Estimation of Some Genetic and Physiological Variables of Iraqi Desert Snake *Cerastes gasperettii*

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Abstract

This study nominate the first blood indicate ranges for desert snake (***Cerastes gasperettii***) , Twenty-eight samples of the species under study were collected during field trips in the study area (Anbar desert located at western sector of Iraq about 400 kilometers west of Baghdad City).The examined hematologic values of the (***Cerastes gasperettii***) snake showed the highest erythrocyte ,WBC , hemoglobin ,MCH , MCHC and hematocrit value in male when a compared with females, The MCV value was found to be the highest in females and the lowest in male, karyological study revealed that the chromosome number is 2n (diploid) and the fundamental number (ZW) is 34 in male and female. DNA fragment was observed by agarose gel electrophoresis for CTNNB1 and WAC specific primers, the derived fragments ranged from approximately 600 bp, Additionally, in the CTNNB1 specific primer amplification of DNA fragments was found in male and female and ranged from approximately 900-bp DNA band.

Keywords: *Cerastes Sp.*, snake, blood, chromosome, PCR

Introduction

Western Iraq desert is home to a unique fauna and flora that has been shaped by the combination of several factors including the harsh climatic conditions of the Sahara (Arabian deserts), the episodic appearance of humid cycles, and by the complex geological evolution of the area. [(1)]. Taxonomy today often relies on molecular data for further support and information, such data are usually preferred over morphology for the reconstruction of evolutionary relationships among organisms. The increasing use and availability of molecular data has led to the development of new methods to study systematics, and has proven to be an invaluable tool for evaluating the evolutionary relationships between both closely and distantly related species [(2)]. Recent studies of Middle Eastern snakes have used molecular data to elucidate the inter- and intra-specific relationships among taxa, revealing high levels of genetic differentiation and cryptic diversity that do not accord with the current taxonomy, such studies have also provided insights into the historical biogeography of the taxa and the processes that triggered their diversification. However, the biodiversity of snakes in the Middle East remains unclear, as systematic and biogeographic data for several genera are still lacking. One such example is that of the colubrid genus *Cerastes sp*.[(3)]. ***Cerastes sp.*** is a venomous viper species occasionally found near human habitations, the information regarding their natural history is scarce are poorly known, it is very similar in appearance to *C. cerastes*, but the geographic ranges of these two species do not overlap, they are mostly daytime but can also be active during the nighttime Morphologically are characterized by Harsh, pressed body and short tail, the head triangular clear from neck, the Females are usually larger than males and average total length (body + tail) is 40–70 cm, with a maximum total length of 100 cm. that are mostly nocturnal but can also be found active during the day, prefer dry areas with little vegetation and are found in desert areas In Iraq desert, Jordan, and Egypt (i.e., the Sinai Peninsula), they are also known from arid and stony steppes, sparsely vegetated rocky slopes and wadis [(4).] The genus is currently comprised of three known species:

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Cerastes cerastes: it is found in arid North Africa (Morocco, Mauritania and Mali, eastward through Algeria, Tunisia, Niger, Libya and Chad to Egypt, Sudan, Ethiopia and Somalia) through Sinai to the northern Negev of Israel. In the Arabian Peninsula, it occurs in Yemen, Kuwait, extreme southwestern Saudi Arabia and parts of the country in Qatar where it is sympatric with *C. gasperettii.* A report of this species being found in Lebanon is unlikely [5]**.**

Cerastes gasperettii: In the Arabian Peninsula it has been found in Kuwait, Bahrain, Saudi Arabia, Oman, Qatar, United Arab Emirates, and Yemen. It is found in the Arava valley, located on the border between southern Israel and Jordan, eastwards through Jordan and Iraq to Khuzestan Province in southwestern Iran. [6].

