

ORIGINAL RESEARCH

Length-weight relationships and mitochondrial COI variation of orange mud crab (*Scylla olivacea*) across four mangrove localities in Peninsular Malaysia

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ABSTRACT. Understanding variation in body-size allometry and genetic diversity of the orange mud crab, *Scylla olivacea*, is important for effective fisheries management and conservation. This study evaluated length-weight relationships (LWRs) and mitochondrial cytochrome c oxidase subunit I (COI) genetic diversity among *S. olivacea* samples collected once from four mangrove ecosystems (four localities; 28 individuals from each locality) along the east and west coasts of Peninsular Malaysia. Carapace width and body weight data were analyzed to characterize locality-specific growth patterns, while mitochondrial (COI) sequences were used to assess the genetic diversity and population genetic structure. The allometric exponent (b) of the LWRs varied among sampling localities, ranging within 2.160-3.694, with coefficients of determination (R²) between 0.809 and 0.971. Analysis of 112 mitochondrial COI sequences revealed 58 haplotypes, with high haplotype diversity (Hd = 0.955-0.973) and relatively low nucleotide diversity (π = 0.009-0.011). Analysis of molecular variance indicated that most genetic variation was distributed within sampling localities (97.66%), while only 2.34% occurred among sampling locations. The low and non-significant fixation index (F_{ST} = 0.023, P = 0.079) suggested weak genetic differentiation based on the mitochondrial marker examined. These findings indicate that spatial variation in growth patterns occurs alongside limited mitochondrial differentiation among sampled localities; however, the observed phenotypic variation cannot be directly attributed to genetic differences without additional environmental and genomic analyses. The findings provide a descriptive baseline for the four sampled locality-dates.

Key words: Mud crab fisheries, body-size allometry, mitochondrial haplotypes, fisheries genetics, mangrove crab.

Relaciones talla-peso y variación del gen mitocondrial COI en el cangrejo naranja de fango (*Scylla olivacea*) en cuatro localidades de manglar de Malasia peninsular

RESUMEN. Comprender la variación en la alometría del tamaño corporal y la diversidad genética del cangrejo de fango naranja (*Scylla olivacea*) es importante para una gestión pesquera y una conservación eficaces. Este estudio evaluó las relaciones longitud-peso (RLP) y la diversidad genética de la subunidad I del citocromo c oxidasa mitocondrial (COI) en muestras de *S. olivacea* recolectadas

en una única ocasión en cuatro ecosistemas de manglar (cuatro localidades; 28 individuos por localidad) a lo largo de las costas Este y Oeste de Malasia peninsular. Se analizaron datos de anchura del caparazón y peso corporal para caracterizar los patrones de crecimiento específicos de cada localidad, mientras que se utilizaron secuencias mitocondriales (COI) para evaluar la diversidad y la estructura genéticas poblacional. El exponente alométrico (b) de las RLP varió entre las localidades de muestreo, oscilando entre 2,160 y 3,694, con coeficientes de determinación (R^2) de entre 0,809 y 0,971. El análisis de 112 secuencias mitocondriales COI reveló 58 haplotipos con una alta diversidad haplotípica ($H_d = 0,955-0,973$) y una diversidad nucleotídica relativamente baja ($\pi = 0,009-0,011$). El análisis de varianza molecular indicó que la mayor parte de la variación genética se distribuyó dentro de las localidades de muestreo (97,66%), mientras que solo el 2,34% se produjo entre las localidades. El índice de fijación bajo y no significativo ($F_{ST} = 0,023$; $P = 0,079$) sugirió una débil diferenciación genética basada en el marcador mitocondrial examinado. Estos resultados indican que existe una variación espacial en los patrones de crecimiento acompañada de una diferenciación mitocondrial limitada entre las localidades muestreadas; sin embargo, la variación fenotípica observada no puede atribuirse directamente a diferencias genéticas sin análisis ambientales y genómicos adicionales. Los hallazgos proporcionan una línea base descriptiva para las cuatro localidades y fechas de muestreo.

Palabras clave: Pesquerías de cangrejo de fango, alometría del tamaño corporal, haplotipos mitocondriales, genética pesquera, cangrejo de manglar.

INTRODUCTION

The orange mud crab, *Scylla olivacea* (Herbst, 1796), is a commercially important portunid crab widely distributed throughout mangrove forests, estuaries, tidal channels, and sheltered coastal waters of the Indo-West Pacific. It supports capture fisheries and aquaculture in many South and Southeast Asian countries and contributes substantially to the livelihoods of coastal communities. In Malaysia, *S. olivacea* is one of the most abundant and widely distributed members of the genus *Scylla*, occurring in mangrove ecosystems along both the east and west coasts of Peninsular Malaysia (Naim et al. 2020). The species is closely associated with mangrove forests and adjacent river channels, where individuals generally occupy relatively restricted intertidal habitats (Fazhan et al. 2022). This dependence on coastal ecosystems, together with its high commercial value, makes wild stocks vulnerable to habitat degradation and intensive harvesting. Recent stock assessments have reported fishing mortality and exploitation rates exceeding recommended biological reference levels in parts of the species' distribution, emphasizing the need for continued biological monitoring of natural populations (Islam et al. 2024).

Body-size allometry provides important biological information for evaluating exploited crustacean populations. Length-weight relationships (LWRs) describe the relationship between body size and body weight and are widely used to estimate biomass, body condition, and allometric patterns within populations (Froese 2006; Islam et al. 2024). Because LWRs are based on cross-sectional measurements, they characterize variation in body-size scaling among individuals rather than individual growth rates. The allometric exponent (b) may vary among localities owing to differences in sex composition, maturity stage, molting condition, food availability, habitat quality, season, and fishing pressure. In Malaysian waters, Waiho et al. (2018) reported geographical variation in size distribution, length-weight relationships, and size at sexual maturity of *S. olivacea*, indicating that body-size allometry may differ among localities. However, morphometric variation alone cannot determine whether locality-specific differences reflect genetic differentiation or phenotypic responses to local environmental conditions. Consequently, genetic analyses provide complementary information for evaluating patterns of diversity, differentiation, and demographic history (Avice 2000; Frankham et al. 2010).

Mitochondrial cytochrome *c* oxidase subunit I (COI) has been widely used to investigate genetic

diversity and population structure in mud crabs and other portunid species because of its relatively high mutation rate and broad applicability in population genetic studies (Rosly et al. 2013; Wang et al. 2020). Nevertheless, COI also has important limitations. As a maternally inherited single-locus marker, it reflects only maternal evolutionary history and may not capture male-mediated dispersal or genome-wide patterns of gene flow. Furthermore, retained ancestral polymorphism and incomplete lineage sorting may reduce its sensitivity for detecting recent population divergence. Therefore, patterns inferred from COI should be interpreted as mitochondrial genetic structuring rather than definitive evidence of contemporary population connectivity.

Peninsular Malaysia provides an appropriate geographical setting for examining spatial variation in *S. olivacea* because its east and west coasts are influenced by contrasting marine systems. The east coast borders the South China Sea, whereas the west coast faces the Strait of Malacca. These regions differ in monsoon regimes, hydrodynamic conditions, circulation patterns, and their connections with adjacent marine waters (Luu et al. 2015), all of which may influence larval transport, settlement, recruitment, and population exchange. At the same time, the planktonic larval stages of *Scylla* species provide considerable dispersal potential that may reduce mitochondrial differentiation among geographically separated mangrove localities.

