Al-Azhar Bulletin of Science

Volume 20 | Issue 1 Article 19

6-1-2009

Section: Botany, Microbiology and Zoology

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BASUONY, MOHAMMAD; ZAKI, ZAKI; EL-SALKH, BOSHRA; and KHEDR, HANAA (2009) "ELECTROPHORESIS STUDY ON SERUM AND SOME TISSUES IN SUBSPECIES OF FAT SAND RAT PSAMMOMYS OBESUS IN EGYPT," Al-Azhar Bulletin of Science: Vol. 20: Iss. 1, Article 19. DOI: https://doi.org/10.21608/absb.2009.7556

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ELECTROPHORESIS STUDY ON SERUM AND SOME TISSUES IN SUBSPECIES OF FAT SAND RAT PSAMMOMYS OBESUS IN EGYPT

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Abstract

Aim of the work: In the present study we revised the taxonomy of the mammalian species Fat Sand Rat *Psammomys obesus* inhabiting the Coastal Desert of Egypt by using protein separation to establishment the pervious taxonomy or not.

Material and methods: The study includes electrophoresis on serum, liver, kidney and muscle proteins of three subspecies of desert rodent Fat Sand Rat *Psammomys obesus*.

Results: The serum protein separation of subspecies *terraesanctae* and subspecies *nicoli* has the same number of proteins while in subspecies *obesus* has the small number of proteins. The liver protein separation of the investigated subspecies is different from each other in the number of proteins. The kidney protein separation of subspecies *nicoli* and subspecies *obesus* showed that the same number of proteins while subspecies *terraesanctae* has the smallest number of proteins. In the muscle of femur bone, the protein separation in subspecies *nicoli*, *terraesanctae* and *obesus* had the same number of proteins. As a result of the high similarity between subspecies *obesus* and *terraescantae*, must be collected in one subspecies called *obesus* and the third subspecies still as it *nicoli*.

Keywords: Electrophoresis, Fat Sand Rat, Psammomys obesus.

Introduction

The electrophoresis was initially used to describe the behaviour of electrically charged colloidal particles in an electric field. The migration of true solutes was originally referred to as onophoresis. Eventually, electrophoresis became the recognized term for the migration of all kinds of particles under the influence of an electric field (Laas, 1998).

Proteins are large molecules varying in molecular weight from 1 to 1000 kDa (Kilodalton) and containing a linear sequence of amino acids covalently linked by peptide bonds (Harrison and Levitt, 1987). Proteins are present in all body fluids, but they are the proteins circulating in the blood that are readily accessible and can be analyzed directly (Fountoulakis *et al.*, 1998).

Sodium Dodecyle Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) still considered as the reference method for the detection of differences at the protein pattern being depend on the molecular weight of the separated proteins (Janson, 1998). It also can be a useful tool for monitoring patients over long periods of time when there are marked alterations in the levels of particular proteins (Jenkins and Guerin, 1997; Crocker and Burnett, 1998).

The success of electrophoresis in separating serum proteins into different fractions results from the heterogenity of charges of these molecules. Analysis of mixture of proteins to determine the number of molecular weight of different polypeptide chain is now almost exclusively carried out using polyacrylamide gel electrophoresis PAGE (Luzio and Thompson, 1990). Also they added that gels either cast in glass tubes (tube gels) or more commonly cast between two glass plates (slab gels).

Polyacrylamide is a polymer which can be poured as a liquid, after pouring, and with the appropriate added catalysts, it sets to a clear flexible, solid support in which electrophoresis of proteins can be carried out (Luzio and Thompson, 1990). Polyacrylamide gels are formed by co-polymerization of acrylamide monomer, CH₂=CH-CO-NH₂ in the presence of smaller amounts of N, N-methylene bisacrylamide which is used as a cross-linking agent, a catalyst, ammonium persulfate and an accelerator, tetramethylene diamine (TEMED) which decompose the persulfate ion to give a free radical. The mechanism of gel formation is free radical vinyl addition (Andrews, 1986). The proteins in Polyacrylamide Gel Electrophoresis (PAGE) migrate in response to the electrical field through the pores in the gel matrix. The porosity of gels is easily adjusted by changing the composition of acrylamide perior to polymerization, where pore size decreases with higher acrylamide concentrations. The combination of gel pore size, protein charge and shape determines the migration rate of the protein (Gallagher and Leonard, 1987).

