GEOGRAPHIC VARIATION IN SKULL SIZE AND SHAPE OF *CROCIDURA DRACULA* (MAMMALIA: SORICIDAE) IN VIETNAM

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Recent studies have shown a great species richness of the Vietnamese white-toothed shrew genus *Crocidura*. To date, a total of 15 species of the genus have been recorded from Vietnamnamely *Crocidura annamitensis, C. attenuata, C. dracula, C. fuliginosa, C. guy, C. indochihensis, C. kegoensis, C. phanluongi, C. phuquocensis, C. rapax, C. sapaensis, C. sokolovi, C. tanakae, C. wuchiensis, C. zaitsevi*, and. However, the taxonomy and distribution pattern of this genus is still incomplete (Bui *et al.*, 2013). For instance, the actual distribution of *C. dracula* is unclear. This species was originally described from Yunnan Province (China), and was supposed to have a wide distribution throughout northern India and southern Asia, including Vietnam. The distribution of *C. dracula* in the mainland and on islands of Vietnam is still questionable because of the confusion between *C. dracula* and *C. fuliginosa*.

The recent studies of *Crocidura* in Vietnam focused on collecting new species and further enhanced the information of species distribution (Lunde *et al*. 2003, 2004; Jenkins *et al*. 2007, 2009, 2010, 2013; Abramov *et al*. 2008, 2013). Although some diagnostic morphological features of these species have been provided, the genetic diversity of *C. dracula* remains unknown (Bannikova *et al*., 2011). In the past, only Jenkins *et al*. (2013) stated the geographical variation of *C. attenuata* from Lao Cai, Ha Tinh, Quang Tri, Kon Tum and Lam Dong provinces.

This study aims to elucidate geographic variation of *C. dracula* by comparing 23 craniodental measurements of the specimens from six localities on the mainland and island of Vietnam. We used principal component analysis (PCA) in order to evaluate variation in the size and shape of *C. dracula*.

I. MATERIALS AND METHODS

A total of 38 skulls of *C. dracula*, collected from Hoa Binh, Phu Tho, Vinh Phuc, Thanh Hoa, Nghe An and Ca Mau (Hon Khoai Island) provinces, were examined from zoological collections of the Institute of Ecology and Biological Resources and Vietnam National Museum of Nature. The specimens were measured for 23 cranial and dental characters by using a digital caliper (Mitutoyo Model 500-197-30 (0-200 mm/0.01 mm) (Figure 1).

All measurements were calculated with mean values, standard errors and standardize data by © 2016 Microsoft ® Excel for Mac version 15.27 software. To investigate the geographic variation in *C. dracula*, a PCA analysis was performed with the software PAST Statistic 3.14 (Hammer *et al.*, 2001) using log-transformed raw data to assess size factor using the PC1 score that represents overall size and log-transformed standardized data to assess shape factor using each of the PC scores that have eliminated size factors.

Differences in the craniodental measurements and the mean values of the principal components among populations were examined by one-way analysis of variance (ANOVA). The statistically significant differences between the mean values of six *C. dracula* groups were determined by using the analysis of variance (ANOVA: Single Factor) on Excel with significance level (α) of 0.01.

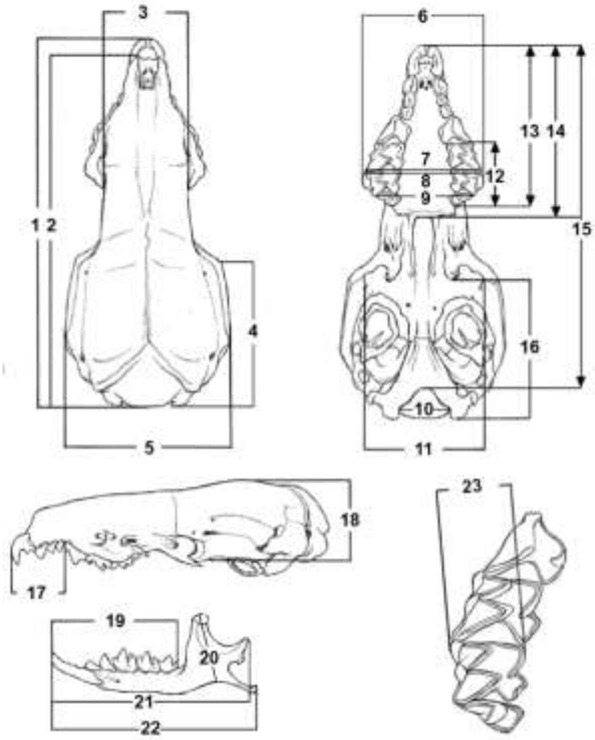

Figure 1: **Dorsal and ventral views of the cranium, lateral views of the cranium and mandible, and details of the postunicuspid dentition showing measurements**

