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Research Article

High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past?

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Abstract Historical sea level fluctuations have influenced the genetic structure and evolutionary history of marine species and examining widespread species across their species' ranges may elucidate some of these effects. *Chlorurus sordidus* is a common and widespread parrotfish found on coral reefs throughout the Indo-central Pacific. We used phylogenetic, phylogeographic, and cladistic analyses to examine the genetic composition and population structure of this species across most of its latitudinal range limits. We sequenced 354 bp of the mitochondrial control region I in 185 individuals from nine populations. Populations of *C. sordidus* displayed high levels of genetic diversity, similar to those recorded for widespread pelagic fish species, but much greater nucleotide diversity values than those previously recorded for other demersal reef fishes. Both phylogenetic and phylogeographic analyses detected strong genetic subdivision at the largest spatial scale (i.e. among oceans). The Pacific Ocean was characterised by weak population genetic structure. Separation of the Hawaiian location from other Pacific and West Indian Ocean sites was evident in phylogenetic analyses, but

not from analysis of molecular variance. NCA and isolation-by-distance tests suggested that the genetic structure of this species was the result of multiple contemporary and historical processes, including long-distance colonisation and range expansion arising from fluctuating sea levels, limited current gene flow, and isolation by distance. This pattern is to be expected when historically fragmented populations come into secondary contact. We suggest the patterns of population genetic structure recorded in *C. sordidus* are caused by large local population sizes, high gene flow, and a recent history of repeated fragmentation and remixing of populations resulting from fluctuating sea levels.

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Introduction

Studies of patterns of connectivity among conspecific populations of marine organisms, including those on coral reef taxa, have yielded complex results. While some studies have provided evidence of long-distance dispersal linking populations at the scale of ocean basins (Benzie [1999](#); Bowen et al. [2001](#)), other studies suggest that in many instances gene flow may be more restricted than previously thought (e.g. Knowlton [1993](#); Palumbi [1994](#); Benzie [1998](#), [1999](#); Planes et al. [1998](#); Taylor and Hellberg [2003](#)). It is increasingly apparent that the genetic structure of marine organisms may be the result of complex current and historical interactions among factors such as habitat connectivity, adult ecology and demography, larval behaviour, and dispersal potential.

Large-scale genetic structure in marine organisms has been found to coincide with biogeographic barriers, such as the Pacific Ocean/Indian Ocean barrier (presumably) located between the coast of Western Australia and Christmas Island (McMillan and Palumbi [1995](#); Lavery et al. [1995](#), [1996](#); Benzie [1998](#); Williams and Benzie [1998](#); Duda and Palumbi [1999](#)) and the Pacific divide situated between Micronesia and Hawaii (Stepien et al. [1994](#); Bernardi et al. [2001](#); but see Williams and Benzie [1996](#)). Genetic structure may also increase gradually with increasing geographic distance when settlement events decline with increasing distance from source areas (isolation by distance, or IBD; Slatkin [1993](#); Palumbi [1994](#); Rousset [1997](#)) or decrease with increasing dispersal ability at any given spatial scale. IBD has been shown to be important over short distances in marine species lacking pelagic larval dispersal (e.g. Hellberg [1994](#); Johnson and Black [1998](#)) but has rarely been recorded in pelagic dispersing marine organisms except over very large distances (Richardson [1983](#); Palumbi et al. [1997](#); Lavery et al. [1996](#); Planes and Fauvelot [2002](#)). A positive relationship between larval duration (a proxy for dispersal ability) and gene flow has been found in some taxa (Doherty et al. [1995](#); Riginos and Victor [2001](#); but see Shulman and Bermingham [1995](#)).

Analysis of genetic structure provides a powerful means of detecting the history of events that have produced present-day patterns of

distribution and connectivity among populations (Benzie [1999](#); Barber et al. [2000](#)). Rapid changes in Pleistocene sea levels driven by cycles of glaciation profoundly affected coral reef habitats and the patterns of connectivity amongst their resident biota, especially in the western Pacific region of the Indo-Australia archipelago (Palumbi [1994](#); Benzie [1999](#); Lambeck and Chappell [2001](#); Yokoyama et al. [2000](#)). This allowed for both restriction and expansion of species ranges producing cycles of genetic isolation, secondary contact, and subsequent merging (Benzie [1999](#)). Widely distributed species whose populations have been subject to the effects of such cycles may exhibit genetic signals characterised by high genetic diversities and complex geographical structures (Grant and Bowen [1998](#)).

Current genetic structure may reflect historical rather than ongoing gene flow (Palumbi [1997](#); Benzie [1999](#)). However, traditional phylogenetic and population genetic analyses have not been able to separate these effects (Crandall et al. [1994](#); Templeton et al. [1995](#); Templeton [1998](#); Posada and Crandall [2001](#)). Nested clade analysis (NCA) uses the genealogical information implicit in the haplotype network to hypothesise on the roles of current and historical gene flow, range expansion, fragmentation, and the spatial and temporal scales at which these processes act (Templeton et al. [1995](#); Templeton [1998](#); Posada and Crandall [2001](#)). NCA does not, however, calculate statistical uncertainty associated with models and does not test to differentiate among alternative outcomes (Knowles and Maddison [2002](#)). While NCA shows promise in elucidating evolutionary processes in the marine environment, especially when interpreted in concert with other analyses, it has so far been applied only to freshwater, anadromous, or temperate marine fish species (e.g. Durand et al. [1999](#); Johnson and Jordan [2000](#); McCusker et al. [2000](#); Gharrett et al. [2001](#)) or recently to tropical invertebrates (e.g. Marquez et al. [2002](#); Rodriguez-Lanetty and Hoegh-Guldberg [2002](#); Worheide et al. [2002](#)).

