June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 161-166

Karyotypes of Four Microhylid Frogs from Xishuangbanna, Southern Yunnan, China

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Abstract: -The karyotypes of *Microhyla butleri, ornata* and *pulchra* and *Kaloula pulchra pulchra* from Xishuangbanna, southern Yunnan are reported. The karyotype of *Microhyla butleri* is reported for the first time. Its diploid number of chromosomes (2n=22) differs from the other species investigated in the same genus which is 24. The results of *M. ornata* and *M. pulchra* are also different from those obtained by the previous authors. The karyotype of *Kaloula p. pulchra* (2n=28) had a slight difference from the result obtained by the previous authors. The further C-banding analysis of this species revealed that an amount of heterochromatin is located in the centric, terminal and interstitial position of chromosomes.

Key Words: Anura, *Microhylidae*, *Microhyla*, *Kaloula*, cytotaxonomy, China

Introduction

The karyotypes of *Microhyla ornata* from Sichuan and Fujian were reported by Chen (1983) and Gao et al. (1985) respectively. The karyotypes of *Microhyla pulchra*, *Kaloula pulchra pulchra* from Guangzhou were reported by He (1986). In the present study, the karyotypes of those species from Xishuangbanna, southern Yunnan, are reported, and they are analyzed by means of C-banding and silver-staining NORs techniques. In addition, the karyotype of *Microhyla butleri* from the same locality is reported for the first time.

Materials and Methods

Microhyla ornata (4 males, 4 females), *M. pulchra* (5 males, 5 females), *M. butleri* (5 males, 1 female) and *Kaloula p. pulchra* (2 males, 1 female) were captured in Xishuangbanna, southern Yunnan, China in May 1991. Chromosome preparations were made from the bone marrow cells by the method of Wu et al. (1981). C-banding and silver-staining NORs were carried out following the methods of Sumner (1972) and Tan et al. (1986).

Results

The karyotypes for the four species are separately shown in Figs. 1-3 and the chromosome measurements in Table 1. The secondary constrictions and results of Ag-NORs are listed in Table 2.

The diploid chromosome number of *M. ornata* and *M. pulchra* is 24, with 18 m, 4 sm and 2 m or 2 sm chromosomes, whereas that of *M. butleri* is 22 with 18 m and 4 sm chromosomes. *Kaloula p. pulchra* had 28 including 20 m, 6 sm and 1 sm or 1 st. In four species, the chromosome length decreased gradually, not forming distinct groups in size. The conspicuous secondary constrictions (SC) were found on the long arm of No. 5 of *K. p. pulchra*, No. 8 of *M. butleri* and Nos. 8, 10 of *M. pulchra*, whereas the unremarkable one can be sought on the long arm of No. 11 of *M. ornata* in a few mitotic metaphases. No consistent heteromorphic pairs were observed in all four species.

The C-banding were successfully obtained in *K. p. pulchra*. The centric positive bands were discovered on all chromosomes, especially present on smaller ones; terminal bands were shown on Nos. 2-4; interstitial bands, as well, can be observed on both the short and long arm of No. 1 and the short arm of No. 4. The result still revealed the highly heterochromatic region possesses two-thirds of the length of No. 5 (Fig. 3). The prominent heterochromatinization are observed in individuals of both sexes, and there are no difference between both sexes, indicating the existence of sex-differentiation.

