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# Chromosomal Analysis of Two Snakehead Fishes, *Channa marulius* (Hamilton, 1822) and *C. marulioides* (Bleeker, 1851) (Perciformes: Channidae) in Thailand

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**Summary** The snakeheads, freshwater perciform fish are the member of the family Channidae. The karyotype diversification was observed in this species. In this study, we investigate the karyotype and chromosomal characteristic of nucleolar organizing regions (NORs) in *Channa marulius* (Hamilton, 1822) and *C. marulioides* (Bleeker, 1851) from Thailand. The metaphase chromosomes were prepared from kidney cells of five male and five female fishes. Conventional and Ag-NOR staining techniques were applied to the chromosomes. The results revealed that: the diploid chromosome number (2*n*) of *C. marulius* was 44, fundamental number (NF) was 56, the karyotype formula should be deduced as $2n=L_4^m+L_6^a+L_{10}^t+M_2^a+M_{22}^t$, the NORs are located at the telomeric position of the short arm of chromosome pairs 3 and 4; the 2*n* of *C. marulioides* was 38, NF was 68, the karyotype formula should be deduced as $2n=L_8^m+L_2^a+M_2^m+M_{18}^a+M_8^t$, the NORs are located at the telomeric position of the short arm of chromosome pairs 10 and 11. The difference of 2*n* and NF in males and females fish was not observed. The number of Ag-NORs in metaphase cell varies between two and four among the cells in the same sample both in *C. marulius* and *C. marulioides*.

**Key words** *Channa marulius*, *Channa marulioides*, Karyotype, Chromosome, Evolution, NOR.

The snakeheads, freshwater perciform fish, are the member of the family Channidae. Two extant genera, *Parachanna* and *Channa*, are included. *Parachanna* is distributed along the central West Africa while *Channa* is distributed along Asia (Courtenay and Williams 2004). In Thailand, seven species of *Channa* have been recorded (Supiwong *et al.* 2009).

The diploid chromosome number and karyotype of 13 *Channa* species have been reported. The 2*n* varies between 32 and 112 such as *C. marulioides*, 2*n*=38 (Magtoon *et al.* 2006), *C. marulius*, 2*n*=44 (Khuda-Bukhsh *et al.* 1986) and *C. micropeltes*, 2*n*=44 (Donsakul and Magtoon 1991). The variation of 2*n* can be found in eight species such as *C. punctata*, *C. orientalis*, *C. gachua* and *C. striata*. The details are as follows, the 2*n* of *C. punctata* from Bangladesh is 32 (Ruma *et al.* 2006) while the population from India both 2*n*=32 and 2*n*=34 can be found (Dhar and Chatterjee 1984). The 2*n* of *C. orientalis* from India are 2*n*=42 (Khuda-Bukhsh *et al.* 1986) and 2*n*=76 (Dhar and Chatterjee 1984) while the population from Bangladesh has 2*n*=78 (Ruma *et al.* 2006). Noticeable, the variations on chromosome number and morphology can be found both between different populations of the same species as reported in *C. orientalis* (Khuda-Bukhsh *et al.* 1986, Dhar and Chatterjee 1984) and within different individuals of the same population as reported in *C. punctata* (Dhar and Chatterjee 1984).

The NORs contain tandem repeat of DNA encode for ribosomal RNA (Pardue and Gall 1969). Silver staining technique (Ag-NOR banding) which detect active NORs was introduced to study the chromosome of many fish species in several families such as Cyprinidae (Nagpure *et al.* 2001), Channidae (Khuda-Bukhsh and Barat 1987, Khakhong *et al.* 2014), Osphronimidae (Furgala-Selezniow *et al.* 2008). In *Channa*, the numbers of NOR-bearing chromosomes vary between one and three pairs.

