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A new species of *Pila* (Gastropoda: Ampullariidae) from Mizoram, India

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ABSTRACT

Family Ampullariidae consists of 11 genera of freshwater snails distributed pan-tropically in the Old and the New World. One of the ampullarid genera, *Pila*, is distributed in Africa and Asia and consists of 28 species. Here we describe the sixth member of the genus *Pila* from India. *Pila mizoramensis* n. sp. was collected from the Northeast Indian state of Mizoram. We adopted an integrative taxonomic approach to describe this species. First, a multilocus phylogeny of the genus was built to determine its placement in the tree. Then we used pairwise distance in the cytochrome oxidase I gene to compare its divergence from its congeners. Finally morphometric data was used to show that this hill stream species of *Pila* does not overlap with other hill stream species in morphometric space. The morphology of the species is also discussed in detail. With the advent of molecular tools in taxonomy a plethora of new species have been described from India in the last few decades. We add this newest member of genus *Pila* to the growing list.

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Endemic; gastropod; hill stream; hotspot; integrative taxonomy; NE India; species discovery

Introduction

The family Ampullariidae consists of a group of omnivorous gastropods, with markedly globose shells (Hayes *et al.* 2015). It has a pantropical distribution spanning South and Central America, Africa, India, southeast and East Asia. There are about 259 species that belong to 11 genera distributed in the New and the Old World (Cowie 2015). The family Ampullariidae is distributed in parts of the Gondwanaland supercontinent that eventually became Africa and South America, and it colonised Asia after the African plate collided with the Eurasian plate (Sil *et al.* 2020). Ampullariids are unique among freshwater snails for being amphibious – they have both functional gills and lungs. The genus *Pila* Röding, 1798 is a tropical Old World genus with a disjunct distribution in Africa and Asia consisting of 28 species (Cowie 2015). It is the only Asian ampullariid genus, colonising India from Africa through Eurasia during the Eocene, and thereafter dispersing into the rest of tropical Asia (Sil *et al.* 2020). Members of the genus are easily identifiable by the presence of a calcified operculum, which assists in retention of moisture during aestivation (Hayes *et al.* 2015). Initially four species of *Pila* were described from India, some of which are represented by only a few specimens (Annandale 1921). The fifth ampullariid species described from India, *Pila saxea* (Reeve, 1856) was placed in the subgenus *Turbinicola* along with the 'Burmese' species '*Pila aperta* (Philippi, 1849)' (Prashad 1925; but see Cowie 2015). This placement was based on their novel habitat, the hill stream, which is unique among *Pila* species, and certain associated morphological features. However, molecular study has shown that *Turbinicola saxea* is nested within the genus *Pila* in the phylogenetic tree and thus does not merit the status of a subgenus (Sil *et al.* 2020). A recent review of global Ampullariidae by Cowie (2015) has placed *P. aperta* under *Incertae sedis* but he is of the opinion that it is a New World species.

Currently the Indian subcontinent has five species of Ampullariidae belonging to the genus *Pila* (Subba Rao 1989): *P. globosa* (Swainson, 1822), *P. virens* (Lamarck, 1822), *P. olea* (Reeve, 1856), *P. saxea*, and *P. scutata* (Wood, 1828). *Pila* is widespread in India, Sri Lanka, Nepal and Bangladesh (Prashad 1925; Jahan *et al.* 2001; Budha 2016), except drier parts of northwestern India and colder regions of North India, especially in the Himalayas. *Pila globosa* is distributed throughout northern Indian plains (Uttarakhand, Uttar Pradesh, Bihar, Jharkhand, West Bengal, Odisha). It was also thought to be distributed in

