



Intra- and Interspecific Variation in Sexual Dimorphism Patterns of Mud Crab Genus *Scylla* Along the Equatorial Region

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Sexual dimorphism is generally obvious in brachyurans, and sexual dimorphism between species is of a higher degree to allow separation of trophic niches compared to the lower intraspecific variations between sexes. Mud crab genus *Scylla* are distributed along the Indo-West Pacific region, and species within this genus often exhibit overlapping niches and similar external morphologies. This study compared the intra- and interspecific sexual dimorphism patterns of three sympatric *Scylla* species from four distinct geographical locations along the equatorial region based on 24 morphometric characters. The consistency of sexual dimorphism patterns between locations was higher than between species. However, reproduction-related characters such as cheliped dimensions and abdomen width (AW) exhibited similar sexual dimorphism patterns across species. Discriminant function analysis based on the 23 morphometric ratios revealed the morphometric intraspecific divergence in all three *Scylla* species from the Asajaya mangrove forest. The cause for this regional intraspecific differentiation of mud crabs from the Asajaya mangrove forest remains unknown.

Keywords: sexual dimorphism, *Scylla*, discriminant function analysis, morphometric ratios, brachyuran

INTRODUCTION

Sexual dimorphism in terms of morphological characters is of functional significance and an outcome of the interaction between natural and sexual selection (Darwin, 1871; Hamasaki et al., 2020). The hard exoskeleton of crustaceans, including brachyurans, allows easy documentation and comparison of external morphological patterns (Barría et al., 2014). The marked difference in morphological characters is pronounced in brachyurans. In general, the larger chela dimensions found in males are associated with agonistic interactions related to mating (Waiho et al., 2015) and male-male competition (Yasuda et al., 2011) whereas females possess larger abdomens due to their reproductive needs, i.e., for brood attachment (Simpson et al., 2016; Parvizi et al., 2017). Although most studies focused on the sexually dimorphic characters linked to reproduction (Parvizi et al., 2017; Hamasaki et al., 2020), other characters might be sexually dimorphic as well, albeit more subtle

and require the aid of quantitative analyses to assess the degree of sexual dimorphism (Bertin et al., 2002). Some of these features that are often used in morphometric discrimination of brachyurans include merus and/or propodus length (PL) of pereopods (Simpson et al., 2016) and supraorbital dimension (Alencar et al., 2014).

Although sexual dimorphism focuses primarily on highlighting the difference in characters between sexes of a species, interspecific and intraspecific variations exist and could be largely due to sexual selection or niche divergence (Lailvaux and Vincent, 2007; Hirst and Kiørboe, 2014; Selz et al., 2016). Interspecific sexual dimorphism is often used to describe the ancestral relationship and phenotypic conservation between species and has been reported in some anomuran (Barría et al., 2014) and brachyuran species (Miyajima and Wada, 2017). Intraspecific or spatial variation in sexually dimorphic characters between populations are linked to mutation or genetic drift (Ritchie et al., 2007), intraspecific mating system evolution (Baur et al., 2019), predation/resource-mediated ecological selection (Scharnweber et al., 2013), or sexual selection for novel/exaggerated signals such as coloration (Selz et al., 2016). Intraspecific latitudinal variations in the magnitude of sexual dimorphism were also reported in both marine (Lima-Filho et al., 2017) and freshwater fish species (Estlander et al., 2017). Variation in environmental conditions is postulated to cause changes in morphological characters and evolutionary patterns and subsequently influence sexual dimorphism patterns between and within a species (Lima-Filho et al., 2017). However, instead of interspecific comparison, intraspecific comparison of sexual dimorphism patterns could highlight variations between populations due to the reduced phylogenetic effects (Shelomi, 2012; Lima-Filho et al., 2017). Thus, the connection between variation in sexual dimorphism patterns and ecological adaptations could be deduced by studying the differences in external morphological characters between populations (Kalate et al., 2017). However, unlike fish (Gunawickrama, 2008; Laporte et al., 2018), intraspecific character difference is less documented in brachyurans (Kalate et al., 2017). Most studies on sexual dimorphism of brachyurans focused primarily on one species or inter-species due to the obvious morphological differences between sexes and species (Peiró et al., 2012; Alencar et al., 2014; Simpson et al., 2016; Parvizi et al., 2017; Fazhan et al., 2021b).

