THE ARBOREAL MICROSNAIL *PUPISOMA DIOSCORICOLA* (C. B. ADAMS, 1845) FROM WEST BENGAL, INDIA: MORPHOLOGY, PLANT PREFERENCES AND DISTRIBUTION

Himangshu Barman[a,b], Pranesh Paul[a], Gautam Aditya[a]*

[a]Department of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Kolkata-700019
[b]Department of Zoology, Ramnagar College, Depal, Purba Medinipur, 721453, West Bengal, India
*Corresponding author. Email: gautamaditya2001@gmail.com

Abstract. The microsnails (Mollusca: Gastropoda), featured by miniscule size (adult shell size < 5mm), are little explored among the Indian terrestrial malacofauna. In the present study, the morphological features, host plant preference and predicted distribution of the arboreal microsnail *Pupisoma dioscoricola* (C. B. Adams, 1845) (Gastropoda: Valloniidae) are characterized from West Bengal, India. The shell features were highly correlated and showed a positive correlation with body weight. The apical angle of the shell of *P. dioscoricola* was negatively correlated with shell height and body weight and revealed a typical value, indicative of the arboreal adaptations. The toothless pupa snail *P. dioscoricola* exhibited a greater preference for the mango tree over China rose, Bengal quince, Indian mahogany and coconut as a host plant. The results of the species distribution modelling suggested that cultivated or managed vegetation and annual precipitation might be the most influential factors for its distribution. Although the present study is a pioneer effort to describe the plant preferences and the morphological features of the microsnail *P. dioscoricola*, further studies should be carried out to decipher the functional roles and conservation management of such an apparently cryptic organism.

INTRODUCTION

Microsnails constitute a unique group of land snails (Mollusca: Gastropoda) characterized by the miniscule size (up to 5mm in length) in the adult stage (Panha and Burch 2005). Different species of microsnails have been encountered from a wide range of habitats, including trees, shrubs, and even in the subterranean environments in China, Croatia, Japan, Malaysia, Thailand and the USA (Pilsbry 1920–21; Hausdorf 2007; Kawakami et al. 2008; Tongkerd et al. 2013; Weigand 2013; Páll-Gergely et al. 2015; Vermeulen et al. 2015; Salvador et al. 2017).The size, morphological variation and habitat utilization pattern are the emergent features of research on microsnails in different regions (Hausdorf 2007; Kawakami et al. 2008; Tongkerd et al. 2013; Páll-Gergely et al. 2015; Vermeulen et al. 2015). Owing to the body size, representatives of the genera *Acmella*, *Gastrocopta*, *Georissa*, *Pupisoma* and *Rahula* constitute the microsnails of India (Pilsbry 1920–21; Mitra et al. 2005; Raheem et al. 2014; Das and Aravind 2021). Apart from the taxonomic details (Rao 1925; Mitra et al. 2005), information on the biology and ecology of these microsnails is yet to be explored from India. However, considerable information on the land snail diversity is available, mainly from the Western Ghats (Aravind et al. 2005, 2008; Sen et al. 2012; Raheem et al. 2014; Madhyastha and Mumbrekar 2016)

that substantiate the functional roles attributable to the land snails. Despite the ecological significance of the land snails, several factors have been identified that contribute to their loss from the terrestrial ecosystem (Aravind et al. 2008). Thus, exploration of the land snails, particularly the microsnails, is required to conserve and sustain their functional roles.

In course of the land snail survey in different regions of West Bengal, India, the microsnail *Pupisoma dioscoricola* (C. B. Adams, 1845) (Gastropoda: Valloniidae) was observed in varying numbers in different plants. The records from Brazil (Cunha et al. 2015; Salvador et al. 2017), China (Páll-Gergely et al. 2015), Singapore (Sow-Yan and Lup 2020) and the USA (Vermeulen and Raven 1988; Hausdorf 2007) justify the worldwide presence of *P. dioscoricola*. Although known to be a part of the malacofauna of West Bengal (Pilsbry 1920–21), information on the morphology and ecology of these microsnails is yet to be deciphered in greater detail. Thus, an attempt to document the host plant preferences and the morphological details of *P. dioscoricola* was carried out to understand the regional scale distribution pattern with respect to the possible influences of the plant species from West Bengal, India. The morphological features of *P. dioscoricola* were also evaluated for comparison with the species occurring elsewhere (Hausdorf 2007).

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The distribution of land microsnails largely depends on environmental factors, such as temperature, humidity and precipitation, along with the vegetation structure and anthropogenic disturbances (Labaune and Magnin 2001; Aravind et al. 2008; Douglas et al. 2013). Depending on the observation of *P. dioscoricola* in different study areas, species distribution modelling was carried out to predict its suitable habitat ranges in West Bengal, India, using the *Maxent* software, which can predict distributions based on presence-only data (Phillips 2017). For instance, *Maxent* modelling was used to predict the potential distribution range, risk of invasion and effect of climate change on several land snail species (Vogler et al. 2013; Beltramino et al. 2015; Hausdorf et al. 2021). The results of the present study will be a valuable contribution to the knowledge of minuscule-sized land snails in the Indian context.