Electrophoresis analysis was used to determine the differences between protein fractions and enzymatic secretion in both *Barbus bynii* and *Bagrus docmac* (Zaki *et al.*, 1995). Also Miguel and Pobles (1993) used polyacrylamide gel electrophoresis in purification of specific protein from the liver of rainbow trout (*Salmo gairdneri*). On the other side Karasinski (1993) declared that electrophoretic analysis of muscle

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myosine revealed a large variety among species in myosin isoform composition. **Tasi and Yang** (1975) concluded that the electrophoretic patteren were consistant with several of body weight, size, sexes or fishing localities, but the electropherograms showed species specific pattern. Assessment of different effects of chemicals in the animal body was carried out using on electrophoretic method.

Gun and Colak (2004) stated that estrase enzymes from 30 species of *Arvicola terrestris* in four localities in Krflehir provience are examined using starch gel electrophoresis and blood serum proteins from 27 speciemens using SDS-PAGE. It is determined that there is a variation in the globulin, prealbumin and postalbumen regions while the albumin region is monomorphic. Nonspecific estrases in the muscle, heart, kidney, and liver tissues of rats (*Rattus norvigicus*) are examined by horizontal starch gel electrophoresis. These findings showed that nonspecific esterases are very polymorphic (Verimli *et al.*, 2000). Blood serum electrophoresis of *Tamias dorsalis* compared to other species of *Tamias* indicated that *Tamias dorsalis* varies from all (Blake, 1992).

In the present study we revised the taxonomy of the mammalian species Fat Sand Rat *Psammomys obesus* inhabiting the Coastal Desert of Egypt by using protein separation to establishment the pervious taxonomy or change it.

Material and Methods

Fat Sand Rat *Psammomys obesus* has three subspecies in Egyptian deserts; *obesus* inhabits Coastal Western Desert, *nicoli* in north Eastern Desert and *terraesanctae* in North Sinai. These rodents collected from natural habitats, weighed and transferred alive to the laboratory in separate cages before classification according to Osborn and Helmy (1980).

For the electrophoretic studies, the samples were taken from different tissues of the rodent specimens. Serum, liver, kidney and muscles are the tissues taken from the specimens for electrophoresis study. The solutions for the gel are:

Stock solution (1)

Acrylamide / bis (30 %): 30 grams of acrylamide and 0.8 grams of N, N-methyleen bisacrylamide (BDH) were dissolved in 100 ml distilled water. The solution was filtered and stored in a dark bottle at 4 C° till used. Stock solution (2)

Stocking buffer: Tris - HCl solution (PH 6.6) was prepared by dissolving 9.0 grams Tris—hydroxymethylaminomethan in 100 ml distilled water. The PH was adjusted using 1 N Hydrochloric acid (HCl) or 1 M Sodium hydroxide (NaOH) then was stored at 4°C.

Stock solution (3)

Separating buffer: Tris–Hcl solution (PH 8.8) was prepared by dissolving 27.3 grams Tris–hydroxymethylaminomethane in 100 ml distilled water and about 20 ml 2N Hcl were added until the optimum PH , then the solution was stored at 4° c

Stock solution (4)

Running buffer: 15.0 grams Tris – base, 72.0 grams glycine and 5.0 grams sodium dodecyl sulfate (SDS) were dissolved in 5 L. distilled water and adjusted to PH 8.3 with 1N Hcl then stored at 4°c.

Stock solution (5)

Sample buffer:

Tris – borate solution PH 8.2 was prepared by dissolving 3.25 grams tris – base and 3.66 grams boric acid in 100 ml distilled water, PH was adjusted with 1N Hcl. The solution was filtered and stored at 4° c

Stock solution (6)

10 % SDS solution: dissolving 10.0 gm sodium dodecyl sulfate in a small amount of water with gentle stirring and thin distilled water was added to 100 ml.

