First Chromosome Analysis of the Humpback Cardinalfish, *Fibramia lateralis* (Perciformes, Apogonidae)

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Received July 27, 2015; accepted November 23, 2015

Summary The first chromosome analysis and nucleolar organizer region (NOR) pattern of the humpback cardinalfish (*Fibramia lateralis*) were studied. Samples from 10 male and 10 female fish were collected from the Andaman Sea and Gulf of Thailand. Mitotic chromosome preparations were prepared directly from kidney tissues. Conventional and Ag-NOR staining techniques were applied to stain the chromosomes. The results showed that the diploid chromosome number of *F. lateralis* was 2ₙ=46, and the fundamental numbers (NF) were 54 in both sexes. The karyotype consisted of 8 large acrocentric, 12 large telocentric, 24 medium telocentric and 2 small telocentric chromosomes. Moreover, the results indicated that the region adjacent to the telomere of the short arm of the second acrocentric chromosome pair showed clearly observable nucleolar organizer regions (NORs). Strange size chromosomes related to sex were not observed. The karyotype formula for *F. lateralis* is as follows:

$$2n(\text{diploid}) = 46 = L_8^a + L_{12}^a + M_{24}^a + S_2^a$$

Key words *Fibramia lateralis*, Chromosome, Karyotype, Nucleolar organizer region.

Cardinalfishes are a family Apogonidae belonging to the class Actinopterygii (ray-finned fishes), superorder Percormorpha, order Perciformes and suborder Percioidei (Nelson 2006). They are chiefly marine, but some species are found in brackish water and a few are found in fresh water. Several 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). Apogonids are widely distributed from warm temperate to tropical areas in the Pacific, Indian and Atlantic Oceans. Most species occur in coral or rocky reefs, while some species inhabit seagrass and coral-line algal meadows, soft-bottom communities, estuaries and lowland freshwater. Eschmeyer and Fong (2015) reported 356 valid species from the listings in the Catalog of Fishes. The family has been traditionally divided into four subfamilies: Apogoninae including most of the species (333 species), Pseudamiinae including only 20 species, Amioidinae including only two species and Paxtoninae including only one species (Eschmeyer and Fong 2015). The Humpback cardinalfish, *Fibramia lateralis* (Valenciennes 1832) has a discreet or diffuse midline body stripe ending in a basicaudal spot smaller than the pupil of the eye (Fig. 1).

Up to the present, basic cytogenetic information is available for the family Apogonidae in only 14 of the 356 species (Table 1). The 2n varied from 34 to 46 chromosomes (mostly 2n=46) and the NF variation occurred between 46 and 92 in this family. Nevertheless, little is known about the karyotypic features of apogonids in the Pacific and Indian Oceans. NOR banding technique was also studied in only one species to detect nucleolar organizer regions (NORs) (Araújo et al. 2010). These regions 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, which were active in previous interphase, form prominent cytogenetic features, namely secondary constrictions (Andraszek et al. 2009). 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 have a simple NOR system characterized by ribosomal cistrons on only a single chromosome pair, whereas others have a multiple NOR system composed of cistrons dispersed over several chromosome pairs (Galetti 1998).

The present study is the first report on chromosomal characteristics of *F. lateralis* using conventional staining and Ag-NOR banding techniques. The obtained results will increase the basic knowledge of the cytogenetics of *F. lateralis*, which could form the basis for future research and provide data to ensure their survival. Moreover, the knowledge on basic cytogenetics could be applied to numerous breeding studies, and this could also provide insight into species conservation and chromosome evolution studies of Apogonidae.

### Materials and methods

**Sample collection**

We collected 10 males and 10 females of *F. lateralis* (20 samples) from the Andaman Sea and the Gulf of Thailand. All specimens were maintained in aerated, flowing seawater aquaria at the Institute of Marine Science, Burapha University, Muang, Chonburi Province until analysis.

**Chromosome preparation**

Chromosomes were prepared *in vivo* (Chen and Ebeling 1968, Nanda *et al.* 1995) as follows. The 0.05%
colchicine was injected into the fish’s intramuscular and then left for one hour. The kidney was cut into small pieces, and then squash mixed with 0.075 M KCl. 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 followed by air-drying.

**Chromosome staining**

Conventional staining was done using 20% Giemsa’s solution for 30 min (Chooseangjaew 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).

**Chromosome analysis**

Metaphase figures were analyzed according to the chromosome classification of 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. The fundamental number, number of chromosome arm (NF), was obtained by assigning a value of two to metacentric, submetacentric and acrocentric chromosomes and one to telocentric chromosomes (Chooseangjaew et al. 2017).

