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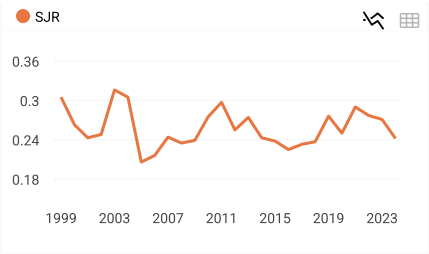

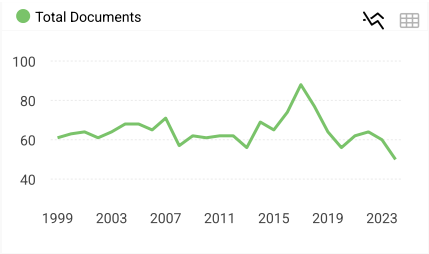

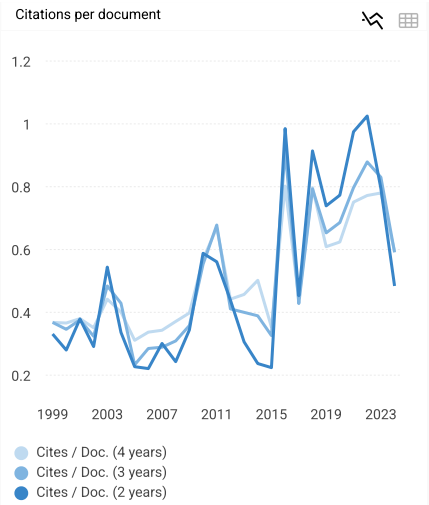

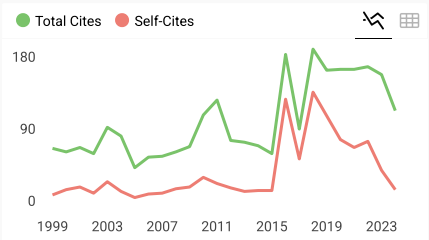

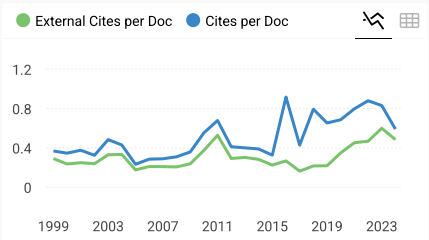

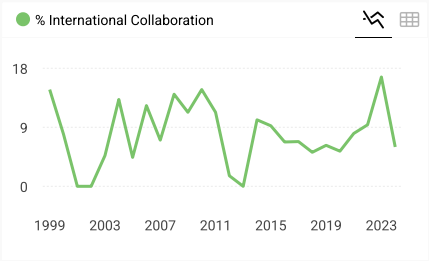

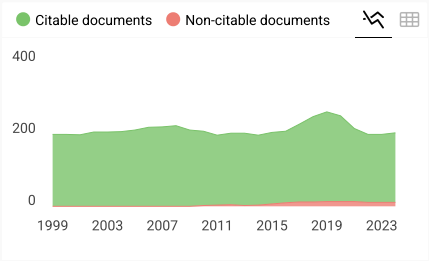

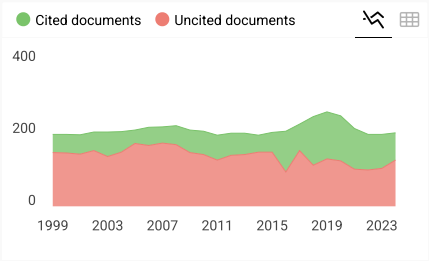

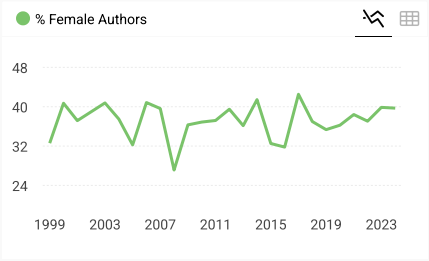

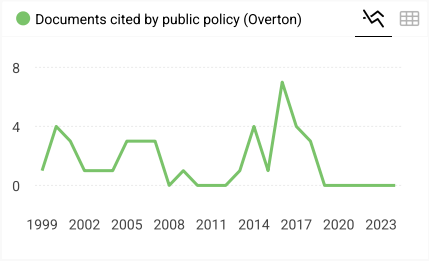

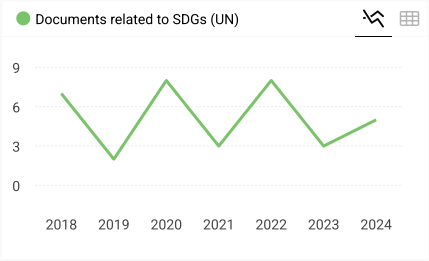

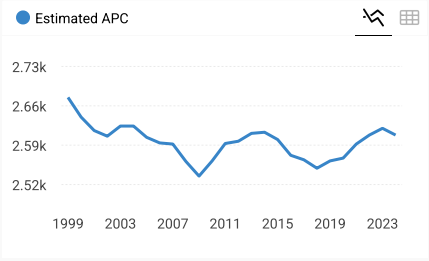

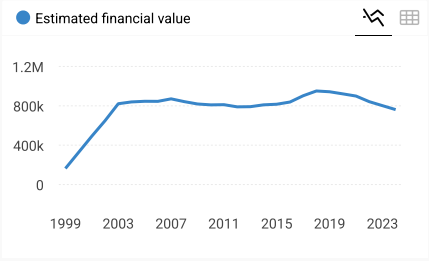

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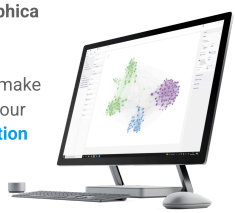

