Standardized Karyotype and Idiogram of Quoy’s Parrotfish, *Scarus quoyi* (Perciformes: Scaridae) by Conventional Staining and Ag-NOR Banding Techniques

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Summary This is the first report on standardized karyotype and idiogram analyses of Quoy’s parrotfish, *Scarus quoyi*, from the Andaman Sea, Southern Thailand. Kidney cell samples were taken from three male and three female fish. Mitotic chromosome preparations were prepared directly from kidney cells. Conventional staining and Ag-NOR banding techniques were applied to stain the chromosomes. The results showed that the diploid chromosome number of *Sc. quoyi* was \(2n=48\), and the fundamental number (NF) was 74 in both males and females. The types of chromosomes were 4 large metacentric, 4 large submetacentric, 16 large acrocentric, 18 large telocentric, 2 medium acrocentric, 2 medium telocentric, and 2 small telocentric chromosomes. There were no irregularly sized chromosomes related to sex. The region adjacent to the short arm near the telomere of acrocentric chromosome pair 6 showed clearly observable nucleolar organizer regions/NORs. The karyotype formula for *Sc. quoyi* is as follows:

\[ 2n \text{ (diploid)} = 48 = L_5^m + L_4^m + L_1^t + L_8^t + M_2^s + M_1^s + S_2^t \]

Key words *Scarus quoyi*, Karyotype, Chromosome, Idiogram.

The family Scaridae is among the best-known groups of typical coral reef fish, containing two subfamilies, the Sparisomatinae and the Scarinae, with a total of 10 genera and 90 species, which are popularly known as parrotfishes (Sale 1991, Parenti and Randall 2000). Parrotfishes are found in coral reef habitats and are morphologically conservative with coloration extremely important for species identification (Choat and Randall 1986, Bellwood 1994).

To date about 8% of the species in the order Perciformes have been karyotyped, revealing a model diploid chromosome number of \(2n=48\) chromosomes (Ohno 1970, Le Grande and Fitzsimons 1988, Affonso *et al.* 2001). However, karyotypes different from the typical order Perciformes pattern have 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).

It has become apparent that a karyological approach to fish systematics has become
increasingly more important. However, chromosomes of only four species of family Scaridae in the
world have been studied to date, namely *Calotomus japonicas* (2n=48) and *Chlorurus sordidus*
(2n=48) from Japan, and *Sparisoma axillare* (2n=46) and *Scarus coelestinus* (2n=48) from Brazil

Our objective was to obtain cytogenetic data for the family Scaridae by analysis of Quoy’s
parrotfish (*Scarus quoyi*), using conventional staining and Ag-NOR banding techniques. In the
future, 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.

Materials and methods

Three males and three females of *Sc. quoyi* were obtained from Phuket Province, Andaman
Sea, Southern Thailand (Fig. 1). The preparation of fish chromosomes was from kidney cells (Chen
and Ehbeling 1968, Nanda et al. 1995). The chromosomes were stained with 10% Giemsa’s for
30 min and identified for NORs by Ag-NOR staining (Howell and Black 1980). 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.

Results and discussion

*Diploid chromosome number, fundamental number and karyotype of Sc. quoyi*

Results showed that the diploid chromosome number was 2n=48 and NF=74 in both males
and females (Fig. 2). Similar to other species in the family Scaridae, no cytologically
distinguishable sex chromosome was observed (Arai and Koike 1980, Ojima and Yamamoto 1990,
Sena and Molina 2007).

