แคริโอไทป์และอิดิโอแกรมของปลาก้าง (*Channa gachua*) จากจังหวัดเพชรบูรณ์ด้วยเทคนิคการย้อมสีแบบธรรมดาและแถบสีแบบนอร์

Karyotype Analysis and Idiogram in the *Channa gachua* from Phetchabun Province by Conventional Staining and Ag-NOR Banding Techniques

สุมาลี พิมพันธุ์[1*] สุรเชษฐ เอี่ยมสำอาง[1] และกาญจน์ คุ้มทรัพย์[2]

Sumalee Phimphan[1*], Surachest Aiumsumang[1] and Kan Khoomsab[2]

[1]สาขาวิชาชีววิทยา คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยราชภัฏเพชรบูรณ์ 67000

[2]สาขาวิชาวิทยาศาสตร์ศึกษา คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยราชภัฏเพชรบูรณ์ 67000

[1]Biology Program, Faculty of Science and Technology, Phetchabun Rajabhat University, Phetchabun, 67000

[2]Education Science Program, Faculty of Science and Technology, Phetchabun Rajabhat University, Phetchabun, 67000

*Corresponding author, E-mail: joodoof@gmail.com

บทคัดย่อ

การวิเคราะห์แคริโอไทป์และอิดิโอแกรมของปลาก้าง (*Channa gachua*) จากอำเภอเขาค้อ จังหวัดเพชรบูรณ์ ใช้ตัวอย่างปลาเพศผู้และเพศเมียอย่างละ 10 ตัว เตรียมโครโมโซมโดยวิธีทางตรงจากไต ย้อมสีโครโมโซมแบบธรรมดาและแถบสีแบบนอร์ ผลการศึกษาพบว่าปลาก้างมีจำนวนโครโมโซมดิพลอยด์เท่ากับ 76 แท่ง มีจำนวนโครโมโซมพื้นฐานเท่ากับ 114 ทั้งเพศผู้และเพศเมีย ตรวจไม่พบความแตกต่างของโครโมโซมเพศในปลาเพศผู้และเพศเมีย แคริโอไทป์ประกอบด้วยโครโมโซมเมทาเซนทริกขนาดใหญ่ 14 แท่ง เมทาเซนทริกขนาดกลาง 4 แท่ง ซับเมทาเซนทริกขนาดใหญ่ 8 แท่ง ซับเมทาเซนทริกขนาดกลาง 2 แท่ง อะโครเซนทริกขนาดใหญ่ 8 แท่ง อะโครเซนทริกขนาดกลาง 2 แท่ง เทโลเซนทริกขนาดกลาง 36 แท่ง และเทโลเซนทริกขนาดเล็ก 2 แท่ง ตำแหน่งโครโมโซมเครื่องหมายนอร์บนแขนข้างสั้นบริเวณปลายเทโลเมียร์ของโครโมโซม จำนวน 2 ตำแหน่ง ปลาก้างมีสูตรแคริโอไทป์ ดังนี้ $2n\ (76) = L^{m}_{14} + M^{m}_{4} + L^{sm}_{8} + M^{sm}_{2} + L^{a}_{8} + M^{a}_{2} + M^{t}_{36} + S^{t}_{2}$

คำสำคัญ: ปลาก้าง แคริโอไทป์ โครโมโซม

Abstract

Karyotype analysis and idiogram of dwarf snakehead (*Channa gachua*) were studied from Khao Kho Distric, Phetchabun Province. Kidney cell samples were taken from ten male and ten female fishes. Mitotic chromosome preparations were conducted using standard protocol. Conventional staining and Ag–NORs banding techniques were applied to stain the chromosomes. The results showed that the diploid chromosome number of *C. gachua* was 2n = 76 and the fundamental number (NF) was 114 in both males and females. No heteromorphic sex chromosomes were found between male and female. Their karyotypes consisted of 14 large metacentric, 4 medium metacentric, 8 large submetacentric, 2 medium submetacentric, 8 large acrocentric, 2 medium acrocentric, 36 medium telocentric and 2 small telocentric chromosomes. The present study of NOR–bearing chromosome

as 2 regions, which showed clearly observable NOR. The karyotype formula is as follows: 2n (76) = $L^{m}_{14} + M^{m}_{4} + L^{sm}_{8} + M^{sm}_{2} + L^{a}_{8} + M^{a}_{2} + M^{t}_{36} + S^{t}_{2}$

