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First report of chromosome and karyological analysis of *Gekko nutaphandi* (Gekkonidae, Squamata) from Thailand: Neo-diploid chromosome number in genus *Gekko*

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Abstract. The karyotypes of red-eyed Gecko are not reported yet. Herein, we describe the karyotypes of red-eyed Gecko (*Gekko nutaphandi* Bauer, Sumontha & Pauwels, 2008) from Thailand. Gecko chromosome preparations were directly conducted from bone marrow and testis. Chromosomal characteristics were analyzed by Giemsa staining, Ag-NOR banding as well as fluorescence in situ hybridization (FISH) using microsatellites $d(GC)_{15}$ probe. The results showed that the number of diploid chromosomes is $2n$=34, while the fundamental number (NF) is 46 in both males and females. The types of chromosomes were 4 large metacentric, 6 large submetacentric, 2 medium telocentric, 2 small metacentric and 20 small telocentric chromosomes. The results of conventional Giemsa staining presented the diploid chromosome number differentiation even in the same genus. NORs are located at the secondary constriction to the telomere on the long arm of chromosome pair 5. There are no sex differences in karyotypes between males and females. FISH with $d(GC)_{15}$ sequences were also displayed at the telomeres of most other chromosomes. We found that during metaphase I the homologous chromosomes showed synapsis, which can be defined as 19 ring bivalents and 17 haploid chromosomes (n=17) at metaphase II as a diploid species. The karyotype formula is as follows: $2n$ (34) = $L_4^m+L_6^{sm}+M_2^t+S_2^m+S_{20}^t$.

Keywords: Chromosome, *Gekko nutaphandi*, Karyotype, Red-eyed Gecko.

INTRODUCTION

Geckos of the genus *Gekko* Laurenti, 1768 are members of a relatively large putatively monophyletic group of lizards that also includes *Lepidodactylus* Fitzinger, 1843, *Luperosaurus* Gray, 1845, *Pseudogekko* Taylor, 1922, and *Ptychozoon* Kuhl & van Hasselt, 1822 (Kluge 1968; Russell 1972; Bauer et al. 2008). The genus *Gekko* itself is species rich (84 species) and is generally characterized by regional endemism across its broad range in tropical Asia. Recently, *G. nutaphandi,* is described from Kanchanaburi Province in central western Thailand (Bauer et al. 2008). *G. nutaphandi* is most similar to *G. gecko*, *G. smithii*, *G. siamensis*, *G. verreauxi*, and *G. albofasciolatus* Günther, 1872, which have previously been considered to be closely related based on their shared possession of a suite of features.

Information about karyotypes in *Gekko* is scarce and fragmented, and usually based on conventional staining technique; only a few studies have been published (19 species of 84 species), all of them recent in genus *Gekko,* namely, *G. gecko*: 2n=38 (Singh 1974; Solleder and Schmid 1984; Wu and Zhao 1984; Trifonov et al. 2011; Qin et al. 2012; Patawang et al. 2014), *G. hokouensis*: 2n=38 (Chen et al. 1986; Kawai et al. 2009; Shibaike et al. 2009), *G. japonicus*: 2n=38 (Yoshida and Itoh 1974; Shibaike et al. 2009; Trifonov et al. 2011), *G. shibatai* and *G. vertebralis*: 2n=38 (Shibaike et al. 2009), *G. Vittatus* and *G. ulikovskii*: 2n=38 (Trifonov et al. 2011), *G. tawaensis*: 2n=38 (Ota 1989a; Shibaike et al. 2009), *G. taylori*: 2n=42 (Ota and Nabhitabhata 1991), *G. monarchus*: 2n=44 (Ota et al. 1990), *G. yakuensis*, *G. petricolus* and *G. smithii*: 2n=38-42 (Ota 1989a), *G. kikuchii*: 2n=44 (Ota 1989a), *G. chinensis*: 2n=40 (Lau et al. 1997), *G. subpalmatus*: 2n=38 (Wu and Zhao 1984), *G. swinhonis*: 2n=38 (Chen et al. 1986), *G. petricolus*: 2n=34 (Thongnetr et al. 2022), *Dixonius hangseesom*: 2n=40, *D. Siamensis*: 2n=40, *D. melanostictus*: 2n=42 (Patawang et al. 2022) and *Cyrtodactylus inthanon*: 2n=40 (Prasopsin et al. 2022) and shown in Table 1.