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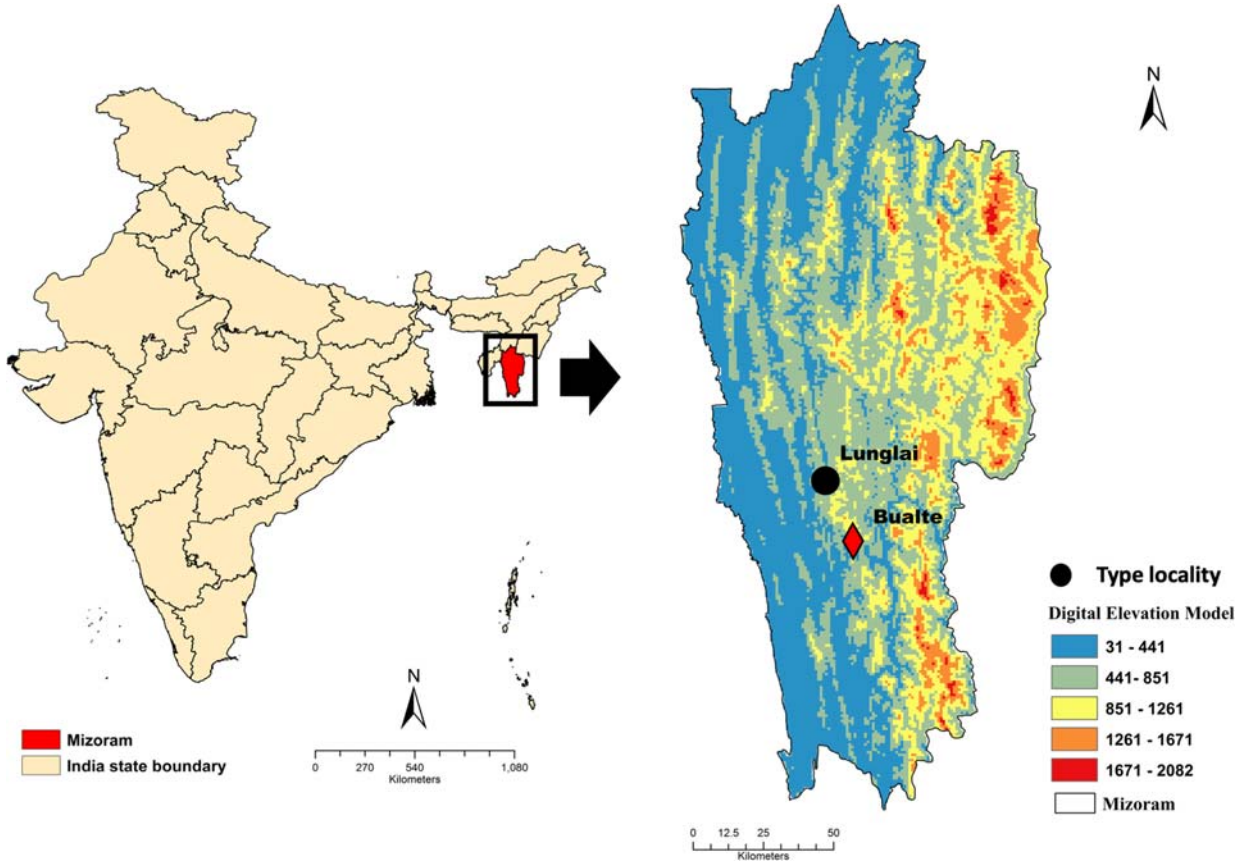

Figure 1. Location of Mizoram in India and digital elevation map of Mizoram State showing collection sites.

northeastern India, but new evidence suggests that the populations from the northeast are allied to *Pila olea,* which was originally described from Cachar, Assam in northeastern India. New molecular evidence suggests that all the populations from northeastern India belong to this species. Further molecular, morphological and anatomical studies are under way. *Pila saxea* is a hill stream species, distributed in the northern Western Ghats (Maharashtra and Gujarat). *Pila virens* is distributed throughout the humid parts of Peninsular India (Andhra Pradesh, Telangana, Karnataka, Tamil Nadu, Kerala, Goa, Southern Gujarat). *Pila scutata* is a Southeast Asian species, with distribution in India restricted to the Andaman and Nicobar Islands (Tan *et al.* 2013). This is understandable since the island chains are geographically proximal to Southeast Asia. All the Indian species of *Pila* are highly divergent at the molecular level. They are not phylogenetically close, thus they do not represent an endemic Indian radiation (Sil *et al.* 2020).

Taxonomic revision of South Asian Ampullariidae is almost a century old (Annandale 1921; Prashad 1925). Cowie (2015) reviewed global ampullarids, including *Pila*. However, there have been no critical taxonomic assessments of Indian *Pila* using molecular tools. The new century has seen a boom in species discovery with the advent of molecular phylogenetics (Deepak and Karanth 2017; Lajmi *et al.* 2016). Many of the descriptions of new species in recent times have employed multiple lines of evidence – molecular tools in combination with morphological, anatomical and ecological data. In this paper, we describe the sixth *Pila* species using multiple lines of evidence, from a hill stream in Mizoram state of Northeast India.

Materials and methods

Sampling site of the holotype

The new species of *Pila* described here was collected from a roadside waterfall four km from Lunglai towards Aizwal in the State of Mizoram, Northeast India in July 2017 (Figure 1). Mizoram is part of an Indo-Burma biodiversity hotspot (Myers *et al.* 2000) and its biota has affiliation with Southeast Asia. The sampling site was at an altitude of 400 m and has moist deciduous forest. This region receives rainfall from the south-west monsoon (June to October), with an annual rainfall of over 2500 mm. The samples were stored in absolute alcohol. The habitat variables such as geo-coordinates, altitude, canopy cover, disturbance, water pH and temperature were also measured.

Morphological measurements

Shells were observed under Carl Zeiss Stemi 508 stereo microscope (Carl Zeiss, Germany). Standard conchological measurements were taken to the nearest 0.01

Table 1. Details of the markers used, its length, PCR annealing temperature and codon position.

Gene name	Primers	Length (bp)	Annealing temperature	Models of sequence evolution	References
Cytochrome c oxidase Subunit 1	LCO and HCO	561	41.9°C	1st codon position K80 +I, 2nd codon position HKY + I, 3rd codon position HKY + G	Folmer *et al.* (1994), Williams *et al.* (2003)
18S ribosomal RNA	18SYLMFOR and 18SYLMREV	383	41.0°C	K80+I	Stothard *et al.* (2000)

mm for all individuals using a digital calliper. A series of photographs at different focal depths were taken using a Nikon D850 DSLR camera mounted with a Nikkor 105 mm macro lens and R1C1 flash unit. Helicon Focus stacking software (Ver 7.0) was used to stack the images. Data on shell measurements for all *Pila* species occurring in India were taken from Annandale (1921), and Prashad (1925). Principal Component Analysis (PCA) analysis was performed in PAST 4 (Hammer *et al.* 2001) to assess the similarity between different species of *Pila* from India and Myanmar.

