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Morphological and Molecular Polymorphism of Snake *Psammophis schokari* (Colubridae) in the Desert-Mountain and Coastal Areas of Egypt

Nahed A. Hussien and Rehab M. Hussein

Department of Zoology, Science Faculty, Cairo University, Egypt

Abstract: Among th e 36 snake-species in Egypt, *Psammophis schokari* is a colubrid snake widely distributed in desert and semi-desert ecosystems. From those different geographical regions, we choose El Maghara Mountain and El Dabaa areas as a desert-mountain and coastal localities respectively for our study. Five snakes from each type (3 males and 2 females) were used to evaluate the morphological and molecular polymorphism between the two types according to their environmental and geographical variation. Our results estimated an increase in the snout-vent length (SVL) and weight of *Schokari* coastal type in comparison with the desert-mountain type. We record the presence of a plenty of fat bodies in both sex of coastal type around the reproductive organs and it is more numerous in females, especially around their eggs, but for the desert-mountain type, males have no fat bodies and the females have only few around their eggs. Genomic DNA isolated from liver of snakes (desert-mountain and coastal types) was subjected to RAPD-PCR analysis using ten random decamer primers to evaluate molecular polymorphism. PCR-RAPD patterns using six out of the ten primers revealed genetic variation among both types. We concluded that, there is a morphological and molecular polymorphism of snake *Psammophis schokari* in different separated geographical areas, desert-mountain and coastal in Egypt. We suggest that variation in there morphology might be affected by food intake and different environmental factors or might be genetically inherited as shown in PCR-RAPD patterns variation.

Key words: *Psammophis schokari* · Polymorphism · PCR-RAPD

INTRODUCTION

The genus *Psammophis* (Psammophiinae) includes 24 species, most of them with an African origin, but also occurs in the Middle East and Asia. The snake *Psammophis schokari* (Colubridae) is widespread in North Africa having a Saharo-Sindian distribution [1], it is also found in the Middle East, Arabia, Iran, a large part of Afghanistan, Uzbekistan and northwest India [2].

The generic name *Psammophis* means simply "sand snake" (Greek), the specific name given in Yemen by Forskal [3], *schokari*, is derived from the Arabic "shigari" (= shajara) meaning "tree" [4], so it is commonly named desert sand snake. This fits the semi-arboreal habits of this snake, which is often found on trees, bushes & shrubs [5].

During the Pleistocene, North Africa experienced alternative humid and drier periods that appear to have influenced genetic subdivisions in this region in terrestrial species ranging from snails [6] to mammals [7]. Therefore several reptiles show deep subdivisions.

In Morocco/Western Sahara, three distinct morphotypes have been recorded for *Psammophis schokari*: the striped form that occurs in the Atlantic Coast and occasionally in the High Atlas Mountains; the uncolored form typically present in the High Atlas; and the Western-Sahara form with a slightly less slender body, striped pattern and greyish belly [1]. The first two co-occur in Southern and Central Morocco, while the Western-Sahara is the only form in this region. The occurrence of striped and uncolored morphotypes has also been recorded in Sinai, Egypt [8].

Corresponding Author: Nahed A. Hussien, Zoology Department, Science Faculty, Cairo University, Egypt. Tel: +20 2 35676781, Fax: +20 2 35727556.

This colubrid species is mostly diurnal and feeds on small vertebrates - lizards, small rodents and songbirds [5, 9] and its known predators include foxes, raptors and *Varanus griseus* [10].

The goal of this research was to illustrate the morphological and molecular polymorphism of snake *Psammophis schokari* in the desert-mountain and coastal areas in Egypt. Moreover, this study was aimed to define correlations between morphological and molecular variations with different environmental factors such as vegetation density, solar radiation intensity and annual rainfall in two different country fields in Egypt.

MATERIALS AND METHODS

Study Site: This study was conducted at two different areas desert- mountain and coastal: 1- El Maghara mountain area (coal mine), 60 kms south of Al-Arish city, Northern Sinai, Egypt, Coordinates: 30°41'10"N-33°27'23"E, It is located at an elevation of 776 meters above sea level; it is characterized by desert moderate climate with rare annual rainfall about 90mm^3/year, therefore people there depends on grazing and few crops on their livelihood. 2- El Dabaa, Matruh city, the extreme northwest of Egypt along the Mediterranean Sea, Coordinates: 30°58'44"N-28°27'46"E, occupies 60 km^2, it is characterized by moderate climate with annual rainfall about 140.4mm^3/year and presence of coastal salt marshes; many crops such as wheat and green barley are famous in Matrouh governorate in Dabaa, respectively. Map of the selected areas are represented in Fig. 1.

