11-1-2010

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Recommended Citation

Gregg, C., Foltz, D., & Fleeger, J. (2010). Genetic diversity in a deep-sea harpacticoid copepod found near two oil-drilling sites in the gulf of mexico. *Journal of Crustacean Biology, 30* (4), 651-657. https://doi.org/10.1651/09-3227.1

JOURNAL OF CRUSTACEAN BIOLOGY, 30(4): 651-657, 2010

GENETIC DIVERSITY IN A DEEP-SEA HARPACTICOID COPEPOD FOUND NEAR TWO OIL-DRILLING SITES IN THE GULF OF MEXICO

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ABSTRACT

Sites adjacent to (near-field) and distant from (far-field) oil-drilling platforms were sampled for harpacticoid copepods at two locations in the northern Gulf of Mexico off the coast of Louisiana, both at depths of ≈ 1100 m. The sites were located at Garden Banks Area Block 602 (GB 602) and Mississippi Canyon Area Block 292 (MC 292). Near-field sites at both locations were characterized by large numbers of a single undescribed species of harpacticoid copepod, *Bathycletopsyllus* sp., but this species was essentially absent from far-field sites. We sequenced a 710 bp portion of the mitochondrial cytochrome c oxidase subunit I gene (COX I) to analyze the genetic diversity and population structure of *Bathycletopsyllus* sp. at the two platforms, and to determine if the harpacticoids examined were either a single species, or a complex of cryptic species. We found that divergence at the COX I gene (maximum 1.6%) was within the range observed for intraspecific variability in previously-studied species of harpacticoid copepods that were well-characterized taxonomically. Thus, the two samples analyzed here were a single species and not a complex of cryptic species. In addition, there was significant genetic heterogeneity between the two samples, suggesting limited gene flow between the two sites. This was expected given the low dispersal potential typical of harpacticoids and the distance between the sites (407 km). Lastly, haplotype diversity and nucleotide diversity were both low in the GB 602 sample, giving a nominally-significant departure from a pure neutral model. This result could indicate the occurrence of selective sweeps, temporal population size variation or other processes not included in the neutral model. In contrast, haplotype diversity and nucleotide diversity were both higher in the MC 292 sample than at Garden Banks, and there was no detectable departure from neutrality. For both samples, diversity at the haplotype and nucleotide levels were within the range seen in shallow-water harpacticoid species inhabiting both uncontaminated and contaminated muddy sediments, so there was no evidence for pollution related effects in the present study.

KEY WORDS: *Bathycletopsyllus*, Copepoda, cytochrome oxidase I, genetic diversity, Harpacticoida

DOI: 10.1651/09-3227.1

INTRODUCTION

The deep-sea in the northern Gulf of Mexico is an increasingly active area for exploration of oil resources. The ecological (Peterson et al., 1996) and genetic (Street and Montagna, 1996) impacts of offshore oil exploration and production have been studied in shallow continental shelf habitats, but there are currently no data on the effects of oil exploration and production on genetic diversity of deep-sea infauna (Creasy and Rogers, 1999). In shallow water, reductions in genetic diversity have been observed in populations of marine organisms exposed to contaminants, compared to those from uncontaminated sites (Street and Montagna, 1996; Ross et al., 2002; Kim et al., 2003). Two mechanisms that may cause the observed reductions in genetic diversity in natural populations exposed to contaminants are natural selection and population bottlenecks (reviewed in Hummel and Patarnello, 1994; Hebert and Luiker, 1996; Bickham et al., 2000; Belfiore and Anderson, 2001; Staton et al., 2001; Van Straalen and Timmermans, 2002; Theodorakis, 2003; Rocha-Olivares et al., 2004; Morgan et al., 2007). Natural selection reduces genetic diversity by the differential survival of tolerant genotypes compared to less tolerant genotypes. Schizas et al. (2001) demonstrated that the harpacticoid copepod *Microarthridion littorale* (Poppe, 1881) that possessed one mitochondrial lineage had greater survival when exposed to a pesticide mixture, compared to individuals from two other lineages, thus bolstering the argument for natural selection, or possibly species selection if the lineages represent cryptic species (see below). Genetic diversity can also be decreased in a population that has undergone a rapid decrease in population size, a bottleneck, through the loss of rare alleles by the random process of genetic drift (Spielman et al., 2004). Undetected cryptic species, morphologically similar but genetically divergent species, may also be a factor in observed reduction in genetic diversity. Molecular analysis within some previously well studied species has revealed genetic divergence suggestive of interspecific differences (Knowlton, 1993; Knowlton, 2000; Bickford et al., 2007). Cryptic species have been found to occur in widely-studied pollution indicator species (Grassle and Grassle, 1976; Lobel et al., 1990) and in harpacticoids (Ganz and Burton, 1995; Rocha-Olivares et al., 2001). The presence of cryptic species may contribute to an observed loss in genetic diversity, if the species: 1) differ in their tolerance to contaminants, 2) are sympatric at uncontaminated sites. This would represent a loss of species diversity as opposed to genotypic selection. Rocha-Olivares et al. (2004) demonstrated that cryptic species within the genus *Cletocamptus*, which co-occur in Louisiana salt marshes, exhibited differential tolerances to heavy metals. The possibility of cryptic species must be accounted for when examining the effect of pollution on genetic diversity.