Table 2. Accession numbers from GenBank for species used for constructing phylogenetic tree.

Species	Location	COI	18S
Outgroup			
Lanistes carinatus	Lake Bisinia, Uganda	EU274560.1	EU274537.1
Lanistes ovum	Lake Mweru, Zambia	EU274564.1	EU274541.1
Lanistes solidus	Chipanda, Malawi	EU274566.1	EU274543.1
Lanistes varicus	Mogtedo, Burkina Faso	EU274567.1	EU274544.1
Species from Africa			
Pila ovata	Uganda	EU274571.1	EU274550.1
Pila speciosa	Kinyasini, Zanzibar, Tanzania	EU274573.1	EU274552.1
Species from Southeast Asia			
Pila ampullacea	Binh Chu, Vietnam	EU528504.1	FJ710254.1
Pila virescens	Vietnam	EU528475.1	EU274551.1
Pila scutata	Hanoi, Vietnam	EU528588.1	EU274547.1
Pila gracilis	Pathumwan, Bangkok	MN104559	MN104528
Pila gracilis	Mueang, Phatthalung	MN104551	MN104526
Pila sp. 1	Mueang, Mae Hong Son	MN104582	MN104531
Pila virescens	Mueang, Trat	MN104567	MN104534
Pila pesmei	Somdet, Kalasin	MN104565	MN104529
Pila turbinis	Thong Pha Phum, Kanchanaburi	MN104538	MN104521
Pila celebensis	Mueang, Phatthalung	MN104543	MN104523
Species from India			
Pila virens	Vallimalai	MN812814	MN733258
Pila virens	Jammalamadagu	MN812806	MN733259.1
Pila virens	Trichy	MN812815	MN733260.1
Pila virens	Valsad	MN812817	MN733261
Pila globosa	Chinsurah	MN812807	MN733244
Pila globosa	Lakhimpur	MN812809	MN733247
Pila globosa	Bhubaneswar	MN812810	MN733248
Pila globosa	Ganjam	MN812811	MN733249
Pila olea	Meleng	MN812812	MN733250
Pila olea	Cachar	MN812813	MN733251
Pila saxea	Saputara	MN812820	MN755936
Pila saxea	Saputara	MN812821	MN755937
Pila mizoramensis n. sp.	Lunglai	MN812824	MN755934
Pila mizoramensis n. sp.	Lunglai	MN812825	MN755935
Samples amplified during this study			
Pila mizoramensis n. sp.	Lunglai	MZ297915	MZ297948
Pila mizoramensis n. sp.	Lunglai	MZ297916	MZ297949

Molecular analysis

Genomic DNA was extracted using the CTAB extraction protocol (Williams *et al.* 2003; Chakraborty *et al.* 2020) from *P. mizoramensis* n. sp. as well as other described species of *Pila* from India. Two molecular markers: one mitochondrial (cytochrome *c* oxidase subunit 1: COI) and one nuclear (18S ribosomal RNA: 18S), were amplified (Table). Each 25 µL reaction consisted of ~60 ng template DNA, 0.3 µM of each primer, 0.25 µM dNTPs mixture, 0.04 µg/mL BSA, 2.5 µM MgCl2, 1X Taq buffer, and 1.5U Taq DNA polymerase. The rest of the volume was made up of Milli-Q ultrapure water. The steps performed during the PCR reactions are as shown: (1) 3 min at 95°C; (2) 20 s at 94°C; (3) 45 s at 41–41.9°C; (4) 2 min at 72°C; and (5) 10 min at 72°C, and finally held at 4°C. Steps 2–4 were repeated 35 times. The annealing temperature at step 3 varied between different primers (see Table 1 for details). Sequencing was carried out at Barcode Biosciences Ltd., Bangalore, India. In addition, sequences of *Pila* species from Southeast Asia and Africa (Table 2) were included in the analyses with four species of *Lanistes* Montfort, 1810, the sister genus of *Pila*, as outgroups.

The sequences were aligned using MUSCLE implemented in MEGA7 (Kumar *et al.* 2016). The uncorrected p-distances of the cytochrome oxidase subunit I (COI) gene between *P. mizoramensis* n. sp. and all other *Pila* species were also calculated in MEGA7. Phylogenetic reconstruction was carried out based on the separate mitochondrial and nuclear gene datasets, as well as the concatenated dataset. Maximum likelihood reconstruction was carried out on the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/) (Trifinopoulos *et al.* 2016). The model of sequence evolution was also assessed with the aid of IQ-TREE web server. Branch support was assessed using 1000 bootstrap replicates and 1000 replicates of SH-aLRT branch test. The best partitioning scheme and the models of sequence evolution for the Bayesian analyses were selected using BIC in PartitionFinder2 (Lanfear *et al.* 2017) (See Table 1). Bayesian reconstruction was performed using MrBayes 3.2 (Ronquist and Huelsenbeck 2003). Two independent runs were carried out, each consisting of four chains that lasted for 5,000,000 generations. The convergence of the chains was assessed by inspecting the lowering of standard deviation of

