Comparative genetic stock structure in three species of commercially exploited Indo-Malay Carangidae (Teleosteii, Perciformes)

T.N.A. Mat Jaafar[1,*] | M. I. Taylor[1,#] | S. A. Mohd Nor[2] | M. de Bruyn[1,+] | G. R. Carvalho[1]

[1]Molecular Ecology and Fisheries Genetics Laboratory, Environment Centre Wales, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK

[2]School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

Correspondence

Gary R.Carvalho, Molecular Ecology and Fisheries Genetics Laboratory, Environment Centre Wales, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK

Email: g.r.carvalho@bangor.ac.uk

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[*]Current address: School of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

[#]Current address: School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK,

[+]Current address: The University of Sydney, School of Life and Environment Sciences, Sydney, NSW 2006, Australia

Abstract

We examine genetic structuring in three commercially important species of the teleost family Carangidae from Malaysian waters: yellowtail scad *Atule mate*, bigeye scad *Selar crumenophthalmus* and yellowstripe scad *Selaroides leptolepis*, from the Indo-Malay Archipelago. In view of their distribution across contrasting habitats, we tested the hypothesis that pelagic species display less genetic divergence compared with demersal species, due to their potential to undertake long-distance migrations in oceanic waters. To evaluate population genetic structure, we sequenced two mitochondrial (mt)DNA [650bp of cytochrome oxidase I (*coI*), 450bp of control region (CR)] and one nuclear gene (910bp of *rag1*) in each species. One hundred and eighty samples from four geographical regions within the Indo-Malay Archipelago including a population of yellowtail from Kuwait were examined. Findings revealed that the extent of genetic structuring among populations in the semi-pelagic and pelagic, yellowtail and bigeye were lower than demersal yellowstripe, consistent with the hypothesis that pelagic species

display less genetic divergence compared with demersal species. The yellowtail phylogeny identified three distinct clades with bootstrap values of 86–99% in mtDNA and 63–67% in *rag1*. However, in bigeye, three clades were also observed from mtDNA data while only one clade was identified in *rag1* dataset. In yellowstripe, the mtDNA tree was split into three closely related clades and two clades in *rag1* tree with bootstraps value of 73–99% and 56% respectively. However, no geographic structure appears in both mtDNA and *rag1* datasets. Hierarchical molecular variance analysis (AMOVA), pair wise F_{ST} comparisons and the nearest-neighbour statistic (S_{nn}) showed significant genetic differences among Kuwait and Indo-Malay yellowtail. Within the Indo-Malay Archipelago itself, two distinct mitochondrial lineages were detected in yellowtail suggesting potential cryptic species. Findings suggests varying degrees of genetic structuring, key information relevant to management of exploited stocks, though more rapidly evolving genetic markers should be used in future to better delimit the nature and dynamics of putative stock boundaries.

KEYWORDS

Carangidae, *coI*, control region, Indo-Malay, population structure, *rag*1

1 | INTRODUCTION

The Indo-Malay Archipelago (IMA) is one of the most globally important commercial fishery regions and, therefore, plays a critical role in providing food resources. According to the Department of Statistics Malaysia (2017), international comparisons in 2016 for production of fish capture showed that Malaysia is ranked 17th in the world and 11th in Asia, with total marine fish landings of 1.5 Mt. There are 100 fishing districts around Peninsular Malaysia and Malaysian Borneo, making the fisheries sector an important economic contributor to local economies (Annual Fisheries Statistics, 2017). Various marine ecosystems can be found in the IMA, which underpin a productive and successful fishing industry due to complex geological features enhancing habitat diversity (Burke *et al*., 2002; Wong, 2004). The South China Sea, Strait of Malacca, Sulu Sea and Celebes Sea also offer opportunities for exploitation of fish. This mega-diverse tropical region (Lohman *et al*., 2011) harbours many species of commercially high-value marine fish for exploitation. Owing to its high levels of biodiversity and accompanying anthropogenic pressures, however, the region has experienced unsustainable exploitation of fisheries and significant habitat destruction (Chong *et al*., 2010). Almost half (48%) of the total of freshwater and marine fishes in Malaysia are currently threatened to some degree, while nearly one third (27%), mostly from marine and coral habitats, require urgent scientific data to evaluate their status (Chong *et al*., 2010).

Limited data on the genetic basis of stock structure for any pelagic fish adds to the complexity in managing regional marine resources. Indeed, it is now well established that an

understanding of the spatial and temporal dynamics of fish-stock structure is supportive of effective stock-management practices (Carvalho & Hauser, 1994; Begg *et al*., 1999; Hauser & Carvalho, 2008). Determining stock or population structure of any fish species is a challenging task as many fish populations vary in distribution and abundance, sometimes across small spatial and temporal scales. Additionally, many fisheries comprise poorly defined mixtures of multiple stocks. Fisheries stocks that differ to varying degrees in their biological and genetic integrity need to be monitored, assessed and managed separately. Failure to recognise stock structure may lead to erosion of some spawning components, over-exploitation and depletion of less productive stocks (Carvalho & Hauser, 1994).

Genetic approaches to fish-stock assessment have been widely used in determining stock structure due to its cost-effectiveness and robustness of results obtained (Hauser & Carvalho, 2008; Dudgeon *et al*., 2012; Ovenden *et al*., 2015). Genetic approaches provide information on levels of genetic diversity in fish populations, degree of genetic differentiation among fish populations and hence population genetic structure. Additional inferences can thereby be made on levels of gene flow among fish populations, such as the effective number of gene migrants that are exchanged among populations. Where significant genetic divergence exists, the information typically indicates demographically independent entities: valuable information in the quest to predict localised response to harvesting. Perrin and Borsa (2001), for example, examined mitochondrial (mt)DNA diversity of the Indian scad mackerel *Decapterus russelli* (Rüppell 1830) (Carangidae) in the IMA. Cytochrome *b* gene (*cytb*) sequence data revealed two major

genetic lineage patterns likely related to historical isolation of the Sulawesi Sea region from other proximate areas in the IMA during Pleistocene climatic cycling. However, Borsa (2003) conducted a study on the genetic structure of round scad mackerel *Decapterus macrosoma* Bleeker 1851 (Carangidae) in the IMA using mitochondrial and nuclear DNA markers and detected no significant heterogeneity in *cytb* haplotype frequencies or in aldolase B-1 allele frequencies, indicating the existence of a single stock in the region. Thus, there is discordance in the evidence available on the extent of structuring across species and regions.

