



# Species-Level Taxonomy and Diversity of Freshwater and Terrestrial Snails, and First Report of *Pseudonapaeus pretiosus* and *Succinea putris* in Azad Jammu and Kashmir, Pakistan, and Evaluation of their Role as Hosts of Trematode-Borne Infections

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## Article Information

Received 02 February 2024

Revised 25 February 2024

Accepted 07 March 2024

Available online 12 August 2024 (early access)

## Authors' Contribution

HA, JC, and SS designed the study. SR conducted the field research activities. SR performed the morphological and statistical analyses. SR and CMW identified the species. SR and FC wrote the manuscript. HA, JC, CMW, HL and SS revised the manuscript. All the authors have approved the final version of the manuscript.

## Key words

Molluscs, Morphometric measurements, Malacofauna

## ABSTRACT

Snails and slugs that belong to the class Gastropoda are also of medical importance because they act as intermediate hosts to trematode parasites. However, few taxonomic studies on snail species in Pakistan have been published to date. This study was designed to identify freshwater and terrestrial gastropods (snails and slugs) that inhabit moist areas or water bodies in three districts (Kotli, Poonch, and Bhimber) of Azad Jammu and Kashmir (AJK), Pakistan. A total of 750 snail samples were collected from agricultural lands and water bodies in the study area. All the sampling areas were wetlands with maximum rainfall and nearby freshwater resources where animals graze and drink water. The snails were tested for trematode infection by using the cercarial emergence test. The emerged cercariae and snails were identified on the basis of their morphology. Ten snail species belonging to seven families were identified. Two of these snail species, *Pseudonapaeus pretiosus* and *Succinea putris*, were reported for the first time in Pakistan. This study provides baseline information and a road map to analyze malacofauna in different regions of Pakistan.

## INTRODUCTION

Slugs and snails are members of the class Gastropoda. Gastropods that have a shell into which they can withdraw their bodies are called snails, and gastropods without a shell are called slugs. The loss of the shell was an evolutionary change that provided slugs with the advantage of being better at maneuvering and squeezing themselves through small crevices or into small spaces such as under rocks and logs (Allmon and Hendricks, 2021). Gastropods

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0030-9923/2024/0001-0001 \$ 9.00/0



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perform various ecological services within an ecosystem. Snails and slugs that inhabit terrestrial habitats constantly recycle nutrients found in the soil and spread seeds or spores imbedded in the mucus covering their body or through waste products such as feces (Abobakr *et al.*, 2022). In addition, snails are used to biologically control harmful plants such as weeds (Barker, 2002). In many parts of the world, snails are consumed as a protein source (Tanyitiku *et al.*, 2022).

Despite being important to the ecosystem, many species of snails or slugs are still detrimental to agriculture and are considered as pests (Gomes *et al.*, 2016). In Azad Jammu and Kashmir (AJK), Pakistan, a few species of snails have been reported to damage crops and ornamental plants (Faiz, 2020). These invertebrates can also serve as intermediate hosts for various parasites. Freshwater gastropods (snails and slugs) are important because they are bio-indicators and intermediate hosts for many trematode-borne infections (Gondal *et al.*, 2020). Trematode parasites share an intimate relationship with their intermediate gastropod hosts and act as a vehicle for their development and transmission (Lockyer *et al.*, 2004).

Generally, freshwater snails can be intermediate hosts to different types of parasites, especially some zoonotic trematodes such as *Schistosoma* spp. or *Fasciola* spp. (Appleton, 1996). Digenean trematodes have a complicated life cycle and use intermediate hosts such as freshwater snails, which are widespread in water sources of most geographical regions, to complete their life cycle; the larval stages, i.e., sporocyst, redia, and cercaria, develop within snails and are excreted to reach their definitive hosts (Skála *et al.*, 2014). Although researchers have studied the prevalence and ecology of freshwater snails (Arshad *et al.*, 2011; Kakar *et al.*, 2017; Gondal *et al.*, 2020) in different regions of Pakistan, very few studies on snail-trematode (larvae) interactions have been conducted. In 2013, a study was conducted in which snails were collected and analyzed for *Schistosoma* cercariae and the correlation between their occurrence and meteorological factors (Niaz *et al.*, 2013), whereas, in 2015, a study was performed to monitor *Fasciola* infection (Qureshi *et al.*, 2015).

