

# GENETIC AND BIOCHEMICAL DIVERSITY BETWEEN TWO NATURAL POPULATIONS OF *Tarentola annularis* INHABITING TWO DIFFERENT HABITATS IN EGYPT

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## ABSTRACT

polyacrylamide gel electrophoreses (Discontinuous) for lactate dehydrogenase (*Ldh*) and Alfa-esterase (*α-Est*) isoenzymes were conducted for biochemical variability between two natural populations of the four-spotted gecko, *Tarentola annularis* inhabiting El-Faiyum and El-Beheira governorates, Egypt respectively. Total lipids and total protein of liver and muscle tissues in both populations were also estimated. Five *Ldh* isoforms in liver tissues were recorded in both populations. *T. annularis* inhabiting El-Beheira showed a higher activity of *Ldhs* isoforms than that inhabiting El-Faiyum. Such higher activity was reflected in the thicker and denser bands as well as their high relative fronts (RF) and could be supported by the significant increase in the total lipids and total protein in liver and thigh muscle tissues of this population. Thus, *T. annularis* inhabiting El-Beheira is more active, energetic and adaptable in its natural habitat than that inhabiting El-Faiyum. *α-Est* in heart tissues showed five isozymic fractions in two samples of *T. annularis* inhabiting El-Faiyum and five isoforms in three samples of such population inhabiting El-Beheira. Heterozygosity for allozyme loci was observed only in *α-Est-5* in *T. annularis* inhabiting El-Faiyum, while it was absent in such population that inhabiting El-Beheira. The presence of such high activity of esterase isoforms as well as heterozygosity for allozyme locus; *α-Est-5* in *T. annularis* inhabiting El-Faiyum may reflect to some extent, the genetic variability and the unsafety of the diet applied to this natural population of gecko, as well as their high ability to accumulate the environmental contaminants in their body tissues than that inhabiting El-Beheira. Thus, the highly polluted natural habitat of El-Faiyum threatens the persistence of the wild natural population of *T. annularis* and this was confirmed by increasing the damage of its DNA that decreases its genomic stability. As a measure of the conservation status, we proposed a Red spot for the natural population of *T. annularis* inhabiting El-Faiyum based on the above mentioned data

## KEYWORDS

*Tarentola annularis*, Gekkonidae, *Ldh* isoenzyme, *Est* isoenzyme, total lipids, total protein

## INTRODUCTION

The squamates are the most diversified group containing the lizards and snakes. Several investigations have been recorded on the fauna of Egyptian reptiles (Vidal and Hedges 2009, Sayed 2012). Lizards are cosmopolitan and geographically distributed over a wide range of habitats and have a striking range of morphological characteristics, ecological habitats and body sizes. In Egypt, most of the gekkonid species are living in and around human habitation. However, some species are free living in Egyptian deserts (Ali 2012). The genus *Tarentola*

comprises 21 species, most of which show low inter-specific morphological variations (Diaz and Hedges 2008, Ali 2012).

The genus *Tarentola* belongs to the family Gekkonidae of the order Squamata that is distributed throughout the world and representing one of the largest vertebrate groups among squamates (Vidal and Hedges 2005). Gekkonidae is divided into four subfamilies: Diplodactylinae, Gekkoninae, Eublepharinae and Sphaerodactylinae with 1130 species and 108 genera (Han et al. 2004).

The genus *Tarentola* lives mainly in arid and semi-arid habitats, widely distributed in Libya, Sinai, Ethiopia and Somali land, Countries and Islands bordering the Mediterranean (Baldo et al. 2008). Moreover, it comprises 21 species with low inter-specific morphological variations (Carranza et al. 2002, Diaz and Hedges 2008). However, genetic variations between them were evidenced by molecular analysis of their protein and their nuclear and mitochondrial genes (Carranza et al. 2002, Jesus et al. 2002, Pereira and Harris 2010, Ali 2012).

The *Tarentola annularis* is distributed in a wide area across Africa, central Sudan, to the north along the Nile to the Nile Delta and Sinai Peninsula (Egypt) (Baha El Din 2006). It is a common rock dwelling species inhabiting rocky wadis, ruins and old buildings.

