Cranial morphometric study of four giant flying squirrels (Petaurista) (Rodentia: Sciuridae) from China

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Abstract: The present study revisited the controversial taxonomic status of Petaurista yunanensis, P. philippensis, P. hainana, and P. petaurista by using a considerably extended set of morphometrical characters (26 cranial variables from 60 adult specimen skulls). The results revealed no sexual dimorphism in any of the four species but confirmed significant craniometric differences among the four species in both the principal components analysis (PCA) and discriminant function analysis (DFA), with the greatest distinction observed between P. petaurista and other Petaurista species. Both univariate and multivariate analysis indicated that the morphological differences between P. yunanensis and P. philippensis were less than that between P. philippensis and P. hainana. The morphometric results were concordant in geographic patterns with mtDNA data from previous studies and indicated that P. petaurista, P. hainana, P. philippensis, and P. yunanensis could be recognized as valid species.

Key words: Petaurista; Cranial variables; Statistical analysis; Species

中国四种鼯鼠的头骨形态学

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摘 要: 针对长期以来有关鼯鼠分类地位的争议，该研究基于查看、测取 60 号鼯鼠成体头骨(每号头骨测取 26 个可量性状)共计 1560 个数据，运用多变量、单变量分析方法，对鼯鼠属(Petaurista)中的 P. yunanensis, P. philippensis, P. hainana 及 P. petaurista 头骨可测量数据进行了统计学分析，以探讨上述 4 种鼯鼠的头骨形态差异以及 P. yunanensis 和 P. hainana 的分类地位。结果揭示: (1)上述可测量头骨性状在该 4 种鼯鼠中不存在性二型现象; (2)上述 4 种鼯鼠在所测量的头骨性状中两两间均存在显著差异; (3)P. philippensis 与 P. hainana 之间的头骨形态差异程度大于 P. yunanensis 与 P. philippensis 之间的差异。该结果在宏观统计分析水平上为上述 4 种鼯鼠的种地位有效性提供了佐证，与前人基于分子水平(mtDNA)的种地位有效性研究结果相似。

关键词: 鼬鼠属; 头骨变量; 统计分析; 物种


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of these species are referenced with very few specimens (Allen, 1940; Corbet & Hill, 1992; Ellerman, 1940; Hoffmann et al, 1993; Wang, 2003; Zhang et al, 1997). However, several of these species are referenced with very few specimens or based solely on skins with no corresponding skulls (Allen, 1940; Ellerman, 1940), and some are actually the synonyms or subspecies of either the P. petaurista complex or the P. philippensis complex due to inraspecific geographic variations across their distributions in Asia.

Corbet & Hill (1992) treated P. albiventer in Pakistan and southwest China as the synonym of P. petaurista and recognized P. philippensis as a distinct species consisting of many forms formerly assigned to P. petaurista, including forms distributed in Taiwan (P. grandis), southwest Yunnan (P. yunanensis), and Hainan (P. hainana). After comparing the pelage and cranial characteristics of P. petaurista and P. hainana, Huang et al (1995) considered P. hainana to be a valid species, but Wang (2003) treated P. hainana as a subspecies of P. yunanensis. Thorington & Hoffmann (2005) treated all Petaurista forms as eight valid species instead of nine as suggested by Corbet & Hill (1992), but they accepted the specific validity of P. philippensis and the subspecies status of P. yunanensis and P. hainana. Patterns of genetic variations observed in the complex of P. philippensis based on cytochrome b genes indicated that P. hainana, P. albiventer, and P. yunanensis could be distinct species (Yu et al, 2006). Some forms included in P. philippensis warranted separate specific rank based on molecular data (Oshida et al, 2000a, b; Yu et al, 2006), but without further evidence from morphometric data, much remains to be done to ascertain conclusively these specific conclusions.