Identification of snails is of value to zoologists, malacologists, and conchologists because of their role as intermediate hosts for the larvae of a variety of trematode parasites. Burch (1982) stated that a large number of generic groups have been classified based on morphological or shell characteristics; however, numerous intervening forms were observed with time. Generally, different species of snails are classified using single, partial, or even juvenile shells; however, complete reliance on shell morphology is confusing when establishing the taxonomy of certain species. Although taxonomy is of extreme importance,

further identification needs to be performed at a molecular level (Aksenova *et al.*, 2018).

Poonch, Bhimber, and Kotli of AJK in Pakistan have different climatic conditions, but precipitation occurs throughout the year. Poonch is one of the coldest regions, where it is cold most of the year; it even has snowy areas. In contrast, Bhimber is one of the hottest regions in the Kashmir Valley. Kotli is moderately hot in summer and moderately cold in winter. Kotli and Poonch are mountainous zones with forests at the top and fast-flowing rivers and winding streams in the valleys, whereas Bhimber districts lay in the foothills (Ali, 2015).

This study comprises a survey of snail fauna, with emphasis on the intermediate hosts of trematode parasites, from different habitats in three districts, Poonch, Bhimber, and Kotli, of AJK in Pakistan. After sampling, the snails were screened for the presence of cercariae; later, taxonomic identification was performed using morphometric measurements of the shell. Each ecosystem has a certain population of snails and slugs, and their diversity and abundance are greatly influenced by a series of climatic factors, such as pollution or the presence of a favorable habitat.

## MATERIALS AND METHODS

### *Study area*

The state of AJK, Pakistan, has numerous livestock, with 1.43 million cattle and buffaloes as well as 1.77 million sheep and goats. A total of 67% of the population are dependent on raising livestock and poultry (GOP, 2021-22). Snails were collected from sites such as natural lakes, rivulets, irrigation canals, agricultural plots, mosses, ravines, stream banks, ponds, and water drainage areas from the three regions (Kotli, Poonch, and Bhimber) of AJK, Pakistan. The snail and slug samples were collected between January 2021 and February 2022. The districts Kotli, Poonch, and Bhimber are located at 33.5008° N latitude and 73.9007° E longitude, 33.8369° N latitude and 73.8889° E longitude, and 32.9753° N latitude 74.0858° E longitude, respectively.

### *Sample collection*

A total of 750 snail samples were collected from the three selected areas. The sampling sites were visited 2-3 times per month during the rainy wet season (July–September) and visited once a month during the cool dry season (October–February). The specimens were collected from wetlands with maximum rainfall and nearby freshwater sources where animals graze and drink water. The snails were individually handpicked and placed in plastic containers. They were strained and repeatedly

dipped in tap water to remove the debris and mud adhering to the surface of the snails and then cleaned in a petri dish by placing them between two layers of moist cotton. This process was followed by an examination under a binocular stereo microscope (ER.59-1990; Wild Heerbrugg) or a magnifying glass.

For associations between species distribution in different seasons, principal component analysis (PCA) was performed using FactoMineR and factoextra packages of R 4.0.5 (R Development Core Team, 2021).

#### *Cercarial emergence test*

The snails were screened for infection by using a standard cercarial emergence test, with cercarial shedding stimulated by exposure of individual snails to artificial light. *Physella acuta*, *Bensonies monticola*, and *Euaustenia monticola* were tested for the presence of cercariae; we tested these species because they were prevalent at hotspot/contaminated areas where animals graze and drink water most of the time. The snails were acclimatized for 24 h before exposure. Then, the snails were placed in distilled water (sometimes in the form of a group of 5–15 and sometimes individually) and exposed to light in an illuminated cabin. The snails were exposed to light for 6 h for each light exposure with multiple exposures (3–5) to ensure cercarial shedding (Frandsen and Christensen, 1984; Thompson, 2004). These repeated light exposures stimulated the emergence of cercaria and metacercaria. Usually, all snail batches were exposed to adequate sunlight for 3–4 h in summer; however, in the absence of sunlight during rains, they were exposed to artificial light. Liquid samples of each snail set-up were examined under a binocular microscope to confirm infection by checking for cercariae (Imani-Baran *et al.*, 2013). The infection status of the snails was recorded according to the shedding of cercariae.

#### *Processing of snails*

The snails were carefully dissected, and the soft tissues were separated from the shell using a sterile surgical blade. Before the soft tissues were removed, photographic images of the snails were taken for preliminary identification. The shell was conserved using a cleaning process that involved placing the shell in a dilute solution of oxalic acid for a few minutes and washing in water while brushing to reveal the shell sculpture. The dry shells were used to obtain all the morphometric measurements. The snail tissues were preserved in ethanol for further analyses (Martello *et al.*, 2008).

