

First Report of NOR Polymorphism and Chromosome Analysis of John's Snapper, *Lutjanus johnii* (Perciformes, Lutjanidae) in Thailand

Sumalee Phimphan¹, Alongklod Tanomtong^{1*}, Sarun Jumrusthanasan¹, Weerayuth Supiwong¹, Pornnarong Siripiyasing², and La-Orsri Sanoamuang¹

¹Applied Taxonomic Research Center (ATRC), Department of Biology, Faculty of Science, Khon Kaen University, Muang, Khon Kaen 40002, Thailand

²Major of Biology, Faculty of Science and Technology, Mahasarakham Rajabhat University, Muang, Mahasarakham 44000, Thailand

Received July 23, 2012; accepted March 15, 2013

Summary The present study reports the first finding of the NOR (nucleolar organizer region) polymorphism and chromosome analysis in the John's snapper (*Lutjanus johnii*) from Thailand. Kidney cell samples were taken from four male and four female fish. Mitotic chromosome preparations were conducted using the standard squash technique, as well as directly from kidney cells. Metaphase spreads were performed on microscopic slides and then air-dried. Conventional and Ag-NOR banding techniques were applied to stain the chromosome. The results showed that the diploid chromosome number of *L. johnii* was $2n=48$, and the fundamental number (NF) was 48 in both male and female. Karyotypes were present as 28 large telocentric, 16 medium telocentric, and 4 small telocentric chromosomes. No strange-sized chromosomes related to sex were observed. The results indicated that the long arm subcentromeric of the telocentric chromosome pair 1 showed clearly observable NORs (satellite chromosomes). In addition, a heteromorphism of one female had different-sized NORs in chromosome pair 1 (1a1b), while three females and four males had equal-sized NORs in chromosome pair 1 with a homomorphism (1a1a). The karyotype formula for *L. johnii* is as follows:

$$2n \text{ (diploid)} 48=L_{28}^t+M_{16}^t+S_4^t$$

Key words John's snapper, *Lutjanus johnii*, Karyotype, NOR polymorphism.

The family Lutjanidae (lutjanid fish), or snappers, is a family of perciform fishes, mainly marine but with some members living in estuaries and entering freshwater to feed. Some of them are important food fish. Species from the family Lutjanidae represent one of the major resources for marine fishery. Lutjanid fish are widespread over the Atlantic, Pacific, and Indian oceans. Fishes of the family Lutjanidae occur in tropical and subtropical seas from shallow waters to depths of approximately 550 m. This family comprises 17 genera with about 105 species, and the genus *Lutjanus* with 64 species is the largest genus including over half of the species in this family (Nelson 2006).

In spite of the high number of species and its worldwide distribution, the family Lutjanidae has been little investigated, and contradictory results have been obtained concerning the phylogenetic relationships and the taxonomic status of some of its genera and species. For example, the validity of the genus *Ocyurus* has been extensively discussed (Chow and Walsh 1992, Domeier and Clarke 1992, Loftus 1992), leading some authors to propose the synonymization of genus

* Corresponding author, e-mail: tanomtong@hotmail.com

DOI: 10.1508/cytologia.78.335

Table 1. Review of cytogenetic reports of snappers in the family Lutjanidae (genera; *Lutjanus*, *Ocyurus*, and *Rhomboplites*).

