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ARTICLE

Intraspecific mitogenomics of three marine species-at-risk: Atlantic, spotted, and northern wolffish (*Anarhichas* spp.)

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Abstract: High-resolution mitogenomics of within-species relationships can answer such phylogeographic questions as how species survived the most recent glaciation, as well as identify contemporary factors such as physical barriers, isolation, and gene flow. We examined the mitogenomic population structure of three at-risk species of wolffish: Atlantic (*Anarhichas lupus*), spotted (*A. minor*), and northern (*A. denticulatus*). These species are extensively sympatric across the North Atlantic but exhibit very different life history strategies, a combination that results in concordant and discordant patterns of genetic variation and structure. Wolffish haplogroups were not structured geographically: Atlantic and spotted wolffish each comprised three shallow clades, whereas northern wolffish comprised two deeper but unstructured lineages. We suggest that wolffish species survived in isolation in multiple glacial refugia, either refugia within refugia (Atlantic and spotted wolffish) or more distant refugia (northern wolffish), followed by secondary admixture upon post-glacial recolonisation of the North Atlantic.

Key words: phylogeography, conservation genetics, Pleistocene glaciations, population genetics, *Anarhichas*, species-at-risk.

Résumé : La mito-génomique à haute résolution des relations intraspécifiques permet de répondre à diverses questions en phylogéographie comme identifier les espèces qui ont survécu à la dernière glaciation ou identifier des facteurs contemporains tels que les barrières physiques, l'isolement et les flux géniques. Les auteurs ont examiné la structure de la population au sein des génomes mitochondriaux chez trois espèces menacées de poisson-loup : atlantique (*Anarhichas lupus*), tacheté (*A. minor*) et gélatineux (*A. denticulatus*). Ces espèces sont largement sympatriques dans l'Atlantique Nord, mais font appel à des stratégies de cycle de vie très différentes, une combinaison qui entraîne des schémas de variation génétique et de structure à la fois concordants et discordants. Les haplotypes de poissons-loup n'étaient pas structurés sur une base géographique; les poissons-loup atlantique et tachetés formaient trois clades peu profonds, tandis que le poisson-loup gélatineux comprenait deux lignages plus profonds mais non-structurés. Les auteurs suggèrent que ces espèces ont survécu en isolement dans de multiples refuges glaciaires, soit des refuges au sein de refuges (poissons-loup atlantique et tacheté) ou au sein de refuges plus distants (poisson-loup gélatineux), avant de connaître de l'admixture lors de la re-colonisation de l'Atlantique Nord. [Traduit par la Rédaction]

Mots-clés : phylogéographie, génétique de conservation, glaciations du pléistocène, génétique des populations, *Anarhichas*.

Introduction

The population genetic structure of marine species is often enigmatic. Many widely distributed fish species display little or no population structure (Ward 1995; Vis et al. 1997), which is attributed to large ranges, high dispersal capability, and few barriers to dispersal (Palumbi 1992). Although population connectivity and the presence or absence of physical barriers significantly affect population genetic structure, species in the Northern Hemisphere were also heavily influenced by the Quaternary glaciations, particularly those of the Pleistocene (ca. 200–10 kya; Hewitt 2000, 2004; Pflaumann et al. 2003; Shaw 2006). Understanding population genetic structure is particularly important for species of conservation concern. Lack of genetic diversity in a species limits their ability to effectively respond to, and recover from, environmental threats such as disease, pollution, parasites, and climate change (Frankham 1995; Amos and Harwood 1998); whereas maintenance or enhancement of variation can mitigate the effects of climatic extremes and environmental changes (Hilborn et al. 2003), and can affect the recovery of species.

The marine environment has been heavily impacted by overexploitation, habitat destruction, and climatic changes (Hutchings 2000). Three species heavily affected by the fisheries of the mid-20th century are the Atlantic wolffish *Anarhichas lupus* (Linnaeus, 1758), the spotted wolffish *Anarhichas minor* (Olafsen, 1772), and the northern wolffish *Anarhichas denticulatus* (Krøyer, 1845). Populations decreased by 91%, 96%, and 98%, respectively, due to a combination of overharvesting as bycatch and habitat destruction (Watling and Norse 1998; O'Dea and Haedrich 2002), leading to these three species being among the first marine fish species to be listed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) under the *Canadian Species At Risk Act* (SARA): Atlantic wolffish as Special Concern (COSEWIC 2000, 2012*a*) and spotted and northern wolffish as Threatened (COSEWIC 2001*a*, 2001*b*, 2012*b*, 2012*c*).

The North Atlantic wolffish species are largely sympatric along the continental shelves of North America and Europe (Fig. 1), although they occur in somewhat different habitats, are stratified by depth, and have adopted different feeding strategies (Barsukov 1959;

