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### Short Communication

## A Note on *Filopaludina martensi martensi* (Frauenfeld, 1865) Artificial Breeding Conditions

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### Abstract

*Filopaludina martensi martensi* Frauenfeld 1865 is an aquatic Viviparidae gastropod found in Thailand, and throughout Southeast Asia. It is popular in the local cousin, a vector of food-borne trematode infections, and used as a bio-indicator of heavy metal contamination, bio-control agent against *Bithynia siamensis* Lea 1856, and a way to reduce the total organic matter content from faeces and feed residue during *Clarias sp.* catfish and Nile tilapia aquaculture. The study aims to establish a protocol for breeding *F. m. martensi* snails in artificial conditions. The snails were maintained in laboratory conditions. The animals gave birth (3 - 4 juveniles) every month. Inflatable, for several hours after the birth, transparent lightly bluish (5 - 6 mm in diameter) spheres are released in which the fully mature juvenile moves, and leaves several hours later after the sphere breaks. All juveniles studied here die after 2 - 3 months failing to grow into a reproductive adults. *F. m. martensi* is an attractive animal that regardless of the current difficulties to maintain and grow its juveniles may be popularised in the ornamental fish trade, and a possible laboratory model animal.

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## 1. Introduction

*Filopaludina martensi martensi* Frauenfeld 1865 is an aquatic gastropod mollusk in the family of Viviparidae. *F. m. martensi* is widely used as food in the cuisine of Thailand, and known from papers dealing with pollution (Piyatiratitivorakul and Boonchamoi, 2008) and parasitic disease dissemination, hosting parasites like *Paragonimus siamensis*, 3 species of Echinostomatidae, and trematode *Multicotyle purvisi* (Sri-aroon et al., 2007; Köhler et al., 2012; Krailas et al., 2012). Although the infection rate of snails is very low (overall trematode cercariae infection of 1.69%), numerous number of the parasite larvae are released (Noikong et al., 2014; Haruay and Piratae, 2019). The need of parasite-free laboratory bred animals for the ornamental fish trade and laboratory model systems serve as the reasons for this work.

*F. m. martensi* was first described by Eduard von Mertens as *Paludina cingulata* in 1860 based on specimen collected by Henri Mouhot (Martens, 1860). George Ritter von Frauenfeld named it *Paludina martensi* (Frauenfeld, 1864). At the moment, 3 species are recognised: *Filopaludina martensi martensi* Fauensfeld 1864, *Filopaludina martensi cambodiensis* Brandt 1974, and *Filopaludina martensi munensis* Brandt 1974.

*F. m. martensi* is found throughout South-east Asia; Thailand, Cambodia, Vietnam, Lao People's Democratic Republic, Malaysia, and Indonesia, and is probably present in southern China (Yunnan) and Myanmar. The characteristic habitats are inland freshwaters and permanent wetlands: canals and ponds, and also rivers, streams, and creeks, including waterfalls. It is abundant and plays important roles in the environment (Köhler et al., 2012). Although described as a filter feeder (Piyatiratitivorakul and Boonchamoi, 2008), it is a scraper accumulating pollutants, and can be used as a bio-indicator of heavy metal contamination. Together with *Pomacea canaliculata* Lamarck 1822, *F. m. martensi* is the most widely distributed mollusk, suggesting its high fitness and good adaptation for new environmental condition (Kwangin et al., 2014). *F. m. martensi* has been suggested as a bio-control agent against *Bithynia siamensis* Lea

1856, first intermediate host of the liver fluke *Opisthorchis viverrini* (Wang et al., 2020). Further, *F. m. martensi* has been suggested (along with *Filopaludina javanica* von dem Busch 1844 and *Sulcospira testudinaria* von dem Busch 1842) as a way to reduce the total organic matter content from organism faeces and feed residue during catfish (*Clarias* sp.) and Nile tilapia culture (Jiwyam et al., 2008; Lailiyah et al., 2021).

