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First report on genetic structure of *lobocheilos rhabdoura* (Fowler, 1934) (Labeoninae, Cyprinidae) in Thailand: Insight the basic knowledge for fish breeding

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Abstract - *Lobocheilos rhabdoura* is a fish of the genus *Lobocheilos* in the family Cyprinidae. It is popular as an ornamental fish in Thailand and is important to fisheries and the economy. Chromosomes and chromosome markers of this species were studied by conventional staining and NOR-banding techniques. Ten male and ten female specimens were collected from the Meklong Basin, Kanchanaburi Province. The chromosomes were directly prepared from kidney tissues. The results showed that diploid chromosome was $2n = 50$ and the fundamental number (NF) was 90 in all specimens. The structure karyotype consisted of 10 metacentric, 18 submetacentric, 12 acrocentric and 10 telocentric chromosomes; 10m + 18sm + 12a + 10t in both sexes without morphologically differentiated sex chromosomes. NOR positive markers were observed in regions adjacent to telomere of long arm of the largest submetacentric chromosome pair. This study provides basic knowledge of the karyotype structure of this species and this basic knowledge that can be applied for breeding of ornamental fish in the future.

Keywords: Chromosome, karyotype, *Lobocheilos rhabdoura*

1. Introduction

Freshwater fishes especially in the order Cypriniformes have been comprise as 2,867 reported species in 35 genera, which are economically important, having high nutritional value and ecological importance, as well as being popular ornamental fish. Thailand has a great variety of freshwater fish. There are numerous wildlife parks and sanctuaries, which give rise to various animal species in the Meklong Basin in Kanchanaburi Province (Water Resources Regional Office 7, 2020). Species of the Cyprinidae that are most popular as ornamentals fish include as *Epalzeorhynchos frenatum* (Fowler, 1934), *E. bicolor* (Smith, 1931), *E. munensis* (Smith, 1934), *Puntigrus partipentazona* (Fowler, 1934), *Puntioplites falcifer* (Smith, 1929) and *Scaphognathops bandanensis* (Phimphan *et al*., 2020), Breeding and improvement of fish breeding in fisheries based on cytogenetics involving studies of the chromosomes as the structural unity of genetic material has been undertaken. Such studies provide information about the type, shape, number, function and chromosomal abnormalities. This data is the basic information to support the breeding by inducing fish to increase the chromosome sets (polyploidy) (Supiwong, 2014), the selection of breeders and classification (Mengampan *et al*., 2004).

Cytogenetics information concerning Cyprinidae is usually presented as the diploid chromosome ($2n$), the fundamental chromosome (NF) and the nucleolar organizer regions (NORs) as shown in the Table 1. *Lobocheilos rhabdoura* (Fowler, 1934) in subfamily Labeoninae is a small fish. It has a single mid-lateral stripe that is darkensed on the caudal peduncle and ends in a large black round or oval spot at base of caudal fin. There are 10 predorsal scales, relatively slender body, depth at 3.7-4.6 times in standard length (SL). It also has relatively wide mouth, occupying the whole width of head and large tubercles on the snout in large individuals (Rainboth, 1996) (Figure 1). Nowadays, it has become popular as an ornamental fish, however, information on cytogenetics has not been reported. Therefore, the present work focused on the study of chromosome structure of *L. rhabdoura* by conventional staining and NOR-banding techniques. This banding technique determines the location on the chromosomes that contains the ribosome genes (rDNAs). These genes are located at the satellite chromosome region or the second chromosome isthmus and are used for transcription tracing or as chromosome markers (Tanomtong *et al*., 2014). The obtained results are basic information for further applied studies on systematics, toxicology, and fish breeding to improve stain of this ornamental fish.

Figure 1. Characteristics of *Lobocheilos rhabdoura*, scale bar indicates 2 cm.

