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Food Habits and Helminth Parasites of Skittering Frog (*Euphlyctis cyanophlyctis*) in the Lower Dir, Pakistan

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ABSTRACT

A total of 30 specimens of Skittering frog (*Euphlyctis cyanophlyctis*), (Anura: Dicroglossidae) were captured from seven selected localities in the Dir Lower, Khyber Pakhtunkhwa province of Pakistan from April to August 2016. They were euthanized with the help of Chloroform (CHCl₃) solution. The biometry of each specimen was measured, then dissected to examine for helminth parasites and foodstuff. All helminths were isolated, washed with normal saline at room temperature, and then preserved in 70% GL ethyl alcohol. Later on, the parasites were classified and studied under the electric compound microscope. In a total of 30 specimens, 17(56.66%) were found infected with 62 helminths parasites belongs to four different species. Among four species, one was Cestodes *Nematotaenia dispar* species and remaining three were Nematodes i.e. *Cosmocerca* species, *Cosmocercoides* (*Raillietnema* species) and *Strongyloides* species. *Cosmocerca* with a percentage of 56.45% was the most dominant. In the total of 30 specimens' alimentary canal, 10 were found empty, containing nothing while in remaining 20 were full of 9 types of food materials which included insects i.e. Ants, Beetles, Wasps, Spiders, Mosquito larvae and cockroaches, and non-living things like stone pebbles and mollusks shells. Among the serial, flies and snails were in the lowest prevalence and ants were recorded the most important component of the frog diet. Among the insects, ants have occurred in higher prevalence 44.50% and flies 1.15% with the lowest prevalence. The results emphasize the significance of further expected studies in the Dir Lower to get a good understanding of the helminth parasites with the ecological relations of their hosts.

1. Introduction

The group of amphibians is unique animals providing evolutionary evidence for the link between aquatic and terrestrial mode of life^[1]. According to

the IUCN (international union for conservation of nature and natural resources) in September 2016, due to a lack of interest in the field, about 545 species of amphibians are critically endangered, including 113 at the risk of possible extinction. The conservation of this group is ignored and

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little work has been conducted on the local threat faced to amphibians in Pakistan^[2]. There are about 8,000 species of amphibians worldwide, while about 1,900 species are listed to be threatened^[3]. Due to arid conditions, Pakistan is less favorable for the survival of amphibian fauna but the only Order Anura is found here^[4]. However, about 24 species of anuran species reported still from Pakistan. Among them, 9 species are endemic^[5]. Many types of diseases and infections found in amphibians due to external or internal parasites i.e. Amoeba, ciliates, flagellates, helminths, etc.

The helminth parasites infect and produce diseases occasionally include Cestode and acanthocephalans. Cestodes are not commonly isolated from amphibian species. But they may produce gastrointestinal lesions, gastrointestinal obstruction, and cause death in case of heavy infection^[6]. Acanthocephalan or thorny headed worms produce a lesion in juveniles and adults' intestine of amphibians^[7]. The helminths are important parasites of free-ranging amphibians with an ecological role in the regulation of the amphibian population. Most of the studies analyzing community structure of helminth parasitizing amphibians were conducted in temperate regions^[8,9], whereas studies on tropical helminths and their amphibian hosts are common in the international literature and mainly confined to the Central and South American study systems^[10]. Various types of helminths parasite infect anuran species^[11].

Anuran amphibians are fertile hosts for Trematodes and Nematodes. Trematodes are may be widely recognized while Nematodes are common helminths parasite which infects amphibians^[12]. Anura amphibians have a rich parasitic fauna due to their typical association with both aquatic and terrestrial habitats and more susceptibility to microbial and parasitic infections. The nematodes parasites of amphibians have usually a direct life cycle without an intermediate host^[13]. Few reports are available on the occurrence of Trematodes, Nematodes, Cestodes, and acanthocephalan parasites from frogs and toads from Pakistan^[14].

