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Short Note

Karyotype of the Emerald Green Tree Frog, *Rhacophorus smaragdinus*

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June-Shiang Lai and Kuang-Yang Lue (1993) Karyotype of the emerald green tree frog, *Rhacophorus smaragdinus*. *Bull. Inst. Zool., Academia Sinica* **32**(3): 214-216. Using bone marrow cell methanol-glacial acetic acid chromosome methodology, we karyotyped the emerald green tree frog *Rhacophorus smaragdinus*. Our results show it has $2n = 26$ chromosomes, including five large and eight small pairs. Eight pairs are submetacentric in shape (Nos. 2, 3, 6, 7, 8, 11, 12, and 13); secondary constrictions were not observed. We compare the karyotype of *R. smaragdinus* with those of the two other *Rhacophorus* species endemic to Taiwan.

Key words: Karyotype, *Rhacophorus smaragdinus*, Taiwan.

Rhacophorus smaragdinus is rhacophorid frog endemic to Taiwan. It was first described by Lue and Mou (1983); tadpole morphology was described by Her *et al.* (1989). Concerning distribution, *R. smaragdinus* is restricted to the northern part of Taiwan (Lue and Lai 1990, Lue *et al.* 1991). Karyological studies of rhacophorid frogs in Taiwan have previously been performed by many researchers (Kuramoto 1989) with the exception of *R. smaragdinus*.

Materials and Methods—The three male and one female specimens used in this study were captured at Datong Shan (大桶山) near Taipei. For chromosome preparation we followed procedures described by Ota (1989). For terminology related to chromosomes we followed Levan *et al.* (1964).

Results—Based on photomicrographs, we found that the 2n chromosome number in *R. smaragdinus* is 26, including five large and eight small pairs (Fig. 1). Sex chromosome is not observed. Pair nos. 1, 4, 5, 9, and 10 are metacentric, while all others are submetacentric (Table 1). Secondary constrictions were not observed in this species.

Pair no. 1 is easily identified by its large size and metacentric form. Pair nos. 2 and 3 are easily defined by their nearly submetacentric shapes. However, pair no. 2 is significantly different from no. 3 in both size and shape (RL:$t = 3.818$, $df = 16$, $p < .05$; AR:$t = 3.35$, $df = 16$, $p < .05$). Pair nos. 4 and 5 are metacentric and also easily defined in terms of size and shape. Among the smaller chromosomes, nos. 9 and 10 were identified as metacentric; they are similar both in the relative length and arm ratio and are not significantly different from each other (RL:$t = 0.795$, $df = 16$, $.2 < p < .5$; AR:$t = -1.57$, $df = 16$, $.1 < p < .2$). Pair nos. 6 and 7 are different in size ($t = 2.014$, $df = 16$, $p < .05$). No. 7 and 8 are similar in shape ($t = .571$, $df = 16$, $p > .5$), as are nos. 11 and 12 ($t = .553$, $df = 16$, $p > .5$); however, pairs 11 and 12 differ in size ($t = 2.61$, $df = 16$, $p < .05$); Pair no. 13 is quite easy to identify due to its small size and submetacentric form.

Using data from both Table 1 and Kuramoto (1985), we drew an idiogram to compare the karyotypes of *R. smaragdinus*, *R. taipeianus*, and *R. moltrechti* (Fig. 2). The *t*-test results revealed that six pairs of chromosomes differed significantly in either relative length or arm ratio (or both) between *R. smaragdinus* and *R. taipeianus* ($p < .05$; pair nos. 3, 5, 6, 7, 8, and 11), as well as in nine pairs between *R. smaragdinus* and *R. moltrechti*. Among the three species, pair nos. 1 and 13 differed significantly in relative length (ANOVA, $df = 2,23$, $p < .05$), and pair nos. 1, 6, and 7 differed

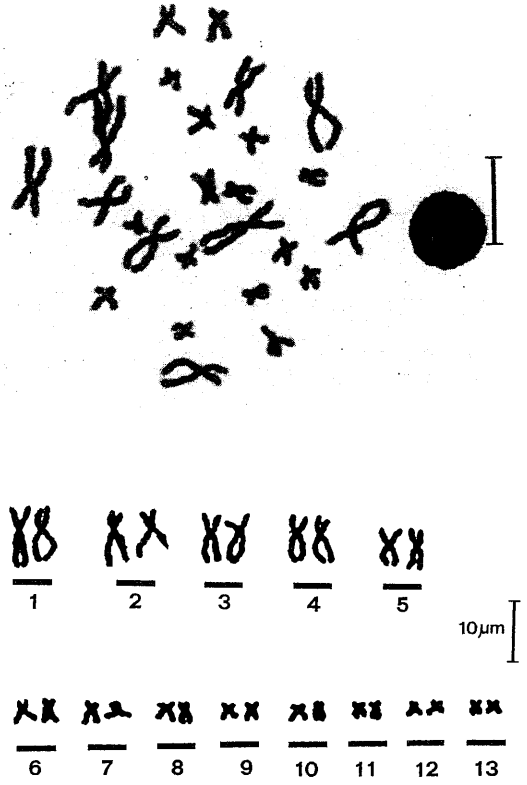

Fig. 1. Karyotype of *Rhacophorus smaragdinus*.

