

INVESTIGATING THE DIVERSITY OF TERRESTRIAL SNAIL AND THEIR HELMINTH PARASITES IN TEHSIL TIMERGARA DISTRICT DIR LOWER

Kareem Ullah¹, Zaheer Khan¹, Attia Rehman¹, Aamir sultan¹, Asad Ali¹, Irfan Ahmad¹, Bibi Zinab¹, Sania Zaman¹, Bushra Bibi¹, Fakhr Ul Islam^{2*}

¹Department of Biology, University of Haripur, Haripur, Khyber Pakhtunkhwa, Pakistan

²Department of Zoology, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan

Corresponding Author: Fakhr Ul Islam

ABSTRACT

Snail is the invertebrate belong to the phylum Mollusca of kingdom Animalia. Snail is cosmopolitan live in a verity of habitats such is terrestrial, freshwater and marine. Molluscs are the second largest group of organism after insects about 35000 land snails' species was recognized. They establish their population on moist and shady places. Their diversity and distribution and their effect on other organism are not much explore in tehsil timergara district dir lower. For this purpose, the present study was conducted to explore the diversity and determine the effect on other organism. Snail were collected from the different localities such is the side of river, green grasses, maize field , tomato field, and from small plant a total 366 specimen were collected and preserved in 80% ethanol in the forensic research lab university of swat. The snails species were identify according the available taxonomic keys. *Oxichilus alliarius* ,*bulimulus guadalapensis*, *trochulus striolatus* and *helicellia itala* were identified. The highest population of *Oxichilus alliarius* which is 110 and the lowest population is *helicellia itala* which is 74. For the presences of helminths parasites both water and alimentary canal were observed under light microscope. The helminths were identified according the available taxonomic keys. Eggs of the helminths parasite encyclostoma duodenals , clonerchis, paragonimus, scihstosoma, toxacara cati, monizia, fasciola hepatica , and larva of helmanths

parasite *Rhabditis*, *skarjabingylus*, *scihstosoma chitwoodorum*, *physaloptera maxillaris*, *microfiliria*.

Key Word: Snail, Diversity, helminths

1. INTRODUCTION

Molluscs are the second largest creatures on the world (Chapman, 2009). The land snail also called gastropods is one of the diverse groups of animals based on the habit and shape about 35000 species of the gastropods have been recognized (Salaam and Al-wakeil, 2012). Snails are nocturnal in nature and they form a well established population on the moist and shady places, because they live in relatively high humidity (Nunes *et al.*, 2012). The fauna structure of the snail is varying with niche and vegetation type (Burch, 1956; Van Es and Boag, 1981). Climate factor that affects the diversity and population of the snail species is the temperature and humidity (Nunes *et al.*, 2012). Generally, the land snail is considering a typical fungi ore, herbivores and detritivores (Burch and Pearce, 1997). Invertebrate is not noticed by the biologist and conservation agencies they mostly do work on the terrestrial vertebrate on their extinctions by (Lydeard *et al.*, 2004). According to the IUCN survey conducted in 2002 there are total of 1222 land and 708 freshwater Molluscs, out of 41% of the 693 extinctions from the animal are the molluscs, which contain the 31 bivalves and 260 gastropods stated by (Zheng *et al.*, 2017). Helminthes are the parasitic worm which causes the infection in their host. The helminthes infection many cause the huge damage and debilitation and also a huge economic loss in the world livestock and human. Helminthes life cycles are very complex, for the different life and developmental stages and they have multiple hosts (Kasl *et al.*, 2018). tropical and sub-tropical area of the world have a high prevalence of the helminths parasite (Weaver *et al.*, 2010; Tinsley *et al.*, 2011) helminths prevalence increases with climatic changes most likely in those area where the social infrastructure is poor and have lack of sanitation (Hotez *et al.*, 2006) snail plays important roles in the life cycle of the parasite they host as an intermediate host, when infected by a miracidia, act as the intermediate host infected by the first stage larva, act as a first intermediate host when the parasite egg is ingested, snail also act a fist intermediate host when penetrated by miracidia (Lu *et al.*, 2018) Current study are design to investigate the diversity of snail and its helminths parasites in Tehsil Timergara Distract Dir lower KP Pakistan.

2. MATERIALS AND METHODS

2.1. STUDY AREA

This study was conducted in Tehsil Timergara of District Dir lower of Khyber Pakhtunkhwa province Pakistan from July 2021 to September 2021. Timergara city is the largest city and district headquarter of Lower Dir. District Dir Lower is located in the northern areas of Pakistan, with Latitudes of $71^{\circ}, 31'$ to $72^{\circ}, 14'$ East and Longitudes of $34^{\circ}, 37'$ to $35^{\circ}, 07'$ North. The elevation of Dir Lower is about 820 meter (2700 feet) above the sea level.

