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ORIGINAL ARTICLE

Additional description of karyotype and meiotic features of *Takydromus sexlineatus* (Squamata, Lacertidae) from northeastern Thailand

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Abstract

This study determined the standardized karyotype and idiogram of *Takydromus sexlineatus* from Surin Province in northeastern Thailand. Mitotic metaphase chromosomes from bone marrow cells and different meiotic stage from testicular cells respectively were studied after staining with conventional Giemsa and Ag-NOR banding techniques for chromosome identification. The study was carried out with samples taken from both male and female *T. sexlineatus*, the sex of which was identified by external examination. The results showed that the diploid chromosome number of *T. sexlineatus* was $2n = 38$ and the fundamental number (NF) was 38 in both male and female. Although chromosome study was done in both sexes of *T. sexlineatus*, this study does not identify the sex chromosomes. This is because of the limitation of the staining techniques used. Therefore, we did not conclude on the sex chromosome system in this species and all chromosomes were taken as autosomes. The types of autosomes observed were 16 large telocentric, 14 medium telocentric, and 6 small telocentric macrochromosomes and 2 microchromosomes. Our result is the new record in Thailand on this species and NOR characteristics by silver staining is the first report on NOR banding in this species. The karyotype formula of *T. sexlineatus* thus was found as: $2n(38) = L^t_{16} + M^t_{14} + S^t_6 + 2$ microchromosomes.

Keywords *Takydromus sexlineatus* · Lacertidae · Karyotype · Chromosome

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Introduction

The Lacertids, Lacertidae family, are a very successful group of lizards that includes about 280 species, widespread in the Palearctic and Paleotropic regions and adapted to various lifestyles [2, 13]. Their cytogenetics have been extensively studied (Table 2). Their diploid number is either 38 or 40, with three exceptions (*Lacerta horvathi*, *L. lepida* and *L. ocellata*) that are 36 [8, 23]. Most species in the Lacertidae family possess karyotypes with 36 to 38 telocentric macrochromosomes and two microchromosomes (Table 2); these are practically indistinguishable by conventional cytological techniques [5].

The *Takydromus* is one of many genera of the Lacertini tribe in the Lacertidae family. Most species of the *Takydromus* genus have slender bodies and long tails – for example, the tail of *T. sexlineatus* is more than three times longer than its snout-vent length (SVL), and about fourfold in *T. sauteri* [1]. Distributed throughout Southeast Asia, southern China, eastern India, Sumatra, Jawa and Borneo [2, 3, 15], *T. sexlineatus* is the only species of the *Takydromus* genus that has

been identified in Thailand. Three previous cytogenetic studies of *T. sexlineatus* in Thailand have been published: Olmo et al. [22, 23] and Odierna et al. [20]. Odierna et al. [20] reported that the karyotype of *T. sexlineatus* was comprised of 36 telocentric macrochromosomes and two microchromosomes. Olmo et al. [22, 23] detected three karyotypic categories, all with the same 36 telocentric macrochromosomes, but with either 2, 4 or 6 macrochromosomes (Table 2). In the present study, we compare and confirm the results from our cytogenetic analyses with those of previous studies.

Materials and methods

Four males and five females of *T. sexlineatus* were collected in September 2016 from Kap Choeng District (14°24′32.5″N, 103°27′11.7″E), Surin Province in northeastern Thailand (Fig. 1). They were transferred to the laboratory and kept under standard conditions for 1 day prior to the experiments. Chromosomes were prepared by the direct method, from bone marrow and testis, followed by a colchicine, hypotonic, fixation, air-drying technique [26]. Conventional staining was done using 20% Giemsa's solution for 30 min. Ag-NOR banding was performed by adding two drops of 50% silver nitrate and 2% gelatin on slides [14]. The lengths of short arm (Ls) and long arm (Ll) chromosomes were measured to calculate the length of the total arm chromosome (LT, LT = Ls + Ll). Relative length (RL) and centromeric index (CI) were estimated. CI was also computed to classify the types of chromosomes according to Chaiyasut [9]. All parameters were used in karyotyping and idiograming.