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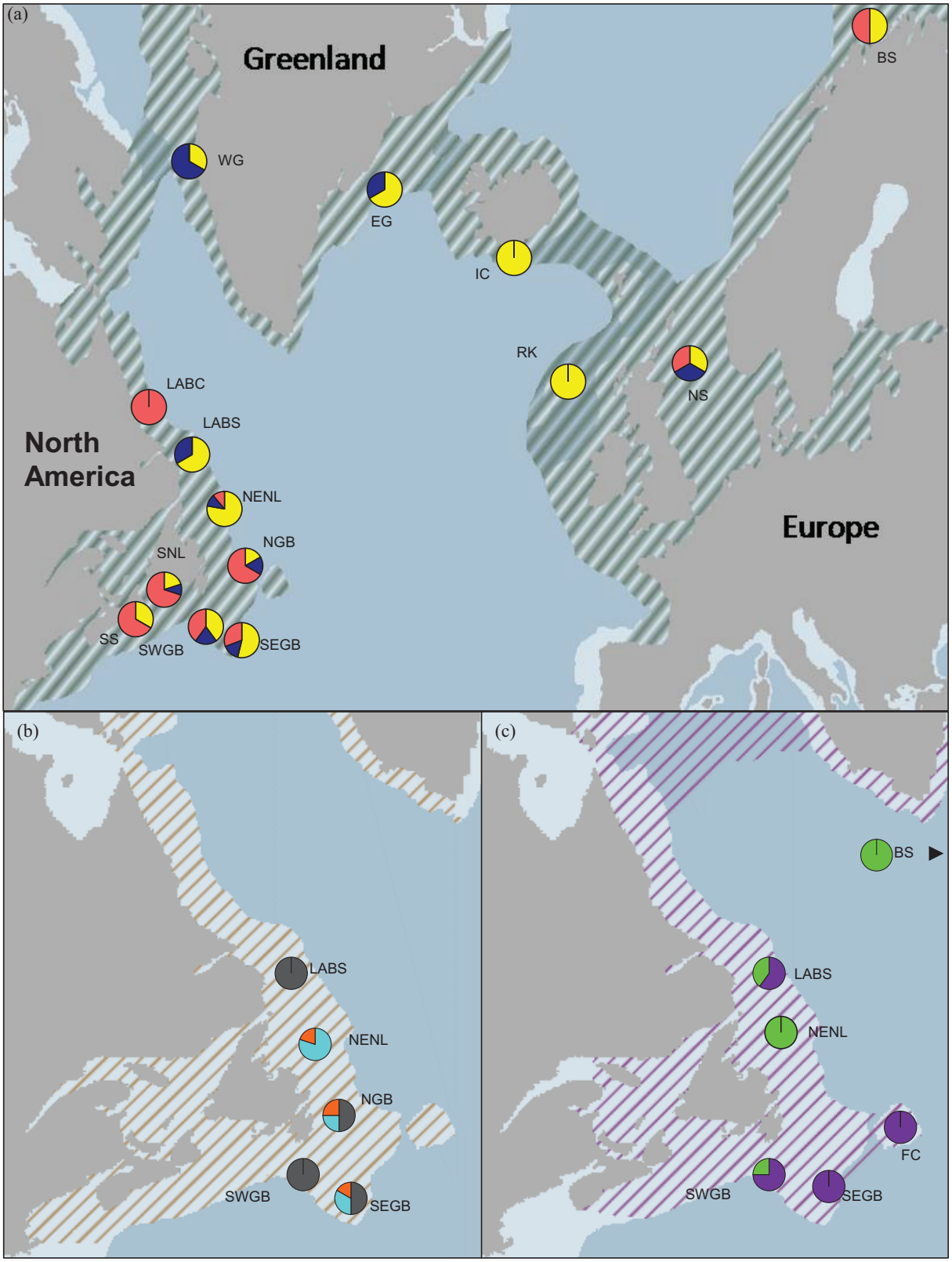
Fig. 1. The approximate species distributions (shaded) and geographic distribution of clusters identified in BAPS v6 for (*a*) Atlantic wolffish, (*b*) spotted wolffish, and (*c*) northern wolffish. Sampling locations are as follows: LABC (central Labrador), LABS (southern Labrador), NENL (northeastern Newfoundland), NGB (northern Grand Banks), FC (Flemish Cap), SEGB (southeastern Grand Banks), SWGB (southwestern Grand Banks), SNL (southern Newfoundland), SS (Scotian Shelf), WG (west Greenland), EG (east Greenland), IC (Iceland), RK (Rockall Bank), NS (North Sea), and BS (Barents Sea). Distributions taken from FishBase (FishBase 2013*a*, 2013*b*, 2013*c*).



Albikovskaya 1983). Wolffish undergo internal fertilisation and lay large demersal egg masses that hatch into well-developed young that often remain close to their hatching site (Johannessen et al. 1993; Pavlov and Novikov 1993). Wolffish are slow-growing species with late maturity and slow population growth, which make them at greater risk of local extirpation and result in decreased recovery potential (Hiddink et al. 2008; Baker et al. 2009). Because of their sedentary nature and specific habitat requirements, it has been suggested that wolffish might show greater population structure than is seen in many marine fish species. Studies to date have offered ambiguous support for such a hypothesis. In Atlantic wolffish a range-wide phylogeographic study of mtDNA sequences found low diversity and a star-like phylogeny (McCusker and Bentzen 2010*a*). In contrast, nuclear microsatellite markers suggested divergence between populations in the Northeast and Northwest Atlantic, with greater structure in the Northwest (McCusker and Bentzen 2010*b*). Nuclear markers found limited structure in spotted wolffish, but identified the Barents Sea population of northern wolffish as genetically isolated (McCusker and Bentzen 2011); no patterns were detected by mtDNA markers in either species (McCusker and Bentzen 2010*a*).

Given the conservation status of these unusual creatures, and the ambiguous population genetic structure identified to date (none with short mtDNA fragments and limited with nuclear markers), higher resolution markers are required. Studies of complete mitochondrial genomes have been successful in clarifying evolutionary relationships in a number of species (Cooper et al. 2001; Inoue et al. 2001; Arnason et al. 2008), and have identified previously hidden diversity in both mammals (Tanaka et al. 2004; Knaus et al. 2011) and fish (Carr and Marshall 2008; Feutry et al. 2014; Lait et al. 2018). Here we aim to measure genetic variability and examine population genetic structure in three at-risk wolffish species by comparing multiple complete mitogenome sequences.

Materials and methods

Sample collection

Wolffish samples were collected by Fisheries and Oceans Canada from eight sampling locations between August 2002 and November 2003 (Fig. 1): LABC (central Labrador), LABS (southern Labrador), NENL (northeastern Newfoundland), NGB (northern Grand Banks), FC (Flemish Cap), SEGB (southeastern Grand Banks), SWGB (southwestern Grand Banks), and SNL (southern Newfoundland). Hearts were removed and stored at –20 °C. Extracted DNA from additional Atlantic and northern wolffish samples was provided by Dr. Paul Bentzen (Dalhousie University), including additional samples from NGB and from seven new sampling locations in Europe (RK (Rockall Bank), NS (North Sea), and BS (Barents Sea)), the mid-Atlantic (WG (west Greenland), EG (east Greenland), and IC (Iceland)), and Nova Scotia (SS (Scotian Shelf)). These latter samples were collected by Fisheries and Oceans Canada between 2002 and 2005, with the exception of the North Sea samples which were obtained from a fish market (see McCusker and Bentzen 2010*b*).

