Morphological analysis of two Asiatic water shrews (Eulipotyphla, *Chimarrogale*) from Vietnam

Bui Tuan Hai*, Masaharu Motokawa, Jadab Kumar Biswas, Alexei V. Abramov, Ly Ngoc Tu, Vu Thuy Duong & Nguyen Truong Son*

ABSTRACT. The patterns of morphometric variation in external body and skull characters of two Asiatic water shrews (*Chimarrogale himalayica* and *C. varennei*) from Vietnam were analysed using different indices of variability. A total of 28 specimens were studied. Univariate, multivariate, and allometric analyses were conducted on 21 measurements of 26 skulls, while univariate analysis was conducted on three external measurements of 24 specimens. An external comparison showed that *C. himalayica* is larger than *C. varennei*. The mean (M) of skull measurements was used as an independent variable to regress the coefficient of variation (CV) and standard deviation (SD). The CVs did not differ significantly between two species and exhibited an inverse relationship with the M of skull measurements. CV trends of major functional parts of the skull showed that incisor length, brain-case size, postglenoid breadth, and mandible length presented opposing differences between the two species. In addition, *C. himalayica* had the highest allometric coefficient for the overall skull length, whereas *C. varennei* had the highest allometric coefficient for the postorbital region. Our study also revealed that the facial musculoskeletal system of *C. himalayica* is more developed than in *C. varennei*.

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Морфологический анализ двух азиатских водяных землероек (Eulipotyphla, *Chimarrogale*) из Вьетнама

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РЕЗЮМЕ. С использованием различных индексов изменчивости проанализированы закономерности морфометрической изменчивости экстерьерных и краниометрических признаков двух видов азиатских водяных землероек (*Chimarrogale himalayica* и *C. varennei*) из Вьетнама. Всего исследовано 28 экземпляров. Одномерный, многомерный и аллометрический анализы были проведены по 21 промеру 26 черепов, а одномерный анализ был проведен по трем промерам тела для 24 экземпляров. Сравнение внешних признаков показало, что *C. himalayica* крупнее *C. varennei*. Среднее значение (M) промеров черепа использовалось в качестве независимой переменной для регрессии коэффициента вариации (CV) и стандартного отклонения (SD). CV существенно не отличались между двумя

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видами и демонстрировали обратную связь со средними размерами черепа (M). Анализ CV основных функциональных частей черепа показал, что длина резца, размер мозговой капсулы, постгленоидная ширина и длина нижней челюсти демонстрируют противоположные различия между двумя видами. Кроме того, у *C. himalayica* самый высокий аллометрический коэффициент был отмечен для общей длины черепа, тогда как у *C. varennei* самый высокий аллометрический коэффициент был отмечен для заглазничной области. Полученные данные свидетельствуют, что костно-мышечная система морды у *C. himalayica* более развита, чем у *C. varennei*.

КЛЮЧЕВЫЕ СЛОВА: Soricidae, аллометрия, коэффициент вариации, одномерный и многомерный анализы, изменчивость.

Introduction

Natural selection is known to allow organisms to adapt to their physical and biological environments (Reiss, 1989). Morphological character displacement, or phenotypic convergence, could result from environmental factors and a competition with closely related sympatric species (Rychlik *et al*., 2006). Skull variation plays an important role in studying taxonomy, evolution, and musculoskeletal function. Several variables have been used to look into the evolutionary significance of variation within or between species (Suzuki *et al*., 2012; Biswas & Motokawa, 2019).

The coefficient of variation (CV), which measures relative variability, has frequently been used as an important and informative index to evaluate a variability of different measurements in several mammalian dentition studies (Suzuki *et al*., 2012; Biswas & Motokawa, 2019). However, CV and the mean variable size show a significant negative correlation, which could artificially overestimate the apparent variability of small traits (Dayan *et al*., 2002; Polly, 2008; Suzuki *et al*., 2012).

Along with CV, the allometry coefficient is yet another important parameter of dimensional variation in studying morphometric variables, function and development of organisms, with a great contribution to evolutionary studies (Szuma, 2000; Meiri *et al*., 2005; Prevosti & Lamas, 2006; Polly, 2008; Suzuki *et al*., 2011, 2012). Allometry would appear to be a more reliable and appropriate morphological method in analysing intra- and interspecific variation in (sub)adults, because it clarifies the interspecific differences in skull shape caused by size variations, thereby limiting the dependence on age variations (Miller *et al*., 2009; Suzuki *et al*., 2011, 2012; Biswas & Motokawa, 2019). Therefore, based on estimating the slope of the allometric regression line, many previous studies have used allometry to evaluate dimensional differences in relation to total length (e.g., CBL) and other cranial parameters (Gould, 1966; Ando *et al*., 1989; Lin & Shiraishi, 1992; Abdala *et al*., 2001; Giannini *et al*., 2004; Suzuki *et al*., 2011, 2012; Cardini & Polly, 2013; Sánchez-Villagra *et al*., 2017; Biswas & Motokawa, 2019). Nevertheless, uni- and multivariate methods remain useful classical analytical approaches in morphological studies.

Water shrews are semi-aquatic small mammals representing a good object for studying the impact of sympatry on special morphological features (Rychlik *et al*., 2006). The tribe Nectogalini (Soricidae) includes six genera: *Episoriculus*, *Soriculus*, *Chodsigoa*, *Chimarrogale*, *Nectogale* and *Neomys*, comprising a total of 29 species (Burgin & He, 2018). The Asiatic water shrew genus *Chimarrogale* is the third largest genus within the tribe Nectogalini, with seven species described to date (Burgin & He, 2018). They are widespread in Asia, ranging from northern India in the west, to China in the north and Japan in the east, and reaching the Sunda Islands in the south (Hutterer, 2005; Burgin & He, 2018).

The composition of *Chimarrogale* and distribution of its species in SE Asia have been controversial for a long time. Hutterer (2005) listed six species with their respective distribution areas (in parentheses) as follows: *C. platycephalus* (Honshu and Kyushu, Japan), *C. sumatrana* (Sumatra, Indonesia), *C. phaeura* (Borneo, Malaysia), *C. hantu* (Malay Peninsula, Malaysia), *C. styani* (central, western and southwestern China, and northern Myanmar), and *C. himalayica* (northern India, southern China, Taiwan, and northern and central Vietnam).

