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ARTICLE

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New haplotypes found in stranded long-finned pilot whales (*Globicephala melas*) in the eastern North Atlantic and adjacent waters

Rachel J. Ball[1] | Ashleigh Kitchiner[1] | Nicholas J. Davison[2,6] | Andrew Brownlow[2,6] | Simon Berrow[3] | Niall J. McKeown[4] | Lonneke L. IJsseldijk[5] | Matthew Geary[1] | Ian McDowall[1] | Anna P. Muir[1]

[1]Conservation Biology Research Group, University of Chester, Chester, UK

[2]Institute of Biodiversity, Animal Health & Comparative Medicine, University of Glasgow, Glasgow, UK

[3]Irish Whale and Dolphin Group, Kilrush, Ireland and/or Marine and Freshwater Research Centre, Galway-Mayo Institute of Technology, Galway, Ireland

[4]Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, UK

[5]Division of Pathology, Department of Biomolecular Health Sciences, Utrecht University, Utrecht, The Netherlands

[6]Scottish Marine Animal Stranding Scheme, SRUC Northern Faculty, An Lòchran, Inverness Campus, Inverness, Scotland, UK

Correspondence
Rachel Ball, University of Chester, Parkgate Road, Chester, CH1 4BJ, UK.
Email: r.ball@chester.ac.uk

Abstract

Long-finned pilot whale (*Globicephala melas*) mitochondrial (mtDNA) genetic diversity is considered low, especially in the North Atlantic, where only seven haplotypes have been recorded in previous studies using a 345 bp control region fragment. Such studies have not included samples from Ireland or the Netherlands. In this study we analyzed a longer sequence of the mtDNA control region (631 bp) from individuals stranded around Ireland, Scotland, and the Netherlands between 1995 and 2019 ($n = 180$). Nine haplotypes were identified, of which five were newly described (haplotype diversity $h = 0.511$). Pairwise tests revealed significant differentiation between the Irish and Scottish samples. Potential confounding factors are discussed but given that failure to recognize population structure may compromise conservation efforts, the findings show the need for further investigation using nuclear markers. Six mass stranding events were included, of which one event reported two haplotypes among individuals confirming a mixing of matrilineal groups. Although the permanence of this combination cannot be determined, this is the first record of such an occurrence within the North Atlantic. This study shows that stranding sample databases are a useful

resource for genetic studies and provides new insights into genetic diversity of long-finned pilot whales in the eastern North Atlantic and adjacent waters.

KEYWORDS
cetacean, genetic diversity, *Globicephala melas*, mass stranding event, matrilineal structure, mitochondrial DNA, stranding

1 | INTRODUCTION

Effective conservation management plans rely on detailed information about the genetic diversity and structure of populations, particularly for species that have no obvious barriers to gene flow (Allendorf et al., 2010; Banguera-Hinestroza et al., 2014). Population structure in cetaceans is often cryptic and can be difficult to detect from observational data due to their high dispersal ability (Foote et al., 2009; Pérez-Alvarez et al., 2015; Pilot et al., 2010). Photo-identification can be used to distinguish individuals and assign them home ranges, which can be suggestive of population dynamics, but this technique is more applicable for species with coastal distributions (Beck et al., 2014; O'Brien et al., 2010; Stevens, 2014). Typically, little is known about pelagic odontocete species' population structure from observational data alone; however, integration of genetic data has offered considerable insight into species' population structure and conservation needs (see Banguera-Hinestroza et al., 2014 and Martien et al., 2017).

Biological information collected from stranded cetaceans represent a cost-effective resource for quantification of baseline trends in population variability and insights into the condition of the at-sea population (ten Doeschate et al., 2018). Additionally, stranded individuals provide opportunistic sampling opportunities for genetic studies that are particularly useful for pelagic species where comprehensive at-sea sampling is difficult or unavailable (Bilgmann et al., 2011; Dalebout et al., 2005; Morin et al., 2017). Geographical referencing of stranded cetaceans can be subject to bias because their exact origin cannot always be known, as individuals that die at sea may drift with the prevailing wind or currents before making landfall (Bilgmann et al., 2011; Peltier et al., 2012). However, genetic data from stranded individuals can be analogous to data obtained from at-sea samples under some circumstances (Dalebout et al. (2005).