Previous studies have produced contrasting conclusions regarding the spatial genetic structure of *S. olivacea*. Rosly et al. (2013) examined mitochondrial COI variation among six sampling localities on the east and west coasts of Peninsular Malaysia and reported high haplotype diversity, low nucleotide diversity, and no clear geographical pattern, while emphasizing that their findings represented a preliminary assessment based on a single mitochondrial marker (Rosly et al. 2013). Habib et al. (2022) reported high haplotype diversity and low nucleotide diversity in *S. olivacea* populations

from the Bay of Bengal and adjacent regions. Their analyses indicated no significant differentiation between the two Bangladeshi populations, although genetic differentiation was observed between the Bay of Bengal populations and populations from the Andaman Sea and Malacca Strait, with broader regional structuring across the Northern Indian Ocean and South China Sea (Habib et al. 2022). In contrast, Mendiola and Ravago-Gotanco (2021), using genome-wide single nucleotide polymorphisms (SNPs), detected weak but statistically significant population differentiation and evidence of local adaptation among *S. olivacea* populations in the Sulu Sea. Together, these studies indicate that inferred population structure depends on both geographical scale and marker resolution and that weak differentiation based on mitochondrial COI should not be interpreted as conclusive evidence of unrestricted contemporary gene flow.

Although biological and genetic characteristics of *S. olivacea* have been investigated previously, these descriptors have generally been examined independently. Studies in Malaysia have described locality-specific size distribution, length-weight relationships, maturity, abundance, and movement (Waiho et al. 2018; Fazhan et al. 2022), whereas genetic studies have primarily focused on mitochondrial diversity or genome-wide differentiation (Rosly et al. 2013; Mendiola and Ravago-Gotanco 2021). Consequently, relatively few studies have evaluated body-size allometry and mitochondrial genetic diversity using the same sampling framework. Integrating these datasets allows comparisons between locality-specific phenotypic variation and maternal genetic patterns, while recognizing that such comparisons do not establish direct genotype-phenotype relationships.

The present study focused on four mangrove ecosystems representing the two principal coastal regions of Peninsular Malaysia: Setiu Wetland Mangrove (SWM) and Tumpat Delta Mangrove (TDM) on the South China Sea coast, and Sungai Merbok Mangrove Forest (SMMF) and Lumut Mangrove Park (LMP) on the Strait of Malacca

coast. These localities were selected to provide a comparative assessment across contrasting marine environments while maintaining balanced sampling among sites. 28 individuals were collected from each locality, providing a consistent sampling framework for estimating locality-specific length-weight relationships and mitochondrial COI diversity. Although this design is suitable for comparative analyses among the selected localities, it is not intended to represent all *S. olivacea* populations throughout Peninsular Malaysia.

The primary question addressed in this study was whether locality-specific variation in body-size allometry is accompanied by detectable mitochondrial genetic differentiation among *S. olivacea* collected from contrasting coastal regions of Peninsular Malaysia. Based on previous mitochondrial studies, we expected COI to reveal high genetic diversity with weak mitochondrial differentiation among sampling localities, whereas locality-specific variation in length-weight relationships might reflect environmental and demographic influences rather than corresponding genetic divergence. Accordingly, this study aimed to characterize locality-specific length-weight relationships using carapace width and body weight, assess mitochondrial COI diversity, population differentiation, and demographic history among four sampling localities, and compare geographical patterns in body-size allometry with patterns of mitochondrial genetic variation within the same sampling framework. By combining morphometric and mitochondrial datasets collected from the same individuals, this study

provides a baseline description of locality-specific biological variation and maternal genetic diversity in *S. olivacea*.

MATERIALS AND METHODS

Sampling and preservation

A total of 112 individuals of wild *S. olivacea* ($n = 28$ for each group) were collected from four distinct geographical areas along the east and west coasts of Peninsular Malaysia in different months in 2025 and 2026 (Table 1; Figure 1). During each sampling occasion, 25 traditional crab traps were deployed in the mangrove waters overnight and retrieved the following morning; 28 individuals were randomly selected for analysis regardless of size. Each locality was sampled on a single occasion (Table 1). Samples were comprised of 19 males and 9 females from Setiu, 17 males and 11 females from Kota Bharu, 24 males and 4 females from Sungai Petani, and 11 males and 17 females from Sitiawan. Subsequently, all specimens were identified based on diagnostic morphological characteristics described previously (Keenan 1999; Fazhan et al. 2021; Keenan et al. 2022). Carapace width (CW) and body weight (BW) were individually recorded. Sex, maturity stage, and molting condition were not recorded during sampling; therefore, individuals were pooled for the length-weight relationship analysis. Because *Scylla* species exhibit

Table 1. Details orange mud crab *Scylla olivacea* samples ($n = 112$) collected from east and west coasts of Peninsular Malaysia from December 2025 to January 2026.

Coast region	Sampling location	State	Latitude (N)	Longitude (E)	Sampling date
East coast	Setiu Wetland Mangrove	Terengganu	5° 41' 41.42"	102° 41' 37.62"	8 December 2025
East coast	Tumpat Delta Mangrove	Kelantan	6° 12' 16.2"	102° 10' 37.30"	8 January 2026
West coast	Sungai Merbok Mangrove Forest	Kedah	5° 37' 51.8"	100° 24' 40.4"	9 December 2025
West coast	Lumut Mangrove Park	Perak	4° 13' 02.9"	100° 38' 48.3"	13 January 2026

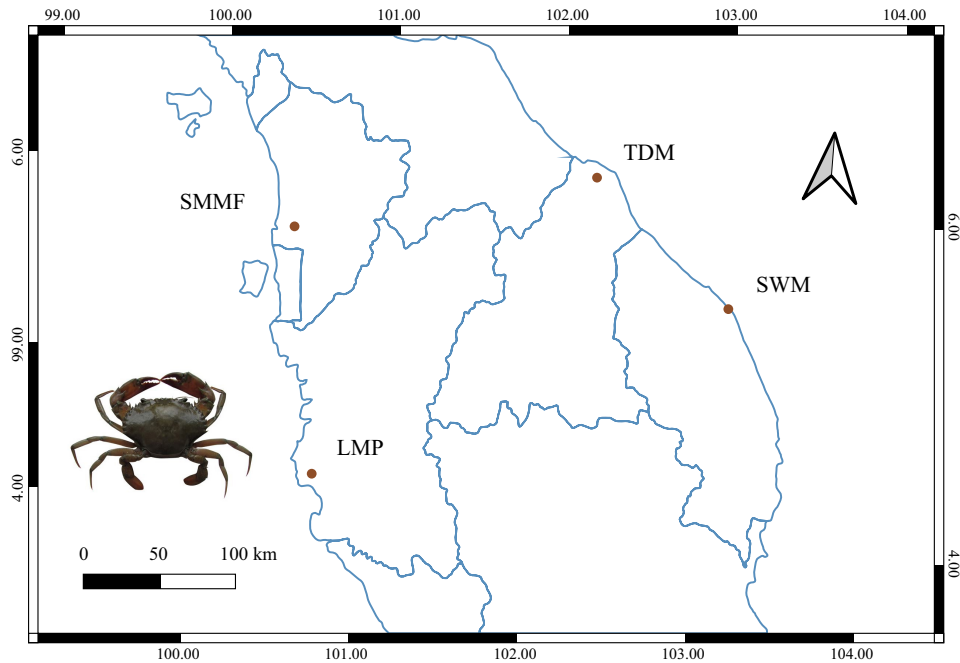


Figure 1. Sampling sites of orange mud crab *Scylla olivacea* in Malaysian waters, and South China Sea on the East Coast of Peninsular Malaysia, and the Strait of Malacca on the West Coast of Peninsular Malaysia. SMMF = Sungai Merbok Mangrove Forest, Kedah, LMP = Lumut Mangrove Park, Perak, SWM = Setiu Wetland Mangrove, Terengganu, TDM = Tumpat Delta Mangrove, Kelantan.

pronounced sexual dimorphism in body size and other morphological traits (Fazhan et al. 2021), differences in sex composition among sampling localities may contribute to variation in the estimated allometric exponent (b). A small muscle tissue sample was excised from the walking leg of each fresh crab specimen, preserved in 95% ethanol, and stored in 1.5 ml microcentrifuge tubes at -20°C until further analysis.