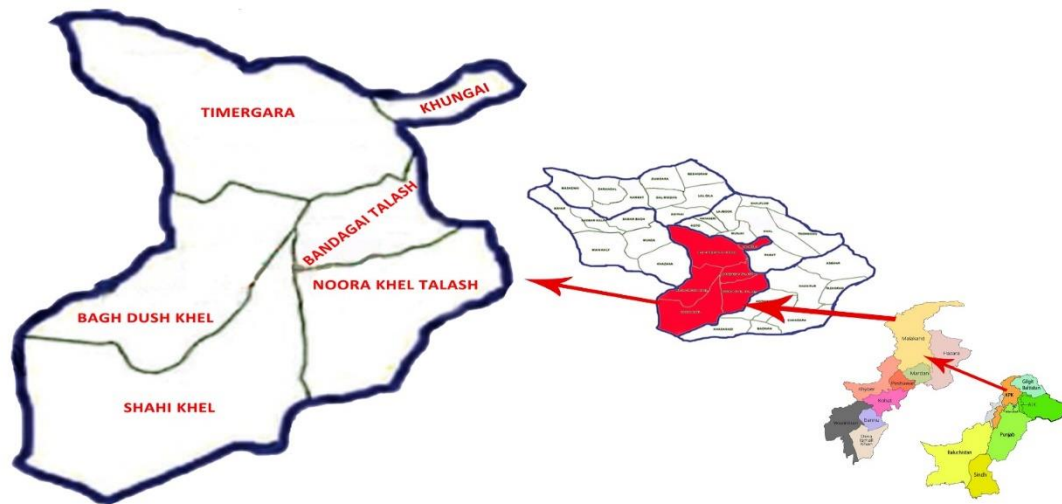


Figure 1. Map of current study area

2.2. SPECIMEN COLLECTION

From different localities of Tehsil Timergara (District Dir Lower) 366 snails specimen were collected by using hair brush (wooden peddle brush) or directly by hand. The snails were collected early in the morning and evening because its mostly available in these times. The number of snails were high in the month of August and September. Their number were increase in rainy season. The snails were collected from six different localities(villages) such as Chinar

Talash, (70 Snails specimens), Shahi Khel, (65 Snails specimen), Bagh otala (61 Snails specimens), Shamshi khan (59 Snails specimen), khungai (50) and Timergara (61Snails specimen). the snails were washed through tap water to remove the debris and then place in water in specimen containers for whole night under light. The specimen was then transported into Forensic Research Laboratory University of Swat for further study.

2.3. SPECIMEN PRESERVATION

The whole body of the snails were preserved in 80% ethanol for further study. The shell which was removed from the snails were preserved in boric acid. The soft body were placed in 80% ethanol and then give number, date of collection, and site of collection. (Murtaza *et al.*,2020).

2.4. IDENTIFICATION OF SNAILS SPECIMEN

The identification of the snails specimens was carried out on the basis of following characteristics such as presence of shell, height of the shell, width of the shell, shape of the shell, number of whorls, shape of the aperture, position of aperture, umbilicus, opacity of shell, eye location, numbers of tentacles, shape of tentacles. The height, width, and aperture of the shell were measure through digital vernier caliper. The height of the shell was measured by placing the shell in the lower jaws of vernier caliper it will show the measurement in digits. Similarly, the width of the shell was measure.Snail specimen was studied under magnifying glass and identified diagram and keys provided by. -IDTools.org. and (Smith And Kershaw, 1979) Identifying land snail.

2.5. TEASING/DISECTING OF SNAIL SPECIMEN

In this method the snails were taken in dissecting dish remove the shell through forceps and dissecting scissors and then dissect the body of the snail through scalpel and dissecting scissors to expose the internal viscera. The alimentary canal was cut into parts through forceps and scalpel in petri dish. The intestine stomach and other organ were cut into sections and then teased to isolate helminths parasites. (Onyishi *et al.*,2018).

2.6. MICROSCOPIC EXAMINATION

First of all, the snails were washed carefully. Then each snail was put in specimen container which is filled about third/ fourth filled with water and then exposed them to light sources for six hours or for whole night. For the presence of parasites take fresh clean slides and take water drops from specimen container put on the slides and then place a cover slip over it and then examine under Light microscope. The examination of slides was carried out with different magnification power. The identification of cercariae was carried by the description by (Qureshi *et al.*, 2015). Microscopic examination of organ was carried out by cutting the organ into slice about 2-3 centimetres (cm) and the place over clean slides place a cover slip over it. The slides were examined under light microscope for helminths parasites with different magnification power. First the slides examined through 5X, then 10X and finally with 40X magnification of microscope. The length and width of parasites is measured in micrometres (μm) through Piximetre.

