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Standardized Karyotype and Idiogram of the Glass Goby Fish (*Gobiopterus chuno*) (Hamilton, 1822) in Thailand

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Abstract-The standardized karyotype and idiogram of the glass goby fish, Gobiopterus chuno (Hamilton, 1822) from the Chao Phraya Basin in Singburi Province, Thailand, were studied. Kidney (and testis in males) tissues were collected from ten female and ten male samples after soaking in the colchicine solution. Conventional staining (20% Giemsa solution) and Ag-NOR banding techniques (50% silver nitrate and 2% gelatin solution) were applied to stain the chromosomes. The results showed that diploid chromosome number of G. chuno was 2n= 48, and the fundamental number (NF) was 50 in both males and females. No strange sized chromosomes associated with sex were identified. Karyotype consisted of two large acrocentric, 24 large telocentric, 20 medium telocentric, and two small telocentric chromosomes. Positions of Nucleolar Organizer regions (NORs) were observed at the region adjacent to the telomeres of short arm of the chromosome pair 2 (acrocentric). The karyotype formula for G. chuno is as follows: 2n (diploid) 48=La2+Lt24+Mt20+St2

Keywords: Gobiopterus chuno, chromosome, karyotype, idiogram

1. Introduction

Oxudercidae (sometimes called the Gobionellidae) is a family of gobies that it was formerly classified under the family Oxudercidae. The species in this family are found in marine and freshwater, which has an ecumenical distribution in temperate and tropical areas and, typically in inshore, euryhaline areas with silt and sand substrates. The Oxudercidae includes 86 genera, which contain about 598 species (Nelson *et al*., 2016). Two genera such as *Brachygobius* and *Gobiopterus* are importance in Thailand. *Gobiopterus chuno* (Hamilton, 1822), commonly known as the glass goby is species in the group of the Oxudercidae family. The maximum size is three centimeters. The distribution of *G. chuno* is found in Asia: India, Myanmar, Malay Peninsula, and Sumatra, Indonesia. The common characteristics have two dorsal fins, the spines in the front part and soft fin in the back. The remarkable feature of *G. chuno* is transparent until the internal structure (Figure 1). *Gobiopterus chuno* in Thailand has distribution in the Mekong and Chao Phraya Basins. Due to the small size, it is unknown to the people. Therefore, there is little basic information. In addition, there is no study on chromosomal data.

Cytogenetics is the study morphology structure, number, and position of the chromosome. Each species has different chromosomes, the specific type and the number of chromosomes in each species (Rooney, 2001). Thus, the chromosomes study can be used classification of animal species from the number and visual appearance of the chromosomes (karyotype). The karyotype is the chromosome complement of an individual or related group of individuals, as defined by chromosome size, morphology, and number. Though for all somatic cells of all individuals of species, the number of chromosomes is used as an indicator of classification of species and interrelationships within families (Tanomtong, 2011). The studies of the karyotypes help to investigate the aquatic structure of the species population in each habitat, so it can determine what species are accurately related to each other. This may help to facilitate the hybridization between them in the future for strain improvement (Sofy *et al.*, 2008), breeding practices of organisms by using chromosome set management (Na-nakhon *et al*., 1980).

The present study, two chromosomes staining techniques: conventional staining and Ag-NOR banding were performed. The knowledge revealed will provide a powerful tool for the next generation of genetics research in Thai gobiids and discovering biodiversity, with useful applications in fish breeding for conservation and commercials, evolution, systematics, phylogenetics, fish fauna management and suitable conservation of river basin.

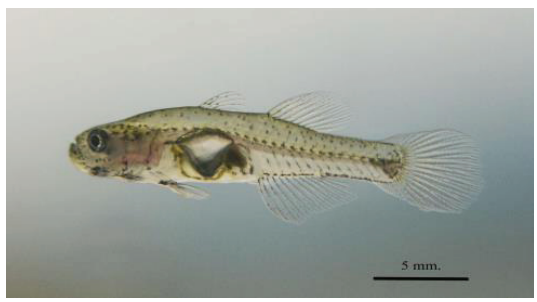

Figure 1. General characteristic of the glass goby fish (*Gobiopterus chuno*)

