Cytotaxonomic Studies of Three Ranid Species
(Amphibia: Anura) from Hong Kong

MASAFUMI MATSUI, HIDETOSHI OTA, MICHAEL W. LAU, AND ANTHONY BOGADEK

Abstract: Karyotypes of three ranid species from Hong Kong were studied. *Amolops hongkongensis*, with $2n=26$ chromosomes consisting of seven metacentric and six submetacentric pairs in both sexes, has secondary constrictions on the longer arms of pair 7. The species is karyotypically closer to *A. ricketti* than to species from western China. *Rana exilispinosa*, also with $2n=26$ chromosomes but consisting of eight metacentric and five submetacentric pairs and lacking secondary constrictions in both sexes, is karyotypically similar to *R. spinosa* and *R. shini*. This supports the validity of the recently proposed subgenus *Quasipaa*. *Rana livida*, again with $2n=26$ chromosomes of eight metacentric and five submetacentric pairs, has secondary constrictions on the longer arms of pair 10 and differs from the Anhui population. This species is suggested to be karyologically closer to members of the subgenus *Odorrana* than to those of *Eburana*.

Key words: Amphibia; *Amolops*; *Rana*; Karyotype; Hong Kong

The anuran fauna of Hong Kong consists of 22 species (Karsen et al., 1986; Zhao and Adler, 1993), of which *Amolops hongkongensis* and *Philautus romeri* are endemic to this region [but see Yang (1991) for an argument against the endemcity of the former] and the others are shared with the southeastern provinces of China. Although karyotypes of Chinese anurans have recently been actively studied (e.g., see Kuramoto, 1990 for review), no chromosomal studies have been done for the Hong Kong frogs and toads, making it difficult to assess their systematic and biogeographical status from a cytotaxonomic point of view. We have karyotyped three ranid species of Hong Kong, including the endemic *Amolops hongkongensis*, for the first time. In this paper, we describe the conventionally Giemsa stained karyotypes of these species and discuss their systematic implications.

**Materials and Methods**

One male and three females of *Amolops hongkongensis*, one male and three females of *Rana exilispinosa*, and four females of *R. livida* were collected in Hong Kong (see Appendix I for detailed sampling data), and were brought back live to the laboratory, where they were injected with colchicine solution (0.1 mg/ml) in their femur muscles at 0.1 ml per gram body weight. Twelve to 15 hr after the injection, they were subjected to hypotonic treatment in KCl solution (0.05 mol/l) for 40–50 min, and then were rinsed and fixed in Carnoy's solution (glacial acetic acid: methanol = 1:3). The cell slides, prepared by the air-dry method, were soaked in 3% Giemsa solution for 30–40 min, and then were observed and photographed with a microscope. The karyotype was determined on the basis of three to five well spread metaphase cells for each individual.

Chromosomes were measured on enlarged photographs and were arranged and numbered in the order of decreasing size. Terminology for the description of centromeric position on each chromosome follows Levan et al. (1964), as modified by Green and Sessions (1991).

**Results**

Both male and female *Amolops hongkongensis* had $2n=26$ homologous chromosomes. Chromosomes forming pairs 1, 5 to 8, 11, and 13 were metacentric, and the remainder were submetacentric. Therefore, the fundamental number (N.F.) equaled 52. The chromosome size decreased very gradually from pairs 1 to 5, and from pairs 6 to 13, but there was a conspicuous size difference between pairs 5 and 6. In some cells, secondary constrictions were evident on the longer arms of pair 7 (Table 1, Fig. 1A).

The karyotypes of male and female *Rana exilispinosa* also consisted of $2n=26$ homologous chromosomes that exhibited a size gap between

Accepted 1 May 1995
Fig. 1. Female karyotypes of (A) Amolops hongkongensis (KUZ 30208, arrows indicate positions of apparent secondary constrictions in pair 7 of another cell), (B) Rana exilispinosa (KUZ 30214), and (C) Rana livida (KUZ 30112, arrows indicate secondary constrictions) from Hong Kong. Thick bar at the right bottom equals 10 μm.
TABLE 1. Chromosomes of three ranid species from Hong Kong. Data are presented as \( \bar{x} \pm SD \). Abbreviations, m and sm, represent metacentric and submetacentric chromosomes, respectively.