Procedure

A set of two glass plates of 4 mm thickness was used for gel 1 mm. Thickness with a total 14×16 cm area; 11 cm, separating plus 3 cm stocking gels. OWL vertical slab was used in protein separation. The separating and stocking Gels were prepared according to Table (1).

Table 1: The separating and stocking gels.

Solutions	Separating gel	Stocking gel
Acrylamide (30 %)	12 ml	3 ml
Tris-HCl buffer (PH 8.8)	6 ml	
Tris–HCl buffer (PH 6.6)		3 ml
Distilled water	10 ml	12 ml
Glycerol	4 ml	
SDS (10 %)	300 ul	150 ul
TEMED	30 ul	15 ul
Ammonium persulphate (10%)	150 ul	75 ul
Total volume	32.48 ml	18.24

Separating gel was poured between the glass plates immediately after adding ammonium persulfate (APS). After polymerization of the separating gel, stocking gel was poured into the glass plates space and then the comb was inserted to form sample wells.

Sample preparation

According to **Stegemman** *et al.* **(1988)**, the blood serum sample were diluted 1: 3 with sample buffer then put in a boiling water path for 5 minutes. The samples were then applied to the gel wells (after cooling to the room temperature). For muscle protein (400 ul sample + 100 ul SDS and 50 ul mercbtoethanol). The samples were applied to the gel wells.

Running condition

The runs were carried out with a constants volt of 200 volt. When the tracking dye becomes one cm behind the end of the gel.

Staining

The separated proteins were stained with coomassie brilliant blue stain R- 250, which had been prepared as follows:

Coomassie brilliant blue R –2500. 1gm

Dist. Water 400 ml

Acetic acid 70 ml

Methanol 200 ml

Trichloroacetic acid 60 ml

The gels were soaked in excess of staining solution until the appearance of the bands.

Destaining

After gel staining, the gel was transferred to destaining solution to remove the excess stain, till the clearance of the background.

Destaining solution

Methanol	150 ml
Acetic acid	50 ml
Dist. Water	300 ml

The slab gel of proteins were photographed analysis percentages of the peaks of each line was carried out using Hoofer scanning densitometer GS 300. Sigma protein marker wide range (MW 6.500-205.000) was used to determinate the approximate molecular weight of protein fractions.

The obtained results were analyzed statistically according to the method of **Sendcor (1956)** and the data obtained from the gel scanning were analyzed using the computer corresponding software, gel scan Vergin 5.1.

Results

Serum protein pattern

Scanning of SDS — PAGE gel of blood serum proteins in three subspecies of *Psammomys obesus* revealed the presence of thirteen types in serum of *Psammomys obesus nicoli* and *Psammomys obesus terraesanctae*. The molecular weights of which are ranged from 229 to 11 KDa in *Psammomys obesus nicoli* and in *Psammomys obesus terraesanctae* they are ranged from and 223 to 28 KDa. The segregation of serum protein in *Psammomys obesus obesus* revealed the presence of twelve types of protein with molecular weights ranged from 179 to 27 KDa which was smaller than that in the other subspecies.

The area percent of *Psammomys obesus nicoli* ranged approximately from 2.04% to 7.16 %, whereas, the area percent of *Psammomys obesus terraesanctae* is smaller where it ranged from 1.31 % to 14.38 %. The area percent of *Psammomys obesus obesus* is greater than that in *Psammomys obesus nicoli* and *Psammomys obesus terraesanctae* which ranged from 6.18 % to 19.96 % (Table 2 and Figure 1)

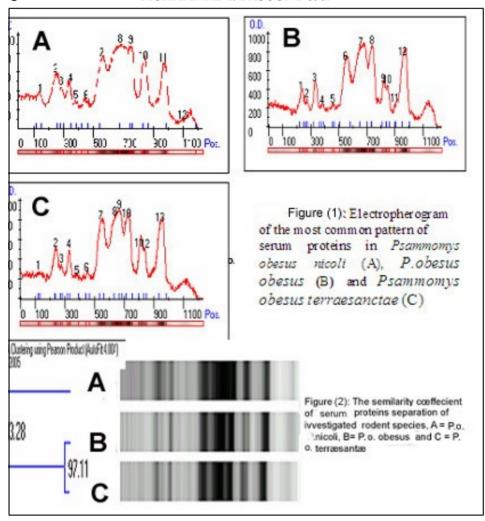
Table (2): The results of SDS gel scanning of the serum of *Psammomys obesus nicoli*, *P. o. Obesus* and *P. o. terraesanctae*