2. Materials and Methods

2.1 Sample Collection

Ten male and ten female samples of *G. chuno* were obtained from the Chao Phraya Basin in Singburi Province, Thailand. Species were identified following an etymological dictionary of taxonomy (Romero, 2002). All specimens were soaked in 0.01% colchicine solution for two hours. Chromosome preparation was conducted directly from grill, testis and kidney tissues (Chen and Ebeling, 1968). The target tissues were cut in a small peace then mixed with 0.075 M KCl in a centrifuge tube and incubated for 30 minutes at room temperature. After that, KCl was discarded from the supernatant after centrifugation at 1,200 rpm for 8 minutes. Cells were fixed in fresh cool fixative (3 absolute methanol: 1 glacial acetic acid) to stop KCl activities. The fixative was gradually added up to 8 mL before centrifuging again at 1,200 rpm for 8 minutes and the supernatant was discarded. The fixation was repeated until the supernatant was clear and the pellet was mixed with 1 mLfixative.

2.2 Chromosome Staining

The slide was conventionally stained with 20 percent of Giemsa's solution for 30 minutes (Chooseangjaew *et al*., 2017) then washed in the distilled water. Ag-NOR staining to identify NORs positions (Sangpakdee *et al*., 2017), this technique was conducted by adding four drops of 50%silver nitrate and two drops of 2% gelatin on slides, respectively. The slides were then sealed with cover glasses and incubated at 60 ^{o}C for 5-7 minutes. After that, the slides were soaked in distilled water until cover glasses were separated.

2.3 Chromosome Analysis

Chromosome counting was performed on mitotic metaphase cells under a light microscope. Twenty clearly observable and well spread chromosomes plates of each male and female were selected and photographed. The length of the short arm chromosome (Ls) and long arm chromosome (Ll) were measured and calculated to the length of the 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. CI was also computed to classify the types of chromosomes according to Chaiyasut (1989). The CI (q/p+q) between 0.50-0.59, 0.60-0.69, 0.70-0.89 and 0.90-1.00 are described as metacentric, submetacentric, acrocentric and telocentric chromosomes, respectively. Fundamental number (number of chromosome arm, NF) is obtained by assigning a value of 2 for metacentric, submetacentric, acrocen-tric chromosomes and 1 for telocentric chromosome. All parameters were used in karyotyping and idiograming.

3. Results

The diploid chromosome number of *G. chuno* was 2n = 48, the fundamental number (NF) was 50 in both sexes (Figs. 2A, 2B). The metaphase I of meiosis can confirm the diploid chromosome number (2n = 48) as 24 bivalents shown in (Figs. 2C, 2D). No heteromorphic chromosomes that determine sex were observed. The standardized karyotypes of chromosomes

composed of two larges acrocentric, 24 larges telocentric, 20 medium telocentric and two small telocentric chromosomes (Table 1). Nucleolar organizer regions/ NORs display at the regions adjacent to the telomeres of the short arms of the large acrocentric chromosome pair 2 (Figure 3). Idiograms showing length and shapes of chromosomes by conventional staining and Ag-NOR banding techniques are presented in (Figure 4). The karyotype formula for *G. chuno* is as follows:

