

**POLYMORPHIC VARIATION IN EDIBLE FRESHWATER SNAIL
SPECIES *Filopaludina bengalensis* (Lamarck, 1822) IN NEPAL**



Entry G 5
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Batch: 2076

A thesis submitted

**In partial fulfillment of the requirements for the award of the degree of Master
of Science in Zoology with special paper Ecology and Environment**

Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

June, 2023

DECLARATION

I hereby declare that the work presented in this thesis “**Polymorphic variation in edible freshwater snail species *Filopaludina bengalensis* (Lamarck, 1822) in Nepal**” has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by the reference to the author(s) or institution(s).

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RECOMMENDATION

This is to recommend that the thesis entitled thesis “Polymorphic variation in edible freshwater snail species *Filopaludina bengalensis* (Lamarck, 1822) in Nepal” has been carried out by Saraswati Bhusal for the partial fulfillment of Master’s Degree of Science in Zoology with special paper Ecology and Environment. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Saraswati Bhusal entitled thesis “**Polymorphic variation in edible freshwater snail species *Filopaludina bengalensis* (Lamarck, 1822) in Nepal**” has been accepted as a partial fulfillment for the requirements of Master’s Degree of Science in Zoology with special paper Ecology and Environment.

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ACKNOWLEDGEMENTS

I am profoundly grateful and highly indebted to my supervisor Prof. Dr. Prem Bahadur Budha for his guidance and encouragement in every step of the work. This work would never be completed without his encouragement.

I am very thankful to Prof. Dr. Kumar Sapkota, Head of Zoology Department, Tribhuvan University for providing me with this privilege. I would also like to extend my gratitude to Prof. Dr. Tej Bahadur Thapa, Former Head of Department of Zoology and all the staffs of Zoology Department, Tribhuvan University for providing me with necessary help and guidance.

I would especially like to extend my gratitude towards my husband Mr. Bipin Aryal for assisting me during the field work and data analysis. I would also like to appreciate Ravi Timilsina for providing his valuable insights in the data analysis.

I appreciate the help and support provided by my family members and friends. Lastly, I would like to express my gratefulness to everyone who assisted me on successful completion of this work.

Saraswati Bhusal

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LIST OF ABBREVIATIONS/ACRONYMS

CDZ	Central Department of Zoology
CDZMTU	Central Department Zoology Museum Tribhuvan University
DNA	Deoxyribonucleic Acid
IOST	Institute of Science and Technology
IUCN	International Union for Conservation of Nature
TU	Tribhuvan University
VDC	Village Development Committee

ABSTRACT

Freshwater snail *Filopaludina bengalensis* (Lamarck, 1822) from 17 different parts of Nepal particularly from terai region including some middle hill districts were analyzed by clustering for the shell morphological variability. This study demonstrates the likelihood of the snail population variations in the shell shape, size, colour bands and operculum structure. In this study, the range of shells was from 7.94 mm to 43 mm with the maximum size 43 mm from Budhi Khola, Itahari. However, the maximum size of operculum was found 20.38 mm from Dhongrahuwa lake, Kailali. All analyzed samples were grouped in four clusters with some exceptions. Most of the samples from East, West and Central Nepal formed separate clusters; however, large numbers of samples from all sites were grouped into fourth cluster. This analysis indicated that *Filopaludina bengalensis* is a species complex with the typical *bengalensis* falls in the largest cluster and their might be more than one species in other clusters. But further analysis with more morphological and anatomical data along with molecular information is required for the conformation of species complex status.

1. INTRODUCTION

1.1. Background

Mollusks are the second most abundant group of invertebrates after insects. The number of valid recent species is currently estimated around 50,000 to 55,000 marine, 25,000 to 30,000 terrestrial and 6,000 to 7,000 freshwater (MolluscaBase eds. 2023). Freshwater mollusca are found in all aquatic habitats such as rivers, lakes, streams, swamps, springs, temporary ponds, drainage ditches and other water bodies. Groombridge (1992) Non-marine molluscs have experienced the highest number of recorded extinctions, with a total of 284 species becoming extinct within the past 300 years. There are about 80 species of freshwater molluscs including 30 species of bivalves and 50 species of gastropods are known from Nepal (Budha 2010). Freshwater gastropods are widely distributed except Antarctica.

The Vivipary snails are found in various regions around the world including North and South America, Europe, Asia, and Africa. Some species have very limited distributions, while others are widespread. The family Viviparidae has a temperate to tropical global distribution but is not native to Antarctica or South America. Vivipary snails or members of the family Viviparidae are freshwater snails that exhibit the reproductive strategy of vivipary. Vivipary snails are a fascinating group of freshwater snails that have evolved a unique reproductive strategy to increase the survival of their offspring. This reproductive strategy in which embryos develop inside the parent's body and are born as live offspring is rare among invertebrates but has evolved in some species of snails as a means of increasing the survival of offspring (Lydeard et al. 2004).

Viviparidae is currently divided into three subfamilies (Bouchet & Rocroi 2005): Viviparinae (Gray 1847), Bellamyinae (Rohrbach 1837) and Lioplacinae (Gill 1863). The African, Asian and Australian genera all belong to the subfamily Bellamyinae and all European genera to the Viviparinae but the genera from North America include representatives of all three subfamilies. Today, approximately 150 species and 31 genera of Viviparidae are recognized (Franke et al. 2007). The taxonomy of viviparids is primarily based on shell morphology and the characters used are mostly shell shape, size and sculpture, whereas the color is of limited use. Within the subfamily Bellamyniae, the shell of *Bellamya* is very variable and due to overlapping shell characters the species are difficult to differentiate (Mandahl-Barth 1954).

There are hundreds of species of gastropod molluscs which exhibit polymorphism for shell colour and pattern, one of them is *Filopaludina bengalensis*. Shell bearing species of molluscs is the highly diverse and widely distributed freshwater gastropod from family Viviparidae (Sengupta et al. 2009; Hirano et al. 2015; Hirano et al. 2019). Viviparid snails are notable for the parallel evolution of phenotypes, such as shell morphology and reproductive strategy which may be the result of repeated local adaptation to specific habitats (Hirano et al. 2015 and Hirano et al. 2019). Viviparidae has a rich fossil record that dates back to the Middle Jurassic which has been used by some previous studies to calibrate diversification ages of viviparid snails based on fossil records (Stelbrink et al. 2020).

F. bengalensis is present in almost all types of lowland water bodies, mainly stagnant water and low saline water resources such as rivers, streams, lakes, ponds, wetlands, marshes, ditches and paddy fields. The most important animals in studying colour polymorphism are freshwater snail species *F. bengalensis*. One benefit of working with this species is that variation in colour and banding seen in the shell. Although phylogeographic and genomic studies are relatively common in other invertebrates, there are remarkably few similar studies in snails or even the wider group of molluscs.

1.1.1 Taxonomy and Distribution of *Filopaludina bengalensis*

Filopaludina bengalensis (Lamarck, 1822) was originally described as *Paludina bengalensis* by Lamarck in 1822. This species has been used as *Bellamya bengalensis* for several years. It is treated as *Filopaludina (Filopaludina) bengalensis* by Brandt (1974). Neesemann (2009) treated this species as *Bellamya (Filopaludina) bengalensis* (Lamarck, 1822). Currently accepted name of the *Bellamya bengalensis* is *Filopaludina bengalensis* (Lamarck, 1822) (MolluscaBase eds. 2023). There exist multiple *Filopaludina* species that exhibit distinguishing characteristics such as variations in shell structures, apex shape, and color patterns whereas identification can prove challenging because of ambiguous and inconsistent depictions in the literature.

They are distributed in India, Bangladesh, Myanmar, and Sri Lanka. Whereas in Nepal, it has been reported from Kailali, Bardiya, Beni, Damauli, Rautahat, Itahari, and Sunsari. It is mostly reported from terai and some parts of middle hills of Nepal. It is distributed all over Asia and Africa. It is found in both lentic and lotic habitat in Nepal.

1.1.2 Food value of *F. bengalensis*

Filopaludina bengalensis is edible freshwater snails and has been consumed by humans in Asia and Africa. The flesh of snails is commonly utilized in both traditional medicine and cuisine within various ethnic communities. Baby et al. (2010) reported that these organisms are the main source of food for 80.81% of households from more than 30 castes of general schedule and tribal peoples. From the immemorial time beings, ethnic people are very conscious about the ethno-medicinal values of *F. bengalensis* (Bar 2020). *F. bengalensis* contains saturated fatty acids (48–60%), monoenic (18–30%), and polyunsaturated fatty acids (21-33%) in its flesh, which has low cholesterol (Misra et al. 2002).

