BIODIVERSITAS
Volume 27, Number 5, May 2026
Pages: 10

ISSN: 1412-033X
E-ISSN: 2085-4722
https://doi.org/10.13057/biodiv/d270512

Morphology and molecular analysis of the Brittle star (*Ophiocoma scolopendrina*) in the coastal waters of Aceh Besar, Indonesia

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Manuscript received: 8 December 2025. Revision accepted: 7 May 2026.

Abstract. *Mursawal A, Wahyuni S, Cahyani NKD, Sarong MA, Effendi I, Kurniawan R. 2026. Morphology and molecular analysis of the Brittle star* (Ophiocoma scolopendrina) *in the coastal waters of Aceh Besar, Indonesia. Biodiversitas 27 (5): d270512. https://doi.org/10.13057/biodiv/d270512.* Brittle stars (Ophiuroidea) are important benthic organisms that play a key role in ecological processes within coastal and coral reef ecosystems. However, information on the combined morphological and genetic characteristics of brittle star populations in the coastal waters of Aceh Besar, Indonesia, remains limited. This study aimed to examine morphological characteristics and genetic diversity of *Ophiocoma scolopendrina* from three coastal sites in Aceh Besar using a combined morpho-molecular approach. Specimens were collected from Lhok Mee (LM), Lhok Redeup (LR), and Lhok Seudu (LS) using purposive sampling of intact adult individuals suitable for morphological and molecular analyses. A total of 96 specimens were analyzed. Morphological identification was based on standard diagnostic characters, while molecular analysis targeted partial mitochondrial 16S rRNA gene sequences (~430 bp). Genetic diversity indices, including haplotype diversity (Hd) and nucleotide diversity (π), were calculated, and population structure was assessed using pairwise FST and Analysis of Molecular Variance (AMOVA). Results showed consistent morphological traits across locations with minor variation in coloration. Genetic analysis revealed high haplotype diversity (Hd = 0.867-0.962) and low nucleotide diversity (π = 0.010-0.016). Pairwise FST values were low (0.002-0.016), and AMOVA indicated weak and non-significant genetic differentiation among populations (FST = 0.012; P = 0.088). These findings suggest weak mitochondrial population structuring among sites. However, results should be interpreted cautiously due to the use of a single mitochondrial marker and purposive sampling design. This study provides baseline data on morphology and genetic diversity of *O. scolopendrina* in Aceh Besar, supporting biodiversity monitoring research in tropical coastal ecosystems.

Keywords: 16S rRNA, coastal biodiversity, haplotype diversity, mitochondrial DNA, population genetics

INTRODUCTION

Ophiuroidea, commonly known as brittle stars, is one of the classes within the phylum Echinodermata and is widely distributed across marine ecosystems in littoral and neritic zones (Aziz 1991; Nugroho et al. 2014). These organisms are found in coral reef ecosystems, seagrass beds, and rocky substrates, where they inhabit crevices or live in association with benthic structures (Boos 2012; Nurdiansah and Supono 2017). Ecologically, Ophiuroidea plays an important role as a benthic organism involved in the transfer of organic matter from the water column to the seabed through suspension and deposit feeding activities (Dauvin et al. 2013). This function highlights its contribution to nutrient cycling and energy flow within coastal marine ecosystems.

Morphologically, brittle stars have a distinct body structure consisting of a central disc and typically five flexible arms. Each arm is composed of repeated segments equipped with spines and podia that support locomotion and environmental interaction (Pechenik 2005; Wilkie 2016; Wakita et al. 2020). Another notable characteristic is their ability to regenerate lost arms, functioning as an adaptive response to environmental stress and predation (Czarkwiani et al. 2016). However, many species exhibit high morphological similarity, making identification difficult using conventional approaches alone. This difficulty is associated with morphological variability influenced by substrate type, depth, and water conditions (Supono 2018). The presence of cryptic species, which are morphologically similar but genetically distinct, further complicates taxonomic classification (O'Hara et al. 2017). Therefore, molecular approaches, particularly DNA barcoding, are increasingly important to complement morphological identification and improve taxonomic resolution (Dharmayanti 2011; Dylus et al. 2018).

Recent advances in molecular techniques have enabled more accurate analyses of population genetics in marine organisms. Mitochondrial markers, such as the 16S rRNA gene, are widely used for species identification,

phylogenetic reconstruction, and assessment of genetic variation within and among populations (Ward et al. 2008). In this study, the mitochondrial 16S rRNA gene was selected due to its utility in echinoderm phylogenetics and species identification, particularly for resolving interspecific and shallow intraspecific relationships. Although Cytochrome C Oxidase subunit I (COI) often provides higher resolution for population-level analyses, 16S remains widely used in marine invertebrates due to its conserved primer sites and reliable amplification across taxa. Moreover, integrated morpho-molecular approaches combining morphological and genetic data can improve taxonomic accuracy and provide a more comprehensive understanding of population structure and evolutionary history in marine species (Lessios and Hendler 2022; Sobczyk et al. 2023).

To date, information on the combined morphological and molecular characteristics of *Ophiocoma scolopendrina* (Lamarck, 1816) in the coastal waters of Aceh Besar, Indonesia, remains limited. Existing studies have mainly focused on morphological descriptions or general biodiversity assessments (Rostikawati et al. 2023), with relatively few addressing population genetics or integrating molecular data. This gap represents a significant limitation in understanding echinoderm diversity in Indonesian coastal ecosystems, which are highly biodiverse yet underexplored in genetic studies.

Aceh Besar, located in northern Sumatra, Indonesia, has a complex coastal environment influenced by the Indian Ocean and the Malacca Strait (Sarong et al. 2014). Differences in currents, salinity, and environmental dynamics between these systems may influence the distribution and genetic connectivity of marine organisms. Oceanographic processes, including current-driven dispersal and larval transport, play a crucial role in shaping gene flow and population structure in marine invertebrates. Planktonic larval stages enable many species to disperse over considerable distances, potentially reducing genetic differentiation among populations (Adams et al. 2019; Melroy and Cohen 2021). However, local environmental conditions may also act as barriers to gene flow, leading to population structuring (Mackenzie et al. 2022; Alemdag et al. 2026).

Considering these ecological characteristics, we hypothesize that *O. scolopendrina* populations in Aceh Besar exhibit low mitochondrial genetic differentiation, with possible shared haplotypes among locations. This study aims to analyze morphological characteristics and genetic diversity of *O. scolopendrina* from three coastal locations using an integrated morpho-molecular approach. It also evaluates genetic variation patterns and assesses whether the data support low population differentiation among sites. The findings are expected to improve understanding of species identification, population connectivity, and marine biodiversity management in Indonesian coastal ecosystems.

MATERIALS AND METHODS

Sample collection

Sampling was conducted in November 2022 in the coastal waters of Aceh Besar District, Aceh Province, Indonesia, at three locations: Lhok Seudu (LS) in the Indian Ocean, and Lhok Redeup (LR) and Lhok Mee (LM), Pasir Putih Lamreh, both in the Malacca Strait (Figure 1).