In the present study, we analyzed genetic diversity in an undescribed harpacticoid copepod species *Bathycletopsyllus* sp. sampled adjacent to (near-field), and 10-20 km distant from (far-field), two post-development drilling sites in the northern Gulf of Mexico, both at a water depth of ≈ 1100 m and separated by approximately 407 km. *Bathycletopsyllus* is a genus of harpacticoid copepod in the family Cletopsyllidae (Huys and Lee, 1998). The species examined in the present study has not yet been described, but it is approximately 0.9 mm body length, and females brood their larvae in an attached egg sac (Fleeger, personal observation), typical of harpacticoid copepods (Hicks and Coull, 1983).

Materials and Methods

As part of a larger study examining the effects of oil drilling on sediment geochemistry and biological communities in the deep sea (Continental Shelf Associates, Inc., 2006), two post-development drilling sites were sampled in July of 2001. Garden Banks Block 602 (GB 602 – latitude 27°22′38″N; longitude 92°27′35.8″W) and Mississippi Canyon Block 292 (MC 292 – latitude 28°42′13″N; longitude 88°35′44″W) are located off the coast of Louisiana, both at ≈ 1100 m depth. A map of the two drilling sites has been published by Gregg et al. (2006). Between 1995-2001, 18 wells were drilled within 10 km of the GB 602 near-field site; similarly, between 1995-2000, 16 wells were drilled within 10 km of the MC 292 site (Phillips, 2006). Sediment samples were collected by box core, from which three 7.6-cm diameter core subsamples were taken from each box core to obtain harpacticoid copepods for genetic analysis. As prior work (Rutledge and Fleeger, 1988) had suggested that subsampling by cylindrical coring of a larger box core may disturb the contained sediments, subsamples within each box core were not analyzed separately but were combined in one collection jar and preserved onboard ship in 95% ethanol. Near-field samples consisted of 12 box cores per drilling site taken from randomly selected locations within a 500 m radius from the center of each drilling site. Far-field samples consisted of 2 box core samples at each of six locations surrounding the drilling site and located 10-20 km from the drilling site (average distance between near-field and far-field samples was 17.6 km for GB 602 and 14.8 km for MC 292). Details of the sampling sites and general sampling methods were given by Phillips and Hart (2006); processing of sediment samples was described by Gregg et al. (2006). *Bathycletopsyllus* sp. appeared to be the most abundant harpacticoid at both the GB 602 and MC 292 near-field sites; more than 100 individuals were found in some box core samples (details in Gregg et al., 2006). At the far-field sites, only one individual of *Bathycletopsyllus* sp. was observed in 24 box core samples from GB 602 and MC 292 combined. At GB 602, 459 *Bathycletopsyllus* sp. were found and removed from all 12 near-field samples, where nine out of 12 samples had 10 or more individuals and four out of 12 had greater than 50 individuals. *Bathycletopsyllus* sp. was less common at MC 292, with 183 individuals sorted from all 12 near-field samples, but was still present in sufficient numbers for genetic analysis. Two out of 12 samples at MC 292 had more than 10 individuals each.

