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Geometric Morphometric Research of the Molars in Tree Shrews (*Tupaia belangeri*) from Different Regions

Cheng-yao Yang[1#], Wen-rong Gao[2#], Na Ning[1], Wan-long Zhu[1]

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ABSTRACT

Background: Biological traits are mainly determined by genotype and are also influenced by environmental factors, especially in the process of adapting to different environments, corresponding phenotypic variations may occur. Even for the same animal, there may be some morphological differences due to its long-term adaptation to different habitat environments. Molar teeth are the direct contact point between animals and the environment, which are of great significance in the study of animal adaptability to different environments.

Methods: To explore the relationship between the morphology of the molars (upper molars and lower molars) of tree shrews (*Tupaia belangeri*) and environmental variations in different regions, we applied geometric morphometric methods to measure the morphological characteristics of the molars of *T. belangeri* in 12 different regions of China (Hainan, Daxin, Leye, Xingyi, Xichang, Hekou, Kunming, Mengla, Dali, Tengchong, Pianma, Laboratory bred F1 generation).

Result: The results showed that the morphological variations of the lower molars of *T. belangeri* in 12 different regions were larger, which was more suitable and reference valuable for studying the morphological differences among different geographical populations. The Principal component analysis showed that *T. belangeri* from 12 regions were clustered into 4 branches: Hainan was clustered into one branch, Daxin was clustered into one branch, Pianma and Tengchong were clustered into one branch and Leye, Xingyi, Hekou, Kunming, Xichang, Mengla, Dali and laboratory bred F1 generation were clustered into one branch. The thin plate spline analysis showed that deformations in the upper molars were mostly concentrated in the alveolar and dental margins. The deformations of the lower molars were mainly concentrated in the alveolar, marginal and occlusal surfaces. Multidimensional scaling showed that there were differences in molars morphology among the 12 locations, with significant variations in Hainan, Daxin, Pianma and Tengchong regions, reflecting the adaptive variation of *T. belangeri* to different ecological environments. The present results can provide a certain basis for the study of phenotypic adaptation in *T. belangeri*.

Key words: Environmental variation, Geometric morphometrics, Molarmorphology, *Tupaia belangeri*.

INTRODUCTION

Phenotypic traits are determined by genotype and are also influenced by environmental factors. Together, organisms evolve the most suitable phenotype to improve their survival fitness (Hadany and Beker, 2003; Rutherford, 2003; Boussange and Pellissier, 2022). In recent years, morphological research has received increasing attention (Fitzpatrick *et al*., 2009). Morphological features, especially skull morphology, have always been the basis for systematic evolution and taxonomic research, which is one of the main bases for exploring phylogenetic, homologous, or heterologous relationships between organisms (Yukibumi, 2002). There was a close relationship between mammals and the environment, especially in the process of adaptation to different environments (temperature, photoperiod, humidity, altitude, food, *etc*.); corresponding phenotypic variations occurred (Wang *et al*., 2022). Mammals with different geographical distributions, even the same species, may exhibit some morphological differences due to their long-term adaptation to different habitat environments, often manifested in their bones and body size (Daniel *et al*., 2019; Zhou and Liu, 2020; Amson and Bibi, 2021; Maher *et al*., 2022; Liao *et al*., 2023). Molar teeth are an important structure in the skull and a direct contact point for animals to interact with the environment, which are of great significance in studying the adaptability of animals to different environments (Zhu *et al*., 2014; Zhang *et al*., 2015; Gao *et al*., 2016; Gao *et al*., 2017; Zhang *et al*., 2019; Ren

[1]Key Laboratory of Ecological Adaptive Evolution and Conservation on Animals-plants in Southwest Mountain Ecosystem of Yunnan Province Higher Institutes College, School of Life Sciences, Yunnan Normal University, Kunming, 650500, China.
[2]School of Biological Resources and Food Engineering, Qujing Normal University, Qujing 655011, China.
[#]These authors contributed equally to this work.