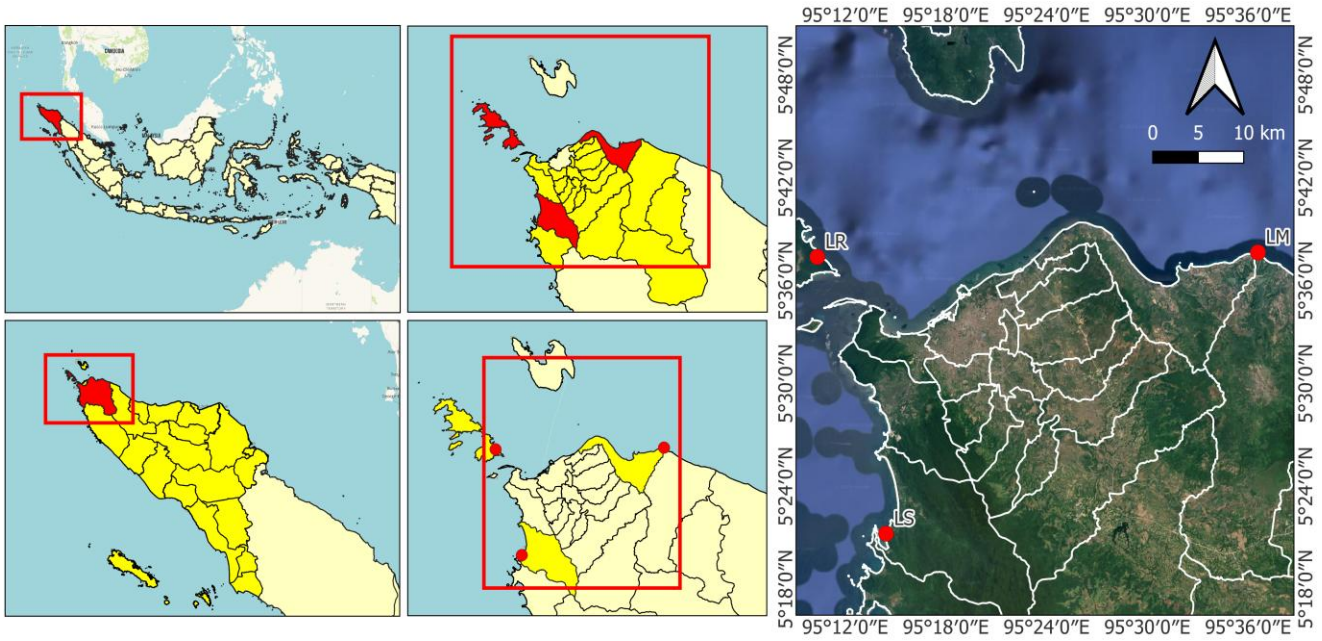

Figure 1. Map of the *Ophiocoma scolopendrina* sampling locations in Lhok Seudu (LS), Lhok Redeup (LR), and Lhok Mee (LM), Aceh Besar District, Aceh, Indonesia

A total of 96 individuals of *O. scolopendrina* were collected, with 32 specimens from each location, and all samples were transported to the Marine Biodiversity and Genetics Laboratory, Universitas Teuku Umar, Indonesia, for further analysis. Representative voucher specimens from each site have been preserved and deposited in the same laboratory under voucher codes OS_LM_001 to OS_LS_032, ensuring availability for future reference and taxonomic verification. These sampling sites represent distinct coastal environments influenced by the Indian Ocean and the Malacca Strait, which may play an important role in larval dispersal and population connectivity.

The use of purposive sampling in this study involved selecting individuals that were intact and suitable for both morphological and molecular analyses. Sampling focused on organisms found in intertidal zones surrounding coral reef areas, with selection criteria including complete body structures and mature life stages to ensure accurate morphological identification and optimal molecular analysis quality. This sampling approach was intended to ensure specimen quality and consistency for morphological and molecular analyses, although it may provide limited representation of natural population variability.

Procedures

Morphological analysis

Morphological identification was carried out using a stereo microscope (Olympus SZ61) to examine external characteristics of each specimen. Identification followed diagnostic keys for Brittle stars (Pomory 2007), including disc shape, arm structure, spine arrangement, granule distribution, and pigmentation patterns (Madduppa 2013). Each specimen was photographed prior to analysis, and morphological sketches were produced using a drawing pad and refined with CorelDRAW software to ensure accurate representation of diagnostic features. Morphological observations were conducted descriptively to confirm species identity across all collected samples. These observations were performed qualitatively for taxonomic identification purposes, and no quantitative morphometric measurements were recorded, as the analysis focused on diagnostic characters rather than intraspecific morphological variation. Representative morphological sketches were also prepared to illustrate key diagnostic structures of *O. scolopendrina*, including both aboral and ventral disc features used for species identification (Figure 2).

Molecular analysis

DNA extraction. Muscle tissue samples were collected from the arms of each specimen, with approximately 5 cm of arm tissue preserved in 96% ethanol. DNA extraction was performed using the gSYNC Geneaid DNA extraction kit following the manufacturer's protocol (Bar et al. 2021). Tissue samples were homogenized and incubated with proteinase K and lysis buffer at 60°C for approximately 4 hours. Following incubation, samples were centrifuged, and the supernatant was transferred into a new tube. Binding buffer and ethanol were added to facilitate DNA binding to the column membrane. Samples were then washed using W1 buffer and wash buffer, followed by DNA elution using elution buffer and distilled water. Extracted DNA was stored at -20°C until further analysis.

Polymerase Chain Reaction (PCR) amplification. PCR amplification targeted a fragment of the mitochondrial 16S rRNA gene using universal primers for echinoderms: forward primer 16SF2 (5'-GTTTCGGTTTACCAAAAACAT-3') and reverse primer 16SR2 (5'-AGGTTTTTCTGATCCAACATCG-3') (de Moura Barboza et al. 2015). PCR reactions were carried out in a total volume of 25 µL containing 2.5 mM $MgCl_2$, 200 µM dNTPs, 0.2 µM of each primer, 1 µL BSA (10 mg/mL), 1 unit of Taq DNA polymerase, 2 µL of DNA template, and nuclease-free water. Amplification was performed using an Eppendorf Mastercycler gradient thermal cycler under the following conditions: initial denaturation at 94°C for 2 minutes; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 90 seconds, and extension at 72°C for 60 seconds; with a final extension at 72°C for 10 minutes (Ward et al. 2008). All 96 collected samples were successfully amplified and used for subsequent sequencing and genetic analyses.

Gel electrophoresis and sequencing. PCR products were visualized using agarose gel electrophoresis containing ethidium bromide. Electrophoresis was conducted at 220 V and 400 mA for approximately 25 minutes. DNA fragments were separated based on their migration rate under an electric field (Ward et al. 2008; Madduppa 2013). Successful PCR products were sequenced using the Sanger sequencing method (Sanger et al. 1977).

Sequence processing and alignment. Raw sequences were evaluated by examining chromatograms to ensure sequence quality. Low-quality regions and ambiguous bases were trimmed prior to analysis. Edited sequences were aligned using ClustalW implemented in MEGA Version 6.0 (Tamura et al. 2013). After trimming and alignment, a fragment of approximately 430 base pairs of the mitochondrial 16S rRNA gene was retained for further genetic analyses. All sequences generated in this study have been submitted to the GenBank database and are currently under review (accession numbers pending). The accession numbers will be made publicly available upon acceptance of the manuscript.

