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Morphological and genetic evidence for vicariance and refugium in Atlantic and Gulf of Mexico populations of the hermit crab *Pagurus longicarpus*

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Abstract The number and wide variety of southeastern United States marine taxa with significant differentiation between Gulf of Mexico and Atlantic Ocean populations suggests that these taxa may have experienced major vicariance events, whereby populations were subdivided by geological or ecological barriers. The present study compared variation in morphology, allozymes, and mtDNA in Gulf of Mexico and western Atlantic populations of the longwrist hermit crab *Pagurus longicarpus* Say collected during 1997 and 1998. Combined Atlantic populations had significantly fewer denticles on the second segment of the third maxilliped than did Gulf of Mexico populations, and the mean ratio of dactyl length to propodus length was significantly greater in the Atlantic crabs than in the Gulf of Mexico crabs. Allozyme allele frequencies at three loci showed genetic differentiation between a Gulf of Mexico population and two Atlantic populations. Analysis of mtDNA sequence data revealed a clear reciprocal monophyly between Gulf and Atlantic populations, with an estimated divergence age of ~0.6 million years ago. This estimated age of divergence is significantly more recent than an age previously estimated for its congener *Pagurus pollicaris* (~4 million years ago), suggesting that species with a similar genetic break between Gulf and Atlantic populations may not necessarily share an identical history. Surprisingly, there is evidence of geographic subdivision within Atlantic populations of *P. longicarpus* along the east coast of North America. This differentiation is especially strong between Nova Scotia and southern populations, suggesting that the Nova Scotia population may represent survivors from a northern refugium during the last glacial maximum.

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Introduction

A wide variety of marine fauna found in the southeastern United States, from hydroids to sturgeon, have been the subject of population genetics studies in the past two decades (see reviews by Avise 1992; Cunningham and Collins 1994, 1998; Felder and Staton 1994; and additional papers by McCommas 1982; Hoagland 1984; Cunningham et al. 1991; Staton and Felder 1995; Foighil et al. 1996; Mangum and McKenney 1996; Tam et al. 1996). Many studies have found significant geographic differentiation between populations in the Gulf of Mexico and along the Atlantic coast, with a hybrid zone in the Cape Canaveral area. Most surprising was the discovery of significant genetic differentiation in taxa that are continuously distributed around the Florida peninsula, and which showed little or no morphological variation (e.g. the American oyster, *Crassostrea virginica*). Perhaps less surprising has been the discovery of significant genetic differentiation in taxa that are found in the Gulf and the Atlantic, but are disjunct in the tropical waters off southern Florida. These disjunct taxa are so numerous that they have been designated the Carolinian biogeographic fauna (Briggs 1974; Cunningham and Collins 1998).

The discovery of so many taxa with significant geographic differentiation between the Gulf and the Atlantic suggests that these taxa may have experienced major vicariance events, whereby populations were subdivided by geological or ecological barriers. For example, Reeb and Avise (1990) suggested that lowering of the sea level and concurrent changes in climate during glacial maxima resulted in a lack of estuarine habitat along the southern portion of the Florida peninsula.

The longwrist hermit crab, *Pagurus longicarpus*, has been collected in the western Atlantic from Mahone Bay

on the east coast of Nova Scotia (Young, personal observation) to Hutchinson Island on the east coast of Florida (Camp et al. 1977) and in the Gulf of Mexico from the Shark River in southwestern Florida (Rouse 1970) to Galveston Bay, Texas (Fotheringham 1976). That is, *P. longicarpus* has a disjunct distribution in the western Atlantic and the Gulf of Mexico (see Fig. 1). Unlike many other taxa with a similar disjunct distribution, Gulf of Mexico and Atlantic populations of *P. longicarpus* exhibit morphological differences (Provenzano 1959). Together with its disjunct distribution around southern Florida, these morphological differences are consistent with distinct evolutionary histories for Gulf of Mexico and Atlantic populations.

The only previous study comparing Gulf of Mexico and Atlantic *P. longicarpus* found very little genetic variation. In a study using 16 S mtDNA, Cunningham et al. (1992) found only a single base pair (bp) substitution distinguishing Gulf of Mexico and Atlantic individuals (0.2% divergence). This contrasts with eight substitutions (2% divergence) in the same gene distinguishing Gulf of Mexico and Atlantic individuals of the hermit crab *Pagurus pollicaris*, which is also disjunct around southern Florida (Cunningham et al. 1992). This large difference in genetic divergence is consistent with a hypothesis that these two hermit crab species have experienced vicariance events at different times. Cases in which taxa have identical disjunctions, but have experienced vicariance at different times have been called examples of "pseudocongruence" (Page 1990; Cunningham and Collins 1994).