Length-weight relationship (LWR) analysis

Carapace width (CW, mm) was measured at the widest part of the carapace using a digital vernier caliper to the nearest 0.01 mm. Body weight (BW, g) was recorded using an electronic balance to the nearest 0.01 g. The LWR for each population was described using the power function:

$$BW = aCW^b$$

where a is the regression intercept, and b is the allometric growth coefficient. The relationship was linearized using logarithmic transformation:

$$\log_{10} BW = \log_{10} a + b \log_{10} CW$$

The parameters a and b were estimated separately for each population using ordinary least-squares linear regression after $\log_{10}$ transformation of body weight (BW) and carapace width (CW). The coefficient of determination (R^2) was used to evaluate the goodness of fit of the length-weight regression models. The allometric exponent (b) was used to describe the scaling relationship between CW and BW. As no formal tests of the null hypothesis $b = 3$ or joint regression tests comparing slopes among localities were performed, the estimated exponents were reported descriptively without statistical classification of the allometric pattern or inference of significant differences among localities.

DNA extraction and quantification

Total genomic DNA was extracted from walking leg muscle tissue using the protocols provided with the PrimeWay Genomic II DNA Extraction Kit (1st BASE Biotechnology, Apical Scientific, Selangor, Malaysia). The purity and concentration of the extracted DNA were assessed using a BioDrop spectrophotometer (Biochrome Ltd., Cambridge, UK). The DNA was stored in 1.5 mL microcentrifuge tubes at -20 °C until PCR amplification.

Polymerase chain reaction (PCR) amplification and sequencing

The DNA samples were amplified by polymerase chain reaction (PCR) targeting the mitochondrial cytochrome c oxidase subunit I (COI) gene using the forward primer Mtd-10 (5'-TTGAT-TTTTGGTCATCCAGAAGT-3') and reverse primer C/N 2769 (5'-TTAAGTCCTAGAAAAT-GTTGRGGA-3'), following (Rosly et al. 2013). Each 25 µL reaction contained 1 µL of genomic DNA (50-100 ng), 0.5 µL of 10 µM forward primer, 0.5 µL of 10 µM reverse primer, 12.5 µL of REDiant II PCR Master Mix (2×) containing Taq DNA polymerase (1st BASE Biotechnology, Apical Scientific, Selangor, Malaysia), and 10.5 µL of double-distilled water. Amplification was performed using an Eppendorf Mastercycler Nexus Thermal Cycler (Eppendorf AG, Hamburg, Germany). The thermal cycling program consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 1 min. A final extension was performed at 72 °C for 5 min, followed by a hold at 4 °C. The PCR products were visualized on a 1.5% agarose gel stained with SYBR Safe to confirm successful amplification and verify the expected fragment size. Successfully amplified products were sent to Apical Scientific Sdn. Bhd., Selangor, Malaysia, for sequencing using an Applied Biosystems ABI 3730xl Genetic Analyzer.

Sequence alignment and editing

Multiple sequence alignment was performed using ClustalW in MEGA 12 (Kumar et al. 2024). The obtained COI sequences were verified by comparison with reference sequences using the Basic Local Alignment Search Tool (BLAST) available through the National Center for Biotechnology Information (NCBI). The quality-checked sequences were aligned using ClustalW implemented in MEGA 12 (Kumar et al. 2024). The aligned sequences were subsequently imported into DnaSP v6 for haplotype identification and estimation of nucleotide and haplotype diversity (Rozas et al. 2017). A total of 112 individual COI sequences were obtained from the four sampling populations and resolved into 58 unique haplotypes. The 58 haplotype sequences were deposited into the NCBI GenBank database under accession numbers PZ794339-PZ794396. The supplementary data provides the complete alignment of all 112 individual sequences and a specimen-level mapping table linking specimen identifiers, sampling localities, haplotype assignments, and corresponding GenBank accession numbers. Multiple specimens sharing the same haplotype are linked to the same representative GenBank accession.

The number of haplotypes, polymorphic sites, and genetic diversity indices, including haplotype and nucleotide diversity, were estimated using DnaSP v6.0 (Rozas et al. 2017). Phylogenetic relationships among the identified haplotypes were inferred using a maximum likelihood (ML) approach implemented in MEGA 12 (Kumar et al. 2024). The Tamura 3 parameter model with a gamma distribution among sites (T92 + G) was selected as the nucleotide substitution model for phylogenetic reconstruction (Tamura 1992). The model was identified to have the lowest Bayesian Information Criterion (BIC) score in MEGA version 12. The statistical support for the branches of the Maximum Likelihood (ML) tree was assessed using 1,000 bootstrap replicates (Felsenstein 1985). Connections among haplotypes were calculated us-

ing a median-joining network (MJN) in the population analysis with reticulate tree (PopART) v1.7 software (Leigh et al. 2015). Pairwise fixation indices (F_{ST} values) for the COI dataset were calculated in Arlequin v3.5, and 10,000 permutations were used to assess the statistical significance of pairwise comparisons. Six pairwise comparisons were possible among the four sampling localities. To account for multiple testing, P values were adjusted using the Bonferroni correction, and adjusted P values were evaluated against a significance threshold of 0.05. In addition, analysis of molecular variance (AMOVA) was performed with Arlequin v3.5 to evaluate regional genetic variation (Excoffier and Lischer 2010). The fixation index (F_{ST}) values were calculated to evaluate population differentiation.

Demographic history and population expansion

The historical demographic pattern of *S. olivacea* populations was examined using Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997). To assess deviations from neutrality. The demographic parameters θ_0 (before expansion), θ_1 (after expansion), and τ (relative time since population expansion) were estimated under the sudden expansion model using Arlequin v3.5 (Excoffier and Lischer 2010). Harpending's raggedness index (H_{RI}) and the sum of squared deviations (SSD) were used to assess the fit of the observed mismatch distribution to the sudden-expansion model. Statistical significance was evaluated using 1,000 bootstrap replicates. Parameter estimates and uncertainty intervals were reported where available. Bayesian Skyline Plot (BSP) analysis was performed in BEAST version 2.5 (Bouckaert et al. 2019) using the Bayesian Skyline coalescent model. The analysis was conducted under a Jukes-Cantor (JC69) substitution model with a strict molecular clock. The mutation rate and clock rate were fixed at 1.0 in the XML configuration file, without an independently calibrated substitution rate. Consequently, the BSP was interpreted as an exploratory analysis of relative changes in the coalescent effective popula-

tion-size parameter through relative time, rather than as an estimate of absolute effective population size or calendar time. The Markov Chain Monte Carlo (MCMC) analysis was run for 10,000,000 generations, with parameters logged every 1,000 generations and states sampled every 5,000 generations. The initial 10% of sampled states were discarded as burn-in. Convergence and effective sample size (ESS) values were assessed using Tracer v1.7.1 with ESS values > 200 considered indicative of adequate sampling (Rambaut et al. 2018). The minimum and parameter-specific ESS values, inspection of trace plots, and evidence of convergence between independent runs were assessed and reported where available.

RESULTS

Length-weight relationship

All populations showed a positive linear relationship between 'log₁₀carapace width' and 'log₁₀body weight', indicating that body weight increases with carapace width following a power-law pattern (Figure 2). Among the studied locations, 'SWM, Terengganu' exhibited the highest growth coefficient ($b = 3.6937$; $R^2 = 0.9711$), closely followed by 'SMMF, Kedah' ($b = 3.5623$; $R^2 = 0.9702$). 'LMP, Perak' showed a moderately high slope ($b = 3.0020$; $R^2 = 0.9388$), while 'TDM, Kelantan' recorded the lowest growth coefficient ($b = 2.1602$; $R^2 = 0.8091$). The coefficient of determination (R^2) values indicated strong model fits for Kedah, Terengganu, and Perak populations, whereas Kelantan showed comparatively higher variability around the regression line. Overall, results suggested that estimated allometric exponents differed descriptively among locality samples.

