

Geographical Variation within the Species *Ratufa bicolor* (Sparrman, 1778) Inferred from External and Cranial Morphology (Sciuridae: Ratufinae) in Thailand

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ABSTRACT:

The giant black squirrel, *Ratufa bicolor* is distributed widely from the south to the north of Thailand. It is easily distinguished from other squirrels owing to its larger size. In this study, the northern population was delimited above the Isthmus of Kra and the southern population below the Isthmus of Kra following the biogeographic transition zone of Hughes *et al.*, 2011. Four external and twenty-four cranial characteristics were studied to determine the geographical variation of this species. Mean measurements suggest that the northern population is larger. In addition, eleven skull characteristics show significant differences between the northern and southern populations. Principal component analysis also identifies two distinct populations.

KEY WORDS: Bergmann's rule, delimitation, Isthmus of Kra, sexual dimorphism.

INTRODUCTION

The family Sciuridae (Squirrels) is cosmopolitan in distribution. They are found throughout the world and are only absent from Madagascar, Australia, New Zealand and the north and south poles. They belong to the order Rodentia.

There are eight non-flying genera comprising twenty-eight species in Southeast Asia. Seventeen species are found in Thailand (Francis, 2008; Thorington *et al.*, 2012) but only nine squirrel species are found on both sides of the Isthmus of Kra including

flying squirrels. The Isthmus of Kra is considered to function as a biogeographical barrier for squirrels (Lekagul & McNeely, 1988; Corbet & Hill, 1992; Hayashida *et al.*, 2006). *Ratufa bicolor* is one of the two extant species of the genus *Ratufa* Gray, 1867 (Ratufinae Moore, 1959) in Thailand. In Asia, it is found in east Nepal through, NE India, Bhutan, Bangladesh, Myanmar, South China (Yunnan, Hainan), Vietnam, Laos, Thailand, Malaysia, Singapore and Indonesia (Lekagul & McNeely, 1988; Corbet & Hill, 1992; Francis, 2008; IUCN, 2012; Thorington *et al.*, 2012). It occurs from coastal plains

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up to 1500 m above sea level (Duckworth *et al.*, 1999). Eleven subspecies are currently recognized from Asia. Probably four races (*Ratufa bicolor angusticeps* Miller, 1901; *R. b. leucogenys* Kloss, 1916; *R. b. melanopepla* Miller, 1900 & *R. b. phaeopepla* Miller, 1913) are widely distributed in Thailand (Moore & Tate, 1965; Thorington *et al.*, 2012). They all have two distinct colours in general: black and white. It is a diurnal and arboreal species that rarely descends to the ground (MacKinnon, 1978; Francis, 2008). Due to habitat fragmentation and excessive hunting by humans in addition to the threat from natural predators, this species is in significant decline (IUCN, 2012). Very little is known about their taxonomic delimitation and as it is expected to be a species complex (IUCN, 2012), further study of the taxonomic status of current populations is recommended by Timmins *et al.* (2008).

MATERIALS AND METHODS

Study Area

This study is limited to specimens from the Natural History Museum, Pathum Thani and to the species of Thai non-flying squirrel (Ratufinae; *Ratufa bicolor*). A total of 20 voucher specimens were studied ($n = 10 \text{ ♂♂}$, 10 ♀♀). The population of *Ratufa bicolor* in Thailand was divided into northern and southern populations delineating at the Isthmus of Kra

(IOK; (11°N - 12°N [Hughes *et al.*, 2011]) to examine the geographical variation within the species (Fig. 1).

Morphometric measurements

Fifteen northern specimens and five southern specimens (see the appendix) were available for morphometric analysis. Four external measurements were carefully noted from the labels on the museum tags and twenty-four cranial characteristics were measured using a digital caliper. In doing so, Lunde & Son (2001) & Hayashida *et al.* (2006) were followed wherever relevant. Sexual dimorphism was tested only for the northern population as the number of specimens from the south was too small to draw any meaningful conclusions ($n = 1 \text{ ♂}$, 4 ♀♀). Illustrated below (Fig. 2) are the dimensions of the skull measurements taken.