One possibility is that 16 S mtDNA is simply evolving too slowly to detect the divergence between Gulf of Mexico and Atlantic populations of *P. longicarpus*, but one cannot rule out the possibility that, despite the disjunction across southern Florida, there has been recent gene flow between the Gulf of Mexico and the Atlantic. In order to distinguish between these possibilities – and in addition to detailed morphological comparisons – nuclear (allozyme) and mitochondrial (cytochrome oxidase I mtDNA sequence, COI) data were collected from several Gulf of Mexico and Atlantic populations of *P. longicarpus*.

Materials and methods

Sample collection and storage

Fifty live *Pagurus longicarpus* Say were obtained between April and September during 1997 and 1998 from each of ten locations (identifying letter in parentheses): near Meisner's Island in Mahone Bay, Nova Scotia (NS); Damariscotta River, Maine (M); Salem Harbor, Salem, Massachusetts (S); Vineyard Sound, Woods Hole, Massachusetts (W) (Marine Biological Laboratory Supply Center); off Beaufort, North Carolina (NC); North Inlet, South Carolina (SC); New College Bayfront in Sarasota Bay, Florida (F); the east end of Shell Point, Apalachee Bay, Panacea, Florida (P) (Gulf Specimen Marine Supply); and East Beach, Red Fish Cove in West Galveston Bay, Texas (T) (see Fig. 1). All specimens other than those from Woods Hole and Apalachee Bay (purchased from commercial suppliers) were collected by the authors or colleagues as listed in the "Acknowledgements". The Mahone Bay population represents an extension of the northern limit to the species' Atlantic range, previously reported as Chignecto Bay between New Brunswick and Nova Scotia (Williams 1984).

To place this study in a larger context, four other species were collected for the purposes of mtDNA analysis. These included samples from Woods Hole, Massachusetts, and Apalachee Bay, Florida, of *Pagurus pollicaris*, whose distribution is similar to that of *P. longicarpus*, as well as three northern species belonging to the *bernhardus* group of hermit crabs (McLaughlin 1974), which participated in the trans-Arctic interchange ca. 3.5 million years ago (Vermeij 1991): *Pagurus armatus* (Bering Sea), *Pagurus acadianus* (Damariscotta River, Maine), and *Pagurus bernhardus* (Roscoff, France).

Live crabs were removed from their shells, and approximately 100–200 µl of crab abdomen tissue was preserved in 1 ml 95% ethanol for examination of DNA signatures. Right chelipeds and third maxillipeds were removed and preserved in a mixture of 70% ethanol plus glycerol for 2 weeks, transferred to 90% ethanol for 1 week, and then maintained in 100% ethanol for examination by scanning electron microscopy; two walking legs from each crab were preserved in 70% ethanol for length and width measurements. The remainder of each crab was homogenized in 1 ml grinding

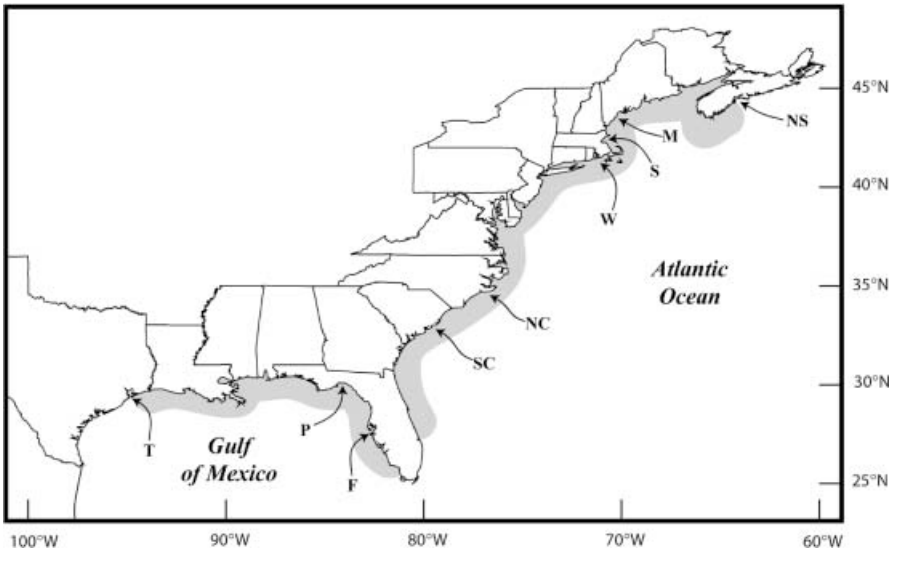

Fig. 1. *Pagurus longicarpus*. Distribution (*shaded regions*) and locations of populations used in the study (*NS* Mahone Bay, Nova Scotia; *M* Damariscotta River, Maine; *S* Salem Harbor, Massachusetts; *W* Woods Hole, Massachusetts; *NC* Beaufort, North Carolina; *SC* North Inlet, South Carolina; *F* Sarasota Bay, Florida; *P* Apalachee Bay, Florida; *T* Galveston Bay, Texas)

