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Detection of Ganeo Species (Trematoda) of the Viscera of *Hoplobatrachus tigerinus* in District Swabi, Pakistan

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ABSTRACT

The purpose of this study is to identify the frog intestinal trematodes that were collected in District Swabi, Khyber Pakhtunkhwa, Pakistan, using their morphological traits. The study has screened 30 Anurans (order) frogs in this study. Two genus Ganeo species of parasite were retrieved out of these frogs. To have permanent slides, specimens were immobilized in F.A.A. (a solution of formalin, acetic acid and 50% alcohol in proportion of 6, 2 and 92). The slides were then dyed with acetocarmine and preserved with Canada balsam. Next, for the purpose of morphological identification Photomicrographs and line drawings were employed. The measurements were recorded in millimeters. Key features of Ganeo sp. Its main characteristics are described as follows: the body is oval in shape, the oral sucker is large in size to which the ventral sucker is small and the latter overlaps with lower testis. The testis has an almost oval form, whereas the ovary is rounded and longer than it is wide. The uterine coils make contact with the ovary. The Ganeo species has been extensively documented in frog hosts, particularly in *Hoplobatrachus tigerinus*. Both of the organisms that were recovered are intestinal trematodes. This is the initial detection of Ganeo sp. in the Swabi area. The discovery made in this study will assist researchers in identifying trematodes in frogs. The categorization of helminths in amphibians, and frogs in particular, requires further molecular research.

INTRODUCTION

The largest order is Anurans of the amphibians. Almost all the major continents and some of the islands found in the ocean have about 5,200 species of this group, except Antarctica. Their special feature is the shortness of the trunk, which in anurans includes only 5-9 presacral vertebrae as compared to other tetrapod's. They also have large flat skulls and have extensive bone reduction and their hind limbs are proportionally much longer than their forelimbs in most species (Zug, Vitt, & Caldwell, 2001).

Even though their long limbs at the back are usually associated with the leaping process. They used it very well to swim, walk, jog and climb. E compatibility Frogs and toads are an example of these modes of movement. As well as being the food of larger organisms, amphibians play an important role within food webs because they also take part in the cycling of helminths. They eat insect species to a large extent and control their population. Frogs are creatures of the water and land giving them the ability to adapt easily and live well in both environments. Amphibians are said to have been the ancestors of any reptile, birds or mammals. These creatures are found at every corner world over in most habitats and act as carriers of numerous parasite diseases (Chikhlyaev, Ruchin, & Fayzulin, 2016).

The decreased population of the frogs around the globe can be defined as the fact of over use and misuse of dangerous chemicals in the human-made environment (Hof, Araújo, Jetz, & Rahbek, 2011). The other reasons are loss of habitat, chemicals toxification, climate alteration, introduction of new species, exposure to increased UV radiation and natural variation in pollution that causes the progressive decline of these species (Gervasi et al., 2013).

The occurrence of disease and health problems poses a significant challenge in frog culture, manifesting in various ways. Frogs have been impacted by many pathogens such as bacteria, viruses, haemo-parasites, protozoans, and worms (Nwosu et al., 2023). In the transmission process of helminthes, amphibians can act as intermediate, and reservoir hosts. It is also possible for human to get infected from these helminths parasitic amphibians (Wakelin, McKechnie, & Woodborne, 2011).

Furthermore, the life cycles of helminths are intricately linked to their host organisms, which means they have the ability to cause an impact. The worldwide decrease in amphibian populations, as well as the documented instances of deformities, have garnered significant interest due to the fact that many species within this group are regarded as markers of environmental health (Johnson, 2010). This provides a valid reason for the rigorous surveillance

of amphibian populations (Morse et al., 2012).

In Nagaland and Meghalaya, which are located in northeastern India, as well as in numerous Far-East nations, the indigenous inhabitants utilized frogs as a source of food and for medicinal purposes (Chakravarty, Bordoloi, Grosjean, Ohler, & Borkotoki, 2011). Helminths, which are invertebrate parasites, are frequently seen in amphibians and can cause many infectious illnesses. An extensively recognized instance in trematodes is found in the monogenean genus Polystoma, which induces infection in the urinary bladder of frogs globally (Sachse et al., 2012).