Molecular methods for extraction, amplification and sequencing of a 710-bp portion of the mitochondrial cytochrome c oxidase subunit I gene (COX I) followed published protocols (Schizas et al., 1997; Rocha-Olivares et al., 2001; Gregg et al., 2006). DNA sequences of the COX I gene were obtained from individuals chosen at random; a total of 33 individuals from GB 602 and 28 from MC 292 was analyzed. A genealogy of the COX I gene in *Bathycletopsyllus* sp. was constructed to show the relationships among the different haplotypes observed in the two populations. A minimum-spanning tree was constructed using Arlequin 2.0 (Schneider et al., 2000) and a statistical-parsimony network was constructed using TCS v 1.13 (Clement et al., 2000); genealogies from both methods produced identical topologies. The 95% connection limit for the TCS analysis was 10 steps. Genetic subdivision of the populations was examined by analysis of molecular variance (AMOVA; Excoffier et al., 1992) using the program Arlequin 2.0. A hierarchical design was implemented to test the null hypotheses of no heterogeneity of haplotype frequencies between drilling sites or among box cores within drilling sites. The distance matrix used in the analysis consisted of pairwise differences among haplotypes; additionally, conventional F-statistics were determined based only on allele frequencies, where distances between all pairs of non-identical haplotypes were set to one. The null distribution for significance tests was obtained by 650 random permutations of the data matrix. Ramos-Onsins and Rozas (2002) have shown that their R_2 statistic, when combined with statistical testing via coalescent simulations, are most powerful in small samples for testing departures from neutrality due to demographic or selective causes. The assumptions of the coalescent simulations are: 1) the sample is drawn from a population of large size, 2) there is no recombination or genetic hitchhiking, 3) the mutation rate is uniform across the sequenced DNA region, and 4) nucleotide substitutions are modeled by the neutral infinite-sites model. We calculated the R_2 statistic and simple population genetics statistics (transition/transversion ratios, nucleotide and haplotype diversities) in the package DNAsp v. 5 (Librado and Rozas, 2009) or in MEGA v. 2.1 (Kumar et al., 2001). Hypothesis testing of various demographic models in a coalescent framework was done with the program IMa (version dated April 21 2008, Hey and Nielsen, 2007).

Results

The sequence alignment consisted of 604 bp of the mitochondrial COX I gene with no alignment gaps or inferred nonsense mutations. We noted 13 polymorphic sites (9 synonymous and 4 nonsynonymous) from 33 GB 602 individuals, while 14 polymorphic sites (10 synonymous and 4 nonsynonymous) were observed from 28 MC 292 individuals. The average transition/transversion ratios for the GB 602 and MC 292 data were 8.9 and 3.6, respectively. The mean p-distance for both samples combined was 0.5% (range, 0-1.6%). For GB 602 alone, the mean p-distance was 0.2% (range, 0-1.0%); the corresponding values for MC 292 were 0.7% (range, 0-1.5%). Both statistical parsimony and minimum-spanning tree approaches yielded identical topologies for the haplotype network with no ambiguities (Fig. 1). The haplotype cladogram revealed the presence of three distinct clades, which were designated clades BI, BII, and BIII. Clade BI was separated from both clades BII and BIII by three mutational steps and clades BII and BIII were separated from each other by a minimum of six mutational steps. The distribution of haplotypes was spatially heterogeneous between the GB and MC sites. Eighty-three percent of the individuals in clade BI were from GB 602, while 88% and 89% of the individuals from clades BII and BIII were from MC 292, respectively. Clade BI consisted of a haplotype that was shared by 21 individuals sampled and five other haplotypes differing from it by a single mutational step. The relationships among the haplotypes in clade BII was more complex in that they did not all radiate from one well-represented haplotype in a ''star phylogeny'' as in clade BI, preventing the identification of a likely ancestral type. Only three haplotypes were observed in clade BIII, none of them numerically dominant. The AMOVA indicated genetic heterogeneity in *Bathycletopsyllus* sp. between the GB 602 and MC 292 sites, for both pairwise distances ($\Phi_{st} = 0.35$; $P \leq 0.0001$) and haplotype frequencies ($F_{st} = 0.21$; $P \leq 0.0001$) (Table 1). There was no evidence of genetic structure among the box core samples within sites in the AMOVA analyses, using either pairwise differences ($\Phi_{sc} = 0.043$; $P > 0.05$) or haplotype frequencies ($F_{sc} = 0.001$; $P > 0.05$).

