Morphological variation of the Diadem Leaf-nosed Bat, *Hipposideros diadema*, Geoffroy, 1813 (Chiroptera: Hipposideridae) in Caves in West Sumatra, Indonesia

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Abstrak

Bukit Barisan sebagai barier fisik diprediksi berpengaruh terhadap populasi di Sumatera Barat sebagaimana terjadi pada kelompok hewan terestrial termasuk kelelawar Hipposideros diadema, yang dikenal memiliki kemampuan dispersal yang tinggi. Pengaruh barier ini diharapkan dapat diamati pada perbedaan morfologi. Sejumlah 58 individu dewasa H. diadema dikoleksi dengan menggunakan Harpa trap pada beberapa goa di Sumatera Barat, yang terdiri dari Goa Kalilawa, dan Goa Lereng di bagian barat Bukit Barisan dan Goa Salamaik di bagian timur Bukit Barisan. Pengkoleksian sampel di lapangan dilaksanakan pada bulan Januari-Desember 2013. Pengukuran dilakukan terhadap 26 karakter tubuh dan 15 karakter tengkorak. Uji Mann-Whitney menunjukkan divergensi karakter antar dua populasi yang berbeda. Hasil PCA sesuai dengan fenogram yang disusun dengan UPGMA yang menunjukkan populasi H. diadema di Goa Salamaik (populasi timur) berbeda dengan populasi dari Goa Kalilawa dan Goa Lereng (populasi barat). Disimpulkan bahwa barier Bukit Barisan memungkinkan berpengaruh terhadap divergensi karakter morfologi antara H. diadema di Sumatera Barat.

Abstract

We assumed that Bukit Barisan as a physical barrier and its acts to population exchanges in West Sumatra, as well as for terrestrial animal group. If it does for bats in case for the Diadem Leaf-nosed Bat, *Hipposideros diadema*, which have superior dispersal powers to many other terrestrial group, then we might expect to see this some how reflected in morphological divergence. A total of 58 adult of *H. diadema* were collected directly using harp traps from several Cave in West Sumatra (Kalilawa Cave, Padang; Lereng Cave, Pariaman; (western of Bukit Barisan) and Salamaik Cave, Sawahlunto (eastern of Bukit Barisan)). The samples were collected on January-December 2013. Kruskall Wallis Test, Mann Whitney U Test, Principal Component Analyses (PCA) and Cluster Analysesdemonstrated that these population could be separated clearly from one to another. This analyses based on 26 external and 15 skull measurement. The result showed that population of H. diadema from Salamaik Cave in Sawahlunto differ from H. diadema in Padang and Pariaman. Divergence characters among three population of H. diadema was found using Kruskall Wallis test. Mann-Whiney U test showed divergence characters between two different population. The result of PCA was congruence to phenogram obtained by UPGMA that showed close relationship between population of H. diadema from Kalilawa Cave, Padang to Lereng Cave, Pariaman and different from Salamaik Cave, Sawahlunto. We conclude that Bukit Barisan barriers could be affected to morphological divergence among *H.diadema* in West Sumatra.

Keywords: Bukit Barisan, cave, Hipposideros diadema,morphology, variation.

Introduction

West Sumatra has the largest limestone outcrops in Indonesia. Caves are known as karsts, and West Sumatra has 114 limestone caves (UKSDA, 1999; Haznan, 2003). Limestone biodiversity consists of three types of ecosystem, as troglobin, troglophil and trogloxene (Dunn,1965). A common cave dweller belongs to a group of bats known as trogloxene (Vermeullen &Whitten, 1999) in the order of chiroptera (Findley, 1993; Kitchener, 1996; Nowak, 1994). Based on echolocation calls chiroptera are divided into two sub order, megachiroptera and microchiroptera (Gunnel &

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Figure 1. *H. diadema* collected from Kalilawa Cave, Padang, West Sumatra.

