Molecular Phylogenetic Relationship Between and Within the Fruit Bat (*Rousettus Aegyptiacus*) and the Lesser Tailed Bat (*Rhinopoma Hardwickei*) Deduced From RAPD-PCR Analysis

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Abstract: The RAPD-PCR in the present study was used to determine the genetic variation within and among two Egyptian bat species, *Rousettus aegyptiacus* and *Rhinopoma hardwickei*. The animals were captured from one locality at Giza governorate, Egypt. A total of 39 bands were amplified by the three primers OPAO2, OPAO8 and OPCO3 with an average 13 bands per primer at molecular weights ranged from 1409 to 107 bp. The polymorphic loci between both species were 34 with percentage 87.18 %. The numbers of monomorphic bands in *Rousettus aegyptiacus aegyptiacus* and *Rhinopoma hardwickei arabium* were 14 and 9 bands, respectively. The two species are sharing 5 (12.8 %) monomorphic bands. The similarity coefficients value between the two bat species was ranged from 0.353 to 0.500 with an average of 0.404 (40.4%). Dendrogram showed that, the two bats genotypes are separated from each other into two clusters and more variation among members of *Rhinopoma hardwickei arabium* was observed in comparison to those of *Rousettus aegyptiacus aegyptiacus*. It is concluded that, the similarity coefficient value between the two bat species indicates that, the two bat species may have the same origin but are not identical and separated into two clusters.

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Key Words: Fruit bat *(Rousettus aegytiacus*); lesser tailed bat (*Rhinopoma hardwickei*), RAPD-PCR, Phylogenetic Relationship.

1. Introduction

The order Chiroptera readily separated into two groups (megabats and microbats) on the basis of the morphological features, the gross anatomical characteristics, feeding habits and behavior (**Anderson, 1902; Sanborn and Hoogstraal, 1955; Hoogstraal, 1962; Gaisler *et al*., 1972; Qumsiyeh, 1985**). The Species *Rousettus aegyptiacus* has two subspecies *Rousettus aegyptiacus aegyptiacus* and *Rousettus aegyptiacus arabicus*. *Rousettus aegyptiacus aegyptiacus* is distributed in Egypt, Lebanon, Jordan, Cyprus, and Turkey and from Levant to the middle of Arabia and Africa (**Corbet 1978; Harrison and Bates, 1991; Bergmans, 1994; Benda and Horacek, 1998; Albayrak *et al*., 2008**) whereas the subspecies *Rousettus aegyptiacus arabicus* distributed in Oman, Aden, and occurred from the South Arabia to Pakistan. While, the lesser tailed bat, *Rhinopoma hardwickei* is represented by two subspecies *Rhinopoma hardwickei arabium* and *Rhinopoma hardwickei cystops* in Egypt (**Qumsiyeh, 1985; Wassif, 1995**).

The megabat *Rousettus aegyptiacus* feeds on a variety of fruits, depending on what is locally available (**Kinzelback, 1986; Harrison and Bates, 1991**). Fruit bats, *Rousettus* spp. are not only a cause of great economic loss in fruit crops but are also vectors of domestic animals and human transmissible ectoparasites (**Parri *et al*., 1971; Kalunda *et al*., 1986; Wellenberg *et al*., 2002; Calisher *et al*., 2006; Reeves *et al*., 2006; Towner *et al*., 2009**) whereas, *Rhinopoma hardwickei* feeds on insects, and acts as a vector for some fungal diseases such as subcutaneous zygomycosis (**Gugnani, 1999**).

Rousettus aegyptiacus is the only bat species recorded in Egypt that belong to the megabats (**Qumsiyeh, 1985**). The species *Rousettus aegyptiacus* contains several subspecies of which the subspecies *Rousettus aegyptiacus aegyptiacus* which presented in Egypt (**Corbet, 1978; Qumsiyeh, 1985; Wassif, 1995; Benda and Horacek, 1998; Kwiecinski and Griffithsi, 1999**). The family Rhinopomatidae in Egypt contains a single genus with two species, *Rhinopoma hardwickei* and *Rhinopoma microphyllum* (**Qumsiyeh, 1985; Wassif, 1995**). In Egypt, *Rhinopoma hardwickei* species has two subspecies; *Rhinopoma hardwickei arabium* in northern Egypt and *Rhinopoma hardwickei cystops* in Southern Egypt (**Qumsiyeh, 1985; Wassif, 1995**). Rhinopomatidae bear a unique set of morphological plesiomorphies for which they were often regarded as the most primitive group of microchiroptera close to the common ancestor of microbats and megabats (**Van Valen, 1979; Qumsiyehi, 198**5).

