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NUCLEOTIDE SEQUENCES OF MITOCHONDRIAL CYTOCHROME B GENE FOR PHYLOGENY OF SOME SPECIES OF RODENTIA

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Abstract

Order Rodentia contains approximately half of all living mammalian species. Most of the living species diversity within rodents is within five monophyletic radiations: Hystricognathi, Sciuridae, Geomyoidea, Dipodidae, and Muroidea. The samples collected across different areas of Egypt were analyzed by means of mitochondrial cytochrome b gene sequence analysis to ascertain the true taxonomic status between the studied species. For phylogenetic analysis, the cytochrome b sequences from this study were combined with previously published data. This study reveals that all species were divided into two main clades, the first for the Gerbillinae taxon and the second for the other studied genera in the molecular phylogenetic trees and demonstrates that *Gerbillus* species form a sister group with *Dipodillus* species. The phylogenetic relationships specified for the murine genera. Clustering was obtained between *Mus* and *Rattus* as well as in all Gerbillinae species. This study provides the first insight into the genetic diversity of some rodents in Egypt.

Keywords: Gene. Gerbils. Monophyly. Myomorpha. Taxon.

1. Introduction

The order Rodentia contains about half of all living mammalian species (Hartenberger 1985). Those classified within the superfamily Muroidea account for approximately one-quarter of all extant mammalian species (Anderson and Jones 1984). Most of the living species diversity within rodents is within five monophyletic radiations: Hystricognathi (213 species), Sciuridae (262 species), Geomyoidea (98 species), Dipodidae (44 species), and Muroidea (11001 species). Additionally, six families (Aplodontidae, Castoridae, Gliridae, Anomaluridae, Pedetidae, and Ctenodactylidae) are either monotypic or represented by fewer than 15 living species. While there is little doubt about the approximately 29 (Wilson and Reeder 1993) to 32 (Chaline and Mein 1979) families to which any extant species belongs, suprafamilial relationships are unclear. The basic high-level taxonomy of Rodentia has remained unchanged since Tullberg (1899) proposed two divisions (Sciurognathi and Hystricognathi) based on the angle of the lower jaw in relation to the plane of the incisors. Hystricognathi are a monophyletic group, but Sciurognathi are monophyletic only if Hystricognathi are sisters to all remaining rodents. This does not appear to be the case, as recent evidence supports a sister group relationship between the Hystricognathi and the sciurognathous family Ctenodactylidae (Meng 1990; Bryant and Mckenna 1995; Huchon et al. 2000).

Generally, rodents are good signals of local biodiversity levels due to their specificity to particular types of environmental systems and their low dispersal potency. However, with intensive advances in modern decades, the alpha-taxonomy of small mammals in Africa remains dynamically changing, and the distribution of taxa defined by integrative taxonomy is lacking (Monadjem et al. 2015). This lack of information is likely to influence the subsequent analyses of spatial biodiversity patterns, and it is subsequently important to have authoritative information on the distributions of particular taxa.

Hoogstral et al. (1963) surveyed 51 species of rodents in Egypt belonging to the suborder Myomorpha and eleven species belonging to the family Muridae, subfamily; Murinae (genera: *Arvicanthis*, *Rattus*, *Acomys*, *Mus* and *Nesoke*) which are native and commensal animals found to be abundant, while five families are low in abundance in deserts and semideserts. Many researchers found that in Upper Egypt, the most distributed species were *Rattus norvegicus*, *Rattus rattus*, *Acomys cahirinus*, *Acomys niloticus*, *Mus musculus*, *Geribilus spp.* and *Juculus spp.* The common species and density are based on habitats, crop installation, nearly reclamation land and abundance shelter and food and seasons (Salit et al. 1982; Abazaid 1990; Hussien 1991; El-Deep and Lokma 1992; Embarak 1997).

Gerbillinae (Gray 1825) contains 103 species and 16 genera of gerbils, jirds and relatives (Musser and Carleton 2005). It is an Old World subfamily that has a much larger geographic distribution than deomyines, covering most of Africa and a larger extent of Asia, which ranges from the Middle East to central Asia. This subfamily occupies mostly arid, unproductive, open regions, including deserts, grasslands and savannahs (Nowak 1999). Many gerbils are characterized by their largely inflated tympanic bullae (cranial chambers that house the auditory ossicles), which function in sound amplification (aids in detecting interspecific vocalizations and foot drumming, as well as sounds from predators) in open, mostly desert, habitats where sound dissipates quickly (Lay 1972). Members of this group share a large suite of morphological and behavioral traits; the majority are diurnal, have varying degrees of ricochetal locomotion (associated elongated tails and narrow hind legs) and are mostly desert adapted, which is evident by their efficient burrowing, well-developed vision (large eyes), and efficient water conservation (Nowak 1999). The genus *Gerbillus* (Desmarest 1804) is one of the most varied groups of rodents living in semiarid and arid areas. Musser and Carleton (2005) revealed that *Gerbillus* has never been completely revised, and its taxonomy still has many mysteries. In fact, *Dipodillus* has been considered a subgenus (Ellerman 1940; Musser and Carleton 1993) or a genus (Osborn and Helmy 1980; Qumsiyeh and Schlitter 1991; Pavlinov 2001). However, Lay (1983), studying the most remarkable characters used to separate these subgenera, identified only one genus, *Gerbillus*.

