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

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1 **Title:** Incipient genetic isolation of a temperate migratory coastal Sciaenid fish (*A.*
2 *inodorus*) within the Benguela Cold Current system

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23 **Abstract**

24 The Benguela Current is considered to be a major biogeographical barrier for tropical
25 and warm-temperate marine fish, but there is limited knowledge regarding its influence
26 on population sub-structuring of cold-tolerant species. Employing genetic variation
27 within the mitochondrial DNA Control Region and six cross-specific nuclear
28 microsatellite markers, a preliminary study was conducted to investigate population
29 substructuring in *Argyrosomus inodorus*, a highly exploited, cool-temperate migratory
30 species, across the Benguela Current region. Results revealed evidence of incipient
31 genetic differentiation (mtDNA $\phi_{ST} = 0.092$; nuclear $F_{ST} = 0.036$ and $D_{ST} = 0.104$,
32 $P < 0.005$) between the two sampling sites, suggesting the presence of two regional
33 populations. Estimates of contemporary migration rates between populations were low,
34 and similar in range to those reported in tagging surveys. Although preliminary, these
35 results suggest that the oceanographic features of the Benguela Current may have
36 influenced the evolutionary history of *A. inodorus*, and that the species is likely to be
37 composed of two populations in the Benguela region. As the species is considered
38 overexploited both in Namibia and South Africa, information on the distribution,
39 population dynamics and long-term dispersal patterns across the Benguela Current
40 region would provide a comprehensive evaluation of genetic structure, which should be
41 incorporated into fishery management arrangements. .

42

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

44

45 **Introduction**

46 The Benguela Cold Current system, located in the southern Atlantic, features cold sea
47 surface temperatures bounded to the north and south by tropical currents (the Angola
48 and Agulhas currents, respectively), and a perennial upwelling cell off central Namibia
49 that divides the region into two sub-systems with different characteristics (Shannon
50 1985; Hutchings et al. 2009). The colder sea surface temperatures of the Benguela
51 Current have been considered an important bio-geographical barrier, isolating tropical
52 and warm-temperate fauna of the Atlantic and Indo-Pacific Oceans (Avisé 2000; Floeter
53 et al. 2008). However, recent studies revealed that other oceanographic features such as
54 the perennial upwelling cell may also play an important role in shaping the population
55 structure of warm-temperate fish populations within the Benguela system, as complete
56 disruption of gene flow was documented both in *Lichia amia* and *Atractoscion*
57 *aequidens* (Henriques et al. 2012; 2014). Little is known, however, regarding the
58 influence of the Benguela system on genetic population connectivity of cold water
59 tolerant species.

60 *Argyrosomus inodorus* is a migratory, benthopelagic sciaenid fish, endemic to the
61 southeastern Atlantic (Griffiths & Heemstra 1995). Distribution range is restricted to
62 cold-temperate waters (13°C-16°C), from the nearshore environment to depths of 100m,
63 between Cape Frio and Meob Bay in Namibia, and between Cape Point and East
64 London in South Africa (Griffiths & Heemstra 1995; Griffiths 1997; Kirchner &
65 Holtzhausen 2001). The species distribution overlaps with those of the congeneric *A.*
66 *coronus* in northern Namibia, and with *A. japonicus* along the southern and Eastern
67 Cape coasts of South Africa (Griffiths & Heemstra 1995). Contrary to *A. inodorus*, *A.*
68 *coronus* and *A. japonicus* are considered warm-temperate species, occurring
69 preferentially in sea surface temperatures of 16°-19°C (Potts et al 2010) and 21°-25°C
70 (Heemstra & Heemstra 2004), respectively. As *A. inodorus* is absent from the west
71 coast of South Africa and there is no evidence for significant migration between the two
72 areas of occurrence (Kirchner & Holtzhausen 2001), the species has been managed as
73 two independent stocks. Life history characteristics appear to corroborate the hypothesis
74 of two isolated and locally adapted populations, as features such as maximum size and
75 size at maturity of Namibian and South African *A. inodorus* are significantly different,
76 although no evidence of differentiation was observed within either region (Griffiths
77 1997; Holtzhausen et al. 2001). *A. inodorus* is a critical component of multiple coastal

