

## Sexual dimorphism and morphometric analysis of *Filopaludina martensi martensi* (Gastropoda: Viviparidae)

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**ABSTRACT.** Sexual dimorphism is the condition where individuals of different sex in the same species exhibit different characteristics beyond the differences in their sexual organs. In this study, individuals of a viviparid species *Filopaludina martensi* from the Kwai Yai River in Kanchanaburi Province (Thailand) were examined for eight shell and operculum characteristics. Sexual differences were observed in size of shell and operculum, with females being larger than males. The results indicated that morphometric analyses are useful to detect subtle differences between sexes in this species.

Половой диморфизм и морфометрический анализ *Filopaludina martensi martensi* (Gastropoda: Viviparidae)

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**РЕЗЮМЕ.** Половой диморфизм – это состояние, когда особи различного пола одного вида имеют различные характеристики помимо половых органов. Восемь признаков раковины и крышечки были исследованы у особей лужанок *Filopaludina martensi* из реки Квай Яй в провинции Канчанабури (Таиланд). Различия между полами были найдены в размерах раковины и крышечки, при этом самки были крупнее самцов. Результаты свидетельствуют, что морфометрический анализ может применяться для выявления незначительных половых различий у этого вида.

been reported, including unionoid mussels [Jass, Glenn, 2004] and some marine, freshwater, and terrestrial snails [Arakawa, Hayashi, 1972; Jokinen *et al.*, 1982; Cazzaniga, 1990; Raven 1990; Brande *et al.*, 1996; Kenchington, Glass, 1998; Estebenet, 1998; Gofas, 2001; Pastorino, 2007]. Most known cases of sexual dimorphism in molluscs are generally related to size differences, where female shells are usually larger than males [Morse, 1876; Warén, 1983; Simone 1996; Pastorino, 2007 and references therein; Riascos, Guzman, 2010].

Freshwater snails for this study were collected from the Kwai Yai River in the Plant Genetic Protection Area of Srinagarind Dam, Sisawat District, Kanchanaburi Province in September 2009, and later identified as *Filopaludina martensi martensi* (Frauenfeld, 1865), which is classified in the family Viviparidae Gray, 1847. Although representatives of the family Viviparidae have been well-studied in India and Burma [Brandt, 1974], investigation of this species has been neglected in Thailand. All Thai genera were previously classified using conchological, radula, and embryonic characteristics [Brandt, 1974] and subsequently by genetic criteria and morphological characteristics [Tarbsripair, 1998].

The presence of sexual dimorphism in snails belonging to family Viviparidae was noted only in the right tentacles of males that are modified into a copulatory organ [Brandt, 1974; Tarbsripair, 1998]. However, the sexual dimorphism in shell morphology of this species was not mentioned in the available literature. The objective of this study was to verify statistically if specimens of *F. martensi martensi*

### Introduction

Sexual dimorphism describes the systematic differences in phenotypic traits between individuals of different sex in the same species [Shine, 1989], and is widespread among living organisms [Shine, 1989; Abouheif, Pairbairn, 1997], including molluscs [Purchon, 1977; Pastorino, 2007]. Many examples of sexual dimorphism in molluscs have