Species	2n	Karyotype formula	NF	NOR banded	Locality	References
<i>L. alexandrei</i>	48	48t	48	2	Brazil	Rocha and Molina (2008)
<i>L. analis</i>	48	48t	48	2	Brazil	Rocha and Molina (2008)
	48	48t	48	2	Venezuela	Nirchio <i>et al.</i> (2008)
<i>L. argentimaculatus</i>	48	48t	48	–	China	Cao <i>et al.</i> (2002)
	48	48t	48	–	India	Patro and Prasad (1979)
	48	48t	48	–	India	Khuda-Bukhsh <i>et al.</i> (1995)
<i>L. bohar</i>	48	48t	48	–	China	Cao <i>et al.</i> (2002)
<i>L. cyanopterus</i>	48	48t	48	2	Brazil	Rocha and Molina 2008
<i>L. erythropterus</i>	48	48t	48	–	China	Cao <i>et al.</i> (2002)
<i>L. griseus</i>	48	48t	48	2–4	Venezuela	Nirchio <i>et al.</i> (2008)
<i>L. jocu</i>	48	48t	48	4	Brazil	Rocha and Molina 2008
<i>L. johnii</i>	48	48t	48	–	China	Li <i>et al.</i> (2005)
	48	48t	48	1	Thailand	Present study
<i>L. kasmira</i>	48	48t	48	–	India	Choudhury <i>et al.</i> (1979)
	48	48t	48	2	Japan	Ueno and Takai (2008)
<i>L. quinquelineatus</i>	48	48t	48	2	Japan	Ueno and Takai (2008)
	47	1m+46t	48	2	Japan	Ueno and Takai (2008)
<i>L. russelli</i>	48	48t	48	2	Thailand	Ueno and Ojima (1991)
<i>L. sanguineus</i>	48	48t	48	–	India	Rio <i>et al.</i> (1973)
<i>L. sebae</i>	48	48t	48	–	China	Yin <i>et al.</i> (2008)
	48	48t	48	1	China	Gou <i>et al.</i> (2011)
<i>L. synagris</i>	48	48t	48	2	Brazil	Rocha and Molina 2008
	48	cytotype I: 48t	48	2	Venezuela	Nirchio <i>et al.</i> (2008)
	47	cytotype II: 1m+46t	48	2	Venezuela	Nirchio <i>et al.</i> (2008)
<i>L. vitta</i>	48	48t	48	–	China	Li <i>et al.</i> (2005)
<i>O. chrysurus</i>	48	48t	48	–	Brazil	Galetti Jr. <i>et al.</i> (2006)
	48	48t	48	2	Brazil	Rocha and Molina 2008
	48	48t	48	2	Venezuela	Nirchio <i>et al.</i> (2009)
<i>R. aurorubens</i>	48	2a+46t	50	2	Venezuela	Nirchio <i>et al.</i> (2009)

Remarks: 2n=diploid chromosome number, NF=fundamental number (number of chromosome arm), m=metacentric chromosome, a=acrocentric chromosome, t=telocentric chromosome, and – =not available.

Ocyurus with the genus *Lutjanus* (Loftus 1992, Clarke *et al.* 1997).

In genus *Lutjanus*, cytogenetic studies have been performed in 16 species, including *L. alexandrei* (Rocha and Molina 2008), *L. analis* (Nirchio *et al.* 2008, Rocha and Molina 2008), *L. argentimaculatus* (Patro and Prasad 1979, Khuda-Bukhsh *et al.* 1995, Cao *et al.* 2002), *L. bohar* (Cao *et al.* 2002), *L. cyanopterus* (Rocha and Molina 2008), *L. erythropterus* (Cao *et al.* 2002), *L. griseus* (Nirchio *et al.* 2008), *L. jocu* (Nirchio *et al.* 2008), *L. johnii* (Li *et al.* 2005), *L. kasmira* (Choudhury *et al.* 1979, Ueno and Takai 2008), *L. quinquelineatus* (Ueno and Takai 2008), *L. russelli* (Ueno and Takai 2008), *L. sanguineus* (Rio *et al.* 1973), *L. sebae* (Guo *et al.* 2011, Yin *et al.* 2008), *L. synagris* (Rocha and Molina 2008, Nirchio *et al.* 2008), and *L. vitta* (Li *et al.* 2005) (Table 1). Most of these species have 2n=48 chromosomes consisting of only telocentric chromosomes and no morphologically distinguished sex chromosomes.

The structure, number, and morphology of a nucleolar organizer region (NOR) may be specific to populations, species, and subspecies. NOR is frequently used to compare variations, as well as to identify and explain specifications. Changes in chromosome number and structure can alter the number and structure of NOR. Robertsonian translocations may cause losses of NOR. Species, which have limited gene exchange due to geographical isolation, have elevated karyotype and NOR

variety. Therefore, different karyotypes are found even in small but isolated populations of these species. The use of NORs in explaining kinships depends on a large extent on the uniformity of this characteristic and on the degree of variety within a taxon (Yüksel and Gaffaroğlu 2008).