The LWR parameters revealed variation in growth patterns among populations. The slope (b) values ranged from 2.1602 in 'TDM, Kelantan' to 3.6937 in 'SWM, Terengganu'. The estimated

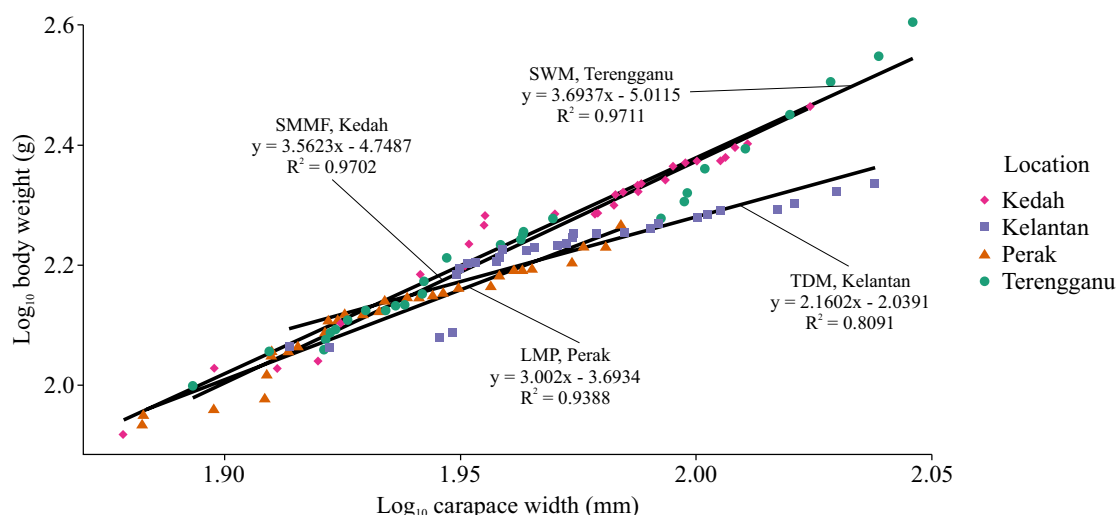


Figure 2. Log₁₀-transformed relationships between carapace width and body weight of *Scylla olivacea* collected from Sungai Merbok Mangrove Forest (SMMF), Setiu Wetland Mangrove (SWM), Lumut Mangrove Park (LMP), and Tumpat Delta Mangrove (TDM). Lines represent population-specific linear regressions.

values were 3.5623 at ‘SMMF, Kedah’, 3.0020 at ‘LMP, Perak’, 2.1602 at ‘TDM, Kelantan’, and 3.6937 at ‘SWM, Terengganu’. These estimates describe variation in the scaling relationship between CW and BW among the sampled localities. However, because formal tests against $b = 3$ and statistical comparisons of slopes among localities were not performed, the estimates are interpreted descriptively and do not establish statistically significant differences in allometric patterns.

Genetic diversity

Haplotype distribution

A total of 112 sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene of *S. olivacea* were successfully obtained from the sampling locations in the mangrove areas of the east and west coasts of Peninsular Malaysia. Analysis of the aligned sequences (543 bp) revealed a total of 58 distinct haplotypes across all samples (Table 2). Fourteen haplotypes were shared among two or more sampling populations, whereas the remaining 44 were restricted to a single population. Among the shared haplotypes, Hap₁₅ was the most fre-

quent, occurring in 12 specimens distributed across ‘SWM, Terengganu’ (1), ‘TDM, Kelantan’ (2), ‘SMMF, Kedah’ (5), and ‘LMP, Perak’ (4). Hap₁ and Hap₂ were also frequent and occurred at all four populations, with 11 and 9 specimens, respectively. Hap₁ was represented by 6, 2, 1, and 2 specimens at SWM, TDM, SMMF, and LMP, respectively, whereas Hap₂ was represented by 2, 3, 2, and 2 specimens at the corresponding populations. Of the 44 population-restricted haplotypes, 43 occurred in a single specimen, while Hap₂₂ occurred in two specimens from SMMF. Overall, the dataset contained several haplotypes shared among populations alongside numerous low-frequency haplotypes restricted to individual sampling localities.

Genetic diversity indices

Genetic diversity analysis based on the mitochondrial cytochrome c oxidase subunit I (COI) gene revealed consistently high levels of haplotype diversity across all four populations of *S. olivacea* (Table 3). A total of 112 individual sequences were analyzed, with 28 specimens representing each sampling location. Across all populations,

Table 2. Distribution of 58 unique mitochondrial COI haplotypes of orange mud crab *Scylla olivacea* from the east (ECPM) and the west coasts (WCPM) of Peninsular Malaysia.

Haplotypes	NCBI accession	Setiu Wetland Mangrove, Terengganu (ECPM)	Tumpat Delta Mangrove, Kelantan (ECPM)	Sungai Merbok Mangrove Forest, Kedah (WCPM)	Lumut Mangrove Park, Perak (WCPM)
Hap_1	PZ794339	6	2	1	2
Hap_2	PZ794340	2	3	2	2
Hap_3	PZ794341	1	-	-	-
Hap_4	PZ794342	1	-	1	2
Hap_5	PZ794343	1	-	-	1
Hap_6	PZ794344	1	-	-	3
Hap_7	PZ794345	1	-	-	-
Hap_8	PZ794346	1	3	-	-
Hap_9	PZ794347	1	-	-	-
Hap_10	PZ794348	1	-	-	-
Hap_11	PZ794349	2	2	-	1
Hap_12	PZ794350	1	-	-	-
Hap_13	PZ794351	1	-	-	-
Hap_14	PZ794352	1	1	-	-
Hap_15	PZ794353	1	2	5	4
Hap_16	PZ794354	1	2	-	-
Hap_17	PZ794355	1	1	2	-
Hap_18	PZ794356	1	-	-	-
Hap_19	PZ794357	1	-	-	-
Hap_20	PZ794358	1	-	-	-
Hap_21	PZ794359	1	-	-	-
Hap_22	PZ794360	-	-	2	-
Hap_23	PZ794361	-	-	1	-
Hap_24	PZ794362	-	-	1	-
Hap_25	PZ79463	-	-	1	-
Hap_26	PZ794364	-	-	1	-
Hap_27	PZ794365	-	-	1	-
Hap_28	PZ794366	-	-	1	-
Hap_29	PZ794367	-	1	1	-
Hap_30	PZ794368	-	-	1	-
Hap_31	PZ794369	-	-	1	-
Hap_32	PZ794370	-	-	1	-
Hap_33	PZ794371	-	-	1	-
Hap_34	PZ794372	-	-	1	2
Hap_35	PZ794373	-	-	1	-
Hap_36	PZ794374	-	-	1	1

Table 2. Continued.

