Shared genomic outliers across two divergent population clusters of a highly threatened seagrass

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The seagrass, *Zostera capensis*, occurs across a broad stretch of coastline and wide environmental gradients in estuaries and sheltered bays in southern and eastern Africa. Throughout its distribution, habitats are highly threatened and poorly protected, increasing the urgency of assessing the genomic variability of this keystone species. A pooled genomic approach was employed to obtain SNP data and examine neutral genomic variation and to identify potential outlier loci to assess differentiation across 12 populations across the ~9600km distribution of *Z. capensis*. Results indicate high clonality and low genomic diversity within meadows, which combined with poor protection throughout its range, increases the vulnerability of this seagrass to further declines or local extinction. Shared variation at outlier loci potentially indicates local adaptation to temperature and precipitation gradients, with Isolation-by-Environment significantly contributing towards shaping spatial variation in *Z. capensis*. Our results indicate the presence of two population clusters, broadly corresponding to populations on the west and east coasts, with the two lineages shaped only by frequency differences of outlier loci. Notably, ensemble modelling of suitable seagrass habitat provides evidence that the clusters are linked to historical climate refugia around the Last Glacial Maxi-mum. Our work suggests a complex evolutionary history of *Z. capensis* in southern and eastern Africa that will require more effective protection in order to safeguard this important ecosystem engineer into the future.

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16 **Abstract**
17 The seagrass, *Zostera capensis*, occurs across a broad stretch of coastline and wide
18 environmental gradients in estuaries and sheltered bays in southern and eastern Africa.
19 Throughout its distribution, habitats are highly threatened and poorly protected,
20 increasing the urgency of assessing the genomic variability of this keystone species. A
21 pooled genomic approach was employed to obtain SNP data and examine neutral genomic
22 variation and to identify potential outlier loci to assess differentiation across 12
23 populations across the ~9600km distribution of *Z. capensis*. Results indicate high clonality
24 and low genomic diversity within meadows, which combined with poor protection
25 throughout is range, increases the vulnerability of this seagrass to further declines or local
26 extinction. Shared variation at outlier loci potentially indicates local adaptation to
27 temperature and precipitation gradients, with Isolation-by-Environment significantly
28 contributing towards shaping spatial variation in *Z. capensis*. Our results indicate the
29 presence of two population clusters, broadly corresponding to populations on the west and
30 east coasts, with the two lineages shaped only by frequency differences of outlier loci.
31 Notably, ensemble modelling of suitable seagrass habitat provides evidence that the
32 clusters are linked to historical climate refugia around the Last Glacial Maximum. Our work
33 suggests a complex evolutionary history of *Z. capensis* in southern and eastern Africa that

34 will require more effective protection in order to safeguard this important ecosystem
35 engineer into the future.

36

Introduction

38 Despite potentially high levels of gene flow, signals of local adaptation to environmental
39 factors such as salinity and temperature gradients, have been described for a diverse set of
40 marine species (Guo et al. 2015, 2016; Dalongeville et al. 2018; Nielsen et al. 2018), and to
41 osmotic niches in freshwater species (Dennenmoser et al. 2016; Attard et al. 2018; Lucek et
42 al. 2018). These and other studies suggest that contemporary spatial patterns of outlier loci
43 significantly contribute towards shaping genetically structured populations (Savolainen et
44 al. 2013; Yeaman 2013; Huang et al. 2014; Tigano & Friesen 2016; Barth et al. 2017; Cure et
45 al. 2017; Marques 2017), although their relevance to local adaptation is often unknown.
46 While standing genomic variation is the material on which selection can act, adaptive
47 variation is expected to increase evolutionary resilience by improving the ability to persist
48 through and adapt to changing environmental conditions (Bible & Sanford 2016). However,
49 in addition to present-day environmental conditions, historical processes should also be
50 considered, as they often play an important role in shaping contemporary patterns of
51 genomic diversity and differentiation (Hewitt 2000; Gaither et al. 2011; Toms et al. 2014;
52 Leprieur et al. 2016; Chefaoui et al. 2017; Hernawan et al. 2017), that could impact the
53 distribution and maintenance of contemporary patterns of neutral and potentially adaptive
54 variation. If the latter is linked to gene regions of known function, this may signal some
55 adaptive importance (Angeloni et al. 2012) and can better our understanding of the
56 mechanisms behind adaptation. RADSeq (Restriction Site Associated DNA Sequencing)
57 methods have been used to investigate outlier loci and have identified both directional
58 (Hohenlohe et al. 2010; Lexer et al. 2014; Gaither et al. 2015) and stabilising selection
59 patterns consistent with adaptation in several studies (Hohenlohe et al. 2010; Gaither et al.
60 2015), providing unique insights into the evolutionary mechanisms of non-model species.
61 However, few studies have addressed spatial variation of outlier loci among populations
62 and how these might contribute towards shaping population divergence in natural
63 systems. In addition, it can be challenging to disentangle the signatures of potential
64 adaptation to different environments from the simple isolating effect of distance, especially
65 as a high degree of collinearity exists between environmental gradients and neutral
66 population structure (Wang & Bradburd 2014; Manthey & Moyle 2015; Prunier et al. 2017;
67 Weber et al. 2017; Rodríguez-Zárate et al. 2018). In broad spatial and environmental
68 contexts, both Isolation By Distance (IBD) and Isolation By Environmental (IBE) will act in
69 differentiating populations. While patterns of IBD have been observed in organisms across
70 a range of life histories and taxonomic groups (Kelly et al. 2006; van Dijk et al. 2009; Harris
71 & Taylor 2010; Moura et al. 2014; Wright et al. 2015), the balance between IBD and IBE in

72   marine systems is becoming more apparent (Limborg et al. 2009; Mendez et al. 2010;
73   Whittaker & Rynearson 2017).

74   Within this context, determining the spatio-temporal patterns of genomic variability
75   that may provide some insights into signals of adaptation of populations, is important for
76   understanding persistence and resilience of species (Sexton et al. 2014; Bernatchez 2016),
77   especially those under threat from environmental pressures. Importantly, detecting
78   potentially adaptive variation can assist in pinpointing conservation units, as local
79   adaptation is an important part of evolutionary diversification, even on a contemporary
80   timescale (Bible & Sanford 2016; Bonin et al. 2007; Carvalho et al. 2011; Funk et al. 2012;
81   Hanson et al. 2017; von der Heyden 2017). Globally, seagrasses are facing persistent
82   declines and habitat fragmentation (Orth et al. 2006; Waycott et al. 2009), both of which
83   have been linked to loss of genetic diversity (Orth et al. 2006; Williams 2017). Decreased
84   population sizes and loss of genetic diversity are particularly important in the face of
85   climate change and habitat alteration facing coastal systems such as the habitat of the
86   southern and eastern African seagrass, *Zostera capensis*.

