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RESEARCH ARTICLE

High similarity of genetic population structure in the false clown anemonefish (*Amphiprion ocellaris*) found in microsatellite and mitochondrial control region analysis

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Abstract Many studies, using various marker systems, have been conducted on the genetic population structure of marine organisms to reveal connectivity among locations and dispersal capabilities. Although mitochondrial sequence markers are widely used, their accuracy is controversially discussed in the context of small scale population genetic discrimination. In the present study, the genetic population structure of the False Clown Anemonefish (*Amphiprion ocellaris*) in the Indo-Malay Archipelago was revealed by screening six microsatellite loci. Results were congruent to previous mitochondrial control region results, with three major genetic breaks within the Indo-Malay Archipelago. Similar to the mitochondrial DNA (mtDNA) analysis, microsatellite data showed a correlation of genetic structure to historical ocean basin separation during Pleistocene sea level low stands, geographic distance, and dominant current patterns. However, microsatellite divergences are not as deep as the mtDNA divergence, suggesting either that admixture of mtDNA lineages is slower than that of nuclear microsatellites, providing a rather historic picture of separation, or the stronger differentiation signal is due to lower effective population sizes presented by mtDNA. As well, the microsatellite analysis did not give a better resolution on the small scale as expected. This study showed that depending on the genetic markers used, different stages of population separation might be illuminated.

Keywords Anemone fish · Coral triangle · Southeast Asia · Indo-Malay archipelago · Clownfish · Genetic markers

Introduction

In modern conservation and sustainable management approaches of species, it is important to have knowledge about biodiversity, population structures and dynamics. Especially in marine species, direct measurements about connectivity and differentiation are difficult to obtain, which is why indirect measures with the help of phylogeographic and population genetic approaches can be very helpful and necessary. The genetic tools available to date, vary from mitochondrial or plastid DNA, to nucleus DNA, sequence based to length or single nucleotide polymorphism markers, and differ in the ways of possible analyses and information gain, all afflicted with different advantages and disadvantages.

Mitochondrial sequence markers are widely used for phylogenetics (e.g., Kochzius et al. 2003; Santini and Polacco 2006; Timm et al. 2008), but also for phylogeographic and population genetic studies of fish (e.g., Nelson et al. 2000; Lourie et al. 2005; Perrin and Borsa 2001; Froukh and Kochzius 2007, 2008; Timm and Kochzius 2008), as certain regions show high variability and the procedure for amplification and sequencing is comparably easy.

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However, the use of mitochondrial DNA (mtDNA) for phylogeographic and population genetic studies is questioned, because within-species diversity does not reflect population size, history, and ecology in a meta-analysis of a data set including about 3,000 animal species. This finding challenges the neutral theory of molecular evolution of mtDNA (Bazin et al. 2006) and was controversially discussed (Berry 2006; Wares et al. 2006; Eyre-Walker 2006; Mulligan et al. 2006; Edwards and Bensch 2009; Barrowclough and Zink 2009). As well, the mitochondrial genome in vertebrates is usually inherited maternally and as a single locus, making it difficult to investigate evolutionary questions for which multiple and/or nuclear loci are necessary (Lee and Edwards 2008; Edwards and Bensch 2009). On the other hand, mtDNA might exhibit a stronger differentiation signal, due to its lower effective population size and therefore rapid coalescence time, enabling the analysis of recently evolved taxonomic groups and fine scale population dynamics (Hoarau et al. 2004; Zink and Barrowclough 2008).

Microsatellites are simple repetitive sequences found throughout the eukaryote nuclear genome (Tautz 1989). They are among the most variable markers, because their mutation rate of 10^{-2}–10^{-6}/locus/generation (Weber and Wong 1993; Vázquez et al. 2000) is higher than that of any other marker system. They are co-dominant and usually show high resolution on the population level (Bentzen et al. 1996; Reilly et al. 1999; Shaw et al. 1999), which can give valuable information for evaluation and conservation issues. Therefore they are frequently used to investigate genetic diversity, patterns of population differentiation (Kochzius 2009 and references therein), as well as for parentage analyses (e.g., Jones et al. 2005; Planes et al. 2009; Saenz-Agudelo et al. 2009).

By comparing the results of mtDNA and ncDNA markers in the same populations, the applicability of mtDNA in phylogeography and population genetics can be validated and additional data can be gained. The main results obtained by different markers were often very similar (Reilly et al. 1999; Williams 2000; Borsa 2003; Rohfritsch and Borsa 2005). Different results were often caused by a higher resolution of the microsatellites (Bentzen et al. 1996; Reilly et al. 1999; Shaw et al. 1999), but as well the contrary could be observed, showing stronger genetic population structure in mtDNA (Zink and Barrowclough 2008 and references therein). Possibly, mtDNA markers may provide rather a historic than a recent picture of gene flow (Fauvelot et al. 2003).

The present study was conducted in the Indo-Malay Archipelago that consists of thousands of different sized islands and peninsulas with a diverse geological origin (Hall 1996), forming complex geographic structures and current patterns (Wyrtki 1961). This region hosts the highest marine shallow water biodiversity worldwide (Briggs 2000; Allen and Werner 2002; Hoeksema 2007). Many population genetic and phylogeographic studies were conducted in the Indo-Malay Archipelago (e.g., Nelson et al. 2000; Lourie et al. 2005; Kochzius and Nuryanto 2008; Nuryanto and Kochzius 2009; Kochzius et al. 2009), including an extensive study on the anemonefish *Amphiprion ocellaris* based on a fragment of the mitochondrial control region (Timm and Kochzius 2008).

Due to its biology *A. ocellaris* has a low dispersal capability, because adults live close to their host, they have demersal eggs, and a short pelagic larval duration (PLD) of only 8–12 days (Fautin and Allen 1994). Furthermore, *A. ocellaris* is a host specialist, living in symbiosis with only three anemone species (*Heteractis magnifica, H. crispa,* and *Stichodactyla mertensii*; Fautin and Allen 1994).

This study showed four major groups of populations, probably derived from lineages that were separated in different ocean basins during sea level low stands in the Pleistocene. Sub-structures on smaller scales due to isolation by distance and currents were also revealed. Within the Spermonde Archipelago (smallest scale of the study), for example, pooling of shelf regions (midshelf, outer shelf, outer rim, and northern outer shelf) resulted in differentiation among these regions and different connectivity to sites out of the Archipelago, although the signals were very low (Timm and Kochzius 2008). It is expected, that the use of highly polymorphic microsatellite markers might give more detailed differentiation patterns on that level. Thus, the separate analysis on different scales, using sub-datasets of the samples, can give more detailed information on connectivity.

In the present study, the genetic population structure of *A. ocellaris* in the Indo-Malay Archipelago is investigated, utilising six microsatellite loci. This study aims (1) to validate previous results based on mtDNA control region sequences (Timm and Kochzius 2008), (2) to reveal ecologically relevant connectivity of reef systems in the Indo-Malay Archipelago, and (3) to find more pronounced substructures on the fine scale analysis.

Materials and methods

Sample locations and collection

A total of 432 tissue samples of the False Clown Anemonefish (*A. ocellaris*) were collected at three different scales for further analysis of genetic diversity and population structure.

On the first scale, 90 individuals were collected at islands of the midshelf (Barranglompo, Barrangcaddi, and

Samalona), outershelf (Lumulumu), northern outershelf (Reangreang and Jangangangang), and outer-rim area (Suranti, Kondongbali, and Kapoposang) of the Spermonde Archipelago (Fig. 1; Table 1). It is located in southeast Sulawesi, comprises around 160 small islands, and covers an area of around 16,000 km^2 (Whitten et al. 2002). The midshelf islands are within the 30 m isobath (20–30 m depth, Moll 1983), 10–20 km away from the shore. The outershelf and northern outershelf islands are 30–40 km away from the coastline and within the 50 m isobath (30–50 m depth, Moll 1983). The outer-rim area is 60–80 km away from the shore and located between shallow waters (≤30 m depth) and the shelf edge (100 m isobath). The shelf edge is exposed to the Indonesian throughflow (ITF) and is exposed to higher hydrodynamic energy than the other areas (Renema and Troelstra 2001). The distance among sample sites at this scale was 10 to 100 km.

On the second scale, the samples from the Spermonde Archipelago were supplemented with 175 samples from six additional sites around Sulawesi (Fig. 1; Table 1). It is located in the centre of the Indo-Malay Archipelago and has a remarkably long coastline (>5,200 km^2; Whitten et al. 2002). Its long and narrow peninsulas originated from different land masses that collided (Hall 1996), and formed the peculiar shape with two large bays (Bone Bay and Tomini Bay). The sample sites on this spatial scale were 40 km (Puntondo to Spermonde) to over 1,100 km (Pulau Sembilan to Manado) apart.

