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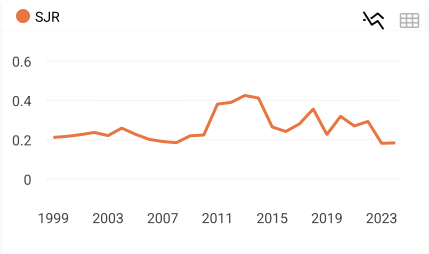

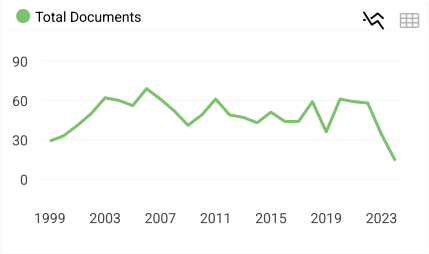

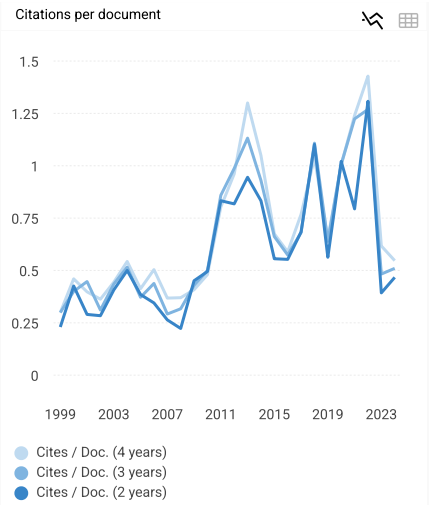

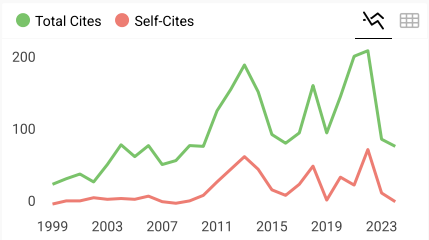

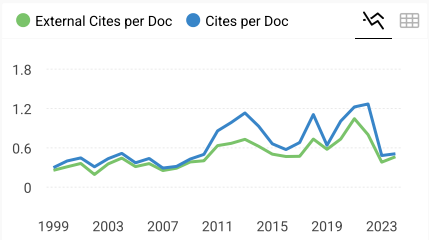

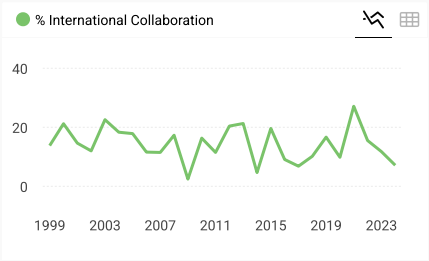

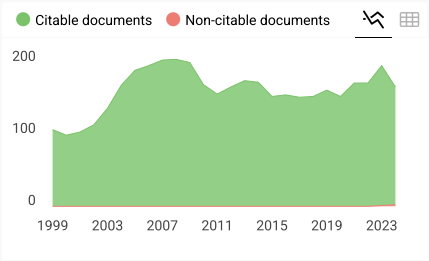

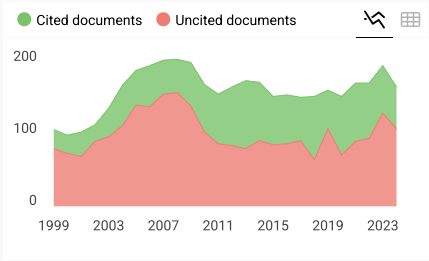

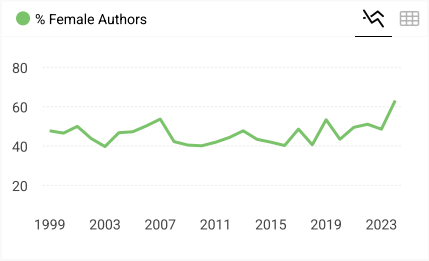

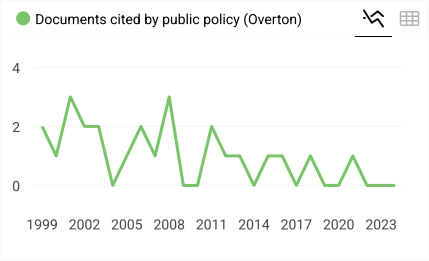

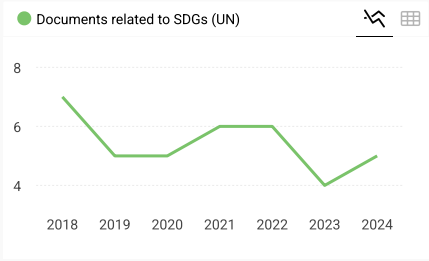

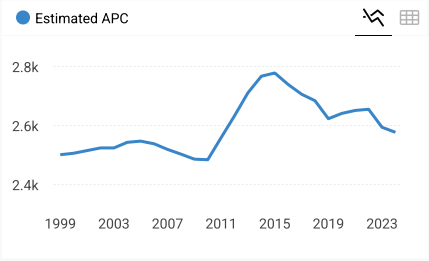

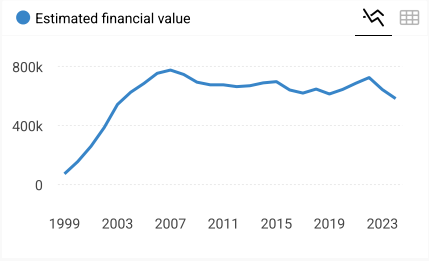

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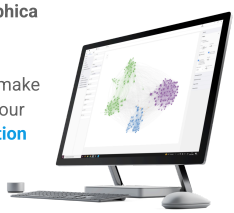