87   *Zostera capensis* has a disjunct distribution limited to estuaries on the cool-
88   temperate biogeographic region on the west coast, the warm-temperate south coast and
89   the tropical east coast of South Africa, where it is the dominant seagrass, and extends up
90   the tropical east African coast to sheltered bays in Kenya. The wide distribution range of
91   this vulnerable species (IUCN; Short et al. 2010) (Green & Short 2003; Fig. 1A)
92   encompasses strong environmental gradients across multiple biogeographic regions
93   providing an excellent opportunity to study the genomic variation of relatively isolated
94   populations along a wide gradient of environmental conditions. *Zostera capensis* likely
95   relies largely on vegetative reproduction (Greve & Binzer 2004; Hall et al. 2006), as
96   flowering in this species has only been recorded once under specific laboratory conditions
97   (McMillan 1980). Unfortunately, the dispersal potential of vegetative fragments is unlikely
98   to provide meaningful connectivity between sites due to harsh coastal conditions, strong
99   currents and often long distances between suitable estuarine habitats (Weatherall et al.
100   2016).

101   Previous studies have shown that the genetic diversity, clonality and connectivity of
102   seagrasses globally is highly context dependent (Jover et al. 2003; Olsen et al. 2004;
103   Procaccini et al. 2007; Sinclair et al. 2014; Arriesgado et al. 2016; Kendrick et al. 2016;
104   Hernawan et al. 2017; Putra et al. 2018), with some studies reporting high genetic diversity
105   and population structuring at regional and local scales (Diekmann et al. 2005; van Dijk &
106   van Tussenbroek 2010; Becheler et al. 2010; Sherman et al. 2016), emphasizing the role of
107   near and off-shore currents (Muñiz-Salazar et al. 2005; Nakajima et al. 2014). Conversely,
108   in a few cases, low levels of genetic diversity and shared genotypes, even across
109   exceptionally large spatial scales, have been recorded (van Dijk & van Tussenbroek 2010;

110   Evans et al. 2014; Nakajima et al. 2014; Phan et al. 2017). So-called 'mega clones' of
111   *Thalassia testudinum* can even have single ramets dispersed over 47km (Bricker et al.
112   2018) and 'millenary clones' of *Posidonia oceanica* are estimated to be hundreds to
113   thousands of years old (Arnaud-Haond et al. 2012). However, how patterns of natural
114   variation and population genomic structure are shaped by adaptive processes remains
115   unknown.

116   Changing African seascapes, through anthropogenic and changing climate pressures
117   (Mead et al. 2013), are severely impacting local populations of *Z. capensis*, prompting calls
118   to monitor and map genomic variation, both for neutral and outlier loci that may indicate
119   some adaptive variation. We utilised a pooled RADseq approach to identify patterns of
120   variation in both neutral and outlier loci for populations throughout the total range of *Z.*
121   *capensis*, with the underlying hypothesis that signals of outliers would vary among
122   populations, given that each site experiences a unique combination of environmental
123   conditions. We also examined the predicted historical distribution by means of hindcast
124   species distribution modelling, as historical conditions are likely to have a strong influence
125   on contemporary patterns of diversity in southern Africa (Toms et al. 2014). Lastly, we
126   examine the role of geographical and ecological distance in shaping patterns of variation of
127   *Z. capensis* in its environmentally heterogeneous habitat and hypothesise that IBE will be at
128   least as important as IBD in driving genomic diversity.

129

130   **Materials and Methods**
131   *Sample collection*
132   Leaf samples (n=336) were collected from 12 sites, including nine estuaries/estuarine bays
133   along the South African coast, one bay in Mozambique and one bay in Kenya (Fig. 1A; Table
134   1). At each location, with the exception of Inhaca, Mozambique, and Shimoni, Kenya, leaf
135   samples were collected over five beds at two distant sites in order to minimise sampling
136   clones. Samples were preserved with silica crystals before being processed. Sampling
137   permits were granted by SanParks and CapeNature (permit number 0028-AAA008-00159);
138   DAFF and DEA (permit number was RES2014/103). Despite intensive questioning of
139   collaborators and other contacts throughout this study, no samples of *Z. capensis* were
140   obtained from Tanzania, where it has not been recorded in recent times (Nordlund, L. pers
141   comm.).

142   *Laboratory protocols*
143   Accurately estimating genome-wide variation and detecting signals of local adaptation in
144   non-model organisms, such as seagrasses, requires many individuals from many sites to be
145   sequenced, which can be prohibitively expensive despite the advances made by high-
146   throughput sequencing methods such as RADseq (Ellegren 2014; Andrews et al. 2016). As
147   such, a pooled sequencing (pool-seq) approach was utilised, combining the genomic DNA of

148   multiple individuals before sequencing (Sham et al. 2002; Kofler et al. 2012). This approach
149   decreases cost whilst increasing the number of individuals analysed and allowing for a
150   more population focussed analysis (Futschik & Schlötterer 2010; Schlötterer et al. 2014).

151   Genomic DNA was extracted from leaf tissue using Qiagen DNeasy plant kit (Qiagen,
152   Valencia, USA) following standard protocols, with the exception of eluting the DNA in
153   nuclease-free water instead of elution buffer. Genomic DNA quality was then assessed
154   using gel electrophoresis and DNA concentrations of each sample determined by Qubit
155   analysis at the Central Analytical Facility of Stellenbosch University (CAF). For each
156   sampling site, genomic DNA of individuals was pooled with equimolar representation to
157   create a total of 12 samples for Illumina sequencing. The two sites at Langebaan, Oostewaal
158   and Geelbek, were kept as separate pools to allow for comparison between the observed
159   morphotypes; one short and stunted on the muddy tidal flats (Geelbek) which experience
160   prolonged exposure to conditions outside the water and the other is longer with a higher
161   biomass on the sandy permanently submerged area (Oostewaal) (Pillay, pers. Comm.).

162   Library preparation and sequencing followed the ezRAD method (Knapp et al., 2016;
163   Toonen et al., 2013; Nielsen et al. 2018), which obtains a reduced representation
164   sequencing library using high frequency restriction enzymes. Pooled genomic DNA was
165   freeze dried before library construction following the protocol of Knapp et al. (2016) and
166   Mi-Seq Illumina sequencing (V3 2x300) conducted at the Genetics Core Facility (GCF) of the
167   Hawaii Institute of Marine Biology (HIMB). These data are stored in the National Center for
168   Biotechnology Information's (NCBI's) Sequence Read Archive (SRA; PRJNA503110) and
169   georeferenced at GeOMe at the project: *Zostera capensis* pooled RADseq.

