

Short Communication

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# Long-range relatives: on the status of the Bengal monitor, *Varanus bengalensis* (Squamata: Varanidae) from southeastern Iran

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Received:  
3 June 2024

Accepted:  
21 June 2024

The Bahu-Kalat (Gando) Protected Area is situated in extreme southeastern Iran, near the border with Pakistan. This area is characterized by arid mountain and semi-arid desert habitats that extend along the Sarbaz River. The climate in this region is consistently hot year-round, with minimal rainfall during the winter season. The fauna and flora in this area is typically Indo-Malayan (BirdLife International, 2024). Specifically, several species of the Iranian reptiles, such as *Calotes versicolor*, *Varanus bengalensis* and *Crocodylus palustris*, are restricted to this particular region (Anderson 1999; Mobaraki et al. 2023).

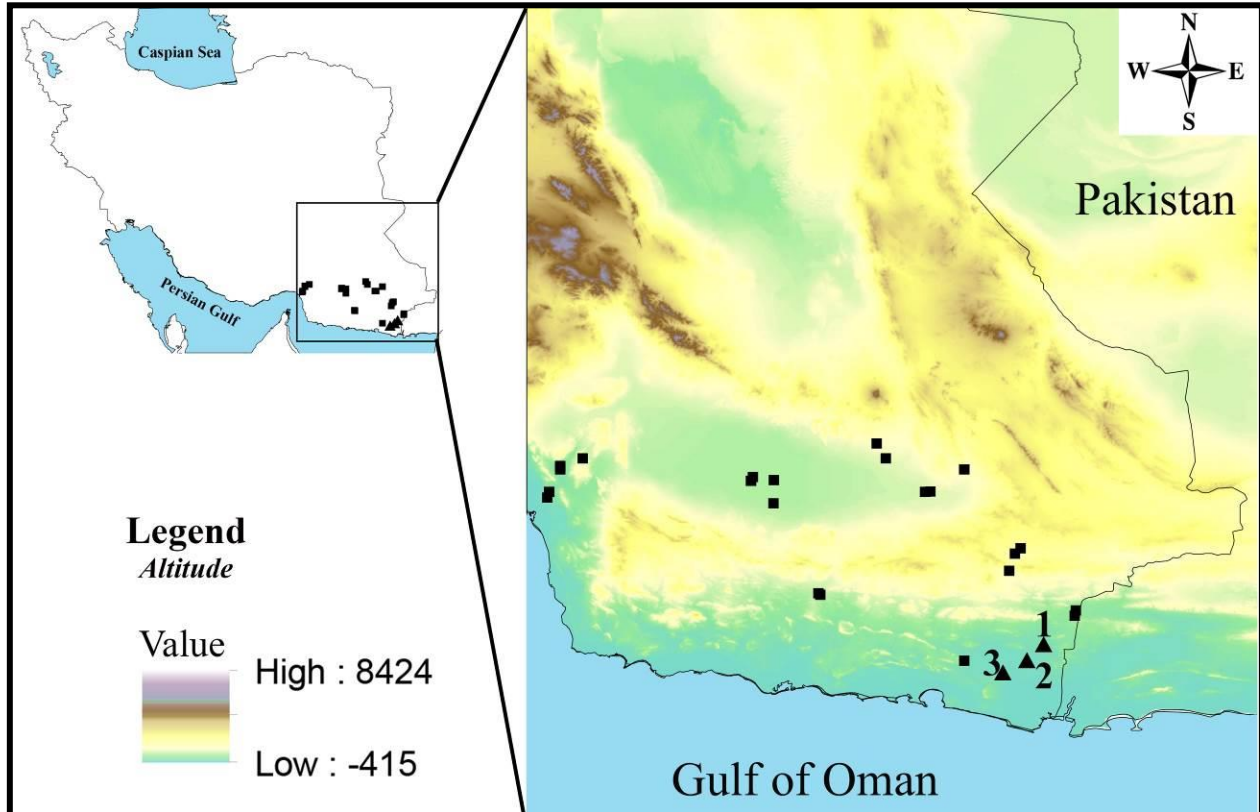
The Bengal monitor lizard, *Varanus bengalensis* (Daudin, 1802) is a species with a broad distribution, ranging from Iran to South Asia and throughout Southeast Asia (Papenfuss et al., 2010). It is a versatile species that can be found in various habitats such as forests, cultivated areas with dense vegetation and near inhabited houses (Shah and Tiwari, 2004; Masroor, 2012). It is classified as Least Concern on the IUCN Red List of Threatened Species (Papenfuss et al. 2010); but is listed in Appendix I of CITES (CITES, 2012). A recent molecular survey has revealed the presence of two separate lineages in *V. bengalensis* distributed across the Himalayan foothills and the rest of mainland India (Gautam et al. 2023). While there are some reports of the Bengal monitor lizard in southeastern regions of Iran (Anderson 1999; <https://www.gbif.org/>; Figure 1), details on the taxonomy and natural history of *V. bengalensis* in Iran are lacking. To address this gap, here we present new data from three distinct locations within the Bahu-Kalat (Gando) protected area in Sistan and Baluchistan, southeastern Iran.