MATERIALS AND METHODS

Study sites and sampling

As a part of the assessment of land snail diversity, regular surveys during the monsoon periods (July to September) of 2017 and 2021were conducted in different districts of West Bengal, India. The study area, West Bengal, is situated in the eastern region of India and consists of diverse geographic features, including Darjeeling Himalayan hill regions, foothills and Dooars in the northern extreme, western plateau and high lands, Sundarbans mangrove forest and coastal area in the extreme south and plain lands in between (Figure 1). The surveys were carried out during the early morning and late evening, including search of snails in the randomly selected multiple quadrats of 10*10 meters of bushes, gardens and shrubbery near the human settlements (n = 42) (Sturm et al. 2006; Fontaine et al. 2007). In a particular sampling quadrat, if a plant was found positive for the presence of the snail *P. dioscoricola*, rigorous visual search was continued through the entire bark, stem and the leaves as the microhabitat spaces up to reachable height. Although the sampling efforts varied in the surveyed quadrats owing to the differences in the number of plants per quadrat, at least 30 minutes time was spend for the search of *P. dioscoricola* in the plants, which remained the same for all the positive quadrats. A repeat scrutiny of the selected plants was also carried out at different times during the survey to ascertain the presence of *P. dioscoricola*. During the surveys, the microsnail was encountered in 10 locations (a total of 16 quadrats) belonging to 8 districts of West Bengal, India (Figure 1). In case of the positive plants, the relative abundance of *P. dioscoricola* in a plant was noted along with the name of the plant. Following this, the snails were collected from plants by the hand-picking method
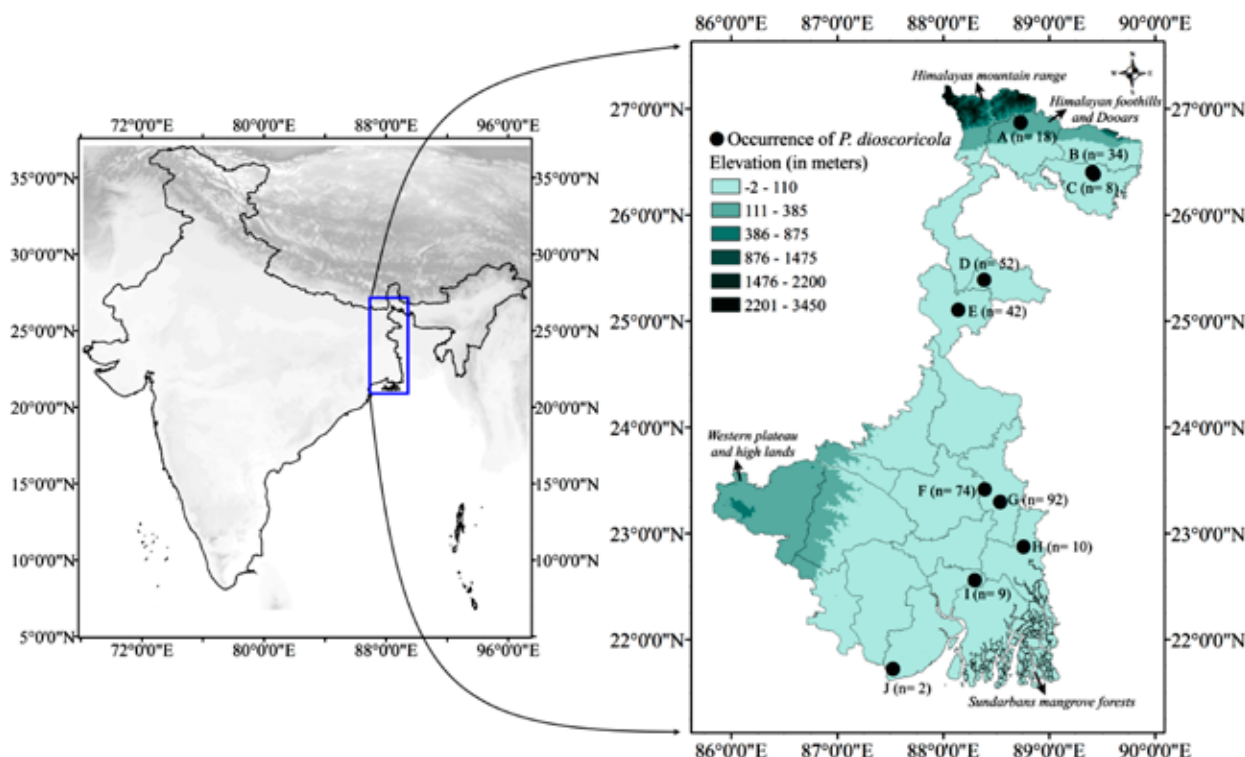

