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Original article

Ontogenetic and sexual patterns in the cranial system of the brown rat (*Rattus norvegicus* Berkenhout, 1769) from Hai'l region, Kingdom of Saudi Arabia

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ABSTRACT

The brown rat, *Rattus norvegicus*, is a model system in ecological and systematic science, but little is known about its skull morphology and developmental patterns. Our objective was to investigate the cranial ontogenetic patterns in the brown rats, from Hai'l, Kingdom of Saudi Arabia.

Quantitative analysis of sexual shape dimorphisms (SShD) and age-classes were investigated using 28 landmarks plotted on two-dimensional images for dorsal and ventral views. Our results detected statistically significant sexual dimorphism (P-value <0.0001) in cranial shape and size for *R. norvegicus*. Nevertheless, males are much larger than females and display variation around the brain-case, while females tend to show greater variation around the occipital bone. In addition, there are subtle age-classes during ontogeny in the skull. However, the older age classes (i.e. age classes 3 and 4) represent well-built crania with an extended case of the brain and shortest nasal, while youngest specimens represent an elongated snout of minimum crania.

Future GMM research should therefore examine the pre-defined age-classes and sex-related individuals in brown rat skulls in relation to genotype to characterize trends in skull shape variation that may affect teeth, zygomatic arches, brain case, and compartments of muscle attachments through its ecological patterns.

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1. Introduction

Rodents, together with bats, have been among the most ecologically diverse mammal groups (Wilson and Reeder, 2005; Presley et al., 2012). Phenotypic variation consists of ontogenetic and ecological variability over lifetime that forms the developmental basis for phenotypic stability and variability (Schradin, 2013). The mechanisms underlying the origins of ontogenetic variation are biological (anatomy and environment) and maternal life cycles (natural history), as all evolutionary changes are intrinsic causes stemming

from ontogeny (Lieberman et al., 2000; Abdel-Rahman et al., 2009; Klingenberg, 2010).

To gain insights into the origin and homology of the derived anatomical patterns and variations in skull shape and size, it is important to understand the development of the cranium from embryo to adult (Herrel et al., 2012). Age-related developmental patterns and sexual dimorphism in crania structure provide the backgrounds for what causes ecological and fitness variations between specific life histories (Weisbecker and Goswami, 2010; Hernandez et al., 2017; Camargo et al., 2019). According to Dos Reis et al. (2002), for detecting population history, adaptation, and limits of quantitative genetics, description of patterns of cranial variation characters within and between populations is important, particularly for species that show seasonal morphological fluctuations (Lieberman et al., 2000; Abdel-Rahman et al., 2009; Herrel et al., 2012).

The genus *Rattus* is widely distributed throughout the world and comprises a mixed taxonomic community of several species and subspecies (Wilson and Reeder, 2005). However, *R. norvegicus*

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and *R. rattus* are the only species occupying Arabia (Harrison and Bates, 1991). Norway rat (*R. norvegicus* Berkenhout, 1769) is native to Asia but has been introduced into all terrestrial ecosystems/habitats worldwide (Harrison and Bates, 1991; Wilson and Reeder, 2005). These may indicate its phenotypic plasticity as its life history preference for each site that provides unique food and shelter in a substantial ecological diversity (Hernandez et al., 2017; Camargo et al., 2019). Nonetheless, these species have been described as carriers of potential public health pathogens and markers of the risk of environmental contamination (Rothenburger et al. 2018; Lee et al., 2019). Bergmann et al. (2006) was studied the differential segmental growth of the *R. norvegicus*' vertebral column and the developmental patterns have been reported to be sexually dimorphic in size, with males having the vertebrae longer than females at different ages. As well as Canals et al. (2000) documented ontogeny changes in the fractal geometry of the lung branchial tree for *R. norvegicus* and Mori et al. (2017), in the same way, the SSD of *R. rattus* and *R. norvegicus* for ecology and eco-evolutionary perspectives in Italy.

While developmental data could provide insights into the origin and homology of derived anatomical patterns, there were globally few studies of the ontogeny of the cranial system in *Rattus* species in general and *R. norvegicus* in particular and locally no studies (Hiimae and Houston, 1971; Canals et al., 2000; Bergmann et al., 2006; Mori et al., 2017). Furthermore, nothing is known about *R. norvegicus* developmental groups and sexual shape dimorphisms (SShD) in KSA. Therefore, this study has been conducted for investigating the cranial system ontogeny (age-classes and SShD) in *R. norvegicus* from KSA by geometric morphometric (GM) software to improve our understanding of Life-Stages-Specific developmental patterns. GM is a common technique for evaluating shape and shape change among biological specimens in biology, ecology, and biodiversity to address a variety of fields including taxonomy, ecomorphology, evolution and population history (Dryden and Mardia, 1998; Rohlf, 2006; Lieberman et al., 2000; Abdel-Rahman et al., 2009; Klingenberg, 2010).

The present study represents the first attempt to determine the intra-specific cranial geometric shape variation within *R. norvegicus* from Hai'l, KSA, which may have important consequences for understanding the sexual and developmental stages growth of this species.

2. Specific objectives

- Standardize approaches to the phenotypic intra-specific variation in the brown rats *R. norvegicus* regarding ontogeny and sexual patterns;
- Determine the degree of the developmental ontogeny of the cranial system of the brown rats;
- Estimate their degree of shape sexual dimorphism;
- Provide a list of cranium variable characteristics that can be implemented in future taxonomic and geographic studies of *R. norvegicus* from the region.
- Standardize a mechanism for implementing the results of ontogenetic and sexual patterns for future studies for any related geographic study of *R. norvegicus* from the region and/or other areas.

3. Material and methods

3.1. Samples

Trapping of brown rats (*Rattus norvegicus* Berkenhout, 1769) was designed to study geometric morphometric analyses of skull shape in the Hai'l region, which is located between latitude 25°

16' 34" and 28° 53' 16"N and longitude 39° 26' 52" and 44° 22' 42" E (Fig. 1), Kingdom of Saudi Arabia from July 2016 to the end of Sept. 2017.