78 fishery sectors, and exploitation pressure throughout its distribution has led to the
79 species becoming severely depleted, with spawning stocks estimated to be 69% of
80 unexploited values (FAO 2012). In Namibia, *A. inodorus* is harvested by the
81 commercial and recreational fishery sectors and although approximately the same
82 numbers of fish are captured in each sector, the average size captured in the commercial
83 sector is larger (Kirchner 1998). While *A. inodorus* is only regulated through an input
84 control (number of permits) the recreational fishery catch, which comprises 70% of the
85 total recreational catch, is regulated through bag- and size-limits (Kirchner & Beyer,
86 1999; Holtzhausen et al. 2001). In South Africa, a 2012 survey reported that total
87 landings of *A. inodorus* exceeded 400t per year (DAFF 2012). To establish sustainable
88 management measures, it is necessary to understand how *A. inodorus* populations are
89 structured across the Benguela Current region and whether migration between the two
90 centers is absent. To date, no comprehensive genetic survey has been carried out in *A.*
91 *inodorus*, with the exception of a genetic identification study in 1997 to differentiate
92 between *A. inodorus* and *A. coronus*, based on allozymes (van der Bank & Kirchner
93 1997), and a more recent study on shifts of abundance of these two species in central
94 Namibia (Potts et al. 2014).

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

103

104 **Methods**

105 **Sampling**

106 A total of 80 fish were captured by rod-and-line fishing from the shore, by local
107 collaborators in two areas: the West Coast Recreational Area in Namibia ($n = 40$) and
108 the Eastern Cape Province in South Africa ($n = 40$), representing the two centres of

109 abundance of the species (Figure 1). A clip of the pectoral fin was removed immediately
110 after capture and stored in 95% ethanol.

111

112 Genetic screening

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

125 Forty individuals per sampling site were screened at six microsatellite loci (UBA5,
126 UBA40, UBA50, UBA91, UBA853 and UBA854). Optimized PCR mixes included 1x
127 NH₄Cl buffer, 2mM of MgCl₂, 0.2mM of dNTPs, 0.5pmol of each primer, 0.2U of Taq
128 polymerase (Bioline UK) and 50-100ng of extracted DNA, in a final volume of 10 µl.
129 The Archangi et al. (2009) protocols were modified to ensure accurate amplification:
130 annealing temperatures and number of cycles (UBA91 T_a = 52°C, remaining loci T_a =
131 48°C, with 35 cycles), and removal of the final extension step of 72°C for 10min. PCR
132 fragments from multiple loci were combined and genotyped on an AB3500 Genetic
133 Analyzer (Applied Biosystems). Alleles were scored as PCR product size in base pairs,
134 and scores were determined against an internal size marker (LIZ 600), using
135 GeneMapper 4.0 (ABIPrism). In order to ensure accurate allele size scoring between
136 runs, individuals with known allele sizes were used in each run as positive controls.

137

138 Data analyses

139 The CR dataset was assessed for levels of haplotype (h) and nucleotide (π) diversity,
140 and fits to neutrality tests: Ewens-Watson's F , Tajima's D and Fu's FS , as
141 implemented in ARLEQUIN (Excoffier et al. 2005). Determination of the most suitable
142 nucleotide substitution model was performed in jModelTest (Posada, 2008). Preliminary
143 inference of population connectivity of *A. inodorus* across the Benguela Current region
144 was estimated as ϕ_{ST} in ARLEQUIN (Excoffier et al. 2005), with a significance level of
145 $P < 0.05$ determined by 10,000 permutations. Haplotype networks were reconstructed to
146 evaluate intraspecific relationships among haplotypes, using the Median-Joining (MJ)
147 algorithm implemented in NETWORK (Bandelt et al. 1999).

