Taxonomic status of the *Rana sauteri* complex: discordance between genetic and morphological traits

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Rana sauteri Boulenger, 1969 and *R. multiderticulata* Chou & Lin, 1997 are two sister species of brown frogs in Taiwan. They are distinguishable by the number of labial tooth rows (LTR) of tadpoles. We investigated morphometric and genetic (mtDNA cyt*b* sequences) traits of 331 tadpoles of the two species and their putative hybrids from 32 locations along two transects. LTR correlated significantly with other morphometric traits and showed a longitudinal cline that increased from west to east across the central mountain range. Genetic differentiation was significant between the two transects, and correlated significantly with geographic distances. However, mtDNA haplotype distributions were indiscernible between the three LTR groups and uncorrelated with other morphometric traits. Individuals of the two sister species also failed to form monophyletic lineages. We argue that LTR is a phenotypically plastic trait related to stream current determined by elevation and monsoon rainfalls, and conclude that *R. sauteri* is the sole representative species, with *R. multiderticulata* being its synonym.

Key words: morphometric traits, mtDNA cyt*b* sequences, *Rana multiderticulata*, tadpole, taxonomy

INTRODUCTION

The analysis of concordance between genetic and morphological traits is an important current subject in evolutionary biology, providing insights into processes that shape divergence among populations (Ballard & Melvin, 2010). Neutral haplotypes and heritable phenotypes provide historical records of population divergence. They are expected to show concordant variation if they share the same evolutionary history (Grady & Quattro, 1999; Crandall et al., 2000), although several recent studies have shown that genetic divergence does not coincide with morphological divergence (Babik et al., 2005; Johnsen et al., 2006; Leaché & Cole, 2007; Richards & Knowles, 2007).

The main evolutionary forces that shape geographical variation in morphological traits are assumed to be genetic drift and natural selection (Grady & Quattro, 1999; Rudh et al., 2007). However, recent divergence (Lorenzen et al., 2006), sex-biased life history, introgression of genes, intense selection (Avise, 1994; 2000; Gompert et al., 2006; Richards & Knowles, 2007) and phenotypic plasticity (Sutherland et al., 2009) may account for a discordance between genetic and morphological traits. Phenotypic plastic traits, or locally adapted traits that are congruent for a species, generally pose difficulties for species delineation (Ghalambor et al., 2007).

Rana sauteri Boulenger, 1909 is a common brown frog from Taiwan. It is widely distributed in the central mountain range and its peripheral hills and adjacent coastal plains at elevations of 100 to 3000 m above sea level (Lue et al., 1990). Mature frogs aggregate in lotic habitats for breeding. Tadpoles possess an abdominal sucker and an enlarged oral disc, an adaptation to fast-flowing streams and to graze algae (Chou & Lin, 1997a). In western Taiwan, *R. sauteri* shows obvious altitudinal clines in life-history traits. As temperature decreases with increased elevation, its breeding season shifts from fall and winter (October–December) to spring (May), the breeding period shortens, and the larval period extends from five to 12 months (Lai et al., 2003).

Chou & Lin (1997a) found a geographical cline in the shape of the upper jaw sheath and the number of labial tooth rows (LTR) of *R. sauteri* tadpoles. They described frogs from the western slope at low elevations of the central mountain range in western Taiwan as *R. sauteri*, frogs from the western slope at high elevations and those from the eastern slope in eastern Taiwan as *R. multidenticulata*, and frogs from the western slope between the ranges of the two species as their hybrids (*R. sauteri* x *R. multidenticulata*; Chou & Lin, 1997b). However, tadpoles can exhibit remarkable phenotypic plasticity (Sutherland et al., 2009), and adults of *R. sauteri* and *R. multidenticulata* are indistinguishable in morphology (Tanaka-Ueno et al., 1998; Che et al., 2007).

Molecular methods are a suitable tool for assessing the taxonomic status of amphibians (Storfer et al., 2009). Studies based on mtDNA sequences of the *R. sauteri* complex have recently shown that genetic variation can differ from the geographical cline of LTR (Tanaka-Ueno et al., 1998; Jang-Liaw & Lee, 2009). However, genetic traits were investigated at small geographic scales, and not compared with morphological traits. The objectives of this study are to examine genetic and morphological traits of tadpoles of the *R. sauteri* complex, to determine their relationships and possible evolutionary processes in differentiation, and to determine the taxonomic status of the two species involved.

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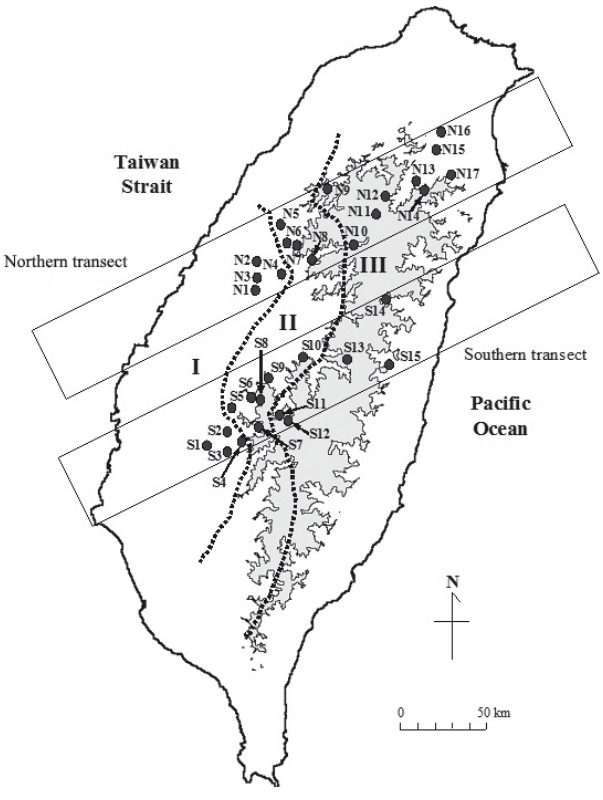

