Aquatic Conservation: Marine and Freshwater Ecosystems

WILEY

ARTICLE OPEN ACCESS

Conservation Implications of Strong Population Structure Despite Admixture in an Endangered African Seagrass

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Received: 8 December 2023 | **Revised:** 22 October 2024 | **Accepted:** 24 October 2024

Funding: This work was funded by project SeaStore (National Research Foundation Marine and Coastal Research Grant UID: 136488) awarded to Sophie von der Heyden and a Western Indian Ocean Marine Sciences Association MARG I grant awarded to Megan J. Jackson, Romina Henriques and Sophie von der Heyden. Charlotte A. Combrink was supported by a free-standing National Research Foundation bursary.

Keywords: Cape dwarf eelgrass | conservation genomics | ddRAD | dispersal | gene flow | genomic diversity | relatedness

ABSTRACT

Zostera capensis is an African seagrass that is endangered throughout its range. In South Africa, it is solely confined to low wave energy estuarine habitats and characterised by two evolutionary lineages that diverge across a biogeographic transition. In this study, we sampled seagrass plants from five populations that span the region of lineage divergence and investigated the extent of lineage overlap. Using 2681 SNP loci, including 32 putative outlier loci, we calculated population structure, genomic diversity and levels of admixture. All populations were significantly different to each other, including those <10 km apart and low levels of admixture indicate limited dispersal of *Z. capensis*. Every population was characterised by a high inbreeding coefficient (F_{IS}), suggesting a limited number of breeding individuals in each population. Given increasing anthropogenic stressors that are linked to declines in seagrass meadow cover in South Africa, our study provides strong support that populations of this endangered seagrass require targeted management and conservation actions of each individual population to avoid further loss of the unique evolutionary dynamics and to safeguard the ecosystem services seagrasses provide. Further, our evidence of significant population structure across geographically close populations highlights that conservation efforts relying on seagrass restoration would risk mixing unique evolutionary signatures of *Z. capensis* in the region when transplanting between estuaries. This represents a critical challenge to using transplants as a potential mechanism of restoring declining populations and highlights the crucial importance of preventing population extinction.

1 | Introduction

Seagrasses are some of the world's most productive ecosystem engineers, providing numerous benefits to humans and biodiversity through supporting vital ecosystem functions that include nutrient cycling, sediment stabilisation and sequestration and storage of blue carbon, as well as providing nursery grounds for many (including commercially important) marine species. Seagrasses also promote ecosystem resilience through supporting high levels of biological diversity, which, in turn, safeguard valuable ecosystem services (Nordlund et al. 2016; do Amaral Camara Lima et al. 2023).

Globally, seagrass meadows continue to experience extensive declines, with ~5602 km^2 of meadow area lost since 1880 (Dunic et al. 2021), including in South Africa (Adams 2016; von der Heyden et al. 2024). Population declines have also been linked to associated losses in genomic diversity (Phair et al. 2020), which, although less apparent than abundance declines, are an important consideration for species persistence and resilience under

environmental change (Reusch et al. 2005; Phair et al. 2020; Nielsen et al. 2023). Given that seagrasses are considered biological indicators in coastal systems (Roca et al. 2016), due to their sessile nature, widespread distribution and ability to integrate perturbations over relatively short timescales worldwide, losses of these 'coastal canaries' (Orth et al. 2006) signal the urgent need to improve management effectiveness. For example, Marine Protected Areas (MPAs) are important management tools that can protect seagrasses from direct anthropogenic impacts and support species persistence (Aller 2018), although it is unlikely that the current global distribution of MPAs is sufficient to protect them (Daru and Rock 2023). Importantly, the effectiveness of conservation efforts can be strengthened through the inclusion of genomic data that are able to, for example, assess connectivity and molecular dynamics of taxa during the identification of priority conservation areas for MPA establishment (von der Heyden 2009; Carvalho et al. 2017; Phair, Nielsen, and von der Heyden 2021; Nielsen et al. 2023).

Molecular studies generate valuable population parameter estimates for integration into conservation and systematic spatial planning (Beger et al. 2014; Nielsen, Beger, et al. 2020; Phair, Nielsen, and von der Heyden 2021), with genomic-level approaches able to identify fine-scale population patterns as well as signals of putative adaptive variation (Nielsen et al. 2023). The ability to distinguish between neutral and putatively selective processes improves our understanding of drivers of adaptation and speciation (Rodríguez-Ezpeleta, Álvarez, and Irigoien 2017; Gaither et al. 2018; Nielsen et al. 2018), and strengthens systematic conservation planning efforts by mapping patterns of putative adaptive variation, which may support species persistence in changing environments (Carvalho et al. 2017; Nielsen, Henriques, et al. 2020b; Nielsen et al. 2021).

Population genetic studies on seagrasses have revealed considerable variation in clonality, genetic diversity and patterns of population structuring patterns within and among species, including *Posidonia oceanica* in the Mediterranean Sea (Arnaud-Haond et al. 2012), *P. australis* (Evans et al. 2014) and *Zostera muelleri* (Sherman et al. 2016) in Australia, *Enhalus acoroides* in the Philippines and Yaeyama (Nakajima et al. 2014), *Cymodocea rotundata* in the Western Pacific (Arriesgado et al. 2016) and *Zostera marina* in the North Sea (Jahnke et al. 2018) and the United States (Allcock et al. 2022). Environmental factors, such as ocean currents and historical environmental variability (Jahnke and Jonsson 2022; Phair et al. 2019), as well as reproductive strategies and dispersal patterns, have been identified as important drivers shaping genetic connectivity and population structure in seagrass species (van Dijk et al. 2009; Nakajima et al. 2014; Sinclair et al. 2014; Hernawan et al. 2017; Kendrick et al. 2017). Further, the integration of metrics derived from molecular data has, for example, informed the delineation of potential conservation priority areas (Phair, Nielsen, and von der Heyden 2021), as well as identified donor–recipient meadow dynamics for seagrass restoration (Pazzaglia et al. 2021).

