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Genetic structure of round scad mackerel *Decapterus macrosoma* (Carangidae) in the Indo-Malay archipelago

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Abstract Round scad mackerel sampled in 1995-1998 were analysed for genetic variation using mitochondrial and nuclear-DNA markers. Sequence variation for a fragment of the *cytochrome b* gene (mitochondrial), amplified by polymerase chain reaction, was screened across individuals using single strand conformation polymorphism (SSCP). Sequence analysis of all SSCP haplotypes indicated two mitochondrial clades separated by 2.3% nucleotide divergence on the average. The geographic distribution of haplotypes was homogeneous (Weir and Cockerham's $\hat{\theta} = -0.002$). Also, no geographical heterogeneity was detected for length polymorphism of Intron 1 of the gene encoding aldolase B ($\hat{\theta} = 0.005$). While homogeneity in allele frequencies throughout the Indo-Malay archipelago conformed to the expectations for a widely distributed pelagic fish in a highly connected habitat, this was at variance with the sharp geographic structure previously uncovered in Indian scad mackerel, *D. russelli*, a fish with life-history characteristics similar to *D. macrosoma*. A remarkable similarity however was the occurrence of two similarly distinct clades within each species, suggesting a common history of geographic isolation. Low sea levels in the Pleistocene might have caused the separation and vicariance of populations within both *D. macrosoma* and *D. russelli*. Subsequent genetic exchange between populations would then have erased allele-frequency differences at the *cytochrome b* and *aldolase B* loci in *D. macrosoma* while some barrier to gene flow was maintained in *D. russelli*.

Introduction

Pelagic marine fishes usually have high fecundity, very large population size, 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. Genetic evidence nevertheless points to sharp population structure in some such organisms (e.g. Kotoulas et al. 1995; O'Connell et al. 1998; Naciri et al. 1999). Such examples are significant from both fundamental and applied perspectives. These indicate that high dispersal ability does not necessarily warrant geographical homogeneity in that unaccounted factors currently impede gene flow between populations, or alternatively that contemporary population structure is at a transient, non-equilibrium state inherited from past allopatric differentiation. Assessing population structure also is the basis for stock delineation in sound fisheries management.

Vicariance in pelagic fishes can result from tectonics, climatic changes and corresponding shifts to refugia, or changes in sea-level. Pleistocene glaciations have had dramatic influence on the geography of the Central Indo-West Pacific region, where the Sunda and Sahul shelves emerged as broad geographic barriers partly isolating the Indian ocean from the West Pacific and enclosing the South China sea, the Sulu sea and the Sulawesi sea (Voris 2001). A growing literature documents the impact of Pleistocene changes of sea level in the central Indo-West Pacific on the genetic structure of Indo-Pacific species (e.g. McMillan and Palumbi 1995; Benzie 1998; Barber et al. 2000). Most of these examples however refer to shore fishes or to sedentary invertebrates whose primary dispersal potential stems from their pelagic larval stage. Pelagic fishes spend their entire life in the open sea. Pleistocene changes in sea level may have caused temporary geographic isolation but it is expected that in the subsequent secondary contact between populations, the active dispersal in this continuous, pelagic habitat is more likely to have erased past genetic discontinuities than in the case of sedentary coastal species.

Round scad mackerel or *layang deles*, *Decapterus macrosoma* Bleeker, 1851, is a widely distributed, abundant pelagic fish of much economic interest to South-East Asian countries. This species and the Indian scad mackerel or *layang biasa*, *D. russelli* (Rüppell, 1830), have received some attention concerning the management of their fisheries (Hardenberg 1937; Widodo 1988; Sadhotomo 1998). Round scad mackerel have pelagic eggs and larvae (Delsman 1926) and adults are thought to undertake extensive seasonal migrations in and out of the shallow seas of the Sunda shelf (Hardenberg 1937). Hardenberg (1937) suggested that at least two stocks of *layang* (a