Data analysis

A non-parametric Mann Whitney U-test was run at a confidence level of 95% to test the significant differences between the northern and southern populations using SPSS version 16.0 for Windows. The same method was applied to test the sexual dimorphism in this species. Principal component analysis (PCA) was also performed to identify the northern and southern populations using PCORD 5 in combination with Microsoft Excel statistics 2007 to clarify the geographical variations.

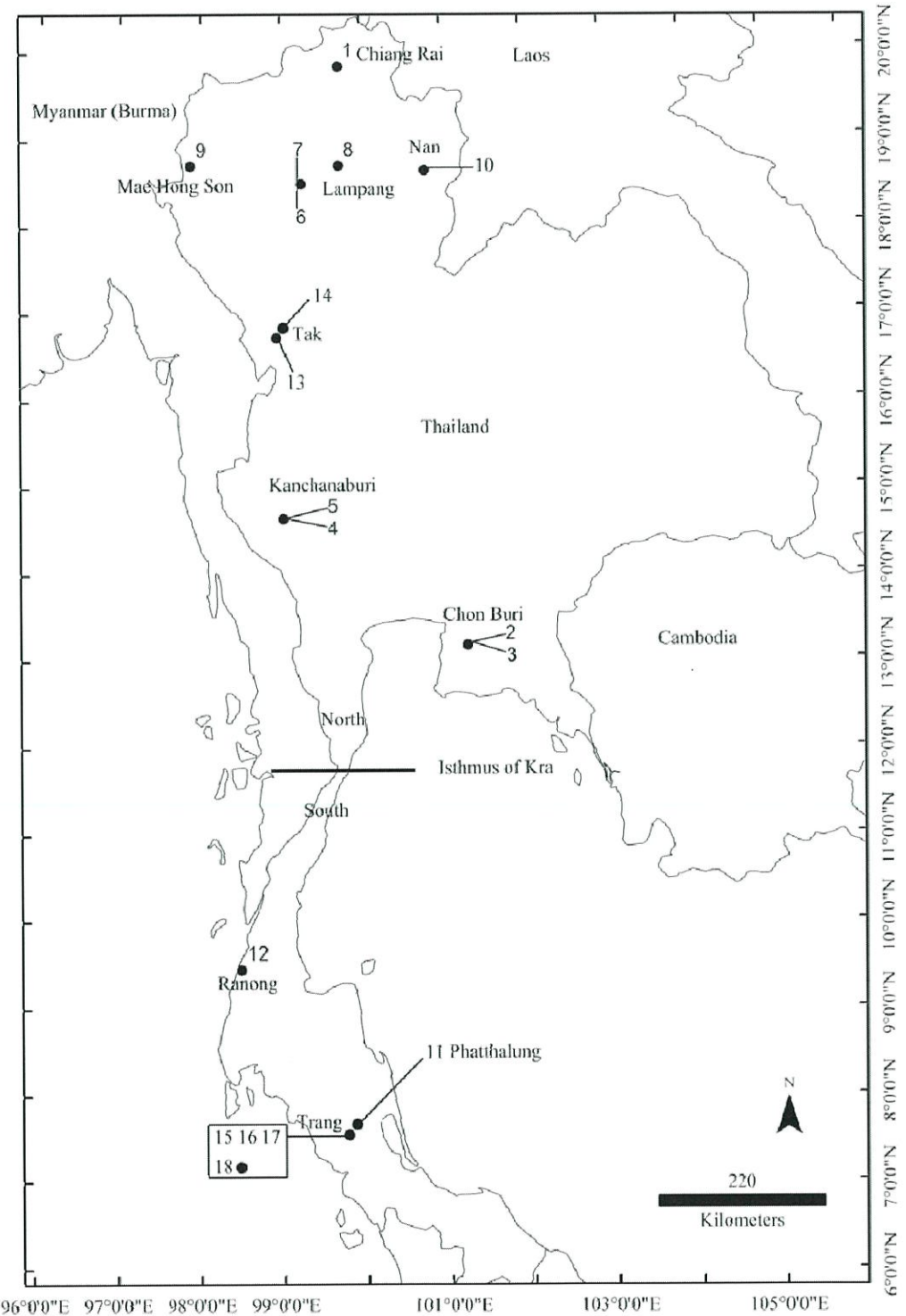


Figure 1. Localities of the specimens of *Ratufa bicolor* from Thailand in the Natural History Museum (THNHM), Pathum Thani. Each number indicates the origin of the voucher specimen.