1. Condyloincisive length (CIL); 2. Condylobasal length (CBL); 3. Least interorbital breadth (LIOB); 4. Superior articular facet to occipital condyle length (SOL); 5. Braincase breadth (BB); 6. Zygomatic process of maxilla breadth (ZZB); 7. M1-M1 breadth (M1M1); 8. M2-M2 breadth (M2M2); 9. M3-M3 breadth (M3M3); 10. Occipital condyle width (OW); 11. Postglenoid breadth (PGB); 12. Upper molar crown length (M1M3L); 13. Upper toothrow length (UTRL); 14. Palate length (PL); 15. Occipital condyle to I1 length (OIL); 16. Braincase length (BL); 17. P3-I1 length (P3-I1); 18. Braincase height (BH); 19. i1 - m3 length (i1m3); 20. Mandible height (MH); 21. Angular to i1 length (MIL); 22. Mandible length (ML); 23. M3 width (M3W).

II. RESULTS

Measurements of the six population of *C. dracula* from Vietnam are presented in Table 1. One-way ANOVA analysis showed that the differences in the size clearly delineated three groups of the six *C. dracula* populations. The PC1 was interpreted to represent a size component, because all character factor loadings were positive, which explains the 66.69% total variance for all specimens. The loadings plot of PC 1 showed relatively large loading in almost all characters, with the highest being ZZB, M1-M3, UTRL, PL, P3-I1, MIL, and M2W (Table 2).

The one-way ANOVA results showed that differences in shape separated five groups of *C. dracula* in this study. The PCA results of log-transformed standardized data (as shape components) showed that PC1 = 38.85%, PC2 = 24.14%. The high factor loadings of the all populations are demonstrated by the following measurements: LIOB, OW, UTRL, PL, BL, P3I1, MIL, and M2W (Table 3).