Although the research targeted brown rats (n = 120), other species were also captured from the fields (Total = 170). Only the target species (i.e., Norway rat, n = 134) were brought to the laboratory and the unknown species (n = 36) were released back to their habitats as they are not relevant for the prediction of this study. The captured rodents were identified by the morphological measurements based on the identification key by Harrison and Bates (1991), and Wilson and Reeder (2005).

The trapped brown rats were kept at the Biology Department, Hai'l University, and the sex data were recorded for each specimen that was euthanized and processed for skull preparations. All the previous processes were carried out in compliance with the Ethical Committee of the Hai'l University, KSA.

Nevertheless, testing repeatability (R), and measurement error (ME) were first undertaken (data not shown) to avoid sources of data variation error and impact on the analyses by GM according to Abdel-Rahman et al. (2009), Fox et al. (2020) and Vrdoljak et al. (2020).

3.2. Geometric morphometric analysis

Geometric morphometric methods (GMM) are excellent candidates to explore the morphospecies' population variation (Dryden and Mardia, 1998; Rohlf, 2006; Klingenberg, 2010; Lawing and Polly, 2010) which may facilitate identification in developmental variation (age and sex). Hence, the two-dimensional cranial landmarks for the images were performed using GMM analysis software, i.e. Tps-package by Rohlf (2006) and Klingenberg (2011) to check for shape outliers, using Generalized Procrustes Analysis (GPA) that compares the distances between tangent space and shape space (Dryden and Mardia, 1998; Klingenberg, 2011).

3.3. Image capture

One person (EH, Abdel-Rahman) took pictures of the brown rat skulls (Fig. 2) and conducted the two-dimensional landmarks to reduce the camera paradox and minimize the digitization error (Abdel-Rahman et al., 2009; Fox et al., 2020).

The images for 134 specimens (males = 68 and females = 66) were taken by a digital Canon 70D camera (Tokyo, Japan) and 28 landmarks (Fig. 3) were digitized and recorded for the dorsal and ventral cranial views by the Tps-Dig (Rohlf, 2006).

3.4. Sex-data

A principal component analysis (PCA) of the covariance total shape matrix between *R. norvegicus* males and females were decomposed into orthogonal, uncorrelated systems (i.e. PCs axes) for age-class 2 (males = 15 and females = 25, for both dorsal and ventral views) in order to summarize the total variation represented across the dataset for multivariate analysis in MorphoJ software (Klingenberg, 2011). Then, sex of specimens' skull centroid size was tested with *t*-test to verify the existence of any significant difference between males and females. Therefore regression of Procrustes coordinates against the log-transformed centroid size was checked with permutation tests against the null hypothesis to remove allometric effects. Finally, to estimate the probabilities for shape characteristics that maximize the distinction between the two groups/classes of *R. norvigcus*, i.e. males and females, a discriminant function analysis (DFA) was also used (Brennan et al., 1991; Zar, 1999).

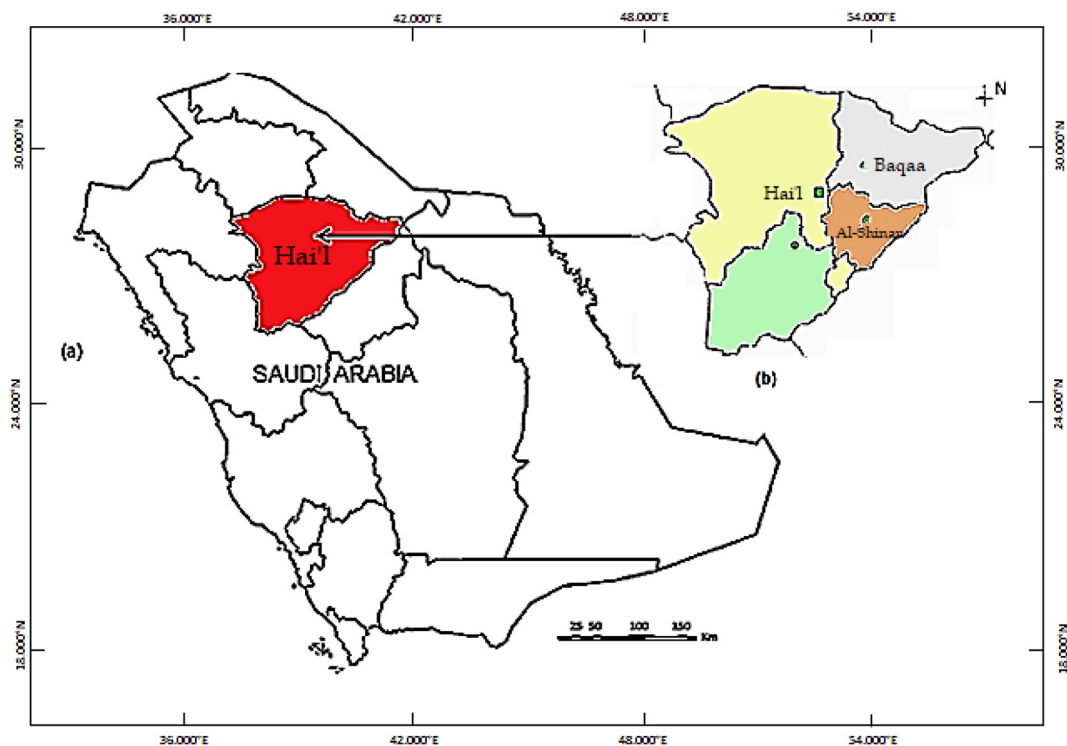


Fig. 1. A map showing the locations of the study sites in Hai'l region (i.e. Hai'l, Baqaa, and Al-Shinan), Saudi Arabia.