Most recent phylogenetic studies have focused on molecular data analysis, but tracing changes in morphological characters is also an important way to evaluate the distribution of the characters on which those taxonomic units are based. Morphometric data are important to understand biological phenomena and have been used to evaluate cranial, dental, and body measurements of many mammals (Muñoz-Muñoz & Perpinan, 2010; Slábová & Frynta, 2007; Zelditch et al, 2004). Quantitative analysis of intra- and inter-specific variations at the morphological level is useful for detecting patterns of geographic variations and delimiting intra- or inter-specific evolutionary units. To date, however, there are currently no published reports of quantitative analysis based on morphological characteristics that would allow the identification of the morphotypes in the complex of P. philippensis and P. petaurista.

To discuss the taxonomic relationships of P. philippensis, P. yunanensis, P. hainana, and P. petaurista and test previous taxonomic hypotheses, the present study conducted a comprehensive morphometric study on the above Chinese Petaurista species based on samples subsequently collected from southwest Yunnan and the Island of Hainan, China. Multivariate analyses were used to produce an overview of the associations between morphological variables and species patterns and discuss the taxonomic implications of these flying squirrels. Our morphometric study could be complementary to studies of variations of DNA sequences in flying squirrels.

1 Materials and Methods

1.1 Specimens and data collection

According to the taxonomic assignments of Allen (1940) and Zhang et al (1997), a total of 60 intact adult skull specimens of P. petaurista, P. yunanensis, P. hainana, and P. philippensis were examined for morphometric study (Append. I). These specimens are from the Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ, CAS) (Kunming, China), the Institute of Zoology, Chinese Academy of Sciences (IOZ, CAS) (Beijing, China), and the Guangdong Entomological Institute (GDEI) (Ganzhou, China).

Twenty-six cranial variables taken with a digital caliper to the nearest 0.01 mm were used in the morphometric analysis as described by Musser (1979), Musser & Heaney (1992), Xia et al (2006), and Yang et al (2005), and depicted in Fig. 1 following Huang’s description (1995). The variables measured included: maximum length of skull (GLS), condylobasal length (CBL), basal length (BL), occipito-nasal length (ONL), palatal length (PL), length of palatal bridge (PBL), length of upper tooth row (LUTR), length of upper molars (LUM), maximum upper molars breadth (GUMB), rostral length (ROL) and breadth (ROB), auditory bulla length (ABL) and breadth (ABB), breadth of zygomatic plate (BZP), breadth of occipital condyles.
(BOO), height of occipital (HO), zygomatic breadth (ZOB), mastoid breadth (MTB), nasal length (NL) and breadth (BN), mandible length (ML), height of mandible (THM), length of lower diastema (LLD), length of lower molar row (LLMR), length of lower tooth row (LTR), and mandibular height (MH). In addition, the head and body length (HB), tail length (TL), hind foot length (HFL), and ear length (EL), which were compared to the original measurements labeled on the skins by the collectors.

1.2 Data analysis

Statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). All variables were transformed into logarithms to eliminate the bias effect of large measurements in multivariate analysis (D’Elía & Pardiñas, 2004). Statistical differences were considered significant at $P<0.05$. In this study, all related data were subjected to one-way ANOVA for calculating mean±SD. T-test was used to assess the sexual dimorphism between male and female groups by comparing the group means of cranial measurements. Multiple comparisons between taxa were made for all 26 cranial measurements to evaluate variations between samples. Multivariate analyses, including principal components analysis (PCA) and discriminant function analysis (DFA), were carried out to evaluate the degree of similarity and dissimilarity in cranial structures between the putative species and to determine how the taxa were related when all cranial characters measured are considered simultaneously.

The PCA is based upon the variance-covariance matrix of the log-transformed variables. The eigenvector scores describing the relative significance of each variable to the principal components were used to compare the cranial morphological similarities and differences. The PCA scatter-plot visually represented the variation among different individuals of the samples. The DFA was performed to investigate the integrity of the pre-defined groups and to predict group membership of specimens with the linear models of variables. Based on the derived discriminant functions, each individual was allocated to the group with nearest centroid, and the proportion of individuals allocated to each group was calculated.