Animal Capture and Data Collection: *Psammophis schokari* represents one of the fastest snakes in the Middle East [11]; it has a thin body with an elongated head, featuring large golden-brown eyes with rounded pupils. The captured snakes (the desert-mountain and coastal types) have a rear-striped form. The background coloration is generally tan or beige (the color of desert) and there are also often dark stripes running from the snout, past the eyes, to the rear of the head (Fig. 2a). They have a yellow or beige color band on the ventral side of the body lined with black line (Fig. 2b). Both snake types have different spotted color pattern on the skin of the lower jaw, in which the coastal type shows more spotted pattern (Fig. 2c, 2d).

Animal dealer captured 8 snakes from desert-mountain area (Fig. 3a). It is diurnal animal and lives under rocks, wood and in rat burrows. It feeds on small lizards (*Acanthodactylus boskianus*). It lives in a surrounding temperature 28-30°C.

Animal dealer captured 6 snakes from coastal area (Fig. 3b); they live in the desert area near the coastline (might crawl 500m near the sea). It is diurnal animal and lives under shrubs and in rat burrows. It feeds on small spotted or big striped lizards (*Acanthodactylus boskianus*, 5 small or one big for a meal) and small migratory birds that land to rest and feed. It lives in a surrounding temperature 25-26°C.

Female of both types holds eggs for 2 months and then lays 5-7 eggs once a year on the middle of June to the first of July and the eggs stay for 60 days (in burrows) until they hatch. Their way of defense is running quickly and hides, not to attack. It is a non-poisonous snake. It hibernates on the end of October with the beginning of raining season. The coastal type is larger in size and more capable of attack or moving quickly than the desert type.

Sex Differentiation by Cloacal Probing: The gender of an adult snake can be determined by introducing a smooth, blunt, lubricated slender small probe into its cloaca and pushing the probe against the posterior wall of the cloaca to see if it can be freely and gently pushed into the base of the tail. This is referred to as the cloacal probing technique [12] (Fig. 4). This technique is based on the fact that a probe introduced into the cloaca can be slid a greater distance into the base of the tail of a male than into the tail of a female, due to probe insertion in the hemipenes of the male.

Body Measurements, Statistical Data Analysis and Dissection: For each snake, we recorded snout-vent length (SVL), head width (HW), tail length (TL, if present) and body weight. Data were expressed as the mean ± standard error (M±SE). Statistical significances of differences between two groups were determined using Student's t-test. The difference between means at the level of $p<0.05$ was considered as significance. Snakes were dissected and liver samples stored at -20°C until used.

DNA Extraction and Quantification: Genomic DNA was extracted from the liver with the Genomic DNA Purification kit (Fermentas) according to the manufacturer's instructions. The quantity of DNA was estimated by absorbance reading at 260 nm and DNA purity was estimated by ratio of absorbance reading between 260 and 280 nm.

RAPD-PCR: Ten primers (Operon Technologies, USA) were used to amplify genomic DNA. Each primer contained 10 base oligonucleotides were used in genetic mapping (Table 1).

Fig. 1: Egypt Map showing location of *psammophish schokari* Capturre. Desert-mountain type captured from 1-Eimaghara mountain area (60 kms south of al-arish city, reoresebted by dotted line ---- , right arrow), while coastal type captured form 2.El-Dabaa (the extreme northwest of egypt along the mediterranean sea, left arrow). Source: Google map 2013.

