Investigation of morphological characters of *Cerastes gasperettii* Leviton & S. Anderson, 1967 (Reptilia: Serpentes: Viperidae) in Iran

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Abstract

Iran has a high diversity of snake species. One of the snake families that has not only environmental but also medical importance in Iran is the Viperidae family, which is known as vipers in the world and most snakebites by members of this family occur in Iran because they are widely distributed in this country. All members of this family are venomous. There are various genera of vipers in Iran, one of which is *Cerastes*, which is represented by only one species in Iran.

C. gasperettii is one of the venomous snakes of the Viperidae family found in Iran, and little research has been done on this species. This species has a limited distribution in Iran, only in two

provinces, and the distinctive appearance of this snake has made it famous. Few and outdated morphological data on this snake are available in Iran, therefore this research was designed to investigate the morphological characters of *C. gasperettii*. For this purpose, the living specimens in the natural habitat and the museum specimens in the Venomous Animals Department Museum of the Razi Institute of Iran were examined for their morphological characteristics. All the live specimens in their natural habitats.

This study provides new data in the field of morphological variation in *C. gasperettii* in Iran including the lower percentage of the single anal plate morph, a high percentage of horned morphs and finding just 31-33 dorsal scales in the midbody in the populations of Horned vipers and also a report of cannibalism in this species in Iran.

Keywords:, *Cerastes gasperettii*, Iran, morphology, Meristic, Viperidae

1. Introduction

Reptiles are one of the most important vertebrate groups that have found special adaptations to live in dry places. One of them is snakes, and the present study focuses on one of their species.

Snakes are considered to be exceptional model organisms [13], in part because of their unique adaptations for feeding and foraging [2].

The desert vipers of the genus *Cerastes* are a monophyletic group of medically important venomous snakes adapted to desert environments [9]. Sand vipers of the genus *Cerastes* are specialized semifossorial snakes [24]. All species of this genus have small to medium body size,

which are adapted to arid environments, commonly known as Horned vipers . This genus is widely distributed across the arid regions of North Africa and the Middle East, particularly in the Sahara Desert and the Arabian Peninsula [18].

Three species of this genus include *Cerastes gasperettii*, *Cerastes cerastes* and *Cerastes vipera* [14]. A fourth species, Bohme's sand viper [21] is sometimes recognized but also disputed. It is based on a single specimen with aberrant horns, allegedly captured in Central Tunisia in 1991 and for which no genetic data is available so far [9].

previous morphological and mitochondrial approaches, which suggested that *C. cerastes* and *C. gasperettii*, two morphologically similar species with shared habitat requirements, were sister taxa while, *C. vipera*, smaller and linked to soft sand habitats, was sister to them [9]. According to other studies, *C. cerastes* and *C. vipera* are sister taxa, while *C. gasperettii* is more distantly related [9]. This was an unexpected result, since *C. cerastes* and *C. gasperettii* exhibit greater morphological similarities, both with horned and hornless populations (Figure1, A&B), a similar body size, and overlapping habitat requirements, since both species are generalists [22]. But *C. vipera* is very different from the other two species as it is smaller, strictly hornless and specialized in burrowing in sandy substrates, with specific adaptations such as the dorsal position of the eyes [2, 15]. Morphological data have led to the description of several subspecies within the two Horned vipers (*C. cerastes* and *C. gasperettii*): *C. c. cerastes* (widely distributed from Western Sahara to Egypt) and *C. c. hoofieni* (in southwestern Arabia based only on morphological data; [22] within *C. cerastes*; and *C. g. gasperettii* (widely distributed in Arabia) and *C. g. mendelssohni* [23,9] within *C. gasperettii*.

Figure 1. *C. gasperettii* in natural habitate in Iran A) Hornless morph B) Horned morph. (Photo by Ali Salemi).

Cerastes gasperettii (Figure 1) known as the Khuzestan Horned viper is one of the venomous snakes of the sandy deserts such as southwestern areas especially Khuzestan province in Iran [25]. In addition to Khuzestan province, this species is also in the checklist of snakes in Ilam province [6]. Therefore, this species only lives in two provinces named Khuzestan and Ilam in Iran [16]. *C. gasperettii* has the easternmost population in Iran.

