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Secondary contacts and genetic admixture shape colonization by an amphiatlantic epibenthic invertebrate

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Abstract

Research on the genetics of invasive species often focuses on patterns of genetic diversity and population structure within the introduced range. However, a growing body of literature is demonstrating the need to study how native genotypes affect both ecological and evolutionary mechanisms within the introduced range. Here, we used genotyping-by-sequencing to study both native and introduced ranges of the amphiatlantic marine invertebrate *Ciona intestinalis*. A previous study using microsatellites analysed samples collected along the Swedish west coast and showed the presence of genetically distinct lineages in deep and shallow waters. Using 1,653 single nucleotide polymorphisms (SNPs) from newly collected samples (285 individuals), we first confirmed the presence of this depth-defined genomic divergence along the Swedish coast. We then used approximate Bayesian computation to infer the historical relationship among sites from the North Sea, the English Channel and the northwest Atlantic and found evidence of ancestral divergence between individuals from deep waters off Sweden and individuals from the English Channel. This divergence was followed by a secondary contact that led to a genetic admixture between the ancestral populations (i.e., deep Sweden and English Channel), which originated the genotypes found in shallow Sweden. We then revealed that the colonization of *C. intestinalis* in the northwest Atlantic was as a result of an admixture between shallow Sweden and the English Channel genotypes across the introduced range. Our results showed the presence of both past and recent genetic admixture events that together may have promoted the successful colonizations of *C. intestinalis*. Our study suggests that secondary contacts potentially reshape the evolutionary trajectories of invasive species through the promotion of intraspecific hybridization and by altering both colonization patterns and their ecological effects in the introduced range.

KEYWORDS
ascidians, gene flow, high-throughput sequencing, introduced species, introgression, population connectivity

1 | INTRODUCTION

It is well established that attributes of nonindigenous species (NIS) such as genetic diversity (Dupont, Jollivet, & Viard, 2003), founder group size (Lockwood, Cassey, & Blackburn, 2005), inbreeding depression (Roman & Darling, 2007) and genetic admixture (Verhoeven, Macel, Wolfe, & Biere, 2011) influence their colonization success. These attributes are not mutually exclusive and often combine to allow or deter species introductions (Rius, Turon, Bernardi, Volckaert, & Viard, 2015). In addition, genetic data are critical for (a) reconstructing invasion routes, (b) identifying the source population(s) and (c) understanding how anthropogenic factors affect colonization success (Cristescu, 2015; Estoup & Guillemaud, 2010). Despite a great deal of recent research on invasion genetics (Bock et al., 2015; Bourne, Hudson, Holman, & Rius, 2018; Rius et al., 2015), there remains a dearth of studies investigating how genetic patterns in the native range influence the introduced range.

Biological invasions act as unique experiments in evolution (Yoshida, Goka, Ishihama, Ishihara, & Kudo, 2007), allowing observations of how NIS spread and adapt to novel environments on a human timescale. The genetic study of NIS furthers our understanding on how contemporary gene flow and local adaptation contribute to colonization success (Verhoeven et al., 2011). In addition, studies of NIS have shown that genetic admixture of divergent lineages can affect fitness of colonizing populations through transgressive segregation (Johansen-Morris & Latta, 2006; Wagner, Ochocki, Crawford, Compagnoni, & Miller, 2017), by masking deleterious mutations (Keller & Waller, 2002), and/or by increasing standing genetic variation on which selection can act (Rius & Darling, 2014). Genetic admixture can also disrupt locally adapted gene pools, which may negatively affect colonization success (Gilk et al., 2004). Therefore, understanding how ecological and evolutionary mechanisms influence colonization success is key for unravelling how genetic patterns found in native and introduced ranges relate. Research progress on the evolutionary effects of NIS has largely been dominated by studies conducted in terrestrial ecosystems (Abbott, 1992; Rius & Darling, 2014), with considerably less effort being devoted to study aquatic organisms.

Ascidians (Chordata, Tunicata, Ascidiacea) are marine sessile invertebrates that are notoriously invasive (Lambert & Lambert, 1998) and frequently foul aquaculture facilities (Fitridge, Dempster, Guenther, & de Nys, 2012; Rius, Heasman, & McQuaid, 2011) and marine infrastructures (Johnston, Dafforn, Clark, Rius, & Floerl, 2017). The early life-history stages of ascidians are ephemeral and represent the only dispersive stages of their life cycle (Millar, 1971), offering only highly restricted natural dispersal. Thus, long-distance dispersal of ascidians is attributed to artificial transport (Hudson, Viard, Roby, & Rius, 2016) or rare rafting events (Carlton et al., 2017). As such, they are relevant and unique models for studying colonization success in marine ecosystems (Zhan, Briski, Bock, Ghabooli, & MacIsaac, 2015). *Ciona intestinalis* is a solitary ascidian with a disjunct amphiatlantic (i.e., inhabiting both sides of the Atlantic) distribution throughout the North Atlantic Ocean (Bouchemousse, Bishop, & Viard, 2016a). It is generally accepted that the northeast

Atlantic coastline is its native range (Bellas, Beiras, & Vázquez, 2003; Bouchemousse, Bishop, et al., 2016a; Gulliksen & Skjæveland, 1973; Nydam et al., 2017), while the introduced range includes the northwest Atlantic coastline (Bouchemousse, Bishop, et al., 2016a; Nydam & Harrison, 2007). As with all solitary ascidians, *C. intestinalis* is hermaphroditic and reproduces through broadcast spawning, with external fertilization. The short-lived pelagic larval stage normally lasts <24 hr, though this stage can be extended to five days (Petersen & Svane, 1995). Larvae of *C. intestinalis* are often retained close to the adults and the production of adhesive mucus strings together with the eggs (Svane & Havenhand, 1993) may result in lower dispersal potential. Consequently, transcontinental dispersal of *C. intestinalis* is attributed to anthropogenic transport or rafting events of individuals only. *Ciona intestinalis* shows a high affinity for marine infrastructures (e.g., pontoons and ropes in harbours and marinas), which are known to concentrate NIS (Aldred & Clare, 2014). This propensity to foul can lead to negative economic and ecological impacts when this species is found in aquaculture facilities (Fitridge et al., 2012; Lutz-Collins, Ramsay, Quijón, & Davidson, 2009; Rius et al., 2011). Consequently, most research studying the extensive distribution of *C. intestinalis* has been performed considering individuals found on artificial structures (e.g., Bouchemousse, Bishop, et al., 2016a; Bouchemousse, Liautard-Haag, Bierne, & Viard, 2016c; Hudson et al., 2016; Zhan, Macisaac, & Cristescu, 2010). This has led to a good understanding of the distribution of *C. intestinalis* on artificial structures, but there is still limited knowledge of the relative importance of natural and artificial habitats for the spread and establishment of this species in new areas.

