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ORIGINAL ARTICLE

GIS-based distribution, population estimation, and morphological variation of Brown sand boa (*Eryx johnii* Russell, 1801) from Cholistan desert, Pakistan

Distribución basada en SIG, estimación de la población y variación morfológica de la boa de arena parda (*Eryx johnii* Russell, 1801) del desierto de Cholistán, Pakistán

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ABSTRACT

Pakistan has a rich diversity of herpetofauna. While numerous diversity studies on snakes have been carried out in Sindh and Baluchistan regions, none exists on the Cholistan desert landscape. Therefore, we aimed to conduct a one-year study on the different microhabitats of the Cholistan desert, to estimate the distribution and density of Brown sand boa (*Eryx johnii*). The sampling techniques include hand-picking, pitfall traps, and opportunistic visual encounters by amateur trackers. During the present study, 36 specimens of *E. johnii* were recorded for the assessment of the GIS-based distribution of species from the study area. *E. johnii* was recorded from 20 localities in the Cholistan desert. Further, we identified five different microhabitats have been identified from the study area for population estimation and morphological variation of *E. johnii*. The relative abundance of males, females, and juveniles was 0.5, 0.33, and 0.16, respectively. Morphologically, the males are significantly larger and heavier than females. We recorded two types of coloration, i.e., yellowish and reddish morphs, from various habitats of the Cholistan desert. This study provides important information on the distribution and density of the Brown sand boa in the understudied Cholistan desert landscape. The current study evaluates the distribution ranges of Brown sand boa to estimate the population density for their conservation in current localities. More investigation is required to evaluate the diversity and distribution of other species in the area because the one-year study timeframe and use of amateur trackers may have produced bias in data collecting.

Keywords: Cholistan desert, *Eryx johnii*, morphological character, population estimation.

RESUMEN

Pakistán posee una rica diversidad de herpetofauna. Aunque se han realizado numerosos estudios sobre la diversidad de serpientes en las regiones de Sindh y Baluchistán, no existen sobre el paisaje desértico de Cholistán. Nos propusimos realizar un estudio durante un año en los distintos microhábitats del desierto de Cholistán, para estimar la distribución y densidad de la boa marrón de arena (*Eryx johnii*). Las técnicas de muestreo incluyen la recolección manual, trampas de caída, y encuentros visuales oportunistas por rastreadores aficionados. Durante el estudio, se registraron 36 ejemplares de *E. johnii* para evaluar la distribución de la especie basado en SIG. Se registró la presencia de *E. johnii* en 20 localidades del desierto de Cholistán. Además, se identificaron cinco microhábitats diferentes para estimar la población y la

variación morfológica de *E. johnii*. La abundancia relativa de machos, hembras y juveniles fue de 0,5, 0,33 y 0,16, respectivamente. Morfológicamente, los machos son significativamente más grandes y pesados que las hembras. Registramos dos tipos de coloración, morfos amarillentos y rojizos, en diversos hábitats del desierto de Cholistán. Este estudio proporciona información importante sobre distribución y densidad de la boa marrón de arena en este poco estudiado paisaje. Este estudio evalúa los rangos de distribución de la boa marrón para estimar la densidad poblacional para su conservación. Se requiere más investigación para evaluar la diversidad y distribución de otras especies en la zona, porque un año de estudio y el uso de rastreadores aficionados pueden haber producido sesgos en la recolección de datos.

Palabras clave: carácter morfológico, desierto de Cholistán, *Eryx johnii*, estimación poblacional.

INTRODUCTION

Pakistan is a geo-ecological landscape that extends from mangrove forests along the southern sea coast to the world's tallest mountain ranges, the western Himalayas, Hindu Kush, and Karakoram ranges. It supports most of the world's eco-biological areas in a relatively small territory of (796,095 km^2) (Chaudhary 2017). Constant overgrazing, forest cutting, salinization, habitat loss, and flooding continue to pose a threat to Pakistan's biodiversity and have severe impacts on the country's fauna and other ecological units. Pakistan has a diverse range of ecosystems that sustain a wide range of animals and plants. Pakistan's fauna and flora are Oriental and Palearctic in origin, with numerous endemic species (BAPP 2004; Khan 2006).