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One pair of NOR-bearing chromosomes were found in *C. lucius* (Khakhong *et al.* 2014), *C. orientalis* (Sobita and Bhagirath 2006) *C. gachua* (Tanomtong *et al.* 2014), *C. micropeltes* (Supiwong *et al.* 2009), *C. striata* (Supiwong *et al.* 2009). Two pairs were found in *C. argus* and *C. striata* (Zhang *et al.* 1992), while three pairs were found in *C. striata* (Sobita and Bhagirath 2006) and *C. punctata* (Khuda-Bukhsh and Barat 1987).

This study was undertaken to investigate the karyotype, and chromosomal characteristic of NORs in *C. marulius* and *C. marulioides* from Thailand. The study would also improve our understanding of karyotype evolution mechanism of Channids.

## Materials and methods

The five male and female fishes of *C. marulius* were obtained from The Khwae Yai River or Si Sawat River, Kanchanaburi Province, Thailand (Fig. 1A) and sample of *C. marulioides* (five males and five females) were obtained from Pru Toh Daeng, a peat swamp in Narathiwat Province, Thailand (Fig. 1B). The fishes were alive when transported to the laboratory and were kept for 72 h prior to processing. The preparation of chromosomes was accomplished after Supiwong *et al.* (2009); however the colchicine added in the incubation period was less. Detection of the NOR was done following the silver staining method of Howell and Black (1980) with slight modification. The chromosomes lengths of 20 cells (males and females) were measured on their short and long arm. The length of short arm (Ls) and long arm (Ll) were calculated for the length of chromosome (LT). Relative length (RL) and centromeric index (CI) were also calculated. The CI was computed to classify the types of chromosomes according to Turpin and Lejeune (1965). All parameters were used in karyotyping and idiograming.

## Results and discussion

*Chromosome number, fundamental number and karyotype*

*C. marulius:* The metaphase plates and karyotypes by conventional staining of males and females *C. marulius* are shown in Fig. 2A–D. The Ls, Ll, LT, RL, CI, type and size of each pair of chromosomes are shown in Table 1. The idiogram of *C. marulius* shows in Fig. 4A. Karyological analysis of *C. marulius* revealed $2n=44$ and NF=56. The same $2n$ and NF were observed in males and females fish. Morphological analysis of each chromosome pair indicated that there are 4 large metacentric, 6 large acrocentric, 2 medium acrocentric, 10 large telocentric, and 22 medium telocentric chromosomes. The karyotype formula should be deduced as follows: $2n=L^{m}_{4}+L^{a}_{6}+L^{t}_{10}+M^{a}_{2}+M^{t}_{22}$.

The results from previous studies on the karyotype of *C. marulius* revealed that $2n$ varied between 40 and 44 (Nayyar 1966, Khuda-Bukhsh *et al.* 1986, Donsakul and Magtoon 1991, Bhatti *et al.* 2013). The $2n$ and types of chromosomes in present and previous studies are not in conformity. Even when $2n$ is equal but the different karyotypes are observed. For example, in the case of $2n=44$, the present study reveals 4m+8a+32t while there were 4m+4a+36t in Donsakul and Magtoon (1991) report and 8m+36t in Bhatti *et al.* (2013).


**Fig. 1.** General characteristics of male fishes of *C. marulius* (A) and *C. marulioides* (B). Scale bars=3 cm.

*C. marulioides*: The metaphase chromosomes and karyotypes by conventional staining of male and female *C. marulioides* are shown in Fig. 2E–H, respectively. The Ls, Ll, LT, RL, CI, type and size of each pair of chromosomes are shown in Table 2. The idiogram of *C. marulioides* shows in Fig. 4B. Karyological analysis of *C. marulioides* revealed $2n=38$ and NF=68. The difference in $2n$ and NF cannot be observed between males and females. Karyotype analysis revealed that there are 8 large metacentric, 2 medium metacentric, 2 large acrocentric, 18 medium acrocentric, and 8 medium telocentric chromosomes. The karyotype formula could be deduced as follows: $2n=L^{m}_{8}+L^{a}_{2}+M^{m}_{2}+M^{a}_{18}+M^{t}_{8}$.

