Vol.7, No.2 pages 9-22 Science Technology and Engineering Journal (STEJ) Research Article

First Karyotypic Study of the Kissing Gourami, *Helostoma temminckii* (Cuvier, 1829) (Anabantiformes, Helostomatidae) from Thailand by Conventional and Ag-NOR Staining Techniques

Boonyada Mingkwan[1], Rattanasuda Chaiyachate[2], Alongklod Tanomtong[1] and Weerayuth Supiwong[2*]

[1] *Department of Biology, Faculty of Science, Khon Kaen University, Muang, Khon Kaen 40002, Thailand*

[2] *Faculty of Interdisciplinary Studies, Khon Kaen University, Nong Khai Campus, Muang, Nong Khai 43000, Thailand*

[*] *Corresponding Author: supiwong@hotmail.com*

(Received: 11th February 2021, Revised: 23rd May 2021, Accepted: 21st June 2021)

Abstract-The first chromosomal study of kissing Gourami, *Helostoma temminckii* from Thailand was karyotypically performed. Kidney cells were obtained from ten male and ten female specimens. Chromosomes were conventionally stained, and Ag-NOR banding was carried out. Conventional staining mainly demonstrated telocentric chromosome with diploid number (2*n*) and fundamental number (NF) as 48. The Ag-NOR banding technique showed the position of nucleolar organizer regions (NORs) at the end of the telomeres of chromosome pair four. The karyotype consisted of 16 large, 30 medium and two small telocentric chromosomes. Thus, the karyotype formula can be represented as follows: $L^t_{16}+M^t_{30}+S^t_2$.

Keywords: *Helostoma temminckii*, karyotype, idiogram, chromosome

1. Introduction

Thailand is located in Southeast Asia and has high species diversity, 6-10% of all known species in the world were recorded (Baimai, 2010). The fish fauna of Thailand is ranked top among other countries and includes more than 2,700 species containing 2,000 marine and 872 freshwater species (Vidthayanon, 2005). The Mekong River system are known to be an international 'hotspot' of high biodiversity. It has been reported as most second biodiverse freshwater habitats with at least 1,200 freshwater fishes (Coates *et al*., 2003). In Thailand, 872 freshwater species have been classified into 255 genera, 55 families and 17 orders found in several areas, and in different habitats (Tan, 2006 ; Nelson *et al*., 2016).

The *Helostoma temminckii* is generally classified into family Helostomatidae, suborder Anabantoidei, order Anabantiformes, and class Actinopterygii (Arai, 2011 ; Nelson *et al*., 2016). It is widely distributed in tropical areas such as central Thailand, Indonesia, Cambodia (Mekong), the Malay Peninsula, Greater Sunda Islands of Borneo, Sumatra, Borneo and the island of Java (Tan, 2006 ; Nelson *et al*., 2016), and is mainly found in restricted habitat as potamodromous and benthopelagic occurring in freshwater at pH 6.0-8.0 (Ferry *et al*., 2012). *H. temminckii* possesses remarkable and unusual jaw characters related to the adaptation of kissing behavior (Ferry *et al*., 2012 ; Nelson *et al*., 2016) (Figure 1). Several studies of *H. temminckii* described the particular characters which differ from other fish groups such as visual sensitivity (Sakai *et al*., 1995), hearing ability (Yan, 1998), meristic and morphometric characteristics (Muryati *et al*., 2016), accessory breathing organ or labyrinth (Ferry *et al*., 2012), genetic diversity (Arifin *et al*., 2017), color change of skin (Kopecký *et al*., 2012), food and feeding habits (Asyari, 2007 ; Prianto *et al*., 2016), and functional morphology of head (Liem, 1967). Moreover, the *H. temminckii* is commercially sold in Southeast Asia (Ahmadi, 2021) and is importance for national economy of Thailand. Accordingly, *H. temminckii* is an interesting freshwater species for further study of its evolutionary diversity and other genetics aspects.

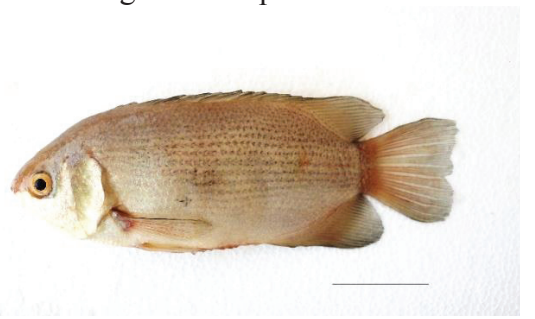

Figure 1. The kissing gourami (*Helostoma temminckii*), scale bar indicates 2 cm.

The study of cytogenetics describes chromosomal information, and is a helpful method to gain knowledges of the genetic diversity, population structure and supports breeding programs in hatcheries as well as aid investigation of evolutionary processes of related species (Esmaeili *et al*., 2015). Although, there have been many studies of fish chromosomes, there is little information of fishes in the suborder Anabantoidei (Arai, 2011). Surprisingly, the chromosomes of fishes in the suborder Anabantoidei have varied diploid number and fundamental chromosome number as $2n$=20-48 and NF=20-86 (Figure 1), respectively (Khuda-Bukhsh *et al*., 1995). However, previous reports of *H. temminckii* prominently suggested telocentric

chromosomes with 2*n*=48 and NF=48 (Hinegardner & Rosen, 1972 ; Arai, 2011). While, cytogenetic studies of *H. temminckii* have never been performed in Thailand.

