QUANTITATIVE ANALYSIS OF ANURAN DIVERSITY, ABUNDANCE, AND DISTRIBUTION IN KHYBER PAKHTUNKHWA, PAKISTAN

Abstract

Aims: This paper presents a comprehensive study of the Anuran fauna of Khyber Pakhtunkhwa, Pakistan, which was conducted across nine districts. After the collection and identification of specimens from the study area, their diversity, relative abundance (RA), and distribution (C) were evaluated to understand their distribution patterns within Khyber Pakhtunkhwa.

Place and Duration of Study: Anurans were collected from the districts of Peshawar, Dir, Bannu, Buner, Charsadda, Chitral, Karak, Lakki Marwat, and Haripur of Khyber Pakhtunkhwa from July to September 2021 and from April to June 2022.

Study design: Visual encounter surveys were conducted during different seasons, encompassing both daytime and nighttime observations near various water bodies such as water channels, small pools, and microhabitats suitable for the anurans. Emphasis was placed on the collection of specimens from diverse habitats. The collected specimens underwent thorough morphological and morphometric analyses to accurately identify the species.

Methodology: In total, 190 specimens were collected and transported to the laboratory for identification. The collected specimens were euthanized, carefully arranged, and preserved in glass jars filled with a 10% formalin solution. Accurate species identification was achieved through the utilization of authentic literature, updated taxonomic keys, and observation and measurement of morphological and morphometric parameters.

Results: The specimens that were examined and identified belonged to two families, Bufonidae and Dicroglossidae, and five genera. Two genera were observed in the Bufonidae family, Duttaphrynus and Bufotes. The Dicroglossidae family comprised three genera: Hoplobatrachus, Euphlyctis, and Allopaa. In the current study, seven different species were identified: *Duttaphrynus stomaticus, Duttaphrynus melanostictus, Bufotes surdus* from the Bufonidae family, and *Hoplobatrachus tigerinus, Euphlyctis cyanophlyctis, Euphlyctis kalasgramensis,* and *Allopaa hazarensis* from the Dicroglossidae family. *Duttaphrynus stomaticus* was the most abundant species found at all the study sites (C=88.8% and RA=44.2%), whereas *Euphlyctis kalasgramensis* and *Allopaa hazarensis* were sporadic (C=11.1% and RA=0.52%), and (C=11.1% and RA=2.10%), respectively. Furthermore, this research unveils the first-ever report of *Euphlyctis kalasgramensis* within Khyber Pakhtunkhwa, specifically in the Peshawar District, while Allopaa hazarensis was solely reported in the Dir district.

Conclusion: The study found that *Duttaphrynus stomaticus* exhibited a vast distribution across all nine districts surveyed. Khyber Pakhtunkhwa, being an ecologically diverse area, holds diverse anuran fauna. Taxonomic revision of the anuran fauna using both classical and molecular approaches may yield several new taxa. This will further help in the study of biology and conservation as well as the ecological role of anurans. The current taxonomic work may require improvement.

Keywords: *Anurans, Khyber Pakhtunkhwa, Species diversity, Relative abundance, Taxonomic identification, Morphological analysis.*

1 INTRODUCTION

Around the globe, there are approximately 6,500 documented species of amphibians (Frost et al., 2006). Among these, 24 species belonging to four families, namely, Bufonidae, Megophryidae, Microhylidae, and Dicroglossidae, have been reported in certain regions of Pakistan (Khan and Mufti, 1994; Frost, 2010–2016; Khan, 2010; Baig et al., 2008). There is no evidence to support the presence of the orders Caudata and Gymnophiona in Pakistan (Hopkins, 2007). As an ancient group of vertebrates, they hold a remarkable distinction as the sole surviving descendants of the earliest terrestrial vertebrates (Wake and Koo 2018). The term "amphibians" comes from "amphibious," reflecting their metamorphosis from aquatic larvae to terrestrial adults (Zug, Vitt, & Caldwell, 2001). Frogs and toads (order Anura) are amphibians, with four legs, short tailless bodies, broad flat heads,

and large mouths. Their muscular elongated hind legs enable them to hop or jump, distinguishing them from other amphibians. They have moist or warty skin but lack scales and claws (Vitt & Caldwell, 2014). Frogs have long legs and smooth mucus-covered skin, whereas toads have short legs and rough skin (Khan, 2010).

Anurans demonstrate adaptability and thrive in diverse habitats, including terrestrial and freshwater ecosystems (Khan 2010; Brode and Burry 1984). Their distribution patterns are influenced by the type of aquatic habitat, substrate, and the surrounding vegetation (Bousbouras and Ioannidis, 1997). They possess a unique reproductive behavior, which involves laying eggs in water, where they remain until hatching and undergo full metamorphosis into adulthood (Tagar et al., 2019). The presence and abundance of anurans at their breeding sites are influenced by a combination of abiotic and biotic factors. Abiotic factors such as temperature (Pope et al., 2000), hydroperiod (Watson et al., 2003), and water quality (Banks and Beebee, 1987) play significant roles in determining the suitability of breeding sites. Biotic factors, such as the structure of vegetation in and around the pond, also contribute to shaping their presence and population at these sites (Bosch and Solano, 2003). A significant number of anurans species adopt a subterranean lifestyle, typically as a strategy to escape harsh environmental factors such as extreme heat, droughts, or moisture deficits (Wells, 2007). They play a vital role in climate change and inhabit diverse habitats worldwide, except on certain islands. Additionally, they serve as a significant food source for various organisms including birds, reptiles, fish, and mammals (Martin, 2000; Madhab and Ashok, 1980). As these amphibians become prey for numerous predators, nutrients are transferred from the aquatic to the aerial ecosphere through nutrient shifting (Christie et al., 2008). They exhibit a predator lifestyle and primarily feed on invertebrates, with larger frogs being capable of capturing vertebrates (Pough et al., 2001). Anurans generally share a common diet, as they primarily consume insects, worms, and occasionally other anurans, while variations exist in reproduction, habitat preference, and feeding methods (Khan 1999; Khan and Malik 1987). They usually exhibit sit-and-wait or active foraging behaviors and primarily consume insects and other invertebrates (Duellman et al., 1986). Their dietary reliance on insects enables them to effectively control insect populations by playing a crucial role in maintaining the ecological balance (Christie et al., 2008; Stuart et al., 2004). Moreover, they are regarded as valuable bioindicators that reflect the health of ecosystems (Duellman and Trueb, 1986; Quaranta et al. 2009).

Pakistan is a man-made northwestern political division of the Indo-Pakistan subcontinent and not a natural geoecological entity (Khan, 1999b). The country covers an area of 796,096 km^2 and is located between 24°N and 37°N latitude, as well as between 61°E and 78°E longitude and falls into two zoogeographical regions: Palearctic and Oriental (Boulenger 1890; Darlington 1957; Smith 1935, 1943; Khan, 2014). The climate in Pakistan is continental, characterized by significant fluctuations in winter and summer temperatures. The monsoon season occurs from July to October, leading to varying rainfall patterns throughout the year, often resulting in both floods and droughts (Ahmad, 1951; Khan, 1999b). Pakistan's anuran fauna is not very diverse. To date, a total of 24 species of anurans have been recorded in Pakistan because of the humid climates in the Indus Valley, streams in mountainous regions of the Himalayas in the North, and underground waterways in the western parts of the Balochistan highlands (Khan, 2002, 2004; Masroor, 2012). In Pakistan, anurans are categorized into four families: Bufonidae, Dicroglossidae, Megophryidae, and Microhylidae (Masroor, 2012), which are distributed across various regions, with elevations ranging from sea level to almost 4000m although no individual species encompass this entire range. Certain species, such as *Duttaphrynus olivaceus*, *Bufotes surdus*, and *Fejervarya syhadrensis*, exhibit a limited distribution and are confined to relatively low elevations. Conversely, species such as *Duttaphrynus himalayanus*, *Scutiger occidentalis*, *Bufotes pseudoraddei*, and *Bufotes baturae* are exclusively found at higher elevations. The remaining species demonstrate broader vertical ranges, primarily spanning from low to moderate elevations. *Duttaphrynus stomaticus* displays the most extensive vertical distribution, ranging from sea level to elevations surpassing 2,400 m (Khan, 2014). Despite this biodiversity richness, there is a severe lack of data concerning the country's distribution of amphibian and reptilian species (Khan 1997,2006; Sindaco and Jerem-čenko, 2008).

The main objective of this study was to describe the relative abundance, diversity, and distribution of anurans in Peshawar, Dir, Bannu, Buner, Charsadda Chitral, Karak, Lakki Marwat, and Haripur, in Khyber Pakhtunkhwa. This study is crucial because it quantifies the diversity, distribution, relative abundance, and surveys of various species of anurans in these districts.

