

RESEARCH ARTICLE

GENETIC DIVERSITY AND POPULATION INSIGHTS OF KAWAKAWA *EUTHYNNUS AFFINIS* (CANTOR, 1849) IN MALAYSIAN BORNEO REVEALED BY MITOCHONDRIAL DNA CYTOCHROME B GENEKhaled Binashikh Abubkr^{1,*}, and Kamal Ahmed Baaom¹¹ Dept. of Biology, Faculty of Science, Hadhramout University, Mukalla, Yemen*Corresponding author: Khaled Binashikh Abubkr; E-mail: khaledsaleh@hu.edu.ye

Received: 13 February 2026 / Accepted: 06 March 2026 / Published online: 31 March 2026

Abstract

Kawakawa (*Euthynnus affinis*) is a commercially valuable pelagic species with a broad distribution across tropical and subtropical waters of the Indo-Pacific region. Despite its economic importance, limited knowledge of stock structure, management, and conservation in Malaysian and adjacent waters has raised concerns regarding overexploitation and population depletion. Comprehensive molecular assessments of population structure are therefore critical for informing effective fisheries management strategies. This study investigated the genetic diversity and population structure of *E. affinis* in Malaysian waters using the mitochondrial cytochrome *b* (Cyt *b*) gene. A 522 bp fragment of the Cyt *b* region was sequenced from 120 individuals collected from eight geographically distinct populations in Malaysian Borneo. Genetic divergence among populations ranged from low to high, accompanied by high overall haplotype diversity ($H_d = 0.9260$) and moderate nucleotide diversity ($\pi = 0.0325$). A total of 32 unique haplotypes were identified across all sampled populations. Phylogenetic reconstruction based on Neighbor-Joining analysis, together with haplotype relationships inferred from a minimum spanning network, revealed the presence of two distinct genetic clades. Analysis of molecular variance (AMOVA) demonstrated strong and statistically significant genetic structuring among populations ($F_{ST} = 0.902$, $P < 0.05$). Neutrality tests further indicated a historical signal of population expansion. This study constitutes the first comprehensive Cyt *b*-based molecular assessment of the genetic structure of *E. affinis* in Malaysian Borneo and provides essential baseline information to support sustainable fisheries management and conservation planning.

Keywords: Cyt *b* gene; *Euthynnus affinis*; fisheries management; Population structure; Conservation genetics.**1. Introduction:**

Globally, ichthyofaunal diversity is exceptionally high, with 37,109 currently recognised fish species distributed across more than 11,000 genera and subgenera [1]. Fishes represent one of the most ancient vertebrate lineages, originating over 500 million years ago, and have since undergone extensive evolutionary diversification. Their remarkable variation in morphology, habitat preference, behaviour, and physiological adaptations, both extant and extinct, renders their evolutionary history and taxonomic classification complex yet scientifically compelling. From primitive agnathans such as hagfishes and lampreys to elasmobranchs, lungfishes, flatfishes, and

highly derived teleosts, fishes exhibit a wide array of adaptive strategies that enable them to occupy nearly all aquatic ecosystems worldwide [2], [3]. Malaysia harbours substantial marine fish diversity, particularly at major fish landing sites where approximately 200–300 species are recorded. Daily fish markets typically offer 50–100 species, reflecting both high biodiversity and strong fisheries activity. While certain species exhibit seasonal availability, others occur year-round in estuarine, coastal, bay, and reef habitats [4], [5].

In recent years, Malaysia's marine fisheries sector has attracted increasing attention due to its growing potential as a key fisheries hub in Southeast Asia [6], [7]. This prominence is largely attributed to the country's strategic

geographic position within several ecologically important marine regions, underpinning its rich maritime heritage and marine biodiversity. Malaysia is geographically situated at the convergence of several major marine systems. The South China Sea lies to the east of Peninsular Malaysia and to the west of East Malaysia (Sabah and Sarawak), while the Strait of Malacca borders the western coast of Peninsular Malaysia, separating it from Sumatra (Indonesia). To the northeast and east of Sabah are the Sulu Sea and the Celebes Sea, respectively, whereas the Andaman Sea is located to the northwest of Peninsular Malaysia [8]. Collectively, these waters support productive fisheries that contribute substantially to national food security, employment, and export revenue [5]

Accurate stock identification is fundamental for effective fisheries management and conservation. To this end, a range of methodological approaches has been employed for marine and freshwater fishes, including traditional morphometric analyses [9-12], geometric morphometrics [13-16], meristic [17], and molecular approaches based on DNA markers, particularly mitochondrial genes [18-20]. Among these, mitochondrial DNA (mtDNA) has been widely applied in species identification and population genetic studies due to its high copy number, lack of recombination, and maternal inheritance. The mitochondrial cytochrome *b* (Cyt *b*) gene is especially valuable for evaluating intraspecific genetic diversity, as it evolves at a moderate rate suitable for taxonomic, phylogenetic, and population-level analyses across a wide range of fish taxa [21], [22]. Consequently, Cyt *b* has been extensively used to investigate population structure, genetic variation, and evolutionary relationships in numerous marine fish species [23-26].

The kawakawa tuna, *Euthynnus affinis*, was first described by Cantor in 1849 as *Thynnus affinis*, with the type locality in the Sea of Penang, Malaysia. This highly migratory species inhabits neritic waters throughout the Indian Ocean and western Pacific, where it forms large schools, often in association with other scombrids. Spawning periods vary geographically, occurring from August to October in Indonesian waters, March to May in the Philippines, and January to July off East Africa [27]. *Euthynnus affinis* undertakes seasonal movements between feeding and spawning grounds and constitutes a key resource for both commercial and artisanal fisheries across the Central and Western Pacific and the Indian Ocean. Its distribution extends from Malaysia northward to China, Taiwan, and southern Japan, and its considerable economic importance has prompted

research into aquaculture development and crossbreeding programmes [28]. Despite its economic significance, *E. affinis* populations in Malaysia and surrounding waters are increasingly threatened by overfishing and illegal, unreported, and unregulated (IUU) fishing activities [29]. Effective monitoring and scientifically informed management strategies are therefore essential to ensure the long-term sustainability of this species. In this context, the present study employs mitochondrial Cyt *b* markers to assess the genetic diversity and population structure of eight *E. affinis* populations from Malaysian Borneo (Sabah and Sarawak). By elucidating genetic patterns within these waters, this study provides critical baseline information that will support fisheries managers and policymakers in developing robust regional and international conservation and management strategies to mitigate further population declines.