Corresponding Author: Wan-long Zhu, Key Laboratory of Ecological Adaptive Evolution and Conservation on Animals-plants in Southwest Mountain Ecosystem of Yunnan Province Higher Institutes College, School of Life Sciences, Yunnan Normal University, Kunming, 650500, China.
Email: zwl_8307@163.com

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et al., 2019a; Li *et al*., 2020). Morphology of molars is closely related to animal feeding habits (Zhang *et al*., 2019). Studying the morphology of molars helps to understand the food spectrum of animals and provide scientific basis for animal feed allocation. There have been many reports on the use of molars to study the relationship between animals and their living environments (Ren *et al*., 2019a), but there is no report on the morphology of the upper and lower molars of *Tupaia belangeri* in different regions.

T. belangeri, belonging to *Tupaiidae*, *Scandentia*, which is an endemic species of the Oriental Realm and is mainly distributed in Yunnan, Guizhou, Sichuan, Guangxi and Hainan Island in China (Hou *et al*., 2020). The distribution areas of *T. belangeri* had significant environmental differences, including high mountains, plateaus and tropical islands (Gao *et al*., 2017). The complex and ever-changing natural environment may lead to genetic and phenotypic variations in the *T. belangeri*. The research results of genomics and metabolomics have found that distinct genetic and metabolic differentiation has occurred in *T. belangeri* in different regions (Ren *et al*., 2023; Hou *et al*., 2023). Studies on some morphological indicators have also found that there were certain variations in body index, fullness, body composition, digestive tract and skull morphology of *T. belangeri* in different regions, which may be related to distribution, altitude and geographical barriers (Gao *et al*., 2017; Ren *et al*., 2019b; Li *et al*., 2020). The present study uses geometric morphology measurement technology to study the morphological characteristics of the molars (upper and lower molars) of *T. belangeri* in 12 different regions of China, exploring the morphological variation characteristics of molars and their relationship with the ecological environment, which to provide more bases for the adaptive variation of *T. belangeri*.

MATERIALS AND METHODS

Experimental materials

T. belangeri used in the present study were collected from Hainan Island (elevation 50-190 m, E109°21′-109°48′, N18°23′-18°53′, n=7), Daxin (elevation 250-500 m, E106°39′-107°29′, N22°29′ -23°05′, n=7), Leye (elevation 1100-1300 m, E106°10′-106°51′, N24°30′-25°03′, n=14), Xingyi (elevation 1100-1200 m, E104°51′ -104°55′, N24°38′-25°23′, n=11), Xichang (elevation 1600-1800 m, E101°46′-102°25′, N27°32′-28°10′, n=11), Hekou (elevation 600-800 m, E103°23′-104°17’, N22°30′-23°02’, n=13), Kunming (elevation 1900-2100 m, E102°10′-103°40’, N24°23′-26°22′, n=16), Mengla (elevation 700-900 m, E101°05′-101°50’, N21°09′-22°23′, n=8), Dali (elevation 2000-2200 m, E98°52 ‘-101°03′, N24°41′-26°42′, n=12) Tengchong (elevation 2000-2100 m, E98°05′-98°45′, N24°38′-25°52′, n=12), Pianma (elevation 2700-3100 m, E98°34′-99°09′, N25°33′-26°32′, n=13) and F1 generation individuals from Institute of Medical Biology, Chinese Academy of Medical Sciences (n=10). After collecting animal samples, the neck was severed and executed. The skull specimens of the *T. belangeri* were prepared using the method of making skull specimens (Zhang and Wu, 2005). When studying sample morphology, the influence of sexual dimorphism can be ignored and the samples can be uniformly counted (Cardini *et al*., 2005).

Image information collection

Using the Sony DSC-Y110 digital camera and the same reference standard, photos of the upper and lower molars of *T. belangeri* were taken using a dissecting microscope at 2.5x magnification. In order to avoid systematic errors as much as possible, attention should be paid to the fixation of various factors during the photography process (the camera is fixed on a tripod, the parameters should be constant and the surrounding light should be roughly the same to ensure that each specimen is photographed at the same position).

