

# *Bathynomus giganteus* (Isopoda: Cirolanidae) and the canyon: a population genetics assessment of De Soto Canyon as a glacial refugium for the giant deep-sea isopod

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**Abstract** Population genetics has gained popularity as a method to discover glacial refugia in terrestrial species, but has only recently been applied to the marine realm. The last glacial maxima occurred 20,000 years ago, decreasing sea levels by 120 m and exposing much of the continental shelf in the northern Gulf of Mexico, with the exception of De Soto Canyon (2100 m depth). The goal of this study was to determine whether population dynamics of the giant deep-sea isopod, *Bathynomus giganteus*, were better explained by habitat diversity or by the past presence of a marine glacial refugium in De Soto Canyon. To accomplish this we (1) measured genetic diversity in De Soto Canyon and adjacent regions, (2) characterized gene flow and connectivity between these regions, and (3) investigated historical changes to population size. We sequenced three mitochondrial

loci (12S, 16S, and COI) from 212 individuals and also performed a next-generation sequencing pilot study using double digest Restriction site-Associated DNA sequencing. We found high genetic diversity and connectivity throughout the study regions, migration between all three regions, low population differentiation, and evidence of population expansion. This study suggests that habitat heterogeneity, rather than the presence of a glacial refugium, has had an historical effect on the population dynamics of *B. giganteus*.

**Keywords** Population genetics/genomics · ddRADseq · *Bathynomus giganteus* · Glacial refugia · Deep-sea · De Soto Canyon · Habitat diversity

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## Introduction

In the last three million years, there have been 11 major glaciation events (Richmond & Fullerton, 1986), with the most recent reducing sea levels by 120–125 m 20,000 years ago. Many studies have focused on the impact on terrestrial species and habitats (reviewed in Avise, 1992; Taberlet, 1998; Taberlet et al., 1998; Hewitt, 2004; Provan & Bennett, 2008), including plants (Lewis & Crawford, 1995; Petit, 2003; Beck et al., 2008), fish (Bernatchez & Dodson, 1991; Bernatchez & Wilson, 1998; Nesbø et al., 1999), and insects (Hewitt, 1996; Knowles,

2001; Trewick & Wallis, 2001). Recently, research focus has turned to the identification and impacts of glaciation on population structure and demography of marine species (Provan et al., 2005; Thatje et al., 2005; Maggs et al., 2008; Mäkinen & Merilä, 2008; Palero et al., 2008; Provan & Bennett, 2008; Zemlak et al., 2008; Campo et al., 2009; Médail & Diadema, 2009; García-Merchán et al., 2012; Kearsse et al., 2012; Dömel et al., 2015). These studies frequently uncovered evolutionary impacts of glacial refugia on populations, such as the establishment and reintroduction of unique lineages (Mäkinen & Merilä, 2008; Zemlak et al., 2008), which is similar to terrestrial studies. However, many studies also concluded that the marine environment imposes unique ecological considerations, such as physical oceanographic characteristics that determine the location and suitability of a refugium (Thatje et al., 2005; Médail & Diadema, 2009; Dömel et al., 2015).

The northern Gulf of Mexico was impacted by the last Pleistocene glaciation as sea levels fell by 120–125 m. This left the majority of the Mississippi–Alabama shelf exposed and substantially decreased the depth over the continental slopes (Sager et al., 1992). De Soto Canyon sits just off of the Mississippi–Alabama shelf and served as the northernmost intrusion of marine abyss during this period. While the canyon has measured the depth and maximum width of 2100 and 5000 m, respectively, it is better defined as an embayment as it lacks steep walls—sloping gently to depth with a network of smaller, more traditional canyons branching off of it (Coleman et al., 2014). The role of this canyon as a potential extension of the deeper, central marine refugium in the Gulf of Mexico has not been investigated, although today the minimum depth of the canyon is 100–150 m (Nguyen, 2014). However, if De Soto Canyon maintained a benthic community in the northernmost Gulf of Mexico during the last glaciation, it would have served as a vital source of biodiversity during recolonization and expansion as sea levels rose to interglacial levels.

The Gulf of Mexico is a highly heterogeneous basin in terms of geology and physical geography. The west Florida slope exhibits some complex topographical features and is primarily comprised of carbonate from ancient coral reefs. Moving north, De Soto Canyon is described as a boundary to this slope. West of De Soto Canyon, the Texas/Louisiana shelf is extremely

intricate, containing intermittent banks, four canyon systems, and a number of substrates, including carbonate, clay, silt, and mud from the Mississippi River. Input from the Mississippi River can disperse as far as the west Florida slope before giving way to the carbonate substrate. The Texas/Louisiana shelf/slope region is considered one of the most geologically and geographically complex in the world (Brooke & Schroeder, 2007). Given this high complexity, and the established relationship between deep-sea habitat heterogeneity and high genetic diversity (Levin et al., 2001; Vanreusel et al., 2010), it is also possible that population dynamics in the Gulf of Mexico may be more heavily influenced by the density of microhabitats in regions of the northern Gulf than by the presence of a glacial refugium. However, it is also important to recognize that regions serve as glacial refugia because they are ecologically and historically suited to do so (Médail & Diadema, 2009). Therefore, a region with many environmental factors that promote or maintain genetic diversity may also predispose it to be a successful glacial refugium. Because of the number of variables that can influence genetic diversity (drift, mutation, selection, effective population size, migration, demographic stability over time, etc.) it can be difficult to find patterns through the noise (Taylor & Roterman, 2017), but is still possible with careful attention and proper analyses (Maggs et al., 2008).