Members of the scarid genus *Chlorurus* are abundant and widespread members of the reef fish fauna and have an inferred history extending into at least the late Pliocene (Bellwood [1994](#)) or earlier (Streelman et al. [2002](#)). *C. sordidus* provides an appropriate study species to examine the role of historical sea level changes. *C. sordidus* is an abundant reef fish at both local and regional scales occupying a variety of habitats within reef systems (Russ [1984a](#), [1984b](#); Choat and Bellwood [1985](#); Gust et al. [2001](#); Sluka and Miller [2001](#)). The species has distinctive initial and terminal colour phases (Choat and Randall [1986](#)), and some differentiation in terminal colouration has been observed between Indian Ocean and Pacific populations (Randall and Bruce [1983](#); Randall et al. [1997](#)). The demographic profile of this species suggests high abundances, early maturity, and extensive reproductive outputs. *C. sordidus* achieves abundances varying between 4 and 18 adults per 100 m² (Gust et al. [2001](#)) and reaches maturity between 1 and 2 years of age with a total life span of 8–9 years (Choat and Robertson [1975](#), [2002](#); Gust et al. [2002](#)). The planktonic larval duration averages 30 days although some individuals may spend up to 40 days in the pelagic environment (Chen [1999](#)). The species is protogynous, with female-biased sex ratios and high reproductive outputs (Choat and Robertson [1975](#)). Spawning episodes are frequent with up to 50 group spawnings being recorded within a 100-m² reef area over a 15-min period (Sancho et al. [2000](#)). Although no individual fecundity figures are available, the reproductive activities in *C. sordidus* were the highest recorded from a suite of 34 reef fish species (Sancho et al. [2000](#)). These demographic and reproductive characteristics infer widespread distribution through larval dispersal.

Here we employed phylogenetic, population genetic, and cladistic approaches to examine the genetic composition and population structure of the common reef fish, *C. sordidus* over a large part of its Indo-central Pacific range. Using a mitochondrial molecular marker we examined the geographic patterns of genetic differentiation in this species to understand the roles of historical and present-day gene flow in producing these patterns. We addressed the following questions: (1) is there any evidence for genetic structure across a large part of the species' range spanning known biogeographic barriers? (2) If so, is that pattern concordant with the distribution of these barriers? (3) Does genetic differentiation in this species appear to be due to biogeographical vicariance events, past fragmentation, limited dispersal (IBD), or elements of all? (4) Do the genetic diversities (nucleotide and haplotype) reveal any information on the evolutionary history of this species? We predicted that *C. sordidus*, having demographic characteristics consistent with high dispersal and slow fixation, would only display strong genetic structure across large spatial scales. Furthermore we hypothesised that populations of *C. sordidus*, by virtue of its generalist ecology, persisted well during cycles of sea level changes and that genetic characteristics and structure of this species will reflect this persistence.

Materials and methods

Sampling locations

Samples were obtained from nine populations distributed within six locations spanning more than 17,000 km (Fig. 1, Table 1). Sampling in the West Indian Ocean was partitioned between the carbonate reefs of the Amirante Island chain and the granitic reefs of the Seychelles Bank. Specimens from eight populations were collected by spearing and sequences from a ninth population (Lizard Island) were provided by C. Dudgeon (Dudgeon et al. 2000). Pectoral fin clips from 10–34 fish were sampled from each population and preserved in 80% EtOH.

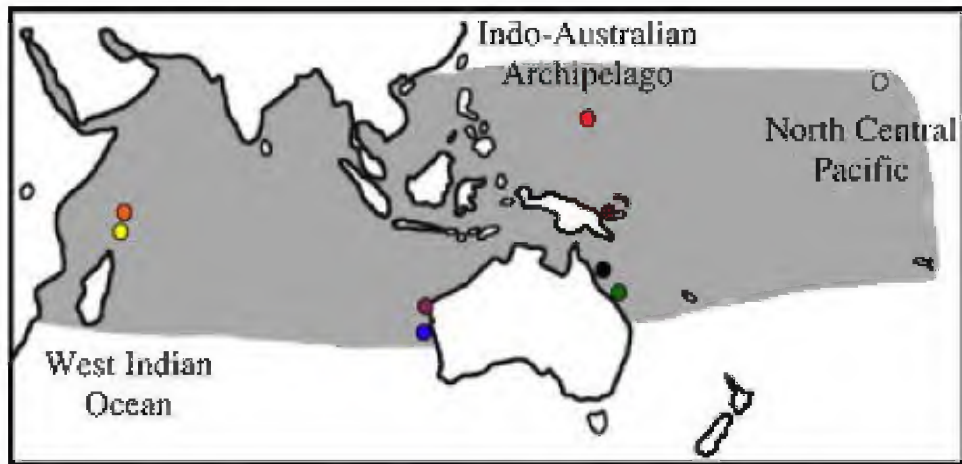


Fig. 1 Map showing the species range of *Chlorurus sordidus* and the nine sampling locations. Locations are colour coded: Amirante *yellow*, Seychelles *orange*, Abrolhos Islands *blue*, Ningaloo Reef *purple*, Papua New Guinea *brown*, Lizard Island *black*, Whitsundays *green*, Rota *red*, Hawaii *grey*. The geographical locations of West Indian Ocean (WIO), Indo-Australian Archipelago (IAA), and north central Pacific (Hawaii) are indicated by the labels

Table 1 Number of individuals sampled (n), number of haplotypes n_h , haplotypic (h) and nucleotide (π , in percent) diversities (\pm SE) in different populations of *Chlorurus sordidus*

	Individuals sampled	Haplotypes sampled	Diversities	
	n	n_h	h	% π
Amirante	15	15	1.00	3.2 \pm 1.7
Seychelles	16	14	0.975	2.3 \pm 1.3

Abrolhos Island	34	27	0.977	2.6±1.4
Ningaloo	10	9	0.978	3.2±1.8
Lizard Island	27	23	0.989	3.0±1.6
Whitsundays	15	13	0.98	2.5±1.4
Papua New Guinea	18	18	1.00	2.6±1.4
Rota	25	22	0.99	3.6±1.8
Hawaii	25	17	0.936	2.8±1.5
Total	185	146	0.993	4.5±1.2

