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Population genetic structure of blue-spotted maskray *Neotrygon kuhlii* and two other Indo-West Pacific stingray species (Myliobatiformes: Dasyatidae), inferred from size-polymorphic intron markers

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ABSTRACT

Exon-primed, intron crossing DNA markers (EPICs) were screened for Mendelian-like allele size polymorphisms in three stingray species (*Himantura gerrardi*, *Neotrygon kuhlii* and *Taeniura lymna*) from the central Indo-West Pacific, where they are commercially exploited. Four to 7 size-polymorphic intron loci were selected in a species, and were subsequently tested as genetic markers of stock structure. Sharp genetic differentiation was observed between populations within each species across the Indo-Malay-Papua archipelago (Weir and Cockerham's $\hat{\theta}$ -values reaching 0.153-0.557 over a few thousand km). A trend of increasing genetic differentiation with increasing geographic distance was apparent in *N. kuhlii*, in which populations distant by 3,000 km were differentiated by an estimated $\hat{\theta} \sim 0.375$. This value was an order of magnitude higher than usually reported in coastal benthic teleost fishes and indicates strong sub-population structure. This is likely, at least partly, a consequence of the sedentary benthic habits of *N. kuhlii* at all life stages. Because replenishment of overexploited populations of *N. kuhlii* and two other stingray species from the central Indo-West Pacific is unlikely at ecological timescales, management should be planned at the local geographic scale.

Keywords: *Himantura gerrardi*; *Taeniura lymna*; population genetic structure; fishery; conservation; Indo-Malay-Papua archipelago

1. Introduction

One of the problems hampering the proper management of commercially exploitable marine species is the trade-off between the effort necessary for the acquisition of scientifically sound information and the increasing pace at which these resources rarefy. This is particularly true for developing nations in the central Indo-West Pacific region, where marine species richness is highest (Briggs, 2005; Bellwood and Meyer, 2009), human pressure is strong (Sadovy, 2005), management is poor (Pauly, 1994), and science is weaker than in other regions in the Indo-Pacific (Haustein et al., 2011; Meijaard, 2011). The low fecundity and slow maturation of sharks and rays (elasmobranchs), in particular, make them highly vulnerable to overfishing. There is increasing concern over their exploitation in Indonesia, the most populated country of the central Indo-West Pacific (Blaber et al., 2009; White and Kyne, 2010). However, little effort for management and conservation has been made thus far (Blaber et al., 2009; White and Kyne, 2010). If unsustainable exploitation is allowed to continue unmonitored and unregulated, it will cause the depletion of stocks, erode genetic diversity, and eventually lead to the extinction of many shark and ray species in this region.

Among the pre-requisites for sound fishery management is the identification of stocks, a goal that can be reached by assessing the geographic structure of populations using genetic markers (Shaklee and Bentzen, 1998; Begg et al., 1999; Ward, 2000). Assessing geographic structure in sharks has been hampered due to low variability of allozyme markers and in mitochondrial DNA (Heist et al., 2003). Recent studies of population genetic structure in elasmobranchs have relied on the hypervariable mitochondrial control region sequences and on microsatellite polymorphisms (Chevolot et al., 2008; Schluessel et al., 2010; Le Port and Lavery, 2012; Portnoy and Heist, 2012). The degree of genetic population subdivision in rays and skates still remains under-investigated (Schluessel et al., 2010) although there is an urgent need for such information for commercially targeted species in the central Indo-West Pacific.

The initial aim of the present study was to select size-polymorphic nuclear markers in several stingray species (Elasmobranchia: Dasyatidae). Exon-primed, PCR-amplified introns (EPIC) were chosen because they can provide such markers of population genetic structure with minimal investment. For instance, a single pair of universal EPIC primers simultaneously allows the amplification of up to several introns in a gene family (Chow and Takeyama, 1998; Hassan et al., 2002; Atarhouch et al., 2003). Compared to microsatellites, another advantage of EPIC markers is that null alleles are less likely, since EPIC primers are anchored in conserved regions. Last, an undesirable property of microsatellites is allele-size homoplasy (Garza and Freimer, 1996; Ellegren, 2004), which is unlikely to occur with EPIC markers when mutations that affect allele size are caused by indels (Creer, 2007). We screened for size polymorphism a number of primer pairs that are known to amplify EPICs in teleost fishes. The rationale for expecting cross-amplification with stingrays comes from the fact that exons of a number of EPIC genes are conserved across orders in Metazoans (e.g. Palumbi et al. 1991; Chenuil et al., 2010). Three stingray species among the most common at fish landing sites in the Indo-Malay-Papua archipelago in the central Indo-West Pacific (White and Dharmadi, 2007) were chosen as test species: the sharpnose stingray, *Himantura gerrardi* (Gray, 1851), the blue-spotted maskray, *Neotrygon kuhlii* (Müller and Henle, 1841) and the ribbontail stingray, *Taeniura lymna* (Forsskål, 1775). Whether the selected intron markers are suitable for assessing population genetic structure was further investigated in *N. kuhlii*, on the basis of an adequate geographic sampling that extended from the Indian Ocean Java coast to the Molucca Sea. Prior to the present survey, cytochrome-oxidase I gene sequences from a barcoding survey of Indo-Australian chondrichthyans have shown strong genetic differences within *H. gerrardi* and *N. kuhlii* (Ward et al., 2008), but no firm evidence that both actually harbour cryptic species within the Indo-Malay archipelago. The present survey may help address this issue because nuclear markers allow the detection of reproductive isolation when cryptic species co-occur sympatrically.

2. Materials and methods

2.1. Sampling

Three stingray species, *Himantura gerrardi*, *Neotrygon kuhlii* and *Taeniura lymna* were sampled from 3-6 landing sites across the Indo-Malay-Papua archipelago (Fig. 1). Sample sizes per species per sampling site ranged from 2 to 40. A piece of flesh and skin (0.05-0.5 cm²) was dissected at the extremity of the disk using surgical scissors, and immediately preserved in ethanol. Total genomic DNA was extracted using the DNeasy® Tissue Kit (Qiagen GmbH; Hilden, Germany), or using Chelex 100 resin beads (Bio-Rad, Hercules CA) according to Walsh et al. (1991).

2.2. PCR conditions and selection of markers

A preliminary series of tests consisted of amplifying by polymerase chain reaction (PCR) the DNA of 2-6 individuals from each of 3 regional samples of each stingray species using 20 EPIC primer pairs available in our laboratory (Supplementary material, Table S1). Those primer pairs that revealed size-polymorphic loci were then selected. PCRs were carried out in a total volume of 10 µL, containing 1 µL 10X *Taq* buffer (Promega Corporation, Madison WI), 2.5mM MgCl₂, 0.2mM of each dNTP (Invitrogen, San Diego CA), 0.5 mM of each primer (MWG-Biotech, Ebersberg, Germany), the forward primer being fluorescently labelled with either 6-carboxyfluorescein (6-FAM), indodicarbocyanine (Cy5), or

tetramethylrhodamine (TAMRA), 0.3 U Taq polymerase and 1 μ L template DNA (at about 150 mg/ml). PCRs were run in a Mastercycler thermal cycler (Eppendorf, Hamburg, Germany), where thermo-cycling conditions consisted of initial heating at 94°C for 3 min, followed by 35 cycles of heating at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min 20 s, before 10 min final extension at 72°C. The PCR products were mixed with 1 vol. loading buffer, denatured for 5 min at 95 °C, loaded into vertical 8% denaturing polyacrylamide gels, and subjected to electrophoresis for 1 hr at 60 W. Fluorescent DNA bands were detected in an FMBIO® II gel scanner (Hitachi Instruments, San José CA).

For each primer pair, a gel exhibited 2 to >8 distinct horizontal series of fluorescent bands in a species. Each series of bands was examined for eventual band-size variation and was interpreted as co-dominant size-polymorphism at a Mendelian locus when the following conditions were met: (1) an individual exhibited either one or two bands; (2) the most commonly encountered bands in two-banded individuals were also observed in at least one single-banded individual; (3) the proportions of single-banded and double-banded individuals in a given sample were reasonably close to those expected for homozygotes and heterozygotes for co-dominant alleles at a Mendelian locus in a panmictic population. Photographs of gels where Mendelian-like intron-length polymorphisms were scored have been presented previously (Borsa et al., 2004; Berrebi et al., 2005; Rohfritsch and Borsa, 2005; Hoareau et al., 2007) and the scan image of a gel illustrating Mendelian-like size polymorphism in *T. hymna* is presented in Fig. 2. The presumptive loci selected after this first step were then scored for all individuals.

Scoring of individual genotypes was done by eye directly from the gel scan. Sizing of alleles was done with the help of the FMBIO® II gel scan analysis software (Hitachi Instruments) using as a reference a fluorescent DNA ladder (Internal Lane Standard 600; 60-600 bp; Promega Corporation) loaded at regular intervals in the gel.

2.3. Genetic data analysis

Estimators of genetic differentiation between populations (F_{st}) were Weir and Cockerham's (1984) multilocus $\hat{\theta}$ and Nei and Chesser's (1983) \hat{G}_{st} , the proportion of genetic diversity that resides among sub-populations. Unlike Weir and Cockerham's (1984) θ which was estimated from genotype frequencies, G_{st} was estimated from allele frequencies, in order to compare the present results with cases from the literature where only allele frequency data are available. Both θ and G_{st} were estimated using GENETIX v 4.02 (Belkhir et al., 2000). The estimated values of θ were compared to pseudo-distributions generated by random permutations of individuals under GENETIX 4.02 as a test of the null hypothesis $\theta = 0$. Wright's F_{is} was estimated using Weir and Cockerham's (1984) multi-allelic \hat{f} estimator. The null hypothesis $f = 0$ (panmixia) was tested using a similar permutation test under GENETIX 4.02. The probability of occurrence of a parameter value larger than or equal to the observed value was estimated as $P = (n+1)/(N+1)$, where n is the number of pseudo-values larger (or lower for negative \hat{f}) than or equal to the observed value, and N is the number of random permutations (Sokal and Rohlf, 1995).

