Taxonomy/Taxinomie

Morphometric analysis of six *Gerbillus* species (Rodentia, Gerbillinae) from Tunisia

Analyse morphometrique de six espèces du genre *Gerbillus* (Rodentia, Gerbillinae) de la Tunisie

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ABSTRACT

Size and shape changes in the skull of the genus *Gerbillus* were investigated using geometric morphometrics. Six species from Tunisia were studied (*G. gerbillus*, *G. campestris*, *G. nanus*, *G. tarabuli*, *G. simoni* and *G. latastei*). Statistical analyses of shape variability allowed us to discriminate three morphological groups which are congruent with the three groups suggested by previous morphological and molecular studies. However, our results contrast with previous molecular investigations. In fact, according to results obtained by the use of principal component analysis, canonical variate analysis and UPGMA, we found a higher degree of divergence between the subgenus *Dipodillus* and the other two subgenera, *Gerbillus* and *Hendecapleura*. This fact suggests that the morphometric differences observed among species within the genus *Gerbillus* are not mainly related to phylogeny. To reconcile the molecular and morphological approaches, we propose a hypothesis of differential rates of phenotypic evolution in the genus *Gerbillus*. In this view, the species belonging to the subgenus *Dipodillus* evolved apomorphic features of the skull likely related to a higher degree of habitat specialization. By contrast, the more generalist *Gerbillus* and *Hendecapleura* subgenera show less differentiated plesiomorphic morphology.

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RÉSUMÉ

La différenciation morphologique au sein du genre *Gerbillus* a été étudiée en utilisant la géométrie morphométrique. Six espèces provenant de la Tunisie (*G. gerbillus*, *G. campestris*, *G. nanus*, *G. tarabuli*, *G. simoni* et *G. latastei*) ont été examinées. Les analyses statistiques permettent la distinction de trois groupes morphologiques qui sont congruents avec les groupes morphologiques et moléculaires antérieurement établis. Cependant, nos résultats sont en partie contradictoires avec les analyses moléculaires. En effet, d’après les résultats obtenus par l’analyse en composant principal, l’analyse canonique des variables et l’UPGMA, on a trouvé une divergence entre le sous-genre *Dipodillus* et les deux autres sous-genres, *Gerbillus* et *Hendecapleura*. Cette divergence suggère que les différenciations observées entre les espèces ne sont pas reliées principalement à la phylogénie. Afin de
The genus *Gerbillus* Desmarest, 1804 is one of the most diversified groups of rodents inhabiting arid and semiarid areas. It is known for its morphological homogeneity [1–3] but high karyotype heterogeneity with a diploid number ranging from \(2n = 34\) to \(2n = 74\) [3–9]. *Gerbillus* has never been comprehensively revised [10] and its taxonomy is still holding a number of controversies. Among these, the genus *Gerbillus* is still a matter for discussion for taxonomists ([10] and reference herein). In fact, whether this genus is holding subgenera or good to split off into several genera is yet debated.

Since its early description, three different subgenera were created for the genus *Gerbillus* [11,12]. These are *Gerbillus*, *Dipodillus* and *Hendecapleura* and this on the basis of some morphological features.

The subgenus *Gerbillus* is characterized by the presence of well developed auditory bullae of which the posterior parts reach or even exceed the level of the occipital bone, a maximum number of five metatarsal tubercules, one carpal tubercule, and the presence of opposite cusps in the first upper molar and haired hind feet. The latter is bare in the species of the subgenus *Hendecapleura* which share some of the other characteristics of the subgenus *Gerbillus* (e.g. the well developed auditory bullae).

On the other hand, the subgenus *Dipodillus* shows a mediocre development of the auditory bullae, a higher number of metatarsal tubercules (six), a first upper molar with alternate cusps and hairless plantar surfaces. Even though these three taxa were mostly accepted by most of authors, there was no general agreement about the taxonomic rank to assign to them, in particular, regarding the *Dipodillus* species. In fact, during the last century, this taxon has been regarded as a subgenus [13–15] or as a genus [16–22]. However, Lay [23], studying the most important characters used to separate these subgenus, recognized only one genus “*Gerbillus*”.