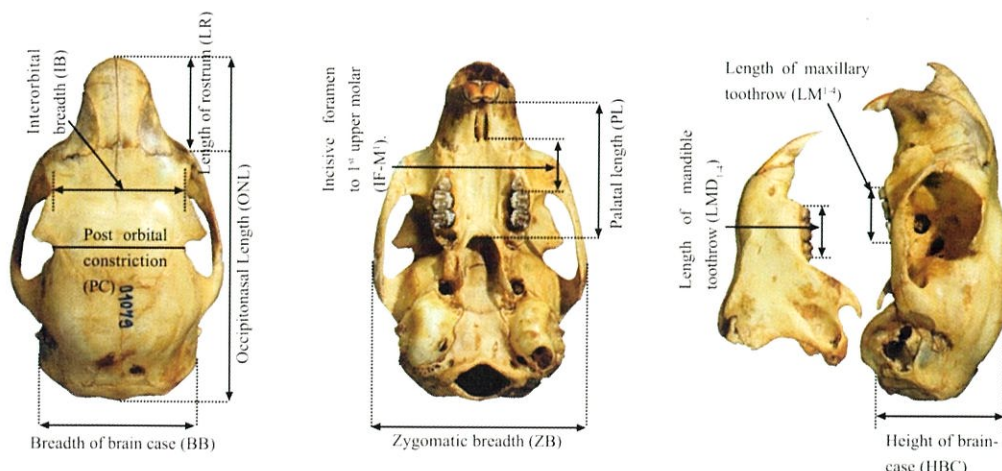


Figure 2. Skulls of *Ratufa bicolor* (THNHM-1079, ♀). The dimensions of the skull measurements. Photo Courtesy (Rassamee & Rattanathavorn, 2012). Note: photos are not to scale.

RESULTS

Sexual Dimorphism

The Mann Whitney U-test performed to determine sexual dimorphism confirmed significant differences between males and females in two skull characteristics: LN ($z = -2.357$, p -value = 0.018), LB ($z = -2.0003$, p -value = 0.045). All these structures were significantly larger in females (♀: LN = 25.77, ± 1.8 , LB = 16.93, ± 0.8 ; ♂: LN = 23.63, ± 1.2 , LB = 16.00, ± 0.9).

Geographic Variation

The skulls are large and broad with depressed frontals (Fig. 3A, 3C). Unlike other genera, this genus does not have a third upper molar ($I = 1/1$, $C = 0/0$ PM = $1/1$ M = $3/3$ = 20). The cusps on the teeth are highly crenulated and individual cusps are scarcely differentiated. It can be

easily distinguished from other similar patterned species by its larger size. However, on careful examination, cusps: paracone, meta-cone, hypocone and protocone can be identified on the upper cheek teeth (Fig. 3B) and also the shallow talonid basin on the lower cheekteeth (Fig. 3E).

Of the four external characteristics and twenty-four skull characteristics tested statistically, eleven skull characteristics (Table 2) were scanty different in the northern and southern populations of *Ratufa bicolor* in Thailand. The skulls of the northern species were significantly larger than the southern species. The significant characteristics were ONL, IB, ZB, LR, BB, HBC, IF-M¹, PL, LMD₁₋₄, LM¹⁻⁴ and PC (Fig. 2). The external characteristics did not show any significant difference statistically, but the mean measurements suggest that, the size of the northern population is

larger (Table 1). Also the principal component analysis performed on the correlation matrix for multivariate

comparisons identified two discrete populations (Table 3 & Fig. 4).