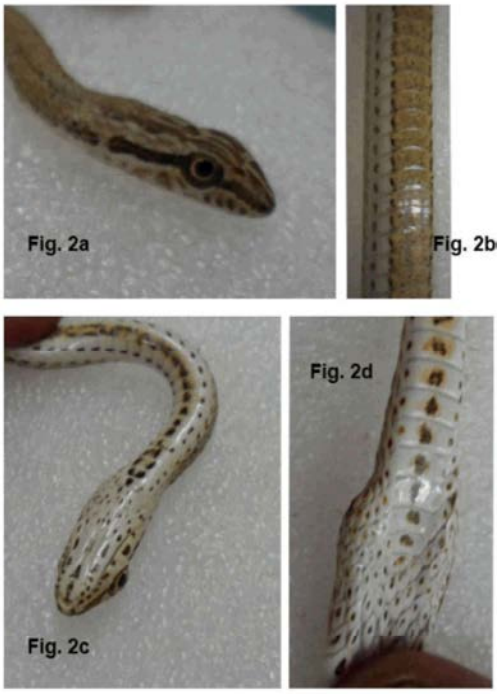

Fig. 2: Morphological features of psammophis schokari, in which it showks dark stirpes running from the snout, past the eyes, to the rear of the head (Fig. 2a) a yellow or beige colored ventral band lined with black line (Fig. 2b). Fig. 2d show spotted color pattern on the lower jaw skin in the desert - mountain and coastal types respectively.

Table 1: Primers sequences used in RAPD-PCR assay.

Primers Code	Primers Sequences
OPB-01	5'-GTTCGCTCC-3'
OPB-20	5'-GGACCCTTAC-3'
OPE-05	5'-TCAGGGAGGT-3'
OPF-02	5'-GAGGTCCCT-3'
OPG-12	5'-CAGCTCACGA-3'
OPH-05	5'-AGTCGTCCCC-3'
OPH-12	5'-ACGCGCATGT-3'
OPH-20	5'-GGGAGACATC-3'
OPO-03	5'-CTGTTGCTCAC-3'
OPX-19	5'-ACGCCGCTGGT-3'

To generate RAPD profiles, The PCR reaction mixture (20 µl) contained: 8 µl sterile water, 1 µl (100 ng/1 µl) extracted DNA, 1 µl primer (20 pmol) and then 10 µl 2× master mix (Promega, USA), added in a 0.2-ml PCR eppendorf tube. Cycling was started in the Thermal Cycler (Programmable Thermal Cycler, PTC-100TM thermal cycler, Model 96; MJ Research, Inc. Watertown, MA, USA), with initial denaturation at 94°C for 4 min, DNA double-strand denaturation at 94°C for 1 min, primer annealing at 36°C for 1 min and primer extension at 72°C for 2 min, for 40 cycles. Final extension at 72°C for 5 min was necessary for complete amplification. RAPD-PCR products were separated and visualized by electrophoresis on a 1.5% ethidium bromide-treated agarose gel (Sigma, UK) according to the standard protocol described by Sambrook *et al*. [13]. RAPD profiles were photographed and molecular weights were scored directly from the photographs.

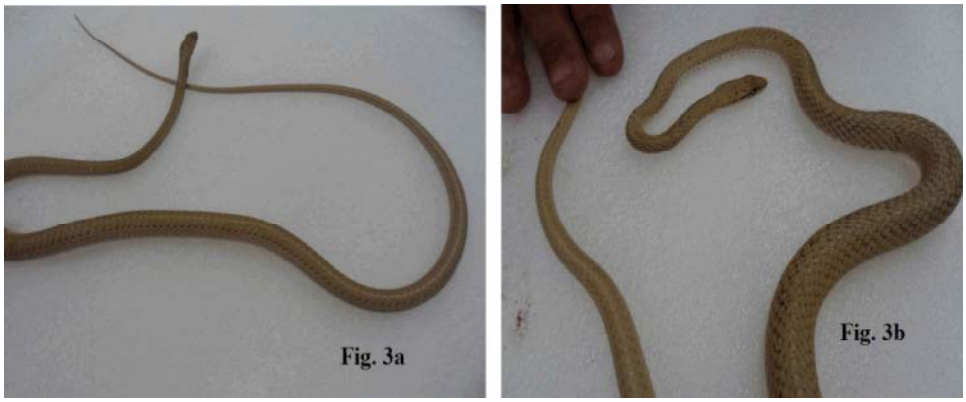

Fig. 3: Psammophis schokari desert - mountain and coastal types (Fig. 3a, Fig. 3b respectively).

Fig. 4: Snake sex differentiation using cloacal probing technique.