Simmons, 2005; Koopman, 1994; Simmons & Geisler, 1998). Based on molecular evidence and evolution of echolocation in bats, Koopman (1994) proposed that *Megachiroptera* (family Pteropodidae) is closely related to *Microchiroptera* (Rhinolipids group includes Rhinolophidae, Megadermatidae, Hipposideridae, Craseonycteridae, Rhinopomatidae) and grouped them as Yinpterochiroptera, whereas Jones and Teeling (2006) grouped Microchiroptera into two infraorders; Yinochiroptera and Yangochiroptera. This group can be found in all habitats with some families preferring caves as roosting sites (Graham, 1994;Vermeullen & Whitten, 1999).

H. diadema belongs to the family Hipposideridae (Roundleaf bats), infraorder Yinochiroptera that is sometimes called Diadem roundleaf bats. Description of this species is large body size, FA 76-87 mm and weight 30-47 gram. Fur of upperparts is dark brown with pale bases, white patches on the shoulders and sides; underparts greyish-white. In adult females orange or orange buff often replaces the white. Noseleaf with 3 or 4 lateral leaflets; posterior noseleaf large and rounded (Francis, 2008) (Figure 1). This species has wide distribution from Burma and Vietnam through Thailand, Laos, West Malaysia and Indonesia (including Sumatra, Borneo, and Bali) to New Guinea, Bismarck Archipelago, Solomon Islands and norteasth Australia; Philippines; Nicobar Islands (Simmons, 2005).

West Sumatra is separated by Bukit Barisan that stretches from south to north of Sumatra Island. This was formed during Miocene when two unequal parts, the narrow west coast and the wider half of hills and alluvial areas. The different ecological conditions east and west of Bukit Barisan is likely to have influenced the morphology and genetic variation of the species (Colombijn, 2005; Whitten, 1989). Studies about morphological traits and genetical variations associated with ecological conditions suggest that *H. diadema* distributed across several small and large islands (include in Lesser Sunda Islands) belong to 16 different subspecies (Kitchener et al., 1992). Rahman and Abdullah (2010) found that *Penthetor lucasi* in three geographical areas of Sarawak (Malaysia) differs in body size and exhibit strong sexual dimorphism in certain characters. Benita (2012) studied morphological variations of *Hipposideros larvatus* from three caves in west Sumatra and concluded that the barrier created by Bukit Barisan mountain range may have lead to the variation in morphological characters of bats in Sumatra.

Currently, there is no other published study that focuses on morphological variation of *H. diadema* from caves in West Sumatra. Tate (1941) summarized information about the subspecies of *H. diadema* in the Indo-Australian region and recognized about 16 subspecies. This, however, does not include subspecies grouping of *H. diadema* populations in west Sumatra. We hypothesized that ecological differences between east and west Bukit Barisan may have induced morphological variation among population of *H. diadema*. Therefore, the aim of this study is to investigate the morphological variation of population *H. diadema* from three caves in West Sumatra separated by Bukit Barisan mountain range.

Figure 1. A diadem leaf-nosed bat, *H. diadema*, from Kalilawa cave, Padang, West Sumatra.

Methods

Bats were captured from three caves in West Sumatra: Kalilawa Cave, Padang (00o56'51.1S, 100o29'50.2E); Lereng Cave, Pariaman (00o92'95.8S, 100o33'89.4E) and Salamaik Cave, Sawahlunto (00o40'11.6S, 100o44'24.1E) (Figure 2). Bats were captured using harp traps (Francis, 1989) setup in entrances of the caves. The traps were deployed in the afternoon and checked in the evening and in the early morning. For each bat were captured, we recorded the age (adult or young) and sex. Presence of growth bands at the finger joints of *H. diadema* were also recorded (Anthony, 1988). Bat trapping took place during January-February 2013 and the specimens were deposited in the Zoological Museum at the University of Andalas (MZUA).

