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Research Article

Nuchal scalation patterns in Iranian Mugger crocodiles (*Crocodylus palustris* Lesson, 1831) point to an overlooked conservation unit

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Abstract

Nuchal scute patterns in crocodilians are important for distinguishing species and populations, particularly isolated ones. The study focuses on nuchal scute numbers and patterns of Mugger Crocodile (*Crocodylus palustris* (Lesson, 1831)) across its distribution in Iran, India, and Sri

Lanka. Data was collected from 146 crocodile photos, analyzing both the numbers and patterns of nuchal scutes. Nonparametric methods were employed due to non-normal data distribution. The Binomial Test indicated a significant deviation from the typical six-scute pattern (about 23–35% of individuals showed variation), with notably higher diversity in Iranian populations (50%). Confidence intervals for scute patterns were 0.41-0.59 in Iran and 0.01-0.13 in India, and Sri Lankan crocodiles conformed to the six-scute pattern. A total of 18 different scute patterns were identified, with unique patterns observed in Iranian crocodiles. The Kruskal-Wallis test revealed significant differences in scute patterns across all data and specifically in Iran. The Mann-Whitney U test indicated differences between the typical scute pattern and others, except for a few specific patterns. Iran exhibited the highest variation in nuchal scute patterns, with diversity increasing towards the north and west. Iranian mugger crocodile populations face threats from flooding, drought, and climate change, necessitating further studies for conservation planning. Also, there is concern about potential genetic bottlenecks due to isolation, highlighting the need for genetic investigation and its possible relationship with morphological diversity.
Keywords: Conservation unit, Identification guide, Morphological diversity, Mugger crocodiles, Nuchal scutes

Introduction

The traditional ecological research approach typically focuses on an individual's responses in a given environment (Hertel et al., 2021). Recent research has emphasized the significance of individual-level estimations, as each organism's reaction is affected by its specific ecological condition, behavioral encounters, or physiological status (Goumas et al., 2020). Collecting data on individuals allows for a better understanding of processes and patterns, which can improve conservation efforts (Petso et al., 2021). Nuchal Scutes number and patterns are used as an identification tool for crocodilian species (Balaguera-Reina et al., 2017; Desai et al., 2022; Forero Sr et al., 2019; García-Grajales et al., 2009; Platt et al., 2010; Seijas, 2002; Treeprapin et al., 2024; Velasco & De Sola, 2022; Velasco Barbieri, 2020) (Fig. 1 (A)). However, not much is known about the morphological variation of the mugger crocodile (*Crocodylus palustris* Lesson 1831) scute patterns (Anderson, 1979; Boulenger & Blanford, 1890; Minton, 1966).

The mugger or Marsh crocodile is a medium-sized crocodile, with adult females attaining lengths from 2.0 to 2.5 m and males from 3 to 3.5 m on average. Endemic to the Indian sub-continent, Marsh crocodiles live in freshwater and brackish water habitats (Deraniyagala, 1953, 1939). The species' distribution extends from southeastern Iran, locally named as Gando, eastward to Pakistan and India, and from Nepal in the north to Sri Lanka in the south (Choudhury & De Silva, 2013; Mobaraki et al., 2021). The conservation efforts for mugger crocodiles are limited as the species mostly lives outside protected areas (Mobaraki et al., 2021; Voluntary Nature Conservancy, 2020; Vyas, 2018), and the muggers are severely at risk due to intense conflict with the adjacent settlements (Vyas, 2018). As a globally threatened species listed as Vulnerable in the IUCN Red List, the species' total population exceeds 10,000 non-hatchlings (Choudhury & De Silva, 2013). It is predicted that the environmental conditions for this species will worsen in the future because of the effects of climate change, leading to more habitat fragmentation (Mobaraki et al., 2023).