In the present study, the population structure of three commercially-important species of Indo-Malay Carangidae was analysed from mtDNA [cytochrome *c* oxidase I (*coI*) and control region] and nuclear DNA [recombination activating gene (*rag1*)]. Being commercially and ecologically important, Carangidae are one of the main capture targets for fisheries in the IMA. Carangidae comprises fishes whose body shapes vary from elongate and fusiform to deeply ovate and strongly compressed. Their habitats range from pelagic to demersal; many are semi-pelagic (Laroche *et al*., 1984). Past studies of Carangidae have focused on their ecology and fishery biology (Blaber & Cyrus, 1983; Dalzell & Penaflor, 1989; Ditty *et al*., 2004; Honebrink, 2000; Roos *et al*., 2007; Smith & Parrish, 2002; von Westernhagen, 1973; Wetherbee *et al*., 2004). Although Pedrosa-Gerasmio *et al*. (2015) studied population genetic structure of bigeye scad *Selar crumenophthalmus* (Bloch 1793), but only focusing in Sulu-Celebes Sea. Little information concerning the assessment of population genetic structure and genetic diversity in high commercial-value species such as yellowtail scad *Atule mate* (Cuvier 1833), bigeye and

yellowstripe scad *Selaroides leptolepis* (Cuvier 1833) in other area of IMA. It is unknown whether these three species in Malaysian waters form single respective stocks, or are genetically subdivided into distinct populations. Such core information can contribute to an effective conservation and management, since fishery yields continue to decline (Abu-Talib *et al*., 2000; Department of Statistics Malaysia, 2017).

2 | MATERIALS AND METHODS

Permission to carry out field work in the country, collection and export of tissue samples was granted by local and national governments in Malaysia and State Planning Unit Sarawak.

2.1 | Sampling and DNA extraction

Three species of Carangidae (yellowtail, bigeye and yellowstripe) that are highly pelagic, moderately pelagic and demersal, respectively, with contrasting habitat distribution, were examined in this study. Multiple samples were collected from four geographic regions within the IMA: South China Sea, Strait of Malacca, Sulu Sea and Celebes Sea (Figure 1). Population samples were collected from several fish-landing sites during two collecting trips; from October to November 2009 and from June to July 2010 (Supporting Information Table S1). All fishes were identified based on morphology, with support from expert local taxonomists in most cases.

Where expert advice was not available, guidance was obtained from FAO-Fisheries Identification Sheets (Fisher & Whitehead, 1974) and identification books published by the Department of Fisheries Malaysia (Annie & Albert, 2009; Mansor *et al*., 1998). Fin clips were taken from the right pectoral fin of each fish and preserved in 99% ethanol. Fish specimens were then placed in ice, frozen on site and transported to South China Sea Museum, University Malaysia Terengganu. Total genomic DNA was extracted from fin clips of 180 specimens using the salting-out method (Miller *et al*., 1988). Isolated DNA was re-suspended in 100 µl deionized water. A fragment of 650 bp of *coI*, 450 bp of the control region and 950 bp of *rag1* were amplified using the list of primers in Supporting Information Table S2.

2.2 | PCR amplification and sequencing

Polymerase reactions were prepared in 11 µl reaction volumes including 1 µl DNA, 6.6 µl ultra pure water, 1.0 µl 10X PCR buffer, 0.2 µl $MgCl_2$ (25 mM), 0.5 µl of each primer (10 µM), 1.0 µL deoxynucleotide triphosphate (dNTP) (2 mM), 0.2 µl Taq polymer ase. The PCR thermal regime for *coI* consisted of an initial step of 5 min at 94^{O}C followed by 35 cycles of 1 min at 94^{O}C, 1 min 30 s at 58.2^{O}C and 1 min at 72^{O}C, followed by 10 min at 72^{O}C. For the control region, the amplification started with an initial step of 2 min at 95^{O}C followed by 35 cycles of 30 s at 94^{O}C, 30 s at 48.3^{O}C and 1 min at 72^{O}C, followed by 10 min at 72^{O}C. The amplification programme for *rag1* was carried out initially at 95^{O}C for 3 min followed by 35 cycles with: 94^{O}C

for 30 s, 52^{O}C for 45 s, 72^{O}C for 1 min 30 s and finally 10min of final extension at 72^{O}C. DNA amplification products were separated in 1.2% (w/v) agarose gels at 100v with 1X Tris-borate-EDTA (TBE) buffer, stained with ethidium bromide and visualised under UV illumination. The PCR product was purified with ExoSAP (exonuclease I and shrimp alkaline phosphatase) to degrade excess primers (Werle *et al*., 1994). The purification thermal conditions consisted of 37^{O}C for 1 *h* and 80^{O}C for 15 min. Bidirectional sequencing was performed by Macrogen Inc., (www.macrogen.com). Once sequencing was completed, nucleotide sequences were checked by eye to ensure sequence information was consistent in both directions. In addition, all sequences were verified by comparison against known specimens from GenBank.

2.3 | Data analysis

Initial editing of ambiguous bases was undertaken with MEGA5 software (Kumar *et al*., 2004). The edited sequences of each locus were aligned using Clustal W implemented in the same software. The alignments obtained were further checked by eye. Amino-acid sequence translation (vertebrate mitochondrial code) was applied and checked for stop codons, to ensure the amplification of mtDNA rather than nuclear copies of *coI* sequences and then translated back for subsequent analysis. Prior to analysis of the data, the program PHASE (Stephens *et al*., 2001) was used to resolve the heterozygous sites in *rag1* sequences to reconstruct haplotypes. DnaSP5.0 (Rozas *et al*., 2003) was used to calculate sequence diversity statistics as well as

determination of identical haplotypes. Arlequin 3.5.1.2 (Excoffier & Lischer, 2010) was used to perform an analysis of molecular variance (AMOVA) to examine population structure of each species. For the regional comparison, populations were divided into four regions in the IMA, South China Sea (SCS), Strait of Malacca (SM), Sulu Sea (SS) and Celebes Sea (CS) and additional Kuwait (KWT) for yellowtail analysis. Pairwise genetic differentiation between populations was evaluated by pairwise Φ_{ST} and the significance of Φ_{ST} was tested by 10,000 permutations for each pairwise comparison in Arlequin. When multiple comparisons were performed, *P*-values were adjusted using the Bonferroni correction. The nearest-neighbour statistic (S_{nn}; Hudson, 2000) was estimated using DnaSP5.0 software, a statistic that measures population differentiation by testing whether low divergent sequences are from the same location. It is particularly useful when populations show high levels of haplotype diversity (Hudson, 2000). A maximum likelihood approach of the mitochondrial and nuclear loci were conducted by determining the highest likelihood tree bootstrapped 1000 times using RAxML 7.2.8 (Stamatakis *et al*., 2008).

3 | RESULTS

3.1 | Mitochondrial DNA analysis

3.1.1 Cytochrome oxidase I

A total of 68, 53 and 57 individuals were assayed from nine populations of yellowtail, bigeye and yellowstripe, respectively, for 650 bp of *coI*. Twenty-four, 23 and 13 unique haplotypes were identified with haplotypic diversity ranging from 0.4–1.0 in yellowtail, 0–0.9722 in bigeye and 0–0.8571 in yellowstripe (Supporting Information Table S3). Although some of the populations recorded high haplotype diversity values, low nucleotide diversities were observed ranging from 0.0065–0.0218 for yellowtail, 0–0.11099 for bigeye and 0–0.19298 for yellowstripe (Supporting Information Table S3). This indicates only a small genetic differences between haplotypes. The haplotype (h) and nucleotide (π) diversity were markedly higher in yellowtail (h = 0.893, π = 0.01424) compared with bigeye (h = 0.758, π = 0.00215) and yellowstripe (h = 0.458, π = 0.00279).

