

## Redefining Dispersal Boundaries of *Siganus fuscescens* In The Coral Triangle Area

Ni Putu Dian Pertiwi<sup>1\*</sup>, Nur Ismu Hidayat<sup>2</sup>, Chloe Henderson<sup>3</sup>, I Nyoman Giri Putra<sup>4</sup>, and  
Andrianus Sembiring<sup>1</sup>

<sup>1</sup>Yayasan Biodiversitas Indonesia,  
Jalan Tukad Balian No.121 Denpasar, Bali, 80226, Indonesia

<sup>2</sup>Conservation International  
Sorong, West Papua 98414, Indonesia

<sup>3</sup>Department of Ecology and Evolutionary Biology, University of California Los Angeles,  
Los Angeles, CA 90095, USA

<sup>4</sup>Faculty of Marine Science, Udayana University  
Jl. Raya Kampus Unud, Jimbaran, Bali 80361 Indonesia  
Email: putudianpertiwi@gmail.com

### Abstract

The increasing demand of fish in the Coral Triangle Area has led to overexploitation of some species of fishes. One of the commercial fishes, which is also known to be the source of food and income for local communities, is the Mottled Spinefoot (*Siganus fuscescens*). Population studies on this species are important in order to manage sustainable stock populations. Genetic variation of the mitochondrial DNA was analyzed to examine the population structure of *Siganus fuscescens* in Indonesia, as part of the Coral Triangle Area. In total, 789 basepairs of control region mtDNA sequences were determined from 133 specimens collected from six localities, including Seribu Islands (n=27), Karimunjawa (n=19), Komodo (n=39), Selayar (n=20), Lembah (n=19) and Luwuk (n=9). From the data, 27 variable sites and 24 haplotypes were detected, with most of the haplotypes unique to each location. Haplotype data show that one haplotype was shared among all populations, three haplotypes were shared between two populations (Komodo & Selayar; Lembah & Seribu; Komodo & Karimunjawa), and 20 were unique to a single population. Haplotype diversity ( $h=0.444$ ) and nucleotide diversity ( $\pi=0.00165$ ) were low. The diversity result, i.e. the  $\Phi_{ST}$  value (0.0658,  $P < 0.0001$ ) revealed genetic structure in *S. fuscescens* populations in Indonesia. A non-dispersal strategy led to restricted gene flow and genetic structuring in *S. fuscescens*. However, both the neutrality test and the mismatch distribution indicated that *S. fuscescens* might have been in populations at demographic equilibrium, with restriction to the population expansion. Although indicating unexpected minor population structure pattern, the overall result still suggest the management of this species population as a single unit across Indonesia.

**Keywords:** Indonesia, genetic, *Siganus* sp.

### Introduction

Indonesia is located at the center of a Coral Triangle, an area with the highest marine biodiversity. Recognized as a biodiversity hotspot, it is home to a remarkable diversity of marine species (Carpenter *et al.*, 2011; Hoeksema, 2007; Veron *et al.*, 2009). Widely known as an archipelagic country, Indonesia has extensive coastal areas that hold high economical value supporting its community. Another wealths of Indonesia's resources is its abundance and diversity of marine species (Hughes *et al.*, 2003; Hoeksema, 2004).

Indonesia is also distinguished by the threats that plague its marine biodiversity (Carpenter *et al.*,

2011). Nowadays, the escalating human population is increasing the demand for fish, which is causing overfishing of some species of marine fishes (Sala & Knowlton, 2006). Directorate General of Capture Fisheries recorded that the volume of fisheries production in Indonesia reached five million tonnes in 2010, while the lowest trend reached three million tonnes in 2000 (KKP, 2011). In order to maintain the supply of fish stock so it can comply with the market demand, marine aquaculture was established in several areas throughout Indonesia. Indonesia is also on the list of the top ten countries with the highest marine aquaculture production in 2010 with 2,304,828 tonnes of fish (FAO, 2012). *Siganus* is one of the economically important species because of its value as food commodity and

aquarium fish (Lam, 1974; Ayson *et al.*, 2002; Kuriwa *et al.*, 2007). Several *Siganus* species have been reported as being overfished in the Philippines, including *Siganus canaliculatus*, *Siganus spinus*, *Siganus argenteus* (Soliman *et al.*, 2009) and *Siganus fuscescens* (Ravago-Gotanco & Juinio-Mendez, 2010). However, information based on a genetic study is still lacking on *Siganus* sp. population stock in Indonesia. This research aims to study the population structure of *Siganus fuscescens* in order to manage sustainable stock populations in Indonesia.

