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Morphometric and molecular analysis of mackerel (*Rastrelliger* spp) from the west coast of Peninsular Malaysia

M.N. Darlina[1,2], A.R. Masazurah[3], P. Jayasankar[4], A.F.J. Jamsari[1] and A.M.N. Siti[1,2]

[1]School of Biological Sciences, Universiti Sains Malaysia, Minden, Penang, Malaysia
[2]Centre for Marine and Coastal Studies, Universiti Sains Malaysia, Muka Head, Penang, Malaysia
[3]Department of Fisheries Malaysia, Fisheries Research Institute, Batu Maung, Penang, Malaysia
[4]Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Orissa, India

Corresponding author: M.N. Darlina
E-mail: darlinamdn@usm.my

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ABSTRACT. Mackerel (Scombridae; *Rastrelliger*) are small commercially important pelagic fish found in tropical regions. They serve as a cheap source of animal protein and are commonly used as live bait. By using a truss morphometrics protocol and RAPD analysis, we examined morphological and genetic variation among 77 individual mackerel that were caught using long lines and gillnets at 11 locations along the west coast of Peninsular Malaysia. Nineteen morphometric traits were evaluated and genetic information was estimated using five 10-base RAPD random primers. Total DNA was extracted from muscle tissue. Morphometric discriminant function analysis revealed that two morphologically distinct groups of *Rastrelliger kanagurta*

and a single group of *R. brachysoma* can be found along the west coast of Peninsular Malaysia. We also found that the head-related characters and those from the anterior part of the body of *Rastrelliger* spp significantly contribute to stock assessment of this population. RAPD analysis showed a trend similar to that of the morphometric analysis, suggesting a genetic component to the observed phenotypic differentiation. These data will be useful for developing conservation strategies for these species.

Key words: Genetic variation; RAPD; Multivariate analysis; Morphometric; *Rastrelliger* spp

INTRODUCTION

Strategies for conservation and sustainable use of biological resources in the coastal zone are crucial for the long-term maintenance of fisheries in these regions (Laikre et al., 2005). Of particular relevance for fishery management is an identification of the geographical ranges of stock units since they are fundamental to population dynamics (Bailey, 1997). Management units are distinct populations of fish that have different population sizes, recruitment patterns, and spawning and nursery grounds, and therefore need to be managed separately for sustainable commercial fishing (Smith and Jamieson, 1986; Begg and Waldman, 1999; Abaunza et al., 2008).

Morphometry is one of the methods used in the multidisciplinary field of stock identification, and by measuring the form, morphometric studies allow the understanding of morphological (or phenotypic) variations between populations (Ihssen et al., 1981). While phenotypic variation is usually associated with the adaptive potential of populations (Hoffmann and Willi, 2008), genetic markers such as DNA and protein detect levels of genetic variation. This information is critical for conservation strategies as the long-term survival and evolution of every species depend on the maintenance of genetic diversity and are closely related to geographic distribution of genotypes (Frankham, 1995; Reed et al., 2002; Reed, 2004). In the last few decades, the advancement of molecular marker techniques, most notably the development of polymerase chain reaction (PCR), coupled with the recent explosion of powerful computer programs and the use of geographical information system (GIS), has greatly assisted in the survey, sampling and assessment of the genetic diversity of populations and higher-level taxa (Rao and Hoskela, 2001), thus offering a wide range of possibilities to study the evolutionary biology and behavior of organisms that were once thought to be impossible (Schlotterer, 2004; Galtier et al., 2009). Some of the molecular markers frequently utilized for population assessment include restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and single sequence repeats (SSR) also known as microsatellites (Lowe et al., 2004).

Mackerel (*Rastrelliger* spp) is one of the most important commercial fish landed by the purse-seine and trawl fisheries in Malaysia (FAO, 2010). This small pelagic fish comprises three main species: *R. brachysoma* (Indo Pacific mackerel), *R. kanagurta* (Indian mackerel) and *R. faughni* (island mackerel) (Matsui, 1967; Froese and Pauly, 2009). They

inhabit coastal waters, form large schools and tend to aggregate around fish aggregating devices. *Rastrelliger brachysoma* is generally distributed in the near-shore coastal areas while *R. kanagurta* and *R. faughni* are more oceanic (FAO, 2000). They can be found throughout the Indo-West Pacific, Red Sea and East Africa to Indonesia, north to the Ryukyu Islands and China, south to Australia, Melanesia and Samoa, entering the eastern Mediterranean Sea through the Suez Canal (FAO, 2000). The overexploitation of the pelagic fishery resources, including the *Rastrelliger* spp, mainly due to the utilization of efficient fishing gear and larger fishing vessels on the west coast of Peninsular Malaysia has been highlighted in Malaysia (FAO, 2000). Additionally, the expansion of fishing grounds into new and non-traditional areas within the Malaysian Exclusive Economic Zone (EEZ) also contributes to this problem. However, *Rastrelliger* spp remains the backbone of the marine fisheries on the west coast of Peninsular Malaysia (FAO, 2000).

Early attempts made to delineate the stock structure of *Rastrelliger* spp (i.e., *R. kanagurta*) from the east and west coasts of India were carried out by Seshappa (1985) based on the traditional morphometry and meristics. Recognizing the importance of the truss morphometrics protocol system, which is more useful than the traditional morphometrics methods, Jayasankar et al. (2004) were the first to discriminate phenotypic stocks of *R. kanagurta* in Peninsular India. These workers used a holistic approach, combining one phenotypic and two genotypic (protein and RAPD) methods to analyze possible population differences in the Indian mackerel sampled from east and west coasts of India. In Malaysia, there are some recorded data on the genetic variation of *R. kanagurta* based on mitochondrium and RAPD markers (Mohd Nor et al., 2008; Jamaluddin et al., 2010); however, to date, no relevant information on stock definition has been produced concerning the genus *Rastrelliger* in Malaysia. Thus, determination of management units is deemed necessary to conserve the stock of the *Rastrelliger* spp in Malaysia.