Genetic data analysis

Sequence similarity was assessed using the BLAST (Basic Local Alignment Search Tool) algorithm against the GenBank database (NCBI/The National Center for Biotechnology Information) to confirm species identity. BLAST results were used as supporting evidence and complemented with phylogenetic analysis. Phylogenetic relationships were reconstructed using Neighbor-Joining (NJ) and Maximum-Likelihood (ML) methods with 1000 bootstrap replications. The Kimura 2-Parameter (K2P) model was applied to maintain comparability with previous mitochondrial DNA studies in echinoderms and DNA barcoding frameworks. However, we acknowledge that model selection based on statistical criteria (e.g., Akaike Information Criterion, AIC) may provide a more accurate fit for the dataset. Therefore, the results of phylogenetic

reconstruction should be interpreted with consideration of this limitation (Kimura 1980). Genetic diversity parameters, including Haplotype number (H), Haplotype diversity (Hd), and nucleotide diversity (π), were calculated using DnaSP software. These parameters were used to assess genetic variation within and among populations. Haplotype networks were constructed using the Median-Joining algorithm implemented in NETWORK software to visualize genetic relationships among haplotypes. The geographic distribution of haplotypes was further analyzed to evaluate spatial patterns of genetic variation across sampling locations. Population genetic structure was assessed using the pairwise Fixation Index (FST) and Analysis of Molecular Variance (AMOVA) implemented in Arlequin software. Statistical significance of AMOVA was tested using 10,000 permutations.

RESULTS AND DISCUSSION

Morphology of Brittle star

A total of 96 Brittle star specimens were collected and identified morphologically. The results showed that all samples from the three sampling locations exhibited similar morphological characteristics and were identified as *O. scolopendrina*. However, a slight variation in body coloration was observed among individuals. The dorsal surface and dorsal shields were covered with granules and exhibited dark coloration patterns consisting of black and brown granular structures. Each arm segment possessed six spines located on the arm plate. The ventral surface consisted of jaws with papillae, two genital slits, and five madreporites distributed on the ventral shields. Each arm segment also possessed two podia located on the arm plate (Figure 2).

The morphological observations revealed that the body of *O. scolopendrina* has a pentagonal disc shape with slightly curved corners, giving the appearance of a circular outline. The aboral disc surface is covered with small, rounded granules. The species possesses five elongated arms with triangular arm plates covered by smaller granules compared to those on the disc surface. The ventral disc contains five oval-shaped shields.

The ventral shields of *O. scolopendrina* contain five madreporites distributed across the shields. The jaws contain eight papillae, where six papillae are larger, and two smaller papillae are located close to the dental papillae. The dental papillae consist of nine structures arranged in three rows, each containing three teeth. Each arm segment contains six spines, divided equally on each side of the arm plate. Four spines are located on the dorsal arm plate and two on the ventral side. The arm plate itself has an oval shape. Podia or tube feet are present on each arm segment and function as part of the water vascular system used for locomotion.

The observed morphological characters are consistent with diagnostic features of *O. scolopendrina* as described by Pomory (2007), including the presence of six arm spines per segment, a granulated dorsal disc, and a characteristic oral papillae arrangement. This species can be distinguished from closely related congeners such as *Ophiocoma erinaceus* (Müller & Troschel, 1842) and *Ophiocoma echinata* (Lamarck, 1816) by differences in spine morphology, disc granulation patterns, and arm plate structure. These diagnostic traits support the identification of all examined specimens as *O. scolopendrina*.

Molecular identification of Brittle star

Molecular identification successfully sequenced all 96 specimens targeting the mitochondrial 16S rRNA gene region. After sequence trimming and alignment, a final fragment of approximately 430 base pairs was obtained for subsequent analyses, containing a moderate number of variable and parsimony-informative sites. No insertions or deletions (indels) were detected, and sequences showed low levels of ambiguity after trimming. Ambiguous bases accounted for less than 1% of the total aligned sequences and were excluded during quality control to ensure reliable downstream analyses. These sequence characteristics indicate adequate variability for assessing mitochondrial genetic diversity at the population level, although the use of a single mitochondrial marker may limit fine-scale resolution. BLAST results showed query cover values ranging from 95% to 100%, with sequence similarity ranging from 97.86% to 100% with *O. scolopendrina* sequences available in GenBank.

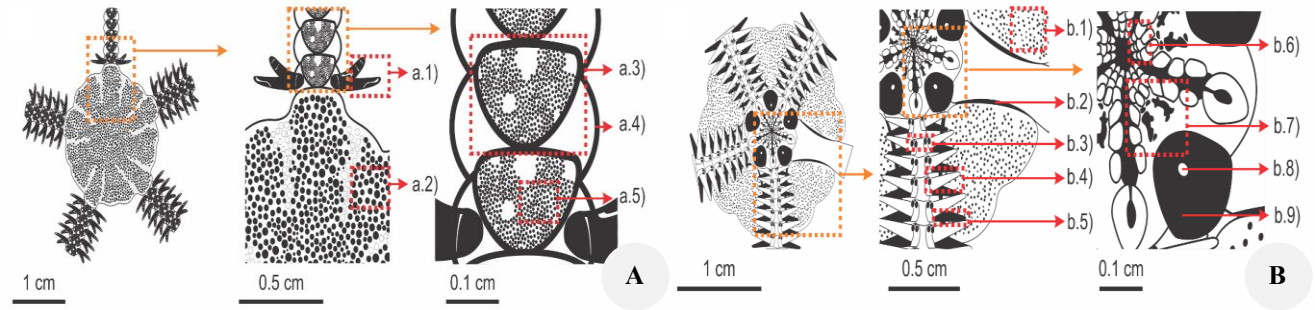

Figure 2. Morphological sketch of *Ophiocoma scolopendrina*: A. Aboral disc showing: a.1. Dorsal arm spines, a.2. Dorsal disc granules, a.3. Dorsal shield arm plate, a.4. Arm plate, a.5. Dorsal shield arm plate granules; B. Ventral disc showing: b.1. Interbrachial granules, b.2. Genital slits, b.3. Podial pore, b.4. Ventral arm spines, b.5. Podia, b.6. Dental papillae, b.7. Jaw with oral papillae, b.8. Madreporite, b.9. Ventral shields. These morphological characters were used for species identification following the diagnostic keys of Pomory (2007)