170   *Data processing and alignment*
171   Quality of raw reads was analysed using FastQC (Andrews 2010) and quality filtering
172   carried out using FastQC Toolkit (Andrews 2010), removing low quality bases (<20 phred
173   score). Additionally, TrimGalore! v 0.4.4 (available at:
174   http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) was used to remove
175   any remaining adapter sequences or reads shorter than 30bp. BWA-MEM (Li 2013) was
176   used to map filtered paired-end reads from each pooled sampling site to the genome of
177   sister species, *Zostera marina* (available from NCBI, BioProject number PRJNA41721,
178   GenBank accession number LFYR00000000, Olsen et al. 2016) with a minimum mapping
179   quality of 20. Ambiguously mapped reads, PCR duplicate reads, reads with less than 20
180   mapping quality and less than 20 base quality were filtered out before converting SAM files
181   to BAM files with SAMtools (Li et al. 2009). Number of mapped and unmapped reads was
182   then calculated using the *idxstats* command in SAMtools. As pools with a higher number of
183   mapped reads may have an artificially inflated number of SNPs, mapped reads were
184   subsampled to median coverage in SAMTools using the *view* command with the '-s' flag.
185   Although subsampling results in a loss of data, it is nonetheless important for correctly

186   interpreting true differences between the sampling sites, as opposed to differences in data
187   quality or quantity (Schlötterer et al. 2014). To confirm that subsampling removed any
188   possible correlation between the number of mapped reads and the number of SNPs and
189   outlier loci identified downstream, Spearman's correlation coefficients were calculated
190   using the *rcorr* function of the 'Hmisc' (Harrell Jr & Dupont 2006) package in R. BAM files
191   were sorted and indexed before being used to creating pileup files for each individual
192   sampling site with the *mpileup* command in SAMTools (Li et al. 2009), using a minimum
193   quality score of 20 and maximum read depth of 10 000. Finally, a pileup file combining all
194   sites was created using the same parameters in SAMtools and converted to a sync file using
195   PoPoolation2 (Kofler et al. 2011b) for downstream use.

196   *Calling SNPs and simulating data*
197   The total number of SNPs and private SNPs were identified using *snp-frequency-diff.pl* in
198   PoPoolation2 (Kofler et al. 2011b) with genomic sites required to have a minimum minor
199   allele count of four, and coverage between 10 and 500 across all 12 sampling sites. SNPs
200   were then filtered to retain only those present among sampling sites, and not those present
201   due to differences between the reference sequence (*Z. marina*) and *Z. capensis*. As many
202   software cannot handle pooled data, requiring individuals to be specified within sampling
203   sites, *subsample_sync2GenePop.pl* in PoPoolation2 was used to simulate a multi-locus
204   dataset of a subset of SNPs identified by PoPoolation2. Because this programme cannot
205   simulate different numbers of individuals across sites, the median sample size of 30
206   individuals was selected for every site. The resulting GenePop file was then converted to
207   various formats in PGDspider (Lischer & Excoffier 2012) for downstream analyses.

208   *Outlier loci identification and functional annotation*
209   Due to the uncertainty surrounding RADseq, Pool-seq and outlier detection methods
210   (Narum & Hess 2011; da Fonseca et al. 2016; Mckinney et al. 2016; Lowry et al. 2017;
211   O'Leary et al. 2018), four outlier detection methods were employed, including Fst-based
212   approaches, genotype-environment correlations and principle component analyses (see
213   supplementary materials for details). Outlier loci identified by two or more methods were
214   considered "candidate outliers" and their functional roles evaluated by subjecting 1000
215   base pairs upstream and downstream of each of the identified outlier SNPs to BLASTx
216   searches, with the non-redundant protein sequence database and an E-value cut off of 10^{-5}
217   (Altschul et al. 1997) using Blast2Go (Conesa et al. 2005). In addition to BLASTing against
218   the general NCBI database, these searches were also carried out against the Zosteraceae
219   family in general, specifically, *Zostera marina* (Olsen et al. 2016) and *Z. muelleri* (Lee et al.
220   2016). Gene Ontology (GO) mapping, Interproscan and annotation were performed with
221   Blast2Go default parameters. The number and proportion of candidate outliers unique to
222   each site and shared between pairwise sites was calculated using a custom script (Table
223   S3).

224   *Neutral variation*
225   All identified outlier loci were flagged as being putatively under selection for the purposes
226   of this analysis and were therefore removed from the dataset to isolate neutral drivers of
227   patterns of population structure. The neutral-only multi-locus dataset set was then re-
228   simulated, using *subsample_sync2GenePop.pl* in PoPoolation2 with 30 individuals per site as
229   described above, and used to calculate measures of neutral variation.

230   *Genome-wide variation and differentiation*
231   To characterise genetic diversity, Tajima's nucleotide diversity (π), Watterson's theta (Θ)
232   and Tajima's D were estimated for the complete and neutral-only datasets using a sliding
233   window approach with *Variance-sliding.pl* in PoPoolation v1.2.2 (Kofler et al. 2011a). For
234   these comparisons, filtering stringency was reduced to a minimum minor allele count of
235   two and coverage between 10 and 500 per sampling site. As the estimation of allele
236   frequencies in pooled individuals is highly reliant on sequence coverage, a high sequence
237   coverage and large sliding windows were used in order to increase accuracy (Kofler et al.
238   2011a). Observed and expected heterozygosity and the inbreeding coefficient (F_{IS}) was
239   estimated from the simulated datasets with the *divBasic* function of the 'DiveRsity' package
240   (Keenan et al. 2013) in R.

241   To investigate genome-wide levels of differentiation, the fixation index (F_{ST}) for
242   pairwise comparisons of populations was estimated using a sliding window approach with
243   *fst-sliding.pl* in PoPoolation2, using a minimum minor allele count of four and a coverage
244   between 10 and 500. Fisher's exact test was carried out with *fisher-test.pl* in PoPoolation2
245   to estimate the significance of allele frequency differences between sites. Patterns of
246   differentiation were visualised on a principle coordinates analysis (PCoA) plot generated in
247   R (R Core Development Team 2008) using the *pco* function of the 'labdsv' package (Roberts
248   2007). The PCoA plot was generated both with and without Kenya in order to account for
249   sampling bias. The simulated neutral dataset was used to investigate population clustering
250   by means of Bayesian Analysis of Population Structure (BAPS) software (Corander &
251   Marttinen 2006; Corander et al. 2006) testing K=1-10.

252   *Habitat suitability for* Z. capensis *in the LGM*
253   In order to understand the influence of historical environmental conditions on the
254   contemporary patterns of genomic variability, the suitable habitat for *Z. capensis* was
255   hindcast to the Last Glacial Maximum (LGM; 21kya). *Zostera capensis* occurrence data was
256   obtained from Adams et al. (2016) and environmental data downloaded from MARSPEC at
257   5 arcminute resolution for both the present-day (Sbrocco et al. 2018) and the LGM (CNRM-
258   CM33 model; Braconnot et al. 2007; Sbrocco 2014). Following Chefaoui et al. (2017), only
259   Sea Surface Temperature (SST) of the coldest month (Biogeo14) and warmest month
260   (Biogeo15) were utilised to avoid using strongly correlated variables for Species
261   Distribution Modelling (SDM; Guisan & Thuiller 2005; Braunisch et al. 2013; Chefaoui et al.

262   2017) and precipitation variables were excluded to decrease model uncertainty (Varela et
263   al. 2015). These variables represent relevant present-day and LGM conditions, which are
264   recognised as important determinants of intertidal seagrass habitat suitability (Short &
265   Neckles 1999; Short et al. 2001; Valle et al. 2014) and they are projected along the present-
266   day (Sbrocco et al. 2018) and LGM coastlines (Braconnot et al. 2007; Sbrocco 2014),
267   respectively. QGIS (QGIS Development Team 2012) was used to crop raster extents, by
268   means of the buffer and crop tools, to focus on the coastal areas including and surrounding
269   the present-day range of *Z. capensis*.

