A Study of Relationships among Ranid Frogs of the Genera *Nanorana* and *Altirana* in the Transhimalaya Mountains of China

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Abstract. *-Nanorana ventripunctata, N. pleskei* and *Altirana parkeri* were examined electrophoretically to investigate the intraspecific genetic relationships. Twelve isozyme loci were assayed and their allele frequencies were calculated. The result of UPGMA clustering, when corrected by the Present-Day Ancestor Method and based on the allele frequencies, detected that the genetic relationship between *N. ventripunctata* and *A. parkeri* is closer than that between *N. ventripunctata* and *N. pleskei*. The authors suggest that the genus *Altirana* should be canceled and that *A. parkeri* be placed in the genus *Nanorana.*

Key words: Anura, Ranidae, *Nanorana, Altirana*, genetic relationships, isozyme, China, Transhimalaya Mountains.

TABLE 1. The location, altitude, date and number of specimens collected.

Species	Locality	Altitude	Date	Number
N. ventripunctata	Zhongdian, Yunnan	3100 m	July 11, 1990	15
N. pleskei	Kangding, Sichuan	3260 m	August 7, 1990	16
A. parkeri	Bashu, Xizang	4100 m	July 4, 1990	15
R. shuchinae	Deqing, Yunnan	3950 m	July 7, 1990	1
R. chensinensis	Mangkang, Xizang	3700 m	July 1, 1990	15

Introduction

The genera *Nanorana* and *Altirana* include three species, which are distributed in the Transhimalaya Mountains of China. They are considered to be closely related, and some identification characters between the two genera have been vague since the description of *N. ventripunctata* (Fei and Huang, 1985). Except for some morphological identification and chromosome studies, there are no other studies published on these genera. In order to re-study the two genera, the authors here use starch gel electrophoresis on extracts from liver and muscle to determine genetic distances in order to better understand the genetic relationships among the three species of frogs.

For comparison, the species of *Rana shuchinae* and *R. chensinensis* were selected as out-groups. Part of the distribution of these two frogs is the same as *Nanorana* and *Altirana.*

Materials and Methods

The collecting locality, altitude, date and number of living specimens of the five species are shown as in Table 1.

The specimens were killed in the field, the liver and thigh muscle of each specimen were taken and placed in 1.5 ml plastic micro centrifuge tubes with several drops of physiological saline, then preserved in liquid nitrogen, and taken back to the laboratory.

Tissues were washed with distilled water and physiological saline, the volume ratio is tissue: physiological saline = 1:1.5, homogenized and centrifuged. These samples were run in horizontal starch gels using gel buffers described by Pasteur et al (1988) as follows: Tris-Borate-EDTA (pH 8.6), Tris-Citrate (pH 6.7). Isozyme stains used were also described by Pasteur et al (1988). The following enzyme systems were stained: alcohol-dehydrogenase

TABLE 2. Allele frequencies detected at polymorphic isozyme loci in the five species.

Locus	Allele	*N. ventripunctata*	*N. pleskei*	*A. parkeri*	*R. shuchinae*	*R. chensinensis*
ADH-1	a		1.0000		1.0000	1.0000
	b			1.0000		
	c	1.0000				
ADH-2	a			0.1667		
	b	0.1000		0.1250		
	c	0.4333	0.5000	0.2917		0.1667
	d	0.0667	0.3125	0.4167		0.8333
	e	0.2667	0.1875		0.5000	
	f	0.1333			0.5000	
EST-2	a					0.6250
	b		0.5714	0.1818	1.0000	0.2500
	c	0.3077	0.3571	0.8182		0.1250
	d	0.6923	0.0715			
EST-3	a		0.4286	0.5000	0.5000	0.2000
	b	0.5000	0.5714	0.5000	0.5000	0.4333
	c	0.5000				
	d					0.3667
EST-4	a	0.5667	0.0667	0.1786		0.4000
	b	0.4333	0.9333	0.8214	1.0000	0.6000
GLC-1	a			0.1250		
	b	0.0357	0.7778	0.6250		0.3750
	c	0.5714	0.2222	0.2500	1.0000	0.3333
	d	0.3929				0.2083
	e					0.0833
LDH-1	a	1.0000	1.0000	1.0000		0.0833
	b				1.0000	0.4167
	c					0.5000
LDH-2	a	1.0000	1.0000	1.0000		
	b				1.0000	
	c					1.0000
MDH-1	a	1.0000	0.0333	0.0667		
	b	0.4333	0.2667	0.6000	0.5000	0.3125
	c	0.3667	0.3000	0.1333		0.3125
	d	1.0000	0.3333	0.1000	0.5000	0.3750
	e		0.0667	0.1000		
MDH-2	a			0.3636		0.5000
	b		0.2333	0.0808	0.5000	0.1250
	c	0.0769	0.4333	0.5000	0.5000	0.3333
	d	0.8077	0.3333	0.0808		0.0417
	e	0.1154		0.0808		
MOD-1	a	0.1250	0.6250	0.2500		
	b	0.0833	0.3750		1.0000	0.4000
	c	0.7917		0.2500		
	d			0.5000		0.6000
SDH-1	a	0.2500	0.2500	0.6250		
	b	0.1000	0.5000	0.3750	0.5000	0.3333
	c	0.1500	0.2500		0.5000	
	d					0.5000
	e	0.4000				0.1667
	f	0.1000				