Zostera capensis Setchell is the most abundant seagrass along the South African coastline, where it grows in sheltered bays and estuaries, encompassing cool-temperate to tropical bioregions (Adams 2016; von der Heyden et al. 2024). It also has a patchy distribution along the East African coastline, with confirmed populations in Mozambique and southern Kenya (Phair et al. 2019), although whether it persists in other locations is uncertain (von der Heyden et al. 2024). Like many seagrasses globally, *Z. capensis* has experienced considerable population losses throughout its range, and in South Africa, numerous populations have been severely eroded or lost, with meadows of *Z. capensis* now only remaining in ~37 estuaries (von der Heyden et al. 2024). Despite the extensive ecological and economic importance of *Z. capensis*, estuaries remain some of the most threatened and least protected ecosystems in South Africa (van Niekerk et al. 2019), with less than a third of estuaries receiving any sort of formal protection, which only indirectly protects a small number of *Z. capensis* populations at best (Adams 2016; van Niekerk et al. 2019).

Several studies have explored the evolutionary dynamics of *Z. capensis* across its distribution range. Notably, Phair et al. (2019) detected two genomically divergent lineages separated into western and eastern regions, with one lineage broadly coinciding with cool and warm-temperate populations and another encompassing plants sampled from subtropical and tropical ecoregions. Based on frequency differences of putative outlier loci, the lineages diverge between the Swartkops and Nahoon estuaries on the southeast coast of South Africa (Figure 1), which is a known biogeographic transition (Griffiths et al. 2010) and also a recognised phylogeographic barrier for many regional marine species (Teske et al. 2011; Wright et al. 2015; Dalongeville et al. 2022), including the kelp genus *Ecklonia* (Madeira et al. 2024). It is possible that the reported frequency differences in putative outlier loci (Phair et al. 2019) reflect differences in adaptive variation because the suite of genes in question exists across an environmental gradient and the population break may have originated at least 25,000 years ago (Phair et al. 2019). However, whether these lineages represent a clear genomic break, or whether populations in the region of overlap show signs of admixture, is unknown.

An important source of genetic variation in seagrasses is admixture, which involves the transfer of genetic material between lineages of the same species, especially when 'pre-adapted' alleles are introduced into new populations (Suarez-Gonzalez, Lexer, and Cronk 2018; Aguillon et al. 2022), which can have important conservation and management implications. In the context of the diverging lineages in *Z. capensis*, admixture may improve resilience potential to future changes in populations where frequencies of adaptive loci from both lineages are present (Phair et al. 2019). Further, the fine-scale population genomic patterns of *Z. capensis* are particularly interesting as previous phylogeographic analyses have suggested strong population structure in *Z. capensis* populations, with most populations showing high levels of divergence (Jackson 2022), even across small spatial scales (< 50 km; Smit et al. 2023).

Understanding the dynamics of population divergence and diversity of seagrass populations across the region where *Z. capensis* lineages overlap is important to mitigate genomic erosion through the loss of population-unique genetic signals, as quantifying the extent of admixture in populations and identifying populations may prove critical for conservation and restoration efforts. As such, using a RAD-Seq approach,

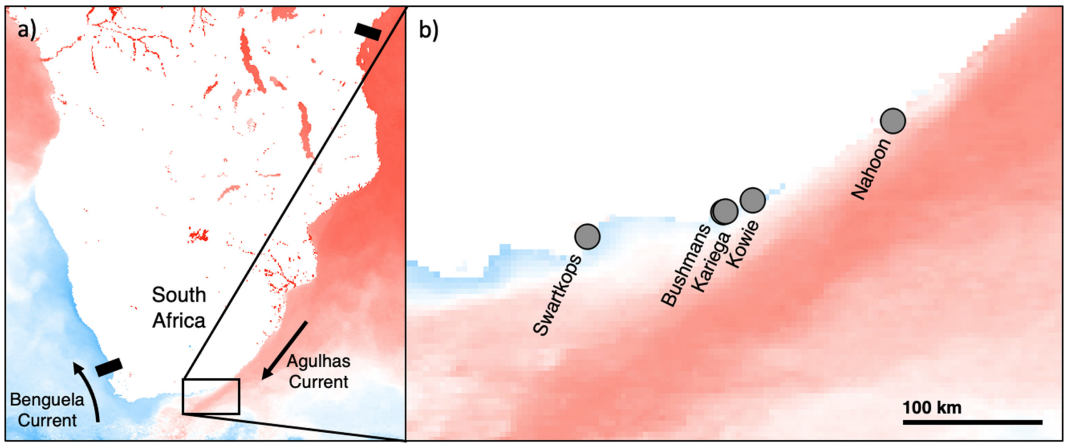

FIGURE 1 | Map of (a) *Zostera capensis* distribution range, spanning from South Africa to Kenya (between the bold black lines, but also see von der Heyden et al. 2024) and (b) locations of *Z. capensis* populations sampled in this study (note that the Bushmans and Kariega estuaries are situated < 3 km apart). Monthly mean sea surface temperatures for August 2023 (NASA Earth Observations, 2009) are shown, with blue indicating cooler waters and red indicating warmer waters, providing a snapshot of the unique oceanographical environment of coastal South Africa (Griffiths et al. 2010).

we explored the genomic signatures of five previously unsampled populations of *Z. capensis* that straddle the region of lineage divergence and estimated levels of admixture and relatedness, as well as genomic diversity and divergence. Given our understanding of population dynamics of *Z. capensis*, we expected to recover signals of population structure, but some levels of admixture between the geographically closest populations. Importantly, the findings of this study are crucial for supporting conservation and management efforts of South African *Z. capensis* through highlighting the unique evolutionary dynamics of seagrass populations, thus ensuring their long-term persistence, particularly under the additive effects of climate and anthropogenic stressors.