270   Ecological niche modelling was implemented through an ensemble approach with
271   the 'biomod2' package (Thuiller et al. 2016) in R. As in Chefaoui et al. (2017), the following
272   six presence-absence algorithms were included in the ensemble models: generalized
273   additive model (GAM), flexible discriminant analysis (FDA), generalized boosting model
274   (GBM), multiple adaptive regression splines (MARS), generalized linear model (GLM), and
275   random forest (RF). Default parameters were used for all algorithms, except for the GLM
276   which was fitted with a quadratic term, the GBM which was run with 1000 trees, and the
277   GAM which was executed with the GAM_mgcv function. As the occurrence data (Adams et
278   al. 2016) included reliable presence and absence records for estuaries along the entire
279   South African coastline, no pseudo-absence selection was required. The data was split into
280   a calibration (80%) and a validation (20%) set and three iterations were performed for
281   each algorithm with three permutations to estimate and weight variable importance, for a
282   total of 18 models. Models were assessed with the true skill statistic (TSS; Allouche et al.
283   2006) and the area under the receiver operating characteristic (ROC) curve (AUC; Fielding
284   and Bell 1997), considering both specificity (true negatives) and sensitivity (true
285   positives). Only models scoring TSS > 0.55 and AUC >0.8 were used to produce ensembles.
286   Retained models were ensembled to produce a weighted mean SDM and first used to
287   project the present-day habitat suitability, in terms of SST, along the South African
288   coastline, and then used to hindcast the habitat suitability to the LGM. The present-day and
289   LGM habitat suitability projections, as well as the changes in habitat suitability between the
290   present-day and LGM were plotted in R.

291   *Disentangling contemporary signals of IBD and IBE*
292   A redundancy analysis (RDA) (Legendre & Legendre 2012) was conducted to evaluate the
293   relative contribution of spatial and environmental variation to genomic variability and
294   patterns of genetic differentiation. RDA can be useful as a multivariate regression
295   technique when running regression analyses with multivariate predictors (space and
296   environment) and multivariate responses (here, minor allele frequencies of SNPs). As
297   spatial distances are not suitable for constrained ordination or regression as implemented
298   in RDA, geographic distances were transformed to Principal Coordinates of Neighbourhood
299   Matrix (PCNM) distances with the *pcnm* function in the 'vegan' package (Oksanen et al.
300   2018) in R. Environmental distances were calculated within the RDA function from the

301 variables in Table 2 (excluding the macrophyte species measure, which were only available
302 for South Africa). The *ordistep* function from the 'vegan' package was used to select the
303 most informative variables and build the 'optimal' model. Four separate RDAs were
304 conducted with minor allele frequency as the response. Predictor variables in the first RDA
305 were transformed geographic distances, and in the second were environmental distances.
306 Lastly, two partial RDAs were performed, partitioning out the effect of transformed
307 geographic distance and environmental variation from the total variation respectively. The
308 *anova* function of the package 'vegan' was performed with 999 permutations (Legendre et
309 al. 2011) to test the significance of RDAs.

310

311 **Results**

312 *Sequencing and mapping*
313 54 982 056 paired reads were obtained, with paired reads from each sampling site ranging
314 from 1 368 372 to 7 429 328 (Table 3). After filtering reads for quality and adapters, and
315 subsampling to a median, 7 432 397 reads, ranging from 222 741 to 750 736 per site, were
316 aligned to the *Z. marina* reference genome (Table 3). The number of filtered subsampled
317 mapped reads had no correlation with the number of SNPs ($r = 0.17$; $p>0.05$) or outlier loci
318 ($r = -0.05$; $p>0.05$) identified.

319 *Neutral and outlier loci*
320 The complete simulated dataset consisted of 308 loci. From this dataset, 101 outlier loci
321 were detected by Lositan, while BayeScan and BayeScEnv detected 25 and five outlier loci
322 respectively. The five outlier loci identified by the ecological association approach in
323 BayeScEnv were correlated with precipitation of the driest quarter and annual mean
324 moisture levels. By analysing allele frequencies of the non-simulated dataset, PCAdapt
325 identified 38 outlier loci. All 169 outlier loci were removed from the complete dataset in
326 order form a neutral-only dataset with which to examine patterns of neutral variation.

327 *Genome-wide variation*
328 The number of SNPs identified by PoPoolation2 in the neutral and complete datasets
329 ranged from 845 to 1683 and 913 to 1784 per sampling site respectively. The number of
330 private SNPs per site was generally low and five populations did not have any private SNPs
331 (Tables 3, 4). The genome-wide average nucleotide diversity (Tajima's π) of the neutral and
332 complete datasets ranged from 0.023 to 0.041 and 0.023 to 0.035, respectively. Allelic
333 richness did not vary much between sites, ranging from 1.23 to 1.36. θ_W of the neutral and
334 complete datasets were the same, ranging from 0.029 to 0.043 (Tables 3, 4). The west and
335 south coast sites, with the exception of Oostewaal (L2), exhibited marginally higher
336 nucleotide diversity and θ_W than the east coast sites. Tests for deviations from neutrality
337 produced genome-wide average Tajima's D that were negative for all sampling sites and

338   ranged from -0.723 to -0.275 and -0.706 to -0.273, for the complete and neutral datasets
339   respectively. Genetic diversity metrics calculated from the simulated datasets included
340   expected heterozygosity (0.04 to 0.06) within each sampling site (Table 3 and 4), and the
341   inbreeding coefficient, F_{IS}, which was uniform across sampling sites and equal to 1, for both
342   the complete and neutral simulated datasets.

343   *Genome-wide differentiation and clustering*
344   F_{ST} values were estimated from the complete non-simulated dataset for pairwise
345   comparisons of sites (Tables S1, S2), with Fisher's exact tests showing no significant
346   differentiation between pairs of sites for either dataset. Similarly, clustering analysis
347   conducted in BAPS on neutral loci revealed no structure across sites, with all sites falling
348   into one cluster (K=1; $p < 0.05$; Fig. S1). Although there is no significant population
349   structuring, the PCoA (Fig. S2) of pairwise F_{ST} values for neutral loci suggests that the west
350   and south coast sites, (except for Oostewaal -L2), are more closely related than the east
351   coast sites. The same pattern was observed for the PCoA generated without Kenya (Fig. S2).

352   However, when the clustering analysis in BAPS included outlier loci, two clusters were
353   detected ($p < 0.05$; labelled cluster one and two), with cluster one comprising samples from
354   the west and south coasts, and cluster two including populations from the east coast of
355   South Africa in addition to Mozambique and Kenya (Fig. 1B). Notably, one west coast site in
356   Langebaan, Oostewaal (L2), groups with cluster two rather than cluster one (Fig. 1B).

