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Research Article

Morphometric analysis of Cantor's Roundleaf Bat, *Hipposideros galeritus* Cantor 1846 from several localities in Sarawak, Malaysia

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ABSTRACT. Changes on the body size of bats due to the ecological and environmental aspects usually acts as the indicator for niche preference. Morphological divergence resulting from the natural selection has contributed to discrimination of the same local population. This study attempts to reveal the population structure of Cantor's roundleaf bat, *Hipposideros galeritus* in Sarawak, using 27 morphometrical measurements of its external body, skull and dental characters. A total of 25 voucher specimens deposited in UNIMAS Zoological Museum representing three different localities at Bako National Park, southern Sarawak and northern Sarawak were examined during which all linear measurements were recorded and analysed using Discriminant Function Analysis (DFA) in the Statistical Package of Social Science (SPSS) programme. The result showed that ear length (EL), second molar tooth crown width (M2W) and mastoid width (MW) had significantly discriminated these populations into their own clusters, respectively. The independent clustering for each locality suggests that the population might be initially isolated and may represent different groups or possibly different subspecies. However, further analysis on molecular genetic studies should be highlighted in order to validate the population structure resulting from this morphological variation.

Keywords: Morphological variation, *Hipposideros galeritus*, Sarawak, Discriminant Function Analysis.

INTRODUCTION

Body size is commonly used as an indicator for niche preference as it is highly associated with many ecological attributes (Peters, 1983). Morphological divergence can occur between local populations due to strong differential natural selection (Maryanto *et al.*, 2005). In order to adapt to different habitat and environmental conditions, a particular bat species or population might undergo some changes or adjustment to their internal and external morphological characters that are associated in determining their ecology and behaviour (Findley, 1993; Birch, 1997; Fenton & Bogdanowicz, 2002). These characters include wing morphology, jaw structure, brain size, general extension dimensions and also geographical variations (Kunz, 1982; Findley, 1993; Rhodes, 2002).

Patterns of variation in morphological characters are essential for describing boundaries of evolutionary units in nature, apart from genetic traits (Reis *et al.*, 2002). Wehausen and Ramey II (2000) noted that morphological variation is a result of genetic and environmental components to individual development that possibly describe the genetic and ecophenotypic variation. In the study of paleontology, morphological variation is one of the key indicators used for taxonomic distinction at species- and genus-level (Stafford & Szalay, 2000).

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Studies on morphological characters of bat species was extensively developed decades ago, basically implementing the classical method of species classification made by various authors including Hill (1963), Corbet and Hill (1992), Koopman (1994) and Simmons (2005). The multivariate statistical analysis using numerical data of morphological characters is very useful in reviewing and studying the relationships among Chiroptera species. In addition, Hillis (1987) noted that morphological methods are very applicable to museum specimens and fossil species.

The morphometric technique is also much cheaper compared to molecular genetics in terms of data collection, requires minimum expenses in terms of supplies and equipment (Hillis, 1987). It is one of the alternatives that has been used to classify species or population as well as for describing new species (Weimin & Robbins, 2004; Nicolas *et al.*, 2008; Sazali *et al.*, 2008a; 2008b). This morphometric analysis is reliable in order to discover misidentified specimens due to overlapping body measurements and similar body characters shared among closely related species (Sazali *et al.*, 2008a; 2008b).

Cantor's roundleaf bat, *Hipposideros galeritus* (Family Hipposideridae; Order Chiroptera), is a well-distributed species that can be found in India, Sri Lanka, Thailand, Peninsular Malaysia, Java and Borneo. They mostly roost in caves, often in small groups along with *H. cervinus*, and sometimes in the colonies of several hundred (Payne *et al.*, 1985). The body weight ranges from 60-85 g, with forearm length ranging from 47-51 mm and tail length of around 30-43 mm. The fur is usually dark grey-brown, and occasionally with a pinkish-grey noseleaf (Payne *et al.*, 1985). As a microchiropteran, *H. galeritus* uses a complicated echolocation system as its primary means of orientation, which enables it to easily fly and catch insects in total darkness (Vaughan, 1986; Feldhamer *et al.*, 1999). It also plays a major role as a natural predator to insects as well as a biological controller (Findley, 1993; Hutson *et al.*, 2001).

Although this understudied species is widely distributed in Malaysia, there is no current study conducted to investigate the morphological variation of *H. galeritus* from Sarawak. The objective of this study is to reveal the population structure of *H. galeritus* based on their morphological variation from three different localities in Sarawak, namely, Bako National Park, southern Sarawak and northern Sarawak using the morphometric technique. The resulted clustering may lead to the recognition of different populations or possibly different subspecies.

