

# Revised phylogeography of the common whelk *Buccinum undatum* (Gastropoda: Buccinidae) across the North Atlantic

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The purpose of this study was to revisit the mitochondrial genetic divergence of North Atlantic populations of the subtidal gastropod *Buccinum undatum*, because previous mitochondrial DNA (mtDNA) analysis by Pálsson *et al.* (2014) included 16S ribosomal RNA sequences that were incorrectly assigned to the species. In the present study, population mtDNA variation is now assessed using *COI* sequences obtained from previous research (Pálsson *et al.*, 2014) and, to increase the geographical cover of the study, data from recently assembled transcriptomes of 96 Icelandic whelks (Jónsson *et al.*, 2019) and GenBank are also used. To estimate the mtDNA divergence in *B. undatum* across the North Atlantic, two species screening indices were used: automatic barcode gap discovery (ABGD; Puillandre *et al.*, 2012a) and species screening threshold index (SSTI; Witt *et al.*, 2006). Phylogenetic reconstruction revealed monophyletic Eastern and Western North Atlantic whelk lineages, which diverged early in the Pleistocene glaciation (2.1 Mya), followed by a subsequent divergence event between Greenlandic and Canadian populations at 1.3 Mya. Species screening indices, ABGD and SSTI, indicated cryptic speciation or allopatric divergence. Genetic distances between populations from the two continents were similar to or greater than interspecific genetic distances across several North Pacific and North Atlantic *Buccinum* species.

ADDITIONAL KEYWORDS: allopatry – *Buccinum* – genetic divergence – marine gastropods – population connectivity – population structure.

## INTRODUCTION

The subtidal gastropod genus *Buccinum* originated in the North Pacific and spread into the Arctic and North Atlantic through the Bering Strait during one of its opening events (Vermeij, 1991, 2005; Vermeij *et al.*, 2019). Intrinsic morphological variation within the genus causes difficulties in species identification despite repeated reviews of species diversity (Golikov, 1980; Tiba & Kosuge, 1984; Higo *et al.*, 1999). This morphological variation is attributable to limited demographic connectivity resulting from low dispersal capacity (direct development without a free-swimming

larval stage; Martel & Larrivé, 1986; Gendron, 1992; Ilano *et al.*, 2003) and a sedentary adult lifestyle (Himmelman, 1988; Jalbert *et al.*, 1989; Himmelman & Hamel, 1993), in addition to shell trait plasticity with respect to environmental heterogeneity (Iguchi *et al.*, 2005; Mariani *et al.*, 2012; Gemmell *et al.*, 2018; Magnúsdóttir *et al.*, 2018). Morphological traits might therefore not be a reliable predictor of species status, and molecular variation should be explored further to resolve evolutionary relationships within this genus.

The common whelk, *Buccinum undatum*, typifies morphological buccinid traits (Golikov, 1980) by exhibiting consistent spatial variation in shell morphology (Golikov, 1968; Ten Happers-Tjabbes, 1979; Thomas & Himmelman, 1988; Kenchington & Glass, 1998; Mariani *et al.*, 2012) and life-history traits (e.g. size at sexual maturity, age and size distribution)

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across its North Atlantic habitat (reviewed by Haig *et al.*, 2015) and even over relatively short distances (~20 km; Magnúsdóttir, 2010; Magnúsdóttir *et al.*, 2018). Population genetic differentiation among geographically proximal sites follows an isolation-by-distance model (Weetman *et al.*, 2006; Mariani *et al.*, 2012; Pálsson *et al.*, 2014).

Previous work on *B. undatum* North Atlantic phylogeography identified clear population structuring based on microsatellite, *COI* and 16S ribosomal RNA (rRNA) variation (Pálsson *et al.*, 2014). Distinct mitochondrial DNA (mtDNA) lineages in the Western and the Eastern North Atlantic indicated that populations from the two continents diverged in correspondence to the onset of the last Ice Age (~2.6 Mya) and are likely to constitute cryptic species (Pálsson *et al.*, 2014). In addition, populations from Greenland and Canada have been isolated for ~1.1 Myr. However, recent analyses have shown that the sequences considered as 16S rRNA by Pálsson *et al.* (2014) were not from *B. undatum* (Jónsson *et al.*, 2019), and thus there is a clear need to revise the phylogeography of *B. undatum* across the North Atlantic.