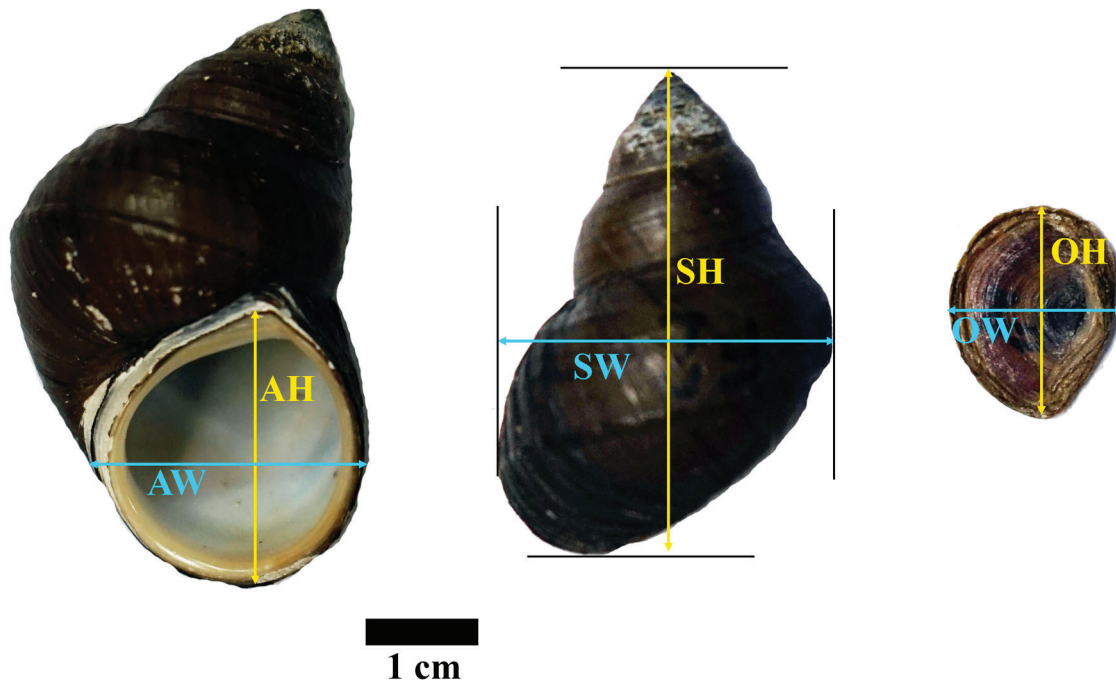


FIG. 1. Distances measured on the shell of *F. martensi martensi*. Shell height (SH), Shell width (SW), Operculum height (OH), Operculum width (OW), Aperture height (AH), and Aperture width (AW).

РИС. 1. Схема измерений раковины *F. martensi martensi*. Высота раковины (SH), ширина раковины (SW), высота крышечки (OH), ширина крышечки (OW), высота устья (AH), ширина устья (AW).

with relatively larger and heavier shells are females, and whether snail sex can be predicted using shell characteristics alone.

### Materials and methods

The specimens of *F. martensi martensi* were collected by hand in September 2009 from a riverbed consisting of gravels and broken concrete near a bank of the Kwai Yai River in the Plant Genetic Protection Area, Srinagarind Dam, Sisawat District, Kanchanaburi Province (14°23'10.3" N, 99°08'33.7"E). To gain more reliable statistical analysis, we decided to do additional snail collection on 30 July 2020 (14°22'48.7" N, 99°08'44.4"E). In total, 104 adult snails (52 females and 52 males) were randomly selected to test the hypothesis of sex-related differences in the laboratory. We classified snails as adult based on their shell size i.e. on assumption that shells width ca. 20 mm and shell length ca. 30 mm belong to mature individuals [Brandt, 1974]. We excluded subadult shells from our analyses in order to avoid size discrepancy resulting from age variation and other causes rather than sexual dimorphism. Subadult shells are green to olive green in contrast to brown to black in adult shells [Tarbsripair, 1998].

*Filopaludina martensi martensi* is a native, common freshwater snail found in canals and ponds in Thailand [Brandt, 1974; Jantataeme *et al.*, 1996;

Piyatiratitivorakula, Boonchamoi, 2008]. *Filopaludina* snails are dioecious, having the male and female reproductive organs in separate individuals. Males have a modified right tentacle which acts as a copulatory organ and can be readily identified. The right tentacle is often longer and curved in contrast to the slender and straight shorter left tentacle of the males and both tentacles of the females [Tarbsripair, 1998]. Females are ovoviviparous. Mature females produce eggs that hatch internally, thereby carrying embryos in the uterine brood pouch before giving birth to live young [Jokinen *et al.*, 1982; Stanisic, 1998]. Sex determination was based on the appearance of the modified right tentacles in males as well as the appearance of embryos in the brood pouches of females. Sex identification was confirmed by exposing the soft bodies after boiling the snails in hot water for 30 minutes and checking the reproductive anatomy. Once the soft bodies were removed, shells and opercula were measured with a Vernier caliper to the nearest 0.01 mm using the measurement scheme modified from Cazzaniga [1990] and Andreeva *et al.* [2017]. Finally, the shells and opercula were weighed to the nearest 0.01 g. Six continuous shell and operculum characteristics were measured (Fig. 1).

