Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(1): 1419-1422

Received: 12-11-2018
Accepted: 17-12-2018

Irfan Tagar
Department of Zoology, University of Sindh, Jamshoro, Pakistan

Kalsoom Shaikh
Department of Zoology, University of Sindh, Jamshoro, Pakistan

Ghulam Sarwar Gachal
Department of Zoology, University of Sindh, Jamshoro, Pakistan

Iqra Arain
Department of Zoology, University of Sindh, Jamshoro, Pakistan

Misbah Arain
Department of Zoology, University of Sindh, Jamshoro, Pakistan

Bibi Abida Sarhindi
Department of Zoology, University of Sindh, Jamshoro, Pakistan

Correspondence
Irfan Tagar
Department of Zoology, University of Sindh, Jamshoro, Pakistan

Assessment of anurans (frogs and toads) inhabiting district Naushahro Feroze, Sindh, Pakistan

Irfan Tagar, Kalsoom Shaikh, Ghulam Sarwar Gachal, Iqra Arain, Misbah Arain and Bibi Abida Sarhindi

Abstract
Anura is the only order of class amphibia that exists in Pakistan where some areas are still unexplored for the confirmation of anuran diversity. In this context, present study explored "district Naushahro Feroze" where no relevant inquisition was ever conducted. Fields were surveyed from January to December, 2018 to record the prevalence of amphibians in different habitats: scrubland, cropland and suburban areas. Amphibians of order Anura were thoroughly examined and identified as *Hoplobatrachus tigerinus* and *Euphlyctis cyanophlyctis*. Morpho-taxonomic variation was utterly absent in members of both distinct species, though minor variations in body coloration and pattern of patches were recorded. Existence of only two anuran species falling in two genera (*Hoplobatrachus* and *Euphlyctis*) indicated extremely poor anuran diversity in Sindh as compared to other areas of Pakistan. Arid climate, habitat destruction and anthropogenic encroachment may be the reasons that district Naushahro Feroze failed to attract majority of anuran species.

Keywords: Frogs, toads, diversity, Naushahro Feroze district, Sindh, Pakistan

Introduction
Anura is order of class amphibia including tailless amphibians such as frogs and toads [1]. There are roughly 5,400 species of order Anura, also called Salientia that have worldwide distribution, hence they are widely familiar and are celebrated mainly for their feeding and medicinal value [2-3]. Anura is the only order of class amphibia that survives with its 4 families including Bufonidae, Dicroglossidae, Megophryidae and Microhylidae [4]. These four families altogether represent 12 genera containing 21 species [4], however some studies claimed presence of 24 amphibian species contained by four families viz: Bufonidae, Megophryidae, Microhylidae, and Ranidae [1].

Frogs possess long legs and smooth, mucus-covered skins, while toad are short-legged and rough skinned anurans. Among frogs, the species of family Ranidae are distinguished as true frogs, meanwhile toad members of family Bufonidae are renowned as true toads. Amphibians of order Anura are adapted to live a variety of habitats such as terrestrial and freshwater ecosystems [1]. Amphibians spawn in water and remain confined there until being hatched, fully metamorphosed and grown into adulthood [5].

According to the morphological studies; conservation status of most frogs and toads is not susceptible to serious threats but some species such as *Hoplobatrachus tigerinus* and *Euphlyctis cyanophlyctis* are confronting habitat contamination due to exposure of fertilizer and pesticide in fields [6], while habitat loss and urbanization are also main factors causing amphibian populations to wane [1-3].

It is recorded that the dissemination of amphibian fauna is very infrequent as entire class amphibia represents merely order Anura with heterogenous assemblage of twenty one distinct species grouped in twelve genera of four major families viz., Bufonidae, Megophryidae, Microhylidae and Dicroglossidae [4]. There is no evidence about existence of orders Caudata and Gymnophiona in entire Pakistan [4]. The diversity and population status of amphibians is also not satisfactory and therefore some measures such as controlling usage of pesticides, restoring wild habitat and establishing appropriate legislation may be convenient to minimize the decline of anuran species in Pakistan [4]. Anurans living in contaminated water may come across with variety of diseases such as retarded growth of body, variety of physical and

physiological abnormalities, aberrations in reproductive organs, sexual deformities, and weak immune system. Contaminants in agricultural fields are highly responsible for the decline in populations of anurans worldwide. In order to sustain the diversity and population of anurans, some international organizations: International Union for the Conservation of Nature (IUCN) and International Society for the Study and Conservation of Amphibians (ISSCA) have been taking convenient efforts for saving amphibian diversity and their population worldwide [6].