2 LOCATION OF THE STUDY AREAS

This study was conducted as a survey in Khyber Pakhtunkhwa (vid. Fig.1), previously known as the Northwest Frontier Province, which is the northernmost province of Pakistan. It shares borders with Afghanistan to the west and north, the northern areas to the east and north, Punjab to the southeast, and Baluchistan to the southwest. Covering a total area of **74,521** km^2, Khyber Pakhtunkhwa is characterized by a variety of physical and climatic conditions, reflecting a broad diversity in terms of stratigraphy, lithology, climate, flora, and fauna. The survey was meticulously executed in the following nine districts, namely district Charsadda, which lies between 34.1682° North and 71.7504° East; district Peshawar, which lies between 34.0151° North and 71.5249° East; district Bannu which lies between 32.9298° North, 70.6693° East; district Buner which lies between 34.3943° North, 72.6151° East; district Dir which lies between 34.9161° North and 71.8097° East; district Chitral which lies between 36.1113° North and 72.1416° East; district Karak which lies between 33.1277° North and 71.0973° East; district Lakki Marwat which lies between 32.5763° North and 70.9071° East; and district Haripur which lies between 34.0403° North and 72.8515° East.

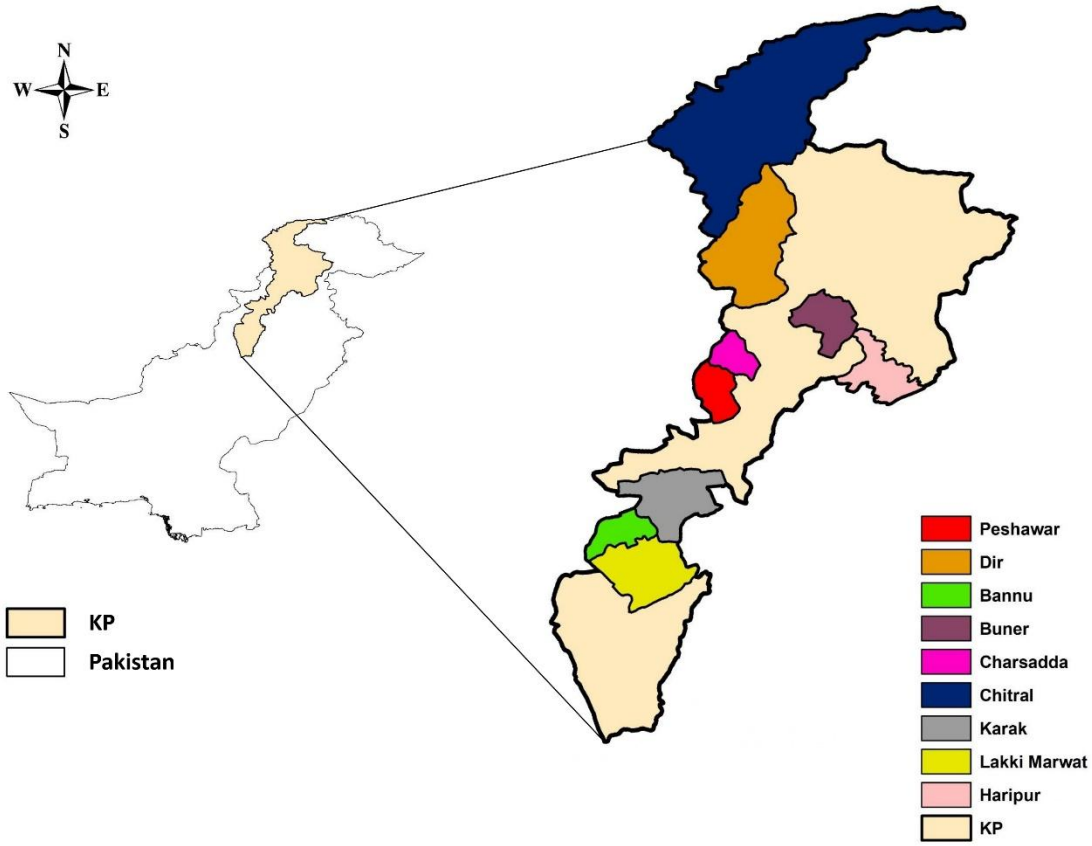


Fig. 1. Map showing locations of the study area- Khyber Pakhtunkhwa, Pakistan.

3 MATERIAL AND METHODS

This study was conducted thoroughly from July to September 2021 and April to June 2022 to collect Anuran specimens. Anurans were sampled using visual encounter surveys conducted during both daytime and nighttime near water bodies, such as water channels, small water pools, and suitable microhabitats like crevices, damp soil, herbs, shrubs, and logs. The specimens were captured using gloves, hand nets, and long forceps. Various equipment, including field books, torch lights, formalin, syringes, large plastic jars, paper tape, and pencils, were used in this study. Active searching was performed to locate specimens under the bushes, logs, and debris. Anurans are typically found near

water, but toads can also be observed in gardens and various habitats. The most effective methods for collecting anurans are hand picking and using hand nets. Handpicking is particularly effective for capturing toads, whereas hand nets are commonly used for collecting frogs because they primarily reside in water and occasionally venture onto land.

3.1 Preservations and identification of the Anurans

All the collected anuran specimens were euthanized instantly by injecting absolute ethanol into their heads using a disposable 3 ml syringe. Subsequently, the specimens were carefully arranged in a tray to facilitate observation of any external abnormalities. They were positioned in a way that highlighted important identification features, such as an open mouth, extended hind legs, and spread fingers and toes. The specimens were subsequently preserved in glass jars filled with a 10% formalin (formaldehyde) solution. Waterproof labels were affixed to the jars containing detailed information, such as the specimen number, collection date, and locality. Taxonomic identification of anuran specimens in this study was aided by authentic literature and updated taxonomic keys (Boulenger, 1890; Minton, 1966; Khan, 2003, 2017; Masroor, 2012).

Two important parameters were used to identify the specimens.

i. Morphological parameters

ii. Morphometrical parameters

During the identification of specimens important morphological parameters, that is, parotid glands present or absent, vomerine teeth present or absent, teeth of the upper jaw present or absent, snout wide or pointed, dorsal and ventral surface characteristics of the skin, presence of warts on the skin, presence or absence of mid-dorsal line, size of tympanum membrane, length of fingers, Webbing, Tibiotarsal articulation, and the presence or absence of the tibial gland were observed in a petri dish using a magnifying glass and stereomicroscope (Leitz Wetzlar, Germany, and Warszawa, Poland).

While Morphometrical parameters such as snout-vent length (SVL), snout length (SL), head length (HL), eye diameter (ED), interorbital distance (IOD), internarial distance (IND), and Foot length (FL) were measured using vernier caliper to the nearest 0.1mm. The methods described by Watters *et al*. (2016) were followed for the morphometric analysis.

Following the identification of the specimens, the whole collection was deposited in the Natural History Museum, Department of Zoology, University of Peshawar, for future studies.

3.2 Data analysis methods

The distribution status of the collected anuran species at different collection sites was calculated using the equation given below (Azim et., 2023):

Eq. 1

$$\text{Distribution (C)} = \frac{\text{n}}{\text{N}} \times 100$$

where n is the number of sites where anurans were found, and N is the total number of sites analyzed. Based on the C values, the species were categorized into the following classes: sporadic species (0 – 20%), infrequent (20-40%), moderate (40-60%), frequent (60-80%), and constant (80-100%).

The relative abundance of the anurans was determined as (Azim et., 2023) follows:

Eq. 2

$$\text{Relative Abundance (RA)} = \frac{\text{n}}{\text{N}} \times 100$$

where n is the number of anurans, and N is the number of anuran species. Based on the values of RA, the species were categorized into the following classes: dominant species (RA >10%), sub-dominant species (3-10 %), and satellite species (RA< 3%).

4 RESULTS

Taxonomic study of the anuran specimens revealed that the collected anurans belonged to two families, Bufonidae and Dicroglossidae, and five genera. Two genera were observed in the Bufonidae family: *Duttaphrynus* and *Bufotes*. The Dicroglossidae family comprised three genera: *Hoplobatrachus*, *Euphlyctis*, and *Allopaa*. In the current study, seven distinct species were identified: *Duttaphrynus stomaticus*, *Duttaphrynus melanostictus*, *Bufotes surdus* from the Bufonidae family, and *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis*, *Euphlyctis kalasgramensis*, and *Allopaa hazarensis* from the Dicroglossidae family (vid. Fig. 2).

