 Cytologia **79**(4): 457–466

Cytogenetics of the Skinks (Reptilia, Scincidae) from Thailand; I: Chromosome Analyses of the Common Sun Skink (*Eutropis multifasciata*)

Sarawut Kaewsri[1], Sirinee Yodmuang[1], Alongklod Tanomtong[2]*, Isara Patawang[2], Sarun Jumrusthanasan[2] and Krit Pinthong[3]

[1] Biology Program, Department of Science, Faculty of Science, Buriram Rajabhat University, Muang, Buriram 31000, Thailand
[2] Applied Taxonomic Research Center (ATRC), Department of Biology, Faculty of Science, Khon Kaen University, Muang, Khon Kaen 40002, Thailand
[3] Department of Fundamental Science, Faculty of Science and Technology, Surindra Rajabhat University, Muang, Surin 32000, Thailand

Received June 26, 2013; accepted November 2, 2013

Summary Chromosomal analyses of the common sun skink (*Eutropis multifasciata*) from mitotic and meiotic cell divisions were studied. Blood samples were taken from five male and five female skinks. Skink chromosome preparations were conducted by the squash technique from the bone marrow and testis. The chromosomes were stained by conventional staining and Ag-NOR banding techniques. The results showed that the diploid chromosome number of *E. multifasciata* was 2*n*=32, the fundamental number (NF) was 48 in both males and females. The types of chromosomes were present as 6 large metacentric, 2 large submetacentric, 6 small metacentric, 2 small submetacentric, 2 small telocentric macrochromosomes and 14 microchromosomes. There was no irregularly sized chromosome related to sex. We also observed distinctive nucleolar organizer regions (NORs) at the region adjacent to the short arms near the telomere of a pair of the largest metacentric chromosomes. We found that during diakinesis (prophase I) the homologous chromosomes showed synapsis, which can be defined as the 16 bivalents and 16 haploid chromosomes at metaphase II as diploid species. The karyotype formula of *E. multifasciata* is as stated:

2*n* (diploid) 32=L_6^m+L_2^{sm}+S_6^m+S_2^{sm}+S_2^t+14 microchromosomes

Key words *Eutropis multifasciata*, Karyotype, Chromosome, Ag-NOR banding.

The common sun skink (*Eutropis multifasciata*) belongs to the class Reptilia, order Squamata, suborder Laccertilia, infarorder Scincomorpha, family Scincidae, and subfamily Lygosominae (Fig. 1). The family Scincidae is the largest family of the extant lizards and includes over 1,200 species ranging from temperate to tropical regions on all continents inhabited by reptiles (Matsui 1992, Zug 1993, Pough *et al.* 2004). Greer (1970) on the basis of external and osteological characters divided this family in to four subfamilies: Acontinae, Feylininae, Scincinae, and Lygosominae.

The subfamily Lygosominae contains over 600 species (Greer 1970, Matsui 1992, Zug 1993). Within this subfamily three evolutionary lineages (*Eugongylus*, *Mabuya*, and *Shenomorphus* groups) are recognized on morphological criteria, karyology, and immunogenetics (Baverstock and Donnellan 1990, Donnellan 1991, Ota and Lue 1994, Ota *et al.* 1991, 1995, 1996).

There is a remarkable paucity of karyotypic information available for the family Scincidae. The karyotypes of fewer than 50 of the 1,200 or so species are recorded (Makino and Asana 1950,

* Corresponding author, e-mail: tanomtong@hotmail.com
DOI: 10.1508/cytologia.79.457

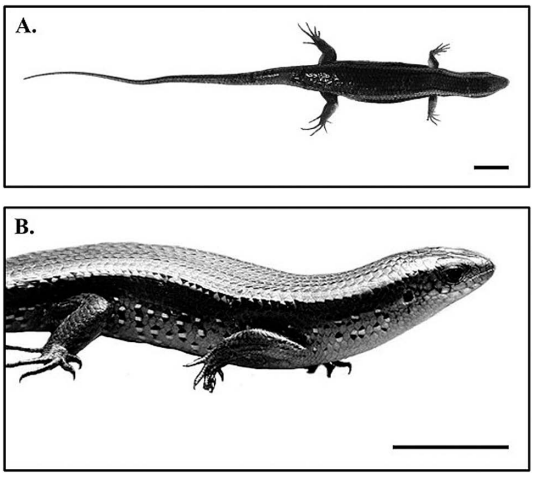

Fig. 1. General characteristics of the common sun skink, *Eutropis multifasciata* (Reptilia, Scincidae) from northeast Thailand, including external dorsal view (A) and lateral view (B). Scale bar=5 cm.