DNA sequences have been beneficial in taxonomic studies, and they now represent an essential source of knowledge in regard to the delimitation of species (Wiens 2007). Used in combination with other sources of data in the frame of integrative taxonomy (Dayrat 2005), they often result in disguising arguments for or against the identification of taxa as distinct species. In the case of cryptic species, DNA is particularly beneficial, where morphological criteria fail to unambiguously recognize specific taxa (Knowlton 1986). For more than 25 years, the retrieval of DNA from ancient paleontological, archaeological and historic study samples has been routinely conducted (Pääbo 1989; Cooper 1994). The analysis of such DNA sequences has contributed to clarifying the systematics of extinct taxa, but it can also be of help in modern taxa that are difficult to sample today. This study aims to discover and estimate the genetic relationship between some Egyptian rodents using DNA sequencing, which is considered a contribution to establishing genetic datasets of rodents.

2. Material and Methods

Phylogenetic sampling

A total of 26 species were included, representing all gerbils (14 species): *Dipodillus campestris*, *Dipodillus mackilligini*, *Dipodillus dasyurus*, *Dipodillus simoni*, *Gerbillus gerbillus*, *Gerbillus andersoni*, *Gerbillus floweri*, *Sekeetamys calurus*, *Gerbillus pyramidum*, *Gerbillus amoenus*, *Psammomys obesus*, *Meriones crassus*, *Meriones shawi* and *Pachyuromys duprasi*, rats (5 species); *Spalax ehrenbergi*, *Arvicanthis niloticus*, *Rattus rattus*, *Nesokia indica* and *Rattus norvegicus*, mouse (3 species); *Acomys cahirinus*, *Acomys russatus* and *Mus musculus*, Glirid (1 species) which is *Eliomys melanurus* and

Dipodoidea (3 species); *Jaculus jaculus*, *Jaculus orientalis* and *Allactaga tetradactyla* species with published sequence data in addition to our newly sequenced species.

DNA techniques

Analysis was executed on 166 specimens from various localities in Egypt (Figure 1). DNA was segregated from rodent tissue using a QIAamp® DNA Mini Kit.

The cyt b gene was amplified using a thermocycler. Pairs of primers, QF (5′- ACG GAG CCT CAA TAT TCT TCA TCT GC -3′) and QR (5′-CAT GAG GAC AAA TAT CAT TCT GAG -3′), were used to target the cyt b gene of 1140 bp size. The polymerase chain reaction (PCR) thermal program was set as described by Khalifa et al. (2018). PCR products were investigated by running in 2.0% agarose gels and stained with ethidium bromide. Successful PCR bands were cut out and purified using the QIAquick PCR purification kit from Quiagen®. The clean PCR products were sequenced using an automated sequencer following the manufacturer’s protocols.

