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Hybridization and Introgression in Black Hakes (*Merluccius polli and M. senegalensis*): Evolutionary Dynamics and Conservation Implications in the Contact Zone Exploited by Multi-Species Fisheries

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ABSTRACT

Hybridization is relatively common between closely related species that share part of their distribution. Understanding its dynamics is important both for conservation purposes and to determine its role as an evolutionary mechanism. Here we have studied the case of black hakes (*Merluccius polli* and *Merluccius senegalensis*) in its contact zone. The area of study is located in the FAO fishing area 34, in Mauritania and Senegal waters, where both species are exploited jointly in multi-species fisheries involving national and foreign fleets. Using a ddRADSeq approach and based on a set of 5820 SNPs and a total of 240 individuals, we identified one F_1 hybrid and several backcrossed individuals among 90 *M. polli* samples and none in 90 *M. senegalensis* samples obtained in 2020, suggesting unidirectional introgression towards *M. polli*. Hybridization signals were not found in any of the 60 historical samples from 2000. Excluding the hybrids and developing two separate sets of SNPs (5093 SNPs for *M. polli* and 2794 SNPs for *M. senegalensis*), our results detected two distinct genetic clusters within *M. polli* that show different genetic diversity estimates, with one of the clusters showing a higher potential vulnerability to exploitation. This pattern was observed in both contemporary and historical samples, and both groups presented subtle depth segregation. Moreover, 109 outlier loci were identified between the two groups, that could be developed into molecular markers to further study differentiation between both clusters and contribute to improved stock assessment and management of these important demersal resources.

1 | Introduction

Hybridization is a known driver of evolution (Abbott et al. 2013; Seehausen 2004). On the one hand, hybridization can negatively affect the involved populations due to post-zygotic barriers, which reduced the probability of producing viable interspecies offspring (Barton 2001; Moran et al. 2021). On the other hand, introgressive hybridization can integrate new advantageous variants in the gene pool of one or the two involved species, in a process called adaptive introgression (Edelman and Mallet 2021; Hedrick 2013). This could be at the origin of events of species radiation (Kautt et al. 2016;

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Meier et al. 2017; Seehausen 2004), or even be the cause of reverse speciation between two diverging species (Seehausen et al. 2008).

Secondary contacts zones, that is, regions where species overlap due to the loss of former barriers, or by expansion of one of them, are zones where hybridization is more likely (Abbott et al. 2013). For this reason, hybridization often occurs in the margins of species distributions, where these species may overlap (Chhatre et al. 2018; Faust et al. 2021). Populations at the edges of a species´ range often exhibit differential characteristics compared to those in the central part of the distribution. Edge populations are typically smaller (abundant-centre-hypothesis), have lower genetic diversity and exhibit reduced connectivity with other parts of the species´ distribution (Cahill and Levinton 2016; Fristoe et al. 2023). Due to the limited availability of partners, there may be higher chances of interspecific reproduction if one of the species is more abundant than the other (Qvarnström et al. 2023; Rohde et al. 2015). This effect is more accentuated in expanding distributions. Currently, the rise of temperatures is causing species displacements, provoking a poleward shift of their distribution ranges (Hastings et al. 2020; Hickling et al. 2006; Oke et al. 2023; Pinsky et al. 2020; Poloczanska et al. 2013). This trend may cause new species overlapping and overall important changes in their relative abundance (Gregory, Christophe, and Martin 2009; Maire et al. 2019; Taylor, Larson, and Harrison 2015; Warren et al. 2016). Accordingly, hybridization rate between species, as well as the zones where it occurs, will also be altered (Kersten et al. 2023). Identifying and understanding these dynamics is necessary for biological conservation, especially when hybrids exhibit reduced fitness compared with the pure species.

Fisheries might have a strong effect on the exploited species. At sea, fishing is a major driver of changes in wild populations (Planque et al. 2010). As many as 35.4% of marine stocks are overfished (FAO 2022). The combined effect of fisheries and species displacement driven by climate change must be carefully considered. Notably, multi-species fisheries exploit several species that are in some cases managed as single stocks. This is often the case of species that are morphologically similar, like hakes (Blanco-Fernandez et al. 2022; Garcia-Vazquez et al. 2012). These fisheries have to deal with changes in the relative abundance of the target species because of distributional shifts (Baudron et al. 2020; Collie, Wood, and Jeffries 2008; Perry et al. 2005). Cryptic species, distinct populations, and the overall genetic characterisation of stocks are rarely integrated into fisheries management (Waples et al. 2008). Consequently, hybridization is seldom considered or reported in fisheries studies. How the differential exploitation of overlapping species (e.g., species with different depth preferences targeted by various fishing techniques (Ward and Myers 2005)) might alter hybridization rates, has not been investigated so far.

Black hakes *Merluccius polli* and *Merluccius senegalensis* are two closely related species that are managed together in mixed fisheries (FAO 2020). They are caught jointly in the area where their distributions overlap, in the northern West African coasts (FAO Area 34) (Pitcher and Alheit 1995). These species are well differentiated, having diverged over a million years ago (Campo et al. 2009; Pérez et al. 2021). However, their morphological similarity makes them difficult for non-experts to distinguish (FAO 2020; Lloris, Matallanas, and Oliver 2003). Differences are subtle, primarily involving general coloration (*M. polli* tends to be darker, while *M. senegalensis* is generally lighter), caudal fin colouration (white in the edges only for *M. polli*), scale size or the number of gill rakers (Fall et al. 2018), among others. The morphological similarity lead to a high level of misreporting in catches, primarily due to a directionality on misidentifications, which could impact the overall conservation status of both species (Blanco-Fernandez et al. 2022).

These species live along West African coast in the East Atlantic Ocean. *M. senegalensis* ranges from 33.00° N to 10.00° N (Fernández-Peralta, Quintanilla, and Rey 2017; Lloris, Matallanas, and Oliver 2003), and the distribution for *M. polli* has been reported between 28.00° N and 18.30° S (Manchih et al. 2018; Lloris, Matallanas, and Oliver 2003). Therefore, they overlap from 28.00° N to 10.00° N latitude. *M. polli* has a preference for deeper waters in comparison to *M. senegalensis*, that is more common in shallower waters (Froese and Pauly 2021). Both species migrate southwards in summer and northwards in winter. They reproduce between October and March, with some overlap in their spawning grounds. *M. senegalensis* primarily spawns between 18°N and 19° N, while *M. polli* spawns between 16° and 18° N. They spawn at different depths: (*M. senegalensis* at 274–316 m, and *M. polli* at 566–653 m) (Fernandez-Peralta et al. 2011). Despite different spawning depth preferences within the *Merluccius* genus, certain species can hybridise under some circumstances. For example, *M. capensis* and *M. paradoxus* occupy different depth niches, but they can spawn together and hybridise in years of drastic environmental alterations like La Niña (Miralles, Machado-Schiaffino, and Garcia-Vazquez 2014). Other species of the *Merluccius* genus also hybridise, i.e. *M. albidus* and *M. bilinearis* (Machado-Schiaffino, Juanes, and Garcia-Vazquez 2010).