The order Chiroptera is an excellent example of how molecular phylogenetics helps in reassessment

of the taxonomy of a well-established group. Genetic data invalidated the traditional subdivision of bats into two divergent suborders; Megachiroptera and Microchiroptera. **Teeling *et al*. (2000, 2002 and 2005)** provided molecular evidence supporting sister position of one clade of microbats, Rhinolophoidea (superfamily) with megabats, Pteropodoidea (superfamily). The family Rhinopomatidae is arranged among superfamily Rhinolophoidea (**Van Den Bussche and Hoofer, 2004; Eicki *et al*., 2005; Teeling *et al*., 2005**). Rhinopomatidae is the most enigmatic group of extant bats. It is monotypic family composed of a single genus *Rhinopoma*. Rhinopomatidae were regarded as the most primitive group of Microchiroptera close to the common ancestor of microbats and megabats (**Hill, 1977; Van Valen, 1979; Koopman, 1993; Eick *et al*., 2005**). Rhinopomatids are the most primitive clade within superfamily Rhinolophoidea and thus, also the most primitive extant clade within the suborder Yinpterochiroptera (**Teeling, 2005**). For all these reasons, Rhinopomatidae are an extremity attractive subject for future detailed studies. **Hulva *et al*. (2007)** suggested that, the taxonomic characteristics of Rhinopomatidae are shared with suborder Yangochiroptera and Rhinolophoidea of suborder Yinpterochiroptera partly resembling the condition in Pteropodidae (suborder Yinpterochiroptera).

The analysis of genetic diversity and relationship between or within different species, populations and individuals is a central task for many disciplines of biological science. The systematic similarity and diversity between *Rousettus aegyptiacus* and *Rhinopoma hardwickei* species was tackled according to chromosomal studies (**Ray-Chaudhuri *et al*., 1968; Qumsiyeh and Baker, 1985**), biochemical data (**Juste *et al*., 1996 and 1997**) and molecular genetics (**Alvarez *et al*., 1999; Juste, 2002; Hulva and Horacek, 2002; Hulva *et al*., 2007; Goodman *et al*., 2010**).

The techniques of molecular genetic markers have an important potential role in detection of genetic differences among and within species. The most common molecular techniques are RAPD-PCR (Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction), SSR-PCR (Simple Sequence Repeat-Polymerase Chain Reaction), RFLP (Amplified Fragment Length Polymorphism), and sequencing of nuclear and mitochondrial genes. **Erlich and Arnheim (1992)** reported that the polymerase chain reaction (PCR) has become one of the most widely used techniques of molecular biology because it is rapid, inexpensive and produces useful amounts of DNA from small quantities of source DNA. RAPD-PCR technique was a powerful tool in detecting genetic variability between species (**Welsh and McClelland, 1990; Williams *et al*., 1993; Yadav and Yadav, 2007**) and within species (**Singh and Sharma, 2002; Hassanien *et al*., 2004; Abdel-Rahman and Hafez, 2007**) based on the amplification of variable regions of a genome.

Many researchers used RAPD-PCR markers to differentiate and determine the genetic variation between and within species of bats (**Sinclair, 1996; Emmanuvel and Marimuthu, 2006; Moreiral and Morielle-versute, 2006; Karuppudurai *et al*., 2007; Karuppudurai and Sripathi, 2010**). Other molecular studies such as cytochrome B, microsatellite, RFLP and DNA sequences had been carried out to solve evolutionary problems in bats (**Mindell *et al*., 1991; Colgan and Flannery, 1995; kirsch *et al*., 1995; Alvarez *et al*., 1999; Rossiter *et al*., 1999; Ditchfield, 2000; Juste *et al*., 2002; Hulva and Horacek, 2002; Sebastien *et al*., 2005; Hulva *et al*., 2007; Levin, 2008; Goodman *et al*., 2010; Chinnasamy *et al*., 2011; Wood *et al*., 2011).**

The present study was mainly focused on clarifying the similarities and divergences within and among individuals of the *Rousettus aegyptiacus aegyptiacus* and *Rhinopoma hardwickei arabium* species commonly present in the local environment by using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique.