Based on the mDNA phylogenetic analysis of *Chimarrogale* from north-west India, southern China, northern Laos, northern Myanmar, and northern and central Vietnam, Yuan *et al*. (2013) discovered that *C. leander* and *C. varennei* were clearly distinct from the Himalayan water shrew *C. himalayica*. Accordingly, *C. leander* is distributed in central and eastern China and Taiwan (also mentioned by Burgin & He, 2018) and *C. varennei* (*sensu* Yuan *et al*., 2013) is known from southern China to northern and central Vietnam. However, Abramov *et al*. (2017) revised *Chimarrogale* from Vietnam and found a new, highly divergent genetic lineage of *Chimarrogale* from the Central Highlands of Vietnam. Based on a morphological analysis, specimens from southern Vietnam were classified as *C. varennei* proper, which is confined to that region, whereas the polymorphic *C. himalayica* containing at least four cytochrome *b* haplogroups (including misidentifying "*C. varennei*" of Yuan *et al*. (2013)) seem to occur in central and northern Vietnam and southern China. Simultaneously, based on molecular and morphological evidence, Abramov *et al*. (2017) proposed a new classification system for all the extant Asiatic water shrews including eight species belonging to two genera — *Chimarrogale* (*C. himalayica, C. leander, C. platycephalus, C. styani, C. varennei*) and *Crossogale* (*C. hantu, C. phaeura, C. sumatrana*). Recently, the genus *Crossogale* was sufficiently supported by Abd Wahab *et al.* (2020).

Several papers on the cranial morphology of *Chimarrogale* have focused mainly on the analysis of variables and geographical variation (Harrison, 1958; Jones & Mumford, 1971; Arai *et al.*, 1985; Motokawa *et al.*, 2006; Abe, 2009; Abramov *et al.*, 2017). To date, there have been no studies investigating the relationship between cranial variability and allometric trends in SE Asian shrews. In this paper, we therefore have examined craniodental parameters of two species of water shrews using different variability indices to investigate the morphological variability of these two species (*C. himalayica* and *C. varennei*) in Vietnam for the first time.

Materials and methods

Measurements — A total of 28 specimens of Asian water shrews (15 individuals of *C. himalayica* and 13 of *C. varennei*) collected from 15 localities in Vietnam were examined (Fig. 1, Table 1). The studied specimens are deposited in the collections of the Institute of Ecology and Biological Resources (IEBR) in Hanoi, Vietnam, and the Zoological Institute of the Russian Academy of Sciences (ZIN) in Saint Petersburg, Russia. Using a digital caliper and partially following Jenkins *et al*. (2009), the twenty-one cranial and dental measurements were taken on 26 skulls as follows: condyloincisive length (CIL); condylobasal length (CBL); least interorbital breadth (LIOB); superior articular facet to occipital condyle length (SOL); braincase breadth (BB); zygomatic process of maxilla breadth (ZZB); M1-M1 breadth (M1M1); M2-M2 breadth (M2M2); M3-M3 breadth (M3M3); occipital condyle width (OW); postglenoid breadth (PGB); M1-M3 length (M1M3); upper toothrow length (UTRL); palate length (PL); occipital condyle to I1 length (OIL); braincase length (BL); i1-m3 length (i1m3); mandible height (MH); angular to i1 length (MIL); mandible length (ML); M2 width (M2W). Additionally, three external measurements were taken on 24 specimens (12 for each species, Table 1): viz., head and body length (HB), tail length (T) and hind foot length (HF).

Statistics — As a multivariate analysis of variance (MANOVA) using log-transformed measurements (both craniodental and external) showed non-significant sexual dimorphism, we combined specimens of both sexes in further analyses. First, we have performed the descriptive statistics, including the arithmetic mean (M) and standard deviation (SD) for three external measurements. Based on 21 skull measurements, we have also calculated M, SD and the coefficient of variation (CV), following several previous studies (Szuma, 2000; Prevosti & Lamas, 2006; Polly, 2008; Suzuki *et al*., 2012; Biswas & Motokawa, 2019). The CV values of skull variables for each species were tested using Wilcoxon's signed-rank test (Pankakoski *et al*., 1987; Biswas & Motokawa, 2019).

To assess the patterns of variation between *C. himalayica* and *C. varennei*, allometric analysis was conducted using the following formula: $\log y = \alpha * \log x + ß$ (with $y = bx^{\alpha}$, $ß = \log b$) (Huxley & Teissier, 1936). Here, CBL (x) was considered as an independent variable, y was the variable of interest and α is an allometric coefficient (Suzuki *et al*., 2011, 2012; Biswas & Motokawa, 2019). To calculate the coefficient of allometry (α), we applied similar methodology of regression [ordinary least-square (OLS) regression] following Kilmer & Rodríguez (2017) and Biswas & Motokawa (2019). We also calculated some pairwise correlations amongst CV, SD, M, and α.

We have compared raw measurements between the two species using the *t*-test. Some informative skull characters,

Table 1. Localities, number of specimens, and voucher IDs of two *Chimarrogale* species from Vietnam used in this study (* — skull only; + — body only).
ZIN — the Zoological Institute of the Russian Academy of Sciences, Saint Petersburg, Russia; NTS, Motokawa, BTH — specimens from the Institute of Ecology and Biological Resources, Hanoi, Vietnam.