TABLE 3. Genetic distances and similarities among the five frogs, based on allozyme data in Table 2. Nei's (1972) genetic distances above diagonal, and genetic similarities below diagonal.

	N. ventripunctata	*N. pleskei*	*A. parkeri*	*R. shuchinae*	*R. chensinensis*
1		0.5709	0.5693	1.4414	1.3817
2	0.5650		0.2979	0.5793	0.6224
3	0.5659	0.7424		1.1775	0.8730
4	0.2366	0.5603	0.3080		0.6559
5	0.2512	0.5367	0.4178	0.5900	

TABLE 4. The corrected distances with *R. shuchinae* as the present-day ancestor.

	N. ventripunctata	*N. pleskei*	*A. parkeri*
N. ventripunctata		-1.4498	-2.0496
N. pleskei			-1.4589
A. parkeri			

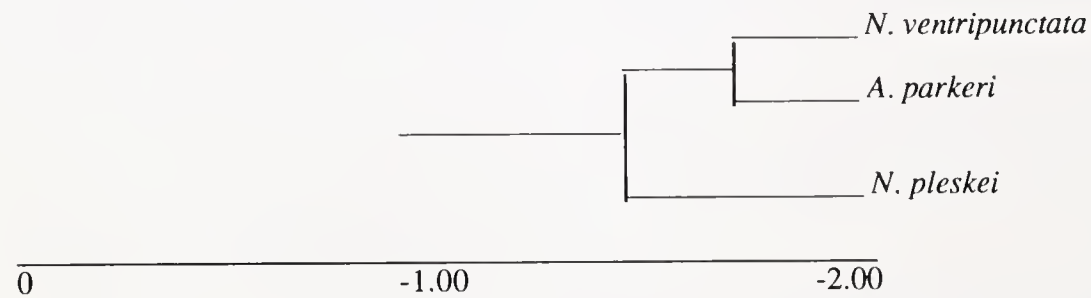

FIG. 1. The UPGMA phenogram of corrected distances with *R. shuchinae* as the present-day ancestor.

(ADH, Ec 1.1.1.1). esterase (EST, Ec 3.1.1.1), NAD-glucose-dehydrogenase (GLC, Ec 1.1.1.1), lactate-dehydrogenase (LDH, Ec 1.1.1.27), malate-dehydrogenase (MDH, Ec 1.1.1.37) malic enzyme (MOD, Ec 1.1.1.40), sorbitol-dehydrogenase (SDH, Ec 1.1.1.14).

Results

Twelve isozyme loci were resolved and scored. Their allele frequencies are shown as in Table 2.

From Table 2, the Nei's (1972) genetic distances and similarities among the five species was calculated and is shown in Table 3.

It is obvious from Table 3 that the evolutionary rates of the five species are unequal, so it is necessary to make a correction before UPGMA clustering. The Present-Day Ancestor Method (Li, 1987) was selected in this paper, the correcting formula is: $D'ij=Dij-Djx$, here D'ij is the corrected distance, the Dij is the original distance, x represents the supposed present-day ancestor. At first, with *R. shuchinae* from the out-group selected as the present-day ancestor, the corrected distances among the three frogs in the genera *Nanorana* and *Altirana* are are shown in Table 4.