Laboratory procedures

DNA was extracted following modified phenol-chloroform extraction and ethanol precipitation procedures (Sambrook et al. [1989](#)). A 400 base pair region of the mitochondrial control region I was amplified using the universal primers L15995 (5'-AATTCTCACCCCTAGCTCCCAAAG-3') and H16498 (5'-CCTGAAGTAGGAACCAGATG-3'; Lee et al. [1995](#)). Amplification using polymerase chain reaction (PCR) was carried out in 20- μ l volumes containing 2.5 mM Tris pH 8.7, 5 mM KCl(NH₄)₂SO₄, 3.0 mM MgCl₂, 200 μ M each dNTP, 10 μ M each primer, 10 ng template DNA, and 0.15 unit of Taq polymerase (Qiagen). Touchdown PCR was performed with a cycling profile of 30 s at 94°C, 30 s at 51°C, and 2 min at 72°C for five cycles, then subsequent 5 cycles were performed as before but with an annealing temperature of 49°C, and finally 25 cycles were performed as before with a 47°C annealing temperature. This was flanked by an initial 2-min denaturing step (94°C) and a 10-min terminal extension phase (72°C). PCR products were cleaned by isopropanol precipitation and sequenced directly in the forward direction with primer L15995. Labelled extension products were cleaned by isopropanol precipitation and analysed with an automated ABI Prism 300 sequencer at Griffith University

Molecular Biology Facility. Approximately 25% of the samples could be fully resolved using only the forward sequence; the reverse sequences did not identify any changes in a number of these samples and were therefore not obtained for the remainder. Seventy-five percent of the samples had ambiguous or unresolved sites and were sequenced in the forward and reverse directions (using primer H16498 for reverse sequences). Sequences were aligned by eye using ESEE (Cabot [1997](#)) and GeneDoc version 2.5.006 (Nicholas et al. [1997](#)). Representative sequences have been deposited in the GenBank nucleotide sequence database under the accession numbers AY392560–AY392744.

Data analysis

Data were explored independently using phylogenetic, population genetic, and cladistic (NCA) approaches.

Phylogenetic analyses

A likelihood approach, implemented in Modeltest version 3.06 (Posada and Crandall [1998](#)) was used to determine which model of evolution best fitted the data. Hierarchical likelihood ratio tests ($-\ln=2,916.47$) and Akaike information criterion (AIC=5,846.95) agreed that the Tamura–Nei model (Tamura and Nei [1993](#)) with invariable sites ($I=0.3523$) and alpha-shape gamma corrections ($\gamma=0.5247$) best fitted the data. The transition (ts)–transversion (tv) ratio was identified using Paup* version 4.0 (Swofford [1998](#)). The role of saturation was examined by comparing the topology of neighbour-joining phylograms with and without transitions included. The topologies of the trees were mostly identical, with all individuals retaining membership of the same supported clades, so transitions were retained in analyses. Neighbour-joining (NJ) and unweighted maximum parsimony (MP) analyses were conducted with 5,000 and 500 bootstraps, respectively, using MEGA version 2.0 (Kumar et al. [2001](#)). For the NJ analysis, the Tamura–Nei substitution model was applied with the alpha-shape gamma parameter specified ($\gamma=0.525$). There were six gaps in the alignment, which were treated as a fifth character.

Maximum likelihood (ML) analyses with 100 bootstrap replicates were performed using Paup* version 4.0 (Swofford [1998](#)). The one-tailed *t*-test option of the Shimodaira–Hasegawa test was selected to compare trees retained by the bootstrapped ML analysis. Trees from the three different methods were found to have similar topologies (with all individuals retaining membership of the same supported clades) and only the ML consensus tree and bootstrap values from the ML analysis will be presented here. Phylogenetic trees were unrooted although using the closely related species *Chlorurus bowseri* and *C. bleekeri* to root the trees produced trees with the same topologies (not shown).

Population genetic analyses

A haplotype tree was constructed based on the minimum spanning tree (Rohlf [1973](#)) implemented in Arlequin version 2.001 (Schneider

et al. [2000](#)). The minimum spanning tree was drawn in preference to the network for clarity of presentation. Comparisons of mean number of base differences and shared haplotype frequencies were made using one-way analysis of variance and chi-square tests and post hoc comparisons were made following Zar ([1984](#), Eqs. 12.4 and 22.71 correcting for unequal sample sizes). Population genetic statistics were calculated in Arlequin version 2.001 (Schneider et al. [2000](#)). Analysis of molecular variance (AMOVA) was used to examine the spatial structure of *C. sordidus* and 10,000 Markov chain permutations were used to test for statistical significance (Weir and Cockerham [1984](#); Excoffier et al. [1992](#)). AMOVA was conducted using two spatial groupings. Structure among major biogeographical regions was tested by comparing populations within three areas: West Indian Ocean (WIO), Indo-Australian Archipelago (IAA), and Hawaii (Fig. [1](#)). Patterns of genetic structure within the Australian and Pacific regions were examined by comparing east and west Australian, Papua New Guinean, and Rotan populations with Hawaii. The role of dispersal was examined using IBD analyses (Slatkin [1993](#); Rousset [1997](#)). The relationship between geographical separation (minimum marine distance between locations estimated from latitude and longitude coordinates) and pairwise F_{st} estimates was examined using a Mantel test with a 1,000-permutation Monte Carlo randomisation test, implemented in PC-ORD version 4.0 (McCune and Mefford [1999](#)). The role of isolation by distance was examined at four spatial scales: (1) across the species range; (2) across Australian and Pacific locations; (3) within the Pacific [Great Barrier Reef (GBR)–Papua New Guinea (PNG)–Rota–Hawaii]; (4) around Australia.

Nested clade analysis

To elucidate further the roles of gene flow and population history on the genetic structure of *C. sordidus* we implemented the NCA (Templeton et al. [1995](#); Templeton [1998](#)). A haplotype network was constructed using TCS version 1.13 (Clement et al. [2000](#)). The network was manually nested following the rules in Templeton et al. ([1987](#), [1992](#)) and Templeton and Sing ([1993](#)). A total of 93 clades containing both geographic and genetic information were entered into GeoDis version 2.0 (Posada et al. [2000](#)). The results were interpreted using the inference key from the GeoDis homepage (http://InBio.byu.edu/Faculty/kac/crandall_lab/geodis.htm).