Given the previous records of hybridization within the genus, along with environmental changes associated with poleward shifts in species distribution (Hickling et al. 2006) and fluctuations in the relative abundance of the two species (Fernández-Peralta, Quintanilla, and Rey 2017), hybridization events between *M. polli* and *M. senegalensis* are likely. Mixed fisheries targeting black hakes are located near the edge of *M. polli*'s distribution, making it essential to consider potential changes in hybridization rates when managing these fisheries. Additionally, the role of hybridization as a potential evolution driver highlights the importance of characterising hybridization events between the two species.

To our knowledge, this is the first study about hybridization in black hakes. Using ddRAD-sequencing approach on historical and contemporary black hakes (*Merluccius polli* and *Merluccius senegalensis*) samples, we aim to (i) evaluate the level and extent of inter-specific gene flow (e.g., hybridization events) between the two species of black hake in their contact zone (Mauritania and Senegal waters), (ii) assess whether there is directionality in crosses and (iii) to characterise within-species genetic differentiation in the overlapping area where the two species are jointly exploited.

2 | Material & Methods

2.1 | Sampling

Tissue samples (fin) or whole individuals from *Merluccius polli* and *M. senegalensis* were obtained from commercial vessels fishing along the coasts of Mauritania and Senegal between 20.762° N and 14.95525° N, covering a distance of approximately 640 km in a straight line from the northernmost to the southernmost point (Figure 1). A total of 90 individuals of each species were sampled between November 2019 and September 2021. Samples were collected within the sampling range and, for analytical purposes, were divided in 3 groups (from north to south) of 30 individuals each to ensure an even distribution along the study region (Figure 1). Two campaigns were involved in the collection of these samples. All *M. senegalensis* samples and 60 *M. polli* samples were self-sampled by fishermen as part of the FARFISH project (H2020) as described in Blanco-Fernandez et al. (2022). Thirty *M. polli* samples were kindly provided by fishing boats landing in the Port of Cádiz. In addition, 30 historical samples from each species, collected in 2000 as part of the EU MARINEGGS project, were included to incorporate a temporal dimension into the analyses. This makes a total of 240 samples in this study.

Sample codes have the two first letters for the species (MP for *M. polli* and MS for *M. senegalensis*), followed by the group (Contemporary (C) or Historical (H)), and the identification number of sample from 001 to 250. For contemporary samples obtained from commercial fisheries (2019–2021), data on sex, length, depth and coordinates of the catch area were recorded; for the samples provided by the Port of Cadiz (2020), data on sex, length and weight were available (Table S1). Unfortunately, none of this data was available for the historical samples (2000). Samples of either fin or muscle were preserved in 100% ethanol and transported to the laboratory for further analyses.

The species identity of all the samples was verified using a barcoding approach on a 450 bp fragment from the mitochondrial Control Region, as published in Blanco-Fernandez et al. (2022).

2.2 | Library Preparation

DNA was extracted from fin or muscle of each sample depending on availability, using a standard DNA extraction protocol of phenol-chloroform. ddRADseq libraries were prepared following a protocol adapted from Peterson et al. (2012) and Recknagel et al. (2015). Briefly, after DNA extraction each sample was digested with the enzymes PstI and MpsI (New England Biolabs). The resulting fragments were ligated with combinations of two different Illumina adaptors (P1 and P2), enabling them to be pooled together. A fragment size range of 200 to 400 pb was set after simulating the number of digested fragments *in silico* using SimRAD (Lepais and Weir 2016), in R (v.4.1.1) (R Core Team 2021), against the *M. polli* reference genome (GenBank Accession No.: GCA_030340625.1) (Mateo et al. 2023). The size selection was performed in an agarose gel, followed by a PCR enrichment consisting of an initial denaturing step of 10s at 98°C, 15 cycles of 10s at 98°C, 30s at 65°C, 30s at 72°C and a final elongation step of 5 min at 72°C. Samples were randomly distributed into three different libraries and were sequenced in three lanes of Illumina HiSeqX in Macrogen Inc. (Korea), for paired-end reads of 150 bp.

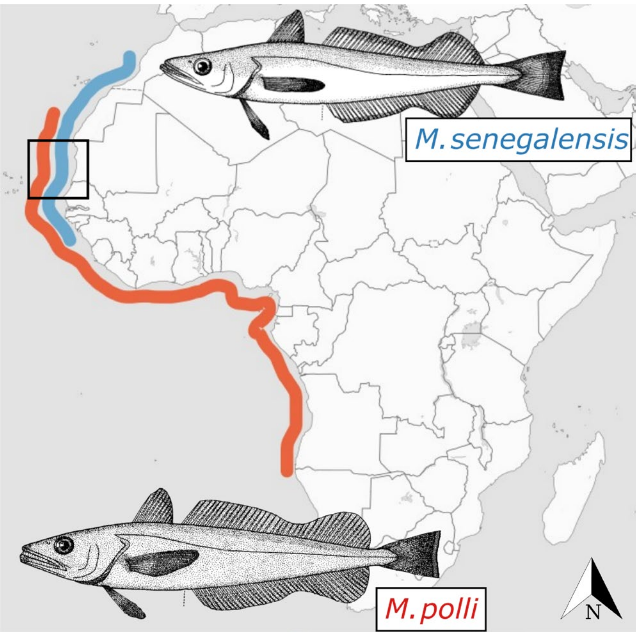

FIGURE 1 | Distribution of black hakes, based on Fernandez-Peralta et al. (2011); Fernández-Peralta, Quintanilla, and Rey (2017); Lloris, Matallanas, and Oliver (2005). *M. polli* in orange and *M. senegalensis* in blue. The black square indicates the sampling area, covering approximately 640 km from north to south.

2.3 | Data Filtering and SNP Calling

Raw reads were demultiplexed using *process_radtags* from STACKS v.2.54 (Catchen et al. 2013). Reads were truncated at 95 bp and those below a quality filter of 20 within a window of 0.15 were discarded. TRIMMOMATIC v.0.39 (Bolger, Lohse, and Usadel 2014) was used to trim the sequences. Then, resulting sequences were aligned to the reference genome for *M. polli* (GenBank Accession No.: GCA_030340625.1) using BWA v.0.7.17 (H. Li and Durbin 2009). Low quality alignments (quality below 25) and partial and secondary alignments were filtered out. After that, final alignments were used to build a catalogue of loci running *ref_map.pl* from STACKS v.2.54. Final SNP filtering was done using the *populations* script from STACKS. Three different catalogues were built. One included all samples from both species, and the other two separated by species were used for population analyses within species. Minimum allele frequency (maf) of 0.05 and keeping common presets were employed for the three different runs of *populations* (both species together, only *M. polli*, and only *M. senegalensis*). For the common analysis (considering both species), *populations* was run with −p 8 and −r 0.75. The analysis of both *M. polli* and *M. senegalensis* were run separately with −p 4 and −r 0.7. In all cases the minimum allele frequency (maf) was kept at 0.05, only one SNP was kept per *locus*, and SNPs out of Hardy–Weinberg equilibrium were filtered out except for the RDA analyses (see below). In all cases, samples with over 30% of missing data were filtered out.