Makino 1951, Gorman 1973, King 1973a, 1973b, Kuprianova 1973, Wright 1973, Krishna and Aswathanarayana 1979, Hardy 1979, De Smet 1981, Bhatnager and Yoniss 1977, Cano *et al.* 1985, Colus and Ferrari 1988, Ota *et al.* 1988, 1995, 1996, 2001, Yang *et al.* 1989, Donnellan 1991, Eremchenko *et al.* 1992, Hernando and Alvarez 2005, Ezaz *et al.* 2009) Chromosome data are too sparse to provide data to meaningfully test the various systematic schemes proposed for the family (Donnellan 1991).

To date, there were only two previous cytogenetic studies of *E. multifasciata* performed by De Smet (1981) and Donnellan (1991). Thus, it is important to conduct an examination of *E. multifasciata*'s cytogenetics. In the present study, we confirm and compare the results from cyctogenetic analyses with those of previous studies and establish a standardized karyotype and idiogram by conventional staining and Ag-NOR banding techniques.

Materials and methods

Sample collection

Five male and female *E. multifasciata* were obtained from Mahasarakham province and Khon Kaen province, northeast Thailand. The skinks were transferred to the laboratory and were kept under standard conditions for seven days before experimentation.

Chromosome preparation

Chromosomes were prepared *in vivo* (Ota 1989, Qin *et al.* 2012) by injecting phytohemagglutinin (PHA) solution into the abdominal cavity of the skinks. After 24 h, colchicines were injected to the skinks' intramuscular and/or abdominal cavity and left for 12 h. Bone marrow and testis were cut into small pieces then mixed with 0.075 M KCl. After discarding all large cell pieces, 15 mL of cell sediments were transferred to a centrifuge tube and incubated for 25–35 min. KCl was discarded from the supernatant after subsequent centrifugation at 1,200 rpm for 8 min. Cells were fixed in fresh cool fixative (3 methanol : 1 glacial acetic acid) gradually added up to 8 mL before centrifuging again at 1,200 rpm for 8 min, whereupon the supernatant was discarded. Fixation was repeated until the supernatant was clear and the pellet was mixed with 1 mL fixative. The mixture was dropped onto a clean and cold slide by micropipette followed by air-drying.

Chromosome staining

Conventional staining by 20% Giemsa's solution for 30 min and Ag-NOR banding were conducted (Howell and Black 1980) by adding two drops of 50% silver nitrate and 2% gelatin on slides, respectively. The slides were then sealed with cover glasses and incubated at 60°C for 5 min. After that, the slides were soaked in distilled water until the cover glasses were separated. The slide was stained with 20% Giemsa's solution for 1 min.

Chromosome checks

Chromosome counting was performed on mitotic metaphase cells under a light microscope. Twenty clearly observable and well spread chromosomes of each male and female were selected and photographed. The length of short arm chromosomes (Ls) and the length of long arm chromosomes (Ll) were measured and calculated to the length of total arm chromosomes (LT, LT=Ls+Ll). The relative length (RL), the centromeric index (CI) and standard deviation (SD) of RL and CI were estimated (Chaiyasut 1989). The CI (q/p+q) between 0.50–0.59, 0.60–0.69, 0.70–0.89 and 0.90–0.99 were described as metacentric, submetacentric, acrocentric and telocentric chromosomes, respectively. Fundamental number (number of chromosome arm, NF) was obtained by assigning a value of two to metacentric, submetacentric and acrocentric chromosomes and one to telocentric chromosomes. All parameters were used in karyotyping and idiograming.

Results and discussion

Chromosome number, fundamental number and karyotype of E. multifasciata

Karyological studies of the *E. multifasciata* using a squash technique and conventional staining procedures revealed that the chromosome number was $2n$=32, which consisted of 18 macrochromosomes and 14 microchromosomes. This karyological feature is similar to that of the subfamily Lygosominae, since other lygosomine species studies, including eight species of the genus *Eutropis* (*E. multifasciata*, *E. trivittata*, *E. carinata*, *E. frenata*, *E. macularia*, *E. longicaudata*, *E. rudis*, and *E. rugifera*), share the remarkably smaller diploid chromosome number (almost $2n \leq 32$), and a distinct size gap between the four largest and the remaining smaller chromosome pairs (Makino and Asana 1950, Makino 1951, Krishna and Aswathanarayana 1979, De Smet 1981, Donnellan 1991, Eremchenko *et al.* 1992, Ota *et al.* 1996, 2001, Hernando and Alvarez 2005) (Table 1). This examination also revealed that the NF of the *E. multifasciata* was 48 in both males and females. This is the same NF as reported by Donnellan (1991) which is different from the report of De Smet (1981) who detected an NF of 56.