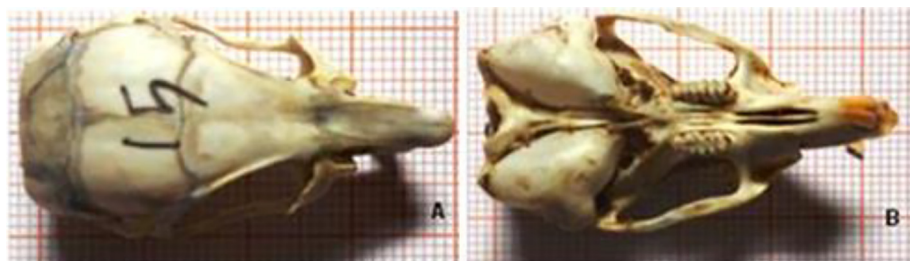


Fig. 2. The brown rat, *Rattus norvegicus* crania images for A. dorsal and B. ventral view from Hai'l region, Kingdom of Saudi Arabia.

3.5. Age-determination

All specimens were aged on the basis of qualitative tooth wear criteria under a binocular microscope (12X). Following Chaplin and White (1969) and Morris (1972) eruption and wear of left molar teeth were examined, and all the specimens were categorized into four non-overlapping age-classes, i.e. juvenile, young, adult and old, respectively (Fig. 4).

Age-class 1 (n = 19) comprised juvenile specimens with visible cusps on M1, M2 and M3. Age-class 2 (n = 40) included young specimens with no cusps on M1, M2 and M3 but moderate molar wear. Age-class 3 had mature specimens (n = 38) with extensive molar wear on M1, M2 and M3. Age-class 4 (n = 37) included old specimens with severe molar wear and completely indistinguishable molar cusps (Fig. 4). Additionally, the age-classes were checked separately as SShD was evident for *R. norvegicus* in this study (in the sexual dimorphism, results section).

In MorphoJ software (Klingenberg, 2011), the variance-covariance matrix generated from the shape data set (affine and non-affine) was then subjected to Principal Component Analysis (PCA) to define any priority groups along the respective PCA axes (Zar, 1999). Finally, in order to statistically evaluate the significance of shape changes across four age groups, a Canonical Variate

Analysis (CVA) was also considered. The data dispersion was then plotted on the first two canonical axes after a total of 10,000 random permutations by morphoJ (Klingenberg, 2011).

4. Results

4.1. Geometric morphometric shape variation

The approximation of the cranial reference shape to the cranial shape space was almost ideal for *R. norvegicus* dorsal views (i.e. Procrustes and Tangent sums of squares are 0.1448 and 0.1442, respectively for males and 0.0840 and 0.0836 for females' dorsal views).

4.2. Sexual dimorphisms

Individuals of *R. norvegicus* (M = 15 and F = 25, age class 2) were separated nicely for both the skull dorsal and ventral views by PCA to explore the main shape feature in the morphospace (Fig. 5).

Sexual dimorphism in shape for *R. norvegicus* was highly significant (P-value <0.00001) by regression of shape onto centroid size for both dorsal and ventral views. However, the mean of centroid

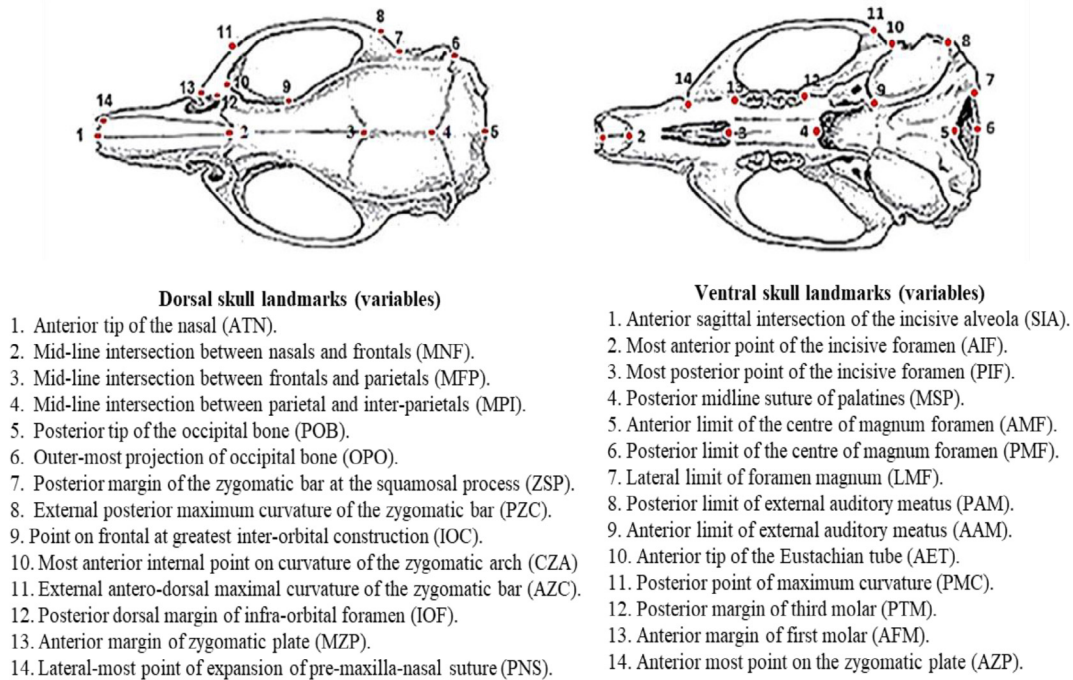


Fig. 3. Positioning of landmarks on the crania of *Rattus norvegicus*. A. Skull ventral side and B. Skull dorsal side, with landmarks that were identified below each one, respectively.

