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Research Article

Cytogenetic study of the redtail bagrid (*Hemibagrus wyckioides*) using conventional staining and NOR-banding techniques

Nuntaporn Getlekha[1] and Kamika Sribenja[2*]

[1] *Department of Biology, Faculty of Science and Technology, Muban Chombueng Rajabhat University, Ratchaburi Province, 70150, Thailand*
[2] *Department of General Science, Faculty of Science and Technology, Muban Chombueng Rajabhat University, Ratchaburi Province, 70150, Thailand*
* *Corresponding author: kamikasri@mcru.ac.th*

Abstract - A karyotype and standard idiogram analysis of the redtail bagrid (*Hemibagrus wyckioides*) from the Mae Klong River Basin, Kanchanaburi province, was conducted using samples from seven males and eight females. Chromosomes were directly extracted from kidney tissue and stained with Giemsa stain and NOR-banding. The results showed that the redtail bagrid had 58 diploid chromosomes (2n) and 102 fundamental chromosomes (NF). The karyotype included 6 large metacentric, 8 large submetacentric, 6 large acrocentric, 16 medium metacentric, 4 medium submetacentric, 4 medium acrocentric, 8 medium telocentric, and 6 small telocentric chromosomes, respectively. No chromosomal differences were observed between the sexes. NOR staining identified one pair of NOR loci located at the telomeric region of the short arm of a metacentric chromosome. This study is the first cellular genetic analysis of the redtail bagrid and provides valuable data for the taxonomic classification of bagrid catfish. Additionally, the findings can serve as a foundation for the conservation of catfish genetic resources and future studies on chromosomal evolution. The karyotype formula of *H. wyckioides* is 2n (58) = $L^{m}_{6}+L^{sm}_{8}+L^{a}_{6}+M^{m}_{16}+M^{sm}_{4}+M^{a}_{4}+M^{t}_{8}+S^{t}_{6}$

Keywords: *Hemibagrus wyckioides*, chromosome, karyotype, idiogram

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1. Introduction

The Mae Klong River Basin is located on the western side of Thailand, encompassing both the Klong River and the Phetchaburi River. It originates in the Tenasserim Mountain Range and is formed by the confluence of the Khwae Yai and Khwae Noi Rivers, which flow from the western forests and merge in Mueang District, Kanchanaburi Province. The river flows through Ratchaburi Province and eventually empties into the sea at Mueang District, Samut Songkhram Province. The Phetchaburi River begins in Tha Yang District, Phetchaburi Province, flows through the province, and empties into the Gulf of Thailand in Ban Laem District, Phetchaburi Province. While the fish species in this basin are largely similar to those in the Chao Phraya River Basin, many endemic species, particularly from the upstream areas, are also found here. Major tributaries include the Phachi River. The fish species in the Mae Klong River Basin share similarities with those found in the Pahang River of Malaysia, suggesting the two rivers may have been connected in the past. Over 200 species of fish inhabit the Mae Klong River Basin (Saenjundaeng, 2014; Phanitwong, 2020).

The redtail bagrid (*Hemibagrus wyckioides*) belongs to the *Hemibagrus* genus. It is characterized by its long body, flat head, distinctively long barbels, and red tail. This species is a large, benthic predator that can grow up to 2 meters in length and weigh as much as 50 kilograms. It inhabits major rivers and large reservoirs (Phanitwong, 2020). The catfish is known for its delicious meat, which has few bones and lacks a fishy odor, making it a highly valued food source. It is an important economic fish and is commonly consumed (Suvarnaraksha & Utsugi, 2023). Additionally, it is a popular game fish. Nowadays, it is extensively bred through artificial insemination, both for meat production and ornamental purposes (Phanitwong, 2020).

The study of chromosomes is a fascinating approach because chromosomes play a crucial role in transmitting genetic traits of living organisms. Any changes in the structure or number of chromosomes can impact the transmission of these traits, leading to variations in the expression of living organisms. These changes can influence their development and evolution (Kamphiranon, 2003).

Building on the previous work, the researchers are interested in investigating the genetic diversity of redtail bagrid from the Mae Klong River Basin, Kanchanaburi Province, by analyzing their karyotypes and developing standard idiograms for the species in this region. This will contribute valuable baseline information about the organisms within this specific system. Furthermore, the Mae Klong River Basin remains an under-researched area, with limited existing data on the subject. The findings from this study will not only support the classification of catfish species but also provide essential information for the conservation of catfish genetic resources in the wild and further studies on chromosome evolution.

