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Original paper

A faunistic survey of snails and their infection with digenean trematode cercariae in Bandar-e Anzali at the littoral of the Caspian Sea

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ABSTRACT. Trematodes are known as a diverse group of endogenous parasites, which snails as their intermediate hosts can dramatically affect parasite transmission dynamics. Snails play a key role in life cycles of digenean trematode. However, there has not been much faunistic surveys in terms of snails' distribution in Iran. The current study was aimed to identify snail's fauna and their current geographic distribution in four regions of Guilan province, Iran. Several species of snails (land and freshwater snails) were obtained from 4 different areas (2018–2019), and then samples were separately transferred to the laboratory. Diagnosis of snails was then performed according to morphometric characteristics including dextral or sinistral shell, shape, color and size of shell. Moreover, radula was stained and then photographed under microscopic examination. Furthermore, 25% of any species were tested for cercarial infection. In total, 2082 snails belonging to 12 species were identified based on the morphological characteristics. Land snails were identified to be *Helicella* sp. (46.4%), *Helix aspersa* (34.1%), *Helicopsis* sp. (8%), *Pomatia* sp. (6.7%) and *Oxyloma elegans* (4.8%). Moreover, freshwater snails were *Physa acuta* (30.6%), *Lymnaea auricularia* (25.8%), *Lymnaea gedrosiana* (18.7%), *Lymnaea palustris* (8.9%), *Lymnaea truncatula* (8.1%), *Planorbis* sp. (6.4%) and *Lymnaea stagnalis* (1.5%). In collected snails, *Lymnaea auricularia* (0.66%) and *Lymnaea gedrosiana* (0.45%) were the only snails harboring cercariae (Gymnocephalus cercariae). There was no statistically significant difference between the cercarial infection and snail species ($P<0.05$). Comprehensive field studies are highly needed for better evaluation of the snail biodiversity in the Caspian Sea region due to the particular climatic conditions and the high prevalence of trematodosis.

Keywords: snail, trematode infection, Caspian Sea, cercariae

Introduction

The molluscs compose the large phylum Mollusca, with known species which have great diversity in freshwater and terrestrial habitats. The greatest diversity of species belongs to the gastropods as a major taxonomic class of the phylum Mollusca. The class Gastropoda has a vast total of many species like snails, slugs, bivalves, and freshwater limpets. Furthermore, the gastropods are considered to live on land, some live in freshwater, but more than two thirds of them are marine gastropods [1]. In general, it can be said that snails and mussels play a variety of roles in biological phenomena, geological issues, culture and civilization [2]. Gastropods can be adapted to almost every kind of existence on earth. Mountain, oceans and desserts are natural barriers that can limit the distribution of freshwater snails. It is likely that a significant part of them passing around an obstacle. Different larval stages of trematodes such as sporocysts, rediae and cercariae can develop within snails. It is worth noting that Pulmonata and Prosobranchia snails play a major role in the life cycle of digenean trematodes. Further understanding of the gastropod carriers also is needed for the control of diseases such as fasciolosis, dicrocoeliosis, paramphistomosis, and schistosomosis in different