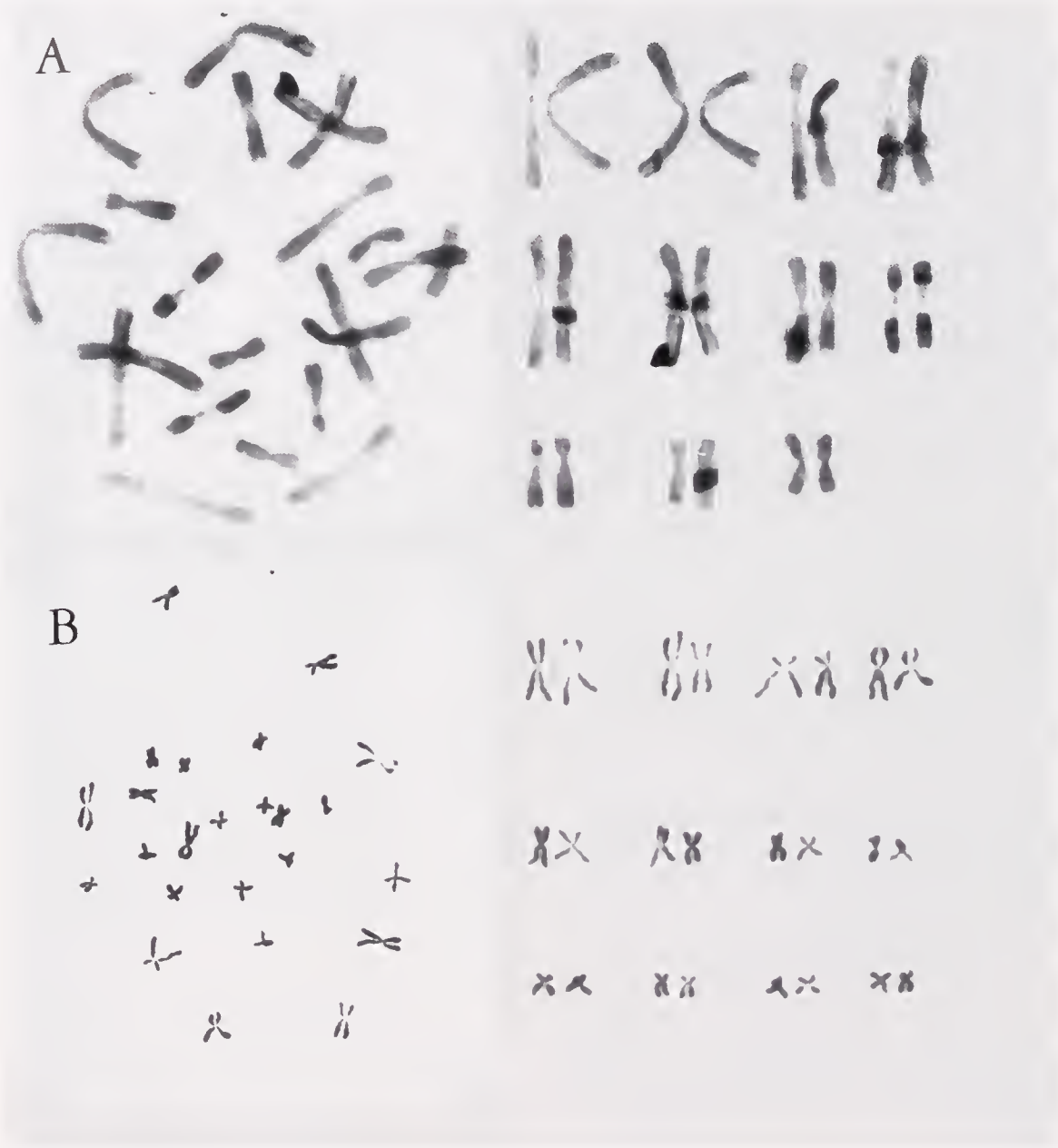

FIG. 1. Karyotypes of *Microhyla butleri* (A) and *M. ornata* (B).

The silver-staining NORs revealed that NOR is located on No. 10, associated with the secondary constriction in *M. pulchra* (Fig. 2). *M. butleri* and *K. p. pulchra* are also treated with the silver-staining NORs technique. Although no perfect spread of silver-staining NORs are in the two species, NOR associated with SC can be sought in some cells.

