ลักษณะโครโมโซมเครื่องหมายและแคริโอไทป์ของปลาหลดจุด (*Macrognathus siamensi*) และปลาหลดภูเขา (*M. circumcinctus*)

Characteristics of Chromosome Marker and Karyotype of Mastacembelid fishes, *Macrognathus siamensis* and *M. circumcinctus* (Synbranchiformes, Mastacembelidae)

กฤติมา เสาวกูล และสำเนาว์ เสากูล

Krittima Saowakoon[1]* and Samnao Saowakoon[1]

คณะเกษตรและเทคโนโลยี มหาวิทยาลัยเทคโนโลยีราชมงคล วิทยาเขตสุรินทร์ อำเภอเมือง จังหวัดสุรินทร์ 32000

[1]Faculty of Agriculture and Technology, Rajamangala University of Technology, Surin Campus, Muang Surin 32000

*Corresponding author, E-mail: saowakoon1970@gmail.com

บทคัดย่อ

การวิจัยครั้งนี้มีจุดมุ่งหมายเพื่อศึกษาโครโมโซมเครื่องหมายและแคริโอไทป์ของปลาหลดจุด (*Macrognathus siamensi*s) และปลาหลดภูเขา (*M. circumcinctus*) ของตัวอย่างปลาเพศผู้และเพศเมียอย่างละ 5 ตัว จากคณะเกษตรศาสตร์และเทคโนโลยี มหาวิทยาลัยราชมงคลอีสาน วิทยาเขตสุรินทร์ จังหวัดสุรินทร์ ซึ่งเตรียมโครโมโซมโดยวิธีทางตรงจากไตด้วยวิธีการสับให้ละเอียด ย้อมโครโมโซมแบบธรรมดาด้วยสีกิมซ่าและย้อมแถบแบบนอร์ (NORs) ผลการศึกษาพบว่าปลาหลดจุดมีจำนวนโครโมโซมดิพลอยด์ (2*n*) เท่ากับ 50 แท่ง มีจำนวนโครโมโซมพื้นฐาน (NF) เท่ากับ 66 ทั้งเพศผู้และเพศเมีย ประกอบด้วยโครโมโซมชนิดเมทาเซนทริกขนาดใหญ่ 10 แท่ง อะโครเซนทริกขนาดใหญ่ 6 แท่ง เทโลเซนทริกขนาดใหญ่ 18 แท่ง เทโลเซนทริกขนาดกลาง 12 แท่ง และเทโลเซนทริกขนาดเล็ก 4 แท่ง พบตำแหน่งนอร์บนปลายแขนข้างสั้นของโครโมโซมคู่ที่ 8 และ 24 เป็นโครโมโซมเครื่องหมาย สำหรับปลาหลดภูเขามีจำนวนโครโมโซมดิพลอยด์เท่ากับ 48 แท่ง มีจำนวนโครโมโซมพื้นฐานเท่ากับ 62 ประกอบด้วยโครโมโซมชนิดเมทาเซนทริกขนาดใหญ่ 12 แท่ง เมทาเซนทริกขนาดกลาง 2 แท่ง เทโลเซนทริกขนาดใหญ่ 14 แท่ง และเทโลเซนทริกขนาดกลาง 20 แท่ง พบตำแหน่งนอร์บริเวณปลายแขนข้างสั้นของโครโมโซมคู่ที่ 8 ปลาทั้งสองชนิดตรวจไม่พบความแตกต่างของโครโมโซมเพศระหว่างปลาเพศผู้และเพศเมีย ปลาหลดจุดมีสูตรแคริโอไทป์ $2n\ (50) = L^{m}_{10} + L^{a}_{6} + L^{t}_{18} + M^{t}_{12} + S^{t}_{4}$ และปลาหลดภูเขามีสูตรแคริโอไทป์ $2n\ (48) = L^{m}_{12} + M^{m}_{2} + L^{t}_{14} + M^{t}_{20}$