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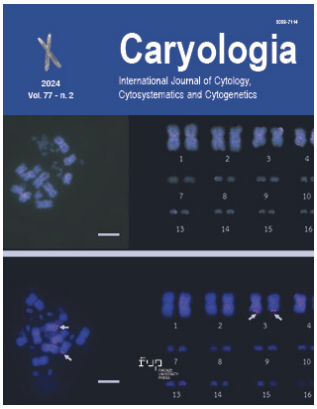

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Parisa Fouroutan, Masoud Sheidai, Fahimeh Koohdar

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First chromosome characterization and repetitive DNA of Barred Gliding Lizard, Draco taeniopterus Günther, 1861 (Draconinae: Agamidae: Squamata)

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Praween Supanuam, Sitthisak Jantarat, Warakarn Khawporntip, Thaintip Kraiprom, Somsak Bauthip, Sarun Jumrusthanasan, Sarawut Kaewsri, Nattasuda Donbundit, Sukhonthip Ditcharoen, Weera Thongnetr, Sumalee Phimphan, Alongklod Tanomtong

Cytotoxic assessment of aqueous extracts of Heliotropium keralense Sivar. & Manilal on Allium cepa root tip cells

DOI: https://doi.org/10.36253/caryologia-2436

Athira R. Mohan, Nisha Joseph

Chromosomal variations and genetic diversity in subpopulations of Senna alexandrina Mill. from Western Thar, India

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Sunita Arora, Monika Vyas, Meena Barupal

Application of Genomic In Situ Hybridization (GISH) and tandem repeat sequence amplification for identification of Erianthus – Saccharum introgression

DOI: https://doi.org/10.36253/caryologia-2670

Valiya Purakkal Sobhakumari, Krishnasamy Mohanraj

Mapping of five classes of repetitive DNAs and microsatellite repeats in the genome of the Rainbow Shark, Epalzeorhynchos frenatum (Fowler, 1934)

DOI: https://doi.org/10.36253/caryologia-2596

Kamika Sribenja, Nuntaporn Getlekha

Natural hybridization between Iris minutoaurea Makino and Iris odaesanensis Y. N. Lee in Korea: evidence from cytological traits

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65-72

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First chromosome characterization and repetitive DNA of Barred Gliding Lizard, *Draco taeniopterus* Günther, 1861 (Draconinae: Agamidae: Squamata)

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Abstract. This research was the first report on karyological analysis and distribution patterns of repetitive DNA using the fluorescence in situ hybridization (FISH) technique on the barred gliding lizard, *Draco taeniopterus* Günther, 1861. The 10 male and 10 female specimens were collected from Than To district, Yala province, Thailand. Chromosome preparation was performed by direct method using bone marrow and testis. The chromosomes were stained using conventional staining, NOR-banded, and FISH technique with $d(GC)_{15}$, $d(TA)_{15}$, $d(CAG)_{10}$, and $d(CAA)_{10}$ microsatellite probes. The karyotype of the barred gliding lizard reveals a diploid chromosome number of 34 and a fundamental chromosome number of 46, comprising of 8 pairs of large metacentric chromosomes, 2 pairs of small metacentric chromosomes, 2 pairs of large submetacentric chromosomes, and 22 pairs of microchromosomes, no sex chromosome detection between male and female karyotype. The metaphase I showed 17 bivalents and metaphase II showed haploid, n=17. The NOR is observed on the telomeric region of the last microchromosome pair 17th. Microsatellite repeat patterns indicate the presence of $d(GC)_{15}$ and $d(CAG)_{10}$ show specific regions, 2qter and 3qter respectively. While $d(TA)_{15}$ and $d(CAA)_{10}$, show cumulative signals dispersed throughout the chromosomes. This research can provide additional fundamental information for future genetic studies. The barred gliding lizard has the following karyotype formula: $2n=34=L^{m}_{8}+L^{sm}_{2}+S^{m}_{2}+22mi$.

Keywords: *Draco taeniopterus*, Chromosome, Cytogenetics, Repetitive DNA.

INTRODUCTION

Flying lizard genus *Draco* are classified in family Agamidae, subfamily Draconinae which consists of 34 genera and 272 species, the important genera such as *Acanthosaura, Calotes, Diploderma, Draco, Gonocephalus, Japalura* and *Sitana*. In the genus *Draco*, 40 species are found, which is distributed from Southwest India through Southeast Asia, including the Malay Peninsula, the Philippines, and Thailand (Honda et al. 2000; McGuire & Heang 2001; Hoser 2014; Denzer et al. 2015; Nampochai et al. 2021).

Barred gliding lizard or spotted flying dragon (*Draco taeniopterus* Günther, 1861), It is a species that indicates the abundance of tropical rainforest ecosystems. The typical characteristics of this lizard are its small body size (66-78 mm from the tip of the mouth to the anus and the tail 136-153 mm long), flat body, small head, the extended dulap (chin flap) is yellow-orange. Patagium has 4-5 distinct dark transverse bands alternative to light transverse bands and presence of light spots in the middle of dark bands. These colors are useful to help camouflage there to match the bark. Tympanum uncovered with scales. Snout without a series of scales forming a Y-shaped figure. Nostril directed upward (Figure 1). The *Draco taeniopterus* is found in Myanmar, Thailand, Cambodia, and Malaysia. The conservation status of this species is least concern (Honda et al. 1999; 2000; Srichairat et al. 2014; 2015; 2017; Visoot et al. 2023)

Cytogenetic review of the genus *Draco* has only 2 reports in 3-4 species with conventional technique. The Draco karyotype is 2n=34 with 16 macrochromosomes and 18 microchromosomes, without sex-chromosomes. The karyotype report of Draconinae also not prevailing. The diploid number of this subfamily has appeared in several genera, *Gonocephalus* (2n=36), *Calotes* (2n=32-34), *Japalura* (2n=34,46), *Sitana* (2n=34, 36, 46) (Ota and Hikida 1989; Sharma and Nakhasi 1980; Li et al. 1981; Ota 1988; Solleder and Schmid 1988; Ota et al. 1992; Kritpetcharat et al. 1999; Diong et al. 2000; Ota et al. 2002; Singh and Banerjee 2004; Zongyun et al. 2004; Patawang et al. 2015a).