On the third scale, 432 samples from different regions of the Indo-Malay Archipelago were included (Fig. 1; Table 1). The maximum geographic distance between sites was more than 4,500 km from Padang (Sumatra, Indian Ocean) to Triton Bay (New Guinea, Banda Sea). Collections were realized spring and autumn 2004 and 2005 at the different sites, while no site was repeatedly sampled.

Fin clips were collected from adult and juvenile individuals under water with aquarium nets and the fish were subsequently released to their host anemone. Since there are usually only 2–5 individuals in one host anemone and the fish differ in size and colouration, the likelihood of a re-capturing was very low. Additionally, the sampled fish were easy to identify through the finclip mark. The tissue samples were preserved in 96% ethanol and finally stored at 4°C.

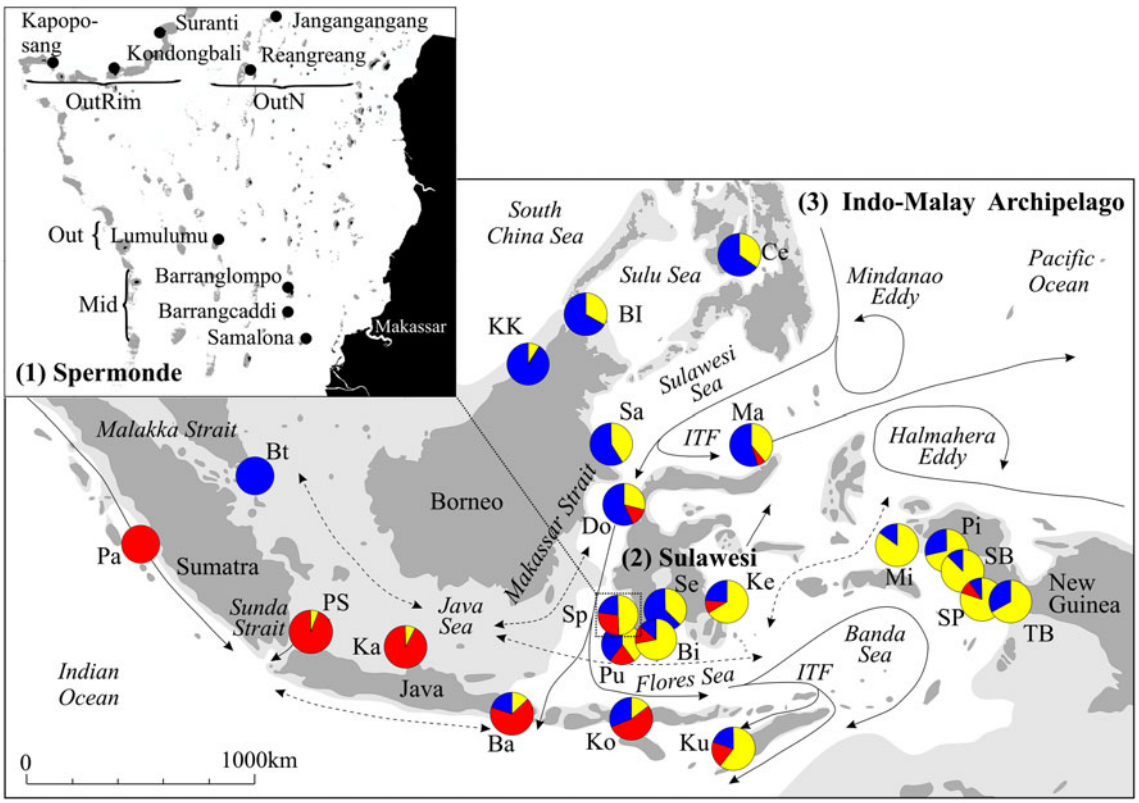

Fig. 1 Genetic structure of the False Clown Anemonefish *A. ocellaris*. Maps of the study area with names of the major islands, seas, straits, and sample sites (for abbreviations refer to Table 1). The frequencies of the different clusters from the analysis with the programme STRUCTURE (ver. 2.2., Pritchard et al. 2000) are indicated by different colours in the pie charts. Major surface currents are given by *arrows* (*solid* uni-directional, *dashed* reversing with the monsoon seasons). The *light grey* shading indicates the land area during the Pleistocene maximum sea level low stand of 120 m (after Voris 2000)

Table 1 Sample sites for *A. ocellaris* in the Indo-Malay Archipelago, and the corresponding abbreviations (Abbr.): number of individuals (N_{ind}), number of alleles ($N_{alleles}$), mean gene diversities (Gene Div.), and allelic richness (Alleleic rich.) over all loci with standard deviations (SD)

Name of sample site(s)	Geographic region (and shelf regions)	Abbr.	N_{ind}	$N_{alleles}$	Gene Div. ± SD	Allelic rich. + SD
Samalona, Barranglompo, Barrangcaddi	Spermonde, midshelf	Mid	16	48	0.79 ± 0.45	4.95 ± 1.71
Lumulumu	Spermonde, outershelf	Out	6	27	0.64 ± 0.40	4.26 ± 1.67
Reangreang, Jangangangang	Spermonde, outershelf (north)	OutN	32	61	0.80 ± 0.44	5.40 ± 1.73
Kapoposang, Suranti	Spermonde, outer-rim	OutRim	36	66	0.79 ± 0.43	5.28 ± 1.65
Spermonde total	South Sulawesi	Sp	**90**	**78**	**0.78 ± 0.42**	**5.05 ± 2.10**
Puntondo	South Sulawesi	Pu	10	46	0.75 ± 0.43	5.78 ± 2.22
Donggala	Central Sulawesi	Do	21	65	0.69 ± 0.39	4.88 ± 3.11
Manado	North Sulawesi	Ma	18	51	0.80 ± 0.47	4.35 ± 2.48
Bira	South Sulawesi	Bi	7	37	0.80 ± 0.46	5.76 ± 2.18
Kendari	Southeast Sulawesi	Ke	21	53	0.75 ± 0.42	5.11 ± 2.31
Pulau Sembilan	Bone Bay, Sulawesi	Se	8	33	0.73 ± 0.43	5.30 ± 2.21
Sulawesi total (Spermonde incl.)			**175**	**109**		
Sangalaki	West Borneo	Sa	29	63	0.70 ± 0.39	4.88 ± 2.79
Bali	South Bali	Ba	15	50	0.80 ± 0.45	5.17 ± 1.88
Komodo	Komodo/Flores	Ko	13	45	0.79 ± 0.45	5.22 ± 1.96
Kupang	Timor	Ku	20	50	0.72 ± 0.41	4.99 ± 2.01
Karimunjava	Java Sea	Ka	24	60	0.81 ± 0.44	5.48 ± 1.48
Pulau Seribu	Java Sea	PS	18	47	0.80 ± 0.45	5.19 ± 1.68
Padang	West Sumatra	Pa	17	54	0.78 ± 0.45	4.99 ± 1.95
Batam	Riau	Bt	23	27	0.49 ± 0.29	3.29 ± 1.50
Kota Kinabalu	Northeast Borneo	KK	23	52	0.64 ± 0.37	3.76 ± 2.94
Banggi Islands	North Borneo	BI	12	46	0.77 ± 0.43	4.51 ± 2.80
Cebu	Cebu, Visayas	Ce	20	47	0.68 ± 0.41	3.47 ± 2.83
Misool	Moluccas	Mi	13	49	0.83 ± 0.47	4.95 ± 2.43
Pisang	West New Guinea	Pi	7	35	0.77 ± 0.45	4.71 ± 2.37
Sebakor Bay	West New Guinea	SB	7	39	0.75 ± 0.43	4.47 ± 2.54
Sanggala/Papisol	West New Guinea	SP	10	45	0.87 ± 0.52	4.80 ± 2.70
Kaimana/Triton Bay	West New Guinea	TB	6	35	0.80 ± 0.47	5.50 ± 2.93
Indo-Malay Archipelago (all sites incl.)			**432**	**124**		

For Spermonde, the values of the total dataset and the pooled shelf regions are shown

Total numbers of the different scales are in bold

DNA extraction, amplification, and fragment analysis

DNA was isolated using Qiagen and Macherey–Nagel extraction kits, following the manufacturers' protocols.