Mud crabs (genus *Scylla*) are naturally occurring in the equatorial region and at least two species co-exist sympatrically in most populations (Overton and Macintosh, 2002; Fazhan et al., 2017a). Thus far, the sexual dimorphism of *Scylla serrata* adults (Presilda et al., 2018) and *Scylla paramamosain* crablets (Shi et al., 2019) have been described. Recently, we characterized and compared the sexual dimorphism of three sympatric species in the equatorial region, i.e., *Scylla olivacea*, *Scylla tranquebarica*, and *S. paramamosain* based on 10 morphological characters. The overall body size and cheliped dimensions were male-biased whereas abdomen size was female-biased in all *Scylla* species. However, spatial and temporal factors were excluded in the previous study to allow a general representation of each species (Fazhan et al., 2021b). To understand the interspecific and intraspecific

variations in the sexual dimorphism patterns of brachyurans, the sexual dimorphism patterns of 24 morphometric characters for three *Scylla* species (*S. olivacea*, *S. tranquebarica*, and *S. paramamosain*) from four geographical locations along the equatorial region were determined and compared in this study. These 24 morphologically defining characters of mud crab were used by Keenan et al. (1998), in combination with molecular data, to separate *S. serrata* into four distinctive *Scylla* species. Further, spatial differences in morphological characters of each sex were analyzed to determine if morphological variations exist among populations of the same species.

MATERIALS AND METHODS

Sampling and Species Identification

Mud crabs of the genus *Scylla* were sampled from four stations along the equatorial region representing three seas in Malaysia, namely the Malacca Strait, Matang Mangrove Forest Reserve, Perak (4°45'N, 100°37'E); South China Sea, Setiu Wetlands, Terengganu (5°39'N, 102°43'E); South China Sea, Asajaya Mangrove Forest, Sarawak (1°32'N, 110°29'E); and the Sulu Sea, Kota Marudu Mangrove Forest, Sabah (6°44'N, 117°1'E) (Figure 1). Sampling was conducted over 3 years from April 2012 until March 2015. All sampling locations are landing grounds of mud crabs and, hence, no specific license is required for their acquisition. To obtain a targeted number ($n = 300$) for each sex per species per location, additional crab samples were also measured from fishermen of the same sampling sites. After acquiring the required morphometric measurements, crabs were either returned alive to the fishermen or released to the wild. Crabs were identified to species level according to the morphological characteristics detailed in Keenan et al. (1998) and Fazhan et al. (2020). Only healthy mature specimens with carapace width (CW) above 95.0 mm (Waiho et al., 2016b), with complete appendages and no visible external abnormalities (Waiho et al., 2017; Fazhan et al., 2018) were included in this study to minimize the effect confounding variables including sexual maturation, allometric growth and compromised immunity. Sex was determined based on the abdominal morphology (Waiho et al., 2016a; Fazhan et al., 2017b). Males were identified based on their triangular-shaped abdomens whereas mature females exhibited prominent globular and darkened abdomens (Ikhwanuddin et al., 2011). Three species, i.e., *S. olivacea*, *S. tranquebarica*, and *S. paramamosain* were present in all four geographical locations (Keenan et al., 1998; Fazhan et al., 2017a). A total of 1,800 crabs were obtained from each sampling station within 3 years.

Morphometric Measurements

Twenty-four (24) morphologically defining characters described by Keenan et al. (1998) were measured for each individual. They are divided into five sections, namely carapace, frontal lobe, sternum, pereopods and chelipeds (Supplementary Table 1). Each character was measured to the nearest 0.01 mm using digital Vernier Calipers. CW was used as a body size index (Hartnoll, 1982; McLain and Pratt, 2011).