2. Materials and methods

2.1 Sampling collection

Fifteen samples of the redtail bagrid (*Hemibagrus wyckioides*) were collected from the Mae Klong River Basin, Kanchanaburi Province (Figure 1), using various fishing equipment, including fish nets, fish traps, fishing rods, and swings. The collected samples (seven males and eight females) were placed in plastic bags filled with oxygen, securely sealed and transported to the laboratory, Science and Applied Science Center Building, Faculty of Science and Technology, Muban Chombueng Rajabhat University. The trials used clove oil as an anesthetic prior to animal slaughter in an effort to minimize pain for the animals. The Muban Chombueng Rajabhat University Ethics Committee and the RGJ Committee,

under reference number U1-04484-2559, approved the procedure. They were classified according to Nelson (2006). This classification was supported by relevant academic references, including works by Vidthayanon (2008), Saenjundaeng (2014), Phanitwong (2020) and Suvarnaraksha and Utsugi (2023).

Figure 1. Characteristics of the redtail bagrid (*Hemibagrus wyckioides*). Scale bar 3 cm.

2.2 Cytogenetic investigation

Chromosomes were prepared using the direct method, adapted from Sumner (1990) and Getlekha et al. (2022). Seven male and eight female fish were injected with a 0.02% colchicine solution (1 ml/100 g body weight) into the ventral cavity and left in water with constant oxygenation for 1 hour. The kidney tissue was surgically removed and immersed in a hypotonic solution (0.075 M KCl). The cell sediment was chopped and incubated in a 15 ml centrifuge tube containing the hypotonic solution for 30 minutes.

Cells were then prefixed by slowly adding a very cold methanol: acetic acid fixative (3:1 ratio) to the centrifuge tube, shaking it until the volume reached 7 ml. The tube was centrifuged at 2,500 rpm for 8 minutes, and the supernatant was aspirated. The cells were fixed again by slowly adding the cooled fixative, shaking to mix the pellet with the fixative, and centrifuging at the same speed for another 8 minutes. The supernatant was aspirated again and the process was repeated until a cloudy white cell supernatant was obtained.

After aspirating the cell fixative, leaving approximately 3-4 ml, the remaining solution was shaken to mix. About 0.2 ml of the cell supernatant was then transferred to a clean, dry slide, with 2 drops applied per slide. The slide was placed on a 75 °C heater to dry. After drying, the slide was stained with 20% Giemsa stain and NOR-banding. Finally, the slide was examined and photographed using a light microscope with a 1,000x magnification. The preparation of the karyotype and idiogram was adapted from the method of Chaiyasut (1989). Metaphase chromosome photographs were selected based on criteria such as well-distributed chromosomes, appropriate length, complete chromosome count, and clearly observable shapes. This research used basic statistical analysis, including mean and standard deviation of chromosome length. The lengths of chromosomes from 20 cells of both male and female fish were measured, including the short arm length (Ls), long arm length (Ll), total length (LT), centromeric index (CI), and relative length (Rl), to identify the type and size of the chromosomes (Table 1). A standard idiogram was then created by using the Adobe Illustrator program, which classified the chromosomes based on their shape and size according to the measured lengths.

3. Results and discussion

This study presents the first karyotype analysis of *Hemibagrus wyckioides* in the area. The results revealed that the catfish has a diploid chromosome number (2n) of 58 chromosomes and a fundamental chromosome number (NF) of 102, which represents the number of chromosome arms, in both males and females. The karyotype is classified as asymmetrical, meaning it includes four types of chromosomes: metacentric, submetacentric, acrocentric, and telocentric (Figure 2). The karyotype consists of 44 bi-armed chromosomes (metacentric, submetacentric

and acrocentric) and 14 mono-armed chromosomes (telocentric).

Based on the preparation of karyotypes and standard idiograms, the chromosomes of *Hemibagrus wyckioides* can be classified into three size categories: large chromosomes, with an average length greater than 0.553 micrometers, including chromosome pairs 1-3, 12-15, 18-20; medium chromosomes, with an average length ranging from 0.380 to 0.553 micrometers, including chromosome pairs 4-11, 16-17, 21-26; and small chromosomes, with a mean length of less than 0.380 micrometers, represented by chromosome pairs 27-29 (Figures 3 and Table 1). The karyotype consists of 6 large metacentric, 8 large submetacentric, 6 large acrocentric, 16 medium metacentric, 4 medium submetacentric, 4 medium acrocentric, 8 medium telocentric, and 6 small telocentric chromosomes (Figures 2 and 3). The Ag-NOR sites are located in the telomeric region of the short arm of a metacentric chromosome. No chromosomal differences were observed between the sexes. The karyotype formula of *Hemibagrus wyckioides* can be written as follows: 2n (58) = $L^{m}_{6}+L^{sm}_{8}+L^{a}_{6}+M^{m}_{16}+M^{s-m}_{4}+M^{a}_{4}+M^{t}_{8}+S^{t}_{6}$

