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Phylogeographic Insights of Five Co-Habiting Grouper Species in The Indo-Malaya Archipelago

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ABSTRACT

Understanding the patterns of genetic diversity of species and precise stock identification are important in fisheries conservation management. However, studies on genetic diversity, connectivity, population structure, and gene flow of groupers within the Indo-Malaya Archipelago (IMA) waters are limited. The objective of the present study was to examine the phylogeographic patterns of the family Epinephelidae in IMA waters based on a parallel study of five selected species utilizing the mitochondrial COI. The grouper species were: areolate grouper (*Epinephelus areolatus*), blacktip grouper (*Epinephelus fasciatus*), six-bar grouper (*Epinephelus sexfasciatus*), blue-lined hind (*Cephalopholis formosa*), and white-edged lyretail (*Variola albimarginata*). Specimens were obtained from fish landing sites and fish markets from 23 locations throughout the IMA waters. This study showed genetic structuring for two species (*Epinephelus areolatus* and *Variola albimarginata*) but genetic homogeneity for the other three investigated species across IMA. Various geological and demographic histories, local and regional oceanographic features, and biological characteristics are hypothesized to shape the present genetic pattern of each species across the IMA waters. The establishment of effective international cooperation is encouraged to manage grouper species stocks in this region.

1. Introduction

The Indo-Malaya Archipelago (IMA) region is recognized to have the highest diversity of grouper species in the world, (Sadovy de Mitcheson *et al.* 2013). The current phylogeographical structure and distribution of marine biodiversity throughout the region (Carpenter *et al.* 2011; Gaither *et al.* 2011), including groupers, is believed to have been shaped by the complex geological and climatic histories of the region. Ma *et al.* (2016) in their study of the historical biogeography of groupers that covered 87% of grouper species globally, revealed that the IMA (referred to as Central Indo-Pacific region-CIP in their study) had the highest diversity of extant grouper species and hypothesized that this region was central to the survival of epinephelids during the Pleistocene epoch. Expanding on this, a population study of three grouper species (*Epinephelus polyphekadion*, *Plectropomus areolatus*, and *P. leopardus*) in the Indo-Pacific revealed signatures of *Pleistocene glacial* cycles in all three species with genetic breaks in the Indo-Pacific Barrier (IPB), coupled with recent demographic expansion to explain the disparity in genetic connectivity (Ma *et al.* 2018).

However, there needs to be more phylogeographic and population structure knowledge of groupers within the IMA region which is critical for the management of this commercially important group. To date, there have been very few comprehensive phylogeographic studies of groupers in the IMA region and its adjacent waters; in *E. coioides* (Antoro *et al.* 2006; Wang *et al.* 2011), *Cephalopholis argus* (Gaither *et al.* 2011) and *E. polyphekadion*, *P. areolatus* and *P. leopardus* (Ma *et al.* 2018). Most molecular

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studies in this region have focused on the IMA groupers' phylogenetics and the taxonomy (DNA barcoding). Alcantara and Yambot (2014) barcoded 27 commercially important grouper species of the Philippines. A study by Ariyanti *et al.* (2015) on the darkfin hind grouper (*C. urodeta*) from South Sulawesi, Indonesia, found monophyly of COI sequences among all *C. urodeta* populations from several regions (the Philippines, Northern Mariana Island, Réunion and Andaman) (GenBank sequences) except the population off the coast of India in the Arabian Sea. Similarly, Jefri *et al.* (2015), based on COI gene sequences from seven grouper species (*Epinephelus areolatus, E. merra, E. fasciatus, E. longispinis, E. coioides, E. ongus,* and *E. coeruleopunctatus*) collected from the local fish markets in Indonesia found that the majority of sequences formed robust clusters within their presumed species with additional samples from various regions (GenBank sequences). For example, *E. merra* clade formed a monophyletic lineage among populations of the Coral Triangle, which is comprised of several seas: Numfor, Karimunjawa, Tanakeke, Kendari, and Lombok (Indonesian waters) with GenBank sequences from the Philippines, Australia, and French Polynesia. Aziz *et al.* (2016) DNA barcoded ten grouper species from the coastal areas of Peninsular Malaysia. Nor Rahim *et al.* (2016) carried out phylogenetic analysis for several Malaysian groupers utilizing mitochondrial and nuclear genes. Fadli *et al.* (2021) recently DNA barcoded 26 commercially important grouper species of Aceh, Indonesia.

Understanding the patterns of genetic diversity of fish species and an accurate fish stock identification are important for developing fisheries and conservation management plan (Ward 2000; Ketchum *et al.* 2016). The inability to precisely identify and manage different fish stocks can result in overfishing and lead to the resource extermination. In the last two decades, molecular approaches have also been widely used to discriminate fish population structure across the globe, mainly focusing on individual grouper species such as the orange-spotted grouper (*E. coioides*) (Antoro *et al.* 2006; Wang *et al.* 2011), the peacock hind *C. argus* (Gaither *et al.* 2011), the Nassau grouper (*E. striatus*) (Jackson *et al.* 2014), blacktip grouper (*E. fasciatus*) (Kuriiwa *et al.* 2014) and a comparative phylogeography of camouflage grouper (*E. polyphekadion*), squaretail coralgrouper (*P. areolatus*), and leopard coralgrouper (*P. leopardus*) (Ma *et al.* 2018).

Research on the genetic diversity, connectivity, population structure, and gene flow of groupers within the IMA waters are limited. Such data hinder the development of a holistic strategy for the conservation of this all-important commercial group. The complex geological and climatic histories, particularly the Pleistocene glacial cycles and several contemporary factors, have been attributed to shape the phylogeographic patterns and population structure of several groupers in the IMA region and its adjacent waters (Antoro *et al.* 2006; Gaither *et al.* 2011; Ma *et al.* 2018; Wang *et al.* 2011). But it is unknown whether similar factors could also influence the phylogeography of other grouper species in the IMA. Furthermore, the few documented studies have been focused on a single or only a few species with limited geographical coverage even within the IMA region. Hence, the objective of the present study was to provide insights into the phylogeographic patterns of five selected species of the family Epinephelidae in IMA waters based on a parallel study utilizing the mitochondrial the cytochrome c oxidase subunit I (COI) gene. This study would also furnish a baseline data to initiate further investigations.

2. Materials and Methods

2.1. Sample Collection

Five grouper species were collected in the IMA region from January to November 2016. The grouper species were areolate grouper (*Epinephelus areolatus*), blacktip grouper (*Epinephelus fasciatus*), six-bar grouper (*Epinephelus sexfasciatus*), blue-lined hind (*Cephalopholis formosa*), and white-edged lyretail (*Variola albimarginata*). Samples were obtained from fish landing sites and fish markets from 23 locations throughout IMA waters (Figure 1). The IMA waters sampled comprised of nine seas: Andaman Sea, Malacca Strait, Indian Ocean (not including the Andaman Sea), South China Sea, Makassar Strait, Celebes Sea, Sulu Sea, Java Sea and Bali Sea (Table 1 and Figure 1). Identification was based on Heemstra and Randall (1993) and Craig *et al.* (2011). Fin tissues were processed, preserved, and documented following Steinke and Hanner (2011). Sample sizes ranged from 1 to 7 individuals per population depending on the availability of the specimens. No specific permissions were required for sampling since tissue samples were obtained from commercial fishers and collaborators.