Haplotypes	NCBI accession	Setiu Wetland Mangrove, Terengganu (ECPM)	Tumpat Delta Mangrove, Kelantan (ECPM)	Sungai Merbok Mangrove Forest, Kedah (WCPM)	Lumut Mangrove Park, Perak (WCPM)
Hap_37	PZ794375	-	-	1	-
Hap_38	PZ794376	-	1	-	-
Hap_39	PZ794377	-	1	-	-
Hap_40	PZ794378	-	1	-	-
Hap_41	PZ794379	-	1	-	-
Hap_42	PZ794380	-	1	-	-
Hap_43	PZ794381	-	1	-	-
Hap_44	PZ794382	-	1	-	-
Hap_45	PZ794383	-	1	-	-
Hap_46	PZ794384	-	1	-	-
Hap_47	PZ794385	-	1	-	-
Hap_48	PZ794386	-	1	-	-
Hap_49	PZ794387	-	-	-	1
Hap_50	PZ794388	-	-	-	1
Hap_51	PZ794389	-	-	-	1
Hap_52	PZ794390	-	-	-	1
Hap_53	PZ794391	-	-	-	1
Hap_54	PZ794392	-	-	-	1
Hap_55	PZ794393	-	-	-	1
Hap_56	PZ794394	-	-	-	1
Hap_57	PZ794395	-	-	-	1
Hap_58	PZ794396	-	-	-	1

104 polymorphic sites and 58 distinct haplotypes were identified, yielding an overall average haplotype diversity (H_d) of 0.965 and nucleotide diversity (π) of 0.010. Among the four populations, the ‘SMMF, Kedah’ population exhibited the highest haplotype diversity ($H_d = 0.973$), despite having the fewest polymorphic sites (22), and contained 21 haplotypes. The ‘TDM, Kelantan’ and ‘LMP, Perak’ populations each possessed 28 polymorphic sites and showed identical haplotype diversity values ($H_d = 0.966$), although ‘LMP, Perak’ contained slightly fewer haplotypes (19) than ‘TDM, Kelantan’ (20). The ‘SWM, Terengganu’ population had

26 polymorphic sites and 21 haplotypes, with the lowest haplotype diversity among the sampled populations ($H_d = 0.955$). Nucleotide diversity was low and relatively uniform across sampling sites, ranging from 0.009 in ‘SWM, Terengganu’ to 0.011 in ‘TDM, Kelantan’, while both ‘SMMF, Kedah’ and ‘LMP, Perak’ recorded values of 0.010.

Population genetic structure

Median joining network

A median-joining haplotype network was constructed from 58 mtDNA COI haplotypes to vis-

Table 3. Genetic diversity indices of orange mud crab *Scylla olivacea* populations based on mtDNA COI gene sequences.

Populations	Number of samples (n)	Polymorphic sites (PS)	Number of haplotypes (NH)	Haplotype diversity (Hd)	Nucleotide diversity (π)
Setiu Wetland Mangrove, Terengganu	28	26	21	0.955	0.009
Tumpat Delta Mangrove, Kelantan	28	28	20	0.966	0.011
Sungai Merbok Mangrove Forest, Kedah	28	22	21	0.973	0.010
Lumut Mangrove Park, Perak	28	28	19	0.966	0.010
Total	112	104	81 (58 unique)	0.965 (avg.)	0.010 (avg.)

ualize relationships based on observed sequence differences (Figure 3). The network showed a complex, multi-star-like pattern, with many low-frequency haplotypes branching from several dominant central nodes. Hap_1, Hap_2, Hap_11 and Hap_15 were found to be the prominent central haplotypes. These nodes were shared by individuals from different sampling locations, as indicated by the multiple locality-specific sectors within the corresponding network nodes. Hap_1, Hap_2, and Hap_15 were represented at all four sampling localities. Most haplotypes were separated by relatively few mutational steps, although some connections involved a greater number of steps. Several closed loops were present in the network, reflecting alternative connections among haplotypes that may arise from homoplasy or ambiguity in the network structure. Median vectors represented inferred intermediate nodes generated by the median-joining algorithm and did not necessarily correspond to observed or ancestral haplotypes. Overall, the network showed haplotypes shared among sampling localities alongside numerous low-frequency haplotypes, many of which were restricted to a single locality. The network did not, by itself, establish the direction of ancestry among haplotypes.

Maximum likelihood tree

The Maximum Likelihood (ML) phylogenetic analysis of the 58 mitochondrial cytochrome c oxidase subunit I (COI) haplotypes revealed a complex branching pattern among the haplotypes of *S. olivacea* (Figure 4). The phylogeny demonstrated hierarchical clustering of haplotypes, with several pairs or small groups (e.g. Hap_13 to Hap_29, Hap_30 to Hap_48, and Hap_39 to Hap_51) exhibiting close genetic relationships. However, the overall tree topology did not reveal distinct major clades that would suggest substantial mitochondrial divergence within the sampled dataset. Overall, the ML tree supports the presence of considerable haplotypic variation while indicating that the haplotypes remain genetically similar to one another.

Analysis of Molecular Variance (AMOVA)

Analysis of Molecular Variance (AMOVA) revealed that most of the genetic variation in *S. olivacea* was distributed within locality samples (97.66%), whereas only 2.34% of the total variation occurred among locality (Table 4). The estimated variance components were 2.641 within locality samples and 0.063 among locality samples. The overall fixation index was low ($F_{ST} = 0.023$) and

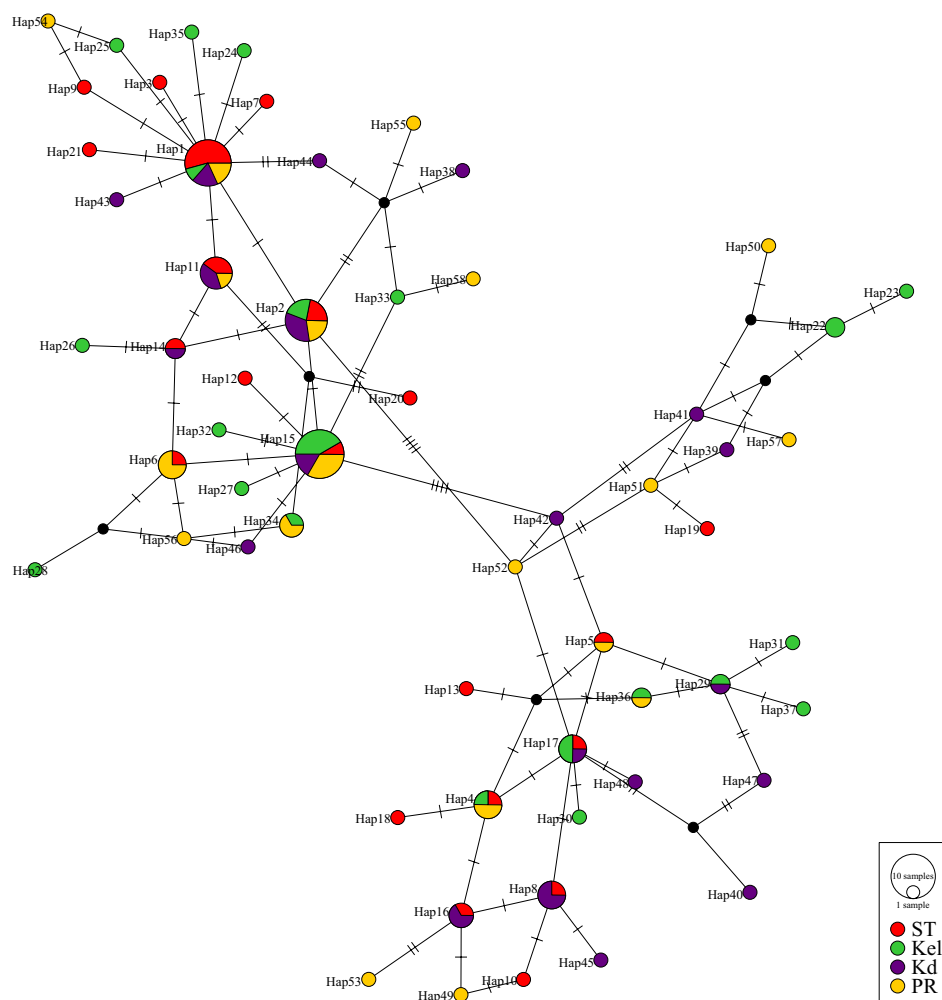


Figure 3. Median-joining haplotype network of orange mud crab *Scylla olivacea* from four mangrove sampling localities along the east and west coasts of Peninsular Malaysia. ST = Setiu Wetland Mangrove, Terengganu; Kel = Tumpat Delta Mangrove, Kelantan; Kd = Sungai Merbok Mangrove Forest, Kedah; and PR = Lumut Mangrove Park, Perak. Each circle represents a unique haplotype, and circle size is proportional to the number of specimens sharing that haplotype. Different colors and symbols indicate the sampling population represented within each haplotype. Connecting lines represent relationships among haplotypes, and small transverse ticks indicate individual mutational steps. Small median-vector nodes represent inferred intermediate nodes generated by the median-joining algorithm and do not necessarily correspond to observed haplotypes.

was not statistically significant ($p = 0.079$), indicating limited detectable genetic differentiation among the four sampled localities based on the mtDNA COI marker. Because the AMOVA treated the four localities as separate sampling units, it did not directly test genetic differentiation between the east and west coasts of Peninsular Malaysia.