#### *Taxonomic identification of snail species*

The snail specimens were identified on the basis of

morphometric data measurements such as shell height, width, or diameter and whorls. Conchological features such as shell shape, texture, height, width, number of whorls, shape and size of the aperture, coiling of the shell, i.e., clockwise (dextral) or anticlockwise (sinistral), presence or absence of columella, operculum, and types of umbilicus (open/close) were examined for snail identification. All morphometric measurements of the shells were obtained using a digital vernier caliper and expressed in millimeters (mm). The biological samples were examined using a magnifying glass and identified with the help of a diagram and identification keys provided by Burch (1982). For the identification of freshwater snails, a protocol reported by Burdi *et al.* (2008) was used. The soft tissues of the specimens were preserved for future studies. Because of the lack of proper guidelines or keys specifically for Pakistan and very little research being undertaken in the field of malacology in Pakistan, the preliminary morphological identification based on the shell was performed using the standardized taxonomic keys designed by (Blandford and Godwin, 1908; Burch, 1980; Bouchet *et al.*, 2005; Anderson, 2005).

## RESULTS

#### *Identification of snail species*

Most of the snails were collected in a period spanning from July to September, which corresponds to the wet season; the snails were more abundant in the wet season than in the dry season (Table I).

For associations between different species distributions, PCA was performed (Horsák *et al.*, 2007), which showed higher association in wet season than in dry season. PCA Dim 1 accounted for 65.6% of the variations in the dataset and demonstrated strong loadings of *Bensonies monticola*, *Indoplanorbis exustus*, *Lymnaea natalensis*, *Milax gagates*, *Physa acuta*, and *S. putris* showing close association with the distribution pattern of the species. Dim 2 accounted for 31.3% of the variations and displayed strong loadings of *Gyraulus convexiusculus*, *Pseudonapaeus pretiosus*, and *Euaustenia monticola* showing close association with the dry and wet seasons (Fig. 1). The snail species were arranged along the first axis in the PCA diagram following the season and distribution gradient. All the species showed differential impact towards the seasons and distribution. If we correlate both season and distribution pattern, most species are dependent on distribution pattern when compared with season.

Overall, 10 species of freshwater and land snails were identified and described. The morphometric measurements of discoidal and conical shells of these species were recorded.

**Table I.** Checklist of species that belong to the class Gastropoda in agro-ecosystems of three districts in AJK collected from different sites during the wet (July–September) and dry (December–February) seasons.

Family	Genus	Species (No. of specimens)	Distribution in the sites (1-10)*	Wet season (July–September)	Dry season (December–February)
Physidae	<i>Physella</i>	<i>Physella acuta</i> (n = 331)	4, 5, 6, 7	300	31
Planorbidae	<i>Gyraulus</i>	<i>Gyraulus convexiusculus</i> (n = 190)	1, 2, 3	170	20
	<i>Indoplanorbis</i>	<i>Indoplanorbis exustus</i> (n = 35)	8, 9	35	-
Enidae	<i>Pseudonapaeus</i>	<i>Pseudonapaeus candelaris</i> (n = 42)	7	42	-
		<i>Pseudonapaeus pretiosus</i> (n = 10)	6, 7	10	-
Ariophantidae	<i>Euaustenia</i>	<i>Euaustenia monticola</i> (n = 38)	1, 2, 3 and 5	23	15
	<i>Bensonies</i>	<i>Bensonies monticola</i> (n = 4)	3, 6 and 7	4	-
Lymnaeidae	<i>Lymnaea</i>	<i>Lymnaea natalensis</i> (n = 70)	5,6,7 and 10	61	9
Succineidae	<i>Succinea</i>	<i>Succinea putris</i> (n = 28)	7	28	-
Milacidae (Slug)	<i>Milax</i>	<i>Milax gagates</i> (n = 2)	7	2	-

Regional sites\*: 1, Saidpur; 2, Killa Karjai; 3, Bhroatgala (Kotli); 4, Darek Dam; 5, Banjonsa Lake; 6, Matyalmera; 7, Hajeera; (Poonch); 8, Fakroat; 9, Morha Nagyal; 10, Mohra Molbian (Bhimber).