Eleven microsatellite loci isolated for the Sattleback Anemonefish (*A. polymnus*, Quenouille et al. 2004) and 13 loci isolated for the eastern Clown Anemonefish (*A. percula*, Buston et al. 2007) were tested in *A. ocellaris*. Finally, six polymorphic microsatellite loci were selected, three of *A. polymnus* (45, 65, and 120) and three of *A. percula* (CF9, CF27, and CF42), and amplified by PCR using the specific primers shown in Table 2. The following PCR conditions were used: the reaction volume of 10 µl contained 1 µl 10× PCR buffer, 0.0125 µmol Mg^{2+}, 0.002 µmol dNTP mix, 5 pmol of each primer (the forward primer was fluorescence labelled with HEX or FAM; see Table 2), 0.1 U Taq polymerase and 10–20 ng genomic DNA of each sample for each locus. The temperature profile of the PCR was 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, the primer specific annealing temperature (Table 2) for 45 s, and 72°C for 60 s. The terminal elongation at 72°C took 2 min. Fragment analyses of the small scale dataset were performed in a 6.5% polyacrylamid gel (PAGE), and bands were detected and analysed by a Li-Cor genetic analyser and SAGA 2 software (Li-Cor, Biosciences).

The fragment analyses for the second and third scale datasets were conducted with an ABI 3100 Automated Sequencer (Applied Biosystems, Darmstadt, Germany) using an internal 500 Rox Size Standard (Applied Biosystems). The fragment lengths were analysed and corrected

Table 2 Microsatellites for *A. ocellaris*: motif, primer sequence (CF9, CF27, CF42: Buston et al. 2007; 45, 120, 65: Quenouille et al. 2004), annealing temperature (Ann. temp.), and the range of the fragment lengths (length), as well as the number of alleles (No. alleles), and the observed (H_o) and expected (H_e) heterozygosites of the whole dataset

Locus	Motif	Primer sequence	Ann. temp. (°C)	Length (bp)	No. alleles	H_o	H_e
CF9	$(TCAA)_8TGAA$ $(TCAA)_{15}$	f:CTCTATGAAGATTTTT r:GTACATGTGTTTCCTC	60	229–301	18	0.74	0.76
CF27	$(TCTA)_{16}$	f:AAGCTCCGGTAACTCAAAACTAAT r:GTCATCTGATCCATGTTGATGTG	62	171–301	34	0.86	0.92
CF42	$(TCTG)_{18}$	f:TGCAATTATGCACCTG r:TGGCCAGATTGGTTAC	60	162–234	30	0.88	0.91
45	$(GT)_{35}$	f:TCAACTGAATGGAGTCCATCTGG r:CCGCCGCTAGCCGTGACATGCAA	54	210–250	19	0.52	0.55
120	$(GT)_{17}N_{20}(GT)_{12}$	f:TCGATGACATAACACGACGCAGT r:GACGGCCTCGATCTGCAAGCTGA	62	450–464	8	0.59	0.61
65	$(GT)_{12}$	f:AGGCCGTGAGTAACTACATTGTT r:GGAGGTGTGGCTGCCATGCCTGT	60	241–261	10	0.75	0.74

with the software Genemapper (Applied Biosystems). To ensure a consistent allele scoring, part of the samples analysed with PAGE were again analysed with the ABI Automated Sequencer and raster-shifting was adjusted.

Data analysis

Various population genetic analyses were conducted to get the most accurate picture of population structuring and genetic diversity in the study area, and profound data for a comparison with mtDNA data ($n = 421$) of the present sample set of *A. ocellaris*. First step was obtaining the input file formats for the various programmes used for analysis, done with the software CONVERT (ver. 1.31, Glaubitz 2004), used with Exel files as input.

Each dataset was tested for the presence of null alleles with Microchecker (ver. 1.0., van Oosterhout et al. 2004). This programme takes into account the heterozygosity, and the frequency and distribution of alleles. The unbiased gene diversity for each population was estimated using FSTAT (ver. 2.9.3.2., Goudet 1995), as well as the allelic richness in proportion to the smallest sample size of six individuals in this dataset. The expected and observed heterozygosities for each population, and the *p* values to test for a significant difference between them, were calculated with the programme Arlequin (ver.3.1, Excoffier et al. 2005), in order to test for the Hardy–Weinberg equilibrium (HWE). The mean heterozygosity over all loci per population and its standard deviations were as well calculated with Arlequin. Genepop (Web-Service, Raymond and Rousset 1995) was used to test for linkage disequilibrium among loci in each population. To reveal if there is a genetic structure within the dataset (Indo-Malay Archipelago) and each subset (Spermonde and Sulawesi), a Bayesian analysis implemented in the software STRUCTURE (ver. 2.2., Pritchard et al. 2000) was performed, testing for different numbers of clusters (k) in the dataset and giving the corresponding probabilities. The settings used were 100,000 replicates and 80,000 burn-ins, but for unstable runs (when the deviation between repeated runs was high), it was increased as high as 1,000,000 replicates and 100,000 burn-ins. Different values of k (number of clusters), one to six for Spermonde and Sulawesi, and one to ten for the Indo-Malay Archipelago, were tested. Each value of k was tested at least three times to estimate the deviation between repeated runs. The number of clusters (k) which showed the highest probability was taken as the most probable number of different genetic lineages, or populations in the data set. If more than one cluster was assumed, all individuals were assigned to the most probable corresponding cluster and the frequencies of each cluster were calculated for each sample site. These frequencies were drawn on a map of the study area as pie charts to visualise the distributions of the clusters/genetic lineages.

Additionally, an analysis of molecular variance (AMOVA) was performed to test for population structures using Arlequin (ver.3.1, Excoffier et al. 2005). In order to reveal detailed patterns of genetic divergences, pairwise *F*-statistics were calculated with the same programme and a hierarchical AMOVA was conducted to test spatial groupings of populations. For all procedures in which multiple tests were conducted (e.g., pairwise comparison of populations for *F*-statistics and population differentiation, as well as linkage disequilibrium), a correction of the significance level was done with the false discovery rate control (Benjamini and Hochberg 1995). It is a statistical method to correct for Type I errors in significance calculations of multiple tests.

Finally, a Mantel-test (Mantel 1967) was calculated with the Isolation by Distance Web-Service (IBDWS, Jensen et al. 2005) in order to test for correlation of geographic and genetic distances.

Results

Microsatellite characterisation

The number of alleles in the six microsatellite loci varied between 8 and 34, whereat the highest numbers of alleles (30 and 34, Table 2) were found in loci CF27 and CF42, which showed also the highest heterozygosities. The lowest numbers of alleles were found in loci 120 and 65. The lowest heterozyosities were observed in loci 45 and 120. The observed heterozygosites were in the same range as the expected heterozygosities in all loci, mostly varying only slightly towards a lower observed heterozygosity. The variability of the loci detected in the present dataset of *A. ocellaris* samples is appropriate for genetic diversity measures and subsequent population genetic analyses.

Genetic variability

The mean gene diversity over all loci per population varied from 0.49 in Batam (Bt) to 0.87 in Sanggala/Papisol (SP). The mean allelic richness varied from 3.29 in Batam (Bt) to 5.78 in Puntondo (Pu). For both diversity measurements of the different populations, Batam (Bt) showed the lowest values (Table 1). Since the six loci show different levels of variability, the standard deviations over all loci are very high. Comparisons of the values in Batam (Bt) for both measurements to the mean value of all other populations with the Mann–Whitney test (Mann and Whitney 1947, Sokal and Rohlf 1981) showed that the values in Batam (Bt) were significantly lower ($p < 0.02$; Fig. 2). Locus 45 revealed an extreme reduced genetic diversity in Batam (Bt) with only one allele present and therefore a gene diversity of 0. This locus showed the highest diversity in the populations of Padang (Pa), the Java Sea (PS, Ka), Bali (Ba), Komodo (Ko), and Kupang (Ku). Locus 120 showed also the highest diversity in Padang (Pa) and the Java Sea (PS, Ka), but not in Komodo (Ko) and Kupang (Ku). Additionally, this locus showed a high diversity in Puntondo (Pu), most sites in New Guinea (Pi, SB, SP), and Misool (Mi). The allele with 464 bp length of locus 120 was present only in the Indian Ocean (Pa, Ku).

Since heterozygosity is correlated to genetic diversity, it showed as well a considerable variation among loci, with the highest values again for loci CF27 and CF42 (Table in Supplementary). Over all loci, the expected heterozygosities varied between 0.491 (Bt) and 0.806 (Ka) and the observed ones between 0.471 (Bt) and 0.832 (Pa). In most cases of differences between the expected and observed heterozygosities, the populations showed a heterozygotes deficit, but only few populations showed a significant deviation from HWE, indicated by significant p values ($\alpha = 0.05$) (Table in Supplementary). The highest number of significant deviations from HWE in single locus calculations was found in loci CF27 (five populations) and 45 (four populations). Locus 65 did not show deviations from HWE. The global test for deviations from HWE over all loci revealed significant p values in Manado (Ma), Bali (Ba), Komodo (Ko), Kupang (Ku), and Triton Bay (TB), all correlated with a heterozygote deficit, although the diversity measurements were high in these populations.