	P. o. nicoli		P. o. obesus		P. o. terraesanctae			
PK	MW (KDa)	Amt %	PK	MW (Kd)	Amt %	PK	MW (KDa)	Amt %
1	229	2.04	1	179	6.18	1	223	1.31
2	213	7.41	2	162	1.06	2	177	1.57
3	172	6.64	3	140	7.79	3	161	1.07
4	157	5.31	4	119	2.49	4	140	3.17
5	133	6.64	5	100	1.32	5	119	7.76
6	119	1.55	6	78	23.17	6	103	2.96
7	102	7.35	7	48	24.06	7	79	10.45
8	75	17.52	8	40	2.36	8	50	20.78
9	46	5.87	9	34	6.12	9	47	6.82
10	39	7.91	10	32	4.56	10	42	16.25
11	33	13.88	11	30	0.76	11	34	9.35
12	26	10.73	12	27	19.96	12	32	4.14
13	11	7.16				13	28	14.38

The examination of similarity coeffecient of serum protein separation between the three studied subspecies is represented as 82.31 %, however, the similarity coeffecient between *Psammomys obesus obesus* and *Psammomys obesus terraesanctae* represents as 88 % (Figure 2).

Liver protein pattern

The liver protein separation showed eleven types of protein in Psammomys obesus terraesanctae and Psammomys obesus obesus while in Psammomys obesus nicoli, the liver protein separation showed only eight types of proteins. The molecular weights of these proteins ranged from 207 to 14 KDa in Psammomys obesus nicoli while in Psammomys obesus terraesanctae and Pammomys obesus obesus they are ranged from 208 to 13 KDa and 209 to 5 KDa respectively. The molecular weights of proteins of Psammomys obesus obesus are greater than that in the other subspecies.

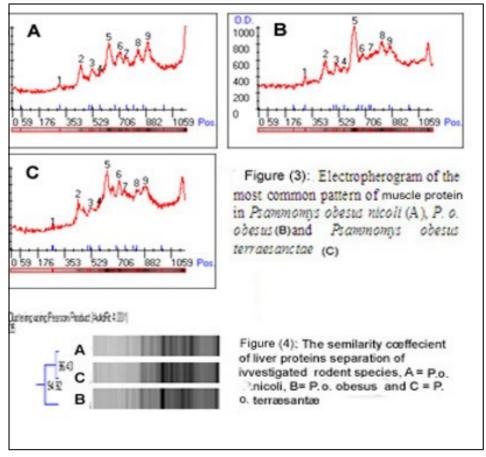


The area percent of *Psammomys obesus terraesanctae* and *Psammomys obesus obesus* ranged approximately from 1.88% to 26.4 % and 3.14 % to 26.81 % respectively while in *Psammomys obesus nicoli*, ranged from 3.22 % to 23.81 % (Table 3 and Figure 3).

The examination of similarity coeffecient of protein separation between *Psammomys obesus terraesanctae* and *Psammomys obesus obesus* is represented 93.69%, while the similarity coeffecient of liver protein separation between *Psammomys obesus nicoli*, *Psammomys obesus terraesanctae and Psammomys obesus obesus* is represented as 58.25% (Figure 4).

