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Original Article

Identification and Determination of the Geographical Distribution of Freshwater Snails in Lorestan, Iran

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ABSTRACT

Snails are creatures present in various ecosystems that, in addition to being present in human surroundings, some of them are also important in veterinary medicine and medicine as the intermediate hosts of Digenean trematodes. The present study was conducted to identify and determine the geographical distribution of freshwater snails and investigate the relationship of variables, such as season and geographical region, with snail species and dispersion in Lorestan in the west of Iran. A total of 4400 samples of freshwater snails were collected using the multistage sampling method (i.e., stratified, cluster, and randomized) from 110 points in five geographical regions in four seasons and then identified based on their morphological characteristics by diagnostic keys. The ArcGIS software (version 10.3) was used to evaluate the spatial distribution of the freshwater snails. In this study, seven species of freshwater snails were identified in six families belonging to six genera, namely *Melanopsis doriae* (6.30% of the variation in species), *Theodoxus doriae* (5.55%), *Bithynia tentaculata* (43.22%, the dominant species), *Physa acuta* (24.98%), *Lymnaea truncatula* (9.75%), *Gyraulus euphraticus* (8.18%), and *Lymnaea gedrosiana* (2.02%). The geographic distribution of freshwater snails was recorded across five regions in 22 points per region for every season. The spatial distribution maps showed that the distribution of freshwater snails varies according to region and season ($P<0.001$). The obtained results revealed the effects of season and geographical region on the distribution and population density of snails in the province. These data can be used for the implementation of control programs against parasitic diseases in the region, including trematodes.

Keywords: Identification; Freshwater snail; Geographical distribution; Lorestan

Identification et Détermination de la Répartition Géographique des Escargots d'Eau Douce dans la Province du Lorestan en Iran

Résumé: Les escargots sont des créatures présentes dans divers écosystèmes qui, en plus d'être répandues dans l'environnement humain, certains d'entre eux s'avèrent être également importants en médecine vétérinaire et humaine en tant qu'hôtes intermédiaires des trématodes digéniens. Cette étude a été menée afin d'identifier et de déterminer la répartition géographique des escargots d'eau douce dans le Lorestan ainsi que d'étudier la relation existante entre la distribution des espèces d'escargots dans l'ouest de l'Iran et différents variables, telles que la saison et la région géographique. Un total de 4400 échantillons d'escargots d'eau douce a été collecté à l'aide de la méthode d'échantillonnage à plusieurs degrés (c'est-à-dire stratifiés, groupés et randomisés) à partir de 110 points repartis sur cinq régions géographiques et les quatre saisons. Ensuite les spécimens ont été identifiés en fonction de leurs caractéristiques morphologiques par des clés de diagnostic. Le logiciel ArcGIS (version 10.3) a

été utilisé pour évaluer la distribution spatiale des escargots d'eau douce. Dans cette étude, sept espèces d'escargots d'eau douce ont été identifiées appartenant à six familles et six genres, à savoir *Melanopsis doriae* (6,30% de la variation des espèces), *Theodoxus doriae* (5,55%), *Bithynia tentaculata* (43,22%, l'espèce dominante), *Physa acuta* (24,98%), Lymnaea truncatula (9,75%), *Gyraulus euphraticus* (8,18%) et *Lymnaea gedrosiana* (2,02%). La distribution géographique des escargots d'eau douce a été enregistrée dans cinq régions (22 points par région pour chaque saison). Les cartes de distribution spatiale ont montré que la distribution des escargots d'eau douce varie selon la région et la saison (P <0,001). Les résultats obtenus ont révélé les effets de la saison et de la région géographique sur la répartition et la densité des populations d'escargots dans la province. Ces données peuvent être utilisées pour la mise en œuvre de programmes de lutte contre les maladies parasitaires dans la région, y compris les trématodes.

Mots-clés: Identification; Escargot d'eau douce; Distribution géographique; Lorestan

INTRODUCTION

Although molluscs have many common characteristics, they can vary in shape and size. Snails belong to the Gastropoda with aquatic and terrestrial species. The most important snails belong to the Pulmonata subclass, which has a significant dispersal (Mansoorian and Rokni, 2004) and lives in different habitats (Magalhães et al., 2008). Freshwater snails play a major role in the life cycle of trematodes (Bargues et al., 2001). Therefore, continuous monitoring and malacological studies are very important in areas where there is a history of contamination transmissible to humans and livestock via snails (Mansoorian, 2000). The present study was designed to identify and obtain information on the geographical distribution of freshwater snails and examine variables, such as season and geographical region, in relation to snails' species and dispersal in Lorestan, Iran. Consequently, the present study sought to elucidate the demographic distribution of snails in each region and meet one of the basic requirements of defeating snails and raising public health.