<table>
<thead>
<tr>
<th>Pair No.</th>
<th>Amolops hongkongensis (N=15; 2n=26)</th>
<th>Rana exilispinosa (N=17; 2n=26)</th>
<th>Rana livida (N=15; 2n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative length Arm ratio</td>
<td>Relative length Arm ratio</td>
<td>Relative length Arm ratio</td>
</tr>
<tr>
<td>1</td>
<td>18.4±0.69 1.07±0.02 m</td>
<td>14.6±0.26 1.27±0.05 m</td>
<td>15.0±0.22 1.39±0.05 m</td>
</tr>
<tr>
<td>2</td>
<td>14.2±0.24 2.52±0.24 sm</td>
<td>12.5±0.27 2.05±0.14 sm</td>
<td>12.3±0.30 1.74±0.11 sm</td>
</tr>
<tr>
<td>3</td>
<td>12.1±0.32 2.34±0.12 sm</td>
<td>11.2±0.26 1.76±0.13 sm</td>
<td>10.8±0.16 1.82±0.05 sm</td>
</tr>
<tr>
<td>4</td>
<td>11.2±0.20 1.70±0.05 sm</td>
<td>10.7±0.23 1.57±0.13 m</td>
<td>10.4±0.14 1.75±0.13 sm</td>
</tr>
<tr>
<td>5</td>
<td>9.8±0.25 1.50±0.06 m</td>
<td>9.7±0.16 1.55±0.07 m</td>
<td>9.5±0.09 1.46±0.07 m</td>
</tr>
<tr>
<td>6</td>
<td>5.0±0.09 1.24±0.05 m</td>
<td>6.1±0.29 1.27±0.05 m</td>
<td>6.3±0.08 1.11±0.02 m</td>
</tr>
<tr>
<td>7</td>
<td>4.9±0.09 1.42±0.07 m*</td>
<td>5.9±0.11 2.01±0.18 sm</td>
<td>5.6±0.09 1.29±0.03 m</td>
</tr>
<tr>
<td>8</td>
<td>4.7±0.12 1.49±0.12 m</td>
<td>5.7±0.11 1.32±0.03 m</td>
<td>5.6±0.09 2.77±0.08 sm</td>
</tr>
<tr>
<td>9</td>
<td>4.4±0.09 2.19±0.09 sm</td>
<td>5.6±0.10 1.78±0.11 sm</td>
<td>5.6±0.10 2.02±0.08 sm</td>
</tr>
<tr>
<td>10</td>
<td>4.3±0.09 1.82±0.13 sm</td>
<td>4.9±0.17 1.18±0.03 m</td>
<td>5.0±0.13 1.49±0.08 m*</td>
</tr>
<tr>
<td>11</td>
<td>4.0±0.06 1.37±0.05 m</td>
<td>4.6±0.19 1.49±0.10 m</td>
<td>4.9±0.13 1.36±0.06 m</td>
</tr>
<tr>
<td>12</td>
<td>3.7±0.09 1.90±0.07 sm</td>
<td>4.3±0.23 1.10±0.03 m</td>
<td>4.7±0.08 1.29±0.05 m</td>
</tr>
<tr>
<td>13</td>
<td>3.3±0.11 1.32±0.07 m</td>
<td>4.1±0.20 1.94±0.14 sm</td>
<td>4.4±0.08 1.38±0.05 m</td>
</tr>
</tbody>
</table>

*Secondary constrictions are evident on longer arms in some cells.

pairs 5 and 6. Chromosomes forming pairs 1, 4 to 6, 8 and 10 to 12 were metacentric, whereas those of the remaining five pairs were submetacentric (Table 1, Fig. 1B). The fundamental number was thus calculated as 52. Secondary constrictions were not detected.

