

Disease of the shells of Indian apple snails (Ampullariidae: *Pila globosa*)

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ABSTRACT. The present investigation was undertaken to study a shell disease of the freshwater snail, *Pila globosa*. Observations were made in June-July in four consecutive years. The disease first appears as blisters in the periostracum and then, once the periostracum is lost from these lesions, dissolution of the underlying calcified layer. The numerically predominant bacterial genera in the lesions included *Aeromonas*, *Pseudomonas*, *Escherichia* and *Listeria*. Communication describes this previously unreported shell disease, which may be a health problem in apple snails.

The apple snail, *Pila globosa* (Swainson, 1822) is a vital component of biodiversity playing an important role in the maintenance of aquatic ecosystems. They are widely distributed in Nepal and south western Asia. Apple snails are exceptionally well adapted to tropical regions with periods of drought alternated with periods of excessive rainfall [Subba Rao, Dey, 1989]. *Pila globosa* occurs in all types of temporary and permanent water bodies like ponds, canals and ditches [Jahan *et al.*, 2001]. Though the species is widespread, in certain regions like Nepal, it is decreasing due to habitat loss, and fish poisoning [Subba Rao, Dey, 1989]. Molluscs are excellent sources of trace and minor elements that are essential for the growth and development of humans and they are also used as nutrient supplements for domestic animals and birds [Baby *et al.*, 2010]. In Bangladesh and some parts of India, *Pila globosa* is used as a protein supplement for humans as well as in aquaculture, such as shrimp and catfish farming [Nath *et al.*, 2008; Wilkins, Lee, 2002]. It has also been tested as a biocontrol agent for the aquatic weed *Salvinia molesta* [Thomas, 1975]. The natural pathogens of apple snails are not well known, though Godan [1983] reported the association of microorganisms especially protozoa, both as parasites and as symbionts or commensals, but was cautious in assessing the role of parasites or pathogens in population regulation. Occurrence of disease in *Pila globosa*

may pose a threat to some groups of people residing within its range.

We report the occurrence of a shell disease in *Pila globosa*. Initially, blister formation in the periostracum is seen (Fig. 1B). As the disease progresses, more blisters appear. Once the protein coat has been lost, the calcified layer appears as white patches. This is followed by deterioration of the shell and cavity formation (Fig. 1C) when exposed to environment factors such as varying pH. Once the pH of the environment drops, the exposed calcium part starts to dissolve. Problems may arise, when holes are formed in the cavity, exposing the soft tissues below. The operculum, which helps to prevent drying out during aestivation [Meenakshi, 1964] is also vulnerable to deterioration (Fig. 1D).

The infected specimens used in this study were collected from paddy fields near brackish water in Kannur district, Kerala, India during June – July of 2010, 2011, 2012 and 2013 on the onset of the monsoon. The study was undertaken with the intention of isolating the causative agent from the infected snails. Diseased snails were brought to the laboratory and maintained under natural temperature and moisture regimes. All experimental procedures were carried out under aseptic conditions. The shell was washed and then surface sterilized with 70% ethanol, carefully without rupturing the blisters. Then with the aid of a surgical blade, the blister was cut open and using a micropipette, 5 µL of the blister fluid was transferred to tubes containing an enrichment broth (10 ml) prepared in snail infusion (prepared by boiling healthy *Pila globosa* in double distilled water) at pH 7, containing 1% tryptone, 0.5% yeast extract and 1% NaCl. We have found this medium to be preferable to other bacteriological media used for studying the bacterial flora of snails. The inoculated tubes were incubated at room temperature (30±2° C) for 3 days. The turbid growth in the liquid medium was then streaked onto solidified snail infusion media and incubated at room temperature for three days before the colonies were counted. Differently looking colonies was isolated in pure cultures. Three gram negative and

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Заболевание раковины у индийской ампулярии (Ampullariidae: *Pila globosa*)

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РЕЗЮМЕ. Настоящая работа была проведена для изучения заболевания раковины у пресноводного моллюска *Pila globosa*. Наблюдения проводились в июне-июле в течение четырех последовательных лет. Заболевание проявляется вначале в появлении пузырей на периостракуме, а после того как периостракум исчезает в местах повреждений, в растворении подлежащих кальцифицированных слоев. Доминирующими по численности родами бактерий в местах повреждений являются *Aeromonas*, *Pseudomonas*, *Escherichia* и *Listeria*. Это первое описание заболевания, которое может вызывать проблемы со здоровьем у ампулярий.