Table 1. The karyotype studies of fish in suborder Anabantoidei

Families/ Species	2*n*	NF1	Karyotype	Ag-NORs	Locality	Reference
Anabus testudineus	46	54	2m+ 6sm+ 6st+ 32a	2	India (Manipur)	Kumar *et al*. (2008)
Belontia hasselti	48	48 49	48t 1m+47t	2	Thailand	Chaiyasan *et al*. (2021)
Ctenopoma acutirostre	48	48	48a	-	Africa	Krysanov and Golubtsov (2001)
Ct. muriei	48	48	48a	-	W. Ethiopia	Krysanov and Golubtsov (2001)
Ct. ocellatum	48	48	48st/a	-	Africa	Krysanov and Golubtsov (2001)
Ct. petherici	48	48	48a	-	W. Ethiopia	Krysanov and Golubtsov (2001)
Helostoma temminckii	48	48	48t	2	Thailand	Present study
Microctenopoma ansorgii	46	48	2m+ 44a	-	Africa	Krysanov and Golubtsov (2001)
M. congicum	46	48	2m+ 44a	-	Africa	Krysanov and Golubtsov (2001)
M. pekkolai	48	52	2m+ 2sm+ 44st/a	-	W. Ethiopia	Krysanov and Golubtsov (2001)
Helostoma temminkii	48	48	48a	-	South East Asia	Arai (2011)
Colisa lalia	46	60	24m + 22a	-	Ornamental fish farm in Port-said	Abu-Almaaty *et al*. (2017)
Luciocephalus pulcher	20	20	20a	-	Thailand	Arai (2011)
Trichogaster chuna	46	86	28m+ 12sm+ 6a	-	India (Assam)	Arai (2011)
T. fasciatus	48	80	16m+ 16sm+ 4st+ 12a	-	India (Manipur)	Sobita and Bhagirath (2007)
T. fasciatus	48	80	16m+ 16sm+ 6st+ 10a	-	India (Manipur)	Arai (2011)
T. labiosus	48	86	22m+ 16sm+ 10a	-	India	Rishi *et al*. (1997)
T. lalius	46	70	24m/sm+ 22st/a	-	Asia	Vinogradov (2005)
T. lalius	46	66	14m+ 6sm+ 26a	-	India (Haryana)	Rishi *et al*. (2001)

Table 1. The karyotype studies of fish in suborder Anabantoidei (cont.)

Families/ Species	2*n*	NF1	Karyotype	Ag-NORs	Locality	Reference
Trichopodus microlepis	46	46	46a		Thailand	Seetapan and Khamma-Ai (2007)
T. leeri	46	46	46a		Thailand	Seetapan and Khamma-Ai (2007)
T. leeri	46	46	46a	-	Ornamental fish farm in Port-said	Abu-Almaaty *et al*. (2017)
T. trichopterus	46	46	46a		Thailand	Magtoon *et al*. (2007)
T. trichopterus	46	46	46st/a	-	Aquarium fish shop in Brazil	Pazza *et al*. (2009)
T. trichopterus	46	46	46a	2	Thailand	Supiwong *et al*. (2010)
T. trichopterus	46	46	46a	-	Ornamental fish farm in Port-said	Abu-Almaaty *et al*. (2017)
Trichosis pumila	46	48	2m+ 44a	-	Thailand	Donsakul *et al*. (2009)
Tr. schalleri	46	46	46a	-	Thailand	Donsakul *et al*. (2009)
Tr. vittatus	46	46	46a	-	Thailand	Magtoon *et al*. (2007)
Beta imbellis	42	64	14m+ 8sm+ 4st+ 16a	-	Thailand	Wattanasirisareekul and Suwannarak (2012)
B. prima	34	42	4m+ 4sm+ 4st+ 22a	-	Thailand	Magtoon *et al*. (2007)
B. simplex	44	52	4m+ 4sm+ 36a	-	Thailand	Donsakul *et al*. (2009)
B. smaragdina	42	48	2m+ 4sm+ 36a	-	Thailand	Donsakul *et al*. (2009)
B. splendens	42	54	4m+ 8sm+ 30a	-	Thailand	Magtoon *et al*. (2007)
B. splendens	42	54	12sm+ 14st+ 16a	-	-	Furgala-Selezniow *et al*. (2008)
Macropodus opercularis	46	62	8m+ 8sm+ 16st+ 14a	-	Asia	Vinogradov (2005)
Osphronemus exodon	48	48	48a	-	Thailand	Donsakul *et al*. (2006)
O. goramy	48	48	48a	-	Thailand	Donsakul *et al*. (2006)
O. laticlavius	48	50	2sm+ 46a	-	Thailand	Donsakul *et al*. (2006)

Notes: 2*n*=diploid chromosome number, NF=fundamental number (number of chromosome arm), m=metacentric, sm=submetacentric, a=acrocentric, t=telocentric, NORs=nucleolar organizer regions, and=not available.

