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Genetic Study on *Apodemus avicennicus* and *Apodemus witherbyi* by RAPD-PCR

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Recently, some specimens of genus *Apodemus* were found from Shirkooh Mountains of central plateau of Iran. In order to certify morphologic and morphometric studies, a study has been carried out on the genome of these specimens using random amplified polymorphism DNA (RAPD) marker. Nine random primers were used. The analysis of data determines the separation of Avicenna's wood mice as a new species. Probably, at the time of ice age, this group of real mouse migrated to central part of Iran. Finally, with the changing weather conditions and appearances of wide deserts around central part of Iran, this group was surrounded there and speciation had occurred.

Key words*: A. avecinnicus*, Shirkooh, RAPD, Speciation

INTRODUCTION

The genus *Apodemus* (Kaup, 1829) consists of 21 currently recognized species (Musser and Carleton, 2005). These species are widely distributed in the temperate zone of the Palearctic region, in Europe, Asia, and Northern Africa (Juskeviciute et al, 2002). Before this, three species of this genus have been found in Iran. These are *A. witherbyi* (=*A. hermonensis* Musser and Carleton, 2005) from Alborz and Zagros mountains in North and West part of Iran, *A. flavicollis* from West of Iran, and *A. hircanicus* from North of Iran (Macholan et al, 2001). For the first time, Darvish (2006) found some specimens of this genus from Shirkooh Mountains of central plateau of Iran. These specimens were very different from other species of this genus which were recorded from Iran on the base of morphologic and morphometric studies. It was named *A. avecinnicus* as a new species (Darvish, 2006). In this study, the presence of this species has been determined using the RAPD marker. RAPD is a multilocus technique which allows obtaining information on the general polymorphism of a genome. Low expense, high efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment, the simplicity and applicability, requirement of small amount of DNA without the requirement of cloning, sequencing or any other form of the molecular characterization of the genome has made the RAPD technique valuable (Bardakci, 2001; Williams et al, 1990).

MATERIAL AND METHODS

Captured specimens were compared with specimens from Alborz and Zagros mountains (Fig.1). Genomic DNA was extracted from 100% ethanol preserved liver or kidney of 11 *A. avicennicus* and six *A. witherbyi* using a genomic DNA purification kit (DNA tissue kit, BILATEST) (Hofstetter et al, 1997; Desall et al. 2001).

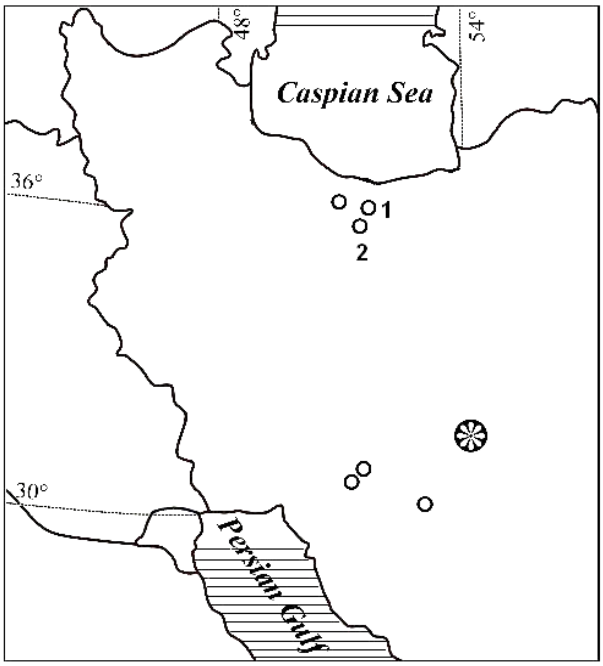

FIG. 1.- Specimen locality, 1 and 2 are indicated with open circle for *A. witherbyi(hermonensis)*; 1- Abnik; 2- Sorkhehesar National Park, Asterisk denotes the geographic place of Fakhrabad village, the type locality of *A. avicennicus* n. sp. (Map after Darvish et al).

The isolated DNA was amplified using nine primers (Table1). Roth-180 set of primers were chosen (Sinagene, Iran) (Juskeviciute, 2002). PCR reactions were performed in a volume of 25 µl containing 2.5 µl PCR buffer (10x), 25 mM $MgCl_2$, and 10 mM dNTPs, and 10µM primer, 14 ng of genomic DNA and 1 unit of *Taq* polymerase (Kohler, 2000). Amplification was done with a programmable thermal cycler (Primus 96 advanced Gradient, Peqlab, Germany) under following conditions: 90˝ at 94° C, 45 cycles (30˝ at 94° C, 60˝ at 42° C, 120˝ at 72° C), with a final extension of 10 min at 72°C (Kohler, 2000). The amplified fragments were separated on 2% agarose gels and stained with ethidium bromide and photographed under UV light. Fragment sizes were estimated by comparison with a 100 bp DNA ladder. Bands were distinguished by Labworks software. The data were used to compute the genetic distance of species. PopGene was used to carry out a cluster analysis by the UPGMA method and to construct the dendrogram.