Isoenzymes are multiple forms of a single enzyme, which often have different isoelectric points and therefore can be separated by electrophoresis. Lactate dehydrogenases (*Ldhs*) isoenzymes are very suitable systems for studying several metabolic, genetic, ecological features, and are very useful in systematic studies (Al-Harbi and Amer 2012). *Ldhs* are a hydrogen transfer enzyme that catalyze the oxidation of L-lactate to pyruvate with nicotinamide-adenine dinucleotide (NAD)<sup>+</sup> as hydrogen acceptor, the final step in the metabolic chain of anaerobic glycolysis. Esterase isoenzymes (*Est*) are one of the lipid-hydrolyzing enzymes, possess high significance in genetics and toxicology (Shahjahan et al. 2008).

Our present study aimed to investigate the patterns of biochemical and genetic diversity between two natural populations of *T. annularis* inhabiting El-Faiyum and El-Beheira governorates in Egypt using the electrophoretic analyses of the two isoenzymes.

## MATERIALS AND METHODS

### Animals' collection and study area

A total of 10 individuals of two natural populations of the four-spotted gecko, *Tarentola annularis* were collected from two different habitats in Egypt.

Morphological identification and classification of the animals as well as scientific and common names of these species were identified according to Baha El Din 2006.

The studied species:-

*Tarentola annularis* (Geoffroy de Saint-Hilaire, 1827) (Baha El Din 2006, Crochet and Renault 2008).

Common name: Egyptian gecko, four-spotted Gecko, Bors Abu Arba'a Noqat

The first population was collected from El-Faiyum governorate (low land = 40 m below sea level) [29° 18' 30.25'' N 30° 50' 34.26'' E] (**Fig. 1a**). It is one of the governorates of Egypt in the middle of the country. Its capital is the city of Faiyum, located about 81 mile (130 km) south west of Cairo. It is characterized by the large fertile Faiyum Oasis, which comprises farmland. South of the Faiyum Oasis, there is a smaller depression called El Gharaq el Sulţāni and irrigated from the Nile. A dry barren depression named Wadi Elrayan covers 280 miles<sup>2</sup> (725 km<sup>2</sup>), west of the El Gharaq el Sulţāni depression. Desert and dry mountains mostly surround the depressions.

While the other population was collected from El-Beheira (near sea level = latitude "coastal" area) [30° 50' 53.16'' N 30° 20' 36.78'' E] (**Fig. 1b**). El-Beheira is located within the Alexandria region, which encompasses Alexandria, Matrouh, and El Beheira govemorates. Geographically, El-Beheira govemorates is characterized by a vast desert in the south and the west, cultivated areas stretching to the eastern borders of the Rosetta branch of the Nile, and Edco Lake and the Mediterranean Sea in the north.

Both natural populations of *T. annularis* have circular pupil of the eye, dilated digits, mid-dorsal tubercles are lower than lateral tubercles with low inter-specific morphological variations between them except only in body coloration where the natural population of *T. annularis* inhabiting El-Faiyum showed a lighter coloration than that inhabiting El-Beheira (**Fig. 2**).

#### **Sample preparation and isoenzyme assay**

Tissue samples of both liver and heart were removed, immediately taken to the lab and stored at -80°C for laboratory use. For isoenzyme extraction, approximately 0.5 g of tissue was homogenized in 10 ml saline solution (PBS, pH=6.8) using a manual Homogenizer. The homogenates were centrifuged at 5000 rpm for 10 minutes and the supernatants were kept at -20°C until use. The enzymes; Alfa-esterase ( $\alpha$ -*Est*) in heart and Lactate dehydrogenase (*Ldh*) in liver supernatants

were separated by discontinuous polyacrylamide gel electrophoresis (Maurer 1968, Shaw and Prasad 1970).

Electrophoresis was carried out conveniently in discontinuous polyacrylamide gels. An amount of 50  $\mu$ l of the clear supernatant of the liver and heart homogenate of each sample was mixed with 20  $\mu$ l of protein dye (1% bromophenol blue) and 20  $\mu$ l of 2% sucrose. 30  $\mu$ l of the mixture per gel slot were used to be applied per each sample for isoenzymes electrophoresis. After electrophoresis, the gel was transferred into a staining solution (50-70 ml) according to Mulvey and Vrijenhoek (1981) which was then replaced by a destaining mixture of methanol, acetic acid and water (5:1:5 v/v/v). A potential gradient of high voltage electrode [(20 v/cm), anode] across the gel was applied for 4 h at 8°C for separation of the enzymes.

