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1. Summary

During routine snail surveillance surveys conducted in Mangochi, Chikwawa and Nsanje Districts, Malawi as part of the HUGS (Hybridisation in Urogenital Schistosomiasis) project, unusual *Lymnaeid* snails were noticed.

Following initial morphological identification and subsequent molecular identification it was confirmed that *Pseudosuccinea columella* is present in Southern Malawi and has the potential to act as an intermediate host of liver fluke in the area, adding an additional dynamic to fluke epidemiology in Malawi.

2. Introduction

The HUGS (Hybridisation in Urogenital Schistosomiasis) Wellcome Trust funded research project incorporates several arms, one of which is malacological surveillance of snail intermediate hosts in areas considered at risk of schistosomiasis transmission within Mangochi, Chikwawa and Nsanje Districts, Malawi.

As part of the study attention is primarily paid to schistosomiasis intermediate snails, however snails that act as intermediate hosts for other trematode parasites are also noted. Here we report the discovery of an alien snail species in Malawi that may be a potential intermediate of liver fluke in the area.

3. Methods

Malacological surveys have been conducted at 12 sites in Mangochi (n=7), Chikwawa (n=2) and Nsanje (n=3) quarterly since October 2021. It was during these surveys that *P. columella* was initially noticed.

Morphological identification was performed initially using the shell microsculpture. Following this the head was incubated in lactic acid to facilitate isolation and photography of the radula¹.

Collected snails were initially screened for trematode cercariae by separating snails in bottled water and exposing to sunlight for 24 hours.

Following cercarial screening snails were transferred to LSTM for molecular identification to confirm species. This was done through amplifying the mitochondrial ribosomal 16S gene. Snails were also screened by qPCR for *Fasciola* spp. to detect prepatent infections².