To solve these controversial allocations, the genus *Gerbillus* was the subject of new molecular studies [24,25]. These investigations allowed an insight in the systematic, the taxonomy and the evolutionary pattern of *Gerbillus*. Particularly, the mitochondrial DNA analysis [25] confirmed the subdivision into three distinct taxa as previously identified on the basis of morphology [11,12] and revealed that the elevation of *Dipodillus* to a genus rank will make *Gerbillus* a paraphyletic genus. On the basis of this analysis, it was concluded that the three taxa *Dipodillus*, *Gerbillus* and *Hendecapleura* must be considered as three distinct subgenera belonging to a unique monophyletic genus [25].

In the present work, we adopted a taxonomic scheme emerging from the molecular investigation and, in order to provide a new insight into the *Gerbillus* systematics and taxonomy, we used a geometric morphometric approach to investigate the patterns of morphological differentiation among species and subgenera. In fact, the geometric morphometric approach has proved to be a useful technique to investigate morphological similarity due to ecological convergence and to solve taxonomic issues in small mammals, particularly in rodents [26–29]. In order to investigate the morphological differentiation among *Gerbillus* species and its implication in systematics, we studied skull size and shape differentiation among six species from Tunisia: *G. simoni* and *G. campestris* belonging to the subgenus *Dipodillus*, *G. gerbillus*, *G. tarabuli* and *G. latastei* belonging to the subgenus *Gerbillus*, and *G. nanus* belonging to the subgenus *Hendecapleura*.

1. Introduction

The genus *Gerbillus* Desmarest, 1804 is one of the most diversified groups of rodents inhabiting arid and semiarid areas. It is known for its morphological homogeneity [1–3] but high karyotype heterogeneity with a diploid number ranging from \(2n = 34\) to \(2n = 74\) [3–9]. *Gerbillus* has never been comprehensively revised [10] and its taxonomy is still holding a number of controversies. Among these, the genus *Gerbillus* is still a matter for discussion for taxonomists ([10] and reference herein). In fact, whether this genus is holding subgenera or good to split off into several genera is yet debated.

Since its early description, three different subgenera were created for the genus *Gerbillus* [11,12]. These are *Gerbillus*, *Dipodillus* and *Hendecapleura* and this on the basis of some morphological features.

The subgenus *Gerbillus* is characterized by the presence of well developed auditory bullae of which the posterior parts reach or even exceed the level of the occipital bone, a maximum number of five metatarsal tubercules, one carpal tubercule, and the presence of opposite cusps in the first upper molar and haired hind feet. The latter is bare in the species of the subgenus *Hendecapleura* which share some of the other characteristics of the subgenus *Gerbillus* (e.g. the well developed auditory bullae).

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In the present work, we adopted a taxonomic scheme emerging from the molecular investigation and, in order to provide a new insight into the *Gerbillus* systematics and taxonomy, we used a geometric morphometric approach to investigate the patterns of morphological differentiation among species and subgenera. In fact, the geometric morphometric approach has proved to be a useful technique to investigate morphological similarity due to ecological convergence and to solve taxonomic issues in small mammals, particularly in rodents [26–29]. In order to investigate the morphological differentiation among *Gerbillus* species and its implication in systematics, we studied skull size and shape differentiation among six species from Tunisia: *G. simoni* and *G. campestris* belonging to the subgenus *Dipodillus*, *G. gerbillus*, *G. tarabuli* and *G. latastei* belonging to the subgenus *Gerbillus*, and *G. nanus* belonging to the subgenus *Hendecapleura*.