Image digitization processing

The images of molars were digitized using TPSDIG2 software (Rohlf, 1990). According to the methods for marking the molars of related mammals (Caumul and Poll, 2005; Renaud *et al.,* 2007) and combined with the actual morphology of the molars of *T. belangeri*, the corresponding images of the molars were marked with dots. Finally, 70 marking points were determined for the upper and 62 marking points for the lower molars (Fig 1). The corresponding coordinate data is obtained after digitizing the boundary points. In order to avoid duplicate coordinates of the marked points, only the occlusal surface was analyzed to study the morphology of the molars. In order to improve the accuracy of boundary markers, each marker point is marked by the same person more than 6 times and the mean of coordinate data is used for subsequent statistical analysis.

Remove non morphological variations

The error in image angle caused by human factors (such as the position, proportion and direction of boundary markers) directly affects morphological analysis. Therefore, it is necessary to remove the interference caused by non morphological variations in the analysis process. The current experiment used the minimum square estimation of translation and rotation parameters and overlaps the layout of boundary markers, namely the generalized procrustes analysis (GPA) method, to remove the impact of non morphological variations on the analysis.

Thin plate spline method (TPS)

TPS method is usually used to draw differences in sample morphology (Bookstein, 1991). This method utilizes grid transformation theory and undergoes rigorous mathematical calculations to reflect morphological differences and its deformation can be used to represent the differences between average markers and target structures (Thompson, 1961). Usually, the parameters that cause these deformations are used as variables for statistical comparison between populations and within populations of morphological variations.

Data analysis

The obtained molar coordinate data was analyzed using Morphologika2 v2.5 software for TPS and PCA. The first three principal component values obtained from molar analysis were analyzed using SPSS 26.0 as discriminant functions. After combining the data information of the upper and lower molars, it can represent the morphological characteristics of the entire molars. The matrix obtained from the combination of the two sets of data is analyzed using SPSS 26.0 for multi-dimensional scale. Finally, the image is drawn using Origin 2018.

RESULTS AND DISCUSSION

Analysis of upper and lower molars

Analysis of the morphological characteristics of upper molars using principal components revealed that the characteristic values of the first, second and third principal components accounted for 84.95% of all variables (Table 1). Using the first and second principal components as scatter plots, the results were shown in Fig 2. Hainan population was mostly distributed on the left side, while Daxin population was mostly distributed above the image, samples from different regions overlap with each other. TPS analysis results were shown in Fig 3. The deformation of the upper molars of *T. belangeri* in different regions mainly occurs at the relative positions of the alveolar and dental margins. Analysis of the morphological characteristics of lower molars using principal components revealed that the characteristic values of the first, second and third principal components accounted for 88.42% of all variables (Table 2). Using the first and second principal components as scatter plots, the results were shown in Fig 4. Hainan population was mostly distributed in the upper right corner, Daxin population was mostly distributed in the lower right corner and Pianma and Tengchong populations were mostly distributed in the left corner. Samples from different regions overlap with each other. TPS analysis results were shown in Fig 5. The deformation of the lower molars of *T. belangeri* in different regions mainly occurs in the relative positions of the alveolar, dental margin and occlusal surface.

Multidimensional scale analysis of molar tooth morphology

Integrating the upper and lower molar tooth data of 12 samples of *T. belangeri* from different regions and conducting multidimensional scale analysis, it was found that the molar tooth morphology of *T. belangeri* varies among different groups, with the populations of Kinma and Tengchong clustered together; Leye, Xingyi, Hekou, Kunming, Xichang, Dali, Mengla and F1 generation populations gather together; Hainan and Daxin populations were separated separately and have significant differences from other populations (Fig 6). The individual dimension weights showed that the upper molar score (0.441, 0.480) has a significant contribution to the second dimension; the lower molar scores (0.490, 0.436) contribute significantly to the first dimension (Fig 7).