Table 1

Measurements of the six populations of *C. dracula* from Vietnam

Character	Hoa Binh	Phu Tho	Vinh Phuc	Thanh Hoa	Nghe An	Ca Mau
CIL	24.30 ± 0.29 23.95-24.72	24.87 ± 0.4 24.46-25.57	26.33 ± 0.28 26.08 - 26.69	25.40 ± 0.68 24.48 - 26.37	24.68 ± 0.33 24.22 - 25.11	23.95 ± 0.53 23.45 - 24.51
CBL	23.48 ± 0.39 23.00 -23.98	23.90 ± 0.36 23.38 - 24.41	25.01 ± 0.09 24.92 - 25.10	24.57 ± 0.66 23.88 - 25.45	23.83 ± 0.23 23.55 - 24.23	22.82 ± 0.71 22.3 - 23.63
LIOB	5.87 ± 0.09 5.76 - 6	5.70 ± 0.18 5.46 - 6.04	5.68 ± 0.04 5.63 - 5.75	5.96 ± 0.25 5.66 - 6.31	5.58 ± 0.19 5.36 - 5.85	5.48 ± 0.15 5.38 - 5.66
SOL	9.35 ± 0.06 9.28 - 9.45	9.52 ± 0.29 9.20 - 9.94	9.81 ± 0.04 9.76 - 9.86	9.82 ± 0.37 9.33- 10.31	9.33 ± 0.28 9.00 - 9.81	9.60 ± 0.25 9.36 - 9.86
BB	10.93 ± 0.03 10.82 -10.99	10.63 ± 0.18 10.40 - 10.89	10.95 ± 0.06 10.88 - 11.01	10.97 ± 0.30 10.62 - 11.6	10.81 ± 0.13 10.65 - 10.98	10.69 ± 0.21 10.46 - 10.85
ZZB	7.57 ± 0.07 7.48 - 7.65	7.53 ± 0.24 7.27 - 7.90	8.76 ± 0.05 8.71 - 8.81	7.99 ± 0.29 7.48- 8.35	7.66 ± 0.20 7.43 - 8.00	7.49 ± 0.22 7.32 - 7.74
M1M1	7.04 ± 0.04 7.01 - 7.10	7.06 ± 0.27 6.66 - 7.36	7.76 ± 0.09 7.68 - 7.89	7.36 ± 0.19 7.11 - 7.69	6.79 ± 0.21 6.54 - 7.12	6.92 ± 0.19 6.72 - 7.10
M2M2	7.15 ± 0.02 7.13 - 7.20	7.21 ± 0.19 7.02 - 7.48	8.17 ± 0.06 8.09 - 8.24	7.52 ± 0.29 7.03- 7.84	7.24 ± 0.17 7.02 - 7.56	7.13 ± 0.24 6.92 - 7.40
M3M3	6.39 ± 0.04 6.36 - 6.46	6.22 ± 0.13 6.06 - 0.45	6.79 ± 0.07 6.69 - 6.86	6.54 ± 0.20 6.26 - 6.88	6.34 ± 0.29 6.00 - 6.76	5.92 ± 0.10 5.80 - 5.99
OW	3.90 ± 0.14 3.75 - 4.03	3.74 ± 0.11 3.61 - 3.92	3.71 ± 0.05 3.66 - 3.76	3.89 ± 0.09 3.75 - 4.03	3.91 ± 0.09 3.75 - 4.02	3.89 ± 0.07 3.82 - 3.96
PGB	7.58 ± 0.09 7.50 - 7.75	7.40 ± 0.17 7.24 - 7.71	7.72 ± 0.04 7.67 - 7.77	7.50 ± 0.19 7.33 - 7.93	7.41 ± 0.22 7.14 - 7.74	7.27 ± 0.22 7.07 - 7.50
M1M3L	4.35 ± 0.11 4.23 - 4.53	4.39 ± 0.16 4.15 - 4.64	4.87 ± 0.05 4.81 - 4.93	4.57 ± 0.21 4.25 - 4.83	4.33 ± 0.30 4.08 - 4.89	4.29 ± 0.24 4.02 - 4.45
UTRL	10.72 ± 0.26 10.43 -11.01	10.94 ± 0.25 10.58 - 11.42	12.4 ± 0.06 12.33 - 12.47	11.33 ± 0.36 10.93 - 12.03	10.48 ± 0.24 10.14 - 10.88	10.76 ± 0.36 10.34 - 11.01
PL	11.13 ± 0.17 10.95 -11.49	11.53 ± 0.22 11.22 - 11.88	13.38 ± 0.09 13.27 - 13.49	11.93 ± 0.35 11.45 - 12.47	11.57 ± 0.27 11.29 - 11.98	11.20 ± 0.369 10.81 - 11.53
OIL	21.99 ± 0.14 21.70 -22.14	22.57 ± 0.33 22.51 - 23.15	23.95 ± 0.08 23.84 - 24.04	23.18 ± 0.56 22.6 - 24.01	21.98 ± 0.10 21.87 - 22.18	21.65 ± 0.64 21.04 - 22.32
BL	9.20 ± 0.11 9.00 - 9.32	9.06 ± 0.19 8.80 - 9.27	9.09 ± 0.07 9.01 - 9.18	9.15 ± 0.34 8.72 - 9.54	9.70 ± 0.13 9.45 - 9.83	8.85 ± 0.24 8.60 - 9.07
BH	5.65 ± 0.08 5.52 - 5.75	5.77 ± 0,15 5.59 - 6.07	5.95 ± 0.06 5.87 - 6.01	5.78 ± 0.23 5.42 - 6.18	5.63 ± 0.22 5.32 - 5.92	5.87 ± 0.10 5.76 - 5.94
P3I1	4.62 ± 0.17 4.35 - 4.93	4.88 ± 0.12 4.55 - 5.12	5.47 ± 0.05 5.41 - 5.52	4.85 ± 0.29 4.33 - 5.24	4.79 ± 0.18 4.53 - 5.03	4.82 ± 0.30 4.53 - 5.13
I1M3	9.95 ± 0.17 9.69 - 10.25	10.10 ± 0.16 9.91 - 10.30	11.34 ± 0.07 11.25 - 11.41	10.40 ± 0.29 10.16 - 11.02	10.08 ± 0.17 9.87 - 10.34	9.75 ± 0.29 9.44- 10.02
MH	5.96 ± 0.03 5.93 - 6.02	5.59 ± 0.10 5.83 - 6.06	6.4 ± 0.07 6.32 - 6.48	6.28 ± 0.22 6.02 - 6.64	5.78 ± 0.19 5.45 - 6.04	5.61 ± 0.30 5.37 - 5.95
MIL	15.17 ± 0.09 15.10 -15.32	15.72 ± 0.23 15.37 - 16.09	17.58 ± 0.06 17.5 - 17.65	16.19 ± 0.37 15.84 - 16.8	15.23 ± 0.28 14.92 - 15.59	14.64 ± 0.38 14.24 - 14.99

