Interpopulational Variation of NOR Positions and Karyotypic Analysis of Siamese Catfish (*Pseudomystus siamensis*) in Thailand

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Summary Interpopulational variation of nucleolar organizer region (NOR) position and karyotypic analysis of Siamese catfish (*Pseudomystus siamensis*) in Thailand were studied. The samples were taken from three locations (8 populations); the mitotic chromosome preparations were conducted directly from kidney cells. Conventional and Ag-NOR staining techniques were applied to stain the chromosomes. The results showed that the diploid chromosomes number was $2n=50$ and the fundamental number (NF) was 96 in both sexes. The karyotype of *P. siamensis* showed two new cytotypes for this species. The chromosome of cytotype A and B showed the presence of metacentric, submetacentric, acrocentric and telocentric autosomes as 22–18–6–4 and 20–20–6–4, respectively, without any apparent sex chromosomal heteromorphism. Data obtained from chromosomal banding technique further suggests an interpopulation NOR polymorphism. Such variation is possibly caused by paracentric inversion. In addition, a pair of the long arms of chromosome pair 2' in cytotype A and chromosome pair 12'' in cytotype B showed clearly observable NORs. The karyotype formulas of cytotype A and B for *P. siamensis* could be deduced as:

Cytotype A: $2n$ (diploid) $50=L_6^m+L_6^{sm}+L_2^a+M_{16}^m+M_{12}^{sm}+M_3^a+M_2^t+S_2^t$

Cytotype B: $2n$ (diploid) $50=L_2^m+L_6^{sm}+M_{14}^m+M_{12}^{sm}+M_6^a+M_2^t+S_4^m+S_2^{sm}+S_2^t$.

Key words Siamese catfish, *Pseudomystus siamensis*, Nucleolar organizer region, Polymorphism.

Thailand is one of the world's richest places of biodiversity, especially for freshwater fish species. With more than 700 species recorded, Thailand is one of the species diversity centers of the world (Vidthayanon 2005). Freshwater fish are especially important as they provide a high quality source of protein as food source for people who live near river basins. Fish in the family Bagridae, belonging to the order Siluriformes, are one of the most important fish consumed as food. Some species are kept as aquarium fish. The aquaculture of several species has recently become booming (Nelson 2006). For these reasons, they play an important role in the national economy.

An important characteristic of nucleolar organizer regions (NORs) in fish is inter- and intra-species polymorphism. NOR characterization can be a cytogenetic marker for cytotaxonomic studies and can even aid in constructing phylogenetic hypotheses (cytosystematics) for several fish groups (Amemiya and Gold 1988, Galetti Jr 1998, Almeida-Toledo 2000). Some fish groups pres-

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Fig. 1. General characteristics of the Siamese catfish, *Pseudomystus siamensis* (Siluriformes, Bagridae). Scale bar indicates 5 cm.

ent a simple NOR system characterized by ribosomal cistrons on only one chromosome pair, whereas others have a multiple NOR system composed of cistrons dispersed over several chromosomes (Galetti Jr. 1998, Capistano *et al.* 2008).

There are five genera and 25 species in family Bagridae in Thailand, including *Bagrichthys hypselopterus*, *Bag. macracanthus*, *Bag. macropterus*, *Bag. majusculus*, *Bag. obscurus*, *Batasio havmollerii*, *Bat. tengana*, *Bat. tigrinus*, *Hemibagrus filamentus*, *H. nemurus*, *H. wyckiioides*, *H. wyckii*, *Mystus albolineatus*, *M. atrifasciatus*, *M. bocourti*, *M. castaneus*, *M. cavasius*, *M. multiradiatus*, *M. mysticetus*, *M. rhegma*, *M. singaringan*, *M. wolffii*, *Pseudomystus bombooides*, *P. siamensis* (Fig. 1), and *P. stenomus* (Ferraris 2007, Rainboth 1996, Vidthayanon 2005).

There has only been one previous attempt at conventional staining analysis in *P. siamensis* from the Mekong basin, Nongkhai Province (five specimens), which detected the occurrence of one cytotype (Donsakul 2002). In the present work, a more detailed analysis is performed, involving Ag-NOR banding technique procedures to establish chromosomal marker, possibly including the nature of the rearrangements related to the present polymorphism. Moreover, the data provides useful basic information for conservation and breeding practices as well as analyses of chromosomal evolution in this species of fish.