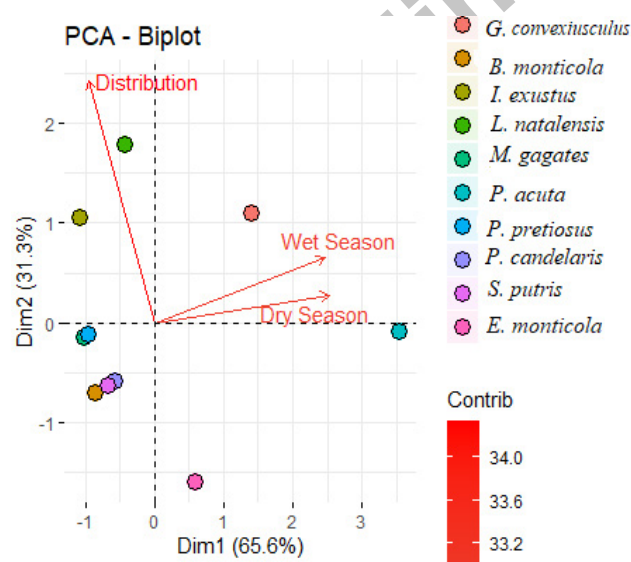


Fig. 1. PCA biplot showing association between species distribution and wet and dry seasons.

#### Family: Physidae

##### *Physella acuta* (Draparnaud, 1805) (Fig. 2A, B)

*Physella acuta* (*Physa acuta*), a freshwater snail has a conically shaped shell of medium thickness and pale horn to dark brown color. The number of whorls is 3-4, and the umbilicus is absent most of the time (if present, imperforated). The shell is slightly shiny and transparent. The shell shape is oval with a peaky apex. The symmetry is sinistral with a left-handed aperture. The aperture is large and comprises 3/4<sup>th</sup> of the shell height, while being narrow at the top side.

#### Family: Planorbidae

##### *Gyraulus convexiusculus* (Hutton, 1849) (Fig. 2C, D)

*Gyraulus convexiusculus* is a land snail found in moist places. The shell is discoid, with 4-5 whorls. The whorls are convex with a well-developed suture, and the last whorl is expanded with rounded margins that do not influence the aperture. The aperture is convex and lunate in shape. This species has a broad and perforated umbilicus. The shell has a pale horn color, non-operculate glossy surface with oblique striation, and dextral coiling.





Fig. 2. *Physella acuta*: ventral view (A); dorsal view (B); *Gyraulus convexiusculus*: ventral view (C); dorsal view (D); *Indoplanorbis exustus*: ventral view (E); dorsal view (F); *Pseudonapaeus candelaris*: ventral view (G); dorsal view (H); *Pseudonapaeus pretiosus*: ventral view (I); dorsal view (J); *Euaustenia monticola*: ventral view (K); dorsal view (L); *Bensonies monticola*: ventral view (M); dorsal view (N); *Lymnaea natalensis*: ventral view (O); dorsal view (P); *Succinea putris*: ventral view (Q); dorsal view (R).

***Indoplanorbis exustus* (Deshayes, 1834)**  
(Fig. 2E, F)

*Indoplanorbis exustus*, another member of the family Planorbidae, is an air-breathing freshwater snail; it also has a discoid shell with four whorls and a wide perforated umbilicus. The shell color is greenish brown, and it is ribbed finely with sinistral coiling. The body whorls are large and flattened concavely on both sides, with a broad concave ear-shaped aperture. The shell surface is non-operculate with thin margins and spiral striation.

**Family: Enidae**

***Pseudonapaeus candelaris* (Pfeiffer, 1834)**  
(Fig. 2G, H)

*Pseudonapaeus candelaris*, a land snail, has an elongated conical shell whitish to light brown in color. The number of whorls is 7. The whorls are round convex and gradually increase in size downwards; the last whorl is a little descending, i.e., it is extended downwards, and a small oval aperture is present at the left side of the shell. The shell has vertically curved ribs and fine spiral striations. The shell is fairly large in height and sinistral (dextral forms occur rarely).

***Pseudonapaeus pretiosus* (Reeve, 1849)**  
(Fig. 2I, J)

*Pseudonapaeus pretiosus*, an endemic land snail species observed by Theobald (1878) has a very limited

range of distribution, and is abundant in the sampling regions of Kashmir. The shell differs from those of other species of its genus, being less ventricose and more elongated with fine striations and thin and reflected peristome. The number of whorls is 6-7, and the shell is convex and muddy brown in color. The shell is dextral (right-handed). This land snail also belongs to the family Enidae.

**Family: Ariophantidae**

***Euaustenia monticola* (Pfeiffer, 1848)**  
(Fig. 2K, L)

*Euaustenia monticola* is a medium-small terrestrial semi-slug (terrestrial pulmonate) with a globular glass shell. It is pale grey with a darker head and tentacles which are larger than the shell. The shell comprises 3-4 large body whorls with a small depressed spire and a large aperture. The umbilicus is closed, and the aperture is convex. The shell is very fragile and transparent yellowish to green in color.