Table (3): The results of SDS gel scanning of the liver of *Psammomys obesus nicoli, P. o. Obesus* and *P. o. terraesanctae*

	P. o. nicoli			P. o. obesus			o. terraesan	ctae
PK	MW	Amt %	PK	MW	Amt %	PK	MW	Amt %
	(KDa)			(Kd)			(KDa)	
1	207	7.76	1	209	4.22	1	208	4.76
2	52	17.50	2	67	3.14	2	120	5.92
3	42	18.11	3	52	6.79	3	68	3.54
4	33	16.60	4	46	15.07	4	62	3.56
5	30	23.81	5	42	5.88	5	52	5.42
6	26	5.37	6	33	6.53	6	41	1.88
7	22	3.22	7	31	5.23	7	34	8.19
8	14	7.63	8	26	4.48	8	31	26.48
			9	23	12.75	9	38	25.79
			10	12	26.81	10	22	11.52
			11	5	9.11	11	13	2.94



Protein patteren of kidneys

Scanning of SDS-PAGE gel of kidney proteins in *Psammomys obesus nicoli* and *Psammomys obesus obesus* had ten types of proteins which are similar to each other. The kidney protein separation in *Psammomys obesus terraesanctae* showed seven types of proteins which are different from the other subspecies. The molecular weight of these proteins in *Psammomys obesus nicoli* is ranged from 66 to 11 KDa. The molecular weight of proteins in *Psammomys obesus terraesanctae* and *Psammomys obesus obesus* ranged from 76 to 11 KDa and 69 to 6 KDa respectively (Table 4 and Figure 5).

The area percent of *Psammomys obesus nicoli*, *Psammomys obesus terraesanctae* and *Psammomys obesus* are ranged from 0.72 % to 11.34 %, 4.30 % to 12.98 % and 2.34 % to 40.40 % respectively. The area percent of *Psammomys obesus terraesanctae* is greater than that in the other subspecies.

The examination of similarity coeffecient of protein separation between *Psammomys obesus terraesanctae* and *Psammomys obesus* is represented 97.22%, while the similarity coeffecient of kidney protein separation between *Psammomys obesus nicoli*, *Psammomys obesus terraesanctae and Psammomys obesus obesus* is represented as 90.32%. However, the similarity coeffecient between *Psammomys obesus terraesanctae*, *Psammomys obesus obesus* and *Psammomys obesus nicoli* is represented as 94.26% (Figure 6).

Protein patteren of muscles

Scanning of SDS-PAGE gel of muscle proteins in femur bone of *Psammomys obesus nicoli*, *Psammomys obesus terraesanctae* and *Psammomys obesus obesus* showed nine types of proteins. The molecular weight of these proteins in *Psammomys obesus nicoli*, *Psammomys obesus terraesanctae* and *Psammomys obesus ranged* from 96 to 23 KDa, 74 to 24 KDa and 70 to 25 KDa respectively. The molecular weight of protein of *Psammomys obesus nicoli* is greater than that in the other subspecies.

The percentages area of *Psammomys obesus nicoli*, *Psammomys obesus terraescantae* and *Psammomys obesus* ranged from 1.36% to 23.33 %, 0.16 % to 23.32 % and 1.48 % to 38.31 % respectively (Table 5 and Figure 7).

Table (4): The results of SDS gel scanning of the kidney of *Psammomys obesus nicoli*, *P. o. obesus* and *P. o. terraesanctae*

	P. o. nicoli			P. o. obesus			o. terraesan	ctae
PK	MW (KDa)	Amt %	PK	MW (KDa)	Amt %	PK	MW (KDa)	Amt %
1	66	6.83	1	69	5.27	1	76	4.30
2	51	14.20	2	63	2.04	2	62	4.56
3	45	0.72	3	52	16.15	3	50	12.98
4	41	2.86	4	40	11.14	4	38	6.06
5	33	43.24	5	33	3.12	5	32	8.94
6	31	11.34	6	32	4.25	6	24	54.26
7	28	5.93	7	30	9.20	7	11	8.90
8	25	4.47	8	24	40.40			
g	22	7 69	q	14	6.08			