## MATERIALS AND METHODS

### Sample collection and Laboratory process

During this research, specimen of *Siganus fuscescens* species were collected from fish markets from six locations, including Seribu Islands (Jakarta) (n=27), Karimunjawa (Central Java) (n=19), Komodo (East Nusa Tenggara) (n=39), Selayar (South Sulawesi) (n=20), Luwuk (Central Sulawesi) (n=9) and Lembeh (North Sulawesi) (n=19) (Figure1).

Samples were collected from muscle tissue or pectoral fin clip and preserved in 95% ethanol. Each sample were morphologically identified as described by Woodland (1990). DNA was extracted using 10%

chelex solution (Walsh *et al.*, 1991). A fragment of control region mitochondrial DNA (mtDNA) was amplified via Polymerase Chain Reaction (PCR) using *S. fuscescens*-specific primers 14F 5'-CTCCCAAAGCTAGGATTCT-3' and 14R 5'-CTTAACATCTTCAGTGTTATGC-3' (Oh *et al.*, 2007). The PCR reaction was carried out in 25 µL volumes, using 1 µL of template. Each reaction included 2,5 µL 10x PCR buffer (Applied Biosystems), 2.5 µL 10 mM dNTPs, 1.25 µL of each primer at 10 mM, 2 µL 25 mM MgCl<sub>2</sub> solution, 0.125 µL AmplyTaq Red™ (Applied Biosystems), 1 µL 1x BSA and 13.5 µL ddH<sub>2</sub>O. The thermocycling profile included an initial denaturation of 94 °C for 15s, 38 cycles of 94 °C for 30s, 50 °C for 30s, and 72 °C for 45s, with a final extension of 72 °C for 5 min. PCR reactions were checked on 1% agarose gels stained with ethidium bromide and then sequenced, applying the PCR primers as sequencing primers.

### Population genetics analysis

Sequences were edited and aligned using the CLUSTALW algorithm in MEGA5.05 (Tamura *et al.*, 2011). Phylogenetic analysis of *S. fuscescens*, *S. canaliculatus*, and *S. argenteus* (accession numbers: GU929566-GU929669, GU929670-GU929673, and GU929674-GU929676, respectively) sequences was performed to further confirm that no sampling errors were made.

