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Genetic variability of the Saudi Arabian *Uromastyx aegyptia microlepis* using protein and isoenzymes electrophoreses

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ABSTRACT

Electrophoresis for SDS-proteins and isoenzymes were conducted to investigate the genetic variations within the agamid lizard *Uromastyx aegyptia microlepis* inhabiting the desert of Saudi Arabia. Samples were collected from four localities: a) Ushayrah near the town of Taif, b) Al Gwaih near Riyadh, c) Nairyah near Dammam and d) Mouileh near Tabok. A range of 7 to 14 protein bands were recorded in the patterns of the studied samples as measured by SDS-polyacrylamide gel electrophoresis. Among them, only one fraction was recorded in all samples as a common protein band. Six arbitrary chosen enzymes were examined by native-polyacrylamide gel electrophoresis. They were *α* and *β* esterase (*Est*), acid phosphatase (*Acph*), Alcohol dehydrogenase (*Adh*), Aldehyde oxidase (*Ao*) and peroxidase (*Px*). Seventeen heterogeneous alleles have been recorded; seven of them were fixed in all populations and 10 were polymorphic. Nearly all recorded alleles were monomeric in all samples. *α-Est2*, *β-Est2*, *Acph2* and *Px1* were restricted to Tabok samples and were not recorded in other localities. *β-Est3*, *Acph3*, *Adh1*, *Adh1*and *Px2* were not recorded in Taif samples and the latter one was not recorded in the Dammam samples. The similarity coefficient that has been calculated according to the number of sharing bands indicated the clustering of Tabok and Dammam populations together. The constructed tree based on the sharing protein bands and isozyme alleles revealed similar results regarding the kinship of both populations. The present results indicated that the populations of this subspecies exhibits high genetic variability among its populations.

Keywords: Uromastyx; Isoenzymes; Population Genetics; Arabia

1. INTRODUCTION

Spiny-tailed *Uromastyx* are small to medium-sized, ground-or rock-dwelling agamid lizards. The animals have a bulky, depressed body and strong, short limbs. The tail is covered by spiny scales, arranged in distinct whorls. *Uromastyx* habitats are generally characterized by high temperatures, low precipitation, sparse vegetation and marked seasonal changes. A detailed discussion of the taxonomic history of the *Uromastyx aegyptia* group is provided that is now recognized as three valid species (*U. aegyptia*, *U. leptieni* and *U. occidentalis*) [1]. However, *Uromastyx aegyptia* has been recognized as a polytypic species with three subspecies (*aegyptia*, *leptieni* and *microlepis*) [2]. The main characters to distinguish the members of the *Uromastyx aegyptia* group from other *Uromastyx* species groups are their large body size combined with very small body scales. The diagnostic features to distinguish the members of the *Uromastyx aegyptia* group are the lack of enlarged tubercular scales on the flanks and the presence of skin folds at the sides of the neck covered with tubercles in *U. a. microlepis* [3]. *U. a. microlepis* was also suggested to be a valid subspecies by many authors [3-7]. It is found in all Arabia and Iraq, commonly, amongst populations of *U.a. aegyptia*. Their potential contact zone is at the east of Wadi Araba (Jordan and Palestine) and at the east of Wadi Sawawin in the Jebel as-Sinfa region (Tabok) where members of both subspecies are found sympatrically, while taxa from Eastern Arabia are exclusively belonging to *U. a. microlepis* [3]. Recent molecular studies investigated the systematics of *Uromastyx* [8-12] but few biochemical studies have been conducted [12,5]. The present investigation aims to study the electrophoretic behavior of several isoenzymes and proteins for *U. a. microlepis* from different localities of its range in order to address the genetic variability within this subspecies.

