DNA Barcoding and Phylogeny of Some Common Rodents of the Family Muridae in the Egyptian Environment

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Abstract: The family Muridae is one of the most ubiquitous invasive families all over the world and in Egypt is particularly common and wide spread. Here we investigated the degree of similarity and divergences between 8 species related to the family Muridae of common occurrence in Egypt through molecular analysis of mitochondrial DNA (COI gene) comparing between *Acomys cahirinus*, *Mus musculus*, *Rattus rattus*, *Rattus norvegicus*, *Albino Rattus norvegicus*, *Gerbillus gerbillus* and *Gerbillus pyramidum* in Egypt. Our result showing a strong link between *Acomys cahirinus* and genus Gerbillus than to genus Mus and Rattus with high bootstrap support.
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1. Introduction

DNA barcoding is a taxonomic method that uses a short genetic marker to identify a certain organism (Hebert *et al*. 2003a). It differs from molecular phylogeny in the main goal as it is not to determine patterns of relationship but to identify an unknown sample (Kress 2005). DNA barcoding and DNA taxonomy have been proposed as solutions to the issues of taxonomy and received significant attention from scientific journals, grant agencies and natural history museums (Meier *et al*. 2006). The most commonly used region for barcoding in animals is a short fragment of 600 base pairs in mitochondrial gene COI (cytochrome oxidase I) (the Folmer region) that was proposed as a potential barcoding region of animals (Hebert *et al.* 2003b). COI gene consistently identifies species where authenticated reference sequence data exists (Dawnay 2007). However, the data obtained from COI barcoding can be efficiently used for phylogenetic analysis between several species and to solve taxonomical problems (Folmer *et al*. 1994). *Murinae* and *Gerbillinae* sub-families were extensively studied using advanced molecular techniques (Steppan *et al*. 2004; Tucker *et al*, 2005; Robins *et al.* 2007, 2008 and Michaux *et al.* 2015). Those advanced molecular techniques for molecular systematic for example: sequenced several DNA regions (e.g. ghr, brca1, rag1, ap5, B2m, Zp3, Tcp1, Sry, Smcx, Smcy and c-myc), nuclear protein-coding genes (e.g. lcat, vWF) and mitochondrial regions (e.g. COII and parts of COI, cytochrome b, D-loop, 12S and ATPase 8) (Michaux & Catzeflis 2001; Adkins *et al*. 2003; Scott *et al*. 2005; Tucker *et al*. 2005; Robins *et al*. 2007, 2008; *Gabriel et al*. 2011 *and* Michaux *et al*. 2015). The superfamily *Muridae* included subfamily *Murinae* and subfamily *Gerbillinae* (Adkins *et al*. 2003). The *Murinae* family was found as a sister group to family *Gerbillinae* (Adkins *et al*. 2003; Steppan *et al*. 2005). However, *Acomys* genus was found to be more related to *Gerbillinae* than to Murinae (Chevert *et al.* 1993; Michaux *et al*. 2001).

2. Material and Methods

Sample collection. In the present work 3 species of *murid* animals have determined the genetic diversity of them by using molecular technique studies. All of them belong to family *Muridae* which are*:* Genus *Acomys* (I. Geoffroy st. Hilaire, 1803) Species *Acomys cahirinus cahirinus* (Desmarest, 1819). This animal has the common local names: Egyptian spiny mouse, Abu shoak. Genus *Gerbillus* (Desmarest, 1804). Two animals belonging to the genus were available for study. Species *Gerbillus gerbillus gerbillus* (Olivier, 1801), this animal has the common local names: Lesser Gerbil Bayoudi. Species *Gerbillus pyramidum pyramidum* (I. Geoffroy st, Hilaire, 1825). This animal has the common local names: Greater Gerbil Demsy.