In the present article, we indicate the first finding of NOR polymorphism and chromosome analysis in the *L. johnii* from Thailand. We provide the very first report on chromosome standardization, including chromosome measurements of shape and size, karyotype formulation, and ideogramming. The results obtained can provide more cytogenetic information for future studies on taxonomy and evolutionary relationships of this genus. Moreover, it provides useful basic information for conservation and breeding practices, as well as for studies on the chromosome evolution of this fish.

Materials and methods

Sample collection

Eight wild *L. johnii* (Fig. 1) individuals (four males and four females) were collected from the coast of Phuket Province, Andaman Sea, 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 24h, colchicines was injected into the fish's intramuscular and/or its abdominal cavity and then left for 2–4h. The kidney and/or gills were cut into small pieces, then squash mixed with 0.075M KCl. After discarding all large pieces of tissue, 8ml 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 1,200 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 1,200 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. Ag-NOR banding (Howell and Black 1980) 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.

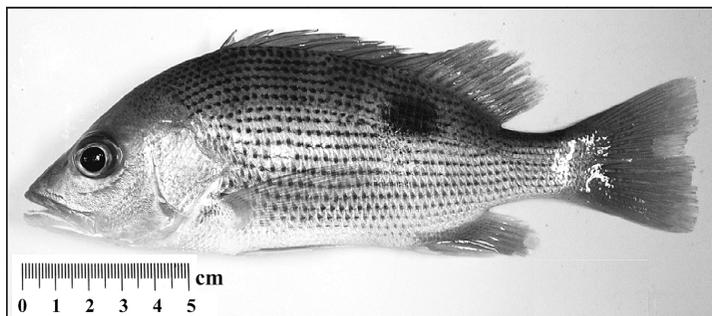


Fig. 1. General characteristics of the John's snapper (*Lutjanus johnii*) from Thailand.

Chromosome checking

Chromosome checking was performed on mitotic metaphase cells under a light microscope. Twenty clearly observable cells with well-spread chromosomes of each male and female were selected and photographed. The length of the short arm chromosome (Ls) and the long arm chromosome (Ll) were measured and the length of the total arm chromosome (LT, $LT=Ls+Ll$) was calculated. The relative length (RL), the centromeric index (CI), and standard deviation (SD) of RL and CI were estimated (Chaiyasut 1989). Values of 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. The fundamental number (NF) was obtained by assigning a value of two to metacentric, submetacentric, and acrocentric chromosomes, and one to telocentric chromosomes. All parameters were used in karyotyping and idiogramming.

Results and discussion

The karyotype and other chromosomal markers as revealed by conventional staining and Ag-NOR banding techniques were studied in *L. johnii*. Conservative karyotypes bearing a diploid chromosome number value of $2n=48$ (NF=48) (Figs. 2 and 4) are usually regarded as a typical condition of a great number of perch-like species, putatively associated with decreased levels of genetic variation among marine populations (Molina and Galetti 2004). Consequently, the occurrence of a large number of migration or dispersion, coupled with weak geographical barriers, may be responsible for an increased gene flow, thus leading to a genetic homogeneity in marine fish population reflected as unchanged karyotype (Molina *et al.* 2002, Rocha and Molina 2008). In the genus