Thirty six characters were measured; twenty one external characters following Rahman and Abdullah (2010), and fifteen skull character following Kitchener and Maryanto (1993). These external characters measurements were as follows, with abbreviations in parentheses; ear length (E), head and body length (HB), tail to ventral length (TV), Forearm length (FA), tibia length (TB), first digit length (PIB), hind foot length (HF), second digit metacarpal (D2MCL), third digit metacarpal (D3MCL), fourth digit metacarpal (D4MCL), fifth digit metacarpal (D5MCL), third digit first (D3P1L) and second phalank length (D3P2L), fourth digit first (D4P1L) and second phalank length (D4P2L), fifth digit first (D5P1L) and second phalank length (D5P2L), antitragus high (TA), eye diameters (DM), posterior nose leaf breadth (LDP) and anterior nose leaf width (LDA). The skull characters measured were the great skull length (GSL), cranial length (PIL), least interorbital width (LI), zygomatic width (LTP), width across caninus to another caninus from outer basal face (CCB), palatal bridge length (PBL), width across molar to another molar from outer mass face (MMB), tymphanic bulla length (TBL), tymphanic bulla width (TBB), cochlea width (CW), cranial heigh (CH), rostrum heigh (RH), rostrum length (RL), lower tooth row length (IML) and dentary length (DL) (Figure 3).

Figure 2. Locality of *H. diadema* specimens used in this study in West Sumatra (insert), Sumatra Island, Indonesia.