The west coast of Sweden is a coastline where *C. intestinalis* is present on natural substrata from the surface to depths of more than 100m (Dybern, 1965, 1967; Svane & Havenhand, 1993). There, the opening of the brackish waters of the Baltic Sea to the Atlantic means that individuals inhabiting shallow water experience a wide range of salinities (10–30 PSU) and variable temperatures (~0–20°C, Dybern, 1965; Renborg, Johannesson, & Havenhand, 2014), whereas individuals at depth live in both more constant temperatures and stable, high salinities (~34 PSU). The difference in density between surface and deeper waters leads to a strong pycnocline separating the less saline surface water of the Baltic Sea from the high salinity bottom water (often more than ~10-15 m in depth) from the Atlantic (Johannesson et al., 2018). There are observable differences in the biology and life history of individual *C. intestinalis* found in different depths. For example, individuals inhabiting shallow waters (<15 m) have two generations a year (each spawning period lasting a couple of months) during boreal spring and late summer, whereas deeper individuals (>15 m) have one generation per year, with spawning lasting approximately one month during boreal summer (Dybern, 1965). Additionally, there appears to be slight morphological variation across depths, with shallow individuals being smaller and more heavily pigmented than deeper individuals (Dybern, 1965; Svane, 1983). This may be due to genetically driven phenotypic variation. A recent study using microsatellites showed that the deep and shallow water populations of *C. intestinalis* along the Swedish west coast are genetically differentiated (Johannesson et al., 2018). Strong pycnoclines

can act as distinct barriers to vertical movement of larvae within the water column (e.g., Gallager, Davis, Epstein, Solow, & Beardsley, 1996), and the existence of genetically distinct populations has tentatively been ascribed to the pycnocline present at ~10-15 m acting as a barrier to reproductive exchange. In addition, local adaptation may contribute to the genetic differences between shallow and deep populations as they are exposed to different conditions, including salinity, temperature, food availability and light. Thus, two distinct populations of *C. intestinalis* separated by an abiotic barrier have evolved along the Swedish west coast.

Here, we used *C. intestinalis* as a model organism to investigate how understanding genetic variability in the native range can help elucidate mechanisms shaping both colonization success and introduction pathways in new ranges. The objectives of the study were to (a) identify fine- and broad-scale population genomic patterns of *C. intestinalis*, (b) reveal evolutionary relationships among individuals collected along coastlines across the range of the species, (c) determine the presence or not of genetic admixture and (d) if admixture was present, infer if it could be associated with successful colonization of novel habitats. We hypothesized that the colonization success of *C. intestinalis* across its introduced range has been affected by the historic divergence of ancestral genotypes, the levels of genetic admixture between divergent lineages, and the intensity of gene flow between native and introduced ranges.

2 | MATERIALS AND METHODS

2.1 | Field sampling

Tissue samples of 285 *C. intestinalis* were collected from 20 sites within the putative native and introduced range of the species (Table 1 and Figure 1). Samples from Sweden were collected from shallow natural, deep natural, and shallow artificial substrata (see details in Table 1), whereas sites outside Sweden were all from shallow artificial substrata. Individuals from natural substrata were sampled by either snorkelling, SCUBA diving or a remotely operated underwater vehicle. Artificial substrata were sampled in marinas by pulling up hanging ropes, submerged buoys and checking the undersides of pontoons. We attempted to leave a distance of at least one metre between each sampled individual to limit the chance of collecting closely related individuals. Once collected, tissue was immediately preserved in 95% ethanol which was periodically replaced until tissue pigment no longer leached into the ethanol. Finally, tissue samples were stored at −20°C until DNA extraction.

2.2 | DNA extraction and genotyping

DNA was extracted from preserved tissue using the Qiagen DNeasy® Tissue Kit (Qiagen) according to the manufacturer's protocol. Gel electrophoresis and the QuantiFluor® dsDNA System (Promega) were used to assess quality and quantity of extracted

DNA, respectively. DNA was shipped to the University of Wisconsin Biotechnology Center where it was genotyped using the genotyping-by-sequencing methodology (GBS; Elshire et al., 2011). Briefly, GBS reduces the complexity of the sample genome by digesting the DNA using methylation-sensitive restriction enzymes and sequencing the ends of the digested fragments using barcoded adapter regions.

2.3 | Analysis of genotyping-by-sequencing data

The GBS assembly was performed using ipyrad v. 0.7.28 (Eaton, 2014), a toolbox for assembly and analysis of restriction site-associated DNA sequencing (RAD-seq) type genomic data sets. We followed the seven sequential assembly steps of ipyrad using parameters based on those recommended for single-end GBS data in the ipyrad documentation (http://ipyrad.readthedocs.io/). As the *C. intestinalis* genome is not yet available, we used the de novo assembly method, which requires no prior genomic resources and used ipyrad to trim Illumina adapter reads. As we were working with only one species, we set the level of sequence similarity for clustering to be 90% (I. Overcast, pers. comm.). Following the iterative filtering framework outlined by O'Leary, Puritz, Willis, Hollenbeck, and Portnoy (2018), we used vcftools v.0.1.13 (Danecek et al., 2011) to first filter for loci with a minimum single nucleotide polymorphism (SNP) call quality of 20, a minimum genotype depth of less than five, and a mean minimum depth (across individuals) of <15. Additionally, we chose to remove loci with a minor allele count of less than three, rather than the commonly used minor allele frequency threshold of 5%, because the latter will remove true rare alleles that are important in elucidating fine-scale structure and accurately drawing inference of past demographic events (O'Connor et al., 2015). We then iteratively increased our stringency for allowing missing data (on both loci and individuals separately), so that our final dataset contained loci with at least 50% call rate (i.e., a locus must be present in at least 50% of individuals), and up to 50% allowed missing data per individual. To remove the confounding effects of linkage disequilibrium, we used vcftools to thin markers so that only one SNP per locus was retained in our dataset.