Long-finned pilot whales (*Globicephala melas*) are a gregarious species with complex social bonds (Augusto et al., 2017b; Evans et al., 2005). Until 2018, long-finned pilot whales were classified on the IUCN red list as "data deficient" and despite reclassification to "least concern," knowledge of population distribution and trends is scarce, requiring further research to bolster our understanding of the conservation needs of this species (Minton et al., 2018). Previous mitochondrial (mtDNA) studies of long-finned pilot whales report haplotype diversity for a 345 bp fragment of the control region (CR) to be generally low worldwide, with only 17 haplotypes recorded (Kraft et al., 2020; Monteiro et al., 2015; Oremus et al., 2009). This is lower than haplotype numbers reported in other worldwide studies of cetaceans with comparable social structure (Alexander et al., 2016; Hoelzel et al., 2002; Morin et al., 2010). Across the Southern Hemisphere, 13 of the known 17 345 bp mtDNA CR haplotypes have been recorded (Kraft et al., 2020; Oremus et al., 2009). Only seven of the 17 345 bp mtDNA CR haplotypes have been recorded in the North Atlantic, of which four are not shared with the Southern Hemisphere (Monteiro et al., 2015; Oremus et al., 2009; Sabatier et al., 2015; Siemann, 1994). Within the North Atlantic, the United Kingdom (UK) was identified as the region with the highest number of 345 bp mtDNA CR haplotypes ($n = 5$) (Monteiro et al., 2015). However, the sample sizes from the three studies that included the UK were limited (Monteiro et al., 2015; Oremus et al., 2009; Siemann, 1994) and they did not include the unusual individuals stranded in the Netherlands or samples from Ireland, which is an understudied coastline of the eastern North Atlantic.

Long-finned pilot whales are the most commonly encountered species in mass stranding events (Augusto et al., 2017b; Evans et al., 2005). Their social structure is based around matrilineal pods (socioecological groups, containing related adult females), as assessed by kinship studies from grinds (drive fishery practice) in the Faroe Islands (Amos et al., 1991, 1993). Photo-identification analysis of pod social structure suggests that they live in small stable pod units that interact with other such pod units, forming wider social associations (Augusto et al., 2017a; de Stephanis et al., 2008). However, these studies have not used genetic markers to infer kin relationships among those stable pod units, and as such, it is still unclear if long-finned pilot whales have a strict matrilineal structure comparable to the resident killer whales (*Orcinus orca*) in the Northwest Pacific (Bigg et al., 1990; Ford, 2002) or a fluid pod structure more similar to Icelandic killer whales (Tavares et al., 2017). Social relations may be an important consideration in understanding mass stranding events, based on observations of pilot whales attempting to retain group cohesion during mass strandings, assumed to be motivated by an effort to remain with closely related maternal kin (Norris & Schilt, 1988; Whitehead et al., 2004). However, if multiple genetically distinct pods are present within a stranding, subsequent refloating of unrelated individuals together as part of rescue efforts may contribute to restranding (Geraci & Lounsbury, 1993). The extended matriline hypothesis predicts that all individuals within a mass stranding event will be from the same female led family socioecological unit and therefore have the same mtDNA haplotype sequence (Oremus et al., 2013). However, this hypothesis has been largely rejected in a study of mass stranding events around New Zealand and Tasmania in favor of the multiple matrilines stranding hypothesis, which is when more than one matriline is present within a mass stranding event (Oremus et al., 2013). The extended matriline hypothesis has never been tested for mass stranding events in the Northern Hemisphere.

The first objective of this study was to investigate mtDNA CR haplotype diversity of long-finned pilot whales stranded on the coasts of the eastern North Atlantic and adjacent waters. The second objective was to assess regional variation and to look for maternal population structure. The third objective was to explore matrilineal social structure of long-finned pilot whale pods involved in mass stranding events.

2 | METHODS

2.1 | Sample collection

The Scottish Marine Animal Stranding Scheme (SMASS) has been recording and collecting samples from stranded and necropsied cetaceans since 1992 (ten Doeschate et al., 2018). In 2006 the Irish Cetacean Genetic Tissue Bank (ICGTB) was set up by the Irish Whale and Dolphin Group (IWDG), which had been recording cetacean strandings since 1990 (Coombs et al., 2019). In addition, within the Dutch stranding investigation program, three stranded long-finned pilot whale samples were available; the atypical presence of this species within a shallow sea permitted their integration into this sample set. Samples of long-finned pilot whale muscle or skin tissue were collected postmortem from stranded individuals between 1995 and 2019. Samples from 180 individuals were included in this study: 81 from SMASS stored at −20°C, 96 from ICGTB stored in ethanol, and three from the Netherlands stored in ethanol. The samples originated from both single stranded individuals ($n = 93$) and individuals involved in six separate mass stranding events ($n = 87$) (Figure 1).