*Bathynomus giganteus* A. Milne-Edwards, 1879 is a deep-sea isopod that can reach lengths of over 36 cm with a described range that includes the Atlantic Ocean and Pacific Ocean at depths ranging from 100–2100 m (Poore & Bruce, 2012). This benthic crustacean is primarily a detritivore, although stomach content analysis has indicated facultative carnivory (Chamberlain et al., 1986; Barradas-Ortiz et al., 2003). Reproduction occurs seasonally, primarily in the winter and spring, and development is direct: an adult female develops a pouch where her offspring brood until they emerge as tiny adults (Briones-Fourzan & Lozano-Alvarez, 1991; Barradas-Ortiz et al., 2003). In the marine environment, populations are usually demographically connected by the exchange of planktonic larvae (Grosberg & Cunningham, 2001; Gaines et al., 2007). Phylogeographic and biogeographic barriers to pelagic larval dispersal tend to be centered on regions where currents no longer provide reliable larval transport along the geographic range (Briggs,

1974); however the adult life stage of marine invertebrates has been found to contribute substantially to gene flow, especially when the adult is pelagic (Cowen & Sponaugle, 2009; Havermans et al., 2013) but also in benthic species (Leese et al., 2010). In this, *B. giganteus* may have an advantage over the majority of other deep-sea isopod species: while deep-sea isopods are primarily (if not exclusively) benthic, swimming behavior has been documented in *B. giganteus* (Chamberlain et al., 1986) and personally witnessed by the authors.

While much attention has been paid to the role of surface production (Campbell & Aarup, 1992), particle flux (Sibuet et al., 1989), and benthic biomass (Rowe, 1983) in determining diversity of abyssal marine invertebrates, the historical effects of a glacial refugium or habitat heterogeneity on population dynamics in the northern Gulf of Mexico requires further investigation. The objective of this study is to determine whether population dynamics are better explained by habitat diversity or by the past presence of a marine glacial refugium in De Soto Canyon. We accomplished this using three mitochondrial loci (12S, 16S, and cytochrome oxidase subunit (I)) and over 2000 SNPs discovered through double digest Restriction site-Associated DNA sequencing (ddRADseq; see Online Resource ddRADseq Supplement). Specifically, we (1) quantified genetic diversity in the De Soto Canyon, as well as a region to the east, near the Mississippi River Delta, and a region to the west along the Florida Slope, (2) characterized gene flow and connectivity between these three regions, and (3) investigated historical changes to population size and tested migration models to elucidate population demography over time. If De Soto Canyon served as a glacial refugium, we expect to see high diversity in the canyon and evidence of population expansion in the east and west. If habitat diversity is a primary driver of population dynamics, we expect to see highest diversity west of De Soto Canyon, near the Mississippi River Delta, as this region is one of the most habitat-heterogeneous in the world (Brooke & Schroeder, 2007). Given the wide dispersal of Mississippi River sediment, managing to make it as far as the west Florida slope, we expect to see a similar west-to-east pattern of decreasing diversity if habitat heterogeneity is driving diversity dynamics. Because the sample distribution is relatively small, and even a migration rate of a few individuals per generation is

enough to prevent differentiation (Hartl & Clark, 1997; Taylor & Roterman, 2017), we do not expect to see population divergence between regions.

## Materials and methods

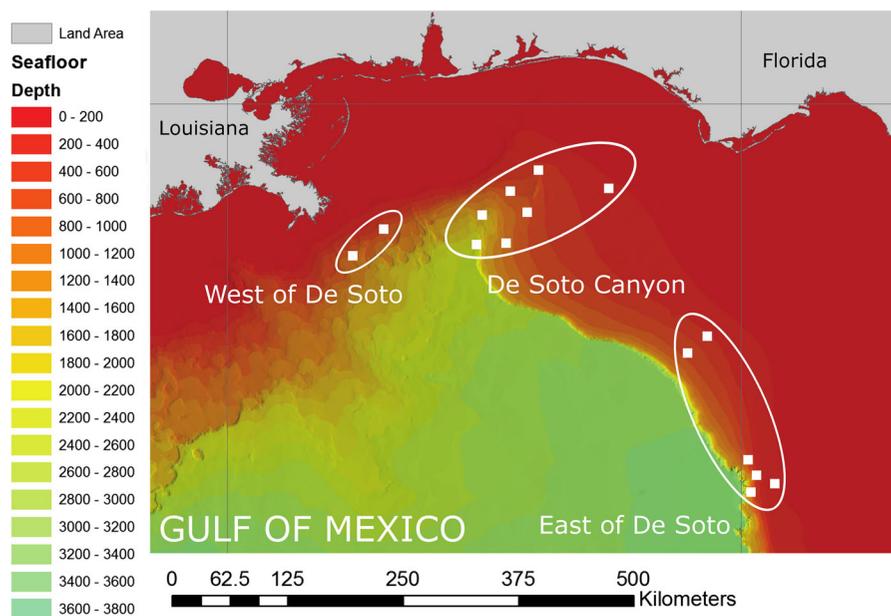
Samples were collected by long-line, in which hooks are baited on-ship and let out to lie on the ocean floor. With this method, we collected over 200 samples (Online Resource Table S1) from the northeastern Gulf of Mexico (Fig. 1) over the course of three Deep-C research cruises carried out on the University of South Florida R/V Weatherbird II in April 2011, August 2011, and August 2012. After collection, specimens were frozen and kept at  $-20^{\circ}\text{C}$  on deck, returned to lab, and stored at  $-20^{\circ}\text{C}$ . Tissue samples were collected in August 2014 and stored at  $-20^{\circ}\text{C}$  in 70% ethanol. Upon returning to lab, they were recorded in the HBG database and archived in the Florida International Crustacean Collection (FICC).

DNA was extracted using the Qiagen DNeasy Blood and Tissue kit following the provided protocol. To increase DNA yield, 40  $\mu\text{l}$  of DL-Dithiothreitol (DTT) was added to the tissue during the initial lysis step and AE buffer was heated to  $56^{\circ}\text{C}$  prior to elution. The quality of every DNA extraction was ascertained by running a 2% agarose gel and through Qubit assay (Life Technologies).

Sixteen of the samples collected for the traditional population genetics study were found to have extractions of high enough quality to be included in a population genomics pilot study. Reduced representation libraries were prepared for these individuals using the double digest Restriction-site Associated DNA sequencing approach (ddRADseq) according to Peterson et al. (2012). Details of library preparation, data filtering, alignment, assembly, and analysis are provided in Online Resource ddRADseq Supplement. Results of ddRADseq analyses are presented within the tables of Sanger results to allow for easy comparison between the two approaches. However, the ddRADseq results are formally compared to Sanger and discussed in the ddRADseq Supplement.