We used BayeScan v.2.0 (Foll & Gaggiotti, 2008) to ensure our dataset contained only putatively neutral loci. BayeScan uses differences in allele frequency between populations to identify candidate loci under natural selection (Foll & Gaggiotti, 2008) and was run using a thinning interval size of 20, with 25 pilot runs of length 10,000 and a burn-in length of 50,000. Prior odds for the neutral model were set to 100 rather than the default 10, to reduce the number of false positives in large datasets (>1,000 SNPs). While commonly used, BayeScan has often been shown to report false positives especially in species undergoing range expansions, while also assuming equal population exchange and evolutionary independence among all populations (Bierne, Roze, & Welch, 2013; Whitlock & Lotterhos, 2015). We therefore also assessed for candidate loci using two newer methods, *OutFlank* v.0.2 (Whitlock & Lotterhos, 2015) and pcadapt v.4.1.0 (Luu, Bazin, & Blum, 2017). Similarly to BayeScan, *OutFlank* groups

TABLE 1 Sampling information for *Ciona intestinalis*

Country	Site name	Code	Latitude (N)	Longitude (E or W)	Depth (category)	Substratum	No. of individuals sequenced	F_{IS}	H_E
Sweden	Vattenholmen	VAT	58.87°	11.09°	60 m (Deep)	Natural	16	0.065	0.218
	Gåseklåvan	GUL	58.31°	11.54°	20–25 m (Deep)	Natural	15	*0.121*	0.267
	Jämningarna	JAM_D	58.26°	11.39°	17–20 m (Deep)	Natural	8	0.086	0.286
	Kåvra	KAV	58.33°	11.36°	18–22 m (Deep)	Natural	16	*0.113*	0.230
	Burholmen	BUH	58.89°	11.13°	5 m (Shallow)	Natural	16	*0.139*	0.202
	South Koster	KOS	58.88°	11.05°	3–4 m (Shallow)	Natural	15	0.078	0.232
	Brattskär	BRA	58.86°	11.07°	1–4 m (Shallow)	Artificial	15	*0.078*	0.238
	Lindholmen	LIN	58.88°	11.15°	0–1 m (Shallow)	Artificial	14	0.088	0.242
	Porsholmen	POR	58.23°	11.40°	2–4 m (Shallow)	Natural	15	0.056	0.233
	Jämningarna	JAM_S	58.26°	11.39°	5–7 m (Shallow)	Natural	16	0.028	0.206
	Fiskebäckskil	FIS	58.24°	11.46°	0.5–2 m (Shallow)	Artificial	15	0.073	0.241
Denmark	Limfjord	DEN	56.78°	9.18°	0.5–2 m (Shallow)	Artificial	20	*0.095*	0.224
England	Hartlepool	HPL	54.69°	−1.20°	0.5–2 m (Shallow)	Artificial	18	*0.089*	0.227
	Town Quay	TNQ	50.89°	−1.41°	0.5–2 m (Shallow)	Artificial	12	*0.121*	0.231
Jersey	St. Helier	JER	49.18°	−2.12°	0.5–2 m (Shallow)	Artificial	15	*0.082*	0.217
France	St. Malo	STM	48.64°	−2.03°	0.5–2 m (Shallow)	Artificial	14	*0.117*	0.232
Canada	Yarmouth	YAM	43.83°	−66.13°	0.5–2 m (Shallow)	Artificial	12	0.069[a]	0.229[a]
	Shelburne	SB	43.76°	−65.32°	0.5–2 m (Shallow)	Artificial	2		
	Brudenell River	BR	46.20°	−62.59°	0.5–2 m (Shallow)	Artificial	9	0.017[b]	0.257[b]
	Sydney	*SD*	46.14°	−60.19°	0.5–2 m (Shallow)	Artificial	2		
							265		

Note: The table includes geographical region, site name abbreviation (Code), coordinates of sampling sites, depth (shallow [<15 m] or deep [>30 m]), substratum collected from and the number of individuals used in genomic analyses. Additionally included are inbreeding coefficient F_{IS} values (values in italics are statistically significant [$p < .05$]) and population mean expected heterozygosity (H_E).

[a]Refers to merged samples known as CAN_1 (Yarmouth and Shelburne).

[b]Refers to merged samples known as CAN_2 (Brudenell River and Sydney).

individuals into predefined populations, before inferring candidate loci based on a trimmed distribution of F_{ST} values for loci deemed to be neutral. As reported in similar studies (see results and Guzinski, Ballenghien, Daguin-Thiebaut, Leveque, & Viard, 2018), *OutFlank* did not recover any F_{ST} outlier loci, so we continued our analyses with other software. Regarding the pcadapt, it ascertains population structure using principal component analysis (PCA) to find candidate loci excessively related to population structure. We classified loci that were recovered by both BayeScan and pcadapt as putatively under natural selection and removed them for the following analyses. Finally, we created a more conservative dataset that excluded all loci recovered by BayeScan and pcadapt and ran the whole set of analyses again (see Appendix A within Supporting Information).

