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Cytogenetics of the skinks (Reptilia, Scincidae) from Thailand; III: the first karyological study of *Sphenomorphus maculatus* and *Jarujinia bipedalis*

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ABSTRACT

This is a karyological study of male and female *Sphenomorphus maculatus* from Khon Kaen Province in Northeastern Thailand, and female *Jarujinia bipedalis* from Kanchanaburi Province in Central Thailand. Chromosomes of two species were prepared by the squash technique from the bone marrow and testis. Conventional staining and Ag-NOR banding techniques were applied to stain the chromosome with Giemsa's solution and silver nitrate solution, respectively. The results showed that the diploid chromosome number of *Sphenomorphus maculatus* and *Jarujinia bipedalis* was 30 and the fundamental number (NF) was 44. There were eight large metacentric, two medium metacentric, four small metacentric and 16 microchromosomes in *Sphenomorphus maculatus* and eight large metacentric, six small metacentric, two small telocentric and 14 microchromosomes in *Jarujinia bipedalis*. There was no irregularly sized chromosome related to sex in either species. For *Sphenomorphus maculatus*, NORs are located at the telomere on the long arms of the second pair of the metacentric chromosome. The karyotype formulae were deduced as: $2n$ (30) = $L^m_8 + M^m_2 + S^m_4$ + 16 microchromosomes and $2n$ (30) = $L^m_8 + S^m_6 + S^t_2$ + 14 microchromosomes for *Sphenomorphus maculatus* and *Jarujinia bipedalis*, respectively.

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Sphenomorphus maculatus; *Jarujinia bipedalis*; karyotype; chromosome

Introduction

Sphenomorphus maculatus, spotted forest skink, is a species of Lygosomine skink found in China, Southeast Asia and eastern India. In the genus *Sphenomorphus* chromosomes are mainly diploid $2n = 30$, e.g. in *S. indicus* and *S. boulengeri* (Ota and Lue 1994), but three old reports in *S. indicus* revealed that it has a diploid chromosome number of $2n = 28$ (Makino and Momma 1949; Yang et al. 1989) and 26 (Guo and Dong 1988). However, the nucleolar organizer region (NOR) of both *S. indicus* and *S. boulengeri* is located at the second pair of the macrochromosome (Guo and Dong 1988; Ota and Lue 1994). *Jarujinia bipedalis*, a new species of Lygosomine skink from Central Thailand, was first reported by Chan-ard et al. (2011) and was designated as a new monotypic genus. The new Lygosomine skink was first discovered in Suan Pung District, Ratchaburi Province in Central Thailand. Its special characteristic is its bipedal nature, with only forearms possessing two clawless vestigial fingers. Chan-ard et al. (2011) suggest that *J. bipedalis* is an evolutionary link between the Lygosomine genera of *Larutia* and *Isopachys*. However, there are no cytogenetic studies of *Larutia*, *Isopachys* and *Jarujinia*.

Scincid skinks, family Scincidae, is the largest group of extant lizards and includes over 1000 species ranging from temperate to tropical regions on all continents, but it is not present in Antarctica (Zug 1993). The Scincidae family has four subfamilies: Acontinae, Feyliniinae, Lygosominae and Scincinae. The Lygosominae subfamily consists of about 82 genera and 769 species that are larger members than other three subfamilies (Lauhajinda 2009; Das 2010). However, a recent report on skink systematics proposed combining all skinks into seven families (Hedges 2014). In the traditional system, *Sphenomorphus* and *Jarujinia* were grouped into the Lygosominae subfamily, which included *Larutia* and *Isopachys* (Lauhajinda 2009; Das 2010; Chuaynkern and Chuaynkern 2012). The new suggested system classifies both of these genera as in the Sphenomorphidae family; this also includes *Larutia* and *Isopachys* (Hedges 2014).

