japan	Murofushi <i>et al.</i> (1980)
	46	54	2m+6sm+38a/t	—	Japan	Ojima and Kojima (1985)
<i>Nectamia fusca</i>	46	—	2m+44sm/a/t	—	Pacific	Rivlin <i>et al.</i> (1986)
<i>Phaeoptyx pigmentaria</i>	38	—	6m+32sm/a/t	—	Atlantic	Rivlin <i>et al.</i> (1986)
<i>Pterapogon kauderni</i>	46	92	4m+14sm+28a	2 (TR)	Thailand	Present study
<i>Sphaeramia orbicularis</i>	46	50	4sm+42a/t	—	Pacific	Ojima and Kojima (1985)

Remarks: 2n=diploid chromosome number, NF=fundamental number (number of chromosome arm), m=metacentric, sm=submetacentric, a=acrocentric, t=telocentric chromosome, NORs=nucleolar organizer regions, TR=subtelomeric region, and —=not available.

acteristics of *P. kauderni* using conventional staining and Ag-NOR banding techniques. The results obtained will increase our basic knowledge of the cytogenetics of *P. kauderni*, which could form the basis for future research and provide data to ensure their survival.

Materials and methods

Sample collection

Twenty *P. kauderni* individuals (10 males and 10 fe-

males) were collected from Phang Nga Coastal Research and Development Center, Thailand. All specimens were maintained in aerated, flowing seawater aquaria until analysis.

Chromosome preparation

Chromosomes were prepared *in vivo* (Nanda *et al.* 1995) as follows. Phytohemagglutinin (PHA) solution was injected into the fish's abdominal cavity. After 24 h, colchicine was injected into the fish's intramuscular

and/or its abdominal cavity and then left for 2–4 h. The kidney was cut into small pieces, and then squash mixed with 0.075 M KCl (Kasiroek *et al.* 2017). After discarding all large pieces of tissue, 8 mL of cell sediments were transferred to a centrifuge tube and incubated for 25–35 min. The KCl was discarded from the supernatant after centrifugation at 1200 rpm for 8 min. Cells were fixed in fresh, cool fixative (3 methanol:1 glacial acetic acid) to which up to 8 mL of fixative were gradually added before being centrifuged again at 1200 rpm for 8 min, at which time the supernatant was discarded. The fixation was repeated until the supernatant was clear, and the pellet was mixed with 1 mL of fixative. The mixture was dropped onto a clean and cold slide by a micropipette, and then an air-drying technique was applied.

Chromosome staining

Conventional staining was done using 20% Giemsa's solution for 30 min (Choo-seangjaew *et al.* 2017). Ag-NOR banding technique was performed by adding four drops of 50% silver nitrate and 2% gelatin on slides. The slides were then sealed with cover glasses and incubated at 60°C for 5 min. Next, the slides were soaked in distilled water until the cover glasses were separated. Then, they were stained with 20% Giemsa's solution for 1 min (Howell and Black 1980, Sangpakdee *et al.* 2017).

Results and discussion

Diploid chromosome number, fundamental number and karyotype of P. kauderni

According to the results, this is the first report on *P. kauderni* cytogenetical knowledge. The present investigation revealed that the somatic chromosome number of *P. kauderni* is $2n=46$, the fundamental numbers (NF) were 92 in both males and females (Fig. 2). The types of chromosomes were 6 large acrocentric, 4 medium metacentric, 14 medium submetacentric, and 22 medium acrocentric chromosomes. Comparative studies with others in family Apogonidae have shown the same chromosome number as those found in *Apogon doederleini*, *A. notatus*, *Sphaeramia orbicularis* (Ojima and Kojima 1985), *A. endekataenia* (Rishi 1973), *A. lineatus* (Murofushi 1986), *A. moluccensis* (Rishi 1973), *A. semilineatus* (Murofushi *et al.* 1980, Ojima and Kojima 1985), and *Nectamia fusca* (Rivlin *et al.* 1986). Similar to other species in family Apogonidae, no cytologically distinguishable sex chromosome was observed (Araújo *et al.* 2010).

According to the previous cytogenetic information of the family Apogonidae, it is known that it has particular cytogenetic features, as they present extremely low diploid values in relation to the order Perciformes, and

Fig. 2. Metaphase chromosome plates and karyotypes of the Banggai cardinalfish (*Pterapogon kauderni*) male (A) and female (B), $2n$ (diploid)=46 by conventional staining technique. Arrows indicates nucleolar organizer regions (NORs) (Scale bar=5 μ m).

Fig. 3. Metaphase chromosome plates and karyotypes of the Banggai cardinalfish (*Pterapogon kauderni*) male (A) and female (B), $2n$ (diploid)=46 by Ag-NOR banding technique. Arrows show three polymorphism patterns of nucleolar organizer regions (NORs) of chromosome pair 13 (13a13a, 13c13a, and 13a13b). Scale bars indicate 5 μm.