A karyotype consisting of 48 acrocentric chromosomes has been considered the basal pattern
for the order Perciformes (Ohno 1970). This tendency has been observed in most of the species of
this order. This karyotypic constitution seems to be more commonly found in marine than in
rearrangements, although rare, have been suggested as an important source of karyotypic
diversification in some groups of fish, such as some species in the Labridae subfamily (Ueno and
Takai 2000, Molina and Galetti 2002). However, a high NF indicates that pericentric inversion
occurred wildly in the karyotypic evolution of these species. Pericentric inversions have been
considered to be the main factor responsible for the divergence among the order Perciformes
(Molina 2006, Sena and Molina 2007).
The *Sparisoma axillare* presented a different diploid chromosome number ($2n=46$, $NF=70$), indicating numerical reduction compared to *Calotomus japonicas* ($2n=48$, $NF=66$), *Chlorurus sordidus* ($2n=48$, $NF=66$), *Scarus coelestinus* ($2n=48$, $NF=88$), and *Sc. quoyi* ($2n=48$, $NF=74$). The presence of a large metacentric chromosome pair 1 in *Sp. axillare*, added to a reduced chromosome number (compare $2n=48$ acrocentric chromosomes for the order Perciformes) suggests a Robertsonian fusion event, which associated with pericentric inversion, may have molded the karyotype of this species (Sena and Molina 2007).

The karyotype of *Sc. quoyi* was composed of 4 large metacentric, 4 large submetacentric, 16 large acrocentric, 18 large telocentric, 2 medium acrocentric, 2 medium telocentric, and 2 small telocentric chromosomes. This was different from the study by Arai and Koike (1980) who found that for *Ca. japonicas* and *Ch. sordidus* from Japan, the number of metacentric, submetacentric,
Table 1. Cytogenetic reviews of parrotfishes in the family Scaridae (Perciformes: Labroidei).

<table>
<thead>
<tr>
<th>Species</th>
<th>2n</th>
<th>Karyotype formula</th>
<th>NF</th>
<th>NOR banded</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calotomus japonicas</td>
<td>48</td>
<td>8m+10sm+30t</td>
<td>66</td>
<td>—</td>
<td>Japan</td>
<td>Arai and Koike (1980)</td>
</tr>
<tr>
<td>Chlorurus sordidus</td>
<td>48</td>
<td>10m+8sm+30t</td>
<td>66</td>
<td>—</td>
<td>Japan</td>
<td>Arai and Koike (1980)</td>
</tr>
<tr>
<td>Sparisoma axillare</td>
<td>46</td>
<td>6m+14sm+4a+22t</td>
<td>70</td>
<td>2</td>
<td>Japan</td>
<td>Ojima and Yamamoto (1990)</td>
</tr>
<tr>
<td>Sparisoma axillare</td>
<td>48</td>
<td>6m+10sm+24a+8t</td>
<td>88</td>
<td>2</td>
<td>Brazil</td>
<td>Sena and Molina (2007)</td>
</tr>
<tr>
<td>Scarus coelestinus</td>
<td>48</td>
<td>6m+4sm+18a+20t</td>
<td>74</td>
<td>S(TR)2</td>
<td>Thailand</td>
<td>Present study</td>
</tr>
<tr>
<td>Scarus quoyi</td>
<td>48</td>
<td>6m+4sm+18a+20t</td>
<td>74</td>
<td>S(TR)2</td>
<td>Thailand</td>
<td>Present study</td>
</tr>
</tbody>
</table>

Remarks: 2n=diploid chromosome number, NF=fundamental number (number of chromosome arm), m=metacentric chromosome, sm=submetacentric chromosome, a=acrocentric chromosome, t=telocentric chromosome, S=short arm, TR=telomeric region, and — =not available.

Fig. 3. Metaphase chromosome plates and karyotypes of male (A) and female (B) of Quoy’s parrotfish (Scarus quoyi), 2n=48 by Ag-NOR banding technique; arrows indicate nucleolar organizer regions (scale bars=10μm).
and telocentric chromosomes were 8-10-30 and 10-8-30, respectively. Sena and Molina (2007) found that for *Sp. axillare* and *Sc. coelestinus* from Brazil, the number of metacentric, submetacentric, acrocentric, and telocentric chromosomes were 6-14-4-22 and 6-10-24-8, respectively (Table 1). The karyotype formula of *Sc. quoyi* is stated below.

\[2n (diploid) = 48 = L_4^{m} + L_n^{sm} + L_{16}^{a} + L_{16}^{t} + M_2^{s} + M_2^{t} + S_2^{t}\]

