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Standardized Karyotype and Idiogram of Two-Spot Glass Catfish, *Ompok bimaculatus* (Siluriformes, Siluridae) in Thailand by Conventional and Ag-NOR Staining Techniques

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Summary Standardized karyotype and idiogram of the two-spot glass catfish (*Ompok bimaculatus*) from Chi basin, Maha Sarakham Province, northeast Thailand, were studied. Kidney cell samples were taken from 5 male and 5 female fish. The mitotic chromosome preparations were prepared directly from kidney cells. Conventional and Ag-NOR staining techniques were applied to stain the chromosomes. The results showed that the diploid chromosome number of *O. bimaculatus* was $2n$=50, the fundamental number (NF) was 90 in both males and females. The types of chromosomes were 10 large metacentric, 2 large submetacentric, 4 large acrocentric, 2 large telocentric, 4 medium metacentric, 18 medium submetacentric, 2 medium acrocentric, 2 medium telocentric, and 6 small telocentric chromosomes. The region adjacent to telomere of the short arm of submetacentric chromosome pair 4 showed clearly observable nucleolar organizer regions (NORs). The karyotype formula for *O. bimaculatus* could be deduced as:

$$2n \text{ (diploid) } 50=L^{m}_{10}+L^{sm}_{2}+L^{a}_{4}+L^{t}_{2}+M^{m}_{4}+M^{sm}_{18}+M^{a}_{2}+M^{t}_{2}+S^{t}_{6}$$

Key words *Ompok bimaculatus*, Karyotype, Chromosome, Idiogram.

Thailand is one of the world's richest places of biodiversity, especially for fresh water fish species. With more than 700 species recorded, Thailand is one of the world's centers of species diversity. Fresh water fish are especially important as they provide a high quality source of protein as well as food source for people who live nearby a river basins (Vidthayonon 2005). The two-spot glass catfish, *Ompok bimaculatus* (Bloch 1794) is commonly found in natural water bodies such as rivers and floodplains in Thailand. The *O. bimaculatus* is a particular favorite food of Thai people and it has a high market value.

Thai fish of the family Siluridae include 27 species, which can be grouped into 9 genera, namely *Belodontichthys*, *Ceratoglanis*, *Hemisilurus*, *Kryptopterus*, *Micronema*, *Ompok*, *Pterocryptis*, *Silurichthys* and *Wallago* (Ferraris 2007, Rainboth 1996, Vidthayonon 2005). In Thailand only 11 species of this family have been cytogenetically investigated where studies report the diploid chromosome number ($2n$) ranging from 40 to 92. Each species has a different diploid number for in-

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stance, *S. phaiosoma* 2*n*=40, *S. schneideri* 2*n*=40 (Magtoon and Donsakul 2009), *O. bimaculatus* 2*n*=40 (Nayyar 1965), 2*n*=41 in male, 42 in female (Das and Khuda-Bukhsh 2007), 2*n*=50 (Donsakul 1992), *W. leerii* 2*n*=56 (Donsakul 1996), *O. fumidus* 2*n*=60 (Magtoon and Donsakul 2009), *B. dinema* 2*n*=62 (Donsakul 1996), *K. macrocephalus* 2*n*=62 (Magtoon and Donsakul 2009), *K. bicirrhis* 2*n*=64 (Donsakul 1996), *M. bleekeri* 2*n*=64 (Donsakul 1992), *W. attu* 2*n*=88 (Donsakul 1996) and *K. cryptopterus* 2*n*=92 (Donsakul 1992).

The present study is an analysis of the karyotype and chromosomal characteristics of nucleolar organizer regions (NORs) in *O. bimaculatus* by conventional and Ag-NOR staining techniques. Only 5 previous cytogenetic studies of the genus *Ompok* have been conducted (Nayyar 1965, Donsakul 1992, Datta *et al.* 2003, Das and Khuda-Bukhsh 2007, Magtoon and Donsakul 2009). Results obtained will increase our basic knowledge of the cytogenetics of *O. bimaculatus* which could form the basis for future research and provide data to ensure their survival.

