CHAPTER V

Karyological Comparison of the Butterfly lizards in the Genus *Leiolepis* (Reptilia: Agamidae)

Abstract

The karyotypes of butterfly lizards collected in Thailand, *Leiolepis belliana ocellata* Peters, 1971, *L. belliana belliana* (Gray, 1827), *L. reevesii rubritaeniata* (Gray, 1831), and *L. boehmei* Darevsky & Kupriyanova 1993 were studied. *L. b. belliana* and *L. r. rubritaeniata* had 2n = 36 chromosomes and the same karyotypic formulae were 10m + 2sm + 24mc (12 macrochromosomes and 24 microchromosomes). *L. b. ocellata* and *L. boehmei* had 2n = 34 chromosomes and their karyotypic formulae were 10m + 2sm + 22mc (12 macrochromosomes and 22 microchromosomes). *L. boehmei* male was never found during the time of the exploration, indicating that this species should be unisexual. The comparisons and relationship of the above species with other congeneric species are discussed from the cytotaxonomic point of view.

5.1 Introduction

The butterfly lizards in the genus *Leiolepis* include seven known species, all restricted to Southeast Asia. Four species are bisexual (*L. belliana* (Hardwicke & Gray, 1827), *L. guttata* Cuvier, 1829, *L. reevesii* (Gray, 1831) and *L. peguensis* Peters, 1971), but three are unisexual (*L. triploida* Peters, 1971, *L.*

guentherpetersi Darevsky & Kupriyanova, 1993 and *L. boehmei* Darevsky & Kupriyanova 1993) (Darevsky and Kupriyanova, 1993). In this genus, five species have been karyotyped (Hall, 1970; Kupriyanova, 1984; Solender and Schmid, 1988; Darevsky and Kupriyanova, 1993). Hall (1970) demonstrated that the karyotype of *L. triploida*, being composed of 3n = 54 chromosomes, differs from that of *L. belliana* (2n = 36). He stated that all 33 specimens of *L. triploida* examined from near the Malaysia-Thailand border were female and he was the first person who suggested that *L. triploida* was another parthenogenetic species. Kupriyanova (1984) determined that *L. reevesii* had a karyotype comprising 2n = 36 chromosomes. Solender and Schmid (1988) reported that the karyotype of *L. guttata* consisted of 2n = 36 chromosomes. Darevsky and Kupriyanova (1993) pointed out that the karyotype of *L. guentherpetersi* consisting of 3n = 54 chromosomes including 18 macrochromosomes and 36 microchromosomes. Yet, karyotypes of some species in genus *Leiolepis* remain unknown, and therefore, it is strongly desirable to accumulate data for such species to clarify the relationships among *Leiolepis* species from a cytotaxonomic viewpoint.

In the present study, karyotypes of three species of *Leiolepis* found in Thailand were investigated. *L. boehmei* and *L. belliana ocellata* karyotypes were described for the first time. Their karyotypes and those of other congeneric species are presented here and discussed in the context of comparative cytogenetic data.

5.2 Materials and Methods

A total of fourteen specimens were collected in Thailand (Fig. 5.1). Locality, sex and sample sizes of them are given in Table 5.1. Identifications of specimens were made on the basis of Peters (1971) and Darevsky and Kupriyanova (1993).

A microtechnique modified from the method of Puangwanttana et al. (2002) for culturing peripheral blood leucocytes was used to obtain metaphase plates. Blood sample was obtained by cardiac puncture after the animal was anesthetized with ice. Then the blood was cultured in the medium consisted of a mixture of RPMI 1640, fetal bovine serum and pokeweed mitogen (10 μg/ml). These lymphocyte cultures were grown at 37 $^{\circ}$C for three days. Fifty minutes prior to harvesting, colchicine (2 mg/ml) was added to the cultures in order to accumulate metaphase cells. After the cell suspension was centrifuged at 1,500 rpm for 10 min, the supernatant was aspirated with a sterile pipette, then slowly added 10 ml of warm 0.075 M KCL and incubated at 37 $^{\circ}$C for 30 min. The cell suspension was recentrifuged, the supernatant was removed. The cells were then immediately mixed in a cold fresh of 3:1 fixative solution of absolute methyl alcohol and glacial acetic acid for 10 min. The fixed cells were centrifuged and washed continuously with fresh fixative for three times before air-dry slides were prepared. Mitotic chromosome slides were investigated after being stained in 10% Giemsa solution for 15 min. The karyotype was determined for each individual on the basis of at least 10 well spread cells. To describe the karyotype, the terminology defined by Green and Sessions (1991) was used.

