

# Genetic Differences Within and Between Species of Deep-Sea Crabs (*Chaceon*) From the North Atlantic Ocean

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**Abstract.** The deep-sea red crab *Chaceon quinquegens* is a commercially important crustacean on the Atlantic continental shelf and slope of North America. To assess genetic subdivision in *C. quinquegens*, we examined the nucleotide sequence of the mitochondrial 16S rDNA gene and the internal transcribed spacers (ITS) of the nuclear ribosomal repeat in samples from southern New England and the Gulf of Mexico. We compared those data to sequences from two congeners, a sympatric species from the Florida coast, *C. fenneri*, and an allopatric eastern Atlantic species, *C. affinis*. The 16S rDNA data consisted of 379 aligned nucleotides obtained from 37 individuals. The greatest genetic difference among geographical groups or nominal species was between *C. quinquegens* from southern New England and *C. quinquegens* from the Gulf of Mexico. Haplotypes from these two groups had a minimum of 10 differences. All 11 *C. fenneri* samples matched the most common haplotype found in *C. quinquegens* from the Gulf of Mexico, and this haplotype was not detected in *C. quinquegens* from southern New England. The three haplotypes of *C. affinis* were unique to that recognized species, but those haplotypes differed only slightly from those of *C. fenneri* and *C. quinquegens* from the Gulf of Mexico. Based on 16S rDNA and ITS data, genetic differences between *C. quinquegens* from southern New England and the Gulf of Mexico are large enough to conclude that these are different fishery

stocks. Our results also indicate that the designation of morphological species within the commercially important genus *Chaceon* is not congruent with evolutionary history. The genetic similarity of *C. affinis* from the eastern Atlantic and *C. quinquegens* from the Gulf of Mexico suggests these trans-Atlantic taxa share a more recent common history than the two populations of “*C. quinquegens*” that we examined.

## Introduction

Many genetic studies of marine populations have been carried out along the Atlantic coast of North America to examine geographical divergence within and between species. Range limits of marine species often coincide with major landforms such as Cape Hatteras (Abbott, 1974; Briggs, 1974; Grosslein and Azarovitz, 1982; Theroux and Wigley, 1983) and the Florida Peninsula (Bert, 1986; Avise, 1992, 2000; Seyoum *et al.*, 2000). Ocean temperatures in the Cape Hatteras region can be stressful to subtropical and boreal species, and there are distinct current systems north and south of this location. In the area that is now Florida, episodes of glaciation during the Pleistocene produced major changes in temperature and sea level, which may have separated populations in the Atlantic Ocean from those in the Gulf of Mexico. Most of the marine biogeographic and genetic studies have focused on species from the intertidal zone and inner continental shelf, where terrestrial and atmospheric effects have a relatively large impact on the marine environment. In the deep sea, however, other environmental factors are likely to control species distributions (Haedrich *et al.*, 1980; Blake and Grassle, 1994; Rhoads and Hecker, 1994; Grassle, 1995).

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Geryonids are a commercially important, cosmopolitan family of marine crabs typically found in mud, sand, and gravel habitats at depths of 200–1200 m (Hastie, 1995). In this study we have examined the population genetic structure of the deep-sea red crab *Chaceon quinquedens* Smith 1879 (Geryonidae). This species occurs on the outer continental shelf and slope of the western Atlantic Ocean, from the Scotian Shelf off Canada, to the Gulf of Mexico (Haefner and Musick, 1974; Williams and Wigley, 1977; Serchuk and Wigley, 1982; Erdman, 1990). *C. quinquedens* is harvested in Canada (Lawton and Duggan, 1998), in New England (Steimle *et al.*, 2001), and in the southeastern United States (Waller *et al.*, 1995).

Little information about population genetic structure in *C. quinquedens* is available to guide fishery management (Diehl and Biesiot, 1994; Waller *et al.*, 1995). To assess genetic subdivision in *C. quinquedens*, we examined the nucleotide sequence of the mitochondrial 16S rDNA gene and the internal transcribed spacers (ITS) of the nuclear ribosomal repeat. Both 16S rDNA (*e.g.*, Ó Foighil and Jozefowicz, 1999; Schubart *et al.*, 2000; Dahlgren *et al.*, 2001) and ITS (*e.g.*, Fritz *et al.*, 1994; Conole *et al.*, 2001) have been used to differentiate between closely related species and to evaluate intraspecific variation.

To provide a broad phylogeographic perspective, we compared sequence data from *C. quinquedens* with data from two congeners: *C. fenneri* Manning and Holthuis 1984, and an eastern Atlantic species, *C. affinis* Milne-Edwards and Bouvier 1894. *C. fenneri*, the golden crab, is distributed from Cape Hatteras to the Gulf of Mexico (Manning and Holthuis, 1984; Erdman, 1990). *C. quinquedens* and *C. fenneri* are the only two *Chaceon* species described from the Atlantic coast of North America. Their ranges overlap in the southeastern United States (Erdman, 1990; Diehl and Biesiot, 1994), and in the Gulf of Mexico they are segregated by depth, with *C. fenneri* occurring at depths of 300–500 m and *C. quinquedens* at 680 m or greater (Erdman, 1990). *C. affinis*, which occurs at 500–1200 m from the Cape Verde archipelago to Iceland, is allopatric with *C. quinquedens* and *C. fenneri* (Manning and Holthuis, 1981; Hastie, 1995; Pinho *et al.*, 2001; López Abellán *et al.*, 2002).

