

Original Article

## Phylogeography of cliff racer (*Platyceps rhodorachis* Jan, 1865) from Punjab, Pakistan

Filografia de penhasco (*Platyceps rhodorachis* Jan, 1865) de Punjab, Paquistão

S. Malik<sup>a</sup> , A. Javid<sup>a\*</sup> , Hamidullah<sup>b</sup> , M. A. Iqbal<sup>c</sup> , A. Hussain<sup>a</sup> , S. M. Bukhari<sup>a</sup> , A. L. Chaber<sup>d</sup> , F. Hemmatzadeh<sup>d</sup> , N. Ismat<sup>e</sup> , A. Ali<sup>f</sup> , S. M. Azam<sup>g</sup> , G. Mustafa<sup>a</sup> , S. Hussain<sup>a</sup> , U. Ahmad<sup>a</sup> , A. Ahmad<sup>h</sup>  and W. Ali<sup>a</sup> 

<sup>a</sup>University of Veterinary and Animal Sciences, Department of Wildlife and Ecology, Lahore, Pakistan

<sup>b</sup>Islamia University Bahawalpur, Department of Zoology, Sub Campus Bahawalnagar, Bahawalpur, Pakistan

<sup>c</sup>Gomal University Sub Campus Tank, Department of Zoology, Dera Ismail Khan, Pakistan

<sup>d</sup>The University of Adelaide, School of Animal and Veterinary Sciences, Adelaide, Australia

<sup>e</sup>The University of Lahore, Department of Molecular Biology, Lahore, Pakistan

<sup>f</sup>The Islamia University of Bahawalpur, Department of Zoology, Bahawalpur, Pakistan

<sup>g</sup>University of Education, Department of Zoology, Lahore, Pakistan

<sup>h</sup>University of Veterinary & Animal Sciences, Para-Veterinary Institute, Karor, Layyah, (Sub-Campus), Lahore, Pakistan

### Abstract

The present study reports the existence of cliff racer, *Platyceps rhodorachis* from the plains of Punjab, Pakistan. A total of 10 specimens were captured during the field surveys from June to September, 2018 from different sites of Punjab. *Platyceps rhodorachis* was identified on the basis of morphology and confirmed through COI gene sequences. The obtained DNA sequences have shown reliable and exact species identification. Newly produced DNA sequences of *Platyceps rhodorachis* were submitted to GenBank and accession numbers were obtained (MK936174.1, MK941839.1 and MT790210.1). N-J tree based on COI sequences of *Platyceps rhodorachis* clearly separated as out-group with other members of family Colubridae based on p-distance. The intra-specific genetic variation ranges from 12% to 18%. The DNA sequences of *Platyceps rhodorachis kashmirensis*, *Platyceps rhodorachis ladacensis*, *Platyceps ventromaculatus*, *Platyceps ventromaculatus bengalensis* and *Platyceps ventromaculatus indusai* are not available at NCBI to validate their taxonomic positions. In our recommendations, a large scale molecular based identification of Pakistan's herpetofauna is required to report more new or subspecies from country.

**Keywords:** DNA barcoding, COI, *Platyceps rhodorachis kashmirensis*, *Platyceps ventromaculatus bengalensis*, *Ptyas mucosus*.

### Resumo

O presente estudo relata a existência de um corredor de penhasco, *Platyceps rhodorachis*, das planícies de Punjab, Paquistão. Um total de 10 espécimes foi capturado durante os levantamentos de campo de junho a setembro de 2018 em diferentes locais de Punjab. *Platyceps rhodorachis* foi identificada com base na morfologia e confirmada por meio de sequências do gene COI. As sequências de DNA obtidas mostraram identificação de espécies confiável e exata. Sequências de DNA de *Platyceps rhodorachis* recém-produzidas foram submetidas ao GenBank e os números de acesso foram obtidos (MK936174.1, MK941839.1 e MT790210.1). Árvore N-J baseada em sequências COI de *Platyceps rhodorachis* claramente separadas como *out-group* com outros membros da família Colubridae com base na distância-p. A variação genética intraespecífica varia de 12% a 18%. As sequências de DNA de *Platyceps rhodorachis kashmirensis*, *Platyceps rhodorachis ladacensis*, *Platyceps ventromaculatus*, *Platyceps ventromaculatus bengalensis* e *Platyceps ventromaculatus indusai* não estão disponíveis no NCBI para validar suas posições taxonômicas. Em nossas recomendações, uma identificação de base molecular em grande escala da herpetofauna do Paquistão é necessária para relatar mais novas ou subespécies do país.