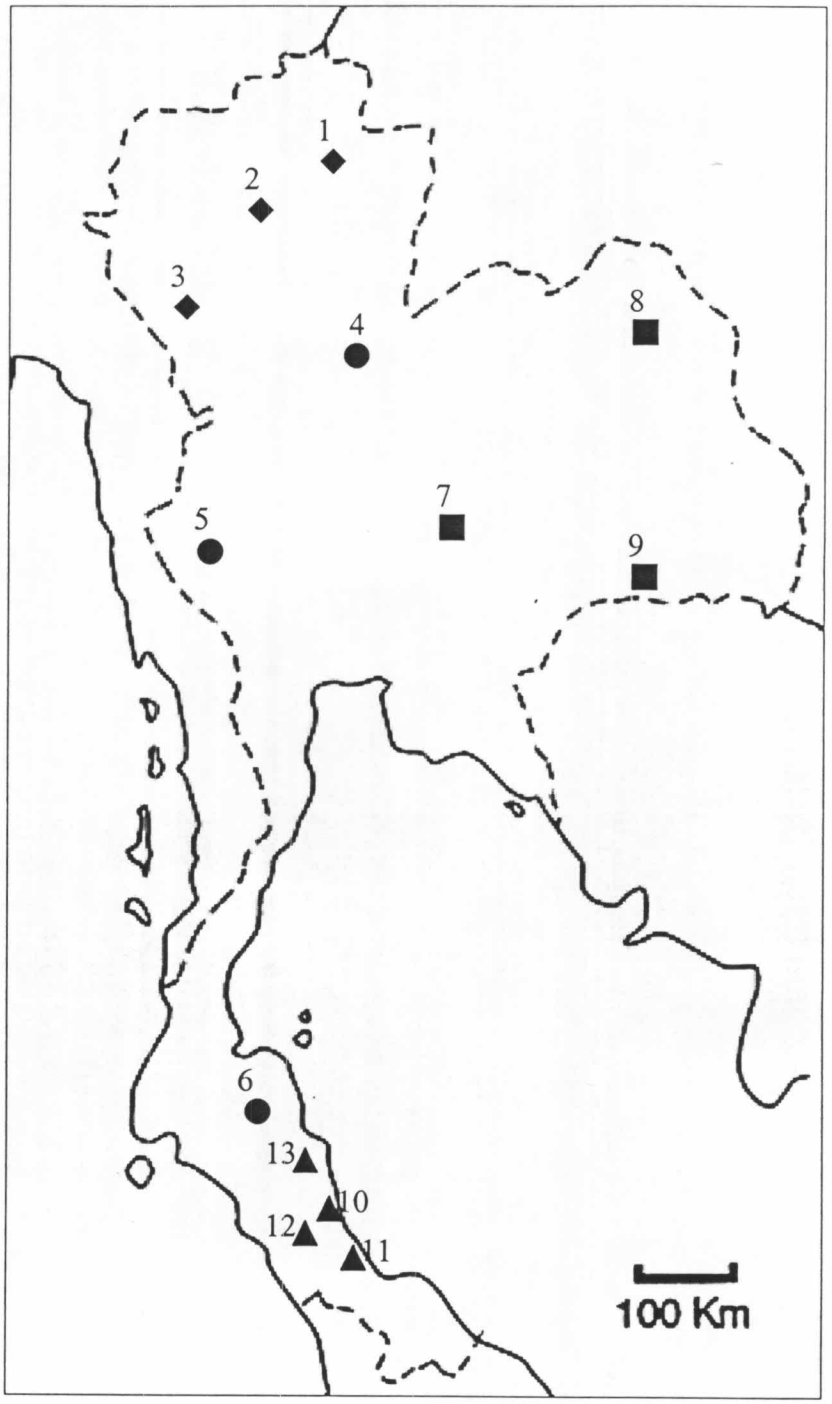

Figure 5.1 Map of Thailand showing sampling localities of *Leiolepis* used in the study. ●, *Leiolepis belliana belliana*; ◆, *Leiolepis belliana ocellata*; ■, *Leiolepis reevesii rubritaeniata*; ▲, *Leiolepis boehmei*.

Table 5.1 Locality, sex and sample sizes of each *Leiolepis* species used in the study.

Species	No.	Locality (District, Province)	Sex		Sample Sizes
			Male	Female	
L. belliana ocellata	1	Chiang Muan Phayao	1	1	2
	2	Mae Phrik, Lampang	1	1	2
	3	Ban Tak, Tak	1	1	2
L. belliana belliana	4	Wang Thong, Phitsanulok	1	1	2
	5	Bo Phloi, Kanchanaburi	1	1	2
	6	Tha Sala, Nakhon Si Thammarat	1	1	2
L. reevesii rubritaeniata	7	Non Sung, Nakhon Ratchasima	1	1	2
	8	Sawang Daen Din, Sakhon Nakhon	1	1	2
	9	Sangkha, Surin	1	1	2
L. boehmei	10	Chana, Songkhla	-	1	1
	11	Thepha, Songkhla	-	1	1
	12	Muang, Songkhla	-	1	1
	13	Hua Sai, Nakhon Si Thammarat	-	1	1

5.3 Results and Discussion

Published data of the chromosome for species belonging to the genus *Leiolepis* were summarized in Table 5.2. Diploid chromosome numbers in most members of this genus were 36. Only two species, *L. boehmei* and *L. b. ocellata* from Thailand, were exception, having 2n = 34. The diploid number and the karyotype of *L. r. rubritaeniata* was similar to those of *L. b. belliana*, comprising 2n = 36 chromosomes in two discontinuous size groups. Of these, six pairs belonged to the larger size-group (Nos. 1-6) whereas the other twelve, assigned to the smaller size-group, were acrocentric elements (Fig. 5.2).