The *E. multifasciata* has three types of macrochromosomes, which are metacentric (12 chromosomes), submetacentric (4 chromosomes) and telocentric (2 chromosomes). The 12 metacentric macrochromosomes were classified by size into 6 large and 6 small chromosomes; the four submetacentric macrochromosomes were classified by size into two large and two small chromosomes, while the two telocentric macrochromosomes were distinguished to be two small chromosomes. Different chromosomal features were reported by De Smet (1981), which showed that they had 24 bi-armed and four uni-armed macrochromosomes. While Donnellan (1991) showed that they had 16 bi-armed and two uni-armed macrochromosomes. The *E. Multifasciata* examined in the present study had 14 microchromosomes in both males and females. This is in accordance with the previous study of Donnellan (1991). However, it is different from the previous study by De Smet (1981), who reported four microchromosomes.

No irregularly sized chromosomes were found that were related to sex of the *E. multifasciata*. Approximately 1,000 species of lizards have been karyotyped and among those, fewer than 200 species have sex-chromosomes, yet they display remarkable diversity in morphology and degree of generation. Lizards with genotypic sex determination (GSD) display remarkable diversity in sex

Table 1. Review of skink cytogenetic reports in the genus *Eutropis* (Reptilia, Scincidae).

Species	2*n*	Karyotype formula	NF	NOR banded	Reference
E. multifasciata	32	24 bi-arms+4 uni-arms+4mi	56	1, 2	De Smet (1981)
	32	16 bi-arms+2 uni-arms+14mi	48	1	Donnellan (1991)
	32	12m+4sm+2t+14mi	48	S(STR)1	Present study
E. trivittata	32	16m+2sm+14mi	50	—	Krishna and Aswathanarayana (1979)
E. carinata	32	16m+2sm+14mi	50	—	Krishna and Aswathanarayana (1979)
E. frenata	30	16m+14mi	46	—	Hernando and Alvarez (2005)
E. macularia	32	8 bi-arms+24mi	40	—	Makino and Asana (1950)
	32	—	—	—	Makino (1951)
	34	12m+4sm+18mi	50	—	Ota *et al.* (2001)
	38	12m+4sm+22mi	54	—	Ota *et al.* (2001)
E. longicaudata	32	18 bi-arms+14mi	50	—	Eremchenko (1992)
E. rudis	32	14m+18mi	46	—	Ota *et al.* (1996)
E. rugifera	32	16m+16mi	48	—	Ota *et al.* (1996)

Remarks: 2*n*=diploid chromosome number, NF=fundamental number (number of chromosome arm), m=metacentric chromosome, sm=submetacentric chromosome, t=telocentric chromosome, mi=microchromosomes, S=short arm, STR=subtelomeric region, and —=not available.