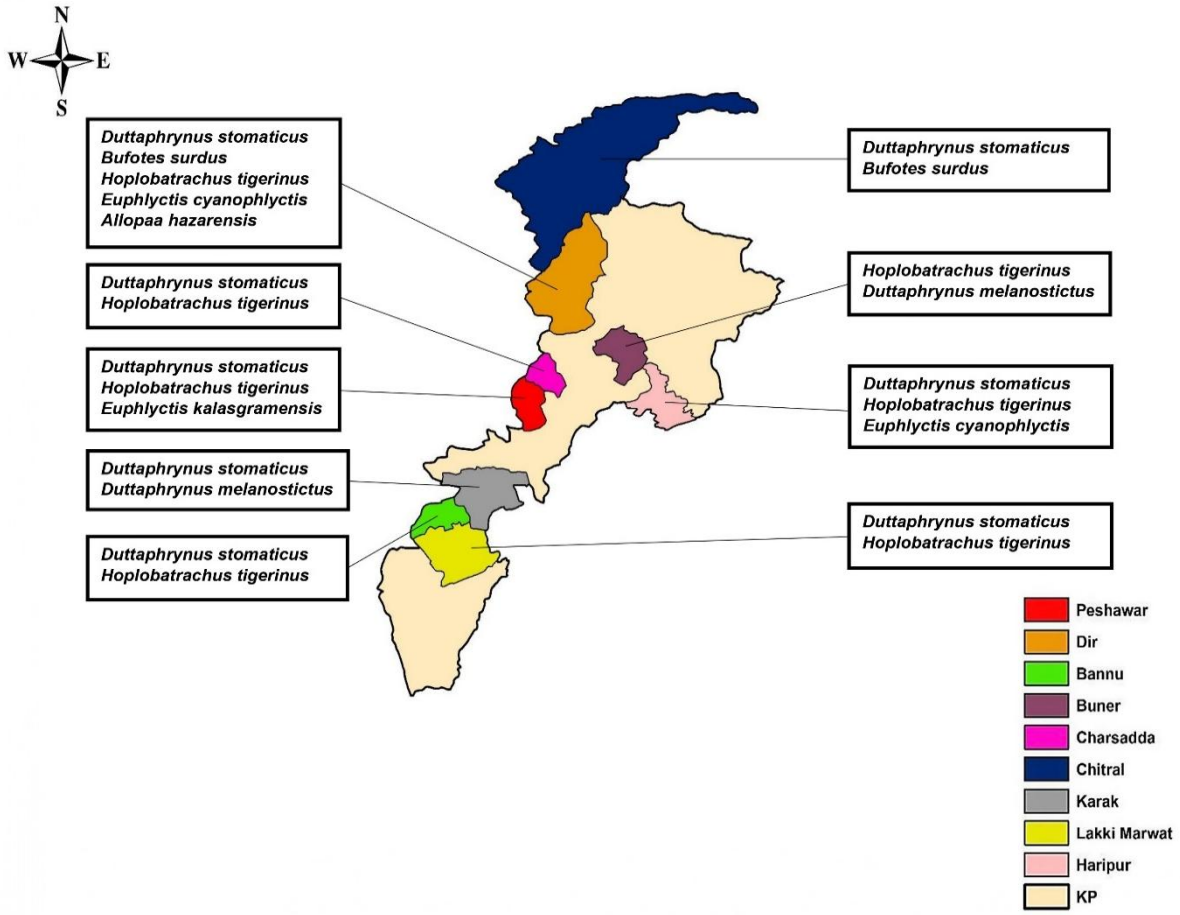


Fig 2. Geographical distribution of Anurans in Khyber Pakhtunkhwa, Pakistan

4.1 Species account

4.1.1 Family Bufonidae

4.1.1.1 Duttaphrynus stomaticus

Common names: Indus Valley toad, Maidani gauk**,** Indian marbled toad**,** Punjab toad.

Morphological characteristics

Duttaphrynus stomaticus, also known as the Indian common toad, is a medium-sized toad with an oblong oval body and a snout-vent length (SVL) typically ranging from 62 to 68 mm. The dorsum is covered with small, flat, and rounded warts that have black tips, with an overall color spectrum that extends from light gray to olive, yellowish green, or near black (vid. Fig. 3.B). Ventrum is dirty white with distinct dark patterns on the throat and forearms (vid. Fig. 3.C). Also, the ventral surfaces of the hands, palms, and legs are covered with numerous creamy white warts along with brown-tipped digits. The

head is characterized by a flat and slightly concave shape, broader than it is long, and lacks cranial crests. Snout is pointed, and interorbital space is slightly broader than that of the upper eyelid. The tympanum is distinct, located at the posterior corner of the eye, and is approximately two-thirds of the size of the eye. Parotoid glands are elongated, elliptical, and not bean-shaped (vid. Fig. 5.A). The first and second fingers are subequal, and fingers follow a length hierarchy of 2=4<1<2<5<3 (vid. Fig. 3.E). Its hind limbs feature partially webbed toes with round fingertips. Toes are arranged in a length sequence of 1<2<5<3<4 and feature single subarticular tubercles and a tarsal spinulated ridge (vid. Fig. 3.D). A distinct tibial gland is also present on the hind limbs. The tibiotarsal articulation aligns between the shoulder and eye when the hind limb is oriented parallel to the body.

Habitat: It is frequently observed in various habitats such as ground, holes, crevices, under rocks, and amidst leaf litter. It is primarily active during the nocturnal hours.

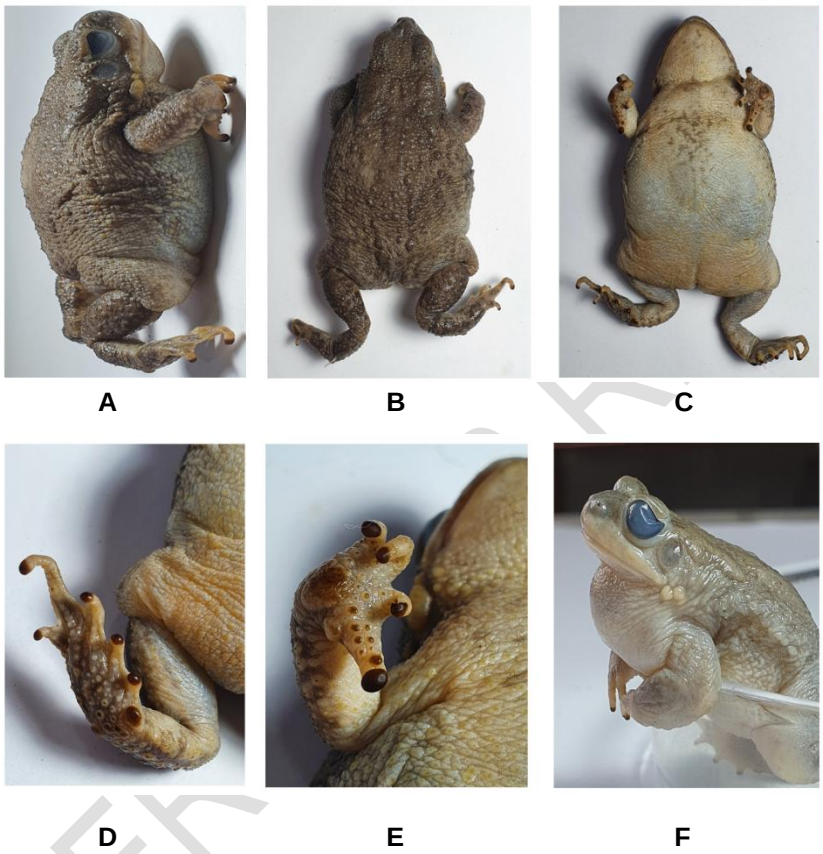


Fig 3. *D.stomaticus* (A-F). (A) Lateral view (B) Dorsal surface (C) Ventral surface (D) Toes (E) Fingers (F) Vocal sac

Table 1. Illustration of statistical analysis of the morphometric characterization of *Duttaphrynus stomaticus*

Morphometric parameters	(n=20)	
	Range	Mean ± S.D
SVL	62.3-68.5	65.6±2.0
SL	5.3-9.2	7.4±1.1
HL	15.2-20.2	17.2±1.4
ED	4.1-7.3	5.7±0.9
IOD	5.1-8.3	6.5±0.9
IND	3.1-5.4	4.1±0.7
FL	19.2-29.3	23.7±2.8

4.1.1.2 Duttaphrynus melanostictus

Common name: Asian common toad, Asian black-spined toad, Asian toad, Black spectacled toad

Morphological characteristics

Duttaphrynus melanostictus also known as the Asian common toad is a prominent toad species in Pakistan, ranking among the country's largest toads with a snout-vent length (SVL) of 83-84 mm. This species exhibits distinct coloration and markings, the dorsum is brownish grey, varying in shade, or brown to reddish with dark spots adorned with horny warts that are black tipped (vid. Fig. 4.A). In contrast, the ventrum is uniformly dirty white, speckled with light brown on the chin and throat (vid. Fig. 4.B). Notably, black cornifications are present on the upper lips and the tips of the fingers and toes. The head, wider than its length, is characterized by the presence of bony ridges and a temporal ridge, whereas the rostral is absent. The interorbital space is nearly broader than the width of the upper eyelid. Crown of the head is smooth, interrupted only by a few tubercles located between the crests. Parotoid glands are kidney-shaped, and the tympanic membrane is distinct (vid. Fig. 4.C). The fingers exhibit double subarticular tubercles under the penultimate phalanx and bear black cornifications (vid. Fig. 4.D). The toes are partially webbed and bear black cornification (vid. Fig. 4.D).

Habitat: It is characterized by its lethargic disposition and primarily nocturnal activities. These toads can often be found on the ground, in holes and crevices, under rocks, and concealed within leaf litter.

