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MORPHOMETRY-BASED IDENTIFICATION OF SNAIL HOST SPECIES FOR HELMINTH PARASITES IN CHITWAN, NEPAL

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ABSTRACT

Snails serve as intermediate hosts for numerous parasitic diseases of humans and animals. This study examined the morphometry-based identification for ten snail species from terrestrial and aquatic habitats in Chitwan, Nepal. In total, 120 snails were identified based on regional taxonomic keys. Morphometric analysis showed interspecific variation in shell dimensions among the studied snails. *Lissachatina fulica* exhibited the largest size with an ovo-conical shell (61.33 ± 2.96 mm height; 31.58 ± 3.59 mm width), while *Indoplanorbis exustus* represented the smallest species (12.33 ± 1.93 mm height; 5.38 ± 0.68 mm width). *Ovate lymnaea* showed moderate size (18.45 ± 1.48 mm height; 8.95 ± 2.99 mm width), whereas *Bellamya dissimilis* had a larger conical form (26.67 ± 1.69 mm × 20.25 ± 1.21 mm). Discoidal *Macrochlamys indica* and elongate-conical species (*Brotia costula*, *Thiara scabra*, *Melanoides tuberculata)* exhibited distinct height-to-width ratios, reflecting habitat-specific adaptations. Globose taxa such as *Pila globosa* (29.50 ± 11.30 mm × 26.83 ± 9.97 mm) and *Cyclophorus* sp. (24.25 ± 1.64 mm × 30.17 ± 1.86 mm) showed broader shells relative to height. Aperture dimensions were widest in *L. fulica* (34.58 ± 2.93 mm × 20.42 ± 0.36 mm) and the narrowest in *T. scabra* (4.92 ± 0.95 mm × 2.38 ± 0.36 mm). These morphometric variations not only reflect ecological adaptations, but also highlight the epidemiological importance of snails as intermediate hosts of human and animal health significant parasites, such as *Schistosoma*, *Fasciola*, and *Angiostrongylus* species.

Key words: *Gastropods, intermediate hosts, morphometry, parasitic diseases*

INTRODUCTION

Nepal's unique geography, extending from the lowland Terai plains to high-altitude Himalayas, supports diverse freshwater and terrestrial ecosystems that harbor a rich gastropod species. These gastropods play essential ecological roles in nutrient cycling and food webs, while also serving as intermediate hosts for helminth parasitic diseases of considerable medical and veterinary importance (Budha et al., 2015; Gurung et al., 1997). The accurate identification of these snail species is fundamental for understanding snail transmitted parasitic disease (STPD) epidemiology. Morphometric analysis provides a reliable and accessible approach for gastropod species identification, particularly when molecular techniques are unavailable or impractical in field conditions (Klingenberg, 2016; Liew & Schilthuizen, 2016). Traditional morphological identification based on shell characteristics has been enhanced by quantitative morphometric approaches that provide standardized, reproducible identification criteria (Liew et al., 2014; Verhaegen et al., 2019). In Nepal, several land and freshwater snail taxa contribute to the malacofaunal diversity of regions such as Chitwan. Notable land snails include *Lissachatina* spp., *Macrochlamys* spp., *Cyclopohorus* spp., while freshwater snails represented by *Pila* spp., *Bellamya* spp., *Lymnea* spp., *Indoplanorbis* spp., *Melanoides* spp., and *Thiara* spp., represent key taxa in Chitwan's malacofauna with varying degrees of medical significance (Bößneck et al., 2016; Devkota et al., 2011). These species differ in their ecological niches and epidemiological significance, with some acting as intermediate hosts for helminths responsible for diseases such as schistosomiasis, fascioliasis, angiostrongyliasis and other snail-borne infections that impose notable public health and veterinary burdens (Devkota, 2011; WHO, 2021). The importance of accurate species identification extends beyond parasitology to encompass ecological studies, biodiversity assessments, and conservation planning (Sarkar & Krupanidhi, 2019). Nepal has checklists and regional guides, but there is no single, standardized morphometric identification key covering all Nepali gastropods that researchers can apply consistently (Budha *et al*. 2015). This creates challenges for scientific research and practical applications during comparative studies of snail species and in predicting their potential roles in helminth parasite transmission.