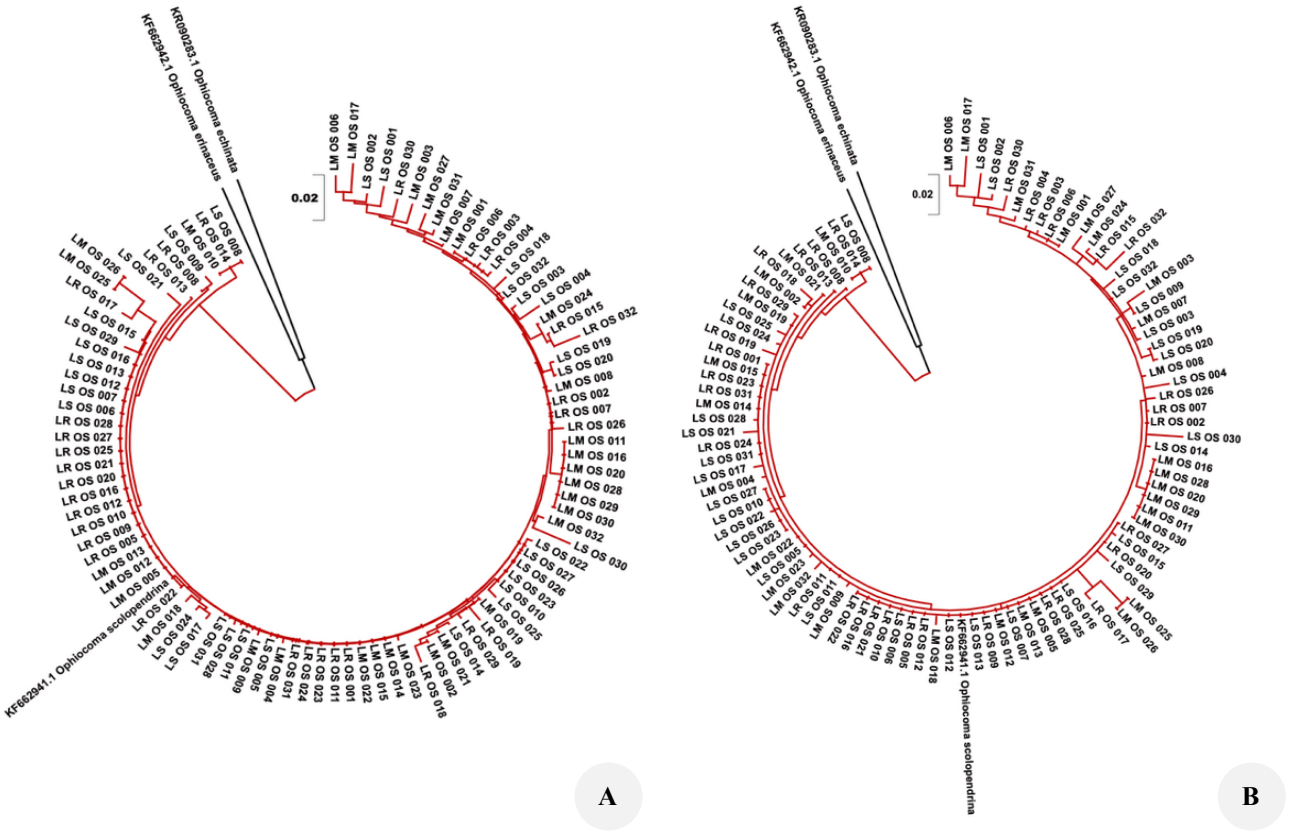

Figure 3. Phylogenetic reconstruction of *Ophiocoma scolopendrina* based on mitochondrial 16S rRNA gene sequences (~430 bp). Phylogenetic trees were constructed using: A. Neighbor-Joining, and B. Maximum-Likelihood methods under the Kimura 2-Parameter model with 1000 bootstrap replications. *Ophiocoma erinaceus* (KF662942.1) and *Ophiocoma echinata* (KR090283.1) were used as outgroup species

Table 1. Genetic diversity indices of *Ophiocoma scolopendrina* populations from three sampling locations in Aceh Besar, including Lhok Mee (LM), Lhok Redeup (LR), and Lhok Seudu (LS). N: Number of individuals analyzed, Hn: Number of haplotypes, Hd: Haplotype diversity, π: Nucleotide diversity

Locate	n	Hn	Hd	π
LM	32	22	0.956	0.016
LR	32	17	0.867	0.010
LS	32	23	0.962	0.013
All Population	96	55	0.928	0.013

The average query cover was 98.82%, and the average sequence similarity reached 99.82%, indicating that the analyzed samples belong to the species *O. scolopendrina* (Table 3). Phylogenetic analysis further confirmed this identification, showing that all *O. scolopendrina* samples formed a well-supported monophyletic clade, clearly separated from the outgroup species *O. erinaceus* and *O. echinata*, with high bootstrap support (>70%) in both Neighbor-Joining and Maximum-Likelihood analyses (Figure 3). No clear geographic clustering corresponding to sampling locations was observed within the phylogenetic tree, suggesting weak mitochondrial differentiation among populations. Genetic distance among *O. scolopendrina* samples ranged from 0.00 to 0.062, indicating low intraspecific divergence. In contrast, the genetic distance between *O. scolopendrina* and *O. erinaceus* was 0.256, while the distance between *O. scolopendrina* and *O. echinata* was 0.229, reflecting clear interspecific separation.

Genetic diversity of Brittle star

The number of haplotypes identified varied among populations. The Lhok Mee population contained 22 haplotypes, the Lhok Redeup population contained 17 haplotypes, and the Lhok Seudu population contained 23 haplotypes (Table 1). Haplotype diversity (Hd) ranged from 0.867 to 0.962, while nucleotide diversity (π) ranged from 0.010 to 0.016, indicating relatively high haplotype diversity but low nucleotide diversity across populations.

Several haplotypes were shared among all sampling locations, indicating genetic overlap among populations. In contrast, a number of haplotypes were private and restricted to single locations, suggesting localized variation within an overall pattern of weak mitochondrial differentiation.

Genetic structure of Brittle star

Population genetic structure was evaluated using the Fixation Index (FST). Pairwise FST values ranged from 0.002 to 0.016, indicating very low genetic differentiation

among the three sampling locations (Table 2). Pairwise FST comparisons were low across all population pairs and were not statistically significant (P>0.05), further supporting the absence of strong genetic structure among sampling sites.

AMOVA results showed that 98.82% of the genetic variation occurred within populations, while only 1.18% occurred among populations, indicating weak population differentiation (Table 4). The AMOVA analysis produced an FST value of 0.012 with a P-value of 0.088.

Haplotype network

Haplotype network analysis identified 55 haplotypes among the 96 analyzed individuals of *O. scolopendrina* collected from three sampling locations in Aceh Besar, indicating high haplotype diversity within the studied populations (Figure 4). Among all haplotypes, haplotype H_5 showed the highest number of individuals, with a total of 19 samples, followed by haplotype H_12 with 11 individuals and haplotype H_11 with 6 individuals. Only a single individual represented most haplotypes.

Geographic distribution of haplotypes

The geographic distribution of haplotypes across the three sampling locations is illustrated in Figure 5. Each sampling location exhibited a mixture of haplotypes rather than being dominated by a single unique haplotype. Lhok Mee (LM), Lhok Redeup (LR), and Lhok Seudu (LS) all contained diverse haplotype compositions, with several haplotypes occurring in more than one location. The most common haplotypes were distributed across all three sampling locations, while less frequent haplotypes were typically restricted to individual sites.

Discussion

The genetic diversity analysis revealed high Haplotype diversity (Hd = 0.867-0.962) but low nucleotide diversity (π = 0.010-0.016) across the studied populations. According to Cui et al. (2025), haplotype diversity values greater than 0.5 indicate high genetic diversity within populations. Patterns of high haplotype diversity combined with low nucleotide diversity have often been interpreted as evidence of large population sizes or recent demographic expansion in marine organisms (Raj et al. 2024; Dash et al. 2025). The combination of high haplotype diversity and low nucleotide diversity observed in this study is a common genetic pattern in marine organisms and has been widely discussed in population genetics theory. This pattern is often associated with populations that have undergone recent expansion, where multiple haplotypes arise from a common ancestral lineage but have not accumulated substantial sequence divergence (Fourdrilis et al. 2016). Such genetic signatures are frequently observed in species with large effective population sizes and high reproductive output, particularly in marine invertebrates inhabiting dynamic coastal environments (Quintero-Galvis et al. 2020).