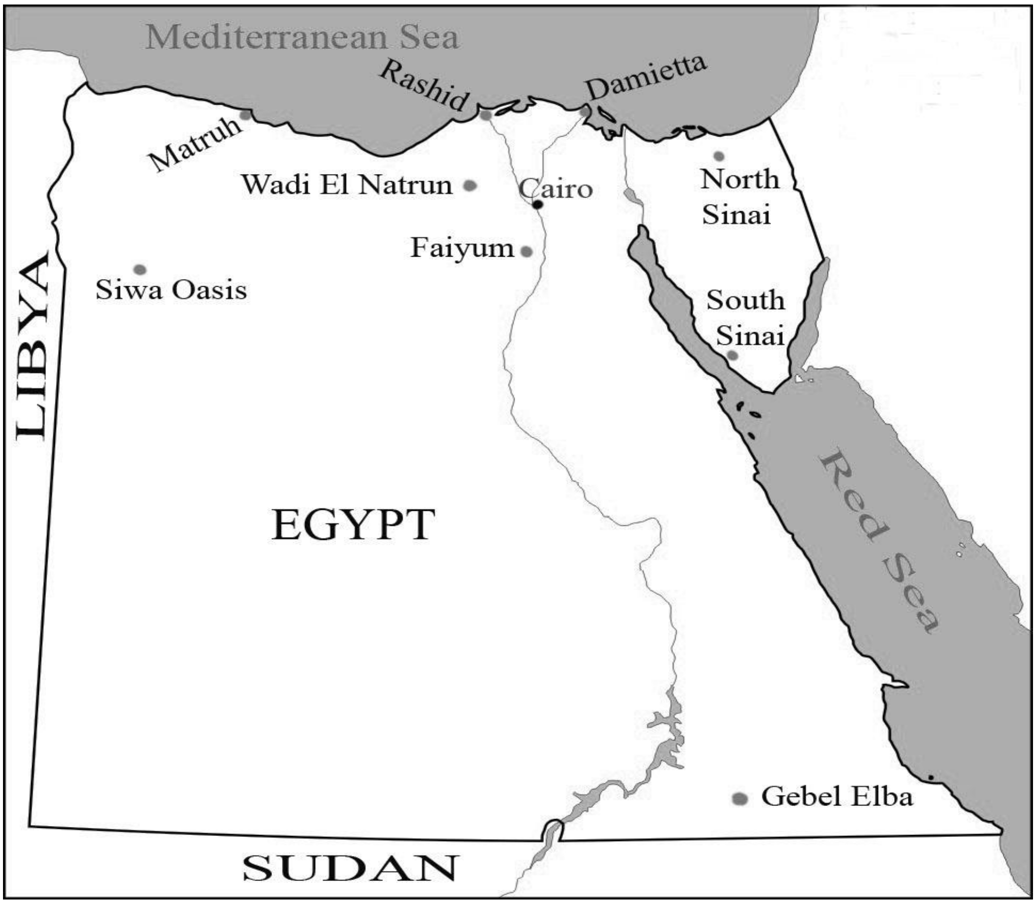

Figure 1. Map of Egypt showing localities (grey circles) of the studied species.

Genetic data analysis

All mtDNA nucleotide sequences were aligned by using Clustal W software, and identical sequences were considered the same haplotype. PhyML v3.0 software (Guindon et al. 2010) was used to construct Maximum Likelihood (ML) and Neighbor-joining (NJ) phylogenetic trees.

MEGA 7.0.14 software (Kumar *et al*. 2016) was used to build a phylogenetic tree based on a maximum-parsimony analysis of complete cytochrome b gene sequences. In the weighting scheme, the transitions at the third codon position and the C ↔ T substitutions for leucine at the first codon position were excluded.

Basic Local Alignment Search Tool (BLAST)

To investigate and recognize the created sequences, each was blast searched as a request through the NCBI (National Center for Biotechnology Information) Blastn tool (www.ncbi.nlm.nih.gov/BLAST/). Sequences with better hits were recovered for further comparison to cyt b sequences from the current study.

3. Results

Cytochrome b Gene Sequences

The cytochrome b gene was specified for 15 genera and analyzed together with previously published muroid rodent sequences. The gene starts within all investigated species with the conserved initiating methionine codon ATG. Within Arvicolinae, the gene has the stop codon TAA. Within Murinae, there is, except for *Rattus norvegicus*, no complete stop codon. The cytochrome b gene ends with a translational termination signal, which is presumably produced by the polyadenylation of the processed mRNA, as in many animal mitochondrial genomes.

Base Composition

The base structure of the cytochrome b gene in the checked gerbillin rodents was identical to that of previously detected mammalian sequences and comparable to values found in some caviomorph rodents. As prospective, the content of G was low (12.3%), while the percentages of A, T, and C were completely identical (27.6–30.3%). The frequency of guanine differs highly among the three codon positions. At the first position, the content is 21.6%; at the second position, it is 13.2%, and at the third position, guanine is rare (2.0%). The second positions have more thymine (43.0%), while the first and third positions are richer in adenine (29.3% and 40.8%). Additionally, these parameters in dipodid rodents were low in G (13.4%), while the percentages of A, T, and C were completely similar (27.8–30.8%). The frequency of guanine differs highly among the three codon positions. At the first position, the content is 23.1%; at the second position, it is 13.8%; and at the third position, guanine is rare (3.3%). Thymine was (28.2%) at the second position, while the first position was richer in adenine (28.2%) and the third position was richer in cytocine (42.1%).

In glirid rodents, G was 13.9%, while the percentages of A, T, and C were completely identical (26.1–30.2%). Additionally, the frequency of guanine differs among the three codon positions. At the first position, it is 21.3%, at the second position, it is 13.9%, and at the third position, guanine is rare (6.6%). The second positions have more thymine (42%), while the first and third positions are richer in adenine (29.2 and 39.5%). Last, the spalacid rodents revealed a low value of G (12.4%). The values of A, T, and C were nearly identical (26.3–32.3%). Guanine differs among the three codon positions. At the first position, it is 21.6%; at the second position, it is 13.4%; and at the third position, guanine is poor (2.1%). Thymine is more abundant in the second position (42%), while adenine is more abundant in the first and third positions (30.3 and 47.9%).

