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Walruses (*Odobenus rosmarus rosmarus*) in the Pechora Sea in the context of contemporary population structure of Northeast Atlantic walruses

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Identifying genetically different groups of animals, occupying specific geographical areas, is a prerequisite for conservation and management priorities. In the present study, the genetic structure of Atlantic walruses (*Odobenus rosmarus rosmarus*) occupying the Pechora Sea (PEC) in the western Russian Arctic, including walruses from Svalbard–Franz Josef Land (SVA–FJL) and East Greenland (EGR) regions, was investigated using 14 microsatellites (N = 159) and mtDNA sequences (N = 212). Bayesian-based clustering analysis identified two clusters: EGR and the other Northeast Atlantic areas. Pairwise F_{ST} analyses based on microsatellites revealed low but significant genetic differences between walruses from the PEC and SVA–FJL groups, which was supported by mtDNA analysis. F_{ST} was not significant for all sampling years, indicating a temporal effect or male-biased gene flow. Extended Bayesian Skyline Plots suggested a constant female subpopulation size (N_{ef}) for EGR and an increase for the SVA–FJL and PEC groups that commenced around 40–30 Kyr ago, indicating different demographic histories for walruses in the EGR. Further, the evolutionary phylogenetic relationship between Atlantic and Pacific walruses (*O. r. divergence*), based on mtDNA sequences, showed a monophyletic Atlantic clade, suggesting that Atlantic and Pacific walruses diverged ~949 Kyr. The principal finding suggests that PEC walruses show low, but significant genetic distinction from walruses in SVA–FJL and should be managed conservatively, as a separate, small population.

ADDITIONAL KEYWORDS: climate change – conservation – divergence – genetics – management – microsatellites – mtDNA.

INTRODUCTION

Identification of genetic population structure and the underlying factors driving it is vital for proper species management (Avise, 1994). Assessment of genetic diversity is also important, given that genetic diversity is linked to a species' ability to adapt to changing environments, survive disease epizootics, etc. (see Avise, 1994; Merilä & Hendry, 2014). Gene flow and changes in effective population size are among the most important factors that determine patterns of genetic diversity, while genetic structure is primarily influenced by past and present gene flow and genetic drift. Hence, understanding the historical demographic trends of populations is important for the evaluation of their current conservation status (Crandall *et al.*, 2000; Hansen *et al.*, 2008; Hansen, 2010).

Several population genetic studies have been conducted on the Atlantic walrus to ascertain evolutionary history and assist in delineating putative populations and subpopulations (Andersen *et al.*, 1998, 2009, 2014; Born *et al.*, 2001; Shafer *et al.*, 2014, 2015). These studies have included samples from Arctic Canada, West,

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Northwest and East Greenland (EGR), Svalbard (SVA) and Franz Josef Land (FJL). The studies have shown that the walruses from SVA and FJL belong to the same population and that these walruses are significantly different from walruses in EGR (Andersen *et al.*, 1998; Born *et al.*, 2001). These genetic studies confirmed the findings suggested by earlier tagging and tracking studies conducted on walruses in these areas. Satellite tracking studies conducted in the 1990s showed that walruses readily moved between SVA and FJL (Wiig, Gjertz & Griffiths, 1996), and recent telemetry studies confirm that this tight linkage remains through to the present (Freitas *et al.*, 2009; Lowther *et al.*, 2015). Consistently, most tagging and tracking studies have shown that EGR walruses remain on the coastal shelf of Greenland, moving seasonally within the region concomitant with changing ice conditions (Born & Knutsen, 1992), although a single walrus from EGR has been sighted on a haul out in SVA (Born & Gjertz, 1993). Potential linkages of the walruses in the northern reaches of the Northeast Atlantic with more southerly haul-out groups in the Russian Arctic are not yet studied.

Walruses in the Pechora Sea (PEC) are listed in the Red Data Book of Russia (Boltunov *et al.*, 2010). Similar to other Atlantic walruses in the Russian Arctic (including FJL), walruses in the PEC have been protected from hunting since 1956 (Wiig, Born & Stewart, 2014 and references therein). Walruses are found in the PEC throughout the year and a recent flying survey suggested that there are some 4000 animals in the region in late summer (Lydersen *et al.*, 2012). Walrus herds that include adult females with calves occupy sea ice-covered areas in the region during the winter, suggesting that it might be a breeding location (e.g. Boltunov *et al.*, 2010 for a review). Recent tracking studies have shown that walruses in the PEC move between Vaygach Island and islands of the Nenets State Nature Reserve as well as moving northward to various haul-out sites on Novaya Zemlya (Semyonova *et al.*, unpublished data). Walruses are capable of migrating long distances (e.g. Born & Knutsen, 1992; Lowther *et al.*, 2015), and there is a geographic continuum of haul outs along Novaya Zemlya (south of FJL) all the way down to the PEC (Semyonova, Boltunov & Nikiforov, 2015). Thus, it is possible that PEC animals might be genetically connected to the other areas within the Barents Sea, as opposed to being a separate genetic and demographic group (NAMMCO, 2006).

The aim of the present study was to analyse the genetic relationships between a putative population of Atlantic walruses occupying waters in the Southern Barents Region (Kara–Pechora–Novaya Zemlya) compared to two neighbouring regions [i.e. SVA–FJL and EGR (see NAMMCO, 2006)] using 14 microsatellite markers and variation in part of the mtDNA genome. The evolutionary relationship between Atlantic and Pacific walrus (*Odobenus rosmarus divergence*) based on mtDNA sequences was also explored using previously published sequences for Atlantic and Pacific walruses (Lindqvist *et al.*, 2009, 2016; Sonsthagen *et al.*, 2012) in addition to new data from this study.

MATERIAL AND METHODS

Sampling

Biopsy samples (skin) from unrestrained Atlantic walruses (N = 50) were collected in 2012 and 2013 from the eastern PEC, at a haul-out site situated on the west coast of Vaygach Island (Fig. 1). Other samples used for comparative purposes in this study include skin biopsies collected from walruses at: SVA collected in 1992 (SVA1992, N = 22) and 2003–2004 (SVA2003, N = 20); FJL collected in 1992 (FJL1992, N = 24); and EGR in 2002 (EGR2002, N = 43), 2004 (EGR2004, N = 20) and 2010 (two sites were used in 2010, delineated by a and b – EGR2010a, EGR2010b, N = 43) (Fig. 1; Table 1). The 2010 samples from EGR were analysed separately in the preliminary phases of analyses due to the possibility of genetic structuring. The samples from EGR2002 and SVA–FJL1992 have been analysed genetically previously (Andersen *et al.*, 1998, 2009; Born *et al.*, 2001; Lindqvist *et al.*, 2009). However, all samples were reanalysed in the present study to avoid any issues that might arise from calibration problems. All sample collections followed the guidelines of the American Society of Mammalogists (Sikes *et al.*, 2011).

Additional mtDNA sequences of Atlantic (N = 12: Lindqvist *et al.*, 2009 and N = 6: Lindqvist *et al.*, 2016) and Pacific (N = 206; Sonsthagen *et al.*, 2012) ancestry were downloaded from the National Center for Biotechnology Information (NCBI) database and used for median-joining network and phylogenetic analyses.

DNA amplification

DNA was extracted using the modified CTAB buffer method (Andersen, Fog & Damgaard, 2004). A total of 14 microsatellite markers were amplified. Ten of these microsatellites were used by Andersen *et al.* (1998, 2009; see these papers for primers and PCR conditions). The last four were developed for grey seals, *Halichoerus grypus* (*HG4.1*, *HG8.10*; Allen *et al.*, 1995), southern elephant seals, *Mirounga leonina* (*M11*; Gemmell *et al.*, 1997) and harbor seals, *Phoca vitulina* (*PV9*; Goodman, 1997), but were found to be polymorphic in the walrus. The markers were PCR multiplexed in three separate runs using the QIAGEN Multiplex PCR kit following the manufactures protocol and a 12.5 µL reaction volume with an annealing temperature of 57 °C

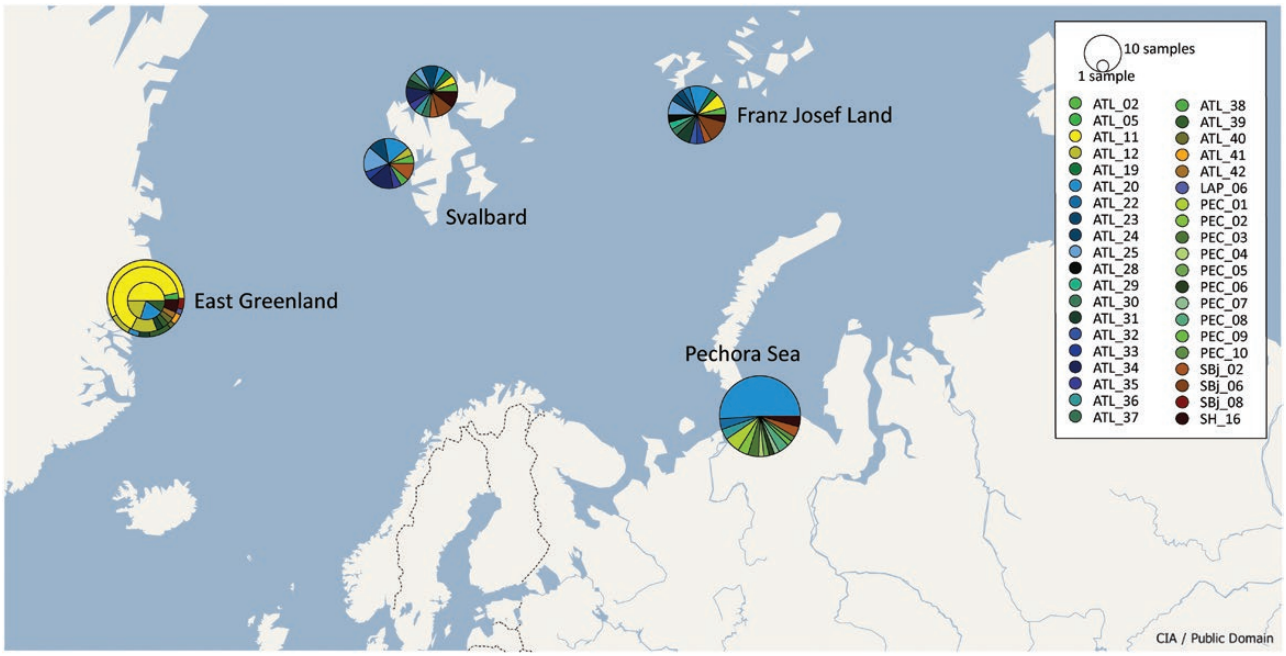


Figure 1. Sampling locations of the Atlantic walruses used in this study from the Barents Sea and adjacent regions, with their respective mtDNA haplotype distributions.