Figure 1. Map of the study area. Among different randomly selected survey areas of West Bengal, India, *P. dioscoricola* was encountered at (A) Malbazar, Jalpaiguri district, (B and C) Basantapur and Konamalli, Cooch Behar district, (D) Buniadpur, Dakhshin Dinajpur district, (E) Gunsharkol, Malda district, (F and G) Swarupganj and Purba Badkulla, Nadia district, (H) Gobardanga, North 24 Parganas district, (I) Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah district, and (J) Hamirpur, Purba Medinipur district ['n' indicates the number of *P. dioscoricola* encountered in the given survey area].

with a fine paintbrush and brought to the laboratory with water-dense tissue paper to maintain higher humidity (Raut and Ghose 1984; Barman and Aditya 2021). In all instances, extreme caution was taken for the picking and subsequent transfer of *P. dioscoricola*, particularly, when these were available in the barks of the trees with considerable camouflage against the background of the bark. The collected *P. dioscoricola* were brought to the laboratory for the assessment of bodyweight, shell height and other parameters to characterize the morphology (Hausdorf 2007; Barman and Aditya 2021).

Morphometric analysis
The shells of the snails were photographed using a microscope digital camera (DGI 510, Dewinter, India) fitted with a binocular microscope (SZ2-ILST, Olympus, Japan) and were used for identification and presentation. Selected shells were dried, placed in carbon tape and further coated with a platinum layer for obtaining the image through scanning electron microscopy (EVO 18 special edition, Zeiss, Germany). Based on the pupiform shell shape, colour, fine incised spiral striae texture, number of whorls and aperture shape, collected arboreal microsnails were identified as the toothless pupa snail, *P. dioscoricola* (Figure 2) (Pilsbry 1920–21; Hausdorf 2007).Using a binocular light microscope fitted with an ocular micrometre (Erma, Japan), the morphological features of the living *P. dioscoricola* were measured to the nearest 0.1 mm. Among the morphological features of *P. dioscoricola*, shell height (SH, in mm), shell width (SW, in mm), aperture length (AL, in mm), aperture width (AW, in mm) and spire ratio (SR- SH/SW) were measured. In addition, the ratio of SW/SH, SH/AL and AW/AL were deduced equivalent to the measures of

morphology of *P. dioscoricola* observed elsewhere (Hausdorf 2007). The apical angle (AA°) of the shell was also calculated using the formula *AA = 2*tan [(0.5X SW)/(SH)]* (Preston and Roberts 2007). The body weight (BW) of living *P. dioscoricola* was measured using a fine pan balance (Afcoset, India) to the nearest 0.1 mg. Using regression equations and correlation coefficients (Zar 1999), the relationship among the morphological variables of live *P. dioscoricola* (n = 110) was deduced using *XLSTAT* software version 9.0 (Addinsoft 2010). The coefficient of determination values (Zar 1999) were considered for selection of the best fit regression equations between any two morphological variables.

Plant preference analysis
Host plant preference was assessed using Jacobs' selectivity index (D_{ia}) (Jacobs 1974), which was calculated based on 341 occurrences using the equation:

$$D_{ia} = \frac{(r_i - p_a)}{(r_i + p_a - 2r_i p_a)}$$

(where D_{ia} is the selectivity index of snail species '*i*' for plant '*a*', 'r_i' is the ratio of plant type '*a*' used to all other plant types used by that species, and 'p_a' is the ratio of plant type '*a*' to all other plants available for the individual to use within the local area). Among the entire surveyed sites of West Bengal, India, the data of the quadrats positive for the snail *P. dioscoricola* (n = 16) were considered to perform the plant preference analysis.

