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New look at the con-specificity of the two shrews, *Crocidura gmelini* and *C. suaveolens* from Iran; geometric morphometrics approach

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New advances in molecular techniques are substantially reshaping our understanding of taxonomy. However, combining molecular and morphologic data is a prevailing trend towards integral taxonomy. In a genetic frame, two nominal species, *Crocidura gmelini* and *C. suaveolens* have recently been lumped into one species. This study aimed to evaluate this hypothesis morphologically. For this, 42 specimens from northern Iran classified either as *C. gmelini* or *C. suaveolens* were subjected to the geometric morphometrics analyses. Landmark based method on labial side of mandible along postcranial morphology showed profound resemblance, provide further supports for genetic results.

Key words: *Crocidura gmelini*, *Crocidura suaveolens*, Geometric morphometrics

INTRODUCTION

The living insectivores roughly comprise of 400 species (most of them are shrews). Currently, shrews are the third largest family of mammals after murid rodents (Symond, 2005). Crocidurinae Milne-Edwards, 1868 belong to the family Soricidae which are known as white-toothed shrews. These micro–mammals are similar morphologically, so that it is often difficult to identify to which species an individual belongs (Leroy et al., 2004; Huttere, 2005). Genus *Crocidura* Wagler, 1832 is one of the nine genera of the shrew in this subfamily showing remarkable diversity (Motokawa et al., 2005, Dubey et al., 2007), with currently 172 established species. In a morphologic point of view, however, taxonomic status of some taxa are left open, proposed to be so far from over at the species level (Corbet, 1978).

Crocidura gmelini Pallas, 1811, Gmelin's white toothed shrew, a member of *suaveolens* group was described from North-Khorasan, 85 kilometers to the west of Bojnord (Dasht area) at the altitude of 975 meters above sea level (Goodwin, 1940; Huttere, 2005; Darvish and Rastegar-Pouyani, 2012). Gmelin's white toothed shrew occurs from Iran through Afghanistan, Pakistan, Turkmenistan, Uzbekistan, Kazakhstan, and China (NW Xinjiang) to Mongolia listed in least concern (LC) category by the International Union for Conservation of Nature (IUCN) (Hutterer, 2008). Lesser white toothed shrew, *Crocidura Suaveolens* Pallas, 1811 with a wide global distribution listed also in LC category by IUCN (Hutterer et al., 2008), has been recorded from north of Iran, Gorgan, Turkmen-sahra and Khorasan (Etemad, 1984; Ziaie, 1996; Darvish and Rastegar-Pouyani, 2012), the southernmost record is available from Fars province (Esmaeili et al., 2008)

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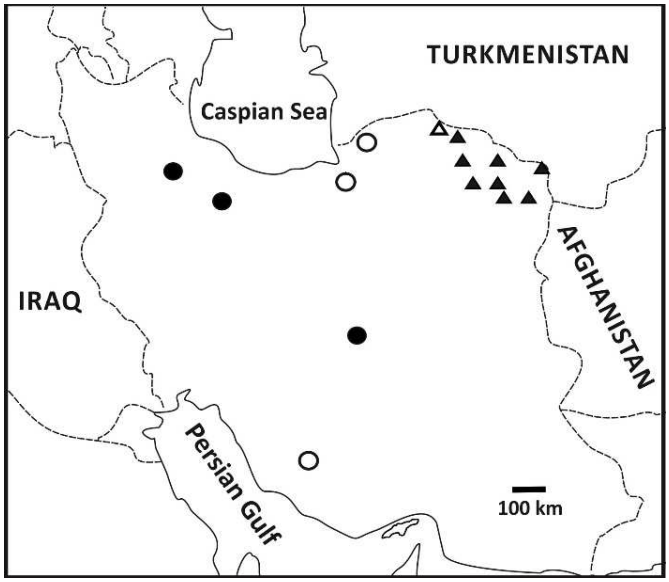

FIGURE 1. The localities of the studied specimens from northern Iran. (●); *C. suaveolens*, (▲); *C. gmelini*. Empty circles and triangle show the previous reports of *suaveolens* and *gmelini,* respectively.