## Sanger sequencing

Three mitochondrial genes were sequenced for all samples: the 12 and 16S mitochondrial ribosomal



**Fig. 1** A bathymetric map of sampling sites. Warmer colors denote shallower depths. Collection sites are marked with white points and circles indicate grouping of collection sites across three geographic areas: western De Soto, De Soto Canyon, and eastern De Soto. This map was derived from the “Bathymetry of the Gulf of Mexico and Adjacent Areas of the Caribbean Sea and

Atlantic Ocean in Shaded Relief” figure within the International Bathymetric Chart of the Caribbean Sea and Gulf of Mexico (IBCCA) map set, under the National Oceanic and Atmospheric Administration’s National Geophysical Data Center (NOAA NGDC)

subunits and cytochrome c oxidase subunit I (COI). Genes were amplified in 25  $\mu$ l PCR reactions. The 12S subunit was amplified using the 12SF (5′-GAAAC-CAGGATTAGATACCC-3′; Mokady et al., 1994) and 12S1R (5′-AGCGACGGGCGATATGTAC-3′; Buhay et al., 2007) primers with an annealing temperature of 52°C. The 16S subunit was amplified using a dual forward primer containing L2 (5′-TGCCTGTTTATCAAAAACAT-3′; Palumbi et al., 2002) and L9 (5′-CGCCTGTTTATCAAAAACAT-3′; Palumbi et al., 2002) and the reverse primer 1472 (5′-AGATAGAAACCAACCTGG-3′; Crandall & Fitzpatrick, 1996), with an annealing temperature of 46°C. COI was amplified using LCOI-1490 (5′-GGTCAACAAATCATAAAGATATTG-3′; Folmer et al., 1994) and HCOI-2198 (5′-TAAACTT-CAGGGTGACCAAAAAATCA-3′; Folmer et al., 1994), with an annealing temperature of 38°C. Sequences were analyzed by Beckman-Coulter Genomics Services single-pass PCR sequencing, cleaned in Geneious v.8.0.5 (Kearse et al., 2012), and aligned with MAFFT (Katoh & Standley, 2013) prior to concatenation (12S + 16S + COI). Sequences were

divided into three regions based on collection locality: west of De Soto Canyon (wDC), De Soto Canyon (DC), and east of De Soto Canyon (eDC).

#### Analysis of Sanger data

Given the maternal inheritance of mitochondrial genes, sequences for each individual were concatenated and analyzed as a single alignment. PartitionFinder results also supported treating the three genes as a single locus. Nucleotide diversity ( $\pi$ ), haplotype diversity ( $h$ ), and the selection coefficient Tajima’s  $D$  (Tajima, 1983) were calculated for each region (wDC, DC, and eDC) in DNAsp v5 (Librado & Rozas, 2009) and significant differences in diversity and selection between regions were tested with ANOVA. Because the number of unique haplotypes can be important indicators of restricted gene flow or past population fragmentation (Allendorf & Luikart, 2009; Campo et al., 2009), we also examined endemicity of haplotypes between regions by measuring the percent of shared haplotypes (haplotypes found across regions) and the percent of endemic

haplotypes (number of haplotypes unique to a region/total number of haplotypes). To measure population differentiation and connectivity, we performed a hierarchical Analysis of Molecular Variance (AMOVA) under the Infinite Allele Model in Arlequin v3.5.1.2 (Excoffier & Lischer, 2010) with 999 permutations to assess statistical significance. Missing data were replaced with randomly drawn alleles determined by overall allele frequencies.

To test for population structure, a multidimensional scaling (MDS) plot was rendered using the R package MASS (Venables & Ripley, 2002). MDS is very similar to Principal Component Analysis (PCA), with the exception that PCA preserves covariance within the data, while MDS preserves distance between points. As genetic distance between individuals is of primary interest in addressing the role of De Soto Canyon during the last glacial maximum, MDS was chosen to better display distances between individuals.

Extended Bayesian Skyline Plot (EBSP) analyses were executed in BEAST2 (Bouckaert et al., 2014) for the purpose of estimating historical changes in population size for each region (wDC, DC, and eDC), as well as the complete dataset (wDC+DC+eDC). Single-locus alignments were loaded individually (12S, 16S, and COI) into BEAUTi2 to set parameters. The COI alignment was divided by codon position (1 + 2 + 3) and site model parameters were set according to the results of PartitionFinder. For all datasets, the clock rate was set by 12S with a clock rate of 0.5, and the clock rates for the 16S and COI alignments were estimated in relation to the 12S alignment. In the absence of estimates of clock rates for 16S and COI, rates were set to 0.005. All additional parameters were set according to the manual, with the exception of the MCMC parameters: 200,000,000 generations were run, logged every 5000th.

In addition, Bayesian inference as implemented in MIGRATE-N (Beerli & Palczewski, 2010) was used to test models of population demography and determine the most likely migration patterns between regions. As per the manual, default settings were used, then the data were reanalyzed using the resultant estimates of  $\theta$  for each population and migration rates between populations to inform parameters to ensure default parameters were appropriate for the dataset. The number of recorded steps was increased from the default (5000) to 20,000, and static heating was used across four chains, swapping every tenth step. To

confirm results and ensure 20,000 steps was adequate, analyses were rerun with 1,000,000 steps and results compared between runs.

## Results

A total of 570 *de novo* sequences were generated across three markers, including 205 12S sequences, 205 16S sequences, and 160 COI sequences. Sequence data are archived under GenBank Accession numbers MG229070-MG229274 (12S), MG229275-MG229479 (16S), and MG229480-MG229639 (COI); and are publicly available in the Gulf of Mexico Research Initiative's Information and Data Cooperative (GRIIDC). The final concatenated alignment contained 1450 bp of sequence data for all three loci across 147 individuals (Timm et al., 2018). Across these three loci, 75 SNPs and 78 haplotypes were identified for analysis (Table 1). Individuals missing data at a locus were not included in the concatenated dataset.