Table 1. Genetic diversity estimates and Hardy–Weinberg expectations

	Microsatellite diversity						Haplotype diversity						
	N	H_E	SD	AR	SD	F_{ISmean}	*N*	*H*	HD	SD	π %	SD %	Fu's F_S
Pechora Sea (PEC)	50	0.624	0.445	7.3	3.62	0.033	47	15	0.74	0.17	0.447	0.279	**–6.535**
Franz Josef Land (FJL1992)	24	0.639	0.328	5.1	2.15	0.097	24	18	0.96	0.02	0.669	0.397	
Svalbard 2003–2004 (SVA2003)	20	0.628	0.295	4.9	2.15	0.009	19	15	0.98	0.02	0.677	0.406	
Svalbard 1992 (SVA1992)	22	0.638	0.286	5.2	2.65	0	18	10	0.92	0.04	0.413	0.272	
SVA–FJLtot	66	0.645	0.528	6.4	3.32	0.037	61	24	0.95	0.01	0.607	0.356	**–11.127**
EGR2010a (EGR2010a)	23	0.576	0.293	4.4	1.62	0.020	31	8	0.73	0.12	0.289	0.274	
EGR2010b (EGR2010b)	20	0.580	0.273	4.8	1.65	0.041	10	4	0.58	0.10	0.363	0.239	
EGR2002 (EGR2002)	n/a	n/a	n/a	n/a	n/a	n/a	43	8	0.65	0.08	0.422	0.267	
EGR2004 (EGR2004)	n/a	n/a	n/a	n/a	n/a	n/a	20	5	0.56	0.11	0.219	0.167	
EGRtot	43	0.584	0.393	5.9	2.31	0.028	104	12	0.61	0.05	0.350	0.227	–3.621

Sample size (*N*) for microsatellites, genetic diversity (H_E) and allele richness (AR), with associated SDs and F_{ISmean} (deviation from Hardy–Weinberg expectations), based on 14 microsatellites (GenAlEx 6.5: Peakall & Smouse, 2006; FSTAT: Goudet, 2001). Sample size (*N*) for mtDNA sequences, number of mtDNA haplotypes (*H*), haplotype diversity (HD), nucleotide diversity (π) (ARLEQUIN 3.5.1: Excoffier & Lischer, 2010), keeping the sampling localities and years separate and for the data pooled by area. Tests for selective neutrality in terms of Fu's F_S (Fu, 1997) were estimated for the data pooled by areas. Bold = significant at the 5% level. n/a, not analysed.

(QIAGEN). Mix 1 consisted of *Orr9*, *SPGV9* and *Orr11*; Mix 2 consisted of *PV9*, *Orr23*, *Orr24*, *Igf-I* and *HGDii*; Mix 3 consisted of *HG8.10*, *M11*, *Orr7*, *Hg4.2*, *Orr16* and *HG6.1*. The PCR products were analysed using an ABI PRISM 3730 DNA sequencer and genotyped in GeneMapper version 4.2 (Applied Biosystems).

A 506-bp-long mtDNA sequence covering part tRNA-thr, tRNA-pro and the hypervariable portion of the control region was amplified using the primers Odro1025L: 5′-ATGAATCGGAGGACAACC-3′ and H00019: 5′-CCACAGTTATGTGTGATCATG-3′ developed for the Pacific walrus (Sonsthagen *et al.*, 2012). Amplification was conducted with a reaction volume of 20 µL using standard PCR and an annealing temperature of 54 °C. Both strands were sequenced using Sanger sequencing at the commercial service provided by Macrogen Inc. (Amsterdam, Holland). All sequences are deposited in GenBank (accession no. MF166700-MF166724).

DATA ANALYSES

Genetic variation

Microsatellites

Genetic variation, estimated as observed and expected heterozygosity, allele richness and tests for goodness of fit to the Hardy–Weinberg equilibrium (HWE) were performed in FSTAT (Goudet, 1995) and GenAlEx 6.5 (Peakall & Smouse, 2006) (Table 1). The possible presence of null alleles in the microsatellite loci was checked using MICRO-CHECKER 2.2.1 (Van Oosterhout *et al.*, 2004). Genotypic linkage disequilibrium was tested (pairwise) between the 14 loci using GENEPOP version 3.4 with 5000 iterations (Raymond & Rousset, 1995).

mtDNA

Sequences were analysed using Sequencher version 5.2.3 (Gene Codes Corp., Ann Arbor, MI, USA). Identical haplotypes among the 212 sequences (Table 1), including downloaded sequences from Lindqvist *et al.* (2009, 2016) (see later for Geneass. No.), were found using POPSTR, a software package developed by H. R. Siegismund (personal communication). Variation in the mtDNA sequences was estimated as haplotype diversity (HD) and nucleotide diversity (π) using ARLEQUIN 3.5.1 (Excoffier & Lischer, 2010).

Population structure

Microsatellites

The number of groups represented in the samples was estimated using STRUCTURE version 2.3.4 (Pritchard, Stephens & Donnelly, 2000). This software uses a Bayesian approach by clustering individuals, minimizing Hardy–Weinberg and gametic phase disequilibrium between loci. The analysis was conducted using the admixture model and the model of correlated allele frequencies between clusters. The results of the tests were based on 1 000 000 iterations, a 100,000 burn-in period and ten independent runs. All samples were combined and assumed to have originated from one to six groups (=K) depending on how samples were combined, without prior information regarding the sample's origin. The clusters of individuals forming the number of populations with the highest likelihood were assigned to sampling locations. ΔK (Evanno, Regnaut & Goudet, 2005) was applied to infer the number of clusters, as this might be difficult due to extensive admixture and isolation by distance (Pritchard *et al.*, 2000; Falush, Stephens & Pritchard, 2003); final estimation was performed using STRUCTURE HARVESTER (Earl & VonHoldt, 2012). Further, CLUMPAK software that automatically processes the structure results across the independent runs of K was applied to visualize the STRUCTURE results (Kopelman *et al.*, 2015).

To investigate possible temporal structure effects, that is, a population unit might enter the sampling areas depending on a seasonal or yearly cycle, population structure was further analysed using the unbiased F_{ST} statistics (Weir & Cockerham, 1984) in ARLEQUIN version 3.5.1 (Excoffier & Lischer, 2010). Two separate analyses were conducted; first samples from a location were treated as units according to their collection times in order to test for temporal effects, and then samples from a given location were combined to test for overall population structure. The degree of population differentiation was analysed using 10 000 permutations to estimate the genetic difference between potential populations/subpopulations. Additionally, discriminant analysis of principal components (DAPC; Jombart, Devillard & Balloux, 2010) was used to further explore the possibility of group structure in the data not detected by STRUCTURE. This method is a multivariate method that uses the genetic relationships among individuals to identify groups. The method is based on allele frequencies of the microsatellite markers and was conducted using the Adegenet package (Jombart, 2008) in R (www.r-project.org; R Development Core Team, 2008).

mtDNA

The possibility for population structure based on variation in the mtDNA sequences was examined using Φ statistics. The genetic distance used between the mtDNA sequences was the pairwise distance between the haplotypes. Both temporal effects and location effects were tested as described above. Estimates

were all run for 10 000 permutations over individual haplotypes among potential populations/subpopulations and tested using ARLEQUIN 3.5.1 (Excoffier & Lischer, 2010). The sequential Bonferroni procedure was applied using a significance level of 5% whenever multiple tests were performed (Rice, 1989).

Finally, relationships among the observed mtDNA haplotypes from the various groups of Atlantic walruses were estimated based on a median-joining network to allow for intermediate haplotypes in the network (Bandelt, Forster & Rohl, 1999). The network was generated using DnaSP (Librado & Rojas, 2009) and POPART (Leigh & Bryant, 2015), a software that constructs haplotype networks. For this analysis, sequences from Lindqvist *et al*. (2009, 2016) were included to identify identical haplotypes.

Migration rates and direction

Microsatellites

A Bayesian method based on multilocus genotypes, implemented in BA3-3.0.3 (Wilson & Rannala, 2003), was used to estimate recent migration rates and directions (first- and second-generation migrant ancestry) between the sampling areas using the microsatellite data. Based on the information from the gametic disequilibrium that is generated by migration, the model assumes that sampling occurs after reproduction and before migration and infers the individual's population ancestry by assigning alleles to the populations of origin. The Markov Chain Monte Carlo (MCMC) mixing migration rates, inbreeding coefficients and allele frequencies used were those recommended by the developer (40–60% of the iterations). Because this study is focused on only a few groups, some of which might be connected geographically while others are recognized as two populations (EGR and SVA–FJL; NAMMCO, 2006) BA3-3.0.3 (Wilson & Rannala, 2003) was used to estimate migration rates and directions following the recommendations by Meirmans (2014). Further, ten runs with different seed numbers were performed with 1×10^8 MCMC iterations and a burn-in phase of 1×10^7 iterations and a sampling interval of $N = 1000$. Convergence was verified using the tracer file option from BA3-3.0.3 and Bayesian deviance was calculated from each run using the R-script provided by Meirmans (2014). The run with the lowest Bayesian deviance was chosen, as recommended by Meirmans (2014). Further, BA3-3.0.3 was also used to infer the ancestry of the individual samples up to two generations back in time. This program allows for testing whether potential migrants, F1 hybrids or backcrossed individuals, exist in the analyzed populations. This was estimated using identical parameters and conditions as described above for BA3-3.0.3 to estimate the migration.

DEMOGRAPHIC HISTORY

Bottleneck

Microsatellites

Walruses in the EGR and SVA are known to have experienced serious reductions in their numbers due to hunting in the 18th century (e.g. Gjertz, Wiig & Øritsland, 1998; Witting & Born, 2014), leading to potential bottlenecks (Lindqvist *et al.*, 2016). During a bottleneck, population size declines abruptly, which affects the number of alleles faster than loss of heterozygosity, which consequently causes a heterozygote excess in the population (Cornuet & Luikart, 1996). In this study, each walrus group was examined using BOTTLENECK 1.2 (Piry, Luikart & Cornuet, 1999) applied to the microsatellite data set to explore potential genetic impacts of historical bottlenecks. This is a non-parametric test that evaluates whether the number of loci with heterozygote excess is larger than that expected to occur by chance alone. The test was performed assuming the two-phase mutation model (TPM) (Di Rienzo *et al.*, 1994), where 90% single-step mutations, 10% multistep mutations and a variance of 12% were allowed.

Demographic inferences using mtDNA

mtDNA

Fu's F_S was calculated for the mtDNA data set for each of the three groups identified by the analysis of molecular variance (AMOVA) analysis of Φ_{ST} – PEC, SVA–FJL and EGR (Fu, 1997) – to explore the possibility of historical population fluctuations. If excess numbers of low-frequency mutations, relative to expectations under the standard neutral model, are detected, this is indicative of recent population growth and is reflected by a significantly negative value of F_S. If large statistically significant positive values of F_S are observed, it indicates a deficiency of rare haplotypes, which suggests that the population has experienced a bottleneck (ARLEQUIN 3.5.1; Excoffier & Lischer, 2010).

Extended Bayesian Skyline Plot (EBSP) analysis (Heled & Drummond, 2008) was applied to investigate long-term fluctuations in female effective population size (N_{ef}), using BEAST (Drummond *et al.*, 2005). Samples identified as migrants in BA3-3.0.3 with a probability score of >0.90 were excluded to avoid violation of the assumption of panmixia of the analyzed populations (Heled & Drummond, 2008). EBSP estimates the number of demographic changes directly from the data and thereby tests for deviations from constant size. Given the results of mtDNA AMOVA analysis of Φ statistics, three data sets were analysed: (1) all individuals sampled from localities in EGR [excluding three migrants from SVA–FJL; Table 1, identified in BA3-3.0.3 (Wilson & Rannala, 2003)], (2)

SVA–FJLtot (excluding one admixed sample identified in BA3-3.0.3 to be a migrant from EGR) and (3) PEC samples (excluding 23 admixed samples identified as migrants from SVA–FJLtot in BA3-3.0.3).

