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Claw and Walking Leg Morphometric of the Fiddler Crab (*Tubuca rhizophorae)* Distributed in the Ca Mau and Bac Lieu Provinces, Vietnam

Anh Ngoc Tran and Quang Minh Dinh

Department of Biology, School of Education, Can Tho University, Xuan Khanh ward, Ninh Kieu district, Can Tho 900000, Vietnam

***Corresponding Author: dmquang@ctu.edu.vn**

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ABSTRACT

This study investigated variations in claw and walking leg morphometric characteristics of the fiddler crab (*Tubuca rhizophorae*) - collected from two sites (Dam Doi, Ca Mau, and Dong Hai, Bac Lieu) in the Mekong Delta, Vietnam. An average of 30 samples per month were manually collected at each site. The samples were randomly collected in different sizes. Subsequently, the samples were fixed and stored in 70% ethanol. The claw morphological parameters were measured such as propodus length (PL), manus length (M), pollex length (P), dactyl length (D), and walking leg length (L). The results did not show significant differences in these claw morphological indices between the two sampling points (Dam Doi, Ca Mau, and Dong Hai, Bac Lieu). However, there were significant variations based on sex; males had larger PL, M, P, D, and L values than females. The PL, M, P, D, and L values in male and female individuals were 5.70±0.05 (mean ± standard error of mean) and 5.07±0.06, 2.54±0.03 and 2.26±0.03, 3.16±0.03 and 2.80±0.04, 3.52±0.03 and 3.17±0.03, 14.5±0.11 and 13.2±0.12. The claw morphological ratios (M/PL, P/PL, D/PL) showed that these meristics had no variations by sampling site or sex. The principal component analysis reveals that environmental factors such as temperature, pH, and salinity (30.46°C, 7.79, and 26.02‰ in DDCM and 30.94°C, 7.74, and 32.97‰ in DHBL) contribute to the variations in some morphological traits according to sex, with temperature and salinity showing the most significant multivariate correlation based on the three principal components studied.

INTRODUCTION

The *Ocypodidae* is one of the dominant fiddler crab families along tropical and near-tropical intertidal zones **(Crane, 2015)**. This species' individuals exhibit high diversity and density and play several significant ecological roles **(Rosenberg, 2001; Skov *et al.*, 2002)**. They feed on mangrove leaves, detritus on the substrate, decaying organic matter from the detrital chain, and initiate food chains with organic fragments. They deepen the substrate for habitation and defense, which alters the pH between the sediment layers and the surface, causing sediment deposition, water retention, and substrate aeration after tide withdrawal **(Tran *et al.*, 2011; Satheeshkumar, 2012)**. Recent literature indicates the presence of 14 species of fiddler crabs belonging to the

Ocypodidae family in Vietnam **(Shih *et al.*, 2022b)**, and *Tubuca rhizophorae* is the common species found in the Mekong Delta **(Shih *et al.*, 2022a)**. As described by **Shih *et al.* (2022a)**, *Tubuca rhizophorae* has a carapace that is not strongly arched, a small size (up to CW ~20mm), a large manus with small tubercles, slender fingers, with a series of slightly enlarged pedestal teeth (approximately 1/2 length of inner margins of fingers) in pollex and dactyl. Furthermore, when describing the external morphology of this species, **Crane (2015)** noted that the carapace's two sides converge less; both sexes' carapaces are dark; they have a prominent marble pattern with lighter tones, from olive green to yellow or light brown. They play an essential role in tropical and subtropical mangrove forests and are a diverse group living in different habitats **(Bezerra & Matthews-Cascon, 2007)**. However, very little information is available on species belonging to the *Ocypodidae* family, especially the *Tubuca rhizophorae*, in coastal areas of the Mekong Delta. There are only a few studies on this species, such as the classification and morphological description studies by **Crane (2015)** and **Shih *et al.* (2022a)**. Furthermore, the characteristics of the morphological criteria of this species have not been studied. The use of differences in morphological criteria of the same genus or species between some regions has gained the interest of researchers, which helps address countless issues, especially the relationship between them **(Wardiatno & Tamaki, 2001; Spivak & Schubart, 2003; Samaradivakara *et al.*, 2012; Qonita *et al.*, 2015)**. Therefore, this study was conducted to provide additional information on claw and walking leg morphological parameters of *Tubuca rhizophorae* in the Mekong Delta, Vietnam.