357   PCoAs of pairwise F_{ST} comparisons from the complete dataset and all outlier loci resulted in
358   a similar, but slighter denser pattern than observed for the neutral dataset (Fig. S2). Sites
359   from cluster one formed a tight group, relatively separate from the remaining sites. Sites
360   from cluster two did not group as closely as those from cluster one, with Mozambique most
361   differentiated. Moreover, Mozambique, followed by Kenya, exhibited much higher outlier
362   allele frequencies than other sites (Table S3).

363   While some outlier loci were identified by more than one method, there was little overlap
364   between outlier loci identified using the four different approaches (Fig. 3), with only three
365   outliers shared between all four methods. However, irrespective of how many outlier loci
366   are included, the frequency at which outlier loci occurred at each site reflects the two
367   clusters identified using BAPS, with higher frequencies observed in the sites comprising
368   cluster 2 than those comprising cluster 1 (Fig. 3). No private outliers were identified as all
369   outlier loci occurred at two or more sites and all candidate outliers (identified by more
370   than one method) occurred at most of the sites (Table S3).

371   *Functional annotation of candidate outlier loci*
372   Two-thousand base pairs surrounding each of 10 candidate outlier loci were subjected to
373   the Blast2Go pipeline. Although all of the 10 candidate outliers yielded significant hits
374   when BLAST searches were conducted against the general NCBI database, Zosteraceae, *Z.*

375   *marina* and *Z. meulleri*, the majority of these hits did not fall within gene regions of known

376   function. GO terms (GO:0016020-IEA 'membrane' and GO:0016021-IEA 'integral

377   component of membrane') were assigned to five of the 10 candidate outlier loci with

378   BLAST matches to hypothetical and predicted proteins (Table S3).

379   *Habitat suitability for Z. capensis in the LGM*

380   Multiple models from each algorithm met the TSS >0.55 and AUC >0.8 criteria and were

381   retained to produce ensembles. Ensemble models obtained the following average

382   validations scores: TSS=0.654, AUC=0.904, sensitivity=92.11, specificity=73.29. Predicted

383   distributions of suitable habitat, in terms of SST, differed between present-day and LGM

384   conditions, in terms of geographic location, extent and probability of occurrence (Fig. S3).

385   The highest probability of occurrence can be seen on the south coast (up to ~25°

386   longitude) and west coast (up to ~18° latitude) for the present-day projection, and on the

387   western-south coast (up to ~21° longitude) and west coast (up to ~18° latitude) for the

388   LGM projection. Ensemble models project an 11.05% loss and a 10.79% gain of suitable

389   habitat from the LGM to present-day, with a 26.1% range shift. These shifts are most

390   evident in the loss of suitable habitat on the south and south-east coasts (~21-27°

391   longitude), southern-west coast (~30-35° latitude), and west coast (~12-18° latitude), as

392   well as the gain of suitable habitat on the northern-east coast of South Africa (~30-35°

393   latitude), the south coast of Madagascar and the northern-west coast of Africa (~3-8°

394   latitude; Fig. 2). Further, within a South African context, the western-south coast

395   represents an area of stable temperature regime, where suitable habitat has occurred from

396   at least as far back as the LGM until the present day (Fig. 2). This can also be seen in

397   patches on the west coast and on the east coast of Africa (~-5-25° latitude).

398   *IBD vs IBE*

399   Of the 11 environmental variables, seven were selected by the RDA as the most informative

400   (Table 2). The pure RDA of genetic variation against transformed geographic distance was

401   not significant (P > 0.05), but was significant when carried out against environmental

402   variation, with 70.4% of the variation in the data explained by the retained environmental

403   variables. Unexpectedly, neither partial RDA analyses, the first conditioned on transformed

404   geographic distance and the other on environmental variation, were significant. Although

405   environmental variation explained such a high percentage of the variation observed in the

406   data, partitioning out the effect of geographic distance on environmental variation

407   rendered the association with genetic variation non-significant.

408

409   **Discussion**

410

411   *Genomic diversity of a threatened seagrass*
412   Genomic variability did not differ greatly between populations with all sites displaying very
413   low heterozygosity and a high inbreeding coefficient (F_{IS}=1; Table 4). However, sites in
414   cluster one did exhibit slightly higher levels of variability than those in cluster two (Table
415   4). With such high inbreeding coefficients, it is likely that this species does indeed rely
416   heavily, if not solely, on clonal growth and vegetative reproduction, rather than sexual
417   reproduction (Table 3 and 4). In terms of reproductive strategy, clonality in seagrasses can
418   vary between species with a continuum from monoclonality to meadows with high clonal
419   diversity (van Dijk & van Tussenbroek 2010), and the predominance of certain clonal
420   lineages may indicate long-term selection on phenotypes. Such selection may be in
421   response to environmental variables, where conditions are more favourable for clonal
422   lineages, but may also represent shared ancestry prior to historic sea-level fluctuations
423   reshaping the topography of the South African coastline (Ramsay & Cooper 2002; Compton
424   2011). In the case of *Z. capensis,* it is likely that a combination of historical (see below) and
425   contemporary factors shape the patterns of observed genomic diversity. Importantly, *Z.*
426   *capensis* is unlikely to be influenced by contemporary gene flow between its fragmented
427   and isolated meadows, being restricted to sheltered and low wave action environments
428   (Van Niekerk et al. 2012). In addition, the lack of recorded sexual reproduction in this
429   species through flowering (McMillan 1980) is likely to contribute to maintaining clonal
430   populations throughout the range, with important implications for potential restoration
431   efforts in the region.

432

433   *Shared adaptive divergence across two genomic clusters shaped by historical dynamics*
434   Although neutral variation can reveal much about a species demographic history, in many
435   cases, patterns revealed from outlier loci can provide unique insights into evolutionary
436   potential and patterns of resilience (Stapley et al. 2010; Guo et al. 2015; Funk et al. 2016;
437   Gaither et al. 2018). Particularly in marine systems where gene flow is generally presumed
438   to be high, signals of outlier loci can help detect population structure (André et al. 2011;
439   Freamo et al. 2011; Hess et al. 2013; Candy et al. 2015; Araneda et al. 2016; Tigano &
440   Friesen 2016; Attard et al. 2018). For example, Atlantic herring in the Baltic and North Sea
441   (André et al. 2011), Atlantic salmon in eastern Canada (Freamo et al. 2011), and Chilean
442   blue mussels (Araneda et al. 2016), all exhibit little to no structure in terms of neutral
443   variation but reveal significant population structure for outliers putatively under selection.
444   In *Z. capensis,* despite the generally low levels of genomic variation detected across its
445   range, clustering analyses revealed differentiation of all populations into two major
446   clusters when outliers that may represent putatively adaptive variation were considered in
447   addition to neutral data (Fig. 1B). Cluster one comprised of sites from the west and south
448   coast, and cluster two sites from the east coast in addition to one west coast site at
449   Langebaan, Oostewaal, also grouping with this cluster (Fig. S2). In addition, PCoAs support

450 the clustering analyses, with both neutral and adaptive variation (Fig. S2). Interestingly, the
451 PCoAs indicate that east coasts sites are more distantly related than the west and south
452 coast sites. Therefore, east coast sites may have had an earlier origin which supports the
453 likelihood of a refugial area for *Z. capensis* on the east coast.