2. Material and methods

A total of 148 specimens were analysed, representing six species of the genus *Gerbillus* from eight localities of Tunisia (Table 1). Samples were unambiguously identified by cytogenetic analysis. Only adult specimens were used in this study. Images of all dorsal and ventral sides of the skulls were digitized using a Nikon D100 camera. Successively, 28 landmarks were collected on the dorsal and 22 on the ventral side (Fig. 1) using the program Tps-Dig2 [30]. The obtained landmark configurations were successfully aligned using the generalized procrustes analysis (GPA) and analysed using the MorphoJ program [31].

Size was computed as the centroid size (CS, the square root of the sum of the square of the distance between landmark and the centroid [32]). Size differences among species were tested by Anova and visualized using a boxplot. The significance of the CS pairwise differences among species was tested through the Tukey HSD test.

Shape differences among species were investigated by a principal component analysis (PCA) and by a canonical variate analysis (CVA). A multivariate analyses of the variance (Manova), computed on principal component scores matrix, was used to test the significance of the observed shape differences. The percentages of correct classifications were calculated using the leave-one-out cross-validation procedure. A regression between shape variables and centroid size was computed in order to investigate the influence of size on shape.

The presence of a significant sexual dimorphism for size and shape was tested by Anova and Manova computed separately for each species. Finally, unweighted pair group method with average (UPGMA) were computed from the
interspecific Procrustes distances computed among refer-
cence configurations of the dorsal and ventral views. The
UPGMA were computed using Mega 4.0 program [33].
All the statistical analyses were performed using the
software R version 2.8.1 [34].

3. Results

3.1. Size

A significant size variation \((F_{\text{sex}} = 70.856, P < 0.001)\) is
found among species (Fig. 2). A significant sexual dimor-
phism is absent in all the species with the exception of
G. simoni. However, the Anova suggests that sexual
dimorphism found in this species did not influence the
observed species differences \((F_{\text{sex}*species}} = 0.977, P = 0.43)\).
The Tukey HSD test suggests that G. nanus
and G. simoni
are significantly smaller than all the other species
\((P < 0.001)\). G. tarabuli is the largest one \((P < 0.001)\), while
G. latastei, G. gerbillus and G. campestris exhibit an
intermediate size value. Among these, G. gerbillus shows
a significant difference with G. campestris
\((P = 0.004)\) while
G. latastei exhibits an intermediate size without significant
differences.

3.2. Shape

The Manova did not reveal the presence of sexual
dimorphism both on dorsal and ventral configurations in
all the species. The regression between shape and size
shows that a significant component of the shape can be
explained by the size both in ventral \((P < 0.0001)\) and
dorsal \((P < 0.0001)\) configurations. However, the variation
of the shape related to the size in ventral and dorsal views
remains low \((2.94\% \text{ and } 7.3\%), \text{ respectively})

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>(n)</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. campestris</td>
<td>Bouhedma</td>
<td>9</td>
<td>(34^\circ 48'N - 09^\circ 65'E)</td>
</tr>
<tr>
<td></td>
<td>chenini</td>
<td>4</td>
<td>(32^\circ 54'N - 10^\circ 17'E)</td>
</tr>
<tr>
<td></td>
<td>Djebil</td>
<td>8</td>
<td>(33^\circ 01'N - 09^\circ 03'E)</td>
</tr>
<tr>
<td></td>
<td>Kondar</td>
<td>10</td>
<td>(35^\circ 55'N - 10^\circ 22'E)</td>
</tr>
<tr>
<td>G. latastei</td>
<td>Sidi toui</td>
<td>11</td>
<td>(32^\circ 44'N - 11^\circ 17'E)</td>
</tr>
<tr>
<td></td>
<td>Bouhedma</td>
<td>15</td>
<td>(34^\circ 48'N - 09^\circ 65'E)</td>
</tr>
<tr>
<td></td>
<td>Faouar</td>
<td>8</td>
<td>(33^\circ 16'N - 08^\circ 29'E)</td>
</tr>
<tr>
<td>G. tarabuli</td>
<td>Dghoumes</td>
<td>12</td>
<td>(34^\circ 04'N - 08^\circ 56'E)</td>
</tr>
<tr>
<td></td>
<td>Faouar</td>
<td>10</td>
<td>(33^\circ 16'N - 08^\circ 29'E)</td>
</tr>
<tr>
<td>G. simoni</td>
<td>Kerkennah</td>
<td>24</td>
<td>(34^\circ 42'N - 11^\circ 11'E)</td>
</tr>
<tr>
<td>G. gerbillus</td>
<td>Faouar</td>
<td>20</td>
<td>(33^\circ 16'N - 08^\circ 29'E)</td>
</tr>
<tr>
<td>G. nanus</td>
<td>Sidi toui</td>
<td>14</td>
<td>(32^\circ 41'N - 11^\circ 44'E)</td>
</tr>
<tr>
<td></td>
<td>Dghoumes</td>
<td>4</td>
<td>(34^\circ 04'N - 08^\circ 56'E)</td>
</tr>
<tr>
<td></td>
<td>Bouhedma</td>
<td>4</td>
<td>(34^\circ 48'N - 09^\circ 65'E)</td>
</tr>
</tbody>
</table>