Considering the threatened status and declining diversity of amphibians worldwide, Present study was designed with focus on recording current diversity of order Anura in Naushahro Feroze district that has been explored for the first time for the discovery of anuran species.

Materials and methods

Study area

Naushahro Feroze district is situated in Sindh province covering 2,945 km² geographical area, located at 26°50'0N 68°7'0E with an altitude of 38 meters. This area has a very hot climate in summer season and mildly low temperature in winter. Variety of habitats including scrubland, cropland, suburban and urban area are found there.

Collection of anurans

Because of amphibian's frequent terrestrial and aquatic habitats, different collecting methods were adapted for collecting them. The simplest technique for collecting amphibians involved a slap of hand over them when they were on the scrubland, but while they were into ponds at cropland or into water reservoirs of suburban areas, a dip net was used as specimens collecting tool. Seining was also as an effective method equally for collecting adult amphibians as well as their larvae in vegetation-free water bodies, however in heavily muddy water or the habitats where thick vegetation was found variety of traps (Funnel trap and pitfall trap) aided in collection of specimens. Funnel trap and pitfall trap were also convenient for collecting amphibians on dry land as well.

Identification of diverse species of order Anura

Amphibian species of order Anura were identified through morphological characteristics, identification key and taxonomic literature [7-10].

Results and discussion

Anurans were abundantly observed in cropland and shrubland as compared to subarban habitats. The morphological characteristics of identified species: *Hoplobatrachus tigerinus* and *Euphlyctis cyanophlyctis* of order Anura (Figure 1-2) are detailed below:

H. tigerinus of family Ranidae exhibited green, olive green and brown coloration on dorsal side of body where prominent dark spots or irregular patches were also observed (Figure 1).

Fig 1: Bullfrog (*Hoplobatrachus tigerinus*)

Fore and hind limbs possessed dark bars or blotches, while their thighs were posteriorly marbled usually with black and sometimes with yellow color. Mid-dorsal line was apparent on their bodies, while they were white ventrally. Feeble pigmentation was also a main characteristic that was found on throat of some specimens of the study area. The characteristics including pointed snout, extensively webbed toes, moderate head and obtuse canthus rostral were also recorded. Nostrils were observed locating nearer to the end of the mouth than to the eye. Inter-orbital space was distinctly narrower than the upper eyelid. Distinct tympanic membrane, short fingers, moderate subarticular tubercles, strongly compressed inner metatarsal tubercle was also observed. Morphometric of *H. tigerinus* is given in table 1.

Table 1: Morphometric of *Hoplobatrachus tigerinus*

S. no	Gender	Body weight (g)	Body length (mm)	Fore limbs length (mm)	Hind limbs length (mm)	Eye diameter (mm)	Tympanum diameter (mm)
44	♂	177.9±60.9	112.5±10.5	37.2±3.2	66.4±5.4	9.3±0.7	9.8±1.3
38	♀	254.0±3.9	135.8±3.0	46.1±2.4	76.3±2.3	10.7±0.5	11.2±1.0

The morphometric of *E. cyanophlyctis* is mentioned in table 2, while observed physical characteristics of this species included dorsal skin of olive, yellowish brown or brown coloration. Entire dorsal side of the body possessed small tubercles and distinct rows of pores along with dark patches which were also abundantly observed in similar pattern on fore and hind limbs (Figure 2).

Fig 2: Skittering frog (*Euphlyctis cyanophlyctis*)

Ventral side of the body was white without any pores or patches. Head of *E. cyanophlyctis* was observed being moderate, while snout was scarcely pointed. Canthus rostral was indistinct, however tympanic membrane was observed as distinct. The inter-orbital space of skittering frogs was much narrower than upper eyelid, while their fingers were observed to be pointed. Toes were extensively webbed and pointed as well; however first digit was not extended beyond the second. Inner metatarsal tubercle was observed to be small and conical in shape.