Eryx Daudin, 1803 is a genus belonging to the family *Erycidae* and comprising about 13 species, some under taxonomic revision, distributed in Africa, SE Europe, Middle East, Central Asia, and Indian Peninsula (Sindaco *et al.* 2013). Due to the secretive habits of most Sand boas, their natural history is currently poorly understood, except for a few studies on their morphology (Tokar 1991; Eskandarzadeh *et al.* 2018; Faraone *et al.* 2019), ecology (Cattaneo 2010; Al-Sadoon & Al-Otaibi 2014; Vignoli *et al.* 2015; Faraone *et al.* 2021), and taxonomy (Eskandarzadeh *et al.* 2013, 2020a, 2020b; Zarrintab *et al.* 2017; Tokar *et al.* 1991).

Morphologically, they have head not distinct from neck; mental grove present; body scales keeled, 51 – 61 body scales at mid body; premaxilla not toothed; head with small scales; subcaudals in a single row. They are slow-moving snakes that are capable of quick movement to escape into loose soil. They are often found close to human habitation owing to their affinity for rodents. The Brown sand boa, *Eryx johnii* (Russell, 1801), is widespread throughout India, Afghanistan and Iran. In Pakistan, this species has been reported from Punjab, Sind, and Baluchistan (Ali *et al.* 2016), below the altitude of 200 m a.s.l. They are predominantly nocturnal, coming out of the burrows at night for prey. Although they are non venomous, people consider them to be fatal for humans and livestock . Therefore, they are killed whenever encountered. Further, they come out of hibernation in March, are active until September, and mate from March to May. They are viviparous, giving birth between April and June (Khan 2006).

Snakes are crucial in many healthy ecosystems and play a dynamic role in various food webs. They maintain ecological balance by acting as prey and predators (Khan 2006; Malik *et al.* 2021). Reptiles circulate food in terrestrial ecosystems, and their absence from any ecosystem will disrupt food dynamics, invertebrate populations, algae biomes, leaf rot, and the nutrient cycle (Baig *et al.* 2006; Ali *et al.* 2017).

Globally snake diversity is in decline due to various reasons such as predation, infection, contamination, acid rain, global warming, and habitat fragmentation (Blaustein *et al.* 2003; Boone & Bridges 2003; Becker *et al.* 2007; IUCN 2009). In addition, snakes need conservation measures because many species are threatened by different human activities such as urbanization, deforestation, fires, use of fertilizers and pesticides, deep tilling, and global warming, which destroy or change their habitats. The snakes have a major impact on the distribution and population density of many bird species, mammals, and other animals (Baig *et al.* 2006; Ali *et al.* 2016). Regardless of the importance of herpetiles as biological resources, very less attention has been given to them in Pakistan. Although numerous investigations of the country's amphibians and reptiles have been carried out, the majority of these investigations have only been undertaken in

the provinces of Sindh and Baluchistan (Ali *et al.* 2018). The current study aims to estimate the population density and morphological variation on the bases of GIS distribution of Brown sand boa (*Eryx johnii*) in the Cholistan desert.

MATERIALS AND METHODS

Study area

The study was conducted at different habitats of the Cholistan desert (28.5062° N, 71.5724° E), Punjab, Pakistan The Cholistan Desert is part of the Great Indian Desert, which also includes the Thar Desert in the province Sindh of Pakistan and the Rajasthan desert in India. It is approximately 80 kilometers long and 32 to 192 kilometers wide (Ahmad, 2005; Ahmad 2007a, 2007b, 2012a, 2012b). It is exposed to the wind and cold, unsheltered, treeless, and bare, with beautiful landscapes of sand dunes. It is situated about 30 kilometers from Bahawalpur city, Punjab, Pakistan (Abdullah *et al.* 2017). The Cholistan Desert is divided into two distinctive geomorphic zones based on the terrain, topography, soil, and floral coverage. Soils are classified as salty or alkaline depending on whether their pH ranges from 8.4 to 9.6. Some part of the desert is an air-sheltered desert with riverbeds, sand dunes, mountains, and depressions. Sand dunes have an average height of roughly 100 meters (Ahmad 2012b; Abdullah *et al.* 2017).

The northern section, known as "Lesser Cholistan," is confined by the canal irrigation zone and spans 7,770 square kilometers, while the southern region, known as "Greater Cholistan," spans 18,130 square kilometers (Ahmed *et al.* 1992; Baig *et al.* 2006; Ahmad 2012a, 2012b). Five different sub-sampling sites were observed for GIS-based distribution, morphology, and population estimation of *E. johnii*. Cholisthan has sparsely distributed xerophytic and halophytic vegetation and comprises *Callotropis*, *Prosopis*, and *Artemisia* grasses. The climate of Cholistan is subtropical, with low rainfall, high temperature, low humidity, high evaporation rate, and strong summer wind (Khan 2006).