The correlation coefficient between genotypes at different loci, R (Garnier-Géré and Dillmann, 1992), was used as a measure of linkage disequilibrium. Random permutations of genotypes at a locus (procedure PERMUTATIONS of GENETIX 4.02) were used to generate a pseudo-distribution of R under the null hypothesis of linkage equilibrium. The correlation coefficient, R , and its associated probability under the null hypothesis, P , were estimated for each locus pair by GENETIX 4.02. Statistical significance of the estimated values was assessed after correcting for multiple tests (Rice, 1989).

Geographic distance between sampling locations was calculated as the shortest ship distance, using the PATH tool implemented in Google Earth (http://www.google.co.uk/intl/en_uk/earth/). The habitat is bi-

dimensional and the correlation of genetic distance with geographic distance was analyzed according to the two-dimensional model of isolation by distance (IBD) (Rousset, 1997).

The number of populations present in the total sample of *N. kublii* was determined and individuals were assigned to these populations using the model-based program STRUCTURE 2.3.1 (Falush et al., 2007). The Markov chain Monte Carlo algorithm implemented in STRUCTURE was run under the admixture model with correlated allele frequencies for 100,000 steps after 50,000 initial burn-in steps, without any a priori information on an individual's sampling location, to estimate individual admixture proportions and assign individuals to populations. Five independent runs were performed for each number of populations (K) ranging a priori from 2 to 6 to determine the number of populations a posteriori by visually comparing the output diagrams.

IBD and Bayesian structure analyses were not carried out in the two other stingray species, *H. gerrardi* and *T. lymna*, because of an insufficient geographic coverage and an insufficient number of samples.

3. Results

Six primer pairs (*Act-2*, *AldoB5*, *Cam-3*, *CK*, *Gpd2*, and *RP*) (Table 1) each allowed the scoring of at least one presumptive polymorphic locus in at least one of three stingray species surveyed for intron-size polymorphism (Tables 2-4; multilocus genotypes in Supplementary material, Tables S2-S4). The *CK* primer pair allowed the scoring of a single polymorphic locus in each of the three species. Fig. 2 illustrates Mendelian-like size-polymorphism at locus *CK* in *Taeniura lymna*, where all three phenotypes expected for a co-dominant gene at a single locus with two size-alleles are present. All primer pairs of Table 1 have been previously selected to amplify size-polymorphic introns in teleost fishes (Chow and Hazama, 1998; Chow and Takeyama, 1998; Hassan et al., 2002; Atarhouch et al., 2003; Berrebi et al., 2005). Up to 5 polymorphic loci were scored with a single primer pair (e.g. *Cam-3*; Table 2), which can be due to either amplification of the paralogous intron in several genes of the family (e.g. Friedberg and Rhoads, 2002; Creer, 2007), or perhaps non-specific amplification within a single gene (i.e. several introns of the same gene might have been amplified). For all the other primer pairs tested (Supplementary material, Table S1), either the DNA band patterns were not variable (thus exhibiting sample monomorphism), or they were variable but we failed to interpret them in terms of size polymorphism at Mendelian loci. In a few cases, the amplification was too weak for scoring, the intensity of DNA bands was highly variable across individuals, or a substantial proportion of individuals could not be scored either because of bands that were too weak or because of overlapping bands presumably from other loci.

The suitability of the selected markers to the analysis of population genetic structure was warranted by the general conformity of the genotype frequencies to Hardy-Weinberg expectations, as Weir and Cockerham's (1984) \hat{f} -estimates were generally not significantly different from 0 (Tables 2-4). There was no evidence of linkage disequilibrium between loci except between *Cam-3* *slow* and *CK* in *N. kublii* from the Banda Sea ($R=0.306$; $N=43$; $P<0.001$). Detailed results of linkage-disequilibrium tests are presented in Supplementary material, Table S5.

The estimate of genetic differentiation between sub-populations was strong in all three species (Table 5), particularly in *Neotrygon kublii* where a trend of increasing genetic differentiation with increasing geographic distance was apparent (Fig. 3) and populations separated by a distance of 3,000 km were genetically differentiated by an estimated Weir and Cockerham's (1984) $\hat{\theta} \sim 0.375$ (Fig. 3).

The most likely outcome from Bayesian structure analysis, which was run for $K=2-6$, was with $K=3$. The case where $K=2$ provided less information than $K=3$, and the structure diagrams obtained when $K>3$

were increasingly noisy as K increased, yet not more informative than with $K=3$ (see Supplementary material, Table S6 for a comparison of the different structure diagrams). Thus, three genetically distinct populations were identified in *N. kublii* by Bayesian structure analysis (Fig. 1A; Fig. 4) and confirmed the strong partition inferred from the overall F_{st} estimate ($\hat{\theta} = 0.336$): one that includes the two samples from the Indian Ocean Java coast; a second that includes the samples from the Bali Strait and the Flores Sea; and a third that was sampled in the Banda Sea only. According to Bayesian structure analysis, the sample from Tomini Bay was close to both the Indian Ocean population and to the Bali Strait / Flores Sea population, although the latter relationship was weaker. Because of the small size of the Tomini Bay sample, its assignment to the Indian Ocean (or Bali Strait / Flores Sea) population should be considered as tentative only. Overall, different oceanic basins seemed to harbour genetically differentiated *N. kublii* populations.

One of the *H. gerrardi* samples was of unknown origin. We observed that genetic differentiation between the two geographically close samples from the Sunda Strait and the Java Sea was much lower than genetic differentiation between the two most distant samples (Indian Ocean *vs.* West Papua) with an intermediate value for intermediate distances (e.g. Java Sea *vs.* Indian Ocean) (Table 5B). Assuming a general trend of increasing genetic differentiation with increasing geographic distance in this species, as in *N. kublii*, it is possible to infer the approximate geographic origin of the ‘Unknown’ sample as geographically intermediate between the Indian Ocean and West Papua, and likely closer to the Indian Ocean than to the other sampled locations (Table 5B).

4. Discussion

Elasmobranchs suffer indiscriminate exploitation in the Indo-Malay-Papua archipelago (Blaber et al., 2009; White and Kyne, 2010). Given the K -selected life-history characteristics of elasmobranchs, the sustainability of the stocks and even the survival of some of the most vulnerable species are becoming more and more a matter of concern (White and Kyne, 2010). Hence, there is an urgent need to analyze stock structure and provide fisheries and environmental managers with information on population genetic structure, which is relevant to designing management policies.

4.1. Suitability of intron markers to assess population genetic structure in stingrays

Screening size polymorphisms using PCR primers known to amplify potentially polymorphic introns in teleost fishes proved an efficient strategy to select a reasonable number of Mendelian-like markers in several stingray species. Here, we showed that these size-polymorphic introns can be employed to assess population geographic structure, which in turn could have useful applications, including the delineation of stocks and the recognition of distinct genetic units of conservation interest.

4.2. Investigating mechanisms of genetic differentiation

The levels of population genetic differentiation in *Himantura gerrardi*, *Neotrygon kublii* and *Taeniura lymna* in the Indo-Malay-Papua archipelago (present study), were comparable to the only figure published thus far for another stingray species, *Dasyatis brevicaudata*. In this species, a significant level of genetic differentiation ($\hat{F}_{st} = 0.160$; mitochondrial DNA control region) has been reported between populations sampled in southwestern Australia and New-Zealand (separated by ca. 5,100 km). However, in the same study, the \hat{F}_{st} -value between populations from southwestern Australia and southeastern Australia, separated by >2,800 km

continuous shelf habitat, was only 0.010, whereas the \hat{F}_{st} -value across the Tasman Sea (ca. 2,200 km deep sea) was 0.030 (Le Port and Lavery, 2012). In other coastal benthic elasmobranchs, genetic differentiation estimates at nuclear loci (using Wright's F_{st} or equivalents) ranged from 0.002 to 0.065 over thousands of km (Fig. 5). These average \hat{F}_{st} -values may mask locally sharp heterogeneities, as in the Milk shark, where populations from Bali and the northern Great Barrier Reef separated by 3,510 km differed by $\hat{F}_{st}=0.268$ (Ovenden et al., 2011).

Comparing the levels of genetic differentiation available from the few nuclear-genetic surveys of coastal benthic elasmobranchs, with those from the literature on teleost fishes, four outliers were visible and they included two Dasyatidae from the Indo-Malay-Papua archipelago (*H. gerrardi* and *N. kuhlii*; present study) (Fig. 5; more details in Supplementary material, Table S7). The two other outliers were two teleost fishes sampled across the Indo-West Pacific, the narrow-barred Spanish mackerel *Scomberomorus commerson* (Fauvelot and Borsa, 2011) and the fourfinger threadfin *Eleutheronema tetradactylum* (Horne et al., 2011). Since all the other \hat{F}_{st} values for coastal elasmobranchs were positioned within the cluster characterizing most coastal teleosts, there is no evidence from these data, that coastal elasmobranchs have higher geographic composition than teleosts sharing similar habitats. However, the four outliers were sampled in, roughly, the same central Indo-West Pacific region, suggesting a possible common history of past geographic isolation in this region, which would have thus translated into a similarly high level of genetic differentiation between populations. The Coral Triangle is a well known area of major biogeographic breaks for marine fauna (Rocha et al., 2007; Carpenter et al., 2011). The phylogeographic patterns uncovered within the Coral Triangle for a proportion of marine animal species with wide Indo-West Pacific distribution point to Plio-Pleistocene vicariance (Barber et al., 2006; Carpenter et al., 2011). The vicariance hypothesis may be invoked to explain the high level of genetic differentiation among stingray populations in the Indo-Malay-Papua archipelago. However, a more exhaustive and finer-grained geographic sampling is required to test this hypothesis in the three stingray species investigated in the present paper.