3. RESULTS AND DISSUCTION

3.1. IDENTIFIED SNAILS SPECIES

A total 366 specimen were collected from Tehsil Timergara Distract Dir Lower. The specimen was collected from different habitat of six villages such as maize field, tomato field, side of canal, and green grasses. The snails species were identified on the basis of their morphological characters. Four species of snails were identified on the basis of morphological characters. *Oxychilus* species, *Bulmulus guadalupensis*, *Trochulus striolatus*, and *Helicella itala*. The oxychillus species were most abundant (110), followed by Bulmulus Guadalupensis (95), Trochulus striolatus (87), Helicella itala (74).

Village	Location	Oxychillus species	Bulmulus guadalupensis	Trochulus striolatus	Helicella itala	Total number
Chinar talash	Maize & Tomato field, canal side	26	19	13	12	70
Shahi khel	Green grasses, vegetable and attached to stone	17	16	17	15	65
Bagh otala katon	Green grasses, road side and maize field.	18	9	18	16	61
Shamshi khan	Spinach, maize field and near spring	13	17	12	17	59
Khungai	Green grasses and attached to stone	20	14	16		50
Timergara	Rice field, shady places and green grasses	16	20	11	14	61

Table 1. Village wise diversity of snail.

Specie name	Chinar talash	Shahi khel	Bagh otala katon	Shamshi khan	khungi	timergara	frequency
<i>Oxychilus alliarius</i>	26	17	18	13	20	16	30.0%
<i>Bulmulus guadalupensis</i>	19	16	9	17	14	20	25.9%
<i>Trochulus striolatus</i>	13	17	18	12	16	11	23.7%
<i>Helicella itala</i>	19	16	9	17		14	20.4%

