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Research Article

Phylogeography on GIS-Based Distribution of Snake Fauna from Cholistan Desert Pakistan

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Abstract

The current abstract revealed a significant portion of the overall ecological zones that exist in the Cholistan Desert are explored by the authors. Snake samples were collected from selected locations inside the Cholistan Desert. There 50 GPS localities were documented for the phylogeography of snake fauna from the study area. The Tail tip biopsies were collected from the specimen with the help of forceps. DNA was extracted from tail tip tissue and internal organs of dead specimens, e.g., liver and spleen, by using Qiagen amp Tissue Genomic Extraction kit according to manufacturer instructions. During present field survey total 11 species and 36 specimens of snakes were recorded from different habitats of study area. However, a total of six species and ten specimens were successfully amplified, sequenced, and attained accession numbers from GenBank. After trimming ambiguous bases, the obtained Cytochrome c oxidase subunit I (COX1) fragments of *Spalerosophis diadema* was 645 bp, *Eryx sistanensis* was 697 pb, *Platyceps rhodorachis* 696bp, *Ptyas macosa* 696bp, *Bungarus caeruleus* 704bp and *Eryx tataricus* was 657 bp. Maximum likelihood trees of Cytochrome c oxidase subunit I (COX1) sequences based on Kimura 2-parameter distance and uncorrected pairwise genetic differences (p-distance) for the studied taxa show clear species identification.

Keywords: Phylogeography; GPS; Cholistan Desert; *Squamata; Serpentes*

Introduction

The study of the concepts and mechanisms influencing the evolution of spatial patterns of genetic variation is known as phylogeography (Avise, 2000; Chaber et al., 2021). DNA polymorphisms

exhibit distinctive geographic patterns due to processes such population growth, range fragmentation, prolonged isolation, and population bottlenecks (Hewitt 2000, 2004; Bukhari et al., 2021). As a result, phylogeographic research helps identify the precise biogeographic occurrences that influenced the geographic range of a species (Maggs et al., 2015). A species' potential responses to present-day or potential future events that could change its geographic range can be inferred from its phylogeography combined with historical climate data (Avise, 2000). The Pleistocene glacial cycles' effects on climate and habitat had a significant impact on the phylogeographic organisation of many animals and plants (Avise, 2000; TurchettoZolet et al., 2013; Chaber et al., 2021).

Taxonomic determination has a direct impact on a species' geographic range (Agapow et al., 2004). The population size and range of the previously recognized species as well as the ranges of the newly formed species, which include parts of the former species' range, fall when a species splits into two or more species (Agapow et al., 2004). Therefore, a species that looks to be low-risk and widely distributed may actually be a complex of many species, some of which are endangered or confined to a narrow range. Such taxonomic modifications necessitate reassessing the priority, listings, and conservation status of the taxa and the affected locations (Mace, 2004). Several new species have been identified within widespread species in recent decades, even among very well-studied organisms like vertebrates. This is mostly because taxonomy and evolutionary research use molecular methods (Bickford et al., 2007; Ceballosa & Ehrlich, 2009; Oliver et al., 2009). Though occasionally these taxonomic classifications result from a "over-splitting" mentality, which is motivated by either a shift in the idea of species (Isaac et al., 2004).

Molecular diversity which also known as genetic variations and gene level bio-diversity demonstrates the occurrence of alleles (variants of genes). The basic components which manipulate the genetic variability of a population are the quantity of gene flow between populations and population size. The determined of the genetic variability at the level of genus, inhabitants and within population is of great significance for conservation practice (Astuti, 2020). At the species level genetic variation help to recognize the taxonomic components and to resolve the species uniqueness. Variability at level of the inhabitants executed diverse heredity classes, the genetic variations among them and their Phylogenetic association with their associations (Weber and May, 2016; Malik et al., 2021).

The variations inside population are very valuable to collect the facts on species identification, breeding design, degree of relatedness and disturbance of genetic diversity between them (Wang et al., 2021). The taxonomy and systematic of bird's knowledge has quickly advanced over the past ten year due to developing and ever improving molecular Phylogenetic tools. For the estimation levels and patterns of genetic diversity the molecular markers are important means and have been beneficial for researching genetic variation across several species (Chang et al., 2007). At the molecular level to study genetic variations there are various available DNA markers that can be used the currently most informatory and polymorphic are microsatellite DNA markers that contain of random DNA repeats of 2–6 base pairs (Hillel et al., 2003; Patzold et al., 2021).

Snakes are the significant element of many healthy ecosystems and show dynamic role in food web. They retain the stability of the nourishment chain in the environment because they eat a lot of insects and are themselves a food source for many species of birds and mammals (Khan, 1998; Khan, 2006; Malik et al., 2021). Reptiles circulate food between terrestrial and aquatic and their absence from any ecosystem will disrupt food dynamics, invertebrate populations, algae biomes, leaf rot as well as the nutrient cycle (Chaudhary, 1992; Baig et al., 2006; Ali et al., 2017; Chaudhary, 2017; Ali et al., 2021).

Global declines of snake diversity are well described in the works, and different numerate of causes containing predation, infection, contamination, acid rain, global warming, habitat fragmentation and manmade features (Blaustein et al., 2003; Boone and Bridges, 2003; Becker et al., 2007; IUCN, 2009a). The snakes have major impact on distribution and population density of many bird species, mammals and other animals. In addition, snakes are important in conservation because many species are endangered by different human activities viz., structure of buildings, cutting of trees, fires, use of fertilizers, use of insecticides, deep tilling and global warming which destroy or change in their habitats. Many anthropogenic hazards causing problems for these taxa and their survival is under threat to extinction of many species (Baig et al., 2006; Ali et al., 2016).

In spite of the fact that snakes are found in abundance, it is very tough to sample them quantitatively. Morphological keys are still thought to be reliable for identifying species in count, but DNA analysis is also necessary for precise species identification., genetic diversity and Phylogeography of the snakes from cholistan desert addition, expansions in molecular biology

have led scientist to identify variations within and among different populations (Vences et al., 2004).