name that refers to both *D. macrosoma* and *D. russelli*) are present in the Java sea and adjacent seas. One stock may originate from the Flores sea and Makassar strait, reaching the central-western part of the Java sea during the East monsoon; the other may originate in the Indian ocean and the Sunda strait, entering the western part of the of the Java sea during the West (wet) monsoon. Hardenberg (1937) also speculated that a third *layang* stock occupies the southern part of the South China sea, entering the northwestern part of the Java sea during the wet monsoon. Seasonal, monsoon-driven circulation from East to West and vice-versa in addition to the intense North-to-South Indo-Pacific throughflow (Fieux et al. 1994), suggest that extensive transportation of eggs and larvae may occur throughout the Indo-Malay archipelago.

Here, the population structure of *Decapterus macrosoma* of the Indo-Malay archipelago was analysed from nuclear-DNA variation at an intron locus and mitochondrial-DNA haplotype frequencies. The phylogeographic structure of *D. macrosoma* was also compared with that of *D. russelli* (Perrin and Borsa 2001). Similarities in genetic architecture in the two species would point to past geological or climatic events of biogeographic significance.

Materials and methods

Round scad mackerel were sampled in 1995, 1997 and 1998 from small-sized purse seiners operating on a daily basis in the vicinity of 7 landing places in the Indo-Malay archipelago [Kelang, Carita, Muara, Kota Kinabalu, Sandakan, Toli-Toli, and Tulehu (Fig. 1; Table 1)] or from medium-sized purse seiners on board of which an observer/collector was present (Sunda, Tambelan, and Lumu-Lumu). Total genomic DNA was extracted using the phenol/chloroform protocol (Sambrook et al. 1989) from alcohol-preserved muscle. Four of these samples (Sunda, Tambelan, Lumu-Lumu, and Toli-Toli) were formerly screened for restriction fragment length polymorphism (RFLP) of their control region (Arnaud et al. 1999).

A nuclear marker, Intron 1 of the *aldolase B* gene was amplified by polymerase chain reaction (PCR) using primers *1F* (5' – CAAGATGACTCACCA GTTCCC – 3') and *1.2R* (5' – CTCGTGGAAGAAGATGATCCCGCC – 3') designed from the alignment of, respectively, the first and the second exon of sea bass, *Dicentrarchus labrax* *aldolase B* cDNA (Lemaire 2001) with that of sea bream, *Sparus aurata* (GenBank access no. X82278) and that of salmon, *Salmo salar* (GenBank access no. AF067796). Just before the PCR, primer *1.2R* was radioactively labelled by incubating for 30 min at 37°C a mixture of 2 µM primer, 1 U *T4* polynucleotide-kinase (Eurogentec, Liège, Belgium), and 1.7 µM (1.85 MBq) [γ -³²P]ATP (Isotopchim, Ganagobie, France). Ten microliters of reaction mixture comprising 4 µl DNA solution, 1.5 mM MgCl₂, 74 µM each dNTP, 0.4 µM *1F* primer, 0.1 µM radioactively labelled *1.2R* primer, and 0.4 U *Taq* polymerase (Promega, Madison WI, USA) in its buffer, was subjected to 35 cycles of DNA denaturing (20 s at 94°C), annealing (12 s at 52°C) and elongating (12 s at 72°C) followed by a final elongation step of 5 min at 72°C. The PCR product was mixed with an equal volume of 95% formamide, 20mM EDTA, 0.05% xylene cyanol and 0.05% bromophenol blue, and heat (95°C)-denatured. Size-polymorphism of *aldolase B* Intron 1 was assessed by subjecting the denatured PCR product to electrophoresis in 0.4-mm thick polyacrylamide gel with 5% acrylamide and 42% urea. After migration, at 50 W for 5 hours, the gel was dried at 80°C for 1 hour in a vacuum drier and exposed against X-Omat autoradiographic film (Eastman-Kodak, Rochester NY, USA) for 12 hours or more depending on the level of radiation. Due to time constraints only 6/10 samples were screened for genetic variation at *aldolaseB-1*.