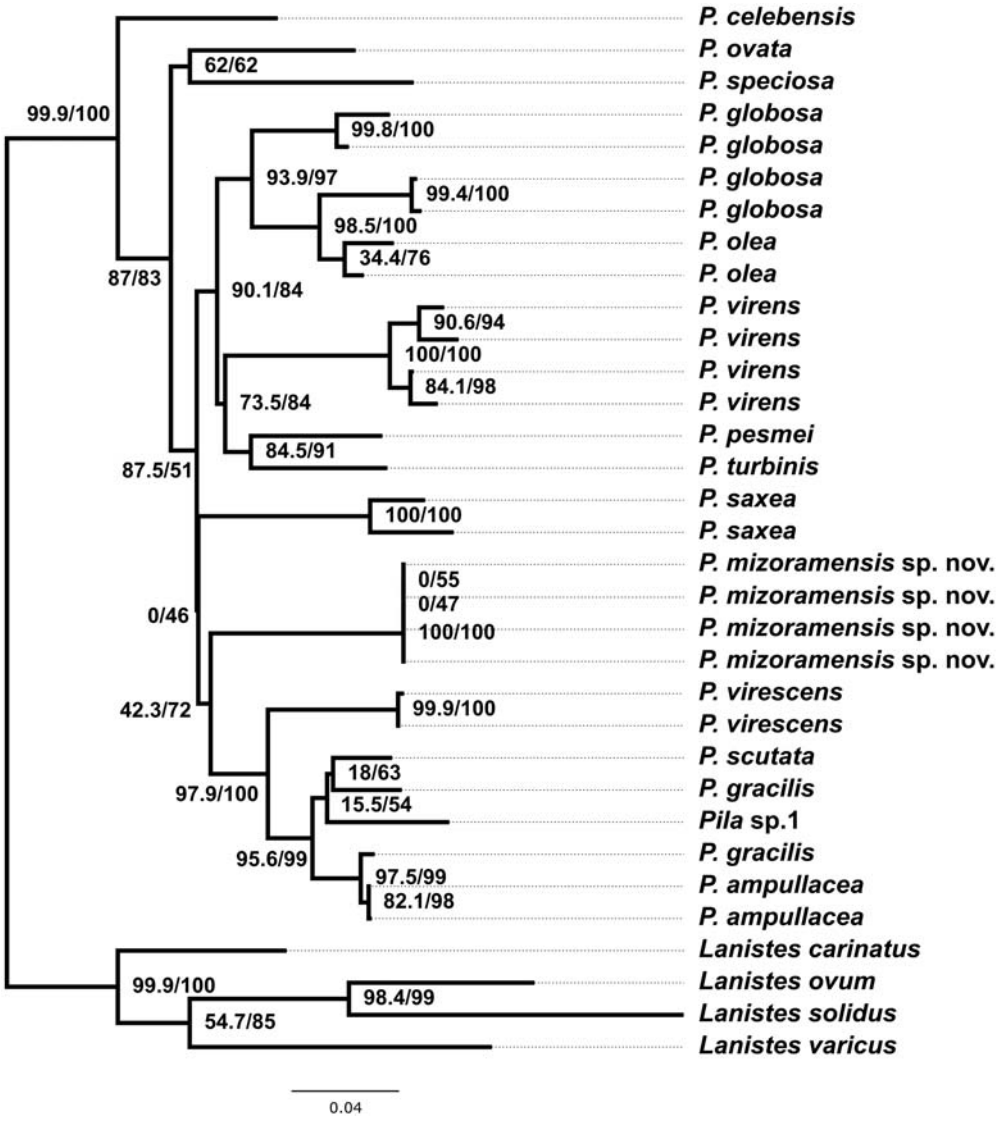

Figure 2. Maximum likelihood tree using COI and 18S for all *Pila* species from India and Southeast Asia for which sequences are available.

split frequency below a value of 0.01. Moreover, we checked the saturation (>200 ESS values) in Tracer v1.7.1 (Rambaut *et al.* 2018). The first 25% of the trees was discarded as burnin.

Abbreviations

ZSI/SRC: Zoological Survey of India, Southern Regional STation, Chennai, India

ATREE: Ashoka Trust for Research in Ecology and the Environment, Bangalore

Results

Phylogenetic analysis

The topologies of trees based on Maximum Likelihood and Bayesian analysis were identical. Furthermore, both concatenated and individual gene trees based on nuclear (18S rRNA) and mitochondrial (COI) were congruent. In all the trees *P. mizoramensis* n. sp. was monophyletic with high support. In the COI (Figure S1 and S4) and concatenated trees (Figure 2 and S3) *P. mizoramensis* n. sp. was sister to a clade containing a majority of species from Southeast Asia [*P. scutata*, *P. ampullacea* (Linnaeus, 1758), *P. gracilis* (I. Lea, 1856), *P. virescens* (Deshayes, 1824)] with moderate levels of support. In the 18S rRNA tree (Figure S2 and S5), however, the *P. mizoramensis* clade formed a polytomy along with three other clades, two of them consisting of Southeast Asian taxa [one clade composed of *P. scutata*, *P. ampullacea*, *P. gracilis* and *P. virescens*; and the second clade consisting of *P. pesmei* (Morlet, 1889) and *P. turbinis* (I. Lea, 1856)]. The third clade was composed of *P. globosa* and *P. olea*. The species *P. mizoramensis* n. sp. was quite distinct from its congeners as evident from the uncorrected p-distance. The uncorrected p-distance in COI between *P. mizoramensis* n. sp. and all other species of *Pila* is comparable to the inter-species divergence seen in this genus (Table 3). The p distance ranged from 10.7% with *P. scutata* to 13-14% with *P. globosa*. Interestingly,

Table 3. Uncorrected p-distance based on COI dataset between all known *Pila* species for which sequence data is available from South and South-east Asia.