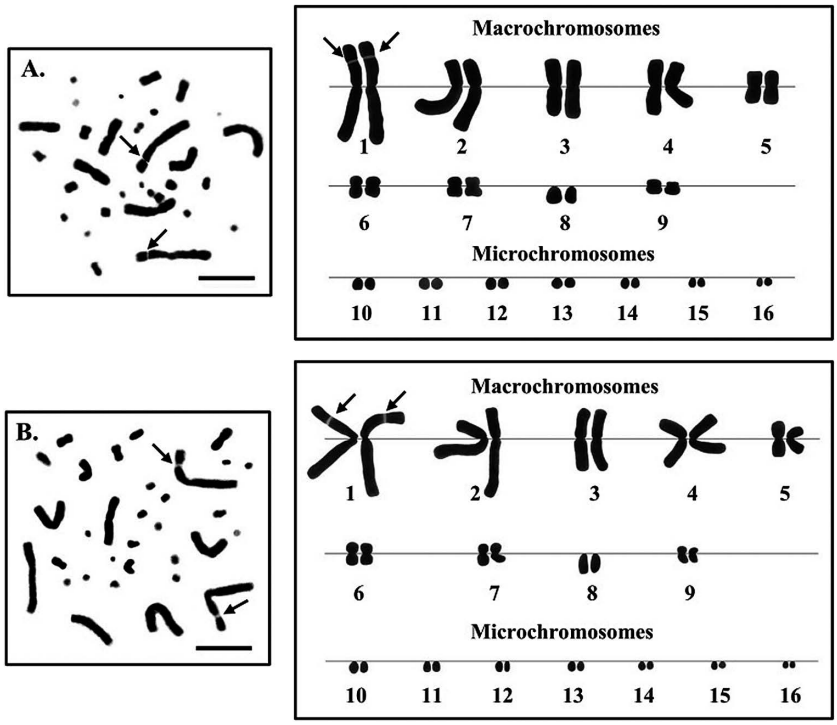

Fig. 2. Metaphase chromosome plates and karyotypes of male (A) and female (B) common sun skink (*Eutropis multifasciata*) 2*n* (diploid)=32 by conventional staining technique. No strange sized chromosomes related to sex were observed. Arrows indicate secondary constriction (scale bars=10 μm).

chromosomes differentiation, ranging from cryptic or homomorphic to highly differentiate. Nevertheless, homomorphic sex chromosomes are certainly likely to be common in GSD lizards, as they are in fish and amphibians (Ezaz *et al.* 2009).

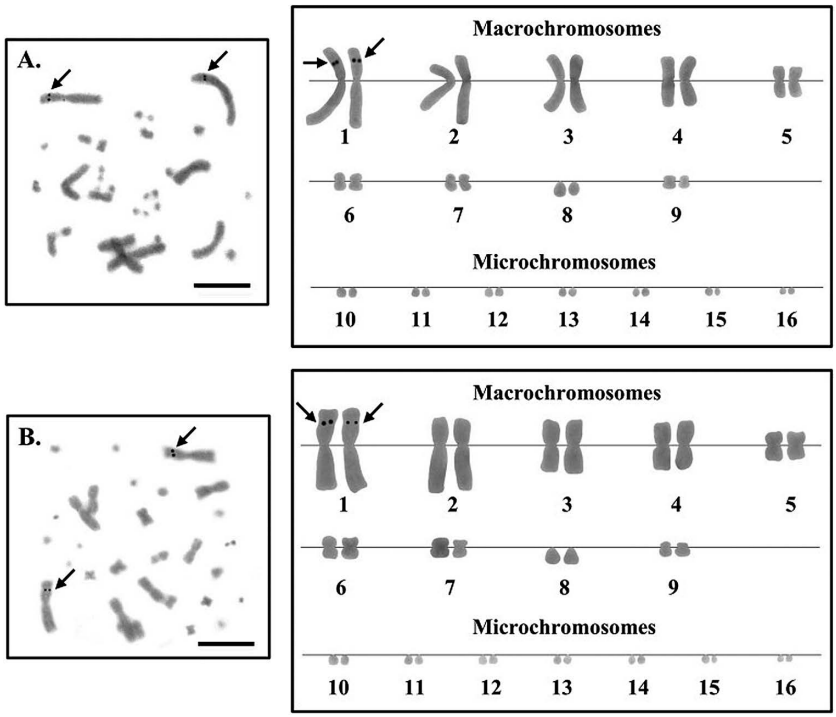

Fig. 3. Metaphase chromosome plates and karyotypes of male (A) and female (B) common sun skink (*Eutropis multifasciata*) 2*n* (diploid)=32 by Ag-NOR banding technique. Arrows indicate nucleolar organizer regions/NORs (scale bars=10 μm).

Table 2. Mean length of short arm chromosomes (Ls), long arm chromosomes (Ll), total arm chromosomes (LT), relative length (RL), centromeric index (CI), and standard deviation (SD) of RL, CI from 20 metaphase cells of male and female common sun skink (*Eutropis multifasciata*), 2*n*=32.

Chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	Chromosome size	Chromosome type
1*	1.520	1.899	3.419	0.169±0.018	0.555±0.017	Large	Metacentric
2	1.123	1.782	2.905	0.142±0.010	0.613±0.017	Large	Submetacentric
3	1.059	1.225	2.283	0.112±0.006	0.536±0.014	Large	Metacentric
4	1.031	1.144	2.175	0.107±0.005	0.526±0.010	Large	Metacentric
5	0.677	0.778	1.455	0.072±0.001	0.535±0.013	Small	Metacentric
6	0.523	0.604	1.127	0.056±0.004	0.536±0.022	Small	Metacentric
7	0.498	0.581	1.079	0.054±0.004	0.539±0.021	Small	Metacentric
8	0.000	0.894	0.894	0.045±0.004	1.000±0.000	Small	Telocentric
9	0.308	0.540	0.848	0.042±0.004	0.637±0.017	Small	Submetacentric
10	—	—	0.684	0.034±0.004	—	Microchromosome	
11	—	—	0.641	0.032±0.003	—	Microchromosome	
12	—	—	0.613	0.031±0.003	—	Microchromosome	
13	—	—	0.589	0.029±0.003	—	Microchromosome	
14	—	—	0.567	0.028±0.003	—	Microchromosome	
15	—	—	0.538	0.027±0.003	—	Microchromosome	
16	—	—	0.488	0.024±0.003	—	Microchromosome	