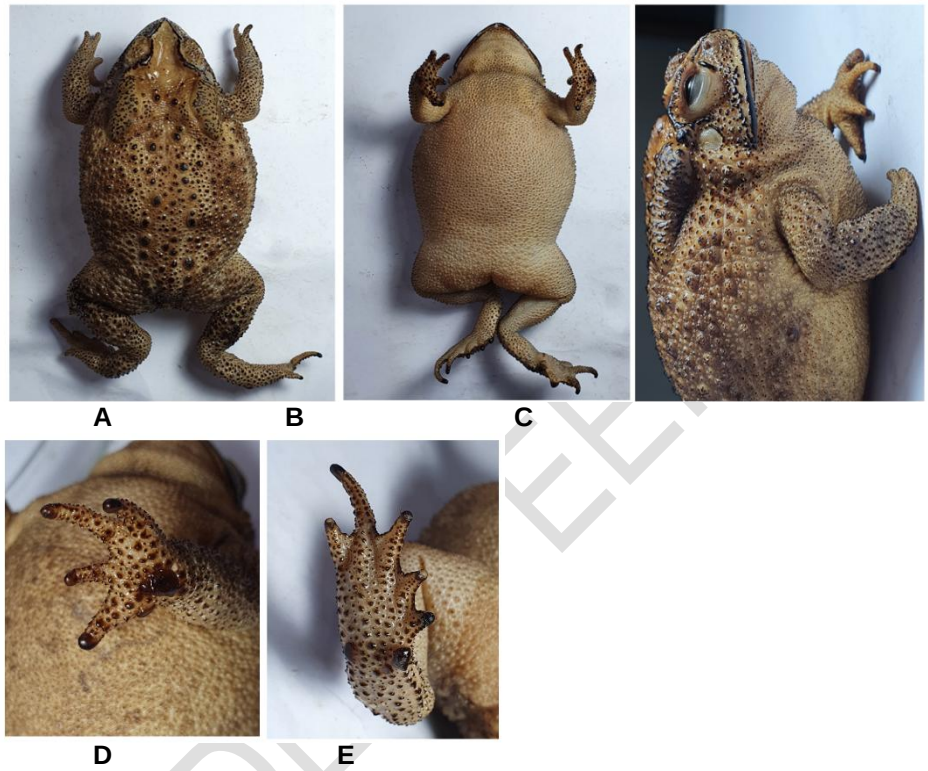


Fig. 4. *D.melanostictus* (A-E). (A) Dorsal surface (B) Ventral surface (C) Lateral view of head showing distinct tympanum (D) Fingers (E) Toes

Table 2. Illustration of statistical analysis of the morphometric characterization of *Duttaphrynus melanostictus*

Morphometric parameters	(n=5)	
	Range	Mean ± S.D
SVL	83.3-84.2	83.8±0.4
SL	8.1-9.2	8.6±0.5
HL	23.2-24.2	23.7±0.5
ED	7.1-7.4	7.2±0.1
IOD	6.1-7.4	6.5±0.5
IND	6.1-6.4	6.3±0.1
FL	28.2-30.2	29.2±1.0

4.1.1.3 Bufotes surdus

Common name: Iranian toad

Morphological characteristics

Bufotes surdus, commonly known as Iranian Toad, is a medium-small toad, with its snout-vent length (SVL) ranging from 55 to 60 mm. A distinctive coloration characterizes this species, where the dorsum is predominantly grey with greenish spotting and bears unispinulated tubercles. A notable feature is the dark blotch that adorns the upper eyelid (vid. Fig. 5.A). The head is without cranial crests, and the interorbital space is narrower than the upper eyelid. Tympanic membrane is distinct, approximately half the eye's diameter. Parotoid glands are squarish, matching the upper eyelid's length. The Fingers lack webbing (vid. Fig. 5.C), while the hind limbs are extensively webbed (vid. Fig. 5.D).

Habitat: It exhibits nocturnal activities. During daylight hours, it remains concealed under stones, vegetation, beneath fallen logs, or retreats into holes and crevices in the ground.

A **B** **C** **D**

Fig 5. *B.surdus* (A-D). (A) Dorsal surface (B) Ventral surface (C) Fingers (D) Toes

Table 3. Illustration of statistical analysis of the morphometric characterization of *Bufotes surdus*

Morphometric parameters	(n=8)	
	Range	Mean ± S.D
SVL	55.3-59.4	57.0±1.4
SL	6.5-7.8	7.2±0.4
HL	6.9-9.1	8.3±0.7
ED	6.0-6.7	6.4±0.2
IOD	4.1-5.1	4.6±0.4
IND	3.0- 4.3	3.5±0.4
FL	23.1-27.0	25.2±1.4

4.1.2 Family Dicroglossidae

4.1.2.1 Hoplobatrachus tigerinus

Common name: Tiger frog, Bullfrog

Morphological characteristics

Hoplobatrachus tigerinus, commonly known as Bullfrog/Tiger Frog is one of the larger and bulkier frog species with snout-vent length (SVL) ranging from 88-125 mm. The dorsum exhibits shades from

yellowish green to olive or greyish-brown and is characteristically marked by an erratic pattern of darker olive or greyish-brown spots. A white or yellowish-white streak originates from each eye's posterior corner, extending to the groin. The dorsal surface also includes a mid-dorsal line, which may be continuous or broken, running from the snout to the cloaca and longitudinal glandular folds (vid. Fig. 6.A). In contrast, the ventrum has a smooth texture with a white or dirty hue (vid. Fig. 6.B). The head is longer than its width and slightly concave, featuring a pointed snout. The interorbital space is narrower than the diameter of the upper eyelid. Tympanic membrane is distinct, and round is equal in size to the eye. Fingers are without webbing and bear blunt tips. The first finger is usually longer than the second, with the overall finger length hierarchy being 3-1-4-2 (vid. Fig. 6.D). Toes are entirely webbed and feebly emarginate, with slightly inflated tips. They are arranged in decreasing order of length, 4-5-3-2-1. A distinctive, long, flat, and blunt shovel-shaped inner metatarsal tubercle is located at the base of the first toe (vid. Fig. 6.C).

Habitat: It is frequently observed in habitats like waterlogged paddy fields, vegetation on the periphery of water bodies, damp grasslands, and marshlands, indicating a preference for damp and partially submerged environments.

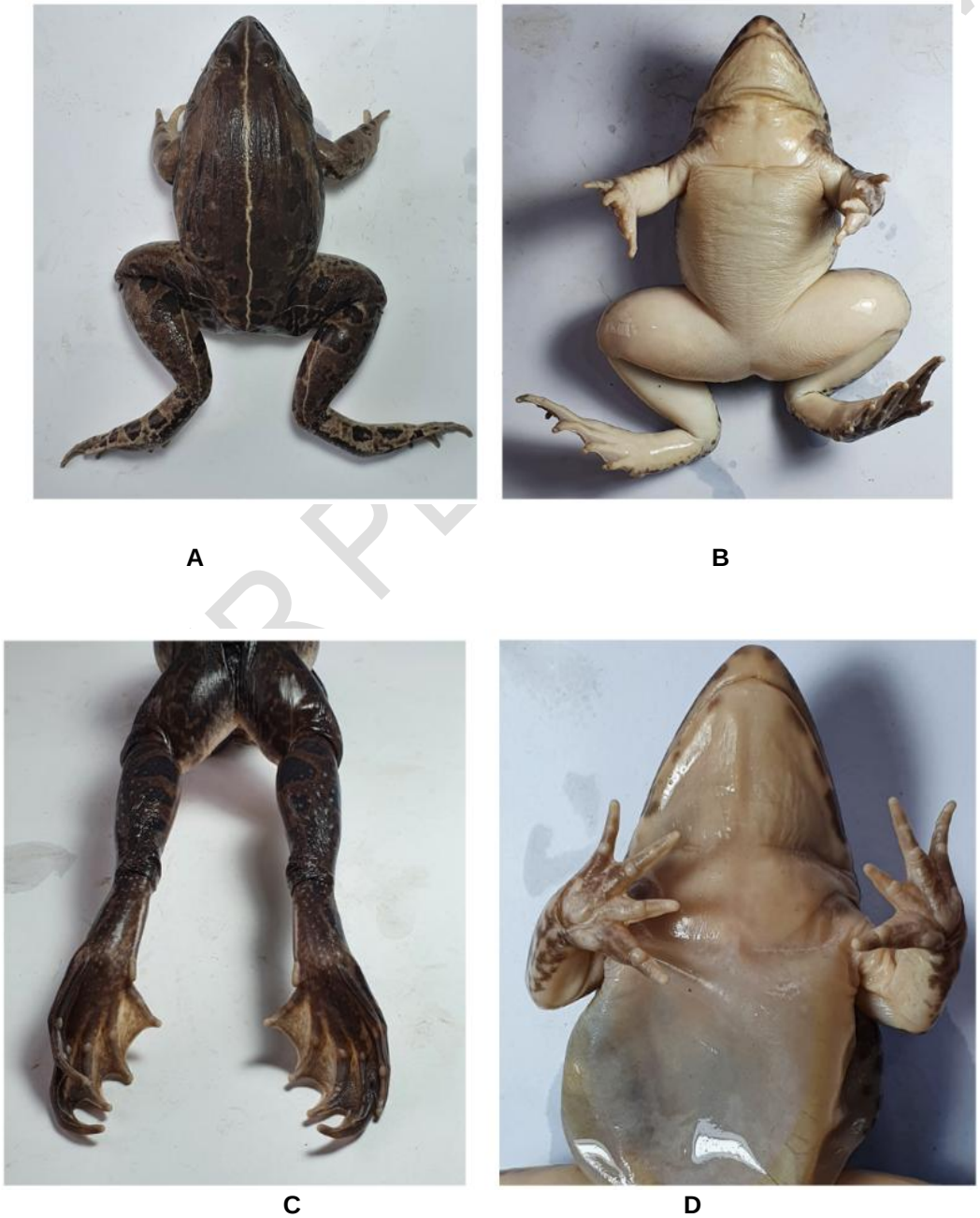


Fig 6. *H.tigerinus* (A-D). (A) Dorsal surface (B) Ventral surface (C) Toes (D) Fingers

