

Is there an evolutionary relict of the Japanese giant flying squirrel *Petaurista leucogenys* on the Asian Continent?

Tatsuo Oshida

Laboratory of Wildlife Ecology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan

In the Japanese archipelago, mammalian fauna is characterized by a high degree of endemism at the species level (40% of the total) (Millien-Parra and Jaeger 1999; Abe et al. 2005). These species may have survived on the Japanese Islands after their continental populations or relatives went extinct, forming what Cronk (1997) called 'relic endemics' in his island biogeographical study. Some of these species may also occur on the Asian Continent, as evolutionary relicts (e.g. Imaizumi 1985). In particular, the Japanese giant flying squirrel *Petaurista leucogenys* (Temminck 1827) was thought to be restricted to three Japanese islands (Honshu, Shikoku, and Kyushu Islands) and a part of southwestern China (from Yunnan and eastern Tibet to Gansu), showing disjunctive distribution (e.g. Imaizumi 1985; Nowak 1991) (Fig. 1). The population in China was treated as a distinct subspecies of *P. leucogenys* (Ellerman and Morrison-Scott 1951). Corbet and Hill (1992) reclassified this form as a distinct species, the Chinese giant flying squirrel *P. xanthotis* (Milne-Edwards 1872), based on its more com-

plex cheek-teeth and lack of a white streak in front of and below the ears. At present, this taxonomic status is broadly accepted (e.g. Wilson and Reeder 1993). Molecular systematic examination to confirm this classification, however, has never been made. If the genetic divergence between *P. leucogenys* and *P. xanthotis* is extremely low, the conspecific status of the species will be supposed. Even if their genetic divergence is high, close clustering in a phylogenetic tree should confirm the closeness of their evolutionary relationship. To test these hypotheses, molecular phylogenetic analysis was carried out using mitochondrial cytochrome *b* gene sequences.

Materials and methods

Sequence data of seven *Petaurista* species from the DNA Data Bank of Japan (DDBJ) were prepared (Table 1) (Oshida et al. 2004; Yu et al. 2004); sequences of *P. leucogenys* from different three main islands of Japan and that of *P. xanthotis* from Gansu of China were

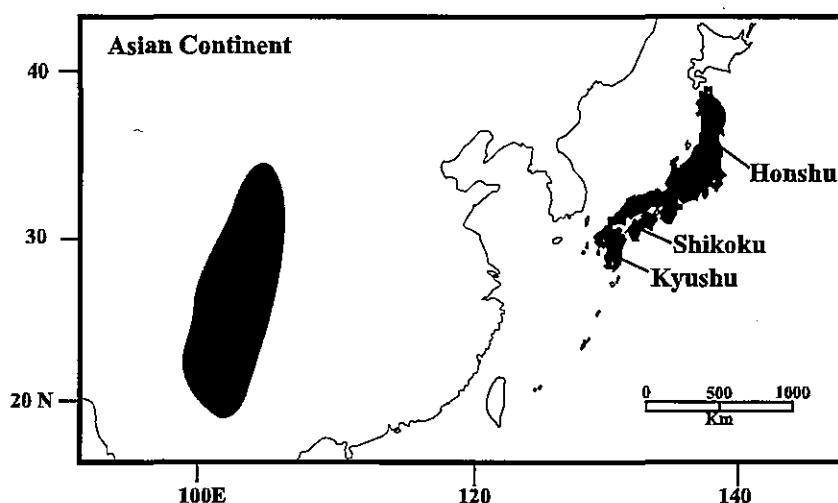


Fig. 1. Disjunct distribution (in gray) of *Petaurista leucogenys* as previously reported by Imaizumi (1985).

Table 1. Species of the genus *Petaurista* examined in the present study.

