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ORIGINAL ARTICLE

Incipient genetic isolation of a temperate migratory coastal sciaenid fish (*Argyrosomus inodorus*) within the Benguela Cold Current system

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Abstract
The Benguela Current is considered to be a major biogeographic barrier for tropical and warm-temperate marine fish, but there is limited knowledge regarding its influence on population sub-structuring of in more cold-tolerant species. Employing genetic variation within the mitochondrial DNA Control Region and six cross-specific nuclear microsatellite markers, a preliminary study was conducted to investigate population sub-structuring in *Argyrosomus inodorus*, a highly exploited, cold-temperate migratory species, across the Benguela Current region. Results revealed evidence of incipient genetic differentiation (mtDNA ϕ_{ST} = 0.092; nuclear F_{ST} = 0.036 and D_{ST} = 0.104, $P < 0.05$) between the two sampling sites, suggesting the presence of two regional populations. Estimates of contemporary migration rates between populations were low, and similar in range to those reported in tagging surveys. Although preliminary, these results suggest that the oceanographic features of the Benguela Current may have influenced the evolutionary history of *A. inodorus*, and that the species is likely to be composed of two populations in the Benguela region. As the species is considered overexploited both in Namibia and South Africa, information on the distribution, population dynamics and long-term dispersal patterns across the Benguela Current region would support a comprehensive evaluation of genetic structure, which should be incorporated into fishery management arrangements.

Key words: Argyrosomus inodorus, *Benguela Current, isolation, population structure*

Introduction

The Benguela Cold Current system, located in the south-eastern Atlantic, features cold sea surface temperatures, bounded to the north and south by tropical currents (the Angola and Agulhas Currents, respectively) and a perennial upwelling cell off central Namibia that divides the region into two sub-systems with different characteristics (Shannon 1985; Hutchings et al. 2009). The colder sea surface temperatures of the Benguela Current have been considered an important biogeographic barrier, isolating tropical and warm-temperate fauna of the Atlantic and Indo-Pacific Oceans (Avise 2000; Floeter et al. 2008). However, recent studies revealed that other oceanographic features, such as the perennial upwelling cell, may also play an important role in shaping the population structure of warm-temperate fish populations within the Benguela system, as complete disruption of gene flow was documented both in *Lichia amia* (Linnaeus, 1758) and *Atractoscion aequidens* (Cuvier, 1830) (Henriques et al. 2012, 2014). Little is known, however, regarding the influence of the Benguela system on genetic population connectivity of cold-water-tolerant species.

Argyrosomus inodorus Griffiths & Heemstra, 1995 is a migratory, benthopelagic sciaenid fish, endemic to the south-eastern Atlantic (Griffiths & Heemstra 1995). Its distribution range is restricted to cold-temperate waters (13—16 °C), from the nearshore

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environment to depths of 100 m, between Cape Frio and Meob Bay in Namibia and between Cape Point and East London in South Africa (Griffiths & Heemstra 1995; Griffiths 1997; Kirchner & Holtzhausen 2001). The species distribution overlaps with those of the congeneric *Argyrosomus coronus* Griffiths & Heemstra, 1995, in northern Namibia, and with *Argyrosomus japonicus* (Temminck & Schlegel, 1843) along the southern and eastern Cape coasts of South Africa (Griffiths & Heemstra 1995). *A. japonicus* and *A. coronus*, however, are considered warm-temperate species, occurring preferentially in areas with sea surface temperatures of 21–25 °C (Heemstra & Heemstra 2004) and 16–19 °C (Potts et al. 2010), respectively. As *A. inodorus* is absent from the west coast of South Africa and there is no evidence for significant migration between the two areas of occurrence (Kirchner & Holtzhausen 2001), the species has been managed as two independent stocks. Life history characteristics appear to corroborate the hypothesis of two isolated and locally adapted populations, as features such as maximum size and size at maturity of Namibian and South African *A. inodorus* are significantly different, while no evidence of differentiation was observed within either region (Griffiths 1997; Holtzhausen et al. 2001). *Argyrosomus inodorus* is a critical component of multiple coastal fishery sectors, and exploitation pressure throughout its range has led to the species becoming severely depleted, with spawning stocks estimated to be 69% of unexploited values (Kirchner 1998; DAFF 2012; FAO 2012). To establish sustainable management measures, it is necessary to understand how *A. inodorus* populations are structured across the Benguela Current region and whether migration between the two centres is absent. To date, no comprehensive genetic survey has been carried out for *A. inodorus*, with the exception of a genetic identification study to differentiate *A. inodorus* and *A. coronus*, based on allozymes (van der Bank & Kirchner 1997), and a more recent study on shifts of abundance of these two species in central Namibia (Potts et al. 2014).