Here, we provide the first chromosomal information of *H. temminckii* in Thailand using conventional banding and Ag-NOR staining techniques. The results

will be used to support and provide general data for genetic research associating the identification of fish taxonomy, evolutionary systematic and genetic control.

2. Methods

2.1 Sample Collection

Twenty individuals of *H. temminckii*, 10 males and 10 females, were obtained from Mekong River in Nong Khai Province in Thailand. Based on morphology, the species identification was followed the methods of Rainboth (1996), Kottelat (2001), Nelson *et al*. (2016). Samples were gently transferred to the laboratory, then, they were kept under standard conditions in a well-aerated aquarium at 20-25 ºC for 5-7 days before analysis.

2.2 Chromosome Preparation

The chromosome preparation was followed the method of Supiwong *et al*. (2010), Tanomtong (2011), Sangpakdee *et al*. (2015) and Sangpakdee *et al*. (2017). Both male and female of *H. temminckii* were intraperitoneally injected with 1 mL/100 g of body weight of 0.05% colchicine and then sacrificed after one hour. The kidneys were removed, chopped, and incubated in 0.075M KCl solution for 30 minutes. Fresh and cold fixative (3:1 ; methanol: glacial acetic acid (vol/vol)) was added and further centrifuged for 10 minutes at 1,200 rpm, discarding the supernatant. The fixative was repeatedly changed until the solution was clear, the cell suspension was then used for chromosomal studies.

2.3 Chromosome Staining

Two classical cytogenetic techniques, conventional staining and Ag-NOR banding were performed as follows. Conventional staining followed the protocol of Rooney (2001), Sreeputhorn *et al*. (2017) and Chaiyasan *et al*. (2018), the chromosomes were stained with 20% Giemsa solution for 30 minutes. Ag-NOR banding techniques were based on the method of Howell and Black (1980) and Getlekha and Tanomtong (2020), using 1: 2 (vol/vol) of 2% gelatin and 50% silver nitrate.

2.4 Chromosomal Analysis

The mitotic metaphase cells were examined under a light microscope and the best of 20 metaphases were chosen for further chromosomal analysis. The chromosomal characterization was performed using Microsoft Excel 2013 software and Adobe Photoshop CS6.5.2.3, providing three parameters ; relative length (RL), centromeric index (CI) and size of chromosomes. The homologous chromosomes were aligned based on their centromere position and CI. Chromosomal identification followed the method of Chaiyasut (1989), Phimphan *et al*. (2013), and Juntaree and Supiwong (2020). Generally, a CI between 0.50-0.59, 0.60-0.69, 0.70-0.89 and 0.90-1.00 implies types of chromosomes as being metacentric (m), submetacentric (sm), acrocentric (a) and telocentric (t). The fundamental number (NF) is commonly elucidated as two values with metacentrics submetacentric and acrocentric, chromosomes and one value with telocentric chromosomes. All parameters were further used for karyotyping and idiograming (Tanomtong *et al*., 2014 ; Jantarat *et al*., 2017).

3. Results and Discussion

This is the first report of chromosomal information of *H. temminckii* in Thailand. Based on chromosome morphology, the karyotype of *H. temminckii* dominantly showed 24 pairs of telocentric chromosomes with 2*n*=48 and NF=48 in both sexes (Figure 2). No difference of sex chromosomes was observed. The standardized karyotype of *H. temminckii* was elucidated and consisted of eight pairs of large, 15 pairs of medium and one pair of small telocentric chromosomes (Table 2). NORs regions were only found at the end the chromosome pair four (Figure 3). The lengths and shapes of chromosomes were precisely described by a standardize idiogram (Figure 4). Therefore, the karyotype formula for this species is described as follows ; $L^t_{16}+M^t_{30}+S^t_2$.