***Bensonies monticola* (Benson, 1838)**  
(Fig. 2M, N)

*Bensonies monticola*, also known as *Macrochlamys monticola*, is a brown terrestrial garden snail. The shell is globular with a reflected lip. The umbilicus is closed, and the aperture presents a convex curvature. The shell has a light brown to yellowish shade. The shell has a series of dark brown spiral bands that can vary in number and are often interrupted by spots or streaks of a lighter color and radial in appearance. It has 4-5 whorls. The animal is medium brown or even gray in color.

**Family: Lymnaeidae**

***Lymnaea natalensis* (Krauss, 1848)**  
(Fig. 2O, P)

*Lymnaea natalensis*, a typical freshwater snail, has a thin conical shell light brownish in color. The shell is very delicate and somewhat squarely ovate. The number of whorls is four; the body whorl abruptly enlarges downwards, and the spire is cone-shaped. The umbilicus is absent or imperforate. The aperture is very broad and ear-shaped. The shell is sculptured with spiral striations and sharply pointed apex, dextral in coiling.

**Family: Succineidae**

***Succinea putris* (Linnaeus, 1758)**  
(Fig. 2Q, R)

*Succinea putris* is an amber snail that inhabits freshwaters, and it has a shell that can vary in color from a yellow-brown to reddish shade. The shell body also has a significant whorl accompanied by a small spire. The total

number of whorls is 3. The whorls are relatively flat with shallow sutures. The umbilicus is absent, and the aperture exceeds 50% of the total shell height. This shell usually is different shades of grey and is, in exceptional cases, blackish. When the snail reaches adulthood, the body becomes considerably larger than those of other amber snails.

#### Family: Milacidae

##### *Milax gagates* (Draparnaud, 1801)

*Milax gagates*, a medium-sized bluish-grey to brown slug, is also known as the green house slug; it is a species of air-breathing, keeled land slug. It is a shell-less terrestrial gastropod of the family Milacidae. The keel runs from the mantle to the tail, with no lateral respiratory orifice. The sole is whitish in color, and the mucus is colorless. The size ranges from 45 to 55 mm.

All species are listed in Table I. To our knowledge, two of the snail species, *P. pretiosus* and *S. putris*, have not yet been reported in Pakistan. The specimens were identified on the basis of their morphometric measurements. Comparative morphometric analysis was performed. The mean height of the shell is considered as a valuable parameter, along with mean shell width or diameter. These values can be used to calculate the height over diameter (H/D) ratio, which is helpful for the classification of snails. Mean aperture size is another major characteristic for the classification of snails. Body weight, number of whorls, umbilicus, symmetry, and shape of the shell are other features needed for classification. All these morphometric

measurements and morphological characteristics of the identified species are shown in Table II.

#### Comparative morphometrics of shells

The shell size of *Bensonies monticola* was quite large (shell height and width, 30.25 mm and 26 mm, respectively), followed by those of *Euaustenia monticola* (14.86 mm and 20.6 mm, respectively), *G. convexiusculus* (14.22 mm and 11.45 mm, respectively), and *I. exustus* (11.40 mm and 9.17 mm, respectively). The conical shell of *P. candelaris* had a maximum height of 20.54 mm and width of 7.76 mm, followed by *L. natalensis* with a shell height of 15.32 mm and width of 7.39 mm. All other conical shells were smaller; the conical snail shells had greater H/D ratios than the discoid or globular shells. Aperture is another characteristic for identification; the symmetry and size of the aperture are noted. The maximum aperture size was found in *Bensonies monticola* and *Euaustenia monticola* (18.5 mm and 12.6 mm, respectively), and the minimum aperture size was found in *G. convexiusculus* (5.2 mm) in the case of discoid shells. In contrast, the aperture size of all conical shells was quite small, except for *L. natalensis* (4.75 mm). All these species had a convex aperture, excluding *I. exustus* (which had a concave aperture). The umbilicus was absent in all conical shells and present in all discoid shells, but only *I. exustus* had an open umbilicus. A few *Bensonies monticola* and *G. convexiusculus* shells also had an open umbilicus.