Fig. 1. Sampling locations of tadpoles of the *R. sauteri* complex in Taiwan (N1–N17, locations in northern transect; S1–S15, locations in southern transect; location codes similar to those denoting in Table 1; light grey area, mountains at elevations above 1500 m; broken lines, putative ranges of *R. sauteri* (I), *R. multidenticulata* (III) and the hybrids (II) proposed by Chou & Lin, 1997b).

MATERIALS AND METHODS

Tadpole collection

In all, 331 tadpoles were collected at 32 locations in the central-northern region of Taiwan between August 2002 and December 2003. A northern (sampling locations N1–N17) and a southern (sampling locations S1–S15) transect were established (Fig. 1, Table 1). Each location was searched for tadpoles in ravine streams. Between seven and 12 individuals were randomly sampled at each location and preserved in 70% ethanol.

Morphometric measurements

We determined the developmental stage of each tadpole collected according to the stages defined by Gosner (1960). All tadpoles were between Gosner stages 27 and 33 with completely developed oral parts; subsequent tadpole development does not affect LTR (Bonacci et al., 2008). A total of 17 morphometric traits were examined. The three traits based on LTR were the number of continuous tooth rows on upper labium (CTR), the number of interrupted tooth rows on the upper labium (ITR) and the number of tooth rows on the lower labium (TRL). Tadpoles were divided into groups I, II and III, representing *R. sauteri*, hybrids and *R. multidenticulata*, respectively (following Chou & Lin, 1997a,b).

Other morphometric traits measured were snout–vent length (SVL), maximum body height (BH), body width at spiracle (BW), snout–nostril distance (SND), snout–spiracle distance (SSD), eye–nostril distance (END), eyeball diameter (EBD), internostrial distance (IND), interorbital distance (IOD), mouth width (MW), tail length (TL), maximum tail height (TH), maximum caudal muscle height (CMH) and maximum caudal muscle width (CMW) (Chou & Lin, 1997a; Altig & McDiarmid, 1999; Grosjean, 2005). SND, END, EBD, IND and IOD were measured with a graduated ocular micrometer under a stereo-microscope, and the other traits were measured with a digital caliper (Mitutoyo: 500–196) to the nearest 0.01 mm.

DNA extraction and sequencing

Genomic DNA was isolated from tail tissue using the phenol-chloroform method (Hoelzel, 1992) and QIAGEN DNeasy extraction kits (QIAGEN Inc.). We sequenced a region of mtDNA including the cytochrome *b* gene (cyt*b*), using primers L14850 (5'-TCTCA TCCTG ATGAA ACTTT GGCTC-3') and H15502 (5'-GGATT AGCTG GTGTG AAATT GTCTG GG-3') (Tanaka-Ueno et al., 1998; Goebel et al., 1999). Amplification was performed in accordance with the following protocol: a hot start at 94 °C for 2 min, followed by 36 cycles at 94 °C for 40 sec, 55 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 10 min. PCR products were purified by Gel-M Gel Extraction Kit (Viogene) and directly sequenced with an ABI3100 automated sequencer (Perkin Elmer Inc.). Sequences were edited with BIOEDIT 5.0.9 (Hall, 1999), aligned with CLUSTAL_X 1.7 (Thompson et al., 1997) and confirmed to be 596 bp in total length from both strands. Unique mtDNA cyt*b* sequences used in this study were deposited at GenBank (accession nos. HM989036–989366).

Genetic traits and phylogenetic analysis

Haplotype diversity (H_d) and nucleotide diversity (π) (Nei, 1987) were calculated with DNASP 3.53 (Rozas & Rozas, 1999). The number of polymorphic sites and the mean number of pairwise differences among the cyt*b* sequences were also estimated. In order to test for deviation from neutrality in the sequence data, Tajima's *D* (Tajima, 1989) was calculated and compared to a beta distribution for significance testing.

Maximum parsimony (MP), neighbour-joining (NJ) and Bayesian methods were employed for phylogenetic reconstruction using *R. longicrus* (accession no. HM989035) as outgroup. The MP tree reconstruction was performed in PAUP 4.0 (Swofford, 2001) with gaps treated as missing data, equal weight for transitions and transversions, heuristic search with tree-bisection-reconnection (TBR) branch swapping and 10 random addition replications. MEGA 4 (Tamura et al., 2007) was used

Table 1. Sampling locations, elevations, sample sizes (*n*), and numbers of labial tooth rows (CTR, continuous tooth rows on upper labium; ITR, interrupted tooth rows on upper labium; TRL, tooth rows on lower labium; sample sizes in parentheses) of the three labial tooth row (LTR) groups (I, II and III) of tadpoles of the *R. sauteri* complex collected from 32 locations from the northern transect (N) and the southern transect (S) on the western slope (W) and the eastern slope (E) of the central mountain range of Taiwan.