The final MCMC sample was based on a run of 50 000 000 iterations and genealogies were sampled every 5000 iterations with 10% discarded as a burn-in. Examination of convergence and effective sample size (ESS) values were conducted using TRACER version 1.5 (Drummond & Rambaut, 2007). All parameters had ESS values >200 and additional runs gave similar results. The HKY substitution model with four gamma categories was assumed based on the AIC (Akaike information criterion) in JMODELTEST version 2.11 (Posada, 2008). A substitution rate of 0.075×10^{-6} substitution/site/year [estimated for southern elephant seal (Slade *et al.*, 1998) to unscale the estimate of effective population size] with a female walrus generation time of 15 years was used, similar to earlier studies of Atlantic walrus genetics (e.g. Andersen *et al.*, 2009).

Phylogenetic analyses and divergence times

mtDNA

Bayesian phylogenetic analysis and estimation of TMRCA (Time of Most Recent Common Ancestor) was performed using BEAST version 1.7.4 (Drummond & Rambaut, 2007). The HKY substitution model used for demographic inference was applied. Preliminary analyses using an uncorrelated log-normal clock model showed no significant evidence for rate heterogeneity among branches (Drummond *et al.*, 2007) and thus subsequent analyses were conducted using a strict clock approach. The data were analysed using three different tree priors, constant size, exponential growth and expansion, and the model showing the highest support based on Bayes factor analyses (conducted in Tracer version 1.5; Drummond & Rambaut, 2007) was subsequently chosen. The timing of divergence from the most recent common ancestor was estimated based on a substitution rate of 0.075×10^{-6} substitution/site/year estimated based on data from southern elephant seals (Slade *et al.*, 1998). The substitution model, the MCMC length and burn-in and the examination of convergence were performed as in the demographic analysis. The maximum clade credibility tree with mean heights for branches was estimated in the program TREEANNOTATOR (Drummond & Rambaut, 2007) with a 10% burn-in, visualized and edited in the program FIGTREE version 1.3.1 (Andrew Rambaut, University of Edinburgh, http://tree.bio.ed.ac.uk/software/figtree/). TMRCA and posterior possibilities for the phylogenetic relationships were extracted directly from this tree.

Assessment of divergence times and general phylogenetic relationships utilized two primary data sets. The first included all the individual mtDNA sequences (including already published Atlantic and Pacific sequences; Lindqvist *et al.*, 2009, 2016; Sonsthagen *et al.*, 2012). The second data set contained only the individual haplotypes of the Atlantic walrus (including sequences from Lindqvist *et al.*, 2009, 2016). This data set was used to infer the genetic relationship between walruses sampled in the PEC compared to walruses from EGRtot and from SVA–FJLtot.

RESULTS

Microsatellite data

Genetic diversity estimates (measured as heterozygosity and allele richness) as well as tests for Hardy–Weinberg expectations are given in Table 1 and Supporting Information, Appendix 1. Levels of polymorphism across the loci varied. Average mean expected heterozygosity based on the 14 loci ranged from 0.576 ± 0.293 (SD) in the EGR2010a sample to 0.645 ± 0.528 (SD) in SVA–FJLtot. Allele richness ranged from 4.4 ± 1.62 (SD) in EGR2010a to 7.3 ± 3.62 (SD) in the PEC sample.

Significant deviations from HWE expectations were observed in the FJL1992 sample, which could be ascribed to a significant heterozygote deficiency at locus *Orr23*. Further, a significant deviation from HWE was observed in the PEC sample at the *Igf-I* locus, similarly caused by heterozygote deficiency, though this situation was not observed in the overall test (Supporting Information, Appendix 1). However, when samples from different time frames were combined for the different sampling areas [i.e. (1) PEC, (2) FJL1992, (3) SVA1992 + SVA2003 and (4) EGRtot; Table 1; Supporting Information, Appendix 1], only a single locus in the PEC showed any significant deviation from HWE after sequential Bonferroni corrections. MICRO-CHECKER did not detect the presence of null alleles in any of the loci from the different combinations of samples (data not shown). Analysis for genotypic linkage disequilibrium revealed that one pair (*SPGV9* and *PV9*) was significantly linked (all populations) (data not shown).

Mitochondrial data

A total of 506 bp in the mitochondrial DNA were sequenced for a total of 212 individuals. For haplotype identification, sequences from Lindqvist *et al.* (2009) and Lindqvist *et al.* (2016) were downloaded from NCBI and aligned and cropped to the 506 bp size. Fifty segregating sites were observed in the 506-bp fragment, representing 44 haplotypes, including three haplotypes previously only found in the Laptev Sea (EU728525, EU728526, EU728527). Twenty-five new haplotypes were among the 44 haplotypes observed (Fig. 1; Fig. 2; Supporting

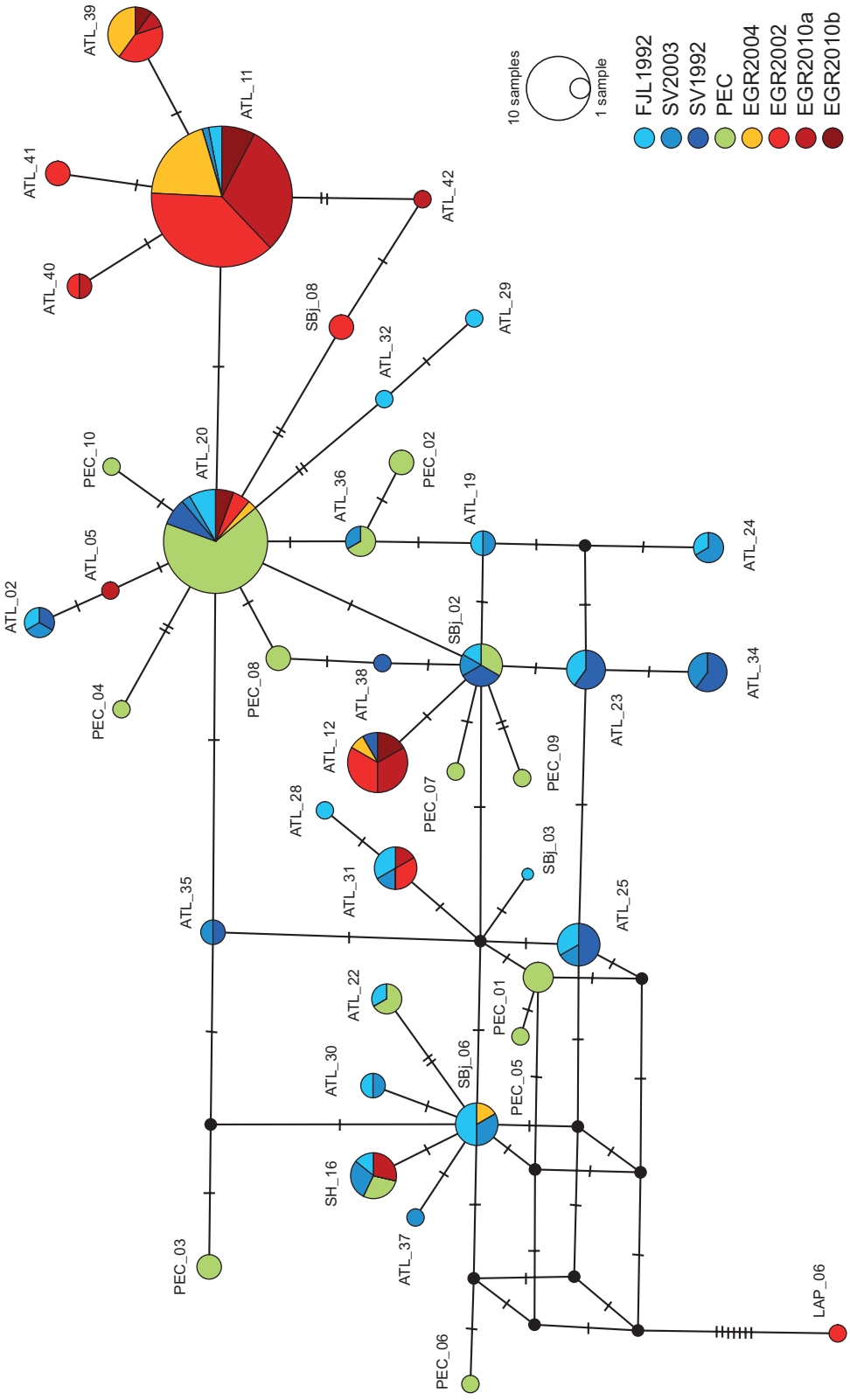


Figure 2. Median-joining network between mtDNA haplotypes of Atlantic walruses in our study region (and an adjacent region) indicating the phylogenetic relationships among the different locations estimated using DnaSP (Librado & Rozas, 2009) and POPART (Leigh & Bryant, 2015). Haplotype names are given next to the circles. The size of the circles indicates the relative frequency of the haplotypes. The number of cross bars on the line connecting haplotypes indicates the number of mutations separating the respective haplotypes. The legend to the right gives sampling areas and years.

Information, Appendix 2a). Overall 14 unique haplotypes were observed in the SVA–FJLtot sample. In EGRtot, four unique haplotypes were observed, when compared to the SVA–FJL and PEC samples, and in the PEC, 10 unique haplotypes were observed (Fig. 2). ATL_20 was the most common haplotype, which was found mostly in samples from SVA, FJL and PEC, though it was observed in all sampling locations. ATL_11 was by far the most frequent haplotype in the EGR walruses. The rest of the haplotypes were shared between different combinations of sampling locations and sampling periods. The HD ranged from 0.56 ± 0.114 (SD) in EGR2004 to 0.98 ± 0.022 (SD) in SVA2003, while nucleotide diversity (ð) ranged from 0.00219 ± 0.00167 (SD) in EGR2004 to 0.00677 ± 0.00406 (SD) in SVA2003.

Population structure

STRUCTURE analysis (based on the 14 microsatellite loci) performed on the samples from the different areas sampled at different times, and on the combined samples from all areas, suggested the presence of two clusters (Fig. 3A–C), with the SVA–FJLtot and PEC forming one cluster and the EGRtot forming the other for all values of *K*. Similarly, testing only SVA–FJLtot and PEC using STRUCTURE (keeping EGRtot out of the analyses), only one group was detected (data not shown). A few loci deviated significantly from HWE in the microsatellite data, and thus the effect of these loci on the population structure was tested by analysing the data set without the markers in STRUCTURE. This analysis did not reveal any difference between the two data sets. Further, the effect of the observed linkage between one microsatellite pair was also tested in STRUCTURE by removing one (*PV9*) from the data set. No effect was observed (data not shown). Thus, the full data set was kept in all analyses based on microsatellite markers.

The F_{ST} results from ARLEQUIN, based on the microsatellite variation focussing on the temporal samples (Table 2a), revealed statistically significant differences between PEC and SVA1992 and PEC, FJL1992, SVA1992, SVA2003 samples and all of the samples from EGR. Combining the temporal samples from the three different areas (EGRtot, SVA–FJLtot and PEC) resulted in three genetically distinct areas (Table 2b). The F_{ST} value between the PEC and SVA–FJLtot was low, but it was statistically significant. The same pattern was apparent in the principal component analysis, DAPC (Fig. 4). Similarly, Φ_{ST} estimates based on mtDNA data suggested that the PEC showed significant differences from all other areas (Table 2a, b).

