

## A preliminary study on molecular phylogeny of giant flying squirrels, genus *Petaurista* (Rodentia, Sciuridae) based on mitochondrial cytochrome *b* gene sequences

Tatsuo Oshida, Chaudhry M. Shafique, Sohail Barkati, Yuki Fujita,  
Liang-Kong Lin & Ryuichi Masuda

**ABSTRACT.** Phylogenetic relationships among five species of the genus *Petaurista* (*P. alborufus*, *P. elegans*, *P. leucogenys*, *P. petaurista*, and *P. philippensis*) were investigated using the complete cytochrome *b* gene sequences (1140 bp). Phylogenetic trees indicated (1) *P. alborufus castaneus* from southern China was closely related to *P. petaurista albiventer* from Pakistan, (2) *P. alborufus lena* from Taiwan, *P. petaurista melanotus* from China and Laos, and *P. philippensis grandis* from Taiwan were grouped, and (3) *P. leucogenys*, which is endemic to Japan, distinctly separated from other species. Our results critically refuse the present classification in *Petaurista*, and suggest that it is reasonable to regard *P. alborufus lena* as a distinct species from *P. alborufus castaneus*, and *P. petaurista albiventer* as a distinct species from *P. petaurista melanotus*.

**KEY WORDS:** *Petaurista*, giant flying squirrel, molecular phylogeny, mitochondrial DNA, cytochrome *b*.

Tatsuo Oshida [oshidata@mail.thu.edu.tw], Laboratory of Molecular Ecology, Department of Life Science, Tunghai University, Taichung, Taiwan 407, R.O.C.; Chaudhry M. Shafique, Department of Zoology, University of Karachi, Karachi 75270, Pakistan; Sohail Barkati, Department of Zoology, University of Karachi, Karachi 75270, Pakistan; Yuki Fujita, Laboratory of Tropical Forest Resources and Environments, Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan; Liang-Kong Lin, Laboratory of Wildlife Ecology, Department of Life Science, Tunghai University, Taichung, Taiwan 407, R.O.C.; Ryuichi Masuda [masudary@ees.hokudai.ac.jp], Laboratory of Genetic Diversity, Center for Advanced Science and Technology, Hokkaido University, Sapporo 060-0810, Japan.

## Предварительное исследование молекулярной филогении гигантских летяг рода *Petaurista* (Rodentia, Sciuridae) на основе последовательностей митохондриального гена цитохрома *b*

Т. Ошида, Ч.М. Шафик, С. Баркати, Ю. Фуджита, Л.-К. Лин, Р. Масуда

**РЕЗЮМЕ.** Филогенетические связи пяти видов рода *Petaurista* (*P. alborufus*, *P. elegans*, *P. leucogenys*, *P. petaurista*, and *P. philippensis*) изучены с помощью полных последовательностей гена цитохрома *b* (1140 пар оснований). Согласно полученным филогенетическим деревьям (1) *P. alborufus castaneus* из южного Китая близкородственен *P. petaurista albiventer* из Пакистана, (2) *P. alborufus lena* из Тайваня, *P. petaurista melanotus* из Китая и Лаоса и *P. philippensis grandis* из Тайваня объединяются вместе и (3) *P. leucogenys*, эндемик для Японии, четко отделен от других форм. Наши результаты позволяют критически пересмотреть существующую классификацию рода *Petaurista*, и позволяют рассматривать *P. alborufus lena* как отдельный вид отличный от *P. alborufus castaneus*, и *P. petaurista albiventer* — как отдельный вид отличный от *P. petaurista melanotus*.

**КЛЮЧЕВЫЕ СЛОВА:** *Petaurista*, гигантская летяга, молекулярная филогения, митохондриальная ДНК, цитохром *b*.

### Introduction

Giant flying squirrels (genus *Petaurista*) are ubiquitous throughout South and Southeast Asia and in southern China, Taiwan, and Japan. They had been split traditionally into five species: *P. alborufus* Milne-Edwards, 1870, *P. elegans* Müller, 1840, *P. leucogenys* Temminck, 1827, *P. magnificus* Hodgson 1836, and *P.*

*petaurista* Pallas, 1766 (Corbet & Hill, 1980), each of which was intricately divided into numerous subspecies (e.g., Lekagul & McNeely, 1988). However, Corbet & Hill (1991, 1992) currently reorganized the classification and recognized five additional species: *P. caniceps* Gray, 1842, *P. nobilis* Gray, 1842, *P. philippensis* Elliot, 1839, *P. sybilla* Thomas & Wroughton, 1916, and *P. xanthotis* Milne-Edwards, 1872 from five species that have been mentioned above.

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

Species name	Identity No.	Common name	Type locality	Collecting locality	Accession No.
<i>Petaurista alborufus castaneus</i>	1 (ALC1)	red and white giant flying squirrel	Ichang, Hubei, China	southern China*	AB092613
<i>Petaurista alborufus castaneus</i>	2 (ALC2)			southern China*	AB092614
<i>Petaurista alborufus lena</i>	(ALL1)		Taiwan	Nantou, Taiwan	AB092615
<i>Petaurista elegans</i>		lesser giant flying squirrel	Java Island, Indonesia	Jambi, Indonesia	AB092610
<i>Petaurista leucogenys leucogenys</i>	1 (LEL2)	Japanese giant flying squirrel	Kyushu Island, Japan	Fukuoka, Japan	AB092616
<i>Petaurista leucogenys leucogenys</i>	2 (LEL1)			Ehime, Japan	AB092617
<i>Petaurista leucogenys nikkonis</i>	1 (LEN1)		Honshu Island, Japan	Wakayama, Japan	AB092618
<i>Petaurista leucogenys nikkonis</i>	2			Nagano, Japan	AB092619
<i>Petaurista petaurista melanotus</i>	1 (PEM1)	red giant flying squirrel	Selangor, Malaysia	Laos*	AB092608
<i>Petaurista petaurista melanotus</i>	2 (PEM2)			southern China*	AB092609
<i>Petaurista petaurista albiventer</i>			Nepal	Ayubia National Park, Pakistan	AB092612
<i>Petaurista philippensis grandis</i>	(PHG)	Indian giant flying squirrel	Taiwan	Nantou, Taiwan	AB092611

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 mean the sample unknown exact collecting locality.