Species	Locality	Symbols on the Fig. 1	*n*	Voucher IDs
C. himalayica	Muong Nhe, Dien Bien Prov.	H1	1	ZIN 101819
	Y Ty, Lao Cai Prov.	H2	1	Motokawa 753+
	Phu Yen, Son La Prov.	H3	2	ZIN 101653, 101654
	Xuan Son, Phu Tho Prov.	H4	1	ZIN 101820*
	Xuan Lien, Thanh Hoa Prov.	H5	3	Motokawa 498, 519, 531
	Tay Con Linh, Ha Giang Prov.	H6	3	Motokawa 643, 686, 715
	Cham Chu, Tuyen Quang Prov.	H7	1	NTS 2019.04.41+
	Tam Dao, Vinh Phuc Prov.	H8	1	Motokawa 572
	Tay Yen Tu, Bac Giang Prov.	H9	1	BTH 13*
	Mau Son, Lang Son Prov.	H10	1	BTH 18*
C. varennei	Phong Nha–Ke Bang, Quang Binh Prov.	V1	1	BTH 187*
	Sao La, Hue Prov.	V2	4	Motokawa 729, 736, 743, 744
	Sa Thay, Dak Lak Prov.	V3	4	ZIN 101815, 103037–103039
	Chu Yang Sin, Dak Lak Prov.	V4	3	ZIN 101816–101818
	Bao Loc, Lam Dong Prov.	V5	1	ZIN 101655

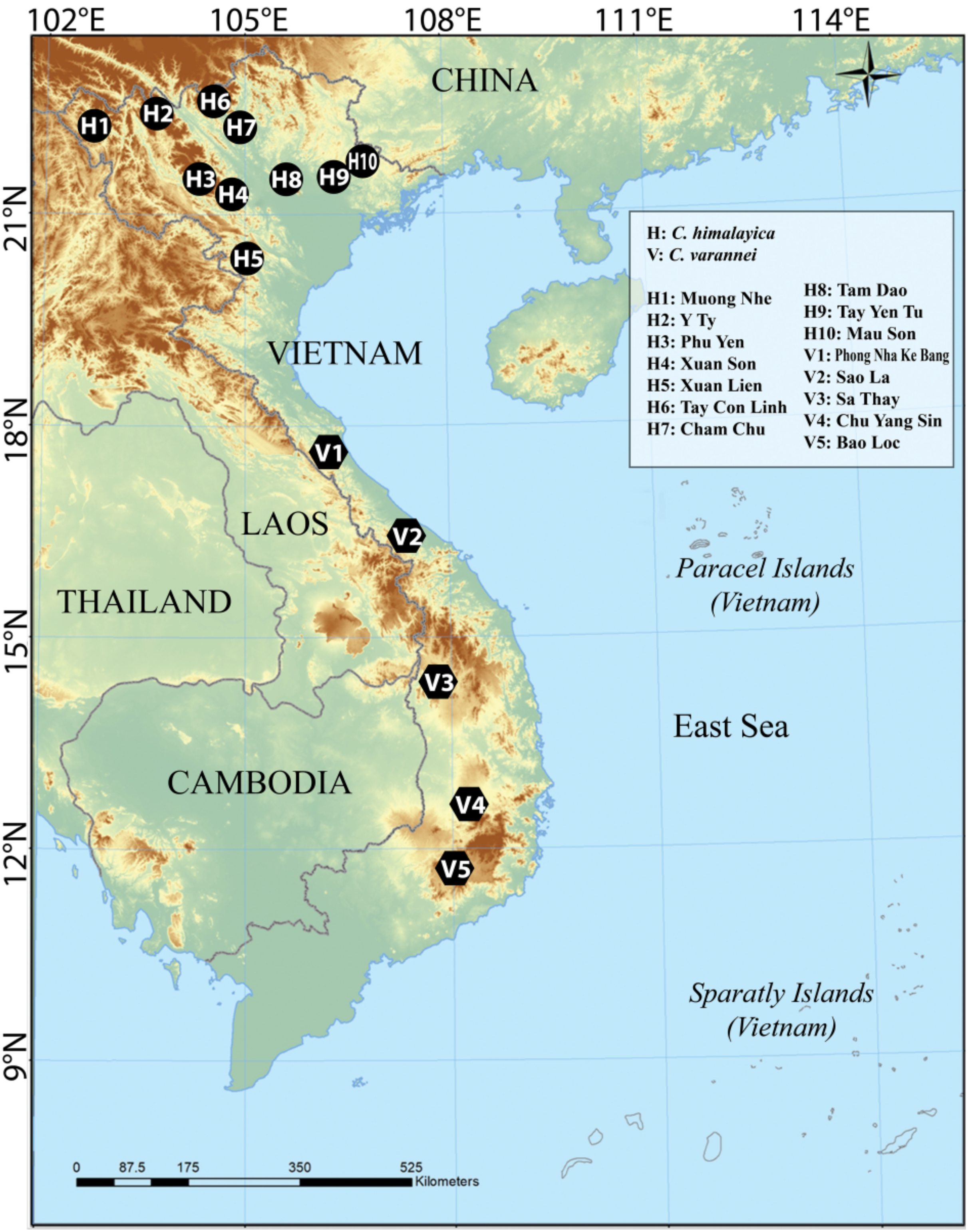

Fig. 1. Map showing the collecting localities of the studied specimens of *Chimarrogale himalayica* and *C. varennei*.

which showed significant correlation with CBL, were also compared by the analysis of covariance (one-way ANCOVA) on skull variables (Motokawa *et al*., 2003). In addition, the principal components analysis (PCA) was conducted based on the covariance matrix of log-transformed skull measurements to evaluate the interspecific morphological variations between *C. himalayica* and *C. varennei* (Motokawa *et al*., 2003). One-way ANOVA and

Tukey's pairwise tests were used to compare the principal components PCs. The Mann-Whitney *U*-test was used in some cases to detect significant differences. The external measurements were also compared using MANOVA and *t*-test. All analyses were performed using the software PAST Statistic 4.0 for Mac OS (Hammer *et al*., 2001).

Results

Variation and variability of skull traits — The CV varied from 0.93 (M1M1) to 4.15 (MH) in *C. himalayica*, and from 1.52 (ZZB) to 3.80 (SOL) in *C. varennei* (Table 2), indicating quite large variability among the skull variables. The CV showed a slightly different pattern in major parts of the cranium, but almost a similar pattern in the mandible between the species (Fig. 2).

The CV showed an inverse relationship with the mean size (M) of skull traits, but the correlation was not significant in both species (*C. himalayica*: $r = -0.309, p = 0.173$; *C. varennei*: $r = -0.295, p = 0.194$). A strong correlation was observed between M and SD in both species (*C. himalayica*: $r = 0.908$, $p < 0.001$; *C. varennei*: $r = 0.888$, $p < 0.001$).