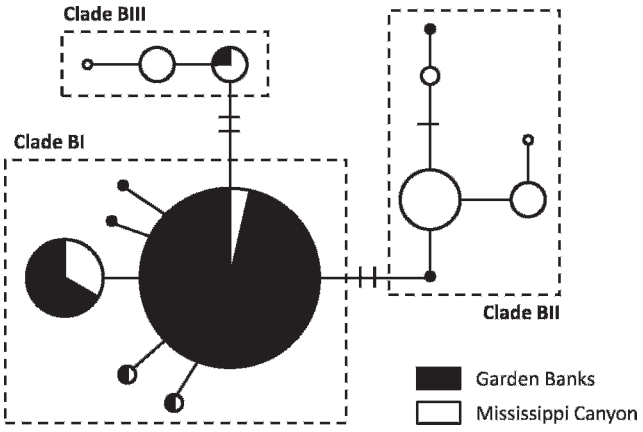

Fig. 1. Haplotype network of 15 unique cytochrome c oxidase subunit I COX I sequences. Circles represent different haplotypes and are drawn with a diameter proportional to the number of individuals sharing a certain haplotype. Each line between circles represents a nucleotide substitution and dashes on lines represent unobserved or extinct haplotypes (each line with two dashes indicates two haplotypes and three mutational steps). Pie wedges within circles are proportional to the number of individuals possessing these haplotypes found at the two sampling sites.

The numerical indices measuring haplotype and nucleotide diversity and the R_2 statistic indicated different genetic structures for *Bathycletopsyllus* sp. at GB 602 and MC 292 (Table 2). These diversity measures reflect the distribution of, and genetic distances, between the haplotypes at the two sites. GB 602 was numerically dominated by two haplotypes represented by 20 and 6 individuals, respectively, in clade BI, and the remaining seven haplotypes were each singletons, i.e., occurred only once in this sample, mostly from clade BI. In contrast, haplotypes were more evenly distributed among clades BI, BII, and BIII at MC 292 with five singletons and six other haplotypes that each occurred 2-7 times in this sample. These differences were reflected in the R_2 analysis, in that the near-absence of moderately-frequent haplotypes in sample GB 602 was responsible for the low value of the test statistic and the nominally significant departure from a pure neutral model. Any of several departures from the assumptions of the neutral model, such as the occurrence of selective sweeps, population size bottlenecks or other temporal population size variation, could be responsible for this result. The more even haplotype frequency spectrum in sample MC 292 resulted in a larger value of the R_2 statistic. Despite the difference in haplotype frequency spectrum between sites, tests of demographic models using the program IMa revealed that a simple model (Model II in Table 3) in which GB 602 and MC 292 had equal migration rates, and each site (plus the ancestral population prior to splitting) had equal mutation-rate-scaled effective population sizes ($\theta = 2Nu$) could not be rejected ($P > 0.05$) in favor of a more complex model (Model I) in which parameter values were different between sites. However, an even simpler model (Model III) in which migration between sites was assumed to be absent (zero rates) could be rejected ($P \leq 0.001$) in favor of Model II. We concluded that, as suggested by the sharing of multiple haplotypes in individuals sampled at both GB602 and MC292 in Fig. 1, there was some historical gene flow between the sites.