Species distribution modelling
During the sampling, the latitudes and longitudes of the study area were obtained from Google Maps for the
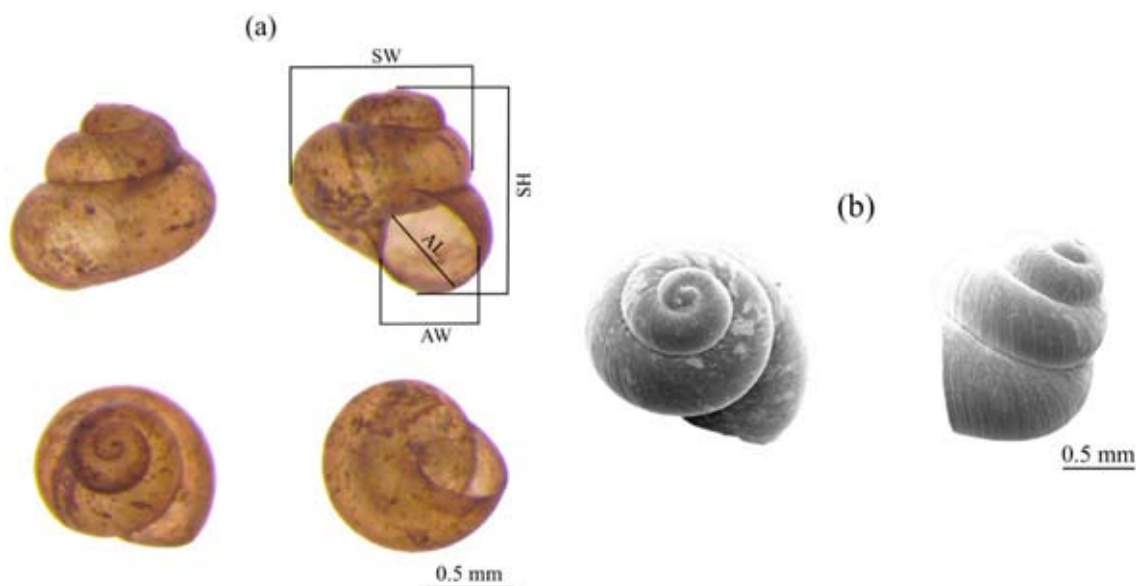

Figure 2. (a) Photographs and (b) SEM images of *P. dioscoricola* shells indicating different morphological variables (SH – shell height, SW – shell width, AL – aperture length, and AW – aperture width.

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Table 1. (a) Different variables of shell morphological variables with range and mean ± SE and (b) the coefficient of correlation among the various morphological variables of live *P. dioscoricola* (n = 110) [SH – shell height in mm, BW – body weight in mg, SW – shell width in mm, AL – aperture length in mm, AW – aperture width in mm, AA – apical angle, and SR – spire ratio]. The values in bold indicate significance at $p < 0.001$ level.

(a)

SH (mm)	SW (mm)	AL (mm)	AW (mm)	BW (mg)
0.64–2.1	0.63–1.88	0.4–1.3	0.26–1.09	0.12–2.62
1.53 ± 0.02	1.5 ± 0.02	0.89 ± 0.01	0.76 ± 0.01	1.28 ± 0.04
AA°	SR	SW/SH	SH/ AW	AL/ AW
0.86–1.85	0.67–1.24	0.81–1.49	1.15–4.32	0.68–1.71
1.08 ± 0.02	1.02 ± 0.01	0.99 ± 0.01	2.08 ± 0.04	1.2 ± 0.02

(b)

Variables	SH (mm)	SW (mm)	AL (mm)	AW (mm)	BW (mg)	AA°	SR	SW/SH	SH/AW
SW (mm)	**0.753**								
AL(mm)	**0.655**	**0.613**							
AW(mm)	**0.397**	**0.626**	**0.550**						
BW (mg)	**0.824**	**0.680**	**0.608**	**0.397**					
AA°	**−0.545**	0.112	**−0.240**	0.185	**−0.329**				
SR	**0.540**	−0.137	**0.210**	**−0.198**	**0.367**	**−0.967**			
SW/SH	**−0.549**	0.118	**−0.235**	0.189	**−0.341**	**0.998**	**−0.982**		
SH/AW	**0.317**	−0.066	−0.126	**−0.695**	**0.194**	**−0.556**	**0.574**	**−0.565**	
AL/AW	0.122	−0.169	**0.236**	**−0.660**	0.090	**−0.418**	**0.411**	**−0.420**	**0.742**

species distribution modelling study. The bioclimatic (Fick and Hijmans 2017) and land cover (Tuanmu and Jetz 2014) variables were downloaded from World Clim global climate (www.worldclim.org) and consensus land cover (www.earthenv.org) database in *GeoTiff* (*.tif*) format (~ 1 km² resolution). Additionally, the *.tif* files were reduced to the extent of our study area using a shape file of West Bengal, India, and converted to *ASCII* raster format using *ArcGIS* software version 10.8 (ESRI 2019). To model and predict the distribution of *P. dioscoricola* in West Bengal, India, selected bioclimatic and land cover (Table 2a) and *P. dioscoricola* occurrence data were used in *Maxent* software version 3.4.4 (Phillips 2017). The modelling was based on the maximum entropy theory, which evaluates the maximised probability distribution by considering a set of constraints in the correspondence of environmental data associated with the species occurrence points (Phillips et al. 2006). The default parameter settings were used to run the model, which used 10 presence records for training and 9999 background points to determine the distribution model. Additionally, the percent variable contribution and *jackknife* procedure were applied to calculate the contribution of each environmental and land cover variable to the model. The output returned a continuous map of presence probability (0 to 1). The model provided the *Area Under the Curve* (*AUC*), which measures the ability of the model to distinguish presence or background data. The values of *AUC* < 0.5 are worse than random, 0.5–0.7 indicate poor performance, 0.7–0.9 indicate moderate performance, and > 0.9 signify high model performance (Peterson et al. 2011).