**Chromosome markers of Sc. quoyi**

The results of a cytogenetic study of *Sc. quoyi* performed by Ag-NOR staining technique are as follows. The objective of this technique is to detect NORs which represent the location of genes that have function in ribosome synthesis (18S and 28S ribosomal RNA). NORs produce numerous gene expressions and they are composed of more non-histone protein than other chromosome regions. A specific dark band (NOR-positive) is induced by the reduction of organic silver by these proteins that change silver to be dark (Sharma et al. 2002). The region adjacent to the short arm near telomere of acrocentric chromosome pair 6 showed clearly observable nucleolar organizer regions/NORs (Fig. 3). This is in agreement with the study by Sena and Molina (2007) who showed that the NORs of the family Scaridae *Sp. axillare* and *Sc. coelestinus* from Brazil were located on the short arms near the telomere of large acrocentric chromosome pairs 11 and 9, respectively.

Over 200 species of fishes have been investigated by the Ag-NOR staining technique. The amount and location of NORs can explain the evolution of each chromosome (Gold et al. 1986). Normally, most fishes have only one pair of small NORs in a chromosome complement. If some fishes have more than two NORs, it may be caused by the translocation between NORs and another chromosome (Sharma et al. 2002).

We have shown that the asymmetrical karyotype of *Sc. quoyi*, which has all four types of chromosomes (metacentric, submetacentric, acrocentric and telocentric) is an important chromosome marker. The idiograms showed continuous length gradation chromosomes. Figs. 3 and
Table 2. Mean length of short arm chromosomes (Ls), long arm chromosomes (Li), total arm chromosomes (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphases of the Quoy’s parrotfish (Scarus quoyi), 2n (diploid)=48.