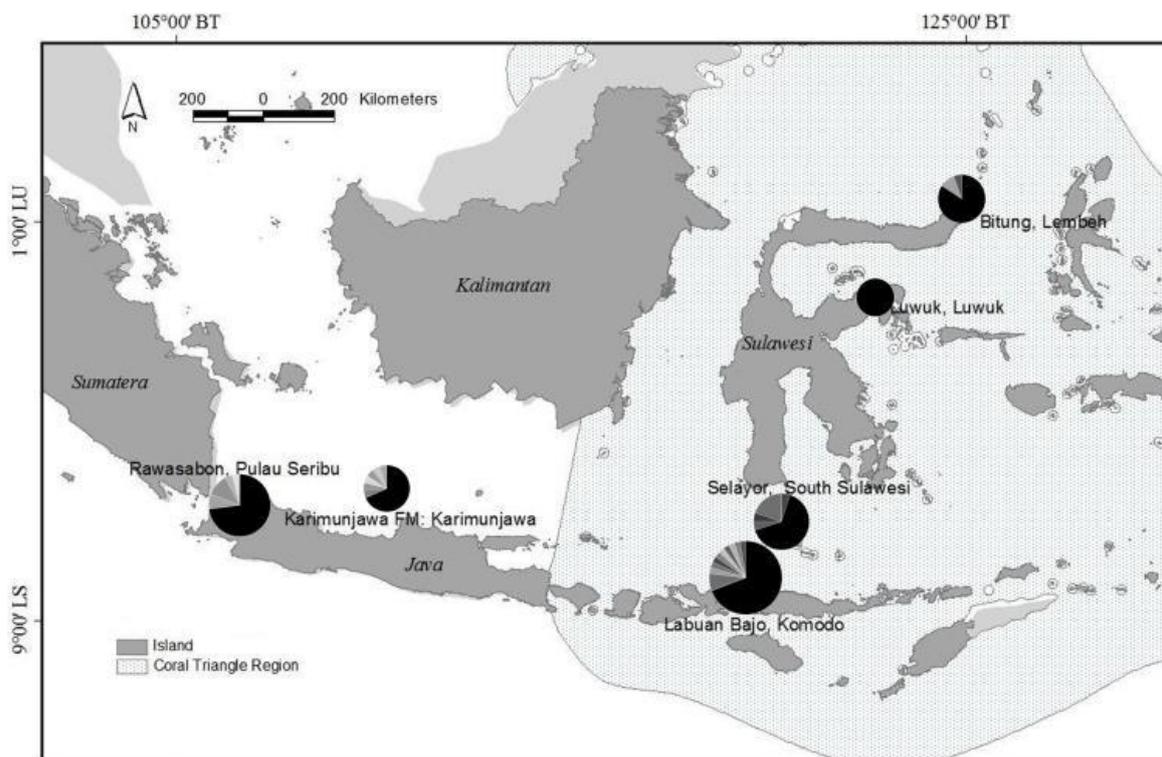


Figure 1. Area of sampling locations

Mitochondrial control region data of *S. fuscescens* were generated from Genbank (GU929566-GU929669) and used to confirm clades with the previous *S. fuscescens* populations study (Ravago-Gotanco & Juinio-Mendez, 2010). Neighbor-joining (NJ) analysis using MEGA5.05 was initially performed to confirm clades, followed by Bayesian analysis using BEAST v1.7.5 (Drummond *et al.*, 2013). Bootstrapping was performed to test the robustness of the resulting tree in NJ tree (Felsenstein, 1985), while Bayesian Information Criterion (BIC) using JmodelTest 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) was used to determine model parameters. Bayesian analysis was run for  $10^7$  generations. The posterior probability estimates from 10,000 tree with a burn-in of 1,000 trees.

Population analyses were conducted only using samples collected from Indonesia. Genetic diversity including the number of haplotypes, haplotype diversity ( $h$ , Nei, 1987), and nucleotide diversity ( $\rho$ , Nei & Li, 1979) were calculated using DnaSP 5.1 (Librado & Rozas, 2009) and Arlequin ver.3.5 (Excoffier & Lischer, 2010). The relationships between haplotypes were calculated using HaploView (Barrett *et al.*, 2005), while the spatial distribution were visualized in the map as pie chart.

Population structure indicated by  $\Phi$ -statistics and pairwise  $\Phi_{ST}$  were calculated by Arlequin, and corrected using false discovery rate (FDR) control (Benjamini & Hochberg, 1995). Regional population genetic structure was examined using analysis of molecular variance (AMOVA) (Weir & Cockerham, 1984; Excoffier *et al.*, 1992; Weir 1996), while population size equilibrium were test using Tajima's D (Tajima, 1989) and mismatch distribution (Li, 1977; Harpending, 1994). The mismatch distribution were test using sum of squares deviation (SSD) and Harpending's raggedness index (Schneider & Excoffier, 1999).

## Results and Discussion

A total of 133 individuals identified as *Siganus fuscescens* were collected and analyses using mitochondrial control region DNA, yielding 24 haplotypes (Genebank accession numbers KU365295-KU365318). The length of each sequence were 812 base pairs (bp) consists of 786 homologous sites, two insertions/deletions (indels), and 27 variable sites.