คำสำคัญ: ปลาหลดจุด ปลาหลดภูเขา โครโมโซม แคริโอไทป์

Abstract

Chromosomal characteristics of nucleolar organizer region (NORs) and karyological analyses of mastacembelid fishes, *Macrognathus siamensis* and *M. circumcinctus* were studies. Kidney cell samples were taken from 5 males and 5 females fish, nourished at the Faculty of Agriculture and Technology Rajamangkala University of Technology Isan Surin campus, Thailand. Mitotic chromosome preparations were prepared directly from the kidneys sampled. Metaphase

spreads were performed on microscopic slides and, thereafter were air-dried. Conventional and Ag-NORs banding techniques were applied to stain the chromosomes. Experimental results showed that *M. siamensis* had the diploid chromosome of 2*n*=50 and the fundamental number (NF) were 66 in both males and females. The karyotype comprised with 10 large metacentric, 6 large acrocentric, 18 large telocentric, 12 medium telocentric and 4 small telocentric. The NORs were found on the short arm of the chromosome pairs, i.e. 8 and 24, which were determined to be chromosome markers for this species. Meanwhile *M. circumcinctus* had the diploid chromosome of 2*n*=48 and the fundamental number were 62 in both sexes. The karyotype comprise with 12 large metacentric, 2 medium metacentric, 14 large telocentric and 20 medium telocentric. The NORs were observed on the short arm of the chromosome pair 8. Sex chromosomes were cytologically indistinguishable for both species. The karyotype formula of *M. siamensis* was designated as: 2*n* (50) = $L^{m}_{10} + L^{a}_{6} + L^{t}_{18} + M^{t}_{12} + S^{t}_{4}$ and *M. circumcinctus* is 2*n* (48) = $L^{m}_{12} + M^{m}_{2} + L^{t}_{14} + M^{t}_{20}$

Keywords: *M. siamensis*, *M. circumcinctus*, chromosome, karyotype

Introduction

Mastacembelids are called "spiny eels" apparently due to its series of detached, depressible spines preceding the soft dorsal fin. The Mastacembelids were belong to perhaps the most highly modified percomorph family, but their relationship to other percomorphs were yet unknown (Plamoottil and Abraham, 2014). Serajddin and Ali (2005) studied and classified the Mastacembelids, and put the thirty-three nominals of freshwater spiny eels under four genera into two subfamilies, namely Mastacembelinae and Afromastacembelinae. These which were belong to the subfamily Mastacembelinae were confined exclusively to Asia while the genera of subfamily Afromastacembelinae were restricted only to Africa. The *Macrognathus siamensis* and *M. circumcinctus* were a genus of Mastacembelid fishes belonging to subfamily Mastacembelinae, family Mastacembelidae suborder Mastacembeloidei and order Perciformes (Tyson, 1986).

Nowadays, *Macrognathus* has been breeding between species. The chromosome studies are considered very important for cytotaxonomy. The study on fish chromosomes is the basic knowledge which can be applied for the several fields such as classification, evolution, heredity, systematic (Gold et al. 1990; Ueda et al. 2001; Barat et al. 2002; Barat and Sahoo 2007), breeding, rapid production of inbred lines and cytotaxonomy (Kirpichnikov, 1981. In genus *Macrognathus*, cytogenetic studies have been performed in 3 species, including *M. aculeatus* (Khuda-Bukhsh and Manna, 1978; Donsakul and Magtoon, 1992), *M. siamensis* and *M. circumcinctus* (Donsakul and Magtoon, 1992) (Table 1). All of these species had 2*n*=48 chromosomes consisting of metacentric, acrocentric and

telocentric chromosomes and no morphologically distinguished sex chromosomes. The structure, number and morphology of nucleolar organizer region (NOR) on chromosome are possibly specific to populations, species and subspecies. NOR has been frequently used to compare between variations, as well as to identify and explain specifications.

In this study, the chromosome marker of *M. siamensis* and *M. circumcinctus* for the first time purposely were investigated. The basic knowledge on cytogenetics can be applied to fish breeding and also used for future studies on taxonomy and evolutionary of Mastacembelid group.