Migration rates and direction

The estimated recent migration rates and directions obtained from microsatellite markers are based on the migration that has occurred within the last two to three generations (Wilson & Rannala, 2003) (Table 3a, b). The ancestral location of most of the walruses in this study was also their sampling location. However, some individuals sampled in PEC had an ancestor from SVA–FJL (mainly from the SVA2003 sample, data not shown) one or two generations ago (F1 hybrid or backcrossed individuals), suggesting a migration direction from SVA–FJL to PEC. This was even more explicit when analysing the ancestry of individuals. Twenty-three of the individuals sampled in PEC showed a first- or second-generation migration ancestry from SVA–FJL. Three individuals from EGR also showed a recent migration from SVA–FJL. EGR showed no recent migrants from PEC (Table 3b). As the observed linkage between two microsatellite markers might affect the estimated migration rate and direction, *PV9* was removed and the data set was reanalysed. This did not alter the results of the analysis based on the complete data set.

Demographic analysis

No bottlenecks were observed for the EGRtot, SVA–FJLtot or PEC data sets (data not shown). However, in PEC, a significant heterozygote deficiency was observed ($P = 0.012$), suggesting that this sample might not be in mutation drift equilibrium (BOTTLENECK 1.2; see Piry *et al.*, 1999).

Fu's F_S (1997) were highly significant for PEC and SVA–FJLtot samples, but not for EGRtot walruses (Table 1). EBSP (Fig. 5) estimated in BEAST indicated different demographic histories for the three areas (EGR, SVA–FJL and PEC), matching the results of the Fu's F_S test.

EGR showed no evidence of population size changes (95% HPD: 0–2) (Fig. 5). Analyses of population size changes of the separate SVA–FJL and PEC walruses demonstrated a possible increase in N_{ef} (Fig. 5). These plots showed a median number of one population size change, but the 95% HPD included zero (95% HPD: 0–3). Median estimated effective female population size N_{ef} for EGR was around one-third (3012, 95% HPD: 37–16 723) of the estimate derived from EBSP analysis of SVA–FJL (11 763, 95% HPD: 43–38 361) or PEC (9477, 95% HPD: 2–67 083).

A single individual from EGR was highly divergent from all the other samples in this study (Figs 2, 6; LAP_06). But, otherwise the median-joining network (Fig. 2) showed a close relationship among all haplotypes, which were only separated by a few mutational steps. Three haplotypes mainly observed in the samples from SVA–FJL and PEC (ATL_20, SBj_02, SBj_06) showed a

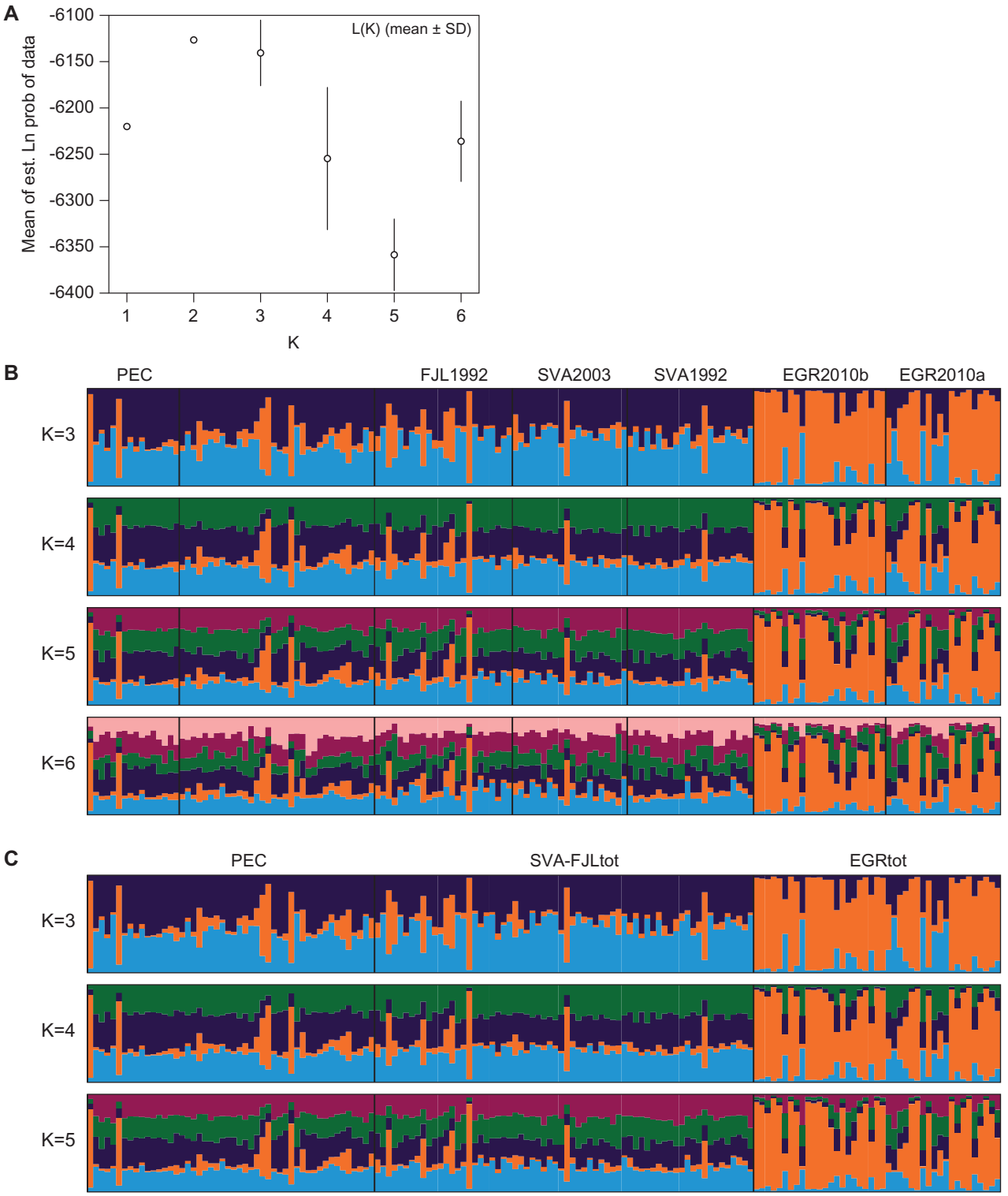


Figure 3. Graphical output from HARVESTER (A) applying ΔK of Evanno *et al.* (2005) to infer the number of clusters and from STRUCTURE (Pritchard *et al.*, 2000), processed in CLUMPAK (Kopelman *et al.*, 2015), (B) K = 3–6, keeping the temporal samples from the same locations separate and (C) K = 3–5, combining all samples from the same location. Each vertical line in (B) and (C) represents an individual, and the colour composition displays the probability of belonging to each of the defined clusters.

Table 2. Pairwise population structure estimates based on variation in 14 microsatellites (below diagonal, seven sampling events) and sequence variation in the mtDNA (above diagonal, eight sampling events)

(a)

	PEC	FJL1992	SV2003	SV1992	EGR2010a	EGR2010b	EGR2002	EGR2004
PEC		**0.083**	**0.119**	**0.169**	**0.219**	**0.160**	**0.224**	**0.298**
FJL1992	0.010		0	0.054	**0.247**	**0.201**	**0.263**	**0.331**
SVA2003	0.011	0		0.055	**0.296**	**0.250**	**0.313**	**0.385**
SVA1992	**0.017**	0.005	0		**0.352**	**0.317**	**0.345**	**0.472**
EGR2010a	**0.055**	**0.027**	**0.033**	**0.025**		0	0	0.007
EGR2010b	**0.031**	**0.044**	**0.051**	**0.047**	0		0	0.016
EGR2002	n/a	n/a	n/a	n/a	n/a	n/a		0

(b)

	PEC	SVA–FJLtot	EGRtot
PEC		**0.099**	**0.314**
SVA–FJLtot	**0.012**		**0.252**
EGRtot	**0.045**	**0.038**	

(a) Different temporal samples of Atlantic walruses from East Greenland (EGR), Svalbard (SVA), Franz Josef Land (FJL) and Pechora Sea (PEC) locations and (b) when combining samples across time for the same location, estimated as F_{ST} (below diagonal) and ϕ_{ST} (using pairwise distance) in ARLEQUIN (Excoffier & Lischer, 2010). Bold = significant after sequential Bonferroni correction; (a) $P < 0.00099$, after sequential Bonferroni correction of P; (b) $P < 0.000001$, after sequential correction of P (Rice, 1989). n/a, not applicable.

star-like pattern typical for a recent population expansion. The star-like pattern was less apparent in EGR where only ATL_11 showed this structure (Fig. 2).

Phylogeny

Bayes factor analyses between the tested models showed highest support for the constant size tree prior, compared to exponential growth (Bayes factor 12.5) or expansion tree priors (Bayes factor 14.6) (Jeffreys, 1961). Thus, time to the most recent common ancestor (TMRCA) was estimated using this tree prior. There was high support for an Atlantic clade with an estimated posterior probability of 1, separated from the Pacific clade represented by Laptev Sea individuals in this study (Fig. 6). The TMRCA between the Atlantic clade and the Pacific clade was estimated to be 949 Kyr (95% HPD: 644–1281). Within the Atlantic clade, posterior support was low and no localities (or subpopulations) showed monophyly of haplotypes. TMRCA of the Atlantic clade was estimated to be 268 Kyr (95% HPD: 150–396 Kyr). Using only the D-loop region (excluding the part of *CytB*, tRNA-thr and tRNA-pro sequenced) did not change the results; TMRCA between Pacific and Atlantic walrus using this subset of data was 1.265 Kyr (95% HPD: 833–1.779 Kyr) and TMRCA of the Atlantic clade was 331 Kyr (95% HPD: 185–497 Kyr).

DISCUSSION

This study provides new insights into the evolutionary history of Atlantic walruses in general, and novel data for walruses from the PEC in particular. The new population structure information is highly relevant to management agencies responsible for conservation planning regarding this species in the Barents Region (NAMMCO, 2010).

Overall population structure

Population structure analyses revealed a pronounced primary population structure separating the EGR walruses from all the other groups within the Barents Region. STRUCTURE, pairwise multilocus F_{ST} tests based on microsatellite markers and Φ_{ST} and the median-joining network based on mtDNA all showed this clear genetic differentiation. Furthermore, the estimates of migration rate and direction indicated only very limited exchange between EGR and SVA–FJL and no exchange between EGR and PEC (also see Andersen *et al.*, 1998).