2.4 | Inter-Species Genetic Differentiation and Hybrids Assessment

Data generated from the joint SNP panel, considering both species, was used to assess hybridization. Three different approaches were used for this process: ADMIXTURE (Alexander, Novembre, and Lange 2009), NEWHYBRIDS (Anderson and Thompson 2002), and *Introgress* (Gompert and Buerkle 2012). Sample clustering was visualised with a PCA using *adegenet* in R (Jombart 2008), and ADMIXTURE was run to analyse clustering among the samples. Given the separation between both species, $k=2$ was considered for the hybrid detection. ADMIXTURE $k=2$ results were used to select 10 putative parental individuals as a reference for the NEWHYBRIDS and *Introgress* analyses. The NEWHYBRIDS (Anderson and Thompson 2002) analysis was run with a burn-in of 50,000 and 500,000 sweeps. It was run 10 times using 10 random subsets of 200 SNPs due to the maximum loci limitation of the software. *Introgress* package was run in R to further categorise and select the hybrid candidates, and to check for the distribution of introgressed regions along the genome in the hybrid candidates. AMOVA was run in ARLEQUIN v.3.5.2.2 (Excoffier and Lischer 2010) to determine what percentage of variation was explained by the species in contrast to the percentage explained by groups and intraindividual variation.

2.5 | Within-Species Characterisation of Populations

Data from each single species was analysed using the same procedure. For these analyses candidate hybrids identified as explained in 2.4 were excluded. General statistics to assess genetic variation were calculated using R packages: *poppr* (Kamvar, Tabima, and Grünwald 2014), *diveRsity* (Keenan et al. 2013), *hierfstat* (Goudet and Jombart 2022). Population differentiation was first visualised with a PCA (*adegenet*) and supported by ADMIXTURE results. The optimal number of clusters was chosen with StructureSelector (Li and Liu 2018; Puechmaille 2016). When more than one genetic cluster was detected (as was the case for *M. polli*), clustering group was assigned by Axis 1 value from the PCA. For *M. polli*, the next analyses were performed to search for differences between genetic clusters. Differentiation between groups was calculated with pairwise F_{ST}'s in ARLEQUIN. The Axis 1 values obtained from PCA were compared across sampling variables to identify potential environmental factors (e.g., depth, proximity to coast) or morphological traits (e.g., length) that differentiate between clusters. These variables were available only for part of the contemporary samples (2019–2021); thus, the analysis was done only on those samples (38 out of 90 samples for *M. polli*). The variables included were length, sex, depth, latitude, sampling date, and distance to the coast. A PCA was run to visualise relationships between the variables, and a multiple regression analysis was run in PAST 4.03 (Hammer, Harper, and Ryan 2001) to assess whether the measured variables partially explained the genetic differentiation found between genetic clusters. For this multiple regression analysis, the values of the Axis 1, previously obtained from the genetic distance PCA (see above), was considered as the dependent variable, representing how genetically distant a sample is from the other group.

We searched for outlier loci between genetic clusters using BayeScan (Foll and Gaggiotti 2008). In order to search for adaptation signatures, Redundancy Analyses (RDA) were performed against Depth, Sex and Length using all SNPs (no HWE filter) to detect loci significantly associated to these variables. Contemporary samples (2019–2021) (See Table S1) were used for this analysis, since the others did not include data on these variables. Significant loci from both the RDA and the BayeScan analysis were mapped to the reference genome and annotated.

Finally, effective population sizes for both species were inferred using the Linkage Disequilibrium Method in NeEstimator2 (Do et al. 2014) on the contemporary samples (2019–2021). Since NeEstimator2 estimates population size from linkage disequilibrium, SNPs from the same contig were filtered out, keeping one at random to avoid bias on the analysis. The analysis was run separately for each genetic cluster (one single analysis for *M. senegalensis*, and two in *M. polli*).

3 | Results

3.1 | Data Analysis and SNPs Filtering

A total of 3,165,470,046 raw reads were obtained. After quality filtering with *process_radtags* and TRIMMOMATIC, 1,653,594,842 reads were retained. Then, the reads were mapped to the reference genome (GenBank Accession No.: GCA_030340625.1), and all resulting alignments were processed jointly with STACKS. For the study of hybridization, a total of 404,923 loci were genotyped with mean depth coverage of 22.0× and standard deviation of 12.9×. After filtering, 5820 SNPs were retained.

STACKS run on *M. polli* samples alone, generated a total of 300,124 markers with mean depth coverage of 25.4× and standard deviation of 11.6×, keeping 5093 SNPs that suited the filtering criteria after running *populations*. Following the same procedure, 244,311 loci were genotyped when STACKS was run with *M. senegalensis* samples only, with mean depth of 20.4×, and standard deviation of 11.7×. After running *populations* 2794 *M. senegalensis* SNPs were finally kept. For the RDA analyses, *populations* was run for each species separately including SNPs out of HWE and excluding hybrid individuals. This analysis kept 6763 SNPs for *M. polli* and 3838 SNPs for *M. senegalensis*.

3.2 | Inter-Species Genetic Differentiation and Hybrid Detection

Taking the joint dataset containing both species, AMOVA results showed that most of the variation in this dataset was explained by the variation among groups (90.60%), supporting that