The mugger crocodile was first described by Lesson in 1831, with the type locality of the Ganges River. In his description of the type specimen, Lesson mentions the nuchal scutes, specifically a pattern on the dorsal region of the neck with four enlarged scales forming a square and with one smaller scale on each side of the square, comprising six nuchal scutes (Lesson, 1831) (Fig. 1 (B)). Over subsequent years, as other authors described further specimens, more detailed accounts of the scalation of the mugger crocodile were presented. Boulenger and Blanford (Boulenger & Blanford, 1890) provided a more detailed description and clearly stated that there are four large square-shaped nuchals with one smaller one on each side, totaling six nuchals. However, Minton (Minton, 1966) mentioned the number of nuchals as five scutes. In the Catalogue of the Tortoise, Crocodiles, and Amphisbaenians in the collection of the British Museum (1844), it has been mentioned that a half-grown stuffed mugger crocodile had 4 nuchal plates, which seems probably the four central plates considered as the nuchals, ignoring the more minor scales on either side. In the diagnosis provided for the species by Anderson (Anderson, 1979), he mentioned four or five large nuchals, referring to Minton. Patterns of nuchal scale variation have been used for the identification of crocodilian species of the world, in which the nuchal scalation for mugger crocodiles is described as four large scales in a square with a small scale on each side, forming a cluster. In general, the taxonomic classification of extant crocodilians (Alligatoridae, Gavialidae, and Crocodylidae) relies on morphometric and meristic characteristics for species differentiation, with particular emphasis on the scalation patterns of the nuchal region (see Wermuth and Fuchs (Wermuth & Fuchs, 1978)).

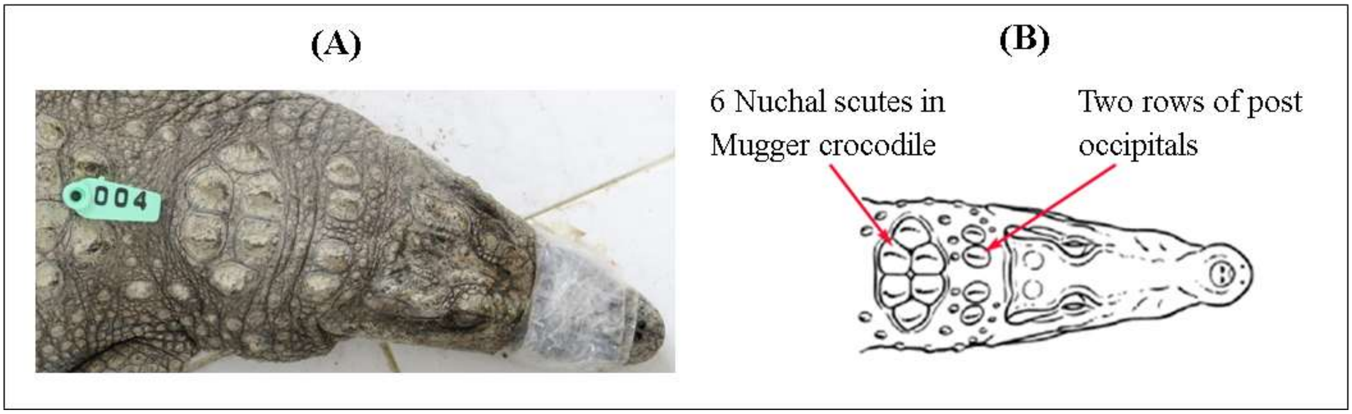

Figure 1 Nuchal scutes pattern in a mugger crocodile from Iran (A) and Identification characters for the mugger crocodile, CITES guidelines, 1995 (B)

Wermuth and Fuchs (Wermuth & Fuchs, 1978) demonstrated the key role of nuchal scutes in crocodile species identification. The related drawing for the Mugger clearly shows the six nuchals, four in the middle and one on each side (Figure 1). This is also reflected in some international guidelines, namely, the CITES identification guide, Crocodilians (CITES, 1995). In this guide, the illustration for the mugger crocodile also shows six nuchals (Fig. 1).

The small population of the Mugger crocodile in Iran, with an estimated size of 500 individuals (Mobaraki & Abtin, 2013) and being the westernmost population of the species, has been isolated from other populations, living in harsh conditions. It is hypothesized that this isolation has resulted in possible genetic or phenotypic variations. Until now, the nuchal scute variation of Mugger crocodiles has not been evaluated using the number and patterns to investigate morphological variations. We herein conducted a morphological study using the nuchal scute patterns as a diagnostic feature to investigate potential variations between the eastern and western populations.