A 355-base pair fragment of the mitochondrial DNA *cytochrome b* gene was amplified by polymerase chain reaction (PCR) using universal primers *CTB* (5' – CATCCAACATCTCAGCATGATGAAA – 3') and *PAT* (5' – CCCTCAGAATGATATTTGTCCTCA – 3') modified from, respectively, primers *CB1-L* and *CB2-H* (Palumbi et al. 1991). PCR amplifications were carried out in 25 µl reaction mixture containing about 50-200 ng DNA, 0.8 mM dNTP mix, 2.5 mM MgCl₂, 1 × *Taq* buffer (Promega, Madison, USA), 0.5 µM each primer and 0.25 unit *Taq* DNA polymerase (Promega). Thirty-five cycles of PCR (denaturation at 94°C for 12 s, primer annealing at 53°C for 12 s, primer extension at 72°C for 20 s) were run in a Crocodile III thermocycler (Appligène, Strasbourg, France). Sequence polymorphism was detected by single-strand DNA conformation polymorphism (SSCP) analysis (Orita et al. 1989), an approach tested in other fish species (e.g. Borsa and Quignard 2001). The single-strand DNAs were run in 1× TBE buffer at 18°C

for 15 hrs at 2 W in non-denaturing polyacrylamide gel 1× MDE (FMC corporation, Rockland, USA), then silver-stained according to Wray et al. (1981). All SSCP variants were sequenced using the Thermosequenase kit (Amersham Life Science, Cleveland OH, USA) with ³³P – labelled dideoxynucleotides (Amersham). The sequencing reaction was done according to the instructions provided with the Thermosequenase kit using either primer *PAT* or *CTB*, on 5 µl of each PCR product purified for 15 min at 37°C by addition of 10 units of exonuclease I and 2 units of shrimp alkaline phosphatase (Amersham).

aldolase B-1 size-allele and *cytochrome b* haplotype frequency differences between populations were estimated using $\hat{\theta}$, Weir and Cockerham's (1984) estimator for the apportioning of genetic diversity among samples relative to the total diversity. Departures from genotype frequencies expected at *aldolase B-1* under panmixia were estimated using Weir and Cockerham's (1984) \hat{f} multiallelic estimator. f and θ are Weir and Cockerham's equivalents of S. Wright's *F_{is}* and *F_{st}* parameters, respectively. Tests of significance were done by comparing the observed \hat{f} - or $\hat{\theta}$ -value with 1000 pseudo- \hat{f} or pseudo- $\hat{\theta}$ calculated by random permutations of alleles using GENETIX (Belkhir et al. 1996). The probability of the observation was estimated as $p = (n+1)/(N+1)$ where n is the number of pseudo- $|\hat{f}|$ or pseudo- $\hat{\theta}$ equal or greater than the observed $|\hat{f}|$ or $\hat{\theta}$, and N , the number of permutations (Sokal and Rohlf 1995).

Phylogenetic inference on *cytochrome b* sequence data was done using the computer packages PHYLIP 3.57 (Felsenstein 1995) and MEGA2 (Kumar et al. 2001). Sequences of two, distinct homologous fragments in *Decapterus macarellus* (Cuvier, 1833) from South Maluku and Makassar were chosen as outgroups. Pairwise nucleotide distances between haplotypes were estimated using Kimura's (1980) 2-parameter model (procedure DNADIST of PHYLIP) with the ratio of transitions to transversions set empirically from sequence data.

Results

One hundred and fifty-one round scad mackerel were screened for allele-size variation at the *aldolase B Intron 1* locus. Eight size-alleles were detected, ranging in size from ca. 290 base pairs (bp) to ca. 310 bp. The most common allele in the total sample (100), of 298 bp, had a frequency per sample ranging from 0.60 to 0.77. Partial nucleotide sequences of two alleles belonging to size-allele 100 have been deposited in GenBank (accession nos. AF500104 and AF500105). Heterozygote deficiencies or excesses were not significant as indicated by \hat{f} values estimated from each sample (Table 1). No heterogeneity in size-allele frequency was evident at the scale of the Indo-Malay archipelago ($\hat{\theta}=0.005$; 1000 permutations, $p>0.24$).