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Regular Article

Classical molecular cytogenetics of spiny-tailed house gecko, *Hemidactylus frenatus* (Squamata, Gekkonidae) from Thailand

Wutthisak Bunnaen[1], Sumalee Phimphan[2], Surachest Aiumsumang[2], Prayoon Wongchantra[3], Suphat Prasopsin[4], Alongklod Tanomtong[5], and Weera Thongnetr[6]*

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Summary The subject of research was a molecular cytogenetic investigation of the *Hemidactylus frenatus* or spiny-tailed house gecko. Ag-nucleolar organizer region (NOR) banding, fluorescence *in situ* hybridization (FISH), and conventional staining were used to mitotic chromosomes prepared directly from bone marrow. According to the findings, there were 40 diploid chromosomes ($2n$). For both sexes, the fundamental number was 74. The karyotype formula, consisting of metacentric, submetacentric, acrocentric, and telocentric chromosomes, is as follows: $2n(40)=10m+4sm+20a+6t$ or $L_2^m+L_4^{sm}+L_8^a+M_6^a+S_8^m+S_6^a+S_6^t$. The NORs appeared at the telomere of the long arm of chromosome pair 16. Using six probes, $(A)_{30}$, $(CA)_{15}$, $(GC)_{15}$, $(CAC)_{10}$, $(GAA)_{10}$, and $(GAG)_{10}$, FISH mapping of microsatellite repeat modes was conducted. The results showed specific signals of $(CA)_{15}$, $(GC)_{15}$, $(CAC)_{10}$, $(GAA)_{10}$, and $(GAG)_{10}$ on chromosome pairs, whereas the $(A)_{30}$ probe spread onto metaphase. *H. frenatus* chromosomes are primarily acrocentric, and all of their chromosomes have distinct patterns in their short and long arms. This characteristic is maintained by the main evolutionary line of the gecko group, including its early predecessors. The evolution of the karyotype in the genus *Hemidactylus* involved the breaking off of chromosomal fragments and the rearrangement of centromeres, resulting in different numbers and shapes of chromosomes within the genus.

Keywords Spiny-tailed house gecko, *Hemidactylus frenatus*, Molecular cytogenetics, Karyotype, Fluorescence *in situ* hybridization.

The spiny-tailed house gecko, *Hemidactylus frenatus*, is a species of house gecko belonging to the family Gekkonidae, It is classified in the class Reptilia, order Squamata, and suborder Lacertilia. The species of house gecko is the most varied and widely distributed. It is native to Southeast Asia and has started to breed in several nations, including Australia, New Guinea, East Africa, Madagascar, and Mexico. It is present throughout Thailand and is frequently observed in rural, agricultural, and forest settings, as well as residential and community locations (Edgren 1950; Darevsky *et al.* 1984; Cook 1990; Das 2010). The spiny-tailed house gecko is resilient to environmental changes and can effectively adapt to various environments. However, human behavior also impacts this species, both directly and indirectly, particularly through the use of chemicals in the home. Consequently, house geckos can serve as an example of how human activities affect the environment. For instance, thorough investigations into the evolution, pharmacology, medicine, epidemiology, population control, and conservation of house geckos have been conducted (Trifonov *et al.* 2011; Subramanean and Reddy 2012).