Keywords: *Channa gachua*, karyotype, chromosome

Introduction

The Channidae family (Actinopterygii, Perciformes) comprises 2 genera (*Channa* and *Parachanna*) and 29 recognized species (Nelson, 2006). There are 7 species found in Thailand (Courtenay and Williams 2004, Vidthayanon 2005). *Channa gachua* is commonly known as dwarf snakehead (Figure 1) (Hamilton, 1822). Dwarf snakeheads are common element of Southeast Asia's freshwater ichthyofauna. They can be found in all habitats, from mountain streams to polluted ponds. They are esteemed food fish for their tasty and firm, muscular flesh, and provide important subsistence fisheries in rural areas. Due to their ability to breathe atmospheric air and can tolerate very stagnant, poorly oxygenated, turbid and even very foul water (Rahman 1989).

There have only been few cytogenetic studies on the *C. gachua*. They showed a different diploid number (2n) and fundamental number (NF) such as 2n=78, NF=102 (Nayyar, 1966; Manna and Prasad, 1973), 2n=78, NF=104 (Sharma and Agarwal, 1981; Banerjee *et al*., 1988), 2n=112 (Donsakul and Magtoon, 1991), 2n=52, NF=74 (Kumar *et al*., 2013), 2n=104, NF=112 (Cioffi *et al*., 2015). However, variations on chromosome number and morphology can be found between different populations of the same species or among different individuals of the same population and even in different cells of the same specimen (Diniz and Bertollo, 2006).

For the present study, karyotypes and other chromosomal markers such as these Ag-stained nucleolar organizer region (Ag-NOR) of *C. gachua* population from Khao Kho District, Phetchabun Province, Thailand were reported. This study was undertaken to investigate the genetic variation among chromosome of *C. gachua* using cytogenetic as well as chromosome markers to increase understanding of the taxonomy and to generate information useful for evolutionary and conservation genetics.

Materials and methods

Sample collection

Twenty males and females of *C. gachua* were obtained from Khao Kho District, Phetchabun Province, Thailand. The fish were transferred to laboratory aquaria and were kept under standard condition for 3 days prior to the experiments.

Chromosome preparation

Chromosomes were directly prepared *in vivo* follow the standard protocols (Supiwong *et al*. 2009; Cioffi *et al*., 2015). Briefly, 0.05% colchicine was injected to fish's abdominal (1 ml:100 g body weight) and left it for 1 hour. Kidney tissue were removed and cut into small pieces then gently mixed with hypotonic solution (0.075 M KCl). After discarding all large piece tissues, 7 mL of cell sediments as transferred to centrifuge tube and incubated for 30 minutes. The supernatant was discarded after centrifugation at 2,000 rpm for 10 minutes. For cell fixation, cell pellet was resuspended with 7 mL of Canoy's fixative solution. The supernatant was removed after centrifugation at 2,000 rpm for 10 minutes. The fixation was repeated until the supernatant was clear. Finally, the cell pellet was resuspended with 1 mL fixative solution. The cell suspension was dropped onto a clean and cold slide by micropipette followed by air-dry technique.

Chromosome staining

Conventional staining was performed by using 20% Giemsa's solution for 30 minutes (Phimphan et al., 2015). Ag-NOR banding was carried out by adding 2 drops of 50% silver nitrate and 2% gelatin on slides, respectively (Howell and Black, 1980). The slides were then sealed with cover glasses and incubated at 60^{o}C for 5 minutes. After that the slides were soaked in distilled water until the cover glasses were separated.

Karyotype analysis

Chromosome counting was performed on mitotic metaphase cells under light microscope (Table 1). Twenty clearly observable and well spread chromosomes plates cells of each male and female were selected and photographed. The metaphase figures were analyzed according to the chromosome classification by Turpin and Lejeune (1965). 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. The fundamental number, number of chromosome arm or NF, is calculated by assigning a value of 2 to metacentric, submetacentric and acrocentric chromosomes and 1 to telocentric chromosome.

Figure 1 Generral charecteristics of the dwarf snakehead (*C. gachua*), scale bar indicates 3 cm.

Table 1 Chromosome number of *C. gachua* from 100 metaphases.