2 | Materials and Methods

2.1 | Sampling

Sampling was conducted in January and February 2023. Individual leaf blades were collected from five estuaries (Swartkops, Bushmans, Kariega, Kowie and Nahoon; Figure 1) spanning the region of *Z. capensis* lineage divergence identified by Phair et al. (2019) in the Eastern Cape, South Africa, which also aligns with known biogeographic (Griffiths et al. 2010) and phylogeographic (Teske et al. 2011; Dalongeville et al. 2022) breaks, including for another marine macrophyte, *Ecklonia sp.* (Madeira et al. 2024). Twenty leaf blades were collected from each estuary at intervals of > 20 m, except in two cases: 10 leaf blades from upper Kariega were collected > 10 m apart due to access constraints, and 20 leaf blades from Nahoon were collected immediately next to each other in batches of five at each of four access points located > 90 m apart. The Sundays and the East and West Kleinemonde estuaries were also visually inspected for *Z. capensis*, as this species has historically been reported there, but no seagrass plants were found during our sampling campaign. All blades were cleaned of epiphytes and stored at −20°C until DNA extraction.

2.2 | Laboratory Protocols

Tissue from all leaf blades was disrupted with liquid nitrogen using a pestle and mortar, and genomic DNA extracted using E.Z.N.A Plant DNA (Omega Bio-tek) and DNeasy Plant (QIAGEN) extraction kits. To increase DNA yield, the E.Z.N.A Plant DNA extraction protocol was modified following Smit et al. (2023). Extraction quality was confirmed with gel electrophoresis and DNA concentration quantified using Qubit fluorometer analysis. DNA extractions with concentrations of > 20 ng/μL were submitted to LGC Genomics in Berlin, Germany, for library preparation using a double digest restriction-site associated (ddRAD) DNA sequencing approach (Peterson et al. 2012) with the PstI and MseI enzymes and sequenced on an Illumina NextSeq 500/550 v2. All leaf blades were sequenced in the same lane to generate ~150 bp paired-end reads.

2.3 | Data Processing and Bioinformatics Pipeline

Bioinformatic computations were performed using Stellenbosch University's HPC2 (http://www.sun.ac.za/hpc). Illumina sequences were received as FASTQ files and raw reads cleaned using the 'process_radtags' program in Stacks v2.59 (Rochette, Rivera-Colón, and Catchen 2019). Quality score filters (-q, process_radtags) and phred score limits (-s 20, process_radtags) were applied to all sequences to discard low-quality reads. Reads with any uncalled base were removed (c-, process_radtags), and barcodes and RAD-Tag cut sites corrected (-r, process_radtags). The quality of filtered reads was checked across leaf blades using FastQC (Andrews 2010) and across all samples using MultiQC (Ewels et al. 2016).

Cleaned reads were processed in the Stacks v2.59 (Rochette, Rivera-Colón, and Catchen 2019) bioinformatics pipeline to build loci using a de novo assembly process. This aproach was used because a reference genome is not available for *Z. capensis*, and Jackson (2022) showed that the de novo approach

performs better than aligning *Z. capensis* to the reference genome of *Z. marina*. A bash script submitted to the HPC2 was used to run the components of the Stacks assembly process (Stacks Manual: Section 4.4) according to best practices guidelines (http://catchenlab.life.illinois.edu/stacks/) and using parameters optimised for *Z. capensis* (Jackson 2022): sequences were aligned using the 'ustacks' program with a minimum depth of 3 and allowing up to two mismatches (--m 3, --M 2, ustacks), and a catalogue of loci was created using the 'cstacks' program allowing one mismatch between loci from different leaf blades (--n 1, cstacks). Individuals were then matched back against the catalogue using the 'sstacks' program and transposed using the 'tsv2bam' program. Paired-end reads were then assembled into a contig, and variant sites were called using the 'gstacks' program. Finally, the 'populations' program of the pipeline was used to calculate population-level statistics. The 'populations' program was run with parameters that specified a minimum minor allele count of three and retained one random SNP per locus (--min-mac 3 --write-random-snp, populations). In order to limit the amount of missing data within the dataset, the 'populations' program was also run with filters which ensured that only loci present in at least 70% of leaf blades across all samples (--min-samples-overall 0.7, populations) and present in at least 70% of individuals within each population (--min-samples-per-pop 0.7, populations) were retained.

In order to further reduce levels of missing data across the dataset, individuals missing more than 40% of detected loci were removed from the dataset following the completion of de novo assembly. These individuals were identified and removed using the --missing-indv and --remove functions in VCFtools v3.0 (Danecek et al. 2011).

As a component of data cleaning, pairwise relatedness was used to examine the dataset for laboratory duplicates that may have resulted from extracting or sequencing errors. Pairwise relatedness was calculated using the --relatedness2 function in VCFtools v3.0 (Danecek et al. 2011) for each sample pair. The --relatedness2 algorithm is based on the Kinship-based INference for Genome-wide association studies (KING) approach developed by Manichaikul et al. (2010), which defines the kinship coefficient (relatedness_phi, ϕ) as the probability that two randomly sampled alleles from two individuals are identical by descent. Pairs of individual leaf blades with high kinship coefficients ($\phi > 0.33$) that had been sequenced in adjacent wells were removed from the dataset.