Code	Location	Latitude	Longitude	Elevation (m)	*n*	CTR	ITR	TRL	LTR groups	Slopes
Northern transect										
N1	Huangjhukeng	24°04'47"	120°45'22"	180	10	2(10)	3(10)	4(10)	I	W
N2	Dakeng	24°10'44"	120°46'27"	250	8	2(8)	3(8)	4(8)	I	W
N3	Dawanjiao	24°06'33"	120°46'51"	250	11	2(11)	3(11)	4(8),5(3)	I	W
N4	Shangping	24°07'04"	120°53'54"	570	10	2(10)	3(10)	4(10)	I	W
N5	Meiyuan	24°19'18"	120°53'19"	750	8	3(8)	3(7),4(1)	5(3),6(3),7(2)	II	W
N6	Heping	24°17'08"	120°54'11"	560	9	2(7),3(2)	3(2),4(7)	4(1),5(8)	II	W
N7	Wushihkeng	24°16'47"	120°56'23"	700	8	2(4),3(4)	3(3),4(5)	4(1),5(2),6(5)	II	W
N8	Jiabaotai	24°11'34"	121°00'51"	570	11	2(1),3(10)	3(10),4(1)	4(1),5(10)	II	W
N9	Guanwu	24°30'06"	121°05'02"	1900	7	3(7)	4(7)	7(7)	III	W
N10	Jiayang	24°15'31"	121°12'42"	1450	9	3(9)	4(9)	6(2),7(3),8(4)	III	W
N11	Sihyuanyakou	24°23'44"	121°21'08"	2000	12	3(12)	4(12)	6(4),7(5),8(3)	III	W
N12	Nanshan	24°27'49"	121°23'32"	840	11	3(8),4(3)	3(2),4(9)	7(9),8(2)	III	E
N13	Renze	24°32'46"	121°30'23"	650	12	3(12)	4(11),5(1)	7(2),8(10)	III	E
N14	Taipingshan	24°29'25"	121°32'06"	1850	12	3(12)	4(12)	7(11),8(1)	III	E
N15	Datong	24°40'51"	121°36'25"	200	11	3(11)	3(1),4(10)	7(11)	III	E
N16	Shuanglianbi	24°45'16"	121°37'20"	410	12	3(12)	3(1),4(11)	6(2),7(9),8(1)	III	E
N17	Gulu	24°33'39"	121°40'10"	1000	12	3(12)	4(12)	7(6),8(6)	III	E
Southern transect										
S1	Guanzihling	23°20'37"	120°30'56"	240	11	2(11)	3(11)	4(11)	I	W
S2	Chukou	23°26'22"	120°36'22"	260	12	2(12)	3(12)	4(12)	I	W
S3	Niaopu	23°19'46"	120°36'44"	270	10	2(10)	3(10)	4(10)	I	W
S4	Shanmei	23°22'07"	120°40'17"	380	12	2(12)	3(12)	4(12)	I	W
S5	Shuangsi	23°32'07"	120°36'51"	350	9	2(9)	3(8),4(1)	4(2),5(7)	II	W
S6	Caoling	23°35'05"	120°42'11"	550	11	2(11)	3(11)	4(3),5(8)	II	W
S7	Dabang	23°27'04"	120°44'22"	790	12	2(12)	3(7),4(5)	4(6),5(6)	II	W
S8	Fongshan	23°34'30"	120°45'08"	700	9	2(8),3(1)	3(9)	5(8),6(1)	II	W
S9	Sitou	23°40'10"	120°47'50"	1150	12	2(12)	3(12)	5(11),6(1)	II	W
S10	Dili	23°46'55"	120°57'12"	450	12	2(10),3(2)	3(2),4(10)	4(9),5(3)	II	W
S11	Alishan	23°31'01"	120°48'36"	2150	8	3(8)	4(8)	7(2),8(6)	III	W
S12	Zihjhong	23°29'57"	120°48'59"	2300	10	3(10)	3(1),4(9)	6(3),7(5),8(2)	III	W
S13	Haitiansih	23°45'24"	121°10'13"	2250	10	3(9),4(1)	3(1),4(9)	5(4),6(3),7(3)	III	W
S14	Muguasi	24°02'53"	121°21'04"	1450	10	3(10)	4(10)	7(3),8(7)	III	E
S15	Wanrong	23°43'17"	121°21'31"	550	10	3(9),4(1)	3(1),4(9)	7(2),8(8)	III	E

to produce an NJ tree. Confidence of phylogenetic relationships was assessed by 1000 nonparametric bootstrap replications. The appropriate DNA substitution model for the Bayesian phylogenetic analysis was obtained by testing alternative models of evolution using Modeltest version 3.7 (Posada & Crandall, 1998). MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003) was used to perform a partition-likelihood Bayesian search. Two simultaneous Metropolis-coupled Markov Chain Monte Carlo analyses were run, each with four chains for

Table 2. Numbers of labial tooth rows of three labial tooth row (LTR) groups (I, II and III) of the *R. sauteri* complex (*n*, number of tadpoles; CTR, continuous tooth row on upper labium; ITR, interrupted tooth row on upper labium; TRL, tooth rows on lower labium; TTR, total tooth rows = CTR + ITR + TRL).