Alternatively, the general pattern of increasing genetic differentiation with geographic distance in *N. kuhlii* may be caused, at least in part, by IBD, as a result of genetic drift at neutral loci locally balanced by low levels of gene flow. The estimates of genetic differentiation (F_{st}) in benthic teleosts for which IBD patterns were observed were ~ 0.008 (*Hypoplectrus puella*; Puebla et al., 2009), ~ 0.020 (*Solea solea*; Borsa et al., 1997b), ~ 0.030 (*Platichthys flesus*; Borsa et al., 1997a), ~ 0.034 (*Psetta maxima*; Blanquer et al., 1992), ~ 0.091 (*Myripristis berndti*; Muths et al., 2011), ~ 0.100 (*Acanthurus triostegus*; Planes and Fauvelot, 2002), and ~ 0.259 (*Eleutheronema tetradactylum*; Horne et al., 2011), for a 3,000 km geographic distance, to be compared with *N. kuhlii* (~ 0.375). Marine teleosts, including species that are sedentary as adults, are often broadcast spawners and have a pelagic larval stage, two traits which are assumed to enhance gene flow between local populations. Stingrays are viviparous (Hamlett and Hysell, 1998) and only the active movement of juveniles or adults potentially translates into gene flow, thus partly explaining a possible higher level of genetic differentiation in *N. kuhlii* than generally observed in coastal benthic teleosts where an IBD pattern has been observed. This is further supported by tagging experiments in *N. kuhlii*, which indicate this as a sedentary species (Pierce et al., 2011).

Also, deep oceanic waters likely constitute a barrier for the movements of stingray species from shallow benthic habitats, like *D. brevicandata* (Le Port and Lavery, 2012) and possibly *N. kuhlii* and the two other stingray species surveyed. The sharp delineation of three *N. kuhlii* populations in the central part of the Indo-Malay-Papua archipelago, as apparent even from the limited sampling of the present study, may be partly explained by the effect of deep-sea barriers restricting the migration of individuals in some regions (e.g., between the Flores Sea and the Banda Sea). However, it is still possible that geographically tighter sampling would reveal smoother transitions between populations. Thus, the present phylogeographic

structure of *N. kublii* in the Indo-Malay archipelago may be caused by the superimposition of genetic differences resulting from past geographic isolation, IBD, and possibly the present effect of deep-sea barriers. Strong population subdivision could also be enhanced by philopatry, as shown in other elasmobranchs (Keeney et al., 2003, 2005; Feldheim et al., 2004; Duncan et al., 2006) and, incidentally, as suspected in both the narrow-barred Spanish mackerel and the fourfinger threadfin (Fauvelot and Borsa, 2011; Horne et al., 2011).

4.3. 'Cryptic species' hypothesis

To be able to assess whether the genetic clusters obtained in this study for *N. kublii* represent distinct evolutionary significant units and therefore constitute incipient species or even, perhaps, cryptic species as envisaged by Ward et al. (2008), would require a geographically denser and broader analysis of population genetic structure, and an extension of the current mitochondrial dataset. There is no evidence, from the available data thus far, that reproductively isolated entities (in short, cryptic species) are present in the three regions sampled in the Indo-Malay archipelago, i.e., the Indian Ocean coast of Java; the Bali Strait / Flores Sea area, and the Banda Sea. Actually, from the present evidence, one cannot discard the hypothesis that *N. kublii* populations are strongly differentiated because of low but geographically continuous gene flow. Neither was there an indication from the limited sample set analysed in this study, that *H. gerrardi* harbours cryptic species.

4.4. Implications for management

Whatever the evolutionary processes that have led to the strong geographic structure observed in *N. kublii* and two other Dasyatidae surveyed from the Indo-Malay-Papua archipelago, our findings indicate that under local overfishing the global genetic diversity of the species is likely to erode, putative locally adapted genetic traits may be lost, and the replenishment of depleted populations is expected to be minimal at the ecological timescale. Consequently, if the absence of management persists, localized extinctions or near-extinctions are predicted to occur in stingray populations throughout the intensively-exploited Indo-Malay-Papua archipelago. It is arguable that in highly mobile elasmobranch species such as pelagic sharks and mobulid rays, conservation efforts have to be conducted at the regional scale (e.g., Ovenden et al., 2009). Because of sharp geographic composition synonymous to limited genetic exchange between local populations, *N. kublii* and other stingray species from the central Indo-West Pacific also need to be managed locally.

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Table 1

PCR primers used to amplify size-polymorphic introns in three Indo-West Pacific stingray species. *Intron no.*: assumed intron number in teleost fishes.

Gene or gene family	Intron no.	Locus (loci), primer name	Primer sequence	Reference
Actin	2	<i>Act-2</i> <i>Act-2-F</i> <i>Act-2-R</i>	5'-GCTATAACCCTCGTAGATGGGCAC-3' 5'-ATCTGGCACCACACCTTCTACAA-3'	Atarhouch et al. (2003)
Aldolase B	5	<i>AldoB5</i> <i>Aldo3F</i> <i>Aldo2R</i>	5'-TCCTGCCTGATGGAACCC-3' 5'-CAGGTACACATGGTGGTC-3'	Hassan et al. (2002)
Calmodulin	3	<i>Cam-3</i> <i>Cam-3-F</i> <i>Cam-3-R</i>	5'-TGACGGAGCTCTGCAGCACTGAC-3' 5'-GTGAGGAGGAGCTCCGTGAGGC-3'	Atarhouch et al. (2003)
Creatine kinase	6	<i>CK</i> <i>CK6-5'</i> <i>CK7-3'</i>	5'-GACCACCTCCGAGTCATCTCSATG-3' ^a 5'-CAGGTGCTCGTTCACATGAA-3'	Palumbi et al. (1991)
Glyceraldehyde-3-phosphate dehydrogenase	2	<i>Gpd2</i> <i>Gpd2F</i> <i>Gpd3R</i>	5'-GCCATCAATGACCCCTTCATCG-3' 5'-TTGACCTCACCCTTGAAGCGGCCG-3'	Hassan et al. (2002)
Ribosomal protein	1	<i>RP</i> <i>RPEX1F</i> <i>RPEX2R</i>	5'-TGGCCTCTTCCTTGGCCGTC-3' 5'-AACTCGTCTGGCTTTTCGCC-3'	Chow and Takeyama (1998)

^a S = C or G, with approximately equal probabilities

Table 2

Himantura gerrardi. Allelic frequencies at 7 presumptive intron loci sampled from the Indo-Malay-Papua archipelago. Alleles were designated from the approximate size of the PCR-amplified fragment (in base pairs). Five different, presumptive intron loci, here designed as *Cam-3 a-e*, were scored using the *Cam-3* primer pair (Table 1). Estimates of genetic differentiation over all samples and loci: $\hat{\theta} = 0.123$ (permutation test, 1000 random permutations: $P < 0.01$) and $\hat{G}_{st} = 0.159$. *Hyphbr*: no data.

Locus, allele	Sample				
	Sunda Strait	Java Sea	Indian Ocean	West Papua	Unknown
<i>Act-2</i>					
238	0.57	0.58	0.70	-	0.56
225	0.43	0.43	0.20	-	0.44
210	0	0	0.10	-	0
(N)	(7)	(21)	(5)	-	(8)
\hat{f}	-0.091	-0.063	0.238	-	-0.750
<i>Cam-3 a</i>					
206	0	0	0.17	-	0.39
203	0.07	0.37	0	-	0
200	0.93	0.63	0.83	-	0.61
(N)	(7)	(19)	(3)	-	(9)
\hat{f}	0	-0.333	0	-	-0.600
<i>Cam-3 b</i>					
178	0	0	0	0	0.17
175	0.71	0.58	0.90	0	0.61
173	0.29	0.39	0.10	0	0.06
171	0	0.03	0	1	0.17
(N)	(7)	(18)	(5)	(1)	(9)
\hat{f}	-0.333	-0.624*	0	-	-0.116
<i>Cam-3 c</i>					
161	0	0.13	0	0	0.06
158	0.43	0.32	0	0	0.44
155	0.57	0.55	0.90	1	0.44
152	0	0	0.10	0	0.06
(N)	(7)	(19)	(5)	(2)	(8)
\hat{f}	-0.714	-0.251	0	-	-0.380
<i>Cam-3 d</i>					
140	0.71	0.80	0.17	-	0.17
138	0.29	0.20	0.83	-	0.67
136	0	0	0	-	0.17
(N)	(7)	(10)	(3)	-	(9)
\hat{f}	0.368	-0.200	0	-	-0.053
<i>Cam-3 e</i>					
127	0	0	0	0	0.17
126	0	0.03	0	0	0
125	1	0.97	1	1	0.83
(N)	(7)	(19)	(5)	(2)	(7)
\hat{f}	-	0	-	-	0.636
<i>CK</i>					
175	0.64	0.63	0.67	1	0.56
173	0.36	0.37	0.33	0	0.44
(N)	(7)	(19)	(3)	(2)	(9)
\hat{f}	-0.500	0.565*	1	-	-0.297

* $P < 0.05$

Table 3

Neotrygon kublii. Allelic frequencies at 4 presumptive intron loci in 6 samples from the Indo-Malay-Papua archipelago. Alleles were designated from the approximate size of the PCR amplified fragment (in base pairs). Two different, presumptive intron loci, here designed as *Cam-3 slow* and *fast* according to the motility of the expressed DNA bands, were scored using the *Cam-3* primer pair (Table 1). Estimates of genetic differentiation over all samples and loci: $\hat{\theta} = 0.336$ (permutation test, 1000 random permutations: $P < 0.001$) and $\hat{G}_{st} = 0.316$.

Locus, allele	Sample					
	Indian Ocean ^a	Indian Ocean ^b	Bali Strait	Flores Sea	Tomini Bay	Banda Sea
<i>Cam-3 slow</i>						
430	0	0	0	0	0	0.05
400	0.96	0.94	0.93	0.82	1	0.91
385	0	0	0	0.11	0	0
370	0.04	0.06	0.08	0.08	0	0.04
(N)	(38)	(9)	(20)	(19)	(3)	(38)
\hat{f}	-0.028	0	-0.056	-0.135	-	0.245
<i>Cam-3 fast</i>						
300	0.18	0.33	0.68	0.94	0	0.88
282	0.74	0.39	0.33	0.06	1	0.12
264	0.08	0.28	0	0	0	0
(N)	(38)	(9)	(20)	(18)	(3)	(39)
\hat{f}	-0.240*	0.297	0	-0.030	-	-0.118
CK						
368	0.46	0	0	0.03	0	0
366	0.01	0	0.43	0	0	0.04
363	0.28	0.67	0.25	0.22	0	0.16
361	0.24	0.33	0.33	0.72	1	0.74
359	0	0	0	0.03	0	0.06
(N)	(37)	(9)	(20)	(16)	(4)	(34)
\hat{f}	0.144	0.543	-0.126	-0.268	-	0.121
<i>Gpd2</i>						
173	0	0	0	0.15	0	0
169	0	0	0	0.03	0	0
167	0.82	0.83	0.89	0.82	1	0.23
164	0.18	0.17	0.11	0	0	0.77
(N)	(38)	(9)	(19)	(17)	(4)	(33)
\hat{f}	-0.037	-0.143	-0.091	-0.150	-	-0.106

^a Binuangen sample (Fig. 1A); ^b Malang sample (Fig. 1A)

* $P < 0.05$

Table 4

Taeniura lymna. Allelic frequencies at 5 presumptive intron loci in 3 samples from the Indo-Malay archipelago. Alleles were designated from the approximate size of the PCR amplified fragment (in base pairs). Three different, presumptive intron loci differing by the mobility of the expressed DNA bands, were scored using the *AldoB-5* primer pair (Table 1). Estimates of genetic differentiation over all samples and loci: $\hat{\theta} = 0.095$ (permutation test, 1000 random permutations: $P < 0.01$) and $\hat{G}_{st} = 0.074$.