The external morphology of an organism is the outcome of the interaction between the organism itself and the habitat in which it lives [2]. Desert environments have been mistakenly characterized as regions extremely poor in biodiversity and with low variability. However, the opposite has been proven [5]. During these warming-cooling cycles, biodiversity was forced to retract or spread its distributional ranges, accordingly to their ecological requirements and life historytraits, in order to survive [7]. During favourable conditions, species expanded their distribution and thrived, with potential reconnections of the existent populations, while during unfavourable conditions, species likely reduced distributional ranges and/or became extinct [2]. Vipers have particular life-history traits such as low growth rates and frequency of reproduction that likely accentuate responses to climatic oscillations [5].

In these dry and desert conditions, snakes have different ways to adapt to the environment, such as the Horned viper, which is a nocturnal true desert snake and prefers sandy soil with some vegetation as shelters. *Cerastes gasperettii* shows a camouflage character e.g. it becomes red on sandy soils and gray in desert plains [2]. The habitat of desert sand is available for Horned viper activities in small areas of southwest Iran. Due to the limited distribution of *C. gasperettii* in Iran, few studies have been done on the specimens of this snake species. Therefore, the aim of this research is to investigate the meristic and mensural characteristics on the available specimens of this species and prepare a comprehensive table of the morphological characteristics of Iranian populations of *C. gasperettii*.

2. Materials and methods :

In this research, the specimens available in the Reptile Laboratory Museum in the Razi Vaccine and Serum Institute from Iran were used in a fixed in ethanol, as well as live specimens in the natural habitat. Live specimens from Khuzestan province, the Albaji region and Bostan city were morphologically examined and the snakes were released in their habitat after examination (Figure 2).

Figure 2. Points studied in the present research in Iran (Red points)

The Razi institute's museum specimens have museum numbers according to table 2, and live specimens were released under supervision of the Environmental Protection Organization in the natural habitat were not given museum numbers. Some specimens were collected from the sand desert of Bostan city (31°43'06.9"N 47°59'38.7"E).The specimens of the museum are all related to the Albaji region of Ahvaz city (31°30'35.7"N 48°37'01.6"E). A total of 11 specimens were morphological studied for this research. Identification keys of Latifi, 2000 and Venchi & Sindaco, 2006 were used for morphological examination. To check, tools such as digital calipers and rulers were used, and to check meristic characters, a form was prepared and used according to Table 1. Photographs were taken of important aspects of the snake, which are important in identification (Figure 3).