Material and Methods

Study area

The research was carried out in various ecosystems across the Cholistan desert (28.5062° N, 71.5724° E), Punjab, Pakistan (Figure 1) which also includes the Thar Desert in Pakistan's Sindh Province and the Rajasthan Desert in India. The Cholistan Desert is around 480 kilometers long and 32 to 192 kilometers wide (Ahmad, 2005; Ahmad, 2007a; 2007b; Ahmad, 2008; Ahmad, 2012a; 2012b)—Cholistan Desert's bleak but lovely landscape. It is situated about 30 kilometers from Bahawalpur City, Punjab, Pakistan (Abdullah et al., 2017). The Cholistan Desert is separated into two distinct geomorphic zones based on terrain, core material, dust, and vegetation cover. The northern section, known as "Les 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).

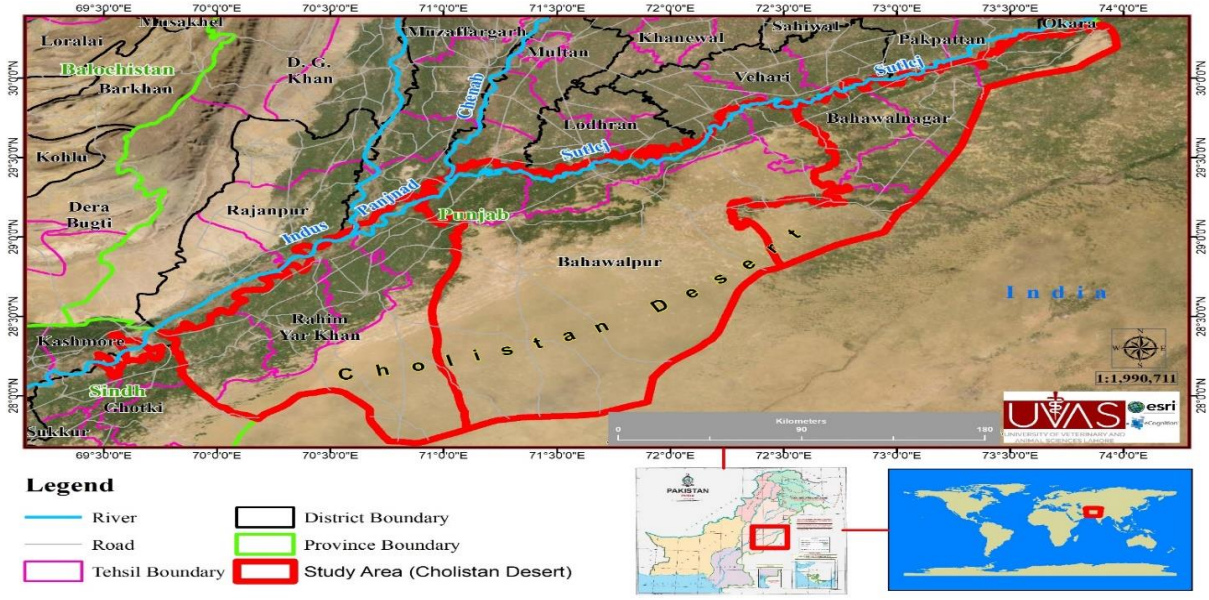

Figure 1. Map of study area.

Collection, preservation, and Identification of Samples

Snake samples were gathered from selected sites of the Cholistan desert. Field surveys were made during the early morning and evening times, and specimens were collected using snake sticks. The Tail tip biopsies were collected from the specimens with the help of forceps (Ashraf et al., 2019)

and brought into post-graduate lab of microbiology, Department of Wildlife and Ecology, C Block, UVAS, Ravi campus. The data related to samples viz, habitat, date, time, species, sex, distribution, GPS coordinates, and location was noted on proformas. Using taxonomic identification keys, each sample was recognized (Khan, 2006).

Sampling Techniques

To determine mitochondrial DNA diversity and distribution of snake species in different habitats of the Cholistan desert. Field surveys were done to collect as many as possible snake species from the study area during dawn and dusk time. A total of (n=5) specimens of each species were collected using hand picking method with snake stick and pitfall traps and indirectly by interviewing the local community, volunteer snake friends, reports on road and community kills and encounters during amateur tracking (Baig et al., 2008; Ali et al., 2017).

Mitochondrial DNA Extraction

DNA was extracted from tail tip tissue and internal organs of dead specimens, e.g., liver, and spleen, using a Qiagen amp Tissue Genomic Extraction kit according to manufacturer instructions. Agarose Gel electrophoresis and NanoDrop (2000 and 2000c Thermo Scientific™) were used to check the presence and concentration of extracted DNA.

PCR Amplification

With the help of Cytochrome C oxidase subunit I (COX1) primer, the polymerase chain reaction was performed. Each experiment used 25µL of reaction mixture and a 200 µL PCR tube. Add 6µl of distilled water, 1µl of primer F and 1µl of primer R (25 mM each), 12 µl of master mix, and 5µl of mixed DNA sample to the 25µL reaction to get it ready (Ali et al., 2020).

Polymerase Chain Reaction (PCR) Conditions

DNA was amplified using primer COX1. The gene was amplification was carried out using a thermal cycler with a denaturing step of three minutes at 94ºC, forty cycles of denaturing for thirty seconds at 94ºC, primer annealing for thirty seconds at 55ºC, depending on the primer, and elongation for one minute at 72ºC, with a final ten minutes at 72ºC and infinity at 4ºC (Vences et al., 2012).

PCR Products Purification

The Qiagen PCR purification kit was used to purify the PCR products. After the purification of gel from agarose gel following steps are performed according to PCR purification kit instructions. First of all, add 5 µL of PB buffer and 1 µL of PCR products. Then, it turns yellow, then mix by

the vortex. The sample was added into a column spin tube and centrifuge for 2 minutes at 14000 rpm. Remove the upper liquid and put the column back in the same tube. Add 750 µl of PE buffer now, then centrifuge at 14000 rpm for one to two minutes. Next, Insert the column tube into a fresh 1.5 ml Eppendorf, Insert the column tube into a fresh 1.5 ml Eppendorf tube, then fill it with 50 µL of EB buffer. Centrifuge it for almost 1 minute. Purified PCR products were collected after centrifugation and washing with EB buffer (Hussain et al., 2020; Malik et al., 2021). Again, purified PCR yields were confirmed and sent to TsentBioscience China for direct sanger sequence.