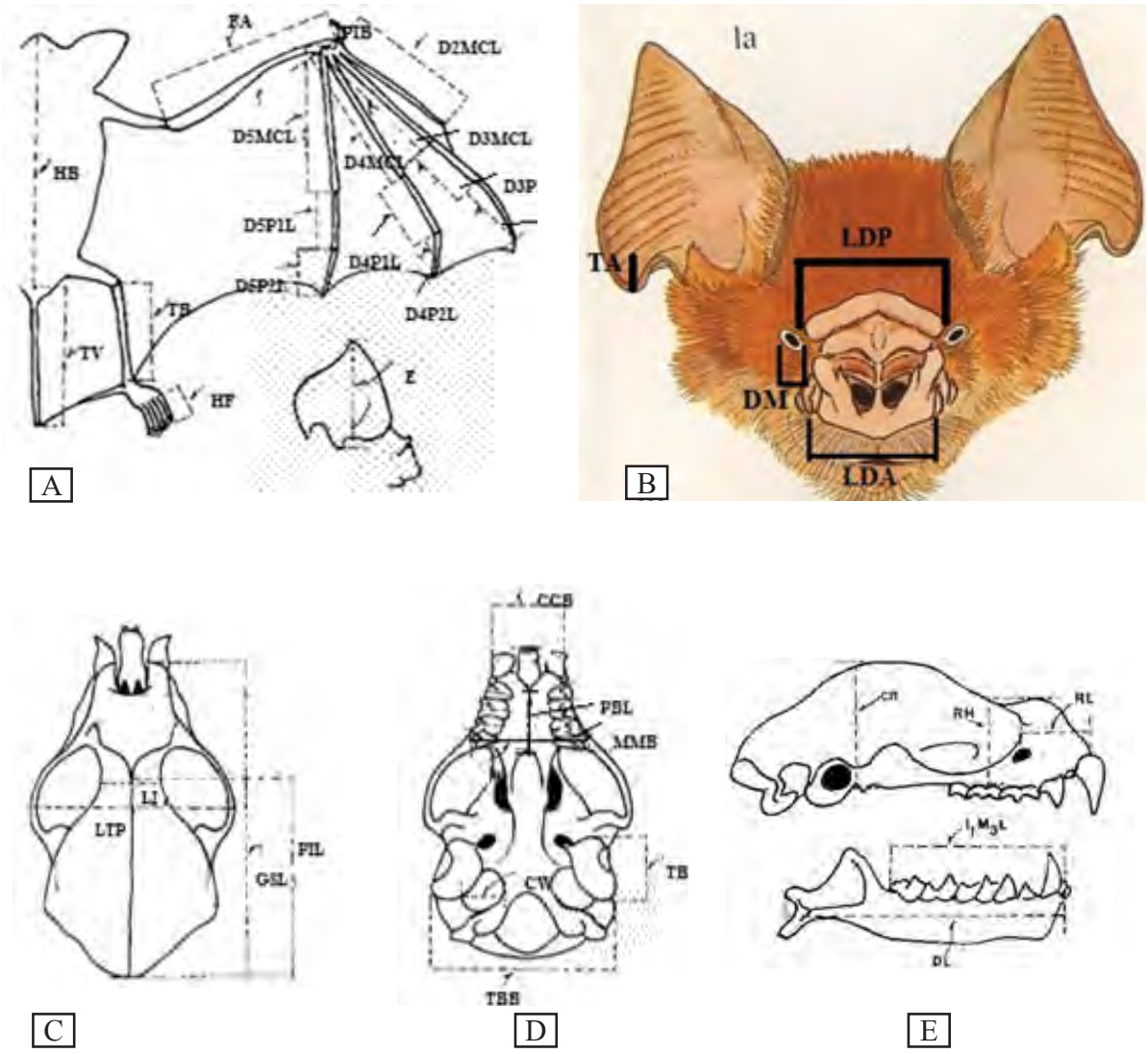

Figure 3. Twentyone external characters (a,b) (modified from Rahman and Abdulllah, 2010) and fifteen skull characters (a;dorsal, b; ventral, c; lateral) (modified from Kitchener and Maryanto, 1993) were used in this study to measure morphological differences.

The data measurements were divided by forearm (external measurements) and great skull length (skull measurements) to standardised body size for all specimens. Morphological variations among the populations were tested using Kruskall-Wallis Test and possible differences between populations were tested using Mann Whitney U Test at a significancy level of 5% using SPSS® software. All data were transformed to $\log_{10}$ values before Principal Component Analyses (PCA) and Cluster analysis shown up by UPGMA (Unweighted Pair Group Method Arithmatic Average) using MVSP 3.1 and NTSyspc Ver 2.0.2i software.

Results

A total of 58 adult *H. diadema* consisting of 31 males and 27 females were collected and measured. The number of specimens collected from each cave was: Kalilawa cave (14 male and 4 female), Lereng cave (3 male and 11 female) and Salamaik cave (14 male and 12 female).

Morphological characters

The morphological characters of male and female specimens from Salamaik Cave population are relative

Table 1. Measurement of male samples in millimeters, for (a) external character measurements (b) Skull character measurement. For each samples, mean ± standard deviation, df=2, N=sample size, p-value, H=Kruskall-Wallis value. *=significance level p≤0.05, ns=not significant)

A) External characters measurements

Characters	Kalilawa cave (N=14)	Lereng Cave (N=3)	Salamaik cave (N=14)	Kruskall-Wallis test
E	31.48 ± 2.36	31.5 ± 3.07	28.44 ± 1.56	H = 10.09; p = 0.006*
HB	100.92 ± 6.9	101.75 ± 6.83	102.66 ± 3.17	H = 0.142; p = 0.9313 ns
TV	56.98 ± 3.34	57.87 ± 3.48	52.40 ± 3.22	H = 10.66; p = 0.005*
FA	78.37 ± 2.48	78.35 ± 2.07	75.92 ± 2.39	H = 8.385; p = 0.015*
TB	43.43 ±2.62	41.09-1.68	40.53 ± 2.32	H = 10.8; p = 0.005*
PIB	12.84 ±1.16	11.60 ± 1.15	10.81 ± 0.86	H = 17.37; p = 0.000*
HF	15.39 ± 1.69	14.65 ±1.19	15.80 ± 1.56	H = 0.431; p = 0.806 ns
D2MCL	77.58 ± 3.27	77.64 ± 3.92	71.03 ± 2.68	H = 18.39; p = 0.000*
D3MCL	74.81 ±1.77	73.79 ±1.26	73.92 ± 2.02	H = 1.273; p = 0.529 ns
D4MCL	72.97 ±3.06	72.35 ±2.46	73.04 ± 2.45	H = 0.264; p = 0.876 ns
D5MCL	66.27 ± 2.10	65.77 ± 2.28	61.53 ± 2.17	H = 16.27; p = 0.000*
D3P1L	33.75 ± 3.01	33.43 ±1.21	27.79 ± 15.85	H = 7.861; p = 0.019*
D4P1L	24.47 ± 2.22	23.59 ±3.03	24.09 ± 2.33	H = 0.188; p = 0.910 ns
D5P1L	26.35 ± 1.93	26.09 ±1.85	26.37 ± 1.17	H = 0.136; p = 0.934 ns
D3P2L	36.33 ± 2.20	34.87 ±1.68	33.45 ± 1.35	H = 11.08; p = 0.003*
D4P2L	21.90 ±7.95	17.21 ±1.71	17.70 ± 1.17	H = 5.768; p = 0.056 ns
D5P2L	20.28 ± 1.96	19.74 ±0.07	18.34 ± 1.26	H = 7.004; p = 0.030*
TA	6.71 ± 1.22	5.27 ±0.31	5.71 ± 0.67	H = 8.149; p = 0.017*
DM	3.12 ± 0.46	2.97 ±0.53	2.40 ±0.34	H = 15.64; p = 0.000*
LDP	14.71 ± 1.41	14.47 ±1.64	16.16 ± 0.54	H = 13.07; p = 0.001*
LDA	14.36 ± 0.86	13.49 ±0.40	14.93 ± 0.60	H = 5.74; p = 0.057ns