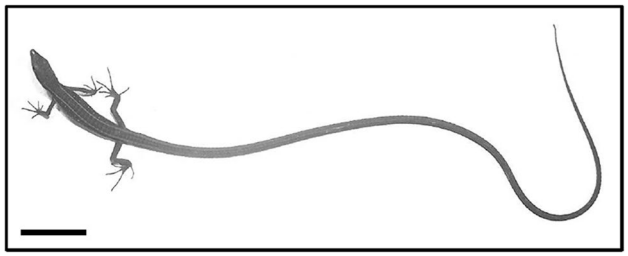

Fig. 1 General characteristics of adult *Takydromus sexlineatus* from Northeastern Thailand, scale bar = 3 cm

Results and discussion

Cytogenetic study of *T. sexlineatus* revealed that the chromosome number was $2n = 38$. The autosomes were composed of 16 large telocentric, 14 medium telocentric, and 6 small telocentric macrochromosomes and 2 microchromosomes; the short arm of 11th pair chromosome had a clearly observable satellite chromosome that had a secondary constriction (Figs. 2, 3, 4 and Table 1). The idiograms by conventional Giemsa's staining and Ag-NOR banding techniques are shown in Fig. 4. There were no sex differences in the karyotypes of the males and females of this

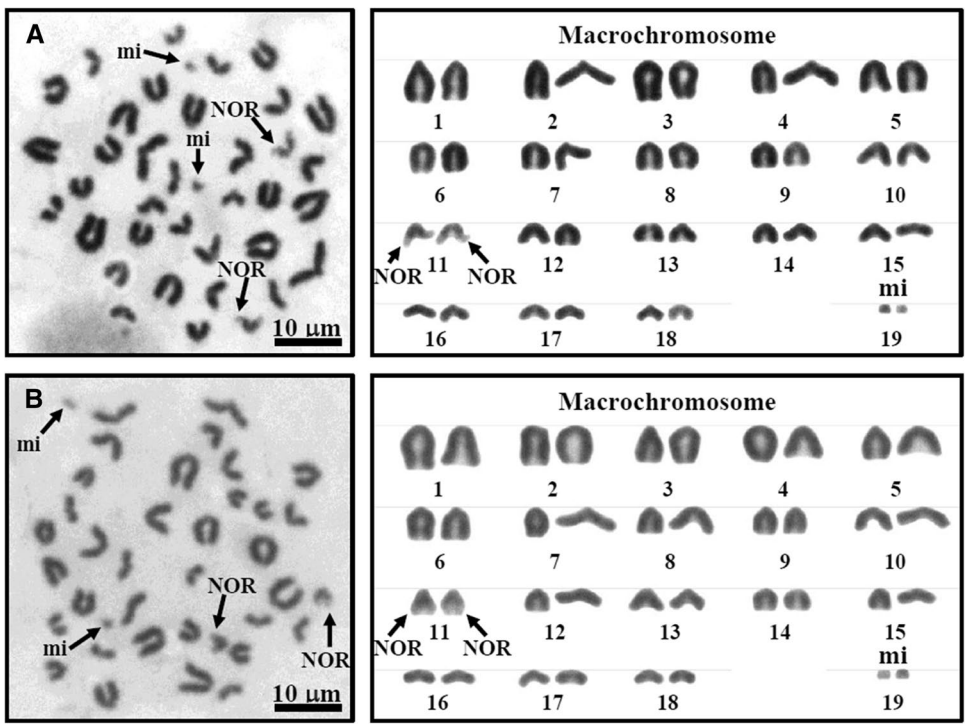

Fig. 2 Metaphase chromosome plates and standardized karyotypes of male (**a**) and female (**b**). *Takydromus sexlineatus*, $2n = 38$ by conventional staining (the arrows indicate nucleolar organizer region/NOR)