454   Temperature-based ensemble models, however, suggest reduced, and more
455 fragmented seagrass habitat along the South African south coast, that is likely to have
456 divided *Z. capensis* into two clusters with the south-western and east coast possibly
457 representing refugial areas during the LGM, with subsequent dispersal into its present-day
458 distribution. A refugial area on the south-western coast may explain the presence of both
459 clusters in Langebaan Lagoon. Notably, this split between the clusters roughly coincides
460 with the split between described temperate and sub-tropical bioregions (Sink et al. 2012)
461 along which phylogeographic breaks have been recorded for marine coastal species (von
462 der Heyden 2009; Teske et al. 2011), including one other saltmarsh plant (Potts et al.
463 2016). Given lack of gene flow and apparent high levels of clonality in *Z. capensis*, the
464 structure detected through outlier loci most likely reflects ancestral adaptation during
465 conditions more conducive to gene flow or incomplete lineage sorting during post-LGM
466 expansion.

467   Interestingly, although our historical models were based on environmental
468 variables, they broadly mirror the findings of changes in topology and composition of the
469 South African coastline during the last 70,000 years (Toms et al. 2014). During the past
470 45,000 years, lowered sea level stands of up to -120m caused significant shifts from rocky
471 to sandy/muddy shorelines which isolated populations of obligate rocky shore species.
472 Although *Z. capensis* is found in present-day estuaries, the latter would also have been
473 affected, although the extent of change is unknown. Our findings, in combination with Toms
474 et al. (2014), do however show that combinations of abiotic changes have the potential to
475 influence the population dynamics of marine and estuarine species in the Atlantic/Indian
476 Ocean transition zone, as they have done in Australia over exceedingly short time scales
477 (Puritz et al. 2012).

478   We provide evidence for a pattern of shared outlier loci for populations across a
479 distinct environmental gradient and large geographic span, despite the presence of two
480 genomic clusters that appear to represent distinct historical lineages. This is in contrast to
481 our hypothesis of distinct population-level signals of adaptive variation as seen in previous
482 studies (Williams & Oleksiak 2008; Perrier et al. 2013; Ravinet et al. 2016). All outlier loci
483 were shared among sites with no populations harbouring private outliers (Table S3),
484 suggesting the same genomic basis for each *Z. capensis* meadow with the same suite of
485 genes under selection across sites in response to the various environmental gradients.
486 However, differences in the frequencies of these outlier loci across sites provides the
487 foundation for the two clusters, with sites from cluster one exhibiting outlier loci at lower

488 frequencies compared to cluster two (Fig. 3). Notably, this pattern of differential outlier
489 allele frequencies could be observed even when only considering three outlier loci (Fig. 3).
490 The same clustering pattern is detected with both non-simulated and simulated datasets,
491 which were necessary in order for the analysis of pooled data with certain software. This
492 similarity demonstrates that simulated datasets can be used to detect biologically
493 significant evolutionary patterns, regardless of the over-simplifications these simulated
494 datasets may introduce, or the number of SNPs one chooses to employ.

495   There have been numerous other studies that also report high levels of shared
496 adaptive variation across sites, such as that in Atlantic salmon of eastern Canada, where the
497 allele frequencies of shared outlier loci were used in to assign individuals to their region of
498 origin, assisting with stock management (Freamo et al. 2011). Similarly, in Pacific and
499 Atlantic sticklebacks different allele frequencies of shared outlier loci have been used to
500 distinguish marine and freshwater populations (Jones et al., 2012). At a smaller scale in
501 western Canada, most outlier loci in sticklebacks were specific to single watershed regions
502 (Deagle et al. 2012). Likewise, few shared outlier loci were observed in the periwinkle,
503 *Littorina saxatilis*, in Sweden (Ravinet et al. 2016), suggesting that the shared or private
504 nature of outlier loci might be highly context specific. Despite the potential for high levels
505 of gene flow and similar selective pressures, *L. saxatilis* populations displayed a
506 considerable amount of unshared genomic divergence, possibly due to complex polygenic
507 traits involved in habitat adaptation.

508 *The contribution of IBD and IBE towards the spatial arrangement of genomic variability in* Z.
509 capensis

510 Despite the low probability of connectivity between sites, due to both the isolated nature of
511 estuaries and the lack of sexual reproduction recorded for this species, geographic distance
512 (IBD) was not a significant driver of the observed genomic variation. However, because of
513 spatial autocorrelation of environmental variables, there appears to be a large spatial
514 component playing a role in genomic variation, which cannot be separated from the effect
515 of environmental variables. IBE plays an important role in shaping genomic variation in
516 this seagrass, with the analysis of IBE revealing dissolved oxygen, annual mean moisture,
517 precipitation and temperature related environmental variables as potentially significant
518 drivers associated with outlier loci that may indicate some level of adaptive variation.
519 Likewise, ecological association analyses correlated precipitation of the driest quarter and
520 annual mean moisture levels with adaptive variation.

521 **Conclusions**
522 *Zostera capensis* along the African coast have not been observed to reproduce sexually, and
523 high clonality combined with low genomic diversity increases their vulnerability to direct
524 human pressures and a changing climate. Genomic similarity between sites however may
525 confer a level of resilience as meadows particularly in the context of restoration (Hughes

526 and Stachowicz 2004; McKay et al. 2005; Reynolds et al. 2012; Baums 2008). Even though
527 there is no significant structure based on neutral loci, the clustering of sites into west and
528 east based on outlier loci may indicate different levels of selection on the same suite of
529 genes, which is not implausible given the environmental and ecological gradients that
530 characterise our study area. Shared outlier loci among independent lineages, coupled with
531 differences in the frequencies of those outlier loci among populations correlated with
532 environmental variability is consistent with the potential for local adaptation. Although we
533 could not directly test for local adaptation, and recognise the need for reciprocal transplant
534 experiments (Kawecki & Ebert 2004), the frequency differences among outlier loci may
535 indicate some functional variation, which could in turn influence how different populations
536 respond to changing environmental conditions. Additionally, the two clusters appear in
537 part to be shaped by historical environmental variation, with each cluster linked to
538 climatically stable refugia on the south-western and east coasts. As such, it is important to
539 protect and maintain distinct populations across the distributional range of keystone
540 species in order to safeguard this seagrass and its vital ecosystem services into the future
541 (Hughes & Stachowicz 2004). In particular, while Langebaan Lagoon may serve as an ideal
542 focal point for conservation on the west coast, areas on the east coast of South Africa,
543 Mozambique and Kenya should also be targeted for conservation in order to increase
544 resilience and reduce the risk of widespread loss.

545

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551

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940

Table 1 (on next page)

Sampling locations, biogeographic zone and number of samples (N) per site.