The distribution range and life history features of this species suggest that, as observed for warm-temperate species, the oceanographic features of the Benguela Current may influence the population structure and gene flow across the region. The aim of this study was to conduct a preliminary assessment of genetic diversity, population sub-structuring and connectivity between the two putative populations of *A. inodorus* across the Benguela Current, using both mitochondrial DNA (mtDNA) and nuclear microsatellite DNA markers, in order to test whether the regional oceanographic features influence population connectivity in this cold-temperate fish species.

Methods

Sampling

A total of 80 fish were captured by rod-and-line fishing from the shore by local collaborators in two areas: the West Coast Recreational Area in Namibia (HEN, n = 40) and the Eastern Cape Province in South Africa (EastC, n = 40), representing the two centres of abundance of the species (Figure 1). A clip of the pectoral fin was removed immediately after capture and stored in 95% ethanol.

Genetic screening

DNA extraction was performed using a standard phenol:chloroform method (Sambrook et al. 1989). Genetic variation was assessed as DNA sequence polymorphism in a fragment of the mtDNA Control Region (CR) and allele frequencies at six microsatellite loci isolated from *Argyrosomus japonicus* (Archangi et al. 2009). A total of 36 *A. inodorus* individuals were amplified by polymerase chain reaction (PCR) for CR, using the primers and protocols of Appleyard et al. (2002). PCR products were purified with an enzymatic digestion, consisting of 0.5 U of EXO1 (New England Biolabs) and 1 U of shrimp alkaline phosphatase (SAP) in 1× supplied buffer (Fermentas), and sequenced in the forward direction using the same amplification primers by Macrogen Inc. (South Korea). Sequences were visually inspected and a multiple alignment was performed in CLUSTAL X (Thompson et al. 1997), as implemented in BioEdit 7.0.1 (accession numbers: JX191998–2033).

Forty individuals per sampling site were screened at six microsatellite loci (UBA5, UBA40, UBA50, UBA91, UBA853 and UBA854). Optimized PCR mixes included 1× NH_4Cl buffer, 2 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.5 pmol of each primer, 0.2 U of Taq polymerase (Bioline UK) and 50–100 ng of extracted DNA, in a final volume of 10 μL. The Archangi et al. (2009) protocols were modified to ensure accurate amplification: annealing temperatures and number of cycles (UBA91 T_a = 52 °C, remaining loci T_a = 48 °C, with 35 cycles), and removal of the final extension step at 72 °C for 10 min. PCR fragments from multiple loci were combined and genotyped on an AB3500 Genetic Analyzer (Applied Biosystems). Alleles were scored as PCR product size in base pairs, and scores were determined against an internal size marker (LIZ 600), using GeneMapper 4.0 (ABIPrism). In order to ensure accurate allele size scoring between runs,