Materials and methods

Sample collection, Chromosome preparation and Chromosome staining

Five male and five female specimens of *M. siamensis* and *M. circumcinctus* individually were obtained from the Faculty of Agriculture and Technology Rajamangkala University of Technology Isan Surin campus, Thailand. Mitotic chromosomes were directly prepared *in vivo* following Supiwong et al. (2013, 2017). Conventional staining was performed using 20% Giemsa's solution for 30 min (Rooney, 2001) and Ag-NOR banding was carried out following the techniques reported by Howell and Black (1980).

Chromosomal checks, Karyotyping and Idiograming

Chromosome counting was carried out on mitotic metaphase cells under the light microscope for 30 cells per specimen to determine the diploid number (2*n*). Twenty clearly observable and well-spread metaphase cells from each male and female were selected and photographed. The short arm length (Ls) and the long arm length (Ll) of individual chromosomes were measured to calculate total length of the chromosome for 20 well-spread metaphase cells. The chromosome types were classified from the methods reported by Turpin and Lejeune (1965) i.e. metacentric, submetacentric, acrocentric and telocentric chromosomes. The karyotyping and idiograming methods were conducted according to Turpin and Lejeune (1965) and Chaiyasut (1989).

Results and discussion

Experimental results showed that *M. siamensis* had diploid chromosome number of 2*n*=50 and had fundamental number (NF)=66 (Fig. 1A-D). The karyotypes of *M. siamensis* comprised with five large metacentric (pairs 1-5), three large acrocentric (pairs 6-8), nine large telocentric (pairs 9-17), six medium telocentric (pairs 18-23) and two small telocentric chromosomes (pairs 24-25). The mean values calculated from twenty mitotic metaphases indicating relative lengths of chromosomes complement ranging from 0.052±0.002 to 0.023±0.004 (Table 2). The NOR was found on the short

arm of chromosome pairs 8 and 24 (Fig. 1E-H). Their respective idiograms of this species from conventional staining and Ag-NOR banding techniques were shown in Fig. 3A. The karyotype formula of *M. siamensis* could be deduced as: 2*n* (50) = L^{m}_{10} + L^{a}_{6} + L^{t}_{18} + M^{t}_{12} + S^{t}_{4}. The numbers of diploid chromosome and NF in this species studied herein are disagree with previous literature (Donsakul and Magtoon, 1992) of which the diploid number reportedly was 2n=48 and NF=58. The differences may be attributed to the specimens utilized in the present work, i.e. collected from local aquarium dealer, which were different from those utilized by the literature and may be due to different criteria used for the chromosome classification. Intra-specific variations of the 2*n* and NF in *M. siamensis* in some ways suggest processes of pericentric inversions between chromosomes during chromosomal evolution (Supiwong, 2012). Furthermore the differences may be attributed to different criteria used for the chromosome classification and inter-populational variation in this genus (Kirpichnikov, 1981).

Table 1. Cytogenetic reviews of fishes in genus *Macrognathus*.

Species	2*n*	NF	Karyotype	Ag-NORs	Locality	Reference
M. aculeatus	48	58	8m+2a+38t	-	-	Manna and Khuda-Bukhsh (1978)
	48	62	14m+4a+30t	-	Thailand	Donsakul and Magtoon (1992)
M. siamensis	48	58	8m+2a+38t	-	Thailand	Donsakul and Magtoon (1992)
	50	66	10m+6a+34t	4	Thailand	Present study
M. circumcinctus	48	64	14m+2a+32t	-	Thailand	Donsakul and Magtoon (1992)
	48	62	14m+34t	2	Thailand	Present study

Remarks: 2*n* = diploid chromosome number, NF = fundamental number (number of chromosome arm), m = metacentric, a = acrocentric, t = telocentric chromosome, NORs = nucleolar organizer regions.