Materials and methods

Sample collection

Specimens of *P. siamensis* (33 males and 33 females) were collected from eight populations of the Chi, Songkhram, and Chao Phraya basins, Thailand (Fig. 2 and Table 1). The fish were transferred to laboratory aquaria and were kept under standard conditions for seven days prior to the experiments.

Chromosome preparation

Chromosomes were directly prepared *in vivo* (Chen and Ehbeling 1968, Nanda *et al.* 1995) as follows. Phytohemagglutinin (PHA) solution was injected to the abdominal cavities of the fish. 24 hours later, 0.05% colchicine solution was injected to the intramuscular and/or abdominal cavities of the fish. The treated samples were left 1–2 h. Kidney and/or gill samples were removed and cut into small pieces before being mixed with 0.075 M KCl. After all the large pieces of cells were discarded, an 8 ml of cell sediments was transferred to a centrifuge tube and incubated for 25–35 min. KCl was discarded from the supernatant after centrifugation at 1,200 rpm for 8 min. Cells were fixed in fresh cool fixative (3 absolute methanol : 1 glacial acetic acid). The fixative was gradually added up to 8 ml before centrifuging again at 1,200 rpm for 8 min and the supernatant was discarded. The fixation was repeated until the supernatant was clear and the pellet was mixed with 1 ml fixative. The mixture was dropped onto clean and cold slides by a micropipette, after which slides

were left to dry following the air-dry technique.

Chromosome staining

Conventional staining was done using 20% Giemsa's solution for 30 min, and Ag-NOR banding was performed by adding 4 drops of 50% silver nitrate and 2% gelatin on slides, respectively. The slides were then sealed with cover glasses and incubated at 60°C for 5 min. After that the slides were soaked in distilled water until cover glasses were separated and stained with 20% Giemsa's solution for 1 min (Howell and Black 1980).

Fig. 2. Map of Thailand showing karyotype cytotype A (telomeric NOR) and B (interstitial NOR) of Siamese catfish (*Pseudomystus siamensis*) from three locations (eight populations).

Chromosome checks

Chromosome counting was performed on mitotic metaphase cells under a light microscope. 20 clearly observable and well-cells spread chromosomes of each male and female were selected and photographed. The length of short arm chromosome (Ls) and long arm chromosome (Ll) were measured to calculate length of total arm chromosome (LT, $LT=Ls+Ll$). The relative length (RL), the centromeric index (CI), and standard deviation (SD) of RL and CI were estimated (Chaiyasut 1989). The CI ($q/p+q$) between 0.50–0.59, 0.60–0.69, 0.70–0.89, and 0.90–0.99 are described as metacentric, sub-metacentric, 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.

Table 1. Eight populations of the Siamese catfish (*Pseudomystus siamensis*) collected in Thailand

Locations (Province)	Populations	Number of specimens		Cytotypes
		Male	Female	
Chi basin	Khon Kaen	4	4	A
	Mahasarakham	6	6	A
	Roi Et	4	4	A
Songkhram basin	Buang Kan	4	4	B
	Nakhon Phanom	3	2	B
	Sakon Nakhon	4	5	B
Chao Phraya basin	Singburi	4	4	B
	Ayutthaya	4	4	B

Remarks: Cytotype A (telomeric NORs) and cytotype B (interstitial NORs).

Fig. 3. Karyotypes of cytotypic A (top) and cytotypic B (below) of the Siamese catfish (*Pseudomystus siamensis*) in Thailand, $2n=50$, by conventional staining technique. Arrows indicate homomorphism chromosome pair 2' and homomorphism chromosome pair 12'' resulting from paracentric inversion.

Results and discussion

Chromosome number, fundamental number and karyotype of P. siamensis

Cytogenetic studies have been carried out on several local populations of *P. siamensis* from different locations in Thailand. The results revealed that the somatic chromosome number of *P. siamensis* is $2n=50$ in both sexes (Fig. 3). This is in accordance with the former study of Donsakul (2002). Cytogenetic studies performed on the family Bagridae have shown a diploid chromosome number ranging from $2n=44$ (*Coreobagrus brevicarpus*; Kim *et al.* 1982) to $2n=80$ (*Batasio havmollerii*; Magtoon and Donsakul 2009). Nevertheless, the most frequent diploid chromosome number is equal to 52 chromosomes (Arai 2011). The fundamental number (NF) of *P. siamensis* is 96 in both male and female, which is different from the report of Donsakul (2002) who detected the NF is 86. This difference was due to different criteria used for the classification of chromosome type is similar to other species in family Bagridae of Thailand, in that no cytologically distinguishable sex chromosome was observed. However, some bagrid catfishes in India, including *M. gulio* and *M. tengara*, have differentiated sex chromosome systems as XX/XY and ZZ/ZW, respectively (Arai 2011).