Molecular Analysis

Bioedit software 7.0 was used to evaluate and edit raw sequences of selected genes. Clustal W was used to align them (Ali et al., 2020; Hussain et al., 2020; Malik et al., 2021). The consensus sequence for every sample was compared to the percentage identity of the sequence by using the National Centre for Biotechnology Information (NCBI) DNA databases through BLAST (basic local alignment tool search) analysis. The closely connected sequences were collected and integrated in Maximum likelihood tree analyses with a bootstrap value of 100 repetitions using MEGA X. Mega X was used to calculate genetic distances within and between species based on p-distance (Tamura et al., 2013; Malik et al., 2021).

Results

Snake species captured from the study area

The specimens were collected throughout a one-year study that extended from March 2022 to March 2023. A significant portion of the overall ecological zones that exist there are explored by the study period. There are attempts being made to reveal the concealed prosperity of a description of the Cholistan Desert with special emphasis on the snake fauna. During the present field survey, total of 11 species and 36 specimens of snakes were recorded from different habitats of the study area. The relative abundance (Pi) of *Eryx johnii* 0.14, *Platyceps rhodorachis* 0.14, *Lycodon aulicus* 0.08, *Ptyas mucosus* 0.05, *Bungarus caeruleus* 0.08, *Naja naja* 0.05, *Echis carinatus* 0.11, *Eryx conicus* 0.11, *Spalerosophis diadema* 0.05, *Eryx tataricus* 0.08 and *Eryx sistanensis* were 0.08 correspondingly (Table 1; Fig. 2).

Table 1. The detail of snake species capture from various habitats types of study area. (n=number of specimen captured).

Species	Agriculture fields (n)	Uncultivated land (n)	Human settlement (n)	Water bodies (n)	Sand Dunes (n)	Total specimens (N)	Pi (Relative abundance)
Eryx johnii	-	1	-	-	4	5	0.14
Platyceps rhodorachis	-	4	-	-	1	5	0.14
Lycodon aulicus	-	1	2	-	-	3	0.08
Ptyas mucosus	1	-	-	1		2	0.05
Bungarus caeruleus	1	-	2	-	-	3	0.08
Naja naja	1	-	1	-	-	2	0.05
Echis carinatus	-	-	-	-	4	4	0.11
Eryx conicus	-	1	-	-	3	4	0.11
Spalerosophis diadema	-	-	-	-	2	2	0.05
Eryx tataricus	-	1	-	-	2	3	0.08
Eryx sistanensis	-	-	1	-	2	3	0.08
Total	**3**	**8**	**6**	**1**	**18**	**36**	**0.89**

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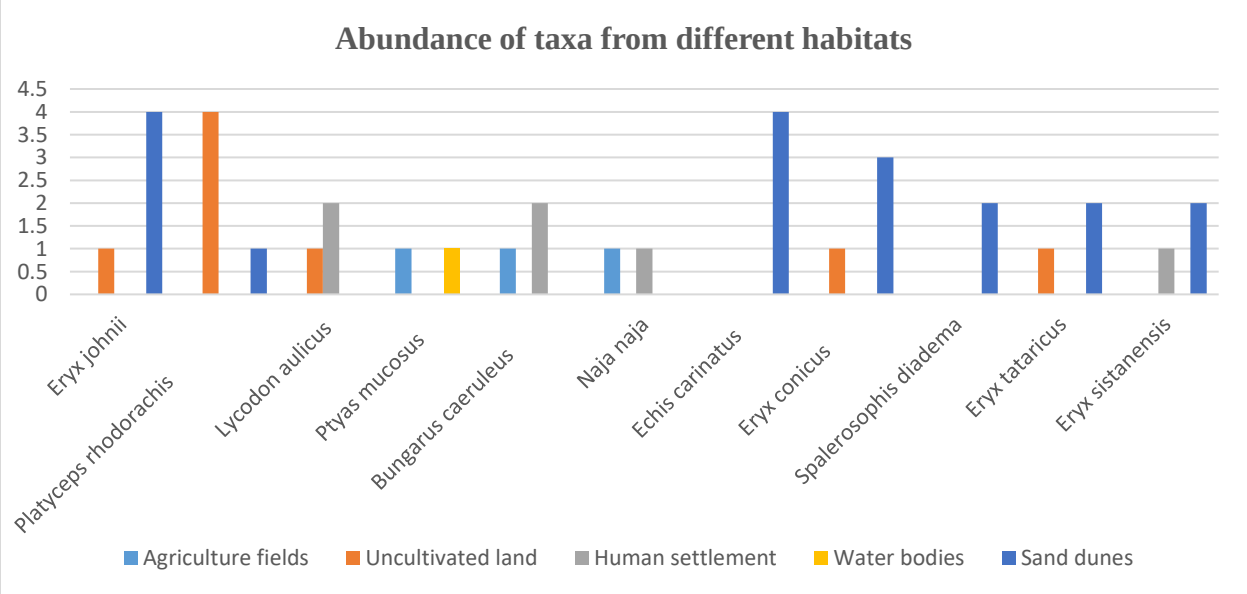

Figure 2. The abundance of snakes from different habitats.

GIS-Based Distribution Records of Species

GPS coordinates was noted from the study area by using the GPS Essential Android application. North latitude N and east longitude E were recorded their values. The highest elevation was 410 ft at Baylawal (N: 29° 23.466´ E: 071º 39.563´). While the lowest elevation was 323 ft at Chasma Dhar. The topography of the desert was also noted for the evaluation of habitat preference of snake fauna. Five types of topography sandal, clayey saline, vegetation, sandy patches, and interdunal sandy were observed, interdunal sandy topography was observed the most favorable for predation and residence of various snakes. Fifty localities were documented for the presence of snake fauna from the Cholistan desert (Table 2). Current distribution records of different snake species were shown on the map (Figure 3).

Table 2. Name, location, elevation and topography of study area.