Typical diploid metaphase chromosome which was complement in both male and female of *M. circumcinctus* was 48 and NF=62 (Fig. 2A-D). The chromosome complement consisted of six large metacentric (pairs 1-6), one medium metacentric (pair 7), seven large telocentric (pair 8-14)

and ten medium telocentric chromosomes (pairs 15 and 24). Categorization of chromosomes with karyometric data was shown in Table 3. The mean value of relative length was in a range from 0.051±0.003 to 0.030±0.001. The NOR was presented at subtelomeric regions on the short arms of chromosome pair 8 (Fig. 2E-H). The idiogram are shown in Fig 3B. The proposed karyotype of *M. circumcinctus* was $2n$ (48) = L^{m}_{12} + M^{m}_{2} + L^{t}_{14} + M^{t}_{20}. The number of chromosomes and NF of *M. circumcinctus* herein coincide with those previously reported by Donsakul and Magtoon (1992) (Table 1).

For both fish species, no cytologically distinguishable sex-chromosome was observed. Such research finding was consistent with previous studies reported by Donsakul and Magtoon (1992). It may be possible that the fish's sex-chromosomes were at the initiation of differentiation and hence these chromosomes containing the sex determination gene could not be detected by the cytogenetic analyses undertaken. The origin and development of sex-chromosomes had been reported for Neotropical fish in Brazil (Bertollo et al., 2004).

The objective of Ag-NOR banding technique is to reach out the nucleolar organizer region which is the representative location of genes (loci) that function in ribosome synthesis (18S and 28S ribosomal RNA) (Sharma et al., 2002). If these regions were active during the interphase prior to mitosis, they could be detected by silver nitrate staining (Howell and Black, 1980) because of its specific stains which were a set of acidic protein related to ribosomal synthesis process. The technique actually revealed active NORs (Ag-NORs), excluding the rDNA associated to NORs (Jordan, 1987). Furthermore, our present study was considered the pioneer reporting NORs bearing chromosome which has been characterized for *M. siamensis* and *M. circumcinctus* that were found on two pair of large acrocentric and small telocentric, and one pairs of large telocentric chromosomes, respectively (Fig. 1E-H and 2E-H). The NOR-bearing pairs represented an efficient cytotaxonomic marker for most of the analyzed species. The research findings importantly highlighted that chromosome morphology and the NOR location are considerably useful cytological characters for taxonomic and evolutionary studies in the Mastacembelid fishes.