2.4 | Population structure

We used the software ADMIXTURE v.1.3 (Alexander, Novembre, & Lange, 2009) to estimate the likelihood that an individual comes from one of a certain number of putative sample populations (K). Like STRUCTURE v.2.3.4 (Pritchard, Stephens, & Donnelly, 2000), ADMIXTURE uses a maximum-likelihood estimation from multilocus SNP genotype datasets, but calculates estimates using a faster numerical optimization algorithm. We performed a discriminant analysis of principal components (DAPC) to visualize between-population genomic variation (Jombart, Devillard, & Balloux, 2010). DAPC transforms the data using PCA before using PCA factors as variables for a discriminant analysis (DA), ultimately maximizing the differences among groups while minimizing variation within groups (Jombart et al., 2010). We used the package *adegenet* v.2.1.1 (Jombart, 2008) for R (R Development Core Team, 2017) to perform the DAPC. We ran the DAPC with and without a priori knowledge of individual populations. We examined pairwise population genetic differentiation using F_{ST} values and their *p* values by running 10,000 permutations with Arlequin v.3.5 (Excoffier & Lischer, 2010). We also used Arlequin to measure the inbreeding coefficient F_{IS} and expected heterozygosity (H_E) per population. Finally, we ran an analysis of molecular variance (AMOVA) test using site clusters as inferred by ADMIXTURE and
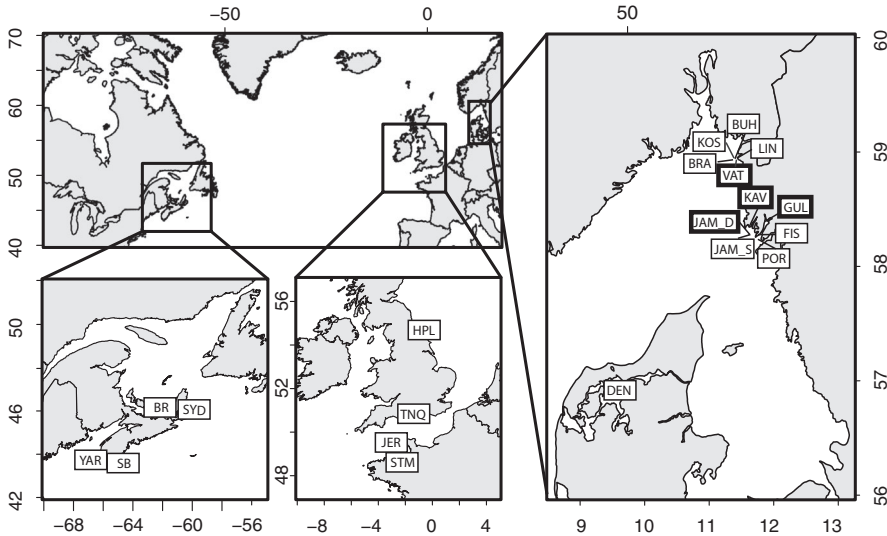
FIGURE 1 North Atlantic coastlines where the samples of *Ciona intestinalis* were collected. Sampling site names are abbreviated as in Table 1, with bold quadrats around site codes representing deep sampling sites. The putative native range in the literature includes Scandinavia, the British Isles and the English Channel, whereas the introduced range includes the northwest Atlantic

DAPC plots and also using only shallow Sweden sites to test whether there was an effect of substratum (natural vs. artificial). AMOVAs were performed in Arlequin v.3.5. (Excoffier & Lischer, 2010).

2.5 | Reconstructing invasion routes

To obtain relevant and detailed information and infer the historical relationship among genotypes of *C. intestinalis* throughout its range, we analysed sets of evolutionary scenarios with the approximate Bayesian computation (ABC) method using DIYABC v.2.0.1 (Cornuet et al., 2014). We grouped sites based on their geographical location and the results of above population structure analyses (shallow Sweden sites plus the Denmark site, deep Sweden sites, England, Jersey and France sites, and Canada sites). The high shipping traffic between the native and introduced ranges (Kaluza, Kölzsch, Gastner, & Blasius, 2010), coupled with the similarity in genetic diversity across our sampled sites (see Results), meant we did not consider the presence of genetic bottlenecks while designing the evolutionary scenarios. Our first two sets of scenarios aimed to infer the evolutionary history within the northeast Atlantic (see details of scenario sets 1 and 2 in Figures S3a,b, respectively). Following the results of this initial analysis, we then added in data from Canada sites to infer the colonization history along the introduced range (scenario set 3, Figure S3c). As specific population sizes, divergence times and potential admixture rates were unknown, we used a uniform distribution with a large interval (population sizes and divergence times: $10–10^7$; admixture rates: 0.001–0.999; Table S5) when setting priors for each parameter (White, Reyes-Betancort, Chapman, & Carine, 2018). We used the mean genic diversity, mean distribution of F_{ST} distances, mean distribution of Nei distances, and whenever an admixture event was included in the scenario, mean admixture estimates for summary statistics. For all scenarios, we used the default 10^6 simulated data per scenario to build reference tables. Upon creation of the reference table, we pre-evaluated scenarios and prior distributions by performing a PCA in the space of the summary

statistics on 1,000 simulated datasets for each scenario and adding the observed dataset to each plane (Cornuet et al., 2014). We used a logistic regression on the 1% simulated datasets that were closest to the observed dataset (using Euclidean distances between simulated and observed datasets) to calculate the posterior probability of each scenario. This approach produces 95% confidence intervals for each scenario's posterior probability, with the most likely scenario defined as the highest estimate without overlapping confidence intervals (Cornuet et al., 2008). For the most probable scenario of scenario set 3 (Figure S3c), we calculated type I (the probability with which this scenario is rejected although it is the true scenario) and type II (the probability of choosing this scenario when simulating data according to other scenarios) error rates. Finally, we assessed the goodness of fit for the final chosen scenario by implementing the model checking feature of DIYABC. We simulated 1,000 datasets using posterior distribution values and compared these with the observed dataset by considering different summary statistics than were used during the generation of the reference table, and visualized this using a PCA (Cornuet et al., 2014).