This fact is probably due to the difficulty in obtaining samples for cytogenetic analysis in some species or to problems in obtaining metaphase cells by cell culture induction. Moreover, cytogenetic studies using conventional staining techniques provide valuable information on the excellent karyotype diversity shown by these animals. Analyses of cytogenetic markers, including the number and karyotype formula, sex determination, B chromosomes, number and location of nucleolar organizer regions (NORs), heterochromatin distribution, G-banding, and R-banding, treatments with base-specific fluorochromes and fluorescence *in situ* hybridization techniques allowed the cytogenetic characterization of populations, species, and supra-specific groups (Affonso and Galetti Jr. 2005). The present study is the first report on the chromosomal characteristics of *G. nutaphandi* determined using conventional staining, Ag-NOR banding, and fluorescence *in situ* hybridization techniques.

MATHERIAL AND METHODS

Sample collection, chromosome preparation and chromosome staining

We obtained specimens of *G. nutaphandi* that were collected from Kanchanaburi Province, Western Thailand. Chromosomes were directly prepared *in vivo* (Ota 1989a; Qin et al. 2012) using the following method. With 20% of Giemsa solution, the slides were conventionally stained for 30 minutes (Patawang et al. 2014). After that, the slides were rinsed thoroughly with running tap water to remove excess stain and placed in air-dry at room temperature. Ag-NOR banding was analysed according to the method of Howell and Black (1980). Two drops each of 50% silver nitrate and 2% gelatine solutions were added to the slides, respectively. Then, they were sealed with cover glasses and incubated at 60°C for 5-10 minutes. They were also soaked in distilled water until the cover glasses were separated. Finally, the slides were placed in air-dry at room temperature. They were observed under the microscope. The use of microsatellite investigations which were described by Kubat et al. (2008), was followed here with slight modifications. These sequences were directly labeled with Cy3 at the 5′-terminal during synthesis by Sigma (St. Louis, MO, USA.) Fluorescence *in situ* hybridization (FISH) was performed under highly rigorous conditions on mitotic chromosome spreads (Pinkel et al. 1986).

Chromosomal checks, karyotyping and idiograming

Chromosome counting was carried out on mitotic metaphase cells under the light microscope for 30 cells per specimen to determine the diploid number (2*n*). Twenty clearly observable and well-spread metaphase cells were selected and photographed from each male and female. The short arm length (Ls) and the long arm length (Ll) of each chromosome were measured to calculate the total length of the chromosome for 20 well-spread metaphase cells. The chromosome types were classified from the method of Turpin and Lejeune (1965) as metacentric, submetacentric, acrocentric, and telocentric chromosomes.

Table 1. The karyotype reviews among the genes *Gekko* (Gekkonidae, Squamata).