TABLE 1.- Primer sequences

Name of primer	*Sequence*
ROTH-180-01	5'-GCACCCGACG-3'
ROTH-180-02	5'-CGCCCAAGC-3'
ROTH-180-03	5'-CCATGGCGCC-3'
ROTH-180-04	5'-CGCCGATCC-3'
ROTH-180-05	5'-ACCCCAGCCG-3'
ROTH-180-06	5'-GCACGCCGGGA-3'
ROTH-180-08	5'-CGCCCTCAGC-3'
ROTH-180-09	5'-GCACGGTGGG-3'
ROTH-180-10	5'-CGCCCTGGTC-3'

RESULT AND DISCUSSION

Juskeviciute (2002) used this set of primers to show genetic diversity among three *Apodemus* species (*A. flavicollis*, *A. uralensis* and *A. agrarius*). They suggested the possibility of using the RAPD method for species identification because the differences among the species were distinct.

In this study, DNA extracted from mice of genus *Apodemus* was amplified using nine random primers. The amplified DNA of *Tatera indica* was used as an outgroup. Each primer provided a

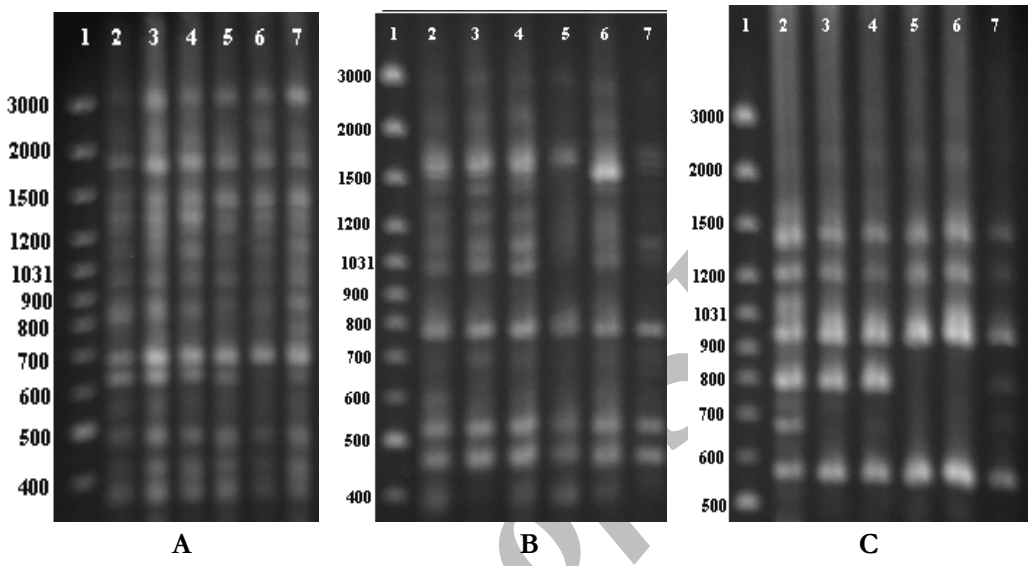

FIG.1.- Three samples of electrophoresis gels of amplified DNA fragments (a- Roth-180-01, b- Roth-180-02 and c- Roth-180-04) in 2% agarose gel. 1-100 bp DNA ladder, 2, 3, 4 - *A. avicennicus*, 5, 6, 7 *A. witherbyi*.

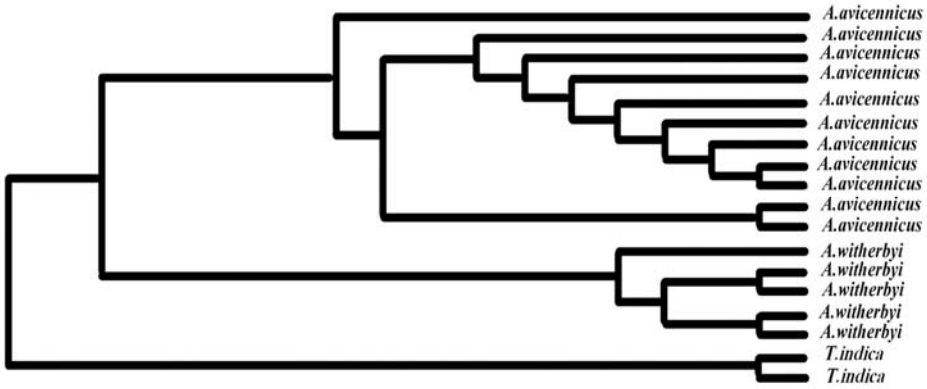

FIG. 2.- Dendrogram showing genetic relation among the *Apodemus* species and outgroup based on RAPD data.

distinct pattern of amplified fragments. The number of fragments and the amount of intraspecific polymorphism were varied among the primers (Fig.2). However, there were several common bands between these two species. Genetic distance between the species based on RAPD data was varied between 0.011 and 0.472 (Table 2). Genetic distance from RAPD data was used to draw the dendrogram (Fig.3). The structure of this tree verifies the results of morphology and morphometry analysis and shows the separation of *A. avicennicus* from very similar species *A. witherbyi*.