148 Microsatellite genotypic frequencies were tested for deviation from Hardy-Weinberg
149 expectations of random mating and from linkage equilibrium, as implemented in
150 GENEPOP (Raymond & Rousset 1995). The occurrence of amplification errors such as
151 large allele drop out and stuttering, and estimation of null allele frequencies were
152 assessed in MICROCHECKER (van Oosterhout et al. 2006). Levels of genetic diversity
153 were estimated as number of alleles (Na), allelic richness (AR), observed and expected
154 heterozygosity (H_O and H_E), and Wright's inbreeding coefficient (F_{IS}), in ARLEQUIN
155 (Excoffier et al. 2005). A preliminary analysis to investigate the statistical power of the
156 dataset to infer population substructuring was conducted in POWSIM (Ryman & Palm
157 2006). Simulations were conducted for six loci and two populations ($n = 40$, $n = 40$),
158 using the estimated allelic frequencies as the baseline for the ancestral population. Runs
159 were performed using multiple combinations of effective population size (N_e) and
160 number of generations (t) to generate a population differentiation of $F_{ST} = 0.05$, $F_{ST} =$
161 0.02 and $F_{ST} = 0.01$. Each simulation was run for 1,000 replicates, and power was
162 estimated as the proportion of tests that indicated significant genetic divergence (Ryman
163 & Palm, 2006). Genetic differentiation was measured as Weir & Cockerham (1984) F_{ST}
164 estimator, as implemented in FreeNA (Chapuis & Estoup 2007), with significance and
165 95% confidence intervals estimated after jackknifing. For comparison purposes, genetic
166 differentiation was also measure using Jost's D_{est} estimator, which is independent of the
167 levels of genetic diversity, in SMOGD (Crawford 2010). Contemporary estimates of
168 long-term average migration rates between the two sampling sites were performed for
169 the microsatellite dataset using two complementary approaches: the classical method
170 based on F_{ST} values ($F_{ST} = 1/(4N_{em} + 1)$) (Excoffier et al. 2005), and by employing the
171 coalescent-based approach of MIGRATE (Beerli 2009). In MIGRATE, the Bayesian
172 approach was implemented, enforcing a full migration model, with three replicates run

173 for each dataset (Beerli 2009). Each analysis was performed with four connected chains,
174 using static heating (1,000,000, 3, 1.5, 1), a burn-in period of 10,000 steps, followed by
175 90,000 steps, and parameters were recorded every 100 steps. Estimates of migration
176 rates (m) were obtained from M ($M = m \cdot \mu$) and Θ ($\Theta = 4N_e\mu$) (Beerli, 2009). In order to
177 obtain estimates of migration rates per generation (and not scaled by mutation) three
178 general mutation rates were used: 0.1%, 0.5% and 1% per generation.

179

180 **Results**

181 Population structure and phylogeography

182 Sequencing of mtDNA CR yielded a fragment of 704 base pairs (bp). The 36
183 individuals screened displayed 32 haplotypes defined by 34 variable nucleotide sites, of
184 which 16 sites were parsimony informative. The Tamura-Nei nucleotide substitution
185 model was identified as the most suitable for the mtDNA dataset. Haplotype diversity
186 was high ($h = 0.991$), whilst nucleotide diversity was low ($\pi = 0.006$), with Namibian
187 samples exhibiting higher values than the South African samples (Table 1). Deviations
188 from the assumptions of selection neutrality were observed in Fu's FS for both
189 populations, but not with either Ewens-Watsonson F or the Tajima's D tests (Table 1). As
190 Fu's FS is known to be sensitive to abrupt demographic changes it is likely that the
191 observed deviation to neutrality resulted from past population size changes, rather than
192 reflecting selection effects. Genetic differentiation (ϕ_{ST}) between samples was low but
193 statistically significant ($\phi_{ST} = 0.092$, $P < 0.05$), although haplotype relationships did not
194 show an obvious geographical pattern (Figure 2): most individuals were represented by
195 unique haplotypes with no obvious clustering of related haplotypes into Namibian or
196 South African groups (Figure 2). The majority of haplotypes were closely related,
197 differing from one another by one to two mutation steps, with the exception of two
198 HEN individuals that were divergent by 10 mutation steps (Figure 2).