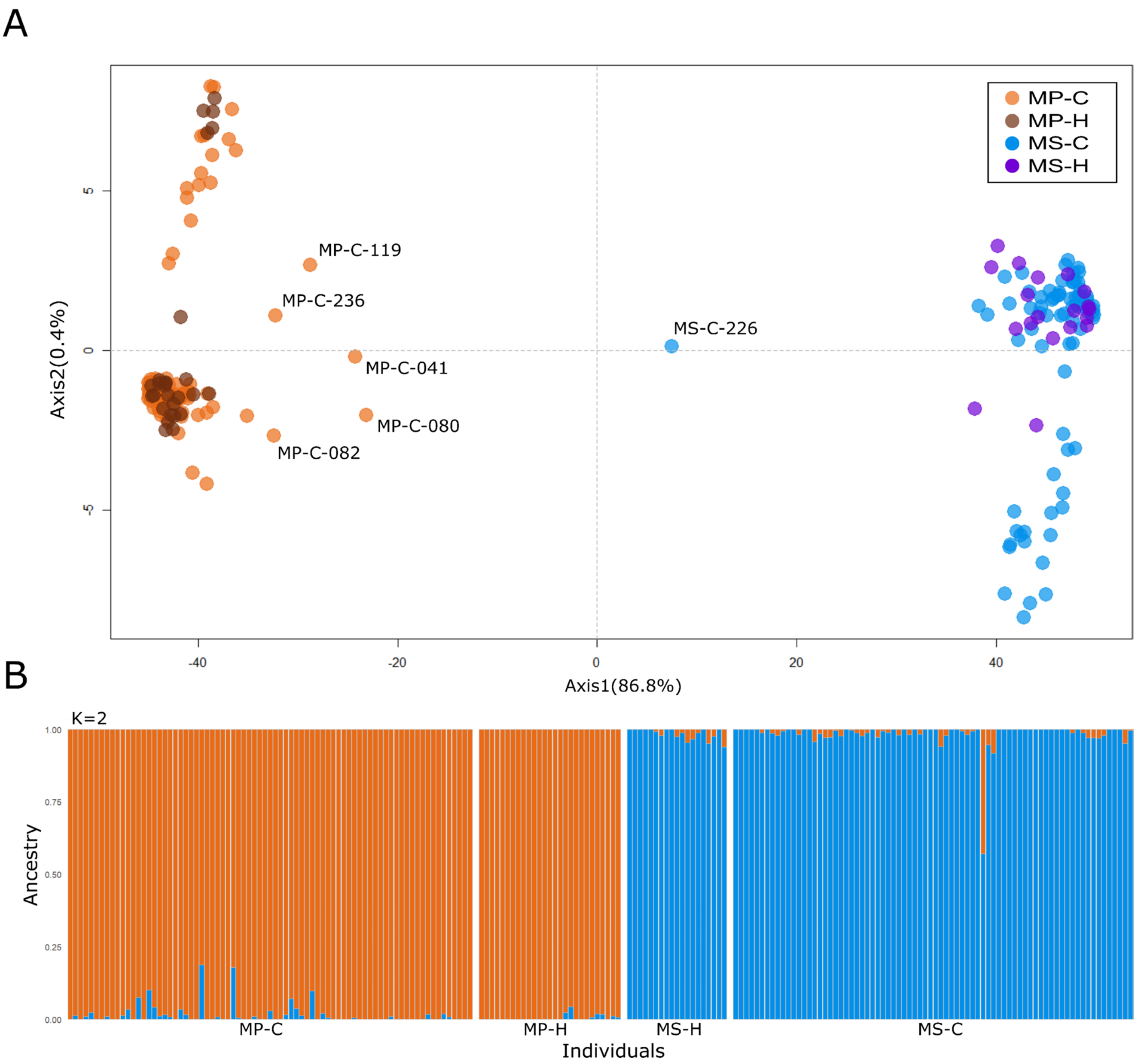

FIGURE 2 | Genetic differentiation between species. (A) PCA plot showing genetic differentiation between both species. *M. senegalensis* are shown in blue (contemporary) and violet (historical) and *M. polli* are shown in orange (contemporary) and brown (historical). (B) Admixture plot ($K=2$) showing genetic differentiation between both species. Each bar represents one individual, and the different colours represent the percentage of contribution of each cluster to each sample. Sample coding: MS and MP, *M. senegalensis* and *M. polli* respectively; C, contemporary and H, historical samples.

these species are well differentiated (Table S2). This was also reflected in the PCA and the ADMIXTURE (Figure 2). The PCA showed a clear separation between species (Figure 2A), with most of the variation explained by Axis 1 (86.8%). Both species formed clusters in opposite sides of the axis, but some of the samples were distributed towards a central position along the axis 1: sample MS-N-226 appeared in the centre of the graph, and five samples assigned as *M. polli*, MP-C-080, MP-C-041, MP-C-119, MP-C-236 and MP-C-082, were also close to the centre in comparison to the rest of the *M. polli* samples (Figure 2A).

Similar results were obtained from ADMIXTURE analysis (Figure 2B). Within all the samples assigned to *M. polli*, samples MP-C-041 and MP-C-080 were assigned to the *M. senegalensis* cluster over a 15%. Within *M. senegalensis* samples, MS-C-226 exhibited evident signs of admixture, with 40.8% assigned to the *M. polli* cluster (Figure 2B).

The results of the NEWHYBRIDS analysis further assigned these intermediate samples as hybrids and categorised them either as F_1, F_2 or introgressed backcrosses. All iterations using different subsets gave the same assignations. Samples MP-C-040, MP-C-80 and MP-C-236 were assigned as a *M. polli* backcross with a probability of 1.0 across all iterations. MP-C-119 had a probability of 0.391 of being a *M. polli* backcross, and MS-N-226 was assigned as F_1 with a 100% in all iterations (Figure S1A).

Accordingly, a similar pattern involving the same samples was obtained from *introgress* analysis (Figure S1B). The combination of hybrid index and heterozygosity are similar to the results from the previous analysis (PCA, Admixture and NewHybrids), suggesting that individuals MP-C-080, MP-C-041, MP-C-119, MP-C-236 and MP-C-082 are introgressed backcrosses for *M. polli* and MS-C-226 is a F_1 between the two *Merluccius* species. In contrast to the rest of the analyses, from *introgress* MS-C-247 could be a *M. senegalensis* backcross. The genomic clines did not show common genomic introgressed regions between the different backcrosses (Figure 3). This pointed to different events of hybridization as the origin of the backcrossed individuals. The hybrid and the introgressed individuals identified were five females (MP-C-080, MP-C-119, MP-C-236, MP-C-082 and MS-C-226) and one male (MP-C-041).

3.3 | Intra-Species Genetic Differentiation

3.3.1 | *Merluccius senegalensis*

We did not find significant genetic differentiation across *M. senegalensis*, either in historical or contemporary samples (Figure 4A). PCA did not show any differentiation between groups, with the first axis explaining only 1.5% of the variation. The ADMIXTURE analysis also suggests all samples belong to the same group (Figure S2). Global expected heterozygosity averaged 0.231 (Table 1). The inbreeding coefficients (F_{IS}) were all close to 0 (F_{IS} range: −0.004; Table 1).

For *M. senegalensis* the estimated Ne was 1999.7, with a 95% confidence interval between 1463.4 and 3143.6.

3.3.2 | *Merluccius polli*

We found genetic structure within *M. polli* samples. PCA analysis showed two differentiated clusters (Figure 4B). The first two axes explained 2.8% and 2.4% of variance respectively, higher percentages than in the case of *M. senegalensis* (Figure 4A). These clusters were further supported by the ADMIXTURE analysis, which showed an optimal clustering of two (Figure S3). *M. polli* samples were then grouped according to their cluster assignation as MP-01 and MP-02. Samples were assigned to clusters regardless of their geographic or temporal distribution (contemporary and historical samples were equally represented in the two clusters; $\chi^2 = 2.035$; $p = 0.154$). We found a higher number of private alleles in MP-01 than in MP-02; 77 and 8, respectively. The rest of the genetic diversity indexes (expected heterozygosity, allele richness, nucleotide diversity) were similar in the two clusters yet slightly higher in MP-1 (Table 1). F_{ST} values between these groups also supported differentiation between the two different genetic clusters regardless the sampling period—2000 or 2020 decade (Table 2).