As heterozygote deficit in microsatellite often results from the presence of null alleles, next to the possibility that the populations are not in HWE, the loci were tested for null alleles in each population. In locus CF27 the possible presence of null alleles was indicated in three populations (Se, PS, and Ba), and in locus 45 in one population (Ko).

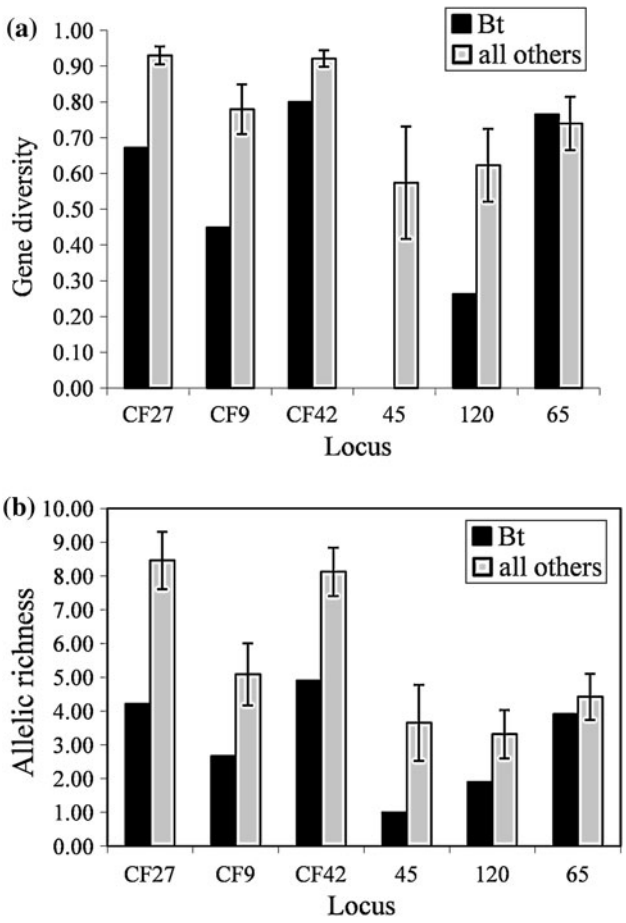

Fig. 2 **a** Gene diversity and **b** allelic richness of the *A. ocellaris* population in Batam and the mean of all other populations (including standard deviations) for each locus

Table 3 Significant loci combinations for linkage disequilibrium and corresponding populations of *A. ocellaris*. The *p* values are given after corrections for false discovery rates (Benjamini and Hochberg 1995)

Loci combination	*p* value	Population
CF9–45	0.008	Kupang
CF9–65	0.017	Karimunjava
CF9–CF27	<0.001	Manado
CF9–CF42	0.011	Manado
CF27–45	0.004	Manado
CF27–65	<0.001	Manado
CF27–120	<0.001	Spermonde
CF27–CF42	<0.001	Manado
CF42–65	0.011	Donggala
CF42–120	0.022	Manado
120–65	0.013	Pulau Seribu

Table 4 Significant results of the hierarchical AMOVA for *A. ocellaris* populations around Sulawesi

Number of groups	Groupings	F_{ct} value	*p* value
2 Groups	[Ma, Do, Sp, Pu][Bi, Se, Ke]	0.01317	0.03519
3 Groups	[Ma, Do, Sp][Bi, Se, Ke][Pu]	0.01188	0.01564
	[Ma, Do, Sp, Pu][Bi, Se][Ke]	0.01068	0.03030
	[Ma, Do][Sp, Pu][Bi, Se, Ke]	0.00847	0.04985

Therefore, the heterozygote deficit in Bali (Ba) and Komodo (Ko) might be due to the presence of null alleles in the loci CF27 and 45, respectively.

The test for linkage disequilibrium among loci showed that some combinations of loci had a significant *p* value after correction for multiple tests. However, combinations with significant *p* values appeared only once, and mostly in single populations. Only in Manado (Ma), six loci combinations were significant, indicating rather a pattern concerning this population than a general linkage of these loci (Table 3). Even though indications of significant linkage disequilibrium were found in some populations for certain loci combinations and the loci CF27 and 45 might have null alleles in a few populations (max. three out of 23), all loci were used for further calculations as none of these disequilibria show trends of selection that could bias population structure analysis.

Population differentiation

Spermonde Archipelago

Bayesian analysis revealed highest probability of population structuring for a single cluster with all individuals, indicating no structure in Spermonde Archipelago. The overall AMOVA ($F_{st} = -0.0009$, $p = 0.603$), as well as hierarchical AMOVA (grouping by shelf area), and pairwise comparisons showed no significant *F*-statistics, underlining a homogeneous panmictic population throughout Spermonde Archipelago. The isolation by distance analysis showed no significant correlation of the geographic and genetic distances between sites ($r = 0.48$, $p = 0.178$). Subsequently, all samples from Spermonde Archipelago were pooled for further analyses (second and third scale).

Sulawesi

In the second analysis, including sites around Sulawesi, the highest probability of the structure analysis was given for one single global cluster, suggesting no population structures around Sulawesi Island. However, the probabilities for two and three clusters were only slightly lower which could indicate existence of substructures.

In fact, the overall AMOVA revealed a significant structure in the dataset, although the F_{st} value was rather low ($F_{st} = 0.0145$, $p < 0.001$). The former CR analysis estimated a significant F_{st} value of 0.039. The hierarchical AMOVA revealed the highest F_{ct} value when separated into two groups (Sulawesi Sea and Makassar Strait versus southeast Sulawesi including Bira, Bi; Table 4). All significant F_{ct} values were lower than the F_{st} value without grouping.

Pairwise comparisons among populations around Sulawesi revealed significant differentiation between Kendari (Ke) and most other sites except its closest neighbours Bira (Bi) and Sembilan Islands (Se), underlining the results of the hierarchical AMOVA. Significant differentiation was also found between Donggala (Do) and Spermonde (Sp), which was not shown by the hierarchical AMOVA. The population in Sembilan Islands (Se) was not significantly different to any other population around Sulawesi, although it is far away from some sites, for example Manado (Ma), and quite remotely located in Bone Bay.

A Mantel-test showed no correlation of geographic and genetic distance ($r = 0.20$, $p = 0.195$).

Indo-Malay Archipelago

The Bayesian analysis revealed a structure of three clusters within the third dataset that included all sample sites. The frequencies of assignments to the clusters in each population showed that the red cluster was mainly present in the Indian Ocean (Pa) and the Java Sea (PS, Ka), but also in large proportions in Bali (Ba) and Komodo (Ko) (Fig. 1). Lower frequencies were found in southwest Sulawesi (Sp, Bi, Pu) and Makassar Strait (Do). Individuals, assigned to

the yellow cluster were found with highest frequencies in the sites along the western coast of New Guinea (Pi, SB, SP, TB), Molukkas (Mi), southeast Sulawesi (Ke), south Sulawesi (Bi) and Timor (Ku) (Fig. 1). Slightly lower frequencies were observed at other sites of Sulawesi (Se, Pu, Sp, Do, Ma), in northern Borneo (Sa, BI) and Cebu (Ce). Low frequencies were as well found further west in the Flores Sea (Ko, Ba), Java Sea (Ka, PS), and Kota Kinabalu (KK). The blue cluster was dominant in the South China Sea (Bt), northern Borneo (KK, BI, Sa) and Cebu (Ce), but also present with high frequencies in North Sulawesi (Ma) and Sembilan Islands (Se) (Fig. 1). Lower frequencies occurred along the ITF (Do), including New Guinea (Pi, SP, SB, TB) and Misool (Mi).