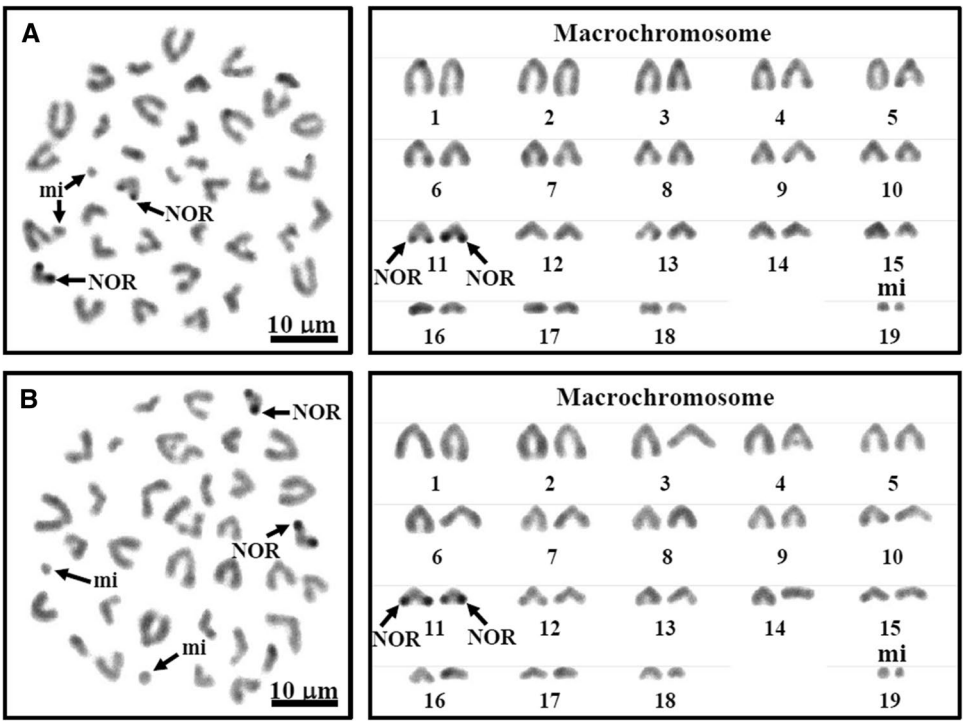

Fig. 3 Metaphase chromosome plates and standardized karyotypes of male (**a**) and female (**b**). *Takydromus sexlineatus*, $2n = 38$ by Ag-NOR banding technique (the arrows indicate nucleolar organizer region/NOR)

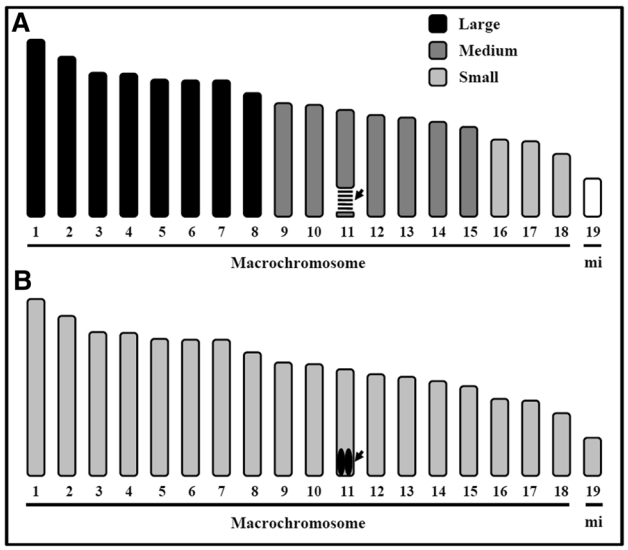

Fig. 4 Standardized idiograms of *Takydromus sexlineatus*, $2n = 38$ by conventional staining (**a**) and Ag-NOR banding (**b**) techniques. The arrows indicate nucleolar organizer region/NOR

species. This result differed from some previous reports that detected sex-chromosomes in the ZZ/ZW system, including *T. amurensis*, *T. sexlineatus* [20, 23], *T. wolteri* [25], *Lacerta mosorensis* [8], *L. viridis* [23], *L. vivipara* [10] and *Gallotia galloti* [23] (Table 2). For *Takydromus sexlineatus*, Olmo et al. [22] described the first case of female heterogamety in which the W-chromosome has the same morphology as the Z-chromosome, but differs from it in being heterochromatic and C-banding positive. Furthermore, Olmo et al. [24] reported the presence of a small euchromatic region at different levels on the W chromosome characterized by C-banding technique of females in five Lacertid lizard species (*T. sexlineatus*, *G. galloti*, *Acanthodactylus erythrurus*, *Meroles cuneirostris* and *L. montieola*). Although chromosome study was done in both sexes of *T. sexlineatus*, this study does not identify the sex chromosomes. This is because of the limitation of the staining techniques used. Therefore, we did not conclude on the sex chromosome system in this species and all chromosomes were taken as autosomes. We reported results based on the techniques that we use in our study.