Female *Rana livida* possessed \( 2n=26 \) chromosomes, of which those forming pairs 1, 5 to 7 and 10 to 13 were metacentric and the others were submetacentric. Thus, N.F. equaled 52. No sex chromosome heteromorphism was evident. There was a recognizable size discontinuity between pairs 5 and 6, whereas size alterations from pairs 1 to 5 and from 6 to 13 appeared very gradual. The sizes of pairs 7, 8 and 9 did not differ from each other significantly \( (p>0.05; \text{Mann-Whitney U-tests}) \). In some cells, secondary constrictions were evident on the longer arms of pair 10 (Table 1; Fig. 1C).

DISCUSSION

Amolops hongkongensis.—The genus *Amolops* is characterized by peculiar larvae that inhabit swift torrents and bear a large abdominal sucker (Inger, 1966). Yang (1991) recently divided this genus into three distinct genera, *Amolops*, *Huia*, and *Meristogenys*. On the other hand, Dubois (1992) relegated Yang’s three genera to subgeneric status of the genus *Amolops* together with another subgenus *Amo*. These classifications, however, are based chiefly on morphology, and many other important characteristics including karyological ones have been utilized insufficiently because of the lack of information.

Compared with members from the other regions, Chinese species of the genus *Amolops* have been well studied karyologically. For example, Wu and Zhao (1985) extensively compared karyotypes of *A. granulosus*, *A. liangshahenansis*, *A. lifanensis*, *A. loloensis*, *A. mantzorum*, all from Sichuan, and *A. viridimaculatus* from Yunnan. Except for *A. viridimaculatus*, all these species have secondary constrictions on the shorter arm of pair 6 and the longer arm of pair 10. They also have pairs 3 and 8 submetacentric, and pairs 1, 6, 10, and 11 metacentric. These characteristics are different from those observed in our *A. hongkongensis* in which pair 8 was metacentric, pair 10 submetacentric, and the secondary constriction was located on the longer arms of pair 7.

Tan (1992) analyzed karyotypic data for the six species mentioned above plus *A. kangtingensis*, *A. wuyiensis*, and *A. ricketti*, and obtained a "cladogram" cytotaxonomically. Some of the character states are not defined in his text, and it is impossible to directly compare their data with ours. However, with regard to the arm ratio, *A. hongkongensis* is quite distinct from the species utilized by Tan (1992). In particular, pairs 2, 9, and 10 in *A. hongkongensis* seem very distinct in arm ratio.

When simply compared with the Chinese members given by Tan (1992) in the values of arm ratios, *A. hongkongensis* is most similar to *A. ricketti*. The original data for *A. ricketti* from Guangdong given by He (1986) reveal that
this species has a secondary constriction on the longer arm of pair 8. In *A. hongkongensis*, the constriction was found on the long arm of pair 7, but the variation range of the relative length overlapped between pairs 7 and 8. Therefore, the position of the secondary constriction may be regarded as actually the same in *A. ricketti* and *A. hongkongensis*, and this suggests that the two species are closer to each other not only in geographical range but also in phylogenetic affinity than to other Chinese congeners.

Kuramoto and Yong (1992) reported that *A. larutensis* from Malaysia has only 2n=24 chromosomes. The species was assigned either to the genus *Amolops* together with Chinese species by Yang (1991), or to a distinct subgenus *Amo* and separated from Chinese members that were included in the subgenus *Amolops* by Dubois (1992). Acoustic evidence (Matsui et al., 1993) favored Dubois' taxonomic treatment rather than Yang's, and after including the data for *A. hongkongensis*, karyological information also supports such an idea.

Neither the sex chromosome found in male *A. (as *Staurois*) mantzorum nor the extra microchromosome in female *A. liangshanensis* (Wu and Zhao, 1984) was found in *A. hongkongensis*. Thus these properties seem to still remain exceptional in the genus *Amolops*.