MATERIAL AND METHODS

Study setting. The present study was set in Lorestan, with an area of 28,560 km^2, located in western Iran. The average rainfall, relative humidity, and temperature are 490 mm, 34-59%, and 17.4°C in this province, respectively. The province is predominantly mountainous with less plain areas. Lorestan has four-season weather with a variety of climates and areas that are suitable for the growth of snails. In this city, most of the drinking water, agriculture water, and water for general use are supplied from springs (i.e., headwaters), some of which have remarkable water flow and constitute suitable environments for the growth of snails (Mansoorian and Rokni, 2004). In this study, Lorestan was divided into five study regions, including north, south, east, west, and center. Twenty-two points were selected from each region for the collection of freshwater snails in every season. A total of 110 points were surveyed in five regions during each season in Lorestan.

Sample size, sampling method, and identification of freshwater snails. The sample size required for the study was determined with a frequency of 15% (according to pilot studies conducted in the province and an expert view on the actual dispersion of snails in the streams, springs, and rivers of the region), 95% confidence interval, and precision of 3%. The sample size was calculated using the following formula and assumptions:

$$n = \frac{(Z_{1-\alpha/2})^2 \times p(1-p)}{d^2} = \frac{(1.96)^2 \times 0.15 \times 0.85}{(0.03)^2} = 545$$

$Z_{0.95} = 1.96 \qquad P = 0.15 \qquad d = 0.2 \times 0.15 = 0.03$

Table 1. Abundance and species diversity of freshwater snails identified in Lorestan, Iran

Species	n	%	95% Confidence interval
Melanopsis doriae	277	6.30	5.6-7.1
Theodoxus doriae	244	5.55	4.9-6.3
Bithynia tentaculata	1902	43.22	41.7-44.7
Physa acuta	1099	24.98	23.7-26.3
Lymnaea truncatula	429	9.75	8.9-10.7
Gyraulus euphraticus	360	8.18	7.4-9
Lymnaea gedrosiana	89	2.02	1.6-2.5

Table 2. Distribution of freshwater snails in different seasons in different geographical regions of Lorestan, Iran

Season Species	Spring n (%)	Summer n (%)	Fall n (%)	Winter n (%)	Total n (%)
Melanopsis doriae	90 (8.18)	30 (2.73)	73 (6.64)	84 (7.64)	277 (6.30)
Theodoxux doriae	91 (8.27)	63 (5.73)	53 (4.82)	37 (3.36)	244 (5.55)
Bithynia tentaculata	392 (35.64)	330 (30.00)	405 (36.82)	775 (70.45)	1902 (43.22)
Physa acuta	137 (12.45)	613 (55.73)	247 (22.45)	102 (9.27)	1099 (24.98)
Lymnaea truncatula	219 (19.91)	7 (0.64)	203 (18.45)	0	429 (9.75)
Gyraulus euphraticus	129 (11.73)	51 (4.63)	89 (8.09)	91 (8.27)	360 (8.18)
Lymnaea gedrosiana	42 (3.82)	6 (0.54)	30 (2.73)	11 (1.00)	89 (2.02)
Total	1100 (100.00)	1100 (100.00)	1100 (100.00)	1100 (100.00)	4400 (100.00)

