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Cryptic Taxonomic Diversity in Two Broadly Distributed Lizards of Thailand (*Mabuya macularia* and *Dixonius siamensis*) as Revealed by Chromosomal Investigations (Reptilia: Lacertilia)

HIDETOSHI OTA[1]*, TSUTOMU HIKIDA[2], JARUJIN NABHITABHATA[3] AND SOMSAK PANHA[4]

[1] *Tropical Biosphere Research Center, University of the Ryukyus, Nishihara, Okinawa 903-0213, JAPAN*
[2] *Department of Zoology, Graduate School of Science, Kyoto University, Kitashirakawa, Sakyo, Kyoto 606-8502, JAPAN*
[3] *Reference Collection Division, National Science Museum, Klong Luang, Pathumthani 12120, THAILAND*
[4] *Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, THAILAND*

ABSTRACT.–Karyological surveys were made for two common lizards, *Mabuya macularia* and *Dixonius siamensis*, in Thailand. Of the *M. macularia* specimens karyotyped, the one from Mae Yom, northwestern Thailand, had 2n=38 chromosomes in a graded series as previously reported for a specimen from southwestern Thailand. The other specimens, all from eastern Thailand, however, invariably exhibited a 2n=34 karyotype consisting of four large, and 13 distinctly smaller chromosome pairs. Of the *D. siamensis* specimens, on the other hand, those from eastern Thailand invariably exhibited a 2n=40 karyotype, whereas three males and one female from Mae Yom had 2n=42 chromosomes. Distinct chromosomal variation was also observed within the Mae Yom sample that may reflect a ZW sex chromosome system. These results strongly suggest the presence of more than one biological species within each of those "broadly distributed species". This further argues the necessity of genetic surveys for many other morphologically defined "common species" in Thailand and its vicinity in order to evaluate taxonomic diversity and endemicity of herpetofauna in this region appropriately.

KEY WORDS: *Mabuya macularia*; *Dixonius siamensis*; cryptic species; karyotype; taxonomy

INTRODUCTION

Southeast Asia accommodates a remarkably diverse herpetofauna in its tropical environment. However, it is almost certain that a large part of its actual diversity is yet to be revealed by future studies. In fact, recent studies of specimens, mostly collected from more or less localized habitats within this region, such as an isolated patch of primary forest, recognized a number of taxa new to science (see Inger et al. [1999], and many other recent publications cited in Manthey and Grossmann [1997]). Most of these discoveries largely or exclusively depend on morphological divergences of

* Corresponding author.
Tel: +81-98-895-8937
Fax: +81-98-895-8966
e-mail: ota@sci.u-ryukyu.ac.jp

TABLE 1. Localities and sizes of samples of *Mabuya macularia* and *Dixonius siamensis* karyotyped.

Species	Locatity	(number in Fig. 1)	Sex	N
Mabuya macularia	Mae Yom	(1)	male	0
			female	1
	Phu Wua	(2)	male	1
			female	3
	Phu Pa Namtip	(3)	male	0
			female	2
	Yot Dom	(5)	male	0
			female	1
	Sakaerat	(6)	male	4
			female	1
Dixonius siamensis	Mae Yom	(1)	male	3
			female	1
	Phu Wua	(2)	male	0
			female	1
	Khao Wang	(4)	male	1
			male	4
	Sakaerat	(6)	male	0
			female	1

samples in problem: very few taxonomic studies on the Southeast Asian herpetofauna have adopted genetic approaches yet.

It is well documented, however, that reproductive isolation sometimes takes place with the absence of discernible morphological changes in amphibians and reptiles, producing so-called cryptic species (e.g., Toda et al., 1998, 2001). Thus, the paucity of genetic approaches to the Southeast Asian herpetofauna is likely to lead to substantial underestimation of its diversity, and erroneous biogeographical and ecological characterization of each regional fauna. Such a failure of appropriate evaluation of regional fauna in terms of taxonomic diversity and endemicity may further lead to insufficient conservation practices.