Phylogenetic analysis of the studied rodents

Phylogenetic trees based on Maximum-Likelihood (Figure 2) and Neighbor-joining (Figure 3) methods were calculated. The various weighting schemes in parsimony analysis revealed identical trees with the same tree topologies. Then, only the results with the exception of the first position C ↔ T changes and without third position transitions are presented (Figure 4). The strength of the phylogenetic signal in the dataset was estimated from the length distribution of 10,000 random trees for both weighting schemes. Deviation was inspected for all sequences and subsets of taxa because significant deviation can result from only one strongly supported clade. The analysis first contained all sequences, but clades were subsequently removed to represent all hierarchical levels in the tree. When all sequences are considered, the g_1 statistic is highly significant ($g_1 = -0.594$; $P < 0.01$). Additionally, with subsets of taxa, there is a highly significant deviation in the distribution, indicating a strong phylogenetic signal.

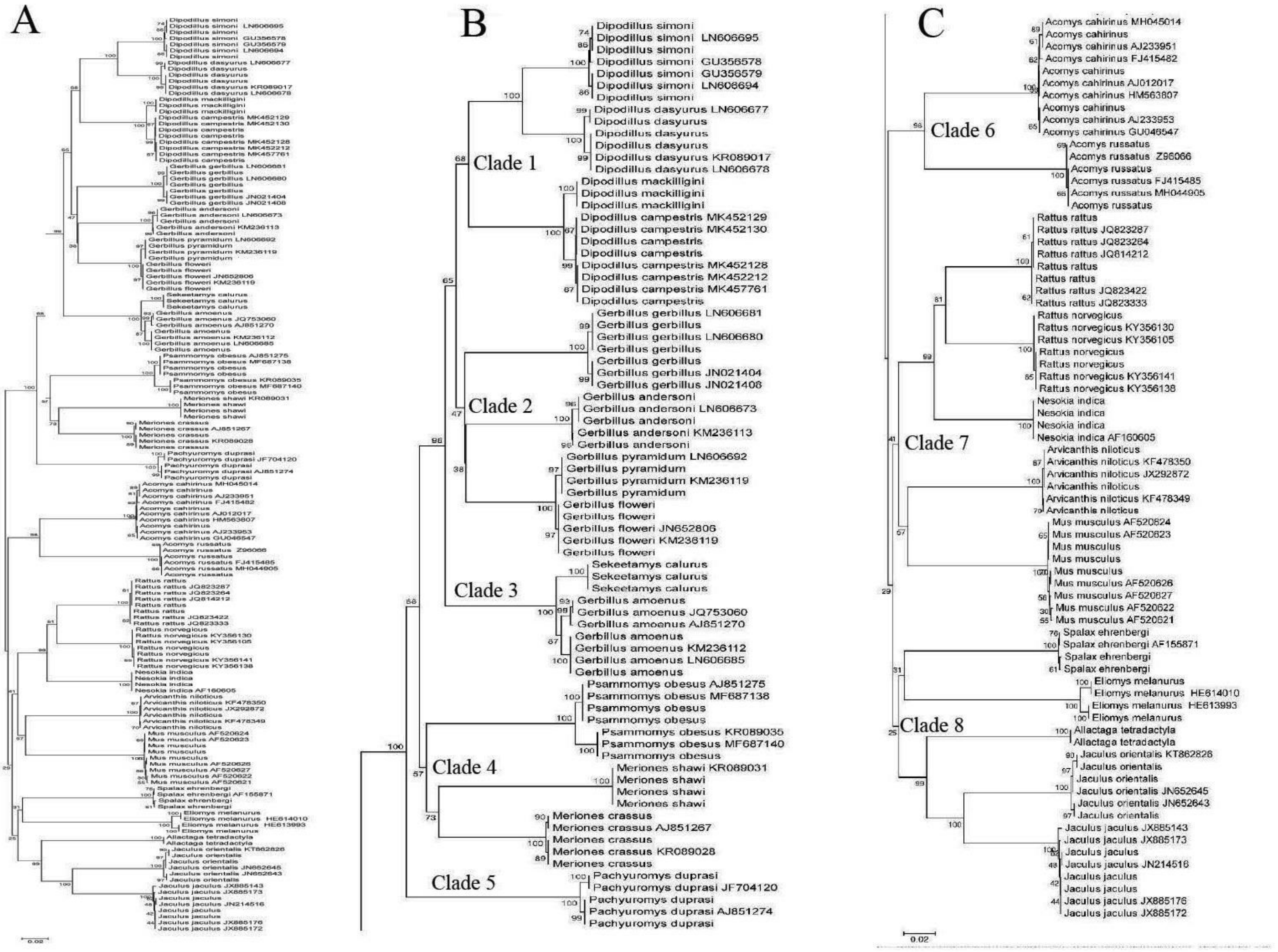

Figure 2. Phylogenetic tree of Maximum Likelihood analyses (Section A) based on the cytochrome *b* gene of rodent species. Clade B contains *Dipodillus*, *Gerbillus*, *Sekeetamys*, *Psammomys*, *Meriones* and *Pachyuromys*; Clade C contains *Acomys*, *Eliomys*, *Rattus*, *Nesokia*, *Mus*, *Arvicanthis*, *Spalax*, *Allactaga* and *Jaculus*. The values at nodes represent the bootstrap confidence level (1000 replicates). The specimen’s number denotes the accession number of GenBank.

Phylogenetic relationships of the studied rodents

