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 quinquedens is a commercially important crustacean on the Atlantic continental shelf and slope of North America. 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 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. quinquedens from southern New England and C. quinquedens 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. quinquedens from the Gulf of Mexico, and this haplotype was not detected in C. quinquedens 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. quinquedens from the Gulf of Mexico. Based on 16S rDNA and ITS data, genetic differences between C. quinquedens 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. quinquedens from the Gulf of Mexico suggests these trans-Atlantic taxa share a more recent common history than the two populations of “C. quinquedens” 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 Wigeley, 1977; Serchuk and Wigeley, 1982; Erdman, 1990). *C. quinquedens* is harvested in Canada (Lawton and Duggan, 1998), in New England (Steinle 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., O 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...
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. quinquedens_ 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. quinquedens_ 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. quinquedens_ 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 Chaceon quinquedens, four in the Gulf of Mexico samples of Chaceon quinquedens, and only one in the C. feni
eri 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. quinquedens from southern New England and C. quinquedens from the Gulf of Mexico. Haplotypes from these two groups had a minimum of 10 differences. All 11 C. feni
eri samples matched the most common haplotype found in the C. quinquedens group. However, haplotype D was not detected in C. quinquedens 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. quinquedens populations. Two ITS2 genotypes were identified (Fig. 2), distinguished by an indel in a microsatellite region close to the 3’ end. The (AAGG)_4 allele was shared by all five southern New England specimens, whereas the (AAGG)_3 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 quinquedens from the New England region of the Atlantic Ocean and C. quinquedens 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. quinquedens from the Gulf of Mexico, C. feni
eri 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. quinquedens suggests these trans-Atlantic taxa share a more recent common history than the two populations of “C. quinquedens” that we examined. This is the first examination of large-scale genetic subdivision in C. quinquedens, 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 quinquedens 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

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

Numbers refer to positions along the 379 base pair 16S rDNA alignment. “.” refers to an identical base, and “—” refers to an indel.
barriers to gene flow (Franz and Merrill, 1980a, b; Avise, 1992, 2000; Bert, 1986; Collin 2001; but see McMillen-Jackson et al., 1994). A recent study by Collin (2001) is the only molecular investigation to date with adequate sampling across all three breakpoints (but see Avise, 1992, 2000). Her results are similar to ours: she found that two traditionally recognized species of the gastropod Crepidula show significant genetic subdivision between New England and the Gulf of Mexico. Because our sampling was limited, we cannot distinguish how the three known breakpoints contributed to the large genetic difference we observed. Alternatively, the observed divergence could represent variation at the two ends of a cline. We are currently trying to obtain samples along the North American east coast to address these issues.

Observing at least 10 nucleotide substitutions between New England and the Gulf of Mexico in a gene as conserved as the 16S was surprising. Although no lineage-specific calibration for substitution rates has been determined for geryonid crab 16S data, grapsid crabs have been shown to have a rate of 0.65% per MY (Schubart et al., 1998). Differences in mtDNA sequences between geminate marine invertebrate taxa on the two sides of the Isthmus of Panama suggest that sequence divergence is in the range of 1%–4% per MY (Bermingham and Lessios, 1993; Knowlton et al., 1993). If one assumes that the Chaceon rate is between 0.5% and 2% per MY, then the New England and Gulf of Mexico populations have been separated for 0.7–2.9 MY (rate homogeneity test not significantly rejected), placing their separation during the Pleistocene or Pliocene. Their genetic isolation may have arisen in association with fluctuating sea level due to glaciation events. However, without additional sampling to understand where genetic breaks occur, determining the exact mechanism or mechanisms of genetic isolation is not possible.

Based on the finding of only a single substitution separating C. affinis from Chaceon in the Gulf of Mexico, we conclude that there has been very recent gene exchange with the Old World at subtropical/tropical latitudes. Although adult C. quinquedens do not migrate extensively (Lux et al., 1982), the larvae have the potential to disperse great distances because they can spend up to 4 months in the plankton (Perkins, 1972; Sulkin and Van Heukelem, 1980; Kelly et al., 1982). Our sample of C. affinis was admittedly limited, and given the cryptic morphology of Chaceon, we do not assume that three individuals from the Canary Islands are a representative sample of C. affinis throughout its extensive range, reported to extend as far north as Iceland (Manning and Holthuis, 1981; Hastie, 1995; Pinho et al., 2001; López Abellán et al., 2002). Nonetheless, our data provide evidence that this species from the continental shelf