Materials and methods

Two males and two females of *S. maculatus* (Figure 1) were obtained from Muang District (16°28′35.4″N 102°49′34.9″E), Khon Kaen Province, Northeastern

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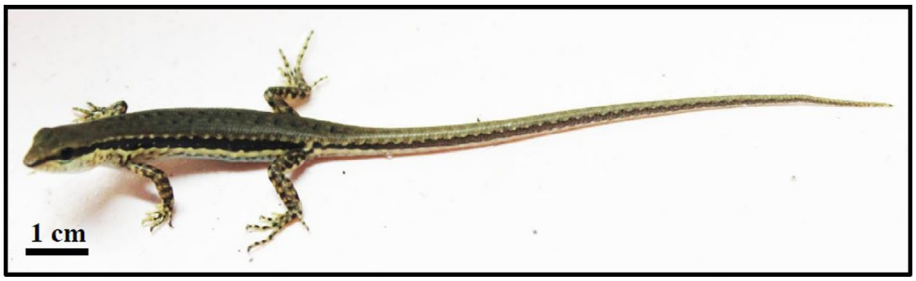

Figure 1. General habitus of *Sphenomorphus maculatus* from Northeastern Thailand.

Figure 2. General habitus of *Jarujinia bipedalis* from Central Thailand: (A) wildlife; (B) dorsal view; and (C) lateral view.

Table 1. Mean length (L) of the short arm chromosome (Ls), long arm chromosome (LI), and total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphase cell of male and female *Sphenomorphus maculatus*, 2*n* = 30.

Chro. pair	Ls	LI	LT	CI ± SD	RL ± SD	Type	Size
1	1.225	1.565	2.789	0.561 ± 0.013	0.153 ± 0.012	m	L
2	1.076	1.459	2.534	0.576 ± 0.018	0.139 ± 0.009	m	L
3	1.017	1.152	2.169	0.531 ± 0.018	0.119 ± 0.006	m	L
4	0.958	1.102	2.060	0.535 ± 0.018	0.113 ± 0.006	m	L
5	0.702	0.792	1.494	0.530 ± 0.013	0.082 ± 0.004	m	M
6	0.614	0.709	1.323	0.536 ± 0.018	0.073 ± 0.004	m	S
7	0.441	0.530	0.970	0.546 ± 0.018	0.053 ± 0.003	m	S
8	–	–	0.799	–	0.044 ± 0.005	Microchromosome	
9	–	–	0.688	–	0.038 ± 0.004	Microchromosome	
10	–	–	0.635	–	0.035 ± 0.004	Microchromosome	
11	–	–	0.604	–	0.033 ± 0.004	Microchromosome	
12	–	–	0.571	–	0.031 ± 0.004	Microchromosome	
13	–	–	0.545	–	0.030 ± 0.004	Microchromosome	
14	–	–	0.523	–	0.029 ± 0.004	Microchromosome	
15	–	–	0.494	–	0.027 ± 0.004	Microchromosome	

Thailand and two females of *J. bipedalis* (Figure 2) were collected from Saiyok District (14°07′51.2″N 99°09′00.1″E), Kanchanaburi Province, Central Thailand. Skink chromosome preparation was accomplished by using the direct method (Kaewsri et al. 2014; Patawang et al. 2017). Samples of bone marrow and

Table 2. Mean length (L) of the short arm chromosome (Ls), long arm chromosome (LI), and total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphase cell of female *Jarujinia bipedalis*, $2n = 30$.

Chro. pair	Ls	LI	LT	CI ± SD	RL ± SD	Type	Size
1	5.380	7.590	12.970	0.585 ± 0.010	0.171 ± 0.012	m	L
2	4.860	6.910	11.770	0.587 ± 0.012	0.155 ± 0.010	m	L
3	4.490	5.240	9.730	0.539 ± 0.012	0.128 ± 0.011	m	L
4	4.080	4.980	9.060	0.550 ± 0.016	0.119 ± 0.009	m	L
5	2.850	3.520	6.370	0.553 ± 0.012	0.084 ± 0.010	m	S
6	2.530	3.190	5.720	0.558 ± 0.014	0.075 ± 0.008	m	S
7	0.000	4.200	4.200	1.000 ± 0.000	0.055 ± 0.008	t	S
8	1.270	1.660	2.930	0.567 ± 0.014	0.039 ± 0.006	m	S
9	–	–	2.260	–	0.030 ± 0.006	Microchromosome	
10	–	–	2.220	–	0.029 ± 0.005	Microchromosome	
11	–	–	1.930	–	0.025 ± 0.004	Microchromosome	
12	–	–	1.830	–	0.024 ± 0.005	Microchromosome	
13	–	–	1.730	–	0.023 ± 0.005	Microchromosome	
14	–	–	1.680	–	0.022 ± 0.004	Microchromosome	
15	–	–	1.650	–	0.022 ± 0.004	Microchromosome	