**Palavras-chave:** código de barras de DNA, COI, *Platyceps rhodorachis kashmirensis*, *Platyceps ventromaculatus bengalensis*, *Ptyas mucosus*.

\*e-mail: arshadjavid@uvas.edu.pk

Received: December 4, 2020 – Accepted: January 20, 2021



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## 1. Introduction

World's almost all eco-biological regions are represented within comparatively little territory of Pakistan (796,095 sq. km), supported by immense variations in its geo-ecology, from mangrove forests along Sea coast in the south to the world's highest chain of mountains: the western Himalayas, Hindu Kush and Karakoram ranges (Ali et al., 2016). Ongoing deforestation, overgrazing, soil erosion, salinity and waterlogging are posing continued threats to Pakistan's biodiversity, have serious implications for the nation's other natural and agricultural ecosystems (Ali et al., 2020). Pakistan is encompassing varieties of habitats supporting rich biodiversity of animals and plants. The fauna and flora of Pakistan is Oriental and Palearctic in nature with many endemic species (Ali et al., 2017).

Punjab province is western stretch of the Indus plain, is characteristically flat and leveled with occasional outcrops of barren hills. The deep stone-free layer of alluvial silt is laid by washed down from the Himalayas through centuries, it extends from the sub-Himalayas in the north to the coast of the Arabian Sea in the south. It has a gradual sink to sea level: Rawalpindi is situated at 500 m of elevation, further south, Multan at 200 m, Sukkhar 100 m, and Hyderabad at 30 m (Khan, 2006). The climate is subtropical continental lowland type, with high summer temperatures and late monsoon rains. Characteristic features are aridity and continentality, annual rainfall ranges 350 to 400 mm, coming mostly in July-August, temperature ranges from 5 °C to 48 °C (Ali et al., 2018a, b).

Reptiles have generally a low ability to withstand changes in habitat and dispersal being particularly dependent on environmental condition for survival; they are therefore indicator species of habitat and climate. Snakes specially play an important role in the ecosystem. They are important in the control of rodents and insects populations in the nature. Venomous snakes are economically and medically important worldwide (Ali et al., 2018c). Snakes in the region have to be conserved as most of them are on the verge of extinction. The present study provides basic information, distribution, ecological description and molecular identification of *Platyceps rhodorachis* from Punjab, Pakistan.

## 2. Materials and Methods

### 2.1. Sampling and identification

Field surveys were done from June to September, 2018 in selected sites of Punjab, Pakistan. During the survey, a

total of 10 specimens were captured from three different sites of study area with the help of snake stick. The captured specimens were identified on the basis of taxonomic keys following Khan (2006). A few (n=5) specimens were euthanized and preserved in 95% alcohol for molecular identification. Preserved specimens were deposited at Zoological Museum, UVAS, Lahore (Table 1).

### 2.2. DNA extraction and Sequencing

Total genomic DNA was extracted from preserved tissues by phenol chloroform method. DNA samples were brought to PC-2 lab at School of Animal and Veterinary Sciences, The University of Adelaide, Australia for molecular identification. COI gene was amplified using universal COI primer set LCO1490 and HCO2198. PCR amplification was done in 25 µl volume reactions with 4-5 µl of DNA following Ali et al. (2020). After purification of PCR products all the samples were Sanger sequenced on AB3730xl sequencer (AGRF, Australia).

### 2.3. Data analysis

DNA sequences were checked and aligned in Bioedit 7.2 (Ali et al., 2020). The closely matched sequences of *Platyceps rhodorachis* and other members of family colubridae were downloaded from GenBank for phylogenetic analysis. The Neighbor-joining tree was constructed using 100 bootstrap replicates in MEGA 10. Genetic identities were calculated using Mega 10 based on p-distance.