Specific taxonomic position of *C. gmelini* has been the source of debate over years, with unstable status regarding to species or subspecies rank. Hoffmann (1996) lumped these two taxa into a single species. Meanwhile, both of these taxa received then its own specific name as two valid species by Jiang and Hoffmann (2001). Recent advances in molecular approaches pointed out con-specificity of these two species, *C. gmelini* and *C. suaveolens* (Bannikova et al., 2006), but no morphologic attempt has been done for, particularly on the specimens from the type locality of *gmelini*. Geometric morphometrics (GM)' approach preserves the geometry of the landmark coordinates throughout the analysis and gives a precise and accurate description of shape variances, a better visualization, interpretation and communication of the results in compare to the traditional morphometrics (Zelditch et al., 2004). It has been proved that skull and mandible consist of quantitative traits with a high heritability (Atchely et al., 1981; Sparks and Jantz, 2003). Therefore, this method as an important revolution in morphometric studies has been frequently used in biology (Adams et al., 2004). The aim of present study is to further understanding of quantitative morphological variations in these two genetically synonymized forms in Iran.

MATERIAL AND METHODS

Morphology

In general, species delimitation is difficult in *Crocidura* which is due to the species conserved phenotype (Leroy et al., 2004; Cornette et al., 2012; Cornette et al., 2015). This study was conducted on the materials deposited in the Zoological Museum of Ferdowsi University of Mashhad (ZMFUM) which were labeled either as *C. suaveolens* or *C. gmelini*. *Crocidura gmelini* is identifiable by paler pelage coloration and shorter tail (tail length to head-body length is almost fifty percent), while *C. suaveolens* has darker coloration and larger tail (tail length to head-body length is higher than fifty percent) (Jiang and Hoffmann, 2001). Geographic location of the analyzed samples supplied further confidence on the species sorting, *gmelinin* collected only in the northeastern part of Iran, while *suaveolens* sampled from central and western Iran (Fig. 1, Table 1). Moreover, postcranial skeleton of two specimens (one sample per species) were cleaned and used for morphologicall comparision.

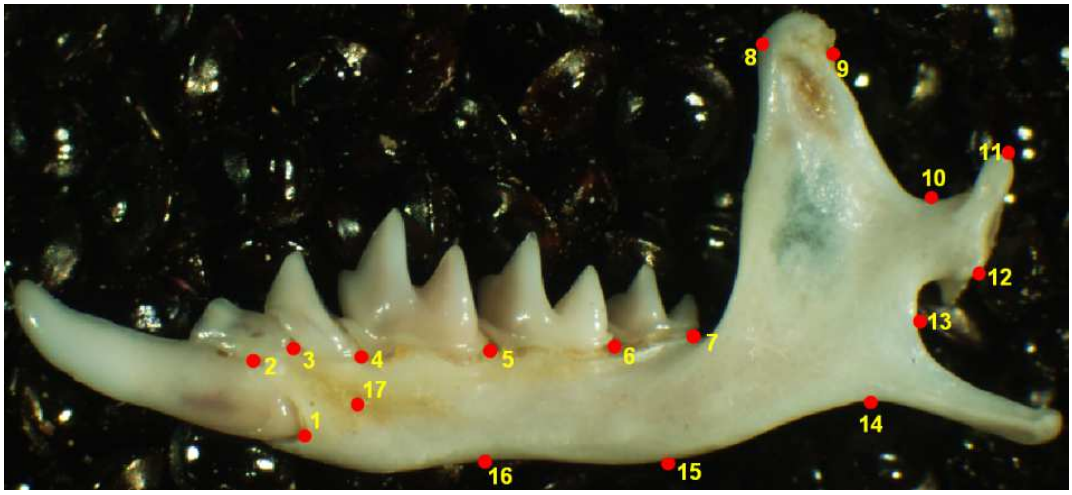

FIGURE 2. Landmarks positioned on the labial side of the left mandible.

Geometric morphometrics

Geometric morphometrics analyses were performed using Landmark method on the labial side of 42 left mandibles (13 belong to *suaveolensis*, 29 belong to *gmelinin*). Images were captured using a digital camera (DP71) connected to a stereoscope (Olympus SZH10) with magnification of 10x. A total of 17 landmarks were defined on the mandible based upon the terminology used by Rychlik et al. (2006), White (2008) and Bernal (2010) (Fig. 2). The digitization was performed on the labial side of the left mandibles, using the software tpsDig (Rohlf, 2010).