There are some fragmentary studies on the systematics of some *Petaurista* species or subspecies based on genetic data. Oshida *et al.* (1992) examined the karyotaxonomy of *Petaurista* and concluded that *P. petaurista melanotus* Gray, 1837 was more closely related to *P. alborufus lena* Thomas, 1907 than to *P. petaurista grandis* (*P. philippensis grandis*) Swinhoe, 1863. In addition, based on the mitochondrial 12S ribosomal RNA sequence data, Oshida *et al.* (1996) reported that *P. petaurista melanotus* was closely related to *P. petaurista grandis* (*P. philippensis grandis*) and that *P. leucogenys* could have early diverged from *P. petaurista*. Recently, a molecular systematic study of the mitochondrial cytochrome *b* partial sequences (1068 bp) showed that *P. alborufus lena* from Taiwan was more closely related to *P. philippensis grandis* and *P. petaurista melanotus* than to *P. alborufus castaneus* from southern China (Oshida *et al.*, 2000a), and that *P. elegans* may be closely related to *P. alborufus castaneus* Thomas, 1923 (Oshida *et al.*, 2001a). In spite of the presentation of these studies, the entire perspective on molecular systematics of the genus *Petaurista* has not been cleared yet.

Giant flying squirrels are arboreal, and depend on the resources of biologically rich forests (e.g., Baba *et al.*, 1982; Lee *et al.*, 1986). So, the recent destruction of rich forests in Asia seems to seriously bring with it decrease of population of giant flying squirrels. However, since even the classification is still confused, at present it is very difficult to plan for conservation of giant flying squirrels, so that, first the basic studies regarding as

systematics and phylogeny of *Petaurista* could be absolutely necessary.

The primary objective of the present study was to examine the complete mitochondrial cytochrome *b* sequences of representative *Petaurista* species (*P. alborufus*, *P. elegans*, *P. leucogenys*, *P. petaurista*, and *P. philippensis*) that represent effectively the geographic distribution pattern of this genus. Specifically, the present study critically addresses (1) the phylogenetic relationships among representative *Petaurista* species including several subspecies and (2) the establishment of appropriate scenario to successfully interpret molecular systematic perspective of this genus.

## Materials and Methods

### Specimen collection

Giant flying squirrels examined in the present study are shown in Tab. 1. According to the descriptions of Wroughton (1911), Thomas (1923), Imaizumi & Miyamoto (1960), Lekagul & McNeely (1988), and Corbet & Hill (1991, 1992) species and subspecies were identified on the basis of their pelage coloration. Specimens of *P. alborufus lena*, *P. philippensis grandis*, *P. petaurista albiventer* Gray, 1834, and *P. elegans* were collected for this study. Two specimens of *P. alborufus castaneus* imported from Hong-Kong to Japan in 1996 were commercially obtained. Muscle tissues of two individuals of *P. leucogenys leucogenys* Temminck, 1827 were pro-

vided from Dr. K. Ikeda of the Fukuoka Prefecture Forest Research and Extension Center, Fukuoka, Japan, and Mr. T. Okayama of the Omogo Mountain Museum, Ehime, Japan. The other *Petaurista* specimens examined in this study were commercially obtained. Except for a sample of *P. petaurista albiventer*, the samples used in previous examinations (Oshida *et al.*, 2000a, 2001a) were analyzed again. In addition, samples of the hairy-footed flying squirrel *Belomys pearsonii* Gray, 1842 and the Russian flying squirrel *Pteromys volans* Linnaeus, 1758, which were employed as an out-group in phylogenetic analyses, were provided from the National Museum of Natural Science, Taichung, Taiwan and the Noboribetsu Bear Park, Noboribetsu, Japan, respectively. To grasp broadly the phylogeographic tendency of *Petaurista*, with respect to the selection of species or subspecies in the present study, we paid attention to the distribution fringe of genus *Petaurista*; *P. petaurista albiventer*, *P. elegans*, and *P. leucogenys* used in the present study are peculiarly distributed in westernmost, southernmost, and northern/easternmost areas, respectively.

### DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from frozen or 99% ethanol-preserved muscles, skins, or liver tissues with the phenol/chloroform method, and then suspended in TE buffer (Sambrook *et al.*, 1989). The whole region of the mtDNA cytochrome *b* gene was amplified with polymerase chain reaction (PCR) using a primer set (L14724: 5'-GATATGAAAACCATCGTTG-3' and H15910: 5'-GATTTTTGGTTTACAAGACCGAG-3') reported by Oshida *et al.* (2000a). Primer names correspond to the light (L) or heavy (H) strand and the 3' end-position of the primers in the human mtDNA sequence (Anderson *et al.* 1981). The 50  $\mu$ l of reaction mixture contained 100 ng of genomic DNA, 25 pM of each primer, 200  $\mu$ M dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, and 2.5 units of r*Taq* DNA polymerase (Takara). Amplification was carried out for 35 cycles using the following cycle program: 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. The extension reaction was completed by incubation at 72°C for 10 min. PCR products were purified with the PCR Clean Up-M (Viogen, Taiwan) and directly sequenced using an automated DNA sequencer (ABI PRISM 377-96 Sequencer, ABI PRISM 3100 Genetic Analyzer Applied Biosystem, CA, USA). For sequencing, four kinds of primer (L14724, H15910, L15576: 5'-CAGAATGATACTTCCTATTTGC-3', and H15554: 5'-GCCTATGAATGCTGTGGCTAT-3') were employed. To determine the sequences of terminals of both 5' and 3', the primers L15576 and H15554 were newly designed in the present study. The processes of purification of PCR products and sequencing were performed with Mission Biotech Co. Ltd. (Taipei, Taiwan).

### Phylogenetic analysis

Sequence alignment was carried out using DNASIS (Hitachi). Genetic distances (*p*-distances) among specimens were calculated in PAUP\* 4.0b10 (Swofford, 2001). In phylogenetic analyses, all trees were rooted using the cytochrome *b* sequences of *Belomys pearsonii* and *Pteromys volans*. Based on nucleotide sequence analyses of interphotoreceptor retinoid-binding protein (IRBP) and 12S and 16S rRNA genes, Mercer & Roth (2003) presented that these flying squirrels were phylogenetically grouped together with *Petaurista* in the subfamily Pteromyinae, so that we chose these species as an out-group for assessment of phylogenetic relationships within the genus *Petaurista*.

The maximum-parsimony (MP) analysis was conducted with the branch-and-bound search algorithm (Hendy & Penny, 1982) in PAUP\* 4.0b10 (Swofford, 2001). In the analysis, the transversional substitution was weighted three times higher than the transitional substitution. For maximum-likelihood (ML) analysis, we used the program Modeltest 3.06 (Posada & Crandall, 1998) to select the most appropriate model of molecular evolution through a nested likelihood ratio test. This test presented the TN93 model of substitution (Tamura & Nei, 1993) taking into account the proportion of invariable sites and following a gamma distribution for variable sites (TN + I + G). The ML tree was then constructed with the heuristic search option (with tree-bisection-reconnection, TBR) under the TN + I + G model implemented by PAUP\* 4.0b10 (Swofford, 2001). To assess the nodal supports, the bootstrapping (Felsenstein, 1985) was performed with 2000 replicates in MP analysis and 200 replicates in ML analysis.

