Phylogenetic Relationships among Asian species of Petaurista (Rodentia, Sciuridae), Inferred from Mitochondrial Cytochrome b Gene Sequences

Authors: Oshida, Tatsuo, Lin, Liang-Kong, Masuda, Ryuichi, and Yoshida, Michihiro C.

Source: Zoological Science, 17(1) : 123-128

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.17.123
Phylogenetic Relationships among Asian species of *Petaurista* (Rodentia, Sciuridae), Inferred from Mitochondrial Cytochrome *b* Gene Sequences

Tatsuo Oshida1*, Liang-Kong Lin2, Ryuichi Masuda1,3 and Michihiro C. Yoshida1,3

1Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan, 2Laboratory of Wildlife Ecology, Department of Biology, Tunghai University, Taichung Taiwan 407, R. O. C., and 3Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan

ABSTRACT—To elucidate the phylogenetic relationships among four species belonging to the genus *Petaurista* (*P. alborufus castaneus, P. alborufus lena, P. leucogenys leucogenys, P. leucogenys nikkonis, P. petaurista melanotus, and P. philippensis grandis*), we investigated the partial sequences (1,068 bp) of the mitochondrial cytochrome *b* gene for these giant flying squirrels. Phylogenetic trees (NJ, MP, and ML trees) constructed from cytochrome *b* sequences indicated that *P. leucogenys* was grouped independently with other species, and that *P. philippensis* was most closely related to *P. petaurista* with 99–100% bootstrap values. In addition, two subspecies of *P. alborufus* did not form a single clade: *P. alborufus castaneus* from China was most distantly related to the other species, whereas *P. alborufus lena* from Taiwan was closely related to *P. petaurista* and *P. philippensis* with 82–90% bootstrap values. This result suggests that it is reasonable to regard *P. alborufus lena* as a distinct species from *P. alborufus castaneus*.

INTRODUCTION

Flying squirrels belonging to the genus *Petaurista* had been classified traditionally into five species: *P. alborufus, P. elegans, P. leucogenys, P. magnificus*, and *P. petaurista* (Corbet and Hill, 1980), each of which was intricately divided into various subspecies (Lekagul and McNeely, 1988). Recently, Corbet and Hill (1991, 1992) renewed the classification and recognized five additional species: *P. caniceps, P. nobilis, P. philippensis, P. sybilla*, and *P. xanthotis*, from five species classified previously (Corbet and Hill, 1980). Such classification disrupts phylogenetic study of the giant flying squirrels. Oshida et al. (1992) investigated the karyotaxonomy of *Petaurista* and concluded that *P. petaurista melanotus* was more closely related to *P. alborufus lena* than to *P. petaurista grandis* (*P. philippensis grandis*). In addition, Oshida et al. (1996) examined the mitochondrial 12S ribosomal RNA sequences on *P. leucogenys* from Japan, *P. petaurista* from Laos (*P. petaurista melanotus*), and *P. petaurista* from Taiwan (*P. philippensis grandis*), and reported that two subspecies of *P. petaurista* were closely related to each other and that *P. leucogenys* could have early diverged from *P. petaurista*.

Mitochondrial DNA (mtDNA) is a valuable molecule in investigating the phylogenetic relationships among populations, subspecies, and species. Features of mtDNA such as the maternal inheritance and rapid evolutionary rate advance the rapid geographic sorting of haplotypes in the absence of gene flow (Avise et al., 1984). Accordingly, using the information of mtDNA, we are able to infer the interspecific relationships, the intraspecific situations of population subdivision, and the genetic differentiation beyond the resolving ability of non-molecular approaches. In the present study, we determined partial sequences (1,068 base pairs: bp) of the mitochondrial cytochrome *b* gene for four species: *P. alborufus, P. leucogenys, P. petaurista*, and *P. philippensis*, and discuss the phylogenetic relationships among them as well as the taxonomic status of *P. alborufus* from continental China and Taiwan.