The karyotypes of *P. siamensis* showed two new cytotypes for this species. The chromosomes of cytotypic A consist of 22 metacentric, 18 submetacentric, 6 acrocentric, and 4 telocentric chromosomes and their frequency in the eight populations was 37.5% (three populations). Cytotypic B chromosomes were composed of 20 metacentric, 20 submetacentric, 6 acrocentric, and 4 telocentric chromosomes and their frequency was 62.5% (five populations). It is inconsistent to the report of Donsakul (2002), which revealed that *P. siamensis* had the following: 20 metacentric, 16 submetacentric, 10 subtelocentric, and 4 telocentric chromosomes.

In the present study, two new cytotypes were found and the cytogenetical analyses revealed a cause of chromosomal rearrangement occurring on *P. siamensis*. That cause is related to paracen-

tric inversion in one of the homologous chromosome pair 2' in cytotype A (telomeric NORs) changing to the first homologous chromosome pair 12'' in cytotype B (interstitial NORs). Since interstitial NORs have been found five populations (Songkham and Chao Phraya basins), they may

Fig. 4. Karyotypes of cytotype A (top) and cytotype B (below) of the Siamese catfish (*Pseudomystus siamensis*) in Thailand, $2n=50$, by Ag-NOR banding technique. Blocks indicate homomorphism chromosome pair 2' (telomeric NORs) and homomorphism chromosome pair 12'' (interstitial NORs) resulting from paracentric inversion.

Fig. 5. Idiogram showing lengths and shapes of chromosomes cytotype A of the Siamese catfish (*Pseudomystus siamensis*) in Thailand, $2n=50$, by conventional staining technique. The arrow indicates telomeric NOR-bearing chromosome pair 2'.

Fig. 6. Idiogram showing lengths and shapes of chromosomes cytotype B of the Siamese catfish (*Pseudomystus siamensis*) in Thailand, $2n=50$, by conventional staining technique. The arrow indicates interstitial NOR-bearing chromosome pair 12''.

Table 2. 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 cytotype A of the Siamese catfish (*Pseudomystus siamensis*) in Thailand, 2n=50.

Chromosome pairs	Ls	Ll	LT	RL±SD	CI±SD	Chromosome type	Chromosome size
1	0.143	0.168	0.311	0.025+0.0009	0.540+0.019	Metacentric	Large
2*	0.132	0.162	0.294	0.023±0.0013	0.546±0.018	Metacentric	Large
3	0.125	0.153	0.278	0.022±0.0008	0.549±0.020	Metacentric	Large
4	0.119	0.143	0.262	0.021±0.0009	0.544±0.020	Metacentric	Medium
5	0.112	0.137	0.249	0.020±0.0005	0.548±0.014	Metacentric	Medium
6	0.109	0.135	0.244	0.019±0.0005	0.552±0.018	Metacentric	Medium
7	0.108	0.130	0.238	0.019±0.0005	0.546±0.018	Metacentric	Medium
8	0.104	0.126	0.230	0.018±0.0005	0.546±0.017	Metacentric	Medium
9	0.102	0.122	0.224	0.018±0.0004	0.545±0.023	Metacentric	Medium
10	0.097	0.116	0.213	0.017±0.0006	0.544±0.020	Metacentric	Medium
11	0.091	0.106	0.197	0.015±0.0012	0.535±0.023	Metacentric	Medium
12	0.116	0.244	0.360	0.028±0.0016	0.677±0.024	Submetacentric	Large
13	0.103	0.194	0.297	0.024±0.0013	0.646±0.057	Submetacentric	Large
14	0.096	0.182	0.278	0.022±0.0010	0.648±0.042	Submetacentric	Large
15	0.095	0.169	0.264	0.021±0.0005	0.637±0.046	Submetacentric	Medium
16	0.091	0.163	0.254	0.020±0.0008	0.642±0.035	Submetacentric	Medium
17	0.089	0.154	0.243	0.019±0.0009	0.626±0.049	Submetacentric	Medium
18	0.083	0.150	0.233	0.018±0.0011	0.642±0.048	Submetacentric	Medium
19	0.069	0.153	0.222	0.017±0.0013	0.687±0.028	Submetacentric	Medium
20	0.072	0.132	0.204	0.016±0.0012	0.646±0.029	Submetacentric	Medium
21	0.069	0.200	0.269	0.021±0.0010	0.738±0.046	Acrocentric	Large
22	0.065	0.176	0.241	0.019±0.0008	0.725±0.024	Acrocentric	Medium
23	0.057	0.155	0.212	0.017±0.0014	0.730±0.052	Acrocentric	Medium
24	0.000	0.221	0.221	0.017±0.0018	1.000±0.000	Telocentric	Medium
25	0.000	0.173	0.173	0.014±0.0021	1.000±0.000	Telocentric	Small