Global Φ_{ST} value was low in bigeye (Φ_{ST} = –0.0054, P > 0.05) compared with yellowtail (Φ_{ST} = 0.03387, P > 0.05) and yellowstripe (Φ_{ST} = 0.1355, P > 0.05; Table 1), as expected based on habitat preferences. Additional samples of yellowtail from Kuwait were also analysed for comparison with the IMA samples (Φ_{ST} = 0.09813, P < 0.01; Table 1). In yellowtail, the pairwise Φ_{ST}s between Kuwait and all IMA sites were significant (pairwise Φ_{ST} = 0.27143–0.41003, P < 0.05). There was also significant differentiation among MKS and SMP (pairwise Φ_{ST} = 0.12281, P < 0.01), as well as between KPJ and SMP (pairwise Φ_{ST} = 0.08163, P < 0.05; Table 2). The pairwise Φ_{ST} in bigeye data primarily suggested no significant differentiation among localities (Table 2). In yellowstripe, significant differentiation was detected between KPJ and six sites:

KBJ (Φ_{ST} = 0.26473, P < 0.05), MGJ (Φ_{ST} = 0.24247, P < 0.05), MR (Φ_{ST} = 0.4385, P > 0.05), SB (Φ_{ST} = 0.4717, P < 0.01), KDT (Φ_{ST} = 0.4385, P < 0.05) and SDK (Φ_{ST} = 0.32897, P < 0.01; Table 2). High pairwise Φ_{ST} values between KPJ and the rest of the IMA samples inflated the yellowstripe global Φ_{ST}. When AMOVA was repeated with the KPJ population excluded, the overall Φ_{ST} value of yellowstripe was low and not significantly different from zero (Φ_{ST} = –0.01360, P > 0.05).

3.1.2 | Control region

A total of 450bp of control region was sequenced in 65, 55 and 56 individuals of yellowtail, bigeye and yellowstripe, respectively. A total of 64, 36 and 43 haplotypes were identified from eight localities of yellowtail and nine localities of each of bigeye and yellowstripe (Supporting Information Table S3). Haplotype diversity ranged from 0.9778–1.0 in yellowtail, 0–1.0 in bigeye and 0.756–1.0 in yellowstripe. While nucleotide diversity ranging from 0.0405–0.5705 in yellowtail, 0–0.43158 in bigeye and 0.00571–0.01834 in yellowstripe. The haplotype (h) and nucleotide (π) diversity were markedly higher in yellowtail (h = 1.0, π = 0.08747) compared with bigeye (h = 0.961, π = 0.01014) and yellowstripe (h = 0.968, π = 0.01763).

Overall Φ_{ST} value was low (Φ_{ST} = 0.00448) and not significantly different from zero (P > 0.05) for Indo-Malay yellowtail compared with bigeye (Φ_{ST} = 0.17983, P < 0.001) and yellowstripe (Φ_{ST} = 0.0466, P < 0.05; Table 3). AMOVA also revealed low Φ_{ST} value (Φ_{ST} =

0.00402) when additional samples from Kuwait were included in the analysis for yellowtail (Table 3). The pairwise Φ_{ST} for yellowtail data suggested no significant differentiation among all localities, even between Kuwait and the IMA (Table 4). For bigeye, significant differentiation was detected between TBJ and all other localities (pairwise Φ_{ST} = 0.25926-0.8, P < 0.01–0.05; Table 4). There were also significant differentiation among TW and five other localities; TSJ (Φ_{ST} = 0.40379, P < 0.05), MKS (Φ_{ST} = 0.55, P < 0.05), KPJ (Φ_{ST} = 0.40379, P < 0.001), SK (Φ_{ST} = 0.5, P < 0.01) and KDT (Φ_{ST} = 0.5, P < 0.01) and between KPJ and SDK (Φ_{ST} = 0.14074, P < 0.01; Table 4). To test whether high pairwise Φ_{ST} values between TBJ and TW, with the rest of the IMA samples may inflate bigeye average Φ_{ST}, AMOVA was repeated with TBJ and TW population excluded. The overall Φ_{ST} value was low but significant (Φ_{ST} = 0.05221, P < 0.05). While for yellowstripe data, significant differentiation was detected between SDK and three other localities: KBJ (Φ_{ST} = 0.14013, P < 0.05), KPJ (Φ_{ST} = 0.13027, P < 0.05) and TW (Φ_{ST} = 0.18367, P < 0.05; Table 4).

The S_{nn} value was 1.0 and significant (P < 0.01) between Kuwait and the IMA yellowtail sequences, suggesting individuals from these two localities are highly differentiated supporting the *coI* data for yellowtail where KWT populations are also significantly different from other IMA samples. The analysis was repeated with the KWT population excluded to test whether genetic differentiation was evident at finer spatial scales in the seas surrounding Malaysia. The S_{nn} value was not significant (S_{nn}= 0.21833, P > 0.05), indicating that no detectable genetic differentiation among yellowtail populations within the IMA. The S_{nn} tests were also significant

for yellowstripe (S_{nn}= 0.31392, P < 0.01) suggesting at least two localities are differentiated. However, non-significant S_{nn} values were evident in bigeye (S_{nn}= 0.0543, P > 0.01).

The maximum likelihood (ML) phylogenetic trees generated from mtDNA *coI* haplotypes indicated three distinct clusters in yellowtail (Figure 2) and yellowstripe (Figure 3) with mean K2P distances within species for yellowtail and yellowstripe were 1.5% (maximum of 4.6%) and 0.3% (maximum of 1.2%) *coI* nucleotide divergence respectively. However, in bigeye, four clusters with a mean of 0.2% K2P distance were identified (maximum of 0.8%) *coI* nucleotide divergence (Figure 4). Control region ML phylogenetic trees also showed the same pattern (tree not shown). For yellowtail, cluster I, the major lineage including most specimens from all sampling sites in Malaysia exhibited no obvious geographic structuring and was supported with a bootstrap value of 99% in ML trees. In contrast, cluster II is a minor lineage, including all individuals from Kuwait (Arabian Gulf; haplotype 01 and 02), while the third cluster included three individuals from Tok Bali (TBJ) and six individuals from Semporna (SMP; haplotypes 15, 16, 17, 19 and 20). For yellowstripe, cluster I included almost all specimens from all sampling sites and was strongly supported with a bootstrap value of 98%, while cluster II included two individuals from Semporna (SMP) and Tawau (TW) with 99% bootstrap value. The third cluster included six individuals from Kuala Perlis (KPJ) supported by 65% bootstrap value. However, for bigeye, cluster I included most specimens from all sampling sites, cluster II consisted of for two individuals from Kuala Perlis (KPJ). Cluster III shared by 1 individual from Kuala Perlis (KPJ) and Semporna (SMP) each and cluster IV consisted of 1

individual each from Tanjung Sedili (TSJ), Kuala Perlis (KPJ) and Semporna (SMP). No geographic pattern was apparent for either bigeye or yellowstripe. The Bayesian trees also had identical topologies for each species (figures not shown).