$$2n \text{ (diploid) } 48 = L_a^{2}+L_t^{24}+M_t^{20}+S_t^{2}.$$

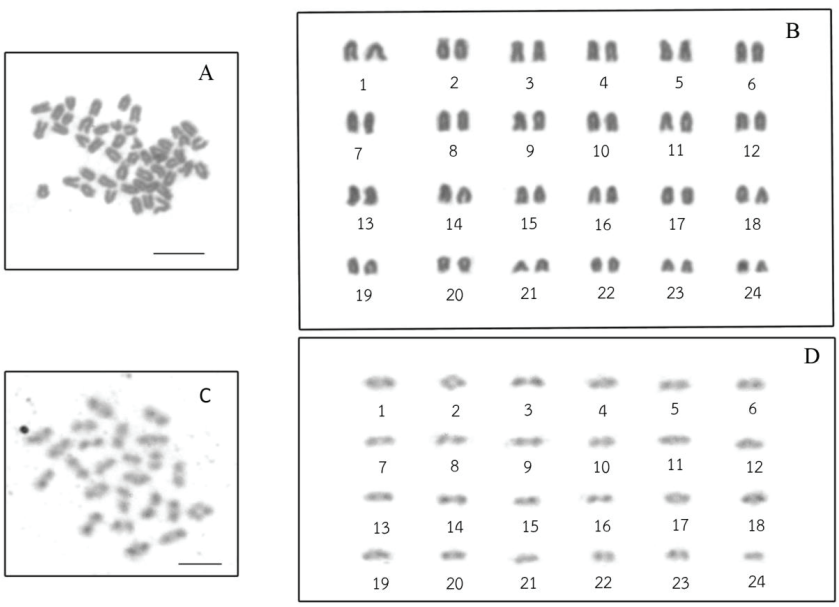

Figure 2. Metaphase chromosome plates (A, C) and karyotypes (B, D) of *Gobiopterus chuno*, 2n = 48 by conventional staining technique, scale bars indicate 5 micrometres. A and B from the metaphase of mitosis, C and D from metaphase I of meiosis

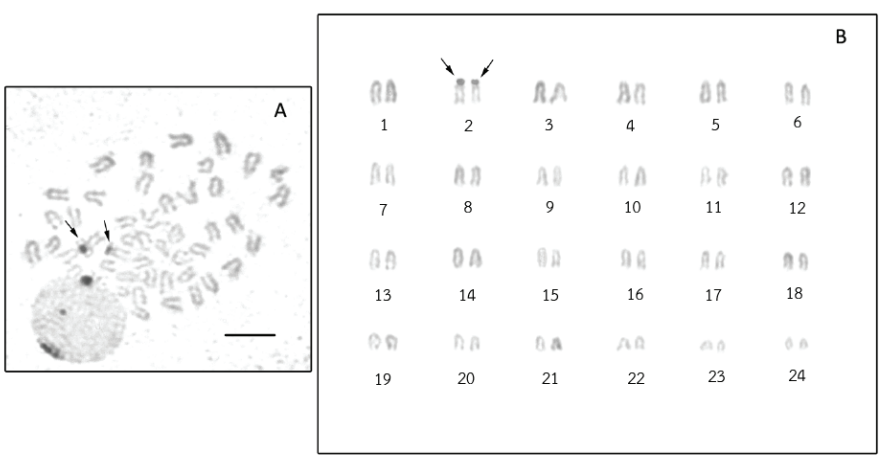

Figure 3. Metaphases chromosome plate (A) and karyotype (B) of *Gobiopterus chuno*, 2n=48 by using Ag-NOR banding technique, scale bar indicates 5 micrometres. Arrows indicate the NOR positions on the chromosomes.