RESULTS

From the external features, it is hard to differentiate between both sexes. Both sexes have the same color, rear-striped pattern and bands on the vertical side. Sex differentiation by cloacal probing technique in both types successfully differentiates only 2 females from each type and the rest are males, which was confirmed by snake dissection and recognize the reproductive organs. We select the 2 females and other 3 males from each type to proceed in our study.

Table 2 shows statistically non-significant increase (at $P \leq 0.05$) in SVL and weight of male *Schokari* coastal type in comparison with the desert-mountain type. In addition, it showed statistically significant increase (at $P \leq 0.05$) in SVL and weight of female *Schokari* coastal type in comparison with the desert-mountain type. While, the head width and tail length didn't show remarkable difference between the two types in both sex. Since the coastal type is larger in size than the desert type due to the plenty of food in the surrounding area, therefore they are more capable of attack or moving quickly than the desert type. During recording external features, we record one female with tail loss in the desert-mountain type and other female with skin dysecdysis, an abnormal skin shedding, in the coastal type.

After the dissection of both *Schokari* types, unsurprisingly, longer snakes were heavier than smaller ones and had larger fat bodies. We record the presence of a plenty of fat bodies in both sex around the reproductive organs in coastal type, but it is more numerous in the female, especially around eggs (Fig. 5c, 5d). But for the desert-mountain type, males have no fat bodies and the females have few around their eggs (Fig. 5a, 5b).

PCR-RAPD was used to explore the genetic variability between the desert-mountain and coastal types of *Psammophis schokari*. Fig. 6 shows the similarity between desert-mountain and coastal types in the PCR-RAPD pattern using 4 different decamer primers, B-20, E-05, F-02 and H-05. The molecular weight of bands obtained from RAPD pattern ranges from 400-1200bp ((with an extra-band in the female of both types at 150bp), 400-2000bp, 900-2000bp, 700-3000bp for primers B-20, E-05, F-02 and H-05 respectively.

Fig. 7 and Fig. 8 show the variation between desert-mountain and coastal types in the PCR-RAPD pattern using other 6 different decamer primers, B-01, G-12, H-12, H-20, O-03 and X-09. As shown in Fig. 7, the molecular weight of bands obtained from RAPD pattern ranges from 400-3000bp but it shows an extra-band at 700bp in both sexes of the desert type using primer B-01, bands range from 500-1500bp with an extra band at 250bp for desert female type using primer G-12, bands

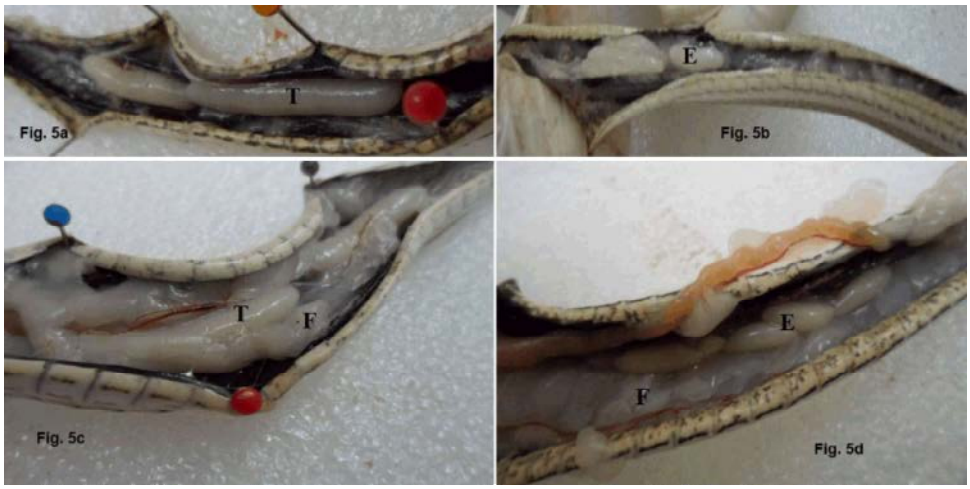

Fig. 5: Dissected psammophis schokari showing the sex and type differentiation. In which, Fig. 5a and Fig. 5b represent male and female desert-mountain typ, Fig. 5c and Fig. 5d represent male and female coastal type respectively. In which: T , testis: E, egg: F, fat body.