*F. m. martensi* is found throughout Thailand except in Khong Chiam District, Ubon Ratchathani Province (Sri-aroon et al., 2007). The characteristic habitat is described as evergreen forests (250 - 1350 m above sea level), medium to large trees allowing filtered sunlight (>10,000 lux at noon), water temperature 22 - 25 °C, dissolved oxygen 7.1 - 9.5 mg/L, swift current in the rainy season, with small-to medium-sized rocks dispersed across. The snails are found on the rough sand, on aquatic plants, and on the rocks. 7.5 % of the collected (549) aquatic mollusks are *F. m. martensi*, contrasting to 65.4 % *Melanoides tuberculata* O. F. Muller 1774 (Krailas et al., 2012). In Cambodia, out of the 153 freshwater mollusk 33, including *F. m. martensi*, are found in Tonle Sap Lake. Accounts for the biodiversity richness of this lake date since 13<sup>th</sup> century (Zhou Dagan, Chinese diplomat), and later in the 19<sup>th</sup> and 20<sup>th</sup> centuries by French-sponsored expeditions (Henri Mouhot, traveller; Auguste Pavie, diplomat; and missionaries like Reeve, Morelet and Morlet). *F. m. martensi* and *F. m. cambodiensis* (lacking the spiral ridges) are sold in local markets, but are not harvested in as large quantities as *Mekongia rattei* Crosse and P. Fischer 1876 and *Mekongia swainsoni* I. Lea 1856, 68 tonnes annually (Ng et al., 2020). *F. m. martensi* is found as alien introduced species in Singapore, and Israel (since 2005). In Israel it is found along *Pomacea maculata* Perry 1810, *Radix luteola* Lamarck 1822, *Physella gyrina* Say 1821, *Planorbella duryi* Wetherby 1879, *Radix luteola* Lamarck 1822, and *Pomacea diffusa* Blume 1957 (Mienis et al., 2015).

*F. m. martensi* has a gill and an operculum, an ovate-conic shell with 5 - 6 convex whorls. The umbilicus is very narrow. The aperture is oblique, ovate and rounded, and cerulean-white in colour (Köhler et al., 2012). Although earlier studies (Bak-

er, 1928; Thiele, 1929) observe sexual dimorphism in the Viviparidae family snails - the right tentacle of males is modified into a copulatory organ (Brandt, 1974; Tarbsripair, 1998), no clear differences are noted in the *F. m. martensi* snails. However, *F. m. martensi* female individuals have larger and heavier shells, with body volume relating to fecundity (Sawangproh *et al.*, 2021). Female viviparid snails bear eggs and embryos (0 - 14) inside their brood pouch until they are fully developed (Berry, 1974).

Abiotic and biotic factors influence the distribution and abundance of *F. m. martensi*. In a study (Wang *et al.*, 2020) aimed to evaluate *F. m. martensi* as a biological control agent against another freshwater snail - *Bithynia siamensis* Lea 1856 (the first intermediate host of liver fluke *Opisthorchis viverrini*), water and soil properties, and snail distribution are studied in Northeast Thailand. While *B. siamensis* has the highest mean dominance in streams and red-yellow podzolic soils, *F. m. martensi* prefers ponds and latosol soils. Both podzolic and latosol soils form under the tropical broadleaf forests (Encyclopedia Britannica, 2016). Red-yellow podzolic soils are of low water holding capacities, low organic matter content and low pH (Dai *et al.*, 1975). Latosol has a medium drainage and sandy or clayey texture with low pH (Ruivo and Cunha, 2003; Suratno *et al.*, 1998). Further, the bacterial communities play crucial functions in soil ecosystems. Proteobacteria are dominant in forest soils - rich in organic matter and clay, with bacterial cell numbers in the range of  $\sim 1.89 \times 10^5$  CFU/gdw of forest soil, whereas Firmicutes dominate in the deforested podzolic areas (Sansupa *et al.*, 2021). The differences observed between biotopes (flora and soil structure), as well as between the river systems (current and sediment load) have been shown to reveal endemism with specific indicator species (Barrios, 2017).

Although the growing number of papers dealing with *F. m. martensi* as an intermediate host of a number of parasites, and an indicator species for environmental pollution, little is known about the basic biology of this widely distributed in Southeast Asia snail. The birth and the growth of juveniles are described, and the reasons for the

inability to grow them to reproductive adults are discussed. The study aims to establish a protocol for breeding *F. m. martensi* snails in artificial conditions.