Remark: *=NOR-bearing chromosome (satellite chromosome).

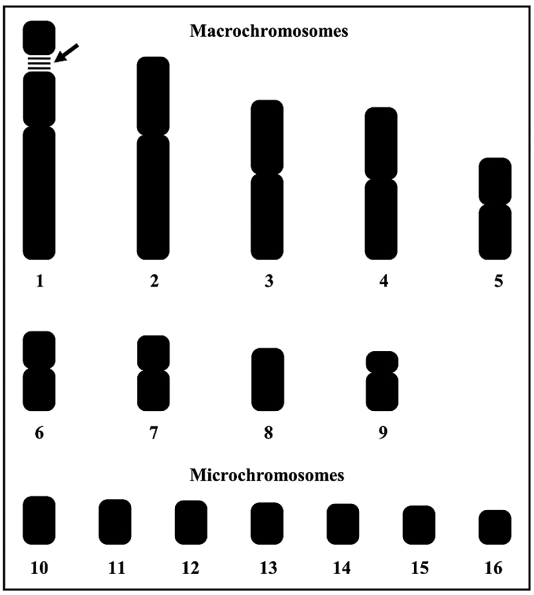

Fig. 4. Standardized idiogram of common sun skink (*Eutropis multifasciata*) 2*n* (diploid)=32 by conventional staining technique. Arrow indicates secondary constriction.

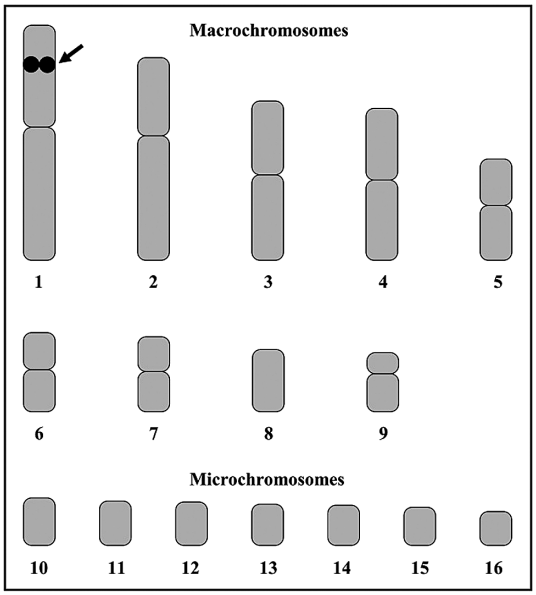

Fig. 5. Standardized idiogram of common sun skink (*Eutropis multifasciata*) 2*n* (diploid)=32 by Ag-NOR banding technique. Arrow indicates nucleolar organizer region (NOR).

Chromosome markers of E. multifasciata

In the present study, the nucleolar organizer regions/ NORs, which can represent chromosome markers, are located only on the short arms near the telomere of the largest chromosome pair (telomeric NORs metacentric chromosome pair 1). This is in accordance with the previous study (Donnellan 1991) but in contrast with De Smet (1981) who showed that the NORs were present on chromosome pairs 1 and 2.