3 | RESULTS

3.1 | Loci assembly and detection of outlier loci

GBS generated a total of 530,157,826 raw reads, with an average of 2,000,596 reads per sample. After filtering and clustering using ipyrad and vcftools, we retained a total of 1,667 putatively unlinked SNPs in the sequence assembly. Twenty individuals were removed from the dataset due to missing data (i.e., >50% missing data), which was likely caused by poor DNA quality or secondary contaminants within the samples (Federman, Donoghue, Daly, & Eaton, 2018). This led to a final dataset of 265 individuals from 20 separate sampling sites. However, the Canadian sites were merged as CAN_1 (sites Yarmouth and Shelburne) and CAN_2 (Brudenell River and Sydney) due to the limited number of individuals obtained

from Shelburne and Sydney. Therefore, the total final number of sites was 18. BayeScan and pcadapt recovered a total of 30 and 61 F_{ST} outlier loci, respectively, of which 14 were found by both software, whereas *OutFlank* recovered no putative loci under selection. We subsequently removed the 14 loci found in both BayeScan and pcadapt from our analyses, leaving a dataset of 1,653 SNPs. We also performed all analyses on a new dataset that excluded all F_{ST} outlier loci recovered, irrespective of program used (77 loci in total, see Appendix A within the Supporting Information for details).

3.2 | Heterozygosity and population structure

Values of F_{IS} ranged from 0.017 to 0.139 (Table 1). Nine sites showed no signs of deviation from Hardy–Weinberg equilibrium, but nine of the sites exhibited significant positive F_{IS} values indicating a deficiency of heterozygotes in these sites (Table 1). Expected heterozygosity ranged from 0.202 to 0.286 (Table 1), with no noticeable differences in genetic diversity between geographical regions (Table 1).

The combination of ADMIXTURE, DAPC and pairwise site comparisons of F_{ST} allowed us to identify fine- and broad-scale population genomic patterns. Cross-validation by ADMIXTURE inferred the most likely number of sampled populations was $K = 4$ (Figure S1) and broadly indicated the structuring of deep Sweden sites (green in Figure 2), shallow Sweden sites (orange) and those found in England, Jersey and France (blue). Individuals from Canada appeared to have a genetic background similar to both individuals found in England, France, Jersey and individuals from shallow Sweden. The Denmark samples clustered with a shallow Sweden site (shallow Jämningarna, purple cluster), and eight individuals from the shallow Sweden site Burholmen were grouped with samples from deep Sweden.

The ADMIXTURE patterns were supported by the DAPC analysis with and without prior sample assignment (Figure 3a,b), which recovered three genetic clusters, one of which (cluster 3) included the shallow Sweden sites (except the eight individuals from BUH) and sites from Denmark and Canada, and the other two clusters including deep Sweden sites and sites from England, Jersey and France, respectively.

Pairwise comparisons of F_{ST} suggested very strong genetic structuring among most sites (Figure 4), with 143 out of 153 comparisons (93%) being significant, including clear structuring between shallow and deep Sweden sites (Figure 4, Table S1). Notably, there was significant genetic differentiation among the deep Sweden sites with the exception of two deep sites (Kåvra vs. deep Jämningarna) that are very close to one another. Pairwise site comparisons among shallow Sweden sites found 15 of 21 comparisons (71%) were significant, while comparison of the two Canadian sites provided a low, but significant, F_{ST} value (Figure 4, Table S1).

The AMOVA test using site clusters as inferred by ADMIXTURE and DAPC plots showed that genetic differentiation was significant among groups, among sites within groups and within sites (Tables S2,S3). The AMOVA test performed using only shallow Sweden sites to test whether there was an effect of substratum (natural vs. artificial) found no significant genetic differentiation between these two groups (Table S4).

3.3 | Reconstructing invasion routes

For all of our ABC analyses, our check of priors showed a good match between simulate datasets and the observed data (Figure S2). We firstly found that within our northeast Atlantic sampling sites, the ancestral population diverged and formed the deep Sweden and England, Jersey, France groups (Figure S3a), with the logistic estimate of posterior probability for this scenario being $p = .996$ (CI = 0.994, 0.997; Table S6). For our next set of scenarios, which assessed the origin of the shallow Sweden group (Figure S3b), we found the scenario with the highest support being an admixture event between deep Sweden and England, Jersey, and France groups ($p = .998$, CI = 0.998–0.999; Table S6) following secondary overlap of the two lineages. Our final set of scenarios, which assessed the scenario that best explains the colonization of the introduced range (Figure S3c), found that the most likely was a recent admixture between shallow Sweden sites and English, Jersey and France sites ($p = .841$. CI = 0.832, 0.851; Table S6; Figure 5). The type I error rate was 0.15, showing that 85% of our datasets simulated with the highest supported scenario (Figure 5) were correctly identified as being produced by the same scenario. Moreover, type II error rate was on average 0.04. Our model checking procedure for the most likely scenario found that for the 57 summary statistics used for model checking, 23 different significantly from the simulated distribution (Table S7, Figure S4), suggesting that even though this is the most strongly supported scenario, there is some discordance between the scenario posterior combinations and the observed dataset.