2.2 | DNA sequencing

DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (QIAGEN Inc., Crawley, UK), following the manufacturer's handbook, the only alteration being an overnight lysis incubation to increase DNA yield from more decomposed samples (Qiagen Handbook, 2019; https://www.qiagen.com/gb/resources/resourcedetail?id=68f29296-5a9f-40fa-8b3d-1c148d0b3030&lang=en). A 710 bp fragment of the control region was amplified using

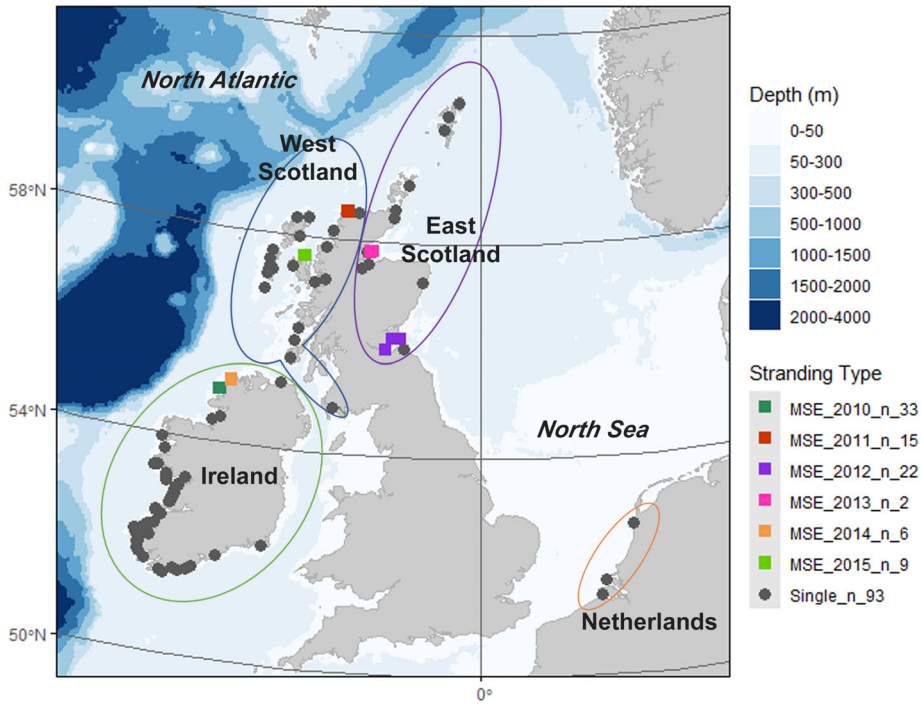

FIGURE 1 Map of all stranding locations included in this study. Single stranded individuals are shown by a gray circle and mass stranding locations are displayed by a square. The color of each square corresponds to the year in which the mass stranding event (MSE) occurred. Number of individuals in each stranding type and event is provided in the map legend. Regional data partitions are shown with colored polygons and place-name labels. The map was drawn in R within the ggOceanMap package (Vihtakari, 2021) and bathymetry spatial data (Amante & Eakins, 2009).

the forward primer, PWCRF (5′-GCTGGAATTCTACATAAACTA-3′; developed at the University of Chester) and reverse primer Dlp-8G (5′-GGAGTACTATGTCCTGTAACCA-3; Dalebout et al., 2005). For all samples, the 25 μl PCR reaction was as follows: one illustra PuReTaq Ready-To-Go PCR bead (200 μM of each dNTP, 10 mM Tris–HCl, pH 9.0, 50 mM KCl & 1.5 mM $MgCl_2$; Thermofisher Scientific, Waltham, UK), 0.5 mM of forward primer, 0.5 mM of reverse primer, 21 μl of sterile water and 3 μl of DNA template. Thermocycler conditions were: 94°C for 5 min, 35 cycles of 94°C, 55°C, and 72°C all for 30 s, with a 10 min 72°C extension. The Qiagen QIAquick PCR Purification Kit (QIAGEN Inc., Crawley, UK) was used to purify the PCR products prior to sequencing. DNA sequencing for each individual was carried out on both the forward and reverse strands using the dideoxy chain termination cycle sequencing method on ABI 3730XL sequencing machines at Eurofins Genomics (Ebersberg, Germany).

Morphological sex was recorded for 94% of individuals, as per Kuiken and Hartman (1991). The remaining individuals were sexed genetically using introns of the ZFX and ZFY genes and the SRY gene, following the methods in de Stephanis et al. (2008).