Table 2: Morphometric of *Euphlyctis cyanophlyctis*

S. no	Gender	Body weight (g)	Body length (mm)	Fore limbs length (mm)	Hind limbs length (mm)	Eye diameter (mm)	Tympanum diameter (mm)
22	♂	31.0±5.6	45.3±6.2	18.8±1.5	26.5±1.1	7.2±0.7	7.3±1.2
26	♀	36.0±2.3	53.1±4.4	22.0±2.3	30.3±3.6	7.5±0.5	7.9±1.0

Climate of Sindh province is arid and therefore it fails to attract majority of amphibian species and it becomes less optimal for amphibian populations to thrive abundantly [10]. Anura is the only order of class amphibia that exists here with 21 diverse species of altogether frogs and toads [7-8]. Oder Anura of Pakistan represents four families: Megophryidae, Microhylidae, Ranidae and Bufonidae which are altogether sorted into 12 genera [4].

In comparison to other parts of Pakistan, Sindh province is recorded to have very poor diversity of amphibian fauna [6] though it is rich with variety of croplands as in Naushahro Feroze district.

Present study confirmed two species of order Anura including *Hoplobatrachus tigerinus* and *Euphlyctis cyanophlyctis* which have previously been recognized as least concern in red data list of IUCN (International Union for the Conservation of Nature). Previous research related with the status of Family Ranidae in Sindh has been documented by 2013. Comparative to previous studies that indicated ranid species having morphological variation in dorsal body coloration at different localities of District Jamshoro [6], this minor morphological variation was also observed by present study as well. The distribution of amphibian fauna in other areas of Sindh such as Karachi and Thatta had also been explored that found habitat degradation and other environmental factors as threatening these wild creatures [11]. The existence of three anuran species i.e. *Hoplobatrachus tigerinus, Euphlyctis cyanophlytis* and *Bufo stomaticus* have been recognized as having deteriorated habitats due to massive use of chemical contaminants in Sindh, Pakistan [11].

The morphological characteristics of *H. tigerinus* and *E. cyanophlyctis* exhibited some difference from those species which exist in Himalayan highlands where most of the anuran species are previously recorded to be dissimilar from the same species of central and eastern areas [4]. Conservation status of most frogs and toads of Pakistan is of least concern as they are not vulnerable to grave threats but some species including *H. tigerinus* and *E. cyanophlyctis* are confronting various ecological problems including habitat loss, water contamination mainly due to fertilizer and pesticide exposure and urbanization [4].

Because of vigorous nature, *H. tigerinus* is commonly called as Indian Bullfrog, Indus valley bullfrog and Asian bullfrog. This amphibian is reported from Afghanistan, Bangladesh, India, Myanmar, and Nepal as a species of least concern [12-18]. From the different areas of Sindh, the studies on systematics and water quality of habitats of *H. tigerinus* had been documented [6, 13-17]. From other provinces of Pakistan, studies on taxonomy have reported the distribution of toad species mainly of *Bufo stomaticus,* but present study revealed that species as unseen in district Naushahro Feroze [1].

The distribution of skittering frog (*E. cyanophlyctis*) had previously been confirmed from south Asia, south east Asia, southeastern Iran, southern Afghanistan, Nepal, India, Bangladesh, Maynmer, Sri Lanka, Thailand, Veitnam, and Bhutan [7]. Alike *H. tigerinus, E. cyanophlyctis* is designated as least concern in red list of threatened species of IUCN [1]. This anuran species had been having broad range of distribution in variety of habitats (Khan, 2010). In district Naushahro Feroze, *E. cyanophlyctis* was found as abundant and widely scattered. It had previously been reported from different parts of Sindh [1, 6].

Conclusion

Present study observed occurrence of only two species of order Anura out of 6,500 species of class amphibia

worldwide. There was no morphological variation of taxonomic level within members of recorded species (*Hoplobatrachus tigerinus* and *Euphlyctis cyanophlyctis)*. Toad species were recorded as absent throughout the study duration (January to December, 2018). In order to enhance the diversity of anuran species in particular and over all amphibian fauna in general, an appropriate legislation is needed to conserve amphibian fauna in the study area "district Naushahro Feroze".

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