3.1.3 | Nuclear DNA analysis

A total of 67, 54 and 45 individuals of yellowtail, bigeye and yellowstripe were assayed for 910 bp of *rag1* gene, respectively. There were four, three and two unique alleles identified in each species respectively (Supporting Information Table S3). Global Φ_{ST} values were high for yellowtail (Φ_{ST} = 0.40156) compared with bigeye (Φ_{ST} = –0.03958) and yellowstripe (Φ_{ST} = –0.07324; Table 5). Additional samples from Kuwait were also analysed for comparison with yellowtail samples from IMA (Φ_{ST} = 0.61104, P < 0.001; Table 5). The pairwise Φ_{ST} analysis revealed a lack of genetic structure among sampled areas and was non-significant for bigeye and yellowstripe (Table 6). However, for yellowtail, comparison between Kuwait and other sampled sites in IMA revealed high pairwise Φ_{ST} values (0.61648–1.0000) and P < 0.01–0.001; Table 6). There were also significant differences among SMP and four other sites (TSJ, MKS, KPJ and SDK). Similar to *coI* and control-region data, the haplotype (h) and nucleotide (π) diversity were markedly higher in yellowtail (h = 0.379, π = 0.00092) compared with bigeye (h = 0.0734, π = 0.0001) and yellowstripe (h = 0.0444, π = 0.00006). The ML phylogenetic tree for *rag1* also

suggested three distinct clusters in yellowtail strongly supported with 63–67% bootstrap values (figure not shown). The pattern observed for yellowtail clusters were consistent with the pattern observed at mtDNA data (Figure 2). However, two clusters were detected in bigeye and yellowstripe with 60–65% and 56% bootstrap values respectively (figure not shown). Mean K2P distances within species were 0.1, 0.2 and 0.1% with 0.6, 0.5 and 0.6% maximum nucleotide divergence for yellowtail, bigeye and yellowstripe, respectively.

4 | DISCUSSION

4.1 | Patterns of population genetic structure in carangid species

Population structure inferred from nuclear as well as mtDNA markers was lower in the pelagic yellowtail and bigeye than in the demersal yellowstripe, which is consistent with the hypothesis that pelagic and semi-pelagic species will display less genetic divergence due to their potential to undertake long-distance migrations in oceanic waters. However, several caveats in data interpretation from the current sampling design must be considered. Due to the high haplotype diversity and small sample sizes examined, most haplotypes in the control-region data appeared in the sample only once and thus the Φ_{ST} analysis is unlikely to reflect actual levels of population structuring for the control-region dataset (Hudson, 2000). Similar patterns of low diversity of control region haplotypes has been identified in other marine taxa (Hauser *et al*., 2001; Ely *et al*.,

2005; Wu *et al*., 2012) and can, in theory be overcome by assaying much larger sample sizes. In an attempt to generate meaningful differentiation measures from the control-region data, we utilised an alternative measure of genetic differentiation, the nearest-neighbour statistic (S_{nn}; Hudson *et al*., 1992). This statistic measures population differentiation by testing whether low divergent sequences are from the same location and it is particularly useful when populations show high haplotype diversity (Hudson, 2000). The S_{nn}-values were high and significant between Kuwait and the IMA yellowtail sequences, suggesting individuals from these two localities are differentiated. Such findings support the *coI* data for yellowtail where Kuwait populations were also significantly differentiated from the rest of the IMA samples. However, when the analysis was repeated with the Kuwait population excluded, to test whether genetic differentiation was evident at finer spatial scales around Malaysia, the S_{nn} value was low and non-significant. Thus, there was no evidence for significant genetic differentiation among yellowtail populations within the IMA. The latter result contradicts the *coI* data, which showed trends of significant differentiation among IMA samples. A combination of slightly higher mutation rates in control region provides more opportunity for drift to vary allele frequencies, combined with insufficient sample sizes may account for slight pairwise genetic differences using the control-region marker for yellowtail specimens. Overall, ML analyses defined two clades (both nuclear and mtDNA markers) in all Indo-Malay specimens, although these clades did not appear to have any obvious geographic structure. The higher mutation rate for the control region than for *coI* and *rag1* was suggested to explain its different topology (Theisen *et al*., 2008). The same discrepancy was also

reported between control region and *cytb* in wahoo *Acanthocybium solandri* (Cuvier 1832) (Theisen *et al*., 2008) and three *Trachurus* species (Karaiskou *et al*., 2004).

4.1 | Population genetic structure

The results of pairwise Φ_{ST} comparisons, AMOVA tests and S_{nn} statistics indicated significant population genetic subdivision among localities in yellowtail, yielding three differentiated mitochondrial lineages. Two lineages comprised haplotypes formerly identified by Mat Jaafar *et al*. (2012), with the major lineage including specimens from all sampling regions across the IMA. However, no geographic structuring was observed in this mitochondrial lineage of yellowtail. The second lineage in Mat Jaafar *et al*.'s (2012) study consisted of only a single specimen from Tok Bali. Here, we included more specimens from Tok Bali and Semporna and three of the Tok Bali specimens (AM12, AM50, AM80) and six Semporna specimens (AM40, AM41, AM42, AM86, AM87, AM88) grouped together, with the formerly identified (Mat Jaafar *et al*., 2012) potential cryptic species. The third lineage included only specimens from Kuwait. A corresponding pattern was also evident in data from the control region and *rag1*. From such patterns, we hypothesise the existence of cryptic species in Indo-Malay yellowtail. The Φ_{ST} *P*-values among IMA populations and the Kuwait population in *coI* and *rag1* data were all significant ($P < 0.05$), indicating limited gene flow among these two regions in the absence of obvious dispersal barriers.

In contrast, the results obtained here indicated no significant heterogeneity in *coI*, control region or in *rag1* across the highly pelagic species, bigeye populations within the IMA, in concordance with data from Pedrosa-Geramsio *et al.* (2015). Findings also indicated a homogeneous population of bigeye in the Sulu Sea. Panmixia of the species in the region is predicted based on their high mobility and dispersal potential of larvae. Pelagic marine fishes usually have high fecundity, very large population sizes and high dispersal potential at egg, larval and adult stages. These life-history features and the continuity of the pelagic environment in theory suggest little genetic divergence over large spatial scales. For a more comprehensive analysis of genetic variation in bigeye, the sampling design of future surveys should address a broader geographic scale and utilise more rapidly evolving nuclear markers, such as microsatellites or single nucleotide polymorphisms (SNP).