**C. quinquedens ITS 2**

Southern New England: CAAAACGAAGAAAGGAAGGAAGG----GACCAGAGACTAAAC

Gulf of Mexico: CAAAACGAAGAAAGGAAGGAAGGAAGGACCAGAGACTAAAC

**Figure 1.** Parsimony network of 16S haplotypes for Chaceon quinquedens, C. fenneri, and C. affinis. Lines crossing branches represent inferred nucleotide substitutions, and thus, the minimum observed number of nucleotide differences between haplotypes. The solid line, dashed line, and shaded area around certain haplotypes indicate three geographical regions: southern New England, Atlantic coast of Florida-Gulf of Mexico, and eastern Atlantic specimens, respectively. Note, 16S position 321 is homoplastic and is represented on the network twice (between A and D, and E and F).

**Figure 2.** A partial alignment of ITS2 (positions 1263-1303) in Chaceon quinquedens, showing the fixed variation in a microsatellite repeat (AAGG) distinguishing between five southern New England samples and 10 Gulf of Mexico samples. The exact position of the indel could be at any of the five (AAGG) sites.
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 Chaceon 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 identified 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. bocourtii 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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## Appendix

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

### Southern New England, *Chaceon quinquedens*, leg muscle: collected by J. Weinberg

| Sample | Sample ID# | Haplotype | Coordinates | Date  | Depth (m) | Carapace Size (mm) |
|--------|------------|-----------|-------------|-------|-----------|--------------------|
| 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 N39° 52.75' W69° 45.29' | | 502 | 112 |
| Female 10 | SNE 180 (#10) | F | Station 180 N39° 51.81' W69° 43.66' | | 646 | 90 |
| Female 03 | SNE 181 (#3) | E | Station 181 N39° 51.81' W69° 43.66' | 5/17/01 | 646 | 113 |
| Male 16 | SNE 181 (#16) | E | Station 182 N39° 51.96' W69° 44.68' | | 694 | 84 |
| Female 07 | SNE 182 (#7) | E | Station 182 N39° 51.96' W69° 44.68' | | 694 | 114 |
| Male 11 | SNE 182 (#11) | E | Station 182 N39° 51.73' W69° 43.66' | | 694 | 68 |
| Female 01 | SNE 183 (#1) | F | Station 183 N39° 51.17' W69° 43.66' | | 731 | 112 |
| Male 02 | SNE 183 (#2) | E | Station 183 N39° 51.17' W69° 43.66' | | 731 | 72 |
| Female 03 | SNE 183 (#3) | E | Station 184 N39° 49.20' W70° 33.19' | 5/18/01 | 914 | 114 |
| Male 01 | SNE 184 (#1) | E | Station 184 N39° 49.20' W70° 33.19' | | 914 | 68 |
| Female 02 | SNE 185 (#2) | F | Station 185 N39° 49.20' W70° 33.19' | 9/18/95 | 931 | 102 |
| Female 06 | SNE 186 (#6) | F | Station 186 N39° 53.32' W70° 36.51' | | 557 | 74 |

### Gulf of Mexico, *Chaceon quinquedens*, gonad: collected by P. Biesiot & H. Perry

| Sample | Sample ID# | Haplotype | Coordinates | Date  | Depth (m) | Carapace Size (mm) |
|--------|------------|-----------|-------------|-------|-----------|--------------------|
| Male 1 | GoM-M1 | D | N27° 49' W85° 24' | 8/23/95 | 951 | 146.9 | 118.8 |
| Male 2 | GoM-M2 | D | N27° 49' W85° 24' | | | |
| Male 3 | GoM-M3 | D | N27° 49' W85° 24' | | | |
| Male 4 | GoM-M4 | D | N27° 49' W85° 24' | | | |
| Male 5 | GoM-M5 | D | N27° 49' W85° 24' | | | |
| Female 51 | GoM-F51 | A | | | | |
| Female 52 | GoM-F52 | B | | | | |
| Female 53 | GoM-F53 | C | | | | |
| Female 54 | GoM-F54 | D | | | | |
| Female 55 | GoM-F55 | D | | | | |

### Atlantic Side of Florida, *Chaceon affinis*, leg muscle: collected by P. Brown-Eyo, R. Nielson, & D. Harper

| Sample | Sample ID# | Haplotype | Coordinates | Date  | Depth (m) | Carapace Size (mm) |
|--------|------------|-----------|-------------|-------|-----------|--------------------|
| 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 |