Four tissue samples were obtained from dead individuals involved in road traffic accidents during surveys conducted in the Bahu-Kalat (Gando) protected area between 2010 and 2015. The specimens were discovered in different habitats at altitudes ranging from 100 to 150 meters above sea level. The names of the localities and their coordinates can be found in the legend of the Figure 1.

The tissue samples were preserved in 96% ethanol and kept at -20° in the Shahrekord University Herpetological Collection (HAC), Iran. Total genomic DNA was extracted using the salting-out method (Green and Sambrook, 2012). The mitochondrial COI gene was amplified using combinations of the following primers: L 1498, 5' -GGT CAA CAA ATC ATA AAG ATA TTG G-3' (Folmer et al. 1994) and RepCOI R, 5' -ACT TCT GGR TGG CCA AAR AAT CA-3' (Nagy et al. 2012). Reactions were set up in 20 µl volumes, each containing: 10 µl ready-to-use 2X Taq Master Mix (Kiagene Fanavar Aria, Cat Number: FPLF007.1000), 1 µl of each primer (10 pM/ml), 1-2 µl of DNA (>50 ng/ml) and 6-7 µl of DD water. The PCR protocol had the following temperature profile: denaturation at 95°C for 4 min; 36 cycles of 94°C for 40 s, primer annealing at 46°C for 40 s and sequence elongation at 72°C for 80 s; 72°C for 10 min, and subsequent storage at 4°C. The amplification products were sequenced on an automated sequencer ABI 3730XL (GeneAzma Genetic Group, Isfahan, Iran) according to standard protocols.

