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Preliminary Molecular Variability Within *Uromastyx aegyptia microlepis* (Reptilia: Agamidae) Inhabiting Saudi Arabia

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Abstract: Approximately 2229 nucleotides representing partial 12S ribosomal RNA, complete NADH dehydrogenase subunit 2 and partial cytochrome b genes from mitochondrial DNA and partial 18S nuclear gene were sequenced in this study. These data were used to investigate the genetic variation within *Uromastyx aegyptia microlepis* from Saudi Arabia. Nine individuals inhabiting four localities were studied and these localities were two from the west (Tabok and Taif) and two from the east (Riyadh and Dammam). The mtDNA sequences showed six substitutions (2 transversions and 4 transitions) among the different haplotypes. Two transitions among them were non-synonymous showing different amino acids. The mutations that have been found in 18S gene were six deletions in the samples of Taif possibly due to habitat difference. The observed genetic structure of *Uromastyx a. microlepis* has management implications for the conservation of this subspecies. Until more information is available, we recommend that *Uromastyx* in Saudi Arabia should not be hunted severely and should receive more attention from conservational point of view.

Key words: Mitochondrial DNA・Nuclear gene・Population・Saudi Arabia・Uromastyx

INTRODUCTION

Uromastyx aegyptia is the largest member of the spiny-tailed mastigure lizards. The Arabian subspecies *Uromastyx a. microlepis* lives in the deserts and semi-deserts of Arabia (Saudi Arabia, Yemen, Oman, United Arab Emirates, Qatar and Kuwait), Jordan, Syria, Iraq and coastal Iran. These habitats show a marked seasonality regarding climatic parameters (temperature, humidity, precipitation) as well as regarding the availability of food.

Uromastyx a. microlepis has a predilection for open habitats with hard, diggable substrates like coarse sand, gravel and sparse vegetation [1]. Mountainous areas and habitat with thick layers of stones and rocks are not suitable for *U. a. microlepis* [2-4]. The common distribution of *U. a. microlepis* in Saudi Arabia extends horizontally in the middle of the Kingdom from the West at the Red Sea costal desert to the east at the Arabian Gulf with some rocky places occupied [5].

This subspecies have been subjected to molecular, morphological [1], biochemical [6], physiological [7, 8], histochemical [9-11] and ecological [12] investigations. To the best of our knowledge, *U. a. microlepis* have not been yet subjected to molecular studies regarding its populations.

The consumption of spiny-tailed lizards in their countries of origin may be considerably higher due to the fact that *Uromastyx* are heavily hunted for food and for the production of souvenirs and traditional medicine [13]. The present study therefore, is the first molecular trial aiming to address the genetic variability within the Saudi Arabian populations of this taxon as a preliminary desired conservation.

MATERIALS AND METHODS

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.

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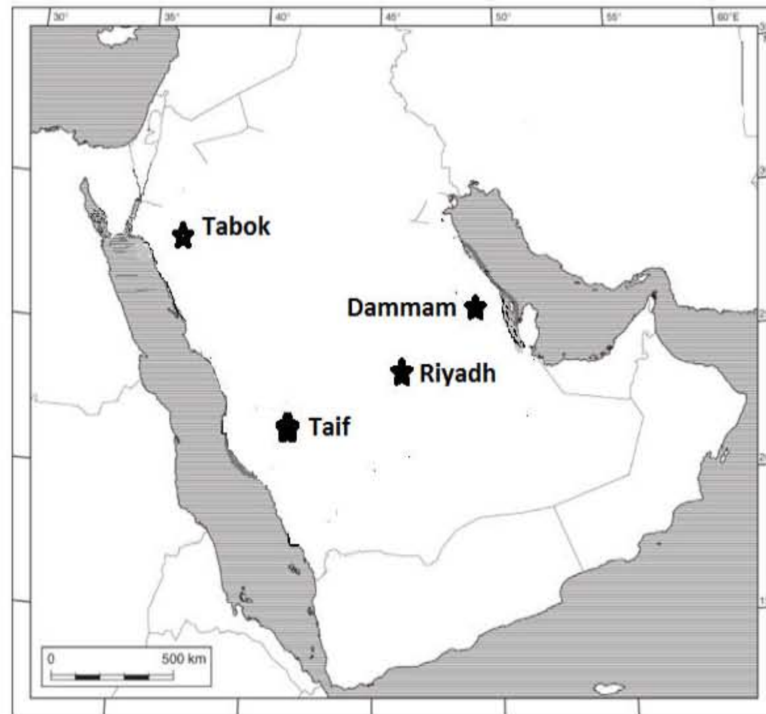