Location	Abbreviation	Coordinates		Biogeographic zone	N
Oifants	O	31.7021° S	18.1876° E	Cool-temperate Namaqua	30
Berg	B	32.7697° S	18.1438° E	Cool-temperate Namaqua	30
Geelbek, Langebaan	L1	33.1941° S	18.1211° E	Cool-temperate Namaqua	30
Oostewaal, Langebaan	L2	33.1214° S	18.0447° E	Cool-temperate Namaqua	30
Breede	BR	34.4074° S	20.8453° E	Warm-temperate Agulhas	30
Knysna	K	34.0791° S	23.0562° E	Warm-temperate Agulhas	30
Swartkops	SK	33.8650° S	25.6333° E	Warm-temperate Agulhas	30
Nahoon	N	32.9864° S	27.9517° E	Warm-temperate Agulhas	30
Mngazana	M	31.6921° S	29.4228° E	Suptropical Natal	30
Richards Bay	RB	28.8105° S	32.0947° E	Suptropical Natal	23
Inhaca, Mozambique	MOZ	26.0500° S	32.9297° E	Tropical Delagoa	10
Shimoni, Kenya	KEN	4.6741° S	39.3440° E	Tropical	3

1

2

Table 2(on next page)

Environmental variables included in BayeScEnv and IBE analyses.

Environmental variable	Source
Macrophyte species measures	
Submerged macrophyte area (ha)	
Number of habitat types	(Adams et al. 2016)
Submerged macrophyte species richness	
the CLiMond dataset	
Annual mean temperature (°C) (Bio1)*	
Max temperature of warmest week (°C) (Bio5)*	
Min temperature of coldest week (°C) (Bio6)	
Annual precipitation (mm) (Bio12)*	
Precipitation of wettest quarter (mm) (Bio16)*	(Kriticos et al. 2012)
Precipitation of driest quarter (mm) (Bio17)	
Annual mean radiation (W m-2) (Bio20)	
Annual mean moisture index (Bio28)*	
World Ocean Atlas	
Salinity (PSS)	Zweng et al. (2013)
Dissolved Oxygen (ml/l)*	Garcia et al. (2013)
Sea Surface Temperature (°C)*	Locarnini et al. (2013)

1   (*) indicates variables selected by the RDA as important contributors

Table 3(on next page)

Summary statistics of RAD data and estimates of genetic diversity metrics per sampling site (refer to Table 1 for full names of abbreviations) for neutral dataset.

Sampling site	Raw reads	Mapped reads	Subsampled mapped reads	SNPs	Private SNPs	π	θ	D	He	Ho	F_{IS}
O	5 862 886	1 457 363	743 255	1 278	2	0.034	0.041	-0.714	0.04	0	1
B	4 314 436	1 114 902	746 984	1 683	3	0.035	0.042	-0.722	0.04	0	1
L1	4 997 550	1 153 894	750 031	1 473	2	0.034	0.041	-0.698	0.04	0	1
L2	1 368 372	222 741	222 741	1 027	0	0.025	0.031	-0.616	0.06	0	1
BR	3 105 804	508 608	508 608	1 624	1	0.034	0.041	-0.705	0.05	0	1
K	5 943 674	1 251 227	750 736	1 342	1	0.035	0.041	-0.673	0.04	0	1
SK	5 882 100	1 360 205	748 113	1 387	0	0.035	0.042	-0.675	0.04	0	1
N	4 296 798	568 703	568 703	845	0	0.028	0.034	-0.654	0.05	0	1
M	3 991 420	475 470	475 470	914	1	0.025	0.032	-0.637	0.05	0	1
RB	7 429 328	781 740	750 470	1 105	0	0.022	0.028	-0.646	0.04	0	1
MOZ	4 136 268	719 319	719 319	598	0	0.026	0.028	-0.276	0.05	0	1
KEN	3 653 420	447 966	447 966	1 480	6	0.029	0.043	-0.324	0.04	0	1
Total	54 982 056	10 062 138	7 432 397	-	16	-	-	-	-	-	-
range	1 368 372-7 429 328	222 741-1 457 363	222 741 – 750 736	845 - 1683	0 - 6	0.023-0.035	0.029 - 0.043	(-0.723) - (-0.275)	0.04 - 0.06	0	1

1   π = Tajima's π; θ = Watterson's θ; D = Tajima's D; He = Average expected heterozygosity; Ho = Average observed heterozygosity

Table 4(on next page)

Estimates of genetic diversity metrics per sampling site (refer to Table 1 for full names of abbreviations) for complete dataset.

Sampling site	Number of SNPs	Number private SNPs	π	θ	D	He	Ho	F_{IS}
O	1 362	2	0.034	0.041	-0.716	0.04	0	1
B	1 784	3	0.035	0.043	-0.723	0.04	0	1
L1	1 577	2	0.034	0.041	-0.700	0.04	0	1
L2	1 091	0	0.025	0.031	-0.616	0.06	0	1
BR	1 726	1	0.034	0.041	-0.706	0.05	0	1
K	1 436	1	0.035	0.042	-0.674	0.04	0	1
SK	1 483	0	0.035	0.042	-0.676	0.04	0	1
N	913	0	0.028	0.034	-0.651	0.05	0	1
M	997	1	0.026	0.033	-0.636	0.05	0	1
RB	1 192	0	0.023	0.028	-0.646	0.04	0	1
moz	668	0	0.027	0.029	-0.273	0.05	0	1
ken	1 580	6	0.029	0.043	-0.323	0.04	0	1
Total	-	16	-	-	-	-	-	-
range	913 - 1784	0 - 6	0.023-0.041	0.029 - 0.043	(-0.706) - (-0.273)	0.04 - 0.06	0	1

1 **π = Tajima's π; θ = Watterson's θ; D = Tajima's D; He = Average expected heterozygosity; Ho = Average**
2 **observed heterozygosity**