Table 5.2 Chromosome data of *Leiolepis* species. (The data of this study is shaded)

Species	References	Chromosome Numbers	Macrochromosome		Microchromosome
			Metacentric	Submetacentric	
L. b. ocellata	**This study**	**2n = 34**	**1,2,5,6,7,8,9,10,11,12**	**3,4**	**22**
L. b. belliana	Hall, 1970	2n = 36	1,2,5,6,7,8,9,10,11,12	3,4	24
	Peters, 1971	2n = 36	1,2,5,6,7,8,9,10,11,12	3,4	24
	Shoubai et al., 1987	2n = 36	1,2,5,6,7,8,9,10,11,12	3,4	24
	Rojchai, 1989	2n = 36	1,2,5,6,7,8,9,10,11,12	3,4	24
	Puangwattana et al., 2002	2n = 36	1,2,5,6,7,8,9,10,11,12	3,4	24
	This study	**2n = 36**	**1,2,5,6,7,8,9,10, 11,12**	**3,4**	**24**
L. r. rubritaeniata	Rojchai, 1982	2n = 36	1,2,5,6,7,8,9,10,11,12	3,4	24
	This study	**2n = 36**	**1,2,5,6,7,8,9,10,11,12**	**3,4**	**24**
L. r. reevesii	Solender & Schmid, 1988	2n = 36	1,2,5,6,7,8,9,10,11,12	3,4	24
L. boehmei	**This study**	**2n = 34**	**1,2,5,6,7,8,9,10,11,12**	**3,4**	**22**
L. triploida	Hall, 1970	3n = 54	1,2,3,7,8,9, 10,11,12,13, 14,15,16,17,18	4,5,6	36
L. guttata	Kupriyanova, 1984	2n = 36	1,2,5,6,7,8,9,10,11,12	3,4	24
L. guentherpetersi	Darevsky & Kupriyanova, 1993	3n = 54	1,2,3,7,8,9, 10,11,12,13,14,15,16, 17,18	4,5,6	36
L. peguensis	?	?	?	?	?

