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Cytogenetic study of the Bent-toed Gecko (Reptilia, Gekkonidae) in Thailand; I: Chromosomal classical features and NORs characterization of *Cyrtodactylus kunyai* and *C. interdigitalis*

Weera Thongnetr[1,2], Alongklod Tanomtong[1,3], Suphat Prasopsin[4], Nuntiya Maneechot[5], Krit Pinthong[5], Isara Patawang[6,7,*]

[1] *Department of Biology, Faculty of Science, Khon Kaen University, Muang, Khon Kaen, Thailand*
[2] *Walai Rukhavej Botanical Research Institute, Mahasarakham University, Kantharawichai, Maha Sarakham, Thailand*
[3] *Toxic Substances in Livestock and Aquatic Animals Research Group, Khon Kaen University, Muang, Khon Kaen, Thailand*
[4] *Research Academic Supports Division, Mahidol University, Kanchanaburi Campus, Saiyok, Kanchanaburi, Thailand*
[5] *Department of Fundamental Science, Faculty of Science and Technology, Surindra Rajabhat University, Muang, Surin, Thailand*
[6] *Department of Biology, Faculty of Science, Chiang Mai University, Muang, Chiang Mai, Thailand*
[7] *Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Chiang Mai University, Muang, Chiang Mai, Thailand*
* Corresponding author: isara.p@cmu.ac.th

Abstract. This study analysed the karyotype of *Cyrtodactylus kunyai* and *C. interdigitalis* from Loei Province in Northeastern Thailand. The metaphase and meiotic chromosome preparations were obtained by squash technique from bone marrow and testes, respectively. The chromosomes were stained by Giemsa staining and Ag-NOR-banding techniques. The results showed diploid chromosome number (2*n*) of 40 for *C. kunyai* and 42 for *C. interdigitalis*. The chromosome types of metacentric, submetacentric, acrocentric and telocentric chromosomes were 8-4-0-28 and 4-2-4-32, respectively. The Ag-NORs banding technique provides the pair of nucleolar organizer regions (NORs) of both two species at telomeric region of the long arm of the pair 12, metacentric type in *C. kunyai* and telocentric type in *C. interdigitalis*. There are no sex differences in karyotypes between males and females of both two species. We found that during metaphase I on meiosis of *C. kunyai* and *C. interdigitalis*, the homologous chromosomes showed synapsis of 20 and 21 bivalents, respectively. Moreover, the meiotic phase on prophase II exhibited 20 and 21 haploid chromosome number (*n*) as respective diploid species. Their karyotype formulas is as follows: *C. kunyai* ($2n = 40$): $L^{m}_{2} + L^{sm}_{4} + L^{t}_{4} + M^{t}_{6} + S^{m}_{6} + S^{t}_{18}$, and *C. interdigitalis* ($2n = 42$): $L^{a}_{4} + L^{t}_{14} + M^{t}_{2} + S^{m}_{4} + S^{sm}_{2} + S^{t}_{16}$.

Keywords. *Cyrtodactylus*, *Cyrtodactylus kunyai*, *Cyrtodactylus interdigitalis*, Karyotype stasis.

INTRODUCTION

The bent-toed gecko, genus *Cyrtodactylus* Gray 1827, belong to the class Reptilia, order Squamata, suborder Lacertilia, and family Gekkonidae. The *Cyrtodactylus* is the most diverse gekkonid genus with ~250 species (Uetz et al. 2018). The species distribution of *Cyrtodactylus* found in mainland of Asia, from boundary between middle east and India to southeast Asia, Archipelago of southeast Asia and Pacific to Australia (Shea et al. 2011; Hartmann et al. 2016). In Thailand, there are about 24 species of *Cyrtodactylus* that were reported: including *C. angularis*, *C. auribalteatus*, *C. brevipalmatus*, *C. chanhomeae*, *C. consobrinus*, *C. dumnuii*, *C. erythrops*, *C. feae*, *C. interdigitalis*, *C. intermedius*, *C. jarujini*, *C. lekaguli*, *C. macrotuberculatus*, *C. oldhami*, *C. papilionoides*, *C. peguensis*, *C. phuketensis*, *C. pulchellus*, *C. quadrivirgatus*, *C. sumonthai*, *C. surin*, *C. thirakhupti*, *C. tigroides*, and *C. variegatus* (Chuaynkern and Chuaynkern 2012). Among the Gekkonidae population in Thailand, the *Cyrtodactylus* is the most diverse group than other gekkonid genera and there is continually new species discovered.

