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Morphological variation in genetically divergent populations of the common whelk, *Buccinum undatum* (Gastropoda: Buccinidae), across the North Atlantic

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The variation in shelled marine gastropod morphology across small spatial scales can reflect restricted population connectivity, resulting in evolution or plastic responses to environmental heterogeneity. The common whelk, *Buccinum undatum*, is a subtidal gastropod, ubiquitous in the North Atlantic, that exhibits considerable spatial variation in shell morphology and colour. Given that species delimitation in shelled marine gastropods is often based on shell characteristics, such morphological variation can lead to taxonomic confusion. Phylogeographical analysis based on mitochondrial DNA and microsatellites suggested cryptic species composed of Western and Eastern North Atlantic common whelk populations, the separation of which dates to the onset of the Pleistocene glaciation ~2.1 Mya. Divergence within the Eastern North Atlantic is more recent and characterized by isolation by distance. In the present study, phenotypic variation in shell morphology across the North Atlantic range is analysed and compared with molecular divergence. The morphological variation of *B. undatum* populations reflected the pattern observed for the molecular markers only for certain comparisons of populations and might, in other cases, reflect larger constraints on the morphological variation and, possibly, the impact of environmental influences.

ADDITIONAL KEYWORDS: allopatry – *Buccinum* – genetic divergence – marine gastropods – phenotypic variance – population connectivity – population structure – shell morphology.

INTRODUCTION

Shelled marine gastropods frequently display remarkable intraspecific variation in shell morphology across relatively small spatial scales. This geographical variation is often a result of evolution owing to limited genetic connectivity or plastic responses to environmental heterogeneity (Valentinsson *et al.*, 1999; Trussell & Etter, 2001; Iguchi *et al.*, 2005; Mariani *et al.*, 2012; Bourdeau *et al.*, 2015; Magnúsdóttir *et al.*, 2018). Given that species delimitation in shelled gastropods has, to a large extent, been based on shell morphology (Schander & Sundberg, 2001; Wagner, 2001; Allmon & Smith, 2011), this can lead to taxonomic confusion, particularly for genera such as the benthic subtidal gastropods *Buccinum* (Shirai *et al.*, 2010) that exhibit great phenotypic variability in shell characteristics across small spatial scales. In addition, the shape or morphology of cones is an important trait in microevolutionary studies, for example applying phylogenies based on palaeontology (cf. Smith & Hendricks, 2013). Assessments of within-species diversity and geographical patterns in subtidal gastropods are needed to understand the evolution of these patterns, but studies on this group have been scarce compared with studies on intertidal and terrestrial snails, such as *Littorina* (e.g. Johannesson, 2015; Galindo *et al.*, 2019), *Nucella* (Pascoal *et al.*, 2012) and *Cepaea* (Cameron *et al.*, 2013).

Similar to other species of the genus *Buccinum*, the common whelk (*Buccinum undatum* L., 1758; Fig. 1A) is known for consistent spatial variation

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in shell morphology (Jeffries, 1867a; Golikov, 1968; Ten Hallers-Tjabbes, 1979; Thomas & Himmelman, 1988; Kenchington & Glass, 1998; Mariani *et al.*, 2012; Magnúsdóttir *et al.*, 2018) and life-history traits, including size at sexual maturity, age and size distribution (cf. Haig *et al.*, 2015), across its North Atlantic range. Golikov (1968) listed how the various morphological forms of the species reflected the hydrological conditions of their habitat throughout the North Atlantic, and Mariani *et al.* (2012) found that shell morphology and environmental variation were correlated at small geographical scales in Ireland. Differences in shell morphology of *B. undatum* between shallow and deep regions were noted from an early time (Jeffries, 1867b), consistent with results from Breiðafjörður Bay, West Iceland (Magnúsdóttir *et al.*, 2018), where thinner, relatively shorter shell spires and increased colour variation were found at greater depths (Magnúsdóttir *et al.*, 2018). Furthermore, increased shell thickness and elongated apertures of Canadian *B. undatum* have been linked with high lobster and crab predation (Thomas & Himmelman, 1988).

Phylogeographical analysis of *B. undatum* in the North Atlantic has revealed clear population structure based on microsatellite and mitochondrial variation on both large and small geographical scales (Weetman *et al.*, 2006; Mariani *et al.*, 2012; Pálsson *et al.*, 2014; Magnúsdóttir *et al.*, 2019). Distinct monophyletic mitochondrial DNA (mtDNA) lineages in Canada, Greenland and the Eastern North Atlantic indicated that populations from the two continents diverged around the onset of the Pleistocene glaciation 2.1 (1.04–3.49) Mya, followed by divergence between Greenland and Canada at 1.3 (0.62–2.14) Mya (Magnúsdóttir *et al.*, 2019). Within Europe, divergence follows the isolation-by-distance model, where small but significant differentiation is observed between sample sites, characteristic of populations with limited demographic connectivity, and even within countries, i.e. around the UK and along the coast of Iceland (Weetman *et al.*, 2006; Mariani *et al.*, 2012; Pálsson *et al.*, 2014; Magnúsdóttir *et al.*, 2019).

Morphological and molecular divergence of allopatric populations is expected to increase with time, although unique patterns may arise through diversifying or stabilizing selection that differentially affects certain traits or molecular variants (Johannesson, 2015; Gemmell *et al.*, 2018). This includes convergent