The second pair of the macrochromosomes was submetacentric while the others were all metacentric. No heteromorphic sex chromosomes were evident. The results indicate that the karyotype of the genus *Leiolepis* is relatively conservative, and that the divergence among species in the genus involves little chromosomal differentiation. Accumulation of data for other species is required to confirm this assumption.

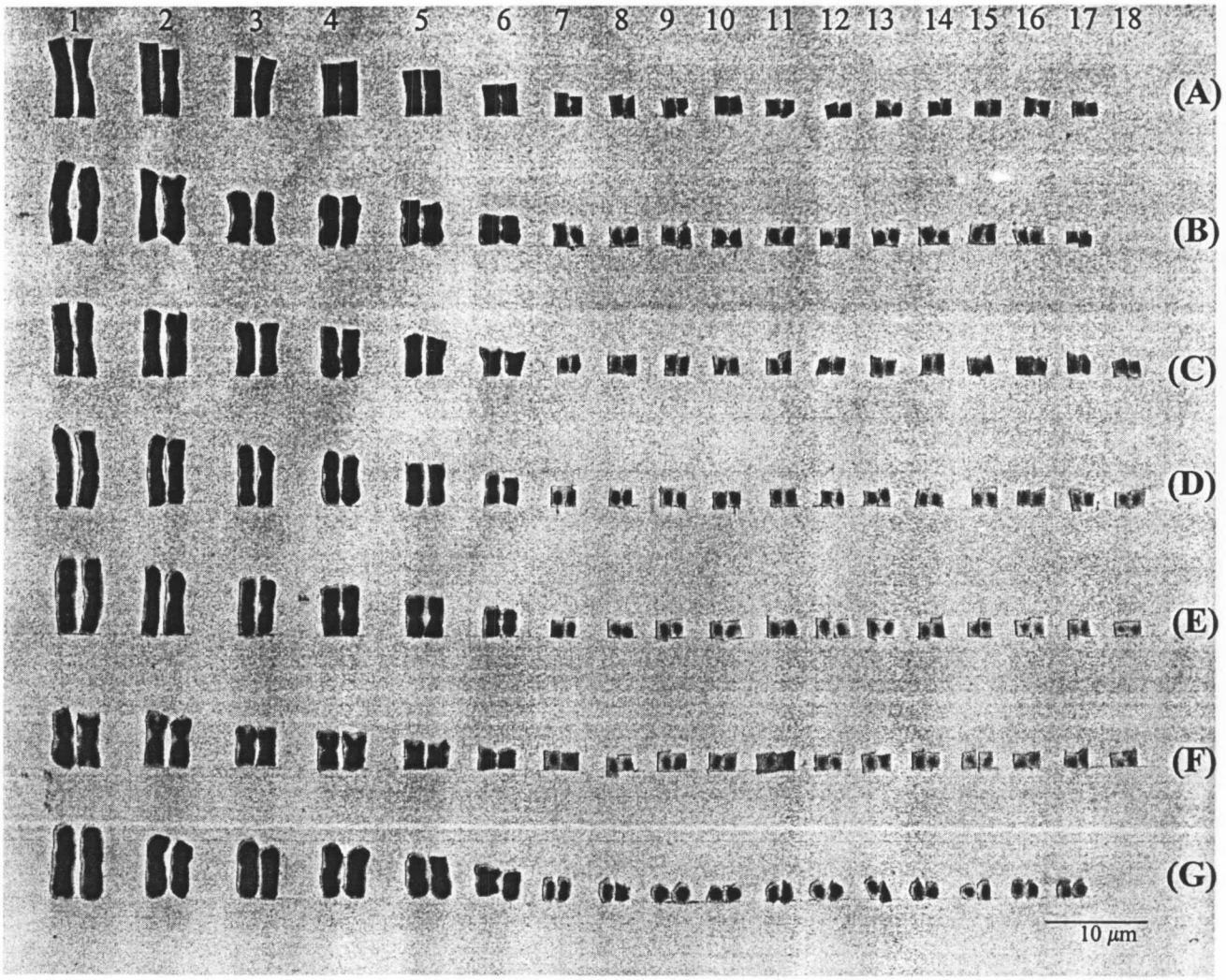

Figure 5.2 Karyotypes of *Leiolepis* species. (A) *L. belliana ocellata* (female); (B) *L. belliana ocellata* (male); (C) *L. belliana belliana* (female); (D) *L. belliana belliana* (male); (E) *L. reevesii rubritaeniata* (female); (F) *L. reevesii rubritaeniata* (male); (G) *L. boehmei* (female).

On the other hand, the karyotypes of both *L. boehmei* and *L. b. ocellata* had 34 chromosomes forming two discontinuous size groups. The larger group accommodated six pairs, of which pairs 1, 3, 4, 5 and 6 were metacentric, and pair 2 was submetacentric (Fig. 5.2). The smaller group consisted of 22 microchromosomes. Several authors argued that the chromosome number of 2n = 36 (consisting of 12

biarmed macrochromosomes and 24 uniarmed microchromosomes) represents the primitive condition of the lizard karyotype, and that the other chromosome counts should derive from this primitive 36 chromosome type through the centric fusion, pericentric inversion, etc. (Moody and Hutterer, 1978; King, 1981; Witten, 1983; Bickham, 1984). Following this hypothesis, it is probable that the 2n = 34 karyotypes of the two *Leiolepis* species, consisting of 12 metacentric macrochromosomes and 22 microchromosomes, have reduced the microchromosome number by Robertsonian fusion or others. The presence of the biarmed microchromosomes in these karyotypes supports this postulation. To substantiate this assumption, further systematic analyses are required for the morphological, as well as more detailed karyological studies.