	P. mizoramensis n. sp.	*P. globosa*	*P. virens*	*P. olea*	*P. saxea*	*P. scutata*	*P. ampullacea*	*P. gracilis 23*	*P. gracilis 15*	*Pila* sp1	*P. virescens*	*P. pesmei*	*P. turbinis*	*P. celebensis*
P. globosa	0.13-0.14													
P. virens	0.128-0.141	0.100-0.130												
P. olea	0.121-0.135	0.070-0.106	0.100-0.114											
P. saxea	0.131-0.153	0.123-0.146	0.119-0.148	0.127-0.143										
P. scutata	0.107	0.132	0.135	0.137	0.137-0.143									
P. ampullacea	0.124-0.127	0.121	0.109-0.121	0.098-0.102	0.125-0.128	0.062-0.064								
P. gracilis 23	0.122-0.128	0.114-0.128	0.112-0.125	0.100-0.108	0.134	0.068	0.012-0.014							
P. gracilis 15	0.119-0.121	0.105-0.135	0.125-0.134	0.105-0.106	0.130-0.132	0.059	0.070-0.071	0.068						
P. sp 1	0.128-0.132	0.125-0.152	0.130-0.145	0.125-0.131	0.134-0.144	0.084	0.078-0.080	0.080	0.087					
P. virescens	0.131-0.132	0.121-0.128	0.130-0.139	0.114-0.122	0.135-0.139	0.096	0.094-0.096	0.100	0.107	0.112				
P. pesmei	0.121-0.122	0.109-0.123	0.107-0.118	0.107-0.111	0.125-0.139	0.121	0.116-0.118	0.123	0.121	0.127	0.111			
P. turbinis	0.143	0.118-0.137	0.116-0.123	0.111	0.144-0.155	0.118	0.114-0.116	0.109	0.128	0.141	0.121	0.114		
P. celebensis	0.134-0.135	0.116-0.141	0.127-0.134	0.124-0.125	0.143-0.153	0.121	0.135-0.137	0.130	0.125	0.150	0.135	0.135	0.137	

the results indicate that *P. mizoramensis* n. sp. is not sister to *P. saxea*, the hill-stream species found in the Western Ghats of India.

Morphometric analysis

The morphometric analysis using the shell characters for all *Pila* species from India and neighbouring regions shows overlap between *P. mizoramensis* n. sp. and *P. olea*. However, *P. mizoramensis* n. sp. is smaller and more globose than *P. olea* (Figure 3).

Systematics

Class: Gastropoda
Clade: Caenogastropoda
Informal group: Architaenioglossa
Superfamily: Ampullarioidea
Family: Ampullariidae J.E. Gray, 1824
Genus: *Pila* Röding, 1798
***Pila mizoramensis* n. sp.**

Figure 4A–D

Holotype: India, Mizoram State, Lunglai District, Lunglai, 401 m a.s.l., 23.0008°N, 92.7237°E, from roadside waterfalls, four km from Lunglai towards Aizawl. The habitat has perennial waterfalls with good amounts of algae and semi aquatic plants on their cliffs. The snails were collected from the base of the waterfalls among algae, locality code 97, leg. N.A. Aravind, 20.vii.2017, ZSI/SRC/FWM 797 (holotype: shell diameter 23.98 mm, shell height 26.03 mm, Figure 4A–F),

Paratype: Same as above, ZSI/SRC/FWM 798 (1 paratype), AT/Type/FW101-104 (4 paratypes); AT/Type/FW105-108 (4 paratypes), India, Mizoram State, Bualte, 871 m a.s.l., 22.807°N, 92.8124°E.

Diagnosis

Shell small, globose to ovate, olive brown, body whorl angular, suture impressed, columellar callus distinct, brown band along the suture between body whorl and penultimate whorl, fine striations present, protoconch invariably eroded.

Description

Shell. Shell small, six whorls, globose-ovate, ochre brown to olive-brown, very solid, longitudinal striations present, suture deeply impressed, body globose, shell higher than broad (H/B ratio: 1.17), part of the whorls next to the suture is distinctly flattened producing a distinct shoulder-shaped appearance, aperture oval, reflexed at the base (in some individuals), shell surface nearly smooth to the naked eye, inconspicuous longitudinal ridges distinguishable under microscope, the apex little raised,