The objectives of this study include: a) determination of genetic variation within and among species of *R. kanagurta* and *R. brachysoma* and b) to provide preliminary assessment on the unit stock that is probably formed by the species along the west coast of Peninsular Malaysia. The present study has potential application in fish stock estimation and proposes measures for sustainable management. If the extent of gene flow is low enough for the stocks to be managed as a single panmictic population, it would be advisable to manage them as a single unit.

MATERIAL AND METHODS

Sample collection

Sampling was carried out at 11 locations situated along the west coast of Peninsular Malaysia during two sampling cruises (February and March 2006; Figure 1). Fish was caught using long lines and gillnets. Immediately after catches, fishes were isolated and both *R. kanagurta* and *R. brachysoma* were identified using morphological identification based on the description of Fisher and Bianchi (1984). For genetic analysis, clipped fins were placed in a preservation buffer of either TNES-urea or 95% ethanol. The remaining whole fish was conveyed in a cool box to the laboratory on each sampling session for morphometric analysis.

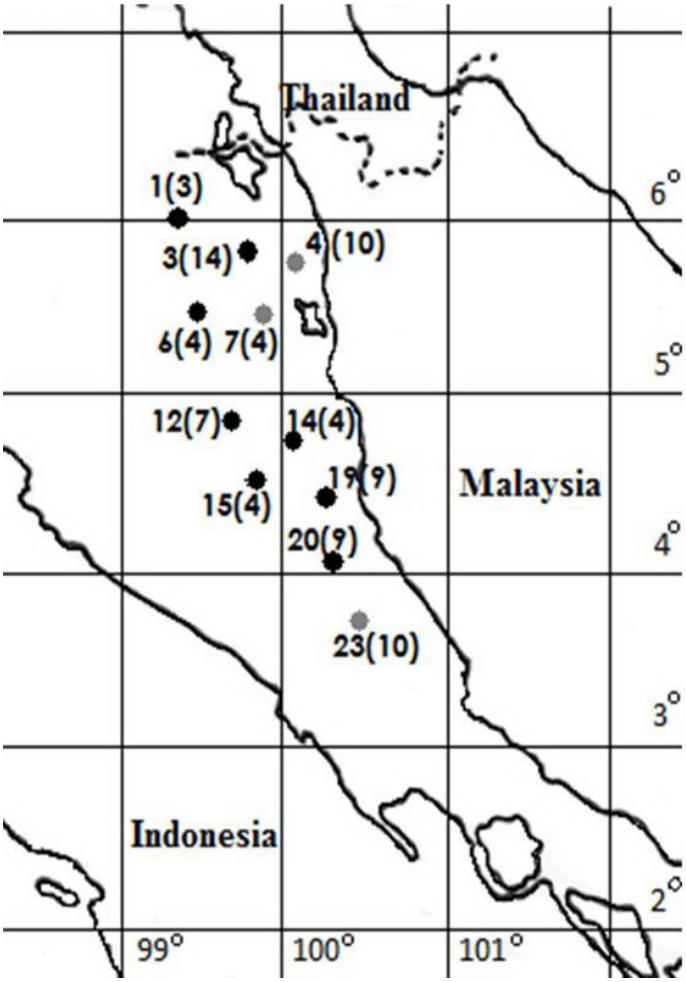

Figure 1. Sampling sites of *Rastrelliger kanagurta* (black circles) and *R. brachysoma* (gray circles) in the northern and central west coast of Peninsular Malaysia. Numbers indicate haul and numbers in parentheses indicate sampling size (*n*) for each haul.

Truss morphometrics

Nineteen morphometric truss measurements, joining homologous points, were taken on the right side of the fish (Humphries et al., 1981), including the standard length for standardization of variables (Figure 2). Measurements were taken by placing the fish on water resistant paperboard and pricking the paper with a vertical dissection needle corresponding to the measurement points. All measurements were taken using a digital caliper to the nearest millimeter. To remove size-dependent variation and to compare shape differences, an allometric approach (Palma and Andrade, 2002) was utilized by transforming the data using the following formula: $M_{trans} = \log M - \beta (\log SL - \log SL_{mean})$, where M_{trans} is the transformed measurement; M, the original measurement; β, the within-group slope regressions of the log M *vs*

log SL; *SL*, the standard length of the fish, and SL_{mean}, the overall mean of the standard length. After size correction, the correlation between truss variables and fish total length should decrease, confirming that the influence of size was effectively eliminated.

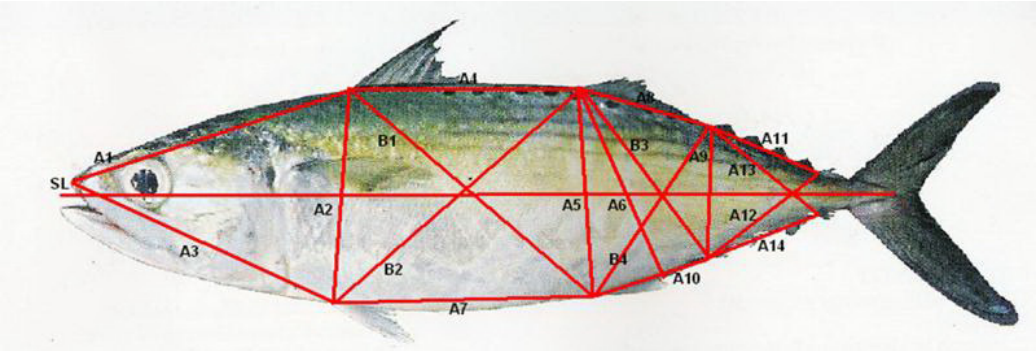

Figure 2. Body-form measures of *Rastrelliger* spp for morphometric analysis. Measures are as follows: A1, tip of snout to first dorsal fin; A2, first dorsal to pelvic fins; A3, tip of snout to pelvic fin; A4, first dorsal to second dorsal; A5, second dorsal to origin of anal fins; A6, second dorsal to insertion of anal fin; A7, pelvic to origin of anal fins; A8, second dorsal fins to first superior finlets; A9; first superior finlets to first inferior finlets; A10, origin of anal fins to first inferior finlets; A11, first superior finlets to fifth superior finlets; A12, first inferior finlets to fifth superior finlets; A13, first superior finlets to fifth inferior finlets; A14, first inferior finlets to fifth inferior finlets; B1, first dorsal to origin of anal fins; B2, pelvic fin to second dorsal fins; B3, second dorsal to first inferior finlets; B4, origin of anal fins to first superior finlets; SL, standard length.