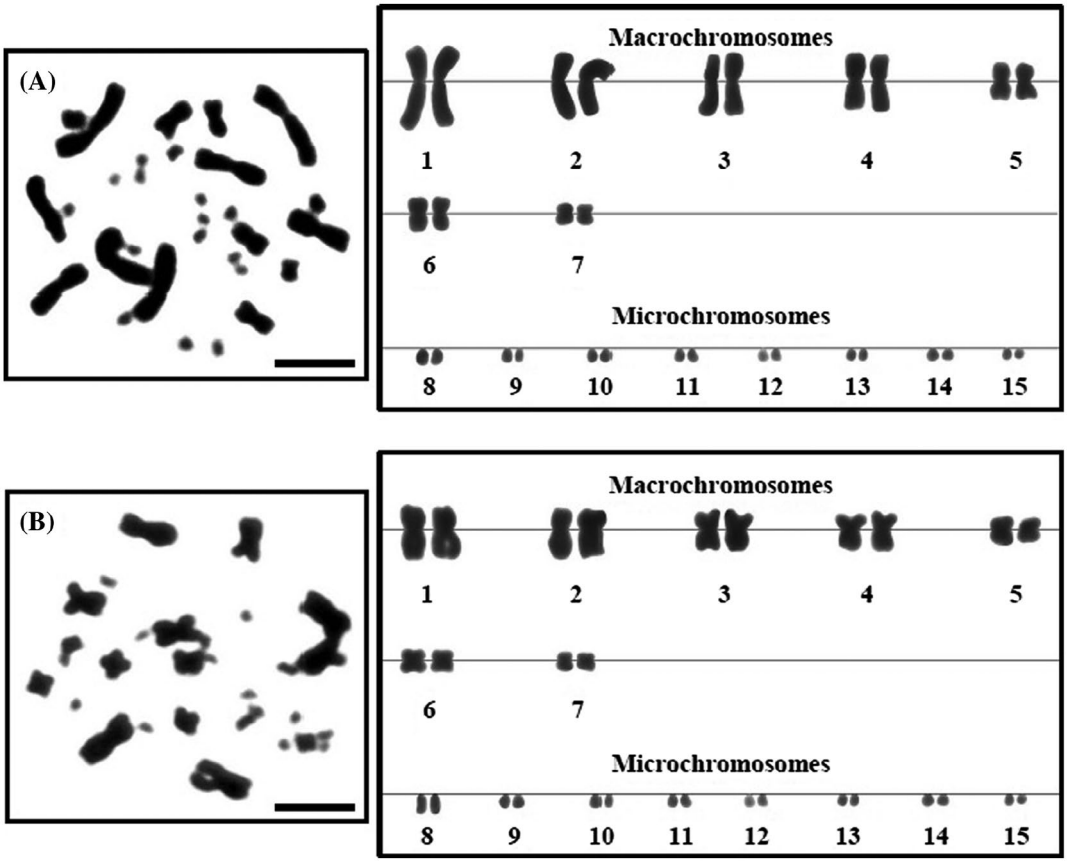

Figure 3. Metaphase chromosome plates and karyotypes of male (A) and female (B) *Sphenomorphus maculatus* 2*n*=30 by conventional staining techniques (scale =10 µm).

intestine tissues were taken from skinks. Conventional staining and Ag-NOR banding techniques were applied to stain the chromosome with 20% Giemsa's solution in conventional staining and 50% silver nitrate solution in Ag-NOR banding technique. The metaphase plates were analyzed according to the chromosome classification after Chaiyasut (1989).