## Results

### Nucleotide composition

In this study, the complete sequences (1140 bp) of cytochrome *b* gene were successively determined from 12 individuals of the genus *Petaurista* and two individuals of out-group. Data on base composition are summarized in Tab. 2. The base composition was biased (28.4%

Table 2. Base composition bias for cytochrome *b* gene of the genus *Petaurista*.

	1st	2nd	3rd	All
A	0.292	0.203	0.352	0.284
C	0.274	0.238	0.388	0.299
G	0.206	0.137	0.032	0.126
T	0.228	0.422	0.228	0.291
Bias	0.088	0.245	0.320	0.167

Values are calculated according to codon position (1st, 2nd, 3rd, and all positions).

The bias is calculated by the formula of Irwin *et al.* (1991).

Table 3. Pairwise transition/transversion number (above diagonal) and uncorrected distance (below diagonal) of complete cytochrome *b* gene sequence (1140 bp) for five species in the genus *Petaurista*.

	<i>P. petaurista albiventer</i>	<i>P. alborufus castaneus</i> 1	<i>P. alborufus castaneus</i> 2	<i>P. alborufus lena</i>	<i>P. elegans</i>	<i>P. philippensis grandis</i>	<i>P. petaurista melanotus</i> 1	<i>P. petaurista melanotus</i> 2	<i>P. leucogenys leucogenys</i> 1	<i>P. leucogenys leucogenys</i> 2	<i>P. leucogenys nikkonis</i> 1	<i>P. leucogenys nikkonis</i> 2
<i>P. petaurista albiventer</i>		107/11	108/10	138/23	145/23	123/27	126/24	126/26	133/25	129/26	134/28	138/26
<i>P. alborufus castaneus</i> 1	10.35		3/1	145/22	143/24	116/26	119/25	117/27	117/26	116/25	119/27	117/25
<i>P. alborufus castaneus</i> 2	10.35	0.35		147/21	146/23	119/25	122/24	120/26	120/25	119/24	122/26	120/24
<i>P. alborufus lena</i>	14.12	14.65	14.74		135/28	116/16	104/13	106/15	129/22	126/21	129/23	131/21
<i>P. elegans</i>	14.74	14.65	14.82	14.30		120/30	126/27	128/29	139/32	139/31	144/33	144/31
<i>P. philippensis grandis</i>	13.16	12.46	12.63	11.58	13.16		37/7	36/9	123/24	126/23	127/25	131/23
<i>P. petaurista melanotus</i> 1	13.16	12.63	12.81	10.26	13.42	3.86		5/2	123/23	124/22	123/24	127/22
<i>P. petaurista melanotus</i> 2	13.33	12.63	12.81	10.61	13.77	3.95	0.61		123/25	124/24	123/26	127/24
<i>P. leucogenys leucogenys</i> 1	13.86	12.54	12.72	13.25	15.00	12.89	12.81	12.98		13/3	16/3	18/1
<i>P. leucogenys leucogenys</i> 2	13.60	12.37	12.54	12.89	14.91	13.07	12.81	12.98	1.40		15/4	17/2
<i>P. leucogenys nikkonis</i> 1	14.21	12.81	12.98	13.33	15.53	13.33	12.89	13.07	1.67	1.67		8/2
<i>P. leucogenys nikkonis</i> 2	14.39	12.46	12.63	13.33	15.35	13.51	13.07	13.25	1.67	1.67	0.88	

A, 29.1% T, 29.9% C, and 12.6% G), with a bias against G, which is usual for the cytochrome *b* sense strand of vertebrates (e.g., Piaggio & Spicer, 2001; Randi *et al.*, 2001). First codon positions did not seem to have significant bias, however, the highest T content at the second codon positions (42.2%) and the lowest G content at the third codon positions (3.2%) were recognized. The compositional bias for our data (0.088, 0.245, and 0.320 for the first, second, and third codon position, respectively) was similar to that of Irwin *et al.* (1991), with the highest bias at the third position.

Tab. 3 shows uncorrected percentage sequence differences (*p*-distances) and numbers of transversions and transitions obtained from pairwise comparison between individuals. Among species of *Petaurista*, *p*-distances and transition/transversion ratios ranged from 3.86 to 15.35% and from 11:1 to 4:1, respectively.

### Phylogenetic relationships of *Petaurista*

A maximum parsimony (MP) analysis was performed using a branch-and-bound search algorithm based on 285 parsimony-informative characters. The analysis

produced a single most-parsimonious tree of length of 1221, consistency index (CI) of 0.665, and retention index (RI) of 0.665 (Fig. 1). Bootstrap analysis (2000 iterations) provided support for three major groups. The first ("clade I") was formed by *P. petaurista albiventer*, *P. alborufus castaneus*, and *P. elegans* with a 59% bootstrap value, the second ("clade II") consisted of *P. elegans*, *P. petaurista melanotus*, *P. alborufus lena*, and *P. philippensis grandis* with a 77% bootstrap value, and the third ("clade III") contained *P. leucogenys leucogenys* and *P. leucogenys nikkonis* Thomas, 1905 with a 100% bootstrap value. The MP analysis caused the clade III to form a sister group to the clades I and II with a low bootstrap value (56%).

A maximum-likelihood (ML) search assuming the TN+I+G model of evolution (score of  $-\ln L = 4950.62$ ) produced a single tree. The topology of ML tree was essentially identical to that of MP tree (Fig. 2). The major clades I, II, and III were supported with 75, 76, and 96% bootstrap values, respectively.

Four specimens of *P. leucogenys* from Japan including two subspecies generated evident monophyly (clade III) (Figs. 1 and 2). However, both *P. alborufus* and *P.*