Analysis of molecular variance of the whole dataset confirmed a significant population structure with a F_{st} value of 0.0478 ($p < 0.001$). The genetic population structure found in the previous CR study of *A. ocellaris* was with a significant F_{st} value of 0.241 four to five fold stronger. In the hierarchical AMOVA, the highest F_{ct} value was given when the dataset was divided into three groups (1) South China Sea (Bt), (2) Indian Ocean and Java Sea (Pa, PS, Ka), and (3) all other populations (Table 6). The red cluster of the Bayesian analysis was dominant in the Indian Ocean/Java Sea populations (Pa, PS, Ka), supporting this group of the hierarchical AMOVA. Batam (Bt) did not form a separate cluster in the Bayesian analysis, but all individuals from this population were assigned to the blue cluster, whereas the other locations showed mixed assignments. To test for substructures within the blue cluster, suggesting a possible differentiation of Batam (Bt), all sample sites that showed a minimum of one-third blue cluster representatives were analysed separately with the structure programme in the same manner as described for the whole dataset. The resulting division into two clusters (blue and light blue, Figure in Supplementary) revealed substructures, undetected in the first Bayesian analysis with all samples. The light blue cluster was dominant in the South China Sea (Batam = 100% and Kota Kinabalu = 78%), while the other tested sample sites showed higher blue cluster frequencies.

Since the yellow cluster was dominant in the eastern sites, it supports a differentiation of New Guinea (Pi, SP, SB, TB) and Misool (Mi), as indicated by a significant, but slightly lower grouping in the hierarchical AMOVA.

The groupings of the hierarchical AMOVA were supported in the pairwise comparisons by high and significant F_{st} values between Batam (Bt) and all other sites. Padang (Pa) showed the only non-significant value to Pulau Seribu (PS), and the lowest significant values to Karimunjava (Ka) and Bali (Ba). Pulau Seribu (PS) and Karimunjava (Ka) exhibited high significant values to nearly all other sites (Table 5, below diagonal). Kota Kinabalu (KK) in northern Borneo showed high F_{st} values, except in pairwise comparisons with sites in northern Sulawesi (Ma, Do), Cebu (Ce), and Sangalaki (Sa). Surprisingly, the F_{st} value to Banggi Islands (BI), which is the closest neighbour, was significant, but not very high. The sites in New Guinea (Pi, SP, SB, TB) and Misool (Mi) had high F_{st} values to all other regions as well, except to southeastern Sulawesi (Bi, Ke, Se) and Timor (Ku). Generally, the northernmost sites of the central group (north of Spermonde, east of Batam) showed many low or non-significant values among each other. Cebu (Ce) exhibited significant F_{st} values to Kendari (Ke) and Misool (Mi), as well as to the other major groups (Batam and the Indian Ocean/Java Sea). The southern sites (south of Donggala, east of Karimunjava) also revealed many non-significant and/or low F_{st} values among each other. The sites in New Guinea (Pi, SP, SB, TB) and Misool (Mi) showed only non-significant F_{st} values among each other (Table 5, below diagonal).

The Mantel-test revealed a significant, but not very strong correlation between geographic and genetic distance ($r = 0.441$, $p < 0.001$).

Discussion

Genetic variability

Genetic diversity, usually expressed as the expected heterozygosity, in *A. ocellaris* (0.491–0.806, Table in Supplementary) was in a similar range as the expected heterozygosities of other marine fish species (0.86 in *Gadus morhua*, Bentzen et al. 1996; 0.69–0.93 in *Dicentrarchus labrax*, García De Leon et al. 1997; 0.75–0.85 in *Epinephelus marginatus*, De Innocentiis et al. 2001; 0.48–0.66 in five other species of *Epinephelus*, Nugroho et al. 1998). Only the population of Batam (Bt) showed a reduced genetic diversity compared to the other populations of *A. ocellaris* as well as to most of the other studies on marine fish species. A reduction in genetic diversity was previously found in Batam (Bt) by the analysis of CR of the same populations of *A. ocellaris* (Timm and Kochzius 2008). Batam seems to have undergone a recent population reduction or local extinction, probably due to over exploitation for the aquarium trade. The demand for *A. ocellaris* in the aquarium trade and its exploitation is very high—it is one of the most traded marine aquarium fish species worldwide (Wabnitz et al. 2003). The island of Batam is located in direct proximity to Singapore, so that fish can be easily shipped to the wholesalers in Singapore for subsequent international trade. Local divers reported that *A. ocellaris* could not be found at their dive sites after its popularity increase due to the movie "Finding Nemo" in 2003. However, during our sampling in 2005, we could

Table 5 Pairwise F_{st} values for 23 populations of *A. ocellaris* in the Indo-Malay Archipelago

	Sp	Pu	Bi	Do	Ma	Ke	Se	Sa	BI	KK	Bt	Pa
Sp		0.103*	0.007	0.066*	0.113*	0.038	0.023	0.076*	0.097*	0.066*	0.232*	0.506*
Pu	0.012		0.051	0.037	0.154*	0.183*	0.132*	0.185*	0.174*	0.156*	0.320*	0.372*
Bi	0.013	0.028		0.026	0.085*	−0.002	−0.011	0.048	0.042	0.089*	0.264*	0.521*
Do	0.012*	0.010	0.036*		0.075*	0.102*	0.070	0.051	0.035	0.090*	0.237*	0.532*
Ma	0.007	0.015	0.019	0.013		0.180*	0.040	0.047	0.095	0.034	0.232*	0.624*
Ke	0.020*	0.035*	0.002	0.034*	0.025*		0.040	0.125*	0.128*	0.147*	0.333*	0.603*
Se	0.006	0.022	−0.007	0.024	−0.006	0.000		0.049	0.096	0.009	0.280*	0.626*
Sa	0.017*	0.013	0.027*	−0.001	0.006	0.032*	0.003		−0.013	0.044	0.173*	0.641*
BI	0.012	0.023	0.007	0.025*	0.005	0.021	−0.009	0.029*		0.114*	0.214*	0.648*
KK	0.029*	0.022	0.076*	0.012	0.011	0.060*	0.034*	0.006	0.045*		0.193*	0.610*
Bt	0.095*	0.078*	0.178*	0.077*	0.103*	0.136*	0.124*	0.070*	0.135*	0.047*		0.708*
Pa	0.090*	0.124*	0.102*	0.137*	0.136*	0.139*	0.137*	0.155*	0.119*	0.186*	0.256*	
PS	0.045*	0.078*	0.057*	0.080*	0.077*	0.085*	0.066*	0.094*	0.067*	0.125*	0.193*	0.009
Ka	0.055*	0.080*	0.063*	0.099*	0.086*	0.093*	0.081*	0.112*	0.069*	0.135*	0.196*	0.015*
Ba	0.011	0.039*	0.003	0.033*	0.029*	0.031*	0.015	0.041*	0.020	0.063*	0.131*	0.045*
Ko	0.002	0.029	0.011	0.017	0.015	0.030*	0.010	0.017	0.017	0.035*	0.101*	0.076*
Ku	0.016*	0.036*	0.005	0.043*	0.016	0.007	0.004	0.028*	0.024	0.055*	0.147*	0.131*
Ce	0.002	0.020	0.009	−0.002	−0.012	0.019*	−0.009	−0.007	−0.005	0.014	0.107*	0.130*
Mi	0.029*	0.045*	0.013	0.050*	0.029*	0.008	0.023	0.049*	0.032*	0.076*	0.193*	0.136*
Pi	0.030*	0.058*	0.039	0.052*	0.038*	0.028*	0.039*	0.048*	0.035	0.065*	0.208*	0.133*
SB	0.033*	0.051*	0.038	0.038*	0.026	0.030*	0.036*	0.035*	0.052*	0.046*	0.178*	0.159*
SP	0.019*	0.035	0.000	0.045*	0.024	0.005	0.000	0.035*	0.020	0.063*	0.176*	0.114*
TB	0.027	0.036	0.001	0.040*	0.041*	−0.003	0.012	0.038*	0.023	0.069*	0.187*	0.140*