199 None of the six microsatellite loci exhibited evidence of amplification errors, and all
200 displayed genotype frequencies that confirmed with Hardy-Weinberg and linkage
201 equilibrium expectations (Table 2). Levels of genetic diversity in terms of
202 heterozygosity and allelic richness were high (overall values of $H_E = 0.774$ and $AR =$
203 13.7), with both samples displaying very similar values at individual loci and overall

204 (Table 2). Number of private alleles varied between 1 and 7, per locus and region (Table
205 2). Analyses of statistical power of the dataset revealed that the loci and sample sizes
206 used in this study could statistically detect genetic differentiation as low as $F_{ST} = 0.001$
207 in 99% of tests. As for the mtDNA data, nuclear genetic differentiation between the
208 Namibian and South African samples was significantly greater than zero ($F_{ST} = 0.036$,
209 $P < 0.05$), with Jost's D_{est} indicating a slightly higher level of differentiation ($D_{est} =$
210 0.104 , $P < 0.05$). Estimates of contemporary migration rates per generation between the
211 two geographical populations were low, independently of the method used or mutation
212 rate considered (F_{ST} -based: $N_{em} = 6$; MIGRATE: $m_{2 \rightarrow 1} = 0.0014$; $m_{1 \rightarrow 2} = 0.0011$ for $\mu =$
213 0.1% per generation).

214

215 **Discussion**

216 Despite the preliminary nature of the present study, due to the limited number of
217 sampling sites available, similarly high levels of genetic diversity and evidence for
218 shallow but significant genetic differentiation between the two regional populations
219 (Namibia and South Africa) of *Argyrosomus inodorus* was found. The observed
220 mitochondrial and nuclear genetic diversity ($h = 0.991$, $\pi = 0.006$; $H_O = 0.771$, $H_E =$
221 0.764) were comparable with other commercially exploited fish species occurring in the
222 Benguela Current region, such as *Argyrosomus japonicus* ($h = 0.96$, $\pi = 0.009$ –
223 Klopper 2005), *Lichia amia* ($h = 0.991$, $\pi = 0.006$ – Henriques et al. 2012), *Atractoscion*
224 *aequidens* ($h = 0.853$, $\pi = 0.005$; $H_E = 0.889$ – Henriques et al. 2014) and
225 *Rhabdosargus holubii* ($h = 0.91$, $\pi = 0.006$ – Oosthuizen 2007). High genetic diversity
226 and shallow population structure are common features of marine teleosts, even in
227 abundant, commercially exploited species. These are thought to result from historically
228 high effective population sizes and/or high levels of gene flow between adjacent
229 populations (Waples 1998). Interestingly, the observed genetic divergence between the
230 Namibian and South African *A. inodorus* populations (mtDNA $\phi_{ST} = 0.092$; nuclear F_{ST}
231 $= 0.036$ and $D_{est} = 0.104$, $P < 0.05$) was higher than that reported for other migratory
232 sciaenids such as *Micropogonias undulatus* ($\phi_{ST} = 0.046$ – Lankford et al. 1999) and
233 *Sciaenops ocellatus* ($\phi_{ST} = 0.057$ – Gold & Richardson 1998), but substantially lower
234 than observed for other fish species with similarly disjunct distributions across the
235 Benguela Current region (*L. amia*, $\phi_{ST} = 0.9$ – Henriques et al. 2012; *A. aequidens*, $\phi_{ST} =$