RAPD primers and PCR amplification

Total genomic DNA from muscle tissue was extracted using the Tissue DNA Kit (Genispin). Ten 10-base RAPD random primers (Operon Technologies Inc., USA) were used to initiate PCR amplifications and were randomly selected based on GC content and annealing temperature for RAPD-PCR amplifications. After initial screening with all the 10 primers, only five (OPC-1, OPC-4, OPC-5, OPC-9, and OPC-13) were used in a final study. The PCR consisted of 10X PCR buffer, 0.4 mM dNTPs mixture, 3.5 mM $MgCl_2$, 5 pmol RAPD primer, 2.0 U *Taq* DNA polymerase, 30 ng template DNA, and distilled water in a final volume of 25 µL. A negative control (without template DNA) was also included in each reaction. The amplification of DNA was conducted using the Peltier Thermal Cycler, PTC-200 (MJ-Research Inc.) that was programmed at 35 cycles for 30 s of denaturation at 94°C, 30 s of annealing at 36°C, 1 min of extension at 72°C, and 2 min of final extension at 72°C. Then, 5 µL PCR product was run alongside a 100-bp PCR ladder (Promega) on a 2% agarose gel at 100 V for 20 min and stained with ethidium bromide (at a final concentration of 0.5 µg/mL). The amplified pattern was visualized on a UV transilluminator and photographed by gel documentation system (Alpha-Innotech, USA).

Data analysis

A stepwise multivariate discriminant function analysis (DFA) was performed on the transformed morphometric variables to identify the combination of characters that optimize

differentiation among the populations. The output generated from this analysis was used to assess the discriminatory effectiveness from the percentage of correct re-classification in discriminant analysis. Mahalanobis distance between population centroids and pairwise *F*-statistics was computed to evaluate significant levels of population difference. The spatial separation between the centroids was represented by the generation of scatter plots. All statistical analysis was performed using SPSS ver. 11.5.

The RAPD fragments were scored by either the presence (1) or absence (0) of fragments on the gel photographs for each individual. The data matrices were entered into Multivariate Statistical Package ver. 3.13 (Kovach Computing Services) and pairwise comparisons between and within populations were made. The index of similarity (SI) values between the RAPD profiles of any two individuals on the same gel were calculated using the equation of Nei and Li (1979): $SI = 2n_{xy} / (n_x + n_y)$, where n_{xy} is the number of fragments shared by individuals x and y, and n_x and n_y are the numbers of fragments scored for each individual (Nei, 1978). This method was chosen because of the greater emphasis placed on fragment matches versus that of non-matches (Nei, 1978). Unweighted pair-group method of arithmetic mean (UPGMA, Sneath and Sokal, 1973) implemented in the Neighbour program of Phylip ver. 3.57c (Felsenstein, 1989) was used to analyze the genetic relationship data resampling procedure (1000 replicates) and matrix calculations for bootstrap analysis were performed using WinBoot, a UPGMA-based program (Yap and Nelson, 1996). We considered values above 75% as significant and the percentage of polymorphism of the RAPD markers was estimated using PopGene ver. 1.31 (Yeh et al., 1999).

RESULTS

Demographic parameters

In total, 77 fishes were sampled along the west coast of Peninsular Malaysia during February and March 2006, which corresponded to 53 specimens of *R. kanagurta* and 24 specimens of *R. brachysoma* (Figure 1). Detailed sampling data are presented in the Supplementary Material 1. As expected, the mean sample size of *R. kanagurta* (6.63 ± 3.79 SD) and *R. brachysoma* (8.0 ± 3.10 SD) differed significantly (*t*-test, $t = 0.5547$, d.f. = 9, $P < 0.05$). Note that the frequency of successful sampling of *R. kanagurta* (N = 8) was greater than in *R. brachysoma* (N = 3) during the same period of sampling.

Truss morphometric analysis

In the stepwise DFA, all variables contributed significantly to the multivariate discriminant analysis of the populations. The first two functions, which contributed significantly to the discriminant analysis accounted for 84.1% of between group variability (Table 1). Based on the DF1 (which explained 70% of the variance) (Table 1), four truss elements were very informative for the distinction of *R. kanagurta* from *R. brachysoma* (A1, A2, A5, and B3). Within each species differentiation was along DF2 (A2, A5, B3) (Table 1). For both intra- and interspecies discrimination the head related and the anterior part of the body seem to be important for discrimination.

Table 1. The first three eigenvectors and percentages of total variance explained by the eigenvalues obtained from discriminant function analysis coefficients.

	DF1	DF2	DF3
A1TRAN	0.769	0.223	-0.163
A2TRAN	1.253	0.508	-0.273
B1TRAN	-0.510	0.036	-0.024
B2TRAN	0.396	0.134	0.556
A3TRAN	-0.264	-0.274	-0.257
A4TRAN	-0.055	-0.069	-0.014
A5TRAN	0.577	0.352	-0.086
A7TRAN	0.268	-0.448	0.736
A8TRAN	-0.231	-0.215	0.440
A9TRAN	0.260	-0.075	-0.304
A10TRAN	0.439	-0.611	0.798
A11TRAN	-0.023	-0.075	0.535
A12TRAN	0.180	0.238	-0.193
B3TRAN	0.526	0.330	0.280
Eigenvalue	14.68	2.94	0.86
% Variance	70.00	14.1	4.1

The overall mean reclassification success within population was high at 82.5% (Supplementary Material 2). The generalized Mahalanobis distances (D2) and *F*-statistics for the two species are presented in Table 2. The *F*-statistics indicated highly significant differences on the truss element Mahalanobis distances with a North-South geographical gradient. Two clusters of homogeneous groups were observed in *R. kanagurta*: i) Hauls 1, 3, 6, off the coast of Kedah and Penang in the north and Hauls 12, 14 and 15 off the coast of central Perak in central Peninsular Malaysia, and ii) Hauls 19 and 20 located off the coast of central Peninsular Malaysia but towards the south (Figure 3).