The M showed a positive correlation with R^2 (*C. himalayica*: $r = 0.754$, $p < 0.001$; *C. varennei*: $r = 0.704$, $p < 0.001$), but showed a non-significant relationship with α (*C. himalayica*: $r = 0.556, p = 0.011$; *C. varennei*: $r = 0.434$, $p = 0.054$) in both species. The α showed a non-significant relationship with *CVs* in both species (*C. himalayica*: $r = -0.065$, $p = 0.784$; *C. varennei*: $r = 0.502$, $p = 0.024$).

Bivariate allometric pattern of skull traits — Amongst 20 craniomandibular characteristics, nine (45%) in *C. himalayica* and eight (40%) in *C. varennei* showed significant correlations with CBL (Table 3). The R^2 varied from 0.007 (MH) to 0.788 (CIL) in *C. himalayica* and from 0.003 (OW) to 0.861 (BL) in *C. varennei* (Table 3). The R^2 values did not show a significant difference between these two species (Mann-Whitney $U = 196.5$, $p = 0.935$).

Out of nine allometric coefficients, seven variables (CIL, ZZB, UTRL, PL, BL, i1m3, MIL) exhibited negative allometry, one variable (M1M1) exhibited isometry and the remaining one (OIL) exhibited positive allometry in *C. himalayica*. In comparison, negative allometry was detected for four measurements

Table 2. Descriptive statistics of cranial measurements (mm) of two *Chimarrogale* species from Vietnam.

Variables	*Chimarrogale himalayica*					*Chimarrogale varennei*				
	Mean	SD	Min	Max	CV	Mean	SD	Min	Max	CV
CIL	27.45	0.61	26.52	28.49	2.21	27.40	0.49	26.69	28.12	1.80
CBL	26.73	0.53	25.93	27.51	1.99	26.56	0.53	25.71	27.27	2.00
LIOB	5.13	0.15	4.94	5.38	3.00	4.80	0.08	4.71	4.96	1.69
SOL	11.54	0.22	11.13	11.79	1.89	11.28	0.43	10.73	11.9	3.80
BB	13.72	0.32	13.13	14.21	2.34	13.62	0.39	12.87	14.29	2.88
ZZB	8.46	0.15	8.22	8.68	1.74	8.22	0.12	8.01	8.5	1.52
M1M1	8.24	0.08	8.14	8.39	0.93	7.97	0.17	7.7	8.25	2.08
M2M2	8.31	0.11	8.15	8.47	1.30	8.05	0.18	7.75	8.42	2.18
M3M3	6.57	0.22	6.32	6.90	3.32	6.56	0.20	6.32	6.96	3.10
OW	4.40	0.17	4.20	4.69	3.87	4.47	0.13	4.21	4.66	2.85
PGB	7.82	0.32	7.35	8.32	4.07	7.74	0.22	7.5	8.29	2.91
M1M3	4.87	0.17	4.54	5.11	3.52	4.89	0.11	4.67	5.06	2.32
UTRL	12.30	0.34	11.89	12.73	2.79	12.37	0.24	11.94	12.75	1.94
PL	13.27	0.37	12.75	13.93	2.80	13.33	0.26	13.01	13.88	1.95
OIL	24.71	0.63	23.94	25.87	2.55	24.58	0.45	24.02	25.29	1.83
BL	12.49	0.32	11.90	12.95	2.60	12.38	0.42	11.79	13.17	3.43
i1m3	11.21	0.23	10.84	11.66	2.06	11.25	0.21	11.01	11.71	1.88
MH	6.61	0.27	6.29	7.03	4.15	6.50	0.18	6.18	6.85	2.76
MIL	17.22	0.55	16.49	17.98	3.18	17.27	0.32	16.82	18.08	1.88
ML	17.17	0.42	16.48	17.67	2.46	16.99	0.39	16.56	17.78	2.28
M2W	2.63	0.07	2.49	2.74	2.84	2.50	0.06	2.42	2.58	2.40

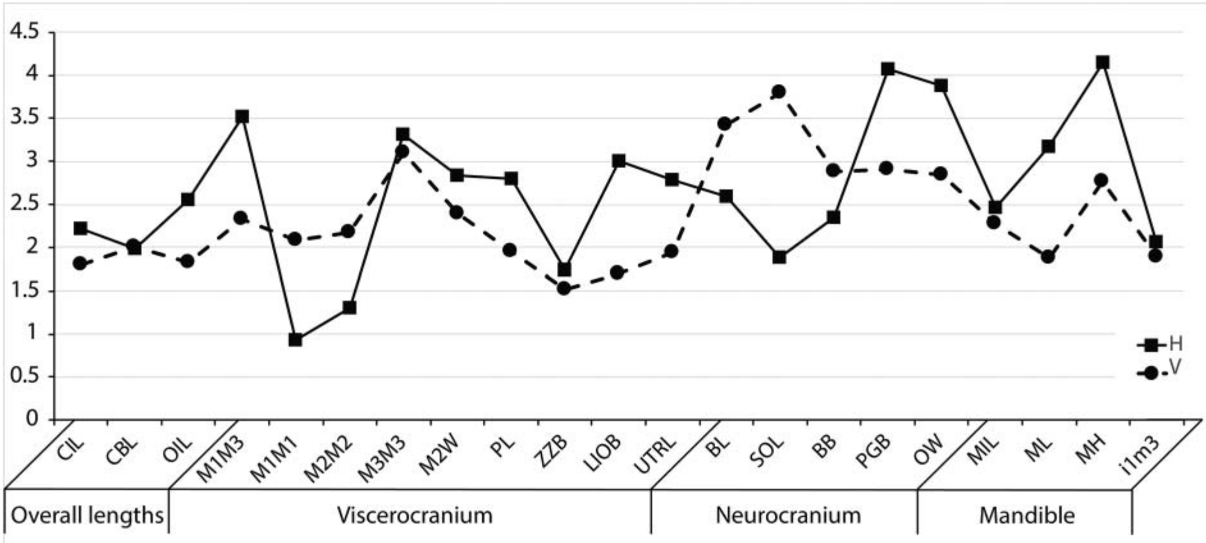

Fig. 2. Trends of coefficients of variation (CV) for the major functional parts of the skull between the two species of *Chimarrogale*. H: *C. himalayica*, V: *C. varennei*.