Fig. 4. The six representative cells displaying polymorphism in size of nucleolar organizer regions (NORs) of chromosome pair 13 (13a, 13b, and 13c) of the 10 male and female Banggai cardinalfish (*Pterapogon kauderni*). Arrows indicate nucleolar organizer regions.

in some species, a remarkable variation in the karyotype formulae is also found. Such reduction in the diploid number might be as low as $2n=34$, as reported in *A. maculatus* (Rivlin *et al.* 1988). Nevertheless, the chromosomal numbers are reduced, suggesting a high incidence of centric fusions, and high fundamental numbers (NF) are also reported, like in *A. nubilus* ($2n=46$, NF=92), which indicates that other rearrangements, such as pericentric inversions, have also played a major role in the chromosomal diversification of this fish group.

The chromosome information of the family Apogon-

nidae revealed that 62.5% of all species analyzed so far ($N=16$ spp.) present diploid values equal to $2n=46$, suggesting this should be an ancestor condition for this family. Among them, the species *A. endekataenia* and *A. moluccensis* are characterized by a karyotype exclusively composed of telocentric chromosomes (Rishi 1973), a symplesiomorphic cytogenetic feature widely observed within the order Perciformes (Molina 2006). Accordingly, the available data indicates a great karyotypic diversity in the evolution of the group regarding both diploid number and chromosomal formulas, resulting

in high fundamental numbers (NF=46–92). This scenario indicates a simultaneous occurrence of different mechanisms of karyotypic diversification in the family Apogonidae, mainly Robertsonian rearrangements and pericentric inversions (Araújo *et al.* 2010). The karyotype formula for *P. kauderni* is as follows:

$$2n \text{ (diploid)} 46 = L_6^a + M_4^m + M_{14}^{sm} + M_{22}^a$$

Chromosome markers of *P. kauderni*

This is the first report on *P. kauderni* accomplished by Ag-NOR banding technique. The technique show dark bands (NOR-position) on the short arm subtelomeric of acrocentric chromosome pair 13 in both males and females (Figs. 3, 4). For other comparative studies, the species in the family Apogonidae, *A. americanus*, had a NOR on the short arm subtelomeric of submetacentric chromosome pair 8 (Araújo *et al.* 2010).

Our obtained results indicated that the short arm subtelomeric of the acrocentric chromosome pair 13 showed clearly observable NORs in all of 20 examined fish (10 male and 10 female fish). It was found that the respective NOR polymorphism patterns were detected in three, six and one of both male and female fish. Three karyotypic patterns of this fish are described below: 1) homomorphic which shows an equal size of both chromosome pair 13 (13a13a), 2) heteromorphic that displays different sizes of NORs of chromosome pair 13 (13a13c) and 3) heteromorphic which is found in only one homologous chromosome pair 13 (13a13b). This is in agreement with several previous reports on *Moenkhausia sanctaefilomenae* (Foresti *et al.* 1989), *Aphanius fasciatus* (Vitturi *et al.* 1995), *Leporinus friderici* (Galetti *et al.* 1995), *Salmo trutta* (Castro *et al.* 1996), *Salvelinus alpinus* (Reed and Phillips 1997), *Chondrostoma lusitanicum* (Collares-Pereira and Ráb 1999), *Hoplias malabaricus* (Born and Bertollo 2000), *Oedalechilus labeo* (Rossi *et al.* 2000), *Astyanax scabripinnis* (Soza *et al.* 2001), *A. altiparanae* (Mantovani *et al.* 2005), *Bryconamericus aff. exodon* (Paintner-Marques *et al.* 2002), *Apareiodon affinis* (Jorge and Filho 2004), *Aphanius fasciatus* (Vitturi *et al.* 2005), *Prochilodus lineatus* (Gras *et al.* 2007), *B. aff. iheringii* (Capistano *et al.* 2008), *Puntioplites proctozystron* (Supiwong *et al.* 2012), and *Lutjanus johnii* (Phimphan *et al.* 2013).

NORs play an important role in the display of perfect markers to display chromosomal polymorphism within and between species in many groups of fish. This variety may affect the NOR number, its localization on the chromosome, size, and active numbers in each genome. The previous studies of NOR exhibited variations between species, within species, and even between individuals (Castro *et al.* 1996). NORs on different homologous chromosomes may have different sizes. Some fish may even indicate a difference of up to a factor of two in size between NORs found on the same homologous chromo-

Fig. 5. Idiogram showing lengths and shapes of chromosomes of the Banggai cardinalfish (*Pterapogon kauderni*), diploid ($2n$)=46, by conventional staining technique. Arrow indicates nucleolar organizer region (NOR).

Fig. 6. Idiogram showing lengths and shapes of chromosomes of the Banggai cardinalfish (*Pterapogon kauderni*), diploid ($2n$)=46, by Ag-NOR banding technique. Arrow indicates nucleolar organizer region (NOR).

some. This is in accordance with previous reports that this extent of variety between NORs may be attributed to the number of cistrons and differences in transcriptional activity (Galetti *et al.* 1984).