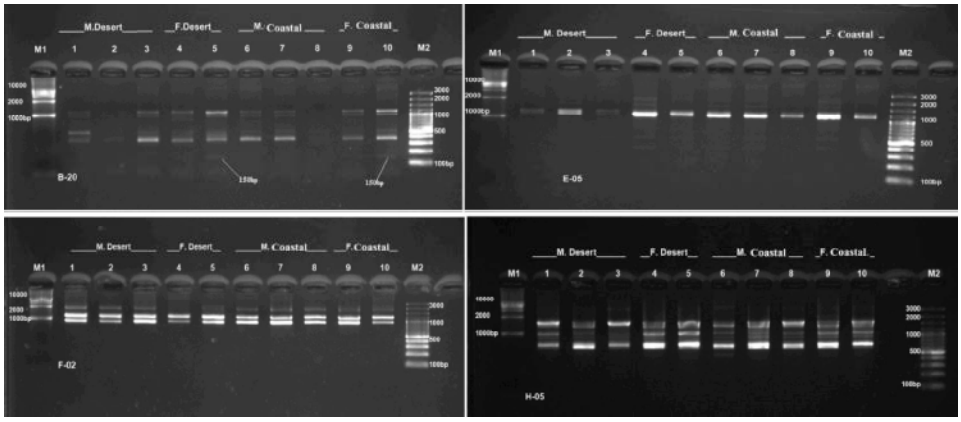

Fig. 6: PCR-RAPD pattern showing similarity between desert-mountain and coastal of psammophis schokari using operon technologies (B-20, E-05, F-02, H-05 respectively). In which : M, male: F, female. M1, 1kbp DNA ladder, M2, 100bp plus DNA ladder.

Table 2: Morphological measurements of desert and cosstal types of schokari reported as mean ± SE. In which : SVL, snout - vent length, HW, head width, TL,tail length, n represents number of snakes.

Schokari type / Sex	Weight	SVL (cm)	HW (cm)	TL(cm)
Desert Male (n=3)	47.00±7.23	57.17 ± 3.24	1.13 ± 0.09	27.33 ± 2.33
Coastal Male (n=3)	61.00±17.35	60.33 ± 6.49	1.03 ± 0.07	26.67 ± 3.28
Desert Female[#](n=3)	31.50±7.50	56.50 ± 0.50	1.00 ± 0	18.00 ± 9.00
Coastal Female[s](n=3)	63.50± 5.50*	66.00 ± 0*	1.25 ± 0.05	32.50 ± 0.50

*. Significant differences at P≤0.05 using students t-test with in the same sex groups at the same measurement.

[#]Tail loss/sankes

[s]Sankes dysecdysis/snskes

range from 300-3000bp with an extra-band at 600bp in both sexes of the desert type and at 250bp for the female desert type, in addition it shows extra-bands at 700bp and 1500bp in both sexes of the coastal type using primer H-12.

Fig. 8 shows the molecular weight of bands obtained from RAPD pattern that ranges from 270-2500bp but it shows an extra-band at 350bp in both sexes of the coastal using primer H-20, bands range from 400-2800bp with additional bands at 800bp and 1200bp in both sex of the