### Study Organism

Manning and Holthuis (1989) revised the taxonomy of geryonid crabs and moved most of the species in the genus *Geryon*, including *G. quinquedens*, into a new genus, *Chaceon*. *C. quinquedens* was first described from specimens collected in the Gulf of Maine (Smith, 1879). *C. quinquedens* reaches a maximum size of about 115–130 mm in carapace width (CW), males grow larger than females, and size at maturity for females is about 70 to 80 mm CW (Wigley *et al.*, 1975; Haefner, 1977; Elner *et al.*, 1987). On

the basis of molting frequency (Lux *et al.*, 1982), adult females probably do not mate every year. After mating, females carry fertilized eggs on their pleopods for up to 9 months until the larvae are released (Haefner, 1977, 1978). Brood sizes range from 160,000 to 270,000 eggs, and fecundity increases with female body size (Hines, 1988). Depending on water temperature, *C. quinquedens* larvae may remain planktonic for up to 4 months before settlement (Perkins, 1972; Sulkin and Van Heukelem, 1980; Kelly *et al.*, 1982).

Geryonid growth rates are lower than those of families of shallow-water crabs (Hastie, 1995). Complete growth curves are not available for *C. quinquedens*, although tagging studies (Lux *et al.*, 1982) indicate that the intermolt period of adults can last as long as 7 years. After studying the growth of juvenile crabs in the laboratory, Van Heukelem *et al.* (1983) projected that *C. quinquedens* could attain a carapace width of 114 mm by age 6 years.

Female and male geryonids tend to segregate by depth (Wigley *et al.*, 1975; Haefner and Musick, 1974; Haefner, 1978; Melville-Smith, 1987), but migration patterns during mating have not been described. Wigley *et al.* (1975) hypothesized that *C. quinquedens* larvae settle to the bottom in deep water and then migrate up the continental slope, where they may obtain more food and grow more rapidly. Tagging studies by Lux *et al.* (1982) and Melville-Smith (1987) showed that adult geryonids travel along and across bathymetric lines. Returns of tagged adult *C. quinquedens* over a 7-year period indicate that most recaptures occurred within 20 km of the release location. Principal movements were along the slope; shorter movements of about 6 km were observed up and down the slope, with depth changes of as much as 500 m (Lux *et al.*, 1982).

### Materials and Methods

The *Chaceon quinquedens* and *C. fenneri* specimens used in the 16S genetic study were collected from the Atlantic Ocean with traps or otter trawls (Table 1). The 13 *C. quinquedens* that were analyzed from southern New England were collected during a National Marine Fisheries Service survey in May 2001 and were obtained from eight tows made in the vicinity of Veatch and Atlantis Canyons, at depths of 465–931 m. The 10 specimens of *C. quinquedens* that were analyzed from the Gulf of Mexico were collected in August 1995 from 951 m. The 11 specimens of the golden crab (*C. fenneri*) that were analyzed from the Atlantic coast of Florida were collected in January 2002 from 335 m. The 16S rDNA data sets from all three sets of samples included both males and females. Three sequences of *C. affinis*, from the eastern Atlantic (Madeira Islands and Canary Islands), were also available in GenBank from analyses by J. Bautista and Y. Alvarez. Thus, a total of 37 individuals were represented in the 16S data (see Appendix

**Table 1**Chaceon quinquegens, *C. affinis*, and *C. fenneri*: distribution of 16S rDNA haplotypes

Haplotype	Sample Locations by Species				Total	GenBank Accession #
	Southern New England <i>C. quinquegens</i>	Gulf of Mexico <i>C. quinquegens</i>	Atlantic Florida <i>C. fenneri</i>	Eastern Atlantic <i>C. affinis</i>		
AFI				1	1	AF100914
AFII				1	1	AF100915
AFIII				1	1	AF100916
A		1			1	AY122641
B		1			1	AY122642
C		1			1	AY122643
D		7	11		18	AY122644
E	9				9	AY122645
F	4				4	AY122646
Sample Size	13	10	11	3	37	

For each haplotype, the number of individuals analyzed is given by locality. The name Eastern Atlantic refers to the Madeira and Canary Islands.

for additional collection details). For all samples, tissue was either frozen immediately or placed in 80% ethanol. Total genomic DNA was extracted using the Qiagen DNA easy tissue kit (Valencia, CA) following manufacturer's protocols.

A fragment of the mitochondrial 16S rDNA gene was amplified using standard PCR protocols. Originally, the Palumbi (1996) 16S rDNA primers (16Sar and 16Sbr) were used. Due to variability in PCR and sequencing results, a new 3' primer was designed (16Sbr.crab; 5'-TAA TTC AAC ATC GAG GTC GC-3'). One of two sets of cycling parameters was used: an initial denaturation at 94 °C for 2 min, 30× (94 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s), and a final extension at 72 °C for 7 min; alternatively, the amplification phase was 35× (94 °C for 20 s, 55 °C for 20 s, 72 °C for 30 s). PCR products were cleaned with the Qiagen PCR cleanup kit (Valencia, CA). The same primers were used in sequencing, following procedures in the Big Dye cycle sequencing kit (ABI, Foster City, CA), except that reactions were 50% Big Dye concentration in 50% volume. After the excess Dye terminators were removed with Sephadex, the reactions were run on an ABI Prism 377 automated sequencer, with samples sequenced in both directions.