The combination of high haplotype diversity and low nucleotide diversity observed in this study suggests that the populations of *O. scolopendrina* consist of numerous closely related haplotypes that differ only by a few mutational steps. Such genetic patterns are frequently interpreted as signals of recent population expansion following a historical bottleneck or colonization event. However, this interpretation remains hypothetical in the present study, as no formal demographic analyses (e.g., Tajima's D, Fu's Fs, or mismatch distribution) were conducted (Kaewmungkoon et al. 2025). This pattern may also reflect rapid demographic expansion after environmental stabilization, where newly arising mutations accumulate within populations without substantial divergence among lineages. In marine invertebrates, this pattern is commonly associated with species that possess large effective population sizes and high dispersal capabilities (Kochanova et al. 2021). Similar patterns of genetic diversity have been reported in other marine invertebrates, including echinoderms and coral-associated species, where high haplotype diversity is accompanied by low nucleotide diversity across geographically separated populations (Otwoma and Kochzius 2016; Waheed et al. 2023). These patterns have been interpreted as evidence of demographic expansion and/or high dispersal potential, although the extent of connectivity often varies depending on species-specific life history traits and environmental conditions.

Table 2. Pairwise Fixation Index (FST) values among populations of *Ophiocoma scolopendrina* from three sampling locations in Aceh Besar, Indonesia, were used to evaluate genetic differentiation between populations

Locate	LM	LR	LS
LM	-	-	-
LR	0.016	-	-
LS	0.016	0.002	-

Table 4. Analysis of Molecular Variance (AMOVA) showing the distribution of genetic variation within and among populations of *Ophiocoma scolopendrina* from Aceh Besar, Indonesia

Source of variation	d.f	Percentage of variation	F_{ST}	P-value
Among populations	2	1.18	0.012	0.088
Within populations	93	98.82		

Table 3. Representative BLAST results of *Ophiocoma scolopendrina* sequences

Sample ID	Closest match	Accession number	Query cover (%)	Identity (%)	E-value
O.S_LM_001	*Ophiocoma scolopendrina*	KU672435.1	99	100	0.0
O.S_LR_025	*Ophiocoma scolopendrina*	KU672435.1	99.3	99	0.0
O.S_LS_017	*Ophiocoma scolopendrina*	KU672435.1	97	99	0.0

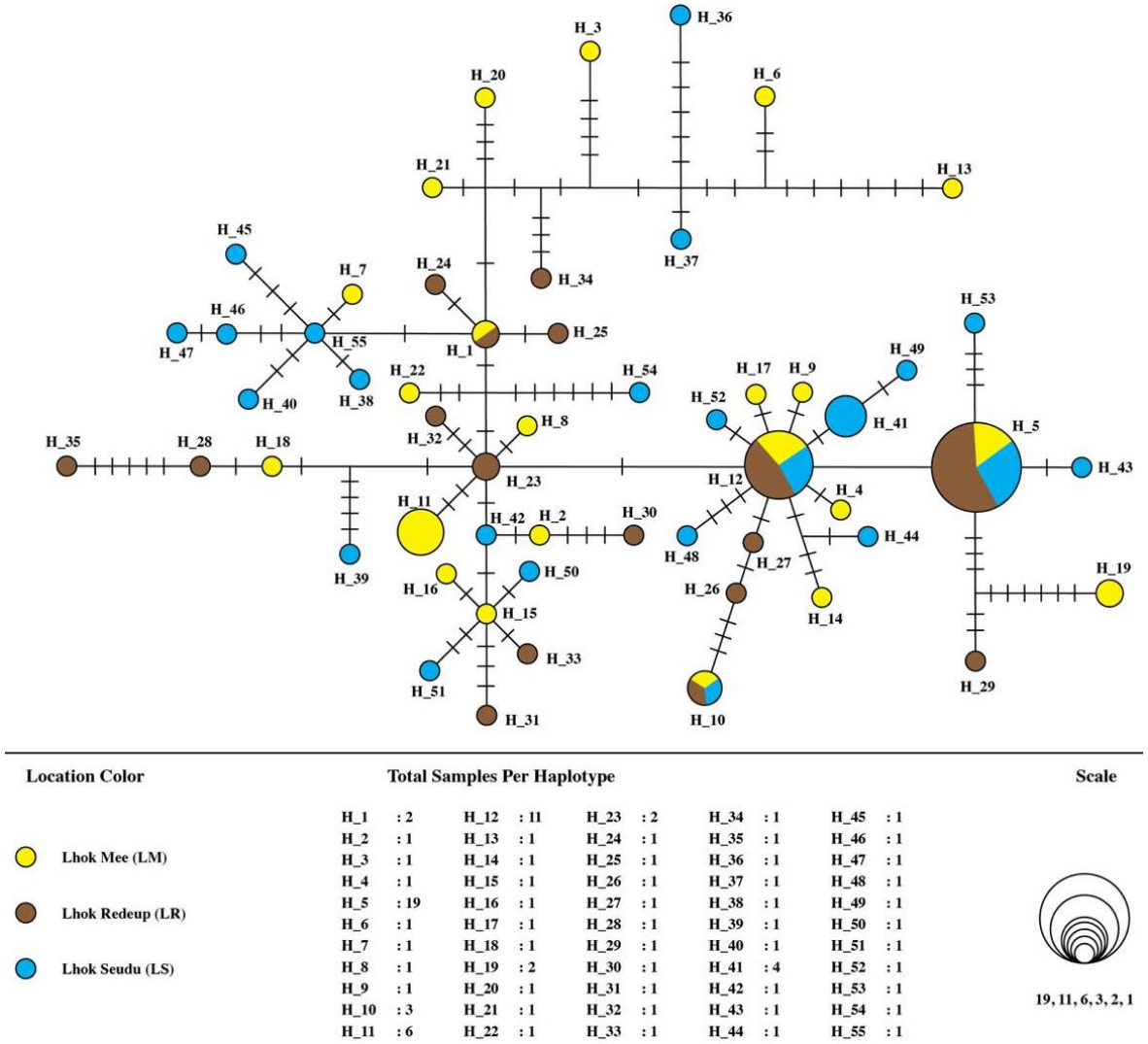

Figure 4. Haplotype network of *Ophiocoma scolopendrina* based on mitochondrial 16S rRNA gene sequences. Each circle represents a haplotype, and the size of the circle corresponds to the number of individuals sharing that haplotype. Colors indicate sampling locations: Lhok Mee (LM), Lhok Redeup (LR), and Lhok Seudu (LS). Lines connecting haplotypes represent mutational steps between haplotypes. The network illustrates the genetic relationships among haplotypes detected from 96 individuals collected in the coastal waters of Aceh Besar, Indonesia

The haplotype network analysis revealed the presence of several dominant haplotypes accompanied by many low-frequency haplotypes. Similar patterns have been reported in marine invertebrates and are often associated with large effective population sizes or historical demographic expansion (Robalo et al. 2020; Sun et al. 2022). Comparable genetic patterns have also been observed in echinoderms such as Brittle stars inhabiting coral reef ecosystems (Lessios and Hendler 2022; Sobczyk et al. 2023). The haplotype network constructed in this study further supports this interpretation, as several dominant haplotypes were connected to numerous rare haplotypes in the network. This configuration indicates that many haplotypes may have originated from a common ancestral haplotype through recent mutations. Such star-like haplotype network structures are widely recognized as genetic signatures of population expansion and have been documented in numerous marine taxa with high dispersal potential (Chen et al. 2025; Li et al. 2026).