The Maximum-Likelihood and Neighbor-joining trees based on analyses of the dataset were fully determined and strongly supported (Figs 2, 3). Both phylogenetic trees of cyt b gene sequences, constructed using the Maximum-Likelihood and the Neighbor-joining methods, resulted in the same topology of branching (Figs. 2, 3). This suggests the reliability of phylogenetic lineages between species shown in these trees. Each of the genera *Dipodillus*, *Gerbillus*, *Sekeetamys*, *Psammomys*, *Meriones* and *Pachyuromys* was grouped into a distinct cluster, supported with high confidence (100% bootstrap value, Figs. 2, 3), and all of them were placed into main clade B called Gerbillinae. Moreover, the genera *Acomys*, *Eliomys*, *Rattus*, *Nesokia*, *Mus*, *Arvicanthis*, *Spalax*, *Allactaga* and *Jaculus* were collectively separated from those of Gerbillinae in another main clade C.

Phylogenetic relationships of Gerbillinae

Clade Gerbillinae contains various clades; clade 1 consists of two branches. The first branch included *Dipodillus campestris,* which formed a sister group with *Dipodillus mackilligini*, supported with a high confidence 100% bootstrap value. The second branch contains *Dipodillus dasyurus* situated in one clade with *Dipodillus simoni*, supported with a 100% bootstrap value. In clade 2, *Gerbillus gerbillus* is laid as a monophyletic group to *Gerbillus andersoni*, *Gerbillus floweri* and *Gerbillus pyramidum*. Additionally, supported by a high bootstrap value (100%), *Gerbillus floweri* was grouped as a sister cluster to *Gerbillus pyramidum*. Another sister group was formed between *Sekeetamys calurus* and *Gerbillus amoenus* with a high bootstrap value (100%), and they were placed in clade 3. Indeed, *Psammomys obesus* formed a monophyletic group into the sister clade, which contained *Meriones crassus* and *Meriones shawi*, as shown in clade 4. Tree topology also revealed that *Pachyuromys duprasi* was laid as a monophyletic clade in all gerbillins (Clade 5). In the current study, multiple alignment of the cyt b gene residues from *Gerbillus* species gave the chance to deepen the perception of the evolutionary relationship between some species within the generic name *Gerbillus*.