The protein content of this food item surpasses that of certain types of fish and red meat. The ethno-pharmacological properties of *F. bengalensis* has been known since ancient times. Baby et al. (2010) found that individuals residing in the northeastern region of India, specifically in Chhattisgarh, Jharkhand, Bihar, West Bengal, Sikkim, Tripura, Manipur, Mizoram, and other areas, who fall below the poverty line depends on *F. bengalensis* as their primary source of food and income. It provides a cost-effective source of protein and address various nutritional deficiencies especially individuals who lack the financial resources to purchase fish and meat on a daily basis (Roy & Singh 2007). Approximately 70% of individuals from ethnic communities residing in the Terai region participate in the consumption of nearly 20 distinct species of freshwater molluscs. *F. bengalensis* exhibits the highest protein content (% dry weight) among all freshwater edible molluscs and possesses notable ethno-medicinal properties. Studies conducted in vivo and in vitro provide evidence against the utilization of the substance as an anti-inflammatory, anti-cancer, anti-oxidative and antimicrobial agent throughout history various ethnic groups have possessed knowledge regarding the ethno-medical advantages associated with *F. bengalensis* (Bar 2020).

The interesting points were noticed that the people who are living beneath the poverty level of north-eastern part of our country (Chhattisgarh, Jharkhand, Bihar, West Bengal, Sikkim, Tripura, Manipur, Mizoram etc) depends directly on *F. bengalensis* as their main earning and edible source (Maity 2015).

Misra et al. (2002) reported that the flesh of molluscs that are fit for consumption is characterized by a low content of cholesterol and saturated fatty acids with monoenoic

fatty acids comprising 18–30% and polyunsaturated fatty acids comprising 21-33% of the fatty acid content. Mukherjee and Basu (2008) noted the widespread availability and affordability, as well as its considerable nutritional benefits. Khalua et al. (2014) reported significant seasonal variations in the protein, carbohydrate and lipid contents of this snail. These fluctuations were found to be associated with variations in environmental factors and aquatic macrophytes. Shells of gastropods contain a rich source of taxonomic information that can be used to interpret evolutionary relationships among taxa. Shell morphological characters are used as primary guidelines for species identification in general handbooks and the taxonomic literature (Chiu et al. 2002).

People frequently eat *F. bengalensis* as a staple food because of its accessibility, flavor, and noteworthy therapeutic properties. Individuals afflicted with serious heart conditions often consume this type of snail with the expectation of improving their health.

1.1.3 Polymorphism and Variability

The study of animal colour has been a key in providing evidence for some of the central tenets of biology, especially with respect to genetics and evolution. For example, early work on the inheritance of colour traits contributed to an understanding of Mendelian genetics (Staples-Browne 1908). Subsequent studies on the distribution and predation of colour morphs shaped our understanding of how natural selection may operate in snails (Cook 2003). Such studies have revealed how connectivity between populations or lack of shapes, the genetic structure of species, how regions of the genome respond differently depending upon the nature of selection and the genetic of a particular colour trait (Poelstra et al. 2014). The land snail *Cepaea nemoralis* (L.) is an iconic example of genetic variation, involving shell colour and pattern polymorphism, almost universally present in populations which shows the most widespread variants and most commonly present as polymorphisms are shell colour (yellow, pink, brown) and banding (basically five-banded or unbanded) (Cook 2017).

While specific studies on polymorphism in *F. bengalensis* appear to be limited, it is likely that this species exhibits some degree of polymorphism, similar to other snail species. Based on general principles of snail polymorphism, it is possible to expect variations in color, shell characteristics and size within *F. bengalensis* populations. The extent and specific forms of polymorphism in this species may be influenced by genetic factors, environmental conditions, and selective pressures. It is possible that *F.*

bengalensis exhibits color polymorphism with individuals displaying different color patterns or shades on their shells or bodies. This could be influenced by genetic variations and environmental factors such as habitat differences or the presence of predators. Shell characteristics including shape, size, and texture can also vary within *F. bengalensis* populations. Some individuals may have shells that are more elongated, while others may have rounder or flatter shells. Shell polymorphism can be influenced by genetic factors and environmental conditions, such as resource availability and predation pressure. Size polymorphism could occur in *F. bengalensis*, with some individuals growing larger or smaller than the average size. Genetic factors, environmental conditions, and individual growth rates can contribute to the observed size variations.

It is important to note that detailed research specific to *F. bengalensis* may be necessary to provide a more comprehensive understanding of the extent and mechanisms of polymorphism in this particular snail species.

There are about 22 forms of this species recognized based on the shell morphology and variations including *B. bengalensis f. balteata*, *B. bengalensis f. typica*, *B. bengalensis f. annandalei*, *B. bengalensis f. mandiensis*, *B. bengalensis f. colairensis*, *B. bengalensis f. doliaris*, *B. bengalensis f. nepalensis* and *B. bengalensis feburnea* (Annandale 1921).

The goal of the current study is to present morphological shell variations on the edible freshwater viviparous snail species *F. benglanensis* from various habitats and geographic locations of Nepal including variation in operculum and anatomical parts of this species.

1.2. General Objective

The general objective of this study is to assess the polymorphic variation in edible freshwater snail species *Filopaludina bengalensis* in Nepal.

The specific objectives are to:

- i.* Measure the shell morphology of *F. bengalensis* in different habitats and location of Nepal
- ii.* Find the variations in color bands in the shell of *F. bengalensis*
- iii.* Illustrate the anatomy of *F. bengalensis*
- iv.* Find the structure of operculum in different populations of *F. bengalensis*

1.3. Significance of the Study

The aim of this study was to investigate the morphometric variability of *F. bengalensis* shells collected from different parts of Nepal. This snail is one of the most important edible snail species in Nepal and neighboring countries. This is also highly abundant species found in lakes, ponds, streams, rivers and paddy fields. The shell shape and size, and color band varies in different population of this species. Due to shell variations many varieties and forms of this species have been recognized (Annandale 1921, Subba Rao 1989). The Nepalese variety and form recognized as *Filopaludina bengalensis* form *nepalensis*. There is very little information about the shell variations or polymorphism of this snail. The purpose of this study is to understand the polymorphic variation of the Nepalese edible freshwater snail species *F. bengalensis* to generate the preliminary database on polymorphism.

1.4. Limitation of the study

There are many limitations to this study. Some of them are mentioned below:

- Limited number of samples from different locations
- Analysis was done by using limited data.
- Insufficient live samples for anatomical data comparisons of all locations.

2. LITERATURE REVIEW

Published journal articles, periodicals, books, and reports on freshwater molluscs were reviewed based on the best available information.

The number of valid recent species is currently estimated around 50,000 to 55,000 marine, 25,000 to 30,000 terrestrial and 6,000 to 7,000 freshwater (MolluscaBase eds. 2023). Subba Rao (1989) reported 285 species of gastropods and bivalves from India and adjacent countries. Non-marine molluscs are important because of their highest recorded extinctions in the last 300 years (Groombridge 1992).

2.1. Distribution of *F. bengalensis* in Nepal

Population size and trends of this species are not well known in Nepal. However, it is a common and abundant species found in high numbers in all kinds of freshwater bodies including ponds, river, streams and paddy fields of southern parts of terai region as well as some middle district of Nepal (Budha et al. 2010; Subba et al. 2011; Nasemaan 2007; Subba 2003; Surana et al. 2004).

Subba (2003) reported 10 freshwater molluscs and one terrestrial species from Ghodaghodi Tal area, Kailali districts. *F. bengalensis* is the most common species of molluscs in the Ghodaghodi Lake area. Surana et al. (2004) reported 10 species of gastropods representing to seven families and four species of bivalves representing two families from Chimdi Lake (Birju Tal), Sunsari district, Eastern Nepal and found that most of the edible genera of molluscs from Nepal are *F. bengalensis*. It is the edible Mollusca of Sunsari area where sustainable utilization of Mollusca is essential because molluscs serve as food for many waterfowls inhabiting the lake area (Surana et al. 2004). In the strata of the Siwalik region of Nepal, fossils of freshwater molluscs were discovered far more frequently than fossils of vertebrates. Molluscan fossils are rather plentiful in the Siwaliks of the Surai Khola section and the Dobato section formation (Gurung 1998). Within these layers, freshwater invertebrate fossils of mollusca are abundant and diverse.

2.2. Habitat and Ecological Importance

F. bengalensis is a snail that lives in freshwater which can be found in practically any sort of lowland water body, mostly stagnant water and low-saline water resources, including rivers, streams, lakes, ponds, wetlands, contaminated roadside marshes and ditches, and paddy fields (Ramakrishna & Dey 2007). *F. bengalensis* have great significance role in aquatic ecology because they form the food of fishes and their productivity play an important link in the food chain. *F. bengalensis* are mainly filter feeder and detritivore that is why they are able to form an important link in the food chain. On account of their ability to convert low quality and low energy detritus into better quality food for higher organisms in the food web with the unfolding of importance of benthos in food chain. Especially, these snail communities are the good indicators of localized conditions indicating the water quality.