The population structure between walruses sampled at PEC and walruses sampled at SVA–FJL was less definitive. STRUCTURE analysis did not detect any separation between these areas. However, studies (Latch *et al.*, 2006; Chen *et al.*, 2007) have reported that for levels of genetic differentiation of F_{ST} <0.02, STRUCTURE has problems inferring the number of clusters correctly and these are the levels of potential genetic separation observed between the SVA–FJL and PEC in the present study. Therefore, lack of differentiation of groups according to STRUCTURE should be interpreted with caution. Pairwise F_{ST} analyses in ARLEQUIN detected small but significant genetic

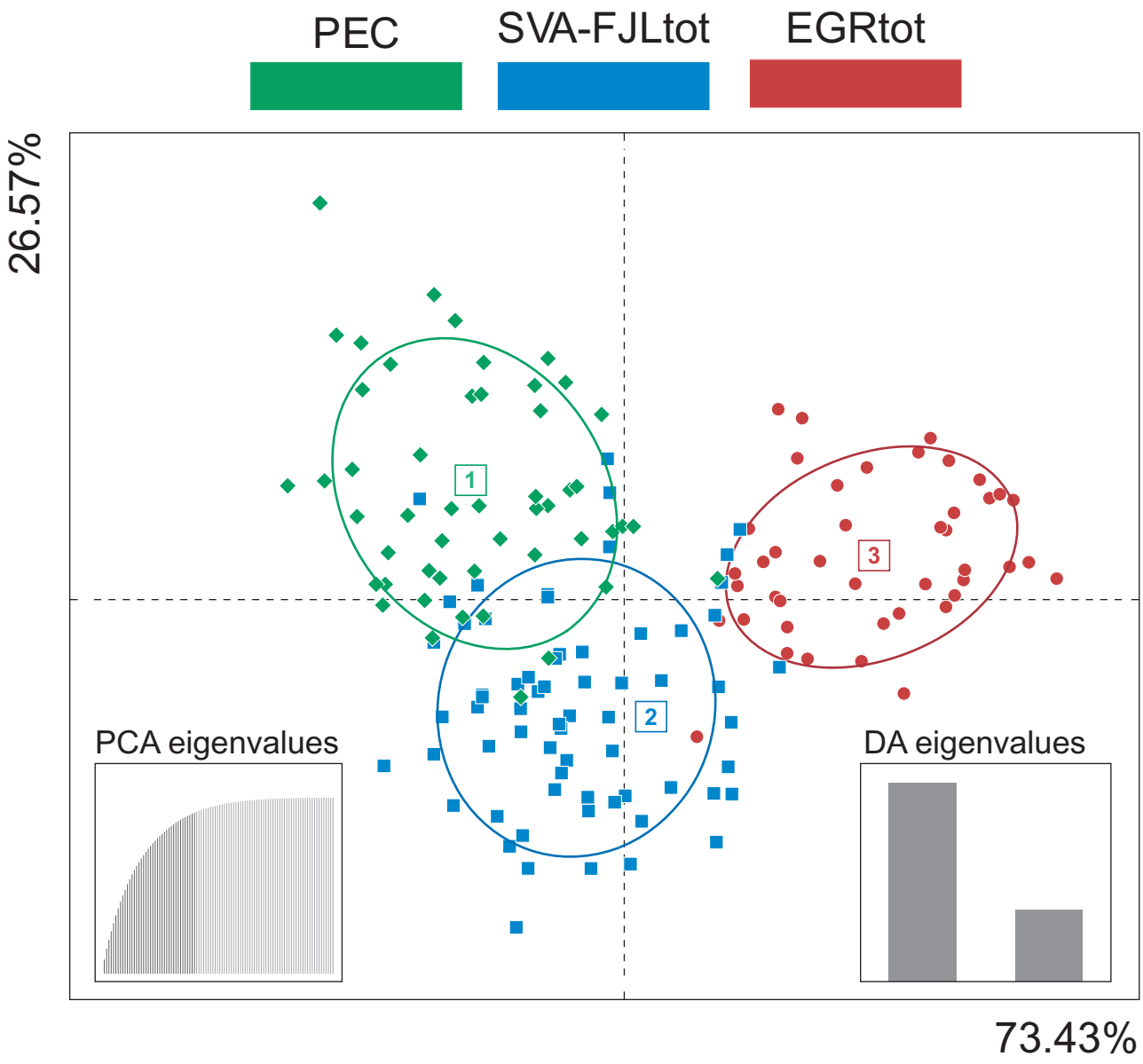


Figure 4. Discriminant analysis of principal components (DAPC; Jombart *et al.*, 2010) identifying the different genetic clusters of the Atlantic walruses based on variation of 14 microsatellites (combining the samples from the same areas).

differences between the PEC and the SVA1992 samples, as well between the PEC and SVA–FJLtot.

Two possible hypotheses might explain the findings of a low, but significant, level of genetic differentiation observed at the microsatellite level: (1) it might be a temporal effect caused by the difference in sampling periods combined with possible migration or (2) the walruses in the two areas are in an early stage of divergence. Addressing the first hypothesis, the sampling interval does include 21 years, which is approximately equivalent to *ca.* 1.5 walrus generations (Andersen *et al.*, 2009; Pacifici *et al.*, 2013). The SVA1992 sample comprised solely adult males (Supporting Information, Appendix 2b), the majority of which would most likely not be alive in 2012 and hence the significant signal could be an effect of genetic drift (i.e. a temporal effect). Moreover, the analysis of migration direction and rate focussed on the individual ancestry suggested that most individuals within the PEC had a migrant ancestry in SVA–FJL. The median-joining network indicated a close genetic relationship between PEC and SVA–FJL haplotypes, and 12 PEC individuals with suggested migrant histories from SVA–FJL had the

Table 3. Means of the posterior distribution and number of individuals with migrant ancestry

(a)

	Migrated from		
Migrated to	PEC	SVA–FJLtot	EGRtot
PEC	**0.758**	**0.225**	0.016
95% CI	**0.699–0.817**	**0.161–0.289**	−0.016–0.048
SVA–FJLtot	0.011	**0.941**	**0.048**
95% CI	−0.009–0.032	**0.892–0.990**	**0.002–0.526**
EGRtot	0.009	**0.061**	**0.930**
95% CI	−0.009–0.027	**0.012–0.11**	**0.879–0.982**

(b)

Sampling area	Migrant origin	Non-migrant*	Migrant†	NS‡
PEC		5		22
	SVA–FJLtot		23	
SVA–FJLtot		39		26
	EGRtot		1	
EGRtot		25		15
	SVA–FJLtot		3	

Means of the posterior distribution of (a) migration rates and directions (m) among the three areas Pechora Sea (PEC), Svalbard–Franz Josef Land (SVA–FJL) and East Greenland (EGR). The populations from which individuals migrated are given in rows while populations where individuals were sampled are given in columns and b) number of individuals with migrant ancestry (first- or second-generation migrants) in one or the other areas (defined with an exclusion probability of ≤0.05 of belonging to the sampling area where they were collected. Other individuals with a higher exclusion probability are not reported) [BA3 (BAYESASS); Wilson & Rannala, 2003]. BOLD = significant 95% CI interval.
*Non-migrant: Number of individuals that were considered non-migrants when they were rejected at $P \leq 0.05$ to have originated from areas other than the sampling area.
†Migrant: Number of individuals that were considered first- or second-generation migrants when they were rejected at $P \leq 0.05$ to have originated from their sampling area.
‡NS: Number of individuals that could not be rejected at $P \leq 0.05$ to have originated from more areas.

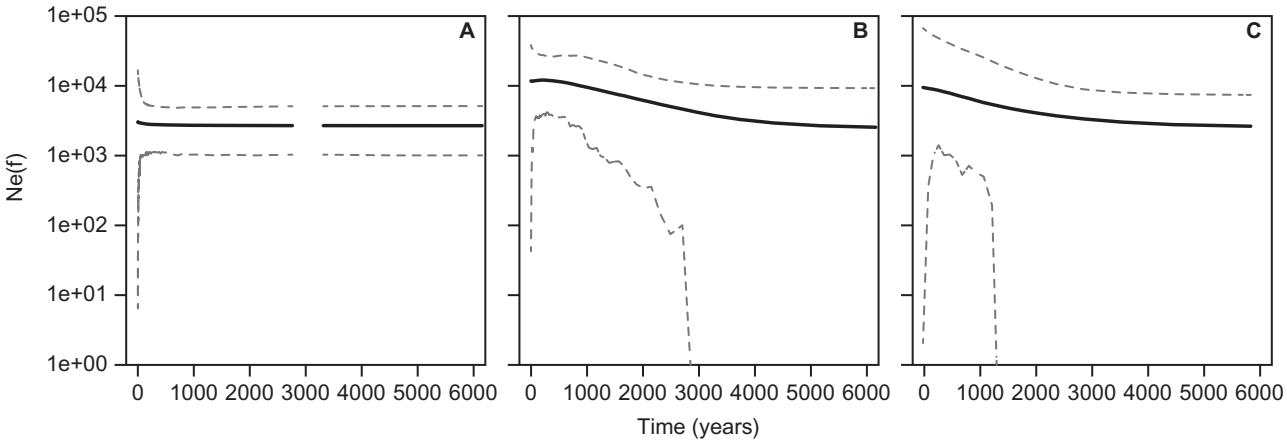
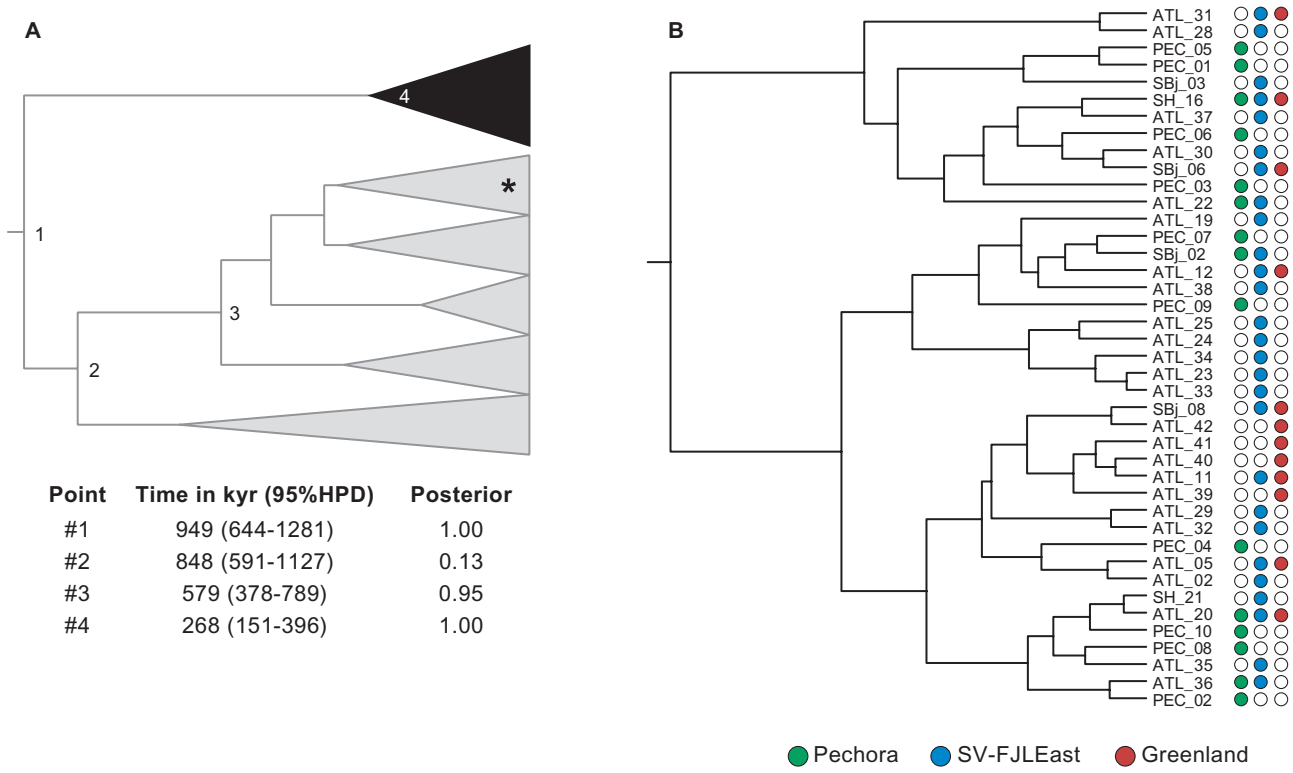