In recent years, there had been many reports on the study of molar morphology, involving different fields and levels, including research on functional dynamics and environmental adaptation, as well as exploration of research methods (Unger, 1998; Teaford, 2000; Zhang *et al*., 2019; Ren *et al*., 2019a). For example, in the process of studying the function of teeth, it was found that the microwear method was more suitable for studying the feeding behavior of animals (Ungar *et al*., 2003; Godfrey *et al*., 2004) and when searching for factors that determine the function of teeth (especially for molars), it is more suitable for qualitative methods (Ungar *et al*., 2003; 2004). Moreover, the research on tooth function mainly focuses on exploring the sources of chewing power and chewing patterns (Evans and Sanson, 2003; Evans, 2005). To explore the effects of genetic effects and island climate on island populations, the molars and mandibles of *Apodemus sylvaticus* were measured, the results showed that different evolutionary patterns appeared

Table 1: Eigen values, percentage of total variance explained and cumulative variance explained of first three principal components for the principal component analysis of upper molars in *Tupaia belangeri*.

Principal component (PC)	Eigen value	Variance explained (%)	Cumulative variance explained (%)
PC1	0.006 125	60.88	60.88
PC2	0.001 632	16.22	77.10
PC3	0.000 789	7.85	84.95

Table 2: Eigen values, percentage of total variance explained and cumulative variance explained of first three principal components for the principal component analysis of lower molars in *Tupaia belangeri*.

Principal component (PC)	Eigen value	Variance explained (%)	Cumulative variance explained (%)
PC1	0.008 054	45.86	45.86
PC2	0.005 325	30.32	76.18
PC3	0.002 149	12.24	88.42

in the molars and mandibles; therefore, they speculated that this phenomenon was related to the distance, size and level of competition of the islands (Renaud and Michaux, 2007).

Geometric morphology measurement technology can not only quantitatively analyze and describe the changes in the morphology of biological samples, but also identify the causes of morphological variations (Renaud *et al.*, 2007). Moreover, when using this method to study biological morphological features, it is not necessary to consider the actual size of the sample, but only the morphological changes of the sample itself (Zhang *et al.*, 2019). This technology has become one of the important methods for studying intra species and inters species morphological differentiation and is widely used in the study of rodents (Zhu *et al.*, 2014; Gao *et al.*, 2016; Renaud *et al.*, 2007). The present study used geometric morphology measurement technology to study the morphology of the upper and lower molars of *T. belangeri* in 12 populations. TPS analysis showed that the deformation of the upper molars of *T. belangeri* in different regions was mainly concentrated in the relative positions of the teeth grooves and edges; the deformation of the lower molars is mainly

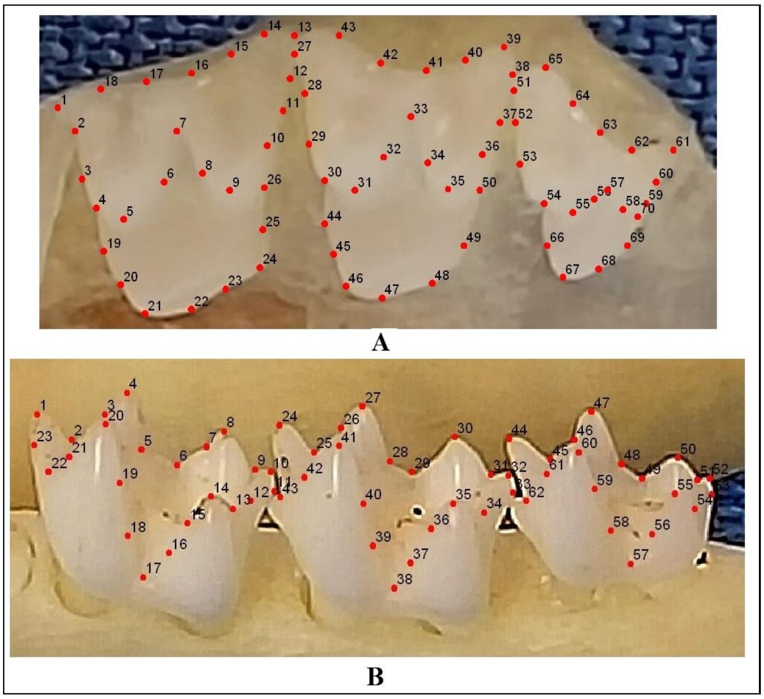


Fig 1: Landmark configurations on the crown of the upper molars (A) and the lower molars (B) of *Tupaia belangeri*.