Several methods have been designed provisionally to assess species status based on the DNA barcoding gap (Hebert *et al.*, 2003, 2004), including the automatic barcode gap discovery (ABGD; Puillandre *et al.*, 2012a) and the species screening threshold index (SSTI; Witt *et al.*, 2006). The SSTI is a conservative threshold for provisional species recognition that has been proposed at ten times the average intrapopulation *COI* haplotype divergence (Witt *et al.*, 2006). Aside from the simplicity of the application of the method, other advantages include the fact that SSTI omits recently diverged taxa and protects against the artificial recognition of intraspecific variants as species (Witt *et al.*, 2006). The ABGD is a more recent method, and is an automatic procedure in which distribution of pairwise distances among aligned sequences is analysed in order to detect a break between intraspecific and interspecific values to assign the sequences to putative species (Puillandre *et al.*, 2012a), and the procedure evaluates a range of threshold values based on the data.

The following analysis addresses a phylogeographical revision using a compilation of previously obtained *COI* sequences (from Pálsson *et al.*, 2014) and, for increased geographical coverage, a longer *COI* region obtained from Icelandic *B. undatum* transcriptomes (Jónsson *et al.*, 2019) and *Buccinum* sequences obtained from GenBank. Given that this is a correction of the mtDNA analysis from the previous paper, the microsatellite data were not included, and their results still stand. To evaluate whether the large divergence in mtDNA between the *B. undatum* from across the Atlantic presents two cryptic species, two species indices, ABGD

and SSTI, were calculated, and the distances between the main lineages were compared with distances between other species within the genus.