buffer (1.21 g Tris, 0.030 g EDTA, 0.040 g NADP, 0.033 g NAD per 100 ml distilled water) and centrifuged for 1–2 min. Individual 50 µl aliquots of supernatant were frozen in 1.5 ml Eppendorf tubes at –80°C for allozyme electrophoresis.

Morphological examination

Right chelipeds and third maxillipeds of crabs from four populations (Nova Scotia; Apalachee Bay, Florida; South Carolina; and Texas) were mounted on metal stubs with double-sided adhesive tape. Using colloidal silver as a ground, the stubs were sputter coated (Fisions Instruments model SC500 sputter coater) with gold for 4 min. Specimens were observed and photographed in either of two Jeol (JeolUSA, Peabody, Massachusetts) scanning electron microscopes operated at 15 kV: a model JSM-35 at Salem State College, Salem, Massachusetts, or a model JSM-840 at the Marine Biological Laboratory, Woods Hole, Massachusetts. The numbers of denticles on each cheliped and on the second segment of the exopod of each third maxilliped were counted. Walking legs of crabs from three populations (Nova Scotia; Salem, Massachusetts; and Sarasota, Florida) were measured using an ocular micrometer, and ratios of total length to maximum width and dactyl length to propodus length were then calculated for each population.

Allozyme electrophoresis

Allozyme electrophoresis was conducted for five enzymes in three populations (Nova Scotia; Salem, Massachusetts; and Sarasota Bay, Florida) using Gelman Optiphor-10 cellulose acetate gels following protocols modified from Richardson et al. (1986). Gels were run horizontally under refrigeration at 15°C for 30 min at 200 V. Enzymes and buffer systems used were as follows: glucose-6-phosphate isomerase (EC 5.3.1.9, Gpi), malate dehydrogenase (EC 1.1.1.37, Mdh), and phosphoglucomutase (EC 2.7.5.1, Pgm) in 0.05 M Tris-maleate at pH 7.8 (buffer C in Richardson et al. 1986); lactate dehydrogenase (EC 1.1.1.27, Ldh) in 0.02 M phosphate at pH 7.0 (buffer B in Richardson et al. 1986); and malic enzyme (EC 1.1.1.40, Me) in 0.1 M Tris-maleate at pH 7.8 (buffer G in Richardson et al. 1986). Following electrophoresis, gels were stained using an agar overlay consisting of 2 ml agar (3.6 g per 250 ml distilled water) added to stain recipes in Richardson et al. (1986).

Allozyme data were analyzed using the phylogeny inference computer package PHYLIP 3.572c (Felsenstein 1999). The GENDIST program was used to compute, from a set of gene frequencies in different populations, the Cavalli-Sforza and Edwards (1967) chord distance, which is most appropriate when the major differences between populations are in frequencies (Cunningham and Collins 1994; Felsenstein 1999). This measure was used to produce an UPGMA dendrogram (Sokal and Michener 1958).

DNA extraction, amplification, and sequencing

Mitochondrial DNA was isolated from *P. longicarpus*, *P. pollicaris*, *P. armatus*, *P. acadianus*, and *P. bernhardus* tissues using a Chelex 100 resin extraction procedure (Goff and Moon 1993). Subsequently, a fragment of the COI gene (645-bp-aligned sequence) was amplified using the primers TrpLF and 9 (Simon et al. 1994). PCR amplification was performed in 50 µl reactions containing 1 µl DNA, 0.4 mM of each primer, 5 µl 10 × pyrostase buffer (0.25 M KCl, 10 mM Tris pH 8.8, 0.125 mM $MgCl_2$, and 1% Triton X), 0.2 mM deoxynucleotide mix (Sigma), 2.5 mM $MgCl_2$ (Promega), and 1.25 U AmpliTaq polymerase (Perkin-Elmer). A Perkin-Elmer 9600 thermocycler was used, with a cycling profile of 94°C (180 s), 35 cycles of 94°C (15 s)/50°C (90 s)/72°C (150 s), and 72°C (300 s).