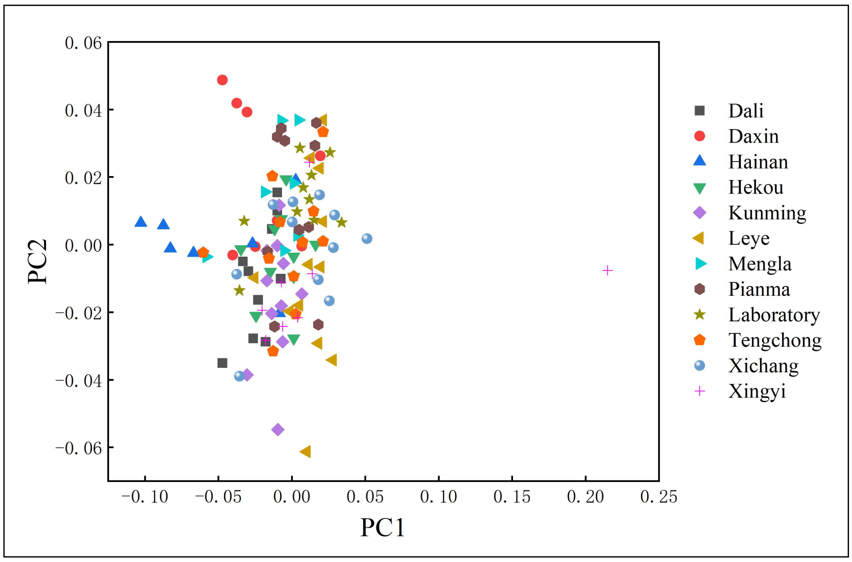


Fig 2: Plots of principal component factors 1 and 2 for upper molars of *Tupaia belangeri*.

concentrated in the relative positions of the alveolar, marginal and occlusal surfaces. The morphology of the upper and lower molars of *T. belangeri* varies to some extent in different regions, with significant variation in the lower molars. A study on *Marmota* found that there was a close relationship between molars and food, but the variation in molar morphology is less affected by food (Avise and Saunders, 1984). There were two main reasons: (1) before the teeth grow out of the gums, the surface enamel of the teeth cannot grow or change anymore; (2) the impact of the environment on molars is relatively small, especially when there is little difference in food. However, in the current study there was a significant variation in the lower molar teeth of *T. belangeri* in different regions, which may be related to the food of *T. belangeri*. It mainly lived in the mountains, forests, or shrubs near villages and farmland, feeding on insects, fruits and seeds of grains. The environmental differences in its distribution areas were significant, including high mountain, plateau and island environments. The crops in Hainan Island were mainly coconuts and mangoes, the crops in Daxin were mainly sugarcane and longans, the crops in the Yunnan-Guizhou Plateau were mainly corn and rice and the crops in Pianma and Tengchong were mainly melons and fruits. It can be seen that the differences in environment and food may be the main reason for the great variation of the lower molar morphology of *T. belangeri*.

(a. Hainan, b. Daxin, c. Leye, d. Xingyi, e. Hekou, f. Kunming, g. Xichang, h. Dali, i. Pianma, j. Tengchong, k. Mengla, l. Laboratory)

Fig 3: Plots of TPS for upper molars of *Tupaia belangeri*.