B) Skull measurements

GSL	31.57 ± 0.71	31.97 ± 1.49	30.56 ± 0.54	H = 12.77; p = 0.002*
PIL	68.25 ± 6.71	66.03 ± 3.85	69.02 ± 7.75	H = 0.195; p = 0.907 ns
LI	12.66 ± 1.08	12.33 ± 1.97	12.63 ± 1.66	H = 0.160; p = 0.923 ns
LTP	53.43 ± 8.20	54.35 ± 2.10	55.78 ± 1.99	H = 2.452; p = 0.294 ns
CCB	25.56 ± 1.51	24.82 ±1.89	24.71 ± 1.89	H = 1.147; p = 0.564 ns
PBL	30.04 ± 0.88	31.19 ± 2.92	30.23 ± 2.83	H = 1.449; p = 0.485 ns
MMB	39.48 ± 1.84	38.31 ± 3.26	39.98 ± 1.71	H = 1.917; p = 0.384 ns
TBL	13.79 ± 1.58	12.86 ± 0.88	13.62 ± 1.47	H = 1.571; p = 0.456 ns
TBB	45.04 ± 0.94	44.83 ± 2.84	45.68 ± 1.75	H = 0.5819; p = 0.747 ns
CW	10.63 ± 1.49	10.95 ± 0.92	10.17 ± 1.20	H = 0.927; p = 0.629 ns
CH	38.06 ± 2.61	35.69 ± 2.28	36.59 ± 20.41	H = 3.555; p = 0.169 ns
RH	30.62 ± 1.19	30.3 ± 1.44	29.72 ± 1.38	H = 2.059; p = 0.357 ns
RL	14.61 ± 1.88	16.03 ± 1.85	13.28 ± 1.17	H = 9.872; p = 0.007*
IML	45.63 ± 2.63	44.42 ± 2.81	46.45 ± 2.05	H = 2.061; p = 0.357 ns
DL	68.66 ± 2.02	64.74 ± 5.83	69.23 ± 2.21	H = 1.831; p = 0.400 ns

Table 2. Measurement of female samples in millimeters for (a) external character measurements (b) Skull character measurement. For each samples, mean ± standard deviation, df=2, N=sample size, p-value, H=Kruskall-Wallis value. *=significance level p≤0.05, ns=not significant)