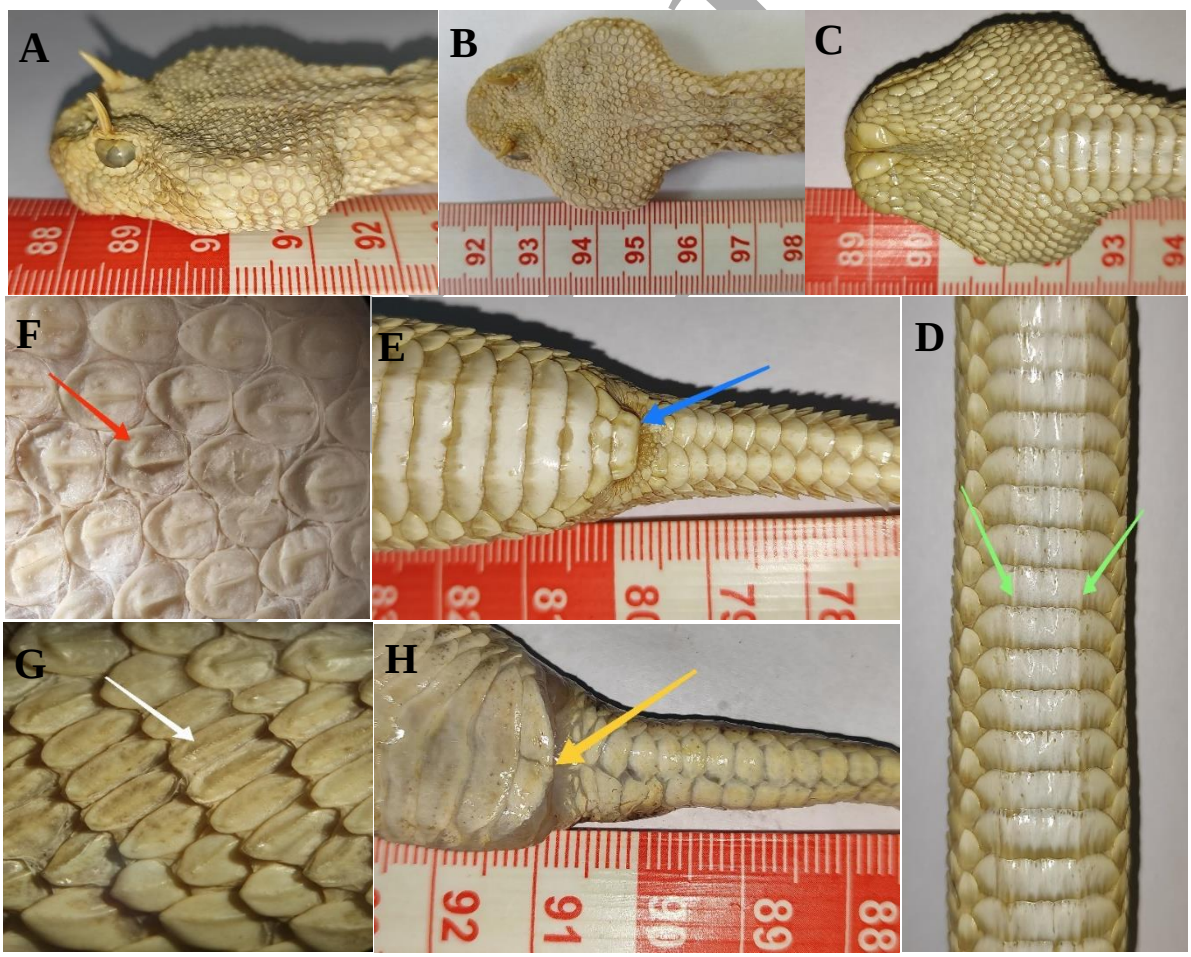

Figure 3. Body aspects of *C. gasperettii* , A)Lateral aspect of head (Horned morph) B) Dorsal aspect of head C) Ventral aspect of head D) Keeled scales of ventral body (Green arrow) E) Single anal plate (Blue arrow) H) Divided anal palte (Yellow arrow) F) Dorsal Keeled similar to the ship's anchor (Red arrow) G) Lateral saw keeled scales (White arrow). (Photo by Ali Salemi).

Table1. **Meristic and mensural characters ,** DS1 (one-head length behind the head), DS2 (rows of scales at mid-body), and DS3 (five ventral scales anterior to anal plate) [4,10].

Meristic and mensural characters	**Abbreviations**
Dorsal scales	DS1 , DS2 , DS3
Ventral scales	VS
Subcaudal scales (Pair)	SCS
Upper labial	ULS
Lower labial	LLS
Interorbital	IOS
Circumorbital	COS
Oblique serated lateral scales(Row)	LOS
Between upper labial and circumorbital scales	ULCO
Body length (mm)	
Tail length (mm)	
Head length (mm)	
Head width (mm)	
Head altitude (mm)	
Sex	
Horn	
Horn length (mm)	
Anal plate type	
Ventral scales type	

3. Result

A total of museum and live specimens of *Cerastes gasperettii* from Khuzestan Province (Albaji region and Bostan area) were examined. Both meristic and mensural characters were recorded and are summarized in Table 2.

Table2. Meristic and mensural characters of Iranian *C. gasperettii*.