MATERIALS AND METHODS

Tubuca rhizophorae (Fig. 1) specimens were manually collected during the day in the intertidal areas along the riverbanks. The sampling was conducted from January to April 2024 with a monthly frequency at two points in Dong Hai, Bac Lieu (DHBL) and Dam Doi, Ca Mau (DDCM). Common plant species at two sites were *Acanthus ebracteatus Vahl*., *Avicen-nia marna* (Forssk.) Vierh., *Bruguiera gymnorrhiza* (L.), *Nypa fruti-cans* Wurmb., *Savigny Sonneratia caseolaris* (L.) A. Engl., and *Rhizophora apiculata* Blume. *Bruguiera gymnorrhiza* were dominant at DHBL, respectively, whereas both *Avicennia marna* and *Bruguiera gymnorrhiza* were dominant at DDCM. An average of 30 samples per month were manually collected at each site. The samples were randomly collected in different sizes. Subsequently, the samples were fixed and stored in 70% ethanol before being transported to the laboratory.

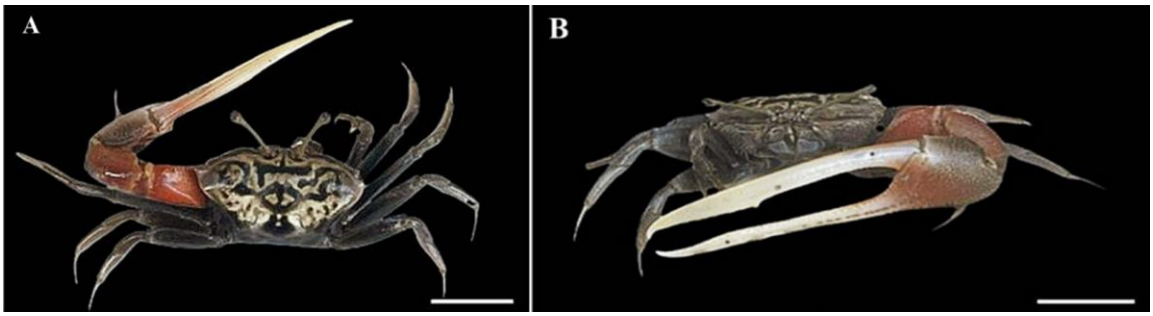

Fig. 1. Male of *Tubuca rhizophorae* (A: top view; B: front view, scale bar: 10mm)

(Source: Dao *et al.*, 2024)

In the laboratory, *Tubuca rhizophorae*, after being identified based on the claw morphological characteristics described by **Tweedie (1950)**, had the following morphological parameters determined: propodus length (PL), manus length (M), pollex length (P), dactyl length (D), the walking leg is in the final position (L) (Fig. 2). Measurements were taken with a caliper that is accurate to the nearest millimeter. The environmental parameters including temperature, pH, and salinity, which are 30.46°C, 7.79, and 26.02 in DDCM and 30.94°C, 7.74, and 32.97‰ in DHBL. The average pH (measured with a pH meter (Hanna HI98127)) and salinity (measured with a Refractometer 0-32 Brix (95000-002)) in each study area were presented; pH and temperature were determined once a month at each sampling point at high tide **(Dinh & Nguyen, 2022)**.

The claw morphological ratios (M/PL, P/PL, D/PL) were used to determine the morphological characteristics of the species.

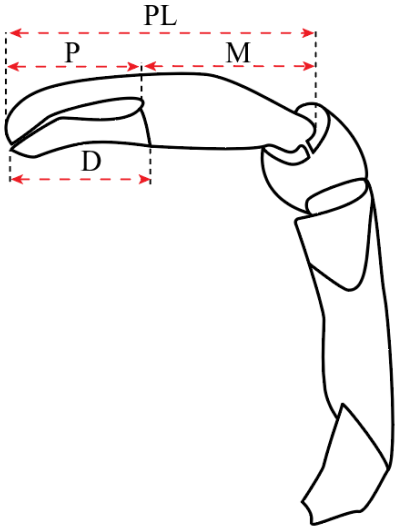

Fig. 2. Illustration depicting the annotations of the morphological characteristics of the *Tubuca rhizophorae* (PL: propodus length, M: manus length, P: pollex length, D: dactyl length)

(Modified from Dao *et al.*, 2024)

The T-test determined differences in propodus length, manus length, pollex length, dactyl length, and walking leg indices between male and female individuals and between

two sites. Principal component analysis (PCA) was used to identify the factors (such as pH, salinity, or water temperature) that influence these variations based on the research methodology of **Nguyen *et al.* (2024)**. The tests were performed at a 95% confidence interval and using Jamovi 2.4.11.