Standard laboratory protocols (*e.g.*, sterile technique and negative controls) were employed to avoid contamination between samples. Because the samples of *C. quinquegens* from the Gulf of Mexico were genetically similar to those of *C. fenneri*, *C. fenneri* DNA was re-extracted and amplified from representatives to verify the results. In all cases, the re-extracted sequences matched the originals.

We also explored the internal transcribed spacer (ITS) regions of the nuclear ribosomal genes. The ITS regions of *C. quinquegens* were screened in 5 crabs from southern New England and in 10 crabs from the Gulf of Mexico. A

1376 base pair (bp) fragment was amplified using universal primers in the flanking 18S and 28S regions (ITS4 and ITS5; White *et al.*, 1990). For sequencing, we used an additional pair of primers located in the 5.8S region (5.8Sc and 5:8Sd; Hillis and Dixon, 1991). PCR and sequencing protocols were as described above, except the PCR annealing temperature was between 55 and 57 °C. We report clean single-read sequences from the two *C. quinquegens* populations (total 15 crabs) for the ITS2 region. When ITS primers were used on *C. fenneri*, we recovered multiple products, which would typically require cloning PCR products, sequencing multiple products per individual, and performing analyses that can handle multiple alleles from one individual. For these reasons, we focused our efforts on the 16S data, which were easier to collect and analyze. However, we still report the ITS data because they are relevant.

The sequence data were aligned with Clustal X (Thompson *et al.*, 1997) and proofread by eye. Two computer programs, PAUP 4.0b10 (Swofford, 2002) and MacClade version 4.0 (Maddison and Maddison, 2000), were used in the analyses. Because the results were unambiguous and involved a limited number of nucleotide substitutions, we present results only from a parsimony network analysis (*sensu* Avise *et al.*, 1979). Other analyses, including an exhaustive parsimony phylogenetic analysis, a neighbor-joining analysis using a general-time-reversible model, and maximum-likelihood analysis using a general-time-reversible model all yielded the same topology, and were entirely consistent with the parsimony network. Indels were counted as characters (not missing data) in the parsimony analyses.

## Results

The 16S rDNA data consisted of 379 aligned nucleotides. Variation was observed at 17 sites, and 12 of these were

parsimony informative (Table 2). Each unique combination of nucleotides was considered a distinct haplotype. Only three indels were observed, of which two were associated with extra bases in the *Chaceon affinis* sequences. Table 1 shows the distribution, occurrence, and GenBank accession number of each 16S haplotype observed in this study. The aligned data set was deposited in TreeBASE (<http://www.treebase.org/treebase/> accession number-SN1246).

Two 16S haplotypes were detected in the southern New England samples of *C. quinquegens*, four in the Gulf of Mexico samples of *C. quinquegens*, and only one in the *C. fenneri* samples from the Atlantic coast of Florida. The *C. affinis* data consisted of three haplotypes (Table 1). The parsimony network (Fig. 1) illustrates the phylogenetic relationships among the nine known haplotypes of *Chaceon*. Position 321 is homoplastic, occurring twice on the network. The largest genetic break observed among geographical groups or nominal species was between *C. quinquegens* from southern New England and *C. quinquegens* from the Gulf of Mexico. Haplotypes from these two groups had a minimum of 10 differences. All 11 *C. fenneri* samples matched the most common haplotype found in the *C. quinquegens* samples from the Gulf of Mexico (haplotype D). However, haplotype D was not detected in *C. quinquegens* samples from southern New England. The three haplotypes of *C. affinis* were unique to that species, although these were very similar to haplotype D, with a maximum of three differences.

The ITS data also show a genetic difference between the two *C. quinquegens* populations. Two ITS2 genotypes were identified (Fig. 2), distinguished by an indel in a microsatellite region close to the 3' end. The (AAGG)<sub>4</sub> allele was shared by all five southern New England specimens, whereas the (AAGG)<sub>5</sub> allele was found among all 10 Gulf of Mexico specimens. In addition to the repeat, a single nucleotide difference (A-T at position 1239) further distinguishes the two populations. ITS data can be found under GenBank accession numbers AY123199-AY123200.

## Discussion

We detected genetic subdivision between *Chaceon quinquegens* from the New England region of the Atlantic Ocean and *C. quinquegens* from the Gulf of Mexico by using sequence data from two genetic loci (16S and ITS). In addition, we found little or no genetic difference (16S data) between *C. quinquegens* from the Gulf of Mexico, *C. fenneri* from Eastern Florida, and *C. affinis* from the Eastern Atlantic. These results suggest that the designation of morphological species within the commercially important genus *Chaceon* is not congruent with evolutionary history. Furthermore, the genetic similarity of *C. affinis* and Gulf of Mexico *C. quinquegens* suggests these trans-Atlantic taxa share a more recent common history than the two populations of "*C. quinquegens*" that we examined. This is the first examination of large-scale genetic subdivision in *C. quinquegens*, and one of the few to genetically characterize a deep-sea organism (*e.g.*, Doyle, 1972; France and Kocher, 1996; Quattro *et al.*, 2001) not at hydrothermal vents. As such, it provides an interesting comparison to intertidal organisms upon which most of our understanding of marine phylogeography is based.