Probably, at the time of ice age, this group of real mouse had migrated to central part of Iran. Finally, with changing weather conditions and appearances of wide deserts around central part of Iran, this group has been surrounded there and speciation has been occurred.

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TABLE 2.- Genetic distance among *Apodemus* species (lower triangle) and genetic similarity (upper triangle).

	A.avicennicus1	*A.avicennicus2*	*A.avicennicus3*	*A.avicennicus4*	*A.avicennicus5*	*A.avicennicus6*	*A.avicennicus7*	*A.avicennicus8*	*A.avicennicus9*	*A.avicennicus10*	*A.avicennicus11*	*A.witherbyi1*	*A.witherbyi2*	*A.witherbyi3*	*A.witherbyi4*	*A.witherbyi5*	*T.indica1*	*T.indica2*
A.avicennicus1	***	0.903	0.777	0.773	.803	.781	0.747	0.788	0.818	0.825	0.766	0.684	0.706	0.673	0.624	0.643	0.584	0.584
A.avicennicus2	0.102	***	0.829	0.825	0.855	0.788	0.732	0.758	0.833	0.825	0.743	0.714	0.743	0.703	0.639	0.680	0.613	0.613
A.avicennicus3	0.252	0.187	***	0.989	0.907	0.714	0.680	0.714	0.780	0.766	0.654	0.818	0.803	0.747	0.684	0.740	0.680	0.680
A.avicennicus4	0.257	0.192	0.112	***	0.911	0.717	0.684	0.717	0.784	0.769	0.658	0.814	0.807	0.758	0.695	0.751	0.691	0.691
A.avicennicus5	0.219	0.157	0.097	0.093	***	0.777	0.729	0.740	0.807	0.799	0.695	0.747	0.777	0.706	0.643	0.706	0.647	0.624
A.avicennicus6	0.248	0.238	0.337	0.332	0.252	***	0.848	0.799	0.836	0.836	0.807	0.665	0.695	0.677	0.643	0.669	0.632	0.610
A.avicennicus7	0.291	0.311	0.385	0.380	0.317	0.165	***	0.840	0.825	0.818	0.825	0.640	0.684	0.680	0.654	0.636	0.636	0.606
A.avicennicus8	0.238	0.277	0.337	0.332	0.301	0.224	0.174	***	0.851	0.851	0.844	0.658	0.665	0.654	0.651	0.617	0.632	0.617
A.avicennicus9	0.201	0.183	0.248	0.243	0.215	0.179	0.192	0.161	***	0.896	0.807	0.703	0.732	0.699	0.643	0.669	0.647	0.610
A.avicennicus10	0.192	0.192	0.267	0.262	0.224	0.179	0.201	0.161	0.110	***	0.799	0.717	0.725	0.684	0.665	0.662	0.610	0.610
A.avicennicus11	0.267	0.296	0.424	0.419	0.364	0.215	0.192	0.170	0.215	0.224	***	0.613	0.628	0.632	0.606	0.624	0.610	0.587
A.witherbyi1	0.380	0.337	0.201	0.206	0.291	0.407	0.447	0.419	0.353	0.332	0.489	***	0.747	0.714	0.673	0.706	0.639	0.639
A.witherbyi2	0.348	0.296	0.219	0.215	0.252	0.364	0.380	0.407	0.311	0.322	0.465	0.219	***	0.773	0.673	0.736	0.647	0.610
A.witherbyi3	0.396	0.353	0.291	0.277	0.348	0.391	0.385	0.424	0.358	0.380	0.459	0.337	0.257	***	0.766	0.769	0.665	0.621
A.witherbyi4	0.471	0.447	0.380	0.364	0.441	0.441	0.424	0.430	0.441	0.407	0.501	0.396	0.396	0.267	***	0.788	0.610	0.624
A.witherbyi5	0.441	0.385	0.301	0.286	0.348	0.402	0.453	0.483	0.402	0.413	0.471	0.348	0.306	0.262	0.238	***	0.628	0.598
T.indica1	0.538	0.489	0.385	0.369	0.436	0.459	0.453	0.459	0.436	0.495	0.495	0.447	0.436	0.407	0.495	0.465	***	0.769