Figure 5. Extended Bayesian Skyline Plots (EBSP) showing changes in effective female population (N_{ef}) size over time. Solid lines represent the median EBSP values while the 95% highest probability density (HPD) intervals are shown as dashed lines (BEAST; Drummond & Rambaut, 2007) for (A) East Greenland, (B) Svalbard–Franz Josef Land and (C) Pechora Sea.

most common haplotype (ATL_20) observed in all areas, suggesting that this is an ancestral haplotype. Two old haplotypes from a study of walruses on SVA using historical DNA samples (Lindqvist *et al.*, 2016) SBj_02 and SH_16, were also found in two immigrant individuals from PEC, which could indicate historical connectivity between the areas or incomplete lineage sorting. Lastly, the observation of the contrasting significant pattern of F_{ST} and Φ_{ST}, (F_{ST} being not significant) between PEC and the more recent SVA2003



Point	Time in kyr (95%HPD)	Posterior
#1	949 (644-1281)	1.00
#2	848 (591-1127)	0.13
#3	579 (378-789)	0.95
#4	268 (151-396)	1.00

Figure 6. Major clades in the phylogenetic tree based on the sequence variation in mtDNA haplotypes of Atlantic and Pacific walruses. A, numbers give the posterior support of the nodes. Information about Time of Most Recent Common Ancestor (TMRCA) and posteriors for the main phylogenetic clades are found in the associated table. Black clades denote the Atlantic walrus and grey clades represent the Pacific walrus. The asterisk shows the position of an unusual individual haplotype in EGR, which showed a Laptev Sea ancestry. B, phylogeny of Atlantic walruses according to BEAST (Drummond & Rambaut, 2007).

sample could imply recent gene flow connecting the areas favouring the first hypothesis. Evidence that supports the second hypothesis is that pairwise Φ_{ST} estimates were all significantly different, suggesting that the PEC constitutes a separate population unit that is diverging slowly from SVA–FJL. Seven individuals with possible migrant histories in SVA–FJL had a unique mtDNA haplotype, found only among PEC, further supporting the existence of a separate population unit. However, since mtDNA is only inherited maternally, and it is probably that females are more stationary than males, similar to many other sexually dimorphic animals, migration connecting the areas might be heavily male biased. Movement studies (Born & Knutsen, 1992; Wiig *et al.*, 1996; Born *et al.*, 2005) have indicated that Atlantic walrus males are more mobile than females. Male walruses from SVA have been documented to temporarily migrate to FJL during the breeding season in winter (Wiig *et al.*, 1996; Freitas *et al.*, 2009; Lowther *et al.*, 2015) supporting an assumption of male-biased gene flow from SVA–FJL. This is further supported by the fact that a substantial fraction of the individuals sampled in PEC had an ancestor from SVA–FJL.

Lydersen *et al.* (2012) conducted an aerial survey of walruses in the PEC and derived an estimate (i.e. accounting for animals at sea during the survey) of the number of walruses occupying this area of 3943 (95% CI 3605–4325). However, no females with calves were seen in this survey, which lead the authors to conclude that the source population of the PEC males hauled out during summer clearly has a distributional area that is larger than the area that was surveyed. However, relatively little is known about the distribution of walruses in the PEC and neighbouring areas. Walruses have been seen on the ice during winter in the PEC (Haug & Nilssen, 1995) and several summering haul-out sites are known in the region. Recent (on-going) tracking studies in PEC suggest some movements between the southern PEC to areas along Novaya Zemlya, but no movements to FJL have been documented.

Genetic diversity

In general, the levels of genetic variation in the PEC (Table 1) were similar to those found in other studies of Atlantic walruses (Atlantic walrus H_E = ranging from 0.59 to 0.66 (Andersen *et al.*, 2009, based on 11 microsatellite loci; Andersen *et al.*, 2014; Shafer *et al.*, 2014). The haplotype variation and nucleotide diversity observed (Table 1) was at a level similar to that observed in other pinniped species that have experienced declines in the past or are declining currently, such as Guadalupe fur seals (*Arctocephalus townsendi*) (pre-bottleneck H = 0.997, π = 5.5%; post-bottleneck H = 0.798, π = 2.5%; Weber, Stewart & Lehman, 2004) and Pacific harbour seals (*Phoca vitulina richardi*) (H = 0.975, π = 1.47%; Westlake & O'Corry-Crowe, 2002), but it was higher than northern elephant seals (*Mirounga angustirostris*) (H = 0.406, π = 0.68%; Weber *et al.*, 2000). Both the Guadalupe fur seal and northern elephant seal have been subjected to extreme levels of commercial hunting, which reduced their numbers and genetic diversity at the mtDNA level (Weber *et al.*, 2000, 2004; Hoelzel *et al.*, 2002). Compared to the Pacific walrus (Sonsthagen *et al.*, 2014), the haplotype and nucleotide diversity documented in this study of the Atlantic walrus were lower, which might be due to the fact that the Pacific walruses have not experienced declines in population size as extreme as those in the Atlantic walrus, which was harvested to near extinction in some stocks/subpopulations (Gjertz, Hansson & Wiig, 1992; Gjertz & Wiig, 1994; Born, Gjertz & Reeves, 1995; Born *et al.*, 1997; Gjertz *et al.*, 1998; Witting & Born, 2014; Lindqvist *et al.*, 2016).

Within the Atlantic walrus populations investigated in this study, genetic diversity was smallest for the EGRtot and largest for SVA–FJLtot samples. This pattern was confirmed independently by analysis of genetic variation obtained from microsatellite markers and mtDNA, and from the N_{ef} estimated by the EBSP method. The reason for the low observed genetic variation in EGRtot might be attributed to the history of the walruses in the area. Backcalculation to historical subpopulation sizes indicated overexploitation since the late 1880s, apparently leading to a severe population depression between the 1920s and late 1950s of the still relatively small and endemic EGR walrus subpopulation (Born *et al.*, 1997; Witting & Born, 2014). However, this study did not detect any bottleneck effect in EGRtot using BOTTLENECK 1.2 (Piry *et al.*, 1999) on the microsatellite data. Although the 95% HPD interval overlapped, the estimated median N_{ef} was smallest for the EGRtot and largest for the SVA–FJLtot.

Demographic population history

Overall Fu's F_S test and EBSPs indicated different trends in the demographic history of the three walrus groups in this study. Both tests supported a constant female population (N_{ef}) size in EGR and expanding populations in SVA–FJL and the PEC, initiated around 30 000–40 000 years ago. The estimated time of population increase is unusual in that population expansion would seem more likely to have occurred after the last Ice Age about 10–15 Kya when re-colonization of the Arctic might have led to an increase in available feeding habitats (*cf.* Born, 2005) and thus an increase in population numbers. This general thinking is supported by analyses of ancient DNA of the bowhead whale which revealed a six-fold increase in female effective population size during the Pleistocene–Holocene transition (Foote *et al.*, 2013). However, timing of population size change in EBSPs is difficult to assess accurately as time is unscaled by substitution rate (Heled & Drummond, 2008; Ho *et al.*, 2008; Ho & Shapiro, 2011). Thus, any uncertainties in substitution rate will translate into uncertainties in the timing(s) of the estimated population size changes, as well as the estimated effective population size (e.g. Jacobsen *et al.*, 2014). In this study, the substitution rate used was estimated using data from southern elephant seals (Slade *et al.*, 1998). Although this species shows some similarities in life-history traits with the walrus, these animals are not closely related; they belong to different pinniped families (Phocidae vs. Odobenidae) and the true substitution rate could be different from the one that was used herein. Moreover, time dependency of substitution rates might also exist (Ho *et al.*, 2005, 2007, 2011; although also see Bandelt, 2008 and Emerson & Hickerson, 2015), which would lead to underestimation of mutation rates over short evolutionary time scales.

The demonstrated increase in N_{ef} in SVA–FJL and PEC is interesting as a previous walrus study analysing 4854 SNPs using Approximate Bayesian Computation (ABC) did not find evidence for expansion in Hudson Bay or West Greenland walruses (Shafer *et al.*, 2015). Instead they found support for a bottleneck model with subsequent constant population size, as is the case in the current study for EGR (Fig. 5). Thus, it is possible that the walruses around SVA–FJL and PEC have a different demographic history compared to the other Atlantic populations, which was reflected in the haplotype network (Fig. 2). Several haplotypes with star-like patterns were observed among walruses from SVA–FJL and PEC in the current study, which is characteristic for expanding populations, in contrast to the pattern observed for EGR where only AT_11 showed a tendency to be star-like. Paleoceanographic conditions during the interstadial periods in Weichselian, when

the sea ice distribution in the Barents Sea varied due to influxes of Atlantic Water, might have caused different availability of food and haul-out sites for the walruses in these different areas (Hughes *et al.*, 2016; Pope *et al.*, 2016). Further, the strong southward-flowing current in the Fram Strait and the deep water of the Greenland Sea (<2000 m deep; Stein & MacDonald, 2004) separating EGR from SVA may act as barriers for walrus migration between these areas. However, Born & Gjertz (1993) documented one case of a walrus moving from Northeast Greenland to northern SVA in 1992 and on occasion walruses are seen on the sea ice over the deep water between Greenland and SVA (NPI, unpublished data). Currently, the SVA–FJL population is increasing at an exponential rate (Lydersen, Aars & Kovacs, 2008; Kovacs, Aars & Lydersen, 2014) following 60+ years of protection from human hunting.

The mtDNA sequences used in this study were short, leading to uncertainties in the estimated number of changes for all populations and the results should, therefore, be interpreted with some caution. Longer mtDNA sequences or preferably extensive SNP data will be needed to address the hypothesis regarding the timing of historical increases in population size suggested in this study.

Phylogeny

BEAST analyses showed a monophyletic Atlantic clade. This result is supported by the study of Lindqvist *et al.* (2009) who used mtDNA sequencing of the partial NADH dehydrogenase 1, cytochrome oxidase 1 and 16S genes and the D-loop and showed monophyly of Atlantic walruses, separate from Pacific walruses. However, Lindqvist *et al.* (2009) did not estimate divergence time between the two subspecies or discuss which external events might have initiated isolation and divergence. Davies (1958) suggested on morphological grounds that the separation between *O. r. divergens* and *O. r. rosmarus* dated from glaciations earlier than the last ice age. A phylogenetic analysis of mtDNA showed that Pacific and Atlantic haplotypes represent separate monophyletic groups and that mtDNA sequence divergence of these subspecies occurred about 500 000 to 785 000 years ago (Cronin *et al.*, 1994).