Results and discussion

Both *S. maculatus* and *J. bipedalis* show the diploid number $2n = 30$ (Tables 1, 2, Figures 3(a), 3(b), and 6(a)) and also have the same fundamental number, which is 44. For *S. maculatus*, there was a conformable diploid number to the previous karyotype studies of *S. indicus* and *S. boulengeri* (Table 3), $2n = 30$ (Ota and Lue 1994). The sex chromosome system was not identified by basic chromosome staining in either sexes of *S. maculatus* and female *J. bipedalis*.

In terms of the types of chromosomes, there were eight large metacentric, two medium metacentric, four small metacentric, and 16 microchromosomes in *S. maculatus* (Table 1 and Figure 5(a)). There were eight large metacentric, six small metacentric, two small telocentric and 14 microchromosomes in *J. bipedalis* (Table 2

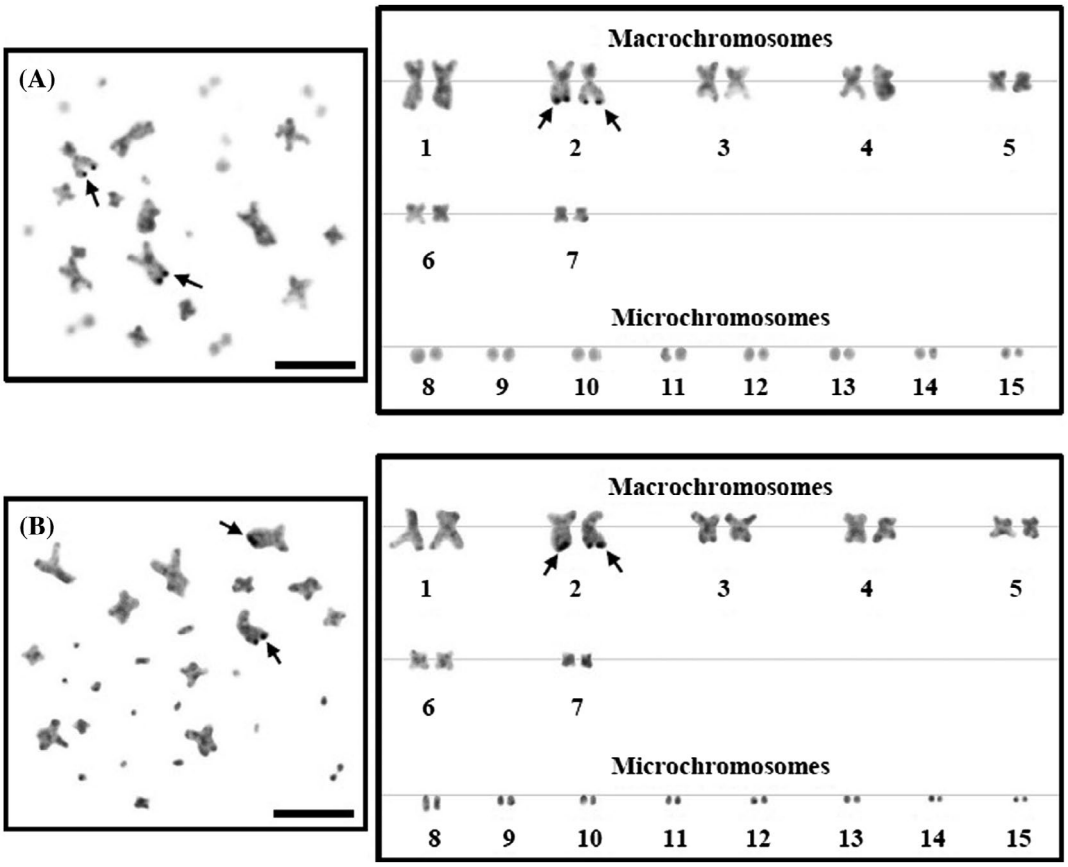

Figure 4. Metaphase chromosome plates and karyotypes of male (A) and female (B) *Sphenomorphus maculatus* 2*n*=30 by Ag-NOR banding technique. Arrows indicate nucleolar organizer regions/NORs (scale =10 μm).

and Figure 6(b)). The karyotype of *S. maculatus* in this study had 14 metacentric macrochromosomes and 16 microchromosomes. This is in accordance with other *Sphenomorphus* species with the previous study of Yang et al. (1989), and Ota and Lue (1994), except for the number of microchromosomes. However, it is different from previous studies by Makino and Momma (1949) and Guo and Dong (1988) which reported differences in karyotype sub-details (Table 3). The nucleolar organizer region of *S. maculatus* investigated by Ag-NOR banding technique was located at the telomeric region on the long arm of the second pair of macrochromosomes (Figures 4(a), 4(b), and 5(b)). The positional characteristics resemble the NOR of *S. indicus* and *S. boulengeri* (Table 3) that were reported by Guo and Dong (1988), and Ota and Lue (1994).