Table 1. Analysis of molecular variance (AMOVA) within and between samples from the GB 602 and MC 292 sites. For each analysis, the percentage of the variance explained (%) and the probability (P) of more extreme values from permutation tests are presented. Values outside of the parentheses are from the AMOVA weighted by pairwise differences between haplotypes; those within parentheses are from the unweighted AMOVA. $\Phi_{st} = 0.35$; $\Phi_{sc} = 0.043$; $\Phi_{ct} = 0.32$; $F_{st} = 0.21$; $F_{sc} = 0.0013$; $F_{ct} = 0.21$.

Source of variation	Degrees of freedom	%	*P*
Among sites	1	32 (21.4)	0.0015 (≤ 0.0001)
Among box cores within sites	11	3 (0.10)	NS (NS)
Within box cores [error]	48	65 (78.5)	≤ 0.0001 (≤ 0.0001)

Discussion

The samples containing *Bathycletopsyllus* analyzed in the present study were not drawn from one large, panmictic population, as shown by the significant AMOVA results in Table 1. Notwithstanding this genetic heterogeneity and the different population structures at the two sites that were revealed in Table 2, there was evidence for some historical gene flow between the sites. This genetic connectedness was shown by the sharing of some sequence haplotypes as well as by the nested log-likelihood ratio tests in Table 3, where a reduced model that included migration rates of 0 could be rejected in favor of a somewhat more complex model that included non-zero but equal migration rates between the sites. The genetic heterogeneity between samples of *Bathycletopsyllus* sp. at GB 602 and MC 292 reflected in the AMOVA analysis was consistent with the generally low dispersal potential of harpacticoid copepods; most species in Copepoda brood eggs in an egg sac that then hatch into a benthic naupliar stage (Hicks and Coull, 1983). The type of development possessed by *Bathycletopsyllus* sp. is presently unknown, but females in our samples had attached egg sacs indicating that this species likely exhibits larval development typical of harpacticoid copepods. Given the likely low-dispersal potential and the distance between the two sites (407 km), the observed genetic heterogeneity between sites was not surprising. Intertidal or shallow subtidal species of harpacticoid copepods have shown comparable, or even larger, levels of genetic heterogeneity among populations, sometimes over smaller geographic distances than in this study (Schizas et al., 2002; Denis et al., 2009; Willett and Ladner, 2009).

There was no evidence within or between sites for the existence of two or more genetically divergent but morphologically similar cryptic species. The maximum uncorrected difference among sequence haplotypes in *Bathycletopsyllus* (1.6%) was within the range previously observed for intraspecific variability of the COX I gene in shallow-water harpacticoid copepods from muddy sediments. For example, when COX I sequence data in four mitochondrial lineages of the nominal species *Cletocamptus deitersi* (Richard, 1897) plus the congener *C. helobius*

Table 2. Genetic diversity measures for the *Bathycletopsyllus* sp. samples from the GB 602 and MC 292 sites, with a comparison to shallow-water species of *Cletocamptus*, *Microarthridion littorale* samples, and *Nannopus palustris* morphs¶.