2. Materials and Methods

2.1. Study area and Sampling

A total of 120 specimens of *Euthynnus affinis* were collected from eight major fish landing sites across Malaysian Borneo, encompassing the states of Sabah and Sarawak. Sampling locations were selected based on fish availability as documented in the annual report of the Department of Fisheries Malaysia [30]. Specimens were obtained from three principal marine regions: the South China Sea, the Sulu Sea, and the Celebes Sea. Within the South China Sea region, samples were collected from four landing sites: Bintawa, Pulau Buit, and Mukah in Sarawak, as well as Kota Kinabalu in Sabah. Two additional landing sites—Kudat and Sandakan—were sampled from the Sulu Sea, while Lahad Datu and Tawau, both located in Sabah, represented the Celebes Sea region (Table 1; Figure 1). All specimens were initially identified based on external morphological characteristics following the taxonomic keys of [31]. The verified samples were then transported to the Molecular Ecology Laboratory, School of Biological Sciences, Universiti Sains Malaysia. For molecular analyses, a tissue fragment approximately 1–2 cm in length was excised from the right pectoral fin of each specimen and preserved in 95% ethanol before DNA extraction.

Table 1: Sampling locations, coordinates, and sample size (N) of eight populations of *E. affinis* based on Cyt *b* genes.

No	Sampling locations	Coordinates	Marine region	N
1.	Sandakan, Sabah (SAN)	5° 50' 24.72" N; 118° 07' 4.44" E	SS	15
2.	Lahad Datu, Sabah (LD)	5° 01' 36.48" N; 118° 19' 37.20" E	CS	15
3.	Tawau, Sabah (TA)	4° 14' 41.35" N; 117° 53' 28.14" E	CS	15
4.	Kudat, Sabah (KU)	6° 53' 14.35" N; 116° 49' 25.10" E	SS	15
5.	Kota Kinabalu, Sabah (KK)	5° 58' 29.64" N; 116° 04' 20.64" E	SCS	15
6.	Bintawa, Sarawak (BIN)	1°33'50.96"N; 110°23'15.6"E	SCS	15
7.	Pulau Bruit, Sarawak (PB)	2° 30' 59.99" N; 111° 25' 59.99" E	SCS	15
8.	Mukah, Sarawak (MUK)	2° 53' 45.5784" N; 112° 6' 13.4820" E	SCS	15
Total				120

Note: South China Sea (SCS), Sulu Sea (SS), and Celebes Sea (CS).

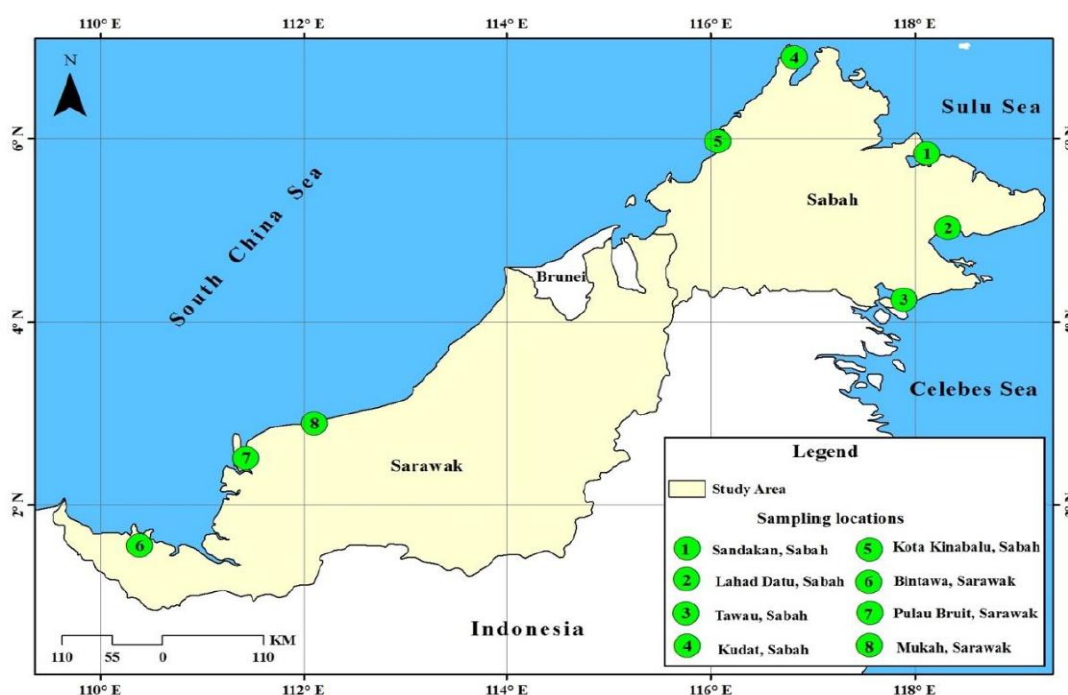


Fig1: Collecting sites of 120 *E. affinis* specimens from the South China Sea, Sulu Sea, and Celebes Sea regions.

2.2. DNA Extraction and PCR Amplification

Total genomic DNA was extracted from fin-clip tissues using the cetyltrimethylammonium bromide (CTAB) protocol described by [5], with minor modifications to the concentration of proteinase K to enhance DNA yield and quality. The resulting DNA pellets were washed, resuspended in deionized water, and visualized by electrophoresis on a 1.7% agarose gel stained with ethidium bromide. DNA quantity and purity were assessed using a Q3000 spectrophotometer (Quawell, Korea), and all samples were stored at -20 °C until further analysis. Polymerase chain reaction (PCR) was performed to amplify a 522-bp fragment of the mitochondrial cytochrome *b* (Cyt *b*) gene. Amplification employed the primer pair F (5'-GCTCACTACTTGGCCTTTGC-3') and R (5'-TGGAGGCTAGGAGGGCTAGT-3') [32]. PCR

reactions were carried out in a final volume of 25 µL containing 16.25 µL molecular-grade water, 2.5 µL 10× PCR buffer, 2.0 µL MgCl₂, 1.0 µL dNTPs, 0.5 µL of each primer (10 mM), 0.25 µL i-Taq DNA polymerase (Intron, South Korea), and 2.0 µL genomic DNA template. Gradient PCR was initially conducted using a T100™ thermal cycler (Bio-Rad, USA) to determine the optimal annealing temperature. Subsequent PCR amplifications were performed using a Major Cycler CyCLER-25 (Major Science, USA) under the following thermal profile: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 47.9 - 48.5 °C for 50 s, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 7 min. PCR products were visualized on a 1.7% agarose gel (Vivantis Sdn. Bhd.) stained with ethidium bromide to confirm successful amplification. Amplified products were purified using the Intron PCR Purification Kit (Intron,

South Korea) to remove residual primers and contaminants. Purified PCR products were subsequently sent to NHK Bioscience (Korea) for bidirectional DNA sequencing.