Chromosome number (2n)	Cells
68	3
69	1
70	1
72	3
73	3
74	6
75	7
76	54
77	7
78	7
79	4
80	3
81	1

Results and discussion

The diploid chromosome number (2n) of *C. gachua* was 76 chromosomes. 2n was count from 100 metaphases (Table 1). This differs from previous studies which is 72 (Nayyar 1966), Manna and Prasad 1973, Sharma and Agarwal 1981, 78 Banerjee *et al*. 1988, 112 Donsakul and Magtoon 1991, 52 Kumar *et al*. 2013 and 104 Cioffi *et al*. 2015 as shown in Table 2. The differences may be attributed to the specimens utilized in the present work is collected from Khao Kho District, Phetchabun Province, which were different from those utilized by the literature (different population).

Intra-specific variations of the 2n and NF in *C. gachua* in some ways suggest processes of pericentric inversions between chromosomes during chromosomal evolution. Furthermore, the differences may be attributed to different inter-populational variation in this species (Kirpichnikov, 1981).

The fundamental number (NF) of *C. gachua* was in this study 114 in both males and females. The comprise with previous reported showed the different NF as those found 102 (Nayyar, 1966; Manna and Prasad, 1973), 104 (Sharma and Agarwal, 1981; Banerjee *et al*., 1988) , 74 (Kumar *et al*, 2013), 112 (Cioffi *et al*., 2015) (Table 2).

No differences between male and female karyotypes were observed (Figure 2) indicate no cytologically sex chromosome. The karyotype consisted of 18 metacentric, 10 submetacentric, 10 acrocentric, 36 medium telocentric and 2 acrocentric chromosomes. It differs from all previous report of Nayyar (1966) that found the karyotype of *C. gachua* consisting of 12 metacentric, 12 submetacentric and 54 telocentric (acrocentric) chromosomes. Sharma and Agarwal (1981) reported the karyotype consists of 10 metacentric, 8 submetacentric, 8 subtelocentric and 52 telocentric chromosomes. Banerjee *et al*. (1988) reported that the karyotype of this species composes of 16 metacentric, 10 submetacentric and 52 telocentric chromosomes. Donsakul and Magtoon (1991) reported that karyotypes of *C. gachua* consist of 2 metacentric, submetacentric and 53 telocentric (acrocentric) chromosomes, while Manna and Prasad (1973) reported the chromosome analysis composing of 12 metacentric, 12 submetacentric, 4 subtelocentric and 50 telocentric chromosomes. Kumar *et al*. (2013) reported the karyotype comprised 12 metacentric, 10 submetacentric, 14 subtelocentric and 16 telocentric chromosomes, then in 2015 Cioffi *et al*. report that the karyotype composes of 8 acrocentric and 96 telocentric chromosomes (Table 2). The length of short arm chromosome, length of long arm chromosome, length of total arm chromosomes, relative length, centromeric index, standard deviation of RL and CI, size and type of chromosome are presented in table 3 and showed that the mean value of relative length ranged from 0.039±0.001 to 0.023±0.001. The present study karyotype formula for *C. gachua* is as follows: 2n (76) = L^{m}_{14} + M^{m}_{4} + L^{sm}_{8} + M^{sm}_{2} + L^{a}_{8} + M^{a}_{2} + M^{t}_{36}+ S^{t}_{2}

Table 2 Comparative karyological variation in *C. gachua*

Species	2n	NF	Karyotype formula	References
C. gachua	78	102	12m + 12sm + 54t/a	Nayyar (1966)
	78	104	10m + 8sm + 8st + 52t	Sharma and Agarwal (1981)
	78	104	16m + 10sm + 52t	Banerjee *et al*. (1988)
	112	-	2m + 1sm + 53t(a)	Donsakul and Magtoon (1991)
	78	102	12m + 12sm + 4st + 50t	Manna and Prasad (1973)
	52	74	12m + 10sm + 14st + 16t	Kumar *et al*. (2013)
	104	112	8a+96t	Cioffi *et al*. (2015)
	76	**114**	**18m+10sm+10a+38t**	**Present study**

Remarks: 2n = diploid chromosome number, NF = fundamental number, m = metacentric chromosome, sm = submetacentric chromosome, a = acrocentric chromosome, t = telocentric chromosome, and - = not available.