Table 2. Pairwise group comparisons based on generalized Mahalanobis distances and *F*-statistics for the linear discriminant functions for all truss characters for the 8 populations of *Rastrelliger kanagurta* and 3 populations of *R. brachysoma*.

Haul		1	3	4	6	7	12	14	15	19	20	23
1	F		3.09	27.622	1.244	22.572	2.021	1.709	0.537	8.349	9.53	18.272
	Sig.		0.022	0	0.301	0	0.102	0.159	0.709	0	0	0
3	F	3.09		49.77	1.52	25.292	2.03	1.758	9.035	13.878	13.94	24.903
	Sig.	0.022		0	0.207	0	0.101	0.149	0	0	0	0
4	F	27.622	49.77		33.538	2.043	42.529	30.948	58.371	51.135	47.278	5.651
	Sig.	0	0		0	0.099	0	0	0	0	0	0.001
6	F	1.244	1.52	33.538		22.473	0.36	0.126	4.176	5.403	6.389	18.48
	Sig.	0.301	0.207	0		0	0.836	0.973	0.005	0.001	0	0
7	F	22.572	25.292	2.043	22.473		25.804	20.903	41.05	26.157	23.083	2.166
	Sig.	0	0	0.099	0		0	0	0	0	0	0.083
12	F	2.021	2.03	42.529	0.36	25.804		0.174	6.896	5.948	7.255	22.61
	Sig.	0.102	0.101	0	0.836	0		0.951	0	0	0	0
14	F	1.709	1.758	30.948	0.126	20.903	0.174		5.48	4.22	5.262	16.59
	Sig.	0.159	0.149	0	0.973	0	0.951		0.001	0.004	0.001	0
15	F	0.537	9.035	58.371	4.176	41.05	6.896	5.48		20.094	21.967	40.253
	Sig.	0.709	0	0	0.005	0	0	0.001		0	0	0
19	F	8.349	13.878	51.135	5.403	26.157	5.948	4.22	20.094		0.453	24.971
	Sig.	0	0	0	0.001	0	0	0.004	0		0.77	0
20	F	9.53	13.94	47.278	6.389	23.083	7.255	5.262	21.967	0.453		21.707
	Sig.	0	0	0	0	0	0	0.001	0	0.77		0
23	F	18.272	24.903	5.651	18.48	2.166	22.61	16.59	40.253	24.971	21.707	
	Sig.	0	0	0.001	0	0.083	0	0	0	0	0	

Hauls 1, 3, 6, 12, 14, 15, 19, 10 = *R. kanagurta*. Hauls 4, 7, 23 = *R. brachysoma.* Significant (Sig.) levels are adjusted for multiple comparisons by multiplying by number of comparisons.

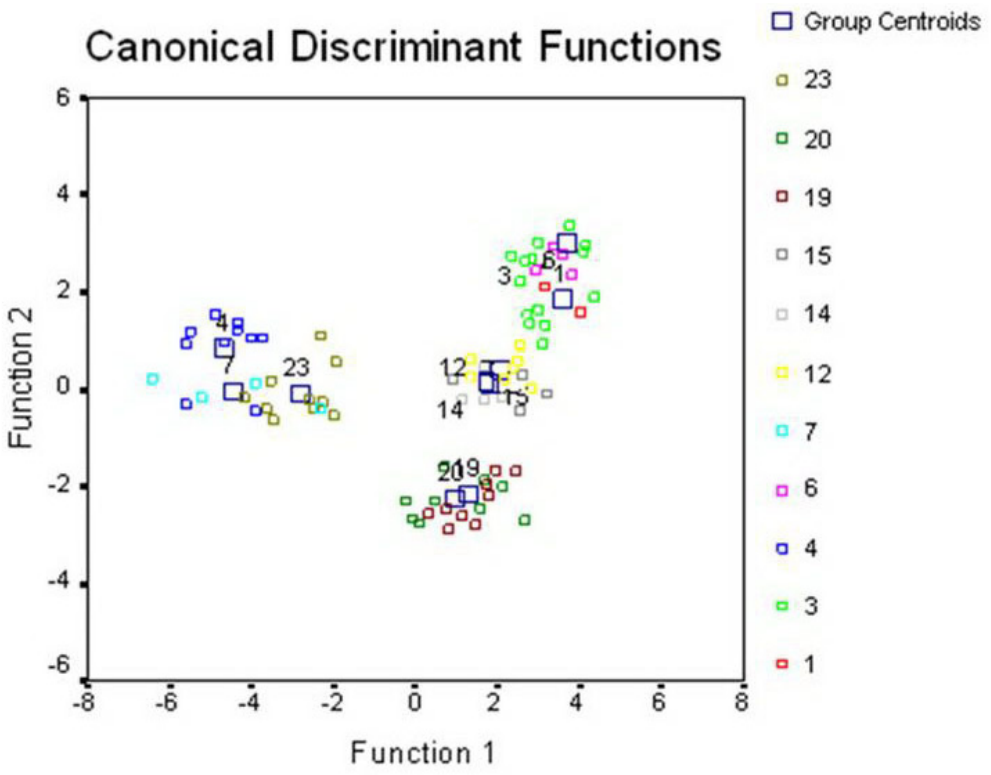

Figure 3. Plots of the coordinates of individuals of *Rastrelliger* spp according to the first two discriminant functions (70 *vs* 14.1% variation) obtained for morphometric data.