The $2n$ of *C. marulioides* from present and previous studies (Magtoon *et al.* 2006) is equal but the karyotypes are different. The present study reveals 10m+20a+8t while there are 2m+2sm+2a+32t in Magtoon *et al.* (2006).

While the conspicuous karyotype diversification is not observed among most Perciformes fish (Molina *et al.* 2002) in the Channidae family, the diversity of chromosome and karyotype has been reported (Li *et al.* 1985, Khuda-Bukhsh *et al.* 1986, Donsakul and Magtoon 1991, Tanomtong *et al.* 2014). The different $2n$, NF and chromosome morphology are observed between *C. marulius* and *C. marulioides*. These two species have been shown to be the most similar to each other in external morphology (Fig. 1), coloration, and may be considered as the species complex (Courtenay and Williams 2004). Moreover, these two species are placed in the same clade on the molecular phylogenetic tree (Li *et al.* 2006).

The discrepancies karyotype in of *C. marulius* and *C. marulioides* observed in present study and previous

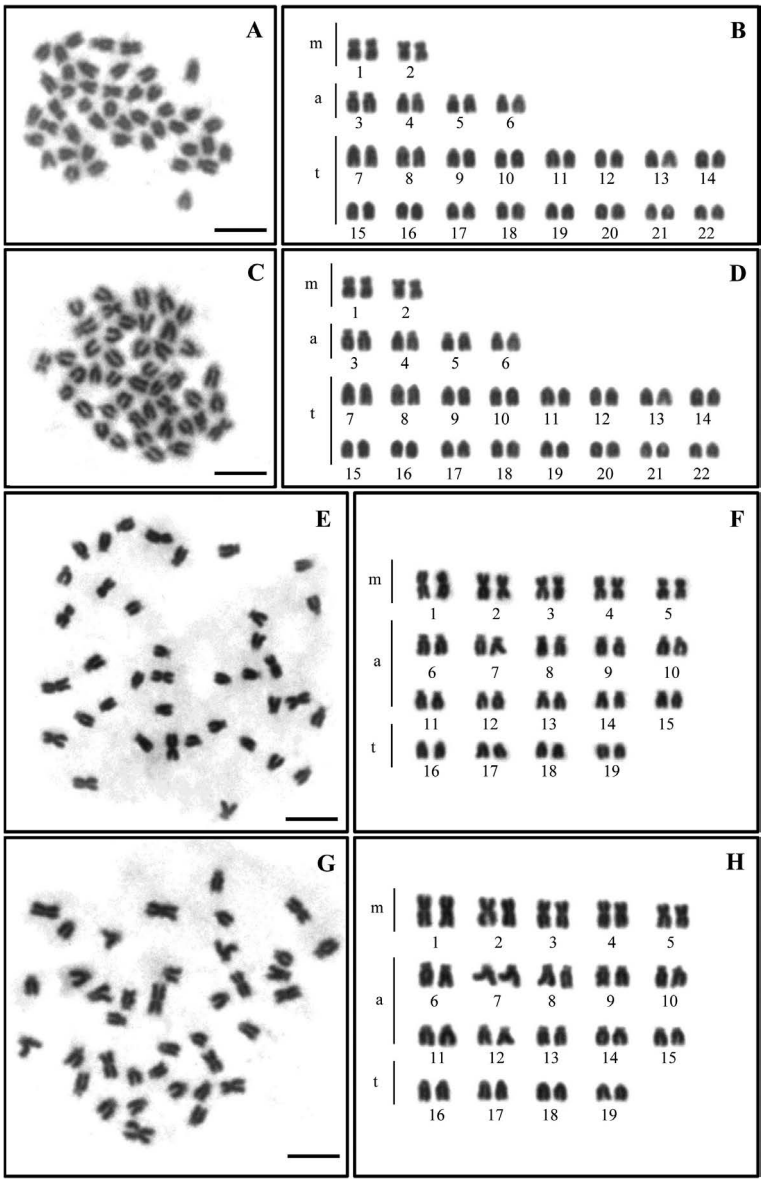


**Fig. 2.** Metaphase chromosome plates and karyotypes by conventional staining of *C. marulius* (male) 2*n*=44 (A, B) *C. marulius* (female) 2*n*=44 (C, D) *C. marulioides* (male) 2*n*=38 (E, F) *C. marulioides* (female) 2*n*=38 (G, H). Scale bars=5 $\mu$m.

studies can be ascribed to the chromosome preparation method, the different criterion used for chromosome type classification and/or the chromosome evolution. The variation in chromosome morphology including size and type of chromosome might be due to the excessive chromosomal contraction