### Population differentiation and connectivity

Results of AMOVA indicate very high gene flow between regions, with among population variance ( $F_{ST}$ ) contributing 1.46% (Table 2). The vast majority of variance (98.54%) is attributed to differences between individuals ( $F_{IT}$ ), regardless of the region from which they were sampled. With a significance value of 0.048, results were determined to be statistically significant. Calculations of  $F_{ST}$  indicate nearly nonexistent population differentiation between regions, with  $F_{ST}$  values ranging from  $-0.003$  (eDC-DC) to 0.001 (eDC-wDC and DC-wDC) (Table 3). All  $P$  values were  $> 0.05$ .

The multidimensional scaling plot does not indicate individuals clustering into groups (Fig. 2). If individuals from each region were more genetically similar (smaller genetic distances), three clusters would be rendered. Instead, we find the majority of individuals cluster together with one or two outliers.

### Genetic diversity and endemism

For every region (wDC, DC, and eDC) and across regions (wDC+DC+eDC), nucleotide diversity ( $\pi$ ) was calculated to be 0.003. That is, regardless of

**Table 1** Sampling effort for each data type and region

	Concatenated Sanger				ddRADseq			
	eDC	DC	wDC	All	eDC	DC	wDC	All
<i>N</i>	62	58	27	147	5	3	2	10
SNPs	52	47	32	75	1891	570	588	2681
Haplotypes	44	35	18	78	5	3	2	10

*N* the number of individuals, *SNPs* number of single nucleotide polymorphisms, and haplotypes are given for *DC* De Soto Canyon, *eDC* and *wDC* the regions lying east and west of the canyon, respectively

**Table 2** Results of the hierarchical AMOVAs conducted to characterize genetic variation among individuals ( $F_{IT} = 98.5\%$ ), among individuals within populations ( $F_{IS} = 0\%$ ), and among populations ( $F_{ST} = 1.5\%$ )

	$F_{ST}$ (%)	$F_{IS}$ (%)	$F_{IT}$ (%)
Concatenated Sanger*	1.5	0.0	98.5
ddRADseq*	1.5	0.0	98.5

The Infinite Allele Model was used with 999 permutations to assess statistical significance. Any missing data were replaced with randomly drawn alleles determined by the overall allele frequencies of the dataset. The Concatenated Sanger AMOVA yielded statistically significant results ( $P = 0.048$ ). AMOVA results indicate the vast majority of variance is due to differences between individuals ( $F_{IT}$ ), regardless of the region from which they were sampled

\* $P$  value < 0.05

**Table 3** Interpopulation genetic distances between the De Soto Canyon (DC) and the regions lying east and west of the canyon (eDC and wDC, respectively), which are reported below the diagonal

	Concatenated Sanger			ddRADseq		
	eDC	DC	wDC	eDC	DC	wDC
eDC	-	0.459	0.394	-	0.257	1.000
DC	0.003	-	0.457	0.068	-	0.208
wDC	0.001	0.001	-	0.000	0.143	-

$P$  values are reported above the diagonal

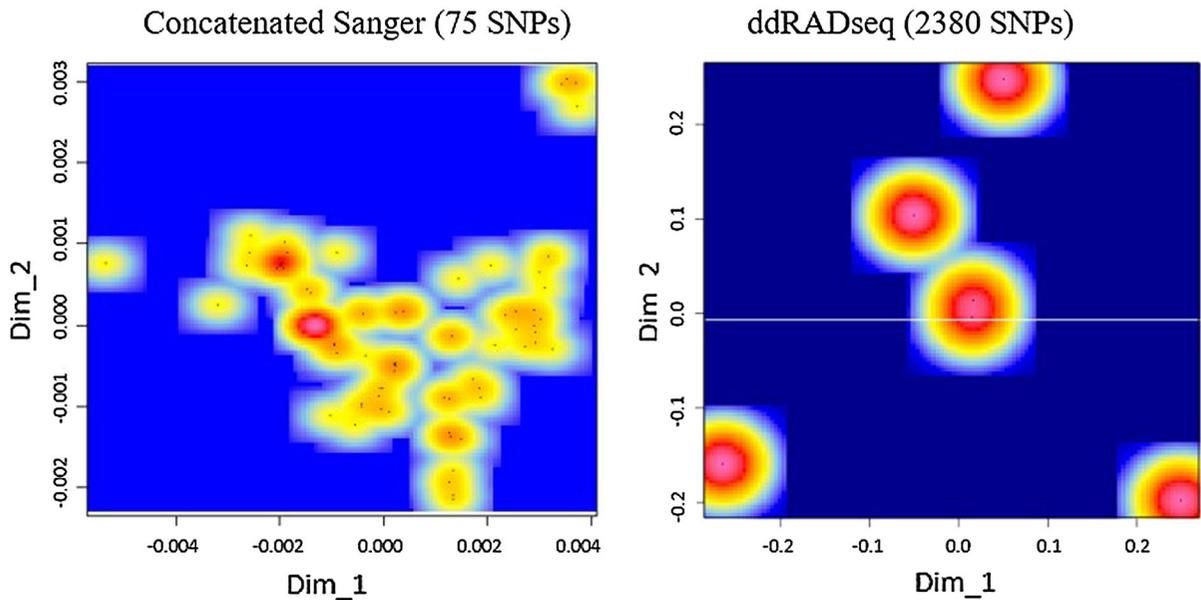
region, the average number of nucleotide differences per site between two randomly chosen sequences from the dataset (Nei & Li, 1979) is 0.003. Haplotype diversity ( $h$ ), defined as the probability of two random haplotypes taken from the dataset being different (Nei, 1987), was also calculated within and across regions

(Table 4). Across regions,  $h$  differed from the trend seen in  $\pi$ : DC yielded the lowest diversity ( $h = 0.880$ ) compared to wDC ( $h = 0.929$ ) and eDC ( $h = 0.942$ ). Overall haplotype diversity fell between the DC and wDC values ( $h = 0.901$ ). While it is notoriously difficult to compare these metrics to other studies (Goodall-Copestake et al., 2012), nucleotide diversity is relatively equal to other studies of marine invertebrates across a similar range; however, haplotype diversity is quite high (here, values are compared to those measured in Etter et al., 2005 and Zardus et al., 2006).