Probable landmark differences due to scale, position and orientation of the specimens during data collection were removed by a generalized procrustes analysis (GPA), based on a least-square algorithm (Rohlf and Slice, 1990; Dryden and Mardia, 1998) in MorphoJ software (Klingenberg, 2011). For better visualization of shape differences between species, mean shape of the two species were calculated in MorphoJ and compared based on Procrustes distances. To explore shape differences among populations we applied two multivariate analysis on shape data, principal component analyses (PCA) and discriminant analysis (DA). In order to the elimination of size factor, it is necessary to quote them using landmarks coordinate. Size information was calculated as the centroid size (CS): the square root of the sum of squared distances between each landmark and the centroid (or geometric center of the object) (Bookstein, 1991). One-way analysis of variance (ANOVA; with unequal post hoc Tukey's HSD test) was performed to asses size differences between two species, the significance level for all statistical analyzes was set at $p < 0.05$.

RESULTS

External morphology. *Crocidura gmelini*. Uniform pelage coloration was not observed in the upper parts of the body. In general, paler pelage is more common, the ventral part ranges from white to yellow. Tail was distinctly bicolour, showing the same coloration to body, and was slightly shorter than half of the head-body length (Table 2).

Crocidura suaveolens. Dorsal pelage coloration varies from greyish brown to dark brown, ventral part was almost yellowish to pale grayish. Tail was either indistinctly bicolour or monochromatic, usually long and the ratio of tail length to head-body length is higher than fifty percent (Table 2).

Postcranial morphology. Exploring postcranial skeleton in two specimens belonging to the two analyzed species were similar; head of the humerus is wide with a small tuberosity and the deltoid of humerus covers more than half of this bone. Head of the femur is apparently separated from the body, and body of the bone becomes wider in the distal ends. Third femoral epicondyle is not so

