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PRIMARY RESEARCH PAPER

Genetic connectivity in a herbivorous coral reef fish (*Acanthurus leucosternon* Bennet, 1833) in the Eastern African region

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Abstract Knowledge of larval dispersal and connectivity in coral reef species is crucial for understanding population dynamics, resilience, and evolution of species. Here, we use ten microsatellites and one mitochondrial marker (cytochrome *b*) to investigate the genetic population structure, genetic diversity, and historical demography of the powder-blue tang *Acanthurus leucosternon* across more than 1000 km of the scarcely studied Eastern African region. The global AMOVA results based on microsatellites reveal a low but significant F_{ST} value ($F_{ST} = 0.00252$ $P < 0.001$; $D_{EST} = 0.025$ $P = 0.0018$) for the 336 specimens sampled at ten sample sites, while no significant differentiation could be found in the mitochondrial cytochrome *b* dataset. On the other hand, pairwise F_{ST}, PCOA, and hierarchical analysis failed to identify any genetic breaks among the Eastern African populations, supporting the hypothesis of genetic homogeneity. The observed genetic homogeneity among Eastern African sample sites can be explained by the lengthy post-larval stage of *A. leucosternon*, which can potentiate long-distance dispersal. Tests of neutrality and mismatch distribution signal a population expansion during the mid-Pleistocene period.

Keywords Western Indian Ocean · Kenya · Tanzania · Mozambique · Seychelles · Surgeonfish

Introduction

Many reef species are sedentary as juveniles and adults and depend on their planktonic larval stage for dispersal. These sedentary species display highly variable dispersal capacity, from having long-lived larvae that drift for months along ocean currents, to short-lived pelagic larvae that have limited dispersal capacity (Hellberg et al., 2002; Thorrold et al., 2006; Jones et al., 2009). Species with long pelagic larval durations (PLDs) tend to exhibit more extensive gene flow and have less structured populations than those with short PLDs (Duda Jr & Palumbi, 1999; Faurby & Barber, 2012; DiBattista et al., 2016). For example, the blue starfish (*Linckia laevigata* Linnaeus, 1758)

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has a long PLD (~22 days) and exhibits a higher gene flow across the Indo-Pacific than the crown-of-thorn starfish (*Acanthaster planci* Linnaeus, 1758), which has a shorter PLD (~14 days) (Benzie, 1999). Nevertheless, evidence is accumulating in marine organisms that show little congruence between observed genetic structure and PLD even in closely related species with comparable life history characteristics (Barber et al., 2002; Imron et al., 2007; DiBattista et al., 2012). This inconsistency in the empirical relationship of PLD and connectivity suggests that various factors may influence gene flow among marine populations, e.g. ocean current systems (Yasuda et al., 2009), larval behaviour (Bird et al., 2007), topographic features (Ahti et al., 2016), historical processes (Gaither et al., 2010), habitat preference (Rocha et al., 2002), and habitat fragmentation (Pellissier et al., 2014). While the relationship between PLD and gene flow still remains ambiguous in some species (Barber et al., 2002; Imron et al., 2007), most fishes with a long PLD tend to have less structured populations, suggesting that besides a long PLD fishes also have reproductive and ecological behaviours capable of enhancing long-distance dispersal (Eble et al., 2011b; Selkoe et al., 2014).

In marine organisms, discordant population structures may also arise due to the transient nature of marine barriers. These anomalous barriers cannot provide absolute vicariance between different populations because dispersal across them is usually possible when conditions are favourable (Mirams et al., 2011). Such porous barriers are found in the Eastern African region, which can be divided into three eco-regions: the North Monsoon Current, the Seychelles, and the East African Coral Coast (Obura, 2012). These eco-regions have biogeographic and oceanographic boundaries that underlie the restriction of gene flow in various coral reef and mangrove species (Ragionieri et al., 2010; Visram et al., 2010; Muths et al., 2015). The biogeographic boundaries correspond to tectonic plate boundaries that shape species distributions by physically preventing or reducing dispersal and recruitment success owing to the distance between suitable habitats (Spalding et al., 2007). Nevertheless, some studies on taxa that disperse through their planktonic phase fail to document genetic discontinuity between the different Eastern Africa eco-regions. These taxa include the fiddler crab *Uca annulipes* Edwards, 1837 (Silva et al., 2010), blue strip snapper *Lutjanus kasmira* Forsskål, 1775 (Muths et al., 2012), and skunk clownfish *Amphiprion akallopisos* Bleeker, 1853 (Huyghe & Kochzius, 2016).

The present-day oceanography in the Eastern African region is dominated by the South Equatorial Current (SEC) that flows westward across the Indian Ocean to the southern coast of Tanzania and northern coast of Mozambique. At the boundary of Mozambique and Tanzania, this current bifurcates to form the permanent northward flowing East African Coast Current (EACC) and complex eddies in the Mozambique Channel. The splitting of the SEC current at the Eastern African coast potentially creates an oceanographic barrier to dispersal between the southern and northern populations. On the other hand, the EACC, travelling up the Eastern African coastline, is strongly influenced by both monsoon winds and the Somali Current. During the northeast monsoon (November to March), the EACC is weakened, causing it to converge with the Somali Current that flows southward. This forms the seasonal eastward flowing South Equatorial Counter Current (SECC) and a strong upwelling wedge in areas North of Kenyan that extends up to the Somali coast. During the southeast monsoon, the Somali Current is weakened and it joins the EACC beyond Malindi in Kenya, where it develops into different gyres and cells that extend to the Horn of Africa (Fig. 1) (Schott & McCreary Jr, 2001; Benny, 2002).