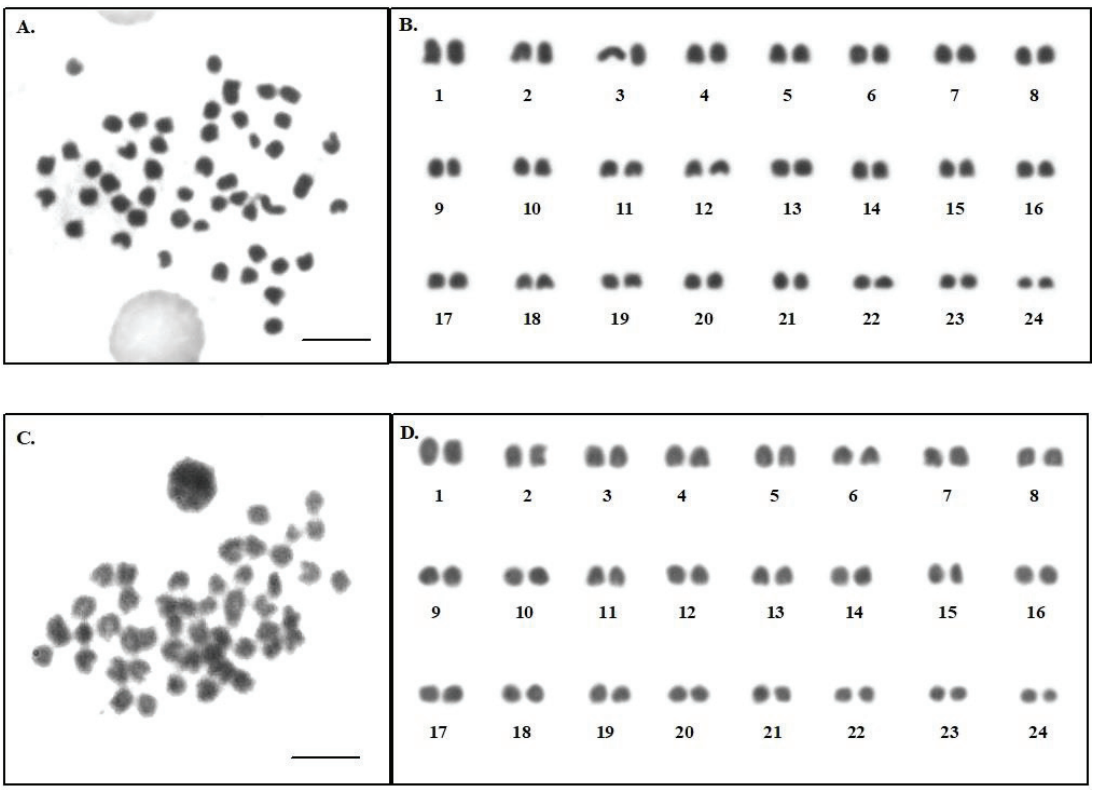

Figure 2. The chromosome plates and karyotypes of male (A, B) and female (C, D) of kissing gouramies (*Helostoma temminckii*, 2n=48) by conventional staining technique. Scale bars indicate 5 µm.

Table 2. The mean values of short arm length (Ls), long arm length (Ll), total arm length (LT), relative length (RL), and centromeric index (CI) of chromosomes, indicating length shape and type of chromosomes from 20 metaphase chromosomes of male and female *H. temminckii* (2*n*=48).

Chromosome pair	Ls	Ll	LT	RL±SD	CI	Size	Type
1	0.000	0.728	0.728	0.0299±0.0015	1.000	Large	Telocentric
2	0.000	0.669	0.669	0.0275±0.0009	1.000	Large	Telocentric
3	0.000	0.626	0.626	0.0257±0.0011	1.000	Large	Telocentric
4*	0.000	0.604	0.604	0.0248±0.0009	1.000	Large	Telocentric
5	0.000	0.579	0.579	0.0238±0.0007	1.000	Large	Telocentric
6	0.000	0.562	0.562	0.0231±0.0008	1.000	Large	Telocentric
7	0.000	0.551	0.551	0.0226±0.0008	1.000	Large	Telocentric
8	0.000	0.539	0.539	0.0221±0.0006	1.000	Large	Telocentric
9	0.000	0.526	0.526	0.0216±0.0002	1.000	Medium	Telocentric
10	0.000	0.518	0.518	0.0213±0.0002	1.000	Medium	Telocentric
11	0.000	0.512	0.512	0.0211±0.0003	1.000	Medium	Telocentric
12	0.000	0.503	0.503	0.0207±0.0003	1.000	Medium	Telocentric
13	0.000	0.494	0.494	0.0203±0.0003	1.000	Medium	Telocentric
14	0.000	0.487	0.487	0.0201±0.0004	1.000	Medium	Telocentric
15	0.000	0.479	0.479	0.0197±0.0005	1.000	Medium	Telocentric
16	0.000	0.470	0.470	0.0194±0.0007	1.000	Medium	Telocentric
17	0.000	0.463	0.463	0.0191±0.0006	1.000	Medium	Telocentric
18	0.000	0.454	0.454	0.0187±0.0006	1.000	Medium	Telocentric
19	0.000	0.444	0.444	0.0183±0.0007	1.000	Medium	Telocentric
20	0.000	0.433	0.433	0.0178±0.0006	1.000	Medium	Telocentric
21	0.000	0.421	0.421	0.0173±0.0006	1.000	Medium	Telocentric
22	0.000	0.393	0.393	0.0161±0.0005	1.000	Medium	Telocentric
23	0.000	0.372	0.372	0.0153±0.0009	1.000	Medium	Telocentric
24	0.000	0.337	0.337	0.0138±0.0010	1.000	Small	Telocentric