Discussion

Karyotypes

The karyotypes of *M. ornata* and *M. pulchra* from Xishuangbanna were different from those of the other localities (Table 2). Firstly, all 12 chromosome pairs, except No. 3 in two species, are metacentric, but Nos. 7-9 from Xishuangbanna changed to, or close to, submetacentric. Next, the

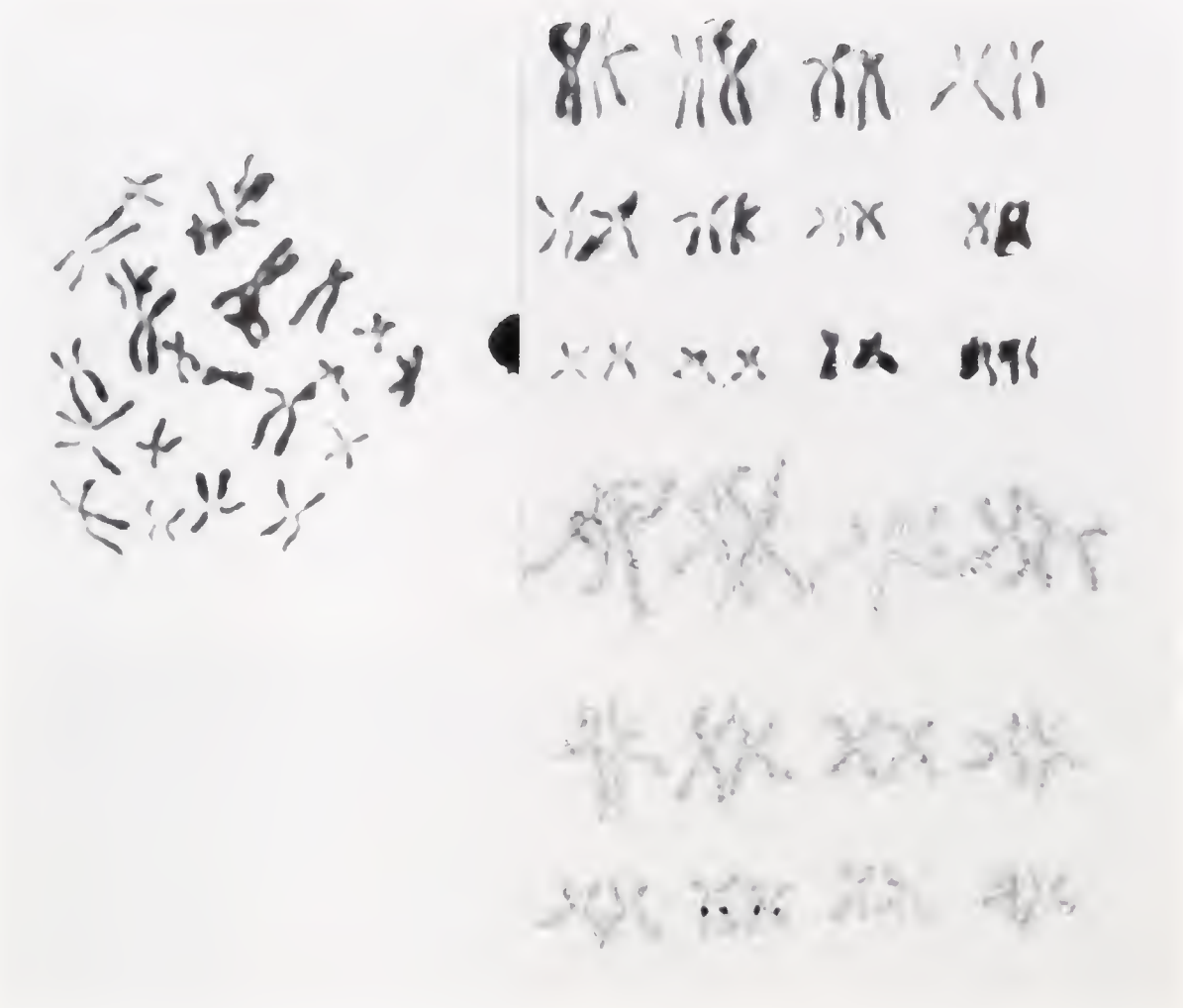

FIG. 2. Karyotype and Ag-NORs of *Microhyla pulchra*.

numbers and positions of SC on the chromosomes are distinctly varied between the same species from Xishuangbanna and the other localities (Table 2). These results seem to show that the karyotypic type of the species are gradually altered due to slowly fitting for the various environments.