Amplification products were purified using Promega Wizard PCR Preps and resuspended in 30 µl ddH_2O. Purified products were used in a cycle-sequencing reaction using fluorescently labeled di-deoxy terminators. Sequencing was performed in the Perkin-Elmer 9600 thermocycler, in 10 l reactions containing 5 µl DNA, 1 mM primer, 3 µl Big Dye buffer and 1 µl Big Dye terminator (Perkin-Elmer), with a profile of 25 cycles of 96°C (10 s)/50°C (5 s)/60°C (240 s). Unincorporated di-deoxynucleotides were removed using CentriSep spin columns (Princeton Preparations) following the manufacturer's protocol. The products were then analyzed using an ABI Prism 377 automated sequencer (Perkin-Elmer).

Phylogenetic and population genetic analysis of mtDNA data

The mtDNA sequences were subsequently analyzed using the Phylogenetic Analysis Using Parsimony* computer program (PAUP* 4.0b8) (Swofford 1998). Phylogenetic analyses were carried out on third codon positions only. This is for several reasons, discussed in detail by Wares and Cunningham (2001). First, third positions are more likely to be neutral. Second, if all three positions are included, a correction for among-site variation is necessary. As noted by Wares and Cunningham (2001), this is problematic for closely related sequences. Third positions are more likely to represent a homogeneous class of rate variation. MODELTEST (Posada and Crandall 1998) was used to find the best-fit model of evolution. The HKY85 distance was chosen with no correction for among-site rate variation. HKY85, which distinguishes between transitions and transversions, is very similar to the F84 model used by Wares and Cunningham (2001). To allow comparison between these studies, the F84 model was used here as well to estimate distances and to search for most likely trees. Heuristic searches were carried out using PAUP* 4.0 with the default search options.

Net genetic divergence (Da) (Nei and Li 1979; Eq. 10.21 in Nei 1987) between populations was calculated using a spreadsheet with F84 distances (not included in AMOVA) calculated using PAUP* 4.0. Da was also calculated for Jukes–Cantor (JC) distances (Jukes and Cantor 1969) using DNAsp (Rozas and Rozas 1999), which also calculates the standard deviation of Da (using Eqs. 10.20–10.24 in Nei 1987). Since DNAsp only uses JC distances, which significantly underestimate divergence, we multiplied the standard deviation of Da calculated for Jukes–Cantor by the proportion DaF84/DaJC.

Arlequin 2.0 (Schneider et al. 1999) was used to determine significance of Da from pairwise populations using 1,000 permutations (since F84 is not offered in Arlequin, the Tamura–Nei distance was used for the permutations).

Results

Morphological examination

Cheliped denticle counts showed no significant variation among populations. There was considerable overlap in range of counts of third maxilliped denticles among populations (see Table 1), but our a priori hypothesis of differences between the Gulf and Atlantic (Provenzano 1959) was strongly supported. The mean number of third maxilliped denticles for the two Atlantic populations examined (Nova Scotia + South Carolina) combined (10.42 mm) was significantly different than the mean for the two Gulf populations (Florida + Texas) combined (11.89 mm) ($P < 0.004$, $t = 0.655$, $df = 19$). In six *t*-tests between the four populations, the only two comparisons that were significantly different after applying a Bonferroni correction were both between Gulf and Atlantic populations (Florida vs. Nova Scotia, Florida vs. South Carolina, both $P < 0.025$ after Bonferroni correction).

Table 1. *Pagurus longicarpus*. Number of denticles on the second segment of the third maxilliped for four populations

Population	*n*	Range	Mean	Variance
Nova Scotia	7	10–12	10.86	0.81
South Carolina	5	9–11	9.80	0.70
Apalachee Bay, Florida	4	12–13	12.75	0.25
Texas	5	10–12	11.20	0.70
Atlantic populations (Nova Scotia + SC)	12	9–12	10.42	0.99
Gulf populations (Florida + Texas)	9	10–13	11.89	1.11

There were no significant differences in the mean overall length to maximum width ratios of walking legs among Nova Scotia; Salem, Massachusetts; or Sarasota, Florida crabs nor in mean dactyl length to propodus length ratios between Nova Scotia and either Massachusetts or Sarasota, Florida crabs (see Table 2). Our a priori hypothesis of differences between the Gulf and Atlantic again was strongly supported by both measurements. The mean overall length to maximum width ratios of walking legs for the two Atlantic populations examined (Nova Scotia + Massachusetts) combined was significantly different than the mean for the Gulf population (Florida) ($P < 0.05$, $t = 1.97$, $df = 148$), and the mean dactyl length to propodus length ratios for the same two Atlantic populations combined was significantly different than the mean for the Gulf population ($P < 0.002$, $t = 1.98$, $df = 148$). Of the various pairwise comparisons, the only comparison that was significantly different after applying a Bonferroni correction was again between a Gulf and an Atlantic population (Massachusetts vs. Florida mean dactyl length to propodus length ratios, $P < 0.001$ after Bonferroni correction).