2.3. Nutritional Value of *F. bengalensis*

Sarkar et al. (2021) reported *F. bengalensis* as a cheap protein rich edible product and supplements several deficiencies. Bar (2020) reported for its importance to treat a variety of illnesses and diseases. Ghobadi and Farahnak (2004) identified a significant quantity of amino acids and proteins in *F. benglanensis*, which are crucial for promoting physiological activities in the human body. Prabhakar and Roy (2009) reported ethno-medical, food and medicinal value. Not only for human beings, this species serves as food item of some carnivore fishes, many aquatic birds and mammals (Datta et al. 2015). *F. bengalensis* contains saturated fatty acids (48–60%), monoenic (18–30%), and polyunsaturated fatty acids (21-33%) in its flesh, which has low cholesterol (Misra et al. 2002).

In other hand this snail is host of many parasites (Hasnath and Khan 2016) had the highest parasite diversity (12 taxa) and also the highest prevalence of infection (51.59%). Baby et al. (2010) reported this snail species as supplementary feed for shrimp culture in Bangladesh. Shells of molluscs are used in industries for manufacturing jewelry, tools and musical instruments as well as poultry feed industries (Williams et al. 1993).

2.4 Polymorphism

Polymorphism refers to the occurrence of different forms or phenotypes within a single species. Snails exhibit various types of polymorphism, including color polymorphism, shell polymorphism, and size polymorphism. While specific studies on polymorphism in

Filopaludina bengalensis appear to be limited, it is likely that this species exhibits some degree of polymorphism, similar to other snail species. Snails can display a wide range of colors and patterns on their shells or bodies. This polymorphism can be attributed to genetic variations, environmental factors, or a combination of both. Snails also exhibit polymorphism in shell characteristics, such as shape, size, and texture. Some species may have shells that are more elongated, while others have rounder or flatter shells. Shell polymorphism can be influenced by genetic factors as well as environmental conditions, including resource availability and predation pressure. Size polymorphism occurs when individuals within a species exhibit significant variation in their overall body size. It can be influenced by genetics, environmental factors, and individual growth rates (Cook 2003).

The extent and specific forms of polymorphism in this species may be influenced by genetic factors, environmental conditions, and selective pressures. These different forms or phenotypes within snail populations can provide advantages in terms of survival and reproduction in various ecological contexts. Polymorphism allows snails to adapt to different environmental conditions, evade predators and occupy distinct niches within their habitats.

Variation can be resolved into ground colour and pattern components and there are associations with habitat. The colours involved are a rich range of yellows, browns, reds, occasionally purple and black. Green is rare but a few examples exist among tree snails from several families (Cook 2014). Rarely, mating between morphs has been observed which, in appropriate conditions, could maintain polymorphism (Rolán-Alvarez et al. 2012). Temporal variation in selective pressure imposed by things like changing weather condition or seasonal progression of vegetation. Among gastropods the groups most likely to contain polymorphic species are snails of intermediate size and density that live temporarily or permanently on vegetation in relatively open and diverse habitats above the ground (Clarke et al. 1978). There are about 22 forms of this species have been recognized due to the shell characteristics by (Annandale 1921).

3. MATERIALS AND METHODS

3.1. Study Area

F. bengalensis were collected from different locations including some middle hills districts of Nepal. Some samples were collected from different location of the local fish/vegetable market in Kathmandu such as Balkhu, Kalimati, Lagankhel. And all samples present in the museum of the Central Department Zoology (CDZ), Tribhuvan University; Central Department Zoology Museum of Tribhuvan University (CDZMTU), from all over Nepal were also used for this study. Different districts along with the location of sample collection in the present work is shown in Figure 1.

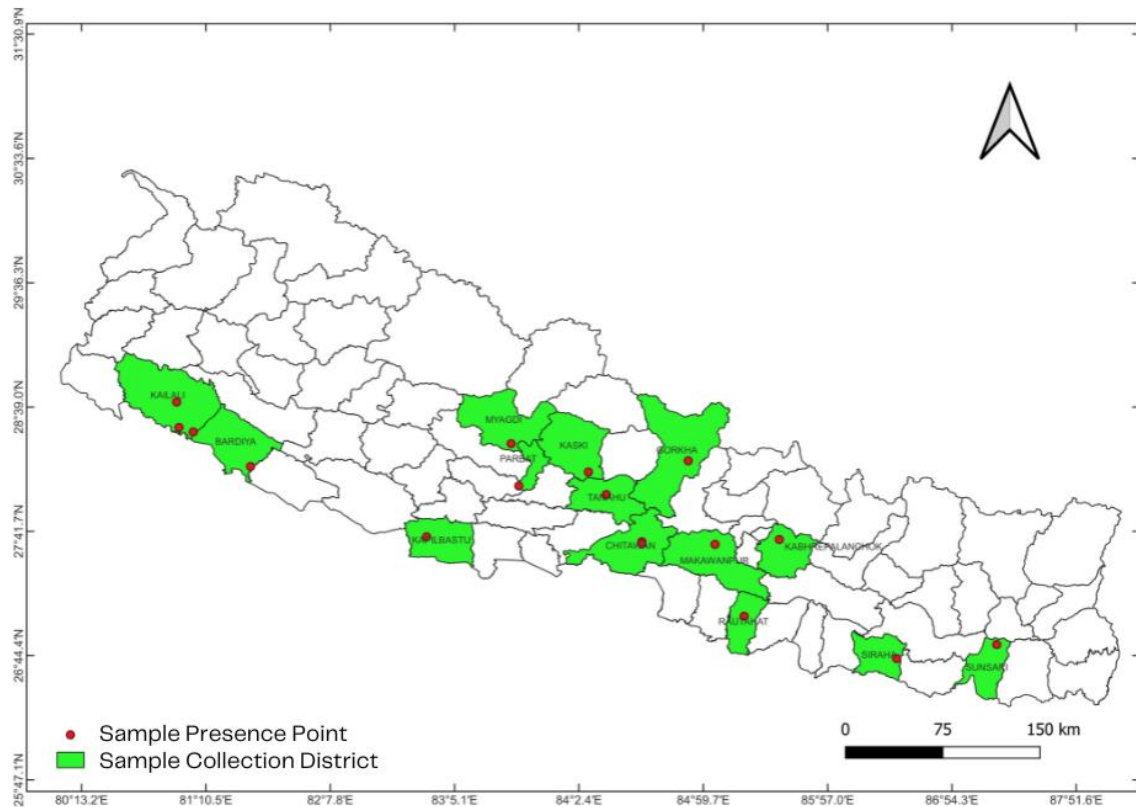


Figure 1. Map of Nepal showing sample collected points

3.2. Sample Collection and Sorting

Samples of *F. bengalensis* were collected by sieving from river. Large sized molluscs were collected by hand picking and small sized molluscs were collected by sieving. Large species were collected by handpicking from paddy fields also. Collected live samples were kept in alcohol and dead shells were stored in plastic zipper bag and brought to

Central Department of Zoology, Kirtipur. Some of the samples were also collected from vegetable and fish market of Kathmandu valley.

3.3. Shell Measurement

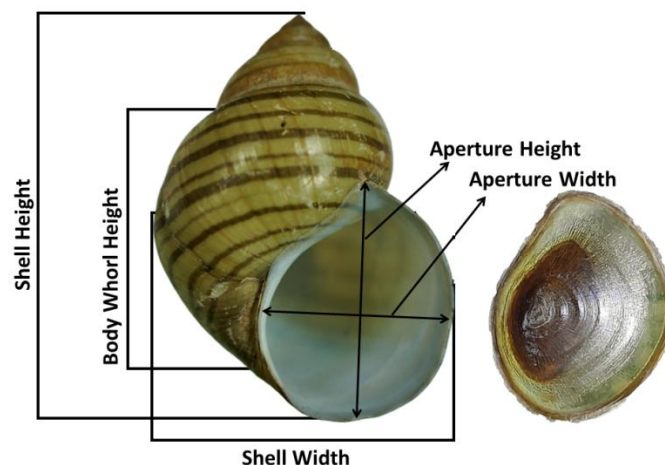


Figure 2. Measurement on shell morphometric parameters of *Filopaludina bengalensis*

Four characters including shell height, shell width, aperture height and aperture width were measured by using digital vernier calipers with accuracy to the nearest of 0.1mm of every location. Number of whorls and bands (major and minor) were noted (see figure 2). Fully grown shells were selected for measurement. Total 51 samples of *F. bengalensis* from 17 different locations were measured and analyzed. Each shell was photographed by using a camera. Operculum were removed from the shell and photographed. Dissection was done under stereo- microscope.