Species	2*n*	NF	Karyotype formulas	NORs	FISH	Locations	References
Gekko gekko	38	50	6m+4sm+2a+26t	P4	-	Thailand	Patawang et al. (2014)
	38	44	6bi-arms+32uni-arms	-	-	-	Singh (1974)
	38	-	-	-	-	-	Wu and Zhao (1984)
	38	46	8bi-arms+30uni-arms	-	-	-	Solleder and Schmid (1984)
	38	-	-	-	-	-	Trifonov et al. (2011)
	38	50	8 m+2sm+2st(a)+26t			Laos	Qin et al. (2012)
	38	48	8 m+2sm+28t	-	-	China	Qin et al. (2012)
G. hokouensis	38	56	4 m+6sm+20t+8bi-arms*	P19	-	China,	Chen et al. (1986)
	38	58/59	2 m+8sm+10a+16t+Z(t)+W(a)	-	-	Japan	Kawai et al. (2009)
	38	58	4 m+8sm+18t+8bi-arms*	P19	-	Taiwan	Shibaike et al. (2009)
	38	58/59	4 m+8sm+16t+8bi-arms*+Z(t)W(sm)	P19	-	Japan	Shibaike et al. (2009)
	38	42	2m+18sm+16a+ZW	P19	FISH mapping	Thailand	Srikulnath (2015)
G. japonicus	38	-	-	-	-	-	Yoshida and Itoh (1974)
	38	58	4m+8sm+8st+18t	P17	-	Japan	Shibaike et al. (2010)
	38	-	-	-	-	-	Trifonov et al. (2011)
G. shibatai	38	58	4m+8sm+18t+8bi-armed	P19	-	Japan	Shibaike et al. (2009)
G. vertebralis	38	62	4m+14sm+14t+6bi-arme*	P19	-	Japan	Shibaike et al. (2009)
G. vittatus	38	-	-			-	Trifonov et al. (2011)
G. ulikovskii	38	-	-			-	Trifonov et al. (2011)
G. tawaensis	38	58	4m+8sm+18t+8bi-armed	P19	-	Japan	Shibaike et al. (2009)
	38	56	-	P19	-	Japan	Ota (1989a)
G. taylori	42	-	-	-	-	Thailand	Ota and Nabhitabhata (1991)
G. monarchus	44	46	-	-	-	Malaysia	Ota et al. (1990)
G. yakuensis	38	56	-	P19	-	Japan	Ota (1989a)
G. petricolus	38	54	-	-	-	Thailand	Ota (1989a)
G. kikuchii	44	50	-	-	-	Taiwan	Ota (1989a)
G. chinensis	40	46	6bi-armed+34uni-armed	-	-	China	Lau et al. (1997)
G. smithii	38	48	-	-	-	-	Ota (1989a)
G. subpalmatus	38	58	-	-	-	-	Wu and Zhao (1984)
G. swinhonis	38	66	-	-	-		Chen et al. (1986)
G. petricolus	38	54	4m+2sm+10a+22t	P17	$(CA)_{15}$, $(GAA)_{10}$	Thailand	Thongnetr et al. (2022)
G. nutaphandi	34	46	6m+6sm+22t	P5	$(GC)_{15}$	Thailand	Present study
Dixonius hangseesom	40	42	2m+38t	P13	-	Thailand	Patawang et al. (2022)
D. siamensis	40	42	2m+38t	P13	-	Thailand	Patawang et al. (2022)
D. melanostictus	42	44	2a+40t	P8	-	Thailand	Patawang et al. (2022)
Cyrtodactylus inthanon	40	58	12m+4sm+2a+22t	P12	$(CA)_{15}$ $(GC)_{15}$ $(CAG)_{10}$ $(GAA)_{10}$	Thailand	Prasopsin et al. (2022)

Note: 2n = diploid chromosome number, NORs = nucleolar organizer regions, NF = fundamental number (number of chromosome arms), bi-arm = bi-armed chromosome, m = metacentric, sm = submetacentric, a = acrocentric, t = telocentric chromosome, *=small bi-arms chromosome, and - = not available.

RESULTS AND DISCUSSION

Diploid chromosome number, fundamental number and karyotype

The *Gecko* is a large genus (84 species) of Gekkonidae family and until now, no study has investigated the karyotype of *G. nutaphandi*. Furthermore, this is the first report on cytogenetic characterization to use conventional Giemsa staining, NOR-banding, and FISH techniques for this species. For *G. nutaphandi,* the results indicated a diploid chromosome number (2*n*) of 34 in all studied samples (Figure 1). This result dif-