Management of the *Chaceon quinquegens* fishery began recently in the United States (Steimle *et al.*, 2001), and Cape Hatteras was designated as the dividing point between a northern and southern stock. Our genetic results lend support for managing this fishery as separate stocks. However, additional research will be required to more accurately determine stock boundaries. The genetic differences we detected probably reflect genetic drift during long periods of isolation. Minimal gene flow between these locations could have been caused by low rates of dispersal of adults between regions and by retention of larvae within regions. For intertidal organisms, at least three faunal breakpoints are known along the east coast of North America: Cape Cod, Cape Hatteras, and near Cape Canaveral. These coastal features serve as both species-range boundaries and genetic

Table 2

List of 17 variable positions in 9 haplotypes from samples of *Chaceon*

	10	30	89	98	104	123	196	219	220	222	275	292	302	321	323	324	379
AFI	G	C	T	G	C	G	C	G	C	T	G	C	—	T	G	C	C
AFII	.	.	.	—	.	.	.	.	.	.	.	.	—	.	.	T	.
AFIII	.	.	.	—	.	.	.	.	.	.	.	.	G	.	.	T	.
A	.	.	.	—	.	.	T	.	.	.	.	.	—	—	.	T	.
B	.	.	.	—	.	A	T	.	T	.	.	.	—	.	.	T	.
C	.	.	C	—	.	.	T	.	.	.	.	.	—	.	.	T	.
D	.	.	.	—	.	.	T	.	.	.	.	.	—	.	.	T	.
E	A	T	.	—	T	.	T	A	T	A	A	T	—	.	A	T	T
F	A	T	.	—	T	.	T	A	T	A	A	T	—	—	A	T	T

Numbers refer to positions along the 379 base pair 16S rDNA alignment. "." refers to an identical base, and "—" refers to an indel.



and slope has experienced transatlantic interchange at lower latitudes, suggesting that North Atlantic biogeography may be more complicated than previously assumed (also see Dahlgren *et al.*, 2000). This finding is in contrast to the widely studied situation of genetic interchange of North Atlantic boreal intertidal organisms at high latitudes (reviewed in Cunningham and Collins, 1998).

Two recognized *Chaceon* species around Florida showed no genetic differentiation even though previous studies have reported various ecological differences between *C. quinquedens* and *C. fenneri*. In the Gulf of Mexico, these two species are segregated by depth, and *C. fenneri* oviposits 3 months later in the year (Erdman, 1990). *C. quinquedens* in southern New England produces larger eggs than *C. fenneri* does in the Gulf of Mexico (Hines, 1988). Given these interspecific differences, we did not expect to find more genetic differences between the two *C. quinquedens* populations than between *C. quinquedens* from the Gulf of Mexico, *C. fenneri*, and *C. affinis*. Several explanations of the genetic similarity between these recognized species are possible, including incomplete lineage sorting, ongoing gene flow, or recent hybridization followed by a mitochondrial sweep (Skibinski *et al.*, 1999). If these lineages diverged recently (which would be consistent with morphology; see below), then the shared haplotype ("D" in *C. fenneri* and Gulf of Mexico *C. quinquedens*) might reflect incomplete sorting of mitochondrial lineages (Hoelzer *et al.*, 1999). Alternatively, the slight differences observed in these three lineages may simply represent within-population variation or environmentally induced variation. For example, *C. fenneri* and *C. quinquedens* from the Gulf of Mexico are geographically sympatric but inhabit different depth zones, which may expose them to different environmental factors. To assess the level of ongoing gene flow between these nominal species, it would be necessary to examine more rapidly evolving molecular markers, such as microsatellites. Lastly, the similarity in the 16S gene of these taxa could be due to hybridization followed by a mitochondrial gene sweep (Skibinski *et al.*, 1999). Studies of hybridization between *Chaceon* species have not been conducted, but hybridization occurs in xanthid crabs in the Gulf of Mexico-Florida region (Bert, 1986; Bert and Harrison, 1988).

*Chaceon* morphology is consistent with a hypothesis of recent speciation. Due to their similar phenotypes, *C. quinquedens*, *C. fenneri*, *C. affinis*, and even a commercial South African species, *C. maritae* Manning and Holthuis 1981, have been confused in the literature. For example, *C. maritae* was called *C. quinquedens* until 1981 (Manning and Holthuis, 1981; Melville-Smith, 1989). *C. fenneri* was identified as *C. quinquedens* by Rathbun (1937), and later as *C. affinis* by Chace (1940). Manning and Holthuis (1984) eventually described *C. fenneri* as a new species. *C. quinquedens* was reported from the coasts of South America

(Rathbun, 1937) and South Africa, but subsequent taxonomic work determined that those crabs were undescribed *Chaceon* species (Manning and Holthuis, 1988; Manning *et al.*, 1989). As in some other crab genera (Schubart *et al.*, 2001a), phenotypic characters used to distinguish among *Chaceon* species are somewhat ambiguous. These characters, which include carapace shape and color, sizes of spines, and shape of the dactyli of the walking legs (Manning and Holthuis, 1984, 1989) are mostly morphometric. Definitive apomorphies are lacking.