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The chromosomal morphology of the mitotic metaphase has been used in karyological investigations of the genus *Hemidactylus* to differentiate species, whereas meiotic metaphase chromosomal morphology has occasionally been reported for species distinction. The published chromosome studies of Gekkonidae family include the following: genus *Cyrtodactylus*, which multiple reports on cytogenetic investigations including *C. doisuthep*: 2*n*=34 (Thongnetr *et al.* 2021), *C. jarujini*: 2*n*=40 (Thongnetr *et al.* 2021), *C. inthanon*: 2*n*=40 (Prasopsin *et al.* 2022), *C. pubisulcus*: 2*n*=42 (Ota *et al.* 1992), *C. consobrinus*: 2*n*=48 (Ota *et al.* 1992), genus *Gekko*, with several reports on cytogenetic studies including *G. gecko*: 2*n*=38 (Patawang *et al.* 2014), *G. hokouensis*: 2*n*=38 (Srikulnath *et al.* 2015), *G. petricolus*: 2*n*=38 (Thongnetr *et al.* 2022a), *G. nutaphandi*: 2*n*=38 (Thongnetr *et al.* 2022b), *G. monarchus*: 2*n*=38 (Ota *et al.* 1990), genus *Hemidactylus*: with studies on *H. frenatus*: 2*n*=40 (King 1978; Darevsky *et al.* 1984; Kupriyanova *et al.* 1989; Ota 1989a, b; Javed *et al.* 2010; Patawang *et al.* 2016), *H. frenatus*: 2*n*=46 (Makino and Momma 1949), *H. brookii*: 2*n*=40 (Bhatnagar 1962), *H. flaviviridis:* 2*n*=40, 46 (Asana and Mahabale 1941; Makino and Momma 1949; Branch 1980), and *H. mabouia*: 2*n*=42 (Beçak *et al.* 1972; McBee *et al.* 1987) (Table 1). Although fluorescence *in situ* hybridization (FISH) has been used to determine microsatellite patterns in other genera of geckos, it has not been documented in *H. frenatus*. According to Thongnetr *et al.* (2021), three species of *Cyrtodactylus* are known: *C. doisuthep*, *C. jarujini* [using $(A)_{20}$, $(TA)_{15}$, $(CGG)_{10}$, $(CGG)_{10}$, $(GAA)_{10}$, $(TA)_{15}$], and *C. inthanon* [using $d(CA)_{15}$, $d(GC)_{15}$, $d(CAG)_{10}$, $d(GAA)_{10}$ (Prasopsin *et al.* 2022)]. The following species have also been studied: *G. petricolus*: $(CA)_{15}$, $(GAA)_{10}$ (Thongnetr *et al.* 2022a); *G. nutaphandi*: $(GC)_{15}$ (Thongnetr *et al.* 2022b) and *G. hokouensis*: FISH mapping (Srikulnath *et al.* 2015) (Table 1). Previous studies on the chromosomes of Gekkonids, particularly *Hemidactylus*, show a diploid number (2*n*) ranging from 40–46, with 40 being the most common (Makino and Momma 1949; King 1978; Darevsky *et al.* 1984; Kupriyanova *et al.* 1989; Ota 1989a, b; Javed *et al.* 2010; Patawang *et al.* 2016). Chromosome mismatches in the number and shape were noted in certain species, and the outcomes of studies vary. Thus, multiple reviews are needed to determine the appropriate standard. Currently, methods such as FISH are employed to study the evolutionary relationships among living organisms. They examine the conservation of chromosomal genes and sex-determining genes in reptiles and chickens (Smith *et al.* 2009) and investigate the development of the gecko's karyotype.

This study used a microsatellite detector with the FISH technique. Microsatellites that have undergone repeated sequencing are used to analyze and contrast the distinct marker chromosomes of the spiny-tailed house gecko (*H. frenatus*). Basic knowledge of molecular cytogenetics and chromosome markers can be obtained from the evolutionary relationship and variation of chromosomes within the cell. This is useful for taxonomic classification, investigating related areas, and researching the development of karyotypes in geckos.

Materials and methods

Sample collection

We collected ten male and ten female *H. frenatus* specimens from five provinces across five regions in Thailand: Yala Province in the South, Saraburi Province in the Central, Kanchanaburi Province in the West, Maha Sarakham Province in the Northeast, and Phetchabun Province in the North. To identify the species, we measured external morphological features following the methods of Zug *et al.* (2007), Das (2010), and Vásquez-Restrepo and Lapwong (2018). This research was conducted under animal use permission of scientific purposes code U1-05662-2559. Before the experiment, all of the spiny-tailed house geckos were transferred to the laboratory and maintained under normal conditions, meaning that they were fed well and kept in good health. Animals were not stressed or subjected to abuse.

Chromosome preparation and chromosome staining

The following technique was used to directly prepare chromosomes *in vivo* (Ota 1989a, b; Qin *et al.* 2012). The slides were traditionally stained for 30 min using a 20% Giemsa solution (Patawang *et al.* 2014). The slides were then thoroughly cleaned with running water to remove any remaining stains and were allowed to air dry at room temperature. The Ag-nucleolar organizer region (NOR) banding was analyzed using the methodology of Howell and Black (1980). The slides were treated with two drops of 2% gelatin solution and two drops of 50% silver nitrate solution. They were then covered with glass coverslips and incubated for 5 to 10 min at 60°C. After incubation, the slides were submerged in distilled water until the coverslips came loose. The slides were allowed to air dry at room temperature before being examined under a microscope. We slightly modified the description of Kubat *et al.* (2008) for the use of microsatellite research. Sigma (St. Louis, MO, USA) directly tagged these sequences at the 5′-terminal with Cy3 during synthesis. Under extremely strict conditions, mitotic chromosomal spreads were subjected to FISH (Pinkel *et al.* 1986).

Chromosomal checks, karyotyping, and drawing idiograms

The diploid number (2*n*) was determined by counting the number of chromosomes in 30 mitotic metaphase cells per specimen using a light microscope. Each male and female specimen had 20 distinct, evenly distributed metaphase cells that were imaged. The total length of

Table 1. Karyotype reviews in the genera *Cyrtodactylus* Gray 1827, *Gekko* Laurenti, 1768, and *Hemidactylus* Goldfuss, 1820 (Gekkonidae, Squamata).