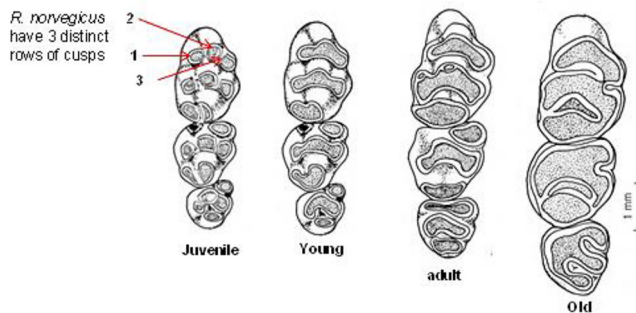


Fig. 4. Diagrammatic representations of *Rattus norvegicus*' tooth eruption and the four recognized age classes (juvenile, young, adult and old).

size in both views (dorsal and ventral) was statistically different according to Student's *t*-test results (Table 1).

For dorsal views males have longer rostrum, shorter and wider zygomatic arch and brain-case (Fig. 6).

Females showed opposite visualization, with shorter rostrum, smooth and forward zygomatic arch and narrower brain case when compared to the males' crania that presenting a more expanded skull in the posterior region (Fig. 6). Therefore, females tend to display greater variation around the occipital bone by contraction of landmarks (6, 7, and 8) and expansion of landmarks (3, 4, and 5) that are proposed in Fig. 6. However, males show opposite variation in the same area (i.e., contraction appears more on landmarks (4, 5, and 6 with expansion on landmarks 6, 7, and 8). Nevertheless, ventral views showed the narrowing of zygomatic arch (base line

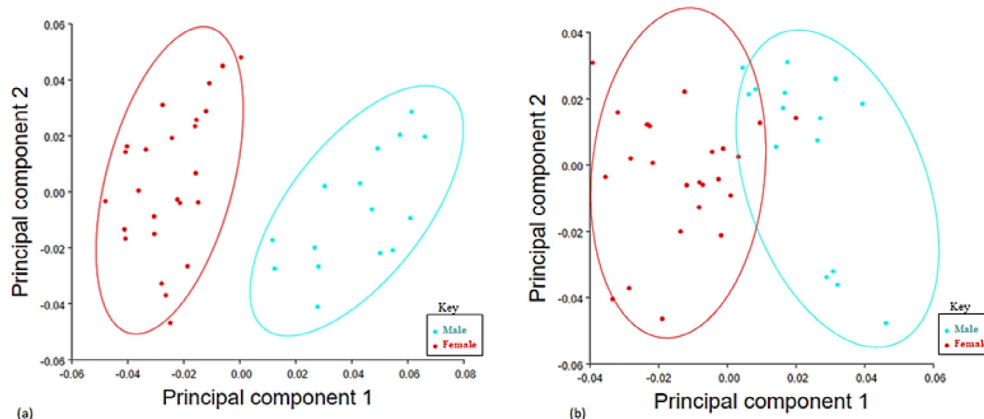


Fig. 5. Scatter plot on a. dorsal view and b. ventral view of the principal component axis 1 and principal component axis 2 for *Rattus norvegicus* sexual dimorphism (female red circles and male blue circles) from the Kingdom of Saudi Arabia.

Table 1
Student t-test results, for sexual dimorphism comparison in centroid size, in both dorsal and ventral views of *Rattus norvegicus* skull from Hai'l area, Kingdom of Saudi Arabia.

| Skull view | Group (n) | Mean ± SD | t-value | Significance |
|------------|--------------|---------------|---------|---------------------|
| Dorsal | Males (15) | 3.4123 ± 0.02 | 5.12 | P-value < 0.00001** |
| | Females (25) | 2.903 ± 0.03 | | |
| Ventral | Males (15) | 3.3256 ± 0.19 | 4.04 | P-value < 0.0001** |
| | Females (25) | 2.733 ± 0.26 | | |

SD = standard deviation, ** P-value is highly significant.

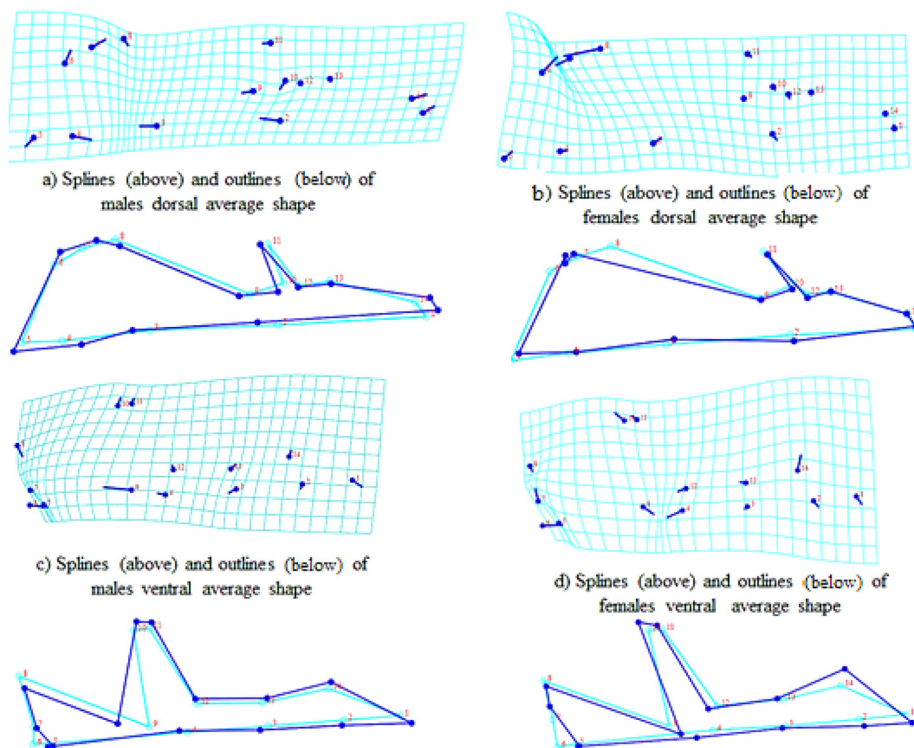


Fig. 6. Shape changes associated with the sexual shape dimorphism (SShD): a. males, and b. Females for dorsal views, as well as, c. males, and d. females' ventral views of *R. norvegicus* skulls from Hai'l region, Saudi Arabia. The variations in the shape are seen as the comparison between the shape of the sex reference (light blue outlines and hollow circles for landmarks) and the sex average (dark blue outlines and solid circles for landmarks). Landmarks are defined in Fig. 2.

