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Patterns of genetic isolation in a widely distributed pelagic fish, the narrow-barred Spanish mackerel (*Scomberomorus commerson*)

CECILE FAUVELOT [1] and PHILIPPE BORSA [2]*

[1] Institut de recherche pour le développement - UR 227, DYNECAR, Laboratoire de Biologie Marine, Université des Antilles et de la Guyane, Pointe-à-Pitre, 97159 Guadeloupe, France

[2] Institut de recherche pour le développement - UR 227, Centre IRD de Montpellier, 911 avenue Agropolis, 34032 Montpellier cedex, France

* Corresponding author. Tel.: +33 4 67636962; E-mail: philippe.borsa@ird.fr

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Although migratory pelagic fishes generally exhibit little geographic differentiation across oceans, as expected from their life-history (broadcast spawning, pelagic larval life, swimming ability of adults) and the assumed homogeneity of the pelagic habitat, exceptions to the rule deserve scrutiny. One such exception is the narrow-barred Spanish mackerel (*Scomberomorus commerson*), where strong genetic heterogeneity at the regional scale has been previously reported. We investigated the genetic composition of *S. commerson* across the Indo-West Pacific range using control-region sequences (including previously published datasets), cytochrome-*b* gene partial sequences, and eight microsatellite loci, to further explore its phylogeographic structure. All haplotypes sampled from the Indo-Malay-Papua archipelago (IMPA) and the southwestern Pacific coalesced into a clade (Clade *II*) that was deeply separated (14.5% nucleotide divergence) from a clade grouping all haplotypes from the Persian Gulf and Oman Sea (Clade *I*). Such a high level of genetic divergence suggested the occurrence of two sister-species. Further phylogeographic partition was evident between the western IMPA and the regions sampled east and south of it, i.e. northern Australia, West Papua, and the Coral Sea. Strong allele-frequency differences were found between local populations in the southwestern Pacific, both at the mitochondrial locus (Φ_{ST}=0.282-0.609) and at microsatellite loci ($\hat{\theta}$=0.202-0.313). Clade *II* consisted of four deeply divergent subclades (9.0-11.8% nucleotide divergence for the control region; 0.3-2.5% divergence at the cytochrome *b* locus). Mitochondrial sub-clades within Clade *II* generally had narrow geographic distribution, demonstrating further genetic isolation. However, one particular haplogroup within Clade *II* was present throughout the central Indo-West Pacific; that haplogroup was found to be sister-group to an haplogroup restricted to West Papua and the Coral Sea, yielding evidence of recent secondary westward colonization. Such a complex structure is in sharp contrast with the generally weak phylogeographic patterns uncovered to date in other widely distributed, large pelagic fishes with pelagic eggs and larvae. We hypothesize that in *S. commerson* and possibly other *Scomberomorus* species, philopatric migration may play a role in maintaining the geographic isolation of populations by annihilating the potential consequences of passive dispersal.

ADDITIONAL KEYWORDS: phylogeography – mitochondrial lineages – microsatellites – Indo-West Pacific – philopatry

INTRODUCTION

Knowledge of population genetic structure and identification of barriers to gene flow in marine organisms are important to understanding genetic-differentiation and speciation processes in the sea. Apart from obvious barriers such as continents, potential barriers to gene flow in the sea include oceanic fronts, temperature and salinity barriers, oligotrophy (as a mortality factor in e.g. drifting larvae), and predation (Palumbi, 1994; Graves, 1998). Genetic differentiation between populations may also arise from spawning asynchrony among populations, retention of eggs and larvae, and adult homing behaviour (Taylor & Hellberg, 2003). An increasing number of marine taxa with high dispersal potential that were once thought to represent a single species distributed over several oceans (references in Briggs, 1960; Briggs, 1974) are now recognized as multiple species, thanks to the development of molecular methods (Knowlton, 1993, 2000; Colborn et al., 2001, and references therein).

Pelagic fishes generally exhibit little geographic differentiation across oceans (Theisen et al., 2008 and references therein), although a few exceptions have been reported (Perrin & Borsa, 2001; Rohfritsch & Borsa, 2005; Lu et al., 2006; Sulaiman & Ovenden, 2009). One such case is the narrow-barred Spanish mackerel (*Scomberomorus commerson* Lacepède 1800) where evidence of strong population structure has been reported (Buckworth et al., 2007) and where preliminary phylogeographic investigations have suggested a possible phylogeographic gap coinciding with Wallace's Line (Sulaiman & Ovenden, 2009). *S. commerson* is an inshore pelagic species capable of long migrations (Collette & Russo 1984). At the basis of important commercial, recreational and artisanal fisheries, the annual world catch of *S. commerson* has steadily increased from less than 70,000 tons in the seventies to over 220,000 tons in 2008 (FAO - Fisheries and Aquaculture Information and Statistics Service; http://www.fao.org/fishery/species/3280/en). Spanish mackerels (genus *Scomberomorus*) constitute the most speciose group in the family Scombridae (Collette & Russo, 1984). Eighteen species are currently recognized in that genus, the majority of which have a geographic distribution limited to a single ocean basin (Collette & Russo, 1984). The distribution of *S. commerson* spans more than 160° longitude and 80° latitude in the Indo-West Pacific (Fig. 1) whereas all other *Scomberomorus* species are distributed within 80° longitude and 80° latitude, and often much less (Collette & Russo, 1984). Therefore, the wide geographic distribution of *S. commerson* contrasts with that of all other species in the genus *Scomberomorus*. Since species in the genus *Scomberomorus* share a similar morphology, similar ecological traits, and apparently similar abilities for migration, this contrast in their respective geographic distributions is remarkable. A possible explanation might be that *S. commerson* actually consists of two or more cryptic species, each with narrower geographic distribution. Significant morphometric differences have been noted between populations across the Indo-Pacific (Collette & Russo, 1984).

Although *S. commerson* is usually regarded as a highly migratory fish (Collette & Russo 1984, and references therein), significant allozyme-genetic differences have also been reported between populations at the regional scale. Three main stocks, centered on northern Australia, Papua New Guinea and Fiji (Shaklee, Phelps & Salini, 1990) and of a fourth stock off Queensland (J.B. Shaklee in Buckworth et al., 2007) have been delineated. Based on sequence polymorphism of the mitochondrial control region, Sulaiman & Ovenden (2009) hypothesized an east-west phylogeographic division within the IMPA. Conversely, no genetic differences were detected at the same locus, among samples from the Persian Gulf and the Oman Sea (Hoolihan, Anandh & van Herwerden, 2006).

So far, no population genetic study of *S. commerson* has covered a significant part of its distribution and, to our knowledge, no attempt has been made to combine the results from studies that shared the same genetic markers. The objective of the present paper is to investigate the phylogeography of *S. commerson* at the scale of the Indo-West Pacific to: (1) test the east-west phylogeographical hypothesis of Sulaiman & Ovenden (2009); and (2) examine the possible occurrence of additional distinct populations of *S. commerson*, from the Persian Gulf to New Caledonia, that is, across over 120° (~71%) of its longitudinal range. For this, we analyzed samples from the Indo-Malay-Papua archipelago (Java Sea, Bali, West Papua) and from New Caledonia using mitochondrial and nuclear genetic markers, and merged the resulting

dataset with previously published mitochondrial sequence datasets from the Persian Gulf and Oman Sea (Hoolihan, Anandh & van Herwerden, 2006) and from the central Indo-West Pacific (Sulaiman & Ovenden, 2009).

MATERIALS AND METHODS

SAMPLING

Fin clips were sampled from *Scomberomorus commerson* obtained from fishermen or from retailers at fish landing places in Indonesia (Java Sea, Bali), West Papua and New Caledonia (Fig. 1) in 2007-2009. The 'Java Sea' sample (*JAVA*; *N*=4) was collected at the Muara Angke fish market, Jakarta; the 'Bali' sample (*BALI*; *N*= 6), consisting of fish from the Bali Strait, was collected at the Jimbaran fish market, southern Bali; the 'West Papua' sample (*WPAP*) consisted of 10 individuals fished in Raja Ampat waters and sold at the Sorong fish market, northwestern West Papua; the 'New Caledonia' sample (*NCAL*) consisted of a total of *N*=194 individuals fished in the Belep islands in the northern lagoon of New Caledonia, and off Nepoui (northwestern lagoon of New Caledonia), Nouméa (southwestern lagoon) and Canala (eastern coast), all obtained from local fishermen. Fin clips were preserved in 95% ethanol and shipped to Perpignan, France, for analysis.