Cerastes vipera: It is found in arid North Africa Mauritania, Morocco , Algeria , Mali , Tunisia , Libya , Niger, Chad and Egypt. Sinai Peninsula: Egypt and Israel. [7]. The current status of the recognized species within *Cerastes sp*., their relationships and distribution, remain relatively unclear, as no study has sampled all known species from the entire distribution range of the genus. In this work, we explore the phylogenetic relationships within *Cerastes sp*. by means of a broad sampling coupled with a morphological revision. Using an integrative taxonomic approach, we seek to produce the most complete phylogeny of *Cerastes sp.* to date, in order to clarify its systematics, describe a new species from Iraq, and elucidate its biogeographically and evolutionary history [8].

Materials and Method

Twenty-eight samples of the species under study were collected during field trips in the study area (Anbar desert located at western sector of Iraq about 400 kilometers west of Baghdad City). Blood samples were collected from (males and females) the ventral tail vein using a 25-gauge needle attached to 1ml disposable syringe containing (EDTA)The red blood cell counts (RBC) and white blood cell counts (WBC) were carried out using a Neubauer hemocytometer, where standard Hayem's solution for red blood cells and Turk's solution for white blood cells were used as a diluting solution.Hematocrit (HCT) was deter-mined using the microhematocrit method [9,10]. The tubes were then spun in a microhemat-ocrit centrifuge for 5 min at 12,000 rpm and the hematocrit (HCT) was calculated with the total blood level divided by the blood cell level. Hemoglobin concentration (Hb) was measured by the Sahli method with a Sahli hemoglobinometer (Tanyer, 1985). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated mathematically, taking the above results into consideration [9]. Chromosome preparations were applied for cytogenetic studies by lymphocyte culture of whole blood samples. The culture cells were treated with a colchicine-hypotonic-fixation-air-drying technique followed by conventional staining, twenty cells of each individual chromosome checks, length measurements, karyotyping were accomplished by using a light microscope [10,11]. Genomic DNA extraction was performed with DNeasy tissue Kit (QIAGEN). Extracted DNA Blood samples were stored in -30ºC to be used as a template for the polymerase chain reaction, DNA were amplified using specific primers WAC and CTNNB1[12, 13]. The descriptive statistics of the data obtained from our study were performed using SPSS (10.0 for Windows Student Version). Hematological variables were summarized as mean, standard deviation (SD), standard error of the mean (SE), and range. Results were considered significant at $P \leq 0.05$.

Table 1. Primers used for the amplification of the CTNNB1 and WAC genes.

WAC	F:5′-CTCAGCCATCTAATCAGTCCCCAA-3
	R:5′-GAACGCTGAAGACTTCGAGGAG-3′
CTNNB1	F1:5′-AGAGACGTCCACAATCGGATTG-3′
	R:5′-CAGACGTTTCTTATAATCTTGTGG-3′

Results

The examined hematologic values of the (***Cerastes gasperettii***) snake showed the highest erythrocyte and WBC count in male whereas the lowest erythrocyte count was found in females , The hemoglobin and hematocrit value was detected to be the highest in male too , The MCV value was found to be the highest in females and the lowest in male , and the MCH and MCHC value was found to be the highest in male and the lowest in females, The hematologic values of (***Cerastes gasperettii***) snake are given in detail in Table 2.

Table 2. Hematological data on the snakes. N: Number of specimens, SD: Standard Deviation, RBC: Red Blood Cell Count, WBC: White Blood Cell Count, Hb: Hemoglobin Value, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration.

Sex	N	RBC (1 mm3)	WBC (1 mm3)	Hb (g/dL)	HCT (%)	MCV (%)	MCH (pg)	MCHC (%)
		Mean ±SD (Min-Max)	Mean ±SD (Min-Max)	Mean ±SD (Min-Max)	Mean ±SD (Min-Max)	Mean ±SD (Min-Max)	Mean ±SD (Min-Max)	Mean ±SD (Min-Max)
Male	13	927,543 ± 135,724 (765,478 -1,120,215)	5530 ± 183,8 (5400-5660)	9.41 ± 2.85 (5.7-12.6)	31.4 ± 4.22 (26-37)	261.18 ± 61.05 (216.62-352.23)	136.11 ± 50.28 (75.10-227.49)	28.98 ± 1.64 (27.77-31.47)
Female	15	768,277 ± 226,125 (393,245 -1,062,223)	5266 ± 141.4 (3400-4240)	8.5 ± 0.81 (7.5-9.3)	27.73 ± 6.86 (20-34)	337.88 ± 60.95 (266.69-412.27)	96.71 ± 38.57 (62.18-137.59)	24.27± 1.39 (22.16-26.24)