Materials and methods

Five males and 5 females of *O. bimaculatus* were obtained from the Chi basin in Maha Sarakham Province, northeast Thailand. The fish were transferred to laboratory aquaria and had

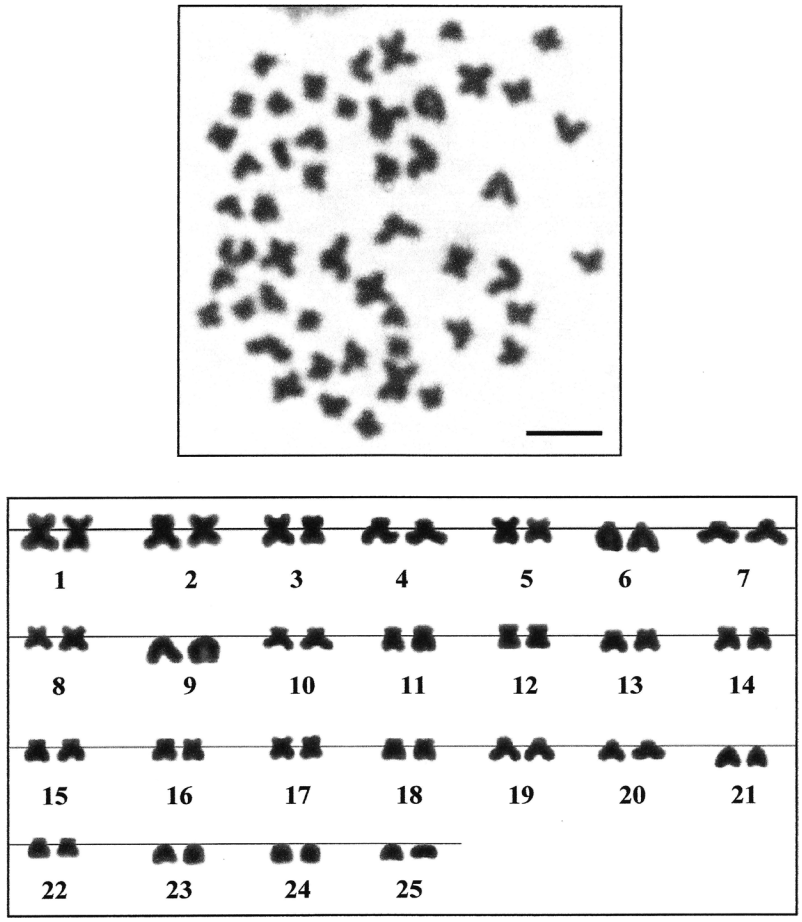

Fig. 1. Metaphase chromosomes plate and karyotype of the two-spot glass catfish (*Ompok bimaculatus*), 2*n* (diploid)=50, by conventional staining technique (scale bar=5 μm).

been kept under standard conditions for 7 d prior to experimentation. 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. Fundamental number, NF (number of chromosome arm) is obtained by assigning a value of 2 to metacentric, submetacentric and acrocentric chromosomes and 1 to telocentric chromosomes.

Results and discussion

Diploid chromosome number, fundamental number and karyotype of O. bimaculatus

Results showed that the diploid chromosome number of *O. bimaculatus* was 2*n*=50 in both males and females (Fig. 1). This is in agreement with the earlier studies conducted in Thailand (Donsakul 1992). Interestingly, it differs from the report of Nayyar (1965) showing a 2*n*=40 for *O. bimaculatus* in India, which differs from studies by Das and Khuda-Bukhsh (2007) who showed that this same species in India had 2*n*=41 and 2*n*=42 in males and females, respectively. The fundamental number (NF, chromosome arm number) was 90 in both males and females. This is different from earlier studies conducted in Thailand and India (Donsakul 1992, Nayyar 1965, Das and Khuda-Bukhsh 2007).

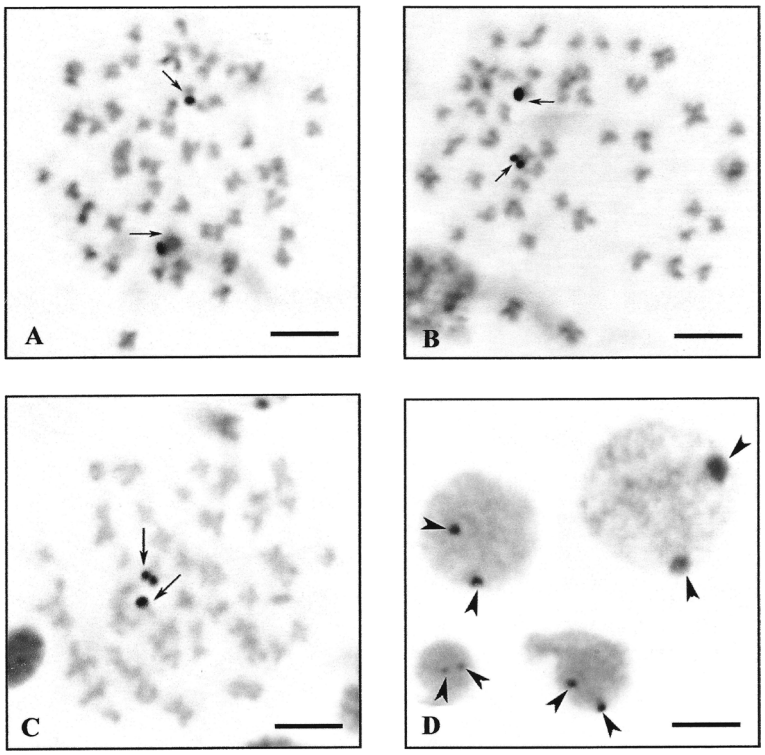

Fig. 2. Metaphase chromosome plates of male (A and B) and female (C and D) of the two-spot glass catfish (*Ompok bimaculatus*), 2*n* (diploid)=50, in Northeast of Thailand by Ag-NOR staining technique. Arrows indicate nucleolar organizer regions, NORs (scale bar=5 μm).