MOLECULAR ANALYSIS

Genomic DNA was isolated from fin clip using the Gentra Puregene tissue Kit (Qiagen, Germantown, USA) following the manufacturer's protocol. Amplification of the highly variable 5' end of the mitochondrial control region (380-383 bp) was done by polymerase-chain reaction (PCR) using the universal *CR-A* (TTC CAC CTC TAA CTC CCA AAG CTA G) and *CR-E* (CCT GAA GTA GGA ACC AGA TG) primers (Lee et al., 1995). Each PCR was done in 25 µL reaction mixture containing 1X PCR buffer (Promega Corporation, Madison, USA), 0.5 mM $MgCl_2$, 0.08 mM of each dNTP, 0.2 µM of each primer, 0.5 U of GoTaq™ DNA Polymerase (Promega Corporation) and about 30 ng genomic DNA. The amplification of the control-region fragment was achieved by 35 cycles of denaturation (30 s at 94°C), annealing (30 s at 51°C), and extension (1 min at 72°C).

A random subsample of individuals from the Java Sea (*N*=4), Bali (*N*=6), West Papua (*N*=7) and a subsample of New Caledonia (*N*=74; including individuals of Haplogroup *ii* and randomly chosen individuals representing each of the three sub-clades *IIA*, *IIb* and *IId*; see Results) were PCR-amplified for an additional 281-bp fragment of the cytochrome *b* gene, using primers *CB1-L* (5' CCATCCAACATCTCAGCATGATGAAA 3') and modified *CB2-H* (5' CCCTCAGAATGATATTGGTCCTCA 3') (Palumbi et al., 1991). The reaction mixture and PCR parameters were the same as those used for the control region. PCR products were sent to GATC Biotech (Konstanz, Germany) for nucleotide sequencing: after purification, the PCR-amplified DNA fragments were sequenced using the *CR-A* primer (for the control-region fragment) or the *CB1-L* primer (for the cytochrome *b* gene fragment) and the sequence reaction products were run on an ABI 3730XL automated sequencer (Applied Biosystems, Foster City, USA). All nucleotide sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/) under accession numbers HQ403255 to HQ403349 (cytochrome *b*) and HQ403350 to HQ403561 (control region).

We further amplified alleles at eight microsatellite loci specifically developed for *S. commerson* from the Persian Gulf and the Oman Sea (van Herwerden et al., 2006) in the individuals from Bali, Java, West Papua, and in a sub-sample of 66 randomly selected individuals from New Caledonia. Forward primers were labeled with ABI fluorescent dyes as follows: C83Sc / 6-FAM, L42Sc / 6-FAM, H96Sc / VIC, D61Sc / VIC, J43Sc / PET, E27Sc / PET, F6Sc / NED, J10Sc / NED. All eight microsatellite marker DNAs were amplified in a single 10 µL multiplex PCR reaction using the Type-it Microsatellite PCR Kit (Qiagen) according to the manufacturer's protocol, and using an annealing temperature of 57°C. Amplified fragments were sent to GATC Biotech where they were separated on an ABI 3730XL

sequencer, with a GeneScan® LIZ-500 internal size standard (Applied Biosystems). The GENEMAPPER software (Applied Biosystems) was used to genotype all individuals screened. Finally, GMCONVERT (Faircloth, 2006) was used to convert the GENEMAPPER table of genotypes into a GENEPOP (Raymond & Rousset, 1995) input file.

DATA ANALYSIS

Nucleotide sequences were aligned visually under BIOEDIT (Hall, 1999). A Median-joining parsimony analysis was performed using NETWORK (Bandelt, Forster & Röhl, 1999) on the nucleotide-sequence matrix of 454 individual control region haplotypes aligned over 310 bp, which comprised the 216 new sequences produced in this study, aligned with sequences available from the literature. The latter included the Persian Gulf / Oman Sea dataset of Hoolihan, Anandh & van Herwerden (2006) (193 sequences; GenBank AM234345-AM234537), the Indo-Malay dataset of Sulaiman & Ovenden (2009) (47 sequences re-constructed from their Table 1 and from the single GenBank sequence deposited by the authors, GenBank EU526382, identified by us as being that of haplotype *ScPHI01*), and two sequences from J.R. Ovenden and R. Street (in Buckworth et al., 2007), *ScEC10* from eastern Australia (AY205242) and *ScNA12* from northern Australia (AY205243) which were added to Sulaiman & Ovenden's (2009) samples '*12*' and '*11*', respectively. The root of the network was determined using an outgroup (*S. niphonius*: GenBank nos. FJ69105-FJ659112) by maximum parsimony analysis in MEGA version 5 (Tamura et al., 2011). The choice of *S. niphonius* as outgroup was motivated by its close systematic proximity with *S. commerson* (Collette & Russo, 1984) and by the availability of control-region sequences for this species in GenBank.

To assess rates of genetic divergence between groups of lineages, nucleotide substitution models that best fit mitochondrial control-region and cytochrome *b* sequence data were tested using a model selection analysis based on the maximum-likelihood method implemented in MEGA. Evolutionary models of nucleotide substitution were different relative to mitochondrial DNA fragments. The tests revealed that the model that best fit the mitochondrial control-region data was HKY85 (Hasegawa, Kishino & Yano, 1985) with gamma correction and a heterogeneous proportion of invariable sites. However, because this model was not proposed in MEGA and ARLEQUIN version 3.11 (Excoffier, Laval & Schneider, 2005) for further analysis, we chose to use the second best fit, which was TN93 (Tamura & Nei, 1993) with gamma correction (G = 0.76) and a heterogeneous proportion of invariable sites (I = 0.42). The model that best fit the cytochrome *b* data was Kimura 2-paramaters (Kimura, 1980). Mean net nucleotide divergences, defined as $d_{xy} - 0.5\ (d_x + d_y)$, that subtracts the average 'within group' divergence from the observed 'between group' estimate (Nei and Li, 1979) were then estimated using the appropriate nucleotide substitution model for each sequence set in MEGA. Finally, the Tamura 3-parameter model (Tamura, 1992) with a heterogeneous proportion of invariable sites (I = 0.84) was found to be the best fit for the merged (control region + cytochrome *b* gene) sequence dataset, i.e. composite haplotypes obtained by merging partial sequences of the cytochrome *b* gene and of the control region for samples from Java Sea, Bali and West Papua, and a subsample (*N*=74) from New Caledonia. The homologous haplotype in *S. niphonius* was obtained by merging the homologous sequences from GeneBank (cytochrome *b*: DQ497887; control region: FJ659108) and used as outgroup.

Genetic diversity within regions was estimated as haplotype diversity (*Hd*: Nei, 1987), nucleotide diversity (π: Nei, 1987), and the average number of nucleotide differences between two sequences (k, Tajima 1983) using DNASP version 5.10 (Librado & Rozas, 2009). Tajima's *D* tests (Tajima 1989) were conducted in DNASP by pooling samples within regions. Regions were defined on a geographical basis as follows: Persian Gulf + Oman Sea (samples *AUH*, *BAH*, *DIB*, *IRN*, *KUW*, *OMN* and *RAK* of Fig. 1); East China Sea (*1*); South China Sea + Malacca Strait (*2-8*); Java Sea + Bali (*JAVA*, *BALI*); Timor Sea + Arafura Sea (*9-11*); West Papua (*WPAP*); Coral Sea (*12*, *NCAL*). Observed haplotype diversities were compared to the empirical distribution of this index generated using coalescent simulations (*H* test; Depaulis & Veuille 1998), performed in DNASP. The computer simulations were based on the coalescent process for a neutral infinite-sites model under the assumption of a large constant population size

(Hudson 1990). DNASP generates the empirical distribution of *Hd*, obtained from 10 000 simulations under the neutral coalescent process and given the number of segregating sites observed in each sampled population (the model therefore considers that the number of segregating sites is fixed and that mutations are uniformly distributed, at random, along lineages). DNASP thus estimates the probability of obtaining lower or higher values of *Hd*, computed from the simulation (Hd_{sim}), than the one observed in each sample (Hd_{obs}). These simulations were conducted within regions, pooling samples as described above.