This research is first report on molecular cytogenetics of the genus *Draco*. Conventional, meiotic configuration, Ag-NOR banding and molecular cytogenetic techniques using microsatellite DNA probes including, $d(TA)_{15}$, $d(CG)_{15}$, $d(CAA)_{10}$ and $d(CAG)_{10}$ were applied to detect. This study is useful for taxonomy, conservation and basic and in-depth cytogenetic information of this species.

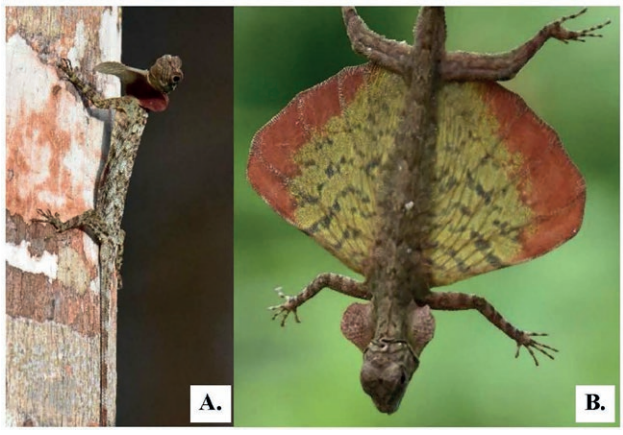

Figure 1. General characteristics (A.) and its patagium (B.) of barred gliding lizard, *Draco taeniopterus*, Draconinae, Agamidae) from Ban Wang Sai, Mae Wat subdistrict, Than To District, in Yala Province, Thailand.

MATERIALS AND METHODS

The 10 male and 10 female specimens, barred gliding lizard (*Draco taeniopterus*) were collected from Ban Wang Sai, Mae Wat subdistrict, Than To District, in Yala Province, Thailand. The flying lizard were transferred to the laboratory and identified according to the morphological criteria of KEY (Chan-Ard et al. 2015; Das 2015). Experiments were performed in accordance with ethical protocols, as approved by the Ethics Committee of Prince of Songkla University (Ref No.AI003/2022).

Chromosomes were directly prepared *in vivo* (Patawang et al., 2018a) as follows. The animals were injected on their abdominal cavity with colchicine. Then leaved for 24 hours. Chromosome preparation containing bone marrow for mitosis and testis for meiosis were conducted by the colchicine-hypotonic-fixation-air drying technique. The chromosomes were stained with 20% Giemsa's for 30 minutes and identified for NORs by Ag-NOR staining according to Howell and Black (1980) and Verma and Babu (1995). Chromosomal checks were performed on mitotic metaphase cells under light microscope.

FISH experiments were performed under high stringency conditions (Yano et al. 2017) to classify microsatellite sequences, specifically $(TA)_{15}$, $(GC)_{15}$, $(CAA)_{10}$, and $(CAG)_{10}$. These sequences were directly labeled by Cy3 at the 5'end during synthesis (Sigma, St. Louis, MO, USA) . FISH was performed under stringent conditions and hybridization occurred overnight in a moist chamber at 37 °C. Chromosomes were counterstained with 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI, 1.2 µg/ml) mounted in antifade solu-

tion (Vector, Burlingame, CA, USA,) (Aiumsumang et al. 2022; Patawang et al. 2022; Prasopsin et al. 2022; Thongnetr et al. 2022).

At least 20 metaphase spreads per individual were analyzed to confirm the diploid number, karyotype structure, NORs and FISH data. Images were captured using an Axioplan II microscope (Carl Zeiss Jena GmbH, Germany) with CoolSNAP and processed using Image Pro Plus 4.1 software (Media Cybernetics, Silver Spring, MD, USA). Chromosomes were classified according to centromere position as metacentric (m), submetacentric (sm) and acrocentric (a) (Tanomtong et al. 2019). For the chromosomal arm number (NF; fundamental number), m, sm, a were scored as bi-armed while t as mono-armed. The microchromosomes are chromosomes that are 5 times less long than the largest pair of chromosomes (Patawang et al. 2016; 2017; 2018b).

RESULTS AND DISCUSSION

Barred gliding lizard (*Draco taeniopterus*) had a diploid number of 34. The karyotype comprised eight large metacentric, two large submetacentric, two small metacentric and 22 microchromosomes. The fundamental number was 46 in both sexes and no sex chromosome heteromorphisms were evident (Table 1 and Figure 2). The karyotype formula of *Draco taeniopterus* is $2n=34=L^{m}_{8}+L^{sm}_{2}+S^{m}_{2}+22mi$. The diploid chromosome number is following previous studies of 4 species of genus *Draco* (Ota and Hikida 1989, Kritpetcharat et al. 1999). The diploid numbers of subfamily Draconinae are 2n=32-46 in 7 genera 21 species (Ota and Hikida 1989; Sharma and Nakhasi 1980; Li et al. 1981; Ota 1988; Solleder and Schmid 1988; Ota et al. 1992; Kritpetcharat et al. 1999; Diong et al. 2000; Ota et al. 2002; Singh and Banerjee 2004; Zongyun et al. 2004; Patawang et al. 2015a). Some species of Draconinae has polymorphism, the *Calotes versicolor* from India has 2n=32 and 34, the *Japarula swinhonis swinhonis* from Taiwan has 2n=36, 40, and 46. This species exhibits no sex differences in karyotypes between males and females, no cytologically distinguishable sex chromosome was observed to be similar to the *Draco cornutus, D. haematopogon, D. quinquefasciatus and D. belliana*. The karyotypes of this genus are quite similar. All species have 12-16 macro-metacentric or submetacentric chromosomes, and 18-22 microchromosomes. The mechanism of chromosomes rearrangement maybe fission, fusion and/or pericentric inversion. Comparative chromosome studies of subfamily Draconinae is show on Table 2 (Ota and Hikida 1989; Sharma and Nakhasi 1980; Li et al. 1981; Ota 1988; Solleder and Schmid 1988; Ota et al. 1992; Kritpetcharat et al. 1999; Diong et al. 2000; Ota et al. 2002;

Table 1. Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosomes (LT), relative length (RL), centromeric index (CI) and standart deviation (SD) from 10 metaphases of male and female of barred gliding lizard (*Draco taeniopterus*), 2*n* (diploid)=34.