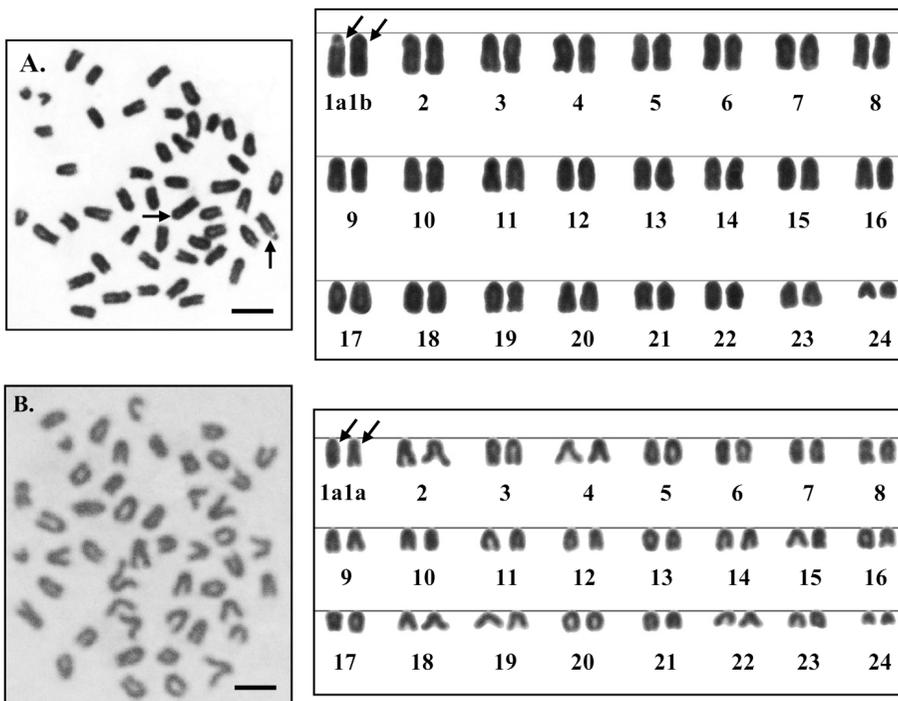


Fig. 2. Metaphase chromosome plates and karyotypes of the John's snapper (*Lutjanus johnii*), $2n=48$, by conventional staining technique. One female's chromosome pair 1 shows a different size of NORs (heteromorphism) (A.), while three females and four males show equal sizes (homomorphism) (B.). Scale bars=10 μ m.

Lutjanus, all species previously studied have a common karyotype consisting of 24 pairs of telocentric chromosomes and no morphologically differentiated sex chromosomes. The cytogenetic features reported here for the examined specimens of *L. johnii* revealed that the species has the 48 telocentric karyotype, which is shared by most of the Lutjaninae species previously analyzed, such as *L. alexandrei* (Rocha and Molina 2008), *L. argentimaculatus* (Cao *et al.* 2002), *L. griseus* (Nirchio *et al.* 2008), *L. kasmira* (Choudhury *et al.* 1979), and *L. russelli* (Ueno and Ojima 1991). However, there are two exceptions; *L. quinquelineatus* (Ueno and Takai 2008) and *L. synagris* (Nirchio *et al.* 2008) have been reported to possess $2n=48$ telocentric chromosomes in females and $2n=47$ (1 metacentric and 46 telocentric chromosomes) in males.

The analysis of the NORs with the Ag-NOR banding technique following Giemsa's staining detected a maximum of two Ag-positive signals in this species. In *L. johnii*, the Ag-positive signals are located along the long arm subcentromeric of the telocentric chromosome pair 1 (Figs. 3 and 5). The NORs are effective cytotaxonomic markers in the family Lutjanidae, and allowed us to distinguish most of the analyzed species, in which the ribosomal sites were similarly located on the same chromosomal pair (chromosome pair 1 or 2). Cytogenetic studies in other groups with conserved karyotypes have also demonstrated the same discriminatory ability of NORs, such as in Anostomidae (Galetti *et al.* 1984) and in some Cichlidae (Brinn *et al.* 2004). Nevertheless, the efficiency of ribosome sites as a cytotaxonomic marker is not applicable to all situations, since this region might remain unchanged on homologous chromosomes of several species (Molina and Galetti 2004).