**Table II. Characterization of the snails on the basis of morphometry.**

Species	Body weight (Mean $\pm$ SD)	Shell length or height (H) mm (Mean $\pm$ SD)	Shell width or diameter (D)mm (Mean $\pm$ SD)	H/D	Aperture mm (Mean $\pm$ SD)	No. of whorls	Shell coiling	Umbilicus	Shape	Color
<i>Physella acuta</i>	0.33 $\pm$ 0.13	11.12 $\pm$ 2.83	7.46 $\pm$ 1.35	1.50	4.21 $\pm$ 1.16	3-4	Sinistral	Absent	Conical with convex aperture	Pale horn color to dark brown
<i>Gyraulus convexiusculus</i>	1.70 $\pm$ 0.83	23.93 $\pm$ 6.1	22.88 $\pm$ 6.3	1.04	17.5 $\pm$ 7.1	4-5	Dextral	Present	Discoid with convex aperture	Light brown
<i>Indoplanorbis exustus</i>	0.26 $\pm$ 2.13	11.44 $\pm$ 2.04	9.17 $\pm$ 2.13	1.24	7.17 $\pm$ 1.93	4	Sinistral	Present	Discoid with concave aperture	Greenish brown
<i>Pseudonapaesus candelaris</i>	0.76 $\pm$ 0.15	20.54 $\pm$ 2.97	7.76 $\pm$ 0.82	2.64	4.19 $\pm$ 0.55	7	Sinistral	Absent	Spiral with convex aperture	Whitish to Light brown
<i>Pseudonapaesus pretiosus</i>	0.16 $\pm$ 0.04	11.1 $\pm$ 2.02	5.2 $\pm$ 0.42	2.13	3.15 $\pm$ 0.47	6-7	Dextral	Absent	Spiral with convex aperture	Mudd brown
<i>Euaustenia monticola</i>	4.31 $\pm$ 2.81	11.45 $\pm$ 2.01	20.7 $\pm$ 3.96	0.55	12.65 $\pm$ 2.81	3-4	Dextral	Present	Globular with convex aperture	Yellowish or greenish
<i>Bensonies monticola</i>	6.12 $\pm$ 0.22	30.25 $\pm$ 1.25	26 $\pm$ 1.25	1.16	18.5 $\pm$ 1.29	4-5	Dextral	Present	Discoid with convex aperture	Medium Brown
<i>Lymnaea natalensis</i>	0.27 $\pm$ 0.12	15.32 $\pm$ 4.29	7.39 $\pm$ 1.66	2.07	4.75 $\pm$ 1.56	4	Dextral	Absent	Conical with convex aperture	Light to dark brownish

### Presence of cercariae

Three snail species, *Physella acuta*, *Bensonies monticola*, and *Euaustenia monticola*, were screened for cercarial shedding. All three species were observed to shed cercariae, which indicated the presence of trematode infection. Cercariae secreted from each snail were pipetted individually and examined under a binocular microscope and placed in 70% ethanol. The trematode species remain unidentified, as cercariae emerging from infected snails cannot be identified precisely to the species level on the basis of morphology alone.

## DISCUSSION

A total of 8 families, 9 genera, and 10 snail species were found in the sampling areas: *Physella acuta* belonging to the family Physidae, *Gyraulus convexiusculus* and *Indoplanorbis exustus* belonging to the family Planorbidae, *Pseudonapaeus candellaris* and *Pseudonapaeus pretiosus* belonging to the family Enidae, *Euaustenia monticola* and *Bensonies monticola* belonging to the family Ariophantidae, *Lymnaea natalensis* belonging to the family Lymnaeidae, *Succinea putris* belonging to the family Succineidae, and *Milax gagates* belonging to the family Milacidae.

All these snails are important because of their great diversity and abundance in moist habitats, such as humid land, freshwater reservoirs, banks of rivers, shallow muddy bottoms of streams, and moist shady vegetation. Their ability to survive is a direct consequence of environments able to produce or provide considerable amounts of nutritive compounds while retaining rainwater and creating optimal proliferation conditions. In Pakistan specifically in the region of AJK, no previous studies have been conducted on the identification of snails.