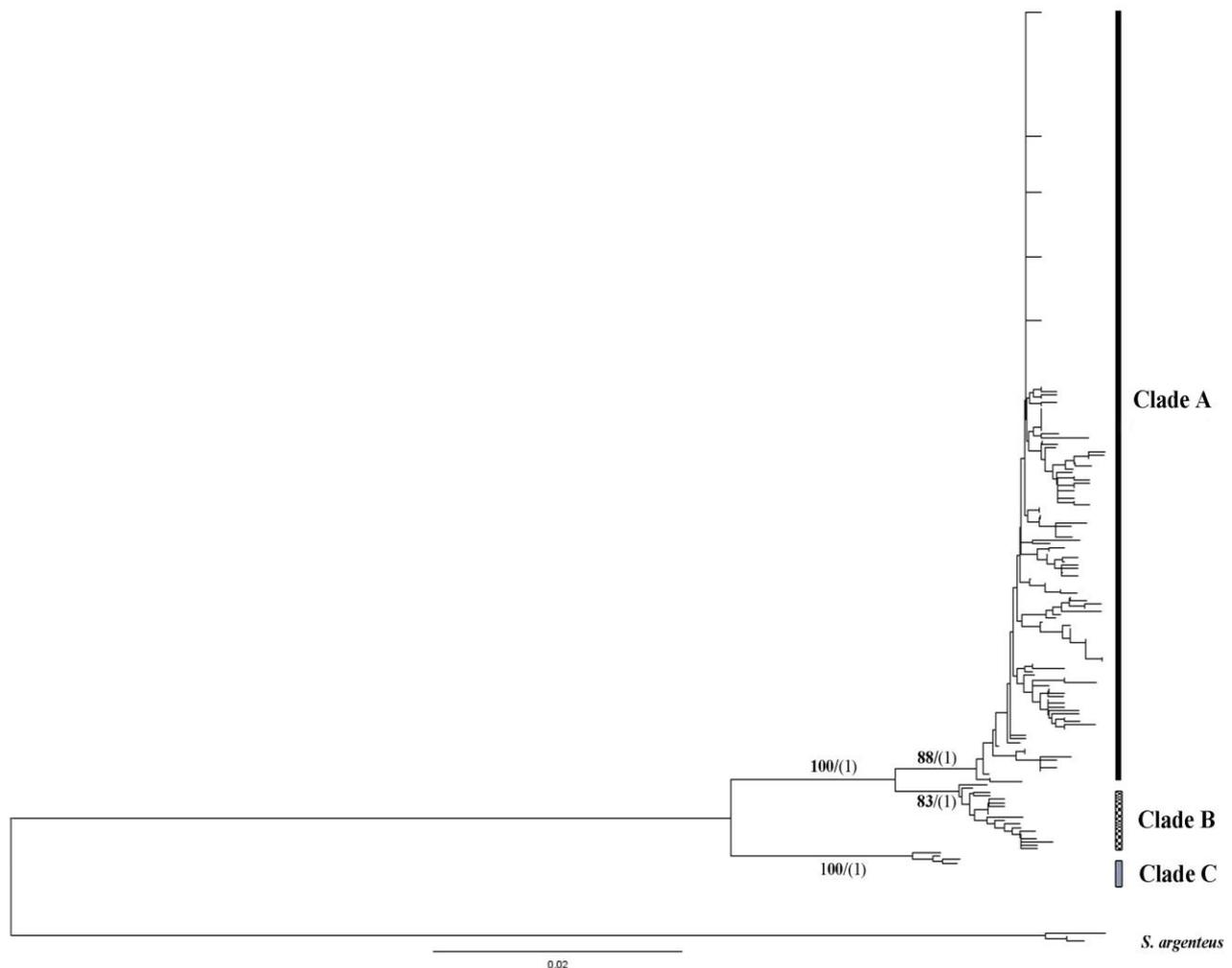
Several reports based on nuclear sequences indicated hybridization between *S. fuscescens* and *S. canaliculatus*, two species of *Siganus* species that has similar morphology (Lam, 1974; Kuriwa *et al.*,

2007). To avoid miss-identification of these two species, GenBank sequences were used as a comparison. Genetic identification using GenBank data confirms that samples on this study were on the same clade as clade A (Figure 2) in comparison with the results of the previous research conducted by Ravago-Gotanco & Juinio-Mendez (2010).

Clade A was reported to be widely distributed across the Coral Triangle, while clade B is restricted to the northwest Pacific, and clade C within the southwest Pacific (Borsa *et al.*, 2007). The phylogenetic tree confirms that all of the samples collected from the Indonesia regions belong to *S. fuscescens* clade A.

The *S. fuscescens* mtDNA control region data set consists of 24 haplotypes that are shared or unique to each of the locations, including a single haplotype that is shared among all populations, three haplotypes that are shared between two populations (Komodo & Selayar; Lembeh & Seribu; Komodo & Karimunjawa), and 20 haplotypes that are unique to a single population (Figure 3). Haplotype distribution within each of geographical sites is indicated in Figure 3a. The minimum-spanning tree concordant with the phylogenetic tree indicated that all haplotypes are nested into one clade with one dominant haplotype, followed by unique haplotypes or shared haplotypes between two populations. The circles size within Figure 3b is proportionally to the frequency of occurrence of each of the haplotypes, with each haplotypes are separated by one mutational step.