2.3. Sequence Processing and Data Analysis

Raw DNA sequences were edited, trimmed, and aligned using MEGA version 11 [33], employing the ClustalW algorithm [34]. Nucleotide composition was calculated in MEGA v11. Species identification was confirmed using the Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) database, with a similarity threshold exceeding 99% applied for assignment to *E. affinis*. Intra- and interpopulation genetic distances were estimated using the Kimura two-parameter (K2P) model [35] implemented in MEGA v11. Genetic diversity indices, including haplotype diversity (Hd) and nucleotide diversity (π) [36], were calculated using DnaSP ver. 6.12 [37]. Phylogenetic relationships among haplotypes were reconstructed using the Neighbor-Joining (NJ) method [38] based on K2P distances in MEGA v11. Node support was assessed through 1,000 bootstrap replicates, and only bootstrap values greater than 50% were reported. The sequence of *Lutjanus erythropterus* (GenBank accession no. AY294204.1) was included as an outgroup to root the phylogenetic tree. A minimum-spanning haplotype network was generated using the median-joining algorithm in PopART version 1.7 [39] to visualize genealogical relationships among haplotypes. Population genetic structure across the eight *E. affinis* populations was evaluated using analysis of molecular variance (AMOVA) [40] implemented in Arlequin version 3.5 [41]. Neutrality tests, including Tajima's D [42] and Fu's Fs [43], were performed in DnaSP v6.12 to assess deviations from neutral evolution and infer historical demographic events. All newly generated sequences were deposited in the GenBank database under accession numbers OP595397–OP595523.

3. Results

3.1. Sampling Data

A total of 120 *E. affinis* specimens were successfully sequenced for the mitochondrial Cyt *b* gene. These samples were collected from eight sampling sites spanning three major marine regions: the South China Sea, the Sulu Sea, and the Celebes Sea (Table 1; Figure 1).

3.2. Genetic Distance and Genetic Diversity

Amplification of mitochondrial DNA successfully generated fragments of 522 base pairs (bp) corresponding to the Cyt *b* gene for all sampled individuals, yielding a 100% amplification success rate. Estimates of genetic distance within and among the eight populations of *E. affinis* are presented in Table 2. Intrapopulation genetic distances ranged from 0.001 to 0.024, with the KK population exhibiting the highest intrapopulation divergence (0.024) and the SAN population showing the lowest (0.001). In contrast, interpopulation genetic distances varied widely, from 0.020 to 0.147. The greatest interpopulation divergence was observed between the BIN and SAN populations (0.147), whereas the lowest value was detected between the TA and LD populations (0.020). Overall, genetic divergence among the eight *E. affinis* populations from Malaysian Borneo waters spanned from low to high levels.

Analysis of nucleotide composition revealed proportions of 26.1% adenine (A), 24.9% thymine (T), 32.8% cytosine (C), and 16.1% guanine (G). The Cyt *b* sequences exhibited a relatively high A+T content (51%), exceeding the G+C content, a pattern consistent with that commonly reported for vertebrate mitochondrial genomes [44]. Haplotype diversity ranged from 0.1330 to 0.9050, with an overall value of 0.9260, while nucleotide diversity varied between 0.0003 and 0.0183, yielding an overall value of 0.0325 (Table 3). These results indicate that all eight populations of *E. affinis* possess appreciable levels of both haplotype and nucleotide diversity.

Table 2: Intra and interpopulation pairwise genetic distance for Cyt *b* gene of eight populations of *E. affinis* from Malaysian Borneo waters based on Kimura 2 parameter model.

	SAN	LD	TA	KU	KK	BIN	PB	MUK
SAN	0.001							
LD	0.028	0.015						
TA	0.034	0.020	0.017					
KU	0.040	0.029	0.032	0.018				
KK	0.051	0.042	0.047	0.035	0.024			
BIN	0.147	0.124	0.105	0.101	0.094	0.011		
PB	0.122	0.103	0.083	0.077	0.067	0.019	0.016	
MUK	0.129	0.108	0.089	0.083	0.073	0.031	0.021	0.002

Note: Bold indicate intrapopulation values.

Table 3: Haplotype diversity, nucleotide diversity, Tajima's D, and Fu's Fs statistics for eight *E. affinis* populations from Malaysian Borneo waters based on Cyt b gene.

No	Population	N	h	Hd	π	Tajima's D	Fu's Fs
1.	Sandakan, Sabah (SAN)	15	2	0.1330	0.0003	-1.1595	-0.6490
2.	Lahad Datu, Sabah (LD)	15	6	0.5710	0.0085	-2.0191*	1.2780
3.	Tawau, Sabah (TA)	15	9	0.9050	0.0115	-1.5455	-0.6440
4.	Kudat, Sabah (KU)	15	5	0.7900	0.0148	-0.5664*	4.6500*
5.	Kota Kinabalu, Sabah (KK)	15	7	0.8760	0.0183	-0.3325	2.6880*
6.	Bintawa, Sarawak (BIN)	15	7	0.7240	0.0081	-1.8426*	0.1790
7.	Pulau Bruit, Sarawak (PB)	15	7	0.8190	0.0139	-0.3212	1.8240
8.	Mukah, Sarawak (MUK)	15	2	0.5140	0.0010	1.3759	1.2530
Total		120	32	0.9260	0.0325	-0.8458*	-1.2550

Notes: N: Number of samples, h: number of haplotypes, Hd: Haplotype diversity, π : Nucleotide diversity.
 *Significant at P < 0.05.

3.3. Phylogenetic Analysis and Minimum Spanning Network (MSN)

Phylogenetic relationships inferred from the neighbor-joining (NJ) tree, together with the minimum spanning network (MSN) analysis based on Cyt b sequences, revealed the presence of two well-defined clades among *E. affinis* specimens from Malaysian Borneo waters (Figures 2 and 3). The first clade comprised individuals from BIN, PULAU, KU, KK, and MUK, encompassing haplotypes Hap 1 to Hap 23. The second clade included samples from KU, TA, LD, and SAN, corresponding to haplotypes Hap 24 to Hap 32. The MSN further demonstrated that Hap 1, Hap 24, and Hap 32 were the most frequent haplotypes across the dataset, whereas the remaining haplotypes showed restricted distributions, occurring either in a limited number of populations or being unique to a single population. The separation of the two major clades (Clades 1 and 2) was strongly supported by the MSN, which indicated nucleotide divergences exceeding five mutational steps between them (Figure 3).