The powder-blue tang surgeonfish (*Acanthurus leucosternon* Bennet, 1833) is widely distributed along reef flats in the Indian Ocean, from the Eastern Indian Ocean (EIO) to the Western Indian Ocean (WIO) (Randall, 2002). The largest densities of *A. leucosternon* are observed in the Maldives, but the primary distribution area is at the Eastern African coastline (Abesamis et al., 2012). *Acanthurus leucosternon* is a prized ornamental species that is heavily traded by Kenyan exporters, in addition to being targeted by artisanal fishing (Okemwa et al., 2016). Fishing pressure results in significant density differences (up to 75%) between adjacent protected and unprotected reefs (McClanahan et al., 1999; McClanahan, 2015). *Acanthurus leucosternon* is considered an ecological indicator species because its abundance correlates with healthy coral reefs (McClanahan et al., 1999). Despite a presumably short generation time of only 3–4 years, a depleted stock needs about 20 years

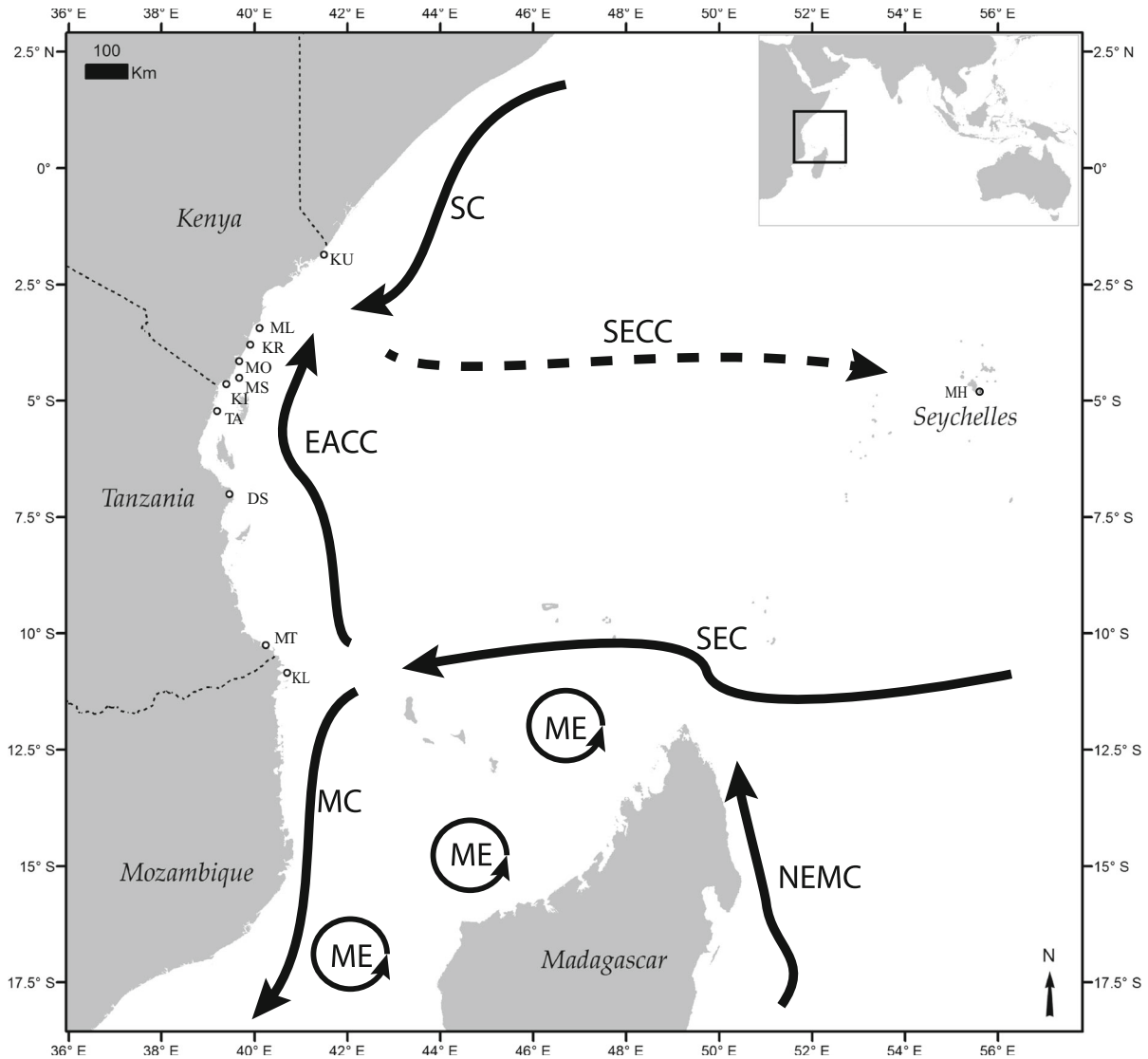

Fig. 1 Map of the eastern African coast with sample sites (for abbreviations see Table 1), main ocean currents (*solid lines*), and seasonal changing current (*dashed lines*). *EACC* East African Coast Current, *SEC* South Equatorial Current, *MC* Mozambique current, *SECC* south equatorial counter current, *NEMC* north equatorial Madagascar current, *ME* Mozambique current eddies (Schott & McCreary Jr, 2001; Benny, 2002)

to recover to its previous density after the closure of a fishing area (McClanahan et al., 2007). Like congener species of the genus *Acanthurus*, its feeding activity not only limits the establishment of algal communities in coral reef ecosystems but also provides a link for energy flow to higher trophic levels (Crossman et al., 2005; Mumby et al., 2007). *Acanthurus leucosternon* has, like many other reef organisms, a bipartite lifestyle, with sedentary adults and planktonic larval phase. Although the PLD of *A. leucosternon* has not yet been estimated, acanthurids are known for their long PLD of approximately 55 days (Thresher, 1984; McCormick, 1999; Fisher et al., 2005). The potentially high dispersal capacity of *A. leucosternon* offers an excellent opportunity to examine the patterns of connectivity across Eastern African biogeographical and oceanographic barriers.