2.3 | Haplotype diversity

Sequence alignment of 710 bp lengths and trimming to 631 bp was completed in MEGAX (Kumar et al., 2018) using ClustalW (Thompson et al., 1994), with default parameters. The maximum likelihood test performed in MEGAX (Kumar et al., 2018) identified the best fit substitution model (Nei & Kumar, 2000) as Tamura (Tamura, 1992). The sequences were confirmed as long-finned pilot whale mtDNA control region using BLAST (Morgulis et al., 2008) and

compared with six previously published 345 bp control region haplotypes recorded in North Atlantic long-finned pilot whales (GenBank accession numbers: FJ513343, FJ513345-46 & FJ513351, Oremus et al., 2009 and KC934933-34, Monteiro et al. 2015). This permitted integration with previous studies and recognition of new haplotypes. A median joining network (MJN; Bandelt et al., 1999) was drawn, for all 180 individuals with mtDNA CR haplotype length of 631 bp, in the software package Network.10 (https://www.fluxus-engineering.com/sharenet.htm) using default parameters.

2.4 | Regional variation

Individuals were grouped into regions based on their sampling locations: Ireland, East Scotland, West Scotland, and the Netherlands (Figure 1). The Netherland individuals were excluded from haplotype frequency-based analysis due to the comparatively small sample size. East and West Scotland were grouped separately as the west coast has high connectivity to the continental shelf edge, known to be an important habitat feature for long-finned pilot whales (Lambert et al., 2014; MacLeod, 2009). In contrast, the east coast of Scotland typically has shallower coastal waters (Figure 1; Lambert et al., 2014; MacLeod, 2009). Long-finned pilot whales are known to exhibit social structure based around family units and, as a result, only one of each haplotype found in each mass stranding event was included in the regional analysis to control for nonindependence as in Kraft et al. (2020) and Oremus et al. (2009).

All analyses were performed in Arlequin version 3.5 (Excoffier & Lischer, 2010) for the 631 bp mtDNA CR alignment. Genetic variability was measured using indices of haplotype (h) and nucleotide (π) diversity and their respective standard deviations (SD). Differentiation between pairs of sampling regions was quantified using Φ_{ST} (with Tamura's interhaplotype distance) and F_{ST} (Weir & Cockerham, 1984), with statistical significance assessed after 10,000 permutations. Pairwise differences in haplotype frequencies were tested using Fisher's exact test (Raymond & Rousset, 1995) with 10,000 permutations. The partitioning of variation among regions versus within regions was also assessed using a hierarchical AMOVA (analysis of molecular variance) based on Φ_{ST} (with Tamura's interhaplotype distance; Excoffier et al., 1992) performed with 16,000 permutations.

3 | RESULTS

All 180 individuals were successfully sequenced for a 710 bp section of the control region. No ambiguities were found between forward and reverse sequences and no evidence of heteroplasmy was present in our data set. All sequences were trimmed following alignment to 631 bp to ensure there were no missing data from any of the samples. The 631 bp sequence of the mtDNA CR defined nine haplotypes (GenBank Accession no: OK247504-12). Genetic sexing was successful in 22 of 27 unknown samples, the sample set contained 95 females, 80 males and five of unknown sex.

3.1 | Haplotype diversity

Comparison to the previously published 345 bp mtDNA CR haplotypes from GenBank revealed that four haplotypes previously recorded in the North Atlantic were present in this sample set: S, R, E, P (defined by Monteiro et al., 2015; Oremus et al., 2009; Table 1). Haplotype S was the most common haplotype in our samples, present in 72% of individuals (Figure 2).

Five new 631 bp mtDNA CR haplotypes are described for the first time in this study: S1, S2, S3, T1, and T2 (Table 1; Figure 2). Within the previously published 345 bp haplotype sequence length (Monteiro et al., 2015; Oremus et al., 2009; Siemann, 1994) we observed three new variable positions, a 2 bp deletion (Haplotype S1) and a

TABLE 1 Variable positions for all mtDNA control region haplotypes (631 bp) found in this study. Plotted against a reference long-finned pilot whale mitogenome (Accession no: HM060334; Morin et al., 2010), the control region starts at position 15475 (not shown in table). Dashed line indicates where the previously published 345 bp haplotype ends.

	Position										Individuals
Reference genome	15621	15622	15623	15624	15642	15679	15749	15992	16067	16080	HM060334
	122	123	124	125	140	177	247	490	565	578	$n = 180$
	Del	Del	In	In	Trs	Trs	Trs	Trv	Trv	Trs	
Haplotype S	T	A	—	—	C	T	C	G	G	G	128
Haplotype S1	—	—	—	—	•	•	•	•	•	•	20
Haplotype S2	•	•	—	—	•	•	•	•	T	•	2
Haplotype S3	•	•	—	—	•	•	•	•	•	A	5
Haplotype T1	•	•	—	—	T	•	•	•	•	•	1
Haplotype T2	—	—	—	—	T	•	•	C	C	•	2
Haplotype R	•	•	—	—	•	C	•	•	•	•	10
Haplotype E	•	•	T	A	•	•	•	•	•	•	9
Haplotype P	•	•	—	—	•	•	T	•	•	A	3

Note: The "." indicates an identical bp to Haplotype S, the "—" indicates the absence of a bp. Del = deletion, In = insertion, Trs = transition & Trv = transversion.