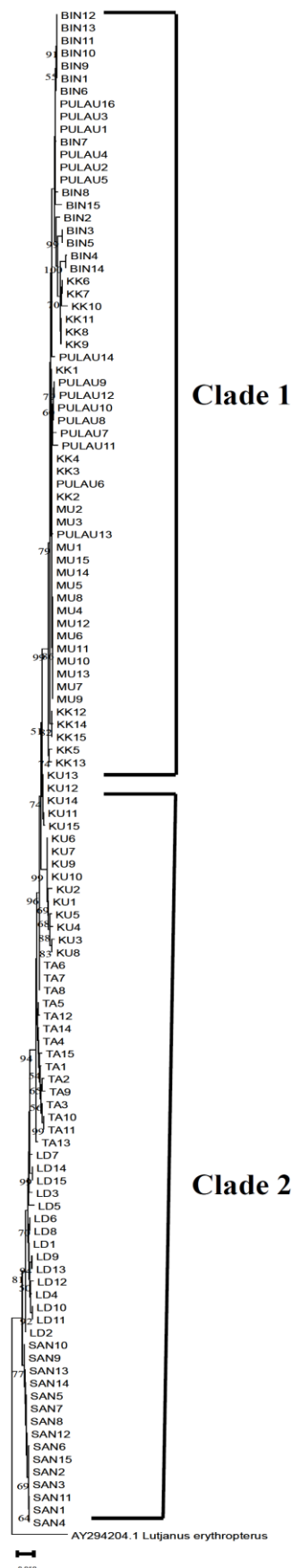


Fig 2: Neighbor-Joining (NJ) tree depicting the relationship between Cyt b sequences of *E. affinis* specimens from Malaysian Borneo. Only bootstrap values exceeding 50% are shown.

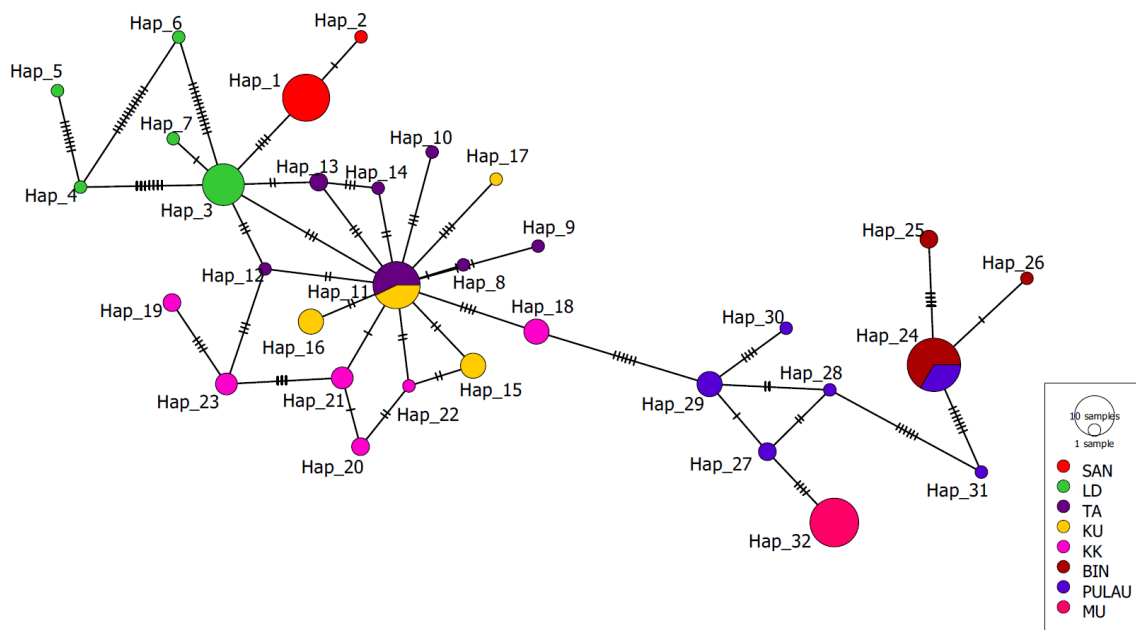


Fig 3: Minimum spanning network among 32 *Cyt b* haplotypes of *E. affinis* collected from eight populations in Malaysian Borneo waters.

3.4. Population Genetic Structure and Neutrality Test

Population genetic structure among the eight *E. affinis* populations was assessed using mitochondrial *Cyt b* data. Analysis of molecular variance (AMOVA) revealed that the majority of genetic variation was attributable to differences among populations (90.23%), while a smaller proportion of variation occurred within populations (9.77%). These results indicate a pronounced and significant genetic structuring among the sampled populations. Consistently, fixation index analysis showed a high and statistically significant level of population differentiation, with an overall F_{ST} value of 0.902 ($P < 0.05$) (Table 4). Neutrality tests based on *Cyt b* sequences produced a significantly negative Tajima’s D value across all populations ($D = -0.8458, P < 0.05$), suggesting deviations from neutrality. Fu’s F_s statistic was also negative (-1.2550), although this result was not statistically significant ($P > 0.05$) (Table 3).

Table 4: Analysis of molecular variance (AMOVA) of eight populations of *E. affinis* collected from Malaysian Borneo waters based on *Cyt b* gene.

Source of variation	Degrees of Freedom	Sum of squares	Variance components	Percentage of variation	F_{ST}	P
Among populations	7	9929.692	82.228 Va	90.23	0.9023	0.0000
Within populations	112	9970.467	8.906 Vb	9.77		
Total	119	10931.58	91.134	100		

4. Discussion

Accurate species identification using traditional morphological approaches relies heavily on the expertise of skilled taxonomists; however, such methods are often constrained by phenotypic plasticity, which can lead to misidentification. In this context, DNA barcoding has emerged as a trustworthy and effective method for species identification, especially when specimens are damaged, incomplete, or represent several life-history phases [2]. Among mitochondrial markers, the cytochrome *b* (*Cyt b*) gene has proven especially effective, enabling accurate identification of approximately 98% of documented marine fish species and being widely applied across diverse geographic regions [7]. In the present study, more than 99% of *E. affinis* specimens were successfully identified using BLAST searches against the NCBI database based on *Cyt b* sequences. These results underscore the reliability of the *Cyt b* gene for species discrimination and for evaluating genetic diversity and population structure of *E. affinis* across three major marine regions in Malaysia, encompassing eight landing sites.

Genetic distance analyses revealed values ranging from low to high (0.001–0.147), indicating measurable genetic differentiation among the eight populations examined. Genetic distance is commonly used in taxonomic and evolutionary studies to assess relationships among closely related taxa. As noted by [45], greater genetic distances reflect reduced relatedness and increased nucleotide divergence. Marine species are often characterized by large effective population sizes,

extensive larval dispersal, and broad geographic distributions [46]. For instance, the relatively low genetic differentiation reported in tuna populations across ocean basins has been attributed to continuous pelagic habitats and widespread spawning grounds [47]. Conversely, genetic structuring in marine fishes may arise from physical and oceanographic barriers, including currents, reefs, and landmasses, which can restrict gene flow and promote population differentiation [5]. While genetic differentiation is a natural evolutionary process, anthropogenic pressures such as overfishing, pollution, and habitat degradation may further intensify population fragmentation and genetic divergence in marine species [48].