Locus, allele	Sample		
	Java Sea	Flores Sea	Molucca Sea
<i>AldoB5 slow</i>			
284	0.21	0.46	0.39
280	0.79	0.54	0.61
(N)	(7)	(12)	(14)
\hat{f}	0.625	0.203	0.285
<i>AldoB5 fast</i>			
123	0.57	0.08	0.17
119	0.43	0.92	0.83
(N)	(7)	(12)	(12)
\hat{f}	-0.091	-0.048	-0.158
<i>AldoB5 faster</i>			
087	0.29	0.13	0.14
085	0.71	0.88	0.86
(N)	(7)	(12)	(14)
\hat{f}	-0.333	0.645	-0.130
CK			
138	0.29	0	0
133	0.71	1	1
(N)	(7)	(12)	(12)
\hat{f}	0.368	-	-
RP			
313	0	0.08	0
296	0.21	0.17	0
290	0.79	0.75	1
(N)	(7)	(12)	(12)
\hat{f}	0.625	0.214	-

Table 5

Population-pairwise multilocus $\hat{\theta}$ (Weir and Cockerham, 1984) (above diagonal) in three stingray species from the Indo-Malay-Papua archipelago, and ship distance between locations (in km, below diagonal, italics). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (permutation test; GENETIX 4.02; 1000 permutations); *na*, not applicable. **A.** *Neotrygon kublii*. **B.** *Himantura gerrardi* and *Taeniura lymna*.

A							
Species, Sample	Sample						
	Indian Ocean ^a	Indian Ocean ^b	Bali Strait	Flores Sea	Tomini Bay	Banda Sea	
<i>Neotrygon kublii</i>							
Indian Ocean ^a	0	0.105**	0.210***	0.360***	0.249**	0.440***	
Indian Ocean ^b	<i>823</i>	0	0.176***	0.326***	0.296	0.430***	
Bali Strait	<i>1 094</i>	<i>254</i>	0	0.153***	0.366*	0.332***	
Flores Sea	<i>1 604</i>	<i>789</i>	<i>638</i>	0	0.486*	0.290***	
Tomini Bay	<i>3 019</i>	<i>2 101</i>	<i>1 920</i>	<i>1 375</i>	0	0.557**	
Banda Sea	<i>2 330</i>	<i>1 433</i>	<i>1 224</i>	<i>695</i>	<i>729</i>	0	
^a Binuangen, Apr. 2010; ^b Malang, Oct. 2010							
B							
Species, Sample	Sample						
	Sunda Strait	Java Sea	Indian O. ^a	Flores Sea	Molucca Sea	West Papua	Unknown
<i>Himantura gerrardi</i>							
Sunda Strait	0	0.009	0.118	-	-	0.455	0.095*
Java Sea	<i>520</i>	0	0.176**	-	-	0.365*	0.136***
Indian Ocean ^a	<i>595</i>	<i>1 075</i>	0	-	-	0.493	0.057
West Papua	<i>4 126</i>	<i>3 592</i>	<i>3 729</i>	-	-	0	0.286
Unknown	<i>na</i>	<i>na</i>	<i>na</i>	-	-	<i>na</i>	0
<i>Taeniura lymna</i>							
Java Sea	0	-	-	0.153*	0.148*	-	-
Flores Sea	<i>1 507</i>	-	-	0	0.003	-	-
Molucca Sea	<i>2 405</i>	-	-	<i>1 277</i>	0	-	-

^a two samples were pooled (Fig. 1)

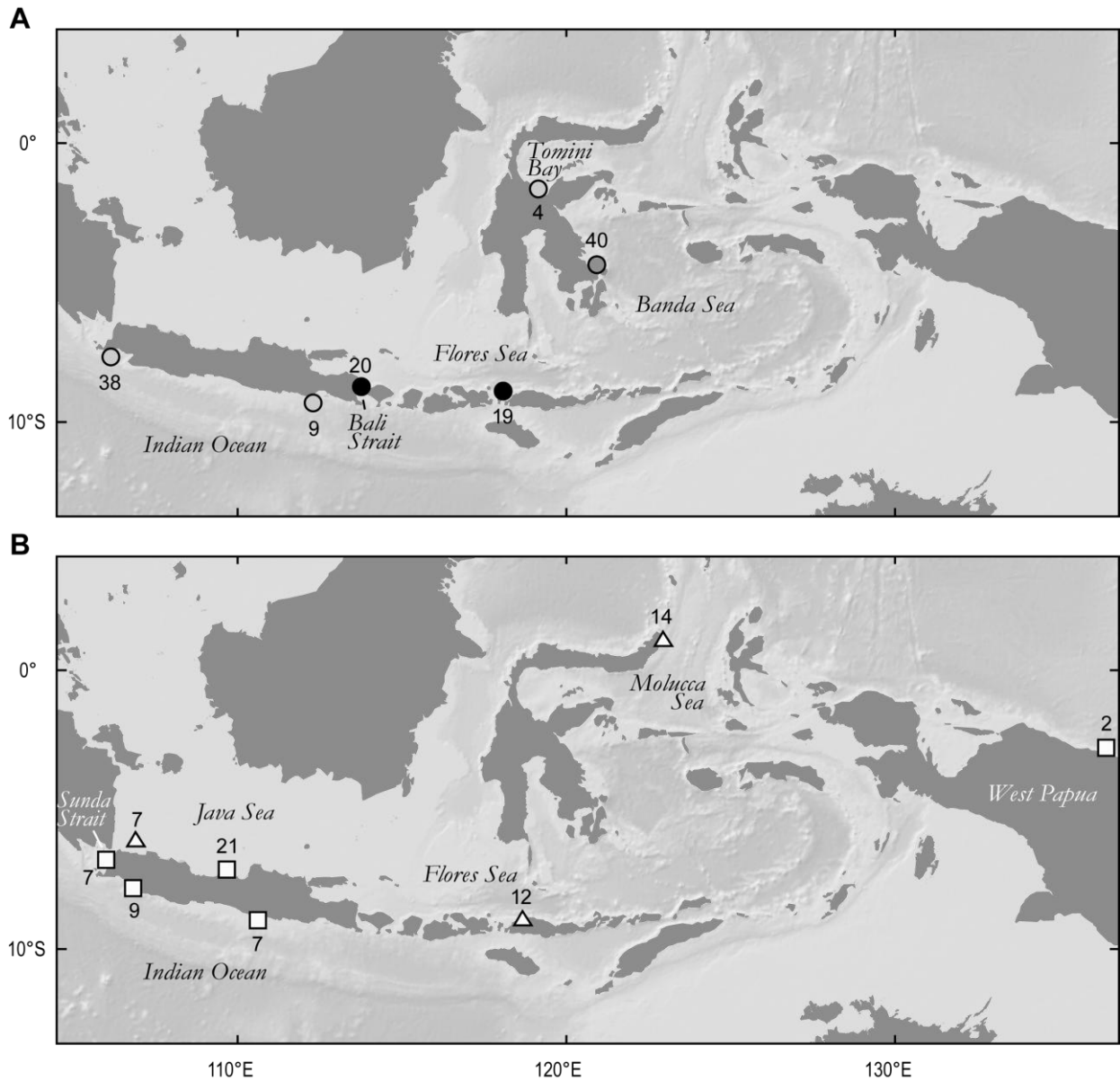


Fig. 1. Map of the Indo-Malay-Papua archipelago with sampling sites for three stingray species. Sample sizes indicated near symbols. Background topographic map from GeoMapApp (Ryan et al., 2009) (<http://www.geomapapp.org>). **A.** *Neotrygon kuhlii* (circles): grey tones (light grey, dark grey, black) illustrate the populations to which individuals were predominantly assigned according to Bayesian structure analysis (STRUCTURE: Falush et al., 2007; $K=3$). Indian Ocean, from West to East: Binuangen (Apr. 2010), Malang (Oct. 2010); Bali Strait: Banyuwangi (Oct. 2010); Flores Sea: Labuan Bajo (Oct. 2010); Tomini Bay: Poso (Jan. 2011); Banda Sea: Kendari (Oct. 2010). **B.** *Himantura gerrardi* (squares) and *Taeniura lymna* (triangles). Sunda Strait: Labuan (Oct. 2010); Indian Ocean, from West to East: Pelabuhan Ratu (Apr. 2010), Sadeng (Oct. 2010); Java Sea, from West to East: Pulau Pari (Nov. 2008) and Batang (Nov. 2007-Jan. 2008); Flores Sea: Labuan Bajo (Oct. 2010); Molucca Sea: Lembah Strait (Nov. 2010); West Papua: Port Numbay (Nov. 2010). Not represented: an *H. gerrardi* sample of unknown origin landed in Batang (Jan. 2008).