Material and methods

Study sites

The photographic survey of nuchal scutes patterns of mugger crocodiles was conducted in three countries: India (at the Madras Crocodile Bank Trust and Centre for Herpetology in the east, along with two additional sites in Gujarat State), Iran (Gando Protected Area), and southern Sri Lanka (Fig. 2). The present study covered the entire known geographic range of Crocodylus palustris, extending from its westernmost populations in Iran to the easternmost populations in India and Sri Lanka, and from northern to southern limits of occurrence. Sampling sites were selected based on the availability of reliable photographic records that allowed clear observation of nuchal scutes.

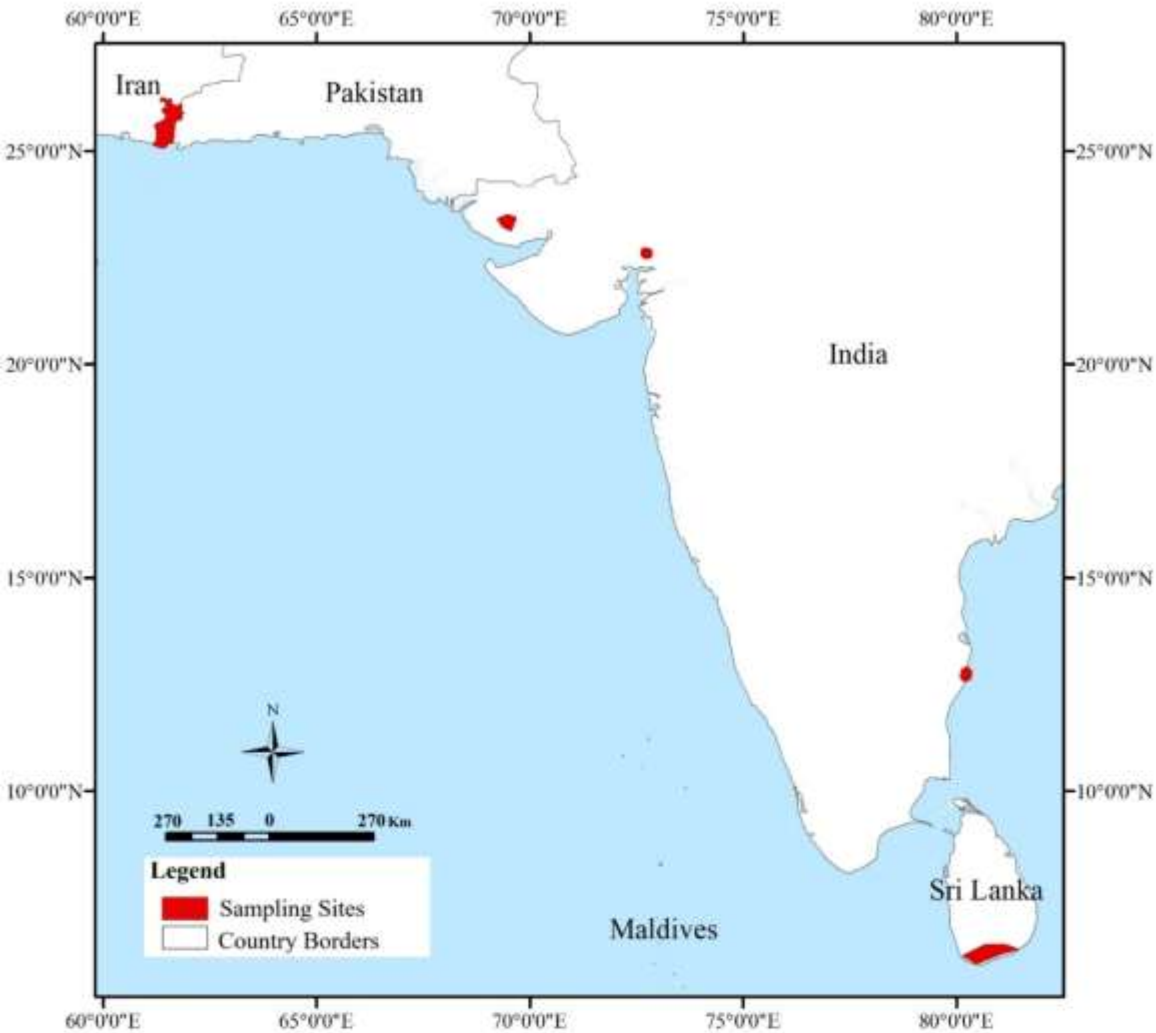

Figure 2. Distribution of study sites

Data acquisition

We collected photographs of the nuchal scutes patterns from mugger crocodiles > 1.5 m in length and some juveniles between 2021 and 2022 across the species' distribution range. A total of 76 individuals were studied based on photographs from Iran, 63 from India, and 12 from Sri Lanka. All photographs used in this study were taken from live mugger crocodile individuals. Most photographs represented wild individuals observed in their natural habitats in Iran, India, and Sri Lanka. In addition, a small number of photographs from India were obtained from captive individuals maintained in recognized facilities. The photographs were improved with Photoshop to enhance the visibility of the numbers and patterns of the nuchal scute clusters for classification. The nuchal clusters were categorized into five groups according to the number of scutes: four, five, six, seven, and eight nuchal scute types. Within each category, scute combination patterns were determined, and the frequency of each pattern was noted in each category.

Data analysis

As a first step, descriptive statistics of the data (number of nuchal scutes in each crocodile) were obtained, followed by checking the normality of data using Kolmogorov-Smirnov and Shapiro-Wilk. The results of the normality tests indicated that the data did not follow a normal distribution. Specifically, the Kolmogorov–Smirnov test yielded a statistic of 0.386 ($df = 151$, $p < .001$), and the Shapiro–Wilk test yielded a statistic of 0.722 ($df = 151$, $p < .001$), both of which were statistically significant. Hence, nonparametric methods were used. As we aimed to find any bias from the combination of the 6-scutes pattern, the Binomial Test of the nonparametric method was utilized.