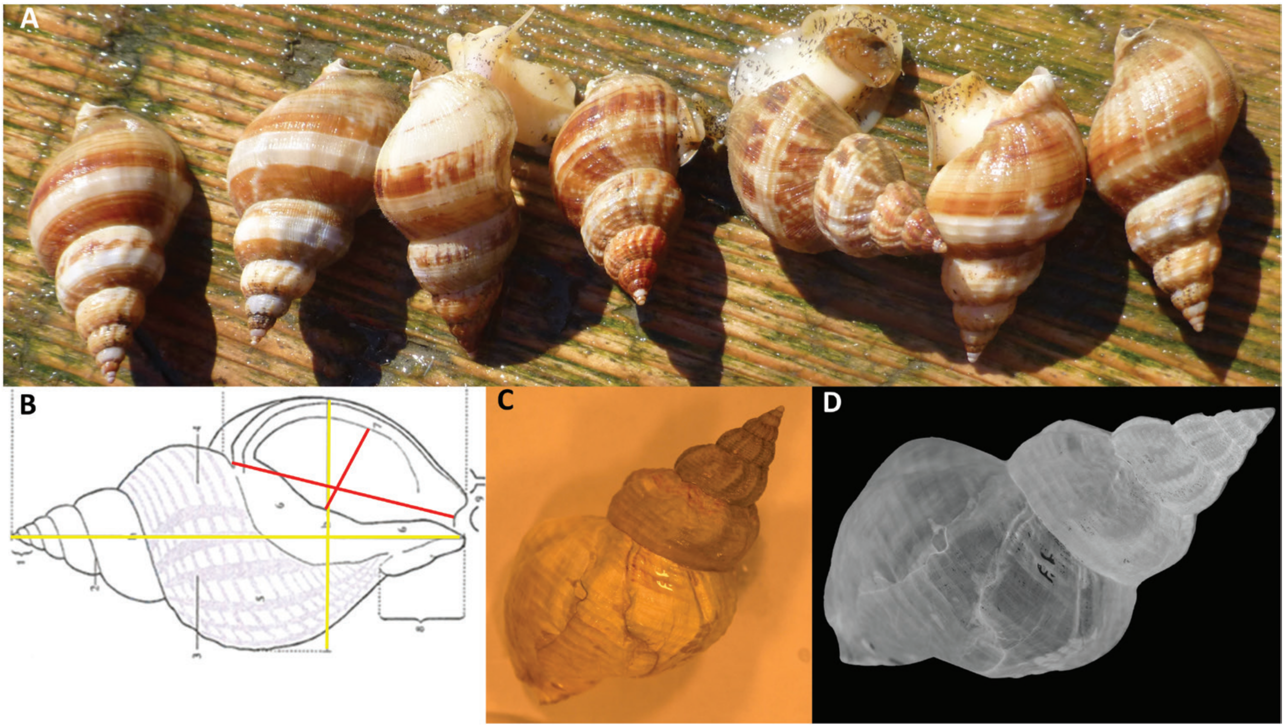

Figure 1. A, *Buccinum undatum* from Breiðafjörður, Iceland. The maximal shell height in Breiðafjörður is ~130 mm. B, traditional morphometric measurements of *B. undatum*. Yellow lines indicate shell height and width, whereas red lines indicate aperture height and width (image adapted from Danske havsnegle, Natur og Museum 33:2, 1994 by Poul Bondesen). C, for the shell outline analysis, *B. undatum* shells were photographed from the dorsal side at a set distance (shell height of the individual is 80.35 mm). D, same individual as in C, but after the background of the image has been removed and the image rotated and turned to monochrome for outline analysis with ShapeR (Libungan & Pálsson, 2015).

evolution owing to similar habitats, or even stasis, characterized by little morphological variation in conjunction with high genetic differentiation or cryptic species (Merilä, 1997; Sæther *et al.*, 2007; Allmon & Smith, 2011; Brommer, 2011). Various metrics address the mismatch between genetic and morphological variation; for example, distance matrices as summarized by the proportion of genetic variation between groups (F_{ST}) and its corresponding additive genetic variance (Q_{ST}). In the case of *Buccinum* species, this could lead to underestimation of the true diversity within the genus in the North Atlantic (Gemmell *et al.*, 2018), highlighted by the results of Magnúsdóttir *et al.* (2019), where genetic distances between *B. undatum* populations from Canada, Greenland and the Eastern North Atlantic exceeded the genetic distances observed between several other *Buccinum* species from the North Pacific and the North Atlantic, and species screening indices indicated cryptic speciation or allopatric divergence. To assess the morphological variation in cones, traditional morphometric analysis of geometric landmarks has often been applied (e.g. Cruz *et al.*, 2012; Mariani *et al.*, 2012; Gemmell *et al.*, 2018; Magnúsdóttir *et al.*, 2018). Additionally, Brönmark *et al.* (2011) analysed the flexible shell shape of *Radix balthica* with respect to predation through outline analysis, and Smith & Hendricks (2013) successfully applied two-dimensional shape analysis to analyse *Conus* gastropods for phylogenetic assessment.

The aim of the present study was to evaluate whether morphological variation within *B. undatum* across the North Atlantic shows similar patterns to those observed for molecular markers. The proportion of phenotypic variance between populations (P_{ST}) of whelk shell traits was estimated as a substitute for the additive genetic variance (Q_{ST}), based on traditional morphometric measurements (Magnúsdóttir *et al.*, 2018) and shape outline analysis (Libungan & Pálsson, 2015), and compared with corresponding genetic divergence (F_{ST}) between populations, based on both mitochondrial and microsatellite variation (Pálsson *et al.*, 2014; Magnúsdóttir *et al.*, 2019).

MATERIAL AND METHODS

SAMPLING

Buccinum undatum samples were acquired from 16 locations across the North Atlantic Ocean (Fig. 2; Table 1) both on the eastern (Iceland, Faroe Islands and UK) and the western (Canada, Greenland) sides. Sampling took place at a range of depths from 10 to 367 m, either with deployment of whelk traps or with dredging, over the period from 2008 to 2015 (Table 1). Two sample

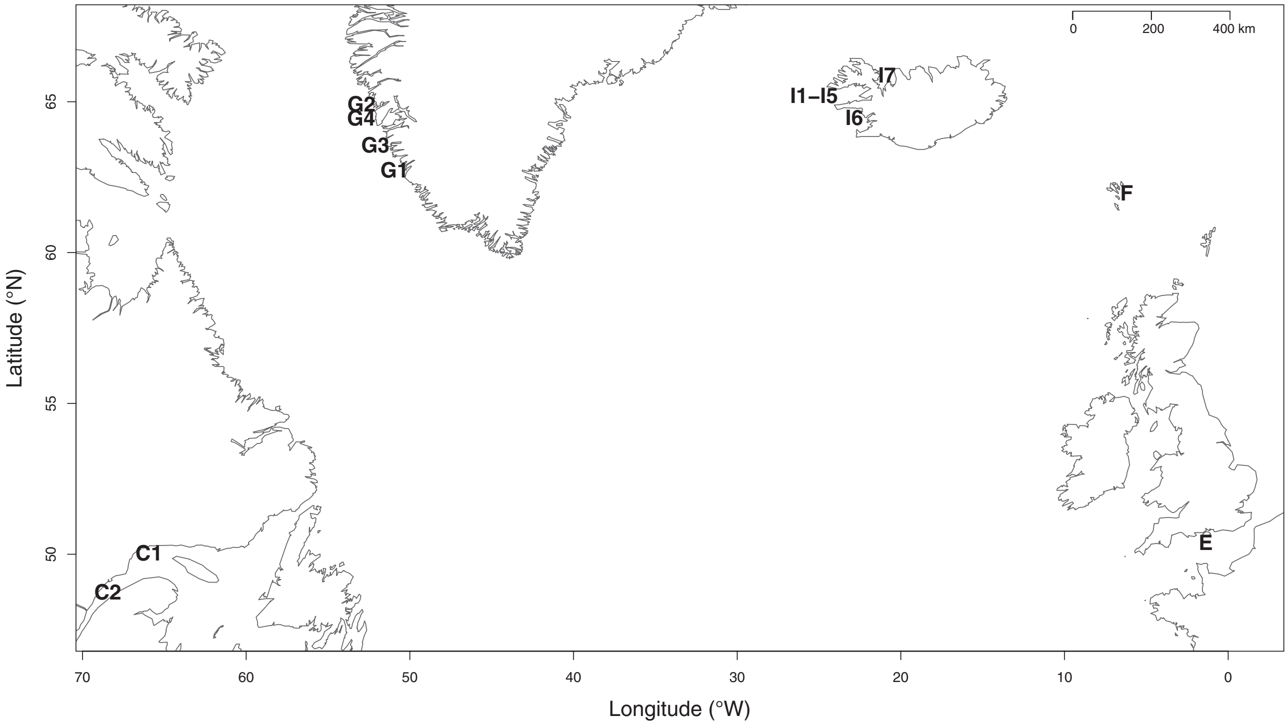

Figure 2. *Buccinum undatum* sampling sites in the North Atlantic. Countries are denoted as follows: C, Canada; E, England; F, the Faroe Islands; G, Greenland; I, Iceland. See Table 1 for details.