(Animalesto, 2020) focused on investigating the variety of trematode worms and their infection patterns in F. cancrivora from Karawang Regency, Indonesia. The study was conducted on around 120 frogs. An examination was conducted on the lungs, stomach, intestine, and caecum to identify the presence of trematodes. The results indicate that *F. cancrivora* was infected by four species of trematodes: *Glypthelmins sp.*, *Pleurogenoides sp.*, *Haematoloechus sp.*, and *Diplodiscus sp.* The prevalence rates for these species were 25.8%, 18.3%, 35.8%, and 23.3%, respectively.

(Chacko & Chacko, 2022) Described That the life-cycle stages from the cercaria to the egg-producing adult take at least two months to complete. It takes 15 days for the infective metacercaria to form within the second intermediate host, and an additional 45 days for the pre-patent phase to occur inside the definitive host. The cercaria of *G. tigrinus* has similarities with that of *G. micracetabulus*, including a body covered in spines, a spear-shaped stylet, and five pairs of penetration glands. However, there are variations in the size of the body, tail, and suckers, as well as the lack of long bristle-like structures on the body and the tail's fin-fold in the dorsoventral direction. The snail hosts utilized by the two species also differ. Moreover, a thorough examination of the current cercaria's characteristics in relation to those of similar non-virgulate xiphidiocercariae documented in existing literature clearly indicates notable differences.

The primary objective is to examine the prevalence of Ganeo trematodes of the viscera of *H. tigerinus* within the villages of District Swabi. To explain the species of the Ganeo in order to consider the morphological characteristics.

MATERIALS AND METHODS

STUDY AREA

This study is carried out at Swabi district of Mardan Division in Khyber-Pakhtunkhwa. The elevation level of the district is 330.85 meters (1085.48 feet). It has latitude 34.12 and the longitude 72.47. Swabi is surrounded by the District Buner on the north, the Haripur District on

the east, the District Attock of the Punjab on the south and the Nowshera District and the Mardan District on the west side. Swabi district lies in the area that is flanked by Indus and Kabul Rivers. It is approximately 100 kilometers away from the Capital of the province Peshawar. The district is 1,543 square kilometers big. Swabi has a climatic condition that is characterized by very high summer (May to August), winter (December to February), enjoyable spring (March to April) and fall (September to November). Rainfall is high during early summer and winter and extra rainfall during spring occasionally. Adult frogs live in freshwater and on land regions, and some species are adapted to underground or tree living. Frogs are very ruthlessly economical in the conversion of food to body weight. Also, they are a prime source of food to the predators and massively involved in the complex food web patterns of various ecosystems around the globe (Ahmad et al., 2022).

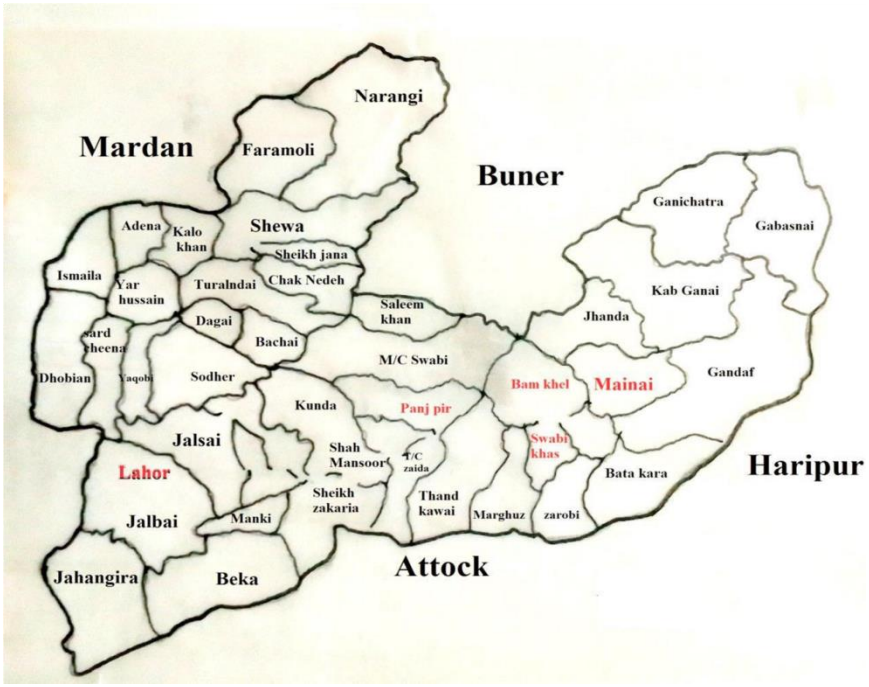

FIGURE 1: MAP OF DISTRICT SWABI

METHODS

SAMPLE COLLECTION

During the months of July to November 2022, frogs were collected in five different locations of Swabi, i.e. Swabi Khas, Panjpir, Chota Lahore, BamKhel and Maini. The numbers of inspected frogs amounted to 30. Others were netted and others were netted manually.