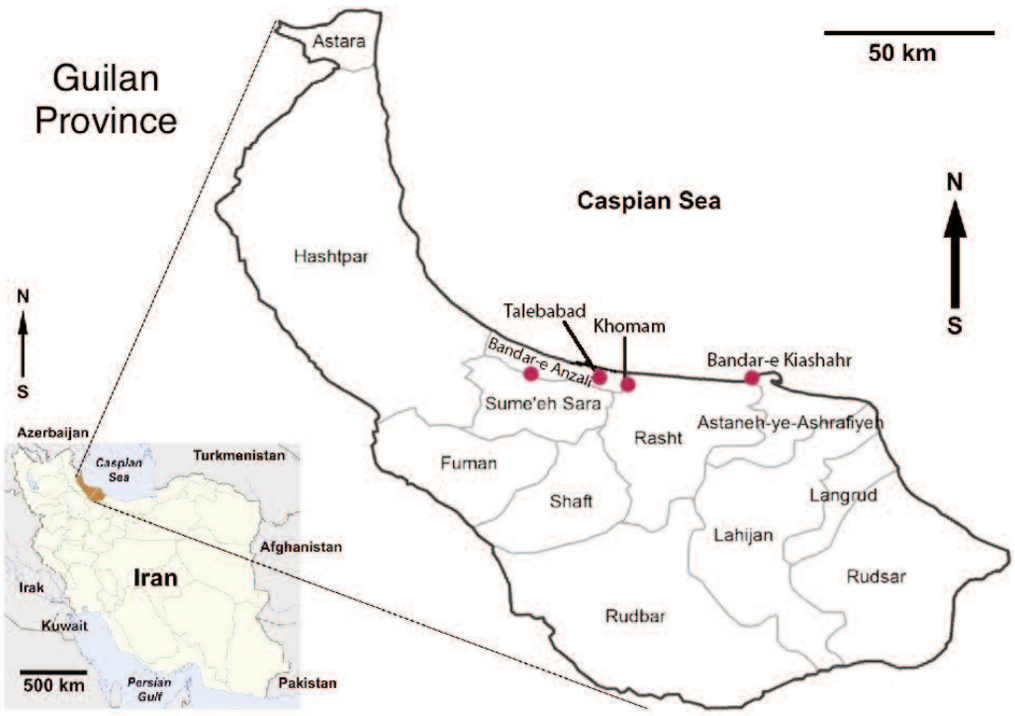

Figure 1. Sampling areas in the present study

environmental conditions [3,4]. Trematodes are regarded as important global parasitic disease of humans and animals [5]. Identification of snail fauna and analysis of their potential distribution are required, especially those that are implicated as intermediate host for the trematodes. To develop interventions against important snails in this region, a comprehensive data is required in study area. Therefore, the present study was aimed to identify snails' fauna and their roles in cercarial transmission of trematodes in Bandar-e Anzali, Guilan province, Iran.

Materials and Methods

Study area

Guilan province is located in northern Iran. It has a humid subtropical climate with the heaviest rainfall in the country. Extensive vegetation coverage of the province has made it a suitable place for the growth and development of snails. The climatic data is provided based on information obtained from the website of Meteorological Organization of Guilan province in one year period (Tab. 1). The geographical location of Guilan province, and study areas, include Bandar-e Anzali, Khomam, Bandar-e Kiashahr and Talebabad are shown in figure 1.

Sampling

Samples were obtained from different areas of Guilan province, between August 2018 and August 2019. It is worth noting that sample collection was mainly conducted at monthly intervals sampling according to the presence of the snail population in the spring, summer and autumn. Labelling included, location name, collecting date, and each sample was also transferred to the parasitology laboratory. Two groups of snails (both land and freshwater snails) were collected from target areas. In the case of land snails, samples were taken by removing them directly from the sand and plant stems, whereas water snails were collected on the sides of ponds, creeks, water canals, and river beds using auxiliary tools such as the phloem and sieve. Sample collection was performed as described by Olivier and Schneiderman [6] according to the time unit with some modification. Afterwards, sampling was conducted for 1 hour in the morning and 1 hour in the evening for 2–3 days at the beginning, middle and end of every month. For mentioned process, it was tried to collect live specimens as much as possible in containers of land or water depending on the type of snail.

Faunistic study

Snail diagnosis was performed on the basis of taxonomic keys and morphological characteristics

Table 1. Meteorological data of studied areas in 2018–2019

Area	Parameters*											
	Rainfall (mm)				Temperature (C°)				Relative humidity (%)			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Bandar-e Anzali	845.3	513.3	264.6	589.7	16.3	10.5	19.3	26.0	86.3	82.2	81.0	76.7
Talebabad	837.0	509.9	263.2	588.1	16.3	10.2	19.0	24.8	88.0	81.3	82.5	76.3
Khomam	801.9	506.1	259.8	574.3	16.6	10.8	19.7	26.9	86.1	79.8	80.1	76.0
Bandar-e Kiashahr	598.2	468.1	169.6	219.3	15.8	10.3	19.1	25.3	86.0	81.5	77.7	78.2

*Data were obtained from local hydro-meteorological organization (www.gilmet.ir)

of the shells such as shape, size and color [7]. In general, shells were divided into two groups including broad and conical and then the color and size of the shells were used for identification in each group. The other features such as dextral or sinistral shell, length of spire, aperture and apex form, number of whorls, grooves and sutures were used for diagnosis of snails. Furthermore, radula was isolated by digesting surrounding tissue with the modified method of Andrade and Solferini [8]. For this purpose, the buccal mass was removed from the snail and transferred to potassium hydroxide 10% as digestive solution for 24 hours at the laboratory temperature. Afterward, digested tissues were removed with microforceps. Radulas were separately transferred to microtubes and stained by eosin, methylene blue, and malachite green. Finally, stained radula was collected under stereo-microscope, and were then photographed under microscopic examination.

Evaluation of parasitic infections in snails

To investigate the prevalence of larval stage of digenean trematodes, live snails were transferred to the laboratory and were put up between two glass slides under pressure smear for light microscopic examination [9]. The cercarial samples were stained with carmine and identified as stated by previous morphological studies [10]. Overall, 25% of every species were tested for infection with larval stage of trematodes.