RESULTS

The study results on 407 individuals of *Tubuca rhizophorae* collected from DHBL and DDCM did not show significant differences in the measured morphological indices PL, M, D, P, and L between the two sampling sites. (Table 1).

Table 1. Claw and walking leg morphometrics' variation in *Tubuca rhizophorae* regarding sampling site

Claw and walking leg morphometric parameters	Site	N	Mean	Standard error of mean	t	p
PL	DDCM	291	5.55	0.04	0.72	0.47
	DHBL	116	5.48	0.09		
M	DDCM	291	2.47	0.02	0.20	0.84
	DHBL	116	2.46	0.06		
P	DDCM	291	3.08	0.03	0.98	0.33
	DHBL	116	3.03	0.05		
D	DDCM	291	3.43	0.03	0.66	0.51
	DHBL	116	3.40	0.04		
L	DDCM	291	14.2	0.10	0.95	0.34
	DHBL	116	14.0	0.18		

Note: PL: Propodus length; M: Manus length; P: Pollex length; D: Dactyl length; L: walking leg length; DDCM: Dam Doi, Ca Mau; DHBL: Dong Hai, Bac Lieu.

Environmental factors such as pH, temperature, and salinity differ between the two sampling sites. At DDCM and DHBL, pH, temperature, and salinity were 7.79±0.00 and 7.74±0.00 (t=-16.16, P<0.001); 30.5±0.02 and 30.9±0.02 (t=12.36, P<0.001); 26.0±0.13 and 33.0±0.09 (t=-32.07, P<0.001). The results of the analysis of the influence of environmental factors on the variation of indices by sampling point in Fig. (3) show a clear distinction between the two research sites through the dispersion of data: DDCM is more concentrated toward the lower side of DIM1, while DHBL dominates the upper side of DIM1. The plot clearly illustrates the dispersion of data based on the two principal components, DIM1 and DIM2, with the total explained variance being 41.9 and 23.2%, respectively. When considering the vectors of the variables, it can be seen that pH, temperature, and salinity have vector lengths at different directions in the PCA space. Among them, temperature and salinity have a more significant impact compared to pH when considering DIM1 and DIM2. Overall, the research points show that each variable has its own distinct but not significant contribution to the claw and walking leg morphological parameters.

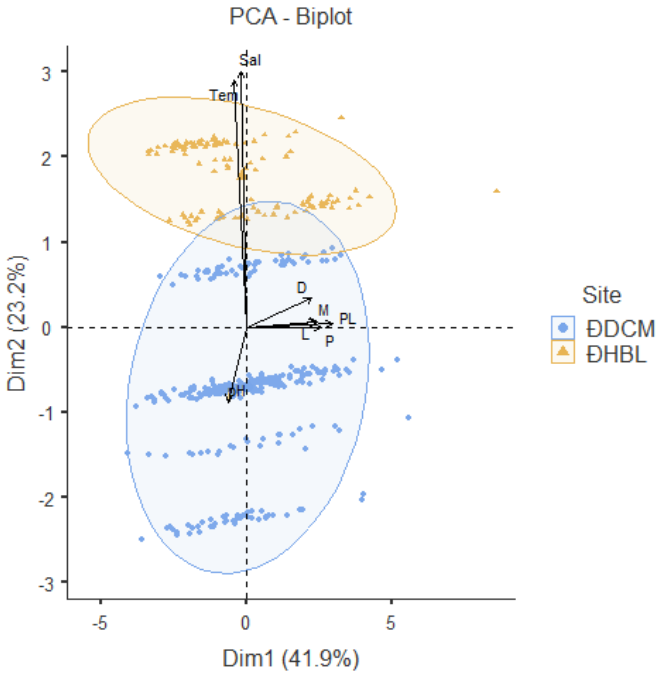

Fig. 3. PCA illustrates relationship between some claw and walking leg morphometrics and environmental factors of *Tubuca rhizophorae* species according to sampling sites (morphological measurements: PL: Propodus length; M: Manus length; P: Pollex length; D: Dactyl length; L: Walking leg is in the final position; Tem: Temperature; Sal: Salinity)

The morphological indices of *Tubuca rhizophorae*, such as PL, M, P, D, and L, showed significant variations according to gender. Specifically, the PL, M, P, D, and L values in males were significantly higher than those measured in females (t-test, $P<0.001$).