## MATERIAL AND METHODS

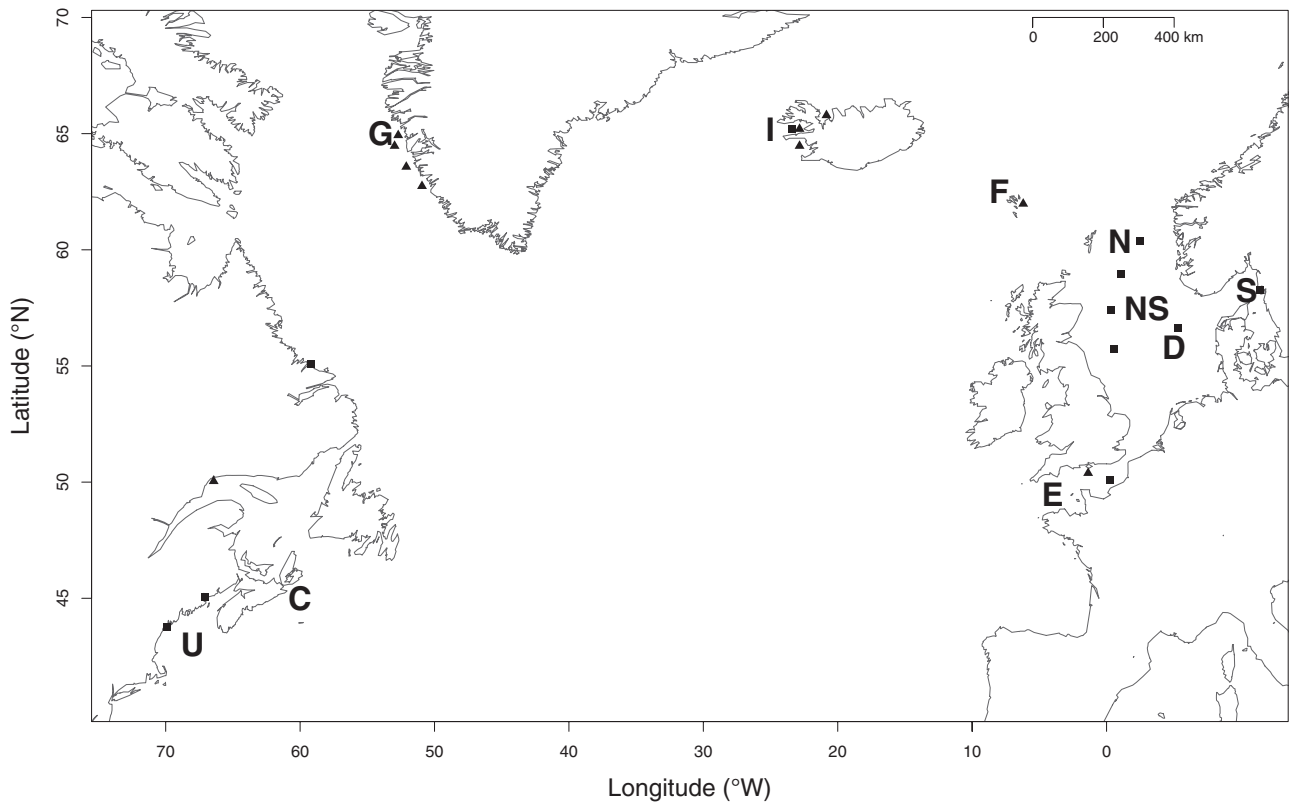
### DATA COLLECTION

Two datasets of the mtDNA *COI* gene were analysed in this study: (1) *COI-1* (369 bases from position 63 to 432) from 599 whelks described by Pálsson *et al.* (2014), sampled at five locations across the North Atlantic Ocean (Fig. 1; Table 1) on both the Northeastern (Iceland, Faroe Islands and UK) and the Northwestern sides (Gulf of St Lawrence, Canada and West Greenland); and (2) to increase the geographical coverage of the study, *COI-2*, the barcode region of 656 bp (from position 39 to 695) obtained from 96 *B. undatum* transcriptomes collected from Iceland (Jónsson *et al.*, 2019) and GenBank sequences comprising four whelks from the Western North Atlantic (Maine to Labrador) and 13 whelks from the Eastern North Atlantic (UK, North Sea and Scandinavia) (Table 1). The segregating sites and location of unique haplotypes of *B. undatum* recovered in the study are listed in the Supporting Information (Tables S1 and S2). Homologous *COI* sequences from species within the *Buccinum* genus were obtained from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) and Barcode of Life Data System (BOLD, <http://boldsystems.org/>) (Supplementary Table S3).

### *COI* MTDNA VARIATION

A phylogenetic tree of the *COI-1* mtDNA within *B. undatum* was reconstructed using BEAST (Suchard *et al.*, 2018) and divergence time estimation as described by Pálsson *et al.* (2014). The topology of the *COI-1* tree was compared with the topology of Pálsson *et al.* (2014) previously named 16S rRNA, by calculating the cophenetic correlation coefficient (Sokal & Rohlf, 1962), i.e. the correlation between patristic distances within each tree, and then tested with a Mantel test using the R package *ape* (Paradis & Schliep, 2019). A second tree, based on *COI-2* sequences, was reconstructed using PhyML implemented in SeaView (Gouy *et al.*, 2010), with *Buccinum pempheus* Dall, 1907 as an outgroup, selecting the best evolutionary model, and estimating branch support by the approximate likelihood ratio test approach (aLRT). The tree was redrawn using *ape*.

Pairwise population differentiation between locations was calculated for the *COI-1* sequences by considering both the haplotype frequencies ( $F_{ST}$ ) and the genetic distances between sequences ( $\Phi_{ST}$ ), tested with 1000 permutations. The association of genetic differentiation with geographical distance (isolation by distance) was



**Figure 1.** *Buccinum undatum* sampling sites in the North Atlantic from the study by Pálsson *et al.* (2014). Areas are denoted as follows: C, Canada; D, Denmark; E, England; F, The Faroe Islands; G, Greenland; I, Iceland; NS, North Sea; N, Norway; S, Sweden; U, USA. Triangles indicate *COI-1* sequences from (Pálsson *et al.*, 2014), and squares indicate *COI-2* sequences from the present study or GenBank (for details, see Table 1).

tested using a Mantel test in *vegan* (Oksanen *et al.*, 2016) in R (R Core Team, 2018) and compared with results from the combined datasets of Pálsson *et al.* (2014) for *COI* and 16S rRNA. Geographical distances were calculated based on latitude and longitude using the *geosphere* R package (Hijmans, 2017).

To explore further the robustness of the geographical groupings and putative species status suggested by the phylogenetic analyses of *COI-1*, the ABGD (Puillandre *et al.*, 2012a) was carried out, in addition to calculation of the SSTI from Witt *et al.* (2006), which proposes a conservative threshold of ten times the ratio of divergence in *COI* between and within populations. The ABGD analysis (Puillandre *et al.*, 2012a) was conducted using the graphic web version (Puillandre *et al.*, 2012b), under the default parameters of prior values for maximum divergence of intraspecific diversity ( $P$ ) range of 0.001–0.1, steps = 10, relative gap width ( $X$ ) = 1.5, Nb bins (for distance distribution) = 20, and the Kimura (K80) evolutionary model.