Results

A total of 354 base pairs of the mitochondrial D-loop were resolved for 185 individuals from nine populations within six geographical locations from three oceanographic realms (Fig. [1](#)). Eighty of 121 polymorphic sites were parsimony informative. Base frequencies were unequal and AT-biased as is commonly found in fish mtDNA (A: 33.45, C: 26.00, G: 14.05, T: 26.50; Wolstenholme [1992](#); McMillan and Palumbi [1997](#)). The ts–tv ratio in *C. sordidus* was 3.4:1. Haplotype and nucleotide diversities were consistently high in all populations (Table [1](#)). Haplotype diversities were close to 1 in all sampled locations (0.97–1.00) although the value for the Hawaiian

population was slightly lower (0.94; Table [1](#)). Likewise nucleotide diversities were high, ranging from 2.3% (Seychelles) to 3.6% (Rota) with 4.5% for the entire data set (Table [1](#)).

Phylogenetic analyses

C. sordidus was monophyletic with respect to the outgroup and two major clades could be distinguished, with strong bootstrap support from the maximum likelihood consensus tree of 144 trees retained after performing the SH test, $P > 0.05$ ($-\ln$ Likelihood score of the best tree was 2,669.101 and of the worst tree retained was 2,998.7882; Fig. [2](#)). Clade A had high bootstrap support (99) in the unrooted phylogram (Fig. [2](#)). It contained the most divergent individuals in the tree and was exclusively composed of fishes from the West Indian Ocean. Clade B contained all individuals from the Pacific Ocean and west Australian locations. Both clades A and B had some substructure. Clade A had two and clade B had three subclades. The largest one, the “Hawaiian-dominated” subclade of clade B, (bs=50) contained primarily north Pacific fishes with 60% of all Hawaiian and 28% of all Rotan individuals. This subclade also contained some fishes from all Australian locations sampled albeit at frequencies of less than 5% but interestingly none of the 18 PNG fishes sampled. The remaining subclades of clades A and B were small, containing three or fewer individuals and did not appear to exhibit any clear geographical structure (Fig. [2](#)).

Clade A

Clade B

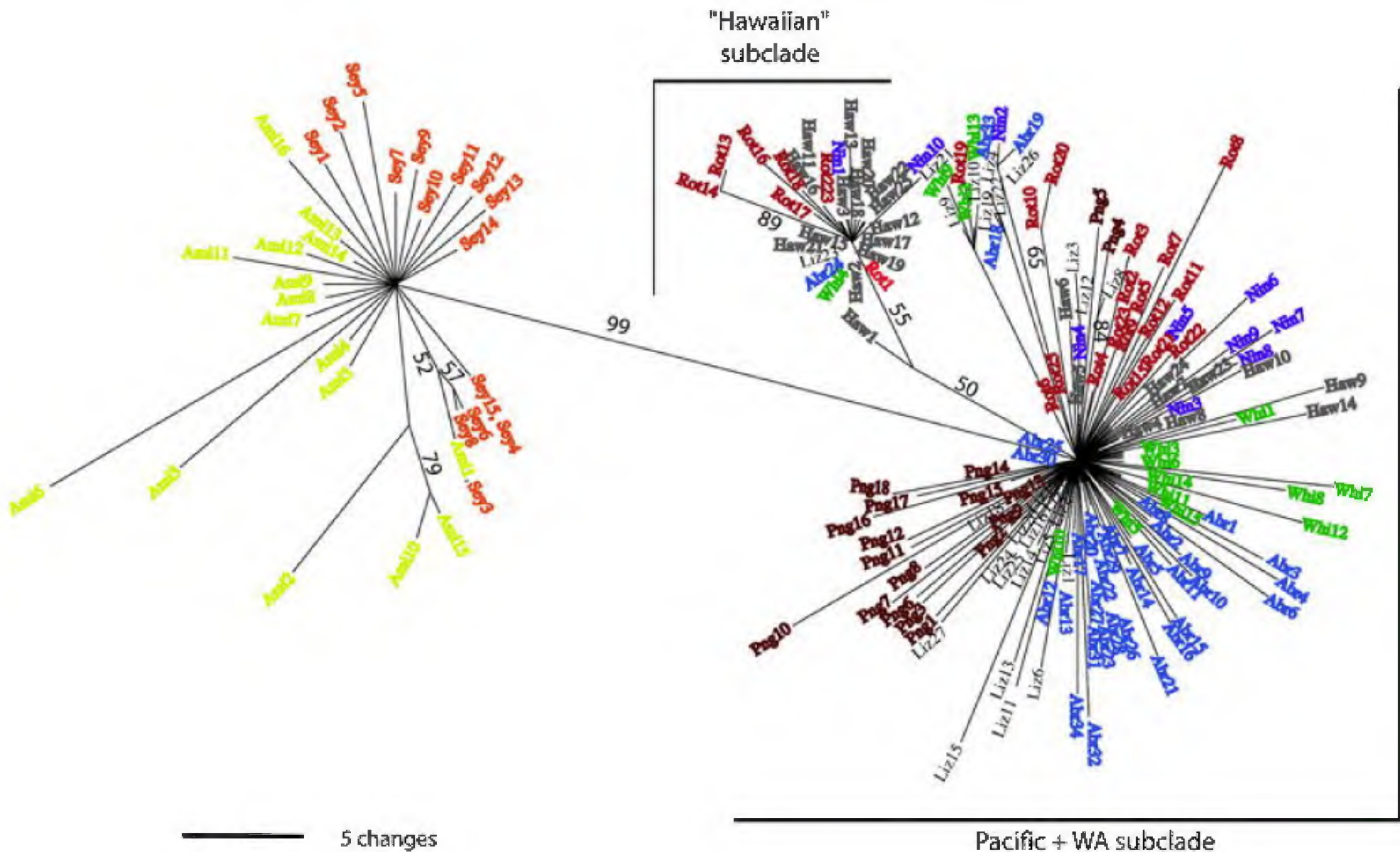


Fig. 2 Consensus maximum likelihood phylogram of *C. sordidus* sequences from the nine populations. Location codes: *Ami* Amirante, *Sey* Seychelles, *Abr* Abrolhos Islands, *Nin* Ningaloo Reef, *PNG* Papua New Guinea, *LIZ* Lizard Island, *Whi* Whitsundays, *Rot* Rota, *Haw* Hawaii. Colour codes follow those employed in Fig. 1