Species	2*n*	NF	NORs	Karyotype formulas	FISH	Locations	References
C. doisuthep	34	56	P9,13	14m+6sm+2a+12t	$(A)_{20}$ $(TA)_{15}$ $(CGG)_{10}$ $(CGG)_{10}$ $(GAA)_{10}$ $(TA)_{15}$	Thailand	Thongnetr *et al.* (2021)
C. jarujini	40	56	P13,14	8m+4sm+4a+24t	$(A)_{20}$ $(TA)_{15}$ $(CGG)_{10}$ $(CGG)_{10}$ $(GAA)_{10}$ $(TA)_{15}$	Thailand	Thongnetr *et al.* (2021)
C. inthanon	40	58	P12	12m+4sm+2a+22t	$d(CA)_{15}$ $d(GC)_{15}$ $d(CAG)_{10}$ $d(GAA)_{10}$	Thailand	Prasopsin *et al.* (2022)
C. pubisulcus	42	44	—	2bi-arm+40t	—	Malaysia	Ota *et al.* (1992)
C. consobrinus	48	50	—	2bi-arm+46t	—	Malaysia	Ota *et al.* (1992)
G. gekko	38	50	P4	6m+4sm+2a+26t	—	Thailand	Patawang *et al.* (2014)
G. hokouensis	38	42	P19	2m+18sm+16a+ZW	FISH mapping	Thailand	Srikulnath *et al.* (2015)
G. petricolus	38	54	P17	4m+2sm+10a+22t	$(CA)_{15}$ $(GAA)_{10}$	Thailand	Thongnetr *et al.* (2022a)
G. nutaphandi	34	46	P5	6m+6sm+22t	$(GC)_{15}$	Thailand	Thongnetr *et al.* (2022b)
G. monarchus	44	46	—	—	—	Malaysia	Ota *et al.* (1990)
H. frenatus	40	—	—	2m+4sm+34t		India	Javed *et al.* (2010)
	40	54	P3	4bi-armed+26t		Austria	King (1978)
	40	40	46	6bi-armed+34t		Vietnam	Darevsky *et al.* (1984)
	40	—	—	—		Taiwan	Kupriyanova *et al.* (1989)
	40	—	—	—		Malaysia	Ota *et al.* (1989a, b)
	40	74	P16	10m+4sm+20a+6t		Thailand	Patawang *et al.* (2015)
	46	—	—	46t		Japan	Makino and Momma (1949)
	60(3*n*)					Oceania	Moritz and King (1985)
	40	74	P16	10m+4sm+20a+6t	$(A)_{30}$ $(CA)_{15}$ $(GC)_{15}$ $(CAC)_{10}$ $(GAA)_{10}$ $(GAG)_{10}$	Thailand	Present study
H. platyurus	46	—	—	—		—	Trifonov *et al.* (2011)
	46	48	P1	2a+44t		Thailand	Patawang *et al.* (2015)
H. brookii	40	44	—	4bi-armed+36t		—	Bhatnagar (1962)
H. flaviviridis	40	60	—	20bi-armed+20t		—	Asana and Mahabale (1941)
	46	46	—	46t		—	Makino and Momma (1949)
	40	52	—	12bi-armed+28t		Oman	Branch (1980)
	44	44	—	—	—	—	Gorman (1973)
H. mabouia	42	56	—	14bi-armed+28t		—	Beçak *et al.* (1972)
	42	54	—	12bi-armed+30t		—	McBee *et al.* (1987)
H. bowringii	46	46	—	—	—	—	Gorman (1973)
H. garnotti	70	76					Gorman (1973)
H. brookii angulatus	40	76	—	—	—	—	Adegoke (1985)
H. fasciatus fasciatus	40	50				Nigeria	Ejere and Adegoke (2001)

Remarks: 2*n*=diploid chromosome number, NORs=nucleolus organiser regions, SCR=subcentromeric regions, NF=fundamental number (number of chromosome arms), bi-arm=bi-armed chromosome, m=metacentric, sm=submetacentric, a=acrocentric, t=telocentric chromosome, L=large, S=small, P=chromosome pair and —=not available

the chromosome for 20 evenly distributed metaphase cells was determined by measuring the short arm length (Ls) and the long arm length (Ll) of each chromosome. Chromosomes were classified as acrocentric, telocentric, metacentric, or submetacentric using the method of Turpin and Lejeune (1965).

Fluorescence in situ *hybridization technique*

In this section, we somewhat modified the description of microsatellite research by Kubat *et al.* (2008). During the synthesis process, Sigma (St. Louis, MO, USA) directly tagged these sequences at the 5′-terminal with Cy3. Under extremely strict conditions, mitotic chromosome spreads were subjected to FISH (Pinkel

et al. 1986). Following chromosomal DNA denaturation in 70% formamide/2×SSC at 70°C, the spreads were incubated in 2×SSC for 4 min at 70°C. The slides were coated with a hybridization mixture, which included 2.5 ng μL^{-1} probes, 2 $\mu g\,\mu L^{-1}$ salmon sperm DNA, 50% deionized formamide, and 10% dextran sulfate. The hybridization process was carried out overnight at 37°C in a moist chamber with 2×SSC. To complete the post-hybridization wash, 1×SSC was used for 5 min at 65°C. A final wash was done in a 4×SSCT for 5 min at room temperature. Subsequently, the chromosomes mounted in antifading solution (Vector, Burlingame, CA, USA) and counterstained with 1.2 $\mu g\,mL^{-1}$ DAPI were analyzed using a Nikon ECLIPSE fluorescent microscope.

Results

The diploid chromosome number and fundamental number

In *H. frenatus*, The diploid chromosome number was 40 in both sexes (Fig. 1A, C), while NF was 74 (Fig. 1). The metacentric, submetacentric, acrocentric, and telocentric chromosomes were 10m+4sm+20a+6t, respectively. No sex-related chromosomal heteromorphism has been found in the examined samples.