Species/sample§	*N*	Nh	R_2	π ± SD	H.D. ± SD	θ ± SD
Bathycletopsyllus sp., GB 602	33	9	0.0503*	0.00208 ± 0.00059	0.612 ± 0.089	0.00552 ± 0.00220
Bathycletopsyllus sp., MC 292	28	11	0.1340	0.00664 ± 0.00042	0.894 ± 0.033	0.00602 ± 0.00241
Mean for *Bathycletopsyllus* sp.				0.00436 ± 0.00228	0.753 ± 0.141	0.00577 ± 0.00025
C. stimpsoni	33	7	0.0578***	0.00067 ± 0.00019	0.428 ± 0.107	0.00209 ± 0.00102
C. fourchensis, Louisiana	17	6	0.0985*	0.00127 ± 0.00035	0.647 ± 0.120	0.00250 ± 0.01300
C. deborahdexterae	32	4	0.1036	0.00391 ± 0.00127	0.333 ± 0.100	0.00455 ± 0.00182
C. helobius	6	1	ND	0	0	0
C. sinaloensis	11	1	ND	0	0	0
Mean for *Cletocamptus*				0.00117 ± 0.00145	0.282 ± 0.251	0.00183 ± 0.00171
M. littorale, Shallot R.	14	3	0.1750	0.00082 ± 0.00046	0.275 ± 0.148	0.00181 ± 0.00136
M. littorale, North Inlet	16	6	0.1324	0.01469 ± 0.00361	0.675 ± 0.117	0.01532 ± 0.00657
M. littorale, Buck Hall, SC	21	8	0.1703	0.01735 ± 0.00182	0.824 ± 0.065	0.01378 ± 0.00555
M. littorale, St. Helena Is.	22	8	0.1862	0.01928 ± 0.00121	0.831 ± 0.059	0.01372 ± 0.00560
M. littorale, Savannah, GA	19	9	0.1434	0.01682 ± 0.00180	0.871 ± 0.051	0.01531 ± 0.00632
Mean, *M. littorale*, uncontaminated sites				0.01379 ± 0.00665	0.695 ± 0.220	0.01199 ± 0.00514
M. littorale, Diesel Ck.	23	7	0.0804*	0.00502 ± 0.00236	0.462 ± 0.128	0.01308 ± 0.00538
M. littorale, Murrell's Inlet	22	10	0.1254	0.01678 ± 0.00266	0.874 ± 0.052	0.01687 ± 0.00663
M. littorale, Newmarket Ck.	16	9	0.1485	0.01868 ± 0.00254	0.892 ± 0.054	0.01700 ± 0.00710
M. littorale, Shipyard Ck.	14	6	0.1076*	0.00317 ± 0.00080	0.747 ± 0.111	0.00544 ± 0.00287
M. littorale, Rathall R.	26	7	0.0669*	0.00647 ± 0.00230	0.471 ± 0.119	0.01478 ± 0.00590
Mean, *M. littorale*, contaminated sites				0.01002 ± 0.00641	0.689 ± 0.189	0.01343 ± 0.00452
N. palustris, notched morph	14	5	0.1122**	0.00301 ± 0.00076	0.659 ± 0.123	0.00478 ± 0.00283
N. palustris, other morphs	28	8	0.0884	0.00617 ± 0.00104	0.794 ± 0.055	0.00832 ± 0.00369

* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.
¶ Data sources: *Cletocamptus* (Rocha-Olivares et al., 2001, and unpublished); *Microarthridion* (Schizas et al., 2002); *Nannopus* (Staton et al., 2005). Taxonomic authorities: *Cletocamptus deborahdexterae* Gomez et al., 2004; *Cletocamptus fourchensis* Gomez et al., 2004; *Cletocamptus helobius* Fleeger, 1980; *Cletocamptus sinaloensis* Gomez et al., 2004; *Cletocamptus stimpsoni* Gomez et al., 2004; *Microarthridion littorale* (Poppe, 1881); *Nannopus palustris* Brady, 1880.
§ *N* = sample size; Nh = number of haplotypes; R_2 = test statistic of Ramos-Onsins and Rozas (2002); π = nucleotide site diversity ± standard deviation; H.D. = haplotype diversity ± standard deviation; θ = theta per site ± standard deviation (Nei, 1987: 255).