Given their phenotypic similarity, it is not surprising that *C. quinquedens*, *C. fenneri*, and *C. affinis* have similar genotypes. The present taxonomic classification of these species, based on phenotypic characters, is not consistent with our genetic results, because we found greater differences between populations of one species than between recognized species. Even though the common names of these species are based on carapace color (*e.g.*, red crab, golden crab), carapace color is not a reliable character for identifying species of *Chaceon*. For example, one of the specimens we genotyped from southern New England had a rare yellowish-tan carapace, which made us think that it might be a golden crab (*C. fenneri*); the *Chaceon* from southern New England are typically deep red to orange in color. The unusual yellowish-tan crab had haplotype F, which is a common haplotype in the southern New England red crab (*C. quinquedens*) population. We should note that the type locality of *C. quinquedens* is from the Gulf of Maine, indicating that if changes are to be made, the Gulf of Mexico individuals should be renamed. Before this can be done, we need a more complete understanding of the morphological and genetic variation along the North American coast.

Similar discrepancies between morphological and molecular genetic data have been noted in other crab species (Schubart *et al.*, 2001a, 2001b). In a study of *Callinectes* spp. in Venezuela, Schubart *et al.* (2001a) found that *C. bocourti* Milne Edwards 1879 and *C. maracaiboensis* Taisson 1969 had no diagnostic molecular characters to distinguish their 16S mtDNA sequences. After considering the genetic and morphological data on those species, Schubart *et al.* (2001a) concluded that one of the species should be reclassified as a junior synonym of the other, rather than considering them as different species. Another study found a lack of genetic variation between two *Brachynotus* species (Schubart *et al.*, 2001b), and concluded that additional work on reproductive isolation, genetic sequences, and morphology is needed to assess whether these are distinct species. Such examples emphasize the need for more information about the morphological and genetic variations that delimit the boundaries between many marine taxa, including *Chaceon*.