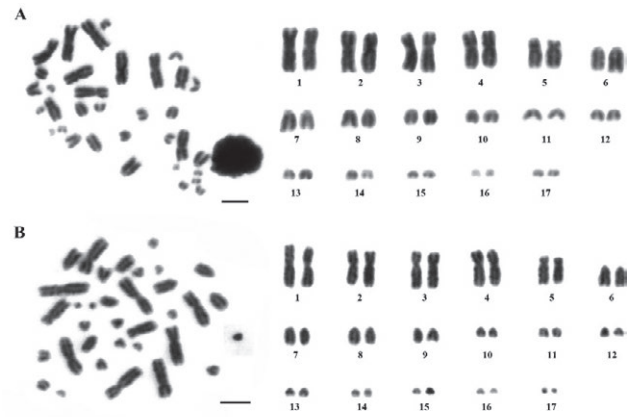

Figure 1. Metaphase chromosome plates and karyotypes of *G. nutaphandi* (A) male (B) female by conventional technique. Scale Bars = 10 μm.

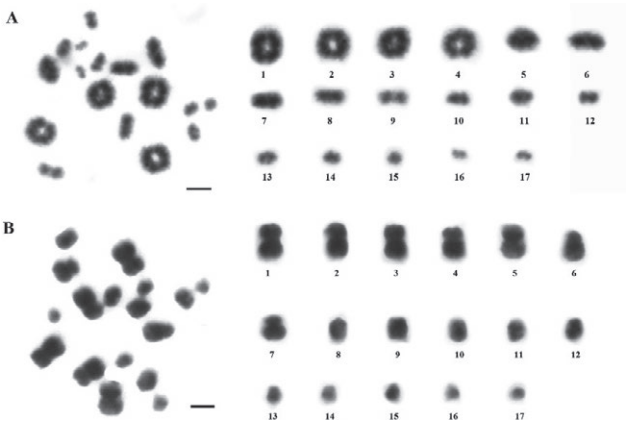

Figure 2. Metaphase chromosome plates and karyotypes of *G. nutaphandi* (A) Metaphase I (B) Metaphase II by conventional technique. Scale Bars = 10 μm.

fers from most species of the genus *Gecko*. The 2*n* of this genus range from 38 to 44. (Singh 1974; Yoshida and Itoh 1974; Solleder and Schmid 1984; Wu and Zhao 1984; Chen et al. 1986; Ota 1989a; Ota et al. 1990; Ota and Nabhitabhata 1991; Lau et al. 1997; Kawai et al. 2009; Shibaike et al. 2009; Trifonov et al. 2011; Qin et al. 2012; Patawang et al. 2014; Patawang et al. 2022 and Prasopsin et al. 2022.

The fundamental number (NF) of *G. nutaphandi* was 46 in both males and females. The karyotype consisted of 4 large metacentric, 6 large submetacentric, 2 medium telocentric, 2 small metacentric, and 20 small telocentric chromosomes (Table 1). These results of NF are agreeable with the previous reports of *G. gecko* (Solleder and Schmid 1984), *G. monarchus* (Ota et al. 1990), and *G. chinensis* (Lau et al. 1997). The NFs of the genus *Gekko* range from 44 to 62, and karyotypes are composed of both mono- and bi-arms chromosomes. Nirchio et al. (2002) proposed that species with high NF is an advanced state or apomorphic character, whereas one with low NF is a primitive state or plesiomorphic character. Thus, the *G. nutaphandi* seems to be a more primitive karyotype than other species. The karyotype formula for this species is $2n$ (34) = $L_4^m+L_6^{sm}+M_2^t+S_2^m+S_{20}^t$. There is no evidence of differentiated sex chromosomes in this species which accord with all species of this genus (Singh 1974; Yoshida and Itoh 1974; Solleder and Schmid 1984; Wu and Zhao 1984; Chen et al. 1986; Ota 1989a; Ota et al. 1990; Ota and Nabhitabhata 1991; Lau et al. 1997; Kawai et al. 2009; Shibaike et al. 2009; Trifonov et al. 2011; Qin et al. 2012; Patawang et al. 2014; Patawang et al. 2022 and Prasopsin et al. 2022.