Population genetic analyses

The relationships among *C. sordidus* haplotypes were complex (Fig. 3). Although 1- and 2-base difference connections among haplotypes were frequent, a large number of haplotypes (35%) were separated by 3 or more base changes. One haplotype was shared by ten individuals from four locations whilst others were separated by up to 8 base changes. The West Indian Ocean haplotypes were separated from all others by 17 base differences and the Hawaiian subclade was separated from other Pacific samples by 7 base changes. The average number of base pair differences between haplotypes differed significantly among each of the three clades ($F=3.045$, $df=2$, $P<0.001$, Tukey's post hoc: WIO vs Hawaii $q=77_{(0.05,182.3)}$, $P<0.05$; WIO vs Pacific $q=52.93_{(0.05,182.3)}$, $P<0.05$; Pacific vs Hawaii $q=35.26_{(0.05,182.3)}$, $P<0.05$). The mean number of base changes within the WIO populations (3.5 ± 0.3) was almost twice and three times the number of base changes in their Pacific (1.9 ± 0.16) and west Pacific (1.1 ± 0.4) counterparts. The frequencies of shared versus unique haplotypes differed significantly among all three regions ($\chi^2=10.567_{0.05,2}$); the "Hawaiian-dominated" subclade had a much higher frequency of shared haplotypes (0.52) than WIO (0.13) and the main Pacific clade (0.34). Post hoc pairwise comparisons found that this difference was evident among all three clades (Tukey's post hoc: Hawaii vs Pacific $q=6.23_{(0.05,Inf,3)}$, $P<0.05$; Hawaii vs WIO $q=9.10_{(0.05,Inf,3)}$, $P<0.05$; Pacific vs WIO $q=9.53_{(0.05,Inf,3)}$, $P<0.05$).

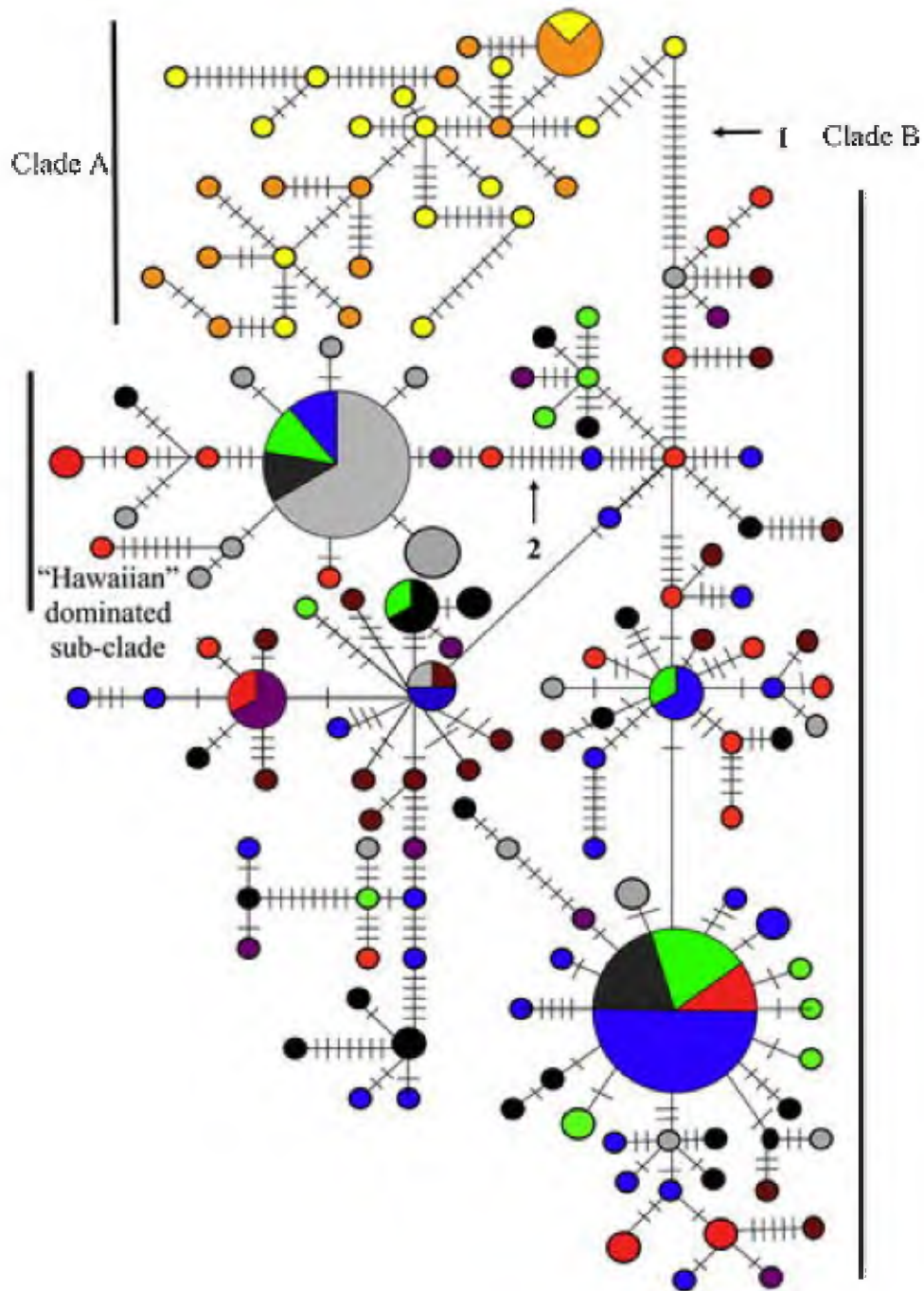


Fig. 3 The relationship among haplotypes of *C. sordidus* expressed in a haplotype network. The size of the *circle* indicates the number of individuals sharing the haplotype (largest circle $n=10$). Locations are *colour coded* following Fig. 1. *Crossbars* specify the number of mutational steps separating

the haplotypes. The approximate locations of clades from Fig. 2 are indicated by the *vertical bars* and the separation of WIO from the rest indicated by *arrow 1*; The separation of the “Hawaiian-dominated” subclade is indicated by *arrow 2*