Species belonging to *Physella* or *Lymnaea* were predominant close to the freshwater reservoirs during the dry and wet seasons in Poonch. Species belonging to *Physa* or *Lymnaea* were predominant during the rainy summer season close to the freshwater reservoirs and more prevalent in Poonch. *Bensonies monticola* is a common garden snail found in both dry and wet seasons. Air-breathing, pulmonate snails of the family Planorbidae (*G. convexiusculus* or *I. exustus*) were found in areas where they could crawl onto water plants or imbed themselves into soil covered by shallow waters. *Pseudonapaeus* snail species were abundant in the dry season and on dry vegetative grounds, where they were attached to the vegetation or climbed onto the walls. To the best of our knowledge this is the first report of *Pseudonapaeus pretiosus*, *Pseudonapaeus candellaris*, and *Succinea putris* from Pakistan. *Pseudonapaeus* snails are endemic

to Asia. This could be one of the reasons for the lack of data on these species in the literature. *Succinea putris* is also present in freshwater reservoirs, and it is very similar to another Succineidae member *Oxyloma elegans* (Risso, 1826). The shell of *O. elegans* is slender and longer, and it is darker than the shell of *S. putris* (which is greenish or amber). Slugs are also very important. *Milax gagates* is a field slug found alongside freshwater reservoirs and lush green fields in both dry and wet seasons.

Afshan *et al.* (2013) identified *Gyraulus convexiusculus*, *Indoplanorbis exustus*, and *Physella acuta* from Pothwar region of Pakistan. *Indoplanorbis exustus* and *Gyraulus convexiusculus* belonging to the family Planorbidae were also reported from Margalla Foothills, Pakistan (Arshad *et al.*, 2011). Recently, in Swat District of Khyber Pakhtunkhwa (KP) Province, situated in the northern part of Pakistan, three families, Bradybaenidae, Hygromiidae, and Zonitidae were identified based on conchological features by using different terrestrial snail keys (Rafiq *et al.*, 2021). Previous studies from India have also shown the presence of these species because of the geographical region. *Gyraulus convexiusculus*, *Indoplanorbis exustus*, and *Lymnaea natalensis* are quite common in central Kashmir, India (Wani *et al.*, 2019; Allaie *et al.*, 2019). *Indoplanorbis* spp. and *Lymnaea* spp. were reported in semi-arid and irrigated areas of Haryana, India (Sangwan *et al.*, 2016). *Gyraulus convexiusculus* is also common in Tamil Nadu, India (Rekha *et al.*, 2021). *Physella acuta* was also collected and identified from the Fars Province of Iran (Abbaspour *et al.*, 2019). *Succinea putris* was also reported from Urmia, Iran (Shamsi *et al.*, 2019). In Australia, freshwater snails of the genus *Gyraulus* (Planorbidae) were identified from dry shells, and a few new species were added to the literature (Brown, 2001). Phylogeographic distribution of the freshwater snail *Indoplanorbis exustus*, the only member of the air-breathing freshwater snail genus *Indoplanorbis*, indicates its prevalence in all geographical regions across the world, including India, Southeast Asia, Central Asia (Afghanistan), Middle East (Oman and Socotra Island), Arabia, and Africa, and it is more prevalent in Africa (Nigeria and the Ivory Coast) than in Asia (Liu *et al.*, 2010).

Medical malacology is the study of molluscs, including snails that serve as intermediate hosts of parasitic diseases of humans and animals. Nearly all snails that harbor diseases of medical importance live in fresh water (Resh, 2009). This is the first study on medical malacology in AJK, Pakistan. The role of snails as intermediate hosts is essential for the transmission of snail-borne parasitic diseases. Freshwater snails are the intermediate hosts of a variety of trematodes and some nematodes that cause



many livestock and human infections (Barbosa, 1968). Certain snail species act as intermediate hosts for a large number of trematode parasites, especially *Fasciola* spp. and *Schistosoma* spp. (Doughty, 1997). We screened three snail species, *Physella acuta*, *Bensonies monticola*, and *Euaustenia monticola*, for cercariae and found all three to be infected. The glass snail *E. monticola* was the most prevalent land snail in the moist habitats of Kotli District throughout the year. In 2013, a similar study was conducted in Punjab, Pakistan, where *Indoplanorbis*, *Physella*, *Bellamya*, *Gyraulus*, *Lymnaea*, *Oncomelania*, and *Bulinus* were screened, and only *Indoplanorbis* snails were found to be positive for *Schistosoma bovis* cercariae (Niaz *et al.*, 2013). In 2015, *Indoplanorbis*, *Bulinus*, *Oncomelania*, *Lymnaea*, *Bellamya*, *Gyraulus*, *Melanoides*, and *Physella* were screened for trematode infection by cercarial emergence, and the only intermediate host for *Fasciola* cercariae were *Lymnaea* snails (Qureshi *et al.*, 2015). Some snail species are the intermediate hosts of *Schistosoma mansoni* in Pakistan (Arijo *et al.*, 2007). Recently, the most prevalent freshwater snails in Pakistan, members of the family Planorbidae, have been found to be intermediate hosts for the transmission of paramphistomosis, a neglected ruminant parasitic disease caused by *Paramphistomum* trematodes (Rafiq *et al.*, 2022).