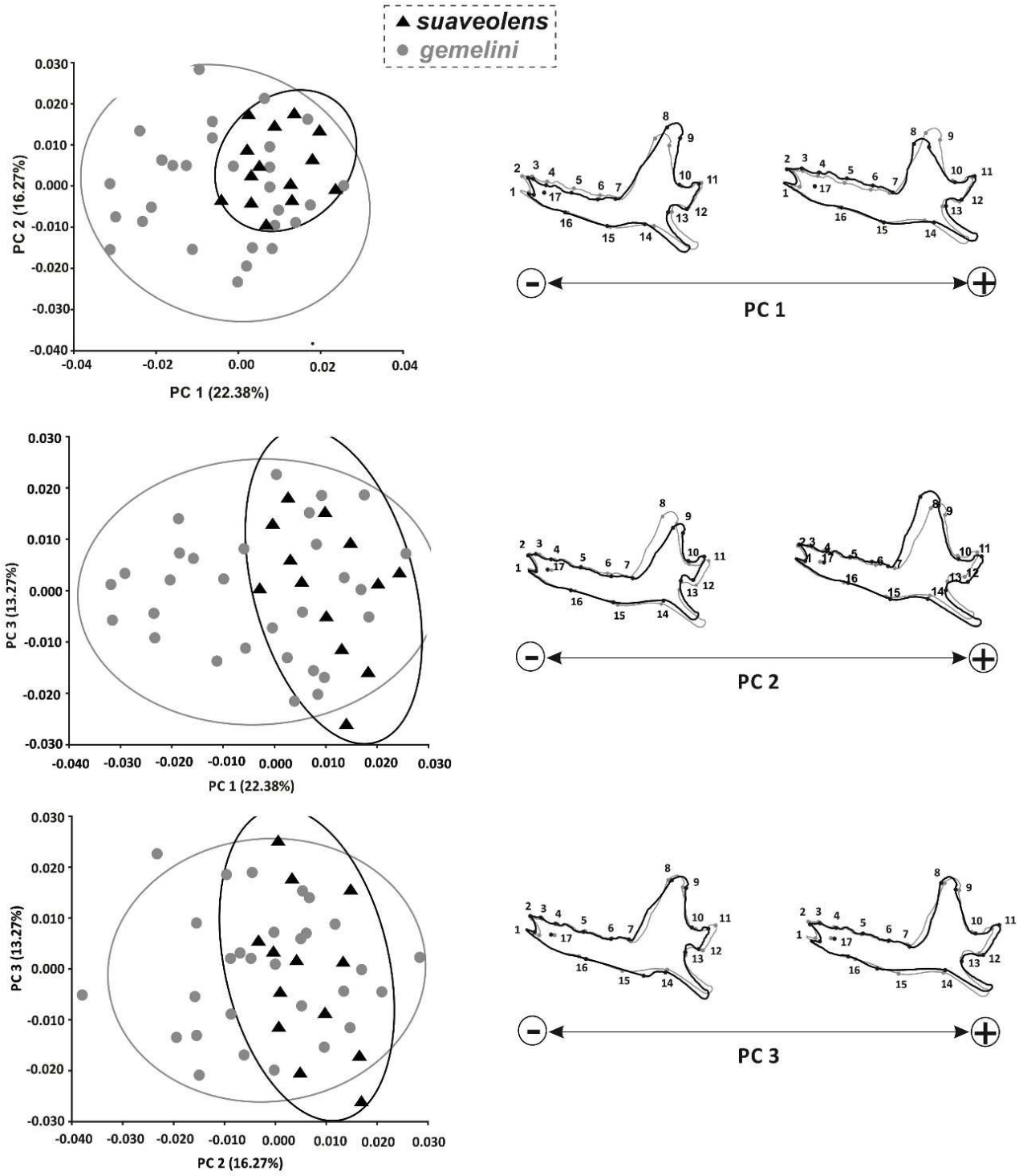

FIGURE 3. The scatter plots of the first three PCs with 95% equal frequency ellipses for *suaveolens* and *gmelini* from Iran. The percentage of explained variance are given in parantheses.

TABLE 1. Number and location of the analyzed specimens of *Crocidura* from Iran.

Species	**Locality** (N)	**Voucher number**	**Geographic coordinate**
Crocidura gmelini	Khorasan, Chahno (2)	751	35°53'N/ 58°22'E
	Khorasan, Chenaran (3)	2462-2463-3080	36°38'N/ 59°06'E
	Khorasan, Khaf (10)	Personal collection	34°34'N/ 60°08'E
	Khorasan, Zoshk (1)	935	36° 16' N/ 59° 46'E
	Khorasan, Sarakhs (7)	1634,1963,3018, 3013, 3014, 3005, 3004	36°33'N/ 61°10'E
	Khorasan, Goushkabad (2)	624, 625	36°40'N/ 59°37'E
	Khorasan, Moghan (3)	277, 705, 706	36°07'N/ 59°22'E
	Khorasan, Neishabour (1)	2183	36°14'N/ 58°44'E
Crocidura suaveolens	Zanjan, Qeydar (1)	3017,	35°55'N/ 48°33'E
	Zanjan, Soltanieh (3)	2014-SO1, 2014-SO2, 2014-SO3	36°22'N/ 48°43'E
	Yazd, Baghestan (6)	3037, 3008, 3129, 3010, 3130, 3011	32°12'N/ 55°32'E
	Qazvin, Buin Zahra (3)	3072-3073, 3074	35°44'N/ 50°05'E

Table 2. Means (±SD) and range of external measurements (mm) of *Crocidura gmelini* and *C. suaveolens* from Iran.

Species	***C.suaveolens*** **(*N*=13)**	***C. gmelini*** **(*N*=29)**
Measurement	Mean±SD	Mean±SD
Head-body length (**HBL**)	72±4.30	58.2±3.20
	53.0-80.0	50.0-67.0
Foot length (**FL**)	11.31±1.37	10.25±1.12
	9.0-13.0	9.0-12.5
Ear length (**EL**)	6.08±0.50	4.09±0.51
	4.0-8.1	3.0-6.50
Tail length (**TL**)	42.10±3.56	29.2±2.90
	38.0-45.0	26.0-33.0