Thirteen SSCP phenotypes were detected in the total sample of 302 round scad mackerel screened for sequence polymorphism at the *cytochrome b* locus. Phenotype *A* was dominant in frequency (≥ 0.80) throughout the Indo-Malay archipelago (Table 1). Of the other 12 haplotypes detected in the total sample, only three (α s, ν , and σ) were encountered in at least two samples. There was no evidence of heterogeneity in the distribution of haplotypes throughout the Indo-Malay archipelago ($\hat{\theta} = -0.002$; 1000 permutations, $p>0.49$).

For testing Hardenberg's (1937) hypothesis of three stocks in the western part of the Indo-Malay archipelago (Java sea, South China sea, Indian ocean), Weir and Cokerham's θ was estimated across samples Carita, Kota Kinabalu and Muara, respectively. No heterogeneity in allele frequency was detected among populations at that geographical scale ($\hat{\theta}=0.010$ for *cytochrome b*; $\hat{\theta} = -0.008$ for *aldolase B-1*; $\hat{\theta} = -0.005$ overall ; none of these values was statistically significant).

All 8 individuals of SSCP phenotype ν , both individuals of SSCP phenotype α ss, and subsamples of respectively 6 individuals of SSCP phenotype *A* and 4 individuals of SSCP phenotype σ were sequenced. There was no sequence variation among individuals within any of these SSCP phenotypes. All individuals characterised by SSCP phenotypes other than any of the foregoing were sequenced and the haplotypes named afterwards.

Nucleotide sequences for ingroup (GenBank accession nos. AF390868 to AF390880) and outgroup taxa for a 309-bp segment of the cytochrome *b* gene were aligned. Of 299 aligned positions for *Decapterus macrosoma* and *D. macarellus* (Fig. 2), 40 were variable, among which 28 were phylogenetically informative. Transitions were more frequent than transversions with an average transition/transversion ratio of 3.1. This empirical value was used to estimate nucleotide distances between haplotypes according to Kimura's 2-parameter model. The Neighbour-Joining phylogenetic tree generated from the matrix of nucleotide distances, rooted by *D. macarellus* (Fig. 3), suggested two distinct mitochondrial lineages (haplotype σ vs. all the others, collectively referred to as '*D. macrosoma* clade A' hereafter). The two lineages were on the average distant from each other by ca. 2.3% (range: 1.6% to 2.7%) nucleotide divergence. *D. macrosoma* clade A was supported by 65% bootstrap score (1000 bootstrap resamplings of nucleotide sites; Felsenstein 1985). The Maximum-Parsimony trees generated using MEGA2 (Kumar et al. 2001) and the Neighbor-Joining tree had identical topologies.

Discussion

No significant heterogeneity in *cytochrome b* haplotype frequencies or in *aldolase B-1* allele frequencies was detected across *Decapterus macrosoma* populations from the Indo-Malay archipelago. In particular, the data failed to support Hardenberg's (1937) hypothesis of three stocks in the western part of the Indo-Malay archipelago. Haplotype-frequency data from a preliminary study using RFLP of the control region of the mitochondrial DNA suggested that the Sunda strait was a transition zone between genetically different populations of round scad mackerel (Arnaud et al. 1999). There was no evidence of genetic differences among Sunda strait samples, or between the Sunda strait region and the rest of the Indo-Malay archipelago when using the *cytochrome b* marker. The nucleotide diversity estimated from RFLPs of the control region [0.000 to 0.005 in 4 *D. macrosoma* samples analysed by Arnaud et al. (1999)] appeared to be slightly larger than nucleotide diversity estimates derived from *cytochrome b* gene sequence data on the same four samples (0.000 to 0.002). Slightly higher mutation rates in the control region combined with insufficient sample size ($N=3$ for the Sunda strait sample) may explain why slight genetic differences went undetected using the *cytochrome b* marker. For a more comprehensive analysis of mitochondrial genetic variation in *D. macrosoma*, the sampling design of future surveys should address a much broader geographical scale, so as to include samples from the Indian ocean in particular. Also, it may be advantageous to use sequence variation of the control region in addition to the *cytochrome b* locus.