2. MATERIALS AND METHODS

2.1. Samples

At least, three samples of *U. a. microlepis* were collected

from each of four localities encompassing the different ecological habitats of the Saudi Arabian desert (**Figure 1**). These localities were Ushayrah, 70 km northeast to Taif, AlGwaih 200 km west to Riyadh, Nairyah 200 km west to Dammam and Mouileh 250 km Southwest to Tabok.

Animals were taken to the lab, killed and dissected. Samples of blood and muscle tissues were taken and immediately frozen at - 80°C.

2.2. SDS-Protein

SDS-polyacrylamide gel electrophoresis was performed in 14 % acrylamide slab gels following the system of [13]. Protein extraction was conducted by homogenizing 1.0 g of tissue in 2 ml saline solution NaCl (0.9%) using a manual homogenizer. Samples were centrifuged at 5000 rpm for 10 min. The supernatants that contain protein extract were kept deep-frozen until use for electrophoresis analysis. A volume of 20 µL protein extract was added to 10 µL of treatment buffer. 20 µL of the mixture was loaded in the gel. After electrophoresis, the gel was stained by commassie brilliant blue. The gel was destained after the appearance of bands and photographed.

2.3. Isoenzymes

Six isoenzymes were used in this study: α and β-esterase (*Est*), acid phosphatase (*Acph*), alcohol dehydrogenase (*Adh*), aldehyde oxidase (*Ao*) and peroxidase (*Px*). Isoenzymes were electrophoresed in 10% native-polyacrylamide gel as described by Stegemann *et al.* [14]. For isoenzyme extraction, 1.0 g of tissue was homogenized in 2 mL saline solution NaCl (0.9%) using a manual homogenizer. The homogenates were centrifuged at 5000 rpm for 10 minutes and the supernatants were kept at -20°C until use. For electrophoresis, 30 µL of the extract was mixed with 10 µL of treatment bufferand 35 µL of this mixture was applied to the well. In gel staining, protocols published for α and *β-Est, Ao* and *Acph, Adh* and *Px* were used [15-18]. Gels were washed two or three times with tap water; fixed in ethanol/ 20% glacial acetic acid (9:11 v/v) for 24 hours and photographed.

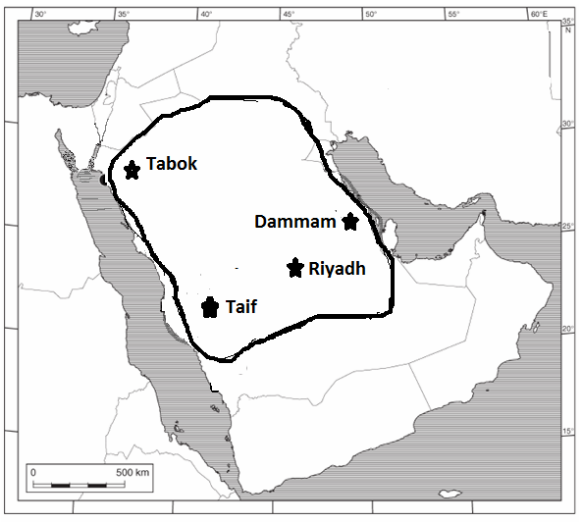

Figure 1. A map with sample sites indicated (stars) and the entire distribution (circled area) of *Uromastyx aegyptia microlepis.*

2.4. Statistics

All gels of protein and isozyme electrophoresis were scanned using Gel Doc-2001 Bio-Rad system. For isoenzymes, the bands of enzyme activity were designated using the known system of nomenclature [19]. An abbreviation which corresponds to the name of the enzyme designated each locus. When multiple loci were involved, the fastest anodal protein band was designated as locus one, the next as locus two and so on. A similarity matrix of allele presence (1) and absence (0) was constructed and the dendrogram was obtained by the UPGMA method from NTSYS-pc package [20]. The similarity coefficient was calculated [21,22] as follows: 2 (number of sharing bands)/ number of bands in population A + number of bands in population B.