Rana exilispinosa.—*Rana exilispinosa*, once described as *R. paraspinosa* (Dubois, 1975), is a member of the subgenus *Paa* Dubois, 1975, which includes "spine frogs". Fei et al. (1990) elevated *Paa* to a distinct genus and recognized three subgenera. They assigned 13 Chinese species [*boulengeri*, *chayuensis*, *conaensis*, *exilispinosa*, *feae*, *jiulongensis*, *liebigi*, *maculosa*, *muta* (=*liui*), *phrynoides* (=*yunnanensis*), *shini*, *spinosa*, and *yadongensis* (=*blanfordii*)] to the nominate subgenus *Paa*. In addition to this subgenus, they established two new monotypic subgenera *Unculuana* and *Quadrana*, on the basis of *R. unculuana* and *R. quadrana*, respectively (Fei et al., 1990).

On the other hand, Dubois (1992) established the tribe Paini in the subfamily Raninae, and similarly regarded *Paa* as one of its distinct genera. The contents of the genus, however, are quite different from those of Fei et al. (1990), although both authors developed their classifications chiefly on the basis of morphological information. He (Dubois, 1992) recognized four subgenera (*Epipaa*, *Gynandropaa*, *Paa*, and *Quasipaa*) in the genus *Paa*. *Rana exilispinosa* was assigned to *Quasipaa* together with *R. boulengeri*, *R. jiulongensis*, *R. shini*, and *R. spinosa*. Of the remaining Chinese species, *R. blanfordii*, *R. chayuensis*, *R. conaensis*, *R. liebigi*, and *R. maculosa* were assigned to the subgenus *Paa*, whereas *R. feae*, *R. liui*, and *R. yunnanensis* to *Gynandropaa* (Dubois, 1992). Further, *Rana unculuana* and *R. quadrana*, respectively, were placed in new subgenera *Chaparana* and *Feirana* of the genus *Chaparana* which was newly established in the tribe Paini.

In assessing the validity of above two taxonomic ideas, we simply use *Rana* as the generic name of the species involved so as to avoid further confusion. Of the four species with which *R. exilispinosa* is assigned to *Paa* (*Quasipaa*) by Dubois (1992), three have been karyotyped. First, the karyotypes of *R. spinosa* from Anhui and Guangxi were reported by Li and Wang (1985a) and Tan and Wu (1987), respectively. The karyotype of this species is composed of 26 chromosomes that are divided into five large and eight small pairs. In the Anhui sample, chromosomes are divided into ten metacentric and three submetacentric pairs and are neither sexually heteromorphic nor with secondary constrictions (Li and Wang, 1985a). On the other hand, one female from Guangxi is reported to have eight metacentric and five submetacentric pairs with a secondary constriction on the shorter arms of pair 6 (Tan and Wu, 1987).

Tan and Wu (1987) also reported the karyotype of *R. shini* from Sichuan. This species again has five large and eight small pairs, but these pairs consist of nine metacentric and four submetacentric elements and have no secondary constrictions. Further, another member of Dubois' subgenus *Quasipaa*, *R. boulengeri* from Sichuan, was karyotyped by Chen et al. (1983) and Wang et al. (1983). The species also has five large and eight small pairs that are composed of ten metacentric and three submetacentric elements. Like *R. spinosa* from Guangxi, there is a secondary constriction on the shorter arms of pair 6.

Of Dubois' (1992) other subgenus, *Paa*, *R. maculosa* has been studied karyologically. Liu et al. (1993) reported that this species has 2n=26 chromosomes with five large and eight small pairs. There is a secondary constriction on the short arm of pair 1, and in addition to six metacentric and six submetacentric pairs, one subtelocentric pair is present. *Rana yunnanensis*, assigned to the subgenus *Gynandropaa* by Dubois (1992), has a very unusual karyotype comprising as many as 2n=64 chromosomes (Liu and Zan, 1984; Wu and Zhao, 1984; Tan and Wu, 1987, all reported the species as *R.*
phrynoides). Likewise, Rana unculuana assigned to a distinct genus Chaparana by Dubois (1992) is characterized by a distinct karyotype with 2n = 40 chromosomes that lack secondary constrictions (Liu et al., 1993).