2. Materials and Methods

Animals

The present work was carried out on the fruit bat, *Rousettus aegyptiacus aegyptiacus* and the lesser tailed bat, *Rhinopoma hardwickei arabium*. Animals were trapped near Abu-Rawash area, Giza governorate, Egypt. Liver samples were obtained from 6 animals of both *Rousettus aegytiacus aegyptiacus* and *Rhinopoma hardwickei arabium* and stored in liquid nitrogen until DNA extraction.

DNA extraction

Genomic DNA was extracted from liver tissue. The extracted DNA quality and concentration was examined with both spectrophotometric analysis and run in 7.0 % agarose gels. In spectrophotometric analysis, each sample of DNA was calculated by their optical density values at 260 and 280 nm. Optical density ratios were evaluated and only good quality DNA samples were used in PCR.

RAPD-PCR

A total of five arbitrary decamer primers from Kits OP-A and OP-C (Operon Technologies, Alameda, CA, USA) were used for RAPD-PCR analysis. The primer sequences and manufacturer codes are given in Table (1). Only three primers

(OPAO2, OPAO8, and OPCO3) were reacted well and used to amplify genomic DNA from all individuals. RAPD-PCR reactions were carried out as described by **Williams *et al*., (1993)**. PCR cycles were performed with 60 s, 94°C initial denaturation and 35 cycles of 20 s, 94°C; 20 s 35°C; and 30 s 72°C. Final extension performed at 72°C for 5 min. PCR amplifications were carried out in 96 well Thermal cycler (Eppendorf Master Cycler) and all amplifications were carried out at two times. A PCR mixture without template DNA was put in each analysis as a control. The PCR products were separated on 1.5 % agarose gels (Sigma) containing ethidium bromide in 0.5 X TBE buffer at 100 V constant voltages. For evaluating the base pair length of bands, DNA ladder (Fermentas) was loaded on middle lane of each gel.

Data and statistical analysis:-

The RAPD banding patterns were scored for the presence (1) and absence (0) of bands for each sample. The scores obtained using all primers in the RAPD analysis were then combined to create a single data matrix. The statistical analysis of the data was performed using the free software, Popgene version 1.31, computer program (**Yeh *et al*., 1999**) including the calculation of allele frequencies according to **Nei (1987)**. This program estimated the number and percentage of polymorphic loci and the genetic diversity according to **Nei (1973)** and **Nei and Li (1979)**. For constructing the dendrogram, the data resulted from RAPD markers banding patterns was introduced to NTSYS-pc package program by Unweighted Pair Group Method using Arithmetic Averages (UPGMA) method (**Rohlf, 2000**).

3. Results

In the present study, three primers (A02, A08 and C03) out of the five random primers produced a PCR product for the investigation of the genetic variation within and between individuals of the two studied bat species. They yielded a sufficient number of bands for comparison and differentiation between the two bat species. The RAPD profile generated from these primers was utilized to estimate the gene frequency. The primer A02 produces much more of amplified fragments of genomic DNA of the two species of bats compared to the other primers (A08 and C03; Figs. 1, 2 & 3 and Table 2). 9 unique bands were found in *Rhinopoma hardwickei arabium* at the molecular weight of 649, 605, 262, 188, 156, 107, 811, 745 and 187 bp and one unique band was characterized in *Rousettus aegyptiacus aegyptiacus* at molecular weights 240 and 210 bp.

Table 3 showed a total of 39 scorable amplified loci and 34 of them were polymorphic (87.18%). The numbers of RAPD loci were 16 for primer OPAO2, 10 for primer OPCO3 and 13 for primer OPAO8 with an average 13 bands/primer at molecular weights ranged from 1409 to 107 bp. The polymorphic bands were 14 (87.5 %), 11 (84.61%) and 9 (90 %) for primers OPAO2, OPAO8 and OPCO3, respectively with an average 11.33 bands/ primer.

Table (4) showed the allele frequencies of different bands in the two species using the three primers at RAPD markers. The numbers of monomorphic bands in *Rousettus aegyptiacus* and *Rhinopoma hardwickei* were 14 (70 %) and 9 (32 %) bands, respectively. The two species are sharing 5 (12.8 %) monomorphic bands (Refer to table 2). The number and percentage of polymorphic bands within the two species of bats are shown in Table (4). From the total loci (39), the numbers of the polymorphic loci were 6 loci with percentage of 30 % in *Rousettus aegyptiacus aegyptiacus* and were 19 loci with percentage of 68 % in *Rhinopoma hardwickei arabium*.