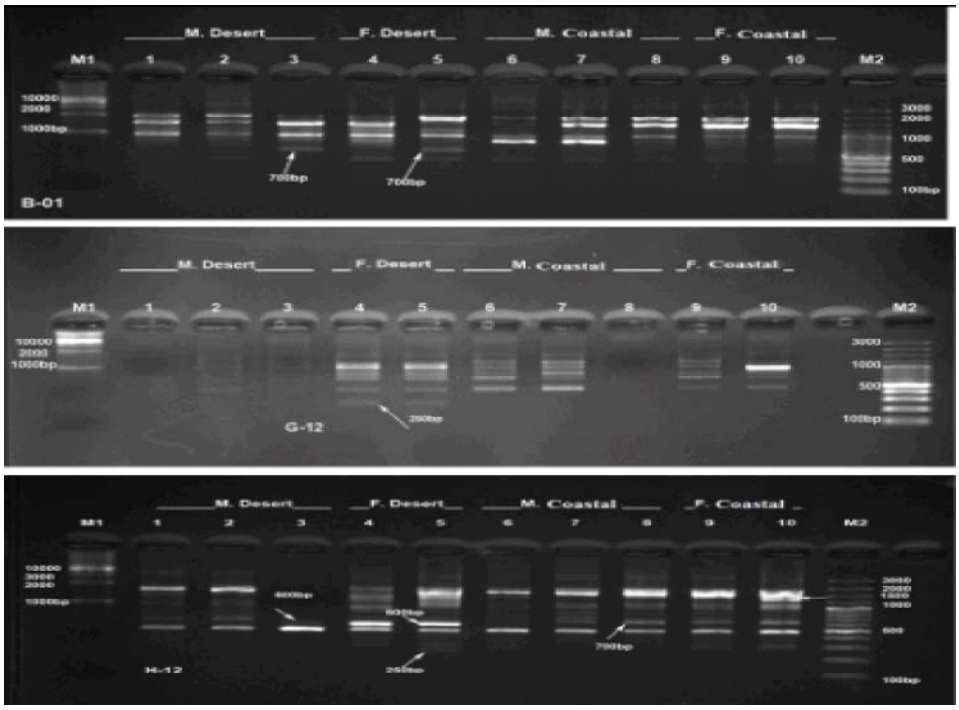

Fig. 7: PCR-RAPD pattern showing variation between desert-mountain and coastal types of psammophis schokari using operon technologies primers (B-01, G-12, H-12 respectively). Arrows are ponts to extra - bands specific of each schokari type with their molecular weight. In which : M, male, F, female, M1, 1kbp DNA ladder, M2, 100nbp plus DNA ladder.

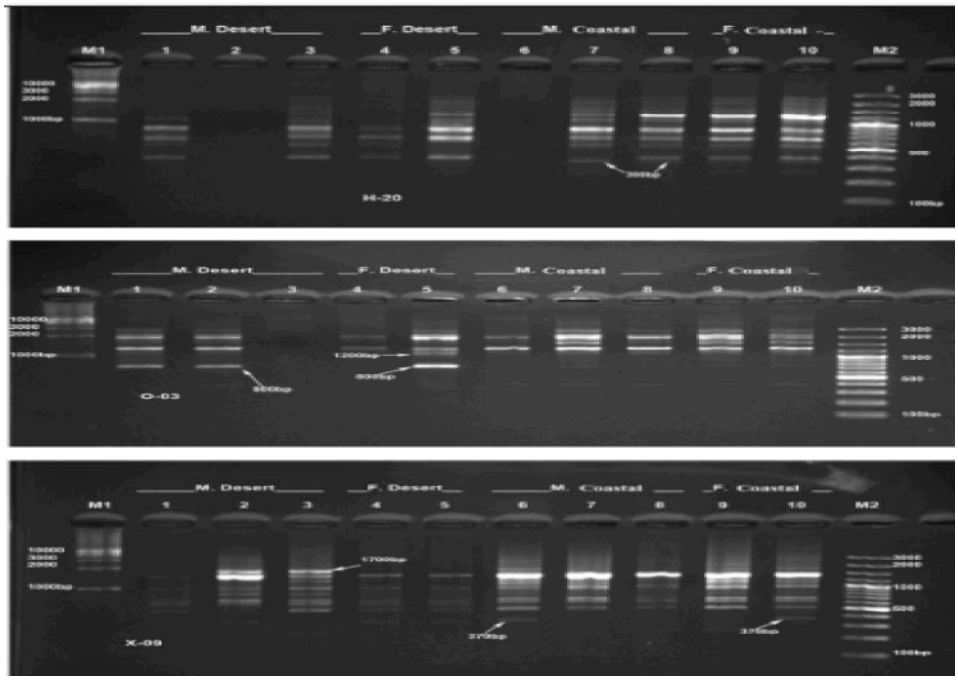

Fig. 8: PCR-RAPD patten showing variation between desert-mountain and coastal types of psammophis schokari using operon technologies primers (H-20, O-03, X-09 respectively). Arrows are pointed to extra - bands specific to each schokari type with their molecular weight. In which: M, male, F, female, M1,1kbp DNA ladder: M2, 100bp plus DNA ladder.

desert type using primer O-03, bands range from 250-3000bp with an additional band at 370bp for both sexes of the coastal type and an extra-band at 1700bp in the male desert type using X-09 primer.