DISSECTION OF HOSTS AND COLLECTION OF HELMINTHS PARASITES

The adult frogs were collected by adopting one of the sample methods referred to as visual

encounter survey as stated by Mendonca et al. Afterwards, the specimens were brought alive into the laboratory and were anesthetized by chloroform (CHCI) solution. The entryways into the abdominal cavity were dissected and opened up and the helminths isolated with the help of the camel brush. The dropper was then used to collect them and the number counted. The analyzed organs are esophagus, stomach, duodenum, liver, and lungs. Dissecting was done on the viscera and they separated in single compartments in Petri dishes containing distilled water. The internal organs were then thoroughly checked under dissecting microscope, to see the parasitic worms (Mendonça, Moser, Oliveira, & Tozetti, 2020).

FIXATION

The samples will be now in hot water. The slides were then fastened with white cottons using moderate pressure which does not damage the specimens. The results of these slides that had been tied were then transferred to a solution of alcohol-formalin-acetic acid (AFA) with overnight [time] at room temperature (Lutz, Jha, Dunne, & Saron, 2015).

STAINING, DEHYDRATION AND MOUNTING OF TREMATODES

The samples were prepared by fixing and preservation, then a specific staining process was applied so that the trematodes could be identify in a morphological manner. A number of ethanol solutions with varying percentages of 30 percent, 50 percent and 70 percent were used to dehydrate the flattened trematodes. They were then stained with Aceto-carmine after 15-30 minutes depending upon the size and permeability of the outer layer of the specimen. In order to achieve the best outcome, over staining of the specimen was performed and re-staining with acid alcohol also was performed in a brief period. The re-stained specimen is re-washed with 70% ethanol and experimented with a sequence of alcohol gradation where the specimen is sequentially immersed in alcohol solutions of ascending concentrations beginning with 70%, flowing to 80%, 90% and lastly to 100 % ethanol. Each of the grades of alcohol is kept about 15-30 minutes. After going through the dehydration, the specimens were then placed in clove oil of 15 to 20 minutes. Clove oil is used as a clearing reagent and it is essential in order to improve the brightness and clarity of internal structure of the specimens. Then the specimens were cleaned with xylene and finally assembled to clean microscope slides with Canada balsam (Bergan, 1955).

RESULTS

In the given case, 30 frogs representing the order anuran are checked on trematode presence. The two species of the parasites of Ganeo genus were reported a total of 30 hosts (frog) Surveyed

altogether. The two species of Ganeo infect the intestine of frog. Everything is expressed in millimeters. Ganeo Klein, 1905. The body is oval, elongate-oval or elongate-pyriform. Tegument are equipped with fine scale-like spines. The oral sucker small, ventro-subterminal. Ventral sucker minute, only a fore part of body. Pharynx are very short, Pharynx small, very much shorter than the length of the oral sucker. Oesophagus is prolonged. Caeca long and slender, taper to a blunt end in hind body posterior region. The testes are globular, oblique or symmetrical, and contiguous and in contact, are intracaecal and lie in forebody, one testis near median, and anterior to the ventral sucker, the other is dextral, and lateral or anterolateral or posterolateral to the ventral sucker. Lack of additional seminal vesicle. Cirrus sac is long-clavate, thinned-walled; extending anterior and lateral, or anterior and posterior and then lateral, in level dorsal below ventral sucker, through genital pore, has rather small looped seminal vesicle, pars prostatica long, highly-developed, Ejaculatory duct short or long. Separate to separate invagination of body wall with genital atrium. Excretory sinistro marginal genital, near oesophagus, or rather posterior. The size of Ovary resembles smaller than testes, posterior/posterior or posterolateral to one of the testis, dextral or posterodextral to single ventral sucker. Ovary, and then seminal receptacle. Uterus long, narrow, much coiled, inhabits intracaecal area behind ovary and extends pretty far on posterior half of trunk; coils not having any dispersion there, however, in rectilinear direction towards genital pore on left hand side of forebody. Eggs propodous, oval, very abundant. Follicular (vitellarium), 2, pre-eminently extracaecal, between the level of the ventral sucker or ovary and just, or even very much anterior to the extremities of the caecae. Terminally pore; one unit of excretory system v-shaped. Usually affect intestine of anurans (mainly amphibians), occasionally fishes and reptiles; species of this genus have been reported in the regions of South Asia that includes the Indian sub-continent, China and Africa. Type-species Excretory system v-shaped, terminally pore. Normally infect intestine of anurans (mostly amphibians), rarely of fishes and reptiles; species of this genus has been reported in South Asia such as Indian sub-continent, in China and Africa. G. glottoides Klein, 1905 type-species. G. glottoides Klein, 1905.