Table 1
List of the specimens included in the analysis; collections are preserved in
the laboratory of Animal Ecology “research unit: Ecology and Population
Biodiversity”; Faculté des Sciences de Tunis, Tunisia.

Fig. 1. Collected landmarks. Dorsal side (a): tip of the nasal (1), front of
the zygomatic plate (2, 24), inferior base of the zygomatic plate (3, 23),
posterior edge of the postorbital bar (4, 22), infraorbital constriction (5,
21), frontal-parietal structure (6, 20), back of the zygomatic notch (7, 19),
squamosal structure (8, 9, 17, 18), junction of parietal and squamosal and
occipital (10, 16), posterior limit of parietal structure (11,15), limit
foramina jugular on the posterior edge of auditory bulla (12, 14), occipital
tip (13), frontal-parietal suture (27), diagonal intersection of frontal
bone (26), frontal-parietal suture (27), naso-frontal structure (25).

Ventral side (b): tip of the nasal (1), inferior margin of infraorbital
foramen (2, 15), anterior extremity of molar row (3, 14), posterior
extremity of molar row (4, 13), back of zygomatic notch (5, 12), tympanic
bulla at the posterior border of the external auditory meatus (6, 11),
posterior extremity of tympanic bulla (7, 10), posterior limit of accessory
bulla (21, 22), anterior extremity of foramen (16), posterior extremity of
foramen (17), junction between tympanic bulla and pterygoid process
(19, 20), anterior limit of mesopterygoid fossa (18), posterior intersection
between foramen magnum and occipital condyle (8, 9).

Fig. 2. Box plot showing the average of centroid size (based on dorsal
configurations) of each species. The inner line represents the median. Box
margins are at 25\(\text{th}\) and 75\(\text{th}\) percentiles, bars extend to 5\(\text{th}\) and
95\(\text{th}\) percentiles, circles represent outliers.
The Manova performed on dorsal configuration suggests the presence of significant shape differences between species (Wilks’ $\lambda = 0.001$, $P < 0.0001$). The scatter plot of the first two principal component axis (Fig. 3a) shows a good discrimination of *G. campestris*, *G. simoni* and *G. nanus*. These species are located on the two extreme points of the variation described by PC1 with *G. campestris* and *G. simoni* characterized by positive values of PC1 while *G. nanus* is characterized by negative values. By contrast, *G. latastei*, *G. tarabuli* and *G. gerbillus* show an intermediate position and are located in the same portion of the morphospace. The second PC axis allows only a partial discrimination between *G. campestris* and *G. simoni*. The CVA performed on the dorsal shape variables (Fig. 4a) shows a good discrimination of *G. campestris*, *G. simoni* and *G. nanus*. Differently from PCA, *G. gerbillus* is also discriminated from other species but it still shows a partial overlap with *G. tarabuli* and *G. latastei*. By contrast, the two latter species are not discriminated by CVA analysis. These results are confirmed by the cross-validation (Table 2) which shows a high percentage of correct classification for all the species (>95%) with the only exception of *G. tarabuli* and *G. latastei* who respectively show a 36.37% and 26.48% of misclassified specimens. Wireframes obtained for the dorsal view (Fig. 4a) show that *G. campestris* and *G. simoni* (positive values of CV1) are characterized by a restriction of the interparietal and of the occipital bones. On the other hand, the remaining species (negative values of CV1) show an opposite trend, with a tendency towards a lateral extension of the parietal bone that is particularly evident.
in *G. nanus*. Wireframes associated to the CV2 (Fig. 4) suggest that *G. gerbillus* and *G. simoni* share a thinner rostrum while *G. campestris*, *G. tarabuli*, *G. latastei* and *G. nanus* have a larger and shorter rostrum.