2.2. DNA Extraction, Primer, and PCR Assay

The genomic DNA was isolated using the CTAB protocol (Grewe *et al.* 1993). Extracted DNA was quantified using the Nano Drop 2000c Spectrophotometer (Thermo Scientific, Waltham, MA). The partial COI gene was amplified using the primer sets of Ward *et al.* (2005) (Fish F1: 5'–TCA ACC AAC CAC AAA GAC ATT GGG AC–3' and 5'–TAG ACT TCT GGG TGG CCA AAG AAT CA–3'). PCR was set up in a 25 µL mixture reaction (2.0 µL DNA template, 0.5 µL of each primer, 2.5 µL of 10x *i*-Taq™ plus PCR Buffer, 2.0 µL of 25mM $MgCl_2$, 1.0 µL of dNTP, 0.25

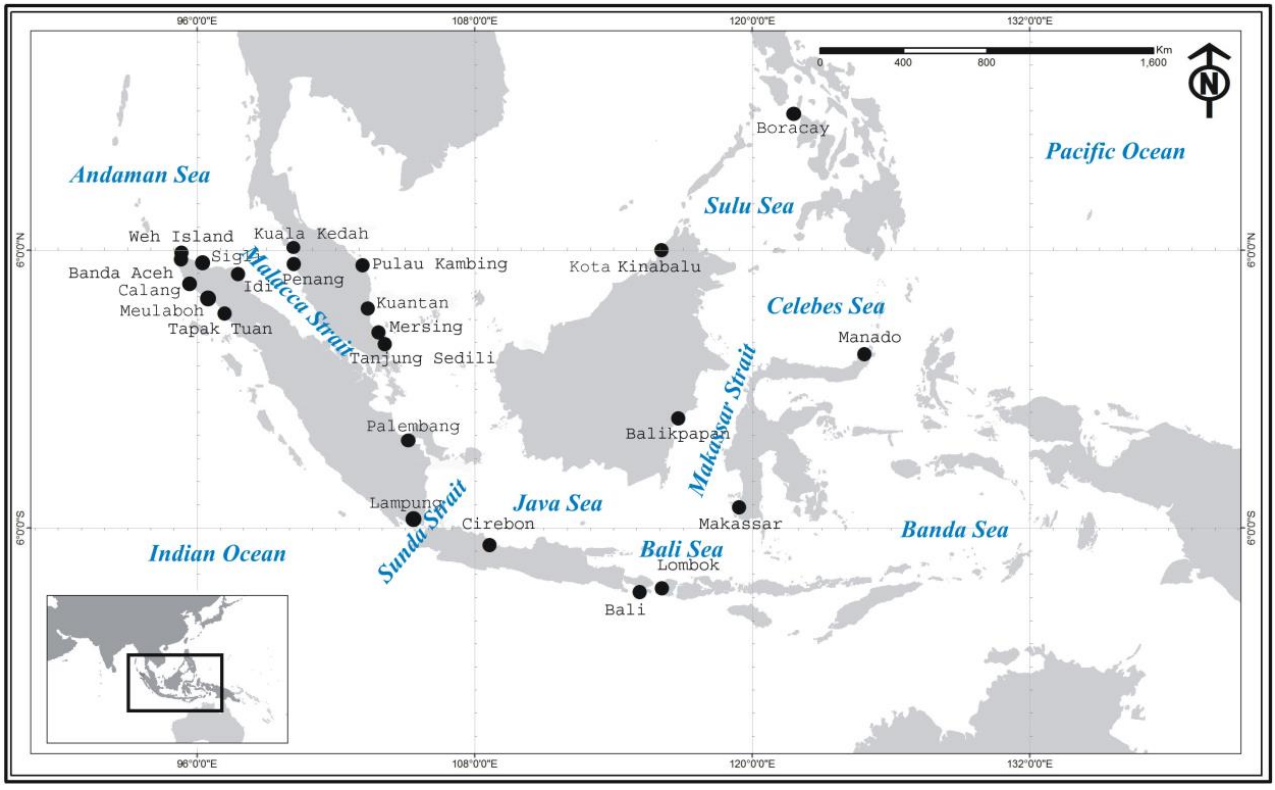

Figure 1. Map of study sites

Table 1. Sampling details of fishes collected and successfully amplified in the study

Species	Location	Seas/Ocean	Geographical coordinates	Number of Samples amplified
Epinephelus areolatus	Penang	MCS	5°23'28.27" N 100°10'59.34" E	2
	Kuala Kedah	MCS	6°6′19.41″ N 100°16′56.44″ E	3
	Palembang	JS	2°20'24.55″ S 105° 3'28.75″ E	3
	Bali	BS	8°45'27.46" S 115° 9'59.76" E	3
	Lombok	BS	8°35'13.09" S 116° 3'59.56" E	3
	Kuantan	SCS	3°47'1.83″ N 103°19'1.59″ E	1
Sub total				15
Epinephelus fasciatus	Weh Island	AS	5°53'9.74" N 95°19'21.49" E	7
	Banda Aceh	AS	5°35'7.80" N 95°18'59.31″ E	5
	Calang	IO	4°37'56.12" N 95°34'17.02" E	3
	Kota Kinabalu	SCS	5°58'58.55″ N 116°4'15.87″ E	5
	Makassar	MKS	5°6'37.33" S 119°25'20.72" E	3
	Manado	CS	1°29'49.06″ N 124°50'18.32″ E	3
	Balikpapan	MKS	1°16'44.80″ S 116°50'29.43″ E	1
	Bali	BS	8°45'27.46" S 115° 9'59.76" E	4
	Lombok	BS	8°35'13.09" S 116° 3'59.56" E	5
	Lampung	JS	5°27'8.34" S 105°16'9.88" E	3
Sub total				39

Table 1. Continued

Species	Location	Seas/Ocean	Geographical coordinates	Number of Samples amplified
Epinephelus sexfasciatus	Sigli	MCS	5°23′20.84″ N 95°57′51.61″ E	3
	Penang	MCS	5°23'28.27" N 100°10'59.34" E	3
	Idi	MCS	4°57'30.30"N 97°46'32.90"E	5
	Meulaboh	IO	4° 8'10.89" N 96° 7'56.21" E	2
	Lampung	JS	5°27'8.34" S 105°16'9.88" E	3
	Cirebon	JS	6°42'48″ S 108°34'36.33″ E	2
	Kota Kinabalu	SCS	5°58'58.55″ N 116°4'15.87″ E	5
	Balikpapan	MKS	1°16'44.80″ S 116°50'29.43″ E	4
	Lombok	BS	8°35'13.09" S 116° 3'59.56" E	2
	TanjungSedili	SCS	1°55′49.18″ N 104°6′47.5″ E	3
	PulauKambing	SCS	5°20'20.18″ N 103° 9'0.01″ E	3
Sub total				35
Cephalopholis formosa	Weh Island	AS	5°53'9.74" N 95°19'21.49" E	5
	Calang	IO	4°37'56.12" N 95°34'17.02" E	3
	Lampung	JS	5°27'8.34" S 105°16'9.88" E	3
	Kota Kinabalu	SCS	5°58'58.55″ N 116°4'15.87″ E	1
	Makassar	MKS	5°6'37.33" S 119°25'20.72" E	1
	Bali	BS	8°45'27.46" S 115° 9'59.76" E	3
	Mersing	SCS	2°26'9.18" N 103°50'25.61" E	3
Sub total				19
Variola albimarginata	Weh Island	AS	5°53'9.74" N 95°19'21.49" E	4
	Calang	IO	4°37'56.12" N 95°34'17.02" E	2
	Tapak Tuan	IO	3°15'12.16″ N 97°11'47.16″ E	3
	Sigli	MCS	5°23′20.84″ N 95°57′51.61″ E	1
	Kota Kinabalu	SCS	5°58'58.55″ N 116°4'15.87″ E	3
	Makassar	MKS	5°6'37.33" S 119°25'20.72" E	3
	Manado	CS	1°29'49.06″ N 124°50'18.32″ E	3
	Lombok	BS	8°35'13.09" S 116° 3'59.56" E	3
	Boracay	SL	11°57'28.73" N 121°55'42.88" E	5
Sub total				27
Total				135

AS: andaman sea; MCS: malacca strait; IO: indian ocean; SCS: south china sea; MKS: makassar strait; CS: celebes sea, SL: sulu sea, JS: java sea, and BS: bali sea

µL of *i*-Taq™ plus DNA Polymerase and 16.25 µL of Milli-Q water) and was amplified in a BIO-RAD T100 Thermal Cycler (BioRad Laboratories Inc., USA). The thermal conditions follow Fadli *et al.* (2021) with initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation at 94°C for 45s; annealing at 47.9-60°C for 45s, elongation at 72°C for 1 min and a final extension of 72°C for 10 minutes before termination of the reaction at 4°C.