Different terminologies used in the shell measurement are as follows:

SH- Shell height

SW- Shell width

AH- Aperture height

AW- Aperture width

OP- Operculum

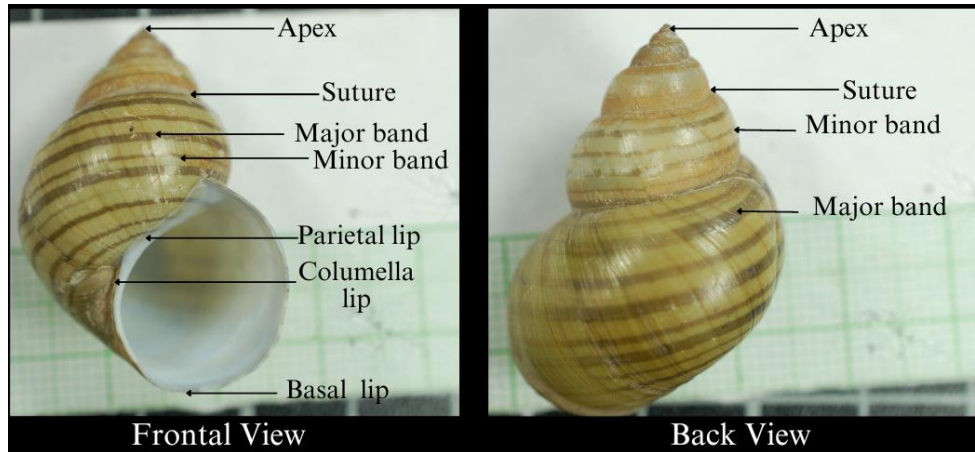


Figure 3. Frontal and back view of *F. bengalensis* (Lamarck, 1822)

3.4. Data Analysis

The morphometric data for the molluscan shell were obtained and recorded in an Excel spreadsheet. The dataset was then loaded into the R programming language (R core team 2021) and the “cluster” “corrplot” and “ggplot2” packages were used to analyze the data. Based on the elbow method, 4 clusters were identified as appropriate for the dataset. Finally, the hierarchical clustering was performed with 4 clusters using the "ward.D2" method. The dendrogram with 4 clusters was plotted and the clusters were visualized using colored rectangles. Mean value from every cluster were also calculated using MS excel 2010.

4. RESULTS

4.1. Shell Morphology of *Filopaludina bengalensis*

Samples from 17 different locations from the southern districts of Nepal were studied and based on the shell morphological characteristic features such as shell height (SH), shell width (SW), aperture height (AH), aperture width (AW), number of whorls, and major and minor bands of *Filopaludina bengalensis* samples were grouped into four different clusters (Figure 4). SH and SW of specimens ranges from 7.94 mm to 43 mm and 6.21 mm to 25.76 mm present within this dendrogram whereas AH and AW of specimens ranges from 4.95 mm to 20.38 mm and 4.09 mm to 16.84 mm respectively. Shell sample from same cluster show similar character and are very close to each other. Samples from Western Nepal form cluster 1, Eastern Nepal form cluster 2, Central Nepal form cluster 3 and remaining samples from all over Nepal form cluster 4 (Figure 4). However, one shell from each cluster falls from outer group in the dendrogram.

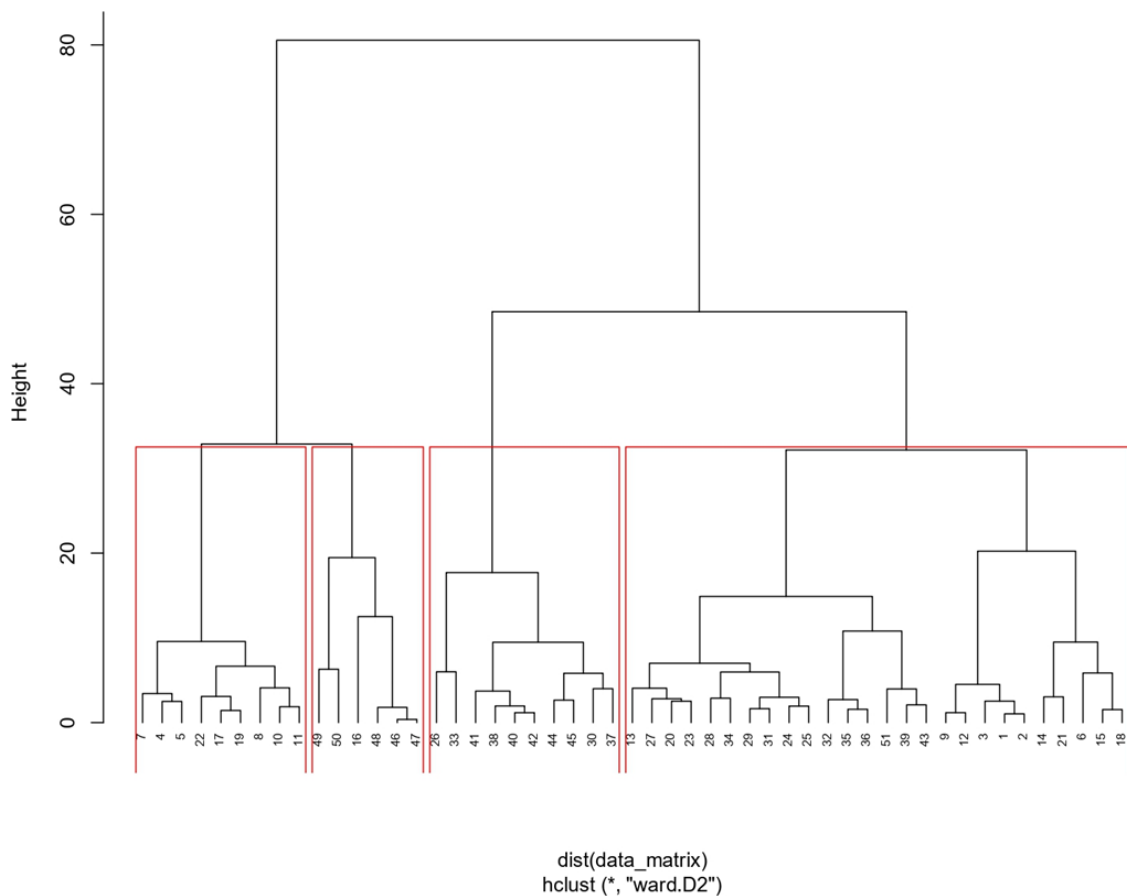


Figure 4. Dendrogram based on shell morphology

Location: A - Ranipani Parbat (1, 2, 3); B - Sunpokhari Ghodaghodi NP (4, 5, 6); C - Barhaiya lake, Bardiya (7, 8, 9); D - Macchi khola (10, 11, 12); E - Satti river, Kailali (13, 14, 15); F - Dhongrahuwa

lake, Kailali (16, 17, 18); G - Damauli (19, 20, 21); H - Jhabahi khola, Chandrauta (22, 23, 24); I - Ratnanagar, Chitwan (25, 26, 27); J - Simvanjyang, KTM (28, 29, 30); K - Rupataal, Pokhara (31, 32, 33); L - Budhi khola, Chitwan (34, 35, 36); M - Dhulikhel (37, 38, 39); N - Rautahat, Simara (40, 41, 42); O - Beni (43, 44, 45); P - Lahan, Sunsari (46, 47, 48); Q - Budhi khola, Itahari (49, 50, 51).

Cluster 1- In this cluster, all the shells were from Western Nepal. All the samples of *F. bengalensis* were from lakes of Western Nepal except Jhabahi khola and Macchi khola (they were collected from rivers). SH, SW, AH and AW ranged from 30.2 mm-37.07 mm, 19.47 mm- 22.32 mm, 15.69 mm - 18.51 mm and 13.6 mm - 16.05 mm with an average value of 33.50 mm, 20.73 mm, 17.25 mm and 14.45 mm respectively. Average value of major and minor bands is 3 and 7.67 respectively. Total specimens of nine locations were present within this cluster with the average no. of whorls 6.25. The highest number of whorls was found from Sunpokhari and Barhaiya which was 6.75 and 6.5 respectively. Sample of Barhaiya and Sunpokhari were from same branch in dendrogram.