Finally, the averages of genetic distances (K2P) between the three main *COI* North Atlantic lineages were calculated for *COI-1* using *ape*, and compared with corresponding pairwise distances between

homologous sequences from 17 other *Buccinum* species from the North Atlantic and the North Pacific oceans obtained from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) and BOLD (<http://boldsystems.org/>) (Supporting Information, Table S3).

## RESULTS

A Bayesian phylogeny based solely on the *COI-1* variation in *B. undatum* (Fig. 2A) recovered two major monophyletic clades (Eastern and Western North Atlantic Ocean), as reported for the mtDNA including the unknown 16S rRNA sequences (Pálsson *et al.*, 2014). The main split between the continents is predicted to have occurred 2.1 Mya (1.04–3.49 Mya), with the Western North Atlantic clade dividing again into two monophyletic clades (Greenland and Canada) 1.3 Mya (0.62–2.14 Mya). The average dates were older in the previous analyses, but the confidence intervals overlap. The overall tree topologies based on mtDNA *COI-1* and 96 of the unknown 16S rRNA sequences (Supporting Information, Fig. S1) were similar, with a high cophenetic correlation of 0.91 ( $P < 0.001$ ).

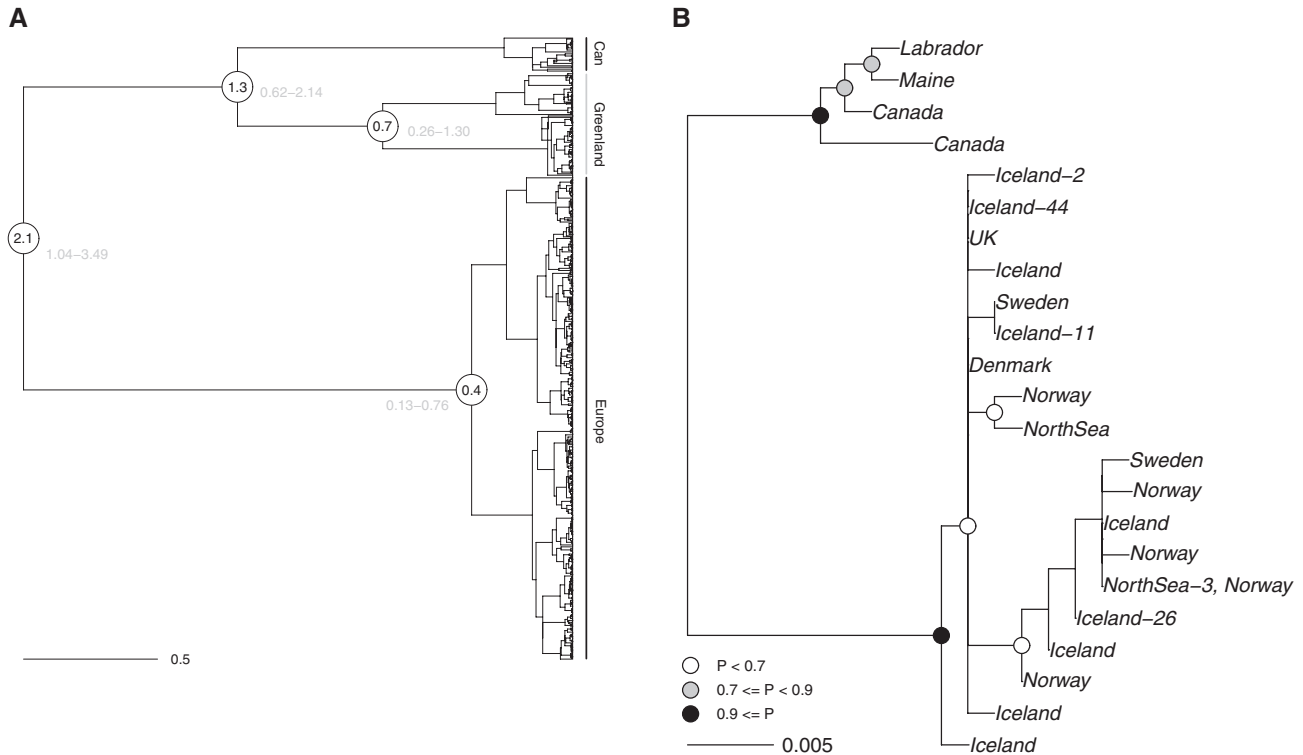
**Table 1.** Location, type and year of sampling of *Buccinum undatum* in the North Atlantic