We used a Binomial test to determine whether the deviations from a theoretically expected distribution of observations into two categories are equivalent to the predicted proportion or not. Using the different proportions of six-scute and non-six-scute samples, we decided on the meaningfulness threshold of the proportion of the two patterns. At a 95% confidence level, a P-value less than 0.05 rejects the null hypothesis, indicating that our suggested proportion for the community should not be correct. After defining the nuchal scute patterns, the frequency of each pattern was determined for different countries (sampling sites). Deviation from the six scutes as the main pattern (4 at center, two up and two down, and one on each side, as described above) was tested using nonparametric Kruskal-Wallis and Mann-Whitney U test methods to compare more than two independent samples and two independent samples, respectively. Also, the difference in the number of nuchal scutes between the countries was compared. Sign Test was used to show the negative (-) and positive (+) deviation from 6 scutes. In this regard, a new variable was defined with a fixed number 6 to show the + or – deviations from the number of 6. In this test, the numbers for six scutes did not affect the result (no deviation from six scutes).

Results

Descriptive statistics for several nuchal samples are presented in Table 1 for all data and all countries, based on which samples from Iran have the highest mean and standard deviation, although the mean of all sample sites is close. This table represents the frequency of numbers of nuchal scutes in different countries. It indicates the frequent representation of six scales in the whole data and also the high diversity of the number of scales in Iran.

Table 1. Descriptive statistics of the number of nuchal scutes

	Number of individual crocodiles	Number of nuchal scutes			
		Minimum	Maximum	Mean	Std. Deviation
Iran	76	4	8	6.15	1.04
India	63	5	7	5.97	.25
Sri-Lanka	12	6	6	6.00	0.00
Total	151	4	8	6.06	.77

Deviation from the six-scute standard pattern

The data distribution did not show normality, so 151 data points were plotted to two main groups: those with six scutes (104 samples with 71.2% frequency) and those with non-6 scutes (more than or less than six scutes) (42 samples with a frequency of 28.8%) and 14 hypotheses (0.50, 0.40 , 0.39 ,0.38 ,0.37, 0.36 ,0.35 ,0.30 ,0.25 ,0.24 ,0.23 ,0.22 ,0.21 and 0.2% of crocodiles do not have six scutes) were tested. The applied test proportions were selected to show the significant thresholds. Based on the results presented in Table 2, the ratio of non-six to six-scute pattern crocodiles in the community may not be larger than 36% as well as less than 22% ($P<0.05$), and we shall state that 23-35% of crocodiles have more than or less than six nuchal scutes. The test was repeated for India and Iran separately; more than 28 hypotheses were tested. In Iran, 50% of crocodiles had more or fewer than six nuchal scutes. These 95% confidence interval thresholds are 0.41 to 0.59 in Iran and 0.03-0.13 in India.