Pairwise population differentiation

Pairwise F_{ST} estimates among populations ranged from -0.014 to 0.064 (Table 5). The highest genetic differentiation was observed between TDM and SWM ($F_{ST} = 0.064$), whereas the comparison between LMP and TDM produced a slightly negative F_{ST} value (-0.014), indicating no detectable

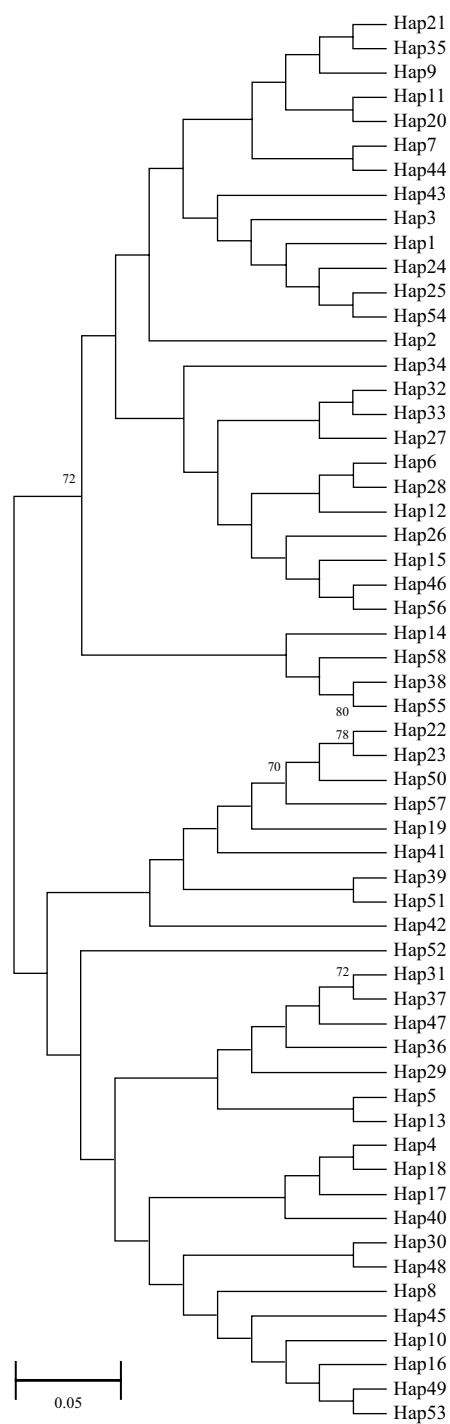


Figure 4. Unrooted maximum-likelihood (ML) phylogenetic tree for the orange mud crab, *Scylla olivacea*, mtDNA COI haplotypes. Numbers at the nodes indicate bootstrap support values from 1,000 replicates. The scale bar represents the number of nucleotide substitutions per site.

Table 4. Analysis of molecular variance (AMOVA).

Source of variations	Degree of freedom	Sum of squares	Variance components	Percentage of variation	p
Among populations	3	13.24	0.06330 Va	2.34	0.079
Within populations	108	285.25	2.64120 Vb	97.66	
Total	111	298.49	2.70451	100	

Fixation index (F_{ST}) = 0.023.

Table 5. Pairwise genetic differentiation (F_{ST}) and associated p-values among the four sampling localities of *Scylla olivacea*. F_{ST} values are presented in the lower triangle, whereas p values $\pm$ standard deviation (p_{adj}) from permutation tests are presented in the upper triangle. SWM = Setiu Wetland Mangrove, Terengganu; TDM = Tumpat Delta Mangrove, Kelantan; SMMF = Sungai Merbok Mangrove Forest, Kedah; LMP = Lumut Mangrove Park, Perak. Padj = Bonferroni adjusted P.

Populations	SWM	TDM	SMMF	LMP
SWM	-	0.117 $\pm$ 0.03 (0.702)	0.684 $\pm$ 0.04 (1.000)	0.081 $\pm$ 0.02 (0.486)
TDM	0.064	-	0.117 $\pm$ 0.03 (0.702)	0.270 $\pm$ 0.04 (1.000)
SMMF	0.012	0.025	-	0.027 $\pm$ 0.01 (0.162)
LMP	0.032	-0.014	0.019	-

genetic differentiation. Overall, pairwise F_{ST} values were low, supporting the conclusion that genetic differentiation among populations is minimal. The associated p-values also showed that most pairwise comparisons were not statistically significant, except SMMF-LMP where the $p = 0.027$. However, no significant was reported in all pairs after the Bonferroni adjustment on P-values, based on the significance threshold, $\alpha_{adj} = 0.0083$.

Historical demography

Neutrality tests

Tajima's D values (Table 6) were negative for all four sampling localities, ranging from -0.09718 at SMMF, to -0.87757 at SWM. However, none of the Tajima's D values were statistically significant ($p > 0.05$), even after adjustment for multiple locality-level tests ($p_{adj} > 0.05$; Table 6), indicating

that significant departures from the neutral equilibrium expectation were not detected by this test. In contrast, Fu's F_s values were strongly negative across all four localities, ranging from -8.01571 at LMP, to -13.34753 at SWM, and all were statistically significant ($p \leq 0.005$). Following Bonferroni correction for multiple testing across localities ($k = 4$, $\alpha_{adj} = 0.0125$), all Fu's F_s indices remained statistically significant with $p_{adj} \leq 0.02$, indicating significant excess of low-frequency haplotypes.

Mismatch distribution analysis

Mismatch distribution analysis was used to further evaluate whether the observed distribution of pairwise nucleotide differences was consistent with a sudden demographic expansion model. The estimated mutation-scaled parameters varied among sampling localities (Table 7). The τ values ranged from 1.000 at SWM to 7.984 at TDM, whereas θ_0

ranged from 0.000 at TDM and SMMF to 4.500 at SWM. The corresponding θ_1 estimates ranged from 12.267 at LMP to 6838.734 at SWM. The parameters θ_0 and θ_1 represent mutation-scaled estimates of the demographic parameter before and after the modeled expansion, respectively, whereas τ represents the timing of the demographic event in units of mutational time.

Goodness-of-fit to the expansion model

The observed mismatch distributions did not show significant departures from the expected sudden expansion model based on the sum of squared deviations (SSD) and H_{RI} statistics. SSD values ranged from 0.00698 to 0.00896, with non-significant P-values ranging from 0.400 to 0.800. H_{RI}

values ranged from 0.00766 to 0.01260, with non-significant P-values ranging from 0.865 to 0.980. These results indicate that the mismatch distributions were statistically compatible with the sudden-expansion model; however, failure to reject this model does not establish that demographic expansion occurred. Therefore, the mismatch results provide no conclusive evidence of expansion on their own and should be interpreted alongside the neutrality tests and other demographic analyses.