Country	Location	Code	Latitude (N)	Longitude (W)	Depth (m)	COI data (N)	Sampling year	Accession numbers	References
Canada	Baie Sainte-Marguerite, Gulf of St Lawrence	C	50.04	66.43	17.5	COI-1 (37)	2010	KC502024–KC502035	(Pálsson <i>et al.</i> , 2014)
Canada	St Andrews, Bay of Fundy	C	45.07	67.04	intertidal	COI-2 (1)	2011	KF643373.1	(Layton <i>et al.</i> , 2014)
Canada	Casco Bay Island, Bay of Fundy	C	44.96	66.93		COI-2 (1)	2011	KF644029.1	(Layton <i>et al.</i> , 2014)
Canada	Makkovik, Labrador	C	55.09	59.17		COI-2 (1)	2014	MG422656.1	
Denmark		D	56.63	-5.30		COI-2 (1)	2010	KR084475.1	(Barco <i>et al.</i> , 2016)
England	Isle of Wight	E	50.39	1.37	10	COI-1 (45)	2010	KC501989–KC501994	(Pálsson <i>et al.</i> , 2014)
England		E				COI-2 (1)	2010	FN677402.1	(Barco <i>et al.</i> , 2016)
Faroe Islands	Nólsoyarfjørður	F	61.98	6.70	40	COI-1 (85)	2008	KC501989–KC501993, KC501995–KC501997	(Pálsson <i>et al.</i> , 2014)
Greenland	Paamiut	G	62.74	50.13	334	COI-1 (57)	2010	KC502000–KC502005, KC502008, KC502009	(Pálsson <i>et al.</i> , 2014)
Greenland		G	64.73	52.70	300	COI-1 (12)	2010	KC502000, KC502001, KC502003, KC502006	(Pálsson <i>et al.</i> , 2014)
Greenland		G	63.57	51.60	290	COI-1 (9)	2010	KC502001, KC502010	(Pálsson <i>et al.</i> , 2014)
Greenland		G	64.47	52.97	367	COI-1 (31)	2010	KC502000, KC502001, KC502003, KC502006, KC502007	(Pálsson <i>et al.</i> , 2014)
Iceland	Brjánslækur, Breiðafjörður	I	65.50	23.14	37	COI-2 (16)	2014, 2015		This study
Iceland	Hempill, Breiðafjörður	I	65.05	23.21	22	COI-1 (76)	2008	KC501989, KC501990, KC501992, KC501995, KC501998, KC501999, KC502011–KC502016	(Pálsson <i>et al.</i> , 2014)
Iceland	Hrútey, Breiðafjörður	I	65.03	22.94	36	COI-1 and -2 (93 and 29)	2008, 2014, 2015	KC501989, KC501992, KC501998, KC502017–KC502023	(Pálsson <i>et al.</i> , 2014) and this study