Table 3. Coefficient of determination (R^2), Pearson's correlation coefficient (r) and bivariate allometric analysis based on log-transformed skull measurements between *C. himalayica* and *C. varennei* using CBL as an independent reference parameter (α: coefficient of allometry; P_{ISO}: deviation form isometry; Right side characteristics (P, N, I, and n.s.) of the α values denote positive allometry, negative allometry, isometry, and non-significant, respectively.

Variables	*Chimarrogale himalayica*						*Chimarrogale varennei*					
	R^2	r	*p*	α	t	P_{ISO}	R^2	r	*p*	α	t	P_{ISO}
CIL	0.788	0.887	< 0.05	0.99 N	6.37	< 0.05	0.764	0.874	< 0.05	0.78 N	5.96	< 0.05
LIOB	0.081	0.285	0.345	0.43 n.s.	–	–	0.1	0.316	0.293	0.27 n.s.	–	–
SOL	0.195	0.441	0.131	0.42 n.s.	–	–	0.683	0.827	< 0.05	1.56 I	4.87	> 0.05
BB	0.077	0.278	0.358	0.33 n.s.	–	–	0.372	0.61	< 0.05	0.88 N	2.55	< 0.05
ZZB	0.567	0.753	< 0.05	0.66 N	3.79	< 0.05	0.207	0.455	0.118	0.34 n.s.	–	–
M1M1	0.376	0.613	< 0.05	0.29 I	2.57	> 0.05	0.367	0.606	< 0.05	0.63 N	2.52	< 0.05
M2M2	0.187	0.433	0.14	0.28 n.s.	–	–	0.245	0.495	0.09	0.53 n.s.	–	–
M3M3	0.247	0.497	0.08	0.82 n.s.	–	–	0.113	0.336	0.262	0.51 n.s.	–	–
OW	0.185	0.43	0.143	0.83 n.s.	–	–	0.003	0.05	0.87	0.07 n.s.	–	–
PGB	0.018	–0.135	0.66	-0.28 n.s.	–	–	0.018	0.135	0.66	0.19 n.s.	–	–
M1M3	0.203	0.45	0.123	0.80 n.s.	–	–	0.086	0.293	0.333	0.34 n.s.	–	–
UTRL	0.312	0.558	< 0.05	0.78 N	2.23	< 0.05	0.035	0.188	0.538	0.18 n.s.	–	–
PL	0.414	0.643	< 0.05	0.91 N	2.79	< 0.05	0.145	0.38	0.2	0.38 n.s.	–	–
OIL	0.613	0.783	< 0.05	1.00 P	4.18	< 0.05	0.681	0.825	< 0.05	0.75 I	4.84	> 0.05
BL	0.392	0.627	< 0.05	0.82 N	2.67	< 0.05	0.861	0.928	< 0.05	1.57 I	8.25	> 0.05
i1m3	0.336	0.579	< 0.05	0.60 N	2.36	< 0.05	0.085	0.292	0.333	0.27 n.s.	–	–
MH	0.007	0.085	0.781	0.18 n.s.	–	–	0.265	0.514	0.072	0.71 n.s.	–	–
ML	0.283	0.532	0.06	0.85 n.s.	–	–	0.47	0.686	< 0.05	0.64 I	3.12	> 0.05
MIL	0.447	0.669	< 0.05	0.83 N	2.98	< 0.05	0.44	0.661	< 0.05	0.75 N	2.92	< 0.05
M2W	0.042	–0.206	0.5	–0.29 n.s.	–	–	0.08	–0.282	0.35	–0.34 n.s.	–	–

(CIL, BB, M1M1, and MIL) and isometry for the remaining four measurements (SOL, OIL, BL, ML) in *C. varennei* (Table 3). The OIL only showed positive allometry in *C. himalayica*, while other measurements showed isometry or negative allometry in both species. Additionally, α values exhibited non-significant differences between the two species (Mann-Whitney $U = 163$, $p = 0.322$).

Variation in skull traits between the species — The descriptive statistics of skull measurements of two species are presented in Table 2. The Mann-Whitney *U*-test showed significant differences between the species in five skull traits, including LIOB (Mann-Whitney $U = 2$, $p < 0.05$), ZZB (Mann-Whitney $U = 18.5$, $p < 0.05$), M1M1 (Mann-Whitney $U = 13$, $p < 0.05$), M2M2 (Mann-Whitney $U = 20$, $p < 0.05$) and M2W (Mann-Whitney $U = 10.5$, $p < 0.05$).

Amongst 21 skull traits, six (LIOB, SOL, ZZB, M1M1, M2M2, M2W) were significantly correlated with CBL in both species (Table 4). One-way ANCOVA indicated significant differences ($p < 0.05$) in slope and the adjusted mean for only SOL between the species (Table 4); however, the plot of SOL against CBL of two species almost overlapped (Fig. 3). Besides, significant differences between the species were observed in the adjusted mean for LIOB, M1M1, M2M2, ZZB and M2W, where the values were higher in *C. himalayica* than in *C. varennei* (Table 4, Fig. 3).

Multivariate analysis: differentiation of skull traits between the species — The factor loadings for the PCA of the log-transformed raw data are provided in Table 5. The one-way ANOVA analysis result, Tukey's pairwise comparisons and Mann-Whitney *U*-test showed significant differences in PC1 and PC2 between the two species. The PC1