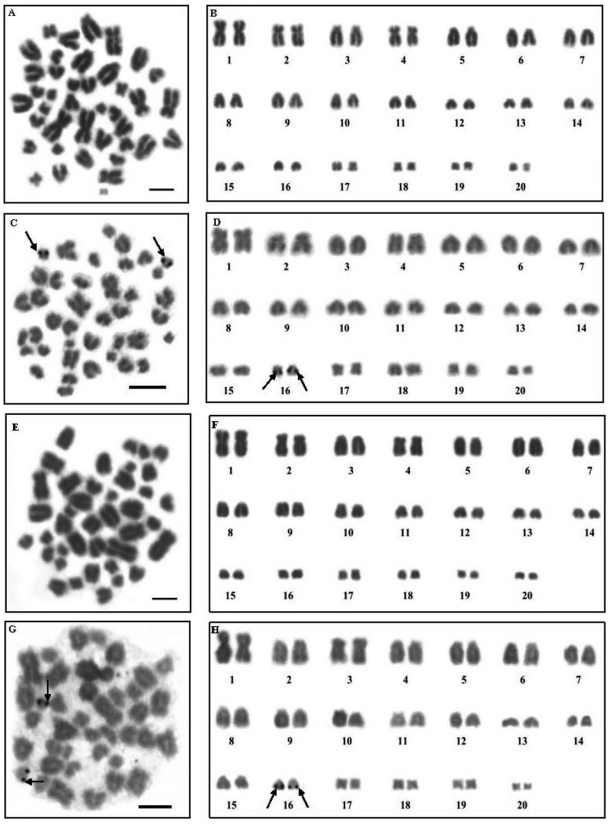

Fig. 1. Metaphase chromosome plates and karyotypes using conventional Giemsa staining. Male (**A**) and female (**C**) *H. frenatus*, and Ag-NOR banding technique of male (**B**) and female (**D**) $2n=40$. The arrows indicate nucleolar organizer regions/NOR. Scale bars=5 µm.

The karyological characteristics

The karyotype of *H. frenatus* consists of 2 large metacentric, 4 large submetacentric, 8 large acrocentric, 6 medium acrocentric, 8 small metacentric, 6 small acrocentric, and 6 small telocentric chromosomes, as shown in Table 2. *H. frenatus* has the following karyotype formula: $2n(40)=L_2^m+L_4^{sm}+L_8^a+M_6^a+S_8^m+S_6^a+S_6^t$.

Ag-NOR banding

H. frenatus has one pair of chromosomes with NOR locations on the long forearm of the 16th pair of small telocentric chromosomes in both males and females, according to staining using the NOR color band technique (Fig. 1B, D).

Microsatellite pattern

The microsatellites are $(A)_{30}$, $(CA)_{15}$, $(GC)_{15}$, $(CAC)_{10}$, $(GAA)_{10}$, and $(GAG)_{10}$. In *H. frenatus*, the signals of the CA_{15}, GC_{15}, CAC_{10}, GAA_{10}, and GAG_{10} specific regions have been observed on chromosome pairs, whereas the A_{30} probe spreads onto metaphase (Fig. 2A). The present study showed that the heteromorphism of the CAC_{10} probe had differenced signal in small metacentric chromosome pair 19a, 19b (Figs. 2D, E and 3). The GAG_{10} probe had two signals in the small metacentric chromosome pair 19 (Figs. 2G and 3), the CA_{15} probes had two signals in the large acrocentric chromosome pair 5 (Figs. 2B and 3). Both GC_{15} and GAA_{10} probes had two signals in the small metacentric chromosome pair 17 (Figs. 2B, C, F and 3), respectively.

Discussion

Karyological data of the genus Hemidactylus

The genus *Hemidactylus* contains a variety of geckos with chromosome counts ranging from 40 to 46; however, 40 and 46 are the most common. There were no chromosomal variations between males and females in the *H. frenatus* investigation, and the species had a diploid chromosome count of 40 and a fundamental number of 74. The study's findings regarding the number of diploid chromosomes align with the reports of King (1978), Darevsky *et al.* (1984), Kupriyanova *et al.* (1989), Ota (1989a, b), Javed *et al.* (2010), and Patawang and Tanomtong (2015), who reported that the spiny-tailed house gecko had 40 diploid chromosomes. This contrasts with Makino and Momma (1949), who reported that the number of diploid chromosomes was 46, and King (1978), who reported that the spiny house lizard had 60 triple chromosomes. Only Patawang and Tanomtong (2015) reported a fundamental chromosome number of 74, consistent with this study. When comparing the outcomes of previously published genome investigations at the *Hemidactylus* genus. Certain species, including *H. frenatus* (King 1978; Darevsky *et al.* 1984; Moritz and King 1985; Kupriyanova *et al.*

Table 2. Karyomorphological characteristics of male and female *H. frenatus*, 2*n*=40.