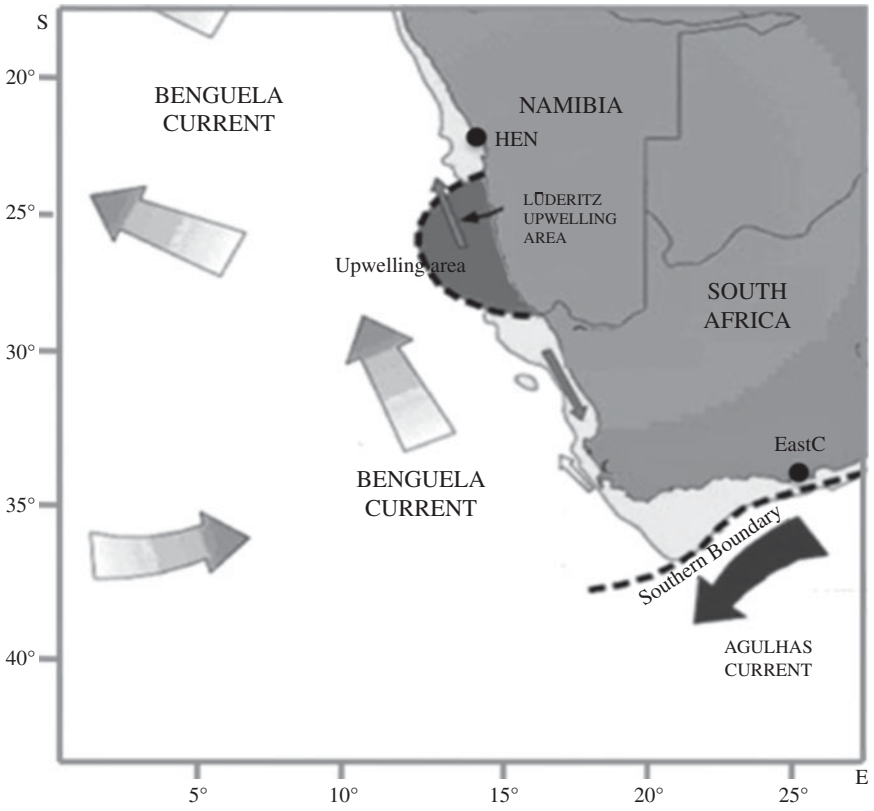

Figure 1. Sampling strategy for *Argyrosomus inodorus* across the Benguela Current region, highlighting sampling sites and their position relative to the major oceanographic features of the system: position of the Benguela and Agulhas Currents, central Namibia upwelling cell, and continental platform width.

individuals with known allele sizes were used in each run as positive controls.

Data analyses

The CR dataset was assessed for levels of haplotype (h) and nucleotide (π) diversity, and fits to neutrality tests: Ewens–Waterson's F, Tajima's D and Fu's Fs, as implemented in ARLEQUIN (Excoffier et al. 2005). Determination of the most suitable nucleotide substitution model was performed in jModelTest (Posada 2008). Preliminary inference of population connectivity of *Argyrosomus inodorus* across the Benguela Current region was estimated as ϕ_{ST} in ARLEQUIN (Excoffier et al. 2005), with a significance level of $P < 0.05$ determined by 10,000 permutations. Haplotype networks were reconstructed to evaluate intraspecific relationships among haplotypes, using the Median-Joining (MJ) algorithm implemented in NETWORK (Bandelt et al. 1999).

Microsatellite genotypic frequencies were tested for deviation from Hardy–Weinberg expectations of random mating and from linkage equilibrium, as implemented in GENEPOP (Raymond & Rousset 1995). The occurrence of amplification errors such as large allele drop-out and stuttering, and estimation of null allele frequencies were assessed in MICROCHECKER (van Oosterhout et al. 2006). Levels of genetic diversity were estimated as number of alleles (Na), allelic richness (AR), number of private alleles (PA), observed and expected heterozygosity (H_O and H_E) and Wright's inbreeding coefficient (F_{IS}), in ARLEQUIN (Excoffier et al. 2005). A preliminary analysis to investigate the statistical power of the dataset for inferring population sub-structuring was conducted in POWSIM (Ryman & Palm 2006). Simulations were conducted for six loci and two populations (n = 40, n = 40), using the estimated allelic frequencies as the baseline for the ancestral population. Runs were performed using multiple combinations of effective population size (N_e) and number of generations (t) to generate a population differentiation of F_{ST} = 0.05, F_{ST} = 0.02 and F_{ST} = 0.01 (N_e = 500–2000; t = 10–51 generations). Each simulation was run for 1000 replicates, and power was estimated as the proportion of tests that indicated significant genetic divergence (Ryman & Palm, 2006). Genetic differentiation was measured as Weir & Cockerham (1984) F_{ST} estimator, as implemented in FreeNA (Chapuis & Estoup 2007), with significance and 95% confidence intervals estimated after jackknifing. For comparative purposes, genetic