Table 1. Reviews of cytogenetic reports in family Cyprinidae in Thailand (note: 2*n* = diploid number, m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric, t = telocentric, NORs = nucleolar organizer regions, NF = fundamental number, and - = not available).

Species	2*n*	NF	Formula	NOR	Reference
Amblyrhynchichthys truncatus (Bleeker, 1851)	50	78	16m+12sm+22a	-	Donsakul *et al*. (2006)
Balantiocheilos melanopterus (Bleeker, 1850)	50	72	10m+12sm+28a	-	Ojima and Yamamoto (1990)
	50	70	14m+6sm+10st+20a	-	Donsakul and Poopitayasathaporn (2002)
Barbonymus gonionotus (Bleeker, 1850)	50	72	2m+20sm+4st+24a	-	Magtoon and Arai (1989)
	50	74	16m+8sm+26a	-	Donsakul and Magtoon (1997)
	50	66	2m+4sm+10st+34a	-	Seetapan (2007)
Barbichthys laevis (Valenciennes, 1842)	50	76	20m+6sm+4st+20a	-	Donsakul *et al*. (2006)
Bangana devdevi (Hora, 1936)	50	86	20m+16sm+14a	-	Donsakul *et al*. (2011)
Catlocarpio siamensis (Boulenger, 1898)	98	150	18m+34sm+46a	-	Saenjundaeng *et al*. (2018[a])
Cirrhinus microlepis (Sauvage, 1878**)**	50	88	22m+8sm+8st+12a	-	Donsakul and Magtoon (1997)
	50	72	12m+10sm+2st+26a	-	Donsakul *et al*. (2007)
Cyclocheilichthys armatus (Valenciennes, 1842)	50	94	12m+18sm+14a+6t	4	Chaiyasan *et al*. (2018)
C. repasson (Bleeker, 1853)	50	96	12m+22sm+12a+4t	8	Chaiyasan *et al*. (2020)
Danio albolineatus (Blyth, 1860)	50	100	8m+14sm+28a	-	Aiumsumang *et al*. (2022)
Devario laoensis (Pellegrin & Fang, 1940)	50	100	6m+10sm+34a	2	Aiumsumang *et al*. (2021)
	50	100	6m+12sm+32a	-	Aiumsumang *et al*. (2022)
D. regina (Fowler, 1934)	50	100	6m+12sm+32a	2	Aiumsumang *et al*. (2021)
Epalzeorhynchos frenatum (Fowler, 1934)	48	72	14m+10sm+8st+16a	-	Donsakul and Magtoon (1993)
	50	78	18m+10sm+10st+12a	4	Phimphan *et al*. (2020)
E. bicolor (Smith, 1931)	50	74	20m+4sm+2st+24a	-	Donsakul and Magtoon (1993)
E. munensis (Smith, 1934)	50	84	22m+12sm+2st+14a	-	Donsakul *et al*. (2012)

Table 1. Reviews of cytogenetic reports in family Cyprinidae in Thailand (note: 2*n* = diploid number, m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric, t = telocentric, NORs = nucleolar organizer regions, NF = fundamental number, and - = not available) (cont.)