MATERIALS AND METHOD

Three different localities were identified (Figure 1) in which, Bako National Park is nominated as locality 1, southern Sarawak as locality 2 consisting of specimens from Kubah National Park, Batang Ai National Park and Jambusan, Bau; and northern Sarawak as locality 3 consisting of specimens from Lambir Hills National Park, Niah National Park and Sg. Murum, Belaga.

A total of 25 voucher specimens deposited at the Universiti Malaysia Sarawak (UNIMAS) Zoological Museum were morphologically examined and identified following the identification keys of Payne *et al.* (1985) and Corbet and Hill (1992). Twenty-seven morphometrical characters of the external body, skull and dental were linearly measured using a digital caliper (Mitutoyo^{TM}; calibrated to 0.01 mm), a steel ruler and with the aid of a microscope following methods by Kitchener *et al.* (1993) and Sazali *et al.* (2008a; 2008b) and recorded appropriately.

Ten external body measurements were taken, including the forearm length (FA), ear length (EL), tibia length (TB), pes length (PES), tail to ventral length (TVL), third digit metacarpal length (D3MCL), third digit first phalanx length (D3P1L), third digit second phalanx length (D3P2L), fourth digit metacarpal length (D4MCL) and fifth digit metacarpal length (D5MCL). In addition, cranial and dental measurements including the

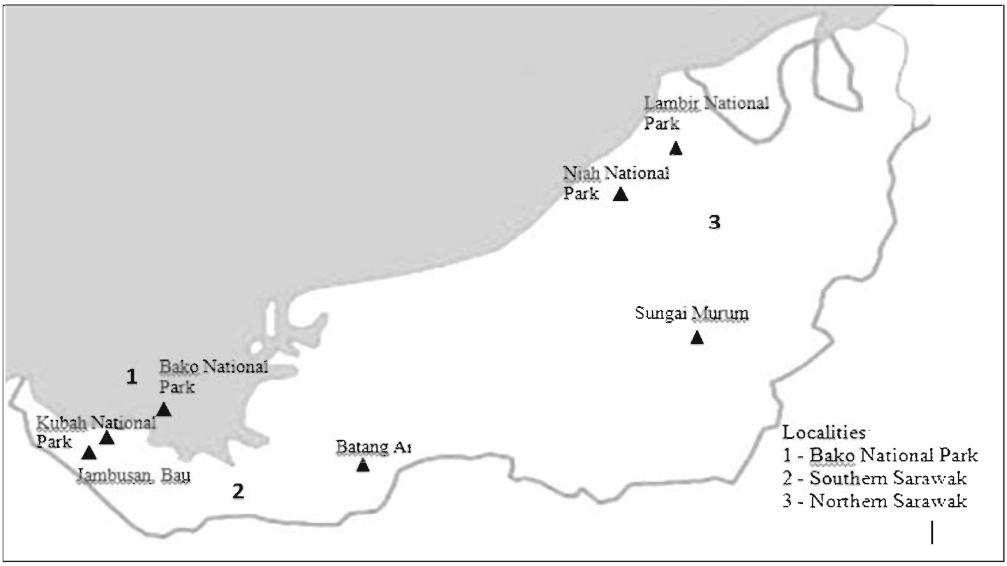

Figure 1 . The known records of *H. galeritus* in Sarawak used in this study.

greatest skull length (GSL), interorbital width (IOW), cranial width (CW), mastoid width (MW), zygomatic width (ZW), post palatal length (PPL), palatal length (PL), distance between cochleae (DBC), bulla length (BL), greatest basial pit length (GBPL) and dentary length (DL) were taken. For dental measurement, measurements taken were of the canine tooth basal width (C1BW), breadth across both canine outside surfaces (C1C1B), breadth across both third molar teeth outside surfaces (M3M3B), canine molar length or maxillary tooth row length (C1M3L), second molar tooth crown length (M2L) and second molar tooth crown width (M2W).

Prior to the analysis, normality tests were run for each of the characters to determine whether the data was normally distributed or not. The data was then analysed using Discriminant Function Analysis (DFA) in Statistical Package of Social Science programme Version 18.0. (SPSS Inc., 2010). DFA was conducted in order to find the significant character(s) that can be use to discriminate these specimens following each population respectively.