RESULTS

Morphometry
The morphological features and body weight of *P. dioscoricola* and the corresponding correlation coefficients are shown in Table 1. Almost all the paired morphological variables remained significant for the values of the Pearson's product moment correlation coefficient values. Apparently, the shell height was a better predictor of the variation in the morphological features owing to its high and significant correlation with variables including body weight. Among the morphological variables, power regression equations (Figure 3) (shell width (y) = 1.115*shell height (x)$^{0.694}$; body weight (y) = 0.486*shell height (x)$^{2.114}$; aperture length (y) = 0.654*shell height (x)$^{0.694}$, and aperture width (y) = 0.585*shell height (x)$^{0.579}$) were the best fit, revealed through the values of the coefficient of determination (R²). The apical angle was a reducing function with the shell height and the body weight of *P. dioscoricola* reflecting the conical pattern of the shell.

Plant preference
The microsnail *P. dioscoricola* was encountered in 16 quadrats, ranged between 2–71 individuals per quadrat (21.31 ± 5.30 SE). Among the total of 21 plant species in the surveyed bushes and gardens (Figure 4a), five host plants, namely *Mangifera indica* (mango), *Hibiscus rosa-sinensis* (China rose), *Aegle marmelos* (Bengal quince), *Chukrasia tabularis* (Indian mahogany) and *Cocos nucifera* (coconut), were identified to host *P. dioscoricola* (n = 2–52) (Figure 4b). The Jacobs' selectivity

Table 2. (a) The list of variables used to predict the distribution of *P. dioscoricola* in West Bengal, India, and (b) relative contributions and permutation importance of the variables to the *Maxent* distribution model in this study.

(a)

	Variables	Source
Bioclimatic variables	Annual mean temperature	Worldclim (Fick and Hijmans 2017) www.worldclim.org
	Mean temp of wettest quarter	
	Annual precipitation	
	Mean diurnal range	
	Temperature seasonality	
Land cover variables	Cultivated and managed vegetation	Consensus land cover (Tuanmu and Jetz 2014) www.earthenv.org
	Deciduous broadleaf trees	
	Herbaceous vegetation	
	Mixed/ other trees	
	Open water	
	Regularly flooded vegetation	
	Shrubs	
	Urban/ built-up	
	Barren	

(b)

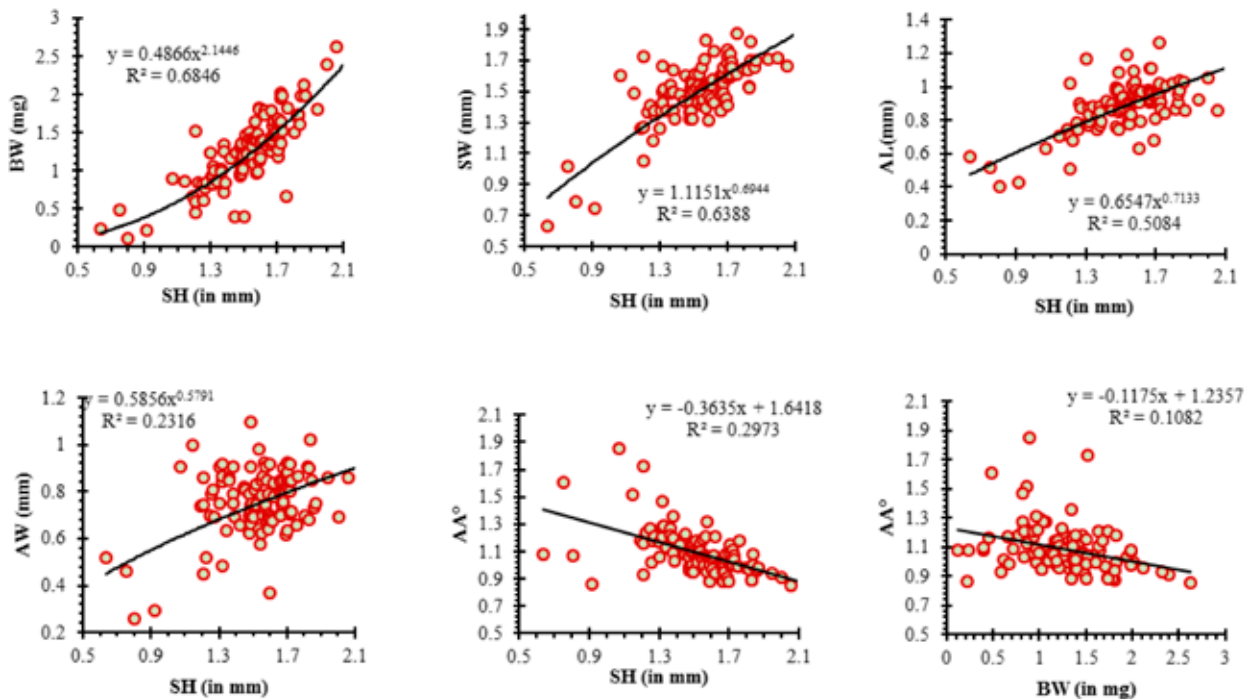| Variables	Percent contribution	Permutation importance
Regularly flooded vegetation	45.6	3.1
Annual precipitation	19.6	45.5
Deciduous broadleaf trees	18.9	2.9
Open water	7.6	0.5
Cultivated/ managed vegetation	6	46.9
Urban/ Built-up	1.7	0
Barren	0.7	1