Table 1. Continued

Country	Location	Code	Latitude (N)	Longitude (W)	Depth (m)	COI data (N)	Sampling year	Accession numbers	References
Iceland	Oddbjarnarsker, Breiðafjörður	I	65.31	23.23	43	COI-1 and -2 (84 and 4)	2008, 2014, 2015	KC501989, KC501990, KC501992, KC501998, KC501999, KC502017, KC502018, KC502036	(Pálsson <i>et al.</i> , 2014) and this study
Iceland	Faxaflói	I	64.37	22.84	30	COI-1 (19)	2008	KC501989, KC501992, KC501998	(Pálsson <i>et al.</i> , 2014)
Iceland	Húnaflói	I	65.79	20.84	27	COI-1 (88)	2008	KC501989, KC501990, KC501992, KC501993, KC501998, KC501999, KC502017	(Pálsson <i>et al.</i> , 2014)
Iceland	Hvammisfjörður, Breiðafjörður	I	65.13	22.38	15	COI-2 (23)	2015		This study
Iceland	Bjarneyjaráll, Breiðafjörður	I	65.14	23.58	125	COI-2 (11)	2015		This study
Iceland	Skor, Breiðafjörður	I	65.34	23.92	53	COI-2 (3)	2015		This study
North Sea	Orkney	NS	58.96	-1.05		COI-2 (1)	2012	KR084589.1	(Barco <i>et al.</i> , 2016)
North Sea	England	NS	55.75	-0.59		COI-2 (1)	2012	KR084601.1	(Barco <i>et al.</i> , 2016)
North Sea	Scotland	NS	57.39	-0.3		COI-2 (1)	2012	KR084450.1	(Barco <i>et al.</i> , 2016)
North Sea	English Channel	NS	50.11	-0.29		COI-2 (1)	2012	KR084521.1	(Barco <i>et al.</i> , 2016)
Norway		N	60.37	-2.48		COI-2 (5)	2010	KR084451.1, KR084587.1, KR084396.1, KR084536.1, KR084432.1	(Barco <i>et al.</i> , 2016)
Sweden	Gullmarsfjorden	S	58.25	11.43	100	COI-2 (1)	2007	EF528303.1	(Bourlat <i>et al.</i> , 2008)
Sweden		S	58.25	11.52	30–35	COI-2 (1)	2004	KT753997.1	(Couto <i>et al.</i> , 2016)
USA	Chebeague Channel, Casco Bay, Maine	U	43.75	70.13		COI-2 (1)	2015	KU564524.1	

COI-1 fragments (369 bp, from position 63 to 432) are from Pálsson *et al.* (2014), and COI-2 fragments (656 bp, from position 39 to 695) come from the transcriptome of the common whelk (Jónsson *et al.*, 2019) and GenBank. Accession numbers and references, when published, are given. Code for areas is denoted as follows: C, Canada; D, Denmark; E, England; F, The Faroe Islands; G, Greenland; I, Iceland; NS, North Sea; N, Norway; S, Sweden; U, USA.



**Figure 2.** Phylogeny of the *COI* mitochondrial DNA variation in *Buccinum undatum* across the North Atlantic. A, based on *COI-1*, a short region of 369 bp (Pálsson *et al.*, 2014). The tree is based on a Bayesian method giving the highest posterior probability using BEAST. The time to the most recent common ancestor of the monophyletic groups (in millions of years) is presented in circles, with the 95% confidence interval adjacent. All marked nodes had a posterior probability of one. B, a maximum likelihood tree based on *COI-2*, 656 bp from GenBank (see Table 1 for references) and Iceland (see Table 1). Branch supports are based on the approximate likelihood ratio test: black,  $P \leq 0.9$ ; grey,  $0.7 \geq P < 0.9$ ; white,  $P < 0.7$ . Number after hyphen indicates number of sequences if more than one.

A BLAST search for a match with the unknown 16S rRNA inconclusively resulted in only an 86% match to other sequences in GenBank, with a very short query cover (26%).

Pairwise genetic differentiation between Canada and the Eastern North Atlantic was high for *COI-1* (Table 2), with  $F_{ST}$  ranging from 0.36 to 0.42 and  $\Phi_{ST}$  from 0.991 to 0.995. The values are similar or slightly larger than those obtained by Pálsson *et al.* (2014) (Table 2). The differences between the samples from Eastern North Atlantic and Greenland, and between Canada and Greenland, were of similar magnitude. Within the Eastern North Atlantic,  $F_{ST}$  ranged from 0.008 to 0.159, whereas  $\Phi_{ST}$  ranged from zero to 0.119.

The average genetic distance (K2P) for the short *COI-1* sequences (369 bp) between Canada and the Eastern North Atlantic was 0.037 (range, 0.030–0.041) and similar for the larger *COI-2* sequences (656 bp) from the Western and Eastern North Atlantic (Table 1) (K2P = 0.030). Furthermore, the maximum likelihood phylogeny (using the GTR model) based on

the *COI-2* sequences substantiates the split between the continents (Fig. 2B).