MATERIALS

Flying squirrels examined in the present study are shown in Table 1. Classification of species and subspecies followed the description of Corbet and Hill (1991, 1992), Imaizumi (1960), and Lekagul and McNeely (1988). Two samples of *P. alborufus lena* and one individual of *P. philippensis grandis* were captured in central Taiwan. Three individuals of *P. alborufus castaneus* imported from Hong-Kong to Japan in 1996 were commercially obtained. Muscle tissues of two individuals of *P. leucogenys leucogenys* were provided from Mr. Koichi Ikeda of the Fukuoka Prefecture Forest Research and Extension Center, Fukuoka, Japan, and Mr. Takehito Okayama of the Omogo...
Table 1. Species of the genera Petarista and Pteromys examined in this study

<table>
<thead>
<tr>
<th>Species name</th>
<th>Code</th>
<th>Common name</th>
<th>Collecting locality</th>
<th>Accession No. of sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petarista alborufus castaneus</td>
<td>ALC1</td>
<td>Red and white giant flying squirrel</td>
<td>southern China</td>
<td>AB023898</td>
</tr>
<tr>
<td></td>
<td>ALC2</td>
<td></td>
<td></td>
<td>AB023899</td>
</tr>
<tr>
<td></td>
<td>ALC3</td>
<td></td>
<td></td>
<td>AB023900</td>
</tr>
<tr>
<td>Petarista alborufus lena</td>
<td>ALL1</td>
<td></td>
<td>Nantou, Taiwan</td>
<td>AB023901</td>
</tr>
<tr>
<td></td>
<td>ALL2</td>
<td></td>
<td>Huaiian, Taiwan</td>
<td>AB023902</td>
</tr>
<tr>
<td>Pteromys leucogenys leucogenys</td>
<td>LE1</td>
<td>Japanese giant flying squirrel</td>
<td>Ehime Pref., Japan</td>
<td>AB023903</td>
</tr>
<tr>
<td></td>
<td>LE2</td>
<td></td>
<td>Fukukura Pref., Japan</td>
<td>AB023904</td>
</tr>
<tr>
<td>Pteromys leucogenys nikkonis</td>
<td>LN1</td>
<td></td>
<td>Wakayama Pref., Japan</td>
<td>AB023905</td>
</tr>
<tr>
<td></td>
<td>LN2</td>
<td></td>
<td>Aomori Pref., Japan</td>
<td>AB023906</td>
</tr>
<tr>
<td>Petarista petarista melanotus</td>
<td>PEM1</td>
<td>Red giant flying squirrel</td>
<td>Laos</td>
<td>AB023908</td>
</tr>
<tr>
<td></td>
<td>PEM2</td>
<td></td>
<td>southern China</td>
<td>AB023909</td>
</tr>
<tr>
<td>Pteromys philipesis grandis</td>
<td>PHG</td>
<td>Indian giant flying squirrel</td>
<td>Nantou, Taiwan</td>
<td>AB023907</td>
</tr>
<tr>
<td>Pteromys volans orii (out-group)</td>
<td>PVO</td>
<td>Russian (Siberian) flying squirrel</td>
<td>Hokkaido, Japan</td>
<td>AB023910</td>
</tr>
</tbody>
</table>

*Sequence data will appear in the DDBJ nucleotide sequence databases with accession numbers.

Table 2. Pairwise comparisons of cytochrome b nucleotide sequences (1,068 bp) between 13 flying squirrel specimens