Fig. 1: A map with localities indicated as stars for the collected samples of *U. a. microlepis* in Saudi Arabia. This map was taken from the published one [5].

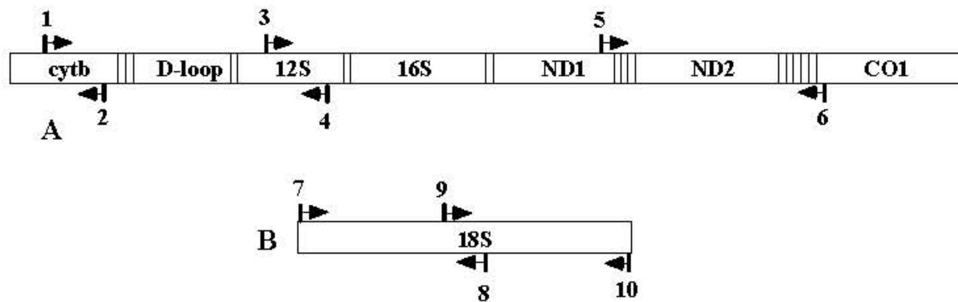

Fig. 2: Position of primers used for amplification and/or sequencing. See Table 1 for the primer sequences. Numbers of primers correspond to those in Table 1.

Table 1: Primers used for PCR amplification and/or sequencing

	Name	Sequence(5' to 3')	Source
1:	Cytb1-1	TCCAACATCTCAGCATGATGAAA	Kocher *et al.* [14]
2:	rcytb-1H	GCGTAGGCRAATAGGAAGTATCA	Kumazawa and Endo [15]
3:	r12S-1L	AGGATTAGATACCCTACTA	Kumazawa and Endo (2004)
4:	12SB-H	GAGGGTGACGGGCGGTGTGT	Kocher *et al.* [14]
5:	rND1-3L	CGATTCCGATATGACCAACT	Kumazawa and Endo [15]
6:	rCO1-3H	GTAYAGGGTGCCRATRTCTTT	Kumazawa and Endo [15]
7:	18S-L1	ACGGGCGTTACAGTGAAACT	This study
8:	18S-H1	GCCTGCTTTGAACACTCTAA	This study
9:	18S-L2	CGATGCTCTTAACTGAGTGTCCC	This study
10:	18S-H2	TAGCCAGTCAGTGAGCGC	This study

DNA Extraction, Amplification and Sequencing: DNA was extracted from 0.5ml blood samples with EZ-10 Spin Column Genomic DNA MiniPreps Kit according to the manufacturer. Extracted DNA was spectrophotmetrically quantified at 260/280nm and was used for PCR.

PCR was performed in 50 µl total volume of reaction buffer containing 0.2mM dNTPs, 1.5 mM $MgCl_2$, 2 µl of DNA solution and 0.25U of DNA *Taq*-polymerase (Invitrogen) and 0.2 µM of each primer. The positions and sequences of the primers that have been used in both amplifications and sequencing are shown in figure 2 and table 1. With respect to the 18S gene, two separate fragments at the extremes of the gene were amplified and sequenced. The reaction mixture was put into a 0.2ml thin-walled PCR tube and amplification was performed in PXE 0.5 thermal cycler (Thermo Electron Corporation Co.) with the following profile: 94°C for 5min, followed by 30 cycles of 94°C for 1min, 58°C for 1min and 72°C for 1min. A final strand elongation at 72°C was done for an additional 7min. was used for amplification.