Sr. No.	Site Name	GPS Location	Elevation	Topography
1	Zahir Pir	N:28.8107° E: 70.5324°	398 ft	vegetation
2	Kalapahar	N: 29º10.430´ E: 072º05.569´	384 ft	Clayey saline
3	Sulleh Wala	N: 28º40.315´ E: 071º35.648´	389 ft	Interdunal sandy
4	Janu wali	N: 29º05.056´ E: 072º09.933´	406 ft	Interdunal sandy
5	Khir sir	N: 29º10.339´ E: 072º08.749´	392 ft	Sandunal
6	Haider Wali	N: 29º02.672´ E: 072º10.200´	383 ft	Clayey saline
7	Moajgh fort	N: 29º01.059´ E: 072º08.106´	392 ft	Sandunal
8	Khan Gargh	N: 28º57.261´ E: 072º03.089´	370 ft	Interdunal sandy
9	Khan Ser	N: 28º59.227´ E: 071º55.299´	351 ft	Sandunal
10	Binot	N: 28º47.988´ E: 071º45.770´	341 ft	Interdunal sandy
11	Denagrgh fort	N: 28º57.454´ E: 071º51.910´	366 ft	sandy
12	Rukan Pur	N: 28º53.182´ E: 071º46.362´	372 ft	Sandy patches
13	Nedamwala	N: 28º52.963´ E: 071º44.270´	356 ft	Clay salin
14	Nawan kot	N: 28º47.939´ E: 071º45.770´	334 ft	Interdunal sandy
15	Lakhan wali	N: 28º52.232´ E: 071º42.731´	351 ft	Clay
16	Chan Peer	N: 28º56.832´ E: 071º40.057´	353 ft	Interdunal sandy
17	Bayla Wala	N: 29º23.466´ E: 071º39.563´	410 ft	Interdunal sandy
18	Derawar Fort	N: 29º23.465´ E: 071º39.560´	345 ft	Interdunal sandy
19	Chasma dhare	N: 28º39.864´ E: 071º15.632´	323 ft	Clay
20	Islam gargh fort	N: 27º50.208´ E: 071º48.129´	334 ft	Sandunal
21	Abbas Nagar	N: 29˚.3170° E: 71˚.9046´	398 ft	vegetation

22	Chak 163/7R	N: 29˚.4082 E: 73˚.080′	384 ft	Clayey saline
23	Fareedkot Tiba	N: 29˚.7800, E: 72˚.9250′	389 ft	Interdunal sandy
24	Danish school HSP	N: 29˚40.717′ E: 72˚30.414′	406 ft	Interdunal sandy
25	Danish school HSP	N: 2940.312′ E: 072˚30.313′	391 ft	Sandunal
26	Danish school HSP	N: 29˚40.427′ E: 072˚29.464′	382 ft	Clayey saline
27	Danish school HSP	N: 29˚39.682′ E: 072˚28.224′	392 ft	Sandunal
28	Danish school HSP	N: 29˚39.681′ E: 072˚27.564′	369 ft	Interdunal sandy
29	Khair Pur Tamiwali	N: 29˚39.490′ E: 072˚27.159′	352 ft	Sandunal
30	KhairPur Tamiwali	N: 29˚38.977′ E: 072˚26.112′	340 ft	Interdunal sandy
31	KhairPur Tamiwali	N: 29˚38.528′ E: 072˚25.102′	365 ft	sandy
32	KhairPur Tamiwali	N: 29˚37.967′ E: 072˚24.025′	371 ft	Sandy patches
33	KhairPur Tamiwali	N: 29˚32.302′ E: 072˚09.277′	355 ft	Clay salin
34	KhairPur Tamiwali	N: 29˚31.426′ E: 072˚08.350′	334 ft	Interdunal sandy
35	Lal Suhanra Park	N: 29˚29.786′ E: 072˚05.523′	351 ft	Clay
36	Lal Suhanra Park	N: 29˚29.345′ E: 072˚04.371′	353 ft	Interdunal sandy
37	NRSP BWP	N: 29˚23.362 E: 071˚42.936	410 ft	Interdunal sandy
38	lawyer housing society	N: 29˚21.355 E: 071˚43.204	345 ft	Interdunal sandy
39	21km away yazman	N: 29˚21.225 E: 071˚42.539	323 ft	Clay
40	chak 21B c yazman	N: 29˚17.755 E: 071˚43.714	334 ft	Sandunal
41	21 B .S east yazman	N: 29˚13.732 E: 071˚42.737	398 ft	vegetation
42	10km aqay from yazman	N: 29˚12.935 E: 071˚42.065	384 ft	Clayey saline
43	44/A DB yazman	N: 29˚13.631 E: 071˚43.543	389 ft	Interdunal sandy
44	New army cant area	N: 29˚13.056 E: 071˚43.114	406 ft	Interdunal sandy
45	New cant area	N: 29˚20.133 E: 071˚42.24	391 ft	Sandunal
46	7BC islam nagar BWP	N: 29˚20.255 E: 071˚42.987	382 ft	Clayey saline
47	10 BC BWP	N: 29˚24.191 E: 071˚47.292	392 ft	Sandunal
48	Jhangi Wala Dera Bakha	N: 29˚26.617 E: 071˚47.451	369 ft	Interdunal sandy
49	IUB	N: 29˚26.376 E: 071˚51.039	352 ft	Sandunal
50	IUB	N: 29˚23.366 E: 071˚45.942	340 ft	Interdunal sandy