Concerning *Ldh*, after electrophoresis, the gel was soaked in 100 ml of 0.2 M Tris-HCl (pH 8.0) containing 30 mg NBT, 25 mg EDTA, 50 mg NAD, 10 mg L-Lactic acid and 2 mg PMS. 0.05 M Tris-HCl pH 8.5 was prepared by dissolving 0.605 g Tris in 50 ml distilled water. The pH was adjusted to 8.5 by HCl. Then the solution was completed to 100 ml by distilled water (Jonathan and Wendel 1990).

Regarding  *$\alpha$ -Est*, after electrophoresis, the gel was soaked in 0.5 M borate buffer (pH 4.1) for 90 minutes at 4°C. This procedure lowers the pH of the gel from 8.8 to about 7 at which the reaction proceeds readily. The low temperature minimizes diffusion of the protein within the gel. The gel then was rinsed rapidly in two changes of double distilled water. The gel was stained for esterase activity by incubation at 37°C in a substrate solution of 100 mg  $\alpha$ -naphthyl acetate and 100 mg fast blue RR salt in 200 ml of 0.1 M phosphate buffer pH 6.5 (Scandaliojs 1964).

After the appearance of the enzyme bands, the reaction was stopped by washing the gel two or three times with tap water. This was followed by adding the fixative solution, which consists of ethanol and 20% glacial acetic acid (9:11 v/v). The gel was kept in the fixative solution for 24 hours and then was photographed.

All gels were scanned using Gel Doc-2001 Bio-Rad system. For isoenzymes, the bands of enzyme activity were designated according to the system nomenclature proposed by Shaklee et al. (1990). An abbreviation which corresponds to the name of the isoenzyme 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.

### **Metabolic study**

Immediately after collection, geckos' were dissected. Pieces of liver and thigh muscles were removed and immediately weighed in grams (g) to the nearest 0.01

g. They were stored frozen at  $-20^{\circ}\text{C}$  till use. Livers and thigh muscles were processed for estimation of total lipids and total protein according to the method of Zöllner and Kirsch (1962) and Gornall et al. (1949), respectively using a kit of Biodiagnostics Company.

### STATISTICAL ANALYSIS

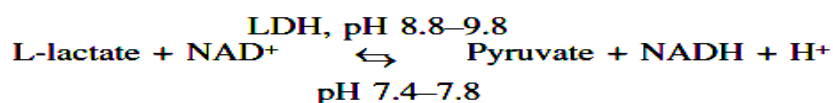
Student *t*-test in the PASW package v. 20 was used to calculate the significance difference of total lipids and total protein within and between both populations of geckos'.

### ETHICAL CONSIDERATIONS

Our Institutional Animal Care and Use Committee (IACUC) at Zoology Department, Faculty of Science, Cairo University has approved this study protocol from the ethical point of view and according to Animal welfare Act of the Ministry of Agriculture in Egypt that enforces the humane treatment of animals and the IACUC permit number is CUFS F Ecol. 4 15.

### RESULTS AND DISCUSSION

*Ldhs* isoenzymes are very useful systems in systematic studies and are very suitable for studying several metabolic, genetic, ecological features (Al-Harbi and Amer 2012, Kadry and Mohamad 2014). *Ldhs* are a hydrogen transfer enzymes that catalyze the oxidation of L-lactate to pyruvate with nicotinamide-adenine dinucleotide (NAD)<sup>+</sup> as hydrogen acceptor, the final step in the metabolic chain of anaerobic glycolysis. The reaction is reversible and the reaction equilibrium strongly favors the reverse reaction, namely the reduction of pyruvate (P) to lactate (L):



The electrophoretic profile of *Ldhs* isoenzymes revealed five isozymic forms in the liver tissues of both natural populations of *T. annularis* inhabiting El-Faiyum and El-Beheira. The natural population of *T. annularis* inhabiting El-Beheira showed a higher activity of *Ldhs* isoforms than in such natural population inhabiting El-Faiyum. Such higher activity was reflected in the thicker and denser bands as well as their high relative fronts (RF) (**Fig. 3**). The apparent increase in the activity of *Ldhs* in the liver tissues of *T. annularis* inhabiting El-Beheira, in the present study, could be supported by the highly significant increase in the total lipids as well as total protein in the in liver and muscle tissues of this natural population (**Table 1**). It is thus possibly reasonable to suppose that *T. annularis* inhabiting El-Beheira is

more active, energetic and adaptable in its natural habitat than that inhabiting El-Faiyum governorate.