Nucleotide-sequence divergences between populations were estimated using Φ-statistics and the Tamura & Nei (1993) nucleotide substitution model with gamma correction (G=0.76) under ARLEQUIN version 3.11 (Excoffier, Laval & Schneider, 2005). *P*-values were obtained using a non-parametric permutation procedure with 10,000 permutations on the original matrix of sequences. A hierarchical analysis of molecular variance (AMOVA: Excoffier, Smouse & Quattro, 1992) was performed using ARLEQUIN to examine the partitioning of the total variance among regional groups of samples. Significance of Φ-statistics and associated variance components was tested by 10,000 random permutations.

For the microsatellite dataset, genetic diversity within samples was estimated at the eight loci as the observed (H_O) and expected (H_E) heterozygosities in GENETIX version 4.05 (Belkhir et al., 2004). Deviations from Hardy-Weinberg (HW) equilibrium were estimated within and over all samples using Weir & Cockerham's (1984) inbreeding coefficient and departures from HW expectations were assessed using the permutation procedure in GENETIX. Null alleles were detected with MICRO-CHECKER (van Oosterhout et al., 2004). Pairwise genetic divergences among samples were estimated using multilocus Weir & Cockerham's (1984) estimator of F_{ST} ($\hat{\theta}$), and their significance tested with 10,000 permutations of individuals among samples in GENETIX. Correspondence analysis (Benzécri, 1973) was performed on the matrix of individual multilocus genotypes using GENETIX. The sequential Bonferroni correction (Rice, 1989) was applied for each test.

RESULTS

The length of the amplified mitochondrial DNA control-region fragment in samples *JAVA*, *BALI*, *WPAP* and *NCAL* (total N=212) varied between 380 and 383 nucleotides. The nucleotide sequences were unambiguously aligned over 385 bp. Those 212 sequences were then aligned with 242 control-region sequences available from the literature (see Material and Methods) producing a total dataset of 454 sequences aligned over 310 bp. We observed 119 variable nucleotide sites: 148 substitutions were counted, 19 of which were singletons. The G+C content was close to 30%. A total of 221 haplotypes were scored; total haplotype diversity was 0.959, nucleotide diversity was 0.088 and the average number of nucleotide differences between two haplotypes was 24.74.

The Median-joining parsimony network connecting all 221 control-region haplotypes is presented in Figure 2. Maximum parsimony analysis conducted using *Scomberomorus niphonius* sequences as outgroup placed the root of the network between a group comprising all haplotypes sampled in the Persian Gulf and the Oman Sea (hereafter designated as Clade *I*) and the rest of the samples (south-east Asia and Oceania: Clade *II*). Mean net divergence between Clade *I* and Clade *II* was 14.5 ± 3.0%. Clade *II* comprised four main sub-clades (hereafter named *IIa*, *IIb*, *IIc* and *IId*) radiating from a central, poorly defined group of haplotypes (here coined 'Haplogroup *ii*' and delineated by dots on Figure 2) with no bootstrap support. Sub-clade *IIa* consisted of haplotypes exclusively found in New Caledonia. Sub-clade *IIb* comprised haplotypes sampled exclusively in the Coral Sea and in West Papua. Sub-clade *IIc* comprised haplotypes sampled exclusively in the East and South China Seas and adjacent Java Sea. Sub-clade *IId* comprised haplotypes exclusively from northern Australia and the Coral Sea. Haplotypes from the central Haplogroup *ii* were distributed from the South China Sea to New Caledonia and were dominant in the eastern part of the Indo-Malay-Papuan archipelago. Sub-clades *IIa*, *IIc*, and *IId* were strongly supported, with bootstrap scores from 81 to 98%. Sub-clade *IIb* was poorly supported statistically, presumably

because of the low number of mutations that separated it from the central Haplogroup *ii*. Nevertheless, Sub-clade *IIb* was distinct, as it mostly consisted of haplotypes arranged in a star-like fashion around a dominant haplotype.

Expanding the length of the mitochondrial fragment on a subsample of individuals allowed us to resolve the phylogenetic placement of haplotypes included in the above, undefined Haplogroup *ii* (Fig. 3). Clade *II* haplotypes thus clustered into four distinct sub-clades, namely *IIa*, (*IIb*+*ii*) (where Haplogroup *ii* appeared as a sister-branch to *IIb*), *IIc* and *IId* (Fig. 3). Mean net divergence between sub-clades, estimated from control-region nucleotide sequences, varied from 9.0 ± 2.3% (between Sub-clades *IIa* and *IId*), to 11.8 ± 2.8% (between Sub-clades *IIa* and *IIc*). Mean net divergence between subclades estimated from partial cytochrome *b* gene sequences alone ranged from 0.3 ± 0.3% (between sub-clades *IIa* and *IId*) to 2.5 ± 0.9% [between sub-clades (*IIb*+*ii*) and *IIc*].

Genetic divergence estimates among sampling locations, expressed as pairwise Φ_{ST}, ranged from 0 to 0.965 (Table 1). Although, some pairwise Φ_{ST} estimates should be taken with caution because of low sample sizes, e.g. between sample 2 from Thailand and sample 9 from Kupang, West Timor (Φ_{ST}=0.965), generally high levels of genetic differentiation were observed between samples (Table 1). The Persian Gulf / Oman Sea samples were very distinct from all other samples (Φ_{ST}=0.735-0.800). Within the latter group, the West Papuan and New Caledonian samples were themselves much differentiated from all the other samples (Φ_{ST}=0.251- 0.851). Pairwise Φ_{ST} estimates were also generally high within the Coral Triangle, though not significant presumably because of low sample sizes. The AMOVA indicated that a large and highly significant proportion (75%) of the total mitochondrial variance resided among regions (Table 2). Excluding the East China Sea sample (because of its low size) and the Indian Ocean samples, the proportion of the total mitochondrial variance attributed among five geographically defined groups of samples across the Indo-Malay-Papuan archipelago and southwestern Pacific Ocean [(South China Sea + Malacca Strait); (Java Sea + Bali); (Timor Sea + Arafura Sea); West Papua; Coral Sea) was high (46%, $P<<0.001$). A small but significant part of the total variance (1.23%) was observed within groups, while 26% of the total variance was attributed among individuals within samples (Table 2). Within geographically defined regions, estimated haplotype diversity ranged from 0.82 in the Coral Sea to 1 in Java Sea/ Bali, and nucleotide diversity ranged from 0.023 in West Papua to 0.017 in the East China Sea (Table 3). The mean number of nucleotide differences between two sequences ranged from 6.72 in West Papua to 15.26 in South China Sea / Malacca Strait (Table 3). Most samples (here grouped by region) showed higher haplotype diversity than expected from the neutral coalescent theory on the basis of the number of observed segregating sites [Depaulis & Veuille's (1998) haplotype diversity test: Table 3], while none of them deviated from neutrality according to Tajima's *D* test (Table 3). Low sample sizes for some samples, or non-equilibrium conditions may explain this discrepancy.

Genetic diversity estimated from the analysis of the eight microsatellite loci is presented in Table 4. Average observed heterozygosity across all eight loci ranged from 0.521 in Bali to 0.571 in New Caledonia. The distribution of genotype frequencies at a locus did not differ significantly from Hardy-Weinberg expectations (standard Bonferroni correction applied) except sample NCAL at locus *D61Sc* (Weir & Cockerham's $\hat{f} = 0.639$; $P < 0.001$). Null alleles were detected at loci *D61Sc* in New Caledonia, *LA42Sc* in Bali and *E27Sc* in West Papua. The graphical illustration of the correspondence analysis conducted on microsatellite multilocus genotypes revealed geographic partition: individuals from West Papua and New Caledonia formed two completely disjunct clusters, themselves clearly separated from an ensemble including all individuals sampled in Bali and in the Java Sea (Figure 4). So, there was no evidence of interchange of individuals among sub-regions in the southwestern tropical Pacific. Estimates of Weir & Cockerham's parameter of genetic differentiation (θ) were $\hat{\theta}$ =0.233 between Java/Bali and West Papua, $\hat{\theta}$ =0.313 between Java/Bali and New Caledonia, and $\hat{\theta}$ =0.202 between West Papua and New Caledonia.