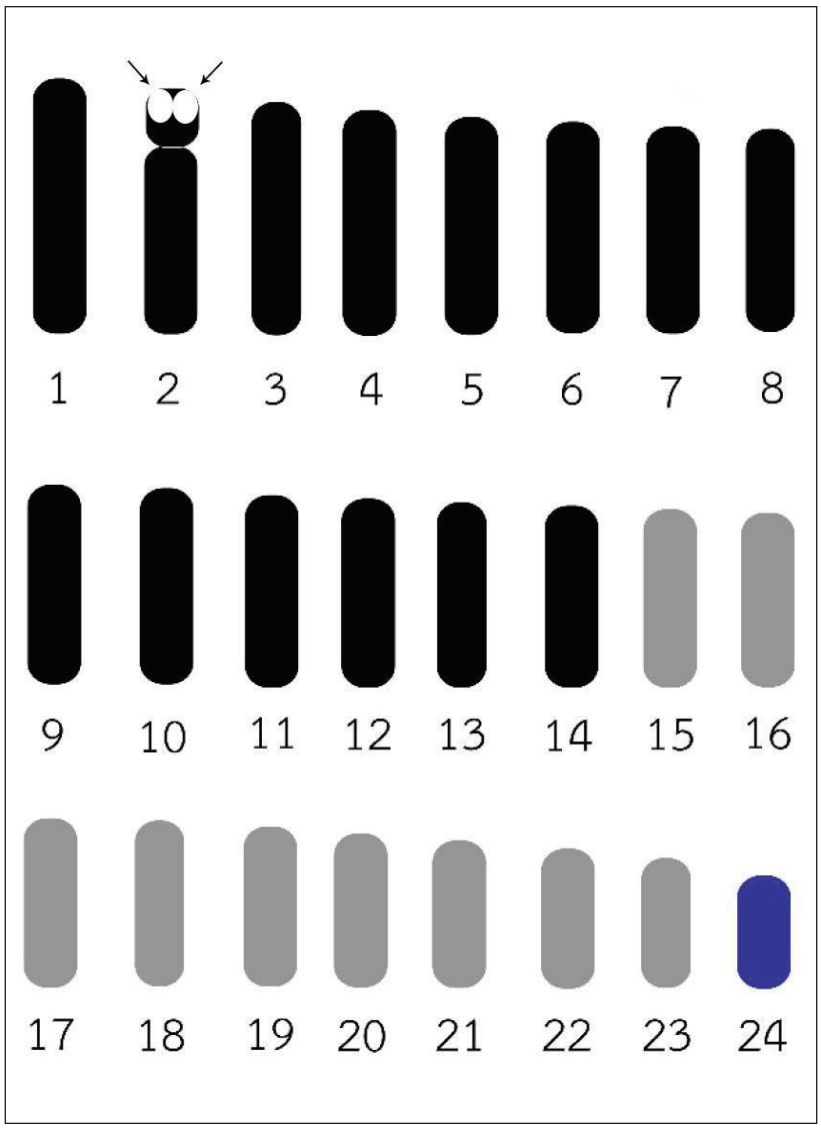

Figure 4. Standardized idiogram of the *Gobiopterus chuno***,** 2n = 48 by conventional and Ag-NOR banding techniques, the arrows indicate the NOR position on the chromosome. The color bars present chromosome sizes: black=large, gray=medium, blue = small.

Table 1. Means of the short arm length (Ls), long arm length (Ll), and total arm length of chromosomes (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from metaphase chromosome of 20 cells in *Gobiopterus chuno*, 2n = 48

Chro. pair	Ls	Ll	LT	RL ± SD	CI ± SD	Chro. Size	Chro. Type
1	0.000	1.123	1.123	0.028 ± 0.002	1.000 ± 0.000	Large	telocentric
2**	0.264	0.826	1.090	0.028 ± 0.002	0.759 ± 0.052	Large	acrocentric
3	0.000	1.028	1.028	0.026 ± 0.001	1.000 ± 0.000	Large	telocentric
4	0.000	0.994	0.994	0.025 ± 0.001	1.000 ± 0.000	Large	telocentric
5	0.000	0.958	0.958	0.024 ± 0.001	1.000 ± 0.000	Large	telocentric
6	0.000	0.933	0.933	0.024 ± 0.001	1.000 ± 0.000	Large	telocentric
7	0.000	0.912	0.912	0.023 ± 0.001	1.000 ± 0.000	Large	telocentric
8	0.000	0.896	0.896	0.023 ± 0.001	1.000 ± 0.000	Large	telocentric
9	0.000	0.878	0.878	0.022 ± 0.000	1.000 ± 0.000	Large	telocentric
10	0.000	0.864	0.864	0.022 ± 0.000	1.000 ± 0.000	Large	telocentric
11	0.000	0.849	0.849	0.022 ± 0.000	1.000 ± 0.000	Large	telocentric
12	0.000	0.833	0.833	0.021 ± 0.000	1.000 ± 0.000	Large	telocentric
13	0.000	0.817	0.817	0.021 ± 0.001	1.000 ± 0.000	Large	telocentric
14	0.000	0.802	0.802	0.020 ± 0.001	1.000 ± 0.000	Medium	telocentric
15	0.000	0.786	0.786	0.020 ± 0.001	1.000 ± 0.000	Medium	telocentric
16	0.000	0.769	0.769	0.019 ± 0.001	1.000 ± 0.000	Medium	telocentric
17	0.000	0.746	0.746	0.019 ± 0.001	1.000 ± 0.000	Medium	telocentric
18	0.000	0.730	0.730	0.018 ± 0.001	1.000 ± 0.000	Medium	telocentric
19	0.000	0.705	0.705	0.018 ± 0.001	1.000 ± 0.000	Medium	telocentric
20	0.000	0.680	0.680	0.017 ± 0.001	1.000 ± 0.000	Medium	telocentric
21	0.000	0.651	0.651	0.017 ± 0.001	1.000 ± 0.000	Medium	telocentric
22	0.000	0.618	0.618	0.016 ± 0.001	1.000 ± 0.000	Medium	telocentric
23	0.000	0.575	0.575	0.015 ± 0.001	1.000 ± 0.000	Medium	telocentric
24	0.000	0.501	0.501	0.013 ± 0.001	1.000 ± 0.000	Small	telocentric