The similarity matrix within and among the individuals of the two species are presented in table (5). The similarity coefficient values among the samples of *Rousettus aegyptiacus aegyptiacus* ranged from 0.824 to 1.00 with an average of 0.919 (91.9%) and the genetic distance equal 0.8 (80%). Whereas, the similarity coefficients value among the samples of *Rhinopoma hardwickei arabium* is ranged from 0.667 to 0.933 with an average of 0.753 (75%) and the genetic distance equal 0.25 (25%). The similarity coefficients value between the two bat species was ranged from 0.353 to 0.500 with an average of 0.404 (40.4%). The genetic distance between the *Rousettus aegyptiacus aegyptiacus* and *Rhinopoma hardwickei arabium* is 0.596 (59.6 %). From the genetic distance, the samples of *Rousettus aegyptiacus aegyptiacus* are closer to each other while the samples of *Rhinopoma hardwickei arabium* demonstrate high polymorphism between them. The UPGMA dendrogram was constructed to show phylogenetic relationships within and among the two bat species based on genetic distance (Fig. 4). This dendrogram represents that the two bats genotypes are separated from each other into two clusters and a variation among members of *Rhinopoma hardwickei arabium* in comparison to those of *Rousettus aegyptiacus aegyptiacus*.

Table (1): Sequence of primers employed in molecular phylogenetic relationship between *R. aegyptiacus* and *R. hardwickei*.

Primers	Sequence	G C %
OPAO2	5'-TGCCGAGCTG-3'	70
OPA03	5'-AGTCAGCCAC-3	60
OPAO8	5'-GTGACGTAGG-3'	60
OPCO3	5'-GGGGGTCTTT-3'	60
OPC06	5'-GAACGGACTC-3'	60

Table (2): Band frequencies of random primers in *R. aegyptiacus* and *R. hardwickei*.

Primer	Band number	Relative front	Molecular weight (bp)	Band frequency *R. aegyptiacus*	Band frequency *R. hardwickei*	Average Band frequency
OPAO2	1	0.059	1409	0.500	0	0.250
	2	0.103	1234	0.333	0.333	0.333
	3	0.190	950	1.000	0.833	0.917
	4	0.231	839	1.000	0.500	0.750
	5	0.296	690	1.000	1.000	1.000
	6	0.348	590	1.000	1.000	1.000
	7	0.438	450	1.000	0	0.500
	8	0.454	429	0	1.000	0.500
	9	0.508	364	0	1.000	0.500
	10	0.539	332	1.000	0	0.500
	11	0.551	320	0	1.000	0.500
	12	0.618	262	0	0.167	0.083
	13	0.646	240	0.167	0.667	0.417
	14	0.727	188	0	0.167	0.083
	15	0.789	156	0	0.167	0.083
	16	0.914	107	0	0.167	0.083
OPCO3	1	0.299	682	0.833	0	0.417
	2	0.316	649	0	0.167	0.083
	3	0.339	605	0	0.167	0.083
	4	0.378	539	0	0.833	0.417
	5	0.388	523	1.000	0	0.500
	6	0.433	457	0	0.333	0.167
	7	0.491	384	1.000	1.000	1.000
	8	0.593	283	1.000	0	0.500
	9	0.678	220	0	0.333	0.167
	10	0.724	192	0	0.667	0.333
OPAO8	1	0.250	928	0	0.667	0.333
	2	0.276	846	1.000	0	0.500
	3	0.288	811	0	0.167	0.083
	4	0.312	745	0	0.167	0.083
	5	0.341	672	0	0.500	0.250
	6	0.357	635	1.000	0	0.500
	7	0.436	480	1.000	1.000	1.000
	8	0.549	322	1.000	1.000	1.000
	9	0.608	261	1.000	0	0.500
	10	0.613	257	0	1.000	0500
	11	0.670	210	0.167	0	0.083
	12	0.672	208	0.333	0	0.167
	13	0.702	187	0	0.167	0.083

Table (3): Total number of bands/primer, polymorphic bands/primer, % of polymorphic bands and their size range (bp) from the random primers.