as a consequence of higher dose or over-exposure to colchicine, improper fixation of tissue, methods of chromosomal preparation and classification (Zhang and Reddy 1991). However, these variations, especially the variation in 2*n*, may also arise from chromosomal aberrations or rearrangements followed by population isolation during evolutionary process (Chu and Bender 1961, Campos and Cuevas 1997) as the karyotype evolution found in *C. gachua and C. striata* (Supiwong *et al.* 2009, Tanomtong *et al.* 2014). The more cytogeographic investigation is needed in these species to reveal and confirm intra- and inter-specific karyotype variations.

*Ag-NOR as chromosome marker*

*C. marulius*: There are two pairs of NOR-bearing chromosomes. The NORs regions are located at the telomeric position of the short arm of the chromosomes pairs 3 and 4 which are the large acrocentric chromosomes. However, the NORs polymorphisms in *C. marulius* have been observed. The number of active NORs in metaphase cell varies between two and four among the cells in the same sample (Fig. 3A). Out of 200 metaphase cells observed, the percentages of cells with two, three, and four active NORs per cell are 17.5% (35 cells), 48% (96 cells), and 34.5% (69 cells), respectively. The polymorphism of Ag-NORs can be observed both in the chromosome pairs three and four. The chromosome markers of *C. marulius* should be the two pairs of the

**Table 1.** Mean of short arm length (Ls), long arm length (Ll), chromosome length (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from metaphase chromosomes in 20 cells of *C. marulius*, 2*n*=44.