Geographical distance limits genetic differences between populations, acting as oceanic barriers that separate habitats. Numerous publications have reported that genetic structure is found in some species due to large geographical distances, including a blennioid fish, *Axoclinus nigricaudus* (Riginos & Nachman, 2001), temperate seagrass, *Zostera marina* (Hammerli & Reusch, 2003) and a giant clam, *Tridacna crocea* (DeBoer *et al.*, 2008). However, it appears that geographical distance does not limit the genetic structure in *S. fuscescens* populations within this study, which is indicated by the high level of gene flow in *S. fuscescens* populations in the central part of Indonesia (Figure 3). High indication of gene flow is shown by a large percentage of shared haplotypes between populations. A single panmictic population is probably common in this area, which was documented by Lourie *et al.* (2009) using three different species of seahorse (*Hippocampus barbouri*, *H. kuda*, and *H. spinosissimus*), coral reef fish, *Caesio cunningg* (Ackiss *et al.*, 2013), blue starfish, *Linckia laevigata* and gastropod



**Figure 2.** Phylogenetic tree of 24 *Siganus fuscescens* mtDNA control region haplotypes from Indonesia in comparison with GenBank sequences and *Siganus argenteus* sequences as outgroup. Node value indicated in bold (bootstrap replicates) and (parentheses) Bayesian posterior probability.

ectoparasite, *Thyca crystalline* (Kochzius et al., 2009), the seastar, *Protoreaster nodosus* (Crandall et al., 2008) and the boring giant clam, *Tridacna crocea* (DeBoer et al., 2008).

Despite the finding of highly shared haplotypes, the result also shows unique haplotypes in each locality (Figure 3a), which may be caused by the isolation of these haplotypes within a single population due to limited dispersal ability and the effect of ocean currents. The adults of *S. fuscescens* probably travel over a short distance as indicated by a short home range and territory that is usually less than three kilometers (Green et al., 2015). Despite the limited dispersal potential of individual adults, long distance dispersal potentially occurred at the larval stages (Shanks, 2009). Although pelagic larval duration (PLD) of this species is not known, the

sibling species, *S. Canaliculatus*, is exposed to three weeks planktonic larval periods (Hasse & Madraisau, 1977). The long pelagic larval period potentially facilitated a long dispersal distance of a species (Shanks et al., 2003). Dispersal can also be affected by ocean currents (Nakajima et al., 2014; Raynal et al., 2014; Lo et al., 2014). The immense passage of water conveyed by the Indonesian Throughflow (ITF), which moves water from the Pacific to Indian Oceans, can facilitate connectivity and enhance lineage mixing between sites, reflected by no obvious differentiation in central Indonesia (Lind et al., 2012; Timm et al., 2012).

It is also notable that a higher gene flow among geographical population was associated with major ocean currents (Imron et al., 2007). Connectivity shown by the population of *S.*

*fuscescens* in Sulawesi (Luwuk, Lembeh, Selayar) and Komodo seemingly follows the ITF pattern. Similar patterns were also found in other fish species such as coral reef fish, *Caesio cunning* (Ackiss *et al.*, 2013), the false clown anemonefish, *Amphiprion ocellaris* (Timm *et al.*, 2012), and the humbug damselfish *Dascyllus aruanus* (Raynal *et al.*, 2014). Meanwhile, connectivity between populations in Java (Seribu and Karimunjawa) and populations in the east (Sulawesi and Komodo) are probably maintained by seasonality reversing current (SRC). Although previous research could not find any relations between a genetic pattern and seasonal current in Sunda shelf (Nelson *et al.*, 2000), this result indicated that the SRC may play an important role in the dispersal of *S. fuscescens*.