Species	2*n*	NF	Formula	NOR	Reference
Esomus metallicus (Ahl, 1923)	50	98	8m+10sm+30a+2t	2	Aiumsumang *et al*. (2021)
Garra cambodgiensis (Tirant, 1883)	50	82	20m+12sm+4st+14t	-	Donsakul *et al*. (2016)
G. fasciacauda (Fowler, 1937)	50	84	18m+14sm+2st+16t	-	Donsakul *et al*. (2016)
G. notata (Blyth, 1860)	50	80	20m+10sm+20t	-	Donsakul *et al*. (2016)
Labeo chrysophekadian (Bleeker, 1850)	50	78	4m+10sm+14st+22a	-	Seetapan (2007)
Labiobarbus leptocheilus (Valenciennes, 1842)	50	86	14m+6sm+16a+14t	2	Saenjundaeng *et al*. (2018[b])
L. lineatus (Sauvage, 1878)	50	80	20m+10sm+20a	-	Magtoon and Arai (1990)
L. spiropleura (Sauvage, 1881)	50	90	34m+4sm+2st+10a	-	Donsakul and Magtoon (1997)
Osteochilus lini (Fowler, 1935)	50	100	22m+24sm+2st+2a	-	Saenjundaeng *et al*. (2020)
O. melanopleura (Bleeker, 1852)	50	100	14m+32sm+4st	-	Saenjundaeng *et al*. (2020)
O. microcephalus (Valenciennes, 1842)	50	100	16m+30sm+4st	-	Saenjundaeng *et al*. (2020)
O. vittatus (Valenciennes, 1842)	50	100	16m+26sm+8st	-	Saenjundaeng *et al*. (2020)
Puntigrus partipentazona (Fowler, 1934)	50	80	6m+24sm+14st+6a	4	Phimphan *et al*. (2020)
Puntioplites falcifer (Smith, 1929)	50	80	14m+16sm+2st+18a	-	Donsakul *et al*. (2007)
	50	92	16m+10sm+16a+8t	-	Sophawanus *et al*. (2017)
P. proctozystron (Bleeker, 1865)	50	82	6m+14sm+12a+4t	2	Supiwong *et al*. (2011)
Probarbus jullieni (Sauvage, 1880)	98	138	26m+22sm+12a+4t	4	Saenjundaeng *et al*. (2018[a])
Rasbora paviana (Tirant, 1885)	50	98	8m+16sm+24a+2t	2	Aiumsumang *et al*. (2021)
R. aurotaenia (Tirant, 1885)	50	98	8m+16sm+24a+2t	-	Aiumsumang *et al*. (2021)
Scaphognathops bandanensis (Boonyaratpalin & Srirungroj, 1971)	50	66	10m+6sm+34a	-	Donsakul *et al*. (2007)
	50	66	10m+6sm+34a	4	Phimphan *et al*. (2020)

2. Methods

2.2 Chromosome preparation and staining

Ten male and ten female individuals of *L. rhabdoura* were collected from the Meklong Basin in Kanchanaburi Province, Thailand. Preparation of fish chromosomes was directly conducted from the kidney tissues (Tanomtong *et al*., 2014). The chromosomes were stained with Giemsa's solution for 30 min. Ag-NOR banding was performed by two drops of 2% gelatin and four drops of 50% silver nitrate on the slides. The slides were then incubated at 60°C for 5 min (Howell & Black, 1980).

2.3 Chromosomal analysis

Chromosomes were analyzed and classified following Turpin and Lejeune (1965), which compose of metacentric (m), submetacentric (sm), acrocentric (a) and telecentric (t). Fundamental number (NF) and the analyzed number of chromosome arms that are commonly elucidated as two values for metacentric, submetacentric and acrocentric chromosomes whereas there is only one value for telocentric chromosome. All parameter values were used for karyotyping and idiograming by the methods of Tanomtong *et al*. (2014).

3. Results

3.1 Karyotype and NORs of *L. rhabdoura*

This is the first report of chromosomal information of *L. rhabdoura* showed the diploid chromosome number as $2n = 50$ and NF = 90 in both male and female specimens. The karyotype was composed of 10 metacentric (m), 18 submetacentric (sm), 12 acrocentric (a) and 10 telocentric (t) chromosomes and was without morphologically differentiated sex chromosomes (Figure 2). The karyotype formula for this species was $2n = 50$; $L^{m}_{10} + L^{sm}_{18} + L^{a}_{8} + M^{a}_{4} + M^{t}_{10}$. 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 from male and female of this fish are showed in Table 2. Positive NOR masks were observed in regions adjacent to the telomere of the long arm of the largest submetacentric chromosome pair (pair 6) in both sexes (Figures 3 and 4).