## 3. Results

### 3.1. Distribution

The present study reports the first record of cliff racer, *Platyceps rhodorachis* from the plains of Punjab, Pakistan. Genus *Platyceps* is represented by six species namely *Platyceps rhodorachis*, *Platyceps rhodorachis kashmirensis*, *Platyceps rhodorachis ladacensis*, *Platyceps ventromaculatus*, *Platyceps ventromaculatus bengalensis* and *Platyceps ventromaculatus indusai* from Pakistan (Khan, 2006). Figure 1 showing the historic distribution map of *Platyceps rhodorachis* in Pakistan. Similarly, Figure 2 showing new locality distribution of *Platyceps rhodorachis* from Punjab.

### 3.2. Taxonomic position

Reptilia Laurenti, 1768  
Squamata Oppel, 1811  
Colubridae Oppel, 1811  
*Platyceps* Blyth, 1860  
*Platyceps rhodorachis* (Jan, 1865)

**Table 1.** Details of voucher specimens captured from study area.

Sampling sites	GPS Co-ordinates	Altitude (meter)	Voucher Number	Accession number
Pattoki	31°02'38.4"N73°52'29.7"E	186	*ZMUVAS22	MK936174.1
Changa manga	31°05'36.5"N73°57'43.4"E	192	*ZMUVAS24	MK941839.1
Lahore	31°36'33.8"N74°16'03.2"E	217	*ZMUVAS27	MT790210.1

\*ZMUVAS: Zoological Museum University of Veterinary and Animal Sciences, Lahore.

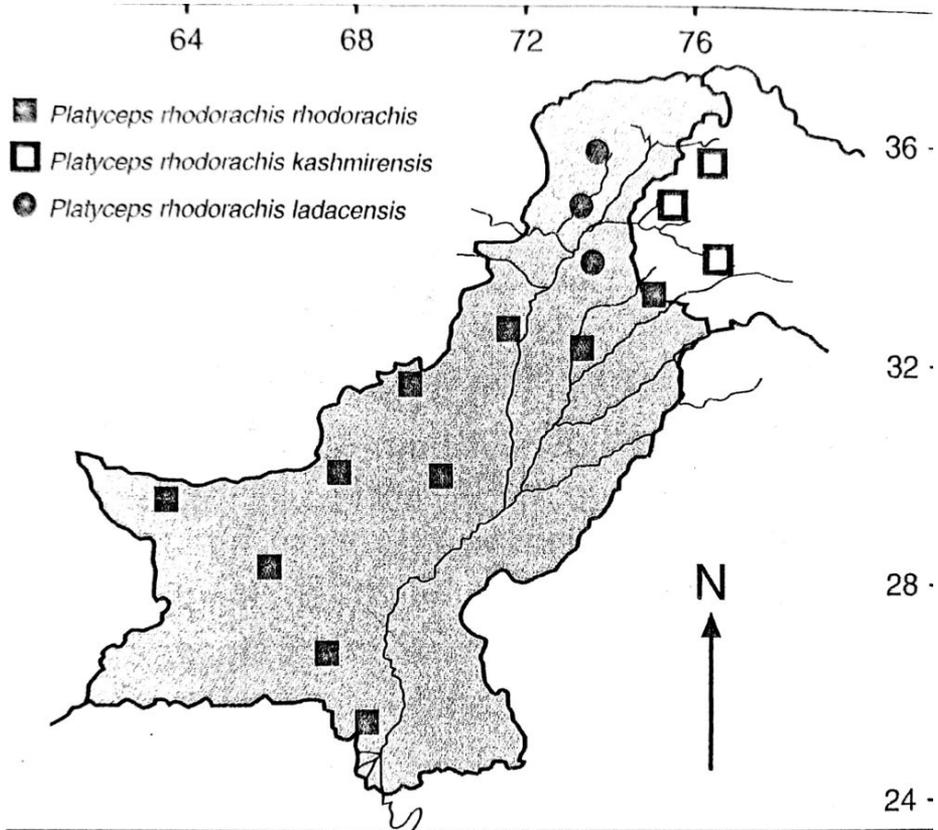


Figure 1. Historic distribution of cliff racer (*Platyceps rhodorachis*) in Pakistan (Khan, 1997).