Species or subspecies name	Identity No. (code)	Common name	Collecting locality	Accession No.
<i>Petaurista alborufus castaneus</i>	1 (ALC1)	red and white giant flying squirrel	southern China*	AB092613
<i>Petaurista alborufus castaneus</i>	2 (ALC2)		southern China*	AB092614
<i>Petaurista alborufus lena</i>	(ALL1)		Nantou, Taiwan	AB092615
<i>Petaurista elegans</i>		lesser giant flying squirrel	Jambi, Indonesia	AB092610
<i>Petaurista leucogenys</i>	1 (LEL2)	Japanese giant flying squirrel	Fukuoka, Japan	AB092616
<i>Petaurista leucogenys</i>	2 (LEL1)		Ehime, Japan	AB092617
<i>Petaurista leucogenys</i>	3 (LEN1)		Wakayama, Japan	AB092618
<i>Petaurista leucogenys</i>	4 (LEN2)		Nagano, Japan	AB092619
<i>Petaurista petaurista melanotus</i>	1 (PEM1)	red giant flying squirrel	Laos*	AB092608
<i>Petaurista petaurista melanotus</i>	2 (PEM2)		southern China*	AB092609
<i>Petaurista petaurista albiventer</i>			Ayubia National Park, Pakistan	AB092612
<i>Petaurista philippensis grandis</i>	(PHG)	Indian giant flying squirrel	Nantou, Taiwan	AB092611
<i>Petaurista xanthotis</i>		Chinese giant flying squirrel	Gansu, China	AY615269

Identity Nos. are corresponded to those in Figs. 1 and 2, and the codes used in the previous report (Oshida et al., 2000) are shown in parentheses. Asterisks indicate that exact collecting locality is unknown.

included. Partial cytochrome *b* gene sequences (435 bases) were based on the shortest DNA fragment data (*P. xanthotis*) (Yu et al. 2004). Sequences were aligned with DNASIS (Hitachi, Tokyo). For maximum-likelihood (ML) analysis, the program Modeltest 3.06 (Posada and Crandall 1998) selected the most appropriate substitution model of molecular evolution through a nested likelihood ratio test. This test selected the general time reversible model of substitution (Rodríguez et al. 1990; Yang et al. 1994), took into account the proportion of invariable sites, and followed a gamma distribution for variable sites (GTR + I + G). I rooted the ML tree with cytochrome *b* sequences of *Belomys pearsonii* (accession number: AB126245) and *Pteromys volans* (accession number: AB097683), since these genera are the most closely related to the genus *Petaurista* in the subfamily Pteromyinae (Mercer and Roth 2003). The ML tree was constructed with the heuristic search option with tree-bisection-reconnection (TBR) under the GTR + I + G model implemented by PAUP* 4.0b10 (Swofford 2001). To assess the nodal supports, the bootstrapping (Felsenstein 1985) was carried out with 100 replications.

Results and discussion

An ML search, assuming the GTR + I + G model of evolution, produced a single tree (Fig. 2). In this tree, there were six lineages, suggesting their polytomic relationships: 1) a group consisting of *P. petaurista albiventer* and *P. alborufus castaneus*, 2) a group consisting of *P. petaurista melanotus* and *P. philippensis grandis*, 3)

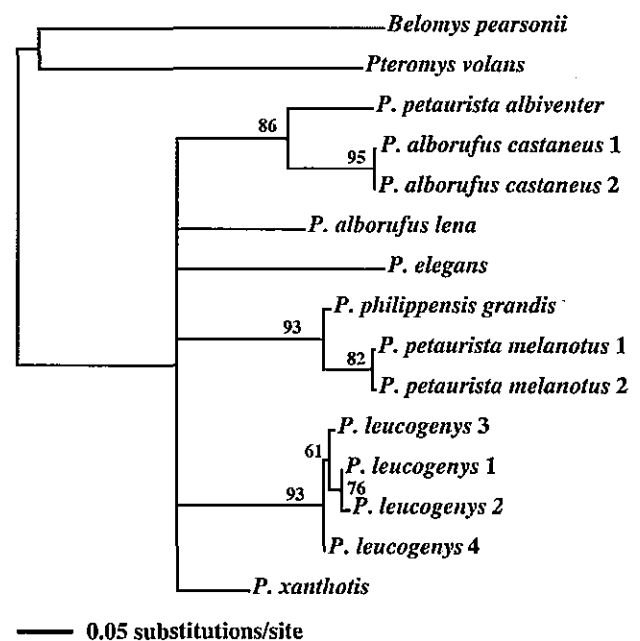


Fig. 2. Phylogeny of *Petaurista* constructed using the maximum-likelihood under GTR + I + G model for the cytochrome *b* sequences. Bootstrap supports are given on branches.

P. alborufus lena, 4) *P. elegans*, 5) *P. leucogenys*, and 6) *P. xanthotis*. Based on cytochrome *b* sequences, several phylogenetic findings of *Petaurista* are previously reported. Oshida et al. (2004) reported the close relationships between *P. petaurista albiventer* and *P. alborufus castaneus* and between *P. petaurista melanotus* and *P. philippensis grandis*. The unique phylogenetic position of *P. leucogenys* is already presented by Oshida et al.