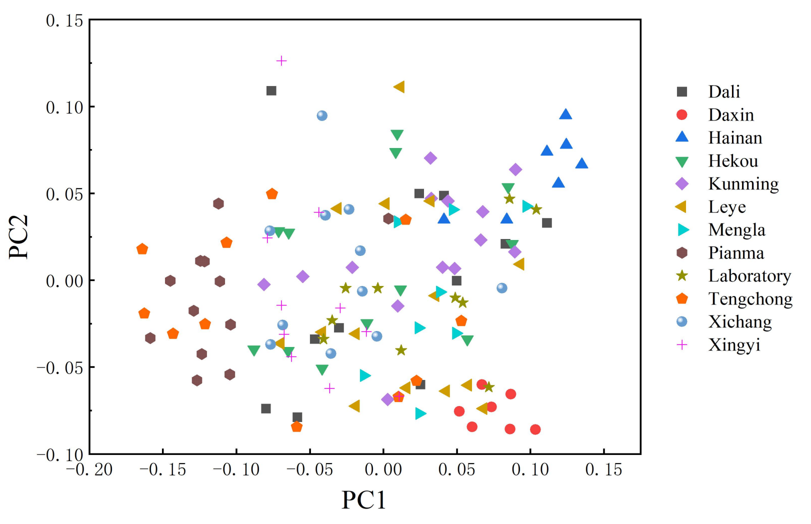
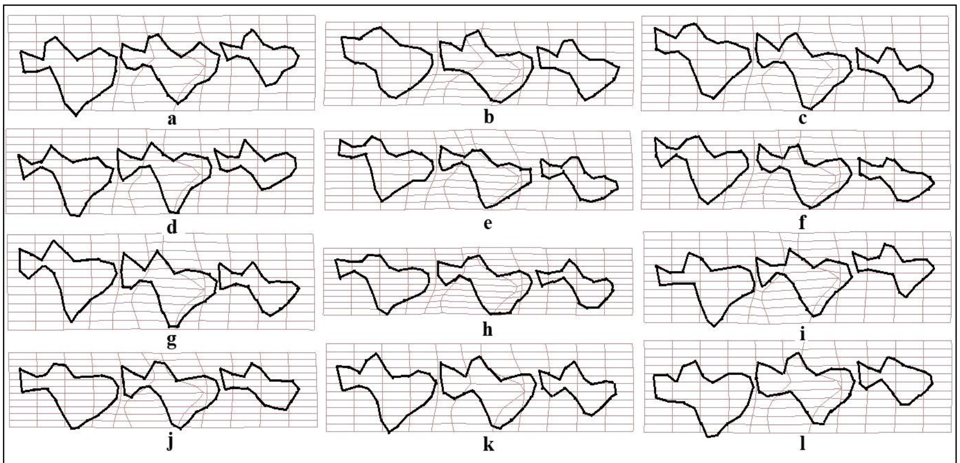


Fig 4: Plots of principal component factors 1 and 2 for lower molars of *Tupaia belangeri*.

Moreover, the mandible attached to the lower molars was closely related to feeding. In the study of the mandible of *T. belangeri*, it was also found that there was significant morphological variations among different populations (Li *et al*., 2020), which was similar to our results.

The results of PCA in our study showed that *T. belangeri* from 12 regions were clustered into 4 branches: Hainan was clustered into one branch, Daxin was clustered into one branch, Pianma and Tengchong were clustered into one branch and Leye, Xingyi, Hekou, Kunming, Xichang, Mengla, Dali and laboratory bred F1 individuals were clustered into one branch. This is related to the natural environment in which the *T. belangeri* lived. Hainan Island had a tropical island climate, the Yunnan-Guizhou Plateau had a northern subtropical plateau climate and Hengduan Mountains had a subtropical alpine climate. The dual effects of temperature and humidity may lead to morphological changes in the molars of the *T. belangeri*. The difference in vegetation in the habitat of *T. belangeri* may also be one of the important reasons for the variation of its molar morphology. Moreover, the results of multidimensional scale analysis also showed that there were certain differences in the morphology of the molars of *T. belangeri* in 12 regions. Among them, there were significant variations in the Hainan, Daxin, Pianma and Tengchong regions. The spatial distribution map of individual weights showed that the upper molars contributed more to the second dimension, while the lower molars contributed more to the first dimension, which may also be related to



(a. Hainan, b. Daxin, c. Leye, d. Xingyi, e. Hekou, f. Kunming, g. Xichang, h. Dali, i. Pianma, j. Tengchong, k. Mengla, l. Laboratory).