LTR		CTR			ITR			TRL			TTR		
groups	*n*	Rows	*n*	%	Rows	*n*	%	Rows	*n*	%	Rows	*n*	%
I	84	2	84	100.0	3	84	100.0	4	81	96.4	9	81	96.4
								5	3	3.6	10	3	3.6
II	101	2	74	73.3	3	71	70.3	4	23	22.8	9	8	7.9
		3	27	26.7	4	30	29.7	5	66	65.3	10	52	51.5
								6	10	9.9	11	31	30.7
								7	2	2.0	12	6	5.9
											13	3	3.0
											14	1	1.0
III	146	2	0	0.0	3	7	4.8	4	0	0.0	12	5	3.4
		3	141	96.6	4	138	94.5	5	4	2.7	13	15	10.3
		4	5	3.4	5	1	0.7	6	14	9.6	14	75	51.4
								7	78	53.4	15	50	34.2
								8	50	34.2	16	1	0.7

40,000,000 generations, sampling trees every 1000 generations. We discarded the first 10,000,000 generations (10,000 trees) on each run as "burn-in" after confirming chain stationarity from plots of likelihood against generation. The remaining trees were used to estimate posterior nodal probabilities and a summary of the phylogeny.

Statistical analysis

We performed a discriminant function analysis (DFA) with STATISTICA version 7.0 to differentiate between the three LTR groups based on *ln*-transformed morphometric variables to address the assumption of normality (Kolmogorov–Smimov test, $P>0.05$). The three LTR traits were discrete and did not follow a normal distribution, and therefore were excluded from the DFA analysis. We conducted an analysis of covariance (ANCOVA) using SVL as covariate and the location's mean of each of the other 13 morphometric traits to identify differences among three LTR groups and between two transects. Spearman rank correlation (r_s) analyses were conducted between total tooth rows (TTR = CTR + ITR + TRL) and morphometric traits.

Geographic patterns of genetic differentiation were evaluated by analysis of molecular variance (AMOVA, Excoffier et al., 1992). This assessed the extent to which genetic variation was attributable to three hierarchical levels of subdivision: among regions (groups of locations), among locations within regions and within locations. AMOVA was first performed on the two geographical transects treated as regions, and using the three LTR groups as regions. The statistical significance of fixation indices (Φ_{CT}, Φ_{SC} and Φ_{ST}) was tested by ARLEQUIN 2.001 in nonparametric permutation with 10,000 permutations against the null hypothesis that all individuals belonged to the same population (Schneider et al., 2001).

Mantel tests were used to determine the relationships between TTR, the other morphometric traits, the genetic data and the geographical distances between sampling locations. The statistical significance of the relationships was based on 10,000 random permutations implemented in PASSAGE (Rosenberg, 2001). TTR distances were calculated as Euclidean, pairwise distances in TTR among the 32 locations. The other morphometric distances, except SVL, were calculated as Mahalanobis distances (Kolbe et al., 2007). Pairwise genetic distances were cal-

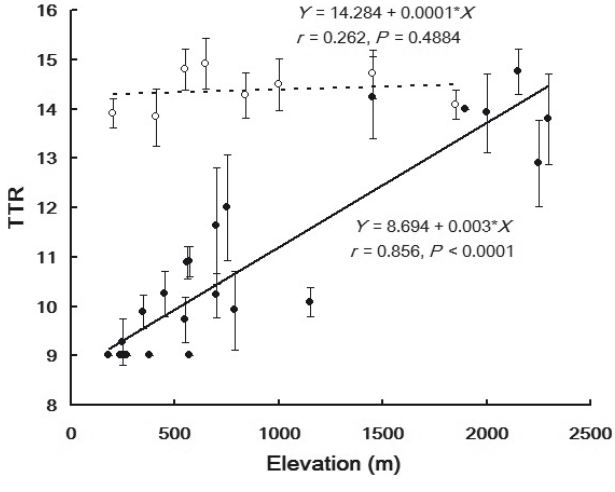

Fig. 2. Relationships between elevations and total labium tooth rows (TTR; location's mean ± SD) of the *R. sauteri* complex on the western slope (solid circles and solid line) and the eastern slope (open circles and dashed line) of central mountain range in Taiwan.

Table 3. Results of ANCOVA with snout–vent length (SVL) as a covariate for testing differences of location's mean of 13 other morphometric traits among three labial tooth row (LTR) groups and between two geographic transects for tadpoles of the *R. sauteri* complex (*n*=32).