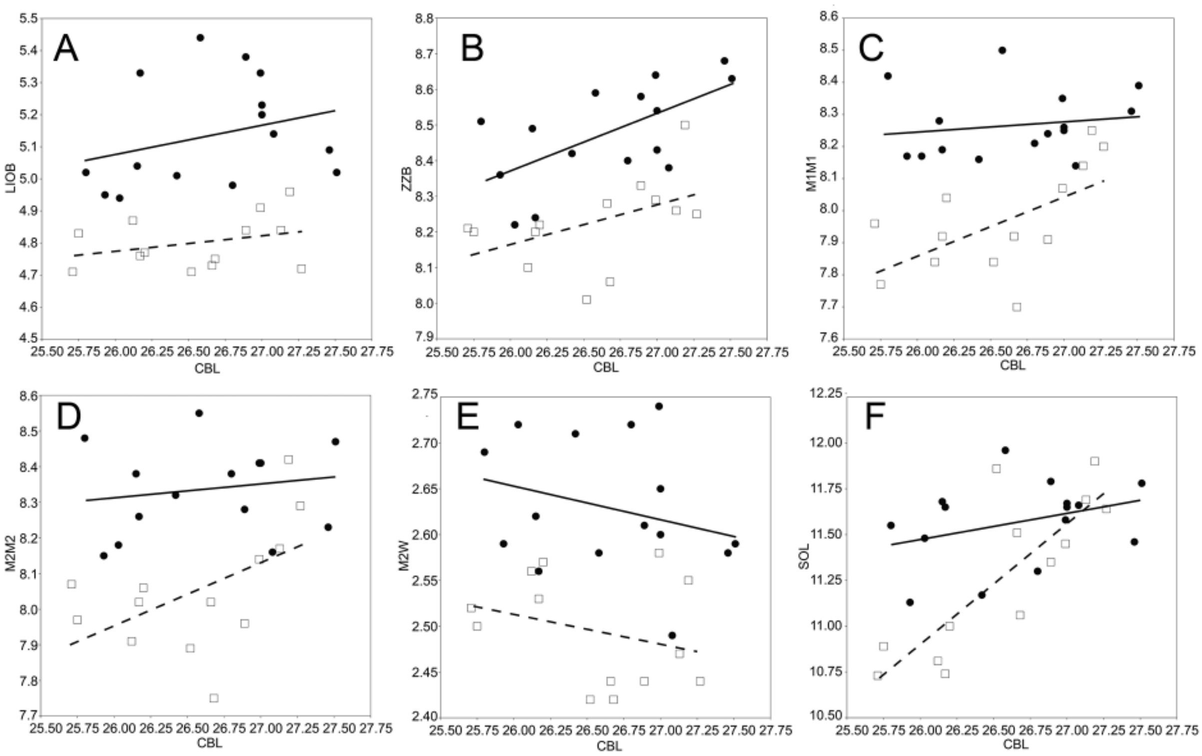

Fig. 3. Plots of condylobasal length (CBL) against LIOB, ZZB, M1M1, M2M2, M2W, SOL. The filled circle and solid regression line represent *C. himalayica*, square and broken regression line represent *C. varennei*.

Table 4. Adjusted means and slopes of skull traits: differences between two species on one-way ANCOVA with CBL as the covariate (H: *C. himalayica* and V: *C. varennei*).

	Adjusted means			Slopes		
Variables	*C. himalayica*	*C. varennei*	Difference	*C. himalayica*	*C. varennei*	*p*
LIOB	5.13	4.80	H > V	0.08	0.05	> 0.05
SOL	11.54	11.28	H > V	0.14	0.66	< 0.05
ZZB	8.46	8.22	H > V	0.16	0.11	> 0.05
M1M1	8.24	7.97	H > V	0.03	0.19	> 0.05
M2M2	8.31	8.05	H > V	0.03	0.16	> 0.05
M2W	2.63	2.50	H > V	-0.03	-0.03	> 0.05

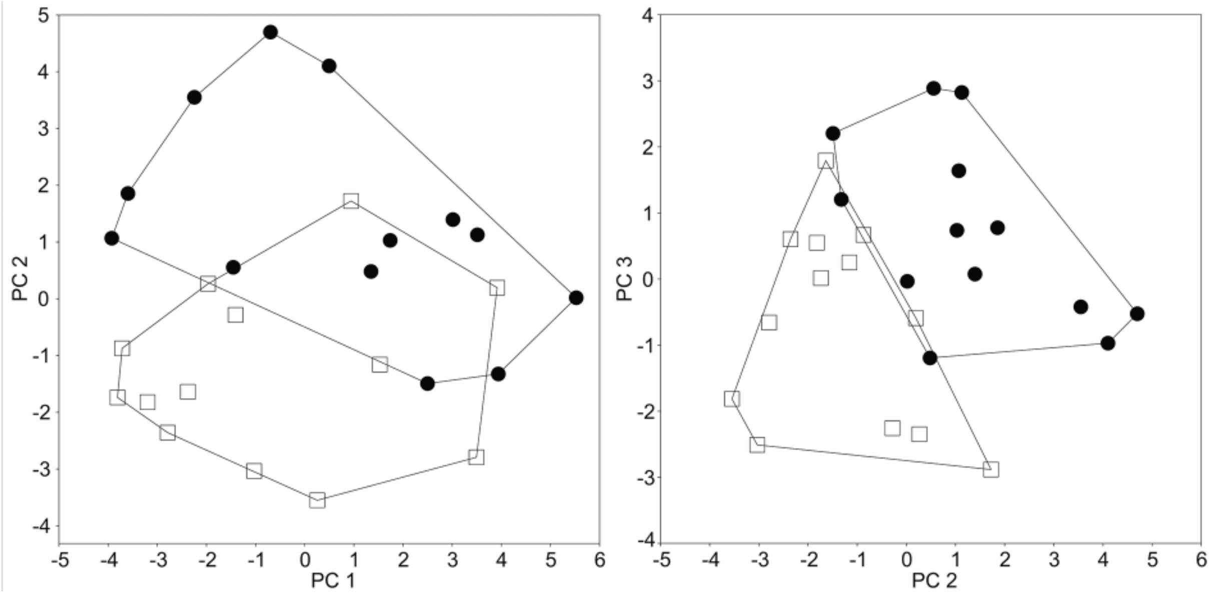

Fig. 4. Scatterplots of scores of the first against the second principal component axes (left) and the second against the third principal component axes (right), based on log-transformed raw data. The filled circle represents *C. himalayica*, the square represents *C. varennei*.

explained 49.45% of the total variance for all specimens and was interpreted to represent size and shape variation, because all character factor loadings were positive and showed higher values in CIL, CBL, OIL and MIL (Table 5).

Table 5. Character factor loadings for principal components analysis of the log-transformed data (PCs 1–3) of two *Chimarrogale* species from Vietnam. High factors (> 0.280) loadings are presented as bold.