Primer	Total No. of bands	No. of polymorphic bands	% of polymorphic bands	Size range (bp)
OPAO2	16	14	87.5 %	1409-107
OPCO3	10	9	90 %	682-192
OPAO8	13	11	84.61 %	928-187
Total (Average)	39 (13)	34 (11.33)	87.18 %	1409-107

Table (4): Number of polymorphic loci and Genetic diversity in two bat Species based on RAPD markers of the three primers (Nei, 1973).

Species	Total scorable bands	Total of monomorphic loci (%)	Total of polymorphic loci (%)
R. aegyptiacus	20	14 (70)	6 (30)
R. hardwickei	28	9 (32)	19 (68)

Table (5): The similarity coefficients within and among the two bat species according to Nei and Li (1979).

Serial	1	2	3	4	5	6	7	8	9	10	11	12
1	1.000											
2	0.971	1.000										
3	0.875	0.909	1.000									
4	0.909	0.941	0.968	1.000								
5	0.875	0.909	1.000	0.968	1.000							
6	0.824	0.857	0.938	0.909	0.938	1.000						
7	0.375	0.364	0.400	0.387	0.400	0.452	1.000					
8	0.353	0.343	0.375	0.364	0.375	0.412	0.813	1.000				
9	0.438	0.424	0.400	0.387	0.400	0.375	0.800	0.750	1.000			
10	0.375	0.353	0.387	0.375	0.387	0.424	0.710	0.667	0.774	1.000		
11	0.500	0.485	0.467	0.452	0.467	0.438	0.800	0.688	0.933	0.77	1.000	
12	0.389	0.378	0.400	0.400	0.412	0.444	0.706	0.667	0.706	0.743	0.765	1.000

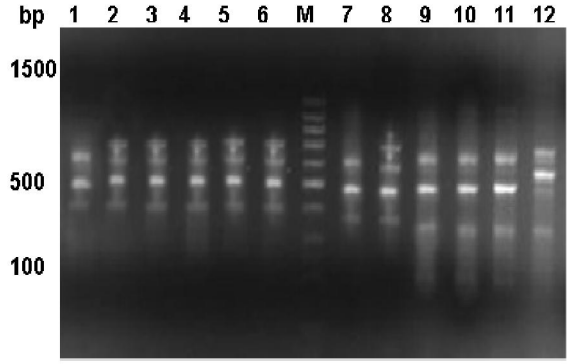

Figure 1: RAPD profiles showing DNA fingerprint patterns with DNA from 1 of 12 with OPCO3 primer. Gel electrophoresis represents PCR products for DNA from samples of *Rousettus aegyptiacus aegyptiacus* (Lanes 1 to 6). Lanes 7 to 12 represent samples of *Rhinopoma hardwickei arabium*. The DNA marker present in lane 7 with molecular size, 100 bp ladder.

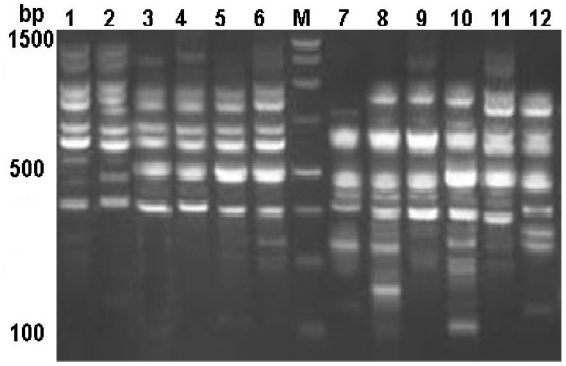

Figure 2: RAPD profiles showing DNA fingerprint patterns with DNA from 1 of 12 with OPAO2 primer. Gel electrophoresis represents PCR products for DNA from samples of *Rousettus aegyptiacus aegyptiacus* (Lanes 1 to 6). Lanes 7 to 12 represent samples of *Rhinopoma hardwickei arabium*. The DNA marker present in lane 7 with molecular size, 100 bp ladder.