Table 1. Location, type and year of sampling of *Buccinum undatum* in the North Atlantic

Country	Location	Code	Latitude (N)	Longitude (W)	Depth (m)	Mitochondrial DNA (*N*)	Microsat. (*N*)	Traditional morphometrics (*N*)	Shell outline (N)	Sampling year
Canada	Baie Sainte-Marguerite, Gulf of St Lawrence	C1	50.04	66.43	17.5	37	56	50	–	2010
Canada	Forestville, Gulf of St Lawrence	C2	48.66	68.95	17.5	–	–	–	32	2015
England	Isle of Wight	E	50.39	1.37	10	45	49	52	–	2010
England	English Channel	E	–	–	–	–	–	–	36	2015
Faroe Islands	Nólsoyarfjørður	F	61.98	6.70	40	85	42	93	23	2008
Greenland	Paamiut	G1	62.74	50.13	334	57	48	60	–	2010
Greenland		G2	64.73	52.70	300	12	11	12	–	2010
Greenland		G3	63.57	51.60	290	9	7	9	–	2010
Greenland		G4	64.47	52.97	367	31	30	30	–	2010
Iceland	Brjánslækur, Breiðafjörður	I1	65.50	23.14	37	–	–	–	27	2014 & 2015
Iceland	Hempill, Breiðafjörður	I2	65.05	23.21	22	76	50	87	–	2008
Iceland	Hrútey, Breiðafjörður	I3	65.03	22.94	36	93	90	100	30	2008, 2014 & 2015
Iceland	Oddbjarnarsker, Breiðafjörður	I4	65.31	23.23	43	84	44	85	30	2008, 2014 & 2015
Iceland	Stykkishólmur, Breiðafjörður	I5	65.08	22.68	18	–	–	–	23	2014
Iceland	Faxaflói	I6	64.37	22.84	30	19	13	20	–	2008
Iceland	Húnaflói	I7	65.79	20.84	27	88	39	92	–	2008
Total						636	479	690	210	

Traditional morphometric measurements were obtained for individuals from Pálsson *et al.* (2014), sampled in 2008–2010. The outline analysis is based on whelks sampled in the years 2014 and 2015. Mitochondrial DNA (*COI*) fragments (369 bp) and microsatellites (Microsat.) are from Magnúsdóttir *et al.* (2019) and Pálsson *et al.* (2014), respectively.

sites were located in the Gulf of St Lawrence, Canada; four along the west coast of Greenland; seven in NW Iceland; one in the Faroe Islands; and two in south England (Fig. 2).

Three types of data were analysed in the study: (1) variation of molecular markers based on 369 bp mtDNA (*COI*) from 636 *B. undatum* (Magnúsdóttir *et al.*, 2019) and five microsatellites from 479 individuals (Pálsson *et al.*, 2014); (2) traditional morphometrics of 690 *B. undatum*; and (3) shell outlines of 210 *B. undatum*.

GENETIC DATA

Genetic data for both microsatellites and *COI* were obtained from *B. undatum* sampled in the period from 2008 to 2010 across the North Atlantic (Table 1). The sampling procedure for both molecular markers and the microsatellite diversity analyses are detailed by Pálsson *et al.* (2014), and results from the analysis of the *COI* diversity are to be found in the paper by Magnúsdóttir *et al.* (2019).

SHELL MORPHOLOGY

Shell variables using traditional morphometrics were obtained from 690 *B. undatum* that were sampled by Pálsson *et al.* (2014) (Table 1); therefore, all individuals from the genetic analysis have shell measurements, and at some sites there were additional shells with morphological measurements. Shell outline analysis was based on 210 individuals sampled from South England, the Gulf of Saint Lawrence, Canada and the Faroe Islands, and from four sites in Breiðafjörður (West Iceland) (Fig. 2). Shell morphology of individuals from sample sites I1, I3, I4 and I5 was analysed with geometric morphometrics in Magnúsdóttir *et al.* (2018).

To minimize allometric effects, morphological analysis was done on individuals considered to be sexually mature or close to sexual maturity. This was based on shell height according to previously published studies on size at sexual maturity of *B. undatum* in the sampling areas (Gendron, 1992; Magnúsdóttir, 2010; Magnúsdóttir *et al.*, 2010; McIntyre *et al.*, 2015; for mean shell variables per site, see Supporting Information, Table S1).

Shell variables

Measurements for traditional morphometrics were carried out as described by Magnúsdóttir *et al.* (2018). Shell and aperture shape ratios were estimated by the ratio of the square root of the shell/aperture width (in millimetres) to the shell/aperture height, respectively (Fig. 1B). Shell thickness was summarized based on the ratio of the square root of the shell weight (in grams) to the shell height (in millimetres), which shows a linear relationship (Magnúsdóttir *et al.*, 2018). The variation in each of the shell variables among countries was tested with ANOVA and posterior comparisons tested with the *TukeyHSD* function in R (R Core Team, 2018). To summarize the difference in all three shell variables between countries, mean Mahalanobis distances, which take into account covariance among the variables, were calculated and their statistical significance assessed with a permutation test (permutations = 1000) applying the R package *HDMD* (R package v.1.2; McFerrin, 2013). Multidimensional scaling (MDS) of the Mahalanobis distances was performed to visualize the variation of the whelk populations, using the *metaMDS* function in the R package *vegan* (R package v.2.5-3; Oksanen *et al.*, 2018). The variation between the populations with respect to sampling depth and geographical distances was assessed with a Mantel test.