Character	Hoa Binh	Phu Tho	Vinh Phuc	Thanh Hoa	Nghe An	Ca Mau
ML	15.99 ± 0.07 15.92 -16.14	16.56 ± 0.25 16.25 - 16.95	17.89 ± 0.09 17.78 - 17.99	17.00 ± 0.42 16.52 - 17.76	16.71 ± 0.15 16.54 - 17.01	15.41 ± 0.43 14.92 - 15.75
M2W	2.12 ± 0.02 2.09 - 2.16	2.24 ± 0.11 2.12 - 2.37	2.34 ± 0.03 2.31 - 2.37	2.36 ± 0.13 2.22 - 2.55	2.37 ± 0.08 2.23 - 2.48	2.62 ± 0.15 2.51 - 2.79

Table 2

Character factor loadings for PCA of the log-transformed raw data

Measurement	PC1	PC2
CIL	0.171	-0.013
CBL	0.155	-0.039
LIOB	0.095	**-0.203**
SOL	0.148	0.099
BB	0.068	-0.009
ZZB	**0.296**	0.032
M1-M1	0.243	-0.078
M2-M2	0.266	0.054
M3-M3	0.199	**-0.164**
OW	-0.020	0.102
PGB	0.104	-0.082
M1-M3	**0.301**	0.039
UTRL	**0.291**	-0.064
PL	**0.300**	0.021
OIL	0.183	-0.059
BL	0.025	0.096
BH	0.121	0.101
P3-I1	**0.295**	0.179
I1-M3	0.242	-0.059
MH	0.238	-0.150
MIL	**0.271**	-0.144
ML	0.207	-0.057
M2W	0.109	**0.885**
Eigenvalue	**0.005**	**0.0009**
%variance	**66.690**	**11.45**

Table 3

Character factor loadings for PCA of the log-transformed standardized data

Measurement	PC 1	PC 2
CIL	-0.0036	0.0266
CBL	-0.0452	0.0767
LIOB	-0.2049	**0.3516**
SOL	-0.1090	-0.0328
BB	-0.2300	0.1195
ZZB	0.1872	-0.0695
M1-M1	0.1253	0.0654
M2-M2	0.1445	-0.0863
M3-M3	0.0155	0.2141
OW	**-0.4681**	0.1084
PGB	-0.1456	0.1673
M1-M3	0.1546	-0.0463
UTRL	**0.2636**	-0.0267
PL	**0.2579**	-0.1133
OIL	0.0472	0.0495
BL	**-0.3600**	0.0611
BH	-0.1058	-0.0629
P3-I1	0.2176	**-0.2702**
i1-m3	0.1695	-0.0006
MH	0.0941	0.1677
MIL	**0.2561**	0.0534
ML	0.0836	0.0356
M2W	**-0.3444**	**-0.7882**
Eigenvalue	**0.0015**	**0.0009**
% variance	**38.852**	**24.143**

We found some high factor loadings and using ANOVA: Single factor, the differences in M2-M2, CIL, PL and MIL from six groups clearly showed significant values among the studied specimens (M2-M2: F29.61, CIL: F15.52, PL: F42.48, MIL: F65.76 > F crit3.65, P = 0.01). In contrast, the differences in OW is not significant (F0.67 < F crit3.65, P = 0.01).