DISCUSSION

Phylogeographic breaks between Indian and western Pacific populations have been reported for several widely distributed Indo-Pacific species (Benzie, 1999; Barber, Erdmann & Palumbi, 2006; Kochzius & Nuryanto, 2008; Carpenter et al., 2011). Barriers to gene flow have been located within the Indo-Malay-Papuan archipelago (IMPA), although the precise location varies extensively across species (Carpenter et al., 2011). In fishes, phylogeographic studies spanning the IMPA have shown evidence of sub-division between Indian Ocean vs. Pacific Ocean populations in a number of benthic shore species (e.g. Lourie, Green & Vincent, 2005; Drew & Barber, 2009; Carpenter et al., 2011) but not in others (e.g. Stepien, Randall & Rosenblatt, 1994; Klanten, van Herwerden & Choat, 2007; Horne et al., 2008; Gaither et al., 2010; Winters et al., 2010). Since, unlike most benthic fishes, pelagic fishes add extreme adult mobility to passive dispersal at the larval and juvenile stages, it is appropriate to consider them separately: genetic studies of large pelagic fishes such as tunas, wahoo and swordfish have not yielded conclusive evidence of geographic partition across the Indo- Pacific (Alvarado-Bremer et al., 1998; Chow & Takeyama, 2000; Durand et al., 2005; Ely et al., 2005; Gonzales, Beerli & Zardoya, 2008; Theisen et al., 2008; Diaz Jaimes et al., 2010), whereas contrasted patterns have been found in small inshore pelagic fishes like scad mackerels (genus *Decapterus*; Carangidae), from broadscale geographic homogeneity (*D. macrosoma*: Borsa, 2003) to sharp population partition (*D. russelli*: Rohfritsch & Borsa, 2005) within the Indo-Malay-Papua archipelago. A summary of genetic- differentiation index estimates in broadcast-spawning Indo-West Pacific bony fishes of various habitats is presented in Table 5. The level of geographic differentiation in *S. commerson* is much higher than all other pelagic species studied so far, with the possible exception of *Decapterus russelli*, and it is matched only by rare examples of benthic shore species (Table 5).

DISTANT MITOCHONDRIAL CLADES *I* AND *II* INDICATE CRYPTIC SPECIES

Here, deep phylogeographic partition was observed between the *Scomberomorus commerson* population sampled in the Persian Gulf and the Oman Sea, and those populations sampled in the Indo-Malay-Papua archipelago and in the western Pacific. The 14.5% control-region sequence divergence observed between Clade *I* and Clade *II* and its correlation with geography indicate a clear separation between a northwestern Indian Ocean (Persian Gulf / Oman Sea) form of narrow-barred Spanish mackerel, and a western Pacific Ocean form. This level of nucleotide divergence is higher than inter-specific sequence divergences estimated using the same genetic marker between blue and chub mackerels (genus *Scomber*; Scombridae) (1.7-9.6%: Catanese, Manchado & Infante, 2010), among scad mackerels (genus *Decapterus*, Carangidae) (2.7-11.7%: Arnaud, Bonhomme & Borsa, 1999) and among horse mackerels (genus *Trachurus*, Carangidae) (1.0-9.6%: Cárdenas et al., 2005). It is also higher than inter-specific genetic divergences for benthic species, e.g. damselfishes (10.1-12.8%; Timm, Figiel & Kochzius, 2008; Bernardi et al., 2002) and more generally higher than genetic divergence at the inter-specific level in fishes (McCune & Lovejoy, 1998; Lessios, 2008).

The present results suggest that the *S. commerson* form sampled in the Indo-Malay-Papua archipelago and in the western Pacific Ocean is a species different from that sampled in the Persian Gulf and the Oman Sea. The relatively large differences in morphology and in meristic characters reported between *S. commerson* sampled in the Indian Ocean and those from the 'East Indies' (Collette & Russo, 1984; their table 15), are consistent with this hypothesis. Therefore, the large distribution of *S. commerson*, an exception among species of that genus, might well be an artefact that results from the taxonomic confusion of cryptic species.

PHYLOGEOGRAPHIC BREAK WITHIN THE INDO-MALAY-PAPUA ARCHIPELAGO AND FURTHER PARTITIONING AT THE REGIONAL SCALE

Further phylogeographic partition was found within the western Pacific Ocean form of *S. commerson*. Clade *II* haplotypes, which characterize that form, clustered into four distinct sub-clades separated by 0.3-2.5% net nucleotide divergence at the cytochrome *b* locus and 9.0-11.8% for control region. Sub-clade *IIc* was exclusively found, and dominant, in the western half of the Indo-Malay-Papua archipelago (i.e. west to a

line running from Timor to West Papua), whereas Sub-clades *IIa*, and *IId* were exclusively found east and south of a line running from Bali to the Philippines. The root of the cytochrome *b* tree presented in Figure 3 was between Sub-clade *IIc* and the rest, suggesting that the genetic differences between *S. commerson* populations of the western half of the Indo-Malay-Papua archipelago and those east and south of it result from vicariance. Sub-clade *IIa* was sampled in New Caledonia only, which suggests that it may be endemic to New Caledonia, or at least geographically restricted to the southeastern extremity of the range of *S. commerson*. The remaining sub-clade, (*IIb+ii*), itself consisted of two sister-branches (hereafter 'Branch *IIb*' and 'Haplogroup *ii*') with different geographic distributions. Branch *IIb* was dominant in New Caledonia and it was also found in West Papua and in northern Australia. In contrast, Haplogroup *ii* haplotypes were found throughout the Indo-Malay-Papua archipelago and the western Pacific Ocean.

The distinctness of *S. commerson* populations from New Caledonia and West Papua was further demonstrated by the strong pairwise divergence estimate over eight microsatellite loci. We are aware of a few genetic studies in other species of the genus *Scomberomorus* that can be used as a basis of comparison with the present results in *S. commerson* at the regional scale (Table 6). Observed F_{ST} -estimates between populations at the regional scale in *S. commerson* were considerably higher (one order of magnitude for nuclear loci) than those estimated for any other *Scomberomorus* species. This indicated that very little, if any gene flow connects *S. commerson* populations in the tropical southwestern Pacific Ocean. Interestingly, F_{ST} estimates at the same microsatellite loci within *S. commerson* sampled in the Persian Gulf and in the Oman Sea were relatively low (range 0-0.078), though sampled over geographic distances similar to that between Bali and West Papua (van Herwerden et al., 2006).

The clover-like structure of Clade *II* and its relationship to the geographic distribution of haplotypes imply that western Pacific *S. commerson* populations have been isolated from each other for a period of time long enough to achieve reciprocal monophyly. The geographic distribution of haplotypes correlates with their phylogenetic structure, with the exception of Haplogroup *ii* haplotypes. A possible explanation to that intriguing pattern is that a proto-Haplogroup *ii* population initially restricted to the eastern part of the distribution of *S. commerson* secondarily diffused westward to colonize the entire Indo-Malay-Papua archipelago, where it entered into secondary contact with populations harbouring Sub-clade *IIc* haplotypes. The question that follows is whether those two branches are reproductively isolated from each other.

TESTING FOR REPRODUCTIVE ISOLATION

It is possible to test for reproductive isolation between groups of individuals that harbour different mitochondrial types by using nuclear markers, when those groups also happen to occur in sympatry. Microsatellite genotypes were available for three *S. commerson* samples (*BALI*, *WPAP* and *NCAL*) with a heterogeneous mitochondrial composition. Heterozygote deficiency was effectively observed at one locus in the *NCAL* sample, but this was ascribed to null alleles. Therefore, there was no conclusive evidence from microsatellites that the groups of individuals characterized by different Clade-*II* mitochondrial lineages at a given location belong to separate gene pools: hence, one cannot reject the hypothesis that they belong to the same one species, but larger sample sizes of both individuals and loci are needed to further explore the question of reproductive isolation in western Pacific *S. commerson*.