Despite their contribution of substantial goods and services to coastal economies (Obura et al., 2017), the genetic connectivity of coral reef species in Eastern

Table 1 Sample information and molecular diversity indices of the microsatellite dataset for *A. leucosternon*: Sample site, location code, number of specimens (n), mean number of alleles (Na), allelic richness (Ar), observed heterozygosity (H_O), expected heterozygosity (H_E), fixation index (F_{IS}), and number of private alleles (PVA). Asterisks indicate significant deviations from the Hardy–Weinberg Equilibrium (HWE)

Sample site	Code	N	$N_a \pm$ SD	Ar	$H_o \pm$ SD	$H_E \pm$ SD	F_{IS} (10 loci)	F_{IS} (6 loci)	PVA
Kiunga	KU	25	10.33 ± 3.14	9.080	0.819 ± 0.040	0.858 ± 0.038	0.140***	0.046^{ns}	1
Malindi	ML	40	13.50 ± 2.88	10.05	0.857 ± 0.076	0.867 ± 0.031	0.068***	0.010^{ns}	1
Kuruwitu	KR	35	11.67 ± 2.58	9.030	0.856 ± 0.116	0.842 ± 0.040	0.057**	-0.016^{ns}	3
Mombasa	MO	33	13.67 ± 4.23	10.82	0.865 ± 0.080	0.886 ± 0.024	0.079***	0.024^{ns}	0
Msambweni	MS	35	13.33 ± 4.50	10.19	0.839 ± 0.070	0.861 ± 0.040	0.070***	0.025^{ns}	3
Kisite-Mpunguti	KI	51	15.00 ± 4.19	10.27	0.819 ± 0.064	0.859 ± 0.045	0.095***	0.047*	7
Tanga	TA	29	11.50 ± 3.08	9.780	0.805 ± 0.116	0.850 ± 0.050	0.195***	0.054*	3
Dar es Salaam	DS	16	11.17 ± 2.14	10.86	0.864 ± 0.072	0.863 ± 0.034	0.061*	-0.001^{ns}	2
Mtwara	MT	41	14.67 ± 4.13	10.28	0.875 ± 0.052	0.859 ± 0.042	0.053**	-0.019^{ns}	5
Kilindi	KL	31	12.00 ± 2.68	9.530	0.818 ± 0.116	0.846 ± 0.078	0.082***	0.034^{ns}	1

Sample sites are arranged from north to south

ns not significant

* $0.05 \geq P \geq 0.01$; ** $0.01 > P \geq 0.001$; *** $P < 0.001$

Africa remains amongst the least studied globally (Gaither et al., 2010; Visram et al., 2010; Muths et al., 2015; Otwoma & Kochzius, 2016). These species are usually managed homogeneously (UNEP-WCMC, 2008; Obura et al., 2017), without taking into account that different populations may have restricted larval exchanges. However, such a uniform management strategy may lead to significant alteration of the genetic subdivisions, with reduced genetic variation and fitness. Therefore, increasing genetic connectivity studies in this region aim to identify a congruent pattern on how ocean currents and other factors interact to influence larval dispersal, which will be essential in devising effective conservation strategies (Almany et al., 2007; Jones et al., 2009). In this study, we investigate the population genetic structure and connectivity of *A. leucosternon* in the Eastern African region using microsatellite markers and the mitochondrial cytochrome *b* gene. In addition, we elucidate the genetic diversity and population expansion of *A. leucosternon* in the context of historical processes. The survey of microsatellite genotypes and mitochondrial sequences of *A. leucosternon* intend to answer two questions: (1) Are there patterns of genetic population structure among populations of *A. leucosternon* in the Eastern African region? (2) Do the structuring patterns coincide with the known Eastern African barriers to dispersal?

Materials and methods

Sampling and DNA extraction

A total of 336 fin clips were taken from adult *A. leucosternon* at ten sampling locations ($n = 16$–51) along the Eastern African coastline between August and December 2015 (Table 1). The fish were obtained from local fishermen who use spear guns, basket traps, and reef seines. The sampled fin clips were preserved in 100% ethanol and stored at 4°C prior to DNA extraction. Total genomic DNA was extracted using the standard salting out protocol (Sunnucks & Hales, 1996).

Mitochondrial DNA amplification and sequencing

We amplified the mitochondrial cytochrome *b* gene using polymerase chain reaction (PCR) with the heavy-strand primer 5′GTGACTTGAAAAACCAC CGTTG 3′ (Song et al., 1998) and the light strand primer 5′AATAGGAAGTATCATTCGGGTTTGAT G 3′ (Taberlet et al., 1992). The PCR reactions were performed in 20 µl volumes containing 2 µl DNA template (50–100 ng), 2 µl PCR buffer B (Roboklon), 13.4 µl H_2O, 400 µm dNTPs, 1 µl BSA (10 mg/ml), 0.4 µl of reverse and forward primers each (10 µM), and a final concentration of 1 µM MgCl. The PCRs

Table 2 Mitochondrial cytochrome *b* diversity characteristics in the eastern African region

Sample site	Code	*N*	*Nhp*	*H*	π	Tajima's *D*	FU's F_S	SSD	HRI	Source
Kiunga	KU	16	12	0.97	0.005	-1.39^{ns}	-7.15^{***}	0.013^{ns}	0.08^{ns}	Present study
Dar es Salaam	DS	16	12	0.96	0.004	-1.67^{*}	-8.39^{***}	0.007^{ns}	0.06^{ns}	Present study
Kilindi	KL	16	10	0.87	0.004	-1.06^{ns}	-5.35^{***}	0.004^{ns}	0.03^{ns}	Present study
Mahe	MH	30	17	0.91	0.005	-1.62^{*}	-10.8^{***}	0.003^{ns}	0.03^{ns}	DiBattista et al. (2016)
All samples		78	35	0.92	0.005	-2.04^{**}	-26.81^{***}	0.0004^{ns}	0.03^{ns}	

Sample size (*n*), number of haplotypes (*Nhp*), haplotype diversity (*h*), nucleotide diversity (π), time since the recent population expansion (*T*), random sequence evolution (Tajima's *D* and FU's F_S), sum of square deviation (SSD), and Harpending's raggedness index (HRI)

ns not significant

* $0.05 \geq P \geq 0.01$; ** $0.01 > P \geq 0.001$; *** $P < 0.001$

were conducted with the following temperature profile: 95°C for 3 min, followed by 35 cycles of 30 s of denaturation at 94°C, 45 s of annealing at 63°C, and 45 s of extension at 72°C. The final extension was done at 72°C for 10 min (DiBattista et al., 2016). The PCR products were analysed using the Dye Deoxy terminator (Applied Biosystems) and sequenced on an automated sequencer (ABI PRISM 310 and 3100, Applied Biosystems).