2.3. Gel Electrophoresis, Staining, and DNA Sequencing

Amplicons were separated and visualized on a 1.7 % agarose gel stained with 2 to 2.5 µL of RedSafe™ Nucleic Acid Staining Solution (IntRON Biotechnology, Gyeonggi-do, Korea). A 2.0 µL volume of PCR product was loaded onto the agarose gel and electrophoresed at 100V for 30 min to assess its quality. Satisfactory PCR products were sent to the First BASE Laboratories, Malaysia, for bidirectional sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit and ABI PRISM 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA) following the manufacturer's protocol.

2.4. Data Analysis

The COI sequences were first aligned in Clustal W and edited in MEGA version 6.06 (Tamura *et al.* 2013) and then translated into protein to ensure accurate alignment and detection of premature stop codons, if present. Haplotype distributions were summarized in DnaSP 5.10 (Librado and Rozas 2009; Rozas *et al.* 2003). Minimum Spanning Network (MSN) version 5.0.03 (Tobias and Siavash 2018) was used to generate a graphical representation of haplotype relationships based on median joining calculation (Bandelt *et al.* 1999). Maximum-Likelihood (ML) tree was constructed using MEGA under the Hasegawa Kishino Yano model with Gamma distribution of

variable (HKY+G) (optimal substitution model under Bayesian Information Criterion using Model Test in MEGA) and bootstrapping of 1000 pseudoreplicates. *Acanthurus mata* (BOLD ID: FADLI005-17) and *Lutjanus johnii* (BOLD ID: FADLI121-17) sequences were used as outgroups to root the tree.

3. Results

3.1. General Findings

In total, 135 COI sequences were generated for all five species combined. The read lengths of COI sequences were 621 bp comprising 426 conserved sites, 195 variable sites, 188 parsimony informative and seven singletons. Mean base compositions were A: 24.2%; C: 27.9%; G: 17.7%; T: 30.2%. Unique haplotypes (numbers in parenthesis) were identified in; *E. areolatus* (7), *E. fasciatus* (9), *E. sexfasciatus* (13), *C. formosa* (3), and *V. albimarginata* (11), respectively (Table 2). For *E. areolatus*, out of seven COI haplotypes, four haplotypes were detected in only a single individual each. Despite the small sample sizes, two distinct clades were detected of *E. areolatus* across IMA. The samples from the western part of IMA (Penang and Kuala Kedah) clustered in one clade. In contrast, the samples from the central and eastern part of IMA (Palembang, Bali, Lombok, and Kuantan) clustered collectively. Both clades were separated by 18 mutations. For *V. albimarginata,* nine out of 12 haplotypes were detected in a single individual only. Hap37, Hap38, and Hap39 were identified as the dominant haplotypes across the study region. Two distinct clades were detected of *V. albimarginata* across IMA, however separated only by

Table 2. Distribution of haplotype frequencies in COI by species

Seas/oceans		AS		MCS				IO			JS			MKS		CS	BS		SCS				SL	
Species	haplotype	Weh Island	Banda Aceh	Sigli	Idi	Penang	Kuala Kedah	Calang	Meulaboh	Tapak Tuan	Palembang	Lampung	Cirebon	Balikpapan	Makassar	Manado	Bali	Lombok	Kota Kinabalu	Mersing	TanjungSedili	Kuantan	PulauKambing	Boracay	Total
E. areolatus	1		1								1						2	2					2		5
	2		2														1								1
	3		3								1							1					1		2
	4		4			2	2																		4
	5		5																			1			1
	6		6								1														1
	7		7				1																		1
E. fasciatus	8	5	2					3								1	1		1				2		13
	9	1																							1
	10	1									1												1		1
	11		1														3	1	4						10
	12		1																			1			1
	13		1								1														1
	14													1	3	1		4							10
	15															1									1
	16													1											1
E. Sexfasciatus	17			1	2									3							1		1		8
	18			2	2	1			2			2						2	3		1		1		16
	19					1																			1
	20					1																			1
	21				1																				1
	22										1														1
	23										1														1
	24										1														1
	25																		1						1
	26																		1						1
	27													1											1
	28																				1				1
	29																						1		1

Table 2. Continued

Seas/oceans		AS	MCS			IO		JS			MKS		CS	BS	SCS				SL	
Species	haplotype	Weh Island Banda Aceh	Sigli	Idi	Penang Kuala Kedah	Calang Meulaboh	Tapak Tuan	Palembang Lampung	Cirebon	Balikpapan Makassar		Manado	Bali Lombok	Kota Kinabalu	Mersing TanjungSedili	Kuantan PulauKambing		Boracay	Total
C. formosa	30	4				3		2											9
	31	1																	1
	32							1		1			3	1	3				9
V. Albimarginata	33	1																	1
	34	1																	1
	35	1																	1
	36	1																	1
	37		1			1	2												4
	38					1	1												2
	39									2		2	3	3				3	13
	40									1									1
	41											1							1
	42																	1	1
	43																	1	1

AS: andaman sea; MCS: malacca strait; IO: indian ocean; SCS: south china sea; MKS: makassar strait; CS: celebes sea, SL: sulu sea, JS: java sea, and BS: bali sea

two mutations. The samples from the western part of IMA (Weh Island, Sigli, Calang, and Tapak Tuan) clustered in one group while the samples from the central and eastern parts of IMA (Makassar, Manado, Lombok, Kota Kinabalu, and Boracay) clustered in another group (Figure 2). For *E. fasciatus*, six out of nine haplotypes were singleton. Three dominant COI haplotypes (Hap08, Hap11, and Hap14) were identified. Six locations shared Hap08 while Hap11 and Hap14 were shared by five locations. For *E. sexfasciatus*, 11 haplotypes were detected in only a single individual each out of 13 haplotypes. Hap17 and Hap18 were found as the dominant haplotypes. The occurrence of star-like network patterns radiating from Hap18 was observed. Hap17 and Hap18 were shared by five and nine locations, respectively. For *C. formosa*, three haplotypes were recognized with Hap30 and Hap32 being the dominant haplotypes, while Hap31 was detected in only a single individual. No distinct geographical structuring was observed for *E. fasciatus, E. sexfasciatus,* and *C. formosa* samples.

The phylogenetic trees showed that all grouper samples were robustly clustered within their putative species (bootstrap ≥98%). *Variola albimarginata* was found to be basal and ancestral among the five species investigated. This was followed by *C. formosa, E. fasciatus*, and *E. sexfasciatus*, with *E. areolatus* as the most recently evolved species. In addition, the ML tree clustered each species in either one or two distinct clades across IMA. Consistent with network analysis, *E. areolatus* and *V. albimarginata* formed two genetic clusters, while *E. fasciatus, E. sexfasciatus* and *C. formosa* were composed of only a single cluster (Figure 3).

Although sample sizes and population numbers were limited to address a comprehensive population genetics investigation, some general pattern could be elucidated with combined samples of each species. The pairwise between-species genetic distances showed the highest value in the *E. sexfasciatus* vs. *V. albimarginata* (21.8%), while the lowest pairwise genetic distance was between *E. areolatus* and *E. fasciatus* (14.7%). The highest intra-species distance was observed in *E. areolatus* (1.7%), while the lowest distance was found in *C. formosa* (0.1%) (Table 3). In addition, the haplotype (h) and nucleotide (π) diversity were higher in *E. areolatus* (h = 0.838, π = 0.017) compared to *E. fasciatus* (h = 0.773, π = 0.002), *E. sexfasciatus* (h = 0.751, π= 0.003), *C. formosa* (h = 0.579, π = 0.001) and *V. albimarginata* (h = 0.792, π = 0.004) (Table 4). However, these are preliminary estimates as sample sizes were low.