In Nepal, limited research on gastropod taxonomy and their role as intermediate host hinders understanding of snail-borne parasitic diseases (Devkota, 2011). Morphological variability and restricted access to molecular tools complicate accurate species identification. As a result, the risk of transmission of neglected diseases (fascioliasis, schistosomiasis, and angiostrongyliasis) while handling or utilizing is higher, particularly in regions like Chitwan (Devkota et al., 2011; Liew et al., 2014; Pathak et al., 2025). In landscapes with intense human, agricultural, livestock, and wildlife interactions, the presence of diverse malacofauna highlights the importance of identifying intermediate host species to better understand disease transmission dynamics (Devkota et al., 2011; Liew & Schilthuizen, 2016; Pathak et al., 2023). Establishing a reliable baseline of gastropod diversity through morphometric characterization will not only strengthen epidemiological studies but also support biodiversity conservation and development of locally adapted cost-effective strategies for disease control. Thus, this study is timely and necessary to advance a one health approach to mitigating burden of snail-borne parasitic diseases in Nepal (Pathak et al., 2023).

The primary objective of this study is to characterize and identify land and freshwater gastropod species in Chitwan, Nepal, using morphometric analysis. Specifically, the study aims to document the diversity and spatial distribution of gastropod species of medical and veterinary importance and to establish standardized morphometric criteria for their reliable identification. The novelty of this study lies in the development of statistically validated, standardized morphometric measurements that effectively distinguish snail species and link morphology variations to their roles in helminth parasite transmission: an approach that has not previously been established in Nepal.

MATERIALS AND METHODS

Study site

Snail samples (n = 120) were collected from nine different spots of Rampur, Chitwan District (27°40′60.0″ N 84°25′60.0″ E). In fact, in total we had more than twelve individuals for some for replicates but we randomly pooled from each species for the preliminary assessment for this study. Therefore, in total single representative species had twelve replicates. Rampur comprises diverse ecological habitats, including freshwater bodies, natural swamplands, and grazing areas, where livestock, birds, wildlife, and humans frequently share common spaces. Such environmental overlaps facilitate the maintenance and transmission of snail-associated helminth parasites by supporting the completion of their life cycles and the persistence of infective stages for definitive hosts. Additionally, frequent human and animal contact with water bodies poses occupational and public health risks, particularly for animal handlers.

Even stall-fed animals may be exposed to infection through contaminated fodder, grass, or vegetation carrying infective parasitic stages.

Sample collection and preparation

Individual samples of snails were collected alive using long handled scoop nets (aquatic snails), hand picking using simple forceps and kept in labelled Ziplock plastic bags and transported to the laboratory at the Department of Microbiology and Parasitology, Agriculture and Forestry University. In laboratory samples were thoroughly washed. The samples were assigned as genera using specific identification keys provided by Budha (2016) for freshwater while Budha *et al*. (2015) for terrestrial snail species and other references (Eversham, 2018; Fiedler, 2019).

Morphometric analyses

Based on the snail shell structure the specific key features were observed for species and measurements were taken using digital vernier caliper (Figure 1). The measurements were noted for individual samples.