For yellowstripe, there was significant differentiation in *coI* and control-region data. The significant differentiation between KPJ with six other localities within the IMA suggested yellowstripe are genetically subdivided into distinct populations although additional studies, possibly with additional nuclear markers (*e.g.*, SNPs), are required to assess patterns more widely. However, no geographical structure was observed in the NJ tree. Overall, low Φ_{ST} values were detected in the Indo-Malay yellowtail and bigeye compared with yellowstripe, indicating extensive gene flow among the former species within the IMA. Seventy per cent of marine organisms have a planktonic stage during the larval phase when larvae may actively disperse (Bonhomme & Planes, 2000; Thorrold *et al*., 2007). In general, fish with longer larval duration

display less genetic differentiation than those with shorter larval duration (Waples, 1987; Bay *et al*., 2006; Bradbury *et al*., 2008; Hauser & Carvalho, 2008). However, gathering evidence suggests the importance of other factors, such as currents and larval retention (Rohfritsch & Borsa, 2005; Carreras-Carbonell *et al*., 2006; Froukh & Kochzius, 2007) that may cause strong differentiation even in species with a long larval phase (Taylor & Hellberg, 2003). Unfortunately, little is known regarding the reproductive biology of yellowtail, bigeye and yellowstripe (Leis *et al*., 2004).

4.3 | Fisheries management in the Indo-Malay Archipelago

Overfishing is one of the most significant threats to the world's marine fishes (Reynolds *et al*., 2002; Dulvy *et al*., 2003), including Malaysian ichthyofauna. The coastal demersal fishes in Sarawak and Sabah were reported to be overfished and heavily overfished, respectively, while the offshore demersal fishes as well as coral-reef fishes in Sabah, were heavily overfished (Oakley *et al*., 2000). Therefore, determination of population genetic structure provides essential information to underpin resource recovery and to aid in delineating and monitoring populations for fisheries management (Han *et al*., 2008). The emergence of two separate lineages in yellowtail (maximum *coI* nucleotide divergences of 4.6%) could suggest that at least two different stocks of this species occur in the IMA waters, although no obvious geographical structure was detected. These putative stocks should be managed separately because of their

likely differential response to harvesting and recruitment, thereby requiring different conservation strategies (Schonrogge *et al*., 2002). For bigeye, the observed homogeneity was interpreted as supporting the view that this species should be managed in the IMA as a single stock, though it is always important to confirm such assertions through temporal analysis of samples to assess stability (Waples, 1998). Even though yellowstripe also showed significant differentiation between IMA localities, the data presented here is still preliminary based on small sample sizes. Additional samples should be collected and more powerful genetic markers (*e.g*., SNPs, microsatellites) should be used to further investigate stock structure and boundaries in Indo-Malay Carangidae. The current population genetic study encompassing three widely distributed and economically important Carangidae species (yellowtail, bigeye, yellowstripe scads) provides a base-line of reference data upon which additional more detailed studies can be conducted. Importantly also, data presented here indicates a complex scenario of genetic structuring that endorses the need to better define the dynamics and putative stock boundaries of regional exploited stocks of marine fish in the IMA and beyond.

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FIGURE1 Distribution locations for 180 specimens of *Atule mate* , *Selar crumenophthalmus* and *Selaroides leptolepis* sampled along the coast of Malaysia. Samples were collected from respective landing sites (●) in four geographical regions of the Indo-Malayan Archipelago; South China Sea, Malacca Strait, Sulu Sea and Celebes Sea. Sample sizes for each species and sample codes are given in Supporting Information Table S1.

FIGURE2 (a) Maximum likelihood phylogenetic relationships among 24 mtDNA *coI* haplotypes in *Atule mate.* Only bootstrap values > 50 are shown. (b) The distribution of haplotypes among populations (Figure 1): KPJ, Kuala Perlis; TBJ, Tok Bali; TSJ, Tanjung Sedili; MKS, Mukah; KDT, Kudat; SDK, Sandakan; SMP, Semporna plus KWT, Kuwait

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FIGURE3 (a) Maximum Likelihood phylogenetic relationships among 13 mtDNA *coI* haplotypes in *Selaroides leptolepis.* Only bootstrap values > 50 are shown. (b) The distribution of haplotypes among populations (Figure 1): KPJ, Kuala Perlis; SB, Kuala Sungai Baru; KBJ, Kuala Besut; MGJ, Mersing; MR, Miri; KDT, Kudat; SDK, Sandakan; SMP, Semporna; TW, Tawau

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1 Change a), b) to (a), (b)

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FIGURE4 (a) Maximum Likelihood phylogenetic relationships among 23 mtDNA *COI* haplotypes in *Selar crumenophthalmus.* Only bootstrap values > 50 are shown. (b) The distribution of haplotypes among populations (Figure 1): KPJ, Kuala Perlis; SK, Sekinchan; TBJ, Tok Bali; MKS, Mukah; KDT, Kudat; SDK, Sandakan; SMP, Semporna; TW, Tawau

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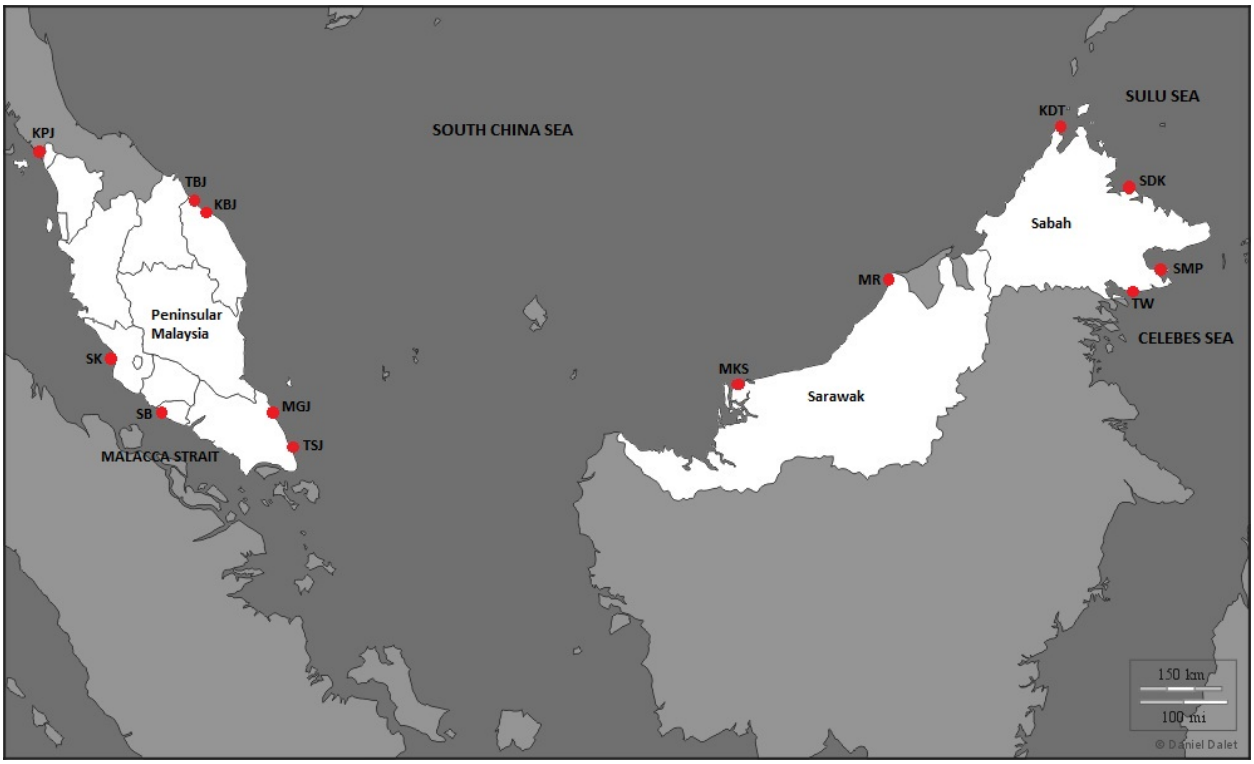