General descriptive statistics were performed for both male and female shells separately for all characteristics. Student's t-test was used to determine the significance of characteristics exhibiting sexual

Table 1. Comparative characteristics of shell and operculum quantitative traits of males and females of *F. martensi martensi*.Табл. 1. Сравнительные характеристики количественных признаков раковины и крышечки самцов и самок *F. martensi martensi*.

Measurement/ index	Females (n=52)		Males (n=52)		Significance of differences between means (Student's t-test)
	Range	Mean±SD	Range	Mean±SD	
Shell height (SH)	33.25-43.45	38.24±2.44	30.50-39.47	35.06±2.06	-7.188, p = 0.000
Shell width (SW)	24.61-33.70	28.40±2.56	20.13-30.40	26.11±2.56	-4.565, p = 0.000
SH/SW	1.21-1.59	1.35±0.12	1.20-1.83	1.35±0.15	0.016, p = 0.987 (NS)
Aperture height (AH)	17.60-23.50	20.49±1.63	16.70-29.21	19.36±1.84	-3.230, p = 0.001
Aperture width (AW)	14.30-19.87	17.23±1.43	14.50-19.71	16.50±1.07	-2.937, p = 0.004
AH/AW	1.10-1.30	1.19±0.05	1.07-1.81	1.17±0.10	-1.019, p = 0.311 (NS)
Operculum height (OH)	14.60-19.59	17.19±1.10	14.00-19.43	15.84±1.16	-6.058, p = 0.000
Operculum width (OW)	11.65-16.33	14.34±0.90	10.85-16.30	13.36±1.01	-5.228, p = 0.000
OH/OW	1.13-1.28	1.20±0.03	1.10-1.29	1.19±0.04	-1.612, p = 0.110 (NS)
Shell weight (SWe)	2.12-5.74	3.82±0.88	1.72-5.18	3.05±0.64	-5.089, p = 0.000
Operculum weight (OWe)	0.03-0.23	0.12±0.04	0.03-0.18	0.10±0.03	-2.579, p = 0.011

dimorphism ( $P \leq 0.05$ ). Data were reported as means  $\pm$  standard deviations. Patterns of shell variation were analyzed using principal component analysis (PCA) to reveal if variations in shell and operculum measurements correlated with two distinctly different morphotypes that would correspond with males and females. PCA analysis was run using the R packages “FactoMineR” [Lé *et al.*, 2008] and “Factoextra” [Kassambara, 2017].

## Results

Eight quantitative shell characteristics except three derived characteristics (i.e., SH/SW, AH/AW, and OH/OW) were significantly different between males and females (Table 1). Females possessed longer and wider shells, longer and wider aperture, longer and wider opercula, and heavier shells and opercula than males. PCA based on shell and operculum data revealed that 79.67% of the observed variations could be explained by the first three factors (PC1=50.59%, mostly summarizing variation in general shell size, apertural size, and opercular size; PC2=17.1% mostly reflecting shell height to shell width ratio and shell width; PC3=11.98% mostly reflecting aperture height to aperture width ratio; Table 2).

A plot of individual component scores along the first principal component (x-axis) and the second principal component (y-axis) showed two clusters of individuals overlapping greatly in the four graph quadrants. Male cluster shifted toward the 2<sup>nd</sup> and 3<sup>rd</sup> quadrants, while females were mainly clustered in the 1<sup>st</sup> and 4<sup>th</sup> quadrants (Fig. 2).