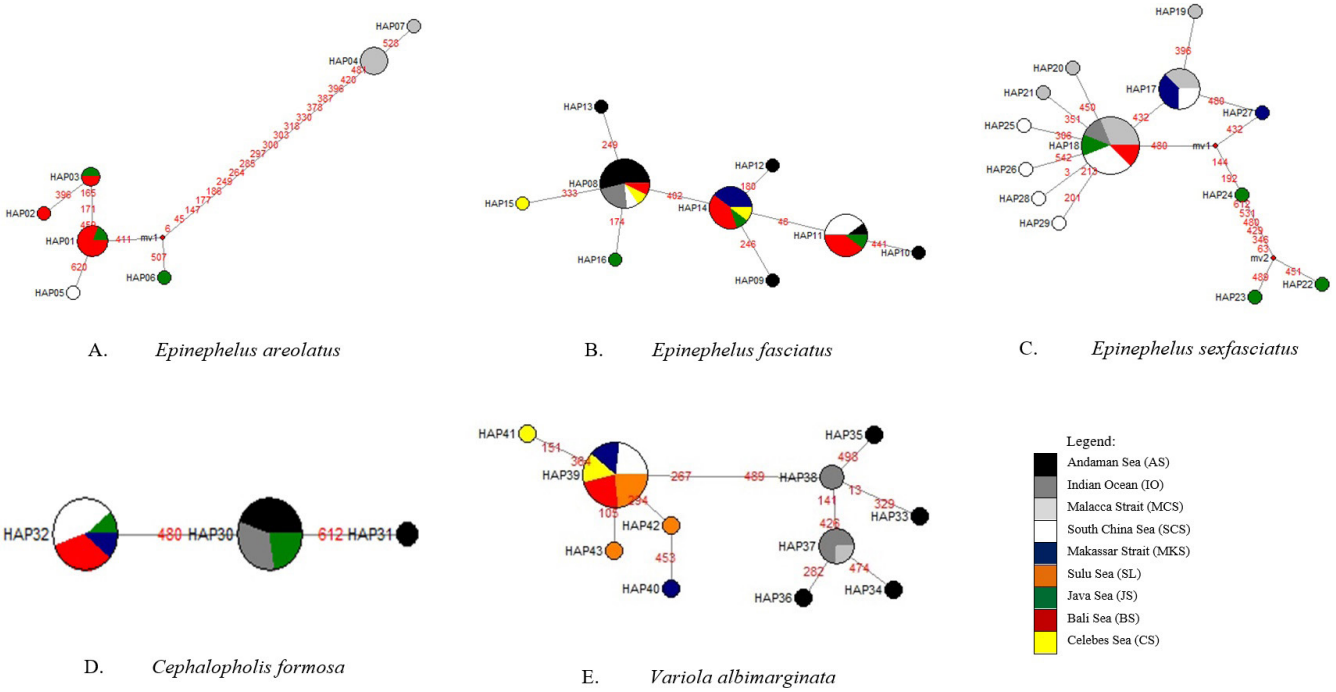

Figure 2. Minimum spanning network (MSN) inferred from COI haplotypes of (A) *Epinephelus areolatus*, (B) *Epinephelus fasciatus*, (C) *Epinephelus sexfasciatus*, (D) *Cephalopholis formosa*, and (E) *Variola albimarginata*. Colours represent different regions. mv = median vectors

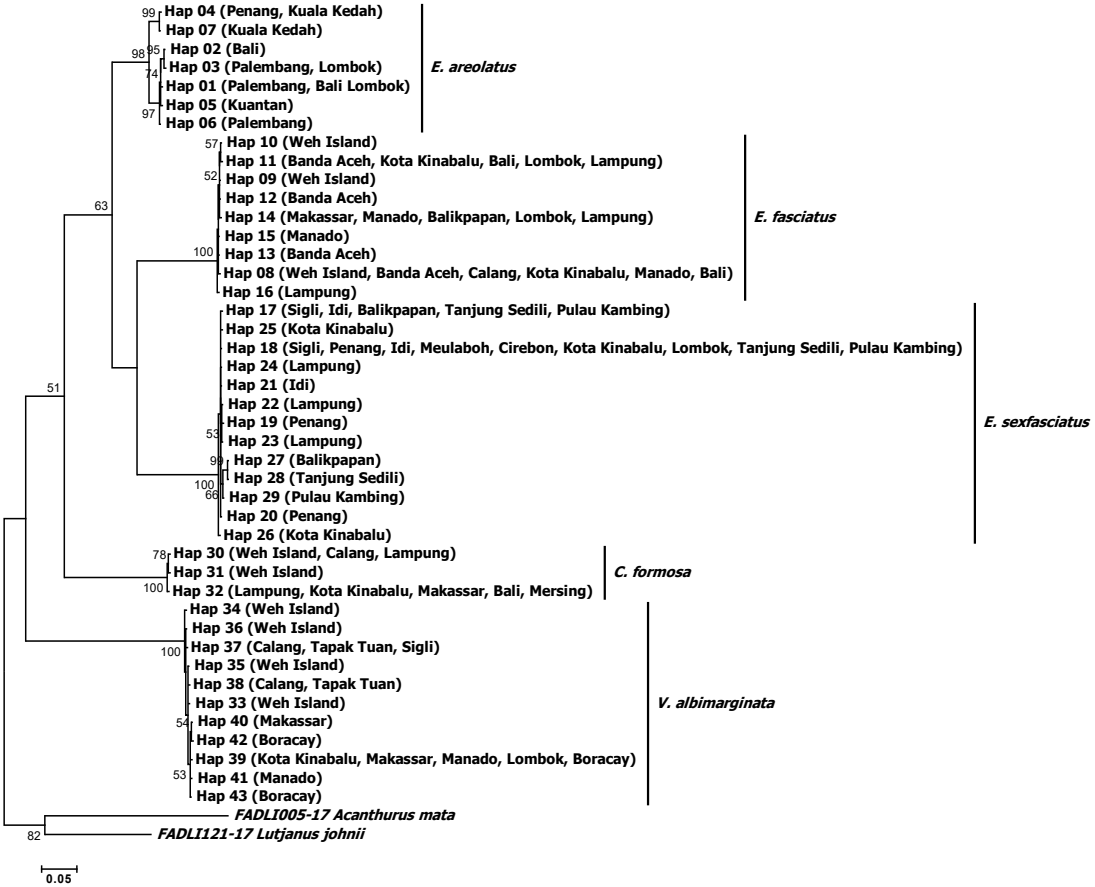

Figure 3. Phylogenetic tree of five grouper species in IMA as inferred using the Maximum-Likelihood method with 1,000 bootstrap replicates (COI). Bootstrap values <50% are not shown. The scale bar indicates percent divergence calculated under the HKY+G model

Table 3. Genetic diversity within species (in bold) and between the species inferred by mtDNA COI.

	1	2	3	4	5
E. areolatus	**0.017**				
E. fasciatus	0.147	**0.002**			
E. sexfasciatus	0.159	0.162	**0.003**		
C. formosa	0.164	0.197	0.188	**0.001**	
V. albimarginata	0.203	0.210	0.218	0.200	**0.004**

Table 4. Number of COI samples (N), haplotypes (H), haplotype diversity (h), and nucleotide diversity (π)

Species	N	H	h	π
E. areolatus	15	7	0.838	0.017
E. fasciatus	39	9	0.773	0.002
E. sexfasciatus	35	13	0.751	0.003
C. formosa	19	3	0.579	0.001
V. albimarginata	27	12	0.792	0.004

4. Discussion

Understanding the patterns of genetic diversity of fish species is important for marine conservation and management (Ketchum *et al.* 2016). In general, values <0.5 for both h and π in marine species indicate low haplotype diversity and low nucleotide diversity (Grant and Bowen 1998). Although the population sizes are relatively low for a comprehensive population genetic analysis of each species but insights into the general pattern could be elucidated.