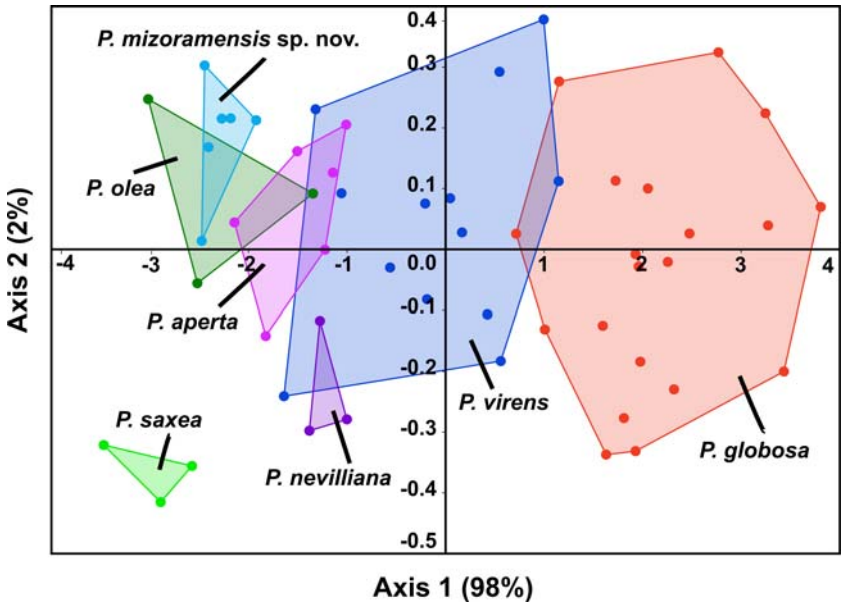

Figure 3. PCA analysis for some species of *Pila* from India and Myanmar

acute, often eroded, protoconch eroded, indistinct, all whorls swollen increasing in size suddenly, columella strongly developed, shell rimate, the umbilicus being almost completely covered by the columellar callus, mouth broadly ovate, the peristome being continuous and the lips not retroverted.

Operculum. Operculum corneous, outline corresponds to that of the mouth and as large as aperture. The external surface is slightly concave and shiny, nucleus large, irregular, growth lines not visible. Inner surface brownish, with large brownish-black boss for the attachment of the muscles. Boss completely surrounded by broad groove. Boss along with groove covers almost half of the operculum (Figure 4E).

Radula. The radula of *P. mizoramensis* n. sp. is typical of the genus, consisting of seven teeth in each transverse row, two marginals and a lateral on each side of a central rachidian tooth. The marginal teeth have three denticulations of which the middle one is the largest, well developed and sharply pointed. The rachidian teeth have five cusps. Of these the central one is the largest, the central region is elevated and the innermost cusp is broad and blunt. The formula is 2, 1, 1, 1, 2 (Figure 5).

Dimensions

Shell height = 22.38-25.76 mm; Shell diameter = 18.92-25.06 mm (n = 6).

Comparative remarks

Pila mizoramensis n. sp. is small compared to *P. olea* (Table 4). Brown bands on the shell are absent as against two in *P. olea,* but present along the suture between body whorl and penultimate whorl, whorls angular as opposed to rounded in *P. olea*, aperture ovate in *P. mizoramensis* n. sp., whereas it is pyriformly ovate in *P. olea*. *P. mizoramensis* n. sp. also differs from *P. olea* in radula structure. The latter has a larger and more pointed rachidian tooth that is narrower than in *P. mizoramensis* n. sp (Figure 4G–L).

Ecology and distribution

Pila mizoramensis n. sp. is known only from two localities in Mizoram, India. This is the third *Pila* species known to inhabit hill streams (Figure 6). The other species are *Pila saxea* from the Northern Western Ghats, India and '*Pila aperta*'*?* from Myanmar (but see Cowie 2015). The new species is found in small groups of up to five individuals at the base of the waterfalls during summer where the water flow is slow and is also seen in the spray zones of the falls amidst algal masses during monsoon. The canopy cover over the falls is around 80%. The type locality is next to the National Highway and habitat is disturbed due to variety of human activities such as dumping of garbage and recreational activities.

Etymology

Named after the state from where the specimens were collected. The specific epithet is a noun in the genitive form.

Suggested common name

Mizoram Apple Snail

Discussion

In this paper we describe sixth species of *Pila* from India. *Pila mizoramensis* n. sp. is the third species of