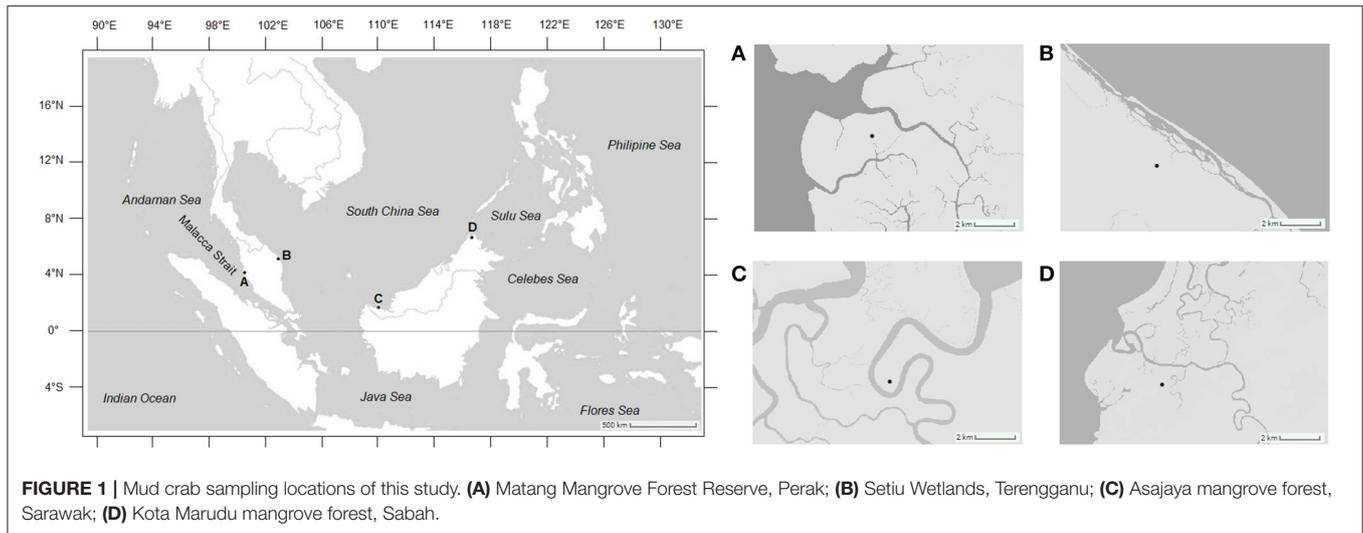


FIGURE 1 | Mud crab sampling locations of this study. (A) Matang Mangrove Forest Reserve, Perak; (B) Setiu Wetlands, Terengganu; (C) Asajaya mangrove forest, Sarawak; (D) Kota Marudu mangrove forest, Sabah.

Data Analysis

All data analyses were performed using IBM SPSS Statistics version 25. As we aimed to characterize sexual dimorphism patterns according to species and locations, possible seasonal variations were not being considered in the analysis. Individuals were grouped according to species, followed by sampled location and sex. All variables were checked for normality using the Kolmogorov-Smirnov test and homogeneity of variance using Levene's test. One-way ANOVA with Welch's correction and subsequent Games-Howell *post-hoc* test were used to compare CW between sex, location and species. Multivariate analysis of covariance (ANCOVA) was used to test for differences in all morphological characters between sexes and locations (independent variables), after controlling for body size, i.e., CW as the covariate. If the multivariate ANCOVA is statistically significant, ANCOVA was performed on each morphological character between sexes based on each location followed by Bonferroni *post-hoc* test, after adjusting for the covariate (i.e., CW). To further characterize the intraspecific differences in characters of specific sex across different locations, 23 morphometric ratios (excluding CW) were divided by CW to ensure values were independent of total size and subsequently analyzed using stepwise discriminant function analysis. A minimum F value of 3.0 (0.05 significance level) was used based on previous studies on the same genus (Keenan et al., 1998; Fazhan et al., 2018). Wilks' lambda (U statistic) was used to determine the significance of the discriminant functions and subsequent V transformation of Bartlett's (Chi-Square statistic) was performed to validate the significance of the lambda value. The robustness of the function was cross-validated using the split-sample validation method available in the SPSS program.

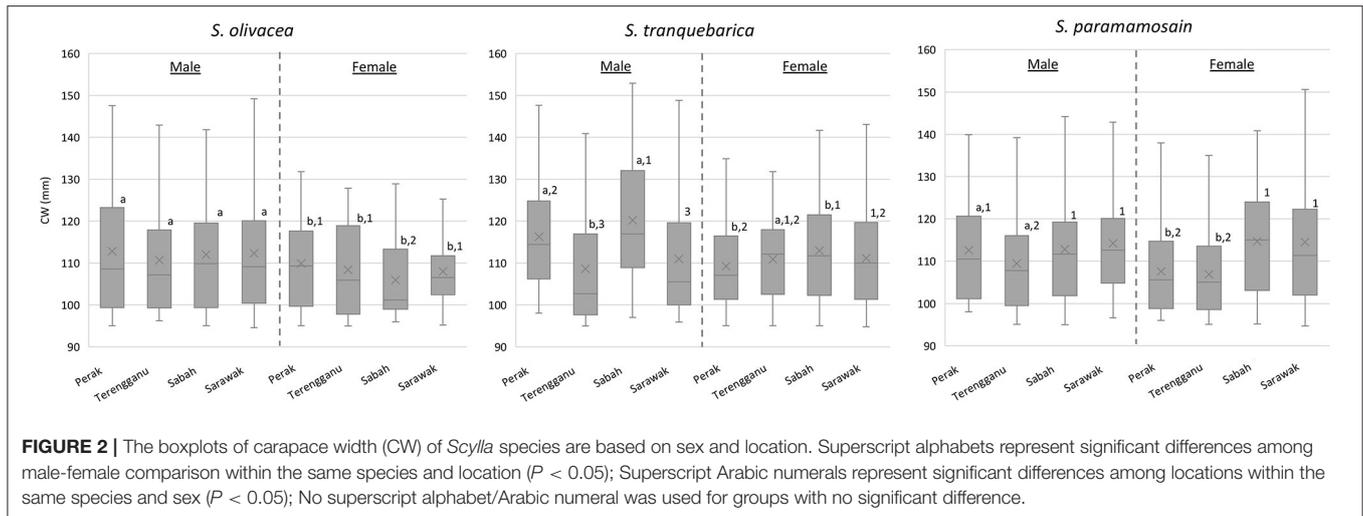
RESULTS

Sexual size dimorphism in terms of CW was consistently male-biased across locations only in *S. olivacea* (all $P < 0.05$; **Figure 2**).