Overall, high haplotype diversity coupled with low nucleotide diversity was observed in this study for all species tested for the mitochondrial genes except for *E. areolatus* (h = 0.838, π = 0.017). These results are comparable with previous studies of several grouper species: the peacock hind *C. argus* (Cyt b: h = 0.38-0.96, π = 0.001-0.009) (Gaither *et al.* 2011), blacktip grouper *E. fasciatus* (combined Cyt b-tRNA-Thr-tRNA-Pro-CR: h = 0.931–0.995, π = 0.003–0.004) (Kuriiwa *et al.* 2014), Nassau grouper *E. striatus* (combined ATPase-Cyt b: h = 0.500–0.954, π = 0.0005–0.0089) (Jackson *et al.* 2014), areolate grouper *E. areolatus* (combined d-loop-12S rRNA: h = 0.944, π = 0.003), orange spotted grouper *E. coioides* (combined d-loop-12S rRNA: h = 0.806, π = 0.005), duskytail grouper *E. bleekeri* (combined d-loop-12S rRNA: h = 0.859, π = 0.002) (Ketchum *et al.* 2016).

Several factors might explain the low level of nucleotide diversity observed in this study. Groupers are among the most commercially important fishes in the world and regarded as one of the first fish groups to be overexploited globally, (Sadovy de Mitcheson *et al.* 2013). Thus, overfishing or contemporary environmental pressures could lead to a severe decline.

Asia (including the IMA region) contributed 80% of grouper production worldwide in 2008 (Sadovy de Mitcheson *et al.* 2013). Based on their review of the 12 overfished marine taxa globally, Pinsky and Palumbi (2014) stated that overfished marine species tend to have lower genetic diversity than stable populations. Similar findings were also reported by Ketchum *et al.* (2016) that reported very low genetic diversity in three *Epinephelus* species (*E. areolatus, E. coioides*, and *E. bleekeri*) in the United Arab Emirates, mainly due to overfishing. In addition, the low level of nucleotide diversity could also be attributed to bottlenecking and founder effect caused by the loss of habitat in the IMA region due to the lowering of sea levels during the Pliocene and Pleistocene. Furthermore, environmental pressures, including habitat destruction might also attribute to the low levels of nucleotide diversity observed.

Most groupers are reef-associated fishes. The IMA region coral reefs are seriously threatened by natural and anthropogenic factors (Burke *et al.* 2011; Hughes *et al.* 2003, 2017), thus destroying extensive grouper habitats. In 2017, only 6.39% of Indonesian corals were classified to be in excellent condition and 23.40% in good condition, while 35.06% in fair condition and 35.15% in a poor state (Giyanto *et al.* 2017). Corals in Malaysia are also facing the same situation, 87% of corals in Malaysia are under medium or high-threat (Praveena *et al.* 2012).

In contrast, haplotype diversities were high in all five species investigated. In general, high haplotype diversity coupled with low nucleotide diversity was observed for the COI gene tested for all species. Based on the classification proposed by Grant and Bowen (1998), marine fish species can be categorized into four categories depending on the combinations of their h and π values to interpret population history. The high h and low π for the gene tested suggests that the studied groupers had undergone a period of population bottleneck followed by significant rapid population growth in the relatively recent past. No data is available on the population trend of the studied species, but the high haplotype diversity indicates that population numbers are still at a high level. This is in the background of a species exposed to high exploitation with loss of nucleotide diversity. Several

factors could account for this. Firstly, the groupers inhabit a refugia region (the IMA), consequential of the *Pleistocene cyclical* high and low sea level events (Hobbs *et al.* 2013). The Central Indo-Pacific region (CIP; referred to as IMA in this study) is hypothesized to be central to the survival for the epinephelids (Ma *et al.* 2016). Marine species genetic diversity has been documented to be high in such areas. For example, Hobbs *et al.* (2013), in their study of coral reef angelfishes (genus: Centropyge) in Christmas and Coccos Islands, Australia (located in the southern part IMA region), revealed high genetic diversity for all studied angelfishes species and hypothesized that these islands are one of the Pleistocene refugia.

The IMA region experienced dramatical changes during the Pliocene and Pleistocene periods. Throughout the periods, sea levels in IMA experienced periodic lowering, which exposed the Sunda Shelf and Sahul Shelf within this region. Sea level in this region reached its minimum (115-130 m below the current level) during the last Glacial Maximum (LGM), reducing approximately 90% of the habitable coastal marine area in the Indo-Pacific (Ludt and Rocha 2015; Voris 2000). However, the sea level lows lasted for relatively short periods (roughly 6% of the time during the last 250 ka) (Ludt and Rocha 2015). The loss of habitats during the lowering of sea level resulted in population bottleneck.

In addition, the lowering of sea levels likely inhibited the dispersal of the studied groupers in the IMA region and led to smaller isolated founder populations. Small populations typically have low genetic diversity (Frankham 1996) because of inbreeding effects and genetic drift (Hobbs *et al.* 2013) as evidenced by the low nucleotide diversity. At the end of the last glacial, the sea level rose again and provided new habitats or rejuvenated the original ones for the marine organisms in this region which would have led to new haplotypes being generated, hence high haplotype diversity.

Secondly, the five investigated species are widespread across the Indo-Pacific region (Heemstra and Randall 1993). Thus, there is enormous potential for high gene flow throughout the IMA ensuring continuous in-flow of genetic variability even from external sources to the IMA. In turn, these gene flows help to maintain the widespread distributions among the species (Hobbs *et al.* 2013). In support of this, is strong evidence of observed genetic homogeneity within the IMA for three of the five species investigated. Although *E. areolatus* and, to a much lower extent *V. albimarginata* showed two distinct groups, but within them, gene flow appeared to be extensive.

The phylogeographic analyses indicated genetic panmixia within the IMA for three species: *E. fasciatus, E. sexfasciatus*, and *C. formosa*. In contrast, *E. areolatus* and *V. albimarginata* formed two genetic lineages across IMA. For both *E. areolatus* and *V. albimarginata*, western IMA was genetically differentiated from central and eastern IMA. This genetic structuring of marine species in the IMA and its adjacent waters has also been reported in other groupers, for example, the orange-spotted grouper (*E. coioides*) (Antoro *et al.* 2006; Wang *et al.* 2011), the peacock hind (*C. argus*) (Gaither *et al.* 2011), the blacktip grouper (*E. fasciatus*) (Kuriiwa *et al.* 2014), and camouflage grouper (*E. polyphekadion*), squaretail coralgrouper (*P. areolatus*), and leopard coralgrouper (*P. leopardus*) (Ma *et al.* 2018). Several marine fish species such as the soldierfish (*Myripristis berndti*) (Craig *et al.* 2007), redbelly yellowtail fusilier (*Caesio cuning*) (Ackiss *et al.* 2013), and several commercial tuna and mackerel (*Auxis thazard, Katsuwonus pelamis*, and *Scomberomorus commerson*) (Jackson *et al.* 2014) have also shown similar genetic structure.

Several historical and current factors have been hypothesized to be responsible for shaping the genetic structuring in numerous marine species inhabiting the IMA region and presumably also in *E. areolatus* and *V. albimarginata*. One of the hypotheses proposed is the exposure of the Sunda Shelf during the *Pleistocene epoch,* which led to the isolation of the Indian Ocean from the Pacific Ocean (which in parallel demarcated the IMA). During this period, the sea level in the IMA region was lowered to 120 m below the present level (Ludt and Rocha 2015; Voris 2000) and caused the restriction of gene flow of numerous marine species between the two oceans.

In addition, the biological characteristics of groupers (e.g., life histories, larval behavior, and larval dispersal) might also have contributed to the present genetic structuring observed in this study. The adult groupers have strong site fidelity such as particular habitat preferences, male territorial behavior, and consistently form spawning aggregations in the same location (Craig *et al.* 2011; Heemstra and Randall 1993; Pet *et al.* 2005), which might increase their population differentiation. Even though there is limited information on the biological characteristics

of *E. areolatus* and *V. albimarginata*, their genetic structuring observed in this study suggest that both species have the same life strategies. Antoro *et al.* (2006) also hypothesized that these life strategies were responsible for the genetic structuring of *E. coioides* in the IMA region.