Because cluster sampling was used in a part of the study, the final number was multiplied by 2 as the cluster sampling impact factor in order to modify the sample. The sample size first reached 1090 in different geographical regions for each season, and the final sample size was obtained at 1100. The stations were located, and the snail samples were collected from Lorestan from April (2015) to February 2016. Multistage sampling was used, and Lorestan was divided into five regions based on geographical direction, namely north, south, east, west, and center (stratified sampling). Then, 22 points were randomly selected from the list of the points in each region (cluster sampling), and from each point, 10 snails were randomly chosen through hand touch (simple random sampling). Eventually, 1100 snails were collected every season. The sampling was conducted in the second month of each season (April 21 to May 21 in spring, July 23 to August 22 in summer, October 23 to November 21 in fall, and January 21 to February 19 in winter). The time and place of collecting the snails were recorded in this study. The snails were stored in flasks containing dechlorinated water. The rubbish and waste material collected with the snails were then separated, and the flasks were transferred to the Parasitology Laboratory of the Faculty of Veterinary Medicine at Shahid Chamran University of Ahvaz, Iran. If it was not possible to begin the laboratory procedures immediately, the snails' survival conditions were facilitated by adding dechlorinated water and lettuce (as food) to their storage space until the time for the study. In order to study the shell characteristics, live snails were boiled in water, and their visceral part (i.e., soft tissues) were then pulled out of their shell using bent forceps (Mansoorian, 2000). The snails were identified based on their shape, size, shell patterns, number of shell turns, navel, shell valve, right- or left-handedness of the shells, gastrointestinal tract (radula), and reproductive system using the Guide to the Identification of Iranian Freshwater Snails (Mansoorian, 2000).

Statistical analysis. The Chi-square test was used to verify the relationships between the variables under the study (i.e., season and geographical region) with snail species and dispersion. In addition, the data were analyzed in Stata software (version 14).

RESULTS

A total of 4400 live snails were collected and identified in four seasons based on the results of the

sampling of 110 stations located in the different regions of Lorestan. A total of seven species belonging to six genera from the six families of gastropods were identified in this study (figures 1a-p).

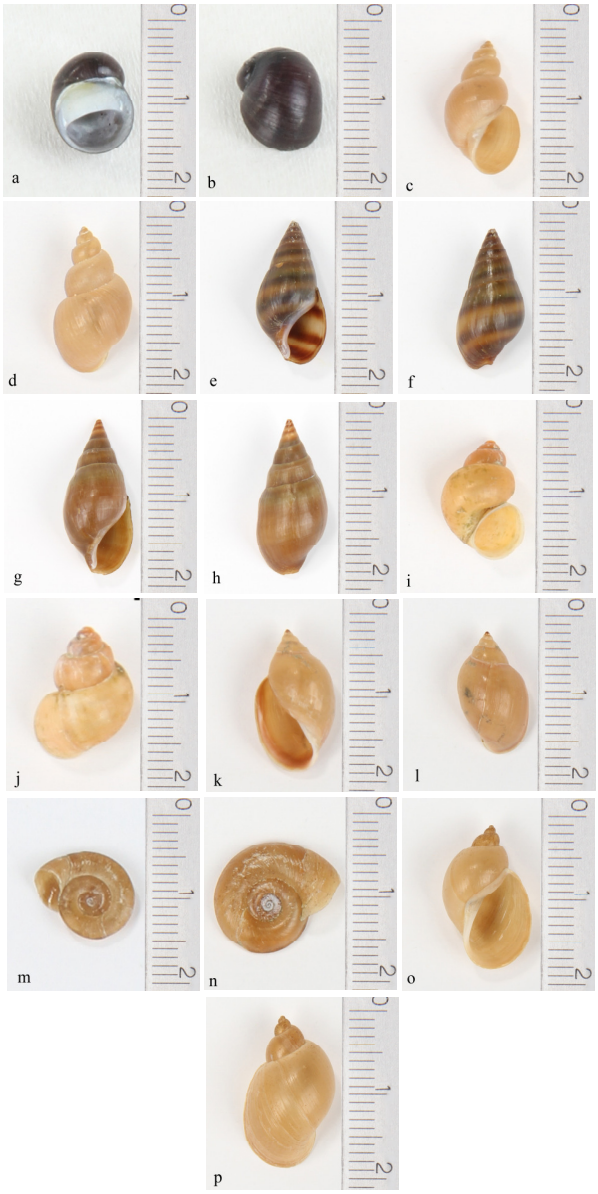

Figure 1. Shells of identified snails in Lorestan, Iran: (a) *Theodoxus doriae* (ventral view); (b) *T. doriae* (dorsal view); (c) *Lymnaea truncatula* (ventral view); (d) *L. truncatula* (dorsal view); (e) *Melanopsis doriae* (ventral view); (f) *M. doriae* (dorsal view); (g) adult *M. doriae* (ventral view); (h) adult *M. doriae* (dorsal view); (i) *Bithynia tentaculata* (ventral view); (j) *B. tentaculata* (dorsal view); (k) *Physa acuta* (ventral view); (l) *P. acuta* (dorsal view); (m) *Gyraulus euphraticus* (ventral view); (n) *G. euphraticus* (dorsal view); (o) *Lymnaea gedrosiana* (ventral view); (p) *L. gedrosiana* (dorsal view).