Interestingly, females of *S. tranquebarica* from Terengganu exhibited larger CW compared to their male counterparts ($P = 0.018$), whereas CW of *S. tranquebarica* from Sarawak ($P = 0.893$) and *S. paramamosain* from Sabah and Sarawak showed no significant difference between sexes ($P_{\text{Sabah}} = 0.087$; $P_{\text{Sarawak}} = 0.793$). Males of *S. olivacea* did not vary greatly in their body size ($P = 0.258$), while males of *S. tranquebarica* and *S. paramamosain* from Terengganu exhibited significantly smaller CW compared to those at different locations (**Figure 2**). Females of *S. tranquebarica* and *S. paramamosain* showed similar patterns, with those from Sabah were of significantly larger body sizes. In contrast, *S. olivacea* females from Sabah were significantly smaller than those from other locations.

The mean and SE of the morphometric measurements for both sexes of *S. olivacea*, *S. paramamosain* and *S. tranquebarica* from four locations are shown in **Supplementary Table 2**. After corrected for body size (CW), there was a statistically significant difference between sex and location on the combined morphological characters in all three *Scylla* species [*S. olivacea*: $F_{\text{sex}(22, 2,370)} = 3710.47$, $P < 0.001$, Wilks' $\Lambda = 0.028$, $F_{\text{location}(66, 7,078)} = 114.88$, $P < 0.001$, Wilks' $\Lambda = 0.114$; *S. paramamosain*: $F_{\text{sex}(22, 2,370)} = 5141.84$, $P < 0.001$, Wilks' $\Lambda = 0.021$, $F_{\text{location}(66, 7,116)} = 122.90$, $P < 0.001$, Wilks' $\Lambda = 0.102$; *S. tranquebarica*: $F_{\text{sex}(23, 2,369)} = 3730.79$, $P < 0.001$, Wilks' $\Lambda = 0.027$, $F_{\text{location}(69, 7,078)} = 67.13$, $P < 0.001$, Wilks' $\Lambda = 0.222$]. The interaction effect between sex and location on the morphological characters was also significant for all three species [*S. olivacea*: $F_{(66, 7,078)} = 106.44$, $P < 0.001$, Wilks' $\Lambda = 0.128$; *S. paramamosain*: $F_{(66, 7,078)} = 117.65$, $P < 0.001$, Wilks' $\Lambda = 0.110$; *S. tranquebarica*: $F_{(69, 7,078)} = 54.90$, $P < 0.001$, Wilks' $\Lambda = 0.278$].

Among the carapace characters, the eighth CW (8CW) and carapace length (CL) were almost exclusively male-biased, except for the 8CW and CL of *S. paramamosain* from Kota Marudu mangrove forest and Asajaya mangrove forest, respectively, were female-biased, and the 8CW of *S. paramamosain* from Asajaya mangrove forest showed no significant difference between sexes.



Although the sexual dimorphism pattern of internal CW (ICW) was inconsistent across species and location, that of posterior width of the carapace (PWC) were consistently based on species, i.e., the PWC of *S. paramamosain* were male-biased whereas the PWC of *S. tranquebarica* and *S. olivacea* were female-biased regardless of location, except for males and females of *S. olivacea* from Asajaya mangrove forest with no apparent dimorphism in PWC character (**Figure 3; Supplementary Table 3**).

The sexual dimorphism patterns of frontal lobe characters were consistent within but not across *Scylla* species (**Figure 3; Supplementary Table 3**), except for *Scylla* species from Asajaya mangrove forest. For example, the frontal width (FW) of *S. paramamosain* from all locations were female-biased but those from the Asajaya mangrove forest exhibited larger frontal width in males. The two sternum characters, i.e., sternum width (SW) and abdomen width (AW), were almost exclusively female-biased in all three *Scylla* species from four locations, except for the male-biased SW of *S. olivacea*.

Inter- but not intraspecific variation in sexual dimorphism of periopod characters was obvious (**Figure 3; Supplementary Table 3**). When comparing between sexes of each species, the third right periopod merus length (3PML) was longer in males of *S. olivacea* and *S. paramamosain*, but shorter in males of *S. tranquebarica*. The third right periopod carpus length (3PCL), however, was male-biased only in *S. olivacea*. The fifth right periopod dactyl length (5PL) was female-biased in *S. olivacea* and *S. tranquebarica*, but male-biased in *S. paramamosain*. The fifth right periopod dactyl width (5PW) was male-biased in *S. paramamosain* and *S. tranquebarica*, but female-biased in *S. olivacea*. The 3PML and 5PW of *S. tranquebarica* and 3PCL of *S. paramamosain* from Asajaya mangrove forest, however, showed opposite sexual dimorphism patterns compared to the same species from other locations.