Statistical analysis

Statistical analyses were carried out by SPSS version 19.0. The findings were analyzed by Chi-Square test. A *P*-value less than 0.05 was statistically considered significant.

Results

In the present study, shell examination of both land and water snails were carried out in different areas. A total of 12 snail species were identified, of which five species were land snail and seven species freshwater snails.

Land snails

Helicella sp. is a species of land snail with medium size. This species has white, tan or pale-yellow shells that are approximately 10–20 mm wide. The shell is flat with open coil forming a convex. Short spire and shallow grooves are other features of the snail shell (Fig. 2A). *Helicella* was found on a variety of plants throughout the sampling area.

Oxyloma elegans is a species of terrestrial pulmonate gastropod. The height of the shell is 6–8 mm. The shell usually has fewer than three whorls. Moreover, the shell is yellowish and the spire is short (Fig. 2B). *Oxyloma elegans* was collected in moist habitats.

Helix aspersa, the garden snail is a species of land snail, that thin calcareous shell is 25–40 mm in diameter, with four or five whorls. The body is soft and covered with slimy mucus. The shell is shown variable in color and shape but generally is dark brown, or cream with brown stripes (Fig. 2C). This species lives on a variety of plants throughout the sampling area.

Helicopsis sp. is a genus of air-breathing land snails. Shell is 5–6 mm in diameter with short spire. The shell is variable in color, but generally white or

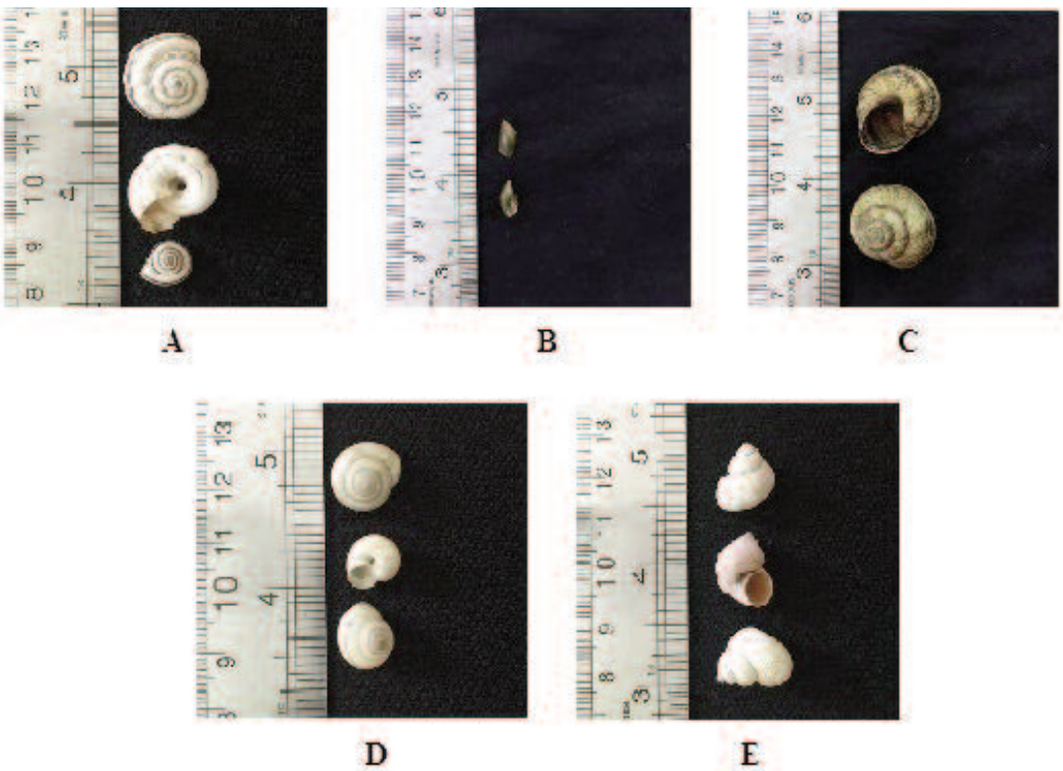

Figure 2. The shell of land snails collected at the present study. A: *Helicella* is a genus of small to medium-sized; B: *Oxyloma elegans*, C: *Helix aspersa*, D: *Helicopsis*, E: *Pomatia* in different sizes

light brown (Fig. 2D). *Helicopsis* was found on a variety of plants throughout the sampling area.

Pomatia sp. this species has big aperture and its margin is white. The shell has five to six whorls and the height of the shell is 30–45 mm (Fig. 2E). *Pomatia* was found on a variety of plants throughout the sampling area.