The present study on the meiotic cell division of *G. nutaphandi* found that during metaphase I (meiosis I), the homologous chromosomes showed synapsis, which can be defined as the 17 bivalent (Figure 2A), and 17 haploid chromosomes at metaphase II (Figure 2B) as diploid species. The largest metacentric chromosome pair 1 is the largest bivalent. No diakinesis and metaphase I cell with partially paired bivalents are speculated to be heteromorphic sex chromosomes, and no metaphase II cells with condensed chromosomes are speculated to be the sex chromosome.

Chromosome markers from Ag-NOR banding

The development of Ag-NOR staining technique (Howell and Black 1980) to detect metaphase chromosome sites of NORs has greatly facilitated comparative studies of NORs variation. Silver staining of NORs is considered as one of the standard banding methods and has assumed considerable importance in characterizing a species' karyotype. The present study was firstly accomplished by using Ag-NOR staining in *G. nutaphandi*. The Ag-NOR positions were shown on the long arm near the centromere of the telocentric chromosome pair 5 (subtelomeric NOR) (Figure 3). The single pair of NOR is the same as in *Gecko* species. Compared with other geckos, the NOR regions showed two NORs appearing near telomeric region of small bi-armed or mono-armed chromosome. An example of the previous reports of the genus *Gekko* had two NORs on one pair of small bi-arms chromosomes. *G. gecko* had two NORs on the near telomere of mono-arms chromosome pair 4 (Patawang et al. 2014), and *G. petricolus* had two NORs on the long arm near telomere of bi-arms chromosome pair 5 (Thongnetr et al. 2022), while there were *G. shibatai*, *G. Yakuensis,*

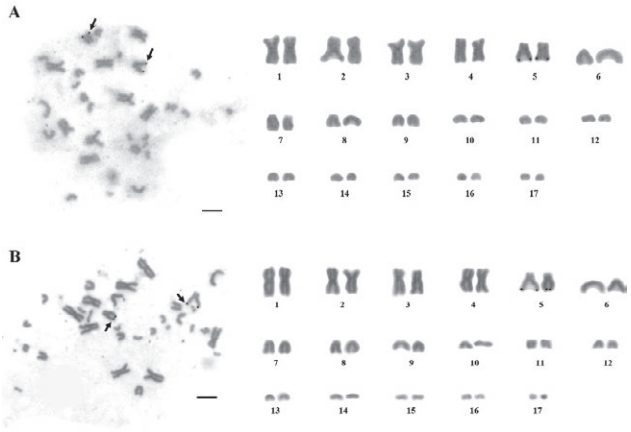

Figure 3. Metaphase chromosome plates and karyotypes of *G. nutaphandi* (A) male (B) female by Ag-NOR banding technique. Scale Bars = 10 μm.

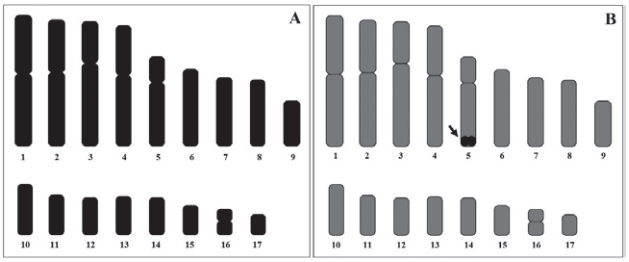

Figure 4. Idiogram represents chromosome type, size, and Ag-NOR banding of *G. nutaphandi*.

G. hokouensis, *G. tawaensis*, *G. vertebralis* and *G. yakuensis* which had two NORs on the long arm near the telomere of small bi-arms chromosome pair 19 (Ota 1989b; Chen et al. 1986; Shibaike et al. 2009). The use of NORs in explaining kinships depends to a considerable extent on the uniformity of this characteristic and the degree of variety within a taxon (Yüksel and Gaffaroğlu 2008).