4. Discussion

4.1 Diploid number, fundamental number and karyotype of L. rhabdoura

The diploid chromosome number ($2n$) of *L. Rhabdoura* was 50 which corresponds to most cyprinid species found in Thailand (see Table 1). However, it differs from *E. frenatum* ($2n$=48) (Donsakul & Magtoon, 1993), *Catlocarpio siamensis* and *Probarbus jullieni* (both $2n$=98) (Saenjundaeng *et al*., 2018[a]), *Tor* spp. ($2n$=100) (Arai, 2011). Although the same diploid numbers were revealed in most Cyprinids, the fundamental number (NF) and karyotype with metacentric, submetacentric, acrocentric and telocentric chromosomes, were different between *L. rhabdoura* and other species. This difference results from a process related to the occurrence of pericentric inversions that the mono-arm chromosomes changed to bi-arm chromosomes. Therefore, the NF is increased but the $2n$ was unchanged (Galetti

Jr. *et al*., 2000). Pericentric inversions seem to be the major event of chromosomal rearrangements responsible for the karyotype variability in many genera of cyprinids such as *Puntius* (Sahoo *et al*., 2007), *Devario* (Sukham *et al*., 2013), *Barilius* (Sukham *et al*., 2014) and *Pethia* (Sukham *et al*., 2015). Like all Thai cyprinids, differently morphological sex chromosomes in *L. rhabdoura* were not observed. Moreover, chromosome diversity is found in the Cyprinidae with 2*n* ranging from 44 to 100 chromosomes. Most species share 2*n*=50 (Arai, 2011). This 2*n* was considered as a primitive state for cyprinid karyotypes (Arai, 1982). Chromosomal studies on fishes have provided new information about karyotypical variability at inter and intraspecific levels, which can be of great interest to phylogenetics, systematics and taxonomy (Centofante *et al*., 2002). The present study is the first report on chromosome structure of *L. rhabdoura*.