<table>
<thead>
<tr>
<th></th>
<th>ALC1</th>
<th>ALC2</th>
<th>ALC3</th>
<th>ALL1</th>
<th>ALL2</th>
<th>LE1</th>
<th>LE2</th>
<th>LEN1</th>
<th>LEN2</th>
<th>PEM1</th>
<th>PEM2</th>
<th>PHG</th>
<th>PVO</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALC1</td>
<td>0.0</td>
<td>0.3</td>
<td>0.1</td>
<td>16.8</td>
<td>16.7</td>
<td>13.9</td>
<td>14.3</td>
<td>14.3</td>
<td>14.1</td>
<td>13.7</td>
<td>13.9</td>
<td>13.9</td>
<td>21.5</td>
</tr>
<tr>
<td>ALC2</td>
<td>0.4</td>
<td>0.7</td>
<td>0.4</td>
<td>16.9</td>
<td>16.8</td>
<td>14.3</td>
<td>14.6</td>
<td>14.6</td>
<td>14.5</td>
<td>13.9</td>
<td>14.1</td>
<td>14.2</td>
<td>21.5</td>
</tr>
<tr>
<td>ALC3</td>
<td>0.4</td>
<td>0.8</td>
<td>0.7</td>
<td>16.9</td>
<td>16.8</td>
<td>14.0</td>
<td>14.4</td>
<td>14.4</td>
<td>14.3</td>
<td>13.4</td>
<td>14.0</td>
<td>14.0</td>
<td>21.6</td>
</tr>
<tr>
<td>ALL1</td>
<td>123</td>
<td>133</td>
<td>133</td>
<td>134</td>
<td>134</td>
<td>134</td>
<td>140</td>
<td>14.6</td>
<td>14.5</td>
<td>12.4</td>
<td>12.2</td>
<td>13.3</td>
<td>23.8</td>
</tr>
<tr>
<td>ALL2</td>
<td>111</td>
<td>111</td>
<td>111</td>
<td>112</td>
<td>112</td>
<td>112</td>
<td>119</td>
<td>15.0</td>
<td>15.0</td>
<td>12.4</td>
<td>12.2</td>
<td>13.3</td>
<td>23.8</td>
</tr>
<tr>
<td>LE1</td>
<td>122</td>
<td>122</td>
<td>122</td>
<td>122</td>
<td>122</td>
<td>122</td>
<td>116</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
<td>21.6</td>
</tr>
<tr>
<td>LEN1</td>
<td>113</td>
<td>113</td>
<td>113</td>
<td>114</td>
<td>114</td>
<td>114</td>
<td>117</td>
<td>15.1</td>
<td>15.1</td>
<td>14.4</td>
<td>14.4</td>
<td>14.4</td>
<td>20.6</td>
</tr>
<tr>
<td>LEN2</td>
<td>112</td>
<td>112</td>
<td>112</td>
<td>112</td>
<td>112</td>
<td>112</td>
<td>112</td>
<td>14.4</td>
<td>14.4</td>
<td>14.9</td>
<td>14.9</td>
<td>14.5</td>
<td>20.6</td>
</tr>
<tr>
<td>PEM1</td>
<td>108</td>
<td>109</td>
<td>109</td>
<td>110</td>
<td>110</td>
<td>110</td>
<td>116</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
<td>0.5</td>
</tr>
<tr>
<td>PEM2</td>
<td>110</td>
<td>112</td>
<td>112</td>
<td>112</td>
<td>112</td>
<td>112</td>
<td>113</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
<td>0.5</td>
</tr>
<tr>
<td>PHG</td>
<td>112</td>
<td>114</td>
<td>114</td>
<td>113</td>
<td>113</td>
<td>113</td>
<td>113</td>
<td>14.9</td>
<td>14.9</td>
<td>14.9</td>
<td>14.9</td>
<td>14.9</td>
<td>20.9</td>
</tr>
<tr>
<td>PVO</td>
<td>129</td>
<td>129</td>
<td>129</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
</tr>
</tbody>
</table>

Data above the diagonal present percentage differences between specimens. Data below the diagonal are the numbers of nucleotide substitutions (transitions/transversions).
ences and numbers of transversions and transitions obtained from pairwise comparison. In addition, to estimate divergence between species, the transversal substitutions at the third codon positions were obtained by pairwise comparison (Table 3). Phylogenetic trees reconstructed using the NJ and the MP methods indicated essentially the same branching patterns (Figs. 1a and b): the first dichotomy isolated _P. alborufus castaneus_ from the other flying squirrels and then _P. leucoogenys_ split from a clade formed by _P. alborufus lena_, _P. philippensis_, and _P. petaurista_. Consequently, the giant flying squirrels analyzed in the present study formed three groups: _P. alborufus castaneus_, _P. leucoogenys_, and the other species or subspecies (82–90% bootstrap values). Although the branching order in the phylogenetic tree constructed with the ML method (Fig. 1c) was different from those of NJ and MP trees, three groups recognized in NJ and MP trees were obviously observed in ML tree (82-100% bootstrap values). However, the bootstrap values to support the branching orders of the groups were low: 34% in NJ tree (Fig. 1a), 75% in MP tree (Fig. 1b), and 43% in ML tree (Fig. 1c). Owing to these low bootstrap values and the branching order differences between NJ and MP trees and ML tree, their phylogenetic relationships were not obvious in the present study.