Due to the low availability of water bodies and rice fields, there are a few anurans species found in the Dir Lower. In the present study, we have work done on food habits and helminth Parasites of Skittering Frog (*Euphlyctis cyanophlyctis*) in the Lower Dir. So it was felt necessary to undertake research work, to recognize the prevalence and diversity of helminth parasites and infection in toads in the study area, to develop literature for the conservation of this ignorant group of vertebrates.

2. Materials And Methods

2.1 Study Area

Dir Lower is a district situated in northern Pakistan, with Longitudes of 34°, 37' to 35°, 07' North and Latitudes

of 71°, 31' to 72°, 14' East. Dir Lower having about 2700 feet height from the sea level. The coldest months are December, January, and February. During this period temperature falls below the freezing point (11.22co and -2.39co-8co). The rainfall is received throughout the year but most rainfall occurs in the month of July, August, and December to March^[15]. During the winter season, the average rainfall is more than the summer. The annual rainfall of 1468.8 mm and 253.7 mm during December and March respectively. Humidity is quiet throughout the year. The district consisted administratively in seven tehsils named Timergara, Adenzai, Balambat, Lal Qila, Khal, Munda, and Samar Bagh^[16]. The plane area of Lower Dir is irrigated by the River Pangkora and three large streams: Asegal, Konhaye, and Rhound with numerous sub tributaries.

2.2 Sample Collection

The frogs were hand-captured from seven selected localities in the Dir Lower i.e. Munda, Kambat, Mayar, Maskeni, Samarbagh, Timargara, and Chakdara, from April to August 2016. The specimens were deposited in iceboxes and then transported to the laboratory of Parasitology, Department of Zoology, University of Malakand,

2.3 External Examination

The frog specimens were brought in boxes to the laboratory of Parasitology, Department of Zoology, University of Malakand, where they were euthanatized with help of Chloroform (CHCl₃) solution. In biometry, the body was measured with the help of graduated scale to the nearest 0.01mm and bodies were weighted with the help of a digital balance to the nearest 0.1g in the laboratory.

2.4 Internal Examination

Each specimen was dissected and the sexes were determined. Subsequently, the digestive tract (Oesophagus, stomach, small and large intestines, and cloaca) was examined for helminth parasites. All helminths were isolated with the help of forceps and fine hairbrush and wash with normal saline (0.9% Sodium chloride solution) at room temperature and then preserved in 70% GL ethyl alcohol. Later on, the parasites were then classified and studied under the electric compound microscope.

2.5 Data Analysis

The collected parasitic data were interpreted and analyzed with the help of computer package MS office.

3. Results

A total of 30 Skittering frogs (*Euphlyctis cyanophlyctis*)

species were collected. In these specimens, 11 (36.66%) were recorded male, while 19 (63.33%) were females. The ratio of female frogs was more than males.

3.1 Biometry

The average body weight (38.90gm) of female frogs were higher than that of male frogs with average body weight is 37.85gm. Females were larger than males. The body length of male and female frogs was measured by a scale in cm which was 5.27 and 5.78cm respectively. The average mean length of forelimbs for males and females was 2.58cm 2cm while the mean average length of hind limbs was 5.38cm and 6.38cm respectively.

3.2 Dietary Habits

In the present study, the food habits of *Euphlyctis cyanophlyctis* was calculated on the basis of total food items recovered from the specimens' stomachs. Total of 30 frogs' stomachs, 10 were found empty, containing nothing while in the remaining 20 were full of 9 types of food materials which included invertebrates, insects, and non-living things like stone pebbles and Molluscs shells. Insects were including ants, beetles, wasps, spiders, mosquito larvae, and cockroaches were found. Some insects were found in crushed forms i.e. their body parts like head, wings, legs, and mouthparts were also isolated (Table 1). The pebbles and snail shells were eaten secondarily and accidentally because frogs are insectivorous. It revealed that arthropods were greater in number than that of other food items. Among arthropods, ants were found 44.50% followed by beetles 27.16%. So it was concluded that ants and beetles were the dominant food items.