	PS	Ka	Ba	Ko	Ku	Ce	Mi	Pi	SB	SP	TB
Sp	0.291*	0.336*	0.146*	0.066*	0.029	0.057*	0.215*	0.107*	0.274*	0.232*	0.293*
Pu	0.102	0.125	−0.006	−0.009	0.082	0.107*	0.142*	0.091	0.190*	0.145*	0.181*
Bi	0.242*	0.300*	0.082	−0.003	−0.018	0.080*	0.134*	0.028	0.190*	0.150*	0.200*
Do	0.301*	0.342*	0.152*	0.023	0.058*	0.043	0.135*	0.028	0.188*	0.129*	0.189*
Ma	0.365*	0.420*	0.208*	0.134*	0.156*	0.141*	0.280*	0.172*	0.346*	0.291*	0.360*
Ke	0.363*	0.416*	0.205*	0.072*	−0.006	0.122*	0.176*	0.068	0.252*	0.205*	0.267*
Se	0.341*	0.398*	0.148	0.095*	0.058	0.059	0.228*	0.091	0.287*	0.243*	0.316*
Sa	0.378*	0.425*	0.223*	0.082*	0.102*	0.083*	0.248*	0.127*	0.322*	0.261*	0.329*
BI	0.365*	0.411*	0.207*	0.051	0.102*	0.106*	0.235*	0.110*	0.312*	0.241*	0.317*
KK	0.376*	0.423*	0.215*	0.130*	0.133*	0.047	0.263*	0.168*	0.314*	0.275*	0.343*
Bt	0.463*	0.487*	0.352*	0.243*	0.302*	0.257*	0.421*	0.365*	0.498*	0.437*	0.511*
Pa	0.170*	0.141*	0.241*	0.471*	0.542*	0.587*	0.598*	0.616*	0.666*	0.614*	0.670*
PS		−0.004	0.018	0.208*	0.288*	0.367*	0.381*	0.348*	0.424*	0.391*	0.422*
Ka	−0.004		0.054	0.253*	0.337*	0.400*	0.416*	0.395*	0.459*	0.424*	0.451*
Ba	0.010	0.018		0.080	0.142*	0.209*	0.247*	0.171*	0.277*	0.249*	0.270*
Ko	0.026	0.037*	−0.012		0.002	0.095*	0.067	0.026	0.124*	0.087*	0.111
Ku	0.073*	0.075*	0.033*	0.018		0.098*	0.081	0.024	0.160*	0.118*	0.158*
Ce	0.063*	0.084*	0.008	−0.003	0.020		0.196*	0.106*	0.242*	0.199*	0.271*
Mi	0.086*	0.095*	0.052*	0.043*	0.000	0.026*		0.070	−0.025	−0.030	−0.035
Pi	0.084*	0.100*	0.053*	0.044*	0.021	0.031	0.001		0.151	0.068	0.077
SB	0.100*	0.120*	0.059*	0.042*	0.015	0.023	−0.008	−0.013		−0.051	−0.012
SP	0.063*	0.078*	0.031	0.023	0.011	0.022	−0.009	0.002	0.003		−0.034
TB	0.092*	0.092*	0.029	0.024	0.013	0.035	0.005	0.006	0.023	0.000	

Below diagonal: microsatellite data of the present study, above diagonal: mtDNA sequence data of previous study (Timm and Kochzius 2008). Significant values (after correction for multiple tests following the false discovery rate procedure, Benjamini and Hochberg 1995) are marked with an asterisk*. For abbreviations see Table 1

find it on coastal coral reefs. This bottleneck due to over exploitation, could explain the low genetic diversity of the population in Batam, as previously also found in other commercially important fish species (e.g., Hauser et al. 2002).

Most populations in the present study were in HWE, with the exception of Manado (Ma), Bali (Ba), Komodo (Ko), Kupang (Ku), and Triton Bay (TB). The deviation from HWE of these populations can be explained by different factors, like presence of Null alleles detected in some loci for Bali (Ba) and Komodo (Ko), or population structuring that causes a violation of the assumptions for the HWE calculation (Rousset and Raymond 1995; Györffy et al. 2004). In Bali and Komodo, as well as Kupang (Ku), the presence of different genetic lineages, shown by high frequencies of the different clusters of the Baysian analysis (red, yellow, blue; Fig. 1) might have led to a Wahlund effect (Wahlund 1928; Hartl 2000), and therefore to an underestimation of heterozygosity. In the population of Manado (Ma), the most probable explanation for a deviation from HWE is the occurrence of linkage disequilibrium in some loci combinations. High degrees of linkage disequilibrium were found in small, relatively isolated and demographically stable populations in humans (Laan and Pääbo 1997; Zavattari et al. 2000). A relative stability in population size could be assumed for the population of Manado, as northern Sulawesi was only little effected by the sea level changes in the Pleistocene, but the assumption that Manado population is notably smaller than many of the other sites would need further studies on population sizes.

The sample size of Triton Bay (TB) is one of the lowest in the dataset, which could have had an influence on the HWE-calculations (Györffy et al. 2004).

Most of the studied populations showed similar levels of genetic variability and heterozygosity compared to other marine fish species (Bentzen et al. 1996; García De Leon et al. 1997; De Innocentiis et al. 2001; Nugroho et al. 1998).

Population differentiation

Spermonde Archipelago

The significant but shallow structure in Spermonde Archipelago shown in the population genetic analysis using CR (Timm and Kochzius 2008), was only revealed by a hierarchical AMOVA, grouping the islands of the different shelf areas together (midshelf, outershelf, northern outershelf, and outer rim). Additionally, two comparisons between pairs of shelf areas (midshelf–outershelf and outershelf–northern outershelf/outer rim) gave significant F_{st} values. When these analyses were applied to the microsatellite dataset, they did not show any significant differentiation.

One of the possible reasons that the previously found shallow population structure in Spermonde Archipelago could not be found with the microsatellite dataset might be homoplasy of alleles, due to limitations of the allele size, which leads to an under-estimation of genetic differentiation (Nauta and Weissing 1996; Garza et al. 1995). However, this is most probably not the case in this study, because on the other scales differentiations could be found, which would not be the case if a saturation of the allele size is present in the dataset. A problematic factor for detecting population structures could also be selection on certain loci (Garza et al. 1995; Barrière and Félix 2007). This possibility is unlikely for this data set, because (1) selection would not affect all loci, but most of them show similar results in the present study, and (2) on the larger scales the results were very similar to the CR data (Timm and Kochzius 2008). For some populations of the present study we could only analyse small sample sizes. Small sample sizes together with a large total number of alleles can lead to the reduction of statistical power, but not to the reduction of the value itself (Ruzzante 1998). The sample sizes in Spermonde were low for some areas (pooled outershelf = 6, and pooled midshelf = 16). Although, these were the most diverged populations on that small scale in the previous study utilising CR, for highly polymorphic markers like microsatellites, sample size can be crucial for a good resolution. Possibly, the shallow differentiation signals, could rather be found with the mtDNA, because of the lower effective population size and therefore faster coalescence time of mtDNA, than with ncDNA markers like microsatellites (Hoarau et al. 2004; Zink and Barrowclough 2008).

Sulawesi

A low but significant population structure was revealed for Sulawesi, although not in all applied analyses. The populations around Sulawesi were assigned to two groups with the hierarchical AMOVA: (1) north-west (Ma, Do, Sp, Pu), and (2) south-east (Bi, Se, Ke) (Table 4). The pairwise comparison revealed that Bira (Bi) in the South seems to be connected to both groups. This population structure follows the major current patterns around the island of Sulawesi, with the strong ITF from the North through the Makassar Strait along the western coast of the island, seasonally reversing currents at the southern coastline, and only very little water movement along the eastern coast of Sulawesi (Wyrtki 1961). These patterns can explain the strong differentiation of Kendari (Ke). Exchange at the eastern coast is common only between close sites, probably through coastal currents or larval movement (Bradbury and

Snelgrove 2001). On the contrary, the strong ITF facilitates a better connectivity among sites along the western coastline.

The CR analysis showed a similar genetic pattern (Table 5, above diagonal), but Manado was differentiated and the isolation by distance analysis was significant. This slight deviation of the microsatellite from the CR results could be due to a different temporal resolution, since microsatellites should detect very recent genetic structures, whereas mitochondrial markers should rather reflect historical pattern (Fauvelot et al. 2003). Manado might have experienced a stronger separation during sea level low stands. Although the ITF was present during the Pleistocene (Kuhnt et al. 2004), it was probably largely reduced by a constriction of Makassar Strait (Voris 2000), reducing the connection between northern and southern Sulawesi. This reduction disappeared with rising sea level and the strong ITF enhanced lineage mixing between sites, reflected by no obvious differentiation among Manado and southern Sulawesi in the microsatellite dataset. Another possible reason for a higher resolution in the CR study could be the lower effective population size of mtDNA, facilitating the fixation of genetic differences and therefore showing stronger population structuring (Hoarau et al. 2004; Zink and Barrowclough 2008), as noticed for the Spermonde data set.

The population of Sembilan Islands was not differentiated to any other site in the microsatellite analysis, which was rather due to low statistical power because of the low sample size (only eight individuals) than to high connectivity. In the CR analysis, the results for Sembilan Islands were similar (Table 5, above diagonal) and low statistical resolution was also expected (Timm and Kochzius 2008).