Remark: *=NOR-bearing chromosome

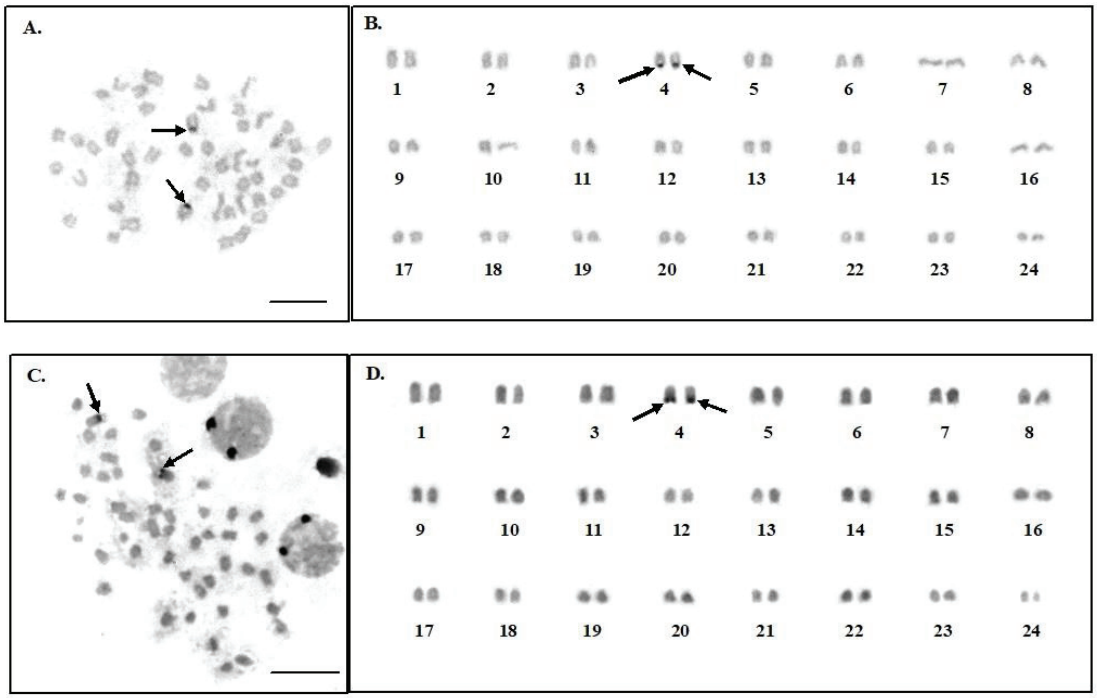

Figure 3. The chromosome plates and karyotypes of male (A, B) and female (C, D) of kissing gouramies (*Helostoma temminckii*, 2n=48) by Ag-NOR banding technique. Arrows indicate NORs-bearing chromosomes and scale bars indicate 5 µm.

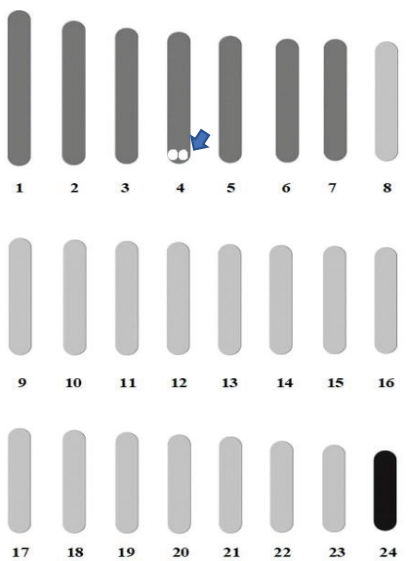

Figure 4. The standardize idiogram of *Helostoma temminckii*, indicating lengths and shapes of chromosomes (n=24) by conventional staining technique. Color blocks describe chromosome sizes as large, medium and small by dark gray, light gray and black, respectively. The arrow indicates Nucleolar Organizer Region (NOR).

The results demonstrate 24 pairs of telocentric chromosomes with 2*n*=48 and NF=48. This is the same result as that reported by Hinegardner and Rosen (1972) and Arai (2011). The Anabantoidei species have been cytogenetically investigated and the karyotypes mostly exhibited a chromosomal type as a telocentric chromosome with 2*n*=48. In karyotype reports of Anabatoidei, they surprisingly indicated variations of diploid number and fundamental number with 2*n*=20-48 and NF=20-86 (Table 1). Remarkably, the karyotypes with 2*n*<48 have mostly demonstrated a significantly higher number of metacentric and submetacentric chromosomes compared to those karyotypes with 2*n*=48. Generally, ray finned fish species have 48 or 50 chromosomes indicating ancestral karyotype (Rishi *et al*., 1997 ; Pazza *et al*., 2009). More over, 46-48 telocentric chromosomes seem to

be common pattern in order Anabantiformes (Rishi *et al*., 1997 ; Pazza *et al*., 2009 ; Nelson *et al*., 2016). The alteration of chromosome number and structure is commonly referred as intraspecific variation among distinct populations and/or chromosomal translocation and rearrangement as deletions, paracentric and pericentric inversions as well as Robertsonian rearrangement, leading to variation of diploid number and fundamental chromosome number (Rishi *et al*., 1997 ; Pazza *et al*., 2009). The sex chromosome of *H. temminckii* was not distinguished in the present study similar to several species in Anabantoidei (Krysanov & Golubtsov, 2001 ; Sobita & Bhagirath, 2007 ; Supiwong *et al*., 2010 ; Arai, 2011 ; Abu-Almaaty *et al*., 2017). Only three species of Anabantoidei were cytogenetically showed sex chromosome system containing ZZ/ Z0 and XX/X0 in *Trichogaster lalius* and ZZ/ ZW in *Trichogaster fasciata* (Arai, 2011) and *Belontia hasselti* (Chaiyasan *et al*., 2021).