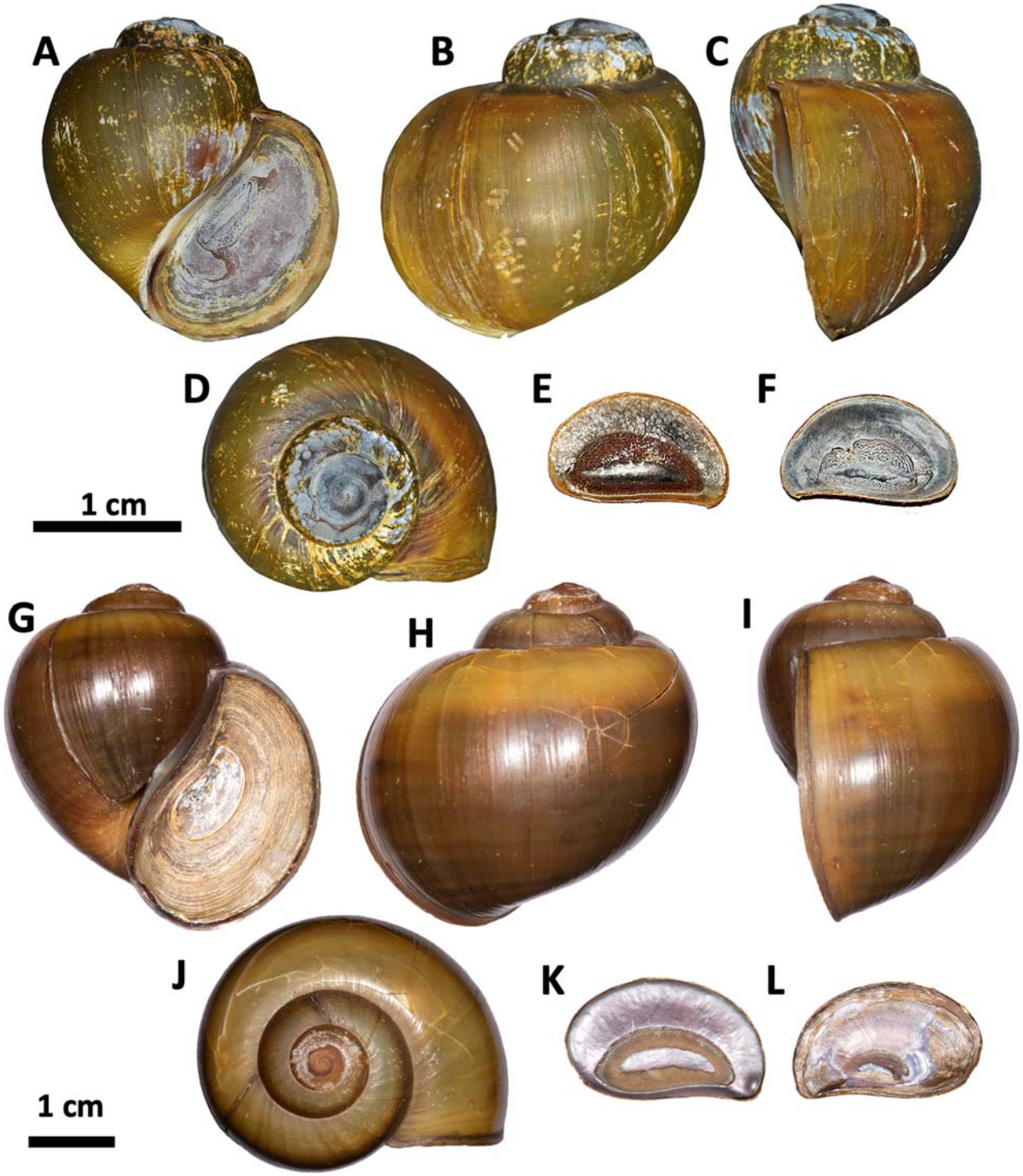

Figure 4. Holotype of *Pila mizoramensis* n. sp. (A–D) and operculum (E–F), *Pila olea* shell (G–J) and operculum (K-L).

Pila that is known to inhabit hill streams and the second hill-stream-dwelling species of *Pila* reported from India. The other two species are *P. saxea*, endemic to northern Western Ghats, India and *Pila* sp. (= '*P. aperta*'?) which is restricted to hill streams of 'Myanmar' (Cowie 2015). All other reported *Pila* spp. are predominantly lentic species, found in ponds, tanks, marshes, paddy fields and other man-made habitats (Annandale 1921; Prashad 1925). North-east India is often considered a part of the Indo-Chinese sub region, along with adjacent Southeast Asia, owing to their biotic similarities. The phylogenetic similarity between *P. mizoramensis* n. sp. and the Southeast Asian taxa further consolidates that theory.

The new species does not show similarity with the other hill streams species of *Pila* and it does not overlap with the other two in morphological space. There is complete overlap with *P. olea* in morphological space. However, the new species is not related to *P. olea* at the molecular level as suggested by both the mitochondrial and nuclear data. The molecular

Table 4. Shell measurements of *Pila* species reported from India and Myanmar.

Species	Height (H)	Diameter (B)	H/B Ratio	Opercular height	Opercular width	Source
P. globosa ($n = 20$)	59.05 ± 5.58	57.15 ± 6.65	1.03	44.85 ± 5.19	28.60 ± 3.28	Prashad (1925)
P. virens ($n = 15$)	41.27 ± 8.24	37.60 ± 7.99	1.10	31.60 ± 6.21	21.53 ± 5.82	Prashad (1925)
P. nevilliana ($n = 3$)	35.33 ± 1.53	30.33 ± 2.36	1.16	26.00 ± 0.87	15.50 ± 0.87	Annandale (1921)
P. aperta ($n = 7$)	30.86 ± 4.38	27.43 ± 3.21	1.13	22.14 ± 2.61	16.00 ± 2.31	Prashad (1925)
P. saxea ($n = 3$)	22.00 ± 4.50	18.00 ± 3.12	1.22	16.00 ± 2.29	08.50 ± 1.50	Annandale (1921)
P. olea ($n = 3$)	25.00 ± 5.29	20.00 ± 9.54	1.25	19.67 ± 4.73	13.67 ± 2.89	Prashad (1925)
P. mizoramensis n. sp. ($n = 6$)	24.33 ± 1.12	20.81 ± 2.36	1.17	18.78 ± 1.05	14.33 ± 1.08	This study