Decapterus macrosoma travel large distances as eggs, larvae (Delsman 1926), and adults (Hardenberg 1937). Lack of significant haplotype-frequency differences may be attributable to genetic homogeneity among putative spawning populations, or to a lack of statistical power to detect a true, subtle difference. In contrast, considerable geographical heterogeneity in *cytochrome b* haplotype frequency [Nei's (1973) $G_{st}=0.416$] was observed in *D. russelli* (Perrin and Borsa 2001). The sampling design in this study was essentially comparable to that of Perrin and Borsa (2001) on *D. russelli*. The geographic range of sampling, the number of samples, and the sample sizes in *D. macrosoma* (this report) and *D. russelli* (Perrin and Borsa 2001) were comparable; the mitochondrial genetic marker and the techniques for the analysis of sequence variation were identical. The differences in genetic structure between *D. macrosoma* and *D. russelli* may therefore be ascribed to differences in biology, or in fine-scale habitat.

The phylogenies of the *cytochrome b* gene of each species nevertheless showed remarkable similarity, which can be explained by a common history of geographic isolation and subsequent secondary contact. Pleistocene lows in sea level have repeatedly left the Sunda shelf above sea-level (Voris 2000). These alterations have reduced the potential habitat of coastal pelagic species including *Decapterus macrosoma* and *D. russelli*, likely causing reduction in population size as well as the geographic isolation of Indian ocean scad mackerel populations from those of the western Pacific ocean and those of enclosed seas amidst the Indo-Malay archipelago. The amount of nucleotide divergence between *D. macrosoma* clades A and σ (2.3%) suggests Pleistocene vicariance (Martin and Palumbi 1993; Johns and Avise 1998). This is of the same magnitude as the divergence value at the *cytochrome b* locus between the two clades, "A" and "M", of *D. russelli* (2.2%; Perrin and Borsa 2001). Sharp geographic heterogeneity in the frequencies of the two distinct *cytochrome b* haplogroups in *D. russelli* led these authors to conclude that two previously isolated *D. russelli* populations came into secondary contact following habitat expansion. *D. macrosoma* populations appear to have undergone a higher degree of secondary population admixture than *D. russelli*.

Genetic analyses of fish species across the Indo-Malay region are still few in number. While no genetic differences were evident for goatfish, *Mulloidichthys vanicolensis*, populations across the Indo-West Pacific (Stepien et al. 1994), two other surveys reported geographic disjunction between Indian and West Pacific mitochondrial lineages and populations of inshore fishes, which was thought to be reminiscent of Pleistocene isolation. A large phylogeographic discontinuity was reported for barramundi, *Lates calcarifer*, on either side of the Torres Straits (Chenoweth et al. 1998), that is, on either side of the Sahul shelf which formed a land barrier connecting Australia and New Guinea through the Pleistocene (Voris 2001). Admixture of haplotypes from two distinct mitochondrial lineages was interpreted as evidence for recent secondary introgression in barramundi (Chenoweth et al. 1998). The phylogeographic structure of anemonefish *Amphiprion ocellaris*, a coastal sedentary fish with short pelagic larval stage, was also better explained by sea level changes in the Pleistocene than contemporary geography (Nelson et al. 2000). Significant differences in *cytochrome b* haplotype frequencies were found between *A. ocellaris* populations of the western edge of the Sunda shelf (West coast of Sumatra, Malacca strait) and those of the rest of the archipelago, including South China sea, Sunda strait, Bali, Sulu sea, and Sulawesi sea. Gene flow in *A. ocellaris* was inferred to be highest between regions connected by a permanent current that flows through the Makassar strait from the Sulawesi sea to the Bali strait (Fieux et al. 1994)