Limited larval dispersal and natal homing have been hypothesized to shape the genetic structuring of several grouper species; *Plectropomus maculatus* in the Keppel Islands, Australia (Harrison *et al.* 2012) and *P. areolatus* larvae in the Manus Island, Papua New Guinea (Almany *et al.* 2013). This behavior might enhance their population differentiation. This larval behavior could be a signature of *E. areolatus* and *V. albimarginata*, leading to significant genetic differentiation observed in this study. Similar characteristic has also been observed for other reef-associated fishes; several anemone species (Dohna *et al.* 2015; Madduppa *et al.* 2014; Timm and Kochzius 2008; Timm *et al.* 2017) thus enhancing their genetic differentiation.

Oceanographic features have also been known to strongly influence the structuring pattern of species in the IMA region. Antoro *et al.* (2006) in their study of the orange-spotted grouper, *E. coioides*, in the IMA region hypothesized that current patterns could shape the larval dispersal in this region. They reported that sea surface currents flowing through the Sunda Strait restricted the dispersal of larvae from Lampung (Indian Ocean) to Jepara (Java Sea), thus increasing genetic differentiation between these two populations. In another study, Kuriiwa *et al.* (2014) studied the influence of the Kuroshio Current (in the northern part of the IMA region up to the Southern of Japan waters) on the population structure of blacktip grouper (*E. fasciatus*). They found that the Kuroshio Current acted as an unseen barrier for larval dispersal restricting the larval dispersal from the Southern of Japan to the northern part of the IMA region. This led to three *E. fasciatus* population lineages.

In contrast, *E. fasciatus*, *E. sexfasciatus*, and *C. formosa* formed a single clade. This lack of genetic structuring in marine species in the IMA region has also been reported in other marine taxa such as; pelagic scads (*Decapterus macrosoma* and *D. macarellus*) (Arnaud *et al.* 1999) and the pelagic Indian mackerel (*Rastrelliger kanagurta*) (Akib *et al.* 2015). Several factors (both historical and current factors) have been hypothesized to be the cause for this lack of genetic structuring in numerous marine species in the IMA region (and apparently in *E. fasciatus, E. sexfasciatus*, and *C. formosa* too). Akib *et al.* (2015) in their study hypothesized that the connection between the Indian Ocean and the South China Sea via the Straits of Singapore during the last Interglacial Period allowed extensive population expansion and permitting free migration leading to low genetic differentiation of *R. kanagurta* populations across IMA region.

In addition, the regional oceanographic conditions in IMA act as effective homogenizing agents for many marine species in the IMA region. Kochzius and Nuryanto (2008) hypothesised that the Indonesian throughflow (ITF) that streams down from Western Pacific through Makassar Strait and empties into the Indian Ocean facilitated the connectivity of the boring giant clam (*Tridacna crocea*) in the Sulawesi Sea, Makassar Strait, and the Flores Sea. Ackiss *et al.* (2013) hypothesized that the ITF also allowed the connectivity of the populations of the redbelly yellowtail fusilier (*Caesio cuning*) from north to south within and between the central and eastern part of the IMA. An inadequate or limited number of samples and geographical coverage could also mask the genetic structures of observed fish samples, resulting in an underestimation of the actual genetic divergence. As noted earlier, the sample sizes and population numbers were small. Thus, more intensive study is required to corroborate the genetic homogeneity observed in the current study.

Genetic structuring was observed in two species, but in contrast genetic homogeneity for the other three investigated species across IMA. Therefore, further studies should be based on this consideration. Despite the low sample sizes, *E. areolatus* and *V. albimarginata*, were observed to be composed of two stocks comprising of western IMA versus the central and eastern parts of IMA. Thus, the immediate strategy is a different management unit of the two clades in a broad sense. Future studies should therefore be targeting on the genetic boundary of each of these species. On the other hand, *E. fasciatus, E. sexfasciatus,* and *C. formosa* each displayed a single gene pool based on the limitation of the study. However, this should be taken cautiously and a conservative approach until more data is acquired. Since these waters transcend across several nations, the establishment of effective international cooperation is critical to managing the grouper stocks in the IMA.

Currently, there are already several past and on-going initiatives addressing these. Examples are the programs initiated by the Southeast Asian Fisheries Development Center (SEAFDEC), which cover many marine taxa including tunas, scads, sharks, and rays. Of late, more efforts are being directed towards genetic considerations. For example, SEAFDEC coordinated a project to study the population genetics of Indian Mackerel utilizing microsatellite markers in the Bay of Bengal (BOBLME), which comprises of four countries in this region (Malaysia, Bangladesh, Maldives, and Myanmar). In addition, SEAFDEC also conducted the population study of *Thunnus tonggol*, which comprises eight member countries throughout Southeast Asian Region, namely Brunei, Cambodia, Indonesia, Malaysia, Myanmar, the Philippines, Thailand and Viet Nam (http://seafdec.org.my/, accessed 17 July 2022).

Furthermore, since most groupers are reef-associated fishes, the establishment of a marine protecting area (MPA) would ensure complete protection of habitat and their resident fishes, including groupers. Until now, 222 MPAs have been established in Indonesia (coverage 182,712 km^2), 71 MPAs in the Philippines (coverage 42,476 km^2), and 93 MPAs in Malaysia (coverage 23,864 km^2) respectively ((http://mpatlas.org/, accessed 18 April 2023). A number of studies have documented the success story of MPA in reviving and increasing fish populations. For example, the establishment of MPA in northwestern Mediterranean increased the fish abundance after three years (Claudet *et al.* 2006). In another study, Raymundo *et al.* (2007) revealed that the fish biomass in the healthy reef sites in the Calagcalag Marine Protected Area in Negros Oriental, central Philippines increased by five-fold within four years from ~2000 g/500 m^2 (2003) to ~17,000 g/500m^2 (2006).

A well-managed MPA could contribute more productive fisheries (e.g. coral trout; *Plectropomus* spp.) as documented in Australia's Great Barrier Reef Marine Park (GBRMP) (Hopf *et al.* 2016). Thus, it is recommended to establish more MPAs in the IMA region. In addition, we could learn from other successful models; Australia applies a number of broad range management tools to manage their *Plectropomus* fisheries, including restriction of size to be caught, catch and effort limits, temporal closures, gear and vessel restrictions, and limited-entry licensing (Frisch *et al.* 2016).

Finally, the data presented in the current study has provided a preliminary window into the phylogeography of five commercially important groupers in the IMA. Nevertheless, from a management point of view, detailed population genetics data is critical. Thus, a more comprehensive and intensive sampling of individuals and populations, as well as the use of complementary nuclear molecular markers, could provide a more complete picture of the studied grouper connectivity in this region.

In conclusion, the present study has contributed baseline knowledge on the phylogeographic patterns of five species of the commercially essential groupers in IMA waters as a prerequisite for further studies and applications. Various past geological, demographic history, local and regional oceanographic features, and species' biological characteristics were hypothesised to shape the present genetic structure of these groupers across the IMA waters. A comprehensive and intensive sampling of individuals and populations and the use of additional molecular markers would provide a more complete picture of grouper connectivity in IMA waters. In addition, the establishment of effective international cooperation is encouraged to manage grouper stocks in this region.