Laboratory protocols

DNA was extracted from heart tissue using the Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's protocols (Qiagen, Hilden, Germany). The complete mtDNA genomes were amplified as series of 18–20 overlapping fragments, or as five long-range PCR fragments. PCR reactions were carried out with a primer-specific annealing temperature (T_A; see Table S1[1] for primer sequences and details; Coulson et al. 2006; Johnstone et al. 2007). For long-range PCRs, the primer pairs used were w18F/g03R, g04F/w07R, g07F/g08R, w08F/wND45R, and w14F2/w18R with a T_A of 58 °C. PCR products for 34 Atlantic, 17 spotted, and 19 northern wolffish were sent to Genome Quebec (McGill University, QC) for sequencing with both forward and reverse primers; long-range PCR products were sequenced with a series of internal primers. An additional 50 Atlantic wolffish samples were sequenced with the Affymetrix GeneChip® CustomSeq® resequencing multispecies microarray, the "ArkChip" (Carr et al. 2009); PCR amplicons were sent to the Centre for Applied Genomics (Toronto, ON) and the microarray data analysed as described by Duggan (2007) and Lait et al. (2018). Four mitogenomes were sequenced with both standard Sanger sequencing and the ArkChip; there were no differences between the two sequencing methods.

Genetic analyses

Sequences were aligned in Sequencher v4.9 (Gene Codes Corporation). Haplotypes were assigned using TCS v1.2.1 (Clement et al. 2000) and confirmed with Arlequin v3.5.2.2 (Excoffier and Lischer 2010). Genetic diversity was measured using nucleotide (π) and haplotype (H_d) diversities in DNAsp v5.10 (Rozas et al. 2003; Librado and Rozas 2009). Population pairwise genetic distances (Φ_{ST} values) were calculated for Atlantic wolffish in Arlequin v3.5.2.2 on a reduced dataset containing only the North American populations with $n \geq 9$ (LABS, NENL, SENL, NGB, SGB, and SNL; n = 63); a modified false discovery rate procedure was applied to correct for multiple tests (FDR; Benjamini and Yekutieli 2001).

Relationships among sequences were visualised as an unrooted statistical parsimony network constructed in TCS v1.21, and as a principal coordinates analysis (PCoA) performed in GenAlEx v6.5 (Peakall and Smouse 2006, 2012). Clustering analysis was performed in BAPS v6 (Corander et al. 2008) with the linked loci option and the codon model of linkage (Corander and Tang 2007). We varied K from 1 to 15, and determined the best number of groups based on the log marginal likelihood of the best visited partitions. The significance of the distribution of groups was tested using both a standard chi-square (χ^2) test and a modified Monte Carlo χ^2 test designed for small population sizes (Roff and Bentzen 1989). In Atlantic wolffish the PCoA and clustering analyses were run on both the complete (n = 84) and reduced (n = 63) datasets.

Bayesian analyses and distance methods were used to evaluate the phylogenetic relationship among sequences within species. Bayesian trees were constructed in BEASTv2.3 (Bouckaert et al. 2014); the analysis was run for 10 000 000 generations, with a 25% burn-in, and final effective sample size (ESS) > 1000 for all parameter estimates. Neighbour-joining trees (NJ) were constructed in PAUP* v4.10 (Swofford 2003) based on the absolute number of nucleotide differences (10 000 bootstrap replications). Each tree was rooted with one of the other two species. Divergence times were estimated using a constant population model in BEAST v2.3 with the HKY + Γ + I model and a strict clock run for 10 000 000 MCMC steps, sampled every 10 000 steps, with a 1 000 000 step burn-in. The trees were calibrated using a normal distribution with a mean divergence time between the congeneric spotted and northern wolffish of 2 million years and a standard deviation of 0.5 million years. A separation date of 1–2 million years ago (mya) has been suggested based on an assumed separation of the wolffish species from the Atlantic and Pacific Oceans 3–4 mya at the last opening of the Bering Strait (Johnstone et al. 2007).

Individual loci

To test whether any of the individual loci could detect population structure, the above analyses were run separately on each of the 13 coding regions, the two rDNAs, and the control region. Results from the NJ analyses are shown.

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/gen-2018-0043.

Table 1. Sample size (n), number of haplotypes (h), haplotype diversity (H_d), nucleotide diversity (π), and haplogroup assignment (A–C) for 84 complete mitochondrial genomes among 14 sampling locations of Atlantic wolffish.

Population	n	h	H_d	π	A	B	C
LABC	1	1	1.000	n/a	1	0	0
LABS	9	9	1.000	0.0010	0	3	6
NENL	9	9	1.000	0.0011	1	1	7
NGB	12	11	0.985	0.0010	8	2	2
SEGB	13	13	1.000	0.0012	4	2	7
SWGB	10	10	1.000	0.0010	4	2	4
SNL	10	7	0.867	0.0007	7	1	2
SS	3	3	1.000	0.0007	2	0	1
WG	3	3	1.000	0.0010	0	2	1
EG	3	3	1.000	0.0009	0	1	2
IC	3	3	1.000	0.0008	0	0	3
NS	3	3	1.000	0.0015	1	1	1
RK	3	2	0.667	0.0007	0	0	3
BS	2	2	1.000	0.0012	1	0	1
A	29	21	0.911	0.0004			
B	15	14	0.990	0.0007			
C	40	39	0.999	0.0008			
Total	84	74	0.989	0.00106	29	15	40

Note: Population abbreviations are as follows: LABC (central Labrador), LABS (southern Labrador), NENL (northeastern Newfoundland), NGB (northern Grand Banks), SEGB (southeastern Grand Banks), SWGB (southwestern Grand Banks), SNL (southern Newfoundland), SS (Scotian Shelf), WG (west Greenland), EG (east Greenland), IC (Iceland), NS (North Sea), RK (Rockall Bank), and BS (Barents Sea).