Such similarities in both the geographic structure and the genetic architecture of mitochondrial lineages in two shore fishes (barramundi and anemonefish) and two closely related pelagic fishes (Indian and round scad mackerels) uncover a general phylogeographic pattern that is compatible with a model of vicariance. Under this biogeographic model, here extrapolated to the infra-specific scale, the high species diversity of the central Indo-West Pacific could be explained by the confluence of species from two areas of endemism which are the Indian ocean and the western Pacific (Woodland 1983; Donaldson 1986). In contrast, other authors have accumulated clues that the Indo-Malay region may be a centre of origin that regularly provides dispersing populations which speciate in the periphery (Briggs 2000, and references therein). Extending the genetic analysis of *Decapterus macrosoma*, *D. russelli*, and other model species with varying degrees of potential mobility to populations from peripheral regions in the Indian and western Pacific oceans will help provide the comprehensive picture of geographic variation that is required to test the confluence hypothesis raised here.

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Table 1 *Decapterus macrosoma*. Details of sampling locations (by increasing longitude) and dates of collection

Location	Oceanic region	Coordinates	Date	Collector
Kelang, Peninsular Malaysia	Malacca Strait	101°17'E; 02°30'N	Dec. 1998	V. Sarpedonti
Sunda Strait, Indonesia	Sunda Strait (Indian Ocean)	105°13'E; 06°05'S	May 1995	M. Potier
Carita, West Java, Indonesia	Sunda Strait (Indian Ocean)	105°42'E; 06°18'S	Oct. 1997	P. Borsa
Tambelan, Indonesia	South China Sea	107°30'E; 01°18'N	April 1995	M. Potier
Muara, Bali, Indonesia	Bali Strait (Indian Ocean)	114°50'E; 08°35'S	Oct. 1997	P. Borsa
Kota Kinabalu, Sabah, Malaysia	South China Sea	115°52'E; 06°12'N	Oct. 1997	P. Borsa
Lumu-Lumu, Indonesia	Makassar Strait	117°15'E; 01°44'S	Feb. 1995	M. Potier
Sandakan, Sabah, Malaysia	Sulu Sea	118°14'E; 05°59'N	Oct. 1997	P. Borsa
Toli-Toli, North Sulawesi, Indonesia	Sulawesi Sea	120°45'E; 01°05'N	May 1995	B. Sadhotomo
Tulehu, Ambon, South Maluku	Banda Sea	128°20'E; 03°42'S	April 1998	R. Andamari