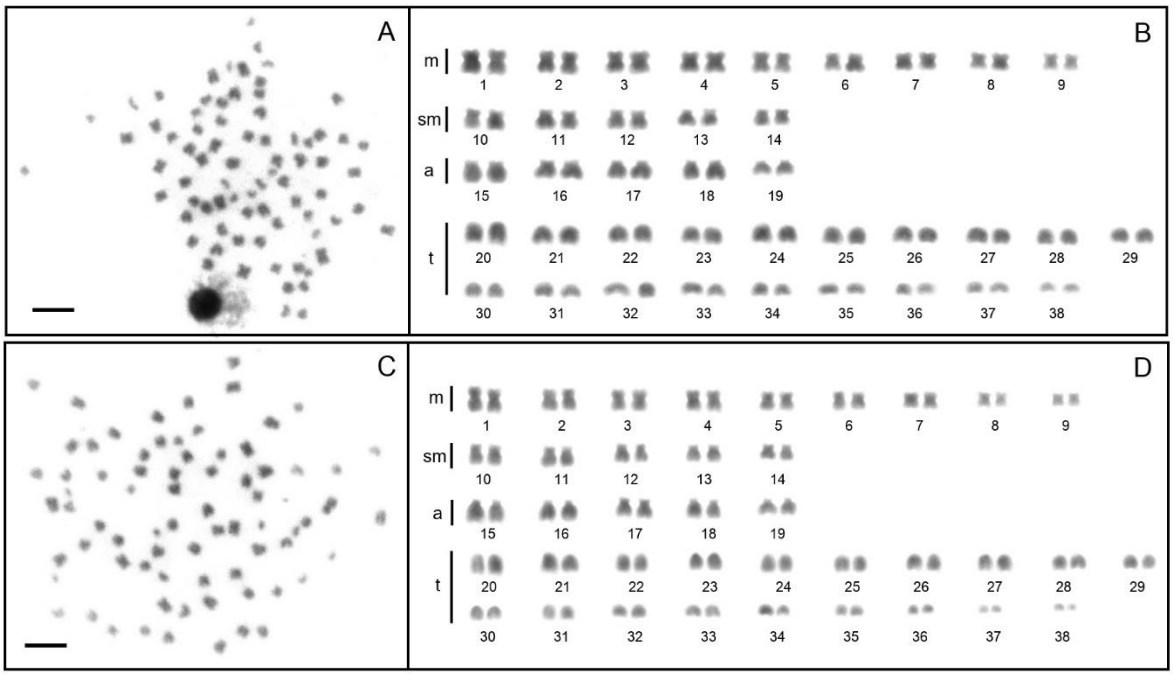

Figure 2 Metaphase chromosome plate of dwarf snakehead (*C. gachua*) male (A), female (C) and karyotype male (B), female (D), 2n (diploid) = 76 by conventional staining, scale bar indicates 2 μm. There is no observation of strange size chromosome related to sex.

The development of silver staining technique to detect metaphase chromosome sites of NORs has greatly facilitated comparative studies of NORs variation. Silver staining of NORs is considered as one of the standard banding methods. The staining displayed terminal Ag-NOR chromosome as 2 regions which are on submetacentric chromosomes (Figure 3). It differs from Cioffi *et al*. (2015) which has interstitial Ag-NOR as 4 regions. This is the first demonstrated Ag-NOR bands in the dwarf snakehead fishes from Northern, Thailand. The location of NOR presented on different chromosome pair in the karyotype implied that those can be a chromosome marker for each fish species. The chromosome length and relative chromosome length were used to construct the standardized idiogram. The idiogram clearly revealed the chromosomes to occur in different size groups of large, medium and small (Figure 4). Such studies are useful in resolving taxonomic ambiguities among closely related fish (Genus *Channa*) species and can also throw light on karyoevolution and speciation of the fish species.