Figure 3. Relationship among the different variables defining the morphology and shell features of live *P. dioscoricola* (n = 110) [AA° – apical angle, SH – shell height in mm, SW – shell width in mm, AL – aperture length in mm, AW – aperture width in mm, and BW – body weight in mg].

index was observed to be highest in case of *M. indica*, and lowest for *C. nucifera* (Figure 4b). Since the Jacobs' selectivity value of +1 denotes complete preference and the –1 value indicates complete rejection, it appears that *P. dioscoricola* preferred *M. indica* the most among the five host plants.

Species distribution modelling
The predicted distribution of the microsnail *P. dioscoricola*, based on the selected bioclimatic and land cover variables (Table 2a) is depicted in Figure 5. The *Maxent* model predicted that the discrete regions of the Himalayan Mountains and foothills, the Dooars region of northern West Bengal and a few parts of Paschim Bardhaman District, Kolkata and its adjacent areas have the highest probability of *P. dioscoricola* occurrence. The lowest probability of occurrence was predicted in a few discrete places of the Himalayan hills, western plateau and Sundarban reserve forest. A moderate to high probability of *P. dioscoricola* occurrence was predicted for the rest of the plains. The results of percent variable contribu-
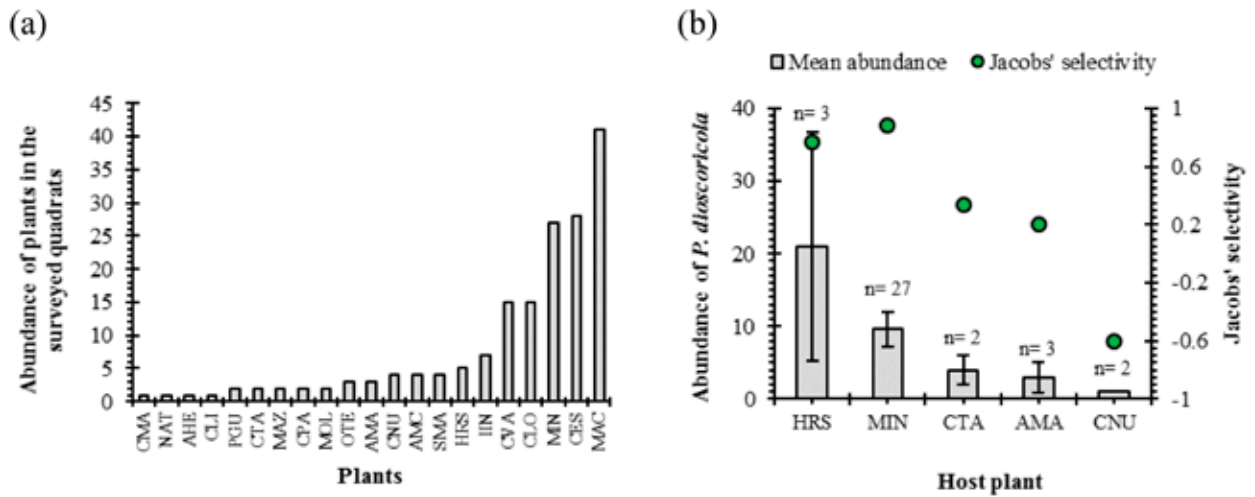
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(a)

(b)