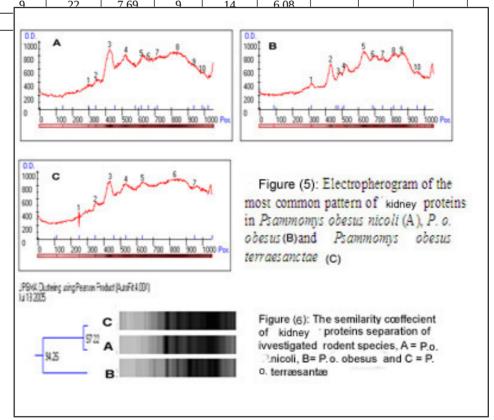
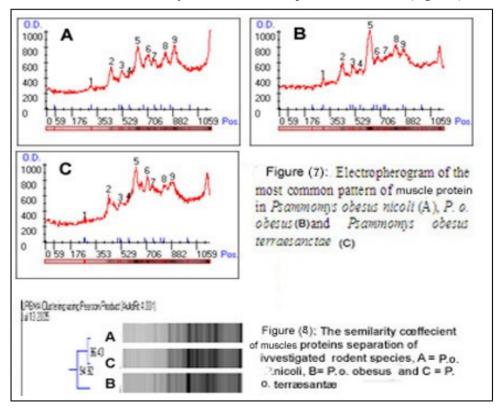


Table (5): The results of SDS gel scanning of the muscles of *Psammomys obesus nicoli*, *P. o. obesus* and *P. o. terraesanctae*

P. o. nicoli			P. o. obesus			P. o. terraesanctae		
PK	MW (KDa)	Amt %	PK	MW	Amt %	PK	MW	Amt %
				(KDa)			(KDa)	
1	69	11.28	1	70	3.90	1	74	0.16
2	53	23.33	2	54	17	2	54	23.22
3	44	1.36	3	45	1.53	3	44	1.72
4	37	4.39	4	38	4.57	4	37	5.36
5	34	16.95	5	34	17.18	5	34	22.43
6	31	14.29	6	32	2.34	6	31	14.24
7	30	5.26	7	30	1.48	7	30	6.21
8	27	7.72	8	27	38.31	8	27	3.34
9	23	15.42	9	25	13.70	9	24	23.32

The examination of similarity coeffecient of muscle protein separation between *Psammomys obesus terraesanctae*, *Psammomys obesus nicoli* and *Psammomys obesus obesus* is represented as 77.75%, while the similarity coeffecient of muscle protein separation between *Psammomys obesus nicoli* and *Psammomys obesus terraesanctae* is represented as 96.43%. However, the similarity coeffecient of muscle protein separation between *Psammomys obesus terraesanctae*, *Psammomys obesus nicoli* and *Psammomys obesus obesus* is represented as 94.92% (Figure 8).



Discussion

Electrophoresis of serum and tissue (liver, kidney and muscle) protein separations of three subspecies of *Psammomys obesus* which were collected from Western, Eastern and Sinai Desert namely, *obesus*, *nicoli* and *terraesanctae* respectively. These are thirteen types of protein in serum of *Psammomys obesus terraesanctae* and *Psammomys obesus* nicoli while in *Psammomys obesus* it showed twelve types of proteins. Similar observations were recorded in speciemens collected from different localities of Turkey (Verimli *et al.*, 2000) in *Arvicola terrestris* (Colak, 2004) in *Tamias dorsalis* (Blake, 1992) in *Apodemus flavicollis* and *Apodemus hermonesis* (Verimli *et al.*, 2001) in *Dryomys nitedula* and *Dryomys laniger* (Yigit *et al.*, 2003).

The liver protein separation of *Psammomys obesus terraesanctae* and *Psammomys obesus obesus* showed eleven types of proteins while in *Psammomys obesus nicoli* showed eight types of proteins. Similar observations were recorded in estrase enzyme in the liver of *Rattus norvigicus* (Verimli *et al.*, 2000).

The kidney protein separation of *Psammomys obesus nicoli* and *Psammomys obesus obesus* showed ten types of proteins while in *Psammomys obesus terraesanctae* showed seven and six protein types respectively. Similar observations were recorded in estrase in the kidney of *Rattus norvigicus* (Verimli *et al.*, 2000).

Protein separation of muscle of femur bone in *Psammomys obesus nicoli*, *Psammomys obesus terraesanctae* and *Psammomys obesus* had nine types of protein. Similar observation had been described in *Rattus norvigicus* (Verimli *et al.*, 2000).

Table 6: Means of external and cranial measurements of *Psammomys obesus* subspecies (Osborn and Helmy, 1980). The numbers between parnesis represent the average numbers and the number after the parnesis is the number of measures animals.