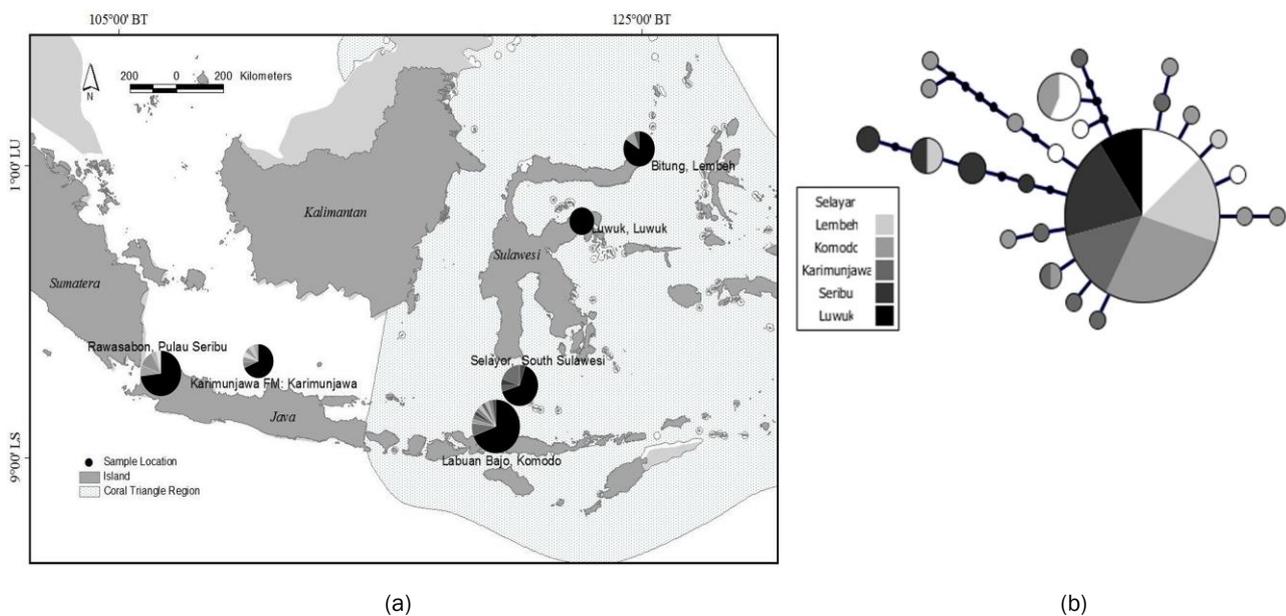
In addition, haplotype diversity (*h*) and nucleotide diversity ( $\pi$ ) for all populations were indicated as low diversity with the value of *h* = 0.4659 and  $\pi$  = 0.00165, respectively. Low levels of nucleotide diversity might be a signal of recent population expansion (Rohfritsch & Borsa, 2005). The number of haplotypes, haplotype diversity, and an estimate of nucleotide diversity for each site are presented in Table 1.

Meanwhile, the  $\Phi$ -statistic value of AMOVA analyses (0.0658,  $p < 0.0001$ ) revealed the genetic structure in *S. fuscescens* population in Indonesia, which is signaled by significant genetic differences in populations on Seribu Islands compared to other populations, including Komodo and Selayar. Significant pairwise  $\Phi_{ST}$  values were indicated in the Table 2. This result also shows the significant

genetic differentiation of two out of 15 population comparisons.

The genetic difference result was not concordant with the hypothesis of the Sunda Shelf Barrier, which is believed to be a geographical genetic break that separates the western and eastern parts of Indonesia. Other significant genetic pairwise differences between distinguishable populations also indicate the same result. Meanwhile, several unique haplotypes revealed in this study may be caused by local isolation due to the effect of sea level rise during Pleistocene glacial events (Carpenter *et al.*, 2011). This phenomenon led to localized extinction and recolonization of *Siganus sp.* populations associated with algal and seagrass meadows in shallow and sheltered reef flats (Woodland, 1990).

The historical demography was analyzed using Tajima's D test and mismatch distribution. The Tajima's D value was significantly negative ( $p < 0.05$ ) for three out of six populations, suggesting an excess of low-frequency haplotypes relative to that expected under mutation-drift equilibrium. Most likely, this indicates a model of rapid population expansion in which several rare alleles have recently derived from one or a few alleles of high frequency. However, the test shows an insignificant negative result for the mean value of all regional populations ( $p > 0.05$ ) (Table 3), indicating the population did not significantly depart from the neutral evolution model. The mismatch distribution for *S. Fuscescens* appeared to be multimodal. This was supported by



**Figure 3.** Map of study area (a) Distribution of *Siganus fuscescens* mtDNA control region haplotypes. (b) Minimum-spanning tree of the specimen's haplotype. Each shades indicated six different regions: Selayar, Lembeh, Komodo, Karimunjawa, Seribu Island, and Luwuk.