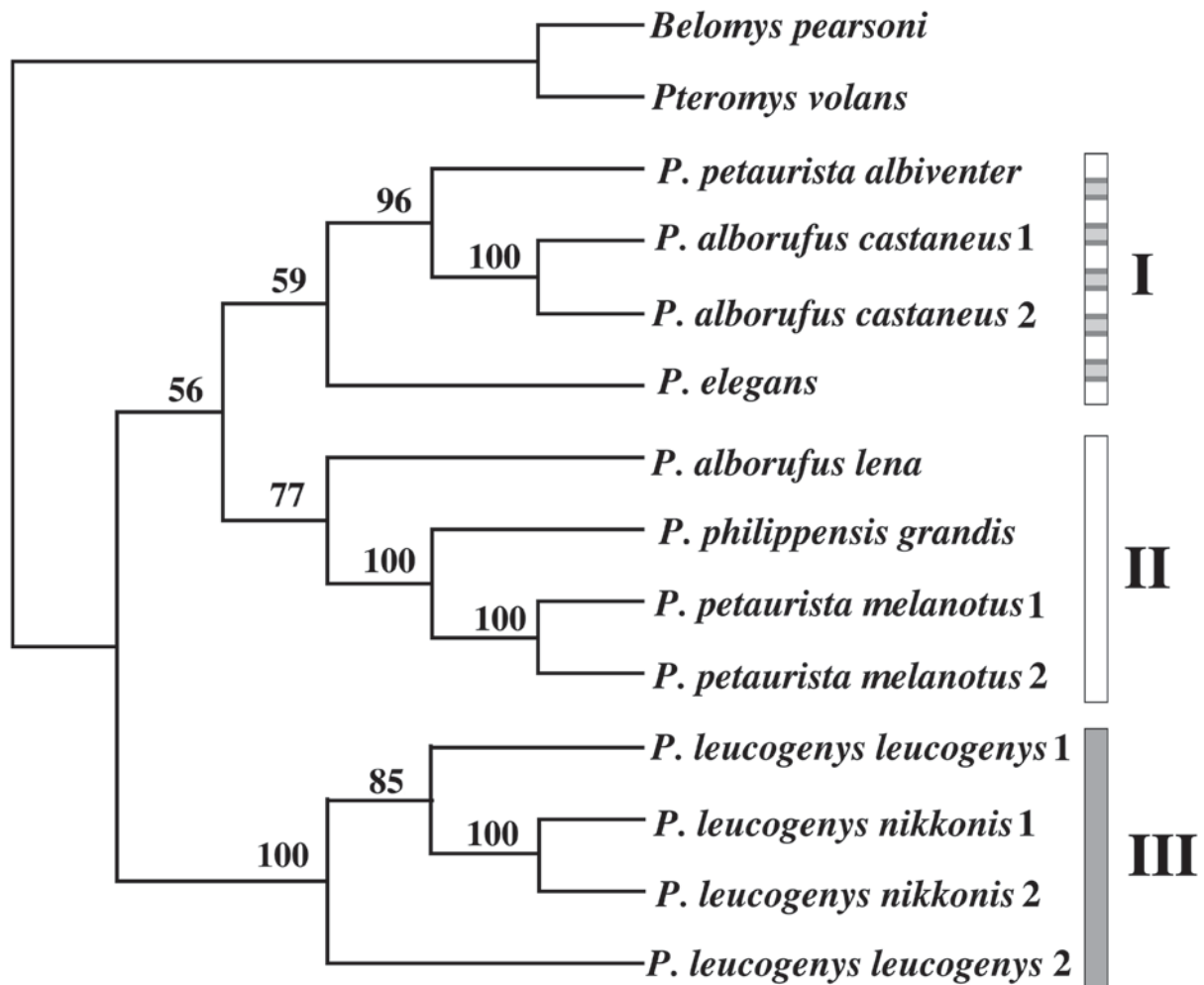


Figure 1. A single most parsimonious tree for *Petaurista* produced with the branch-and bound search algorithm for the cytochrome *b* sequences. Bootstrap supports for the tree are given on branches.

*petaurista* did not form an each unique clade. *Petaurista petaurista albiventer* from Pakistan was closely related to *P. alborufus castaneus* from China in clade I with high nodal supports (96% in MP tree and 98% in ML tree). In the clade II, *P. petaurista melanotus* from China and Laos and *P. philippensis grandis* from Taiwan formed a single clade, to which *P. alborufus lena* from Taiwan was closely related, although the nodal supports were not so high (77% in MP tree and 76% in ML tree). *Petaurista elegans* from Indonesia was contained in the clade I, but the nodal supports for phylogenetic position of this species were significantly low in both trees (56% in MP tree and 75% in ML tree).

## Discussion

### Phylogeny of *Petaurista alborufus*

*Petaurista alborufus*, which occurs in southern China, Taiwan, Burma, and Thailand, had been divided into seven subspecies: *barroni* Kloss, 1916, *casta-*

*neus*, *candidula* Wroughton, 1911, *lena*, *leucocephalus* Hilzheimer, 1905, *ochraspis* Thomas, 1923, and *taylori* Thomas, 1914. However, Corbet & Hill (1992) have currently recognized only four variations as subspecies of *P. alborufus*: *castaneus*, *lena*, *leucocephalus*, and *ochraspis*. This species has a white color head and ventral and rufous color back and tail as common characteristics. Using partial sequences of the cytochrome *b* gene (1068 bp), Oshida *et al.* (2000a, 2001a) reported that *castaneus* from China was distantly related to *lena* from Taiwan, and also, *lena* was closely related to *P. petaurista melanotus* and *P. philippensis grandis*. Moreover, on the basis of chromosomal characteristics, it was underpinned that *P. alborufus lena* was more closely related to *P. petaurista melanotus* than to *P. petaurista grandis* (*P. philippensis grandis*) (Oshida *et al.*, 1992).

In the present study, it is noteworthy that *castaneus* was closely related to *P. petaurista albiventer* with high nodal supports (96% in MP tree and 98% in ML tree). Moreover, as described previously by Oshida *et al.*