	Psammomys obesus	Psammomys obesus	Psammomys obesus
	obesus	nicoli	terraescantae
HBL	73 (151-187) 168.4	41 (199 -160)178.6	20 (144-168) 157.5
TL	69 (100-144) 125.4	38 (157 -122) 143.5	19 (115-131) 122.8
%TL/HBL	71 (82.5 -60.9) 73.5	38 (90.1 -60.1) 80.2	19 (89.6 -73.6) 78.0
FL	78 (40 -32) 36.9	46 (43 -38) 40.2	21 (40 -35) 36.8
EL	77 (16 -14) 14.8	41 (14-18) 16	21 (17 -13) 14.6
Wt	37 (205.1 -116.3) 141.8	27 (223 -106.6) 130	12 (135.3 -92.1) 114.5
ONL	68 (45.4 -38.8) 41.7	40 (48.2 -42.8) 45.4	22 (44.9 -37.7) 41.0
ZW	59 (27.4 -23.7) 24.9	31 (28.8 -25.4) 26.7	18 (26.6 -22.2) 24.4
NL	67 (17.6 -14) 16.2	38 (20.9 -17) 18.6	21 (18.5 -15) 16.6

where: HBL: Head and body lengthy

TL: Tail length
FL: find foot length
EL: Ear length

Wt: body weigh ingans
ONL: occipitenasal length
ZW: zygomatic width.
NL: Nasal length

This study revealed the similarity of proteins in serum, liver and kidney is very high between subspecies *obesus* and *terraescantae* inhabiting Western and Sinai Deserts respectively. The similarity is higher between subspecies *obesus* and *terraescantae* than that between each of them and the third subspecies *nicoli*. This observation is typical to that found by Osborn and Helmy (1980) on the external and cranial measurements of these subspecies (Table 6). However, the third subspecies *nicoli* inhabiting the northern region of Eastern Desert is somewhat different from the other two subspecies.

Psammomys obesus is one of the Saharo-Sindian species. This group of species occurs in the phytogeographic regions designated by Zohary (1973) as Saharo-Arabian and Irano-Turanian. Many of these species have Egypt as the center of this range and occur in the desert and subdesert regions extending from Mauritania to northwestern India (Qumsiyeh, 1985). The first population of Psammomys obesus appears to enter Africa from the east, following the end of the Saharan wet episodes, extending its range westwards along the Mediterranean seafront. The invasion of the Sahara by Psammomys obesus must have taken place prior to the evolution of the Neonile (Said, 1981), the Nile valley and delta then cut across the range of the species separating their eastern and western populations.

The subsequent breakdown of the *Psammomys obesus* populations into those of the Western Mediterranean Coastal Belt, the northern region of the Eastern Desert and the Sinai, is essentially a consequence of the development of the river Nile system. The delta and valley of the Neonile present a complete barrier that has completely separated the eastern and western populations of *Psammomys obesus*. Associated with the development of the river system is the extent of inundation of the Isthmus of Suez by the river water, which presents a barrier that seemed to the river water, which presented a barrier that seemed to block this Afro-Asian gateway. This barrier often partly separated the Eastern Desert and Sinai populations of this species (Nour El-Din, 2006). To the east of the Nile, in the northern part of Eastern desert, climatic barrier, to a lesser extent the lack of suitable habitats, and desertification seem to prevent the southward dispersion of *Psammomys obesus* and differentiation of the individuals of subspecies *nicoli* in this area.

So, as the high similarity in the proteins in the serum, liver, kidney and muscles between the two subspecies *obesus* and *terraescantae* must be collected in one subspecies called *obesus* and the third subspecies still as it *nicoli*.