In a view of both macro and microevolutionary points, NORs are very dynamic regions in evolutionary terms. These regions have been frequently used as phylogenetic

Table 2. Mean length of short arm chromosome (Ls), length long arm chromosome (LI), length total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphase cells of the male and female Banggai cardinalfish (*Pterapogon kauderni*), $2n=46$.

Chro. pair	Ls	LI	LT	RL $\pm$ SD	CI $\pm$ SD	Chro. type	Chro. size
1	0.700	0.801	1.501	0.0230 $\pm$ 0.0016	0.535 $\pm$ 0.016	Metacentric	Medium
2	0.613	0.705	1.318	0.0201 $\pm$ 0.0015	0.535 $\pm$ 0.033	Metacentric	Medium
3	0.523	1.022	1.545	0.0235 $\pm$ 0.0015	0.660 $\pm$ 0.030	Submetacentric	Medium
4	0.465	0.974	1.439	0.0219 $\pm$ 0.0008	0.674 $\pm$ 0.032	Submetacentric	Medium
5	0.422	0.982	1.404	0.0214 $\pm$ 0.0005	0.696 $\pm$ 0.038	Submetacentric	Medium
6	0.450	0.939	1.389	0.0212 $\pm$ 0.0006	0.675 $\pm$ 0.036	Submetacentric	Medium
7	0.423	0.891	1.315	0.0200 $\pm$ 0.0007	0.675 $\pm$ 0.048	Submetacentric	Medium
8	0.418	0.842	1.261	0.0192 $\pm$ 0.0008	0.667 $\pm$ 0.022	Submetacentric	Medium
9	0.392	0.787	1.179	0.0180 $\pm$ 0.0010	0.667 $\pm$ 0.032	Submetacentric	Medium
10	0.548	1.456	2.004	0.0306 $\pm$ 0.0020	0.724 $\pm$ 0.024	Acrocentric	Large
11	0.448	1.147	1.596	0.0243 $\pm$ 0.0013	0.715 $\pm$ 0.020	Acrocentric	Large
12	0.402	1.191	1.594	0.0244 $\pm$ 0.0010	0.745 $\pm$ 0.055	Acrocentric	Large
13*	0.442	1.142	1.584	0.0241 $\pm$ 0.0026	0.722 $\pm$ 0.030	Acrocentric	Medium
14	0.414	1.102	1.516	0.0231 $\pm$ 0.0007	0.725 $\pm$ 0.034	Acrocentric	Medium
15	0.442	1.037	1.479	0.0226 $\pm$ 0.0007	0.700 $\pm$ 0.034	Acrocentric	Medium
16	0.371	1.064	1.435	0.0219 $\pm$ 0.0007	0.740 $\pm$ 0.047	Acrocentric	Medium
17	0.400	0.993	1.394	0.0213 $\pm$ 0.0009	0.711 $\pm$ 0.030	Acrocentric	Medium
18	0.315	1.067	1.382	0.0211 $\pm$ 0.0008	0.770 $\pm$ 0.036	Acrocentric	Medium
19	0.363	1.008	1.371	0.0208 $\pm$ 0.0006	0.732 $\pm$ 0.033	Acrocentric	Medium
20	0.350	0.987	1.338	0.0203 $\pm$ 0.0005	0.737 $\pm$ 0.040	Acrocentric	Medium
21	0.372	0.939	1.311	0.0200 $\pm$ 0.0005	0.713 $\pm$ 0.039	Acrocentric	Medium
22	0.348	0.905	1.253	0.0190 $\pm$ 0.0011	0.719 $\pm$ 0.022	Acrocentric	Medium
23	0.331	0.872	1.204	0.0183 $\pm$ 0.0009	0.726 $\pm$ 0.035	Acrocentric	Medium

Remarks: * NOR-bearing chromosome and chro.=chromosome.

markers (Amemiya and Gold 1988), and consequently led to differences in chromosome location being detected even between sibling species (Volleth 1987). These changes in position during evolution have been quite often attributed to chromosome rearrangements (Hall and Parker 1995). In another way, conventional cytogenetic and the most recent hybridization techniques have shown NOR regions to be also polymorphic both in number and location within species (Schmid *et al.* 1995). Although one NOR-bearing chromosome pair is usually considered plesiomorphic in most groups analyzed, some vertebrate species show a multichromosomal location of NORs (Suzuki *et al.* 1990). A constant number of several stable NOR sites has been usually observed in these species, but in some cases, the multichromosomal pattern appears to be unstable (Castro *et al.* 2000).

The asymmetrical karyotype of *P. kauderni* with three type of chromosomes (metacentric, submetacentric, and acrocentric chromosomes) found in this study is the important chromosome marker. The idiogram shows continuous length gradation chromosomes (Figs. 5, 6). The size difference between the largest and the smallest chromosomes is approximately twofold. The chromosome marker of *P. kauderni*, chromosome pair 10, is the largest acrocentric chromosome. Data of the chromosomal checks on mitotic metaphase cells of the *P. kauderni* are shown in Table 2.

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