As many seagrass species are known to exhibit clonal reproduction, two approaches were used to examine the dataset for highly similar genotypes, either due to duplicated samples (same individual sampled twice, e.g., from the Nahoon) or clones. First, levels of pairwise relatedness for each sample were re-estimated with --relatedness2 in VCFtools v3.0 (Danecek et al. 2011). The similarity of genotypes was interpreted according to the kinship coefficient (ϕ) inference criteria delineated by Manichaikul et al. (2010), where sample pairs with kinship coefficients (ϕ) > 0.354 were flagged as clones/duplicated samples. Second, the Jaccard index (Yu et al. 2023) was used to examine the dataset for putative clones. In contrast with --relatedness2 outputs, which are based on shared alleles, the Jaccard index calculated in this investigation were based on shared heterozygosity using scripts adapted from Yu et al. (2023; https://github.com/leiyu37/Detecting-clonemates.git). According to best practice in the absence of technical replicates, the mode close to SH = 1 was used to determine the threshold values to distinguish between putative clone pairs and non-clone pairs.

Highly similar genotypes identified by both detection methods were considered to be potential clones in Swartkops, Bushmans, Kariega and Kowie. In Nahoon, highly similar genotypes were considered to be potential duplicate samples if they were collected directly next to each other during sampling, as this possibility could not be excluded (see Section 2.1 for sampling constraints). In order to remove the signal of clones and duplicate samples from the dataset while retaining the maximal amount of data, only one individual leaf blade from each potential clone or duplicate group was retained for further analyses. After the dataset was controlled for quality, missing data, duplicate samples and clonality, the de novo assembly pipeline was run in Stacks v2.59 (Rochette, Rivera-Colón, and Catchen 2019) for 72 samples, and 'population' program outputs generated for downstream analyses. The same parameters were applied to the pipeline (--m 3, --M 2, ustacks; --n 1, cstacks) and 'populations' program (--min-mac 3, --write-random-snp, --min-samples-overall 0.7, --min-samples-per-pop 0.7, populations) as in the initial pipeline execution.

2.4 | Detection of Putative Outliers

Due to uncertainties associated with RAD-seq and statistical methods of outlier detection (Lowry et al. 2017; McKinney et al. 2017; Nielsen, Henriques, et al. 2020), putative outlier loci were detected using three outlier detection methods: BayeScan v2.1 (Foll and Gaggiotti 2008), PCadapt (Luu, Bazin, and Blum 2017) and the sNMF function in the R package LEA (Frichot et al. 2014). BayeScan and sNMF are F_{ST}-based outlier tests, whereas PCadapt represents a distinct PCA-based outlier detection method. BayeScan was executed using default parameters (-thin 10, -nbp 20, -pilot 5000, -burn 50,000, -pr_odds 10), and diversifying loci assigned false discovery rate q-values > 0.95 were considered to be putative outlier loci. PCadapt and *sNMF* assign p-values to loci, and *p*-values above the 95th percentile were considered to be putative outlier loci. Only putative outlier loci identified by at least two outlier detection methods were retained for the 'outlier dataset'. The candidate outliers were removed to create a 'neutral dataset', whereas the 'full dataset' included both neutral and putative outlier loci.

2.5 | Genomic Diversity

Genomic diversity and inbreeding levels were investigated on the full, neutral and outlier datasets to assess variation across populations, with statistical analyses conducted in R (R Core Team 2024). Observed heterozygosity (H_O), expected heterozygosity (H_E) and the inbreeding coefficient (F_{IS}) were calculated using the R package *hierfstat* (Goudet 2005). Private alleles were determined using the R package *poppr* (Kamvar, Brooks, and

Grünwald 2015). For the full dataset, normality and pairwise significance of H_O and H_E were assessed using the R commands 'shapiro.test' and 'kruskal.test' and the R package *dunn.test* (Dinno 2024); F_{IS} 95% confidence intervals (CI) were calculated in *hierfstat* using the 'boot.ppfis' command (nboot = 100) to determine whether F_{IS} values were significantly different from zero.

2.6 | Population Structure

Population structure analyses were also performed on all datasets. The fixation index (F_{ST}) was calculated using the R package *STaMPP* (nboots = 100, percent = 95) (Pembleton, Cogan, and Forster 2013) to compare pairwise differentiation between populations. Principal component analyses (PCAs) were generated using the R package *adegenet* (Luu, Bazin, and Blum 2017) to assess genetic differentiation across populations. Individual blade ancestry was determined for the full, neutral and outlier datasets for optimal K using the R package *LEA* (Frichot et al. 2014). Optimal K, which describes the number of clusters that minimises within-cluster variation, was identified for each dataset as the K value with the lowest cross-entropy score (full and neutral datasets: K = 1:12, repetitions = 1000, entropy = TRUE, alpha = 100; outlier dataset: K = 1:7, repetitions = 600, entropy = TRUE, alpha = 100). In order to verify lineage divergence between the two *Z. capensis* lineages identified by Phair et al. (2019) across the study area, individual blade ancestry was also investigated at K = 2 for the full dataset (K = 2, repetitions = 1,000, entropy = TRUE, alpha = 100; Frichot et al. 2014).

3 | Results

3.1 | Data Processing and Bioinformatic Analyses

A total of 355,472,888 raw reads, with an average of 3,702,843 reads per leaf blade was received. The de novo pipeline assembly executed in Stacks v2.59 (Rochette, Rivera-Colón, and Catchen 2019) on the original dataset of 96 individuals retained 2401 variant sites for downstream analyses (hereafter 'loci'). Six of the 96 original samples were missing more than 40% of detected loci and were removed from the dataset (one each from Swartkops, Bushmans and Kariega and three from Nahoon), resulting in 90 individuals retained.