At the largest spatial scale AMOVA detected significant genetic subdivision due to location [WIO vs the Pacific and west Australia (WA)], which explained the majority of variation (59%), although a considerable amount of variation was attributable within populations (39%) (Table 2). The lack of geographical structuring across the Pacific Ocean (including west Australia) indicated by the phylogenetic analysis was supported by AMOVA when Hawaii and other non-WIO locations were compared (Table 2). Only 17% of the variation ($P=0.14$) could be attributed to location whilst a large amount of variation was found within populations (80%, $P=0.00$).

Table 2 Results of hierarchical analysis of molecular variance (AMOVA) of populations of *C. sordidus*. Samples were analysed among oceans [West Indian Ocean (WIO) vs Indo-Australian Archipelago (IAA) vs Hawaii] and between Hawaii and other non-WIO locations (IAA and Rota vs Hawaii)

Source of variation	df	SS	Variance components	Percent of Variation	Φ	<i>P</i>
WIO vs IAA vs Hawaii						
Among locations	2	692.21	7.79	59.16	0.591	0.017±0.001
Among populations within locations	6	53.59	0.19	1.42	0.035	0.000±0.000
Within populations	176	913.97	5.19	39.42	0.606	0.000±0.000
Total	184	1,659.78	13.17			
IAA and Rota vs Hawaii						
Among locations	1	57.15	1.14	17.42	0.174	0.144±0.003

Among populations within location	5	43.67	0.17	2.56	0.031	0.000±0.000
Within populations	147	769.71	5.24	80.02	0.200	0.000±0.000
Total	153	870.53	6.54			

There was some evidence of isolation by distance across the largest geographical scale examined (17,000 km; Mantel $r=0.726$; $P=0.007$, Fig. 4A). Some, although not statistically significant, effects of IBD were suggested from WA across the Pacific to Hawaii (Hawaii–Rota–PNG–GBR–WA Mantel $r=0.554$, $P=0.076$; Fig. 4B) and across the Pacific Ocean alone (Hawaii–Rota–PNG–GBR Mantel $r=0.802$, $P=0.082$ Fig. 4C). There was no evidence of IBD between east and west Australia (GBR–WA Mantel $r=-0.492$, $P=0.157$; Fig. 4D). Thus the IBD relationship across the entire range (Fig. 4A) evidently was due to low or absent levels of migration between the WIO and other populations.

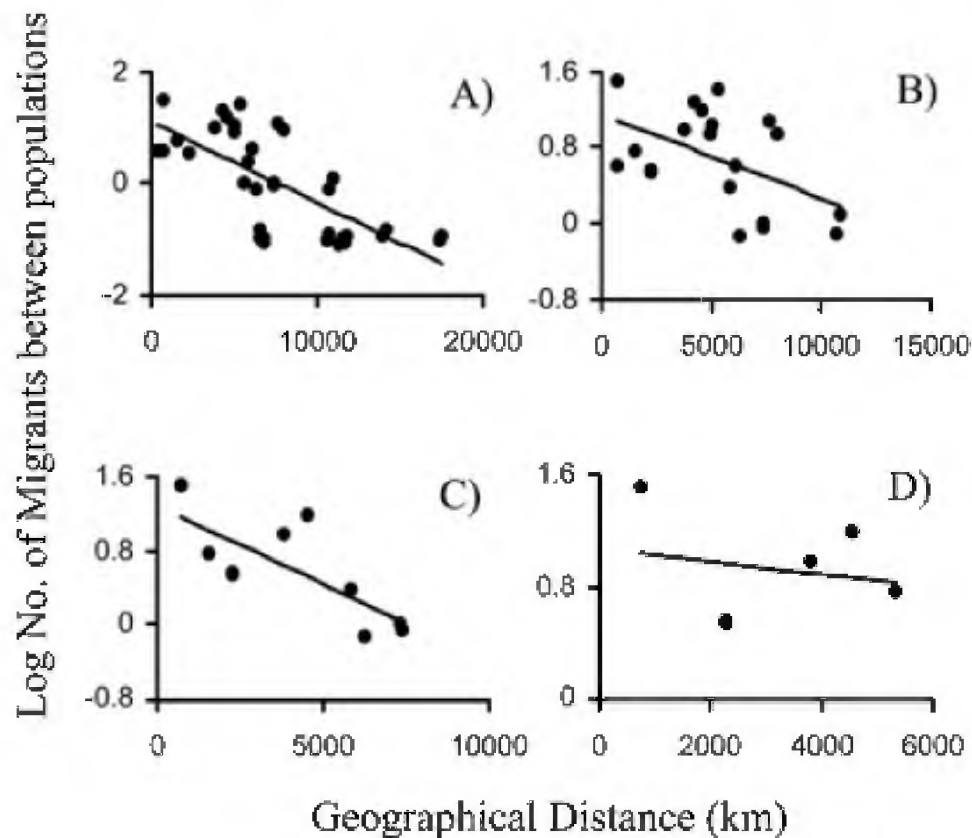


Fig. 4A–D Isolation by distance of populations of *C. sordidus* expressed as the number of migrants versus minimum marine geographical distance (km) at four spatial scales. **A** Across the entire species range (WIO to Hawaii); **B** across Australian and Pacific locations; **C** east Australia-PNG-Rota; and **D** around Australia

Nested clade analysis

Significant relationships between geography and genetic structure were found in only 6 clades out of 93 (Table 3) and appeared to be driven primarily by the inclusion or exclusion of Hawaiian samples (Table 4). Significant structure was found at the lowest level of the design (Clade 1.87, Table 4) and was attributed to long-distance colonisation. This appeared to be driven by one subclade (one haplotype) with geographical structure containing only individuals from Hawaii. Restricted gene flow with some effect of isolation by distance was significant at two levels (Table 4). Clades 3.3 and 5.3 both contained a large proportion of Hawaiian individuals and appear to drive the

pattern in both clades. The separation of the Hawaiian population from the rest of the Pacific populations corroborated results of the phylogenetic and IBD analyses. Contiguous range expansion appeared to be an important structuring process across the full range of the Pacific and west Australia (clade 6.1). It was not possible to determine which processes are important in structuring the genetic composition of *C. sordidus* at the highest nesting level (Table 4). The two subclades (Pacific and Indian Ocean) were so different that a connection between them could not be assigned within the 95% parsimony criterion and hence the tip-interior status could not be determined.