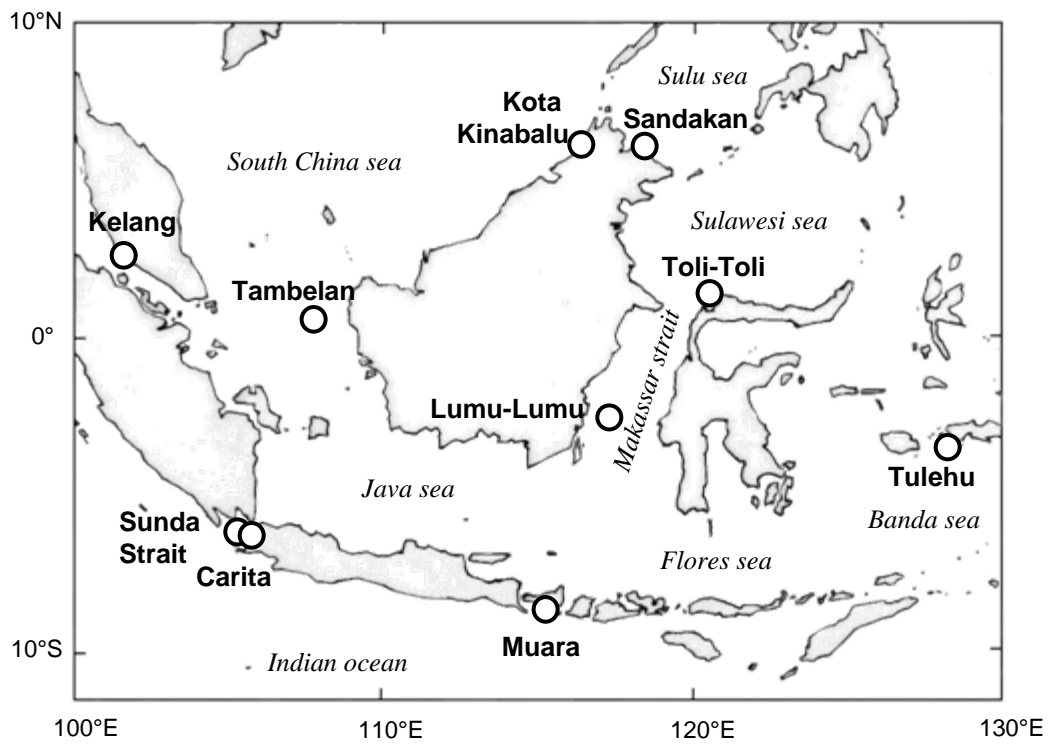
Table 2 *Decapterus macrosoma*. SSCP phenotype frequencies at the *cytochrome b* locus and length-allele frequencies at the *aldolase B-1* locus in 6-10 samples from the Indo-Malay archipelago. Size alleles at Locus *aldolase B-1* were denominated by their electrophoretic mobility relative to the two most common size alleles in the Tulehu sample, arbitrarily chosen as 098 and 100, respectively. *N* sample size; \hat{f} Weir and Cockerham's (1984) estimator of fixation index in a population at Locus *aldolase B-1*; $\hat{\pi}$ nucleotide diversity estimate at Locus *cytochrome b* (Kumar et al. 2001)

Locus, Allele	Sample									
	Kelang	Sunda	Carita	Tambelan	Muara	Kota Kinaba	Lumu-Lumt	Sandakan	Toli-Toli	Tulehu
<i>aldolase B-1</i>										
095			–		–	–		–	–	0.02
098			–		0.16	0.25		0.13	0.32	0.19
100			0.63		0.68	0.60		0.77	0.61	0.67
102			0.25		0.05	0.07		0.05	–	0.05
104			0.13		0.10	0.05		0.03	0.05	0.03
108			–		0.02	0.02		–	–	0.02
110			–		–	0.02		–	0.02	0.02
120			–		–	–		–	0.04	0.02
(<i>N</i>)			(4)		(31)	(30)		(32)	(22)	(32)
\hat{f}			-0.286 ^{NS}		-0.138 ^{NS}	0.023 ^{NS}		-0.158 ^{NS}	0.153 ^{NS}	0.034 ^{NS}
<i>cytochrome b</i>										
<i>A</i>	0.80	1.00	0.88	0.83	0.95	0.89	0.96	0.85	0.92	0.91
<i>aB</i>	–	–	–	–	0.02	–	–	–	–	–
α	–	–	–	–	–	–	–	–	0.03	–
α^{ff}	–	–	–	0.17	–	–	–	–	–	–
α_s	–	–	0.06	–	–	–	–	–	–	0.02
α_{ss}	–	–	–	–	–	–	–	0.04	–	–
α_{LB}	–	–	–	–	–	–	–	–	–	0.02
χ	–	–	–	–	–	0.03	–	–	–	–
κ	–	–	–	–	–	0.03	–	–	–	–
ν	–	–	0.03	–	0.02	0.03	0.02	0.07	0.05	0.02
ν_{LB}	–	–	–	–	–	–	–	–	–	0.02
<i>n</i>	–	–	–	–	–	–	0.02	–	–	–
σ	0.20	–	0.03	–	–	0.03	–	0.04	–	–
(<i>N</i>)	(5)	(3)	(32)	(6)	(42)	(36)	(49)	(46)	(39)	(44)
$\hat{\pi}$	0.0077	0.0000	0.0014	0.0020	0.0002	0.0010	0.0002	0.0016	0.0003	0.0006