The main cheliped characters such as the dactyl length (DL), propodus depth (PD), PL, merus length (ML), and propodus width (PW) were exclusively larger in males regardless of species and locations, except for DL, PD and ML of *S. olivacea* from

Asajaya mangrove forest with no significant difference among sexes (**Figure 3; Supplementary Table 3**). The inner and outer propodus spines (IPS, OPS) and carpus spines (ICS, OCS) of *S. tranquebarica* were almost exclusively longer in females, except the IPS of *S. tranquebarica* from Kota Marudu mangrove forest that showed a male-biased pattern. Similarly, the IPS, OPS, and OCS of *S. paramamosain* were longer in females. However, except for OCS, the IPS and OPS of *S. olivacea* were almost male-biased.

Stepwise discriminant function analysis was used to discern if spatial differences in terms of morphometric characters exist in males and females of *Scylla* species. Three significant canonical discriminant functions were obtained for females of *S. olivacea* and *S. tranquebarica* and males of *S. olivacea*, whereas two significant functions were obtained for males and females of *S. paramamosain* and males of *S. tranquebarica* (all $P < 0.005$; **Table 1; Supplementary Tables 4, 5**). When each species was analyzed according to sex, we noticed intraspecific differences in characters that significantly discern females or males across the four sampling locations. For example, ICW and one of the frontal lobe characters, i.e., the distance between median spines (DFMS), were useful in separating either males or females of all three species across four locations (**Table 1**). Interestingly, the spinations on cheliped and cheliped dimensions were important discerning criteria of the intraspecific differences of the three *Scylla* species (**Table 1; Supplementary Table 4**). In all three *Scylla* species, the PD and inner propodus spine (IPS) of the right cheliped of females were important criteria to discriminate the corresponding species among locations; in males, spination patterns, especially outer carpus spine (OCS) of the right cheliped of *S. olivacea* and *S. paramamosain* and most cheliped dimensions of *S. tranquebarica* helped discriminate intraspecific dimorphism (**Table 1; Supplementary Table 4**). Based on the group centroids (**Supplementary Table 4**), the first function of males and females of all species was specifically useful to discern specimens from the Asajaya mangrove forest from the other three locations. The remaining functions allowed more general discrimination among the four locations. When the functions