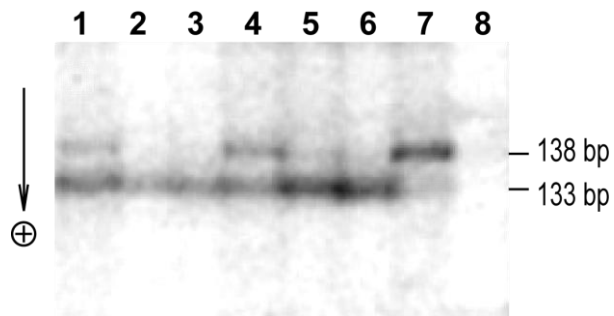


Fig. 2. Mendelian-like allele-size polymorphism detected from an electrophoresis gel of CY5-labelled, PCR-amplified introns at the *CK* locus in ribbontail stingray (*Taeniura lymna*). The gel was scanned under laser light (FMBIO® II Fluorescence Imaging System, Hitachi Instruments, San José CA, U.S.A.) to detect fluorescent DNA bands. Two size-alleles were scored, with size indicated on the left margin of the image. Lanes 1, 4: heterozygotes 138/133; lanes 2, 3, 5, 6: homozygotes 133; lane 7: homozygote 138; lane 8: PCR control with no DNA template.

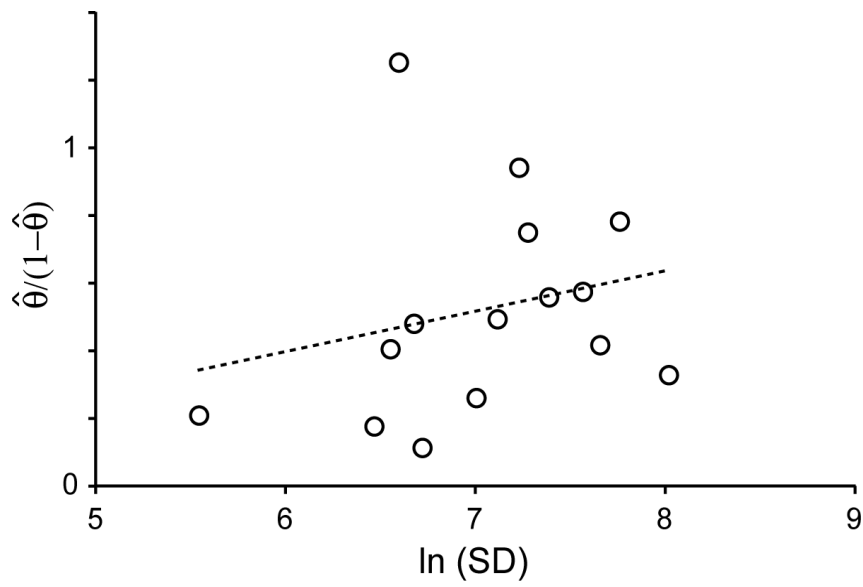


Fig. 3. Population genetic structure in *Neotrygon kublii* in the Indo-Malay-Papua archipelago. Pairwise multiple-locus estimates of genetic differentiation (ordinates) plotted against the logarithm of ship distance (*SD*; abscissa). The regression equation was $\hat{\theta}/(1-\hat{\theta}) = 0.118 \ln(SD) - 0.309$ [$R^2 = 0.058$; Mantel's test (Belkhir et al., 2000): $P=0.13$]. Pairwise multiple-locus θ were estimated from genotypic data (Supplementary material, Table S3) according to Weir and Cockerham (1984).

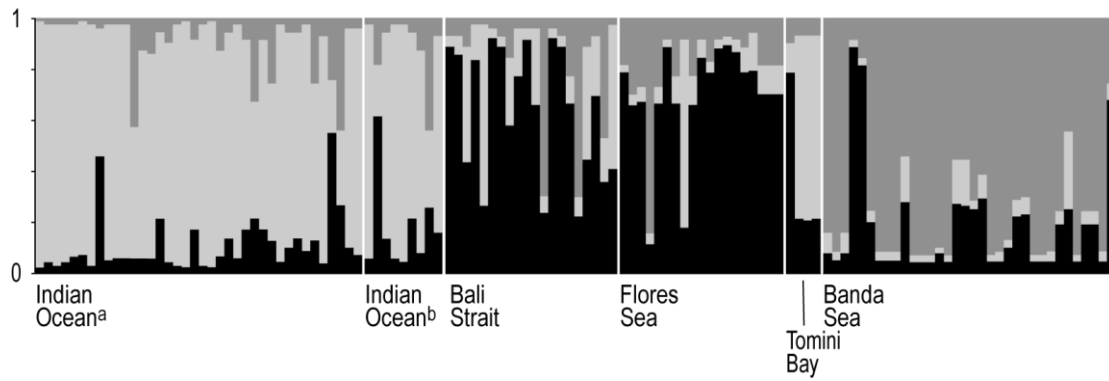


Fig. 4. *Neotrygon kuhlii*. Output of Bayesian structure analysis (STRUCTURE: Falush et al., 2007) with $K=3$ populations, on individual genotypes at 4 presumptive intron loci for 6 samples from the Indo-Malay-Papua archipelago (Supplementary material, Table S3). Individuals are represented by vertical bars; the different grey tones correspond to different genetic clusters and their proportion in an individual (ordinate) indicates its degree of assignment to the corresponding cluster. ^a Binuangen sample (Fig. 1); ^b Malang sample (Fig. 1).

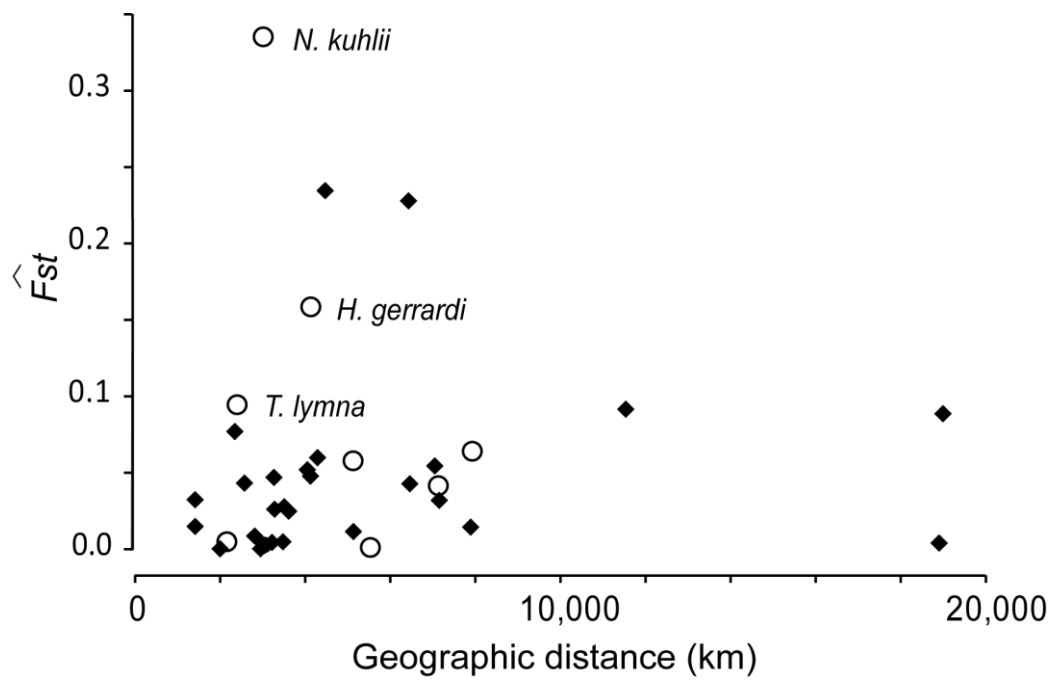


Fig. 5. Plot of genetic-differentiation estimates (overall \hat{F}_{st} value) against geographic distance in coastal elasmobranchs and teleosts. Data from nuclear-marker based phylogeographic surveys of 8 coastal elasmobranch species (open circles) (Chevolot et al., 2006; Dudgeon et al., 2009; Plank et al., 2010; Ovenden et al., 2011; present study) and 27 coastal teleost fish species (black diamonds) (Blanquer et al., 1992; Kotoulas et al., 1995; Borsa et al., 1997a; Roldán et al., 1998; Hoarau et al., 2002; Planes and Fauvelot, 2002; Borsa, 2003; Rohfritsch and Borsa, 2005; Hemmer-Hansen et al., 2007; Rolland et al., 2007; Magsino and Juinio-Meñez, 2008; Matschiner et al., 2009; Puebla et al., 2009; Purcell et al., 2009; González-Wangüemert et al., 2010; Jones et al., 2010; McCusker and Bentzen, 2010; Mobley et al., 2010; Fauvelot and Borsa, 2011; Francisco et al., 2011; Gaither et al., 2011; Horne et al., 2011; Muths et al., 2011; Timm et al., 2012). Other details in Supplementary material, Table S7.

Table S1

Introns tested for size polymorphism in *Himantura gerrardi*, *Neotrygon kuhlii*, and *Taeniura lymna*. Intron no.: assumed intron number in teleost fishes. Mendelian-like allele-size polymorphisms were detected using primer pairs (*Act-2-F*, *Act-2-R*), (*Cam-3-F*, *Cam-3-R*) and (*CK6-5'*, *CK7-3'*) in *H. gerrardi*, (*Cam-3-F*, *Cam-3-R*), (*CK6-5'*, *CK7-3'*) and (*Gpd2F*, *Gpd3R*) in *N. kuhlii*, and (*Aldo3F*, *Aldo2R*), (*CK6-5'*, *CK7-3'*) and (*RPEX1F*, *RPEX2R*) in *Taeniura lymna*. For the other primer pairs tested, either the DNA band patterns were not variable (thus exhibiting sample monomorphism), or they were variable but we failed to interpret them in terms of size polymorphism at Mendelian loci. In a few cases, the amplification was too weak for scoring, or the intensity of DNA bands was highly variable across individuals, or a substantial proportion of individuals could not be scored either because of bands that were too weak or because of overlapping bands presumably from other loci. Since a number of factors, e.g. unsuitable annealing temperature, quality of the primers, quality of the DNA extracts, etc. may lead to unsatisfactory results of this kind, we consider that more tests are needed before definitely discarding the primer pairs that failed to produce Mendelian-like polymorphisms in our study.