Shell outline analysis

Shell outline analysis was conducted on 210 *B. undatum* shells from Canada, the Faroe Islands, England and four sites in Iceland (Table 1). The empty shells were photographed dorsally on a level surface (Fig. 1C) using a Nikon D3200 with a 55 mm lens at a set distance following a standardized protocol. Photographs for each group were taken shortly after sampling of each batch, and the remainder of the image processing was randomized with regard to sample site. The background of the images was removed with Burner Bonanza (Bonanza.com, 2014), and the resulting images were rotated and converted to monochrome (Fig. 1D) in GIMP v.2.8.18 (Kimball *et al.*, 2016). Outlines and reciprocal Wavelet coefficients were generated for the shells using the R package *ShapeR* (Libungan & Pálsson, 2015), which allows an automated procedure to analyse variation in two dimensions. In studies of otolith shape variation, similar results have been obtained with Wavelets and Fourier analyses (S. Pálsson and L. A. Libungan, unpublished results). However, Wavelet coefficients offer an additional assessment of which parts of the shape contribute to the differentiation between populations. To analyse the differences in shape between populations, variation in the Wavelet coefficients was analysed using canonical analysis of principal coordinates (CAP) in *vegan* (R package version 2.5-3; Oksanen *et al.*, 2018) and their signficance tested using an ANOVA-like permutation test (anova.cca) in the same R package. To test for allometric effects, a linear model (lm) was run with CAP1 and CAP2 as response variables and site * shell height as predicting variables. The relationship of shell shape with depth, latitude, longitude and geographical distance was assessed with correlation tests with

CAP1 and CAP2, and Mantel's test with Euclidean distances between coordinates of the canonical axes.

PHENOTYPIC AND GENOTYPIC DIVERGENCE ACROSS POPULATIONS (P_{ST} VS. F_{ST})

In our study, the phenotypic variance (P_{ST}) of whelk shell traits was estimated as a substitute for additive genetic variance (Q_{ST}) (Merilä, 1997; Sæther *et al.*, 2007; Brommer, 2011; Defaveri & Merilä, 2013), incorporating the uncertainities of heritability (h^2) and the between-population additive genetic component (c) following Brommer's (2011) formulation:

$$P_{\mathrm{ST}} = \frac{\frac{c}{h^2}\sigma_{\mathrm{B}}^2}{\frac{c}{h^2}\sigma_{\mathrm{B}}^2 + 2\sigma_{\mathrm{W}}^2}$$

where σ_{B}^2 and σ_{W}^2 are phenotypic variances between and within populations, respectively. When $c/h^2 = 1$, P_{ST} equals Q_{ST}, and when $c/h^2 = 2$ it equals the intraclass correlation. Variances were based on the average of squared distances, d, between individuals i and j as $s^2 = \sum\left(d_{ij}^2/N_iN_j\right)$, where N is the sample size, and the variance components were summarized from the output from pairwise comparisons using the *adonis* function in the R package vegan (R package version 2.5-3; Oksanen *et al.*, 2018). To account for variation between samples within the Eastern North Atlantic and in Greenland, in addition to the fine-scale spatial patterns in shell morphology previously observed in whelk populations in Iceland (Magnúsdóttir *et al.*, 2018), the P_{ST} estimates were based on average variance between sites, e.g. for Canada (C) vs. all sites separately within the Eastern North Atlantic (E_i), i.e the average of $s^2_{\mathrm{Cvs.E}_i}$, and the average of variances within sites (s^2_{W}).

An adjustment for h^2 and c is necessary for *B. undatum* because the species exhibits late sexual maturity (~5 years), internal fertilization and a long lifespan, which are not ideal characteristics for the detailed common garden studies needed to estimate genetic components affecting shell traits and the partition of genetic variance among groups (Q_{ST}) (Magnúsdóttir, 2010; Mariani *et al.*, 2012). Different *B. undatum* populations are likely to be subjected to different environmental conditions that can create an inequality of c and h^2 (Sæther *et al.*, 2007; Pujol *et al.*, 2008; Brommer, 2011; Magnúsdóttir *et al.*, 2018), thus the sensitivity of P_{ST} comparisons with the neutral expectation of genetic drift and migration, based on microsatellites (Pálsson *et al.*, 2014) and *COI* (Magnúsdóttir *et al.*, 2019), was evaluated by performing a selection of simulated values of c/h^2 (from 0.2 to 2.0, as described by Brommer (2011)) for the phenotypic divergence between Canada, the Eastern North Atlantic and Greenland. The F_{ST} for both microsatellites and *COI* was estimated based on Weir & Cockerham (1984).

To ensure robustness in our analysis of phenotypic variance, the P_{ST} was calculated both for the Mahalanobis distances based on the traditional morphometric measurements and the shape outline analysis. Traditional morphometrics can be useful for delineating gastropod populations based on shell characteristics (Thomas & Himmelman, 1988; Hollyman, 2017; Woods & Jonasson, 2017; Magnúsdóttir *et al.*, 2018), but methods such as outline analysis and landmark-based geometric morphometrics can provide higher resolution (Brönmark *et al.*, 2011; Magnúsdóttir *et al.*, 2018) because they use a large number of independent variables to evaluate patterns of shape variation within and between populations (Rohlf & Marcus, 1993; Stransky, 2005).

RESULTS

SHELL MORPHOLOGY

Shell variables

The Mahalanobis distances indicated a clear morphological distinction between Canadian, Greenlandic and Eastern North Atlantic populations based on the three shell variables (Fig. 3; Supporting Information, Table S2). Greenland and Canadian whelk clustered separately (Fig. 3) on the extreme opposite sides (MDS stress = 0.09) of the Eastern North Atlantic populations ($P < 0.006$, permutation test; Supporting Information, Table S2).

Variation along the first MDS coordinate was correlated with all three shell variables; negatively with thickness ($r = -0.74$, $P < 0.001$), but Positively with shell ratio: ($r = 0.79$, $P < 0.001$) and aperture ratio ($r = 0.95$, $P < 0.001$). The second MDS coordinate was also correlated with all three shell variables, negatively with thickness ($r = -0.44$, $P < 0.001$) and shell ratio ($r = -0.57$, $P < 0.001$), but positvely with aperture ratio ($r = 0.16$, $P < 0.001$). Accordingly, the Canadian whelks have more elongate shapes and apertures than whelks from the other sample sites (Fig. 4; Supporting Information, Table S2; Tukey's HSD *post hoc* test, $P < 0.0001$). The thinnest shells were from Greenland and the thickest from Canada (Fig. 4; Supporting Information, Table S2; Tukey's HSD *post hoc* test, $P < 0.001$). Depth at the sample sites was correlated with axis 1 ($r = 0.47$, $P < 0.001$) and axis 2 ($r = 0.44$, $P < 0.001$). Morphological variation across the North Atlantic did not increase with distance between the populations (Mantel's $r = 0.29$, $P = 0.088$).