**Table 1.** Diversity measures for populations of *Siganus fuscescens* based on 812 base pairs of control region mitochondrial DNA sequence. The number of individuals sampled per site is indicated by *a*, number of haplotypes *n*, haplotype diversity *h* and nucleotide diversity  $\pi$ .

Sampling site	<i>a</i>	<i>n</i>	<i>h</i> ( $\pm$ sd)	$\pi$ ( $\pm$ sd)
Seribu	27	5	0.4986 ( $\pm$ 0.1103)	0.081882 ( $\pm$ 0.051957)
Karimunjawa	19	7	0.5439 ( $\pm$ 0.1364)	0.04635 ( $\pm$ 0.034212)
Komodo	39	11	0.5223 ( $\pm$ 0.0972)	0.066477 ( $\pm$ 0.043584)
Selayar	20	5	0.5579 ( $\pm$ 0.1144)	0.038402 ( $\pm$ 0.029813)
Luwuk	9	1	0 ( $\pm$ 0)	0 ( $\pm$ 0)
Lembeh	19	3	0.2924 ( $\pm$ 0.1274)	0.042285 ( $\pm$ 0.032362)

**Table 2.** Matrix of pairwise  $\Phi_{ST}$  (below diagonal) and *p*-value (above diagonal) among *S. fuscescens* populations based on mitochondrial control region sequence data

	Seribu	Karimunjawa	Komodo	Selayar	Luwuk	Lembeh
Seribu		<b>0.01074</b>	<b>0.00098*</b>	<b>0.00488*</b>	0.13379	0.19238
Karimunjawa	<b>0.13533</b>		0.66211	0.57422	0.93945	0.24609
Komodo	<b>0.12615*</b>	-0.01282		0.32715	0.58105	0.08105
Selayar	<b>0.15657*</b>	-0.00853	0.00274		0.54883	<b>0.02832</b>
Luwuk	0.11408	-0.03854	-0.01861	-0.00175		0.53906
Lembeh	0.03158	0.02899	0.03047	<b>0.05398</b>	-0.00547	

Numbers in bold indicate significant values ( $P < 0.05$ ) prior to multiple test corrections while bold numbers with an asterisk mark (\*) indicate significant values after applying false discovery rate corrections (Benjamini & Hochberg, 1995).

**Table 3.** Tajima's D neutrality test based on 812 base pairs (bp) of the mitochondrial control region sequence

	Seribu	Karimunjawa	Komodo	Selayar	Luwuk	Lembeh	All Populations
Sample Size	27	19	39	20	9	19	22.16667
S	6	9	14	6	0	6	6.83333
Pi	2.21083	1.25146	1.79487	1.03684	0	1.09942	1.23224
Tajima'S D	0.96345	<b>-2.20521</b>	<b>-1.7672</b>	<b>-1.60994</b>	0	-1.16396	-0.96381
Tajima'S D p-value	0.864	<b>0.001*</b>	<b>0.016*</b>	<b>0.039*</b>	1	0.130	0.34533

\* Significant Tajima's D p-value ( $P < 0.05$ ) is indicated in bold.

the non-significant low values for SSD (0.06778,  $p$ -value=0.24167) and Harpending's raggedness index (0.17631,  $p$ -value=0.58333) under the sudden expansion model. This result indicated that the *S. fuscescens* population in this study is stable. The minimum-spanning tree shape also supported that the population of *S. fuscescens* is at demographic equilibrium.

This suggests that the population range expansion may have been restricted to a non-dispersal strategy that may have restrained *S. fuscescens* from demographic expansion. Based on these findings, the population of *S. fuscescens* in

Indonesia should be managed as a single natural management unit throughout Indonesia

### Conclusion

There is a minor structure in *Siganus fuscescens* populations in Indonesia. However, this population structure is not limited by the geographical distance shown by a high indication of gene flow and haplotypes shared among populations. The population also shows low haplotype and nucleotide diversity and a stable demographic population. The population structure of

*Siganus fuscescens* in this study is not concordant with the Sunda Shelf Barrier hypothesis, which acts as a geographical genetic break that separates the western and eastern parts of Indonesia.

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