## Discussion

Sexual dimorphism involving shell and operculum sizes of *F. martensi martensi* was observed in our study. The earlier studies such as Baker [1928] and Thiele [1929] found similar observation that snails belonging to family Viviparidae are sexually dimorphic. In this study, a consensus of morphologi-

Table 2. Factor loadings for the first three principal axes of a PCA based on eight shell and operculum measurements of *F. martensi martensi*. Characteristics with highest scores are shown in bold.Табл. 2. Факторные нагрузки первых трех осей анализа главных компонент восьми измерений раковины и крышечки *F. martensi martensi*. Полу жирным шрифтом выделены характеристики с наивысшими значениями..

Character	Factor loadings		
	PC1	PC2	PC3
Shell height (SH)	<b>0.94</b>	0.00	-0.01
Shell width (SW)	0.47	<b>-0.83</b>	0.22
SH/SW	0.23	<b>0.90</b>	-0.27
Aperture height (AH)	<b>0.80</b>	0.29	0.44
Aperture width (AW)	<b>0.85</b>	0.13	-0.23
AH/AW	0.11	0.25	<b>0.89</b>
Operculum height (OH)	<b>0.92</b>	0.15	-0.00
Operculum width (OW)	<b>0.94</b>	0.05	-0.13
OH/OW	-0.00	0.26	0.33
Shell weight (SWe)	<b>0.78</b>	-0.33	-0.06
Operculum weight (OWe)	<b>0.83</b>	-0.15	-0.14
Eigenvalue	5.57	1.88	1.32
Cumulative variance (%)	50.59	67.69	79.67