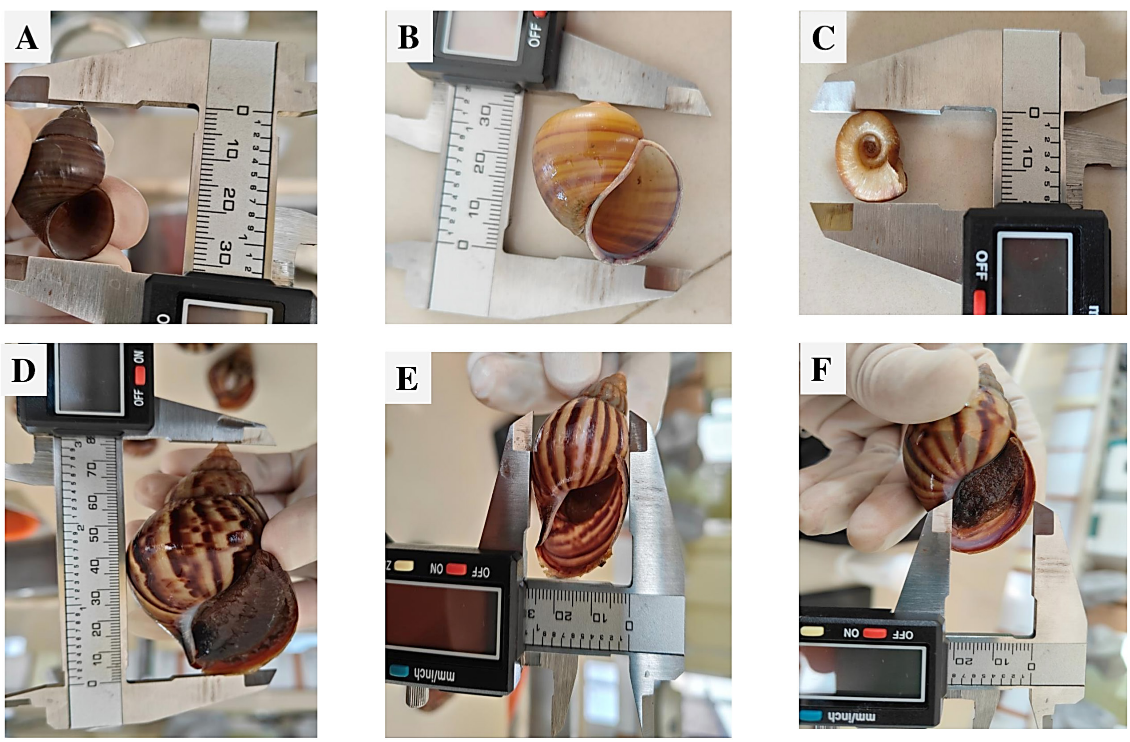

Figure 1. Measurement of different morphometric parameters of snail shells using a digital Vernier caliper. A = Shell height, B = Shell height, C = Shell diameter, D = Shell height, E = Shell width, and F = Aperture width.

Data management

Recorded data were entered into the excel spreadsheet [Microsoft® Excel® for Microsoft 365 MSO (Version 2507)]. Calculations for mean and range were performed using simple data calculations for key components such as snail shell length/height, width/diameter, aperture length, aperture width, aperture diameter, and number of whorls presented. The data were visualized in the tabular forms.

RESULTS AND DISCUSSION

All about ten different genera were identified based on the morphological keys. Among ten three species were terrestrial and remaining seven species from the freshwater settings. Under the terrestrial setting *Lissachatina* spp., *Macrochlamys* spp., *Cyclophoria* spp. Whereas in freshwater *Bellamya* spp., *Brotia* spp., *Indoplanorbis* spp., *Melanoides* spp., *Pila* spp., *Lympnea* spp., and *Thira* spp. (Figure 2).

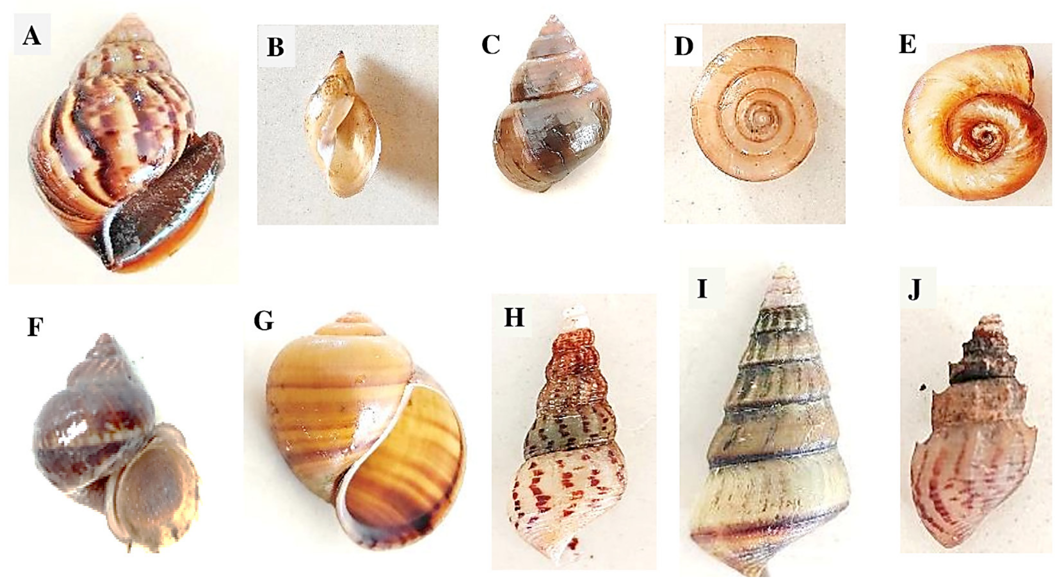

Figure 2. A = *Lissachatina fulica*, B = *Lymnaea* sp., C = *Bellamya dissimilis*, D = *Macrochlamys indica*, E = *Indoplanorbis exustus*, F = *Cyclophorus* sp., G = *Pila globosa*, H = *Brotia costula*, I = *Thiara scabra*, and J = *Melanoides tuberculatus*.