Remarks: *=NOR-bearing chromosome.

be regarded as derivatives of telomeric NORs (three populations in Chi basin) by a process of paracentric inversion (Fig. 7).

The occurrence of a local population with more than 1 cytotype has been reported for several fish species, such as those of the genus *Bryconamericus* (Wasko *et al.* 1996) and *Aphanius fasciatus* (Vitturi *et al.* 1995), in which different cytotypes had the same diploid number and different chromosome formulae. In other genera such as *Corydoras* (Oliveira *et al.* 1990, 1993), *Gymnotus* (Fernandes-Matioli 1996) and *Hoplias* (Bertollo *et al.* 1997), cytotypes with different diploid numbers have been described. Among 37 populations of *Astyanax scabripinnis* analyzed (Souza *et al.* 1995, Maistro *et al.* 1998), specimens with different diploid numbers were found in sympatry only in two, namely the populations from Canta Galo brooks (Souza *et al.* 1995). The karyotype formulae for *P. siamensis* are as stated:

Cytotype A: 2n (diploid) 50=L₆^m+L₆sm+L₂^a+M₁₆^m+M₁₂sm+M₄^a+M₂^t+S₂^t

Cytotype B: 2n (diploid) 50=L₂^m+L₆sm+M₁₄^m+M₁₂sm+M₆^a+M₂^t+S₄^m+S₂sm+S₂^t

Table 3. Mean length of short arm chromosome (Ls), long arm chromosome (LI), total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphases of cytotype B of the Siamese catfish (*Pseudomystus siamensis*) in Thailand, $2n=50$.

Chromosome pairs	Ls	LI	LT	RL $\pm$ SD	CI $\pm$ SD	Chromosome type	Chromosome size
1	0.150	0.182	0.332	0.025 $\pm$ 0.0016	0.546 $\pm$ 0.021	Metacentric	Large
2	0.131	0.160	0.291	0.022 $\pm$ 0.0013	0.550 $\pm$ 0.027	Metacentric	Medium
3	0.123	0.151	0.274	0.021 $\pm$ 0.0011	0.552 $\pm$ 0.025	Metacentric	Medium
4	0.121	0.139	0.260	0.020 $\pm$ 0.0007	0.535 $\pm$ 0.016	Metacentric	Medium
5	0.113	0.139	0.252	0.019 $\pm$ 0.0006	0.553 $\pm$ 0.021	Metacentric	Medium
6	0.110	0.133	0.243	0.018 $\pm$ 0.0008	0.549 $\pm$ 0.025	Metacentric	Medium
7	0.101	0.129	0.230	0.017 $\pm$ 0.0010	0.559 $\pm$ 0.029	Metacentric	Medium
8	0.095	0.123	0.218	0.016 $\pm$ 0.0009	0.565 $\pm$ 0.027	Metacentric	Medium
9	0.091	0.114	0.205	0.015 $\pm$ 0.0008	0.559 $\pm$ 0.027	Metacentric	Small
10	0.087	0.103	0.190	0.014 $\pm$ 0.0010	0.543 $\pm$ 0.028	Metacentric	Small
11	0.140	0.287	0.427	0.032 $\pm$ 0.0022	0.672 $\pm$ 0.030	Submetacentric	Large
12''*	0.151	0.265	0.416	0.031 $\pm$ 0.0016	0.637 $\pm$ 0.033	Submetacentric	Large
13	0.105	0.214	0.319	0.024 $\pm$ 0.0009	0.670 $\pm$ 0.026	Submetacentric	Large
14	0.096	0.199	0.295	0.022 $\pm$ 0.0001	0.674 $\pm$ 0.030	Submetacentric	Medium
15	0.093	0.194	0.287	0.021 $\pm$ 0.0009	0.674 $\pm$ 0.027	Submetacentric	Medium
16	0.087	0.181	0.268	0.020 $\pm$ 0.0010	0.675 $\pm$ 0.025	Submetacentric	Medium
17	0.088	0.166	0.254	0.019 $\pm$ 0.0010	0.653 $\pm$ 0.030	Submetacentric	Medium
18	0.081	0.155	0.236	0.018 $\pm$ 0.0007	0.658 $\pm$ 0.030	Submetacentric	Medium
19	0.072	0.143	0.215	0.016 $\pm$ 0.0011	0.666 $\pm$ 0.033	Submetacentric	Medium
20	0.067	0.130	0.197	0.015 $\pm$ 0.0012	0.660 $\pm$ 0.032	Submetacentric	Small
21	0.080	0.201	0.281	0.021 $\pm$ 0.0015	0.717 $\pm$ 0.024	Acrocentric	Medium
22	0.064	0.181	0.245	0.018 $\pm$ 0.0013	0.741 $\pm$ 0.023	Acrocentric	Medium
23	0.060	0.162	0.222	0.016 $\pm$ 0.0015	0.727 $\pm$ 0.021	Acrocentric	Medium
24	0.000	0.219	0.219	0.016 $\pm$ 0.0020	1.000 $\pm$ 0.000	Telocentric	Medium
25	0.000	0.166	0.166	0.012 $\pm$ 0.0011	1.000 $\pm$ 0.000	Telocentric	Small