Similarly to the dorsal configuration, the ventral one shows a significant shape difference between the species (Manova: Wilks’ $\lambda = 0.0027$, $F = 12.1$, $P < 0.0001$). However, both PCA and CVA analyses suggest that these shape differences observed within the same subgenus are of a lower magnitude regarding the ventral configuration (Figs. 3 and 4). This is particularly evident for the species of the subgenus *Gerbillus*. Conversely, a higher phenetic divergence is observed between the subgenus *Dipodillus* and the subgenera *Gerbillus* and *Hendecapleura*. In fact, the PCA (Fig. 3b) performed on the ventral configurations shows that *G. campestris* and *G. simoni* are well discriminated from the other species while *G. nanus* partially overlaps with *G. latastei*, *G. tarabuli* and *G. gerbillus*. The
three latter species are completely overlapping. The CVA (Fig. 4b), explaining the 86.97% of the total shape variation, differently from the CVA obtained for the dorsal configuration, does not allow any discrimination between the three taxa. *G. nanus* is well discriminated by CVA but it is located close to the species belonging to the subgenus *Gerbillus*. The other two species belonging to the subgenus *Dipodillus*, i.e. *G. simoni* and *G. campestris*, are largely differentiated from the other species but show a higher degree of overlapping if compared to the results obtained from the CVA performed on the dorsal shape variables (Fig. 4a). Cross-validation (Table 3) shows a high percentage of correct classification for *G. campestris*. The other species show a higher number of misclassified specimens regarding the dorsal configuration with *G. tarabuli* which is nearly reaching 50% of misclassified individuals (Table 3). The wireframes obtained for the ventral view (Fig. 3b) suggest that the major deformations are associated to the shape of the tympanic bullae and the maxillary bone. The first CV axis describes clearly an opposite tendency in shape characterizing respectively the subgenera *Gerbillus* and *Hendecapleura* versus the subgenus *Dipodillus*. In *G. campestris* and *G. simoni*, the auditory bullae are of mediocre development while *G. nanus* shows a particularly hypertrophic tympanic bullae and a very well developed accessory bulla. The second CV axis describes shape difference in the position of the junction between tympanic bullae and pterygoid process (landmarks 19 and 20), more backward in both *G. simoni* and *G. campestris* comparing to the other species (Fig. 4). These two species, according to the wireframes described by CV2, show also a larger palate due to the enlargement of the posterior extremity of the molar row.

### 3.3. UPGMA

The UPGMA trees based on Procrustes distances (Fig. 5) show the same topology both for dorsal and ventral views highlighting the occurrence of two clusters reflecting the subgeneric assignment of the species. One cluster includes *G. campestris* and *G. simoni* while the other includes the three species belonging to the subgenus *Gerbillus*, i.e. *G. gerbillus*, *G. tarabuli* and *G. latastei*. *G. nanus*, the sole species of the subgenus *Hendecapleura*, shows a phenotypic similarity with the subgenus *Gerbillus*. According to the PCA and CVA, the UPGMAs suggest that the subgenus *Dipodillus* has a higher phenetic distance from the other subgenera, particularly in the ventral configuration. On the other side, the Procrustes distances between species belonging to the same subgenus are lower in the ventral configuration with respect to the dorsal one.