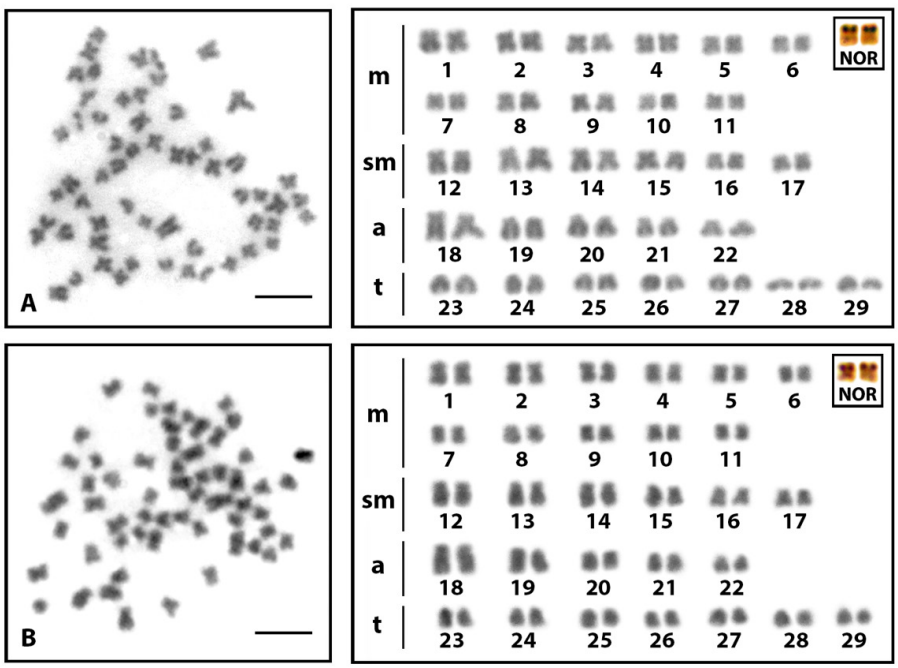

Figure 2. Metaphase cells and karyotype of male (a) and female (b) catfish (*Hemibagrus wyckioides*, 2n=58) using the conventional staining technique. Scale bar, 2 μm.

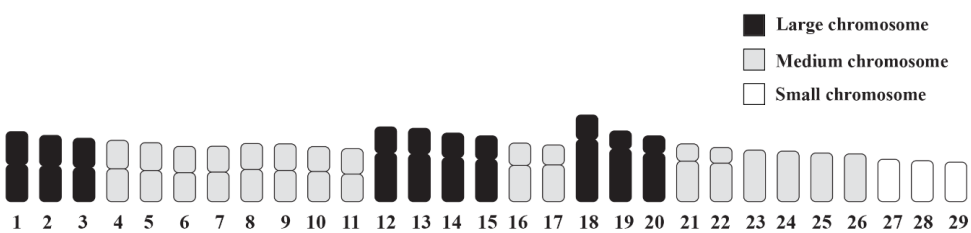

Figure 3. Standard idiogram of catfish (*Hemibagrus wyckioides*, 2n=58) using the conventional staining technique.

Table 1. Mean length of short chromosome arm (Ls), long chromosome arm (Ll), length of each chromosome pair (LT) in micrometers, centromeric index (CI), relative length (RL), and standard deviation (SD) of CI and RL of catfish (*Hemibagrus wyckioides*, 2n=58).