## 2. Materials and Methods

### 2.1 Sample Collecting

*F. m. martensi* Frauenfeld 1865 snails were obtained from European trader. The snails were maintained in clean laboratory conditions: local tap water with pH 7.42; GH 0.89 meq/L; conductivity 115.4 uS/cm, and Cl 0.11 mg/L (Sofiyiska voda Ltd., Sofia, Bulgaria), and general aquarium consumables: Prime, AmGuard, and Stability (SeaChem Laboratories Inc., Madison, USA) according to the manufacturers recommendations at 25°C and 12/12hr light/dark cycle with 947 lm, colour 7700 K light lux illumination (EHEIM Classic LED lights, EHEIM GmbH & Co. KG, Germany).

## 3. Results and Discussion

*F. m. martensi* snails studied in the laboratory were obtained from European dealer, with uncertain origin - probably Thailand (Figure 1). They have an ovate-conic shell, which apex is acute, violet-black in colour (2 - 3 whorls, 4 - 5 in the older animals) (Figure 2). The colour of the shell of the animals discussed here is white (white velvet, silk and opaque, matt - not gloss). In the literature (Köhler *et al.*, 2012), the colour of the shell is green or dark brown to blackish, with fine spiral lines on the whorls. The umbilicus of the shell is very narrow. The shell surface is clear and even with black traverse, not spiral lines. There are no fine spiral lines on the shell as in the reference literature. The last whorl is swollen. Shell width 29 +/-2 mm, and height 43 +/-4 mm. The aperture is oblique, ovate and rounded, cerulean-white in colour with a black lip. The upper part of the aperture is not acute, with length of 22 +/-1 mm. The operculum (15 x 12 mm) is ovate with concentric lines, brown to black, colour of horn with golden shining. Although Viviparidae snails are recognised as sexually dimorphic (Baker, 1928; Thiele, 1929; Brandt, 1974; Tarbsripair, 1998), *F. m. martensi* snails studied here are not sexually dimorphic with males having functional tentacles, and a penis as seen in the copulat-

ing couple (Figure 3). Female individuals are described to have larger and heavier shells, with body volume relating directly to fecundity (Sawangproh et al., 2021).

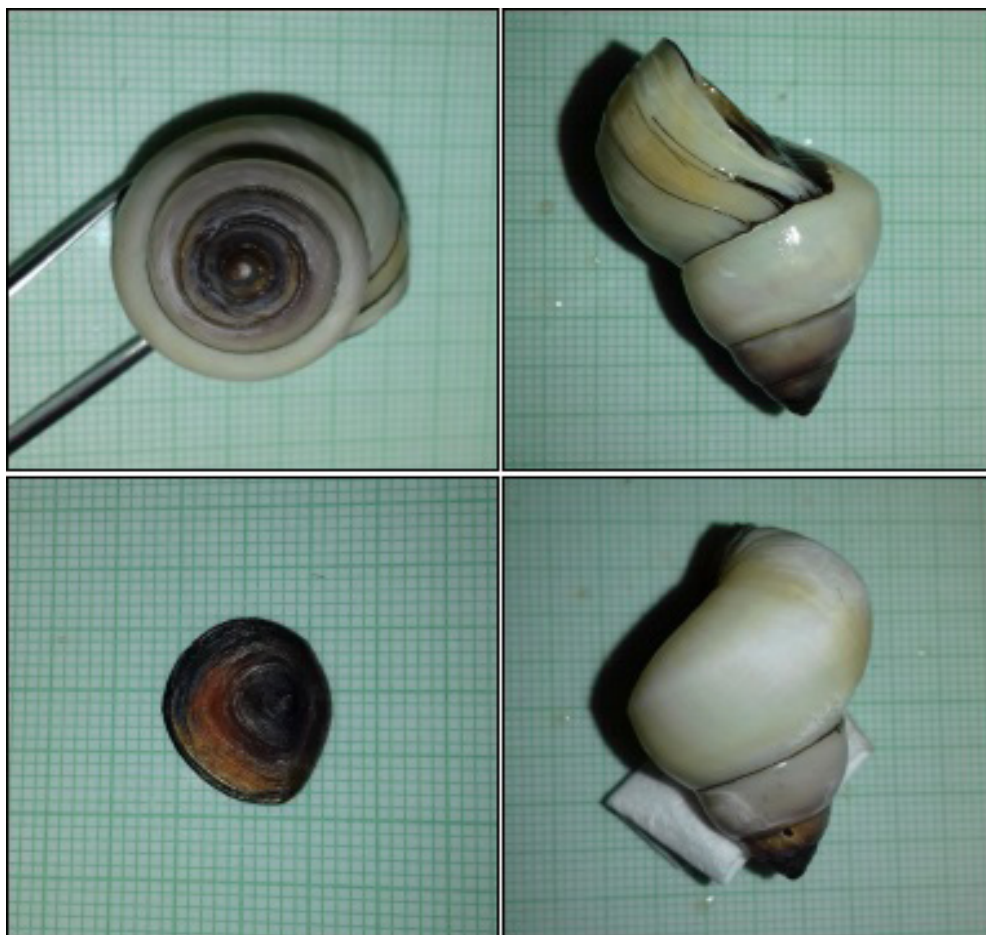
Female Viviparidae snails bear their eggs and brood the embryos inside a uterine brood pouch until they are fully developed. There are 0 - 14 developing juveniles in the brood-pouch of a female. The female gives birth to fully developed juveniles mainly at night (Berry, 1974). The animals studied in the laboratory gave birth regularly, every month or two, to several (3 - 4) juveniles, regardless of the time of the day. Inflatable for several hours after