Table 2. Results of the binomial test with different test prop.

Total data		Iran		India	
Test Prop.	p-value (1-tailed)	Test Prop.	p-value (1-tailed)	Test Prop.	p-value (1-tailed)
0.50	0.00	0.64	0.01	0.18	0.01
0.40	0.00	0.63	0.01	0.17	0.01
0.39	0.01	0.62	0.02	0.16	0.02
0.38	0.01	0.61	0.03	0.15	0.03
0.37	0.02	0.60	0.05	0.14	0.05
0.36	0.04	0.59	**0.07**	0.13	**0.08**
0.35	**0.07**	0.50	**1**	0.12	**0.11**
0.30	**0.41**	0.41	**0.07**	0.1	**0.23**
0.25	**0.17**	0.40	0.05	0.08	**0.43**
0.24	**0.11**	0.39	0.03	0.06	**0.53**
0.23	**0.06**	0.38	0.02	0.04	**0.25**

0.22	0.03	0.37	0.01	0.03	**0.12**
0.21	0.02	0.36	0.01	0.02	0.04
0.2	0.01	0.35	0.01	0.01	0.00

Sig. %5	Sig. %1	**Bold=non sig.**

Deviation from the typical, common pattern (6 scutes)

In total, 18 distinct patterns were identified (Fig. 3; in all figures, the head of the animal is above the highlighted scutes). The number of scutes ranged from four to eight. Scutes numbers four and eight were reported only from Iran, with a high pattern variety (Fig. 4). The samples from India and Sri Lanka, with six scutes, had the same usual pattern (P6-1). Very few samples had five or seven scutes, but with only one pattern. So, the highest variety in the number of scutes and patterns was observed in Iran (Figs 3 and 4).