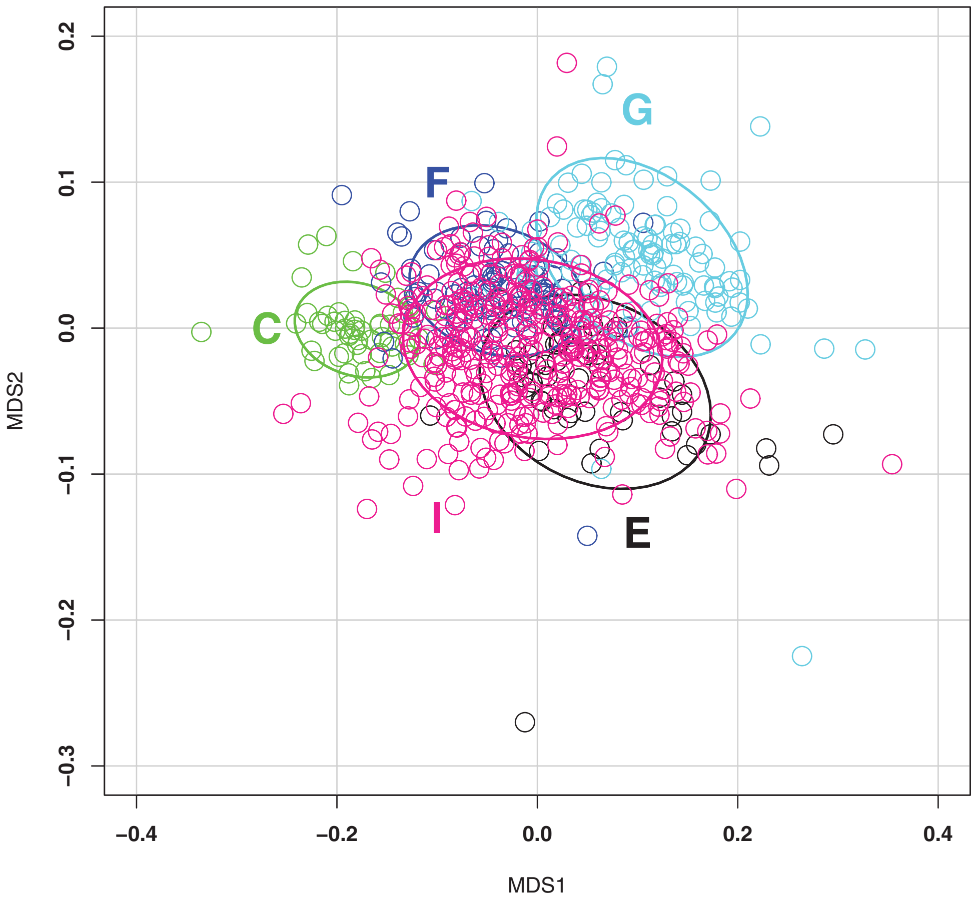

Figure 3. Multidimensional scale (MDS) plot based on Mahalanobis distances of shell variables of *Buccinum undatum* between the countries sampled. Individual values are indicated by open circles, and variance within each country is indicated by data ellipses covering 68% of the distribution or one standard deviation from the mean. Samples from Greenland and Iceland are pooled within the respective country. Countries are denoted as follows: C, Canada; E, England; F, the Faroe Islands; G, Greenland; I, Iceland. Stress was 0.09.

Shell outlines

The ordination of shell shape population averages along the first two canonical axes (CAP1 and CAP2), based on Wavelet coefficients, clearly places whelk shells from Canada and England in the same cluster (Fig. 5). The two populations differ significantly from the whelks from Iceland and the Faroe Islands along the first axis (CAP1 explains 88%; Fig. 5), having both a proportionally higher and wider body whorl and a shorter spire (Fig. 6; $F = 33.801$, $P = 0.001$). There was no significant effect of shell height on the observed differences in shape between sites.

The first canonical axis was significantly correlated with both depth ($r = 0.81$, $P < 0.05$) and latitude ($r = 0.89$, $P < 0.01$), and the variation in shell shape decreased with geographical distance between the populations (Mantel's $r = -0.63$, $P = 1$), owing to the similar shape of whelks from England and Canada.

COMPARISONS OF P_{ST} AND F_{ST}

The phenotypic divergence (P_{ST}) of shell shape of the common whelk across the North Atlantic was either similar to or lower than genetic divergence (F_{ST}) for the mtDNA and microsatellites (Fig. 7). In the sensitivity analysis of the shell ratios based on Mahalanobis distances, with respect to P_{ST} and F_{ST}, for combined comparisons between Canada and each of the Eastern North Atlantic countries (Iceland, the Faroe Islands and England; Fig. 7A), the upper confidence interval of P_{ST} overlapped with the lower confidence interval of mtDNA F_{ST} and with microsatellite F_{ST} at a value of $c/h^2 = 0.8$ or greater. Confidence intervals of P_{ST} values from comparisons of Mahalanobis distances of shell variables between Canada and Greenland (Fig. 7B) and between Greenland and Eastern North Atlantic (Fig. 7C) overlapped with mtDNA F_{ST} and microsatellite F_{ST} at a value of $c/h^2 \geq 0.4$ and 1.0, respectively. The upper confidence interval of the combined P_{ST} between