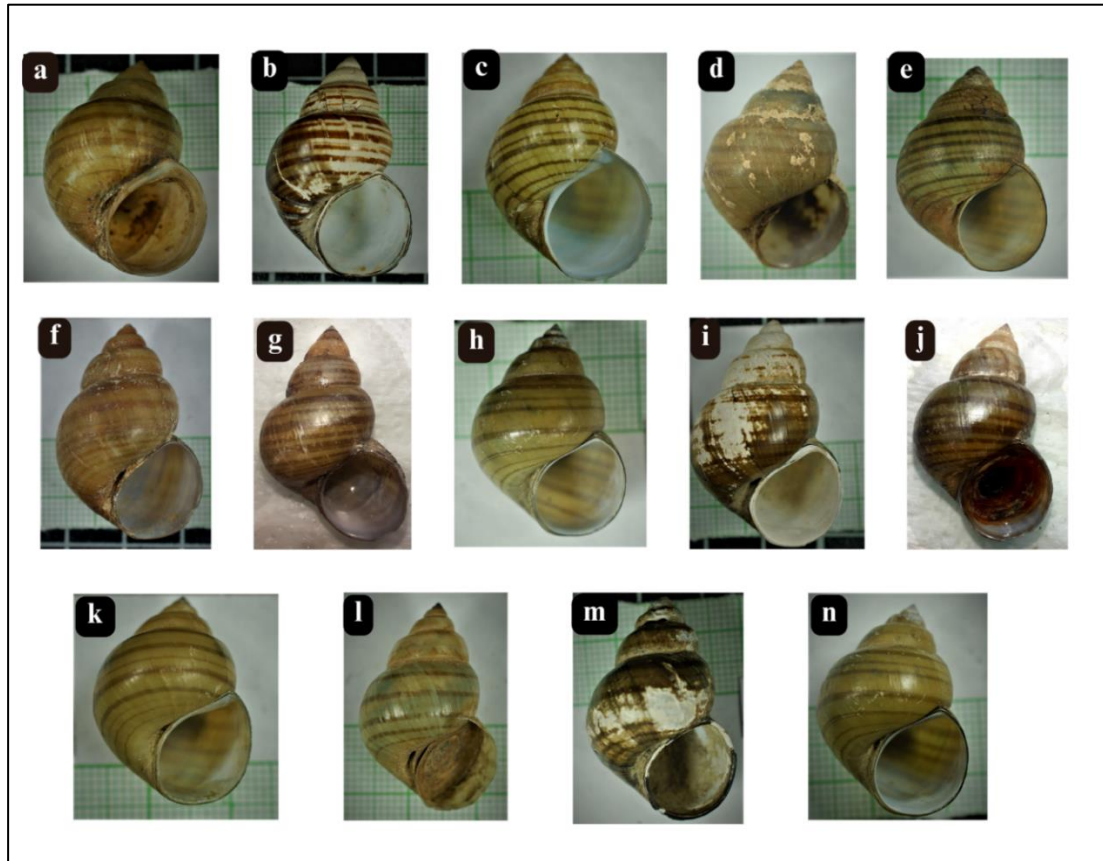


Figure 5. Morphological variation of *F. bengalensis*

Location: a- Ranipani, Parbat; b- Barhaiya lake; c- Macchi khola; d- Satti river, Kailali; e- Damauli; f- Ratnanagar, Chitwan; g- Budhi khola, Itahari; h- Rupa lake, Pokhara; i- Simvanjyang, Kathmandu; j- Simara, Rautahat; k- Beni; l- Lahan, Sunsari; m- Dhulikhel; n- Jhabahi khola, Chandrauta.

Clusters 2- Most of the samples in this cluster were from Eastern part of Nepal except Dhongrahuwa Lake, Kailali. SH ranged from 29.74 mm to 43 mm, SW from 20.11 mm to 25.76 mm, AH from 15.62 mm to 20.38 mm and AW from 13.43 mm to 17.6 mm with an average value of 39.05 mm, 23.48 mm, 18.88 mm and 15.99 mm respectively. All the shells from second cluster contain 6 whorls which is also average value of number of whorls. The mean value of no. of major and minor bands was found to be 4 and 7.67 respectively. Largest shell from Budhi Khola, Itahari is also present within this cluster.

Cluster 3- Specimens from 6 different location were present in third clusters. Most of the samples were from Central Nepal except Ratnanagar, Chitwan. SH ranged from 7.94 mm to 20.07 mm, SW from 6.21 mm to 13.51 mm, AH from 4.95 mm to 12.03 mm and AW from 4.09 mm to 9.39 mm with an average value of 15.25 mm, 10.92 mm, 9.19 mm and 7.25 mm respectively. Mean value of major and minor bands are 2.80 and 5.10 respectively. Average number of whorls present in third cluster is 6.

Cluster 4- Highest number of specimens from 15 different location were present in this cluster. This cluster contains the samples from all over Nepal. SH, SW, AH and AW ranged from 15.17 mm to 28.84 mm, 10.64 mm to 18.78 mm. 9.31 mm to 15.9 mm and 7.24 mm to 12.67 mm with an average value of 24.33 mm, 16.03 mm, 13.33 mm and 10.83 mm respectively. The average value of no. of whorls present in this cluster is 5.69. The average of no. of major and minor bands was 3.27 and 5.96 respectively.

4.2 Shell Colour Bands of *F. bengalensis*

The dendrogram was prepared based on only shell colour band patterns of *F. bengalensis* from 17 different locations. Specimens from same clusters show similar characters and are very close to each other.

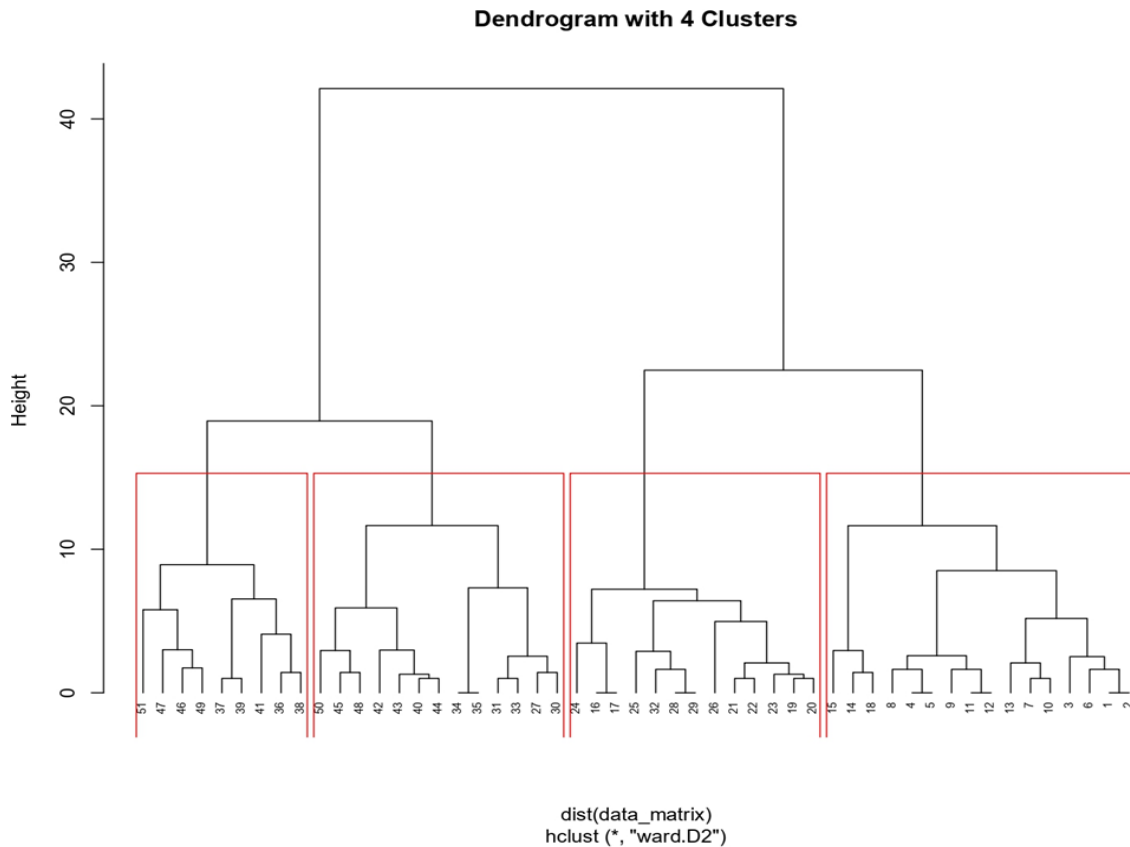


Figure 6: Dendrogram based on colour bands

[Location: A- Ranipani Parbat (1, 2, 3) B - Sunpokhari lake, Ghodaghodi NP (4, 5, 6); C - Barhaiya lake, Bardiya (7, 8, 9); D - Macchi Khola Gorkha (10, 11, 12); E - Satti river, Kailali (13, 14, 15); F - Dhongrahuwa lake, Kailali (16, 17, 18); G - Damauli (19, 20, 21); H - Jhabahi khola, Chandrouta (22, 23, 24); I - Ratnanagar, Chitwan (25, 26, 27); J - Simvanjyang, Kathmandu (28, 29, 30); K - Rupataal, Pokhara (31, 32, 33); L - Budhi khola, Chitwan (34, 35, 36); M - Dhulikhel (37, 38, 39); N - Rautahat, Simara (40, 41, 42); O - Beni (43, 44, 45); P - Lahan, Sunsari (46, 47, 48); Q - Budhi khola, Itahari (49, 50, 51).]