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

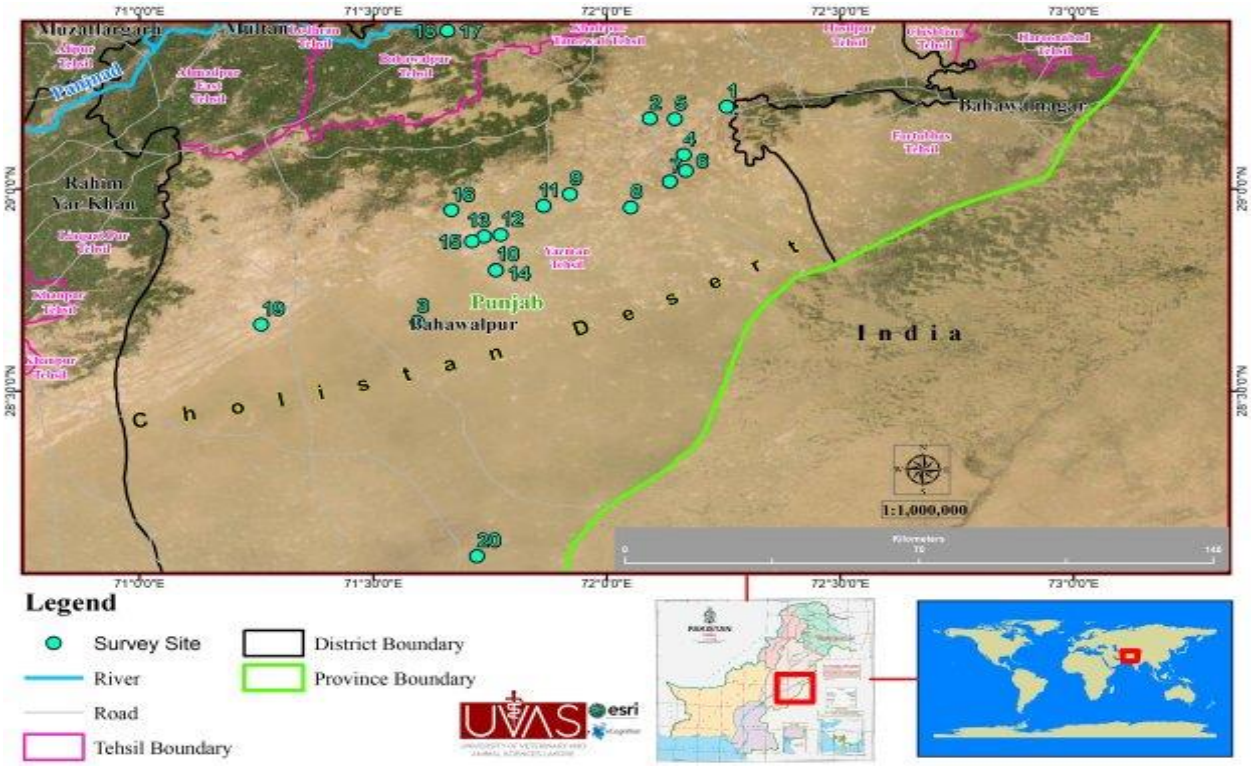

Figure 3. Current distribution records of snake species from study area.

Amplification and sequence

A total of six species and ten specimens were successfully amplified, sequenced, and attained accession numbers from GenBank. After trimming ambiguous bases, the obtained Cytochrome c oxidase subunit I (COX1) fragments of *Spalerosophis diadema* was 645 bp, *Eryx sistanensis* was 697 pb, *Platyceps rhodorachis* 696bp, *Ptyas macosa* 696bp, *Bungarus caeruleus* 704bp and *Eryx tataricus* was 657 bp. The summaries of successful amplified species and the GenBank accession numbers are shown in (Table 3).

Table 3. List of successful amplified species and the GenBank accession numbers of specimens used in this study.

Genus	Family	Species	Label	Base pair (bp)	Gene bank accession number
					Cytochrome c oxidase subunit I (COX1)
Class *Reptelia*					
Spalerosophis	*Colubridae*	*Spalerosophis diadema*	ZM UVAS-50	645bp	OR026593
Eryx	*Boidae*	*Eryx sistanensis*	ZM UVAS-51	697bp	OR026594

Platyceps	*Colubridae*	*Platyceps rhodorachis*	ZM UVAS-52	696bp	OR026595
Spalerosophis	*Colubridae*	*Spalerosophis diadema*	ZM UVAS-53	645bp	OR026596
Spalerosophis	*Colubridae*	*Spalerosophis diadema*	ZM UVAS-54	645bp	OR026597
Spalerosophis	*Colubridae*	*Spalerosophis diadema*	ZM UVAS-55	645bp	OR026598
Spalerosophis	*Colubridae*	*Spalerosophis diadema*	ZM UVAS-90	698bp	OR229681
Ptyas	*Colubridae*	*Ptyas macosa*	ZM UVAS-56	696bp	OR026599
Bungarus	*Elapidae*	*Bungarus caeruleus*	ZM UVAS-68	704bp	OR186190
Eryx	*Boidae*	*Eryx tataricus*	ZM UVAS-68	657bp	N/A

Phylogeography of Snakes and Phylogenetic analysis

In the course of the investigation, six DNA sequences of *Spalerosophis diadema*, *Eryx sistanensis*, *Platyceps rhodorachis*, *Ptyas macosa*, *Bungarus caeruleus* and *Eryx tataricus* were obtained. Nearly all of the species specimens have had their identities clearly and reliably confirmed by these DNA sequences. However, few specimens were morphologically identified as *Eryx johnii* but sequence analysis of cytochrome c oxidase subunit I (COX1) identifies as *Eryx sistanensis* that is first record of the specie from Pakistan. Although, closely matched sequences of Cytochrome c oxidase subunit I (COX1) were often taken from open databases during blast searches to determine how closely related species are to one another using Maximum likelihood trees. There have been a few recent DNA barcoding investigations of Asian snakes, and the NCBI has sequences for snakes that are related. Maximum likelihood trees of Cytochrome c oxidase subunit I (COX1) sequences are shown in Figures 4, 5, 6, 7, 8, and 9 based on Kimura 2-parameter distance. Uncorrected pairwise genetic differences (*p*-distance) for the studied taxa are presented in Table 4, 5, 6, 7, 8 and 9.

Table 4. Uncorrected pairwise genetic differences (*p*-distance) for COX1 gene among studied species of *Bungarus caeruleus*.

Taxa	1	2	3	4	5	6	7	8
1.*Bungarus caeruleus*		0.02629	0.02623	0.02736	0.02640	0.03838	0.02751	0.03757
2.*Bungarus multicinctus*	0.11518		0.00496	0.03001	0.00577	0.04784	0.01453	0.03542
3.*Bungarus candidus*	0.11522	0.01144		0.02963	0.00335	0.04527	0.01532	0.03630
4.*Bungarus fasciatus*	0.12314	0.13088	0.12931		0.03005	0.04274	0.02928	0.03912
5.*Boiga dendrophila*	0.11512	0.01436	0.00570	0.13256		0.04436	0.01506	0.03579
6.*Elaphe quadrivirgata*	0.15852	0.19092	0.18367	0.18008	0.17842		0.04506	0.02067
7.*Bungarus suzhenae*	0.12163	0.05634	0.06100	0.12931	0.05944	0.18148		0.04143
8.*Elaphe schrenckii*	0.15594	0.14967	0.15511	0.16546	0.15134	0.08728	0.17485	

Table 5. Uncorrected pairwise genetic differences (*p*-distance) for COX1 gene among studied species of *Eryx sistanensis.*

Taxa	1	2	2	4	5	6	7
1.*Eryx_sistanensis*		0.0120477587	0.0160715357	0.0160715357	0.0201510959	0.0200828639	0.0192082670
2.*Eryx_johnii*	0.0743241126		0.0163672362	0.0163672362	0.0227672682	0.0211359964	0.0195219790
3.*Eryx_tataricus*	0.1220996538	0.1324042815		0.0000000000	0.0211555599	0.0199568575	0.0196886846
4.*Eryx_miliaris*	0.1220996538	0.1324042815	0.0000000000		0.0211555599	0.0199568575	0.0196886846
5.*Python_molurus*	0.1889686962	0.2163025626	0.2013479586	0.2013479586		0.0223699297	0.0215334962
6.*Corallus_caninus*	0.1857531674	0.1954294288	0.1819678434	0.1819678434	0.2231328985		0.0211272548
7.*Eryx_jayakari*	0.1720753205	0.1773920466	0.1750269744	0.1750269744	0.1934198262	0.1888465912	

Table 6. Uncorrected pairwise genetic differences (*p*-distance) for COX1 gene among studied species of *Eryx tataricus*.