	Three LTR groups			Two transects		
Variables	R^2-values	*F*-values	*P*-values	R^2-values	*F*-values	*P*-values
Body height	0.81	2.93	0.070	0.78	1.04	0.317
Body width	0.90	1.17	0.324	0.90	3.65	0.066
Snout–nostril distance	0.79	25.26	<0.001	0.45	1.77	0.193
Snout–spiracle distance	0.95	19.47	<0.001	0.45	6.36	0.017
Eye–nostril distance	0.76	0.01	0.997	0.77	0.43	0.519
Eyeball diameter	0.45	5.91	0.007	0.27	2.20	0.149
Internostrial distances	0.88	0.97	0.391	0.87	0.03	0.862
Interorbital distance	0.91	9.36	0.001	0.85	0.01	0.917
Mouth width	0.78	16.33	<0.001	0.63	8.41	0.007
Tail length	0.91	0.26	0.774	0.93	4.84	0.036
Tail height	0.75	6.03	0.007	0.65	0.75	0.394
Caudal muscle height	0.85	4.01	0.029	0.81	0.40	0.531
Caudal muscle width	0.81	2.63	0.090	0.77	0.01	0.914

culated as Slatkin's linearized F_{ST} represented by $F_{ST}/(1 - F_{ST})$ among the locations using ARLEQUIN 2.001. Geographical distances (latitudes and longitudes) among locations were calculated using the online distance calculator (http://vldb.gsi.go.jp/sokuchi/surveycalc/bl2stf.html).

RESULTS

LTR traits

Based on LTR traits, individuals of the *R. sauteri* complex were divided into three groups: group I with 2 CTR, 3 ITR and 4 (mode ranges, 4–5) TRL; group II with 2 (2–3) CTR, 3 (3–4) ITR and 5 (4–7) TRL and group III with 3 (3–4) CTR, 4 (3–5) ITR and 7 (5–8) TRL (Table 2). The number of LTR increased longitudinally (eastward) from group I to group III with the increase in elevation on the western slope of the central mountain range, but not on the eastern slope (Figs 1, 2; Table 1).

Morphometric variation

There was a significant difference in SND, SSD, EBD, IOD, MW, TH and CMH (significant slope heterogeneity in TH and CMH) among all LTR groups; significant differences in SSD, MW and TL were also found between southern and northern transects (ANCOVA, $P<0.05$, Table 3).The positive correlation of TTR with MW, SND and SSD suggested that the number of labial tooth rows is associated with the size of the oral disc, related to MW and snout size represented by SND and SSD (Fig. 3). The regression coefficient of 1.92 (Fig. 3c) indicates that TTR is related to the square of MW, proportional to the size of the oral disc.

The DFA yielded two significant canonical variables. The first was strongly influenced by SND and MW and explained 87.8% of the total variance. The plot of the first two axes showed a remarkable separation of the mor-

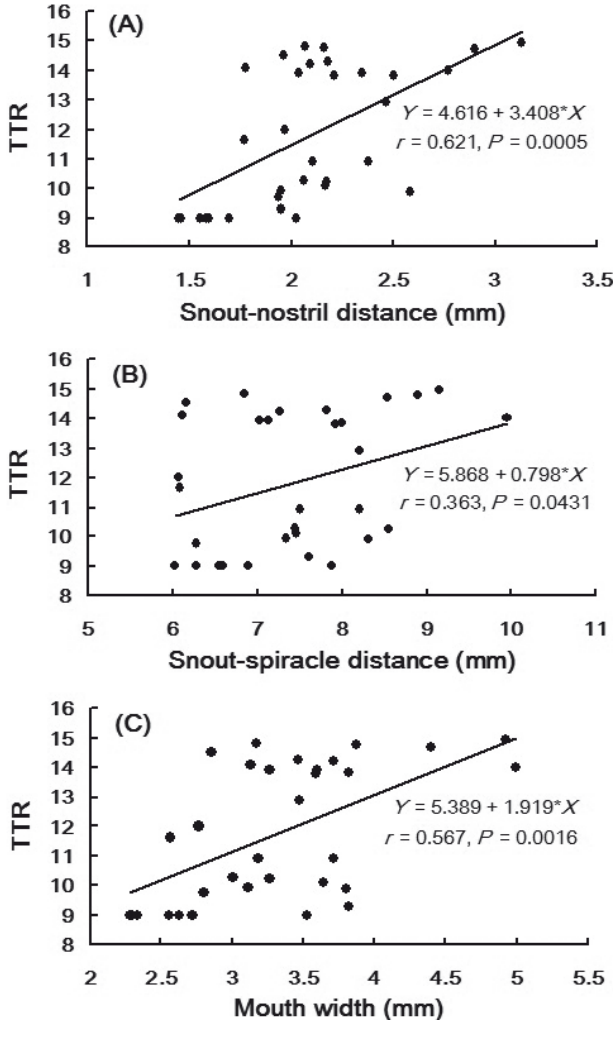

Fig. 3. Relationships of total tooth rows (TTR) to snout–nostril distances (A), snout–spiracle distances (B) and mouth widths (C) of tadpoles of the *R. sauteri* complex.

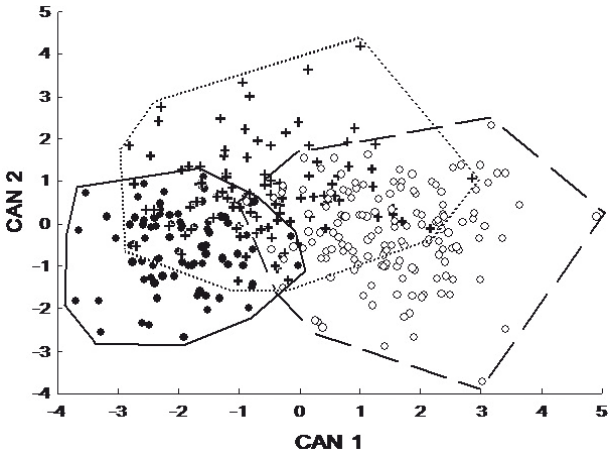

Fig. 4. Canonical plot between the first two canonical scores for 14 morphometric traits of tadpoles of the *R. sauteri* complex (solid circles, labial tooth rows (LTR) group I; crosses, group II; open circles, group III).

phometric traits between groups I and III, while group II overlapped widely with groups I and III (Fig. 4).