3. RESULTS AND DISCUSSION

The number of protein bands that were obtained from this study varied between and within populations. A range of 7 to 14 bands were recognized in the pattern of the studied populations with minimum numbers in the samples of Taif and Riyadh and maximum numbers in Tabok (12 bands) and Dammam populations (12 bands) as measured by SDS-polyacrylamide electrophoresis (Table 1). The molecular weights of these bands showed a wide range between a maximum of 89 kDa and a minimum of 14 kDa. All samples studied recorded one shared band with a molecular weight of 33.534 kDa. There were no shared bands between populations at the extremes of the protein profile. The number of shared bands were 4, 9, 5 and 11 within Taif, Dammam, Riyadh and Tabok populations, respectively. When the different localities were compared, the number of shared bands were 3 between Taif and Dammam populations, 2 between Taif and Riyadh populations, 4 between Taif and Tabok populations, 4 between Dammam and Riyadh populations, 9 between Dammam and Tabok populations and 5 between Riyadh and Tabok populations. Three unique bands were found in the Taif population at the molecular weight of 89, 88 and 60 kDa and two unique bands were shown in the Dammam population at 68 and 14 kDa. The electrophoretic banding of proteins indicated that the populations of the present study have similar bands within the molecular weight of the range be-

tween 73 and 63 kDa (as shown in **Table 1**) and in the range between 46 and 16 kDa. The former range is comparable to vertebrate albumin (65 -70 kDa) [23,24] that is considered as a good indicator for species differentiation [25]. These similarities, therefore, could be considered as an indication of gene flow among the studied samples and do not refer to any fragmentation. The similarity coefficients that were calculated according to the number of sharing protein bands are shown in **Table 2**.

The Tabok population recorded the highest similarity to the Dammam population with a coefficient of 0.69 and to the Riyadh population with a coefficient of 0.48. The similarity coefficients between the Taif population and each of the three other populations were 0.32, 0.29 and 0.38 for the Dammam, Riyadh and Tabok populations, respectively. The elctrophoretic behavior of the six studied isoenzymes was shown as alleles and their relative mobility in **Tables 3 to 8**. Seventeen presumptive heterogeneous alleles have been recorded in the present study. Among the twelve *microlepis* samples studied herein, the *α* and *β-Est*, *Acph, Ao* and *Px* isozymes recorded three putative genotypes and three allelic products for each (**Tables 3-5, 7, 8**). All genotypes were identified as monomers (the complete enzyme consists of only one polypeptide) with one allele. *Adh* isoezyme was identified by two genotypes with two monomeric alleles (**Table 6**). Seven alleles for *α-Est3*, *β-Est1*, *Acph1, Adh2, Ao1, Ao3* and *Px3* were shown to be fixed in all samples. The samples from Tabok recorded the highest diversity of phenotypes where *α-Est2, β-Est2, Acph2* and *Px1* were characteristic to this population and were not recorded in other populations. On the other hand, Taif samples were shown to be the lowest in phenotypic diversity where *β-Est3*, *Acph3*, *Adh1*, *Adh1* and *Px2* were not recorded in this population. The samples from the vicinity of Tabok were collected from Mouileh (sandy desert) which is located close to the Red Sea in the northwest of the Kingdom and is found in sympatry with *U. a. aegyptia*. On the other hands, Taif is located in the Sarawat mountains (2000-3000 m altitude) with rocky nature that could have exerted some effect on the genetic variability of the population inhabiting this area.

The heterogeneity of the isozyme electrophoreses among populations was also revealed in earlier investigations. Esterase and alcohol dehydrogenase showed four fractions in their loci and these fractions were proposed to be obtained by the combinations of trimer polypeptides [26]. A trimeric structure was reported for esterase in human, pig, rat, and guinea pig [27]. Similar to the present study, multiple forms were reported for aldehyde oxidase in *Uromastyx* and other vertebrates [12].