Fleeger, 1980 originally analyzed by Rocha-Olivares et al. (2001) were reanalyzed by us as separate species, based on the taxonomic work of Gómez et al. (2004), *Cletocamptus deborahdexterae* Gomez, Fleeger, Rocha-Olivares & Foltz, 2004 had an average intraspecific pairwise p-distance value of 0.4% (range, 0-1.6%). The corresponding values for *C. fourchensis* Gomez, Fleeger, Rocha-Olivares & Foltz, 2004 and *C. stimpsoni* Gomez, Fleeger, Rocha-Olivares & Foltz, 2004 were 0.1% (range, 0-0.4%) and 0.1% (range, 0-0.3%), respectively. Samples of *C. helobius* and *C. sinaloensis* Gomez, Fleeger, Rocha-Olivares & Foltz, 2004 each had only a single sequence haplotype present, so the minimum, mean and maximum intraspecific pairwise p-distance values for those two species were all 0 (see Table 2 for more details). The average interspecific Kimura 2-parameter distance among congeners in *Cletocamptus* was 26.1% (range, 9.5-33.0%). As noted by Costa et al. (2007), using COX I sequence data for species-level resolution requires that the genetic divergence between species be much greater than the genetic divergence within species. These interspecific distances in *Cletocamptus* generally equaled or exceeded the interspecific but congeneric Kimura 2-parameter distances reported by Costa et al. (2007) for decapods, amphipods, and cladocerans. To the extent that these results from a shallow-water genus of harpacticoid copepod that has been studied taxonomically using both molecular and morphological characters can be extrapolated to the less well-characterized deep-water genus *Bathycletopsyllus*, the molecular data collected in the present study would appear to have adequate resolving power to detect the presence of cryptic species among the sampled individuals, yet none was found. High levels of genetic differentiation at several mitochondrial loci between putatively conspecific populations have been noted in samples of *Tigriopus californicus* (Baker, 1912) from rocky intertidal habitats in southern California over relatively small spatial scales (Willett and Ladner, 2009, and references therein). Also, in a genetic survey of populations of *M. littorale* from the southeastern United States, Schizas et al. (1999) found high genetic divergence at the mitochondrial cytochrome b (cyt b) gene. Populations from Louisiana showed uncorrected nucleotide divergence of as much as 36% from the remaining sites, while 10% divergences were observed among populations from South Carolina, Georgia, and Florida. Genetic surveys

Table 3. Tests of nested models of θ = 2Nu (Nei, 1987: 255) and migration rate (m) parameter values between the GB 602 and MC 292 sites, using log-likelihood-ratio (LLR) test statistics in the program IMa. Model I is the most complex model (i.e., has the most parameters estimated from the data) and model III is the least complex (fewest parameters).

Model§	No. of free parameters	LLR†	Degrees of freedom	*P*
I. $\theta_1, \theta_2, \theta_A, m_1, m_2$	5	–		–
II. $\theta_1 = \theta_2 = \theta_A, m_1 = m_2$	2	2.30	3	NS
III. $\theta_1 = \theta_2 = \theta_A, m_1 = m_2 = 0$	1	11.70	1	≤ 0.001

§ θ_A refers to the genetic diversity in the ancestral population from which the two samples are assumed to have diverged at some time in the past. Because nothing is known about mutation rates in deep-sea copepods, all parameters estimated from the data were scaled to the same (unknown) mutation rate.
† LLR test statistics are calculated as twice the difference in log-likelihood between the indicated model and the one immediately above it in the table, with degrees of freedom equal to the difference in the number of parameters free to be estimated from the data. P values are obtained by comparison to the Chi-square distribution.

of benthic invertebrates from the deep sea using mitochondrial genes have also revealed high genetic divergence over sometimes small spatial scales in morphologically similar taxa (Creasy and Rogers, 1999; Etter et al., 1999).

Overall, the observed diversity values were similar to those observed for shallow-water species of harpacticoid copepods that live in muddy habitats (Gregg et al., 2006, see also Table 2). Demonstrating an apparent effect of contaminant exposure on genetic diversity ideally involves a comparison of samples from contaminated and uncontaminated sites (Street and Montagna, 1996). Here, the near absence of *Bathycletopsyllus* sp. from the far-field sites meant that the only possible comparison was to studies of shallow water harpacticoid species living in muddy sediments. The average value of both nucleotide site diversity and haplotype diversity in *Bathycletopsyllus* sp. was not significantly different (when analyzed as in Nei, 1987: 183) for three comparisons from Table 2: *Bathycletopsyllus* vs. *Cletocamptus*, *Bathycletopsyllus* sp. vs. *M. littorale* from uncontaminated sediments and *Bathycletopsyllus* sp. vs. *M. littorale* from contaminated sediments. In addition, although a significant departure from a pure neutral model was observed at GB 602, similar results have been seen in comparably-sized samples from four shallow water harpacticoid species (Table 2). This inter-sample variability in population genetic structure means that larger numbers of samples from different habitats and possibly different species will be required to determine the factors that influence the structure of deep-sea harpacticoid species. One possible avenue for future research is an approximately 10-fold difference between GB 602 and MC 292 near-field sites in concentration of total petroleum hydrocarbons (TPH) indicative of synthetic-based drilling mud (SBM). At GB 602, the average concentration in μg TPH dry g^{-1} of SBM petroleum hydrocarbons was 3378.5 (SD $\pm$ 3942.0) at the near-field sites and 5.75 (SD $\pm$ 2.0) at the far-field sites. At MC 292, the average concentration in μg TPH /dry g of SBM petroleum hydrocarbons was 411.2 (SD $\pm$ 712.6) at the near-field sites and 6.95 (SD $\pm$ 8.4) at the far-field sites (all hydrocarbon data were from McDonald and Brooks, 2006). Analysis of abundance data suggested that *Bathycletopsyllus* sp. probably responds to disturbance/hydrocarbons by increasing in population size but only up to a concentration of approximately 2500 μg TPH dry g^{-1} of SBM petroleum hydrocarbons; above this concentration, the species decreases in number (Gregg et al., 2006).