The resultant PCR products were electrophoresed on a 1.5% agarose gel in TAE (40mM Tris, 40mM acetic acid and 1mM Ethylenediamine-tetra acetic acid) and then the gels were stained with ethidium bromide. 100bp DNA Ladder (Biolabs) was used as a marker for the molecular weight size. The PCR-products were then purified from gel with the use of QIAquick Gel Extraction Kit according to the Kit Manual.

Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using an ABI PRISM. BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq-DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer were used. Single-pass sequencing was performed on each template using the abovementioned PCR-primers. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

The obtained sequences were analyzed with the DNASIS-Mac program v3.5 (Hitachi, Tokyo). After mtDNA sequence was obtained, their features were identified by MacClade program v 4.08 (Sinauer Associates, Inc.). The obtained sequences for protein coding genes (ND2 and cytb) were aligned separately and manually and the sequences after alignment were translated into amino acid sequences using the vertebrate mitochondrial genetic code as implemented in DNASIS program. The unalignable and gap-containing sites were deleted and the aligned data were concatenated so that 1236 bp were left for the analysis. The aligned nucleotide sequences could be obtained from author for correspondence upon request. We used maximum-parsimony (MP) method with PAUP* 4.0b10 [16] by heuristic searches with the TBR branch swapping and 10 random taxon additions. We set the bootstrapping replicates to 1000 with simple additions.

RESULTS AND DISCUSSION

Sequences from mitochondrial DNA of the individuals from each locality tested were identical indicating that primers amplified the mitochondrial DNA only. All sequences used in this study will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB619807 - AB619831. In order to sample within-and between-population variation in haplotype diversity, two and in some cases more individuals per locality were sequenced for 12S rRNA, cytb and ND2 genes (mtDNA) and for 18S gene (nuclear DNA). These results constitute the first investigation of population genetics for *U. a. microlepis.*

Using 1236 unambiguous sites from ND2 and cytb genes, the base frequencies were A=34.1%, C=31.4%, G=11.6% and T=22.9%. Of these nucleotides, 1217 were constant and 19 were variables. 16 of the variable sites were parsimony-uninformative and 3 were informative under parsimony criterion. The estimated pairwise genetic distance was lower among the different haplotypes of *U. a. mic*rolepis (D range= 0.002-0.0008) compared to that between this subspecies and its conspecific *U. a. aegyptia* (D= 0.013).

Nucleotide substitutions are generally considered in terms of changes within the two structural classes of nucleotides (purines and pyrimidines), that is, in terms of transitions and transversions. 231 bp sequenced from 12S rRNA gene, in this study, did not show any difference among all samples. When this sequence was compared to the same published fragment for *U.a. aegyptia*, only one base substitution (C to T) between the two subspecies was found (data not shown).