Cluster 1- The number of major bands were from 0 to 4 in this cluster. Major bands were absent in sample from Rautahat but minor bands were present. Number of minor bands ranged from 5 to 13. The highest number of minor bands was found from the sample of Itahari, Eastern Nepal. The mean value of number of major and minor bands was 2.22 and 8.67 respectively.

Cluster 2- The number of major bands ranges from 3 to 6 and minor bands from 0 to 6. Minor bands were absent from the sample of Budhi khola, Chitwan but major bands were present. The mean value of major and minor bands was 4.38 and 3.46 respectively.

Cluster 3- In this cluster the no. of major bands ranged from 0 to 5 whereas minor bands ranged from 4 to 11. Major bands were absent from sample of Ratnanagar, Chitwan but highest no. of minor bands was present from Dhongrahuwa lake, Kailali. The mean value of no. of major and minor bands was 3 and 8.29 respectively.

Cluster 4- In this cluster the no. of major bands ranged from 3 to 4 and minor bands from 0 to 8. Minor band was absent from sample of Satti River, Kailali. The mean value of no. of major and minor bands was 13 and 5.38 respectively.

Overall, The no. of major bands ranges from 0 to 6 and minor bands ranges from 0 to 13. Highest number of major bands were found in sample from Itahari, Beni and Rupataal, Pokhara. Study found that the highest number of major and minor bands were found from Budhi khola, Itahari.

4.3. Anatomy of *F. bengalensis*

The front part contains the head with a pair of tentacles, eyes and mouth. The soft part of snail is attached permanently to the columella of a shell by a great retractor muscle. A number of internal organs can be located in a visceral mass in the body whorl and are surrounded by a large fold of skin called mantle. Organs such as the lung, kidney and heart are situated along the inner wall of mantle. The mantle border provides for the growth of shell. Parts of the digestive and reproductive system are found from head to foot region. It contains the greater part of digestive tract, including the liver, the heart, nephridium and the reproductive system. The position of the intestine and rectum close to a major blood vessel. Respiration is mainly carried out by gills known as ctenidium. The circulation is very open and completed through body sinuses. Before running to the heart, the blood passes through gills and the nephridia.

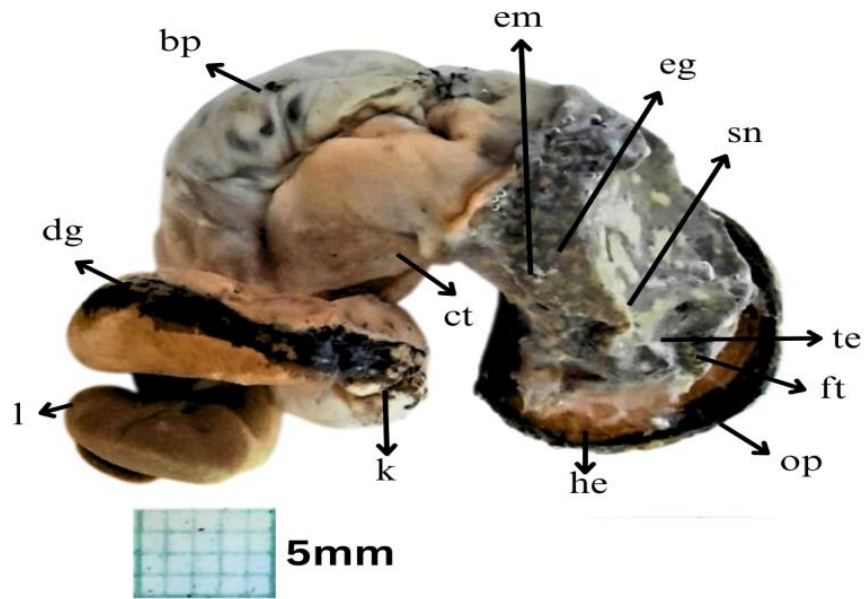


Figure 7. Internal features of *Filopaludina bengalensis*

Abbreviations: l-liver, eg-egg shell gland, te-tentacle, sn-snout, ft-foot, op- operculum, ct-ctenidium, ag-albumen gland, dg-digestive gland, k-kidney, he-head

Out of 12 samples, 8 were females and 4 were males. Males and females were identified by their brood pouches and tentacles. Most of the females are filled with embryos in their brood pouches.

The snail sex was determined by shape of right tentacles. Differences in shell morphology of *F. bengalensis* were explained by differences of sizes of mantle section of male and female reproductive system present in the whorl body. External differences between male and female were found in relative size and the form of right tentacles. The male genital consists of well-developed testis lying in the mantle cavity, the distal vas deferens extending within the right tentacles and opening at its tip. The female genital organ is composed of a remarkably small ovary, a thin oviduct, a U-shaped glandular and thick oviduct and an albumen gland. The male genital opening is found near the tentacles while female opening is found on the same side close to the mantle border.

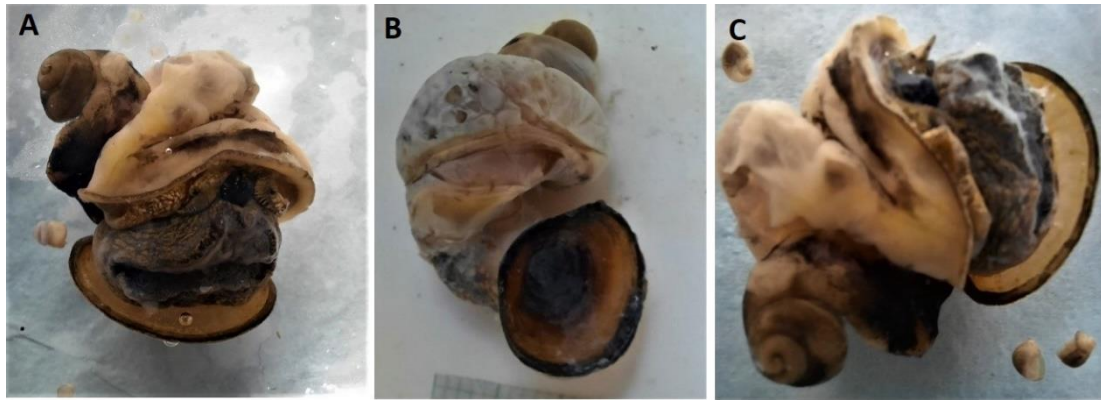


Figure 8. Anatomy of *F. bengalensis* from sample of different location (A: Budhi khola, Itahari, B: Lahan, Sunsari, C: Rautahat)

Number of eggs capsules with embryos ranges above 100. Pregnant females have a large brood pouch, filled with egg capsules with embryos is quite numerous. The brood pouch is located in the upper part of the mantle cavity and occupies almost the entire body volume. The oviduct increases in size and runs to egg shell gland along brood pouch. Brood pouch located in dorsal of oviduct (Figure 9).



Figure 9. Embryonic shells released from female *F. bengalensis* brood pouch

Dissection of female *Bellamya* reveals that it has a brood pouch from head to foot, which contained numerous small eggs but no shelled embryos. Hatching into the brood pouch presumably takes place with a shell size of 2-3 whorls measuring about 0.17 mm across. Embryos of *F. bengalensis* different developmental stages could be extracted from the brood pouch in the head-foot. Metamorphosis already occurs when the first whorl of the shell is completed. Each egg is surrounded by a spherical capsule (Figure 9).