The low pairwise FST values (0.002-0.016) and AMOVA results (FST = 0.012; P = 0.088) indicate weak and statistically non-significant genetic differentiation among the three sampling locations. Therefore, the results should be interpreted as evidence of limited or undetectable mitochondrial differentiation rather than definitive proof of population connectivity or ongoing gene flow (Wu et al. 2024). The presence of shared haplotypes among sampling locations may suggest potential genetic exchange or recent common ancestry among populations. However, shared haplotypes can also arise from incomplete lineage sorting or limited resolution of the mitochondrial marker used in this study (Cerca et al. 2021). Thus, the observed pattern should be interpreted cautiously and not solely attributed to active gene flow or larval dispersal.

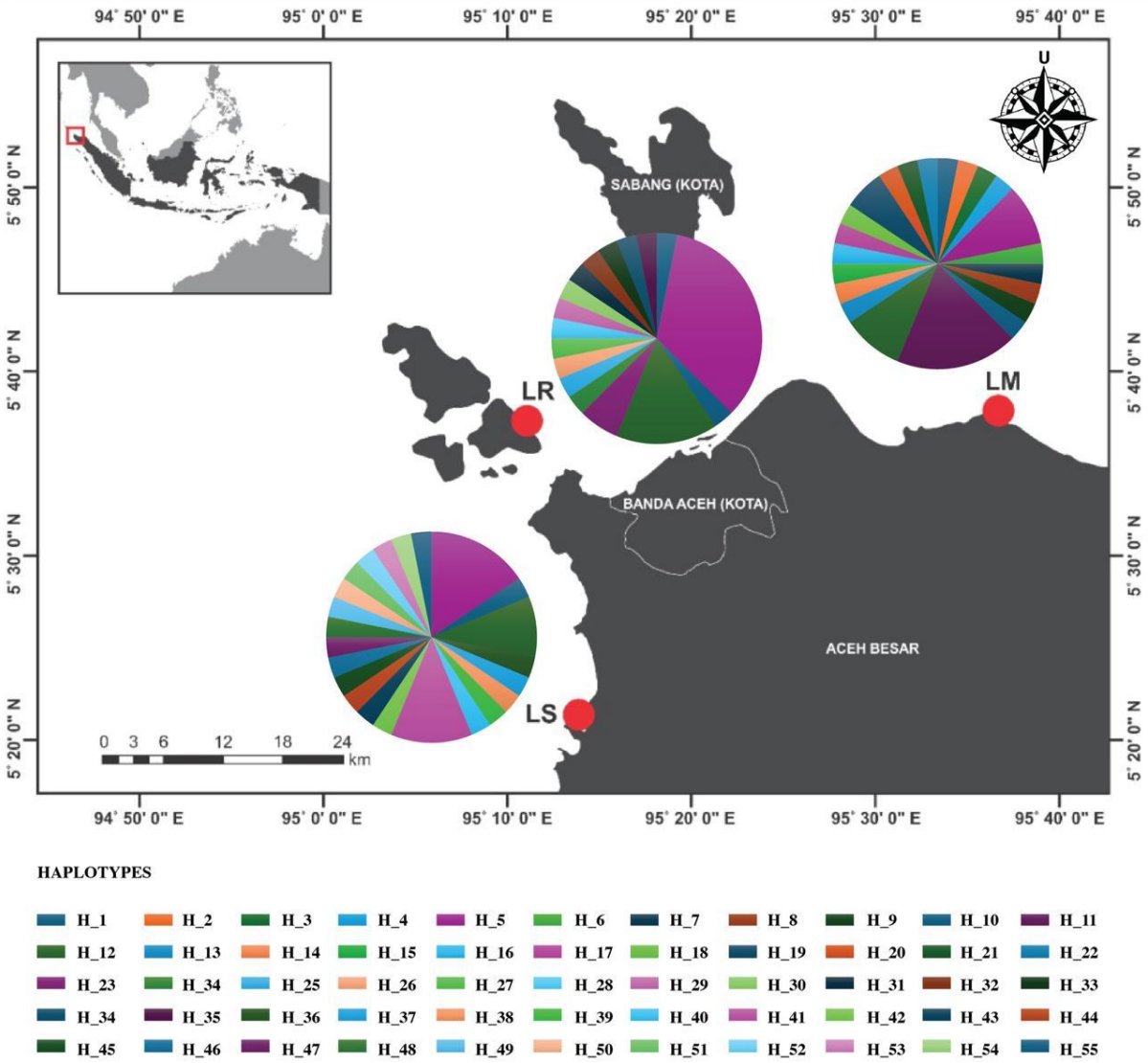

Figure 5. Geographic distribution of haplotypes of *Ophiocoma scolopendrina* across three sampling locations in Aceh Besar, Indonesia: Lhok Mee (LM), Lhok Redeup (LR), and Lhok Seudu (LS). Pie charts represent the relative frequency of haplotypes detected in each population based on mitochondrial 16S rRNA gene sequences

Marine invertebrates with planktonic larval stages often exhibit low genetic differentiation across geographic regions due to dispersal facilitated by ocean currents (Melroy and Cohen 2021). Larval dispersal is therefore a plausible biological mechanism that could contribute to the observed genetic pattern. However, this mechanism was not directly tested in the present study, as no oceanographic data, larval duration measurements specific to *O. scolopendrina*, or dispersal modeling were included (Michie et al. 2024). In the context of Aceh Besar, the interaction between the Indian Ocean and the Malacca Strait may influence dispersal pathways and population structure. However, the role of these oceanographic processes remains speculative in this study and should be interpreted as a potential explanation rather than a confirmed mechanism.

Taken together, the results of haplotype diversity, nucleotide diversity, haplotype network structure, and population genetic analysis indicate that *O. scolopendrina* populations in Aceh Besar exhibit weak mitochondrial differentiation across sampling sites. These findings are consistent with a scenario of genetic homogeneity at the mitochondrial level; however, they do not provide direct evidence of panmixia or high levels of ongoing gene flow (Jose et al. 2023). These findings contribute to the broader understanding of population genetic patterns in marine invertebrates, particularly in tropical coastal ecosystems (Farhadi et al. 2024). The observed genetic structure highlights the importance of considering both genetic diversity and population connectivity when evaluating marine biodiversity and ecosystem resilience.

From a conservation perspective, the observed genetic pattern suggests that populations across the studied sites may not be strongly differentiated at the mitochondrial level. However, given the limited marker resolution and spatial coverage, conservation recommendations should be made

cautiously and primarily viewed as baseline information rather than definitive management guidance. This study is subject to several limitations. First, the analysis relied on a single mitochondrial marker (16S rRNA, ~430 bp), which may not provide sufficient resolution to detect fine-scale population structure. Second, the spatial coverage was limited to three sampling locations within a relatively narrow geographic range. These limitations may reduce the ability to detect subtle genetic differentiation and restrict broader biogeographic inference (Glück et al. 2022).

In addition, the use of purposive sampling in this study ensured that only intact adult individuals suitable for both morphological and molecular analyses were selected. However, this sampling approach may limit the representativeness of the sampled populations and may not fully capture the complete genetic variation present in natural populations. Consequently, the population genetic inferences presented in this study should be interpreted with caution, as they may reflect patterns within the sampled individuals rather than the entire population. Future studies employing random or stratified sampling designs would be beneficial to improve representativeness and strengthen population-level conclusions.