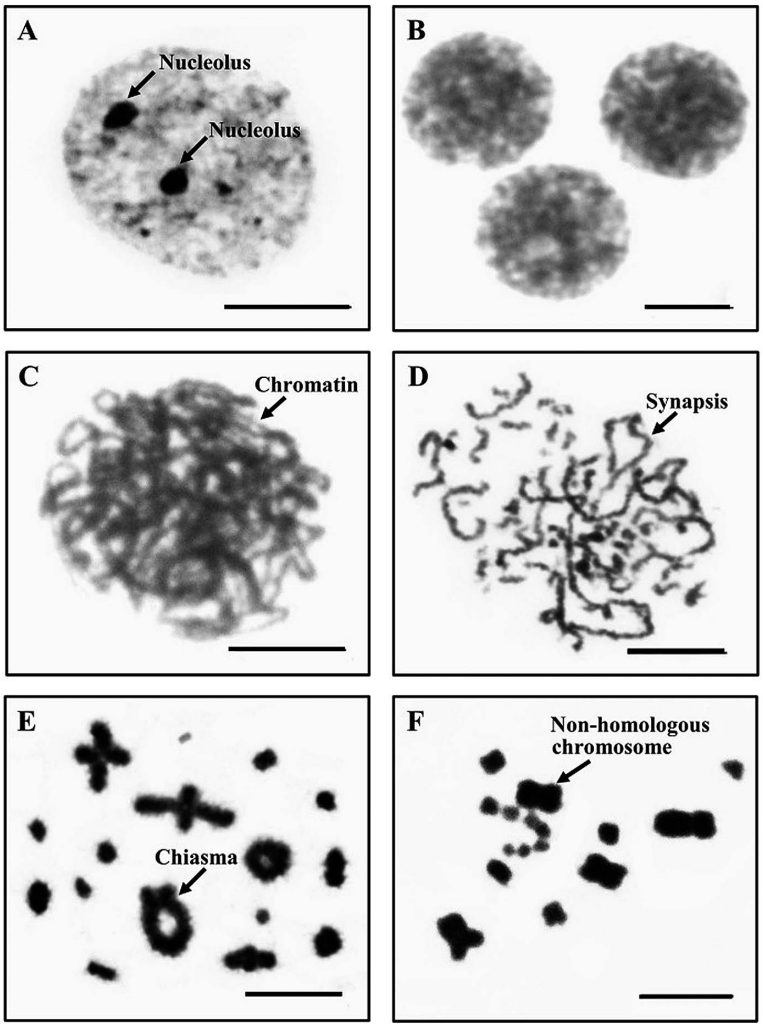

Fig. 6. Meiotic cell division of the common sun skink (*Eutropis multifasciata*) 2*n* (diploid)=32 on interphase by Ag-NOR banding (A) and conventional staining (B), pachytene (C), diplotene (D), diakinesis (E), and metaphase II (F). Scale bar=5 μm.

The karyotypes of mitotic chromosomes (metaphase plate) and the karyotype of meiotic chromosomes (Metaphase II plate) are shown in Fig. 2, 3 and 7. The chromosome length in centimetres of 20 cells (males and females) in mitotic metaphase was measured. The mean length of short arm chromosomes (Ls), length of long arm chromosomes (Ll), total length of arm chromosomes (LT), relative length (RL), centromeric index (CI), and type of chromosome were presented in Table 2. Figures 4 and 5 show the standardized ideograms from conventional staining and Ag-NOR banding techniques. The idiograms show gradually decreasing length of the macrochromosomes and microchromosomes.

Results for *E. multifasciata* show that chromosome markers are the macrochromosome pair 1 which is the largest metacentric chromosomes (satellite chromosomes). The important karyotype feature is the asymmetrical karyotype, which was found in three types of macrochromosomes (metacentric, submetacentric, and telocentric chromosomes). The largest macrochromosome is four times larger than the smallest macrochromosomes. The karyotype formula for *E. multifasciata*