Esterases are used as bio-indicators to measure the toxic potency of pesticide and heavy metal residues usually applied in the field (Shahjahan et al. 2008, Al-Harbi and Amer 2012). While  $\alpha$ -Est showed five isozymic fractions in two samples of natural population of *T. annularis* inhabiting El-Faiyum, it revealed five isoforms in three samples of such population inhabiting El-Beheira. Heterozygosity for allozyme loci was observed only in  $\alpha$ -Est-5 in the natural population of *T. annularis* inhabiting El-Faiyum, while it was absent in such population that inhabiting El-Beheira.  $\alpha$ -Est-4 appeared in two samples of natural population of *T. annularis* inhabiting El-Faiyum, while it revealed in three samples of such population inhabiting El-Beheira. The other isoforms;  $\alpha$ -Est-1,  $\alpha$ -Est-2 and  $\alpha$ -Est-3 were dense and thick by nearly the same extent and also their relative front was nearly close to each other in both natural populations *T. annularis* inhabiting El-Faiyum and El-Beheira (**Fig. 4**). The present results revealed the higher activity of alfa-esterases in the examined heart tissues of *T. annularis* inhabiting El-Faiyum than inhabiting El-Beheira. The presence of such high activity of esterase isoforms as well as heterozygosity for allozyme locus;  $\alpha$ -Est-5 in the natural population of *T. annularis* inhabiting El-Faiyum may reflect to some extent, the genetic variability and the unsafety of the diet applied to this natural population of gecko and its high ability to accumulate the environmental contaminants in their body tissues than that inhabiting El-Beheira governorate. Thus, the highly polluted natural habitat of El-Faiyum threatens the persistence of the wild natural population of *T. annularis* and this was confirmed by increasing the damage of its DNA that decreases its genomic stability (Mohamed and Kadry 2015)

**Table (1)** recorded the mean and standard error values of total lipids and total protein in liver and thigh muscle tissues of both natural populations of *T. annularis* inhabiting El-Faiyum and El-Beheira. The natural population of *T. annularis* that inhabiting El-Beheira showed a very highly significant increase in liver total lipids (major) and muscle (minor) ( $5.32 \pm 0.52$ ,  $P < 0.001$  and  $3.06 \pm 0.30$ ,  $P < 0.001$  respectively) than that inhabiting El-Faiyum ( $3.70 \pm 0.50$ ,  $P < 0.001$  and  $1.79 \pm 0.32$ ,  $P < 0.001$  respectively). While the total protein recorded a highly significant increases in the liver tissues of *T. annularis* that inhabiting El-Beheira ( $101.86 \pm 16.95$ ,  $P < 0.01$ ), it revealed a very highly significant increases in the muscle tissues of that population ( $13.02 \pm 1.16$ ,  $P < 0.001$ ) when compared with the other population that inhabiting El-Faiyum. Within each natural population of *T. annularis*, while the total lipids showed a very highly significant increases ( $P < 0.001$ ) in the liver than in muscle tissues, total protein revealed a highly

significant increase ( $P < 0.01$ ) in the liver than in muscle tissues in the natural population that inhabiting El-Beheira compared with that inhabiting El-Faiyum.

## CONCLUSION

In conclusion, the natural population of *T. annularis* inhabiting El-Beheira acquired a high physiological performance, genetic variability and activity than that natural population inhabiting El-Faiyum, where *Ldh* isoenzyme expression in the first natural population was higher than in the second. The accumulation of total lipids and total protein were also significantly higher in the first population than in the second. The present data also revealed a high activity of the esterase isoforms as well as heterozygosity for allozyme locus;  $\alpha$ -*Est-5* in the heart tissue of the natural population of *T. annularis* inhabiting El-Faiyum which may reflect the high ability of that natural population to accumulate the environmental contaminants in their body tissues than in such population inhabiting El-Beheira governorate. Thus, the highly polluted natural habitat of El-Faiyum threatens the persistence of the wild natural population of *T. annularis* and this was confirmed by increasing the damage of its DNA that decreases its genomic stability.