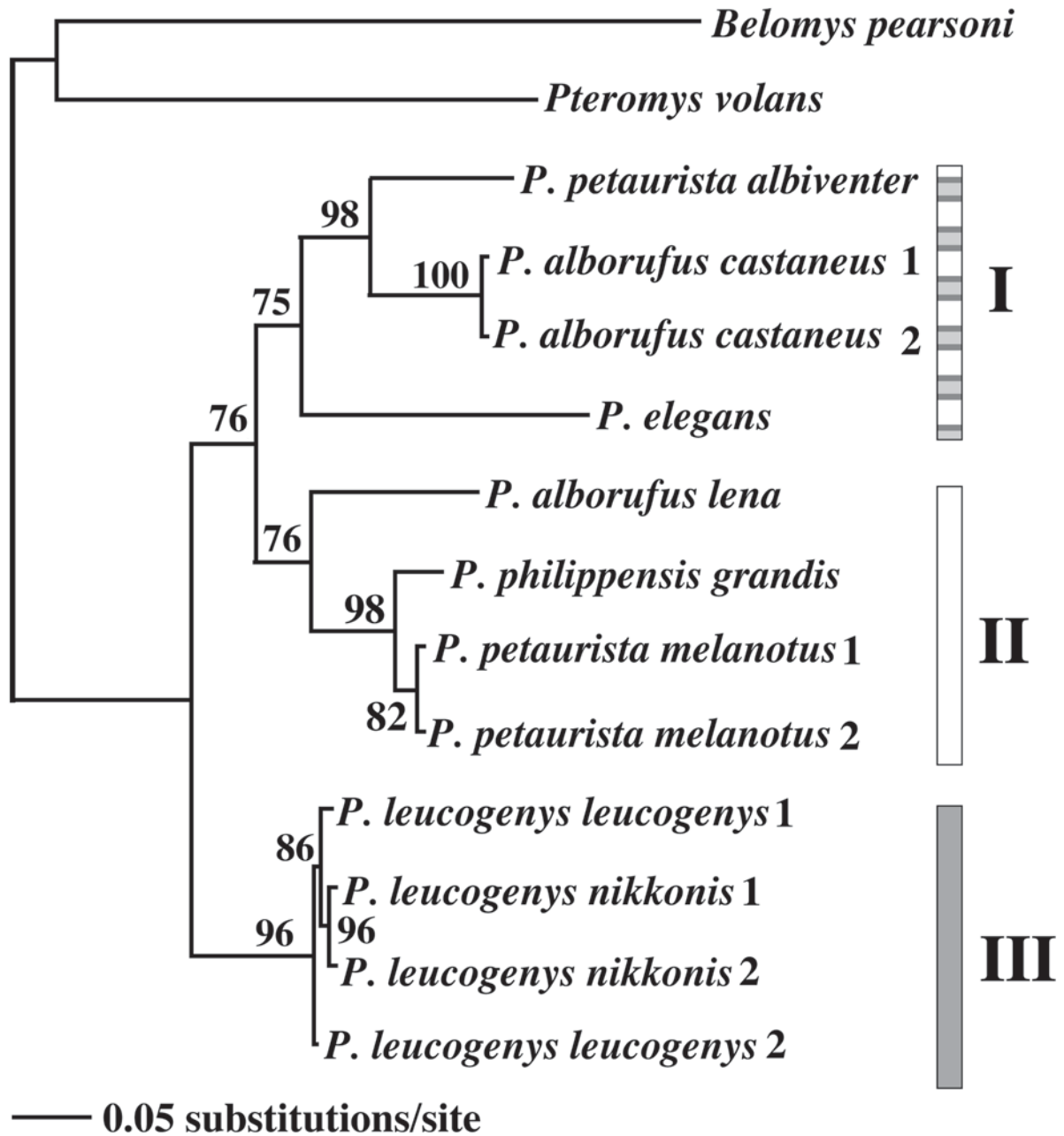


Figure 2. Phylogeny for *Petaurista* constructed using maximum-likelihood under the TN93 + I + G model for the cytochrome *b* sequences. Bootstrap supports for the tree are given on branches.

(2000a, 2001a), it is of interest that *lena* was closely related to the clade consisted of *P. petaurista melanotus* and *P. philippensis grandis*, although the bootstrap values supported the relationship were not high (77% in MP tree and 76% in ML tree). The *p*-distances between two *alborufus* subspecies were 14.65% and 14.74% (Tab. 3), supporting that the different species status of two *alborufus* subspecies should be recognized. Based on the characteristics of pelage color, *P. alborufus lena* was once treated as a distinct species *P. pectoralis* Swinhoe, 1871. Also, Corbet & Hill (1992) suggested

that *P. alborufus lena* is distinct enough to merit specific rank. Phylogenetic results obtained in the present study did not conflict with the phylogenetic position of *lena* proposed by Swinhoe (1870) and Corbet & Hill (1992). In addition, Oshida *et al.* (2000b) reported that the banded karyotypes of two *alborufus* subspecies were remarkably different from each other. On the assumption that *castaneus* distributed more widely in the mainland is a representative subspecies of *P. alborufus*, we conclude that *lena*, which is endemic to Taiwan, is a distinct species from *P. alborufus*.

With respect to the geographic history of Taiwan, Hsu (1990) reported that the Taiwan had risen from the sea floor on the Eurasian Continent approximately 4.0 million years (Myr) ago. Based on faunistic and geological investigations, Kano (1940) and Liu & Ding (1984) concluded that the connection between Taiwan and the Eurasian Continent had occurred at least twice due to the glacial eustasy, initially during the Pliocene and subsequently during the Pleistocene. It is highly probable that the multiple faunistic exchanges between the Taiwan and the mainland had been taken place by these geographic events. From a paleoenvironmental point of view, Lin & Lin (1983) hypothesized that the first mammal fauna which immigrated from the Eurasian Continent into the Taiwan during the glacial period of the Pliocene had adapted themselves to the cold environment, but, after the glacial period, to refuge from the warm climate, they had to move to the high elevation areas of Taiwan. Lin & Lin (1983) added, subsequently the second mammal fauna that immigrated into the Taiwan during the glacial periods of the Pleistocene had expanded their ranges throughout the low and middle elevation areas of Taiwan.

*Petaurista alborufus lena* is confined to the high elevation area (1200 to 3750 m) in Taiwan, while the distribution area of *P. philippensis grandis* in Taiwan widely ranges from 700 to 2600 m elevation (Chang, 1985). So, it is likely that the invasion of *P. alborufus lena* was earlier than that of *P. philippensis grandis*. As described by Oshida *et al.* (2000a), after the divergence between *P. alborufus lena* and the lineage of *P. petaurista* and *P. philippensis* in the Eurasian Continent during the late Miocene, *P. alborufus lena* might have immigrated to Taiwan and adapted itself to the alpine region in Taiwan. In other words, *P. alborufus lena* might have independently evolved from other *Petaurista* species in the alpine region of Taiwan, owing to the absolute geographic isolation.

On the other hand, it is really surprising that *P. alborufus castaneus* was also closely related to *P. petaurista albiventer* from Pakistan with high nodal supports (Figs. 1 and 2), although the *p*-distance between two species was sufficiently distant (10.35%, Tab. 3). Based on the morphological characteristics such as externals and dental forms, three subspecies (*barroni*, *candidula*, and *taylori*) of *P. alborufus* were referred to as *P. petaurista* by Corbet & Hill (1992). Conceivably, *P. alborufus* might have been sometimes confused with *P. petaurista* due to the complicated morphological variation of two species. In the present study, the close phylogenetic relationships between *P. alborufus castaneus* and *P. petaurista albiventer* and between *P. alborufus lena* and *P. petaurista melanotus* might be interpreted as a result of the confused classification relied on pelage coloration. In the light of molecular systematics of mammals (e.g., Irwin *et al.*, 1991), the genetic distances of the present study (Tab. 3) would be enough to consider *P. alborufus castaneus*, *P. alborufus lena*, *P. petaurista melanotus*, and *P. petaurista albiventer*, as each distinct species.