Taxa	1	2	3	4	5	6	7
1.*Eryx tataricus*		0.98286	0.00170	0.02959	1.64461	1.75008	1.72842
2.*Eryx sistanensis*	0.00158		2.24006	0.92607	1.80728	1.51968	1.58726
3.*Eryx johnii*	0.07264	0.13796		0.03033	1.64841	1.75408	1.72949

4.*Xenopeltis hainanensis*	0.24254	0.14174	0.07446		1.67599	1.76047	1.73949
5.*Eunectes notaeus*	0.27316	0.25008	0.24581	0.25875		1.91840	1.78656
6.*Chilabothrus argentum*	0.25394	0.21057	0.27665	0.26045	0.26433		1.30662
7.*Hypsiglena torquata*	0.27029	0.22209	0.25671	0.25283	0.25213	0.19001	

Table 7. Uncorrected pairwise genetic differences (*p*-distance) for COX1 gene among studied species of *Platyceps rhodorachis*.

Taxa	1	2	3	4	5	6	7	8
1.*Platyceps rhodorachis*		0.0166	0.0172	0.0171	0.0172	0.0183	0.0169	0.0172
2.*Spalerosophis diadema*	0.1524		0.0171	0.0177	0.0189	0.0189	0.0175	0.0173
3.*Hypsiglena ochrorhyncha*	0.1584	0.1729		0.0187	0.0190	0.0114	0.0177	0.0181
4.*Lytorhynchus diadema*	0.1613	0.1755	0.1797		0.0200	0.0190	0.0172	0.0177
5.*Rhinocheilus lecontei*	0.1625	0.1878	0.1780	0.2036		0.0185	0.0159	0.0185
6.*Hypsiglena sp.*	0.1729	0.1947	0.0794	0.1956	0.1772		0.0185	0.0195
7.*Stegonotus batjanensis*	0.1594	0.1651	0.1634	0.1706	0.1490	0.1649		0.0159
8.*Masticophis flagellum*	0.1571	0.1629	0.1741	0.1671	0.1759	0.1861	0.1403	

Table 8. Uncorrected pairwise genetic differences (*p*-distance) for COX1 gene among studied species of *Ptyas mucosa*.

Taxa	1	2	3	4	5	6	7
1.*Ptyas mucosa*		0.0159	0.0173	0.0156	0.0158	0.0180	0.0163
2.*Cyclophiops major*	0.1364		0.0151	0.0128	0.0085	0.0153	0.0166
3.*Euprepiophis perlacea*	0.1450	0.1264		0.0161	0.0151	0.0160	0.0163
4.*Ptyas dhumnades*	0.1330	0.0932	0.1432		0.0125	0.0169	0.0164
5.*Ptyas major*	0.1324	0.0456	0.1238	0.0910		0.0155	0.0162
6.*Coluber constrictor*	0.1657	0.1371	0.1356	0.1560	0.1422		0.0176
7.*Ptyas korros*	0.1460	0.1489	0.1460	0.1501	0.1478	0.1633	

Table 9. Uncorrected pairwise genetic differences (*p*-distance) for COX1 gene among studied species of *Spalerosophis diadema*.

Taxa	1	2	3	4	5	6
1.*Spalerosophis diadema*		0.0147450097	0.0181534888	0.0185529413	0.0183874249	0.0185482714

2.*Spalerosophis dolichospilus*	0.1179145202		0.0190090898	0.0178042687	0.0190988749	0.0188357607
3.*Platyceps rhodorachis*	0.1519734755	0.1778192973		0.0201570183	0.0193619131	0.0207529899
4.*Coluber constrictor*	0.1611748363	0.1629271705	0.1914240240		0.0145716730	0.0171944355
5.*Masticophis flagellum*	0.1541845438	0.1821733420	0.1728102334	0.1054490656		0.0179931351
6.*Euprepiophis mandarinus*	0.1566709232	0.1667596759	0.1846132175	0.1316200720	0.1472414137	

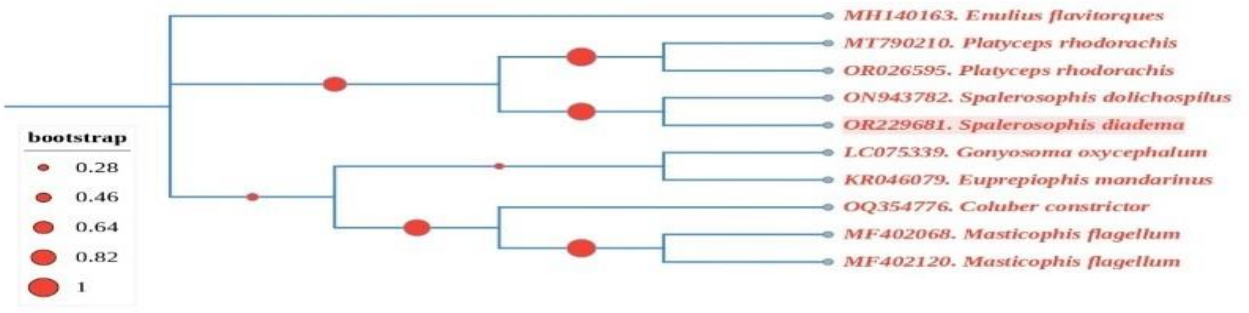

Figure 4. Maximum likelihood tree of *Spalerosophis diadema* based of cytochrome c oxidase subunit I (COX1) sequence using Kimura 2-parameter distances. With 100 Bootstrap values.