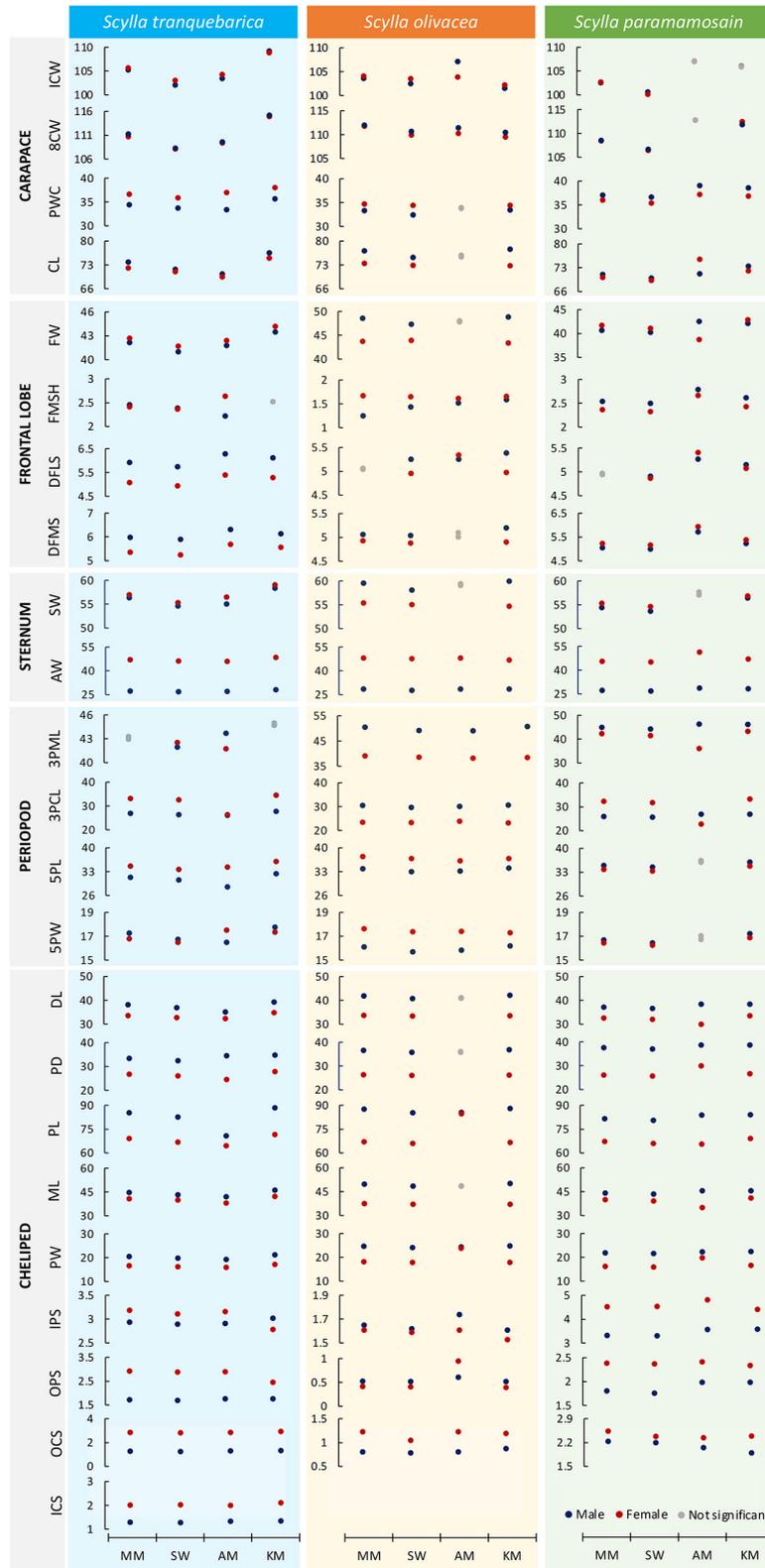


FIGURE 3 | Comparison of sexual dimorphism patterns across geographical locations for three *Scylla* species based on the estimated marginal means after controlling for the covariate (carapace width, CW) using ANCOVA. For detailed statistical values, refer to **Supplementary Tables 6–8**. MM, Matang Mangrove Forest Reserve, Perak; SW, Setiu Wetlands, Terengganu; AM, Asajaya mangrove forest, Sarawak; KM, Kota Marudu mangrove forest, Sabah.

TABLE 1 | Standardized canonical discriminant function coefficients of *Scylla* spp.

Sex	Characters		Canonical functions								
			<i>S. olivacea</i>			<i>S. paramamosain</i>			<i>S. tranquebarica</i>		
			1	2	3	1	2	3	1	2	3
Female	Carapace	ICW	0.014	-0.509*	0.157	0.093	0.253		0.005	0.299	0.308*
		8CW	-	-	-	-0.030	0.850*		-0.020	-0.442	0.806*
		PWC	-0.167	-0.115	-0.156*	0.067	-0.229*		0.167	-0.071*	-0.073
		CL	-0.166	-0.061	0.023*	0.541	-0.038*		-0.212	-0.015	0.201*
	Frontal lobe	FW	-	-	-	-0.547	-0.046*		0.146	-0.029	0.054*
		FMSH	-	-	-	0.274*	0.051		0.278*	-0.060	-0.106
		DFLS	-	-	-	0.200	0.301*		0.257*	-0.041	-0.042
		DFMS	-0.144	-0.028	-0.144*	0.508	-0.105*		0.294*	-0.060	0.048
	Sternum	SW	-	-	-	0.243	-0.156*		0.010	-0.056	-0.385*
		AW	-	-	-	0.702	-0.331*		0.137*	0.180	0.280
	Periopod	3PML	-0.156	0.033	0.136*	-0.768*	-0.011		-	-	-
		3PCL	-0.105	-0.145*	0.086	-1.373*	-0.119		-0.934*	0.066	-0.037
		5PL	-0.279	0.063	-0.099*	0.342	0.078*		0.122	-0.067	-0.244*
		5PW	-0.122	0.051	-0.023*	-	-		0.254*	-0.055	0.100
	Cheliped	DL	0.192*	0.064	-0.190	-0.368*	0.211		-	-	-
		PD	0.407*	-0.134	-0.093	0.700*	0.239		-0.163*	0.021	0.186
		PL	0.305*	0.065	-0.300	-	-		-0.145*	-0.048	-0.098
		ML	0.368*	-0.018	0.146	-0.595*	0.050		-	-	-
		PW	0.285*	-0.002	-0.051	0.257*	-0.102		-	-	-
		IPS	0.010	-0.031	0.867*	0.012	-0.399*		0.060	0.564*	0.119
OPS		0.647*	-0.006	0.208	-	-		0.043	0.644*	0.130	
OCS		0.028	0.858*	0.022	0.008	-0.192		-	-	-	
ICS	-	-	-	-	-		-	-	-		
Male	Carapace	ICW	0.917*	-0.155	-0.114	-0.086	0.171		0.071	0.950*	
		8CW	0.041	0.334*	0.145	-0.015	0.147		-0.023	0.346*	
		PWC	0.484	-0.153	0.422*	-0.181	0.066		-	-	
		CL	-0.400	-0.113	0.339*	0.372	-0.686		-	-	
	Frontal lobe	FW	-	-	-	-	-		0.140	-0.057*	
		FMSH	0.170	0.724*	0.320	-0.276	0.277*		-	-	
		DFLS	0.078	0.887*	-0.768	-0.122	0.167		0.316*	0.142	
		DFMS	-0.113	0.343	-0.164*	-0.502	0.867*		0.395*	-0.011	
	Sternum	SW	-0.012	-0.243	0.364*	-	-		-	-	
		AW	0.211	-0.065	0.243*	-	-		-	-	
	Periopod	3PML	-0.336	-0.220	-0.042*	-	-		0.306	-0.015	
		3PCL	0.242	-0.203	0.068*	-	-		-	-	
		5PL	-	-	-	0.192	-0.105		-0.365	0.053	
		5PW	-	-	-	0.142	-0.147		-	-	
	Cheliped	DL	-	-	-	-	-		-0.279*	-0.164	
		PD	-	-	-	-	-		0.891	0.154	
		PL	-0.491	-0.114	0.090*	-	-		-1.115*	0.050	
		ML	-	-	-	-	-		-0.128*	-0.143	
		PW	0.152	0.152	0.020*	0.195*	-0.257		-	-	
		IPS	0.178*	-0.117	0.122	-	-		-	-	
OPS		0.152*	-0.053	0.104	-	-		-	-		
OCS		0.209*	-0.031	0.060	0.812*	0.575		-	-		
ICS	-	-	-	-	-		-	-			