For cytb gene, there was no difference among the samples from different localities except one synonymous base transversion (C to G) in the samples from the locality of Riyadh. The sequenced fragment of cytb gene (558 bp) showed 9 third position substitutions (5 transitions of A to G and 4 transitions of C to T) between *U.a. aegyptia* and *U. a. microlepis* and that all substitutions were

```
U.a.  aegyptia        1 LPPSAMLTFS  TGLVMSTVIV  MSSHHWLTAW  VGLELNTLSI  VPIISNPKHP      50
Riyadh                1 LPPSAMLTFS  TGLVMSTVIV  MSSHHWLTAW  VGLELNTLSI  VPIISNPKHP      50
Taif                  1 LPPSAMLTFS  TGLVMSTVIV  MSSHHWLTAW  VGLELNTLSI  VPIISNPKHP      50
Tabok1                1 LPPSAMLTFS  TGLVMSTVIV  MSSHHWLTAW  VGLELNTLSI  VPIISNPKHP      50
Tabok                 1 LPPSAMLTFS  TGLVMSTVIV  MSSHHWLTAW  VGLELNTLSI  VPIISNPKHP      50
Dammam                1 ----------  ----------  ----------  ----------  ----------      50

U.a.  aegyptia       51 RATEATMKYF  LTQAIASALL  LFSGTLNAWQ  TGQWDTTQLS  NEYACMIMTI     100
Riyadh               51 RATEATMKYF  LTQAIASALL  LFSGTLNAWQ  TGQWDTTQLN  NEYACMIMTI     100
Taif                 51 RATEATMKYF  LTQAIASALL  LFSGTLNAWQ  TGQWDTTQLS  NEYACMIMTI     100
Tabok1               51 RATEATMKYF  LTQAIASALL  LFSGTLNAWQ  TGQWDTTQLS  NEYACMIMTI     100
Tabok                51 RATEATMKYF  LTQAIASALL  LFSGTLNAWQ  TGQWDTTQLS  NEYACMIMTI     100
Dammam               51 ----------  ----------  ----------  ----------  ----------     100

U.a.aegyptia        101 ALTMKLGAAP  FHFWLPEVLQ  GSTMQASLLI  LTWQKIAPIT  LLYTTANHLP     150
Riyadh              101 ALTMKLGAAP  FHFWLPEVLQ  GSTMQASLLI  LTWQKIAPIT  LLYTTANHLP     150
Taif                101 ALTMKLGAAP  FHFWLPEVLQ  GSTMQASLLI  LTWQKIAPIT  LLYTTANHLP     150
Tabok1              101 ALTMKLGAAP  FHFWLPEVLQ  GSTMQASLLI  LTWQKIAPIT  LLYTTANHLP     150
Tabok               101 ALTMKLGAAP  FHFWLPEVLQ  GSTMQASLLI  LTWQKIAPIT  LLYTTANHLP     150
Dammam              101 ----------  ----------  ---MQASLLI  LTWQKIAPIT  LLYTTANHLP     150

U.a. aegyptia       151 TQMMLAIGIL  STVVGGLGGL  NQTQLRKILA  YSSISNLGWT  ISAMALAPNT     200
Riyadh              151 TQMMLAIGIL  STVVGGLGGL  NQTQLRKILA  YSSISNLGWT  ISAMALAPNT     200
Taif                151 TQMMLAIGIL  STVVGGLGGL  NQTQLRKILA  YSSISNLGWT  ISAMALAPNT     200
Tabok1              151 TQMMLAIGIL  STVVGGLGGL  NQTQLRKILA  YSSISNLGWT  ISAMALAPNT     200
Tabok               151 TQMMLAIGIL  STVVGGLGGL  NQTQLRKILA  YSSISNLGWT  ISAMALAPNT     200
Dammam              151 TQMMLAIGIL  STVVGGLGGL  NQTQLRKILA  YSSISNLGWT  ISAMALAPNT     200

U.a. aegyptia       201 AILNITIYIL  LSTPTMLLLT  KTSTKTLKDT  TTMWTTNPTA  SVLLALLLLS     250
Riyadh              201 AILNITIYIL  LSTPTMLLLT  KTSTKTLKDT  TTMWTTNPTA  SVLLALLLLS     250
Taif                201 AILNITIYIL  LSTPTMLLLT  KTSTKTLKDT  TTMWTTNPTA  SVLLALLLLS     250
Tabok1              201 AILNITIYIL  LSTPTMLLLT  KTSTKTLKDT  TTMWTTNPTA  SVLLALLLLS     250
Tabok               201 AILNITIYIL  LSTPTMLLLT  KTSTKTLKDT  TTMWTTNPTA  SVLLALLLLS     250
Dammam              201 AILNITIYIL  LSTPTMLLLT  KTSTKTLKDT  TTMWTTNPTA  SVLLALLLLS     250

U.a. aegyptia       251 TGGLPPFTGF  LPKLLIMNEL  SSQNLTLLGT  LMAMASLLNL  MYYLRIVYLA     300
Riyadh              251 TGGLPPFTGF  LPKLLIMNEL  SSQNLTLLGT  LMAMASLLNL  MYYLRIVYLA     300