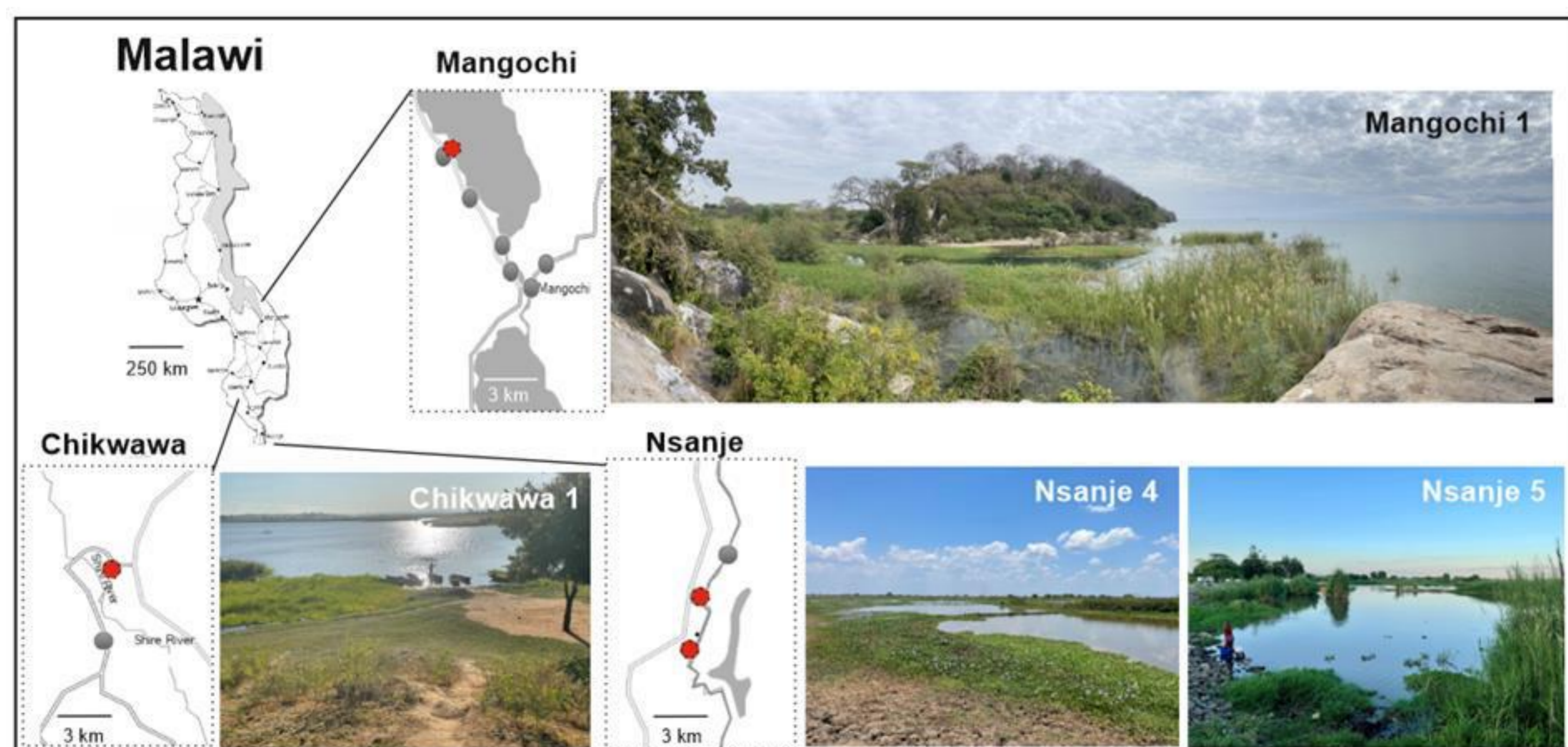


Figure 1. Map showing the sites inspected in Malawi with sites indicated where *Pseudosuccinea columella* has been found, with corresponding pictures. Of note is Mangochi 1 which connects directly to Lake Malawi

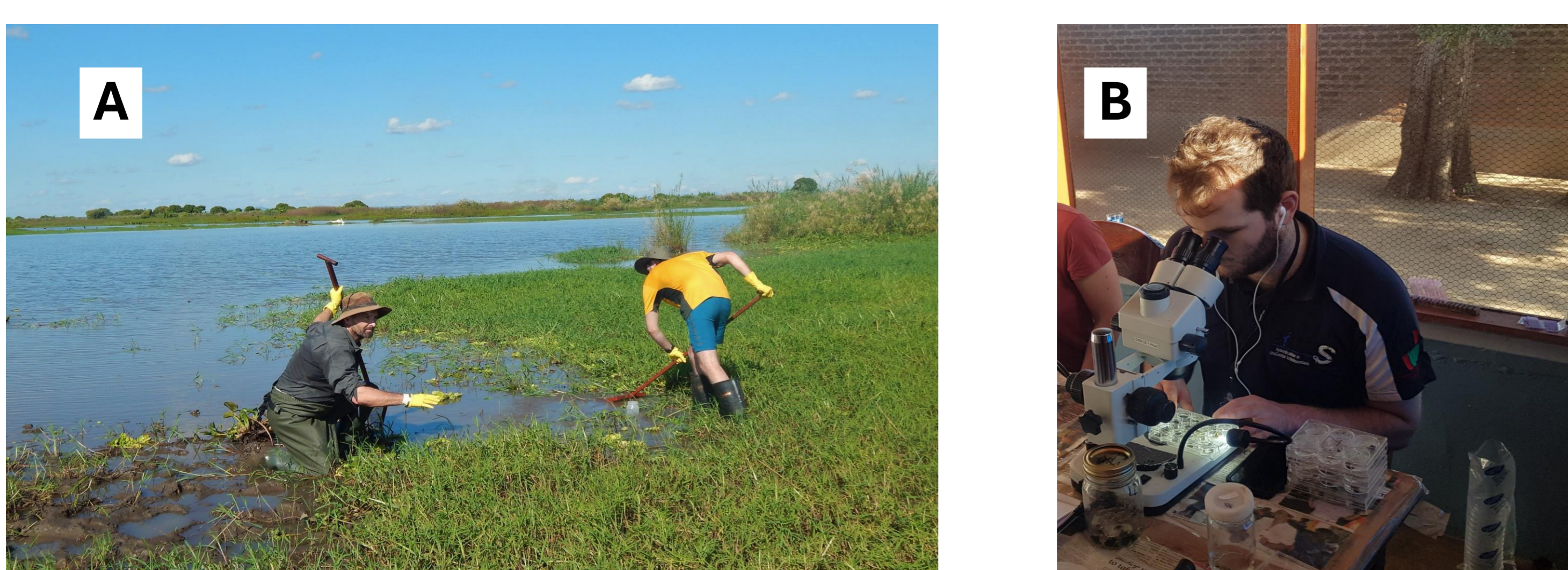


Figure 2. Freshwater snail collection at site Nsanje 4 (A); snail shedding analysis using a dissecting microscope (B)

4. Results

P. columella snails have been found at four of the 12 sites surveyed (33%) with examples found in all three districts, with one site in Mangochi, one site in Chikwawa and 2 sites in Nsanje (fig.1).

Environmental parameters of the locations snails were found were broadly similar as permanent water bodies with muddy substrate with various vegetation present.

Morphological identification chiefly noted the unique shell microsculpture (fig. 3) which is used to differentiate *P. columella* from the more commonly encountered *Radix natalensis*, a similar *Lymnaeid* snail.

It was also noted that there were slight differences in the radula structure between examples of *P. columella* and *R. natalensis*.