DNA, PCR and phylogenetic analysis. Total DNA was extracted from 0.25 g grinded liver tissue of samples using Bioline ISOLATE II genomic DNA kit using BENCH-TOP protocol. An approximately 600bp fragment of cytochrome *c* oxidase subunit I (COI) mitochondrial gene was PCR-amplified using LCO1490 and HCO2198 primers (Folmer et al. 1994). DNA amplified in 50ul reactions using 1x MyTaq™ Red Mix (cat. #BIO-25043, Bioline, UK), 10 pmol of each primer and 50-100ng DNA. The PCR amplification was performed using Techne 512 programed as follows: 5 min denaturation at 94 °C, followed by 30 cycles of strand denaturation at 94 °C,

annealing at 48 °C and extension at 72 °C, a final extension at 72 °C for 10 min. Amplicons were tested on 1.5% w/v agarose gel electrophoresis supplemented by 1x Ethidium Bromide (EtBr), samples were loaded along with 2.5ul GeneRuler 100bp DNA Ladder (cat. # SM0243, Fermentas, Lithuania). When successful amplicos were purified directly from the PCR product using DNA Clean & Concentrator™ -25 Kit (cat. # D4033, Zymo Research, USA). Purified COI fragments were sequenced using Macrogene, Inc. services (Seoul, South Korea).

Chromatograms of the bidirectional sequence the were refined, aligned and assembled using Geneious V8.1. The haplotypes from the samples were Blasted and aligned with the Blast result sequences. Phylogenetic tree was generated using maximum likelihood method, while tested using bootstrap method of 1000 times, while the consensus tree was generated applying the majority rule.

3. Results

After trimming, COI sequence length varied according to the species type, for *Acomys* was 379 bp, *G. pyramidum* 686 bp, *G. gerbillus* 683bp. COI sequences of the sampled taxa were blasted and aligned with resulted GenBank accessions G. sp KF422711, *G. nanus* KF422708, *M. musculus domesticus* GQ905751, *M. musculus* (2: KC617840, KC617857), *R. tanzuny* GQ793910 and *R. rattus* (3: GF446035, EF186584 and GF444222).

All sequences examined were typical of our genera. Therefore, our phylogenetic tree was based purely on family Muridae polytypes and clades were labeled according to previous practice for these genera (Fig. 1). The majority of sequences were evenly divided among two clades with the highest bootstrap support value (1.00). The Murinae sequences harbored 2 distinct polytypes belonging to 2 sub-clades which are sub-clade for genus *Rattus* and another sub-clade for the genus *Mus*. In the second clade the *Acomys* of the subfamily Acominae was found in the same clade with *Gerbillus* of the subfamily Gerbillinae.

The existence of unknown *G.* sp and proximity to *Acomys* is likely due to the lack of the present database in the definition of this value, where it tends to being *Acomys* more than being *Gerbillus* with a lower bootstrap support 0.82.

Phylogenetic arrangement of family tree between these members revealed that *A. cahirinus*, *G. gerbillus* and *G. pyramidum* do not belong to Murinae. On the other hand, the second clade which included the remaining genus which are *Rattus* and *Mus* genera which are do not belong to Gerbillinae but to other murid rodents which is Murinae. That means the spiny mouse *A. cahirins* is not a mouse and should not be a member of murinae.

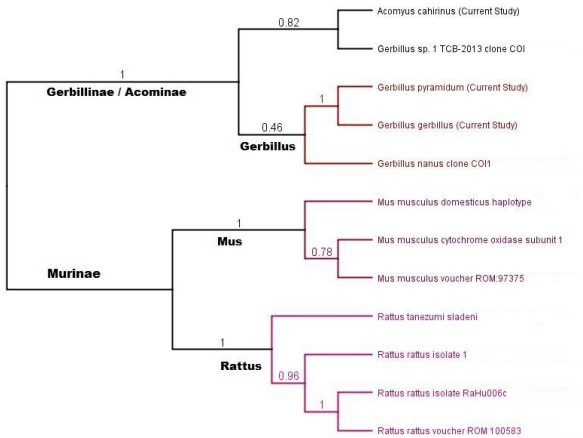

Figure (1). Maximum likelihood based phylogenetic tree. Two major clades are defined and supported with the maximum bootstrap support 1.00. Each sub-clade related to a certain species is colored accordingly. However, *Acomyus cahirinus* COI sequence found to be highly supported within the Gerbillus genus clade (0.82) along with unknown species from the genus.