All morphometric characters (except carapace width, CW) were divided by CW.

- variables that were not included in the discriminant function; * largest absolute correlation of a variable with one of the canonical functions. Function 3 of male and female *S. paramamosain* and male *S. tranquebarica* were not included ($P > 0.05$).

were integrated (**Supplementary Figure 1**), the differences in morphometric characters of mud crabs (all *Scylla* species) in both sexes, especially prominent in females, between Asajaya mangrove forest and the other three geographical locations were highlighted. All combination of functions has a correct classification rate of more than 60% and a cross-validation rate of more than 58% (**Supplementary Figure 1**).

DISCUSSION

Consistent male-based sexual size dimorphism patterns among *Scylla* species have been reported in previous studies (Fazhan et al., 2021a,b) when geographical and seasonal factors were excluded. However, based on CW, 8CW and CL, this study further shows that although the general pattern was male-biased, intraspecific variation in sexual size dimorphism within *Scylla* species exist as well, at least for *S. tranquebarica* and *S. paramamosain*. The differences in growth-promoting environmental parameters and sex-specific phenotypic plasticity could be a plausible explanation (Cox and Calsbeek, 2010). Lengkeek et al. (2008) also postulated that the intraspecific variation in sexual size dimorphism of Mediterranean blennies might be due to phenotypic plasticity based on the demonstration that intraspecific variation exceeded interspecific variation. For example, if the intensity of combat is related to resource availability (e.g., food, territory or mate) and the degree of competition directly influences energy investment in body size, then the difference in resource availability across population could result in intraspecific body size differences in *Scylla* species. In addition, high predation pressure could also cause variation in sexual size dimorphism patterns between populations, as observed in the lower degree of sexual size dimorphism of freshwater fish *Salaria fluviatilis* from lake populations compared to the river counterparts (Laporte et al., 2018). Another possible explanation of the variation of sexual dimorphism pattern in carapace dimensions of *Scylla* species across locations could be due to the different age structures in different populations (Cox and Calsbeek, 2010). However, this could not be verified due to the lack of data on the mortality and age of each *Scylla* species in every sampling location.

Sexual dimorphism patterns of reproduction-related characters such as sternum and cheliped characters were almost exclusively female- and male-biased, respectively, across geographical locations of all three *Scylla* species (**Figure 3**). This is expected as both cheliped dimensions and AW were obvious sexually dimorphic characters in almost all brachyuran species (Simpson et al., 2016; Parvizi et al., 2017), including in *Scylla* spp. (Waiho et al., 2016b; Fazhan et al., 2021b). The conserved patterns in reproduction-related morphological characters between *Scylla* spp. could be explained by the genetic variation that exists between them, as high interspecific but low intraspecific genetic distances were reported for the three *Scylla* species along the equatorial region (inclusive of the sampling sites in this study) by Naim et al. (2020).