Gene or gene family	Intron no.	Abbreviation	Primer pair	Reference
Actin	2	<i>Act-2</i>	<i>Act-2-F</i> , <i>Act-2-R</i>	Atarhouch et al. (2003)
Aldolase B	2	<i>AldoB2</i>	<i>Aldob2F</i> , <i>Aldexon3R</i>	Hassan et al. (2002)
Aldolase B	4	<i>AldoB4</i>	<i>Aldo5F</i> , <i>Aldo3.1R</i>	Hassan et al. (2002)
Aldolase B	5	<i>AldoB5</i>	<i>Aldo3F</i> , <i>Aldo2R</i>	Hassan et al. (2002)
Aldolase C	1	<i>AldoC1</i>	<i>Aldoc1F</i> , <i>Aldoc2R</i>	Hassan et al. (2002)
α -Amylase	1	<i>Am2B-1</i>	<i>Am2b1F</i> , <i>Am2b2R</i>	Hassan et al. (2002)
α -Amylase	3	<i>Am2B-3</i>	<i>Am2b3F</i> , <i>Am2b4R</i>	Hassan et al. (2002)
Calmodulin	3	<i>Cam-3</i>	<i>Cam-3-F</i> , <i>Cam-3-R</i>	Atarhouch et al. (2003)
Calmodulin	4	<i>CaM</i>	<i>CALMex4F</i> , <i>CALMex5R</i>	Chow (1998); Chow and Takeyama (2000)
Creatine kinase	6	<i>CK</i>	<i>CK6-5'</i> , <i>CK7-3'</i>	Palumbi et al. (1991)
Creatine kinase	7	<i>Ck7</i>	<i>Ck7F</i> , <i>Ck8R</i>	Hassan et al. (2002)
Glyceraldehyde-3-phosphate dehydrogenase	2	<i>Gpd2</i>	<i>Gpd2F</i> , <i>Gpd3R</i>	Hassan et al. (2002)
Gonadotropin-releasing hormone 3	3	<i>GnRH3-3</i>	<i>GnRH3F</i> , <i>GnRH3R</i>	Hassan et al. (2002)
Growth hormone	2	<i>Gb2</i>	<i>Gb2F</i> , <i>Gb3R</i>	Hassan et al. (2002)
Growth hormone	5	<i>Gb5</i>	<i>Gb5F</i> , <i>Gb6R</i>	Hassan et al. (2002)
Major histocompatibility complex class II antigen	1	<i>MhcII</i>	<i>Mhc1F</i> , <i>Mhc2R</i>	Hassan et al. (2002)
Myosin, light chain	3	<i>Mlc-3</i>	<i>Mlc-3-F</i> , <i>Mlc-3-R</i>	Atarhouch et al. (2003)
Opsin	1	<i>Ops-1</i>	<i>Ops-1-F</i> , <i>Ops-1-R</i>	Atarhouch et al. (2003)
Ribosomal protein	1	<i>RP</i>	<i>RPEX1F</i> , <i>RPEX2R</i>	Chow and Takeyama (1998)
α -Tropomyosin	1	<i>Tr1</i>	<i>Tr1F</i> , <i>Tr1R</i>	Hassan et al. (2002)

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Table S2

Individual genotypes in 5 *Himantura gerrardi* samples from the Indo-Malay-Papua archipelago, at 7 presumptive intron loci. Alleles at a locus are designated by their approximate size (in bp).

Sample, individual no.	Locus						
	<i>Act-2</i>	<i>Cam-3 a</i>	<i>Cam-3 b</i>	<i>Cam-3 c</i>	<i>Cam-3 d</i>	<i>Cam-3 e</i>	<i>CK</i>
Indian Ocean							
DY 1	225/210	-	175	155	-	125	173
SY 1	238	200	175	155/152	138	125	175
SY 2	238/225	-	175	155	-	125	-
PLRQ 1	238	200	175	155	140/138	125	175
PLRC 2	238	206/200	175/173	155	138	125	-
Java Sea							
BT07 1	238/225	203	175/173	158/155	140	125	175
BT07 2	238/225	200	175	155	140/138	125	175/173
BT07 3	238/225	203/200	175/173	158/155	140/138	125	175/173
BT07 4	238/225	203/200	175/173	161/158	-	125	175/173
BT07 5	225	203/200	175/173	158/155	140/138	125	175/173
BT07 6	238/225	203/200	175/173	161	140	125	175
BT08 1	225	203/200	175	155	-	125	175
BT08 2	225	200	175/173	161/155	-	125	175/173
BT08 3	238	200	175/173	158/155	-	125	175/173
BT08 4	238	203/200	175/173	155	140	125	175
BT08 5	238	200	175/173	155	140	125	-
BT08 6	238/225	203/200	175/171	158/155	140	125	175/173
BT08 7	238/225	203/200	175	161/155	-	126/125	0
BT08 8	238	203/200	175/173	158/155	-	125	175/173
BT08 9	238/225	203/200	175/173	158/155	-	125	175/173
BT08 10	238	203/200	175/173	158/155	-	125	175/173
BT08 11	238	203/200	-	158/155	-	125	175/173
BT08 12	238/225	-	-	-	-	-	175/173
BT08 13	238	-	-	-	-	-	175/173
BT08 14	238/225	200	175/173	158/155	140/138	125	175/173
BT08 15	238/225	200	175/173	158/155	140	125	175
Unknown							
BLTR 18	238/225	206/200	178/175	158/155	140/138	125	175/173
BLTR 19	238/225	206/200	175	158/155	138	125	175/173
BLTR 20	238/225	206/200	175	158/155	138	125	175/173
BLTR 21	-	200	175/171	-	140/136	127	173
BLTR 22	238/225	206/200	175/171	158/155	138/136	125	175
BLTR 23	238/225	206/200	175	158	138	125	175/173
BLTR 24	238/225	200	173/171	155/152	138/136	127/125	175/173
BLTR 25	238/225	206/200	178/175	158/155	140/138	125	175/173
BLTR 26	238	206/200	178/175	161/155	138	125	175
Sunda Strait							
LBN 1	238/225	200	175/173	158/155	140	125	175/173
LBN 2	238/225	203/200	175/173	155	140	125	175/173
LBN 3	238/225	200	175/173	158/155	138	125	175/173
LBN 4	238	200	175	158/155	140/138	125	175
LBN 5	238/225	200	175/173	158/155	140/138	125	175/173
LBN 6	238	200	175	158/155	140	125	175/173
LBN 7	225	200	175	158/155	140	125	175
West Papua							
HNSM1	-	-	171	155	-	125	175
HNSM2	-	-	-	155	-	125	175

Table S3

Individual genotypes in 6 *Neotrygon kuhlii* samples from the Indo-Malay-Papua archipelago, at 4 presumptive intron loci. Alleles at a locus are designated by their approximate size (in bp).

Sample, individual no.	Locus				Sample, individual no.	Locus			
	<i>Cam-3 fast</i>	<i>Cam-3 slow</i>	<i>CK</i>	<i>Gpd2</i>		<i>Cam-3 fast</i>	<i>Cam-3 slow</i>	<i>CK</i>	<i>Gpd2</i>
Indian Ocean ^a					Flores Sea				
BNG1	282	400	368	167	MB18	300/282	400/370	363/361	167
BNG2	282/264	400	368/361	167	MB19	300/282	400	361	-
BNG4	282/264	400	363/361	167	MB20	282	400	366/361	167
BNG5	282/264	400	368/363	167	LB1	300	400	361	173/167
BNG7	282/264	400	363	167	LB2	300	400	361	169/167
BNG8	282	400	363	167	LB3	300	400/370	363/361	-
BNG9	282	400/370	368	167	LB4	300	400	361/359	-
BNG10	282	400	368/363	167	LB5	300	400	361	167
BNG11	282	400/370	361	167	LB6	300	400/370	361	173/167
BNG12	-	-	368	-	LB7	300	400	363/361	167
BNG13	282	400	368/361	167	LB8	300/282	400	368/361	167
BNG14	282	400	363	167	LB9	300	400	363/361	167
BNG15	282	400	363	167	LB10	300	400/370	363/361	167
BNG16	300/282	400	368/363	164	LB11	300	400	361	173/167
BNG17	300/282	400	368	167/164	LB12	300	400/385	361	167
BNG18	300/282	400	368	167/164	LB13	300	400/385	363/361	173/167
BNG19	282	400	361	167	LB14	300	400/385	363/361	167
BNG20	282/264	400	368/361	167/164	LB15	300/282	400	361	173/167
BNG21	282	400	368/363	167	LB16	-	400/385	363/361	167
BNG22	282	400	368	167	LB17	300	400	-	167
BNG23	300/282	400	368/361	167	LB18	300	400	-	167
BNG24	282	400	368/363	167	LB19	300	400	-	167
BNG25	282	400	368	167	Tomini Bay				
BNG26	300/282	400	368	167/164	LPPS1	-	-	361	167
BNG27	282	400	368/366	167/164	LPPS2	282	400	361	167
BNG28	282	400	363	167	LPPS3	282	400	361	167
BNG29	300/282	400	368/361	167	LPPS4	282	400	361	167
BNG30	300/282	400	363	167/164	Banda Sea				
BNG31	300/282	400	368/361	167	KDI4	-	430/400	-	-
BNG32	300/282	400	368/361	167/164	KDI5	300/282	400	361	164
BNG33	282/264	400	368/361	167	KDI6	300	400	363/361	164
BNG34	300/282	400	368/363	167	KDI7	300/282	400	361	164
BNG35	282	400	368/366	167/164	KDI8	300	400/370	366	167/164
BNG36	282	400	-	167	KDI9	300	400/370	366/361	167/164
BNG37	300/282	400	368/361	167/164	KDI10	300	-	-	-
BNG38	282	400	368/363	167/164	KDI11	300	400	361	167/164
BNG39	300/282	400/370	363/361	167/164	KDI12	300	400	363/361	164
BNG40	300/282	400	363/361	167/164	KDI13	300	400	363/361	164
BNG41	282	400	363/361	167	KDI14	300	400	363/361	164
BNG42	300/282	400	368	167	KDI15	300	400	-	-
Indian Ocean ^b					KDI16	300/282	400	361	167/164
SB1	282	400	363	167	KDI17	300	400	361	164
SB2	300	400	363	167	KDI18	300	400	361	164
SB3	282/264	400	361	167	KDI19	300	400	361	164
SB4	282	400	363	167	KDI20	300	430/400	361/359	164
SB5	282/264	400	363	167	KDI21	300	430	-	-
SB6	282	400	361	167	KDI22	300	400	361	164
SB7	282/264	400	363/361	167/164	KDI23	300/282	400	361	167/164
SB8	300/282	400	363/361	167/164	KDI24	300/282	400	-	-
SB9	264	400/370	363	167/164	KDI25	300/282	400	361	167/164
Bali Strait					KDI26	300	400	361	-
MB1	300	400	366/361	167	KDI27	300/282	400/370	361	164
MB2	300/282	400/370	366	167/164	KDI28	300	400	361	164
MB3	300/282	400	363/361	167	KDI29	300	400	363/361	164
MB4	300/282	400	366	167	KDI30	300	400	359	-
MB5	282	400	366/363	167	KDI31	300	400	363/361	167/164
MB6	300	400	366	167	KDI32	300	400	363/361	167/164
MB7	300	400	366/361	167	KDI33	300	400	361	164
MB8	300/282	400	366/363	167/164	KDI34	300	400	361	164
MB9	300/282	400	366/361	167	KDI35	300	400	363/361	164
MB10	300	400/370	366/363	167	KDI36	300	400	361	167/164
MB11	300/282	400	366/363	167	KDI37	300/282	400	363/361	167/164
MB12	300	400	363/361	167/164	KDI38	300	400	363/359	164
MB13	300	400	366	167	KDI39	300	400	361	167/164
MB14	300	400	366/361	167	KDI40	300	400	361	167/164
MB15	300	400	363/361	167	KDI41	300	400	363/361	164
MB16	300	400	363/361	167/164	KDI42	300	400	361	167
MB17	300/282	400	363/361	167	KDI43	300/282	-	-	167/164