Karyological analyses in bent-toed gecko thus far has differentiated species based on mitotic metaphase chromosomal morphology while sporadic reports have based the species differentiation based on meiotic metaphase chromosomal morphology. Thus the basic diploid number of the bent-toed gecko is in the range of 42-48 (Ota et al. 1992). Example of chromosome study of other gekkonid that have been reported such as; *Gekko*: diploid number ranging from 38-42 and mostly 38 (Ota 1989; Shibaike et al. 2009; Trifonov et al. 2011; Patawang et al. 2014; Patawang and Tanomtong 2015a), *Hemidactylus*: diploid distance from 40-56 and mostly 40 or 46 (De Smet 1981; Patawang and Tanomtong 2015b), *Gehyra*: mostly 44 (King 1984), *Ptychozoon*: $2n$ = 34 and 42 (Ota and Hikida 1988), *Paroedura*: diploid number ranging from 31-38 and mostly 36 (Aprea et al. 2013; Koubová et al. 2014), *Phelsuma*: $2n$ = 36 (Aprea et al. 1996), and *Dixonius*: $2n$ = 42 (Ota et al. 2001). Most gekkonid chromosome complements consist of acrocentric or telocentric chromosomes which gradually decrease in size, and karyotype evolution within the group is accompanied by Robertsonian fissions, fusions and pericentric inversions (Gorman 1973). The report of King (1987) presented eight putative ancestral karyomorphs ($2n$ = 32, 34, 36, 38, 40, 42, 44, and 46 all acrocentric or telocentric chromosomes) and assigned the genus *Cyrtodactylus* to a group sharing an ancestral karyomorph consisting of $2n$ = 42 uni-armed chromosomes.

In Thailand, there are no studies of *Cyrtodactylus*'s chromosome or karyotypic analyses. The present study of the cytogenetic of two *Cyrtodactylus* provides the first report of both two species, overall on the conventional Giemsa, Ag-NOR banding, and meiotic cell division. Data provided here will increase our knowledge of cytogenetic information which can be used as a basis to comprehensively examine the taxonomy and evolutionary relationship of *Cyrtodactylus* species and other gekkonid.

MATERIALS AND METHOD

Five male and four female specimens of *C. kunyai* (Figure 1a) and five male and six female of *C. interdigitalis* (Figure 1b) were collected from Puan Phu Sub-district, Nong Hin District, Loei Province, Northeastern Thailand. Chromosome preparation was conducted by the squash technique, from bone marrow for mitotic cell and testis for male meiotic cell, and followed with colchicine-hypotonic-fixation-air-drying technique (Patawang et al. 2014). The chromosomes were stained with 10% Giemsa for 30 min and NORs were identified through Ag-NOR staining (Howell and Black 1980; Rooney 2001). The length of short arm (Ls) and long arm (Ll) chromosomes were measured and calculated for the length of total arm chromosomes (LT, LT = Ls+Ll). Relative length (RL, RL = LT/∑LT) and centromeric index (CI, CI = Ll/LT) were estimated. CI was also computed to classify the types of chromosomes (Turpin and Lejeune 1965; Chaiyasut 1989). All parameters were used in karyotyping and idiograming.