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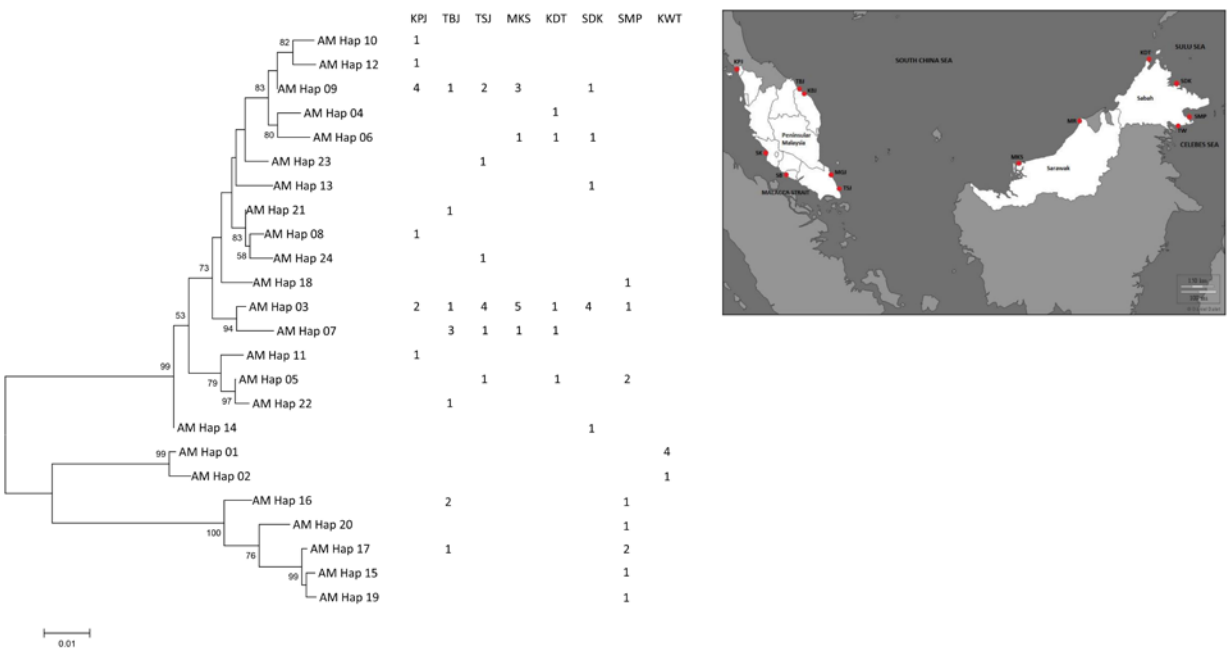
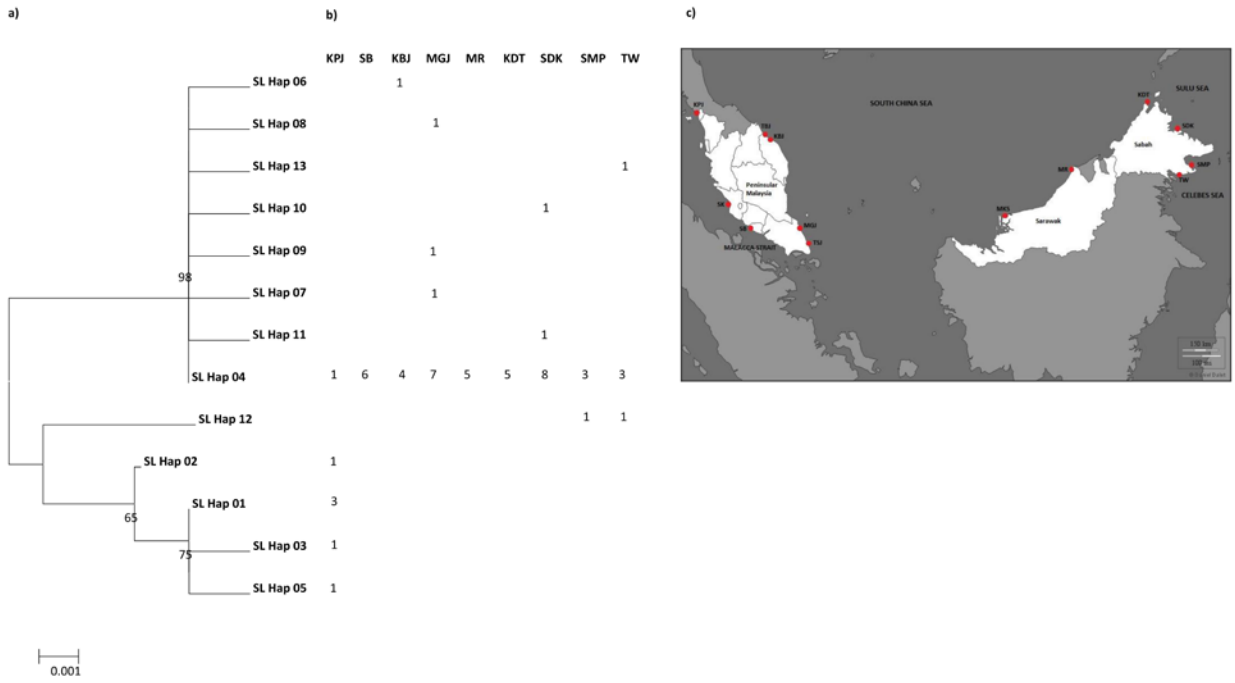
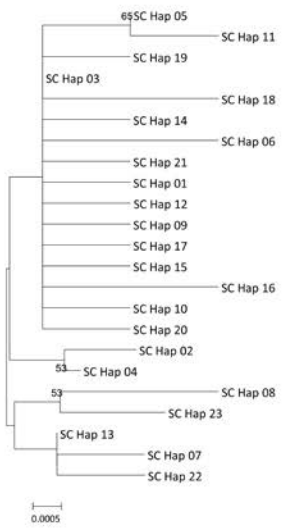




a)



b)

