

Distinctive shell and radula features of the native apple snail, *Pila globosa* and the exotic alien invasive species of golden apple snail, *Pomacea diffusa* from selected locations in Sri Lanka

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ABSTRACT

Apple snails of the genus *Pomacea* are natives of tropical and sub tropical South, North and Central America. Over the past few decades, several species of *Pomacea* have been introduced to many countries in Asia. Sri Lanka is home to a single species of native apple snail, *Pila globosa*. But, in the 1980s an apple snail of the genus *Pomacea* had been introduced to Sri Lanka through the aquarium trade. However, there is ambiguity as to the species identity, whether it is *P. diffusa* or the rice pest, *P. canaliculata*. Both species are considered invasive and potential threat to aquatic habitats. The present study was carried out to identify the *Pomacea* sp. recorded from Kandy and Colombo in Sri Lanka through comparison of its shell and radula morphology with those of the native apple snail, *Pila globosa* as well as to *P. canaliculata*. Live samples of the two native and exotic apple snails were collected from Kandy, Colombo and Kurunegala. Shell morphological parameters and indices as well as radulae teeth patterns showed significant differences between the two species. Furthermore, shell and radula features helped confirm the locally present *Pomacea* to be distinct from *P. canaliculata* and it was further confirmed as *P. diffusa*.

Key words: Apple snail, *Pila*, *Pomacea*, shell, SEM, radula

INTRODUCTION

Apple snails are members of Family Ampullariidae spread throughout different freshwater bodies in the tropics and sub tropics of Africa, America and Asia. Reportedly there are approximately 120 different species of apple snails represented in ten genera (Hayes et al., 2009; Hayes et al., 2015). Out of these, *Pila*, *Pomacea*, and *Lanistes* represent the majority of the apple snail species. The genus *Pila* is native to Africa and Asia, *Lanistes* to Africa and *Pomacea* to South, Central and North America (Hayes et al., 2015). The correct identification of some of the different species is complicated due to subtle variations in shell morphology among species and high variations within species. These variations could arise due to variations in habitat characteristics, snail density and the available food quality and quantity (Martin and Estebenet, 2002).

Over the past few decades several species of *Pomacea* has been introduced to countries in North America and Asia as a food source or for the aquarium trade (Halwart, 1994; Yusa and Wada, 1999; Cowie, 2002; Cowie et al, 2007; Cowie and Hayes, 2012). But, in a short period of time these species have spread rapidly and established themselves in natural and agricultural

ecosystems (Cowie, 2002, Cowie and Hayes, 2012). They received much attention due to the serious damage caused to agriculture crops such as rice and taro and due to the threats posed to wetland ecosystems through habitat alterations. *Pomacea canaliculata* is implicated in most of these events and hence considered to be among the world's worst top 100 invaders (Lowe et al., 2000). Over the past few decades this species has become a major rice pest in Asian countries like Japan, Taiwan and the Philippines and it has spread across much of East and Southeast Asia (Halwart, 1994; Cowie, 2002; de Brito and Joshi, 2016). With increased global trade and commerce, there is imminent danger of this species becoming established in Sri Lanka as well.

A single species of apple snail, *Pila globosa* is native to Sri Lanka. It is generally found in stagnant water bodies, slow flowing rivers and streams, and paddy fields (Mordan et al, 2003). It is a grazer of algae and detritus and not considered to damage rice plants or other aquatic plants. But in the early 1980s exotic apple snails of the genus *Pomacea* were first observed in water bodies of Sri Lanka in Colombo and Galle. It is believed that these snails were either purposely or accidentally released from aquaria (Epa, 2006). At present these snails have expanded their range in the wet zone in areas like Colombo, Galle, Gampaha, Kalutara, Matara, Rathnapura and Kandy. Thus far it has not been reported to cause damage to agriculture in the island (Epa, 2006; Perera and Epa, 2008).