In this study, divergence time (TMRCA) was estimated to be 949 Kyr (95% HPD: 644–1281 Kyr) between Pacific and Atlantic haplotypes, which corresponds to the middle of the Pleistocene (0.011–2.7 Mya; Larsen, 2006). During the glaciations in the Pleistocene, sea ice cover was extensive and might have represented a barrier to gene flow between the two regions, promoting allopatric divergence. However, gene flow may still occur between the regions, as observed in this study, where one individual sampled in EGR showed an mtDNA sequence matching a haplotype otherwise found only in the Laptev Sea (part of the Pacific clade, see Fig. 6). Given the presence of only one such haplotype within 212 Atlantic walruses, it is reasonable to assume that such long distance migration is rare. However, the finding of two historic haplotypes in bones of animals thought to have been harvested during the mid 1800s on SVA (SBj_08 and SH_16) (Lindqvist *et al.*, 2016), matching sequences from EGR, suggests occasional connections between the areas and moreover supports observations that walruses sometimes do migrate long distances (Born & Gjertz, 1993; Wiig *et al.*, 1996; Freitas *et al.*, 2009; Born *et al.*, 2014).

CONCLUSION

In conclusion, most of the analyses performed in this study (especially the analysis based on mtDNA variation) suggest that Atlantic walruses from PEC show low, but significant genetic distinction from SVA–FJL. Additional genetic analysis, involving RAD sequencing (Baird *et al.*, 2008; Hohenlohe *et al.*, 2010; Peterson *et al.*, 2012) should be conducted to more fully understand the fine-scale population structure and migration patterns between these areas (e.g. Pujolar *et al.*, 2014a, b; Hand *et al.*, 2015; Shafer *et al.*, 2015). However, in the meantime, it is recommend that the PEC population should be managed conservatively as an independent, small population unit based on the finding of this study, because of the high level of anthropogenic activities in the PEC, such as the major developments associated with extraction of oil and gas (Semyonova *et al.*, 2015).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix 1. The 14 microsatellite markers used to study walruses in the Barents Sea Region and adjacent areas. Observed (H_O) and expected (H_E) heterozygosity, allele richness (AR), deviations from HWE (F_{IS}), P values for F_{IS} estimates, number of individuals (N) and allele-range for sampling areas.
Appendix 2a. Walrus haplotypes observed in the different areas at different times within the Northeast Atlantic.
Appendix 2b. Gender of the Atlantic walruses from the four different areas.

Appendix 1. The 14 microsatellite markers used to study walruses in the Barents Sea Region and adjacent areas. Observed (H_o) and expected (H_e) heterozygosity, Allele richness (AR), deviations from HWE (F_{IS}), P-values for F_{IS} estimates, number of individuals (N) and allele-range for sampling areas.

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Walruses (*Odobenus rosmarus rosmarus*) in the Pechora Sea in the context of contemporary population structure of Northeast Atlantic walruses

Locality & sampling year	Variable	*Orr9*	*SPGV9*	*PV9*	*Orr23*	*HGDii*	*Orr24*	*Hg4.2*	*HG6.1*	*HG8.10*	*M11*	*Orr16*	*Orr7*	*Orr11*	*Igf-I*	Average
SVA	H_o	0,750	0,750	0,750	0,700	0,350	0,700	0,850	0,350	0,500	0,650	1,000	0,900	0,684	0,000	
2003	H_e	0,759	0,773	0,774	0,799	0,335	0,604	0,844	0,301	0,660	0,663	0,804	0,803	0,676	0,000	0,628
	AR	5	6	6	7,95	3	4,95	8,9	3	3	3,95	6,95	9,8	5	1	
	F_{IS}	0,037	0,055	0,055	0,149	-0,019	-0,134	0,018	-0,137	0,266	0,044	-0,22	-0,096	0,015	NA	0,009
	P-values	0,4649	0,422	0,4107	0,1125	0,5774	0,8946	0,5435	1	0,0774	0,4869	1	0,919	0,5667	NA	
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	
FJL	H_o	0,583	0,750	0,750	0,571	0,273	0,625	0,750	0,250	0,667	0,792	0,875	0,667	0,708	0,000	
1992	H_e	0,748	0,733	0,733	0,777	0,376	0,713	0,859	0,262	0,656	0,684	0,847	0,819	0,734	0,000	0,639
	AR	5	5,75	5,75	8,71	3	5,58	9,7	3,78	3	4,79	8,54	7,75	5,75	1	
	F_{IS}	0,241	-0,002	-0,002	0,287	0,296	0,144	0,147	0,068	0,005	-0,137	-0,012	0,206	0,056	NA	0,097
	P-values	0,0327	0,6054	0,6012	**0,0054**	0,0988	0,1482	0,047	0,3976	0,5548	0,9107	0,6649	0,0268	0,4054	NA	
	N	24	24	24	21	22	24	24	24	24	24	24	24	24	24	
SVA	H_o	0,636	0,727	0,727	0,909	0,364	0,591	0,864	0,273	0,773	0,682	0,864	0,955	0,773	0,000	
1992	H_e	0,682	0,724	0,724	0,832	0,417	0,628	0,880	0,449	0,632	0,625	0,807	0,823	0,702	0,000	0,638
	AR	5,86	4,98	4,98	10,68	2,98	4	11,69	3,85	3	3,98	8,46	9,57	4,98	1	
	F_{IS}	0,09	0,019	0,019	-0,07	0,152	0,082	0,042	0,413	-0,2	-0,068	-0,047	-0,137	-0,077	NA	0
	P-values	0,2958	0,5131	0,519	0,8857	0,3054	0,3357	0,3714	0,0196	0,9458	0,769	0,7917	0,9869	0,8179	NA	
	N	22	22	22	22	22	22	22	22	22	22	22	22	22	22	
EGR	H_o	0,522	0,870	0,870	0,609	0,261	0,783	0,478	0,304	0,652	0,696	0,565	0,826	0,652	0,000	
2010a	H_e	0,625	0,685	0,685	0,690	0,302	0,763	0,570	0,258	0,658	0,734	0,597	0,784	0,714	0,000	0,576
	AR	4,97	4,65	4,65	6,65	2,996	5	6,478	2	4,946	5,652	5,988	8,571	4,826	1	
	F_{IS}	0,186	-0,248	-0,248	0,14	0,157	-0,004	0,182	-0,158	0,031	0,075	0,076	-0,031	0,108	NA	0,02
	P-values	0,1387	0,9863	0,9887	0,1506	0,2821	0,5994	0,1024	1	0,5077	0,3649	0,3173	0,7131	0,2607	NA	
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	

Locality & sampling year	Variable	*Orr9*	*SPGV9*	*PV9*	*Orr23*	*HGDii*	*Orr24*	*Hg4.2*	*HG6.1*	*HG8.10*	*M11*	*Orr16*	*Orr7*	*Orr11*	*Igf-I*	
EGR	H_o	0,650	0,800	0,800	0,450	0,300	0,700	0,650	0,250	0,850	0,650	0,650	0,650	0,600	0,000	
2010b	H_e	0,640	0,693	0,692	0,550	0,265	0,756	0,640	0,301	0,683	0,696	0,740	0,754	0,711	0,000	0,580
	AR	4,999	4,95	4,95	6,9	2,99	5,95	8,85	2,99	6,8	4,95	6,95	6,95	5,9	1	
	F_{IS}	0,01	-0,13	-0,13	0,206	-0,107	0,1	0,01	0,195	-0,221	0,092	0,147	0,163	0,181	NA	0,041
	P-values	0,5476	0,8774	0,9024	0,0988	1	0,2655	0,6024	0,2601	0,9774	0,3405	0,1577	0,1125	0,1315	NA	
	N	23	23	23	23	23	23	23	23	23	23	23	23	23	23	
Pechora	H_o	0,700	0,755	0,760	0,720	0,220	0,720	0,760	0,367	0,600	0,698	0,792	0,796	0,660	0,000	
Sea	H_e	0,753	0,711	0,695	0,714	0,296	0,720	0,825	0,399	0,649	0,659	0,866	0,869	0,546	0,039	0,624
	AR	5,98	6,76	4,99	12,51	3	6,86	12,4	4,99	5,72	6	12,69	12,97	4,99	1,98	
	F_{IS}	0,08	-0,057	-0,084	0,002	0,267	0,011	0,089	0,09	0,086	-0,047	0,097	0,094	-0,198	1	0,033
	P-values	0,194	0,8071	0,8667	0,5583	0,0208	0,5196	0,1095	0,2161	0,2339	0,7161	0,0631	0,0613	0,9923	0,0095	
	N	50	49	50	50	50	50	50	49	50	43	48	49	47	50	
SVA-	H_o	0,652	0,742	0,742	0,730	0,328	0,636	0,818	0,288	0,652	0,712	0,909	0,833	0,723	0,000	
FJLtot	H_e	0,741	0,750	0,750	0,816	0,380	0,669	0,881	0,347	0,665	0,667	0,831	0,821	0,716	0,000	0,645
	AR	5,65	5,99	6	10,75	3	5,65	13,13	4,61	3	4,95	9,37	11,05	5,66	1	
	F_{IS}	0,128	0,018	0,018	0,113	0,145	0,057	0,079	0,177	0,028	-0,06	-0,086	-0,007	-0,002	NA	0,037
	P-values	0,0321	0,4833	0,4548	0,0357	0,1262	0,256	0,0417	0,0571	0,4131	0,819	0,9714	0,6179	0,5881	NA	
	N	66	66	66	63	64	66	66	66	66	66	66	66	65	66	
EGR	H_o	0,581	0,837	0,837	0,535	0,279	0,744	0,558	0,279	0,744	0,674	0,605	0,744	0,628	0,000	
tot	H_e	0,640	0,694	0,694	0,634	0,285	0,774	0,605	0,279	0,670	0,718	0,680	0,787	0,717	0,000	0,584
	AR	5	6	6	8	3	6	10	3	7	6	7	9	6	1	
	F_{IS}	0,103	-0,194	-0,194	0,167	0,032	0,051	0,09	0,012	-0,099	0,073	0,123	0,067	0,135	NA	0,028
	P-values	0,1702	0,9905	0,9905	0,0393	0,4726	0,3107	0,1726	0,5905	0,8798	0,2595	0,0988	0,2345	0,0952	NA	
	N	43	43	43	43	43	43	43	43	43	43	43	43	43	43	
Allele-range		199-213	154-168	159-173	102-132	118-122	171-183	143-169	142-150	143-169	127-147	190-214	199-223	148-160	100-104	

Appendix 2a. Walrus haplotypes observed in the different areas at different times within the Northeast Atlantic.