In consideration of this problem, we conducted a preliminary cytogenetic survey on two broadly distributed lizards in Thailand. The structural change of chromosomes is considered to often play a crucial role in the establishment and maintenance of reproductive isolation between sympatric or parapatric bisexual populations (King, 1993). Thus, the chromosomal investigation is expected to be effective for the elucidation of cryptic species (e.g., King, 1983; Ota, 1988, 1989a). Our survey has detected an unexpectedly prominent taxonomic diversity in each of those species, which has obviously been concealed under low variability in commonly used taxonomic characters.

MATERIALS AND METHODS

Of the two species surveyed, *Mabuya macularia* belongs to the family Scincidae and is broadly distributed in India, Indochina, and adjacent regions (Smith, 1935; Welch et al., 1990; Cox et al., 1998). Smith (1935) reported geographic variation recognized in external characters of this species, and Taylor and Elbel (1958) recognized three subspecies from Thailand, *M. m. quadrifasciata* and *M. m. postnasalis* from the north-central mountain range, and *M. m. malcolmi* from the north-central lowland, on the basis of a few external characteristics observed in small numbers of specimens (also see Taylor, 1963). They, however, did not provide ranges of those three subspecies in the other part of this country. Indeed, for a number of specimens from the eastern hilly and low altitude regions, diagnostic characters of the three subspecies proposed by those authors failed to determine their subspecific identities (Ota, unpublished observations).

Dixonius siamensis, formerly referred to as *Phyllodactylus siamensis* (see Bauer et al., 1997), belongs to the family Gekkonidae and is also broadly distributed in Indochina, including the whole Thailand exclusive of high mountains (Taylor, 1963; Welch et al., 1990). No subspecies are recognized for this species.

Field sampling was carried out in northern and eastern Thailand, and 13 specimens of *M. macularia* and 11 specimens of *D. siamensis* were collected from six localities within these regions (Fig. 1; Table 1). These specimens were karyotyped by a bone marrow air dry method in the field following Ota (1989b), or after being brought back to the laboratory following Ota et al. (1987). Slides were stained in 3% Giemsa solution for 30 min, rinsed in running tap water for 1 min, dried for 24 hrs, and then were subjected to microscopial investigation and photographing.

For most individuals, karyotype was determined on the basis of five or more well spread metaphase cells. For two males of *M. macularia* from Sakaerat (KUZ R40699 and 40711) and one female of *D. siamensis* from Phu Wua (KUZ R40524), however, only two and three metaphase cells were respectively obtained, on which their karyotypes were determined. In the description of chromosome morphology below, terminology follows Green and Sessions (1991).

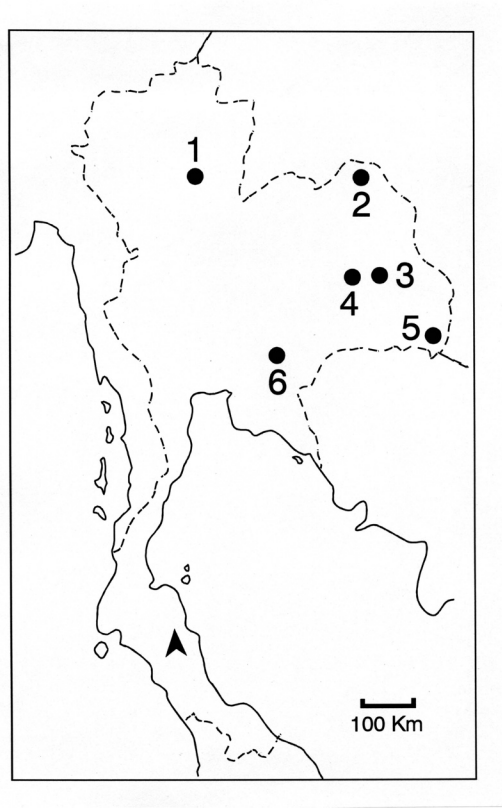

FIGURE 1. A map of Thailand showing sampling localities of specimens karyotyped. 1, Mae Yom. 2, Phu Wua. 3, Phu Pa Namtip. 4, Khao Wang. 5, Yot Dom. 6, Sakaerat. Arrow indicates Khao Luang, where a female *Mabuya macularia* karyotyped by Ota et al. (1996) was collected.