Overall, the present study provides integrated morphological and mitochondrial evidence that *O. scolopendrina* populations in the coastal waters of Aceh Besar exhibit high haplotype diversity but weak genetic differentiation across sampling locations. These findings suggest that the observed genetic pattern is consistent with limited mitochondrial structuring; however, the extent of population connectivity and gene flow cannot be conclusively determined based on the current dataset. As such, this study serves as a baseline for future research and highlights the need for more comprehensive genetic and ecological approaches to understand population dynamics in marine invertebrates better. Future studies should incorporate additional genetic markers, such as COI, nuclear DNA, or genome-wide approaches (e.g., SNPs), to provide higher resolution insights into population structure. Expanding geographic sampling and integrating oceanographic and ecological data would also improve understanding of dispersal processes and connectivity patterns in this species. These findings provide baseline genetic information that may support future monitoring and conservation planning in coastal ecosystems of Aceh Besar. This information may help identify coastal habitats that maintain important genetic diversity, support habitat protection efforts in intertidal areas where *O. scolopendrina* occurs, and inform sustainable coastal biodiversity management in Aceh Besar.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the Department of Marine Science, Faculty of Fisheries and Marine Sciences, Universitas Teuku Umar, Aceh, Indonesia, for providing laboratory facilities to conduct the molecular analyses in this study. We also thank all field assistants who contributed to sample collection in the coastal waters of Aceh Besar. The authors acknowledge the valuable support provided by their respective institutions during the completion of this research. All sampling procedures were conducted in accordance with local regulations and permitted by the relevant authorities in Aceh Province. The authors declare that there are no conflicts of interest regarding the publication of this study.

REFERENCES

Adams NL, Heyland A, Rice LL, Foltz KR. 2019. Procuring animals and culturing of eggs and embryos. Methods Cell Biol 150: 3-46. https://doi.org/10.1016/bs.mcb.2018.11.006.

Alemdag M, Öztürk RÇ, Firidin SI, Altinok I. 2026. Genetic structure, species boundaries, and evidence of hybridization in Turkish brown trout populations. Hydrobiologia 853: 2643-2667. https://doi.org/10.1007/s10750-025-06101-y.

Aziz A. 1991. Some notes on ophiurids as benthic fauna. Oseana 16 (1): 13-22.

Bar S, Vershkov D, Keshet G, Lezmi E, Meller N, Yilmaz A, Yanuka O, Nissim-Rafinia M, Meshorer E, Eldar-Geva T, Benvenisty N. 2021. Identifying regulators of parental imprinting by CRISPR/Cas9 screening in haploid human embryonic stem cells. Nat Commun 12: 6718. https://doi.org/10.1038/s41467-021-26949-7.

Boos K. 2012. Tooth morphology and food processing in *Ophiothrix fragilis* (Abildgaard, in O.F. Müller, 1789) and *Ophiura albida* Forbes, 1839 (Echinodermata: Ophiuroidea). Zoosymposia 7: 111-119. https://doi.org/10.11646/zoosymposia.7.1.11.

Cerca J, Rivera-Colón AG, Ferreira MS, Ravinet M, Nowak MD, Catchen JM, Struck TH. 2021. Incomplete lineage sorting and ancient admixture, and speciation without morphological change in ghost-worm cryptic species. PeerJ 9: e10896. https://doi.org/10.7717/peerj.10896.

Chen Z, Chen W, Du H. 2025. Phylogeographic pattern of *Sargassum hemiphyllum* var. *chinense* (Phaeophyceae, Ochrophyta) in Chinese coastal waters. Plants 14 (9): 1269. https://doi.org/10.3390/plants14091269.

Cui J, Zhang J, Liu J, Wang Z, Guo Y, Yang J, Wang Z. 2025. Establishment of a *COI* haplotype baseline and genetic diversity evaluation of *Vespa soror* (Hymenoptera: Vespidae) in Southern China based on mitochondrial gene sequences. Diversity 17 (11): 756. https://doi.org/10.3390/d17110756.

Czarkwiani A, Ferrario C, Dylus DV, Sugni M, Oliveri P. 2016. Skeletal regeneration in the brittle star *Amphiura filiformis*. Front Zool 13: 18. https://doi.org/10.1186/s12983-016-0149-x.

Dash SS, Golive P, Parameswaran C, Rath PC, Chatterjee H, Mukherjee AK, Tripathy PS, Nayak AK, Mohapatra S, Behera BK, Das Mohapatra S. 2025. Genetic diversity and population structure of *Cnaphalocrocis medinalis* across India and South Asia: Insights from *COI* and *ITS2* gene analyses. Curr Res Biotechnol 9: 100281. https://doi.org/10.1016/j.crbiot.2025.100281.

Dauvin J, Méar Y, Murat A, Poizot E, Lozach S, Beryouni K. 2013. Interactions between aggregations and environmental factors explain spatio-temporal patterns of the brittle-star *Ophiothrix fragilis* in the Eastern Bay of Seine. Estuarine, Coastal and Shelf Science 131: 171-181. http://doi.org/10.1016/j.ecss.2013.07.005.

de Moura Barboza CA, Mattos G, Paiva PC. 2015. Brittle stars from the Saint Peter and Saint Paul Archipelago: Morphological and molecular data. Mar Biodivers Rec 8 (e16): 1-9. https://doi.org/10.1017/S1755267214001511.

Dharmayanti N. 2011. Molecular phylogenetic: Organism taxonomy method based on evolution history. Wartazoa 21: 1-10.

Dylus DV, Czarkwiani A, Blowes LM, Elphick MR, Oliveri P. 2018. Developmental transcriptomics of the brittle star *Amphiura filiformis* reveals gene regulatory network rewiring in echinoderm larval skeleton evolution. Genome Biol 19: 26. https://doi.org/10.1186/s13059-018-1402-8.

Farhadi A, Vazirzadeh A, Jeffs AG, Lavery SD. 2024. Genetic insights into the population connectivity, biogeography, and management of fisheries-important spiny lobsters (Palinuridae). Rev Fish Sci Aquac 32 (4): 579-611. https://doi.org/10.1080/23308249.2024.2355576.

Fourdrilis S, Mardulyn P, Hardy OJ, Jordaens K, de Frias Martins AM, Backeljau T. 2016. Mitochondrial DNA hyperdiversity and its potential causes in the marine periwinkle *Melarhaphe neritoides* (Mollusca: Gastropoda). PeerJ 4: e2549. https://doi.org/10.7717/peerj.2549.

Glück M, Geue JC, Thomassen HA. 2022. Environmental differences explain subtle yet detectable genetic structure in a widespread pollinator. BMC Ecol Evol 22: 8. https://doi.org/10.1186/s12862-022-01963-5.

Jose A, Sukumaran S, Roul SK, Azeez PA, Kizhakudan SJ, Raj N, Nisha K, Gopalakrishnan A. 2023. Genetic analyses reveal panmixia in Indian waters and population subdivision across the Indian Ocean and the Indo-Malay Archipelago for *Decapterus russelli*. Sci Rep 13: 22860. https://doi.org/10.1038/s41598-023-49805-8.

Kaewmungkoon S, Pradit N, Pilap W, Ninlaphay S, Chatiwong T, Saijuntha J, Jaroenchaiwattanachote C, Tawong W, Suksavate W, Pramual P, Tantrawatpan C, Saijuntha W. 2025. Genetic diversity, genetic structure and demographic history of the leaf beetle *Platycorynus peregrinus* (Herbst, 1783) (Coleoptera: Chrysomelidae) from Thailand. Biology 14 (9): 1266. https://doi.org/10.3390/biology14091266.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111-120. https://doi.org/10.1007/bf01731581.