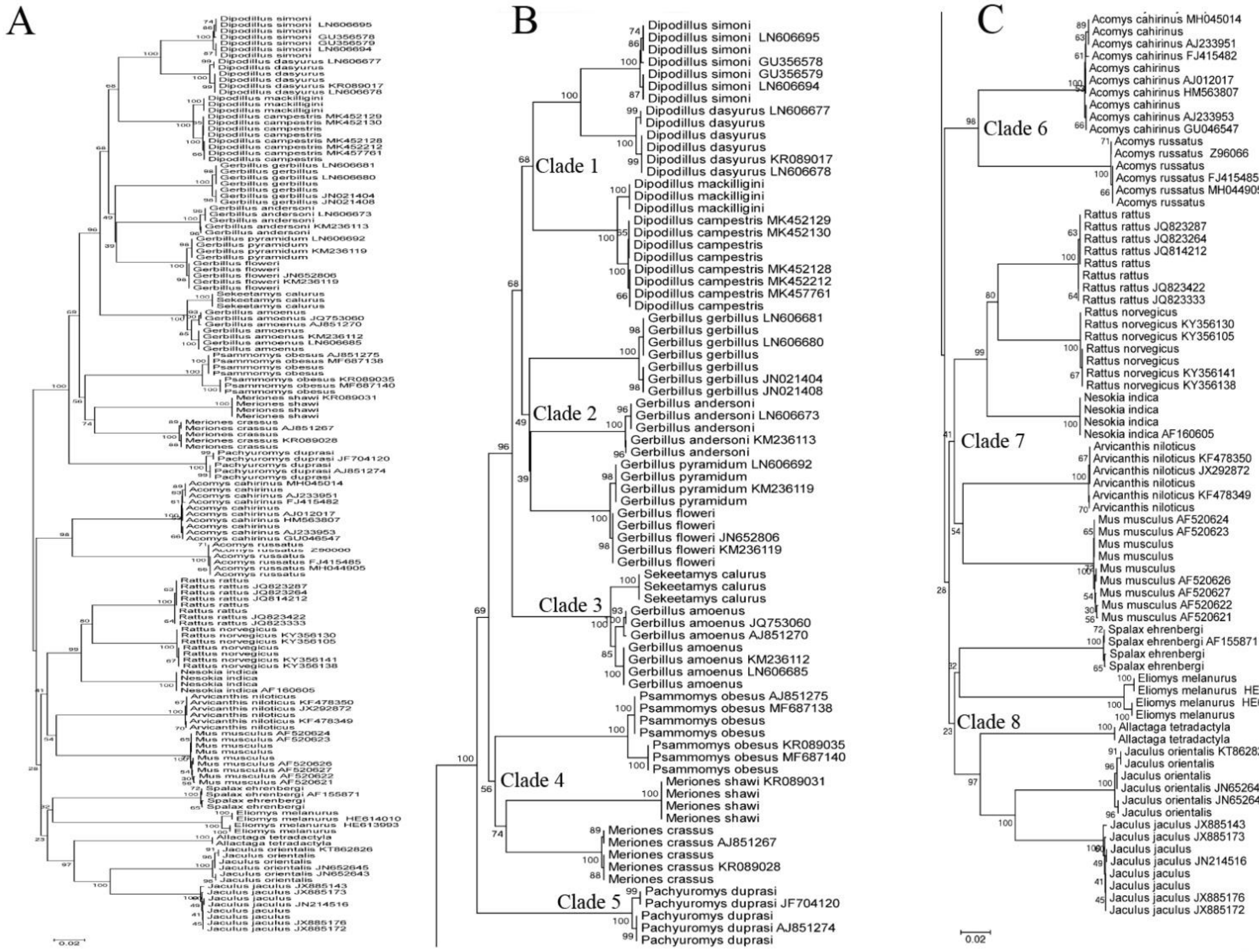

Figure 3. Neighbor-joining phylogenetic tree based on cytochrome *b* gene sequences of rodent species (Section A). Clade B contains *Dipodillus*, *Gerbillus*, *Sekeetamys*, *Psammomys*, *Meriones* and *Pachyuromys*; Clade C contains *Acomys*, *Eliomys*, *Rattus*, *Nesokia*, *Mus*, *Arvicanthis*, *Spalax*, *Allactaga* and *Jaculus*. The values at nodes represent the bootstrap confidence level (1000 replicates). The specimen's number denotes the accession number of GenBank.

Phylogenetic relationships of Deomyinae

Among the Deomyinae, the resolution was good, and *Acomys cahirinus* and *Acomys russatus* formed a sister clade, supported by a high bootstrap value (97% in the ML tree and 98% in the NJ tree) (Clade 6).

Phylogenetic relationships of Murinae

Within the Old World mice and rats Murinae (Figs. 2, 3), we identified *Nesokia indica* as a strong support bootstrap value (100%) monophyletic group into a strong support bootstrap value (81% in the ML tree and 80% in the NJ tree) sister clade, which contains *Rattus rattus* and *Rattus norvegicus*. Furthermore, *Mus musculus* formed a sister group to *Arvicanthis niloticus,* although it did not have strong supporting value (Clade 7).