Variables	PC 1	PC 2	PC 3
CIL	**0.317**	–0.081	–0.186
CBL	**0.297**	0.053	–0.181
LIOB	0.123	**0.338**	0.193
SOL	0.240	0.203	–0.198
BB	0.126	0.252	–0.360
ZZB	0.217	0.249	0.219
M1M1	0.220	**0.285**	0.238
M2M2	0.216	**0.288**	0.234
M3M3	0.180	0.116	–0.016
OW	0.122	–0.13	–0.16
PGB	0.006	0.244	0.004
M1M3	0.211	–0.242	0.170
UTRL	0.242	**–0.279**	0.110
PL	0.241	**–0.288**	0.070
OIL	**0.311**	–0.113	–0.096
BL	0.219	0.159	–0.316
i1m3	0.233	–0.221	0.152
MH	0.079	0.237	–0.294
ML	0.264	–0.223	0.031
MIL	**0.305**	–0.092	0.073
M2W	0.059	0.188	0.521
% Variance	49.45%	34.29%	18.11%

The PC2 explained 34.29% of the variance. The characters LIOB, M1M1, M2M2 (positive), UTRL and PL (negative) showed much higher loadings values than others (Table 5). The ANOVA showed significant difference in PC2, Tukey's pairwise comparisons and the Mann-Whitney *U*-test showed differences between *C. himalayica* and *C. varennei.* In the 2-dimensional plots of PC1 versus PC2 and PC2 versus PC3 (Fig. 4), the two species were almost clearly separated, with only a small overlapping zone of a few plots. Along PC2, *C. himalayica* may contain a larger average size (Fig. 4).

Variation in external traits between the species — The descriptive statistics showed that *C. himalayica* is larger than *C. varennei* in all external measurements (Table 6). MANOVA showed significant differences between the two species (Wilk's lambda = 0.347, F = 12.56, $p < 0.001$). The *t*-test showed significant differences between the species in head and body length ($t = 7.918$, $p < 0.05$) and hind foot length ($t = 3.016$, $p < 0.05$), but non-significant difference in tail length ($t = 0.436$, $p > 0.05$).

Discussion

Initially, our result showed that the CV values did not differ significantly between *C. himalayica* and *C. varennei* in Vietnam. Similar trends in variability amongst the variables were also mentioned in several studies on rodents (Sather, 1956; Panakoski *et al.*, 1987; Biswas & Motokawa, 2019; Biswas *et al.*, 2020). However, the ratio of CV and the mean of skull measurements in both species similarly showed the trends of inverse dependence which were detected in

Table 6. Descriptive statistics of external measurements (mm) of two *Chimarrogale* species from Vietnam.

Variables	*Chimarrogale himalayica*				*Chimarrogale varennei*			
	Mean	SD	Min	Max	Mean	SD	Min	Max
HB	119.58	4.8	113	127	108.5	5.38	96	115.5
T	87.29	7.34	76.5	102	86.4	6.55	76	95
HF	23.41	1.84	20.94	28	22	1.48	20	24.3

other rodents and mammals (Biswas & Motokawa, 2019; Biswas *et al*., 2020). This phenomenon is a mathematical constraint when comparing the whole variation and variability to its parts (Lande, 1977; Dayan *et al*., 2002; Biswas & Motokawa, 2019). Although 66.67% of the skull variables between *C. himalayica* and *C. varennei* showed a similar pattern in terms of the CV, our results indicated that size differences were not completely responsible for the variation and variability of skull characteristics. The trends of the CV for the major functional parts of the skull (Fig. 2) indicated that the differences between the two species presented in the length of incisors (trends of CIL and UTRL), brain-case size (trends of BL, SOL, BB), postglenoid breadth (trend of PGB) and mandible length (trend of ML). The huge differences of CV values showed the obvious differences in some characters including: M1M3 (H > V), M1M1 (H < V), LIOB (H > V), SOL (H < V), PGB (H > V), OW (H > V), MIL (H > V), and MH (H > V). Besides, the PCA results also demonstrated high factor loadings for the CIL, CBL, OIL, and ML in PC 1 as well as for the LIOB, M1M1, M2M2, UTRL and PL in PC 2. Thus, the skulls of *C. himalayica* and *C. varennei* can be identified by the size (by the ratio with CBL) and the shape of interorbital and postorbital regions. The first upper molars were superior, followed by the nasal, inferior and superior articular facets, occipital condyle and mandible. This pattern could reflect the developmental trends during ontogeny in different climatic regions between north (*C. himalayica*) and south (*C. varennei*) populations. Furthermore, *C. himalayica* had the highest allometric coefficient in the overall length of skull (CIL, OIL), while *C. varennei* had the highest allometric coefficient in the post-orbital region (SOL, BL) (Table 2). Yet, the MANOVA test did not show any differences between the two species in overall measurements. Along with this, a larger CV and isometric allometry were observed in the post-orbital region, while the variables in the pre-orbital region showed the smaller CV and negative allometry in *C. varennei*. Accordingly, these data indicate a general trend towards correlations of cranial traits in *C. himalayica*, but in *C. varennei* the facial region tends to be less developed as compared to its braincase. Additionally, the differentiating effects of geographically distant haplogroups of *C. varennei* in Vietnam should also be considered (see Abramov *et al*., 2017).

Character displacement, or phenotypic convergence, in closely related sympatric species seems to reflect the effect of environmental factors and competition (Rychlik *et al*., 2006). In addition, different growth rates in given species are shown to result in the discrepancies in morphological characteristics that are correlated with the life mode (Ando *et al*., 1989). In our study, we have found out that M1M1 and OIL exhibit an isometric and positive relationship with CBL in *C. himalayica*, suggesting that incisors and first molars increase in size with increasing CBL. In *C. varennei*, even OIL showed the isometric relationship with CBL, but the length of brain-case (effect on OIL) tends to increase the value simultaneously with the skull length, while M1M1 showed a negative allometric relationship with CBL. Moreover, the values for facial traits (LIOB, M1M1, M2M2, ZZB, M2W) were significantly higher for *C. himalayica* as compared to *C. varennei*. The ANCOVA test based on the highest factor loadings of PCA (SOL, LIOB, M1M1 and M1M3) compared with CBL as the covariate also showed the significantly larger LIOB and M1M1 for *C. himalayica* than for *C. varennei*. The size of the upper molar rows and interorbital breadth were correlated with the shape of the posterior palate and glenoid fossa. Our results thus indicated that the facial musculoskeletal system of *C. himalayica* is more developed than that of *C. varennei*. This part is associated with mastication, and so their variation could be related to food selection (Rychlik, 1997; Rychlik & Jancewicz, 2002; Churchfield & Rychlik, 2006; Rychlik *et al*., 2006).