Results

Variation in mitogenome sequences

Complete mtDNA genome sequences were obtained for 84 Atlantic, 17 spotted, and 19 northern wolffish (GenBank Accession Numbers KX117921–KX118041). The mitogenomes varied from 16 512 bp in Atlantic wolffish, to 16 510 – 16 515 bp in spotted wolffish, and 16 510 – 16 516 bp in northern wolffish. Variation in length was due to a homopolymer run of cytosine residues between the tDNA aspartic acid and the cytochrome oxidase II coding regions; Atlantic wolffish are invariable for five C residues, whereas individual spotted and northern wolffish varied between 7–12 and 7–13 residues, respectively.

A total of 257 variable sites (SNPs) were identified in Atlantic wolffish (111 parsimony informative), 61 in spotted wolffish (17 parsimony informative), and 81 in northern wolffish (37 parsimony informative; Table S2[1]). Coding regions contained 64%–75% of the variable sites, where the majority of the changes were third position transitions (137/193, 28/39, and 48/57, respectively). There were no frameshift or nonsense mutations, and all insertion-deletion events occurred in non-coding regions (e.g., 16S rDNA).

Species-specific genetic analyses

Atlantic wolffish

The 257 identified SNPs defined 74 distinct mitogenomic sequences, of which 71 were found only in a single individual and three of which were shared by two, two, and nine fish (Table 1; Fig. 2*a*). No haplotypes were shared between North American and European populations. Haplotype diversity (H_d) was high in all populations (0.667–1.000) while nucleotide diversity (π) ranged from 0.0007 in RK to 0.0015 in NS (Table 1). Population pairwise Φ_{ST} values ranged from 0.000 between NGB and SNL and among the three adjacent Grand Banks populations (NGB, SEGB, and SWGB) to 0.236 between LABS and SNL (Table 2*a*). The lowest Φ_{ST} values occurred between geographically proximal locations, and no neighbouring populations were significantly differentiated.

The statistical parsimony network showed two divergent groups radiating from a central variable group (Fig. 2*a*); these are hereinafter referred to as Haplogroups A (red), B (blue), and C (yellow). There was no correlation between haplogroup membership and geographical origin (Table 1; Fig. 1*a*; standard $\chi^2 = 38.35$, df = 26, $p = 0.056$; mean modified $\chi^2 = 41.6$, $p = 0.027$), with the exception that we did not find Haplogroup A in LABS or the three mid-Atlantic populations. Haplotype diversity was high in all three haplogroups ($H_d > 0.9$), and nucleotide diversity was high in Haplogroups B and C (Table 1). Pairwise Φ_{ST} comparisons among haplogroups were highly significant ($p < 0.0001$; Table 2*b*). The PCoA separated the sequences into the same three groups (Fig. 3*a*): Haplogroup A separated from the other two along the first coordinate (30.8% of the variation), while Haplogroups B and C separated along coordinate 2 (21.3% of the variation); coordinate 3 explained 6.3% of the variation. Results were similar in the reduced dataset (not shown), with 79.3% of the variation explained by coordinates 1 (42.6%), 2 (26.8%), and 3 (9.9%). Bayesian clustering also identified three groups found across all populations (prob K = 3 > 0.999; Table 1; Fig. 1*a*), with highest log maximum likelihood values of –3247.6, –3309.1, and –3390.4 for K = 3, 4, and 5, respectively.

The Bayesian (Fig. 4) and neighbour-joining (Fig. S1[1]) analyses distinguished Haplogroups A and B from Haplogroup C, with Haplogroup C containing at least three statistically-significant sub-haplogroups. Based on the calculated separation of spotted and northern wolffish ca. 2 mya (Johnstone et al. 2007), the three haplogroups coalesced ca. 219 kya (95% Highest Posterior Density (HPD) = 177–270 kya; Fig. 4), placing the separation of haplogroups well within the Pleistocene glaciations.

Spotted wolffish

The 61 variable sites defined 17 haplotypes in spotted wolffish; there were no shared haplotypes ($H_d = 1.000$). Pairwise differences varied from 2 to 28, and nucleotide diversity was low ($\pi = 0.0005$). All analyses identified three haplogroups, designated Haplogroup D (grey), E (cyan), and F (orange). Bayesian clustering gave log likelihood values of –449.6, –472.2, and –488.5 for K = 3, 4, and 5, respectively (prob K = 3 is 1.000; Fig. 1*b*). The statistical parsimony network identified the same groups separated by three to five substitutions (Fig. 2*b*). Haplogroups D and E were of similar abundance and pattern of variation, arranged around a missing haplotype, whereas Haplogroup F was more divergent. In the PCoA analysis (Fig. 3*b*), Haplogroup F separated from the other two along coordinate 1 (35.1% of the variation), and Haplogroups D and E separated from each other along coordinate 2 (20.8% of the variation); coordinate 3 explained 17.1% of the variation.

Bayesian and NJ analyses identified the same three haplogroups (Figs. 5*a* and S1[1]). There was strong statistical support for Haplogroup F with both Bayesian and NJ analyses, Haplogroup E had strong support in the Bayesian tree, and Haplogroup D consisted of the remaining individuals. The three haplogroups were estimated to have diverged approximately 81 kya (95% HPD = 33–132 kya), with the divergence time between Haplogroups D and E at 63 kya (95% HPD = 28–104 kya), towards the end of the Pleistocene glaciations.