Although diversity was hypothesized to be significantly higher in the canyon, ANOVA results did not indicate significant differences in diversity in any region for either metric (for  $\pi$ ,  $P = 0.79$ ; for  $h$ ,  $P = 0.96$ ). An analysis of the percent of unique endemic haplotypes (number of unique endemic haplotypes/total number of unique haplotypes) within each region found eDC had the highest percent of unique endemic haplotypes (70.5% of the haplotypes found in eDC are unique to eDC), followed by DC (65.7%) and wDC (61.1%) (Fig. 3). In addition, we noted that 46.2% of the 13 shared haplotypes were found in all regions and an additional 46.2% were found to be shared between DC and eDC. No shared haplotypes were found between wDC and DC, and only a single haplotype was shared between wDC and eDC (representing 7.7% of all shared haplotypes) (Fig. 3). All 13 shared haplotypes were found to be present in eDC, compared to wDC (7 shared haplotypes) and DC (12 shared haplotypes).

#### Selection and historical demography

Tajima's  $D$  was estimated for each region (Fig. 4). All values were negative, and most were significant:



**Fig. 2** Multidimensional scaling plots as heat maps built from MAFFT-aligned concatenated Sanger data (left); as well as the plot rendered from 2380 SNPs identified with ddRADseq (right). See Supplementary Materials for Methods and Results of ddRADseq. In the heat maps, higher densities of individuals

are denoted with warmer colors. In both plots, individuals are clustered based on genetic distance. Note the difference in scale between plots. Across plots, we do not see evidence of genetic differentiation

**Table 4** Diversity metrics, nucleotide diversity ( $\pi$ ) and haplotype diversity ( $h$ ), and Tajima's  $D$  and significance value for each population in each dataset

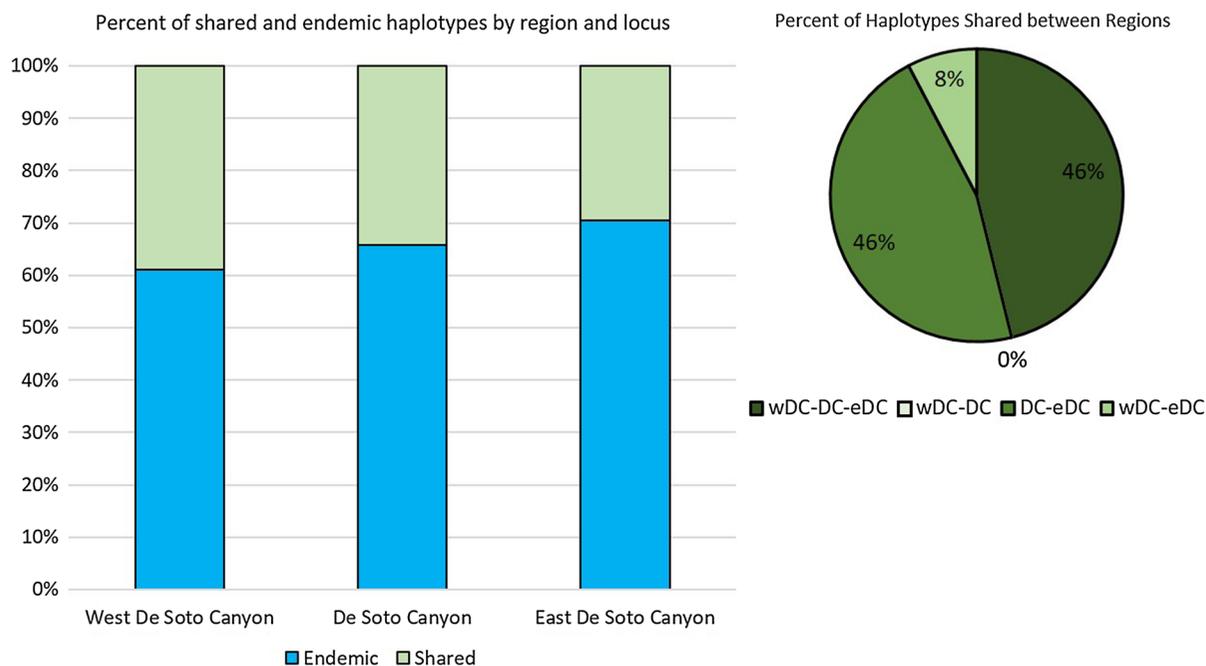
	Concatenated Sanger				ddRADseq				Etter et al. (2005) $N = 268$	Zardus et al. (2006) $N = 130$
	eDC	DC	wDC	All	eDC	DC	wDC	All		
$\pi$	0.003	0.003	0.003	0.003	0.261	0.171	0.262	0.234	0.0029–0.0175	0.0217
$h$	0.942	0.880	0.929	0.901					0.277–0.783	0.731
$D$	-2.122*	-1.976*	-1.585	-2.210**						

Diversity values from previous studies of molecular diversity in marine invertebrates, specifically bivalves, are also reported. For these previous studies, the sample size ( $N$ ) is given in place of "region"

\* $P$  values < 0.05; \*\* $P$  values < 0.01 ( $D_{sim} < D_{obs}$ , 1000 simulations)

- 1.585 in wDC ( $P > 0.05$ ), - 1.976 in DC ( $P < 0.05$ ), - 2.122 in eDC ( $P < 0.05$ ), and - 2.210 overall ( $P < 0.01$ ). Negative Tajima's  $D$  values indicate a deficiency of rare alleles. Typically, this deficiency is associated with recovery following a population bottleneck. ANOVA testing of Tajima's  $D$  values across loci by region indicate that selection is not significantly different between regions ( $P = 0.96$ ). These results were confirmed by modeling changes in population sizes with EBSPs. Overall, the rate of population expansion was highest when the entire

dataset was analyzed as a whole (increasing by a factor of  $\sim 40$  in the last 15,000 years). By population, eDC had the highest growth rate (increasing by a factor of  $\sim 17$  in the last 18,000 years), followed by DC (increasing by a factor of  $\sim 11$  over the last 20,000 years), and finally wDC (increasing by a factor of  $\sim 8$  in the last 30,000 years). The fact that all three regions experienced statistically similar selection pressures, combined with high connectivity and resultant low population differentiation, suggests