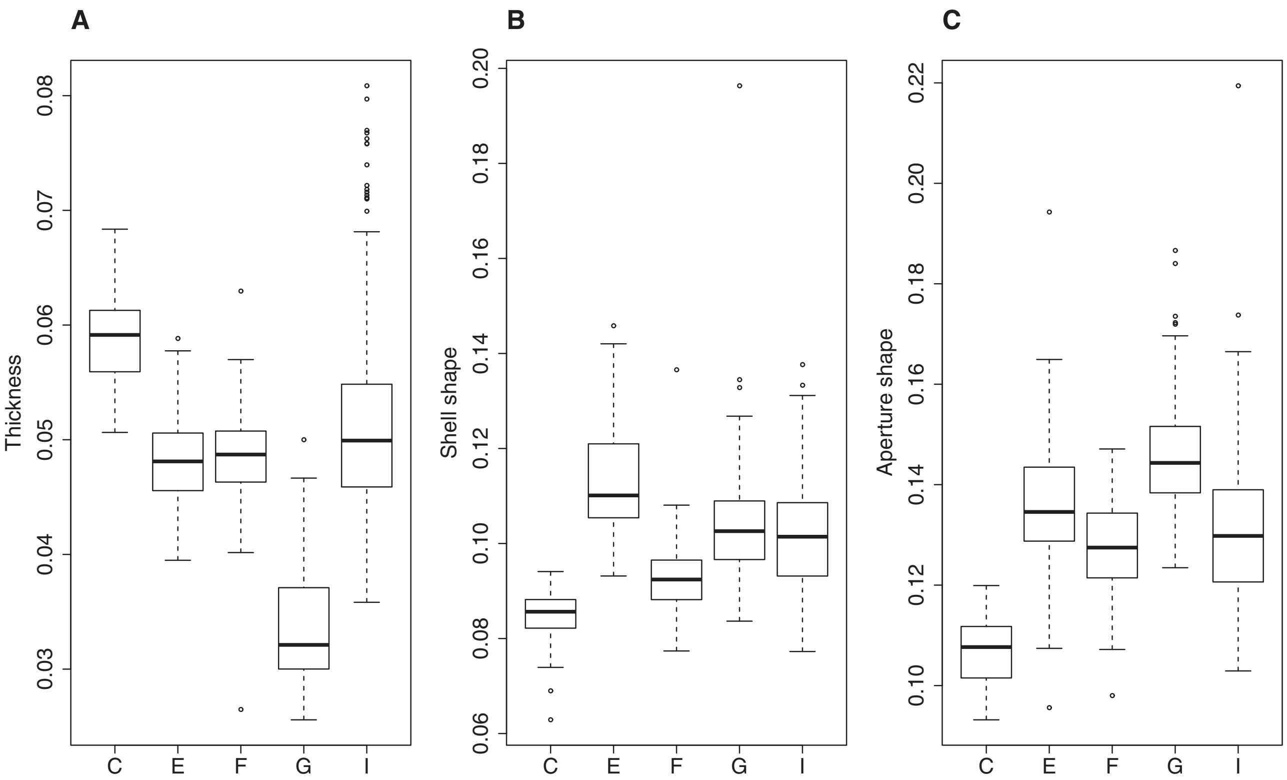

Figure 4. Shell variables of *Buccinum undatum* across the North Atlantic based on traditional morphometrics: A, thickness estimated as √(shell weight)/shell height; B, shell shape estimated as √(shell width)/shell height; and C, aperture shape estimated as √(aperture width)/aperture height. Country of origin is denoted as follows: C, Canada; E, England; F, the Faroe Islands; G, Greenland; I, Iceland.

Canada and each of the Eastern North Atlantic countries, based on the shell outlines, overlapped with the F_{ST} values at a value of $c/h^2 \geq 0.4$, except for the P_{ST} between whelks from England and Canada, which was always lower than the confidence interval for mtDNA F_{ST} and the microsatellite F_{ST} (Fig. 7D).

DISCUSSION

The pattern of phenotypic divergence of *B. undatum* across the North Atlantic, described here by two different morphological methods, provides partial support for the existence of distinct species on either side of the North Atlantic, as suggested by genetic analysis. To some extent, the morphological differentiation reflects the genetic split across the Atlantic, but in other cases a discordance is observed where local environmental variation might have shaped the shell morphology. Morphological convergence might have occurred by natural selection, or the similarity might have been shaped during development, whereby genetically different individuals respond in a similar manner to similar environments. The observed mismatch is consistent with the results obtained by Mariani *et al.* (2012) on *B. undatum* in Ireland, where phenotypic and genetic patterns based on microsatellites were not in agreement, yet the P_{ST} vs. F_{ST} comparisons indicated diversifying selection to some extent, e.g. for shell thickness.

The phenotypic differentiation of the Northern Atlantic populations in Iceland and the Faroe Islands from whelks from Canada reflected the genetic differentiation, whereas the results for *B. undatum* from the English channel and Canada were inconclusive because the two morphological methods were not in agreement. *Buccinum undatum* from England and the Faroe Islands were consistently dissimilar in morphology, although genetic differentiation between them is fairly low. Finally, between the distinct genetic North American populations from Canada and Greenland, a larger differentiation was observed in the traditional morphometrics than between whelks from Canada and the Eastern North Atlantic.

Some of the inconsistency between the two morphological methods, which mostly encompasses Canada and England, might be attributable to the fact that the populations in these locations were not

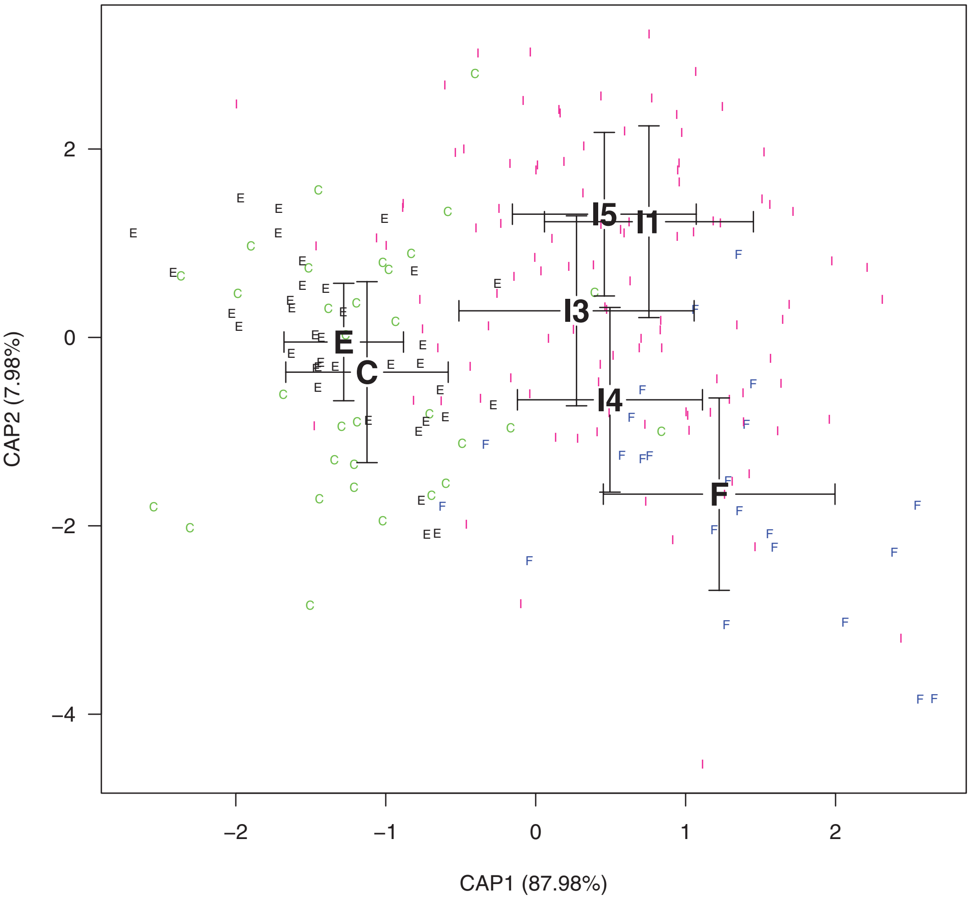

Figure 5. Canonical analysis of principal coordinates (CAP1 and CAP2) of Wavelet coefficients of shell shape of *Buccinum undatum* from sampling locations across the North Atlantic. Bold black letters represent the mean canonical value for each population, and coloured letters indicate individual whelks. Country of origin is denoted as follows: C, Canada; E, England; F, the Faroe Islands; G, Greenland; I, Iceland.

sampled in exactly the same place and year between methods (Table 1). In fact, traditional morphometrics for the shells used in the shell outline analysis show the same phenotypic results (Supporting Information, Fig. S1A, B); that is, shells from England that were sampled in 2015 (shell outline analysis) were significantly more elongate than the shells from England sampled in 2010 (shell variable analysis), whereas the shells from Canada sampled for the outline analysis were signficiantly more rotund than the shells sampled from Canada for the shell variable analysis. This explains why the outlines of whelks from the two countries overlap, whereas the shell variables do not, and emphasizes the importance of taking fine-scaled population structure into account in this type of comparison (Magnúsdóttir *et al.*, 2018).