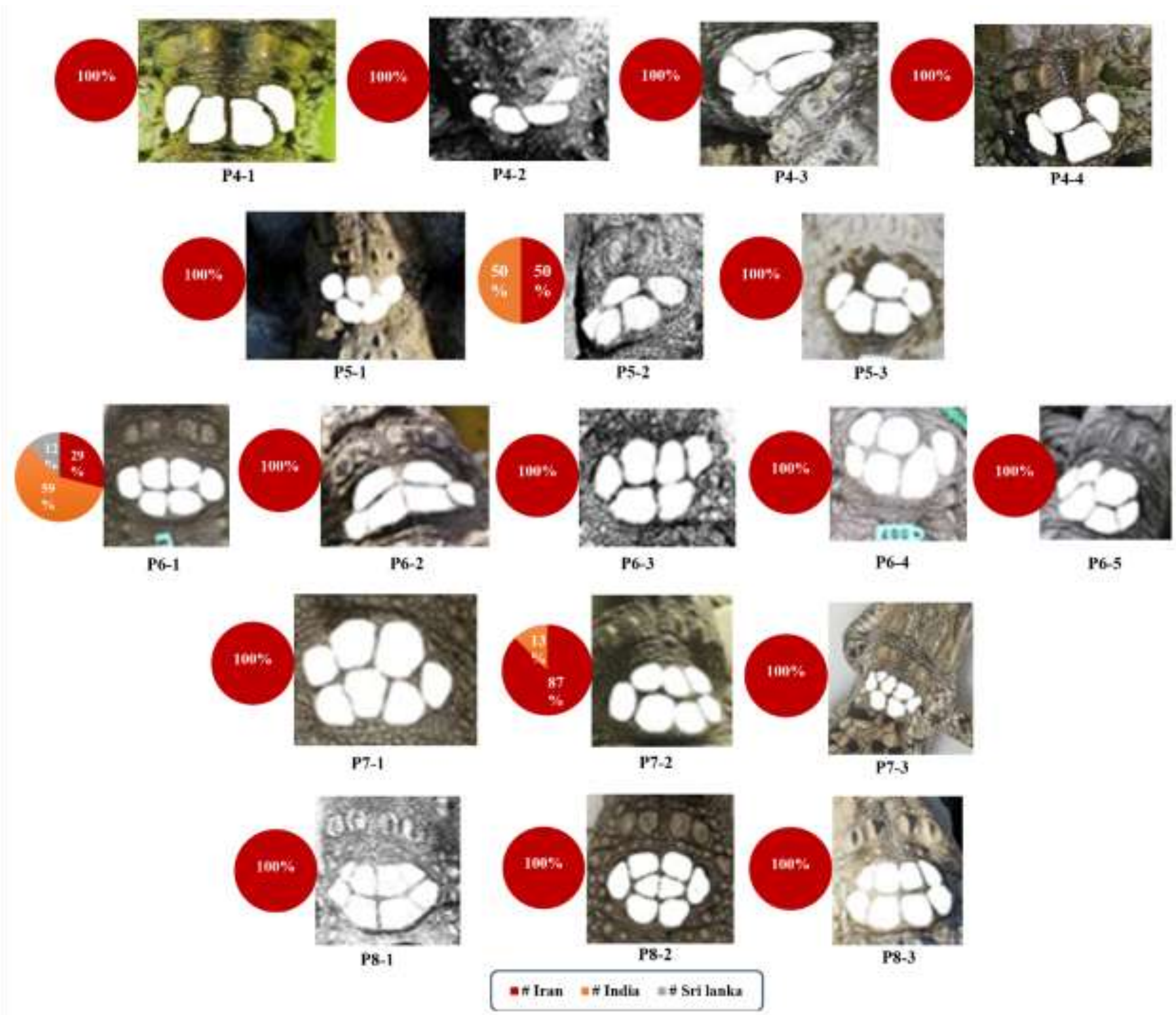

Figure 3. Patterns and frequency of nuchal scutes for specimens with four scutes

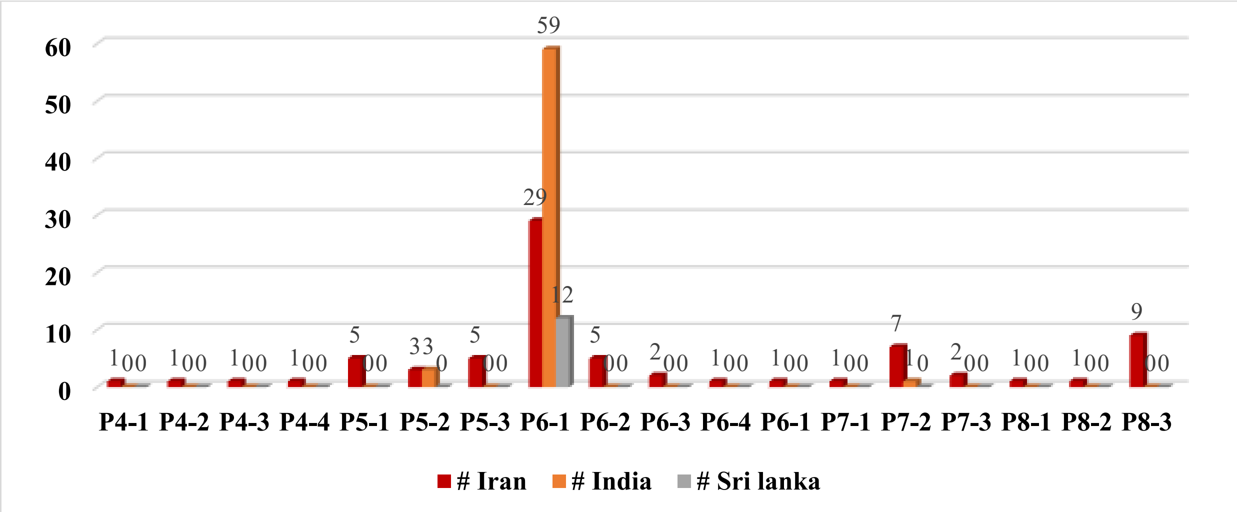

Figure 4. Frequency of nuchal scutes pattern in mugger crocodile specimens

Kruskal-Wallis test showed that there is a significant difference between 18 scale patterns for all data (Kruskal-Wallis H=145.000, df=17, P=0.000) and Iran (Kruskal-Wallis H=75.000, df=17, P=0.000). Mann-Whitney U test shows the difference between the typical scutes pattern (P6-1) and other scutes patterns except P6-2, P6-3, P6-4, and P6-5 in the whole data and Iran (Table 3).