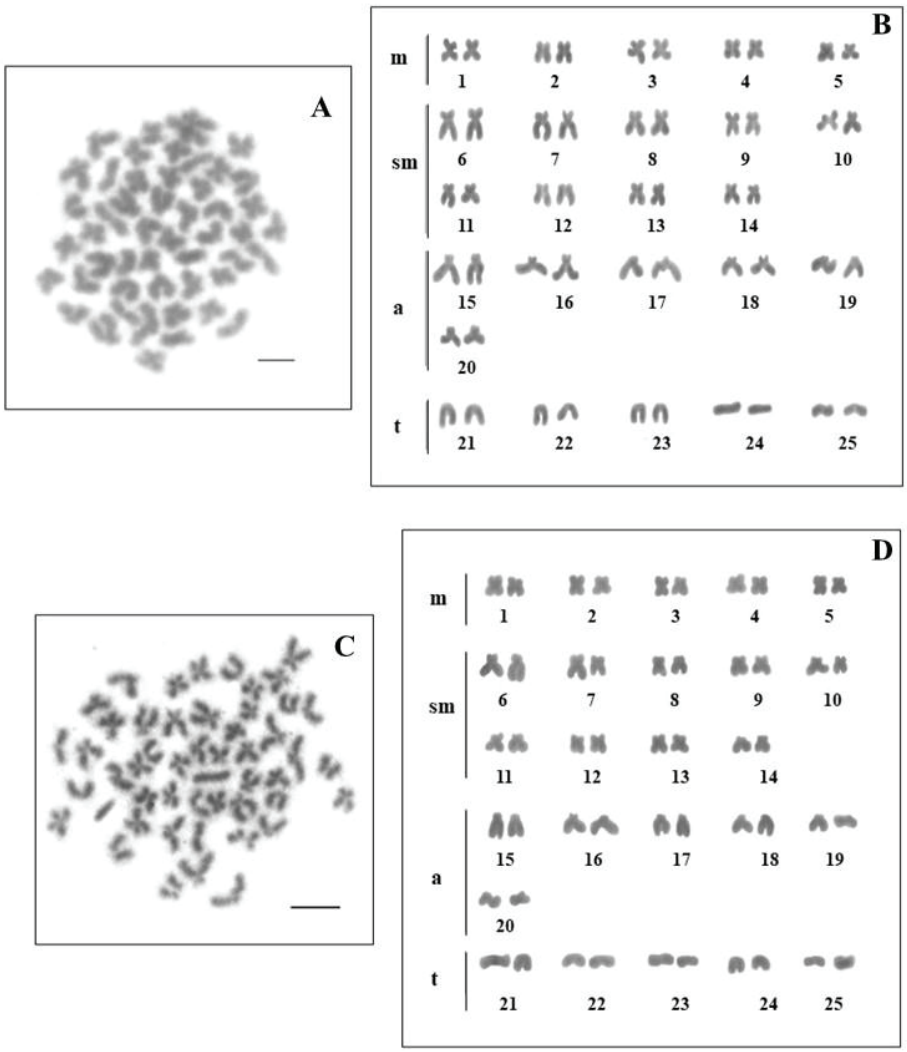

Figure 2. The metaphase chromosome plates and karyotypes of male (A, B) and female (C, D) of *Lobocheilos rhabdoura* (2n=50) by conventional technique. Scale bars indicate 2 μm.

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 types of chromosomes from 20 metaphase chromosomes of both sexes in *Lobocheilos rhabdoura* (2*n* = 50).

Chromosome pair	Ls	Ll	LT	RL ± SD	CI ± SD	Chromosome type	Chromosome size
1	1.076	1.345	2.422	0.047 ± 0.004	0.557 ± 0.023	metacentric	Large
2	1.007	1.224	2.231	0.043 ± 0.003	0.552 ± 0.024	metacentric	Large
3	0.987	1.192	2.178	0.042 ± 0.002	0.550 ± 0.020	metacentric	Large
4	0.944	1.149	2.093	0.040 ± 0.002	0.551 ± 0.022	metacentric	Large
5	0.912	1.135	2.046	0.040 ± 0.004	0.555 ± 0.034	metacentric	Large
6*	0.907	1.571	2.478	0.048 ± 0.006	0.627 ± 0.063	submetacentric	Large
7	0.826	1.571	2.396	0.047 ± 0.004	0.654 ± 0.030	submetacentric	Large
8	0.800	1.490	2.290	0.044 ± 0.003	0.653 ± 0.029	submetacentric	Large
9	0.800	1.390	2.183	0.042 ± 0.003	0.636 ± 0.028	submetacentric	Large
10	0.780	1.337	2.116	0.041 ± 0.003	0.633 ± 0.033	submetacentric	Large
11	0.738	1.346	2.084	0.040 ± 0.003	0.647 ± 0.021	submetacentric	Large
12	0.731	1.274	2.005	0.039 ± 0.003	0.637 ± 0.032	submetacentric	Large
13	0.721	1.316	2.037	0.039 ± 0.006	0.645 ± 0.037	submetacentric	Large
14	0.653	1.412	2.065	0.040 ± 0.006	0.679 ± 0.055	submetacentric	Large
15	0.600	1.838	2.438	0.047 ± 0.007	0.746 ± 0.047	acrocentric	Large
16	0.566	1.749	2.315	0.045 ± 0.005	0.750 ± 0.040	acrocentric	Large
17	0.546	1.717	2.263	0.044 ± 0.003	0.757 ± 0.035	acrocentric	Large
18	0.476	1.638	2.113	0.041 ± 0.004	0.770 ± 0.060	acrocentric	Large
19	0.351	1.518	1.869	0.036 ± 0.004	0.799 ± 0.107	acrocentric	Medium
20	0.239	1.641	1.879	0.036 ± 0.003	0.874 ± 0.122	acrocentric	Medium
21	0.055	1.762	1.817	0.035 ± 0.004	0.965 ± 0.085	telocentric	Medium
22	0.000	1.724	1.724	0.033 ± 0.003	1.000 ± 0.000	telocentric	Medium
23	0.000	1.648	1.647	0.032 ± 0.003	1.000 ± 0.000	telocentric	Medium
24	0.000	1.600	1.590	0.031 ± 0.003	1.000 ± 0.000	telocentric	Medium
25	0.000	1.362	1.362	0.027 ± 0.004	1.000 ± 0.000	telocentric	Medium