Table 1. Food items with number and percentage recovered from the stomach of *E. cyanophlyctis*

S/No	Prey Order	Each Prey type	No of each prey	Total number	Total %age
1	Hymenoptera	Ants	77	92	53.17%
		Wasp	15		
2	Coleoptera	Beetles	47	47	27.16%
3	Cepidoptera	Butterflies	7	7	4.04%
4	Araneae	Spiders	9	9	5.20%
5	Diptera	Mosquito larvae	10	12	6.93%
		Flies	2		
6	Blattodea	Cockroaches	4	4	2.312%
7	Molluscs	Small snails	2	2	1.15%
Total			173	173	100%

3.3 Helminth Parasites

A total of 30 frogs collected and examined for helminth parasites (Figure 1), 17(56.66%) were found to be infected and harbored helminths parasites in larval or adults form (Table 2). Among the infected specimens 6 (35.29%) out of 11 were male and 11(64.70%) out of 19 were female. A total of 62 worms of helminths parasites were collected from the digestive tract of *E. cyanophlyctis*. Among these 8(12.90%) were identified to be single Cestode (*Nematotania dispar*) specie and the remaining 54(87.10%) were identified to be Nematodes. The nematodes belong to the different genera; they were *Cosmocerca* (56.45%). *Cosmocercoides* (*Raillietnema* species) (25.80%), and *Strongyloides* (4.83%).

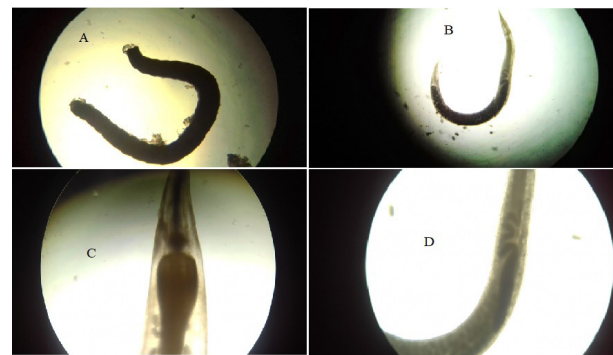


Figure 1. Showed the helminth parasites collected from *E. cyanophlyctis* stomachs (A) *Nematotania dispar* (B) *Cosmocerca* spp.(C) *Cosmocercoides* spp. (Male) (D) *Cosmocercoides* spp. (Female)

Table 2. Prevalence of Helminth parasites found in *E. cyanophlyctis*

S/No	Type of parasite	No of parasite	Total No of frog	No of infected frog	Prevalence	Site of infection
1	<i>Cosmocerca</i> spp.	35	30	9	56.45%	Large intestine
2	<i>Cosmocercoides</i> (<i>Raillietnema</i> spp).	16	30	5	25.80%	Large intestine
3	<i>Strongyloides</i> spp	3	30	2	4.83%	Large intestine
4	<i>Nematotania dispar</i> .	8	30	1	12.90%	Small intestine
Total	4	62	30	17	100%	2 Organs

4. Discussion

Our study reflects that *E. cyanophlyctis* feed on snails and insects like ants, beetles, cockroaches, wasps, spi-

ders, butterflies, flies, mosquitoes, and cockroaches. So the present study agrees with the observations of Boulenger (1897)^[17] and that of Noble (1918) that frogs are carnivorous.

The different varieties of prey consumed suggest that the species feeds on some wide varieties of invertebrates' species and each frog diet are depended on the size and locality of frogs. The availability of food is different for each individual frog. It is the point of interest in this generalist species that ants constitute an important part (44.50%) of its diet. In other species of Malagasy frogs, such as *Mantella*, the proportion of mites and ants are much higher, ranging from 14% to 74%^[18] and these frogs are considered as microphagous specialists. Other frogs (e.g. *Pelophylax nigromaculatus*) that inhabit rice fields in other parts of the world are also generalists^[19], because ants constituting a major component of their diet followed by beetles. The lowest percentage was of that of flies and snails in the present study.