Kochanova E, Nair A, Sukhikh N, Väinölä R, Husby A. 2021. Patterns of cryptic diversity and phylogeography in four freshwater copepod crustaceans in European lakes. Diversity 13 (9): 448. https://doi.org/10.3390/d13090448.

Lessios HA, Hendler G. 2022. Mitochondrial phylogeny of the brittle star genus *Ophioderma*. Sci Rep 12: 5304. https://doi.org/10.1038/s41598-022-08944-0.

Li Y-Q, Huang X-P, Li D, Wu T, Fu X-Y, Zhang Y-N, Huang Q, Wei G-F, Wan L-L, Zhang Q. 2026. Mitochondrial *Cyt b* reveals low diversity and basin-scale population structure in black carp (*Mylopharyngodon piceus*) from the Yangtze, Pearl, and Red River Basins. Animals 16 (5): 768. https://doi.org/10.3390/ani16050768.

Mackenzie CL, Kent FEA, Baxter JM, Gormley KSG, Cassidy AJ, Sanderson WG, Porter JS. 2022. Genetic connectivity and diversity of a protected, habitat-forming species: Evidence demonstrating the need for wider environmental protection and integration of the marine protected area network. Front Mar Sci 9: 772259. https://doi.org/10.3389/fmars.2022.772259.

Madduppa HH. 2013. Bioekologi dan Biosistematika Ikan Terumbu. IPB Press, Bogor. [Indonesian]

Melroy LM, Cohen CS. 2021. Temporal and spatial variation in population structure among brooding sea stars in the genus *Leptasterias*. Ecol Evol 11 (7): 3313-3331. https://doi.org/10.1002/ece3.7283.

Michie C, Lundquist CJ, Lavery SD, Penna AD. 2024. Spatial and temporal variation in the predicted dispersal of marine larvae around coastal Aotearoa, New Zealand. Front Mar Sci 10: 1292081. https://doi.org/10.3389/fmars.2023.1292081.

Nugroho W, Ruswahyuni R, Suryanti S. 2014. Kelimpahan bintang mengular (Ophiuroidea) di perairan Pantai Sundak dan Pantai Kukup, Kabupaten Gunungkidul, Yogyakarta. J Management of Aquatic Resources 3 (4): 51-57. [Indonesian]

Nurdiansah D, Supono. 2017. Diversity of brittle stars (Ophiuroidea) in Talise Waters, North Sulawesi. J Ilmu Teknologi Kelautan Tropis 9 (2): 709-716. https://doi.org/10.29244/jitkt.v9i2.19303.

O'Hara T, Hugall A, Thuy B, Stöhr S, Martynov AV. 2017. Restructuring higher taxonomy using broad-scale phylogenomics: The living Ophiuroidea. Molecular Phylogenetics and Evolution 107: 415-430. http://doi.org/10.1016/j.ympev.2016.12.006.

Otwoma LM, Kochzius M. 2016. Genetic population structure of the coral reef sea star *Linckia laevigata* in the Western Indian Ocean and Indo-West Pacific. PLoS One 11 (10): e0165552. https://doi.org/10.1371/journal.pone.0165552.

Pechenik JA. 2005. Biology of the Invertebrates. McGraw-Hill Higher Education, Boston.

Pomory CM. 2007. Key to the common shallow-water brittle stars (Echinodermata: Ophiuroidea) of the Gulf of Mexico and Caribbean Sea. Caribb J Sci Spec Publ 10: 1-42.

Quintero-Galvis JF, Bruning P, Paleo-López R, Gómez D, Sánchez R, Cárdenas L. 2020. Temporal variation in the genetic diversity of a marine invertebrate with a long larval phase, the muricid gastropod *Concholepas concholepas*. J Exp Mar Biol Ecol 530-531: 151432. https://doi.org/10.1016/j.jembe.2020.151432.

Raj N, Sukumaran S, Jose A, Nisha K, Roul SK, Rahangdale S, Kizhakudan SJ, Gopalakrishnan A. 2024. Population genetic structure of randall's threadfin bream, *Nemipterus randalli*, in Indian waters based on mitochondrial and nuclear gene sequences. Sci Rep 14: 7556. https://doi.org/10.1038/s41598-024-58047-1.

Robalo JI, Francisco SM, Vendrell C, Lima CS, Pereira A, Brunner BP, Dia M, Gordo L, Castilho R. 2020. Against all odds: A tale of marine range expansion with maintenance of extremely high genetic diversity. Sci Rep 10: 12707. https://doi.org/10.1038/s41598-020-69374-4.

Rostikawati RT, Susanto LH, Ichsan IZ, Marhento G. 2023. Development of biology learning media based on echinoderm diversity for supporting environmental sustainability. J Penelitian Pendidikan IPA 9 (3): 1217-1225. https://doi.org/10.29303/jppipa.v9i3.3098.

Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74 (12): 5463-5467. https://doi.org/10.1073/pnas.74.12.5463.

Sarong MA, Saputrie M, Maslim. 2014. Wajah Pesisir Aceh. Pena, Banda Aceh. [Indonesian]

Sobczyk R, Presler P, Czortek P, Serigstad B, Pabis K. 2023. Diversity, distribution patterns, and indicatory potential of echinoderm communities of the tropical East Atlantic (Gulf of Guinea): Influence of multiple natural and anthropogenic factors along a 25-1000 m depth gradient. Ecol Indic 156: 111108. https://doi.org/10.1016/j.ecolind.2023.111108.

Sun C-H, Yang F, Huang Q, Zeng X-S, Zhang Y-N, Li S, Yu J-F, Zhang Q. 2022. Genetic population structure and demographic history of the endemic fish *Paralichthys olivaceus* of the Northwest Pacific Ocean. Ecol Evol 12 (11): e9506. https://doi.org/10.1002/ece3.9506.

Supono. 2018. Difficulties in the identification of deep-sea Ophiuroidea post-larvae from Makassar Strait, Indonesia, and their implication for taxonomic studies. Oseanologi dan Limnologi di Indonesia 3 (1): 11-18. https://doi.org/10.14203/oldi.2018.v3i1.127.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30 (12): 2725-2729. https://doi.org/10.1093/molbev/mst197.

Waheed Z, Bos AR, Kochzius M, Hoeksema BW. 2023. Genetic population structure of the blue sea star (*Linckia laevigata*) and the boring giant clam (*Tridacna crocea*) across Malaysia. Hydrobiologia 850: 81-95. https://doi.org/10.1007/s10750-022-05044-y.

Wakita D, Kagaya K, Aonuma H. 2020. A general model of locomotion of brittle stars with a variable number of arms. J R Soc Interface 17 (162): 20190374. https://doi.org/10.1098/rsif.2019.0374.

Ward RD, Holmes BH, O'Hara TD. 2008. DNA barcoding discriminates echinoderm species. Mol Ecol Resour 8 (6): 1202-1211. https://doi.org/10.1111/j.1755-0998.2008.02332.x.

Wilkie IC. 2016. Functional morphology of the arm spine joint and adjacent structures of the brittle star *Ophiocomina nigra* (Echinodermata: Ophiuroidea). PLoS One 11 (12): e0167533. https://doi.org/10.1371/journal.pone.0167533.

Wu H, Qi S, Fan S, Li H, Zhang Y, Zhang Y, Xu Q, Chen G. 2024. Analysis of the mitochondrial *COI* gene and genetic diversity of endangered goose breeds. Genes 15 (8): 1037. https://doi.org/10.3390/genes15081037.