The hierarchical clustering axes of size and shape with similarity index of correlation and 1000-time repetitions are shown in Figures 2 and 3. There are three separated clades, the clade 1 includes four groups: Phu Tho, Hoa Binh, Thanh Hoa and Nghe An. Of this clade, the correlation of *C. dracula* population in Phu Tho, Hoa Binh and Thanh Hoa are closer than Nghe An (subclade 1 and subclade 2). Clade 2 and clade 3 have only one group from Vinh Phuc and Ca Mau (Hon Khoai Island), respectively and they are completely separated from the others.

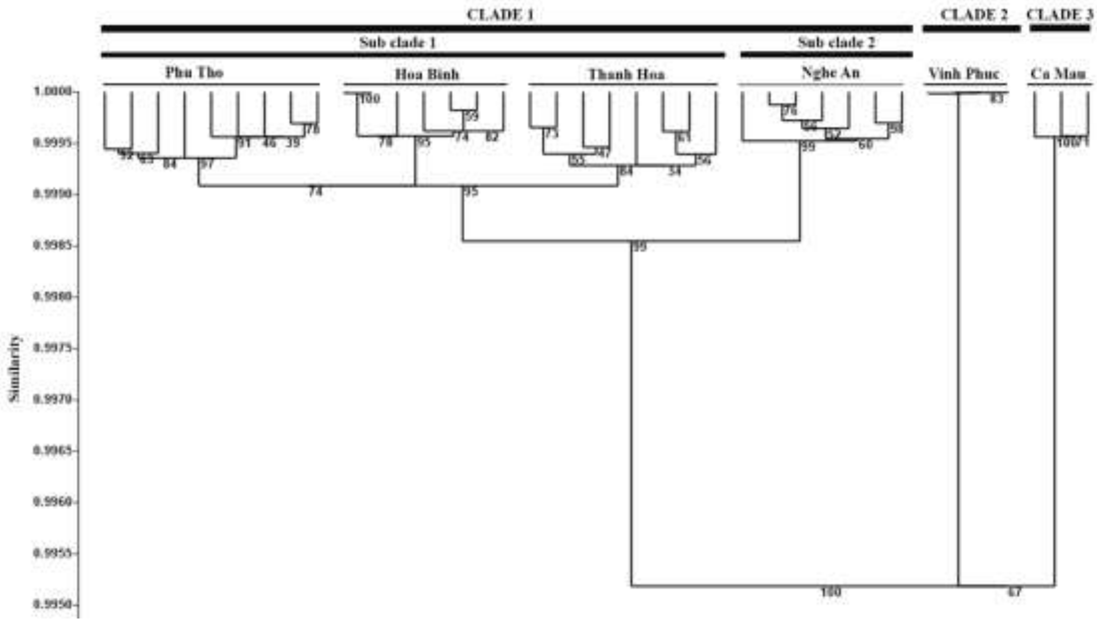

Figure 2: **The hierarchical clustering axes of size**

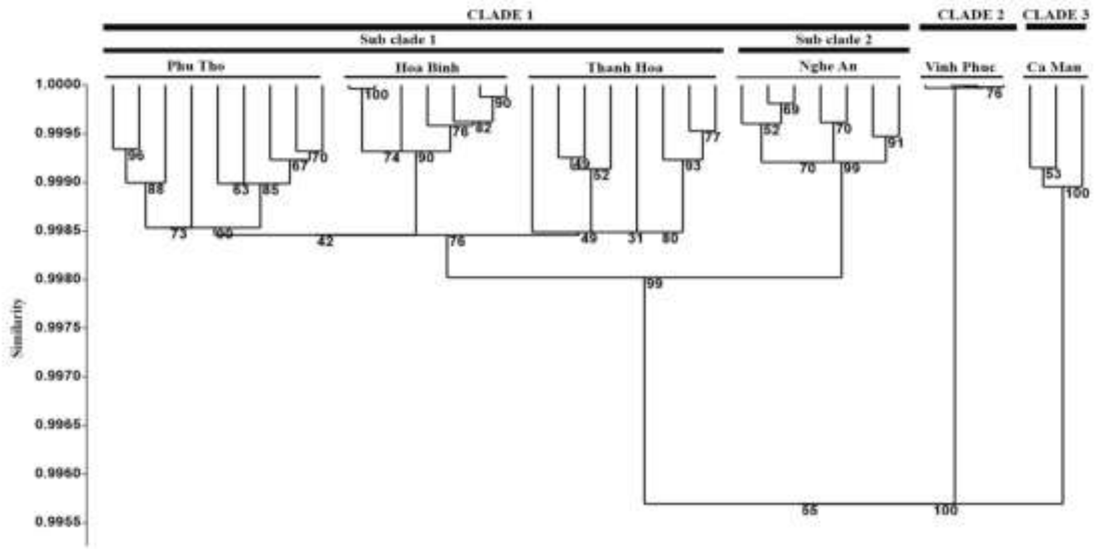

Figure 3: **The hierarchical clustering axes of shape**