Increased shell thickness, in addition to elongated apertures and shells, in gastropods have been linked previously to decapod predation (Vermeij, 1978; Thomas & Himmelman, 1988; Bourdeau, 2010; Brönmark *et al.*, 2011; Johannesson, 2015), whereas thinner gastropod shells have been associated with lower availability of calcium in the deep sea (Vermeij, 1978). In our data, the variation of the individuals along the axis of the ordination plot (MDS1 and MDS2) for the shell variables is correlated with thickness, shell and aperture shape and depth. The thick shells and elongate apertures/shells of Canadian whelks sampled from the sheltered shallow waters of the Gulf of Saint Lawrence might, to some extent, reflect heavier crab predation (Thomas & Himmelman, 1988), whereas the thin shells of the Greenlandic whelks might reflect the considerable depth at which they were collected (> 200 m).

Neither the thickness of the shells nor the aperture shape is taken into account by the shell outline analysis, which could explain some of the finer differences in the results between the two morphological methods. In fact, exclusion of the aperture shape and thickness, which are highly correlated (thicker shells have significantly narrower apertures) and known often to be affected by the environment, might be expected to yield

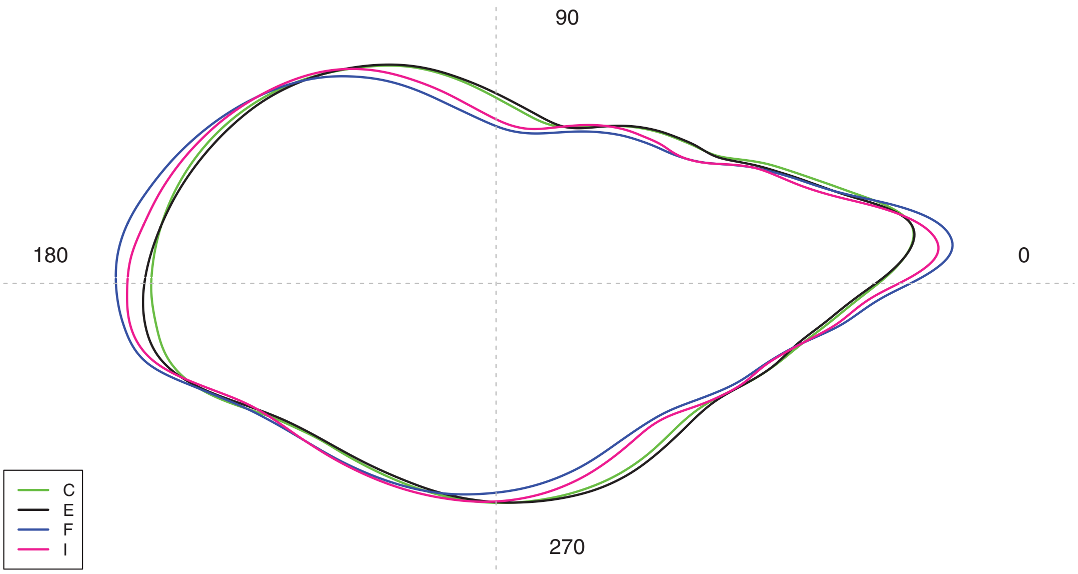

Figure 6. Mean shell shape (dorsal view) based on wavelet reconstruction for *Buccinum undatum* from Canada (C), England (E), the Faroe Islands (F) and Iceland (I). Numbers represent angles (in degrees), with the centre point indicated by the centre of the cross.

morphological results that may be more informative of the true heritable differences in shell shape. The shell outline analysis, however, was only marginally correlated with genetic differentiation but showed a significant correlation with both depth and latitude. Given that latitude and depth are confounded, where the whelks in England and Canada were sampled in shallow areas and at lower latitudes than the whelks in Iceland and the Faroe Islands, it is difficult to tease apart the contribution of these two variables in the present dataset.

Comparison of P_{ST} vs. F_{ST} indicated neither diversifying nor convergent evolution for *B. undatum* across the North Atlantic. Assessment of P_{ST} vs. F_{ST} for such a phenotypically variable species as *B. undatum* appears to be more sensitive over shorter distances/more similar environments, as described by Mariani *et al.* (2012), than when comparisons are made on a larger scale/in variable environments, such as in the present study. This might be attributable to the inequality of additive genetic effects (*c*) and heritability (h^2) across phenotypically plastic populations (Sæther *et al.*, 2007; Pujol *et al.*, 2008; Brommer, 2011). Moreover, genetic differences could be caused by drift over such a long time, whereas selection pressure would tend to constrain the shape (Johannesson, 2015; Gemmell *et al.*, 2018). Several important concerns have been raised regarding the comparison of F_{ST} vs. Q_{ST}, in addition to the assumptions behind the substitution of Q_{ST} with P_{ST} (i.e. the unknown c/h^2) (Galindo *et al.*, 2019). A mismatch between F_{ST} and P_{ST} can arise owing to differences in variation within groups for the two statistics, e.g. in cases where F_{ST} is limited by high heterozygosity (Hedrick, 1999; Edelaar & Björklund, 2011). Variable mutation rates in genetic markers, such as microsatellites, and over haplotypes of different lengths will thus affect the F_{ST}. This might be of more concern when evaluating whether the ratio of F_{ST} and P_{ST} deviates from one, rather than the overall association of the two pairwise distances which, for neutral markers, is expected to increase with time for separated populations. Distances based on nucleotide diversity, which are independent of the sequence length, could be more informative, but in the case of *B. undatum*, where distinct monophyletic mtDNA lineages are found in Canada, Greenland and the Eastern North Atlantic, such distances are > 0.9 (Magnúsdóttir *et al.*, 2019).

The evident discrepancy between genetic and phenotypic differentiation in *B. undatum* detailed here underlines the importance of taking fine-scaled geographical variability into account when considering phenotypic variation in shell-bearing gastropods, in addition to species Delimitation based on these traits, because phenotypic traits can be under selection or influenced by environmental effects during development. In fact, shared environmental factors seem to influence the variation in shell shape in multiple, unrelated lineages in a similar way, whether the gastropods in question are freshwater, intertidal or subtidal, often but not always regardless of genotypic differentiation (Thomas & Himmelman, 1988; Trussell & Etter, 2001; Bourdeau *et al.*, 2015; Johannesson, 2015; Kosloski *et al.*, 2017; Magnúsdóttir *et al.*, 2018).