| Chromosome pair | Ls | Ll | LT | RL±SD | CI±SD | Chromosome size | Type |
|---|---|---|---|---|---|---|---|
| 1 | 1.136 | 1.386 | 2.522 | 0.057±0.003 | 0.55±0.019 | L | Metacentric |
| 2 | 0.944 | 1.244 | 2.188 | 0.050±0.002 | 0.568±0.023 | L | Metacentric |
| 3* | 0.597 | 1.870 | 2.466 | 0.056±0.003 | 0.755±0.043 | L | Acrocentric |
| 4* | 0.542 | 1.724 | 2.266 | 0.051±0.003 | 0.760±0.037 | L | Acrocentric |
| 5 | 0.466 | 1.626 | 2.093 | 0.048±0.003 | 0.777±0.029 | L | Acrocentric |
| 6 | 0.441 | 1.556 | 1.996 | 0.045±0.003 | 0.779±0.024 | M | Acrocentric |
| 7 | 0.000 | 2.336 | 2.336 | 0.053±0.002 | 1.000±0.000 | L | Telocentric |
| 8 | 0.000 | 2.232 | 2.232 | 0.050±0.001 | 1.000±0.000 | L | Telocentric |
| 9 | 0.000 | 2.154 | 2.154 | 0.049±0.002 | 1.000±0.000 | L | Telocentric |
| 10 | 0.000 | 2.099 | 2.099 | 0.047±0.001 | 1.000±0.000 | L | Telocentric |
| 11 | 0.000 | 2.043 | 2.043 | 0.046±0.001 | 1.000±0.000 | L | Telocentric |
| 12 | 0.000 | 2.010 | 2.010 | 0.045±0.001 | 1.000±0.000 | M | Telocentric |
| 13 | 0.000 | 1.966 | 1.966 | 0.044±0.001 | 1.000±0.000 | M | Telocentric |
| 14 | 0.000 | 1.941 | 1.941 | 0.044±0.001 | 1.000±0.000 | M | Telocentric |
| 15 | 0.000 | 1.889 | 1.889 | 0.043±0.001 | 1.000±0.000 | M | Telocentric |
| 16 | 0.000 | 1.843 | 1.843 | 0.042±0.001 | 1.000±0.000 | M | Telocentric |
| 17 | 0.000 | 1.812 | 1.812 | 0.041±0.001 | 1.000±0.000 | M | Telocentric |
| 18 | 0.000 | 1.776 | 1.776 | 0.040±0.001 | 1.000±0.000 | M | Telocentric |
| 19 | 0.000 | 1.752 | 1.752 | 0.040±0.001 | 1.000±0.000 | M | Telocentric |
| 20 | 0.000 | 1.703 | 1.703 | 0.038±0.001 | 1.000±0.000 | M | Telocentric |
| 21 | 0.000 | 1.650 | 1.650 | 0.037±0.001 | 1.000±0.000 | M | Telocentric |
| 22 | 0.000 | 1.556 | 1.556 | 0.035±0.002 | 1.000±0.000 | M | Telocentric |

L=large chromosome (LT>2.039 $\mu$m), M=medium chromosome (LT=1.261–2.039 $\mu$m) *=NOR-bearing chromosome.

**Table 2.** Mean of short arm length (Ls), long arm length (Ll), chromosome length (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from metaphase chromosomes in 20 cells of *C. marulioides*, 2*n*=38.

| Chromosome pair | Ls | Ll | LT | RL±SD | CI±SD | Chromosome size | Type |
|---|---|---|---|---|---|---|---|
| 1 | 1.575 | 1.958 | 3.533 | 0.076±0.003 | 0.554±0.016 | L | Metacentric |
| 2 | 1.427 | 1.763 | 3.191 | 0.068±0.003 | 0.552±0.019 | L | Metacentric |
| 3 | 1.335 | 1.679 | 3.014 | 0.064±0.002 | 0.557±0.020 | L | Metacentric |
| 4 | 1.274 | 1.595 | 2.869 | 0.061±0.002 | 0.556±0.020 | L | Metacentric |
| 5 | 1.207 | 1.453 | 2.660 | 0.057±0.003 | 0.547±0.024 | M | Metacentric |
| 6 | 0.647 | 2.131 | 2.778 | 0.059±0.002 | 0.767±0.023 | L | Acrocentric |
| 7 | 0.612 | 1.970 | 2.581 | 0.055±0.002 | 0.763±0.024 | M | Acrocentric |
| 8 | 0.568 | 1.891 | 2.459 | 0.053±0.001 | 0.768±0.030 | M | Acrocentric |
| 9 | 0.555 | 1.841 | 2.396 | 0.051±0.001 | 0.767±0.028 | M | Acrocentric |
| 10* | 0.551 | 1.797 | 2.347 | 0.050±0.001 | 0.765±0.022 | M | Acrocentric |
| 11* | 0.500 | 1.766 | 2.265 | 0.048±0.001 | 0.779±0.037 | M | Acrocentric |
| 12 | 0.489 | 1.728 | 2.216 | 0.047±0.001 | 0.778±0.022 | M | Acrocentric |
| 13 | 0.487 | 1.692 | 2.179 | 0.047±0.001 | 0.776±0.019 | M | Acrocentric |
| 14 | 0.465 | 1.643 | 2.108 | 0.045±0.001 | 0.779±0.021 | M | Acrocentric |
| 15 | 0.470 | 1.581 | 2.051 | 0.044±0.001 | 0.770±0.028 | M | Acrocentric |
| 16 | 0.000 | 2.218 | 2.218 | 0.047±0.003 | 1.000±0.000 | M | Telocentric |
| 17 | 0.000 | 2.093 | 2.093 | 0.045±0.003 | 1.000±0.000 | M | Telocentric |
| 18 | 0.000 | 1.974 | 1.974 | 0.042±0.002 | 1.000±0.000 | M | Telocentric |
| 19 | 0.000 | 1.825 | 1.825 | 0.039±0.003 | 1.000±0.000 | M | Telocentric |