**Results and discussion**

**Diploid chromosome number, fundamental number and karyotype of F. lateralis**

This is the first report on *F. lateralis* cytogenetical knowledge. The present study revealed that the diploid chromosome number of *F. lateralis* was 2n=46, and the fundamental numbers (NF) were 54 in both males and females (Fig. 2). The types of chromosomes were 8 large acrocentric, 12 large telocentric, 24 medium telocentric, and 2 small telocentric chromosomes. Comparative studies with others in the family Apogonidae have shown the same chromosome number as those found in *Jaydia lineata* (Murofushi 1986), *Nectamia fusca* (Rivlin et al. 1986), *Ostorkinhus doederleini*, *O. notatus*, *Sphaeramia orbicularis* (Ojima and Kojima 1985), *O. endekataenia*, *O. moluccensis* (Rishi 1973) and *O. semilineatus* (Murofushi et al. 1980, Ojima and Kojima 1985). However, it differs from *Phaeoptyx pigmentaria* (2n=38), *A. americanus*, *A. binotatus*, *A. imberbis*, *A. pseudomaculatus* (2n=36) and *A. maculatus* (2n=34) (Rivlin et al. 1986, 1987, 1988, Alvarez et al. 1991). Although *F. lateralis* has the same 2n as most of the species, its NF is different except *O. doederleini* and *O. semilineatus* (Ojima and Kojima 1985). In addition, the

Fig. 2. Metaphase chromosome plates and karyotypes of male (A.) and female (B.) humpback cardinalfish (*Fibramia lateralis*), 2n=46 by conventional staining technique (scale bars 10 μm).
results showed that cytologically distinguishable sex chromosomes were observed. It is similar to other species in the family Apogonidae (Araújo et al. 2010).

The chromosome data of the family Apogonidae revealed that 60% of all species analyzed so far (N=15 spp.) present diploid values equal to 2n=46, suggesting this should be an ancestor condition for this family. According to the chromosome diploid, the family Apogonidae is divided into two groups: first, 2n=46 found in the genera Fibramia, Jaydia, Nectamia, Ostorrhinchus and Sphaeramia; second, 2n=34–38 found in the genera Apogon and Phaeoptyx. It is known that it has particular cytogenetic features, as they present extremely low diploid values in relation to the order Perciformes and, 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 N. fusca (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 (Araújo et al. 2010).

Among species in the family Apogonidae, some species, such as O. endekataenia and O. moluccensis, are characterized by a karyotype exclusively composed of telocentric chromosomes (Rishi 1973), a sympleiomorphic 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 F. lateralis is as follows:

\[ 2n (diploid) = 46 = L_1^4 + L_1^4 + M_{12}^{14} + S_1^{14} \]

**Chromosome markers of F. lateralis**

This is the first report on F. lateralis accomplished by the Ag-NOR banding technique. The technique shows dark bands (NOR positions) on the subtelomeric short arm of the second acrocentric chromosome pair in both males and females (Fig. 3). For other comparative studies of the species in the family Apogonidae, A. americanus had a NOR on the subtelomeric short arm of the submetacentric chromosome pair 8 (Araújo et al. 2010).

Our obtained results indicated that the subtelomeric short arm of the second acrocentric chromosome pair

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**Fig. 3.** Metaphase chromosome plates and karyotypes of male (A.) and female (B.) humpback cardinalfish (Fibramia lateralis), 2n=46 by Ag-NOR banding technique; scale bars indicate 10 µm.
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Fig. 4. Standardized idiogram showing lengths and shapes of chromosomes of the humpback cardinalfish (*Fibramia lateralis*), $2n=46$ by conventional staining technique.

Fig. 5. Standardized idiogram of chromosomes of the humpback cardinalfish (*Fibramia lateralis*), $2n=46$ by Ag-NOR banding technique. The arrow indicates nucleolar organizer regions on the short arm of acrocentric chromosome pair 2.

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 male and female humpback cardinalfish (*Fibramia lateralis*), $2n=46$.