Thus, a high degree of karyotypic differentiation has evolved in "spine frogs", and the tribe Paini (Dubois, 1992) can not be simply characterized karyologically. In order to generalize karyotypic evolution of this group, further studies including additional species from outside of China, and using differential staining, are required. However, from the data currently available, *R. exilispinosa* is karyologically most similar to the Anhui population of *R. spinosa* and *R. shini* in the absence of secondary constrictions. Thus these two species may be sister taxa of *R. exilispinosa* among Quasipaa proposed by Dubois (1992).

*Rana livida*.—Li and Wang (1985b) briefly reported the karyotype of *R. livida* from Anhui. According to them, the 26 diploid chromosomes are divided into five large and eight small pairs that are meta- or submetacentric, except for pair 13 which is subtelocentric. Chromosomes are not sexually heteromorphic and secondary constrictions are found on the longer arms of pair 6. In our samples, five pairs were submetacentric but no comparable data are available for the number of submetacentric pairs in the Anhui sample (Li and Wang, 1985b). On the other hand, pair 13 was submetacentric and a secondary constriction was detected on the longer arms of pair 10. In our samples, five pairs were submetacentric but no comparable data are available for the number of submetacentric pairs in the Anhui sample (Li and Wang, 1985b). On the other hand, pair 13 was submetacentric and a secondary constriction was detected on the longer arms of pair 10 in our female sample. Thus geographic differentiation in the karyotype seems to be present in this species even within China. Distribution of the species is wide, ranging from India through Indochina to eastern China (Frost, 1985), and further studies especially using samples from more southern areas are needed to clarify the pattern of karyotypic differentiation in this species.

Systematic relationships of *R. livida* are not clear. Fei et al. (1990) placed this species in a distinct genus *Odorrana* which has *R. margaretae* as the type species. In addition to these two species, *O. Andersonii*, *O. anlungensis*, *O. grahami*, *O. kuangwuenis*, *O. lungshengensis*, *O. schmackeri*, *O. swinhoana*, *O. tiannanensis*, *O. versabilis*, and *O. wuchuanensis* are included in this genus. Dubois (1992) admitted *Odorrana* of Fei et al. (1990), but moved *R. livida* and *R. swinhoana* to the subgenus *Eburana* that has *R. narina* as the type species. Similarly, Wei et al. (1993) did not include *R. livida* in their "stink frogs" which encompass all species of *Odorrana* (of Dubois, 1992) except for *R. versabilis*.

Six species of *Odorrana* (sensu Dubois, 1992) have been karyotyped, and all are reported to have five large and eight small pairs as do many other ranid species. *Rana margaretae* is reported to have karyotypes that vary among populations. Namely, the number of submetacentric pairs varies from three (samples from Guizhou: Wei et al., 1993) to five (samples from Sichuan: Chen et al., 1983, and the secondary constrictions are located on the longer arms of pair 10 (Wei et al., 1993) or 11 (Chen et al., 1983; samples from Sichuan: Wang et al., 1983). Chen et al. (1983), however, noted that the position of the constriction is not fixed even within the sample from a single locality.

Of other species of *Odorrana*, *R. Andersonii* from Yunnan and Guizhou have been karyotyped by Liu et al. (1993) and Wei et al. (1993), respectively. The species have four to five submetacentric pairs and constrictions on the longer arms of pair 10. Additional constrictions are also found on the shorter arms of pair 3 in the Yunnan sample. On the other hand, Wei et al. (1993) could not detect secondary constrictions in *R. kuangwuenis* from Sichuan, but they found a nucleolar organizer region (NOR) on the longer arms of pair 10. Five submetacentric pairs were found in this species.