Helostoma temminckii was further analyzed using Ag-NOR staining technique. This procedure is used to determine secondary constriction site as chromosome marker (Crocker, 1990). The secondary constriction site is detected at NORs, it mainly appears as a phenomenon in vertebrates, particularly in teleost fishes (Juntaree & Supiwong, 2020). The NORs region is commonly composed of ribosomal RNA genes, argilophelic protein and nucleolus. The ribosomal RNA gene mainly includes a cluster of 5S, 18S and 28S rDNA genes for synthesis of ribosomal RNA which is the subunit of ribosomes involved in protein synthesis. Due to the 5S rDNA is mostly distributed over genome and 18S rDNA is located at NORs position, leading to define 18S rDNA as secondary constriction site (Crocker, 1990). In our work, the NORs region of *H. temminckii* was firstly reported on one chromosome pair at the region adjacent to the telomere of the telocentric chromosome pair four. However, a few NORs studies in the suborder Anabantoidei had been performed as follows. Only two species *Anabus testudinous* and *Tricopodus tricopterus*, have been reported with one pair of NORs sites. In *A. testudinous*, NORs were located at the terminal of the short arm chromosome of a submetacentric chromosome (Arai, 2011), while NORs of *Tr. tricopterus* were demonstrated at a position adjacent to the telomere of telocentric chromosome pair two (Supiwong *et al*., 2010). The number of chromosome carrying NORs is specific in related species, it is used for referring gene marker, for example in several cyprinid and salmonids (Amemiya & Gold, 1986). The single NOR-bearing chromosome seem to be a common characteristic in fish groups and was considered as plesiomorphic or primitive state found in several fish taxa. While multiple NORs in fishes was referred to be apomorphic or advance condition (Kumar *et al*., 2013). Thus, NOR-harbouring chromosome can be used as marker and tool for identification of fish group (Khakhong *et al*., 2014 ; Sochorová *et al*., 2018).

Moreover, the chromosomal information of *H. temminckii* will be used to support the basic knowledge of chromosome morphology, cytotaxonomic markers and to further describe karyotype evolution and phylogenetic relationship of fish in this suborder.

3. Acknowledgement

This work is supported by Science Achievement Scholarship of Thailand (SAST), Department of Biology, faculty of Science ; and Research

and Technology Transfer Affairs of Khon Kaen University.

4. References

Abu-Almaaty, A.H., Hassan, M.K., Bahgat, I.M. & Suleiman, M.E.E. (2017). Inter simple sequence repeat (ISSR) and cytogenetic analysis of three fish species of family osphronemidae. *Egyptian Journal of Aquatic Biology and Fisheries, 21* (2), 1-15.

Ahmadi. (2021). Length-weight relationship and relative condition factor of the Kissing Gourami (*Helostoma temminckii*) from Sungai Batang River, Indonesia. *Songklanakarin J. Sci. Technology, 43* (1), 210-217.

Amemiya, C.T. & Gold, J.R. (1986). Chromomycin A 3 Stains Nucleolus Organizer Regions of Fish Chromosome. *Copeia, 1986* (1), 226.

Arai, R. (2011). *Fish karyotypes: a check list*. Springer.

Arifin, O.Z., Cahyanti, W., Kristanto & A.H., (2017). Genetic diversity of three generations of kissing gourami (*Helostoma temminckii*) under a domestication program. *Jurnal Riset Akuakultur,12* (4), 295-305.

Asyari, E. (2007). Food habit of Biawan fish (*Helostoma temminckii*) at Sababila, Barito South Kalimantan. *Journal of Protein, 14*, 1-11.

Baimai, V. (2010). Biodiversity in Thailand. *The Journal of the Royal Institute of Thailand, 2*, 107-118.

Chaiyasan, P., Mingkwan, B., Jantarat, S., Suwannapoom, C., Cioffi, M.B., Liehr, T., Talumphai, S., Tanomtong, A. & Supiwong, W. (2021). Classical and molecular cytogenetics of Belontia hasselti (Perciformes: Osphronemidae): Insights into the ZZ/ZW sex chromosome system. *Biodiversitas, 22* (2), 548-556.

Chaiyasan, P., Supiwong, W., Saenjundaeng, P., Seetapan, K. Pinmongkhonkul, S., Tanomtong, A. (2018). A report on classical cytogenetics of highfin Barb fish, Cyclocheilichthys armatus (Cypriniformes, Cyprinidae). *Cytologia, 83* (2), 149-154.

Chaiyasut, K. (1989). *Cytogenetics and Cytotaxonomy of the Family Zephyranthes*. Department of Botany, Chulalongkorn University.

Coates, D., Ouch, P., Suntornratana, U., Nguyen, T.T. & Viravong, S. (2003). Biodiversity and fisheries in the Mekong River Basin. *Mekong Development Series, 2*, 1-30.

Crocker, J. (1990). Nucleolar organiser regions. *Current Topics in Pathology Ergebnisse Der Pathologie, 82*, 91-149.