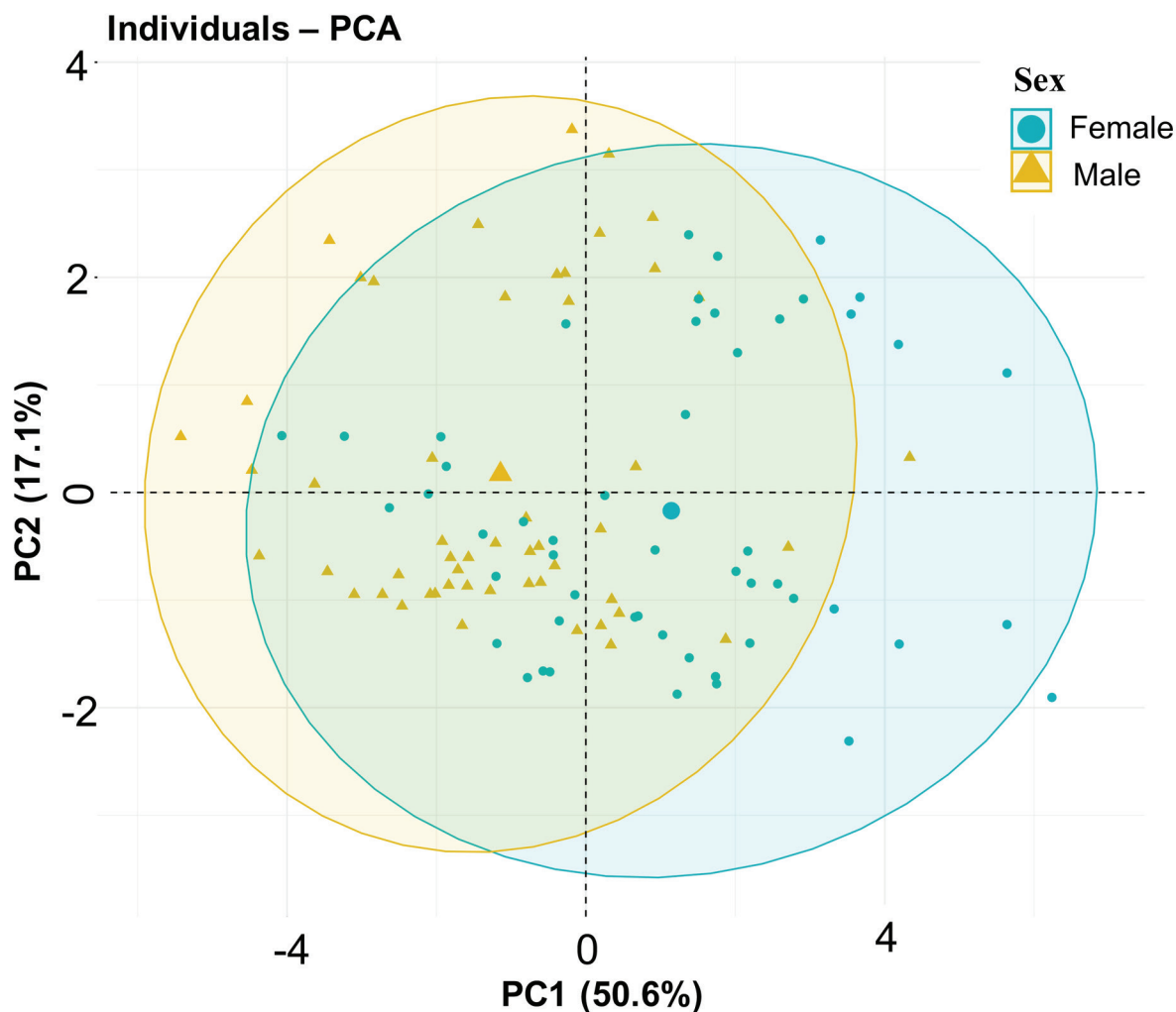


FIG. 2. Principal component analysis of eight shell and operculum characteristics from 104 specimens representing males and females of *F. martensi martensi*. The first two axes (PC1 and PC2) represent 67.69% of the variations. The encircled surfaces represent 95% confidence level of the sample means with the center of each cluster marked by a larger symbol. Factor loadings are presented in Table 2.

РИС. 2. Анализ главных компонент восьми признаков раковины и крышечки 104 экземпляров самцов и самок *F. martensi martensi*. Первые две оси (PC1 и PC2) объясняют 67.69% дисперсии. Обозначенные эллипсами области соответствуют 95% доверительному интервалу, центр каждого кластера обозначен более крупным значком. Факторные нагрузки представлены в Табл. 2.

cal differences between males and females (Table 1) confirmed that shells of females were relatively larger than those of males. Tarbsripair [1998] studied systematic relationships of Thai viviparid snails of the genus *Filopaludina* using comparative morphology, anatomy and biochemistry but failed to show sexual dimorphism because of a lack of morphometric comparison between male and female individuals. In spite of sexual difference, sex identification of empty shells using shell and operculum characters is difficult since the values of all measured parameters overlap.

*Filopaludina martensi martensi* is one of a few species of viviparid snails in which a sex difference has been observed. Similar shell dimorphism

was also observed in other viviparid snails such as *Lioplax subcarinata occidentalis* Pilsbry, 1935 [Cleave, Chambers, 1935], *Viviparus georgianus* (I. Lea, 1834) [Jokinen *et al.*, 1982], *Sinotaia angularis* (O.F. Müller, 1774) [Pagulayan, Cepillo, 1991], *Cipangopaludina chinensis* (Gray, 1833) [Jokinen *et al.*, 1982; Chiu *et al.*, 2002], *Viviparus subpurpureus* (Say, 1829) [Minton, Wang, 2011], and *V. viviparus* (Linnaeus, 1758) [Berezkina, Zotin, 2013]. Other gastropod examples are *Ficus subintermedia* (d'Orbigny, 1852) [Arakawa, Hayashi, 1972], *Melanoides tuberculata* (O. F. Müller, 1774) [Brandt *et al.*, 1996], *Pomacea canaliculata* (Lamarck, 1819) [Estebenet, 1998], and *Olivella plata* (Ihering, 1909) [Pastorino, 2007].



The morphological variation documented here may represent a sexual dimorphism of subspecies *F. martensi martensi* because shells and opercula of adult snails were analysed. Thus, we have reason to believe that morphological difference results from sexual dimorphism. Sexual dimorphism is expressed in the development of distinctive shell features that are generally related to reproduction or spawning in viviparid snails [Pastorino, 2007]. In viviparid snails, females bear eggs and brood embryos inside the uterine brood pouch until they are fully developed. Females usually have a larger body volume than males when mature because body volume relates directly to fecundity [Jakubik 2006; Bocxlaer, Strong, 2016].

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