2 Results

Mean±SD of 4 external and 26 cranial variables for the four taxa are presented in Tab. 1.
2.1 Univariate analysis

Univariate comparison revealed that the means of all variables were significantly different and, in general, tended to become progressively larger from *P. petaurista*, *P. hainana*, *P. yunanensis*, to *P. philippensis*. The t-tests of Equality of Group Means on 54 (30 males, 24 females) out of 60 specimens indicated there was no sexual dimorphism in the 26 cranial variables in the four *Petaurista* groups (Tab. 2). Quantitative pairwise comparisons of all cranial variables between taxa indicated that *P. yunanensis* was morphologically similar to *P. philippensis*, with 11 cranial measurements showing no significant difference (*P*>0.05) (Tab. 3).

2.2 Multivariate analysis

In PCA, the eigenvalues for the first three principal components were 20.62, 1.64 and 0.85, respectively, accounting for 88.91% of the total variance (Tab. 4). Most characteristics with high positive loadings on the first principal component suggested that this component (79.32% of the total variance) represented size variation within the samples. All specimens on the first principal component were clustered as three groups, *P. petaurista*, *P. hainana*, and the group of *P. yunanensis* and *P. philippensis* with considerable overlaps. The second principal component (6.32% of the total variance) was strongly correlated with ROB, ABL, ABB, and BZP (loadings>0.50), and the third principal component (3.27% of the total variance) was correlated primarily
with HO (loadings > 0.50) (Tab. 4). The first two principal components separated all specimens as four distinct groups (Fig. 2).

The DFA identified the major patterns of morphological divergence in the crania among the four Petaurista groups. The variation pattern reflected by the first two discriminant functions was consistent with the morphological variations observed in the PCA, and all samples were clearly clustered as four distinguishable groups based on the 1st and 2nd discriminant functions (Fig. 3). In specimen reclassification by DFA, all individuals were properly assigned to their original groups on the basis of the studied measurements. Fig. 4 is the geographic distributions of all Petaurista samples used in this study.

### 3 Discussion

One contentious issue regarding the Chinese Petaurista is the taxonomic status of P. yunanensis, P. philippensis, P. hainana, and the populations of P. petaurista in China, which have long been controversial (Corbet & Hill, 1992; Ellerman, 1940; Ellerman & Morrison-Scott, 1950; Hoffmann et al, 1993; Huang et al, ...
Tab. 4 Factor loadings and percentage of variance explained for principal component analysis (variable codes are given in the text and Fig. 1)

| Variables | PC1     | PC2     | PC3     |
|-----------|---------|---------|---------|
| GLS       | 0.898   | 0.347   | 0.221   |
| CBL       | 0.868   | 0.379   | 0.212   |
| BL        | 0.876   | 0.388   | 0.227   |
| ONL       | 0.904   | 0.336   | 0.201   |
| PL        | 0.841   | 0.422   | 0.224   |
| PBL       | 0.872   | 0.347   | 0.024   |
| LUTR      | 0.889   | 0.375   | 0.192   |
| LUM       | 0.944   | 0.164   | -0.016  |
| GUMB      | 0.839   | 0.250   | 0.330   |
| ROL       | 0.820   | 0.420   | 0.151   |
| ROB       | 0.535   | 0.586   | 0.470   |
| ABL       | 0.129   | 0.895   | 0.207   |
| ABB       | 0.393   | 0.766   | 0.085   |
| BZP       | 0.833   | -0.608  | -0.028  |
| BOO       | 0.847   | 0.279   | 0.258   |
| HO        | 0.084   | 0.191   | 0.943   |
| ZOB       | 0.852   | 0.250   | 0.370   |
| MTB       | 0.882   | 0.307   | 0.265   |
| NL        | 0.745   | 0.357   | 0.317   |
| BN        | 0.757   | 0.339   | 0.389   |
| ML        | 0.919   | 0.291   | 0.219   |
| THM       | 0.875   | 0.260   | 0.208   |
| LLD       | 0.709   | 0.401   | 0.372   |
| LLMR      | 0.914   | 0.275   | -0.002  |
| LTR       | 0.883   | 0.391   | 0.157   |
| MH        | 0.838   | 0.288   | 0.242   |