The genetic patterns observed in this study are consistent with previous investigations of *E. affinis* conducted in the North Indian Ocean [28], the Straits of Malacca [49], and the coastal waters of Tanzania [50], all of which reported the presence of distinct genetic clades. Similarly [51], documented minimal levels of genetic differentiation among 11 populations of *Thunnus tonggol* in Malaysian waters. Comparable findings were reported by [52] for *Thunnus obesus* populations from the North and South Moluccas Seas, with genetic distances ranging from 0.023 to 0.027. In addition, analysis of GC content provides insights into nucleotide composition, mutation pressures, and evolutionary lineages, facilitating the selection of appropriate substitution models [53]. Higher GC content has been associated with increased nucleotide diversity and elevated mutation potential within populations [6].

Haplotype diversity (H_d) and nucleotide diversity (π) are informative indicators of demographic history [54]. The combination of high haplotype diversity and low nucleotide diversity observed in this study suggests large population sizes and recent population expansion, with insufficient time for extensive nucleotide divergence among haplotypes [54]. High genetic diversity in marine fishes is often attributed to their large effective population sizes [28]. The substantial haplotype diversity detected in *E. affinis* likely reflects its wide geographic distribution and large population size. These findings are in agreement with earlier studies conducted on various species of tuna, including Kawakawa (*E. affinis*) ($H_d = 0.69$, $\pi = 0.0011$) [55], longtail tuna (*Thunnus tonggol*) ($H_d = 0.990$, $\pi = 0.0195$) [54]. The high number of haplotypes may also be partially explained by the elevated mutation rate characteristic of mitochondrial DNA [56], a conclusion supported by the minimum spanning network (MSN) analysis, which indicated that at least four mutational steps preceded the emergence of novel haplotypes.

Phylogenetic reconstruction and MSN analysis grouped the eight *E. affinis* populations from Malaysian Borneo into two distinct clades. Such intraspecific genetic

structuring may result from secondary contact and ongoing interbreeding among populations that were historically geographically isolated [49]. The MSN revealed that several populations shared common haplotypes (Hap 1, Hap 24, and Hap 32), suggesting recent gene flow among these locations [57]. Long-term genetic exchange and individual movement between distant populations have similarly been shown to promote haplotype sharing in marine species [58]. Nevertheless, the present findings also suggest a potential reduction in gene flow among certain locations, likely influenced by oceanic current systems [59].

Results from the AMOVA further supported the phylogenetic and MSN analyses, confirming significant genetic structuring among *E. affinis* populations. Fixation index (F_{ST}) values are widely used to infer gene flow, with higher values indicating greater genetic differentiation [60]. In this study, AMOVA revealed a high and statistically significant F_{ST} value (0.902; $P < 0.05$), indicating strong genetic differentiation among populations.

Demographic history analyses provide insights into historical population size fluctuations by examining deviations from mutation–drift equilibrium [42]. Neutrality tests conducted in this study indicated a significant negative Tajima's D value across populations, suggesting historical population expansion. Although Fu's F_s was also negative, it was not statistically significant. Fu's F_s is considered more sensitive than Tajima's D in detecting population growth driven by an excess of recent mutations [43], which may explain the observed discrepancy between the two statistics. Negative Tajima's D values are generally associated with population expansion, purifying selection, or recent growth, whereas positive values indicate balancing selection, population subdivision, or recent bottlenecks [61]. Overall, the neutrality test results collectively suggest that *E. affinis* populations from Malaysian Borneo have undergone historical population expansion.

5. Conclusion

This study delivers the initial comprehensive, multi-location assessment of the genetic diversity and population structure of *E. affinis* in Malaysian Borneo waters using the mitochondrial Cyt *b* gene. The Cyt *b* marker proved effective in resolving population structure, identifying eight populations of *E. affinis* across the region. The analyses further revealed the presence of two distinct genetic lineages distributed among three major marine regions: the South China Sea, Sulu Sea, and Celebes Sea. These findings establish a robust scientific foundation for the effective management and conservation of neritic tuna resources in Malaysian Borneo. Future research on *E. affinis* would benefit from the incorporation of additional genetic

markers, along with expanded geographic coverage and larger sample sizes, to further refine understanding of population connectivity and evolutionary dynamics.

Acknowledgement

The authors gratefully acknowledge Hadhramout University for providing financial support for the authors' studies. The authors also extend sincere appreciation to Universiti Sains Malaysia (USM) and the School of Biological Sciences (SBS) for granting access to research facilities and for the academic support that enabled the successful completion of this study.