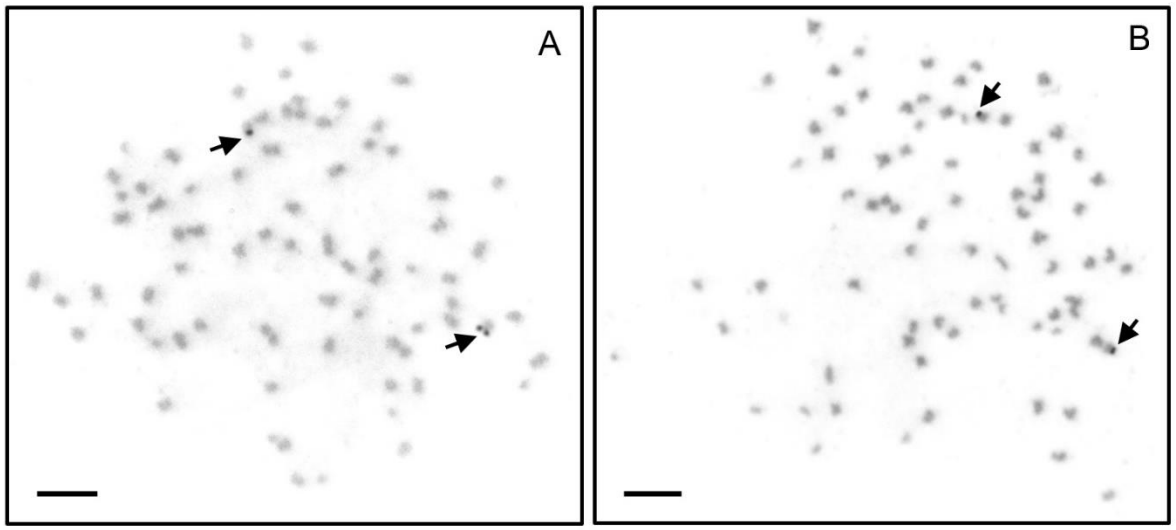

Figure 3 Metaphase chromosome plates of male (A) and female (B) dwarf snakehead (*C. gachua*), 2n (diploid) = 76 by Ag-NORs staining technique, scale bars indicate 2 μm. The clearly observable nucleolar organizer regions/NORs, (arrows).

Table 3 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosomes (LT), relative length (RL), centromeric index (CI) from 20 metaphases of male and female dwarf snakehead (*C. gachua*), 2n (diploid) = 76.

Chromosome pair	Ls (μm)	Ll (μm)	LT (μm)	CI±SD	RL±SD	Chromosome Size	Chromosome Type
1	1.630	2.266	3.896	0.582±0.005	0.039±0.001	Large	metacentric
2	1.438	2.092	3.530	0.593±0.021	0.035±0.001	Large	metacentric
3	1.561	2.017	3.577	0.566±0.024	0.035±0.001	Large	metacentric
4	1.413	1.896	3.309	0.575±0.023	0.033±0.000	Large	metacentric
5	1.229	1.847	3.076	0.593±0.033	0.030±0.000	Large	metacentric
6	1.287	1.704	2.990	0.571±0.021	0.030±0.001	Large	metacentric
7	1.168	1.534	2.702	0.565±0.019	0.027±0.002	Large	metacentric
8	1.249	1.345	2.595	0.519±0.006	0.026±0.001	Medium	metacentric
9	1.062	1.284	2.347	0.544±0.034	0.023±0.001	Medium	metacentric
10	1.195	2.013	3.209	0.628±0.003	0.032±0.001	Large	submetacentric
11	1.160	2.029	3.189	0.637±0.017	0.032±0.000	Large	submetacentric
12	1.068	1.854	2.921	0.634±0.001	0.029±0.001	Large	submetacentric
13	1.011	1.758	2.769	0.636±0.010	0.027±0.000	Large	submetacentric
14	0.853	1.498	2.351	0.639±0.020	0.023±0.001	Medium	submetacentric
15	0.789	2.829	3.618	0.781±0.009	0.036±0.000	Large	acrocentric
16	0.856	2.382	3.238	0.736±0.009	0.032±0.003	Large	acrocentric
17	0.828	2.119	2.947	0.718±0.029	0.029±0.001	Large	acrocentric
18	0.850	2.233	3.083	0.723±0.015	0.030±0.000	Large	acrocentric
19	0.679	1.806	2.485	0.727±0.004	0.025±0.002	Medium	acrocentric
20	0.000	3.420	3.420	1.000±0.000	0.034±0.001	Medium	telocentric
21	0.000	3.070	3.070	1.000±0.000	0.030±0.001	Medium	telocentric
22	0.000	2.917	2.917	1.000±0.000	0.029±0.000	Medium	telocentric
23	0.000	2.688	2.688	1.000±0.000	0.027±0.000	Medium	telocentric
24	0.000	2.858	2.858	1.000±0.000	0.028±0.001	Medium	telocentric
25	0.000	2.646	2.646	1.000±0.000	0.026±0.000	Medium	Telocentric
26	0.000	2.537	2.537	1.000±0.000	0.025±0.000	Medium	telocentric
27	0.000	2.414	2.414	1.000±0.000	0.024±0.001	Medium	telocentric
28	0.000	2.505	2.505	1.000±0.000	0.025±0.000	Medium	telocentric
29	0.000	2.176	2.176	1.000±0.000	0.021±0.001	Medium	Telocentric
30	0.000	2.178	2.178	1.000±0.000	0.022±0.001	Medium	telocentric