The karyotype of *S. maculatus* and *J. bipedalis* showed a close relationship to other skinks that revealed diploids mainly of 28, 30 and 32 (Kaewsri et al. 2014; Patawang et al. 2017). *S. maculatus* has only one type of metacentric macrochromosome, and *J. bipedalis* has most types of macrochromosomes to be metacentric – except only one pair of telocentric pair 7th. These characteristics are classified as symmetrical karyotype. Symmetrical karyotype has been found in many skinks, e.g. *Eutropis*, *Lipinia*, *Lygosoma* and *Sphenomorphus* (Yang et al. 1989; Ota and Lue 1994; Aranyavalai et al. 2013; Kaewsri et al. 2014; Patawang et al. 2017). The symmetrical karyotype of macrochromosomes in most skinks represents a close evolutionary relationship between various members in the skink group, although they have differences in other sub-details.

We observed other conserved co-characteristics in the skink group, in the first, second, third and fourth macrochromosome pairs, including in the karyotype

Table 3. Comparative chromosome studies between the genera of *Sphenomorphus* and *Jarujinia* (Scincidae, Squamata).

Species	2*n*	Type	NF	NORs	Reference
Sphenomorphus indicus	28	10bi + 18mi	38	–	Makino and Momma (1949)
	26	–	–	2nd	Guo and Dong (1988)
	28	14 m + 14 mi	44	–	Yang *et al.* (1989)
	30	14 m + 16mi	44	2nd	Ota and Lue (1994)
S. boulengeri	30	14 m + 16mi	44	2nd	Ota and Lue (1994)
S. maculatus	30	14 m + 16mi	44	2nd	Present study
Jarujinia bipedalis (female)	30	14m+2t+14mi	44	–	Present study

Abbreviations: 2*n*, diploid chromosome; *NF*, fundamental number; *bi*, bi-armed chromosome (metacentric, submetacentric or acrocentric); *m*, metacentric; *t*, telocentric; *mi*, microchromosome.