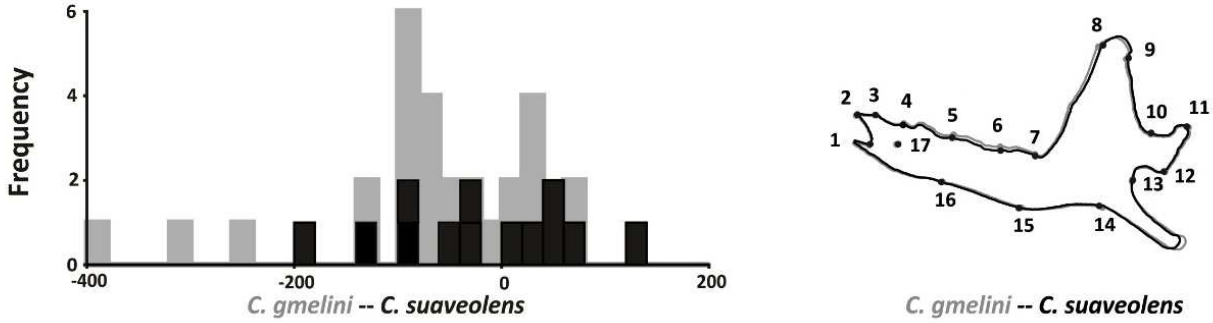

FIGURE 4. Discriminant analysis (DA) of mean shape in mandible between *gmelini* (grey) and *suaveolens* (black) from northern Iran.

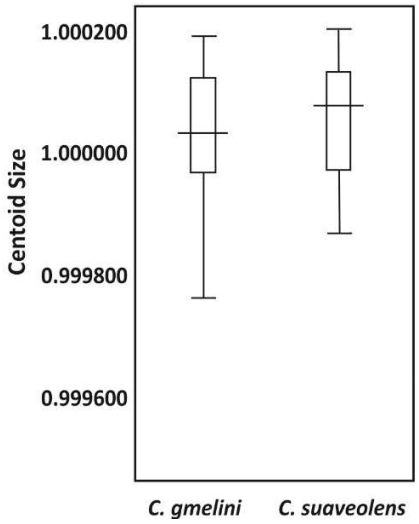

FIGURE 5. Distribution of centroid sizes between *suaveolens* and *gmelini*.

large, and the patellar surface of it, has smooth and flat edges. The middle spine of fibula is clear and the terminal end is wide. The proximal end is slightly curved. The end of bone horn is a solid bony bridge attached to the lateral and medial malleolus (Appendix 1).

Geometric morphometrics. Scatter plots of all specimens on the first few principal components (PCs) were used as a preliminary step to exclude outliers, and summarize relationships in the shape space (explained 22.38 %, 16.27% and 13.27% of the total variances, respectively) (Fig. 3). No obvious division was found on the first three PCs, two species completely overlap to each other. In the case of DA, due to uneven sample size in the two analyzed species, we performed non-parametric permutation test (cross-validated) to assess percentage of correctly classified specimens. The DA showed that *C. gmelini* and *C. suaveolens* were not statistically separated (Wilks's $\lambda = F_{8, 28} = 1.4 = p = 0.2377$), based on mean shape differences (Fig. 4), and the proportion of correct classification for cases was low (54%). Although, size comparison revealed no statistically significant difference between two species (p=0.98), *gmelini* showed a wide range of size variation as shown in boxplot (Fig. 5).

DISCUSSION

Despite burgeoning interest to molecular techniques utilization in taxonomy, shape analysis is still an essential part of biological researches, due to that shape of an organism has more similarity to its genetic than size (Atchely et al., 1988). It's not possible that shape variation could be related to the environmental fluctuates as much as size or external morphology. Common morphology of analyzed specimens of *Crocidura* from Iran (*suaveolens* **vs** *gmelini*) is consistent with those from south-east and Middle Asia (Jiang and Hoffmann, 2001; Jenkins et al., 2009). In case of our study, despite observed differences in external morphology between analyzed specimens (see results section), the GM analysis did not provide significant variation for shape and size of the mandible for the previously nominal species of interest, *gmelini* and *suaveolens*. This idea previously received strong support by molecular data suggesting that such variations do not make species sense and probably demoted to the intraspecific rather than intfraspecific variation (Bannikova et al., 2006). Although, molecular evaluation of *C. suaveolens* group in Iran, as it has done partly by Dubey et al., (2008) elucidate high genetic variation for the country, this group is remained heavily under-studied. Both genetic and morphologic researches through dense sampling are needed to well-comprehend the taxonomy and relationships of these shrews in Iran.

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APPENDIX 1. Postcranial skeleton in *Crocidura* from Iran.

Skeleton	*C. suaveolens*	*C. gmelini*
Fibula and Tibia		
Arm		
Femur		
Pelvis