The correct identification of exotic apple snails present in the country has been difficult due to the variability in shell morphology. Earlier reports identified the species to be *P. bridgesii* (Nugaliyadde et al., 2001). However, *P. bridgesii* is a rather rare species not known to be available in the aquarium trade (applesnail.net). Other reports have either identified the species as *P. diffusa* or as *P. canaliculata*. (Kumara et al., 1999; Epa, 2006). Hence, more studies in the correct taxonomic identification of these snails are necessary before suitable measures could be adopted to control these species.

The following study was carried out to verify the species of *Pomacea* found in selected locations in Sri Lanka, using its external shell characteristics and radula teeth pattern. Shell morphometry and shell indices of *Pomacea* sp. was compared with the native species of apple snail, *Pila globosa* as a means of distinguishing the two species. In addition, the radula of this exotic species was characterized and compared with that of *Pila globosa* and *P. canaliculata* for further confirmation of the species.

MATERIALS AND METHODS

a. Field sampling

Samples of *Pila globosa* and *Pomacea* sp. were collected from Kandy, Kurunegala (*Pila* samples) and Colombo based on previous studies. The GPS coordinates of each sampling site were recorded using a GPS receiver (Magellan GPS eXplorist 310) (Figure 1). Snails were hand picked and few live snails were brought to the laboratory for further analysis.

b. Shell morphometric parameters

Shell morphometric analysis was carried out to determine species-specific shell features and indices for identification. The following shell morphometric parameters were taken using a digital Vernier caliper. Shell height (SH) was measured as the maximum length from tip of shell apex to base of shell aperture. Similarly, shell width (SW) was measured as the maximum width perpendicular to SH. The aperture height (AH) was recorded as the maximum length from the shoulder of the aperture to bottom of the aperture and aperture width (AW) was measured as the maximum width perpendicular to aperture height. At each study site, morphometric measures of 10 individuals from each species were recorded. Morphometric indices of SW/SH, AW/AH, AH/SH, AW/SW were calculated and used in statistical analysis for species characterization. Both morphometric measurements and shell indices were subjected to Wilcoxon Rank Sum Test using statistical software RStudio (R-3.3.2 version).

c. Radula analysis

Three to five individuals of each species were left overnight (12-15 hrs) in 5% ethanol to anesthetize. The snails were then fixed in Bouin's and dissected to extract the radula in the buccal cavity. The radulae were then immersed in 1M NaOH to soften and remove the surrounding tissues. The radula samples were thoroughly rinsed in distilled water and after dehydrating in an alcohol series (50%, 70%, 90% and 100%) the samples were observed using a scanning electron microscope (Zeiss EVOLS 15).

RESULTS

a. Shell morphology and indices

Typically, live snails of *Pila globosa* have a darker shell color than the *Pomacea* sp. However, due to the variability observed in shell color and patterns this cannot be used as a reliable distinguishing feature of the two species. At first glance, live snails of *Pila globosa* and *Pomacea* sp. could be easily distinguished by looking at the operculum covering the aperture of the snail when the snail retracts into its shell. In *Pila globosa* the operculum firmly seals around the perimeter of the aperture while in *Pomacea* sp. the operculum retracts partway into the aperture (Figure 2A and B). Additionally *Pila globosa* has a more rounded (globose) shell compared to the more ovate shell of *Pomacea* sp., which has a prominent conical shell apex. On the other hand, available literature on *P. canaliculata* indicates the shells to be more globose compared to the ovate shells of *P. diffusa*. When the angles of the shell sutures were compared it was evident that while the collected *Pomacea* sp. shell specimens had a 90° suture at the shoulders, *P. canaliculata* sutures are more indented with the angle being less than 90° (Figure 2B and C). Therefore, it was concluded that the specimens of *Pomacea* collected from Kandy and Colombo were not that of *P. canaliculata*. but of *P. diffusa*.