Freshwater snails

Lymnaea auricularia a species of air-breathing freshwater snails that is classified as sinistral. The shell is roundly ovate and the last whorl comprises 90% of its volume. Spire is rounded and broad that pinches in steeply at the apex. The width of the shell is from 10–15 mm (Fig. 3A). This species was collected from the ponds throughout the sampling area.

Lymnaea gedrosiana is a species of freshwater snail and is also categorized as dextral snail. The shell of the species can grow to ~30 mm in height. The body whorl is large with the short and sharp spire (Fig. 3B). *L. gedrosiana* was found from the ponds and puddles in all areas.

Lymnaea palustris a species of air-breathing freshwater snails that is classified as sinistral snail. Spire is short and the color of the shell is light brown. The dimensions of the shell are observed from about 10 to 17 mm in length, and about 5 to 10 mm in width (Fig. 3C). This species was isolated from the ponds, puddles of water, river bank or the terrain alongside the bed of a river in Talebabad region.

Lymnaea truncatula is a species of air-breathing freshwater snail, the height of the shell is 5–10 mm and the width of the shell is 2–5 mm. Spire and body are usually equal in size. The shell is variable in color it can vary from light to dark brown (Fig. 3D). *L. palustris* was collected from the ponds, puddles of water, river bank or the terrain alongside the bed of a river in Bandar-e Anzali and Talebabad region.

Lymnaea stagnalis is classified as dextral snail. The shell color is brown. Spire is short and the color of the shell is light brown. Furthermore, the dimensions of the shell range from 45 to 60 mm in length, and about 20 to 30 mm in width. The last whorl is suddenly inflated (Fig. 3E). *L. stagnalis* was seen in the river bed and stagnant water in Kiashahr area.

Physa acuta is known to be a species of left-handed, air-breathing freshwater snail. The height of the shell is 10 mm. Shell is held, and the spire pointing up (Fig. 3F). This species lives in freshwater rivers, lakes, streams and swamps.