Furthermore, PCA results also presented correlation in skull size and shape of six *C. dracula* groups (Figures 4 and 5). The scatter plot of scores of the fist principal component axes base on log-transformed raw data evidenced the distinct differences in size of the three groups. The populations in Ca Mau (Hon Khoai Island) and Vinh Phuc belong to two separated groups. The populations in Nghe An, Phu Tho, Thanh Hoa and Hoa Binh together belong to one group, but the divergence among these populations is probably high and the variation range may slightly overlap.

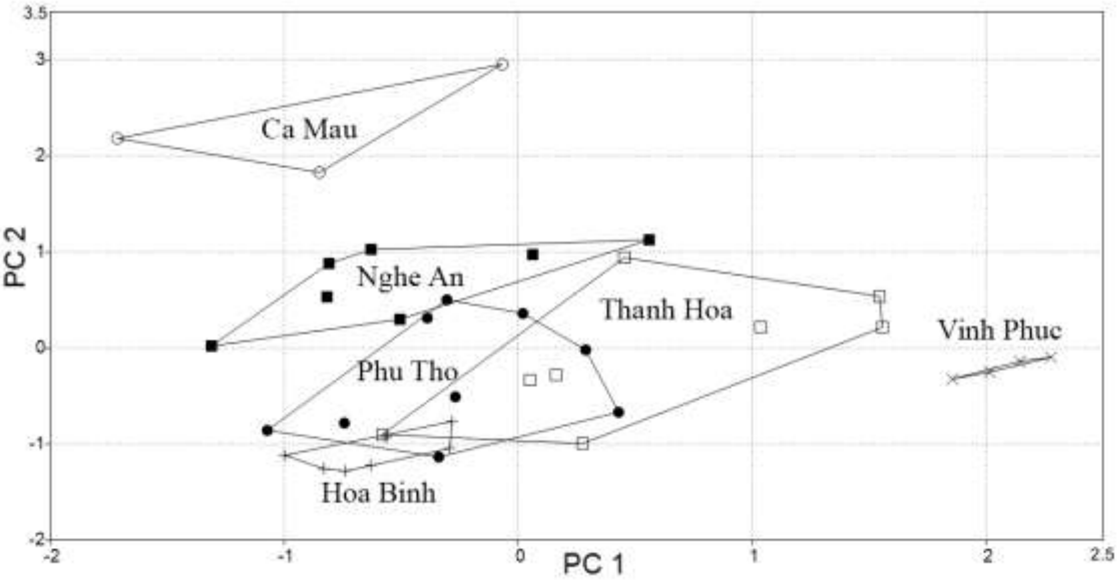

Figure 4: **The scatter plot of scores of the fist principal components axes base on log-transformed raw data**