RESULTS

Of the *M. macularia* specimens karyotyped, a female from Mae Yom, northwestern Thailand (Fig. 1), had 2n=38 chromosomes in a graded series (Fig. 2A). Of these chromosomes, pairs 1, 2, 4 and 6-8 were metacentric, whereas pairs 3 and 5 appear submetacentric or subtelocentric. Due to the failure in making sufficient elongation for each chromosome in our preparation, centromeric positions in the remaining pairs were not determined with certainty. The other specimens, all from eastern Thailand (Fig. 1; Table 1), invariably had a 2n=34 karyotype consisting of four pairs of distinctly enlarged chromosomes and 13 much smaller pairs forming a graded series (Fig. 2B). Of these, all pairs of the larger group, and pairs 5 and 6 of the smaller group were metacentric. Like in the above karyotype, centromeric positions in the remaining pairs remained uncertain.

Both males and females of *D. siamensis* from eastern Thailand (Fig. 1) invariably had 2n=40 chromosomes in a largely graded series, of which pair 1 was metacentric and the remainder telocentric (Fig. 3A). So, the fundamental number (N.F.) equaled 42. Specimens from Mae Yom (Fig. 1), on the other hand, had 2n=42 chromosomes that also constitute a largely graded series. In three males from this locality, all chromosomes,

including the largest pair, were telocentric (Fig. 3B), and thus the N.F. value was also 42. However, karyotype of the one Mae Yom female contained one metacentric chromosome as a component of the largest pair (Fig. 3C), making the N.F. 43. Such a prominent heteromorphism in pair 1 was evident in all of the 17 metaphase cells obtained from this specimen.

DISCUSSION

Above results indicate the presence of extensive chromosomal variation in each of the two broadly distributed lizards within Thailand. With respect to *M. macularia,* Ota et al. (1996) already reported a 2n=38 karyotype for a female from Khao Luang, southwestern Thailand (Fig. 1). Karyotype of a female from Mae Yom obtained this time shares with the Khao Luang karyotype most features, such as the diploid chromosome number and the absence of distinct subdivision into size groups. These karyological features are quite exceptional as of the subfamily Lygosominae, since the other lygosomine species hitherto studied, including 17 species of *Mabuya*, share the remarkably smaller diploid number ($\leq$ 32), and a distinct size gap between the four largest and the remaining smaller chromosome pairs (Olmo, 1986; Ota et al., 1996). By contrast, the karyotype of *M. macularia* from eastern Thailand fits to such a general lygosomine format