### 4. Discussion

Although skull features were one of the main arguments used to assess the systematics and the taxonomy of the genus *Gerbillus*, most previous studies regarded only a limited data set of measures mainly related to the dental

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### Table 2

Classification results of the discriminant analyses performed on the dorsal configuration according to the leave-one-out cross-validation procedure. In the diagonal there are the percentages of correct classification for each species.

<table>
<thead>
<tr>
<th></th>
<th><em>G. campestris</em></th>
<th><em>G. gerbillus</em></th>
<th><em>G. latastei</em></th>
<th><em>G. nanus</em></th>
<th><em>G. simoni</em></th>
<th><em>G. tarabuli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. campestris</em></td>
<td>26 (96.29%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>G. gerbillus</em></td>
<td>0</td>
<td>20 (95.23%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>G. latastei</em></td>
<td>0</td>
<td>1</td>
<td>25 (73.52%)</td>
<td>2</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><em>G. nanus</em></td>
<td>0</td>
<td>0</td>
<td>21 (95.45%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>G. simoni</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>23 (95.83%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>G. tarabuli</em></td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>14 (63.63%)</td>
</tr>
</tbody>
</table>

### Table 3

Classification results of the discriminant analysis performed on ventral configuration according to the leave-one-out cross-validation procedure. In the diagonal there are the percentages of correct classification for each species.

<table>
<thead>
<tr>
<th></th>
<th><em>G. campestris</em></th>
<th><em>G. gerbillus</em></th>
<th><em>G. latastei</em></th>
<th><em>G. nanus</em></th>
<th><em>G. simoni</em></th>
<th><em>G. tarabuli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. campestris</em></td>
<td>26 (96.29%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>G. gerbillus</em></td>
<td>0</td>
<td>18 (85.71%)</td>
<td>24 (68.57%)</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td><em>G. latastei</em></td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>18 (85.71%)</td>
<td>0</td>
<td>2</td>
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<tr>
<td><em>G. nanus</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20 (83.33%)</td>
<td>0</td>
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<tr>
<td><em>G. simoni</em></td>
<td>4</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>12 (54.5%)</td>
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<tr>
<td><em>G. tarabuli</em></td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>14 (63.63%)</td>
</tr>
</tbody>
</table>

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Fig. 5. UPGMA phenograms based on Procrustes distances for dorsal (a) and ventral (b) configurations of the skull.
and bullae morphology [9,20,35–37]. This is the first study which investigated the systematic relationships within the genus *Gerbillus* by a statistical quantification of the skull shape differences and suggests the study that the magnitude of differentiation among species and subgenera is different in ventral or dorsal configurations of the skull. The latter aspect should indeed be taken into account when morphology is used to assign a taxonomic rank to the different taxa.

Recent molecular data [25] proved that the subgenera *Dipodillus* and *Gerbillus* are actually sister taxa which comes across the view of two subgenera or even two different genera. However, according to our results and in particular those obtained for the ventral configuration (Figs. 3b and 4b), *G. campestris* and *G. simoni*, the two species belonging to the subgenus *Dipodillus*, appear as the most differentiated. Moreover, we found a close phenotypic similarity between the subgenus *Gerbillus* and *G. nanus* (subgenus *Hendecapleura*). These results contrast with the molecular systematics of *Gerbillus* [25] and agree with the previous morphological investigations that supported the separation of *Dipodillus* from the rest of the genus *Gerbillus*.