** Nucleolar organizer regions/NORs, Chro. = Chromosome

4. Discussion

This is the first report on chromosome analysis in both mitosis and meiosis divisions in *Gobiopterus chuno* and this genus. The chromosome number counted was 2n = 48. This result is confirmed by metaphase and metaphase I stage more than 100 observed cells. When compare with other genera/ species in the family Oxudercidae, the same of diploid chromosome number as 2n = 48 was found in *Bathygobius soporator* (Lima-Filho *et al*., 2012), *Bathygobius fuscus* (Arai and Kobayasi, 1973), *Bostrichthys sinensis* (Arai and Sawada, 1974), *Gobius nigerjozo* (Cataudella, 1973), *Gobius paganellus* (Caputo *et al*., 1997). However, the fundamental number (NF) of *G. chuno* equal to 50 which is different from the fundamental number from *Ba. soporator* (NF = 56), *Ba. fuscus* (NF = 48), *Bo. sinensis* (NF = 52) and *G. nigerjozo* (NF = 56). Only *Gobius paganellus*, has the same diploid and fundamental number with *Gobiopterus chuno* (Table 2.). The diploid chromosome numbers in the family Oxudercidae vary from 2n = 40 in *Aboma lactipes* (Arai and Kobayasi, 1973) to 2n = 50 in *Gobius niger* (Vitturi 1989) whereas the fundamental numbers are ranged between NF = 40 in *A. lactipes* and NF = 90 in *Dormitator maculatus* (Maldonado, 1985).

The chromosome types of *G. chuno* were two acrocentric and 46 telocentric chromosomes. There are different on chromosome types with species in this family that has the same of diploid chromosome number as 2n = 48. The asymmetrical karyotype of *Gobiopterus chuno*, only two types of chromosomes (acrocentric, telocentric chromosomes) was observed. The idiogram shows a continuous length gradation of chromosomes. The largest and smallest chromosomes show approximately two-fold size differences.

Nucleolar Organizer region (NOR) is a pervasive phenomenon in both vertebrates and invertebrates especially in teleost fishes (Caputo, 1998). It was not sex-linked and was present in both males and female. The NOR positions of *G. chuno* are the regions adjacent to the telomeres of short arms of the chromosome pair 2 (two NOR-bearing chromosomes). It is different from *Ba. soporator* (Lima-Filho *et al*., 2012) and *Gobius paganellus* (Caputo, 1998) that four NOR-bearing chromosomes were presented. Normally, most fishes have only one pair of small NOR (single NOR) on chromosomes. However, some fishes have more than two NORs, which may be caused by the translocation between some part of the chromosome having a NOR and another chromosome (Nagpure *et al*., 2006). The localization of NOR sites is an important tool in certain studies, such as those on evolution and cytotaxonomy, and those on gene expression (Galetti, 1998).

Table 2. Review of cytogenetic reports in the family Oxudercidae with 2n=48

Species	2n	m	sm	a	t	NF	Reference
Bathygobius soporator	48	2	6	40	-	56	Lima-Filho *et al*. (2012)
Bathygobius fuscus	48	-	-	-	48	48	Arai and Kobayasi (1973)
Gobius niger	48	2	6	8	32	56	Cataudella (1973)
Bostrichthys sinensis	48	2	2	44	-	52	Arai *et al*. (1974)
Gobius paganellus	48	-	2	46	-	50	Caputo *et al*. (1997)
Gobiopterus chuno	**48**	**-**	**-**	**2**	**46**	**50**	Present study

Remarks: 2n = diploid chromosome number, NF = fundamental number, m = metacentric chromosome, sm = submetacentric chromosome, a = acrocentric chromosome, t = telocentric chromosome, NOR = nucleolar organizer region, and - = not available

5. Conclusion

The present study is the first report on the karyotype of *G. chuno*. The number of diploid chromosomes (2n) was found as 48 chromosomes. The fundamental number (NF) was 50 in both sexes. The karyotype consisted of two larges acrocentric, 24 larges telocentric, 20 mediums telocentric, and 2 small telocentric chromosomes. Positions of Nucleolar Organizer region (NORs) are the regions adjacent to the telomeres of short arms of the chromosome pair 2. The karyotype formula for *G. chuno* is as follows:

$$2n\ (\text{diploid})\ 48 = L_a{}^{2}+Lt_{24}+Mt_{20}+St_{2}.$$

6. Acknowledgement

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