RESULTS

The mean, standard deviation and range (minimum and maximum) of 27 morphological measurements are summarised in Table 1. From the analysis (Table 2), two functions have been extracted, in which Function 1 explained 78.7% and Function 2 explained 21.3%. This showed that Function 1 has a high variability of characters in this analysis. The Wilk's Lambda statistics (Table 3) for Function 1 through 2 functions is 0.074 (p=0.000), meanwhile for Function 2 through 2 functions is 0.433 (p=0.02), which are significant at P = 0.05. Overall, there were five characters used to distinguish each population (Table 4). The highest characters observed from Function 1 was ear length (EL) followed by second molar tooth crown width (M2W), while from Function 2, mastoid width (MW) was the highest character observed. The canonical discrimination function (Figure 2) shows clear separation of these *H. galeritus* based on each respective population, where locality 1 of Bako National Park is apparently distinct from localities 2 and 3, of southern and northern Sarawak, respectively. From the analysis,

Table 1. Descriptive statistics for the studied population of *H. galeritus.*

Locality	Bako National Park (n=6)			Southern Sarawak (n=7)			Northern Sarawak (n=12)		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
External									
FA	45.71 ± 1.78	42.90	48.51	49.30 ± 1.43	47.18	51.26	48.06 ± 1.38	46.11	51.43
EL	9.08 ± 0.61	8.24	9.67	11.57 ± 1.36	9.65	13.81	11.67 ± 0.81	10.18	13.12
TB	17.82 ± 1.69	14.92	20.10	19.50 ± 1.03	18.81	21.51	19.15 ± 1.48	16.28	21.37
PES	4.55 ± 0.39	4.02	5.00	5.16 ± 0.48	5.46	5.85	4.84 ± 0.53	4.01	4.94
TVL	25.78 ± 2.46	22.51	29.67	32.30 ± 5.80	25.32	40.87	29.93 ± 4.06	24.45	37.87
D3MCL	34.04 ± 1.38	31.48	35.16	37.40 ± 2.98	31.30	39.76	37.20 ± 1.62	34.80	40.05
D4MCL	34.14 ± 0.95	32.34	35.03	36.44 ± 3.68	30.91	40.64	36.79 ± 1.16	34.19	37.78
D5MCL	31.21 ± 1.30	29.40	32.48	35.17 ± 4.28	29.76	42.22	33.11 ± 1.03	30.79	34.49
D31PL	15.12 ± 1.27	13.48	16.60	16.36 ± 1.03	14.66	18.09	15.94 ± 0.55	14.95	16.55
D3P2L	18.00 ± 1.58	16.06	20.72	17.97 ± 3.53	10.63	20.49	18.12 ± 1.67	15.53	19.46
Cranial									
GSL	17.57 ± 0.51	17.02	18.34	18.13 ± 0.4	17.47	18.59	18.20 ± 0.42	17.47	19.04
DL	10.86 ± 0.40	10.43	11.37	11.12 ± 0.37	10.57	11.79	11.33 ± 0.42	10.72	11.98
IOW	2.90 ± 0.13	2.73	3.03	2.98 ± 0.23	2.79	3.36	2.96 ± 0.21	2.61	3.22
CW	7.11 ± 0.39	6.57	7.76	7.18 ± 0.31	6.83	7.68	7.30 ± 0.26	6.92	7.89
MW	8.92 ± 0.23	8.64	9.24	9.03 ± 0.23	8.56	9.25	9.32 ± 0.15	9.08	9.57
ZW	7.88 ± 0.50	7.38	8.54	7.72 ± 0.3	7.34	8.14	7.70 ± 0.57	6.30	8.39
PPL	7.93 ± 0.16	7.66	8.10	8.17 ± 0.17	7.86	8.13	8.23 ± 0.30	7.88	8.69
PL	5.81 ± 0.30	5.47	6.32	6.05 ± 0.20	5.78	6.25	6.13 ± 0.16	5.92	6.40
DBC	4.41 ± 0.13	4.24	4.56	4.51 ± 0.26	4.18	4.92	4.69 ± 0.24	4.41	5.16
BL	2.53 ± 0.18	2.19	2.68	2.75 ± 0.13	2.61	2.97	2.74 ± 0.15	2.35	2.93
GBPL	6.32 ± 0.18	6.05	6.54	6.54 ± 0.34	6.25	7.26	6.55 ± 0.32	6.12	7.12
Dental									
C1BW	2.25 ± 0.09	2.15	2.39	2.21 ± 0.25	2.00	2.59	2.35 ± 0.21	2.05	2.70
C1C1B	3.38 ± 0.29	2.89	3.75	3.46 ± 0.26	3.00	3.82	3.50 ± 0.25	3.17	3.89
M3M3B	6.19 ± 0.30	5.96	6.77	6.58 ± 0.25	6.23	6.85	6.45 ± 0.23	6.17	6.87
C1M3L	6.03 ± 0.10	5.86	6.14	6.33 ± 0.20	6.07	6.70	6.35 ± 0.15	6.14	6.64
M2L	1.22 ± 0.11	1.01	1.31	1.29 ± 0.20	1.10	1.69	1.20 ± 0.11	1.02	1.39
M2W	1.57 ± 0.12	1.41	1.78	1.68 ± 0.11	1.59	1.83	1.71 ± 0.09	1.61	1.86

Table 2: Eigenvalues for Discriminant Function Analysis.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	4.828[a]	78.7	78.7	0.910
2	1.307[a]	21.3	100.0	0.753

[a]First two canonical discriminant functions were used in the analysis

Table 3: Wilk's Lambda for Discriminant Function Analysis.