(Note; * = NOR- bearing chromosome)

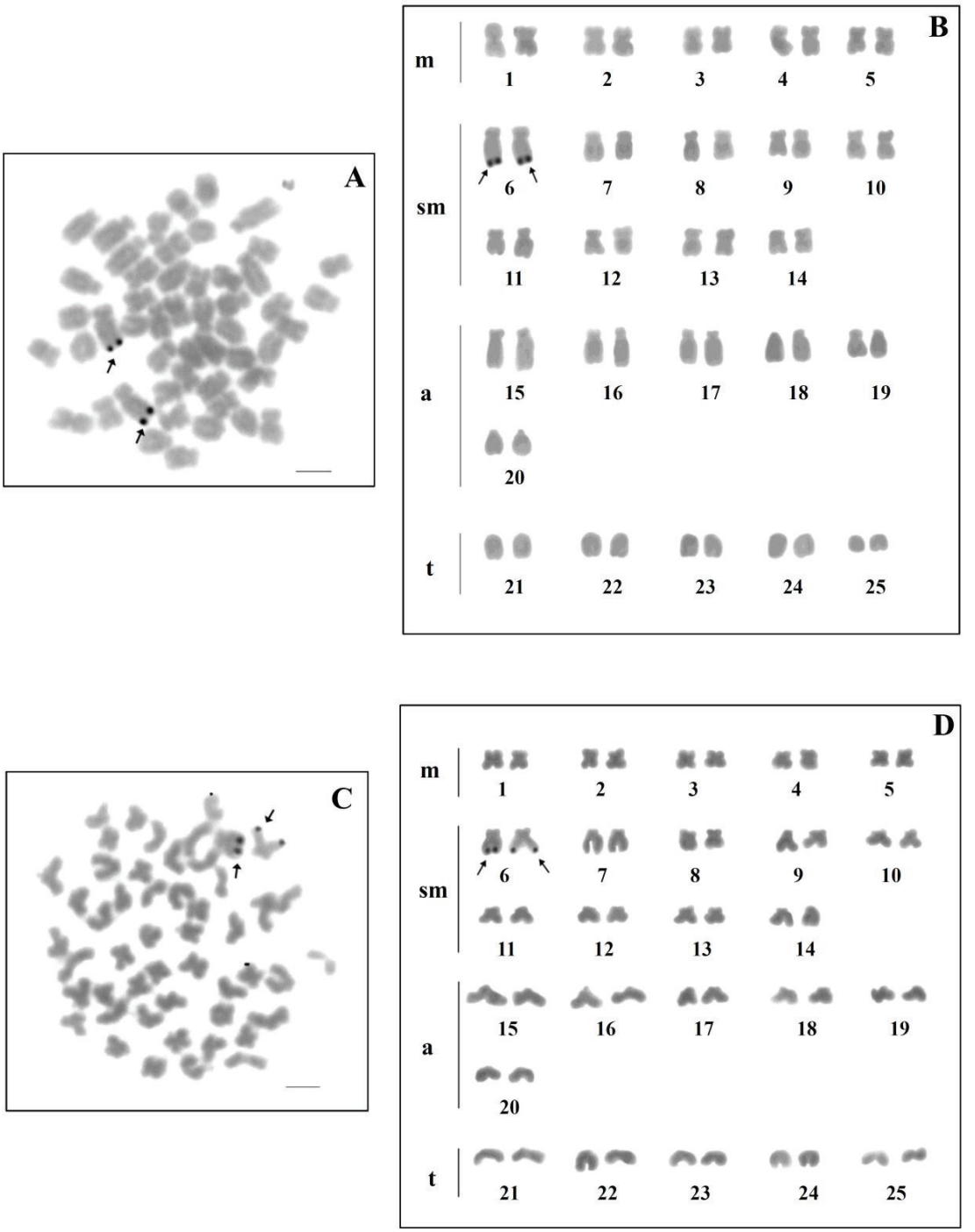

Figure 3. The metaphase chromosome plates and karyotypes of male (A, B) and female (C, D) of *Lobocheilos rhabdoura* (2*n*=50) by NOR-banding technique. Scale bars indicate 2 μm. (Arrows indicate NORs)

