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Preliminary Study of Skull Polymorphims of *Tupaia glis* in Peninsular Malaysia by using MorphoJ

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Graphical abstract

Abstract

A global geographic fragmentation has caused the diversity of animals and plants worldwide, mostly to the population of small mammals. In Malaysia, there are a lot of natural barriers that can separate the population of small mammals including common treeshrew, *Tupaia glis*. In this study, the polymorphism of ninety (90) *Tupaia glis* in Peninsular Malaysia is carried out by comparing the skulls of the species from different states by using MorphoJ analysis. This is carried out to know the landmark of the species and the shape changes.

Keywords: Small mammals; polymorphism; skulls; shape changes; MorphoJ

■1.0 INTRODUCTION

Small mammals are mammals that have a maximum weight of less than 5 kg and are mainly consist of mammals from the order Rodentia, Chiroptera, Lagomorpha, Erinacepmorpha, Soricomorpha, and Scandentia [1, 2]. They are the most diverse group of mammals but yet little information is known about them as compared to larger enigmatic mammals such as tigers and elephants. Fifteen percent of mammals are categorized as data deficient in the IUCN Red List of Threatened Species, with bats and rodents dominating the list of data deficient species [3]. Thus there is a big gap of knowledge on the distribution, abundance and conservation status of small mammals. This is confounded by the fact that small mammals are hard to be identified and need to be captured for such purposes, making surveys very costly due to transport of trap and field equipments.

Tupaia (derived from a Malay word ‘tupai’, meaning ‘squirrel’. *T. glis* can be differentiated from the small tree squirrels in many details of anatomy and behaviour. They have very long muzzle with a total of 38 teeth, with pointed incisors and several premolars. All feet of common tree shrew have five well-developed digits with claws [4]. According to [4] Francis (2005), *T.glis* have hair that are banded dark and pale on the upper parts and buff on the under parts. It appears finely speckled with a strong reddish tint and usually has a pale stripe on each shoulder.
According to [5] Boonsong, 1977 there are 8 recognized subspecies found in mainland Thai which are: *T. g. ferruginea*, *T. g. wilkensoni*, *T. g. clarissa*, *T. g. belangeri*, *T. g. chinensis*, *T. g. laotum*, *T. g. olivacea and T. g. concolor*. This all subspecies has their own colour variations that differentiate them from each other, the pelage colour is widely variable for subspecies. [6] Hill (1960) has demonstrated a cline of colour for the mainland tree shrews, with the ones in the South brighter and more reddish then the ones in the North. The colour variation seems to be related to changes in rainfall and temperature, with the brighter, redder colours correlated with high humidity and temperature and grey or grey-brown colours correlated with lower temperature and humidity.

Figure 1.0 The distribution of common treeshrew

Figure 1.0 showed the distribution of common treeshrew which mainly cover East Asia including Malaysia. The geography of Malaysia is particularly distinctive owing to its various geological and hydrological features. Peninsular Malaysia is primarily divided into east and west coast by the Titiwangsa range [7, 8]. Spanning 480 km from the north to the south of Peninsular Malaysia, this mountainous and forested area would probably be an effective geographical barrier to the dispersal of small mammals from the east coast to the west coast of Peninsular Malaysia and is a good source of landscape variation for testing of the morphology within species of *Tupaia glis*.

The effect of genetic isolation due to fragmentation can pose as threat to small mammals if they are effectively isolated from other populations. Isolation may subject a particular small population to loss of genetic variation via genetic drift, directional selection, problems associated with effective population size**,** inbreeding, reduced gene flow with other populations and reduced variation in niche width [9]. These can be further aggravated by a bottleneck event [10]. Thus a landscape genetic study may reveal habitat quality from the conservation genetics point of view and can be used as a guide for future conservation actions. The study on the habitat fragmentation will utilize the common treeshrew *Tupaia glis* (Order Scandentia) as its organism of choice as it is a slow moving organism that is widely distributed in Peninsular Malaysia.

■2.0 EXPERIMENTAL

The sampling areas for those *Tupaia glis* are as follow, Perlis, Kedah, Pulau Pinang, Terengganu, Pahang, Kelantan, and Johor. The total of 90 individuals is captured by using random sampling cage trap methods. These cage traps are equipped with banana as the bait due to its strong colour preferences and as well as for their odour. These traps and nets were deployed for at least three consecutive days to maximize capture and to accumulate species number. Captured animals were identified using keys from [4] Francis (2008) and standard measurements were recorded. Voucher specimens (three individuals per species) were collected in this study and these specimens were euthanized using cloroform. All samples were preserved in the form of wet specimens and were deposited in UMK Zoological Museum.

The skulls of the samples were then extracted and cleansed before the measurements of the skull are measured. The explanation of the anatomical terms associated with cranial morphology is well explained by both [11] Rosevear (1969) and [12] Harrison (1972). The soft parts were removed immediately after dissection. The soft parts that removed are the brain, eyes, tongue and other organs of the sample before move boiling session and drying for the long term preservation. The data of the measurement were recorded and the data gathered were analysed using the Discriminant Function Analysis (DFA). The skulls were captured into raw image and processed to build into tps.file (by using software tpsUtil and tpsDig2) before being analyzed into MorphoJ software.