Allozyme electrophoresis

Of the five loci studied, two were monomorphic (*Ldh* and *Mdh*) and three were polymorphic (*Gpi*, *Pgm*, and *Me*). Each of the three polymorphic loci had only two alleles. Of the 120 crabs examined at five loci, only two individuals showed heterozygosity at any locus; one individual from Massachusetts and one from Florida were heterozygous, both at the *Gpi* locus. At polymorphic loci the χ^2 test for Hardy–Weinberg equilibrium could not be performed because the expected values for genotype frequencies were too small. The UPGMA (unweighted pair-group method using arithmetic averages) dendrogram produced using allele frequencies (Fig. 2a) shows a pronounced differentiation between the Gulf of Mexico and Atlantic populations.

Phylogenetic analysis of mtDNA sequence data

A maximum-likelihood analysis of 49 individuals from two Gulf of Mexico and five Atlantic populations showed a pattern of clear reciprocal monophyly between these areas (Fig. 2b, GenBank numbers AF483107–AF483148 and AF483156–AF483171). Like *P. longicarpus*, *P. pollicaris* shows clear reciprocal monophyly between the Gulf of Mexico and the Atlantic ($n = 6$ for P, 3 for SC, and 3 for W, GenBank numbers pending). However, Da (net nucleotide divergence) for COI third positions between Gulf of Mexico and Atlantic populations is much higher for *P. pollicaris* than for *P. longicarpus* (0.49 ± 0.11 vs. 0.074 ± 0.007).

The date of divergence between the two North Atlantic species (*P. acadianus* and *P. bernhardus*) and their North Pacific sister taxon (*P. armatus*) can be roughly estimated as the age of the trans-Arctic interchange (approximately 3.5 million years ago, Vermeij 1991; Wares and Cunningham 2001). Because the molecular clock could not be rejected (likelihood ratio test as described by Felsenstein 1988), the phylogeny is shown as a maximum-likelihood phenogram constrained to fit the molecular clock (Fig. 3).

Testing for geographic subdivision in the mtDNA data

Five pairs of populations were compared for significance of Da in Arlequin 2.0. All reported *P*-values have been corrected for five comparisons, and did not differ for any of the several genetic distances available in AMOVA. There is no significant geographic subdivision between the Texas and Florida populations ($P > 0.4$), but as expected from reciprocal monophyly, there is significant subdivision between the Florida and Carolina populations ($P < 0.001$). In contrast, there were two significant geographic "breaks" along the Atlantic coast between Nova Scotia and Maine ($P < 0.001$); and between Mas-

Table 2. *Pagurus longicarpus*. Walking leg measurement ratios for three populations ($n = 50$ for all measurements)

Population	Range	Mean	Variance
Total length: maximum width			
Nova Scotia	11.0–16.8	13.58	1.32
Salem, Massachusetts	11.3–19.2	13.98	3.41
Sarasota, Florida	11.5–16.8	13.58	1.53
Atlantic populations (Nova Scotia + Massachusetts)	11.0–19.2	13.78	2.38
Dactyl length: propodus length			
Nova Scotia	0.9–2.5	1.45	0.09
Salem, Massachusetts	1.0–2.3	1.52	0.06
Sarasota, Florida	1.0–1.7	1.35	0.03
Atlantic populations (Nova Scotia + Massachusetts)	0.9–2.5	1.49	0.08