Test of Function(s)	Wilks' Lambda	Chi-square	Df	Sig.
1 through 2	0.074	51.971	10	0.000
2	0.433	16.717	4	0.002

Table 4. Standardized Canonical Discriminant Function Coefficients. Higher character loadings for each function were indicated with an asterisk.

Characters	Function	
	1	2
M2W	*0.893	0.151
MW	-0.032	*-1.034
FA	0.603	0.589
EL	*0.896	-0.212
PES	0.274	0.797

*Diagnostic character(s) in each function.

96.0% of original grouped cases were correctly classified.

DISCUSSION

Swartz *et al.* (2003) noted that the body size of bats influences their flight behaviour, diet selection, roosting, reproductive behaviour and physiology. Moreover, some factors that lead to the differences between mainland and island environments are competition, predation and resource availability (Palkovacs, 2003). In this study, the locality 1 of Bako National Park is regarded as an island, whereas locality 2 of southern Sarawak and locality 3 of northern Sarawak are classified as mainland, respectively.

According to Raia and Meiri (2006), a smaller body size would result from the adjustment taken to cope for space and resource shortage. It is possible that the smaller body size for the island population is associated with limited food resources due to the small size of the area, compared to the mainland. Resource limitation coupled with reduced predation pressure on islands could confer higher fitness to smaller-bodied individuals because they require less energy to survive and reproduce (McClain *et al.*, 2006). In addition to

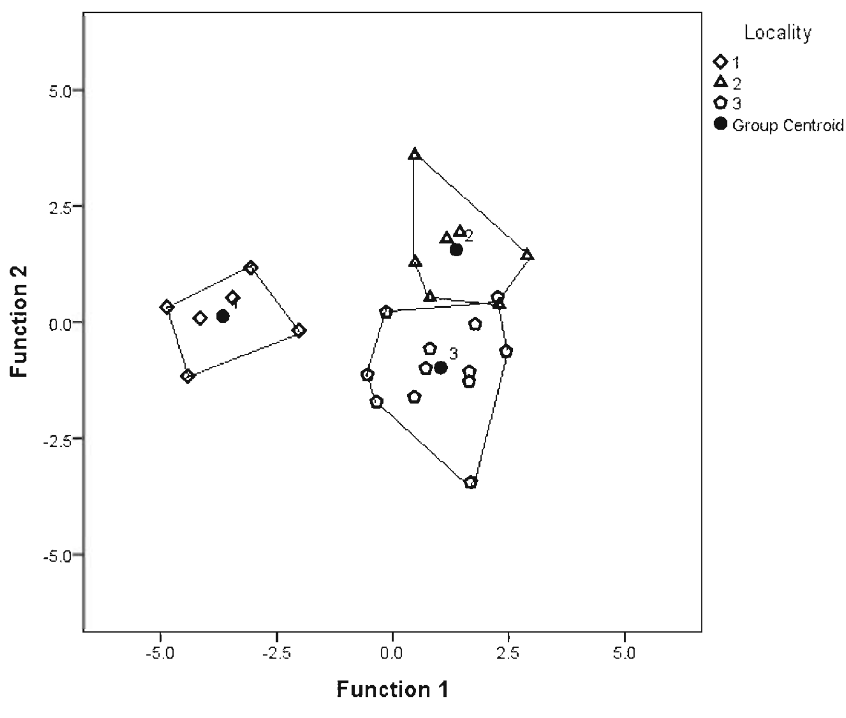

Figure 2. Canonical variate analysis plot of Functions 1 and 2 for three different populations of *H. galeritus*. 1 = Bako National Park, 2 = southern Sarawak, 3 = northern Sarawak.

imposing metabolic constraints, body size also influences the foraging behaviour of species (Belovsky, 1997; Ritchie, 1998).