**Fig. 3** Percent of shared haplotypes (found across regions) and endemic haplotypes (number of unique endemic haplotypes/total number of haplotypes) found within the study area, which are presented in the bar chart on the left. Note that East De Soto Canyon contains the highest percent of endemic haplotypes across loci. Shared haplotypes are further divided in the pie

chart to the right. The 13 shared haplotypes found in the data are expressed as percentages shared between: all regions (wDC-DC-eDC = 6), West De Soto Canyon and De Soto Canyon (wDC-DC = 0), De Soto Canyon and East De Soto Canyon (DC-eDC = 6), and West De Soto Canyon and East De Soto Canyon (wDC-eDC = 1)

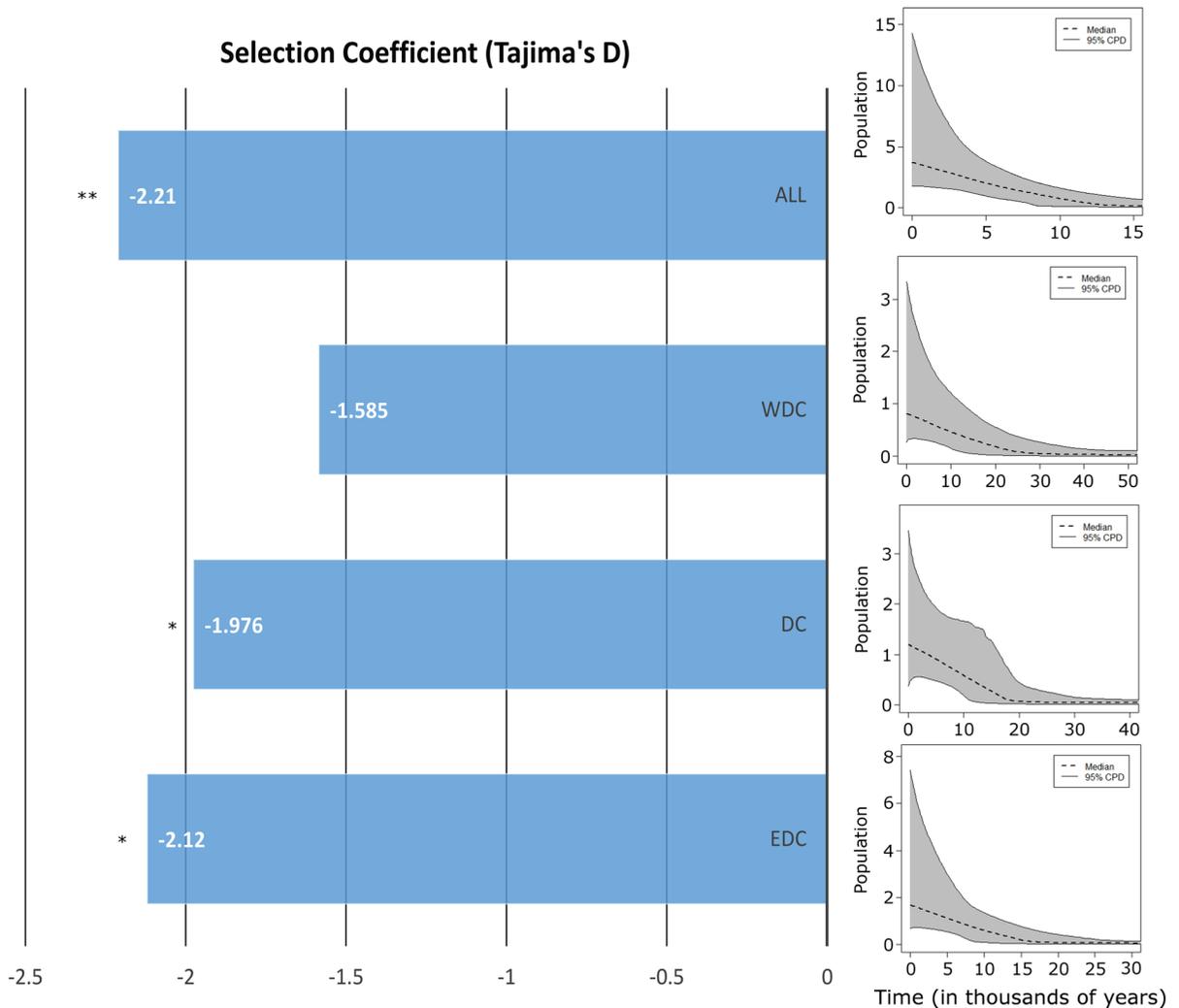
migration of *Bathynomus giganteus* between the tested regions in northern Gulf of Mexico (Table 4).

In MIGRATE-N, the posterior probabilities of 18 models were estimated using Bayesian inference. The parameters and thermodynamically integrated log marginal likelihood of each model are presented in Table 5. The 18 models ranged from a single panmictic population, to three, entirely separate populations. All models were tested with 20,000 and 1,000,000 steps, but the results did not change substantially between runs, so the results from the 20,000 step analyses are reported (Table 5). The model indicated to be the most likely, given the data (highest log marginal likelihood), supported three populations (wDC, DC, and eDC) and bidirectional migration between all three.