### Phylogeny of *Petaurista petaurista* and *P. philippensis*

*Petaurista petaurista*, which is one of the most dominant species in genus *Petaurista*, is widely distributed in southern parts of the Eurasian Continent and Southeast Asia (Corbet & Hill, 1980; Lekagul & McNeely, 1988). The variation of this species in color is considerable, and the subspecific descriptions based on the pelage color patterns are full of ambiguities. However, Corbet & Hill (1992) currently described nine subspecies: *albiventer*, *batuana* Miller, 1903, *candidula* Wroughton, 1911, *marchi* Thomas, 1908, *melanotus*, *nitidula* Thomas, 1900, *petaurista* Pallas, 1766, *taylori* Thomas, 1914, and *terutaus* Lyon, 1907. Of them, in the present study, two subspecies were phylogenetically examined. *Petaurista petaurista albiventer* was closely related to *alborufus castaneus*, while *P. petaurista melanotus* was closely related to *P. philippensis grandis* (Figs. 1 and 2). Both *petaurista* subspecies did not generate monophyletic clade each other, and the *p*-distances between two subspecies were 13.16 and 13.33% (Tab. 3), suggesting *albiventer* could be appreciably different from *melanotus* at the species level.

The distribution of *P. petaurista albiventer* is confined in Himalayan moist temperate forests in northern Pakistan (and probably in a part of the eastern Afghanistan), and this subspecies occurs from about 1353m elevation to the upper limit of the tree line at about 3050 m (Roberts, 1997). Therefore, it is reasonable that *P. petaurista albiventer* could have been geographically isolated from other *P. petaurista* subspecies for a long time, as the status of *P. alborufus lena* in the alpine regions of Taiwan. In contrast, *P. petaurista melanotus* is widely distributed in southern China, Malay and Indochina Peninsulas, and Borneo, Bungan, Riau, and Penang islands (Corbet & Hill, 1992). The *p*-distances between *P. petaurista melanotus* and *P. philippensis grandis* were 3.86 and 3.95% (Tab. 3), indicating significantly close kinship between both flying squirrels.

*Petaurista philippensis* had been treated as a subspecies of *P. petaurista* until Corbet & Hill (1991, 1992) established it as a distinct species. This species occurs in Sri Lanka, western India, southern China, Indochina and Malay Peninsulas, and Taiwan. Corbet & Hill (1992) proposed seven subspecies of *P. philippensis*, but their geographical variations are very extensive. *Petaurista philippensis grandis*, which is endemic subspecies to Taiwan, was previously treated as *P. petaurista grandis* by Swinhoe (1870). From sequence data of the 12S rRNA gene, Oshida *et al.* (1996) reported that the genetic distance between *P. philippensis grandis* (*P. petaurista grandis*) and *P. petaurista melanotus* was almost equal to intraspecific differences within *P. leucogenys*. In the present cytochrome *b* data, the *p*-distances between *P. philippensis grandis* and *P. petaurista melanotus* corresponded to approximately twice of intraspecific differences within *P. leucogenys* (Tab. 3). However, *P. philippensis* is most closely related to *P. petaurista*

with high nodal supports (100% in MP tree and 98% in ML tree). So, further studies, using the molecular data from other subspecies of *P. petaurista* and *P. philippensis*, could test the hypothesis whether *P. petaurista* and *P. philippensis* deserve of separate species status within genus *Petaurista*.

### Phylogeny of *Petaurista elegans*

*Petaurista elegans* is mainly distributed in the Sunda land (Sumatra, Java, and Borneo islands), and also occurs in a part of Indochina and Malay Peninsulas (Corbet & Hill, 1992). Corbet & Hill (1992) described that, although the extremes are very difficult for identification of each variant, principle five subspecies of *P. elegans* can be recognized. However, it was also difficult to identify *elegans* individual examined here with the subspecific rank.

In the present study, this species was felt in the clade I with *P. petaurista albiventer* and *P. alborufus castaneus*, but the bootstrap values to support its phylogenetic position were significantly low in both MP and ML trees (Figs. 1 and 2). Based on the neighbor-joining analysis with transversal distance of cytochrome *b* partial sequence, Oshida *et al.* (2001a) reported that *P. elegans* is closely related to *P. alborufus castaneus*. The present study also revealed the close relationship between *P. elegans* and *P. alborufus castaneus*.

The *p*-distances between *elegans* and other *Petaurista* species were 13.16–15.35% (Tab. 3). Although some parts of the distribution of *P. elegans* are overlapped with that of *P. petaurista* in Southeast Asia, the patterns are different from each other. *Petaurista petaurista* is widely distributed in both of peninsulas and islands, but *P. elegans* biased its major distribution area toward islands. So, after *P. elegans* had been separated from lineage of *P. alborufus castaneus* and *P. petaurista albiventer*, in the islands of Southeast Asia, this species might have independently evolved from other *Petaurista* species.

### Phylogeny of *Petaurista leucogenys*

*Petaurista leucogenys* is indigenous to three Japanese main islands (Kyushu, Shikoku, and Honshu islands) (Corbet & Hill, 1991). Based on the fossil records, Kawamura (1988, 1990) and Kawamura *et al.* (1989) hypothesized that *P. leucogenys* had immigrated from the Eurasian Continent to the Japanese islands through the land bridges around the early to the middle Pleistocene, and then had been isolated due to the separation of the Japanese islands from the Eurasian Continent in the Pleistocene. Corbet & Hill (1980) described that *P. leucogenys* is distributed on the Japanese islands and central China, but, recently they categorized the *leucogenys* population of central China as a distinct species *P. xanthotis* (Corbet & Hill, 1991, 1992). Based on the pelage coloration, Imaizumi & Miyamoto (1960) described three subspecies of *P. leucogenys* (*leucogenys*,

*nikkonis*, and *oreas* Thomas, 1905) within Japan. However, it seems that the subspecific classification has not been generally accepted, owing to the presence of ambiguous color variations. In addition, based on the mitochondrial control region sequences, Oshida *et al.* (2001b) reported that there are two major *leucogenys* lineages (Honshu-Shikoku and Kyushu lineages), suggesting that the *leucogenys* population could not be classified into three subspecies. In the present study, *P. leucogenys* clearly formed a single clade (clade III) with high bootstrap values (100% in MP tree and 96% in ML tree) (Figs. 1 and 2), indicating that *P. leucogenys* is the most distantly related to other *Petaurista* species. The *p*-distances between *P. leucogenys* and other *Petaurista* species were ranging from 12.37 to 15.35% (Tab. 3). This species would have also independently evolved from other *Petaurista* species in Japanese islands. Additionally, in the clade III, two individuals from Honshu Island were closely related to each other with the high nodal supports (Figs. 1 and 2). However, to further discuss the phylogeography of *P. leucogenys* populations as a whole is beyond the scope of this paper, because of the inadequate sample number examined here.