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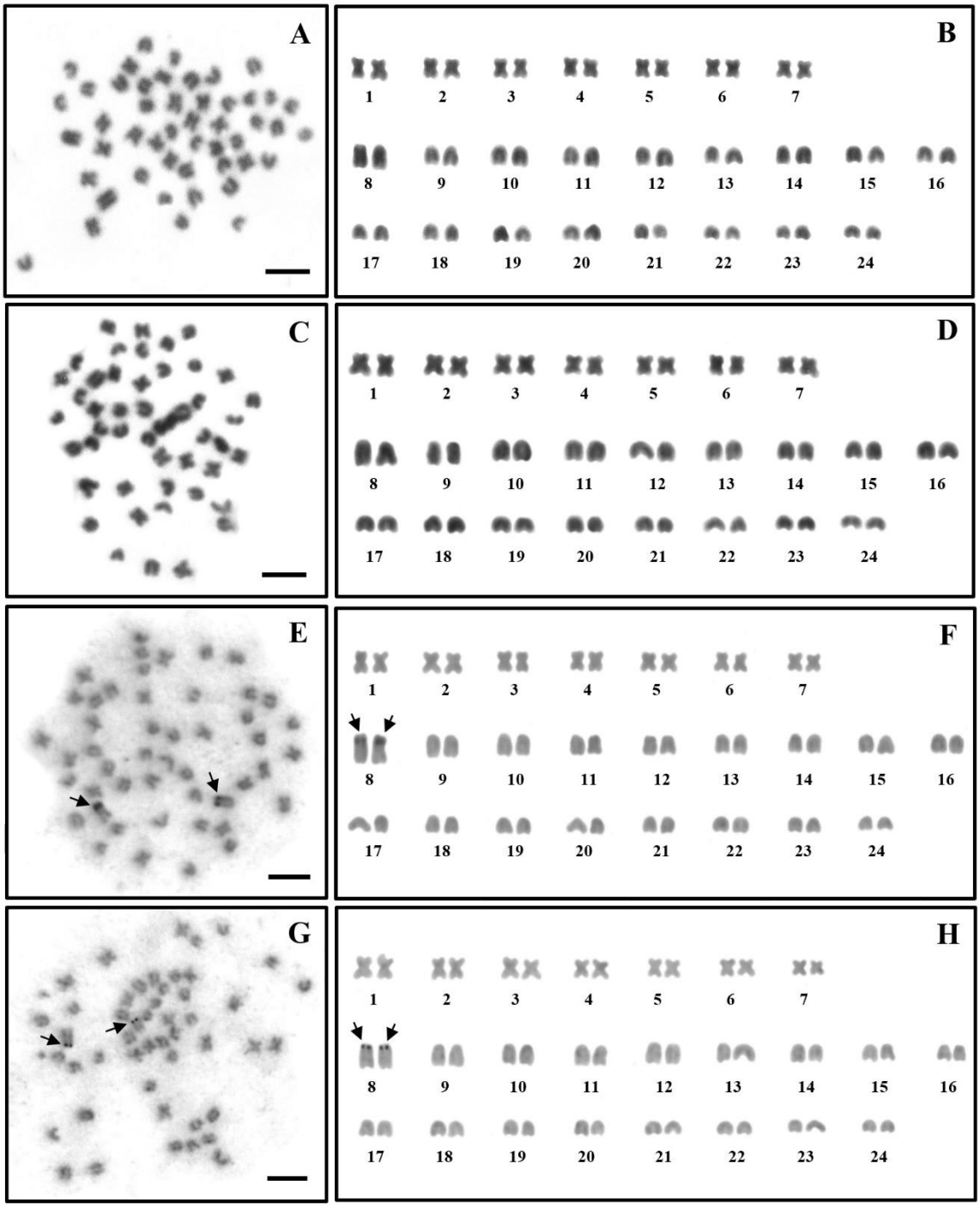

Figure 1 Metaphase chromosome plates and karyotypes of *Macrognathus siamensis* male (A, B, E and F), female (C, D, G and H) $2n=50$ by conventional staining and Ag-NOR banding techniques. Arrow indicates nucleolar organizer regions/NORs (scale bars indicate 5 micrometers).