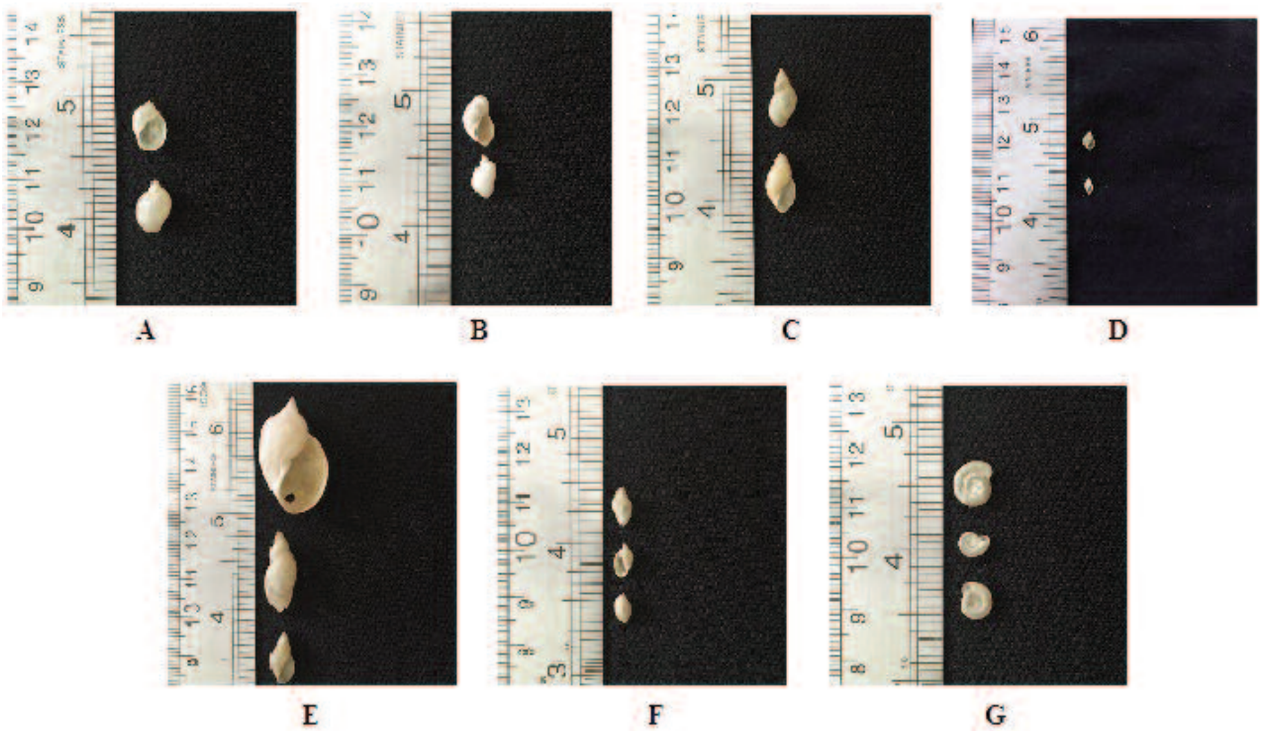

Figure 3. The shell of water snails collected at the present study. A: *L. auricularia*, B: *L. gedrosiana*, C: *L. palustris*, D: *L. truncatula*, E: *L. stagnalis*, F: *Physa acuta*, G: *Planorbis* sp. in different sizes

Planorbis sp. has a sinistral shell. The width of the shell was 15–20 mm. The keel on the periphery of the shell was located in near the edge closest to the spire side. Its color varies from white to light brown (Fig. 3G). This genus was found from the ponds, and mud substrate in throughout the sampling area.

Five species of land snail identified in the Bandar-e Anzali. Frequency of land snail species varies, between 46.4% for *Helicella* sp. to 4.8% for *Oxyloma elegans*. A total of 1171 samples were collected in regard to freshwater snails, the *Physa acuta* was found to be the highest (30.6%), followed by *L. auricularia* (25.8%), and *L. gedrosiana* (18.7%), respectively (Tab. 2).

Lymnaea auricularia and *Lymnaea gedrosiana*

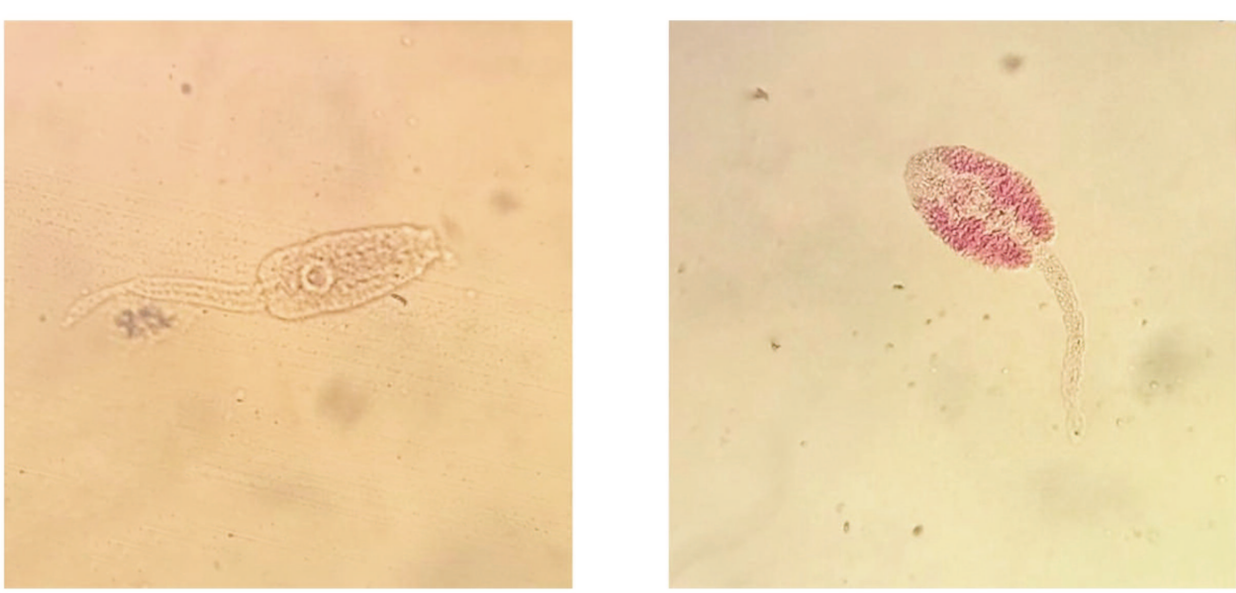

Figure 4. One type of cercariae was found. A: non-stained, B: stained with carmine; 100× magnification

Table 2. The frequency of snails and trematodes cercariae infection in the intermediate host

Snail species	Frequency of snails		Status of infection*	
	Number	%	Number	%
Land snails				
Helicella sp.	423	46.4	–	–
Helix aspersa	311	34.1	–	–
Helicopsis sp.	72	8	–	–
Pomatia sp.	61	6.7	–	–
Oxyloma elegans	44	4.8	–	–
Total	911	–	–	–
Freshwater snails				
Lymnaea auricularia	302	25.8	2	0.66
Lymnaea gedrosiana	219	18.7	1	0.45
Lymnaea palustris	104	8.9	–	–
Lymnaea truncatula	95	8.1	–	–
Lymnaea stagnalis	17	1.5	–	–
Physa acuta	359	30.6	–	–
Planorbis sp.	75	6.4	–	–
Total	1171	–	–	–

*About 25 percent of each species were investigated for the presence of cercariae

were infected with larval stage (Gymnocephalus cercariae) of digenetic trematodes (Fig. 4). Not only was there no statistically significant difference between the two infected snails, but also between the infected and non-infected ones ($P<0.05$).