### Conclusion

From the standpoint of molecular systematics, there is no reason to support the present classification of *Petaurista*. Especially, the phylogenetic relationship between *P. petaurista* and *P. alborufus* obtained in the present study is quite different from the present taxonomic status. At the least, it could be reasonable to regard *P. alborufus lena* as a distinct species from *P. alborufus castaneus*, and *P. petaurista albiventer* as a distinct species from *P. petaurista melanotus*. So far, characteristics of pelage coloration have been applied for the classification of *Petaurista* as an important index. However, in fact, it would be difficult to identify each species or subspecies on the basis of pelage coloration, because of the presence of numerous color variations within genus *Petaurista*. So, we need to postulate a different scenario to clearly explain the classification and phylogeny of *Petaurista*.

The genus *Petaurista* may have some 'geographical evolutionary units (groups)' originated from the primitive divergence or radiation. In each the geographical evolutionary unit, secondary speciation would have independently and locally taken place. The clades I, II, and III observed in the present study may be regarded as typical examples of geographical evolutionary units; particularly the clades II and III demonstrated obviously geographical bias (Fig. 3). Therefore, in the further study, to establish reliable scenario on systematics and phylogeny of *Petaurista*, it should be carefully consider two steps, namely, the detection of geographical evolutionary units and the analysis of phylogenetic relationships within each unit.



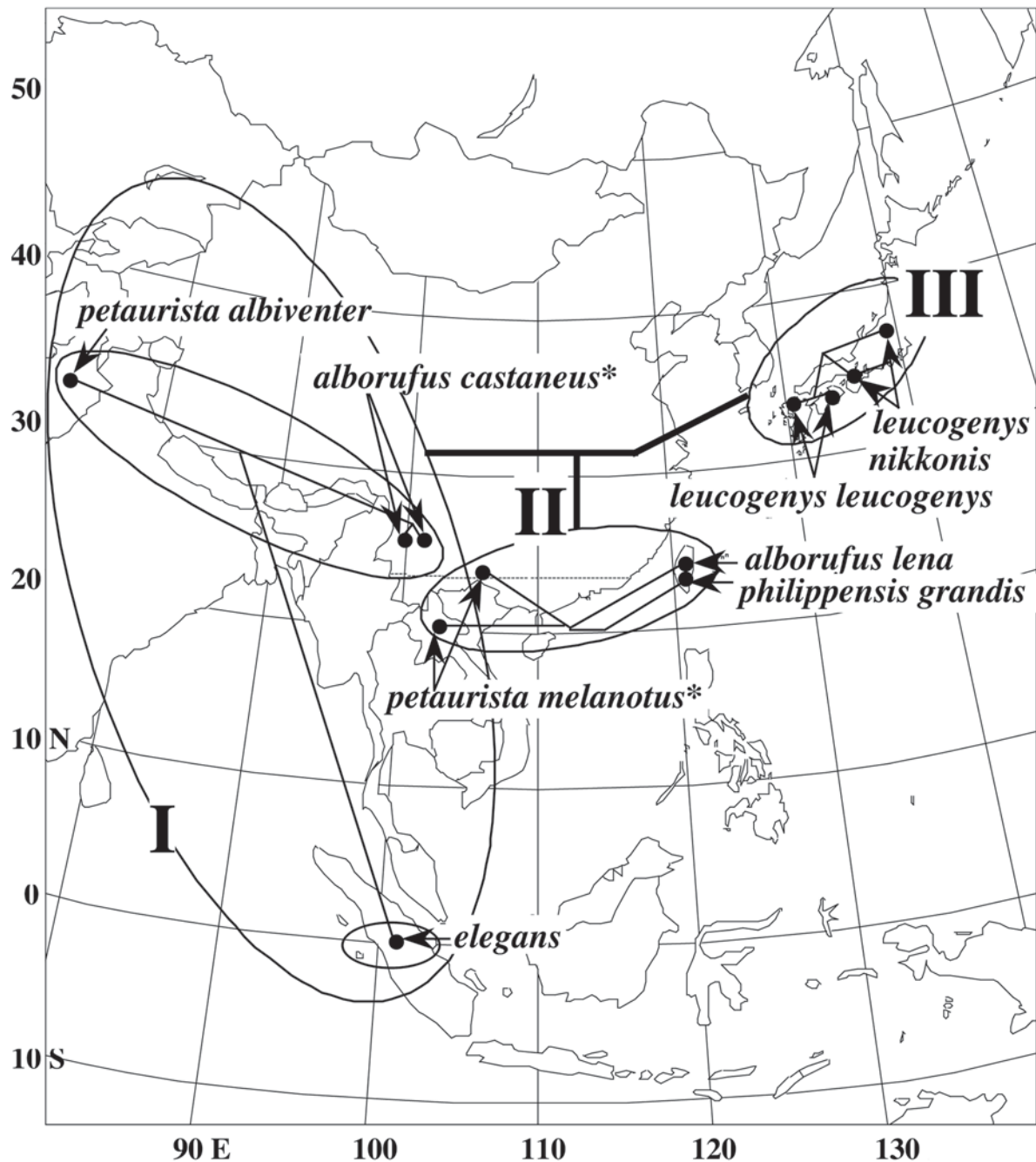


Figure 3. Correlation between the geographic distribution and molecular phylogeny of *Petaurista*. Asterisks mean the samples unknown exact collecting locality, and their localities were roughly plotted on the basis of present distribution pattern.

**ACKNOWLEDGMENTS.** We would like to thank Mr. L.-S. Tzen, Dr. K. Ikeda (Fukuoka Prefecture Forest Research and Extension Center), Mr. T. Okayama (Omogo Mountain Museum), Drs. K. Gouda and M. Satoh (Noboribetsu Bear Park), Ms. Y.-J. Chen (National Museum of Natural Science), and Dr. Suhardi and technical staffs of Faculty of Forestry, Gadjah Mada University for supplying specimens. This study was partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture, Japan.