During meiotic cell division of male *T. sexlineatus*, this study found that the homologous chromosomes showed synapsis during metaphase I (meiosis I), with 19 bivalents (18 macrochromosome bivalents and 1 microchromosome bivalent), and 19 haploid chromosomes at metaphase II

Table 1 Mean length of the short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), centromeric index (CI), relative length (RL) and standard deviation (SD) of CI, RL from metaphase chromosomes in 20 cells of *Takydromus sexlineatus*, $2n = 38$

Chro	Ls	Ll	LT	CI ± SD	RL ± SD	Type	Size
1	0.000	5.860	5.860	1.000 ± 0.000	0.083 ± 0.007	t	L
2	0.000	5.290	5.290	1.000 ± 0.000	0.075 ± 0.005	t	L
3	0.000	4.750	4.750	1.000 ± 0.000	0.068 ± 0.006	t	L
4	0.000	4.730	4.730	1.000 ± 0.000	0.067 ± 0.009	t	L
5	0.000	4.530	4.530	1.000 ± 0.000	0.064 ± 0.004	t	L
6	0.000	4.510	4.510	1.000 ± 0.000	0.064 ± 0.004	t	L
7	0.000	4.500	4.500	1.000 ± 0.000	0.064 ± 0.006	t	L
8	0.000	4.080	4.080	1.000 ± 0.000	0.058 ± 0.007	t	L
9	0.000	3.760	3.760	1.000 ± 0.000	0.053 ± 0.005	t	M
10	0.000	3.700	3.700	1.000 ± 0.000	0.053 ± 0.004	t	M
11*	0.000	3.540	3.540	1.000 ± 0.000	0.050 ± 0.003	t	M
12	0.000	3.350	3.350	1.000 ± 0.000	0.048 ± 0.005	t	M
13	0.000	3.280	3.280	1.000 ± 0.000	0.047 ± 0.006	t	M
14	0.000	3.140	3.140	1.000 ± 0.000	0.045 ± 0.004	t	M
15	0.000	2.980	2.980	1.000 ± 0.000	0.042 ± 0.005	t	M
16	0.000	2.540	2.540	1.000 ± 0.000	0.036 ± 0.006	t	S
17	0.000	2.490	2.490	1.000 ± 0.000	0.035 ± 0.004	t	S
18	0.000	2.080	2.080	1.000 ± 0.000	0.030 ± 0.005	t	S
19	–	–	1.250	–	0.018 ± 0.002	mi	

Chro chromosome pair, *t* telocentric chromosome, *mi* microchromosome, *L* large, *M* medium, *S* small, * satellite chromosome/NOR

as diploid species, $2n = 38$ (Fig. 5f). The largest telocentric chromosome (1st pair) was the largest bivalent and the single microchromosome pair was the smallest bivalent (Fig. 5e). This study did not detect any diakinesis (Fig. 5d) or metaphase I cells with partially paired bivalents that are speculated to be male heteromorphic sex-chromosomes; nor did we detect any metaphase II cells with condensed chromosomes that are speculated to be the Y or Z chromosome. In prophase I, we found that *T. sexlineatus* had the distinctness of the observable interphase by Ag-NOR banding technique, which showed observable NORs (Fig. 5a), pachytene (Fig. 5b) and diplotene (Fig. 5c) by conventional staining technique.

The mainly diploid characterized and karyotypic features of each genera of Lacertid lizard are as follows: $2n(40) = 38t + 2$ mi in *Gallotia* [6, 16, 23]; $2n(38) = 36t + 2$ mi in *Lacerta dugesii*, *L. mosorensis*, *L. muralis*, *L. trilineata*, *L. viridis* [8, 10, 20]; $2n(36) = 36t$ in *L. horvathi* [7]; $2n(36) = 2$ m/sm + 32t + 2 mi in *L. lepida* and *L. ocellata* [18, 23]; $2n(38) = 36t + 2mi$ in *Ophisops*, *Podarcis*, *Psammodromus* and *Takydromus* [4, 10–12, 18–20, 22, 23, 25]. The Lacertid lizards mostly share a highly conservative karyotype that is comprised of two virtually unchanged characters. Two types of highly conserved karyotype exhibit the first of 36 telocentric macrochromosomes series and the second of 2 microchromosomes. These karyological characteristics of the Lacertid lizards differ from most other lizards; moreover, most of its chromosomal features have changed little during the evolution line of the family Lacertidae. However, there is a little data available on the *T. sexlineatus*. I hope this information will be useful to other research in the future.