Examination of *radula*

In the present study, radula structure was evaluated in some snails as far as possible. Moreover, an explanation was provided in each case because of structural differences in *Physa acuta*, *Helicella* sp., *L. auricularia*, *L. palustris* and an unknown slug.

Radula in *Physa acuta* is rhipidoglossan type. The teeth are small and completely uniform in each row. There is one central tooth, flanked by some laterals, and numerous marginal teeth (Figs 5A, 5B). Central tooth is multi-dentate in the Physidae family. Type of radula in *Helicella* sp. show a simpler structure with a central tooth, flanked on each side by several lateral teeth (22–26), numerous tiny dentations are visible on marginal teeth (Fig. 5C). *Helix aspersa* radula type was similar to *Helicella* sp. (Fig. 5D). *L. auricularia* as are described in the results of *Physa acuta*, radula type of this species is rhipidoglossan (Fig. 5E). In contrast to what is observed for *Physa acuta, L. auricularia* has one usually central tooth in each row with an indentation. Type of radula in *L. palustris* was similar to *L. auricularia* (Fig. 5F). In the slug, central teeth, lateral and marginal teeth (shell-less terrestrial gastropod mollusc), are slightly stretched and angled in slug compared with the *Helix aspersa* (Fig. 5G).

Discussion

Previous studies have demonstrated the key role of lymnaeid snails in transition of parasitic trematodes including fasciolosis [11,12]. The major implications of lymnaeid intermediate hosts for fasciolosis transmission, revealed the importance of applying faunistic surveys, populations dynamic, systematics and taxonomy to determine specimen distribution for developing disease control strategies. Different lymnaeid species demonstrated to be associated with various transmission and epidemiological patterns. Therefore, lymnaeids can be considered as indicator of fasciolosis for modelling and remote sensing-geographical