The constructed dendrogram (**Figure 2(a)**) showed clustering of Tabok, Dammam and Riyadh populations. The Taif population came basal in the dendrogram since

Table 2. The estimated similarity coefficients calculated according to the shared protein bands among the studied populations.

	Taif	Dammam	Riyadh	Tabok
Taif		0.32	0.29	0.38
Dammam			0.42	0.69
Riyadh				0.48

Table 1. Protein band molecular weights measured in kDa in samples of the different populations studied.

MW	**Taif** (Ushayrah)			**Dammam** (Nairyah)		**Riyadh** (AlGwaih)		**Tabok** (Mouileh)	
	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9
89.463	-	-	-	-	-	-	-	-	+
88.218	-	-	-	-	-	-	-	-	+
85.951	-	-	-	+	+	-	-	+	+
83.910	-	-	-	+	+	-	-	+	+
74.559	+	+	+	+	+	-	+	+	+
68.134	-	-	-	-	+	-	-	-	-
63.015	+	+	+	-	+	+	+	+	+
60.784	-	-	-	-	-	-	-	-	+
46.946	+	+	+	+	+	+	-	+	+
42.303	+	-	-	+	+	-	-	+	+
33.534	+	+	+	+	+	+	+	+	+
29.382	+	-	+	+	+	+	+	+	+
22.422	-	+	+	+	+	+	+	+	+
16.471	-	+	+	+	+	+	+	+	+
14.636	-	-	-	+	-	-	-	+	-

Table 3. The recorded phenotypes and the relative mobility (RF) for the electrophoretic pattern of *α-Est* isoenzyme in the studied populations of *U. a. microlepis*. Lanes are as follow: 1-4 (Taif samples), 5-6 (Dammam samples), 7-9 (Riyadh samples) and 10-12 (Tabok samples).

RF	Alleles	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10	Lane11	Lane12
0.033	*α-Est3*	+	+	+	+	+	+	+	+	+	+	+	+
0.890	*α-Est2*	-	-	-	-	-	-	-	-	-	-	+	-
0.946	*α-Est1*	+	+	-	-	-	-	-	-	+	+	+	+

Table 4. The recorded phenotypes and the relative mobility (RF) for the electrophoretic pattern of *β-Est* isoenzyme in the studied populations of *U. a. microlepis*. Lanes are as follow: 1-4 (Taif samples), 5-6 (Dammam samples), 7-9 (Riyadh samples) and 10-12 (Tabok samples).

RF	Alleles	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10	Lane11	Lane12
0.024	*β-Est3*	-	-	-	-	+	+	-	+	-	+	+	+
0.867	*β-Est2*	-	-	-	-	-	-	-	-	-	-	+	-
0.917	*β-Est1*	+	+	+	+	+	+	+	+	+	+	+	+

Table 5. The recorded phenotypes and the relative mobility (RF) for the electrophoretic pattern of *Acph* isoenzyme in the studied populations of *U. a. microlepis*. Lanes are as follow: 1-4 (Taif samples), 5-6 (Dammam samples), 7-9 (Riyadh samples) and 10-12 (Tabok samples).

RF	Alleles	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10	Lane11	Lane12
0.028	*Acph3*	-	-	-	+	+	+	-	+	-	+	+	+
0.858	*Acph1*	-	-	-	-	-	-	-	-	-	-	+	-
0.931	*Acph1*	+	+	+	+	+	+	+	+	+	+	+	+

Table 6. The recorded phenotypes and the relative mobility (RF) for the electrophoretic pattern of *Adh* isoenzyme in the studied populations of *U. a. microlepis*. Lanes are as follows: 1-4 (Taif samples), 5-6 (Dammam samples), 7-9 (Riyadh samples) and 10-12 (Tabok samples).