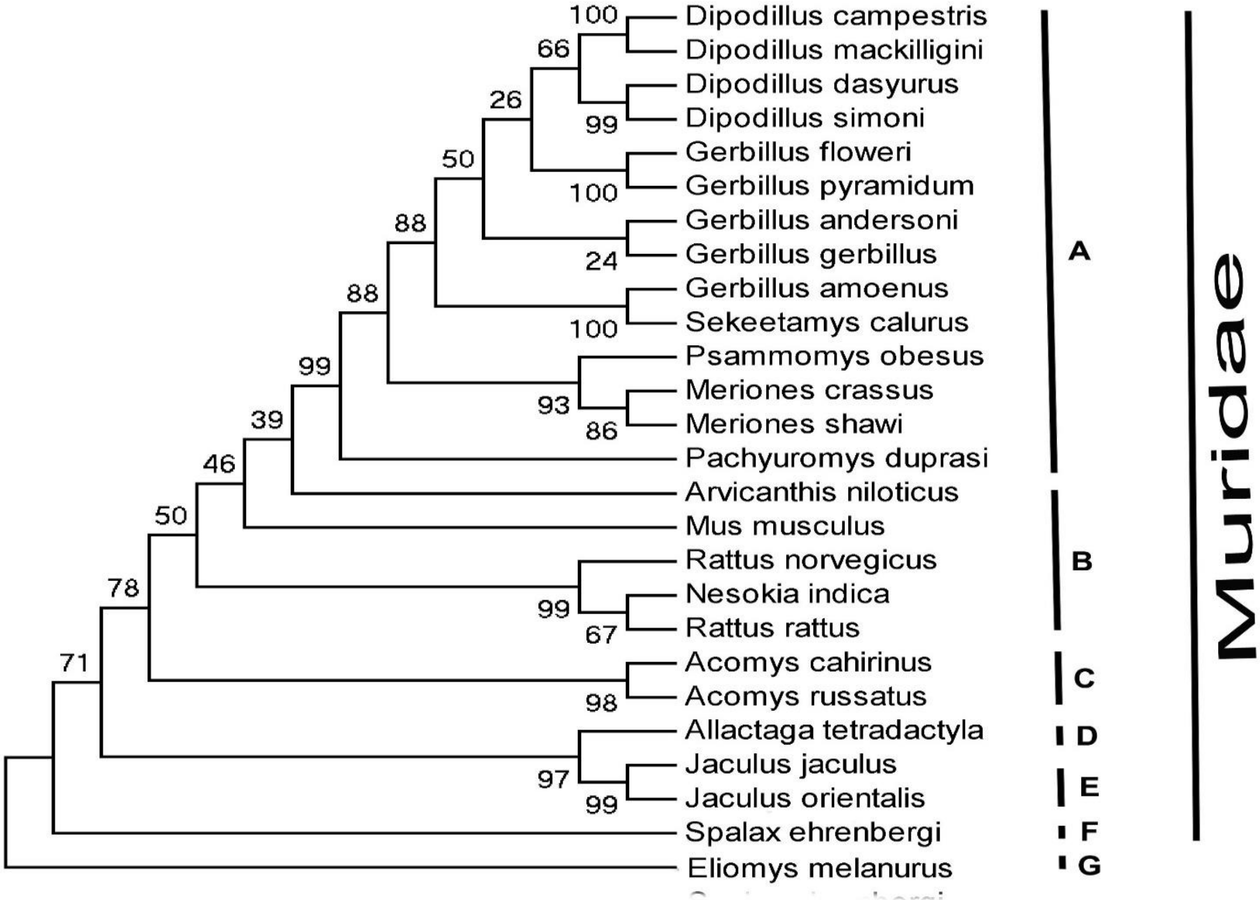

Figure 4. Phylogenetic tree showing the relationships of the different species of the subfamilies, A - Gerbillinae, B - Murinae, C - Acomyminae, D - Allactaginae, E - Dipodinae, F - Spalacinae and G - Leithiinae. It is based on a maximum-parsimony analysis of complete cytochrome *b* gene sequences. In the weighting scheme, the transitions at the third codon position and the C ↔ T substitutions for leucine at the first codon position were excluded. The numbers above branches are bootstrap values obtained in 500 replicates.

Phylogenetic relationships of the Dipodidae

The phylogenetic results reinforce confidence in the high supported value (98%) placement of *Allactaga tetradactyla* as a monophyletic clade with the sister clade that contains *Jaculus orientalis* and *Jaculus jaculus*, supported with a high confidence 100% bootstrap value. Moreover, only Leithiin representative *Eliomys melanurus* (Sciuromorpha: Gliridae) is consistent, although not strong, support for *Spalax ehrenbergi* (only representative spalacinae member) to be a sister group with a value of 31% (Clade 8).

4. Discussion

It was expected that molecular studies would clarify the relationships among rodents. Early studies that depended on molecular data and the understanding of rodent evolution complicated the understanding of rodent evolution by reporting that rodents are paraphyletic (Graur et al. 1991; D'Erchia et al. 1996; Reyes et al. 2000). These findings led to lively debates regarding evolutionary lineages among rodents and their place among placental mammals (Graur 1993; Novacek 1993). The phylogenetic relationship supporting rodent paraphyly has been criticized because it was established on very restricted taxonomic sampling.

In this study, the values of base composition are similar to basic compositional patterns formerly observed in several species of mammals, including some caviomorph rodents (Irwin et al. 1991; Lara et al. 1996; Lessa and Cook 1998). At the first and second positions, the compositional bias is smaller (0.0513 and 0.2840) than at the third position (0.3125). As expected, the first and second codon positions display lower variability than the third codon positions (Irwin et al. 1991).