Table 2. Village wise frequency of snail species

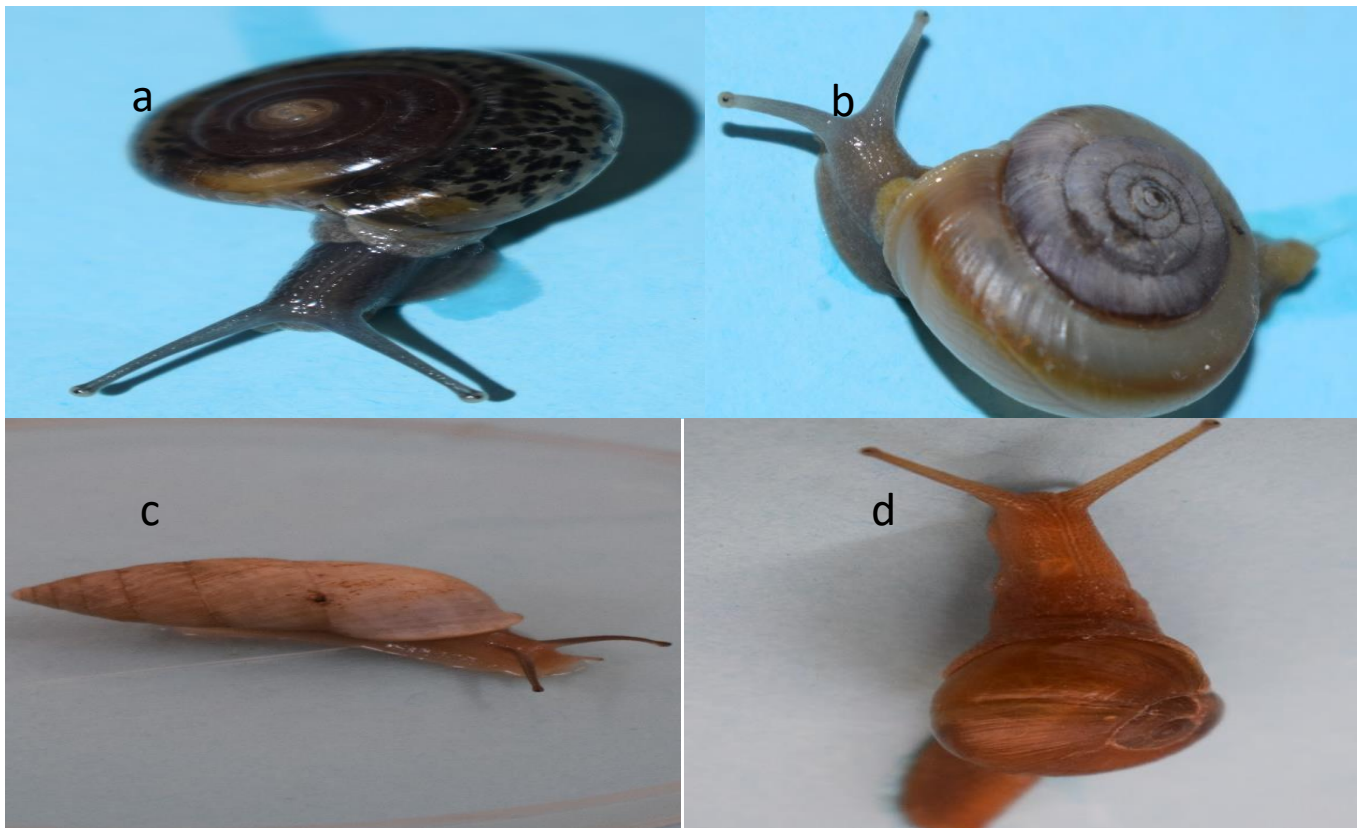


Figure 2. (a) *Trochilus striolatus* (b) *Helicella itala* (c) *Bulimulus guadalupensis* (d) *Oxchilus alliarius*

3.2. RECOVERY OF HELMINTHS PARASITES EGGS FROM SNAILS

Total number of snails is 366 Out of these snails 120 were examined for helminths parasites 30 from each species. The highest prevalence of helminth parasites were found in *Oxychillus* species which having 138 different helminth parasite eggs (*Clonorchis* egg 32, *Fasciola* egg 26, *Moniezia* egg 25, *Schistosoma* egg 10, *Paragonimus* 9) which are followed by *Bulimulus guadalupensis* 131 different helminth parasite eggs (*Clonorchis* egg 28, *Paragonimus* egg 26, *Fasciola* egg 24, *Schistosoma* egg 17 *Taxocara cati* egg 11) then *Trochulus striolatus* having 116 different helminths parasites eggs (*Encyclostoma duodenalis* 15, *Clonorchis* 20, *Paragonimus* egg 17, *Schistosoma* egg 31, *Taxocara cati* 14) while the least followed by *Helicella itala* 86 different helminth parasite egg (*Fasciola* egg 24, *paragonimus* egg 21, *Schistosoma* 20).

s.no	Description of parasite	Availability in host				Important characters of parasite	Measurable character			
							Picture no	Length in μm	Width in μm	Figure no
1	Trematode <i>Clonorchis</i> egg	<i>Oxychillus alliaris</i>	<i>Bulimulus guadalupensis</i>	<i>Trochulus striolatus</i>	-	- Chinese vase shape - Well defined external shell without any ornaments	A, B	25.2	14.6	4.10
2	<i>Paragonimus</i> egg	<i>Oxychillus alliaris</i>	<i>Bulimulus guadalupensis</i>	<i>Trochulus striolatus</i>	<i>Helicella itala</i>	- shell is thick. - operculum is visible. - colour are Gray-brownish golden.	C, D	88.7	71.6	4.11
3	<i>Schistosoma</i> egg	<i>Oxychillus alliaris</i>	<i>Bulimulus guadalupensis</i>	<i>Trochulus striolatus</i>	<i>Helicella itala</i>	- shell are thin and transparent. - A prominent lateral spine.	E, F	140.4	60.5	4.12

4	Fasciola egg	Oxychillus alliarius	Bulimulus guadalupensis	-	Helicellia itala	-Operculum are small and not well define. -Some time irregular and thicken. -Yellowish light brownish-Gray colour.	G, H	132.8	69.7	4.13
5	Nematode Encyclostoma duodenal egg	-	-	Trochulus striolatus	-	- egg are yellowish brown in colour. -round in regular.	I, J	65.6	34.1	4.14
6	Taxocara cati egg	-	-	Ttochulus striolatus	Helicellia itala	-zygote cell is present when release. -eggs are morphological identical.	K, L	67.8	72.7	4.15
7	Cestode Moniezia egg	Oxychillus alliarius	-	-	-	-shell are irreglar and thick. -quadrangular in shape.	M, N	54.7	60.3	4.16