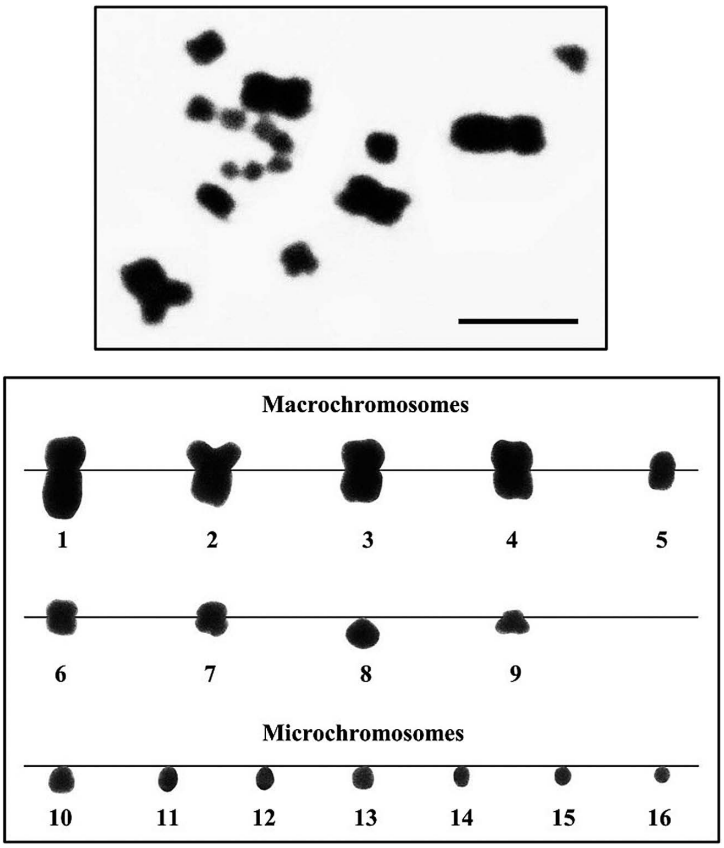

Fig. 7. Metaphase II chromosome plate (meiotic cell division) and karyotype of common sun skink (*Eutropis multifasciata*) contain haploid chromosome set (n=16) by conventional staining technique (scale bar=10μm).

could be deduced as:

$2n$(diploid) $32=L_6^m+L_2^{sm}+S_6^m+S_2^{sm}+S_2^t+14$ microchromosomes

Meiotic cell division of E. multifasciata

The present study on meiotic cell division of *E. multifasciata* found that during diakinesis (meiosis I) the homologous chromosomes showed synapsis, which can be defined as the 16 bivalents (9 bivalents of macrochromosomes and 7 bivalents of microchromosomes), and 16 haploid chromosomes at metaphase II as diploid species, $2n$=32 (Fig. 6F). The largest metacentric chromosome pair 1 is the largest bivalent. No diakinesis cells with partially paired bivalents that are speculated to be heteromorphic sex-chromosomes, and no metaphase II cells with condensed chromosomes that are speculated to be the Y or Z chromosome were detected.

In prophase I (meiosis I), we found that *E. multifasciata* had the distinctness of the observable interphase by Ag-NOR banding technique, which showed observable NORs (Fig. 6A), interphase by conventional staining technique (Fig. 6B), pachytene (completion of chromosome synapsis, Fig. 6C), diplotene (chiasma and crossing over, Fig. 6D), and diakinesis (terminalisation, Fig. 6E).

Acknowledgments

This work was supported by Buriram Rajabhat University and the Applied Taxonomic

Research Center (ATRC), Khon Kaen University grant ATRC_R5801. We wish to acknowledge the support of the Khon Kaen University Publication Clinic, Research and Technology Transfer Affairs, Khon Kaen University, for their assistance.

References

Baverstock, P. B. and Donnellan, S. C. 1990. Molecular evolution in Australian dragons and skinks: A progress report. Mem. Queensl. Mus. **29**: 323–331.

Bhatnager, A. N. and Yoniss, Y. T. 1977. Chromosome cytology of two lizards, *Agama rederata* and *Mabuya aurataseptem taeniata*. Caryologia **30**: 399–409.

Cano, J., Pretel, A. and Lopez-Jurando, L. F. 1985. Studies of the herpetofauna of the Canary Islands. II. The karyotype of the lizard *Chalcides sexlineatus*. J. Herpetol. **19**: 416–417.

Chaiyasut, K. 1989. Cytogenetics and Cytotaxonomy of the Family Zephyranthes. Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok.

Colus, I. M. and Ferrari, I. 1988. Mitotic chromosomes of *Mabuya* (Scincidae: Reptilia). Genetica **77**: 105–111.

De Smet, W. H. O. 1981. Description of the orcein stained karyotypes of 36 lizard species (Lacertilia, Reptilia) belonging to the families Teiidae, Scincidae, Lacertidae, Cordylidae and Varanidae (Autrachoglossa). Acta Zool. Pathol. Antverp. **76**: 37–118.

Donnellan, S. C. 1991. Chromosomes of Australian lygosomine skinks (Lacertilia: Scincidae) I. The Egernia group: C-banding, silver staining, Hoechst 33258 condensation analysis. Genetica **83**: 207–222.

Eremchenko, M., Panfilov, A. M. and Tsarinenko, E. I. 1992. Some Aspects of Cytogenetical and Systematical Research of Some Asian Species of Scincidae and Lacertidae (in Russian). Ilim, Bishchkek. pp.1–181.

Ezaz, T., Sarre, S. D., O'Meally, D., Marshall Graves, J. A. and Georges, A. 2009. Sex chromosome evolution in lizards: Independent origins and rapid transitions. Cytogenet. Genome Res. **127**: 249–260.