Table 1: Shell shape and morphometric characteristics (mean ± SD) of selected terrestrial and freshwater gastropod species

Snail species	Shell shape	Height/ Diameter (mm)	Width/ Thickness (mm)	AP length (mm)	AP width (mm)
Lissachatina fulica	Ovo-conical	61.33±2.96	31.58±3.59	34.58±2.93	20.42±0.36
Lymnaea (Lamarck, 1799)	Ovate	18.45±1.48	8.95±2.99	11.95±0.70	5.41±0.33
Bellamya dissimilis (Mueller, 1774)	Conical	26.67±1.69	20.25±1.21	13.17±1.03	8.33±0.24
Macrochlamys indica	Discoidal	18.00±1.21	8.00±0.00	7.00±0.00	5.00±0.20
Indoplanorbis exustus (Deshayes, 1834)		12.33±1.93	5.38±0.68	6.19±0.86	3.29±0.50
Brotia costula (Rafinesque, 1833)	Elongate-conical	36.83±5.32	11.00±1.47	10.00±1.00	8.00±1.63
Thiara scabra (Mueller, 1774)		13.42±0.95	6.08±0.64	4.92±0.95	2.38±0.36
Melanoides tuberculata (Muller 1774)		21.17±2.54	6.75±1.07	6.25±0.80	3.25±0.56
Pila globosa (Swainson, 1822)	Globose	29.50±11.30	26.83±9.97	22.67±8.01	13.83±5.84
Cyclophorus sp.		24.25±1.64	30.17±1.86	12.08±1.20	9.33±0.94

Note: AP; Aperture. AP length = Aperture length (distance from the top to the base of the shell opening), and AP width = Aperture width (maximum breadth of the shell opening), mm = millimeter.

Morphometric analysis supported by robust statistical tests can strengthen the interpretation of results from parasitic disease perspectives. However, due to limited time and resources, it was not possible to conduct experiments on a large number of snail species in the present study. If parasitic agents are detected within specific snail species, comparisons of body size and shape could be used to assess morphometric variations associated with infection. Greater accuracy could be achieved by evaluating morphometric changes under controlled laboratory conditions using experimentally infected snails.

A minimum of 20-30 individuals per snail species is recommended for reliable morphometric analyses; however, small sample sizes (≥10 individuals) may be used for preliminary assessments (González-Casarrubios et al., 2023). Morphometric analysis revealed substantial interspecific variation in shell shape and dimensions among the ten gastropod species examined in this study (Table 1).

Lissachatina fulica exhibited the largest overall size, with an ovo-conical shell averaging 61.33 ± 2.96 mm in height/diameter and 31.58 ± 3.59 mm in width/thickness and these findings are supported by Vogler & Beltramino (2013). In contrast, *Indoplanorbis exustus*, a discoidal freshwater snail, represented the smallest species, with a mean height/diameter of 12.33 ± 1.93 mm and width/thickness of 5.38 ± 0.68 mm which agrees with Chontananarth et al., (2017). The morphometric variability observed among species corresponds closely to their ecological adaptations and taxonomic affiliations. The large ovo-conical shell of *Lissachatina fulica* reflects its terrestrial lifestyle and invasive potential, providing protection and water retention in diverse environments. Conversely, the discoidal shells of *Indoplanorbis exustus* and *Macrochlamys indica* are adaptive for freshwater or semi-aquatic habitats, facilitating buoyancy and lateral movement. *Lissachatina fulica* is considered as potential intermediate host for transmission of zoonotic nematode parasite *Angiostrongylus cantonensis* (lungworm of rat) and responsible for eosinophilic meningitis in humans (Pathak et al., 2023; Sohal et al., 2024). *Indoplanorbis exustus* is responsible for harboring and developing the intermediate stages of trematode parasites like blood flukes (*Schistosoma* spp.) (Chontananarth et al., 2017; Dumidae et al., 2024).

Globose species such as *Pila globosa* and *Cyclophorus* sp. displayed broad shell widths relative to height, with *P. globosa* showing high intraspecific variability (height/diameter 29.50 ± 11.30 mm; width/thickness 26.83 ± 9.97 mm) which is supported by previous studies (Budha, 2016; U.S. Fish and Wildlife Service, 2022). *Cyclophorus* sp. had a relatively consistent morphology (height/diameter 24.25 ± 1.64 mm; width/thickness 30.17 ± 1.86 mm) and agree with the findings of Nantarat et al., (2014) and Budha et al. (2015). Both the *Pila* sp. of snail have potential to transmit the trematode parasites (Sanil & Janardanan, 2016). However, in the case of *Cyclophorus* records for parasitic diseases are not directly available however they have potential to transmit rat lungworm to humans through consumption of raw or undercooked snails or contaminated vegetables (Morassutti & Da Silva, 2020).