**Phylogeny of _Petaurista alborufus_**

_P. alborufus_ from southern China, Taiwan, Burma, and Thailand had been divided into seven subspecies: _barroni_ (Kloss, 1916; Ellerman and Morrison-Scott, 1951), _castaneus_ (Thomas, 1923), _candidula_ (Wroughton, 1911; Ellerman and Morrison-Scott, 1951), _lena_ (Thomas, 1907), _leucocephalus_ (Hilzheimer, 1905), _ochraspis_ (Thomas, 1923), and _taylori_ (Thomas, 1914; Ellerman and Morrison-Scott, 1951). However, Corbet and Hill (1992) have recognized only four variations as subspecies of _P. alborufus_: _castaneus_, _lena_, _leucocephalus_, and _ochraspis_. In the present study, it is noteworthy that _P. alborufus castaneus_ was distantly related to _P. alborufus lena_ (Fig. 1). Based on palaeoecological characteristics, _P. alborufus lena_ was once treated as a distinct species _P. pectoralis_ (Swinhoe, 1870). In addition, Corbet and Hill (1992) also suggested that _P. alborufus lena_ is distinct enough to merit specific rank. Phylogenetic trees (Fig. 1) obtained in the present study did not conflict with the phylogenetic position of the form _lena_ proposed by Swinhoe (1870) and Corbet and Hill (1992). Assuming that _castaneus_ distributed in the mainland is a representative subspecies of _P. alborufus_, _lena_ living only in Taiwan may be regarded to be distinct from _P. alborufus_. Moreover, _P. alborufus lena_ was closely related to the clade of _P. petaurista_ and _P. philippensis_ with high bootstrap values (82% in NJ tree of Fig. 1a, 90% in MP tree of Fig. 1b, and 82% in ML tree of Fig. 1c). Based on the morphological characteristics such as external and dental forms, Corbet and Hill (1992) regarded three subspecies ( _barroni, candidula_, and _taylori_ ) of _P. alborufus_ as _P. petaurista_. _P. alborufus_ has often been confused with _P. petaurista_ owing to the complicated morphological variation of these species. Based on the chromosomal characteristics, Oshida et al. (1992) have reported that _P. alborufus lena_ was more closely related to _P. petaurista melanotus_ than to _P. petaurista grandis_ ( _P. philippensis grandis_). The present molecular findings support their view.

Hsu (1990) reported that the Taiwan island rose from the sea floor on the Eurasian Continent approximately 4.0 Myr ago. Moreover, based on the faunistic and geological analyses, Kano (1940) and Liu and Ding (1984) concluded that the Taiwan island had been connected with the Eurasian Continent at least twice due to the glacial eustacy, initially during the Pliocene and subsequently during the Pleistocene. It is likely that the multiple faunistic exchanges between the Taiwan island and the Eurasian Continent had occurred through these connections. Lin and Lin (1983) explained the complicated zoogeographical exchanges of Taiwanese mammals as follows: from a paleoenvironmental point of view, the first mammal group which immigrated from the Eurasian Continent to the Taiwan island during the glacial period of the Pliocene had adapted themselves to the cold environment. However, after the glacial period, to avoid the environment being warm, they had to move to the high elevation areas of Taiwan. Subsequently, the mammal group which immigrated to the island during the
glacial periods of the Pleistocene had succeeded in expanding their ranges throughout the low elevation areas of Taiwan.

*P. alborufus lena* inhabits areas with elevation of 1,200 to 3,750 meters above sea level (Chang, 1985). In contrast, the distribution area of *P. philippensis grandis* living in Taiwan widely ranges from 700 to 2,600 meters above sea level (Chang, 1985). Accepting the hypothesis of Lin and Lin (1983), it is likely that the incursion of *P. alborufus lena* was earlier than that of *P. philippensis grandis*. In the present study, by using the available divergence rate estimated from the mammalian cytochrome b genes (Irwin et al., 1991) and our data.

Fig. 1. Phylogenetic trees constructed by (a) the neighbor-joining (NJ), (b) the maximum parsimony (MP), and (c) the maximum likelihood (ML) methods based on the cytochrome b nucleotide sequences. Scale bars for the NJ and the ML trees represent branch length in terms of nucleotide substitutions per site. Numbers above branches indicate bootstrap values (%) derived from 1,000 replications for NJ and MP trees and 100 replications for ML tree.
(Table 3), the divergence between P. alborufus lena and P. petaurista melanotus and that between P. alborufus lena and P. philippensis grandis were estimated to have occurred approximately 6.2–6.8 Myr ago and 7.4–7.8 Myr ago, respectively. It seems reasonable to suppose that, after the deviation from the lineages of P. petaurista and P. philippensis in the Eurasian Continent during the late Miocene, the lineages of P. alborufus lena immigrated to Taiwan island and adapted itself to the alpine region in Taiwan. P. alborufus lena might have evolved independently from other Petaurista species due to the geographic isolation. Thus, our results support that this giant flying squirrel should be treated as a distinct species, as described originally by Swinhoe (1870) and Thomas (1907).