It is generally hypothesized that highly divergent intra-specific lineage originate either from recent admixture of formerly isolated populations or from hybridization and introgression of mtDNA from one species into another (Avise et al., 1987). The co-occurrence of discontinuous mtDNA lineages at a given geographic site ("Category II" of Avise et al., 1987) is not common. This has nevertheless been observed in two other Scombridae species, the bigeye tuna *Thunnus obesus* (Durand et al., 2005; Gonzales, Beerli & Zardoya, 2008) and the carite, *Scomberomorus brasiliensis* (Gold et al., 2010) as well as a few other pelagic fishes (Magoulas et al. 1996; Rosel & Block, 1996; Nesbø et al., 2000; Graves & McDowell, 2003; Viñas, Alvarado Bremer & Pla, 2004; Rohfritsch & Borsa, 2005), and generally is thought to result from secondary contact between formerly geographically isolated populations, although the alternative hypothesis of sympatric divergence has been also proposed. Assuming a mutation rate of approximately

10% per million years for the control-region (Bowen et al., 2006), intraspecific net divergences among sub-clades within western Pacific *S. commerson* (9.0% to 11.8%) would imply that the lineages started to diverge between ca. 450,000 and ca. 590,000 years ago. This dating designates the lowering of sea level within the last five or six Milankovitch glacial cycles as a possible cause for the geographic isolation of *S. commerson* populations in the Indo-West Pacific region. Secondary contact would have occurred during one or another of the subsequent rises in sea level.

DO MIGRATORY CAPABILITIES ENHANCE GEOGRAPHIC STRUCTURE IN *S. COMMERSON*?

The present survey of genetic variation in *S. commerson* revealed deeply divergent mitochondrial clades associated with strong geographic structure at both mitochondrial and nuclear-DNA loci, not only at the scale of the geographic distribution of the species, but also at the regional scale. At the Indo-Pacific scale, highly divergent clades in *S. commerson* and correlated morphological differences are indicative of cryptic species. At the regional scale, our results showed the occurrence of independent populations within the Indo-Malay-Papua archipelago, confirming Sulaiman and Ovenden's (2009) preliminary results, and also within the southwestern Pacific region. Despite having a pelagic lifestyle in both adults and larvae, the degree of geographic differentiation in *S. commerson* was much higher than generally observed in pelagic species, with the notable exceptions of *Decapterus russelli* (Table 5). In particular, this degree of geographic partition contrasts with the patterns generally observed in other *Scomberomorus* species, where little or no genetic variation has been detected distribution-wide (Table 6). Although genetic differences were evident between populations of both *S. munroi* and S. *queenslandicus* sampled along the northeastern Australian shores (Begg, Keenan & Sellin, 1998), the magnitude of those differences as expressed by Wright's *F*st, was the same as that in *S. commerson* sampled in the Persian Gulf and Oman Sea, but still one order lower than *F*st values for *S. commerson* in the tropical southwestern Pacific (Table 6).

It might be sensible to assume that the extreme migrating ability of pelagic fishes entail wide-scale geographic homogeneity in allele frequencies. Although *S. commerson* occurs in inshore waters and presumably does not cross large expanses of ocean as do tunas and billfishes, the level of geographic difference observed at the regional scale in *S. commerson* (present results) is striking: this suggests that migrating ability might rather be associated with increased potential for homing, hence for reproductive isolation.

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Table 1. Pairwise Φ_{ST} estimates based on sequences of the 5'end mitochondrial control region among narrow-barred Spanish mackerel (*Scomberomorus commerson*) samples (above diagonal). Super-sample 'Persian Gulf+Oman Sea' (*PG+OS*) comprises samples *AUH*, *BAH*, *DIB*, *IRN*, *KUW*, *OMN* and *RAK* of Hoolihan, Anandh & van Herwerden (2006); samples *1-12* as in Sulaiman & Ovenden (2009); else: see Fig. 1. *n*, number of sequences (in brackets). Significance levels (based on 10,000 permutations of haplotypes among samples) are indicated below diagonal: *NS* non significant; * $P<0.05$; ** $P<0.010$; *** $P<0.001$. Above diagonal, bold: values remaining significant after sequential Bonferroni correction (Rice 1989)

Sample	(*n*)	*PG+OS*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*JAVA*	*BALI*	*9*	*10*	*11*	*12*	*WPAP*	*NCAL*
PG + OS	(193)	-	**0.772**	**0.770**	**0.784**	**0.746**	**0.735**	**0.763**	**0.744**	**0.780**	**0.788**	**0.747**	**0.800**	**0.773**	**0.749**	**0.785**	**0.787**	**0.765**
1	(2)	***	-	0.002	0.091	-0.166	0.081	-0.059	0.470	-0.077	0.352	0.213	0.932	0.684	0.692	0.645	0.829	**0.670**
2	(2)	***	NS	-	0.326	-0.102	0.133	0.071	0.526	0.009	0.433	0.275	0.965	0.715	0.729	0.664	0.851	**0.657**
3	(2)	***	NS	NS	-	-0.197	0.064	0.051	0.438	-0.215	0.299	0.241	0.895	0.667	0.678	0.623	0.811	**0.673**
4	(4)	***	NS	NS	NS	-	-0.092	-0.119	0.197	-0.124	0.275	0.139	0.651	0.481	0.449	0.493	0.657	**0.573**
5	(5)	***	NS	NS	NS	NS	-	-0.055	-0.033	0.118	0.343	0.144	0.466	0.309	0.195	0.335	0.477	**0.421**
6	(5)	***	NS	NS	NS	NS	NS	-	0.139	0.056	0.327	0.149	0.510	0.451	0.361	0.485	0.567	**0.569**
7	(4)	***	NS	NS	NS	NS	NS	NS	-	0.473	0.590	0.307	0.332	0.264	-0.077	0.355	0.376	0.332
8	(3)	***	NS	NS	NS	NS	NS	NS	NS	-	0.314	0.268	0.865	0.669	0.674	0.655	0.804	**0.675**
JAVA	(4)	***	NS	NS	NS	*	*	*	*	*	-	0.028	0.860	0.735	0.713	0.712	0.764	**0.668**
BALI	(6)	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	0.587	0.517	0.436	0.546	0.463	**0.518**
9	(5)	***	*	*	*	*	**	**	*	*	**	*	-	0.629	0.432	0.626	0.533	**0.465**
10	(5)	***	*	*	*	*	*	**	*	*	**	**	**	-	0.295	0.308	0.585	0.367
11	(5)	***	*	*	*	*	NS	*	NS	*	**	*	**	*	-	0.424	**0.332**	0.251
12	(5)	***	*	*	*	**	**	**	*	*	**	**	**	*	**	-	**0.609**	0.375
WPAP	(8)	***	*	*	*	**	***	***	**	**	**	*	***	***	***	***	-	0.282
NCAL	(194)	***	***	***	***	***	***	***	**	***	***	***	***	**	**	***	**	-

Table 2. Analysis of molecular variance (AMOVA) among samples of *Scomberomorus commerson* based on partial sequences (310 bp) of the mitochondrial control region. Samples were grouped on a geographic basis (see Fig. 1) as following: Persian Gulf + Oman Sea (samples *AUH*, *BAH*, *DIB*, *IRN*, *KUW*, *OMN* and *RAK*); East China Sea (*1*); South China Sea + Malacca Strait (*2-8*); Java Sea + Bali (*JAVA*, *BALI*); Timor Sea + Arafura Sea (*9-11*); West Papua (*WPAP*); Coral Sea (*12*, *NCAL*)

Source of variation	d.f.	Sum of squares	Variance	% variation	*P*-value
Among groups	6	5787.59	Va=20.49	72.79	<0.0001
Among populations within groups	20	266.97	Vb= 0.35	1.23	0.0014
Within groups	424	3100.91	Vc= 7.31	25.98	<0.0001
Total	450	9155.48	28.15		

Table 3. Genetic diversity and results of neutrality tests in populations of *Scomberomorus commerson* across the Indo-Pacific. *N* = number of individuals, *H* = number of haplotypes, *Hd* = haplotype diversity (Nei, 1987), *S* = number of polymorphic sites, π = nucleotide diversity (Nei, 1987), and k = average number of nucleotide differences between two sequences (Tajima, 1983). For the *H*-test (Depaulis & Veuille, 1998), the probability that $Hd_{sim}<Hd_{obs}$ is reported. The value of Tajima's *D* test (Tajima, 1989) is reported. Samples were grouped as in Table 2