Taif                251 TGGLPPFTGF  LPKLLIMNEL  SSQNLTLLGT  LMAMASLLNL  MYYLRIVYLA     300
Tabok1              251 TGGLPPFTGF  LPKLLIMNEL  SSQNLTLLGT  LMAMASLLNL  MYYLRIVYLA     300
Tabok               251 TGGLPPFTGF  LPKLLIMNEL  SSQNLTLLGT  LMAMASLLNL  MYYLRIVYLA     300
Dammam              251 TGGLPPFTGF  LPKLLIMNEL  SSQNLTLLGT  LMAMASLLNL  MYYLRIVYLA     300

U.a. aegyptia       301 SMTAPPTITT  MTMKWRLKQY  HPSSTLLLIA  TFTTAALLLT  PAAPTITI..     350
Riyadh              301 SMTAPPTITT  MTMKWRLKQY  HPSPTLLLIA  TFTTAALLLT  PAAPTITI..     350
Taif                301 SMTAPPTITT  MTMKWRLKQY  HPFPTLLLIA  TFTTAALLLT  PAAPTITI..     350
Tabok1              301 SMTAPPTITT  MTMKWRLKQY  HPSPTLLLIA  TFTTAALLLT  PAAPTITI..     350
Tabok               301 SMTAPPTITT  MTMKWRLKQY  HPFPTLLLIA  TFTTAALLLT  PAAPTITI..     350
Dammam              301 SMTAPPTITT  MTMKWRLKQY  HPFPTLLLIA  TFTTAALLLT  PAAPTITI..     350
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Fig. 3: Aligned amino acid sequences for ND2 mitochondrial gene for the studied individuals.

synonymous. The translated amino acids of these sequences showed complete identities among all samples of *U. a. microlepis* and between the two subspecies (data not shown).

ND2 gene showed variations among the different localities and therefore, it could be considered as an important marker to study the possible polymorphisms in *U. a. microlepis.* The polymorphism of the 1046 base pair

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GenBank      1 GTTCCTTTGG  TCGCTCCAAC  CGTTACTTGG  ATAACTGTGG  TAATTCTAGA    50
Dammam       1 GTT-CTTTGG  TCGCTCCAAC  CGTTACTTGG  ATAACTGTGG  TAATTCTAGA    50
Tabok        1 GTT-CTTTGG  TCGCTCCAAC  CGTTACTTGG  ATAACTGTGG  TAATTCTAGA    50
Taif         1 GTT-CTTTGG  TCGCTCCA-C  CGTTACTTGG  ATAACTGTGG  TA-TTCTAGA    50

GenBank     51 GCTAATACAT  GCCAACGAGC  GCTGACCTCC  GGGGATGCGT  GCATTTATCA   100
Dammam      51 GCTAATACAT  GCCAACGAGC  GCTGACCTCC  GGGGATGCGT  GCATTTATCA   100
Tabok       51 GCTAATA---  ----------  ----------  ----------  ----------   100
Taif        51 GCTAATA---  ----------  ----------  ----------  ----------   100

GenBank    101 GACCAAAACC  AACCCGGGCT  CGCCCGGCCG  CTTTGGTGAC  TCTAGATAAC   150
Dammam     101 GACCAAAACC  AACCCGGGCT  CGCCCGGCCG  CTTTGGTGAC  TCTAGATAAC   150
Tabok      101 ----------  ----------  ----------  ----------  ----------   150
Taif       101 ----------  ----------  ----------  ----------  ----------   150

GenBank    151 CTCGGGCCGA  TCGCACGCCC  CCGTGGCGGC  GACGACGCAT  TCGAATGTCT   200
Dammam     151 CTCGGGCCGA  TCGCACGCCC  CCGTGGCGGC  GACGACGCAT  TCGAATGTCT   200
Tabok      151 ----------  ----------  ----------  ----------  ----------   200
Taif       151 ----------  ----------  ----------  ----------  ----------   200

GenBank    201 GCCCTATCAA  CTTTCGATGG  TACTTTCTGT  GCCTACCATG  GTGACCACGG   250
Dammam     201 GCCCTATCAA  CTTTCGATGG  TACTTTCTGT  GCCTACCATG  GTGACCACGG   250
Tabok      201 ----------  ----------  ----------  ----------  ----------   250
Taif       201 ----------  ----------  ----------  ----------  ----------   250

                                      A272        1
GenBank    251 GTAACGGGGA  ATCAGGGTTC  GA------AA  TTCCGATAAC  GAACGAGACT    22
Dammam     251 GTAACGGGGA  ATCAGGGTTC  GA------AA  TTCCGATAAC  GAACGAGACT    22
Tabok      251 ----------  ----------  ----------  ----------  ----------