Table 4. Illustration of statistical analysis of the morphometric characterization of *Hoplobatrachus tigerinus*		
Morphometric parameters	(n=20)	
	Range	Mean ± S.D
SVL	88.0-125.3	108.5±10.2
SL	15.1-22.1	18.8±1.8
HL	31.1-39.1	35.7±1.9
ED	7.1-10.2	9.4±0.8
IOD	3.5-6.0	5.1±0.7
IND	4.6-6.5	5.9±0.5
FL	38.5-61.1	50.9±6.1

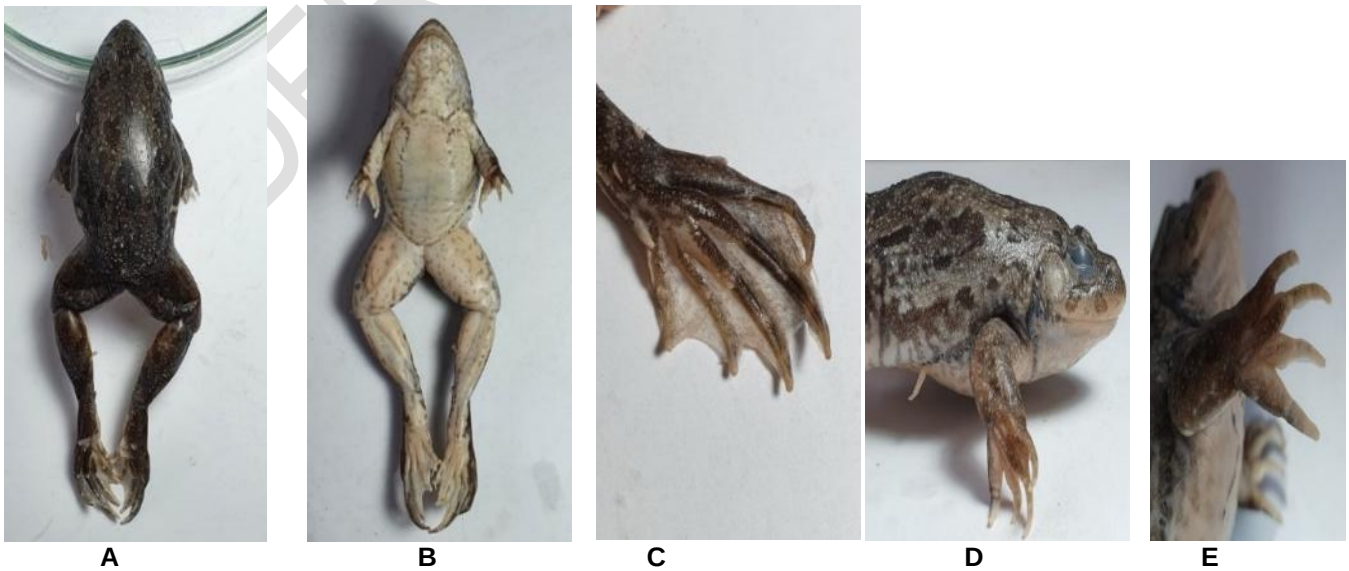
4.1.2.2 Euphlyctis cyanophlyctis

Common name: Skittering frog

Morphological characteristics

Euphlyctis cyanophlyctis commonly known as skittering frog, is a medium size frog with a snout-vent length (SVL) ranging from 57-61 mm. The dorsum coloration varies from light gray to olive-green, dark olive-brown, or occasionally black, featuring irregular, darker rounded spots on the back (vid. Fig. 7.A). The patterns of patches on the thighs and legs mirror those on the dorsal side of the body. Its Dorsal surface is adorned with Numerous scattered, small smooth tubercles. The ventrum is white, either immaculate or displaying dark speckling or reticulation, belly is smooth: presenting a single row of porous warts on each flank (vid. Fig. 7.B). The snout is rounded, and the nostril, projecting upwards, is equidistant between the snout's tip and the eye. The interorbital space is narrower than the upper eyelid. Tympanic membrane is distinct, and its size is approximately two-thirds of the eye (vid. Fig. 7.D). Their fingers are slender, either pointed or slightly swollen, and lack webbing (vid. Fig. 7.E). The second finger does not extend beyond the first, and the fingers' relative length is 4<2<1<3. The toes are pointed and extensively webbed, resembling those of ducks (vid. Fig. 7.C).

Habitat: This highly aquatic and littoral frog species is a permanent resident in various habitats featuring pooled water and is often observed floating in open water. These frogs typically spend most of their time either in the water or in the vicinity of water bodies.



A **B** **C** **D** **E**

Fig 7. ***E.cyanophlyctis* (A-E).** (A) Dorsal surface (B) Ventral surface (C) Toes (D) Lateral view (E) Fingers

Table 5. Illustration of statistical analysis of the morphometric characterization of *Euphlyctis cyanophlyctis*		
Morphometric parameters	(n=6)	
	Range	Mean ± S.D
SVL	57.1-61.0	59.4±1.4
SL	7.8-8.2	8.0±0.1
HL	16.4-17.0	16.8±0.2
ED	6.0-6.3	6.2±0.1
IOD	2.7-3.2	3.0±0.2
IND	3.0-4.1	3.4±0.4
FL	24.0-26.0	25.0±0.7

4.1.2.3 Euphlyctis kalasgramensis

Species: *Euphlyctis kalasgramensis* Howlader, Nair, Gopalan, and Merilä, 2015

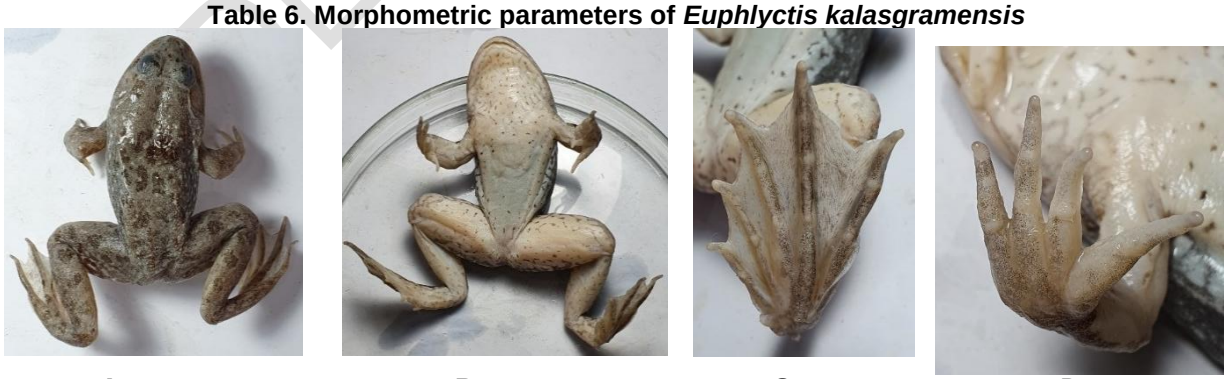
Morphological characteristics

Euphlyctis kalasgramensis is a medium-large size frog, with a snout-vent length of about 69 mm. Its dorsum is greenish-brown or grey, textured with small tubercles and warts, which are also present on the upper side of the forelimbs, thighs, and tarsus (vid. Fig. 8.A). The ventrum is smooth, featuring irregular black spots that create a net-like appearance (vid. Fig. 8.B). The head is large and triangular. Snout is almost pointed, and the interorbital space is significantly narrower than the upper eyelid. Tympanic membrane is round and distinct. The fingers are relatively small and free of webbing, while the tips of the fingers are round. Relative length of fingers, from shortest to longest, is 1 = 2 < 4 < 3. Subarticular tubercles are prominent, rounded, with a single tubercle per digit (vid. Fig. 8.D). Hind limbs are longer and thinner than the forelimbs. The tips of the toes are round and have well-developed webbing. The relative lengths of the toes, from shortest to longest, are 1 < 2 < 5 < 3 < 4. There is a faint, indistinct fringe of skin on the outer side of the toe 5. Additionally, the elongated and compressed inner metatarsal tubercle is located at the base of toe 1 (vid. Fig. 8.C).