Table 3 Nested contingency analysis of geographical associations of different clades of *C. sordidus*

Clade	Chi square	Significance
1.87	21.54	0.041
3.3	19.00	0.036
4.3	7.22	0.040
5.3	17.44	0.002
6.1	47.07	0.000
Total	33.23	0.000

Table 4 Significant clades from nested contingency analysis, their geographical affinity and approximate location on the maximum likelihood tree (Fig. 2), the number of haplotypes included in the clade, the significant distances, inference key path and outcome (Templeton 1998). WA west Australia; GBR Great Barrier Reef; IBD isolation by distance

Clade	Geographical affinity of haplotypes	Approximate location on Fig. 2	Number of individuals in clade	Significant distances (<i>P</i>)	Inference key and outcome
1.87	WA, GBR, and north Pacific locations	B	13	D _c Int S (0.048)	1Y-2N-11Y-12Y-13Y
				D _n Int S(0.026)	
				D _c Tip S (0.044)	Long-distance colonisation
				D _n Tip L(0.004)	
				D _n I-T S (0.008)	
3.3	Predominantly Hawaii but few individuals from GBR, WA, and Rota	“Hawaiian-dominated” subclade	19	D _c Int S (0.025)	1Y-2N-11N-17Y-4N
				D _n Int L (0.025)	
				D _n Int L (0.025)	Restricted gene flow with some IBD
5.3	Many Hawaiian individuals	“Hawaiian-dominated” subclade vs B	30	D _c Tip S (0.012)	1Y-2Y-3N-4N
				D _n Int L (0.000)	
				D _c I-T L (0.012)	Restricted gene flow with some IBD

6.1	No Hawaiian individuals in first subclade, some in second and many in the third	“Hawaiian-dominated” subclade vs B	154	D _c Int S (0.027)	1Y-2N-11Y-12N
				D _c Tip S (0.009)	
				D _n Tip S (0.007)	Contiguous range expansion
				D _n Tip L (0.000)	
				D _n I-T S (0.032)	
Total	Pacific samples vs Indian Ocean samples	A vs B	185	D _c S (0.000)	Inconclusive outcome (Int/tip status could not be determined)
				D _n S (0.000)	
				D _c S (0.000)	
				D _n L (0.000)	

Discussion

Major findings

Our analyses produced three major results concerning the phylogeographic history of *C. sordidus*. First, we found strong evidence for population subdivision only at the largest spatial scale. Populations were different among oceans with no haplotypes shared between the West Indian Ocean and the rest. Second, we did not find evidence of a central Pacific barrier. The Pacific locations were characterised by a high degree of similarity and only some geographical structure of the Hawaiian population could be detected. Third, we found

extraordinarily high levels of both nucleotide and haplotype diversity in populations of *C. sordidus*. These levels were similar to values recorded for widely distributed pelagic species such as sardines (e.g. Bowen and Grant [1997](#)) rather than other reef fishes (e.g. Bowen et al. [2001](#)). These findings will be discussed in turn below.

Genetic structure among oceans

Populations of *C. sordidus* were genetically subdivided at the scale of oceans with a strong break between WIO and Pacific plus WA populations, a result evident from both phylogenetic and population genetic analyses. This divergence between populations in different oceans was close to 3 times deeper (7.1%) than that recorded between Hawaii and other Pacific locations (2.5%) based on pairwise comparisons, however less than that between in- and outgroups. We found no sharing of haplotypes between WIO and other locations, suggesting that effective gene flow between these locations is absent.

The separation of the WIO from other Indo-Pacific locations is perhaps the most concordant result of any large-scale inter-ocean studies of marine organisms (Benzie [1998](#)). It is reflected in both the distribution of species and the genetic patterns within species (Benzie [1998](#); Briggs [1999](#)). This pattern of separation has been found in taxonomically diverse organisms such as fishes (McMillan and Palumbi [1995](#); Lacson and Clark [1995](#)), crustaceans (Lavery et al. [1995](#), [1996](#)), and echinoderms (Williams and Benzie [1998](#); *Diadema setosum* but not *D. paucispinum-b* and *D. savignyi* Lessios et al. [2001](#)) and has been associated with morphological differentiation in some species (e.g. Benzie [1992](#); Williams and Benzie [1998](#)), including *C. sordidus*. Such congruence in the evolutionary history of organisms from different taxa, with variable demographic attributes and probably dispersal abilities, strongly indicates that an extremely effective environmental mechanism that is not taxon specific blocks gene flow between the WIO and other parts of the Indo-Pacific (Neigel and Avise [1993](#); Gordon and Fine [1996](#); Gordon [1998](#); Avise [2000](#); Arbogast and Kenagy [2001](#)). It is, however, unclear exactly where this barrier is located and which evolutionary processes may operate within the Indian Ocean. Most studies (e.g. McMillan and Palumbi [1995](#); Lavery et al. [1996](#); Williams and Benzie [1998](#); Duda and Palumbi [1999](#); present study) have included only one or two sites in the Indian Ocean. It will prove interesting to examine the genetic structure of widespread species among several locations within the Indian Ocean. This is currently being undertaken for *C. sordidus* and will be the focus of a future investigation.