Sampling

For specimen collection, we divided the study region of the desert into five different microhabitats: (a) agricultural fields, (b) uncultivated lands, (c) sand dunes, (d) human settlement, and (e) water bodies (Fig. 1). The data was collected from various field surveys were carried out between dawn and dusk, and both active and passive approaches were used to collect the specimens. Mark-recapture method was used for population estimation and morphometric characters of *E. johnii*.

The active capturing field survey was done during the early mornings and evenings times. During summer (March to September), the morning survey was conducted between 6.00 am and 9.00 am, and evening surveys were carried out from 5.00 pm to 7.00 pm. The specimens were directly captured with snake sticks from different habitats. Another active capturing method involved digging burrows to extract the snakes. The technique was useful in tracking *E. johnii*, which mostly used long, zigzag burrows of desert lizards *Uromastyx hardwickii* as a hideout and safe refuge to avoid daylight and harsh weather conditions (Ali *et al.* 2017).

The passive capturing method was mostly used for nocturnal species for lizards and snakes. Pitfall traps were also used to capture snakes from the study area. The captured specimens were tagged, and various records were noted as GPS coordinates, locational, habitat, and sex of species. Few captured (n=4) specimens were preserved in 70% alcohol for further genomic research and donated to the Zoological Museum of the University of Veterinary & Animal Sciences, Lahore.

Morphometric measurements

The morphometric measurements and morphological variation documentation were conducted by following Khan (2006) and Ali *et al.* (2017). Abbreviations: Snout to vent length (SVL); Tail length (TAL); from the back of the mandible to the tip of snout (HL); head width (HW); widest point of the mid-body (BW); body weight (W, g).

Analysis

Diversity software PAST 3.0 was used to calculate the relative abundance (Pi) of *E. johnii* by using the formula;

$$Pi = TS/TP \times 100$$

- Where Pi is the relative abundance of species
- TS is the total number of species in an area
- TP is the total sum of the population of all species in the area

(divide the total number of species in an area by the total sum of all populations of species in an area, then multiply by 100) from study area (Shannon & Weaver 1963; Pielou 1966). The mean ± SD results of Weight (g) and external body measurements (mm) of Brown Sand Boa for juvenile, female, and male groups were compared using one-way analysis of variance (ANOVA) followed by Tukey's HSD. The statistical analysis was performed using SPSS software (Version 20; SPSS Inc., Chicago, IL, USA) with a significance level set at $P < 0.05$.

All the data of GPS coordinates incorporate into an Excel sheet then ArcGIS software 10.5.1 was used to make the maps of distribution records against the GPS coordinates.