The idiogram shows a continuous length gradation of chromosomes. The size differences between the largest and smallest chromosomes exhibit approximately two-fold. The data of the chromosome measurement on mitotic metaphase cells (from all specimens) are shown in Table 2. Idiograms by conventional Giemsa staining and Ag-NOR banding are shown in Figure 4.

Microsatellite pattern

Microsatellites or simple sequence repeats (SSRs) are oligonucleotides of 1–6 base pairs in length, forming excessive tandem repeats of usually 4 to 40 units (Tautz and Renz 1984; Ellegren 2004; Chistiakov et al. 2006). They show ample distribution throughout eukaryotic genomes, scattered or clustered in euchromatin and heterochromatin. They are highly polymorphic regarding

Table 2. Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosomes (LT), relative length (RL), centromeric index (CI), and standard deviation (SD) from 20 metaphases of male and female Red-eyed Gecko (*Gekko nutaphandi*), 2*n* (diploid)=34.

Chro.pairs	Ls	Ll	LT	CI±SD	RL±SD	Chro. size	Chro. type
1	0.932	1.131	2.035	0.548±0.012	0.132±0.007	Large	metacentric
2	0.835	1.160	1.961	0.602±0.015	0.120±0.005	Large	submetacentric
3	0.769	1.303	2.001	0.626±0.023	0.115±0.007	Large	submetacentric
4	0.784	1.113	1.833	0.586±0.020	0.113±0.007	Large	metacentric
5*	0.410	1.003	1.372	0.673±0.019	0.084±0.004	Large	submetacentric
6	0.000	1.216	1.216	1.000±0.000	0.066±0.003	Medium	telocentric
7	0.000	1.016	1.016	1.000±0.000	0.060±0.003	Small	telocentric
8	0.000	1.049	1.049	1.000±0.000	0.055±0.002	Small	telocentric
9	0.000	0.720	0.720	1.000±0.000	0.046±0.003	Small	telocentric
10	0.000	0.800	0.800	1.000±0.000	0.037±0.004	Small	telocentric
11	0.000	0.629	0.629	1.000±0.000	0.032±0.003	Small	telocentric
12	0.000	0.595	0.595	1.000±0.000	0.029±0.003	Small	telocentric
13	0.000	0.615	0.615	1.000±0.000	0.026±0.003	Small	telocentric
14	0.000	0.596	0.596	1.000±0.000	0.023±0.004	Small	telocentric
15	0.000	0.464	0.464	1.000±0.000	0.021±0.003	Small	telocentric
16	0.195	0.220	0.374	0.520±0.038	0.024±0.004	Small	metacentric
17	0.000	0.326	0.326	1.000±0.000	0.016±0.003	Small	telocentric

Note: Chro.=chromosome, * NORs bearing chromosomes (satellite chromosome).

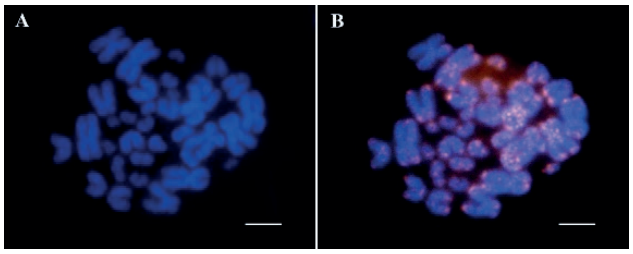

Figure 5. Metaphase chromosome plate of *G. petricolus* (**A**) by FISH technique of DAPI (**B**) microsatellite probe $(GC)_{15}$. Scale Bars = 10 μm.

copy number deviation (Ellegren 2004). Fluorescence hybridization indicates the $(GC)_{15}$ repeat showing abundance at the telomeric ends of most chromosomes (Figure 5), verifying the findings from other gekko groups investigated to date (Srikulnath 2015). The distribution of microsatellites was not only restricted to heterochromatin but also dispersed in euchromatic regions of the chromosomes (Getlekha et al. 2016). Nonetheless, closely related fish species involved in recent speciation events could present a differential pattern in the distribution and quantity of microsatellite sequences on chromosome.