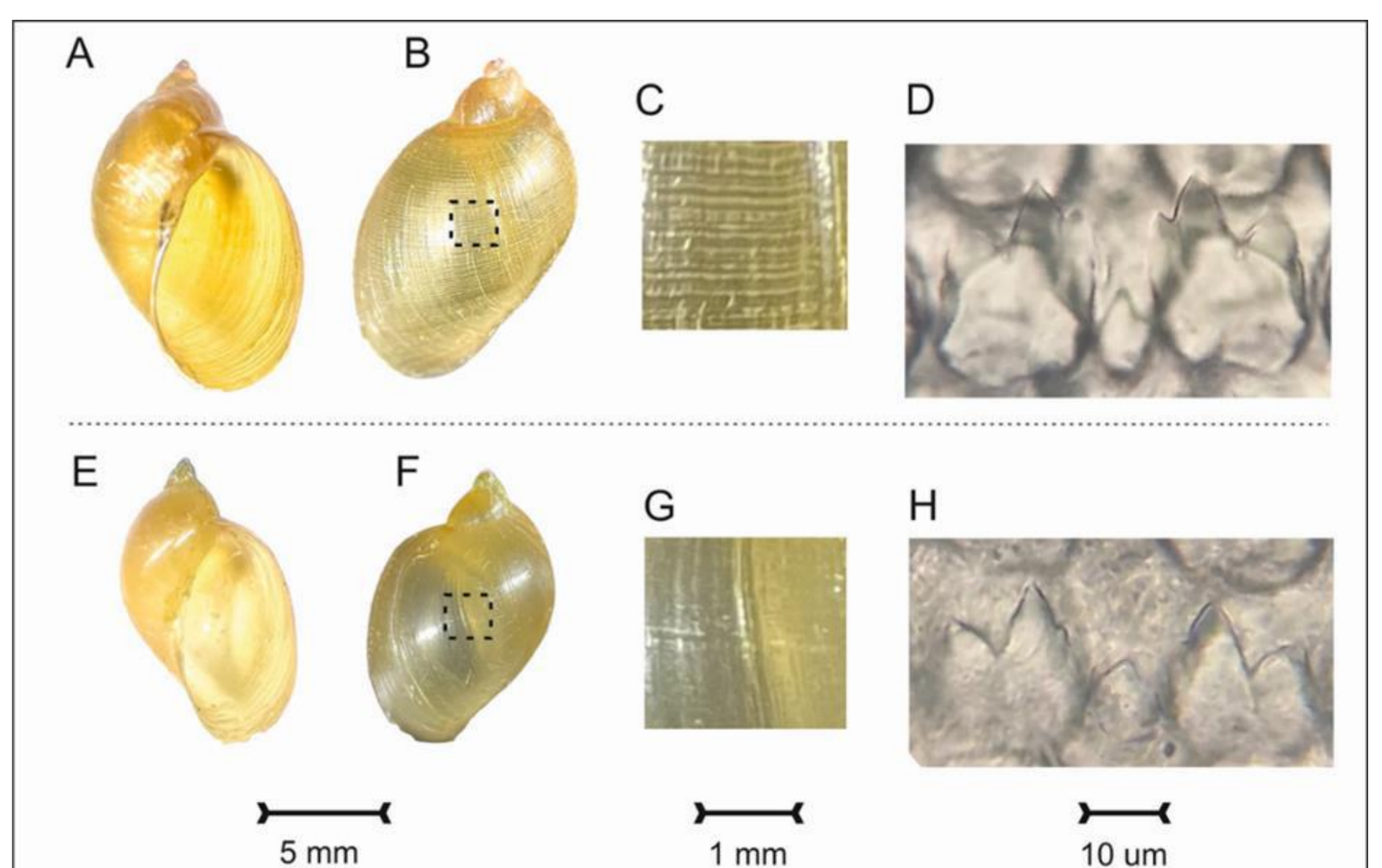


Figure 3. Conchological and anatomical comparison of *Pseudosuccinea columella* (top row) compared with *Radix natalensis* (bottom row); *P. columella* conchology (2A & 2B), shell microsculpture of the black square hatched area (2C) and radular teeth (2D) and *R. natalensis* conchology (2E & 2F), shell microsculpture of the black square hatched area (2G) and radular teeth (2H). Although there is minor variation in the shape of the inner cusp of the first lateral teeth, the discriminatory feature is the periostracum's spiral ridges.

Molecular identification using the 16S gene confirmed the snails as *P. columella* following a BLAST search. Sequences were subsequently deposited in GenBank (accession number OR801605).

Cercarial shedding analysis found that no specimens collected during the survey were actively shedding trematode cercariae.

After performing qPCR assays to screen for *Fasciola hepatica* and *Fasciola gigantica* no conclusive evidence was seen that our samples were infected.

5. Discussion

This report of *P. columella* expands the known geographic range of this intermediate host across Southern Africa.

This adds to the growing list of invasive freshwater snails in Lake Malawi and other Malawian waterbodies.

While no snails were shown to be infected with *Fasciola* spp. parasites, this snail is a well-known intermediate host for both *F. hepatica* and *F. gigantica* in other parts of the world.

There is no formal reporting of animal or human liver fluke in Malawi and local knowledge of the infection is scant.

This discovery further highlights the need to increase surveillance of liver fluke in Malawi due to the newly established presence of an intermediate host alongside *R. natalensis*.