characters	RIC01	RIC02	RIC03	RIC04	RIC05	RIC06	RIC07	RIC08	RIC09	RIC10	RIC11
DS	24:31:22	23:31:23	24:31:24	26:33:23	24:31:25	25:31:25	24:33:25	23:31:25	25:33:25	26:33:23	25-31-24
VS	147	155	156	164	156	166	164	158	142	147	158
SCS	35	35	34	35	35	35	34	35	36	35	30
ULS	13-13	15-14	15-14	12-12	13-15	16-16	16-15	15-15	12-13	16-16	16-16
LLS	14-14	16-16	15-15	15-16	14-15	17-16	16-15	15-15	15-14	16-16	16-16
IOS	18	10	13	11	12	15	11	11	12	12	12
COS	15-15	14-14	13-13	15-14	17-16	17-17	14-14	15-15	15-14	15-15	14-14
LOS	5	5	4	4	5	5	4	4	7	6	6
ULCO	4-4	4-4	4-4	4-5	5-5	4-5	5-4	5-5	4-4	4-4	4-4
Body length (mm)	600	570	700	530	640	740	620	530	660	700	340
Tail length (mm)	69.5	66.6	82.5	57	70.8	20	66.6	73.2	70	70.5	30
Head length (mm)	33.6	28.2	37.4	29.5	36.1	37.1	32.8	28.4	30.6	30	10.6
Head width (mm)	29.3	24.4	30.9	26.6	19.6	28.9	27.1	25.2	30.1	20.8	10.7
Head altitude (mm)	13.2	8. 8	15.7	12	13.5	11.4	10.5	10. 7	10. 3	10	3
Sex	♂	♀	♂	♀	♂	♀	♀	♂	♂	♂	♀
Horn	-	+	+	+	-	-	+	+	+	-	-
Horn length (mm)		6.4	6.6	5.1			6.5	5.7	5		
Anal plate type	Single	Divided	Divided	Single	Divided	Divided	Divided	Divided	single	Divided	Divided
Ventral scales type	keeled	keeled	keeled	keeled	keeled	keeled	keeled	keeled	keeled	keeled	keeled

3.1 Anal Plate Condition

Two types of anal plates were observed among the examined specimens: single (entire) and divided. The divided anal plate was the predominant condition. Based on the examined material, 27.2% of specimens possessed a single anal plate, whereas 72.8% exhibited a divided anal plate (Table 3). Both conditions occurred within the same geographic population.

Table3. characters differences

characters	Number of specimens	Percent (%)
Anal single plate	3	27.2
Anal divided plate	8	72.7
Horn	6	54.5
Hornless	5	45.4
DS2	31	63.6
	33	36.3

3.2 Supraocular Horn Morphology

Both horned and hornless morphs were present in the studied population (Figure 4; Figure 1). Horned individuals constituted 54.5% of the examined specimens, while 45.5% lacked supraocular horns. No additional morphological differences were detected between horned and hornless individuals aside from the presence or absence of supraocular projections. Both morphs were found sympatrically within the same sandy habitats.

3.3 Dorsal Scale Rows at Midbody (DS2)

All examined specimens possessed either 31 or 33 dorsal scale rows at midbody (DS2). No specimens with 32, 34, 35, or 36 dorsal scale rows were recorded. Among the studied individuals, 63.6% exhibited 31 dorsal scale rows, while 36.4% had 33 dorsal scale rows (Table 3).

3.4 Ventral Scales

Ventral scale counts were consistent with published identification keys for *C. gasperettii*. All examined specimens possessed more than 147 ventral scales in males and more than 152 in females, corresponding to diagnostic criteria for the species.

3.5 Habitat Observations

All live specimens were recorded from sandy habitats without mountainous terrain. Individuals were observed on low sand dunes as well as flat sandy substrates. No specimens were found in rocky or mountainous areas. Microhabitats included shifting sand and semi-stabilized sandy areas with sparse vegetation.

3.6 Cannibalism Observation

During field examination, one live specimen regurgitated a smaller individual of *C. gasperettii*. This observation confirms the occurrence of cannibalistic behavior in this species within Iran.

3.7 Diagnostic Characters from Shed Skins

Shed skins collected from the natural habitat showed strongly keeled dorsal scales with an anchor-shaped appearance. Ventral scales exhibited longitudinal keels, a distinctive feature observable in the shed integument. These characters allow identification of *C. gasperettii* from shed skins found in sandy habitats.

4. Discussion

This research was designed in order to raise the level of morphological information from different specimens of *C.gasperettii* in distribution areas in Iran.

According to Latifi, (2000) and Rajabizadeh, (2018) there are single and divided anal plates (Figure 4, E&H) in C. gasperettii, both types of scales were observed in this research, and according to table3, 27.2% of the specimens had single scales. This issue identifies an important issue only in this research that most of the specimens are of divided type. If we consider this characteristic as an individual trait, then according to the data of this research, divided anal plate appear more than single ones. Of course, Wagner and Wilms, (2010) only mentioned anal plate entire in this species.