There are about 20 species described in the genus *Microhyla* that range over Asia only. Up to now, seven species have been analyzed karyologically. The karyotypic character proved their obvious interspecific differentiation. In *M. nornata* and *mixtura*, st chromosomes can be observed. The positions of SC on the chromosomes are quite different between these species: *ornata* on Nos. 3, 9 and 11; *heymonsi* on No. 2 (Gao et al., 1985; Guo et al., 1987); *pulchra* on several pairs; *inornata* (Zhao, 1988) and *mixtura* (Guo et al., 1991) on No. 9 and *butleri* on No. 8. Moreover, this evident differentiation is reflected on the various diploid number in the genus. Most of them have 2n=24 except for *inornata* and *rubra* with 2n=26 and *butleri* with 2n=22 (the present study). Diploid number of 22, 24, 26 and 28 are known for *Microhylidae*. Usually, most species in the same genus have the same diploid number in anurans. On the other hand, if we supposed the 24 was the diploid number of the genus *Microhyla*, it would be possible to consider whether *butleri*, *inornata* and *rubra* might be separated from the genus.

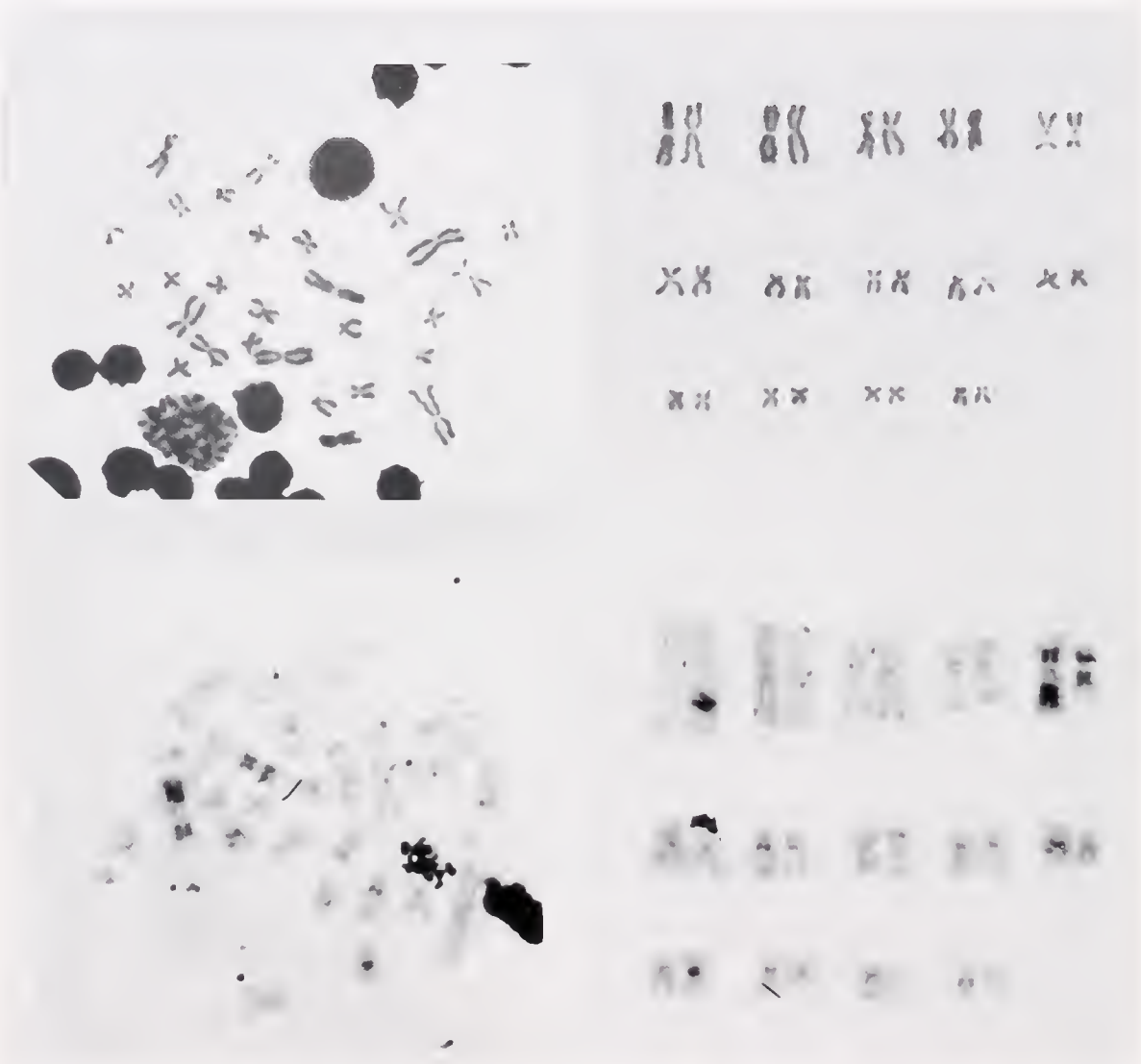

FIG. 3. Karyotype and C-bands of *Kaloula p. pulchra*.

The karyotypes of *K. p. pulchra* from Xishuangbanna and Guangzhou are compared (Table 2) and the difference between them are not obvious. Unlike in *Microhyla*, five species in *Kaloula* whose karyotype are known have 2n=28, and the conspicuous SC located on No. 5 except *K. picta* (Kuramoto, 1980). The interspecific differentiation is less clear. The differences between these species are shown on the variety of centromere type of a few corresponding chromosome pairs.

C-bands and NORs

Although many *microhylids* are analyzed karyologically, their C-banding is rarely reported. The C-bands of *K. rugifera* are even mentioned (Zeng et al., 1989) in which the interstitial and terminal bands except centric are observed. In *K. p. pulchra*, not only interstitial and terminal but also centric bands are easily seen (Fig. 3). In the two species, the interstitial bands associated with the main SC on No. 5 are enhanced, which indicates the genetic stability in this genus. The C-bands of the two species present the

TABLE 1. Chromosome measurements of four microhylid species from Xishuangbanna, southern Yunnan, China.