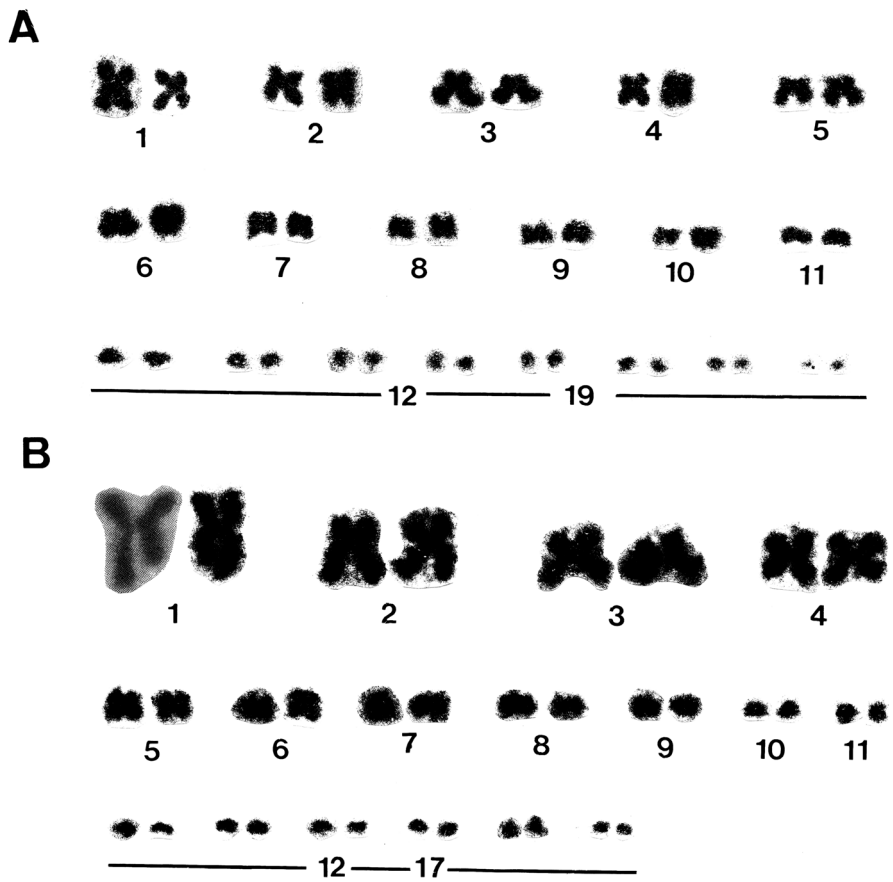

FIGURE 2. Karyotypes of *Mabuya macularia*. (A) female from Mae Yom. (B) female from Phu Wua.