the birth, transparent lightly bluish spheres 5-6 mm in diameter are released in which the fully mature juvenile moves, and leaves several hours later after the sphere breaks (Figure 4). Unfortunately, all juveniles studied here die after 2 - 3 months failing to grow into a reproductive adults (Figure 5).

In order to overcome this problem and establish the proper laboratory conditions for care and breeding *F. m. martensi* snails, several experimental setups are underway; long-term maintenance at different pH (5 and 8), water hardness, and various food. Local mud sample (a generous gift from Dr. Duangduen Krailas, Department of Biology, Silpakorn



Figure 1. *Filopaludina martensi martensi* Frauenfeld 1865



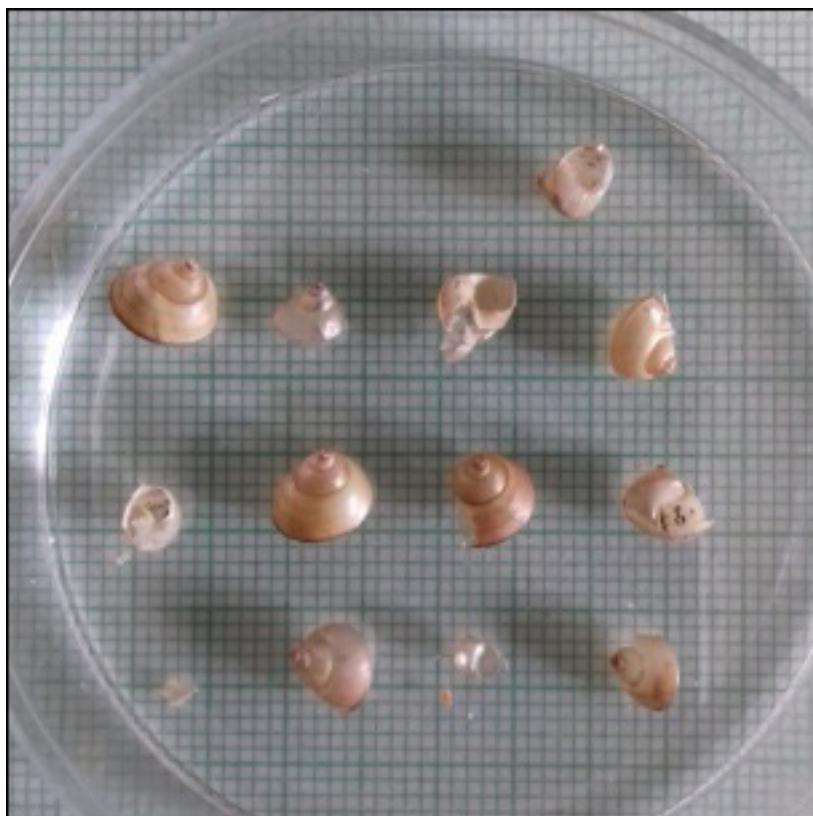
**Figure 2.** *F. m. martensi* shell and operculum