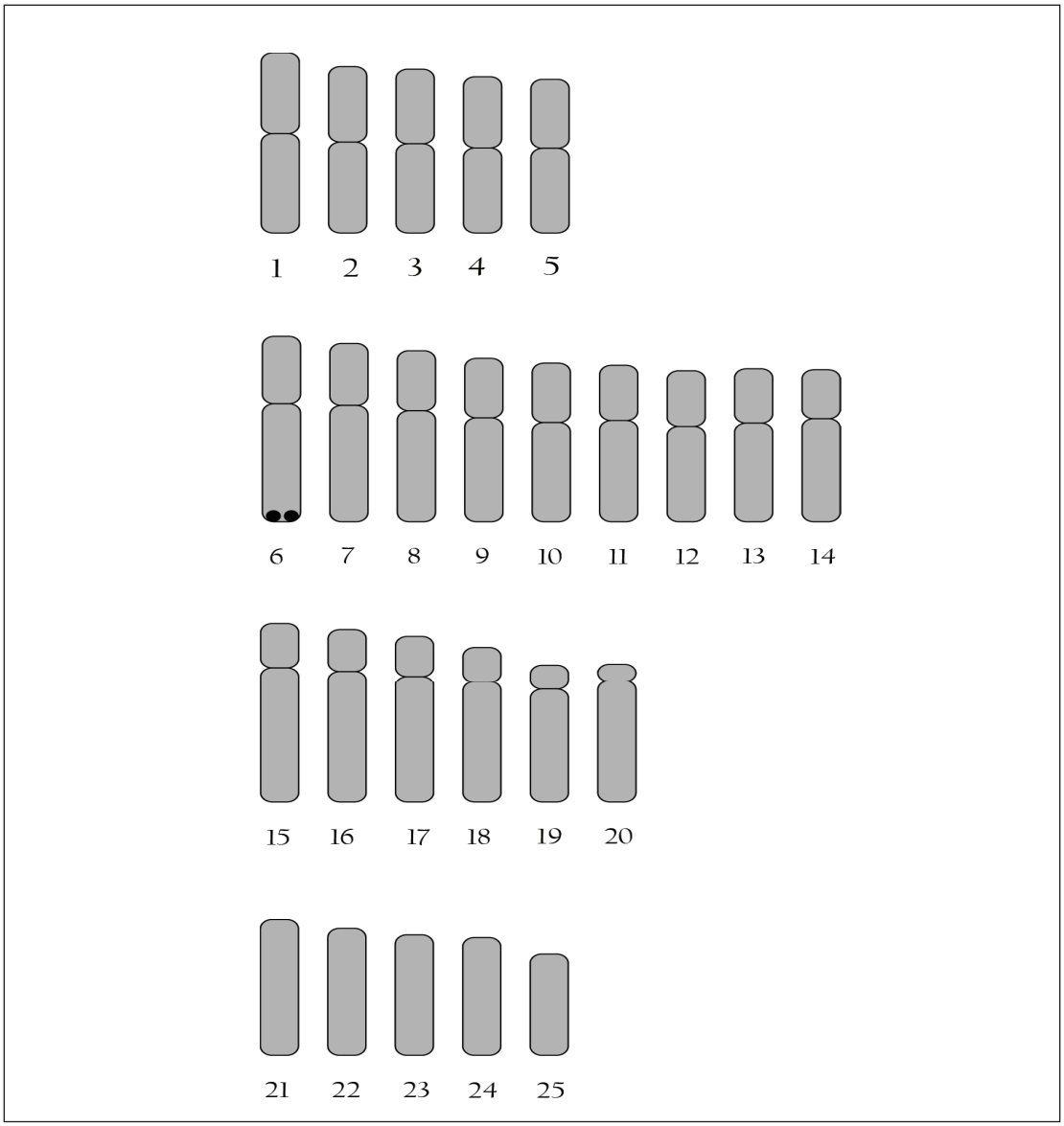

Figure 4. The standardize idiogram of *Lobocheilos rhabdoura* showing lengths and shapes of chromosomes (n = 25) by NOR-banding technique. Black dots indicate telomeric NOR-bearing chromosome pair 6.

4.2 Chromosome marker of Lobocheilos rhabdoura

Silver nitrate staining can detect active rRNA, which is the complementary sequences of DNA that is coded for RNA (rDNA) on the chromosome (Howell & Black, 1980). In *L. rhabdoura*, the NOR locations of active rRNA is on chromosome in the telomere region on the first submetacentric chromosome pair. The single NOR pair corresponds with many cyprinids such as *Puntioplites proctozysron* (Supiwong *et al*., 2012), *Puntius brevis* (Nitikulworawong & Khrueanet, 2014), *Labiobarbus leptocheilus* (Saenjundaeng *et al*., 2018[b]), and seven

Minnows (Aiumsumang *et al*., 2021; 2022). This is a common feature found in many fish groups and other mammals as well as vertebrates (Supiwong *et al*., 2013). However, the species in family Cyprinidae had NORs on more than one pair on chromosomes such as two pairs in *E. frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis* (Phimphan *et al*., 2020), *Cyclocheilichthys armatus* (Chaiyasan *et al*., 2018), three pairs in *Hypsibarbus wetmorei* (Chantapan, 2015), four pairs in *Cyclocheilichthys repasson* (Chaiyasan *et al*., 2020) and *Puntius denisonii* (Nagpure *et al*., 2004). NORs can be the perfect markers to display wide chromosomal polymorphism within and between species in many groups of fish. This