For mitochondrial DNA analysis, a total of 48 sequences were sub-sampled from the 336 individuals. The 48 sequences from Kiunga, Dar es Salaam, and Kilindi were supplemented by 30 published sequences from Mahe, Seychelles (DiBattista et al., 2016), altogether representing the three Eastern African eco-regions that are separated from each other by oceanographic and/or biogeographic boundaries known to disrupt gene flow in marine organisms (Ragionieri et al., 2010; Visram et al., 2010; Muths et al., 2015).

Microsatellite amplification and genotyping

Individuals were genotyped at 10 published microsatellite loci: Ahy49, Ahy54, Ahy65, Ahy75, Ahy112, Ahy119, Ahy170, Ahy178, Ahy182, and Ahy203 (DiBattista et al., 2011), using an M13-tailed primer PCR protocol (Schuelke, 2000). PCR amplification was conducted in 10 µl reaction volume containing 1 µl DNA template (50–100 ng), 1 µl PCR buffer B (Roboklon), 6.5 µl H_2O, 200 µm dNTPs, 0.5 µl BSA (10 mg/ml), 0.2 µl of M13 fluorescent labelled tail primer (10 µM), 0.2 µl of reverse primer (10 µM), 0.2 µl of forward primer (2.5 µM) with M13 tail, and 500 nM of MgCl. The temperature profile consisted of 95°C for 3 min, followed by 35 cycles of 30 s of denaturation at 94°C, 45 s of annealing at a locus-specific temperature, and 45 s of extension at 72°C. The final extension was done at 72°C for 7 min (DiBattista et al., 2011).

The PCR products were labelled with different dye colours and pooled for genotyping along with an Alexa Fluor 660 (IBA GmbH)-labelled oligo as an internal size standard. Generation of the LIZ size marker followed the protocol described in DeWoody et al. (2004) using pUC19 as a template and resolved with an ABI 3730 genetic analyser (Applied Biosystems), at the Ludwig-Maximilians-Universität München, Germany. The software Geneious version 8.1.6 (Kearse et al., 2012) was used to manually assign allele sizes of the microsatellite loci. In total, 336 individuals were genotyped from 10 sample sites (Table 1) along the Eastern African mainland coastline, while the published sequences from Mahe, Seychelles were only used in the cytochrome *b* dataset (Table 2).

Data analysis

Mitochondrial DNA

The cytochrome *b* sequences were edited using Geneious version 8.1.6 (Kearse et al., 2012) and aligned in BIOEDIT version 7.0.4.1 (Hall, 1999). To ensure that only functional mitochondrial DNA was used and not pseudogenes, the sequences were translated into amino acids by the software Squint Alignment Editor version 1.02 (Goode & Rodrigo, 2007). The online services of FABOX (Villesen, 2007) were

used to collapse sequences into haplotypes. Haplotype and nucleotide diversity were calculated in Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010).

The null hypothesis of neutral evolution of cytochrome *b* was tested using the Tajima *D* test (Tajima, 1989) and Fu's *Fs* tests (Fu, 1997). Significant negative Tajima's *D* values indicate population expansion following either selective sweeps, genetic bottleneck event, or purifying selection (Tajima, 1989). Besides, population expansion was tested by comparing the observed sequence mismatch distributions within sampling sites and those simulated under Rogers' (1995) sudden population expansion model (Schneider & Excoffier, 1999), and the goodness-of-fit of the observed to simulated distributions was tested using both the sum of square deviation (SSD) and Harpending's raggedness index (HRI) (Rogers, 1995). A multimodal mismatch distribution indicates a population under a demographic equilibrium, while unimodal distribution suggests a recent and fast demographic expansion.

The time (*T*) since the recent population expansion was determined using the formula $T = \tau/2u$ (Rogers & Harpending, 1992), where Tau (τ) is the expansion parameter estimate and u equals the mutation rate × generation time × sequence length. The cytochrome *b* divergence rate range of 1 to 2% per million years in reef fish was used (Bowen et al., 2001; Lessios, 2008; Reece et al., 2010) together with a generation time of 3.4 years (estimated from Eastern African *A. leucosternon* length-frequency data; T.R McClanahan pers. comm.). The parameter Tau (τ) was estimated from Arlequin under a sudden population expansion hypothesis.

We used Arlequin to run an analysis of molecular variance (AMOVA) to estimate the genetic differentiation and pairwise Φ_{ST} values among populations of *A. leucosternon* (Excoffier et al., 1992). A network of haplotypes was constructed with the program TCS version 1.21 (Clement et al., 2000), to infer the evolutionary relationships between populations of *A. leucosternon*, with the proportion of haplotypes found at each sample site being reflected in the pie diagrams.

Microsatellites

Genetic diversity was estimated as the mean number of alleles (N_a), observed heterozygosity (H_O), expected heterozygosity (H_E), and private alleles in the program Arlequin version 3.5.1.3 (Excoffier & Lischer, 2010). The program FSTAT version 2.9.3.2 (Goudet, 1995) was used to estimate the mean allelic richness (*Ar*) and fixation index (F_{IS}). For each locus, an exact test for the departure from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) was estimated using Arlequin. The software MICRO-CHECKER version 2.2.3 (Van Oosterhout et al., 2004) was used to screen for possible genotypic errors, large allele dropout, and null alleles. Genotypic errors were further minimised by repeating PCR and fragment analysis in 132 randomly selected individuals (39.3% of all analysed specimens) at all the 10 loci.