Table 2. Claw and walking leg morphometrics' variation in *Tubuca rhizophorae* regarding sex

Claw and walking leg morphometric parameters	Sex	Number	Mean	Standard error of mean	T	p
PL	Male	296	5.70	0.05	-7.45	< 0 .001
	Female	111	5.07	0.06		
M	Male	296	2.54	0.03	-5.50	< 0.001
	Female	111	2.26	0.03		
P	Male	296	3.16	0.03	-6.76	< 0.001
	Female	111	2.80	0.04		
D	Male	296	3.52	0.03	-6.50	< 0.001
	Female	111	3.17	0.03		
L	Male	296	14.5	0.11	-6.72	< 0.001
	Female	111	13.2	0.12		

Note: PL: Propodus length; M: Manus length; P: Pollex length; D: Dactyl length; L: Walking leg length.

The plot clearly illustrates the dispersion of data based on the two principal components, DIM1 and DIM2, with the total explained variance being 41.9 and 23.2%, respectively. The comparison by points shows a clear distinction between male and female individuals, as indicated by the data dispersion: male individuals are more concentrated on the right side of DIM1, while female individuals dominate the left side of DIM1. When considering the vectors of the variables, it can be seen that pH, temperature, and salinity have vector lengths pointing at different directions in the PCA space. Regarding DIM1 and DIM2, temperature and salinity have a more significant impact than pH on the variation of the indices PL, M, P, D, and L. Overall, the research points show that each variable has its distinct contribution and influence on the principal components (Fig. 4).

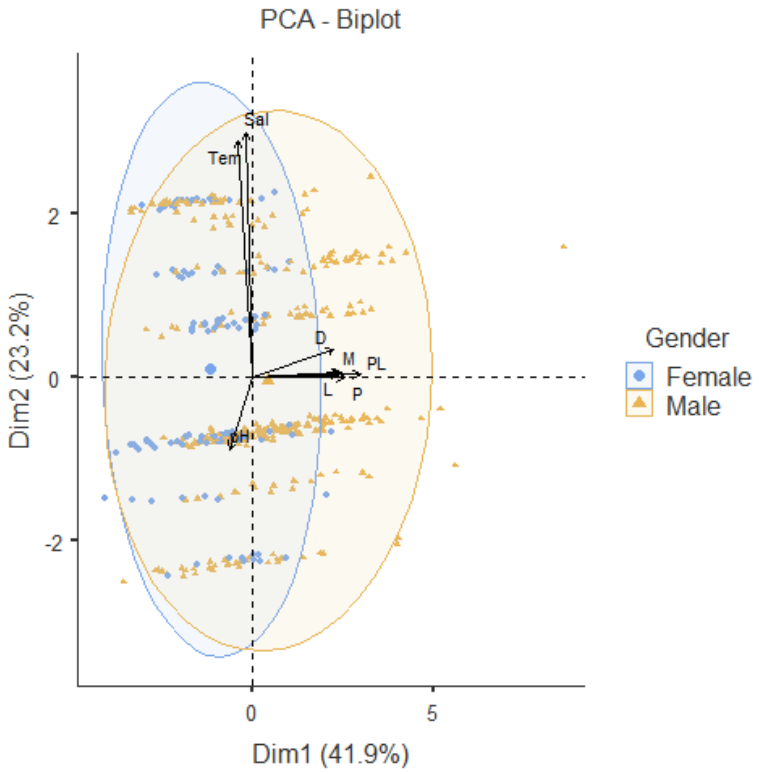

Fig. 4. PCA illustrates relationship between some claw and walking leg morphometrics and environmental factors of *Tubuca rhizophorae* species according to sex (morphological measurements: PL: Propodus length; M: Manus length; P: Pollex length; D: Dactyl length; L: Walking leg is in the final position; Tem: Temperature; Sal: Salinity).