Chromosome pairs	Ls (μm)	Ll (μm)	LT (μm)	CI±SD	RL±SD	Chromosome size	Chromosome type
1	7.69	8.61	16.30	0.528±0.000	0.184±0.000	Large	metacentric
2	5.97	8.45	14.41	0.586±0.000	0.163±0.000	Large	metacentric
3	5.37	6.09	11.46	0.532±0.000	0.129±0.000	Large	metacentric
4	5.25	5.59	10.84	0.515±0.000	0.122±0.000	Large	metacentric
5	2.87	6.15	9.02	0.683±0.000	0.102±0.000	Large	submetacentric
6	2.83	3.44	6.27	0.550±0.000	0.070±0.000	Small	metacentric
7	0.00	2.22	2.22	1.000±0.000	0.025±0.000	microchromosome	
8	0.00	2.13	2.13	1.000±0.000	0.024±0.000	microchromosome	
9	0.00	2.06	2.06	1.000±0.000	0.023±0.000	microchromosome	
10	0.00	2.04	2.04	1.000±0.000	0.023±0.000	microchromosome	
11	0.00	1.96	1.96	1.000±0.000	0.022±0.000	microchromosome	
12	0.00	1.89	1.89	1.000±0.000	0.021±0.000	microchromosome	
13	0.00	1.67	1.67	1.000±0.000	0.019±0.000	microchromosome	
14	0.00	1.66	1.66	1.000±0.000	0.019±0.000	microchromosome	
15	0.00	1.71	1.71	1.000±0.000	0.019±0.000	microchromosome	
16	0.00	1.58	1.58	1.000±0.000	0.018±0.000	microchromosome	
17*	0.00	1.45	1.45	1.000±0.000	0.016±0.000	microchromosome	

* = NORs bearing chromosomes.

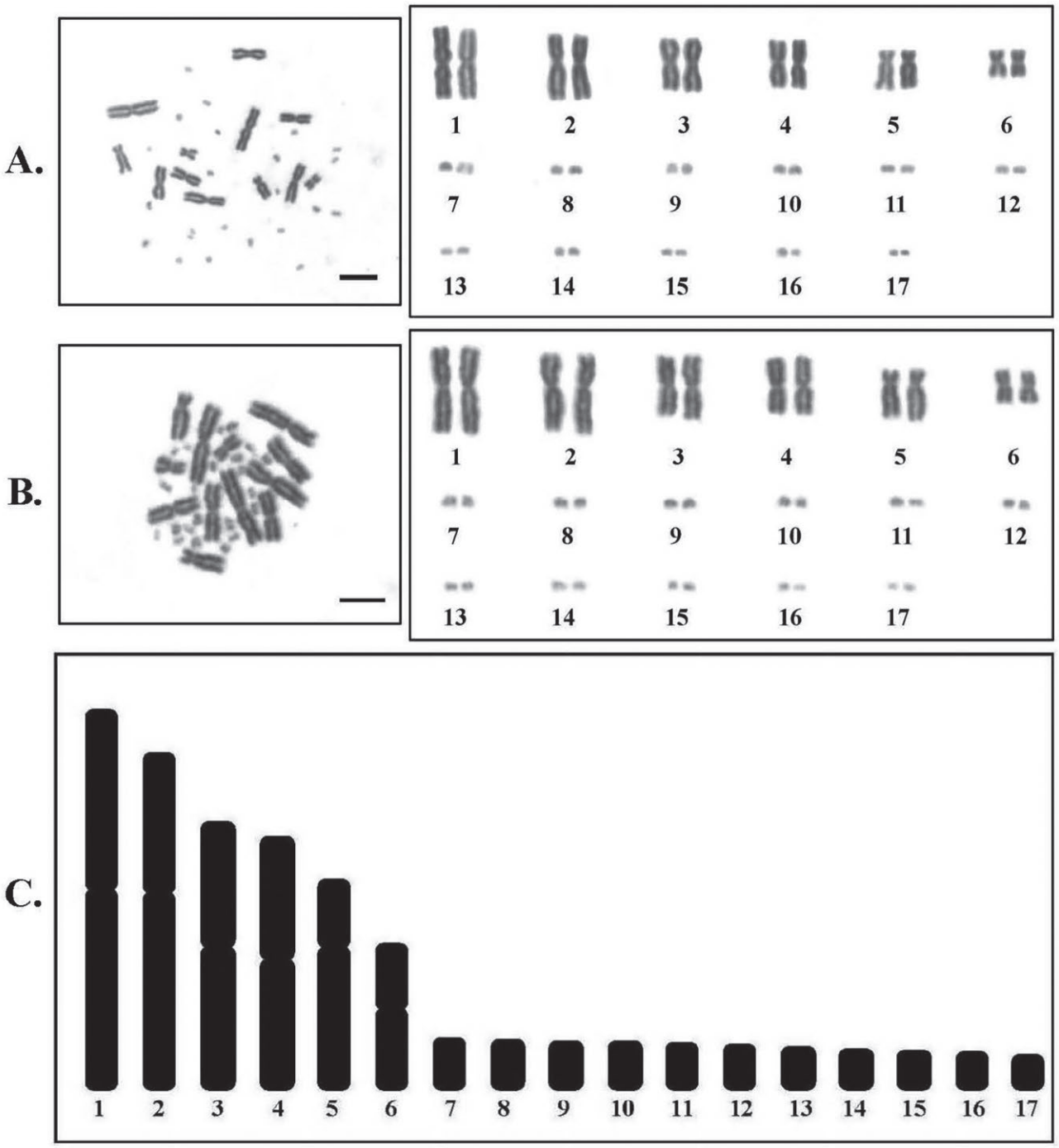

Figure 2. Metaphase plates and standardized karyotypes of male (A.), female (B.) and Idiogram (C.) of barred gliding lizard, *Draco taeniopterus*, 2n=34 by conventional staining (Scale bars = 10 μm).