Chr. pair	Ls	Ll	LT	RL±SD	CI±SD	Chr. size	Chr. type
1	1.689	2.081	3.770	0.091±0.004	0.551±0.013	Large	metacentric
2	1.036	2.026	3.062	0.075±0.003	0.666±0.020	Large	submetacentric
3	0.435	2.460	2.895	0.071±0.003	0.844±0.029	Large	acrocentric
4	0.861	1.875	2.736	0.062±0.003	0.680±0.015	Large	submetacentric
5	0.470	2.121	2.591	0.059±0.002	0.816±0.045	Large	acrocentric
6	0.392	2.069	2.461	0.058±0.003	0.842±0.032	Large	acrocentric
7	0.412	1.914	2.326	0.059±0.002	0.822±0.039	Large	acrocentric
8	0.381	1.747	2.128	0.056±0.001	0.823±0.048	Medium	acrocentric
9	0.377	1.657	2.033	0.054±0.001	0.810±0.059	Medium	acrocentric
10	0.354	1.580	1.935	0.051±0.002	0.818±0.053	Medium	acrocentric
11	0.279	1.368	1.647	0.048±0.002	0.829±0.046	Small	acrocentric
12	0.000	1.468	1.468	0.048±0.003	1.000±0.000	Small	telocentric
13	0.240	1.158	1.399	0.048±0.003	0.802±0.047	Small	acrocentric
14	0.246	1.048	1.294	0.032±0.003	0.800±0.022	Small	acrocentric
15	0.000	1.028	1.028	0.031±0.003	1.000±0.000	Small	telocentric
16*	0.000	0.925	0.925	0.027±0.003	1.000±0.000	Small	telocentric
17	0.421	0.428	0.849	0.026±0.003	0.506±0.036	Small	metacentric
18	0.391	0.412	0.803	0.023±0.003	0.517±0.025	Small	metacentric
19	0.374	0.374	0.747	0.020±0.004	0.514±0.033	Small	metacentric
20	0.327	0.329	0.656	0.018±0.004	0.525±0.021	Small	metacentric

Remark: Chr.=chromosome; *=satellite chromosome (NOR); Ls=Mean length of short arm chromosome; Ll=length of long arm chromosome; LT=length of total chromosomes; RL=relative length; CI=centromeric index; SD=standard deviation.

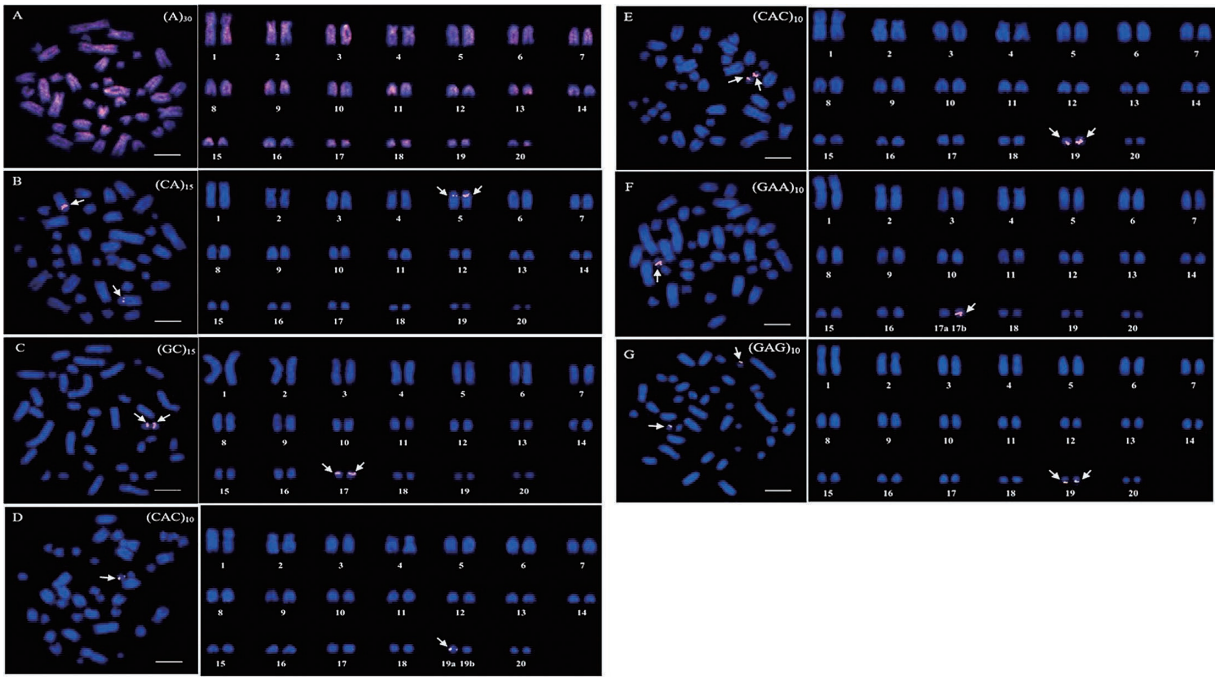

Fig. 2. Metaphase chromosome plate and karyotypes of *H. frenatus* by FISH technique of microsatellite probes. Probes were A_{30} (**A**), CA_{15} (**B**), GC_{15} (**C**), CAC_{10} (**D, E**), GAA_{10} (**F**), and GAG_{10}. (**G**). Scale bars=10 μm.