4. Discussions

Despite the difference in the length of the fragment of COI (Former region) of the genus *Acomys* in comparison to the rest of samples but It passed in defining the sample by comparing them to the database and considered *A. cahirinus* and that's where kinship based on mutations and their types to reflect the variation between under test samples. Whereas Gerbillus sample recorded twice the length of the pieces obtained from *Acomys* but the kinship relationship tree reflected value between the specimens regardless of the length of region which confirms the importance, stability and efficiency of COI in the definition of the samples.

The tree confirms that the genus *Acomys* close to Gerbillus but not attributed to the same genus, since the morphological and chromosomal studies confirm the extent of the difference between 2 genera as already mentioned confirming studies carried out by Frynta *et al*. (2010) and Michaux *et al*. 2015.

Acomys genetically not much different from Gerbillus, *M. musculus* and *R. rattus* where all of whom belong to the same family. Where COI has been proven success in inventory genetic differences between them and proven the importance of using it in any future studies related to this family in agreement with previous research (Chevret*et al*. 1993; Agulnik and Silver 1996; Barome*et et al*. 1998, 2002, 2001a and b; Volobouev*et al*. 2002 and 2007).

In the present study *A. cahirinus*, *G. gerbillus* and *G. pyramidum* do not belong to Murinae but

rather belong to a clade of the Murid rodents represented in this study by the Gerbillinae. On the other hand, the second clade which included the remaining genus which are *R. rattus*, *R. norvigicus*, white *R. norvigicus*, *M. musculus* and white *M. musculus* which are not belong to Gerbillinae but to other murid rodents which is Murinae. That means the spiny mouse *A. cahirins* is not a mouse and should not be a member of murinae. This is Molecular evidence that the spiny mouse (*Acomys*) is more closely related to gerbils (*Gerbillinae*) than to true mice (Murinae). This view has been challenged by immunological studies and DNA-DNA hybridization that have suggested that *Acomys* is as distantly related to mice (*Mus*) as are other subfamilies of the muroid rodents (Chevert *et al.* 1993). Also Phylogenetic trees based on 1,962 nucleotides from the two genes indicate that the 14 *Muridae* subfamilies Lead to present of evidence that the sister group of Acomyinae is Gerbillinae.

Recent molecular techniques by using the pericentric satellite DNA (Kunze*et al*. 1999) and cytochrome *b* mitochondrial gene (Barome*et al*.1998, 2002, 2001a and b; Volobouev*et al*. 2002 and 2007), revealed that *A. cahirirnus* from Egypt is closely related to *Acomys* species that included, *A. dimidiatus* (from Palestine, Sinai and Saudi Arabia), *A. russatus* (from Jordan), *A. ignites* (from Kenya), *A. airensis* (from Niger), *A. minous* (from Crete), *A. nesiotes* (from Cyprus) and *A. cilicicus* (from Turkey).

Phylogenetic arrangement of family tree between members of the genus *Acomys* and the closely related taxa of the family Muridae was studied by using protein polymorphism (Janecek*et al*., 1991). The mitochondrial cytochrome *b* gene is well known as a protein-coding marker. This gene was sequenced for specimens from most *Acomys* species and proved to be a useful tool for investigating inter-specific relationships within this genus (Irwin *et al*., 1991; Barome *et al*., 1998, 2000 and 2001a & b; Volobouev*et al*., 2002 & 2007). Recent molecular genetic studies showed that *Acomys* species are more closely related to gerbils (Gerbillinae) than to the true mice (Murinae) (Chevret*et al*., 1993; Agulnik and Silver, 1996).

In the current study, the COI sequencing confirmed the close relation of the *Acomyus* sp. to the Gerbilline than to the other Rodents with high bootstrap support (0.82/1 = 82%).

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