RF	Alleles	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10	Lane11	Lane12
0.029	*Adh2*	+	+	+	+	+	+	+	+	+	+	+	+
0.061	*Adh1*	-	-	-	+	+	+	+	+	+	+	+	+

Table 7. The recorded phenotypes and the relative mobility (RF) for the electrophoretic pattern of *Ao* isoenzyme in the studied populations of *U. a. microlepis*. Lanes are as follow: 1-4 (Taif samples), 5-6 (Dammam samples), 7-9 (Riyadh samples) and 10-12 (Tabok samples).

RF	Alleles	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10	Lane11	Lane12
0.030	*Ao3*	+	+	+	+	+	+	+	+	+	+	+	+
0.060	*Ao2*	-	-	-	-	+	+	+	+	+	+	+	+
0.082	*Ao1*	+	+	+	+	+	+	+	+	+	+	+	+

Table 8. The recorded phenotypes and the relative mobility (RF) for the electrophoretic pattern of *Px* isoenzyme in the studied populations of *U. a. microlepis*. Lanes are as follow: 1-4 (Taif samples), 5-6 (Dammam samples), 7-9 (Riyadh samples) and 10-12 (Tabok samples).

RF	Alleles	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10	Lane11	Lane12
0.634	*Px3*	+	+	+	+	+	+	+	+	+	+	+	+
0.830	*Px2*	-	-	-	-	-	-	+	+	+	+	+	-
0.926	*Px1*	-	-	-	-	-	-	-	-	-	-	+	-

it showed the lowest similarities to the other populations (**Table 2**). The application of the UPGMA clustering on the raw data obtained from isoenzymes and SDS proteins split the populations (**Figure 2(b)**) into two main clusters: the populations of Taif and AlGwaih (Riyadh) as one group and the populations of Tabok and Dam-

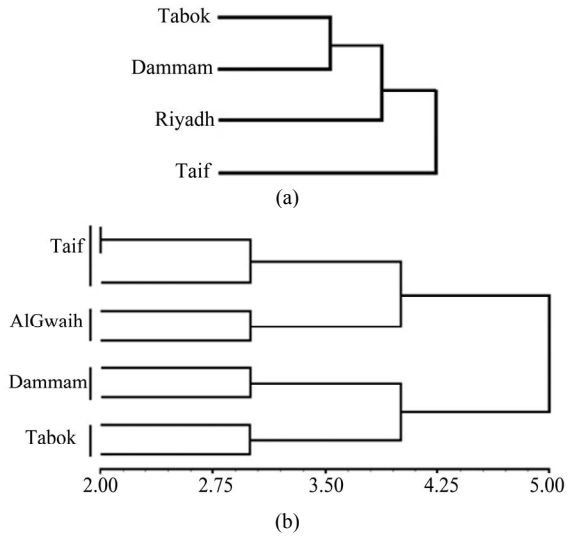

Figure 2. Dendrogram based on similarity coefficients (A) and UPGMA (B) analysis of genetic similarity obtained from isozyme and protein data showing relationships among the studied populations of *U. a. microlepis*.

mam as another group. By comparing this tree to that of **Figure 2(a)**, it was obvious that there was a concordance regarding the kinship of Tabok and Dammam populations. The disconcordance between the two dendrograms was found in the relationship of both Taif and Riyadh populations. This could be attributed to the extremes in the raw data applied for constructing **Figure 2(b)**. Both results indicated that *U. a. microlepis* have a wide genetic variability between populations that are explained by adaptations to different environmental conditions. *Uromastyx a. microlepis* from Taif inhabits mountainous areas and habitats with thick layers of stones and rocks which are not suitable for this subspecies [28]. The common habitats for other populations are the open areas with hard, diggable substrates like coarse sand, gravels and sparse vegetation [29].

4. CONCLUSION

I conclude that protein and isoenzyme electrophoreses is a powerful tool in targeting the genetic variability within the *Uromastyx aegyptia microlepis* populations. This technique provides for further molecular investigations that are necessary to address the genetic differentiation of this subspecies more accurately.

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