L=large chromosome (LT>2.679 $\mu$m), M=medium chromosome (LT=1.767–2.679 $\mu$m) *=NOR-bearing chromosome.

large metacentric chromosomes (pairs 1 and 2) in Fig. 4A.

*C. marulioides*: The NORs regions are located at the telomeric position of the short arm of the chromosome pairs 10 and 11 which are the medium acrocentric chromosomes. The NORs polymorphisms have been observed as in *C. marulius*. The number of active NORs in metaphase cell varies between two and four among the cells in the same sample (Fig. 3B). Out of 200 metaphase cells observed, the percentages of cells with two, three, and four active NORs per cell are 30% (60 cells), 44.5% (89 cells), and 26.5% (53 cells), respectively. The polymorphism of Ag-NORs observed only in the chromosome pair 11. The chromosome markers of *C. marulioides* should be the five pairs of the metacentric chromosomes (pairs 1–5) in Fig. 4B.

In the normal karyotype, the number of NOR-bearing chromosomes is species-specific and constant (Derjusheva *et al.* 1998). However, the variation in the number of NORs at the intraspecific level has been noted for

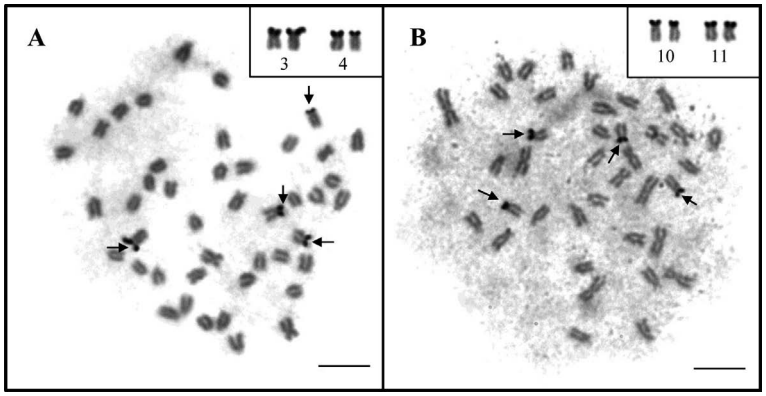


**Fig. 3.** Metaphase chromosome plates stained with NOR staining and Ag-NORs bearing chromosomes in *Channa* species. Arrows indicate Ag-NORs on the chromosome pairs 3 and 4 in *C. marulius* (A) and the chromosome pairs 10 and 11 in *C. marulioides* (B). Scale bars=5 $\mu$m.