Other subtle characters, such as the dimensions of third and fifth right periopods and frontal lobe dimensions, however,

were more similar within than between species (**Figure 3**). The longer ML of the third periopod in males of Japanese mitten crab *Eriocheir japonica* was linked to active mate searching and female-cradling and -guarding during the mating process (Kobayashi, 2002). This could hold true for *S. olivacea* and *S. paramamosain* as similar male-biased MLs of their third periopods were observed in this study. The mating behavior of *S. olivacea* whereby males were involved in mate selection and guarding further supports this postulate (Waiho et al., 2015). Comparatively, *S. tranquebarica* females exhibited longer ML of their third periopods. In sand fiddler crab *Uca pugilator*, the long rear legs of females were believed to be involved in egg mass support and digging out of breeding burrows (McLain and Pratt, 2008). However, future research on the mating and brooding behaviors of *S. tranquebarica* is warranted to verify the involvement of female-biased 3PML in the breeding or spawning processes. In addition, future works on the genetic differentiation among geographically close populations of *Scylla* and the differences in environmental factors could reveal the potential involvement of adaptive phenotypic plasticity in the inter- and intraspecific variation of sexual dimorphism patterns in *Scylla* species along the equatorial region.

Cheliped spinations were female-biased across locations for *S. paramamosain* and *S. tranquebarica*, whereas those of *S. olivacea* showed male-biased patterns in some locations. Cheliped spinations serve as important defense accessories and we postulate that the female-biased sharp spination patterns (inner and outer propodus and carpus spines on the cheliped) protect females from predators, especially when they are carrying eggs externally during their migration toward offshore to spawn (Meynecke and Richards, 2014), in compensating with the lesser cheliped size compared to their male counterparts (Fazhan et al., 2021b). The variation in dimorphism patterns of cheliped spinations observed in *S. olivacea*, however, might be ecologically related. *S. olivacea* is known to prefer habitats of lower salinity, such as upstream of river mouths and mangrove forests, whereas *S. tranquebarica* and *S. paramamosain* are often found in estuaries and intertidal and subtidal zones of higher salinities with less salinity fluctuation (Fazhan et al., 2017a, 2021b). A less protruding propodus spines in females of *S. olivacea* might facilitate easier movement in between pneumatophores and enable convenient burrowing to escape from predators. This hypothesis, however, requires further verification *via* observing their behavior and movement in the natural environment.

When comparing each species according to sex using discriminant function analysis, *Scylla* spp. from Asajaya mangrove forest, especially females, were distinct from the other three remaining locations. Various interplaying factors, including evolutionary selection pressure (Kim et al., 2017), diet (Papacostas and Freestone, 2016) and predation pressure (Laporte et al., 2018) might be involved in causing this phenomenon. The morphological divergence between populations (intraspecific variation) was also reported in eight species of *Uca* along the coast of Brazil and ecological modulation of phenotype was proposed as a possible inducing factor (Hampton et al., 2014). However, it is beyond the scope of this study to identify potential causative agents to the

intraspecific morphological variations of *Scylla* spp. due to the lack of environmental data. Another possible explanation is that the distinct variation in morphological characters of *Scylla* spp. from the Asajaya mangrove forest could be linked with the latitudinal factor. Among the four locations, Asajaya mangrove forest is the closest to the equator (latitude: 1°32'N) whereas other locations are at 4°45'N, 5°39'N and 6°44'N, respectively. Instead of reduced intraspecific sexual dimorphism with increasing latitude as reported in other aquatic species (Estlander et al., 2017; Lima-Filho et al., 2017), this study shows that increasing latitude resulted in reversal of some morphometric characters. Latitudinal variation influences temperature and other biotic and abiotic factors, which in turn, could impact trophic interactions between species (Estlander et al., 2017). Future studies on the life-history traits and their correlation with biotic and abiotic factors are needed to validate this pattern.

CONCLUSIONS

The interspecific variation in sexual dimorphism patterns was stronger than intraspecific among the three *Scylla* species. However, the results also highlight a divergence in phenotype in all *Scylla* species of Asajaya mangrove forest compared to the other three geographical locations. Future characterization of the ecological niche and molecular characteristics could aid in identifying the mechanisms behind the phenotypic divergence and further the understanding of the potential causative factors of morphological variation between and among brachyurans.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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AUTHOR CONTRIBUTIONS

KW: methodology, investigation, formal analysis, writing the original draft, and writing the review and editing. MI: methodology, validation, and writing the review and editing. MA and AS-C: formal analysis, validation, and writing the review and editing. SI: visualization and formal analysis. MJ and GA: investigation and formal analysis. HF: conceptualization, investigation, writing the original draft, writing the review, and editing and supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.690836/full#supplementary-material>

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