Pairwise relatedness analyses revealed three pairs of leaf blades (two from Nahoon and one from Bushmans and Kariega) with high kinship coefficients ($\phi > 0.33$) that were sequenced in adjacent wells (Table S1). All six individual blades were removed from the dataset as a precaution against including laboratory duplicates in the analyses, bringing the dataset to $n = 84$ individuals.

During clone and sampling duplicate detection, pairwise relatedness kinship coefficients (ϕ) identified 10 pairs and one trio of individual leaf blades (11 groups; Table S2) that were related above the threshold of identical genotypes ($\phi > 0.354$; Manichaikul et al. 2010). These groups were found in four estuaries: two in Swartkops, three in Bushmans, two in Kariega and four in Nahoon; no identical genotypes were detected across estuaries. Additionally, 52 second-degree relationships ($\phi = 0.088–0.177$) and 214 third-degree relationships ($\phi = 0.044–0.088$) relationships were detected within the dataset (Table S3). The only occurrences of second- or third-degree relationships between samples from different estuaries were observed between Bushmans and Kariega (15 second-degree relationships, $\phi = 0.088–0.177$; 12 third-degree relationships, $\phi = 0.044–0.088$) and Kariega and Kowie (2 third-degree relationships, $\phi = 0.044–0.088$; Table S3). The Jaccard Index approach (Yu et al. 2023) identified the same set of 10 pairs and one trio of individual leaf blades (11 groups; Table S4) as having highly similar genotypes using a threshold of SH > 0.90 (Figure S1).

Of the 11 groups with highly similar genotypes, seven were considered putative clones, as they were detected in Swartkops, Bushmans, Kariega and Kowie estuaries, whereas four were detected in Nahoon and were thus considered putative duplicate or triplicate samples because these individual leaf blades were sampled directly next to each other. One leaf blade from each of these 11 groups was retained in the dataset with other blades removed. As such, the final dataset consisted of 72 individuals, with between eight to 17 individuals per population (Table 1). The de novo pipeline, when run on the final dataset ($n = 72$), retained 2681 variant sites (hereafter 'loci'). The distribution of missing data at each locus was visualised across all populations (Figure S2) and considered for each population (Table S5) and genotype (Table S6).

TABLE 1 | Population statistics of genomic diversity across five *Zostera capensis* populations in the Southeastern Cape calculated from the full SNP dataset (2681 SNPs) in *hierfstat* (Goudet 2005) and *poppr* (Kamvar, Brooks, and Grünwald 2015).

Population	*N*	Private	H_o (±SE)	H_E (±SE)	F_{IS} (±SE)
Swartkops	17	344	0.15 (±0.00)	0.29 (±0.00)	0.49 (±0.02)
Bushmans	14	20	0.17 (±0.00)	0.32 (±0.00)	0.47 (±0.02)
Kariega	16	108	0.20 (±0.00)	0.27 (±0.00)	0.26 (±0.02)
Kowie	17	124	0.18 (±0.00)	0.28 (±0.00)	0.36 (±0.02)
Nahoon	8	121	0.13 (±0.00)	0.27 (±0.00)	0.51 (±0.02)

Note: All inbreeding coefficients (F_{IS}) were significantly different from zero.
Abbreviations: F_{IS} = inbreeding coefficient, H_E = expected heterozygosity, H_O = observed heterozygosity, N = number of individuals, Private = number of private SNPs, SE = standard error.

3.2 | Detection of Putative Outlier SNPs

A total of 201 outlier SNPs were detected by at least one outlier detection method, of which 32 were detected by both PCadapt and sNMF analysis and retained as the 'outlier dataset'. No outliers were detected by BayeScan (Figure S3).

3.3 | Genomic Diversity

For the full dataset, comprising both putative neutral and outlier loci, H_O ranged from 0.13 to 0.20 and was significantly higher in the central three populations (Bushmans, Kariega and Kowie; Kruskal–Wallis, H = 362.5, df = 4, $p = 0$; Dunn's test with Benjamini–Hochberg correction, $p < 0.05$ for all pairwise population comparisons; Table 1). Expected heterozygosity (H_E) ranged from 0.27 to 0.32 and was significantly higher in the two westernmost populations (Swartkops and Bushmans; Kruskal–Wallis, H = 111.0, df = 4, $p = 0$; Dunn's test with Benjamini–Hochberg correction, $p < 0.05$ for all pairwise comparisons with easterly populations; Table 1). Estimates of F_{IS} were always positive and significantly different from zero (95% CIs: SW = 0.47–0.51; BU = 0.45–0.49; KA = 0.24–0.28; KO = 0.34–0.38; NA = 0.49–0.54), with F_{IS} highest in the edge populations and lowest in populations at the centre of the sampled distribution (Table 1). The results for the neutral and the outlier datasets showed overall similar patterns to that of the full dataset (Table S1).