Species characteristics.

Theodoxus doriae: In this species, the shell is almost black, and the inner surface of the shell cap has a protrusion called the rip. The spout is semicircular, and the size of the snail is 4.4 × 4.8 × 5.8 mm.

Lymnaea truncatula: The maximum length of the shell is 10 mm. The shell is right-handed with a fairly broad vertex, 5 to 6 twists, deep sutures between the twists, clear and well-defined navel, protrusion on the columella, as well as a hatch half the shell height.

Melanopsis doriae: This species has a right-handed smooth shell and size of 21 × 9.7 mm, with a sharp vertex and dark transverse ribbons. The adult shell has 7 to 8 twists, with a horn-shaped cap and fairly dark-brown color.

Bithynia tentaculata: This conical globose shell is right-handed with dark brown background in the live specimens. The shell size is 10.5 × 6.2 mm with 5 to 6 twists, as well as a lime cap and spiral nucleus with concentric circles around it.

Physa acuta: The left-handed shiny shell of this snail lacks axial patterns. It has a sharp vertex, with a size of 15 × 9 mm. The transverse ribbon of the radula is v-shaped with multidentate denticles. In addition, a glandular swelling is observed on the surface of the preputium.

Gyraulus euphraticus: It is a fairly broad shell with no noticeable dents, a smooth surface, and no intersecting patterns. The shell diameter rarely reaches 7 mm, and its height is less than 1.5 mm. The number of twists ranges from 3.5 to a maximum of 4.5 (less than 5 twists) with a slight curvature in the last and biggest twist.

Lymnaea gedrosiana: The shell is 7 × 13.4 mm in size and right-handed, with a small spire (the length of the twists is shorter than the hatch height), sharp tip, 4 upper end forming an obtuse angle with the shell body.

Table 1 tabulates the species abundance and diversity of freshwater snails identified in the studied stations in spring, summer, fall, and winter. In the analysis of the data, *M. doriae*, *T. doriae*, B. *tentaculata*, *P. acuta*, *L. truncatula*, *G. euphraticus*, and *L. gedrosiana* showed

the abundances of 277 (6.30%), 244 (5.55%), 1902 (43.22%), 1099 (24.98%), 429 (9.75%), 360 (8.18%), and 89 (2.02%), respectively (Table 1). Table 2 shows the distribution of freshwater snails in different seasons among different geographical regions of Lorestan. If the geographical distribution of freshwater snails in Lorestan was determined in different regions according to season, the results as shown in Table 2 revealed that *M. doriae* was observed in both western and central regions of the province throughout the year, with the highest abundance noticed in the west and center of the region in spring (n=90; 8.18%). Similarly, *T. doriae* was noticed in the western and central regions of the province throughout the year, with the highest abundance observed in both regions in spring (n=91; 8.27%). *B. tentaculata* was detected year-round in all geographical regions of the province, with the highest abundance in winter (n=775; 70.45%).

P. acuta was noticed in all seasons in the west, east, center, and south of the regions. In the northern region, *P. acuta* was absent in winter and present in the other seasons, and the highest abundance was observed in summer (n=613; 55.73%) in all regions. *L. truncatula* was present in the central region in spring and fall, as well as in the eastern region in spring, summer, and fall, with the highest abundance in spring (n=219; 19.91%) in these two regions. *G. euphraticus* exhibited an all-season presence in the northern, central, and southern regions, with the highest abundance in spring (n=129; 11.73%) in all three regions. Finally, *L. gedrosiana* was observed in the central region in all seasons, as well as in the north of the province in spring and fall, with the highest abundance in spring (n=42; 3.82%) in both regions (Table 2). The relationship between season and geographical region with the distribution of freshwater snails using the Chi-square test showed that there were statistically significant differences between the distribution of the surveyed species of freshwater snails in Lorestan in different geographical regions according to season or in different seasons based on geographical region (P<0.001). In this study, it was demonstrated that *B. tentaculata* species was more dispersed than other species, and *L. gedrosiana* was reported with less dispersal. Figures 2, 3, 4, and 5 depicted the spatial distribution of the snails in this study.