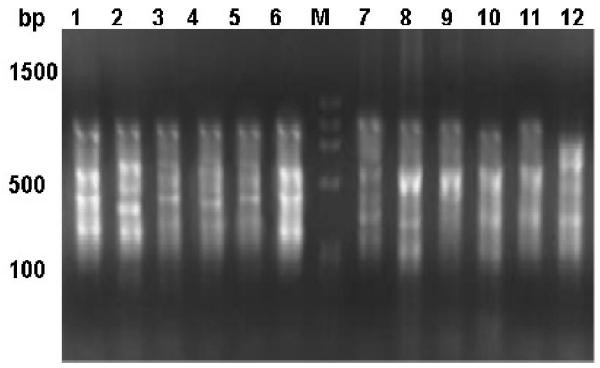

Figure 3: RAPD profiles showing DNA fingerprint patterns with DNA from 1 of 12 with OPAO8 primer. Gel electrophoresis represents PCR products for DNA from samples of *Rousettus aegyptiacus aegyptiacus* (Lanes 1 to 6). Lanes 7 to 12 represent samples of *Rhinopoma hardwickei arabium*. The DNA marker present in lane 7 with molecular size, 100 bp ladder.

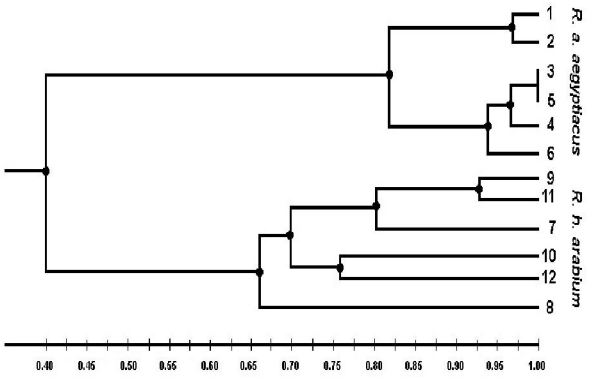

Figure 4: UPGMA based Dendrogram showing phylogenetic relationships among *Rousettus aegyptiacus aegyptiacus* (1-6) and *Rhinopoma hardwickei arabium* (7-12) based on RAPD-PCR by OPAO2, OPAO8 and OPCO3 primers.

4. Discussion

In the present study, the intra- and inter-specific genomic polymorphisms in two bat species, *Rousettus aegyptiacus aegyptiacus* and *Rhinopoma hardwickei arabium* were analyzed by using RAPD-PCR technique. The molecular technique RAPD-PCR analysis was used mostly to differentiate the genomes of the closely related species in order to determine the genetic distance and genetic diversity (**Welsh and McCelland, 1990; Bowditch *et al*., 1993; Karuppudurai and Sripathi, 2010**). Different molecular genetic technique like microsatellite markers; *cyt* b; mitochondrial DNA and protein polymorphism were carried out to resolve the genetic relationship between and within the individuals of *Rousettus aegyptiacus* and *Rhinopoma hardwickei* species (**Alvarez *et al*., 1999; Juste *et al*., 2002; Hulva and Horacek, 2002; Hulva *et al*., 2007; Goodman *et al*., 2010; Lamb *et al*., 2011**).

Bats are the second largest order of mammals, after rodents (**Cantrell *et al*., 2008**). Nowadays, the families Rhinopomatidae and Pteropodidae are belong to the suborder Yinpterochiroptera (**Teeling *et al*., 2000 and 2005; Hulva *et al*., 2007**). In the traditional view, Rhinopomatidae was regarded as the most primitive group of extant bats and considered as the closest to the common ancestor of microbats and megabats (**Van Valen, 1979; Eicki *et al*., 2005; Hulva *et al*., 2007**).

The primer OPAO2 produces a large number of amplified fragments of genomic DNA in the two species of the bats compared to the other primers OPAO8 and OPCO3 (Refer to Figs. 1, 2 & 3 and Table 2). As demonstrated by **Dinesh *et al.* (1995)**, primers with high G+C bases contents generate more amplified products.