Remarks: *=NOR-bearing chromosome.

Chromosome markers of *P. siamensis*

Recently, chromosomal studies on fish have been providing increasingly details information about karyotypical variability at inter- and intra-specific levels, which can be of great interest to phylogenetic, systematics and taxonomy (Centofante *et al.* 2002). The present study is the first report on *P. siamensis* accomplished by the Ag-NOR banding technique. The technique shows dark bands (NOR-positions) on long arms near telomere of metacentric chromosome pair 2' in cytotype A and interstitial on long arms of submetacentric chromosome pair 12'' in cytotype B (Figs. 4 and 8).

Chromosomal rearrangements involving inversions have been reported in some fish species. In *Ilyodon furcoides* from the Coahuayana River basin in Southern Mexico, Turner *et al.* (1985) detected a diploid chromosome number of $2n=48$ but with numerical variation of the metacentric chromosomes, thus identifying 4 cytotypes. In *Hoplerythrinus unitaeniatus*, from the Negro River, Giuliano-Caetano and Bertollo (1988) detected a number of $2n=48$ and identified four related cytotypes with or without acrocentric chromosomes. In *S. spilopleura*, from the Paraná Paraguay basin, Cestari and Galetti (1992a), detected a number of $2n=60$ identifying three cytotypes related to the

presence of metacentric and acrocentric chromosomes, in allopatry. Nakayama *et al.* (2000) also observed the occurrence of two cytotypes (cytotype A and B) with equal diploid numbers ($2n=60$), but in sympatry at Catalão Lake for this same species. Most chromosomal inversions determine a genetic variation caused by gene segregation, and they are disadvantageous when in homozygosity.

Among piranhas, *Serrasalmus* might be considered the most well studied genus, in terms of chromosomal analysis. Multiple NORs systems, located on subtelo-acrocentric chromosomes, in which number, size and intensity of markings vary intra-individually, seem to be a rule (Galetti *et al.* 1985, Cestari and Galetti 1992a, b, Martins-Santos *et al.* 1994, Cestari 1996, Nakayama 1997, Centofante *et al.* 2002). Since variability due to chromosome rearrangements can determine change

only in the chromosome structure (by deletions, duplications, inversions, and/or translocations), understanding these alterations and their association to the speciation process, is a major challenge in karyoevolutionary studies (White 1977, John 1980, Guerra 1988, Centofante *et al.* 2002).

The important chromosome marker of the *P. siamensis* is the asymmetrical karyotype in which all four types of chromosomes (metacentric, submetacentric, acrocentric, and telocentric chromosomes) were found. The idiograms demonstrate a continuous length gradation of chromosomes (cytotype A and B). The largest and smallest chromosomes show size difference (approximately two fold). The chromosome marker

Fig. 7. The chromosome pair 12'' cytotype B formation model of the Siamese catfish (*Pseudomystus siamensis*) in Thailand resulting from paracentric inversion.

Fig. 8.

of *P. siamensis* is chromosome pair one, which is the largest metacentric chromosome, and chromosome pair 25, which is the smallest telocentric chromosome. The data of the chromosomal checks on mitotic metaphase cells of *P. siamensis* are shown in Tables 2 and 3, and the idiograms are shown in Figs. 5 and 6.

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