Singh and Banerjee 2004; Zongyun et al. 2004; Patawang et al. 2015a).

The first cytogenetic study of *Draco taeniopterus* carried out by Ag-NOR banding technique was obtained from this research. We found NORs observed in the region adjacent the last smallest microchromosomes (pair 17th) (Figure 3). The report of NOR position in Draconinae was located on telomeric region of q-arm of pair 2nd in 4 species of *Calotes* including *C. cristatellus, C. emma, C. mystaceus,* and *C. versicolor* (Solleder and Schmid

Table 2. Comparative chromosome studies of subfamily Draconinae.

Species	2n	Karyotype	NOR	Locality	References
Acanthosaura armata	32	12m+20mi	-	Malaysia	Ota et al. (2002)
Bronchocela cristatella	34	14m+20mi	-	Singapore	Ota et al. (2002)
	34	12m/sm+22mi	2qter	Asia	Solleder and Schmid (1988)
Calotes cristatellus	34	12m/sm+22mi	2qter	Asia	Solleder and Schmid (1988)
C. emma					
	34	12m/sm+22mi	-	Thailand	Kritpetcharat et al. (1999)
	34	12m+22mi	-	Malaysia	Ota et al. (2002)
	34	-	-	India	Singh and Banerjee (2004)
C. jerdoni	34	12m/sm+22mi	-	India	Sharma and Nakhasi (1980)
	34	-	-	India	Singh and Banerjee (2004)
C. mystaceus	34	12m/sm+22mi	2qter	Asia	Solleder and Schmid (1988)
	34	12m/sm+22mi	-	Thailand	Kritpetcharat et al. (1999)
	34	-	-	India	Singh and Banerjee (2004)
	34	10m+2m+22mi	2qter	Thailand	Patawang et al. (2015a)
C. versicolor	34	12m/sm+22mi	-	India	Sharma and Nakhasi (1980)
	34	12m/sm+22mi	2qter	Asia	Solleder and Schmid (1988)
	34	12m/sm+22mi	-	Thailand	Kritpetcharat et al. (1999)
	34	12m+22mi		Singapore	Ota et al. (2002)
	32, 34	-	-	India	Singh and Banerjee (2004)
	34	12m/sm+22mi	2qter	Thailand	Patawang et al. (2015a)
Draco cornutus	34	16m+18mi	-	Malaysia	Ota and Hikida (1989)
D. haematopogon	34	16m+18mi	-	Malaysia	Ota and Hikida (1989)
D. quinquefasciatus	34	16m+18mi	-	Malaysia	Ota and Hikida (1989)
D. belliana	34	12m/sm+22mi	-	Thailand	Kritpetcharat et al. (1999)
D. taeniopterus	34	10m+2sm+22mi	17	Thailand	This study
Diploderma splendidum (as *Japarula splendida*)	34	12m+22mi	-	China	Zongyun et al. (2004)
	36	10bi+26a	-	Central Taiwan	
Di. swinhonis (as *Japarula swinhonis swinhonis*)	40	6bi+34a	-	Central Taiwan	Ota (1988)
	46	46a	-	Northern Taiwan	
Gonocephalus chamaeleontinus	42	22m+20mi	-	Malaysia	Diong et al. (2000)
G. liogaster	42	22m+20mi	-	Malaysia	Diong et al. (2000)
G. bellii	42	22m+20mi	-	Malaysia	Diong et al. (2000)
G. grandis	42	30m/sm+12t	-	Boeneo	Ota et al. (1992)
	42	22m+20mi	-	Malaysia	Diong et al. (2000)
G. myotympanum	42	30m/sm+12t	-	Boeneo	Ota et al. (1992)
G. robinsonii	32	12m+20mi	-	Malaysia	Diong et al. (2000)
Japalura variegata	34	-	-	India	Singh and Banerjee (2004)
J. varcoae	34	12m+22mi	-	China	Li et al. (1981)
Ptyctolaemus gularis	34	12m/sm+22mi	-	India	Sharma and Nakhasi (1980)

Remark: 2n=diploid number, m=metacentric, sm=submetacentric, a=acrocentric, t=telocentric, mi=microchromosome, qter=terminal region of long arm.

1988; Patawang et al. 2015a). The NOR position of *Draco taeniopterus* was more conserved than the genus *Calotes*.

Chromosomes of barred gliding lizard testis for meiosis was observed. The metaphase I has 17 bivalents comprising 6 ring bivalents of macrochromosomes and 11 small rod bivalents of microchromosomes. The metaphase II has n=17 haploid comprising 5 metacentric, 1 submetacentric macrochromosomes and 11 microchromosomes (Figure 4). The meiosis karyotypes of other species in Agamidae are showed in lndo-Chinese water dragon, *Physignathus cocincinus* which has 2n =36 with 6 ring bivalents of metacentric or submetacentric macrochromosomes and 12 rod bivalent of microchromosomes (Patawang et al. 2015b). In addition, the meiotic