Pairwise nucleotide differences

The pairwise nucleotide mismatch distribution was generated using the pooled mitochondrial COI dataset comprising sequences from all four sampling localities (Figure 5). The observed distribu-

Table 6. Neutrality tests (Tajima's D and Fu's F_s) for *Scylla olivacea* across four sampling localities, presenting p-values and Bonferroni-adjusted p (p_{adj}) values ($k = 4$). SWM = Setiu Wetland Mangrove (Terengganu); TDM = Tumpat Delta Mangrove (Kelantan); SMMF = Sungai Merbok Mangrove Forest (Kedah); LMP = Lumut Mangrove Park (Perak).

Populations	Tajima's D			Fu's F_s		
	Value	p	p_{adj}	Value	p	p_{adj}
TDM	-0.662	0.273	0.716	-11.165	0.001	< 0.001
SMMF	-0.0978	0.517	1.000	-10.169	< 0.001	0.004
LMP	-0.361	0.425	1.000	-8.016	0.005	< 0.001
SWM	-0.876	0.179	1.000	-13.347	< 0.001	0.020

Table 7. Mutation-scaled mismatch-distribution parameters (θ_0 , θ_1 , and τ) estimated under the demographic expansion model for *Scylla olivacea* from four sampling localities. The sampling localities are presented in a consistent order: SWM, TDM, SMMF, and LMP. θ_0 and θ_1 represent mutation-scaled demographic parameters before and after the modeled expansion, respectively, whereas τ represents the timing of the demographic event in units of mutational time. SWM = Setiu Wetland Mangrove, Terengganu; TDM = Tumpat Delta Mangrove, Kelantan; SMMF = Sungai Merbok Mangrove Forest, Kedah; LMP = Lumut Mangrove Park, Perak.

Populations	θ_0	θ_1	τ
SWM	4.500	6,838.734	1.000
TDM	0.000	13.057	7.984
SMMF	0.000	14.966	6.973
LMP	0.021	12.267	7.840

tion showed a multimodal pattern, with fluctuations across different pairwise-difference classes, rather than a single smooth unimodal distribution. The expected distribution under the sudden demographic expansion model was comparatively smoother. Although the pooled mismatch distribution showed departures from the idealized unimodal expansion pattern, the locality-specific goodness-of-fit anal-

yses based on the SSD and H_{RI} did not indicate significant deviations from the sudden expansion model for any of the four sampling localities (Table 8). Therefore, the pooled mismatch distribution provides a visual summary of mitochondrial variation across the entire sampled dataset, whereas the demographic expansion tests and mismatch parameters presented in Tables 7 and 8 were es-

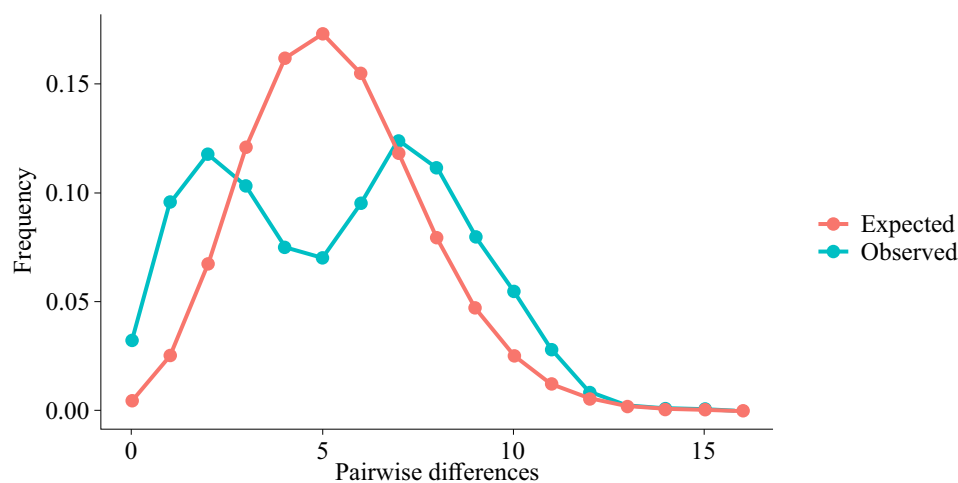


Figure 5. Pairwise nucleotide mismatch distribution of mitochondrial COI sequences of *Scylla olivacea* based on the pooled dataset from the four sampling localities on the east and west coasts of Peninsular Malaysia. The observed mismatch distribution is compared with the expected distribution under the sudden demographic expansion model.

Table 8. Sum of squared deviations (SSD) and Harpending’s raggedness index (H_{RI}) for testing the fit of mitochondrial COI sequence dataset ($n = 112$) to a sudden demographic expansion model across four localities of the orange mud crab (*Scylla olivacea*) in Peninsular Malaysia. P-value denotes the proportion of simulated mismatch distributions from 10,000 parametric bootstrap replicates that yielded an SSD or H_{RI} value $\geq$ the observed value under the null hypothesis of sudden demographic expansion. SWM = Setiu Wetland Mangrove, Terengganu; TDM = Tumpat Delta Mangrove, Kelantan; SMMF = Sungai Merbok Mangrove Forest, Kedah; LMP = Lumut Mangrove Park, Perak.

Populations	SSD		H_{RI}		H_0 fitness
	Value	p	Value	p	
SWM	0.009	0.746	0.011	0.87	Compatible
TDM	0.007	0.80	0.012	0.95	Compatible
SMMF	0.007	0.69	0.013	0.95	Compatible
LMP	0.009	0.40	0.008	0.98	Compatible

Pairwise nucleotide differences.

timated separately for each sampling locality. The multimodal pattern should be interpreted cautiously and does not, by itself, demonstrate a departure from demographic expansion.

DISCUSSION

Body-size allometry

The length-weight analysis demonstrated positive relationships between carapace width and body weight at all four sampling localities, but the estimated allometric exponent (b) varied among locations. Values ranged from 2.1602 at TDM to 3.6937 at SWM, indicating negative allometry at TDM, approximately isometric scaling at LMP, and positive allometry at SWM and SMMF. These estimates should be interpreted as location-specific size-weight allometry rather than individual growth rates, because cross-sectional length-weight data do not measure growth through time. Length-weight relationships describe how body mass scales with body size and are influenced by the size structure and biological composition of the sampled individuals (Froese 2006).

The observed variation in ' b ' is broadly consistent with previous observations of *S. olivacea* in Malaysian waters. Earlier studies reported geographical variation in carapace width and body weight and found that the LWRs differed between males and females, with males showing positive allometry and females negative allometry (Fazhan et al. 2021, 2022). This finding is particularly relevant to the present study because sex, maturity and molting status can influence body mass relative to carapace width. Therefore, differences in the composition of individuals sampled at each locality may contribute to variation in the estimated ' b ' values. Because the present analysis did not formally test differences in slopes among localities and did not incorporate sex as a covariate, the observed differences should be regarded as descriptive rather

than evidence of statistically different locality-specific allometric relationships.

The relatively high R^2 values at SWM, SMMF and LMP indicate that carapace width explained a substantial proportion of the variation in body weight within those samples, whereas the lower R^2 at TDM indicates greater dispersion around the fitted relationship. Such variation may arise from differences in the biological composition of samples, including sex, maturity, reproductive condition and molting stage. However, because these variables were not incorporated into the present analysis, their relative contributions cannot be determined.

Importantly, the present study did not measure environmental variables such as salinity, temperature, food availability, productivity or habitat quality. Consequently, these factors should be considered as possible explanations rather than demonstrated causes of the observed differences. Likewise, fishing pressure was not quantified at the four localities and therefore cannot be directly linked to the observed LWRs patterns. The most appropriate conclusion from the present data is that body-size allometry varied among the sampled localities, while the biological and environmental mechanisms responsible for this variation require further investigation.