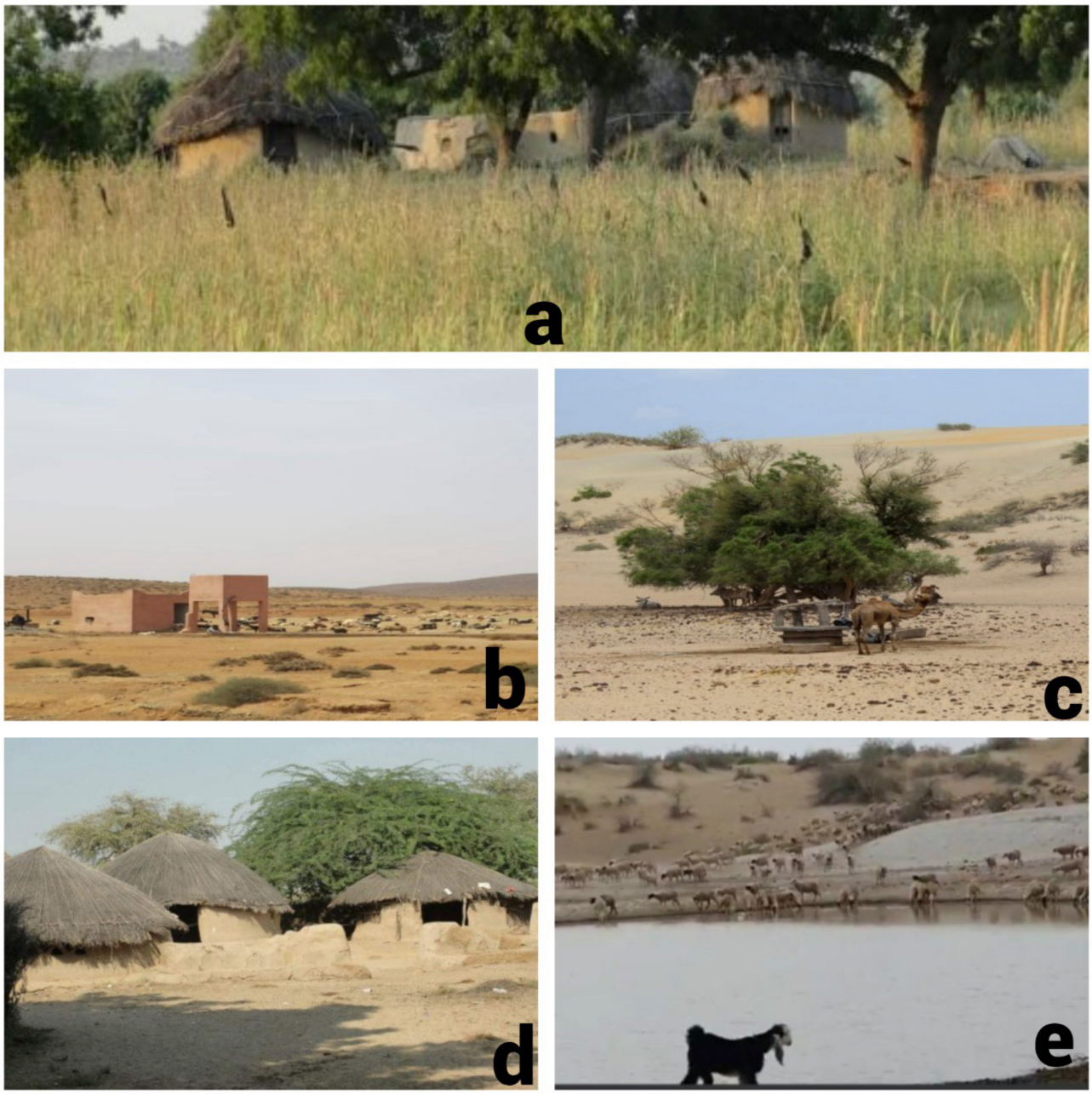

Figure 1. Different Habitats of Cholistan desert: (a) agricultural fields, (b) uncultivated lands, (c) sand dunes, (d) human settlement, and (e) water bodies. / Diferentes hábitats del desierto de Cholistán: (a) campos agrícolas, (b) tierras no cultivadas, (c) dunas de arena, (d) asentamientos humanos, y (e) cuerpos de agua.

RESULTS

Population estimation, morphological variation, and comparison

The records of specimens collected throughout the one-year study duration characterize a substantial section of the total ecological zones of that region. During the present field survey, 36 specimens of Brown sand boa (18 males, 12 females, and 6 juveniles) were recorded from five different habitats. While the (Pi) of males was 0.5, that of females and juveniles were 0.33 and 0.16, respectively (Table 1; Fig. 2). The average snout to vent length of male, female and juveniles showed significant differences among them and showed sexual dimorphism in the taxa. Morphologically males are significantly larger in size and heavier in weight than females. Two types of coloration, yellowish and reddish, were observed from various habitats of the Cholistan desert.

GIS-based distribution records of species

The values of North latitude N and east longitude E were recorded. While the highest elevation of 410 ft was recorded at Baylawal (29° 23.466' N, 71° 39.563' E), the lowest was recorded at Chasma Dhar (323 ft, 28° 39.864' N, 71° 15.632' E) were recorded. The topography of the desert was also noted for the evaluation of the habitat of *E. johnii*. Three types of topography sandunal, clayey saline, and interdunal sandy, were observed. Interdunal sandy topography was observed to be the most favorable habitat for Brown sand boa. We documented 20 localities for the presence of Brown sand boas from the Cholistan desert (Table 3). Historical (a) and current distribution (b) records of *E. johnii* are shown in (Fig. 3).

Table 1. Relative abundance of Brown Sand Boa captured from different habitats of the study area. (n = number of specimens recorded). / Abundancia relativa de la boa marrón de arena capturada en diferentes hábitats de la zona de estudio. (n = número de especímenes registrados).