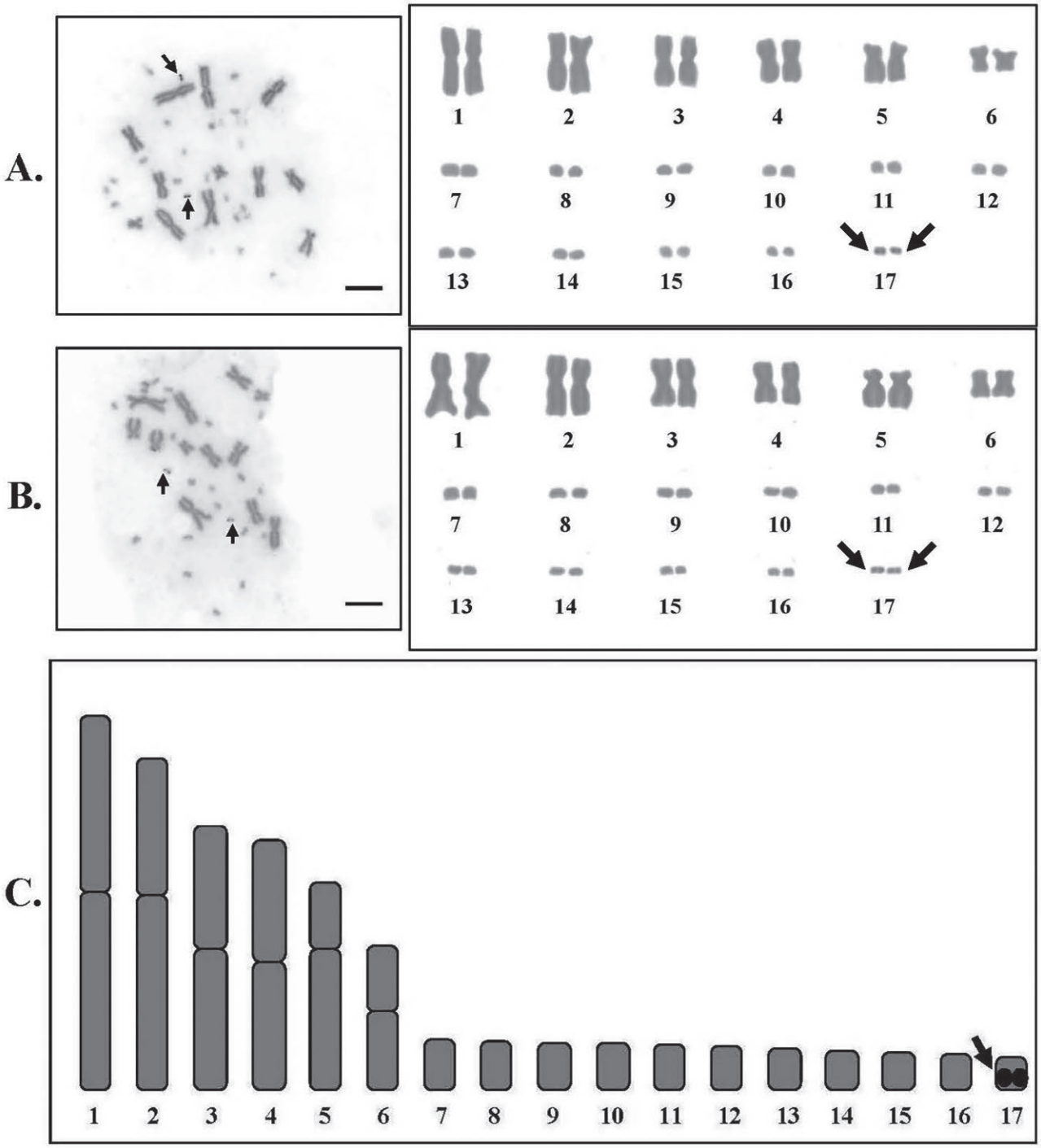

Figure 3. Metaphase plates and standardized karyotypes of male (A.), female (B.) and Idiogram (C.) of barred gliding lizard, *Draco taeniopterus*, 2n=34 by Ag-NOR banding, arrows indicate NORs (Scale bars = 10 μm).

configurations of another lizard were revealed in butterfly lizard, *Leiolepis reevesii rubritaeniata* (Agamidae) and long-tailed grass lizard, *Takydromus sexlineatus* (Lacertidae) (Phimphan et al. 2013; Patawang et al. 2018b).

Microsatellite repeat patterns of *Draco taeniopterus* indicated the presence of $d(GC)_{15}$ and $d(CAG)_{10}$ showed specific regions, 2qter and 3qter respectively. While $d(TA)_{15}$ and $d(CAA)_{10}$, showed cumulative signals dis-