The karyotype was composed of 10 large metacentric, 2 large submetacentric, 4 large acrocentric, 2 large telocentric, 4 medium metacentric, 18 medium submetacentric, 2 medium acrocentric, 2 medium telocentric, and 6 small telocentric chromosomes. This was different from the study by Donsakul (1992) who found that *O. bimaculatus* in Thailand had 34 metacentric, 2 submetacentric, 2 subtelocentric and 12 acrocentric chromosomes. In a comparative study, we found different results when we compared *O. fumidus* in Thailand namely a 2*n*=60, made up of 20 metacentric, 2 submetacentric, 2 subtelocentrc, and 36 telocentric chromosomes (Magtoon and Donsakul 2009). No cytologically distinguishable sex chromosome similar to *O. fumidus* and *O. pabda* was observed (Datta *et al.* 2003, Magtoon and Donsakul 2009) and other silurid fishes in Thailand (Donsakul 1992, 1996, Magtoon and Donsakul 2009). The karyotype formula of *O. bimaculatus* is stated below.

$$2n \text{ (diploid) } 50=L_{10}^{m}+L_{2}^{sm}+L_{4}^{a}+L_{2}^{t}+M_{4}^{m}+M_{18}^{sm}+M_{2}^{a}+M_{2}^{t}+S_{6}^{t}$$

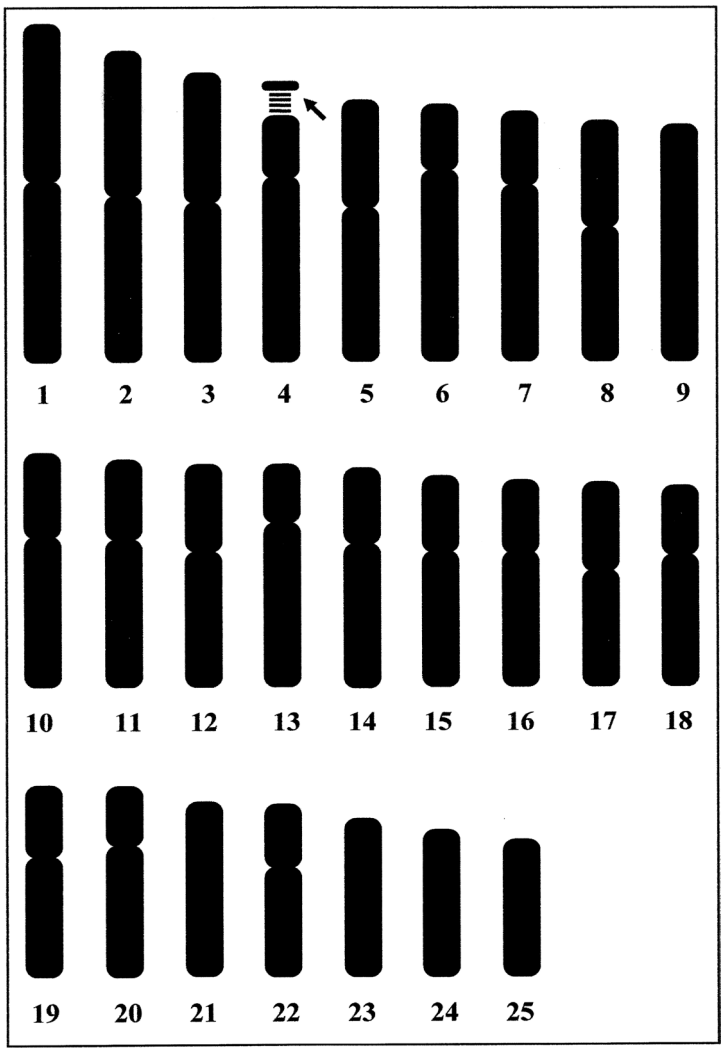

Fig. 3. Idiogram of the two-spot glass catfish (*Ompok bimaculatus*), 2*n* (diploid)=50, by conventional staining technique. Arrow indicates nucleolar organizer region (NOR).