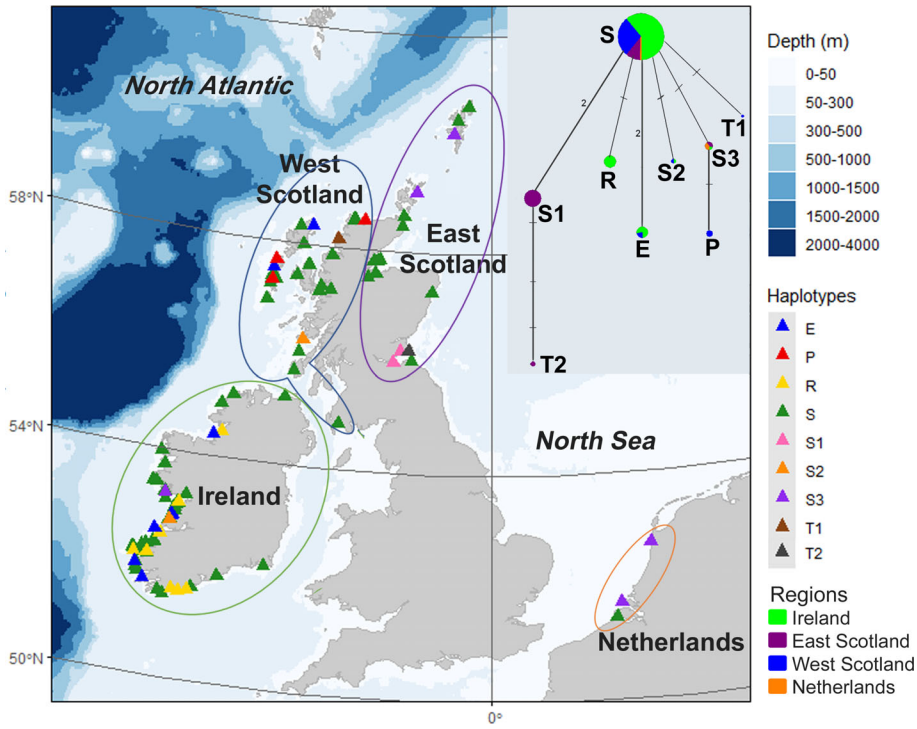

FIGURE 2 Map of all haplotypes, based on 631 bp of the control region, reported in this study. Each haplotype corresponds to a different color. Regional data partitions are shown with colored polygons and placename labels. The median joining haplotype network is provided in the top right corner. Node size is proportional to the frequency occurrence of each haplotype. Single nucleotide variable bp positions are marked with a crossbar and indel variations of a 2 bp change is indicated with the number 2. The median joining network was drawn with the 631 bp control region haplotype for all 180 individuals. The map was drawn in R within the ggOceanMap package (Vihtakari, 2021) and bathymetry spatial data (Amante & Eakins, 2009).

single transition (Haplotype T1) (Table 1). Haplotype T2 had both aforementioned variable sites and an additional two variable positions located in the 631 bp haplotype sequence length (Table 1). Within the extended 631 bp sequence, Haplotypes S2 and S3 had one variable position each (Table 1). Additionally, the individuals that matched previously published 345 bp Haplotype P (Monteiro et al., 2015; Oremus et al., 2009; Siemann, 1994) also had a variable position in the 631 bp haplotype length (Table 1). Two of the new haplotypes (S2 and S3) were only distinguished by a substitution in this extended length of sequence.

Haplotypes T1 and S2 were found in the West Scotland region, with S2 also recorded in one stranded long-finned pilot whale in Ireland (Figure 2). Haplotype S3 was found in Ireland, East Scotland, and the Netherlands. Haplotypes S1 and T2 were only recorded in the East Scotland region (Figure 2).

There were more singularly stranded male ($n = 55$) long-finned pilot whales than females ($n = 37$). Seven of the nine haplotypes reported in this study were found in the single stranded individuals (Figure 3a). Haplotypes S2 and P were only found in individually stranded males (Figure 3a). There were more female long-finned pilot whales than males in the mass stranding events ($n = 58$ and $n = 29$, respectively; Figure 3b). Haplotypes S1 and T2 were only found in the 2012 mass stranding event, S1 was observed in both males and females but, Haplotype T2 was only found in female individuals.