The ear length (EL) is the strongest character found in separating these populations that is associated with echolocation. Most bats species from the families of Emballonuridae, Mormoopidae, Noctilionidae, Vespertilionidae, Molossidae, Rhinolophidae and Hipposideridae are aerial feeders which use intense echolocation calls (>110 dB SPL) to detect, track and assess moving targets, usually flying insects (Bogdanowicz *et al.*, 1998).

Due to the echolocation system, microbats usually have tragus, a cartilaginous projection from the base of the ear, which sits inside the pinna. The ear and tragus combination may be designed to improve the directionality or sensitivity to incoming echoes (Altringham, 1996). Bats which are sensitive to lower frequency sounds (<10 kHz) generally have large ears associated with prey movement or prey calling (Arita & Fenton, 1997). Echolocation, similar to morphology, is a flexible character that is often shaped more by ecological demands rather than by phylogeny (Jones & Teeling, 2006).

In addition to that, there is a correlation between the body size and ear length (Table 1). According to Arita and Fenton (1997), bats with smaller body size tend to have smaller ears and they also have low wing loadings, thus making their flight more maneuverable. This relationship implies that smaller bats should be more efficient at capturing airborne prey. On the other hand, larger bats tend to use low frequency of echolocation calls that are well suited for detecting large targets. This might be one of the reasons that explain the separation between island and mainland populations. It is assumed that food consumed by *H. galeritus* at islands and the mainland are different due to habitat and vegetation types.

On top of that, two individuals from southern Sarawak and northern Sarawak are found overlapping probably due to their similar environment of having a mixed dipterocarp forest, where some parts are from the limestone area. Meanwhile, Bako National Park has various vegetation types, namely, kerangas, mangrove, mixed dipterocarp, riverine and beach forests, as well as cliff vegetation (Hazebroek & Abang Kashim, 2000). The abundance of insects such as fireflies, ants and mosquitoes around mangrove areas in this park (Mackinnon *et al.*, 1996) might lead to the adaptation of needing a smaller body size for this insectivorous bat in order to accommodate itself in this dense forest and to utilize available food resources.

A study on the morphometrical variations of *Penthetor lucasi* from Sarawak by Rahman and Abdullah (2010) suggested that different ecological forces between populations, such as breeding, foraging behaviour, crowding effects and resource availability, could influence morphological variations. Moreover, the effect of constant crowding could lead to changes in body size due to competition for space and food resources. They also noted that differences in foraging behaviour could affect the external characters where wider foraging radius will favour larger external characters to endure long flights.

The northern and southern Sarawak populations are found to have larger external attributes that correlate with their wing size. According to Jennings *et al.* (2004), bats with long wings could perform powerful flights and they also use low frequency calls of echolocation to search for food. This is consistent with the longer ear length (EL) and wings size by these mainland populations, which are known to be sensitive to lower frequency calls, compared to the island population of Bako National Park. In combination, the echolocation call structure and wing morphology are important indicators of the foraging ecology of bats, as they may constrain the foraging habitats that bats can use, the types of food items that they can detect and how those resources are perceived (Jennings *et al.*, 2004).

Apart from that, diet can also influence dental characteristics. The number and size of teeth, the size of the jaw and size of cranial crest where the chewing muscle are attached can be related to diet habit (Freeman, 1981; Findley & Black, 1983). Hard feeding in insectivore bats have been found to correlate with skull morphology including the size of the molar teeth (larger M1 and M2, but smaller M3). Accordingly, the cranial structure in bats is thought to reflect specialisations for feeding (Freeman, 2000) and echolocation (Pedersen, 2000).

Convergence of species with comparable dietary specialisation onto a similar dental form suggests that there is a functional relationship between the tooth form and diet (Evans & Sanson, 2005). Locality 1 was recorded to have the smallest size of second molar tooth crown width (M2W), compared to the mainland populations (localities 2 and 3). This suggests that the types of food consumed at islands might be different from the mainland, possibly due to different types of insects consumed, in terms of hardness, softness and brittleness (Phillip, 2000). Additionally, the difference in dietary composition is also correlated with the difference in habitat used by the population (Barlow *et al.*, 1997).

CONCLUSION AND RECOMMENDATION

In summary, there is a significant difference in the morphological variations of *H. galeritus* at the three different localities in Sarawak. It is found that the ear length (EL), second molar tooth crown width (M2W) and mastoid width (MW) are the diagnostic characters for discriminating these three populations (p-value < 0.05). The independent clustering for different populations suggests that the population might be initially isolated and may represent different groups or possibly different subspecies. However, phylogenetic studies should be highlighted for reviewing and to compare the molecular genetics finding with this morphometrical result in order to provide a comprehensive study on *H. galeritus*, as well as to validate the recognition of the population structure resulting from this morphological variation.

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