Haplotype variation

A total of 76 unique haplotypes were identified from the mtDNA cyt*b* sequences of 331 tadpoles; 58 were restricted to a single location, and 47 to a single tadpole. There were 79 polymorphic sites, 53 of which were parsimony informative and had no length variation. The H_d-values among locations ranged between 0 and 0.924, and the π-values ranged between 0 and 0.0167. Global H_d-values were 0.935±0.007 (mean±SD), and global π-values were 0.01287±0.00076. The neutral expectation of the *D* values was not rejected (Tajima's test, D= –1.169, P>0.1), suggesting that the cyt*b* gene evolved under neutral expectations.

Forty-seven haplotypes were more abundant in the northern transect, and 32 haplotypes were more abundant in the southern transect; H_d -values were similar in the two transects. The π-values and mean numbers of pairwise differences (k) were two-fold higher for the southern transect than for the northern transect, indicating a higher divergence in haplotypes in the former than the latter. For three LTR groups, the number of haplotypes (H) was highest for Group III, while the π and k-values were highest for Group II (Table 4).

Phylogeny

The average pairwise sequence divergences among haplotypes was 0.0165±0135, with a range between 0.0017 and 0.0503. The MP analysis yielded a single most parsimonious tree with a length of 248, a consistency index of 0.59 and a retention index of 0.74. The NJ tree and Bayesian 50% majority-rule consensus tree had a topology identical with the MP tree.

For the MP tree (Fig. 5), there were three major lineages (A, B, C) with a stepwise differentiation. Lineage A represents the clade with the largest number of haplotypes (H1–H61) divided into three sub-lineages (Aa, Ab and Ac); each contained haplotypes from three LTR groups. Sub-lineage Aa encompassed 48 haplotypes (H1–H48) from 167 tadpoles of groups I, II and III in the northern transect, and 19 tadpoles of groups II and III in the southern transect. All tadpoles of the sub-lineages Ab and Ac were from groups I, II and III of the southern transect, and consisted of 6 haplotypes from 20 tadpoles and 11 haplotypes from 90 tadpoles, respectively (Table 4). Lineage B had 4 haplotypes with 20 tadpoles from group III and lineage C had 8 haplotypes with 15 tadpoles from groups I and II. Evidently, the three LTR groups were nested together phylogenetically.

Table 4. Genetic variations of mtDNA cytb sequences of 331 tadpoles of the *R. sauteri* complex in two geographical transects and three labial tooth row (LTR) groups (*n*, sample size of tadpoles; H, number of total haplotypes; S, number of polymorphic sites; k, mean number of pairwise differences; Hd, haplotype diversity; π, nucleotide diversity; number of haplotypes and number of tadpoles (in parentheses) in haplotype groups Aa, Ab, Ac, B and C corresponding to those denoting lineages in the phylogenetic tree (Fig. 5).

							Haplotype groups				
Partitions	*n*	H	S	K	H_d	π (×100)	Aa	Ab	Ac	B	C
Transects											
Northern	173	47	57	3.581	0.891	0.601	43(167)				4(6)
Southern	158	32	60	8.193	0.894	1.375	7(19)	6(20)	11(90)	4(20)	4(9)
LTR groups											
Group I	84	19	41	5.041	0.902	0.846	8(38)	1(1)	9(44)		1(1)
Group II	101	25	46	8.826	0.866	1.481	11(40)	2(3)	5(44)		7(14)
Group III	146	48	52	6.827	0.910	1.145	38(108)	4(16)	2(2)	4(20)	
Total	331	76	79	7.669	0.935	1.287	48(186)	6(20)	11(90)	4(20)	7(15)