Chromosome marker of O. bimaculatus

The results of a cytogenetic study of *O. bimaculatus* 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 non-histone protein more than other chromosome regions. Accordingly, the specific dark band (NOR-positive) is induced by the reduction of organic silver by these proteins that change from silver to dark (Sharma *et al.* 2002). The region adjacent to the telomere of the short arm of submetacentric chromosome pair 4 showed clearly observable nucleolar organizer regions (Fig. 2). This is in the agreement with the study by Das and Khuda-Bukhsh (2007) which showed that the NORs of *O. bimaculatus* in India were located on the short arms in submetacentric chromosome pair 4.

Over 200 species of fish have been investigated by the Ag-NOR staining technique, and some of those studies were conducted to elucidate taxonomic and morphological affinities. Moreover, the amount and location of NORs can explain the evolution of each chromosome (Gold *et al.* 1986). Normally, most fishes have only 1 pair of small NORs in a chromosome complement. If some fish have more than 2 NORs, this may have been caused by translocation between NORs and another chromosome. Furthermore, NORs are usually closely located to the telomere of the chromosome arm. If NORs appear between the centromere and telomere, this may have been caused by centric fusion or pericentric inversion between 2 telocentric chromosomes of which one chromosome has a NOR at the telomere (Sharma *et al.* 2002).

We have shown that the asymmetrical karyotype of *O. bimaculatus*, which has all 4 types of

Table 1. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphases of the two-spot glass catfish (*Ompok bimaculatus*), 2*n* (diploid)=50.

Chromosome pairs	Ls	Ll	LT	RL±SD	CI±SD	Chromosome size	Chromosome type
1	0.188	0.216	0.404	0.030±0.001	0.536±0.035	Metacentric	Large
2	0.172	0.201	0.373	0.027±0.000	0.539±0.031	Metacentric	Large
3	0.155	0.192	0.347	0.025±0.001	0.553±0.027	Metacentric	Large
4*	0.116	0.221	0.337	0.025±0.001	0.655±0.042	Submetacentric	Large
5	0.129	0.185	0.314	0.023±0.001	0.590±0.043	Metacentric	Large
6	0.080	0.230	0.310	0.023±0.001	0.743±0.037	Acrocentric	Large
7	0.090	0.211	0.301	0.022±0.001	0.700±0.069	Acrocentric	Large
8	0.127	0.163	0.290	0.021±0.001	0.562±0.034	Metacentric	Large
9	0.000	0.286	0.286	0.021±0.001	1.000±0.000	Telocentric	Large
10	0.102	0.179	0.281	0.021±0.000	0.638±0.045	Submetacentric	Medium
11	0.097	0.175	0.272	0.020±0.000	0.642±0.040	Submetacentric	Medium
12	0.107	0.160	0.267	0.019±0.000	0.598±0.028	Metacentric	Medium
13	0.071	0.195	0.266	0.019±0.000	0.732±0.051	Acrocentric	Medium
14	0.090	0.172	0.262	0.019±0.000	0.656±0.047	Submetacentric	Medium
15	0.091	0.163	0.254	0.019±0.000	0.641±0.040	Submetacentric	Medium
16	0.085	0.162	0.247	0.018±0.000	0.656±0.035	Submetacentric	Medium
17	0.106	0.139	0.245	0.018±0.000	0.567±0.041	Metacentric	Medium
18	0.082	0.158	0.240	0.017±0.001	0.659±0.038	Submetacentric	Medium
19	0.085	0.143	0.228	0.017±0.001	0.626±0.070	Submetacentric	Medium
20	0.071	0.156	0.227	0.016±0.000	0.690±0.081	Submetacentric	Medium
21	0.000	0.209	0.209	0.015±0.001	1.000±0.000	Telocentric	Medium
22	0.075	0.131	0.206	0.015±0.000	0.640±0.082	Submetacentric	Medium
23	0.000	0.190	0.190	0.014±0.000	1.000±0.000	Telocentric	Small
24	0.000	0.175	0.175	0.013±0.000	1.000±0.000	Telocentric	Small
25	0.000	0.165	0.165	0.012±0.000	1.000±0.000	Telocentric	Small

Remarks: *=NOR-bearing chromosome.

chromosome (metacentric, submetacentric, acrocentric and telocentric chromosomes), is an important chromosome marker. The idiogram show continuous length gradation chromosomes. Figure 3 shows the idiogram of *O. bimaculatus* from conventional staining. The largest and smallest chromosomes show an approximately 2 fold size difference. Data of the chromosomal checks on mitotic metaphase cells of *O. bimaculatus* are shown in Table 1. Regarding, the chromosome marker of *O. bimaculatus*, chromosome pair 1 is the largest metacentric and chromosome pair 25 is the smallest telocentric chromosome.

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