RESULTS AND DISCUSSION

Mitotic chromosome features from Giemsa staining

Karyomorphology of the *C. kunyai* and *C. interdigitalis* revealed that the diploid chromosome number ($2n$)

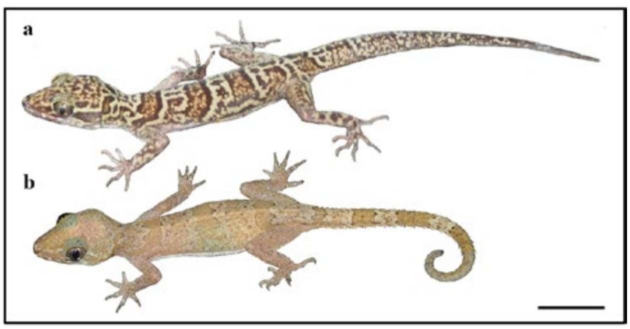

Fig. 1. General characteristic of male *Cyrtodactylus kunyai* (a) and female *C. interdigitalis* (b) (scale bar = 2 cm).

is 40 and 42, respectively. The karyotype of *C. kunyai* composed of 8 metacentric, 4 submetacentric, and 28 telocentric (Table 1 and Figures 2a-b), while the karyotype of *C. interdigitalis* comprised 4 metacentric, 2 submetacentric, 4 acrocentric, and 32 telocentric (Table 2 and Figures 2c-d). Both two species exhibit no sex differences in karyotypes between males and females (Figures 2a-d). Chromosome sizes of *C. kunyai* have pairs 1st to 5th to be large, pairs 6th to 8th to be medium, and pairs 9th to 20th to be small (Figure 3a). For the size of *C. interdigitalis* included large size in pairs 1st to 9th, medium size in the 10th, and small size in pairs 11th to 21st (Figure 3b). The diploid number of *C. kunyai* (2*n* = 40) and *C. interdigitalis* (2*n* = 42), both showed difference and accordance with others *Cyrtodactylus* that have been reported (Table 3). However, overall of these karyotypes of *C. kunyai* and *C. interdigitalis* resemble to other *Cyrtodactylus* and other gekkonid, which comprised many gradient mono-armed (telocentric) and few bi-armed chromosomes (meta- or submetacentric). Proximity of chromosome number and karyotype feature within genus *Cyrtodactylus* represents a close evolutionary line in the group.

Nucleolar organizer region and meiotic cell characteristics

The objective of the Ag-NOR banding technique is to detect nucleolar organizer regions (NORs) which represent the location of genes that have a function in ribosome synthesis (18S and 28S ribosomal RNA). The first cytogenetic study of *C. kunyai* and *C. interdigitalis* performed by Ag-NOR banding technique was obtained from this research. We found the clearly observable NORs on the region adjacent to telomere of long arm of the metacentric chromosome pair 12th (Figures 4a-b) for *C. kunyai* and on the region adjacent to telomere of long arm of the telocentric chromosome pair 12th (Figures 5a-b) for *C. interdigitalis*. Compared with other geckos, most showed two NORs appearing near telomeric region of small bi-armed or small mono-armed chromosome. An example of the previous reports of the geckos' NOR

Table 1. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total chromosomes (LT), centromeric index (CI), relative length (RL) and standard deviation (SD) of CI and RL from 20 metaphases of male and female *Cyrtodactylus kunyai*, 2*n* = 40.