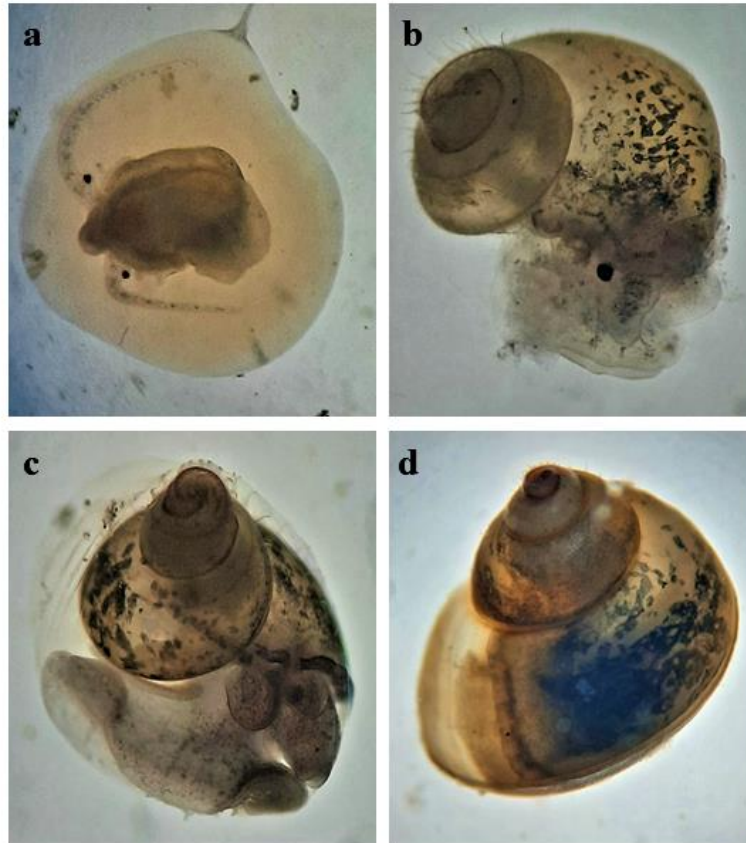


Figure 10. Different embryonic stages of *F. bengalensis*

The brood pouch is adapted for incubation of egg till after hatching where the young snails emerge from the female genital opening singly. Newly born *F. bengalensis* are comparatively well-developed and show 3-4 shell whorls. Juvenile *bengalensis* contain spines in their shells but absent in adult snails(Figure 10). They are present inside the eggs during their first stage.

F. bengalensis modified their pallial oviduct into a brood chamber or sac, where eggs and embryos are retained until shelled juveniles hatch. This brood pouch can be called subhaemocoelic brood pouch. This unique incubatory chamber is situated in the right head-foot and extends posteriorly into the neck region of the female.

4.4. Structure of Operculum

Operculum from different location form different in colour variation and shape. The size of operculum also determines the size of shell. Colour variation was seen in operculum from different location and habitats of Nepal.

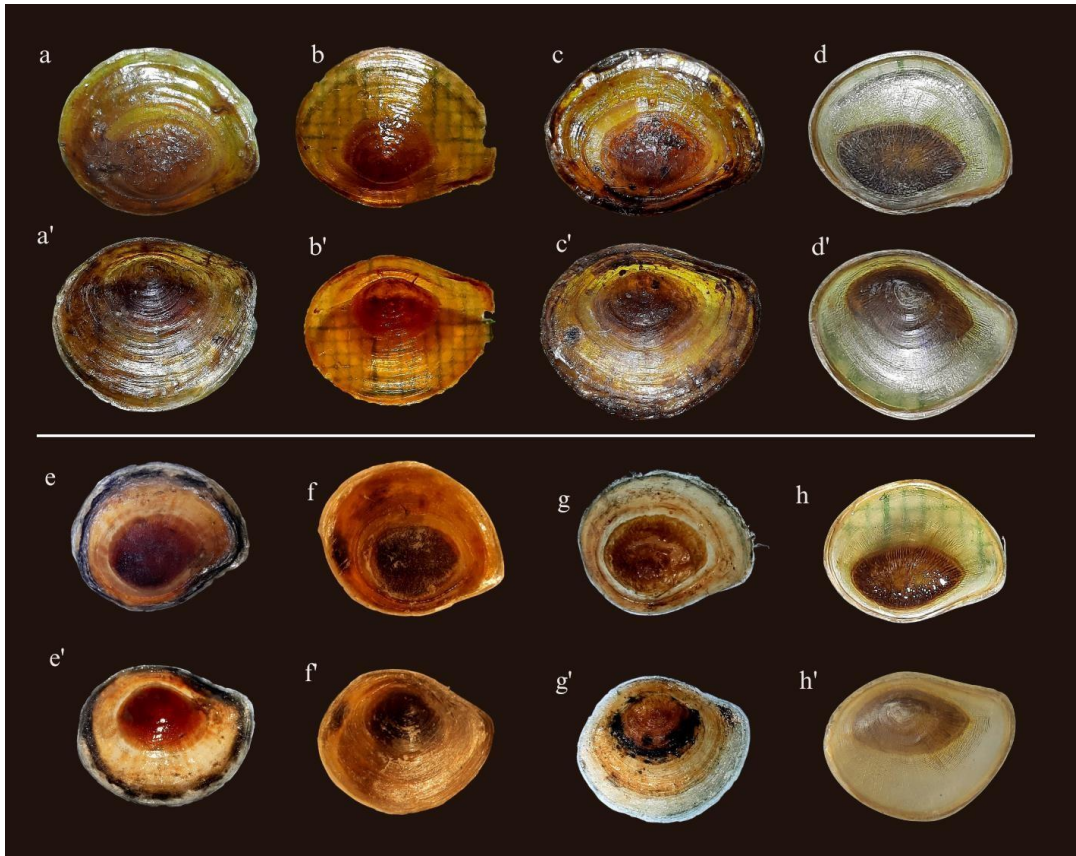


Figure 11. Structure of the operculum from different habitats and locations in Nepal (dorsal and 'ventral views) Location: a) Ranipani Parbat, b) Lahan Sunsari, c) Rautahat Simara, d) Beni, e) Budhi Khola Chitwan, f) Rupataal Pokhara, g) Ratnanagar Chitwan, h) Jhabahi Khola Chandrauta

Operculum of Ranipani, Parbat (a) and Rautahat, Simara (c) is of same colour and size. Dark concentric band form in operculum from Beni and Jhabahi khola, and Chandrauta show transparent characters. *F. bengalensis* has an operculum that is moderately thin and has a deep brownish complexion with a teardrop shaped disk of flexible protein that forms a door close like aperture. Growth marks of operculum are different from different habitats. The operculum is a disc-shaped structure attached to the dorsal surface of the foot. The external surface of the operculum is convex, the outer margin is strongly curved, the inner margin is slightly sinuate and the posterior margin is bluntly pointed. The operculum is moderately thin and of a deep brownish complexion. It has two long cephalic tentacles which are located dorsally beside the base of the snout. Some operculum changes from spiral to concentric during growth. The operculum itself attached into the opercular disc. This is a conspicuous disc-like epithelium attached to the dorsal surface of the foot, slightly posterior to the mantle edge and quite distinct from the surrounding foot.

5. DISCUSSION

This study found the variations in shell size, shape and colour bands of *Filopaludina bengalensis*. Samples were studied from 17 different locations from the southern districts of Nepal. Shell sample from same cluster show similar character and are very close to each other. The growth relation between shell height and operculum height does not support my study because the largest shell height and largest operculum height was not found in same specimen. Specimens from same clusters show similar characters and are very close to each other.

Naser (2010) found that maximum age of *F. bengalensis* was four year reaching maximum height of operculum was 35 mm from Bogra, Bangladesh. The maximum height of the operculum in this study was only 20.38 mm from Dhongrahuwa lake, Kailali. Number of whorls is not affected by location and shell size as it remains same in all specimen of every location. Number of major and minor bands changes with change in location and habitats. Number of major bands were absent from Rautahat and Ratnanagar, Chitwan but no. of minor bands was present in shell from this location. From Satti, Kailali and Budhi Khola Chitwan minor bands were absent but major bands were present. Vail (1978) found one fertilized female is capable of carrying up to 50-80 embryos and may release 50-80 juvenile but during my study, a fertilized female carry above 100 of embryos inside their brood pouch.

Gu et al. (2019) found that molecular phylogenetics indicate *Bellamya* in China and East Africa represent genetically unique phylogenetic clades where molecular dating suggested that the *Bellamya* in East Africa diverged from China suggesting that dispersal rather than vicariance was responsible for the extant biogeographic pattern of *Bellamya* in Africa and Asia. The different divergence patterns of *Bellamya* on the different continents are mainly attributed to the contrasting climatic and geological events.

Rao (1985) support my study showing that the biometric characteristics of the shell varied between the two species of *Filopaludina* collected from the two distinct habitats, not only between the two species but also between males and females of each species, there were noticeable differences in shell shape. The shell characters such as shape, spire length and shape, mouth opening, opercular shape, umbilicus shape and size, color and ornamentation of the shell are used mainly for the identification of gastropods apart from

the internal characters of which the important one is radula.

Because of the dramatic change in environment in the current study, there was a major variation in the symmetry of shell form and size for species. Kitthawee and Rungsri (2011) who referred that not matching between the identical landmark as because of the differences of environment between the studied area and variation in species shell size was observed, indicating a substantial variation in *Filopaludina* species which may be due to geographical location, which induces variation in species as well as differences in environmental variables between locations. Differences in food supply, particularly when it is abundant have an impact on the shell growth rate (Minton et al. 2008). They support that to environmental stress, so the bigger shell defends the snail from exposure in current water and as the thickness of the shell increases the harm caused by the swift current is reduced.