Indo-Malay Archipelago

The populations across the Indo-Malay Archipelago could be assigned to three groups based on the microsatellite data, instead to four revealed by the CR analysis (Timm and Kochzius 2008). The samples from New Guinea were included into the central group, and the groups of Batam and the Indian Ocean/Java Sea were identical (highest F_{ct} value in the hierarchical AMOVA). Again, this deviation between the two datasets could be due to different resolutions in the marker systems. While the CR analysis detected the separation of a genetic lineage in the eastern region of the archipelago, the microsatellite dataset revealed mixing of populations from the east and central part of the archipelago. Batam and Padang showed a strong differentiation in the present study, very similar to the separation revealed in the CR analysis (Timm and Kochzius 2008), although these two populations have very different genetic structures. Batam is strongly separated to all other populations studied and additionally it has a reduced genetic diversity.

Padang showed high genetic diversity in the microsatellite analysis and strong differentiation to other populations. The CR study (Timm and Kochzius 2008) revealed high haplotype diversity, but low nucleotide diversity, underlining the separation of a population in the Indian Ocean during sea level low stands. Since Padang and the Java Sea populations are strongly differentiated to the other studied populations in both marker systems (Table 5), exchange between this genetic lineage and the rest of the archipelago is restricted.

The differentiation of the populations from the western coast of New Guinea and Misool, and most other parts of the archipelago found in the CR study was supported in the present study by many significant and high pairwise F_{st} values (Table 5, below diagonal), as well as the distribution of the yellow cluster of the Bayesian analysis, most prominent in the New Guinea/Misool populations. However, the hierarchical AMOVA could not detect this separation. When four groups were tested, the two highest F_{ct} values occurred with the New Guinea/Misool populations and Padang as separate groups (Table 6). The less pronounced differentiation of the New Guinea/Misool group in the microsatellite study could indicate contemporary gene flow along the ITF from the central Indo-Malay Archipelago, while the stronger separation in the CR might reflect historic restrictions in exchange, probably caused by the reduced ITF during sea level low stands. Contrariwise, the differences in the markers could be due to a higher fixation of mtDNA differences, due to the lower effective population size of the marker, as suggested earlier.

Generally, the results of the microsatellite analysis on the second and third scale support the findings of the CR study done with mostly the same individuals of *A. ocellaris*. Although estimations of the genetic structure are based on two different marker systems and to some extent different algorithms, both analyses revealed a very similar

Table 6 Hierarchical AMOVA for *A. ocellaris* populations in the Indo-Malay Archipelago

Number of groups	Groupings	F_{ct} value
3 Groups	[Bt][Pa, PS, Ka][all others]	0.08229
	[Bt][Pa, PS, Ka, Ba][all others]	0.07296
4 Groups	[Bt][Pa, PS, Ka][Mi, Pi, SB, SP, TB][all others]	0.06745
	[Bt][Pa][PS, Ka][all others]	0.06745
5 Groups	[Bt][Pa][PS, Ka][Mi, Pi, SB, SP, TB][all others]	0.06714

All shown groupings were significant (p values < 0.001)

population structure in the Indo-Malay Archipelago. The differentiation of populations of different ocean basins in the Indo-Malay Archipelago was also shown in other studies on marine organisms (Barber et al. 2002; Lourie et al. 2005; Rohfritsch and Borsa 2005; Kochzius and Nuryanto 2008; Timm et al. 2008; Nuryanto and Kochzius 2009; Knittweis et al. 2009; Kochzius et al. 2009).

Interestingly, the CR shows a high potential for population genetic studies in anemonefish, although mitochondrial markers were criticised not to be suitable because of possible selective sweeps. Therefore, it was suggested that results based on mitochondrial markers should be interpreted with caution (Bazin et al. 2006). However, the structure revealed by the previous study based on CR (Timm and Kochzius 2008) was supported by the present microsatellite analysis. Even more, the CR showed stronger population differentiations in many cases, either due to different effective population sizes of the markers, promoting a faster accumulation of differentiation signal in mtDNA (Hoarau et al. 2004), or the slower mutation rate of the mtDNA sequence marker, compared to polymorphic microsatellites, is reflecting the traces of former isolations of lineages and increasing admixture shown by the microsatellite analysis. The latter scenario is coherent with the geographic history of the study area, and the most probable population dynamics after the sea basin separations during the Pleistocene. Low sample size in some of the analysed populations is probably another main factor for the lower resolution of microsatellites compared to CR. In order to reach a higher resolution, especially on the small scale, more loci should be tested and it would be important to have a larger sample size in order to increase the statistical power. It underlines though, that mitochondrial markers can be powerful tools for phylogeography and population analyses, but generally the use of multiple markers is very important to draw correct conclusions and get additional information about demographic conditions on historic and contemporary time scales.

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Conflict of interest The authors declare no conflict of interest.

References

Allen GR, Werner TB (2002) Coral reef fish assessment in the "coral triangle" of southeastern Asia. Environ Biol Fish 65:209–214

Barber PH, Palumbi SR, Erdmann MV, Moosa MK (2002) Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. Mol Ecol 11:659–674

Barrière A, Félix M (2007) Temporal dynamics and linkage disequilibrium in natural *Caenorhabditis elegans* populations. Genetics 176:999–1011

Barrowclough GF, Zink RM (2009) Funds enough, and time: mtDNA, nuDNA and the discovery of divergence. Mol Ecol 18: 2934–2936

Bazin E, Glémin S, Galtier N (2006) Population size does not influence mitochondrial genetic diversity in animals. Science 312:570–572

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 57:289–300

Bentzen P, Taggart CT, Ruzzante DE, Cook D (1996) Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Can J Fish Aquat Sci 53:2706–2721

Berry OF (2006) Mitochondrial DNA and population size. Science 314:1388

Borsa P (2003) Genetic structure of the round scad mackerel *Decapterus macrosoma* (Carangidae) in the Indo-Malay archipelago. Mar Biol 142:575–581

Bradbury IR, Snelgrove PVR (2001) Contrasting larval transport in demersal fish and benthic invertebrates: the role of behaviour and advective processes in determining spatial pattern. Can J Fish Aquat Sci 58:811–823

Briggs JC (2000) Centrifugal speciation and centres of origin. J Biogeogr 27:1183–1188

Buston PM, Bogdanowicz AW, Harrison RG (2007) Are clownfish groups composed of close relatives? An analysis of microsatellite DNA variation in *Amphiprion percula*. Mol Ecol 16: 3671–3678

De Innocentiis S, Sola L, Catandella S, Bentzen P (2001) Allozyme and microsatellite loci provide discordant estimates of population differentiation in the endangered dusky grouper (*Epinephelus marginatus*) within the Mediterranean Sea. Mol Ecol 10:2163–2175

Edwards S, Bensch S (2009) Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008. Mol Ecol 18:2930–2933

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinf Online 1:47–50

Eyre-Walker A (2006) Size does not matter for mitochondrial DNA. Science 312:537–538

Fautin DG, Allen GR (1994) Anemonenfische und ihre Wirte. Tetra-Verlag, Melle, pp 1–168

Fauvelot C, Bernardi G, Planes S (2003) Reductions in the mitochondrial DNA diversity of coral reef fish provide evidence of population bottlenecks resulting from Holocene sea-level change. Evolution 57:1571–1583

Froukh T, Kochzius M (2007) Genetic population structure of the endemic fourline wrasse (*Larabicus quadrilineatus*) suggests limited larval dispersal distances in the Red Sea. Mol Ecol 16:1359–1367

Froukh T, Kochzius M (2008) Species boundaries and evolutionary lineages in the blue green damselfishes *Chromis viridis* and *Chromis atripectoralis* (Pomacentridae). J Fish Biol 72:451–457

García De Leon FJ, Chikhi L, Bonhomme F (1997) Microsatellite polymorphism and population subdivision in natural populations of European sea bass *Dicentrarchus labrax* (Linnaeus 1758). Mol Ecol 6:51–62

Garza JC, Slatkin M, Freimer NB (1995) Microsatellite allele frequencies in humans and chimpanzees, with implications for constraints on allele size. Mol Biol Evol 12:594–603

Glaubitz JC (2004) CONVERT: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol Ecol Notes 4:309–310

Goudet J (1995) Fstat version 1.2: a computer program to calculate F-statistics. J Hered 86:485–486

Györffy B, Kocsis I, Vásárhelyi B (2004) Biallelic genotype distributions in papers published in gut between 1998 and 2003: altered conclusions after recalculating the Hardy-Weinberg equilibrium. Int J Gastroenterol Hepatol 53:614–616

Hall R (1996) Reconstructing cenozoic SE Asia. In: Hall R, Blundell D (eds) Tectonic evolution of southeast Asia. Geological Society of London Special Publication No. 106, pp. 153–184

Hartl DL (2000) A primer of population genetics, vol 3rd. Sinauer Associates, Inc. Publishers, Sunderland, pp 70–74

Hauser L, Adcock GJ, Smith PJ, Bernal Ramírez JH, Carvalho GR (2002) Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagus auratus*). Proc Natl Acad Sci USA 99: 11742–11747