	Habitat types					
Taxa	**Agriculture Fields** (n)	**Uncultivated lands** (n)	**Sand dunes** (n)	**Human settlements** (n)	**Water bodies** (n)	**Relative abundance (Pi)**
Male	1	3	12	2	-	0.5
Female	2	2	4	3	1	0.33
Juveniles	-	2	4	-	-	0.16

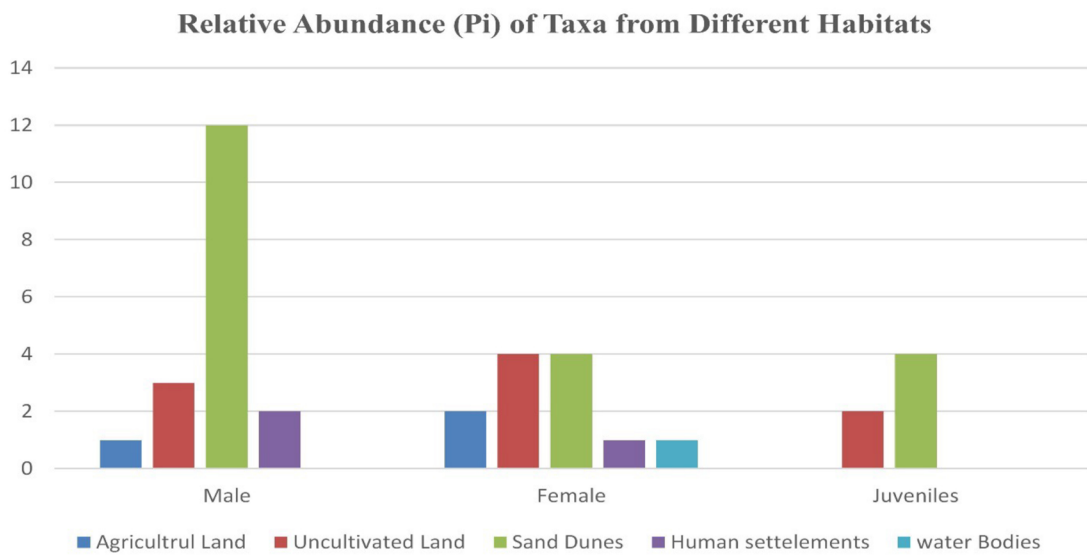

Figure 2. The counts of *Eryx johnii* from different habitats. / Recuentos de *E. johnii* de diferentes habitats.

Table 2. Weight (g), body measurements (mm) and min-max range of *Eryx johnii* captured from different habitat types of the Cholistan Desert. / Peso (g), medidas corporales (mm) y rango min-max de *E. johnii* capturado en diferentes tipos de hábitat del desierto de Cholistan.

Body Parameters	Males (n=18)		Females (n=12)		Juveniles (n=6)	
	Mean ±SD	(Min-Max)	Mean ±SD	(Min-Max)	Mean ±SD	(Min-Max)
SVL	306.9 ± 51.60a	290-305	270.50 ± 40.72b	265-270	186.25 ± 25.72c	177-180
TAL	125.9 ± 21.48a	115-120	110.92 ± 11.50a	105-111	74.96 ± 7.68a	71-74
HL	26.0 ± 2.13a	18-26	20.14 ± 1.14 b	16-20	14.58 ± 1.10c	11-14
HW	15.7 ± 1.67a	10-15	13.87 ± 1.10a	10-11	09.63 ± 1.00a	0.7-0.9
BW	21.0 ± 1.71a	17-21	18.22 ± 2.00b	15-18	10.45 ± 1.18c	8-10
W	130.3 ± 18.48a	120-130	95.16 ± 7.95b	90-95	70.16 ± 4.30c	66-70
TL	452.3 ± 45.72a	440-452	374.22 ± 40.81b	368-374	110.22 ± 24.54c	107-110

Note: Values in the same row not sharing the same subscript are significantly different at $p<0.05$.

Table 3. Name, location, and topography of study area. / Nombre, ubicación y topografía de la zona de estudio.