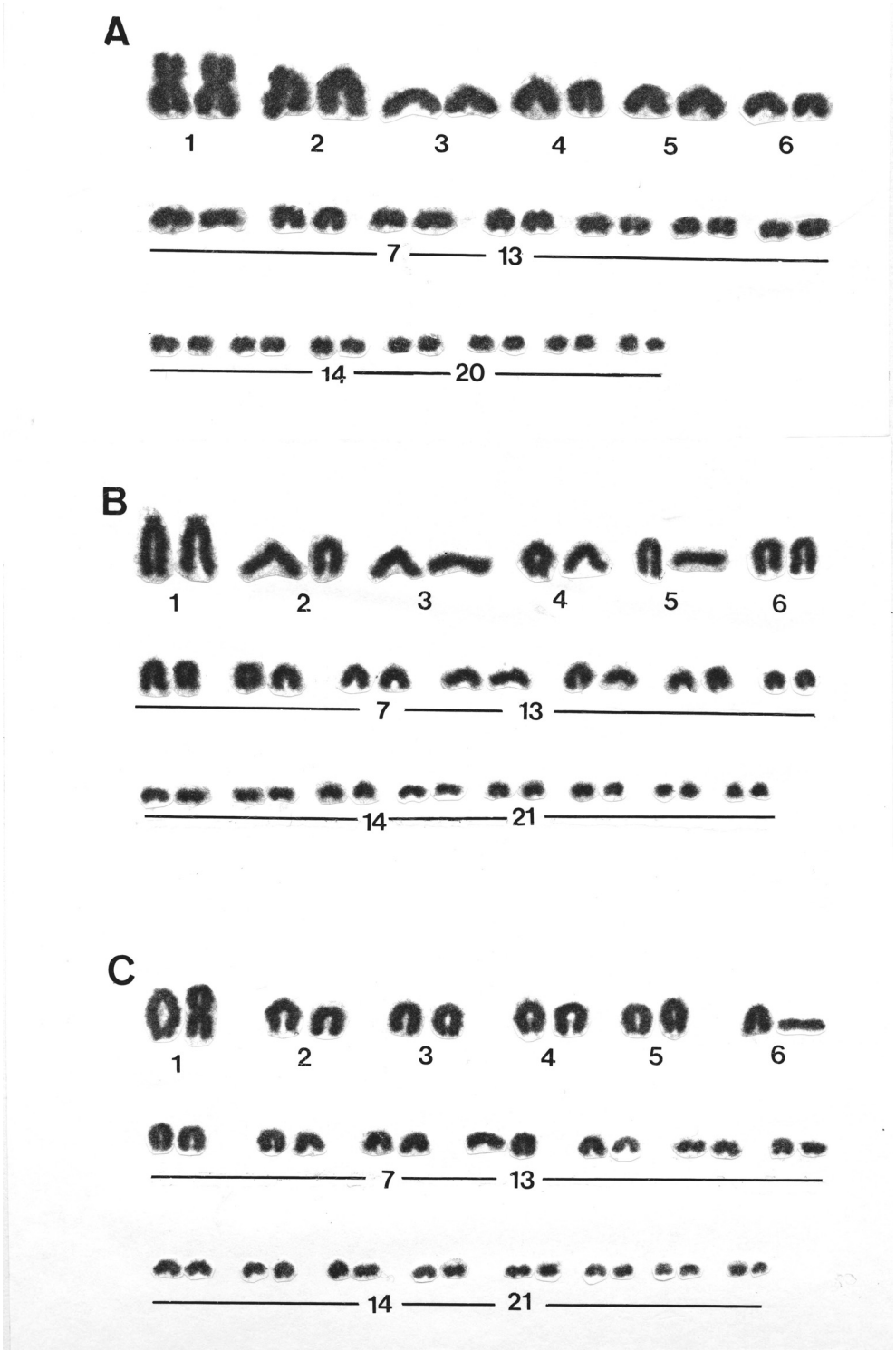

FIGURE 3. Karyotypes of *Dixonius siamensis*. (A) male from Phu Wua. (B) male from Mae Yom. (C) female from Mae Yom. Note the heteromorphism prominent in pair 1 in (C).

except for the presence of one extra-pair of microchromosomes. Furthermore, Makino and Asana (1950) described karyotype of this species from India as consisting of eight large biarmed chromosomes and 24 distinctly smaller chromosomes. It is thus highly likely that populations of *M. macularia* from the northern and southwestern Thailand are historically exclusively close to each other.

In the karyotype of the Khao Luang female, pairs 3 and 4 are described as metacentric and subtelocentric (Ota et al., 1996), whereas those in the present karyotypes were submetacentric or subtelocentric and metacentric, respectively. Detailed comparisons of clearer metaphase preparations with more elongated chromosomes for both of those populations are needed to see whether these differences reflect their actual differentiations or mere artifacts.

No chromosomal descriptions have ever been made for the genus *Dixonius* (or Asiatic *Phyllodactylus* sensu lato: Bauer et al., 1997), and this is the first report of the karyotype at generic level. Identical N.F. value (42) in karyotypes of *D. siamensis* from eastern and northern Thailand suggests that one of them was derived from the other through a simple Robertsonian rearrangement like several other species complexes of lizards showing extensive chromosomal variations (King, 1981; Bickham, 1983).

The heteromorphic karyotype of the Mae Yom female may have been derived from the homologous, all telocentric karyotype as possessed by the Mae Yom male through pericentric inversion involving one of the largest chromosomes. Several authors already reported heteromorphisms in gekkonid karyotypes, and interpreted most of them as reflecting heteromorphic sex chromosomes (e.g., Moritz, 1990; Ota et al., 1992). We thus assume at this moment that the above result indicates the presence of female heteromorphic sex chromosomes (i.e., ZW system) in the Mae Yom population of *D. siamensis*. Considering that only one female and three males from this locality were karyotyped and that the balanced chromosome polymorphism was also documented for a different gekkonid population (Lau et al., 1997; Ota, unpublished observations), this postulation definitely needs careful verification on the basis of additional materials.

From a taxonomic view point, present results strongly suggest the presence of more than one biological species within each of the two "broadly distributed species". This further argues the necessity of surveyal studies by use of genetic approaches for a number of other morphologically defined "broadly distributed species" in Thailand and its vicinity in order to evaluate taxonomic diversity and endemicity of herpetofauna in this region appropriately.

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