Fig 5: Plots of TPS for lower molar of *Tupaia belangeri*.

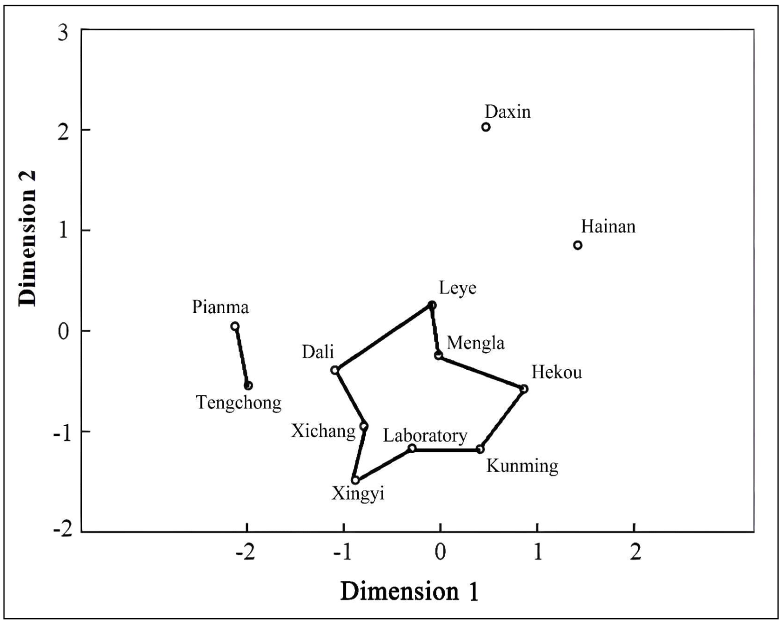


Fig 6: Diagram of Multidimensional scaling for combined datasets of molar in different regions of *Tupaia belangeri*.

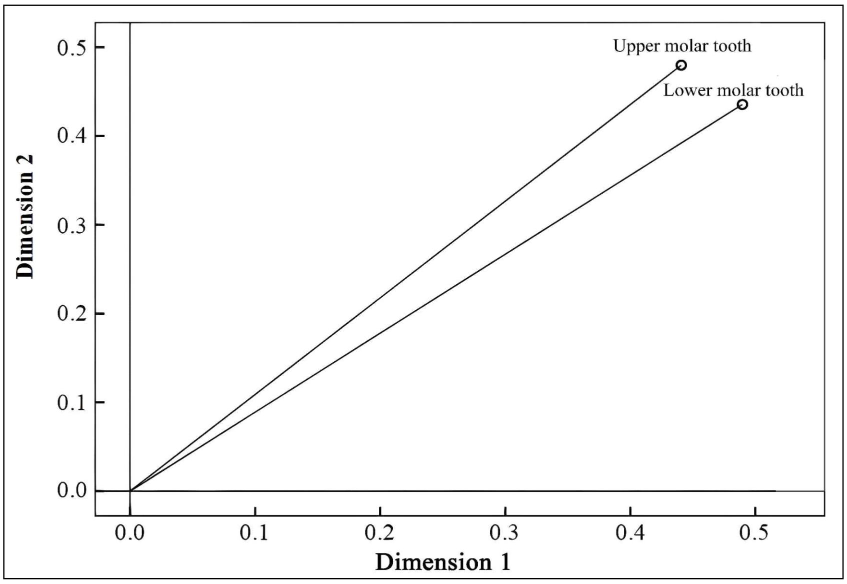


Fig 7: Diagram of dimension weights for upper molar and lower molar of *Tupaia belangeri*.

the climate and geographical environment (temperature, humidity, food, *etc*.) in which *T. belangeri* habitat.

CONCLUSION

In conclusion, there were significant differences in the morphology of the molars of *T. belangeri* from 12 regions, with significant variations in the upper and lower molars. Based on the morphological differences between the upper and lower molars, *T. belangeri* in 12 regions can be divided into 4 subgroups, reflecting the adaptive variation of *T. belangeri* to different ecological environments.

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Conflict of interest statement

The authors declare no conflict of interest.

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