Taif       251 ----------  ----------  ----------  ----------  ----------

GenBank     23 CTGGCATGCT  AACTAGTTAT  GCGACCCCCG  AGCGGTCGGC  GTCCAACTTC    73
Dammam      23 CTGGCATGCT  AACTAGTTAT  GCGACCCCCG  AGCGGTCGGC  GTCCAACTTC    73
Tabok       23 ----------  ----------  ----------  ----------  ------CTTA    73
Taif        22 ----------  ----------  ----------  ----------  ------CTTA    73
                                                                     B
GenBank     74 TTAGAGGGAC  AAGTGGCGTT  CAGCCACCCG  AGATTGAGCA  ATAACAGGT.   123
Dammam      74 TTAGAGGGAC  AAGTGGCGTT  CAGCCACCCG  AGATTGAGCA  ATAACAGGT.   123
Tabok       74 TTAGAGGGAC  AAGTGGCGT-  CAGCCACCCG  AGATTGAGCA  ATAACAGGT.   123
Taif        74 ---GAGGGAC  AAGTGGCGTT  CAGCCACCCG  AGAT-GAGCA  ATAACAGGT.   123
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Fig. 4: Aligned sequences for short segments at the upstream (A) and the downstream (B) of the 18S nuclear gene. The underlined sequences indicate the regions with deletions.

representing the complete sequence of the ND2 gene in 7 individuals of *U. a. microlepis* was analyzed. Five polymorphic sites in the gene defined four haplotypes. These sites included four transitions and one transversion, which accounted for two synonymous and three non-synonymous substitutions. The non-synonymous changes involved a substitution of serine with asparagine at $G_{269} \rightarrow A_{269}$ and a substitution of serine with phenylalanine at $C_{968} \rightarrow T_{968}$ (Figure 3). When *U. a. microlepis* was compared to its conspecific *U. a. aegyptia*, eight polymorphic sites were defined. These sites included seven transitions and one transversion. These variations accounted for three synonymous and five non-synonymous substitutions. Only one more non-synonymous transition involved a substitution of serine to proline at $C_{970} \rightarrow T_{970}$. Both conspecific taxa were previously compared in a similar molecular study [17].

Among 394 bp sequenced from 18S nuclear gene, 6 deletions were found in the samples from Taif, while in other samples this site is occupied by either T or A (Figure 4). This mutation could be attributed to that samples from Taif are inhabiting mountainous areas while other samples are found in open habitats with coarse, sand, gravel and sparse vegetation [1].

The level of genetic variation within this subspecies (0.5%) was similar or higher for other *Uromastyx* taxa [1]. The authors found that within taxa genetic distances are extremely low. It was 0.1 within *U. a. microlepis* and 0.13 within U. *a. leptieni.* Within other agamid populations, the genetic variability was lower than ours (0.2) [18]. This lower genetic variability was perhaps due to the small molecular data collected by the authors. Comparing the results presented herein to recent biochemical investigations [6] for this subspecies, a concordance regarding the genetic difference between Taif population and other localities was revealed.

In conclusion, the present study showed that the population genetic structure is concordant with the geographic distribution and different habitat types. This distribution suggests a restricted gene flow rather than isolation by distance among the different localities. A range-wide survey to elucidate inter- and intra-population genetic diversity is clearly necessary.