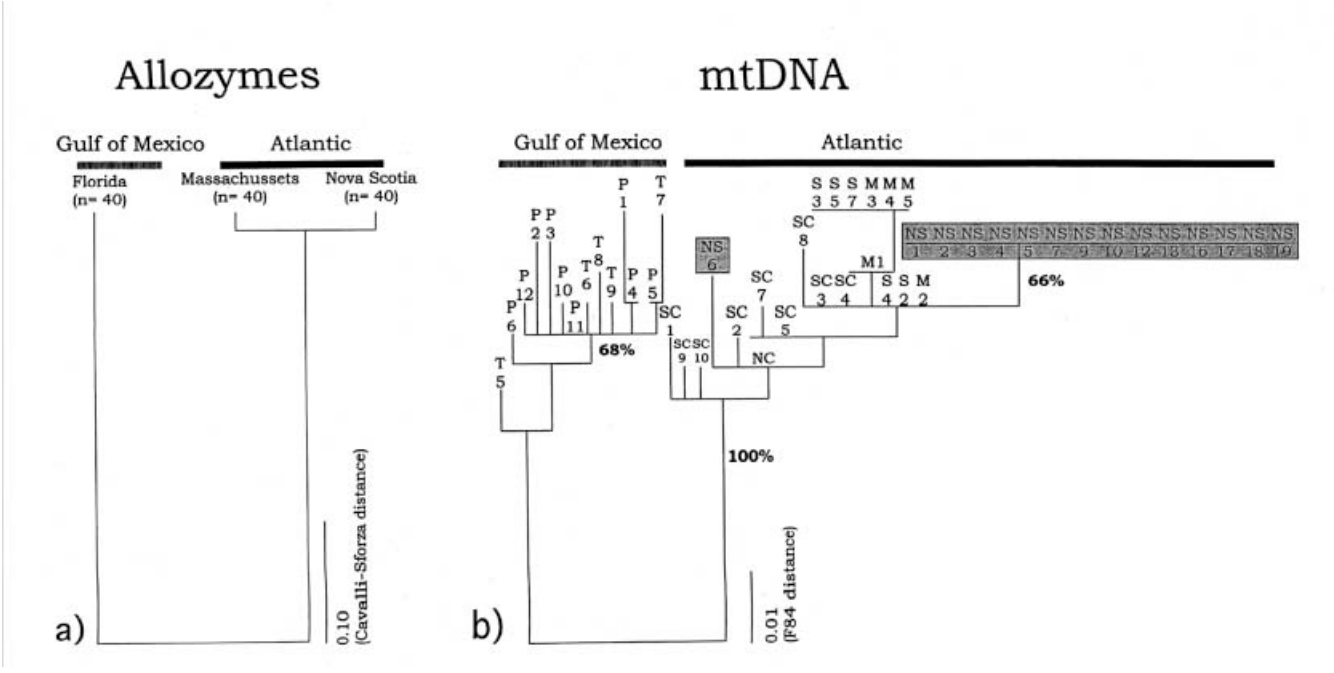

Fig. 2a, b. *Pagurus longicarpus*. **a**. UPGMA dendrogram based on Cavalli-Sforza chord measure calculated from allele frequencies measured at five loci in Atlantic coast and Gulf of Mexico populations. Distances between Gulf and Atlantic populations were 0.755 (Cavalli-Sforza and Edwards 1967 chord distance, shown in figure) and 0.163 (Nei's 1972 distance, not shown). **b**. Maximum-likelihood tree (F84 model) of third positions from mitochondrial cytochrome oxidase I sequences from Atlantic (*NC*; *NS*; *M*; *S*; *SC*) and Gulf (*P*; *T*) populations (site abbreviations see Fig. 1). *Numbers below location letters* refer to individuals. *Shaded haplotypes* from Nova Scotia showed no shared haplotypes with southern populations. Bootstrap percentages >50% from 1,000 equally weighted parsimony pseudoreplicates shown

sachusetts and the Carolinas ($P < 0.05$). When Maine and Massachusetts were combined, the subdivision between New England and the Carolinas was highly significant ($P < 0.001$).

Estimating times of population subdivision

Estimating mutation rates is an inexact science, so several calibrations have been used, including several crustacean taxa thought to have been divided by the Isthmus of Panama, and hermit crab species thought to have been subdivided since the trans-Arctic interchange (Vermeij