Pairs	Ls	Ll	LT	CI ± SD	RL ± SD	Size	Type
1	0.290	0.324	0.614	0.529±0.008	0.041±0.002	Large	Metacentric
2	0.268	0.313	0.581	0.542±0.018	0.039±0.001	Large	Metacentric
3	0.258	0.298	0.556	0.538±0.016	0.037±0.001	Large	Metacentric
4	0.250	0.287	0.537	0.536±0.027	0.036±0.002	Medium	Metacentric
5	0.245	0.272	0.518	0.529±0.019	0.035±0.001	Medium	Metacentric
6	0.231	0.253	0.483	0.525±0.013	0.033±0.001	Medium	Metacentric
7	0.227	0.259	0.486	0.534±0.027	0.032±0.002	Medium	Metacentric
8	0.229	0.281	0.510	0.553±0.019	0.034±0.002	Medium	Metacentric
9	0.233	0.273	0.506	0.542±0.020	0.034±0.001	Medium	Metacentric
10	0.218	0.264	0.482	0.547±0.009	0.032±0.002	Medium	Metacentric
11	0.222	0.242	0.464	0.523±0.010	0.031±0.001	Medium	Metacentric
12	0.230	0.426	0.656	0.653±0.024	0.044±0.001	Large	Submetacentric
13	0.226	0.417	0.643	0.653±0.030	0.042±0.003	Large	Submetacentric
14	0.214	0.387	0.601	0.649±0.025	0.040±0.003	Large	Submetacentric
15	0.206	0.371	0.578	0.648±0.031	0.039±0.001	Large	Submetacentric
16	0.195	0.318	0.514	0.626±0.044	0.035±0.003	Medium	Submetacentric
17	0.175	0.322	0.497	0.656±0.042	0.033±0.002	Medium	Submetacentric
18	0.215	0.545	0.761	0.720±0.030	0.051±0.001	Large	Acrocentric
19	0.158	0.462	0.619	0.743±0.029	0.042±0.003	Large	Acrocentric
20	0.152	0.425	0.576	0.735±0.023	0.039±0.002	Large	Acrocentric
21	0.151	0.355	0.506	0.700±0.015	0.034±0.001	Medium	Acrocentric
22	0.138	0.337	0.475	0.709±0.012	0.032±0.001	Medium	Acrocentric
23	0.000	0.453	0.453	1.000±0.000	0.030±0.002	Medium	Telocentric
24	0.000	0.442	0.442	1.000±0.000	0.029±0.003	Medium	Telocentric
25	0.000	0.426	0.426	1.000±0.000	0.028±0.001	Medium	Telocentric
26	0.000	0.418	0.418	1.000±0.000	0.028±0.001	Medium	Telocentric
27	0.000	0.371	0.371	1.000±0.000	0.024±0.002	Small	Telocentric
28	0.000	0.359	0.359	1.000±0.000	0.024±0.001	Small	Telocentric
29	0.000	0.346	0.346	1.000±0.000	0.023±0.002	Small	Telocentric

Chromosomal data from the *Hemibagrus* genus show that the diploid chromosome number ranges from 48 to 62, with most species having 58 chromosomes. The karyotype analysis of *H. wyckioides* in this study revealed a diploid number (2n) of 58, which is consistent with earlier findings by Donsakul (2001) and Supiwong et al. (2014), but differs in the fundamental

chromosome number (Table 2). Variations in the fundamental number may arise from differences in the chromosome preparation process. For instance, if chromosomes are overly condensed, the type of chromosome might be misclassified, such as identifying an acrocentric chromosome as a telocentric chromosome, which can influence the calculation of the basic chromosome number. Alternatively, the process of chromosome break-and-rejoin (pericentric inversion) involving the centromere can lead to changes in chromosome type, from telocentric (NF=1) to metacentric, submetacentric, or acrocentric (NF=2). These processes may not affect the overall chromosome number but can increase the number of basic chromosomes (Getlekha et al., 2018). Such chromosomal evolution is commonly observed in fish species (Getlekha et al., 2016). Therefore, the karyotype data obtained in this study will significantly contribute to supporting the taxonomic classification of catfish.

Table 2. Comparative of karyotype studies of fish in the genus *Hemibagrus*.

species	2n	NF	Karyotype	References
Hemibagrus guttatus	60	108	20m +12sm +16st +12a	Yu et al. (1989)
H. filamentus	58	104	20m+26sm+12st/a	Supiwong et al. (2014)
H. macropterus	60	108	20m +12sm +16st +12a	Yu et al. (1989)
H. menoda	48	72	12m+12st+24a	Lakra and Rishi (1991)
	58	100	22m+20sm+16a	Das and Khuda-Bukhsh (2007a)
H. menoda menoda	56	108	36m +16sm + 4a	Khuda-Bukhsh et al. (1995)
H. nemurus	58	100	22m+20sm+16a	Barat and Khuda-Bukhsh (1986)
	56	100	20m +14sm +10st +12a	Sharma and Tripathi (1986)
	56	102	32m +14sm +10a	Khuda-Bukhsh et al. (1995)
H. spilopterus	58	94	28m+8sm+20st+2a	Donsakul (2001)
	58	102	18m+26sm+14st/a	Supiwong et al. (2014)
H. wyckii	54	84	16m+14sm+24a	Magtoon and Arai (1988)
	62	106	34m+10sm+8st+10a	Donsakul (2001)
	62	102	14m+26sm+22st/a	Supiwong et al. (2014)
	62	110	22m+18sm+8a+14t	Supiwong et al. (2017)
H. wyckioides	58	92	24m +10sm + 6st +18a	Donsakul (2001)
	58	100	22m+20sm+16st/a	Supiwong et al. (2014)
	58	102	22m +12sm + 10a +14t	Present study

Additionally, reports have indicated that the large catfish genus *Hemibagrus* has a chromosome count of 2n=58, though the number of chromosomes varies among different species, such as *H. filamentus*, *H. menoda*, *H. nemurus*, and *H. spilopterus*. This suggests that these species may have evolved from a common ancestor, leading to the same number of chromosomes.