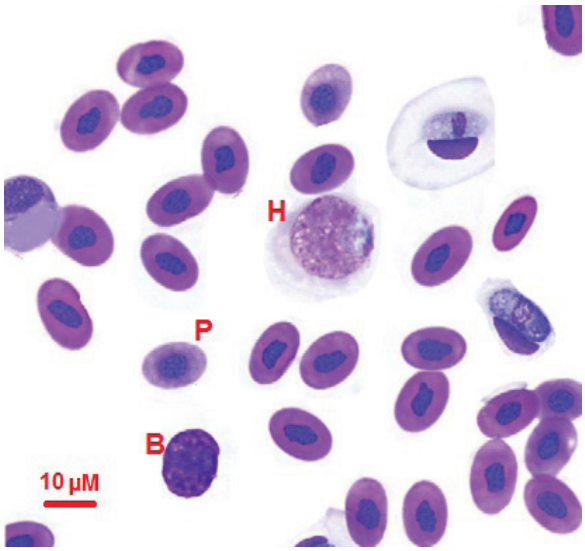
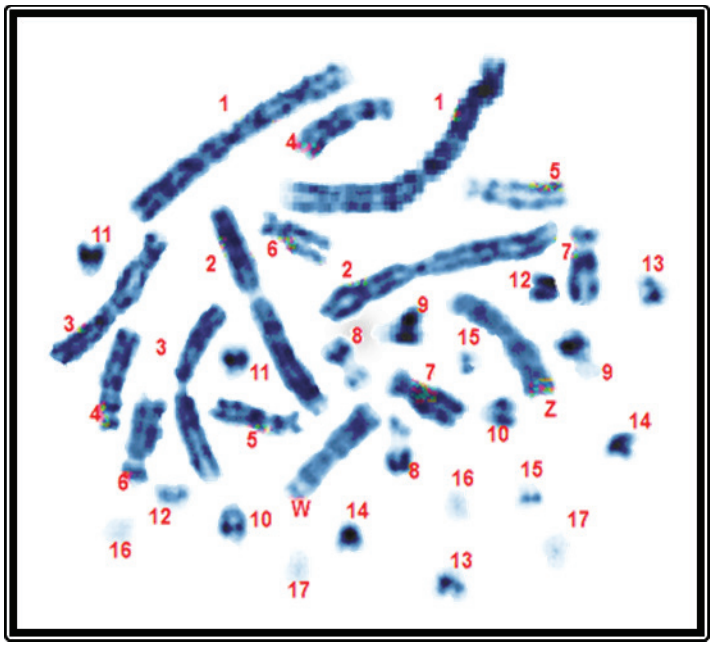


Figure. 1. Peripheral blood from (*Cerastes gasperettii*) with erythrocytes and B, basophil; H, heterophil; P, polychromatophils Wright-Giemsa, x100 objective

Karyological study of the (***Cerastes gasperettii***) snake using lymphocyte revealed that the chromosome number is 2n (diploid) and the fundamental number (ZW) is 34 in male and female. The diploid chromosome complements (Fig.1) consisted of pairs of metacentric or submetacentric macrochromosomes, gradually decreasing in size and pairs of microchromosomes.