Habitat: This newly identified species is commonly found in various types of aquatic environments, including ponds, rice fields, crop fields, and temporary pools.

S.no.	Museum No	SVL	SL	HL	ED	IOD	IND	FL
1.	02	69.0	11.3	21.0	7.1	2.5	4.0	37.3

Table 6. Morphometric parameters of *Euphlyctis kalasgramensis*



A **B** **C** **D**

Fig 8. *E.kalasgramensis* (A-D). (A) Dorsal surface (B) Ventral surface (C) Toes (D) Fingers

Remarks: This study marks the first recorded instance of *Euphlyctis kalasgramensis* in Khyber Pakhtunkhwa, specifically in the Peshawar District. The species, frequently misidentified as *Euplyctis cyanophlyctis* due to their similarity, is distinguishable through features such as a rounder snout and

lack of continuous warty lines on its ventral surface. The confusing photographic evidence from previous studies indicates a need for a comprehensive taxonomic revision of these two species, which share the same distribution range. Furthermore, it suggests the necessity of molecular and cytogenetic studies to examine potential hybridization between these closely related species.

4.1.2.4 Allopaa hazarensis

Common name: Hazara Torrent Frog

Morphological characteristics

Allopaa hazarensis is medium size frog commonly known as Hazara Torrent Frog, has a snout-vent length of approximately 59-60 mm. Its dorsum is greyish, overlaid with a network of a darker color, and marked with spinulated tubercles on short longitudinal folds (vid. Fig. 9.A, F). The upper parts of the hind limbs consist of dark cross bars, while the rear parts of the thighs have a network of small blackish spots (vid. Fig. 9.C). Ventrum is whitish and throat is speckled with grey, and webbing of the feet exhibits a greyish hue (vid. Fig. 9.B). The head is longer than it is wide. Snout is round and slightly projects beyond the mouth. The naris, situated above the canthus, is accompanied by fewer or no tubercles in the interorbital region. Interorbital space is narrower than the diameter of the upper eyelid, while the internarial distance equals the diameter of the upper eyelid. Fingers are slender with rounded tips. Nuptial spines are present on the inner finger and metacarpal tubercle while sub-articular tubercles of fingers are longer than wide (vid. Fig. 9.E). The relative length of the fingers is 2<1<4<3. Hind limbs are slightly long and extensively webbed, with distinct single subarticular tubercles on the toes, inner metatarsal is longer than wide (vid. Fig. 9.D).

Habitat: This species is typically observed in calm, clear water pools that are part of rapidly flowing streams.

Figure 9. *A.hazarensis* (A-E). (A) Dorsal surface (B) Ventral surface (C) Lateral view (D) Toes (E) First finger showing nuptial spines (F) Spines on dorsal surface of the body.

Table 7. Illustration of statistical analysis of the morphometric characterization of *Allopaa hazarensis*		
Morphometric parameters	(n=4)	
	Range	Mean ± S.D
SVL	59.3-60.4	59.9±0.5
SL	8.8-10.3	9.6±0.7
HL	18.2-19.3	18.8±0.5
ED	5.8-6.3	6.1±0.2
IOD	3.1-3.4	3.3±0.1
IND	6.1-6.3	6.2±0.1
FL	24.3-26.4	25.5±0.9

4.2 Abundance of anuran species

Overall, the bar graph illustrates the distribution and abundance of different anuran species within the nine districts. The data highlights the dominance of *Duttaphrynus stomaticus*, the notable presence of *Hoplobatrachus tigerinus*, *Bufotes surdus,* and the comparatively smaller populations of *Duttaphrynus melanostictus, Euphlyctis cyanophlyctis, Euphlyctis kalasgramensis,* and *Allopaa hazarensis.*

Based on the scrutiny of the 190 collected anurans, the species that displayed the highest prevalence was *Duttaphrynus stomaticus*, with a count of 84 individuals (vid. Fig. 10). This particular species accounted for 44.2% of the total number of anurans collected. This is one of the most common species found in the eight districts. Its prominence in the dataset indicates its prevalence and ecological importance within the studied area. Its widespread occurrence across the eight districts underscores its prevalence and ecological importance. The second most abundant species was *Hoplobatrachus tigerinus*, with a noteworthy count of 74 individuals (vid. Fig. 10). This species demonstrated a widespread presence in seven districts, indicating its adaptability and successful establishment within the local ecosystem. Taking the third position in terms of abundance, *Bufotes surdus* was observed with a count of 13 individuals (vid. Fig. 10). Though its population size was relatively smaller compared to the dominant species, its presence signifies its ecological significance within the studied regions.

Furthermore, *Duttaphrynus melanostictus* was observed with a count of 8 individuals in districts Karak and Buner (vid. Fig. 10). Although relatively fewer in number, the identification of this species enriches the overall species richness and underscores its existence within the sampled area. Additionally, the dataset recorded the presence of *Euphlyctis cyanophlyctis* with 6 individuals observed from districts Dir and Haripur (vid. Fig. 10). *Allopaa hazarensis* was observed with a count of 4 individuals, exclusively in district Dir (vid. Fig. 10), indicating its relatively low population density. Lastly, a lone representative *Euphlyctis kalasgramensis* was observed in the district of Peshawar (vid. Fig. 10). This solitary presence in the dataset signifies the occurrence of this species within a specific locale, underscoring its unique ecological niche and the need for further investigation.

These findings contribute to the understanding of the local anuran fauna and provide valuable insights for ecological studies and conservation efforts in the area.

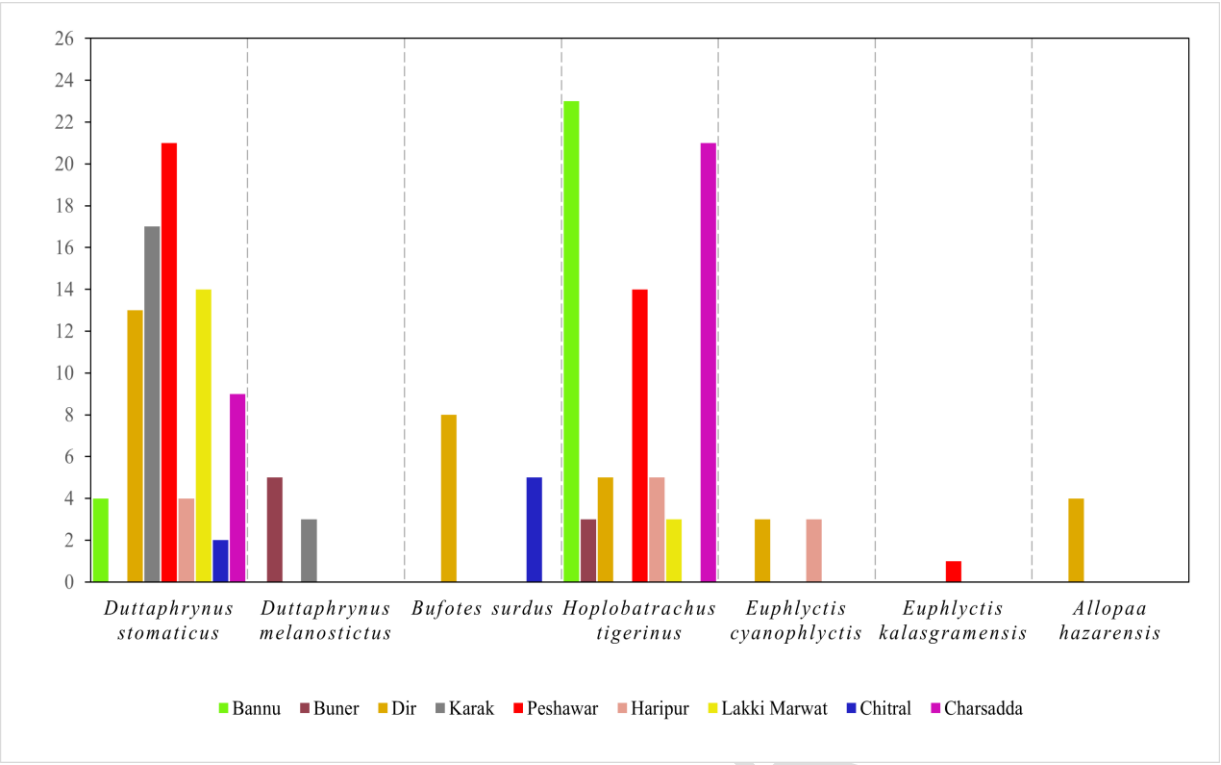


Fig. 10. Relative abundance of Anurans found in the nine districts

4.3 Monthly distribution of anuran species

4.3.1 Duttaphrynus stomaticus

Fig. 11 illustrates the distribution of *D. stomaticus* by month and location. From the figure, it is obvious that the distribution of *D. stomaticus* varies depending on the month (i.e., season) and location. Overall, it can be noted that the highest number of *D. stomaticus* appeared in the district Peshawar, while the lowest number of species were found in the district Chitral. Further, the highest frequency of the species was shown in the month of July, while few species were found in the months of April and May. This indicates that July is a peak month for this species, possibly due to specific environmental conditions or favorable breeding patterns during that time. Conversely, the months of April and May show relatively few species recorded, which implies that these months are less favorable for the presence of the species. In contrast to other districts, no species of *D. stomaticus* has been found in the Buner district.