Northern wolffish

There were 19 haplotypes defined by the 81 SNPs; all individual northern wolffish mitogenomes were unique ($H_d = 1.000$). Pairwise differences varied from 1 to 28, and nucleotide diversity was higher than that seen in spotted wolffish ($\pi = 0.0009$). All five analyses methods identified two distinct haplogroups: G (green) and H (purple). Bayesian clustering gave log likelihood values of –752.7, –760.8, and –770.0 for K = 2, 3, and 4 respectively (prob K = 2 was 0.999). Haplogroup G occurred primarily in waters off northern Newfoundland and Europe, and Haplogroup H on the Grand Banks, Flemish Cap, and in Labrador (Fig. 1*c*). The statistical parsimony network identified the same two groups, with no significant phylogeographic structure (Fig. 2*c*). PCoA analysis separated Haplogroups G and H along coordinate 1 (52.8% of the variation), while coordinate 2 showed separation within Haplogroup H (17.0%

Fig. 2. Statistical parsimony networks of complete mitogenomes for (*a*) Atlantic wolffish, (*b*) spotted wolffish, and (*c*) northern wolffish. Each symbol represents an individual, samples are colour-coded by sampling location, and shared haplotypes are encased by black boxes. The black dots are inferred or unsampled haplotypes, and each connection represents one nucleotide change. The dashed boxes correspond to the haplogroups identified by BAPS v6. Sample locations are defined in Fig. 1.

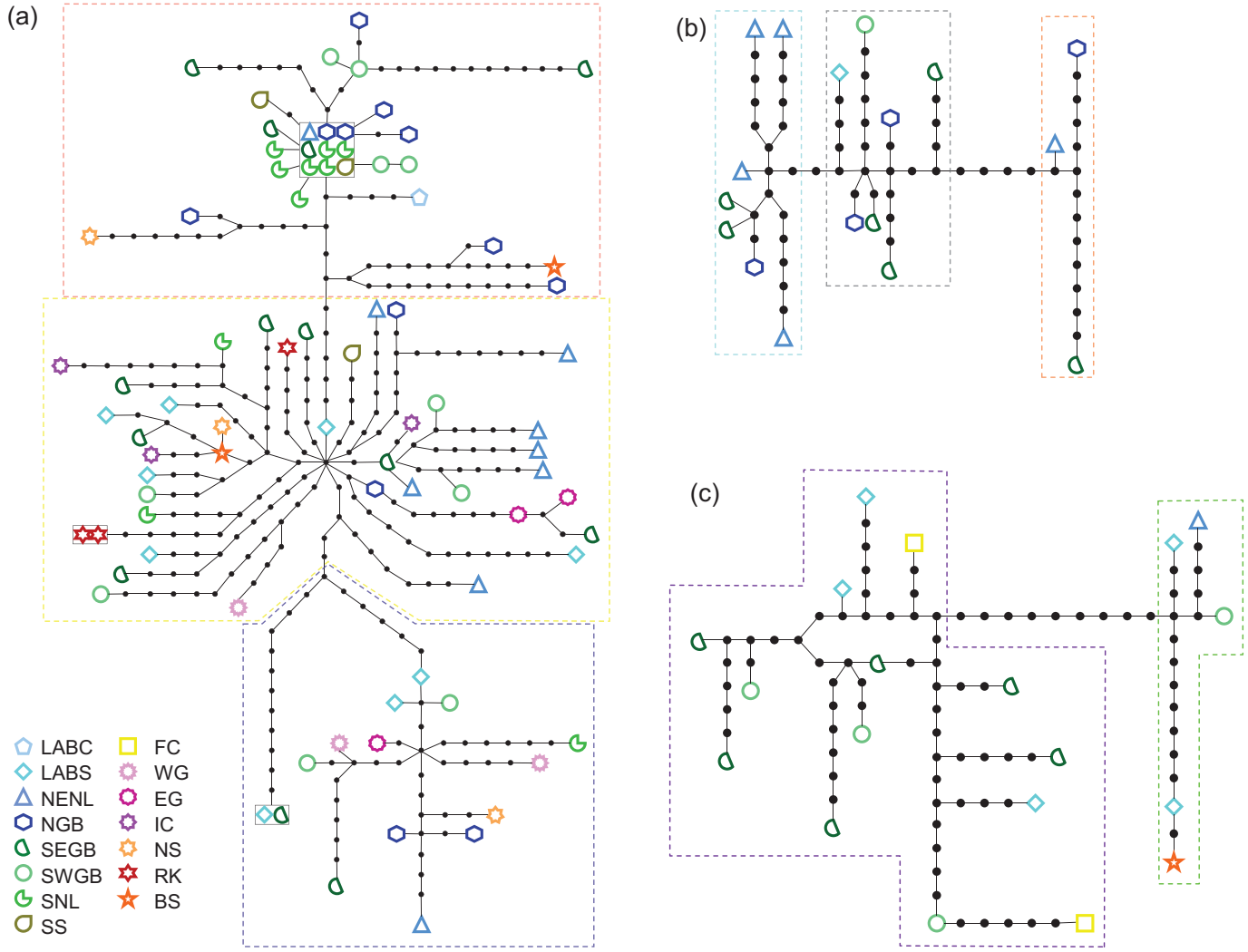


Table 2. Population pairwise differences based on 100 000 permutations (Φ_{ST}; below diagonal) and corresponding p-values (above diagonal) for complete mitogenomes from (*a*) six North American populations of Atlantic wolffish, and (*b*) three haplogroups (A–C).

(*a*)	LABS	NENL	NGB	SEGB	SWGB	SNL
LABS	—	0.1174	**0.0036**	0.4275	0.1646	**0.0027**
NENL	0.0366	—	0.0306	0.3133	0.3693	**0.0102**
NGB	0.1530	0.0966	—	0.1448	0.4377	0.4346
SEGB	0.0013	0.0058	0.0273	—	0.8678	0.0936
SWGB	0.0400	0.0062	0.0000	0.0000	—	0.1533
SNL	0.2355	0.1891	0.0000	0.0527	0.0425	—

(b)	A	B	C
A	—	**<0.0001**	**<0.0001**
B	0.6084	—	**<0.0001**
C	0.4610	0.4014	—

Note: Significant p-values after modified FDR (P_{crit} = 0.0151) are shown in bold.

of the variation). Bayesian and NJ analyses identified the same two groups with strong statistical support (Figs. 5*b* and S1[1]). The estimated divergence time of Haplogroups G and H was 126 kya (95% HPD = 57–204 kya), well within the period of Pleistocene glaciations.