As a measure of the conservation status, we proposed a Red spot for the natural population of *T. annularis* inhabiting El-Faiyum based on the above mentioned data .

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

Author stated that there is no conflict of interest.

## REFERENCES

- Al-Harbi, M.S.; Amer, S.A.M. (2012) Comparison of energy-related isoenzymes between production and racing Arabian camels. *Advances in Bioscience and Biotechnology* 3: 1124-1128. <http://dx.doi.org/10.4236/abb.2012.38138>.
- Ali, R.A.M. (2012) Genetic Variation among nine Egyptian Gecko Species (Reptilia: Gekkonidae) Based on RAPD-PCR. *Life Science Journal* 9(1): 154-162.
- Baha El Din, M. (2006) *A Guide to Reptiles and Amphibians of Egypt*. Cairo: American University in Cairo Press. 359 pp.

Baldo, D.; Borteiro, C.; Brusquetti, F.; García, E.; Prigioni, C. (2008) Notes on geographic distribution, Reptilia, Gekkonidae, *Hemidactylus mabouia*, *Tarentola mauritanica*: Distribution extension and anthropogenic dispersal. Check List, 4: 434-438.

Carranza, S.; Arnold, N.; Mateo, A.; Geniez, P. (2002) Relationships and evolution of the North African geckos, *Geckonia* and *Tarentola* (Reptilia: Gekkonidae), based on mitochondrial and nuclear DNA sequences. Molecular Phylogenetics and Evolution 23: 244-256.

Crochet, P.-A.; Renoult, J.P. (2008) *Tarentola annularis annularis* (Geoffroy de Saint-Hilaire, 1827) preying on a mammal. Herpetology Notes 1: 58-59.

Diaz, M.; Hedges, B. (2008) A new gecko of the genus *Tarentola* (Squamata: Gekkonidae) from eastern Cuba. Zootaxa 1743: 43-52.

Gornall, A.E.; Bardawill, C. J.; David, M.M. (1949) Determination of serum total protein by means of the biuret reaction. Journal of Biological Chemistry 177: 751-766.

Han, D.; Zhou, K.; Bauer, A.M., (2004) Phylogenetic relationships among gekkotan lizards inferred from C-mos nuclear DNA sequences and a new classification of the Gekkota. Biological Journal of Linnean Society 83: 353-368.

Jesus, J.; Brehm, A.; Harris, J. (2002) Relationships of *Tarentola* (Reptilia: Gekkonidae) from the Cape Verde islands estimated from DNA sequence data. Amphibia-Reptilia 22: 235-242.

Jonathan, F.W.; Wendel, N.F. (1990) Visualization and interpretation of plant isoenzymes. In: Soltis, D. E. and Soltis, P. S., Eds., Isoenzymes in plant biology Champan and Hall, London, 5-45.

Kadry, M.A.M.; Mohamad, H.R.H. (2014) Genetic and metabolic variability between two subspecies of *Chamaeleo chamaeleon* (Reptilia: Chamaeleonidae) in Egypt. Advances in Bioscience and Biotechnology 5(8): 699-703. <http://dx.doi.org/10.4236/abb.2014.58083>.

Maurer, R. (1968) Disk electrophorese. W. de Gruyter and Co., Berlin, 222pp.

Mohamed, H.R.H.; Kadry, M.A.M. (2015) Study of genomic DNA damage in four-spotted gecko, *Tarentola annularis* one species inhabiting two different habitats. Ciência e Técnica Vitivinícola 30(8): 164-175.



Mulvey, M.; Vrijenhoek, R.C. (1981) Genetic variation among laboratory strains of the planorbid snail; *Biomphalaria glabrata*. *Biochemistry and Genetics* 19 (11-12): 1169-1182. <http://dx.doi.org/10.1007/BF00484572>.