Table 2 Comparative chromosome study in the family Lacertidae

Species	2*n*	NF	Karyotype	NOR	Locality	References
Gallotia galloti	40	40	38t + 2 mi	–	Canary Islands	Cano et al. [6]
	40	40	38t + 2 mi (ZZ/ZW)	–	Canary Islands	Olmo et al. [23]
G. atlantica ssp.	40	40	38t + 2mi	–	Canary Islands	Lopez-Jurado et al. [16]
G. simonyi stehlini	40	40	38t + 2mi	–	Canary Islands	Lopez-Jurado et al. [16]
Lacerta dugesii	38	38	36t + 2mi	–	island of Madeira	Odierna et al. [20]
L. horvathi	36	36	36t	*P* 7th	(1) Udine, NE Italy (2) Ogulin, Croatia, C Jugoslavia	Capula et al. [7]
L. lepida	36	36	2m/sm + 32t + 2mi	–	Spain	Olmo et al. [23]
L. mosorensis	38	38	36t + 2mi (♂, ZZ) 35t + 3mi (♀, ZW)	–	Mostar, Prenj Mountains, SW Jugoslavia	Capula and Lapini [8]
L. muralis	38	38	36t + 2mi	–	France	Chevalier et al. [10]
L. ocellata	36	36	2m/sm + 32t + 2mi	–	–	Matthey [18]
L. trilineata	38	38	36t + 2mi	–	Balkans	Odierna et al. [20]
L. viridis	38	38	36t + 2mi	–	France	Chevalier et al. [10]
	38	38	36t + 2mi (ZZ/ZW)	–	(1) Hungary (2) Italy	Olmo et al. [23]
L. vivipara	36	–	–	–	–	Matthey [17]
	36	–	–	–	–	Oguma [21]
	36♂ 35♀	36	36t (♂, $Z_1Z_1Z_2Z_2$) 34t + 1 m (♀, Z_1Z_2W)	–	France	Chevalier et al. [10]
Ophisops elegans	38	38	36t + 2mi	–	Mosul of Iraq	Bhatnagar and Yoniss [4]
Podarcis metisellensis	38	38	36t + 2mi	–	Islet of Mclisello, Yugoslavia	Olmo et al. [23]
Po. sicula campestris	38	38	36t + 2mi	–	–	Dallai and Baroni-Urbani [11]
	38	38	36t + 2mi	–	Italy	Chevalier et al. [10]
	38	38	36t + 2mi	–	–	De Smet [12]
	38	38	36t + 2mi	–	NE Italy	Olmo et al. [23]
Po. sicula klemmeri	38	38	36t + 2mi	–	Islet of Licosa, Salerno, Italy	Olmo et al. [23]
Po. sicula sicula	38	38	36t + 2mi	–	surroudings of Naples	Odierna et al. [20]
	38	38	36t + 2mi	–	Scafati and Puma Licosa, Salerno, Italy	Olmo et al. [23]
Po. tiliguerta	38	38	36t + 2mi	–	Island of La Maddalena, Sassari, Italy	Olmo et al. [23]
Psammodromus algirus	40	40	38t + 2mi	–	–	De Smet [12]
	38	38	36t + 2mi	–	Spain	Olmo et al. [23]
Ps. hispanicus	38	38	36t + 2mi	–	–	Matthey [18]
Takydromus amurensis	38	38	36t + 2mi (ZZ/ZW)	–	Heilongjiang, China	Qin and Zhao [25]
T. formosanus	38	–	–	–	–	Nakamura [19]
T. wolteri	38	38	36t + 2mi (ZZ/ZW)	–	Heilongjiang, China	Qin and Zhao [25]
T. septentrionalis	38	–	–	–	–	Nakamura [19]
T. sexlineatus	38	–	–	–	–	Nakamura [19]
	38	38	36t + 2mi (ZZ/ZW)	–	Thailand	Odierna et al. [20]
	38	38	36t + 2mi (ZZ/ZW)	–	Thailand	Olmo et al. [22]
	38	38	36t + 2mi	–	Thailand	Olmo et al. [23]
	40	40	36t + 4mi	–	Thailand	
	42	42	36t + 6mi (ZZ/ZW)	–	Thailand	
	38	38	36t + 2mi	*P* 11th	NE Thailand	Present study