This clear differentiation between the populations from Western and Eastern North Atlantic is confirmed by the two species indices. The ABGD analysis partitioned the *COI-1* sequences from 33 samples with a  $P_{min} = 0.0010$  to a  $P_{max} = 0.0215$  for two groups consisting of either whelks from the Eastern or the Western North Atlantic Ocean. The second largest  $P$ -value obtained with the ABGD analysis (0.0129) delimited three groups: one in the Eastern North Atlantic and two in the Western North Atlantic, i.e. Canada and Greenland. The ratio of divergence between and within the three species groups also exceeded the ten times *COI* SSTI for Canada vs. Eastern North Atlantic (16.5 times). The extensive variation within Greenland led to a lower ratio when all the Greenlandic samples were pooled: Greenland vs. Eastern North Atlantic = 6.7 times, Greenland vs. Canada = 4.9 times; but the average of pairwise comparisons of the Eastern North Atlantic with

**Table 2.** Pairwise genetic differentiation of *COI-1* mtDNA region in *Buccinum undatum* across the North Atlantic

Comparison	$\Phi_{ST}$	Comparison	$\Phi_{ST}$ from Pálsson <i>et al.</i> (2014)
Canada vs. Eastern North Atlantic	0.991–0.995	Canada vs. Greenland vs. Eastern North Atlantic	0.870
Canada vs. Greenland	0.872		
Eastern North Atlantic vs. Greenland	0.916–0.943		
Within Eastern North Atlantic	0–0.42	Within Eastern North Atlantic	0.039
<hr/>			
Comparison	$F_{ST}$	Comparison	$F_{ST}$ from Pálsson <i>et al.</i> (2014)
Canada vs. Eastern North Atlantic	0.36–0.42	Canada vs. Greenland vs. Eastern North Atlantic	0.273
Canada vs. Greenland	0.36		
Eastern North Atlantic vs. Greenland	0.39–0.45		
Within Eastern North Atlantic	0.008–0.159	Within Eastern North Atlantic	0.096

Both the haplotype frequencies ( $F_{ST}$ ) and the genetic distances between sequences ( $\Phi_{ST}$ ) are tested with 1000 permutations. Values based on *COI* and 16S rRNA from Pálsson *et al.* (2014) are included for comparison.

each of the Greenlandic sites was 20.9 times and the average of pairwise comparisons between Greenland vs. Canada was just below the ten times threshold average (9.5 times).

The genetic distances between each of the Canada, Greenland and Eastern North Atlantic populations were similar to or greater than between several other *Buccinum* species (Supporting Information, Fig. S2). Six of the 136 pairwise comparisons (between the 17 species) had smaller genetic distances than between Greenlandic and Canadian *B. undatum*, eight comparisons were less than between Eastern North Atlantic and Canadian *B. undatum*, and 11 comparisons were less than between Eastern North Atlantic and Greenlandic *B. undatum*.

## DISCUSSION

A clear genetic split between Eastern North Atlantic, Greenlandic and Canadian *B. undatum* populations is evident based on *COI* differentiation, confirming previous results (Pálsson *et al.*, 2014). The larger mtDNA region (*COI-2*) from both Iceland and the newly sampled geographical regions confirms the previous split between the continents. The large divergence between the continents is further supported by molecular species indices, with the ratio of divergence between and within all three populations exceeding the SSTI that has been proposed for *COI* by Witt *et al.* (2006) and the groups suggested by the ABGD. These results support cryptic species or clear evolutionary divergence among the populations caused by allopatric separation of whelk populations on either side of the North Atlantic.

In marine environments, gene flow may be restricted by transient or consistent allopatry (Mayr, 1999), non-planktonic development and/or ecological divergence (Krug, 2011). In the case of *B. undatum*, internal fertilization, direct development and a relatively sedentary adult lifestyle contribute to limited demographic connectivity among populations that are also separated by great distances. Low or zero connectivity is reflected in the phylogenetic analysis of *COI* variation from trans-North Atlantic populations and supported by the SSTI and the ABGD. These results reaffirm our previous analysis of mtDNA (including the erroneous uncharacterized '16S rRNA' marker) and microsatellite differentiation (Pálsson *et al.*, 2014), in addition to results published by Mariani (2012) and Weetman (2006), in which analysis of microsatellite variation showed that genetic differentiation follows an isolation-by-distance model over shorter geographical distances.