DISCUSSION

The Colubrid snakes of the subfamily Psammophiinae have been the subject of several phylogenetic studies, including that of Broadley [14]; Kelly *et al*. [15] and most recently as part of a wider study by Pyron *et al*. [16]. *Psammophis schokari* Forskal has been considered a distinct species by Parker [17].

The geographic range of *Psammophis schokari* is continuous throughout northwestern Africa, across northern Africa south through Somaliland, through southwestern Asia to central Asiatic Russia, Baluchistan and Sind [18]. *Psammophis schokari* distributed in Egypt widely on the western Mediterranean coastal plain, extending south to about 29°N, but appears to be lacking from the interior of the Western Desert. It occurs locally along the margins of the Nile Valley and Delta. In the Eastern Desert it is widespread in the north, but appears more confined to the Red Sea littoral in the south. Widespread over much of Sinai, including the dune fields of the north [19].

From this geographic range of the widely distributed *Psammophis schokari* in Egypt, we choose two different localities to illustrate variation in the present study, El Maghara, Sinai and El Dabaa, Matruh, as a desert-mountain and coastal areas respectively. In the present study, initially we record only 2 females from total captured snakes per type (total 6 desert-mountain and 8 coastal types) because the time of snake capture begins just before time of laying eggs that starts from middle of June to the first of July, although females were hidden in burrows to prepare for laying eggs.

It seems that there are only subtle morphological differences between the desert-mountain and coastal types. Although the two types are from the same species, only few morphological features variations are present. We record that the coastal type is much longer and heavier than the desert type in both sexes. These records are in agreement with that reported by Dugan and Hayes [20]; they observed that Rattlesnakes from coastal populations averaged longer in body length than snakes from desert populations. They concluded that coastal snakes consumed a higher proportion of rodents and prey of larger body mass when controlling for snake length, than snakes from desert populations. Therefore, due to moderate climate and much more annual rainfall about 140.4mm^3/year in the coastal area, there is a variety of vegetations in coastal area and in turn increases the number of preys to feed on and snakes are longer and heavier and become more capable of attack or moving quickly than that of the desert-mountain area. In addition, Bronikowski [21] concluded that growth of rattlesnakes appears to be affected most heavily by energy intake. This is not to say that there is no genetic basis for body size in snakes; indeed, Garter Snake body size has a heritable component.

In the present study we record one coastal femalesnake with dysecdysis. As a snake approaches dysecdysis, the skin pattern becomes dull and dark and is characterized by the skin coming off or shedding in pieces and or single scales. In which, dysecdysis may be associated with external parasitism or inappropriate humidity [22]. In addition, we reported one female from desert type with tail loss, as with past studies [23; 24], tail stub frequency tended to be sex-biased with more females exhibiting such wounds than males. This bias exists is not known, but it may be the result of more females surviving tail attacks than males. Male tails differ from female tails in that reproductive organs are found there, but it is not known if damage to these organs can be fatal.

Due to plenty of food for *Schokari* coastal type, we observe the presence of many fat bodies in both sex around the reproductive organs, but it is more numerous in the female, especially around eggs. But for the desert-mountain type, males have no fat bodies and the females have few around their eggs. This result was in agreement with Vigil [25], he concluded that female brown water snakes (*Nerodia taxispilota*) have to significantly increase their body fat (to about 50%) in order to ovulate and produce eggs.

Genomic DNA isolated from liver of snakes (desert-mountain and coastal types) was subjected to RAPD-PCR analysis using ten random decamer primers. PCR-RAPD patterns using six out of the ten primers revealed genetic variation among both types but they are similar in RAPD patterns using the rest four primers. We suggest that, there is a genetic variation between geographically separated desert and coastal populations of *Psammophis schokari*. Our results were in agreement with Prior *et al*. [26], they estimated genetic differences between geographically separated populations of black rat snakes using seven variable RAPD markers. In addition, regional populations appear to be strongly divergent from east to west across the species' range.

We concluded that, there is a morphological and molecular polymorphism of snake *Psammophis schokari* in different separated geographical areas, desert-mountain and coastal in Egypt. We suggest that variation in there morphology might be affected by food intake and different environmental factors or might be genetically inherited as shown in PCR-RAPD patterns variation. However, further in-depth study is required before reaching to a final conclusion.

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