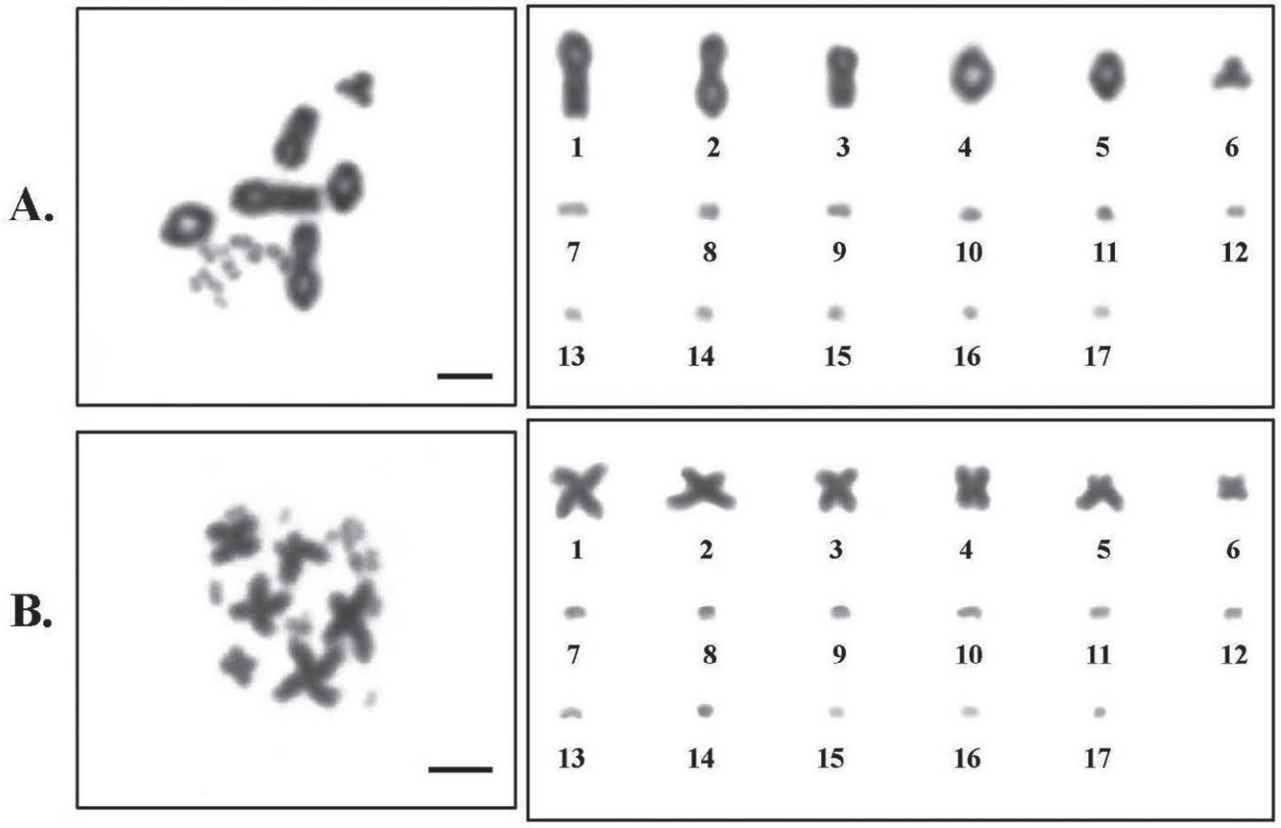

Figure 4. Meiotic cell divisions and karyotypes of metaphase I (A.) and metaphase II (B.) of barred gliding lizard, *Draco taeniopterus*, 2n=34 by conventional staining (Scale bars = 10 μm).

persed throughout the chromosomes (Figure 5 and 6). The microsatellite loci were highly evolved loci. Therefore, in many species there may be different forms of repetitive sequences. Most of them were found distributed throughout the genome. But in some species it was found on the telomere position. However, in some species it may be found in a specific location. This study, the short tandem repeats of $d(GC)_{15}$ was found on q-arm telomeric of pair 2nd and $d(CAG)_{10}$ was found on q-arm telomeric of pair 3rd. The molecular cytogenetics applying microsatellite probe of family Agamidae in previous study has *Leiolepis reevesii rubritaeniata*, 2n=36 (12bi+24mi) using $(TTAGGG)_n$ probes presented on telomeric and interstitial some chromosomes and *Tympanocryptis lineata* and *Rankinia diemensis*, 2n=32 (12bi+ 20mi) using (TTAGGG)7 presented on centromeric and telomeric region in some chromosomes (Jantarat et al. 2018; Srikulnath et al. 2009; Alam et al. 2021). We suggest employing GC and CAG probes in different Drago to have a deeper understanding of the relationship.

The barred gliding lizard, *Draco taeniopterus* from Than To district, Yala province, Thailand has 2n=34, NF=46. The karyotype comprises four pairs of large metacentric chromosomes, one pairs of small metacentric chromosomes, one pairs of large submetacentric chromosomes, and 11 pairs of microchromosomes. The metaphase I showed 17 bivalents and metaphase II showed haploid, n=17. The NOR was located on the last microchromosome pair 17th. Microsatellite repeat patterns indicated the presence of $d(GC)_{15}$ and $d(CAG)_{10}$ showed specific regions, 2qter and 3qter respectively. While $d(TA)_{15}$ and $d(CAA)_{10}$, showed cumulative signals dispersed throughout the chromosomes. This study is useful in supporting our understanding of the evolution of flying lizards and promote conservation of wildlife resources in tropical rainforests.

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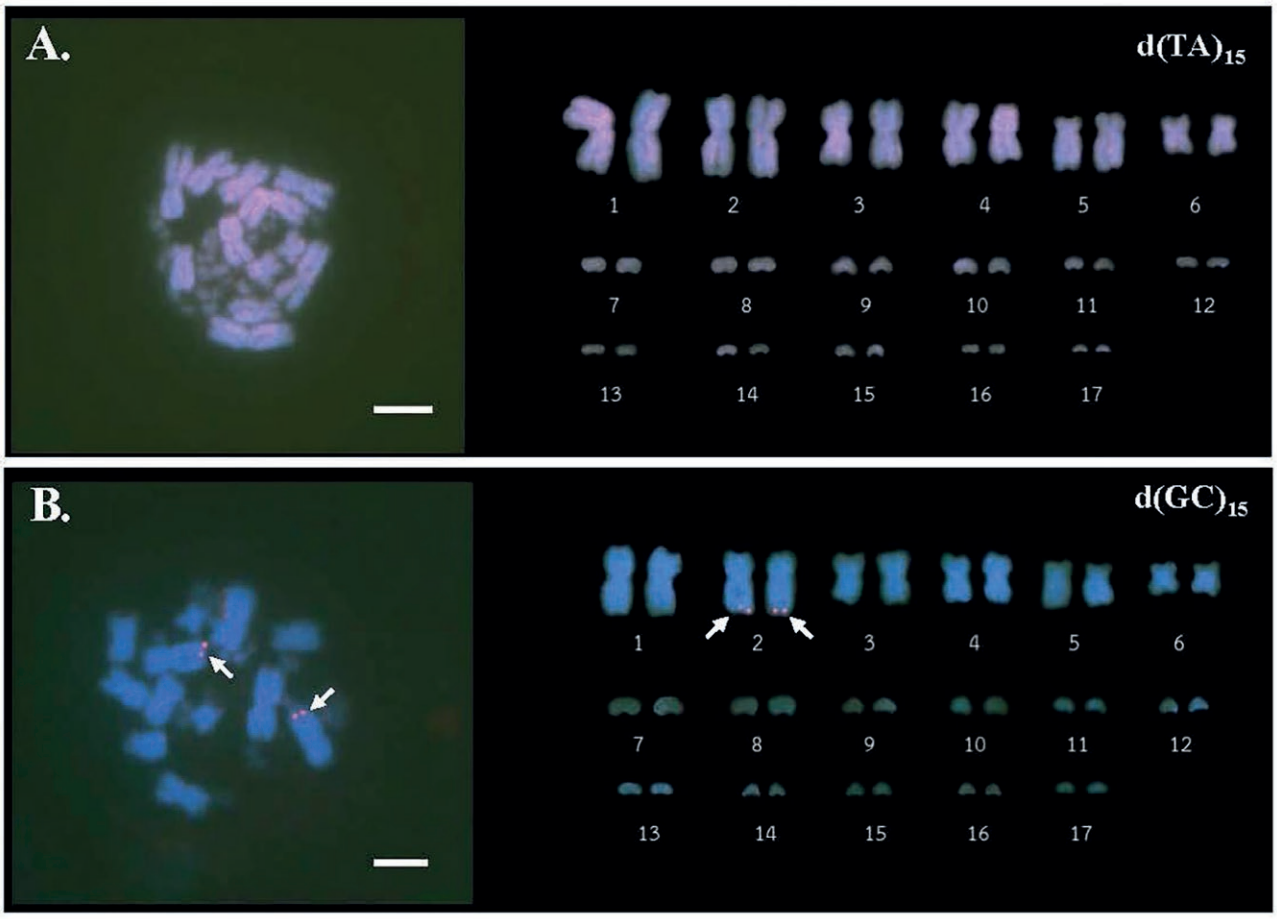