Because null alleles have the likelihood of inflating F_{ST} values, the null allele-corrected global AMOVA and pairwise F_{ST} values were estimated in the software FreeNA (Chapuis & Estoup, 2007). FreeNA uses the ENA (Excluding Null Alleles) method to efficiently correct for null allele bias and F_{ST} overestimation. Since the estimates of F_{ST} have been observed to decline with increasing microsatellite polymorphism, Jost's D_{EST} was also estimated in this study in GenAlEX version 6.5 (Hedrick, 2005; Jost, 2008; Peakall & Smouse, 2012). The correlation between geographical and genetic distances in the *A. leucosternon* dataset was tested using the Mantel test in GenAlEX by utilising the pairwise F_{ST} and D_{EST} values.

A hierarchical AMOVA was carried out, testing for significant differentiation among groups of sites in Arlequin with composing groupings based on oceanographic conditions and the geographical locations of sites in the Eastern African region. In addition, a principal coordinate analysis (PCoA) was done in GenAlex, to examine the spatial variation among *A. leucosternon* populations using the unbiased Nei genetic distance. The software STRUCTURE version 2.3.4 (Pritchard et al., 2000) was used to define genetic clusters (K) without a priori information on the geographical origin of specimens. To estimate the optimal number of homogeneous genetic units (*K*), STRUCTURE was run under the admixture model for $K = 1$–10, using 10 iterations, a burn-in length of 100,000, and 1,000,000 MCMC (Markov chain Monte Carlo) replications. The most probable value of K was determined using the software STRUCTURE HARVESTER web version 0.6.94 (Earl & vonHoldt, 2012) by plotting log probability L (*K*) and ΔK (Evanno et al., 2005).

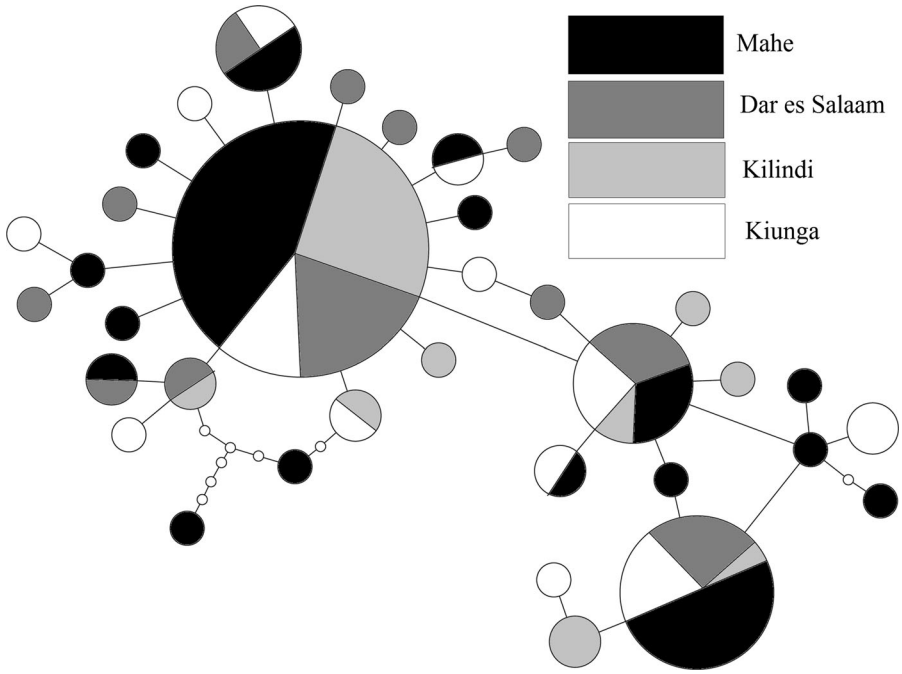

Fig. 2 Haplotype network of cytochrome *b* sequences of *A. leucosternon*. Each *circle* represents a haplotype and its size is proportional to the total frequency. The *lines* show mutational steps, while the *smallest circles* represent intermediate missing haplotypes

Results

Genetic diversity

Mitochondrial DNA

A 491-base pair (bp) fragment of cytochrome *b* was obtained after a sequence alignment, which did not contain indels and stop codons. The 78 sequences from Eastern Africa yielded 35 haplotypes, of which 24 were unique and eleven were shared by 19 to 2 individuals (Fig. 2). There were 35 polymorphic sites that included 33 transitions and 2 transversions. Overall, haplotype and nucleotide diversity estimates were similar among the Eastern African sampling sites, ranging from 0.87 to 0.97 and from 0.004 to 0.005, respectively (Table 2).

Microsatellites

Based on within-site comparisons, around a quarter of the loci (27 out of 100, $P \leq 0.025$) deviated significantly from the expectations of the HWE, these differences being mostly represented by four markers (Ahy54, Ahy75, Ahy182, and Ahy203) (Supplementary Table S1). Further analysis with MICRO-CHECKER indicated that the deviations at these four loci could be due to the presence of null alleles. The re-amplification and re-genotyping results indicated negligible evidence of misamplification and genotyping disagreement, 0.76% of all re-genotyped loci (10 out 1320). Low levels of linkage disequilibrium were noted after the removal of the four loci not conforming to HWE (14 out of 150 within-site comparisons, $P \leq 0.04$).

Over all populations, mean number of alleles (N_a) and allelic richness (Ar) varied from 10.33 to 15.00 and 9.03 to 10.86, respectively. The expected heterozygosity values ranged between 0.842 and 0.886, while the observed heterozygosity values ranged between 0.805 and 0.875. Private alleles were detected in all sample sites, with the exception of Mombasa, which shared all its alleles with the other sample sites. Populations from two sample sites (Kisite-Mpunguti and Tanga) exhibited significant F_{IS} values even after the exclusion of the four loci (Ahy54, Ahy75, Ahy182, and Ahy203) not in HWE (Table 1).