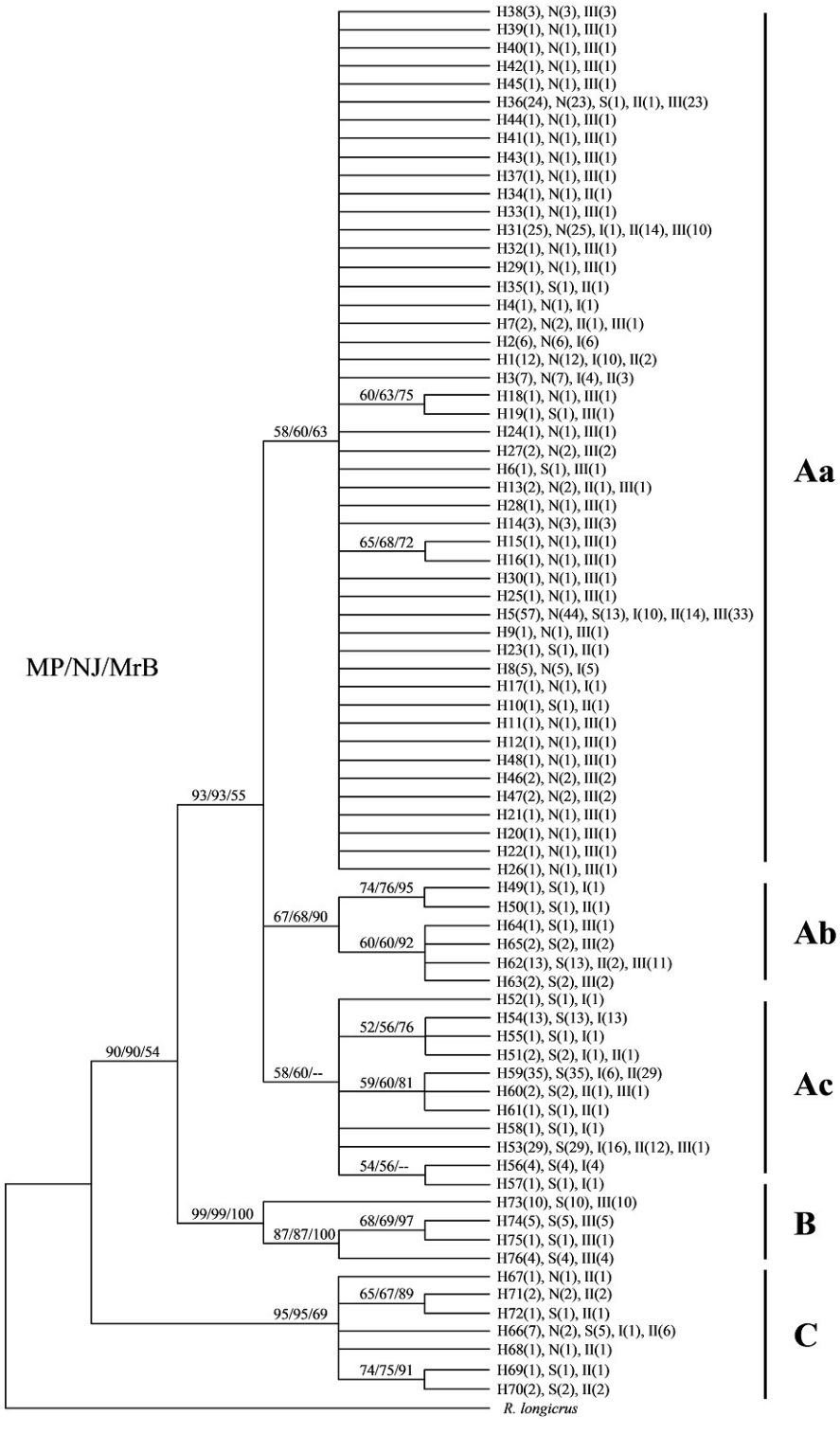

Fig. 5. A phylogenetic tree of the *R. sauteri* complex reconstructed from the mtDNA cytb sequences with *R. longicrus* as outgroup, using maximum parsimony (MP) method (numbers above branches, bootstrap values (>50%) supported by MP/NJ/Bayesian posterior probabilities; H1–H76, haplotypes; Aa, Ab, Ac, B, and C, haplotype groups (clades) of lineages or sublineages; N, northern transect; S, southern transect; I, II, III, labial tooth row groups I, II, and III; number of tadpoles in parentheses).

Genetic differentiation

The AMOVA results are shown in Table 5. There was a significant difference in genetic variances at each of the three hierarchical levels (between transects, among locations within transects and within locations). The largest amount of genetic variance (55.3%) was explainable by differences among locations within transects. There was non-significant difference in the genetic variances among

Table 5. Analysis of molecular variances (AMOVA) of mtDNA cytb sequences of the *R. sauteri* complex for two transects (northern and southern) and three labial tooth row (LTR) groups (I, II, and III).

Structure	Source of variance	d.f.	Variance (%)	Fixation index	*P*-value
Two transects	Between transects	1	25.85	Φ_{CT}=0.259	0.0001
	Among locations/within transects	30	55.3	Φ_{SC}=0.746	<0.0001
	Within locations	299	18.84	Φ_{ST}=0.812	<0.0001
Three LTR groups	Among groups	2	7.67	Φ_{CT}=0.077	0.0673
	Among locations/within groups	29	71.33	Φ_{SC}=0.773	<0.0001
	Within locations	299	20.99	Φ_{ST}=0.790	<0.0001

three LTR groups, but significant difference among locations within groups and within locations. In other words, the genetic differentiation was significant for tadpoles between the two transects but not among the three LTR groups.

Relationships among genetic, morphometric and geographical variations

Table 6 shows the results of Mantel tests for correlation among pairwise TTR distances, morphometric distances and genetic as well as geographic distances. The pairwise genetic distances were significantly correlated with geographic distances but not with TTR and other morphometric distances. There were significant positive correlations among geographic distances, TTR distances and other morphometric distances. Pairwise F_{ST} values among locations were 0.564±0.322, suggesting high genetic differentiation among geographic populations.