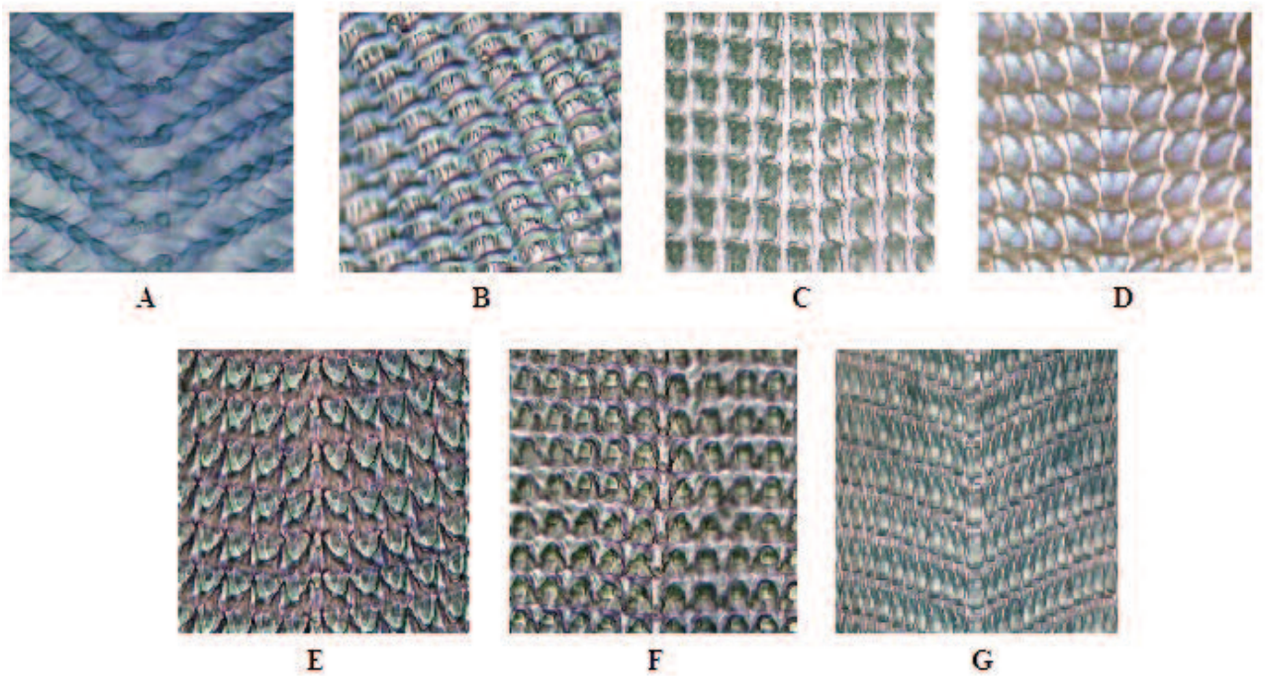

Figure 5. The shape of radula in different species snails. A: distribution of the central, lateral and marginal teeth of radula in *Physa acuta*, B: marginal teeth with a large number of indentations in *P. acuta* (stained with methylene blue), C: central and marginal teeth of radula in *Helicella* sp. (stained with malachite green), D: a portion of the radula of *Helix aspersa* showing marginal and central teeth (stained with methylene blue), E: a portion of the radula of *L. auricularia* showing marginal teeth, and immediately next to them lateral teeth, central teeth, F: the radula of the *L. palustris*, G: the radula of the slug (stained with malachite green)

information system [13,14].

The various ecological factors, including season, temperature, and rainfall are very effective on lymnaeid snails population. Ecologically, in the Caspian Sea regions, especially Guilan province, weather condition is more suitable for the growth and development of freshwater snails (from April to November) than other parts of Iran, because of an average annual temperature (26–28°C), high annual rainfall (140–150 cm) and high relative humidity [13–15].

It is worth noting that faunistic survey is a relatively long tradition in Iran. Despite a growing number of literature records over the last years, snail's fauna in Guilan province have remained unreviewed. Furthermore, large part of Iran remains unexplored.

Although taxonomy of snails is important for biological and molecular studies, it should be noted that they can play an important role in transmission of parasitic infection. Trematoda, a class within the phylum Platyhelminthes, consist of different species that cause infection in molluscs and vertebrates such as *Fasciola*, *Schistosoma*, *Opisthorchis*, *Clonorchis*, *Echinostoma, Heterophyes*, and etc. [16,17].

The high incidences of *Fasciola* species and *Dicrocoelium dendriticum* have been reported in many areas of Iran. Several studies have already showed that coastal strip of the Caspian Sea is endemic area for fasciolosis in both human and animals [13,14,18,19]. In order to develop prevention against important snails, a faunistic survey is required to provide a clear aspect of the snail fauna.

According to what was mentioned briefly, the aim of this study was to determine snail's diversity and their infection with trematode cercariae in a region with specific climate condition. Although samples were collected from four regions including Bandar-e Kiashahr, Talebabad, Khomam and Bandar-e Anzali, but ecosystem, soil structure, vegetation, temperature, rainfall and humidity (the Caspian Sea region) are similar in all sampling areas. It is worth noting that the shrubs, vegetable plants, old trees and clay soil with high humidity, are dominant in our study regions. Moreover, ponds, rivers, water canals, and other water reservoirs are usually seen throughout study areas. Therefore, it should be noted that these areas are ideal for growth and reproduction of snails and slugs as an

intermediate host of free-living organisms [13,14].

Of the total 2082 snails, less than half of them were identified to be land snails. *Helicella* (46.4%) and *Helix aspersa* (34.1%) were most widespread snails and *Helicopsis*, *Pomatia* and *Oxyloma* were less widespread snails (ranged between 4.8–8%) in all sampling areas. *Helix aspersa* has been described as the first intermediate host for *D. dendriticum* and *Brachylaima*, which demonstrated a high potential for fertility and growth [20].

This species has been distributed in many regions of word including Australia, New Zealand, Mexico, Europe, Britain, and Iran, as well as the coastal strip of the Mediterranean and Black Sea [21–23]. Investigations have indicated occurrence of different parasites infecting *H. aspersa* under farm environments, indicating the need for epidemiological studies and faunistic survey to control pathogenic species under farming conditions [23–25]. This species can be of great importance as the intermediate host of the *D. dendriticum* in Iran.