variety may affect the NOR number, its localization on the chromosome, size, and active numbers in each genome. Previous NOR studies have shown variations between species, within species, and even between individuals (Galetti *et al*., 1984; Gold *et al*., 1993; Castro *et al*., 1996; Supiwong *et al*., 2012). Structure and number of NOR may be specific to populations, subspecies and species (Supiwong *et al*., 2012). NOR is frequently used to compare variations, as well as to identify and explain specifications (Gold *et al*., 1993). Losses of NOR may result in chromosome rearrangements by Robertsonian translocations (centric fusion), suggesting that rDNA clusters are highly mobile components of the genome (Britton-Davidian *et al*., 2012). The use of NORs in explaining relationships among species depends to a large extent on the uniformity of this characteristic and on the degree of variety within a taxon (Yüksel & Gaffaroðlu, 2008).

5. Conclusion

The present research is the first report of the 2*n*, NF, karyotype and NORs in *L. rhabdoura* which showed 2*n* = 50, NF = 90 that corresponded to fishes in the family Cyprinidae (2*n* = 50). However, there are differences in both the NF and karyotype. *L. rhabdoura* had one pair of NORs but several species in family Cyprinidae showed more than one chromosome pair. The cytogenetics data indicated that this knowledge could be used for classification and as basic information for fish breeding in fisheries.

6. Acknowledgment

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