Effective population sizes were estimated separately for each group with the disequilibrium linkage method. Within *M. polli*, the cluster MP-01 was estimated to have 2318.5 Ne with a 95%

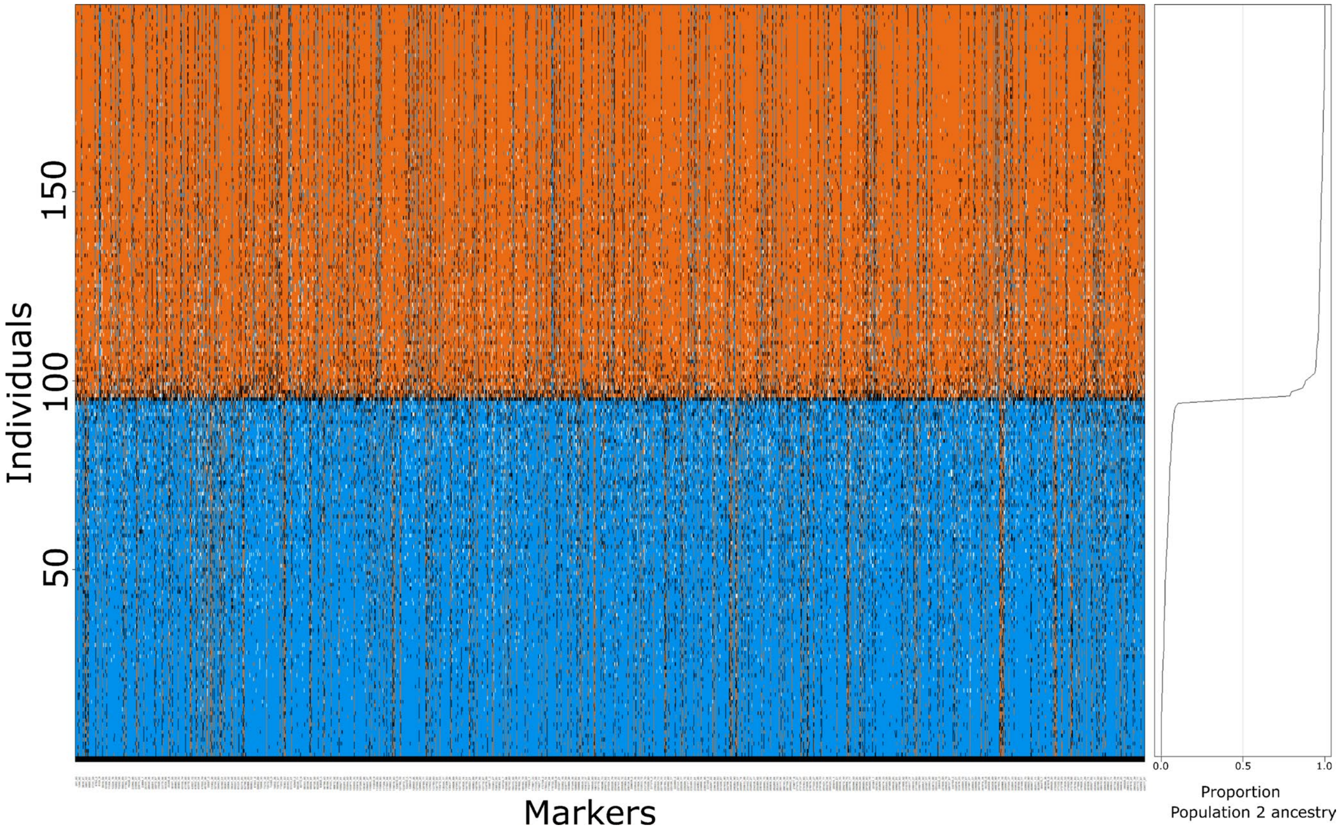

FIGURE 3 | Genomic Cline showing the assignation of every bi-allelic locus (columns) as assigned to one of the species for each sample (rows). For each locus, one of the alleles is assigned to one of the species, and the second one is assigned to the second species. Orange is assigned to homozygotes of the allele associated to *M. polli*, blue represents homozygotes for the allele associated to *M. senegalensis*, and black represents heterozygotes.

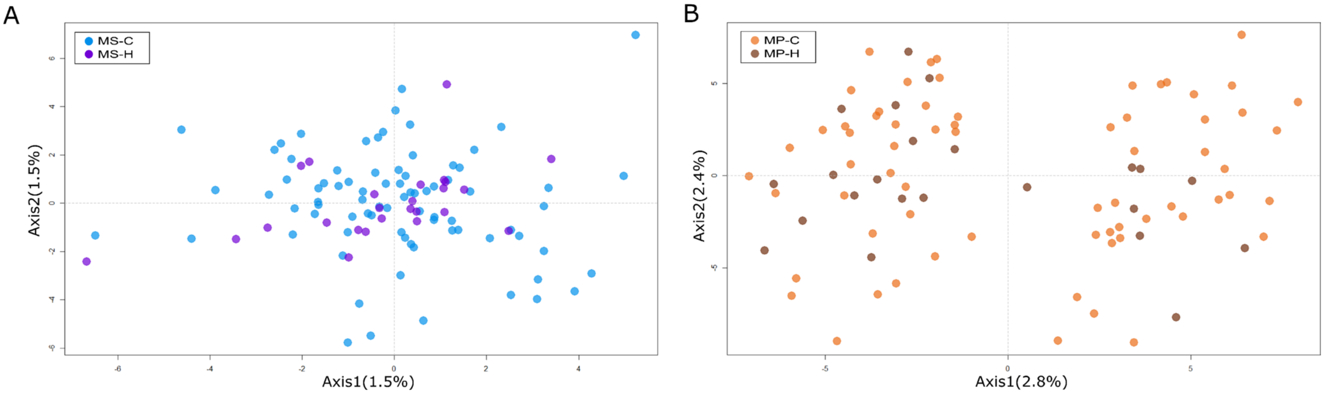

FIGURE 4 | Within species genetic differentiation. PCA plot showcasing genetic distances between (A) *Merluccius senegalensis* (MS) and (B) *Merluccius polli* (MP) samples. The samples are presented as follows: MS-C in blue, MS-H in violet, MP-C in orange and MP-H in brown. C, contemporary; H, historical samples.

TABLE 1 | Genetic diversity statistics for *Merluccius senegalensis* (MS) and each cluster of *Merluccius polli* (MP).

Pop ID	Private alleles	Allelic richness	Observed heterozigosity	Expected heterozygosity	π	F_{IS}
MS	—	1.975	0.232	0.231	0.233	−0.004
MP-01	77	1.783	0.275	0.258	0.262	−0.065
MP-02	8	1.756	0.251	0.249	0.254	−0.005

Note: From left to right private alleles between *Merluccius polli* clusters, allelic richness, observed heterozygosity, expected heterozygosity, nucleotide diversity (π), and inbreeding coefficient (F_{IS}).

TABLE 2 | Calculated F_{ST} values for genetic differentiation between different groups of *M. polli*.