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الفصل الكهربى للبروتينات فى مصل الدم وبعض الأنسجة لتحت الأنواع فى فأر الرمل السمين (ساموميس أوبيساس) فى مصر

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الملخص العربي

تتضمن هذه الدراسة مراجعة الوضع التقسيمى لفأر الرمل السمين (سامومس أوبيساس) والذى يندرج تحت النوع أوبيساس ثلاثة تحت نوع، أحدهم يقطن الصحراء الشرقية (نيكولى) والثانى يقطن الصحراء الغربية (أوبيساس) والثالث يقطن صحراء سيناء (تراسكانتى). وتستخدم طريقة الفصل الكهربائى للبروتينات الموجودة فى الدم والكلية والكبد والعضلات من تحت الأنواع المذكورة لمعرفة مدى التشابه والاختلاف بينهم. ولإجراء الدراسة تم تجميع العينات من بيئاتها الطبيعية بواسطة مصائد خاصة وتم نقلهم إلى المعمل وتخديرهم المحلول الكلوروفورم وأجريت عليهم الدراسات السابقة.

وقد أوضح كت الدراسة أن سيرم الدم يحتوى على 13 نوعاً من البروتينات في تحت نوعى تراسكانتي ونيكولى اللذين يقطنان صحراء سيناء والصحراء الشرقية على الترتيب، بينما يقل عدد البروتينات في مصل الدم لتحت النوع أوبيساس الذي يقطن الصحراء الغربية. ويتشابه الوزن الجزيئي للبروتينات في تحت النوعين الأوليين حيث يتراوح الوزن الجزيئي للبروتينات من 229 إلى 11 كيلودالتون في تحت نوع نيكولي، ومن 223 إلى 28 في تحت نوع تراسكانتي، بينما كان هناك اختلاف في الوزن الجزيئي لبروتينات الدم في مصل الدم في تحت النوع الثالث أوبيساس والذي يقطن الصحراء الغربية حيث يتراوح الوزن الجزيئي للبروتينات من 179 إلى 27 كيلودالتون، والتشابه بين أوبيساس وتراسكانتي 179 %.

ويحتوى الكبد على 11 نوعاً من البروتينات في تحت نوعي تراسكانتي وأوبيساس اللذين يقطنان صحراء سيناء والصحراء الغربية على الترتيب, بينما يقل عدد البروتينات في الكبد لتحت النوع نيكولي الذي يقطن الصحراء الشرقية إلى ثمانية فقط. ويتراوح الوزن الجزيئي للبروتينات من 209 إلى 13 كيلودالتون في تحت نوع تراسكانتي, ومن للبروتينات من 209 إلى 5 في تحت نوع أوبيساس، وفي نيكولي يتراوح الوزن الجزيئي

للبروتينــات من 207 إلى 14 كيلودالتــون، والتشــابه بين أوبيســاس وتراسكانتي 93.69 % بينما التشابه بين نيكولي وأي منهما 58.25 %.

وتحتوى الكلية على 10 أنواع من البروتينات فى تحت نوعى أوبيساس ونيكولى اللذين يقطنان الصحراء الغربية والصحراء الشرقية على السترتيب، بينما يقل عدد البروتينات فى الكلية لتحت النوع تراسكانتى الذى يقطن صحراء سيناء إلى سبعة أنواع فقط. ويتراوح السوزن الجزيئي للبروتينات من 69 إلى 6 كيلودالتون فى تحت نوع أوبيساس، ومن 66 إلى 11 كيلودالتون فى تحت نوع نيكولى، ومن 11 إلى 76 كيلودالتون فى تحت نوع نيكولى، ومن أوبيساس وتراسكانتى، والتشابه بين أوبيساس وتراسكانتى وأى منهما 94.26 %.

وتحتـوى العضـلات على 9 أنواع من البروتينـات فى تحت الأنـواع الثلاثة، ويتراوح الوزن الجزيـئى للبروتينـات من 69 إلى 23 كيلودالتـون فى تحت نـوع فى تحت نـوع نيكـولى، ومن 70 إلى 25 كيلودالتـون فى تحت نـوع أوبيسـاس، ومن 74 إلى 24 كيلودالتـون فى تحت نـوع تراسـكانتى، والتشابه بين نيكولى وتراسكانتى 97.43% بينما التشابه بين أوبيسـاس وأى منهما 94.92%.

ونظــراً للتشــابه الواضح بين تحت نــوع تراســكانتى وتحت نــوع أوبيساس ويبقى أوبيساس ويبقى تحت النوع الثالث كما هو تحت اسم نيكولى.