Table 2. Helminths parasites eggs from snails

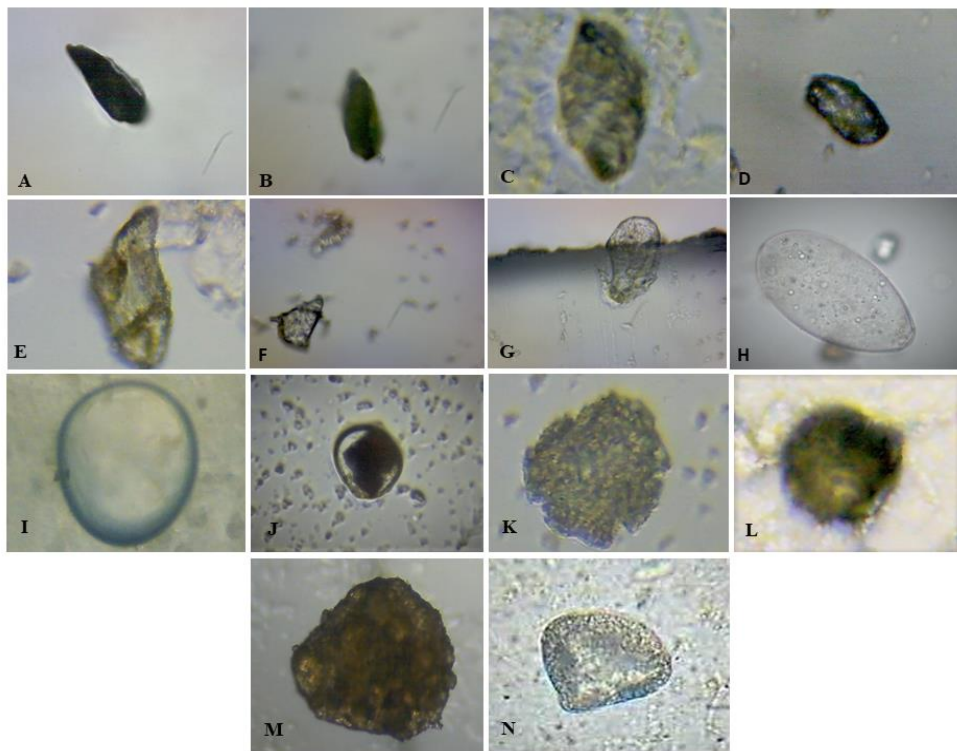


Figure 3. (A, B) are the egg of Clonorchis were observed with 40X magnification of microscope in the water sample and digestive system of snail.(C, D) are the egg of Paragonimus were observed with 40X magnification of microscope in the water sample and the digestive system of snail.(E, F): are the egg of Schistosoma were observed with 40X magnification of microscope in the water sample and digestive system of snail. (G, H) are the egg of Fasciola egg were observed with 40X magnification of microscope in the water sample and digestive system of snail. (I, J)Ancylostoma Duodenale egg were observed with 40X magnification of microscope in the water sample and digestive system of snail. (K, L) Toxocara cati egg were observed with 40X magnification of microscope in water sample and digestive system of snail. (M, N) Moniezia egg were observed with 40X magnification of microscope in water and digestive system of snail.

In this research study we find out four different species of snail in our study area. A very little work is done on snail in our study area almost non touchable. There are few biologists they were know very little about the importance's of snail. Snail is an important intermediate host for most of helminth parasites. We used microscopic analysis of both the water samples and a snail's alimentary canal to look for helminth parasites transmitted by the snail in this investigation. mostly Larvae and eggs of Helminth parasites were observed. Similar research was conducted on the diversity of Snail in the agroecosystem of Faisalabad Pakistan. They were collected 19290 Snails specimen from different villages of Faisalabad. They found fifteen (15) different species of snail. A single species of genus oxychilus is similar to the current study (Altaf *et al.*, 2016). The reason of difference between our study and above mention study it may be due to the average temperature of the area. another research was carried out on the identification and isolation of helminths having zoonotic potency (snail). They identified helminth parasites on the basis of morphological characters. They were isolated helminths parasites from snail. They isolated different types of helminths parasites from snail. the parasites which is similar to our research study is clonorchis, Paragonimus, and Fasciola hepatica. They are found in snail because snail act as intermediate host of helminth parasites. which can transmit into higher animals such as cattle, sheep and goat (Panda and Dash, 2016).

4. CONCLUSION

It was concluded from this study that the snail having greater diversity in study area. the number of snails were high in moist and shady places. Their number is also increase in the month of September. Snail act as intermediate host of helminths parasites the most dominant species in the study area was *Oxichilus alliarius*. It is found about 30% in the study area. Its number is high in rainy season. *Oxichilus alliarius* also having high number of parasites larvae and egg. The most dominant helminths parasites are schistosome species followed by the larvae strongyloides.

Conflict of Interest:

The authors declare no conflict of interest regarding the data presented in the manuscript

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