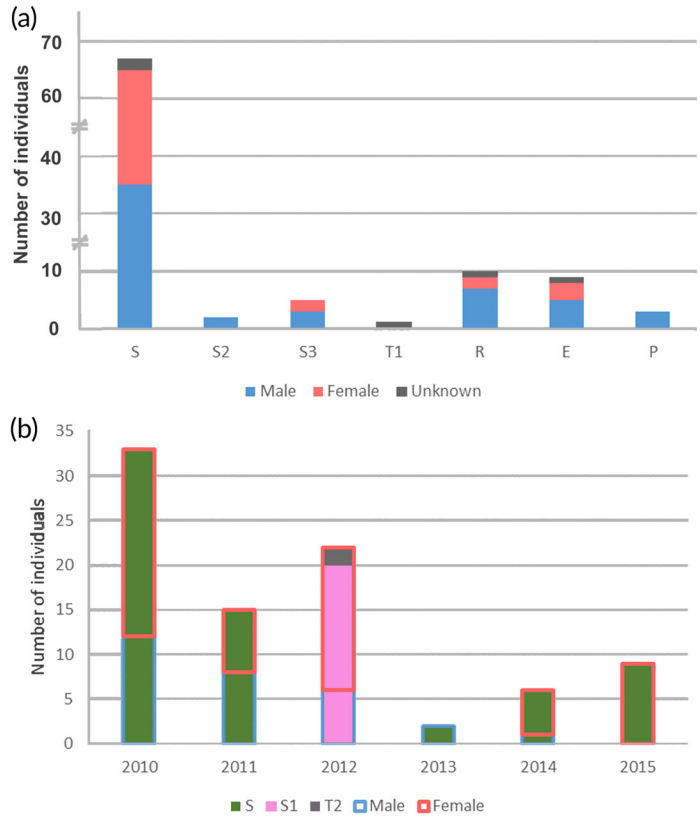

FIGURE 3 (a) numerical plot of every single stranded individual ($n = 93$) categorized by haplotype to show sex split. (b) numerical plot of the six mass stranding events, categorized by the year the mass stranding took place showing, haplotype and sex of each individual ($n = 87$).

TABLE 2 Overall diversity indices and those for the three regions in the adjusted data set (631 bp), which includes only single stranded individuals, one individual of each haplotype from each mass stranding event, and the Netherlands samples are excluded. Shown are sample number (*n*), number of haplotypes (No. Haps) and number of variable bp positions (Variable sites). Standard deviation (*SD*) is shown after the ± for haplotype (*h*) and nucleotide diversity (π).

	Ireland	West Scotland	East Scotland	Overall	Netherlands
n	59	24	14	97	3
No. Haps	5	5	4	9	2
Variable sites	5	6	6	10	1
h	0.506 ± 0.067	0.493 ± 0.117	0.495 ± 0.151	0.510 ± 0.059	
π %	0.056 ± 0.062	0.099 ± 0.090	0.111 ± 0.099	0.078 ± 0.075	

3.2 | Regional variation

The overall haplotype diversity from the eastern North Atlantic was 0.511 (*SD* ± 0.059) and nucleotide diversity was 0.078 (*SD* ± 0.075) (Table 2). By region, haplotype diversity varied between 0.493 ± 0.117 (West Scotland) and 0.506 ± 0.067 (Ireland) (Table 2). Nucleotide diversity was lower for Ireland (0.056 ± 0.062) than West Scotland

TABLE 3 Pairwise differentiation values for the three regions, calculated using the adjusted data set with the 631 bp sequence length. Φ_{ST} (calculated with Tamura's interhaplotype distance) and *p*-values, in parentheses, are shown below the diagonal. Fishers exact test is shown above the diagonal with significant values marked in bold (significance level = .05).

	Ireland $n = 59$	West Scotland $n = 24$	East Scotland $n = 14$
Ireland	—	**0.008 ± 0.002**	**0.009 ± 0.001**
West Scotland	**0.096 (.007)**	—	0.092 ± 0.004
East Scotland	**0.089 (.022)**	−0.019 (.562)	—

(0.099 ± 0.090) and East Scotland (0.111 ± 0.099). The hierarchical AMOVA based on pairwise Φ_{ST} (with Tamura's interhaplotype distance) reported most genetic variation occurred within regions (94.44%), with a significant proportion of variation (5.56%; $p = .01$) between region groups. AMOVA based on pairwise F_{ST} reported the proportion of variation between regions to be nonsignificant ($p = .148$). Fisher's exact test of differentiation reported significant differentiation between Ireland and West Scotland (0.007 *SD* ± 0.001) as well as between Ireland and East Scotland (0.017 *SD* ± 0.002) (Table 3). The pairwise Φ_{ST} values mirrored those of Fisher's exact test, with Ireland being significantly different from the two Scottish regions (Table 3). No pairwise F_{ST} results were statistically significant (Table S1).