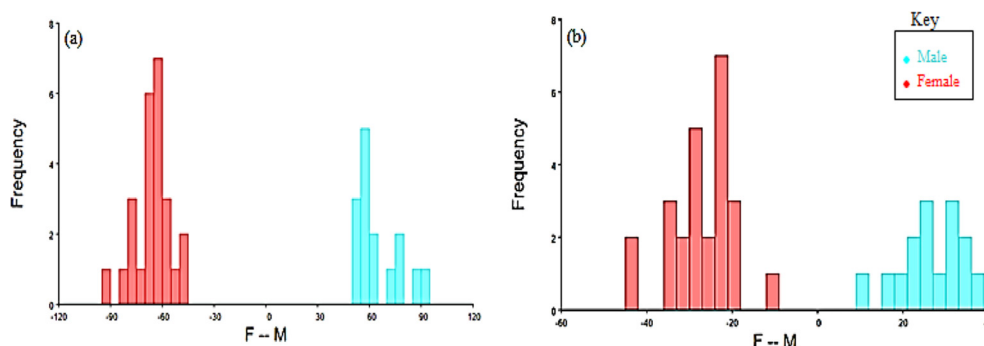


Fig. 7. Discriminant histogram on a. dorsal view and b. ventral view of the *Rattus norvegicus* crania. Female red bars and male blue bars from the Kingdom of Saudi Arabia.

for landmarks 10 and 11); the smellers meatus (landmarks 8–9); and the larger magnum foramen (landmarks 5, 6, and 7) for females and vice versa for males (Fig. 6).

Finally, the histograms from Discriminant Function Analysis (DFA) scores of *R. norvegicus* males and females for each view (i.e., dorsal and ventral views) are shown in Fig. 7.

In this analysis, however, 100% were correctly sexed by DFA for all specimens (males and females, n = 40) which indicate a higher

significant ($P < 0.0001$) discrimination between groups (i.e. males and females) for both ventral view and dorsal views (Fig. 7).

4.3. Age-determination

Since we found SShD (Shown in the previous section), sexes were studied separately for all our analysis. However SSD was evident by Canals et al. (2000) and Mori et al. (2017) for *R. norvegicus*

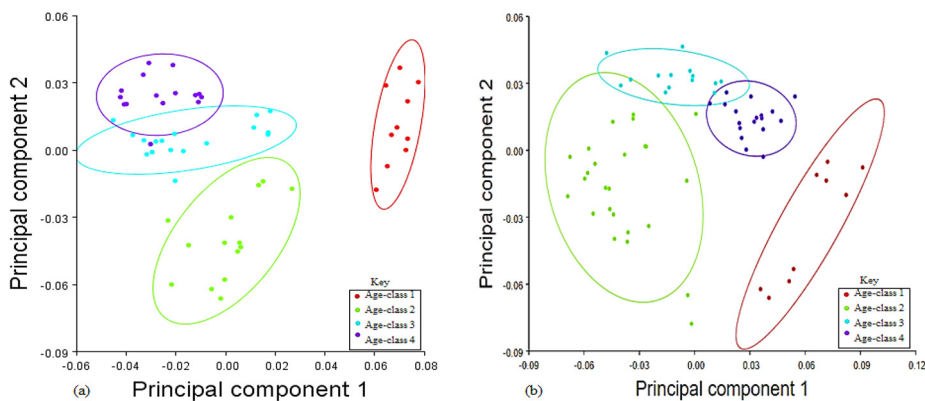


Fig. 8. Scatter plot on the principal component axis 1 and principal component axis 2 (a) males and (b) females dorsal view for age-classes (age-class 1 red circles, age-class 2 green circles, age-class 3 light blue circles and age-class 4 purple circles) of *Rattus norvegicus* from the Kingdom of Saudi Arabia. Landmarks are defined in Fig. 2.

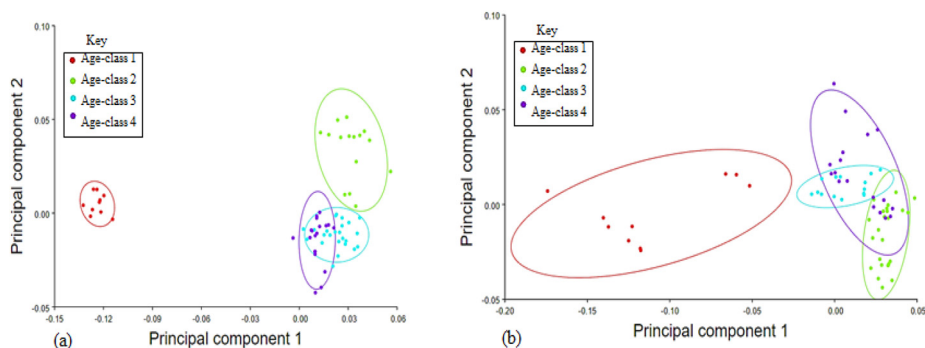


Fig. 9. Scatter plot on the principal component axis 1 and principal component axis 2 (a) males and (b) females ventral view for age-classes (age-class 1 red circles, age-class 2 green circles, age-class 3 light blue circles and age-class 4 purple circles) of *Rattus norvegicus* from the Kingdom of Saudi Arabia. Landmarks are defined in Fig. 2.