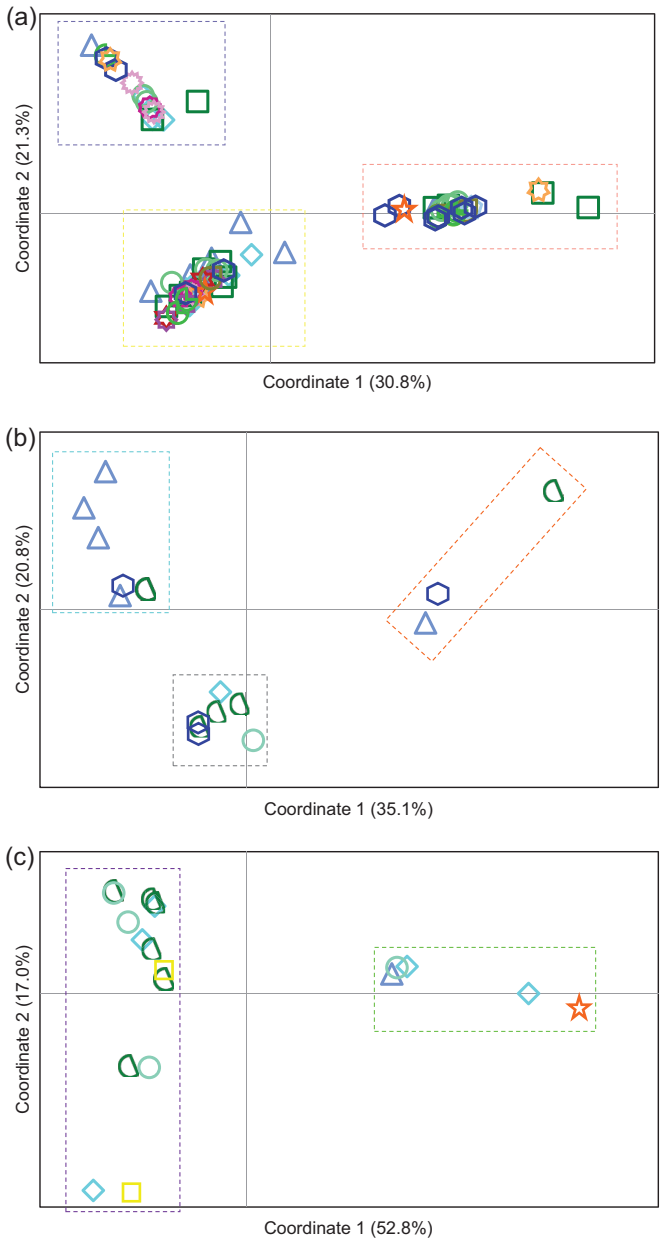
Individual loci

Analysis of the individual loci found that only one marker could identify all three haplogroups in Atlantic (COX1) or spotted wolffish (ND2), although with low statistical support (Figs. S2[1] and S3[1]), while several markers could detect one of the three haplogroups in Atlantic wolffish (e.g., ND3 and ND5 could detect Haplogroup A but not B or C). In contrast, seven of the 16 regions could identify the two deeper haplogroups in northern wolffish with varying levels of bootstrap support (Fig. S4[1]).

Discussion

Analysis of multiple complete mitogenome sequences within species identifies previously undetected genetic structure in Atlantic, spotted, and northern wolffish based on multiple divergent haplogroup lineages. The distribution of variation within the genome (Table S2[1]) and the analyses of individual loci (Figs. S2[1]–S4[1]) show that the markers used in the previous mitochondrial DNA study (McCusker and Bentzen 2010*a*), ND1 and the control region, were not highly variable in any of the species. The lack of phylogeographic structure despite low migration rates (Barsukov 1959; Templeman 1984) may be attributed to the young evolution-

Fig. 3. Principal coordinates analyses (PCoA) of complete mitogenomes for (*a*) Atlantic wolffish, (*b*) spotted wolffish, and (*c*) northern wolffish. Sampling locations are coded as in Fig. 2, and dashed boxes outline the haplogroups identified by BAPS v6.



ary age of the species (Johnstone et al. 2007), or recent recovery from loss of variation in glacial refugia.

Population genetic structure

Analysis of complete wolffish mitochondrial genomes identified a lack of geographical structure in all three species; however, each showed evidence of multiple divergent haplogroups: three in the Atlantic and spotted wolffish, and two in the northern wolffish. Distribution of these groups did not support separation of individual populations, nor did it support separation across the Atlantic basin. McCusker and Bentzen (2010*a*), based on 1830 bp from the mitochondrial ND1 and control region, reported little variation and no population structure in any of the species, but did find limited geographic structure in Atlantic and northern wolffish with nuclear microsatellites and AFLPs (McCusker and Bentzen 2010*b*, 2011). The absence of population genetic structure could be the result of extensive gene flow among populations or incomplete lineage sorting due to recent colonisation of the North Atlantic. Although the distribution of Atlantic wolffish is continuous along the continental shelves from Europe to North America, it is unlikely that movement or gene flow is extensive between distant populations or regions. Tagging studies have found only occasional long-distance movements in wolffish, with no evidence of trans-Atlantic movement (Barsukov 1959; Templeman 1984).