Sr. No.	Site Name	GPS Location	Elevation	Topography
1	Zahir Pir	28.8107° N 70.5324° E	398 ft	Sandunal
2	Kalapahar	29°10.430' N 72°05.569' E	384 ft	Clayey saline
3	Sulleh Wala	28°40.315' N 71°35.648' E	389 ft	Interdunal sandy
4	Januwali	29°05.056' N 72°09.933' E	406 ft	Interdunal sandy
5	Khirsir	29°10.339' N 72°08.749' E	391 ft	Sandunal
6	Haider wali	29°02.672' N 72°10.200' E	382 ft	Clayey saline
7	Mojgarh Fort	29°01.059' N 72°08.106' E	392 ft	Sandunal
8	Khangarh	28°57.261' N 72°03.089' E	369 ft	Interdunal sandy
9	Khanser	28°59.227' N 71°55.299' E	352 ft	Sandunal
10	Bijnot	28°47.988' N 71°45.770' E	340 ft	Interdunal sandy
11	Dingarh Fort	28°57.454' N 71°51.910' E	365 ft	Clayey saline
12	Rukanpur	28°53.182' N 71°46.362' E	371 ft	Sandunal
13	Nidamwala Toba	28°52.963' N 71°44.270' E	355 ft	Clayey saline
14	Nawankot	28°47.939' N 71°45.770' E	334 ft	Interdunal sandy
15	Lakhan	28°52.232' N 71°42.731' E	351 ft	Clayey saline
16	Chananpir	28°56.832' N 71°40.057' E	353 ft	Interdunal sandy
17	Baylawala	29°23.466' N 71°39.563' E	410 ft	Interdunal sandy
18	Derawar fort	29°23.465' N 71°39.560' E	345 ft	Interdunal sandy
19	Chasma Dhar	28°39.864' N 71°15.632' E	323 ft	Clayey saline
20	Islamgarh Fort	27°50.208' N 71°48.129' E	334 ft	Sandunal

N: North latitude, **E**: East longitude, **ft**: Foot (is a unit of length in the Imperial)