1989; Ota 1989a, b; Javed *et al.* 2010; Patawang and Tanomtong 2015), *H. brookii* (Bhatnagar 1962) and *H. flaviviridis* (Asana and Mahabale 1941), have all been reported to have mismatched numbers of diploid chromosomes (Table 1). Overall, though the karyotypes of *H. frenatus* are similar to those of other *Hemidactylus* species and gekkonids, consisting primarily of a few mono-armed (telocentric) and many bi-armed (metacentric, submetacentric, and acrocentric) chromosomes. Regarding the gekkonid chromosomes that have been previously documented, most species revealed that the karyotype consists of many bi-armed chromosomes and a few mono-armed chromosomes. Within the gekkonid group, the current data for *H. frenatus* supports the concept of chromosomal evolution (Trifonov *et al.* 2011). The karyotype of *H. frenatus* showed a gradient of small bi-arm and a few mono-armed chromosomes. These are comparable to species found in the genus *Hemidactylus*: for *H. brookii*, *H. fasciatus fasciatus*, *H. mabouia*, *H. bowringii*, *H. platyurus*, and *H. garnotti*,

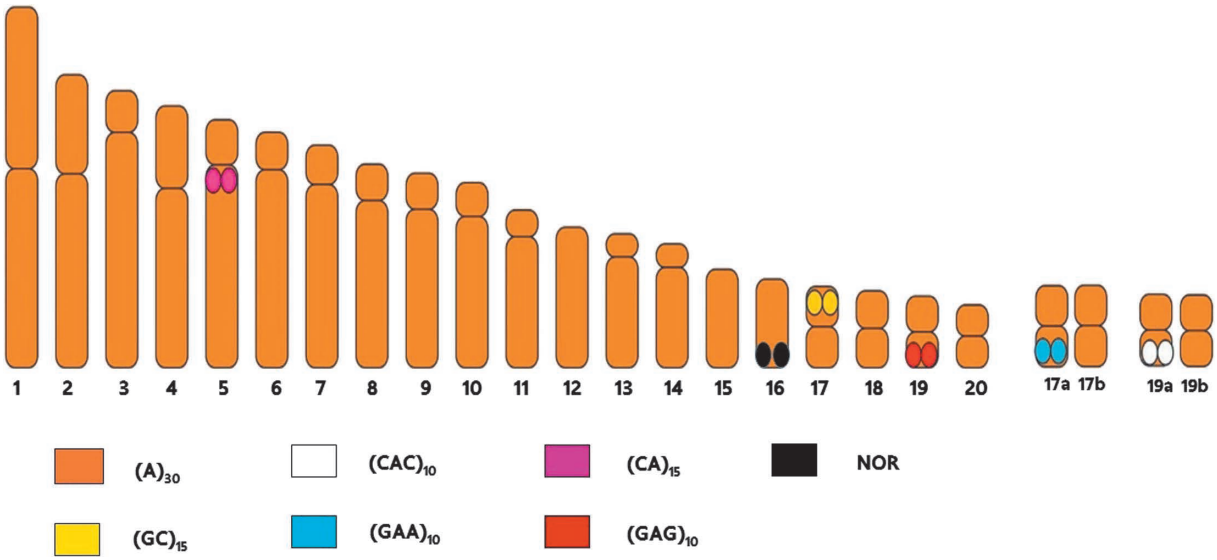

Fig. 3. Idiograms representing the $(A)_{30}$, $(CA)_{15}$, $(GC)_{15}$, $(CAC)_{10}$, $(GAA)_{10}$, $(GAG)_{10}$, and NOR mapping on the chromosomes of *H. frenatus*.

respectively, chromosomal numbers 2*n*=40, 40, 42, 46, 46, and 70 correspond to fundamental numbers 44, 50, 54, 46, 48, and 76 (Mahabale 1941; Bhatnagar 1962; Asana and Gorman 1973; Adegoke 1985; McBee *et al.* 1987; Patawang *et al.* 2016). Based on several studies (Gorman, 1973; Ota *et al.* 1992; Organ *et al.* 2008), these species are categorized as metacentric, submetacentric, acrocentric, and telocentric. These characteristics are consistent with the theory that reorganization from the original karyotype resulted from Robertsonian fissions, fusions, or pericentric inversions (Gorman 1973; King 1978). Our results confirmed 2*n* for *H. frenatus* species but with small differences in the karyotype composition. This incongruence reflects probably the small size of the gekonid chromosomes, especially those of polyploids. Furthermore, gekonid chromosomes also exhibit a gradual decrease in size, with the centromere positions ranging step-wisely from median to nearly terminal. Indeed, these features make it difficult to assess the chromosomal categories with accuracy.

Nucleolar organizer region

NORs are chromosome sites that contain the 18S and 28S ribosomal RNA genes. The objective of this technique is to identify the NORs, which represent the location of genes that function in ribosome synthesis (Sharma *et al.* 2002). If these regions were active during the interphase prior to mitosis, they can be detected by Ag-NOR banding (Howell and Black 1980). In the present study, the chromosome markers of *H. frenatus* were determined by using the Ag-NOR banding technique as shown in a small telocentric chromosome pair 16, in both males and females. This is similar to the previous reports of the genus *Hemidactylus* in the Gekkonidae family by King (1978) and Moritz and King (1985), genus *Cyrtodactylus*, including *C. doisuthep, C. jarujini, C. inthanon, Dixonius hangseesom, D. siamensis, D. melanostictus, Gekko gecko, G. hokouensis, G. petricolus, G. nutaphandi, H. frenatus*, and *H. platyurus* (King 1978; Darevsky *et al.* 1984; Patawang *et al.* 2014; Srikulnath *et al.* 2015; Patawang *et al.* 2016; Thongnetr *et al.* 2021; Patawang *et al.* 2022; Prasopsin *et al.* 2022; Thongnetr *et al.* 2022a, b). The most striking variation is seen in the morphology of the secondary constrictions. Generally, one major NOR is present per genome (*n*), which may vary in its position between species. However, closely related and often morphologically very similar species share the same type and location of their nucleolar organizer regions, which can therefore provide an effective taxonomic.