Eigenvalues: 20.62, 1.64, 0.85
Variance explained (%): 79.32, 6.32, 3.27

Fig. 2 Scatterplots of the samples in PCA space
Fig. 3 Plot of the samples of the four Petaurista species on discriminant canonical function 1 and 2
Fig. 4 Geographic distributions of samples used in the study
A: Dulongjiang; B: Bijiang; C: Yunlong; D: Yingjiang; E: Lianghe; F: Tengchong; G: Longling; H: Luxi; I: Cangyuan; J: Xishuangbanna; K: Xiping; L: Mile; M: Lvchun; N: Pingbian; O: Xiangzhou; P: Northern Guangdong; Q: Hainan.
The sharing of morphological characteristics between *P. philippensis* and *P. yunanensis* is related to their similar living conditions.

*Petaurista hainana* was considered a valid species based on both molecular and morphological data (Huang et al., 1995; Yu et al., 2006). Our morphometric results were concordant with mtDNA data of previous research (Oshida et al., 2000a; Yu et al., 2006) and demonstrated the significant differences between *P. hainana* and *P. yunanensis/P. philippensis*. In both PCA and DFA, *P. hainana* was clearly separated from other three groups (Fig. 2, 3), with 21/26 cranial variables being significantly different (*P*<0.05) (Tab. 3). *Petaurista hainana* is confined to tropical forests on Hainan Island of China and *P. philippensis* and *P. yunanensis* are distributed in mountainous coniferous, dry deciduous and evergreen forests at different elevations in western Yunnan of China. The phenotypic divergence of *P. hainana* in relation to *P. philippensis* and *P. yunanensis* is likely associated with their geographical distributions and living conditions and could be viewed as a reflection of adaptations to various ecological niches. The differences in skull morphology suggest that *P. hainana* is neither the synonym of *P. philippensis* nor a subspecies of *P. yunanensis* or *P. petaurista* (Corbet & Hill, 1992; Thorington & Hoffmann, 2005; Wang, 2003), but a valid species in its own right.

The greatest distinction observed was between *P. petaurista* and other *Petaurista* forms. *Petaurista petaurista* displayed a relatively high level of diversity in skull morphology, with 22/26 cranial variables significantly different from *P. hainana*, *P. philippensis*, and *P. yunanensis* at *P*<0.001 level (Tab. 3). Based on 26 morphological cranial variables, the specimens of *P. petaurista* formed a distinct aggregate in both PCA and DFA (Fig. 2,3), consistent with the results of Oshida et al. (2000a) and Yu et al. (2006). It is obvious that *P. petaurista*, *P. hainana*, *P. philippensis*, and *P. yunanensis* are taxonomically distinct and distinct valid species.
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Append I: specimens examined

Petaurista yunanensis n=15

Yingjiang, Yunnan: IOZ 25849(♀). Gongshan, Yunnan: KIZ 73442(♀), 73445(♂), 73744(♂), 73745(♀), 73823(♂), 830207(♀), 90039(♂), 90043(♀), 90051(♂), 90407. Tengchong, Yunnan: KIZ 76348(♀). Lianghe, Yunnan: KIZ 650236(♂), 650237(♂). Bijiang, Yunnan: KIZ 780102(♀).

Petaurista philippensis n=21

Xishuangbanna, Yunnan: IOZ 10457(♂), 10458(♀), 10460(♀), 15041(♀), 15042(♀).

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