Elongate-conical species including *Brotia costula*, *Thiara scabra*, and *Melanoides tuberculata* demonstrated marked variation in shell height, with *B. costula* reaching 36.83 ± 5.32 mm, considerably larger than *T. scabra* (13.42 ± 0.95 mm) and *M. tuberculata* (21.17 ± 2.54 mm). These findings agree with previous research (Budha, 2016; Budha et al., 2015). *Brotia costula, Thiara scabra,* and *Melanoides tuberculata* act as the intermediate host of trematodes like *Paragonimus* spp. and intestinal flukes *Haplorchis* spp. (Lu et al., 2018; Pathak et al., 2023; Pinto & De Melo, 2011; Pratumsrikajorn et al., 2017; Thompson et al., 2009). The *Lymnaea* species exhibited an ovate shell with relatively smaller height (18.45

± 1.48 mm) and aperture dimensions (aperture length 11.95 ± 0.70 mm; width 5.41 ± 0.33 mm). From a public health perspective, *Lymnaea* snails are well-established intermediate hosts for trematodes of veterinary and medical significance, particularly *Fasciola hepatica* and *F. gigantica*, which cause fascioliasis in livestock and humans (Pathak et al., 2023; Vázquez et al., 2018).

The number of whorls varied markedly among species, reflecting taxonomic and ecological differences. *Indoplanorbis exustus* had the fewest (3–4, mean 3.5), while *Cyclophorus* sp. showed moderate values (3.5–5, mean 4.18). *Macrochlamys indica* also remained low (around 4), whereas *Bellamya dissimilis* (5–5.5, mean 5.17) and *Lissachatina fulica* (5–6.25, mean 5.83) displayed higher counts. *Lymnaea* spp. averaged 5.5–6, and *Pila globosa* ranged from 5–6 with notable intraspecific variability. *Thiara scabra* and *Melanoides tuberculata* showed intermediate ranges (5–7), while *Brotia costula* reached the maximum (8–9, mean 8.5). Overall, whorl numbers proved useful in distinguishing species and reflect adaptive variation across habitats.

Aperture (AP) dimensions followed a similar trend, being widest in *L. fulica* (AP length 34.58 ± 2.93 mm, AP width 20.42 ± 0.36 mm) and narrowest in *T. scabra* (AP length 4.92 ± 0.95 mm, AP width 2.38 ± 0.36 mm). Elongate-conical species (*B. costula*, *T. scabra*, and *M. tuberculata*) displayed pronounced shell height relative to aperture dimensions. Such forms are often associated with riverine habitats, where streamlined shapes help withstand water currents and reduce predation risk. The smaller aperture of *T. scabra* suggests a defensive adaptation against desiccation and predators.

These research findings highlight how shell shape and dimensions are strongly shaped by ecological niche, habitat type and evolutionary lineage. Comparative morphometric profiles provide baseline data for future taxonomic, ecological, and parasitological studies, particularly in relation to their roles as intermediate hosts of medical and veterinary important parasites. Thus, the morphometric findings not only highlight interspecific differences but also help explain their contrasting epidemiological importance.

CONCLUSION

The present morphometric study on snails shows the interspecies variability in shell shape, size, and aperture dimensions among the terrestrial and freshwater settings, reflecting their ecological adaptations evolutionary affiliations. The differences are not only taxonomically important but also have direct implications for their role as intermediate hosts of parasitic diseases of medical and veterinary concerns. Lager species such as *Lissachatina fulica* exhibit adaptation that help their invasive potential and capacity to transmit zoonotic nematodes like *Angiostrongylus cantonensis*, while *Indoplanorbis planorbis* are major vectors of trematodes including *Schistosoma* spp (Pathak et al., 2023; Sohal et al., 2024). Similarly, *Brotia costula*, *Thiara scabra*, *Melanoides tuberculata* and *Lymnaea* spp. Show potential of transmitting trematode parasites like blood fluke, lung fluke, and intestinal fluke (Sanil & Janardanan, 2016). Overall, this study identified some genera of snails based on morphometry of shell which has not only structural attributes but are also predictive indicators of epidemiological risk, providing essential baseline data for snail surveillance, control strategies and future experimental parasitological investigations.

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