References

- [1] R. Fricke, W. N. Eschmeyer, and R. der Laan, "Eschmeyer's catalog of fishes: genera, species, references." [Online]. Available: <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp,2025>.
- [2] L. Wang *et al.*, "DNA barcoding of marine fish species from Rongcheng Bay, China," *PeerJ*, vol. 2, no. 6, pp. 1–19, 2018, doi: 10.7717/peerj.5013.
- [3] P. Marchetti, A. Mottola, R. Piredda, G. Ciccarese, and A. Di Pinto, "Determining the Authenticity of Shark Meat Products by DNA Sequencing," *Foods*, vol. 9, no. 9, pp. 1–16, 2020.
- [4] K. Binashikhubkr, A. A. Malik, S. Mahboob, and D. Naim, "Geometric morphometric discrimination between seven populations of Kawakawa *Euthynnus affinis* (Cantor, 1849) from Peninsular Malaysia," *J. King Saud Univ. - Sci.*, p. 101863, 2022, doi: 10.1016/j.jksus.2022.101863.
- [5] A. A. Bakar *et al.*, "DNA barcoding of Malaysian commercial snapper reveals an unrecognized species of the yellow-lined Lutjanus (Pisces:Lutjanidae)," *PLoS One*, vol. 13, no. 9, 2018, doi: 10.1371/journal.pone.0202945.
- [6] A. Imtiaz, D. T. Yen, S. Azizah, M. Nor, and D. Naim, "Molecular identification of commercially important species of Nemipterus (Perciformes : Nemipteridae) in surrounding seas of Malaysia," *Biodiversitas*, vol. 17, no. 2, pp. 571–577, 2016, doi: 10.13057/biodiv/d170226.
- [7] K. Binashikhubkr, F. Al-Misned, and D. Md. Naim, "Genetic diversity and population structure of Kawakawa *Euthynnus affinis* (Cantor, 1849) in Malaysia and its surrounding waters inferred by mitochondrial DNA cytochrome oxidase I and cytochrome b genes," *J. King Saud Univ. - Sci.*, vol. 36, no. 6, p. 103193, 2024, doi: 10.1016/j.jksus.2024.103193.
- [8] E. M. Faizal, S. Jamon, N. A. Jamaludin, and N. H. A. Halim, "Status of neritic tuna fishery and some biological aspects of Kawakawa (*Euthynnus affinis*) in the northern part of Peninsular Malaysia," *IOTC–2019–WPNT09–13*, no. July, pp. 1–5, 2019.
- [9] S. P. Griffiths, G. C. Fry, F. J. Manson, and R. D. Pillans, "Morphometric relationships for four Scombridae fish species in Australian waters," *J. Appl. Ichthyol.*, vol. 33, no. 3, pp. 583–585, 2017, doi: 10.1111/jai.13136.
- [10] H. El Mghazli, M. Znari, A. Mounir, H. Benaissa, and H. El Ouizgani, "Does the Atlantic horse mackerel *Trachurus trachurus* (Teleostei: Carangidae) differentiate morphologically within the putative Moroccan-Saharan stock?," *Mar. Biol. Res.*, vol. 17, no. 4, pp. 341–349, 2021, doi: 10.1080/17451000.2021.1957935.
- [11] K. Binashikhubkr, J. Babangida Kachi, F. Al-Misned, and D. M. Naim, "Stock structure delineation of Kawakawa *Euthynnus affinis* (Cantor, 1849) from Malaysian Borneo using multivariate morphometric analysis," *J. King Saud Univ. - Sci.*, vol. 36, no. 8, p. 103278, 2024, doi: 10.1016/j.jksus.2024.103278.
- [12] D. B. Čihoric A. Maric, D. Mrdak, and P. Simonovic, "Morphometric and meristic differentiation among five populations of *Delminichthys ghetaldii* (Actinopterygii , Cyprinidae) from five karst fields in Eastern Herzegovina," *Turkish J. Zool.*, vol. 48, no. 5, 2024, doi: 10.55730/1300-0179.3184.
- [13] A. Kasinath *et al.*, "Are Indian mackerel (*Rastrelliger kanagurta*) populations in the eastern Indian Ocean truly homogeneous? Insights from geometric morphometric analysis," *Reg. Stud. Mar. Sci.*, vol. 75, no. April, p. 103555, 2024, doi: 10.1016/j.rsma.2024.103555.
- [14] S. M. Shukri, K. Binashikhubkr, A. D. W. I. Setyawan, and D. Naim, "Geometric morphometric divergence of five populations of *Pampus argenteus* (Euphrasen , 1788) from Malaysian waters," *Nusant. Biosci.*, vol. 16, no. 1, pp. 1–12, 2024, doi: 10.13057/nusbiosci/n160101.
- [15] K. De Arvind and K. Dwivedi, " Geometric Morphometric ' Approach To Detect Body Shape Variations among Three Indian Shads," pp. 41–185, 2025.
- [16] J. B. Kachi, K. Binashikhubkr, and D. M. Naim, "Morphological differentiation of *Pennahia aneus* (Bloch , 1793) populations from Northern Peninsular Malaysia using geometric morphometrics," *Nusant. Biosci.*, vol. 17, no. 1, pp. 30–38, 2025, doi: 10.13057/nusbiosci/n170104.

- [17] D. Gain *et al.*, “Landmark-based morphometric and meristic variations of endangered mrigal carp, *Cirrhinus cirrhosus* (Bloch 1795), from wild and hatchery stocks,” *Sains Malaysiana*, vol. 46, no. 5, pp. 695–702, 2017, doi: 10.17576/jsm-2017-4605-03.
- [18] N. Syahida Kasim *et al.*, “Recent population expansion of longtail tuna *Thunnus tonggol* (Bleeker, 1851) inferred from the mitochondrial DNA markers,” *PeerJ*, vol. 8, p. e9679, 2020, doi: 10.7717/peerj.9679.
- [19] N. A. Jamaludin, J. Amirul, F. Jamaluddin, W. M. Arshaad, S. Azizah, and M. Nor, “Mitochondrial marker implies fishery separate management units for spotted sardinella, *Amblygaster sirm* (Walbaum, 1792) populations in the South China Sea and the Andaman Sea,” *PeerJ*, 2022, doi: 10.7717/peerj.13706.
- [20] A. Y. Selcuk, A. Kaya, and H. Kefelioglu, “Morphological, linear, and geometric morphometric differences (skull, mandible, and pelvis) among subspecies of *Talpa levantis* (Eulipotyphla: Talpidae) with molecular divergence (mtDNA and nuclear DNA),” *Turkish J. Zool.*, vol. 49, no. 2, 2025, doi: 10.55730/1300-0179.3216.
- [21] R. Lalitha and V. R. Chandavar, “Analysis of genetic diversity in CYTB and control region sequences of *Melanochelys trijuga* (Schweigger, 1812) from Karnataka,” *J. Asia-Pacific Biodivers.*, vol. 11, no. 3, pp. 346–352, 2018, doi: 10.1016/j.japb.2018.05.001.
- [22] M. N. Findra, G. M. Samadan, A. Syazili, and M. Irfan, “Species confirmation of freshwater prawns in Ternate Island, Indonesia, through DNA barcoding: *Not Macrobrachium rosenbergii*,” *Int. J. Aquat. Biol.*, vol. 13, no. 6, pp. 71–79, 2025.
- [23] N. A. Jamaludin, W. Mohd-Arshaad, N. A. Mohd Akib, D. H. Zainal Abidin, N. V. Nghia, and S. A. M. Nor, “Phylogeography of the Japanese scad, *Decapterus maruadsi* (Teleostei: Carangidae) across the Central Indo-West Pacific: evidence of strong regional structure and cryptic diversity,” *Mitochondrial DNA Part A DNA Mapping, Seq. Anal.*, vol. 0, no. 0, pp. 1–13, 2020, doi: 10.1080/24701394.2020.1799996.
- [24] A. Kurniawan, A. M. Hariati, A. Kurniawan, and D. G. R. Wiadnya, “First genetic record and the phylogenetic relationship of *osteochilus spilurus* (Cyprinidae: Labeoninae) originating from Bangka and Belitung islands, Indonesia,” *Biodiversitas*, vol. 22, no. 2, pp. 794–802, 2021, doi: 10.13057/biodiv/d220233.
- [25] J. Wei *et al.*, “Validity of pampus liuorum liu & li, 2013, revealed by the dna barcoding of pampus fishes (Perciformes, stromateidae),” *Diversity*, vol. 13, no. 12, pp. 1–15, 2021, doi: 10.3390/d13120618.
- [26] K. Abbas, X. Zhou, and W. Wang, “Mitochondrial diversity and phylogenetic structure of Yellowcheek (*Elopichthys bambusa*) in the Yangtze River,” *J. Appl. Ichthyol.*, vol. 38, no. 6, pp. 596–603, 2022, doi: 10.1111/jai.14362.
- [27] M. Syamsuddin, Sunarto, and L. Yuliadi, “Seasonal Variations of Oceanographic Variables and Eastern Little Tuna (*Euthynnus affinis*) Catches in the North Indramayu Waters Java Sea,” in *IOP Conference Series: Earth and Environmental Science*, 2018, doi: 10.1088/1755-1315/116/1/012073.
- [28] G. Kumar, S. P. Kunal, M. R. Menezes, and R. M. Meena, “Single genetic stock of kawakawa *Euthynnus affinis* (Cantor, 1849) along the Indian coast inferred from sequence analyses of mitochondrial DNA D-loop region,” *Conserv. Genet.*, vol. 13, no. 4, pp. 1119–1131, 2012, doi: 10.1007/s10592-012-0359-5.
- [29] S. Mardijah *et al.*, “The Fishing Grounds and the Exploitation Status of Kawakawa (*Euthynnus affinis*) in Java Sea, Indonesia,” *HAYATI J. Biosci.*, vol. 29, no. 2, pp. 255–265, 2022, doi: 10.4308/hjb.29.2.255-265.
- [30] DoFM—Department of Fisheries Malaysia, 2021. Annual Fisheries Statistics 2021, vol. 1. Kuala Lumpur: Ministry of Agriculture and Agro-industry.
- [31] B. B. Collette and C. E. Nauen, FAO Species Catalogue Vol. 2 Scombrids of the world an annotated and illustrated catalogue of Tunas, Mackerels, Bonitos and related species know to date, vol. 2, no. 125, 1983, doi: FAO Fish. Synop. 125(2).
- [32] V. Terio *et al.*, “Identification of tuna species in commercial cans by minor groove binder probe real-time polymerase chain reaction analysis of mitochondrial DNA sequences,” *Mol. Cell. Probes*, vol. 24, no. 6, pp. 352–356, 2010, doi: 10.1016/j.mcp.2010.07.006.
- [33] K. Tamura, G. Stecher, and S. Kumar, “MEGA11: Molecular Evolutionary Genetics Analysis Version 11,” *Mol. Biol. Evol.*, pp. 1–12, 2021, doi: 10.1093/molbev/msab120.