Historical demography

Overall, tests of neutral evolution of the cytochrome *b* marker revealed negative and significant FU *FS* and Tajima's *D* values, supporting population expansion

following selective sweeps, genetic bottleneck, or purifying selection (Table 2). The analysis of the sequence mismatch distribution revealed that the model of sudden expansion could not be rejected in the Eastern African population, using both SSD and HRI goodness-of-fit (Table 2). The range of mutation rates and τ estimated for all sample sites revealed a demographic expansion that began between 143,000 and 287,000 years ago.

Genetic population structure

Mitochondrial DNA

The AMOVA results of cytochrome *b* gene showed no genetic differences among Eastern Africa populations, even after inclusion of published sequences from Mahe, Seychelles (DiBattista et al., 2016) ($\Phi_{ST} = -0.021$, $P = 0.96$), with 100% genetic variation being observed within populations. Similarly, pairwise Φ_{ST} estimates were low and not significant, showing genetic homogeneity among all four sampling sites at the mitochondrial marker (Kiunga, Dar es Salaam, Kilindi, and Mahe) (Supplementary Table S2). The evolutionary relationship of 35 *A. leucosternon* haplotypes found in Eastern Africa is presented in the haplotype network (Fig. 2), showing a distinct star-like pattern of three common haplotypes surrounded by singletons. The distribution of the shared haplotypes throughout all four sample sites provides further evidence of a single panmictic population.

Microsatellites

Global F_{ST} ($F_{ST} = 0.00252$ and F_{ST} ENA $= 0.00249$) and pairwise F_{ST} (Table 3) estimates from the ENA-corrected and uncorrected dataset were not significantly different (*t* test: $P = 0.45$), suggesting that null alleles had little influence on genetic differentiation estimates. The global AMOVA revealed a low but significant F_{ST} value ($F_{ST} = 0.00252$, $P < 0.001$, $D_{EST} = 0.025$ $P = 0.0018$), with most of the genetic differences being observed within locations (99%). Similarly, all pairwise F_{ST} and D_{EST} estimates among populations were low and nonsignificant after Bonferroni correction ($P < 0.001$) (Table 3 and Supplementary Table S3). The hierarchical AMOVA grouping based on ocean currents and geographical location of sample sites was not significant, supporting the hypothesis of panmixia in the Eastern African region (data not shown). The Mantel test revealed no significant correlation between geographic distance and pairwise F_{ST} ($R^2 = 0.081$, $P = 0.32$) and D_{EST} ($R^2 = 0.0006$, $P = 0.42$) estimates.

The software STRUCTURE HARVESTER identified the optimum ΔK at $K = 2$ (Supplementary Fig. S1), with few individuals within sample sites KR, MO, and TA being genetically distinct from the other Eastern African populations (Fig. 3). On the other hand, the PCoA based on unbiased Nei genetic distances did not reveal any genetic breaks between the geographical locations, but two sample sites (KU and DS) were slightly separated from the other sample sites (Supplementary Fig. S2).

Discussion

This is the first study that examines genetic diversity and structure among populations of powder-blue tang surgeonfish along the Eastern African coastline. The findings using microsatellite and cytochrome *b* markers complement previous studies on coral reef fish species such as *Scarus ghobban* Forsskål, 1775, *L. kasmira*, and *A. akallopisos* (Visram et al., 2010; Muths et al., 2012; Huyghe & Kochzius, 2016). As expected, for a species with a lengthy post-larval stage, our results based on microsatellites revealed a weak genetic differentiation ($F_{ST} = 0.00252$ $P < 0.001$, $D_{EST} = 0.025$ $P = 0.0018$) among populations of *A. leucosternon*. However, pairwise F_{ST}, PCOA, and hierarchical analysis could not identify any genetic breaks among the Eastern African populations, suggesting a homogeneous connectivity pattern.

Genetic diversity and historical demography

Marine species traditionally have high genetic diversity, which can be attributed to historically large population sizes and high reproductive potential (Carvalho & Hauser, 1994). Findings on *A. leucosternon* do not appear to deviate greatly from this generalisation, both historically ($h = 0.87$–0.97) and contemporarily ($Ar = 9.03$-10.86, $H_E = 0.842$–0.886). The high levels of microsatellite genetic

Table 3 Raw and ENA-corrected pairwise F_{ST} values for populations of *Acanthurus leucosternon* in the Eastern African region (for sample site abbreviations see Table 1)

	KU	ML	KR	MO	MS	KI	TA	DS	MT	KL
KU		0.00287^{ns}	0.00826^{ns}	−0.00006^{ns}	0.00605^{ns}	0.00473^{ns}	0.00634^{ns}	0.00366^{ns}	0.00139^{ns}	0.00597^{ns}
ML	0.00349^{ns}		0.00444^{ns}	0.00035^{ns}	0.00073^{ns}	−0.00127^{ns}	0.00281^{ns}	0.00233^{ns}	−0.00085^{ns}	−0.00068^{ns}
KR	0.00775^{ns}	0.00481^{ns}		0.00826^{ns}	0.00239^{ns}	0.00369^{ns}	0.00216^{ns}	0.00569^{ns}	0.00484^{ns}	0.00505^{ns}
MO	0.00033^{ns}	0.00029^{ns}	0.00808^{ns}		0.00231^{ns}	0.00369^{ns}	0.00209^{ns}	0.00729^{ns}	0.00257^{ns}	0.00308^{ns}
MS	0.00632^{ns}	0.00046^{ns}	0.00286^{ns}	0.00191^{ns}		−0.00172^{ns}	−0.00221^{ns}	−0.00021^{ns}	0.00261^{ns}	0.00085^{ns}
KI	0.00495^{ns}	−0.00185^{ns}	0.00381^{ns}	0.00367^{ns}	−0.00237^{ns}		0.00155^{ns}	0.00408^{ns}	0.00254^{ns}	0.00018^{ns}
TA	0.00661^{ns}	0.00409^{ns}	0.00145^{ns}	0.00361^{ns}	−0.00133^{ns}	0.00291^{ns}		0.00224^{ns}	0.00204^{ns}	0.00159^{ns}
DS	0.00267^{ns}	0.00209^{ns}	0.00717^{ns}	0.00776^{ns}	−0.00022^{ns}	0.00332^{ns}	0.00181^{ns}		0.00521^{ns}	0.00686^{ns}
MT	0.00163^{ns}	−0.00091^{ns}	0.00491^{ns}	0.00224^{ns}	0.00247^{ns}	0.00232^{ns}	0.00183^{ns}	0.00496^{ns}		0.00256^{ns}
KL	0.00571^{ns}	−0.00039^{ns}	0.00577^{ns}	0.00271^{ns}	0.00116^{ns}	0.00021^{ns}	0.00128^{ns}	0.00692^{ns}	0.00221^{ns}	