Rastrelliger brachysoma showed similar gradient with the northern Hauls 4 and 7 showing some degree of distinction from the more southern Haul 23 found off the coast of Selangor (Figure 3). However, adjustment for multiple group comparison shows non-significance for all comparisons. Haul 15 was significantly different from other populations in its cluster but after similar adjustment its group centroid is not significantly different from Hauls 1, 6 and 14 (Figure 3).

Genetic analysis

Of the 10 RAPD primers screened from Kits OPC (Operon Technology Inc., USA), five produced repeatable amplification products that yielded a total of 47 scorable markers with molecular sizes ranging from 300 to 1200 bp. The number of scorable bands amplified by each primer varied from 8 to 9 in *R. brachysoma* and from 8 to 10 in *R. kanagurta*. The percentage of polymorphic loci showed a high value of 87.2% in *R. kanagurta* but a low of 36.34% in *R. brachysoma* (data not shown). As expected, the UPGMA cluster analysis using Nei and Li's similarity coefficient (Nei and Li, 1979) shows two main clusters comprising *R. kanagurta* and *R. brachysoma* (Figure 4). Within the latter species, a fair degree of structuring was observed, although with some overlap, most of which was

between individuals in the northern area in Hauls 4 and 7 (Figure 4). The extent of structuring was more evident in *R. kanagurta*, almost congruent with those observed in the morphometric analysis with those populations to the north and south off the coast of Perak that form separate cohesive groups (Figures 3 and 4). The main difference is found in Haul 15, which showed different morphometric data (where it grouped with the northern group), but in the phylogenetic analysis, three of its individuals clustered with the southern Hauls 19 and 20, while a single individual was clustered with Haul 12 in the central west coast of Peninsular Malaysia (Figure 4). This suggests that Haul 15 is more genetically related to the southern populations although gene flow to the neighboring Hauls of 12 and 14 still occurs.

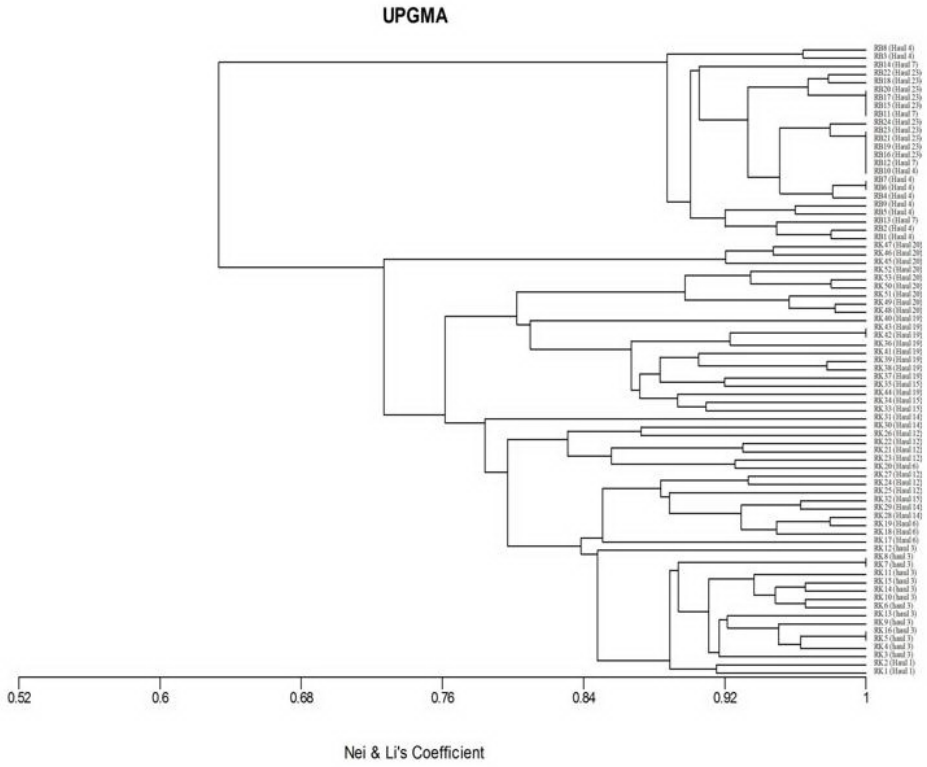

Figure 4. UPGMA dendrogram of 77 individuals from 11 populations of two *Rastrelliger* spp based on Nei and Li's coefficient.

The principal coordinate analysis (PCOA) chart (Figure 5) confirmed the findings shown by the UPGMA analysis. The first principal coordinate axis, which accounted for 39.6% of the total variance, separated the two species but not at the intra-species level. However, the second coordinate axis, which explained 11.7% of the total variation (data not shown), not only differentiated populations north of the coast of Perak from the more southern populations, but also to a large extent from the northern populations (Hauls 1 and 3) and from the central populations (Hauls 12 and 14).