Figure 4. (a) The number of different plants in the surveyed quadrats (n = 16) where *P. dioscoricola* was encountered, which was further used in the plant preference analysis and (b) mean abundance (± SE) of *P. dioscoricola* ('n' indicates the number of plants with the occurrence of *P. dioscoricola*) and Jacobs' selectivity (Jacob's selectivity value for the rest of the plants was –1, hence excluded from the graph) for different host plants (Acronyms: CMA – *Citrus maxima*, NAT – *Nyctanthes arbor-tristis*, AHE – *Artocarpus heterophyllus*, CLI – *Citrus limon*, PGU – *Psidium guajava*, CTA – *C. tabularis*, MAZ – *Melia azedarach*, CPA – *Carica papaya*, MOL – *Moringa oleifera*, OTE – *Ocimum tenuiflorum*, AMA – *A. marmelos*, CNU – *C. nucifera*, AMC – *Alocasia macrorrhizos*, SMA – *Swietenia macrophylla*, HRS – *H. rosa-sinensis*, IIN – *Ipomoea indica*, CVA – *Codiaeum variegatum*, CLO – *Curcuma longa*, MIN – *Mangifera indica*, CES – *Colocasia esculenta*, MAC – *Musa acuminata*).
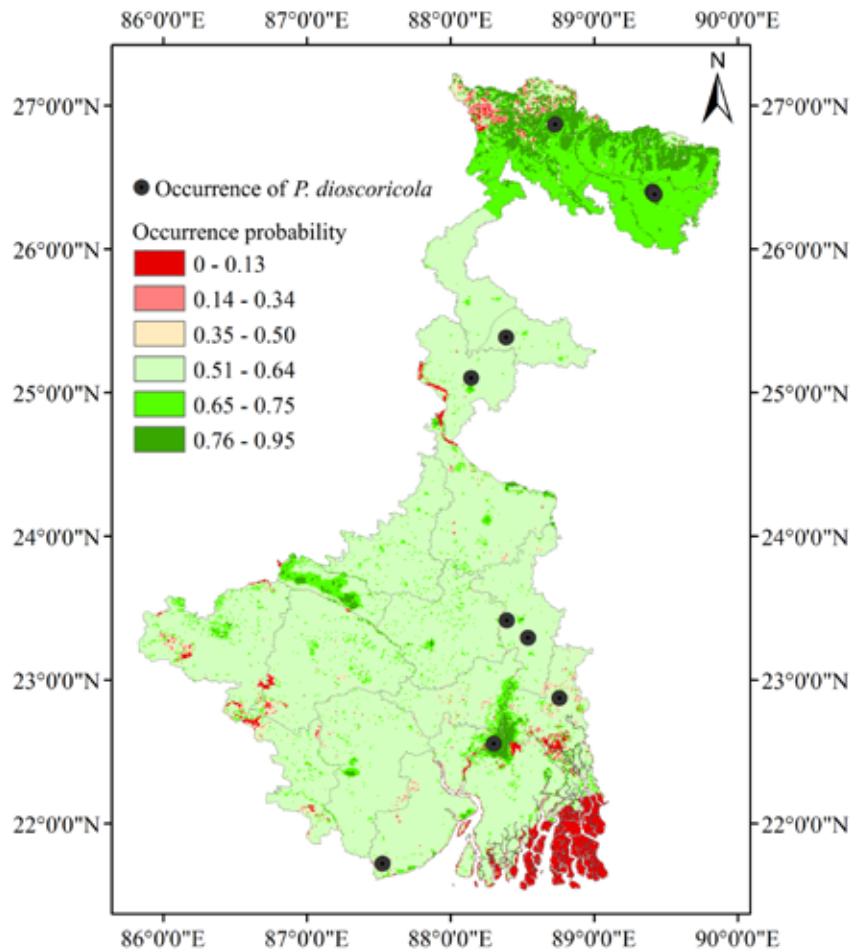

Figure 5. Predicted distribution of *P. dioscoricola* in West Bengal, India, based on *Maxent* species distribution modelling. The green regions represent areas with suitable habitats, while red regions represent areas less suitable for the microsnail *P. dioscoricola*.

tion (Table 2b) and *jackknife* procedure suggested that, among the 14 bioclimatic and land cover variables, only 7 variables had a significant contribution to the model. Regularly flooded vegetation had the highest gain when used in isolation, therefore, appears to provide the most useful factor for the predicted *P. dioscoricola* distribution. Among the bioclimatic variables used in this model, annual precipitation had the highest contribution to the *Maxent* model. Based on the model, cultivated or managed vegetation and annual precipitation were the most influential variables for the distribution of *P. dioscoricola* (Table 2b). However, the *AUC* value of this model was 0.720, indicating its moderate performance based on the occurrence points and the environmental variables.

DISCUSSION

The shell morphology of the snail *P. dioscoricola* observed in the present study appears to comply with the observations made for specimens from the USA (Hausdorf 2007). In the present observation, the mean shell width was 1.5 ± 0.02 (mean ± SE) mm and the shell height was 1.53 ± 0.02 (mean ± SE) mm, which is comparable to the mean shell height of 1.55 mm and shell diameter of 1.68 mm observed in the USA (Hausdorf 2007). The spire ratio of *P. dioscoricola* shell was 1.02 ± 0.01 (mean ± SE), indicating a high spired mode like in other documented arboreal snail families such as Achatinillidae and Amastridae and supporting its arboreal mode of life (Cowie 1995).This value of the spire ratio was comparable to *P. dioscoricola* observed from the USA (1.09, Hausdorf 2007). Land snail habitat utilization and adaptability depends on its morphometric characteristics such as shell shape and size. The portrayal of the body size through the relationship between shell length and shell weight is observed in many terrestrial snails (Cain 1977; Cain and Cowie 1978; Cowie 1995; Preston and Roberts 2007). In this study, the shell height and body weight relationship of live *P. dioscoricola* (n = 110) complied with the allometric equation (power regression was the best fit). Arboreal snails of family Achatinellidae and Pupillidae that can climb vertically, generally exhibit flat shape and bear a very large mouthed shell and high spired mode (h > d) (Cain 1977; Cain and Cowie 1978; Cowie 1995), different from discoidal, pointed and narrow shell shapes. With the increase in the shell height the spire ratio increased, while the apical angle decreased. Such relationships would mean that with increase in age, the shell changes from globular to the pointed form at the apical end which is also substantiated through the spire ratio. An increment in the spire ratio would mean the increase in height over the width of the shell. These observations show similarity with the observations on *Calliostoma zizyphinum* (Preston and Roberts 2007),