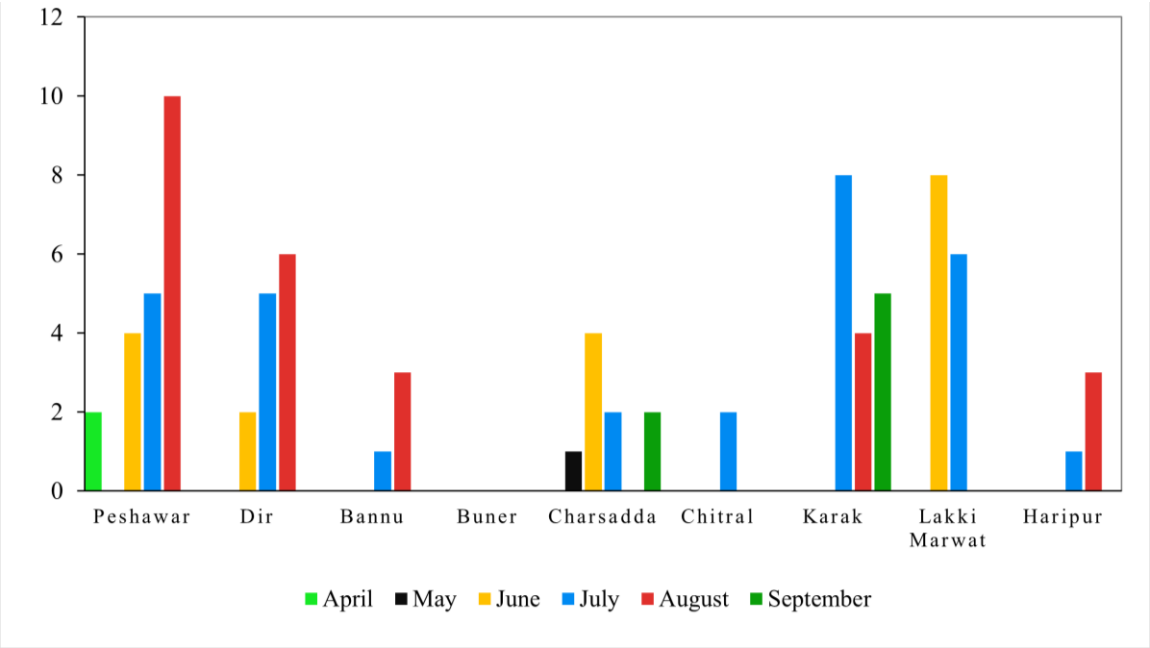


Fig. 11. *D. stomaticus* monthly and location wise distribution

4.3.2 Hoplobatrachus tigerinus

The monthly and location-wise distribution of *H. tigerinus* is shown in Fig.12. The figure highlights the abundance of this species in district Bannu, followed by district Charsadda. On the other hand, the lowest number of species was found in the districts Buner and Lakki Marwat. However, no species were found in Chitral and Karak districts. The highest number of species was observed in August, followed by July. In contrast, April had the lowest number of recorded species. While June and September exhibited a moderate number of species. Interestingly, in District Buner, only a few species were specifically found during the month of August.

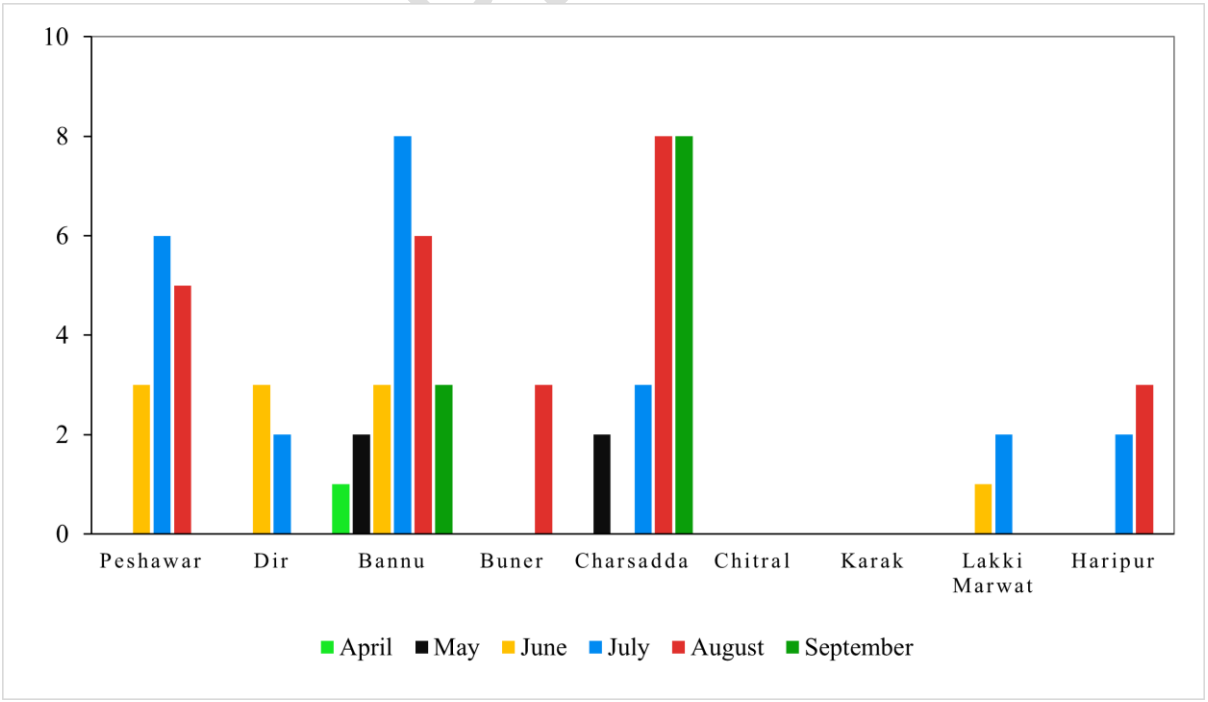


Fig. 12. *H. tigerinus* monthly and location wise distribution

4.3.3 Bufotes surdus

The graph reveals that *B. surdus* was exclusively detected in two districts, namely Dir and Chitral. The total count of species observed amounted to thirteen, all of which were confined to the months of June and July. Interestingly, an equal number of species were recorded in the month of July from both districts Fig.13. This suggests a balanced distribution of this species during that month across the two districts.

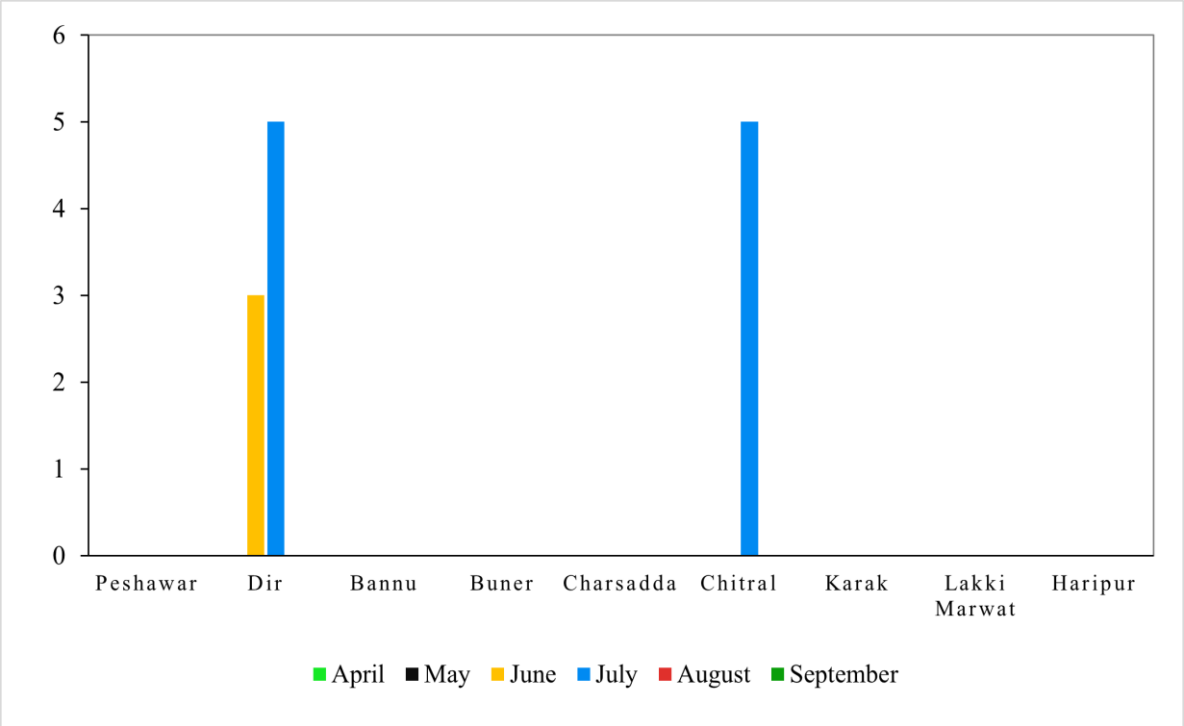


Fig. 13. *B. surdus* monthly and location wise distribution

4.3.4 Species with limited abundance

In addition to the dominant species mentioned above, the dataset also includes several species with limited abundance. It should be kept in mind that species numbering less than thirteen are not shown in the graph but will be explained here.