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). This study is the first report of the microsatellites $(A)_{30}$, $(CA)_{15}$, $(GC)_{15}$, $(CAC)_{10}$, $(GAA)_{10}$, and $(GAG)_{10}$ on the chromosomes of. *H. frenatus*. In the *H. frenatus*, the signals of the $(CA)_{15}$, $(GC)_{15}$, $(CAC)_{10}$, $(GAA)_{10}$, and $(GAG)_{10}$ specific regions have been observed on chromosome pairs, whereas the $(A)_{30}$ probe spreads onto metaphase. The present study showed that a heteromorphism of $(CAC)_{10}$ probe had a differentiated signal in chromosome pair 19 and 19a, 19b, while $(CA)_{15}$, $(GC)_{15}$, $(GAA)_{10}$, and $(GAG)_{10}$ probes had two signals (Fig. 3). This agrees with previous reports in *G. chinensis* (Lau *et al.* 1997). Earlier research on microsatellites revealed patterns in a few species, such as *C. jarujini* and *C. doisuthep* [the dinucleotides $d(A)_{20}$, $d(CAG)_{10}$, $d(CGG)_{10}$, $d(GAA)_{10}$, $d(TA)_{15}$]; *C. inthanon* [$d(CA)_{15}$, $d(GC)_{15}$, $d(CAG)_{10}$, $d(GAA)_{10}$]; *G. petricolus* [$(CA)_{15}$, $(GAA)_{10}$]; *G. nutaphandi* $(GC)_{15}$ and FISH mapping on

G. hokouensis exclusively accumulated in telomeric and subtelomeric chromosomal regions, dispersed over the whole genomes including chromosomes, with some having signals specific regions on pairs of homologous chromosomes (Srikulnath *et al.* 2015; Thongnetr *et al.* 2021; Prasopsin *et al.* 2022; Thongnetr *et al.* 2022a, b) as shown in Table 1. In this study, a comparison of the cytogenetic maps of *C. jarujini*, *C. doisuthep*, *C. inthanon*, *G. petricolus*, *G. nutaphandi*, and *G. hokouensis*, enabled us to describe the processes of chromosomal reorganization in Gekkota (Luu *et al.* 2015). However, the results clearly indicate that the microsatellite repeats are in high copy number on some chromosome pairs, according to previous reports on reptile groups (Shibaike *et al.* 2009; Pokorná *et al.* 2011; Matsubara *et al.* 2013). In the gekonid genomes, microsatellites are usually abundant in the centromeric and telomeric regions, Otherwise, the dinucleotides $(CA)_{15}$ and $(GC)_{15}$ accumulated exclusively in telomeric and subtelomeric regions, corroborating findings from other geko groups studied to date (Luu *et al.* 2015; Thongnetr *et al.* 2022a, b). These molecular cytogenetic data could also be a substantial prerequisite for future reptile genome projects. This study discovered that the cytogenetic maps of *H. frenatus* allowed us to map out the steps involved in this species' chromosomal rearrangement. This is the first report on the FISH study of this species in Thailand. The motifs $(CA)_{15}$, $(GC)_{15}$, $(CAC)_{10}$, $(GAA)_{10}$, and $(GAG)_{10}$ are in the telomeric, centromeric, and pericentromeric regions of the chromosomes, with more extensive blocks in different sizes of the chromosome pairs of karyotypes. In summary, at least five microsatellite motifs are accumulated in one pair of chromosomes involved in rearrangements. Therefore, the molecular cytogenetic data collected is a necessary precondition for future genome projects of gecko groups and can be applied to the field of cytotaxonomy and the study of gecko evolution.

Author contributions

Conceptualization, [Weera Thongnetr, (W.T.)]; Data curation, [Wutthisak Bunnaen, (W.B.) and Alongklod Tanomton, (A.T.)]; Formal analysis, (W.T. and W.B.); Funding acquisition, (W.T. and W.B.); Investigation, [W.T., Sumalee Phimphan, (S.P.), Surachest Aiumsumang, (S.A.) Prayoon Wongchantra, (P.W.) Suphat Prasopsin, (S.P.)]; Methodology, (W.B., S.P., S.A., P.W., S.P., A.T. and W.T.); Project administration, (W.B. and W.T.); Supervision, (W.B., S.P., S.A., P.W., S.P., A.T. and W.T.); Validation, (W.B., A.T. and W.T.); Visualization, (S.P., S.A., P.W., A.T. and W.T.); Writing-original draft, (W.B., S.P., A.T. and W.T.); Writing-review and editing, (W.B., S.P., S.A., P.W., S.P., A.T. and W.T.). All authors have read and agreed to the published version of the manuscript.

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