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### Literature Cited

- Abbott, R. T. 1974.** *American Seashells*. Van Nostrand Reinhold, New York.
- Avise, J. C. 1992.** Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos* **63**: 62–76.
- Avise, J. C. 2000.** *Phylogeography, the History and Formation of Species*. Harvard Univ. Press, Cambridge, MA.
- Avise, J. C., R. A. Lansman, and R. O. Shade. 1979.** The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus *Peromyscus*. *Genetics* **92**: 279–295.
- Bermingham, E., and H. Lessios. 1993.** Rate variation of protein and mtDNA evolution as revealed by sea urchins separated by the Isthmus of Panama. *Proc. Natl. Acad. Sci. USA* **90**: 2734–2738.
- Bert, T. M. 1986.** Speciation in western Atlantic stone crabs (genus *Menippe*): the role of geological processes and climatic events in the formation and distribution of species. *Mar. Biol.* **93**: 157–170.
- Bert, T. M., and R. G. Harrison. 1988.** Hybridization in western Atlantic stone crabs (genus *Menippe*): evolutionary history and ecological context influence species interactions. *Evolution* **42**: 528–544.
- Blake, J. A., and J. F. Grassle. 1994.** Benthic community structure on the U.S. Atlantic slope off the Carolinas: spatial heterogeneity in a current dominated system. *Deep-Sea Res.* **41**: 835–874.
- Briggs, J. C. 1974.** *Marine Zoogeography*. McGraw Hill, New York.
- Chace, F. A., Jr. 1940.** The brachyuran crabs. Reports on the scientific results of the *Atlantis* expeditions to the West Indies, under the joint auspices of the University of Havana and Harvard University. *Torrea* **4**: 1–67.
- Collin, R., 2001.** The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). *Mol. Ecol.* **10**: 2249–2262.
- Conole, J., N. B. Chilton, T. Jarvis, and R. B. Gasser. 2001.** Mutation scanning analysis of microsatellite variability in the second internal transcribed spacer (precursor ribosomal RNA) for three species of *Metastrongylus* (Strongylida: Metastrongyloidea). *Parasitology* **122**: 195–206.
- Cunningham, C. W., and T. M. Collins. 1998.** Beyond area relationships: extinction and recolonization in molecular marine biogeography. Pp. 297–321 in *Molecular Approaches to Ecology and Evolution*, R. DeSalle and B. Schierwater, eds. Birkhäuser Verlag, Basel.
- Dahlgren, T. G., J. R. Weinberg, and K. M. Halanych. 2000.** Phylogeography of the ocean quahog (*Arctica islandica*): influences of paleoclimate on genetic diversity and species range. *Mar. Biol.* **137**: 487–495.
- Dahlgren, T. G., B. Åkesson, C. Schander, K. M. Halanych, and P. Sundberg. 2001.** Molecular phylogeny of the model annelid *Ophryotrocha*. *Biol. Bull.* **201**: 193–203.
- Diehl, W. J., and P. M. Biesiot. 1994.** Relationships between multilocus heterozygosity and morphometric indices in a population of the deep-sea red crab *Chaceon quinque-dens* (Smith). *J. Exp. Mar. Biol. Ecol.* **182**: 237–250.
- Doyle, R. W. 1972.** Genetic variation in *Ophiomusium lymani* (Echinodermata) populations in the deep sea. *Deep-Sea Res.* **19**: 661–664.
- Elner, R. W., S. Koshio, and G. V. Hurley. 1987.** Mating behavior of the deep-sea red crab, *Geryon quinque-dens* Smith (Decapoda, Brachyura, Geryonidae). *Crustaceana* **52**: 194–201.
- Erdman, R. B. 1990.** Reproductive ecology and distribution of deep-sea crabs (Family Geryonidae) from southeast Florida and the eastern Gulf of Mexico. Ph.D. dissertation. University of South Florida. 147 pp.
- France, S. C., and T. D. Kocher. 1996.** Geographic and bathymetric patterns of mitochondrial 16S rRNA sequence divergence among deep-sea amphipods, *Eurythenes gryllus*. *Mar. Biol.* **126**: 633–645.
- Franz, D. R., and A. S. Merrill. 1980a.** Molluscan distribution patterns on the continental shelf of the Middle Atlantic Bight (Northwest Atlantic). *Malacologia* **19**: 209–225.
- Franz, D. R., and A. S. Merrill. 1980b.** The origins and determinants of distribution of molluscan faunal groups on the shallow continental shelf of the northwest Atlantic. *Malacologia* **19**: 227–248.
- Fritz, G. N., J. Conn, A. Cockburn, and J. Seawright. 1994.** Sequence analysis of the ribosomal DNA internal transcribed spacer 2 from populations of *Anophelese nuneztovari* (Diptera: Culicidae). *Mol. Biol. Evol.* **11**: 406–416.
- Grasle, J. F. 1995.** Deep-sea biodiversity. *Bull. Mar. Sci.* **57**: 281–285.
- Grosslein, M. D., and T. R. Azarovitz. 1982.** *Fish Distribution*. MESA New York Bight Atlas Monograph 15. Marine Ecosystems Analysis [MESA] Program. New York Sea Grant Institute, Albany, NY.
- Haedrich, R. L., G. T. Rowe, and P. T. Polloni. 1980.** The megabenthic fauna in the deep-sea south of New England. *Mar. Biol.* **57**: 165–179.
- Haefner, P. A., Jr. 1977.** Reproductive biology of the female deep-sea red crab, *Geryon quinque-dens*, from Chesapeake Bight. *Fish. Bull.* **75**: 91–102.
- Haefner, P. A., Jr. 1978.** Seasonal aspects of the biology, distribution and relative abundance of the deep-sea red crab *Geryon quinque-dens* Smith, in the vicinity of the Norfolk Canyon, western North Atlantic. *Proc. Natl. Shellfish. Assoc.* **68**: 49–62.
- Haefner, P. A., and J. A. Musick. 1974.** Observations on the distribution and abundance of red crabs in Norfolk Canyon and the adjacent continental slope. *Mar. Fish. Rev.* **36**: 31–34.
- Hastie, L. C. 1995.** Deep-water geryonid crabs: a continental slope resource. *Oceanogr. Mar. Biol. Annu. Rev.* **33**: 561–584.
- Hillis, D. M., and M. T. Dixon. 1991.** Ribosomal DNA: molecular evolution and phylogenetic inference. *Q. Rev. Biol.* **66**: 411–453.
- Hines, A. H. 1988.** Fecundity and reproductive output in two species of deep-sea crabs, *Geryon femeri* and *G. quinque-dens* (Decapoda: Brachyura). *J. Crustac. Biol.* **8**: 557–562.
- Hoelzer, G. A., J. Wallman, and D. J. Melnick. 1999.** The effects of social structure, geographical structure, and population size on the evolution of mitochondrial DNA: II. Molecular clocks and the lineage sorting period. *J. Mol. Evol.* **47**: 21–31.
- Kelly, P., S. D. Sulkin, and W. F. Van Heukelem. 1982.** A dispersal model for larvae of the deep sea red crab *Geryon quinque-dens* based on behavioral regulation of vertical migration in the hatching stage. *Mar. Biol.* **72**: 35–43.
- Knowlton, N., L. A. Weight, L. A. Solórzano, D. K. Mills, and E. Bermingham. 1993.** Divergence of proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science* **260**: 1629–1632.
- Lawton, P., and D. Duggan. 1998.** *Scotian Shelf Red Crab*. DFO