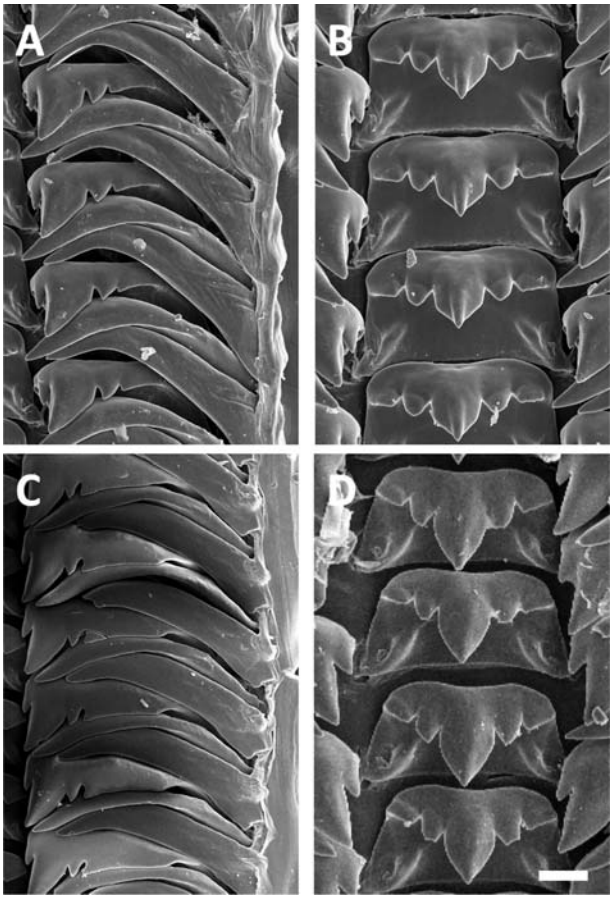

Figure 5. Radulae of Pila species. A. closeup view of rachidian teeth of *Pila mizoramensis* n. sp.; B. Lateral teeth of *P. mizoramensis*; C. closeup view of rachidian teeth of *Pila olea*; and D. Lateral teeth of *P. olea*. Scale bar = 100 microns.

data suggests that *Pila mizoramensis* n. sp. is related to the Southeast Asian species of *Pila*. This is not surprising given that much of northeastern India is part of the Indo-Chinese biogeographic division which covers parts of Southeast Asia as well (Elwes 1873; Mani 1974; Wallace 1876). The phylogenetic analysis also suggests that *P. olea* is a distinct species and closely related to *P. globosa* with a genetic difference of 7–10.6% for COI gene, which is comparable to inter-species divergence observed in this genus (Table 3). Prashad (1925) in his review of ampullariids suggested that *P. olea* might be variety of *P. virens*, here we refute that conjecture.

It should be noted that *P. mizoramensis* n. sp. is highly divergent from the Southeast Asian as well as the Indian taxa, at the molecular and morphological level, as well as at an ecological scale. Both the gene trees and the species tree are highly congruent (See Figure 2 and the figures in Supporting Information) arguing against hybridisation or incomplete lineage sorting (Maddison 1997; Degnan and Rosenberg 2006; Arekar *et al.* 2019). *Pila mizoramensis* n. sp. is retrieved as monophyletic in the nuclear as well as the mitochondrial tree (See Supporting Information). This shows that *P. mizoramensis* n. sp. has not hybridised with any other species. It also rules out any possibilities of incomplete lineage sorting. In both trees, it is sister to the same clade consisting of a handful of Southeast Asian species (see Supporting Information). *P. mizoramensis* n. sp. is highly isolated geographically.

Figure 6. Habitat of *P. mizoramensis* n. sp. A. General view; B. Closeup of the habitat from where the samples were collected.

No other *Pila* species is known to inhabit the hills of Mizoram state. Even at a local scale these hill streams are quite isolated which further reduces the possibilities of range overlap with any other species. Thirdly, the new species dwells in hill streams. All other *Pila* species found in Mizoram are largely lentic or occupy slow-moving rivers. Hence, it is less likely for a specialist species like *P. mizoramensis* n. sp. to encounter a congeneric. All of this evidence suggests that *P. mizoramnesis* n. sp. is a highly divergent and specialist species, whose distribution and habitat specialisation contributed to its isolation.

The Western Ghats and Northeast India exhibit similarity in climate and consequently the forest type is also similar. They constitute what is loosely called the Indian wet zone. The faunal groups from these regions also show phylogenetic and morphological similarity. There have been several theories speculating about the similarities between the fauna of northeast India and the Western Ghats. Such similarities could have resulted from vicariance, dispersal or morphological convergence (Karanth 2003). *P. saxea* and *P. mizoramensis* n. sp. are the only hill stream dwelling species of genus *Pila* distributed in the Indian wet zone but are found in the Western Ghats and Northeast India, respectively. Hence, it is interesting to note that their similarity is limited to the habitat type they occupy. The two species are phylogenetically distinct. They do not show morphological convergence, as they do not overlap in morphological space. Further research has to be carried out to establish how similar is the ecology of these species.

Most *Pila* species are generalists and can tolerate moderate to high human disturbance such as pollution. *Pila mizoramensis* n. sp. is found in roadside waterfalls in the moist deciduous forest. This habitat is experiencing a moderate level of threat from recreational activities and garbage dumping in the type locality. *Pila mizoramensis* n. sp. occurs in low densities in the type locality (<5 individuals per m^2). Extensive survey of similar habitats in Mizoram and adjoining areas might yield more locations.

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Disclosure statement

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