<table>
<thead>
<tr>
<th>Chro. pair</th>
<th>Ls</th>
<th>Ll</th>
<th>LT</th>
<th>RL±SD</th>
<th>CI±SD</th>
<th>Chro. type</th>
<th>Chro. size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.333</td>
<td>1.054</td>
<td>1.388</td>
<td>0.0268±0.0017</td>
<td>0.753±0.049</td>
<td>Acrocentric</td>
<td>Large</td>
</tr>
<tr>
<td>2*</td>
<td>0.305</td>
<td>0.996</td>
<td>1.301</td>
<td>0.0254±0.0020</td>
<td>0.761±0.033</td>
<td>Acrocentric</td>
<td>Large</td>
</tr>
<tr>
<td>3</td>
<td>0.307</td>
<td>0.951</td>
<td>1.258</td>
<td>0.0243±0.0013</td>
<td>0.753±0.033</td>
<td>Acrocentric</td>
<td>Large</td>
</tr>
<tr>
<td>4</td>
<td>0.296</td>
<td>0.870</td>
<td>1.166</td>
<td>0.0225±0.0015</td>
<td>0.743±0.035</td>
<td>Acrocentric</td>
<td>Large</td>
</tr>
<tr>
<td>5</td>
<td>0.000</td>
<td>1.585</td>
<td>1.585</td>
<td>0.0307±0.0025</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Large</td>
</tr>
<tr>
<td>6</td>
<td>0.000</td>
<td>1.347</td>
<td>1.347</td>
<td>0.0260±0.0011</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Large</td>
</tr>
<tr>
<td>7</td>
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<td>1.275</td>
<td>1.275</td>
<td>0.0246±0.0009</td>
<td>1.000±0.000</td>
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<td>Large</td>
</tr>
<tr>
<td>8</td>
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<td>1.231</td>
<td>1.231</td>
<td>0.0238±0.0007</td>
<td>1.000±0.000</td>
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<td>Large</td>
</tr>
<tr>
<td>9</td>
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<td>1.201</td>
<td>1.201</td>
<td>0.0232±0.0008</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Large</td>
</tr>
<tr>
<td>10</td>
<td>0.000</td>
<td>1.178</td>
<td>1.178</td>
<td>0.0227±0.0007</td>
<td>1.000±0.000</td>
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<td>Large</td>
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<tr>
<td>11</td>
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<td>1.157</td>
<td>0.0223±0.0006</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Medium</td>
</tr>
<tr>
<td>12</td>
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<td>1.134</td>
<td>0.0219±0.0006</td>
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<td>1.112</td>
<td>1.112</td>
<td>0.0215±0.0004</td>
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<td>Medium</td>
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<tr>
<td>14</td>
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<td>1.090</td>
<td>1.090</td>
<td>0.0211±0.0004</td>
<td>1.000±0.000</td>
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<td>15</td>
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<td>1.072</td>
<td>1.072</td>
<td>0.0207±0.0005</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Medium</td>
</tr>
<tr>
<td>16</td>
<td>0.000</td>
<td>1.050</td>
<td>1.050</td>
<td>0.0203±0.0004</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Medium</td>
</tr>
<tr>
<td>17</td>
<td>0.000</td>
<td>1.026</td>
<td>1.026</td>
<td>0.0198±0.0005</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Medium</td>
</tr>
<tr>
<td>18</td>
<td>0.000</td>
<td>0.981</td>
<td>0.981</td>
<td>0.0190±0.0008</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Medium</td>
</tr>
<tr>
<td>19</td>
<td>0.000</td>
<td>0.945</td>
<td>0.945</td>
<td>0.0183±0.0007</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Medium</td>
</tr>
<tr>
<td>20</td>
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<td>0.911</td>
<td>0.911</td>
<td>0.0177±0.0008</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Medium</td>
</tr>
<tr>
<td>21</td>
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<td>0.875</td>
<td>0.875</td>
<td>0.0170±0.0010</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Medium</td>
</tr>
<tr>
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<td>0.828</td>
<td>0.0161±0.0011</td>
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<td>Medium</td>
</tr>
<tr>
<td>23</td>
<td>0.000</td>
<td>0.734</td>
<td>0.734</td>
<td>0.0144±0.0021</td>
<td>1.000±0.000</td>
<td>Telocentric</td>
<td>Small</td>
</tr>
</tbody>
</table>

Remarks: * NOR-bearing chromosome and chro.=chromosome
showed clearly observable NORs in all of 20 examined fish (10 male and 10 female fish). The number and location of NORs in chromosomes can be variable among populations and/or closely related species, thus representing a useful cytotaxonomic marker for phylogenetic reconstruction in some groups (Cross et al. 2006). Structural rearrangements might be a cause of variation in either chromosomal location or frequency of these regions (Gu and Hua 2003, Shan et al. 2003). Although the karyotypes in Apogonidae have been related to a great number of pericentric inversions and Robertsonian fusions, variation in the number or position of NORs were absent between both analyzed populations of *F. lateralis*. The lack of information in other species restrains any further studies about the evolutionary pattern of these regions within Apogonidae.

NORs play an important role in displaying the perfect markers to display chromosomal polymorphism within and between species in many groups of fish. This variety may affect 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 chromosome. 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 markers (Amemiya and Gold 1988), and consequently lead to differences in chromosome location being detected even between sibling species (Vooleth 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 *F. lateralis* with two types of chromosomes (acrocentric and telocentric chromosomes) found in the present study is an important chromosome marker. The idiograms show continuous length gradation chromosomes (Figs. 4, 5). The size difference between the largest and the smallest chromosomes is approximately twofold. The chromosome markers of *F. lateralis* are chromosome pairs 5 and 23, which are the largest and the smallest telocentric chromosomes, respectively. Data of the chromosomal checks on mitotic metaphase cells of the *F. lateralis* is shown in the Table 2. Further studies in other species of the Apogonidae family are required in order to provide a better understanding about the dynamic scenario of karyotype diversification in this family.

Acknowledgements

This work was supported by the doctoral thesis support grant from the Faculty of Science, Burapha University, fiscal year 2015, the Institute of Marine Science, Burapha University and the Toxic Substances in Livestock and Aquatic Animals Research Group, Khon Kaen University.

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