The WIO populations were as isolated from the nearest populations in western Australia (approx 6,700 km) as they were from central Pacific populations (a separation of 12,000–17,000 km). This suggests that the significant effect of IBD across the species range was a result of complete isolation of the WIO populations rather than a gradual dilution of settlement events with increasing geographical distance. This pattern is similar to that found among pelagic larval dispersing marine organisms, which generally show signs of IBD only at the largest spatial scales (Richardson [1983](#); Palumbi et al. [1997](#); Planes and Fauvelot [2002](#)).

Genetic structure within oceans

The genetic relationships amongst Pacific and Australian populations of *C. sordidus* were complex and the only structure we detected was a partial separation of the Hawaiian population from the rest (2.5%). This separation was evident in the phylogenetic, mean base separation, frequencies of shared haplotypes, and cladistic analyses but not detected by the AMOVA. A number of studies on reef fishes have found genetic structure among Pacific locations (Planes [1993](#); Bernardi et al. [2001](#); Nelson et al. [2000](#); Planes and Fauvelot [2002](#)). In particular, Hawaiian populations have been found to be genetically distinct from other Pacific locations (e.g. Stepien et al. [1994](#); Bernardi et al. [2001](#)). While lack of statistically significant genetic structure may be the result of low statistical power, the patterns observed in *C. sordidus* may also be due to high current and past gene flow.

Our results suggest that *C. sordidus* has experienced a complex evolutionary past. NCA indicated that several historical processes have influenced the genetic composition of *C. sordidus* at several nesting levels. This pattern would be expected if these processes acted repeatedly (Templeton et al. [1995](#); Templeton [1998](#)) due to repeated changes in sea level. The high genetic diversity and widespread distribution of many haplotypes was most likely a result of both current and historical gene flow mixing haplotypes among different Pacific locations, with genetic differentiation being retained at only the most distant location (Hawaii) through isolation by distance. Our results suggest that current gene flow is substantial (Fig. [4](#)) and bi-directional across the Pacific. Australian and Rotan haplotypes were found in the Hawaiian clade and Hawaiian haplotypes cluster with other Pacific locations (Figs. [2](#), [3](#)). This finding corroborates findings of other studies (e.g. Bernardi et al. [2003](#)) that found low but significant levels of gene flow at a similar spatial scale in *Dascyllus trimaculatus*.

While IBD was found to be an important process restricting gene flow across the Pacific by NCA, the IBD analysis produced only near-significant results ($P=0.076$) at this spatial scale. This lack of statistical significance could be due to sampling error in the small F_{st} estimates (Waples [1998](#)), which formed the basis of this analysis. Interpreting the results of both NCA and IBD analyses in concert suggested that this may be the case and that IBD operated both in historical and present time to produce the present pattern of genetic structure we found in *C. sordidus* across the Pacific.

The western Australian populations of *C. sordidus* showed strong Pacific affinities and appeared to have been established by range expansion of Pacific populations. It appears likely that western Australian populations were founded by individuals from the Indo-Australian Archipelago and are isolated from the wider Indian Ocean. This corroborates patterns found for marine invertebrates (Benzie [1998](#); Williams and Benzie [1998](#); Lessios et al. [2001](#)) and indicates that general environmental processes such as inshore upwelling zones (Fleminger [1986](#); Wells and Wells [1994](#); Wells et al. [1994](#)) may be important in maintaining the isolation of western Australian coastal populations from populations in the rest of the Indian Ocean.

Patterns of genetic variability

Many marine taxa exhibit high genetic diversities and populations of fishes often display high haplotype but generally medium to low nucleotide diversities [reviewed in Grant and Bowen [1998](#); see also Dudgeon et al. [2000](#) (D-loop sequences); Rocha-Olivares et al. [2000](#) (D-loop sequences); Muss et al. [2001](#) (Cyt b sequences); Rocha et al. [2002](#) (Cyt b sequences); but see Bowen et al. [2001](#) (Cyt b sequences)]. Grant and Bowen ([1998](#)) suggest that high levels of both nucleotide and haplotype diversities are indicative of either a long stable evolutionary history or secondary contact among differentiated lineages. We found extraordinarily high levels of both nucleotide and haplotype diversity in populations of *C. sordidus* scattered throughout most of the species range. Overall haplotype diversities of *C. sordidus* were high (0.993) and amongst the greatest recorded for benthic and reef fishes [e.g. 0.41–1.00: Shulman and Bermingham [1995](#), (RFLP whole mtDNA); 0.96: Dudgeon et al. [2000](#); 0.99–1.00: Rocha-Olivares et al. [2000](#)]. *C. sordidus* displayed very high nucleotide diversities (total=4.5%), which were more similar to those recorded for widely distributed pelagic species such as sardines [5.1%: Bowen and Grant [1997](#), (D-loop sequences)] and Atlantic menhaden [3.2%: Bowen and Avise [1990](#), (RFLP whole mtDNA)] rather than other reef fishes. While the high genetic diversities of some of these species may be the result of a long stable evolutionary history (Grant and Bowen [1998](#)), the geological history of Indo-Pacific coral reefs suggests that this is unlikely to be the case for *C. sordidus*. It is more plausible that high genetic diversities in this species were the result of secondary contact among differentiated lineages. Genetic differentiation may have taken place during historical fragmentation events, and subsequent mixing by gene flow among persisting lineages produced the very high nucleotide and haplotype diversities. The significantly different genetic composition (mean number of base differences and frequency of shared haplotypes) among the three clades suggests that these locations have undergone different processes in the past. This further corroborates the hypothesis that the populations differentiated in allopatry.

Conclusion

C. sordidus possess a number of demographic characteristics (high abundances and spawning rates, rapid population turnover), which, in concert, are instrumental in producing the pattern of genetic structure recorded by this study. *C. sordidus* has great potential dispersal ability, which may have caused genetic subdivision at small and medium spatial scales, evident in other reef fish species (e.g. Doherty et al. [1995](#); Planes et al. [1997](#); Nelson et al. [2000](#)), to be lost. Large local population sizes and reproductive outputs affect rates of gene flow, genetic drift, and fixation, acting to reduce genetic differentiation across the species range. These demographic features may be characteristic of a number of reef fish species and imply that the phylogeographic pattern of *C. sordidus* may be widespread in broadly distributed species.

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