4 | DISCUSSION

Our results showed high levels of genomic differentiation between the main regions of the northeast Atlantic (i.e., English Channel and North Sea) and identified the presence of historical genetic admixture among individuals from these regions. This seems to have resulted in genotypically and phenotypically distinctive individuals that are currently found in shallow sites in Sweden. In addition, we revealed genomic patterns suggesting secondary contacts and postulate that this may have promoted intraspecific hybridization. Our result supported the presence of genetic admixture during the spread to and colonization of the northwest Atlantic. More specifically, we found evidence of genetic admixture between genotypes from the English Chanel and genotypes from the shallow North Sea. While we found here no direct evidence that intraspecific hybridization influences colonization, our results indicate that this may be a possible mechanism promoting successful colonization of sites with new environmental conditions, such as trans-oceanic introductions.
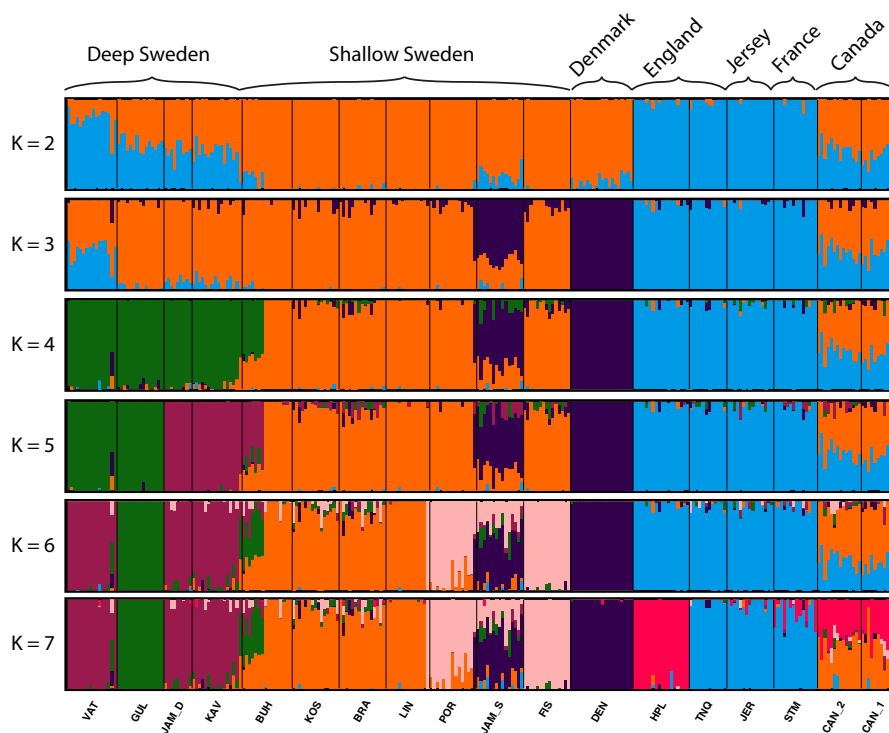
FIGURE 2 ADMIXTURE plots representing all sampled populations of *Ciona intestinalis*. The main regions are highlighted above. The different colours represent putative genetic clusters with *K* ranging from 2 to 7, with *K* = 4 being found to be the most optimal value (Figure S1). Sampling site names are abbreviated as in Table 1

This builds on a growing number of studies showing that the mixing of divergent genotypes as a result of human mediated transport of species has the potential to fundamentally alter colonization patterns and to unprecedentedly alter ecological and evolutionary patterns (Bouchemousse, Liautard-Haag, et al., 2016c; Mooney & Cleland, 2001; Pineda, López-Legentil, & Turon, 2011).

The presence of high genetic subdivision among genotypes found in deep sites off Sweden and in England, Jersey and France suggests that individuals found in these sites represent native populations (Figure 3). This is supported by the ABC analyses, which indicated an initial divergence between these two groups (Figure 5). This accords with the expectation that native ranges will show a highly defined population structure, often involving two main groups of ancestral genotypes (Boubou et al., 2012; Reusch, Bolte, Sparwel, Moss, & Javidpour, 2010; Rius, Clusella-Trullas, et al., 2014a). Divergence of these *C. intestinalis* populations may reflect adaptation to differing local conditions and/or earlier periods of allopatric isolation leading to the generation of genetic divergence through selection or genetic drift. Previous research has shown that high plasticity in *C. intestinalis* allows acclimatization of deep water individuals to shallow water salinities (Renborg et al., 2014), which suggests that local adaptation and primary divergence are less likely. Rather, it seems more likely that during the last glacial maximum deep sites off Sweden and sites in England, Jersey and France were isolated as separate glacial refugia, leading to the divergence that we see today. ABC analyses suggest that secondary contacts leading to genetic admixture between the England, Jersey, France and the deep Sweden genotypes formed the genotypes found in shallow Sweden and Denmark. Thus, either historic artificial transport or postglacial expansion may have promoted such secondary contacts, as reported

for other marine invertebrates (Pérez, Nirchio, Alfonsi, & Muñoz, 2006). A similar situation has been identified with the ascidian *Pyura chilensis* in the southwest Pacific Ocean, where historical divergence of *P. chilensis* populations occurred due to isolation associated with glacial periods. This was followed by secondary contacts and genetic admixture between these previously isolated populations (Haye & Muñoz-Herrera, 2013). Our results suggest that genetic admixture may have had fitness effects that enabled *C. intestinalis* to expand to previously uninhabitable substrata and conditions within its native range. The ability of individuals from shallow Sweden populations to survive relatively high temporal variability in environmental conditions such as temperature and salinity compared to individuals found in England, Jersey, France and deep Sweden may be explained by the fitness benefits of genetic admixture (Wagner et al., 2017), allowing survival in the face of strong selective pressures (Verhoeven et al., 2011).

In contrast to the Sweden populations, individuals from England, Jersey and France formed a relatively homogeneous genetic cluster in both ADMIXTURE and DAPC analyses (Figures 2–4). Earlier work showed that samples from these locations were subdivided into two genetic groups (Hudson et al., 2016), but this differentiation was weaker than what we found between samples from deep Sweden and those from England, Jersey and France. The native range of *C. intestinalis* has been previously described as the northeast Atlantic (e.g., Bouchemousse, Bishop, et al., 2016a; Hudson et al., 2016), and here, we show that this range comprises most of the genomic differentiation among populations, with more complex demographic histories among populations along the northeast coast of the Atlantic than the northwest coast. In line with previous studies that identified admixture within native ranges (Gillis, Walters, Fernandes, & Hoffman,