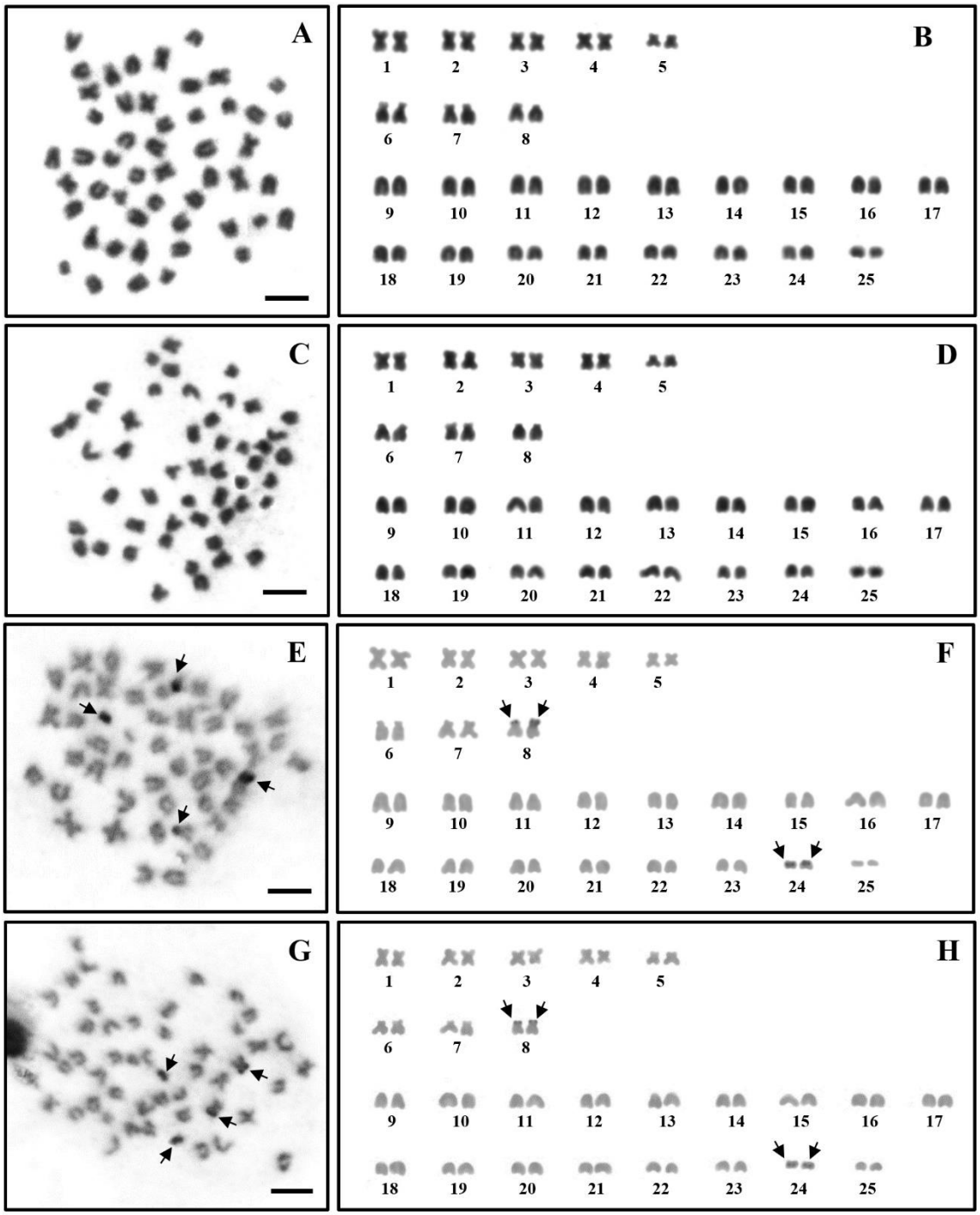

Figure 2 Metaphase chromosome plates and karyotypes of *Macrognathus circumcinctus* male (A, B, E and F), female (C, D, G and H) $2n=48$ by conventional staining and Ag-NOR banding techniques. Arrow indicates nucleolar organizer regions/NORs (scale bars indicate 5 micrometers).