The differences observed between morphological and molecular data could be explained by a different rate of phenotypic evolution among subgenera. According to molecular data [25], *G. nanus*, which is phenotypically closely related to the subgenus *Gerbillus*, is the most basal species of the genus. This suggests that the subgenus *Gerbillus*, which is from a molecular point of view a sister taxon of *Dipodillus*, retained ancestral morphological features while *Dipodillus* has got a different phenotypic evolution and shows an apomorphic shape comparing to both the subgenus *Gerbillus* and *Hendecapleura*. It is worth mentioning that, even if the dorsal and the ventral configurations analyses have led to similar results, we observed a different degree of differentiation among subgenera when ventral or dorsal configurations are considered. In fact, our analyses suggest that the ventral side of the skull is more different in the subgenus *Dipodillus* compared to the subgenera *Gerbillus* and *Hendecapleura* (Figs. 3b and 4b) while a lower degree of differences in the ventral configuration were observed among species within the same subgenera. On the other hand, when the dorsal configuration is considered, we observed a higher degree of differentiation between species belonging to the same subgenus while, especially in the PCA (Fig. 3a), the subgenus *Dipodillus* appears less differentiated from the subgenus *Gerbillus*. These different degrees of interspecific differentiation observed when ventral and dorsal configurations are considered could suggest the action of different selective pressures or functional constraints in the morphological evolution of the skull of *Gerbillus* species. Notably, the phenotypic diversification observed between *Dipodillus* and *Gerbillus* subgenera appears mostly related to the ventral side of the skull and the nature of these modifications should be considered when a higher taxonomic rank for *Dipodillus* is claimed.

Shapes analyses clearly demonstrated that species belonging to the subgenus *Dipodillus* show modifications in the shape of the rostrum, in the zygomatic plate and especially in the tympanic bullae and in the accessory bullae. In fact, the tympanic bullae in *G. campestris* and *G. simoni* show a mediocre development compared to other species and the posterior extremity of the accessory bullae is reduced. Moreover, a narrow zygomatic length which accentuates the angle between the anterior edge of posterior part of zygomatic arc and the dorsal root of squamosal, was observed in *G. campestris* and *G. simoni*. Some of these modifications were suggested to have an adaptive value related to auditory and feeding behaviour [28,37–39]. It has also been suggested that different degree of bullae hypertrophy in gerbils is inversely proportional to the population density [38,40] and that different degrees of hypertrophy could influence the efficiency of con-specific mate recognition [38] and the recognition of predators [40], especially in open habitat. The specimens involved in this study were all trapped in southern Tunisia, which is characterized by an arid bioclimate. In general, all species studied here are known to feed on seeds, insect larva and plants stems growing in the desert all over the year but with different proportionality [38,41,42]. Since all these species occur in similar habitats, the morphological features cannot be easily interpreted in the light of different pattern of trophic adaptations. However, with the exception of *G. latastei*, all of the subgenera *Gerbillus* and *Hendecapleura* have a wide distribution range while *G. campestris* and *G. simoni* (subgenus *Dipodillus*) are restricted to North Africa. Evolution of increased specialization in habitat or resources use occurred frequently in animals and it might be regarded as a trend where species with morphological adaptation to specialized habitat evolved from more generalist ancestors [43]. Thus, in the genus *Gerbillus*, this fact apparently favours a model of phenotypic evolution likely related to a higher habitat (and possibly trophic) specialization of *Dipodillus* species versus the *Gerbillus* and *Hendecapleura* species. In this view, differential selective pressures in the genus *Gerbillus* led to a peculiar (apomorphic) phenotype of *Dipodillus* species that can explain the observed incongruence between morphological and molecular data. Thus, phenetic difference could not be representative of the correct systematic relationships among the three subgenera.

In conclusion, the present geometric morphometrics study based on skull shape allowed to discriminate three morphological groups which are congruent with the three subgenera suggested by molecular analyses and by early morphological classification. Moreover, the skull structure, investigated by geometric morphometric study, may play an important role in taxonomy and could be of enough significance to be a valid identification criterion. Furthermore, we suggest that the integration of different techniques might provide a powerful tool to investigate phenotypic evolution and taxonomic issues in the genus *Gerbillus* and might help to solve systematic issues due to contrasting results from different sources.

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