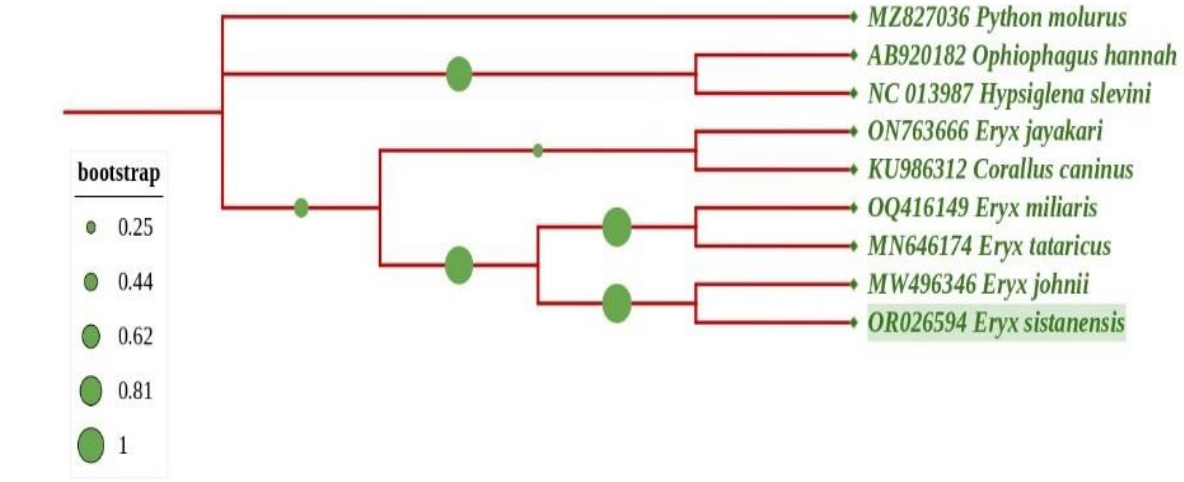

Figure 5. Maximum likelihood tree of *Eryx sistanensis* based of cytochrome c oxidase subunit I (COX1) sequence using Kimura 2-parameter distances. With 100 Bootstrap values.

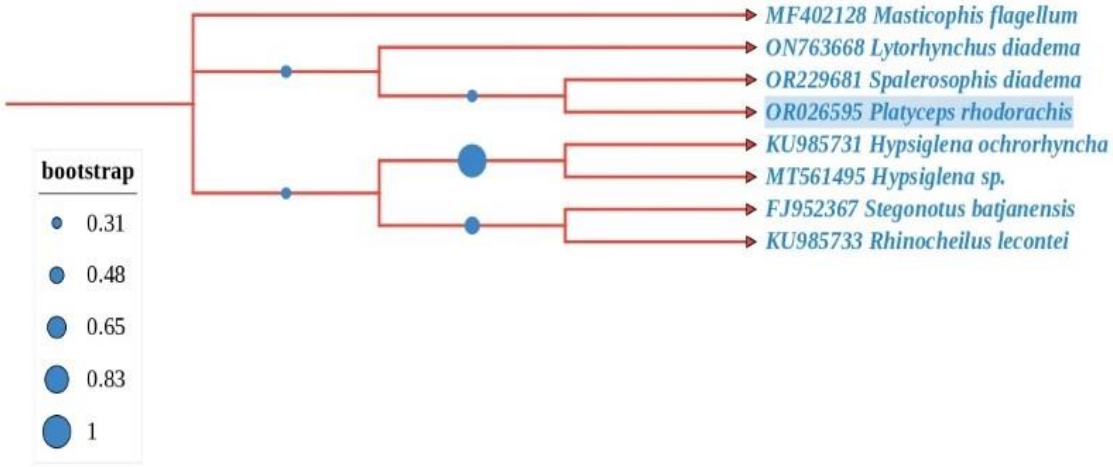

Figure 6. Maximum likelihood tree of *Platceps rhodorachis* based of cytochrome c oxidase subunit I (COX1) sequence using Kimura 2-parameter distances. With 100 Bootstrap values.

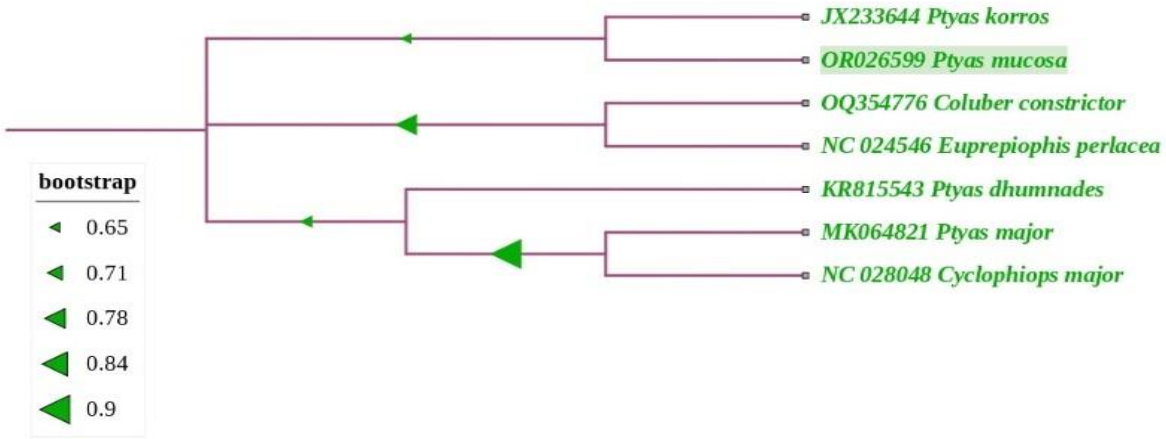

Figure 7. Maximum likelihood tree of *Ptyas mucosa* based of cytochrome c oxidase subunit I (COX1) sequence using Kimura 2-parameter distances. With 100 Bootstrap values.