Multiple NORs have rarely been reported in the order Perciformes (Galetti *et al.* 2006), although they are common in Characiformes and Siluriformes (Mantovani *et al.* 2000, Paintner-Marques *et al.* 2002). In a previous report, Rocha and Molina (2008) revealed that a single NOR

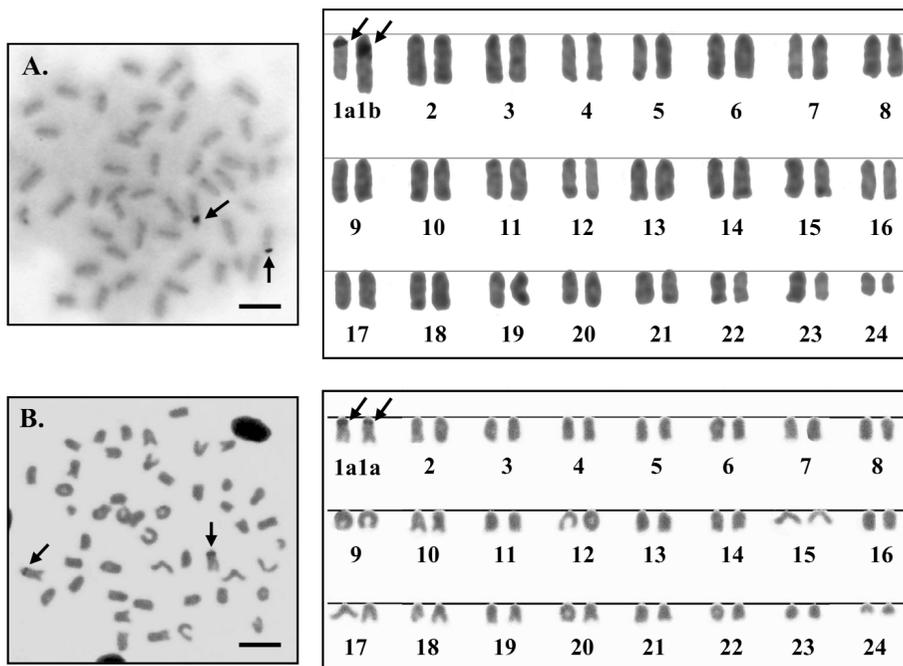


Fig. 3. Metaphase chromosome plates and karyotypes of the John's snapper (*Lutjanus johnii*), $2n=48$, by Ag-NOR banding technique. One female's chromosome pair 1 shows different sizes of NORs (heteromorphism) (A.), while three females and four males show equal sizes (homomorphism) (B.). Scale bars= $10\mu\text{m}$.

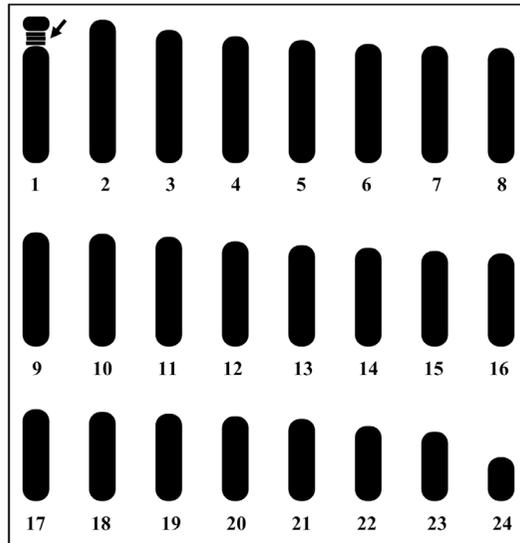


Fig. 4. Idiogram showing lengths and shapes of chromosomes of the John's snapper (*Lutjanus johnii*), $2n=48$, by conventional staining technique. The arrow indicates NOR-bearing chromosome pair 1.

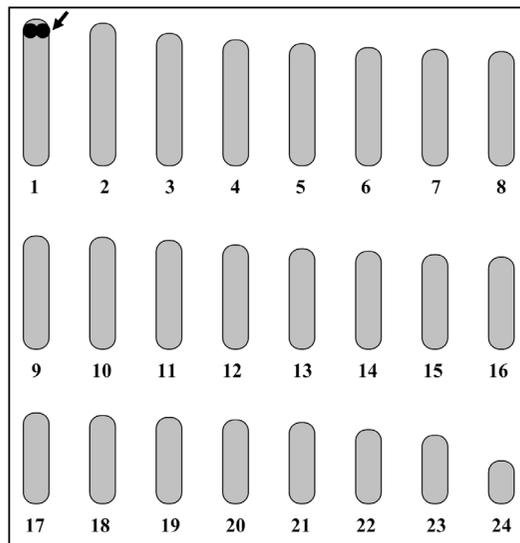


Fig. 5. Idiogram of chromosomes of the John's snapper (*Lutjanus johnii*), $2n=48$, by Ag-NOR banding technique. The arrow indicates NOR-bearing chromosome pair 1.