TABLE 1. COMPARISON OF GANEO TIGRINUM WITH GANEO KUMAONENSIS PANDE, 1937, GANEO GIBSONI.

Description	Ganeo tigrinum	Ganeo karachiensis	Ganeo bicadatum	Ganeo gibsoni
Body	2 ×1.24	2.1 × 1.17	2.7 × 0.9	1.82-1.84 × 0.88-0.89
Oral sucker	0.24 × 0.11	0.13 × 0.17	0.15 × 0.1	0.10-0.11 ×0.10-0.11
Phyranx	0.5 ×0.5	0.6 ×0.8	0.15 ×0.11	0.04 × 0.05
Esophagus	0.11	0.25	0.5 ×0.01	0.51-0.20 × 0.04-0.045
Ventral sucker	0.22 × 0.2	0.13 ×0.15	0.13 ×0.1	0.009-0.1 × 0.08-0.09
Anterior testis	0.24 × 0.2	-0.2 dem in position	5 ×0.27, -ovarian	0.21- 0.22 Diagonal Tandem in position
Posterior testis	0.24 × 0.2	0.25 ×0.22	0.25 ×0.23	0.21-0.23
Ovary	0.23 × 0.25 nearly oval in shape	6 × 0.19,round intercecal	15 × 0.15,post testicular	Oval 0.16-0.17 × 0.24-0.24 below the testes
Egg	Immature	0.05 × 0.05	0.01 ×0.005	Oval 0.028-0.029 × 0.014-0.015
Location	a Lahore District Swabi	Karachi Sindh	Karachi Sindh	Oderolal station Sindh

TABLE 2. COMPARISON OF *GANEO* SP. WITH *GANEO GIBSONI* GHAZI *ET AL.*, 2005, *GANEO KARACHIENSIS* KHATOON *ET AL.*, 2011 AND *GANEO KUMAONENSIS* PANDE, 1937

Description	*Ganeo* sp.	*Ganeo gibsoni* Ghazi *et al.*, 2005	*Ganeo karachiensis* Khatoon *et al.*, 2011	*Ganeo kumaonensis* Pande, 1937
Body	1.5 × 0.72	1.82-1.84 × 0.88-0.89	2.1 X1.17	0.82-0.90 X01.5-0.08
Oral sucker	0.08 × 0.13	0.10-0.11 ×0.10-0.11	0.13 × 0.17	0.09 × 0.08
Phyranx	0.04 × 0.04	0.04 × 0.05	0.6 × 0.8	0.06 × 1.0
Esophagus	0.08	0.51-0.20×0.04-0.045	0.25	0.27
Ventral sucker	0.9 × 0.10	0.009-0.1 × 0.08-0.09	0.13 × 0.15	0.07 × 008
Anterior testis	0.14 × 0.18	Tandem in position 0.21-0.22	-0.2 Tandem in position	-0.17 tandem in position
Posterior testis	0.19 × 0.14	0.21-0.23	0.25 × 0.22	0.19-0.18
Ovary	0.13 × 0.14	Oval 0.16-0.17 × 0.24-0.24 below the testes	16×0.19,round intercostal	0.7×0.15,near acetabulum
Egg	Immature	val 0.028-0.029 × 0.014-0.015	0.05 × 0.05	Oval 0.01 × 0.005
Location	Swabi khas, District Swabi	Oderolal station Sindh	Karachi Sindh	Lucnow India

DISSCUSSION

The definition of the amphibian species is that they are tetrapod, ectothermic, and predominantly carnivorous vertebrate animals belonging to the amphibian class (Hallinger, Taubert, &

Hermosilla, 2020). The modern amphibians start living in diverse habitats with the majority of living in terrestrial, fossorial, arboreal or in freshwater aquatic environments.