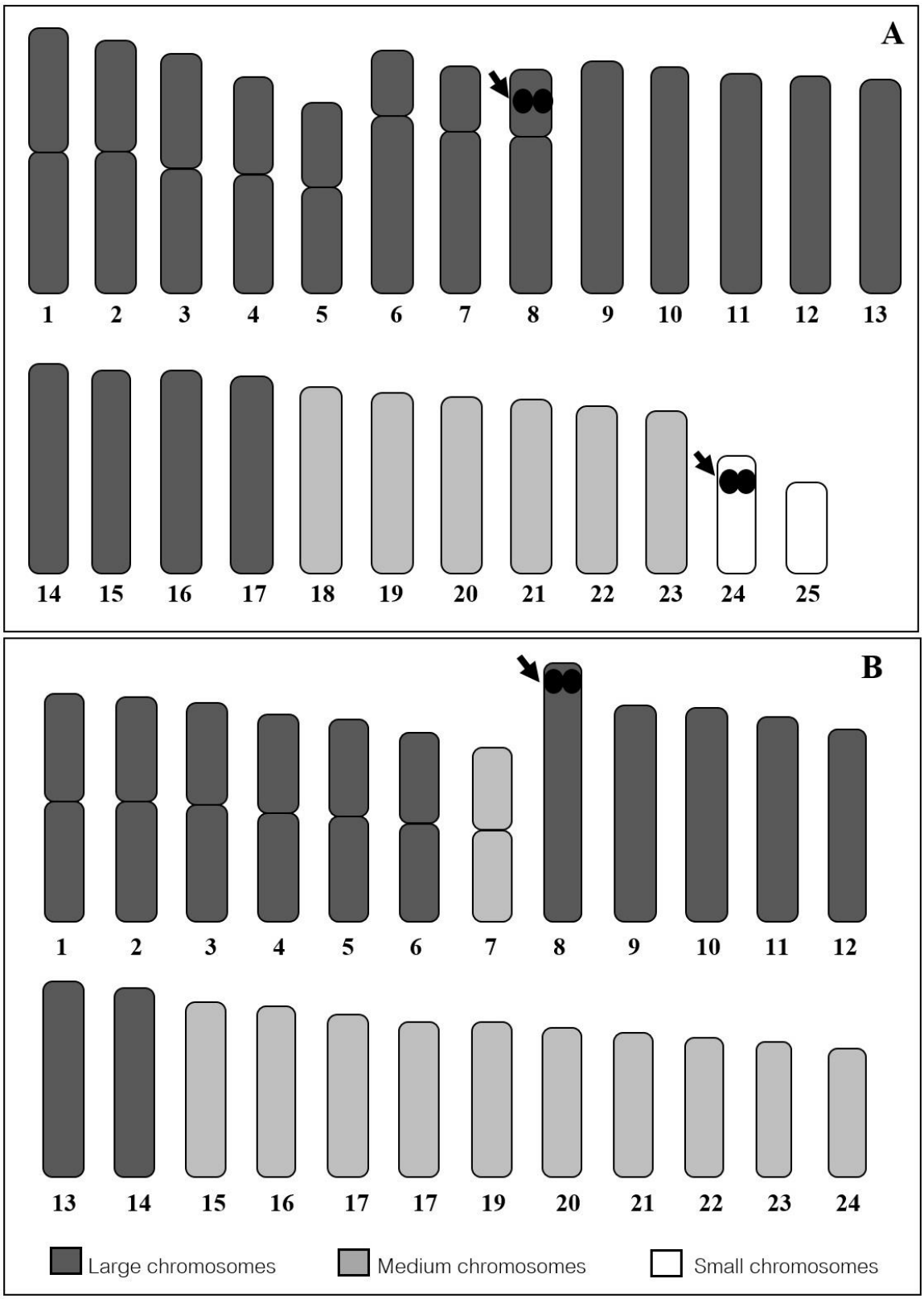

Figure 3 Idiograms of showing lengths and shape of chromosomes of the *Macrognathus siamensis* (A) and *M. circumcinctus* (B). Arrow indicates nucleolar organizer regions/NORs.

Table 2 Mean length of short arm chromosome (Ls), length of long arm chromosome (LI), length of total chromosomes (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphases of male and female *Macrognathus siamensis*, 2*n* = 50.

Chromosome pair	Ls (μm)	LI (μm)	LT (μm)	RL±SD	CI±SD	Chromosome size	Chromosome type
1	1.678	1.910	3.588	0.052±0.002	0.533±0.039	Large	Metacentric
2	1.515	1.908	3.423	0.050±0.002	0.558±0.026	Large	Metacentric
3	1.540	1.681	3.221	0.047±0.002	0.523±0.033	Large	Metacentric
4	1.308	1.601	2.909	0.042±0.001	0.549±0.018	Large	Metacentric
5	1.151	1.435	2.587	0.038±0.005	0.555±0.018	Large	Metacentric
6	0.887	2.400	3.286	0.048±0.003	0.730±0.032	Large	Acrocentric
7	0.884	2.204	3.088	0.045±0.002	0.714±0.005	Large	Acrocentric
8*	0.900	2.117	3.017	0.044±0.001	0.702±0.046	Large	Acrocentric
9	0.000	3.132	3.132	0.045±0.003	1.000±0.000	Large	Telocentric
10	0.000	3.057	3.057	0.045±0.001	1.000±0.000	Large	Telocentric
11	0.000	2.962	2.962	0.043±0.001	1.000±0.000	Large	Telocentric
12	0.000	2.928	2.928	0.043±0.001	1.000±0.000	Large	Telocentric
13	0.000	2.885	2.885	0.042±0.002	1.000±0.000	Large	Telocentric
14	0.000	2.836	2.836	0.041±0.001	1.000±0.000	Large	Telocentric
15	0.000	2.749	2.749	0.040±0.001	1.000±0.000	Large	Telocentric
16	0.000	2.744	2.744	0.040±0.002	1.000±0.000	Large	Telocentric
17	0.000	2.668	2.668	0.039±0.000	1.000±0.000	Large	Telocentric
18	0.000	2.517	2.517	0.037±0.001	1.000±0.000	Medium	Telocentric
19	0.000	2.438	2.438	0.036±0.002	1.000±0.000	Medium	Telocentric
20	0.000	2.378	2.378	0.035±0.001	1.000±0.000	Medium	Telocentric
21	0.000	2.350	2.350	0.034±0.001	1.000±0.000	Medium	Telocentric
22	0.000	2.264	2.264	0.033±0.002	1.000±0.000	Medium	Telocentric
23	0.000	2.193	2.193	0.032±0.007	1.000±0.000	Medium	Telocentric
24*	0.000	1.745	1.745	0.026±0.002	1.000±0.000	Small	Telocentric
25	0.000	1.583	1.583	0.023±0.004	1.000±0.000	Small	Telocentric