Figure 5. Metaphase plates and hybridization patterns with microsatellite probes $d(TA)_{15}$ (A.) and $d(CG)_{15}$ (B.) (red signals) on metaphase plates of barred gliding lizard, *Draco taeniopterus*, 2n=34, chromosomes were counterstained with DAPI (blue) (Scale bar = 10 μm).

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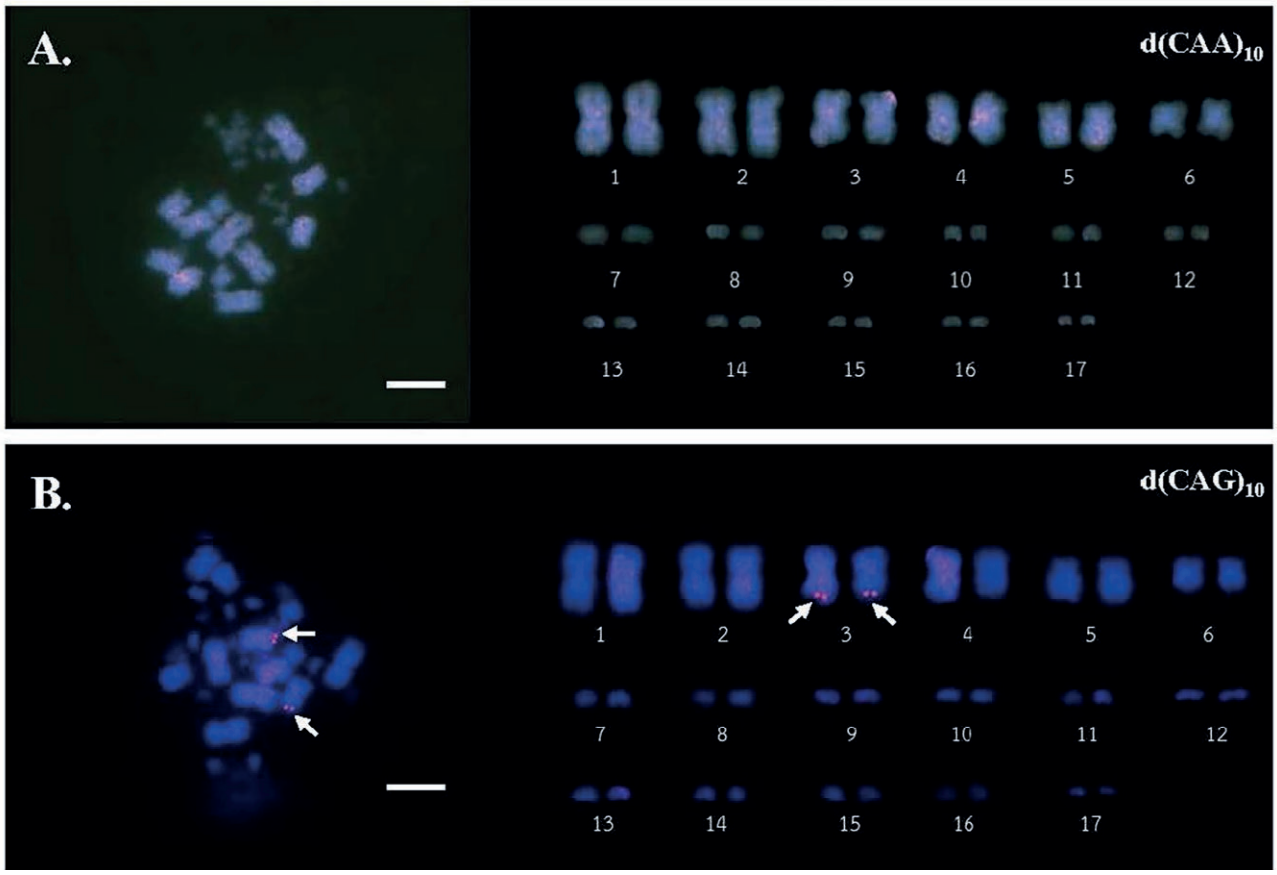

Figure 6. Metaphase plates and hybridization patterns with microsatellite probes $d(CAA)_{10}$ (A.) and $d(CAG)_{10}$ (B.) (red signals) on metaphase plates of barred gliding lizard, *Draco taeniopterus*, 2n=34, chromosomes were counterstained with DAPI (blue) (Scale bar = 10 μm).

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