FIGURE 3 (a) Discriminant analysis of principal components using unlinked loci with no a priori population information. The first axis explains 58.2% of the variation, and the second axis explains 41.8%. (b) Discriminant analysis of principal components using unlinked loci with a priori population information. The first axis explains 23.1% of total variation, and the second axis explains 18.1%. Sampling site names are abbreviated as in Table 1. Sites in (a) are assigned to clusters as follows; Cluster 1: FIS, KOS, BRA, LIN, POR, BUH, JAM_S, DEN, CAN_1, CAN_2, and eight individuals from BUH; Cluster 2: JER, TNQ, HPL, STM; Cluster 3: VAT, JAM_D, GUL, KAV, and eight individuals from BUH
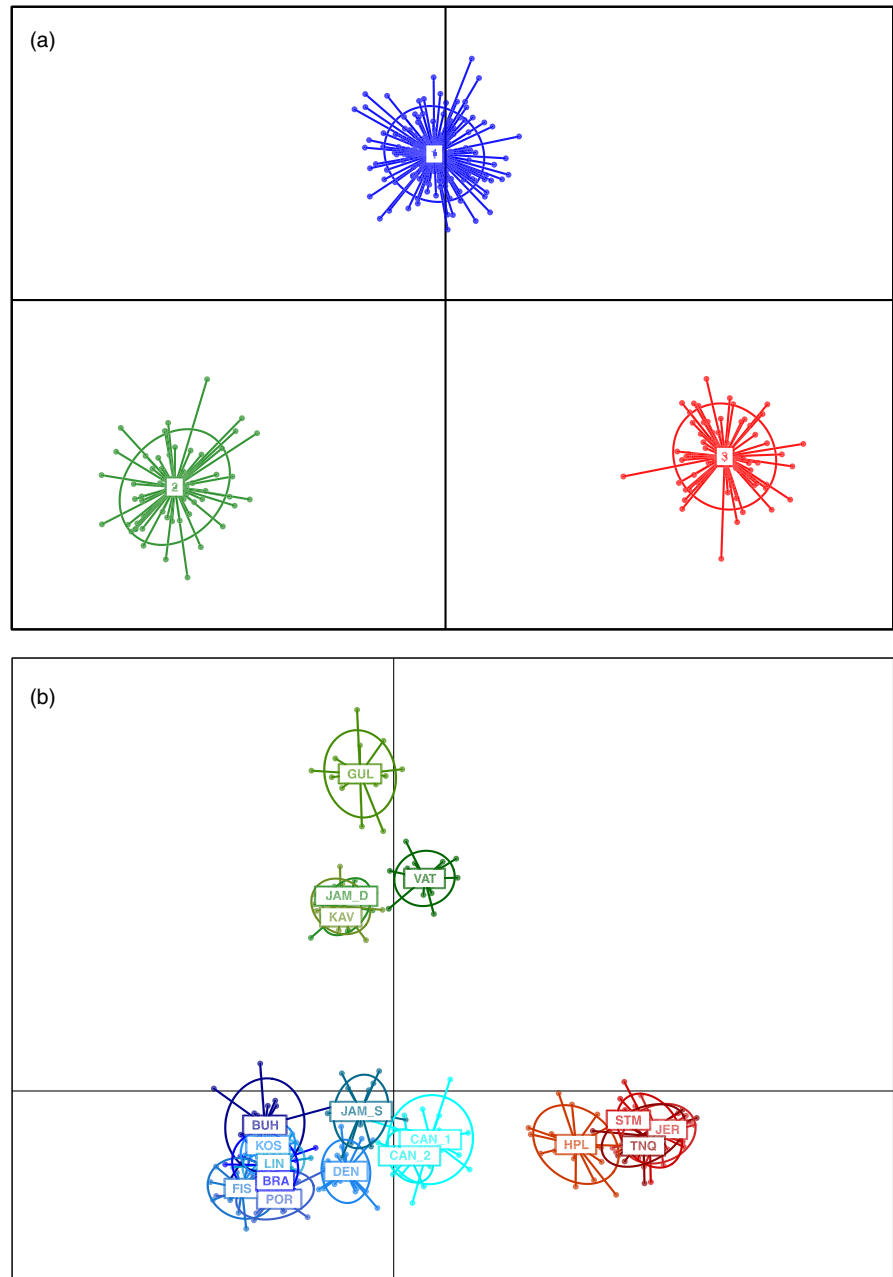
2009; Rius, Turon, Ordóñez, & Pascual, 2012), our findings suggest that historic artificial transport may have facilitated the admixture of the genotypes from deep Sweden and the English Channel.

It is well established that the recent spread of *C. intestinalis* has been promoted by the proliferation of man-made structures along coastlines that act both as stationary substrata and as vectors (Clarke Murray et al., 2014). Throughout the study area, this species is widespread in harbours and marinas in much of its current distribution (Bouchemousse, Bishop, et al., 2016a; Hudson et al., 2016), but rare on natural substrata except along the Swedish coast (Johannesson et al., 2018). This raises the issue of the origin and evolutionary background of the many populations living on artificial substrata that may represent either extensions of large natural populations or completely new introduced populations. In the sampled shallow Sweden sites, comparison of genetic differentiation between natural

and man-made sites showed no significant differences (Table S4), with individuals sampled on artificial substrata being generally more closely related to nearby shallow natural sites than to individuals from other artificial sites along the Swedish coast (Figure 4; Table S1). This suggests that the nature of natural and artificial substrata does not, in itself, create a barrier to local gene flow. In shallow waters off Sweden, the sampled natural substratum included seagrass beds where *C. intestinalis* lives at modest densities attached to blades of *Zostera marina*. Below the pycnocline, at depths of 20 m or more, dense populations occur on the natural vertical rock walls (see also Svane & Havenhand, 1993), whereas in the English Channel and south-western North Sea area, very few individuals have been documented on natural substrata. To our knowledge, there are no reports of *C. intestinalis* inhabiting natural substrata in the English Channel. However, small numbers have been recovered during
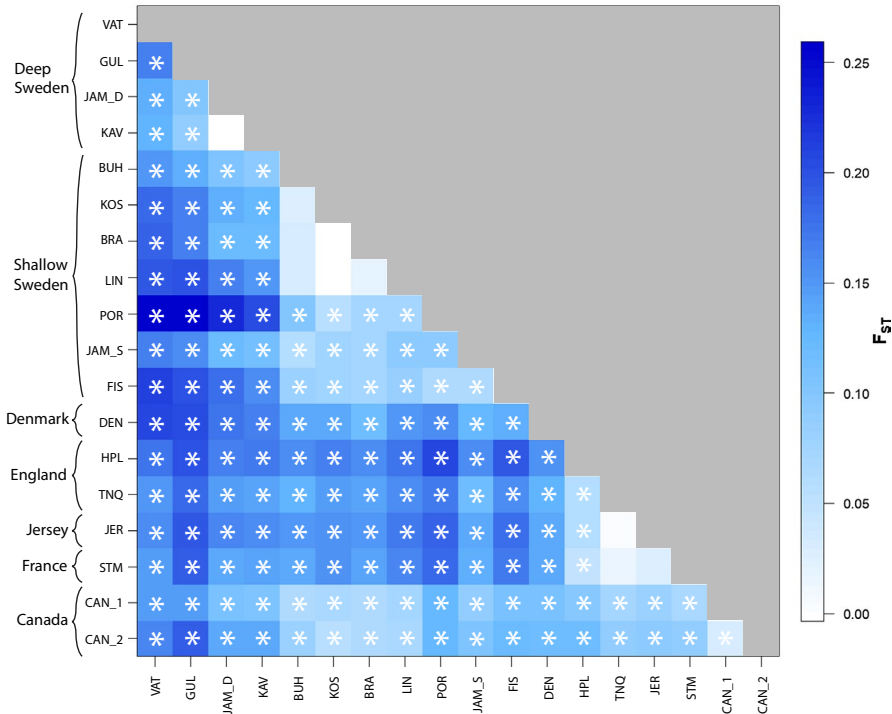
FIGURE 4 Matrix of F_{ST} values. Asterisks represent significant values, after Bonferroni correction. Sampling site names are abbreviated as in Table 1
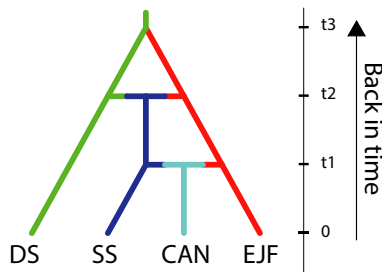