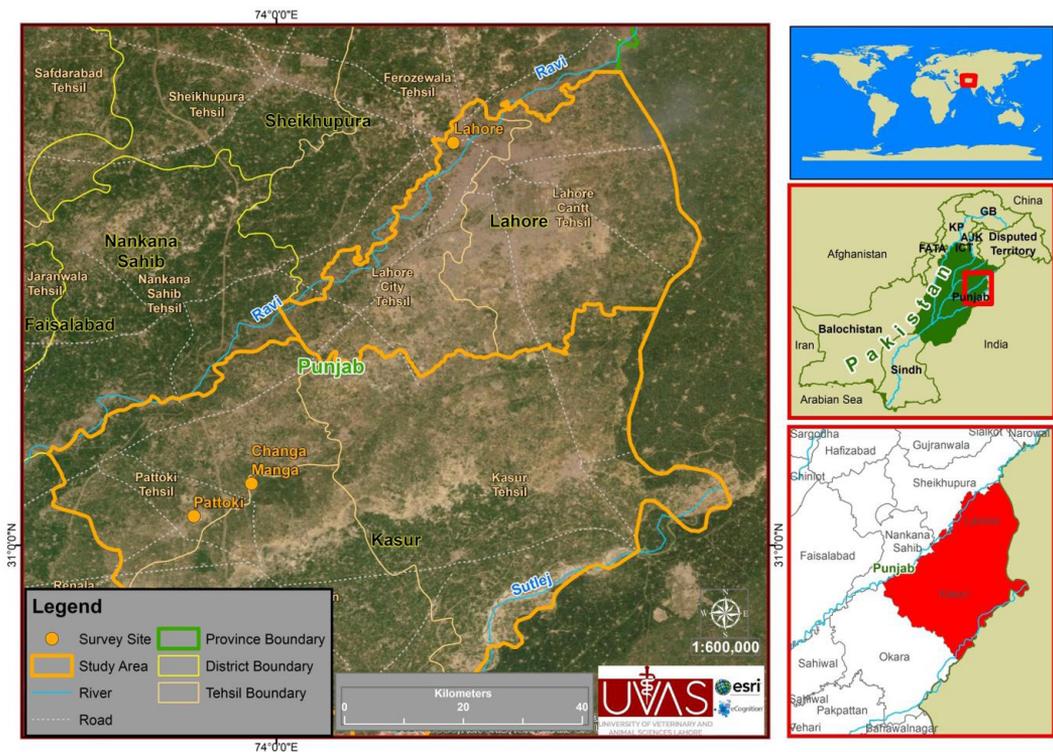


Figure 2. Collection sites of cliff racer (*Platyceps rhodorachis*) from Punjab, Pakistan.

### 3.3. Morphology

*Platyceps rhodorachis* (Figure 3) has a frail body with head being recognizable. The head is a bit brownish and the body is covered with circular spots at varying distance. The mean snout to vent length was  $337.88 \pm 1.19$  mm, head length  $17.18 \pm 1.09$  mm, tail length was  $419.15 \pm 10.16$  mm and weight was  $35.25 \pm 13.18$  g.

### 3.4. Coloration

*Platyceps rhodorachis* has dark grey to brown coloration.

### 3.5. Natural history

It inhabits rocky places with light vegetation. It occupies narrow spaces such as cracks and cervices of rocks. Being diurnal in nature it roams the areas occupied by birds and rats. It feeds on small lizards, rats, nestlings, small birds and sometimes insects. When cornered it hisses and can bite. The breeding season remains from April to May. The eggs are laid in rock breaches and are somewhat outstretched.