differentiation was also measured using Jost's D_{est} estimator, which is independent of the levels of genetic diversity, in SMOGD (Crawford 2010). Contemporary estimates of long-term average migration rates between the two sampling sites were performed for the microsatellite dataset using two complementary approaches: the classical method based on F_{ST} values ($F_{ST} = 1/(4N_{em} + 1)$) (Excoffier et al. 2005), and by employing the coalescent-based approach of MIGRATE (Beerli 2009). In MIGRATE, the Bayesian approach was implemented, enforcing a full migration model, with three replicates run for each dataset (Beerli 2009). Each analysis was performed with four connected chains, using static heating (1,000,000, 3, 1.5, 1), a burn-in period of 10,000 steps, followed by 90,000 steps, and parameters were recorded every 100 steps. Estimates of migration rates (m) were obtained from M ($M = m.\mu$) and θ ($\theta = 4N_e\mu$) (Beerli 2009). In order to obtain estimates of migration rates per generation (and not scaled by mutation) three general mutation rates were used: 0.1%, 0.5% and 1% per generation (Ellegren 2000).

Results

Population structure and phylogeography

Sequencing of mtDNA CR yielded a fragment of 704 base pairs (bp). The 36 individuals screened displayed 32 haplotypes defined by 34 variable nucleotide sites, of which 16 sites were parsimony informative. The Tamura–Nei nucleotide substitution model was identified as the most suitable for the mtDNA dataset. Haplotype diversity was high ($h = 0.991$), whilst nucleotide diversity was low ($\pi = 0.006$), with Namibian samples exhibiting higher values than the South African samples (Table I). Deviations from the assumptions of selection neutrality were observed in Fu's *Fs* for both populations, but not with either Ewens–Waterson's *F* or Tajima's *D* tests (Table I). As Fu's *Fs* is known to be sensitive to abrupt demographic changes, it is likely that the observed deviation from neutrality resulted from past population size changes rather than reflecting selection effects. Genetic differentiation (ϕ_{ST}) between samples was statistically significant ($\phi_{ST} = 0.092$, $P < 0.05$), although haplotype relationships did not show an obvious geographic pattern (Figure 2): most individuals were represented by unique haplotypes with no obvious clustering of related haplotypes into Namibian or South African groups (Figure 2). The majority of haplotypes were closely related, differing from one another by one to two mutation steps, with the exception of two HEN individuals that were divergent by 10 mutation steps (Figure 2).

Table I. Estimates of mitochondrial genetic diversity levels and neutrality tests for *A. inodorus* CR. *n*, number of individuals; *H*, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity; *F*, Ewens–Waterson neutrality test; *D*, Tajima neutrality test; *Fs*, Fu neutrality test.

	HEN	EastC	Overall
n	18	18	36
H	18	14	32
h	1.000	0.968	0.991
π	0.008	0.004	0.006
F	–	0.862	0.966
D	−1.486	0.324	−1.554
Fs	**−14.762**	**−10.099**	**−25.652**

Note: Significant departures from expectations ($P < 0.05$) in bold.