The results showed that the number of amplified bands for the two bats were 39 bands, 34 of them were polymorphic (87.18 %). The percentage of the polymorphic loci within the samples of *Rousettus aegyptiacus aegyptiacus* was 15.38 %. The low genetic variation within the samples of *Rousettus aegyptiacus aegyptiacus* in the present study was previously reported among other members of Pteropodidae family. **Sinclair *et al.* (1996)** reported very little genetic variation among samples of the fruit bat, *Pteropus scapulatus* (% 5) by using RAPD technique and allozyme electrophoresis. **Webb and Tidemann (1996)** found low genetic variation within the fruit bats, *Pteropus scapulatus* (0.028); *Pteropus alecto* (0.023) and *Pteropus poliocephalus* (0.014) by using protein electrophoresis. Add to that, **Bastian *et al.* (2001)** found very small genetic divergence between the samples of *Rousettus amplexicaudatus, Eonycteris spelaea* and *Cynopterus brachyotis* in the Philippines by using DNA sequences of the cytochrome b gene. Also, **Campbell *et al.* (2007)** reported that the mean genetic distance among populations of fruit bat, *Cynopterus brachyotis* was low (2.7 %) and mean genetic distance among samples of the fruit bat *Thoopterus nigrescens* was (7.9 %) by using cytochrome b and microsatellite. On the other hand, **Karuppudurai *et al.* (2007)** presented 73.77 % polymorphic bands and considerable genetic diversity (51.4 %) between the species of short nosed fruit bat *Cynopterus sphinx* from different zonal population by using RAPD-PCR analysis. Also, **Andrianaivoarivelo (2009)** found polymorphic loci ranging between 0.757 and 0.916 in 37 individuals from single population of *Rousettus*

madagascariensis by using microsatellite loci. **Goodman *et al*. (2010)** found that, the high genetic diversity within the *Rousettus madagascaruensis* was 0.901 and *Rousettus obliviosus* was 0.740 in Indian Ocean islands by using cyt-b and microsatellite loci. Moreover, they reported that the monophyly group of *Rousettus leschenaultia, Rousettus madagascaruensis* and *Rousettus obliviosus* were strongly supported as sisters to each other (70% of the phylogenetic tree).

In the present work, the samples of *Rhinopoma hardwickei arabium* showed high polymeric loci (48.72 %). These results may indicate that, the samples of *Rhinopoma hardwickei arabium* may be migrated from various populations and had random mating (hybrid origin) (panmictic) within an interbreeding population **(Rivers *et al*., 2005)** and alteration of their habitat). Another possible cause for such variation in *Rhinopoma hardwickei arabium* samples due to low population sampling which confirms high genetic divergence **(Chan *et al*., 2011)**. **Hulva *et al*. (2007)** found deep genetic divergences within *Rhinopoma hardwickei* lineages of Upper Egypt, northern Libya, Jordan, Syria, Yemen and Iran by using cytochrome b technique. Also, they found no genetic difference in *Rhinopoma hardwickei cystops* between Upper Egypt and northern Libya but there is low genetic distance in *Rhinopoma hardwickei arabium* between Jordan, Syria and Yemen (0.5%). Moreover, the genetic distance between the Iranian species (*Rhinopoma hardwickei hardwickei*) and the Jordan, Syria, Yemen, Upper Egypt and Libya species showed high variation in the genetic distance (8-9%).

On the other hand, **Van Cakenberghe and De Vree (1994)** showed that the Iranian specimens, greater mouse-tailed bat (*Rhinopoma microphylum harrisoni*) differ from Levant and Moroccan specimens (*Rhinopoma microphylum microphylum*). On the other hand, **Hulva *et al*. (2007)** and **Levin *et al*. (2008)** found a high genetic similarity (low genetic diversity) within the greater mouse-tailed bat, *Rhinopoma microphyllum* from several populations by using mitochondrial markers.

The genetic distance and genetic similarity between the *Rousettus aegyptiacus aegyptiacus* and *Rhinopoma hardwickei arabium* are 0.596 (59.6%) and 0.404 (40%) respectively. The low genetic similarity and the high significant genetic distance between the two species indicate that the two species are separated from each other. According to **Baker *et al*. (2006)** the two species were distinct and separate from each other because the genetic distance is greater than 5% between the two populations. This outcome, is verified with UPGMA dendrogram which separated into two clusters that, quite expected in the light of traditional systematics is in good concordance with our recent understanding of relationships between these clades as first indicated by **Kirsch *et al*., 1995; Teeling *et al*., 2002**)

In conclusion, the RAPD bands profile of the *Rousettus aegyptiacus* and *Rhinopoma hardwickei* were clear and sharp. And, the variation in bands number and shape between and within the species may indicate the hybrid origin (mating) of these strains and alteration of their habitat. From these results, the two species may have the same origin and separated from each other into two closely related clads.

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