^a Binuangen sample (Fig. 1A); ^b Malang sample (Fig. 1A)

Table S4

Individual genotypes in three *Taeniura lymna* samples from the Indo-Malay-Papua archipelago, at 5 presumptive intron loci. Alleles at a locus are designated by their approximate size (in bp).

Sample, individual no.	Locus				
	<i>AldoB5 slow</i>	<i>AldoB5 fast</i>	<i>AldoB5 faster</i>	<i>CK</i>	<i>RP</i>
Java Sea					
KS 1	280	123/119	085	138/133	290
KS 2	284/280	123/119	085	133	290
KS 3	280	123	087/085	133	296
KS 4	280	123/119	087/085	138/133	290
KS 5	284	123/119	087/085	133	290
KS 6	280	119	085	133	290
KS 7	280	123	087/085	138	296/290
Flores Sea					
LB 1	280	123/119	085	133	290
LB 2	284/280	119	085	133	290
LB 3	284	119	087	133	296/290
LB 4	284	119	085	133	290
LB 5	284/280	119	085	133	290
LB 6	284/280	119	085	133	296
LB 7	284	119	085	133	296/290
LB 8	284/280	119	085	133	290
LB 9	280	119	085	133	313/290
LB 10	280	119	085	133	313/290
LB 11	280	119	087/085	133	290
LB 12	284/280	123/119	085	133	290
Molucca Sea					
LSNS 1	284	-	085	-	-
LSNS 2	284/280	-	085	-	-
LSNS 3	280	123/119	087/085	133	-
LSNS 4	284/280	119	085	133	290
LSNS 5	284/280	119	085	133	290
LSNS 6	284/280	119	085	133	290
LSNS 7	280	123/119	087/085	133	290
LSNS 8	280	123/119	087/085	133	290
LSNS 9	280	123/119	087/085	133	290
LSNS 10	284	119	085	133	290
LSNS 11	280	119	085	133	290
LSNS 12	284/280	119	085	133	290
LSNS 13	280	119	085	133	290
LSNS 14	284	119	085	133	290

Table S5

Locus-pairwise linkage disequilibrium values (R ; Belkhir et al., 2000) in samples of three stingray species from the Indo-Malay-Papuan archipelago. Probability of encountering a value equal or higher than the observed value under the null hypothesis (P) is given (in brackets) when $P \leq 0.050$. Only one P -value remained significant after correction for multiple tests (Rice, 1989). **A.** *Himantura gerrardi*. Sample “Unknown” exhibited significant D values (before correction for multiple tests) in 7/21 pairwise comparisons, which we interpret as linkage disequilibrium in the sample, due to admixture of individuals from different locations. **B.** *Neotrygon kuhlii*. One R value remained significant after correction for multiple tests: sample ‘Banda Sea’ at locus pair (*Cam-3 slow*, *CK*). **C.** *Taeniura lymna*. 3/3 D -values significant (before correction for multiple tests) in sample ‘Molucca Sea’: this may be ascribed to admixture.

A							
Locus	Sample	Locus					
		<i>Cam-3 a</i>	<i>Cam-3 b</i>	<i>Cam-3 c</i>	<i>Cam-3 d</i>	<i>Cam-3 e</i>	<i>CK</i>
<i>Act-2</i>	Indian Ocean	-	0.434	0.434	-	-	0
	Java Sea	0.032	0.228	0.189	0.590	0.058	0.149
	Sunda Strait	0.107	0.301	0.107	0.153	-	0.165
	West Papua	-	-	-	-	-	-
	Unknown	0.163	0.294	0.946	0.313	0.163	0.748
<i>Cam-3 a</i>	Indian Ocean	-	-	0	0	-	-
	Java Sea	-	0.125	0.193	0.283	0.119	0.296
	Sunda Strait	-	0.852	1.000	0.374	-	0.301
	West Papua	-	-	-	-	-	-
	Unknown	-	0.158	0.946	0.673	1.000 ($P=0.016$)	0.649
<i>Cam-3 b</i>	Indian Ocean	-	-	0.313	0	-	-
	Java Sea	-	-	0.271	0.296	0.391	0.296
	Sunda Strait	-	-	0.412	0.330	-	0.301
	West Papua	-	-	-	-	-	-
	Unknown	-	-	0.695 ($P=0.039$)	0.748 ($P=0.049$)	0.550 ($P=0.013$)	0.649
<i>Cam-3 c</i>	Indian Ocean	-	-	0	0	-	0
	Java Sea	-	-	0.221	0.240	0.240	0.288
	Sunda Strait	-	-	0.374	-	-	0.301
	West Papua	-	-	-	-	-	-
	Unknown	-	-	-	0.355	0.946 ($P=0.019$)	0.446
<i>Cam-3 d</i>	Indian Ocean	-	-	-	-	-	-
	Java Sea	-	-	-	-	-	0.900
	Sunda Strait	-	-	-	-	-	0.072
	West Papua	-	-	-	-	-	-
	Unknown	-	-	-	-	0.723 ($P=0.004$)	0.465
<i>Cam-3 e</i>	Indian Ocean	-	-	-	-	-	-
	Java Sea	-	-	-	-	-	-
	Sunda Strait	-	-	-	-	-	-
	West Papua	-	-	-	-	-	-
	Unknown	-	-	-	-	-	0.772 ($P=0.018$)

B				
Locus	Sample	Locus		
		<i>Cam-3 slow</i>	<i>CK</i>	<i>Gpd2</i>
<i>Cam-3 fast</i>	Indian Ocean ^a	0.080	0.136	0.356 ($P=0.025$)
	Indian Ocean ^b	0.607 ($P=0.031$)	0.195	0.349
	Bali Strait	0.011	0.050	0.108
	Flores Sea	0.200	0.342 ($P=0.044$)	0.124
	Tomini Bay	-	-	-
<i>Cam-3 slow</i>	Banda Sea	0.085	0.199	0.179
	Indian Ocean ^a	-	0.146	0.020
	Indian Ocean ^b	-	0.325	0.563
	Bali Strait	-	0.162	0.138
	Flores Sea	-	0.242	0.114
<i>CK</i>	Tomini Bay	-	-	-
	Banda Sea	-	0.306 ($P=0.0001$)	0.109
	Indian Ocean ^a	-	-	0.138
	Indian Ocean ^b	-	-	0
	Bali Strait	-	-	0.148
<i>RP</i>	Flores Sea	-	-	0.354
	Tomini Bay	-	-	-
	Banda Sea	-	-	0.196

^a Binuangen sample (Fig. 1A); ^b Malang sample (Fig. 1A)