No	*Microhyla ornata* Arm Ratio	Relative Length	Type	*Microhyla pulchra* Arm Ratio	Relative Length	Type
1	1.34± 0.10	13.71± 0.75	m	1.21± 0.13	13.68± 0.92	m
2	1.49± 0.25	11.71± 0.51	m	1.42± 0.18	11.69± 0.59	m
3	2.36± 0.35	11.06± 0.64	sm	2.24± 0.38	10.38± 0.47	sm
4	1.54± 0.31	10.24± 0.78	m	1.34± 0.12	10.01± 0.41	m
5	1.28± 0.25	9.10± 0.43	m	1.24± 0.21	9.25± 0.57	m
6	1.18± 0.09	8.58± 0.48	m	1.33± 0.33	8.47± 0.46	m
7	1.87± 0.40	7.05± 0.56	sm	1.60± 0.39	7.40± 0.61	sm
8	1.42± 0.26	6.48± 0.23	m	1.65± 0.35	6.73± 0.31	sm/m
9	1.64± 0.43	5.94± 0.28	m/sm	1.32± 0.28	6.19± 0.48	m
10	1.39± 0.23	5.61± 0.26	m	1.55± 0.30	5.61± 0.48	m
11	1.37± 0.22	5.26± 0.39	m	1.45 - 0.27	5.46± 0.42	m
12	1.24± 0.10	5.06± 0.45	m	1.25± 0.14	5.13± 0.22	m
	Microhyla butleri			*Kaloula p. pulchra*		
1	1.29± 0.15	14.18± 0.92	m	1.16± 0.13	14.52± 0.84	m
2	1.34± 0.25	12.63± 0.85	m	1.23± 0.09	12.07± 0.71	m
3	1.90± 0.26	11.07± 0.45	sm	1.60± 0.18	10.21± 0.38	sm
4	1.40± 0.18	10.47± 0.42	m	1.15± 0.10	9.04± 0.38	m
5	1.19± 0.12	9.68± 0.52	m	1.28± 0.25	8.28± 0.40	m
6	1.22± 0.15	8.81± 0.43	m	1.35± 0.16	7.01± 0.33	m
7	1.39± 0.28	8.31± 0.55	m	2.56± 0.51	6.10± 0.25	sm/st
8	1.32± 0.16	7.24± 0.44	m	1.53± 0.19	5.82± 0.16	m
9	1.96± 0.36	6.54± 0.34	sm	1.95± 0.48	5.24± 0.35	sm
10	1.41± 0.21	6.00± 0.36	m	1.34± 0.26	4.87± 0.26	m
11	1.23± 0.20	5.07± 0.66	m	1.37± 0.27	4.58± 0.41	m
12				1.19± 0.17	4.46± 0.37	m
13				1.28± 0.33	4.11± 0.40	m
14				2.28± 0.34	2.28± 0.34	sm

TABLE 2. Karyotypes of four microhylid species from different localities

Species	Locality	1	2	3	4	5	6	7	8	9	10	11	12	13	14	S.C.	Ag. NOR's
M. ornata	Sichuan	m	m	sm	m	m	m	m	m	m	m	m	m				
	Fujian	m	m	sm	m	m	m	m	m	m	m	m	m			Nos. 3, 9q	
	Xishuangbanna	m	m	sm	m	m	m	sm	m	m/sm	m	m				No. 11q	
M. pulchra	Guangzhou	m	m	sm	m	m	m	m	m	m	m	m	m			Nos. 1,3, 6, 10, 11q	
	Xishuangbanna	m	m	sm	m	m	m	sm	sm/m	m	m	m				Nos 8, 10q	No. 10q
M. butleri	Xishuangbanna	m	m	sm	m	m	m	m	m	sm	m	m				No. 8q	No. 8q
K. pulchra	Guangzhou	m	m	sm	m	m	m	m	sm	m	m	m	m	m	m	Nos. 5, 14p	
	Xishuangbanna	m	m	sm	m	m	m	sm/stn		sm	m	m	m	m	sm	No. 5q	No. 5q

heterochromatin of *microhylids* are widely spread on the centric, interstitial and terminal positions of chromosomes. The results are very similar to those in the higher anurans. It suggests that the evolutionary level of *microhylids* correspond to that of the higher anurans from cytogenetics. The obvious heterochromatinization of No. 5 in *K. pulchra* does not show sex differentiation, and it acts as part of a special sign to distinguish it from other species.

The stable and conspicuous SC is always the location of NORs. In fact, silver-staining NORs shows that NORs of *K. pulchra*, *K. rugifera*, *M. pulchra*, *M. butleri*, *M. ornata*, *M. mixtura*, and *M. heymonsi* are just located in the position of their main SC. Tymowska (1977) concluded these species in the genus show a close relationship due to having the same NORs. From this point, the close relationship exist between species in the genus *Kaloula* for they have the same SC on No. 5. On the contrary, those species in the genus *Microhyla* reveal their higher interspecific differentiation level because of their different NOR association with the main SC.

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