236 0.902, $F_{ST} = 0.055$ – Henriques et al. 2014). These results, combined with estimates of
237 the number of contemporary migrants ($N_{em} = 0.0014 - 6$ per generation, depending on
238 the method used), suggest a limited level of gene flow between Namibian and South
239 African *A. inodorus* populations, and support the presence of incipient population
240 differentiation. The present findings concur with tagging studies conducted for the
241 species, where only two of 17,353 *A. inodorus* tagged in Namibia were recaptured in
242 South Africa, suggesting that connectivity between populations may be limited
243 (Kirchner & Holzhausen 2001). Therefore, the low but significant genetic
244 differentiation displayed by *A. inodorus* is likely to result from a present-day disjunct
245 population distribution, with occasional migrants, and historically high levels of
246 effective population size, rather than substantial gene flow between Namibia and South
247 Africa.

248 As with other fish species distributed around southwestern Africa (e.g. *L. amia* –
249 Henriques et al. 2012; *A. aequidens* – Henriques 2012; *Albula* spp. – Colborn et al.
250 2001), the distribution break in *A. inodorus* appears to correspond with the areas of cold
251 water upwelling off southern Namibia and the west coast of South Africa (Griffiths &
252 Heemstra, 1995; Griffiths 1997; Kirchner & Holtzhausen 2001). Although the limited
253 sampling precludes the drawing of definitive conclusions, the reported genetic
254 divergence and breakdown of gene flow across the Benguela Current suggests that the
255 oceanographic features of the system, namely the cold water region, may be
256 contributing to disrupt both adult and larval dispersal of *A. inodorus*, and supports the
257 hypothesis of two isolated populations with limited migration between them. As the
258 species is considered overexploited both in Namibia and South Africa, information on
259 the distribution, population dynamics and long-term dispersal patterns across the
260 Benguela Current region would provide a comprehensive evaluation of genetic
261 structure, which should be incorporated into fishery management arrangements.

262

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269

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376

377 **Tables**

378

379 **Table 1:** Estimates of mitochondrial genetic diversity levels and neutrality tests for *A.*
380 *inodorus* CR: n – number of individuals; H – number of haplotypes; h – haplotype
381 diversity; π - nucleotide diversity; F – Ewens-Waterson neutrality test; D – Tajima
382 neutrality test; FS – Fu neutrality test. Significant departures from expectations ($P <$
383 0.05) in bold.

	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

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387 **Table 2:** Genetic diversity in *A. inodorus* samples at six cross-specific microsatellite
 388 loci: n – number of individuals genotyped; NA – number of alleles; AR – allelic
 389 richness; PA – number of private alleles H_E – expected heterozygosity; H_O – observed
 390 heterozygosity; F_{IS} – inbreeding coefficient. Significant deviations to Hardy-Weinberg
 391 expectations in bold.

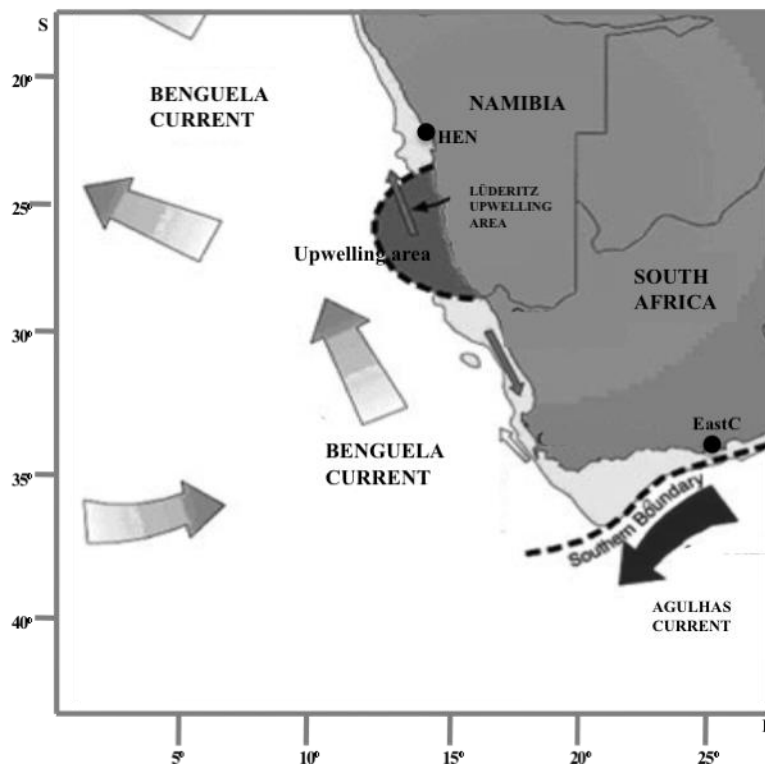
	HEN	EastC	Overall
	40	40	80
	11	11	13
	10.803	10.925	10.52
UBA5	1	2	3
	0.819	0.825	0.839
	0.875	0.825	0.850
	-0.047	0.003	-0.007
	40	39	79
	8	7	8
	7.951	7.000	7.452
UBA40	1	0	1
	0.765	0.807	0.790
	0.750	0.795	0.772
	0.038	0.037	0.028
	39	40	79
	14	15	16
	13.974	14.899	14.846
UBA50	1	2	3
	0.887	0.896	0.914
	0.821	0.800	0.810
	0.066	0.038	0.120