Remark: * = NORs bearing chromosomes (satellite chromosome)

Table 3 Mean length of short arm chromosome (Ls), length of long arm chromosome (LI), length of total chromosomes (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphases of male and female *Macrognathus circumcinctus*, 2*n* = 48.

Chromosome pair	Ls (μm)	LI (μm)	LT (μm)	RL±SD	CI±SD	Chromosome size	Chromosome type
1	1.614	1.801	3.416	0.051±0.003	0.538±0.024	Large	metacentric
2	1.563	1.804	3.367	0.051±0.001	0.540±0.009	Large	metacentric
3	1.527	1.757	3.284	0.049±0.003	0.539±0.007	Large	metacentric
4	1.468	1.636	3.104	0.046±0.001	0.535±0.017	Large	metacentric
5	1.465	1.573	3.038	0.045±0.001	0.517±0.010	Large	metacentric
6	1.366	1.472	2.838	0.043±0.001	0.526±0.023	Large	metacentric
7	1.306	1.378	2.684	0.040±0.002	0.525±0.019	Medium	metacentric
8*	0.000	3.890	3.890	0.058±0.004	1.000±0.000	Large	telocentric
9	0.000	3.249	3.249	0.049±0.001	1.000±0.000	Large	telocentric
10	0.000	3.215	3.215	0.048±0.002	1.000±0.000	Large	telocentric
11	0.000	3.068	3.068	0.046±0.004	1.000±0.000	Large	telocentric
12	0.000	2.887	2.887	0.043±0.001	1.000±0.000	Large	telocentric
13	0.000	2.832	2.832	0.042±0.002	1.000±0.000	Large	telocentric
14	0.000	2.713	2.713	0.041±0.003	1.000±0.000	Large	telocentric
15	0.000	2.633	2.633	0.039±0.002	1.000±0.000	Medium	telocentric
16	0.000	2.648	2.648	0.040±0.002	1.000±0.000	Medium	telocentric
17	0.000	2.365	2.365	0.036±0.000	1.000±0.000	Medium	telocentric
18	0.000	2.404	2.404	0.036±0.002	1.000±0.000	Medium	telocentric
19	0.000	2.408	2.408	0.036±0.001	1.000±0.000	Medium	telocentric
20	0.000	2.313	2.313	0.035±0.001	1.000±0.000	Medium	telocentric
21	0.000	2.228	2.228	0.033±0.002	1.000±0.000	Medium	telocentric
22	0.000	2.163	2.163	0.032±0.001	1.000±0.000	Medium	telocentric
23	0.000	2.088	2.088	0.031±0.002	1.000±0.000	Medium	telocentric
24	0.000	1.997	1.997	0.030±0.001	1.000±0.000	Medium	telocentric

Remark: * = NORs bearing chromosomes (satellite chromosome)

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