## Discussion

Previous studies have established four metrics as evidence for a region to be classified as a glacial

refugium: connectivity, diversity, endemism, and population expansion. First, connectivity must exist between the hypothetical/purported refugium population and nearby populations (Bernatchez & Dodson, 1991; Treweek & Wallis, 2001; Petit, 2003). Second, diversity is typically higher within the refugium population, although in species with limited/low dispersal, the highest diversity tends to be found in populations between refugia (Lewis & Crawford, 1995; Petit, 2003; Thatje et al., 2005; Beck et al., 2008; Provan & Bennett, 2008). Third, and relatedly, the refugium population is likely to contain the highest number of unique, endemic haplotypes (haplotypes which occur in one population but are not present in any others) (Knowles, 2001; Provan & Bennett, 2008). The final line of evidence comes from analysis of historical selection: all populations should exhibit a signal of expansion following a bottleneck (Provan et al., 2005; Maggs et al., 2008; Campo et al., 2009). Glaciation events cause dramatic and quick range changes (GRIP Project Members, 1993), which many species are unable to cope with in real time (Atkinson



**Fig. 4** On the left: Tajima's  $D$  values for each species. \* $P < 0.05$ . \*\* $P < 0.01$ . All values indicate population growth and, with the exception of wDC, all are statistically significant. To the right of the Tajima's  $D$  graph are four Extended Bayesian Skyline Plots (EBSPs) generated in BEAST. Top to bottom: West De Soto Canyon + De Soto Canyon + East De Soto Canyon, West De Soto Canyon, De Soto Canyon, and East De Soto Canyon. The horizontal axis describes time (in thousands of years), and the vertical axis measures population size. In these

et al., 1987). Such range contractions cause high mortality along distribution margins, but also allow for population expansion as range increases during interglacial periods (Nesbø et al., 1999; Knowles, 2001). The role of habitat heterogeneity in shaping population dynamics is deduced through associations of genetic diversity and habitat diversity (Levin et al., 2001; Vanreusel et al., 2010), where we expect the

visual representations of the EBSP posterior samples for each analysis: the solid lines define the 95% central posterior density (CPD), and the dotted line traces the median value over time. Note that all regions experienced population growth, individually and overall (in agreement with the Tajima's  $D$  values). Population growth was the most dramatic in the analysis of all samples (ALL, top), as was expected. By region, population growth was the fastest in the east (EDC, bottom) and the slowest in the west (WDC, second from the top)

highest genetic diversity to be associated with the most complex habitat. However, it is important to note that these two drivers, the hypothesized presence of a glacial refugium and habitat diversity, may themselves be interrelated (Médail & Diadema, 2009).

Across regions and data types, we find (1) low differentiation and high population connectivity, indicating strong gene flow between regions; (2)

**Table 5** Models tested in MIGRATE-N and their associated thermodynamically integrated log marginal likelihood (l ml)

Model	Description	l ml
8	<> (wDC) <> (DC) <> (eDC)	- 3163.25
6	> (wDC) x (DC) < (eDC)	- 3168.97
7	x (wDC) <> (DC) <> (eDC)	- 3171.19
5	x (wDC) < (DC) > (eDC)	- 3178.6
16	(wDC+eDC) > (DC)	- 3709.45
15	(wDC+eDC) < (DC)	- 3709.88
13	(wDC) > (DC+eDC)	- 3711.17
9	(wDC+DC) < (eDC)	- 3713.53
12	(wDC) < (DC+eDC)	- 3715.95
4	< (wDC) > (DC) x (eDC)	- 3721.9
14	(wDC)<>(DC+eDC)	- 3726.54
10	(wDC+DC)>(eDC)	- 3726.97
3	x (wDC) < (DC) < (eDC)	- 3727.37
17	(wDC+eDC) <>(DC)	- 3732.62
2	x (wDC) > (DC) > (eDC)	- 3733.77
11	(wDC+DC) <> (eDC)	- 3735.55
18	(wDC+DC+eDC) panmictic	- 3739.52
1	x (wDC) x (DC) x (eDC)	- 4157.71

Results presented here are from the 20,000-step runs. Populations are put in parentheses and the symbols between them indicate the direction of migration (<, >, or <>) or its absence (x). The direction listed before (wDC) indicates direction between eDC and wDC. When two populations are listed within the same set of parentheses, e.g., (wDC+DC), it means that individuals collected from these two regions are treated as a single population. Models are listed in order of decreasing l ml

relatively high genetic diversity across regions; (3) slightly elevated levels of endemism in East De Soto Canyon compared to adjacent regions; and (4) evidence that a bottleneck was experienced and recovery is underway across all regions in the northern Gulf of Mexico, which may correlate to the last glaciation event of the Pleistocene. In light of these findings, we will discuss the potential impact of habitat heterogeneity and/or the presence of a glacial refugium on the current and historical population dynamics of the deep-sea isopod *Bathynomus giganteus* in the northern Gulf.

#### Population differentiation and connectivity

Across the De Soto Canyon, *Bathynomus giganteus* exhibits similar genetic diversity values, regardless of

geographic location. It seems that this high diversity and low population differentiation is sustained through high population connectivity. However, it is also possible that low divergence and  $F_{ST}$  values are evidence for recent population expansion (Stamatis et al., 2004) out of the putative De Soto Canyon refugium or recent reacquaintance of separated populations (Taylor & Roterman, 2017). Given the small geographic distance between sites and the low migration rate required to prevent genetic divergence, we are inclined to interpret these results as evidence of moderate, historical gene flow.

*Bathynomus giganteus* lacks a pelagic larval phase, which could potentially impede migration (see Marko, 2004 for a more thorough investigation of this often-incorrect inference). Our results indicate that not only are individuals capable of traversing the canyon, but they apparently do so quite freely. This is not too surprising considering *Bathynomus giganteus* are known to be quite efficient swimmers (per observation). No unique genetic signature was found on either side of the canyon, nor within the canyon itself. Moreover, multidimensional scaling does not cluster individuals by collection location to any appreciable extent.

Our analyses of population differentiation suggest that high connectivity contributes to the even distribution of diversity in the northern Gulf of Mexico. This was somewhat unexpected as many studies in the Atlantic deep sea have found strong differentiation corresponding to depth in motile taxa (Doyle, 1972; Siebenaller, 1978; Wilson, 1983; France & Kocher, 1996; Taylor & Roterman, 2017), although the swimming ability of *B. giganteus* may help explain high connectivity between regions. In addition, a study of the bathyal gastropod *Bathybembix bairdii* indicated low population differentiation as well (Siebenaller, 1978), and more recent studies of gastropods and bivalves found population-level differences in diversity decreased with depth, as factors associated with population differentiation, such as environmental heterogeneity and topographical complexity, also tend to decrease along a depth gradient (Etter & Rex, 1990; Rex et al., 1993; Etter et al., 2005).