	<i>n</i>	40	40	80
	<i>NA</i>	5	3	5
	<i>AR</i>	4.902	3.000	3.962
UBA91	<i>PA</i>	1	1	2
	<i>H_E</i>	0.361	0.387	0.375
	<i>H_O</i>	0.275	0.475	0.375
	<i>F_{IS}</i>	0.182	-0.207	0.006
	<i>n</i>	40	40	80
	<i>NA</i>	13	14	17
	<i>AR</i>	12.799	12.924	14.530
UBA853	<i>PA</i>	3	4	7
	<i>H_E</i>	0.831	0.872	0.876
	<i>H_O</i>	0.925	0.900	0.913
	<i>F_{IS}</i>	-0.100	-0.036	-0.035
	<i>n</i>	40	40	80
	<i>NA</i>	9	7	19
	<i>AR</i>	7.604	11.899	15.152
UBA854	<i>PA</i>	7	1	8
	<i>H_E</i>	0.881	0.776	0.860
	<i>H_O</i>	0.975	0.675	0.825
	<i>F_{IS}</i>	-0.038	0.130	0.047
	<i>n</i>	40	40	80
	<i>NA</i>	10	9.500	11.333
	<i>AR</i>	9.004	9.833	13.667
Average all loci	<i>PA</i>	14	10	24
	<i>H_E</i>	0.757	0.760	0.776
	<i>H_O</i>	0.770	0.745	0.758
	<i>F_{IS}</i>	0.000	0.012	0.027

393 **Figure Legends**

394

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



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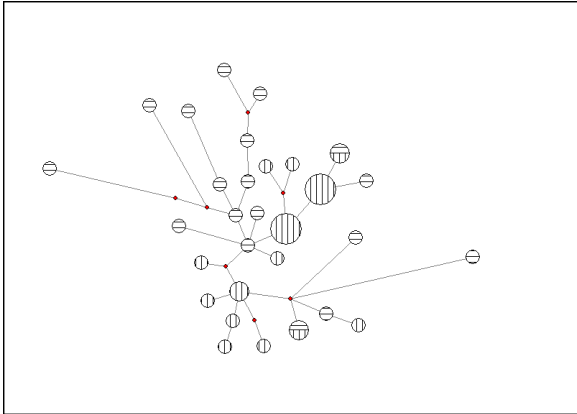
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408 **Figure 2:** Haplotype network for *A. inodorus* across the Benguela Current region, based
409 on 704bp of mtDNA CR sequences: \ominus = HEN; \oplus = EastC. Branch lengths are
410 proportional to number of nucleotide differences, and node sizes are proportional to the
411 number of individuals. Red dots represent unsampled inferred haplotypes.



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