Donsakul, T., Magtoon, W. & Rungsiruji, A. (2006). Karyotypes of three giant goramy, Osphronemus goramy, O. exodon and O. laticlavius (Pisces: Osphronemidae). *Proceedings of 44th Kasetsart University Annual Conference Fisheries* (pp. 461-468). *Bangkok*.

Donsakul, T., Magtoon, W. & Rungsiruji, A. (2009). Karyotypes of five belontiid fishes (Family Belongtiidae): Betta smaragdina, B. simplex, B. sp., Trichopsis pumila and T. Schalleri. *The 35th Congress on Science and Technology of Thailand (*pp.77). Chonburi, Thailand.

Esmaeili, H.R., Pirvar, Z., Ebrahimi, M. & Matthias, F. (2015). Karyological and molecular analysis of three endemic loaches (Actinopterygii: Cobitoidea) from Kor River basin, Iran. *Molecular Biology Research Communications, 4* (1), 1-13.

Ferry, L.A., Konow, N. & Gibb, A.C. (2012). Are Kissing Gourami Specialized for Substrate-Feeding? Prey Capture Kinematics of *Helostoma temminckii* and Other Anabantoid Fishes. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 317* (9), 571-579.

Furgala-Selezniow, G., Fopp-Bayat, D., Jankun, M., Krejszeff, S. & Mamcarz, A. (2008). Note on the karyotype and NOR location of Siamese fighting fish Betta splendens (Perciformes, Osphronemidae). *Caryologia, 61* (4), 349-353.

Getlekha, N. & Tanomtong, A. (2020). First Cytogenetic Study of Green Chromis, Chromis viridis (Perciformes, Pomacentridae) by Conventional Staining and Ag-NOR Banding Techniques. *Chiang Mai University Journal of Natural Sciences, 19* (2), 164-175.

Hinegardner, R. & Rosen, D.E. (1972). Cellular DNA content and the evolution of teleostean fishes. *The American Naturalist, 106* (951), 621-644.

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

Jantarat, S., Supiwong, W., Phintong, K., Sonsrin, K., Kong-ngarm, N. & Tanomtong, A. (2017). First Analysis on the Cytogenetics of Painted Sweetlip, Plectorhinchus pictus (Heamulidae: Perciformes) from Thailand. *Cytologia, 82* (2), 145-150.

Juntaree, S. & Supiwong, W. (2020). Standardized Karyotype and Idiogram of the Glass Goby Fish (Gobiopterus chuno) (Hamilton, 1822) in Thailand. *Science Technology and Engineering Journal (STEJ), 6* (2), 72-81.

Khakhong, S., Supiwong, W., Tanomtong, A., Sriuttha, M., Jearranaiprepame, P., Soemphol, W. & Jiwyam, W. (2014). A First Chromosomal Characterization of NORs in Splendid Snakehead Fish, Channa lucius (Perciformes, Channidae). *Cytologia, 79* (2), 133-139.

Khuda-Bukhsh, A.R., Rahman, A., Chanda T., Nayak, K., Khuda-Bukhsh, A.R. (1995). *Diploid numbers and chromosome formulae of some 29 species of Indian teleosts (Pisces).*

Kopecký, J., Král, M., Čurlej, J. & Mrázová, L. (2012). Influence of Astaxanthin and β-carotene on Kissing Gourami (*Helostoma temminckii*) Colouring. *Scientific Papers: Animal Science and Biotechnologies, 45* (2), 57-60.

Kottelat, M. (2001). *Fishes of Laos*. WHT Publications Ltd.

Krysanov, E.Y. & Golubtsov, A.S. (2001). Karytypes of eight species of African climbling gouramie from the genera Ctenopoma and Microctenopoma (Anabantidae, Perciformes) and their probable phylogenetic relationships. *Voprosy Ikhtiologii, 41* (9), 737-741.

Kumar, R., Nagpure, N.S., Behera, B.K. & Lakra, W.S. (2008). Chromosome banding in two freshwater Gouramies from North Eastern India. *Nucleus, 51* (1,2), 61-72.

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

Liem, K.F. (1967). Functional morphology of the head of the anabantoid teleost fish Helastoma temmincki. *Journal of Morphology, 121*, 135-157.

Magtoon, W., Rangsiruji, A. & Donsakul, T. (2007). Karyotypes of Betta splendens, B. prima, Trichopsis vittatus and Trichogaster trichopterus (family Belontiidae) from Thailand. *The 33rd Congress on Science and Technology of Thailand (*pp. 94*),* Thailand.

Muryati, S., Putra, R.M. & Efizon, D. (2016). A Study on morphometric and meristic of *Helostoma temminckii* from swamp area in the Bencah Kelubi Village, Tapung Kiri Sub-Regency, Kampar Regency, Riau Province. *Jurnal Online Mahasiswa Fakultas Peri-kanan dan Ilmu Kelautan Universitas Riau, 3*, 1-10.

Nelson, J.S., Grande, T.C., Wilson, M.V.H. (2016). *Fishes of the World* (5th ed). John Wiley & Sons, Inc.