- [34] J. . Thompson, D. G.Higgins, and T. J.Gibson, "CLUSTAL W (improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice)," *Nucleic Acids Res.*, vol. 22, no. 22, pp. 4673–4680, 1994, doi: 10.1007/978-1-4020-6754-9_3188.
- [35] M. Kimura, "A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences," *J. Mol. Evol.*, vol. 16, no. 2, pp. 111–120, 1980, doi: 10.1007/BF01731581.
- [36] M. Nei, *Molecular Evolutionary Genetics*. Columbia University Press, New York., 1987.
- [37] J. Rozas *et al.*, "DnaSP 6: DNA sequence polymorphism analysis of large data sets," *Mol. Biol. Evol.*, vol. 34, no. 12, pp. 3299–3302, 2017, doi: 10.1093/molbev/msx248.
- [38] N. Saitou and M. Nei, "The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees'," *Mol. Biol. Evol.*, vol. 4, no. 4, pp. 406–425, 1987.
- [39] J. W. Leigh and D. Bryant, "POPART: Full-feature software for haplotype network construction," *Methods Ecol. Evol.*, vol. 6, no. 9, pp. 1110–1116, 2015, doi: 10.1111/2041-210X.12410.
- [40] L. Excoffier, P. E. Smouse, and J. M. Quattro, "Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data," *Genetics*, vol. 131, no. 2, pp. 479–491, 1992, doi: 10.1093/genetics/131.2.479.
- [41] L. Excoffier and H. E. L. Lischer, "Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows," *Mol. Ecol. Resour.*, vol. 10, no. 3, pp. 564–567, 2010, doi: 10.1111/j.1755-0998.2010.02847.x.
- [42] F. Tajima, "Statistical methods for testing the neutral mutation hypothesis by DNA polymorphism. Genetics," *Genet. Soc. Am.*, vol. 123, pp. 585–595, 1989.
- [43] Y. Fu, "Statistical Testsof Neutrality of Mutations Against Population Growth, Hitchhiking and Background Selection," *Genet. Soc. Am.*, vol. 14, pp. 915–925, 1997.
- [44] S. Nei and M. Kumar, *Molecular phylogenetics*. Oxford University Press, New York, 2000.
- [45] P. D. N. Hebert, S. Ratnasingham, and J. R. DeWaard, "Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species," *Proc. R. Soc. B Biol. Sci.*, vol. 270, no. SUPPL. 1, pp. 96–99, 2003, doi: 10.1098/rsbl.2003.0025.
- [46] Palumbi S.R., "Marine speciation on a small planet," *Trends Ecol. Evol.*, vol. 7, no. 4, pp. 114–118, 1992.
- [47] J. D. Durand, A. Collet, S. Chow, B. Guinand, and P. Borsa, "Nuclear and mitochondrial DNA markers indicate unidirectional gene flow of Indo-Pacific to Atlantic bigeye tuna (*Thunnus obesus*) populations, and their admixture off southern Africa," *Mar. Biol.*, vol. 147, no. 2, pp. 313–322, 2005, doi: 10.1007/s00227-005-1564-2.
- [48] D. O. Conover, L. M. Clarke, S. B. Munch, and G. N. Wagner, "Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation," *J. Fish Biol.*, vol. 69, no. SUPPL. C, pp. 21–47, 2006, doi: 10.1111/j.1095-8649.2006.01274.x.
- [49] A. R. Masazurah, S. A. M. N, and B. Samsudin, "A preliminary study of population structure of kawakawa, *Euthynnus affinis* (Cantor 1849) in the straits of Malacca," *IOTC-2012-WPNT02-23*, pp. 1–10, 2012.
- [50] M. Johnson, Y. Mgaya, and Y. Shaghude, "Genetic Stock Structure and Phylogenetic Relationship of Kawakawa *Euthynnus affinis* – Cantor (1849) in the Northern Coastal Waters of Tanzania Using Mitochondrial DNA Control Region," *Indian Ocean Tuna Comm.*, no. 1849, pp. 1–17, 2016.
- [51] N. S. Kasim *et al.*, "Recent population expansion of longtail tuna *Thunnus tonggol* (Bleeker, 1851) inferred from the mitochondrial DNA markers," *PeerJ*, vol. 8, pp. 1–23, 2020, doi: 10.7717/peerj.9679.
- [52] N. Akbar, M. Irfan, and M. Aris, "Population Genetics and Phylogeography of Bigeye Tuna in Moluccas Seas, Indonesia," *ILMU Kelaut. Indones. J. Mar. Sci.*, vol. 23, no. 4, pp. 145–155, 2018, doi: 10.14710/ik.ijms.23.4.145-155.
- [53] E. Figuet, M. Ballenghien, J. Romiguier, and N. Galtier, "Biased gene conversion and GC-content evolution in the coding sequences of reptiles and vertebrates," *Genome Biol. Evol.*, vol. 7, no. 1, pp. 240–250, 2014, doi: 10.1093/gbe/evu277.
- [54] W. S. Grant and B. W. Bowen, "Shallow Population Histories in Deep Evolutionary Lineages of Marine Fishes: Insights From Sardines and Anchovies and Lessons for Conservation," *J. Hered.*, vol. 89, no. 5, pp. 415–426, 1998.