A) External characters measurements

Characters	Kalilawa cave (N=14)	Lereng Cave (N=3)	Salamaik cave (N=14)	Kruskall-Wallis test
E	33.75 ± 2.17	31.99 ±1.83	29.15 ± 1.92	H = 13.87; p = 0.000*
HB	104.45 ± 4.19	105.73 ± 2.91	104.38 ± 4.11	H = 0.8009; p = 0.67 ns
TV	58.44 ± 0.40	57.48 ± 4 49	54.20 ± 3.68	H = 4.325; p = 0.115 ns
FA	75.03 ± 0.77	78.39 ± 1.79	78.33 ± 1.91	H = 8.62; p = 0.013*
TB	44.40 ± 0.79	42.22 ± 2.47	39.40 ± 0.93	H = 14.18; p = 0.000*
PIB	12.95 ± 1.76	12.67 ± 1.55	11.63 ± 1.35	H = 3.819; p = 0.148 ns
HF	18.57 ± 0.43	16.76 ± 1.52	15.41 ± 1.23	H = 11.43; p = 0.003*
D2MCL	82.79 ± 2.30	79.1 ± 2.91	82.69 ± 3.40	H = 15.51; p = 0.000*
D3MCL	77.18 ± 1.38	75.16 ± 2.67	73.66 ± 1.43	H = 7.528; p = 0.023*
D4MCL	73.69 ± 0.99	74.01 ± 2.23	71.18 ± 1.68	H = 11.67; p = 0.003*
D5MCL	69.19 ± 2.08	68.72 ± 3.29	60.85 ± 1.39	H = 16.09; p = 0.000*
D3P1L	34.66 ± 0.26	33.29 ± 11.12	31.31 ± 1.95	H = 15.62; p = 0.000*
D4P1L	23.85 ± 1.84	26.56 ± 4.03	24.95 ± 0.88	H = 3.155; p = 0.206 ns
D5P1L	27.02 ± 0.54	25.93 ± 1.96	26.81 ± 1.28	H = 1.194; p = 0.551 ns
D3P2L	38.25 ± 0.18	37.07 ± 5.81	32.25 ± 1.40	H = 15.73; p = 0.000*
D4P2L	17.60 ± 0.75	20.92 ± 9.12	17.20 ± 0.86	H = 4.216; p = 0.122 ns
D5P2L	21.03 ± 0.15	19.79 ± 1.87	18.88 ± 8.73	H = 12.94; p = 0.002*
TA	7.14 ± 0.26	7.16 ± 3.61	5.49 ± 1.17	H = 4.283; p =0.118 ns
DM	3.33 ± 0.05	3.28 ± 1.17	2.50 ± 0.39	H = 10.13; p = 0.006*
LDP	16.60 ± 0.99	14.06 ± 0.67	15.98 ± 0.75	H = 17.33; p = 0.000*
LDA	14.57 ± 0.98	14.50 ± 5.12	14.95 ± 0.54	H = 4.109; p = 0.128 ns

B) Skull measurements

GSL	32.13 ± 0.51	31.99 ± 0.44	31.75 ± 0.58	H = 1.869; p = 0.393 ns
PIL/GSL	64.42 ± 1.21	66.02 ± 7.32	63.83 ± 1.49	H = 4.242; p = 0.119 ns
LI/GSL	12.59 ± 0.60	13.12 ± 2.36	11.15 ± 1.46	H = 5.717; p = 0.057 ns
LTP/GSL	53.72 ± 2.27	55.32 ± 2.15	52.66 ± 1.53	H = 7.225; p = 0.027*
CCB/GSL	24.69 ± 0.35	25.99 ± 1.84	23.19 ± 1.53	H = 14.61; p = 0.000*
PBL/GSL	29.23 ± 0.80	31.03 ± 1.67	27.76 ± 1.26	H = 15.15; p = 0.000*
MMB/GSL	39.29 ± 0.42	38.81 ± 1.99	37.71 ± 1.20	H = 2.165; p = 0.339 ns
TBL/GSL	11.71 ± 0.50	13.47 ± 2.39	9.94 ± 1.00	H = 17.2; p = 0.000*
TBB/GSL	44.32 ± 0.78	45.15 ± 2.68	43.92 ± 1.63	H = 1.022; p = 0.600 ns
CW/GSL	12.14 ± 1.44	11.26 ± 2.88	11.31 ± 7.02	H = 10.52; p = 0.005*
CH/GSL	35.16 ± 1.70	38.21 ± 2.80	34.21 ± 2.19	H = 13.44; p = 0.001*
RH/GSL	30.46 ± 0.53	30.99 ± 2.18	26.73 ± 4.82	H = 14.5; p = 0.000*
RL/GSL	12.80 ± 1.77	29.56 ± 14.81	12.80 ± 1.22	H = 13.75; p = 0.001*
IML/GSL	41.97 ± 4.49	47.58 ± 2.52	40.73 ± 0.70	H = 15.61; p = 0.000*
DL/GSL	66.13 ± 0.60	66.17 ± 4.06	65.70 ± 1.55	H = 0.914; p = 0.6343 ns