The three haplogroups identified in Atlantic wolffish showed weakly non-random distribution (Figs. 1–4). The two smaller haplogroups (A and B) were found predominantly in the western Atlantic (27/29 and 14/15, respectively); the scarcity in European waters may either be due to the small sample sizes or a North American origin for these groups (see below). McCusker and Bentzen (2010*b*) separated three broad areas based on nuclear data — Atlantic Canada, the North Atlantic, and Rockall Bank — which do not correspond to those identified here. The microsatellite data suggest that Atlantic Canada forms a single somewhat heterogeneous group, the mid-Atlantic and Europe a second homogeneous group, and Rockall Bank a third isolated group. The present study places the Rockall Bank (RK) samples alongside those from both the eastern and western Atlantic, and not in a monophyletic or isolated group. The identification of Rockall Bank as a separate population is likely due to the contemporary isolation of this location rather than its historical sequestration.

Spotted wolffish, the sister species to Atlantic wolffish, also showed three major, shallow haplogroups that were found across sampling locations, suggesting a similar evolutionary history. In contrast, northern wolffish, the outgroup to the other two species examined here, occurred as two deeper haplogroups of pre- or mid-glacial origin, with Haplogroup G more common in northern and eastern locations, and Haplogroup H in the south and west. The different pattern seen in the northern wolffish may result from the different life history strategies, particularly the more pelagic nature of this species (Barsukov 1959).

The pattern seen in the three wolffish species, of multiple clades that do not correspond to geographic origin, has been seen in a number of mitogenomic studies in marine (Carr and Marshall 2008; Teacher et al. 2012; Carr et al. 2015; Lait et al. 2018) and terrestrial species (Wang et al. 2010). For example, Teacher et al. (2012) identified three distinct but widespread clades in 98 Atlantic herring in the Baltic region, while Carr et al. (2015) found six major clades among 52 harp seals from four discrete trans-Atlantic breeding grounds. In both species the structure was explained by historical isolation pre-dating the post-glacial expansion of the species. Isolation of multiple groups of wolffish prior to the end of the last glacial maximum could explain the separation seen in these species.

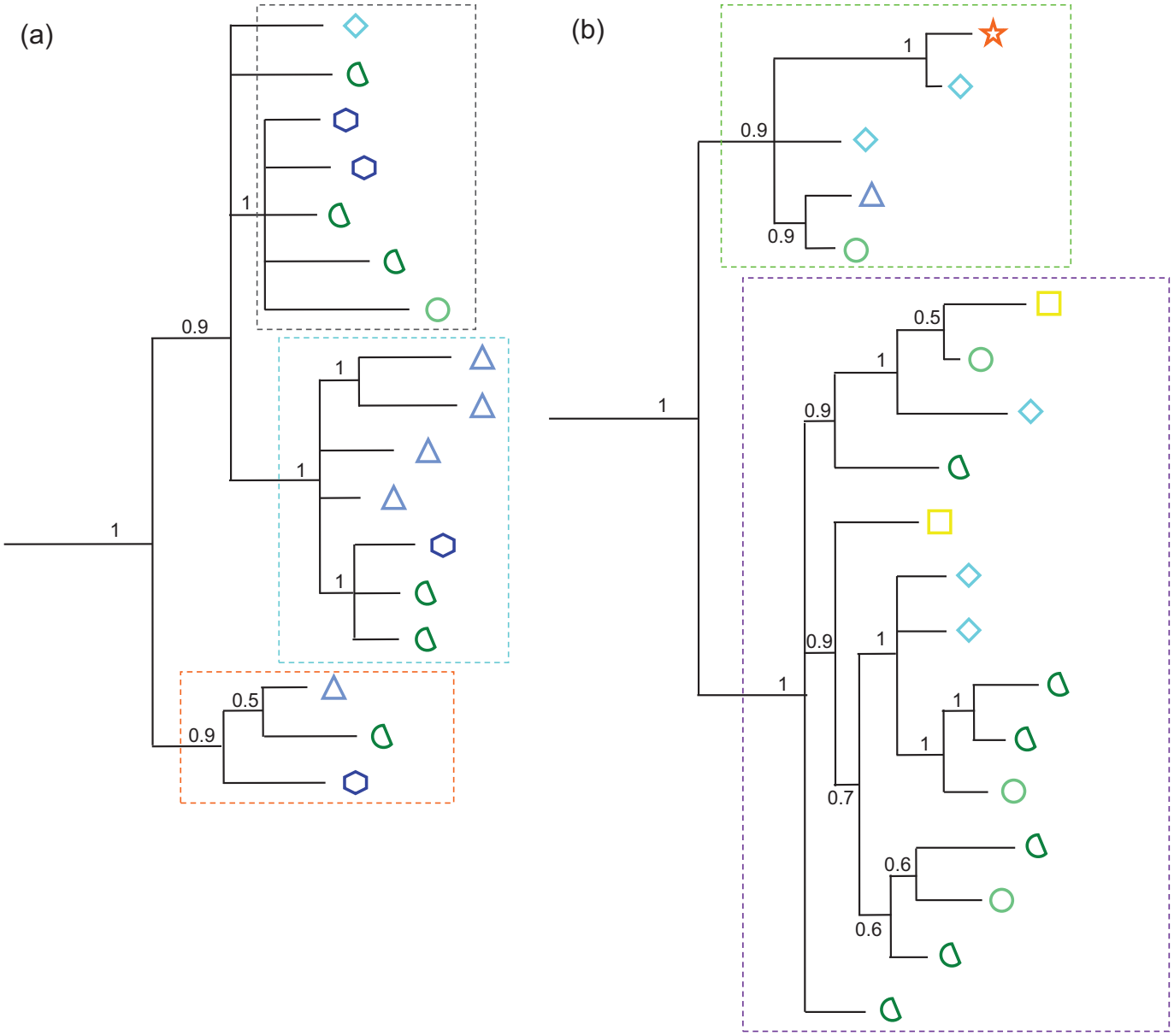
Consequences of Pleistocene biogeography

At the height of the last glacial maximum (ca. 20 kya) there was a large drop in both sea surface temperatures and sea levels which reduced the available marine habitat and disrupted the spatial distribution of marine species (Pielou 1991; Rohling et al. 1998; Hewitt 2000). Some marine fish species survived in small northern refugia on either side of the North Atlantic, or in more extensive refugia to the south (Maggs et al. 2008; Provan and Bennett 2008). The pattern found in Atlantic wolffish, of three distinct lineages of relatively recent divergence and no strong biogeographic structure, may be explained by a number of hypotheses:

(1) multiple refugia on both sides of the North Atlantic;
(2) a single glacial refugium occupied by three genetically divergent groups;
(3) a single glacial refugium occupied by a genetically panmictic population, with subsequent post-glacial diversification;

Fig. 4. Clock-calibrated Bayesian analysis of complete mitogenomes from Atlantic wolffish. Posterior probabilities above 0.75 are given, all others fell between 0.5 and 0.749. The *x*-axis shows estimated separation time in millions of years. Sampling locations are coded as in Fig. 2, and dashed boxes outline haplogroups identified by BAPS v6. The rooting is the same when either of the other two Northwest Atlantic species is used.