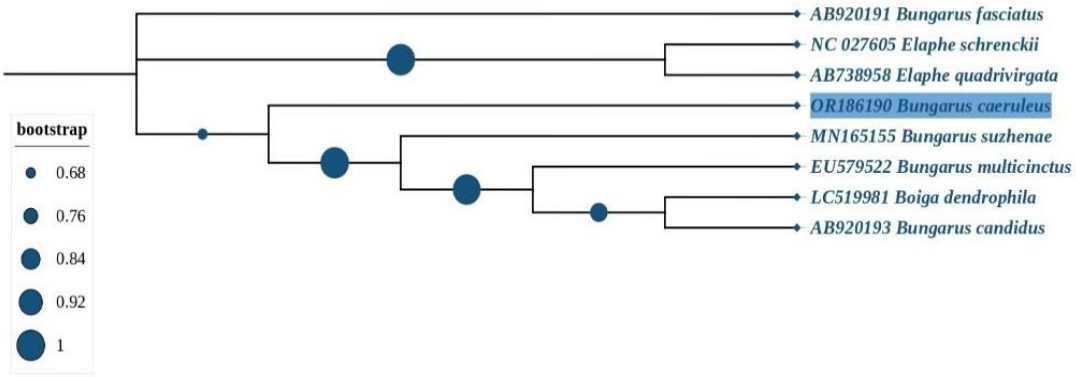

Figure 8. Maximum likelihood tree of *Bungarus caeruleus* based of cytochrome c oxidase subunit I (COX1) sequence using Kimura 2-parameter distances. With 100 Bootstrap values.

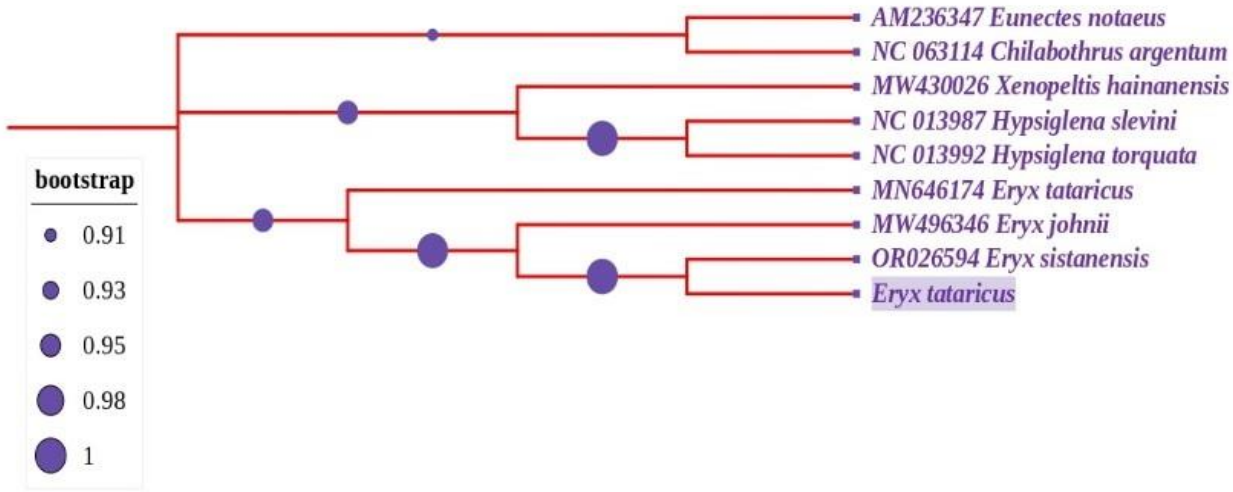

Figure 9. Maximum likelihood tree of *Eryx tatricus* based of cytochrome c oxidase subunit I (COX1) sequence using Kimura 2-parameter distances. With 100 Bootstrap value.

Discussion

The outcomes of our study on the phylogeography of snake fauna in the Cholistan desert provide valuable insights into the distribution and population of this species in an understudied region. Our results add to the existing knowledge on the snake fauna of Pakistan. During the present field survey, 11 species and 36 specimens of snakes were recorded from different habitats of the study area. The current study documented fifty localities for the presence of snake fauna, which is important for the phylogeography of snakes from any ecosystem (Khan, 2006).

The study of the concepts and mechanisms influencing the evolution of spatial patterns of genetic variation is known as phylogeography (Mertens, 1969; Avise, 2000; Chaber et al., 2021). GIS-based distribution records were kept up to date in a recent survey. As a result, phylogeographic research helps identify the precise biogeographic occurrences that influence the geographic range of a species (Maggs et al., 2015). In light of recent climatic data, the phylogeography of the species *Ptyas macosa*, *Eryx sistanensis, Platyceps rhodorachis, and Spalerosophis diadema* offers insight into how those species may react to events that could change their geographic range in the future (Avise, 2000; Guyomarc'h, 2003). Five different microhabitats were identified from the study area for the presence of snake species viz., Agriculture fields where crops, trees, and water are available. Uncultivated land flat stretches of land locally called "Dahars" were mostly barren areas; sand dunes contain mostly the desert area having no water available is the most populated and preferred habitat for snakes. Human settlements where the local population lived in towns and villages and tribes. Water bodies have different rain water ponds are present (Baig et al., 2008).

According to the current study, the region has an arid subtropical continental climate with strong summer winds, high temperatures, low relative humidity, low and intermittent rainfall, and a high rate of evaporation (Abdullah et al., 2017). At an altitude of 112 meters above sea level with an average annual temperature of 28.33°C, it is one of Pakistan's driest and warmest regions. June is the hottest month, with daily maximum temperatures typically above 45°C and occasionally surpassing 50°C (Ahmad, 2002). During the current study, molecular analysis according to subunit I of cytochrome c oxidase (COX1) gene sequence confirmed the identification of four different populations of snakes viz, *Spalerosophis diadema*, *Eryx sistanensis*, *Platyceps rhodorachis* and *Ptyas macosa* from Cholistan desert, Punjab, Pakistan. The results suggested the existence of four distinct molecular operational taxonomic units of *Spalerosophis diadema*, *Eryx sistanensis, Platyceps rhodorachis* and *Ptyas macosa* from study area (Baig et al., 2008; Mughal,

2017). However, *Eryx sistanensis* was the first recorded species from the study area. Before this *Eryx sistanensis* was considered as endemic species to Iran (Eheskandarzadeh et al., 2020). Neighbor-joining tree of *Eryx sistanensis* based on cytochrome c oxidase subunit I (COX1) sequence using Kimura 2-parameter distances with 100 Bootstrap values show that *Eryx sistanensis* was 697 bp clade of Pakistan while *Eryx sistanensis* clade of Iran have 627 bp show the remarkable closeness of species (Eskandarzadeh et al., 2020). Sanger sequencing has historically been utilized for the molecular identification of snake species. However, DNA barcoding of snake fauna for species identification should be preceding using standardized markers and protocols should be proceeding for practical reasons.

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