In 2012, a similar study was conducted in Myanmar, where *G. convexiusculus* was identified to play a role in the transmission of *Paramphistomum* spp. (Ichikawa *et al.*, 2013). *Gyraulus convexiusculus* is widespread throughout Africa, Asia, and Latin America and serves as the intermediate host of *Fasciola hepatica*, *Fasciola buski*, *Schistosoma haematobium*, *S. mansoni*, and *Schistosoma intercalatum* (Guo *et al.*, 2015). A study from Iran documented *Physa* species as the intermediate hosts of *Schistosoma* spp. (Dodangeh *et al.*, 2019). In another study, lymnaeid snails were reported as the hosts of liver fluke species *F. hepatica* and *Fasciola gigantica* worldwide (Vázquez *et al.*, 2018). Members of the freshwater snail family Planorbidae are the intermediate hosts for trematode parasites that belong to the genus *Schistosoma* (Huot *et al.*, 2020). In an extensive Indo-Australian study in which *Indoplanorbis* specimens were collected from Southeast Asia and the Indian subcontinent, including Indonesia, India, Myanmar, and Thailand, as well as Australia, the results showed that *Indoplanorbis exustus* is the sole intermediate host of *Schistosoma indicum* (Gauffre-Autelin *et al.*, 2017). Besides their wide occurrence, amber snails are known as the hosts of unique parasites. During a study in the Leningrad Region (Russia), *Succinea putris* was found to be the intermediate host of trematodes from the genus *Leucochloridium*. *Succinea*

*putris* was found to be infected with the sporocysts of three species: *Leucochloridium perturbatum*, *Leucochloridium paradoxum*, and *Leucochloridium vogtianum* (Ataev *et al.*, 2016). Snails also act as one of the two most common intermediate hosts for *Dicrocoelium dendriticum* (Lancet fluke) and the other definitive host is typically a ruminant (i.e., cows, sheep, and goats), although *D. dendriticum* can infect other organisms as well, such as human beings (Schweiger and Kuhn, 2008). A study was conducted in Turkey, where *Helix aspersa*, commonly known as the brown garden snail, was screened and found to be infected with *D. dendriticum* and *Brachylaima* spp. (Köse *et al.*, 2015). The same edible land snail *Helix aspersa* (*Cornu aspersum*) was analyzed in France and in the UK, and found to be positive for *Brachylaima* spp. (Gérard *et al.*, 2020). In 2012, *Angiostrongylus cantonensis* was detected in the giant African land snail *Achatina fulica* in French Polynesia by using molecular methods (Fontanilla and Wade, 2012). In 2014 *Angiostrongylus cantonensis* and other nematodes were isolated from *Achatina fulica* populations of Metro Manila, The Philippines (Constantino-Santos *et al.*, 2014). Slugs and semi-slugs were analyzed in Germany and found as intermediate hosts of parasitic nematodes from the genus *Angiostrongylus* (Lange *et al.*, 2018).

## CONCLUSION

A variety of snails are distributed in the marshlands of AJK, Pakistan, but they have not been studied previously. This study provides new data on the diversity of terrestrial snail species and their role in trematode infection in the AJK region of Pakistan. Our findings will provide ways for the scientific community to perform in-depth taxonomic studies that involve the Gastropoda families described to date. To date, few studies have been conducted on snails as intermediate host of parasites in Pakistan. Moreover, this study provides baseline information on the malacofauna of the AJK region. Elimination of trematode parasite infections will receive a great boost when snail host studies and effective snail control programs are prioritized in the country.

## ACKNOWLEDGEMENT

The authors would like to thank Prof. Dr. R.A. (Ruud) Bank, Faculty of Medical Sciences, The University Medical Center, University of Groningen, The Netherlands, for his assistance in the identification of land snails. The authors would also like to thank Mr. Zia ul Rahman, Department of Zoology, University of Poonch, Azad Jammu and Kashmir, for his help in the collection of snail samples.



**Funding**

This study was supported by the Three-Year Public Health Action Plan of Shanghai (No. GWV-10.1-XK13 to JC). The funders had no role in the study design, the data collection, and analysis, the decision to publish, or the preparation of the manuscript.

**IRB statement**

This work has been approved by the institutional review board under CUI/Bio/ERB/2021/44.

**Statement of conflict of interest**

The authors have declared no conflict of interest.

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