Gorman, G. C. 1973. The chromosomes of the Reptilia. In: Chiarelli, A. B. and Capana, E. (eds.). A Cytotaxonomic Interpretation. Academic Press, London, New York. pp. 349–424.

Greer, A. F. 1970. A subfamilial classification of scincid lizards. Bull. Mus. Comp. Zool. **139**: 151–183.

Hardy, G. S. 1979. The karyotypes of two scincid lizards, and their bearing on relationships in genus *Leiolopisma* and its relatives (Scincidae: lygosominae). N.Z. J. Zool. **6**: 609–612.

Hernando, A. and Alvarez, B. 2005. Estudios cromosómicos en saurio syanfisbénidos del litoral fluvial argentino y área de influencia. INSUGEO Miscelánea **14**: 427–440.

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

King, M. 1973a. Chromosomes of two Australian lizards of the families Scincidae and Gekkonidae. Cytologia **38**: 205–210.

King, M. 1973b. Karyotypic studies of some Australian Scincidae (Reptilia). Aust. J. Zool. **21**: 21–32.

Krishna, S. and Aswathanarayana, N. V. 1979. Karyological studies on four species of lizards from peninsular India. Curr. Sci. **48**: 667–671.

Kuprianova, L. 1973. The karyotype characteristics of two species of the Scincidae family. Tsitologiya **15**: 1135–1142.

Makino, S. 1951. An Atlas of the Chromosome Number in Animals. Iowa State College Press, Ames.

Makino, S. and Asana, J. J. 1950. A karyotypic study in some Indian species of Lacertilia. Integrative Res. Genet. **1**: 75–78.

Matsui, M. 1992. Systematic Zoology Vol. 9 Vertebrate IIb2, Reptilia. Nakayama Shoten, Tokyo.

Ota, H. 1989. Karyotype of five species of *Gekko* (Gekkonidae: Lacertilia) from East and Southeast Asia. Herpetologica **45**: 438–443.

Ota, H., Hikida, T. and Hasegawa, M. 1995. Karyotypes of two lygosomine lizards of the genus *Emoria* (Squamata: Scincidae) from Malaysia and Micronesia. Russ. J. Herpetol. **2**: 43–45.

Ota, H., Hikida, T., Matsui, M. and Hasegawa, M. 1988. Karyotype of a scincid lizard, *Carlia fusca*, from Guam, the Mariana Island. Zool. Sci. **5**: 901–903.

Ota, H., Hikida, T., Matsui, M., Hasegawa, M., Labang, D. and Nabhitabhata, J. 1996. Chromosomal variation in the scincid genus *Mabuya* and its arboreal relative (Reptilia: Squamata). Genetica **98**: 87–94.

Ota, H., Hikida, T., Matsui, M. and Mori, A. 1991. Karyotypes of two water skinks of the genus *Tropidophorus* (Retilia: Squamata) from Borneo. J. Herpetol. **25**: 488–490.

Ota, H., Hikida, T., Nabhitabhata, J. and Panha, S. 2001. Cryptic taxonomic diversity in two broadly distributed lizards of Thailand (*Mabuya macularia* and *Dixonius siamensis*) as revealed by chromosomal investigations (Reptilia: Lacertilia). The Natural History Journal of Chulalongkorn University **1**: 1–7.

Ota, H. and Lue, K. Y. 1994. Karyotypes of two lygosomine skinks of the genus *Sphenomorphus* from Taiwan. J. Herpetol. **28**: 253–255.

Pough, F. H., Andrews, R. M., Cadle, J. E., Crump, M. L., Savitzky, A. H. and Wells, K. D. 2004. Herpetology. 3rd ed. Pearson Education, Inc., Upper Saddle River.

Qin, X. M., Li, H. M., Zeng, Z. H., Zeng, D. L. and Guan, Q. X. 2012. Genetic variation and differentiation of *Gekko gecko* from different populations based on mitochondrial cytochrome *b* gene sequences and karyotypes. Zool. Sci. **29**: 384–389.

Wright, J. W. 1973. Evolution of the X_1X_2Y sex chromosome mechanism in the scincid lizard *Scinicella laterale* (Say). Chromosoma **43**: 101–108.

Yang, Y., Gao, Z. and Zhao, E. 1989. Karyotypic studies of *Sphenomorphus indicus* (Scincidae) and *Takydromus septentrionalis* (Lacertidae). Chinese Herpetological Research **2**: 56–59.

Zug, G. R. 1993. Herpetology. Academic Press, San Diego.