Pereira, A.; Harris, J. (2010) Genetic variability within the Oudri's fan-footed gecko *Ptyodactylus oudrii* in North Africa assessed using mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution* 54: 634–639.

Sayed, N.H.M. (2012) Genetic diversity among eight Egyptian snakes (Squamata-Serpentes: Colubridae) using RAPD-PCR. *Life Science Journal* 9(1): 423-430.

Scandaliojs, E. (1964) Tissue-specific isozyme variations in maize. *Journal of Heredity* 55: 281-285.

Shahjahan, R.M.; Karim, A.; Begum, R.A.; Alam, M.S.; Begum, A. (2008) Tissue specific esterase isozyme banding pattern in Nile Tilapia (*Oreochromis niloticus*). *Universal Journal of Zoology (Rajshahi University)* 27: 1-5.

Shaklee, J.B.; Allendorf, F.W.; Morizot, D.C.; Whitt, E.S. (1990) Gene nomenclature for protein coding loci in fish. *Transactions of the American Fisheries Society* 119: 2-15. [http://dx.doi.org/10.1577/1548-8659\(1990\)119<0002:GNFPLI>2.3.CO;2](http://dx.doi.org/10.1577/1548-8659(1990)119<0002:GNFPLI>2.3.CO;2).

Shaw, C.R.; Prasad, R. (1970) Starch gel electrophoresis of enzymes: A compilation of recipes. *Biochemistry and Genetics* 4: 297-329. <http://dx.doi.org/10.1007/BF00485780>.

Vidal, N.; Hedges, S.B. (2005) The phylogeny of squamate reptiles (lizards, snakes, and amphisbaenians) inferred from nine nuclear protein coding genes. *Comptes Rendus Biologies* 328: 1000–1008.

Vidal, N.; Hedges, S.B. (2009) The molecular evolutionary tree of lizards, snakes, and amphisbaenians. *Comptes Rendus Biologies* 332: 129-139.

Zöllner, N.; Kirsch, K. (1962) Colorimetric method for determination of total lipids. *Journal of Experimental Medicine* 135: 545-550.

### EXPLANATIONS OF FIGURES

**Fig. 1** Study areas of the collected four-spotted gecko, *T. annularis* in Egypt.

**Fig. 2** Photos of *Tarentola annularis* inhabiting El-Faiyum (a) and El-Beheira (b) in Egypt.

**Fig. 3** The electrophoretic profile of *Ldh* isoenzymes in liver tissues. Lanes are as follow: 1-5 (*T. annularis* inhabiting El-Faiyum) and 6-10 (*T. annularis* inhabiting El-Beheira).

**Fig. 4** The electrophoretic profile of  $\alpha$ -*Est* isoenzymes in heart tissues. Lanes are as follow: 1-5 (*T. annularis* inhabiting El-Faiyum) and 6-10 (*T. annularis* inhabiting El-Beheira). H= Heterozygosity.