Region	*N*	*H*	*S*	*Hd*	π	k	*H*-test	*D*
Persian Gulf / Oman Sea	193	123	79	0.986	0.040	11.74	0.999	-0.407
East China Sea	2	2	5	1.000	0.017	5.00	-	-
South China Sea / Malacca Strait	25	20	55	0.980	0.053	15.26	0.974	0.185
Java Sea / Bali	10	10	42	1.000	0.052	15.18	0.999	0.109
Timor Sea / Arafura Sea	16	15	39	0.992	0.038	11.36	0.999	-0.041
West Papua	8	6	17	0.929	0.023	6.72	0.726	0.124
Coral Sea	200	62	66	0.817	0.041	11.94	0.011	0.191

Table 4. Genetic diversity at eight microsatellite loci in *Scomberomorus commerson* samples; sample size in brackets; H_E: expected heterozygosity according to HWE; H_O: observed heterozygosity; ^*f*: Weir and Cockerham's (1984) estimate fixation index. * indicate significant deviation after standard Bonferroni correction (Rice 1989)

Locus, Parameter	Sample *NCAL* (*N*=66)	*BALI* (*N*=6)	*JAVA* (*N*=4)	*WPAP* (*N*=10)
C83Sc				
H_E	0.488	0.583	0.531	0.725
H_O	0.515	0.667	0.500	1.000
^*f*	-0.049	-0.053	0.200	-0.333
D61Sc				
H_E	0.342	0.486	0.563	0.500
H_O	0.125	0.167	0.750	0.143
^*f*	0.639*	0.706	-0.200	0.750
E27Sc				
H_E	0.498	0.153	0.000	0.580
H_O	0.500	0.167	0.000	0.200
^*f*	0.003	0.000	-	0.684
F6Sc				
H_E	0.499	0.375	0.219	0.185
H_O	0.585	0.500	0.250	0.200
^*f*	-0.164	-0.250	0.000	-0.029
H96Sc				
H_E	0.618	0.694	0.688	0.595
H_O	0.667	1.000	1.000	0.700
^*f*	-0.071	-0.364	-0.333	-0.125
J10Sc				
H_E	0.510	0.569	0.219	0.505
H_O	0.561	0.667	0.250	0.500
^*f*	-0.091	-0.081	0.000	0.063
J43Sc				
H_E	0.565	0.667	0.688	0.560
H_O	0.710	0.500	0.750	0.800
^*f*	-0.249	0.333	0.053	-0.385
L42Sc				
H_E	0.884	0.847	0.781	0.895
H_O	0.906	0.500	1.000	0.900
^*f*	-0.017	0.483	-0.143	0.047
Multilocus				
H_E	0.550	0.547	0.461	0.568
H_O	0.571	0.521	0.563	0.555
^*f*	-0.030	0.138	-0.080	0.080

Table 5. Estimates of population genetic (mtDNA) differentiation (*F*st or equivalent) reported for broadcast-spawning Indo-Pacific bony fishes of various habitats

Habitat, Species	Geographic range considered	Marker locus	F_{ST}	Reference
Reef-associated				
Parrotfish, *Chlorurus sordidus*	Western Pacific + northern Australia[1]	CR, sequence	0.031	Bay et al. (2003)
Bigscale soldierfish, *Myripristis berndti*	Indo-Pacific	cyt *b*, sequence	0.580	Craig et al. (2007)
Bignose unicornfish, *Naso vlamingii*	Western Pacific[2]	CR, sequence	0.079	Klanten et al. (2007)
Forktail rabbitfish, *Siganus argenteus*	Western Pacific	cyt *b*, sequence	0.031	Lemer et al. (2007)
Lined unicornfish, *Naso brevirostris*	Indo-Pacific	CR, sequence	0.000-0.163	Horne et al. (2008)
Bluespine unicornfish, *Naso unicornis*	Indo-Pacific	CR, sequence	0.000-0.033	Horne et al. (2008)
Coral trout, *Plectropomus leopardus*	Western Pacific + Northwestern Australia[3]	CR, sequence	0.892	van Herwerden et al. (2009)
Blacktail snapper, Lutjanus fulvus	Indo-Pacific	cyt *b*, sequence	0.640 (0.110*)	Gaither et al. (2010)
Bluestripe snapper, Lutjanus kasmira	Indo-Pacific	cyt *b*, sequence	0.000-0.634 (0.062*)	Gaither et al. (2010)
Demersal				
Crimson snapper, *Lutjanus erythropterus*	Western Pacific	CR, sequence	0.086	Zhang et al. (2006)
Golden snapper, *Pristipomoides multidens*	Indo-Malay region	CR, RFLP	0.055	Ovenden et al. (2004)
Balloon alfonsino, *Beryx mollis*	Western Pacific	cyt *b*, sequence	0.000	Akimoto et al. (2006)
Slender alfonsino, *Beryx splendens*	Western Pacific	cyt *b*, sequence	0.003	Akimoto et al. (2006)
Pelagic				
Round scad mackerel, *Decapterus macrosoma*	Indo-Malay region	cyt *b*, SSCP	0.000	Borsa (2003)
Bigeye tuna, *Thunnus obesus*	Indo-Pacific	RFLP	0.000	Durand et al. (2005)
Indian scad mackerel, *Decapterus russelli*	Indo-Malay region[4]	cyt *b*, SSCP	0.370	Rohfristch & Borsa (2005)
Bigeye tuna, *Thunnus obesus*	Western Pacific	CR, sequence	0.004	Chiang et al. (2006)
Swordfish, *Xiphias gladius*	Indian O. vs. western Pacific	CR, sequence	0.038	Lu et al. (2006)
Wahoo, *Acanthocybium solandri*	Indo-Pacific	cyt b, sequence	0.000-0.054	Theisen et al. (2008)
Dolphinfish, *Coryphaena hippurus*	Indo-Pacific	NAD1, sequence	0.000-0.016	Diaz Jaimes et al. (2010)
Narrow-barred Spanish mackerel, *Scomberomorus commerson*	Indo-Pacific	CR, sequence	0.699-0.743	present study

Abbreviations: *CR* control region; *cyt b* cytochrome *b* locus; *RFLP* restriction fragment length polymorphism of a PCR-amplified fragment of mtDNA

[1] includes samples from Rota (Micronesia), Papua New Guinea, Great Barrier Reef and Western Australia

[2] includes Philippines, Papua-New Guinea, eastern Australia and Hawai'i

[3] includes Taiwan

[4] including West Papua

* Marquises population excluded

Table 6. *Scomberomorus* spp. Reported estimates of genetic differentiation (*F*st or equivalent) at the regional scale. *S. commerson* appeared as an outlier for both mitochondrial and nuclear markers [Dixon's test for detecting outliers (Sokal & Rohlf, 1969): Q=0.693, P<0.05, and Q=0.833, P<0.01, respectively]

Species	Geographic range considered	Marker type	F_{ST}	Reference
		Mitochondrial		
S. cavalla	W Atlantic and Gulf of Mexico	Whole mtDNA, RFLP	0.000	Gold, Kristmundsdóttir & Richardson (1997)
S. commerson	Persian Gulf – Oman Sea	CR, RFLP	0.010	Hoolihan, Anandh & van Herwerden (2006)
S. commerson	Tropical SW Pacific	CR, sequence	0.306-0.586	present study
S. maculatus	W Atlantic and Gulf of Mexico	ND4, RFLP	0.000-0.065	Buonaccorsi, Starkey & Graves (2001)
S. niphonius	East China Sea – Yellow Sea	CR, sequence	0.000-0.047	Shui et al. (2009)
S. sierra	Pacific coast of Tropical America	CR, sequence	0.004-0.094	Domínguez López, Uribe Alcocer & Díaz Jaimes (2010)
		Nuclear		
S. brasiliensis	Southern Caribbean Sea	8 microsatellite loci*	0.002	Gold et al. (2010)
S. cavalla	W Atlantic and Gulf of Mexico	5 microsatellite loci*	0.000-0.013	Broughton, Stewart & Gold (2002)
S. cavalla	Around peninsular Florida	5 microsatellite loci*	0.000-0.005	Gold, Pak & DeVries (2002)
S. commerson	Persian Gulf – Oman Sea	5 microsatellite loci*	0.029	van Herwerden et al. (2006)
S. commerson	Tropical Australia – Papua New Guinea	9 allozyme loci*	0.002	J.B. Shaklee in Buckworth et al. (2007)
S. commerson	Tropical SW Pacific	8 microsatellite loci*	0.228	present study
S. maculatus	W Atlantic and Gulf of Mexico	1 intron locus*	0.000	Buonaccorsi, Starkey & Graves (2001)
S. munroi	Northern and Eastern Australia	7 allozyme loci*	0.038	Begg, Keenan & Sellin (1998)
S. queenslandicus	Northern and Eastern Australia	7 allozyme loci*	0.025	Begg, Keenan & Sellin (1998)