FST/*p*-Value	MP-C1	MP-C2	MP-H1	MP-H2
MP-01-C		**< 0.0001**	0.516	**< 0.0001**
MP-02-C	**0.014**		**< 0.0001**	0.129
MP-01-H	−0.003	**0.017**		**< 0.0001**
MP-02-H	**0.017**	0.001	0.000	

Note: Bottom diagonal shows pairwise F_{ST} values for each comparison, and top diagonal shows their corresponding *p*-values. Significant values are shown in bold. MP-01 and MP-02 represent the two genetic clusters. C, contemporary samples (2020); H, historical samples (2000).

confidence interval between 1347.0 and 8195.7, and the genetic cluster MP-02 had 198.2 Ne with a 95% confidence interval between 184.1 and 214.5.

3.3.3 | Inferred Factors Contributing to *M. polli* Cluster Differentiation

In the multiple regression analysis, the dependent variable was genetic distance (between *M. polli* clusters: MP-01 and MP-02) as measured from the values of the first component of the PCA (Figure 4B), and the independent variables geographical location (latitude), distance to the coast, depth, date of capture, length and sex of the individual. Genetic differentiation between *M. polli* clusters was significantly explained by depth and sampling date (Table 3). From the sign of the coefficient, MP-01 would contain individuals caught at a greater depths. For MP-01, the average depth was 515.61 ± 107.62 m and for MP-02, the average depth was 473.80 ± 118.73 m.

The relationships among the variables included in the multiple regression can be seen in the PCA scatter plot (Figure 5A). PC1 of the PCA explains 40.1% of the variance and PC2 explains 21.7% (See eigenvalues and factor loadings for the first two components in Table S3).

BayeScan analysis revealed 109 outliers between the two genetic clusters of *M. polli* (Figure S4). Mapping of these outliers to the reference genome showed that 16 of them are located in exon regions. Out of this, were synonymous changes and five were non-synonymous changes and fell in exons of the genes Harbi1_156, znf598, Gga3_1, cdr2l_1, radil_1 (full list in Table S4).

RDA analysis contrasted SNPs variation to depth, length and sex. Different SNPs were significantly correlated to each of these variables for each genetic cluster (See Figure 5B,C for *M. polli* and D for *M. senegalensis*). Most remarkably, 64 SNPs (27 of them fall in exons, four of which are non-synonymous changes) were correlated to Sex in *M. senegalensis* (see Figure 5C). Ten SNPs (four in exons) were related to length of the fish, and 11 SNPs (one in an exon) had a significant correlation to the depth of the samples. Regarding *M. polli* cluster MP-01, 12 SNPs (one in an exon) were significantly correlated to depth, seven SNPs (one fell in an exon and was a non-synonymous change) were

TABLE 3 | Multiple regression analysis with genetic distance as a dependent variable, and explanatory variables length and sex of the individuals, and depth, latitude, date and distance to coast of the capture as independent variables.

	Coefficient	SE	T	*p*	R^2
Constant	8.153	6.203	1.314	0.198	
Length	−0.017	0.030	−0.552	0.585	0.022
Sex	0.290	0.404	0.717	0.479	0.009
Depth	−0.007	0.003	−2.312	**0.028**	0.028
Latitude	−0.216	0.295	−0.731	0.470	0.002
Date	0.025	0.011	2.310	**0.028**	0.008
Distance to coast	0.000	0.000	−0.366	0.717	0.001

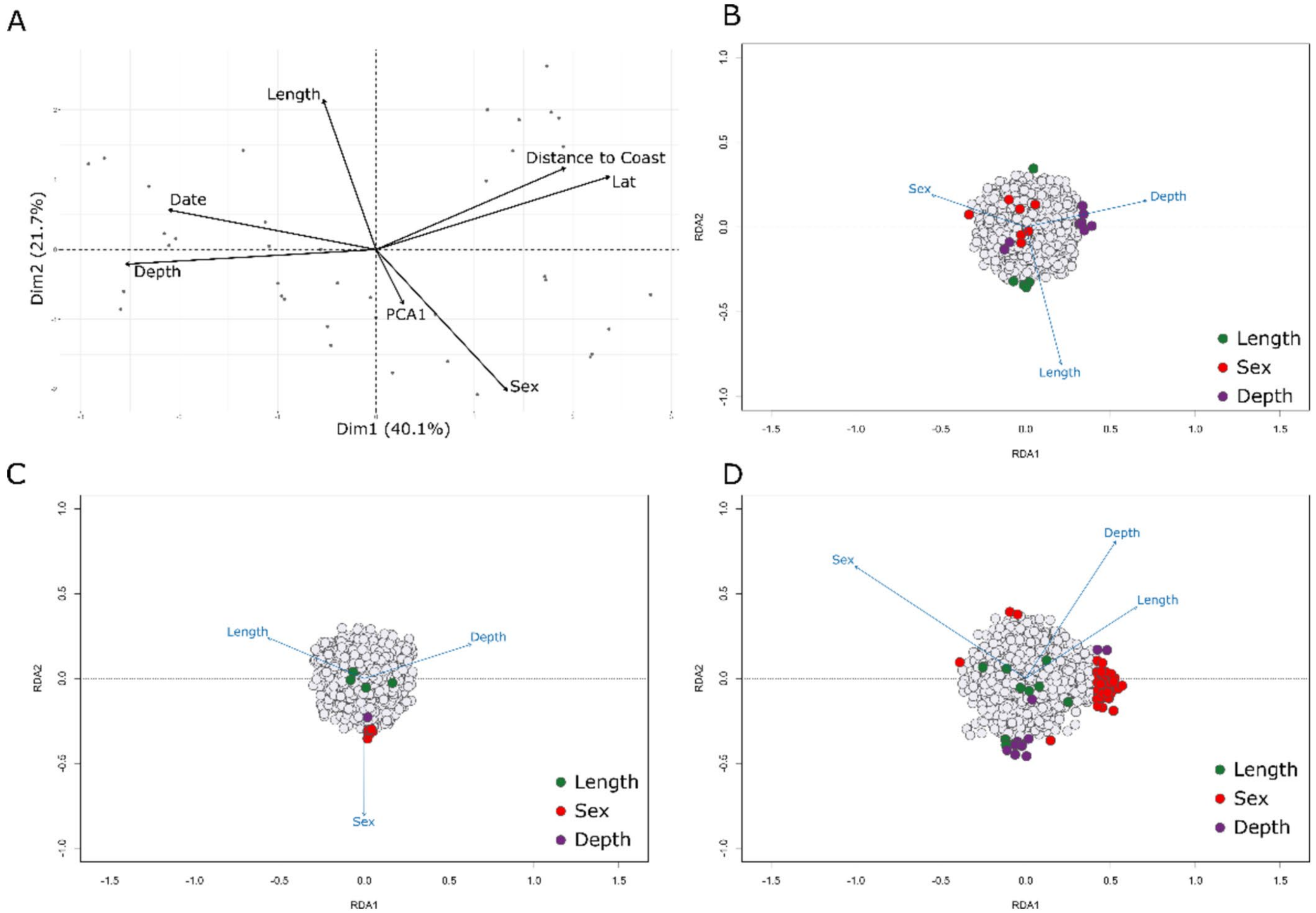

FIGURE 5 | Genetic differentiation among clusters and environmental variables. (A) Biplot showing the two first components of a PCA. Each arrow represents one of the explanatory variables (PCA_1 as a proxy for genetic differentiation, length and sex of the individuals, and depth, latitude, date, and distance to coast of the capture). Dots represent the individuals analysed. (B–D) Results from the RDA analysis. Plot showing the two main axes for both species (B. *Merluccius polli* genetic cluster 1, C. *M. polli* genetic cluster 2, and D. *M. senegalensis*). Each SNP is represented by a dot. Grey dots indicate non-significant SNPs for any of the variables. SNPs significantly correlated to Length are shown in green, those significantly correlated to Sex are in red, and SNPs significantly correlated to depth are highlighted in purple.

correlated to sex, and six SNPs (three in exons) were correlated to length. In *M. polli* genetic cluster MP-02, 20 SNPs (eight in exons, four of them associated with non-synonymous changes) were associated to sex, four SNPs had a significant correlation to length and only one SNP was associated with depth (full list in Table S4).