### 3.6. Molecular identification and phylogenetic relationship

After trimming ambiguous bases, the obtained COI sequences were 660 bp. The DNA sequences have shown

reliable and exact species identification. Newly produced DNA sequences of *Platyceps rhodorachis* were submitted to GenBank and accession numbers were obtained as mentioned in Table 1.

Recently few molecular identification studies of Asian herpetofauna have been carried out and DNA sequences of *Platyceps rhodorachis* and few other members of family colubridae were available at NCBI. Closely related DNA sequences were retrieved in blast searches and Neighbor-joining tree was constructed based on p-distance. Figure 4 showing Neighbor-joining tree of family colubridae and bootstrap values are given above the nodes.

*Platyceps rhodorachis* and closely related sequences were clustered together in the Neighbor-joining tree. *Platyceps rhodorachis* clearly separated as out-group with other members of family colubridae in N-J tree. Table 2 summarizes the comparison the genetic identities of family colubridae. The intra-specific genetic variation ranges from 12% to 18%.

The DNA sequences of *Platyceps rhodorachis kashmirensis*, *Platyceps rhodorachis ladacensis*, *Platyceps ventromaculatus*, *Platyceps ventromaculatus bengalensis* and *Platyceps ventromaculatus indusai* are not available at NCBI to validate their taxonomic positions.



Figure 3. Cliff racer (*Platyceps rhodorachis*).

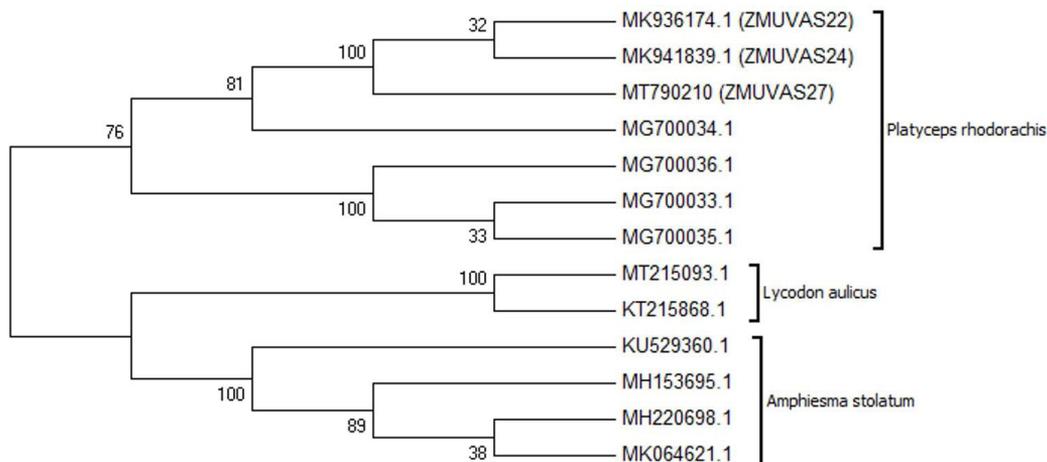


Figure 4. Neighbor-joining tree of family colubridae. Bootstrap values are given above the nodes.

**Table 2.** The interspecific and intraspecific genetic identities of family colubridae calculated based on p-distance.

Species	Sequences	<i>Lycodon aulicus</i>			<i>Platyceps rhodorachis</i>				<i>Amphiesma stolatum</i>					
		MT215093.1	KT215868.1	MK936174.1	MK941839.1	MT790210	MG700034.1	MG700036.1	MG700033.1	MG700035.1	MH153695.1	MH220698.1	MK064621.1	KU529360.1
<i>Lycodon aulicus</i>	MT215093.1	ID												
	KT215868.1	0.974	ID											
	MK936174.1	0.825	0.831	ID										
	MK941839.1	0.825	0.831	1										
	MT790210	0.825	0.831	1	1	ID								
<i>Platyceps rhodorachis</i>	MG700034.1	0.825	0.831	0.886	0.886	0.886	ID							
	MG700036.1	0.831	0.831	0.868	0.868	0.868	0.839	ID						
	MG700033.1	0.831	0.831	0.868	0.868	0.868	0.839	1	ID					
	MG700035.1	0.831	0.831	0.868	0.868	0.868	0.839	1	1	ID				
	MH153695.1	0.823	0.823	0.819	0.819	0.819	0.829	0.829	0.829	0.829	ID			
<i>Amphiesma stolatum</i>	MH220698.1	0.823	0.823	0.819	0.819	0.819	0.829	0.829	0.829	0.829	1	ID		
	MK064621.1	0.823	0.823	0.819	0.819	0.819	0.829	0.829	0.829	0.829	1	1	ID	
	KU529360.1	0.823	0.823	0.821	0.821	0.821	0.827	0.831	0.831	0.831	0.996	0.996	0.996	ID