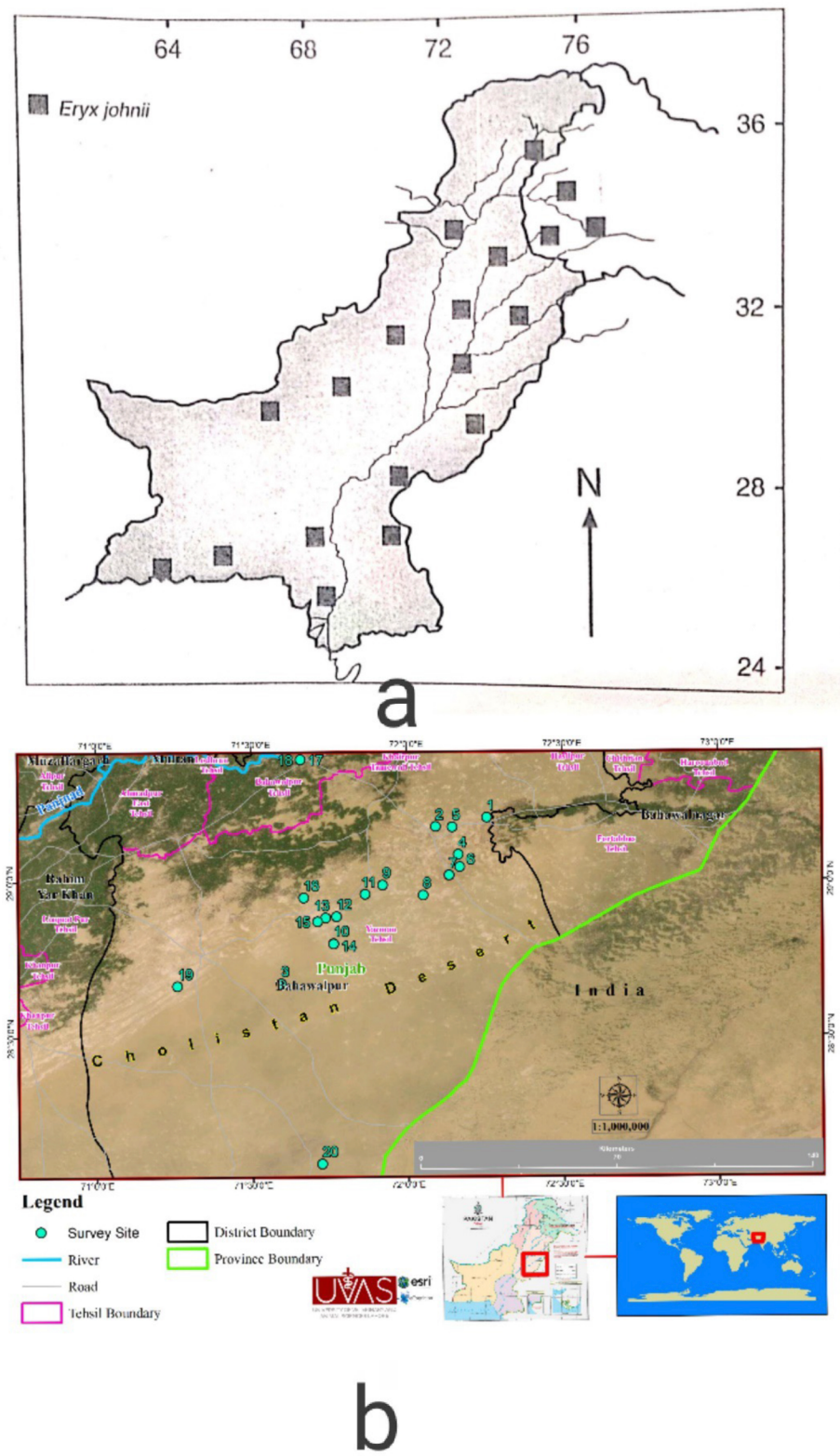

Figure 3. Historic (a) (Khan 2006) and current (b) distribution records of *E. johnii.* / Registros de distribución históricos (a) (Khan 2006) y actuales (b) de *E. johnii.*

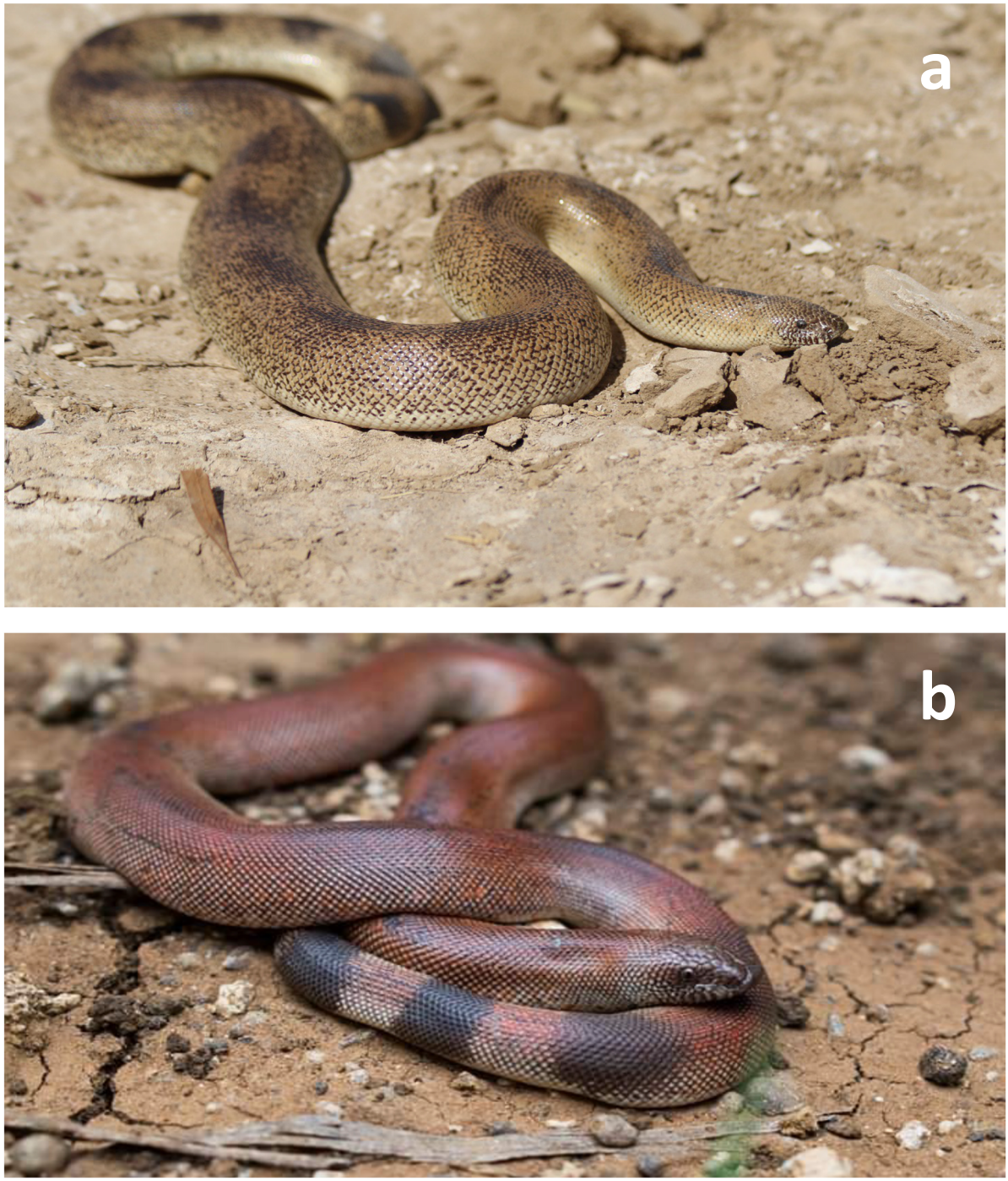

Figure 4. a) *E. johnii* yellowish color morphology, b) *E. johnii* reddish color morphology. / a) *E. johnii* morfología de color amarillento, b) *E. johnii* morfología de color rojizo.