According to Mochales-Riano et al, (2023); Latifi (2000); Werner, (1991) *C. gasperettii* and *C. cerastes* have two morphs, horned and hornless,(Figure 4, A & Figure1, A&B) while the species *C. vipera* is hornless in all morphs. This trait is considered one of the most important traits to identify these species. In this research, both horned and hornless morphs were present in the investigated specimens. According to Table 3, 54.5% of the specimens studied here had horns above their eyes. Due to the fact that the presence of horns above the eyes creates a different appearance, there may be a mistake in identification that these are different species and not two morphs of the same species, but both belong to the same species. Therefore, the number of specimens found in this research was higher for horned individuals than those without horns.

In this study, all the specimens had 31 and 33 DS2, among them, 63.6% of the specimens had 31 DS2 (Table3). Latifi (2000) and Rajabizadeh (2018) stated the number of dorsal scales in the middle of the body in the range of 31-36. Of course, the DS2, 31-33 are mentioned by Fathinia et al (2010). It should be mentioned that a specimen with numbers different from 31 and 33 was not found in this research. According to Rhadi et al (2015), *C. gasperettii* in the middle of the body is in the range of 32-35, and the specimen studied in that study was the 35 DS2, According to the Amr et al (2022) the number of DS2 in C. gasperettii is stated to be 31 to 35. In general, according to Rhadi et al (2015), it can be said that the genus *Cerastes* has 25 to 35 DS2. According to these data, it can be concluded that numbers other than 31 and 33 are found less in Iran. More specimens are needed to see different results.

In terms of natural environment, all living specimens in this research were found in sandy areas without mountains. Some were on short sand dunes and some were in a sandy flat environment. I must mention that Sand dunes often comprise three distinct microhabitats: shifting sand free of vegetation, vegetated semistabilized areas and fully vegetated stabilized ones [17] And in each of these places, adapted fauna lives.

According to the Barros et al (2019), *C. cerastes* and *C. gasperettii* are found in sandy areas without high mountains. According to this issue, sand plays a very important role in the life process of *C.gasperettii*. Because the diet of this snake also lives in the sand, Such as Scincus, *Stenodactylus*, *Acanthodactylus* [3] which are considered sand lizards in Iran. For this reason, when the sands are destroyed and they are contaminated with oil particles, both the prey and the predator are harmed.

In this research, we also want to report on cannibalism in this species. One of the living specimens in the natural habitat vomited up another C. gasperettii of small size during a

morphological study in the natural environment. Based on this, we found out that this species also has a type of cannibalism in Iran.

To identify *C. gasperettii* from the shed skins as a result of molting, in the first step, you can look at the keeled dorsal scales on the skin, which should be similar to a ship's anchor, and in the next step, the keeled scales on the abdominal aspect (Figure 4, F,G&D). In this species, are present on the ventral scales (VS), longitudinally, and the traces of these scales are left on the desert sands. These ventral keeled scales are only found in *C.gasperettii* and *Eristicophis macmahonii* of snakes in Iran (Figure 4, D). So, as a result, *C. gasperettii* can be identified from the skin found in the natural habitat.

Key identification to the Genus *Cerastes* :

1a- Eyes on the upper part of head; average size 35 cm (max 50),
less than 120 ventral scales ..... *C. vipera*
1b- Eyes lateral; average size 50-60 cm, sometimes > 80 cm;
more than 130 ventrals ……..… 2
2a- Ventral scales less than 147 in males, less than 152 in females;
at least 1 pair of enlarged tubercles in the occipital area
on medial line, just before the eyes; 3 dark spots above the maxilla ……*C. cerastes*
2b- Ventral scales more than 147 in males, more than 152 in females; no enlarged tubercles in the occipital area; dark spots on upper maxilla
absent……. *C. gasperettii* [19]

5. Conclusion:

This research was based on the collected specimens as well as the specimens available in the collection of Razi vaccine and serum research institute, Karaj-Iran and the results provided us with valuable morphological characteristics for future studies. For further studies, it is suggested to conduct molecular research on this species in Iran. because the zoological data on this species is very scarce.

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Ethics:

Ethics committee of the Razi Vaccine and Serum Research Institute (No. RVSRI.REC.98.016).

Data availability:

All data during this study are included in this article.

Authors' contributions:

Conceptualization: Specimens collection, Methodology, analysis, writing, Review and editing: Ali Salemi

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