4 | Discussion

4.1 | Hybridization Between Black Hake Species

We found potential hybrids between the two hake species considered. All performed analyses delimited the species clearly, as expected, having diverged a long time ago (Pérez et al. 2021), but also the occurrence of intermediate genotypes at a genomic level. One potential F_1 hybrid with mitochondrial DNA of *M. senegalensis* lineage, and four backcrossed individuals between *M. polli* and hybrids point to introgression from the parental populations (e.g., *M. polli*), indicating viable offspring between hybrids and *M. polli*. Introgression was only found within *M. polli*, suggesting directionality in the crosses, as no clear introgressed individuals were found within *M. senegalensis*. This is the first time introgressive hybridization is found in black hakes. This discovery has profound implications at both the evolutionary and conservation levels, considering that these species are heavily over-fished (FAO 2020).

Incomplete lineage sorting can often be mistaken for hybridization (Wang et al. 2018), which is particularly relevant in sister species with short divergence times. However, in the specific case of *M. polli* and *M. senegalensis*, this scenario is unlikely. Several studies strongly support the early divergence (~1.9 MYA, Pérez et al. 2021) of two Euro-African lineages of hakes: one comprising *M. polli* and *M. paradoxus*, and another including *M. senegalensis*, *M. merluccius* and *M. capensis* (Roldán et al. 1999; Quinteiro, Vidal, and Rey-Méndez 2000; Campo et al. 2007).

Hybridization does not seem to be rare in the genus *Merluccius*. Moreover, a species within the genus, *M. australis*, has been suggested to have a hybrid origin (Campo et al. 2009; Pérez et al. 2021). Using microsatellites, hybrids were found between *M. albidus* and *M. bilinearis* along the East coast of North America (Machado-Schiaffino, Juanes, and Garcia-Vazquez 2010), and between *M. capensis* and *M. paradoxus* in southern Africa (Miralles, Machado-Schiaffino, and Garcia-Vazquez 2014). One of the overlapping species of each pair prefers shallower waters (i.e., *M. bilinearis, M. capensis*, *M. senegalensis*) and the other greater depths (i.e., *M. albidus*, *M. paradoxus*, *M. polli*). Perhaps hybridization occurs between other overlapping species within the genus, like *M. productus* and *M. angustimanus*, *M. capensis* and *M. polli* and *M. gayii* and *M. australis*. New genomic technologies will allow to study this genus in depth, since the use of panels of several thousand SNPs (over 5000 markers) contributes to a higher confidence in the hybrid assignment, allowing higher resolution and precision (Miralles et al. 2023; Szatmári et al. 2021; Zimmerman, Aldridge, and Oyler-Mccance 2020). In any case, results to date suggest that depth could be a barrier between *Merluccius* species, and that the barrier can be broken at least in some circumstances. Miralles, Machado-Schiaffino, and Garcia-Vazquez (2014) attributed hybridization to alterations of oxygen concentration in water, related with La Niña, that would force the species to move and spawn in similar depths. In the case of black hakes, the weakening of between-species barriers could be also related with climate, but it could be due to very different factors, as we will explain next.

Although based on a limited number of samples, there was a clear unidirectionality in the introgression, the five introgressed individuals having *M. polli* mitochondrial DNA only; thus, the mother in a hybrid cross, either the F_1 or the backcross, was *M. polli*. Directionality can be caused by asymmetrical pre- and/or post-mating barriers between the involved species (Li, Ohadi, and Mesgaran 2021; While et al. 2015), but also to the scarcity of conspecifics (Lepais et al. 2009; Mckelvey et al. 2016). This could be the case here. Surveys from the sampled area report a higher abundance of *M. polli* than of *M. senegalensis* (in a 4:1 ratio) (Fernández-Peralta, Quintanilla, and Rey 2017). Perhaps hybrids are more likely to reproduce with more abundant *M. polli* simply because of a higher probability of encounters (Lepais et al. 2009). The hypothesis of asymmetric barriers (mating only *M. polli* females with *M. senegalensis* males, or only hybrids of *M. polli* mother being viable) could be more difficult to explain, because the F_1 found with *M. senegalensis* mitochondrial DNA demonstrates that hybridization between *M. senegalensis* females and *M. polli* males is also possible, and the offspring can reach adulthood.

Interestingly, hybrids were only found in the modern set of samples, perhaps suggesting a potential increase of the hybridization over the last 20 years. It is worth noting that the sample size of historical samples was relatively small (27 *M. polli* and 19 *M. senegalensis* after all sample filtering) and this potential increase in hybridization rates must be taken with caution, as even if the rates are maintained, the chance of no finding any hybrid is not negligible. Given the percentage of introgression estimated within *M. polli* modern samples (five out of 77, 6.5%), finding around two introgressed individuals in the historical set would be expected ($0.065 \times 27 = 1.755$) outcome, if the rates were maintained over time. Furthermore, the introgressed samples do not show a common pattern of introgression (See Figure 3), suggesting that they come from different crosses instead of being issued from a common hybrid cross. An increase in recent hybridization would be consistent with northward shift of the *M. polli* distribution, supported from the distribution records of *M. polli*, whose northern boundary was first described as 20.00° N (Pitcher and Alheit 1995) and later widened to 28.00° N (Manchih et al. 2018). New records of black hake species landings in the Canary Islands for the first time have been described in González-Lorenzo et al. (2020). Fernández-Peralta, Quintanilla, and Rey (2017) also describe a relatively lower abundance of *M. senegalensis* than that reported 30 years earlier in Senegalese waters (López Abellán and Ariz Telleria 1993). Studying the relative abundance of these two species along the contact area could be useful to further understand the introgression patterns revealed in our study.

Indeed, climate variability affects black hakes. The North Atlantic Oscillation (NAO) has been linked to changes in the abundance of these species within Northwest African fisheries (Meiners et al. 2010). Likewise, temperature is key for fish reproduction, whose effects have been seen in different fishes (Fincham, Rijnsdorp, and Engelhard 2013; Jansen and Gislason 2011; Morgan, Wright, and Rideout 2013; Sandström et al. 1997). Increments of temperature could affect spawning areas, depths, and seasonality of both species, leading to greater overlapping in spawning and thus to an increase of introgressive hybridization.