* Only polymorphic loci (where estimated allele frequencies <0.95 in at least one population) were considered in this count

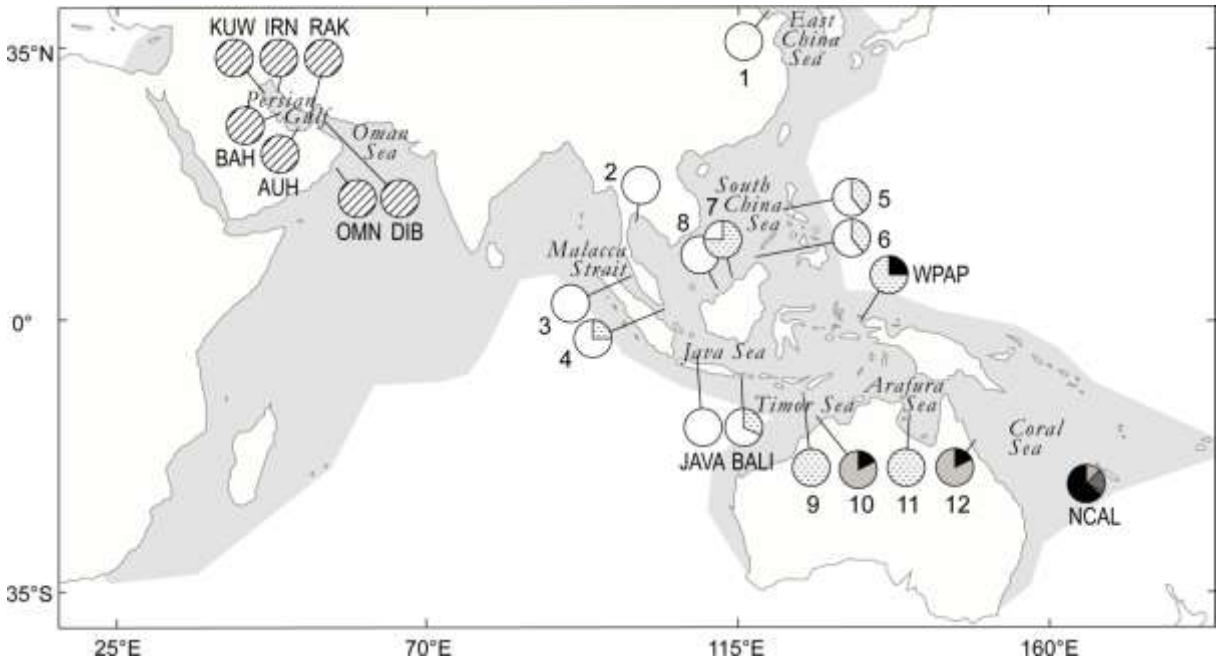

Figure 1. Narrow-barred Spanish mackerel, *Scomberomorus commerson*. Sampling locations across the Indo-West pacific. Pie diagrams represent control-region haplotype frequencies (*hatchured* Clade *I*; *black*: Haplogroup *ii*; *dark grey*: Subclade *IIa*; *dotted*: Subclade *IIb*; *white*: Subclade *IIc*; *pale grey*: Subclade *IId*; see Fig. 2). Abbreviations for samples: *JAVA*, Java Sea; *BALI*, Bali; *WPAP*, West Papua; *NCAL*, New Caledonia. Samples *AUH*, *BAH*, *DIB*, *IRN*, *KUW*, *OMN* and *RAK* from Hoolihan, Anandh & van Herwerden (2006); samples *1-12* from Sulaiman & Ovenden (2009). *Shaded area*: distribution range of *S. commerson* (Collette & Russo, 1984; Carpenter & Niem, 2001).

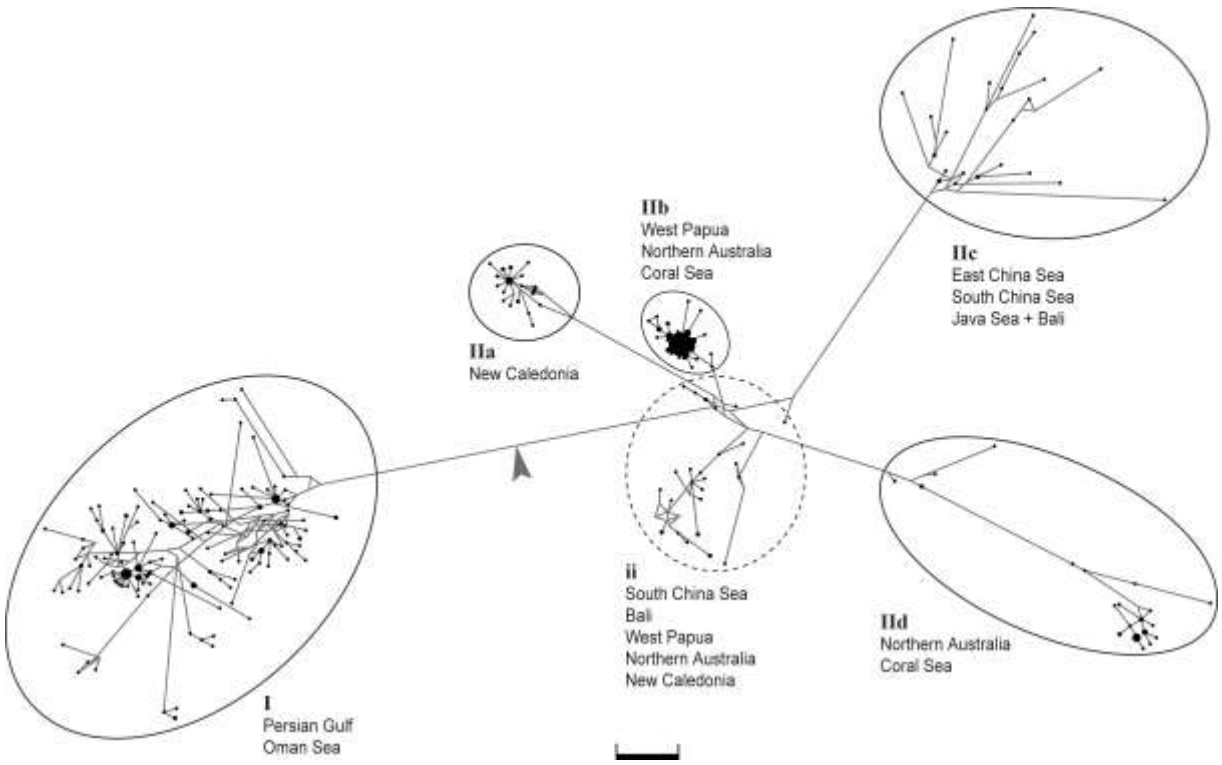

Figure 2. Narrow-barred Spanish mackerel, *Scomberomorus commerson*. Median-joining parsimony network (Bandelt, Forster & Röhl, 1999) of control region haplotypes. Groups of haplotypes delineated according to genetic proximity, with indication of area of occurrence; two main clades numbered *I* and *II*, the latter with sub-clades *IIa*, *IIb*, *IIc* and *IId* radiating from a central haplogroup *II*. Branch length proportional to number of mutational steps; *closed circles* represent individual haplotypes, their area being proportional to their frequency in the total sample; *arrow* indicates root. Bootstrap support (Neighbour-Joining algorithm; Kimura-2 parameter distances; 1000 resampling runs; Tamura et al., 2007) was: 100% for clade *I*; 98% for sub-clade *IIa*; 32% for sub-clade *IIb*; 81% for sub-clade *IIc*; 98% for Sub-clade *IId*. Scale bar: 5 mutational steps.