Duttaphrynus melanostictus was found exclusively only in two districts, Buner, and Karak. Eight specimens were collected from both districts, with five species collected from Buner in the month of July and three species in the month of August from Karak. The next species, *Euphlyctis cyanophlyctis*, was found in District Dir and Haripur. Overall, only six species were identified from these two districts. These species were exclusively collected during the months of July and August. The *Allopaa hazarensis* species were collected from District Dir in the month of August. In total, only four species were collected. Lastly, *Euphlyctis kalasgramensis* was the species of which only one individual was captured from District Peshawar.

4.4 Diversity Indices

The information about the diversity, relative abundance (RA), and distribution (C) of the species are shown in Table 8. The table illustrates the relationship between the two parameters for each month. The calculations are based on the total population of seven species across different months.

Duttaphrynus stomaticus was the most abundant species found in most of the study sites, with C=88.8% and RA=44.2%. While *Euphlyctis kalasgramensis* and *Allopaa hazarensis* were found to be sporadic species, with C=11.1%, RA=0.52%, and C=11.1%, RA=2.10%, respectively. Further, *Duttaphrynus melanostictus*, *Bufotes surdus*, and *Euphlyctis cyanophlyctis* were found to be infrequent species, with C=22.2% and RA=4.21%, C=22.2% and RA=6.84%, and C=22.2% and RA=3.15%, respectively. Lastly, *Hoplobatrachus tigerinus* falls in the category of frequent species, with C=77.7% and RA=38.9%.

S.No.	Species name	Total number of specimens	Distribution (C)	Status	Relative abundance (RA)	Status
1.	***D.stomaticus***	84	88.8%	Constant	44.21%	Dominant
2.	***D.melanostictus***	8	22.2%	Infrequent	4.21%	Sub dominant
3.	***B.surdus***	13	22.2%	Infrequent	6.84%	Sub dominant
4.	***H.tigerinus***	74	77.7%	Frequent	38.94%	Dominant
5.	***E.cyanophlyctis***	6	22.2%	Infrequent	3.15%	Sub dominant
6.	***E.kalasgramensis***	1	11.1%	Sporadic	0.52%	Satellite
7.	***A.hazarensis***	4	11.1%	Sporadic	2.10%	Satellite

Table 8. Distribution and relative abundance of anurans species in the nine districts.

5 DISCUSSION

The present study was conducted to identify and explore the Anuran fauna of Khyber Pakhtunkhwa. As discussed above, the taxonomic identification revealed the presence of seven anuran species belonging to five genera. *Duttaphrynus*, *Bufotes*, *Hoplobatrachus*, *Euphlyctis*, and *Allopaa*, each represented by one species except *Duttaphrynus* and *Euphlyctis* which contained two species. The species are *Duttaphrynus stomaticus*, *Duttaphrynus melanostictus*, *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis*, *Euphlyctis kalasgramensis*, and *Allopaa hazarensis*. The most abundant species was *Duttaphrynus stomaticus*, collected from eight districts, followed by *Hoplobatrachus tigerinus*, found in seven districts. While *Bufotes surdus*, *Duttaphrynus melanostictus*, and *Euphlyctis cyanophlyctis* were found only in two districts. Lastly, *Allopaa hazarensis*, and *Euphlyctis kalasgramensis* being the least abundant species could be only found in one district.

Several existing studies have confirmed the presence and abundance of *Duttaphrynus stomaticus* in various districts in KP province, including Lakki Marwat, Dera Ismail Khan, Haripur, and Abbottabad. The studies were conducted by (Hamid et al.,2021; Bibi et al., 2020; and Rehman et al.,2020). Furthermore, no studies have been conducted in districts Peshawar, Charsadda, Bannu, Dir, and Chitral. As for *Hoplobatrachus tigerinus*, previous studies (Bibi et al., 2020; Arsalan et al., 2018; Ullah et al., 2022; Younas et al., 2017a; Younas et al., 2017b; Zaryab et al., 2016; and Noureen et al., 2018) has shown its presence in district Haripur, Bannu, Kohat, and Karak though it has not been studied yet in district Buner, Lakki Marwat, Dir, Peshawar, and Charsadda as compared to the ones studied in the current study. However, no studies could be found about the presence and distribution of the species in these five districts. *Bufotes surdus* was found to be an infrequent species in the study sites considered in this study which was only found in district Dir and Chitral. Previous studies in KP show the presence of this species only in Karak (Younas et al., 2017a).

As shown above (vid. Table 8), *Duttaphrynus melanostictus* was found to be an infrequent species in the study sites considered in this research study. Existing published literature shows that the species is widely distributed in Haripur (Bibi et al., 2020), Abbottabad (Rehman et al., 2020), Bannu (Arsalan et al., 2018), Karak (Younas et al., 2017b; Noureen et al., 2018), and Kohat (Ullah et al., 2022), while in our study this species was infrequently distributed in Karak and Buner. *Euphlyctis cyanophlyctis* was found to be an infrequent species, only occurring in districts Haripur and Dir in the present study. Previous literature shows that the species is also present in several other districts of KP, including Karak, Kohat, Dir, Haripur, Dera Ismail Khan, and Lakki Marwat. (Younas et al., 2017a; Younas et al., 2017b; Noureen et al., 2018; Ullah et al., 2022; Khan et al., 2021; Bibi et al., 2020; and Hamid et al., 2021). *Allopaa hazarensis* presence was only restricted to district Dir in which only four species were found. From

previous literature, it is reported, from Hazara, Mansehra, Larkana, Jamshoro (Dubois and Khan, 1979), Sindh (Shaikh et al., 2014), *A. hazarensis* was first time recorded from Rush Valley of Hazara Division of Pakistan. It has also been discovered in Jammu and Kashmir and India however its occurrence in Nepal and Bhutan is reported but not confirmed (Khan et al., 2004). Faiz et al., 2018 reported *Allopaa barmoachensis* from Toli Pir National Park, Pakistan. Dubois (1992) considered *A. barmoachensis* synonymous with *Allopaa hazarensis*, but (Khan, 2004b) regarded the two as distinct. However, (Ohler and Dubois, 2006) reiterated that the species is conspecific with *A. hazarensis*.

Lastly, *Euphlyctis kalasgramensis* presence was only restricted to the district of Peshawar. In Pakistan it was reported for the first time from Punjab (Ali et al., 2020). Prior to this, it had been documented in Bangladesh by (Howlader et al.,2015), who suggested the possibility of its range extending into Pakistan and India. In our present investigation, we provide the inaugural record of *Euphlyctis kalasgramensis* from Khyber Pakhtunkhwa (KP), specifically from district Peshawar. This novel finding expands the known distribution of the species in the region, further contributing to the understanding of its geographical presence. Limited work has been done on the identification and distribution of the last two species in the literature.

6 Conclusion

In conclusion, this investigation presents an illuminating study on the rich and diverse Anuran fauna within the ecologically diverse Khyber Pakhtunkhwa (KP) region of northwestern Pakistan. Our pivotal findings reveal *Duttaphrynus stomaticus* as the predominant species throughout KP, with *Hoplobatrachus tigerinus* following closely, thus providing meaningful insights into the distribution of Anuran fauna within the region. We observed the highest concentration of Anurans during the months of July, August, and June across all studied areas. The district of Dir displayed the greatest diversity in terms of Anuran species, followed by the districts of Peshawar and Haripur. Significantly, our study presents the first documented occurrence of *Euphlyctis kalasgramensis* in the KP region, specifically in the Peshawar district.

These findings highlight the remarkable potential of KP in discovering potentially unknown amphibian species, possibly adding new species categories to scientific knowledge. Therefore, there's a need for comprehensive taxonomic revisions using both traditional and molecular approaches, which could unearth numerous new taxa. The insights gained from this study serve as a robust foundation for future in-depth exploration and identification of amphibians in this region. Furthermore, these findings accentuate the need for effective legislation aimed at conserving the amphibian fauna within KP, which will not only enhance the biodiversity of Anuran species, but also contribute to our understanding of their biological and ecological roles.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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