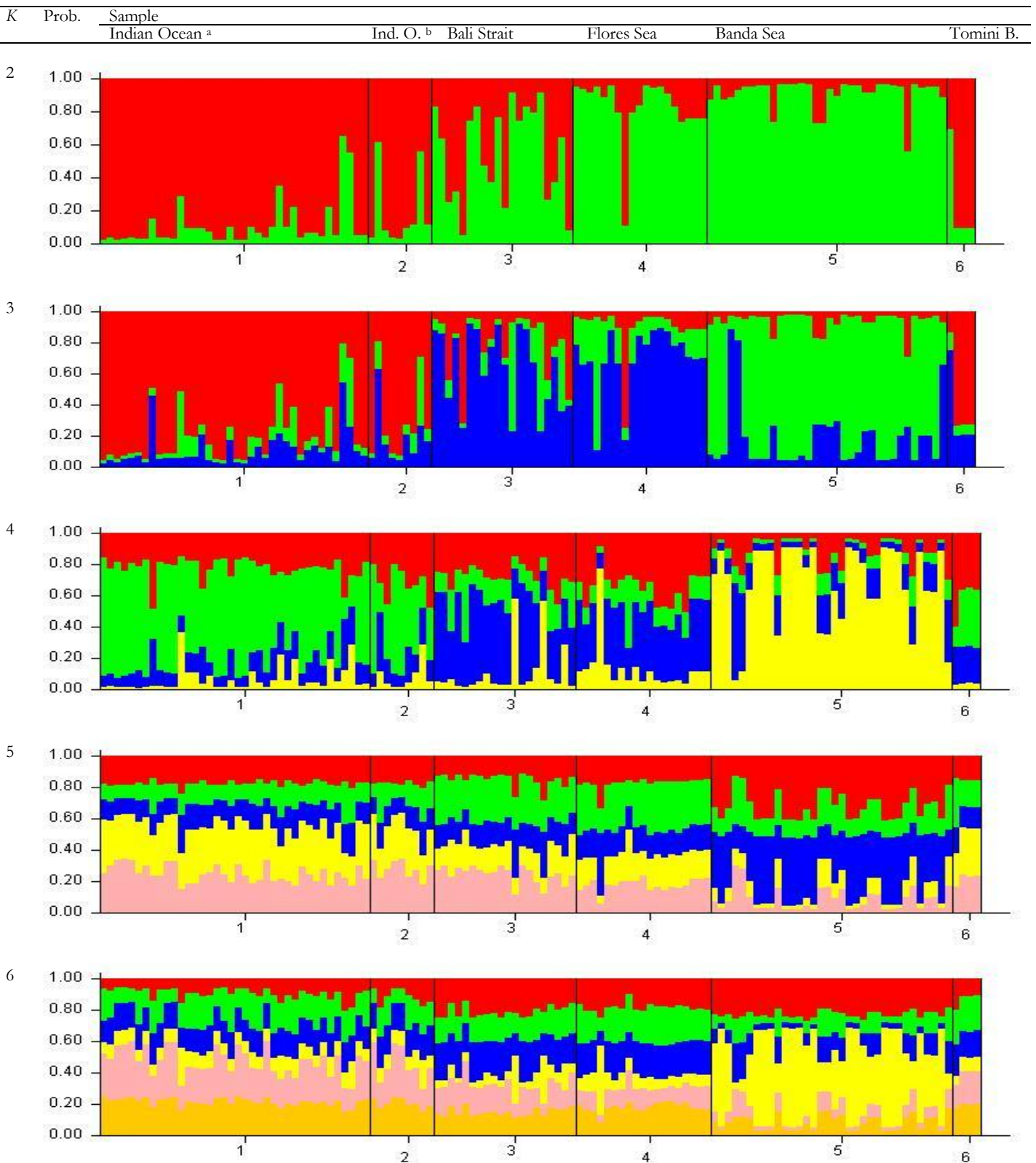
C					
Locus	Sample	Locus			
		<i>AldoB5 fast</i>	<i>AldoB5 faster</i>	<i>CK</i>	<i>RP</i>
<i>AldoB5 slow</i>	Java Sea	0.153	0.132	0.538 ($P=0.041$)	0.404
	Flores Sea	0.268	0.252	-	0.395 ($P=0.026$)
	Molucca Sea	0.690 ($P=0.017$)	0.693 ($P=0.008$)	-	-
<i>AldoB5 fast</i>	Java Sea	-	0.753	0.511	0.920 ($P=0.005$)
	Flores Sea	-	0.205	-	0.287
	Molucca Sea	-	1.000 ($P=0.003$)	-	-
<i>AldoB5 faster</i>	Java Sea	-	-	0.330	0.594
	Flores Sea	-	-	-	0.190
	Molucca Sea	-	-	-	-
<i>CK</i>	Java Sea	-	-	-	0.090
	Flores Sea	-	-	-	-
	Molucca Sea	-	-	-	-

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Table S6

Neotrygon kublii. Output of Bayesian structure analysis (STRUCTURE: Falush et al., 2007) run with $K=2-6$ populations, on individual genotypes at 4 presumptive intron loci for 6 samples from the Indo-Malay-Papua archipelago (Supplementary material, Table S3). Individuals are represented by vertical bars; the different grey colours correspond to different genetic clusters and their proportion in an individual (ordinate) indicates its degree of assignment to the corresponding cluster. Visual observation of the graphs indicates that maximum segregation of the total sample into different clusters was reached with $K=3$. *Prob.* Posterior probability of assignment of a given individual to a cluster. Individuals grouped by sample. Samples separated by vertical traits.



^a Binuangen sample (Fig. 1A); ^b Malang sample (Fig. 1A)

Reference.

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Table S7

Regional-scale population differentiation estimates at nuclear loci in coastal elasmobranchs and teleost fishes. R , number of marker loci. Distance calculated as ship distance (i.e. the shortest maritime path) using the PATH menu of Google Earth (http://www.google.co.uk/intl/en_uk/earth/).

Species	Genetic markers	r	Geographical range considered	Max. distance between samples (km)	Overall (max.) \hat{F}_{st} or equivalent	Reference	
Scientific name	Vernacular name						
Elasmobranchs							
<i>Himantura gerrardi</i>	Sharpnose stingray	introns	7	Indo-Malay-Papua archipelago	4 130	0.159 (0.493)	present study
<i>Neotrygon kuhlii</i>	Blue-spotted maskray	introns	4	Indo-Malay archipelago	3 020	0.336 (0.557)	present study
<i>Raja clavata</i>	Thornback ray	microsatellites	5	NE Atlantic + Mediterranean	7 130	0.042 (0.231)	Chevolut et al. (2006)
<i>Rhizoprionodon acutus</i>	Milk shark	microsatellites	6	Indonesia and eastern Australia	5 130	0.058 (0.223)	Ovenden et al. (2011)
<i>Sphyrna lewini</i>	Scalloped hammerhead	microsatellites	8	Indonesia and eastern Australia	5 540	0.002 (0.007)	Ovenden et al. (2011)
<i>Stegostoma fasciatum</i>	Zebra shark	microsatellites	13	Central Indo W Pacific	7 940	0.065 (0.098)	Dudgeon et al. (2009)
<i>Taeniura hymna</i>	Ribbontail stingray	introns	5	Indo-Malay archipelago	2 410	0.095 (0.153)	present study
<i>Urobatis balleri</i>	Round stingray	microsatellites	7	California + Gulf of California	2 170	0.002-0.007 (0.025)	Plank et al. (2010)
Teleosts							
<i>Acanthurus triostegus</i>	Manini	allozymes	12	W Pacific	11 540 ^a	0.092 (0.096) ^a	Planes and Fauvelot (2002)
<i>Amphiprion ocellaris</i>	False clownfish	microsatellites	6	Indo-Malay-Papua archipelago	4 120	0.048 (0.208)	Timm et al. (2012)
<i>Anarhichas lupus</i>	Atlantic wolffish	microsatellites	12	N Atlantic	5 140	0.012 (0.037)	McCusker and Bentzen (2010)
<i>Cephalopholis argus</i>	Peacock grouper	introns	2	Indo-West Pacific	18 970	0.089 (0.496)	Gaither et al. (2011)
<i>Decapterus macrosoma</i>	Round scad mackerel	intron	1	Indo-Malay archipelago	3 490	0.005 (0.100)	Borsa (2003)
<i>Decapterus russelli</i>	Indian scad mackerel	introns	2	Indo-Malay-Papua archipelago	4 280	0.061 (0.165)	Rohfritsch and Borsa (2005)
<i>Diplodus sargus</i>	White seabream	microsatellites	9	NE Atlantic + Mediterranean	3 540	0.029 (0.115)	González-Wangüemert et al. (2010)
<i>Eleutheronema tetradactylum</i>	Fourfinger threadfin	microsatellites	5	Northern Australia	4 480	0.235 (0.581)	Horne et al. (2011)
<i>Gobionotothen gibberifrons</i>	Humped rockcod	microsatellites	8	SW Atlantic	2 000	0.001 (0.005)	Matschiner et al. (2009)
<i>Hypoplectrus puella</i>	Barred hamlet	microsatellites	10	Gulf of Mexico	3 200	0.005 (0.017)	Puebla et al. (2009)
<i>Lipophrys pholis</i>	Shanny	intron	1	NE Atlantic + Mediterranean	2 940	0.000 (0.048)	Francisco et al. (2011)
<i>Lutjanus kasmira</i>	Common bluestripe snapper	introns	2	Indo-West Pacific	18 900 ^b	0.004 (0.048) ^b	Gaither et al. (2010)
<i>Merluccius merluccius</i>	Hake	allozymes	10	NE Atlantic + Mediterranean	7 170	0.033 (0.052)	Roldán et al. (1998)
<i>Myripristis berndti</i>	Blotcheye soldierfish	microsatellites	8	SW Indian Ocean	2 580	0.043 (0.119)	Muths et al. (2011)
<i>Platichthys flesus</i>	Flounder	allozymes	5	NE Atlantic	3 260	0.047±0.016 (0.058)	Borsa et al. (1997)
<i>Platichthys flesus</i>	Flounder	microsatellites	9	NE Atlantic	3 640	0.024 (0.091)	Hemmer-Hansen et al. (2007)
<i>Pleuronectes platessa</i>	Plaice	microsatellites	6	NE Atlantic	2 840	0.009 (0.046)	Hoarau et al. (2002)
<i>Pomacentrus amboinensis</i>	Ambon damselfish	microsatellites	9	Coral Sea to Bismarck Sea	2 360	0.077 (0.214)	Jones et al. (2010)
<i>Psetta maxima</i>	Turbot	allozymes	5	NE Atlantic + Mediterranean	7 050	0.055 (0.101)	Blanquer et al. (1992)
<i>Scomberomorus commerson</i>	Narrow-barred Spanish mackerel	microsatellites	8	Central Indo-West Pacific	6 450	0.228 (0.311)	Fauvelot and Borsa (2011)
<i>Scophthalmus rhombus</i>	Brill	allozymes	7	NE Atlantic + Mediterranean	4 050	0.053 (0.084)	Blanquer et al. (1992)
<i>Siganus argenteus</i>	Forktail rabbitfish	allozymes	14	Eastern Philippines	1 420	0.015	Magsino and Juinio-Meñez (2008)
<i>Siganus fuscescens</i>	Mottled rabbitfish	allozymes	9	Eastern Philippines	1 420	0.033 (0.081)	Magsino and Juinio-Meñez (2008)
<i>Solea solea</i>	Common sole	allozymes	12	NE Atlantic + Mediterranean	7 900	0.015±0.003 (0.126)	Kotoulas et al. (1995)
<i>Solea solea</i>	Common sole	introns	3	NE Atlantic + Mediterranean	6 460	0.043 (0.176)	Rolland et al. (2007)
<i>Stegastes partitus</i>	Bicolour damselfish	microsatellites	5	Caribbean Sea	3 020	0.003 (0.008)	Purcell et al. (2009)
<i>Syngnathus floridae</i>	Dusky pipefish	microsatellites	6	NW Atlantic	3 290	0.026 (0.054)	Mobley et al. (2010)

^a Hawaii, Marquesas and Clipperton samples excluded; ^b Marquesas sample excluded

References to Table S7

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