None of the six microsatellite loci exhibited evidence of amplification errors, and all displayed genotype frequencies that conformed to Hardy–Weinberg and linkage equilibrium expectations (Table II). Levels of genetic diversity in terms of heterozygosity and allelic richness were high (overall values of $H_E = 0.774$ and $AR = 13.7$), with both samples displaying very similar values at individual loci and overall (Table II). The number of private alleles varied between one and seven, per locus and region (Table II). Analyses of the statistical power of the dataset revealed that the six loci and sample sizes used in this study could statistically detect genetic differentiation as low as $F_{ST} = 0.01$ in 99% of tests. As with the mtDNA data, nuclear genetic differentiation between the Namibian and South African samples was significantly greater than zero ($F_{ST} = 0.036$, $P < 0.05$), with Jost's D_{est} indicating a slightly higher level of differentiation ($D_{est} = 0.104$, $P < 0.05$). Estimates of contemporary migration rates

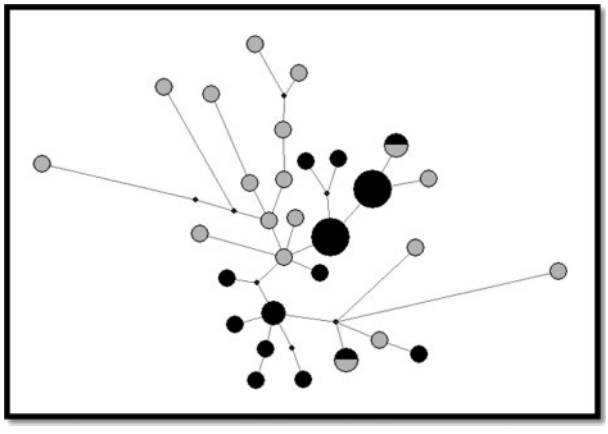

Figure 2. Haplotype network for *Argyrosomus inodorus* across the Benguela Current region, based on 704 bp of mtDNA CR sequences: ◯, HEN; ●, EastC. Branch lengths are proportional to number of nucleotide differences, and node sizes are proportional to the number of individuals. Smaller dots represent unsampled inferred haplotypes.

Table II. Genetic diversity in *A. inodorus* samples at six cross-specific microsatellite loci. *n*, number of individuals genotyped; *NA*, number of alleles; *AR*, allelic richness; *PA*, number of private alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, inbreeding coefficient.

		HEN	EastC	Overall
UBA5	*n*	40	40	80
	NA	11	11	13
	AR	10.803	10.925	10.52
	PA	1	2	3
	H_E	0.819	0.825	0.839
	H_O	0.875	0.825	0.850
	F_{IS}	−0.047	0.003	−0.007
UBA40	*n*	40	39	79
	NA	8	7	8
	AR	7.951	7.000	7.452
	PA	1	0	1
	H_E	0.765	0.807	0.790
	H_O	0.750	0.795	0.772
	F_{IS}	0.038	0.037	0.028
UBA50	*N*	39	40	79
	NA	14	15	16
	AR	13.974	14.899	14.846
	PA	1	2	3
	H_E	0.887	0.896	0.914
	H_O	0.821	0.800	0.810
	F_{IS}	0.066	0.038	0.120
UBA91	*n*	40	40	80
	NA	5	3	5
	AR	4.902	3.000	3.962
	PA	1	1	2
	H_E	0.361	0.387	0.375
	H_O	0.275	0.475	0.375
	F_{IS}	0.182	−0.207	0.006
UBA853	*n*	40	40	80
	NA	13	14	17
	AR	12.799	12.924	14.530
	PA	3	4	7
	H_E	0.831	0.872	0.876
	H_O	0.925	0.900	0.913
	F_{IS}	−0.100	−0.036	−0.035
UBA854	*n*	40	40	80
	NA	9	7	19
	AR	7.604	11.899	15.152
	PA	7	1	8
	H_E	0.881	0.776	0.860
	H_O	0.975	0.675	0.825
	F_{IS}	−0.038	0.130	0.047
Average all loci	*n*	40	40	80
	NA	10	9.500	11.333
	AR	9.004	9.833	13.667
	PA	14	10	24
	H_E	0.757	0.760	0.776
	H_O	0.770	0.745	0.758
	F_{IS}	0.000	0.012	0.027

Note: No significant deviations from Hardy-Weinberg expectations ($P < 0.05$) were found.

per generation between the two geographic populations were low, independent of the method used, or mutation rate considered (F_{ST}-based: $N_{em} = 6$; MIGRATE: $m_{2\to1} = 0.0014$; $m_{1\to2} = 0.0011$ for $\mu = 0.1\%$ per generation).