Table 2

Variables (i.e., landmarks) loadings of the 1st and 2nd principle component loadings from a principle components analysis (PCA) of the dorsal view of the cranium of male and female *Rattus norvegicus* from Hai'l region, Kingdom of Saudi Arabia. Landmarks are defined in Fig. 2. Bold absolute values > 0.05.

| Variable | Males | | Females | |
|----------|----------------|----------------|----------------|----------------|
| | PCA axis 1 | PCA axis 2 | PCA axis 1 | PCA axis 2 |
| 1. ATN | 0.4227 | -0.0507 | 0.4110 | -0.0527 |
| 2. MNF | 0.1338 | -0.0629 | 0.1336 | -0.0645 |
| 3. MFP | -0.1326 | -0.0779 | -0.1161 | -0.0823 |
| 4. MPI | -0.2941 | -0.0932 | -0.2930 | -0.0964 |
| 5. POB | -0.4008 | -0.1035 | -0.4067 | -0.1084 |
| 6. OPO | -0.3281 | 0.0623 | -0.3307 | 0.0598 |
| 7. ZSP | -0.2586 | 0.0830 | -0.2618 | 0.0875 |
| 8. PZC | -0.1928 | 0.0939 | -0.2034 | 0.1019 |
| 9. IOC | 0.0575 | 0.0020 | 0.0681 | 0.0055 |
| 10. CZA | 0.1276 | 0.0246 | 0.1348 | 0.0288 |
| 11. AZC | 0.4830 | 0.1067 | 0.4844 | 0.1050 |
| 12. IOF | 0.1589 | 0.0156 | 0.1670 | 0.0167 |
| 13. MZP | 0.2219 | 0.0221 | 0.2219 | 0.0221 |
| 14. PNS | 0.4016 | -0.0220 | 0.3908 | -0.0230 |
| % Trace | 38.476 | 21.610 | 36.888 | 18.825 |

in other countries. Principal component analysis were then run separately for *R. norvegicus* males and females groups and the scatter plot for the PCA1 and PC2 axis are shown independently for dorsal views (Fig. 8) and for ventral views (Fig. 9).

The scatter plots (Figs. 8 and 9) displayed clustering of individuals based on age group. Also a slight overlap between age classes 2, 3 and 4 was evident for dorsal and ventral views but no overlap between age class 1 and the remaining age classes. However, the separation of the youngest age groups (age-class 1) was meaning-

ful as it showed high variability in youngest brown rats crania shape, while the skull shape is comparatively fixed for adults and old ones (2, 3 and 4).

The First two PCs accounted for 38.48% and 21.61%, respectively for the males and 36.88% and 18.83%, respectively, for the females' dorsal views (Table 2, Fig. 8).

Furthermore, 60.19% and 10.31%, respectively for the males and 54.39% and 8.98%, respectively for the females' ventral views (Table 3, Fig. 9).

Table 3

Variables (i.e., landmarks) loadings of the 1st and 2nd principle component loadings from a principle components analysis (PCA) of the ventral view of the cranium of male and female *Rattus norvegicus* from Hai'l region, Kingdom of Saudi Arabia. Landmarks are defined in Fig. 2. Bold absolute values > 0.05.

| Variables | Males PCA axis | | Females' PCA axis | |
|-----------|-----------------|-----------------|-------------------|-----------------|
| | PCA axis 1 | PCA axis 2 | PCA axis 1 | PCA axis 2 |
| 1. SIA | 0.4794 | −0.0215 | 0.4685 | −0.0226 |
| 2. AIF | 0.3567 | −0.0325 | 0.3540 | −0.0320 |
| 3. PIF | 0.1789 | −0.0469 | 0.1829 | −0.0470 |
| 4. MSP | 0.0056 | − 0.0573 | 0.0112 | − 0.0567 |
| 5. AMF | − 0.2850 | − 0.0782 | − 0.2787 | − 0.0812 |
| 6. PMF | − 0.3172 | − 0.0813 | − 0.3188 | − 0.0860 |
| 7. LMF | − 0.3155 | −0.0386 | − 0.3237 | −0.0315 |
| 8. PAM | − 0.3311 | 0.0508 | − 0.3359 | 0.0469 |
| 9. AAM | − 0.0782 | −0.0426 | − 0.0758 | −0.0445 |
| 10. AET | − 0.1247 | 0.1422 | − 0.1221 | 0.1482 |
| 11. PMC | − 0.3818 | 0.1516 | − 0.4801 | 0.1558 |
| 12. PTM | 0.0412 | 0.0037 | 0.0391 | 0.0002 |
| 13. AFM | 0.1726 | 0.0104 | 0.1797 | 0.0108 |
| 14. AZP | 0.2991 | 0.0402 | 0.2997 | 0.0397 |
| % Trace | 60.917 | 10.305 | 54.395 | 8.98 |