*Rana schmackeri* from Guizhou is reported to have three submetacentric pairs and constrictions on the longer arms of pair 10 (Wei et al., 1990). On the other hand, *R. versabilis* from Hainan, studied by Wu et al. (1989), had four submetacentric pairs and constrictions on the longer arms of pair 9 and the shorter arms of pair 6. Further, *R. grahami* from Yunnan, with three submetacentric pairs, are said to have many small NORs in addition to ones on the secondary constrictions on longer arms of pair 10 (Wei et al., 1990).

Thus, the karyotype of *Odorrana* (sensu Dubois, 1992) seems to be quite variable in minor points, although they are conservative fundamentally (i.e., compared with "spine frogs"). In the number of submetacentric pairs and positions of secondary constrictions, our *R. livida* from Hong Kong is identical to some populations of *R. margaretae* or *R. Andersonii*.

According to Kuramoto (1980), *R. swinhoana* (as *R. narina* from Taiwan), which is a member of *Eburana* (Dubois, 1992), had four submetacentric pairs and secondary constrictions on the longer arms of pairs 6, 8, and 10. Karyotypes are sexually dimorphic in this
species, and the male pair 8 of this species is conspicuously heteromorphic. Other members of Dubois' "Eburana, R. anamensisis (reported as R. narina) and R. ishikawai., have six and five submetacentric pairs, respectively, and both have constrictions on the longer arms of pair 9 (Maeda and Matsui, 1989). These characteristics are dissimilar to those of R. livida. As already pointed out by Matsui (1994), R. livida has a smelly skin and is placed better in Odorrana than in Eburana. The karyological evidence shown here seems to support this idea.

Acknowledgments.—We thank L. Fei, D.-T. Yang and E.-M. Zhao for providing literature cited here. H. Ota is also grateful to Frs. S. C. W. Lam, J. Zen and other staff members of St. Louis School for a comfortable accommodation during his visit to Hong Kong, and to S. Iwanaga and M. Toda for laboratory assistance. In Hong Kong, handling of Amolops hongkongensis is strictly regulated by the law (Wild Animal Protection Ordinance Cap. 170). The present research was carried out under a special permit from the Director of Agriculture and Fisheries of the Hong Kong Government issued to M. W. Lau (Ref.: AF CON 09/51).

Literature Cited


Wei, G., N. Xu, D.-J. Li, G.-F. Wu, and X.-Q. Song.

APPENDIX I.
Specimens examined.—Voucher specimens are deposited in the herpetological collection of Department of Zoology, Kyoto University (KUZ) under catalogue numbers listed below.
Amolops hongkongensis: KUZ 30110, a male from Lui Kung Tin, New Territory; KUZ 30208-20, three females from Shing Mun, New Territory.
Rana exilispinosa: KUZ 30113, a female from Shing Mun, New Territory; KUZ 30214, 30215 and 30228, one male and two females from Lantau Island.
Rana livida: KUZ 30112 and 30216, two females from Shing Mun, New Territory; KUZ 30230 and 30231, two females from Lantau Island.

要旨 香港産アガエル科 3 種の細胞分類学的研究
松井正文・太田英利・ミカエル W. ラウ・アンソニー・ポダゲ
香港産アガエル科 3 種の核型を調査した。Amolops hongkongensis は、雌雄とも中部動原体型 7 対と次中部動原体型 6 対からなる染色体数 2n = 26 本で、第 7 番目の対の長腕に二次狭帯をもつ。この種は核型からみて中国西部産の同属諸種よりも A. ricketti に近い。Rana exilispinosa もまた、雌雄とも染色体数 2n = 26 本であるが、中部動原体型は 8 対、次中部動原体型が 5 対で、二次狭帯を欠く。核型からみてこの種は R. spinosa と R. shini に近く、最近提示された Quasipaa 亜属の妥当性を支持する。
Rana livida もまた、中部動原体型 8 対、次中部動原体型 5 対からなる染色体数 2n = 26 本だが、第10番目の対の長腕に二次狭帯をもつ点で安徽省産と異なる。この種は核型からみてEburana 亜属よりも Odorana 亜属の種に近いと思われる。