The UPGMA clustering phenogram of the corrected distances among the three species in Table 4 is shown in Figure 1.

When *R. chensinensis* is selected as the present-day ancestor, the corrected distances are shown in Table 5.

TABLE 5. The corrected distances with *R. chensinensis* as the present-day ancestor.

	N. ventripunctata	*N. pleskei*	*A. parkeri*
N. ventripunctata		-1.4332	-1.6854
N. pleskei			-1.1975
A. parkeri			

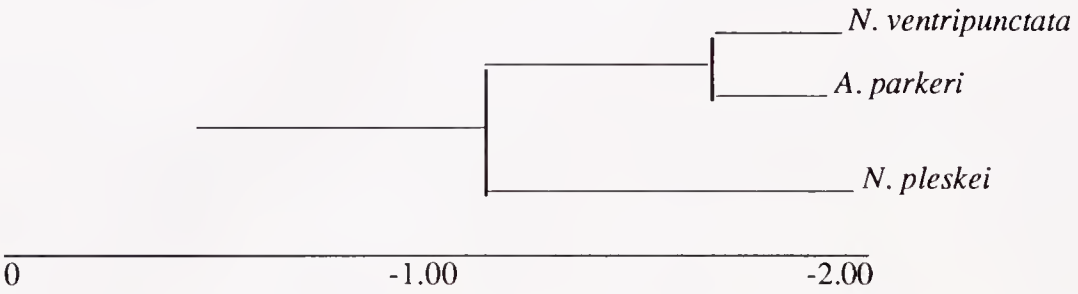

FIG. 2. The UPGMA phenogram of corrected distances with *R. chensinensis* as the present-day ancestor.

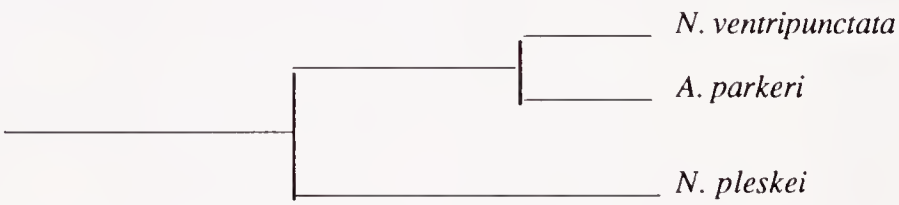

FIG. 3. The genealogical phenogram among the three species.

Based on Table 5, we prepared a UPGMA phenogram which is shown in Figure 2.

Not minding the difference of distances among the frogs, we find that they are similar in both Figure 1 and Figure 2, though they are based on the different present-day ancestors, so we synthesized and simplified them as shown as in Figure 3.

Figure 3 shows that the genetic relationship between *N. ventripunctata* and *A. parkeri* is closer than that between *N. ventripunctata* and *N. pleskei*.

Discussion

Up to the present, the differences between *Nanorana* and *Altirana* described by Tian and Jiang (1986) are the most detailed, but the genus *Nanorana* of their meaning does not contain *N. ventripunctata*. It is just *N. ventripunctata* that confuses the distinction between the two genera, and the study of morphological similarities among the three species of the two genera shows the same result that *N. ventripunctata* and *A. parkeri* are more similar than *N. ventripunctata* and *N. pleskei* (Lu and Yang, 1994). Both of the results of biochemical systematics and morphological similarity studies do not support the presently recognized generic assignments and we suggest that *N. ventripunctata* should be taken out of the genus *Nanorana* and placed in the genus *Altirana*.

From Table 3, we know Nei's (1972) genetic distances among the three species are 0.5709, 0.5693 and 0.2979. This is larger than 0.15, but much smaller than 1.05. These differences are at the species level,

but not the generic level (Thorpe, 1983. Also, we know that there is a principle: in order to avoid more mongenera, the interruption of a genus with other genera should be anti-relative with the number of species in this genus. Thinking of these and the vague line between *Nanorana* and *Altirana*, according to the principle of priority of the International Code of Zoological Nomenclature, the authors suggest that it is perfect to cancel the genus *Altirana*, and that the species *parkeri* should be placed in the genus *Nanorana*.

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