was found at a pericentromeric position on the long arms of the 2nd pair in *O. chrysurus*, *L. alexandrei* and *L. cyanopterus*, on the 5th pair in *L. analis* and on the 23rd pair in *L. synagris*. In contrast, *L. jocu* presented multiple NORs located on a pericentromeric region of the 2nd pair and a telomeric region of the 5th pair. Typically, such variation results from chromosomal rearrangements, but transposition events have also been indicated as one major factor for the numerical variability of NORs in fishes (Galetti *et al.* 1995, Castro *et al.* 1996).

The present study showed that a heteromorphism of one female had different-sized NORs in chromosome pair 1 (1a1b), while three females and four males had equal-sized NORs in chromosome pair 1 with a homomorphism (1a1a) (Fig. 6). This is in agreement with several previous

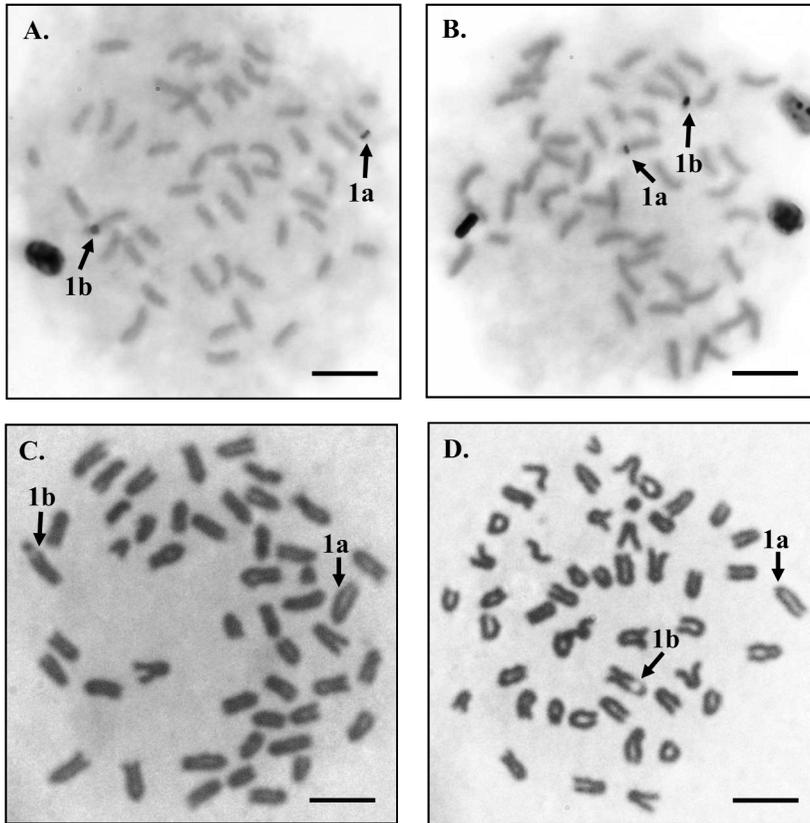


Fig. 6. A new discovery of polymorphism in size of nucleolar organizer regions (NORs) on chromosome pair 1 (1a1b) from four sample cells of a female John's snapper (*Lutjanus johnii*) in Thailand by Ag-NOR banding (A., B.) and conventional staining techniques (C., D.). Arrows indicate satellite chromosomes/NORs. Scale bars=10 μ m.