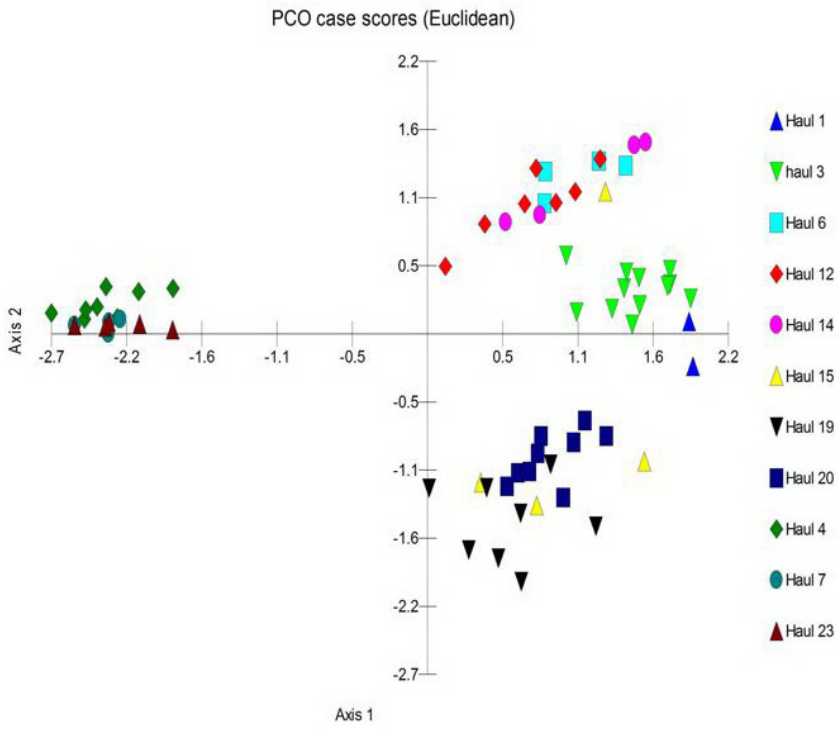

Figure 5. Principal coordinate analysis of RAPD data, based on RAPD profiles of 77 individuals of *Rastrelliger* spp.

DISCUSSION

The present results on truss morphometric analysis indicate the possible existence of two morphologically distinct groups of *R. kanagurta* along the west coast of Peninsular Malaysia. The first group was comprised of populations in the north and the central region off the coast of central Perak and the second of populations just further south off the coast of southern Perak. Within each area the overlapping distribution of samples is probably attributable to extensive migration in these waters. The same trend was observed for *R. brachysoma* (although not as marked, perhaps due to limited sample size). The parallel pattern of differentiation shown by both species suggests the presence of natural or artificial geographical barriers (Palumbi, 1994), which has contributed largely to the emergence of differences between populations existing between the areas in the north and south off the coast of central Perak.

The present study indicated a significant contribution of head-related characters and the anterior part of the body for stock differentiation in *R. kanagurta*. Jayasankar et al. (2004) reported high component loadings for the area encompassing depth between origin of anal fin and that of second dorsal fin and caudal peduncle depth in the same species from India. *Rastrelliger kanagurta* and *R. brachysoma* share similar morphological characters but show differences in head-related variables and anterior part of the body. Such head-related differences would normally suggest the influence of habitat differences between the populations (Palma and Andrade, 2002). Variation in pattern of head morphology is often attributed to

the exploitation of different ecological niches as a result of food availability and type of prey (Hyndes et al., 1997; Delariva and Agostinho, 2001). Water temperature, prolonged swimming and currents (Sarà et al., 1999) have also been suggested to account for these differences although the temperature may not have any bearing in this study. As morphology is especially dependent on environmental conditions during early life-history stages (Lindsey, 1988; Turan, 2004), phenotypic differentiation may indicate that the majority of fish spend their entire lives in separate regions (Turan, 2004; Turan et al., 2006).

The detection of high morphometric differentiation indicates that extensive mixing does not occur between the two zones to the north and south of the coast of central Perak. This is confirmed by the highly significant Mahalanobis distance values observed between the areas (Table 2). Nevertheless, in general, fishes demonstrate greater variance in morphological traits both within and between populations than other vertebrates, and are more susceptible to environmentally induced morphological variation (Allendorf and Leary, 1988; Wimberger, 1992) as mentioned above. However, although environmental factors play a major role in phenotypic discreteness of aggregations, restricted movement may also lead to reproductive isolation resulting in genetic differentiation (Bailey et al., 1999; Begg and Waldman, 1999). Truss morphometric analysis indicated a high degree of homogeneity among populations of Indian mackerel from three different geographical locations (Jayasankar et al., 2004).

The RAPD method suggests that there was a genetic component to the phenotypic differentiation observed between geographic regions. Analysis of the RAPD marker profiles obtained for the *R. kanagurta* samples shows a similar trend to the morphometric studies, although population structuring was more evident and spatially correlated. Both the UPGMA (Figure 4) and PCO analysis (Figure 5) showed three clusters (north, central and another cluster just south of the second cluster off the coast of south Perak), suggesting the existence of a genetic demarcation of populations to the north and south of Central Peninsular Malaysia. A break in the coastal continuum and the complexity of local current systems, which may retard larval dispersal, could explain the degree of heterogeneity observed (Russell et al., 1993; Collins et al., 1994). Oceanographic (currents) and topographical features, physical barriers, spatio-temporal spawning time are some of the factors that have been proposed to prevent gene flow among adjacent and contiguous populations (Wimberger, 1992). Assessment of such factors could act as a starting point for future investigation. In the study reported from India, RAPD markers could not differentiate populations from three geographical locations (Jayasankar et al., 2004).

Another interesting feature of our data is that the genetic structure of the individuals in Haul 15 that was located on the border between the two zones was overlapped (Figure 5). This certainly raises the issue of the formation of a ‘transient’ population within the sampling area, that was thought to move over a broader geographic range (Dahlheim et al., 1997; Ford et al., 1998; Hoelzel et al., 2007). This trend (although insignificant) is also observed in *R. brachysoma* with more overlapping of individuals from those in close proximity (Hauls 4 and 7, Hauls 7 and 23) (Figures 4 and 5). However, the precise mechanism(s) underlying this phenomenon remain unknown, but could be investigated by more intensive sampling around this area in combination with other studies.

Data of genetic variation are now recognized as an important prerequisite towards planning conservation strategies (Darlina et al., 2009). Genetic richness can be assessed by estimating the genetic diversity parameters such as percentage of polymorphic loci and gene

diversity index (Yeh, 2000). The high percentage of polymorphism in *R. kanagurta* compared to *R. brachysoma* suggests that the former species has healthy levels of genetic variation although steps have to be initiated to prevent erosion. The high genetic variability among individuals of *R. kanagurta* was also evident in studies by Jayasankar et al. (2004) and Jamaluddin et al. (2010). However, in the case of *R. brachysoma*, levels of variation were alarmingly low (although based on only five RAPD primers). The observed homogeneity, which generally would suggest extensive migration between the northern region and the coasts off south Perak, is attributed to the sharing of many monomorphic markers. The continual overexploitation of this resource has led to a drastic reduction in population size, as a consequence of monomorphism. During the course of the survey, population numbers and sizes were observed to be very low for *R. brachysoma* (Supplementary Material 1). The presence of variability within species (among populations, and also between individuals within populations) is essential to their ability to survive and to successfully respond to environmental changes (Ryman et al., 1995). Thus, immediate steps should be taken to address the current situation.