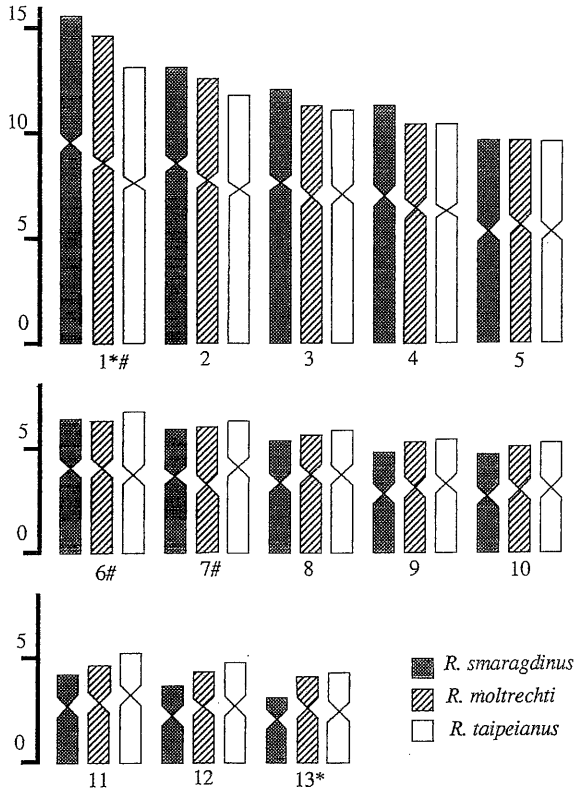

Fig. 2. Idiogram for comparing karyotypes among *Rhacophorus smaragdinus, R. moltrechti*, and *R. taipeianus*. Data for *R. moltrechti* and *R. taipeianus* are from Kuramoto (1985). The symbols * and # indicate significant differences in relative length and arm ratio, respectively.

Table 1. The relative length and arm ratio (X ± SD) of *Rhacophorus smaragdinus* chromosomes (n = 9)

Pair No.	Relative Length	Arm Ratio
1	15.5 ± 0.64	1.6 ± 0.03 m
2	13.2 ± 0.75	2.0 ± 0.21 sm
3	12.1 ± 0.46	1.7 ± 0.12 sm,m
4	11.4 ± 0.81	1.6 ± 0.07 m
5	9.6 ± 1.03	1.3 ± 0.12 m
6	6.4 ± 0.55	1.7 ± 0.12 sm,m
7	5.9 ± 0.48	1.7 ± 0.17 sm,m
8	5.4 ± 0.34	1.7 ± 0.20 sm,m
9	4.9 ± 0.46	1.4 ± 0.19 m
10	4.7 ± 0.55	1.5 ± 0.16 m
11	4.2 ± 0.46	1.8 ± 0.23 sm,m
12	3.7 ± 0.47	1.7 ± 0.23 sm,m
13	3.1 ± 0.43	2.1 ± 0.27 sm

(m and sm indicate metacentric and submetacentric, respectively.)

significantly in arm ratio (ANOVA, $df = 2,23$, $p < .05$). These findings suggest that *R. smaragdinus* is karyologically different from the other two Taiwanese *Rhacophorus* species.

Discussion—To date, nine *Rhacophorus* species have been karyotyped (Kuramoto 1989). These species all have five pairs of large chromosomes and eight pairs of small ones. They can be separated by differences in relative chromosome length, the number of secondary constrictions and their positions, and centromere location.

R. smaragdinus has eight submetacentric chromosomes (nos. 2, 3, 6, 7, 8, 11, 12, and 13) which are quite different from those in *R. chenfui* (7 pairs, nos. 2, 3, 4, 6, 11, 12, and 13) (Tan *et al.* 1987), *R. taipeianus* (4 pairs, nos. 3, 7, 8, and 11) and *R. moltrechti* (3 pairs, nos. 6, 8, and 13). It is worthy of note that *R. smaragdinus* has the largest number of submetacentric

chromosomes; in addition, *R. smaragdinus* has six submetacentric chromosome pairs which are similar to those in *R. chenfui* (nos. 2, 3, 6, 11, 12, and 13), but only 4 pairs to *R. taipeianus* (nos. 3, 7, 8, and 11) and three in *R. moltrechti* (nos. 6, 8, and 13). The karyological evidence discussed in this note and the morphological comparisons provided previously made by Lue and Mou (1983) confirms that *R. smaragdinus* is a distinct species.

There are more than fifty species of *Rhacophorus* frogs throughout the world, many which are widely distributed in East Asia (Frost 1985). Evolutionary relationships among *Rhacophorus* species or between *Rhacophorus* and other groups of frogs invite further study.

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翡翠樹蛙(*Rhacophorus smaragdinus*)之核型

賴俊祥　呂光洋

以骨髓細胞，甲醇—冰醋酸法，我們製作了翡翠樹蛙的核型。其具有2n＝26個染色體，五對大的八對小的。八對是亞中位染色體（Nos. 2, 3, 6, 7, 8, 11, 12, 13），未觀察到二次縊縮及性染色體。文中我們比較了其和台灣產另兩種綠樹蛙屬(*Rhacophorus*)種類，在核型上的差異。