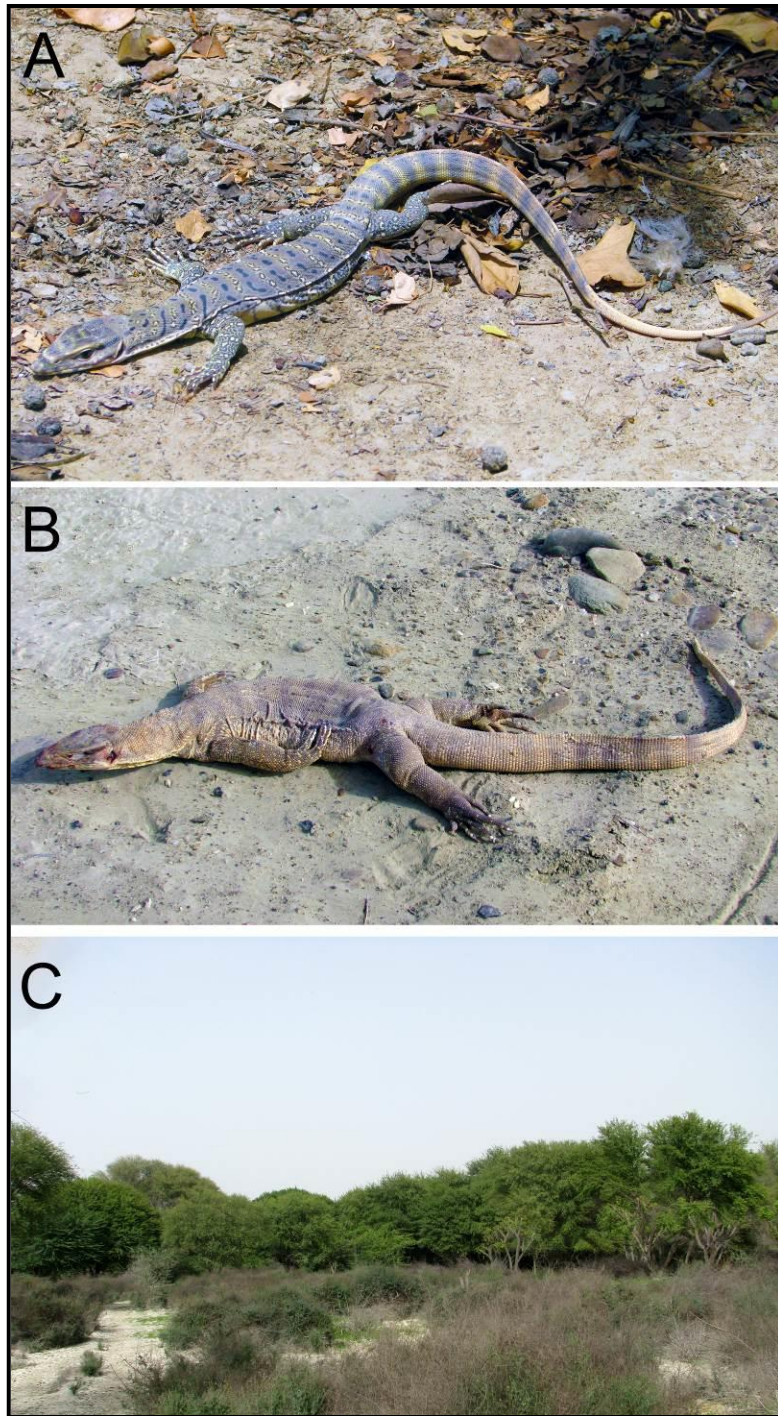
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**FIGURE 1.** Distribution of *Varanus bengalensis* in Iran. Squares, previous records (based on Anderson 1999; <https://www.gbif.org/>), triangles, sampling sites of the current study in Bahu Kalat (Gandu) Protected Area, southeastern Iran. 1: Around the village of Dompak ( $25^{\circ}42'09.0''\text{N } 61^{\circ}21'25.5''\text{E}$ ), 2: Around the village of Dargas ( $25^{\circ}50'39.4''\text{N } 61^{\circ}30'17.2''\text{E}$ ), 3: Around the village of Sedigh Zehi ( $25^{\circ}35'40.5''\text{N } 61^{\circ}08'36.9''\text{E}$ ).

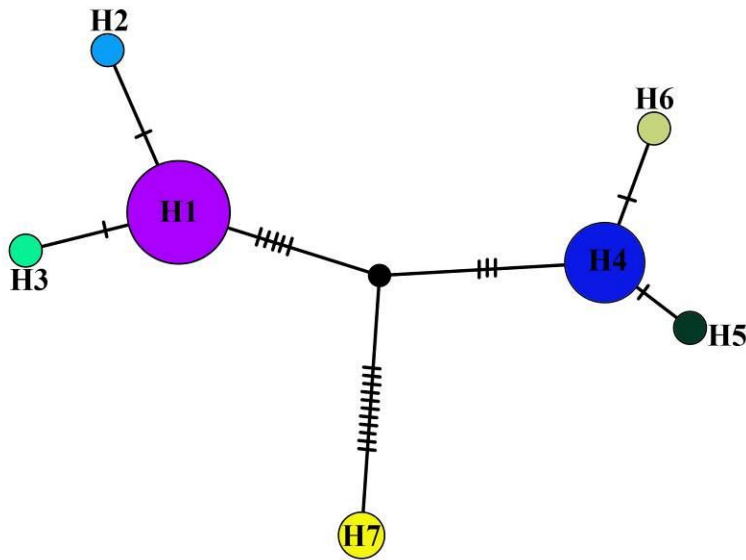
A 635 base pair segment of the mitochondrial COI gene was generated in this research and then deposited in the National Center for Biotechnology Information (NCBI) (PP400452 (HAC 1356); PP400453 (HAC 1357); PP400454 (HAC 1355); PP400455 (HAC 1359)). The novel sequences underwent similarity searches against the reference sequences using a BLAST search on NCBI (<http://blast.ncbi.nlm.nih.gov/>) and Barcode of Life Data (BOLD) platforms. We recovered 18 sequences of *V. bengalensis* samples that displayed an aligned length of 635 nucleotides with no insertions or deletions (Table S1). The final dataset was checked for stop codons using the vertebrate mitochondrial genetic code in the program MEGA v11.0.10 (Tamura et al. 2021). Mitochondrial haplotype networks were examined and drawn using PopArt (Leigh and Bryant 2015) (<http://www.popart.otago.ac.nz>) and the implemented parsimony network algorithm (Clement et al. 2000), with a 95% connection limit. Our analysis recognizes two widespread haplotypes, H1 and H4 (Table S1, Figure 3). Haplotype H1 shows extensive geographical spread beyond the borders of India, with points of presence spanning over a distance of more than 1000 kilometers. Additionally, our results indicate the presence of this haplotype in Southeastern Iran. This record is situated over 1500 km away from the documented range of this particular haplotype within India. Based on the results, all specimens examined belong to the clade mentioned as "the remainder of mainland India" of the Bengal monitor lizard (Gautam et al. 2023).