DISCUSSION

The findings of our study on *E. johnii* in the Cholistan desert provide valuable insights into the distribution and population of this species in an understudied region. Our results add new data to the existing knowledge on the herpetofauna of Pakistan. The results of the one-way ANOVA statistical analysis showed that there were significant differences in the morphological measurements of the Brown Sand Boa (*Eryx johnii*) captured from different habitat types of the Cholistan Desert. The mean body length (SVL, TAL, HL, and HW), body weight (BW and W), and total length (TL) of males were significantly greater than those of females and juveniles. Additionally, the mean body length (SVL, TAL, HL, and HW), body weight (BW and W), and total length (TL) of females were significantly greater than those of juveniles. The higher values of subcaudal scales and a relative tail length of males may be associated with the advantages that a longer tail can provide more space for the housed hemipenes inside the tail base and also can help males to increase the ability to translocate the tails of other rival males from the proximity of the female´s cloaca during the courtship to increase the chance of success in competition between males (King 1989; Shine *et al.* 1999). Longer snout-vent length in females than males would lead to increased fecundity in the sand boa. The larger female size can be an adaptation to the low reproductive frequencies. Larger females usually produce larger clutch sizes, and it is advantageous for females that produce a single clutch each year; however, some males show higher SVL in *E. johnii* that may depend on age and growth factors that vary between habitats (Fitch 1981; Bertona & Chiaraviglio 2003).

These findings indicate that the habitat types of Cholistan Desert may have an impact on the morphological characteristics of *Eryx johnii* and that males have larger body sizes than females and juveniles. The average snout to vent length of male were (306.9 ± 51.60) mm, and female were (270.50 ± 40.72) mm, while average SVL of female *Eryx jayakari have (*38.20 ± 12.68) and males have (55.20 ± 14.60) (Eskandarzadeh *et al.* 2018). The coloration of the species varies between habitats: yellowish (Fig. 4a) and reddish (Fig. 4b). This information may be useful in population estimation and management of the Brown Sand Boa from the Cholistan Desert, Pakistan.

The Current study identified five different microhabitats from the study area viz. Agricultural fields with crops, trees, and water availability; Uncultivated, flat stretches of land locally called “Dahars” were mostly barren areas; Sand dunes in the desert area which do not have water available; Human settlements; and water bodies in the form of rainwater ponds, which are present in the study area Baig *et al.* (2008).

The study revealed that sand dunes were the most populated habitat of sand boas in the study area, where the habitat has an arid subtropical climate of the continental variety with high temperatures, little to no relative humidity, a high rate of evaporation rate, and strong summer winds, as described by Khan (1957). This region is one of the hottest and driest deserts, with an elevation of 112 m a.s.l. and a mean annual temperature of 28.33 °C. The hottest month is June, during which the daily maximum temperature often surpasses 45 °C and occasionally crosses 50 °C. (Ahmad 2002). GIS-based distribution of sand boa presents that the study area was relatively medium to high populated as the sandy soil was favorable for the breeding sites for species (Khan 2006).

In previous studies, Minton (1966), Mertens (1969), and Khan (1985) conducted surveys on the herpetofauna of the South Punjab and Sindh regions, which indicated the presence of a Brown sand boa population in the Cholistan desert. However, Baig *et al.* (2008) provided a checklist of the herpetofauna of the Cholistan desert without estimating the population of the Brown sand boa (*Eryx johnii*). In contrast, the present study significantly contributes to understanding the morphological variation, population estimation, and GIS-based distribution of the Brown sand boa in the study area. Therefore, this study fills a critical gap in the existing knowledge on this species in the Cholistan desert.

Conclusion

The herpetofauna of Pakistan, especially in the Cholistan desert, is not well explored. Existing data from this region is either outdated or limited. In our study, we explored the density of *E. johnii* and evaluated its habitat by GIS-based distribution ranges in the desert to point out the threat faced by this species due to habitat loss. Furthermore, a comprehensive survey is required to assess Pakistan’s snake fauna for the conservation status of the species.

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