In conclusion, we present the first Karyotype, NOR phenotype, and microsatellite patterns $(GC)_{15}$ on the chromosomes are specific to species in the *G. nutaphandi*. More species and techniques should be further studied for more information about the chromosomal diversity and chromosomal evolution in this genus.

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REFERENCES

Affonso PRAM, Galetti Jr PM. 2005. Chromosomal diversification of reef fishes from genus *Centropyge* (Perciformes, Pomacanthidae). Genetica 123: 227–233.

Bauer AM, Sumontha M, Pauwels SGO. 2008. A new red-eyed Gekko (Reptilia: Gekkonidae) from Kanchanaburi Province, Thailand. Zootaxa 1750(1750): 32–42.

Chen J, Peng X, Yu D. 1986. Studies on the karyotype of three species of the genus *Gekko*. Acta Herpetologica Sinica 5: 24–29.

Chistiakov DA, Hellemans B, Volckaert FAM. 2006. Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. Aquaculture 255: 1–29. https://doi.org/10.1016/j.aquaculture.2005.11.031

Ellegren H. 2004. Microsatellites: simple sequences with complex evolution. Nat Rev Genet 5: 435–445. https://doi.org/10.1038/nrg1348

Getlekha N, Molina WF, Cioffi MB, Yano CF, Maneechot N, Bertollo LAC, Supiwong W, Tanomtong A. 2016. Repetitive DNAs highlight the role of chromosomal fusions in the karyotype evolution of *Dascyllus* species (Pomacentridae, Perciformes). Genetica 144(2): 203–211. https://doi.org/10.1007/s10709-016-9890-5

Howell WM, Black DA. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36: 1014–1015.

Kawai A, Ishijima J, Nishida C, Kosaka A, Ota H, Kohno S, Matsuda Y. 2009. The ZW sex chromosomes of *Gekko hokouensis* (Gekkonidae, Squamata) represent highly conservedhomology with those of avian species. Chromosoma 118: 43–51.

Kluge, A.G. 1968. Phylogenetic relationships of the gekkonid lizard genera *Lepidodactylus* Fitzinger, *Hemiphyllodactylus* Bleeker, and *Pseudogekko* Taylor. *Philippine Journal of Science*, 95: 331–352.

Kubat Z, Hobza R, Vyskot B, Kejnovsky E. 2008. Microsatellite accumulation in the Y chromosome of *Silene latifolia*. Genome 51: 350–356.

Lau MW, Ota H, Bogadek A. 1997. Chromosomes polymorphismand karyotype of *Gekko chinensis* (Tokaynidae: Reptilia) from Hong Kong. J Herpetol 31: 137–139.

Nirchio M, Turner BJ, Perez JE, Gaviria JI, Cequea H. 2002. Karyotypes of three species of toadfish (Batrachoididae: Teleostei) from Venezuela. Sci Mar 66(1): 1–4.

Ota H. 1989a. Karyotypes of five species of *Gekko* (Gekkonidae: Lacertilia) from East and Southeast Asia. Herpetologica 45 (4): 438-443.

Ota H. 1989b. A review of the geckos (Lacertilia: Reptilia) of the Ryukyu Archipelago and Taiwan. In: Matsui M, Hikida T, Goris RC. (eds.). Current Herpetology in East Asia. Herpetological Society of Japan, Kyoto.

Ota H, Hikida T, Matsui M, Mori A. 1990. Karyotype of *Gekko monarchus* (Squamata: Gekkonidae) from Sarawak, Malaysia. Japanese Journal of Herpetology 13: 136–138.

Ota H, Nabhitabhata J. 1991. A new species of *Gekko* (Gekkonidae: Squamata) from Thailand. Copeia 1991: 503–509.

Prasopsin S, Muanglen N, Ditcharoen S, Suwannapoom C, Tanomtong A, Thongnetr W. 2022. First Report on Classical and Molecular Cytogenetics of Doi Inthanon Bent-toed Gecko, *Cyrtodactylus inthanon* Kunya et al., 2015 (Squamata: Gekkonidae) in Thailand. Caryologia, 75(2): 109–117.