Raw microsatellite estimates (below the diagonal) and ENA-corrected estimates (above the diagonal)

ns not significant

* $P < 0.001$ (after Bonferroni correction)

diversity (H_O and H_E) are similar in range to those reported for *A. leucosternon* populations in the Eastern Indian Ocean and its congeners *Acanthurus nigricans* Linnaeus, 1758, *Acanthurus achilles* Shaw, 1803, and *Acanthurus japonicus* Schmidt, 1931 in the Pacific and Indian Oceans (DiBattista et al., 2016). The similarity in contemporary genetic diversity estimates could suggest analogous population dynamics in these relatively young species that have akin morphology, ecology, and biology (DiBattista et al., 2016). However, unlike the Eastern Indian Ocean populations where only two loci (Ahy54 and Ahy203) deviated from the HWE (DiBattista et al., 2016), in the Eastern African populations four loci (Ahy54, Ahy75, Ahy182, and Ahy203) deviated from the HWE.

The ranges of haplotype and nucleotide diversity estimates observed in *A. leucosternon* are comparable to the estimates found in other coral reef fish in the Eastern African region, such as *L. kasmira* (Muths et al., 2012) and *Epinephelus merra* Bloch, 1793 (Muths et al., 2015), which also used the cytochrome *b* marker. On a global scale, these estimates are similar to those obtained for *A. leucosternon* in the Eastern Indian Ocean and other *Acanthurus* species in the Atlantic, Indian, and Pacific Oceans (Rocha et al., 2002; DiBattista et al., 2016). The high haplotype and low nucleotide diversity may suggest an expansion of the Eastern African populations after a bottleneck (Grant & Bowen, 1998), which is consistent with the results of the mismatch distribution of HRI and SSD tests as well as the star-like topology of the haplotype network. Based on the mismatch distribution analysis, this population expansion is estimated to have begun between 143,000 and 287,000 years ago, which corresponds to the mid-Pleistocene. However, *A. leucosternon* does not have a well-calibrated molecular clock, suggesting that these estimates may not accurately reflect the absolute demographic expansion time of this species. Nevertheless, the range of these estimates is useful to indicate the epoch and the period in which the expansion most likely occurred. During the Pleistocene glacial sea level low stands, a large proportion of the continental shelf became emergent, leading to loss of habitats and increased fragmentation within coral reef ecosystems of the Western Indian Ocean and Indo-Pacific (Grant & Bowen, 1998; Voris, 2000; Pellissier et al., 2014). The loss of habitats and increased fragmentation may have led to the extirpation and drastic reduction of the *A. leucosternon*

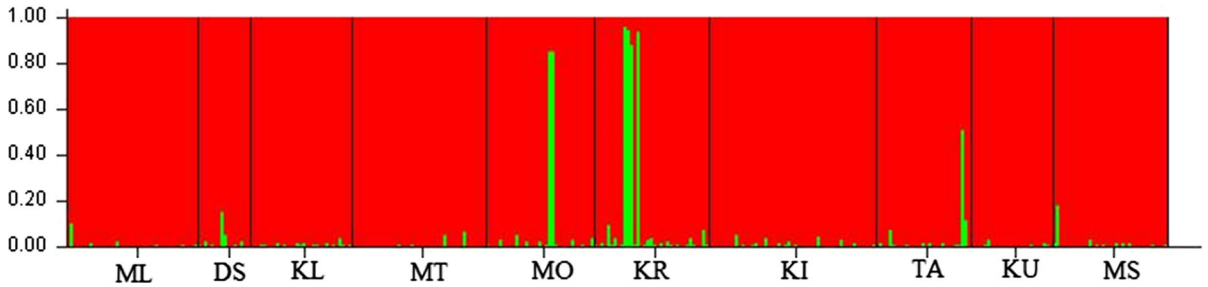

Fig. 3 Structure analysis performed on 10 microsatellite loci with K = 2 for *A. leucosternon* populations. For abbreviations, see Table 1

population in Eastern Africa. Decline in fish population due to a shortage of habitats can occur on very short time scales and has been shown in contemporary reef monitoring studies, which indicate that 62% of fish disappeared within 3 years of reduction of at least 10% coral cover (Wilson et al., 2006). When the sea level subsequently rose, increased suitable coral reef habitats could have enabled the population growth of *A. leucosternon*. Evidence of demographic expansion after a bottleneck has been reported in other surgeonfish species, e.g. *Acanthurus nigrofuscus* Forsskål, 1775 (Eble et al., 2011a), *Zebrasoma flavescens* Bennett, 1828 (Eble et al., 2011b), and *Ctenochaetus strigosus* Bennett, 1828 (Eble et al., 2009). For Eastern Africa, the hypothesis of population expansion after a bottleneck has also been supported in other reef fish such as the blue-barred parrotfish *S. ghobban* (Visram et al., 2010) and the skunk anemonefish *A. akallopisos* (Huyghe & Kochzius, 2016).