This paper further supports the need for gathering and combining genetic with physiological, ecological and oceanographic information when assessing the genetic structure of highly abundant, widely distributed and high gene flow marine fish to facilitate the development of management recommendations. Additional DNA markers, such as mtDNA cytochrome *b* and microsatellites shall be used to analyze genetic polymorphism in mackerel populations from this region. In summary, by the combination of the morphometric protocol and molecular genetics (RAPD) method, two fairly self-contained stocks are observed along the west coast of Peninsular Malaysia to the north off the coast of southern Perak for *R. kanagurta*. As such, conservation strategy should aim at preserving the diversity in each area, as there may already be local adaptation that will be lost if the population is mixed with others.

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REFERENCES

Abaunza P, Murta AG, Campbell N and Cimmaruta R (2008). Considerations on sampling strategies for an holistic approach to stock identification: the example of the HOMSIR project. *Fish. Res.* 89: 104-113.

Allendorf FW and Leary RF (1988). Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conserv. Biol.* 2: 170-184.

Bailey KM (1997). Structural dynamics and ecology of flatfish populations. *J. Sea Res.* 37: 269-280.

Bailey KM, Quinn TJ II, Bentzen P and Grant WS (1999). Population structure and dynamics of walleye Pollock, *Theragra chalcogramma*. *Adv. Mar. Biol.* 37: 179-255.

Begg GA and Waldman JR (1999). An holistic approach to fish stock identification. *Fish. Res.* 43: 35-44.

Collins TM, Wimberger PH and Naylor GJP (1994). Compositional bias, character-state bias, and character-state reconstruction using parsimony. *Syst. Biol.* 43: 482-496.

Dahlheim ME, Ellifrit D and Swenson J (1997). Killer Whales of Southeast Alaska: A Catalogue of Photo Identified Individuals. Day Moon Press, Seattle.

Darlina MN, Kemp SJ, Telfer S and Watts PC (2009). Isolation and characterization of 10 microsatellite loci in the common dormouse *Muscardinus avellanarius*. *Mol. Ecol. Res.* 9: 1010-1012.

Delariva RL and Agostinho AA (2001). Relationship between morphology and diets of six Neotropical loricariids. *J. Fish Biol.*832-847.

FAO (2000). Report: Workshop on the Fishery and Management of Short Mackerel (*Rastrelliger* spp.) on the West Coast of Peninsular Malaysia. Food and Agricultural Organization, Rome.

FAO (2010). Report: First Workshop on the Assessment of Fishery Stock Status in South and Southeast Asia. Food and Agricultural Organization, Rome.

Felsenstein J (1989). PHYLIP, phylogeny interference package (version 3.2). *Cladistics* 5: 164-166.

Fisher W and Bianchi G (1984). FAO Species Identification Sheets for Fishery Purposes. Western Indian Ocean (Fishing Area 51). Vol. 4. Food and Agricultural Organization, Rome.

Ford JKB, Ellis GM, Barrett-Lennard LG and Morton AB (1998). Dietary specialization in two sympatric populations of killer whales (*Orcinus orca*) in coastal British Columbia and adjacent water. *Can. J. Zool.* 76: 1456-1471.

Frankham R (1995). Conservation genetics. *Annu. Rev. Genet.* 29: 305-327.

Froese R and Pauly D (2009). FishBase. World-Wide Web Electronic Publication. Available at [http://www.fishbase.org/search.php]. Accessed October 2009.

Galtier N, Nabholz B, Glemin S and Hurst GD (2009). Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol. Ecol.* 18: 4541-4550.

Hoelzel AR, Hey J, Dahlheim ME and Nicholson C (2007). Evolution of population structure in a highly social top predator, the killer whale. *Mol. Biol. Evol.* 24: 1407-1415.

Hoffmann AA and Willi Y (2008). Detecting genetic responses to environmental change. *Nat. Rev. Genet.* 9: 421-432.

Humphries SE, Whittall R, Minty A and Buckingham M (1981). There are approximately 20 acting genes in the human genome. *Nucleic Acids Res.* 9: 4895-4908.

Hyndes GA, Platell ME and Potter IC (1997). Relationships between diet and body size, mouth morphology, habitat and movement of six sillaginid species in coastal waters: implications for resource partitioning. *Mar. Biol.* 128: 585-598.

Ihssen PE, Bodre HF, Casselman JM and McGlade JM (1981). Stock identification: materials and methods. *Can. J. Fish Aquat. Sci.* 38: 1838-1855.

Jamaluddin JAF, Ahmad AT, Basir S and Rahim MA (2010). *Rastrelliger* systematics inferred from mitochondrial cytochrome *b* sequences. *Afr. J. Biotechnol.* 9: 3063-3067.

Jayasankar P, Thomas PC, Paulton MP and Mathew J (2004). Morphometric and genetic analyzes of Indian mackerel (*Rastrelliger kanagurta*) from peninsular India. *Asian Fish. Sci.* 17: 201-215.

Laikre L, Palm S and Ryman N (2005). Genetic population structure of fishes: implications for coastal zone management. *Ambio* 34: 111-119.

Lindsey CC (1988). Factors Controlling Meristic Variation. Academic Press, San Diego.

Lowe A, Harris S and Ashton P (2004). Ecological Genetics: Design, Analysis, and Application. Blackwell Publishing, Oxford.

Matsui T (1967). Review of the mackerel genera *Scomber* and *Rastrelliger* with description of a new of *Rastrelliger*. *Copeia* 1: 71-83.