	KPJ	SK	TBJ	TSJ	MKS	KDT	SDK	SMP	TW
SC Hap 05	1		1		1		1		
SC Hap 11		1							
SC Hap 19						1			
SC Hap 03	2	2	3	4	2	3	3	2	5
SC Hap 18					1				
SC Hap 14				1					
SC Hap 06	1								
SC Hap 21							1		
SC Hap 01	1								
SC Hap 12			1						
SC Hap 09		1							
SC Hap 17					1				
SC Hap 15				1					
SC Hap 16				1					
SC Hap 10		1		1					
SC Hap 20						1			
SC Hap 02	1			1					
SC Hap 04	1								
SC Hap 08	1								
SC Hap 23								1	
SC Hap 13				1					
SC Hap 07	1								
SC Hap 22								1	

c)

Significance Statement

Comparative genetic stock structure in three species of commercially exploited Indo-Malay Carangidae (Teleosteii: Perciformes)

T.N.A. Mat Jaafar[1,3], M. I. Taylor[1,4], S. A. Mohd Nor[2], M. de Bruyn[1,5], G. R. Carvalho[1*]

Population genetic structuring of commercial marine fishes from Malaysian waters is understudied, despite the potential thereof to significantly contribute to the management of these natural resources. We examined population genetic structure in three commercially important scad (teleost) fishes. Our study shows that pelagic and semi-pelagic species display less population genetic structuring than demersal fishes, according with predictions based on life-history strategies and vagility.

TABLE 1 Results of the analysis of molecular variance (AMOVA) for *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis* showing Φ-statistics analysis for *coI*

Hierarchical level	*A. mate*		*S. crumenophthalmus*		*S. leptolepis*	
	***Φ*-statistic**	***P***	***Φ*-statistic**	***P***	***Φ*-statistic**	***P***
Among IMA localities						
Among all regions (Φ_{CT})	0.02055	ns	0.02830	ns	–0.01543	ns
Among populations within regions (Φ_{SC})	0.01359	ns	–0.03472	ns	0.14864	*
Among individuals within populations (Φ_{ST})	0.03387	ns	–0.00544	ns	0.13550	**
Between IMA and KWT						
Among all regions (Φ_{CT})	0.08144	ns				
Among populations within regions (Φ_{SC})	0.01817	ns				
Among individuals within populations (Φ_{ST})	0.09813	**				

IMA, Indo–Malay Archipelago; KWT, Kuwait

ns, Not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.01$

TABLE 2 Population pairwise Φ_{ST} (below the diagonal) for *coI* and corresponding significance level (above the diagonal; after Bonferroni correction) for three species of scad from the Indo-Malayan Archipelago and Kuwait

	Population							
	KWT	**TBJ**	**TSJ**	**MKS**	**SMP**	**KPJ**	**KDT**	**SDK**
...ile mate								
...WT		**	**	***	***	**	*	**
...BJ	0.29598		ns	ns	ns	ns	ns	ns
TSJ	0.33333	0.03541		ns	ns	ns	ns	ns
MKS	0.41003	0.08864	–0.06545		**	ns	ns	ns
SMP	0.27143	0.01754	0.04255	0.12281		*	ns	ns
...PJ	0.33333	0.06619	–0.00529	0.00285	0.08163		ns	ns
KDT	0.30000	–0.03390	–0.03943	0.02439	–0.03789	0.04918		ns
...DK	0.37747	0.09264	–0.05253	–0.06810	0.08046	0.04003	–0001064	
...iar crumenophthalmus								
	TBJ	**TSJ**	**MKS**	**SMP**	**TW**	**KPJ**	**SK**	**KDT**	**SDK**
TBJ		ns	ns	ns	ns	ns	ns	ns	ns
...SJ	–0.04126		ns	ns	ns	ns	ns	ns	ns
...KS	–0.11111	–0.04972		ns	ns	ns	ns	ns	ns
...MP	–0.09053	–0.06543	–0.08541		ns	ns	ns	ns	ns
TW	0.12500	0.16667	0.25000	0.23077		ns	ns	ns	ns
KPJ	–0.00544	–0.02097	–0.05649	–0.02683	0.27419		ns	ns	ns
...	–0.05263	–0.07547	–0.07143	–0.08541	0.25000	–0.03096		ns	ns
...DT	–0.09375	–0.04126	–0.05263	–0.09053	0.12500	0.01941	–0.05263		ns
SDK	–0.16667	–0.04126	–0.11111	–0.09053	0.12500	–0.00544	–0.05263	–0.09375	
Selaroides leptolepis									

	KBJ	MGJ	MR	KPJ	SB	KDT	SDK	TW	SMP
KBJ		ns	ns	*	ns	ns	ns	ns	ns
MGJ	–0.07383		ns	*	ns	ns	ns	ns	ns
MR	–0.00000	0.01235		**	ns	ns	ns	ns	ns
KPJ	0.26473	0.24247	0.43850		**	**	**	ns	ns
SB	0.04000	0.04000	0.00000	0.47170		ns	ns	ns	ns
KDT	–0.00000	0.01235	0.00000	0.43850	0.00000		ns	ns	ns
SDK	–0.07759	–0.03535	–0.03659	0.32897	–0.00990	–0.03659		ns	ns
TW	–0.05769	–0.04972	0.12500	0.14137	0.16832	0.12500	–0.00324		ns
SMP	–0.11913	–0.09233	0.06250	0.20731	0.11111	0.06250	–0.07633	–0.20787	

KBJ, Kuala Besut; KDT, Kudat; KPJ, Kuala Perlis; KWT, Kuwait; MGJ, Mersing; MKS, Mukah; MR, Miri; SB, Kuala Sungai Baru; SDK, Sandakan; SK, Sekinchan; SMP, Semporna; TBJ, Tok Bali; TSJ, Tanjung Sedili; TW, Tawau

ns, Not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

TABLE 3 Results of the analysis of molecular variance (AMOVA) for *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis* showing Φ-statistics analysis for control region

Hierarchical level	***A. mate***		***S. crumenophthalmus***		***S. leptolepis***	
	Φ-statistic	***P***	**Φ-statistic**	***P***	**Φ-statistic**	***P***
Among IMA localities						
Among all regions (Φ_{CT})	0.00402	*	0.03994	ns	0.02975	ns
Among localities within regions (Φ_{SC})	0.00047	ns	0.14571	***	0.01736	ns
Among individuals within localities (Φ_{ST})	0.00448	ns	0.17983	***	0.04660	*
Between IMA localities and Kuwait (KWT) for *Atule mate*						
Among all regions (Φ_{CT})	0.00358	*				
Among localities within regions (Φ_{SC})	0.00043	ns				
Among individuals within localities (Φ_{ST})	0.00402	ns				

IMA, Indo–Malay Archipelago; KWT, Kuwait

ns, Not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

TABLE 4 Population pairwise Φ_{ST} (below the diagonal) for control region and corresponding *P*-values (above the diagonal; after Bonferroni correction) for three species of scad from the Indo-Malayan Archipelago and Kuwait***Atule mate***