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References

Ackiss, A.S., Pardede, S., Crandall, E.D., Ablan-Lagman, M.C.A., Ambariyanto, Romena, N., Barber, P.H., Carpenter, K.E., 2013. Pronounced genetic structure in a highly mobile coral reef fish, *Caesio cuning*, in the Coral Triangle. *Marine Ecology Progress Series*, 480, 185-197. https://doi.org/10.3354/meps10199

Akib, N.A.M., Tam, B.M., Phumee, P., Abidin, M.Z., Tamadoni, S., Mather, P.B., Nor, S.A.M., 2015. High connectivity in *Rastrelliger kanagurta*: influence of historical signatures and migratory behaviour inferred from mtDNA Cytochrome b. *Plos One,* 10, e0119749. https://doi.org/10.1371/journal.pone.0119749

Alcantara, S.G., Yambot, A.V., 2014. DNA barcoding of commercially important Grouper species (Perciformes, Serranidae) in the Philippines. *Mitochondrial DNA*, 1-9. https://doi.org/10.3109/19401736.2014.958672

Almany Glenn, R., Hamilton Richard, J., Bode, M., Matawai, M., Potuku, T., Saenz-Agudelo, P., Planes, S., Berumen Michael, L., Rhodes Kevin, L., Thorrold Simon, R., Russ Garry, R., Jones Geoffrey, P., 2013. Dispersal of grouper larvae drives local resource sharing in a coral reef fishery. *Current Biology*, 23, 626-630. https://doi.org/10.1016/j.cub.2013.03.006

Antoro, S., Na-Nakorn, U., Koedprang, W., 2006. Study of genetic diversity of Orange-Spotted Grouper, *Epinephelus coioides*, from Thailand and Indonesia using microsatellite markers. *Marine Biotechnology*. 8, 17-26. https://doi.org/10.1007/s10126-005-5026-0

Ariyanti, Y., Farajallah, A., Arlyza, I.S., 2015. Phylogenetic analysis of the darkfin hind, *Cephalopholis urodeta* (Serranidae) using partial mitochondrial CO1 Gene Sequences. *Indonesian Journal of Marine Sciences*. 20, 38-44. https://doi.org/10.14710/ik.ijms.20.1.38-44

Arnaud, S., Bonhomme, F., Borsa, P., 1999. Mitochondrial DNA analysis of the genetic relationships among populations of scad mackerel (*Decapterus macarellus, D. macrosoma,* and *D. russelli*) in South-East Asia. *Marine Biology*. 135, 699-707. https://doi.org/10.1007/s002270050671

Aziz, N.M.A., Esa, Y., Arshad, A., 2016. DNA barcoding and phylogenetic analysis of Malaysian groupers (Subfamily: Epinephelinae) using mitochondrial Cytochrome c oxidase I (COI) gene. *Journal of Environmental Biology*. 37, 725-733.

Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*. 16, 37-48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Burke, L., Reytar, K., Spalding, M., Perry, A., 2011. *Reefs at Risk Revisited*. World Resources Institute (WRI), Washington D.C.

Carpenter, K.E., Barber, P.H., Crandall, E.D., Ablan-Lagman, M., Carmen, A., Mahardika, G.N., Manjaji-Matsumoto, B.M., Juinio-Meñez, M.A., Santos, M.D., Starger, C.J., 2011. Comparative phylogeography of the Coral Triangle and implications for marine management. *Journal of Marine Biology*. 2011, 1-14. https://doi.org/10.1155/2011/396982

Claudet, J., Pelletier, D., Jouvenel, J.Y., Bachet, F., Galzin, R., 2006. Assessing the effects of marine protected area (MPA) on a reef fish assemblage in a northwestern Mediterranean marine reserve: identifying community-based indicators. *Biological Conservation*, 130, 349-369. https://doi.org/10.1016/j.biocon.2005.12.030

Craig, M., Eble, J., Bowen, B., Robertson, D.R., 2007. High genetic connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis berndti* (Holocentridae). *Marine Ecology Progress Series*. 334, 245-254. https://doi.org/10.3354/meps334245

Craig, M.T., Sadovy de Mitcheson, Y., Heemstra, P.C., 2011. *Groupers of the World: A Field and Market Guide*. NISC, Grahamstown, South Africa.

Dohna, T.A., Timm, J., Hamid, L., Kochzius, M., 2015. Limited connectivity and a phylogeographic break characterize populations of the pink anemonefish, Amphiprion perideraion, in the Indo-Malay Archipelago: inferences from a mitochondrial and microsatellite loci. *Ecology and Evolution*. 5, 1717-1733. https://doi.org/10.1002/ece3.1455

Fadli, N., Muchlisin, Z.A., Siti-Azizah, M.N., 2021. DNA barcoding of commercially important groupers (Epinephelidae) in Aceh, Indonesia. *Fisheries Research*. 234, 105796. https://doi.org/10.1016/j.fishres.2020.105796

Frankham, R., 1996. *Relationship of genetic variation to population size in wildlife*. *Conservation Biology*. 10, 1500-1508. https://doi.org/10.1046/j.1523-1739.1996.10061500.x

Frisch, A.J., Cameron, D.S., Pratchett, M.S., Williamson, D.H., Williams, A.J., Reynolds, A.D., Hoey, A.S., Rizzari, J.R., Evans, L., Kerrigan, B., Muldoon, G., Welch, D.J., Hobbs, J.P.A., 2016. Key aspects of the biology, fisheries and management of Coral grouper. *Reviews in Fish Biology and Fisheries*. 26, 303-325. https://doi.org/10.1007/s11160-016-9427-0

Gaither, M.R., Bowen, B.W., Bordenave, T.R., Rocha, L.A., Newman, S.J., Gomez, J.A., van Herwerden, L., Craig, M.T., 2011. Phylogeography of the reef fish *Cephalopholis argus* (Epinephelidae) indicates Pleistocene isolation across the Indo-Pacific Barrier with contemporary overlap in the Coral Triangle. *BMC Evolutionary Biology*. 11, 189. https://doi.org/10.1186/1471-2148-11-189

Giyanto, G., Muhammad, A., Hadi, T., Budianto, A., Hafizt, M., Salatalohy, A., Yulia Iswari, M., 2017. *Status Terumbu Karang Indonesia 2017*. Puslit Oseanografi-LIPI, Jakarta.

Grant, W.A.S., Bowen, B.W., 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*. 89, 415-426. https://doi.org/10.1093/jhered/89.5.415

Grewe, P.M., Krueger, C.C., Aquadro, C.F., Bermingham, E., Kincaid, H.L., May, B., 1993. Mitochondrial DNA variation among lake trout (*Salvelinus namaycush*) strains stocked into lake ontario. *Canadian Journal of Fisheries and Aquatic Sciences*. 50, 2397-2403. https://doi.org/10.1139/f93-264

Harrison Hugo, B., Williamson David, H., Evans Richard, D., Almany Glenn, R., Thorrold Simon, R., Russ Garry, R., Feldheim Kevin, A., van Herwerden, L., Planes, S., Srinivasan, M., Berumen Michael, L., Jones Geoffrey, P., 2012. Larval export from marine reserves and the recruitment benefit for fish and fisheries. *Current Biology*. 22, 1023-1028. https://doi.org/10.1016/j.cub.2012.04.008

Heemstra, P.C., Randall, J.E., 1993. *FAO Species Catalogue vol. 16 Groupers of the World (Family Serranidae, Subfamily Epinephelinae): An Annotated and Illustrated Catalogue of the Grouper, Rockcod, Hind, Coral Grouper, and Lyretail Species Known to Date.* Food and Agriculture Organization of the United Nations, Rome.