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* (Rodrigues and Collares-Pereira 1996, Collares-Pereira and Ráb 1999), *Hoplias malabaricus* (Born and Bertollo 2000), *Oedalechilus labeo* (Rossi *et al.* 2000), *Astyanax scabripinnis* (Mantovani *et al.* 2000, Marco-Ferro *et al.* 2001, Soza *et al.* 2001), *A. altiparanae* (Pacheco *et al.* 2001, 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), and *Puntiplites proctozysron* (Supiwong *et al.* 2012). NORs can be the perfect markers to display wide 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. Previous NOR studies showed variations between species, within species, and even between individuals (Galetti *et al.* 1984, Gold *et al.* 1993, Castro *et al.* 1996). NORs on different homologous chromosomes may have different sizes. Some fish may even show a difference of up to a factor of two in size between NORs found on the same homologous chromosome. It has been reported that this extent of variety between NORs may be attributed to the number of cistrons and differences in transcriptional activity (Galetti *et al.* 1984).

The asymmetrical karyotype of *L. johnii* with only one type of chromosomes (telocentric chromosomes) found in this study is the important chromosome markers. The idiogram shows continu-

Table 2. Mean short arm chromosome length (Ls), long arm chromosome length (Ll), total arm chromosome length (LT), relative length (RL), centromeric index (CI), and standard deviation (SD) of RL from 20 metaphases of the John's snapper (*Lutjanus johnii*), $2n=48$.

Chromosome pairs	Ls	Ll	LT	CI±SD	RL±SD	Chromosome type	Chromosome size
1*	0.00	0.99	0.99	1.00	0.060±0.005	Telocentric	Large
2	0.00	0.96	0.96	1.00	0.058±0.005	Telocentric	Large
3	0.00	0.89	0.89	1.00	0.054±0.005	Telocentric	Large
4	0.00	0.85	0.85	1.00	0.051±0.004	Telocentric	Large
5	0.00	0.83	0.83	1.00	0.050±0.004	Telocentric	Large
6	0.00	0.80	0.8	1.00	0.048±0.003	Telocentric	Large
7	0.00	0.78	0.78	1.00	0.047±0.002	Telocentric	Large
8	0.00	0.77	0.77	1.00	0.047±0.001	Telocentric	Large
9	0.00	0.76	0.76	1.00	0.046±0.001	Telocentric	Large
10	0.00	0.75	0.75	1.00	0.046±0.001	Telocentric	Large
11	0.00	0.73	0.73	1.00	0.044±0.001	Telocentric	Large
12	0.00	0.70	0.7	1.00	0.042±0.001	Telocentric	Large
13	0.00	0.68	0.68	1.00	0.041±0.001	Telocentric	Large
14	0.00	0.66	0.66	1.00	0.040±0.002	Telocentric	Large
15	0.00	0.64	0.64	1.00	0.039±0.001	Telocentric	Medium
16	0.00	0.62	0.62	1.00	0.037±0.001	Telocentric	Medium
17	0.00	0.61	0.61	1.00	0.037±0.001	Telocentric	Medium
18	0.00	0.60	0.6	1.00	0.036±0.001	Telocentric	Medium
19	0.00	0.58	0.58	1.00	0.035±0.001	Telocentric	Medium
20	0.00	0.57	0.57	1.00	0.034±0.002	Telocentric	Medium
21	0.00	0.55	0.55	1.00	0.033±0.001	Telocentric	Medium
22	0.00	0.50	0.5	1.00	0.030±0.003	Telocentric	Medium
23	0.00	0.46	0.46	1.00	0.028±0.003	Telocentric	Small
24	0.00	0.29	0.29	1.00	0.017±0.004	Telocentric	Small

Remark: *=NOR-bearing chromosome (satellite chromosome).

ous length gradation chromosomes. The size difference between the largest and the smallest chromosomes is approximately threefold. The chromosome marker of *L. johnii*, chromosome pair 1, is the largest telocentric chromosome, while chromosome pair 24 is the smallest telocentric chromosome. Data of the chromosomal checks on mitotic metaphase cells of the *L. johnii* are shown in Table 2. The karyotype formula for *L. johnii* is as follows: $2n$ (diploid) $48=L_{28}^I+M_{16}^I+S_4^I$.

Acknowledgments

This work was supported by the Applied Taxonomic Research Center (ATRC), Khon Kaen University grant; ATRC-R5304 and by a grant of the Faculty of Science, Khon Kaen University.

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