#### 4. Discussion

Relatively few surveys have been conducted on the herpetofauna of Pakistan. There is scanty of knowledge regarding diversity and distribution of various reptilian species as very few species and fewer areas have been comprehensively studied (Ali et al., 2017). Most of the species in Pakistan have been identified on the basis of morphology by Khan (2006) but there is ambiguity in the taxonomic position of many species due to lack of molecular based identification (Ali et al., 2020).

Pakistan is represented by 72 snakes species and venomous species belongs to families Elapidae and Viperidae (Ali et al., 2018c). Previously Punjab province is represented by *Duttaphrynus stomaticus*, *Microhyla ornata*, *Euphylyctis cyanophlyctis*, *Hoplobatrachus tigerinus*, *Sphaerotheca breviceps*, *Fejervarya limnocharis*, *Lissemys punctata*, *Eublepharis macularius*, *Hemidactylus brookii*, *Hemidactylus flaviviridis*, *Trapelus agilis*, *Laudakia melanura*, *Acanthodactylus cantoris*, *Mesalina watsonana*, *Ophisops jerdonii*, *Ablepharus pannonicus*, *Eurylepis taeniolatus*, *Eutrophis dissimilis*, *Varanus bengalensis*, *Varanus griseus*, *Ramphotyphlops braminus*, *Typhlops ductuliformes*, *Eryx johnii*, *Boiga trigonata*, *Lycodon striatus*, *Amphiesma stolatum*, *Xenochrophis piscator*, *Oligodon arnensis*, *Oligodon taeniolatus*, *Psammophis leithii*, *Psammophis schokari*, *Ptyas mucosus*, *Spalerosophis diadema*, *Bungarus caeruleus*, *Naja naja*, *Naja oxiana*, and *Daboia russelii* (Minton, 1966; Mertens, 1969; Khan, 2006). Endemic species of Punjab are *Varanus griseus*, *Laudakia melanura*, *Ophisops jerdonii*, *Typhlops ductuliformes* (Khan, 2010).

*Platyceps rhodorachis* commonly known as cliff racer is widely spread in Middle East countries Syria, Iran, Saudi Arabia and Somalia. Previously, it has been reported from Baluchistan and Karachi in Pakistan (Khan, 1997). During the study, DNA of 3 specimens was successfully amplified using universal COI primer set.

Morphology-based identification of species and their description are slow, and mostly lead to vague results. In recent years, using of short DNA sequences for the formalized detection of species has become prominent under the components of DNA barcoding or DNA taxonomy (Hussain et al., 2020). The DNA based identification of species has been started in 2009 however effective protocols of amphibians and reptiles barcoding are relatively new (Che et al., 2012; Nagy et al., 2012; Xia et al., 2012). The COI has a high success rate as compare to Cytb in identification of many taxa including birds, fish and invertebrates' species (Hebert et al., 2003).

Traditionally Sanger sequencing is used in molecular identification of reptiles. However, there are efforts to apply high-throughput sequencing methods and environmental sequencing based on sampling of DNA without any contact with the actual organisms (Hajibabaei et al., 2011; Thomsen et al., 2012).

#### 5. Conclusions and Recommendations

All the newly produce DNA sequences have shown reliable and clear species identifications of all the

captured specimens. The diversity and distribution of many reptiles species are not well known in the Pakistan due to lack of molecular based taxonomic information. In our recommendations, a large scale molecular based identification of Pakistan's herpetofauna is required to report more new and subspecies from country.

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