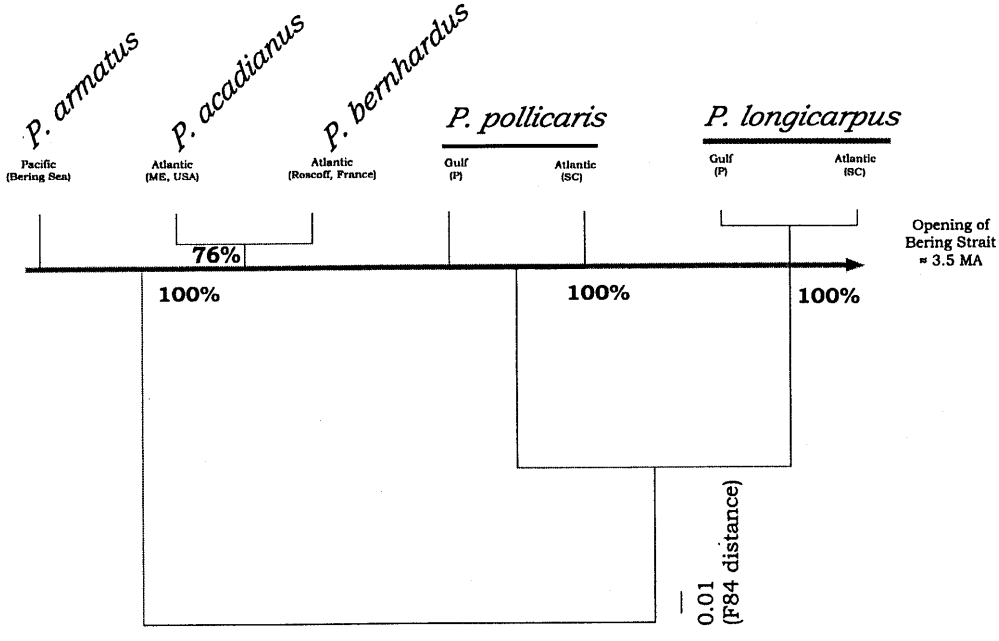

Fig. 3. *Pagurus* spp. Phylogenetic analysis of five hermit crab species, including two from southeastern United States (*P. longicarpus* and *P. pollicaris*), and three cold–temperate species (*P. acadianus*, *P. bernhardus*, and *P. armatus*) from the *bernhardus* group of hermit crabs that participated in the trans-Arctic interchange (*P* Apalachee Bay, Florida; *SC* North Inlet, South Carolina; *MA* million years ago)

1991). For the previously published datasets, we reanalyzed the original sequences from GenBank. This was necessary because the divergences reported in the literature are for all three codon positions, whereas our analyses were confined to third positions only (as in Wares and Cunningham 2001). For trans-Isthmian divergences, we used only the smallest Atlantic/Pacific divergence for any taxon. For COI third positions under the F84 model, this approach gave us rates of the substitutions per site per generation ranging from 2.3×10^{-8} for *Alpheus* sp., a shrimp (Knowlton and Weigt 1998), to 2.9×10^{-8} for *Sesarma* sp., a crab (Schubart et al. 1998), to 4.5×10^{-8} for *Euraphia* sp., a barnacle (Wares 2001a). These values were calculated by Wares and Cunningham (2001).

Because rates of molecular evolution can vary between crustacean lineages, we estimated a lineage-specific rate for hermit crabs that participated in the trans-Arctic interchange (Fig. 3, see above). The average Da between *P. armatus* and the two Atlantic species is 0.418, giving a rate of 6.0×10^{-8}, which is higher than the rates reported for other crustaceans.

The calculations presented in Table 3 use the maximum rate of evolution calculated for COI third positions (for *Pagurus* hermit crabs) and the minimum rate (*Alpheus*) to evaluate the age of divergence for the three pairs of populations showing significant geographic subdivision in *P. longicarpus*, and for the Gulf/Atlantic break in *P. pollicaris*. The age estimates for the hermit-crab-specific rates appear most realistic, especially for the Gulf/Atlantic divergence reported in *P. pollicaris* (4.1 vs. 10.7 million years ago), and these dates will be used in the "Discussion".

Discussion

Both allozyme and mitochondrial DNA sequence data confirm that there is significant genetic divergence between *Pagurus longicarpus* populations in the Gulf of Mexico and on the Atlantic coast of the United States, with an estimated age of divergence of 0.62 ± 0.054 million years ago (Table 3). This divergence is considerably more recent than the divergence between populations of the hermit crab *P. pollicaris* (4.1 ± 0.95 million years ago) (Table 3). Although *P. longicarpus* and *P. pollicaris* share an identical disjunction across southern Florida, and both show substantial genetic divergence between the Gulf of Mexico and the Atlantic, it is very unlikely that they were divided by the same vicariance event. This difference in histories in species with similar disjunctions is an example of pseudocongruence (Page 1990; Cunningham and Collins 1994). This underscores the complex history of vicariance between Gulf of Mexico and Atlantic populations of marine invertebrates, with each glacial maximum having the potential of interrupting gene flow (Reeb and Avise 1990; Felder and Staton 1994).

The long estimated age of divergence between Gulf and Atlantic *P. pollicaris* is worth noting, since a vicariance 4.1 million years ago would be pre-glacial. Although this is based on a hermit-crab specific calibration, estimates based on Panamanian crustaceans would be even older (10.72 million years ago, Table 3). Mechanisms for a pre-glacial vicariance for *P. pollicaris* include the closure of the Suwannee straits across northern Florida ~4 million years ago, as suggested by Cunningham et al. (1991).