Genetic population structure

Microsatellite data showed a low but significant F_{ST} value ($F_{ST} = 0.00252$ $P < 0.001$, $D_{EST} = 0.025$ $P = 0.0018$) across our sampling area, which spans potential biogeographic and oceanographic barriers in the Eastern African region. On the other hand, the mtDNA cytochrome *b* did not show significant structuring. The weak genetic differentiation revealed by microsatellites among *A. leucosternon* Eastern African populations is in agreement with the findings reported for the Eastern Indian Ocean populations, albeit with a lower magnitude of genetic differentiation (Eastern Africa $F_{ST} = 0.00252$: distance of $\sim$1500 KM and Eastern Indian Ocean $F_{ST} =$ 0.0063: distance of $\sim$6000 KM) (DiBattista et al., 2016). However, despite the significant F_{ST} and D_{EST} values, results for pairwise F_{ST}, PCOA, and hierarchical AMOVA failed to identify any genetic break among the eastern African population, supporting the hypothesis of genetic homogeneity. It is likely that a homogeneous genetic pattern in *A. leucosternon* is facilitated by its lengthy post-larval stage (Randall, 2002), which can provide a mechanism for long-distance dispersal. Furthermore, estimates of gene flow in marine fish with a PLD greater than 2 days have shown that over 50% of the variance in spatial genetic patterns can be attributed to the duration of planktonic phase (Kinlan & Gaines, 2003). The finding of weak genetic differentiation broadly matches previous studies on other reef fish such as *L. kasmira* (Muths et al., 2012), *A. akallopisos* (Huyghe & Kochzius, 2016), and *S. ghobban* (Visram et al., 2010). However, contrary to these findings, *Myripristis berndti* Jordan & Evermann, 1903 (Muths et al., 2011) and *E. merra* (Muths et al., 2015) show pronounced genetic structures despite having relatively long PLDs (30–80 days), which suggests that mechanisms that lead to genetic differentiation among reef fish populations are expected to be different between species. On the other hand, the lack of a significant relationship between the genetic and geographic distance (F_{ST} $R^2 = 0.081$, $P = 0.32$ and D_{EST} $R^2 = 0.0006$, $P = 0.42$) confirms that the observed weak genetic differentiation is not attributed to distance-restricted dispersal. The general congruence between mitochondrial DNA and nuclear markers in inferences of genetic homogeneity may suggest that connectivity among Eastern African population of *A. leucosternon* occurred deep in the past and has persisted to contemporary times.

Besides having a lengthy post-larval stage, *A. leucosternon* populations are often found on the outer reef (seaward) habitats of Eastern African lagoons (pers. obs), an ecological characteristic that can enhance genetic homogeneity because the spawned larvae have a high chance of occurring in the path of the permanent flowing EACC, which can facilitate long-distance dispersal along the Eastern African coastline. Long-distance dispersal among offshore Eastern African marine population has been supported by a recent model-based survey, which indicates that these populations exhibit a higher connectivity compared to their counterparts found in sheltered lagoons (Mayorga-Adame et al., 2017). However, recruitment and settlement of these dispersed larvae depend on the availability of suitable habitats as Alberto et al. (2010) showed that genetic distance increased with increasing habitat discontinuity and/or fragmentation. This suggests that the continuous Eastern African fringing reef that runs parallel to the Eastern African coastline from northern Mozambique (KL) to northern Kenya (KU) may also play a critical role in promoting larval exchange among different marine populations, preventing the deleterious outcomes of inbreeding and genetic isolation (Bowler & Benton, 2005; Keyghobadi, 2007). It is thus reasonable to argue that other factors (not only dispersal capacity) might also be responsible for the large-scale connectivity of *A. leucosternon* in Eastern Africa.

Interestingly, the STRUCTURE results of $K = 2$ showed that a few individuals from KR, MO, and TA were genetically distinct from the other populations (Fig. 3). This pattern of differentiation is not consistent with the effect of barriers to dispersal in the Eastern African marine realm and could suggest the occurrence of chaotic genetic patchiness in *A. leucosternon* as a result of pre-settlement selection, post-settlement selection (Johnson & Black, 1984), sweepstake reproduction success (Hogan et al., 2010), variable source of larvae (Selkoe et al., 2006), or kinship aggregations (Selwyn et al., 2016). These phenomena can create an admixture of genetically differentiated individuals within a single local population by facilitating the accumulation of genetically distinct cohorts (Pusack et al., 2014). Alternatively, the few differentiated individuals in these sample sites could be potential hybrids between *A. leucosternon* and *A. nigricans* as these two species are known to hybridise beyond their suture zone (DiBattista et al., 2016). However, no morphological differences were detected among the 336 individuals analysed in this study, suggesting that the potential hybrids will likely be backcrossed offsprings of F1 (without excluding F2 or later generations) hybrids and pure *A. leucosternon* parents. Most morphologically different hybrids were identified as F1 generation, while no morphological difference was observed between backcrossed offsprings and pure parental species (DiBattista et al., 2016).

In conclusion, the genetic homogeneity established among *A. leucosternon* populations separated by more than 1000 km suggests that substantial larval exchange occurs among distant populations. Therefore, it is possible to manage these populations as a single unit, following a trans-boundary approach among the coral reef ecosystem of the four countries (Kenya, Tanzania, Mozambique, and Seychelles). This indicates that networks of marine protected areas (MPAs) are likely to be successful if they are implemented following a regional approach rather than a national approach because highly dispersive species such as *A. leucosternon* might have source and sink populations located in jurisdictions of two different Eastern African countries. The consistency between the findings of our study and a recent larval based modelling study (Mayorga-Adame et al., 2017) underpins the need to consider species life history characteristics (e.g. PLD) in marine species conservation.

The high level of genetic diversity displayed by *A. leucosternon* parallel to other regional studies on coral reef fishes indicates the ability of this species to withstand environmental changes among the sample sites. Nevertheless, the recovery of acanthurids after fisheries closure was remarkably slower compared to other fish families (McClanahan et al., 2007), which underscores the importance of monitoring and assessing artisanal and aquarium fisheries in Eastern Africa, especially with catch composition records from Kenyan reefs showing that *A. leucosternon* contribute up to 16% of all Acanthuridae caught for ornamental export (Okemwa et al., 2016).

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