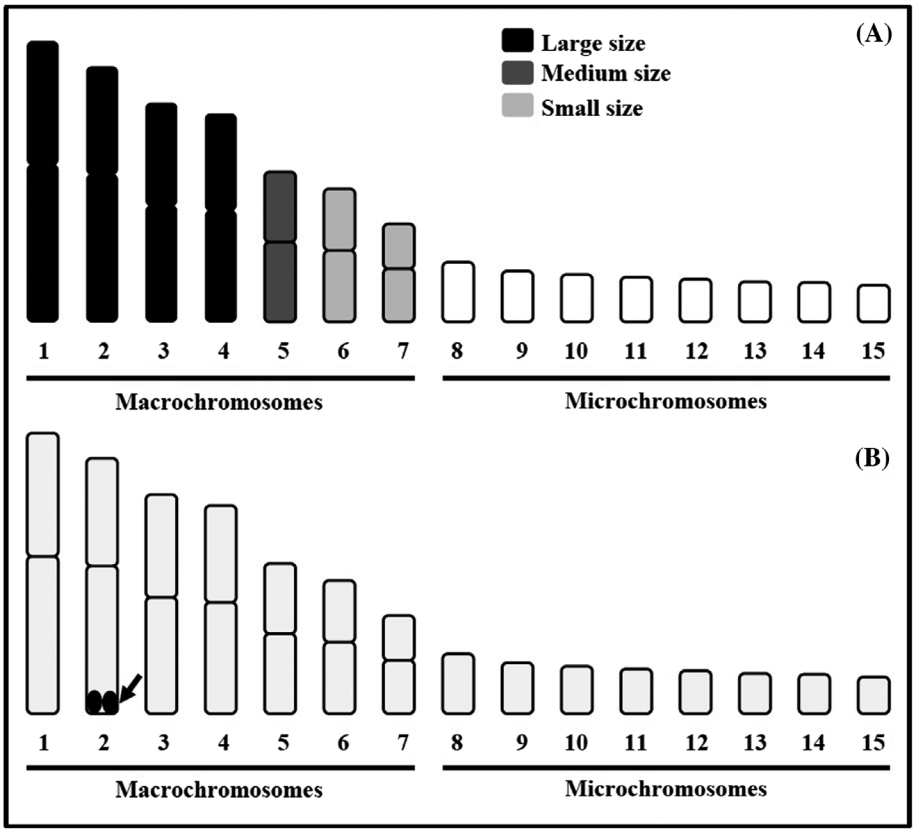

Figure 5. Standardized idiogram of *Sphenomorphus maculatus* 2*n*=30 by conventional staining (A) and Ag-NOR banding (B) techniques. Arrow indicates nucleolar organizer regions/NORs.

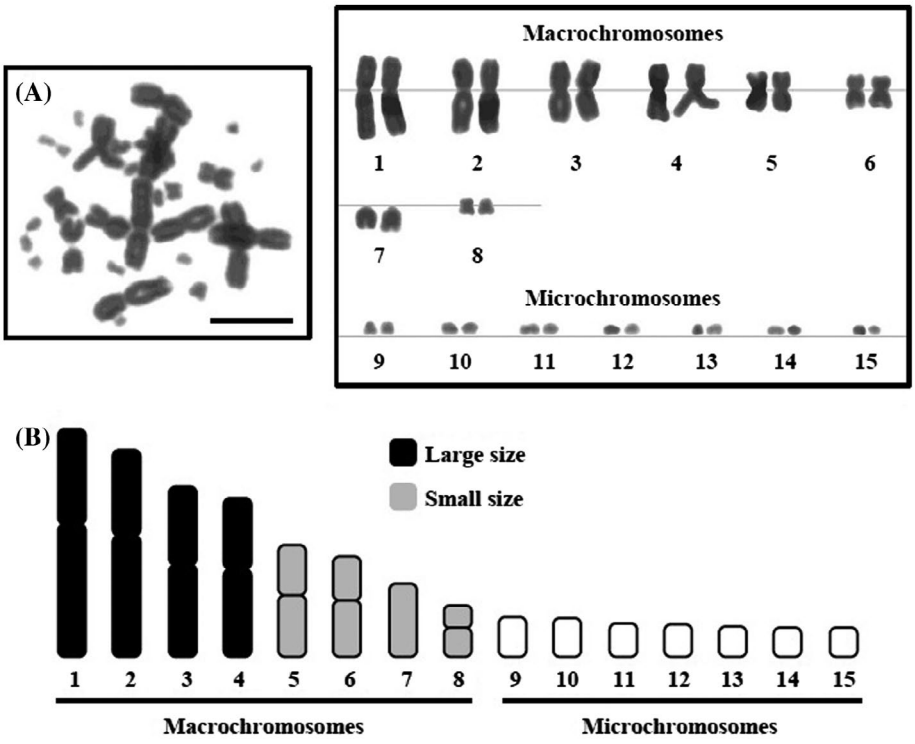

Figure 6. Metaphase chromosome plate and karyotype (A) of female *Jarujinia bipedalis* 2*n*=30 by conventional staining technique, and standardized idiogram (B) of female *Jarujinia bipedalis* by conventional staining.

of *S. maculatus* and *J. bipedalis* (Tables 1 and 2). The co-characteristics comprise metacentric type – except in some pairs of some species that are submetacentric – and size of the large chromosome. Examples of skink species in Thailand that maintain the conserved co-characteristics include *Eutropis multifasciata*, *E. longicaudata*,

E. macularia, *Lipinia vittigera*, *Lygosoma bowringii*, *L. khoratense* and *L. quadrupes* (Eremchenko et al. 1992; Ota et al. 2001; Aranyavalai et al. 2013; Kaewsri et al. 2014; Patawang et al. 2017).

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Disclosure statement

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