- [55] K. Binashikhubkr and D. M. Naim, "Lack of genetic differentiation of Kawakawa Euthynnus affinis (Cantor, 1849) in Peninsular Malaysia based on mitochondrial DNA cytochrome oxidase I Lack of genetic differentiation of Kawakawa Euthynnus affinis (Cantor, 1849) in Peninsular Malaysia b," in *IOP Conf. Series: Earth and Environmental Science*, 2022. doi: 10.1088/1755-1315/1139/1/012004.
- [56] S. Vandewoestijne, M. Baguette, P. M. Brakefield, and I. J. Saccheri, "Phylogeography of *Aglais urticae* (Lepidoptera) based on DNA sequences of the mitochondrial COI gene and control region," *Mol. Phylogenet. Evol.*, vol. 31, no. 2, pp. 630–646, 2004, doi: 10.1016/j.ympev.2003.09.007.
- [57] W. J. M. Koopman *et al.*, "Linked vs. unlinked markers: Multilocus microsatellite haplotype-sharing as a tool to estimate gene flow and introgression," *Mol. Ecol.*, vol. 16, no. 2, pp. 243–256, 2007, doi: 10.1111/j.1365-294X.2006.03137.x.
- [58] J. B. Horne, L. van Herwerden, J. H. Choat, and D. R. Robertson, "High population connectivity across the Indo-Pacific: Congruent lack of phylogeographic structure in three reef fish congeners," *Mol. Phylogenet. Evol.*, vol. 49, no. 2, pp. 629–638, 2008, doi: 10.1016/j.ympev.2008.08.023.
- [59] L. J. Halim, I. Rahim, S. Mahboob, K. A. Al-Ghanim, A. AMAT, and D. Md. Naim, "Phylogenetic relationships of the commercial red snapper (*Lutjanidae* sp.) from three marine regions," *J. King Saud Univ. - Sci.*, vol. 34, no. 2, p. 101756, 2022, doi: 10.1016/j.jksus.2021.101756.
- [60] D. M. Jose, P. R. Divya, and K. K. Lal, "Panmictic stock structure of milkfish (*Chanos chanos*, Forsskål 1775) from Indian waters determined using mtDNA marker," *J. Genet.*, vol. 102, no. 1, 2023, doi: 10.1007/s12041-022-01395-6.
- [61] T. S. Korneliussen, I. Moltke, A. Albrechtsen, and R. Nielsen, "Calculation of Tajima's D and other neutrality test statistics from low depth next-generation sequencing data," *BMC Bioinformatics*, vol. 14, no. 1, 2013, doi: 10.1186/1471-2105-14-289.

التنوع الوراثي والرؤى المتعلقة بالبنية السكانية لسمكة الكاواكاوا *EUTHYNNUS AFFINIS* (كانتور، 1849) في بورنيو الماليزية كما تُكشف عنها جين السيتوكروم ب في الحمض النووي للميتوكوندريا

خالد بن الشيخ أبوبكر^{1*}، و كمال أحمد باعوم¹

¹ قسم علوم الحياة، كلية العلوم، جامعة حضرموت، المكلا، اليمن

* الباحث الممثل: خالد بن الشيخ أبوبكر؛ البريد الإلكتروني: khaledsaleh@hu.edu.ye

استلم في: 13 فبراير 2026 / قبل في: 06 مارس 2026 / نشر في 31 مارس 2026

المُلخَص

تُعد سمكة الكاواكاوا من الأنواع السطحية ذات القيمة التجارية العالية، وتتميز بانتشار واسع في المياه الاستوائية وشبه الاستوائية لمنطقة المحيطين الهندي والهادئ. وعلى الرغم من أهميتها الاقتصادية، فإن محدودية المعرفة ببنية المخزون وإدارته وحفظه في المياه الماليزية والمجاورة أثارت مخاوف بشأن الاستغلال المفرط وتناقص الأعداد. وعليه، تُعد التقييمات الجزيئية الشاملة للبنية السكانية ضرورية لاستراتيجيات فعّالة لإدارة المصائد. هدفت هذه الدراسة إلى استقصاء التنوع الوراثي والبنية السكانية لسمكة الكاواكاوا في المياه الماليزية باستخدام جين الميتوكوندريا سيتوكروم ب. تم تسلسل جزء بطول 522 زوجاً قاعدياً من منطقة السيتوكروم ب من 120 عينة جُمعت من ثماني تجمعات سكانية متميزة جغرافياً في بورنيو الماليزية. تراوح التباين الجيني بين المجموعات السكانية من منخفض إلى مرتفع، مصحوباً بتنوع عالٍ في الأنماط الفردية ($Hd = 0.9260$). وتنوع متوسط في النيوكليوتيدات ($\pi = 0.0325$)، تم تحديد 32 نمطاً فردياً في جميع المجموعات السكانية التي شملتها الدراسة. أظهر تحليل إعادة بناء العلاقات التطورية بالاعتماد على تحليل الأنماط الفردية المستخلص من شبكة الامتداد الأذني، وجود سلاتين وراثيتين. وأظهر تحليل التباين ($AMOVA$) إلى وجود بنية وراثية قوية ذات دلالة إحصائية بين التجمعات ($FST = 0.902, P < 0.05$). وأظهرت اختبارات الحيادية إلى وجود إشارة تاريخية لتوسع سكاني. تمثل هذه الدراسة أول تقييم جزيئي شامل يعتمد على جين السيتوكروم ب للبنية السكانية لسمكة الكاواكاوا في بورنيو الماليزية، وتوفر هذه الدراسة معلومات مرجعية أساسية لدعم الإدارة المستدامة لمصايد الأسماك وخطط الحفظ.

الكلمات المفتاحية: إدارة المصائد؛ البنية السكانية؛ جين السيتوكروم ب؛ الكاواكاوا؛ علم الوراثة الحفظي.

How to cite this article:

K. B. Abubkr, and K. A. Baaoom, "GENETIC DIVERSITY AND POPULATION INSIGHTS OF KAWAKAWA EUTHYNNUS AFFINIS (CANTOR, 1849) IN MALAYSIAN BORNEO REVEALED BY MITOCHONDRIAL DNA CYTOCHROME B GENE", *Electron. J. Univ. Aden Basic Appl. Sci.*, vol. 7, no. 1, pp. 34-45, Mar. 2026. DOI: <https://doi.org/10.47372/ejua-ba.2026.1.497>



Copyright © 2026 by the Author(s). Licensee EJUA, Aden, Yemen. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY-NC 4.0) license.