Discussion

Despite the preliminary nature of the present study due to the limited number of sampling sites available, sample sizes and microsatellite markers used, similarly high levels of genetic diversity and evidence for shallow, but significant, genetic differentiation between the two regional populations (Namibia and South Africa) of *Argyrosomus inodorus* were found. The observed mitochondrial and nuclear genetic diversity ($h = 0.991$, $\pi = 0.006$; $H_O = 0.771$, $H_E = 0.764$) were comparable with other commercially exploited fish species occurring in the Benguela Current region, such as *Argyrosomus japonicus* ($h = 0.96$, $\pi = 0.009$; Klopper 2005), *Lichia amia* ($h = 0.991$, $\pi = 0.006$; Henriques et al. 2012), *Atractoscion aequidens* ($h = 0.853$, $\pi = 0.005$; $H_E = 0.889$; Henriques et al. 2014) and *Rhabdosargus holubi* (Steindachner, 1881) ($h = 0.91$, $\pi = 0.006$; Oosthuizen 2007). High genetic diversity and shallow population structure are common features of marine teleosts, even in abundant, commercially exploited species. These are thought to result from historically high effective population sizes and/or high levels of gene flow between adjacent populations (Waples 1998). Interestingly, the observed genetic divergence between the Namibian and South African *A. inodorus* populations (mtDNA $\phi_{ST} = 0.092$; nuclear $F_{ST} = 0.036$ and $D_{est} = 0.104$, $P < 0.05$) was higher than that reported for other migratory sciaenids such as *Micropogonias undulatus* (Linnaeus, 1766) ($\phi_{ST} = 0.046$; Lankford et al. 1999) and *Sciaenops ocellatus* (Linnaeus, 1766) ($\phi_{ST} = 0.057$; Gold & Richardson 1998), but substantially lower than observed for other fish species with similarly disjunct distributions across the Benguela Current region (*L. amia*, $\phi_{ST} = 0.9$; Henriques et al. 2012; *A.aequidens*, $\phi_{ST} = 0.902$, $F_{ST} = 0.055$; Henriques et al. 2014). These results, combined with estimates of the number of contemporary migrants ($N_{em} = 0.0014$–6 per generation, depending on the method used), suggest a limited level of gene flow between Namibian and South African *A. inodorus* populations and support the presence of incipient population differentiation. The present findings concur with tagging studies conducted for the species, where only two of 17,353 *A. inodorus* tagged in Namibia were recaptured in South Africa, suggesting that connectivity between populations may be limited (Kirchner & Holtzhausen 2001). Therefore, the low but significant genetic differentiation displayed by *A. inodorus* is likely to result from a present-day disjunct population distribution, with occasional migrants, and a historically large effective population size, rather than from substantial gene flow between Namibia and South Africa.

As with other fish species distributed around southwestern Africa (e.g. *L. amia*, Henriques et al. 2012; *A. aequidens*, Henriques et al. 2014; *Albula* spp., Colborn et al. 2001), the distribution break in *A. inodorus* appears to correspond with the areas of cold water upwelling off southern Namibia and the west coast of South Africa (Griffiths & Heemstra 1995; Griffiths 1997; Kirchner & Holtzhausen 2001). Although the limited sampling precludes the drawing of definitive conclusions, the reported genetic divergence and breakdown of gene flow across the Benguela Current suggest that the oceanographic features of the system, namely the cold-water region, may be contributing to disrupt both adult and larval dispersal of *A. inodorus*, and support the hypothesis of two isolated populations with limited migration between them. As the species is considered overexploited both in Namibia and South Africa, information on the distribution, population dynamics and long-term dispersal patterns across the Benguela Current region would inform a comprehensive evaluation of genetic structure, which should be incorporated into fishery management arrangements.

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