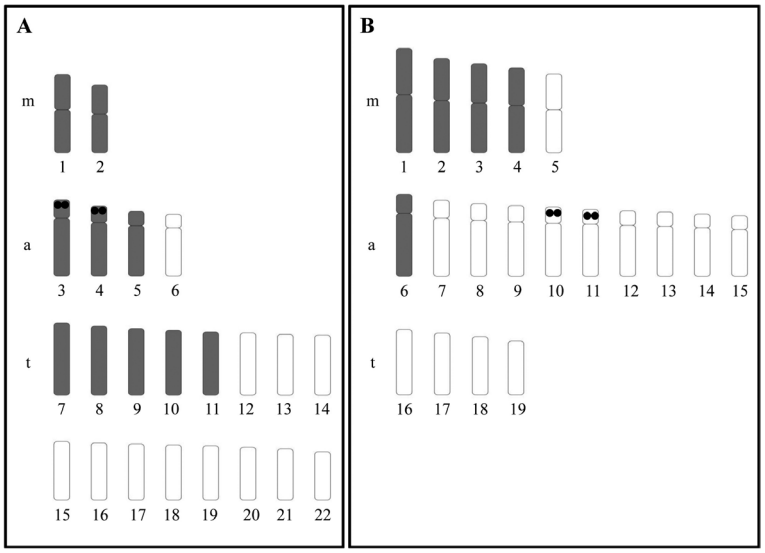


**Fig. 4.** Standardized idiograms represented the haploid set of chromosome showing size, shape and Ag-NORs regions of *C. marulius* (A) and *C. marulioides* (B). Gray bars indicate the large size of chromosome (L) and white bars indicate the medium size of chromosome (M). Black dots indicate the Ag-NORs on the short arm of acrocentric chromosomes.

the fishes in the family Sparidae (Garrido-Ramos *et al.* 1995). The number and location of NORs have been used as chromosome markers in fish cytotaxonomy (Pereira *et al.* 2012, Rocchi *et al.* 2012). In *C. marulius* and *C. marulioides*, the NORs were located at the telomeric position of the short arm of acrocentric chromosomes corresponds to those observed in many of the *Channa* species (Sobita and Bhagirath 2006, Khakhong *et al.* 2014, Tanomtong *et al.* 2014). The two pairs of NORs-bearing chromosomes in these two species are different from the majority of fishes which have only one pair of NORs on the chromosome. The increasing of NOR-bearing chromosome may be caused by the translocation between some parts of chromosomes having NOR and another chromosome (Sharma *et al.* 2002). In *Channa* species, the number of NOR-bearing chromosome varies between one and three pair; one pair in *C. gachua* (Tanomtong *et al.* 2014), two pairs in *C. marulius* and *C. marulioides* and three pairs in *C. striata* (Sobita and Bhagirath 2006) and *C. punctata* (Khuda-Bukhsh and Barat 1987). This variation indicated the NOR division by chromosome rearrangements or DNA magnification. Furthermore, the NOR polymorphism was observed in *Channa* species in this study. This variation may be caused by the chromosome rearrangements which separate these genes from large blocks of heterochromatin result in their variegated expression.

*Chromosome evolution*

Channidae fishes show remarkable karyotype diversity (Cioffi *et al.* 2015). The variation in karyotype caused by consequences of events that might alter number, size and shape of chromosomes has been proposed (Schubert 2007). The decrease in the 2*n* number with concomitant changes in the number of the bi-armed chromosomes (ba) in the *Channa* species, suggests that fusions and pericentric inversions were the main chromosomal rearrangements related to the karyotypic evolution of this genus (Cioffi *et al.* 2015). Furthermore, Robertsonian rearrangements and polyploidy also appear to be the main

sources of karyotypic variation among species and populations of this genus (Rishi and Haobam 1990, Singh and Barman 2013). A reference to the karyotypes of *Channa* species indicates that species with large number of chromosomes have acrocentric centromere. For example, the 2*n* of *C. marulioides* is 38 of which are 10 metacentric chromosomes while the 2*n* of *C. marulius* is 44 of which are four metacentric chromosomes. This observation supports the hypothesis of origin of bi arm chromosome from telocentric counterparts in *Channa* species.

Further study including additional species of *Channa* of different geographical locations, employing different staining techniques such as fluorescence *in situ* hybridization to investigate the distribution of repetitive DNA sequences in genome (Feldman and Levy 2005), the knowledge obtained should provide a better understanding of the chromosomal evolution in the genus *Channa*.

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