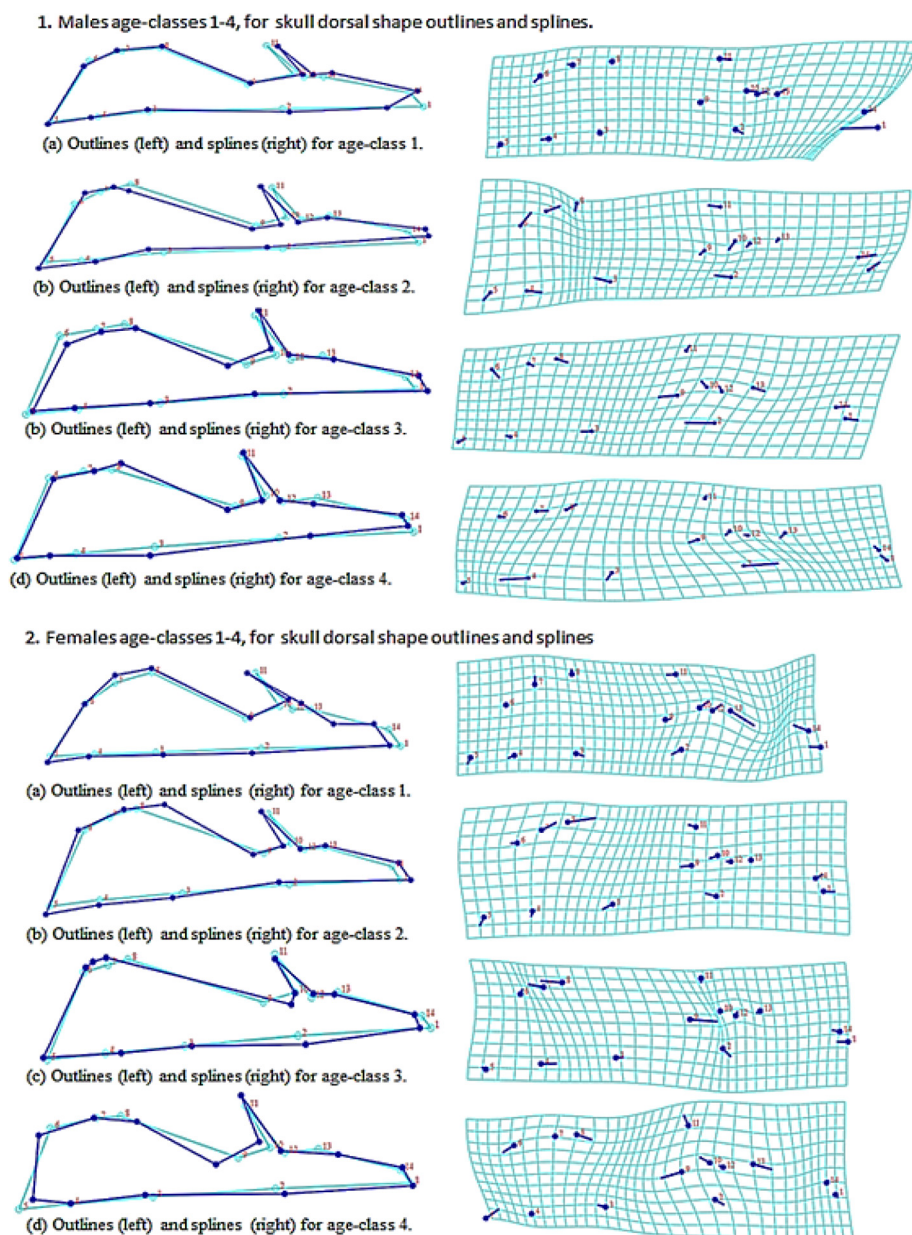
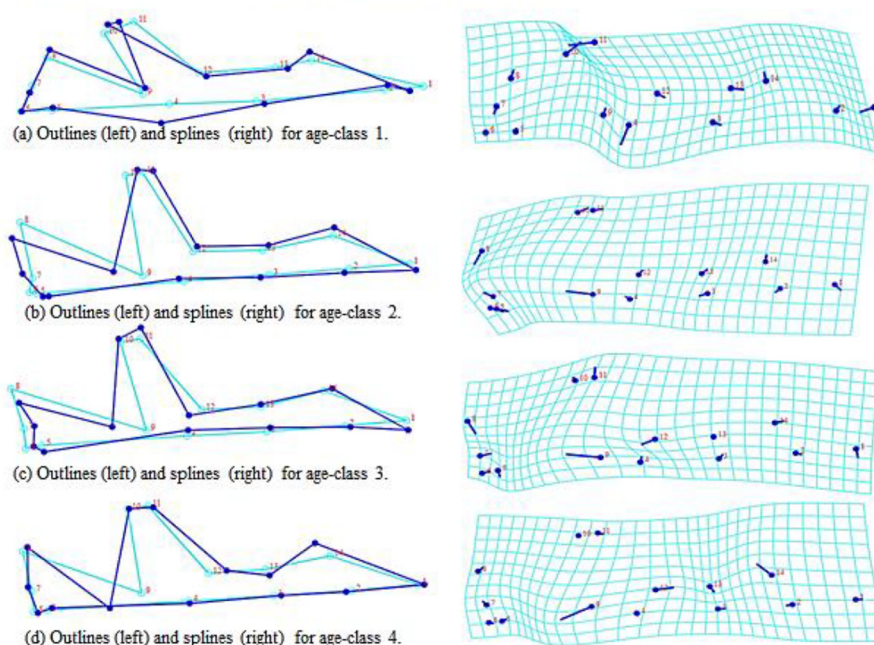


Fig. 10. Shape changes associated in 1. Males and 2. Females dorsal views of *Rattus norvegicus* skulls. The variations in form are seen as the comparison between the sex shape reference (light blue outlines and hollow circles) and the sex shape (dark blue outlines and solid circles). Landmarks are defined in Fig. 2.