The study used the ratio of the manus length, pollex length, and dactyl length sizes to the propodus length. Based on the statistical results, the values of M/PL, P/PL, and D/PL at DDCM and DHBL for the species do not vary according to the research site.

Table 3. Meristics' variation in *Tubuca rhizophorae* regarding sampling site

Claw and walking leg morphometric parameters	Site	Number	Mean	Standard error of mean	t	p
M/PL	DDCM	291	44.50	0.24	-0.19	0.85
	DHBL	116	44.61	0.59		
P/PL	DDCM	291	55.50	0.24	0.19	0.85
	DHBL	116	55.39	0.59		
D/PL	DDCM	291	62.27	0.49	-0.86	0.39
	DHBL	116	63.09	0.89		

(PL: Propodus length; M: Manus length; P: Pollex length; D: Dactyl length; L: Walking leg length; DDCM: Dam Doi, Ca Mau; DSHBL: Dong Hai, Bac Lieu).

The values of M/PL, P/PL, D/PL showed no variation when considered by gender (Table 4).

Table 4. Meristics' variation in *Tubuca rhizophorae* regarding sex

Claw and walking leg morphometric parameters	Sex	Number	Mean	Standard error of mean	t	p
M/PL	Male	296	44.44	0.27	0.65	0.52
	Female	111	44.78	0.49		
P/PL	Male	296	55.56	0.27	-0.65	0.52
	Female	111	55.22	0.49		
D/PL	Male	296	62.20	0.50	1.13	0.26
	Female	111	63.30	0.86		

(PL: Propodus length; M: Manus length; P: Pollex length; D: Dactyl length; L: Walking leg length).

DISCUSSION

According to the research conducted by **Pramithasari *et al.* (2017)** on the species *Albunea symmysta*, the recorded results showed propodus length; the Aceh population had a longer dactylus length than the Bengkulu population. Another study by **Thurman *et al.* (2021)** on the *Minuca burgers* species also showed morphological variations between two regions: the Caribbean Sea and the Atlantic coast of South America. Furthermore, some researchers have indicated that variation in morphometric characteristics of any species may be caused by various factors, such as geographic region **(Hepp *et al.*, 2012)**, including elevation and latitude. Environmental conditions **(Waldman *et al.*, 1988; Hausch *et al.*, 2013; Qonita *et al.*, 2015)** showed that variations in morphology were due to environmental conditions in the pile ark cockle (*Anadara pilula*), and this finding strengthened the argument of **Barría *et al.* (2011)**, who hypothesized that adaptive responses to environmental conditions brought about morphological variations. Overall, the study points out that each variable contributes separately but insignificantly to the main components. Based on the study of **Thurman *et***

***al.* (2021)**, the results indicate that the site, substrate, and salinity are essential factors that influence specific morphological characteristics.

According to the recorded results, the claw and walking leg morphological characteristics of males are larger than those of females, demonstrating that the characteristics of PL, M, P, D, and L varied depending on sex. The study of **Araújo *et al.* (2012)** also indicates that adult males have larger sizes than females in both mangrove forest areas, possibly because they focus more on body growth, while females allocate energy to the reproductive process. Therefore, it can be concluded that the morphological ratio of species does not vary by sex. Besides, based on the study by **Thurman *et al.* (2021)** analyzing the morphological ratio of *Minuca burgersi* species, differences were observed between males and females.

CONCLUSION

Tubuca rhizophorae did not display significant differences in the claw and walking leg morphological indices propodus length (PL), manus length (M), pollex length (P), dactyl length (D), and walking leg length (L) between the two sampling sites. However, there were significant variations in these traits based on sex, with males having higher PL, M, P, D, and L values than females. Analysis of the morphological ratios (M/PL, P/PL, D/PL) found no variations by sampling site or sex. The principal component analysis revealed that environmental factors such as pH, temperature, and salinity contribute to variations in some morphological traits, with temperature and salinity show the most significant multivariate correlation based on the three principal components studied. The study highlights how the morphological characteristics of *Tubuca rhizophorae* can vary according to site and sex, providing information on this species of fiddler crabs in the Mekong Delta region. Understanding morphological variations is vital to address taxonomic and ecological issues related to this species.

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