Table 3. Results of the Mann-Whitney U test

Paired pattern with P6-1	Total		Iran	
	Mann-Whitney U	P value	Mann-Whitney U	P value
P4-1	0.00	0.00	0.00	0.00
P4-2	0.00	0.00	0.00	0.00
P4-3	0.00	0.00	0.00	0.00
P4-4	0.00	0.00	0.00	0.00
P5-1	0.00	0.00	0.00	0.00
P5-2	0.00	0.00	0.00	0.00
P5-3	0.00	0.00	0.00	0.00
P6-2	237.50	1.00	72.50	1.00
P6-3	95.00	1.00	29.00	1.00
P6-4	47.50	1.00	14.50	1.00
P6-5	47.50	1.00	14.50	1.00
P7-1	0.00	0.00	0.00	0.00
P7-2	0.00	0.00	0.00	0.00
P7-3	0.00	0.00	0.00	0.00
P8-1	0.00	0.00	0.00	0.00
P8-2	0.00	0.00	0.00	0.00
P8-3	0.00	0.00	0.00	0.00

Positive and negative deviation from six scutes

Based on the results of the Sig test (Table 4), the number of positive and negative deviations from 6 scute deviations for all data and for Iran was not significant; hence, there is no specific tendency to increase or decrease the number of scutes. This test does not consider the number of deviations from 6 scutes, and only the number of + and – is interpreted. Of the total sum of samples with more than six scutes (33) and less than six scutes (24), the number of samples for Iran was 32 and 21, and for India, 1 and 3, respectively. This indicates that changes in the number of nuchal scutes in Iran are ascending and, in India, vice versa, descending.

Table 4. Sign Test results

Regions	# Negative Differences (value < 6)	# Positive Differences (value > 6)	Ties (value = 6)	Z	P value
Total	22	20	104	-0.15	.88
Iran	21	17	38	-0.49	0.63
India	1	3	59	-	-

Discussion

Nuchal scutes patterns in mugger crocodiles in their distribution range in Iran, India, and Sri Lanka were studied, and the highest deviation from the diagnostic feature of six scutes was observed in Iran, as the northwestern most marginal range for the species. In crocodiles with a 6-scute nuchal arrangement, the pattern of four at the center and one on either side was observed in all samples from India and Sri Lanka; in contrast, the samples with six scutes from Iran had five different patterns, as well as a variety of patterns with either more or less than six scutes. Crocodiles with four and eight nuchal-scutes arrangements were only observed in samples from Iran, indicating that mugger populations in Iran have the highest variation in the number and pattern of nuchal scutes as a diagnostic feature.

Sri Lanka had minimal variation in nuchal scute patterns at the lowest latitude. However, we had just a small number of samples (N=12). Limited data from Sri Lanka and lack of Pakistan, thus clearly is a limitation to this study. According to our sample size, moving to the north and west, the diversity of the patterns increases, too.

Scutes pattern variation is also known from the American Crocodile (*Crocodylus acutus*), where it is used to identify individuals (Balaguera-Reina et al., 2017; Forero Sr et al., 2019; García-Grajales et al., 2009; Platt et al., 2010; Seijas, 2002), with analogous studies conducted on Siamese crocodiles (*Crocodylus siamensis*) (Treeprapin et al., 2024) and Orinoco crocodile *(Crocodylus intermedius)* as well (Velasco Barbieri, 2020). Also, an investigation into the diversity of scute patterns in the Orinoco Crocodile (*Crocodylus intermedius*) has been undertaken (Velasco & De Sola, 2022). The patterns of nuchal scalation in mugger crocodiles compared to other crocodile species especially American crocodile, remains unknown and this research is the first attempt to address this issue. Moreover, it raises the interesting question whether the deviating nuchal scute pattern in Iranian mugger crocodiles is due to individual variation or rather because of separation from the remaining populations and thus also reflected by genetic divergence. The Iranian population has also been divided into several scattered sub-populations (Mobaraki et al., 2015). Also in this respect, genetic research will be important, as it could show whether there is still exchange and thus gene flow between subpopulations, or not, then resulting in potential or already existing genetic bottleneck effects due to inbreeding, which also could lead to diverging scute patterns.