Fig. 1 Map of Indo-Malay archipelago with sampling sites for *Decapterus macrosoma*

Fig. 2 *Decapterus macrosoma*. Alignment of partial sequences of the cytochrome *b* gene for all 13 haplotypes detected by SSCP in 299 individuals from the Indo-Malay archipelago. These sequences have been deposited in GenBank under accession numbers AF390868 to AF390880 (1st column). Aligned partial sequences of *D. macarellus* haplotypes 01 and 05 (P. Borsa and C. Poux, unpublished data) are also given. *Dash* no data; *dot* identity to reference sequence

Fig. 3 *Decapterus macrosoma*. Neighbour-joining tree [NEIGHBOR procedure in the PHYLIP package (Felsenstein 1995); tree drawn under TREEVIEW (Page 1996)] of Kimura's (1980) nucleotide distance estimates from partial nucleotide sequences (309 bp) of the cytochrome *b* gene, for 13 haplotypes detected in 10 samples from the Indo-Malay archipelago. Haplotypes 01 and 05 of *D. macarellus* (P. Borsa and C. Poux, unpublished data) were used as outgroups. All *D. macrosoma* sequences were deposited in GenBank (see Legend to Fig. 1). Numbers at a node are scores out of 1000 bootstraps, given as percentages; *above*: bootstrap scores on the Neighbour-Joining tree using procedure SEQBOOT of PHYLIP; *below, italics*: bootstrap scores on the Maximum-Parsimony trees generated by MEGA2 (Kumar et al. 2001). Bootstrap scores <50% have not been reported. Scale bar = 1% nucleotide divergence



A AF390868 ACTTTGGTTC CCTCCTCGCI CTCTGCCTA~~A~~ TTACCCAAA~~A~~ CCTCACTGGG CTCTTCCTCG CCATACACT~~A~~
a_B AF390869
χ AF390870 .T.....
n AF390871 ---.....
α_{ff} AF390872 -.....T.
α AF390873 -----.....
α_{ss} AF390874 -----.....
α_s AF390875
α_{LB} AF390876
κ AF390877 --.....
σ AF390878
ν AF390879
ν_{LB} AF390880
O1 - ---...C...T...C...G...T...
O5 - ---...C...T...C...G...T...

A CACCTCAGAC ATTGCAACTC CTTTTACATC CGTTGCCAC ATCTGCCGAC ACGTAAAT~~T~~ CGGCTGACTC ATCCGAAAC~~A~~
a_B
χ
n
α_{ff}
α
α_{ss}A.....
α_s
α_{LB}A.....
κ
σA.....
ν
ν_{LB}
O1C...C...T...C...A...G...
O5C...C...T...C...A...

A TGCACGCCA~~A~~ CGGCGCATCC TTCTTCTTT~~A~~ TCTGTATTT~~A~~ CCTTCACATC GGTCGAGGCC TTTACTACGC CTCATACCTC
a_BC.....
χ
n
α_{ff}
αC.....
α_{ss}
α_sC.....
α_{LB}
κG.....
σC.....T
ν
ν_{LB}C.....
O1T...C...G...T...A.....
O5 ...T.....C...G...T...A...T...

A TACAAAGAA~~A~~ CCTGAAATAC AGGAGTTGTT CTCTCCTCC TCCTTATGGC AACTGCTTTC GTAGGATATC TCCTTCCTC
a_B
χ
n ...G.....
α_{ff}T.....
α
α_{ss}G.....
α_sG.....
α_{LB}G.....A.....
κ
σA...A...G.....
νA.....
ν_{LB}A.....
O1 ...T...C...C...C...T...A...C...A...C...T...C...C...
O5 ...T...C...C...C...T...A...C...A...C...T...C...C...