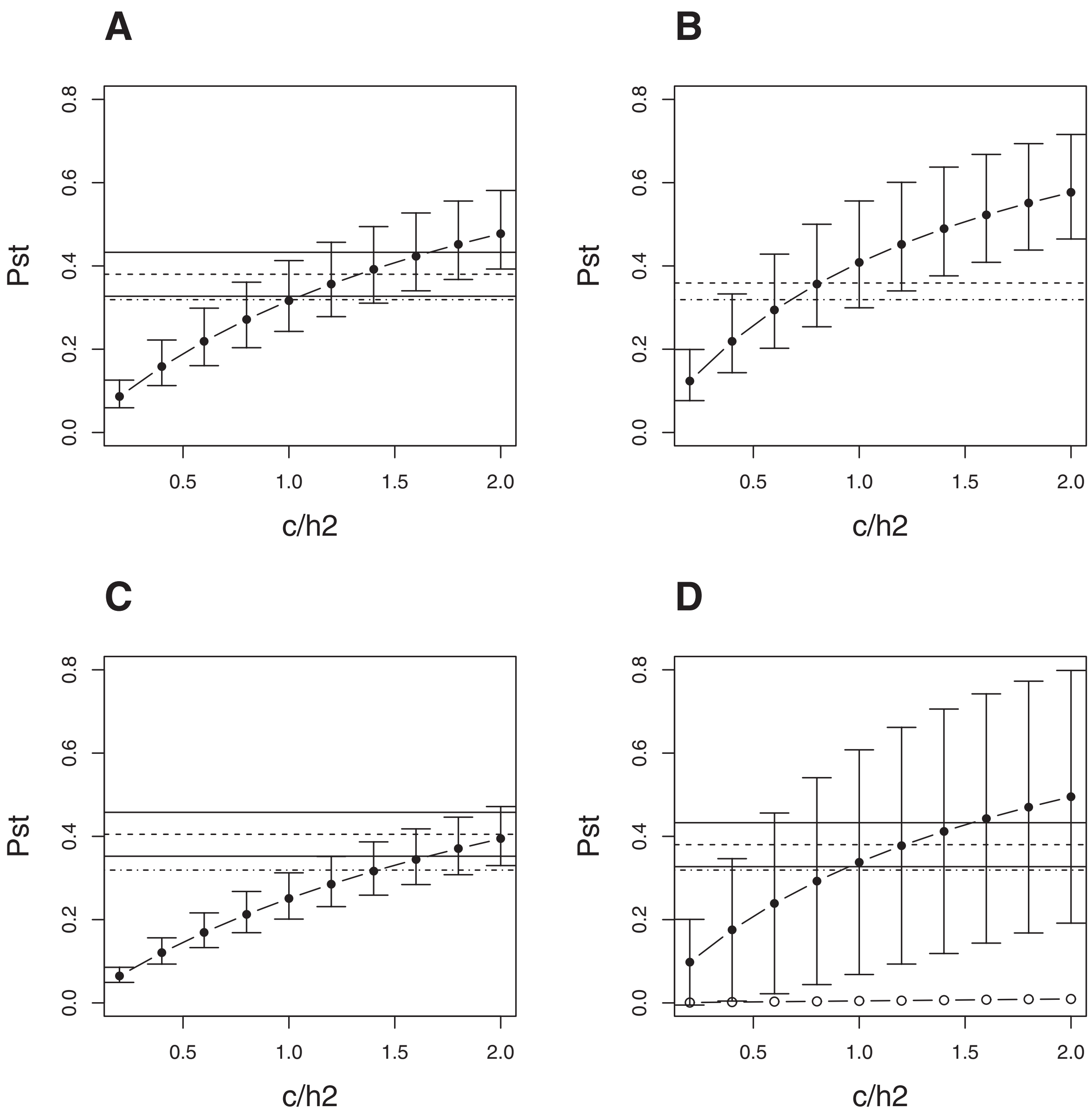

Figure 7. Phenotypic divergence in *Buccinum undatum* shell shape (P_{ST}) at c/h^2 (where c, the between-population additive genetic component and h^2, heritability) from 0 to 2.0 based on: A, Mahalanobis distances of shell variables between Canada and the Eastern North Atlantic; B, Mahalanobis distances of shell variables between Canada and Greenland; C, Mahalanobis distances of shell variables between the Eastern North Atlantic and Greenland; and D, Euclidean distances of CAP1 and CAP2 of the shell outlines between Canada and the Eastern North Atlantic sites (N.B. here, the comparison between Canada and England is portrayed by open circles). Vertical lines indicate the 95% confidence interval. The dashed horizontal line indicates the mean mtDNA divergence (F_{ST}) (see Magnúsdóttir *et al.*, 2019), the continuous horizontal lines indicate the confidence interval of the mtDNA F_{ST}, where available, and the horizontal dot–dash line indicates the microsatellite divergence (F_{ST}) (0.32; see Pálsson *et al.*, 2014).

With regard to the question of cryptic speciation within *B. undatum*, in addition to species delimitation within the genus, it would be of value to compare morphological distances between *Buccinum* species, because genetic distances between populations of *B. undatum* have been found to exceed the genetic distances between several *Buccinum* species (Magnúsdóttir *et al.*, 2019). Anatomical characteristics of the soft body, when available, are also used for gastropod species delimitation (Schander & Sundberg, 2001; Kantor, 2003; Ponder *et al.*, 2008), yet many lineages display considerable enough levels of homoplasy in both some anatomical and shell characteristics (Schander & Sundberg, 2001) to diffuse their usefulness in construction of a species phylogeny, when not supplemented by genetic data. Therefore, a combination of morphological and genetic studies is required before making assumptions on the degree of cryptic speciation. On a broader scale, comparison with other buccinid genera, such as *Colus* and *Neptunea*, that share the North Atlantic/North Pacific distribution with *Buccinum* would shed light on whether the phenotypic patterns observed for *B. undatum* are replicated in other long-lived predatory whelk genera.

For *B. undatum*, additional studies are needed to assess how well the external morphology reflects the genomic variation of *B. undatum* throughout the North Atlantic. Morphological studies on juvenile whelks raised in controlled common garden conditions and on adults from different environments are needed to control for the possible effect of the environment on the morphological divergence of the populations. To resolve these issues, we have initiated further studies assessing genome-wide variation and transcriptome analysis in our laboratory to understand how development, natural selection and historical isolation have shaped the observed morphological diversity of *B. undatum*.

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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:

Table S1. Descriptive statistics of *Buccinum undatum* collected at sample sites across the North Atlantic. See Table 1 for codes and site detail.
Table S2. Pairwise Mahalanobis distances (×1000) between *Buccinum undatum* populations across the North Atlantic. Significance was estimated with 1000 permutations. For codes and details, see Table 1. ^{NS}Not significant, according to Holm–Bonferroni corrections ($P = 0.006$).
Figure S1. Shell variables of the combined datasets (2008–2010 and 2014–2015) of *Buccinum undatum* across the North Atlantic based on traditional morphometrics: A, shell shape estimated as √(shell width)/shell height; and B, aperture shape estimated as √(aperture width)/aperture height. Country of origin is denoted as follows: C, Canada; E, England; F, the Faroe Islands; G, Greenland; I, Iceland; and *O* stands for old (2008–2010) dataset, whereas *N* stands for new (2014–2015) dataset.