3.4 | Population Structure

Pairwise F_{ST} values for the full, neutral and outlier datasets ranged from $F_{ST} = 0.03–0.17$ ($p = 0.00$), $F_{ST} = 0.05–0.34$ ($p = 0.00$) and $F_{ST} = 0.13–0.70$, respectively ($p < 0.05$; Table 2). Overall, pairwise F_{ST} values were lowest between the three central population pairs, with the least differentiation observed between the two populations located closest together (Bushmans and Kariega; Table 2). Swartkops had the highest number of private SNPs (Tables 1 and S7). The PCA plots showed a similar pattern to that retrieved by pairwise F_{ST} analyses, with the central populations clustering and in an intermediated position between the margin populations, Swartkops and Nahoon (Figure 2a). Admixture and optimal clustering (K) analyses showed that all populations had unique population genomic signals and showed varying levels of admixture between populations, with the highest levels of admixture seen between Bushmans and Kariega (Figure 2b). Patterns of genomic variation differed between the full (K = 5), neutral (K = 4) and outlier (K = 5) datasets (Figure 2) but support multiple, divergent populations. An admixture analysis run for K = 2 for the full dataset verified the separation of two major *Z. capensis* lineages identified across the study region

TABLE 2 | Pairwise F_{ST} values between five *Zostera capensis* populations pairs in the Eastern Cape calculated from the (a) full (2681 SNPs), (b) neutral (2649 SNPs) and (c) outlier (32 SNPs) SNP datasets in *STaMPP* (Pembleton, Cogan, and Forster 2013).

a. Full dataset (2681 SNPs)				
	Bushmans	**Kariega**	**Kowie**	**Nahoon**
Swartkops	0.16	0.16	0.14	0.17
Bushmans		0.03	0.08	0.16
Kariega			0.07	0.16
Kowie				0.14
b. Neutral dataset (2649 SNPs)				
	Bushmans	**Kariega**	**Kowie**	**Nahoon**
Swartkops	0.18	0.20	0.19	0.33
Bushmans		0.05	0.10	0.31
Kariega			0.09	0.34
Kowie				0.34
c. Outlier dataset (32 SNPs)				
	Bushmans	**Kariega**	**Kowie**	**Nahoon**
Swartkops	0.59	0.13	0.52	0.70
Bushmans		0.13	0.22	0.64
Kariega			0.24	0.55
Kowie				0.66

Note: All pairwise comparisons for all datasets were significantly different ($p < 0.05$).

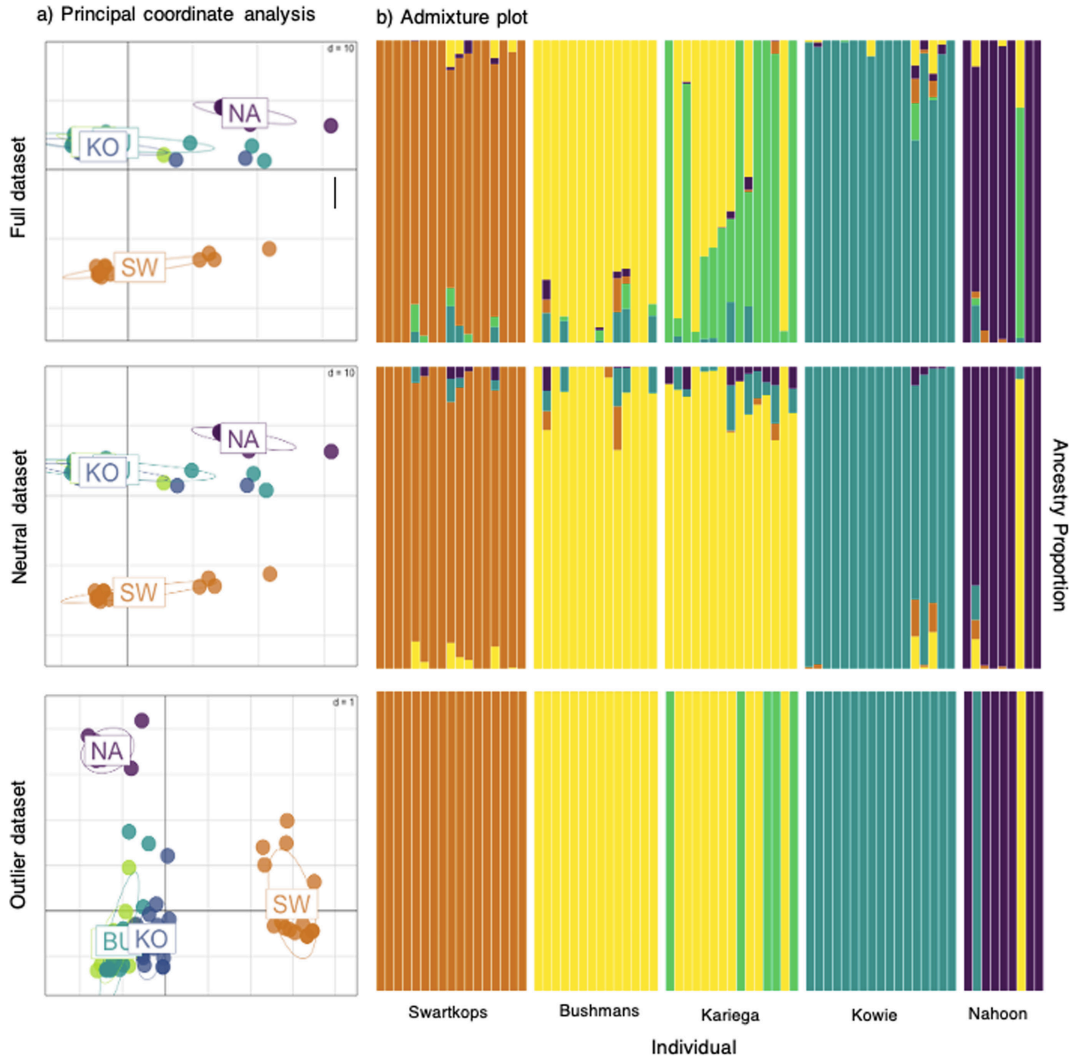

FIGURE 2 | (a) PCA and (b) admixture plots for five *Zostera capensis* populations in the Southeastern Cape, constructed from full (2681 SNPs), neutral (2649 SNPs) and outlier datasets (32 SNPs) at optimal K for each dataset.

by Phair et al. (2019), with Swartkops falling into a 'western' cluster and all remaining populations comprising a distinct 'eastern' cluster (Figure 3).