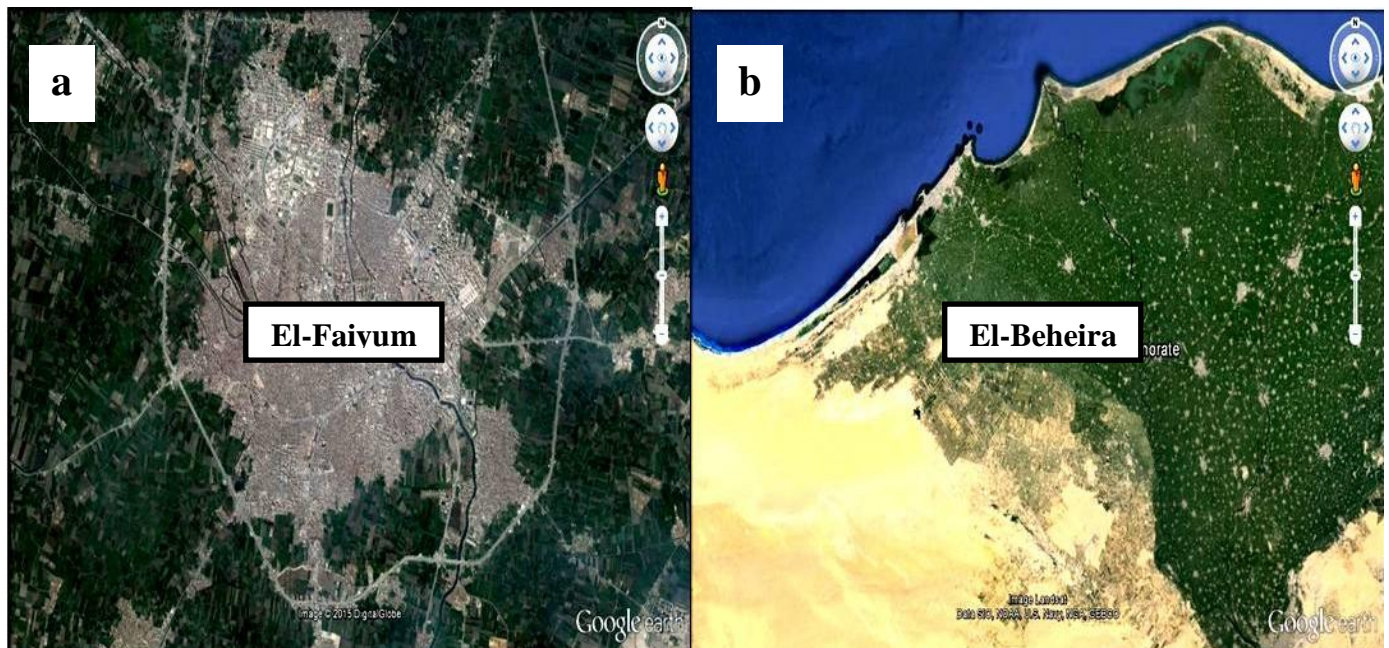


Fig.1. Study areas of the collected four-spotted gecko, *T. annularis* in Egypt.



Fig. 2. Photos of *Tarentola annularis* inhabiting El-Faiyum (a) and El-Beheira (b) in Egypt.