The average SH and SW of *Pomacea diffusa* was approximately 40 mm and 32.5 mm while in *Pila globosa* it was 32 mm and 28 mm respectively. The average AH and AW for *Pomacea*

diffusa was about 28 mm and 19 mm while those of *Pila globosa* was about 24 mm and 16 mm respectively (Table 1). Statistical analysis of these shell measurements as well as their different shell indices showed a significant difference between the two species (Table 2). Hence, these morphometric measurements could be reliably used in the identification of the two species.

b. Radula morphology

The ampulleriid radula typically is made up of seven rows of chitinous teeth indicated using a radula formula of 2.1. c. 1.2. Typically, these snails have a central tooth bordered by two lateral teeth. Two rows of marginal teeth (inner and outer) flank the lateral teeth on either side of the radula (Martin and Negrete, 2005). The scanning electron micrograms (SEM) of radulae of *Pomacea diffusa* and *Pila globosa* indicated clear, easily distinguishable differences between the two species and with the radulae of *P. canaliculata*:

1. *Pila globosa* radula ultrastructure

The central tooth of *Pila globosa* had a broad main trapezoidal cusp, which was flanked by two obtuse, smaller cusps that were shorter than broad (Figure 3A). The lateral teeth were multicuspid with the middle cusp being the largest. The outer and inner marginal teeth were relatively shorter with broad, bifid distal ends (Figure 3B).

2. *P. diffusa* radula ultrastructure

Two smaller cusps on either side flank the main cusp of the central tooth of *P. diffusa*. Characteristically the main cusp is taller and triangular and the smaller cusps are seen to curve slightly toward the main cusp (Figure 3C). Two broad, multicuspid lateral teeth border the central tooth. The main cusp of this tooth has two smaller cusps toward the central tooth while one smaller cusp is found toward the marginal teeth. The outer and inner marginal teeth are long and face the row of central teeth and each ends in two sharp cusps (Figure 3D). In contrast, the central tooth of *Pomacea canaliculata* has a main trapezoidal cusp flanked by three cone-shaped smaller cusps on either side (Martin and Negrete, 2005). Furthermore, the multicuspid lateral tooth has one main cusp, which is flanked by two smaller cusps on either side (Martin and Negrete, 2005).

The distinctly different teeth pattern of the analyzed pomacid snail in this study further confirmed that the species found was not *Pomacea canaliculata*. We conclude that *Pomacea diffusa* present in the island has a unique radula teeth pattern that could be used for species level identification.

CONCLUSTIONS

The present study indicates the occurrence of *Pomacea diffusa* in Kandy and Colombo. The shell and opercular features could be used to distinguish this exotic apple snail from the native apple snail in the island. This is important in order to make sure that the native species is not harmed during implementation of any control measures of the exotic species.

The SEMs of the radulae of *P. diffusa* are uniquely different from *P. canaliculata* and *Pila globosa*. Hence it allows a reliable method of identification and confirmation of the species.

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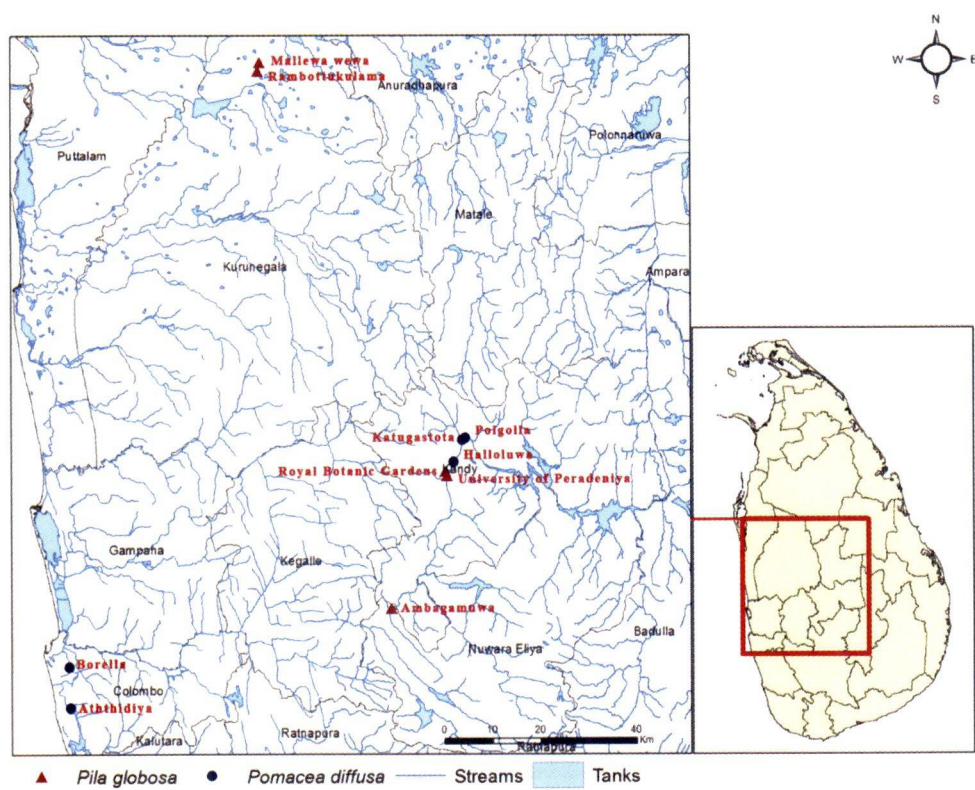