Mohd Nor SA, Abu Talib A, Mohd Ghows MA and Samsudin B (2008). A preliminary genetic investigation of *Rastrelliger kanagurta* based on RAPD and mitochondrial ND2 gene. *Wetland Sci.* 6: 518-525.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.

Nei M and Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. U. S. A.* 76: 5269-5273.

Palma J and Andrade JP (2002). Morphological study of *Diplodus sargus*, *Diplodus puntazzo*, and *Lithognathus mormyrus* (Sparidae) in the Eastern Atlantic and Mediterranean Sea. *Fish. Res.* 57: 1-8.

Palumbi SR (1994). Genetic divergence, reproductive isolation, and marine speciation. *Ann. Rev. Ecol. Syst.* 25: 547-572.

Rao R and Hoskela J (2001). Actions Plan and Research Needs to Conserve Forest Genetic Resources in Asia. In: Forest Genetic Resources: Status, Threats and Conservation Strategies (UmaShaanker R, Ganeshaiah KN and Bawa KS, eds.). Oxford and IBH Publishing Co. Pvt. Ltd., Calcutta, 283-301.

Reed DH (2004). Extinction risk in fragmented habitats. *Anim. Conserv.* 7: 181-191.

Reed DH, Briscoe DA and Frankham R (2002). Inbreeding and extinction: the effect of environmental stress and lineage. *Genetics* 3: 301-307.

Russell JR, Hosein F, Johnson E, Waugh R, et al. (1993). Genetic differentiation of cocoa (*Theobroma cacao* L.) populations revealed by RAPD analysis. *Mol. Ecol.* 2: 89-97.

Ryman N, Jorde PE and Laikre L (1995). Supportive breeding and variance effective population size. *Conserv. Biol.* 9: 1619-1628.

Sarà M, Favarolo E and Mazzola A (1999). Comparative morphometrics of sharpsnout seabream (*Diplodus puntazzo*

Cetti, 1777), reared in different conditions. *Aquacult. Eng.* 19: 195-209.
Schlotterer C (2004). The evolution of molecular markers - just a matter of fashion? *Nat. Rev. Genet.* 5: 63-69.
Seshappa G (1985). On the homogeneity of the mackerel population at Calicut during the years1969 to 1976 as determined on the basis of C/L, C/W and TL/SL ratios. *Indian J. Fish.* 32: 359-374.
Smith PJ and Jamieson A (1986). Stock discreteness in herrings: a conceptual revolution. *Fish. Res.* 4: 223-234.
Sneath PHA and Sokal RR (1973). Numerical Taxonomy: The Principles and Practice of Numerical Classification. W.H. Freeman & Co., San Francisco.
Turan C (2004). Stock identification of Mediterranean horse mackerel (*Trachurus mediterraneus*) using morphometric and meristic characters. *ICES J. Mar. Sci.* 61: 774-781.
Turan C, Oral M, Öztürk B and Düzgünes E (2006). Morphometric and meristic variation between stocks of Bluefish (*Pomatomus saltatrix*) in the Black, Marmara, Aegean and northeastern Mediterranean Seas. *Fish. Res.* 79: 139-147.
Wimberger PH (1992). Plasticity of fish body shape: the effects of diet, development, family and age in two species of *Geophagus* (Pisces: Cichlidae). *Biol. J. Linn. Soc. Lond.* 45: 197-218.
Yap IV and Nelson RJ (1996). WinBoot: A Program for Performing Bootstrap Analysis of Binarydata to Determine the Confidence Limits of UPGMA-Based Dendrograms. International Rice Research Instititute, Manila.
Yeh FC (2000). Population Genetics. In: Forest Conservation Genetics (Young A, Boshier D and Boyle T, eds.). CSIRO Publishing, United Kingdom.
Yeh FC, Boyle TYZ and XIYAN JM (1999). POPGENE Version 131. Microsoft Window-Based-Freeware for Population Genetic Analysis. University of Alberta and Center for International Forestry Research, Edmonton, 29. Available at [http://www.ualberta.ca/~fyeh/]. Accessed August 19, 2006.

SUPPLEMENTARY MATERIAL

Supplementary Material 1. Sampling details of *Rastrelliger kanagurta* and *R. brachysoma* used in the study.

Species	Sample size	Location (Haul No.)	Date of capture
Rastrelliger kanagurta	2	1	24/02/06
	14	3	25/02/06
	4	6	27/02/06
	7	12	04/03/06
	4	14	05/03/06
	4	15	05/03/06
	9	19	08/03/06
	9	20	10/03/06
Total specimens	53	-	-
Rastrelliger brachysoma	10	4	26/02/06
	4	7	27/02/06
	10	23	13/03/06
Total specimens	24	-	-
Grand total of specimens	77	-	-

Supplementary Material 2. Percentage of individuals reclassified in each group.

Haul	Predicted group membership (%)										
	1	3	4	6	7	12	14	15	19	20	23
1	2	0	0	0	0	0	0	0	0	0	0
3	0	13	0	0	0	1	0	0	0	0	0
4	0	0	10	0	0	0	0	0	0	0	0
6	0	0	0	4	0	0	0	0	0	0	0
7	0	0	0	0	4	0	0	0	0	0	0
12	0	1	0	0	0	6	0	0	0	0	0
14	0	0	0	0	0	2	2	0	0	0	0
15	0	0	0	0	0	1	0	3	0	0	0
19	0	0	0	0	0	2	0	0	5	2	0
20	0	1	0	0	0	0	0	0	1	7	0
23	0	0	0	0	3	0	0	0	0	0	7
1	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	92.9	0.0	0.0	0.0	7.1	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	14.3	0.0	0.0	0.0	85.7	0.0	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0	0.0	50.0	50.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0	25.0	0.0	75.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	22.2	0.0	0.0	55.6	22.2	0.0
20	0.0	11.1	0.0	0.0	0.0	0.0	0.0	0.0	11.1	77.8	0.0
23	0.0	0.0	0.0	0.0	30.0	0.0	0.0	0.0	0.0	0.0	70.0