FIGURE 5 The most likely evolutionary scenario involving the sample sites as calculated using DIYABC. The y-axis represents time (not to scale). Included are shallow Sweden sites (SS), Canada sites (CAN), the Denmark site (DEN), England, Jersey, and France sites (EJF), and deep Sweden sites (DS). The full set of scenarios can be found in Figure S3

dredging estuaries in the English Channel (authors pers. obs.) and the south-western North Sea (Rees, Waldock, Matthiessen, & Pendle, 2001). Such low densities may be due to the effects of predation on different life-history stages as seen for closely related species in other parts of the world (Dumont, Gaymer, & Thiel, 2011; Rius, Potter, Aguirre, & Stachowicz, 2014b).

Our study corroborated the findings of Johannesson et al. (2018) by identifying strong genetic differentiation between shallow and deep populations of *C. intestinalis* along the west coast of Sweden. While this genomic differentiation among populations appears surprising as some deep and shallow sites are geographically close to one another, this can be explained due to the effect of the aforementioned pycnocline promoting depth-defined divergence that has also been observed in corals (Prada & Hellberg, 2013). Admixture between deep Sweden and England, Jersey, France genotypes, as indicated by our ABC analysis, suggests that the pycnocline may not

have always been the impenetrable barrier to gene flow as currently observed (Johannesson et al., 2018). Taken together, our results suggest that although historically the pycnocline may have allowed the mixing of divergent genotypes, it currently provides a strong barrier to gene flow, maintaining contemporary genomic differentiation between deep and shallow sites.

The northwest Atlantic range of *C. intestinalis* is restricted to the east coast of North America. It has been documented in eastern Canada since at least the mid-1800s (Carver, Mallet, & Vercaemer, 2006), but its population size and range have only recently expanded (Ramsay, Davidson, Landry, & Arsenault, 2008). Our ABC analyses suggest that the origin of the Canadian sites was due to secondary contact between populations from England, Jersey, France and shallow Sweden. This is supported by ADMIXTURE analysis, which indicates high similarity between the individuals from Canada and the ones from both the English Chanel and shallow sites within the North Sea (Figure 2). In addition, the DAPC analyses indicated that Canadian individuals were similar to individuals found in shallow Swedish waters (Figure 3). This interpretation accords with previous studies showing that multiple introductions facilitate marine biological invasions (Rius et al., 2015; Simon-Bouhet, Garcia-Meunier, & Viard, 2006) and that recurrent introductions of large numbers of individuals explain patterns of genetic diversity within introduced ranges (Uller & Leimu, 2011). Indeed, our results do not show a noticeable change in genetic diversity between Canadian and European sites (Table 1) and are compatible with contemporary introgression among divergent English Channel and North Sea genotypes followed by multiple introductions to Canadian sites.

Heterozygote deficiency at nine of our sites (F_{IS} values significantly greater than zero; Table 1) could reflect either selection against heterozygotes or non-random mating. We can reject

selection against heterozygotes as we excluded loci putatively under selection, and we can also exclude selfing as self-fertilization success is generally low in *C. intestinalis* (Bouchemousse, Lévêque, Dubois, & Viard, 2016b; Byrd & Lambert, 2000). A more likely explanation is a Wahlund effect (Wahlund, 1928), a reduction of expected heterozygosity due to mixing of two genetically differentiated populations, which has been reported in other studies (Dupont, Viard, Dowell, Wood, & Bishop, 2009; Marescaux et al., 2015) including studies of *Ciona* spp. (Hudson et al., 2016; Zhan et al., 2010).

The history of the introduction of *C. intestinalis* to the western Atlantic coast is complex, starting with historical divergence in the native range involving two groups (England, Jersey, France and deep Sweden lineages, Figure 5), which was likely due to genetic drift during a period of isolation (allopatry) in different glacial refugia. More recent historic gene flow between these populations appears to have led to the formation of the admixed genotypes found in shallow Sweden and Denmark sites. Finally, the Canada specimens originated from secondary contacts between individuals from these sites and individuals from the western North Sea and English Channel. Our findings suggest that admixture between genetically diverse native genotypes preceded successful trans-oceanic colonization, in line with previous studies showing that genetic admixture facilitates the colonization of new habitats (Abbott, Barton, & Good, 2016). We suggest that artificial transport of species facilitates secondary contacts and intraspecific admixture among divergent native genotypes, strongly altering NIS evolutionary trajectories and influencing their ecological impacts within the introduced range.

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CONFLICT OF INTEREST

None declared,

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.