- Science Stock Status Report C3-11*. Dept. of Fisheries and Oceans, Dartmouth, Nova Scotia, Canada.
- López Abellán, L. J., E. Balguerías, and V. Fernandez-Vergaz. 2002.** Life history characteristics of the deep-sea crab *Chaceon affinis* population off Tenerife (Canary Islands). *Fish. Res.* **58**: 231–239.
- Lux, F. E., A. R. Ganz, and W. F. Rathjen. 1982.** Marking studies on the red crab *Geryon quinquegens* Smith off southern New England. *J. Shellfish Res.* **2**: 71–80.
- Maddison, W. P., and D. R. Maddison. 2000.** *MacClade: Analysis of Phylogeny and Character Evolution version 4.0*. Sinauer Associates, Sunderland, MA.
- Manning, R. B., and L. B. Holthuis. 1981.** West African brachyuran crabs (Crustacea: Decapoda). *Smithson. Contrib. Zool.* **306**: 379.
- Manning, R. B., and L. B. Holthuis. 1984.** Two new genera and nine new species of geryonid crabs (Crustacea, Decapoda, Geryonidae). *Proc. Biol. Soc. Wash.* **97**: 666–673.
- Manning, R. B., and L. B. Holthuis. 1988.** South African species of the genus *Geryon* (Crustacea, Decapoda, Geryonidae). *Ann. S. Afr. Mus.* **98**: 77–92.
- Manning, R. B., and L. B. Holthuis. 1989.** *Geryon fenneri*, a new deep-water crab from Florida (Crustacea: Decapoda: Geryonidae). *Proc. Biol. Soc. Wash.* **102**: 50–77.
- Manning, R. B., M. S. Tavares, and E. F. Albuquerque. 1989.** *Chaceon ramosae*, a new deep-water crab from Brazil (Crustacea: Decapoda: Geryonidae). *Proc. Biol. Soc. Wash.* **102**: 646–650.
- McMillen-Jackson, A. L., T. M. Bert, and P. Steele. 1994.** Population genetics of the blue crab *Callinectes sapidus*: modest population structuring in a background of high gene flow. *Mar. Biol.* **118**: 53–65.
- Melville-Smith, R. 1987.** Movements of deep-sea red crab (*Geryon maritae*) off South West Africa/Namibia. *S. Afr. J. Zool.* **22**: 143–152.
- Melville-Smith, R. 1989.** A growth model for the deep-sea red crab (*Geryon maritae*) off South West Africa/Namibia (Decapoda, Brachyura). *Crustaceana* **56**: 279–292.
- Ó Foighil, D., and C. J. Jozefowicz. 1999.** Amphi-Atlantic phylogeography of direct-developing lineages of *Lasea*, a genus of brooding bivalves. *Mar. Biol.* **135**: 115–122.
- Palumbi, S. R. 1996.** Nucleic acids II: the polymerase chain reaction. Pp. 205–248 in *Molecular Systematics*, D. M. Hillis, C. Mortiz, and B. K. Mable, eds. Sinauer Associates, Sunderland, MA.
- Perkins, H. C. 1972.** The larval stages of the deep sea red crab, *Geryon quinquegens* Smith, reared under laboratory conditions (Decapoda: Brachyrhyncha). *Fish. Bull.* **71**: 69–82.
- Pinho, M. R., J. M. Goncalves, H. R. Martins, and G. M. Menezes. 2001.** Some aspects of the biology of the deep-water crab, *Chaceon affinis* (Milne-Edwards and Bouvier, 1894) off the Azores. *Fish. Res.* **51**: 283–295.
- Quattro, J. M., M. R. Chase, M. A. Rex, T. W. Greig, and R. J. Etter. 2001.** Extreme mitochondrial DNA divergence within populations of the deep-sea gastropod *Frigidivalvania brychia*. *Mar. Biol.* **139**: 1107–1113.
- Rathbun, M. J. 1937.** The oxystomatous and allied crabs of America. *U. S. Natl. Mus. Bull.* **166**: 1–278.
- Rhoads, D. C., and B. Hecker. 1994.** Processes on the continental slope off North Carolina with special reference to the Cape Hatteras region. *Deep-Sea Res.* **41**: 965–980.
- Schubart, C. D., R. Diesel, and S. B. Hedges. 1998.** Rapid evolution to terrestrial life in Jamaican crabs. *Nature* **393**: 363–365.
- Schubart, C. D., J. E. Neigel, and D. L. Felder. 2000.** Use of mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *Crustac. Issues* **12**: 817–830.
- Schubart, C. D., J. E. Conde, C. Carmona-Suárez, R. Robles, and D. L. Felder. 2001a.** Lack of divergence between 16S mtDNA sequences of the swimming crabs *Callinectes bocourti* and *C. maracaiboensis* (Brachyura: Portunidae) from Venezuela. *Fish. Bull.* **99**: 475–481.
- Schubart, C. D., J. A. Cuesta, and A. Rodriguez. 2001b.** Molecular phylogeny of the crab genus *Brachynotus* (Brachyura: Varunidae) based on the 16S rRNA gene. *Hydrobiologia* **449**: 41–46.
- Serchuk, F. M., and R. L. Wigley. 1982.** Deep-sea red crab, *Geryon quinquegens*. Pp. 125–129 in *Fish Distribution*, M. D. Grosslein and T. R. Azarovitz, eds. MESA New York Bight Atlas Monograph 15. N. Y. Sea Grant Institute, Albany, NY.
- Seyoum, S., M. D. Tringali, T. M. Bert, D. McElroy, and R. Stokes. 2000.** An analysis of population genetic structure in red drum, *Sciaenops ocellatus*, based on mtDNA control region sequences. *Fish. Bull.* **98**: 127–138.
- Skibinski, D. O. F., C. Gallagher, and H. Quesada. 1999.** On the roles of selection, mutation and drift in the evolution of mitochondrial DNA diversity in British *Mytilus edulis* (Mytilidae; Mollusca) populations. *Biol. J. Linn. Soc.* **68**: 195–213.
- Smith, S. I. 1879.** The stalk-eyed crustaceans of the Atlantic coast of North America north of Cape Cod. *Trans. Connecticut Acad. Arts Sci.* **5**: 27–138.
- Steimle, F. W., C. A. Zetlin, and S. Chang. 2001.** Red deepsea crab, *Chaceon (Geryon) quinquegens*, life history and habitat characteristics. *NOAA Tech. Memo. NMFS-NE-163*. U.S. Dept. of Commerce, NOAA, NMFS, Woods Hole, MA.
- Sulkin, S. D., and W. F. Van Heukelem. 1980.** Ecological and evolutionary significance of nutritional flexibility in planktotrophic larvae of the deep sea red crab *Geryon quinquegens* and the stone crab *Menippe mercenaria*. *Mar. Ecol. Prog. Ser.* **2**: 91–95.
- Swofford, D. L. 2002.** *PAUP\* 4.0 Phylogenetic Analysis Using Parsimony*. Sinauer, Sunderland, MA.
- Theroux, R. B., and R. L. Wigley. 1983.** Distribution and abundance of east coast bivalve mollusks based on specimens in the National Marine Fisheries Service Woods Hole Collection. *NOAA Tech. Rept. NMFS SSRF-768*. U.S. Dept. of Commerce, NOAA, NMFS, Seattle, WA.
- Thompson, J., T. Gibson, F. Plewniak, F. Jeanmougin, and D. Higgins. 1997.** The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **22**: 4876–4882.
- Van Heukelem, W., M. C. Christman, C. E. Epifanio, and S. D. Sulkin. 1983.** Growth of *Geryon quinquegens* (Brachyura: Geryonidae) juveniles in the laboratory. *Fish. Bull.* **81**: 903–905.
- Waller, R., H. Perry, C. Trigg, J. McBee, R. Erdman, and N. Blake. 1995.** Estimates of harvest potential and distribution of the deep sea red crab, *Chaceon quinquegens*, in the north central Gulf of Mexico. *Gulf Res. Rep.* **9**: 75–84.
- White, T. J., T. Burns, S. Lee, and J. Taylor. 1990.** Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in *PCR Protocols: A Guide to Methods and Applications*, M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, New York.
- Wigley, R. L., R. B. Theroux, and H. E. Murray. 1975.** The deep-sea red crab. *Mar. Fish. Rev.* **37**: 1–21.
- Williams, A. B., and R. L. Wigley. 1977.** Distribution of decapod crustacea off northeastern United States based on specimens at the Northeast Fisheries Science Center, Woods Hole, Massachusetts. *NOAA Tech. Report NMFS Circular 407*. NOAA, Woods Hole, MA.