Phylogeny of Petaurista leucogenys

P. leucogenys is indigenous to the Japanese main islands except for Hokkaido (Corbet and Hill, 1991). Although Corbet and Hill (1980) described that P. leucogenys is distributed on the Japanese islands and central China, recently they changed the classification and newly treated the leucogenys population of central China as a distinct species P. xanthotis (Corbet and Hill, 1991, 1992). Based on the poor information about the pelage, Imaizumi (1960) classified P. leucogenys into three subspecies: leucogenys, nikkonis, and oreas, although this classification of subspecies has not been generally accepted. In the present study, P. leucogenys clearly formed a single clade with high bootstrap values (100% in NJ, MP, and ML trees). Meanwhile, the divergence time estimated from our data (Table 3) was approximately 10.2–10.6 Myr ago between P. leucogenys and P. petaurista, 8.4–9.0 Myr ago between P. leucogenys and P. philippensis, 9.0–10.2 Myr ago between P. leucogenys and P. alborufus lena, and 9.0–9.6 Myr ago between P. leucogenys and P. alborufus castaneus. Although it is hard to determine which species is most closely related to P. leucogenys, this species could be an independent lineage in the genus Petaurista at least in the late Miocene. Based on the fossil records, Kawamura (1988, 1990) and Kawamura et al. (1989) showed that P. leucogenys immigrated from the Eurasian Continent to the Japanese islands through the land bridges around the early to the middle Pleistocene, and that this species had been isolated due to the separation of the Japanese islands from the Eurasian Continent in the Pleistocene. Accepting Kawamura’s hypothesis, our results suggest that an ancestral stock of P. leucogenys had diverged from the other Petaurista species within the Eurasian Continent prior to its immigration to Japan.

Phylogeny of Petaurista petaurista and P. philippensis

P. petaurista melanotus and P. philippensis grandis formed a single clade with high bootstrap values (100% in NJ and MP trees and 99% in ML tree, Fig. 1). P. petaurista, which is one of the most dominant species in the genus Petaurista, is distributed throughout southern parts of the Eurasian Continent and Southeast Asia (Corbet and Hill, 1980; Lekagul and McNeely, 1988). On the other hand, P. philippensis had been treated as a subspecies of P. petaurista until Corbet and Hill (1991, 1992) established it as a distinct species. A Taiwanese form (P. philippensis grandis) examined here was previously considered as P. petaurista grandis by Swinhoe (1870). Based on sequence data of the 12S rRNA gene, Oshida et al. (1996) reported that the genetic distance between P. philippensis grandis (P. petaurista from Taiwan) and P. petaurista melanotus (P. petaurista from Laos) was almost parallel to intraspecific differences within P. leucogenys. Cytochrome b data in the present study supported that, although the genetic distance between P. philippensis grandis and P. petaurista melanotus corresponded to approximately twice of intraspecific differences within P. leucogenys (Fig. 1a), P. philippensis is most closely related to P. petaurista.

ACKNOWLEDGMENTS

We would like to thank Mr. L. S. Tzen, Mr. K. Ikeda (Fukuoka Prefecture Forest Research and Extension Center), Mr. T. Okayama (Omogo Mountain Museum), and Dr. K. Gouda and Dr. M. Satoh (Noboribetsu Bear Park) for supplying specimens. We thank Dr. T. Tanaka-Ueno (Kyoto University) for invaluable suggestion on the phylogenetic analysis. This study was partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture.

REFERENCES


Hsu V (1990) Seismicity and tectonics of a continent-island arc collision zone at the island of Taiwan. J Geophysical Res 95 (B4);
4725–4734


Kano T (1940) Zoogeographic Studies of Tsugitaka Mountains of Formosa. Institute Ethnogeographical Researchers, Tokyo, pp 1–145


Thomas O (1914) Scientific results from the mammal survey VIII. J Bombay Nat Hist Soc 23: 197–205


(Received June 4, 1999 / Accepted August 9, 1999)