■3.0 RESULTS AND DISCUSSION

Figure 2.0 showed the UPGMA tree of Discriminant Function Analysis of all the external body and skull measurement of *Tupaia glis* in Peninsular Malaysia. This showed that there were grouping and differences of *Tupaia glis* in Peninsular Malaysia due to the geographical barriers which is Range of Titiwangsa.

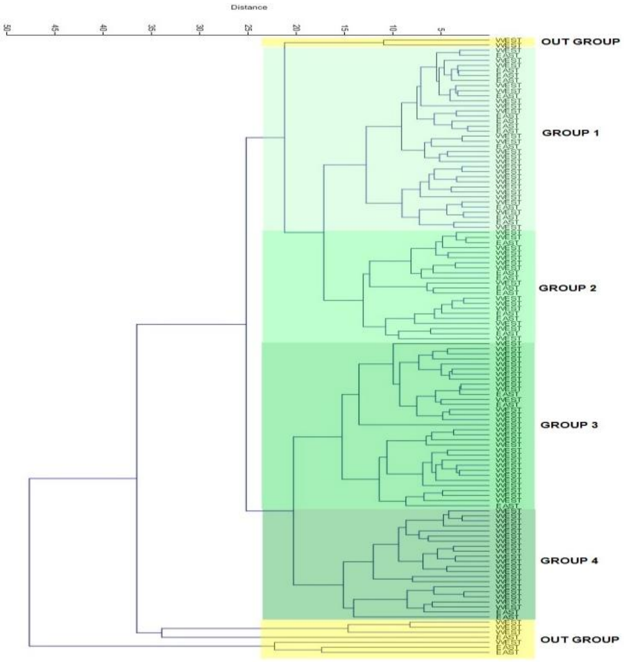


Figure 2.0 UPGMA analysis of *Tupaia glis*

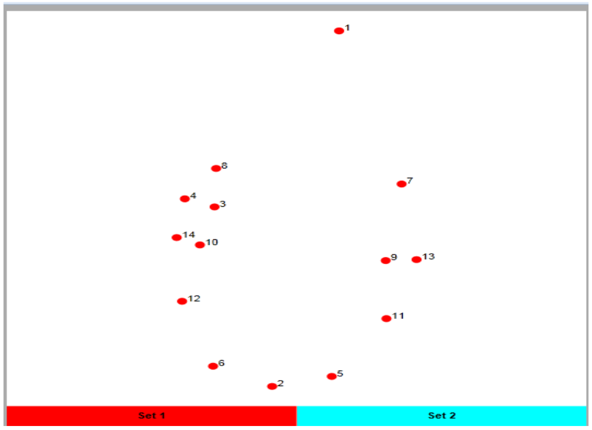
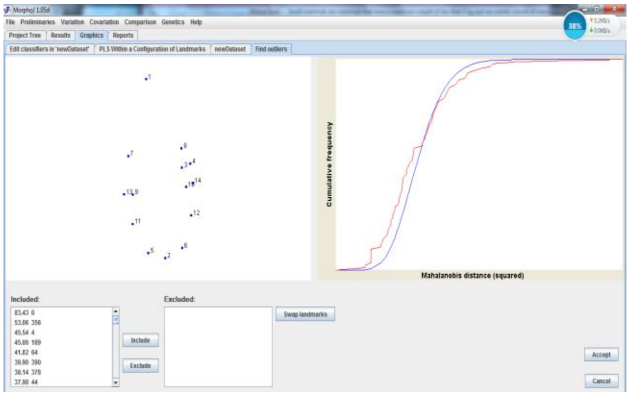


Figure 3.0 PLS Within a configuration of landmarks



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83.43 0
53.06 356
45.54 4
45.00 189
41.82 64
39.90 390
38.14 378
37.90 44
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Figure 4.0 Outliers of landmarks

In morphometric analysis by using MorphoJ, there were many types of methods can be analyzed concerning the integration of pattern within a single structure or the strength of integration between different parts of the organisms.

Partial Least Sequare (PLS) analysis that being showed in the Figure 3.0 is a common analysis done to look into variation pattern between two or more sets of variables. However, in this study, since it is a preliminary data, the variables are not develop enough to do two-block PLS hence its configurate into within group only.

In order to do a single study there will be several different analyses included so that all the results can be combined to provide a comprehensive understanding of integration in the structure under study. According to [13] Goswami and Polly (2010) and also [14] Zelditch *et al*., (2012), the methods that are usually being used are the standard tools of geometric morphometrics, kind as the principal component analysis; meanwhile others are specialized methods that were developed specifically for this purpose. Hence, the overview will focus on the landmark methods only.

■4.0 CONCLUSION

The nature of the aggregates present in dilute solution, below the gelation threshold concentration, was investigated using atomic force microscopy (AFM). When the two components were present in a 2:1 (dendrimer:diamine) ratio, rod like aggregates were observed in the AFM images (Figure 4a–shows aligned rods running from upper left to lower right). The lngth of these rods was approximately 100 nm, and their diameters were ca. 9 nm (depth ca. 1 nm).

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