**Figure 3.** *F. m. martensi* copulating couple. The penis of the male animal is clearly visible.



**Figure 4.** *F. m. martensi* juveniles. 3-4 juveniles were born regularly, every month or two, regardless of the time of the day. Inflatable (several hours after the birth) transparent lightly bluish spheres 5-6 mm in diameter are released in which the fully mature juvenile moves, and leaves several hours later after the sphere breaks.



**Figure 5.** *F. m. martensi* juvenile shells. 12 shells of the juveniles studied - all die after 2-3 months, fail-ing to grow into a reproductive adults.

University, Bangkok, Thailand) was obtained in order to investigate the possible algae and microorganisms that could influence the development, growth and survival of the juveniles. Although *F. m. martensi* has a wide distribution range it has a habitat preference for ponds and latosol soils (Wang *et al.*, 2020), constant water quality, water temperature (22-25°C), and dissolved oxygen (7.1-9.5 mg/L) (Krailas *et al.*, 2012). Bottom sediment (Nile tilapia aquaculture) and 'green water' (phytoplankton, chlorophyll-a 392.8 +/-384.3 ug/L) are an important source of nutrients. Interestingly, in this study the temperature (34.8 +/-1.9°C) and pH (8.9 +/-0.8) values are higher (Jiwyam *et al.*, 2008). Similarly, Piyatiratitivorakul and Boonchamoi (2008) maintain *F. m. martensi* juvenile snails (weight 1 - 4 g, height 0.7 - 1.0 cm and width 0.5 - 0.7 cm) at 22-29°C and pH 7-9 with mortality not exceeding 10%. The more acidic environment could be the reason for the inability to maintain and grow the juveniles (Figure 4). An alkaline habitat with a higher GH value (lack of plants), and a convenient biofilm could be the solution of the problem.

The differences observed between biotopes (flora, soil structure and sediment load) have been shown to reveal endemism with specific indicator species (Barrios, 2017). Although *F. m. martensi* has been shown to have habitat preference, its wide distribution throughout Thailand, and the suggested use as a bio-control agent against *B. siamensis* make it difficult to understand the reason behind the failure to maintain and grow *F. m. martensi* juveniles in artificial habitat. The importance of the differential bacterial load (Proteobacteria / Firmicutes ratio) as suggested by the alteration of the bacterial communities during the process of transformation of clayey latosols into white-sand podzols (Sansupa *et al.*, 2021) will be subject to future investigation.

#### 4. Conclusion

*Filopaludina martensi martensi* Frauenfeld 1865, a common viviparid snail inhabiting the ponds and the canals in Thailand, and throughout Southeast Asia, can be maintained and reproduce successfully in artificial conditions. Further, the

birth and the initial growth of *F. m. martensi* juveniles are observed and described. All juveniles were lost when maintained at pH 7.42, GH 0.89 meq/L, conductivity 115.4 uS/cm, temperature 25°C and 12/12hr light/dark cycle with 947 lm, colour 7700 K light lux illumination. *F. m. martensi* is a beautiful animal that may be popularised in the ornamental fish trade, and a possible laboratory model animal. In order to establish the proper laboratory conditions for care and breeding, several experimental setups are underway: long-term maintenance at different pH and water hardness, and various food. Culture and 16S DNA analysis methods are used to investigate the possible algae and microorganisms that could influence the development, growth, and survival of the juveniles.

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#### Authors' Contributions

The author is the only contributor to the work presented in this manuscript, and to the manuscript itself.

#### Conflict of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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