3

Figure 1

Sampling sites and clustering of *Z. capensis* populations

A) Map showing the sampling sites and sea surface temperature across the range of Z. capensis (indicated by the solid lines). An inset of the two sites at Langebaan Lagoon is provided. B) Clustering analysis of the twelve sites estimated in BAPS for the complete dataset, with the twelve sites grouped into two clusters.
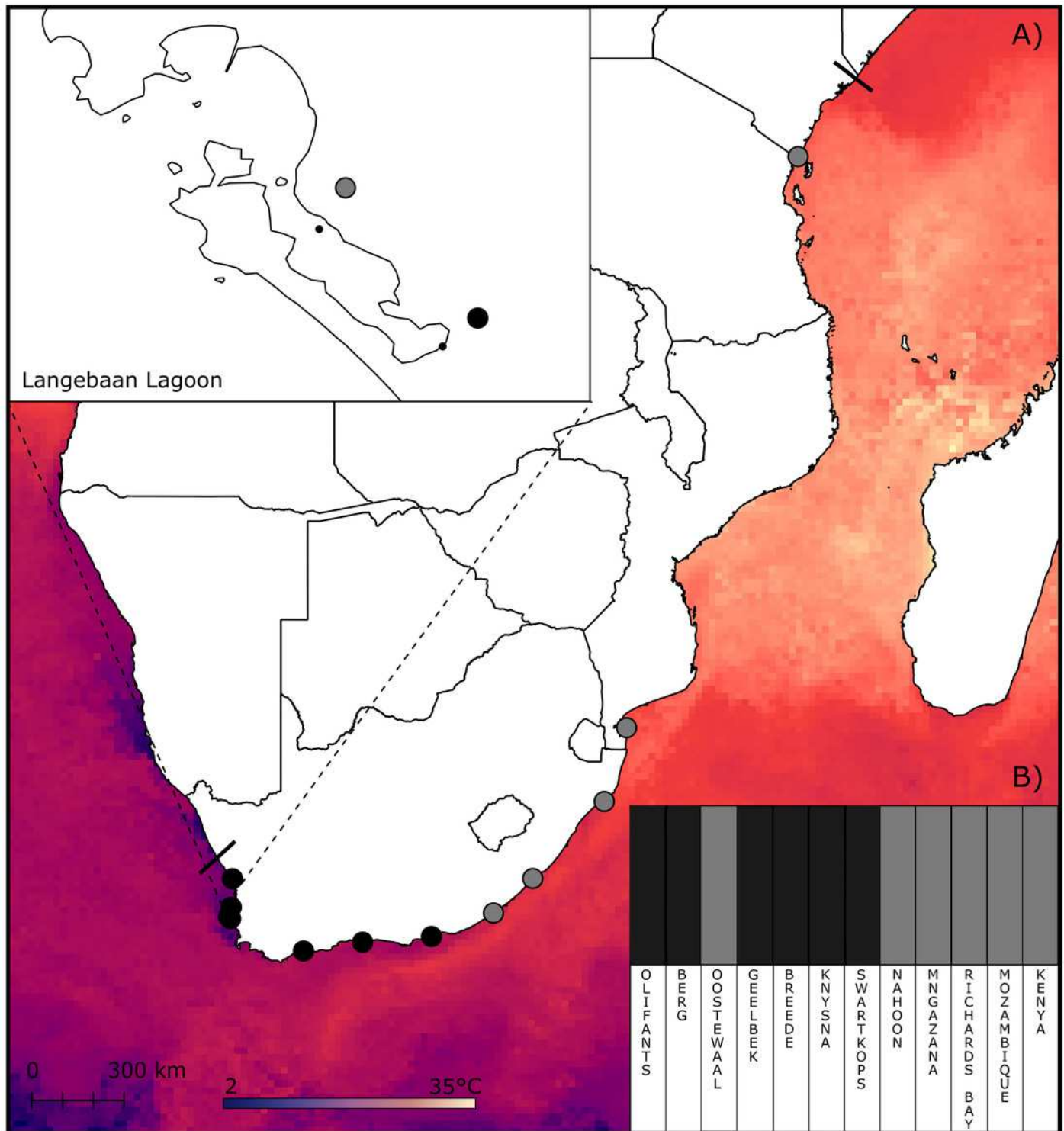
Figure 2(on next page)

Projected changes in suitable habitat, in terms of SST, from the LGM to present with the probability of occurrence graphically represented along the x and y axes.

Niche shift from LGM to present
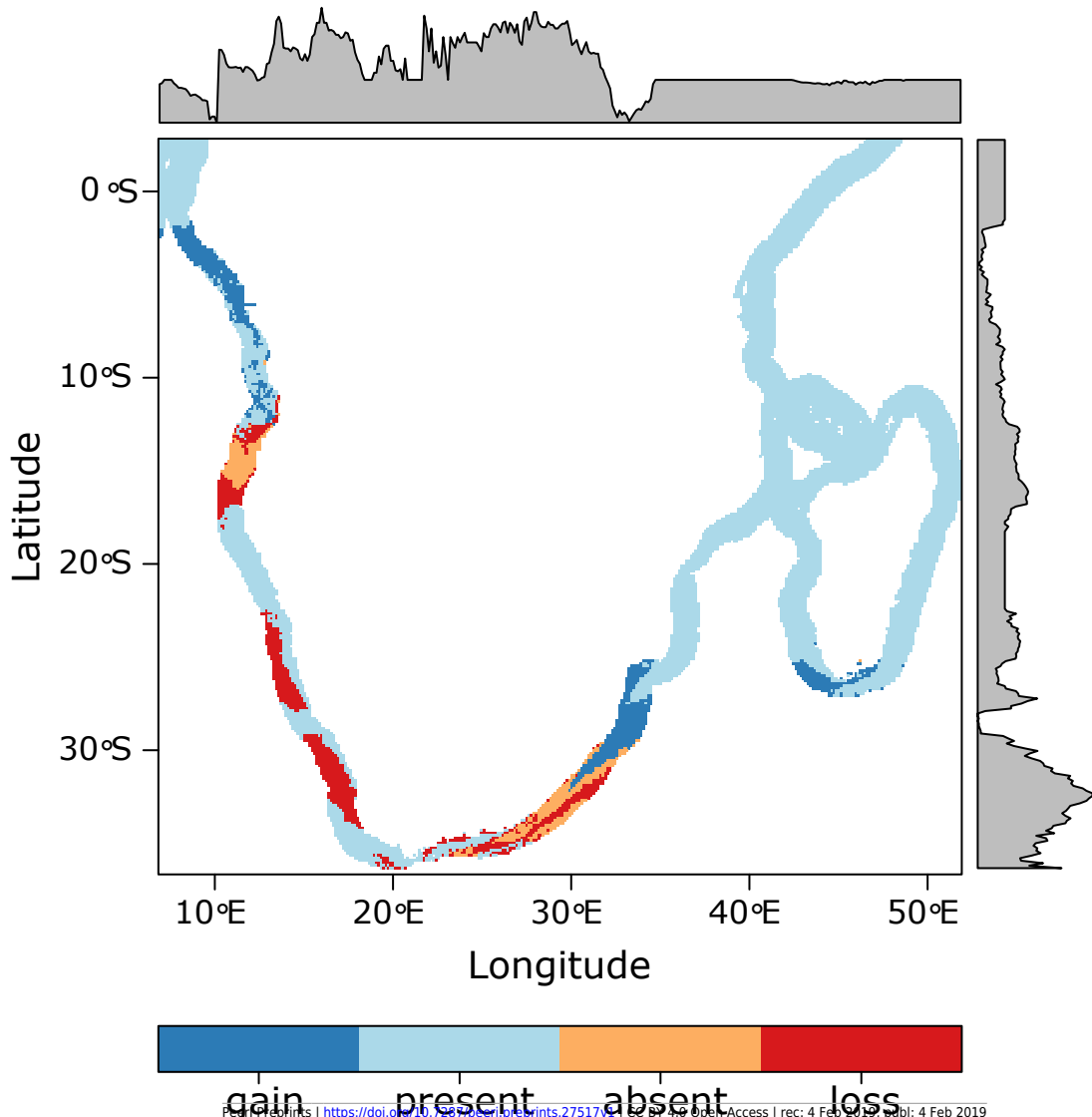
Figure 3(on next page)

Outlier frequency and identification overlap.

A) The frequency of the three outlier loci identified by all four approaches (Lositan, BayeScan, BayeScEnv and PCAdapt) across sampling sites. B) Venn diagram illustrating the overlap between outlier loci identified using the four different approaches