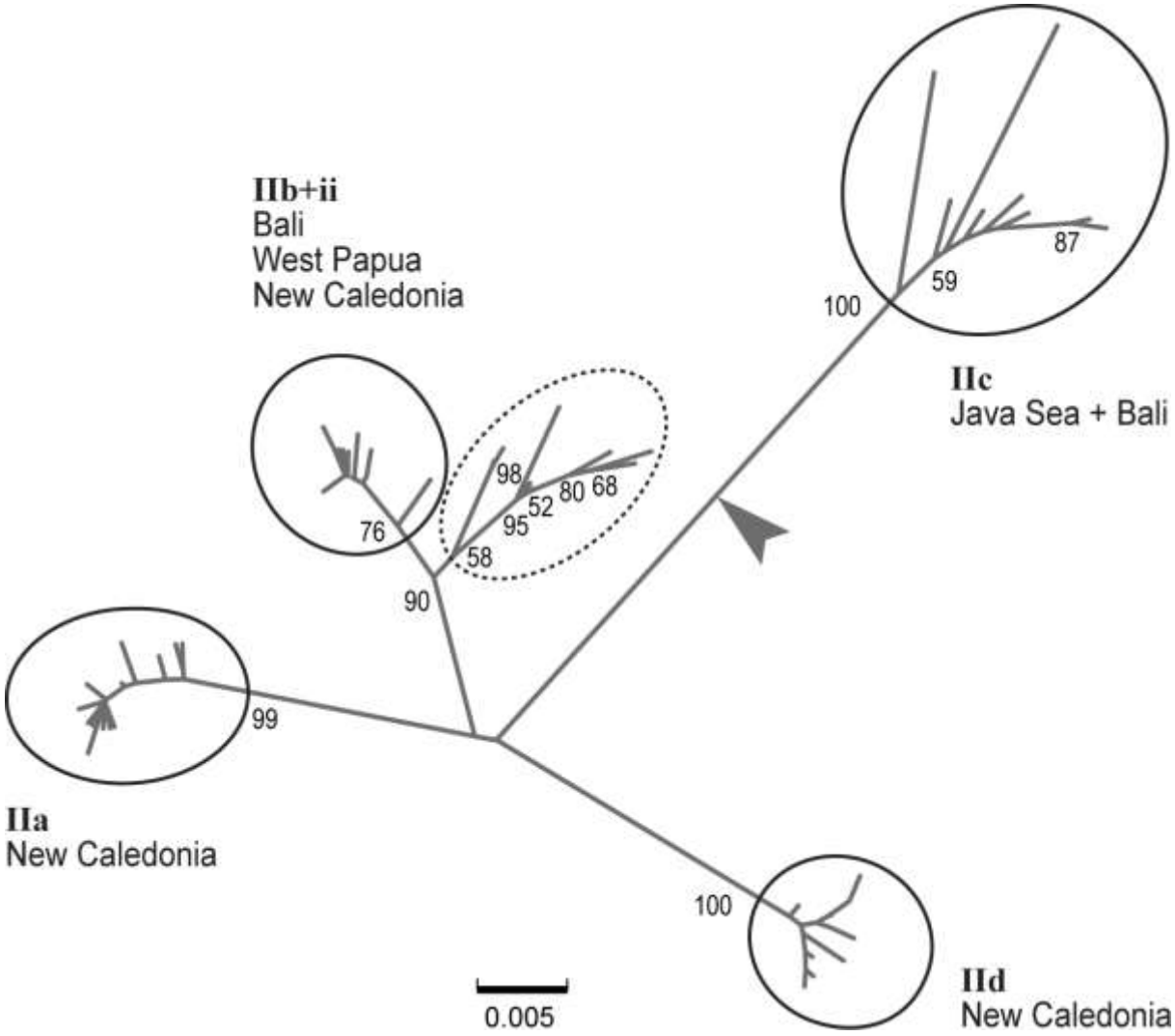

Figure 3. Narrow-barred Spanish mackerel, *Scomberomorus commerson*. Neighbor-Joining tree (Tamura-3 parameter distances; MEGA 5) of composite mitochondrial sequences (281 bp cytochrome *b* gene + 392 bp control region). *Arrow* indicates placement of root.

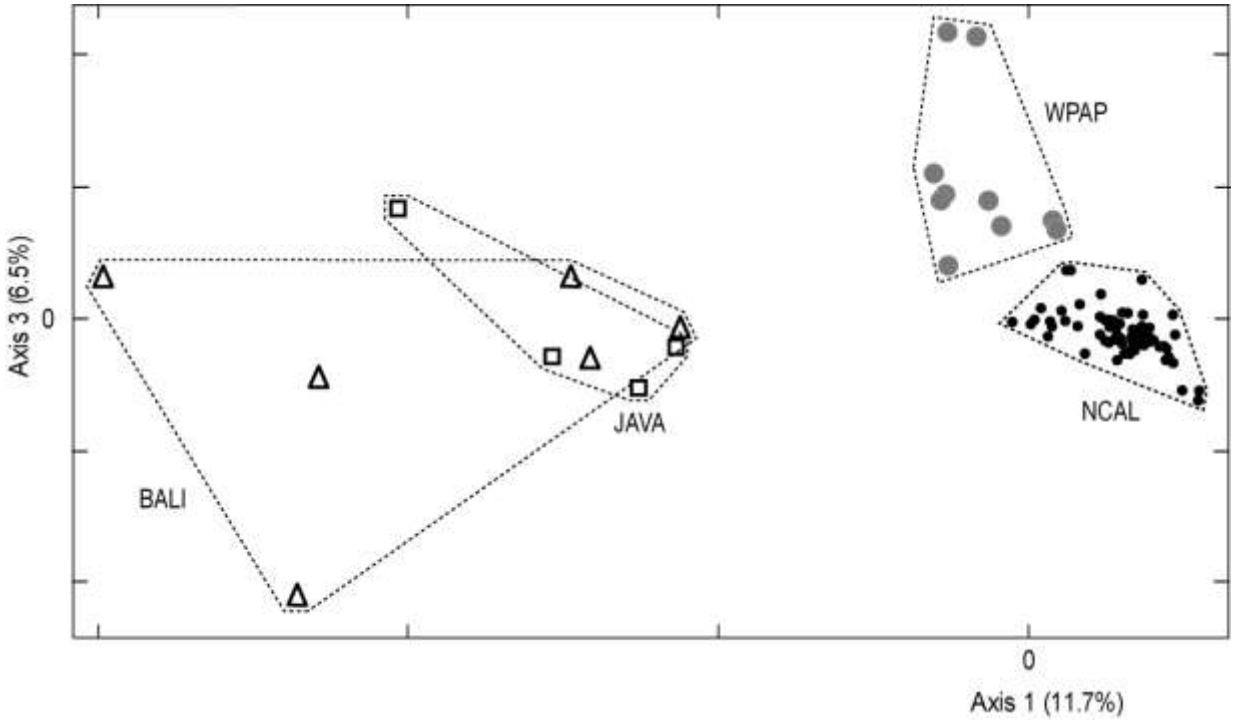

Figure 4. Narrow-barred Spanish mackerel, *Scomberomorus commerson*. Correspondence analysis: projection on the two dimensions defined by axis 1 and axis 3, of 86 individuals sampled from Bali (*BALI*: *N*=6), Java Sea (*JAVA*: *N*=4) and West Papua (*WPAP*: *N*=10), and New Caledonia (*NCAL*: *N*=66), characterized by their genotype at eight microsatellite loci. Multiple-nuclear locus estimates of genetic differentiation were the following: *JAVA* vs. *BALI*: $\hat{\theta}$=0.014, *P*=0.369; (*JAVA* + *BALI*) vs. *WPAP*: $\hat{\theta}$=0.231, *P*<0.001; (*JAVA* + *BALI*) vs. *NCAL*: $\hat{\theta}$=0.311, *P*<0.001; *WPAP* vs *NCAL*: $\hat{\theta}$=0.202, *P*<0.001.