Over the past decades, amphibians positioned themselves in all regions of the globe as domestic exotic pets/ zoo animals (Henle & Dubois, 2017). The wild amphibian species have been subjected to a drastic decline in the last decades (primarily caused by man himself, including environmental pollution, UV-B irradiation, effect of alien/introduced species, direct maltreatment, habitat destruction, climate changes, and the emergence of diseases, e.g., chytridiomycosis, Ranavirus) (Nguyen, Van Nguyen, Ziegler, Pasmans, & Martel, 2017), and have been under specific media and community consideration. This media interest, among other things, has aroused the interest in these ectothermic animals to lead to a greater popularity of amphibians as privately keep animals too, also in Germany. In most of the countries, international trade in amphibians is increasingly becoming restricted by governments (Krautwald-Junghanns, 2018).

The free-ranging amphibians appear to be obligate hosts of parasites and, therefore, high prevalence of up to 90 percent of free-ranging amphibians have already been reported. As an example,(Rizvi, Bursey, & Bhutia, 2011) sampled free-ranging amphibians in an Indian Wildlife Sanctuary (Haryana); and demonstrated that endemic common dicroglossid frogs (*Euphlyctis cyanophlyctis*) were often infected (52.9%) by nematodes. Unlike this study on wildlife, very little information exists on parasitic infection of dicroglossid frogs (*E. cyanophlyctis*) in captivity. When giving comparisons of our data on prevalence against that previously published, the aspect that most of such surveys have taken place on wild animals ought to be taken into account and this difference may lead to such variable prevalence. Probably most noteworthy to dicroglossid frogs, other of the free ranging amphibians are also proving to have higher prevalence of parasitism when it is compared with captive kept amphibians. In addition, sensitivity and specificity of applied DSFS used to identify helminth and protozoan stages could have affected seen prevalence since various diagnostic approaches that have been applied in previous studies in wildlife have been adopted in the past (León-Règagnon, 2019).

The major parasites of the freshwater fishes (Pakistan) particularly Sindh province are trematodes. In the current research abnormal specimens of family were retrieved in the intestine of Channa striatus of Haleji Lake in Sindh. They would not fit in any of the current subfamilies of the family due to significantly variant diagnostic characteristics. Thus, a new subfamily Heckmanninae and a new genus Heckmannia and a new species (Heckmannia channai) is proposed. It is named as the subfamily after the honour of Prof. Dr. Richard A. Heckmann,

Department of Biology, Brigham Young University, Provo, Utah, USA who has several publications on fish parasite and a well-recognized parasitologist (Hussain, 2022).

Nor are there at this date known to any of the species of the genus Brachycoelium (Dujardin, 1845) Stiles and Hassall, 1898, of which there are 10 species, any life history. An account of this genus may be found in Cheng (1958) and that the systematic position of Brachycoelium, whether in the Plagiorchiidae, Dicrocoeliidae or Brachycoeliidae may be settled by an understanding of the life history patterns. On the adult morphology he made the decision of regarding the genera Brachycoelium, Cymatocarpus Braun, 1901, Leptophallus Liihe, 1909, Glypthelmins Stafford, 1905, Margeana Cort, 1919, and Mesocoelium Odhner, 1911, as representatives of the same taxon as, the Brachycoeliinae (Dicrocoeliidae). In a subsequent paper (Cheng, 1959) he added the new genus Reynoldstrema Cheng, 1959 though he said that the subfamily Brachycoeliinae should be given familial status as proposed by Johnston (1912) since such a separation appears to be natural as being based upon the life histories of Glypthelmins quieta (Stafford, 1900), as described by Rankin (1944) and Leigh (1946), and Mesocoelium brevicaecum O (Bursey, Goldberg, Telford, & Vitt, 2012).

CONCLUSIONS

Ganeo species had been commonly reported both in amphibians host and mainly with Rana tigrina. The total of the recovered species are the trematodes of intestine. This is a recovery of Ganeo sp. in the district Swabi to be first time. Identification of trematodes of frog will assist the researchers because of the finding of the present study. Future Molecular work should be carried out to develop further classification of amphibian helminths and that of frogs specifically.

RECOMMENDATIONS

The suggestions are as below.

1. In order to assess seasonal and ecological conditions of trematodes occurrence.
2. A huge study concerning trematodes as well as other helminthes on other amphibious hosts should be conducted.
3. To carry out a molecular work on the basis of nuclear or mitochondrial genome of trematodes fauna of frogs and other amphibians, who are the hosts.

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