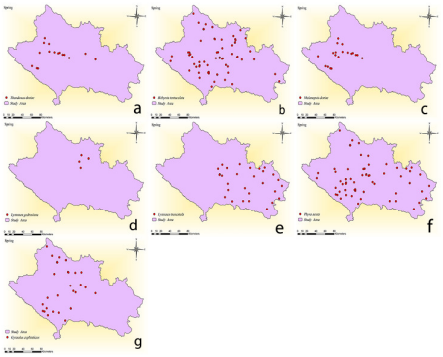

Figure 2. Distribution maps of freshwater snails in different geographical regions of Lorestan, Iran, in spring: (a) *Theodoxus doriae*; (b) *Bithynia tentaculata*; (c) *Melanopsis doriae*; (d) *Lymnaea gedrosiana*; (e) *Lymnaea trucatula*; (f) *Physa acuta*; (g) *Gyraulus euphraticus*.

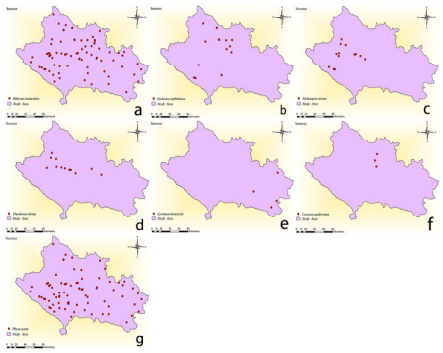

Figure 3. Distribution maps of freshwater snails in Lorestan, Iran, in different geographical regions in summer: (a) *Bithynia tentaculata*; (b) *Gyraulus euphraticus*; (c) *Melanopsis doriae*; (d) *Theodoxus doriae*; (e) *Lymnaea truncatula*; (f) *Lymnaea gedrosiana*; (g) *Physa acuta*.

DISCUSSION

There are reports of a variety of species of freshwater snails in most regions of Iran (Mansoorian, 2000). The role of Lymnaeid snails in the establishment of the transmission cycle of parasitic diseases is of interest to parasitologists (Brown, 1978). Other snails in the country may play a separate role in human or zoonotic diseases (Salahi-Moghaddam, 2010). *B. tentaculata* is

of more importance in the genus *Bithynia*, which is a global intermediate host of *Clonorchis* and *Opisthorchis* species (Salahi-Moghaddam, 2010).

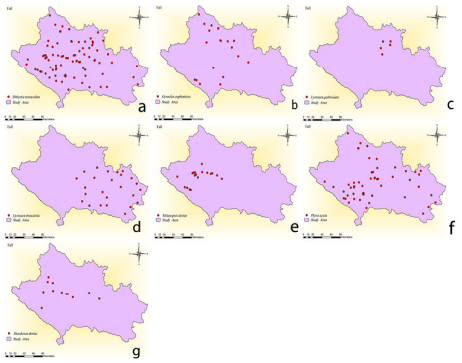

Figure 4. Distribution maps of freshwater snails in Lorestan, Iran, in different geographical regions in fall: (a) *Bithynia tentaculata*; (b) *Gyraulus euphraticus*; (c) *Lymnaea gedrosiana*; (d) *Lymnaea trucatula*; (e) *Melanopsis doriae*; (f) *Physa acuta*; (g) *Theodoxus doriae*.

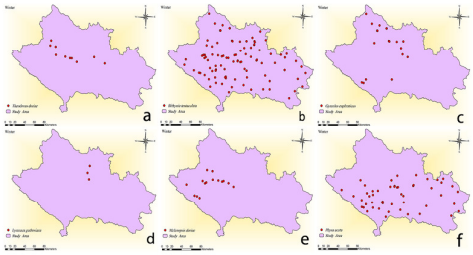

Figure 5. Distribution maps of freshwater snails in Lorestan, Iran, in different geographical regions in winter: (a) *Theodoxus doriae*; (b) *Bithynia tentaculata*; (c) *Gyraulus euphraticus*; (d) *Lymnaea gedrosiana*; (e) *Melanopsis doriae*; (f) *Physa acut*