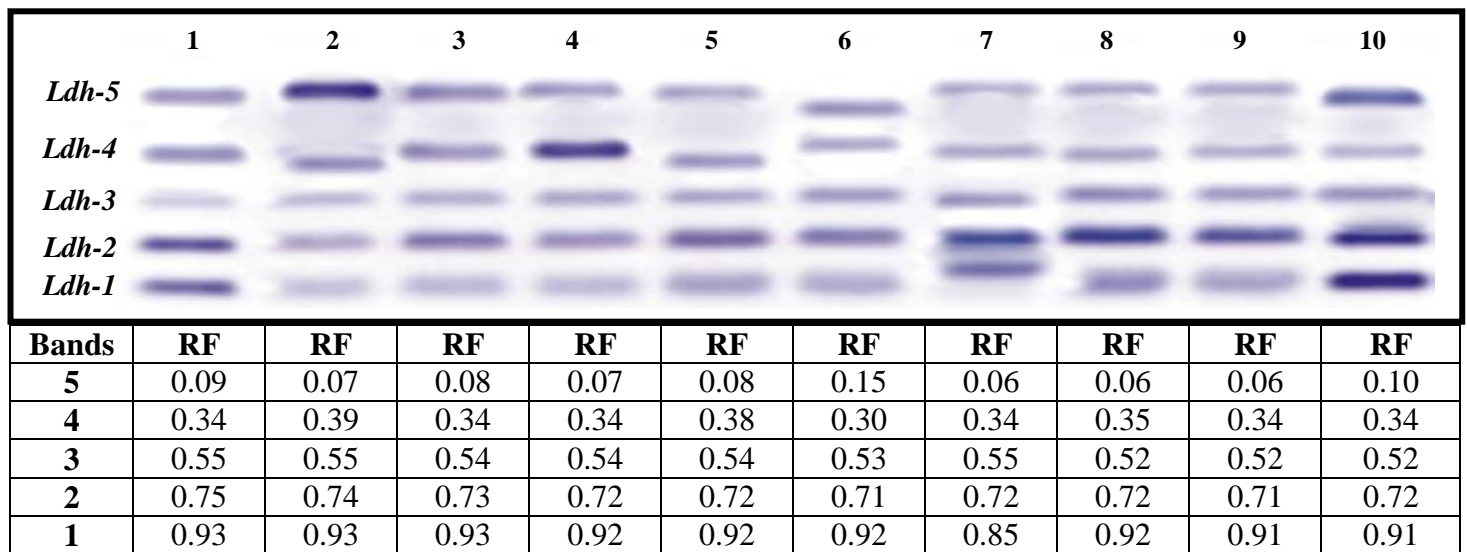


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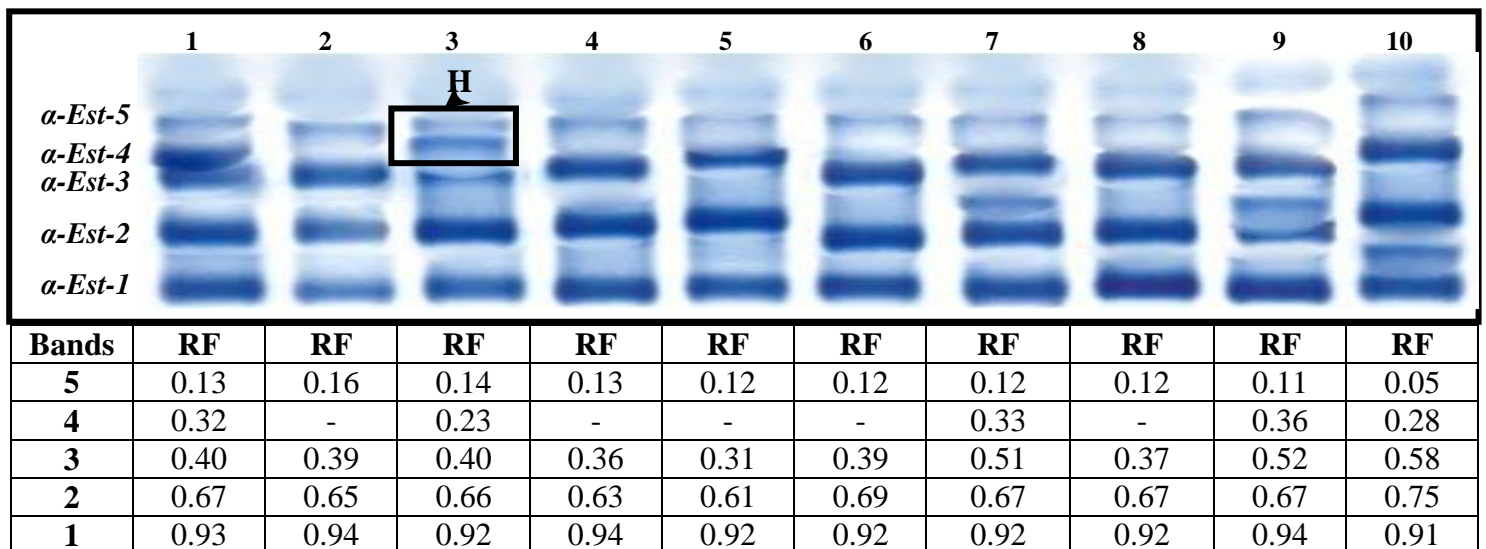


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**Table 1** Comparison of total lipids and total protein in liver and muscle tissues of *T. annularis* inhabiting El-Faiyum and El-Beheira. Data are expressed as mean  $\pm$  standard error. Number of individuals between parentheses.

Parameters	<i>Tarentola annularis</i>		<i>t-test</i>
	El-Faiyum	El-Beheira	
Liver total lipids (mg/100 mg)	3.70 $\pm$ 0.50 (5)	5.32 $\pm$ 0.52 (5)	10.39***
Thigh muscle total lipids (mg/100 mg)	1.79 $\pm$ 0.32 (5)	3.06 $\pm$ 0.30 (5)	8.19***
<i>t-test</i>	6.50***	8.86***	---
Liver total proteins (mg/100 mg)	27.19 $\pm$ 8.93 (5)	101.86 $\pm$ 16.95 (5)	4.20**
Thigh muscle total protein (mg/100 mg)	6.97 $\pm$ 0.57 (5)	13.02 $\pm$ 1.16 (5)	8.48***
<i>t-test</i>	3.16**	3.41**	

\*\* Highly significant at  $P < 0.01$

\*\*\* Very highly significant at  $P < 0.001$