In addition to Abramov *et al*. (2017), who analysed some of the same skulls used in the present study (indicated as "ZIN" vouchers in Table 1), our PCA results also reported a similarity in skull sizes between these species. Furthermore, we have identified differences in the ratio between the pre-orbital and post-orbital regions. Abramov *et al*. (2017) suggested that the fourth upper premolar (P4) and the first upper molar (M1), as well as the shape and distal tip of the palatal suture, could be used to tell apart these two water shrew species. Yet, additional diagnostic features have been identified in the inferior and superior articular facets and rostrum. *C. varennei* has the angular inferior and superior articular facets, in contrast to their more rounded appearance in the skull of *C. himalayica*. The occipital foramen of *C. himalayica* is uniformly round, whereas that of *C. varennei* has curved segments. Compared to *C. himalayica*, *C. varennei* has a more slender rostrum. In the mandible, *C. varennei* has a more curved anterior edge of the ramus compared to *C. himalayica* (Fig. 5).

The external comparison between the two species indicated that *C. himalayica* is larger than *C. varennei*.

The ratio of tail length to head and body length is greater in *C. varennei* than in *C. himalayica*. It is possible that *C. himalayica* has a longer body length than *C. varennei*, despite having the same tail length.

Conclusion

Our results suggest that there are different trends in the interspecific static variation and variability in major skull parts between two Asiatic water shrews from Vietnam (*C. himalayica* and *C. varennei*), despite having similar overall skull sizes. Therefore, the skull morphology trends of water shrews in Vietnam seem to have been influenced by a functional adaptation associated with a diet selection.

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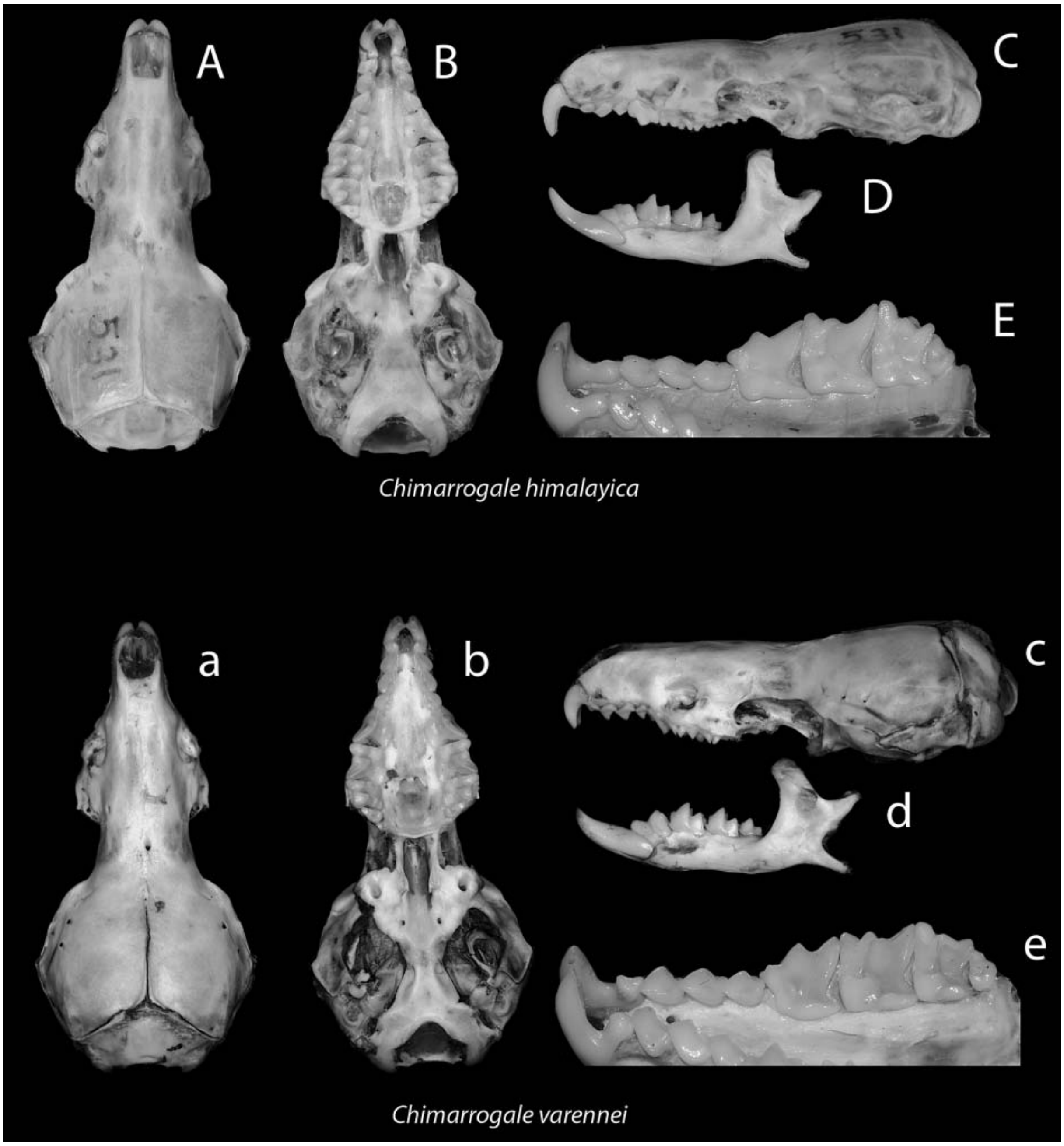

Fig. 5. Dorsal views (A, a), ventral views (B, b), lateral views (C, c) of craniums, lateral views (D, d) of mandibles, lingual views of upper toothrows (E, e) of *Chimarrogale himalayica* (upper) and *C. varennei* (lower).

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