Nevertheless, the number of introgressed individuals found, as well as the low density of markers is not enough for an in-depth study to detect signs of adaptive introgression. A higher of density of markers would allow to identify introgressed regions, if any are present (Edelman and Mallet 2021; Upadhyay et al. 2021).

The case of black hakes is not unique but is likely to occur in other fish groups, as the distribution ranges shift polewards, hybridization windows shift as well (Taylor, Larson, and Harrison 2015). For instance, in the case of horse mackerels (*Trachurus*), various species (*Trachurus trachurus*, *T. picturatus*, *T. mediterraneus*, and *T. capensis*) partially overlap their distributions in certain areas, making them potentially vulnerable to new hybridization events (Healey et al. 2020). Moreover, regardless of the effects on hybridization rates, changes on distribution may affect the management efforts of joint fisheries, accentuating the need for correct identification of stocks as the basic unit for management, and the damaging effects of pooled data for two or more morphologically similar species as is the case with black hake fisheries (Blanco-Fernandez et al. 2022).

4.2 | Implications of the Different Pattern of Genetic Structuring in Black Hakes

Within species, we expected to find no genetic differentiation due to the high mobility of hake. Generally, fishes with high mobility as such as is the case for many species within the Gadiformes order are characterised by low F_{ST}s between populations due to the high dispersal capabilities (Drinan et al. 2018; Salmenkova 2011). In other studies, exploring genetic structure in hakes, differentiation was found over long distances and often associated to obvious oceanographic barriers (García-De León et al. 2018; Longo et al. 2024; Machado-Schiaffino, Campo, and Garcia-Vazquez 2009; Machado-Schiaffino, Juanes, and Garcia-Vazquez 2011; Westgaard et al. 2017). The geographical scale of this study is much smaller (i.e., black hake fisheries from the coast of Mauritania and Senegal) and does not include any apparent oceanographic barrier that could imply genetic differentiation. As expected, we did not find any differentiation among the *M. senegalensis* samples. However, *M. polli* showed two differentiated genetic clusters. This clustering was not associated to geographic distance but to depth and date of capture that contributed significantly to explain the variance between clusters, suggesting some degree of segregation between the two groups. Notably, this structuring was found both in the contemporary and the historical set of samples, indicating consistency of this differentiation over time. In marine environments, depth is often a driver of genetic differentiation (Costantini et al. 2016; Piertney et al. 2023). In fishes, depth is one more spatial dimension that has to be considered when looking at spatial segregation within species (Stefánsson et al. 2009). Nevertheless, the differences in depth found between *M. polli* clusters are subtle and both groups have a great overlap. Spatial differences between the two groups may vary depending on seasonality and migration patterns (Goethel, Berger, and Cadrin 2023).

Despite these differences, significantly differentiated loci between the two clusters were not associated with any known depth-linked genes. Likewise, the same happened within the different groups (both clusters of *M. polli* and the population of *M. senegalensis*) in the RDA analysis, where most significant loci were associated with sex. However, it is not possible to fully discard adaptive differences between the groups, since ddRADseq is a reduced sequencing strategy, and many parts of the genome are left uncovered (Lowry et al. 2017). Further research into adaptative signals with techniques covering a higher percentage of the genomes (e.g., WGS) could identify stronger signals.

Genetic diversity was slightly higher in cluster MP-01 than in MP-02 (Table 1). Notably, MP-01 exhibited a higher number of private alleles than MP-02, and an effective population size (2318.5) one order of magnitude greater than the one estimated for MP-02 (198.2). This could have implications for the conservation of hakes within the fishery. Generally, populations of demersal fishes like hake are characterised by large censuses. Even if the effective population size is magnitudes smaller (Hauser and Carvalho 2008), the effect of genetic drift in these populations is expected to be minimal. However, with smaller effective population sizes, as seems to be the case of MP-02 (the shallower cluster), drift could have a stronger effect in the population (Willi et al. 2013). Overall, the occurrence of genetic differentiation within *M. polli* indicates that its fishery should be reconsidered taking into account a higher vulnerability (at least partially) of the smaller genetic group. It is advisable to integrate this structuring in the management of black hake fisheries, perhaps establishing differential quotas not only according to species, but also to the fishing depths. As seen for the case of identification between the two black hake species, due to the differences in depth preference for both, the depth of capture would influence the capture of one species over the other (Blanco-Fernandez et al. 2022). A similar pattern may be found between the two genetic clusters of *M. polli*. While there is evidence of limited gene flow, it is necessary to understand the extent of this differentiation: Distinct units may converge in time only seasonally (Cadrin 2020; Goethel, Berger, and Cadrin 2023). Moreover, different adaptations and life strategies may be reflected on the genetic structure. Genetic structure can be associated to differences in habitat, depth and different life strategies (Grabowski et al. 2011; Knutsen et al. 2009). Moreover, outlier loci has been previously associated with different ecotypes and the detection of fine scale population structure (Milano et al. 2014). We found 109 outlier loci between the two clusters that could be applied in fisheries management of Black hakes. They could be used for identification of the groups, and a marker set could be developed for further routine monitoring. Indeed, it is advisable to study this differentiation in more detail, determining its main drivers as well as its extension with more samples from other zones within the distribution range of *M. polli* and controlling for seasonality.

To our knowledge, this is the first genetic study conducted on either of these species. Both species are fairly understudied in all aspects, especially outside of the main area where they are exploited in these multi-species fisheries. Details about the biology of *M. polli* along most part of its distribution are generally unknown. There is an urgent need for a better understanding of the genetic structure of these species along their entire distribution, to ensure the sustainable management, not only of *M. polli*, but also of *M. senegalensis*.

5 | Conclusions

Our study aimed to disentangle underlying dynamics within the contact zone of black hakes, where mixed fisheries take place, and the interaction between the two species. We have found signs of hybridization between both species, and a directionality that suggests that introgression occurs mostly towards *M. polli*, most likely for opportunistic reasons.

In a Poleward shift of distributions scenario, it is likely that the rates of introgression in *M. polli* will increase in this area due to the relative increase of *M. polli* in its northern distribution area, where it overlaps with *M. senegalensis*. According to these results and other studies, hybridization is likely to be a common occurrence between overlapping *Merluccius* hake species.

We found differentiation in two genetic clusters within *M. polli*, partially associated with depth. It suggests different population units that should be further explored for a better management of the fishery.

Author Contributions

C.B.-F.: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft. J.R.-R.: Investigation, Methodology, review and editing. J.L.M.: Investigation, Methodology, review and editing. K.E.: Methodology, review and editing. E.G.-V.: Conceptualization, Formal analysis, Writing – review and editing, Funding acquisition. G.M.-S.: Conceptualization, Methodology, Writing – review and editing, Supervision, Funding acquisition.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All sequencing data generated in this study have been submitted to the NCBI BioProject database (http://www.ncbi.nlm.nih.gov/bioproject/) under accession number PRJNA1160467.

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Supporting Information

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