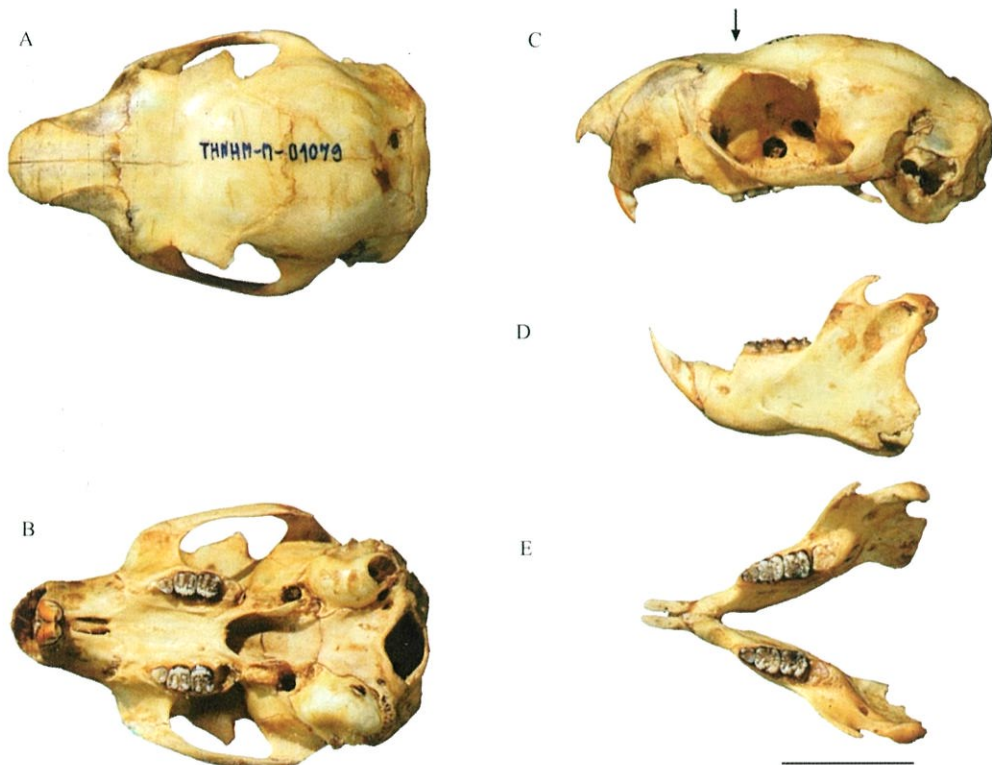


Figure 3. Representative skulls of *Ratufa bicolor* (THNHM-1079, ♀). **A** = Dorsal view, **B** = Ventral view, **C** = Lateral view of maxillary, **D** = Lateral view of mandible, **E** = Occlusal view of mandible. Scale = 20 mm.

Table 1. External measurements (Mean & Standard deviation in mm) of *Ratufa bicolor*. n = Sample size; N=Northern population; S = Southern population HB = Head body length; T = Tail length; E = Ear length; HF = Hind foot length (claws excluded); Sex. dim = Sexual dimorphism; ns = not significant.

Species	n	N/S	HB	T	E	HF
<i>R. bicolor</i>	15	N	387.6, 50.17	475.3, 36.81	30.61, 13.71	81.65, 7.17
Sex. dim			ns	ns	ns	ns
<i>R. bicolor</i>	5	S	373.2, 28.78	462, 43.81	30.6, 6.38	73, 8.36
Sex. dim			ns	ns	ns	ns

Table 2. Cranial measurements (Mean & Standard deviation [mm]) and the results of the Mann Whitney U-test showing the significant difference in the size of the skulls of *Ratufa bicolor* between the northern and southern populations (For the abbreviations of the skull characteristics used, refer to the method section Fig. 2). n= Sample sizes, SD = Standard deviation.