The high prevalence of *Helicella* and *Helix* is probably due to the soil type and dense vegetation of the region and also arises from morphological resistance and a high potential for reproduction of snails. These species were distributed in all part of study areas. Among land snails, *Oxyloma* was only found in Kiashahr area, especially in sand and rubble habitats. Taken together, land snails are most important in transmission of *D. dendriticum*, where up to now more than 70 species of snails were identified as a first intermediate host of *Dicrocoelium* spp. [26]. Among freshwater and amphibian snails, many species were also identified in the present study including *Lymnaea* spp., *Physa acuta* and *Planorbis* sp. As a matter of fact, *Physa acuta* was the most prevalent species (30.6%), followed by *L. auricularia* (25.8%) and *L. gedrosiana* (18.7%), respectively. Furthermore, *Lymnaea* and *Physa* were found in all part of sampling sites, while *L. truncatula* as an intermediate host of *Fasciola hepatica* was predominantly observed in Bandar-e Anzali and Talebabad. Freshwater snails have been reported in various regions of Iran including northern area (Mazandaran) and southern areas (Khuzestan) [27–29].

Freshwater snails are rich in variety. Many freshwater snails are important in the course of their biological life as first and second intermediate hosts in the life cycle of trematodes and some pathogenic nematodes. Most parts of Iran have many species of freshwater snails, some of which are widely distributed throughout the country, and some have local distribution. *L. truncatula* and *L. gedrosiana* species have been observed in highlands and plains, respectively, in most parts of the country. Furthermore, species such as *Planorbis planorbis* and *Physa acuta* have been reported from almost all provinces of the country [30]. Southern littoral of Caspian Sea, especially Guilan province, is ideal for development of freshwater snails due to special ecological and geo-climatic conditions such as water, ponds, free waters and presence of aquatic vegetation and wild grown plants where 60% of Guilan province is covered by wetlands and rice field [13–15].

Based on the data presented, *Physa acuta as* ubiquitous freshwater snails was found to be most prevalent species among freshwater snails in the present study, which has been previously reported from different parts of Iran such as Khuzestan, and Lorestan Provinces, as well as Guilan, and Mazandaran provinces in southern littoral of Caspian Sea [31–34]. *Physa* spp. has been revealed to act as the first intermediate host to a variety of digenean parasitic helminths, such as white grub in many fish species, *Posthodiplostomum minimum* [35,36].

Seven species of lymnaeid snails has been reported in Iran, among which *L. auricularia* can be considered to be one of the major pond snails. This freshwater snail has been found in many areas of Iran such as northwestern Iran. A study has found two groups of fluke's cercariae in *L. auricularia* including furcocercariae and Echinostome cercariae in wetlands of North West of Iran, where there has been wild aquatic birds and is also consistent with the present study because of the presence of *L. auricularia* in wetlands (slow streaming waters) with presence of aquatic vegetation and wild grown plants [29,37].

L. truncatula has been collected from various provinces of Iran including Guilan, Mazandaran, Lorestan and other regions where is determined to be the main intermediate host for *F. hepatica* in Iran [34,38–41]. In the present study, the frequency of this species appears to be low in comparison to other lymnaeid snails.

L. gedrosiana and *L. palustris* have been previously found from northern Iran [38,42,43]. *L. gedrosiana* is known to be the main intermediate host for *F. gigantica* transmission in Iran, this species has been revealed to be a suitable

intermediate host for a number of parasites and zoonotic diseases in Iran including *Fasciola gigantica*, *Trichobilharzia* spp., *Ornithobilharzia turkestanicum*, echinostomes as well as cercarial dermatitis, and *Clinostomum* infections and plagiorchids infections [27,28,38,42,44–46].

Moreover, it should be noted that among identified land snails, only *Helicella* is capable to transmit cercariae (mainly *D. dendriticum*) [26], while *L. auricularia* and *L. gedrosiana* showed infection with cercariae (Gymnocephalus). In a study to identify cercariae fauna of *Bellamya* (*Viviparus*) *bengalensis* in Khuzestan province, they indicated that of the total 1143 collected samples, 5 snails (0.4%) were infected with Xiphidocercariae [45].

Lowest cercarial infection rate has been frequently reported in different studies. For instance, in a study from Bangladesh among 864 tested snails, the most prevalent species were *Indoplanorbis exustus* (31.6%) followed by *L. luteola* (23.7%) and *L. auricularia* (16.8%), respectively [47]. The prevalence of Gymnocephalus cercariae in *L. luteola* and *L. auricularia* were identified to be 5.8% and 6.2%, while Echinostome cercariae was identified to occur in *L. auricularia* (1.4%) and *Indoplanorbis exustus* (1.8%).

In conclusion, Guilan province is located in southern Caspian Sea littoral with a suitable climate for development of snails and trematodes. In the present study, different species of snails were identified in four regions of Guilan province. There were five species of land snails and seven species of freshwater snails, which latter group has mostly belonged to *Lymnaea* genus. Although cercarial infection was very low in snails, they can be considered as first intermediate hosts for digenean trematodes. This study provides new findings on the distribution of snails and the intermediate hosts of trematodes in the study area.

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