Mitochondrial COI genetic diversity and population structure

The COI dataset showed high haplotype diversity ($H_d = 0.955$ to 0.973) and comparatively low nucleotide diversity ($\pi \approx 0.010$) across the four sampling localities. The combination of high haplotype and low nucleotide diversity is commonly observed in marine populations and can arise under demographic expansion, retention of ancestral polymorphism, or other historical demographic scenarios (Grant and Bowen 1998). Therefore, although the observed diversity is compatible with a history of demographic expansion, it should not be interpreted as evidence for a single specific demographic process without additional evidence.

The haplotype network further showed extensive sharing of haplotypes among sampling localities, together with numerous low-frequency or private haplotypes. The absence of strongly geographically restricted haplotype groups is consistent with limited mitochondrial differentiation across the sampled region. Similar patterns of weak spatial structure have previously been reported for *S. olivacea*, whereas higher-resolution genome-wide analyses have detected weak but significant differentiation at broader or different geographical scales (Mendiola and Ravago-Gotanco 2021). This contrast illustrates the importance of marker resolution when evaluating population structure.

AMOVA showed that 97.66% of the molecular variation occurred within localities samples and 2.34% among localities samples. The overall F_{ST} was low and non-significant ($F_{ST} = 0.023$, $p = 0.079$). These results indicate limited mitochondrial differentiation among the sampled locations, but they do not by themselves demonstrate unrestricted contemporary gene flow, panmixia, or the existence of a single stock. Low differentiation can also reflect shared ancestral polymorphism, historical connectivity, incomplete lineage sorting and the limited resolution of a single mitochondrial marker (Waples and Gaggiotti 2006; Meirmans and Hedrick 2011).

This limitation is particularly important because COI is maternally inherited and represents only a single mitochondrial locus. Consequently, the present dataset cannot evaluate male-mediated gene flow or provide a genome-wide assessment of population connectivity. Mendiola and Ravago-Gotanco (2021), using genome-wide markers, detected weak but significant differentiation and signatures of local adaptation in *S. olivacea* populations in the Sulu Sea, demonstrating that fine-scale population structure may not be detectable from mitochondrial data alone. Thus, the present results should be described as evidence of weak mitochondrial structuring, rather than conclusive evidence of high contemporary gene flow.

Relationship between body-size allometry and genetic structure

The principal pattern emerging from the two datasets is that variation in body-size allometry occurred among sampling localities despite limited differentiation in the mitochondrial COI dataset. However, this pattern should not be interpreted as evidence that environmental factors caused the morphometric differences or that phenotypic plasticity was responsible. No environmental variables were measured, and no genotype-phenotype association, common-garden experiment or reaction-norm analysis was conducted.

The lack of strong mitochondrial differentiation nevertheless demonstrates that the observed variation in body-size allometry is not accompanied by corresponding differentiation at the mitochondrial COI locus. Several mechanisms could potentially produce this pattern, including environmental effects, differences in sex or maturity composition, phenotypic plasticity, or genetic differentiation that is not detectable using COI. Previous work on *S. olivacea* has demonstrated that sex can substantially influence LWR parameters (Waiho et al. 2016), emphasizing that biological composition should be considered when comparing location-specific morphometric relationships. Accordingly, the present findings are best interpreted as a descriptive comparison of morphometric and mitochondrial patterns within the same sampling framework, rather than as a direct test of genotype-phenotype correspondence. Future studies incorporating environmental measurements, sex-specific analyses and nuclear or genome-wide markers would be required to distinguish among these possible mechanisms.

Historical demographic patterns

The neutrality analyses showed negative Tajima's D values at all four localities, although none were statistically significant, whereas Fu's F_s values were strongly negative and statistically significant. Negative neutrality statistics can be associ-

ated with demographic expansion, but they can also arise from selection or other departures from the neutral equilibrium model (Fu 1997). Fu's F_s is particularly sensitive to demographic expansion, but interpretation should nevertheless be combined with other demographic evidence rather than treated as definitive proof of expansion.

The mismatch analyses produced low SSD and Harpending's raggedness values with non-significant goodness-of-fit tests. These results indicate that the observed mismatch distributions were not significantly inconsistent with the sudden-expansion model. However, the mismatch distribution itself should be interpreted cautiously, particularly when the observed distribution is not strongly unimodal. Mismatch-based inference can be affected by population structure and other demographic histories, and a failure to reject a sudden-expansion model does not establish that a sudden expansion actually occurred (Rogers and Harpendings 1992; Schneider and Excoffier 1999).

Implications and limitations

From a fisheries perspective, the principal finding is that the four sampled locations showed variation in body-size allometry but limited mitochondrial differentiation. This information provides a useful biological baseline for *S. olivacea* in the sampled Malaysian mangrove systems, but it is insufficient to define a single management stock or demonstrate contemporary connectivity across the entire east and west coasts of Peninsular Malaysia.

The morphometric results indicate that location-specific LWR parameters may be useful for describing the size-weight characteristics of sampled crabs. However, comparisons among localities should account for sex, maturity and molting stage because these factors can influence body size and weight (Waiho et al. 2016). The genetic results indicate limited differentiation at the mitochondrial COI locus, but the absence of significant differentiation should not be interpreted as evidence that management units are absent.

The study also has several important limita-

tions. First, the LWR analysis was cross-sectional and therefore does not provide direct estimates of individual growth rates. Second, sex, maturity and molting status were not incorporated into the statistical comparison of LWRs. Third, environmental and fishing-pressure data were not collected, preventing direct evaluation of their effects on body-size allometry. Fourth, the genetic analysis was restricted to a single 543-bp mitochondrial COI fragment, limiting inference about genome-wide population structure, contemporary gene flow and male-mediated dispersal. Finally, the demographic analyses should be considered exploratory because of the single-locus dataset and the absence of a calibrated substitution rate. Overall, the study demonstrates that spatial variation in body weight-size allometry can occur alongside weak mitochondrial differentiation in *S. olivacea*. Results therefore support the need to consider both morphometric and genetic information when characterizing mud crab populations, while avoiding the assumption that mitochondrial similarity necessarily indicates unrestricted contemporary connectivity or that morphometric differences are environmentally driven. Future research combining standardized sex-specific sampling, environmental measurements and nuclear or genome-wide markers would provide a stronger basis for evaluating the mechanisms underlying spatial variation and for determining whether the sampled localities constitute meaningful management units.

CONCLUSIONS

Although estimated growth (b) and genetic diversity parameters (H_d , π) differed descriptively among the four locality-dates, these variations cannot be interpreted as definitive population-level differences due to the lacking demographic variables and single-locus limitation. Furthermore, low pairwise F_{ST} values and insignificant goodness-of-

fit tests indicate weak maternal lineage structure, with no clear evidence on panmixia or a single management stock. Further works shall focus on in-depth stock assessments, including the use of multiple genetic markers, complete demographic information as well as multitemporal sampling.

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Ethics statement

The procedures were carried out in accordance with the guidelines outlined by the Animal Ethics Committee of UMT (animal ethics approval reference: UMT/JKEPMHK/2024/114) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

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Declaration of generative AI in scientific writing

The authors declare that they did not use generative AI, and took full responsibility for the content of the published article.

Declaration of competing interest

The authors declare no financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The haplotype sequences were deposited into the NCBI GenBank database under accession PZ794339-PZ794396.

Author contributions

Murak: investigation; methodology; analysis; writing-original draft. Mohammad Ekramul Haque: analysis; writing-original draft. Cher Chien Lau: data analysis; writing-review and editing. Muhammad Sajjad Ahmad: conceptualization; analysis; writing-original draft; review and editing. Mohammad Asmat-Ullah: analysis; review. Hanafiah Fazhan: supervision; review. Kuo-Wei Lan: supervision; review. Khor Waiho: supervision; review. Hongyu Ma: supervision; review. Mhd Ikhwanuddin: conceptualization; supervision; funding acquisition; project administration; review.

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