Characters	Northern population		Southern Population		Mann Whitney U-test.
	Mean, SD	n	Mean, SD	n	
ONL	74.8, 3.7	15	68.7, 3.9	5	Z = -2.31 P = 0.021
ZB	45.0, 2.7	15	41.5, 2.3	5	Z = -2.22 P = 0.026
IB	29.5, 2.4	15	26.6, 1.4	5	Z = -2.13 P = 0.032
LR	31.6, 2.04	15	28.2, 1.8	5	Z = -2.31 P = 0.021
BB	32.1, 1.5	15	30.5, 0.8	5	Z = -2.13 P = 0.032
HBC	22.2, 3.1	15	20.4, 0.7	5	Z = -2.05 P = 0.040
IF-M ¹	12.8, 1.4	15	11.4, 0.8	5	Z = -2.05 P = 0.040
PL	28.8, 1.7	15	26.1, 1.4	5	Z = -2.48 P = 0.013
LMD ₁₋₄	14.84, 0.5	15	14.1, 0.1	5	Z = -2.14 P = 0.032
LM ¹⁻⁴	14.3, 0.5	15	13.5, 0.3	5	Z = -2.83 P = 0.005
PC	25.1, 1	15	23, 0.8	5	Z = -3.01 P = 0.003

Table 3. Character loading factors from the Principal Components Analysis of seventeen cranial measurements from eighteen specimens (10 ♂♂, 8 ♀♀) of *Ratufa bicolor* (See also Fig. 4).

	ONL	ZB	IB	LR	BR	BB	HBC	LN	LD	IF-M ¹
PCA-1	-18.4799	-11.3368	-9.7869	-9.4873	-3.9583	-5.3260	-4.1807	-5.6303	-2.6097	-2.6127
PCA-2	2.5804	-1.5765	-0.6895	0.6041	2.6979	0.6667	-2.5628	0.7540	-11.8494	-3.1972

	PPL	PL	ML	LM ¹⁻⁴	IUI	LLI	PC	E.V	P.V.E
PCA-1	-7.6345	-7.6529	-8.7182	-2.1280	-2.8989	-3.1697	-3.5173	1004.118	60.9
PCA-2	0.7690	0.6238	-1.0267	0.3994	0.4492	0.3888	-1.1154	180.462	10.9

E. V: Eigen Value. P. V. E: Percentages of total variation explained by PC1 and PC2 factors.

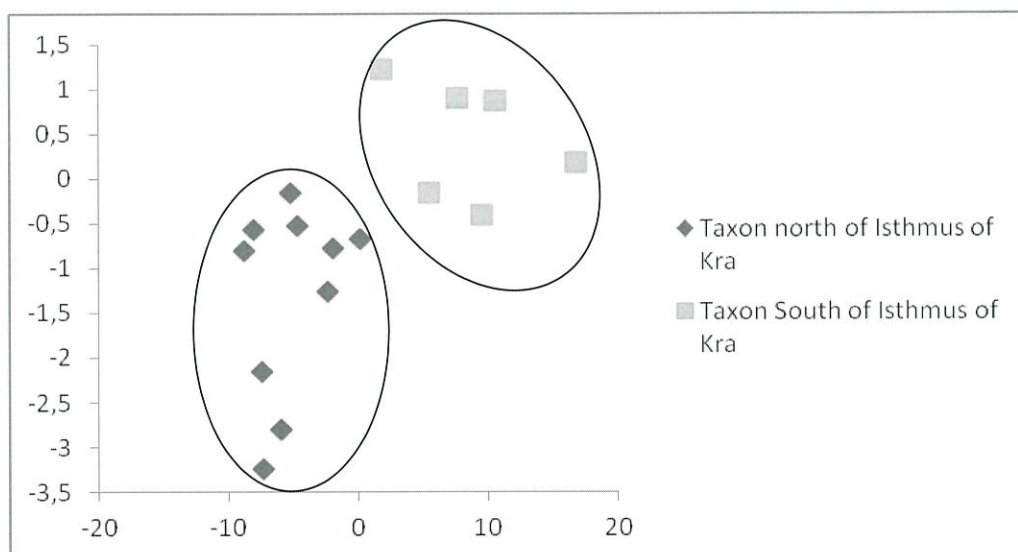


Figure 4. The Principal Component Analysis chart of skulls of *Ratufa bicolor* between PCA-1 and PCA-2 showing the division of the northern and southern populations in Thailand. The first principal component comprises 61 % of variance and the second comprises 11 % (n= 18).