On the basis of a morphological comparison, Peters (1971) stated that *L. b. belliana* was very closely allied to *L. b. ocellata*. However, he suggested that the taxonomic conclusion on the validity needs further detailed studies. The present results indicate the occurrence of extensive karyological differentiation between *L. b. belliana* and *L. b. ocellata* and strongly support that they are separate species.

The absence of males in all of the *L. boehmei* samples strongly suggested the occurrence of a unisexual form. No chromosomal variation was evident between specimen from Songkhla and Nakhon Si Thammarat Provinces. Both groups possessed a karyotype consisting of 34 chromosomes forming a discontinuous series in size. Of these, the secondary constrictions were located in the distal zone of the long arm of the largest metacentric chromosomes (chromosome no. 1). Normally in bisexual populations of lizards, males are collected more frequently than females and

the absence of males is considered one of the evidences for parthenogenetic reproduction (Darevsky *et al.*, 1985).

Darevsky and Kupriyanova (1993) stated that the charactersistics of coloration and some morphological characters of *L. triploida* were intermediate between *L. belliana* and *L. boehmei* which might support Böhme's hypothesis. Böhme (1982) proposed that *L. triploida* was an allotriploid hybrid clone between the diploid-parthenogenetic female and normal bisexual males of *L. belliana* species which widely distributed. The present results showed that the diploid chromosome numbers of *L. boehmei* was 34 which did not support Böhme's hypothesis. There is probability to assume that the appearance of *L. triploida* may be originally based on autopolyploidy, as a consequence of spontaneous reorganization of an originally diploid karyotype. Moreover, it is possible that the parthenogenetic *L. boehmei* might have originated from various diploid bisexual forms of the *L. belliana* group.

Further investigations on chromosomes of *L. b. belliana* and *L. b. ocellata*, as well as with advanced banding techniques, are required to correctly outline the karyological relationship within the genus *Leiolepis*.