Sarkar et al. (2021) found a large number of *F. bengalensis* in paddy fields during the monsoon season but due to water scarcity during the winter, the species abundance was very low. Numerous reports state that differences in the habitat, environmental conditions (Balapameswara Rao & Ganapati 1971; Balapameswara Rao & Bhavanarayana 1976), differential feeding or the degree of habitat exposure are the causes of the variation in shell shape observed between different populations of gastropods. De Bernardi et al. (1976) reported shell variation in two populations of *Vivipartis ater* living in two distinct habitats of the same lake, Lake Alserio (Northern Italy).

Shelled animals are used as the best biomonitoring tools worldwide (Khatoon & Kumari 2022). Testing hypotheses about the variables related to production and reproduction statistically is a key goal of morphometrics. The body shape of an animal is determined by its length and width, so morphometric analyses can be used as a tool for establishing relationships between morphological parameters like shell length, shell width, spire length, aperture length, and aperture width that may be helpful for taxonomy in aquatic organisms in general and snails in particular (Dominguez & Abdala 2019). In order to identify species and quantify the type of morphological variation within a species, the study of shell is a crucial technique to identify species (Caley et al. 1995).

Subba Rao (1989) made the following observation: while juvenile *F. bengalensis* tend to

cling to floating weeds and sticks, adults are typically restricted to the muddy bottom of ponds and other bodies of water. According to Patnaik and Ray (1968) different species of snails occupy different freshwater habitats according to their needs for living. Khan and Chaudhuri (1984) and Subba Rao (1989) conducted studies on the distribution, bionomics, seasonal variation, and population ecology of *F. bengalensis* in a few natural and artificial freshwater bodies in west Bengal, India. Saha et al. (2017) studied a good habitat for viviparous gastropods but *F. bengalensis* appeared to be unfavorable to deep water and a lot of aquatic vegetation which abundance exhibited a positive correlation with sunlight, and this species inhabits both permanent water bodies and temporary ditches as well as irrigated paddy fields. Boycott (1934) came to the conclusion that clean and clear water in Britain was the main factor for aquatic molluscs, almost all aquatic environments, including rivers, streams, lakes, and swamps, contain gastropods with the exception of Antarctica (Strong et al. 2008). Khatoon and Kumari (2022) reported that the pre monsoon, monsoon, and post monsoon seasons are when *F. bengalensis* of the family viviparied is most prevalent. Allometry is a possible cause of morphological shell variation within populations not only due to growth patterns but also to sexual dimorphism (Chiu et al. 2002).

Pulmonate snails commonly express variation in shell morphology due to phenotypic plasticity in relation to different environmental conditions. Therefore, species identification based only on shell morphology may be prone to error (Wulfschleger & Jokela 2002). Wilbur and Owen (1964) noted that different body dimensions may not grow at the same rates, changing the relative proportions of the body as size increases. They also emphasized how various environmental factors, including seasonal and regional variations affect the allometric relations. The two species of *Filopaludina* have different growth rates for the shell various dimensions, which may be a result of the two habitats various environmental factors, particularly the water flow and food availability. However, a genetic component cannot be ruled out. Kitching et al. (1966) showed that forms with a large mouth have stronger adhesion properties which is possible that the short shells provide less resistance to water current. *F. bengalensis* that lives in standing water is not affected by water current and may as a result produce taller shells with a smaller aperture. Male members of many animal populations are less resilient to the effects of unfavorable environmental factors. The nutrient i.e. protein, carbohydrate and lipid content in the flesh of *F. bengalensis* showed the great seasonal variation and were

correlated with the changes in environmental factors and aquatic macrophytes also (Khalua et al. 2014)

The age of the mollusks was determined by the number of concentric dark structure on the operculum, which marks the slower snail growth in winter (Uvayeva & Utevsky 2021). The river snails under a year in age do not have specific sculpture elements on their shells and dark structure on the opercula, because they have not experienced a winter period of slowed growth. The operculum of shell of all age classes including the one year old snails, bear embryonic mark. Opercula have been used in both extant and fossil species and genus identification for a long time. Checa and Jimenez-Jimenez(1998) comprehensively studied operculum function, formation and types and discussed the systematic and evolutionary significance of the operculum. All species of Viviparidae have a concentric operculum with a subcentral nucleus. However, we found that the interior surface of the operculum provides more diagnostic characters and this is especially true for the nuclear, enamel and marginal regions (Zhang & Von Rintelen 2021). Opercular characters should be routinely included in addition to shell morphology and anatomy in future morphology- based taxonomic and systematic studies. The morphological identification of many groups of gastropods is mostly based on shell morphology and genital structures (Zhang & Von Rintelen 2021).

Naser (2010) reported growth mark in operculum, the maximum age of the snail was found to be four years in reaching a maximum length of 35 mm and weight of 6.8 g. This snail is ovoviviparous and its operculum is a thin teardrop-shaped disk of flexible protein that forms a door close the aperture and two long cephalic tentacles are located dorsally beside the base of the snout (Ghobadi & Farahnak 2004). Most previous studies on freshwater snail only focused on the general shape of the operculum and the position of the nucleus on the exterior surface. A striking morphological fact is that spiral and concentric opercula follow a completely different growth pattern, while both usually perform a similar function (Checa & Jimenz-Jimenz 1998). The allometric growth or environmental effects could result in intraspecific variations in shell morphology and operculum (Chiu 2002) and these anatomical characteristics could be used to identify young specimen.

Neumann (1928) suggested that the comparatively larger brood pouch of *Viviparus* may relate to its Palaearctic distribution, with winters being unfavorable for reproduction. The

results indicate that fecundity increases markedly with female shell size and hence body volume (Bocxlaer & Strong 2016). Intestine and rectum are the main areas of nutrient resorption. (Vail 1978) reports in *Viviparus* the testis are divided into anterior and posterior parts near to the digestive gland. Differences in fecundity between males of different sizes is probably small in comparison to that between females of different sizes, which may explain different life expectancies for the sexes (Stańczykowska et al. 1971). Vail (1978) for example mentioned that the reproductive system was a useful characteristic to delineate viviparid subfamilies and genera by anatomical studies of mature male and female.

Bellamyinae are poorly documented although substantial differences have been observed in comparison to other viviparid subfamilies i.e., *Viviparinae* and *Lioplacinae* (Bouchet & Rocroi 2005), particularly in reproductive anatomy (Vail 1978). For many of the approximately 125- 150 known viviparid species (Strong et al. 2008), limited anatomical and molecular data are available leaving their position within the family and genus level relationships uncertain. Because of this lack of knowledge no hypotheses exist as to how these differences in body plan among the subfamilies have arisen how the sub- families are phylogenetically related to one another what the ancestral states were in the earliest viviparids (Starobogatov 1992).

The anatomy, functional morphology and evolutionary ecology of the *Viviparidae* and the subfamily *Bellamyinae* in particular are completely unknown where genealogical relationships within the family remain poorly understood. Because of this lack in knowledge few informed hypotheses exist on ancestral states how differences in body plans between the subfamilies evolved and how the peculiar biogeographic distribution patterns of viviparids have arisen (Bocxlaer & Strong 2016).

This study also demonstrates the variation in body structure, shell shape, size, anatomical structure and shape of operculum within the same location in the same species. Such variation in molluscan shells may be caused by various environmental factors, seasonal variations and food availability.

6. CONCLUSION

This study shows that same species from same location show great variation in shell size and body structure. Specimens from same clusters show similar character and are very close to each other. Each cluster was formed from specific location of Nepal. Habitats of Kailali and Itahari was found appropriate geographical region for growth and development of edible freshwater molluscan species *F. bengalensis* because most of large sized snails were found from these locations of Nepal. Large shell snail was found from Budhi khola, Itahari which is 43 mm in size. The specimens within same cluster shows the same shell morphologies with closely related individuals meanwhile the different cluster shows that individuals possessed different shell morphologies. Maximum height of operculum was found 20.38 mm from Dhongrahuwa lake, Kailali.

7. RECOMMENDATION

- The numbers of shells were limited in the present study; similar work with high number of samples in the shell morphology can be more informative.
- Relation of shell height/shell width vs. aperture height/aperture width may give the good taxonomic information for the delineation of species complex.
- The future work is recommended for the study of detail anatomical as well as morphometric analysis of various clustered species.
- For the confirmation of the shell variations whether they belong to the same or different species, analysis of DNA barcoding of populations from multiple locations is recommended.

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