Ag-NOR sites are the only pair of relevant chromosomal markers shared by all *H. wyckioides* in this study. NORs are chromosomal regions composed of repetitive ribosomal gene sequences (rRNA). Each unit in eukaryotes consists of three genes that code for 18S, 5.8S, and 28S ribosomal RNA (Sharma et al., 2002). Because these chromosomal features are

often species-specific, the number and arrangement of rDNA clusters have been widely used in systematics and phylogenetic studies (Britton-Davidian et al., 2012). The results here are consistent with previous studies on chromosomes containing the nucleolar organizer region (Phimphan et al., 2020). This feature is found in several fish species and other vertebrates (Supiwong et al., 2012, 2013). In this study, no chromosomal differences were observed between the two sexes, which aligns with the findings of Donsakul (2001) and Supiwong et al. (2014). They proposed that fish were among the first vertebrates to evolve, which led to the initial stage of differentiation of sex chromosomes in fish. This may explain why the chromosomes responsible for sex determination could not be identified in the cellular genetic analysis, as the genes for sex determination might be scattered across different chromosomes.

Further research on the origin and development of sex chromosomes in Neotropical fish in Brazil, as reported by Bertollo et al. (2004), also suggests that sex chromosome differentiation is a complex process that is still evolving in some fish species.

From the collection of chromosomal studies on fish in the *Hemibagrus* genus (Table 2), differences were observed in the number of diploid chromosomes, basic chromosomes, and karyotypes. These differences are influenced by the evolutionary patterns of chromosomes, the adaptability of each fish species, and the environmental and habitat conditions of the species.

The study identified three major processes involved in chromosomal rearrangement, which contribute to the diversity of freshwater fish chromosomes: Pericentric inversion, Fusion and Fission. These processes are likely involved in the chromosomal changes observed in various freshwater fish families. Each family may exhibit unique chromosomal characteristics as a result of one or more of these mechanisms (Kasirik, 2014).

The *Hemibagrus* genus exhibits a chromosome number ranging from 2n=48 to 62 chromosomes. This group of fish shows significant changes in their diploid chromosome count compared to the ancestral chromosomes of freshwater fish (2n=50), indicating considerable alterations in their chromosome structure. Most chromosomes in this group are bi-armed chromosomes, which are considered an advanced evolutionary stage. These fish are typically found in large water bodies and reservoirs, environments that can act as geographical barriers, limiting gene transfer and promoting a high level of chromosomal evolution. As a result, the chromosomes of this genus have diverged significantly from those of ancestral freshwater fish, both in number and structure (Maneechot et al., 2016).

These chromosomal changes may confer certain advantages, such as increased survival, better adaptability to new environments, and enhanced reproductive success. Consequently, *Hemibagrus* species can thrive in a variety of habitats, including both still an d flowing waters. The rapid chromosomal changes lead to genetic diversification, and new species often exhibit similar morphology to their ancestors, making them difficult to classify based on morphological features alone. This highlights the need for cellular genetic data to better understand chromosomal evolution and the patterns of change that drive the evolution of living organisms (Tanomtong, 2011).

4. Conclusion

The karyotype study of *Hemibagrus wyckioides* revealed that the catfish has a diploid chromosome number (2n) of 58 and a fundamental number (NF) of 102, composed of 44 bi-armed chromosomes and 14 mono-armed chromosomes. These

include 20 large chromosomes, 32 medium-sized chromosomes, and 6 small chromosomes. The karyotype consists of four types of chromosomes: 22 metacentric, 12 submetacentric, 10 acrocentric, and 14 telocentric chromosomes. This research represents the first cytogenetic study of the catfish in the Mae Klong River Basin, Kanchanaburi Province. However, genetic studies on catfish in Thailand have been limited to only a few regions. Chromosomes play a crucial role in expressing the inheritance of genetic traits and determining the unique characteristics of each organism. The data obtained from this study can be used in cytogenetics to support taxonomic work on catfish, investigate the evolutionary relationships of the species, and contribute to further molecular genetic studies.

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