Zooarchaeological information confirms that *B. undatum* was found on the Eastern US coast in the middle Pliocene (3.1–3.7 Mya) (Campbell, 1993) and

in the UK during the Miocene and Pliocene (Wood, 1848). Colder ocean temperatures and shifting ocean levels during the Pleistocene glaciation (2.58 Mya) might have caused the separation of *B. undatum* populations throughout the North Atlantic, resulting in the allopatric divergence of the two larger clades on each side of the North Atlantic during, or even before, the onset of glaciation. The isolation might also have occurred as a consequence of colonization of southerly regions on both sides of the Atlantic, possibly enforced by colder climate, as is the case for other marine organisms, e.g. some starfish species (*Asterias rubens* and *Asterias forbesi*; Wares & Cunningham, 2001). The later divergence between the Greenlandic and Canadian clades might reflect secondary contact during oscillating glacial periods of the Pleistocene epoch. Likewise, divergence between clades might have resulted from genetic differentiation within populations as a function of sample collection depth, because the depth of the samples from the shallow area in the Gulf of St Lawrence and the deep Greenland sites differs by ~300 m.

The high cophenetic correlation between the unknown '16S rRNA' and the *COI* data from *B. undatum* indicates that this part of DNA co-evolved with the mtDNA and might be maternally transmitted. We did not observe any signs of diploidy or recombination between the *COI* and the formerly assigned 16S rRNA, as one might expect if it presents a nuclear mtDNA segment (Lopez *et al.*, 1994). Whether it is an 16S rRNA from an endoparasite, such as the haploid stage of an apicomplexan species (e.g. *Piridium sociabile*; Patten, 1936), or bacteria associated with the foot of the whelk, where the DNA samples were taken from (Pálsson *et al.*, 2014), needs further studies.

In shelled marine gastropods, species delimitation is largely based on shell morphology (Schander & Sundberg, 2001, Wagner, 2001, Allmon & Smith, 2011), which has led to some taxonomic confusion, particularly for genera such as *Buccinum*, which exhibit great phenotypic variability in shell characteristics (Shirai *et al.*, 2010). When individuals of one morphologically defined species are found to be genetically divergent, it could indicate the occurrence of cryptic species (Allmon & Smith, 2011). In this case, genetic distances between populations of *B. undatum* from Canada, Greenland and the Eastern North Atlantic were similar to or greater than distances observed between several other *Buccinum* species from the North Pacific and the North Atlantic oceans (Supporting Information, Fig. S2).

In the present study, we revise the mtDNA-based phylogeny of *B. undatum* and gain additional information on its species status by using two species indices. The results from these analyses warrant more detailed analysis of the taxonomic status of the *B. undatum* populations with respect to morphology and other genetic markers. These questions are

addressed in our ongoing work. Morphological divergence of *B. undatum* across the North Atlantic in comparison with genetic patterns has been studied in a recently submitted paper (Magnúsdóttir H, Pálsson S, Westfall KM, Jónsson ZO, Örnólfsdóttir EB, unpublished observations), and studies of the morphological divergence of juvenile whelks raised in a common garden experiment (Magnúsdóttir H, Pálsson S, Westfall KM, Jónsson ZO, Örnólfsdóttir EB, unpublished observations) and the RADseq-based population structure of *B. undatum* across the North Atlantic (Goodall J, Westfall KM, Magnúsdóttir H, Jónsson ZO, Pálsson S, Örnólfsdóttir EB, unpublished observations) are also underway.

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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.** Unique *COI* haplotypes of *Buccinum undatum* recovered in the study. The *COI* sequence is consistent with sites 63–696 in the *COI* sequence of *B. undatum* (Jónsson *et al.*, 2019).

**Table S2.** Locations of *COI* haplotypes recovered in the study. For details of sample locations, see Table 1.

**Table S3.** Homologous *COI* sequences from species within the *Buccinum* genus were obtained from GenBank and BOLD. Species, accession numbers and references, if published, are given.

**Figure S1.** Phylogenies of *COI-1* mtDNA (A) and of the incorrectly assigned mtDNA 16S rRNA sequence genotypes in *Buccinum undatum* (B), presented as a single tree by Pálsson *et al.* (2014). Abbreviations: C, Canada; E, England; F, The Faroe Islands; G, Greenland; I, Iceland. Numbers after hyphen refer to numbers of individuals. Cophenetic correlation of the two phylogenies was 0.91,  $P = 0.001$ . For detailed site information, see Table 1.

**Figure S2.** Species tree based on *COI-1* for *Buccinum undatum* populations in Western North Atlantic (Canada and Greenland) and Eastern North Atlantic and several species within the *Buccinum* genus from the North Atlantic and Pacific Oceans. The tree is based on a 386 bp alignment. See Supporting Information, Table S3 for references and accession numbers.