4 | Discussion

4.1 | Strong Population Genetic Structure Despite Admixture and Potentially Unsampled Populations

The patterns of extant genomic variation of natural populations are shaped both by historical and contemporary processes (Phair et al. 2019; Edgeloe et al. 2022) and in the Anthropocene are important considerations for the conservation and management of species (von der Heyden 2009; Carvalho et al. 2017; Nielsen et al. 2023). Historical and contemporary drivers of genomic diversity in seagrass include somatic mutation and gene flow, which can introduce genomic variation (Yu et al. 2020), changes in ploidy level (Edgeloe et al. 2022) and stochastic events such as palaeoclimatic and tectonic developments that are linked to population bottlenecks and/or disruption of gene flow (Procaccini, Olsen, and Reusch 2007; van Dijk et al. 2009; Phair et al. 2019), as well as adaptation to local climatic conditions (Ruocco et al. 2022). Further, in seagrasses, levels of gene flow within and between populations are influenced by dispersal patterns of pollen, seeds and vegetative fragments, as well as their recruitment success (van Dijk et al. 2009; Kendrick et al. 2017). Given that admixture between populations and lineages is an important source of genomic variation in seagrasses (Suarez-Gonzalez, Lexer, and Cronk 2018; Aguillon et al. 2022), the presence of two divergent lineages in the South African *Z. capensis* (which were resolved with additional SNP data in this study, as well as highlighted previously; Phair et al. 2019) provides a unique opportunity not only to disentangle population structure but also to provide insights into the processes driving these patterns.

Overall, strong population genomic structure was detected across all populations, which were also characterised by a number of private alleles (with private alleles per population ranging from 0.75% to 12.8%), suggesting negligible gene flow between most populations. This is likely a consequence of the confinement of *Z. capensis* to sheltered estuarine habitats and the absence of propagules that can withstand transport along South Africa's high-energy coastal environment

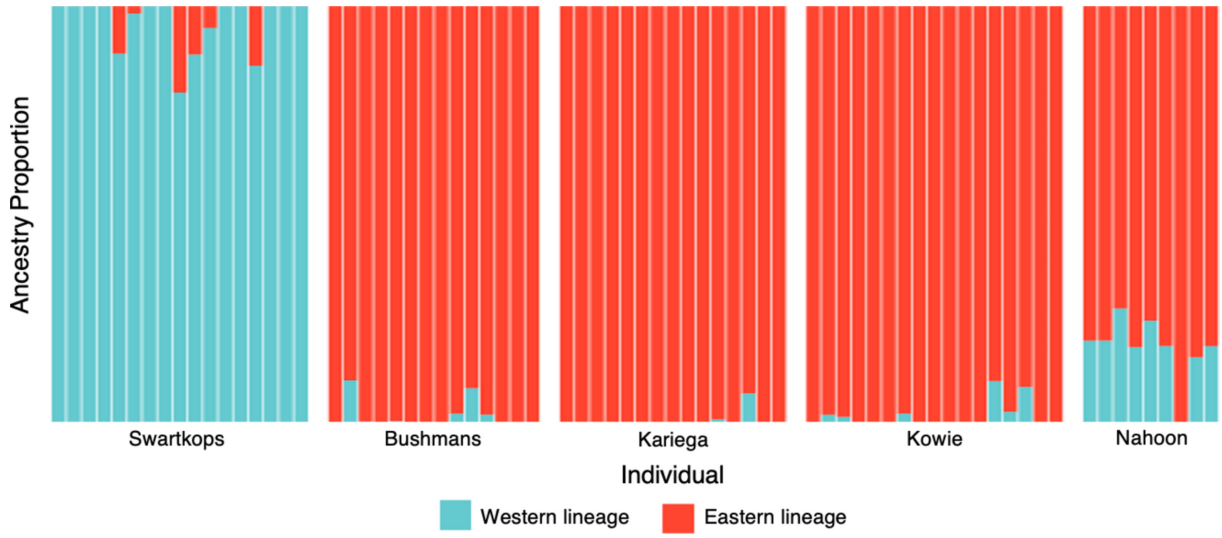

FIGURE 3 | Admixture plots of five *Zostera capensis* populations for the full SNP dataset (2681 SNPs) implemented in *LEA* (Frichot et al. 2014) with clustering set at K = 2 to confirms the divergence of two major *Z. capensis* lineages identified by Phair et al. (2019).

(Smit et al. 2023). The assignment of as many genetic clusters as estuaries (K = 5) for the full and outlier datasets corroborates previous findings that show strong population differentiation in *Z. capensis* even at small geographic scales and in adjacent estuaries (Phair et al. 2019; Jackson 2022; Smit et al. 2023). Further, the signal of two populations with admixture in Kariega suggests that multiple genetic populations of *Z. capensis* may coexist within one estuary. Several studies have called into question the appropriateness of applying the traditional concept of populations to partially clonal species, which can exist as highly differentiated clusters even at small spatial scales or as entire populations comprised of a single individual (Becheler et al. 2010; Kendrick et al. 2017; Bricker et al. 2018; Arnaud-Haond, Stoeckel, and Bailleul 2020; Edgeloe et al. 2022).