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Walruses (*Odobenus rosmarus rosmarus*) in the Pechora Sea in the context of contemporary population structure of Northeast Atlantic walruses

Haplotype ID	FJL1992	SVA2003	SVA1992	SVAHIS*	PEC	EGR2004	EGR1992*	EGR2002	EGR2010a	EGR2010b	LAPTEV	GenBank accession	Reference
ATL_20	x	x	x	x	x	x		x		x		EU728566, KU710183	Lindqvist *et al.*, 2009
ATL_28	x											-	Present study
ATL_25	x	x	x									EU728571	Lindqvist *et al.*, 2009
ATL_29	x											-	Present study
SBj_02	x	x	x	x	x							KU710184	Lindqvist *et al.*, 2016
ATL_23	x		x									EU728569	Lindqvist *et al.*, 2009
ATL_24	x	x										EU728570	Lindqvist *et al.*, 2009
ATL_30	x	x										-	Present study
ATL_31	x	x						x	x			-	Present study
ATL_32	x											-	Present study
SH_16	x	x		x	x				x			KU710193	Lindqvist *et al.*, 2016
ATL_11	x	x				x	x	x	x	x		EU728554	Lindqvist *et al.*, 2009
ATL_19	x	x										EU728565	Lindqvist *et al.*, 2009
ATL_02	x	x	x									EU728565	Lindqvist *et al.*, 2009
ATL_33	x		x									-	Present study
SBj_06	x	x		x		x						KU710187	Lindqvist *et al.*, 2016
ATL_22	x				x							EU728568	Lindqvist *et al.*, 2009
ATL_34		x	x									-	Present study
ATL_35		x	x									-	Present study
ATL_36		x			x							-	Present study
ATL_37		x										-	Present study
ATL_05	x								x			EU728548	Lindqvist *et al.*, 2009
ATL_38			x									-	Present study
ATL_12			x	x		x	x	x	x	x		EU728555	Lindqvist *et al.*, 2016
SBj_03				x								KU710185	Lindqvist *et al.*, 2016
SBj_08				x				x				KU710189	Lindqvist *et al.*, 2016
SH_21				x								KU710198	Lindqvist *et al.*, 2016
PEC_01					x							-	Present study
PEC_02					x							-	Present study
PEC_03					x							-	Present study
PEC_04					x							-	Present study
PEC_05					x							-	Present study
PEC_06					x							-	Present study
PEC_07					x							-	Present study
PEC_08					x							-	Present study
PEC_09					x							-	Present study
PEC_10					x							-	Present study
ATL_39						x		x	x	x		-	Present study
ATL_40								x	x			-	Present study
ATL_41								x				-	Present study
ATL_42									x			-	Present study
LAP_05											x**	EU728525	Lindqvist *et al.*, 2009
LAP_06											x	EU728526	Lindqvist *et al.*, 2009
LAP_07											x**	EU728527	Lindqvist *et al.*, 2009

*samples from Lindqvist *et al.* studies

** only in Lindqvist *et al.*, 2009

Appendix 2b. Gender of the Atlantic walruses from the 4 different areas.

Andersen LW, Jacobsen MW, Lydersen C, semenova V, Boltunov A, Born EW, Wiig Ø, Kovacs KM. : Walruses (*Odobenus rosmarus rosmarus*) in the Pechora Sea in the context of contemporary population structure of Northeast Atlantic walruses

Id	Location	Year	Sex
P1	Pechora-Vaygach	2010	M
P2	Pechora-Vaygach	2010	M
P3	Pechora-Vaygach	2010	M
P4	Pechora-Vaygach	2010	M
P5	Pechora-Vaygach	2010	M
P6	Pechora-Vaygach	2010	M
P7	Pechora-Vaygach	2010	?
P8	Pechora-Vaygach	2010	M
P9	Pechora-Vaygach	2010	?
P10	Pechora-Vaygach	2010	M
P11	Pechora-Vaygach	2010	M
P12	Pechora-Vaygach	2010	M
P13	Pechora-Vaygach	2010	M
P14	Pechora-Vaygach	2010	?
P15	Pechora-Vaygach	2010	M
P16	Pechora-Vaygach	2010	M
VAY1	Pechora-Vaygach	2013	M
VAY2	Pechora-Vaygach	2013	M
VAY3	Pechora-Vaygach	2013	M
VAY4	Pechora-Vaygach	2013	M
VAY5	Pechora-Vaygach	2013	M
VAY6	Pechora-Vaygach	2013	M
VAY7	Pechora-Vaygach	2013	
VAY8	Pechora-Vaygach	2013	M
VAY9	Pechora-Vaygach	2013	M
VAY10	Pechora-Vaygach	2013	M
VAY11	Pechora-Vaygach	2013	M
VAY12	Pechora-Vaygach	2013	M
VAY13	Pechora-Vaygach	2013	M
VAY14	Pechora-Vaygach	2013	M
VAY16	Pechora-Vaygach	2013	M
VAY17	Pechora-Vaygach	2013	M
VAY18	Pechora-Vaygach	2013	M
VAY19	Pechora-Vaygach	2013	M
VAY20	Pechora-Vaygach	2013	M
VAY21	Pechora-Vaygach	2013	M
VAY22	Pechora-Vaygach	2013	M
VAY23	Pechora-Vaygach	2013	M
VAY24	Pechora-Vaygach	2013	M
VAY25	Pechora-Vaygach	2013	M

VAY26	Pechora-Vaygach	2013	M
VAY27	Pechora-Vaygach	2013	M
VAY28	Pechora-Vaygach	2013	M
VAY29	Pechora-Vaygach	2013	M
VAY30	Pechora-Vaygach	2013	M
VAY31	Pechora-Vaygach	2013	M
VAY32	Pechora-Vaygach	2013	M
VAY33	Pechora-Vaygach	2013	M
VAY34	Pechora-Vaygach	2013	M
VAY35	Pechora-Vaygach	2013	M
FJL1	Franz Josef Land	1992	M
FJL11	Franz Josef Land	1992	F
FJL13	Franz Josef Land	1992	M
FJL14	Franz Josef Land	1992	M
FJL15	Franz Josef Land	1992	M
FJL16	Franz Josef Land	1992	M
FJL17	Franz Josef Land	1992	F
FJL18	Franz Josef Land	1992	F
FJL19	Franz Josef Land	1992	F
FJL2	Franz Josef Land	1992	F
FJL20	Franz Josef Land	1992	F
FJL21	Franz Josef Land	1992	F
FJL23A	Franz Josef Land	1992	F
FJL23A?	Franz Josef Land	1992	
FJL26	Franz Josef Land	1992	M
FJL27	Franz Josef Land	1992	F
FJL28	Franz Josef Land	1992	M
FJL29	Franz Josef Land	1992	F
FJL3	Franz Josef Land	1992	F
FJL35	Franz Josef Land	1992	M
FJL36	Franz Josef Land	1992	M
FJL4	Franz Josef Land	1992	F
FJL5	Franz Josef Land	1992	F
FJL9	Franz Josef Land	1992	M
N1	Svalbard	2004	M
N12	Svalbard	2004	M
N14	Svalbard	2004	M
N16	Svalbard	2004	M
N18	Svalbard	2004	M
N21	Svalbard	2002	M
N22	Svalbard	2003	M
N23	Svalbard	2003	M
N24	Svalbard	2003	M
N25	Svalbard	2003	M

N26	Svalbard	2003	M
N27	Svalbard	2003	M
N29	Svalbard	2003	M
N3	Svalbard	2003	M
N30	Svalbard	2003	M
N31	Svalbard	2003	M
N32	Svalbard	2002	M
N5	Svalbard	2004	M
N7	Svalbard	2004	M
N9	Svalbard	2004	M
SV1	Svalbard	1992	M
SV12	Svalbard	1992	M
SV14	Svalbard	1992	M
SV15	Svalbard	1992	M
SV16	Svalbard	1992	M
SV17	Svalbard	1992	M
SV20	Svalbard	1992	M
SV2	Svalbard	1992	M
SV21	Svalbard	1992	M
SV22	Svalbard	1992	M
SV24	Svalbard	1992	M
SV25	Svalbard	1992	M
SV26	Svalbard	1993	M
SV27	Svalbard	1993	?
SV28	Svalbard	1993	?
SV29	Svalbard	1993	M
SV30	Svalbard	1993	F
SV5	Svalbard	1992	M
SV6	Svalbard	1992	M
SV7	Svalbard	1992	M
SV8	Svalbard	1992	M
SV9	Svalbard	1992	
E100	East Greenland	2010	M
E101	East Greenland	2010	M
E104	East Greenland	2010	M
E106	East Greenland	2010	M
E107	East Greenland	2010	M
E108	East Greenland	2010	M
E71	East Greenland	2010	M
E74	East Greenland	2010	M
E75	East Greenland	2010	M
E77	East Greenland	2010	M
E79	East Greenland	2010	M
E81	East Greenland	2010	M

E82	East Greenland	2010	M
E83	East Greenland	2010	M
E86	East Greenland	2010	M
E88	East Greenland	2010	M
E91	East Greenland	2010	M
E92	East Greenland	2010	M
E94	East Greenland	2010	M
E95	East Greenland	2010	M
E96	East Greenland	2010	M
E98	East Greenland	2010	M
E99	East Greenland	2010	M
Y10	East Greenland	2010	M
Y12	East Greenland	2010	M
Y13	East Greenland	2010	M
Y16	East Greenland	2010	M
Y26	East Greenland	2010	M
Y28	East Greenland	2010	M
Y29	East Greenland	2010	M
Y33	East Greenland	2010	M
Y34	East Greenland	2010	M
Y39	East Greenland	2010	M
Y45	East Greenland	2010	M
Y48	East Greenland	2010	M
Y49	East Greenland	2010	M
Y59	East Greenland	2010	M
Y63	East Greenland	2010	M
Y64	East Greenland	2010	M
Y65	East Greenland	2010	M
Y66	East Greenland	2010	M
Y70	East Greenland	2010	M
Y8	East Greenland	2010	M
NE1	East Greenland	2004	M
NE10	East Greenland	2004	M
NE11	East Greenland	2004	M
NE12	East Greenland	2004	M
NE13	East Greenland	2004	M
NE16	East Greenland	2004	M
NE19	East Greenland	2004	M
NE20	East Greenland	2004	M
NE21	East Greenland	2004	M
NE23	East Greenland	2004	M
NE24	East Greenland	2004	M
NE28	East Greenland	2004	M
NE29	East Greenland	2004	M

NE30	East Greenland	2004	M
NE37	East Greenland	2004	M
NE38	East Greenland	2004	M
NE39	East Greenland	2004	M
NE40	East Greenland	2004	M
NE6	East Greenland	2004	M
ORR1	East Greenland	2002	M
ORR10	East Greenland	2002	M
ORR11	East Greenland	2002	F
ORR12	East Greenland	2002	M
ORR13	East Greenland	2002	M
ORR14	East Greenland	2002	M
ORR15	East Greenland	2002	M
ORR17	East Greenland	2002	M
ORR18	East Greenland	2002	M
ORR19	East Greenland	2002	M
ORR20	East Greenland	2002	M
ORR2	East Greenland	2002	M
ORR21	East Greenland	2002	M
ORR22	East Greenland	2002	M
ORR23	East Greenland	2002	M
ORR25	East Greenland	2002	M
ORR26	East Greenland	2002	M
ORR27	East Greenland	2002	M
ORR28	East Greenland	2002	M
ORR29	East Greenland	2002	M
ORR30	East Greenland	2002	M
ORR31	East Greenland	2002	M
ORR32	East Greenland	2002	M
ORR33	East Greenland	2002	M
ORR36	East Greenland	2002	M
ORR37	East Greenland	2002	M
ORR38	East Greenland	2002	M
ORR40	East Greenland	2002	M
ORR41	East Greenland	2002	M
ORR42	East Greenland	2002	M
ORR44	East Greenland	2002	M
ORR46	East Greenland	2002	M
ORR48	East Greenland	2002	M
ORR50	East Greenland	2002	M
ORR51	East Greenland	2002	M
ORR53	East Greenland	2002	M
ORR60	East Greenland	2002	M
ORR70	East Greenland	2002	M

ORR74	East Greenland	2002	M
ORR9	East Greenland	2002	M
ORR3	East Greenland	1999	M
SCO18	East Greenland	1992	M
SCO22	East Greenland	1992	M