Ch.p	Ls	Ll	LT	CI±SD	RL±SD	Ch.s	Ch.t
1	4.306	6.392	10.698	0.597±0.005	0.106±0.005	L	m
2	2.753	5.639	8.392	0.672±0.036	0.083±0.000	L	sm
3	2.911	5.120	8.031	0.637±0.042	0.080±0.003	L	sm
4	0.000	7.179	7.179	1.000±0.000	0.071±0.003	L	t
5	0.000	6.838	6.838	1.000±0.000	0.068±0.003	L	t
6	0.000	6.452	6.452	1.000±0.000	0.064±0.002	M	t
7	0.000	5.837	5.837	1.000±0.000	0.058±0.003	M	t
8	0.000	5.443	5.443	1.000±0.000	0.054±0.003	M	t
9	0.000	5.210	5.210	1.000±0.000	0.052±0.002	S	t
10	0.000	4.799	4.799	1.000±0.000	0.048±0.003	S	t
11	0.000	4.208	4.208	1.000±0.000	0.042±0.004	S	t
12*	1.946	2.147	4.093	0.525±0.017	0.041±0.003	S	m
13	0.000	3.319	3.319	1.000±0.000	0.033±0.002	S	t
14	0.000	3.300	3.300	1.000±0.000	0.033±0.003	S	t
15	0.000	3.189	3.189	1.000±0.000	0.032±0.003	S	t
16	1.570	1.580	3.150	0.547±0.030	0.035±0.003	S	m
17	0.000	2.793	2.793	1.000±0.000	0.028±0.003	S	t
18	0.000	2.654	2.654	1.000±0.000	0.026±0.002	S	t
19	0.000	2.443	2.443	1.000±0.000	0.024±0.003	S	t
20	1.136	1.156	2.292	0.552±0.020	0.025±0.002	S	m

Abbreviations: *Ch.p*, chromosome pair; *Ch.s*, chromosome size; *Ch.t*, chromosome type; *, nucleolar organizer region; *L*, large size; *M*, medium size; *S*, small size; *m*, metacentric; *sm*, submetacentric; *t*, telocentric.

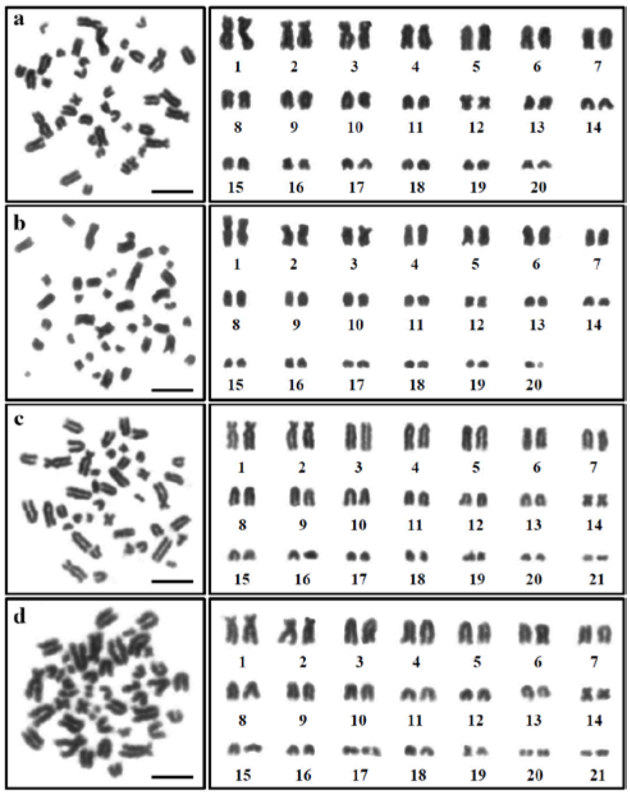

Fig. 2. Conventionally stained somatic metaphase complement and karyotypes of male (a) and female (b) of *Cyrtodactylus kunyai*, 2*n* = 40, and male (c) and female (d) of *C. interdigitalis*, 2*n* = 42, (scale bars = 10 μm).

Table 2. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total chromosomes (LT), centromeric index (CI), relative length (RL) and standard deviation (SD) of CI and RL from 20 metaphases of male and female *Cyrtodactylus interdigitalis*, $2n = 42$.