Figure 1:Map of collection sites of *Pila globosa* and *Pomacea diffusa* in Kandy, Kurunegala and Colombo.

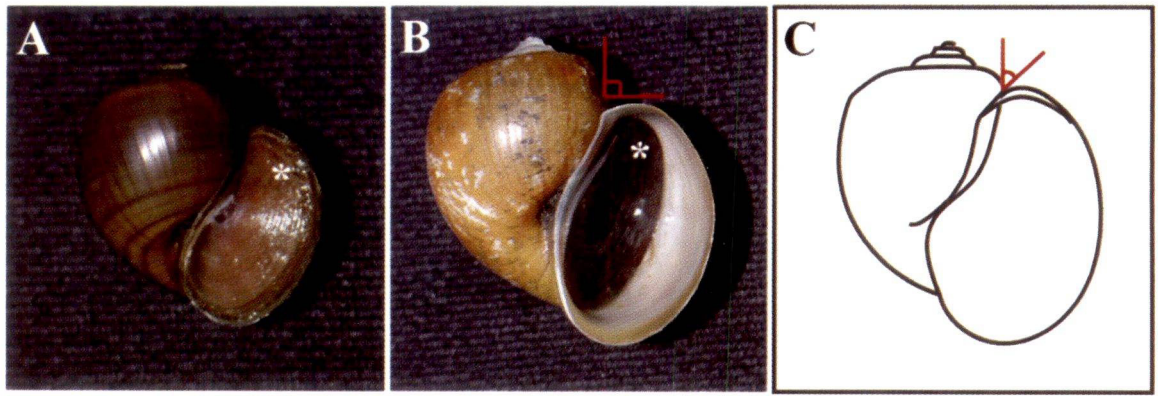


Figure 2: Comparison of shell and opercular features of (A) *Pila globosa*, (B) *Pomacea diffusa* and (C) diagrammatic representation of *P. canaliculata*. (A) *Pila globosa* has a more globose shell where the operculum (*) seals the aperture perimeter whereas (B) *P. diffusa* has an ovate shell and the operculum dips into the aperture partway. The angles marked in red indicate that the shoulder of the suture has a 90° angle in *P. diffusa* whereas in *Pomacea canaliculata* it is less than 90°.

Table 1: Direct Measurements (SH, SW, AH, AW) and Morphometric Indices (SW/SH, AW/AH, AH/SH, AW/SW) of *Pila globosa* and *P. diffusa* (Mean $\pm$ SD in millimeters).

Species	<i>Pila globosa</i>	<i>Pomacea diffusa</i>
Measurements		
SH	32.02 $\pm$ 6.65	40.17 $\pm$ 3.36
SW	28.26 $\pm$ 6.03	32.50 $\pm$ 2.04
AH	24.23 $\pm$ 5.21	27.94 $\pm$ 3.76
AW	16.47 $\pm$ 3.68	19.04 $\pm$ 1.22
Indices		
SW/SH	0.88 $\pm$ 0.02	0.81 $\pm$ 0.02
AW/AH	0.68 $\pm$ 0.01	0.69 $\pm$ 0.06
AH/SH	0.76 $\pm$ 0.03	0.69 $\pm$ 0.06
AW/SW	0.58 $\pm$ 0.02	0.59 $\pm$ 0.02
Number examined	10	10

Table 2: Wilcoxon Rank sum test for comparative shell morphometric data and shell indices.
 ** Statistically significant ($p < 0.05$); n.s. non-significant ($p > 0.05$).