Hoarau G, Piquet AM-T, van der Veer HW, Rijnsdorp AD, Stam WT, Olsen JL (2004) Population structure of plaice (*Pleuronectes platessa* L.) in northern Europe: a comparison of resolving power between microsatellites and mitochondrial DNA data. J Sea Res 51:183–190

Hoeksema BW (2007) Delineation of the Indo-Malayan centre of maximum marine biodiversity: the coral triangle. In: Renema W (ed) Biogeography, time and place: distributions, barriers and islands, vol 29. Springer, Dordrecht, pp 117–178

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. BMC Genet 6:1–6

Jones GP, Planes S, Thorrold SR (2005) Coral reef fish larvae settle close to home. Curr Biol 15:1314–1318

Knittweis L, Krämer WE, Timm J, Kochzius M (2009) Genetic structure of *Heliofungia actiniformis* (Scleractinia: Fungiidae) populations in the Indo-Malay Archipelago: implications for live coral trade management efforts. Conserv Genet 10:241–249

Kochzius M (2009) Trend in fishery genetics. In: Beamish R, Rothschild B (eds) The future of fisheries science in north America, fish & fisheries series. Springer, Dordrecht, pp 453–493

Kochzius M, Nuryanto A (2008) Strong genetic population structure in the boring giant clam *Tridacna crocea* across the Indo-Malay Archipelago: implications related to evolutionary processes and connectivity. Mol Ecol 17:3775–3787

Kochzius M, Söller R, Khalaf MA, Blohm D (2003) Molecular phylogeny of the lionfish genera *Dendrochirus* and *Pterois* (Scorpaenidae, Pteroinae) based on mitochondrial DNA sequences. Mol Phylogenet Evol 28:396–403

Kochzius M, Seidel C, Hauschild J, Kirchhoff S, Mester P, Meyer-Wachsmuth I, Nuryanto A, Timm J (2009) Genetic population structures of the blue starfish *Linckia laevigata* and its gastropod ectoparasite *Thyca crystallina*. Mar Ecol Prog Ser 396:211–219

Kuhnt W, Holbourn A, Hall R, Zuvela M, Käse R (2004) Neogene history of the Indonesian throughflow. In: Clift P, Hayes D, Kuhnt W, Wang P (eds) Continent-ocean interactions in the East Asian marginal seas, vol 149. AGU Monograph, Washington, pp 299–320

Laan M, Pääbo S (1997) Demographic history and linkage disequilibrium in human populations. Nat Genet 17:435–438

Lee JY, Edwards SV (2008) Divergence across Australia's carpentarian barrier: statistical phylogeography of the red-backed fairy wren (*Malurus melanocephalus*). Evolution 62:3117–3134

Lourie SA, Green DM, Vincent ACJ (2005) Dispersal, habitat differences, and comparative phylogeography of Southeast Asien seahorses (Syngnathidae: *Hippocampus*). Mol Ecol 14:1073–1094

Mann HB, Whitney DR (1947) On a test of whether one of two random variables is stochastically larger than the other. Ann Math Stat 18:50–60

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

Moll H (1983) Zonation and diversity of *Scleractinia* on reef off S.W. Sulawesi, Indonesia. PhD Thesis, University of Leiden, The Netherlands

Mulligan CJ, Kitchen A, Miyamoto MM (2006) Comment on "populatin size does not influence mitochondrial genetic diversity in animals". Science 314:1390a

Nauta MJ, Weissing FJ (1996) Constraints on allele size at microsatellite loci: implications for genetic differentiation. Genetics 143:1021–1032

Nelson JS, Hoddell RJ, Chou LM, Chan WK, Phang VPE (2000) Phylogeographic structure of false clownfish, *Amphiprion ocellaris*, explained by sea level changes on the Sunda shelf. Mar Biol 137:727–736

Nugroho E, Takagi M, Sugama K, Taniguchi N (1998) Detection of GT repeats microsatellite loci and their polymorphism for grouper of the genus *Epinephelus*. Fish Sci 64:836–837

Nuryanto A, Kochzius M (2009) Highly restricted gene flow and deep evolutionary lineages in the giant clam *Tridacna maxima*. Coral Reefs 28:607–619

Perrin C, Borsa P (2001) Mitochondrial DNA analysis of the geographic structure of Indian scad mackerel in the Indo-Malay archipelago. J Fish Biol 59:1421–1426

Planes S, Jones GP, Thorroldd SR (2009) Larval dispersal connects fish populations in a network of marine protected areas. Proc Natl Acad Sci USA 106:5693–5697

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Quenouille B, Bouchenak-Khelladi Y, Hervet C, Planes S (2004) Eleven microsatellite loci for the saddleback clownfish *Amphiprion polymnus*. Mol Ecol Notes 4:291–293

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Reilly A, Elliott NG, Grewe PM, Clabby C, Powell R, Ward RD (1999) Genetic differentiation between Tasmanian cultured Atlantic salmon (*Salmon salar* L.) and their ancestral Canadian population: comparison of microsatellite DNA and allozyme and mitochondrial DNA variation. Aquaculture 173:459–469

Renema W, Troelstra SR (2001) Larger foraminifera distribution on a mesotrophic carbonate shelf in SW Sulawesi (Indonesia). Palaeogeogr Palaeoclimatol Palaeoecol 175:125–146

Rohfritsch A, Borsa P (2005) Genetic structure of Indian scad mackerel *Decapterus russelli*: Pleistocene vicariance and

secondary contact in the central Indo-West Pacific seas. Heredity 95:315–326

Rousset F, Raymond M (1995) Testing heterozygote excess and deficiency. Genetics 140:1413–1419

Ruzzante DE (1998) A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. Can J Fish Aquat Sci 55:1–14

Saenz-Agudelo P, Jones GP, Thorrold SR, Planes S (2009) Estimating connectivity in marine populations: an empirical evaluation of assignment tests and parentage analysis under different gene flow scenarios. Mol Ecol 18:1765–1776

Santini S, Polacco G (2006) Finding Nemo: molecular phylogeny and evolution of the unususal life style of anemonefish. Gene 385:19–27

Shaw PW, Pierce GJ, Boyle PR (1999) Subtle population structuring within a highly vagile marine invertebrate, the veined squid, Loligo forbesi, demonstrated with microsatellite DNA markers. Mol Ecol 8:407–417

Sokal RR, Rohlf FJ (1981) Biometry: the principles and practice of statistics in biological research. W.H. Freeman and Company, New York

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 17:6463–6471

Timm J, Kochzius M (2008) Geological history and oceanography of the Indo-Malay Archipelago shape the population structure in the false clown anemonefish (*Amphiprion ocellaris*). Mol Ecol 17:3999–4014

Timm J, Figiel M, Kochzius M (2008) Contrasting patterns in species boundaries and evolution of anemonefishes (Amphiprioninae, Pomacentridae) in the centre of marine biodiversity. Mol Phylogenet Evol 49:268–276

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Vázquez JF, Pérez T, Albornoz J, Dominguez A (2000) Estimation of microsatellite mutation rates in *Drosophila melanogaster*. Genet Res 76:323–326

Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and durations. J Biogeogr 27:1153–1167

Wabnitz C, Taylor M, Green E, Razak T (2003) From ocean to aquarium. UNEP-WCMC, Cambridge

Wahlund S (1928) Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas 11:65–106

Wares JP, Barber PH, Ross-Ibarra J, Sotka EE, Toonen RJ (2006) Mitochondrial DNA and population size. Science 314:1388–1389

Weber JL, Wong C (1993) Mutation of short tandem repeats. Hum Mol Genet 2:1123–1128

Whitten T, Mustafa M, Henderson GS (2002) The EcOLOGY Of Sulawesi. ecology of Indonesia series, vol IV. Periplus Editions Ltd, Hong Kong

Williams ST (2000) Species boundaries in the starfish genus *Linckia*. Mar Biol 136:137–148

Wyrtki K (1961) Physical oceanography of the Southeast Asien waters. In: NAGA Report, vol. 2, University of California, Scripps Institution of Oceanography, LaJolla, California

Zavattari P, Deidda E, Whalen M, Lampis R, Mulargia A, Loddo M, Eaves L, Mastio G, Todd JA, Cucca F (2000) Major factors influencing linkage disequilibrium by analysis of different chromosome regions in distinct populations: demography, chromosome recombination frequency and selection. Hum Mol Genet 9:2947–2957

Zink RM, Barrowclough GF (2008) Mitochondrial DNA under siege in avian phylogeography. Mol Ecol 17:2107–2121