	Population							
	KWT	**TBJ**	**TSJ**	**MKS**	**SMP**	**KPJ**	**KDT**	**SDK**
Atule mate								
KWT		ns	ns	ns	ns	ns	ns	ns
TBJ	0.00000		ns	ns	ns	ns	ns	ns
TSJ	0.00000	0.00000		ns	ns	ns	ns	ns
MKS	0.00000	0.00000	0.00000		ns	ns	ns	ns
SMP	0.01235	0.01111	0.01111	0.01142		ns	ns	ns
KPJ	0.00000	0.00000	0.00000	0.00000	0.01111		ns	ns
KDT	0.00000	0.00000	0.00000	0.00000	0.01235	0.00000		ns
SDK	0.00000	0.00000	0.00000	0.00000	0.01164	0.00000	0.00000	
Selar crumenophthalmus								
	TBJ	**TSJ**	**MKS**	**SMP**	**TW**	**KPJ**	**SK**	**KDT**	**SDK**
TBJ		**	*	*	*	**	*	*	*
TSJ	0.25926		ns	ns	***	ns	ns	ns	*
MKS	0.35000	0.05632		ns	*	ns	ns	ns	ns
SMP	0.35000	0.05632	0.10000		0.15723	ns	ns	ns	ns
TW	0.80000	0.40379	0.55000	0.25000		***	**	**	ns
KPJ	0.25926	0.02222	0.05632	0.05632	0.40379		ns	ns	**
SK	0.30000	0.01235	0.05000	0.01042	0.50000	0.01235		ns	ns
KDT	0.30000	0.01235	0.01042	0.05000	0.50000	–0.00787	0.00000		ns
SDK	0.45000	0.14074	0.20000	–0.05263	0.12500	0.14074	0.11458	0.15000	
Selaroides leptolepis									

	KBJ	MGJ	MR	KPJ	SB	KDT	SDK	TW	SMP
KBJ		ns	ns	ns	ns	ns	*	ns	ns
MGJ	0.03727		ns	ns	ns	ns	ns	ns	ns
MR	0.00000	–0.04730		ns	ns	ns	ns	ns	ns
KPJ	0.00000	0.03504	0.00000		ns	ns	*	ns	ns
SB	0.00000	0.01743	0.00000	0.00000		ns	ns	ns	ns
KDT	0.00000	–0.0279	–0.04167	0.00000	0.00000		ns	ns	ns
SDK	0.14013	0.00654	0.04085	0.13027	0.14013	0.04085		*	ns
TW	0.05000	0.08116	0.05000	0.04654	0.05000	0.05000	0.18367		ns
SMP	0.00000	0.03919	0.00000	0.00000	0.00000	0.00000	0.14820	0.05308	

KBJ, Kuala Besut; KDT, Kudat; KPJ, Kuala Perlis; KWT, Kuwait; MGJ, Mersing; MKS, Mukah; MR, Miri; SB, Kuala Sungai Baru; SDK, Sandakan; SK, Sekinchan; SMP, Semporna; TBJ, Tok Bali; TSJ, Tanjung Sedili; TW, Tawau

ns, Not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

TABLE 5 Results of the analysis of molecular variance (AMOVA) for *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis* showing Φ-statistics analysis for *rag1*

Hierarchical level	*A. mate*		*S. crumenophthalmus*		*S. leptolepis*	
	***Φ*-statistic**	***P***	***Φ*-statistic**	***P***	***Φ*-statistic**	***P***
Among IMA localities						
Among all regions (Φ_{CT})	0.25620	ns	–0.03423	ns	0.03696	ns
Among localities within regions (Φ_{SC})	0.19543	*	–0.05170	ns	–0.11443	ns
Among individuals within localities (Φ_{ST})	0.40156	***	–0.03958	ns	–0.07324	ns
Between IMA localities and Kuwait (KWT) for *Atule mate*						
Among all regions (Φ_{CT})	0.50635	ns				
Among localities within regions (Φ_{SC})	0.21206	*				
Among individuals within localities (Φ_{ST})	0.61104	***				

IMA, Indo–Malay Archipelago; KWT, Kuwait

ns, Not significant ($P > 0.05$); *, $P < 0.05$; ***, $P < 0.001$

TABLE 6 Population pairwise Φ_{ST} (below the diagonal) for *rag1* corresponding *P*-values (above the diagonal; after Bonferroni correction) for three species of scad from the Indo-Malayan Archipelago and Kuwait***Atule mate***

	Population							
	KWT	**TBJ**	**TSJ**	**MKS**	**SMP**	**KPJ**	**KDT**	**SDK**
Atule mate								
KWT		***	***	***	***	***	**	**
TBJ	0.61648		ns	ns	ns	ns	ns	ns
TSJ	1.00000	0.23295		ns	*	ns	ns	ns
MKS	1.00000	0.25000	0.00000		**	ns	ns	ns
SMP	0.65616	0.02299	0.53905	0.55556		**	ns	*
KPJ	1.00000	0.25000	0.00000	0.00000	0.55556		ns	ns
KDT	1.00000	0.14013	0.00000	0.00000	0.45205	0.00000		ns
SDK	1.00000	0.21426	0.00000	0.00000	0.52096	0.00000	0.00000	
Selar crumenophthalmus								
	TBJ	**TSJ**	**MKS**	**SMP**	**TW**	**KPJ**	**SK**	**KDT**	**SDK**
TBJ		ns	ns	ns	ns	ns	ns	ns	ns
TSJ	–0.08434		ns	ns	ns	ns	ns	ns	ns
MKS	0.00000	–0.08434		ns	ns	ns	ns	ns	ns
SMP	0.00000	–0.08434	0.00000		ns	ns	ns	ns	ns
TW	0.00000	–0.12150	0.00000	0.00000		ns	ns	ns	ns
KPJ	0.00000	–0.00000	0.00000	0.00000	0.00000		ns	ns	ns
SK	–0.00000	–0.03659	–0.00000	–0.00000	–0.05263	0.14894		ns	ns
KDT	0.00000	–0.08434	0.00000	0.00000	0.00000	0.00000	0.00000		ns
SDK	0.00000	–0.08434	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	
Selaroides leptolepis									

	KPJ	SB	SMP	TW	KBJ	MGJ	MR	KDT	SDK
KPJ		ns	ns	ns	ns	ns	ns	ns	ns
SB	–0.05528		ns	ns	ns	ns	ns	ns	ns
SMP	–0.09804	0.00000		ns	ns	ns	ns	ns	ns
TW	–0.05528	0.00000	0.00000		ns	ns	ns	ns	ns
KBJ	–0.09804	0.00000	0.00000	0.00000		ns	ns	ns	ns
MGJ	–0.09804	0.00000	0.00000	0.00000	0.00000		ns	ns	ns
MR	–0.05528	0.00000	0.00000	0.00000	0.00000	0.00000		ns	ns
KDT	–0.05528	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000		ns
SDK	–0.02439	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	

KBJ, Kuala Besut; KDT, Kudat; KPJ, Kuala Perlis; KWT, Kuwait; MGJ, Mersing; MKS, Mukah; MR, Miri; SB, Kuala Sungai Baru; SDK, Sandakan; SK, Sekinchan; SMP, Semporna; TBJ, Tok Bali; TSJ, Tanjung Sedili; TW, Tawau

IMA, Indo–Malay Archipelago; KWT, Kuwait

ns, Not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$