In characterizing connectivity between regions, we find there are functionally no barriers to gene flow between regions in the northern Gulf of Mexico. This suggests that, if De Soto Canyon served as a glacial refugium during the last Pleistocene glaciation event,

individuals of *B. giganteus* migrated out of the canyon into adjacent regions as sea levels rose. However, lack of population differentiation also suggests that differences in habitat do not impede gene flow between regions.

### Genetic diversity and endemism

Previous population genetics studies of deep-sea invertebrates provide context for our findings of relatively high genetic diversity (Doyle, 1972; Siebenaller, 1978; Etter & Rex, 1990; France & Kocher, 1996; Etter et al., 2005; Zardus et al., 2006; Raupach et al., 2007). Studies of deep-sea mollusk population genetics found similar haplotype diversity values; however, our analyses indicate much higher diversity in *B. giganteus* than in mollusks targeted in previous studies (Etter et al., 2005; Zardus et al., 2006), despite larger sample sizes (see Table 4 for comparison with previous studies of diversity in marine bivalves). This difference may be due in part to the loci analyzed: in the Etter et al. study, 16S was sequenced (225 bps); our study sequenced a larger portion of 16S (527 bps) in addition to 12S (336bps) and COI (596 bps).

The high haplotype diversity may be explained by the species' dispersal ability: while it lacks a pelagic larval stage, individuals migrate great distances over the course of their lives, perhaps even into adjacent oceanic basins. This may be facilitated by strong swimming behavior. High diversity within *Bathynomus giganteus* is likely maintained through the unique habitat conditions of the northern Gulf of Mexico, as suggested by previous studies of genetic diversity in the marine benthos (Rex, 1983; Sibuet et al., 1989; Campbell & Aarup, 1992; Levin et al., 2001; Vanreusel et al., 2010).

Analyses of molecular diversity revealed non-De Soto sites (wDC and eDC) had very similar haplotype diversity values, slightly higher than values measured for De Soto Canyon. This seems to support the habitat diversity hypothesis, instead of the De Soto Canyon refugium: if the canyon had served as a refugium, we would expect diversity values to be substantially higher within it and for it to contain the highest proportion of endemic haplotypes (see Introduction). Instead, we find eDC contains the highest proportion of endemic haplotypes. Moreover, every haplotype sampled can be found in eDC. Differences in diversity between regions may be better explained by habitat

diversity: the high degree and variety of organic particulate influx from the Mississippi River contributes to habitat heterogeneity, a crucial feature for the sustenance of diversity in the deep-sea benthos (Etter & Grassle, 1992; Grassle & Maciolek, 1992). This riverine input flows directly over the wDC sites and is known to disperse as far as the west Florida slope (Brooke & Schroeder, 2007), from which the eDC samples were collected. De Soto Canyon site, by contrast, is hard-bottomed, high relief, and primarily the result of erosion (Nowlin, 1971; Gore, 1992; Brooke & Schroeder, 2007).

### Selection and historical demography

Our results indicate a bottleneck was experienced across regions in the northern Gulf of Mexico, but populations are expanding. This may be indicative of sea-level rise, range expansion, and concomitant population growth. Given the relative dearth of information available on major disruptions in the benthic deep sea, it is difficult to definitively deduce the cause of this bottleneck. However, the last glaciation is indicated for three reasons: first, sea levels were 120–125 m lower causing dramatic range contraction in the northern Gulf of Mexico, which includes the distributional range of *B. giganteus*; second, periods of glaciation are also associated with decreased precipitation, which in turn depress the input of organic particulate matter into the deep Gulf of Mexico and could increase microhabitat homogeneity and decrease diversity through mortality (Etter & Grassle, 1992; Grassle & Maciolek, 1992); third the timing of population expansion indicated by EBSPs suggest expansion began approximately 15,000–30,000 years ago. This correlates well with the retreat of the last glacial maximum, with the exception of the population west of De Soto Canyon. The last glacial maximum of the Pleistocene occurred approximately 20,000 years ago, alongside the estimated beginning of expansion for the De Soto Canyon population and that east of the canyon. However, the population west of De Soto appears to have begun increasing 30,000 years ago. Not only does the wDC population expand at a much lower rate, but the Tajima's  $D$  values associated with this region ( $D = -1.585$ ) were the lowest measured in this study and were not statistically significant. This provides justification for an intriguing inference: the

population west of De Soto seems to have been relatively unimpacted by the last glacial maximum, suggesting the population is relatively stable. In nonmarine environments, long-term stability of a population can be a predictor of higher genetic diversity (Carnaval et al., 2009). Thus, regional stability in the wDC, combined with the bidirectional gene flow indicated by migration analysis and lack of population differentiation, may contribute to high genetic diversity for the entire northern Gulf.

Selection coefficient values and rates of population expansion exhibited an increasing trend from east to west, away from the Mississippi River Delta, the most geologically, topographically, and geographically diverse region included in the study. Rather than supporting De Soto Canyon as a glacial refugium, for which we would expect the coefficient to be highest and the expansion rate lowest for the region, we instead find those characteristics in the region west of De Soto. This provides evidence for the influence of habitat diversity on population demography in the northern Gulf of Mexico.

## Conclusions

Our investigation into the historical role of De Soto Canyon and the habitat diversity in the northern Gulf of Mexico illuminates population dynamics of a charismatic deep-sea invertebrate in the region and increases our understanding of an often overlooked environment. Despite low population differentiation, high connectivity, and a strong signal of population expansion, we find diversity to be lowest in the canyon. Our results lend support to the intriguing hypothesis that population dynamics have historically been influenced by the unique habitat diversity found in the northern Gulf, rather than by the presence of a putative glacial refugium. To more confidently evaluate the role of the De Soto Canyon in the past glaciation events, a more inclusive ddRADseq study should be undertaken to include samples from a broader geographic range.

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