Table 3 Continue.

Chromosome pair	Ls (μm)	LI (μm)	LT (μm)	CI±SD	RL±SD	Chromosome Size	Chromosome Type
31	0.000	2.031	2.031	1.000±0.000	0.020±0.000	Medium	telocentric
32	0.000	2.050	2.050	1.000±0.000	0.020±0.000	Medium	telocentric
33	0.000	1.879	1.879	1.000±0.000	0.019±0.002	Medium	telocentric
34	0.000	1.840	1.840	1.000±0.000	0.018±0.000	Medium	telocentric
35	0.000	1.683	1.683	1.000±0.000	0.017±0.002	Medium	telocentric
36	0.000	1.657	1.657	1.000±0.000	0.016±0.001	Medium	telocentric
37	0.000	1.392	1.392	1.000±0.000	0.014±0.001	Medium	telocentric
38	0.000	1.377	1.377	1.000±0.000	0.014±0.002	Small	telocentric

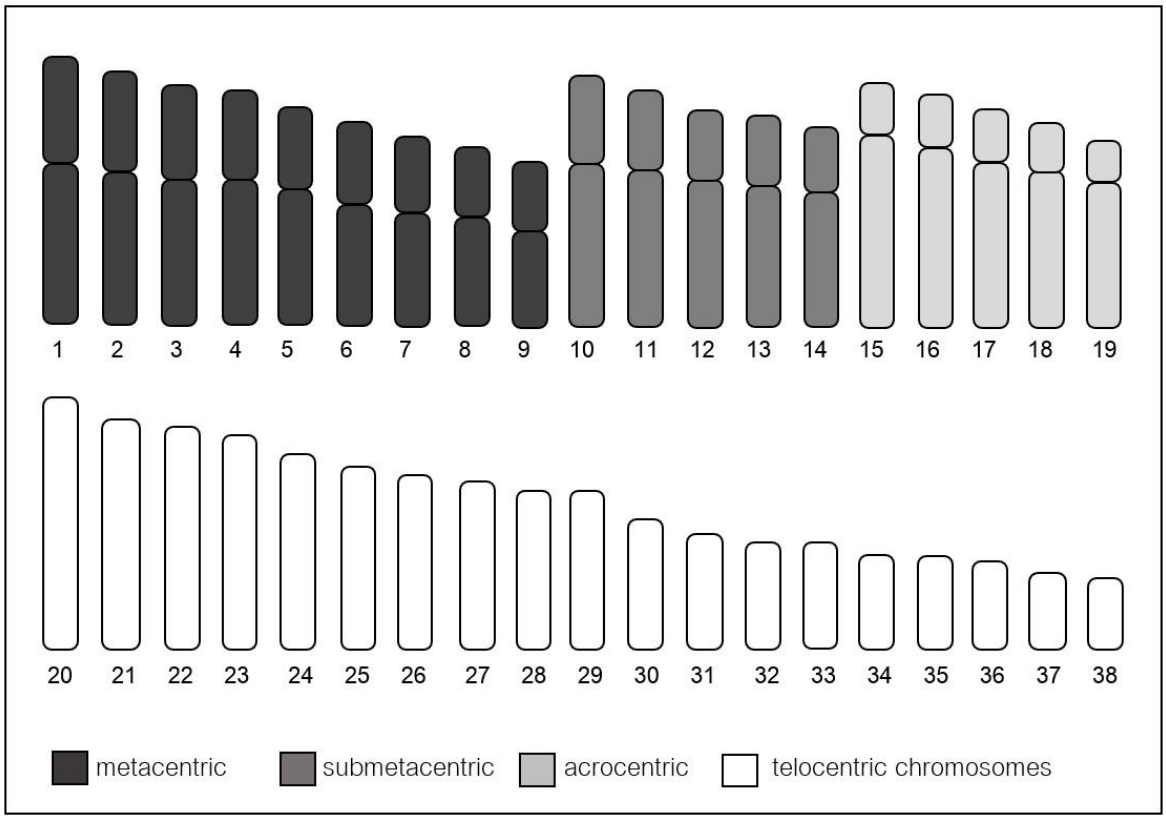

Figure 4 Standard idiogram showing length and shape chromosome of dwarf snakehead (*C. gachua*) demonstrated the haploid set (n=38) by conventional staining techniques.

Acknowledgments

This work was supported by research grants of new researcher budget year 2019, Phetchabun Rajabhat University and grateful to Prof. Dr. Alongklod Tanomtong for suggestion.

References

Banerjee, S.K., Mishra, K.K., Banerjee, S. and Ray-Chaudauri, S.P. 1988. Chromosome number, genome sizes, cell volumes and evolution of snake-head fish (family Channidae) Fish Biology 33(5): 781–789.

Cioffi, B.M., Bertollo, L.N.C, Villa, M.A., Oliveira, E.A., Tanomtong, A., Yano, C.F., Supiwong, W. and Chaveerach, A. 2015. Genomic Organization of Repetitive DNA Elements and Its Implications for the Chromosomal Evolution of Channid Fishes (Actinopterygii, Perciformes). PLOS one DOI:10.1371/journal.pone.0130199.

Courtenay, W. R. and Williams, J. D. 2004. Snakeheads-A Biological Synopsis and Risk Assessment. U.S. Geological Survey, Denver.

Diniz, D. and Bertollo, L.A.C. 2006. Intra- and inter-individual chromosome variation in *Hoplerythrinus unitaeniatus* (Pisces, Erythrinidae). A population from the Brazilian São Francisco river basin. Genetics Molecular Biology 29: 453–458.