Finding the crocodiles out of the water in the right position, or their head completely exposed out of the water surface, to take proper photographs of nuchal scutes, as well as the lack of proper museum specimen remains, as the most important limitations for such studies (Balaguera-Reina et al., 2017).

In addition, obtaining photographic data across the wide geographic range of mugger crocodiles was another key challenge. Due to limited accessibility, sampling sites in India and Sri Lanka—although representing major populations—did not fully encompass the species' entire distribution and were restricted to locations where suitable photographic data were available. This was particularly pronounced in Sri Lanka, where the species is known to have high population sizes, but only a small number of individuals could be included in the dataset. Hence, insufficient availability of quality photographs and lack of full geographic coverage were the major limitations of this study. While for the other species like American Crocodile (*Crocodylus acutus*), many studies proved the diversity in scute patterns which could be used for identification at the individual level (Balaguera-Reina et al., 2017; Desai et al., 2022; García-Grajales et al., 2009; Platt et al., 2010; Seijas, 2002), this study may be is the first attempt to prove that mugger crocodile has divers nuchal scute numbers and patterns.

The lack of range-wide genetic assessment of muggers makes it challenging to determine whether scute variability between different populations also indicates variation at the molecular level. An analysis using a limited number of samples and 750 bp of the mitochondrial control region showed several substitutions and indels between the Iranian population and other samples from previous studies, while all sequences from Iran were found to be identical (Mobaraki et al., 2015). Unfortunately, as the data obtained from GenBank did not have associated locality and morphological information, it is still unclear what parts of the species range the samples are from and if the genetic divergence underlines morphological differences. We recommend that future studies investigate the genetic diversity of muggers across its range using both mitochondrial and microsatellite markers (e.g., Aggarwal et al., 2015; Campos et al., 2018).

Mugger crocodile populations in Iran are under severe stress from flooding, drought, and other effects of climate change, and these stressors are predicted to increase in the future too (Mobaraki et al., 2023). Thus, molecular analyses seem really important to be conducted in the future, because if the Iranian population turns up to be a distinct taxonomic lineage and thus a distinct conservation unit, or rather consists of isolated subpopulations developing a genetic bottleneck, both findings will have an impact on conservation as different conservation strategies will have to be developed. The use of other Scutes, which has been extensively utilized in studies concerning the other crocodiles such as Dorsal (Balaguera-Reina et al., 2017; Desai et al., 2022), back, and post occipital (Forero Sr et al., 2019; Velasco & De Sola, 2022) are suggested for subsequent observational research. It is also recommended to use deep machine learning methods to identify similar recent researches (Balaguera-Reina et al., 2017; Desai et al., 2022; Ghamemi RaeiniNejad & Sadeghi, 2017) and less dangerous photography methods for sampling.

Conclusion

Nuchal scute patterns in the Mugger crocodile across its distribution range in Iran, India and Sri Lanka were studied and the highest deviation from the diagnostic feature of six scutes was observed in Iran, as the northwestern most and marginal range for the species. The pattern of six scutes, a cluster of four enlarged scales at the center and one of variable size on either side (P6-1), was observed in all samples from India and Sri Lanka. In samples from Iran, those with 6 scutes had five different patterns, as well as a variety of patterns with more than or less than six scutes. Four- and eight-scute patterns only were observed in samples of Iran, indicating that Mugger populations in Iran have the highest variation in number and pattern of nuchal scutes as a diagnostic feature, making it worthy for further study to determine possible genetic variations between Muggers in Iran and populations from Sri Lanka and India. Sri Lanka, with the southern-most Mugger populations, had the lowest variety in nuchal scute patterns; moving further north and west within the range of the Mugger, the diversity of these nuchal patterns in the tested samples increases. Mugger crocodile populations in Iran are under severe stress of flooding, drought, and other effects of extreme habitat and climate change, and these stressors are predicted to increase in the future. Lack of data from Sri Lanka and Pakistan was a limitation to this study, and the tiny number of samples is to show a preliminary idea about the countries. Indeed more detailed comparison with adequate samples from Sri Lanka and Pakistan would make the situation clearer.

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