There are reports on the contamination of *P. acuta* with trematode species in other parts of the world. However, the meta-analysis of the data revealed no evidence of such infections in Iran (Salahi-Moghaddam et al., 2004). In the present study, seven species of freshwater snails were identified in Lorestan. The distribution of freshwater snails varied in different regions of the province in different seasons. The studied variables, including the season and geographical region, had a significant impact on the demographic distribution of the snails. In addition, the total abundance of *B. tentaculata* rendered this species a dominant population in different regions and seasons of Lorestan. A review of the literature showed several studies on the identification and determination of the distribution of freshwater snails around the world, which will be discussed in this section. In a study conducted in Msambweni, a coastal province in Kenya, 67% and 25% of the collected snails were *Bulinus nasutus* and *Lanistes purpureus*, respectively; however, *Bulinus forskalii* and *Melanoides tuberculata* showed lower abundances. In the aforementioned study, rainfall was significantly associated with the temporal distribution of all the species; nevertheless, *B. nasutus* was observed in a large number after heavy rainfall, followed by *Schistosoma haematobium* (Kariuki et al., 2004). In another study on the distribution of freshwater snails performed by Ofoezie from the Oyan, Nigeria, in 1999, the distribution of snails was also affected by rainfall, and in this study, the species *Indoplanorbis exustus* was most abundant among the species (Ofoezie, 1999). The results of the present study are not in line with the findings of Ofoezie (1999) and Kariuki et al. (2004) in relation to the reported snails. In the above-mentioned studies, the increase in the number of *B. nasutus* and *I. exustus* snails was observed after a heavy rainfall; however, in the present study, an increase in the number of all species was observed in spring except *P. acuta*, which increased in summer, and the species *B. tentaculata*, which increased in winter. This can be for all species except *P. acuta* snail due to rain in spring and winter. A decrease in the amount of precipitation leads to the decrease of snails' activity periods and is considered a leading factor in the reduction of the population of most animal species at present and in the future (Gerlach, 2007). This result disagrees with the findings of the present study only in relation to *P. acuta*. The dense number of snails in spring may be due to favorable temperatures and ample vegetation in this season (El-Kady et al., 2000). However, in a study conducted on

two rivers in Nigeria, *M. tuberculata* snail was the dominant species, and it was also shown that snail density decreased in the rainy season. Because in the dry season (i.e., summer), the flow of water is expected to be low and stable, the snail density is higher (Omonijo et al., 2016). This is in contrast with the findings of previous studies, except in the case of *P. acuta* snail that could be due to the unstable water flow in the rivers. In a study in two regions in Cuba, *Pseudosuccinea columella* species were most abundant among other species in both regions in April and May 2004 (Gutiérrez et al., 2005). The results of a study conducted by Gutiérrez are not consistent with the findings of the present study in terms of the reported species; however, they are in line with the observation that all the species were more abundant in April-May except for *P. acuta* and *B. tentaculata*. A study of Lake Uluabat in the northwest of Turkey reported a new species of *Bithynia* called *Bithynia timmii*; nevertheless, *B. tentaculata* was also reported in the aforementioned study (Odabaşı and Arslan, 2015). In the aforementioned study and another study by Yıldırım (1999), it was observed that *Bethyina tentaculata* had a large dispersal in Turkey, which is consistent with the present findings in relation to *B. tentaculata*. Other studies also presented evidence on the varying distribution of freshwater snails depending on water quality factors, such as pH, dissolved oxygen, and temperature (Żbikowska et al., 2006). In previous years, there have been numerous studies on the identification and examination of the dispersal of freshwater snails in Iran, some of which will be discussed in this section. A collection of freshwater snails' fauna in Iran has been gathered based on the information available at the Basel Museum of Natural History, Berlin Zoology Museum, and Vienna Natural History Museum. The collection includes 73 species of 34 genera and 14 families, including 27 (37%) species native to Iran (Glöer and Pešić, 2012). The known species may only be part of the total population of freshwater snails in Iran (Glöer and Pešić, 2009). The following species were identified and reported in different regions of Iran: *L. gedrosiana*, *Lymnaea stagnalis*, *Lymnaea auricularia*, *L. truncatula*, and *L. palustris* in West Azarbaijan (Imani-Baran et al., 2013), *L. gedrosiana*, *L. truncatula*, and L. palustris in Mazandaran (Moghaddam et al., 2004), *L. gedrosiana*, *L. palustris*, and *L. truncatula* in Gilan (Ashrafi, 2015). The species observed in the present study are similar to those reported in West Azarbaijan, Mazandaran, and Gilan in relation to *L. gedrosiana* and *L. truncatula* from the Lymnaeid family. Therefore, as noted in a study by Mansoorian (2000), the two species, namely *Lymnaea gedrosiana* and *Lymnaea truncatula*, have the highest dispersion throughout Iran. In a study conducted in West Azarbaijan, Iran, spring and fall were noticed to offer favorable temperatures for the activity of Lymnaeid snails, except for *L. palustris*, which was not present in fall (Imani-Baran et al., 2013). Another study carried out in West Azarbaijan showed that season and certain chemical factors affect the distribution of Lymnaeid snails in aquatic habitats and demonstrated the highest abundance of this family of snails in spring and fall (Imani-Baran et al., 2015). The present study showed that L. *truncatula* was mainly observed in spring, summer, and fall and was most abundant in spring. Similarly, *L. gedrosiana* was most abundant in spring; nevertheless, it had a year-round presence. Therefore, the results of the present study in Lorestan are in line with the findings of two studies conducted in West Azarbaijan, as the two species were observed to have the highest abundance in spring and fall. Furthermore, the temperature has a proven direct effect on vital parameters (i.e., growth, maturity, and fertility) in freshwater pulmonates, including Lymnaeid snails, due to the increased metabolic processes (Brown, 1978; Sharif et al., 2010). Other studies have also been carried out in Lorestan, which will be discussed in this section. In a comprehensive study of Iranian freshwater snails, *L. stagnalis*, *L. auricularia*, *L. truncatula*, *P. acuta*, *T. doriae*, *M. doriae*, *B. tentaculata*, *G. euphraticus*, and