1. Males age-classes 1–4, for ventral shape outlines and splines.



2. Females age-classes 1–4, for ventral shape outlines and splines

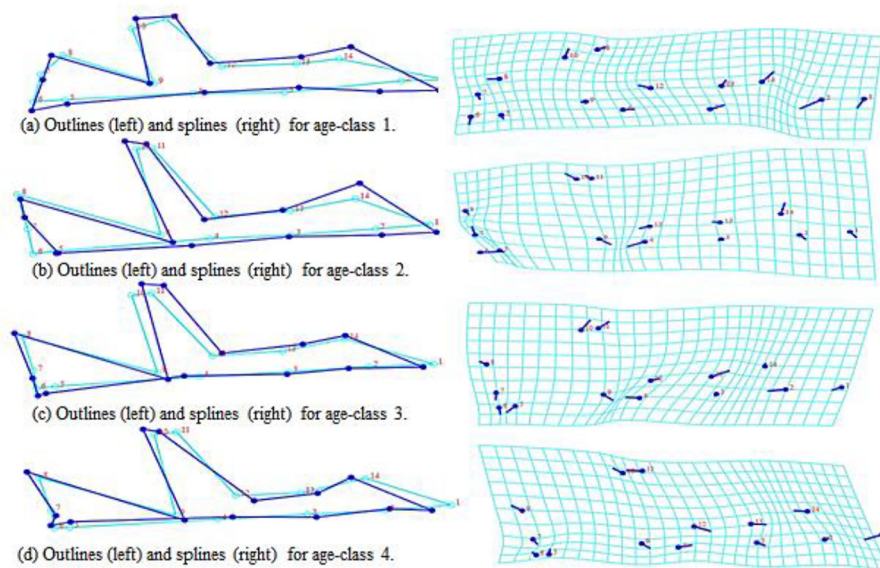


Fig. 11. Shape changes in 1. Males and 2. Females ventral views of *Rattus norvegicus* skulls. The shape changes are shown as the difference between the reference shape (light blue outlines and hollow circles) and each age-class average shape (dark blue outlines and solid circles). Landmarks are defined in Fig. 2.

Dorsal view PC1 (Table 2, Fig. 8) was very closely correlated with landmarks 1 and 11 and displayed a shape gradient specifically associated with age.

Figures (10, 11) display PCA outlines and splines in the references and tangent shapes for the landmarks shift as the grids are distorted after superimposition due to the change in the landmark positions.

Positive PC1 values associated with juveniles and negative values associated with young, old, and adults ones (Fig. 8), but vice versa for the ventral views (Fig. 9). Nevertheless, nasal sections (landmarks 1, 2 and 14), zygomatic arches (landmarks 8, 9, 10, 11) and brain-cases (landmarks 3, 4, 5, 6, 7, 8) in dorsal views were had showed the largest amount of shape variation (Fig. 10). Never-

theless, the corresponding areas on the ventral views (Fig. 11) showed the same variation. However, due to the formation of the surrounding muscle attachments on the zygomatic arch, the oldest age groups (i.e. age-classes 3 and 4) represent well-built crania, with extended brain-case and shorter nasal case, whereas youngest specimens represent subtle crania with elongated snout and narrow brain-case (age-classes 1 and 2, Figs. 10 and 11).

Furthermore, age-classes CVA analyses run on the relative warp scores of the dorsal and ventral projections of males and females and showed a complete distinction between juvenile age-class 1, the developmental age-class 2 and old age-classes 3–4 (Figures not shown as PCA findings are so similar to, Figs. 6, 7). However, the age groups in different sexes indicate higher discrimination with

respect to ventral views between age groups for character reported on the dorsal views as P-values from permutation tests < 0.0001.

5. Discussion

5.1. Geometric morphometric shape variation

The approximation of the cranial reference shape to the cranial shape space was evaluated for dorsal and ventral views. The results was in alignments with others (Abdel-Rahman et al., 2009; Klingenberg, 2011) as variation in shape occupies only a small fraction of shape space due to similar magnitude as there were no outliers in the corresponding datasets.

5.2. Sexual dimorphisms

SShD was significant of dorsal and ventral views (P-value < 00001, and P-value < 0001, respectively) for the brown rats (*R. norvegicus*), from Hai'l, Kingdom of Saudi Arabia. These results are similar to other vertebrate studies using the cranial occipital basal area due to dietary and environmental pressure constraints for human sexual dimorphisms (Wescott and Moore-Jansen, 2001; Ukoha et al., 2011; Jansky et al., 2016). Nevertheless, dimorphisms of the sexual type are common characteristics among rats, in particular in certain species of polygons (Mori et al., 2017).

The relationship between the sample size and cut-off intervals from the DFA by Brennan et al (1991) was consistent with our findings, i.e. higher significant difference (P-value < 00001) for a sample size of 40 specimens. As Brennan et al (1991) stated that a small sample size (n < 50) is typically associated with a high cut-off interval of > 0.9 and a large sample size (>140–170) with a lower cut-off interval of 0.7–0.8 according to the DFA classification.

5.3. Age-determination

There is subtle phenotypic variation in age-classes of the brown rats in this study, during development in the age groups (Figs. 8 and 9) as cranium bone develops under the guidance of disorganized muscle movements and decreases as neuromuscular control increases due to signals transmitted to bone by the brain and sensory organs and the developmental system of the skull (Hiimae and Houston, 1971). However, little modifications has occurred with the same age-group regarding sexual dimorphism as a results of selections for males more than females as they will get massive muscular generations around the zygomatic arch. According to Klingenberg (2010), Abdel-Rahman et al. (2009) and Adams et al. (2013) shape analysis is an approach for understanding the causes of variation and morphological growth of animals due to evolution, age, and selective breeding. Most intense dental and muscular specializations among mammals are demonstrated by rodents to promote antero-posterior jaw movements (Hiimae and Houston, 1971; Landry, 1970; Samuels and Van Valkenburgh, 2008; Cox and Jeffery, 2011). Therefore, differences within the masseter system of the jaw musculature and related skull bone modifications have historically been used as diagnostic characters to classify rodents (Herrel et al., 2012; Flynn et al., 2019).

6. Conclusions

The phenotypic variation of age variation and sexual selection has been determined by the skull-shape based system as an important qualitative and quantitative assessment of morphological characteristics and shape affinities for the brown rats (*R. norvegicus*), from Hai'l, Kingdom of Saudi Arabia.

Sexual shape dimorphism (SShD) was evident in KSA. In addition, there is subtle phenotypic variation in age-classes during development in the phenotypic shape of age groups but the accessory macular have remained surprisingly uniform in adults age-groups but the developing muscles can be seen more in the youngest group (age-class 1), by totally separating from the other developmental stages/groups.

Future GMM research should therefore examine the pre-defined age-classes and sex-related individuals in brown rat skulls in relation to genotype to characterize trends in skull shape variation that may affect teeth, zygomatic arches, brain case, and compartments of muscle attachments through its ecological patterns.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declarations

The author(s) declares that all the works are original and no other journals have published this manuscript.

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