Hobbs, J.P.A., Van Herwerden, L., Jerry, D.R., Jones, G.P., Munday, P.L., 2013. High genetic diversity in geographically remote populations of endemic and widespread coral reef angelfishes (genus: centropyge). *Diversity*. 5, 39. https://doi.org/10.3390/d5010039

Hopf Jess, K., Jones Geoffrey, P., Williamson David, H., Connolly Sean, R., 2016. Synergistic effects of marine reserves and harvest controls on the abundance and catch dynamics of a coral reef fishery. *Current Biology*, 26, 1543-1548. https://doi.org/10.1016/j.cub.2016.04.022

Hughes, T., Barnes, M., Bellwood, D.E., Cinner, J., Cumming, G., Jackson, J., Kleypas, J.A., van de Leemput, I., Lough, J., Morrison, T.R., Palumbi, S.H., van Nes, E., Scheffer, M., 2017. *Coral reefs in the Anthropocene. Nature*, 546. 82-90. https://doi.org/10.1038/nature22901

Hughes, T.P., Baird, A.H., Bellwood, D.R., Card, M., Connolly, S.R., Folke, C., Grosberg, R., Hoegh-Guldberg, O., Jackson, J.B.C., Kleypas, J., Lough, J.M., Marshall, P., Nyström, M., Palumbi, S.R., Pandolfi, J.M., Rosen, B., Roughgarden, J., 2003. Climate change, human impacts, and the resilience of coral reefs. *Science*. 301, 929-933. https://doi.org/10.1126/science.1085046

Jackson, A.M., Semmens, B.X., Sadovy de Mitcheson, Y., Nemeth, R.S., Heppell, S.A., Bush, P.G., Aguilar-Perera, A., Claydon, J.A.B., Calosso, M.C., Sealey, K.S., Schärer, M.T., Bernardi, G., 2014. Population structure and phylogeography in nassau grouper (*Epinephelus striatus*), a mass-aggregating marine fish. *Plos One,* 9, e97508. https://doi.org/10.1371/journal.pone.0097508

Jefri, E., Zamani, N.P., Subhan, B., Madduppa, H.H., 2015. Molecular phylogeny inferred from mitochondrial DNA of the grouper *Epinephelus* spp. in Indonesia collected from local fish market. *Biodiversitas*. 16, 254-263. https://doi.org/10.13057/biodiv/d160221

Ketchum, R.N., Dieng, M.M., Vaughan, G.O., Burt, J.A., Idaghdour, Y., 2016. Levels of genetic diversity and taxonomic status of *Epinephelus* species in United Arab Emirates fish markets. *Marine Pollution Bulletin*. 105, 540-545. https://doi.org/10.1016/j.marpolbul.2015.11.042

Kochzius, M., Nuryanto, A., 2008. Strong genetic population structure in the boring giant clam, *Tridacna crocea*, across the Indo-Malay Archipelago: implications related to evolutionary processes and connectivity. *Molecular Ecology*. 17, 3775-3787. https://doi.org/10.1111/j.1365-294X.2008.03803.x

Kuriiwa, K., Chiba, S.N., Motomura, H., Matsuura, K., 2014. Phylogeography of Blacktip Grouper, *Epinephelus fasciatus* (Perciformes: Serranidae), and influence of the Kuroshio Current on cryptic lineages and genetic population structure. *Ichthyological Research*. 61, 361-374. https://doi.org/10.1007/s10228-014-0408-9

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*. 25, 1451-1452. https://doi.org/10.1093/bioinformatics/btp187

Ludt, W.B., Rocha, L.A., 2015. Shifting seas: the impacts of Pleistocene sea-level fluctuations on the evolution of tropical marine taxa. *Journal of Biogeography*. 42, 25-38. https://doi.org/10.1111/jbi.12416

Ma, K.Y., Craig, M.T., Choat, J.H., van Herwerden, L., 2016. The historical biogeography of groupers: clade diversification patterns and processes. *Molecular Phylogenetics and Evolution*. 100, 21-30. https://doi.org/10.1016/j.ympev.2016.02.012

Ma, K.Y., van Herwerden, L., Newman, S.J., Berumen, M.L., Choat, J.H., Chu, K.H., Sadovy de Mitcheson, Y., 2018. Contrasting population genetic structure in three aggregating groupers (Percoidei: Epinephelidae) in the Indo-West Pacific: the importance of reproductive mode. *BMC Evolutionary Biology*. 18, 180. https://doi.org/10.1186/s12862-018-1284-0

Madduppa, H.H., Timm, J., Kochzius, M., 2014. Interspecific, spatial and temporal variability of self-recruitment in anemonefishes. *Plos One*. 9, e90648. https://doi.org/10.1371/journal.pone.0090648

Nor Rahim, N.S., Esa, Y., Arshad, A., 2016. Molecular phylogeny of some Malaysian groupers (Subfamily: Epinephelinae, Family: Serranidae) inferred from mitochondrial and nuclear gene sequences. *Journal of Sustainability Science and Management*. 11, 1-10.

Pet, J.S., Mous, P.J., Muljadi, A.H., Sadovy, Y.J., Squire, L., 2005. Aggregations of *Plectropomus areolatus* and *Epinephelus fuscoguttatus* (groupers, Serranidae) in the Komodo National Park, Indonesia: monitoring and implications for management. *Environmental Biology of Fishes*. 74, 209-218. https://doi.org/10.1007/s10641-005-8528-8

Pinsky, M.L., Palumbi, S.R., 2014. Meta-analysis reveals lower genetic diversity in overfished populations. *Molecular Ecology*. 23, 29-39. https://doi.org/10.1111/mec.12509

Praveena, S.M., Siraj, S.S., Aris, A.Z., 2012. Coral reefs studies and threats in Malaysia: a mini review. *Reviews in Environmental Science and Bio/Technology*. 11, 27-39. https://doi.org/10.1007/s11157-011-9261-8

Raymundo, L.J., Maypa, A.P., Gomez, E.D., Cadiz, P., 2007. Can dynamite-blasted reefs recover? a novel, low-tech approach to stimulating natural recovery in fish and coral populations. *Marine Pollution Bulletin*. 54, 1009-1019. https://doi.org/10.1016/j.marpolbul.2007.02.006

Rozas, J., Sánchez-DelBarrio, J.C., Messeguer, X., Rozas, R., 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics.* 19, 2496-2497. https://doi.org/10.1093/bioinformatics/btg359

Sadovy de Mitcheson, Y., Craig, M.T., Bertoncini, A.A., Carpenter, K.E., Cheung, W.W.L., Choat, J.H., Cornish, A.S., Fennessy, S.T., Ferreira, B.P., Heemstra, P.C., Liu, M., Myers, R.F., Pollard, D.A., Rhodes, K.L., Rocha, L.A., Russell, B.C., Samoilys, M.A., Sanciangco, J., 2013. Fishing groupers towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery. *Fish and Fisheries*. 14, 119-136. https://doi.org/10.1111/j.1467-2979.2011.00455.x

Steinke, D., Hanner, R., 2011. The FISH-BOL collaborators' protocol. *Mitochondrial DNA*. 22, 10-14. https://doi.org/10.3109/19401736.2010.536538

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*. 30, 2725-2729. https://doi.org/10.1093/molbev/mst197

Timm, J., Kochzius, M., 2008. Geological history and oceanography of the Indo-Malay Archipelago shape the genetic population structure in the False Clown Anemonefish (*Amphiprion ocellaris*). *Molecular Ecology*. 17, 3999-4014. https://doi.org/10.1111/j.1365-294X.2008.03881.x

Timm, J., Kochzius, M., Madduppa, H.H., Neuhaus, A.I., Dohna, T., 2017. Small scale genetic population structure of coral reef organisms in Spermonde Archipelago, Indonesia. *Frontiers in Marine Science*. 4, 1-14. https://doi.org/10.3389/fmars.2017.00294

Tobias, P., Siavash, V.D., 2018. NETWORK. Available at: http://www.fluxus-engineering.com. [Date accessed: 28 December 2020]

Voris, H.K., 2000. Maps of *Pleistocene* sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography*. 27, 1153-1167. https://doi.org/10.1046/j.1365-2699.2000.00489.x

Wang, L., Meng, Z., Liu, X., Zhang, Y., Lin, H., 2011. Genetic diversity and differentiation of the orange-spotted grouper (*Epinephelus coioides*) between and within cultured stocks and wild populations inferred from microsatellite DNA analysis. *International Journal of Molecular Sciences*. 12, 4378. https://doi.org/10.3390/ijms12074378

Ward, R.D., 2000. Genetics in fisheries management. *Hydrobiologia*. 420, 191-201. https://doi.org/10.1023/A:1003928327503

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N., 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*. 360, 1847-1857. https://doi.org/10.1098/rstb.2005.1716