Patawang I, Tanomtong A, Jumrusthanasan S, Kakampuy W, Neeratanaphan L, Pinthong K. 2014. Chromosomal characteristics of NORs and karyological analysis of tokay gecko, Gekko gecko)Gekkonidae, Squamata) from mitotic and meiotic cell division. Cytologia 79(3): 315–324.

Patawang I, Prasopsin S, Suwannapoom C, Tanomtong A, Keawmad P, Thongnetr W. (2022). Chromosomal description of three Dixonius (Squamata, Gekkonidae) from Thailand. Caryologia, 75(2): 101–108.

Pinkel D, Straume T, Gray J. 1986. Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. Proceedings of the National Academy of Sciences of the United States of America 83: 2934–2938.

Qin XM, Li HM, Zeng ZH, Zeng DL, Guan QX. 2012. Genetic variation and differentiation of *Gekko gecko* from different populations based on mitochondrial cytochrome *b* gene sequences and karyotypes. Zoological Science 29: 384–389.

Russell AP. 1972. The Foot of Gekkonid Lizards: A Study in Comparative and Functional Anatomy. [Dissertation]. University of London, London.

Singh L. 1974. Study of mitotic and meiotic chromosomes in seven species of lizards. Proceedings of the Zoological Society 27: 57–79.

Solleder E, Schmid M. 1984. XX/XY sex chromosomes in *Gekko gecko* (Sauria, Reptilia). Amphibia-Reptilia 5: 339–345.

Srikulnath K, Uno Y, Nishida C, Ota H, Matsuda Y. 2015. Karyotype reorganization in the Hokou Gecko (*Gekko hokouensis*, Gekkonidae): The Process of Microchromosome Disappearance in Gekkota. PLoS ONE. DOI:10.1371/journal.pone.0134829

Shibaike Y, Takahashi Y, Arikura I, Iiizumi R, Kitakawa S, Sakai M, Imaoka C, Shiro H, Tanaka H, Akakubo N, Nakano M, Watanabe M, Ohne K, Kubota S, Kohno S, Ota H. 2009. Chromosome evolution in the lizard genus *Gekko* (Gekkonidae, Squamata, Reptilia) in the East Asian islands. Cytogenet Genome Res 127: 182–190.

Solleder E, Schmid M. 1984. XX/XY sex chromosomes in *Gekko gecko* (Sauria, Reptilia) amphibia-Reptilia 5: 339–345.

Tautz D, Renz M.1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res 25: 4127–4138. https://doi. org/10.1093/nar/12.10.4127

Thongnetr W, Aiumsumang S, Tanomtong A, Phimphan S. 2022. Classical chromosome features and microsatellites repeat in *Gekko petricolus* (Reptilia, Gekkonidae) from Thailand. *Caryologia* 75(2): 81–88.

Trifonov VA, Giovannotti M, O'Brien PCM, Wallduck M, Lovell F, Rens W, Parise-Maltempi PP, Caputo V, Ferguson-Smith MA. 2011. Chromosomal evolution in Gekkonidae. I. Chromosome painting between *Gekko* and *Hemidactylus* species reveals phylogenetic relationships within the group. Chromosome Res 19: 843–855.

Turpin R, Lejeune J. 1965. Les Chromosomes Humains. Gauther Villars, Paris.

Wu G, Zhao E. 1984. Studies on karyotypes of *Gekko gecko* and *Gekko subpalmatus*. Acta Herpetologica Sinica 3: 61–64.

Yoshida M, Itoh M. 1974. Karyotype of the gecko, *Gekko japonicus*. Chromosome Information Service 17: 29–31.

Yüksel E, Gaffaroğlu M. 2008. The analysis of nucleolar organizer regions in *Chalcalburnus mossulensis* (Pisces: Cyprinidae). Journal of Fisheriessciences.com 2: 587–591.