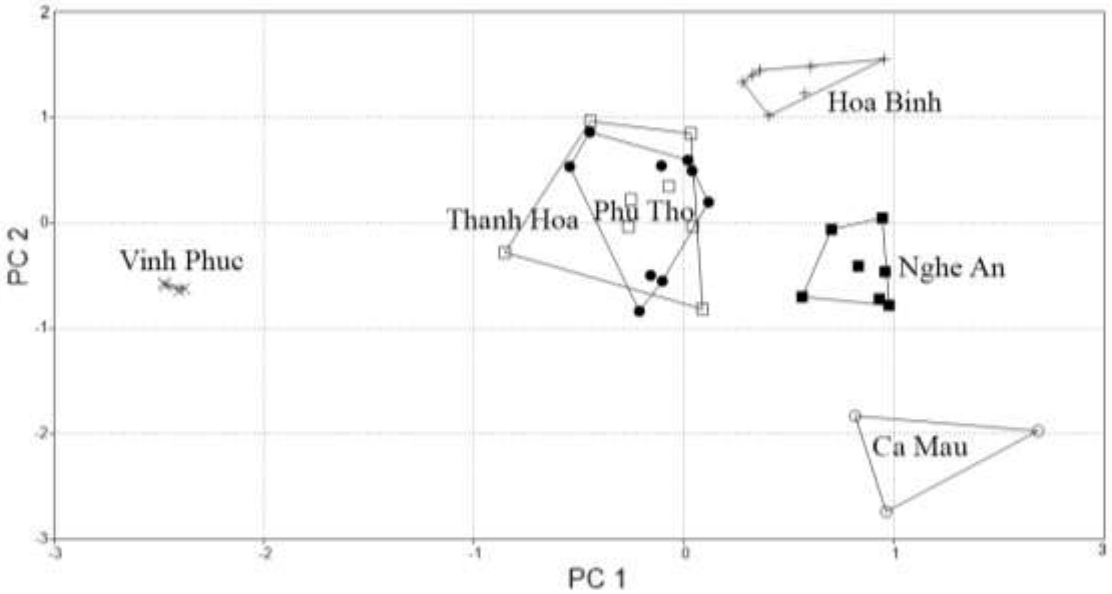

Figure 5: **The scatter plot of scores of the fist principal components axes base on log-transformed standardized data**

On the other hand, the variation of skull shape from six localities was indicated in the scatter plots of scores of PC 1 and PC 2 axes based on log-transformed standardized data (Fig. 5). The plots of Hoa Binh, Nghe An, Ca Mau (Hon Khoai Island) and Vinh Phuc from the continent were strongly separated from the others. The plot of Thanh Hoa approximately overlapped with the plot of Phu Tho.

III. DISCUSSION

Our results showed appreciable geographic variation in the populations of *C. dracula* from Vietnam. Overall skull size variation demonstrated a close relationship among *C. dracula* populations in Hoa Binh, Thanh Hoa, Nghe An and Phu Tho, and a clear separation from the populations in Ca Mau and Vinh Phuc. This pattern is reflected in the geographic distribution of these groups, for example, the specimens from Vinh Phuc were collected in Tam Dao where the elevation is approximately 900m a.s.l. and represents a montane island and specimens from Ca Mau were collected in Hon Khoai isolated Island. There are significant differences in skull shape among Hoa Binh, Ca Mau, Nghe An, Vinh Phuc and the group including Thanh Hoa and Phu Tho. Our analysis indicated that the characteristics: M2-M2, CIL, PL and MIL contribute the highest impact to variation of skull size and shape. It is possible that the palate structure is the important morphological characteristic for taxonomy of this species.

Skull size and shape of specimens collected from Ca Mau (Hon Khoai Island) are clearly distinguished from each other and represent a separated population. Specifically, the differences between *C.* cf. *dracula* in Hon Khoai Island and Vinh Phuc. These results may provide an interesting explanation for the observed geological variation.

This study also suggested that the multivariate analysis of skull size and shape is useful for assessing geographic variations, and probably for taxonomy in mammal research. This approach may consequently have material implications for understanding the mechanisms of morphological evolution.

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SỰ SAI KHÁC VỀ HÌNH DẠNG VÀ KÍCH THƯỚC SỌ GIỮA MỘT SỐ QUẦN THỂ LOÀI *CROCIDURA DRACULA* Ở VIỆT NAM

Bùi Tuấn Hải, Lý Ngọc Tú, Vũ Thuỳ Dương và Nguyễn Trường Sơn

TÓM TẮT

Kết quả phân tích 38 mẫu sọ của loài Chuột chù răng trắng miền Bắc (*C. dracula*) thu thập ở các tỉnh Phú Thọ, Vĩnh Phúc, Hoà Bình, Thanh Hoá, Nghệ An và Cà Mau (đảo Hòn Khoai) cho thấy sự khác biệt của 3 nhóm kích thước sọ và 5 nhóm hình thái sọ. Đặc biệt, sự sai khác về kích thước và hình dạng sọ của hai quần thể tại Vĩnh Phúc và Hòn Khoai ẩn chứa nhiều điều thú vị về phân bố, cách ly địa lý và cần được nghiên cứu kỹ lưỡng hơn, đặc biệt là phân tích quan hệ di truyền.