These phylogenetic results indicated that *Dipodillus* formed a sister group with *Gerbillus*, and all analyses showed robust support for *Gerbillius* being more closely related to *Dipodillus*. Here, again, *Dipodillus* could be returned to a subgenus of *Gerbillus*. Given the morphological variety in this group. The situation with *Meriones* is stronger because support for monophyly is high in Maximum-Likelihood and Neighbor-joining analyses. At the end of the Pliocene, the separation of subgenus *Dipodillus* and subgenus *Gerbillus* likely occurred. Throughout this period, there is a marked climate fluctuation in the African continent (Demenocal 2004). Naked soled gerbils are less adapted to high temperature and desert habitats, and they occupy moderately sandy and gravelly areas. In contrast, haired soled gerbils are well adapted to sandy areas (Choumowitch 1954; Wassif 1956; Petter 1961; Maddalena et al. 1988; Harrison and Bates 1991). The presence of this feature may have allowed the species belonging to the subgenus *Gerbillus* to best live in desert habitats that underwent expansibility at the end of the Pliocene.

The genus *Sekeetamys* (Ellerman 1947) sets an intermediate position between advanced members of Gerbillinae and generalized Rhombomyinae in many morphological features. This is reflected in its “inconstancy” through these two clades in different classifications: some authors set it close to *Gerbillus* and its allies (Chevret and Dobigny 2005), in particular to *Microdillus* (Tong 1989), others place it with *Meriones*, up to including it in the latter genus (Ellerman 1941, 1947, 1948; Charworth-Musters and Ellerman 1947). The morphological characteristic accountable for such an uncertainty is the dental crown pattern found in *Sekeetamys*. This makes it equiprobable to consider the latter genus as either the most advanced member of Gerbillinae or the most primitive member of Rhombomyina.

Heptner (1933) placed *Psammomys* into the family Merioninae, as his taxonomy reflected grades defined by the evolution of tooth crown height. Petter (1959) also grouped *Psammomys* close to *Meriones* rather than to *Rhombomys*. In our findings, *Psammomys* takes a basal position in the group of “higher gerbils”, which is compatible with the molecular phylogenetic tree of Chevret and Dobigny (2005). Tong (1989) placed *Pachyuromys* within the clade of “higher gerbils”, as reported in the results of the current study.

In the molecular phylogenetic tree of Balakirev and Rozhnov (2012), *Mus musculus* takes a basal position and forms a monophyly to *Rattus* species. Indeed, *Mus musculus* was more related to *Rattus norvegicus* than *Rattus rattus*. These results are in agreement with the findings of this study. Moreover, the phylogenetic relationships specified for the murine genera *Mus*, *Nesokia*, *Arvicanthis* and *Rattus* in this work are in agreement with the findings of Catzeflis et al. (1987) and Nikoletopoulos et al. (1992). Good resolution was obtained between *Mus* and *Rattus*.

Based on their similar molar structure, it was supposed that *Acomys* belongs to Murinae and was a close relative to *Mus* (Jacobs 1978). Nevertheless, the immunological results of Sarich (1985) indicated that *Acomys* is as far from *Mus* as it is from other subfamilies of Muridae. Chevret et al. (1993) investigated the relationships of *Acomys* to other rodent species with DNA/DNA hybridization data and obtained a closer relationship of *Acomys* to Gerbillinae than to Murinae. In a recent study by Dubois et al. (1999), a subfamily level for *Acomys* and *Uranomys* (Acomyinae) was proposed based on sequences of the nuclear pancreatic ribonuclease A gene. The cytochrome b data also suggest an early separation of *Acomys* from the other murine genera.

Deomyinae was separated from Gerbillinae supported by some morphological traits, including pelage texture, palatal and molar occlusal patterns, and reproductive biology (Musser and Carleton 2005). The present results support the sister group of the two studied Deomyinae species. The present findings robustly support the hypotheses that Dipodidae is sister to Muroidea. This hypothesis is now supported by morphological (e.g., Luckett 1985; Lavocat and Parent 1985; Meng 1990) and multiple sources of molecular data (Nedbal et al. 1996; Huchon et al. 1999; Huchon et al. 2000).

5. Conclusions

The present study supports the utilization of mitochondrial genes in helping to resolve the phylogeny of Gerbillinae and provides further support for different clades, including the paraphyletic nature of Acomyinae and the position of the *Mus* and *Rattus* genera. This study also shows that the mtDNA control region can be useful in further understanding the genetic diversity of some Egyptian rodents. This work provides the first insight into the genetic diversity of these species of Rodentia in Egypt, a framework for further study and can be considered a core dataset for mammalian species in Egypt.

Authors' Contributions: DEEF, L.E.M.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The author declares no conflicts of interest.

Ethics Approval: All animal experiments involved in this study were approved by the Laboratory Animal Welfare and Animal Experimental Ethical Committee of IACUC Cairo University. We followed guidelines of the Committee for experimental animals during this study.

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