Ch.p	Ls	Ll	LT	CI±SD	RL±SD	Ch.s	Ch.t
1	2.715	6.857	9.572	0.716±0.012	0.090±0.005	L	a
2	2.193	6.374	8.567	0.744±0.018	0.081±0.003	L	a
3	0.000	8.136	8.136	1.000±0.000	0.077±0.004	L	t
4	0.000	7.822	7.822	1.000±0.000	0.074±0.003	L	t
5	0.000	7.505	7.505	1.000±0.000	0.071±0.005	L	t
6	0.000	6.513	6.513	1.000±0.000	0.062±0.002	L	t
7	0.000	6.066	6.066	1.000±0.000	0.057±0.003	L	t
8	0.000	5.874	5.874	1.000±0.000	0.056±0.003	L	t
9	0.000	5.677	5.677	1.000±0.000	0.054±0.002	L	t
10	0.000	5.337	5.337	1.000±0.000	0.050±0.003	M	t
11	0.000	4.539	4.539	1.000±0.000	0.043±0.004	S	t
12*	0.000	4.031	4.031	1.000±0.000	0.038±0.003	S	t
13	0.000	3.808	3.808	1.000±0.000	0.036±0.003	S	t
14	1.824	1.830	3.654	0.501±0.027	0.035±0.004	S	m
15	1.102	2.235	3.337	0.670±0.041	0.032±0.004	S	sm
16	0.000	3.113	3.113	1.000±0.000	0.029±0.003	S	t
17	0.000	2.856	2.856	1.000±0.000	0.027±0.003	S	t
18	0.000	2.820	2.820	1.000±0.000	0.027±0.001	S	t
19	1.399	1.410	2.809	0.502±0.038	0.027±0.003	S	m
20	0.000	2.017	2.017	1.000±0.000	0.019±0.003	S	t
21	0.000	1.712	1.712	1.000±0.000	0.016±0.002	S	t

Abbreviations: *Ch.p*, chromosome pair; *Ch.s*, chromosome size; *Ch.t*, chromosome type; *, nucleolar organizer region; *L*, large size; *M*, medium size; *S*, small size; *m*, metacentric; *sm*, submetacentric; *a*, acrocentric; *t*, telocentric.

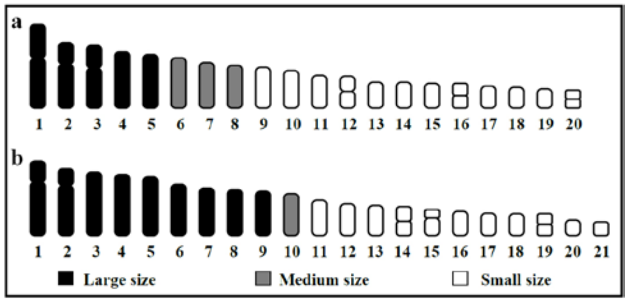

Fig. 3. Standardized idiogram of *Cyrtodactylus kunyai* (a) and *C. interdigitalis* (b) by conventional staining.

localization included in the genus *Gehyra* (King 1983), *Gekko* (Chen et al. 1986; Shibaike et al. 2009; Patawang et al. 2014), *Hemidactylus* (Patawang and Tanomtong 2015b), and *Lepidodactylus* (Trifonov et al. 2015). These previous studies showed the NOR appearing near terminal region of one homologous small chromosome.

The present study on male meiotic cell division in *C. kunyai* and *C. interdigitalis* found the late interphase to early prophase that the cell of each species showed two signals of nucleolus by positive silver staining (Figures 4c, 5c). The characteristics of two nucleolus structure at early phase of cell division supported the appearing of two NORs on one homologous at metaphase chromosome in both two *Cyrtodactylus* species. We found diplotene phase in meiotic cell of both two species (Figures 4d, 5d), which showed synapsis between two of homologous and compacted chromosome. The metaphase I (meiosis I, reductional division) was found in two species, which can be defined as the 20 bivalents for *C. kunyai* (Figure 4e) and 21 bivalents for *C. interdigitalis* (Figure 5e). No metaphase I cells with partially paired bivalents, which are speculated to be male heteromorphic sex chromosomes in both two *Cyrtodactylus* species. Moreover, $n = 20$ in *C. kunyai* (Figure 4f) and $n = 21$ in *C. interdigitalis* (Figure 5f) were found at metaphase II (meiosis II, equational division) of spermatid cells. Form these results, behavior and number of chromosome in metaphase I and metaphase II confirmed of each other's accuracy and also verified the accuracy of diploid chromosome in somatic cells.