3.3 | Mass strandings

The mass stranding events were typically characterized by the presence of more females than males, although the 2013 event was an exception as the two individuals that stranded were male (Figure 3b). The mass stranding that occurred in 2012 in Fife (East Scotland) contained two new haplotypes, S1 ($n = 20$) and T2 ($n = 2$). The mass stranding events of long-finned pilot whales in 2010, 2011, 2013, 2014, and 2015 all exhibited a single haplotype in each respective event (Haplotype S; Figure 3b).

4 | DISCUSSION

The sample size of our study is the largest to date, consisting of 180 long-finned pilot whale individuals from the eastern North Atlantic and adjacent waters, almost doubling the 95 individuals from the UK, Iberian Peninsula, and Faroe Isles in the study by Monteiro et al. (2015). Our study included individuals collected from unanalyzed areas of Scotland and from Ireland and the Netherlands, which have not previously been included in assessments of long-finned pilot whale genetic diversity. Five new mitochondrial control region (mtDNA CR) haplotypes were identified by this study, along with four of the seven haplotypes previously reported in the North Atlantic (S, P, R, and E; Monteiro et al., 2015; Oremus et al., 2009; Sabatier et al., 2015; Siemann, 1994). Our study nearly doubles the number of known long-finned pilot whale mtDNA CR haplotypes to a total of 12 for the North Atlantic. These results highlight the utility of large tissue banks, such as those managed by the Irish Whale and Dolphin Group and the Scottish Marine Animal Stranding Scheme, for genetic studies.

Haplotype S was the most common mtDNA CR haplotype found in this study and was recorded in 72% of individuals. This is in line with previous studies of long-finned pilot whale genetic diversity in the North Atlantic that found between 62% and 93% of individuals sampled were Haplotype S (Monteiro et al., 2015; Sabatier et al., 2015; Siemann, 1994). Haplotype S has been recorded in every location sampled around the North Atlantic, from Cape Cod in the USA to the Faroe Islands and the Iberian Peninsula (Monteiro et al., 2015; Oremus et al., 2009; Sabatier et al., 2015; Siemann, 1994).

The discovery of nine long-finned pilot whale mtDNA CR haplotypes along the coasts of Ireland and Scotland is higher than previously described for the entire North Atlantic (Monteiro et al., 2015; Oremus et al., 2009; Siemann, 1994). Two of these newly found haplotypes were distinguished by substitutions occurring outside the standard 345 bp alignment used in previous studies and emphasizes the utility of increased genome coverage. However, three new haplotypes were also found within the standard 345 bp fragment. This direct comparison merits consideration as to whether the Irish and Scottish waters could be an area of high genetic diversity for long-finned pilot whales within the North Atlantic. The continental shelf adjacent to the western shores of Ireland and Scotland is known to be an important habitat feature associated with the distribution of long-finned pilot whales and their primary food source, cephalopod species (Barile et al., 2021; de Pierrepont et al., 2005; MacLeod et al., 2007; Pike et al., 2019; Santos et al., 2014; Spitz et al., 2011; Waggitt et al., 2020). Although, the results may also reflect the larger sample size used than in previous studies, they add to evidence suggesting that northern Atlantic waters may harbor high levels of mtDNA control region diversity in long-finned pilot whales that have not been identified due to certain areas (e.g., Norway) being understudied.

4.1 | Regional variation

The North Atlantic Sighting Surveys (NASS) and satellite tracking[1] of long-finned pilot whales show long distance movements from the Faroe Islands to as far south as the Bay of Biscay (Bloch et al., 2003; Pike, et al., 2019). Such data, alongside the observed distribution, range movements,[1] and predicted density of long-finned pilot whales in the eastern North Atlantic (2008; Pike et al., 2019; Waggitt et al., 2020) are compatible with high dispersal across the studied region. Interestingly, while F_{ST} yielded an overall nonsignificant interregional variance component, Φ_{ST}, AMOVA, and exact tests reported a clear pattern of differentiation wherein the Irish sample was significantly differentiated from the two Scottish samples. Interpretation of this result in terms of restricted connectivity should be made with caution given the potential for nonindependence among individuals within a defined group (i.e., related individuals within a group biasing between group comparisons; O'Connell et al., 2019). To minimize the potential for nonindependence bias we included only one of each haplotype from each mass stranding event; however, it is possible that some consanguineous individuals were included from the single stranded samples (Bilgmann et al., 2011; IJsseldijk et al., 2015; Peltier et al., 2012). Dalebout et al. (2005) reported similar genetic patterns (F_{ST}) inferred among samples from stranded versus bycaught Cuvier's beaked whales (*Ziphius cavirostris*), indicating that stranded samples may be representative of at sea populations. Significant population isolation linked to sea surface temperature has previously been reported for long-finned pilot whales in the North Atlantic (Fullard et al., 2000). As failure to identify distinct population units will fundamentally compromise future spatial conservation strategies, the statistically significant results for the mtDNA control region haplotypes reported here need to be further assessed with nuclear markers and individual-based approaches before a lack of long-finned pilot whale population isolation within the region is assumed.