small compared to the specimens from Kalilawa cave and Lereng cave. The sample size, mean, standard deviation, maximum and minimum values for all characters measurements, including external and skull characters measurements of *H. diadema* are presented in Table 1 and 2. Comparison of adult specimens among the tree population showed significant differences among them in 15 morphometric characters among male consist of 13 external characters and 2 skull characters, and 21 morphometric character measurements of female, consist of 13 external characters and 9 skull characters. We recorded significant differences in male externall characters for TV, FA, TB, PIB, D2MCL, D5MCL, D3P1L, D3P2L, D5P2L, TA, DM, LDP, and for skull characters GSL and RL. For females we recorded significant differences for D2MCL, E, FA, TB, HF, D3MCL, D5MCL, D3P1L, D3P2L, D5P2L, DM, LDP, and for skull characters LTP, CCB, TBL, CW, CH, RH, RH, RL, and IML. Significant differences in both characters measurements indicates high divergence of external and skull characters of *H. diadema* between the three populations (Kalilawa cave, Lereng cave, and Salamaik cave).

A Mann Whitney U-test was used to compare remaining characters. The *H. diadema* populations from Lereng and Salamaik caves differs significantly for both males and females, This was also the case between the Kalilawa and Lereng cave populations, and Kalilawa and Salamaik cave populations. The males from Kalilawa cave and Lereng cave only differed significantly on one character, whereas females differed on two 2 characters. Males and females from Kalilawa and Salamaik caves differed signficantly on two characters. The male populations of Lereng and Salamaik caves differed significantly on three characters and in five characters for females (Table 3).

Unweighted Pair Group Method Arithmatic Average and Principall Component Analysis

Euclidian distances showed up in a UPGMA analysis as clusters between three population with males and females analysed separately. PCA of 36 characters revealed a clear separation between the three different cave populations (Fig. 4).

UPGMA analysis revealed a close relationship between Padang and Pariaman populations (0.25 (male) and 0.14 (female)), and PCA showed that of *H. diadema* populations from Padang were closely related to the Pariaman populations, and clearly distinct from the Sawahlunto populations.

Discussion

H. diadema populations from three caves separated from each other by Bukit Barisan in West Sumatra revealed variations and morphological character divergences. Our data suggest that the individuals from Kalilawa cave are more closely related to individuals from the Lereng cave, whereas it differed from Salamaik cave. From 21 external and 15 skull characters used in this analysis the *H. diadema* population from Salamaik cave are significantly smaller than conspecifics from Kalilawa and Lereng caves. Ecological circumstances related with breeding, foraging, crowding and resources avaibility may differ between the three populations due to their separation by the Bukit Barisan range. Kalilawa and Lereng caves are located on the western side of Bukit Barisan and at a lower altitude than the Salamaik cave on the eastern side. The ecological different conditions, combined with a lower inter-population migration, may have required different behavioural adaptation and resulting morphological variations. Euclidian distance among *H. diadema* populations from the three study sites showed that geographic distance is reflected in the relationship distance. Kalilawa cave is closer geographically and in relationship distance to Lereng cave than to Salamaik cave.

Rahman and Abdullah (2010) reported morphological variations between geographical separated populations of *Penthetor lucasi* in Sarawak and suggested that ecological conditions as the likely main cause of the differentiation. Kitchener and Suyanto (1996) suggest that the Pleistocene- modern time island arrangement have caused relatively recent morphological changes. Kitchener, Konishi and Suyanto (1996) assumed that longitude was the most important variable when predicting overall skull and body size. In contrast Whitten (1987) argued that Bukit Barisan was formed already during Miocene, and therefore separated populations of *H. diadema* in West Sumatra at a much earlier stage. Kitchener et al., (1992) noted that *H. diadema* in Lesser Sunda Island was divided into three phenetic grouping based on external and skull measurements: *H.d. diadema*, *H.d. reginae* and *H.d. masoni* in one group; *H.d. griseus* and *H.d. oceanitis* in a second group, and *H.d.nobilis* in a separate cluster. The study indicated that the eastern form of *H. d. diadema* is smaller than the western form, suggesting different ecological conditions had required different adaptational strategies and eventually morphological unique forms.

Whereas morphological variation could give rise to speciation, we were unable to determine from morphological characters alone that individuals of *H. diadema* from the three different study sites belong to different subspecies. Further studies on the genetic variation of *H. diadema* in West Sumatra is needed to confirm if there are indeed three different subspecies.

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