The divergence between Gulf and Atlantic *P. longicarpus* populations is also reflected in morphological differences. We observed significant differences in dactyl size and third maxilliped denticle counts between the Gulf and the Atlantic, and confirmed the consistent color differences reported by Provenzano (1959). Given the strong molecular and morphological evidence for independent histories between Gulf of Mexico and Atlantic populations of *P. longicarpus*, these two populations most likely represent separate species.

The two significant genetic breaks in *P. longicarpus* along the Atlantic coast were not expected, since the 2–3 week planktonic larval development period (Roberts 1970) should be sufficient time for dispersal and gene flow among populations. Of the two breaks, the one between Massachusetts and the Carolinas represents a more recent divergence, with a Da that is only barely significant (0.006 ± 0.004), giving an estimated date of $\sim 50{,}000 \pm 30{,}000$ years ago (see Table 3). This divergence appears somewhat older than the last glacial maximum (~18,000 years ago). An entire clade is composed entirely of Maine/Massachusetts individuals, suggesting restricted gene flow between New England and the Carolinas (Fig. 2b). The only haplotype shared between Maine/Massachusetts and Carolina is deeply nested, and therefore is likely to be shared due to ancestral polymorphism. Given the relatively large geographic distance between Massachusetts and the Carolinas, it is possible that the divergence simply rep-

Table 3. *Pagurus* spp. Estimated ages of divergence for significant genetic breaks in populations of *P. longicarpus* and *P. pollicaris* [millions of years, using Da (Nei and Li 1979)]

Genetic break	Species	Da (F84 distance) (substitutions per site)	Age according to calibration with:	
			Trans-Arctic hermit crab	Panamanian shrimp
Nova Scotia/ New England	*P. longicarpus*	0.013 ± 0.004	0.11 ± 0.03	10.28 ± 0.08
New England/ Carolinas	*P. longicarpus*	0.006 ± 0.004	0.05 ± 0.03	0.12 ± 0.08
Gulf of Mexico/ Atlantic	*P. longicarpus*	0.074 ± 0.007	0.62 ± 0.054	1.41 ± 0.12
Gulf of Mexico/ Atlantic	*P. pollicaris*	0.486 ± 0.11	4.10 ± 0.95	10.72 ± 2.50

resents isolation by distance since the last glacial maximum (Slatkin 1993; Hellberg 1996). To test this further it will be necessary to sample populations in the intervening region.

More striking is the genetic break between Maine and Nova Scotia. Although the 500 km distance between these populations is much smaller than between Massachusetts and the Carolinas, the estimated age of divergence is twice as large (108,000 vs. 50,000 years ago, see Table 3). This population subdivision is apparent from inspecting Fig. 2b. Although not enough time has passed for full reciprocal monophyly, there are no haplotypes shared between Maine and Nova Scotia. This is in contrast to the aforementioned shared haplotype between Maine, Massachusetts, and the Carolinas (Fig. 2b).

A pre-glacial divergence between Maine and Nova Scotia suggests that the Nova Scotia population might be descended from a population that occupied a northern refugium during the last glacial episode. This hypothesis is supported by the very low genetic diversity in the Nova Scotia population, with all but one individual sharing a single haplotype (Fig. 2b). This low diversity cannot be explained by a post-glacial colonization from the south, even though it may appear so in the figure from the fact that Nova Scotia haplotypes are nested within a paraphyletic southern population. This paraphyly is one of the expected stages that populations pass through on the way to achieving full reciprocal monophyly (Neigel and Avise 1986). In this case, recent post-glacial expansion is unlikely, because such a colonization would be expected to result in many shared haplotypes between the source populations in the south and a newly founded population in the north, as is the case between New England and the Carolinas. Contrary to this prediction, neither of the two Nova Scotia haplotypes is shared with any southern population.

P. longicarpus represents one of the few species to have a range extending from the Gulf of Mexico to Nova Scotia, which may be related to its ability to survive in a northern refugium. Interestingly, there are hints of a Nova Scotia refugium in two other studies. Wares (2001b) found evidence for a cryptic species of the isopod *Idotea balthica* confined to Nova Scotia, which is distinct not only from most *I. balthica* individuals in Nova Scotia and the Maritimes, but from another cryptic species of *I. balthica* found further south; and a study of the quahog *Arctica islandica* found a unique and strongly divergent haplotype in Nova Scotia that may represent a signal from an ancient refugium (Dahlgren et al. 2000). Further studies of western Atlantic phylogeography will be necessary to test the hypothesis of a northern refugium.

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