4.2 | Mass strandings

In five of the six mass stranding events that have occurred in Ireland and Scotland between 2010 and 2015, a single 631 bp mtDNA CR haplotype was present (Haplotype S). This means that the extended matriline hypothesis cannot be rejected in these cases (Oremus et al., 2013). However, due to the dominance of Haplotype S in this sample set and the low overall levels of mtDNA CR variation in the species (Kraft et al., 2020; Monteiro et al., 2015; Oremus et al., 2009), the number of distinct matrilineal groups may be underestimated. Such a scenario has been observed in short-finned pilot whales in the Hawaiian Islands, where a single mtDNA CR haplotype dominates in the majority of individuals across the region even though, distinct female led pod units may never interact (Van Cise et al., 2017). Mitogenomic sequencing of

cetacean species has shown that there is more variation throughout the mitochondrial genome than just in the control region (Alexander et al., 2013; Archer et al., 2013; Bachmann et al., 2021; Foote et al., 2011; Louis et al., 2020; Van Cise et al., 2019). Therefore, full mitogenome sequences have the potential to reveal greater matriline variation in long-finned pilot whales.

The finding of two haplotypes, S1 ($n = 20$) and T2 ($n = 2$), within the 2012 Scottish mass stranding confirms mixing of matrilines at some stage; it is possible that these two matrilines were combined in a social association prior to stranding, like the observations of interactions between pod units in Nova Scotia (Augusto, et al., 2017a), or that the pod units converged in the narrowing estuary during this mass stranding event. The social relationships of long-finned pilot whale pod structure are still unclear, their social structure could be similar to the complex patterns observed in Icelandic killer whales (Tavares et al., 2017). However, this pattern of mixed matrilines among stranded individuals aligns with Oremus et al. (2013), who detected multiple haplotypes and used microsatellites to reveal disruption of kinship bonds, in nine of the 12 long-finned pilot whale mass stranding events examined in the Southern Hemisphere. This is the first evidence of matrilines mixing within North Atlantic mass strandings and emphasizes that it cannot be assumed that individuals within a mass stranding event are from a single pod.

5 | CONCLUSION

Overall, our study found the seaboard of Ireland and Scotland to contain new mitochondrial control region haplotypes for long-finned pilot whales within the eastern North Atlantic and that extensive stranding records are a useful tool for conducting genetic analysis of deep water distributed species. Despite the low level of mtDNA control region variation, geographically congruent significant haplotype differentiation was detected. Genomic based approached are required to assess the biological significance of this pattern (Attard et al., 2018) and inform conservation. Our study also provides an observation of mixed matrilines within a mass stranding event. This could reflect a confluence of biological, environmental, and/or anthropogenic influences that may have caused the mass stranding event itself or be reflective of a more complex social society than assumed of long-finned pilot whales. Analysis of nuclear markers and unstranded individuals will be key to disentangling such factors with this information being of great relevance to rescue strategies.

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AUTHOR CONTRIBUTIONS

Rachel Ball: Conceptualization; formal analysis; investigation; writing – original draft; writing – review and editing. **Ashleigh Kitchiner:** Investigation; writing – review and editing. **Nicholas J Davison:** Resources; writing – review and editing. **Andrew Brownlow:** Resources; writing – review and editing. **Simon Berrow:** Resources; writing – review and editing. **Niall J Mckeown:** Resources; writing – review and editing. **Lonneke Liza IJsseldijk:** Resources; writing – review and editing. **Matthew Geary:** Supervision; writing – review and editing. **Ian McDowall:** Conceptualization; supervision; writing – review and editing. **Anna P Muir:** Conceptualization; supervision; writing – review and editing.

ORCID

Rachel J. Ball https://orcid.org/0000-0002-9940-8684

ENDNOTE

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