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REFERENCES

1. Wilms, T., W. Böhme, P. Wagner, N. Lutzmann and A. Schmitz, 2009. On the phylogeny and taxonomy of the genus *Uromastyx* Merrem, 1820 (Reptilia: Squamata: Agamidae: Uromastycinae) - resurrection of the genus *Saara* Gray, 1845. Bon. Zool. Beit., 56: 55-99.
2. Kevork, K. and H.S. Al-Uthman, 1972. Ecological observations on the Egyptian spiny-tailed lizard *Uromastyx aegyptius*. Bull. Iraq Nat. Hist. Mus., 5: 26-44.
3. Arnold, E.N., 1980. The Reptiles and Amphibians of Dhofar, Southern Arabia. J. Oman Stud. Special Report, 2: 273-332.
4. Al-Ogily, S.M. and, A. Hussain, 1983. Studies on the ecology of the Egyptian spiny-tailed lizard, *Uromastix aegyptius* (Forskål, 1775) in the Riyadh region, Saudi Arabia. J. College of Sci., King Saud Univ., 14: 341-351.
5. Wilms, T. and W. Böhme, 2007. Review of the taxonomy of the spiny-tailed lizards of Arabia (Reptilia: Agamidae: Leiolepidinae: *Uromastyx*). Fauna Arabia, 23: 435-468.
6. Amer, S.A.M., 2011. Genetic variability of the Saudi Arabian *Uromastyx aegyptia microlepis* using protein and isoenzymes electrophoreses. Adv. Biosci. Biotechnol., 2: 27-32.
7. Zari, T.A., 1991. Effect of temperature on resting metabolic rate of the spiny-tailed lizard, *Uromastyx aegyptius microlepis*. J. Egypt. Ger. Soc. Zool., 4: 9-18.
8. Elamin, A., 2004. Dynamics of blood biochemistry and haematology in the spiny-tailed lizard (*Uromastyx microlepis*). Saudi J. Biol. Sci., 11(1): 55-65.
9. Jarrar, B.M. and N.T. Taib, 1985. Histochemical studies on the lingual salivary glands of the spiny-tailed lizard *Uromastyx microlepis* (Blandford). Bull. Instit. Zool. Acad. Sin., 24(2): 203-212.
10. Jarrar, B.M. and N.T. Taib, 1987. The histochemistry of labial salivary glands of the spiny-tailed lizard *Uromastyx microlepis* (Blandford). Amph-Rept., 8: 59-67.
11. Taib, N.T., 1988. Histochemical characterization of the sublingual salivary glands of the spiny-tailed lizard, *Uromastyx microlepis*. Copeia, 2: 308-313.

12. Wilms, T., P. Wagner, M. Shobrak, Lutzmann and N.W. Böhme, 2010. Aspects of the ecology of the Arabian spiny-tailed lizard (*Uromastyx aegyptia microlepis* Blanford, 1875) at Mahazat as-Sayd protected area Saudi Arabia. Salamandra, 46(3): 130-141.
13. Wilms, T., 2007. Dornschwanzagamen der Gattung *Uromastyx* - Einführung in Taxonomie und Ökologie einer auf Wüsten spezialisierten Echsengruppe. Draco, 8(31): 6-19.
14. Kocher, T.D., W.K. Thomas, A. Meyer S.V. Edwards, *et al.,* 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA, 86: 6196-6200.
15. Kumazawa, Y. and H. Endo, 2004. Mitochondrial genome of the Komodo Dragon: EYcient sequencing method with reptile-oriented primers and novel gene rearrangements. DNA Res., 11: 115-125.
16. Swofford, D.L., 2003. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Ver 4 Sinauer Sunderland MA.,
17. Amer, S.A.M. and Y. Kumazawa, 2005. Mitochondrial DNA sequences of the Afro-Arabian spinytailed lizards (genus *Uromastyx*; family Agamidae): phylogenetic analyses and evolution of gene arrangements. Biol. J. Linn. Soc., 85: 247-260.
18. Ozdemir, N. Gul and C.M. Tusonuglo, 2011. Genomic variation within *Laudakia stellio* (Linnaeus 1758) (Sauria: Agamidae) in Turkey, based on analysis of mitochondrial 12S rRNA sequences. J. Animal. Vet. Adv., 10(4): 415-420.