## References

- Anderson S., Bankier A.T., Barrel B.G., de Bruijn M.H.L., Coulson A.R., Drouin J., Eperon I.C., Nierlich D.P., Roe B.A., Sanger F., Schreier P.H., Smith A.J.H., Staden R. & Young I.G. 1981. Sequence and organization of the human mitochondrial genome//Nature. Vol.290. No.5806. P.457-465.
- Baba M., Doi T. & Ono Y. 1982. Home range utilization and

- nocturnal activity of the giant flying squirrel, *Petaurista leucogenys* // Japanese Journal of Ecology. Vol.32. No.2. P.189–198.
- Chang W. 1985. Ecological study of the flying squirrel in central Taiwan // Proceedings Seminar on the Control of Squirrel Damage to Forest Trees, Council of Agriculture, Forestry Series. Vol.2. P.45–68 [in Chinese, with English abstract].
- Corbet G.B. & Hill J.E. 1980. A World List of Mammalian Species. First Edition. London: Cornell University Press. 226 p.
- Corbet G.B. & Hill J.E. 1991. A World List of Mammalian Species. Third Edition. Oxford: Oxford University Press. 243 p.
- Corbet G.B. & Hill J.E. 1992. The Mammals of the Indomalayan Region: A Systematic Review. Oxford: Oxford University Press. 488p.
- Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap // Evolution. Vol.39. No.4. P.783–791.
- Hendy M.D. & Penny D. 1982. Branch and bound algorithms to determine minimal evolutionary trees // Mathematical Biosciences. Vol.59. No.2. P.277–290.
- Hsu V. 1990. Seismicity and tectonics of a continent-island arc collision zone at the island of Taiwan // Journal of Geophysical Research. Vol.95. No.4. P.4725–4734.
- Irwin D.M., Kocher T.D. & Wilson A.C. 1991. Evolution of the cytochrome *b* gene of the mammals // Journal of Molecular Evolution. Vol.32. No.2. P.128–144.
- Imaizumi Y. & Miyamoto T. 1960. Colored Illustrations of the Mammals of Japan. Osaka: Hoikusha. 196 p. [in Japanese].
- Kano T. 1940. Zoogeographic Studies of Tsugitaka Mountains of Formosa. Tokyo: Institute Ethnogeographical Researchers. 145 p.
- Kawamura Y. 1988. Quaternary rodent faunas in the Japanese Islands (Part 1) // Memoirs of the Faculty of Science, Kyoto University, Series Geology and Mineralogy. Vol.53. Nos.1–2. P.31–348.
- Kawamura Y. 1990. The origin of rodents in Japan, based on fossil records // Abstract of Annual Meeting of the Mammalogical Society of Japan. Tokyo: Mammalogical Society of Japan. P.70 [in Japanese].
- Kawamura Y., Kamei T. & Taruno H. 1989. Middle and Late Pleistocene mammalian faunas in Japan // Quaternary Research. Vol.28. No.4. P.317–326 [in Japanese with English abstract].
- Lee P.-F., Progulsk D.R. & Lin Y.-S. 1986. Ecological studies on two sympatric *Petaurista* species in Taiwan // Bulletin of the Institute of Zoology, Academia Sinica. Vol.25. No.2. P.113–124.
- Lekagul B. & McNeely J.A. 1988. Mammals of Thailand. Second Edition. Bangkok: Darnsutha Press. 758 p.
- Lin J.-Y. & Lin L.-K. 1983. A note on the zoogeography of the mammals in Taiwan // Science Annals of the (Taiwan) Provincial Museum. Vol.26. P.53–62 [in Chinese, with English abstract].
- Liu D.-S. & Ding M.-L. 1984. The characteristics and evolution of the palaeoenvironment of China since the late Tertiary // White R.O. (ed.). The Evolution of the East Asian Environment. Hong Kong: Center of Asian Studies, University of Hong Kong. P.11–40.
- Mercer J.M. & Roth V.L. 2003. The effects of Cenozoic global change on squirrel phylogeny // Science. Vol.299. No.5612. P.1568–1572.
- Oshida T., Fujita Y., Lin L.-K. & Masuda R. 2001a. A preliminary note on phylogenetic position of the lesser giant flying squirrel *Petaurista elegans* in the genus *Petaurista*, inferred from mitochondrial cytochrome *b* gene sequence // Mammal Study. Vol.26. No.2. P.149–152.
- Oshida T., Ikeda K., Yamada K. & Masuda R. 2001b. Phylogeography of the Japanese giant flying squirrel *Petaurista leucogenys* based on mitochondrial DNA control region sequences // Zoological Science. Vol.18. No.1. P.107–114.
- Oshida T., Lin L.-K., Masuda R. & Yoshida M.C. 2000a. Phylogenetic relationships among Asian species of the genus *Petaurista*, inferred from mitochondrial cytochrome *b* gene sequences // Zoological Science. Vol.17. No.1. P.123–128.
- Oshida T., Masuda R. & Yoshida M.C. 1996. Phylogenetic relationships among Japanese species of the family Sciuridae (Mammalia, Rodentia), inferred from nucleotide sequences of mitochondrial 12S ribosomal RNA genes // Zoological Science. Vol.13. No.4. P.615–620.
- Oshida T., Satoh H. & Obara Y. 1992. A preliminary notes on the karyotypes of giant flying squirrels *Petaurista alborufus* and *P. petaurista* // Journal of Mammalogical Society of Japan. Vol.16. No.2. P.59–69.
- Oshida T., Obara Y., Lin L.-K. & Yoshida, M. C. 2000b. Comparison of banded karyotypes between two subspecies of the red and white giant flying squirrel *Petaurista alborufus* (Mammalia, Rodentia) // Caryologia. Vol.53. No.3–4. P.261–267.
- Piaggio A.J. & Spicer G.S. 2001. Molecular phylogeny of the chipmunks inferred from mitochondrial cytochrome *b* and cytochrome oxidase II gene sequences // Molecular Phylogenetics and Evolution. Vol.20. No.3. P.335–350.
- Posada D. & Crandall K.A. 1998. Modeltest: testing the model of DNA substitution // Bioinformatics. Vol.14. No.9. P.817–818.
- Randi E., Lucchini V., Hennache A., Kimball R.T., Braun E.L. & Ligon D. 2001. Evolution of the mitochondrial DNA control region and cytochrome *b* genes and the inference of phylogenetic relationships in the avian genus *Lophura* (Galliformes) // Molecular Phylogenetics and Evolution. Vol.19. No.2. P.187–201.
- Roberts T.J. 1997. The Mammals of Pakistan. Second Edition. Oxford: Oxford University Press. 525 p.
- Sambrook J., Fritsch E.F. & Maniatis T. 1989. Molecular Cloning: A Laboratory Manual. Second Edition. New York: Cold Spring Harbor Laboratory Press.
- Swinhoe R. 1870. Catalogue of the mammals of China // Proceedings of the Zoological Society of London. P.615–653.
- Swofford D.L. 2001. PAUP\* Phylogenetic Analysis Using Parsimony (\*and other Methods). Version 4.0 beta version. Sinauer, Sunderland, MA.
- Tamura K. & Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees // Molecular Biology and Evolution. Vol.10. No.3. P.512–526.
- Thomas O. 1923. Geographical races of *Petaurista alborufus* // Annals and Magazine of Natural History, Series 9. Vol.12. P.171–172.
- Wroughton R.C. 1911. Oriental flying squirrels of the *Pteromys* group // Journal of the Bombay Natural History Society. Vol.20. P.1012–1023.