<table>
<thead>
<tr>
<th>Chromosome pair</th>
<th>Ls</th>
<th>Li</th>
<th>LT</th>
<th>RL±SD</th>
<th>CI±SD</th>
<th>Chromosome size</th>
<th>Chromosome type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.460</td>
<td>1.835</td>
<td>3.295</td>
<td>0.042±0.004</td>
<td>0.556±0.023</td>
<td>Large</td>
<td>Metacentric</td>
</tr>
<tr>
<td>2</td>
<td>1.340</td>
<td>1.765</td>
<td>3.105</td>
<td>0.040±0.002</td>
<td>0.568±0.011</td>
<td>Large</td>
<td>Metacentric</td>
</tr>
<tr>
<td>3</td>
<td>1.162</td>
<td>1.663</td>
<td>2.826</td>
<td>0.036±0.000</td>
<td>0.588±0.016</td>
<td>Large</td>
<td>Metacentric</td>
</tr>
<tr>
<td>4</td>
<td>1.195</td>
<td>2.225</td>
<td>3.420</td>
<td>0.044±0.000</td>
<td>0.650±0.008</td>
<td>Large</td>
<td>Submetacentric</td>
</tr>
<tr>
<td>5</td>
<td>1.118</td>
<td>1.896</td>
<td>3.015</td>
<td>0.039±0.001</td>
<td>0.628±0.024</td>
<td>Large</td>
<td>Submetacentric</td>
</tr>
<tr>
<td>6*</td>
<td>0.779</td>
<td>2.995</td>
<td>3.774</td>
<td>0.049±0.004</td>
<td>0.793±0.056</td>
<td>Large</td>
<td>Acrocentric</td>
</tr>
<tr>
<td>7</td>
<td>0.867</td>
<td>2.952</td>
<td>3.820</td>
<td>0.049±0.002</td>
<td>0.772±0.021</td>
<td>Large</td>
<td>Acrocentric</td>
</tr>
<tr>
<td>8</td>
<td>0.830</td>
<td>2.845</td>
<td>3.675</td>
<td>0.047±0.001</td>
<td>0.774±0.016</td>
<td>Large</td>
<td>Acrocentric</td>
</tr>
<tr>
<td>9</td>
<td>0.790</td>
<td>2.747</td>
<td>3.537</td>
<td>0.046±0.001</td>
<td>0.776±0.015</td>
<td>Large</td>
<td>Acrocentric</td>
</tr>
<tr>
<td>10</td>
<td>0.760</td>
<td>2.677</td>
<td>3.437</td>
<td>0.044±0.001</td>
<td>0.778±0.015</td>
<td>Large</td>
<td>Acrocentric</td>
</tr>
<tr>
<td>11</td>
<td>0.705</td>
<td>2.620</td>
<td>3.325</td>
<td>0.043±0.001</td>
<td>0.788±0.019</td>
<td>Large</td>
<td>Acrocentric</td>
</tr>
<tr>
<td>12</td>
<td>0.655</td>
<td>2.497</td>
<td>3.152</td>
<td>0.041±0.002</td>
<td>0.792±0.026</td>
<td>Large</td>
<td>Acrocentric</td>
</tr>
<tr>
<td>13</td>
<td>0.612</td>
<td>2.330</td>
<td>2.942</td>
<td>0.038±0.003</td>
<td>0.791±0.023</td>
<td>Large</td>
<td>Acrocentric</td>
</tr>
<tr>
<td>14</td>
<td>0.530</td>
<td>1.927</td>
<td>2.457</td>
<td>0.032±0.000</td>
<td>0.784±0.029</td>
<td>Medium</td>
<td>Acrocentric</td>
</tr>
<tr>
<td>15</td>
<td>0.000</td>
<td>4.105</td>
<td>4.105</td>
<td>0.053±0.001</td>
<td>1.000±0.000</td>
<td>Large</td>
<td>Telocentric</td>
</tr>
<tr>
<td>16</td>
<td>0.000</td>
<td>3.757</td>
<td>3.757</td>
<td>0.048±0.001</td>
<td>1.000±0.000</td>
<td>Large</td>
<td>Telocentric</td>
</tr>
<tr>
<td>17</td>
<td>0.000</td>
<td>3.410</td>
<td>3.410</td>
<td>0.044±0.001</td>
<td>1.000±0.000</td>
<td>Large</td>
<td>Telocentric</td>
</tr>
<tr>
<td>18</td>
<td>0.000</td>
<td>3.275</td>
<td>3.275</td>
<td>0.042±0.002</td>
<td>1.000±0.000</td>
<td>Large</td>
<td>Telocentric</td>
</tr>
<tr>
<td>19</td>
<td>0.000</td>
<td>3.157</td>
<td>3.157</td>
<td>0.041±0.001</td>
<td>1.000±0.000</td>
<td>Large</td>
<td>Telocentric</td>
</tr>
<tr>
<td>20</td>
<td>0.000</td>
<td>3.097</td>
<td>3.097</td>
<td>0.040±0.002</td>
<td>1.000±0.000</td>
<td>Large</td>
<td>Telocentric</td>
</tr>
<tr>
<td>21</td>
<td>0.000</td>
<td>3.022</td>
<td>3.022</td>
<td>0.039±0.002</td>
<td>1.000±0.000</td>
<td>Large</td>
<td>Telocentric</td>
</tr>
<tr>
<td>22</td>
<td>0.000</td>
<td>2.772</td>
<td>2.772</td>
<td>0.036±0.000</td>
<td>1.000±0.000</td>
<td>Large</td>
<td>Telocentric</td>
</tr>
<tr>
<td>23</td>
<td>0.000</td>
<td>2.522</td>
<td>2.522</td>
<td>0.032±0.001</td>
<td>1.000±0.000</td>
<td>Medium</td>
<td>Telocentric</td>
</tr>
<tr>
<td>24</td>
<td>0.000</td>
<td>1.867</td>
<td>1.867</td>
<td>0.024±0.004</td>
<td>1.000±0.000</td>
<td>Small</td>
<td>Telocentric</td>
</tr>
</tbody>
</table>

Remark: *=NOR-bearing chromosome.

4 show the ideograms from conventional staining and Ag-NOR banding techniques. The largest and smallest chromosomes show an approximately twofold size difference. Data of the chromosomal checks on mitotic metaphase cells are shown in Table 2. Our results show that chromosome markers of Sc. quoyi, chromosome pair 1 is the largest metacentric and chromosome pair 24 is the smallest telocentric chromosome.

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2014 Standardized Karyotype and Idiogram of Quoy's Parrotfish, *Scarus quoyi* (Perciformes: Scaridae) 435


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