DISCUSSION

Discordance of genetic and morphometric traits

LTRs of tadpoles of the *R. sauteri* complex showed a longitudinal cline in both northern and southern transects (Figs 1, 2; Table 1), but such a cline was not observed for cyt*b* haplotypes. Instead, genetic differentiation differed significantly between the two transects but not among the three LTR groups. In addition, genetic distances were significantly correlated with geographical distances but not LTR or other morphometric distances. Such discordance between genetic and phenotypic differentiations has also been detected in, for example, newts (Babik et al., 2005), frogs (Richards & Knowles, 2007), lizards (Leaché & Cole, 2007; Kolbe et al., 2007) and birds (Johnsen et al., 2006).

Adaptive plastic phenotypes

Differences in oral structure of tadpoles among geographical populations have been attributed to differences in phylogeny, feeding habits and dietary specializations (Bonacci et al., 2008). *Rana dalmatina* shows fewer upper labial tooth rows in smaller ponds, because metamorphosis is induced before attaining full development of oral structures (Bonacci et al., 2008). Tadpoles of the *R. sauteri* complex that have a shorter tadpole period at low elevations (Lai et al., 2002) may develop fewer tooth rows than those that have a longer tadpole period. Larger oral discs have more tooth rows (McDiarmid & Altig, 2010) and the length of labial tooth rows is related to the microhabitat (Altig & Johnston, 1989).

Individuals of the *R. sauteri* complex breed in streams of low current and low elevations during the dry season (autumn and winter), and at high elevations in strong current during the rainy season (spring and summer; Lai et al., 2003). In eastern Taiwan, due to steep mountain slopes with northeast monsoon rainfalls, streams are on average characterized by faster currents than in western Taiwan over the entire year. Substrates in the streams were fairly homogeneous with mixtures of rubble and pebbles at low elevations, while they were highly variable with more large boulders at the high elevations and in eastern Taiwan. The longitudinal cline of LTR was found to correspond with longitudinal changes in stream current and substrate condition. LTR traits are known to be associated

Table 6. Results of Mantel tests for correlations among genetic distances, geographical distances, total tooth row distances, and other morphometric distances (*r*, Mantel correlation coefficient; italic, *P*<0.05). *ln (km).

Matrix 1	Matrix 2	*r*-values	*P*-values
Genetic distance	Geographical distance*	0.119	*0.0118*
Genetic distance	Total tooth row distance	0.068	0.0614
Genetic distance	Other morphometric distance	0.124	0.0965
Geographical distance*	Total tooth row distance	0.401	*<0.0001*
Geographical distance*	Other morphometric distance	0.262	*0.0001*
Total tooth row distance	Other morphometric distance	0.402	*<0.0001*

with the size of the oral disc, which is important for feeding in lotic habitats (Altig & Johnston, 1989).

Based on our results it is reasonable to consider that LTRs of tadpoles are a consequence of phenotypic plasticity. It increases with enlargement of the oral disc corresponding with elevation and monsoon rainfalls. The increase in LTR and size of the oral disk enables the tadpoles to grasp and graze on substrates in fast-flowing streams (Richards, 2002).

Distribution and differentiation

The *R. sauteri* complex is one of the most common and widespread riverine frogs in Taiwan. It forms breeding colonies of up to several thousand individuals. It is common at all elevations in western Taiwan, but rare and sporadic in the east (Lue et al., 1990). The higher H_d -value in the southern transect than in the northern one suggests that populations in the southern transect had a longer evolutionary history of local differentiation (Avise, 2000; Chen et al., 2006). They also had high F_{ST} values and a large proportion of private haplotypes, suggesting that they were more restricted geographically, shaping their genetic differentiation. Mountains might act as barriers for gene flow among geographical populations (Toda et al., 1998; Huang et al., 2002). Amphibians also exhibit a wider population variation than other major terrestrial animal taxa (Beebee, 2005), due to lower gene flow and high genetic differentiation among geographical populations resulting from site fidelity and limited dispersal. However, current ecology, demography and the historical pattern of both vicariance and dispersal may act in concert to produce a very complex population structure (Avise, 1994). The weak positive correlation between genetic and geographical distance probably resulted from the interplay between modern and vicariant forces that restrict contemporary gene flow and isolate populations (e.g. Bossart & Prowell, 1998). Pleistocene glacial refugia have been proposed for some high-elevation species of animals in Taiwan (Hsu et al., 2001; Yuan et al., 2006). A rapid colonization from multiple intermountain refugia after the last glaciation might result in the present mosaic geographic distribution of genotypes in the *R. sauteri* complex.

Taxonomic status

The structure, arrangement and configuration of mouthparts of tadpoles are regularly used as diagnostic traits (Haas, 2003; Altig, 2006; Bonacci et al., 2008), and the LTR formula has been considered to be species-specific for the genus *Rana* (Vences et al., 2002). The formula was used by Chou & Lin (1997a; 1997b) to distinguish the *R. sauteri* complex into two species. The results of this study, however, suggest that LTR represents a phenotype associated with size differences in the oral disc determined by environmental factors. Therefore, it is an unsuitable trait for species delimitation. Phylogenetically, *R. sauteri* and *R. multidenticulata* were nested together with their putative hybrids without representing monophyletic lineages. Accordingly, we regard *Rana sauteri* Boulenger, 1909 as the sole representative species of the *R. sauteri* complex, and *Rana multidenticulata* Chou and Lin, 1997 as its synonym.

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