**FIGURE 2.** Photograph of a juvenile Bengal monitor lizard (*Varanus bengalensis*) captured in Bahu Kalat (Gandu) Protected Area (A). The unfortunate demise of the *V. bengalensis* was the result of a road accident (B). The natural habitats of *V. bengalensis* are located about 20 km north of the Dompak village area, in the Bahu Kalat (Gandu) Protected Area (C). (Photos: A. Mobaraki).

The large-bodied lizards of the genus *Varanus* found in Iran, especially *V. bengalensis* in the southeastern region, have not been thoroughly studied in zoological research for several reasons. This study represents the initial effort to elucidate the phylogenetic relationships of Iranian specimens of *V. bengalensis* using a genetic marker. Our analysis of COI sequences yields compelling evidence that Iranian *V. bengalensis* specimens belong to a clade widely distributed throughout India, excluding Himalayan foothills (Gautam et al. 2023). Nevertheless, it is crucial to emphasize that due to the limited sampling geographical area, additional extensive research is required to clarify the genetic variability of *Varanus bengalensis* in the southeastern region of Iran.





**FIGURE 3.** Statistical parsimony network analysis (TCS algorithm) based on 22 mtDNA *COI* sequences (635 bp) in *Varanus bengalensis*. Circle size corresponds to haplotype frequency. Dashes at branches indicate the number of mutational steps. The black circle represents the hypothetical haplotype. For more information see Table S1.

Based on our observations in the Bahu-Kalat (Gando) Protected Area, Iranian specimens of *Varanus bengalensis* appears to be well adapted to living inside and near the villages and human settlements. They don't seem to be harmed by local people; and even benefit from scavenging on waste and debris. On some occasions, they have been seen entering locally constructed water ponds called "Hootak" near or inside villages, and also climbing the trees. Moreover, considering the agricultural practices conducting in the region, it is common to see monitors within the agricultural plots and orchards. These areas sustain abundant populations of rodents, offering a valuable source of nourishment for the monitors. Nevertheless, in light of our findings, it is noteworthy to highlight that a significant concern for *V. bengalensis* in Iran pertains to the issue of road accidents (Figure 2B). Besides the variety of negative significant ecological impacts of roads, they are also accountable for the direct mortality of herpetofauna (e. g. Kouris et al. 2024; Jones et al. 2024). Recently a noticeable surge in the frequency of encountering deceased monitors on roads due to vehicular collisions has been observed in southeastern Iran, attributable to the expansion of road networks and human settlements (Figure 2B). It is possible that as a consequence of this factor, the probability of encountering *V. bengalensis* in its natural environment has significantly decreased over the last decade.

In conclusion, though *Varanus bengalensis* has been categorized as an "endangered species" according to national law and regulations in Iran, with punishment determined for its illegal hunting and killing, it appears that these measures have not been sufficient to halt the potential extinction of this species.

#### ACKNOWLEDGMENTS

We acknowledge the partial financial support provided by Shahrekord University grant 02GRD1M31721, awarded to H. Oraie.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

**TABLE S1.** Information of all sequences included in this study along with their localities and NCBI accession numbers.

**DATA AVAILABILITY STATEMENT**

The data for this study, including accession numbers for genetic sequences deposited on NCBI GenBank, are recorded in the [Supporting Information, Table S1](#).