Table 2. Pairwise uncorrected distances of partial cytochrome *b* gene sequences (435bp) for six species in the genus *Petaurista*.

	<i>P. petaurista</i> <i>albiventer</i>	<i>P. alborufus</i> <i>castaneus</i> 1	<i>P. alborufus</i> <i>castaneus</i> 2	<i>P. alborufus</i> <i>lana</i>	<i>P. elegans</i>	<i>P. philippensis</i> <i>grandis</i>	<i>P. petaurista</i> <i>melanotus</i> 1	<i>P. petaurista</i> <i>melanotus</i> 2	<i>P. leucogenys</i> 1	<i>P. leucogenys</i> 2	<i>P. leucogenys</i> 3	<i>P. leucogenys</i> 4
<i>P. alborufus</i> <i>castaneus</i> 1	9.89											
<i>P. alborufus</i> <i>castaneus</i> 2	9.66	0.23										
<i>P. alborufus</i> <i>lana</i>	13.33	12.87	13.10									
<i>P. elegans</i>	14.94	14.25	14.48	12.64								
<i>P. philippensis</i> <i>grandis</i>	12.87	11.49	11.49	11.26	13.56							
<i>P. petaurista</i> <i>melanotus</i> 1	14.02	11.26	11.26	10.58	15.40	4.37						
<i>P. petaurista</i> <i>melanotus</i> 2	13.56	11.26	11.26	10.12	14.94	4.37	0.46					
<i>P. leucogenys</i> 1	12.41	11.95	12.18	13.56	13.56	12.64	13.79	13.79				
<i>P. leucogenys</i> 2	12.18	11.72	11.95	12.87	13.33	12.87	13.56	13.56	1.15			
<i>P. leucogenys</i> 3	12.18	11.26	11.49	13.33	14.48	12.41	13.10	13.10	1.61	1.84		
<i>P. leucogenys</i> 4	12.87	11.49	11.72	13.56	14.71	13.10	13.79	13.79	2.30	2.53	0.69	
<i>P. xanthotis</i>	10.81	11.49	11.72	10.35	11.03	9.89	11.03	10.58	10.12	10.35	10.81	11.03

(2000, 2001, 2004). The ambiguous phylogenetic position of *P. elegans* is suggested by Oshida et al. (2001, 2004). Unlike previous reports (Oshida et al. 2000; 2001; 2004), *Petaurista alborufus lana* did not cluster with *P. philippensis grandis* and *P. petaurista melanotus* in the present study. Therefore, I limited the discussion to the phylogenetic relationships between *P. xanthotis* and the other *Petaurista* species (especially, *P. leucogenys*).

In the ML tree, *P. xanthotis* was not clustered with other *Petaurista* species including *P. leucogenys*, indicating that *P. xanthotis* could be a distinct species. This means that *P. xanthotis* should not be regarded as an evolutionary relict of *P. leucogenys*, supporting strongly the taxonomy of Corbet and Hill (1992). Uncorrected genetic distances (*p*-distances) among *Petaurista* specimens are presented in Table 2. Genetic distances (10.12–11.03%) between *P. xanthotis* and *P. leucogenys* were similar to those (9.89–11.72%) between *P. xanthotis* and the other *Petaurista* species except for *P. leucogenys*. Therefore, *P. xanthotis* should be considered a distinct species and not an evolutionary relict of *P. leucogenys*.

The phylogenetic position of *P. xanthotis* was still unclear in this molecular phylogenetic analysis. Oshida et al. (2004) proposed that some geographical evolutionary units (groups) in the genus *Petaurista* may have originated from the primitive divergence or radiation with secondary speciation taking place independently and locally within each geographical evolutionary unit. Corbet and Hill (1992) described that externally *P. xanthotis* is similar to some Chinese form of *P. caniceps*. Since both species are sympatrically distributed in southwestern China (eastern Yunnan to eastern Tibet), the hypothesis of Oshida et al. (2004) may be supported by the further comparative molecular phylogenetic examination between *P. xanthotis* and *P. caniceps*. In addition to this examination, to find the other phylogenetic relatives of *P. xanthotis* and to identify its placement in the phylogenetic tree require phylogenetic analysis of more *Petaurista* forms (species and subspecies) from central and southern parts of the Asian Continent.

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