2*n* diploid chromosome number, *NF* fundamental number (number of chromosome arm), *m* metacentric, *sm* submetacentric, *t* telocentric chromosome, *NOR* nucleolar organizer region, *mi* microchromosome, *Z* Z chromosome, *W* W chromosome, *P* pair, *NE* northeastern, *C* central, *SW* southwestern, – not available

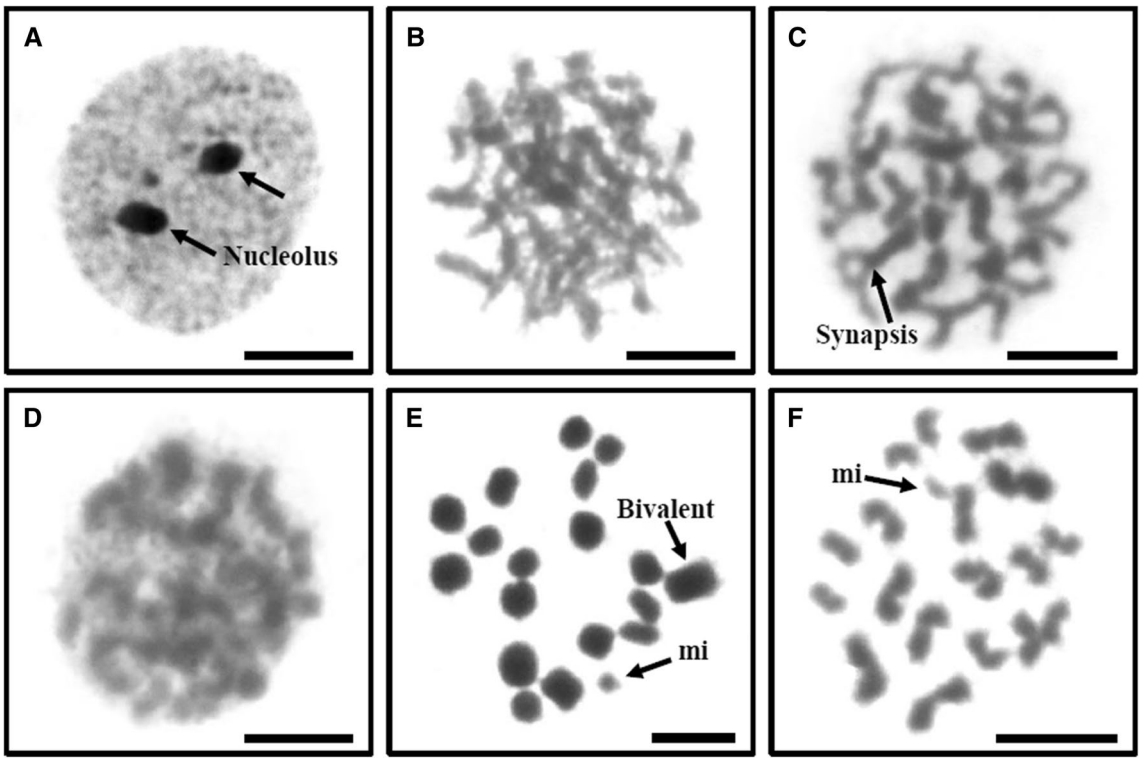

Fig. 5 Meiotic cell division of *Takydromus sexlineatus* on interphase by Ag-NOR banding (**a**), pachytene (**b**), diplotene (**c**), late diakinesis (**d**), metaphase I (**e**) and metaphase II, $n = 19$ (**f**). Scale bars indicate 10 μm

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