Fig. 5. Bayesian analysis of complete mtDNA genomes from (*a*) spotted wolffish and (*b*) northern wolffish. Posterior probabilities above 0.5 are given. Sampling locations are coded as in Fig. 2, and dashed boxes outline haplogroups identified by BAPS v6. The rooting of each species is the same when either of the other two Northwest Atlantic species is used.



(4) multiple refugia in the eastern North Atlantic, followed by recolonisation of the western North Atlantic;
(5) multiple refugia in the western North Atlantic, followed by recolonisation of the eastern North Atlantic;

If Atlantic wolffish survived the LGM on both sides of the Atlantic Ocean we would expect to see fixed transatlantic differences and high levels of diversity in both eastern and western Atlantic populations. There were no significant differences between populations from the Northeast and Northwest Atlantic, and, while haplotype diversity was high in all populations, nucleotide diversity was generally higher in eastern populations (Table 1). It is possible, however, that while the majority of the wolffish survived in European refugia, a remnant population survived in a small periglacial refugium in Atlantic Canada with little post-glacial movement into the mid- and eastern Atlantic. It has been suggested that the Flemish Cap may have acted as a marine glacial refugium during this time (Pflaumann et al. 2003; Shaw 2006), and it has been shown that small refugia can result in low diversity levels due to founder effects and persistent small population sizes (Maggs et al. 2008). The next two scenarios are also unlikely: if the separation had occurred prior to the last glaciation we would expect to see much greater divergence among haplogroups; while if a panmictic population had survived in a single glacial refugium and separated post-glacially, we would see either strong correlation between haplogroup membership and population of origin, or limited variation in a starburst pattern. Neither of these resembles the pattern seen here.

The use of multiple glacial refugia in a single region is most likely given the observed distribution of haplotypes (Figs. 2–4) and pattern of diversity (Table 1). The presence of all three haplogroups in all regions suggests admixture following isolation but before recolonisation of glaciated regions. Genetic diversity is high in the two European populations (BS and NS; Table 1), which may suggest a European or Mediterranean refugial source. Several

glacial refugia have been suggested for marine species in the Northeast Atlantic: the Azores, the Iberian Peninsula, the Mediterranean Sea, the English Channel, southwestern Ireland, Iceland and the Faroe Islands, and the Lofoten Coast of Norway (Maggs et al. 2008). Alternatively, the wolffish may have persisted in separate pockets within a single refugium (i.e., refugia within refugia; Gómez and Lunt 2007). The use of multiple refugia has been suggested in a number of marine species including the green crab *Carcinus maenas* (Roman and Palumbi 2004), red algae *Palmaria palmata* (Provan et al. 2005), and brown algae *Fucus serratus* (Hoarau et al. 2007). While there is evidence supporting two North American marine refugia, one in or near Atlantic Canada and a large region in the south (Pflaumann et al. 2003; Shaw 2006), it has been suggested that the southern refugium did not have the solid substrate required for the feeding habits of Atlantic wolffish, and therefore, despite being unglaciated, this region may not have provided suitable habitat for this species (Maggs et al. 2008).

Spotted wolffish also showed separation of three haplogroups within the last glaciation, likely in the same region (i.e., in three nearby refugia, or in pockets of a single larger refugium). As the ice sheets melted and additional habitat became suitable the refugial populations may have converged before recolonising previously glaciated regions. It has been suggested that variation in the yak *Bos grunniens*, where three differentiated lineages show no correlation to geographical origin, may have arisen in isolated Pleistocene refugia with a subsequent reunion into a single gene pool following deglaciation (Wang et al. 2010).

Northern wolffish exhibited a deeper separation that suggests that the two haplogroups may have persisted in multiple isolated refugia on one or both sides of the North Atlantic Ocean. Unlike Atlantic and spotted wolffish, northern wolffish do not require a hard, stony bottom to feed; they can instead catch mobile prey such as comb jellies and jellyfish (Albikovskaya 1982, 1983). This lack of dependence on a solid substrate may have facilitated their survival in a southern refugium in the western North Atlantic.

Conclusions

The three North Atlantic wolffish species are sedentary marine species whose life history characteristics suggest a priori a strong population structure, with little or no movement during either larval or adult stage; however, this has been shown not to be the case. Examination of multiple complete mitogenomes sequences instead shows an absence of phylogeographic structure. There is, however, evidence of an interesting evolutionary history: we identified multiple distinct haplogroups supported by traditional population statistics, distance methods, and Bayesian analyses. The pattern was similar in the Atlantic and spotted wolffish, sister species with similar habitat and feeding requirements, while that in the northern wolffish differed, likely due to the differences in life history traits. In each case historical isolation with subsequent admixture is likely responsible for the lack of geographical structure.

Despite limited sampling, particularly in the mid- and eastern North Atlantic, mitogenomics was able to identify previously undetected genetic structure in all three species. The fact that the pattern of variation in the threatened northern wolffish differs from that seen in the spotted and Atlantic wolffish suggests that these species should be managed differently.

Data availability

DNA sequences have been deposited in GenBank (Accession Numbers KX117921–KX118041).

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