## Appendix

Summary of collection data for specimens of *Chaceon* (deep-sea red crab) used in the 16S genetic study

Southern New England, <i>Chaceon quinquegens</i> , leg muscle: collected by J. Weinberg							
Sample	Sample ID#	Haplotype	Coordinates Lat/Long	Date	Depth (m)	Carapace Size (mm)	
						Width	Length
Female 17	SNE 179 (#17)	E	Station 179 N39° 53.42' W69° 42.18'	5/16/01	465	102	
Male 05	SNE 180 (#5)	E	Station 180		502	112	
Female 10	SNE 180 (#10)	F	N39° 52.75' W69° 45.29'			84	
Female 03	SNE 181 (#3)	E	Station 181	5/17/01	646	90	
Male 16	SNE 181 (#16)	E	N39° 51.81' W69° 43.66'			113	
Female 07	SNE 182 (#7)	E	Station 182		694	84	
Male 11	SNE 182 (#11)	E	N39° 51.96' W69° 44.68'			114	
Female 01	SNE 183 (#1)	F	Station 183		731	112	
Male 02	SNE 183 (#2)	E	N39° 51.17'			72	
Female 03	SNE 183 (#3)	E	W69° 51.73'			68	
Male 01	SNE 184 (#1)	E	Station 184	5/18/01	914	114	
			N39° 49.20' W70° 33.19'				
Female 02	SNE 185 (#2)	F	Station 185 N39° 49.20' W70° 33.19'		931	102	
Female 06	SNE 186 (#6)	F	Station 186 N39° 53.32' W70° 36.51'		557	74	
Gulf of Mexico, <i>Chaceon quinquegens</i> , gonad: collected by P. Biesiot & H. Perry							
Sample	Sample ID#	Haplotype	Coordinates Lat/Long	Date	Depth	Carapace Size	
						Width	Length
Male 1	GoM-M1	D	N27° 49' W85° 24'	8/23/95	951	146.9	118.8
Male 2	GoM-M2	D				134.2	108.1
Male 3	GoM-M3	D				131.7	104.2
Male 4	GoM-M4	D				135.4	105.8
Male 5	GoM-M5	D				122.5	97.2
Female 51	GoM-F51	A				118.5	94.1
Female 52	GoM-F52	B				110.9	90.3
Female 53	GoM-F53	C				133.0	104.2
Female 54	GoM-F54	D				110.1	89.4
Female 55	GoM-F55	D				115.9	93.7
Atlantic Side of Florida, <i>Chaceon affinis</i> , leg muscle: collected by P. Brown-Eyo, R. Nielson, & D. Harper							
Sample	Sample ID#	Haplotype	Coordinates	Date	Depth	Carapace Size	
						Width	Length
01 Male	GC-1M	D	So. FL., Lat 25° Long 79°	1/11/02	335	146	
02 Male	GC-2M	D				172	
03 Male	GC-3M	D				137	
04 Male	GC-4M	D				171	
05 Male	GC-5M	D				125	
01 Female	GC-1F	D				137	
02 Female	GC-2F	D				111	
03 Female	GC-3F	D				117	
04 Female	GC-4F	D				120	
05 Female	GC-5F	D				137	
06 Female	GC-6F	D				121	