Pazza, R., Kavalco, K.F., Penteado, P.R., Kavalco, S.A.F. & De Almeida-Toledo L.F. (2009). Gene mapping of 18S and 5S rDNA genes in the karyotype of the three-spot gourami Trichogaster trichopterus (perciformes, osphronemidae). *Zebrafish, 6* (3), 219-222.

Phimphan, S., Tanomtong, A., Jumrusthanasan, S., Supiwong, W., Siripiyasing, P. & Sanoamuang, L. (2013). First Report of NOR Polymorphism and Chromosome Analysis of John's Snapper, Lutjanus johnii (Perciformes, Lutjanidae) in Thailand. *Cytologia, 78* (4), 335-344.

Prianto, E., Husnah, N.S. & Asyari. (2016). Food habit of Biawan fish (*Helostoma temminckii*) at Sababila, Barito South Kalimantan. *Jurnal PROTEIN, 14* (2), 161-166.

Rainboth, W.J. (1996). *Fishes of the Cambodian Mekong*. Food and Agriculture Organization of the United Nation.

Rishi, S., Gill, P.K. & Rishi, K.K. (1997). Karyotypic studies in eight species of fishes belonging to family Belontiidae (Pisces, Perciformes). *Journal of Chromatographic Science, 1*, 55-59.

Rishi, S., Gill, P.K. & Rishi, K.K. (2001). Heterochromatin localization in the chromosomes of five belontid fishes (Belontiidae, Perciformes). *Chromosome Science, 5* (1), 35-38.

Rooney, D.E. (2001). *Human Cytogenetics: Constutitional Analysis: A Practical Approach*. Oxford University Press.

Sakai, H.M., Wang, J.L. & Naka, K. (1995). Contrast gain control in the lower vertebrate retinas. *The Journal of General Physiology, 105* (6), 815-835.

Sangpakdee, W., Phimphan, S., Tanomtong A., Patawang, I., Pinthong, K. & Neeratanaphan, L. (2015). Karyological study of Lutjanus ehrenbergii and L. carponotatus (perciformes, lutjanidae) by classical and Ag-NOR staining techniques. *Nucleus (India), 58* (3), 1-7.

Sangpakdee, W., Phimphan, S., Tengjaroenkul, B., Pinthong, K., Neeratanaphan, L., Tanomtong, A. (2017). Cytogenetic study of tree microhylid species (Anura, Microhylidae) from Thailand. *Cytologia, 82*, 67-74.

Seetapan, K. & Khamma-Ai, M. (2007). Karyotypes of the genus Trichogaster from Thailand. *The Proceedings of Fisheries Annual Conference* (pp. 323-329). Bangkok.

Sobita, N. & Bhagirath, T. (2007). Colisa fasciata (Schneider) (Perciformes, Anabantoidei, Belontidae) karyotype is divergent and ZW sex chromosomes are allocyclic and asynaptic. *Nucleus, 50*, 45-49.

Sochorová, J., Garcia, S., Gálvez, F., Symonová, R. & Kovaří, A. (2018). Evolutionary trends in animal ribosomal DNA loci: Introduction to a new online database. *Chromosoma, 127* (1), 141-150.

Sreeputhorn, K., Mangumphan, K., Muanphet, B., Tanomtong, A., Supiwong, W. & Kaewmad, P. (2017). The First Report on Chromosome Analysis of F1 Hybrid Catfish: Mekong Giant Catfish (Pangasianodon gigas)x Striped Catfish (Pangasianodon hypophthalmus) and Spot Pangasius (Pangasius larnaudii)×Pangasianodon hypophthalmus (Siluriformes, Pangasiidae). *Cytologia, 82* (4), 457-463.

Supiwong, W., Tanomtong, A., Kenthao, A., Seetapan, K., Kaewsri, S. & Sanoamuang L. (2010). Standardized karyotype of the three-spot gourami, Trichogaster trichopterus (Perciformes, Belontidae) from Thailand by conventional and Ag-NOR staining technique. *The Nucleus, 53* (3), 103-107.

Tan, S.H. (2006). The Raffles Bulletin of Zoology. *Raffles Bulletin of Zoology*, *54*.

Tanomtong, A. (2011). *Cytogenetics*. Khon Kaen University press.

Tanomtong, A., Supiwong, W., Jearranaiprepame, P., Khakhong, S., Kongpironchuen, C. & Getlekha, N. (2014). A new natural autotetraploid and chromosomal characteristics of dwarf snakehead fish, Channa gachua (Perciformes, Channidae) in Thailand. *Cytologia, 79* (1), 15-27.

Vidthayanon, C. (2005). *Freshwater Fish in Thailand (5th ed.)*. Nanmeebook Press.

Vinogradov, A.E. (2005). Genome size and chromatin condensation in vertebrates. *Chromosoma, 113* (7), 362-369.

Wattanasirisareekul, R. & Suwannarak, A. (2012). Karyotype of peaceful betta, Betta imbellis (perciformes, Osphronemidae) from Thailand. *Journal of Fisheries Technology Research, 2*, 35-45.

Yan, H.Y. (1998). Auditory role of the suprabranchial chamber in gourami fish. *Journal of Comparative Physiology A, 183*, 325-333.