Species	Direct measurements				Morphometric indices			
	SH	SW	AH	AW	SW/SH	AW/AH	AH/SH	AW/SW
<i>P. globosa</i> – <i>P. diffusa</i>	**	**	**	**	**	**	**	n.s.

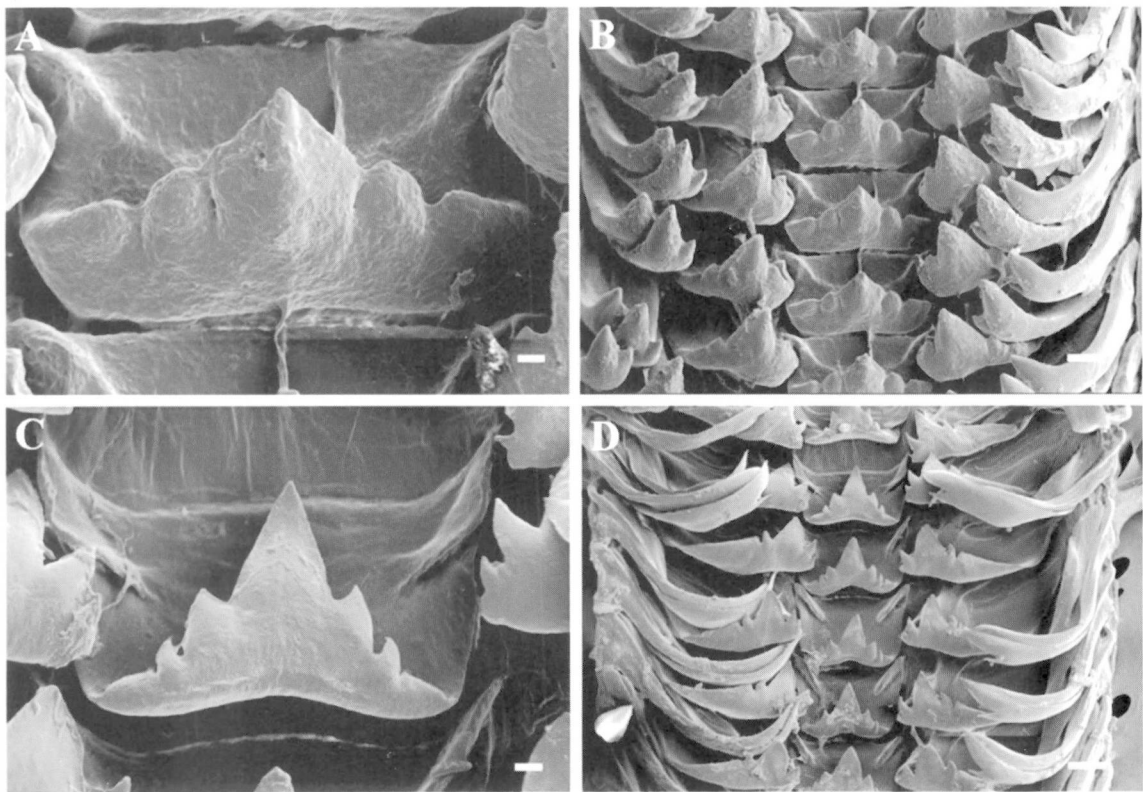


Figure 3: Radula ultrastructure of (A-B)*Pila globosa* and (C-D) *Pomacea diffusa*. (A) Enlarged view of the central tooth of *Pila globosa* with a large trapezoidal cusp in the middle and (B) section of the seven rows of radula teeth. (C) The central tooth of *P. diffusa* with a prominent triangular middle cusp flanked by two smaller cusps each on either side. (D) The seven rows of radula teeth in *P. diffusa* are significantly different from that of *Pila*. (Scale bars indicate 20 μ m in A and C and 100 μ m in B and D).

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