Generally, we detected low levels of admixture across populations, except for Bushmans and Kariega, which are geographically close (~2.5 km) and show considerable admixture with each other, although each is also characterised by private alleles. However, to fully understand drivers of population structuring of *Z. capensis* in this highly dynamic oceanographic area, biophysical models considering the effects of currents, winds, seascapes and biotic factors on propagule movement, in combination with genetic approaches and estimated distance within and between estuaries, will further our understanding of seagrass population dynamics in a region with unique oceanographic conditions (Griffiths et al. 2010). In several seagrass species, biophysical models have already demonstrated the important role of dispersal in accounting for genetic structuring (Jahnke and Jonsson 2022) and showed that dispersal can be limited even in seed-bearing marine angiosperms (Evans et al. 2021; Jahnke and Jonsson 2022) or can be mediated through other species (Tavares et al. 2022). As such, a stronger understanding of the basic biology of *Z. capensis*, such as mechanisms of reproduction, is vital for contextualising findings from molecular studies (von der Heyden et al. 2024).

4.2 | *Z. capensis* Populations May Be Limited in Sexual Reproduction

Although high levels of genetic diversity may be important facets of species persistence to changing climates (Reusch et al. 2005), numerous seagrass species globally are defined by a range of levels of clonality, where some clonal meadows can be thousands of years old (Bricker et al. 2018; Edgeloe et al. 2022). For *Z. capensis* in this study, the high and positive F_{IS} observed for all populations, coupled with the high level of relatedness found in some populations, suggests that individual plants within each population are more related to each other than expected under Hardy–Weinberg equilibrium and that only a limited number of genotypes may be contributing to the gene pool in each estuary. We also detected evidence of clones in several estuaries, which is not surprising given that the rhizomes of *Z. capensis* can potentially spread several metres (although the spread of *Z. capensis* rhizomes remains very poorly understood). However, the extent of clonality in this species remains unexplored and requires more targeted regional genomic efforts across numerous populations. Notably, seeds and flowers of *Z. capensis* are not regularly observed in the field (von der Heyden et al. 2024), which, in conjunction with the high inbreeding coefficients and the detection of seven putative clones in this dataset and no shared clones between populations, in addition to the strong signal of population structure, strongly suggest limited sexual reproduction in *Z. capensis*.

4.3 | *Z. capensis* in South Africa Urgently Requires Conservation and Management Efforts at the Individual Population Level

In South Africa, using a systematic conservation planning approach, Phair, Nielsen, and von der Heyden (2021) showed that the contemporary MPA network is not sufficient to adequately conserve the genomic diversity of *Z. capensis*, including their adaptive capacity. However, protecting genomic diversity in seagrasses is vital given the positive links with population resilience,

such as recovery from marine heatwaves and restoration success (Reusch et al. 2005; Phair et al. 2020; Pazzaglia et al. 2021). There is overwhelming evidence that populations of *Z. capensis* reflect unique genomic signals even at small spatial scales, such as in this study and Smit et al. (2023). Thus, it is crucial to urgently implement targeted conservation actions for seagrasses in South Africa before these unique signals are lost (Watson, Pillay, and von der Heyden 2023). This is especially true as *Z. capensis* is solely restricted to estuarine environments, which are highly threatened due to pressures from both marine and terrestrial environments (van Niekerk et al. 2022) and include a wide range of both climatic and anthropogenic pressures with several records of seagrass populations declines and regional extinctions (Adams 2016; von der Heyden et al. 2024). Currently, none of the extant 37 populations receive any directed management or conservation efforts, with only two populations in Langebaan and Knysna indirectly protected, although the Knysna meadows are subject to disturbance through activities such as bait collection even in protected areas due to, for example, poor enforcement of bait collection restrictions (Claassens et al. 2020; Mokumo, Adams, and von der Heyden 2023).

Within a South African context, to avoid further loss of seagrass meadows, their evolutionary potential and ecosystem services, relevant policy and legislation, through, for example, estuarine management plans, a requirement of the National Estuarine Management Protocol (NEMP) need to specifically identify and include seagrass habitats in their planning processes. This is difficult, given the extensive but entangled policy and legal mechanisms that currently underpin South Africa's coastal management (Taljaard, van Niekerk, and Weerts 2019). As such, given the combined findings of Phair et al. (2019), Jackson (2022), Smit et al. (2023) and this study, we strongly call for a precautionary approach where the management of individual seagrass populations and their estuaries is warranted. As such, estuarine management plans, which focus on specific estuaries, need to specifically zone seagrass protection areas (Janine Adams, pers. comm.). To facilitate protection, identifying stable meadows, i.e. those that are permanent for > 5 years in each estuary, is essential as it will provide a baseline for protection. These meadows could then also be targeted for screening of genomic metrics to understand key metrics such as clonality, diversity and divergence in order to better understand intra-estuarine, fine-scale evolutionary dynamics. However, the success of this approach is strongly tied to building a monitoring network, which can account for seagrass extent and changes in cover, information that is currently not available (von der Heyden et al. 2024). Overall, given the intense threats and dire outlook for the long-term persistence of *Z. capensis* throughout southern Africa, only a proactive and rapid response will increase the likelihood that seagrasses are 'here to stay' in the region.

Author Contributions

Charlotte A. Combrink: investigation–sampling, laboratory and data analysis, writing – original draft, writing – review and editing. **Romina Henriques:** supervision, investigation – data analysis, writing – review and editing, funding acquisition. **Megan J. Jackson:** writing – review and editing, funding acquisition. **Sophie von der Heyden:** conceptualisation, supervision, writing – review and editing, funding acquisition, project administration.

Acknowledgements

We thank Breyten van der Merwe for his help in conducting sampling and Dr Andrew Ndhlovu for his help with data handling and analysis.

Ethics Statement

All sampling was conducted under a permit issued by the Department of Environment, Forestry and Fisheries (2023/49).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw sequence data used in this study have been submitted to the Sequence Read Archive (SRA) database under accession number PRJNA1050843. Additional files (.vcf and R scripts) are available at www.github.com/vonderHeydenLab/Combrink_Zostera-capensis-scripts-and-files.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.