Donsakul, T. and Magtoon, W. 1991. Chromosome study on five species of channid fishes (Channa, family Channidae), from Thailand. Proceedings of the 29th Kasetsart University Annual Conference: Animal Science, Veterinary Science, Aquaculture; 1991 Feb 4–7; Kasetsart University, Bangkok (Thailand). p. 561–574. Published by FAO and NACA at Bangkok.

Howell, W.M. and Black, D.A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36: 1014–1015.

Kirpichnikov, V.S. 1981. Genetic Bases of Fish Selection. Springer Verlag, Berlin, Heidelberg, New York.

Kumar, R., Kushwaha, B., Nagpure, S.N., Behera, K.B. and Lakra, S.W. 2013. Karyological and molecular diversity in three freshwater species of the genus *Channa* (Teleostei, Perciformes) from India. Caryologia 66(2): 109–119.

Manna, G.K. and Prasad, R. 1973. Chromosome in three fishes (*Channa*). Nucleus 16(3): 150–157.

Nayyar, R.P. 1966. Karyotype studies in thirteen species of fishes. Genetica 37(1): 78–92.

Nelson, J.S. 2006. Fishes of the World. 4th ed. New York: John Wiley & Sons.

Phimphan, S., Supiwong, W. and Khakhong, S. 2015. Standardized Karyotype and Idiogram of False Clown Anemonefish, *Amphiprion ocellaris* Cuvier, 1830 (Amphiprioninae, Perciformes). Journal of Fisheries Technology Research 9(1): 43-52.

Rahman, A.K.A. 1989. Freshwater fishes of Bangladesh. Published by Zoological Society of Bangladesh. Department of Zoology, University of Dhaka. Bangladesh 364 p.

Sharma, O.P. and Agrawal, A. 1981. Cytogenetic studies in two species of genus *Channa*. Perspective Cytology Genetics 3: 57–61.

Supiwong, W., Jearranaiprepameand, P. and Tanomtong, A. 2009. A new report of karyotype in the chevron snakehead fish, *Channa striata* (Channidae, Pisces) from Northeast of Thailand. Cytologia 74(3): 317–322.

Turpin, R. and Lejeune, J. 1965. Les Chromosomes humans. Cornell University: Gauthier-Villars.

Vidthayanon, C. 2005. Handbook of Freshwater Fish. 2nd ed. Sarakadee press, Bangkok.