DISCUSSION

Squirrels are believed to have no external sexual dimorphism. Gorbell *et al.* (2012) used molecular methods to determine the sex of squirrels. This technique could help the sexing of unidentified specimens and fossils, but the application is limited to a few experts only. In this study we examined cranial characteristics that could be applied by non-experts in the field or in the laboratory/museum. There were two cranial characteristics that showed a significant difference between males and females of the northern population. However, it would not be appropriate at this point to determine / predict this same pattern for the southern population as a result of: (1) small sample size, (2) significant geographical variation between these two (N & S) populations, (3) lack of data of climatic conditions and, (4) laxity of other possible factors. The significant difference in the skull size of the northern and southern populations, suggests that there is a latitudinal effect on this species; meaning that the overall size of the body tends to increase from the south to north (Bergmann's rule).

The geographic variation of *Callosciurus caniceps* around the two phytogeographic transitions were elucidated by Hayashida *et al.*, (2012). Avian transitions were noted along the northern phytogeographical boundaries Hughes *et al.*, (2003), but our current results for *Ratufa bicolor* may be affected by the Isthmus of Kra that divides two rich

biogeographic regions: the Indochina area and the Sunda area. A contributing factor could be that there exists a change in climatic zonation gradually as we move along the latitudinal gradient. Hughes *et al.*, (2011) also illustrated this same pattern of zoogeographic transition in this area in numerous bat species. Future studies could be carried out to see if this wide morphological plasticity is attributable to short term adaptation or caused by genetic drift or caused by repeated changes in the breadth of the Sunda Shelf during the recent glacial cycles, that led to higher extinction of the biota, both temporally and spatially. This species could be a good living model to test for the validity of Allen's rule in this region (Thailand). Moreover, larger sampling covering all of its range would further help to define the complex geographic variation in this species.

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APPENDIX

List of Specimens

Ratufa bicolor - [Skins = skulls] THNHM-1077-80, 82-83, 86-87, 98-99, THNHM-1105, 08-09, 12, 15, 26, 28, 30, 34, 39, 10♂♂ + 10♀♀, **Chiang Rai Province** (Mae Taeng, Doi Um Paa, Wiang Chai District, 19° 28.750'N, 98° 58.629'E, 1437 m), **Chon Buri** (Changburi SE, Nong Yai District, 13° 12.105'N, 101° 15.143'E, 65 m), **Kanchanaburi Province** (Bo Phloi, Nong Yung Chang Siam, Sri Sawat District, 14° 40.207'N, 99° 0.774'E, 643 m), **Lampang Province** (Khun Tan, Mae Ta District, 18° 31.713'N, 99° 15.264'E, 466 m), **Mae Hong Son** (Kiew Kor Mah, Mae Nah, Pai District, 18° 44.222'N, 97° 52.330'E, 914 m), **Nan Province** (Pua Phu Kha, Phu Phiang District, 18° 41.089'N, 100° 48.000'E, 425 m), **Phatthalung Province** (Khao Phub Pa, 7° 40.747'N, 99° 52.356'E, 119 m), **Ranong Province** (Ban Bang Mon, Kapur District, 9° 28.000'N, 98° 29.000'E, 17 m), **Tak Province** (Mae Sot, Doi Mu Soer: NW Siam, 18° 18.613'N, 102° 0.313'E, 429 m), **Trang Province** (Muang Khao Chong, Na Yong District, 7° 33.427'N, 7° 33.427'E, 423 m).

Note: Geographic coordinates are approximate where original descriptions were imprecise.