though studies using cohort-based individuals would provide a better picture of the variation in the shell shape in the course of ontogeny.

Empirical evidence suggests that the land snails utilize microhabitats such as rock crevices, litter, live trees, and deadwood due to high humidity and food resources. Among these varied habitat elements, rock crevices and deadwood were observed to be more preferred microhabitats (Sólymos et al. 2009; Kemencei et al. 2014). More specifically, the microsnails of the genus *Pupisoma* use various habitats, including aerial parts of trees, bird nests (Miquel et al. 2015), lichens and bryophytes (Pérez et al. 2008). In the USA, *P. dioscoricola* occurred in abandoned *Citrus* trees, on the undersides of leaves, leaf litter and carbonate rock outcrops (Nekola and Coles 2010). However, in the present study, *P. dioscoricola* was found on the leaves, stem and under the bark of the mentioned trees, but was not observed in *Citrus* trees and detritus. The selectivity of *P. dioscoricola* to its possible host plants may be due to the presence of microbiota, algae and fungi as a food source (Bledsoe and Minnick 1982), and grooves and crevices as sites for hiding from predators and for reproduction (Raut and Biswas 1991). In case of the microsnails, several factors like body size and biomass are considerably minuscule in dimension, which may limit their inclusion in the typical sample of the land snails. Therefore, suitable sampling methods (Sturm et al. 2006) are required for the collection and estimation of the relative abundance of microsnails as illustrated in several studies (Liew et al. 2008; Foon et al. 2017). Exploration of the detritus, soil and the allied terrestrial ecosystem elements provides an option to evaluate the distribution and the abundance of the land snails in the respective habitat (Sturm et al. 2006). However, in the present study, the time spent for the observations and sampling was increased for unit area within a quadrat along with visual scanning of the crevices created by the bark of the plants. The detritus and soil were also scanned thoroughly for the presence of living or shells of dead individuals.

As the microsnails are susceptible to anthropogenic disturbances such as deforestation and urbanization, and due to low dispersal ability, utilization of specialized habitats and small home ranges (Sen et al. 2012), conservation efforts should focus on population distribution and habitat preferences. As shown in this study, the abundance, plant preference patterns and predicted distribution range of *P. dioscoricola* may prove helpful in conservation. The observations on the distribution of the snails in the geographical area concerned also provide an impression about the possible spaces preferred. Exploration of the areas predicted by the model for a greater probability of encounter should be given priority for the habitat-based abundance and preference of *P. dioscoricola*. In recent times, priority

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areas for the conservation of the snails are highlighted in several studies that include forests (Fontaine et al. 2007; Ovando et al. 2019; Gheoca et al. 2021) as well as urban habitats (Barbato et al. 2017). On the basis of the prediction of the models, further assessment should be made to justify the status of *P. dioscoricola* as a dominant microsnail or not. Consequently, the determination of the conservation status can be made for the snail. In addition, the morphometric study of *P. dioscoricola* provides information about the prospective growth pattern in natural conditions. It is relevant to point out that the shell architecture of microsnails is highly diverse, as observed in China, Croatia, Japan and Malaysia (Kawakami et al. 2008; Weigand 2013; Cunha et al. 2015; Páll-Gergely et al. 2015; Vermeulen et al. 2015) as well as from India (Das and Aravind 2021). Although a few studies have highlighted the role of the microsnails in a community, the diversity, significance and population ecology of the microsnails at large are yet to be deciphered with greater details. In many instances, the shell characteristics (Liew and Schilthuizen 2014) and the habitat preferences provide key information on the population growth and trophic interactions (Panha and Burch 2005; Páll-Gergely et al. 2015) of the microsnails in the community. Considering the minuscule *Pupisoma* snail of India, studies encompassing trophic interactions, habitat preference and function roles should be initiated to address the issues related to population sustenance and conservation in nature. Nonetheless, the present information is a pioneer effort to highlight the habitat preferences and morphology of the snail *P. dioscoricola,* which may be helpful in the conservation management of the snail.

ACKNOWLEDGEMENTS

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