Overview of chromosomal feature of the two Cyrtodactylus

Gekkonid chromosome that has been reported in the past, most species show the gradient karyotype, which comprising of many mono-armed chromosomes

Table 3. Review of cytogenetic study of the genus *Cyrtodactylus*.

Species	*2n*	NF	Karyotype	NOR	Locality	Reference
C. consobrinus	48	50	2bi-armed+46t	-	Sarawak, Malaysia	Ota et al. (1992)
C. interdigitalis	42	52	4m+2sm+4a+32t		Loei, Thailand	Present study
C. kunyai	40	52	8m+4sm+28t		Loei, Thailand	Present study
C. pubisulcus	42	44	2bi-armed+40t	-	Sarawak, Malaysia	Ota et al. (1992)

Abbreviations: *2n*, diploid chromosome; *NF*, fundamental number; *NOR*, nucleolar organizer region; *bi-armed*, bi-armed chromosome; *m*, metacentric; *sm*, submetacentric; *a*, acrocentric; *t*, telocentric.

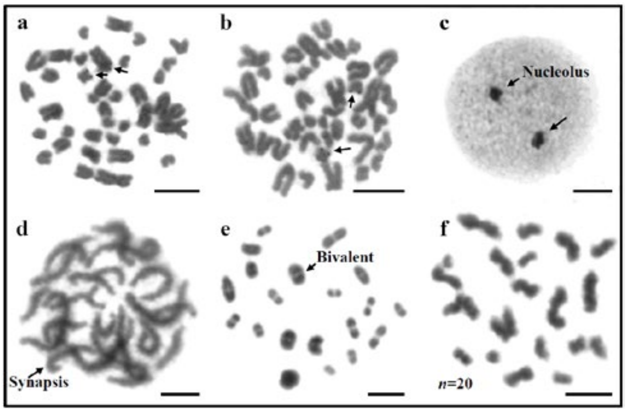

Fig. 4. Silver stained somatic metaphase complement of male (a) and female (b) of *Cyrtodactylus kunyai*, arrows indicate nucleolar organizer region (NOR), and meiotic cell division of the male *C. kunyai* on interphase by silver NOR staining (c), diplotene (d), metaphase I (e), and metaphase II (f) (scale bars = 10 μm).

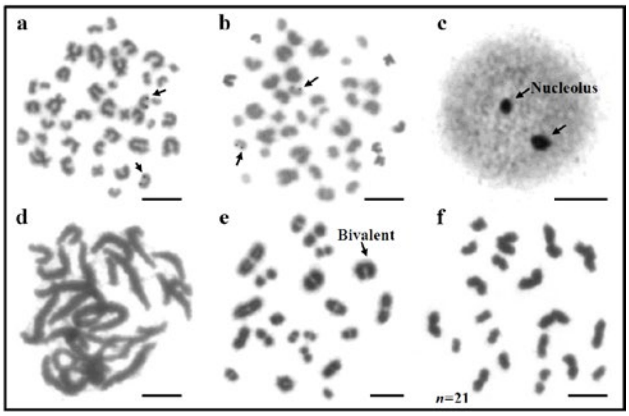

Fig. 5. Silver stained somatic metaphase complement of male (a) and female (b) of *Cyrtodactylus interdigitalis*, arrows indicate nucleolar organizer region (NOR), and meiotic cell division of the male *C. interdigitalis* on interphase by silver NOR staining (c), pachytene (d), metaphase I (e), and metaphase II (f) (scale bars = 10 μm).

and few bi-armed chromosomes. Present results of *C. kunyai* and *C. interdigitalis* agree with chromosomal evolution line hypothesis within the gekkonid group. The karyotype of *C. kunyai* and *C. interdigitalis* showed the gradient of most telocentric while comprised of a few bi-armed chromosome, 12 chromosomes in *C. kunyai* and 10 chromosomes in *C. interdigitalis*. These features conform to the hypothesis of rearrangement from ancestral karyotype by Robertsonian fissions, fusions or pericentric inversions (Gorman 1973; King 1987).

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