Planorbis intermixtus were reported in Lorestan (Mansoorian, 2000). The present study observed *L. gedrosiana* from the Lymnaeid family, while, *L. stagnalis*, *L. auricularia*, and *P. intermixtus* were absent from the records. However, they were previously reported in Lorestan. This disparity could be due to the drying up of the water resources most suitable for the growth of these snails as a result of sequential droughts and human interventions in the habitats of snails (Imani-Baran et al., 2015). This part of the present findings is consistent with the results obtained by Imani-Baran et al. (2015); nevertheless, the rest of the findings are only consistent with the results reported by Mansoorian (2000). Freshwater snails' fauna in Iran were also studied at five locations in Lorestan and two species were reported, namely *Pseudobithynia irana* and *P. acuta* (Glöer and Pešić, 2012). However, the present study observed seven species and only corresponds to the aforementioned study in terms of the presence of *P. acuta*. A study in Gahar Lake in Lorestan investigated ten locations and identified *L. truncatula*, *P. acuta*, *P. intermixtus*, *Lymnaea peregra*, *M. tuberculata* species, and *Melanopsis genus* and reported *M. tuberculata* as the most frequent one (Valipour Nouroozi, 2014). The present study conforms to the aforementioned study on reporting *P. acuta*, *L. truncatula,* and *Melanopsis genus*. Moreover, Karimi et al. (2016) introduced *L. gedrosiana* as the dominant Lymnaeid species in Lorestan; however, *B. tentaculata* was the dominant species in the present study, and *L. gedrosiana* showed the lowest abundance. The distribution of freshwater snails is believed, as noted by Kariuki et al. (2004), to vary in specific habitats over a given period depending on prevailing, biological, biochemical, and physical conditions.

The results of the present study and access to information on the geographical distribution of snails can help prevent probable parasitic diseases. Due to the different biochemical properties of water in different aquatic resources, it is necessary to investigate the effect of the biochemical properties of water, including temperature, pH, electrical conductivity, and salinity, on the distribution of freshwater snails in Lorestan.

Ethics

The observation of ethical issues in this study was confirmed at the Graduate Council, which plays the role of the Ethics Committee, in the Faculty of Veterinary Medicine at Shahid Bahonar University of Kerman.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Drafting of the manuscript: Jahangiri Nasr

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