Further study of *Bathycletopsyllus* sp. is also warranted to help explain the high abundance of this species near the two drilling sites. The association between synthetic-based mud (SBM) petroleum hydrocarbon concentration and abundance of *Bathycletopsyllus* sp. in the near-field samples, and the near-absence of this species from the far-field samples, suggest that this species possibly responds to disturbance by increasing in population size. These data must be interpreted with caution, however, because no independent information is available on the biology of this copepod species, let alone its tolerance to contaminants. It is possible that SBM concentration may serve as an indicator of physical disturbance to which *Bathycletopsyllus* responds. For example, a change in sediment granulometry may accompany the increased SBM associated with drilling activities. Laboratory experiments and additional field sampling are necessary to determine cause-effect relationships. Informal comparison of the near-field samples to the far-field samples indicated that two very different harpacticoid communities, with few species in common, were present (Fleeger, personal observation). Near-field sites were characterized by relatively low species diversity and high dominance. Far-field sites were characterized by high species diversity and low dominance, more typical of undisturbed deep-sea communities. *Bathycletopsyllus* sp. is thus possibly a disturbance specialist, present in low numbers in undisturbed deep-sea habitats, but able to respond to disturbance associated with oil drilling and production activities. For example, this species might be common at natural hydrocarbon seeps in the northern Gulf of Mexico and therefore might have evolved a tolerance to hydrocarbons prior to oil and gas exploration and extraction. Additionally, other disturbances in the deep sea, which lead to localized carbon enrichment in the sediment, e.g., whale carcasses and phytoplankton settlement events, could provide suitable environmental conditions for opportunistic colonization by this species. If further sampling at other deep-sea oil platforms and organically enriched habitats shows similar results, then *Bathycletopsyllus* sp. may prove to be a useful marine pollution indicator species in the deep sea.

The geographic ranges of species in the deep sea are difficult to measure, partly because relatively few taxa are formally described. Species diversity is high in the deep sea and specialists identify taxa to ''working species,'' making it almost impossible to estimate ranges by comparing species lists generated by different investigators. This problem is compounded by cryptic species that increase the uncertainty of species identity, especially when species diversity is apparently high. Genetic data have proven valuable to answer questions about proposed endemism at deep-sea seamounts (Samadi et al., 2006) and are important in addressing questions regarding distributional ranges by assuring that conspecifics are used in analyses. Our results suggest that *Bathycletopsyllus* sp. is a single species found over a large portion of the Gulf of Mexico, and that it displays genetic heterogeneity over the scale of hundreds of km suggestive of limited gene flow. More such studies using molecular tools are needed to better describe the geographic ranges of deep-sea species.

Acknowledgements

Sequences have been deposited with GenBank with accession numbers AY327328-AY327388. Morphological vouchers have been deposited with the British Museum of Natural History under accession number BMNH 2004.2883-2888. This article was prepared under contract 1435-01-00-CT-31034 between the Minerals Management Service (MMS) and Continental Shelf Associates, Inc., 759 Parkway Street, Jupiter, Florida 33477-9596 USA. This article has been technically reviewed by the MMS and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Service, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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RECEIVED: 28 September 2009.
ACCEPTED: 5 February 2010.