The presence of some stone pieces and pebbles along with plants leaves have been reported from the stomach contents of some anuran species^[20, 21], and its ingestion has been considered to be accidentally ingested when they feed and swallows the prey among the dead leaves or in the sand (e.g. Van Sluys et al. 2001, Martinez-Coronel and Perez-Gutierrez 2011), which seems the same to be in the case of *E. cyanophlyctis*.

Our results indicate that *E. cyanophlyctis* mostly feed on arthropods and have a generalized diet. From the observations recorded in the present study, it can be noticed that insects make the major food items of *E. cyanophlyctis* because the insects isolated from the bulk of the diet appear to be the favored food. Insects in the frogs' diet have great economic importance. Issac and Rege (1975) and Abdullali (1985) have been reported that *R. tigrina* plays a significant role in controlling agricultural and other pests. Crabs are found in the field of this frog. They are present in the paddy fields and cause damage to the bunds in the fields because they make bores in them. Crabs are the one of pest found in the paddy fields^[22] and they are also seeming to eat on the seedling of rice. Thus frog is very helpful in the keeping control crab population and check-in agricultural field crabs' harmful effects.

In the present study, a total of 5 different helminths parasites were collected from *E. cyanophlyctis*. These 5 species belong to two different groups of helminths i.e. Nematoda and Cestoda. Out of five species, one species belongs to Cestoda: *Nematotania dispar* and other 4 species are belonging to the three nematode genera: *Cosmocerca*, *Cosmocercoides*, and *Strongyloides* spp. All of

these helminths species were identified previously and reported from several anurans species from the world^[23]. However, a research study of parasitic fauna of *E. cyanophlyctis* in Pakistan was not conducted previously by anyone. In our study cosmocera was the most common species of *E. cyanophlyctis* in the study area which were isolated from the intestine of the host frog.

Nematotania dispar is the helminth species belonging to the family Nematotaniidae, and it is also has been reported from the *Rhinella icterica* toad intestine by (Yildirimhan et al., 2005) as well as by Mohammad and Ahmad (2010)^[24]. In addition, with that, it also has been reported that there is a greater incidence of infection of anurans with nematodes than that of Cestodes infections (Mohammad, 2010)^[24]. It has been also reported from North Central Texas that nematodes are dominant in infection as compare to Cestode in Spotted Chorus frog, *Pseudacris clarkii* frog^[25]. *Nematotania dispar* was found to be the most dominant species of Cestodes. *Nematotania dispar* covers broad geographical regions throughout the countries of North Africa, the Middle East, Europe, India, and Southeast Asia^[26]. Host records to date imply that *N. dispar* is primarily a parasite of Bufonidae^[27] and it is also recorded from the other anuran families (Ranidae and Hylidae), and from caudated amphibians as well^[28]. *Lanfrediella* spp of nematode has been recorded from the toad in Shendi Sudan^[29] and *Baerietta jaegerskioelii* Cestode has been reported from African common toad^[30]. *N. dispar* Cestode was isolated and recorded from some Iraqi Amphibians species i.e. *Rana ridibund*, *Bufo viridis*, and *Hyla Arborea* species^[31].

5. Conclusion

The present study of *E. cyanophlyctis* in the region of the Dir Lower, Khyber Pakhtunkhwa province of Pakistan, the feeding habits of *E. cyanophlyctis* indicates that they are insectivorous in nature and prey for insects. Among insects, ants were dominant food prey followed by beetles. Helminths that parasitized the frogs are nematodes of the genera cosmocercoides, *Cosmocerca*, and *strongyloides* were frequently found. In Cestode, *Nematotania dispar* was detected in *E. cyanophlyctis* while there was no single species of Trematods and Acanthocephalan were found. The results emphasize the significance of further studies in the Dir Lower to get a well understanding of the helminth parasites with ecological relations of their hosts.

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