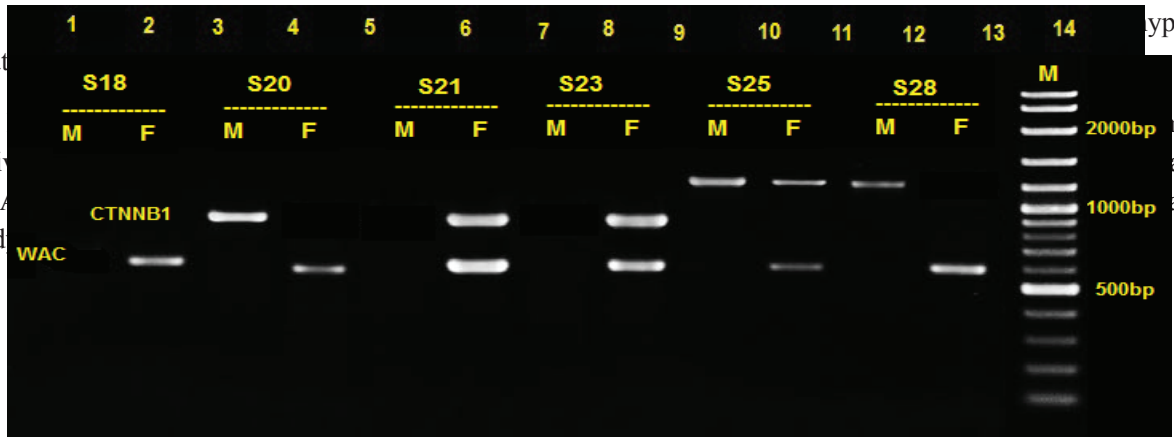


Figure.4. Agarose gel electrophoresis of PCR products in males and females of six (*Cerastes gasperettii*) snake using CTNNB1 and WAC M, male F, female

Discussion

The recent study has Recorded the first set of haematological parameters for any Iraqi snake species and provides normal reference intervals may be applied when examining other snakes, particularly species in the same family as those lived in the same desert. The coccygeal vein blood sampling of snakes is the easiest and safest method for clinicians blood collection from venomous snakes[14]. We adjust our method of collection Because lymph can contaminate samples collected in the syringe via aspiration, One of the most uncertainty aspects of diagnostic hematology of snake is the adjust the cell counts, Because RBCs are nucleated manual methods must be used to quantify leukocytes[15].

This study reported significantly Other snakes may have higher PCVs as in other environments such as percutaneous oxygen uptake, increased oxygen storage capacity in the lungs and lowered metabolic rates may provide increased oxygen availability therefore reduce the need for higher PCV for oxygen carrying capacity. [16,17]. male had higher RBC counts, MCV , MCH , and MCHC values than females, Because of the inverse relationship between erythrocyte number and size, species with higher MCV, such as turtles and snakes, have lower RBC counts than lizards, which have a lower MCV and higher RBC count, The average erythrocyte lifespan ranges from 600 to 800 days in reptiles[17]. This extremely slow turnover of erythrocytes (relative to human erythrocytes, which have a 120-day lifespan)

is thought to be associated with the slow metabolic rate of reptiles[15]. The conventional G-banding techniques revealed good number of G-bands on one set of haploid, which includes autosomes, Z and W chromosomes, Our present study showed that eight chromosome pairs show the same patterns (pairs 10, 11, 12,13, 14 ,15 ,16 and 17) and ten pairs share similarities (pairs 1, 2, 3, 4, 5, 6, 7,8, 9 and ZW chromosomes).This indicates that maybe there is evolutionary relationship between the desert Snake and the other snakes[18]. For further studies, more information about genetic differences is needed which may be accomplished by using molecular biology or molecular genetics. We try to developed novel PCR-based molecular sexing methods with two primer sets to identify sex chromosome systems by molecular method utilizing sex-specific sequences, thus, more advantageous than cytogenetic analyses to identify individual (*Cerastes gasperettii*) snake sex , based on the nucleotide sequence differences of two gametologous genes CTNNB1 and WAC the males with the homogametic sex chromosome (ZZ) were characterized by a single DNA fragment band from the two Z homologs, and the females with the heterogametic sex chromosome (ZW) were identified by two bands differing in fragment sizes from the one Z and one W homologs. The two primer sets CTNNB and WAC were available for molecular sexing in (*Cerastes gasperettii*) snakes, respectively. These two markers exhibited co-dominant DNA pattern type. This suggests that the Z and W forms of the CTNNB or WAC genes were differentiated by the cessation of recombination in the (*Cerastes gasperettii*) lineages [19,20].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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